Вавиловский журнал генетики и селекции. 2023;27(2):146-152
DOI 10.18699/VJGB-23-20

Original Russian text https://vavilovj-icg.ru/

The mitochondrial genome of *Dendrobaena tellermanica* Perel, 1966 (Annelida: Lumbricidae) and its phylogenetic position

S.V. Shekhovtsov[1, 2] ✉, G.V. Vasiliev[1], R. Latif[3], T.V. Poluboyarova[1], S.E. Peltek[1], I.B. Rapoport[4]

[1] Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
[2] Institute of Biological Problems of the North of the Far Eastern Branch of the Russian Academy of Sciences, Magadan, Russia
[3] Semnan University, Semnan, Iran
[4] Tembotov Institute of Ecology of Mountain Territories of Russian Academy of Sciences, Nalchik, Russia
✉ shekhovtsov@bionet.nsc.ru

Abstract. Earthworms are an important ecological group that has a significant impact on soil fauna as well as plant communities. Despite their importance, genetic diversity and phylogeny of earthworms are still insufficiently studied. Most studies on earthworm genetic diversity are currently based on a few mitochondrial and nuclear genes. Mitochondrial genomes are becoming a promising target for phylogeny reconstruction in earthworms. However, most studies on earthworm mitochondrial genomes were made on West European and East Asian species, with much less sampling from other regions. In this study, we performed sequencing, assembly, and analysis of the mitochondrial genome of *Dendrobaena tellermanica* Perel, 1966 from the Northern Caucasus. This species was earlier included into *D. schmidti* (Michaelsen, 1907), a polytypic species with many subspecies. The genome was assembled as a single contig 15,298 bp long which contained a typical gene set: 13 protein-coding genes (three subunits of cytochrome *c* oxidase, seven subunits of NADH dehydrogenase, two subunits of ATP synthetase, and cytochrome *b*), *12S* and *16S* ribosomal RNA genes, and 22 tRNA genes. All genes were located on one DNA strand. The assembled part of the control region, located between the *tRNA-Arg* and *tRNA-His* genes, was 727 bp long. The control region contained multiple hairpins, as well as tandem repeats of the AACGCTT monomer. Phylogenetic analysis based on the complete mitochondrial genomes indicated that the genus *Dendrobaena* occupied the basal position within Lumbricidae. *D. tellermanica* was a rather distant relative of the cosmopolitan *D. octaedra*, suggesting high genetic diversity in this genus. *D. schmidti* turned out to be paraphyletic with respect to *D. tellermanica*. Since *D. schmidti* is known to contain very high genetic diversity, these results may indicate that it may be split into several species.
Key words: earthworms; Lumbricidae; *Dendrobaena tellermanica*; mitochondrial genomes.

For citation: Shekhovtsov S.V., Vasiliev G.V., Latif R., Poluboyarova T.V., Peltek S.E., Rapoport I.B. The mitochondrial genome of *Dendrobaena tellermanica* Perel, 1966 (Annelida: Lumbricidae) and its phylogenetic position. *Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding*. 2023;27(2):146-152. DOI 10.18699/VJGB-23-20

Митохондриальный геном *Dendrobaena tellermanica* Perel, 1966 (Annelida: Lumbricidae)

С.В. Шеховцов[1, 2] ✉, Г.В. Васильев[1], Р. Латиф[3], Т.В. Полубоярова[1], С.Е. Пельтек[1], И.Б. Рапопорт[4]

[1] Федеральный исследовательский центр Институт цитологии и генетики Сибирского отделения Российской академии наук, Новосибирск, Россия
[2] Институт биологических проблем Севера Дальневосточного отделения Российской академии наук, Магадан, Россия
[3] Университет Семнана, Семнан, Иран
[4] Институт экологии горных территорий им. А.К. Темботова Российской академии наук, Нальчик, Россия
✉ shekhovtsov@bionet.nsc.ru

Аннотация. Дождевые черви – важная экологическая группа, которая оказывает значительное влияние как на состав почвенной фауны, так и на растительность. Генетическое разнообразие и филогения дождевых червей при этом остаются относительно слабо изученными. В настоящее время большинство работ по генетическому разнообразию дождевых червей основывается на единичных митохондриальных и ядерных генах. В связи с этим для реконструкции филогенетических отношений у дождевых червей перспективными становятся митохондриальные геномы. Почти все работы по этой теме посвящены видам из Западной Европы или Восточной Азии, другие регионы практически не затронуты. В настоящей работе мы провели секвенирование, сборку и анализ митохондриального генома *Dendrobaena tellermanica* Perel, 1966. Этот вид ранее входил в состав кавказского вида *D. schmidti* (Michaelsen, 1907) – политипического вида, в пределах которого выделяли множество таксонов. Геном был собран в виде одного контига длиной 15 298 п. н., содержащего типичный набор генов: 13 белок-кодирующих генов (три субъединицы цитохромоксидазы, семь субъединиц NADH дегидрогеназы, две субъединицы АТФ синтетазы, цитохром *b*), гены *12S* и *16S* рибосомальной РНК и 22 гена тРНК. Все гены были расположены на

одной цепи ДНК. На контрольный регион, находящийся между генами *tRNA-Arg* и *tRNA-His*, приходилось 727 п. н. Контрольный регион содержал множество шпилек, а также тандемные повторы мономера AACGCTT. Филогенетический анализ на основе полных митохондриальных геномов показал, что род *Dendrobaena* является базальным в семействе Lumbricidae. *D. tellermanica* оказалась довольно далеким родственником космополитного вида *D. octaedra*, что говорит о высоком генетическом разнообразии в этом роде. Вид *D. schmidti* был парафилетичным по отношению к *D. tellermanica*. Поскольку для *D. schmidti* характерна очень высокая генетическая изменчивость, можно рассматривать эти данные как свидетельство в пользу разделения *D. schmidti* на несколько видов.
Ключевые слова: дождевые черви; Lumbricidae; *Dendrobaena tellermanica*; митохондриальные геномы.

Introduction

Earthworms are an important ecological group that accounts for the highest biomass among the soil fauna in many habitats (Hendrix et al., 2008). Its representatives process plant detritus to soil humus and return organic matter to the global cycles (Blouin et al., 2013). Earthworms also form soil structure, which has high impact on both soil fauna composition and vegetation (Lavelle et al., 2016). Therefore, this group defines ecosystem productivity in many respects.

Genetic diversity and phylogeny of earthworms remain insufficiently studied (Marchán et al., 2018). Currently, most works on earthworm genetic diversity are based on single mitochondrial and nuclear genes (Jamieson et al., 2002; Marchán et al., 2022). Construction of multigene nuclear datasets is impeded by frequent polyploidy characteristic for this group (Viktorov, 1997; Vsevolodova-Perel, Bulatova, 2008; Mezhzherin et al., 2018), which makes it hard to detect suitable orthologs and amplify them by PCR.

Mitochondrial genomes are thus a promising tool for reconstruction of phylogenetic relationships in earthworms. A lot of mitochondrial genomes were sequenced and published in recent years (Zhang L. et al., 2014–2016a, b; Wang et al., 2015; Conrado et al., 2017; Hong et al., 2017; Shekhovtsov, Peltek, 2019; Zhang Q. et al., 2019; Liu et al., 2020; Seto et al., 2021; Csuzdi et al., 2022; Kim, Hong, 2022), and studies on phylogenetic relationships of certain groups were also conducted (Shekhovtsov et al., 2020a; Liu et al., 2021). However, almost all of these studies were made on species from West Europe and East Asia, with almost no representatives of other regions.

In this study, we performed sequencing, assembly, and analysis of the mitochondrial genome of *Dendrobaena tellermanica* Perel, 1966. This species was earlier included into *D. schmidti* (Michaelsen, 1907), a polytypic species that was considered to contain multiple subspecies (Michaelsen, 1907; Kvavadze, 1985). *D. tellermanica* was believed to be a parthenogenetic form of *D. schmidti* (Perel, 1966). T.S. Vsevolodova-Perel (2003) demonstrated that many populations of *D. tellermanica* are amphimictic (sexual) and so isolated it into a separate species. *D. tellermanica* differs from *D. schmidti* by the lack of pigmentation, different position of the clitellum and the form of tuberculae pubertatis (Vsevolodova-Perel, 2003).

Currently, there is only one complete mitochondrial genome of the genus *Dendrobaena* in GenBank belonging to the cosmopolitan *D. octaedra* (Savigny, 1826). The mitochondrial genome of *D. tellermanica* will be the first sequenced mitochondrial genome of a Caucasian earthworm and will be important for studying the phylogeny of lumbricids.

Materials and methods

Specimens of *D. tellermanica* were collected in the Karachay-Cherkess Republic (right bank of r. Uchkulan, road to the Chiper pass, 1483 a. s. l., 4–5 km from the Aktyube town, *Alchemilla* and *Geranium* meadow, N 43.410944, E 42.174538). Worms were fixed in ethanol. Morphological identification was performed according to the key of T.S. Vsevolodova-Perel (1997).

DNA was extracted using the standard phenol-chloroform method and sonicated on Covaris M220 to the target fragment length of 350 bp. The fragments were purified by 1.2 volume of AMPureXP (Beckman Coulter, USA) and quantified using fluorometry on a Qubit device. Genomic libraries were obtained from 100 ng of DNA using Roche KAPA Hyper Prep according to the manufacturer's protocol using KAPA UDI Adapter double barcodes. Quality and molarity of the obtained genomic library was assessed on a BA2100 bioanalyzer using the Agilent DNA High Sensitivity Kit and sequenced on an Illumina NextSeq550 with the Mid Output Kit v. 2.5 (300 Cycles) for 2×150 bp paired reads.

The obtained data were processed by TrimmomaticPE (Bolger et al., 2014) with the ILLUMINACLIP:TruSeq3-SE:2:30:10 SLIDINGWINDOW:4:15 MINLEN:36 options. SPAdes v. 3.14.1 was used for contig assembly (Bankevich et al., 2012) with the --isolate option. The assembled contigs were aligned with mitochondrial earthworm genomes from the NCBI database with blastn (https://blast.ncbi.nlm.nih.gov) in order to search for mitochondrial sequences.

Preliminary annotation was done by MITOS 2 (Bernt et al., 2013) with subsequent manual comparison with annotated earthworm genomes. The mitochondrial genome of *D. tellermanica* was deposited in GenBank under accession number ON960857. Map of the genome was constructed using Benchling (https://www.benchling.com/).

Secondary structures of tRNAs were visualized using MITOS 2 (Bernt et al., 2013); of the control region, using RNAfold Web Server (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi) and forna (http://rna.tbi.univie.ac.at/forna/forna.html) (Gendron et al., 2001). Search for tandem repeats was done by Tandem Repeats Finder (Benson, 1999). For phylogenetic reconstructions, mitochondrial genomes were aligned with Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/); control regions were not included into the alignments. Ambiguously aligned regions were removed with gblocks 0.91b (Castresana, 2000). Earthworm mitochondrial genomes and sequences of the *COX1* gene of representatives of the *Dendrobaena* genus were extracted from GenBank. Phylogenetic trees were built using the Maximum Likelihood approach in RAxML v. 8.2.12 (Stamatakis,

2014) with the GTRCAT substitution model; 1000 bootstrap replicates were performed.

Results

We obtained 4.2 million paired reads for the *D. tellermanica* genomic library; 3.4 million remained after processing. Median coverage of genome contigs longer than 500 bp was estimated at 6 (average – 20), median coverage for the mitochondrial contig was 20 (average – 30).

The assembled mitochondrial contig was 15,298 bp long and contained the typical set of genes: 13 protein-coding genes (three subunits of cytochrome oxidase, seven subunits of NADH dehydrogenase, two subunits of ATP synthase, and cytochrome *b*), *12S* and *16S* ribosomal RNA genes, and 22 tRNA genes. All genes were located on one DNA strand (Fig. 1). AT-content was 65.3 %. The leading strand contained 31.1 % A, 34.2 % T, 13.9 % G, and 20.8 % C. The *ND4* and *ND4L* genes overlapped by 7 bp. ATG was the only start codon used. Three protein coding genes (*COIII*, *ND1* и *ND2*) had an abbreviated stop codon. Transport RNA genes were 60 to 69 bp long, their predicted structures are shown on Fig. 2.

The region between the *tRNA-Arg* and *tRNA-His* genes is known as the control region. A total of 727 bp were assigned to it. The control region could not be assembled, so the final sequence contained a gap. Its AT-content (63.5 %) was close to the genome average, and its sequence contained multiple hairpins (see Fig. 2). It also included 11 tandem repeats of the AACGCTT monomer.

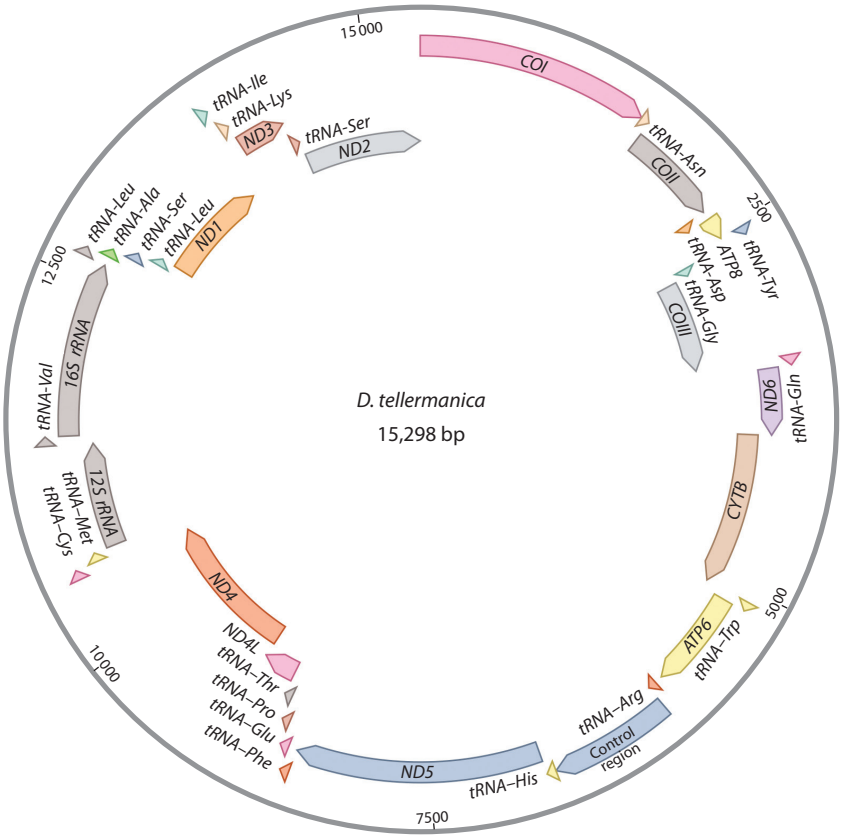

Fig. 1. The organization of the *D. tellermanica* mitochondrial genome.

Discussion

Organization of mitochondrial genome in earthworms

For a long time, the study of J.L. Boore and W.M. Brown (1995) on *Lumbricus terrestris* was the only description of an earthworm mitochondrial genome. It was 14,998 bp long and contained a set of genes usual for animal mitochondria. The hallmark of earthworm mitochondrial genomes, as well of Annelida as a whole, with few exceptions, is that all genes are located on a single strand (Weigert et al., 2016). All mitochondrial genes in earthworms are presumably expressed as a single transcript (Vallès, Boore, 2006). In this case, any inversions and most of the translocations will be non-viable, which leads to fixed gene positions in the genome. Indeed, mitochondrial gene order in annelids is highly conserved, and all Clitellata have identical gene order. We also failed to find any deviations from this rule.

While mtDNA gene order in earthworms is conserved, its sequence is highly variable, which is especially pronounced for the control region (also referred to as the D-loop). The control region acts as the replication origin, promotor, and the regulatory region for the mitochondrial gene expression (Clayton, 1992).

Organization of the control region varies in different earthworm taxa. Among the representatives of Glossoscolecidae and Megascolecidae they are short, usually less than 500 bp (Zhang L. et al., 2016a; Hong et al., 2017; Zhang Q. et al., 2019; Seto et al., 2021; Kim, Hong, 2022), while in two species of *Drawida* (Moniligastridae) these sequences were completely absent (Liu et al., 2020). In Lumbricidae, the length of the control region varies from 400 bp in *L. terrestris* to 2000 bp in *Eisenia fetida*. For many species, control regions could not be amplified (Shekhovtsov et al., 2020a) or even recovered using NGS methods (Zhao et al., 2022). Here, we also failed to amplify the lacking part of the control region of *D. tellermanica*. This could be caused by its length or complex secondary structure: Fig. 2 demonstrates that almost all of the control region forms hairpins.

The phylogeny of earthworms based on mitochondrial genomes and the position of *D. tellermanica*

Phylogenetic analysis based on complete mitochondrial genomes (Fig. 3) suggests that Moniligastridae is distantly related to other earthworm families. Glossoscolecidae, represented here by a single species *Pontoscolex corethrurus*, occupied the basal position within the order Crassiclitellata. Megascolecidae and Lumbricidae, which were the most densely sampled, turned out as sister groups.

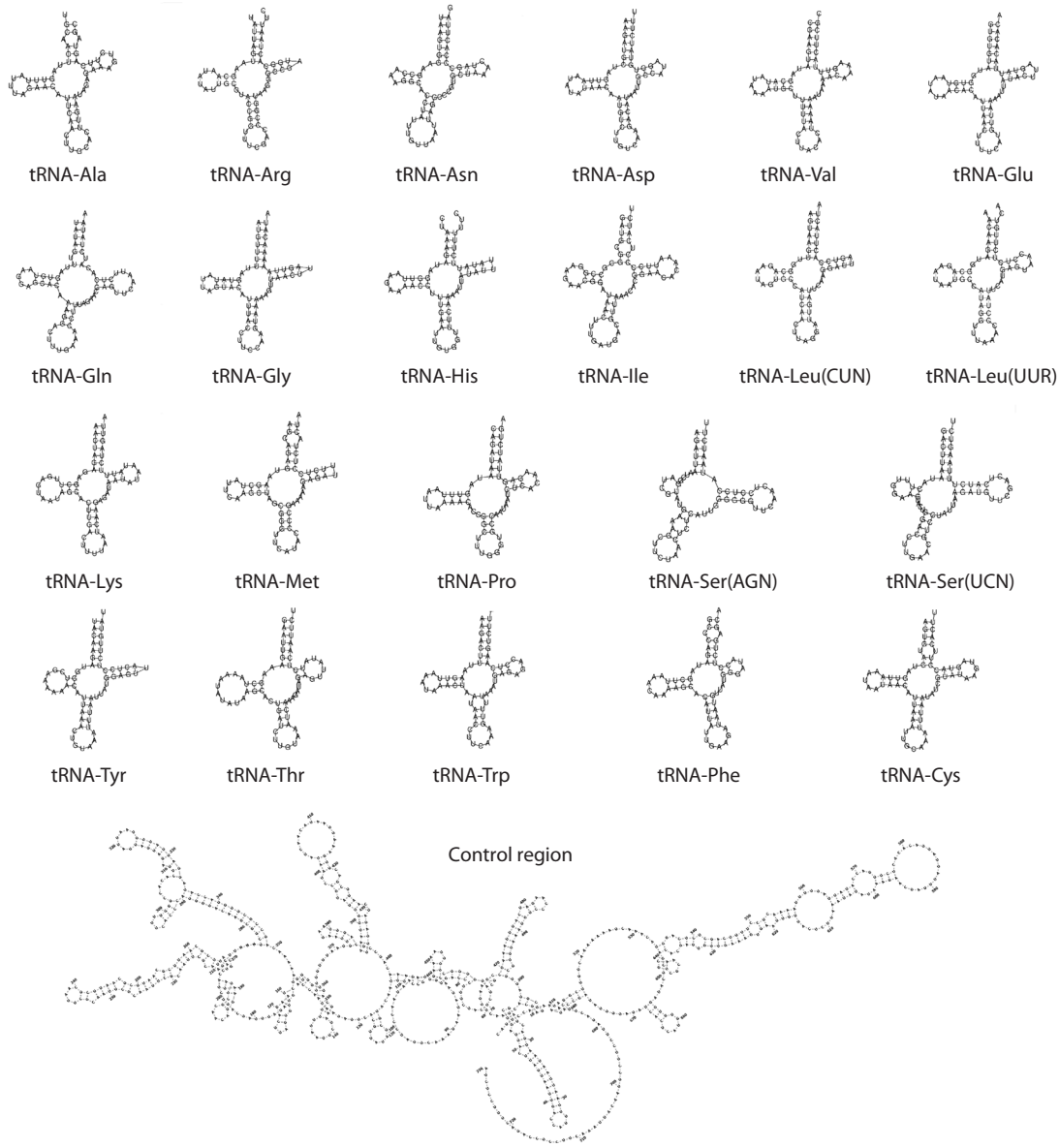

Fig. 2. Secondary structures of tRNAs and the control region of the mitochondrial genome of *D. tellermanica*.

Within the family Lumbricidae, which includes *D. tellermanica*, the genus *Dendrobaena* was the sister group to all other genera of the family. There are only two known mitochondrial sequences for the genus *Dendrobaena*, the cosmopolitan *D. octaedra* and *D. tellermanica* obtained in this study. Expectedly, *D. tellermanica* forms a clade with *D. octaedra*, but they are rather distantly related.

We can conclude that this work is a first step in the study of the basal branches of Lumbricidae. Representatives of the genus *Dendrobaena* from the Caucasus are particularly interesting in this respect, because they account for a large part of its species diversity.

Earlier we performed a genetic analysis of morphological forms of *D. schmidti* (Shekhovtsov et al., 2020b), demonstrating that it represents at least two separate species. On the phylogenetic tree constructed using the *COX1* gene (Fig. 4), *D. tellermanica* was inside one of the branches of *D. schmidti*. We should note that single mitochondrial genes, including *COX1*, are unsuitable for phylogenetic reconstruction on the family level (Klarica et al., 2012; Shekhovtsov et al., 2016, 2020c), since they demonstrate poor resolution of the relationships between species and do not support the monophyly of most genera. *COX1* is however of much use in the search for closely related species or genetic lineages. Moreover, there are thousands of *COX1* sequences in the public databases and only a few mitochondrial genomes; e. g., mtDNA of *D. schmidti* has not been sequenced yet. Therefore, the tree on Fig. 4 is given only to demonstrate the close relationship of *D. tellermanica* and *D. schmidti*.

Conclusion

The obtained preliminary results indicate that *D. tellermanica* could be treated as a subspecies of *D. schmidti*, as was believed earlier, or split *D. schmidti* into several species. The latter

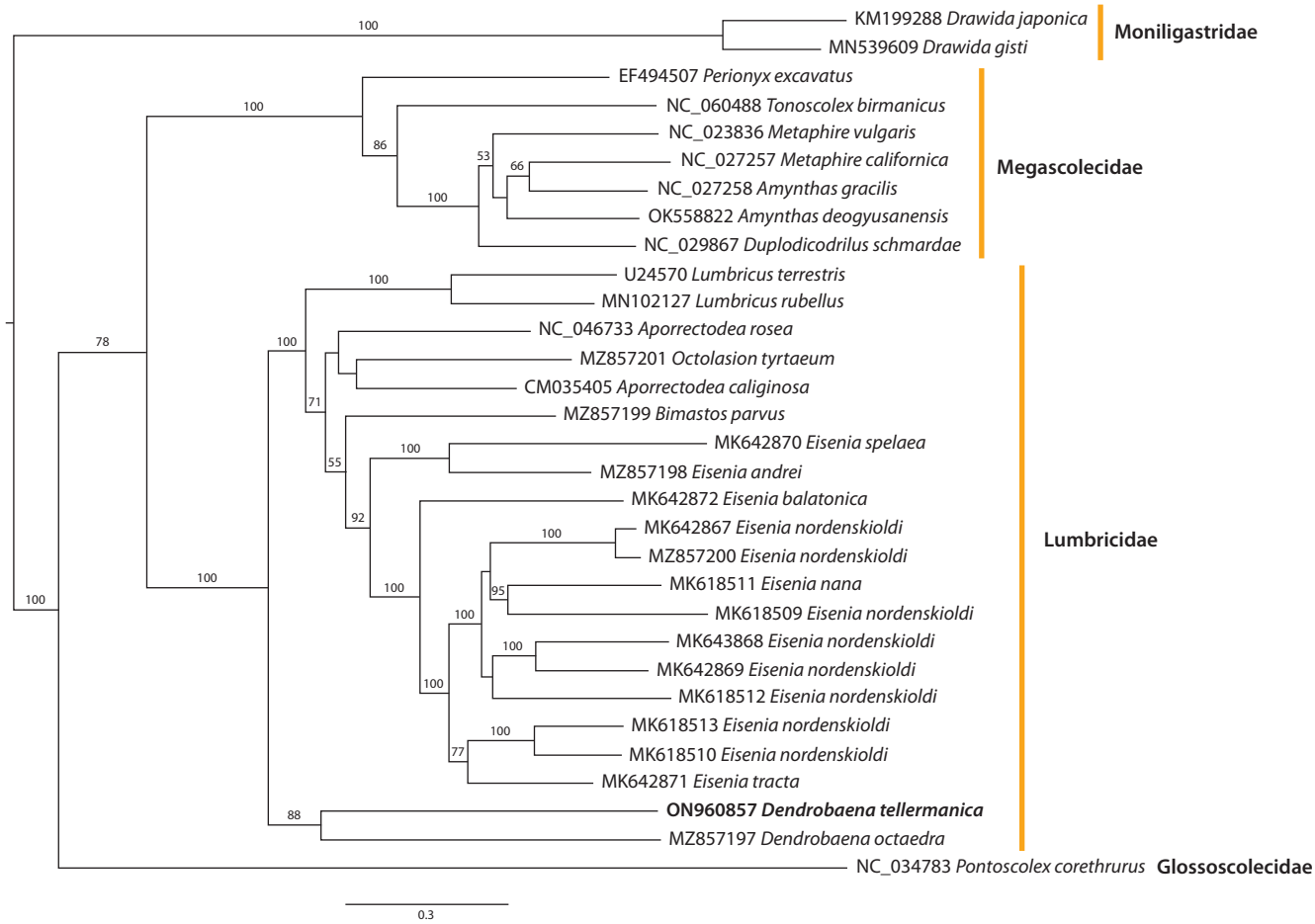

Fig. 3. Phylogenetic tree based on earthworm mitochondrial genomes using the Maximum Likelihood method.
Here and in Fig. 4: Numbers near the branches indicate bootstrap support.

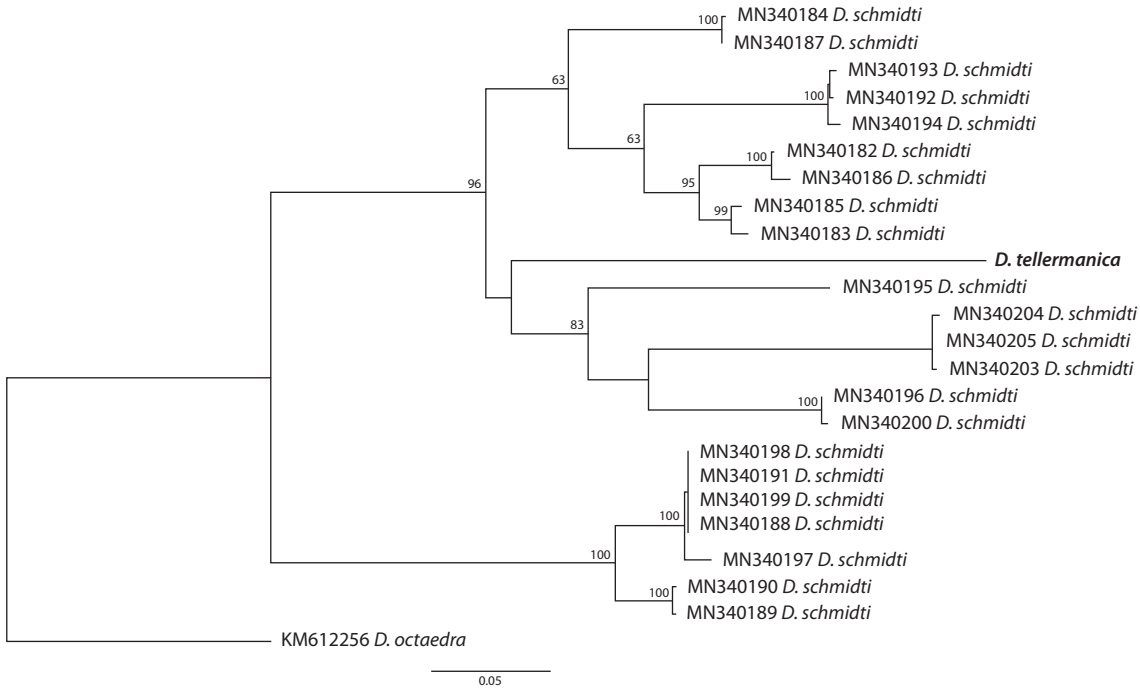

Fig. 4. Phylogenetic tree based on the *COX1* gene for the genus *Dendrobaena* using the Maximum Likelihood method.

option is supported by the high genetic and morphological variation within this species. However, such conclusions would require an analysis based on several loci, including nuclear ones.

References

Bankevich A., Nurk S., Antipov D., Gurevich A.A., Dvorkin M., Kulikov A.S., Lesin V.M., Nikolenko S.I., Pham S., Prjibelski A.D., Pyshkin A.V., Sirotkin A.V., Vyahhi N., Tesler G., Alekseyev M.A., Pevzner P.A. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol*. 2012;19(5): 455-477. DOI 10.1089/cmb.2012.0021.

Benson G. Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Res*. 1999;27(2):573-580. DOI 10.1093/nar/27.2.573.

Bernt M., Donath A., Jühling F., Externbrink F., Florentz C., Fritzsch G., Pütz J., Middendorf M., Stadler P.F. MITOS: Improved *de novo* metazoan mitochondrial genome annotation. *Mol. Phylogenet. Evol*. 2013;69(2):313-319. DOI 10.1016/j.ympev.2012.08.023.

Blouin M., Hodson M.E., Delgado E.A., Baker G., Brussaard L., Butt K.R., Dai J., Dendooven L., Peres G., Tondoh J.E., Cluzeau D., Brun J.-J. A review of earthworm impact on soil function and ecosystem services. *Eur. J. Soil Sci.* 2013;64(2):161-182. DOI 10.1111/ejss.12025.

Bolger A.M., Lohse M., Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 2014;30(15):2114-2120. DOI 10.1093/bioinformatics/btu170.

Boore J.L., Brown W.M. Complete sequence of the mitochondrial DNA of the annelid worm *Lumbricus terrestris*. *Genetics*. 1995;141(1): 305-319. DOI 10.1093/genetics/141.1.305.

Castresana J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol. Biol. Evol*. 2000;17(4): 540-552. DOI 10.1093/oxfordjournals.molbev.a026334.

Clayton D.A. Transcription and replication of animal mitochondrial DNAs. *Int. Rev. Cytol*. 1992;141:217-232. DOI 10.1016/S0074-7696(08)62067-7.

Conrado A.C., Arruda H., Stanton D.W.G., James S.W., Kille P., Brown G., Silva E., Dupont L., Taheri S., Morgan A.J., Simõe N., Rodrigues A., Montiel R., Cunha L. The complete mitochondrial DNA sequence of the pantropical earthworm *Pontoscolex corethrurus* (Rhinodrilidae, Clitellata): Mitogenome characterization and phylogenetic positioning. *ZooKeys*. 2017;688:1-13. DOI 10.3897/zookeys.688.13721.

Csuzdi C., Koo J., Hong Y. The complete mitochondrial DNA sequences of two sibling species of lumbricid earthworms, *Eisenia fetida* (Savigny, 1826) and *Eisenia andrei* (Bouché, 1972) (Annelida, Crassiclitellata): comparison of mitogenomes and phylogenetic positioning. *ZooKeys.* 2022;1097:167-181. DOI 10.3897/zookeys.1097.80216.

Gendron P., Lemieux S., Major F. Quantitative analysis of nucleic acid three-dimensional structures. *J. Mol. Biol*. 2001;308(5):919-936. DOI 10.1006/jmbi.2001.4626.

Hendrix P.F., Callaham M.A., Drake J.M., Huang C.-Y., James S.W., Snyder B.A., Zhang W. Pandora's box contained bait: the global problem of introduced earthworms. *Annu. Rev. Ecol. Evol. Syst*. 2008;39(1):593-613. DOI 10.1146/annurev.ecolsys.39.110707.173426.

Hong Y., Kim M.J., Wang A.R., Kim I. Complete mitochondrial genome of the earthworm. *Amynthas jiriensis* (Clitellata: Megascolecidae). *Mitochondrial DNA A DNA Mapp. Seq. Anal*. 2017;28(2): 163-164. DOI 10.3109/19401736.2015.1115491.

Jamieson B.G.M., Tillier S., Tillier A., Justine J.-L., Ling E., James S., McDonald K., Hugall A.F. Phylogeny of the Megascolecidae and Crassiclitellata (Annelida, Oligochaeta): combined versus partitioned analysis using nuclear (28S) and mitochondrial (12S, 16S) rDNA. *Zoosystema*. 2002;24(4):707-734.

Kim M.J., Hong Y. Complete mitochondrial genome of the earthworm *Amynthas seungpanensis* (Clitellata: Megascolecidae). *Mitochondrial DNA B Resour*. 2022;7(6):989-991. DOI 10.1080/23802359.2022.2080604.

Klarica J., Kloss-Brandstätter A., Traugott M., Juen A. Comparing four mitochondrial genes in earthworms – Implications for identification, phylogenetics, and discovery of cryptic species. *Soil Biol. Biochem*. 2012;45:23-30. DOI 10.1016/j.soilbio.2011.09.018.

Kvavadze E.Sh. The Earthworms (Lumbricidae) of the Caucasus. Tbilisi: Metsniereba Publ., 1985. (in Russian)

Lavelle P., Spain A., Blouin M., Brown G., Decaëns T., Grimaldi M., Jiménez J.J., McKey D., Mathieu J., Velasquez E., Zangerlé A. Ecosystem engineers in a self-organized soil. *Soil Sci*. 2016;181(3/4): 91-109. DOI 10.1097/SS.0000000000000155.

Liu H., Xu N., Zhang Q., Wang G., Xu H., Ruan H. Characterization of the complete mitochondrial genome of *Drawida gisti* (Metagynophora, Moniligastridae) and comparison with other Metagynophora species. *Genomics*. 2020;112(5):3056-3064. DOI 10.1016/j.ygeno.2020.05.020.

Liu H., Zhang Y., Xu W., Fang Y., Ruan H. Characterization of five new earthworm mitogenomes (Oligochaeta: Lumbricidae): mitochondrial phylogeny of Lumbricidae. *Diversity*. 2021;13(11):580. DOI 10.3390/d13110580.

Marchán D.F., Cosín D.J.D., Novo M. Why are we blind to cryptic species? Lessons from the eyeless. *Eur. J. Soil Biol*. 2018;86:49-51. DOI 10.1016/j.ejsobi.2018.03.004.

Marchán D.F., Decaëns T., Domínguez J., Novo M. Perspectives in earthworm molecular phylogeny: recent advances in Lumbricoidea and standing questions. *Diversity*. 2022;14(1):30. DOI 10.3390/d14010030.

Mezhzherin S.V., Garbar A.V., Vlasenko R.P., Onishchuk I.P., Kotsyuba I.Yu., Zhalai E.I. The Evolutionary Paradox of Parthenogenetic Earthworms. Kiev: Naukova Dumka Publ., 2018. (in Ukrainian)

Michaelsen W. Die Lumbriciden des Kaukasischen Museums in Tiflis. *Mitteilungen des Kaukasischen Museums*. 1907;3:81-93.

Perel T.S. Earthworms in forest soils of the Northwestern Caucasus. In: The Impact of Animals on the Productivity of Forest Cenoses. Moscow: Nauka Publ., 1966;146-165. (in Russian)

Seto A., Endo H., Minamiya Y., Matsuda M. The complete mitochondrial genome sequences of Japanese earthworms *Metaphire hilgendorfi* and *Amynthas yunoshimensis* (Clitellata: Megascolecidae). *Mitochondrial DNA B. Resour.* 2021;6(3):965-967. DOI 10.1080/23802359.2020.1830728.

Shekhovtsov S., 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*. 2016;75:151-156. DOI 10.1016/j.ejsobi.2016.06.004.

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., Peltek S.E. The complete mitochondrial genome of *Aporrectodea rosea* (Annelida: Lumbricidae). *Mitochondrial DNA Part B*. 2019;4(1):1752-1753. DOI 10.1080/23802359.2019.1610091.

Shekhovtsov S.V., Rapoport I.B., Poluboyarova T.V., Geraskina A.P., Golovanova E.V., Peltek S.E. Morphotypes and genetic diversity of *Dendrobaena schmidti* (Lumbricidae, Annelida). *Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding*. 2020b;24(1):48-54. DOI 10.18699/VJ20.594. (in Russian)

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*. 2020c;11:1508. DOI 10.3389/fgene.2020.598196.

Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*. 2014;30(9): 1312-1313. DOI 10.1093/bioinformatics/btu033.

Vallès Y., Boore J.L. Lophotrochozoan mitochondrial genomes. *Integr. Comp. Biol*. 2006;46(4):544-557. DOI 10.1093/icb/icj056.

Viktorov A.G. Diversity of polyploid races in the family Lumbricidae. *Soil Biol. Biochem*. 1997;29(3-4):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. Addition to the fauna of earthworms (Oligochaeta, Lumbricidae) of the Northern Caucasus. *Zoologicheskiy Zhurnal = Zoological Journal*. 2003;82(2):275-280. (in Russian)

Vsevolodova-Perel T.S., Bulatova N.Sh. Polyploid races of earthworms (Lumbricidae, Oligochaeta) in the East European Plain and Siberia. *Biol. Bull. Russ. Acad. Sci.* 2008;35(4):385-388. DOI 10.1134/S1062359008040092.

Wang A.R., Hong Y., Win T.M., Kim I. Complete mitochondrial genome of the Burmese giant earthworm, *Tonoscolex birmanicus* (Clitellata: Megascolecidae). *Mitochondrial DNA.* 2015;26(3):467-468. DOI 10.3109/19401736.2013.830300.

Weigert A., Golombek A., Gerth M., Schwarz F., Struck T.H., Bleidorn C. Evolution of mitochondrial gene order in Annelida. *Mol. Phylogenet. Evol*. 2016;94(Pt. A):196-206. DOI 10.1016/j.ympev.2015.08.008.

Zhang L., Jiang J., Dong Y., Qiu J. Complete mitochondrial genome of an *Amynthas* earthworm, *Amynthas aspergillus* (Oligochaeta: Megascolecidae). *Mitochondrial DNA A DNA Mapp. Seq. Anal.* 2014; 27(3):1-2. DOI 10.3109/19401736.2014.971267.

Zhang L., Jiang J., Dong Y., Qiu J. Complete mitochondrial genome of four pheretimoid earthworms (Clitellata: Oligochaeta) and their phylogenetic reconstruction. *Gene.* 2015;574(2):308-316. DOI 10.1016/j.gene.2015.08.020.

Zhang L., Jiang J., Dong Y., Qiu J. Complete mitochondrial genome of a Pheretimoid earthworm *Metaphire vulgaris* (Oligochaeta: Megascolecidae). *Mitochondrial DNA*. 2016a;27(1):297-298. DOI 10.3109/19401736.2014.892085.

Zhang L., Sechi P., Yuan M., Jiang J., Dong Y., Qiu J. Fifteen new earthworm mitogenomes shed new light on phylogeny within the *Pheretima* complex. *Sci. Rep*. 2016b;6:20096. DOI 10.1038/srep20096.

Zhang Q., Liu H., Zhang Y., Ruan H. The complete mitochondrial genome of *Lumbricus rubellus* (Oligochaeta, Lumbricidae) and its phylogenetic analysis. *Mitochondrial DNA Part B*. 2019;4(2):2677-2678. DOI 10.1080/23802359.2019.1644242.

Zhao H., Fan S., Aspe N.M., Feng L., Zhang Y. Characterization of four earthworm mitogenomes from Northeast China and phylogenetic implication (Oligochaeta: Lumbricidae, Moniligastridae). *Diversity*. 2022;14(9):714. DOI 10.3390/d14090714.

ORCID ID

S.V. Shekhovtsov orcid.org/0000-0001-5604-5601
R. Latif orcid.org/0000-0002-7201-5426
T.V. Poluboyarova orcid.org/0000-0002-5652-0553
S.E. Peltek orcid.org/0000-0002-3524-0456
I.B. Rapoport orcid.org/0000-0002-6766-1482

Acknowledgements. This study was supported by the grant of the Russian Foundation for Basic Research No. 20-54-5603_Iran_t and of the Iran National Science Foundation No. 99003929, as well as by the State Budget Projects No. AAAA-A18-122011900453-0 and FWNR-2022-0022.

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

Received July 29, 2022. Revised September 30, 2022. Accepted September 30, 2022.