A cytogenetic study on the rodent tapeworm *Rodentolepis myoxi*

J.C. Casanova^{1*}, M. Spakulová² and N. Laplana¹

¹Laboratori de Parasitologia, Facultat de Farmàcia, Universitat de Barcelona, Avda Diagonal s/n, 08028 Barcelona, Spain: ²Parasitological Institute SAS, Hlinkova 3, 04001 Košice, Slovak Republic

Abstract

The karyotype of glirid tapeworm *Rodentolepis myoxi* (Rudolphi, 1819) (Cestoda: Hymenolepididae) comprises six pairs of small bi-armed chromosomes (2n=12). All pairs of chromosomes possess uniform morphology, i.e. metacentric, submetacentric or meta-submetacentric types of structures. The formula of the karyotype structure is n=2m + 1m-sm + 3sm. The absolute chromosome length ranges from 3.78 to $2.00 \ \mu m$. The mean total length of the haploid complement is $15.98 \ \mu m$. The first pair (group A) is the largest, pairs 2 and 3 can be grouped into group B while pairs 4-6 are smaller and can be classified as group C. The number of chromosomes of *R. myoxi* is the same for the congeneric species, however, karyological characteristics differ from all recently known karyotypes of rodent hymenolepidids.

Introduction

Major problems exist in the taxonomy and systematics of the rodent hymenolepidids both at generic and species levels. A tapeworm, originally described as Taenia myoxi Rudolphi, 1819 from the rodent genus Myoxus was subsequently transferred into various genera, such as Hymenolepis, Dicranotaenia, Armadolepis and Staphylocystis (Janicki, 1904; 1906; Baer, 1932; Lopez-Neyra, 1942; Spaskii, 1950; Spasskii, 1954; Pojmanska & Czaplinski, 1998). Two other rodent cestodes were recognized as synonyms of R. myoxi: Armadolepis spasskyi (Tenora & Barus, 1958) and Taenia (Hymenolepis) sulcata von Linstow, 1879 (Yamaguti, 1959; Vaucher & Quentin, 1975). To date, R. myoxi has been identified from the rodent hosts Myoxus glis, M. xilensis, Glis glis, Dryomis nitedula and Eliomys quercinus (Gliridae), Rattus rattus alexandrinus (Muridae) and Clethrionomys glareolus (Arvicolidae). At present, R. myoxi is the only armed hymenolepidid species parasitic in European glirids.

There are no data on additional characteristics of this morphologically distinct species and thus, studies on the chromosomes of *R. myoxi* are presented here.

Materials and methods

Adult cestodes *R. myoxi* were collected from *Eliomys quercinus* in the Pyrenean Mountains (northeastern Spain and southern France) and Sierra de Gredos (central Spain) during 1995–1996. Cestodes were fixed in 70% ethanol, stained in iron acetocarmine according to the method of Georgiev *et al.* (1986) and mounted in Canada balsam. All morphological features of *R. myoxi* analysed were similar to those previously described for this species.

Three cestodes were processed cytogenetically following the method of Petkeviciuté & Ieshko (1991) with modifications published by Spakulová & Casanova (1998). Karyological analysis was carried out on photographs of 54 well-spread gametogonial and embryonic mitotic metaphase plates. Metric characteristics (absolute and relative length and cetromeric index) were calculated from measurements of ten best spreads. A statistical comparison of metrical characteristics among individual chromosome pairs was made using analysis of variance (P < 0.01). The classification of the chromosome pairs followed that of Levan *et al.* (1964). For fitting our data to the system of Levan *et al.* (1964), we used a mean centromeric index \pm SD.

Results

* Fax: 934024504 E-mail: casanova@farmacia.far.ub.es Mitotic cell divisions were most often found in developing embryos. The diploid number of 98% of

Chromosome number	Absolute length (μm)	Relative length (%)	Centromeric index	Classification
1	3.78 ± 0.36	23.55 ± 1.09	32.97 ± 3.47	sm
2	2.99 ± 0.56	18.74 ± 0.73	46.59 ± 2.96	m
3	2.77 ± 0.56	17.33 ± 1.09	29.19 ± 3.68	sm
4	2.33 ± 0.46	14.61 ± 1.18	37.84 ± 4.17	m-sm
5	2.11 ± 0.40	13.24 ± 0.69	29.60 ± 2.35	sm
6	2.00 ± 0.40	12.52 ± 0.72	43.86 ± 3.11	m

Table 1. Measurements (means \pm SD) and classification of chromosomes of *Rodentolepis myoxi*.

Abbreviations: m, metacentric chromosomes; sm, submetacentric chromosomes.

analysed cells was 2n = 12 and 1 cell was an uploid. Mean karyometrical data are reported in table 1. The karyotype was composed of six pairs (figs 1, 2), of which the first pair was the largest (group A), pairs 2 and 3 were of similar length (group B) and pairs 4-6 were small, gradually decreasing in length and classified as group C (P < 0.01). Pairs 1, 3 and 5 had submetacentric type of structure while pairs 2 and 6 were metacentric. Pair 4 was intermediate between the metacentric and submetacentric types. Pair 2 was characterized by a small unstained pericentromeric region. The formula of the karyotype structure was n=2m+1m-sm+3sm. The mean total length of the haploid complement was 15.98 μ m. The most clearly defined chromosome structure was seen in the prometaphase and metaphase chromosomes of the first cleavage of eggs. Meiotic cells at the stage of diplotene-diakinesis were found sporadically and spreads of 6 bivalents within primary oocytes were seldom observed.

Discussion

Spasskii (1954) erected the genus Armadolepis for one species, the former Staphylocystis myoxi according to

Spasskii, 1950 or Dicranotaenia myoxi according to Lopez-Neyra (1942) and Skryabin & Matevosyan (1948). As its main differentiating feature, the presence of minute hooks on the suckers, has not been confirmed subsequently, Czaplinski & Vaucher (1994) synonymized Armadolepis with Rodentolepis Spasskii, 1954. This genus comprises rodent parasites characterized by a retractile armed rostellum and the mature proglottid with three testes in an elongated triangle, separated into two groups by the female gonads. However, more recently Pojmanska & Czaplinski (1998) proposed to transfer R. myoxi back into the genus Staphylocystis. The reason for this suggestion was stated: 'some features of this species resemble Staphylocystis Villot, 1877, rather than Rodentolepis. They are: rostellar hooks with rather short guards, testes arranged in triangle, and not forming two separate groups.' Nevertheless, these authors suggested that further studies are needed to resolve this problem and thus we retain the name Rodentolepis myoxi.

Considering the current information on karyotypes of *Rodentolepis* species, all of them possess the same diploid number of elements (2n=12). Conformally, all karyologically studied hymenolepidids except the genus *Microsomacanthus* possess six pairs of homologues (for review see Petkeviciute & Regel, 1994; Spakulova &

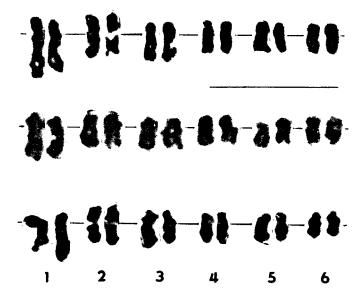


Fig. 1. Diploid sets of three mitotic cells of *Rodentolepis myoxi*. Bar = $10 \,\mu$ m.

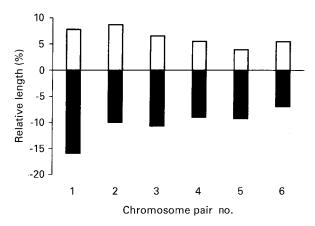


Fig. 2. Idiogram of Rodentolepis myoxi chromosomes.

Casanova, 1998). Characteristics of the chromosome morphology were revealed in only six species of the genus Hymenolepis s. l.: Rodentolepis microstoma (Profitt & Jones, 1969), Hymenolepis citelli (Ward et al., 1981), Hymenolepis diminuta (Liu & Lin, 1987; Mutafova & Gergova, 1994), Rodentolepis nana and Rodentolepis erinacei (Mutafova & Gergova, 1994) and Rodentolepis straminea (Spakulova & Casanova, 1998). The morphology of the chromosomes differed largely in individual species. In fact, R. microstoma, H. citelli and R. erinacei were characterized by telocentric chromosomes. Rodentolepis nana from spontaneously infected mice and H. diminuta possessed five pairs of telocentrics and one pair of metasubmetacentric structure according to Mutafova & Gergova (1994). Interestingly, Liu & Lin (1987) reported a different morphology for the H. diminuta chromosome set with predominating meta- and submetacentric chromosomes. Mutafova & Gergova (1994) explained this discrepancy in terms of variability between local populations. However, the classical taxonomy in Hymenolepis s. l. tapeworms is still a problem and the range of morphological variability of most species is not ascertained. Therefore, the tapeworms studied could hypothetically belong to different species. Unfortunately, the scarcity of karyological studies of tapeworm species and particularly various populations of a single species has not confirmed the possibility of intraspecific variability in cestode karyotypes. Rodentolepis straminea, which has recently been studied by Spakulova & Casanova (1998), is also mainly characterized by biarmed chromosomes, i.e. four metacentric or submetacentric and two subtelocentric pairs. The present results indicate that the karyotype of R. myoxi is composed only of meta- or submetacentric chromosomes. Conflicting hypotheses were proposed concerning the relationship between karyotype symmetry and the degree of speciation in various plant and animal groups. Generally, a hypothesis proposed by White (1973), suggesting that more evolved species possess symmetrical karyotypes with less number of biarmed, mostly metacentric chromosomes, has often been assumed in studies on several helminth families and genera (e.g. Grossman & Cain, 1981; Barsiene, 1993; Mutafova, 1994; Petkeviciute & Regel, 1994; Petkeviciute

et al., 1995; Bell *et al.*, 1998). According to the high degree of symmetry of the *R. myoxi* karyotype, this species could be considered a young member of the genus. However, an assessment of the likely route for chromosome evolution in karyotypically conservative groups with equal numbers of chromosomes can only be speculative, and therefore further detailed analyses of other congeneric species are required.

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References

- Baer, J.G. (1932) Contribution a la faune helmintologique de Suisse, II. *Revue Suisse de Zoologie* 39, 1–56.
- Barsiene, J. (1993) *The karyotypes of trematodes*. 370 pp. Vilnius, Academia (in Russian).
- Bell, A.S., Sommerville, C. & Gibson, D.I. (1998) Karyological studies on three strigeid digeneans: *Ichthyocotylurus erraticus* (Rudolphi, 1809), *I. variegatus* (Creplin, 1825) and *Apatemon gracilis* (Rudolphi, 1819). *Systematic Parasitology* 41, 169–178.
- Czaplinski, B. & Vaucher, C. (1994) Family Hymenolepididae Ariola, 1899. pp. 595–664 in Khalil, L.F., Jones, A. & Bray, R.A. (Eds) Keys to the cestode parasites of vertebrates. Wallingford, UK, CAB International.
- Georgiev, B., Biserkov, V. & Genov, T. (1986) In toto staining for cestodes with iron acetocarmine. *Helminthologia* 23, 279–281.
- Grossman, A.I. & Cain, G.D. (1981) Karyotype and chromosome morphologies of *Megalodiscus temperatus* and *Philophthalmus gralli*. Journal of Helminthology 55, 71– 78.
- Janicki, C. (1904) Zur Kenntnis einiger Saugetiercestoden. Zoologischer Anzeiger 27, 770–782.
- Janicki, C. (1906) Studien an Saugetiercestoden. Zeitschrift für Wissenschaftliche Zoologie 81, 505–597.
- Levan, A., Fredga, K. & Sandberg, A.A. (1964) Nomenclature for centromeric position on chromosomes. *Hereditas* 52, 201–220.
- Liu, G. & Lin, H. (1987) Studies on the cytogenetics of Cestoda I. The karyotype of *Hymenolepis diminuta*. *Hereditas* (*Beijing*) 9, 26–27 (in Chinese).
- Lopez-Neyra, C.R. (1942) Revision del genero *Hymenolepis* Weinland. *Revista Ibérica de Parasitología* 2, 113–256.
- Mutafova, T. (1994) Karyological studies on some species of the families Echinostomatidae and Plagiorchiidae and aspects on chromosome evolution in trematodes. *Systematic Parasitology* 28, 229–238.
- Mutafova, T. & Gergova, S. (1994) Cytological studies on three hymenolepidid species. *Journal of Helminthology* 68, 323–325.
- Petkeviciuté, R. & Ieshko, E.P. (1991) The karyotypes of Triaenophorus nodulosus and T. crassus (Cestoda: Pseudophyllidea). International Journal for Parasitology 21, 11–15.
- Petkeviciuté, R. & Regel, K.V. (1994) Karyometrical analysis

of Microsomacanthus spasskii and M. spiralibursata. Journal of Helminthology **68**, 53–55.

- Petkeviciuté, R., Staneviciuté, G. & Kiseliené, V. (1995) Mitotic chromosomes of Sphaerostomum bramae (Müller, 1776) Szidat, 1944. Journal of Helminthology 69, 347–349.
- Pojmanska, T. & Czaplinski, B. (1998) Some further proposition of new generic combinations or emendations for tapeworms recorded in Poland. Acta Parasitologica 46, 36–38.
- Proffitt, M.R. & Jones, A.W. (1969) Chromosome analysis of Hymenolepis microstoma. Experimental Parasitology 25, 72– 84.
- Skryabin, K.I. & Matevosyan, E.N. (1948) Hymenolepidids of mammals. *Trudy Gelmintologicheskoi Laboratorii AN* SSSR 1, 15–92.
- Špakulová, M. & Casanova, J.C. (1998) Chromosome analysis of *Rodentolepis straminea* (Cestoda: Hymenolepididae) parasitizing wood mice (*Apodemus* spp.) in Spain. *Helminthologia* 35, 185–188.
- Spaskii, A.A. (1950) New data on morphology and systematics of hymenolepidids (Cestoda: Hymenolepididae).

Doklady Akademii Nauk SSSR, Seria Biologija **75**, 895–898.

- Spasskii, A.A. (1954) Classification of hymenolepidids of mammals. *Trudy Gelmintologicheskoi Laboratorii AN SSSR* 7, 120–167.
- Vaucher, C. & Quentin, J.C. (1975) Présence du cysticercoide de Hymenolepis myoxi (Rud., 1819) ches la puce du lerot et redescription du ver adulte. Bulletin de la Societé Neuchateloise des Sciences Naturelles 98, 27–34.
- Ward, E.J., Evans, W.S. & Novak, M. (1981) Karyotype of Hymenolepis citelli (Cestoda: Cyclophyllidea). Canadian Journal of Genetics and Cytology 23, 449–452.
- White, M.J.D. (1973) Animal cytology and evolution. London, Cambridge University Press.
- Yamaguti, S. (1959) Systema helminthum. Vol. II. The cestodes of vertebrates. New York and London, Interscience Publishers.

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