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A karyotype of *Nippotaenia mogurndae*: the first cytogenetic data within the order Nippotaeniidea (Cestoda)

M. BOMBAROVÁ, M. ŠPAKULOVÁ, M. OROS

Parasitological Institute of the Slovak Academy of Sciences, Hlinkova 3, 040 01 Košice, Slovak Republic;
E-mail: *bertova@saske.sk*

Summary

A karyotype of *Nippotaenia mogurndae* Yamaguti et Myiata, 1940, a cestode parasite of the perciform fish, Chinese sleeper *Perccottus glenii* (Odontobutidae), has been studied for the first time. A chromosome set consists of 14 pairs of metacentric and submetacentric chromosomes (2n = 28; n = 7m + 3sm-m + 4sm). All pairs are small, measuring 1.16 – 2.74 μm. The length of individual pairs decreased gradually and the differences between neighbouring pairs were insignificant. Karyological characteristics correspond with the latest phylogenetic hypotheses that nippotaeniids belong to the higher tetrafossate tapeworms and are closely related to the Cyclophyllidea and especially Mesocestoididae.

Introduction

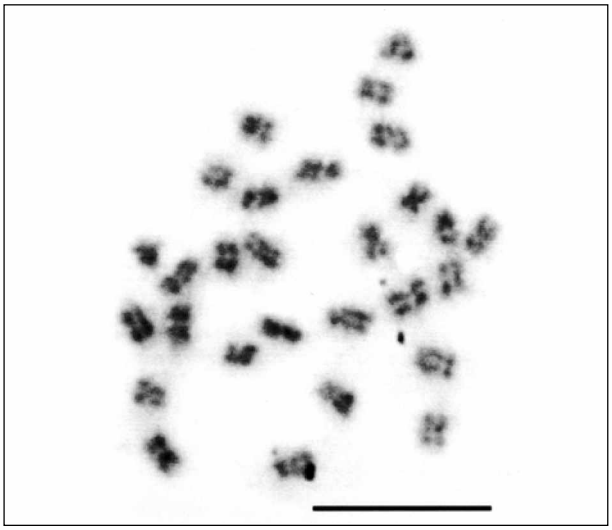
The cestode order Nippotaeniidea is markedly low in number of taxa. Up to now, only six species from freshwater teleosts have been described. Nippotaeniids parasitic in fish hosts of the family Odontobutidae are indigenous in East Asia (Bray, 1994; Nelson, 1994). Recently, the Chinese sleeper *Perccottus glenii* Dybowski, 1877, which serves as a host of the tapeworm *Nippotaenia mogurndae* Yamaguti et Myiata, 1940, has expanded to Central Asia and the eastern part of Europe (Kautman, 1999; Koščo, Košuth, 2002; Koščo *et al.*, 2003). The host fish has apparently carried its parasite *N. mogurndae* to the eastern Slovakia where it has been found for the first time by Košuthová *et al.* (2004).
No information on karyotype of any nippotaeniid cestode is available to date. This study provides the first description of the chromosome complement of *N. mogurndae* based on the analysis of both colchicine-treated and untreated chromosomes.

Material and Methods

Hosts and parasites
A total of 51 Chinese sleepers *Perccottus glenii* were captured by means of electrofishing in the channel backwater of the Latorica River near the village of Svätá Mária (eastern Slovakia). The tapeworm *Nippotaenia mogurndae* occurred in the intestine of 49 % of fish with the intensity of 1 – 6 worms. Some specimens were fixed in 4 % hot formaldehyde and identified on the basis of morphological traits according to Dubinina (1987).

Chromosome preparations
For karyological study, 17 alive strobila *N. mogurndae* and 18 detached hermaphroditic or gravid proglottides were used. A part of the material was treated with 0.025 % colchicine in saline solution for 30 min and then with hypotonic solution of 0.6 % sodium citrate for 1 hr. During the hypotony, the tegument was slightly destroyed using needles. Thereafter, a modified method of Petkevičiuté, Ieshko (1991) was used. Worms were fixed by methanol-acetic acid mixture (3:1) and homogenised mechanically within the Eppendorf tube with few drops of 60 % acetic acid by means of plastic plunger. The cell suspension was dropped onto a clean slide and the surplus of the fluid was sucked from the centre of each drop.
The second part of strobila or proglottides was processed without colchicine treatment following the spreading procedure described by Frydrychová, Marec (2002) with slight modifications. Individual worms were swollen for 10 min in a hypotonic solution (0.075 M KCl) and fixed in Carnoy's fluid (ethanol, chloroform, acetic acid, 6:3:1) for 15 min. Fixed tissues were transferred into a drop of 60 % acetic acid on a slide. After tearing up the tissue and its dissociation, a drop of cell suspension was spread throughout the slide using a heating plate at 45°C. Each of the slides was made from a single or two individuals.

Karyological analysis
Slides were stained with 4 % Giemsa solution (pH 6.8) for 30 – 45 min. An Olympus BX microscope supplied with the digital camera DP50 was used for taking photographs



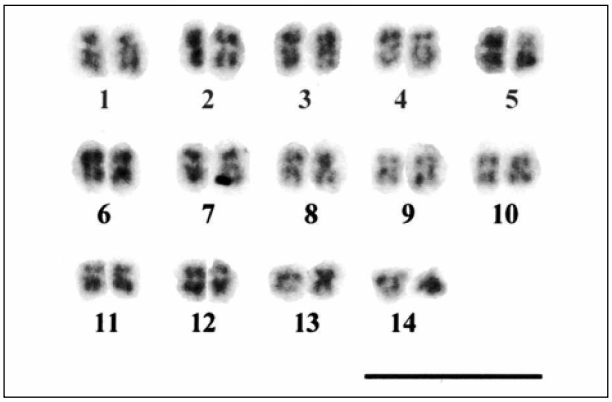


Fig. 1. Karyotype of *Nippotaenia mogurndae*. Mitotic metaphase spread of spermatogonial cell and corresponding idiogram
Scale-bars = 10 μm

of well-spread mitotic metaphases. Karyological data (absolute and relative length and centromeric index) were calculated from 10 best mitotic spreads chosen from 84 evaluated cells. Metric characteristics are given in micrometres.

Results

A karyotype of *Nippotaenia mogurndae* consists of 14 chromosome pairs (2n = 28, Fig. 1). The complete set of 28 chromosomes was found in 66 % of evaluated cells; the shortage of chromosomes in the rest of evaluated cells was most probably attributed to the loss of elements during the preparation of slides, predominantly when using the "heating plate" method. The diploid number of 28 elements was confirmed by the occurrence of dividing spermatocytes in diakinesis – first meiotic metaphase, comprising 14 bivalents (Fig. 2).

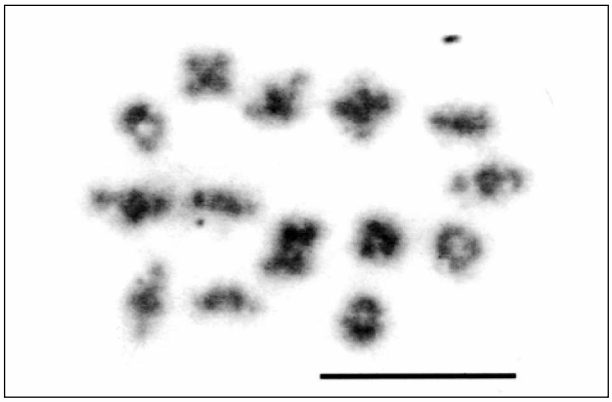


Fig. 2. Meiotic chromosomes of *Nippotaenia mogurndae*: spermatocyte in diakinesis
Scale-bar = 10 μm

Table 1. Measurements (means ± SD) and classification of chromosomes of *Nippotaenia mogurndae*

Chromosome number	Absolute length (μm)	Relative length (%)	Centromeric index	Classification
1	2.74 ± 0.61	9.52 ± 0.61	28.53 ± 3.22	sm
2	2.60 ± 0.57	9.02 ± 0.48	46.25 ± 2.37	m
3	2.47 ± 0.49	8.60 ± 0.31	39.90 ± 2.73	m
4	2.39 ± 0.46	8.32 ± 0.20	30.32 ± 2.37	sm
5	2.30 ± 0.42	8.03 ± 0.27	45.80 ± 2.63	m
6	2.24 ± 0.39	7.80 ± 0.12	42.40 ± 1.84	m
7	2.13 ± 0.41	7.41 ± 0.29	33.67 ± 2.92	sm
8	2.09 ± 0.36	7.29 ± 0.15	37.98 ± 2.25	sm-m
9	1.93 ± 0.28	6.77 ± 0.25	38.72 ± 3.74	sm-m
10	1.86 ± 0.26	6.52 ± 0.20	46.18 ± 2.08	m
11	1.69 ± 0.25	5.86 ± 0.51	38.70 ± 4.04	sm-m
12	1.58 ± 0.22	5.62 ± 0.48	45.30 ± 1.21	m
13	1.47 ± 0.14	5.18 ± 0.58	30.72 ± 5.19	sm
14	1.16 ± 0.17	4.07 ± 0.51	43.22 ± 4.12	m

m – metacentric; sm – submetacentric chromosomes

The chromosomes are small, measuring from 1.16 to 2.74 µm (Table 1). No differences were found between the lengths of colchicine-treated and untreated chromosomes. The mean of the total length of haploid set (TCL) was 28.65 µm. The length of individual pairs decreased gradually and the differences between neighbouring pairs were insignificant (Table 1). Homologues of the smallest pair 14 were sometimes situated one alongside the other. According to the centromeric index, all pairs were biarmed, classified as metacentric and submetacentric (n = 7m + 3sm-m + 4sm, Table 1).

Discussion

Within the order Nippotaeniidea, no data on chromosomes have been available to date. Scarce information exists also on the life-cycle and genetic characteristics of this cestode group (Demschin, 1985; Olson, Caira, 1999; Olson *et al.*, 2001). The most recent phylogenetic studies based on molecular, morphological, ontogenetic and ultrastructural data concur that monofossate nippotaeniids have evolved together with tetrafossate groups of higher tapeworms and are highly derived (Beveridge, 2001; Hoberg *et al.*, 2001; Mariaux, Olson, 2001). Unequivocal support was found for the placement of the orders Cyclophyllidea, Nippoteaniidea and Tetrabothriidea into one clade which is called as higher acetabulates (Olson *et al.*, 2001). Among cyclophyllideans, the unique family Mesocestoididae has been excluded from Cyclophyllidea in some analyses, being closely clustered either together with tetrabothriids or nippotaeniids (Olson *et al.*, 2001).

Out of the above cestode groups, chromosome numbers are known in 58 cyclophyllideans including one species of the genus *Mesocestoides* (for review see Petkevičiuté, 2002). The chromosome number of the studied Cyclophyllidea species varies from 2n = 6 in *Microsomacanthus* species (Hymenolepididae) (Petkevičiuté, Regel, 1994) to 28 in *Nematotaenia dispar* (Nematotaeniidae) (Vijayaraghavan, Subramanyam, 1980); the most frequent numbers are 12, 16 and 18. *Nematotaenia dispar* has long time been the only cestode possessing, similarly as *N. mogurndae*, 14 chromosome pairs. However, data on the morphology of chromosomes are lacking in this species and chromosome morphology is well described only in less than 30 cyclophyllidean tapeworms. A majority of these taxa have relatively small chromosomes up to 5 µm long; regarding the centromere location, biarmed chromosomes predominate. In comparison, tapeworms belonging to the most primitive groups (e.g. Caryophyllidea and Spathebothriidea) and majority of trematodes posses longer chromosomes up to 7 – 15 µm (Baršiené, 1993; Petkevičiuté, 2002).

The only karyological analysis of *Mesocestoides vogae* (syn. *M. corti*) revealed the diploid chromosome number to be 14 metacentric or submetacentric chromosomes 0.5 – 2 µm long (Raghunathan, Voge, 1974). Considering a close phylogenetic relationship between mesocestoidean (2n = 14, small biarmed chromosomes) and nippotaeniid (2n = 28, small biarmed chromosomes) cestodes, the two-fold chromosome number and similar morphology evoke speculations that processes of polyploidy played a role in the origin of *N. mogurndae* karyotype in the course of evolution. Multiplication of chromosome number represents, indeed, a frequent route in speciation of plants and sometimes also in animals (White, 1973). Hypothetically, species with higher number of chromosomes are considered to have a better chance of adaptation to unfavourable and unstable conditions due to the higher combinatorial gene variability (Petkevičiuté, Ieshko, 1991). Indeed, the recent invasion of *N. mogurndae* to the Europe manifests a good adaptative potential of this parasite.

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