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Diversity of Dilepididae (Cestoda: Cyclophyllidea) revealed by cytogenetic analysis

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Abstract

Karyotypes of three dilepidid species: *Molluscotaenia crassiscolex*, *Anomotaenia bacilligera* and *Dilepis undula,* which have not been recorded previously, were studied using conventional Giemsa staining and comparative karyometric analysis. Twelve small biarmed chromosomes were observed in mitotic cells of *M. crassiscolex*, 16 biarmed chromosomes of gradually decreasing size were found in cells of *A. bacilligera*, while 18 elements were characteristic for *D. undula*. These data, together with information available in literature, prove the heterogeneity and possible polyphyletic nature of the family Dilepididae.

Introduction

The family Dilepididae comprises a diverse group of cestodes, differing in morphological and ecological traits and in patterns of ontogeny. Consequently, the taxonomy of the classical Dilepididae *sensu lato* has long been controversial. Freeman (1973) even considered a complete dissolution of the family. Revision of the group has resulted in the recognition of the families Dilepididae Railliet & Henry, Metadilepididae Spasskii, Paruterinidae Fuhrmann, and Dipylidiidae Stiles (see Bona, 1994; Georgiev & Kornyushin, 1994; Jones, 1994; Kornyushin & Georgiev, 1994). Nevertheless, diversity within the remaining Dilepididae is considerable and, as Bona (1994) has suggested, the family does not form a monophyletic assemblage. It is evident that dilepidids require further evaluation. Given that karyotypes provide a phenotypic view of the genotype, it is not surprising that comparative chromosome analysis found an early use for understanding the systematic and phylogenetic relationships of species. Knowledge on cestode cytogenetics is still rather scanty, but existing data lead to the conclusion that cestodes, in general, are karyotypically conservative, with related species on generic and even family levels differing by a few chromosome rearrangements. Monophyletic groups seem to be characterized by 'typical' karyotypes (see Petkevičiūtė, 2002) and hence a karyological approach can improve the hypotheses formulation of phylogenetic studies of dilepidids, as well as other cestode taxons.

In the present study, three dilepidid species were analysed: *Molluscotaenia crassiscolex* (von Linstow), from shrews (Soricidae), *Anomotaenia bacilligera* (Krabbe) Fuhrmann, from sandpipers (Scolopacidae), and *Dilepis undula* (Schrank), from terrestrial birds (chiefly Turdidae), together with the information available in the literature. The chromosome sets of all three species are described for the first time.

Materials and methods

Five entire adult specimens of *M. crassiscolex*, used for cytogenetic analysis, were collected during the dissection of the common shrew, *Sorex araneus*, trapped in a park in Vilnius, Lithuania. Ten adults of *D. undula* were obtained from the intestine of the blackbird, *Turdus merula*, caught in the Kuronian spit (Biological station Rybachy of the Zoological Institute of the Russian Academy of Sciences). Six adults of *A. bacilligera* were collected from the intestine of the short-billed dowitcher, *Limnodromus griseus,* in Chauna lowland, Northwestern Chukotka, Russia.

Entire living tapeworms were treated with 0.01% colchicine in physiological solution for 3–4 h at about 38–39°C, and with distilled water for hypotony for 1 h at room temperature. Material was fixed in a freshly prepared mixture of ethanol–glacial acetic acid (3:1) with three changes, 20 min duration each time, and stored at −20°C until use. Slides were prepared from cell-suspensions using an air-drying technique (Petkevičiūtė

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Fig. 1. Mitotic metaphase and karyotype of *Molluscotaenia crassiscolex*. Scale bar = 10 μm.

& Ieshko, 1991), stained with 4% Giemsa in Sörensen's buffer (pH 6.8) for 30–40 min, rinsed in tap water and allowed to dry. Chromosome counts were made directly by microscope (Zeiss) observations on intact and well-spread metaphases. The best mitotic and meiotic plates were photographed under an oil-immersion system and karyograms were constructed by arranging the chromosomes in order of decreasing size and centromere position. The lengths of the short and long arms of chromosomes were measured in metaphases from different specimens. Measurements (absolute length in micrometres, relative length in percent, and centromeric indices) are given as mean values and standard deviations (S.D.). The classification of chromosomes followed that of Levan *et al.* (1964).

Results

All specimens of *M. crassiscolex* show a modal diploid chromosome set composed of 12 biarmed chromosomes (fig. 1). This number was found in 271 (92.81%) dividing cells from 292 examined. Eighteen (6.16%) aneuploid spreads, with 2n = 11, were encountered and part of these might be related to technical shortcomings. Three (1.03%) tetraploid cells (4n = 23–24) were found. Mitotic cell divisions were most often observed in developing embryos and gametogonial cells. The chromosomes are small, the largest measuring 3.26 μm and the smallest 1.47 μm. They can be divided into two length groups: large elements of pairs 1–3, and smaller elements of pairs 4–6. Mean karyometrical data are reported in table 1. The karyotype includes five metacenric and one submetacentric (no. 2) chromosome pairs.

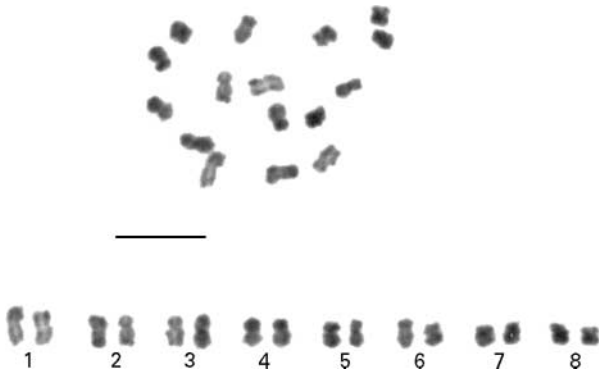

Fig. 2. Mitotic metaphase and karyotype of *Anomotaenia bacilligera*. Scale bar = 10 μm.

Chromosomes of 167 mitotic metaphases of *A. bacilligera* were counted; 154 (92.22%) cells had 2n = 16 (fig. 2), 13 cells were aneuploid, containing fewer chromosomes (14 or 15). All chromosomes were biarmed, but individual pairs were distinguishable. Pairs 1–4, 6 and 8 were metacentric, pairs 5 and 7 represented an intermediate between the meta- and submetacentric type of structure. They decreased in size gradually from 4.01 μm to 2.13 μm (table 2).

A total of 72 counts were made on chromosome spreads at metaphase from specimens of *D. undula*. The modal count was 2n = 18, with 93.05% of cells examined having this number of chromosomes. Five (6.95%) spreads were aneuploid, displaying a chromosome number lower than the mode (2n = 16–17). Seventeen haploid sets with n = 9 at the stage of diplotene-diakinesis confirmed the diploid number (fig. 3). Unfortunately, the location of the centromere was not clearly visible in some metaphase chromosomes and therefore only measurements of the absolute length of whole mitotic chromosomes have been

Table 1. Measurements (means ± SD) and classification of chromosomes of *Molluscotaenia crassiscolex*.

Chromosome number	Absolute length (μm)	Relative length (%)	Centromeric index	Classification
1	3.26 ± 0.60	23.15 ± 0.69	44.30 ± 2.46	m
2	3.03 ± 0.62	21.40 ± 1.14	31.33 ± 3.57	sm
3	2.77 ± 0.56	19.57 ± 1.02	42.62 ± 3.58	m
4	1.93 ± 0.36	13.69 ± 0.60	43.61 ± 1.81	m
5	1.66 ± 0.28	11.69 ± 0.70	45.45 ± 1.72	m
6	1.47 ± 0.24	10.46 ± 0.62	45.12 ± 1.92	m

m, metacentric; sm, submetacentric chromosomes.

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Table 2. Measurements (means ± SD) and classification of chromosomes of *Anomotaenia bacilligera*.

Chromosome number	Absolute length (μm)	Relative length (%)	Centromeric index	Classification
1	4.01 ± 1.04	16.71 ± 1.49	43.11 ± 2.28	m
2	3.48 ± 0.75	1458 ± 0.67	42.58 ± 2.35	m
3	3.25 ± 0.71	13.59 ± 0.61	42.02 ± 1.89	m
4	3.00 ± 0.63	12.61 ± 0.71	40.44 ± 3.32	m
5	2.81 ± 0.49	11.85 ± 0.43	37.03 ± 2.33	sm-m
6	2.70 ± 0.54	11.32 ± 0.38	42.59 ± 2.69	m
7	2.42 ± 0.41	10.24 ± 0.65	37.61 ± 2.40	m-sm
8	2.13 ± 0.26	9.10 ± 1.14	46.04 ± 1.73	m

m, metacentric; sm, submetacentric chromosomes.

made. These values ranged from 3.76 μm to 2.17 μm (table 3). Nevertheless, it could be seen that the karyotype is composed mainly of biarmed elements: pairs 1, 2, 4 and 5 are metacentric, pairs 6, 7 and 9 have submedian centromeres, pairs 3 and 8 are likely to have subterminally located centromeres.

Discussion

Most karyological studies on cyclophyllidean cestodes have focused primarily on parasites of economic or medical importance belonging to the families Hymenolepididae and Taeniidae (Petkevičiūtė, 2002). Because of the necessity to process living worms, karyological analyses are limited in studies on the taxonomy of cestodes. Small chromosomes and the scarcity of mitotic activity is another problem noted by the workers in the field (Wikgren & Gustafsson, 1965; Grey & Mackiewicz, 1974; Mutafova & Gergova, 1994). Chromosomal data for dilepidids are very limited. An early karyological study by Jones (1945) suggests that Dilepididae *sensu lato* is cytologically diverse, while hymenolepidid worms are very similar in number and 'general appearance' of chromosomes. Information available from this early study relates to the number and some notes on the morphology of chromosomes of six species of Dilepididae *sensu lato*, recently recognized as representatives of families Dilepididae, Dipylidiidae and Paruterinidae (see table 4). Despite the small number of species examined, a wide variation in chromosomal numbers was revealed, ranging from 10 to 16 in diploid sets. The information on *Dipylidium. caninum*, 2n = 10, was only tentative, as little material was examined and only meiotic chromosomes were observed. Later investigations revealed the diploid number to be 2n = 16 for this species (Liu & Lin, 1988; Margarian, 1989). Only two species of Dilepididae *sensu* Bona (1994) were studied: *Liga brasiliensis*, 2n = 14, and *Choanotaenia* sp., 2n = 16. Despite the different diploid number, marked similarities between the complements were noted. Most chromosomes of both species were biarmed with submedian centromeres; one large pair of homologues with subterminal centromere was present in both karyotypes (Jones, 1945).

The present results confirm the chromosomal variability of dilepidid species. *Molluscotaenia crassiscolex* showed a diploid number of 2n = 12, while 2n = 16 was determined in *A. bacilligera* and 2n = 18 in *D. undula*. Twelve chromosomes is the basic diploid number for the species of Hymenolepididae (Petkevičiūtė & Regel, 1994). Although chromosome sets of species of the genus *Hymenolepis* sensu lato and of most other hymenolepidids comprise the same diploid number, 2n = 12, the morphology of the chromosomes is different.

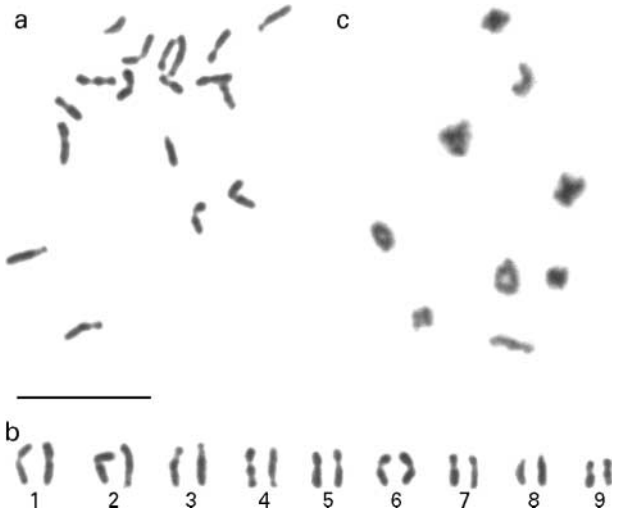

Fig. 3. Chromosomes of *Dilepis undula*: a, mitotic metaphase and b, karyotype; c, diakinesis, showing 9 bivalents. Scale bar = 10 μm.

Table 3. Measurements (means ± SD) of chromosomes of *Dilepis undula*.

Chromosome number	Absolute length (μm)	Relative length (%)
1	3.76 ± 0.71	13.73 ± 1.04
2	3.58 ± 0.60	13.12 ± 0.69
3	3.52 ± 0.67	12.86 ± 1.07
4	3.13 ± 0.57	11.43 ± 0.45
5	3.10 ± 0.73	11.26 ± 1.00
6	2.90 ± 0.43	10.66 ± 0.97
7	2.62 ± 0.40	9.65 ± 0.85
8	2.52 ± 0.40	9.28 ± 1.13
9	2.17 ± 0.38	8.00 ± 0.89

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Table 4. Chromosome number and morphology in the family Dilepididae *sensu lato.*

Species	No. and morphology of chromosomes	References
Dilepididae		
Liga brasiliensis	2n = 14	Jones (1945)
Choanotaenia sp.	2n = 16	Jones (1945)
Molluscotaenia crassiscolex	2n = 12 = 10m + 2sm	Present study
Dilepis undula	2n = 18	Present study
Anomotaenia bacilligera	2n = 16 = 12m = 4m/sm	Present study
Dipylidiidae		
Dipylidium caninum	2n = 10	Jones (1945)
	2n = 16 = 2sm + 8m + 6t	Liu & Lin (1988)
	2n = 16	Margarian (1989)
Paruterinidae		
Anonchotaenia globata	2n = 12	Jones (1945)
Anonchotaenia sp.	2n = 16	Jones (1945)
Rhabdometra similis	2n = 12	Jones (1945)

m, metacentric; sm, submetacentric; t, telocentric chromosomes.

Hymenolepis citelli and *Vampirolepis erinacei* possess telocentric chromosomes, *H. diminuta* is characterized by two first pairs of metacentric, three pairs of submetacentric and one, the last, pair of telocentric elements, *V. nana* possesses ten chromosomes with terminal localization of centromeres and two (pair no. 3) meta-submetacentrics, and the chromosomal complement of *Rodentolepis straminea* consists of two subtelocentric, two metacentric and two submetacentric pairs (Ward *et al.*, 1981; Liu & Lin, 1987; Mutafova & Gergova, 1994; Špakulova & Casanova, 1998). The karyotype of *M. crassiscolex* is conspicuous by the presence of only biarmed, metacentric and submetacentric (pair no. 2), chromosomes. The diploid number 2n = 12 was also revealed in both *Anonchotaenia globata* and *Rhabdometra similis*, representatives of the recently recognized family Paruterinidae (see table 4). The morphology of the chromosomes was not described in detail, but Jones (1945) noted that the species are cytologically close, with three pairs with median or subterminal centromeres and three smaller pairs.

The diploid number 2n = 16, characteristic for *A. bacilligera*, is frequently encountered in different cestode species and this number is also found in some representatives of Dilepididae *sensu lato* (see table 4).

The diploid number 2n = 18 established for *D. undula* is the most widespread among cestodes. It has been reported in about 25% of species so far investigated, belonging to different orders (Petkevičiūtė, 1996, 2002). Among cyclophyllideans this number occurred in species of the families Davaineidae and Taeniidae.

Dilepididae *sensu* Bona (1994) exceed one hundred genera. But a study of even such a small portion of species is likely to reveal a trend that exists in the entire group, with the Dilepididae being a heterogeneous taxon.

The question arises as to what extent could karyotype features be suitable for phylogenetic inference. In our viewpoint, if karyotype features are plotted over a phylogenetic tree reconstructed on molecular or morphological data, the processes involving chromosome evolution might be clarified. The congruence between chromosomal and molecular data obtained on diphyllobothriids, characterizing them as a distinct clade within the Pseudophyllidea (see Petkevičiūtė, 2002, 2003), shows that chromosomal changes could be valuable phylogenetic characters. It is notable that phylogenetic analysis of families of the Cyclophyllidea based on comparative morphology revealed extensive polymorphism within the Dilepididae *sensu* Bona (1994) (Hoberg *et al.*, 1999). In recent years molecular systematic studies on cestodes have accumulated with increasing speed (Mariaux & Olson, 2001). Despite much interest in molecular phylogeny, molecular studies dealing with families and genera of tapeworms are scarce. The results of von Nikisch-Rosenegk *et al.* (1999), based on a rather short partial sequence of the mitochondrial SSU gene (314 bp), placed *D. undula* with the hymenolepidids. Mariaux (1998) examined relationships of five dilepidid species in the context of a higher systematic study based on 1.2 kb of the nuclear SSU gene, and concluded that dilepidids remain a relatively homogenous group, the only exception being the relationship between the Dilepididae and *Neogryphorhynchus*.

It seems that a considerable amount of sampling of taxa is needed before revision based on molecular or cytogenetic results can be justified.

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