

## Karyotypes of three species of the genus *Aporrectodea* Örley (Oligochaeta: Lumbricidae) from the Ukraine

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**Abstract.** The karyotypes of *Aporrectodea caliginosa*, *A. rosea*, and *A. longa* were studied. The diploid sets of chromosomes comprise 18 pairs of two-armed chromosomes (FN = 72) in each species. A triploid form was discovered in *A. rosea* ( $3n = 54$ , FN = 108). Considerable variations of chromosome number were noted within the same preparation.

**Key words:** Oligochaeta, Lumbricidae, *Aporrectodea*, karyotypes, chromosome numbers.

### INTRODUCTION

Karyological studies of earthworms were started in the middle of XX century (Omodeo, 1951; Muldal, 1952). Then, different taxa of this group were investigated karyologically (Vedovini, 1973; Casellato, 1987). However, the widely used method of squashed preparations did not allow study of the morphology of chromosomes adequately and even to determine chromosome numbers in some cases. The more convenient method of air-drying preparations was applied to lumbricids by Grafodatsky et al. (1982) for the first time. In particular, polyploidy series of *Eisenia nordenskioldi* (Eisen, 1879) were studied by this method. At present, about 60 species of earthworm species from 12 genera have been karyotyped.

The species of the genus *Aporrectodea* Örley 1885, *A. caliginosa* (Savigny, 1826), *A. rosea* (Savigny, 1826), *A. longa* (Ude, 1826), and some others, play an important role in soil-formation. The chromosome numbers of these species were defined without information on chromosome morphology (Victorov, 1993). Diploid races ( $2n$

= 36) were discovered in *A. longa* and *A. caliginosa*; triploid ( $2n = 54$ ) and tetraploid races ( $2n = 72$ ) were found in *A. caliginosa* subsp. *trapezoides* (Duges, 1828). *A. rosea* has a wide variety of polyploid forms; seven different forms from  $2n = 36$  to  $2n = 160-174$  have been described in this species. Information on karyotypes of above mentioned species from the territory of the Ukraine was absent till now. So, we have decided to investigate the range of polyploid forms from this territory.

### MATERIAL AND METHODS

Individuals of *A. caliginosa*, *A. rosea* and *A. longa* were collected in Zhytomyr, Kyiv, Chernigiv and Sumy Provinces during April-September 2005.

Testicular tissues were prepared by the method, known for mollusks (Garbar, 1998). Colchicine 0.02% was injected to the animals 24 hours before the dissection of the testicular bags. Testicular tissues were dissected out and incubated in distilled water for 60 minutes, and fixed in acetoethanol (1 : 3). The cellular suspension was placed on warm



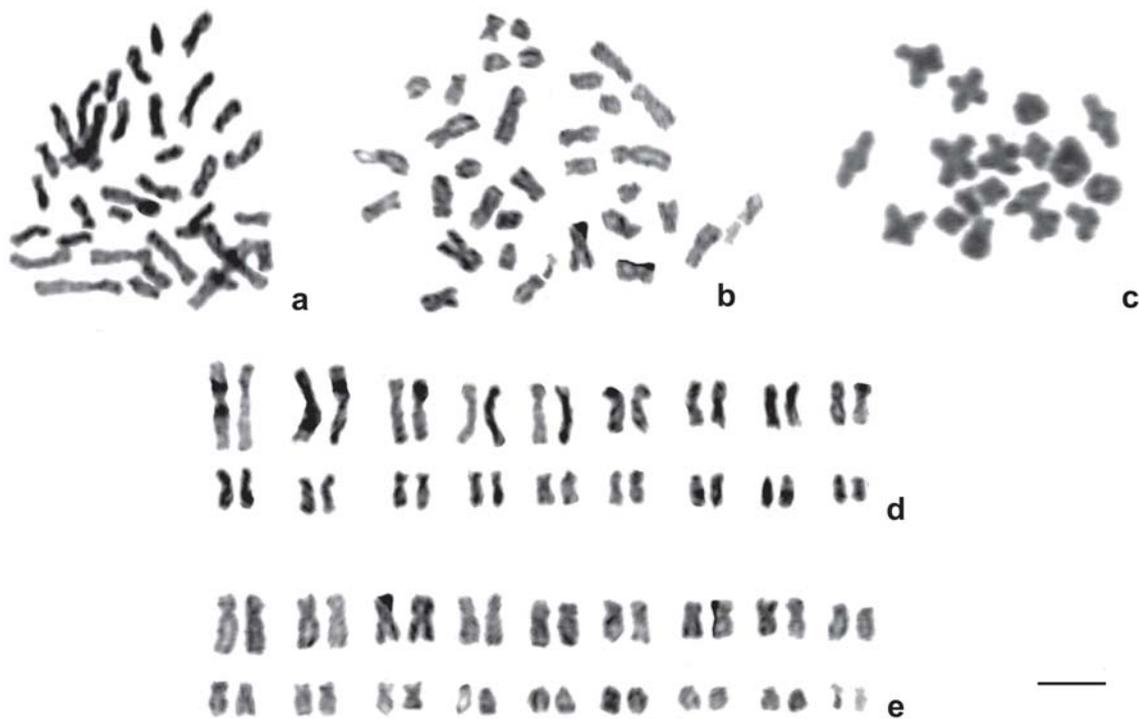


Fig. 1, a-e. Chromosomes of *Aporrectodea caliginosa*. a, b - mitotic metaphase ( $2n = 36$ ); c - diakinesis ( $n = 18$ ); d, e - karyograms. Bar = 10  $\mu\text{m}$ .

slides (50° C) with a capillary dropper. The dried preparations were stained in 10% azure-eosin in 0.01M phosphate buffer (pH 6.8) by Romanovsky. Stained slides were mounted in Canada balsam.

The morphology of chromosomes is given according to Levan et al. (1964).

## RESULTS AND DISCUSSION

### *Aporrectodea caliginosa*

As a whole, 70 chromosome preparations with 246 mitotic metaphases and 26 meiotic prophase (diakinesis) were studied. The species has 36 chromosomes in its diploid set, as it was reported earlier (Muldal, 1952 and other authors). All chromosomes are two-armed (Fig. 1), therefore, the fundamental number of the species is 72. On the plates with less condensed chromosomes (Fig. 1,

a, d) two groups of chromosomes are clearly visible; the first 5 pairs are large meta- and submetacentrics; the other 13 pairs are smaller meta-, submetata- and subtolocentrics. In each of the 8 specimens collected in Nizhyn (Chernigiv Province) and Romaniv (Zhytomyr Province) the apparent diploid chromosome number varied from 36 to 100 and above, but the majority of worms from these localities had the modal chromosome number.

In diakinesis 18 bivalents were found in the most of the analyzed specimens (Fig. 1, c).

### *A. rosea*

48 preparations with 106 mitotic metaphases were studied. Diploid ( $2n=36$ ) and triploid ( $3n=54$ ) chromosome races were discovered. Similar races were found earlier in this species by other authors

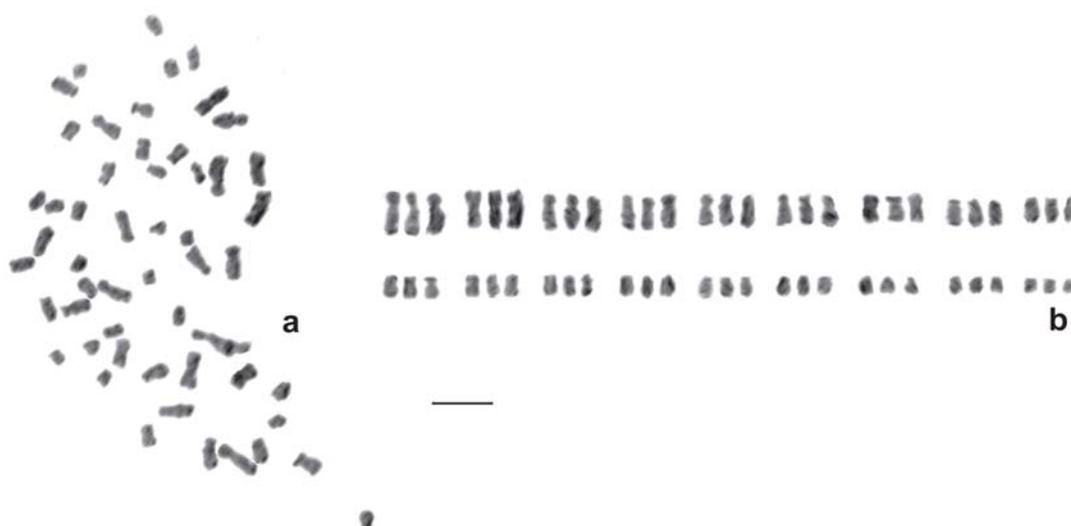


Fig. 2, a, b. Chromosomes of triploid race of *Aporrectodea rosea*. a - mitotic metaphase ( $3n = 54$ ); b - karyogram. Bar = 10  $\mu\text{m}$ .

in Europe (Muldal, 1952; Vedovini, 1973). In the Ukraine we have found diploid worms only in Chernigiv Province; triploid representatives were found in Zhytomyr, Chernigiv, and Kyiv Provinces. The chromosomes of the triploid race are slightly smaller than in diploid one. All chromosomes are two-armed (NF = 108) (Fig. 2).

#### *A. longa*

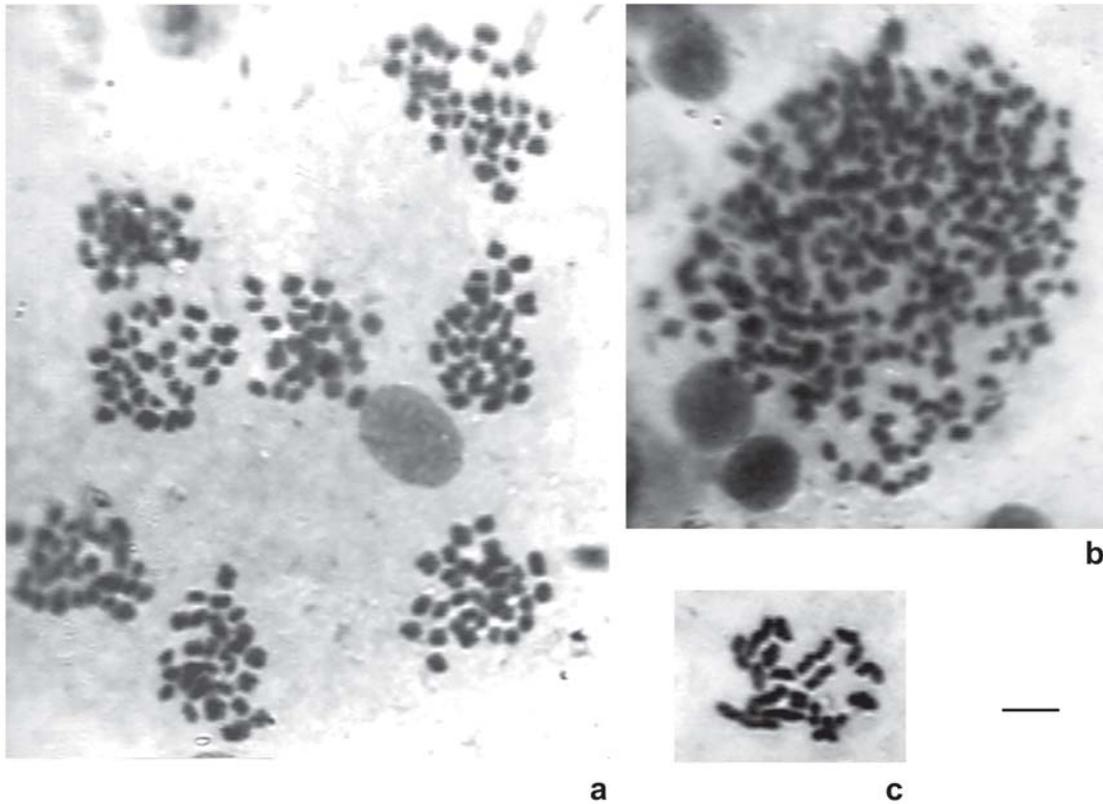
44 chromosome preparations with 78 mitotic metaphases were analyzed. The diploid chromosome number is 36, as it was reported earlier (Muldal, 1952; Vedovini, 1973) (Fig. 3, a). All chromosomes are two-armed; the fundamental number is 72. In diakinesis 18 bivalents were found in the most of the analyzed specimens (Fig. 3, c).

The determination of the chromosome numbers in all the species mentioned above was difficult because of the considerable variation of the chromosome number on many slides (Fig. 3, b). However, we do not know any reasons or rules of this variability. Perhaps, in some cases it is a

result of mixoploidy of the animals. But most probably, the variability connects with the peculiarities of lumbricid spermatogenesis. In the early stages of spermatogenesis the spermatocytes are connected with cytoplasmic canals to each other, and as a result, the groups of 16, 32, 64, and 128 cells are formed (Roosen-Runge, 1980). Such formations on squash chromosome preparations can look as large accumulation of metaphase plates (Fig. 3, a) or a large accumulation of chromosomes (Fig. 3, b).

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**Fig. 3, a-c.** Chromosomes of *Aporrectodea longa*. **a** - accumulation of metaphase plates ( $2n = 36$ ); **b** - chromosome accumulation from some metaphase plates; **c** - diakinesis ( $n = 18$ ). Bar = 10  $\mu$ m.

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