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

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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 be cryptic taxa. The *Eisenia nordenskioeldi* 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. nordenskioeldi* 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 nordenskioeldi*; genome size; flow cytometry; phylogeny.

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Введение

Наличие геномных вариаций в геномах изучаемых видов имеет большое значение в генетике и биологии, так как могут указывать на наличие дополнительных видов или линий внутри вида. Одним из примеров, иллюстрирующих это, является комплекс Eisenia nordenskioldi, включающий несколько генетических линий, которые отличаются по размеру генома.

Целью данной работы было изучение геномных вариаций у представителей комплекса Eisenia nordenskioldi, обитающих в различных регионах России.

Материалы и методы

Материалом исследования служили земноводные Eisenia nordenskioldi, собранные в различных регионах России.

Результаты и обсуждение

Изученные образцы варьировались по размеру генома, что может указывать на наличие дополнительных форм или линий внутри вида. Полученные данные показывают, что геномные вариации имеют большое значение в понимании биологии и генетики исследуемых видов.
**Species specimens**

| Species/lineage | Location | Genome size, Mbp | SE | n |
|-----------------|----------|-----------------|----|---|
| *E. nordenskioldi* lineage 9 | Magadan oblast, Magadan | 3284 | 168 | 4 |
| *Eisenia* sp. 1 lineage 1 | Novosibirsk oblast, Kibernia | 2351 | 124 | 4 |
| *Eisenia* sp. 1 lineage 2 | Altai Republic, Biryuzova Katun | 343 | 30 | 4 |
| *Eisenia* sp. 1 lineage 3 | Kemerovo oblast, Kuzedeevo | 2746 | 126 | 3 |
| *Eisenia* sp. 1 lineage 5 | Kemerovo oblast, Zolotoy Kitat | 3499 | 227 | 4 |
| *Eisenia* sp. 1 lineage 7 | Altai krai, Makarievka | 2780 | 9 | 4 |
| *Eisenia* sp. 1 f. *pallida* lineage 1 | Magadan oblast, Magadan | 2494 | 18 | 4 |
| *Eisenia* sp. 1 f. *pallida* lineage 2 | Khabarovsk krai, Lesopilnoye | 487 | 3 | 3 |
| *Eisenia* sp. 1 f. *pallida* 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 amphiimictic, the ancestor of the complex was amphiimictic 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/S1062359019050442.
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). Zoomkeys. 2013;285:1-21. DOI 10.3897/zoomkeys.285.4502.
Blommaert J. Genome size evolution: towards new model systems for old questions. Proc. Royal Soc. B. 2020;287:20201441.
Cavalier-Smith T. Nuclear volume control by nucleoskeletal DNA, segregation and the C-value enigma. J. Cell Sci. 1978;34:247-278.
Galbraith D.W., Harkins K.R., Maddox J.M., Ayres N.M., Sharma D.P., Firoozabad Y. 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.tb0059.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 rediscription of Eisenia koreana (Zies). In: The Evolution of the Genome. San Diego: Elsevier, 2005;3-87. DOI 10.1016/B978-012301463-4:50003-6.
Shekhovtsov S.V., Golovanova E.V., Peltek S.E. Cryptic diversity within the Nordenskiold’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., Vasilev 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/fgen.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., Studler 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: Cadaster 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)