

Original Russian text www.bionet.nsc.ru/vogis/

Variation in nuclear genome size within the *Eisenia nordenskioldi* complex (Lumbricidae, Annelida)

S.V. Shekhovtsov^{1,2}✉, Ya.R. Efremov¹, T.V. Poluboyarova¹, S.E. Peltek¹

¹ Kurchatov Genomic Center of ICG SB RAS, Novosibirsk, Russia

² Institute of Biological Problems of the North of the Far-Eastern Branch of the Russian Academy of Sciences, Magadan, Russia

✉ shekhovtsov@bionet.nsc.ru

Abstract. The size of the nuclear genome in eukaryotes is mostly determined by mobile elements and noncoding sequences and may vary within wide limits. It can differ significantly both among higher-order taxa and closely related species within a genus; genome size is known to be uncorrelated with organism complexity (the so-called C-paradox). Less is known about intraspecific variation of this parameter. Typically, genome size is stable within a species, and the known exceptions turn out to be cryptic taxa. The *Eisenia nordenskioldi* complex encompasses several closely related earthworm species. They are widely distributed in the Urals, Siberia, and the Russian Far East, as well as adjacent regions. This complex is characterized by significant morphological, chromosomal, ecological, and genetic variation. The aim of our study was to estimate the nuclear genome size in several genetic lineages of the *E. nordenskioldi* complex using flow cytometry. The genome size in different genetic lineages differed strongly, which supports the hypothesis that they are separate species. We found two groups of lineages, with small (250–500 Mbp) and large (2300–3500 Mbp) genomes. Moreover, different populations within one lineage also demonstrated variation in genome size (15–25 %). We compared the obtained data to phylogenetic trees based on transcriptome data. Genome size in ancestral population was more likely to be big. It increased or decreased independently in different lineages, and these processes could be associated with changes in genome size and/or transition to endogeic lifestyle.

Key words: earthworms; *Eisenia nordenskioldi*; genome size; flow cytometry; phylogeny.

For citation: Shekhovtsov S.V., Efremov Ya.R., Poluboyarova T.V., Peltek S.E. Variation in nuclear genome size within the *Eisenia nordenskioldi* complex (Lumbricidae, Annelida). *Vavilovskii Zhurnal Genetiki i Seleksii* = *Vavilov Journal of Genetics and Breeding*. 2021;25(6):647–651. DOI 10.18699/VJ21.073

Изменчивость размеров ядерных геномов у представителей комплекса *Eisenia nordenskioldi* (Lumbricidae, Annelida)

С.В. Шеховцов^{1,2}✉, Я.Р. Ефремов¹, Т.В. Полубаярова¹, С.Е. Пельтек¹

¹ Курчатовский геномный центр ИЦиГ СО РАН, Новосибирск, Россия

² Институт биологических проблем Севера Дальневосточного отделения Российской академии наук, Магадан, Россия

✉ shekhovtsov@bionet.nsc.ru

Аннотация. Размеры ядерного генома у большинства эукариот определяются преимущественно содержанием мобильных элементов и некодирующих последовательностей и варьируют в широких пределах. Они могут значительно различаться как между крупными таксонами (причем размер генома не коррелирует со сложностью организма – так называемый С-парадокс), так и между близкородственными видами в пределах рода. В то же время размах внутривидовой изменчивости по этому параметру изучен значительно хуже. Комплекс *Eisenia nordenskioldi* объединяет несколько близкородственных видов дождевых червей, широко распространенных на Урале, в Сибири и на Дальнем Востоке России, заходящих своими ареалами и в сопредельные регионы. Для этого комплекса характерна значительная морфологическая, кариотипическая, экологическая и генетическая изменчивость. Целью настоящей работы было оценить размеры ядерного генома у нескольких филогенетических линий комплекса *E. nordenskioldi* при помощи проточной цитофотометрии. Получены данные о размерах генома для 13 популяций, относящихся к семи филогенетическим линиям *E. nordenskioldi*. Наши результаты показали, что между линиями комплекса наблюдается заметный разброс по размерам, что является еще одним подтверждением их видовой самостоятельности. В целом по размеру генома выборки разделены на две группы. В одну вошли три популяции с небольшим (250–500 м.п.н.), во вторую – с крупным (2300–3500 м.п.н.) размером генома. Кроме того, разные популяции в пределах одной филогенетической линии также имели заметные различия в размере генома (15–25 %). Полученные данные были сопоставлены с филогенетическими деревьями, построенными на основе транскриптомных данных.

Судя по топологии филогенетических деревьев, предковые популяции комплекса с большей вероятностью имели большой размер генома, а уменьшение или увеличение его размера происходило в разных линиях независимо и, возможно, было связано с изменением размеров тела и/или переходом к собственно почвенному образу жизни.

Ключевые слова: дождевые черви; *Eisenia nordenskioldi*; размер генома; проточная цитофотометрия; филогения.

Introduction

The amount of nuclear DNA in eukaryotes varies widely and does not correlate with the complexity of an organism (Cavalier-Smith, 1978; Gregory, 2001). This phenomenon was dubbed the “C-paradox” (Thomas, 1971). Patterns of genome size variation are currently well studied both for higher-level taxa and for groups of closely related species from many diverse phyla (Gregory, 2005). The patterns of intraspecific diversity are generally less known. It is generally believed that genome size and architecture must be common in different populations so they remain genetically and reproductively compatible, i. e. remain a species. There are certain deviations from this rule: differences between males and females due to sex chromosomes; the presence of additional B-chromosomes or large blocks of heterochromatin (Gregory, 2005; Biémont, 2008). However, in most cases intraspecific diversity does not exceed several percent (Blommaert, 2020). The known cases of high variation in intraspecific genome size (Alvarez-Fuster et al., 1991; Marescalchi et al., 1998; Neiman et al., 2011; Stelzer et al., 2011; Jeffery et al., 2016) are often explained by the presence of the so-called cryptic, or sister, species, which were not detected earlier.

The *Eisenia nordenskioldi* (Eisen, 1874) complex is a group of species/genetic lineages of earthworms from the Lumbricidae family widespread in Asian Russia and also found in the East European Plain and certain adjacent countries (Perel, 1979; Zhukov et al., 2007; Blakemore, 2013; Hong, Csuzdi, 2016; Shekhovtsov et al., 2017b). This complex is known for its enormous morphological (Malevich, 1956; Perel, 1979; Vsevolodova-Perel, 1997), karyotypic (Graphodatsky et al., 1982; Vsevolodova-Perel, Bulatova, 2008), ecological (Berman et al., 2019), and genetic (Malinina, Perel, 1984; Shekhovtsov et al., 2013, 2016a, b, 2017a, 2018a, b) diversity. Phylogenetic studies using genomic and transcriptomic data confirmed deep divergence between the lineages of this complex (Shekhovtsov et al., 2019, 2020a, b) and suggested that it could be divided in at least two distinct species.

Remarkable differences between the nuclear and mitochondrial genomes of *E. nordenskioldi* genetic lineages indicate that they diverged long ago (Shekhovtsov et al., 2013, 2015). Significant variation in genome size not associated with polyploidy could thus have accumulated in this complex. To elucidate this question we studied genome size in several genetic lineages of *E. nordenskioldi* using flow cytometry.

Materials and methods

Live earthworms were collected in 2020 in various locations from the Urals, Siberia, and the Far East (see the Table). The worms were rinsed, placed individually in Petri dishes with wet paper and kept for 3–7 days. Genome size was estimated according to the fluorescence of DAPI-stained nuclei of individual cells according to the technique of D.W. Galbraith

et al. (1997). Nuclei were isolated either from several posterior segments of a live earthworm (100–300 µg) or from the whole animal if it was small. A part of the material (about 50–100 µg) was fixed in ethanol for DNA extraction as described below.

Live material was placed in a Petri dish with 500 µl of Galbraith buffer: 45 mM MgCl₂, 20 mM 3-[N-morpholino] propanesulfonic acid (MOPS), 30 mM sodium citrate, 0.1 % Triton X-100 (Galbraith et al., 1983). Material was grinded by multiple strokes with a razor blade. Liquid phase was transferred into an Eppendorf tube. Another 500 µl of Galbraith buffer was added to the Petri dish, and liquid phase was again transferred to the Eppendorf tube. The sample was incubated for 15–60 min, filtered through a 40 µm mesh, and placed on top of 2 ml Galbraith buffer with 3 % glycerol. The tube was centrifuged for 10 min at 200 g; supernatant was discarded, the sediment was dissolved in 500 µl of Galbraith buffer with 10 µl RNase (1 u/µl). The sample was incubated for 30 min, mixed with 100 µl of propidium iodide (1 mg/ml) and analyzed on a FACSaria III flow cytometer (BD Biosciences, USA). We used chicken blood cells (2C = 1250 Mbp) (Kasai et al., 2012) and mouse spleen cells (2C = 3280 Mbp) (Redi et al., 2005) as the reference.

To determine genetic lineage, we sequenced a fragment of the mitochondrial cytochrome oxidase I gene as described in (Shekhovtsov et al., 2018c). Phylogenetic trees built using the Maximum Likelihood and Bayesian inference algorithms were taken from S.V. Shekhovtsov et al. (2020b).

Results and discussion

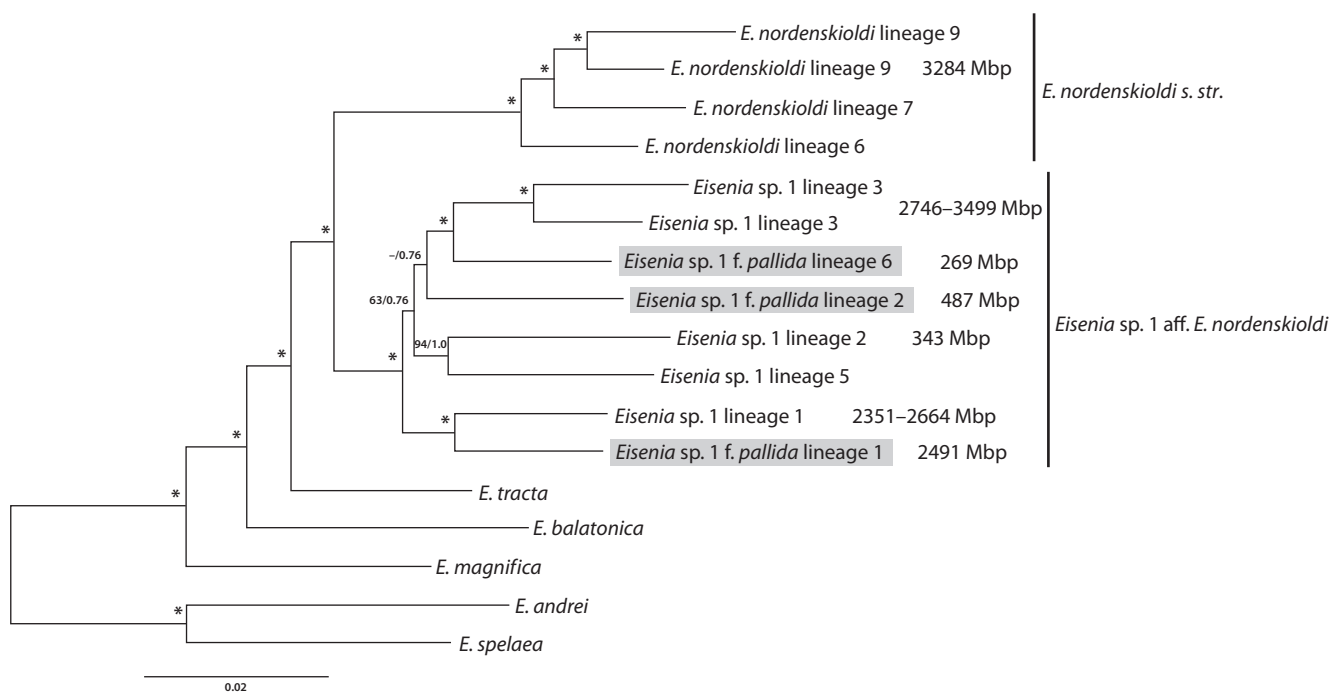
In this study we determined genome size for several genetic lineages of the *E. nordenskioldi* complex (see the Table and Figure). The obtained data indicate high variation in genome size in this complex. We could distinguish two size classes: small (250–500 Mbp) and large (2350–3500 Mbp) genomes. Small genomes were observed in three cases, for two non-pigmented lineages of *Eisenia* sp. 1 aff. *E. nordenskioldi* and for the pigmented lineage 2 of this species. Large genomes (2350–3500 Mbp) were found in the rest of the lineages.

Thus, different genetic lineages of the *E. nordenskioldi* have strongly diverged genomes. This could imply that these lineages represent distinct species (Shekhovtsov et al., 2020a, b), or that this is the result of polyploidy in this complex. It is known that *E. nordenskioldi* consists of races with different ploidy: $2n$, $4n$, $6n$, $7n$, $8n$, with the chromosome number ranging from 36 to 142–152 (Viktorov, 1997). Diploid chromosome set is believed to be characteristic for the non-pigmented *pallida* form (Viktorov, 1997; Vsevolodova-Perel, Leirikh, 2014). Based on this, it would be reasonable to suggest that the diploid non-pigmented forms are ancestral to this complex. However, transcriptomic data demonstrated (see the Figure) (Shekhovtsov et al., 2020b) that these forms are not at the basis of the tree, and the ancestral forms were pigmented.

Studied specimens

Species/lineage	Location	Genome size, Mbp	SE	n
<i>E. nordenskioldi</i> lineage 9	Magadan oblast, Magadan	3284	168	4
<i>Eisenia</i> sp. 1 lineage 1	Novosibirsk oblast, Kiternia	2351	124	4
	Sverdlovsk oblast, Khomutovka	2664	128	4
<i>Eisenia</i> sp. 1 lineage 2	Altai Republic, Biryuzovaya Katun	343	30	4
<i>Eisenia</i> sp. 1 lineage 3	Kemerovo oblast, Kuzedeevo	2746	126	3
	Kemerovo oblast, Zolotoy Kitat	3499	227	4
	Altai krai, Makarievka	2780	9	4
	Novosibirsk oblast, Kiternia	3120	49	3
	Khabarovsk krai, Tigrovoye	3215	43	3
<i>Eisenia</i> sp. 1 f. <i>pallida</i> lineage 1	Magadan oblast, Magadan	2494	18	4
<i>Eisenia</i> sp. 1 f. <i>pallida</i> lineage 2	Khabarovsk krai, Lesopilnoye	487	3	3
<i>Eisenia</i> sp. 1 f. <i>pallida</i> lineage 6	Altai krai, Makarievka	269	28	3

Note. SE – standard error; n – number of individuals.



Phylogenetic tree constructed for the *E. nordenskioldi* complex based on transcriptomic data, taken from (Shekhovtsov et al., 2020b).

Grey squares denote the non-pigmented *pallida* form. Numbers near the branches indicate Maximum Likelihood bootstrap support/Bayesian posterior probabilities; asterisks stand for 100/1.0.

Moreover, one of the *pallida* lineages had a large genome while one pigmented lineage had a small one. Therefore, one cannot state that all non-pigmented forms are diploid and pigmented ones are always polyploid. Moreover, the *pallida* form arose independently several times.

The same arguments apply to genome size: it seems more probable that the ancestral genome was large. Moreover, since the majority of *E. nordenskioldi* populations are amphimictic,

the ancestor of the complex was amphimictic and diploid. For *Eisenia* sp. 1, the tree topology also implies that large nuclear genome was the ancestral state, and some branches (lineages) subsequently went through genome compaction.

Several populations from diverse geographic locations were sampled for two genetic lineages (lineages 1 and 3 of *Eisenia* sp. 1). Our analysis demonstrated that there is a certain genome size diversity within these lineages, approximately

13 and 27 % for lineages 1 and 3, respectively. It is well known (Viktorov, 1997; Vsevolodova-Perel, Bulatova, 2008) that chromosome number in octaploid *E. nordenskioldi* populations varies widely, and we may suggest a similar mechanism in this case.

Polyploidy results in increased body size in many animals (Otto, 2007). Earthworms, however, may not conform to this pattern: T.V. Malinina and T.S. Perel (1984) found no size differences between *E. nordenskioldi* of different ploidy. Here we could not measure body size, because the studied animals were completely or partially grinded. However, rough estimates suggest that genetic lineages with small genomes were small or average in size (4–7 cm long), while those with large genomes could be either large (to over 10 cm for *Eisenia* sp. 1 lineage 3) or average (5–10 cm for other lineages). Therefore, although we did not observe a clear pattern, we could hypothesize that genome size partially accounts for body size.

Conclusion

In this study we demonstrated that nuclear genome size varies widely among genetic lineages of the *E. nordenskioldi* complex. This corroborates the remarkable differences among them demonstrated by molecular genetic methods. Moreover, there was also some variation between different populations of the same lineage. Both genome expansion and contraction occurred during the evolution of the complex.

References

- Alvarez-Fuster A., Juan C., Petitpierre E. Genome size in *Tribolium* flour-beetles: inter- and intraspecific variation. *Genet. Res.* 1991;58: 1-5. DOI 10.1017/S0016672300029542.
- Berman D.I., Bulakhova N.A., Meshcheryakova E.N., Shekhovtsov S.V. Cold resistance and the distribution of genetic lineages of the earthworm *Eisenia nordenskioldi* (Oligochaeta, Lumbricidae). *Biol. Bull.* 2019;46:430-437. DOI 10.1134/S1062359019050042.
- Biémont C. Genome size evolution: Within-species variation in genome size. *Heredity.* 2008;101:297-298. DOI 10.1038/hdy.2008.80.
- Blakemore R.J. Earthworms newly from Mongolia (Oligochaeta, Lumbricidae, *Eisenia*). *ZooKeys.* 2013;285:1-21. DOI 10.3897/zookeys.285.4502.
- Blommaert J. Genome size evolution: towards new model systems for old questions. *Proc. Royal Soc. B.* 2020;287:20201441. DOI 10.1098/rspb.2020.1441.
- Cavalier-Smith T. Nuclear volume control by nucleoskeletal DNA, selection for cell volume and cell growth rate, and the solution of the DNA C-value paradox. *J. Cell Sci.* 1978;34:247-278.
- Galbraith D.W., Harkins K.R., Maddox J.M., Ayres N.M., Sharma D.P., Firoozabady E. Rapid flow cytometric analysis of the cell cycle in intact plant tissues. *Science.* 1983;220:1049-1051.
- Galbraith D.W., Lambert G.M., Macas J., Dolezel J. Analysis of nuclear DNA content and ploidy in higher plants. *Curr. Protoc. Cytom.* 1997;2:6-7.
- Grafodatsky A.S., Perel T.S., Radzhabli S.I. Chromosome sets of two forms of *Eisenia nordenskioldi* (Eisen) (Oligochaeta: Lumbricidae). *Doklady Akademii Nauk SSSR = Reports of the Academy of Sciences of USSR.* 1982;262:1514-1516. (in Russian)
- Gregory T.R. Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. *Biol. Rev.* 2001;76:65-101. DOI 10.1111/j.1469-185X.2000.tb00059.x.
- Gregory T.R. Genome size evolution in animals. In: *The Evolution of the Genome.* San Diego: Elsevier, 2005;3-87. DOI 10.1016/B978-012301463-4/50003-6.
- Hong Y., Csuzdi C. New data to the earthworm fauna of the Korean peninsula with redescription of *Eisenia koreana* (Zicsi) and remarks on the *Eisenia nordenskioldi* species group (Oligochaeta, Lumbricidae). *Zool. Stud.* 2016;55:1-15. DOI 10.6620/ZS.2016.55-12.
- Jeffery N.W., Hultgren K., Chak S.T.C., Gregory T.R., Rubenstein D.R. Patterns of genome size variation in snapping shrimp. *Genome.* 2016;59:393-402. DOI 10.1139/gen-2015-0206.
- Kasai F., O'Brien P.C.M., Ferguson-Smith M.A. Reassessment of genome size in turtle and crocodile based on chromosome measurement by flow karyotyping: close similarity to chicken. *Biol. Lett.* 2012;8:631-635. DOI 10.1098/rsbl.2012.0141.
- Malevich I.I. On the study of earthworms in the Far East. *Uchenye Zapiski MGPI im. V.P. Potemkina = Transactions of the V.P. Potemkin Moscow City Pedagogical Institute.* 1956;61:439-449. (in Russian)
- Malinina T.V., Perel T.S. Characterization of *Eisenia nordenskioldi* (Oligochaeta, Lumbricidae) chromosome races using allozyme markers. *Doklady Akademii Nauk SSSR = Reports of the Academy of Sciences of USSR.* 1984;279:1265-1269. (in Russian)
- Marescalchi O., Scali V., Zuccotti M. Flow-cytometric analyses of intraspecific genome size variations in *Bacillus atticus* (Insecta, Phasmatodea). *Genome.* 1998;41:629-635. DOI 10.1139/g98-064.
- Neiman M., Paczesniak D., Soper D.M., Baldwin A.T., Hehman G. Wide variation in ploidy level and genome size in a New Zealand freshwater snail with coexisting sexual and asexual lineages. *Evolution.* 2011;65:3202-3216. DOI 10.1111/j.1558-5646.2011.01360.x.
- Otto S.P. The evolutionary consequences of polyploidy. *Cell.* 2007; 131:452-462. DOI 10.1016/j.cell.2007.10.022.
- Perel T.S. The Range and Regularities in the Distribution of Earthworms of the USSR Fauna. Moscow: Nauka Publ., 1979. (in Russian)
- Redi C.A., Zacharias H., Merani S., Oliveira-Miranda M., Aguilera M., Zuccotti M., Garagna S., Capanna E. Genome sizes in Afrotheria, Xenarthra, Euarchontoglires, and Laurasiatheria. *J. Hered.* 2005;96: 485-493. DOI 10.1093/jhered/esi080.
- Shekhovtsov S.V., Bazarova N.E., Berman D.I., Bulakhova N.A., Golovanova E.V., Konyaev S.V., Gerova T.M., Lyubchanskii I.I., Peltek S.E. DNA barcoding: How many earthworm species are there in the south of West Siberia? *Russ. J. Genet. Appl. Res.* 2017a;7: 57-62. DOI 10.1134/S2079059717010130.
- Shekhovtsov S.V., Berman D.I., Bazarova N.E., Bulakhova N.A., Porco D., Peltek S.E. Cryptic genetic lineages in *Eisenia nordenskioldi pallida* (Oligochaeta, Lumbricidae). *Eur. J. Soil Biol.* 2016a;75: 151-156. DOI 10.1016/j.ejsobi.2016.06.004.
- Shekhovtsov S.V., Berman D.I., Bulakhova N.A., Vinokurov N.N., Peltek S.E. Phylogeography of *Eisenia nordenskioldi nordenskioldi* (Lumbricidae, Oligochaeta) from the north of Asia. *Polar Biol.* 2018a;41:237-247. DOI 10.1007/s00300-017-2184-2.
- Shekhovtsov S.V., Berman D.I., Bulakhova N.A., Makarova O.L., Peltek S.E. Phylogeography of earthworms from high latitudes of Eurasia. *Acta Zool. Acad. Sci. Hungaricae.* 2018b;64:369-382. DOI 10.17109/AZH.64.4.369.2018.
- Shekhovtsov S.V., Berman D.I., Golovanova E.V., Peltek S.E. Genetic diversity of the earthworm *Eisenia nordenskioldi* (Lumbricidae, Annelida). *Vavilovskii Zhurnal Genetiki i Seleksii = Vavilov Journal of Genetics and Breeding.* 2017b;21(5):588-595. DOI 10.18699/VJ17.24-o. (in Russian)
- Shekhovtsov S.V., Berman D.I., Peltek S.E. Phylogeography of the earthworm *Eisenia nordenskioldi nordenskioldi* (Lumbricidae, Oligochaeta) in northeastern Eurasia. *Dokl. Biol. Sci.* 2015;461:85-88. DOI 10.1134/S0012496615020039.
- Shekhovtsov S.V., Ershov N.I., Vasiliev G.V., Peltek S.E. Transcriptional analysis confirms differences among nuclear genomes of cryptic earthworm lineages living in sympatry. *BMC Evol. Biol.* 2019; 19:50. DOI 10.1186/s12862-019-1370-y.
- Shekhovtsov S.V., Golovanova E.V., Ershov N.I., Poluboyarova T.V., Berman D.I., Bulakhova N.A., Szederjesi T., Peltek S.E. Phylogeny of the *Eisenia nordenskioldi* complex based on mitochondrial genomes. *Eur. J. Soil Biol.* 2020a;96:103137. DOI 10.1016/j.ejsobi.2019.103137.

- Shekhovtsov S.V., Golovanova E.V., Peltek S.E. Cryptic diversity within the Nordenskiöld's earthworm, *Eisenia nordenskioldi* subsp. *nordenskioldi* (Lumbricidae, Annelida). *Eur. J. Soil Biol.* 2013;58: 13-18. DOI 10.1016/j.ejsobi.2013.05.004.
- Shekhovtsov S.V., Golovanova E.V., Peltek S.E. Mitochondrial DNA variation in *Eisenia n. nordenskioldi* (Lumbricidae) in Europe and Southern Urals. *Mitochondrial DNA A DNA Mapp. Seq. Anal.* 2016b;27:4643-4645. DOI 10.3109/19401736.2015.1101594.
- Shekhovtsov S.V., Shipova A.A., Poluboyarova T.V., Vasiliev G.V., Golovanova E.V., Geraskina A.P., Bulakhova N.A., Szederjesi T., Peltek S.E. Species delimitation of the *Eisenia nordenskioldi* complex (Oligochaeta, Lumbricidae) using transcriptomic data. *Front. Genet.* 2020b;11:1508. DOI 10.3389/fgene.2020.598196.
- Shekhovtsov S.V., Sundukov Y.N., Blakemore R.J., Gongalsky K.B., Peltek S.E. Identifying earthworms (Oligochaeta, Megadrili) of the southern Kuril islands using DNA barcodes. *Anim. Biodivers. Conserv.* 2018c;41:9-17. DOI 10.32800/abc.2018.41.0009.
- Stelzer C.-P., Riss S., Stadler P. Genome size evolution at the speciation level: The cryptic species complex *Brachionus plicatilis* (Rotifera). *BMC Evol. Biol.* 2011;11:90. DOI 10.1186/1471-2148-11-90.
- Thomas C.A.J. The genetic organization of chromosomes. *Annu. Rev. Genet.* 1971;5:237-256.
- Viktorov A.G. Diversity of polyploid races in the family Lumbricidae. *Soil. Biol. Biochem.* 1997;29:217-221. DOI 10.1016/S0038-0717(96)00086-7.
- Vsevolodova-Perel T.S. The Earthworms of the Russian Fauna: Catalogue and Key. Moscow: Nauka Publ., 1997. (in Russian)
- Vsevolodova-Perel T.S., Bulatova N.Sh. Polyploid races of earthworms (Lumbricidae, Oligochaeta) in the East European Plain and Siberia. *Biology Bulletin.* 2008;35(4):385-388. DOI 10.1134/S1062359008040092.
- Vsevolodova-Perel T.S., Leirikh A.N. Distribution and ecology of the earthworm *Eisenia nordenskioldi pallida* (Oligochaeta, Lumbricidae) dominant in southern Siberia and the Russian Far Eastern. *Entomol. Rev.* 2014;94(4):479-485. DOI 10.1134/S0013873814040034.
- Zhukov A.V., Pakhomov A.Ye., Kunach O.N. Biological Diversity of Ukraine. The Dnipropetrovsk region. Earthworms (Lumbricidae). Dnipropetrovsk, 2007. (in Ukrainian)

ORCID ID

S.V. Shekhovtsov orcid.org/0000-0001-5604-5601
Ya.R. Efremov orcid.org/0000-0002-0649-7543
T.V. Poluboyarova orcid.org/0000-0002-5652-0553
S.E. Peltek orcid.org/0000-0002-3524-0456

Acknowledgements. This study was supported by the Russian Foundation for Basic Research (grant No. 19-04-00661_a), the Budget project No. 0259-2021-0010 of ICiG SB RAS, and the Kurchatov Genomic Center of ICiG SB RAS (075-15-2019-1662). DNA sequencing was performed in the SB RAS Genomics Core Facility (Novosibirsk, Russia). The authors are grateful to D.I. Berman, N.A. Bulakhova, and E.V. Golovanova for the live earthworm specimens.

Conflict of interest. The authors declare no conflict of interest.

Received February 19, 2021. Revised March 30, 2021. Accepted March 31, 2021.