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Karyotype analysis of five species of earthworms (Oligochaeta: Lumbricidae)

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Abstract. Karyotypes of five species of earthworms from southeast edge of the West Siberian plain are described. Haploid chromosome sets of all species comprise of 18 bi-armed chromosomes. Four of these species, *Eisenia atlavinyteae* (Perel, Graphodatsky, 1984), *Eisenia nordenskioldi* (Eisen, 1879), *Eisenia balatonica* (Pop, 1943), and *Aporrectodea caliginosa* (Savigny, 1826) are diploid (2n=36, FN=72) and one, *Octolasion lacteum* (Örley, 1885) – triploid (3n=54, FN=108). Relative chromosome length and centromere index are presented.

Key words: Oligochaeta, Lumbricidae, karyotypes, chromosome numbers, chromosome structure.

Introduction

Chromosome analysis is an essential part of systematic studies of any group of organisms, and can play an important role, especially in taxonomically complicated genera. Description of the relative size of chromosomes and position of centromeres provide an objective information, suitable to compare the species and geographically remote populations. It was successfully used for many species of invertebrates, for example, in comparative cytogenetic of trematodes (Barsene, 1993). However, only a few such examples can be found among descriptions of karyotypes of subterranean earthworms (see Muldal, 1952 and Vitturi et al., 1991 as examples of karyotype description of Eisenia foetida (Savigny, 1826)). Available information is usually limited to the basic chromosome numbers and a level of ploidy (Omodeo 1952, 1955; Vedovini, 1973; Viktorov, 1993; Bakhtadze et al., 2008 and other papers) and nearly everywhere it does not include the statistical measurements of chromosomes (Vitturi et al., 1991).

Here we present the description of karyotypes of five species of earthworms from three genera: *Eisenia atlavinyteae* (Perel, Graphodatsky, 1984), *Eisenia nordenskioldi* (Eisen, 1879), *Eisenia balatonica* (Pop, 1943), *Aporrectodea caliginosa* (Savigny, 1826), and *Octolasion lacteum* (Örley, 1885). First two species are aboriginal to Siberia (Bulatova et al., 1984; Perel et al., 1985; Perel, 1987). Another species of the same genus – *E. balatonica* – is distributed from the West Siberia to Hungary (Vsevolodova-Perel, 1997). The latter two species occupy practically all continents (Vsevolodova-Perel, 1997; Reynolds, 1995; James, Hendrix, 2004).

Karyotype of *A. caliginosa* had been studied in different regions of Europe and everywhere it was diploid, composed of 36 chromosomes (Omodeo, 1951; Muldal, 1952; Vedovini, 1973; Viktorov, 1989; Garbar, Vlasenko, 2007). Another species – *O. lacteum*, has diploid (2n=38) (Muldal, 1952), triploid (3n=54) and tetraploid (4n=72) (Omodeo, 1955) forms in the West Europe.

Two Siberian species were found to have a series of chromosome races with different ploidy: *E. atlavinyteae* – 2n=36 and 72 (Perel, Graphodatsky, 1984; Perel et al., 1985) and *E. nordenskioldi* c 2n=36, 72, 108 and 144 (Graphodatsky et al., 1982; Perel, Graphodatsky, 1983; Bulatova et al., 1987; Viktorov, 1989).

Karyotype of *E. balatonica* is described here for the first time.

Material and methods

Earthworms were collected during the summer of 2005 and 2006 at the right bank of the Ob' river (54°49'N/83°06'E) in vicinity of Novosibirsk (West Siberia, Russia) (Table 1). Species were identified according to Vsevolodova-Perel (1997). Air-drayed spreads of mitotic and meiotic chromosomes were prepared from testis (Graphodatsky et al., 1982) and stained with Giemsa (Macgregor, Varley, 1986). The preparations were analyzed with an Axioplan 2 Imaging microscope (Carl Zeiss, Germany) equipped with a CCD camera (CV M300, JAI Corporation, Japan). Brightness and contrast of all the images were enhanced using PaintShopPro 7.0.

The length of the chromosomes was measured in micrometers at the images using MicroMeasure 3.3 (http://www.colostate.edu/Depts/Biology/MicroMeasure/) and the centromere positions were recorded. Relative size (percentage of chromosome within the total length of the haploid complement) and centromere index (ratio between the length of the short arm and the total chromosome length) was scored for each chromosome.

Table 1. Chromosome numbers of five species of earthworms.

Species of earthworms under study	2n	Number of specimens examined
Eisenia balatonica	36	7
Eisenia nordenskioldi	36	7
Eisenia atlavinyteae	36	16
Aporrectodea caliginosa	36	13
Octolasion lacteum	54	26

Results and Discussion

Haploid chromosome sets of five investigated species include each 18 bi-armed chromosomes (Table 1 and Fig. 1). Four species – *Eisenia atlavinyteae*, *E. nordenskioldi*, *E. balatonica* and *Aporrectodea caliginosa* – are diploid (2n=36, FN=72). In diakinesis of these species we observed 18 bivalents. Karyotype of O. lacteum contained three haploid chromosome sets (2n=54, FN=108) and in the spreads of its meiotic chromosomes we detected 36 elements – 18 bivalents and 18 univalents. These data (except of *Eisenia balatonica* which is described here for the first time) are in agreement with the previously published observations (Perel, Graphodatsky, 1984; Bulatova et al., 1987; Garbar, Vlasenko, 2007; Omodeo, 1955).

A useing of a new for invertebrates method of air-drying preparations adopted by Graphodatsky et al. (1982) allowed to produce very clear karyograms of *Eisenia atlavinyteae* (Perel et al., 1985) and *Aporrectodea caliginosa* (Garbar, Vlasenko, 2007). Because of the lack of measurements in these publications we can conclude only that the structures of chromosomes of these two species are very similar in cited articles and presented work.

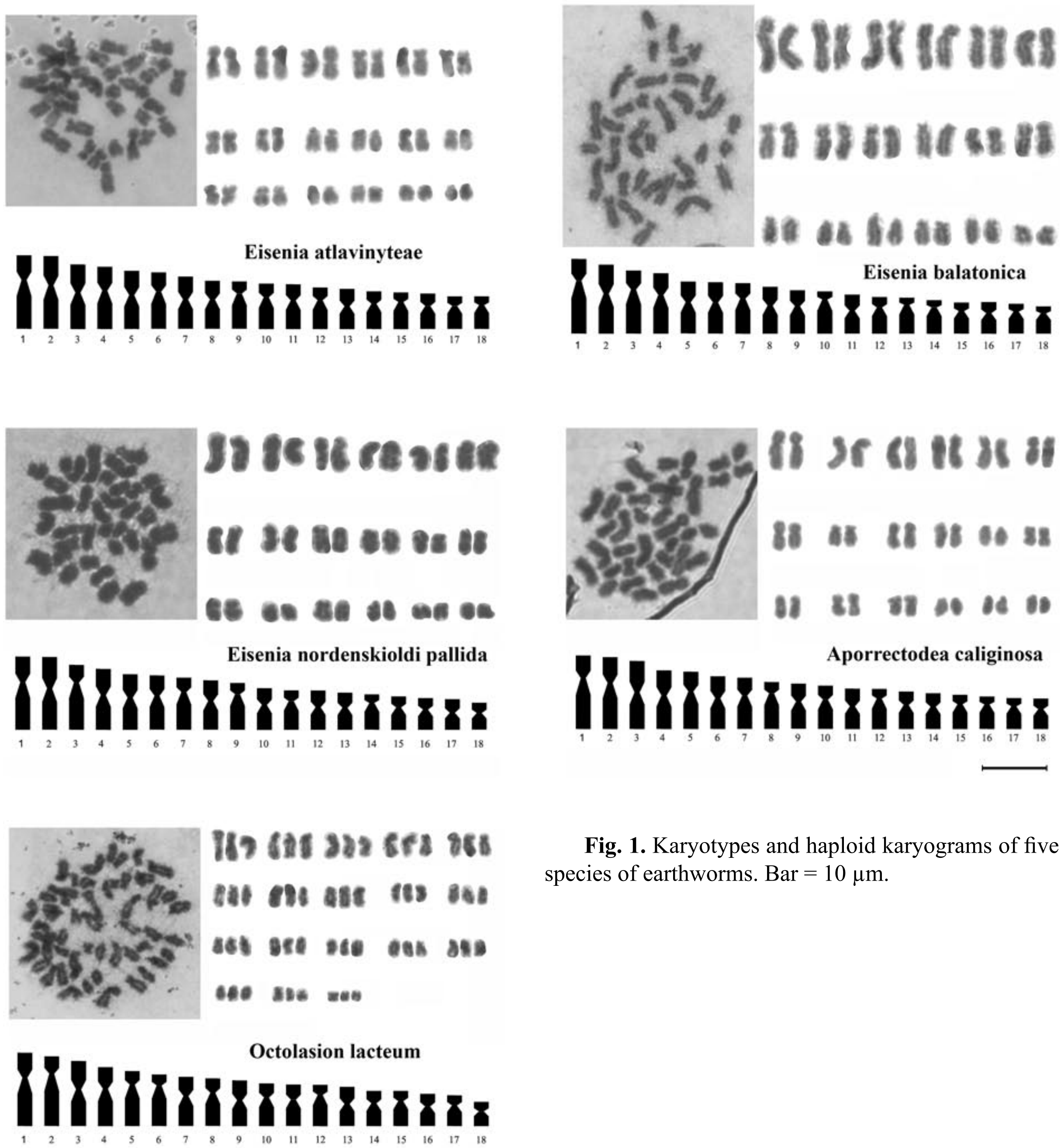

Fig. 1. Karyotypes and haploid karyograms of five species of earthworms. Bar = 10 µm.

It is interesting that representatives of tetraploid race of *E. atlavinyteae* (2n=72) were observed earlier near Novosibirsk (West Siberia, Russia) (Perel et al., 1985) at the same place where we collected diploid specimens. This finding can indicate the possibility for existence of the hybrid zone or zone of sympatry of diploid and tetraploid worms somewhere on this area.

In conclusion it is necessary to say that presented data on the relative length and centromere index (Table 2 and karyograms in Fig. 1) reveal a continuous gradation in the size of chromosomes. In general, chromosomes are

Table 2. Relative length and centromere index of chromosomes of five species of earthworms. RL - relative length, CI – centromere index, SD - standard deviation.

№	*E. atlavinyteae*		*E. balatonica*		*E. nordenskioldi*		*A. caliginosa*		*O. lacteum*	
	RL ± SD	CI ± SD	RL ± SD	CI ± SD	RL ± SD	CI ± SD	RL ± SD	CI ± SD	RL ± SD	CI ± SD
1	8.66 ±0.83	33.44 ±0.33	9.13 ±0.88	29.15 ±0.34	8.75 ±1.22	34.97 ±0.46	8.55 ±0.94	35.97 ±0.41	8.75 ±1.34	34.01 ±0.37
2	8.22 ±0.73	40.00 ±0.31	8.39 ±0.88	42.02 ±0.37	8.52 ±1.61	43.10 ±0.19	8.27 ±1.00	30.58 ±0.69	8.35 ±1.28	24.81 ±1.02
3	7.29 ±0.74	43.67 ±0.18	7.69 ±0.54	29.94 ±0.49	7.74 ±0.97	27.62 ±0.99	7.99 ±0.65	46.08 ±0.16	7.71 ±1.39	43.67 ±0.22
4	7.11 ±0.71	38.31 ±0.33	7.42 ±0.85	35.84 ±0.62	7.20 ±0.82	42.92 ±0.23	6.88 ±0.68	42.74 ±0.24	7.03 ±0.98	36.63 ±0.43
5	6.57 ±0.76	41.67 ±0.23	6.34 ±0.45	39.68 ±0.39	6.54 ±1.06	41.84 ±0.24	6.77 ±0.66	34.25 ±0.31	6.55 ±0.56	24.69 ±0.97
6	6.39 ±0.48	34.13 ±0.46	6.26 ±0.59	39.06 ±0.34	6.46 ±0.82	37.59 ±0.35	6.21 ±0.65	41.49 ±0.25	6.20 ±0.94	21.10 ±1.29
7	5.91 ±0.57	44.05 ±0.22	6.20 ±0.55	30.30 ±0.32	6.14 ±0.57	27.47 ±0.64	6.04 ±0.68	34.72 ±0.37	5.85 ±0.64	39.06 ±0.34
8	5.42 ±0.48	39.06 ±0.38	5.75 ±0.42	40.00 ±0.19	5.88 ±0.88	42.19 ±0.31	5.54 ±0.91	24.04 ±0.71	5.66 ±0.63	30.21 ±0.51
9	5.39 ±0.59	25.38 ±0.89	5.29 ±0.58	36.36 ±0.46	5.44 ±0.66	23.92 ±0.76	5.32 ±0.56	39.68 ±0.64	5.49 ±0.58	43.67 ±0.19
10	5.12 ±0.56	29.85 ±0.56	5.18 ±0.32	19.01 ±1.74	4.95 ±0.49	40.00 ±0.37	5.06 ±0.56	32.26 ±0.44	5.07 ±0.41	32.89 ±0.69
11	5.06 ±0.43	36.10 ±0.46	4.77 ±0.55	41.32 ±0.35	4.66 ±0.37	42.37 ±0.25	4.78 ±0.48	23.87 ±1.37	4.96 ±0.51	24.15 ±0.92
12	4.67 ±0.52	30.58 ±0.88	4.50 ±0.72	28.33 ±0.77	4.65 ±0.38	26.88 ±0.58	4.75 ±0.65	43.67 ±0.30	4.94 ±0.61	36.50 ±0.49
13	4.52 ±0.59	43.48 ±0.26	4.39 ±0.73	19.57 ±2.60	4.42 ±0.44	40.16 ±0.33	4.42 ±0.53	35.59 ±0.55	4.67 ±0.53	43.86 ±0.35
14	4.24 ±0.48	32.68 ±0.89	4.11 ±0.25	21.37 ±1.82	4.16 ±0.46	24.45 ±0.79	4.15 ±0.35	37.88 ±0.93	4.18 ±0.59	28.99 ±0.76
15	4.15 ±0.31	25.91 ±0.73	3.85 ±0.32	35.09 ±0.98	3.96 ±0.39	32.68 ±0.55	4.02 ±0.52	27.62 ±0.92	4.16 ±0.68	35.21 ±0.57
16	3.98 ±0.43	35.09 ±0.89	3.81 ±0.34	27.55 ±0.78	3.68 ±0.41	35.84 ±0.38	4.02 ±0.41	24.39 ±0.72	3.83 ±0.56	39.06 ±0.52
17	3.65 ±0.46	29.15 ±1.09	3.61 ±0.21	25.71 ±0.94	3.68 ±0.68	44.64 ±0.17	3.63 ±0.49	34.84 ±0.62	3.71 ±0.49	32.68 ±0.68
18	3.64 ±0.55	26.88 ±0.88	3.32 ±0.27	25.25 ±2.66	3.17 ±0.47	38.46 ±0.51	3.60 ±0.43	26.60 ±0.70	2.90 ±0.51	38.91 ±0.51

rather similar in the centromere index (about 35). It means that it would be difficult to use karyograms as a quick and easy feature for distinction among species with equal ploidy and basic numbers; at least in regard to species described in this paper. Nevertheless, presented data will be useful for precise analysis of karyotype and its geographic variability within species, as it was performed by Vitturi et al. (1991) for *Eisenia foetida*.

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