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NEW SPECIES OF RED-BACKED VOLE (MAMMALIA: RODENTIA: CRICETIDAE) IN FAUNA OF RUSSIA: MOLECULAR AND MORPHOLOGICAL EVIDENCES

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ABSTRACT

The new species for the fauna of Russia, Hokkaido red-backed vole (*Myodes rex*), has been identified at the south of Sakhalin Island (Dolinsk District). Its identification was reliably confirmed by molecular and morphological methods. Undoubtedly, this species is much more widespread in islands of the Far East. Some records of *M. sikotanensis* from Sakhalin including the so-called "*microtinus*" form, actually, should be reidentified as *M. rex*. The voles with complex molars from Shikotan Island and probably those from Zelenyi (= Sibotsu) Island also belong to *M. rex*.

Key words: *Myodes rex*, mitochondrial DNA, Sakhalin, teeth pattern

РЕЗЮМЕ

На южной оконечности о. Сахалин (Долинский р-н) обнаружен новый для фауны России вид рыжих полевок – *Myodes rex*. Достоверность определения этого вида подтверждена как молекулярным, так и морфологическим методами. Несомненно, этот вид имеет более широкое распространение на островах Дальнего Востока. Часть находок *M. sikotanensis* с о. Сахалин, включая и т.н. форму "*microtinus*", должны быть переопределены как *M. rex*. Полевки со сложным строением зубов с о. Шикотан и, вероятно, с о. Зеленый (= Шиботцу) также должны быть отнесены к *M. rex*.

INTRODUCTION

The genus *Myodes*, or red-backed voles, is widely distributed in Northern Hemisphere and includes 12 species (Musser and Carleton 2005). The territory of Russia is inhabited by 3 species of *Myodes*: the bank vole, *M. glareolus* (Schreber, 1780), northern red-backed vole, *M. rutilus* (Pallas, 1779), and gray red-backed vole, *M. rufocanus* (Sundevall, 1846). Many Russian scientists believe Shikotan red-backed vole *M. sikotanensis* (Tokuda, 1935) distributed in Sikotan, Sakhalin and Zelenyi (or Sibotsu) islands is a separate species (Gromov and Erbaeva 1995; Pavlinov et al. 1995; Frisman et al. 2002; Kostenko et al. 2004). The taxonomic status of this form for a long time was controversial, but recently Motokawa (2008) analyzed the type specimens of *M. sikotanensis* and demonstrated this name is a junior synonym of *M. rufocanus*.

During the mammalogical survey in Sakhalin Island in 2008, we collected *M. rufocanus* in few localities in southern part of the island. Based on the molecular and morphological analyses, we found a new species of vole, previously not recorded for the fauna of Russia.

MATERIAL AND METHODS

Fieldwork was conducted between 19 and 29 August 2008 near Novoaleksandrovsk (vicinity of Yuzhno-Sakhalinsk), in the lower reach of Yablochnaya River (Kholmsk District), and near Sokol Research Station (Sokol) in Dolinsk District of southern Sakhalin. Totally 11 specimens of *M. rufocanus* were collected. A variety of small mammal traps, including pit-fall traps and snap-traps, were used to collect voles, mice and shrews. Specimens were fixed in 70% ethanol. Tissue samples were preserved in 96% etha-

Fig. 1. Localities from which specimens of *Myodes* spp. were genetically analyzed. Locality numbers refer to those in Table 1.

nol. Skulls were extracted and cleaned from ethanol-preserved vole specimens.

Vole skulls were compared with material from Sakhalin (n = 21), Kunashir (n = 50), Shikotan (n = 23) and Hokkaido (n = 1) islands kept in the collections of the Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia (ZIN) and Zoological Museum of Moscow State University, Moscow, Russia (ZMMU). We also studied the original descriptions and figures for skulls of Far Eastern *Myodes* kept in the collections of the Kyoto University Museum, Kyoto, Japan (KUZ) and National Science Museum, Tokyo, Japan (NSM) (Tokuda 1935; Imaizumi 1971; Motokawa 2008).

Total genomic DNA was extracted from ethanol-preserved muscles or liver using proteinase K digestion, NaCl precipitation of proteins, and DNA precipitation with ethanol, following a modification of Miller et al. (1988). We analyzed the samples collected during our field work as well as ethanol-preserved tissues housed in the tissue collection of ZIN and sequences available in the Genbank (see Table 1). The collecting localities are shown in Fig. 1. The arvicoline sequences new to this study have been deposited in the EMBL data bank.

A portion of cytochrome *b* (910 bp) was amplified using primers UCBU/LM (Abramson and Rodchenkova 2007). Double-stranded polymerase chain reaction (PCR) usually entailed 95°C 5 min, followed by 95°C 30 sec, 55°C 30 sec, 72°C 1 min (30 times), with a final extension at 72°C 5 min. All PCR experiments included negative controls. PCR products were visualized on 1.5% agarose gel and then purified using the Qiagen QIAquick kit and sequenced on both strands using automatic sequencing (Big Dye Terminator cycle kit) on an ABI 3130 (PE Applied Biosystems). Sequences were aligned and compared manually using the Bioedit v.7.0.3 (Hall 1999).

Estimates of divergence between haplotypes were calculated by using Kimura's two parameter method, and a neighbor-joining (NJ) phylogenetic tree (Saitou and Nei 1987). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4 (Tamura et al. 2007).

RESULTS

DNA analysis. A total of 23 haplotypes were identified among 41 animals. Of the 817 base pairs (after alignment and removing ambiguous sites), 102 sites were variable and 80 parsimony informative. The NJ reconstruction of phylogenetic relationships between haplotypes is shown in Fig. 2. All studied *M. rufocanus* specimens from island populations clearly split into two groups with mean divergence between them 2%. The first group includes animals from

Table 1. Collecting localities, specimens numbers, samples codes and Genbank accession numbers of *Myodes* spp. genetically examined in this study.

Collecting locality	Specimen No.	Sample code	Locality No. in Fig.1	Accession No.
		M. rufocanus		
Russia, Moneron	91*	Mon-1	1	FJ792779**
Russia, Moneron	93*	Mon-2	1	FJ792780**
Russia, Moneron	241*	Mon-3	1	FJ792781**
Russia, Sakhalin, Okha		Sakh-1	2	AB031573
Russia, Sakhalin, Okha		Sakh-2	2	AB031574
Russia, Sakhalin, Okha		Sakh-3	2	AB031575
Russia, Sakhalin, Okha		Sakh-4	2	AB031576
Russia, Sakhalin, Sokol	235*	Sakh-5	3	FJ792775**
Russia, Sakhalin, Sokol	921*	Sakh-6	3	FJ792776**
Russia, Sakhalin, Sokol	922*	Sakh-7	3	FJ792777**
Russia, Sakhalin, Sokol	920*	Sakh-8	3	FJ792778**
Russia, Sakhalin, Sokol	1322*, ZIN C.98137	Sakh-13	3	FJ792792**
Russia, Sakhalin, Yablochnaya River	1318*, ZIN C.98187	Sakh-9	4	FJ792788**
Russia, Sakhalin, Yablochnaya River	1321*, ZIN C.98125	Sakh-10	4	FJ792789**
Russia, Sakhalin, Novoaleksandrovsk	1314*, ZIN C.98123	Sakh-11	5	FJ792790**
Russia, Sakhalin, Novoaleksandrovsk	1317*, ZIN C.98188	Sakh-12	5	FJ792791**
Russia, Kunashir, Filatovka	1325*, ZIN C.98127	Kun-1	6	FJ792782**
Russia, Kunashir, Filatovka	1326*, ZIN C.98128	Kun-2	6	FJ792783**
Russia, Kunashir, Filatovka	1328*, ZIN C.98130	Kun-6	6	FJ792787**
Russia, Kunashir, Alekhino	1329*, ZIN C.98131	Kun-3	7	FJ792784**
Russia, Kunashir, Alekhino	1331*, ZIN C.98133	Kun-4	7	FJ792785**
Russia, Kunashir, Alekhino	1333*, ZIN C.98135	Kun-5	7	FJ792786**
Russia, Polonskogo Is. (=Taraku Is.)		Tarak	8	AB031564
Japan, Hokkaido, Kuromatsunai		Kuro-1	9	AB031557
Japan, Hokkaido, Kuromatsunai		Kuro-2	9	AB031558
Japan, Hokkaido, Nemuro		Nem-1	10	AB031555
Japan, Hokkaido, Nemuro		Nem-2	10	AB031556
Japan, Hokkaido, Takinoue		Tak-1	11	AB031561
Japan, Hokkaido, Takinoue		Tak-2	11	AB031562
Japan, Hokkaido, Takinoue		Tak-3	11	AB031563
Japan, Hokkaido, Tobetsu		Tobet	12	AB031560
Japan, Hokkaido, Naganuma		Nagan	13	AB031559
Japan, Hokkaido, Ishikari-shi		Hok-1	14	AY309416
Japan, Hokkaido, Ishikari-shi		Hok-2	14	AY309417
Japan, Hokkaido, Ishikari-shi		Hok-3	14	AY309418
Japan, Rebun		Rebun	15	AB031554
Japan, Rishiri		Rishi-1	16	AB031553
		M. rex		
Russia, Sakhalin, Sokol	1323*, ZIN C.98136	Sakh-14	3	FJ792793**
Japan, Hokkaido, Takinoue		Tak-4	11	AB031582
Japan, Rishiri		Rishi-2	16	AB017239
Japan, Hokkaido, Teshio		Tesh	17	AB017240

* number in tissue collection of ZIN
** sequences obtained in this study

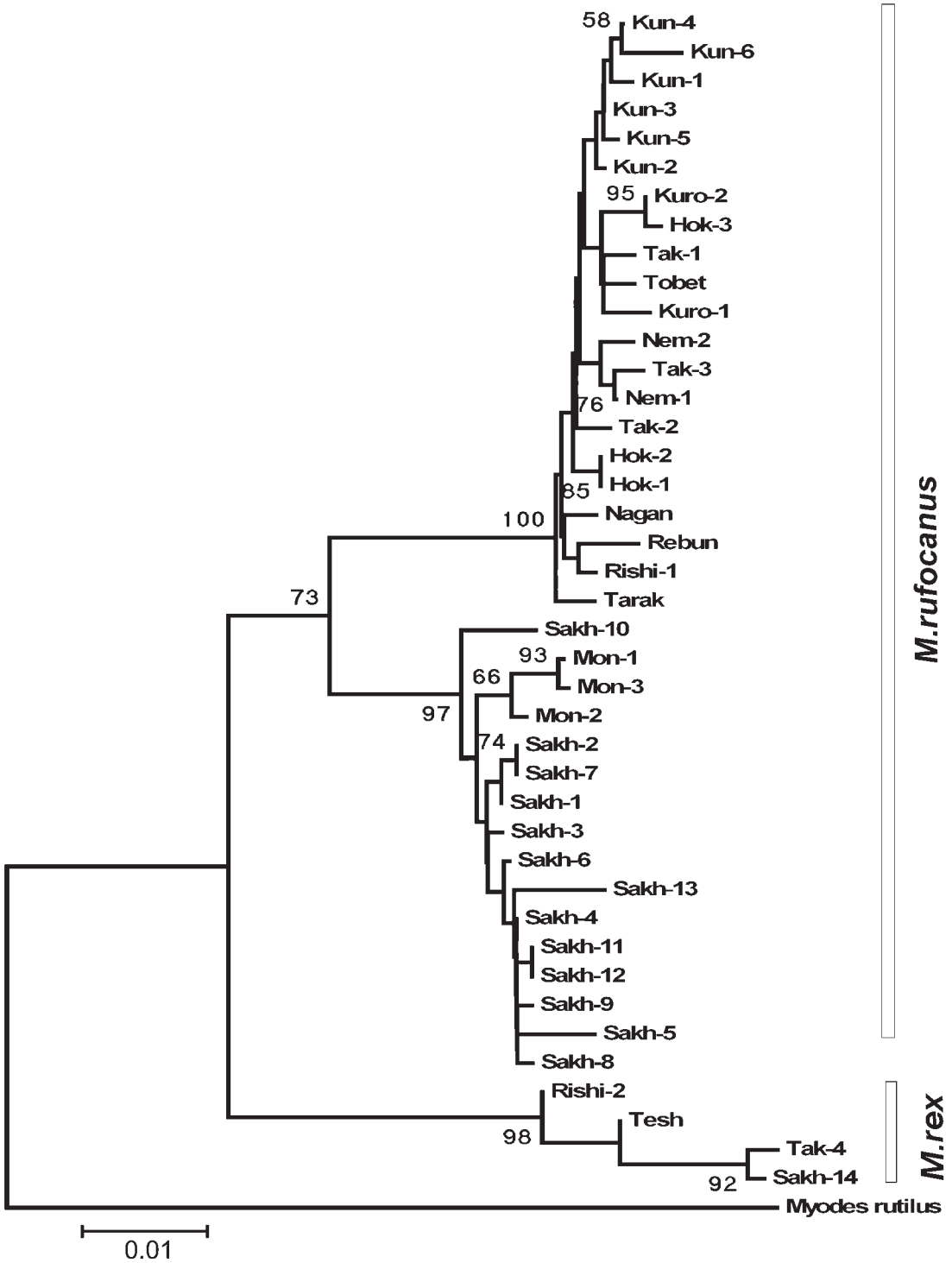

Fig. 2. Cladogram resulted from the phylogenetic analysis of the mitochondrial cytochrome *b* gene (817 bp) in *M. rufocanus* and *M. rex* from the Far East. A neighbor-joining tree was constructed with pairwise sequence divergences that were calculated by the Kimura's two parameter method considering all substitutions at all codon positions. Bootstrap values (over 50) related to the nodes are indicated (1000 replicates). Sources of the haplotypes are listed in Table 1.

Japanese islands and Kunashir, the second one – from Moneron and Sakhalin.

Up to now cytochrome *b* have been sequenced only in three individuals of *M. rex*, with only partial sequences (402 bp) available in Genbank for two of them. However it is remarkable that one individual collected in Sakhalin, near Sokol (ZIN C.98136, see Table 1), has nearly identical haplotype with the known *M . rex*. Two species (*M. rufocanus* and *M. rex*) differ by 50 informative substitutions and mean divergence between *M. rex* and *M. rufocanus* comprises 4%.

Morphology. Most of the studied skulls of red-backed voles from Sakhalin, Kunashir, Shikotan and Hokkaido islands have simple occlusal pattern of the upper third molar, i.e. third upper molars with two deep reentrant and three prominent salient angles both on inner and outer sides (Fig. 3 A–D). This molar pattern is typical of *M. rufocanus*. The type specimens of *M. sikotanensis* also have the same pattern (Tokuda 1935; Motokawa 2008; see Fig. 3A). The voles from Shikotan Island display different patterns on the upper third molar. Some specimens have "simple" molars as *M. shikotanensis* and typical *M. rufocanus* (Fig. 3), whereas others have "complex" molar.

Hokkaido red-backed vole, *M. rex* (Imaizumi, 1971), has complex shape of the upper third molar, i.e. third upper molars with three deep reentrant and four prominent salient angles both on inner and outer sides (Fig. 3 E-H). The specimen ZIN C.98136 from Sokol has the complex pattern as typical *M. rex* (Fig. 3F). It is interesting to note that another specimen from Sokol (ZIN C.98137), captured in the same trap-line, has simple molar pattern and typical *rufocanus*-haplotype of cytochrome *b* (see Table 1, Fig. 2).

We have studied 8 specimens from Sakhalin labeled as "*Clethrionomys sikotanensis*" from the collection of ZMMU. Four of them have a simple upper molar pattern, whereas other specimens from Krilion Peninsula in southern Sakhalin have the complex pattern of the upper third molar similar to that in typical *M. rex* (Fig. 3G). Two of the latter (ZMMU S-67985 and ZMMU S-77229) are labeled by Kuzyakin as type specimens of *Clethrionomys microtinus* (Kuzyakin 1963, nomen nudum).

DISCUSSION

Species taxonomy of red-backed voles in the Far East had been very confusing. Tokuda (1935) described the new species *Neoashizomys sikotanensis* from Shikotan Island, and pointed out the simple molar pattern for this form (see Fig. 3A). However later, the Japanese authors attributed this name to voles with complex molar pattern, distributed in Daikoku and Rishiri islands of eastern and northern coasts of Hokkaido and, probably, also in mainland Hokkaido (Imaizumi 1949, 1960; Abe 1973a, b). Later, Imaizumi (1971) described a new form from Rishiri Island, named *Clethrionomys rex* and distinguished by coloration, larger size and complex molar pattern. Some authors regarded *M. sikotanensis* most likely conspecific with *M. rex* (Iwasa et al. 2000, 2001), but according to the current opinion, this name is a junior synonym of *M. rufocanus* (Kaneko et al. 1998; Musser and Carleton 2005; Motokawa 2008).

Indeed, two forms of voles co-occur in Shikotan Island. In the common taxonomic practice of Russian scientists, *M. sikotanensis* was regarded as a valid species with complex third upper molars, whereas voles with simple molar pattern were referred to *M. rufocanus* (Kostenko and Allenova 1978; Kostenko et al. 2004). According to this point of view, the voles with

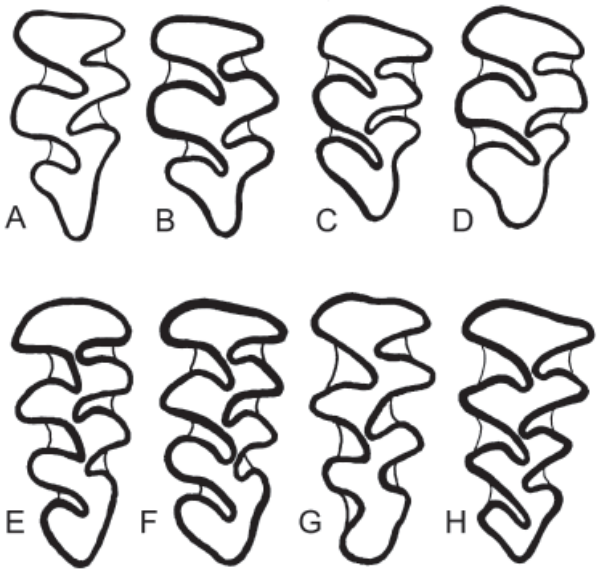

Fig. 3. Pattern of the upper third molar in red-backed voles: *Myodes rufocanus* (A–D), *Myodes rex* (E–H). A – holotype of *Neoaschizomys sikotanensis* Tokuda, 1935, Shikotan Island, KUZ M.9144 (after Tokuda [1935, fig. 4] and Motokawa [2008, fig. 2]); B – Sakhalin Island, Novoaleksandrovsk, ZIN C.98123; C – Kunashir Island, Alekhino, ZIN C.98135; D – Shikotan Island, ZIN C.83150; E – holotype of *Clethrionomys rex* Imaizumi, 1971, Rishiri Island, NSM M.10823 (after Imaizumi 1971, fig. 3); F – Sakhalin Island, Sokol, ZIN C.98136; G – Sakhalin Island, Krilion Peninsula, Shebunino, ZMMU S-67985; H – Shikotan Island, ZIN C.87096. Not to scale.

complex molar pattern from Sakhalin were also classified as *M. sikotanensis* (Gromov and Erbaeva 1995; Pavlinov et al. 1995; Kostenko et al. 2004).

Based on our results, the vole with complex molars from Dolinsk District in Sakhalin should be identified as *M. rex*. This is the first reliably confirmed record of *M. rex* in Russia. Undoubtedly, this species is much more widespread in islands of the Far East. Some records of *M. sikotanensis* from Sakhalin Island including the so-called "*microtinus*" form are related to *M. rex*. Voles with complex molars from Shikotan Island and probably from Zelenyi (or Sibotsu) Island belong to *M. rex* too (see Iwasa et al. 2001).

Proceeding from the data available now the evolutionary scenario suggested earlier by Iwasa et al. (2000) seems very probable. *M. rex* appears to be a derivative of the common ancestor of *M. rufocanus* that colonized the Far-Eastern islands in the Early Pleistocene and evolved in isolation during the Middle Pleistocene interglacial. The populations of *M. rufocanus* inhabiting the islands nowadays colonized them much later during the Late Pleistocene and ecologically superior than the former species (Nakata 1995). Few lines of evidence support this scenario. Based on molecular data, *M. rex* derivates earlier than *M. rufocanus* (Lebedev et al. 2007), and despite of insufficient genetic data for this species all studied specimens of *M. rex* from different localities are dramatically monomorphic in genetic structure. The latter fact suggests not only a small number of founders but also a long time of isolation.

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