Karyotypic characterization of *Apatemon gracilis*

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Abstract

The cytotaxonomical characteristics of parthenitae of *Apatemon gracilis* (Rudolphi, 1819) Szidat, 1928 were studied using karyometric analysis to extend our knowledge of chromosome morphology and karyosystematics among trematodes. The karyotype, reported here for the first time, consists of ten pairs (2n = 20) of chromosomes divided into two size groups: five pairs of comparatively large and five pairs of small chromosomes. Biarmed chromosomes prevail in the chromosome set. According to centromere index values, chromosome 1 is submetacentric to metacentric, 2 is subtelocentric, 3 and 5 are acrocentric, 4, 9 and 10 are metacentric, 6 is submetacentric and 7 and 8 are submeta-subtelocentric. The small uniarmed B-chromosome was found in the chromosome set of parthenitae of *A. gracilis* from one snail. Data are discussed with reference to the karyotypes previously described within the Strigeidae.

Introduction

Although the strigeid species have been the subject of intensive morphological, biological and ecological studies over a considerable period of time, the taxonomic status of a number of species remains unclear (see Dubois, 1968; Niewiadomska, 1973; Sudarikov, 1984). The identification of larval trematodes of the family Strigeidae on morphological criteria remains difficult and few life-cycles are known. Also there are cercariae which could be ascribed to strigeid trematodes on the basis of their morphological features, but the adult forms are still unknown and consequently their systematic position unclear. Cytogenetic studies can therefore supplement morphological, biological and other characteristics used for systematic analysis. Unfortunately, cytogenetic studies on parasitic helminths have lagged behind those of free living animals, especially the insects and vertebrates, largely because of the difficulties in obtaining sufficient good material for cytological preparation.

The purpose of the present study is to provide karyological information on the strigeid species, *Apatemon gracilis*, and to compare its chromosome set structure with that of other strigeid species. *Apatemon gracilis* is the type species of the genus *Apatemon* Szidat, 1928. According to Sudarikov (1984) there are only three valid species of this genus recorded in the former Soviet Union. While both the number and morphology of chromosomes have been described in *A. fuligulae* and *A. minor* (Baršienė *et al.*, 1990; Baršienė, 1992), there has been no published information to date on the chromosomes of *A. gracilis*.

Materials and methods

Mitotic chromosome preparations were made from the cells of parthenitae obtained from the snail *Lymnaea ovata*. Three snails naturally infected with *A. gracilis* were collected from a pond in the village of Zhelabovka (Crimea, Ukraine).

Infected snails were treated with 0.01% colchicine in well water at room temperature for 4–5 h. They were dissected and the digestive glands containing trematode larvae removed and treated with distilled water for 40–50 min for hypotony. The material was fixed in freshly prepared ethanol–acetic acid (3:1) and stored at 4°C. Microscope preparations were made using the air-dried method of Kligerman & Bloom (1977) with some modifications. Pieces of tissue were transferred to some drops of 45% acetic acid on pre-cleaned slides, smeared on them and air-dried for at least one day. Preparations were then hydrolysed in 1 N HCl for 10–15 min, rinsed in distilled water and stained with 4% Giemsa solution in Sörensen's buffer (pH 6.8) for 30–40 min. The well

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spread metaphase plates were photographed under an oil-immersion system using Mikrat-300 film. For karyotyping, chromosomes were cut out of the photographs and paired on the basis of their size and centromeric position.

Morphometric measurements on chromosomes in the karyotypes of eight metaphasic cells of karyomorph 2n = 20 and five cells of karyomorph 2n = 21 were made. For each pair the mean absolute and relative length and mean centromere index with their standard deviation (SD) were calculated. The relative length of chromosomes was calculated as follows: absolute chromosome length × 100/total length of haploid genome; the centromere index was calculated as length of short arm × 100/length of the whole chromosome. The length of the B-chromosome was not included in the total length of haploid complement in the measurements of the relative length of chromosomes of karyomorph 2n = 21. The terminology relating to the centromere position follows that of Levan *et al.* (1964). A chromosome is considered metacentric (m) if the centromere index falls within the range 37.5–50.0, submetacentric (sm) 25.0–37.5, subtelocentric (st) 12.5–25.0 and telocentric (t) 0.0–12.5. When the centromere position was on the borderline between two categories, two chromosome categories are listed.

Results

A diploid complement of 2n = 20 was found in 29 mitotic metaphases (out of 32 studied) of somatic cells of *A. gracilis* from two snails *L. ovata*. The percentage of aneuploid cells was 9.4, but these are presumably artefacts resulting from cell rupture and loss of chromosomes during preparation. One snail was infected with parthenitae containing 21 chromosomes in the diploid sets of 18 cells out of 22 studied (fig. 1).

The chromosomes are medium sized and range in length from 2.12 to 8.11 μm (table 1). Two distinct size groups of chromosomes are revealed in the karyotype. The first five pairs consist of comparatively large elements and constitute 68–69% of the total complement length. The remaining five pairs (Nos 6–10) consist of comparatively small chromosomes. Biarmed elements predominate in the karyotype. According to the centromere position, chromosome pair 1 is considered submeta-metacentric, pair 2 is subtelocentric, pairs 3 and 5 are acrocentric, pairs 4, 9 and 10 are metacentric, pair 6 is submetacentric and pairs 7 and 8 are submeta-subtelocentric.

The 21st additional chromosome detected in the karyotype of the parthenitae from one snail *L. ovata*, is a small uniarmed element, measuring 1.2 μm, non-homologous to the regular chromosomes. The regular chromosome set of this karyomorph did not differ from the standard complement (table 1).

Discussion

The karyotype of *A. gracilis*, reported here for the first time, shows the same chromosome number (2n = 20) as other karyologically studied strigeid species. To date, the chromosome set structure among strigeids is known for

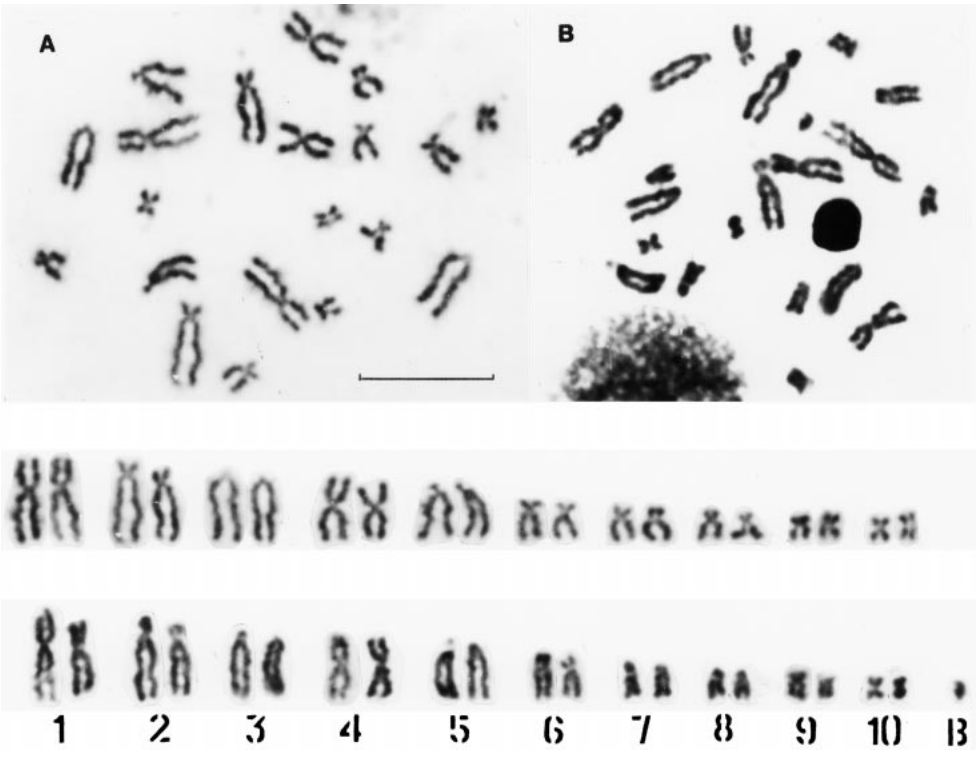

Fig. 1. Mitotic metaphases and karyotypes of two karyomorphs of *Apatemon gracilis* with 2n = 20 (A) and 2n = 21 (B). Scale bar = 10 μm.

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Table 1. Measurements (means ± SD) and classification of chromosomes of two karyomorphs of *Apatemon gracilis*.

Chromosome number		Absolute length (μm)	Relative length (%)	Centromere index	Classification
1	A	7.10 ± 0.99	16.36 ± 1.15	36.52 ± 2.30	sm-m
	B	8.11 ± 0.96	17.65 ± 1.00	35.53 ± 1.25	sm-m
2	A	6.71 ± 0.78	15.37 ± 0.41	19.54 ± 2.91	st
	B	7.13 ± 0.63	15.57 ± 1.00	20.03 ± 2.07	st
3	A	5.56 ± 0.83	12.72 ± 0.72	6.54 ± 3.15	a
	B	5.70 ± 0.38	12.46 ± 0.45	8.44 ± 3.04	a
4	A	5.39 ± 0.51	12.38 ± 0.43	44.31 ± 2.67	m
	B	5.64 ± 0.38	12.32 ± 0.69	43.29 ± 2.42	m
5	A	5.01 ± 0.60	11.59 ± 0.48	8.22 ± 4.03	a
	B	5.18 ± 0.49	11.30 ± 0.40	6.24 ± 5.35	a
6	A	3.41 ± 0.27	7.84 ± 0.33	28.42 ± 1.92	sm
	B	3.52 ± 0.14	7.71 ± 0.37	27.61 ± 2.07	sm
7	A	3.15 ± 0.31	7.24 ± 0.20	28.09 ± 2.64	sm
	B	3.13 ± 0.27	6.84 ± 0.47	26.30 ± 1.96	sm-st
8	A	2.61 ± 0.22	5.99 ± 0.30	27.17 ± 2.49	sm-st
	B	2.76 ± 0.20	6.02 ± 0.23	26.72 ± 3.17	sm-st
9	A	2.45 ± 0.32	5.61 ± 0.38	42.86 ± 5.57	m
	B	2.50 ± 0.17	5.45 ± 0.20	40.17 ± 4.16	m
10	A	2.22 ± 0.20	5.11 ± 0.29	46.78 ± 1.88	m
	B	2.12 ± 0.16	4.63 ± 0.18	43.47 ± 0.52	m

Abbreviations: A, measurements of karyomorph with 2n = 20; B, measurements of karyomorph with 2n = 21; m, metacentric; sm, submetacentric; st, subtelocentric; a, acrocentric chromosomes.

six species of the genera *Apatemon*, *Ichthyocotylurus* and *Cotylurus* (Baršienė *et al.*, 1990; Baršienė, 1992). In all these six species, the chromosomes of the complement can be arranged into two distinct size groups: five pairs of comparatively large and five pairs of small elements. Such an arrangement is probably typical of strigeid trematodes. Trematodes are fairly conservative karyotypically, with related species differing by a few chromosome rearrangements. Chromosome sets with 20 or 22 elements in diploid complements predominate in the Trematoda. But the existence of generalized karyotypes usually does not exclude identification of species by karyotypic characters.

A striking similarity was revealed in comparison of the chromosome sets of *A. gracilis* and *Cercaria globocaudata* U. Szidat, 1940. *Cercaria globocaudata* is a species of the 'bulbocauda' group (Szidat, 1940), characterized by a strongly developed tail with a coloured aggregation near bifurcation. Szidat (1940) suggested a close relationship between this cercaria and the genus *Apatemon* Szidat, 1928. The morphology of *C. globocaudata* from the Nemunas delta was examined by Niewiadomska & Kiselienė (1993), whereas that of the cercariae of *A. gracilis* (syn. *A. cobitidis* Linstow, 1890) from the Crimea was described by Stenko (1977). The cercaria of *A. gracilis* resembles that of *C. globocaudata* in such characters as the structure of the intestine, flame cell formula, as well as the number and position of the penetration glands (Niewiadomska & Kiselienė, 1993). These characters are of generic or subgeneric significance (after Dubois, 1968) and consistent with the characteristics of subgenus *Apatemon* Szidat, 1928 but differ from the subgenus *Australapatemon* Sudarikov, 1959 in the flame cell formula and structure of the intestine. With reference to the tail structure, the cercariae do not fit either subgenus. Karyological observations support the close taxonomic affinity between *C. globocaudata* and *A. gracilis* and support the comments of Cable (1965) that the use of cercarial tails as phylogenetic indicators is very misleading.

The karyotype of *C. globocaudata* was described by Stanevičiūtė (1993) and by Baršienė (1993). The material for both studies was collected from the same focus in the Nemunas Delta. Baršienė (1993) pointed out the karyotypical similarity between *C. globocaudata* and *A. fuligulae*. Although no differences were revealed in the measurements of the relative length of chromosomes, the two species clearly differ in the position of the centromere in chromosome pairs 1, 3 and 5. The karyotypes of *C. globocaudata* (according to data of Stanevičiūtė, 1993) and *A. gracilis* appear almost identical both with respect to the relative length and the centromeric indices of the corresponding chromosome pairs and they could not be differentiated by conventional Giemsa staining. Small differences, noted in the centromere index values of chromosomes pair 3 and 5, may arise due to differential condensation among the metaphases. In chromosomes with subterminal centromeres, the short arm may contract only moderately, whereas the long arm contracts to a much greater extent (Drouin *et al.*, 1991). Therefore, variation in the centromere index values may be greater in chromosomes with unequal arms. Taking this into

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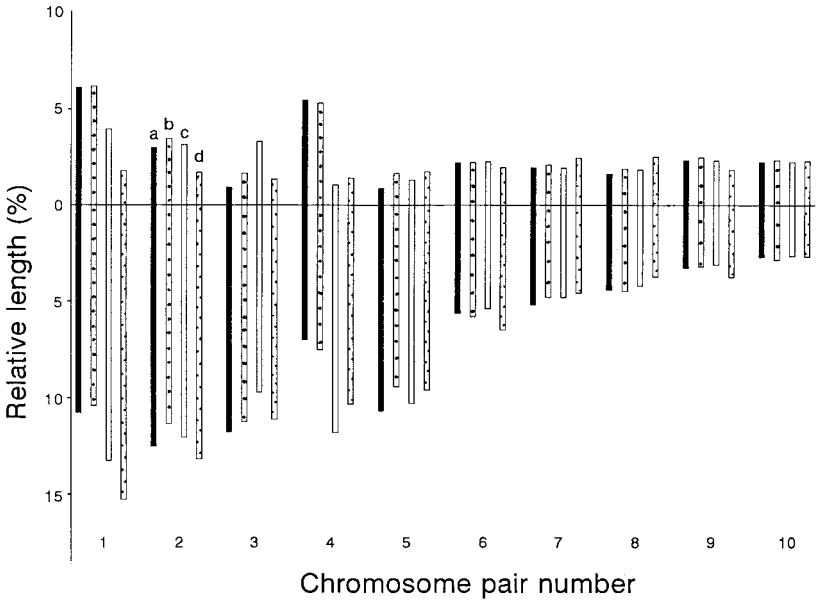

Fig. 2. Idiograms representing the haploid sets of four species: a, *Apatemon gracilis*; b, *Cercaria globocaudata* (data of Stanevičiūtė, 1993); c, *A. fuligulae* (data of Baršienė *et al.*, 1990); d, *A. minor* (data of Baršienė, 1992).

account, the observed differences could result from differential condensation of metaphases rather than from any structural changes. The only difference between the karyotype of *A. gracilis* and that of *C. globocaudata* described by Baršienė (1993) arises from the different determination of the chromosome pair number of chromosomes 4 and 5. The length of these chromosomes does not differ significantly and therefore their ordinal numbers in the karyotype could be established otherwise.

It is generally accepted that karyotypic similarity among related organisms indicates phylogenetic relationship. In any case, karyotypic similarities do not originate *de novo* among unrelated species, since the karyotype does not respond to selective pressures in the same way as anatomical and physiological characteristics.

To demonstrate these karyometrical peculiarities, the idiograms of karyotypes of *C. globocaudata* and three *Apatemon* species were constructed (fig. 2) based on the mean values presented in table 1 and previously published data (Baršienė *et al.*, 1990; Baršienė, 1992; Stanevičiūtė, 1993). The main interspecific differences were observed in the morphology of the large elements (1st–5th pairs), while the group of small elements (6th–10th) pairs are more stable. The variation in the centromere position of corresponding chromosomes in genus *Apatemon* is most easily explained by pericentric inversions of a different entity. Patterns of karyotypic evolution in Strigeidae generally seem to fit two categories, namely, conservatism and karyotypic orthoselection (White, 1975). Taxa that are conservative have slow rates of karyotypic evolution and related species differ by few or no chromosomal rearrangements. Taxa that evolve by karyotypic orthoselection often have a few or even numerous karyotypic rearrangements when closely related species are compared, but only one or few kinds of rearrangements are involved.

Despite the comparatively conservative nature of the karyotype structure of trematodes, intraspecific strain differences have been documented in several species. For example, differences between seven strains of *Schistosoma mansoni* could be accounted for by relatively small pericentric inversions and/or translocations (Short *et al.*, 1989). The similarity of the karyotypes of two such clearly morphologically distinct species, *C. globocaudata* and *A. gracilis*, is a striking example of karyotypic conservatism among trematodes, and presumably the proportion of chromosomal differences is not directly related with the degree of divergence between the species.

The presence of an additional 21st chromosome, which is likely to be a B-chromosome, is not very rare, since B-chromosomes have been recorded in several species of trematodes of the genera *Notocotylus* (Petkevičiūtė & Baršienė, 1988; Petkevičiūtė *et al.*, 1989a; Baršienė *et al.*, 1990), *Diplodiscus* (Petkevičiūtė *et al.*, 1989b), *Echinostoma* (Baršienė & Kiselienė, 1991) and in all studied species of the genus *Apatemon* (Baršienė *et al.*, 1990; Baršienė, 1992). The additional or B-chromosomes and polymorphism for them are widely known in plants and animals (Jones, 1975). B chromosomes differ from the members of the normal karyotype in several features, particularly as they are present in different numbers, with an absence of clear effects on the carrier individual. B chromosomes of trematodes, described so far, are the elements of different size and morphology. More frequently they are mitotically stable and exist by one or two in the cells of the carrier individual, but the number can differ from cell to cell and from individual to individual (see Baršienė,

1993). A larger amount of material should be examined to reveal the patterns of prevalence of B chromosomes in populations of *A. gracilis*.

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