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New Data on the Northern Limit of the Range and the Chromosomal Set of the Ussuri White-Toothed Shrew (*Crocidura lasiura*, Soricidae, Lipotyphla)

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Abstract—In an intermontane kettle in the southwestern spurs of the Bureinskii Mountain Range, in the Bureya River basin, the Ussuri white-toothed shrew has been recorded for the first time. This locality lies north of the previously supposed species distribution range in the southern part of the Russian Far East. Study of the female karyotype has allowed us to describe the morphology of the chromosomes of *Crocidura lasiura* ($2n = 40$, NF = 54). The karyotype differs from the earlier published information ($2n = 40$, NF = 56) in the number of subtelocentric autosomes. The data obtained expand the northern range limit of the Ussuri white-toothed shrew and show variability in the number of chromosome arms (NFa = 50, 52).

Keywords: distribution, karyotype, chromosomal variability

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The genus *Crocidura* (shrew) has 198 species distributed in Africa and Eurasia (Burgin et al., 2018). Nine species have been found on the territory of Russia and adjacent territories (Zaitsev et al., 2014). Two types of shrews, *C. shantungensis* Miller 1901 and *C. lasiura* Dobson 1890, live in the southern part of the Far East (FE), are morphologically well differentiated, and occupy different biotopes. Both species have the same diploid number of chromosomes, $2n = 40$, but differences were found between species in the chromosome morphology, expressed by the values of the basic number of arms, NF. In all populations of *C. shantungensis* studied, the number of autosome arms (NFa) is 46, and variability in the Y chromosome of males has been revealed (Iwasa et al., 2001; Kartavtseva and Park, 2010). As will be shown below, conflicting information about the characteristics of the chromosome set of *C. lasiura* NFa = 54 (Orlov and Bulatova, 1983) or NFa = 52 (Zima et al., 1998) refers to the same specimen from southern Primorye. This makes a new description of the karyotype of this species based on our find from the northern border of its range relevant.

The Ussuri white-toothed shrew (*C. lasiura*) is the largest species of the genus found in Russia, the Korean Peninsula, and Northeast China. The species inhabits coastal and floodplain biotopes, and in forests it is found in clearings or in the floodplains of small rivers and streams (Zaitsev et al., 2014). The northern border runs on Russian territory along the valley of the northern bank of the Amur River in the Jewish Autonomous Oblast (about 133^0 E) and follows along it east to the city of Khabarovsk (Nesterenko, 1999). The northernmost record of the species (Fig. 1*d*) is marked in a small intermountain basin in the southwestern part of the Bureinskii Range (49°25′17.15 N, 133°17′15.31 E) in Khabarovsk krai, on the bank of the Urmi River, beyond the village of Dogoron (Frisman et al., 2019). Further east, the species was recorded along both banks of the Amur in the Middle Amur Lowland to the city of Komsomolsk-on-Amur in Khabarovsk krai (Nesterenko, 1999). Through the territory between the Ussuri River and the river valleys of the western spurs of Sikhote-Alin, it descends south into the Khanka lowland, the Razdolnaya River valley, and to the extreme southwestern part of Primorskii krai, to the mouth of the Tumannaya River. The species is found in wet areas of the coast of the Sea of Japan and treeless areas of river valleys on the eastern slope of Sikhote-Alin in Primorskii krai, up to approximately 43° N (Okhotina, 1984). It is found on two islands, Russkii and Vera, in Peter the Great Bay (Nesterenko, 1999).

Rare encounters of the Ussuri white-toothed shrew in the northern part of its range did not allow us to delineate clearly the boundaries of the species' distribution (Fig. 1); the northwestern habitats were conditionally drawn along the left bank of the Amur River in Amur oblast (Nesterenko, 1999). In 2009 and 2020,

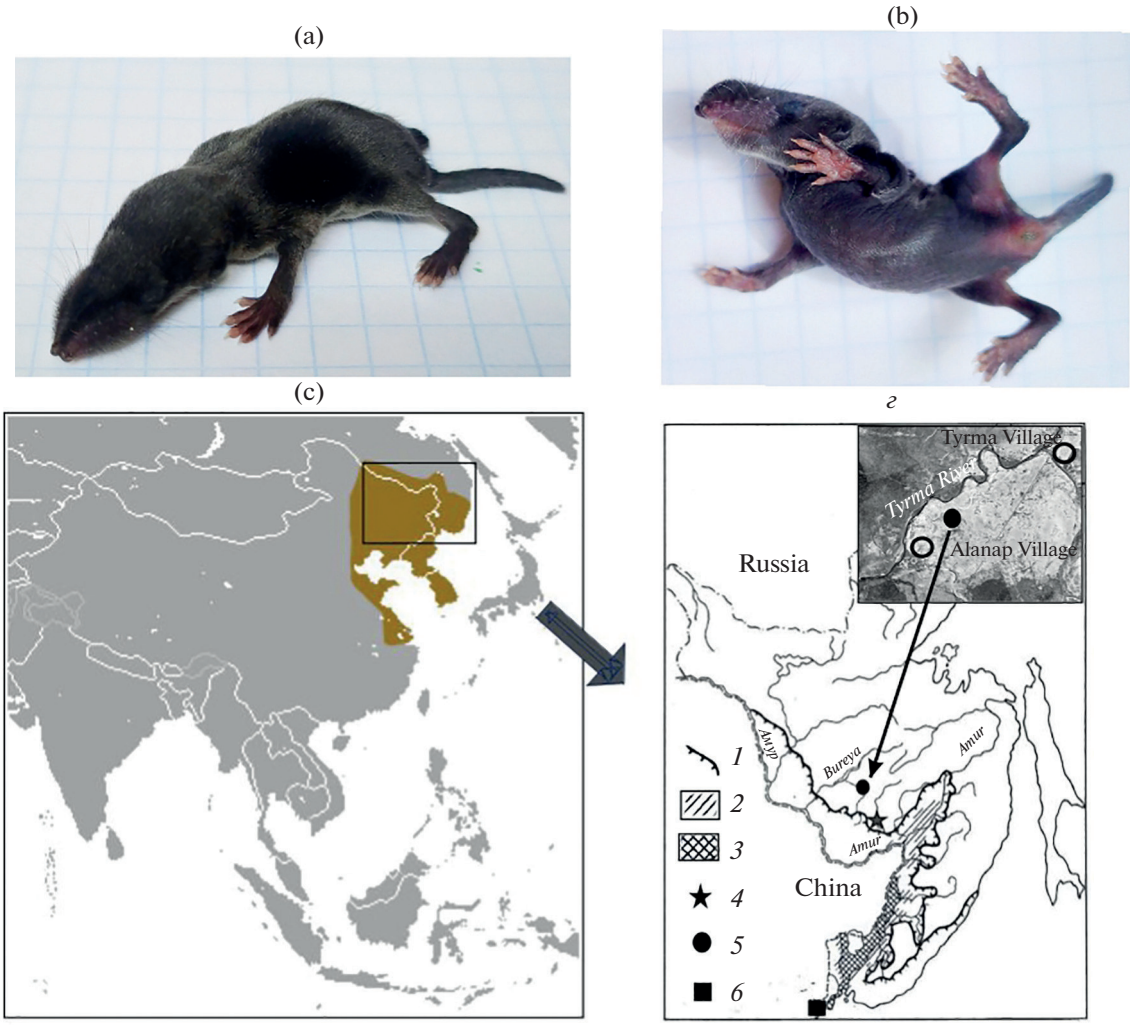

Fig. 1. Distribution and appearance of a karyotyped specimen of the Ussuri white-toothed shrew (*Crocidura lasiura*) (no. 4712): (a) view from above, (b) bottom view. Species range: (c) on the territory of Eurasia (from https://Ussuri White-toothed Shrew area – Ussuri white-toothed shrew – Wikipedia), (d) on the territory of the Russian Far East (according to Nesterenko, 1999). (*1*) Northern border of the range; (*2*) rare encounters; (*3*) frequent sightings, unshaded areas – possible sightings of shrews; (*4*) a recent find from the valley of the Urmi River in Khabarovsk krai (Frisman et al., 2019). Karyotyped individuals: (*5*) valley of the Tyrma River, Khabarovsk krai (our data), (*6*) the environs of the village of Khasan, Primorskii krai (Zima et al., 1998).

the species, as rare and penetrating into the Magdagachinsk district, was included in the *Red Book of Amur Oblast* (status 3, rare), but there are no references to points, collection specimens, and years of specific finds and publications. The latest systematic index of species "Insectivorous fauna of Russia and adjacent territories" states that "the distribution of the species in Amur oblast requires clarification" (Zaitsev et al., 2014, p. 341).

Species of the genus *Crocidura* are rarely widespread, which is probably why their karyotypes have been studied in less than half of the species (Zima et al., 1998). Information about the chromosome set of *C. lasiura* was included for the first time by E.Yu. Ivanitskaya in the list of karyotyped mammal species in the book *Sravnitel'naya tsitogenitika i kariosistematika mlt-kopitayushchikh* (Comparative Cytogenetics and Karyosystematics of Mammals) (Orlov, Bulatova, 1983). In tabular form, without indicating the point of capture, the values of diploid chromosome number $2n = 40$ were published, the total number of arms of autosomes NFa = 54, and the designation of the morphology of sex chromosomes X and Y is A (acrocentrics). Taking into account the acrocentric X chromosome, the total number of arms in this karyotype (not indicated in the table (Orlov and Bulatova, 1983)) will be NF = 56.

Later, in a review of the chromosomal evolution of Soricidae (Zima et al., 1998), apparently updated information was provided to the published description of the karyotype of *C. lasiura*, with reference to additional data from E.Yu. Ivanitskaya with the designa-

tion: Far East, Russia. The chromosome layout was presented without indicating the point of geographical origin of the materials and without comments on the morphology of chromosomes. For *C. lasiura* (in the table of chromosome numbers of the species *Crocidura*), the chromosomal characteristics are given ($2n$ = 40, NF = 56), similar to those indicated in the previous description, but with changes in the designations of the sex chromosome morphology. Thus, the X- and Y-chromosomes are defined differently, as submetacentric (SM), i.e., with a pronounced second arm in comparison with the conditionally one-armed acrocentrics (A) of the previous description (Orlov, Bulatova, 1983). Only in the case of a double-armed X chromosome will the NFa value no longer be 54, but 52, which does not correspond to the values in the original message.

Here it is worth making the following clarifications. One of the authors of this work was directly related to the materials on the karyotype of *C. lasiura* published in 1998. Earlier I.V. Kartavtseva obtained photographs of the metaphase plates of the Ussuri shrew (male) and submitted them to E.Yu. Ivanitskaya, who then used them when preparing a summary in the publication by Orlov and Bulatova (1983), as well as by Zima et al. (Zima et al., 1998). The data on the author of the collections and the place of capture were lost for a number of reasons. Thus, conflicting data on the morphology of the chromosome set of the Ussuri shrew, published in two works, refer to the same specimen of this species, caught in the Khasanskii district of Primorskii krai, near the village of Khasan (1976), in the valley of the Tumannaya River at 42°26′07.7″ N, 130°38′44.4″ E (Fig. 1g). E.Yu. Ivanitskaya (personal communication) confirmed to us that she had only this one specimen of *C. lasiura* at her disposal.

Information about the karyotype of *C. lasiura* ($2n$ = 40, NF = 56) in subsequent works (Motokawa et al., 2000, 2001, 2004, etc.) is given with reference only to the publication of 1998. It seemed that the karyotype of the species was stable and well studied. However, a review of the evolutionary history of East and South Asian species and their comparison with West Asian and European taxa (Motokawa et al., 2005) provides conflicting information. Thus, in the table of chromosomal characteristics of the species *Crocidura* for *C. lasiura* in Korea, China, and Russia, the indicated values are $2n$ = 40, NF = 54 (X chromosome is metacentric, Y chromosome is acrocentric), and in the text, $2n$ = 40, NF = 56. However, the references to specimens from Korea and China were unfounded, because we were talking about a karyotype studied only on the territory of Russia.

This message is devoted to the discovery of the Ussuri shrew north of the known range limits of this species, in the intermountain basin of the southwestern spurs of the Bureinskii ridge, in the valley of the Tyrma River in Khabarovsk krai, and a description of its karyotype.

As a result of field work in August 2022, 5 km southwest of the village of Tyrma and 2 km northeast of the village of Alanap, Khabarovsk krai, on a swampy area of the bend of the left bank of the Tyrma River of the same name (50°01′17.55″ N, 132°03′02.60″ E) (Figs. 1*c*, 1*d*), a Ussuri shrew juvenis was found in thickets of rose hips and wormwood, which, perhaps, accidentally became detached from the caravan when the mother was leading the brood (collection no. 4712, stored in alcohol). The cub was alone and attracted our attention with a thin squeak, apparently beckoning its mother. We placed it in the breast pocket of the encephalitis suit, where it stayed until the end of checking the traps we had set up and arriving at the camp, i.e., about four hours. The animal was alive and active. The color of the cub's fur is black above and dark silver below, silky (Figs. 1*a*, 1*b*). The tail is dense, thick, pubescent, slightly two-colored, black above. The foot is black above, white below, and the claws are white. The head is large, and the anterior nasal part, on which the whiskers are well expressed, is elongated in length and width. The eyes are closed; the ears are not pronounced.

Between the points of the new find in the valley of the Tyrma River and the place of the previous discovery of the shrew in the valley of the Urmi River, it is about 100 km in a straight line (Fig. 1*d*). The Tyrma River originates on the southwestern slopes of the Bureya Range and flows into the Bureya River, which in turn flows into the Amur River in the area of the Zeya-Bureya Plain in Amur oblast. The Urmi River originates in the southeastern slopes of this ridge and, merging with the Kur River, flows into the Amur near the city of Khabarovsk. It can be assumed that the shrew penetrates into more northerly regions along the swampy winding river valleys of Khabarovsk krai and Amur oblast. The biotope of the study area is typical for the previously described habitat of the Ussuri shrew with marshy lowlands and tussock meadows overgrown with grass forbs (Gamaleev and Novik, 1964; Nesterenko, 1999). Despite the 353 trap-nights we worked, we were unable to catch adult shrews, although 20 shrews of the genus *Sorex* were caught (in rodent traps in Primorskii krai, both these shrews and white-toothed shrews were caught in traps). This study used Sherman live traps baited with bread and butter. The use of the method of short-term culture of liver cells (Grafodatskii and Rajabli, 1988) made it possible to obtain a suspension of chromosomes and study the karyotype. Metaphase plates were analyzed using an Axio Imager 1 microscope at the Center for Collective Use of the Federal Scientific Center for Biodiversity, Far East Branch, Russian Academy of Sciences.

The chromosomes in our layout (Fig. 2) are divided into three groups (meta-submetacentrics (M-SM), subtelocentrics (ST), and acrocentrics (A)), as was

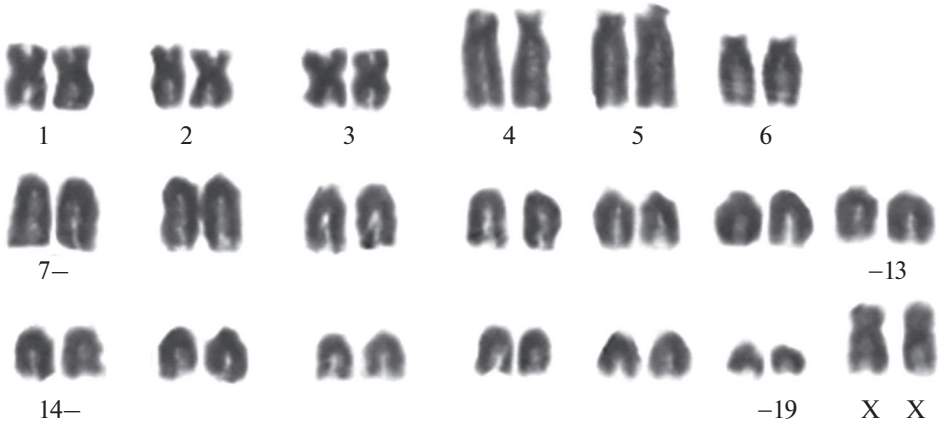

Fig. 2. Female karyotype of *Crocidura lasiura* (No. 4712) from the valley of the Tyrma River, Khabarovsk krai. $2n = 40$, NF = 54, NFa = 50. Three groups of chromosomes: nos. 1, 2, and 3, meta-submetacentrics (M-SM); nos. 4, 5, 6, subtelocentrics (ST); nos. 7–19, acrocentrics (A).

done in works on shrews of Asia and Indochina (Ruedi and Vogel, 1995; Motokawa et al., 1997, 2001, 2004; Kartavtseva and Park, 2010).

The female karyotype consists of 19 pairs of autosomes: three pairs of small meta-submetacentrics (nos. 1–3 in Fig. 2), two pairs of large and one pair of medium-sized subtelocentrics (nos. 4–6), and 13 pairs of acrocentrics (nos. 7–19), gradually decreasing in size from medium to small ($2n = 40$, NFa = 50). The pair of X chromosomes, represented by medium-sized submetacentrics, is slightly larger than the submetacentrics of autosomal pairs. The morphology of the X chromosome is consistent with that of the previously published layout (Zima et al., 1998), but this earlier description and our description give different arm counts, indicating distinct differences in the autosome morphology. In our case (in a female from Khabarovsk krai, Tyrma River) NF = 54 (Fig. 2), and in another (in a female and a male in Primorskii krai, Tumannaya River) NF = 56 (Zima et al., 1998), although on the layout in the cited article the morphological characteristics of the chromosomes are inexpressive and it is not clear how this happened in publication, the number of arms is 56, which, minus the four arms of the double arms of a pair of X chromosomes, for the autosomal set will be NFa = 52. In the new karyotype we described, the autosomal number of arms is less, NFa = 50. The larger number of arms in the original publication (NFa = 54: Orlov, Bulatova, 1983) is clearly erroneous due to the incorrect assignment of a pair of submetacentric sex chromosomes to autosomes, and an acrocentric pair of autosomes to XX chromosomes.

In a comparative analysis of the karyotypes of East Asian and Indochinese 40-chromosomal species, similarity was noted in the number of arms, NF = 56 (*C. lasiura*, *C. fuliginosa* Blyth 1855, *C. dsinezumi* (Temminc 1842), *C. tanakae* Kuroda 1938, and *C. tadae kurodae* Jameson et Jones 1977) (Motokawa et al., 2001). In some cases, intraspecific variation in this trait has been described. Thus, *C. fuliginosa* from western Malaysia has NF = 54–58 as a result of variability in the number of ST chromosomes from four to six pairs with the presence of two meta-submetacentric pairs (Ruedi et al., 1990). For another species, *C. tadae* Tokuda et Kano 1936 from the island of Taiwan and the small islands of Green and Orchid, NF varies from 54 to 64 (the number of ST chromosomes varies from three to eight pairs). At the same time, there are three pairs of meta-submetacentric medium sizes (nos. 1, 2, 3) and three pairs of large ST chromosomes (nos. 4, 5, 6) in the karyotype with $2n = 40$, NF = 54. The increase in the number of autosome arms is associated with the appearance of short arms on chromosomes of pairs of smaller sizes, numbers 12, 13, 14, 16, and 17. Variation in the morphology of the Y chromosome (metacentric or acrocentric) has also been described (Fang and Lee, 2002). Later, karyotypes with $2n = 40$, NF = 56, where the short arms of only four pairs of large ST chromosomes were taken into account, while the short arms of smaller chromosomes were ignored. The use of G-staining of chromosomes (Motokawa et al., 2004) made it possible to assign numbers to pairs of autosomes and suggest that the variability in Y-chromosome morphology is due to pericentric inversion. Taking into account our data, the group of species with a variable number of chromosome arms should also include *C. lasiura*. However, it is worth paying attention to the ambiguity in the interpretation of a feature such as the number of arms, which is either taken into account or ignored in the analysis of subtelocentrics.

As a result of this work, it was shown that the Ussuri shrew, in its distribution in Khabarovsk krai and the southern part of the Russian Far East, can reach more northerly regions than previously thought. At the same time, the possibility of its penetration into Amur oblast

cannot be ruled out. A study of the karyotype of this species reveals signs of intraspecific variability in the number of autosome arms ($2n = 40$, NFa = 50, 52). The submetacentric morphology of the X chromosome is confirmed, and there is reason to assume the presence of cytogenetic differences between geographical populations of *C. lasiura*.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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