Current Status of Rare Species of Earthworms (Clitellata: Lumbricidae) in Kuznetsk-Salair Mountain Area (Kemerovo Region, Russia)

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Abstract. The article presents data on the fauna and ecology of earthworms in the Kuznetsk-Salair mountain area. The paper describes the current condition and activities of the conservation of the two rare endemic species listed in the Red Data Book of the Russian Federation – Eisenia salairica and Eisenia malevici. The characteristics of their habitats are given. Data on molecular genetic analysis of Eisenia salairica are presented.

1. Introduction

For the territory of the Russian Federation, 52 species of earthworms of the family Lumbricidae are known. At the same time, 22 species were noted in the Asian part of Russia. Some species of earthworms have a wide cosmopolitan distribution; others inhabit a large part of Eurasia. In addition, quite a large number of species are endemic. The centers of endemism of earthworms in Russia are mountain systems such as the Caucasus and the Urals. In Siberia, such a center is the Altai-Sayan mountain system, where 5 endemic species are noted [1].

The Kuznetsk-Salair mountain area is the north-western tip of the Altai-Sayan mountain region. The first studies of the fauna of lumbricids were conducted in the 1960s when two endemic species were described: Eisenia malevici Perel, 1962 and Eisenia salairica Perel, 1968. In 1984 both species were included in the Red Data Book of the USSR [2] and then in the Red Data Book of the Russian Federation [3] with the categories of conservation status 1 – for Eisenia malevici and 2 for Eisenia salairica, and the Red Data Book of the Kemerovo region [4] with a status of 1 – a species being under the threat of extinction. Initially, both species were found only in one point within the Kuznetsk-Salair mountain area – the vicinity of Zhernovo in Novokuzeisk district of the Kemerovo region on the slope of the valley of the Chernovoy Naryk River, the left tributary of the Tom River, under the canopy of high-grass abies-aspen forest (relict dark coniferous taiga). This territory was within the limits of the regional zoological reserve "Naryk" with an area of about 35 thousand hectares, created for the protection of re-acclimatized beavers, and also elks, and sables. Most of the reserve’s territory was located on the areas of Yerunakovskoy coal Deposit.

In 2001, the reserve was liquidated in the interests of coal companies and soon coal mining began on its territory.

The first surveys of this territory were carried out in 1999 and 2001 when there was no threat to the habitat of species in the vicinity of the Zhernovo. The decision on the coal deposit development was made outside of Kemerovo region. Their implementation became known only from design and survey
or even construction works. The information to scientists and the environmental community has not been reported in advance.

We started monitoring studies of rare earthworm populations in 2013 with the support of gas and coal mining companies. Surveys were conducted in areas potentially suitable for rare species in the middle reaches of the Tom River and directly in the basin of the Chernovoy Naryk River. We found that the habitat of *Eisenia salairica* and *Eisenia malevici* and the Zernovo as the village itself was destroyed by the construction of the mine. At the same time, as a result of studies conducted in the basin of the Chernovoy Naryk River and its tributary Berezovaya River, we found 5 new habitats of *Eisenia salairica* and only one place of occurrence of the species on the left bank of the Tom River on the slope of the Azhenarovsky Ridge. *Eisenia malevici* was not detected.

The question of restoration of the security status at least in part of the territory of the former "Naryk" reserve was raised repeatedly at different levels. After a lot of effort in 2018 managed to negotiate the creation of regional reserve "Chernovoy Naryk" on an area of 283 hectares in one of the known habitats of *Eisenia salairica* in the basin of Chernovoy Naryk River.

The habitat of *Eisenia salairica* found in 2016 on the slope of the Azhendarovsky Ridge near the Burgas River, is under protection, on the territory of the regional complex reserve "Bungarapsko-Azhendarovsky".

2. Methodology

Earthworms of the family Lumbricidae were collected in 2013-2018 in natural biocenoses of the Kuznetsk-Salair mountain area.

Description of plant communities was carried out by conventional methods [5, 6].

Worms were manually sorted out from 25×25 cm patches according to conventional methods. To create a collection fund, the material was preserved in 4% formaldehyde with the addition of glycerin (1:99) and in 70% ethanol according to the generally accepted method of preservation of soil invertebrates [7]. Identification of earthworms was carried out by the Key of T. S. Vsevolodova-Perel [1].

Samples for molecular genetic studies were preserved with 70% ethanol. Genomic DNA was isolated using a DNA extraction kit ("Biosilica", Novosibirsk). Amplification of the cox1 gene fragment was performed using universal primers (HCO2198, 5’-TAAAC-TTCAG-GGTGA-CCAAA-AAATC-A-3’; LCO1490m, 5’-TACTC-AAACAA-ATCAC-AAAGA-TATTG-G-3’) [9]. DNA sequencing was carried out at the SB RAS Genomics Core Facility. The obtained data were processed by the Chromas Lite 2.0 program (Technelysium Pty Ltd). GenBank databases (www.ncbi.nlm.nih.gov/genbank) and BOLD (v3.boldsystems.org) databases, as well as our unpublished data, were used for sequence comparison.

3. Results and discussion

As the result of research conducted in the 2013-2018 on the territory of the Kuznetsk-Salair mountain region, we found 10 species of earthworms, including 4 subspecies: *Dendrodrilus rubidus tenuis* (Eisen, 1874); *Octolasion lacteum* (Örley, 1885); *Aporrectodea caliginosa caliginosa* (Savigny, 1826); *Eisenia fetida* (Savigny, 1826); *Eisenia nordensioldi nordensioldi* (Eisen, 1879); *Eisenia nordensioldi pallida* Malević, 1956; *Eisenia balatonica* (Pop, 1943); *Eisenia salairica* Perel, 1968; *Eisenia sibirica* Perel et Graphodatsky, 1984; *Eisenia atlavinyteae* Perel et Graphodatsky, 1984; *Dendrobaena octaedra* (Savigny, 1826).

In the research conducted on the territory of the Kuznetsk-Salair mountain area, the *Eisenia malevici* was not detected. It is possible that this species had very limited distribution in the Kemerovo region at the Zherново and was completely destroyed by the construction of the coal mine. However, unlike *Eisenia salairica*, *Eisenia malevici* was also found in the Altai: in the vicinity of Gorno-Altaysk, near Ulus-Cherga [9], and on the Western slope of the Salair ridge, in the "Togul'skiy" natural reserve in the Altai region [10].

New habitats of *Eisenia salairica* was discovered in the 2013-2018 in the Chernovoy Naryk River basin in the Novokuznetsk and Prokopyevsk districts (54°15’ N, 87°19’ E), and in 2016-2017 near the
The estuary of the Bugas River in the "Bungarapsko-Azhendarovsky" natural reserve in the Krapivinsky district of the Kemerovo region (54°42’ N, 87°02’ E) (Fig. 1).

Figure 1. The map of the Kuznetsk-Salair mountain area with earthworm sampling sites
(○ – sampling by the authors in 2013-2018; ■ – the origin of the holotypes of Eisenia malevici and Eisenia salairica [11, 12]; ▲ – localities of Eisenia malevici [9, 10]; ● – localities of Eisenia salairica (1 – Chernovoy Naryk, 2 – Bugas; ? – Toguchin district of the Novosibirsk region without specifying the exact habitat [13])

3.1 Characteristic of habitats of earthworms in the basin of Chernovoy Naryk River

The Chernovoy Naryk River originates from the hills around the Aba Mountain (564 m) and flows South-South-East for over 30 km. The right bank is flat forest-steppe, and the left bank is occupied by relict dark coniferous taiga. Further, the river makes a sharp bend to the East, turns to the North-North-East and after 30 km flows into the Tom River. Starting from the bend, both banks of the river are covered with dark coniferous taiga, but rare species of earthworms were noted only in the left-bank part inside the U-shaped bend of the river.

In general, the Chernovoy Naryk River basin is a transition zone from the highlands of the Kuznetsk Alatau to the lowlands of the Kuznetsk Depression. According to the geobotanical zoning of the Kemerovo region, proposed by A. V. Kuminova [14], the studied area is located in the Northern sub-district of the Tom-Kondoma transition region. Mixed secondary aspen-abies and abies-aspen forests are widespread in the most part of the district, with a smaller contribution of birch and aspen forests. The transitional nature of the landscape-geographical complex causes the mixing of forest and forest-steppe zones. Within the study area there are at least 484 species of embryophyte vascular plants belonging to 79 families and 287 genera.

The analysis of scientific literature shows that the right-bank part of the basin of the Chernovoy
Naryk River is characterized by sod-podzolic loamy soils, and the left-bank part, including the habitat of *Eisenia salairica*, by gray soils in complex with dark gray soils [15].

In general, mountain-taiga pseudo-podzolic, brown, and sod-podzolic soils covering all elements of the relief from watersheds to river valleys is characteristic both for the entire area of the taiga foothills of the Western macro-slope of Kuznetsk Alatau and for the basin of Chernovoy Naryk River. Some of the variable features are caused by the proximity of the Kuznetsk Depression and the distribution of patches of pure birch and mixed birch-pine stands.

In the process of studies in the habitats of rare species of earthworms we identified two soil variations. The first variation is gray pseudo-podzolic leached soils. According to genetic links, such soils occupy a transitional position between podzolic soils and chernozems. In the foothills, they are adjacent and combined with forest soils, to varying degrees podzol soils and directly bordered and integrated with mountain-taiga soils. For the area of the basin of Chernovoy Naryk River these soils are not typical, but their presence in sufficient extent may explain the disjunction of the population of the *Eisenia salairica* in the study area.

The second variation, more typical for the basin of the Chernovoy Naryk River, is a complex of light-brown and brown taiga medium-podzolic soils. This type of soil cover was formed under the canopy of high forest grass with a thick layer of litter.

Comparison of morphological descriptions of soil profiles shows that the signs of eluvial-diluvial differentiation are very weak. This is mainly due to the hydrothermal regime of soils, the specificity of which determines the set of factors that form the living conditions of earthworms.

Dense and tall-grass forest stand of dark coniferous taiga, representing a powerful barrier to wind, prevents significant convection under its canopy. Taiga tall grass, shading the soil, covered with a loose layer of forest litter, significantly reduces heat consumption for physical evaporation of moisture from its surface. In winter, sufficiently high precipitation and dense vegetation contribute to the accumulation of relatively high snow cover, reduce the effect of winter temperatures and limit the depth of soil freezing, which favors preservation of relics of flora and fauna, including rare species of earthworms.

3.2. Characteristic of habitats of *Eisenia salairica* in the area of the estuary of the Bugas River

Geographically the "Bungarapsko-Azhendarovsky" natural reserve is located at the junction of two physiographic provinces of the Kuznetsk-Salair mountain area of the Altai-Sayan mountain region – the Kuznetsk Depression and the Kuznetsk Alatau.

Phytocoenotic appearance of the territory is formed by mixed forests with large participation of small-leaved species. On the considerable area, secondary forests were formed at logging sites. At present, mixed forests prevail among forests, in which aspen-abies and abies-aspens plantations are formed under the canopy of birch forests. Less than 1 % of the area is occupied by pine forests, which grow on the ridges. Thus, the most typical plant communities are aspen-abies high-grass and birch motley grass forests.

Due to the transitional nature of the area (at the junction of the mountainous areas of the Kuznetsk Alatau and plain relief of the Kuznetsk Depression), there is a great diversity of soils. Its components in their spatial arrangement are subject not only to zoning but also to the vertical zonality characteristic of mountain countries. The main soil types in the study area are gray soils in combination with dark gray. West of the forest-steppe adjacent to them have podzolic black soil. Throughout the floodplain of the Tom River, alluvial meadow soils are typical [15].

The discovered habitat of *Eisenia salairica* is situated on the Eastern tip of the Azhendarovsky Ridge, at the estuary of the Bugas River. The nature of the forest cover is close to that in the places of the initial description of the species (typical territory) in the basin of the Chernovoy Naryk River. Habitats of relict species *Eisenia salairica* are also situated under the canopy of aspen and abies-aspens forests.

The new site is already under the protection, as it is located on the territory of the regional "Bungarapsko-Azhendarovsky" complex reserve.

The analysis of earthworm abundance in the basin of the Chernovoy Naryk River in the Prokopyevsk and Novokuznetsk districts and the estuary of the Bugas River in the Krapivinsky district of the
Kemerovo region (see Table 1) showed that the invasive species *Octolasion lacteum* is predominant in habitats of *Eisenia salairica*. This factor indicates that earthworm communities are changing and endemic species are at risk of decreasing numbers.

**Table 1.** The fauna and the average number of earthworms in sites of the discovery of new micro-populations of *Eisenia salairica*.

| Species                     | Average number, specimens/m² |
|-----------------------------|------------------------------|
|                             | Chernovoy Naryk | Bugas        |
| *Eisenia nordenskioldi*     | 6.9±0.8          | 13.3±1.0     |
| *Eisenia nordenskioldi*     | 9.6±1.2          | -            |
| *Octolasion lacteum*        | 60.5±7.7         | 56.8±3.6     |
| *Eisenia salairica*         | 0.5±0.3          | 1.6±0.7      |
| *Eisenia sp.* juv.*         | 22.4±3.2         | 16.0±1.4     |

3.3. Molecular genetic studies of *Eisenia salairica*

The question of the species independence of *Eisenia salairica* has recently emerged and is gaining urgency. To address this issue, molecular genetic studies have been conducted. We obtained *cox1* gene sequences of three specimens of *E. salairica* from the Prokopyevsk district of the Kemerovo region (the basin of the Chernovoy Naryk River, aspen-abies forest). As they turned out to be identical, we deposited only one sequence into the GenBank database under the accession number MK118721. Searching the GenBank and BOLD databases and our unpublished data revealed no closely related sequences. *E. salairica* was not genetically close to any of the Siberian (*E. nordenskioldi, E. tracta, E. nana, E. magnifica, E. balatonica*), or Ural (*E. intermedia, E. uralensis*) species of the genus *Eisenia*, for which the sequences of the *cox1* were known, and were more than 15% different from them (Fig. 2).

Thus, *Eisenia salairica* is not a close relative of the Siberian or Ural species of the genus *Eisenia*.

**Figure 2.** Phylogenetic tree based on the *cox1* gene depicting the position of *Eisenia salairica* among Siberian species of the genus *Eisenia*.
4. Conclusions

As a result of our research on the territory of the Kuznetsk-Salair mountain area, we found 10 species of earthworms, including 4 subspecies. *Eisenia malevici*, previously reported from the Kemerovo region, was not detected. It is possible that this species in the Kemerovo region was completely destroyed as a result of coal mining in its habitats. As a result of molecular genetic studies, we found that *Eisenia salairica* is not a close relative of the Siberian or Ural species of the genus *Eisenia*. New habitats of *Eisenia salairica* were discovered by us in the basin of the Chernovoy Naryk River and middle reaches of the Tom River about 45 km North of the previously known habitats of the species. All places of discovery of *Eisenia salairica* are located in the area of grey soil in complex with dark gray soils. One of the habitats of *Eisenia salairica* in the basin of the Chernovoy Naryk River has been brought under protection through the establishment of a comprehensive "Chernovoy Naryk" regional reserve with an area of 283 hectares. The habitat of the species in the middle reaches of the Tom River is in a protected area in the "Bugarapsko-Azhendarovsky" regional reserve.

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