



## Original article

Genetic differentiation of black and grey colored forms of the earthworm *Drawida ghilarovi* Gates, 1969 (Moniligastridae, Oligochaeta) on Russian Far EastDmitry M. Atopkin <sup>a, b, \*</sup>, Gennady N. Ganin <sup>c</sup><sup>a</sup> Department of Cell Biology and Genetics, Far Eastern Federal University, 690051 Vladivostok, Russia<sup>b</sup> Institute of Biology and Soil Science, Far Eastern Branch of Russian Academy of Sciences, 690022 Vladivostok, Russia<sup>c</sup> Institute Water and Ecology Problems, Far Eastern Branch of Russian Academy of Sciences, 680000 Khabarovsk, Russia

## ARTICLE INFO

## Article history:

Received 18 June 2014

Received in revised form

12 November 2014

Accepted 17 December 2014

Available online 18 December 2014

## Keywords:

*Drawida*

Oligochaeta

Mitochondrial DNA

Earthworm

Russian Far East

## ABSTRACT

The endemic earthworm *Drawida ghilarovi* Gates 1969, which is found in the Primorye and the south of the Khabarovsk Krai, is represented in the forest by a live form of aneciques (burrowing) and in the wetland by soil-litter (epigeic) worms. These moniligastrida species noticeably differ both in pigmentation (grey and black forms, respectively) and in ecology specifics. Molecular analysis of these specimens has been performed using partial sequences of the mitochondrial COI and 16S genes. The genetic p-distances between the two colored forms of *D. ghilarovi* and within the black form of this species have been estimated and the phylogenetic relationships of these different forms reconstructed using the maximum parsimony and maximum likelihood algorithms. Genetic differentiation within the genus *Drawida* ranged from 16.3 to 23% when using the COI gene sequence data. Genetic differences between the colored forms of *D. ghilarovi* were 14.8–16.9%, suggesting species level differentiation between these forms. Phylogenetic relationships, based on the combined molecular data, showed obvious differentiation between the grey and black forms of *D. ghilarovi*. In addition, there was also considerable differentiation between different samples of the *D. ghilarovi* black forms, collected from different geographical locations.

© 2014 Elsevier Masson SAS. All rights reserved.

## 1. Introduction

The earthworm *Drawida ghilarovi* Gates, 1969 (Moniligastridae, Oligochaeta) is an endemic species of the south of the Russian Far East. These exclusive specimens of tropical genus are included in the Red Book of the Russian Federation (2001) [1] and the Khabarovsk region (2008) [2]. The earthworm specimens were first found at the territory of the Far Eastern forest reserves [3], and were later described by Gates (1969) as a new species [4]. Little biological or ecology data are available on this species [5–10]. Members of the family Moniligastridae occupied South-Western Asia after the collision of the Indian and Asian geological plates during the Tertiary period (i.e., about 70 million years) [11]. The *Drawida* worms are the most broad among other genera of earthworms and

includes the Russian Primorye region, the territories of China, the Korean peninsula, Japan and the Indostan peninsula [4]. In a report by Blakemore (2003), six species of the genus *Drawida* were found to inhabit north-eastern China, four species on the Korean peninsula, and eight species in Japan [12]. Kurchaeva (1977) was the first to identify *Drawida* earthworms in the south of the Russian Far East [13]. Within this territory a color variation of among different specimens was found for *D. ghilarovi*, which was dependent on their environments [4,5,10,14,15]. There are three forms of earthworms, which are differentiated by the morphology and the ecology specifics: endogeic, burrowing and epigeic forms [17]. The burrowing forms live underground in the coprolite capsule. These worms migrate vertically from the capsule upwards towards the decomposed tree waste. Burrowing worms are better adapted to the soil temperature extremes, but their natural habitat is limited by the soil drainage. Epigeic worms live on the soil surface or under the grass waste and selectively feed on plant residues or rootlets within the humus. Epigeic species are hydrophilic and better adapted to flooding. Burrowing and epigeic species are considered

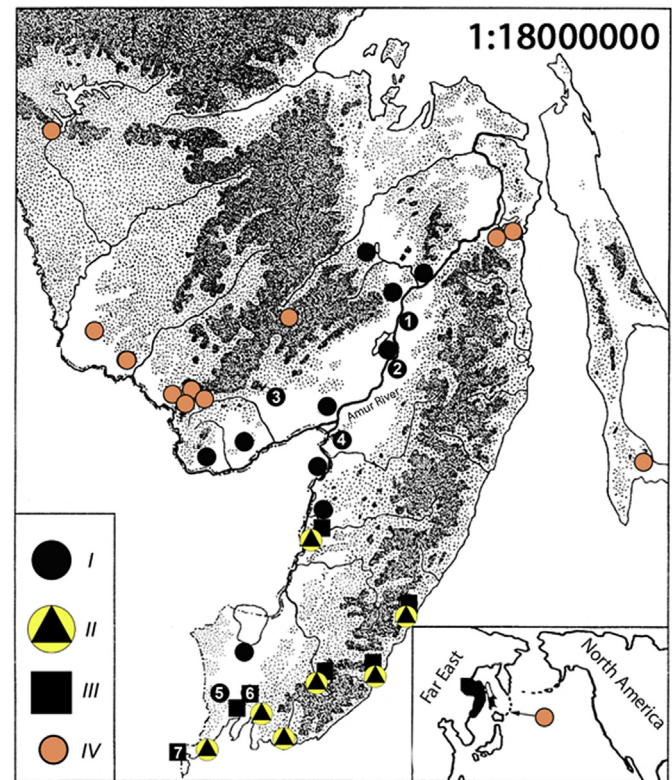
\* Corresponding author. Institute of Biology and Soil Science, Far Eastern Branch of Russian Academy of Sciences, 690022 Vladivostok, Russia.

E-mail address: [atop82@gmail.com](mailto:atop82@gmail.com) (D.M. Atopkin).

as ecologically equal groups because of the great differences of extreme dampness conditions. Burrowing species are able to inhabit the subtropical climatic environments and the epigeic species are found in the northern swampy soils [18]. Each of these species differ in their body size and color, which are either expressed more on the preclitellar part of the body (burrowing species) or evenly distributed throughout the body (epigeic species). Based on the morphological and ecological data, certain ecological forms of *D. ghilarovi* could be recognize as different species [15,16]. Moreover, there is currently no data on the interbreeding abilities of the various color morphs. For this reason, the aim of our study was to clarify the taxonomical status of the different ecological forms of *D. ghilarovi* using molecular tools. The lack of molecular studies addressing *Drawida* species taxonomy using mitochondrial nucleotide sequences [19] allowed us to perform a detailed comparative analysis, using molecular data for the different specimens.

## 2. Materials and methods

Fifteen specimens of the *D. ghilarovi* were collected from the different locations of the Primorye region and the south of Khabarovsk Krai (Table 1, Fig. 1). Specimens were identified morphologically (Ganin et al., 2014) and then fixed in the 96% ethanol for the genetic analysis. Total genomic DNA extracted using the Invitrogen Genomic DNA extraction kit by the manufacturer protocol. Amplification of the COI gene fragment was performed using the polymerase chain reaction with primers LCO1490 (5' GGT CAA CAA ATC ATA AAG ATA TT 3') and HCO2198 (5' TAA ACT TCA GGG TGA CCA AAA AA 3'), described earlier by Folmer et al. (1994) [20]. Amplification of the 16S rRNA gene fragment performed using primers 16S ar (5'CGC CTG TTT ATC AAA AAC AT3') and 16S br (5'CCG GTC TGA FCT CAG ATC ACG T3') [21]. PCR contamination control performed by including the negative control alongside the positive control, using both primers. PCR products directly sequenced using the Big Dye Terminator v.3.1 Cycle Sequencing kit (as instructed by manufacturer) using primers for amplification, described above. Reading of the sequence products performed with the ABI 3130xl genetic analyzer at the Department of Cell Biology and Genetics, FEPU. The resulting sequences submitted to the



**Fig. 1.** Different earthworm morphs of *Drawida ghilarovi* Gates, 1969 (Oligochaeta: Moniligastridae) in the south of the Russian Far East. I – black, II – brownish, III – greenish-blue, bluish-grey, grey *Drawida*, IV – only red Lumbricidae earthworms. 1 – Wetland, Nanaian region, Khabarovsk Krai (Nch); 2 – Wetland, Anyui Park, Khabarovsk Krai (Ach); 3 – Wetland, Bastak Reserve, Jewish Autonomous Region (Bch); 4 – Wetland, Chirki river, Khabarovsk Krai (Chch); 5 – Wetland, Razdol'naja river, Primorje (Pch); 6 – Forest, Ussurijskij Reserve, Primorje (Uz); 7 – Forest, Kedrovaja Pad' Reserve, Primorje (Ps). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Table 1**  
List of taxa, incorporated into analysis.

№	Species (form)	n	Location (Abb.)	Acc. Number	
				COI	16S
1	<i>D. ghilarovi</i> (black)	3	Wetland, Nanaian region, Khabarovsk Krai (Nch)	HG970197 –HG970199	HG970212 –HG970214
2	<i>D. ghilarovi</i> (black)	1	Wetland, Anyui Park, Khabarovsk Krai (Ach)	HG970203	HG970218
3	<i>D. ghilarovi</i> (black)	3	Wetland, Bastak Reserve, Jewish Autonomous Region (Bch)	HG970191 –HG970193	HG970206 –HG970208
4	<i>D. ghilarovi</i> (black)	3	Wetland, Chirki River, Khabarovsk Krai (Chch)	HG970194 –HG970196	HG970209 –HG970211
5	<i>D. ghilarovi</i> (black)	3	Wetland, Razdol'naja River, Primorje (Pch)	HG970200 –HG970202	HG970215 –HG970217
6	<i>D. ghilarovi</i> (grey)	1	Forest, Ussurijskij Reserve, Primorje (Uz)	HG970205	HG970220
7	<i>D. ghilarovi</i> (grey)	1	Forest, Kedrovaja Pad' Reserve, Primorje (Ps)	HG970204	HG970219
8	<i>D. bullata</i>	1	Kumar et al., 2011	JN887894	No data
9	<i>D. gracilis</i>	2	Kumar et al., 2011	JN887887 –JN887888	JN887899 –JN887900
10	<i>D. hattamimizu</i>	2	Minamiya et al., 2010	AB543206 AB543227	No data
11	<i>D. j. japonica</i>	4	Chang et al., 2009	EF077597 –EF077600	No data

European Nucleotide Archive (ENA), accession numbers presented in Table 1.

Nucleotide sequences initially assembled with the SeqScape v.2.6 software and aligned using the MEGA 5.22 alignment explorer with default options [22]. Regions that aligned ambiguously, excluded from the analysis. Calculation of a number of variable and parsimony-informative sites, and of genetic *p*-distances performed using the MEGA 5.22. Genetic divergence estimated by calculation. Saturation analysis performed by calculation of the rate of number of variable sites on the 3-rd codon position and the *p*-distances. Phylogenetic relationships analyzed with the maximum parsimony and the maximum likelihood algorithm using the MEGA 5.0 software. Resulting trees were rooted with species of the genus *Eisenia* (family Lumbricidae), which were used as outgroup taxa. Phylogenetic relationships significance estimated using the bootstrap analysis with 1000 replications [23].

Phylogenetic relationships of the *D. ghilarovi* inferred from our data and the nucleotide sequences of mitochondrial COI and 16S genes of other species of the genus *Drawida* from the NCBI GenBank database (Table 1).

## 3. Results

A total of 581 and 457–460 alignable characters were available for the analysis of the COI and 16S rRNA mitochondrial gene datasets of *D. ghilarovi*, respectively. Of these, 446 (76.8%) and 373

(81.1–81.6%) were invariant and 79 (13.6%) and 64 (13.9–14%) were parsimony informative, for COI and 16S rRNA respectively. Analysis of the *D. ghilarovi* specimens from seven distinct samples revealed obvious genetic differentiation between the two ecological forms by both COI and 16S mitochondrial gene sequences. It is known that mitochondrial genes, such as COI gene or cytochrome B gene are saturated by nucleotide substitutions on the third codon position. This feature impeded to perform an adequate estimation of genetic variation. In this case the third codon position forcibly excluded from the analysis. We performed saturation analysis for COI gene sequence data. Results showed that saturation on the third codon position appear on 13% of genetic differentiation (Fig. 2a). Thus we excluded the third codon position to perform genetic analysis of the *Drawida* species and different morphological forms of *D. ghilarovi*. Nevertheless, genetic p-distances for local population of *D. ghilarovi* were calculated with the third codon position, because there is no saturation on this level of differentiation (Fig. 2b). Thus we were able to make taxonomical conclusions based on comparative analysis of genetic p-distance mean values estimated for different colored forms of the *D. ghilarovi* and different species of the genus *Drawida*.

The genetic differentiation between the black and grey colored forms was 3.7% and 12.6% by COI and 16S rRNA gene sequence data, respectively (Table 2). There is little data available on the 16S rRNA gene sequences of *Drawida* species, so that the interspecific genetic differentiation values were calculated using the COI gene sequences data only. Interspecific genetic differentiation values ranged from 4.64% [*Drawida bullata*/*Drawida gracillis*] to 6.96% [*Drawida gracilis*/*Drawida hattamimizu*] (Table 3). Species *D. ghilarovi* was genetically closer to *D. j. japonica* (5.39%).

There was also genetic variation between the geographically distant samples of the *D. ghilarovi* epigeic form (Table 3, Fig. 1).

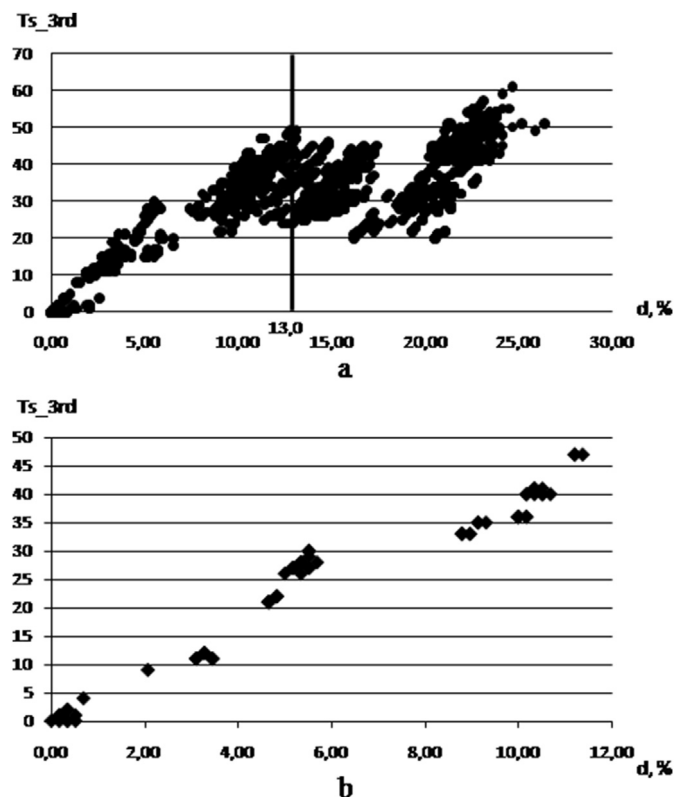


Fig. 2. Results of saturation analysis performed for COI gene nucleotide sequences of a – different species of the genus *Drawida* and b – different populations of *D. ghilarovi*.

**Table 2**  
Genetic differentiation (%) between different species of the genus *Drawida* by COI gene partial sequences data, calculated with exclusion of the third codon position.

		1	2	3	4	5	6
1	<i>D. ghilarovi</i> black						
2	<i>D. ghilarovi</i> grey	3.66					
3	<i>D. bullata</i>	5.49	6.06				
4	<i>D. gracillis</i>	6.34	5.28	4.64			
5	<i>D. hattamimizu</i>	5.69	5.54	5.67	6.96		
6	<i>D. j. japonica</i>	5.39	5.03	6.44	6.19	6.44	

**Table 3**  
Genetic differentiation (%) between samples of *D. ghilarovi* by COI gene (below diagonal) and 16S gene (above diagonal) sequences data. B – black colored form, G – grey colored form.

		1	2	3	4	5	6	7
1	Nch (B)		1.29	1.77	4.42	2.28	13.69	10.82
2	Ach (B)	4.65		2.10	4.38	2.50	14.35	11.48
3	Bch (B)	3.27	2.87		5.30	3.46	15.16	12.29
4	Chch (B)	10.38	10.73	10.04		5.15	14.20	11.77
5	Pch (B)	5.28	5.22	4.65	8.95		13.91	11.04
6	Uz (G)	16.58	15.92	15.55	15.32	14.80		4.19
7	Ps (G)	16.52	16.35	15.89	15.83	14.92	2.07	

Genetic differentiation between samples ranged from 3.3% (Nch/Bch) to 10.7% (Ach/Chch) by COI gene sequences data and from 1.3% (Nch/Ach) to 4.9% (Bch/Chch) by 16S rRNA gene sequences data. The sample from Chch was more genetically distant in comparison with the other specimens. Moreover, the 16S rRNA gene sequences of the Chch specimens was characterized by the highest fragment length (473 bp) due to insertion of the nucleotide triplet, as compared with specimens from other locations. The Nch and Bch samples were genetically closer to each other using the COI gene sequence data. Whereas Nch was closer to Ach when using the 16S rRNA sequence data. For this reason, we performed the analysis of the molecular variation of *D. ghilarovi* specimens using the combined sequence data. The results of this combined analysis showed the same result as the COI sequence data analysis, indicating that the highest genetic identity existed between the Bch and Nch samples (2.8%). The genetic differentiation between specimens of the burrowing form (samples Ps and Uz) was 2.07 and 4.3% by the COI gene and 16S rRNA gene sequences, respectively.

Analysis of the phylogenetic relationships of *D. ghilarovi* with other species from this genus were made using the partial sequencing of the mtDNA COI gene (Fig. 3), together with species data found shown in the international GeneBank database. The *D. ghilarovi* specimens were closely related to the Chinese *Drawida japonica japonica* (GeneBank data) that correspond to genetic p-distances data. Species *D. hattamimizu* included into the Red Book of Japan (2007) [24] was related to the *D. ghilarovi*/*D. j. japonica* cluster, whereas *D. bullata* and *D. gracilis* formed distinct branches.

The phylogenetic analysis of the *D. ghilarovi* specimens using the

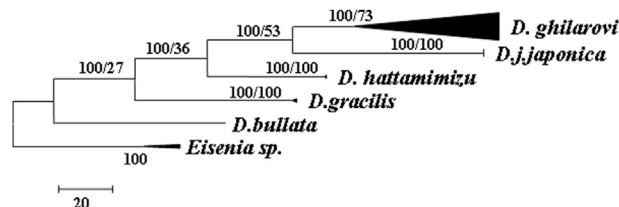


Fig. 3. Phylogenetic tree of the species of the genus *Drawida* based on COI gene partial sequences. Nodal labels indicate bootstrap statistics, calculated for the MP/ML algorithms. Branch length measured by amount of substitutions.



combined COI gene and 16S rRNA sequence data showed obvious differentiation between the black and grey colored forms (Fig. 4). The phylogenetic relationships of the black colored specimens strongly corresponded with their geographical distribution. Each geographical sample formed a distinct cluster with high bootstrap support. Specimens from Nch and Bch were closely related to each other, whereas samples from Pch, Chch and Ach formed distinct clusters. The specimens from Ach were closely related to the Bch/Nch cluster.

#### 4. Discussion

Our molecular data, the first reported for this species, reveal a high genetic differentiation (p-distances) between the black and grey colored forms (up to 16.5–16.6% by the COI sequence data), as well as the various phylogenetic relationships between the studied forms. The p-distance values between the black and grey colored earthworms are close to the genetic differentiation of *D. ghilarovi* and *D. hattamimizu* (16.6%). Moreover, these results corresponded with the molecular differentiation of the Chinese *D. j. japonica* and the Japanese *D. cf. japonica* (17.13%), reported by Blakemore et al. (2010) [19]. These authors concluded that the mutual conspecificity of these two specimens may be questioned. However, the Chinese *D. japonica* is probably a misidentification as its DNA sequence differs from the Japanese specimens reported in Blakemore et al. (2010) [19]. Moreover, molecular analysis of these species was performed including the third codon position that could provide an adequate estimation of genetic differentiation. Nevertheless, our molecular data suggest the necessity to perform a taxonomical revision for the different forms of *D. ghilarovi* because molecular differentiation of these worms is relatively high. The grey worm is represented in the forest by an anecic (burrowing) form, while the black worm is found in the wetland and is a soil-litter (epigeic) form. These moniligastrida differ noticeably by both their pigmentation and ecology specifics. Meadow-swamp form of *D. ghilarovi* inhabit the far north along the Amur floodplain. Experiments have shown that the grey worms could also survive in peat soils, whereas the black *D. ghilarovi* die in the forest soils. The sympatric habitations and interbreeding for these oligochaeta

species remain unknown [15].

Intraspecific variation of the *D. ghilarovi* black form, as revealed by molecular data, is associated with the geographical origin of the investigated specimens. Each geographical sample has a unique haplotype of both the COI and 16S rRNA gene and formed a distinct cluster on the phylogenetic tree. Such genetic differentiation suggests local geographical isolation of the various groups of these earthworms. These results suggest further study is required on the phylogeography of *D. ghilarovi* with a the more representative sample. Nevertheless, there is a hypothesis that the Amur River has a thread of stream that runs from north to the south, across the Khanka Lake, and flows into the Amur Bay of the Japan Sea [25]. Distribution of the *D. ghilarovi* from east and west is thought to be limited by ancient watersheds, e.g., the Small Khingan and Sikhote-Alin mountain ridges (about 12.5 Mya). This part of the pre-Amur River is therefore considered the ancient natural habitat of the black form of *D. ghilarovi*. Now such inhabitations are mostly noted for the Ussury River basin, the Middle and Lower Amur River and, partially, lowland near the Khanka Lake and the floodplain of the Razdolnaya River. The present inhabitations and phylogenetic relationships, obtained in our study, therefore correspond with the shape of the Amur River basin in the Late Neogene.

#### 5. Conclusion

The earthworm *D. ghilarovi*, belonging to the large Indo-oriental family Moniligastridae Claus 1880, is the endemic species of the south of the Russian Far East. The phylogenetic analysis of this specimen by combined COI gene and 16S rRNA gene sequence data showed obvious differentiation between the meadow-swamp black form and the forest grey form. These two forms of *D. ghilarovi* differ from each other as much as the epigamic. *ghilarovi* differs from *D. hattamimizu* from Honshu Island. The molecular data therefore suggest the necessity to perform a taxonomical revision for the black and grey colored forms of *D. ghilarovi*. The phylogenetic relationships of the black colored specimens were strongly associated with their geographical distribution, which is possibly associated with the shape of the pre-Amur River basin in the Late Neogene.

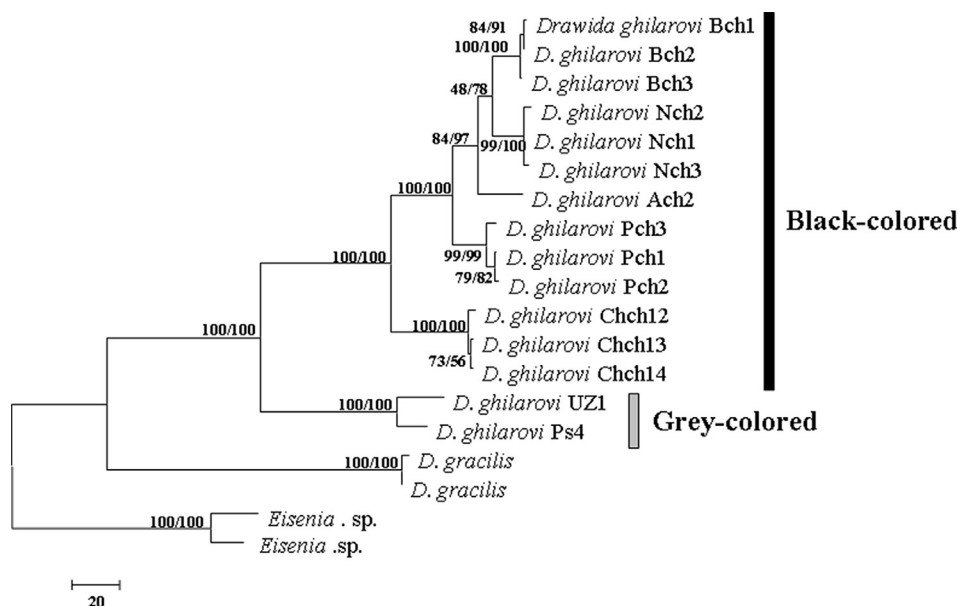


Fig. 4. Phylogenetic tree of the species *D. ghilarovi* from different locations based on COI gene partial sequences. Nodal labels indicate bootstrap statistics, calculated for the MP/ML algorithms. Branch length measured by amount of substitutions.

## Acknowledgments

Partial funding support for this research by grant № 12-04-00221-a.

## References

- [1] The Ministry of Natural Resources of the Russian Federation, Russian Academy of Sciences, The Red Book of the Russian Federation (Animals), ACT Press, Astrel Press, Moscow, 2001.
- [2] The Ministry of Natural Resources of the Khabarovsk Territory, the Institute of Water and Ecological Problems FEB RAS, The Red Book of the Khabarovsk Territory: Rare and Endangered Species of Plants and Animals: the Official Edition, Publishing House "Amur lists", Khabarovsk, 2008.
- [3] M.S. Ghilarov, T.S. Perel, Soil invertebrate complexes of conifer and broad-leaved forests of Far East as an indicator of the soil type, *Soil Inv. Ecol.* (1973) 40–59.
- [4] G.N. Gates, A new species of earthworm of the family Moniligastridae of the genus *Drawida* Michaelsen, 1900, *Russ. J. Zool.* 48 (1969) 674–676.
- [5] T.S. Perel, Geographical Distribution and Patterns of Distribution of Earthworms of USSR Fauna, Nauka, Moscow, 1979.
- [6] G.N. Ganin, Soil Animals of the Ussuriysk Region, Dalnauka Press, Vladivostok, 1997.
- [7] D.I. Berman, E.N. Mescheryakova, A.N. Leirykh, D.K. Kurenschikov, Natural habitat and cold-resistance of the earthworm *Drawida ghilarovi* (Oligochaeta, Moniligastridae), *Russ. J. Zool.* 89 (2010) 1027–1036.
- [8] G.N. Ganin, E.N. Sokolova, On the biology of the earthworm *Drawida ghilarovi* Gates, 1969 (Moniligastridae, Oligochaeta): cocoons development, in: *Proceedings of International Conference "Mountain Systems and Their Components"*, vol. 1, 2012, pp. 138–139.
- [9] G.N. Ganin, E.N. Sokolova, On the biology of the earthworm *Drawidaghilarovi* Gates, 1969 (Moniligastridae, Oligochaeta): Amur population survival under laboratory conditions, in: *Proceedings of International Conference "Ecology, Evolution and Systematic of Animals"*, vol. 1, 2012, pp. 59–60.
- [10] G.N. Ganin, A.P. Anisimov, D.M. Atopkin, G.V. Roslik, E.K. Berezkin, Distribution and genetic studying of the earthworms *Drawida ghilarovi* Gates, 1969 (Moniligastridae, Oligochaeta) – a Russian Far Eastern endemic, in: *Proceedings of International Conference "Animals: Ecology, Biology and Protection"*, vol. 1, 2012, pp. 82–85.
- [11] E.G. Easton, Japanese earthworms: a synopsis of the Megadrile species (Oligochaeta), *Bull. Brit. Mus. Zool.* 40 (1981) 33–65.
- [12] R.J. Blakemore, Japanese earthworms (Annelida: Oligochaeta): a review and checklist of species, *Org. Div. Evol.* 3 (2003) 241–244.
- [13] G.F. Kurcheva, Far Eastern Soil Invertebrates, Nauka, Moscow, 1977.
- [14] T.S. Vsevolodova-Perel, Earthworms Fauna of Russia: the Inventory and Keys, Nauka, Moscow, 1997.
- [15] G.N. Ganin, Earthworms *Drawida ghilarovi* Gates, 1969 (Oligochaeta, moniligastridae): 1. Polymorphism, geographic range, ecology specifics, *Amur. J. Zool.* 4 (2013) 401–405.
- [16] R.J. Blakemore, E.K. Kupriyanova, Unravelling some Kinki worms (Annelida: Oligochaeta: Megadrili: Moniligastridae) Part I, *Opusc. Zool.* 40 (2010) 3–18.
- [17] T.S. Perel, Differences of the earthworm species organization, *Adapt. Soil. Anim. Env. Cond.* (1977) 129–144.
- [18] K.E. Lee, Earthworms. Their Ecology and Relationships with Soils and Land Use, Academic Press, Moscow, 1985.
- [19] R.J. Blakemore, E.K. Kupriyanova, M.J. Grygier, Neotipification of *Drawida hattamimizu* Hatai, 1930 (Annelida, Oligochaeta, Megadrili, Moniligastridae) as a model linking mtDNA (COI) sequences to an earthworm type, with a response to the 'Can of Worms' theory of cryptic species, *ZooKeys* 41 (2010) 1–29.
- [20] O. Folmer, M. Black, W. Hoeh, R. Lutz, R. Vrijenhoek, DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates, *Mol. Mar. Biol. Biotech.* 3 (1994) 294–299.
- [21] C.D. Schubart, J.E. Neigel, D.L. Felder, Molecular phylogeny of mud crabs (Brachyura: Panopeidae) from the northwestern Atlantic and the role of morphological stasis and convergence, *Mar. Biol.* 137 (2000) 11–18.
- [22] K. Tamura, D. Peterson, N. Peterson, G. Stecher, M. Nei, S. Kumar, MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods, *Mol. Biol. Evol.* 28 (2011) 2731–2739.
- [23] J. Felsenstein, Confidence limits on phylogenies: an approach using bootstrap, *Evolution* 39 (1985) 783–791.
- [24] Anon, The Red Data Book of Japan, 2007. <http://www.biodic.go.jp/emglish/J-IBIS.html>.
- [25] Y.F. Chemekov, A history of development of the drainage of Amur River basin, *Izv. RAN* 1 (1964) 81–93.