ISSN 1062-3590, Biology Bulletin, 2019, Vol. 46, No. 5, pp. 430–437. © Pleiades Publishing, Inc., 2019.
Russian Text © The Author(s), 2019, published in Izvestiya Akademii Nauk, Seriya Biologicheskaya, 2019, No. 5, pp. 457–465.

Cold Resistance and the Distribution of Genetic Lineages of the Earthworm *Eisenia nordenskioldi* (Oligochaeta, Lumbricidae)

D. I. Berman[a], N. A. Bulakhova[a, b, *], E. N. Meshcheryakova[a], and S. V. Shekhovtsov[a, c]

[a] *Institute of Biological Problems of the North, Far East Branch, Russian Academy of Sciences, Magadan, 685000 Russia*
[b] *Research Institute of Biology and Biophysics, Tomsk State University, Tomsk, 634050 Russia*
[c] *Federal Research Center Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, Novosibirsk, 630090 Russia*
**e-mail: sigma44@mail.ru*

Received March 4, 2019; revised April 15, 2019; accepted April 15, 2019

Abstract—It has been noted that the taxonomic status of genetic lineages isolated within the widespread earthworm species *Eisenia nordenskioldi* is not clear; differences in their cold resistance indicate in favor of advanced intraspecific differentiation. It was found that there are both moderately resistant (tolerating a cooling of –10...–12°C) and surviving at significantly lower temperatures (–28...–34°C) among the four *E. n. nordenskioldi* subspecies lines studied and the two *E. n. pallida* subspecies lines. It was established that the state of ranges of some genetic lines can be explained by worm resistance to negative temperatures, while in some other cases the ranges of genetic lines does not coincide with the expected spatial limits.

DOI: 10.1134/S1062359019050042

INTRODUCTION

Wide use of molecular methods to study the structure of taxa with vast ranges and the development of phylogeography as a powerful direction are the signs of modern biology. Recently, there have been publications about the genetic heterogeneity of a number of earthworm species (King et al., 2008; Novo et al., 2009; Porco et al., 2013; etc.), including *Eisenia nordenskioldi* Eisen, 1879, which is distributed in Eurasia from Eastern Europe to the Pacific Ocean (Shekhovtsov et al., 2016, 2017, 2018a) (Fig. 1). This species is a key species to Siberia, and, as it was believed (Perel', 1979), the only earthworm found on most of its territory, including in regions with extremely low winter temperatures.

It is known that the *E. nordenskioldi* is divided into two subspecies: *E. n. nordenskioldi* (Eisen, 1879) and *Eisenia n. pallida* Malevič, 1956. Molecular genetic studies have detected at least nine genetic lines within the nominative subspecies and at least five lines within the *E. n. pallida* (Shekhovtsov et al., 2016, 2017). Judging by the large number of pairwise nucleotide substitutions between the lines (reaching 20% in mtDNA and 1.2–3.5% in nuclear genes), at least some of them can claim species status. Our study on the *E. nordenskioldi* genetic structure over vast territory (from the Kola Peninsula to Chukotka) has allowed us to outline the ranges of genetic lines only schematically so far (Fig. 1). However, it can now be argued that a number of lines are allopatric (Shekhovtsov et al., 2017).

The allopatry can be determined by differences in the adaptive potential, particularly, different cold resistance (cryoresistance), that is, the ability to tolerate low negative temperatures required for existence in cold regions. Apparently, the range of *E. nordenskioldi* is located completely within territories with low winter temperatures air; however, the conditions in the soil differ significantly in its various geographical parts. The minimal soil temperatures depend not so much on the position of the regions in the geographic coordinate system as on the combination of the degree of winter air cooling and the snow-cover height. As a similar example, we recall that the second "soil cold pole," which is not inferior in severity to the first "pole" in Oymyakon, was allocated in southern Siberia (in Transbaikalia) (Alfimov and Berman, 2006).

On a number of invertebrate taxons, it has been demonstrated that taxonomic proximity does not guarantee similarities in cryoresistance (Berman and Leirikh, 2017). The resistance to cold in worms of different lines of the same subspecies may not be the same, which will indicate in favor of advanced intraspecific differentiation and can allow us to consider the boundaries of the ranges of genetic lineages as a function of winter soil temperature.

To check these assumptions, the cold resistance was determined in the worms of four *E. n. nordenskioldi* genetic lines (1, 3, 7, and 9th) and two *E. n. pallida*

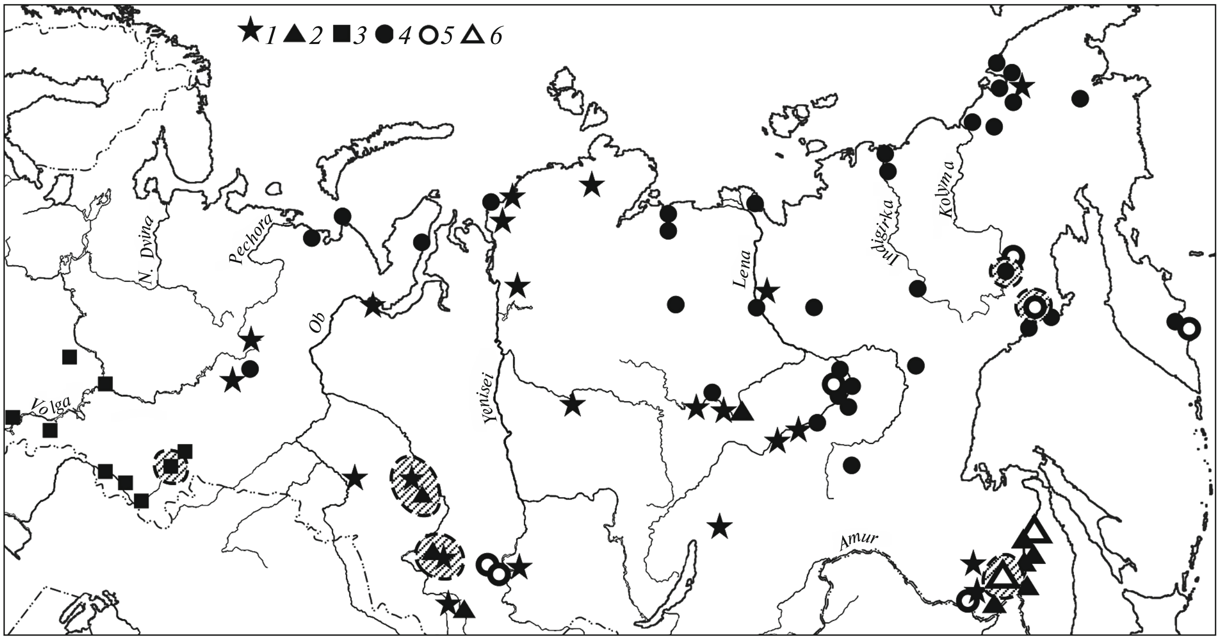

Fig. 1. Distribution of genetic lines of earthworms of two *Eisenia nordenskioldi* subspecies (Shekhovtsov et al., 2017, with additions). *E. n. nordenskioldi* lines: *1,* first; *2,* third; *3,* seventh; *4,* ninth. *E. n. pallida* lines: *5,* first; *6,* third. Shaded areas, places of worm collection for the experiments.

lines (1st and 3rd), and it was compared with winter temperature conditions in the soil within range boundaries previously detected to date (Shekhovtsov et al., 2017) (Fig. 1).

MATERIALS AND METHODS

Worm collection points for experiments. E. n. nordenskioldi of the first and third lines were collected near the village of Podgornoe, Tomsk oblast (57° N, 82° E) and in Novosibirsk Akademgorodok (55° N, 82° E); those of the seventh line, in the vicinity of the village of Faizullino, Republic of Bashkortostan (52° N, 58° E); and those of the ninth, in Magadan oblast (Aborigen Field Station of IBPN FEB RAS, 62° N, 159° E). *E. n. pallida* of the first line was collected in the suburbs of Magadan (59° N, 150° E); that of the third, 5 km from the village of Kazakevichevo, 30 km south of Khabarovsk (48° N, 134° E).

The *E. n. nordenskioldi* worms of first and third lines live together at the points mentioned above and are not clearly distinguished by color; the genetic affiliation of individuals surviving at the threshold temperatures was confirmed after the experiment.

Habitats of worms. Animals were collected just before wintering (at the end of August and in September). In the vicinity the village of Podgornoe worms of first and third lines of the nominative subspecies are common (up to 10 ind./m^2) in the forests of different types along the valley side and in the floodplains (except for long flooded places). The number of worms of the first line is higher in the forests; the number of worms of third line is greater along the forest edges and in places with meadow vegetation. In spring and summer, the worms either live under the litter on the soil surface or sink into the soil of 15–20 cm, but they are not found at specified depths after the first snow falls; that is, their places of wintering are probably deeper than 20 cm.

In the vicinity of Novosibirsk Akademgorodok, worms of both lines of the *E. n. nordenskioldi* are found in the forest litter and upper soil layers in a pine–birch–aspen forest. Before wintering (September–October), the worms of first line are absent in the surface soil horizons (likely evidence that and in the South of the region they go deep). On the contrary, individuals of the third line are found together with *Dendrobaena octaedra* (Savigny, 1826) and *Lumbricus rubellus* Hoffmeister, 1843 in the litter and in the upper centimeters of the mineral horizon.

In the Southern Urals, worms of seventh line were collected in a dry birch forest outlier on the border with the steppe. In late September, they were concentrated at a depth of 10–15 cm.

The worms of the ninth line occupy a many types of habitats: edges of the steppe lots, varuous plant formations on a permafrost and without it, from valleys to highlands in forest and tundra zones. They do not inhabit both stably dry and soils with pH <5 (Berman and Meshcheryakova, 2013).

The *E. n. pallida* first line was collected in the vicinity of Magadan, where it was found only in vege-

Table 1. Steps for determining lower lethal temperature (LLTs, °C) by earthworms of different genetic lines of two *Eisenia nordenskioldi* subspecies

Subspecies	Line	Temperature steps*****
Eisenia nordenskioldi nordenskioldi	1_P*	–5 (30), –8 (42), –10 (13), –12 (28), –13 (15)
	1_N**	–5 (30), –8 (45), –10 (15), –12 (67), –13 (16)
	3_P*	–5 (8), –8 (16), –10 (6), –12 (18), –13 (21)
	3_N**	–5 (0), –8 (0), –10 (0), –12 (19), –13 (15)
	7	–8 (12), –12 (12), –20 (14), –23 (22), –24 (40), –26 (45)
	9***	–12 (25), –14 (25), –16 (25), –17 (25), –19 (25), –21 (25), –22 (25), –23 (25), –25 (25), –28 (25), –30 (25), –34 (25)
Eisenia nordenskioldi pallida	1****	–10 (25), –12 (25), –15 (25), –18 (25), –20 (42), –25 (44), –28 (40), –30 (64)
	3	–5 (18), –7 (15), –10 (30), –12 (30)

* 1_P and 3_P, village of Podgornoe, ** 1_N and 3_N, Novosibirsk; *** (Berman and Leirikh, 1985); **** (Berman and Meshcheryakova, 2013); ***** the sample size is indicated in brackets; for Tables 1 and 2.

table gardens and has been never found on uncultivated land (Berman and Meshcheryakova, 2013).

Near Khabarovsk, the *E. n. pallida* worms of the third line were found on a high places of flood plain of the Amurskaya duct of the Ussuri River occupied by secondary broadleaf forest. They are common here, sometimes extremely numerous (>100 ind./m^2). At the end of September, the worms are no deeper than 10 cm in the soil.

Acclimation and determination of the cold resistance strategy and lower lethal temperature (LLTs) were carried out in the Laboratory of Biocenology of the Institute of Biological Problems of the North, Far East Branch, Russian Academy of Sciences (Magadan), by standard methods developed for other earthworm species (Berman and Leirikh, 1985; Leirikh and Meshcheryakova, 2015).

In preparation for the experiments the acclimation of animals (only mature individuals were used) was carried out in ventilated plastic containers (200 mL) with soil moisture ~35% at the following temperatures: 14 days at 5 and 3°C, then 7 days at 1, 0, and –1°C. The worms already stop feeding at the soil temperatures below 5°C; being disturbed, they move sluggishly. Their integuments remain wet until 0°C and become dry and sandpapery at negative temperatures, and the body becomes almost flat.

To clarify the cold resistance strategy (the tolerance of negative temperatures in a supercooled or frozen state), the supercoling point (*SCP*) was measured and related with LLT. If LLT was lower than *SCP*, it was concluded the worm had the ability to withstand freezing (Block, 1995).

The lower lethal temperature was determined by assessing the survival of individuals at the lowest temperature tested (Table 1). The number and values of temperature steps differ for different lines, since the cold resistance of the worms was not known even approximately before the beginning of the experiments. It was necessary to "grope" it starting from small values. The sample size when studying *SCP* was 10–12 individuals; LLT, from six individuals at intermediate steps up to 67 individuals at close to the lower lethal temperature (Table 1). The worms that survived in the experiments after their heating up to 10°C became active after approximately two days.

Materials on the cold resistance of the ninth line of the *E. n. nordenskioldi* and the first line of the *E. n. pallida* subspecies have been published previously (Berman and Leirikh, 1985; Berman and Meshcheryakova, 2013).

The temperature conditions in places of worm wintering were determined in the vicinity of Podgornoe and near Kazakevichevo by means of autonomous electronic loggers (DS1922L model, accuracy ±0.5°C) at the depths of 2 and 10 cm two times a day. The logger were calibrated before the installation; deviations from the true values did not exceed 0.5°C.

The characteristics of the temperature habitat conditions of the ninth line of *E. n. nordenskioldi* in the Kolyma River headwaters was published previously (Berman and Leirikh, 1985). The long-term average air and soil temperatures at the depths of 20 and 40 cm, as well as maximum snow-cover height, are given according to the USSR Climate Handbooks (1955, 1965, 1966, 1968).

RESULTS AND DISCUSSION

The supercooling point of the worms of all the studied lines are close; their mean values are –4.6...–3.1°C (Table 2). Despite close *SCP* values, LLTs are in the range –34...–10°C; that is, they differ very significantly in different lines (Fig. 2). Judging by the ratio of *SCP* and LLTs, the worms of all considered lines of both *E. nordenskioldi* subspecies can stay for a long time at temperatures that are far below the supercooling point, indicating the ability to withstand freezing. We recall that besides *E. nordenskioldi*, only four freeze tolerant earthworm species are known in the fauna of Russia (perhaps in the world): *Dendrobaena octaedra*, *Drawida ghilarovi* Gates, 1969, *Aporrectodea caliginosa* (Savigny, 1826), and *Eisenia sibirica* Perel et Graphodatsky, 1984 (Berman et al., 2016b).

LLT and distribution of the E. n. nordenskioldi ninth line. Among the worms lines studied, these worms are the most resistant to negative temperatures (Fig. 2): tolerate to –34°C (Berman and Leirikh, 1985) and have one of the most extensive and of the most northern distribution (Shekhovtsov et al., 2017) (Fig. 1). A significant part of their range covers regions with extremely hard winter conditions: Chukotka, River basins Kolyma, Indigirka, Lena, Olenek, Anabar, Yamal Peninsula, etc. Judging by the detected cold resistance of the worms even in an extremely cold region, such as the Kolyma River headwaters have only one type of habitat unsuitable for its wintering - completely snowless lots, in which the soil temperature in the layer 0–5 cm can fall below –40°C at an air temperature –58°C (Alfimov, 1985). Outside continental regions of the Northeast and Yakutia, *E. n. nordenskioldi* can winter in snowless places (Tikhomirov, 1937).

Having among the lumbricide not only outstanding cryoresistance, but also resistance to drought and high acidity of the soil (Berman et al., 2002), worms of ninth line were meanwhile not found in our large collections (Fig. 1) neither in Amur basin, nor in Central Siberia, nor in most of Western Siberia. However, it is obvious that the absence of these animals in relatively well-studied regions of the southern part of Siberia and the Far East cannot be the result of insufficient cold resistance of the worms and their wintering egg cocoons.

LLT and distribution of the seventh line of E. n. nordenskioldi. Among the lines tested, this line of this subspecies is the second by resistance to negative temperatures. Most of the studied individuals sustained cooling to –23°C (Fig. 2). Worms of the seventh line were found in the southeastern European part of Russia and in the Southern Urals (Fig. 1)—in the Republics of Tatarstan and Bashkortostan and Nizhny Novgorod, Orenburg, Saratov, and Chelyabinsk oblasts (Shekhovtsov et al., 2016)—and are not yet known in the Asian part of Russia. No worms of other lines *E. nordenskioldi* were found within their range.

The territory named is located in the area of isotherms of the mean air temperatures in January –12...–16°C (Alisov, 1956). The soil temperature at a depth of 3 cm under a snow cover of average height does not fall below –17°C in the regions mentioned (Alfimov, 2005), while the mean monthly temperatures even in the coldest month are –6.4°C at a depth of 20 cm and –3.5°C at a depth 40 cm (*Spravochnik* ..., 1955, 1965).

Thus, the cold resistance of the worms of the seventh line is much higher than the background winter temperature of the regional soils. The available cryoresistance could allow them to spread both to the west and north and far to the east. Only continental regions of Yakutia and northeastern Asia would be unsuitable for it here. However, this does not happen, since the cold resistance is not the only factor determining the distribution of the *E. nordenskioldi* genetic lines. It can be noted that the seventh line is related to the ninth by mtDNA and is combined with it into a single cluster (Shekhovtsov et al., 2016), which can conditional an affinity of high cold resistance.

Table 2. Supercooling point (*SCP*, °C) of worms of different genetic lines of two *Eisenia nordenskioldi* subspecies

Subspecies	Line	*n*	*SCP* ± *SEM*
E. nordenskioldi nordenskioldi	1_P*	10	–3.6 ± 0.1
	1_N**	10	–3.5 ± 0.3
	3_P*	10	–3.4 ± 0.1
	3_N**	10	–3.1 ± 0.4
	7	10	–4.4 ± 0.4
	9***	48	–3.5 ± 0.2
E. nordenskioldi pallida	1****	11	–4.6 ± 0.3
	3	12	–3.6 ± 0.2

LLT and the distribution of the first and third lines of E. n. nordenskioldi. The worms of these lines are almost two times less resistant to negative temperatures than those of the seventh and almost three times less than the ninth line, but are close to each other by this trait. A quarter to a fifth of animals from the vicinities of the village of Podgornoe and the city of Novosibirsk withstood cooling to –12°C, but did not survive at –13°C. Thus, –12°C should be considered as the LLT (Table 1, Fig. 2).

The worms of the first line were found at 25 localites on the territory that we studied (Fig. 1), mainly in

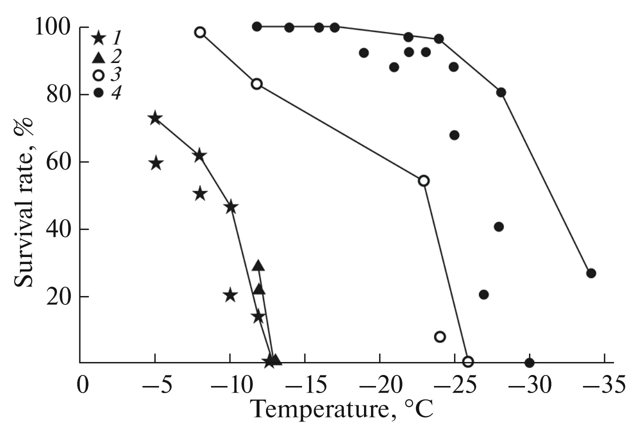

Fig. 2. Survival of worms of four *Eisenia nordenskioldi nordenskioldi* genetic lines after three-day exposure at various temperatures: *1–4*, first, third, seventh, ninth (Berman and Leirikh, 1985) lines, respectively.

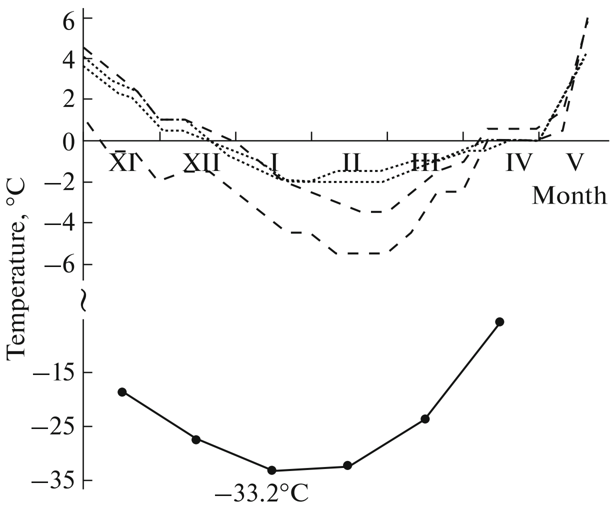

Fig. 3. Course of mean monthly air temperatures (solid curve) and minimal soil temperatures for a decade at the depths of 2 cm (dashed curves) and 10 cm (point curves) from autumn 2013 to spring 2014 in two habitats of the third genetic line of *Eisenia nordenskioldi pallida* worms in Bol'shekhekhtsirskii Reserve. The air temperature is given according to Khabarovsk weather station (distance 27 km).

natural habitats. Three markedly different groups within this line can be allocated according to molecular genetic data: western, which includes the worms from the Middle Urals; Arctic regions (Vaygach Island, Labytnangi village, Taimyr Peninsula), Novosibirsk and Tomsk oblasts; and two eastern found in Khakassia and Yakutia (Shekhovtsov et al., 2018a). Despite the significant range, the western group is characterized by limited genetic variability (~2% variable positions), which probably indicates its recent expansion; the variability of eastern groups is not clear, since there are few points studied.

This genetic "picture" is very different from those for the seventh and ninth lines that have a significant "intralinear" diversity (Shekhovtsov et al., 2017, 2018b). The first line, apparently, expanded its range to huge sizes relatively recently (in the Holocene).

The worms of the third line are distributed less wide. They were found in the southern part of Western Siberia (Tomsk and Novosibirsk oblasts, Altai Republic (Artybash village), on the Markha River (Vilyuy River inflow) near the mouth, in the Jewish Autonomous Region, and along the lower reaches of the Amur (Shekhovtsov et al., 2017).

The worm LLT of −12°C is enough for safe wintering almost everywhere in the southeastern part of Western Siberia and in the middle altitude forest of Altai taking into account the relatively high background of minimal temperatures of the soil surface horizon (Alfimov et al., 2012; Berman et al., 2016b). The temperature minima in the soil at a depth 10 cm did not fall below −6°C near Podgornoe and Tomsk in the vast majority of 12 studied habitats, where the worms were found; they were −12...−13°C only in two habitats (Alfimov et al., 2012). The winter temperature in the vicinity of Khabarovsk in the first 10 cm of the soil was also much higher than −12°C (Fig. 3).

The worms of the first and third lines of the *E. n. nordenskioldi* reach large sizes, which is usually a sign of Lumbricidae-anecic (living in burrows). It is likely that Perel' (Perel', 1982) and a number of other authors after her had in mind the individuals of some of these lines (or both), mentioning especially large octaploid worms *E. n. nordenskioldi* found in Taimyr tundra, on Salair Ridge, and in Moscow and Kursk oblasts (Striganova and Tiunov, 1994; Vsevolodova-Perel' and Bulatova, 2008). "A morph giant for worms three times higher than usual..." was noted by G.N. Ganin (Ganin, 1997, p. 25) for the Far East.

It is very likely that the distribution of worms of the first line in such severe locations as the basin of the upper Bureya River (vicinity of the village of Urgal), where the mean air temperature in January is −31.1°C with a maximum height of the snow cover of only 29 cm, can be explained namely by wintering deep in the soil, which is typical for anecic worms. But there is no permafrost in this region, which probably allows the worms to burrowing into deep soil horizons for the winter.

The finds of the first and third lines worms of *E. n. nordenskioldi* in territories with a cold winter and permafrost (such as Southwest Yakutia or Central Yakutia) seems strange at first glance. Indeed, for example, the mean air temperature of the coldest month is −33.5°C in the vicinity of Olekminsk. But minimal soil temperatures in this territory, as between the Lena and Vilui rivers, can be significantly increased in so-called talik zones (*Geokriologiya...*, 1989) (areas with nonfreezing and close to the surface groundwater located near riverbed river flow, shores of nonfreezing lakes, deep water outlets, and etc.). The highest winter soil temperatures at the depth 20 cm (−11...−5.5°C) were registered within talik zones. They were not lower than −12°C even at a depth of 2–3 cm (Berman et al., 2016a), and mean long-term temperatures at this depth do not fall below −10.4°C in the region (*Spravochnik...*, 1966). Thus, the worms from the territories of Southwestern Yakutia studied can winter in comfortable conditions for them.

However, the worms of the first line were found in the lower reaches of Ob, near Zhigansk (on Lena), the mouth of the Markha River (Vilui inflow), Sobach'e Lake near Norilsk, and on Taimyr Peninsula (Fig. 1). The climate of these regions is extremely harsh, the depth of the seasonally thawed soil layer sometimes barely exceeds a few tens of centimeters. For example, the minimal soil temperature at the depth of 20 cm at Dixon Weather Station in 2002–2010 fell to −21.8°C (probe thermomete) with a snow height of 20–25 cm in February–March (All-Russia Scientific Research

Institute of Hydrometeorological Information—World Data Center site http://meteo.ru/data).

Assuming that the cold resistance of the same earthworm species by the range is the same (Meshcheryakova and Berman, 2014), it is difficult to explain the presence of the first line *E. n. nordenskioldi*, which has a low cold resistance, in the harsh locations named in the previous paragraph. However, the absence of geographic variability of cold resistance was demonstrated on limited material and needs more thorough evidence.

At present, the biology and ecology of the worms of the first line *E. n. nordenskioldi* are completely unexplored. The characteristics its life history were transferred from the ninth line or even from *E. n. nordenskioldi* of undefined lines without any reason. As a minimum, the standard ecological characteristics of the worms of the first line, including the biotopic types, the temperature conditions of wintering, the cold resistance of cocoons and worms of northern populations, etc., should be clarified for objective judgments. We reiterate that the difference in cold resistance in worms and cocoons reaches 30–40°C in some earthworm species (Meshcheryakova and Berman, 2014). Other biological indices of these worms (for example, the possibility of parthenogenesis, development rates) contributing to the formation of small local populations are also important. Such micropopulations can be timed to especially warm winter places (microrelief, position relative to the direction of prevailing winds, etc.) and persist in them for a long time without gene exchange with other populations.

LLT and distribution of the first and third lines of E. n. pallida. The worms of first line from the suburbs of Magadan have an amazingly high cryoresistance at –28°C (Berman and Meshcheryakova, 2013) (Fig. 4). They were found here only in vegetable gardens and do not settle in natural habitats, while worms of the ninth line of the *E. n. nordenskioldi* have never been found in anthropogenic biotopes. For wintering, the animals burrow deep into the cultivated soil down to 60 cm (Berman and Meshcheryakova, 2013), where the temperatures are higher than they tolerate. Thus, the minimal soil temperatures at the depths of 2, 20, and 40 cm at three points in the vegetable garden in the vicinity of Magadan city in autumn 2011–winter 2012 were –10...–13.5°C, –8...–11.5°C, and –6.5...–8.5°C, respectively. The worms of the 1st line of *E. n. pallida* also inhabit the vegetable gardens in the village of Seymchan located in the continental part of the region, where January temperatures are about 10°C lower than in Magadan. The worms of the first line were found only in anthropogenically modified biotopes in Kamchatka, Amur oblast and in the Republic of Khakasia. The remoteness of places of finding (Fig. 1) of these worms with insignificant genetic variation and live in to anthropogenic habitats (unlike the first line

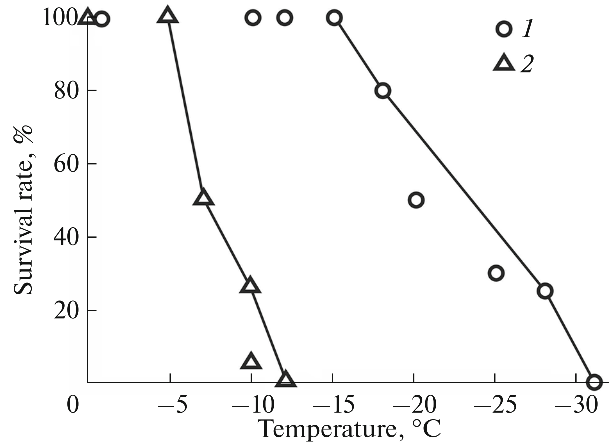

Fig. 4. Survival of worms of (*1*) the first (Berman and Meshcheryakova, 2013) and (*2*) third *Eisenia nordenskioldi pallida* genetic lines after three-day exposure at various temperatures.

of *E. n. nordenskioldi*) indicates a recent expansion with the involvement of humans.

The worms of the third line of *E. n. pallida* found so far only in Khabarovsk krai (Shekhovtsov et al., 2017) (Fig. 1) tolerate temperatures no lower than –10°C. At this temperature, three individuals in the sample out of 12 individuals (25%) survived, while only one (<6%) did in the sample out of 18 individuals (Fig. 4). Thus, these worms are close in LLT values to the animals of the first and third lines of *E. n. nordenskioldi*.

The temperature near Khabarovsk during the winter at the places of the collection of animals did not fall lower than –5.5°C in the horizon of 0–2 cm, or lower than –2°C at a depth of 10 cm; that is, it did not reach the limits of their cryoresistance. The ratio of the soil temperatures and cold resistance of the worms indicates that the population is safe in the studied Ussuri floodplain terrace. But the temperature conditions outside the floodplain are no worse. Thus, the minima in the winter 2007/2008 at a depth of 2 cm were not lower than –11.8°C, and at the depth 25 cm, they were not lower than –8.2°C between the Amur and its tributary, the Tunguska River, near Nikolaevka (our unpublished data).

The LLT *and ranges of genetic lines of the worms—a synthesis*. Based on the above, it would appear that the dependence of the position and size of the ranges on the cold resistance of worms is traced. Indeed, the most resistant ninth line (–34°C) is extremely widespread in the coldest regions of the North and Yakutia. Very low LLT values also have the first line *E. n. pallida* (–28°C) and seventh line of nominative subspecies (–23°C). The first of these lines inhabits cultivated lands near Magadan, but it could be ubiquitous with such cold resistance not only on the coast, but also in continental regions (which is not happening). It is surprising that the seventh line was not found in Asia, as it has clearly excessive and nonadaptive cold resistance relative to the temperature habitat conditions in the southeastern part of Eastern Europe. The cryoresistance of these worms is so great that it indicates the complete independence of their geographical distribution on the temperature conditions of wintering within the whole territory populated by *E. nordenskioldi*.

On the contrary, the cold resistance in the third line of *E. n. pallida* worms is minimal out of those studied (–10°C). It is only slightly higher (–12°C) at the first and third lines of the nominative subspecies. It would seem that the distribution of these three lines should be limited by winter soil temperatures; however, the picture obtained of the association of the position of ranges and the worm cold resistance is much more difficult.

The absence of the ninth line in the south, as well as the seventh line in northern Europe or anywhere in Asia, and, on the contrary, the extremely wide distribution of the first line *E. n. nordenskioldi* with its insignificant cold resistance in Siberia (including in the Subarctic)—all these are examples of the discrepancy between the ecophysiological possibilities of the worms and range occupied by them. Thus, the pattern of the ranges of only a part of the lines can be explained by their resistance to negative temperatures.

CONCLUSIONS

As it turns out, the ability of the *E. n. nordenskioldi* to tolerate temperatures of –34°C, earlier determined in worms from the basin of the Kolyma headwaters, refers not to the species as a whole, but exclusively to its ninth genetics line. Among the four *E. n. nordenskioldi* subspecies lines and two *E. n. pallida* lines studied, there are both those that are moderately resistant (tolerating temperatures of –10...–12°C) and those that survive at significantly lower temperatures (–28...–34°C). The cold resistance values are close in three lines (first and third of the *E. n. nordenskioldi* and third of the *pallida* subspecies) out of six and differ sharply in the three others. The result obtained indicates an intraspecific ecophysiological differentiation while maintaining the ability to tolerate continuous freezing (a common feature for *E. nordenskioldi*).

The pattern of the ranges of the seventh and ninth lines of the *E. n. nordenskioldi* is probably determined by paleogeographic reasons or by other factors unrelated to the temperature, since their cold resistance cannot limit the modern distribution. The worms of three lines of both subspecies are moderately resistant to negative temperatures, which is in accordance with the position of the range in southern Siberia.

It is most difficult to interpret the discrepancy between the low cryoresistance of the worms of the first line of the *E. n. nordenskioldi* (–12°C) measured in the animals in the southern part of Western Siberia and their distribution in the north in the regions with permafrost. This can be clarified by analyzing, first of all, the biotopic types, wintering temperature conditions, and cold resistance of the worms and egg cocoons from northern populations.

ACKNOWLEDGMENTS

The authors are grateful to T.S. Vsevolodova-Perel' (deceased) for clarifying the species affiliation of the worms, A.B. Babenko, I.N. Kornienko, A.A. Kotov, O.L. Makarova, N.A. Petrunina, N.N. Tridrikh, S.P. Kharitonov, Z.A. Yanchenko, and S.A. Yarosheva for collected and the delivery of the worms from remote regions; and Deputy Director of Bol'shekhekhtsirskii Reserve R.S. Andronova for organizational assistance.

FUNDING

This work was supported by the Russian Foundation for Basic Research, project no. 16-04-00082.

COMPLIANCE WITH ETHICAL STANDRDS

Conflict of Interests

The authors declare that they have no conflict of interest.

Statement of the Welfare of Animals

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

REFERENCES

Alfimov, A.V., Thermal regime of the upper soil layers in the main ecosystems of the sparse forest belt of the Upper Kolyma basin, in *Poyas redkolesii verkhovii Kolymy (raion stroitel'stva Kolymskoi GES)* (The Sparse Forest Belt of the Upper Reaches of the Kolyma River (the Kolyma HPP Construction Range)), Vladivostok: Dal'nevost. Nauch. Tsentr Akad. Nauk SSSR, 1985, pp. 9–29.

Alfimov, A.V., Distribution of minimum temperatures in the surface soil layer under the snow in the Northern Eurasia, *Pochvovedenie*, 2005, no. 4, pp. 438–445.

Alfimov, A.V. and Berman, D.I., Two cold poles under the snow cover in Eurasia, *Priroda* (Moscow, Russ. Fed.), 2006, no. 6, pp. 27–33.

Alfimov, A.V., Berman, D.I., and Bulakhova, N.A., Winter temperature conditions in the root zone of soils in Siberia and north-east Asia, *Vestn. SVNTs DVO RAN*, 2012, no. 3, pp. 10–18.

Alisov, B.P., *Klimat SSSR* (Climate of the USSR), Moscow: Mosk. Gos. Univ., 1956.

Berman, D.I. and Leirikh, A.N., On the ability of the earthworm *Eisenia nordenskioldi* (Eisen) (Lumbricidae, Oligochaeta) to tolerate negative temperatures, *Dokl. Akad. Nauk SSSR*, 1985, vol. 285, no. 5, pp. 1258–1261.

Berman, D.I. and Leirikh, A.N., The cold hardiness of mass soil invertebrates of Northeastern Asia: 2. The cold hardiness of soil invertebrates as adaptation to climate, *Biol. Bull.* (Moscow), 2017, vol. 45, no. 7, pp. 680–690.

Berman, D.I. and Meshcheryakova, E.N., Ranges and cold hardiness of two earthworm subspecies (*Eisenia nordenskioldi*, Lumbricidae, Oligochaeta), *Biol. Bull.* (Moscow), 2013, vol. 40, no. 9, pp. 719–727.

Berman, D.I., Leirikh, A.N., and Alfimov, A.V., The hardiness of the earthworm, *Eisenia nordenskioldi* (Oligochaeta, Lumbricidae), to extremely low soil moisture in northeast Asia, *Zool. Zh.*, 2002, vol. 81, no. 11, pp. 1308–1318.

Berman, D.I., Bulakhova, N.A., Alfimov, A.V., and Meshcheryakova, E.N., How the most northern lizard, *Zootoca vivipara*, overwinters in Siberia, *Polar Biol.*, 2016a, vol. 39, pp. 2411–2425.

Berman, D.I., Bulakhova, N.A., and Meshcheryakova, E.N., Cold hardiness and range of the earthworm *Eisenia sibirica* (Oligochaeta, Lumbricidae), *Contemp. Probl. Ecol.* (Moscow), 2016b, vol. 9, no. 1. pp. 45–52.

Block, W., Insects and freezing, *Sci. Progress*, 1995, vol. 78, no. 4, pp. 349–372.

Ganin, G.N., *Pochvennye zhivotnye Ussuriiskogo kraya* (Soil Animals of the Ussuri Region), Vladivostok: Dal'nauka, 1997.

Geokriologiya SSSR (Geocryology of the USSR), Moscow: Nedra, 1989.

King, R.A., Tibble, A.L., and Symondson, W.O.C., Opening a can of worms: unprecedented sympatric cryptic diversity within British lumbricid earthworms, *Mol. Ecol.*, 2008, vol. 17, pp. 4684–4698.

Leirikh, A.N. and Meshcheryakova, E.N., The methods of study of cold hardiness of invertebrates, *Zool. Zh.*, 2015, vol. 94, no. 8, pp. 972–984.

Meshcheryakova, E.N. and Berman, D.I., Cold hardiness and geographic distribution of earthworms (Oligochaeta, Lumbricidae, Moniligastridae), *Biol. Bull.* (Moscow), 2014, vol. 94, no. 4, pp. 486–497.

Novo, M., Almodóvar, A., and Díaz-Cosín, D.J., High genetic divergence of hormogastrid earthworms (Annelida, Oligochaeta) in the central Iberian Peninsula: evolutionary and demographic implications, *Zool. Scripta*, 2009, vol. 38, pp. 537–552.

Perel', T.S., *Rasprostranenie i zakonomernosti raspredeleniya dozhdevykh chervei fauny SSSR* (Occurrence and Distribution Patterns of Earthworms in the Fauna of the USSR), Moscow: Nauka, 1979.

Perel', T.S., Geographical features of breeding of earthworms of the family Lumbricidae (Oligochaeta), *Zh. Obshch. Biol.*, 1982, vol. 43, no. 5, pp. 649–658.

Porco, D., Decaëns, T., Deharveng, L., James, S.W., Skarżyński, D., Erséus, C., Butt, K.R., Richard, B., and Hebert, P.D.N., Biological invasions in soil: DNA barcoding as a monitoring tool in a multiple taxa survey targeting European earthworms and springtails in North America, *Biol. Invasions*, 2013, vol. 15, pp. 899–910.

Shekhovtsov, S.V., Berman, D.I., Golovanova, E.V., and Peltek, S.E., Genetic structure of the earthworm *Eisenia nordenskioldi* (Lumbricidae), *Vavilov. Zh. Genet. Selekts.*, 2017, vol. 21, no. 5, pp. 588–595.

Shekhovtsov, S.V., Golovanova, E.V., and Peltek, S.E., Mitochondrial DNA variation in *Eisenia n. nordenskioldi* (Lumbricidae) in Europe and Southern Urals, *Mitochondrial DNA*, 2016, vol. 27, pp. 4643–4645.

Shekhovtsov, S.V., Berman, D.I., Bulakhova, N.A., Makarova, O.L., and Peltek, S.E., Phylogeography of earthworms from high latitudes of Eurasia, *Acta Zool. Acad. Sci. Hung.*, 2018a, vol. 64, pp. 369–382.

Shekhovtsov, S.V., Berman, D.I., Bulakhova, N.A., Vinokurov, N.N., and Peltek, S.E., Phylogeography of *Eisenia nordenskioldi nordenskioldi* (Lumbricidae, Oligochaeta) from the north of Asia, *Polar Biol.*, 2018b, vol. 41, pp. 237–247.

Spravochnik po klimatu SSSR (Handbook on the Climate of the Soviet Union), Leningrad: Gidrometeoizdat, 1955, 1965, 1966, 1968, nos. 9, 12, 24, 25, 33, pts. II, IV.

Striganova, B.R. and Tiunov, A.V., Foraging strategy of earthworms in the mountain soils in the permafrost, *Izv. Akad. Nauk, Ser. Biol.*, 1994, no. 3, pp. 435–445.

Tikhomirov, B.A., On the habitats of earthworms in tundra soils, *Priroda* (Moscow, Russ. Fed.), 1937, no. 5, pp. 52–58.

Vsevolodova-Perel, T.S. and Bulatova, N.Sh., Polyploid races of earthworms (Lumbricidae, Oligochaeta) in the East European Plain and Siberia, *Biol. Bull.* (Moscow), 2008, vol. 35, no. 4, pp. 385–388.

Translated by A. Barkhash