A KARYOLOGICAL STUDY OF THE SPIRURID NEMATODE *MASTOPHORUS MURIS* (NEMATODA: SPIROCERCIDAE)

ŠPAKULOVÁ M.*, CASANOVA J.C.** , LAPLANA GUILLÉN N.** & KRÁL'OVÁ I.*

Summary :

The karyotype of *Mastophorus muris* (Gmelin, 1790) comprises four pairs of small autosomal chromosomes and two larger sex X chromosomes in females or one X chromosome in males ($2n = 8 + XX/XO$). All pairs of chromosomes possess rather uniform morphology without distinct primary or secondary constrictions. No heterochromatin bands were found by C-banding analysis. The absolute chromosome length ranges from 4.02 to 2.24 μm . The mean total length of the haploid complement is 14.34 μm . The course of gametogenesis represents a typical pattern common in the order Spirurida. The recently available karyotypes of spirurid nematodes have been reviewed.

KEY WORDS : Nematoda, *Mastophorus muris*, Spirurida, karyotype, chromosomes, *Mus musculus*.

Résumé : ÉTUDE DU CARYOTYPE DU NÉMATODE *MASTOPHORUS MURIS* (NEMATODA: SPIROCERCIDAE)

Le caryotype de *Mastophorus muris* (Gmelin, 1790) comprend quatre paires de courts chromosomes autosomaux et deux chromosomes X chez la femelle, ou un X chez le mâle ($2n = 8 + XX/XO$). Toutes les paires de chromosomes possèdent à peu près la même morphologie, sans constriction primaire ou secondaire. L'analyse par C-banding n'a pas permis de révéler la présence de bande d'hétérochromatine. La longueur totale du chromosome est comprise entre 4,02 et 2,24 μm . La plus grande longueur totale du chromosome haploïde est de 14,34 μm . Le cycle de la gamétogenèse représente un modèle commun dans l'ordre des Spirurida. Le caryotype d'autres spirurid nématodes a été revu.

MOTS CLÉS : Nematoda, *Mastophorus muris*, Spirurida, caryotype, chromosomes, *Mus musculus*.

INTRODUCTION

Mastophorus muris (Gmelin, 1790) is the cosmopolitan spirurid nematode parasitizing stomach of small rodents, often in associations with synantropic mice populations (Yamaguti, 1961, Genov 1984). It was originally described as *Ascaris muris* Gmelin, 1790 and was later combined with the genus *Mastophorus* by Chitwood (1938). This species was well characterized by its morphology and biology (Skryabin & Sobolev, 1963). Since its systematic status changed several times, the present paper adopts the classification of the Nematoda published by Anderson *et al.* (1974-1983). The only information on the chromosome number of *M. muris* was published by Walton (1924 ex Walton, 1974), as the part of the tabular review: $2n = 9-10$, $X = 1$. As no other information has been available so far, the aim of this work is to describe the chromosome complement of both sexes and visible events of gametogenesis of *M. muris*, and to compare available karyological data on other nematodes of the order Spirurida.

MATERIALS AND METHODS

Nematodes *M. muris* were recovered from stomachs of two mice *Mus musculus* L. collected in the farm Les Franqueses near Granollers (Spain) in July 15, 1998. Immediately after dissection, six males and nine females were treated with colchicine (0,5mg.ml⁻¹) in physiological saline for 30 min at 37° C. Then the worms were subjected to a hypotonic treatment (distilled water, 60 min), processed according to the air-drying method and stained by conventional Giemsa and C-banding methods as described by Špakulová *et al.* (1994). Karyological analysis of mitotic cells was carried out according to photographs of 30 well-spread spermatogonial plates and 24 oogonial plates. Metric characteristics (absolute and relative lengths) were calculated from the measuring of the 10 best metaphase spreads.

RESULTS

A chromosomal complement consisted of nine elements in males (Fig. 1A-C) and 10 in females (Fig. 2A, B). The sex was determined by two XX chromosomes in female and one X chromosome in male ($2n = 8 + XX/XO$). The sex chromosome X was the largest one out of all and measured $4.02 \pm 0.92 \mu\text{m}$. The absolute lengths of autosomal pairs were $2.98 \pm$

* Parasitological Institute SAS, Hlinkova 3, 04001 Košice, Slovak Republic.

** Laboratori de Parasitologia, Facultat de Farmacia, Universitat de Barcelona, Avda Diagonal s/n, 08028 Barcelona, Spain.

Correspondence: Marta Špakulová.

Tel.: +421 95 6334455 – Fax: +421 95 6331414.

E-mail: spakulma@saske.sk

Fig. 1 – Chromosomes of *Mastophorus muris* male. A-C, three spermatogonial cells in a mitotic division. A, prometaphase, C-banding. B, C, metaphase. D, primary spermatocyte in the first meiotic metaphase. Complement comprising four bivalents and one univalent sex chromosome X. E, primary spermatocyte in the first meiotic anaphase. Univalent sex X chromosome located between two haploid sets of autosome chromosomes. F, two secondary spermatocytes comprising haploid sets of four ($n = 4$, down) and five ($n = 4 + X$, upward) chromosomes. Sex chromosome X arrowed. B-F Giemsa staining. Scale bar 10 μ m.

Fig. 2 – Chromosomes of *Mastophorus muris* female. A-B, two oogonial cells in mitotic prometaphase (A) and metaphase (B). C, oocyte in meiotic metaphase II. Chromosomes X arrowed. A-C Giemsa staining. Scale bar 10 μ m.

0.55 μm , $2.65 \pm 0.45 \mu\text{m}$, $2.45 \pm 0.49 \mu\text{m}$ and $2.24 \pm 0.43 \mu\text{m}$. A gross chromosome morphology was better remarkable in the early metaphase cells (Figs 1A, B, 2A) than in the late metaphase cells (Figs 1C, 2B). All pairs of chromosomes possessed rather uniform morphology without distinct primary or secondary constrictions and were classified according to their length. C-banding analysis revealed no heterochromatin bands (Fig. 2A).

A number of spermatocytes (Fig. 1D-F) were found in males being in various phases of meiotic division, predominantly in the metaphases I and II. In females, oocytes in the metaphase II were rarely observed containing five bar-form chromosomes (Fig. 2C). The subsequent stages of cleavage were not observed.

DISCUSSION

Walton (1959) showed the haploid chromosome number of *M. muris* to be $n = 5$. Later, he specified the data more accurately ($2n = 9-10$, $X = 1$) and published the figure of two secondary spermatocytes with haploid sets of four and five chromosomes (Walton, 1974). No information on the gross morphology of mitotic male and female complements has been available so far.

Present results correspond well with the above data on the sex-determining mechanism XX in females and XO in males. This fact has been proved now by the evaluation of gametogonial mitotic divisions in both sexes. Dividing oogonial cells comprised 10 chromosomes while spermatogonial cells nine chromosomes. The heterochromosome X was the largest of all and measured up to 4.02 μm .

In our study, mitotic chromosomes neither showed clear primary constrictions in metaphase nor the pericentromeric heterochromatin. The similar rod-shaped mitotic chromosomes were found in many other nematode species belonging to the subclass Secernentea. The holokinetic chromosome structure was anticipated in the majority of them (e.g. Walton, 1974; Triantaphyllou, 1983; Král, 1994). However, diffuse or polycentric centromere was proved in only several nematode species using ultrastructural, immunocytochemical and other special methods studying kinetochore structure (for review see Špakulová *et al.*, 1995).

Within the order Spirurida, mitotic chromosomes showing no clear secondary constrictions have been reported in several species: *Brugia pahangi* and *B. malayi* (Sakaguchi *et al.*, 1983), *Dirofilaria immitis* (Sakaguchi *et al.*, 1980), *Gongylonema pulchrum* (Valero *et al.*, 1982), *Physaloptera clausa* (Mutafova, 1995), *Onchocerca volvulus* and *O. gutturosa* (Hirai *et al.*, 1985, 1987; Procunier & Hirai, 1986) and *Thelazia callipaeda* (Sakaguchi & Kihara, 1984).

However, this feature was interpreted in the different ways. Some authors did not comment the chromosomal

structure at all (Valero *et al.*, 1982; Hirai *et al.*, 1985). In some other cases, the monocentric character of chromosomes was supposed and the location of centromere was inferred from the formation of bivalents during meiotic prophase (Sakaguchi *et al.*, 1980; Sakaguchi & Kihara, 1984; Procunier & Hirai, 1986). Finally, Goswami (1974), Podgornova *et al.* (1979) and Sakaguchi *et al.* (1983) discussed the possibility of the holokinetic character of chromosomes in the members of the genera *Camallanus*, *Setaria*, *Cystidicola* and *Brugia*. According to the *M. muris* karyotype, the peculiarities of mitotic and meiotic chromosomes determined by a light microscopy enable us to anticipate their hypothetic holokinetic structure. Thus, the majority of mitotic chromosomes have a rod-shaped structure without a clear location of centromere. During meiotic divisions, bivalents behave as telocentrics. Similar features were demonstrated also in above mentioned spirurid species and some other oxyurid nematodes (e.g. Mutafova & Vasilev, 1987; Špakulová *et al.*, 1994; Mutafova, 1995). However, further study is required for the decision on the character of attaching of mitotic chromosomes to the spindle fibers.

Recently available karyological data on the spirurid nematode species (Table I), showed that their karyotypes are composed predominantly of 10 (sixteen species) or 12 chromosomes (thirteen species). According to Triantaphyllou (1983), the same numbers of elements characterised also most species of Strongylida and Oxyurida, whereas the diploid number $2n = 12$ and XX/XO mechanism of sex determination is the most widespread among all nematodes.

There is a certain consistency of the type of the chromosomal sex determination in spirurid species comprising various genera. The heterogametic male configuration of sex pair occurring in spirurids is either XO or XY, and the former type was considered to be evolutionary older (Triantaphyllou, 1983). For instance, males of phylogenetically older genera of the family Onchocercidae (including *Litosomoides*) have XO sex pair while more advanced genera have XY (e.g. *Brugia* and *Onchocerca*, Table I). Phylogenetic relationships of these spirurid worms have been assessed according to rRNA sequence comparison (Gill *et al.*, 1988). Concerning higher taxonomic categories, both XO and XY configurations have been found within the phylogenetically older families (Thelasiidae and Gongylonematidae) as well as within the younger ones (Onchocercidae) (see Table I). The elucidation of the role of Y chromosome in the sex determination in nematodes has recently been started by the identification of the TOY locus specifically associated with the Y chromosome of *Brugia malayi* (Underwood & Bianco, 1999). Unfortunately, karyological data of the spirurid nematodes are still rather scarce and insufficient for assessing of pathways of karyotype evolution within this systematic group or the phylogenetic relations in general.

Superfamily, Family	Species	Chromosome No. + sex pair ♀/♂	References
Camallanoidea, Camallanidae	<i>Camallanus baylisi</i>	2n = 8 + XX/X0*	Goswami, 1974
	<i>Camallanus lacustris</i>	2n = 12	Walton, 1959, 1974
Gnathostomatoidea, Gnathostomatidae	<i>Ancyracanthus cistidicola</i>	n = 6/5 (XX/X0)**	Mulsow, 1912 ex Terry <i>et al.</i> , 1961
Physalopteroidea, Physalopteroidae	<i>Physaloptera clausa</i>	n = 5/4 (XX/X0)	Mutafova, 1995
	<i>Physaloptera turgida</i>	2n = 8 + XX/X0	Walton, 1959, 1974
	<i>Proleptus robustus</i>	2n = 16	Walton, 1959, 1974
Thelazioidea, Thelaziidae	<i>Thelazia callipaeda</i>	2n = 6 + XX/XY	Sakaguchi & Kihara, 1984
Spiruroidea, Gongylonematidae	<i>Gongylonema pulchrum</i>	2n = 8 + XX/XY	Valero <i>et al.</i> , 1982
Spiruridae	<i>Spirura talpae</i>	2n = 16	Walton, 1959, 1974
Spiroceridae	<i>Mastophorus muris</i>	2n = 8 + XX/X0	Walton, 1959, 1974, present results
Habronematoidea, Cystidicolidae	<i>Cystidicola cristiwomeri</i>	n = 6/5	Boyes & Anderson, 1961
	<i>Cystidicola farionis</i>	2n = 10 + XX/X0	Walton, 1959, 1974, Podgornova <i>et al.</i> , 1979
	<i>Cystidicola stigmatura</i>	n = 6/5 (XX/X0)	Boyes & Anderson, 1961
Acuarioidea, Acuriidae	<i>Synbimathus (Dispharynx) spiralis</i>	2n = 10 + XX/X0	Walton, 1959, 1974
Filarioidea, Onchocercidae	<i>Setaria cervi</i>	2n = 10 + XX/X0	Podgornova <i>et al.</i> , 1979
	<i>Setaria digitata</i>	2n = 10 + XX/X0	Sakaguchi <i>et al.</i> , 1980
	<i>Setaria equina</i> (syn. <i>Filaria papillosa</i>)	2n = 10 + XX/X0	Meves, 1915 ex Walton, 1959, 1974, Podgornova <i>et al.</i> , 1979
	<i>Setaria labiato-papillosa</i>	2n = 4 + XX/X0 or XY (6-14)	Podgornova <i>et al.</i> , 1979
	<i>Loa loa</i>	2n = 10 + XX/X0	Post & Pinder, 1995
	<i>Foleyella agamae</i>	2n = 4, 6, 8	Obiamiwe <i>et al.</i> , 1995
	<i>Dirofilaria immitis</i>	n = 5/4 2n = 8 + XX/XY	Taylor, 1960 Sakaguchi <i>et al.</i> , 1980, Delves <i>et al.</i> , 1986
	<i>Litosomoides carinii</i>	n = 5/4	Taylor, 1960
	<i>Dipetalonema witei</i>	n = 6/5 (XX/X0)	Terry <i>et al.</i> , 1961
	<i>Brugia malayi</i>	2n = 8 + XX/XY	Sakaguchi <i>et al.</i> , 1993
	<i>Brugia pabangi</i>	2n = 8 + XX/XY	Sakaguchi <i>et al.</i> , 1983, Delves <i>et al.</i> , 1989
	<i>Wuchereria bancrofti</i>	n = 5	Miller, 1966
	<i>Onchocerca armillata</i>	2n = 8 + XX/XY	Post <i>et al.</i> , 1989
	<i>Onchocerca dukei</i>	n = 5 (XX/XY)	Post <i>et al.</i> , 1991
	<i>Onchocerca gibsoni</i>	2n = 6 + XX/XY + B	Post <i>et al.</i> , 1989
	<i>Onchocerca gutturosa</i>	2n = 8 + XX/XY	Post <i>et al.</i> , 1989, Hirai <i>et al.</i> , 1985
	<i>Onchocerca linealis</i>	2n = 8 + XX/XY	Post <i>et al.</i> , 1989
	<i>Onchocerca ochengi</i>	2n = 8 + XX/XY	Post <i>et al.</i> , 1989
	<i>Onchocerca tarsicola</i>	n = 3 or 5	Post <i>et al.</i> , 1991
	<i>Onchocerca volvulus</i>	n = 2 2n = 8 + B	Salazar <i>et al.</i> , 1962 Miller, 1966, reinterpreted by Procunier & Hirai, 1986
		2n = 6 + XX/XY + B	Basáñez <i>et al.</i> , 1983, reinterpreted by Procunier & Hirai, 1986, Post <i>et al.</i> , 1989
		2n = 6 + XX/XY	Hirai <i>et al.</i> , 1985, 1987; Procunier & Hirai, 1986

Notes: * diploid number and sex-pair reported; ** haploid number reported and sex-pair inferred.

Table I. – A review of karyological characteristics of species of the order Spirurida.

ACKNOWLEDGEMENTS

This work was supported by the projects PB96-0401-CO2-01 of the DGICYT of Spain and the Commissionat per Universitats i Recerca (1996 SGR 0003) of the Government of Catalonia (Spain), by the project No. 2/5018/98 of the Science Grant Agency VEGA and the Agency Literárny Fond of the Slovak Republic.

REFERENCES

- ANDERSON R.C., CHABAUD A.G. & WILLMOTT S. (eds.): C.I.H. Keys to the Nematode Parasites of Vertebrates. Nos. 1-10. CAB, Farnham Royal, Bucks, England, 1974-1983.
- BÁSÁÑEZ M.G., BOTTO C. & YARZABAL L. Características du un deme de *Onchocerca volvulus* s.l. del alto Orinoco (Territorio Federal Amazonas, Venezuela). Estudio citogené-

- tico preliminar, in: Las filarias humanas en la Territorio Federal Amazonas (Venezuela). Proicet Amazonas (ed.), Caracas Público Científico, 1983, 2, 79-82.
- BOYES J.W. & ANDERSON R.C. Meiotic chromosomes of *Cystidicola stigmatura* and *C. cristivomeri* (Nematoda: Spiruroidea). *Canadian Journal of Genetics and Cytology*, 1961, 3, 231-236.
- CHITWOOD B.G. The status of *Protospirura mastophorus*, with a consideration of the species of these genera, in: Livro Jubilar do prof. L. Travassos. Rio de Janeiro, 1938, 115-118.
- DELVES C.J., HOWELLS R.E. & POST R.J. Gametogenesis and fertilization in *Dirofilaria immitis* (Nematoda: Filarioidea). *Parasitology*, 1986, 92, 181-197.
- DELVES C.J., REES H.H. & HOVELLS R.E. Egg production in *Brugia pabangi* (Nematoda: Filarioidea). *Parasitology*, 1989, 98, 105-113.
- GENOV T. Helminths of Insectivorous Mammals and Rodents in Bulgaria. Izdatelstvo BAN, Sofia, 1984, pp. 347.
- GILL L.L., HARDMAN N., CHAPPELL L., QU L.H., NICOLOSO M. & BACHELLERIE J.-P. Phylogeny of *Onchocerca volvulus* and related species deduced from rRNA sequence comparisons. *Molecular and Biochemical Parasitology*, 1988, 28, 69-76.
- GOSWAMI U. An unequal pair of chromosomes in *Camallanus baylisi* (Nematoda). *Cytologia*, 1974, 39, 321-325.
- HIRAI H., SAKAGUCHI Y. & TADA I. Chromosomes of *Onchocerca volvulus* and *O. gutturosa*. *Zeitschrift für Parasitenkunde*, 1985, 71, 135-139.
- HIRAI H., TADA I., TAKAHASHI H., NWOKE B.E.B. & UFOMADA G.O. Chromosomes of *Onchocerca volvulus* (Spirurida: Onchocercidae): A comparative study between Nigeria and Guatemala. *Journal of Helminthology*, 1987, 61, 43-46.
- KRÁL J. Holokinetic chromosomes. *Biol. Listy, Praha*, 1994, 58, 191-217.
- MILLER M.J. Observations on spermatogenesis in *Onchocerca volvulus* and *Wuchereria bancrofti*. *Canadian Journal of Zoology*, 1966, 44, 1003-1006.
- MUTAFOVA T. & VASILEV I. Comparative karyological investigation of *Ascaridia columbae* (Gmelin, 1970) Travassos, 1913 and *Ascaridia compar* (Schränk, 1790) Travassos, 1913. *Kbelmintologia*, 1987, 23, 44-49.
- MUTAFOVA T. Meiosis and some aspects of cytological mechanisms of chromosomal sex determination in nematode species. *International Journal for Parasitology*, 1995, 25, 453-462.
- OBIAMIWE B.A., OHAGI I.E. & UGBOMOIKO U.S. Morphology and cytology study of *Foleyella agamae* complex (Nematoda: Filarinae) infecting the agamid lizard *Agama agama* in Nigeria. *Parasitology Research*, 1995, 81, 527-530.
- PODGORNOVA G.P., TROFIMENKO V.J., DMITRIEVA T.I., LOMAKIN V.V., SHLIKAS A.V. & SHOL V.A. On the karyology of some nematodes of the suborders Adenophorea and Secernentea. *Trudy gelmintologicheskoi laboratorii AN SSSR*, 1979, 29, 112-118.
- POST R.J. & PINDER M. Oogenesis and embryogenesis in *Loa loa*. *Journal of Helminthology*, 1995, 69, 351-356.
- POST R.J., BAIN O. & KLÄGER S. Chromosome numbers in *Onchocerca dukei* and *O. tarsicola*. *Journal of Helminthology*, 1991, 65, 208-210.
- POST R.J., MCCALL P.J., TREES A.J., DELVES C.J. & KOUYATE B. Chromosomes of six species of *Onchocerca* (Nematoda: Filarioidea). *Tropical Medicine and Parasitology*, 1989, 40, 292-294.
- PROCUNIER W.S. & HIRAI H. The chromosomes of *Onchocerca volvulus*. *Parasitology Today*, 1986, 2, 307-309.
- SAKAGUCHI Y. & KIHARA S. A karyotype study of the oriental eye worm, *Thelazia callipaeda* (Thelaziidae: Nematoda). *Japanese Journal of Parasitology*, 1984, 33, 221-223.
- SAKAGUCHI Y., KIHARA S. & TADA I. The chromosomes and gametogenesis of *Dirofilaria immitis*. *Japanese Journal of Parasitology*, 1980, 29, 377-381.
- SAKAGUCHI Y., TADA I., ASH L.R. & AOKI Y. Karyotypes of *Brugia pabangi* and *Brugia malayi* (Nematoda: Filarioidea). *Journal of Parasitology*, 1983, 69, 1090-1093.
- SALAZAR M.M., GOZÁLEZ D.B. & SÁMANO A. Cromosomas de *Onchocerca volvulus*. *Salud Pública Méx*, Epoca V4, 1962, 983-984.
- SKRYABIN K.I. & SOBOLEV A.A. Spirurids of Animals and Man and Diseases Causing by Them, in: Essentials of Nematology. Skryabin K.I. (ed.), Izdatelstvo AN SSSR, Moskva, 1963, p. 511.
- ŠPAKULOVÁ M., KRÁL'OVÁ I. & CUTILLAS C. Studies on the karyotype and gametogenesis in *Trichuris muris*. *Journal of Helminthology*, 1994, 68, 67-72.
- ŠPAKULOVÁ M., MUTAFOVA T. & KRÁL J. Cytogenetic study of *Heterakis gallinarum* (Nematoda, Heterakidae). *Biologia*, 1995, 50, 605-609.
- TAYLOR A.E.R. The spermatogenesis and embryology of *Litomosoides carinii* and *Dirofilaria immitis*. *Journal of Helminthology*, 1960, 34, 3-12.
- TERRY A., TERRY R.J. & WORMS M.J. *Dipetalonema witei*, filarial parasite of the jird, *Meriones libycus*. II. The reproductive system, gametogenesis and development of the microfilaria. *Journal for Parasitology*, 1961, 47, 703-711.
- TRIANTAPHYLLOU A.C. Cytogenetic aspects of nematode evolution, in: Concepts in Nematode Systematics. A.R. Stone, H.M. Platt & L.F. Khalil (eds.), Academic Press, London and New York, 1983, p. 55-71.
- UNDERWOOD A.P. & BIANCO A.E. Identification of a molecular marker for the Y chromosome of *Brugia malayi*. *Molecular and Biochemical Parasitology*, 1999, 99, 1-10.
- VALERO LÓPEZ A., ROMERO RODRÍGUEZ J., PRETEL MARTÍNEZ A. & GUEVARA BENÍTEZ D.C. Estudios cariologicos de *Gongylonema pulchrum* Molin, 1857. *Revista Ibérica de Parasitología*, 1982, 42, 33-43.
- WALTON A.C. Some parasites and their chromosomes. *Journal of Parasitology*, 1959, 45, 1-20.
- WALTON A.C. Gametogenesis, in: Introduction to Nematology. B.G. Chitwood & M.B. Chitwood (eds.), University Park Press, Baltimore, 1974, pp. 191-201.
- YAMAGUTI S. Systema Helminthum. Vol. III. The Nematodes of Vertebrates. Interscience Publishers, New York and London, 1961, p. 1261.

Reçu le 2 mars 2000
 Accepté le 30 juin 2000