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Aneuploidy Occurrence in Oligochaeta

Tomáš Pavlíček[1, *], Tova Cohen[1], Shweta Yadav[3], Michèle Glasstetter[4], Petr Král[5], Oren Pearlson[1, 2]

[1]Institute of Evolution, University of Haifa, Haifa, Israel

[2]School of Science and Technology, Tel Hai Academic College, Upper Galilee, Israel

[3]School of Biological Sciences, Dr. H S Gour Central University, Sagar, India

[4]Department of Environmental Sciences, Biogeography, University of Basel, Basel, Switzerland

[5]Departments of Chemistry, Physics and Biopharmaceutical Sciences, the University of Illinois at Chicago, Chicago, USA

Email address:

pavlicek@research.haifa.ac.il (T. Pavlíček)

[*]Corresponding author

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Abstract: Appearance of aneuploidy in the germ and somatic lines is usually associated with chromosome and genome rearrangements leading to polysomies and cancer. However, aneuploidy plays an important role in chromosome evolution and in the regulation of the ontogenetic development and phenotypic expression. The latter is known as chromosome diminutions. In Oligochaeta (mainly family Naididae but also Lumbricidae, Erpobdellidae and Branchiobdellidae), we have equated the variability of the chromosome count numbers with aneuploidy based on the results of our analyses and identified chromosome-like nondisjunctions as a major mechanism responsible for it. Another author detected Robertsonian-like translocations producing aneuploidy in *Eisenia fetida* (Lumbricidae, Oligochaeta). Our observations, nevertheless, show that, among karyotyped haploid/diploid cells, the most frequent were haploid (1n) or diploid (2n) chromosome counts connected by multiples. The number of aneuploidy counts was decreasing with the increase of x in expressions 1n + x/1n – x or 2n + x/2n – x. Noteworthy is that not all frequencies of chromosomes in a pair have the same probability. For example, odd aneuploidy numbers of chromosomes are significantly less frequent than the even ones. The wide spread of aneuploidy among Oligochaeta supports the punctuated equilibria model of evolution.

Keywords: Oligochaeta, Earthworm, Aneuploidy, Evolution, Macroevolution, Microevolution

1. Introduction

Aneuploidy is a state in which the number of chromosomes in a cell or organism deviates from multiples of the haploid number of chromosomes [1]. As a matter of fact, in the Robertsonian translocations found in *Eisenia fetida* [2], the decrease in the number of chromosomes is combined with changes in their size and structure. Various mechanisms produce aneuploidy [1]. A consequence of this complexity resembles the chicken or the egg causality dilemma between aneuploidy and chromosome and genome instabilities [3]. Nevertheless, from the evolutionary point of view, aneuploidy might be regarded as a "macromutation" associated with chromosomal and genome rearrangements and phenotypic changes [4], [5]. The saltation phenotypic changes seem to be common in Oligochaeta [6]. They might be associated with hybridizations [7] and asymmetry in the transmission of male and female autosome complements from generation to generation (e.g. sperm-dependent parthenogenesis and hybridogenesis) [8], [9].

Looking at the role of aneuploidy, we cannot however ignore its part in the ontogenetic (mostly embryonic) regulation of development, known as chromosome diminutions. The benchmark of chromosome diminutions is a deliberate loss of chromosomes during the progression of embryogenesis. From the time of discovery by Theodor Boveri [10] of the chromosome diminutions in the *Ascaris* (Nematoda) embryo evidence of their wider presence was gathered. Chromosome diminutions, including chromatin ones which have similar regulatory expression, are known in rotifers, ciliates, crustaceans, insects, nematodes, mammals and other chordates, and even in plants [11].

2. Material and Methods

2.1. Data Origin

We surveyed literature, searching for a recorded variability in the number of chromosomes in Oligochaeta. For different reasons, such records are rare (see below). The majority of data correspond to family Naididae (formerly Tubificidae) in which the somatic chromosomes were counted in cells from the bud [12], *i.e.* the mitotically active structure [13]. A few analyses of haploid metaphases have also been done [12]. In other taxa, different authors [14] - [19] counted either the haploid numbers of chromosomes in metaphase I or the chromosome numbers in diploid cells (Tabs. 1, 2). Multiple haploid and diploid cells in the same organism have been analyzed in the leeches *Erpobdella punctata* and *Nephelpsis obscura* [20].

2.2. Statistical Analyses

We statistically examined the obtained dataset employing the following tests available online [21]:

- Jarque-Bera goodness-of-fit test. The rejection of the H_0 at $p < 0.05$ is interpreted as a rejection of the non-normal distribution of a tested sample due to excess of kurtosis and/or skewness.
- Kendall tau correlation. Statistical probability $p < 0.05$ is interpreted as the rejection of H_0, stating that correlated variables are independent.
- Chi-square test. Statistical probability $p < 0.05$ is interpreted as a rejection of H_0, stating that the variances of the data representing two samples are independent.
- Binomial test. Statistical probability $p < 0.05$ is interpreted as a rejection of H_0, stating that there is no difference from the expected distribution observations in two categories of events.

3. Results

3.1. Dataset

Our survey yielded six species from the family Lumbricidae (Tabs. 1, 2), two species from the leech-like taxa (Branchiobdellidae, Erpobdellidae) (Tab. 1) and 36 species from the family Naididae (Appendix 1). Chromosomes count variabilities and variability between single chromosomes (cytotypes) frequencies are recorded in these species. The dataset (Tabs. 1, 2; Appendix 1) has been gathered during the survey from various works and karyotyping methods.

Table 1. *Variability in the chromosomes number counts in Oligochaeta, except Naididae. (f - sample size, fc – frequency of the given diploid number of chromosomes from the total number of counted cells, dt – diploid number of chromosomes in testes, do – diploid number of chromosomes in ovaries). The given ploidy (n) corresponds to the one suggested by the authors.*

Family or species + author of species description	Ploidy: chromosome range [reference]	Family or species + author of species description	Ploidy: chromosome range [reference]
Lumbricidae		Lumbricidae	
Aporrectodea rosea (Savigny, 1826)	3n: 54- 56- 58, 10n: 167- 174 [16]	*Dendrobaena rubida* (Savigny, 1826)	8n: 120- 126 [14]
Dendrobaena octaedra (Savigny, 1826)	6n: 97- 106 (f = 3) [14]	*Eisenia nordenskioldi* (Eisen, 1879)	6n: 96- 102, 7n: 110-115, 8n: 142- 152 [17]
		Lumbricus terrestris Linnaeus, 1758	2n: 30- 34, 36, 38 [18], [19]
Erpobdellidae		Branchiobdellidae	
Erpobdella punctata (Leidy, 1870)	dt/do: 15 (fc = 8/54) / 15 (fc = 7/77), dt/do: 16 (fc = 36/54) / 16 (fc = 57/77), dt/do: 17 (fc = 10/54) / 17(fc = 13/77) [20]	*Nephelopsis obscura* (Verrill, 1872)	dt/do: 21(fc = 15/70) / 21 (fc = 10/97), dt/do: 22 (fc = 42/70) / 22 (fc = 72/97), dt/do: 23 (fc = 13/70) / 23 (fc = 15/97) [20]

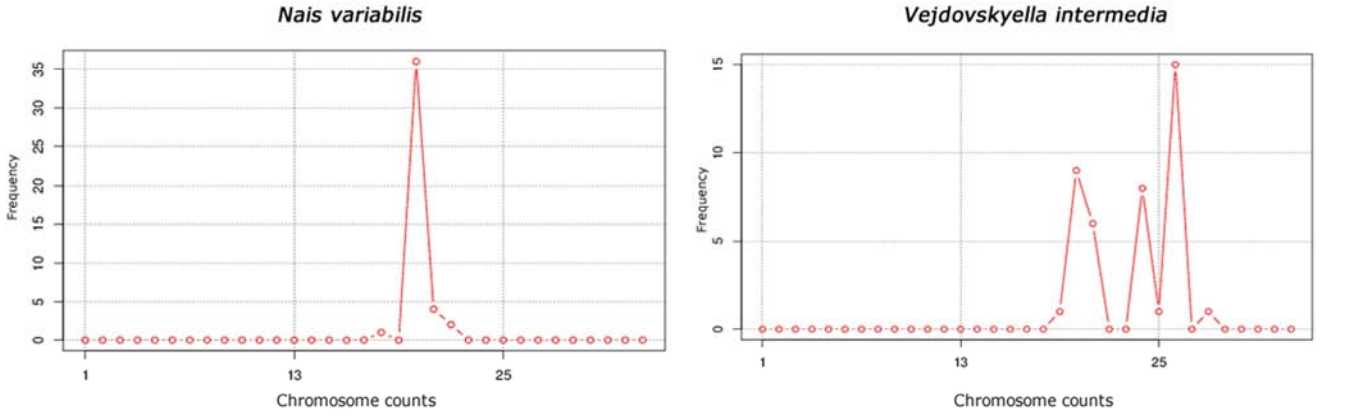

Figure 1. *Monomodal and trimodal distributions of the chromosomes count variability in Nais variabilis, and Vejdovskyella intermedia which might indicate mixing of different lineages (species) in one sample. Source of data [12].*

3.2. Chromosome Patterns in Naididae

3.2.1. Chromosome Variability

In the group of 36 species of Naididae, we found variability among and in the chromosomes number counts in all studied species and all studied samples / populations with the exception of samples No. 3 (*Dero nivea*) and No. 5 (*Nais variabilis*) (Appendix 1). The observed lack of chromosome variability in both samples could be coincidental since only three and six specimens were karyotyped, respectively.

The following modal values of the chromosome counts were observed in Naididae:

a) 2n = 32 in *Pristina* (2 species).
b) 2n = 34 in *Pristina* (1 sp.).
c) 2n = 42 in *Chaetogaster* (3 sp.).
d) 2n = 46 in *Pristina* (1 sp.) and *Stylaria* (2 sp.).
e) 2n = 48 in the genera *Amphichaeta* (2 sp.), *Arcteonais* (1 sp.), *Dero* (4 sp.), *Homochaeta* (1 sp.), *Nais* (7 sp.), *Ophidonais* (1 sp.), *Paranais* (2 sp.), *Piguetiella* (1 sp.), *Pristina* (1 sp.), *Ripister* (1 sp.), *Slavina* (2 sp.) and *Vejdovskyella* (1 sp.).
f) 2n = 52 in *Chaetogaster* (1 sp.), and *Uncinais* (1 sp.).
g) 2n = 54 in *Vejdovskyella* (1 sp.).

The modal values of the chromosomes count variability correspond to the designated diploid chromosomes numbers (2n) in the respective species [12]. The difference between the number of species with the most frequent diploid value 2n = 48 (24 cases) was significantly higher than the number of species with 2n < 48 (9 cases) and 2n > 48 (3 cases) (chi-square test, df = 2, chi-square = 9.83, $p = 0.007$).

3.2.2. Character of Modality

As far as we can judge, the distribution of most variabilities in the chromosome counts was monomodal and some bimodal or polymodal (Fig. 1). Unfortunately, the relatively small sample size did not allow a statistical treatment of this phenomenon. Nevertheless, a relationship between sample size and mode size is indicated by the significant positive correlation between chromosome count variability and sample size (Pearson Product Moment Correlation: the number of observation, n = 36, correlation = 0.68, $p = 0.00006$).

3.2.3. Proportion of Even and Odd Cytotypes

The sum of odd cytotypes (n = 248) in Naididae has been significantly smaller than the sum of even cytotypes (n = 1282).

The probability of odd/even cytotypes being 0.5/0.5 is $p < 0.000001$ (Binomial test).

The H_0 expecting a normal distribution of chromosome variability in counted number of chromosomes in Naididae was rejected (Jarque-Bera normality test, skew = 1.57, z = 3.55, $p = 0.00000001$).

3.2.4. Differences Between Testes and Oogonia in Leeches

We tested differences in frequencies of cytotypes between testes and oogonia (Table 2) in two species of leeches.

Table 2. *Variability in the haploid chromosomes number counts (h) in Oligochaeta (f = sample size or frequency, fc' – frequency of the given haplotype from the total number of counted cells, h – haploid number of chromosomes, ht – haploid number of chromosomes in testes, ho – haploid number of chromosomes in ovaries, S - sample).*

Family or species + author of species description	Ploidy: chromosome range [reference]	Family or species + author of species description	Ploidy: chromosome range [reference]
Lumbricidae		Naididae	
Octolasion croaticum (Rosa, 1895)	h: 55-60 [16]	*Dero digitata* O. F. Müller, 1773	h(S1): 24 (f = 9), 26 (f = 6) [12]
Erpobdellidae		*Dero digitata* O. F. Müller, 1773	h(S2): 26 (f = 3), 27 (f = 1) [12]
Erpobdella punctata (Leidy, 1870)	ht/ho: 7 (fc' = 13/126) / 7 (fc' = 13/239), ht/ho: 8 (fc' = 78/126) / 8 (fc' = 182/239), ht/ho: 9 (fc' = 24/126) / 9 (fc' = 33/239), ht/ho: 10 (fc' = 11/126) / 10 (fc' = 13/239) [20]	*Nais elinguis* O. F Müller, 1773	H(S1): 23 (f = 4), 24 (f = 8), 26 (f = 1) [12]
Branchiobdellidae			
Nephelopsis obscura (Verrill, 1872)	ht/ho: 9 (fc' = 12/155) / 9 (fc' = 11/228), ht/ho: 10 (fc' = 23/155) / 10 (fc' = 20/228), ht/ho: 11 (fc' = 91/155) / 11 (fc' = 171/228), ht/ho: 12 (fc' = 29/155) / 12 (fc' = 26/228) [20]		

In *Erpobdella punctata* and *Nephelopsis obscura*, the differences in frequencies of the chromosome count variability were significant between haploid counts in testes and haploid counts in oocytes ($X^2 = 30.81$, df = 3, $p < 0.0001$) and ($X^2 = 11.3$, df = 3, $p < 0.01$), respectively. The differences between diploid counts in testes and diploid counts in oocytes were significant in *E. punctata* ($X^2 = 15.11$, df = 2, $p = 0.0005$) and not significant ($X^2 = 1.06$, df = 2, $p = 0.59$) in *N. obscura*.

3.2.5. The Pattern in Lumbricid Earthworms

The variability in number of chromosomes (Tab. 1) encompasses five additional widely distributed species of earthworms of the family Lumbricidae. We did not analyse them because cytogenetics had been done by non-comparable methods and the sample was too small.

4. Conclusions and Discussion

The regularities we found in the chromosome number counts representing different samples/populations, individuals or cells allow us to reject the possibility that they are caused by error or chance alone. We interpret this

as evidence for the presence of aneuploidy since the numbers of chromosomes in samples/populations and cells deviate from multiples of the haploid number of chromosomes. Surprisingly, we found evidence of aneuploidy even in lineages in which only diploidy, and not polyploidy, is expected, for instance in genus *Lumbricus* (2n = 36) [17]. In the analyzed Naididae, heteroploidy is not suspected since all modal values were even. However, in a few cases, we cannot exclude the admixture of different lineages (species) showing bimodal or polymodal patterns (Fig. 1) of the chromosomes count variability distribution.

Interestingly, the pattern we found in Naididae (see below) was similar to the more advanced Oligochaeta taxon *Lumbricus terrestris* (Lumbricidae) as reported by M. P. Walsh: "*Eighteen was the number observed in late diakinesis and the first meiotic metaphase plates. In the spermatogonial divisions, 36 chromosomes were found in the vast majority of metaphase stages. However, there were some first meiotic metaphase plates and late diakinesis figures that showed variations from the number 18. A few very clear metaphase plates showed 17 or 19 bivalents. In some spermatogonial divisions, variations of 34 and 38 were observed. In a few animals, 17 bivalents were seen in some cells while other cells within the same individual showed the usual number*" [19].

The inter-individual variability in chromosome counts was documented earlier in family Lumbricidae (Tabs. 1, 2) and intra-individual variability in the number of counted chromosomes in *L. terrestris* [19].

Our results based on literature data indicate a widespread aneuploidy in Oligochaeta if taking into account that (a) karyotype data is missing in the majority of higher taxa (genera, families), (b) the chromosome count variability is ignored as an artefact [22], and (c) its presence is mentioned without providing data [14]. If the fluctuations in the staining intensity caused the variability, one would expect to get an asymmetric distribution of the chromosome count variability due to some chromosomes being stained differently. If simple technical or human errors would generate this variability, then one would expect a Gaussian distribution of errors around the modal value and a reasonable number of cases with no error made. Moreover, our data set combines results of karyotyping in multiple taxa done by different authors using dissimilar techniques. Besides, aneuploidy was suggested to explain the inter- and intra-individual variability in chromosome numbers in *L. terrestris* [19] and to explain the relatively low chromosome numbers (2n = 22) in *Eisenia fetida* (Lumbricidae, Oligochaeta) [2].

The observed pattern in the chromosomes count variability indicates that the diploid (2n) or haploid (1n) number of chromosomes is most probably transferred to the next mitotic division or to the next generation diploid (2n) or haploid (1n) number of chromosomes. The probability of transferring 2n + x and 2n - x or 1n + x and 1n - x sets of chromosomes decreases with the increase of x, where x = 1, 2, 3, 4, 5…. However, we do not know if there is a limit for n other than the total number of chromosomes. Certainly, chromosome nondisjunctions are not the only aneuploidy-generating mechanism present in Oligochaeta. Another, so far suggested, mechanism are Robertsonian-like translocations proposed to explain the low haploid chromosome number (1n = 11) in *E. fetida* [2]. A negative relationship might exist between Robertsonian translocations and chromosome non-disjunctions because aneuploidy was not detected in present-day populations of *E. fetida* despite numerous studies [2], [15], [16], [23]. Whereas both aneuploidy-generating mechanisms can explain the decrease in the number of chromosomes, as compared to the haploid or diploid values, the Robertsonian translocations cannot explain their increase. Still, both increasing and decreasing numbers of chromosomes were observed in Oligochaeta species (e.g. [24]).

In spite of the fact that chromosome or chromatin diminutions have not been found in Oligochaeta, as far as we know, they cannot be ruled out. An indication of such a possibility might be triploid (3n = 39) sperm-dependent parthenogenetic *Lumbriculus lineatus* [25]. In this lineage the following chromosome complements are generated in the first reduction division: 19-20 (f = 19), 18-21 (f = 10), 17-22 (f = 7), 16-23 (f = 3), 15-24 (f = 2), 14-25 (f = 1), 13-26 (f = 1), where "f" is the frequency. In the 3n *L. lineatus*, this variability is eliminated in the second division by the spindle of unique shape and function [25]. However, the mechanism and reason for the generating (and maintenance) of the chromosome variability during the first reduction division in this lineage are unknown.

Nevertheless, the presence of aneuploidy indicates that the evolutionary process is near to the punctuated equilibrium model [26] in Oligochaeta. In a first step, the aneuploidy associated with chromosome and genome instabilities produces macroscopic phenological changes through rearrangement of genomic, chromosomal, cellular and host-parasite interactions. An example of the two-step punctuated equilibrium process might be gutless marine oligochaetes belonging to the genera *Inanidrilus* and *Olavius* (Oligochaeta: Naididae). They are relying on the "food" provided by the chemosynthetic bacteria metabolizing sulphur from hypoxic marine sediments [27].

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Appendix

Appendix 1. *Variability in the somatic chromosomes number counts in Naididae (f - frequency, S – sample). The source of data: [12]. The given ploidy corresponds to the one suggested by the author.*

Species	Sample: ploidy: No. chromosomes (frequency)	Species	Sample: ploidy: No. chromosomes (frequency)
Amphichaeta leydigi Tauber, 1879	S1: 2n: 45 (f = 1), 46 (f = 2), 47 (f = 2), 48 (f = 8), 50 (f = 3), 52 (f = 6)	*Amphichaeta sannio* Kallstenius,1892	S1: 2n: 46 (f = 1), 47 (f = 2), 48 (f = 11), 49 (f = 3), 52 (f = 1)
Arcteonais lomondi Martin, 1907	S1: 2n: 47 (f = 1), 48 (f = 6), 49 (f = 2)	*Chaetogaster cristallinus* Vejdovský, 1883	S1: 2n: 36 (f = 1), 40 (f = 4), 41 (f = 2), 42 (f = 15), 43 (f = 2), 44 (f = 2)
Chaetogaster cristallinus Vejdovský, 1883	S2: 2n: 40 (f = 2), 42 (f = 6), 43 (f = 1)	*Chaetogaster cristallinus* Vejdovský, 1883	S3: 2n: 40 (f = 2), 42 (f = 4), 44 (f = 2)
Chaetogaster diaphanus Gruithuisen, 1828	S1: 2n: 41 (f = 1), 42 (f = 6), 43 (f = 1), 44 (f = 1)	*Chaetogaster diaphanus* Gruithuisen, 1828	S2: 2n: 40 (f = 1), 42 (f = 3), 43 (f = 1)
Chaetogaster diaphanus Gruithuisen, 1828	S3: 2n: 38 (f = 1), 42 (f = 11), 43 (f = 3), 44 (f = 2)	*Chaetogaster diaphanus* Gruithuisen, 1828	S4: 2n: 40 (f = 1), 41 (f = 3), 42 (f = 15), 43 (f = 4), 44 (f = 3)
Chaetogaster diaphanus Gruithuisen, 1828	S5: 2n: 40 (f = 1), 41 (f = 1), 42 (f = 8), 43 (f = 3), 44 (f = 1)	*Chaetogaster diastrophus* Gruithuisen, 1828	S1: 2n: 41 (f = 1), 42 (f = 14)
Chaetogaster limnaei von Baer, 1827	S1: 2n: 51 (f = 1), 52 (f = 3)	*Chaetogaster limnaei* von Baer, 1827	S2: 2n: 46 (f = 1), 48 (f = 3), 52 (f = 16), 54 (f = 2), 56 (f = 5)
Chaetogaster limnaei von Baer, 1827	S3: 2n: 52 (f = 2), 53 (f = 1)	*Chaetogaster limnaei* von Baer, 1827	S4: 2n; 52 (f = 1), 54 (f = 1), 58 (f = 5), 60 (f = 1)
Chaetogaster limnaei von Baer, 1827	S5: 2n: 56 (f = 1), 58 (f = 1), 59 (f = 3), 60 (f = 5), 61 (f = 1)	*Dero digitata* O. F. Müller, 1773	S1: 2n: 42 (f = 1), 43 (f = 2), 44 (f = 3), 45 (f = 2), 46 (f = 8), 47 (f = 5), 48 (f = 39), 49 (f = 1)
Dero digitata O. F. Müller, 1773	S2: 2n: 42 (f = 1), 44 (f = 1), 45 (f = 1), 46 (f = 1), 48 (f = 3)	*Dero digitata* O. F. Müller, 1773	S3: 2n: 47 (f = 1), 48 (f = 7), 49 (f = 1)
Dero digitata O. F. Müller, 1773	S4: 2n: 46 (f = 1), 47 (f = 3), 48 (f = 12), 49 (f = 4), 50 (f = 1)	*Dero furcata* O. F. Müller, 1773	S1: 2n: 47 (f = 1), 48 (f = 7), 49 (f = 1), 50 (f = 1)
Dero nivea Aiyer, 1930	S1: 47 (f = 2), 48 (f = 13), 49 (f = 4), 50 (f = 2)	*Dero nivea* Aiyer, 1930	S2: 46 (f = 4), 48 (f = 22)
Dero nivea Aiyer, 1930	S3: 48 (f = 6)	*Dero obtusa* d'Udekem, 1855	S1: 2n: 46 (f = 1), 47 (f = 1), 48 (f = 7), 50 (f = 3), 52 (f = 3)
Dero obtusa d'Udekem, 1855	S2: 2n: 46 (f = 3), 47 (f = 2), 48 (f = 6), 49 (f = 1)	*Dero obtusa* d'Udekem, 1855	S3: 2n: 46 (f = 1), 47 (f = 2), 48 (f = 8), 49 (f = 4), 50 (f = 1)
Dero obtusa d'Udekem, 1855	S2: 2n: 46 (f = 1), 47 (f = 5), 48 (f = 16), 49 (f = 5)	*Homochaeta naidina* Bretscher 1896	S1: 2n: 47 (f = 2), 48 (f = 6), 49 (f = 2)
Nais barbata O. F. Müller, 1773	S1: 2n: 46 (f = 3), 48 (f = 16), 50 (f = 1), 52 (f = 1)	*Nais barbata* O. F. Müller, 1773	S2: 2n: 44 (f = 1) 47 (f = 2), 48 (f = 8), 49 (f = 1), 50 (f = 1)
Nais barbata O. F. Müller, 1773	S3: 2n: 46 (f = 4), 47 (f = 4), 48 (f = 17), 49 (f = 3)	*Nais bretscheri* Michaelsen, 1899	S1: 2n: 46 (f = 3), 47 (f = 2), 48 (f = 15), 49 (f = 1)
Nais bretscheri Michaelsen, 1899	S2: 2n: 46 (f = 4), 47 (f = 2), 48 (f = 4), 49 (f = 2)	*Nais bretscheri* Michaelsen, 1899	S3: 2n: 46 (f = 1), 47 (f = 1), 48 (f = 6), 49 (f = 1)
Nais communis Piguet, 1906	S1: 2n: 47 (f = 2), 48 (f = 5), 49 (f = 2), 50 (f = 50)	*Nais communis* Piguet, 1906	S2: 2n: 47 (f = 1), 48 (f = 5)
Nais communis Piguet, 1906	S3: 2n: 46 (f = 1), 47 (f = 1), 48 (f = 14), 50 (f = 2), 52 (f = 1)	*Nais communis* Piguet, 1906	S4: 2n: 46 (f = 1) ,47 (f = 2), 48 (f = 15), 49 (f = 3)
Nais elinguis Müller, 1773	S1: 2n: 46 (f = 3), 47 (f = 2), 48 (f = 24), 50 (f = 1)	*Nais elinguis* Müller, 1773	S2: 2n: 46 (f = 1), 47 (f = 1), 48 (f = 3), 49 (f = 2)
Nais elinguis Müller, 1773	S3: 2n: 46 (f = 2), 47 (f = 1), 48 (f = 8), 52 (f = 2)	*Nais elinguis* Müller, 1773	S4: 2n: 46 (f = 2), 47 (f = 3), 48 (f = 9), 49 (f = 1), 50 (f = 1), 52 (f = 4)
Nais pardalis Piguet, 1906	S1: 2n: 46 (f = 9), 47 (f = 4), 48 (f = 26), 49 (f = 1), 50 (f = 2), 52 (f = 1)	*Nais pardalis* Piguet, 1906	S2: 2n: 46 (f = 1), 47 (f = 2), 48 (f = 9)
Nais pardalis Piguet, 1906	S3: 2n: 46 (f = 2), 48 (f = 11)	*Nais pseudobtusa* Piguet, 1906	S1: 2n: 47 (f = 1), 48 (f = 11), 49 (f = 2)
Nais variabilis Piguet, 1906	S1: 2n: 48 (f = 2), 49 (f = 1), 50 (f = 1)	*Nais variabilis* Piguet, 1906	S2: 2n: 48 (f = 19), 49 (f = 1)
Nais variabilis Piguet, 1906	S3: 2n: 46 (f = 1), 48 (f = 6), 50 (f = 1)	*Nais variabilis* Piguet, 1906	S4: 2n: 48 (f = 2), 49 (f = 1)
Nais variabilis Piguet, 1906	S5: 2n: 48 (f = 3)	*Nais variabilis* Piguet, 1906	S6: 2n: 46 (f = 1), 48 (f = 4), 49 (f = 1)
Ophidonais serpentina Müller, 1773	S1: 2n: 46 (f = 3), 47 (f = 4), 48 (f = 18), 50 (f = 2)	*Ophidonais serpentina* Müller, 1773	S2: 2n: 46 (f = 2), 48 (f = 4)
Ophidonais serpentina Müller, 1773	S3: 2n: 46 (f = 4), 47 (f = 2), 48 (f = 15), 49 (f = 1), 50 (f = 1), 52 (f = 1)	*Paranais friči* Hrabě, 1941	S1: 2n: 47 (f = 2), 48 (f = 6), 50 (f = 2)
Paranais friči Hrabě 1941	S2: 2n: 46 (f = 1), 47 (f = 2), 48 (f = 11), 49 (f = 1)	*Paranais litoralis* O. F. Muller, 1784	S1: 2n: 44 (f = 1), 46 (f = 2), 47 (f = 1), 48 (f = 1)
Paranais litoralis O. F. Muller, 1784	S2: 2n: 46 (f = 3), 47 (f = 3), 48 (f = 21), 49 (f = 1), 50 (f = 1), 52 (f = 3)	*Paranais litoralis* O. F. Muller, 1784	S3: 2n: 44 (f = 1), 46 (f = 1), 47 (f = 2), 48 (f = 25), 49 (f = 1), 52 (f = 2)

Species	Sample: ploidy: No. chromosomes (frequency)	Species	Sample: ploidy: No. chromosomes (frequency)
Paranais litoralis O. F. Muller, 1784	S4: 2n: 46 (f = 9), 47 (f = 9), 48 (f = 24), 49 (f = 5), 50 (f = 1)	*Piguetiella blanci* Piguet, 1906	S1: 2n: 48 (f = 4), 50 (f = 1)
Pristina aequiseta Bourne, 1891	S1: 2n: 31 (f = 2), 32 (f = 2), 33 (f = 1), 34 (f = 17)	*Pristina aequiseta* Bourne, 1891	S2: 2n: 31 (f = 1), 32 (f = 1), 33 (f = 3), 34 (f = 8)
Pristina foreli (Piguet,1906)	S1: 2n: 46 (f = 1), 47 (f = 1), 48 (f = 4), 49 (f = 1), 50 (f = 1)	*Pristina foreli* (Piguet,1906)	S2: 2n: 46 (f = 1), 47 (f = 3), 48 (f = 16), 49 (f = 2), 50 (f = 1)
Pristina foreli (Piguet,1906)	S3: 2n: 48 (f = 4), 50 (f = 1)	*Pristina jenkinae* Stephenson, 1931	S1: 2n: 30 (f = 1), 32 (f = 11)
Pristina jenkinae Stephenson, 1931	S2: 2n: 31 (f = 1), 32 (f = 2), 34 (f = 1)	*Pristina longiseta* Ehrenberg, 1828	S1: 2n: 44 (f = 7), 45 (f = 1), 46 (f = 7), 47 (f = 4), 48 (f = 9)
Pristina longiseta Ehrenberg, 1828	S2: 2n: 44 (f = 1), 45 (f = 1), 46 (f = 6), 47 (f = 4), 48 (f = 2)	*Pristina longiseta* Ehrenberg, 1828	S3: 2n: 45 (f = 2), 46 (f = 7), 47 (f = 4), 48 (f = 3)
Pristina osborni Walton, 1906	S1: 2n: 29 (f = 1), 30 (f = 1), 31 (f = 2), 32 (f = 17), 33 (f = 3)	*Ripistes parasita* (Schmidt, 1847)	S1: 2n: 47(f = 2), 48 (f = 7), 49 (f = 1)
Slavina appendiculata d'Udekem, 1855	S1: 2n: 46 (f = 1), 48 (f = 11), 49 (f = 1)	*Slavina appendiculata* d'Udekem, 1855	S2: 2n: 42 (f = 1), 46 (f = 3), 47 (f = 1), 48 (f = 18), 50 (f = 1)
Slavina appendiculata d'Udekem, 1855	S3: 2n: 42(f = 3), 46 (f = 2), 48 (f = 5), 52 (f = 1)	*Slavina appendiculata* d'Udekem, 1855	S4: 2n: 48 (f = 4), 50 (f = 2), 52 (f = 3)
Slavina appendiculata d'Udekem, 1855	S5: 2n: 46 (f = 1), 48 (f = 7), 49 (f = 2), 54 (f = 1)	*Specaria josinae* Vejdovský, 1883	S1: 2n: 44 (f = 1), 47 (f = 2), 48 (f = 16), 50 (f = 4)
Stylaria fossularis Leidy, 1852	S1: 2n: 44 (f = 2), 45 (f = 2), 46 (f = 5), 47 (f = 1)	*Stylaria lacustris* Linnaeus, 1767	S1: 2n: 45 (f = 2), 46 (f = 13), 47 (f = 1)
Stylaria lacustris Linnaeus, 1767	S2: 2n: 46 (f = 11), 47 (f = 1)	*Stylaria lacustris* Linnaeus, 1767	S3: 2n: 44 (f = 2), 45 (f = 2), 46 (f = 6), 47 (f = 1), 48 (f = 1)
Uncinais uncinata Ørsted, 1842	S1: 2n: 48 (f = 4), 50 (f = 2), 52 (f = 12), 54 (f = 6)	*Uncinais uncinata* Ørsted, 1842	S2: 2n: 48 (f = 1), 50 (f = 2), 52 (f = 23)
Vejdovskyella comata Vejdovský, 1883	S1: 2n: 47 (f = 1), 48 (f = 21)	*Vejdovskyella comata* Vejdovský, 1883	S2: 2n: 46 (f = 2), 47 (f = 1), 48 (f = 3)
Vejdovskyella intermedia Bretscher, 1896	S1: 2n: 48 (f = 2), 49 (f = 2), 52 (f = 1)	*Vejdovskyella intermedia* Bretscher, 1896	S2: 2n: 47 (f = 1), 48 (f = 6), 49 (f = 1), 52 (f = 3), 53 (f = 1), 54 (f = 5), 56 (f = 1)
Vejdovskyella intermedia Bretscher, 1896	S3: 2n: 48 (f = 1), 49 (f = 3), 52 (f = 4), 54 (f = 10)		

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