

PHYLOGEOGRAPHY OF EARTHWORMS FROM HIGH LATITUDES OF EURASIA

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Earthworms are an important component of soil fauna even in high latitudes, in the taiga and tundra biomes. It is yet unclear if earthworm populations from these regions are autochthonous or recent invaders. We collected earthworms from approximately from 64° to 73°N from the Kola Peninsula to Chukotka and genotyped it using the COI gene. We found *Dendrobaena octaedra*, *Lumbricus rubellus*, and *Eisenia nordenskioldi nordenskioldi*. Within *E. n. nordenskioldi*, two cryptic phylogenetic lineages were detected, namely lineages 1 and 9 that were characterized in our previous studies. The western part (from the Kola Peninsula to the Taimyr Peninsula) contained *D. octaedra*, *L. rubellus* and both lineages of *E. n. nordenskioldi*; their COI sequences were closely related to those from very remote (up to several thousand km) populations. On the contrary, in the east (from the basins of the Anabar River to the Chukotka Peninsula) we found solely *E. n. nordenskioldi* belonging mostly to lineage 9 and its haplotype groups from various parts of this region differed significantly, indicating long-term divergence. Thus, our data suggests that earthworms recolonized northwestern Eurasia in the Holocene, while the climate in its eastern part was sufficient for earthworm survival even during glaciation maximums.

Key words: earthworms, Lumbricidae, DNA barcoding, tundra, taiga.

INTRODUCTION

Although earthworms are rather delicate creatures, they are still able to survive in cold and dry climates (MESHCHERYAKOVA & BERMAN 2014). Naturally, they are usually smaller in size and not as numerous in extreme environments as in the much more favorable soil conditions of the temperate zone: in tundra and in acidic taiga soils there are about 2-4 individuals per m² (TIKHOMIROV 1937, STEBAEV 1959). However, in certain intrazonal habitats of these biomes, e.g., on southern slopes with meadow vegetation, earthworm density may be as much as 150 individuals per m² (CHERNOV 1965).

Not all northern regions have equally favorable conditions for earthworms. Fennoscandia, which is heated by the Gulf Stream, harbors the highest earthworm diversity; a wide range of cosmopolitan species that live far to the south (*Lumbricus rubellus* Hoffmeister, 1843, *L. terrestris* L., 1758, *Aporrectodea caliginosa* (Savigny, 1826), *A. rosea* (Savigny, 1826), *Eiseniella tetraedra* (Savigny, 1826), *Dendrodrilus rubidus* (Eisen, 1874), *Dendrobaena octaedra* (Savigny, 1826), *Octolasion cyaneum* (Savigny, 1826)) was found to the north of the 65 parallel (HARALDSEN & ENGELSTAD 1998), and most of these species were detected as far as the northern coast of the Scandinavian Peninsula (TERHIVUO 1988). On the Kola Peninsula, *A. caliginosa*, *D. octaedra*, *D. rubidus*, and *L. rubellus* were found beyond the Polar Circle (ZENKOVA *et al.* 2011, RYBALOV & KAMAEV 2012). Similar earthworm fauna consisting of cosmopolitan species only was detected in Iceland and Greenland (a total of 15 species: BLAKEMORE 2007) and North America (approximately ten species from the 60th to the 64th parallels; no earthworms were found further to the north yet: BERMAN & MARUSIK 1994, REYNOLDS 1995, 2017).

Less is known about the north of Eurasia. In the tundra biome, the prevalent earthworm species is *D. octaedra* in Fennoscandia and the European Russia (MICHAELSEN 1903), and *E. n. nordenskioldi* in the Asian Russia (VSEVOLODOVA-PEREL 1997); ranges of these species partly overlap. *Eisenia atlavinyteae*, a close relative of *E. n. nordenskioldi*, is found in the north of West Siberia and on the Taimyr Peninsula (VSEVOLODOVA-PEREL 1997, STRIGANOVA & PORJADINA 2005). For European tundra, besides the mentioned species, the cosmopolitan *A. caliginosa*, *L. rubellus*, and *L. terrestris* were also reported by several studies (VSEVOLODOVA-PEREL 1997, KONAKOVA *et al.* 2017); however, we should note that to our opinion reports on the latter species are results of misidentification. On the whole, both faunistic records listed above and experimental research on cold tolerance (MESHCHERYAKOVA & BERMAN 2014) suggest that several earthworms can survive beyond the Polar Circle. Although winter air temperatures are very low there, temperature fluctuations in soil are significantly milder.

In addition to harsh climate, soil fauna in the North was significantly affected by the Pleistocene glaciation cycles. It is generally believed that the north of Western Europe and North America were covered by glacial sheets that erased the majority of the fauna (HEWITT 2000), but some species could survive in nunataks (PROVAN & BENNETT 2000). The latter variant seems unlikely for earthworms, however, such hypotheses were proposed for Fennoscandia (FRIDOLIN 1936, STÖP-BOWITZ 1969) and Greenland (HANSEN *et al.* 2006). Northeastern Eurasia, however, had only limited glaciation but harsher climate. Thus it is unclear if the earthworm populations currently inhabiting high latitudes survived glaciations *in situ* or colonized these regions only recently. On the one hand, many earthworm species found in the North, especially *D. octaedra*, are capable of rapid dispersal (JAMES 2011). On the other

hand, certain studies (SHEKHOVTSOV *et al.* 2015, 2018a) suggest that at least some earthworm population living beyond the Polar Circle could survive several Pleistocene glaciations *in situ*.

It is impossible to determine which of the aforementioned hypotheses is correct based on morphological data alone. However, molecular genetic data could reveal that, since earthworms are characterized by very high genetic diversity. Currently no such studies were performed, although populations of various species from more southern regions of Scandinavia do not show significant differences from those of Southern Europe (MARTINSSON & ERSÉUS 2017, MARTINSSON *et al.* 2017). We collected a sample of earthworms from high latitudes of Eurasia (approximately from 64° to 73°N) from the Kola Peninsula to Chukotka and compared their COI mtDNA haplotypes to those from southern regions.

MATERIAL AND METHODS

Earthworm samples were collected in 2010–2016 in 30 locations from the Kola Peninsula to Chukotka (Table 1, Fig. 1) and fixed by ethanol. Morphological identification was performed according to VSEVOLODOVA-PEREL (1997). DNA was extracted from several caudal segments using BioSilica DNA extraction kits (Novosibirsk). A fragment of the COI gene was amplified in 20 µl of mixture containing 60 mM Tris-HCl, 1.5 mM MgCl₂, 25 mM KCl, 10 mM 2-mercaptoethanol, 0.1% Triton X-100, and 1 u of TaqSE polymerase (SibEnzyme, Novosibirsk).

The COI fragment of *E. n. nordenskioldi* was amplified using universal primers LCO1490m (5'-TACTC-AACAA-ATCAC-AAAGA-TATTG-G-3'; modified from FOLMER *et al.* 1994) and HCO2198 (5'-TAAAC-TTCAG-GGTGA-CCAAA-AAATC-A-3'; FOLMER *et al.* 1994); for *D. octaedra* and *L. rubellus* we used LCO1490m and COI-E (5'-TATAC-TTCTG-GGTGT-CCGAA-GAATC-A-3'; BELY & WRAY 2004).

The GenBank database and our own barcoding collection were used for sequence identification. Haplotype networks were constructed using Network 5.0 (fluxus-engineering.com) with the Median Joining algorithm.

RESULTS

We obtained a total of 321 COI sequences belonging to the following species: *D. octaedra*, *L. rubellus*, and *E. n. nordenskioldi* (Table 1); all were 658 bp in length and contained no indels.

Lumbricus rubellus (n = 24) was detected only in the two westernmost locations (no. 1 and 2) in Karelia and the Archangelsk oblast. Six haplotypes were found in 24 studied individuals, all of them belonged to lineage 2 of this species that was earlier found in Western Europe and North America (KING *et al.* 2008, MARTINSSON *et al.* 2018). They differed by up to nine substitutions from the most closely related GenBank entries, i.e. from Great Britain (LT900525), New Zealand (KX790515), USA (JQ909121, JQ909117), and Canada (JQ909105).

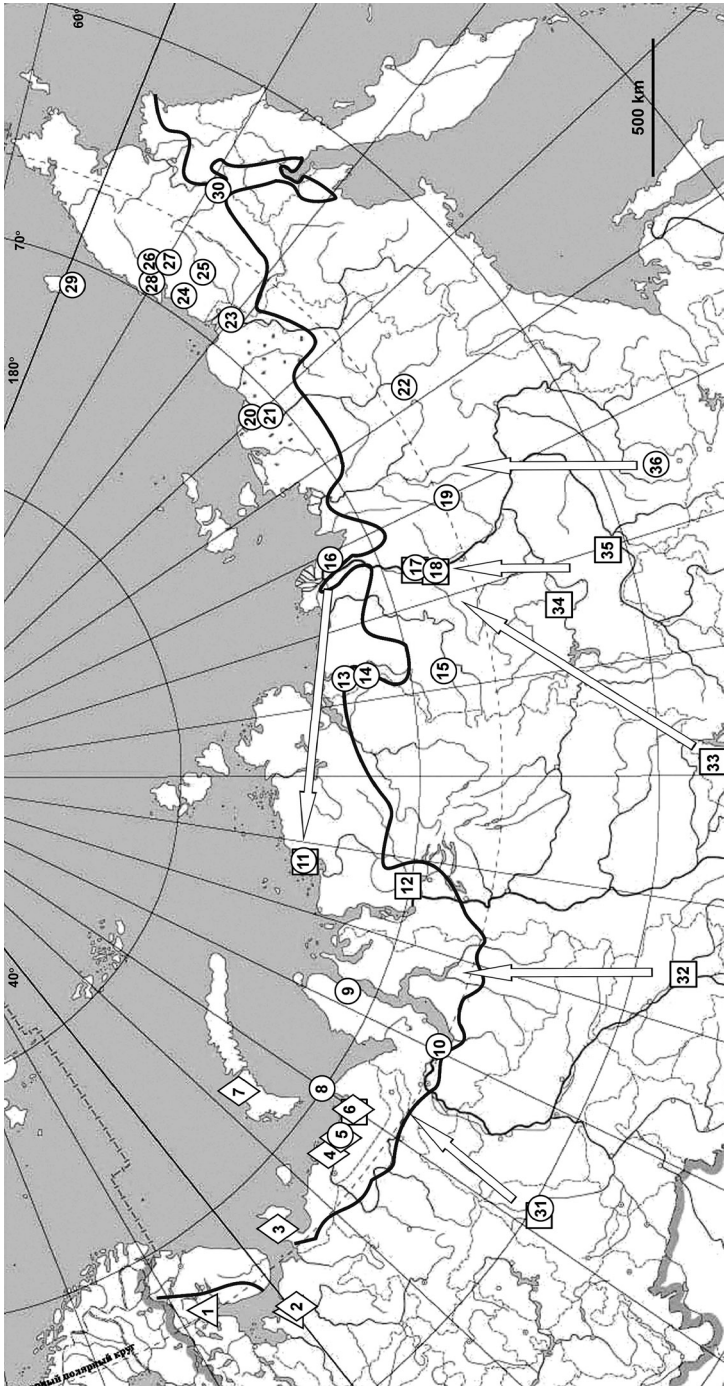


Fig. 1. Localities studied in this paper. Triangle = *L. rubellus*, diamond = *D. octaedra*, square = *E. n. nordenskioldi* lineage 1; circle = *E. n. nordenskioldi* lineage 9. Bold line indicates the boundary of taiga and tundra. Southern locations (31–36) were used as outgroups; arrows indicate probable dispersal directions

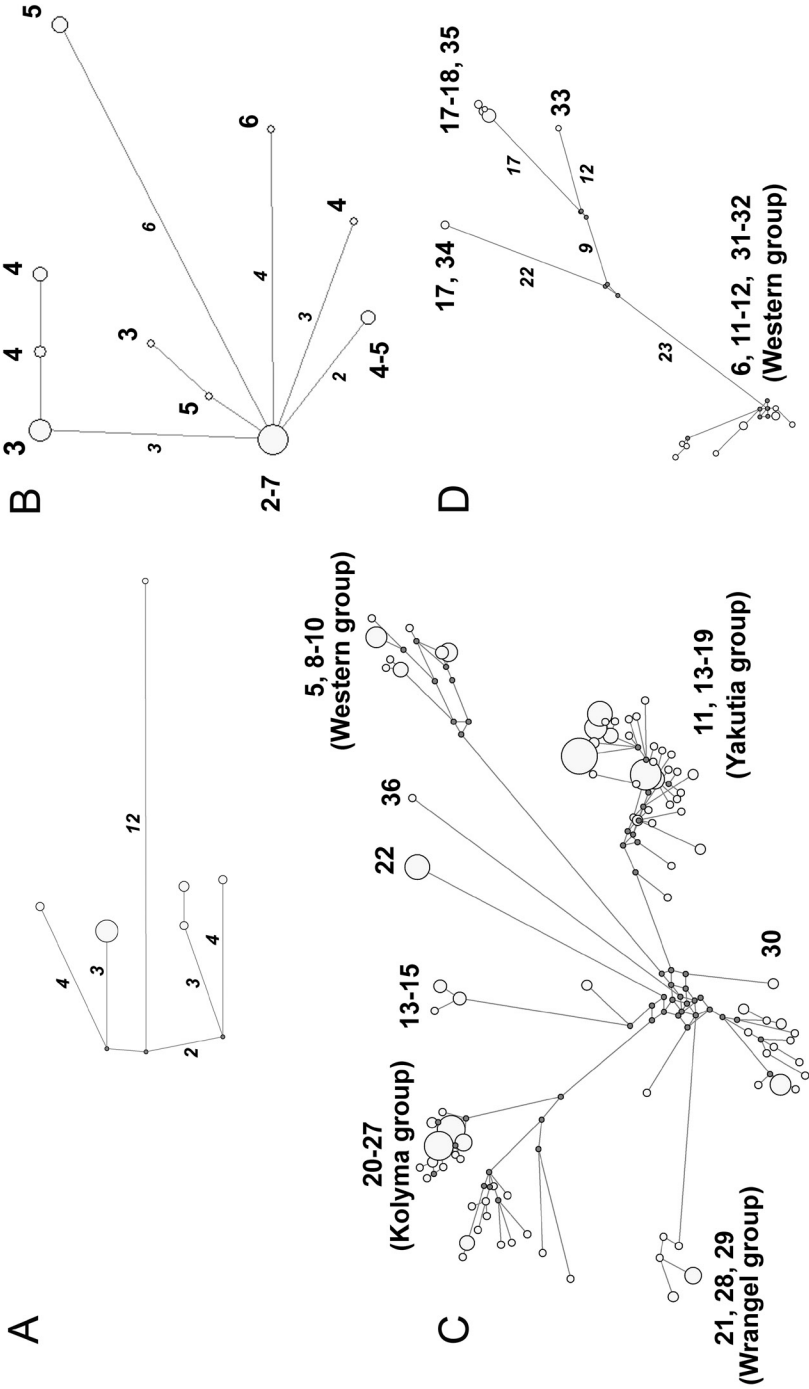


Fig. 2. Haplotype networks of the studied species: a = *L. rubellus*, b = *D. octaedra*, c = *E. n. nordenskioldi* lineage 9, d = *E. n. nordenskioldi* lineage 1. Dots indicate missing haplotypes; circle diameter is proportional to the number of individuals with this haplotype. Numbers near branches (in italics) indicate the number of substitutions; numbers near circles (regular font) refer to location (Fig. 1, Table 1). On (c) and (d), the number of substitutions is given only for long branches due to the lack of space

Table 1. Sampling locations. Location nos. refer to Fig. 1.

no.	Location	N	Species	Coordinates	GenBank acc. nos.
1	Zabornoye lake, Karelia	12	<i>L. rubellus</i> L2	66N 32.8E	MH755618–MH755629
2	Babonegovo, Arkhangelsk oblast	15	<i>L. rubellus</i> L2, <i>D. octaedra</i>	64.427N 41.028E	MH755630–MH755641, MH755667–MH755669
3	Shoina, Nenets AO	7	<i>D. octaedra</i>	67.9N 44.2E	MH755671–MH755677
4	Bolvanskaya Bay, Nenets AO,	7	<i>D. octaedra</i>	68.086N 54.794E	MH755642–MH755647, MH755678
5	Pakhancheskaya bay, Nenets AO	30	<i>D. octaedra</i> , <i>E. n. nordenskioldi</i> L9	68.335N 57.434E	MH755648–MH755665, MH755702
6	Khaipudyr bay, Nenets AO	3	<i>D. octaedra</i> , <i>E. n. nordenskioldi</i> L1	68.281N 59.950E	MH755666, MH755707–MH755708
7	Bezmyannaya bay, Novaya Zemlya islands, Arkhangelsk oblast	1	<i>D. octaedra</i>	72.4N 53.9E	MH755670
8	Vaigach isl., Nenets AO	3	<i>E. n. nordenskioldi</i> L9	69.69N 60.25E	KX601639–KX601641
9	Sabetta, Yamalo-Nenets AO	12	<i>E. n. nordenskioldi</i> L9	71.25N 72.10E	KX601445–KX601456
10	Labytnangi, Yamalo-Nenets AO	1	<i>E. n. nordenskioldi</i> L1	66.66N 66.38E	JX531501
11	Dikson, Krasnoyarsk krai	19	<i>E. n. nordenskioldi</i> L1, <i>E. n. nordenskioldi</i> L9	73.24N 80.39E	KX601286, MH755684–MH755701
12	Taymyr, Sobachye lake	2	<i>E. n. nordenskioldi</i> L1	69.10N 90.90E	MH755709–MH755710
13	Yuryung-Khaya, Yakutia	3	<i>E. n. nordenskioldi</i> L9	72.8N 113.3E	KX601412–KX601414
14	Saskylakh, Yakutia	8	<i>E. n. nordenskioldi</i> L9	71.95N 114.2E	KX601507–KX601513, KX601544
15	Olenek, Yakutia	30	<i>E. n. nordenskioldi</i> L9	68.5N 112.5E*	KX601514–KX601543
16	Tiksi, Yakutia	15	<i>E. n. nordenskioldi</i> L9	73N 126E	KX601593–KX601601, KX601633–KX601638
17	Agrafena, Yakutia	10	<i>E. n. nordenskioldi</i> L1, <i>E. n. nordenskioldi</i> L9	66.1910N 123.8070E	KX601319–KX601326, KX601602–KX601603
18	Zhigansk, Yakutia	7	<i>E. n. nordenskioldi</i> L1, <i>E. n. nordenskioldi</i> L9	66.70N 123.37E	MH755703–MH755706, MH755711–MH755713

Table 1 (continued)

no.	Location	N	Species	Coordinates	GenBank acc. nos.
19	Arkachan, Yakutia	4	<i>E. n. nordenskioldi</i> L9	65.6186N 130.2887E	KX601604–KX601607
20	Russkoye Ustie, Yakutia	8	<i>E. n. nordenskioldi</i> L9	72.2N 149.5E	KX601359–KX601366
21	Kresty, Yakutia	6	<i>E. n. nordenskioldi</i> L9	68.2N 145.3E	KX601415–KX601420
22	Chumpu-Kytyl, Yakutia	25	<i>E. n. nordenskioldi</i> L9	65.35N 143.15E	KX601564–KX601588
23	Cherskiy, Chukotka AO	14	<i>E. n. nordenskioldi</i> L9	68.75N 161.40E	KX601367–KX601380
24	Rauchua, Chukotka AO	4	<i>E. n. nordenskioldi</i> L9	69.15N 166.75E	KX601427–KX601430
25	Bilibino, Chukotka AO	32	<i>E. n. nordenskioldi</i> L9	68.05N 166.50E	KX601422–KX601426, KX601431– KX601444, KX601642–KX601654
26	Valkumei, Chukotka AO	1	<i>E. n. nordenskioldi</i> L9	69.6N 170.3E	KX601421
27	Aiopechan isl., Chukotka AO	8	<i>E. n. nordenskioldi</i> L9	68.85N 170.57E	KX601351–KX601358
28	Pevek, Chukotka AO	3	<i>E. n. nordenskioldi</i> L9	69.849N 170.479E	KX601381–KX601383
29	Wrangel isl., Chukotka AO	5	<i>E. n. nordenskioldi</i> L9	**	MH755679–MH755683
30	Markovo, Chukotka AO	26	<i>E. n. nordenskioldi</i> L9, <i>D. rubidus</i>	64.8N 169.5E	KX601457–KX601482
Outgroups					
31	Karpinsk, Sverdlovsk oblast	3	<i>E. n. nordenskioldi</i> L1, <i>E. n. nordenskioldi</i> L9	59.8N 60.0E	KX601327, JX531499, JX531500
32	Tomsk, Tomsk oblast	2	<i>E. n. nordenskioldi</i> L1	56.4N 85.5E	JX531503–JX531504
33	Solenoozernoye, Khakasia	3	<i>E. n. nordenskioldi</i> L1	54.7N 90.2E	JX531495–JX531497
34	Nakyn, Yakutia	10	<i>E. n. nordenskioldi</i> L1	65.05N 117.15E	KX601306–KX601315
35	Olekminsk, Yakutia	5	<i>E. n. nordenskioldi</i> L1	60.5N 120.2E	KX601287–KX601291
36	Neryungreika, Yakutia	1	<i>E. n. nordenskioldi</i> L9	56.75N 124.59E	KX601619

* collected in unknown location within the Ust-Lena natural reserve

** Two pooled locations, 70.945N 178.7175E and 71.07N 178.9193E

Dendrobaena octaedra (n = 37) was found in six locations (nos. 2–7) from the Arkhangelsk oblast to the coast of the Pechora Sea, as well as on the Southern Island of Novaya Zemlya. We detected eleven haplotypes of this species that were also found in other parts of the worlds. The most widespread haplotype that was present in about a third of individuals was identical to a GenBank accession from Denmark (FJ214235) and differed by one nucleotide substitution from specimens from Austria (DQ092896) and North America (KU496868, KM611907, JQ909028, JQ909013).

Our sample of *Eisenia n. nordenskioldi* was represented by two genetic lineages, 1 (n = 17) and 9 (n = 243) (see SHEKHOVTSOV *et al.* 2013, 2018a). Sequences of lineage 1 could be divided into two groups (Fig. 2d), one from the west (Nenets Autonomous okrug and the north of the Krasnoyarsk krai (Nenets Autonomous Okrug and the north of the Krasnoyarsk oblast; locations 6, 11, 12), and two from the east (middle reaches of the Lena River; locations 17–18). Worms belonging to the second eastern groups were found only on the Agrafena island (location 17). It is noteworthy that each group has close relatives in more southern regions: the sequences of the western group are very similar to those from the Middle Urals and Tomsk (locations 31 and 32) that were found in our previous studies (SHEKHOVTSOV *et al.* 2013); whereas sequences of the eastern group are close to those from Khakassia Republic and the Olekminsk town (northwestern Yakutia) (Figs 1, 2d).

The 9th lineage of *E. n. nordenskioldi* had the highest genetic diversity. In part it may be explained by larger sample size. However, it demonstrated the most pronounced phylogeographic patterns (Fig. 2c). We detected several geographically restricted clusters, i.e., the western groups (from the Nenets Autonomous okrug and Yamalo-Nenets autonomous okrug; locations 5, 8–10); the Olenek group (locations 13–15) with the haplotypes restricted to the basins of Olenek River and Anabar River; the Yakutia group (locations 11, 13–19) that encompassed locations throughout Yakutia; the Kolyma group (locations 20–27) that also included populations from the East Siberian Sea shore; and the Wrangel group (locations 21, 28, 29) with populations from the Wrangel island and the coast of the East Siberian Sea. Several minor branches were detected as well (Fig. 2c). Boundaries between the observed groups were not strict, and highly diverged haplotypes could be found within a single location.

DISCUSSION

Intraspecific diversity

Among our northern samples, *L. rubellus* was found only in the two westernmost locations (1 and 2). Cold tolerance of this species is low (MESHCHERYAKOVA & BERMAN 2014), and its northern records are mostly associated

with human settlements and fishing sites (TIUNOV *et al.* 2006) that suggests that it was introduced there recently. Our genetic data corroborate this viewpoint, as the haplotypes from the studied locations were closely related to those from the West European populations.

Dendrobaena octaedra COI haplotypes from various regions of the world are closely related, and no cryptic phylogenetic lineages can be discerned, as opposed to the majority of cosmopolitan species, e.g. *L. rubellus* (MARTINSSON & ERSÉUS 2017), *A. rosea* (KING *et al.* 2008), *A. longa* (Ude, 1826) (MARTINSSON *et al.* 2017), and *A. caliginosa* (SHEKHOVTSOV *et al.* 2016c). Judging by GenBank entries, *D. octaedra* contains significant intraspecific diversity, but does not demonstrate any geographical patterns: North European populations were found to be very similar to other European ones (CAMERON *et al.* 2008). This is true for Asian populations of this species as well (SHEKHOVTSOV *et al.* 2014, 2018b). Individuals sampled by us were also identical or closely related to those from West Europe and America, which indicates recent colonization, as in the case of *L. rubellus*. It is noteworthy that COI of *D. octaedra* from Novaya Zemlya (location 7) was also very close to West European haplotypes. The Kara Strait is deeper than 100 m, so the archipelago obviously was not connected to the mainland during Pleistocene glaciations. Therefore, *D. octaedra* was most likely introduced to Novaya Zemlya only recently.

However, in contrast to *L. rubellus*, cold tolerance of *D. octaedra* is high both on the adult and cocoon stages (BERMAN *et al.* 2002), so existence of other lineages that survived Pleistocene glaciations in the north and were not yet detected is quite possible. So, HANSEN *et al.* (2006) suggested that for *D. octaedra* from Greenland, which seems to be genetically distant from European and American populations. Unfortunately, this study was performed using allozymes, and its results thus cannot be directly compared with DNA data.

Eisenia nordenskioldi nordenskioldi, like many other earthworm species, was found to contain at least nine genetic lineages that have different distribution patterns and are sometimes associated with different physiographic regions (SHEKHOVTSOV *et al.* 2013, 2016a,b). Each of the lineages is also characterized by significant diversity with deep phylogeographic patterns.

Haplotypes of *E. n. nordenskioldi* lineage 1 from our sample belonging to the western group were closely related to each other (differed by only 1–2 substitutions), as well as to those from the Middle Urals (location 31) and West Siberia (location 32). This suggests their recent dispersal, most probably during the Holocene. It would be reasonable to suggest that earthworms dispersed from the south to the north, and not vice versa, probably along the course of the Ob' River. It is impossible to hypothesize anything about eastern haplotypes of lineage 1 yet, because very few locations were sampled.

The situation with *E. n. nordenskioldi* lineage 9 is more complicated. We do not know where the ancestral range of this lineage may be, but phylogeog-

graphic analysis indicates that populations forming the basal branches on its tree reside the southern Yakutia (location 36) (SHEKHOVTSOV *et al.* 2018a) suggesting that the ancestral range might be in more southern regions. In North-eastern Asia (Yakutia and Chukotka), populations of lineage 9 demonstrate high level of differences among geographic regions (Fig. 2c). Certain clusters are found exclusively in the northernmost locations. Judging by the significant number of substitutions, one can consider that their dispersal occurred long before the LGM.

For example, haplotypes of the Wrangel group (locations 21, 28, 29) differ from those of the Kolyma group (locations 20–27) by about as many substitutions as the western and eastern groups of *E. n. nordenskioldi* lineage 1. This implies that clusters of lineage 9 from the high latitudes survived at least several glaciation cycles (SHEKHOVTSOV *et al.* 2018a).

In the west, from the coast of the Barents Sea to Taimyr (locations 5, 8–11), phylogeographic patterns are dramatically different. Haplotypes from locations 5 and 8–10 are all very closely related to that from location 31 that is far to the south (Fig. 1), and those from Taimyr (location 11) appeared to be very similar to the Yakutia group. Similarly to *D. octaedra* and *E. n. nordenskioldi* lineage 1 from the same regions, we can hypothesize that northern populations of lineage 9 dispersed here from the south and east, respectively.

Global patterns of earthworm distribution in the northern Eurasia

Patterns of current earthworm dispersal in the North were influenced by paleogeographic events. It is well known that during the LGM a big part of northern Europe was covered by a solid ice sheet (SVENDSEN *et al.* 2004). Although Northern Asia had colder climate, precipitation was low, which is why this region underwent only scattered mountain glaciation. The Urals, as well its northern enclave, Novaya Zemlya, was undoubtedly either covered by glaciers (SVENDSEN *et al.* 2004) or had strongly changed environments (MANGERUD *et al.* 2008). Thus it is reasonable to assume that locations 1 to 8 of our sample are from the territories whose soil fauna was altered or “erased” during the LGM.

The observed patterns of earthworm distribution were in accordance with this viewpoint. Northern territories west of the Urals were populated by cosmopolitan species of European origin. In Northern Europe, climate gets harsher toward the east and only the most resistant species remain. *D. octaedra* had the largest distribution among North European invaders because of its high propensity to dispersal (partly caused by small size and obligate parthenogenesis) and high cold tolerance (up to -16°C ; BERMAN *et al.* 2002).

Although Northeast Asia had extremely harsh climate during glaciation periods, the sufficient conditions for earthworm survival seem to have maintained, and *E. n. nordenskioldi* was ubiquitous here (we failed to detect *E. atlavyniteae* in our sample).

Genetic data obtained in this study is in agreement with the abovementioned data. Our sample can be divided in two parts, western (locations 1–12) and eastern (locations 13–30) ones. The western set contains several species that are similar in that all of them have haplotypes very close (98–100% sequence similarity) to those from populations sampled in thousands kilometers from them, i.e., in West Europe (*D. octaedra* and *L. rubellus*) or southwestern Siberia (*E. n. nordenskioldi* lineage 1). Populations of *E. n. nordenskioldi* lineage 9 from locations 5, 6, and 9 are very close to that from location 31 from Middle Urals (Fig. 1), while that from the Taimyr, are resembling haplotypes from Yakutia (locations 16–19). It is reasonable to suggest that such dispersal patterns reflect recent, most probably Holocene dispersal.

The eastern part of our sample was mostly represented by lineage 9 of *E. n. nordenskioldi*. The observed patterns were geographically structured and contained significant genetic differences among population groups, indicating that they diverged long ago, at least before the LGM.

The obtained molecular data are in good accordance with the conventional paleogeographic data, that suggest that northern Europe biota suffered strong disturbance during glaciation maximums, while northern Asia had only limited glaciation and environmental changes.

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