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ORIGINAL ARTICLE /ARTÍCULO ORIGINAL

KARYOTYPE OF *ADENOSCOLEX OREINI*: THE FIRST CYTOGENETIC DATA WITHIN THE ORDER CARYOPHYLLIDEA (CESTODA) FROM *SCHIZOTHORAX* SPECIES OF KASHMIR VALLEY, INDIA

CARIOTIPO DE *ADENOSCOLEX OREINI*: PRIMER DATO CITOGENÉTICO DEL ORDEN CARYOPHYLLIDEA (CESTODA) DE ESPECIES DE *SCHIZOTHORAX* DEL VALLE DE KASHMIR, INDIA

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ABSTRACT

A karyotype of *Adenoscolex oreini* Fotedar, 1958 (Capingentidae), a cestode parasite of the cyprinid fish, Kashir Gaad, *Schizothorax niger* (Cypriniformes) was studied for the first time. A chromosome set consisted of 20 pairs of metacentric, submetacentric and acrocentric chromosomes (2n=20; n= 8m+1sm+1a). All pairs are small, measuring 1.86-7.22 µm. It seems that the chromosome number of *A. oreini* is higher than those of the members of family Caryophyllaeidae. The variation in size of smallest bivalents and other bivalents indicates that the somatic chromosomes will show a marked variation in the length of longest and shortest chromosome in *A. oreini*. So far no one has undertaken the cytological studies of this parasite; its chromosome number is being reported for the first time.

Keywords: *Adenoscolex oreini* - Chromosomes - India - Kashmir - *Schizothorax*.

RESUMEN

Un cariotipo de *Adenoscolex oreini* Fotedar, 1958 (Capingentidae), un parásito céstodo de carpas, Kashir Gaad, *Schizothorax niger* (Cypriniformes) ha sido estudiado por primera vez. Consiste en un conjunto de cromosomas de 20 pares de cromosomas metacéntricos, submetacéntricos y acrocéntricos (2n = 20; n = 8m+1sm+1a). Todos los pares son pequeños, miden 1,86-7,22 µm. Parece que el número de cromosomas de *A. oreini* es mayor en comparación con la de los miembros de la familia Caryophyllaeidae. La variación en el tamaño de los más pequeños bivalentes y otros bivalentes indican que los cromosomas somáticos mostrarán una marcada variación en la longitud del cromosoma más largo y más corto en *A. oreini*. Hasta ahora nadie ha emprendido los estudios citológicos de este parásito y en la presente investigación se está divulgando su número cromosómico por primera vez.

Palabras clave: *Adenoscolex oreini*- Los cromosomas- India- Kashmir- *Schizothorax*.

INTRODUCTION

Caryophyllids are unique among Eucestoda in having a monopleuroid body plan that is they have neither internal proglottidization nor external segmentation and have a single set of reproductive organs. The embryo has six hooks; scolex is highly variable, with acetabula, loculi, bothria, folds, fimbriae, terminal introvert or without special attachment structures (Mackiewicz, 1972, 1994). They are intestinal parasites of cypriniform and siluriform freshwater fishes; intermediate hosts are aquatic annelids (Mackiewicz, 1982). Some progenetic representatives, such as *Archigetes*, may also have a complete life cycle with aquatic annelids. Phylogenetically, Caryophyllidea and Diphyllobothriidea are regarded as sister lineages; both belong to basal tapeworm groups (Olson *et al*., 2008). Knowledge of chromosome sets of caryophyllidean tapeworms has increased within the past 40 years (Table 1). To date, 23 species of all four existing families have been studied cytogenetically and karyotypes of 14 species have been completed (Table 1). The diploid chromosome number of all, but one species ranges from 14 to 20; *Caryoaustralus sprenti* Mackiewicz *et* Blair, 1980, represents the exception, having only six chromosomes (Grey, 1979: listed as "*gen. et sp. n.*"). Congeners may have constant chromosome numbers as, for example, four species of the genus *Khawia* with 2n = 16 (Grey, 1979; Petkeviciute, 1998; Mutafova & Nedeva, 1999; Orosova *et al*., 2010b); or they may differ in diploid number as in *Glaridacris*, with diploid number 2n =16 in two species and 20 in two others (Grey, 1979; Grey & Mackiewicz, 1974, 1980). This order has the greatest range in chromosome numbers, 6–30, if one includes triploids (Table 1). *Adenoscolex oreini* Fotedar, 1958 is an intestinal parasite of *Schizothorax niger* (Ale gad), a fresh-water fish in Kashmir, India. So far no body has undertaken the cytological studies of this parasite and in the present investigation its chromosome number is being reported for the first time.

The objective of the present study is to describe the karyotype of *A. oreini*, a cestode parasite of Cyprinid fish, Kashir Gaad, *Schizothorax niger* (Ale Gad) (Cypriniformes) of Kashmir. Next, these results will be compared with published data obtained by molecular studies of different species and with cytogenetic information available for other Caryophyllaeidae species.

MATERIAL AND METHODS

Specimens of *A. oreini* of different sizes and maturity were obtained by the dissection of *Schizothorax niger* (Ale Gad), caught in Dal Lake of Kashmir, India in the year 2014. Living specimens were incubated for 3–4 h in 0.01% colchicines in physiological solution at room temperature and were then transferred to distilled water for 1 h for hypotony. Fixation was in a freshly prepared mixture of ethanol and glacial acetic acid (3:1). Specimens were kept refrigerated until they could be processed in the laboratory. Slides were made from cell-suspensions using an air-drying technique (Petkeviciute & Ieshko, 1991), stained with 4% Giemsa, pH 6.8, rinsed in tap water and allowed to dry.

Suitable mitotic metaphases were photographed with a 100X objective under oil immersion using Mikrat-300 film. For karyotyping, chromosomes were cut out of the photographs and paired on the basis of size and centromeric position. Means and standard deviations of the absolute length in micrometres, relative length (100x absolute chromosome pair length/total length of haploid complement) and the centromeric index (100x length of short arm/ total length of

chromosome) were calculated for each chromosome pair. The centromere position on the chromosomes was classified according to the nomenclature of Levan *et al*. (1964). When a centromere position was on the borderline between two categories, the confidence limits of the means were calculated and two chromosome categories are reported.

RESULTS

Analysis of 57 mitotic metaphase spreads from seven specimens showed that the modal diploid complement of *A. oreini* contains 20 chromosomes (2n=20). The karyotype (Fig. 1a, b) included eight metacentric; one submetacentric and one acrocentric chromosome pair. First three pairs of metacentric elements are distinctly larger than the remaining chromosomes and contributed 48.62% to the total chromosome length. The karyotype formula of *A. oreini* can be summarized as 2n=20=8m+1sm+1a. A summary of the results obtained after measuring the Giemsa-stained chromosomes of ten complete metaphase plates is given in Table 2. The chromosomes are middle sized; the largest measured 7.22 µm and the smallest were 1.86 µm. The total chromosome length of the haploid complement was 39.88 µm. The homologues of pairs 5 and 6 could not be distinguished clearly. There are no statistically significant differences in their sizes and centromeric indexes.

In order to better visualize the existing differences in chromosome morphology, ideograms were constructed using the centromere indexes and relative length values (Fig. 2).

Table 1. Measurements (means + SD) and classification of chromosomes of *Adenoscolex oreini.*

Chromosome Number	Absolute length (µm)	Relative length (%)	Centromeric Index	Classification
1	7.22+1.35	26.09+2.06	46.13+1.31	Metacentric
2	6.63 + 0.98	26.77 + 1.43	44.31 + 1.71	Metacentric
3	5.42 + 0.82	21.85 + 0.89	45.67 + 1.38	Metacentric
4	3.45 + 0.44	13.95 + 0.56	46.51 + 1.54	Metacentric
5	2.93 + 0.37	11.87 + 0.89	44.46 + 1.29	Metacentric
6	2.68 + 0.30	10.87 + 0.49	44.68 + 2.30	Metacentric
7	1.77 + 0.22	7.15 + 0.27	43.79 + 2.98	Metacentric
8	5.73+1.08	20.70+1.05	42.24+3.66	Metacentric
9	1.86 + 0.22	7.52 + 0.35	37.69 + 3.74	Submetacentric
10	2.19+0.53	7.83+0.58	3.14+2.88	Acrocentric

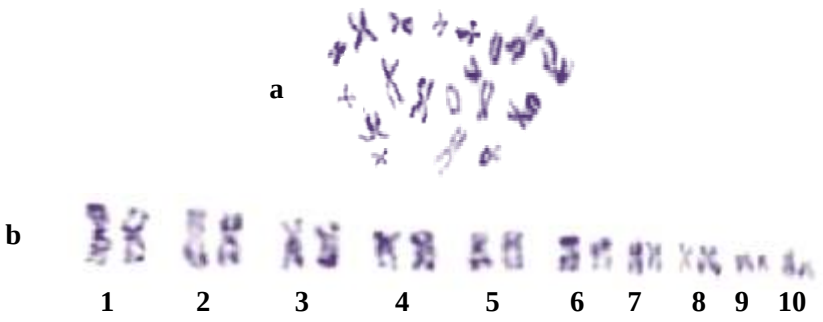

Figure 1. a–b Mitotic chromosomes of *Adenoscolex oreini.* Scale Bar 10 µm.

Table 2. Summary of chromosomes and karyotype data of Caryophyllidea Cestodes (Tapeworms) (1929–till Date).

Order/ Family	Number 2n [3n]	Morphology	TCL (mm)	Absolute length (mm)	References
CARYOPHYLLIDEA					
Balanotaeniidae					
Balanotaenia bancrofti Johnston, 1924	14				Grey (1979)
Capingentidae					
Capingens singularis Hunter, 1927	14				Grey (1979)
Adenoscolex oreini Fotedar, 1958	20	8m+1sm+1a	39.88 µm		Present Study
Caryophyllaeidae					
Hunterella nodulosa Mackiewicz *et* McCrae, 1962	14	3m + 1sm +3a		2.5–8.0	Mackiewicz & Jones (1969)
	14	3m + 4a	56.6	1.9–7.0	Grey (1979)
Archigetes sp. (=*appendiculatus*)	18			<4	Motomura (1929)
Biacetabulum biloculoides Mackiewicz *et* McCrae, 1965	20				Grey (1979)
Caryophyllaeus laticeps (Pallas, 1781)	[30]				Grey (1979)
	20 [30]	10m	87.8	3.0–12.9	Petkeviciute & Kuperman (1992)
	20	10m			Bombarova *et al*. (2009)
Glaridacris laruei Lamont, 1921	16	3m + 1sm + 4a	37.7	2.0–12.0	Grey &Mackiewicz (1974), Grey (1979)
Glaridacris confusus Hunter, 1927	16				Grey (1979)
Glaridacris catostomi Cooper, 1920	20 [30]	8m + 2sm	55.7	3.2–7.6	Grey (1979), Grey & Mackiewicz (1980)
Glaridacris vogei Mackiewicz, 1976	20	8m + 1sm + 1a	60.5	3.0–8.6	Grey (1979)
Monobothrium hunter Mackiewicz, 1963	20	9m + 1a	61.2	2.1–8.7	Grey (1979)
Isoglaridacris folius Fredrickson *et* Ulmer, 1965	18	1m + 8a	24.1	1.5–3.8	Grey (1979)
Isoglaridacris jonesi Mackiewicz, 1972	18	2m + 7a	25.9	1.7–4.3	Grey (1979)
Isoglaridacris bulbocirrus Mackiewicz, 1965	18 [27]				Grey (1979)
Lytocestidae					
Atractolytocestus huronensis					

Table 2. Continuation

Order/ Family	Number 2n [3n]	Morphology	TCL (mm)	Absolute length (mm)	References
Anthony, 1958	[24]	4m + 3a+ 1 minute	24.9	0.8–7.2	Jones & Mackiewicz (1969)
	[24]	4m + 3a + 1	37.0	0.9–9.6	Kralova-Hromadova *et al.* (1979)
Caryoaustralus sprenti Mackiewicz *et* Blair, 1980	6				
Khawia iowensis Calentine *et* Ulmer, 1961	16	5m + 3a	35.5	3.0–6.1	Grey (1979)
Khawia rossittensis (Szidat, 1937)	16				Grey (1979)
Khawia sinensis Hsu, 1935	16	3m + 5a	59.3	5.2–10.8	Petkeviciute (1998)
	16	3m + 5a	41.2	3.8–6.8	Mutafova & Nedeva (1999)
Khawia saurogobii Xi *et al*., 2008	16	3m + 5a	56.3	3.9–10.0	Orosova *et al*. (2010b)
Lytocestus indicus (Moghe, 1925)	16				Vijayaraghavan & Subramanyam (1977)
Caryophyllaeides fennica (Schneider, 1902)	20				Bombarova *et al*. (2009)
	20	10m	58.6	2.8 – 8.2	Orosova *et al*. (2010a)
Notolytocestus minor Johnston *et* Muirhead, 1950	12	6a	17.0	1.7–4.2	Grey (1979)

TLC = Total Chromosome Length; m = Metacentric; sm=Submetacentric; t=Telocentric; a=Acrocentric.

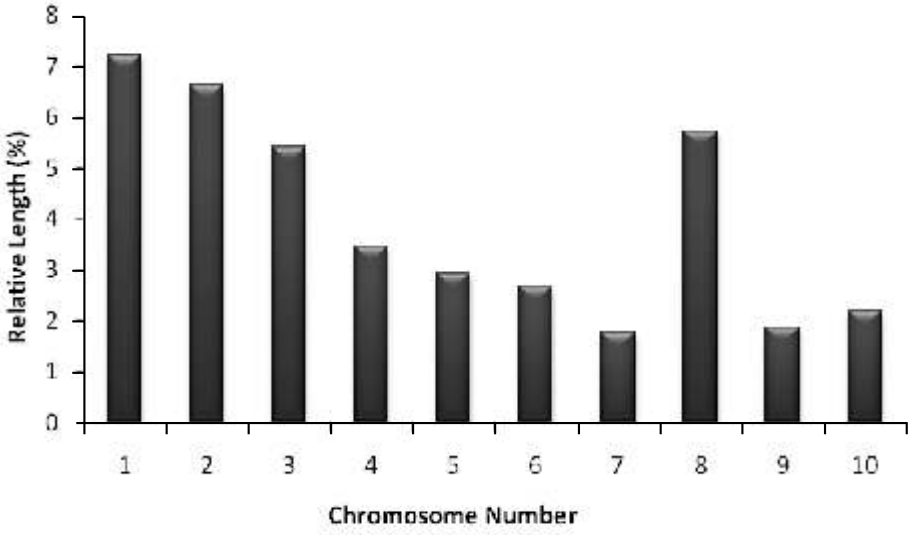

Figure 2. Idiogram of *Adenoscolex oreini*.

DISCUSSION

It may be concluded that the diploid chromosome number of *A. oreini* is 20 and it is perhaps the first report of chromosome number of a genus from family Capingentidae of order Caryophyllidea. However, in the family Caryophyllaeidae the chromosome number has been reported by Motomura (1929); Mackiewicz & Jones (1969), Grey & Mackiewicz (1974); Vijayaraghavan & Subramanyam (1977); Grey (1979); Grey & Mackiewicz (1980); Petkeviciute & Kuperman (1992); Petkeviciute (1998); Bombarova *et al.* (2009) and Orosova *et al.* (2010a,b) for *Archigetes appendiculatus* (2n=18); *Hunterella nodulosa* (2n=14); *Glaridacis laruei* (2n=16); *Lytocestus indicus* (2n=16); *Capingens singularis* (2n=14); *Glaridacris catostomi* (2n=20); *Caryophyllaeus laticeps* (2n=20); *Khawia sinensis* (2n=16); *Caryophyllaeides fennica* (2n=20) and *Khawia saurogobii* (2n=16), respectively. It seems that the chromosome number of *A. oreini*, a member of family Capingentidae is higher as compared to that of the members of family Caryophyllaeidae. The variation in size of smallest bivalent and other bivalents indicates that the somatic chromosomes will show a marked variation in the length of longest and shortest chromosome in *A. oreini.* The same has been reported by Motomura (1929) in *Archigetes appendiculatus* and Mackiewicz & Jones (1969) in *Hunterella nodulosa.* Therefore, this type of chromosome complement may be characteristic of Caryophyllidea in general.

Chromosomes of 14 caryophyllidean species are of mid or large length, up to 12.9 mm long in *C. laticeps* (Table 2). They are among the largest of the Cestoda, a feature they share with *Eubothrium crassum* Bloch, 1779, of the Bothriocephalidea. The mean TCL in Caryophyllidea is 47.8 mm, with a high of 87.8 for *C. laticeps*, the highest yet recorded from any tapeworm. Satellites (*i.e.* secondary constrictions), rare with cestode chromosomes, have been found in *H. nodulosa*, *A. huronensis* and *K. saurogobii* (Grey, 1979; Kralova-Hromadova *et al*., 2010; Orosova *et al*., 2010b).

In individual species, chromosome morphology differs significantly; a predominance of bi-armed elements (symmetrical karyotypes) was detected in four species of the family Caryophyllaeidae and one lytocestid species (Table 2), one-armed acrocentric chromosomes (asymmetrical karyotypes) prevailed in four species of Caryophyllaeidae and Lytocestidae and the rest of five karyotypes with rather balanced chromosome morphology occurs in both families. Because of this variation, no relevant hypothesis on karyotype evolution can be made on basis of traditional karyological analyses among the four families of the Caryophyllidea. It is noteworthy that recent cladistic analysis by Oros *et al.* (2008), based on unweighted morphological characters, are only partly congruent with the existing classification into four families that is based on the placement of the internal, longitudinal musculature. However, paraphyly of Caryophyllidean families should be corroborated by molecular studies.

It is apparent that classical karyological data are hardly sufficient to resolve phylogenetic and systematic relationships within insufficiently investigated animal groups such as the Caryophyllidea. However, the recent inputs of molecular approaches into the cytogenetics of Caryophyllidea (Kralova-Hromadova *et al*., 2010; Orosova *et al*., 2010a,b) raises hopes that new data will substantially help in elucidating the phylogenetic relationships within this unique group of monozoic tapeworms.

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