Journal of Helminthology (2001) **75,** 295–297 DOI: 10.1079/JOH200050

Research Note

Chromosomes of *Aspidogaster conchicola*

R. Petkevičiūtė*

Institute of Ecology, Lithuanian Academy of Sciences, Akademijos 2, Vilnius 2600, Lithuania

Abstract

Analysis of mitotic and meiotic chromosome spreads of *Aspidogaster conchicola* (Trematoda: Aspidogastrea) reveals a diploid number of 10 for this species. Haploid sets containing 5 bivalents confirm the diploid number. The karyotype consists of one pair of comparatively long subtelocentric chromosomes and four pairs of shorter acrocentric elements. The results are discussed in comparison with existing data on chromosomes of aspidogastrean species and other related helminth groups.

Aspidogastrea, which is a small platyhelminth taxon with fewer than 40 species parasitizing both molluscs and poikilothermous vertebrates (Rohde, 1972), combines characteristics of both the Digenea and Monogenea. The most conspicuous characteristics of aspidogastreans are the ventral disc and direct development without alternation of generations. The systematics of aspidogastreans are complex, as they have been classified as monogeneans, digeneans or considered as intermediate between the Digenea and Monogenea. La Rue (1957), Ginetzinskaya (1968) and Bychovskaya-Pavlovskaya & Ginetzinskaya (1975) are of the opinion that in view of the morphological and life-cycle differences between the Aspidogastrea and the Trematoda, the aspidogastreans represent a separate class of platyhelminths. Rohde (1972) suggests that the Aspidogastrea possess many archaic features, form a group of their own and are closely related to Digenea. Gibson (1987) concludes that the aspidogastreans are the sister group of the Digenea. Recent phylogenetic analyses, based on morphology and molecules, demonstrate that trematodes (Aspidogastrea plus Digenea) are monophyletic (Littlewood *et al*., 1999).

Cytogenetic studies can contribute an array of information independent of morphological, biochemical and other characters that are used for phylogenetic analysis. *Aspidogaster conchicola* Baer, 1827 is one of the best known aspidogastrean species with respect to its morphology, development and biology but there has been no published information to date on the chromosomes of this species. A karyological study on the mitotic and meiotic chromosomes of *A. conchicola* was undertaken to supplement the characteristics of this species and to extend our limited knowledge of the karyotypes of aspidogastreans.

Living specimens of adult *A. conchicola*, used for karyological analysis, were recovered from the pericardial cavity of the freshwater mussels *Unio pictorum, U. tumidus, Crassiana crassa* and *Anodonta piscinalis*, collected from the river Nėris and the cooling basin of the Lithuanian hydro-electric power station.

Mussels prior to dissection were injected with 0.01% colchicine solution and maintained for 10–15 h in well water. Recovered worms were placed in physiological solution, containing 0.01% colchicine for 1–2 h at room temperature, then transferred to distilled water for 30–40 min for hypotony, fixed in freshly prepared ethanol–glacial acetic acid (3:1) and stored at 4°C. For slide preparations, fixed worms were stained with aceto-orcein for 10–12 h, briefly soaked in 45% acetic acid and squashed under a coverslip. Squashes were sealed with Canada balsam and examined for cell divisions. The best mitotic and meiotic plates were photographed under an oil-immersion system using Mikrat-300 film. Measurements were made on karyotypes from photographs of seven well-spread mitotic metaphases. Terminology relating to centromere position follows that of Levan *et al*. (1964).

Karyological analysis revealed the modal diploid number $2n = 10$ at the stage of mitotic metaphase.

*Fax: (370 2) 72 92 57
E-mail: helmi@ekoi.lt

Table 1. Measurements (means ± SD) and classification of chromosomes of *Aspidogaster conchicola*.

Chromosome number	Absolute length (μm)	Relative length (%)	Centromeric index	Classification*
1	3.56±0.15	33.06±0.58	24.36±0.59	st
2	2.84±0.21	23.14±0.70	7.48±1.52	a
3	1.96±0.06	16.52±0.42	10.28±2.01	a
4	1.62±0.05	14.23±0.35	11.16±1.45	a-st
5	1.49±0.07	13.04±0.44	9.09±2.11	a

* st, subtelocentric; a, acrocentric chromosomes.

Haploid sets with n = 5 at various stages of meiotic division confirmed the diploid number. Four hundred and twenty-three dividing cells were studied, 79.2% of them contained the modal chromosome number. The percentage of aneuploid cells was 10.2 and 10.6% of cells were polyploid.

Mean karyometrical data are reported in table 1. At the mitotic metaphase the diploid complement is characterized by a comparatively long pair of subtelocentric chromosomes, averaging 3.56 μm in length and four pairs of shorter acrocentric elements 2.84–1.49 μm in length (fig. 1a,b). The total chromosome length of the complement (TCL) is 11.47 μm. Meiosis was observed in spermatogenesis and in intrauterine eggs. Metaphase II configurations (fig. 1c) were frequently seen in eggs; diakinesis (fig. 1d) was more often observed in the testes. The chiasma frequency has not been worked out but it appears to be from 1 to 3 per bivalent.

Information on chromosomes of members of the Aspidogastrea has been reported for only three species. Rohde (1973) observed a haploid number of 7 in the dividing egg cells of *Lobatostoma manteri.* LoVerde & Fredericksen (1978) described the karyotype of *Cotylogaster occidentalis* (2n = 12) with chromosomes 2–6 μm in length at the mitotic metaphase. There were two pairs of acrocentrics, two pairs of submetacentrics and two pairs of metacentrics. Only meiotic cells from the testes of *Cotylaspis insignis* with a haploid number 11 were observed (LoVerde & Fredericksen, 1978).

All karyologically studied aspidogastrean species are members of one family – Aspidogastridae. Each of the four species has a different chromosome number and it is likely that this diversity is due to the archaic nature or long history of the group.

Digenetic trematodes are karyotypically conservative. Their karyotypes tend to have the same number and

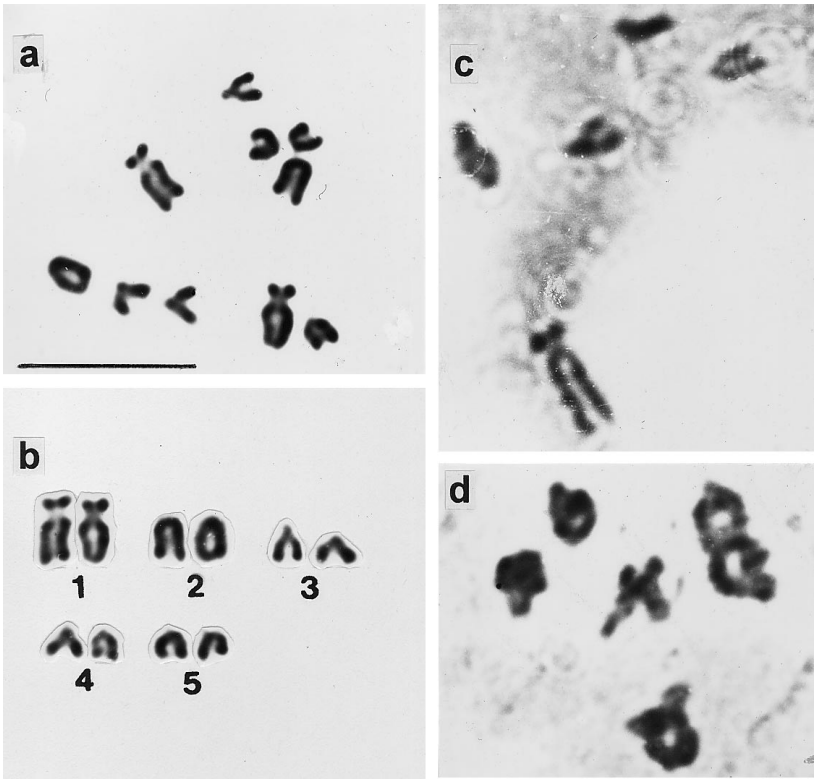

Fig. 1. Mitotic and meiotic chromosomes of *A. conchicola*: a, mitotic metaphase plate and b, karyotype, 2n = 10; c, metaphase II of meiosis, n = 5; d, diakinesis, showing 5 bivalents. Scale bar = 10 μm.

https://doi.org/10.1079/JOH200050 Published online by Cambridge University Press

closely related gross chromosome morphology at the genus and even family taxonomic levels. The diploid chromosome numbers range between 12 and 28 (Baršienė, 1993). Chromosome sets with 22 or 20 elements predominate. The karyotype of primitive trematodes was probably composed of 10 (or 11) pairs of telocentric or subtelocentric chromosomes (Grossman *et al.*, 1981).

There are very few data on the karyology of Monogenea. Species of the karyologically best-known monogenean genus *Diplozoon* (family Diplozoidae) have different diploid numbers, i.e. 8, 10 or 14 (Koroleva, 1969). A comparison of the number and morphology of chromosomes indicates that a reduction in chromosome number results from the centromeric fusion of acrocentrics. The chromosomes of two species of the genus *Kuhnia* (family Mazocraeidae), 2n = 16, resemble each other closely (Rohde, 1994).

The diploid number 10, found in *A. conchicola* is below the lower range of the Digenea. Unfortunately, the lack of information on karyotypes and chromosome morphologies for aspidogastreans, as well as for many other groups of platyhelminths, has made it impossible to place the karyotypic data in any larger evolutionary context.

References

Baršienė, J. (1993) *The karyotypes of trematodes.* Vilnius: Academia, 370 pp. (in Russian).

Bychovskaya-Pavlovskaya, I.E. & Ginetzinskaya, T.A. (1975) The current state of knowledge of Trematoda and main aims of their investigation. *Parazitologiya* **9**, 3–16 (in Russian).

Gibson, D.I. (1987) Questions in digenean systematics and evolution. *Parasitology* **95**, 429–460.

Ginetzinskaya, T.A. (1968) [*Trematodes, their life cycles, biology and evolution*.] Leningrad, Nauka, 412 pp. (in Russian).

Grossman, A.I., Short, R.B. & Cain, G.D. (1981) Karyotype evolution and sex chromosome differentiation in schistosomes (Trematoda, Schistosomatidae). *Chromosoma* **84**, 413–430.

Koroleva, Y.I. (1969) Karyology of some species of *Diplozoon*. *Parazitologiya* **3**, 411–414 (in Russian).

La Rue, G.R. (1957) The classification of digenetic Trematoda: a review and a new system. *Experimental Parasitology* **6**, 306–349.

Levan, A., Fredga, K. & Sandberg, A. (1964) Nomenclature for centromere position on chromosomes. *Hereditas* **52**, 201–220.

Littlewood, D.T.J., Rohde, K. & Clough, K.A. (1999) The interrelationships of all major groups of Platyhelminthes: phylogenetic evidence from morphology and molecules. *Biological Journal of the Linnean Society* **66**, 75–114.

LoVerde, P.T. & Fredericksen, D.W. (1978) The chromosomes of *Cotylogaster occidentalis* and *Cotylaspis insignis* (Trematoda: Aspidogastrea) with evolutionary considerations. *Proceedings of the Helminthological Society of Washington* **45**, 158–161.

Rohde, K. (1972) The Aspidogastrea, especially *Multicotyle purvisi* Dawes, 1941. *Advances in Parasitology* **10**, 77–151.

Rohde, K. (1973) Structure and development of *Lobatostoma manteri* sp. nov. (Trematoda: Aspidogastrea) from the Great Barrier Reef, Australia. *Parasitology* **66**, 63–83.

Rohde, K. (1994) Chromosomes of *Kuhnia scombri* and *K. sprostonae* (Monogenea, Polyopisthocotylea, Mazocraeidae). *Acta Parasitologica* **39**, 117–119.

(Accepted 9 October 2000)

https://doi.org/10.1079/JOH200050 Published online by Cambridge University Press