DOI 10.2478/s11687-010-0012-0

HELMINTHOLOGIA, 47, 2: 69 – 75, 2010

A description of karyotype of the giant liver fluke *Fascioloides magna* (Trematoda, Platyhelminthes) and a review of Fasciolidae cytogenetics

M. REBLÁNOVÁ[1*], M. ŠPAKULOVÁ[1], M. OROSOVÁ[1, 2], E. BAZSALOVICSOVÁ[1], D. RAJSKÝ[3]

[1]Parasitological Institute of the Slovak Academy of Sciences, Hlinkova 3, 040 01, Košice, Slovak Republic, E-mail: *reblan@saske.sk*; [2]Institute of Parasitology, Biology Centre ASCR, Branišovská 31, 37005 České Budějovice, Czech Republic; [3]Faculty of Forestry, Technical University in Zvolen, T.G. Masaryka 20, 060 53 Zvolen, Slovakia

Summary

The study describes a karyotype of a common parasite of cervids, the giant liver fluke, *Fascioloides magna* (Trematoda, Platyhelminthes). The chromosome set of *F. magna* comprises 11 pairs of chromosomes, all classified as subtelocentric except for the submeta-metacentric pair No. 8 and the submetacentric pair No. 10 (2n = 22, n = 1sm + 1sm-m + 9st). The first longest pair is 4.65 μm long and the length decreases continuously to the 1.92 μm length of the last pair No. 11. No distinct secondary constriction has been observed in mitotic preparations. Fluorescent DAPI-staining reveals distinct heterochromatin bands on all 11 chromosome pairs in the centromeric regions; another DAPI-positive bands are localized at the end of the long arms of chromosomes No. 5 and the last less distinct signals appear interstitially on the long arms of the pair No. 6. Synchronous meiotic divisions of 8-spermatocyte groups have been observed during spermatogenesis, similarly with a development of spermatocytes in other trematodes. In the first two stages of heterotypic spermatocyte division, 11 bivalents (n = 11) are regularly observed, confirming the diploid number of 22 elements. Furthermore, the present analysis summarises and discusses available cytogenetic data on Fasciolidae flukes suitable for future studies on taxonomy or phylogenetic interrelationships within the family.

Keywords: fasciolid flukes; chromosome; fluorescent staining; heterochromatin

Introduction

Fascioloides magna (Bassi, 1875) (Fasciolidae) is a veterinary important liver fluke of a variety of wild and domestic ruminants, namely cervids, cattle, sheep, goats and a series of other hosts. It is generally accepted that the trematode has its original territory in the North America (Pybus, 2001), and was introduced to Europe along with its hosts, wapiti deer, in the 19th century (Bassi, 1875). Till now, the reports on occurrence of *F. magna* in Europe were documented from German-Polish border, Czech Republic, Spain, Slovakia, Hungary, Austria, and Croatia appeared (for review see Novobilský *et al*., 2007). *Fascioloides magna* is a single-species genus and differs from other fasciolid liver flukes by conspicuously larger body size being 80 – 100 mm long, 20 – 35 mm wide and 2.0 – 4.5 mm thick, as well as by other specific morphological traits (Špakulová *et al*., 2003). Its life cycle includes the aquatic snails of the family Lymnaeidae as intermediate hosts within which several generations of larvae develop by asexual multiplication. This intramolluscan period results in releasing of free-swimming cercariae which encapsulate on water or marsh plants and wait out to be grazed by final ruminant host (Erhardová-Kotrlá, 1971). The number and shape of chromosomes represent important characteristics of biological species. The first raw information on the chromosome number in *F. magna* (2n = 22) was published by Špakulová *et al*. (2003) but detailed characteristics of a chromosomal structure has not been published yet.

In this paper, we present the original description of chromosome set of the giant liver fluke assessed on the basis of mitotic divisions of spermatogonial cells isolated from fluke testes. A course of spermatogenesis, as inferred from meiotic divisions of spermatocytes, was studied as well. Besides, so far available cytogenetic data on Fasciolidae flukes are summarized and cytogenetic markers are discussed as valuable tool in future taxonomic and phylogenetic studies of the family.

Materials and methods

Parasites

Twelve living flukes, processed immediately after dissection of seven red deer (*Cervus elaphus*) livers, were used

for cytogenetic study. Red deer were hunted in Danube basin near the village Bodíky (southern Slovakia, Dunajská Streda district) through the years 2006-2008; one deer was shot near Beroun, central Bohemia, Czech Republic, in November 2008.

Chromosome preparations

Immediately after dissection, whole living flukes were placed into saline solution with 0.025 % colchicine for 1 – 4 hours by room temperature. Small portions of tissue, situated in the middle third of the body and containing parts of tubular testes, were dissected and processed according to the spreading method described by Fuková *et al.* (2005) with some modifications. Shortly, the small pieces of testes were incubated in a hypotonic solution of 75 mM KCl for 15 – 30 min, fixed in freshly prepared Carnoy (ethanol:chloroform:acetic acid 6:3:1) for 15 – 30 min. After fixation, samples were transferred into a drop of 60 % acetic acid on a slide and torn into fine pieces using tungsten needles. Then the slide with a drop of cell suspension was placed on a heating plate at 45 °C and fluid was slowly drawn along the slide until it evaporated. Preparations were screened in a phase contrast microscope, the best of them were dehydrated in ethanol series (70, 80 and 100 %, 1 min each) and kept at -20 °C until further use.

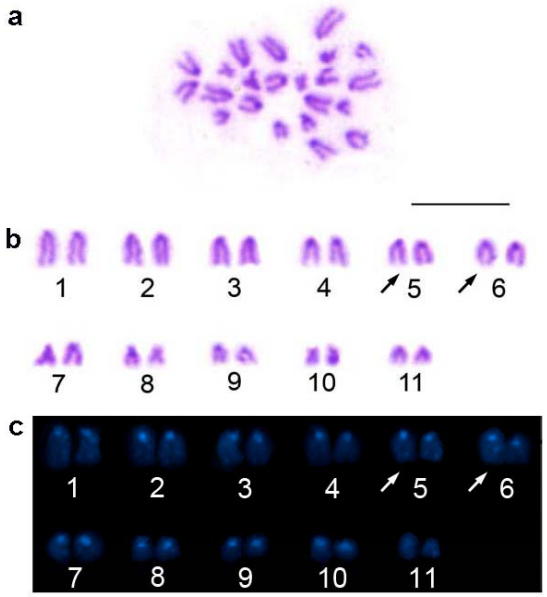

Fig. 1. Mitotic chromosomes of *Fascioloides magna*. a – spread mitotic metaphase stained by Giemsa; b – karyotype derived from the cell shown in Fig. 1a; c – another mitotic set stained by DAPI. Scale bar indicates 10 µm; arrows indicate chromosome pair with extra-centromeric heterochromatin blocks.

Karyological analysis

Slides were stained in 5 % solution of Giemsa-Romanowski dye (Merck, New Jersay, USA) in buffer phosphate solution (pH 6.8) for 15 – 30 min and flushed with flowing water.

Another preparations were stained with the DNA binding dye, 0.5 µg/ml DAPI (4',6-diamino-2-phenylidole; Sigma-Aldrich, St. Louis, USA) and mounted in antifade based on DABCO (Sigma-Aldrich). Preparations were observed and photographed in the combined light and fluorescent microscope Olympus BX 51 with digital camera DP70. Digital images were processed with Adobe Photoshop, version 7.0. Karyological data (absolute and relative lengths and centromeric index) were calculated from 10 best spermatogonial spreads out of 69 evaluated mitoses. The classification of chromosomes followed the nomenclature of Levan *et al*. (1964).

Results

The diploid chromosome number of *F. magna* is 2n = 22 (Fig. 1, Tab. 1). Out of 69 observed mitotic spermatogonial cells, 15 (21.7 %) were aneuploid; the random lack of one or two chromosomes can be explained as methodological problem caused by the spreading technique. In first meiotic division of spermatocytes, a haploid set of n = 11 bivalents was regularly observed which is in agreement with the diploid number. All 11 chromosome pairs were relatively similar in their length and morphology. The length

Table1. Measurements (means ± SD) and classification of chromosomes of *Fascioloides magna*

Chromosome number	Absolute length (µm)	Relative length (%)	Centromeric index	Classification
1	4.65 ± 0.41	13.25 ± 0.96	16.46 ± 3.60	st
2	4.24 ± 0.41	12.06 ± 0.37	17.98 ± 3.13	st
3	4.03 ± 0.36	11.32 ± 0.37	18.34 ± 2.64	st
4	3.70 ± 0.12	10.52 ± 0.31	20.47 ± 4.49	st
5	3.42 ± 0.34	9.70 ± 0.47	21.00 ± 3.97	st
6	3.12 ± 0.35	8.82 ± 0.37	20.90 ± 3.52	st
7	2.77 ± 0.43	7.98 ± 0.59	21.98 ± 3.59	st
8	2.69 ± 0.41	7.63 ± 0.54	37.09 ± 2.73	sm-m
9	2.36 ± 0.24	6.79 ± 0.57	24.16 ± 5.97	st
10	2.27 ± 0.31	6.47 ± 0.63	28.94 ± 2.70	sm
11	1.92 ± 0.33	5.43 ± 0.55	24.24 ± 2.62	st

Note: st - subtelocentric, sm-m - submeta-metacentric, sm - submetacentric chromosome pair

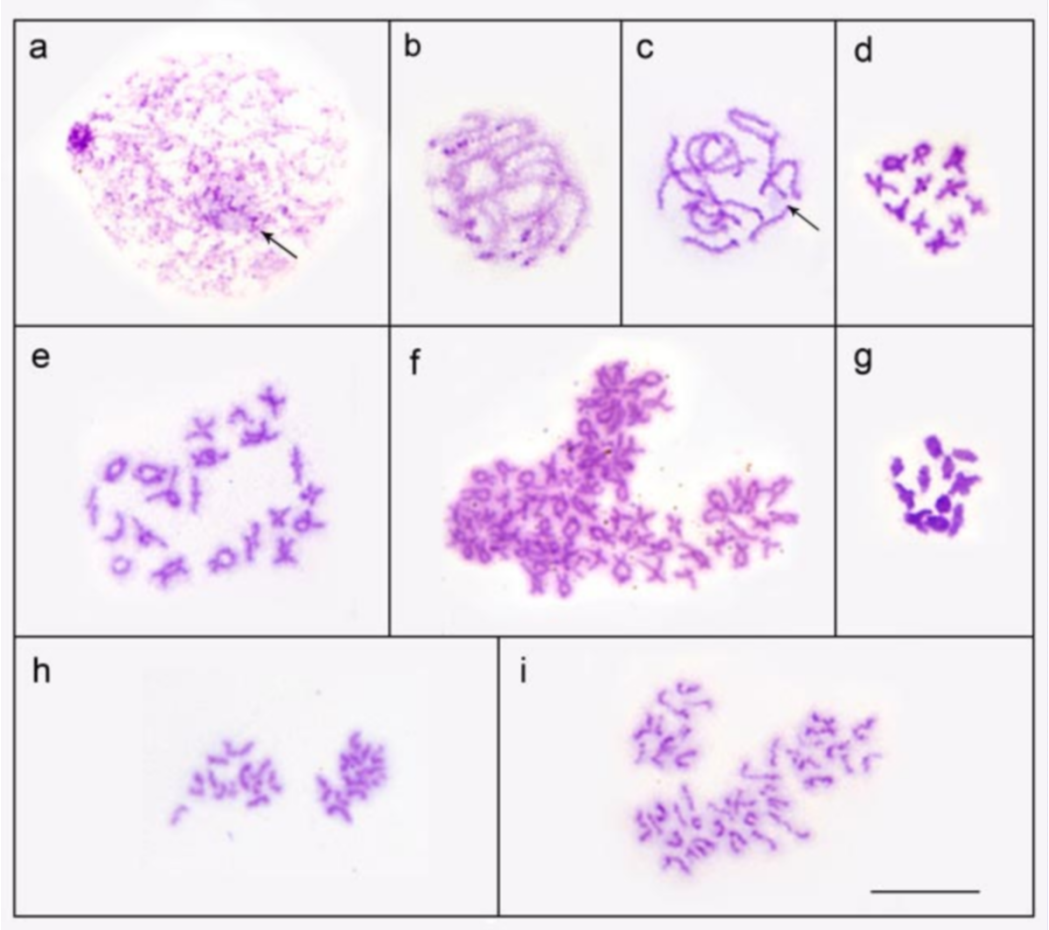

Fig. 2. Meiotic chromosomes of *Fascioloides magna* stained by Giemsa. a – leptotene; b, c – pachytene; d, e, f – diakinesis, one cell (d), two cells (e), eight cells (f); g – metaphase I; h – metaphase II; i – anaphase II, four cells. Scale bar indicates 10 µm; arrow indicates nucleolus.

descended continuously with small differences between the neighbouring pairs (Tab. 1) and the mean total length of the haploid genome (TCL) reached 35.17 µm. The largest pair was 4.65 µm long occupying 13.25 % of TCL, the smallest one measured 1.92 µm which represented 5.43 % of TCL. Regarding morphology, nearly all chromosome pairs were classified as subtelocentric; the only exceptions were the submeta-metacentric pair No. 8 and submetacentric pair No. 10 (Tab. 1). The karyotype formula is 2n = 22, n = 1sm + 1sm-m + 9st. No distinct secondary constriction was observed in mitotic preparations (Fig. 1a – c). Fluorescent DAPI-staining revealed distinct heterochromatin bands on all 11 chromosome pairs in their centromeric regions (Fig. 1c). Another DAPI-positive band was localized at the end of the long arm of the pair No. 5 and a last less distinct signal appeared interstitially on the long arm of the pair No. 6 (see arrows in Fig. 1b, c).

In meiotically dividing spermatocytes (Fig. 2a – c), a single nucleolus was observed from leptotene (Fig. 2a) to pachytene (Fig. 2c) stages. Pachytene nuclei showed clumps of 11 bivalents with darkly stained pericentromeric heterochromatin blocks (Fig. 2 b, c). A synchronous meiotic division of up to 8-spermatocyte groups was observed; during diakinesis, one to maximum eight cells were often found in slides (Fig. 2d, e, f). Clusters of diverse number of synchronously dividing secondary spermatocytes were seen, as well (Fig. 2h, i).

Discussion

The family Fasciolidae comprises six genera (*Protofasciola*, *Fasciolopsis*, *Parafasciolopsis*, *Fascioloides*, *Fasciola*, *Teniufasciola*) with nine species occurring in various artiodactyls, primarily in wild and domestic ruminants, but also in hogs and pigs, elephants and hippos (Jones, 2005). Out of them, *Fasciola hepatica* L., 1758, *F. gigantica* Cobbold, 1869 and *Fasciolopsis buski* (Lankester, 1857) may parasitize also humans and have high medical importance (Lotfy & Hillier, 2003; Mas-Coma *et al.*, 2005; Dorko *et al.,* 2009). Therefore, these flukes have been studied most intensively for a variety of biological features including chromosomes (for review see Baršiené, 1993). In addition to medically and veterinary important fasciolid species, a karyotype of *Parafasciolopsis fasciolaemorpha* Ejsmont, 1932, the parasite of elk and cervids, was also described (Baršiené, 1990). The survey on cytogenetic data of fasciolid species, except for the papers dealing exclusively with ploidy, is provided in Table 2.

Table 2. Survey of cytogenetic data of flukes of the family Fasciolidae

Species	Number of chromosomes 2n / 3n	Classification and absolute length of chromosome pairs (μm)	Bi-armed and one-armed chromosomes	References
Protofasciola robusta	–	–	–	–
Fasciolopsis buski	2n = 14	m/m/m/m/m/m/t	n = 6X+1Λ	Gao (1985)
F. buski	2n = 14	m/m/m/sm/m/sm/t	n = 6X+1Λ	Dai (1990)
Parafasciolopsis fasciolaemorpha	2n = 20	m/t/st/st/st/st/st/sm/sm 8.7/6.4/5.8/5.4/4.9/4.4/3.8/3.4/3.0/2.6	n = 3X+7Λ	Baršiené (1990)
Fascioloides magna	2n = 22	st/st/st/st/st/st/st/sm-m/st/sm/st 4.6/4.2/4.0/3.7/3.4/3.1/2.8/2.7/2.4/2.3/1.9	n = 2X+9Λ	Present results
Fasciola jacksoni	–	–	–	–
Fasciola hepatica	2n = 20	sm-m/st/st/st/st/st/sm/m/m-sm/sm 4.8/3.4/3.1/2.9/2.6/2.4/2.3/2.2./1.9/1.9	n = 5X+5Λ	Romanenko & Pleshanova (1975)
F. hepatica	2n = 20	n = 5sm+4st+1t	n = 5X+5Λ	Li & He (1988)
F. hepatica	2n = 20 3n = 30	m/st/st/st/m/st/m/m/st/st	n = 4X+6Λ	Li *et al.* (1988)
F. hepatica	2n = 20	sm-m/st/st/sm*/st/st/sm/sm/sm/sm 9.4 – 2.9	n = 6X+4Λ	Špakulová & Kráľová, 1991
Fasciola gigantica	2n = 20	sm/m-sm/st/sm/st/sm/st/sm/sm/sm 6.6/5.2/4.5/4.3/3.9/3.5/2.9/2.6/2.3	n = 7X+3Λ	Venkat Reddy & Subramanyam (1973); Subramanyam & Venkat Reddy (1977)
F. gigantica	2n = 20	sm/t/st/st/st/st/st/st/sm 7.3/5.1/4.1/3.6/3.3/2.9/2.6/2.2/2.0	n = 2X+8Λ	Romanenko & Pleshanova (1975)
Fasciola sp.	3n = 30	sm/st/st/sm/sm/sm/sm/sm/sm/	n = 8X+2Λ	Sakaguchi & Nakagawa (1975)
Fasciola sp.	2n = 20 3n = 30 mixopoid	sm/st/t/st/st/st/sm-st/st/st-sm/sm sm/st/t/st/st/st/sm-st/st/st-sm/sm sm/st/t/st/st/st/sm-st/st/st-sm/sm	n = 3X+7Λ n = 3X+7Λ n = 3X+7Λ	Moriyama *et al.* (1979)
Fasciola sp.	2n = 20 3n = 30 mixopoid	m/st/st/st/st*/st/sm/sm/sm/st (9.0 – 2.8) m/st/st/st/st*/st/sm/sm/m/st (9.0 – 2.8)	n = 4X+6Λ n = 4X+6Λ	Sakaguchi (1980)
Fasciola sp.	2n = 20 3n = 30 mixopoid	m/st/st/st/st/sm/sm/sm/sm/sm m/st/st/st/st/sm/sm/sm/sm/st	n = 6X+4Λ n = 5X+5Λ	Rhee *et al.* (1987)
Fasciola sp.	2n = 20 3n = 30	n = 2m+4sm+3st+1t n = 2m+4sm+3st+1t	n = 6X+4Λ n = 6X+4Λ	Yin & Ye (1990)
Fasciola nyanzae	–	–	–	–
Teniufasciola tragelaphi	–	–	–	–

Note: * – satellited pair; m – metacentric, sm – submetacentric, st – subtelocentric, t – telocentric chromosome pair; X – bi-armed chromosome pairs (m + sm) ; Λ – one-armed chromosome pairs (t + st).

The fasciolids are aligned according to their hypothetical evolutionary origin (Lotfy *et al*., 2008; Prasad *et al*., 2008). The karyotype of *F. magna*, described here in detail for the first time, differs from chromosome sets of all other fasciolids by higher diploid number 2n = 22. Namely, *F. hepatica* and *F. gigantica* have 2n = 20; triploid forms, reported often in both species and in *Fasciola* sp., have 3n = 30 (Sanderson, 1953; Terasaki *et al*., 2000; Itagaki *et al*., 2009 and references therein). The chromosome number 2n = 20 is characteristic also for *P. fascioaemorpha* (Baršiené, 1990). *Fasciolopsis buski* differs from all fasciolids by low number of chromosomes, having 2n = 14 (Lo, 1969; Gao, 1985; Dai *et al*., 1990). Except of chromosome number, the karyotype of *F. magna* varies from other fasciolid species chiefly by morphology of the first chromosome pair. It is of medium length and clearly subtelocentric in the giant liver fluke but longer and metacentric or submetacentric in all other species (see Tab. 2).

A comparison of ratio of telocentric and subtelocentric (i.e. one-armed) and metacentric and submetacentric (bi-armed) chromosome pairs in karyotypes of individual fasciolid species (Table 2, column 4) reveals that one-armed chromosomes predominate in *P. fasciolaemorpha* and *F. magna,* both belonging to evolutionary basal or intermediate fasciolid species, as ascertained by Lotfy *et al*. (2008) by molecular phylogenetic study. This fits well with a hypothesis that less advanced species of a specific group usually possess non-symmetric karyotype with higher number of one-armed chromosomes and lower proportion of bi-armed elements (White, 1973). On the other hand, the karyotype of another, presumably basal, fasciolid species *F. buski* (Lotfy *et al*., 2008; Prasad *et al.,* 2008) apparently represents an exception having only 14 chromosome pairs, all but one being metacentric (Gao, 1985; Dai, 1990). It seems that karyotype evolution of this Asian parasite accelerated rapidly after it had derived from the common ancestor and acquired human and domesticated pig hosts.

Regarding multiple records on karyotypes of *F. hepatica*, *F. gigantica* and *Fasciola* sp., the cytogenetic data are often contradictory. While *F. hepatica* seems to have balanced incidence of one- and bi-armed chromosome types (Romanenko & Pleshanova, 1975; Li *et al*., 1988; Špakulová & Kráľová, 1991), the data referred to *F. gigantica* and *Fasciola* sp. are much more discrepant (see Table 2). In general, genetic variation within the *Fasciola* spp. throughout the world is outstanding. In many Asian countries from Iran and India as well as in Pacific islands Taiwan, the Philippines and Hawaii, *F. hepatica, F. gigantica* and *Fasciola* sp. exhibit not only diploid, but also triploid populations and even mixoploid (2n/3n) specimens (e.g. Moriyama *et al*., 1979; Sakaguchi, 1980; Terasaki *et al*., 1998, 2000; Itagaki *et al*., 2009; Srimuzipo *et al*., 2000; Ichikawa & Itagaki, 2010). Additionally, triploid *F. hepatica* has been found in Britain and Ireland (Fletcher *et al.,* 2004). Recent molecular studies have suggested that the origin of Asian triploids may be hybridization between *F. hepatica* and *F. gigantica* (Itagaki & Tsutsumi, 1998; Agatsuma *et al*., 2000; Lin et al., 2007; Itagaki *et al.,* 2009; Peng *et al.,* 2009). Moreover, Terasaki *et al.* (2000), Fletcher *et al*. (2004) and Dreyfuss & Rondelaud (2008) suggest that triploidy in *Fasciola* spp. probably arises repeatedly and occasionally in various regions by a variety of mechanisms and facultative gynogenesis is thus widespread in *Fasciola*. Local populations could be outbreeding and diverse, or clonal with few genotypes present (Fletcher *et al*., 2004). A rather broad genetic diversity among geographically distinct *Fasciola* populations was also discussed by Semyenova *et al*. (2006) and Dreyfuss & Rondelaud (2008). It seems likely that divergent intraspecific lineages might differ also in their karyotypes (see discrepancies in Table 2). In this respect, it would be worthwhile to supplement future molecular studies of *Fasciola* spp. with thorough determination of chromosome characteristics and vice versa.

Except of chromosome number and shape, additional chromosomal characteristics like distribution of heterochromatin can help us to clarify phylogenetic relationships. As usual in hermaphroditic platyhelminthes (e.g. Mutafova *et al*., 1986; Špakulová & Kráľová, 1991; Orosová *et al*., 2010), the karyotype of *F. magna* possess rather small amount of heterochromatin distributed mainly near the centromeres. Interestingly, slight interstitial bands were found in two middle-sized chromosome pairs. However, their specification requires further analysis with a use of molecular cytogenetic methods (e.g. fluorescent in situ hybridization, FISH) in higher number of Fasciolidae species.

Development of spermatocytes of *F. magna* corresponds with that of other hermaphroditic digenean trematodes (Gresson, 1965). It is known that in the testis of a fluke, primary, secondary and tertiary spermatogonia are present and that their mitotic divisions are mostly synchronized. Hence, we have often found groups up to eight primary spermatocytes in various stages of meiosis (mainly in diakinesis). During the second meiotic division, 16 secondary spermatocytes give a group of 32 haploid spermatids. We have not observed any deviations from this standard (see Fig. 2). The process of degradation of nucleolus during meiotic prophase (between pachytene and metaphase I) in *F. magna* also confirms related data in other Platyhelminthes (e.g. Orosová *et al*., 2010)

In spite of the fact that our present knowledge on cytogenetics of Fasciolidae flukes is not sufficient to draw general lines of chromosome evolution within the group, the present analysis provides new valuable markers suitable for future comparative evaluation of respective taxonomic or phylogenetic questions.

Acknowledgements

We acknowledge Dr. Martin Kašný (Faculty of Science, Charles University, Prague, Czech Republic) for a kind providing us with a Czech material of *F. magna.* The work was supported by grants of the Slovak Research and Development Agency No. LPP-0126-07 and APVV-51-

062205, the National Science Foundation, USA (PBI award Nos. 0818696 and 0818823), the Grant Agency of the Czech Republic (No. 524/08/0885), research projects of the Institute of Parasitology BC ASCR (Z60220578, LC 522), and it was realized within a frame of Centre of Excellence for Parasitology (Code ITMS: 26220120022) based on the support of the Operational Programme "Research & Development" funded from the European Regional Development Fund (rate 0.2).

References

AGATSUMA, T., ARAKAWA, Y., IWAGAMI, M., HONZAKO, Y., CAHYANINGSIH, U., KANG, S-Y., HONG, S-J. (2000): Molecular evidence of natural hybridization between *Fasciola hepatica* and *F. gigantica*. *Parasitol. Int*., 49(3): 231 – 238. DOI: 10.1016/S1383-5769(00)00051-9

BARŠIENÉ, J. (1990): Chromosome sets of trematodes *Parafasciolopsis fasciolaemorpha* (Ejsmont, 1932) and *Cathemasia hians* (Rudolphi, 1809) Looss, 1899. *Helminthologia,* 27(3): 145 – 152

BARŠIENÉ, J. (1993): *The karyotypes of trematodes*. Vilnius, Lithuania: Academia, 370 pp. (In Russian)

BASSI, R. (1875): Sulla cachessia ittero-vermicosa, o marciaia dei Cervi, causata dal *Distomum magnum*. *Med. Vet.Torino*, 4: 497 – 515

DAI, X. (1990): Karyotype analysis of *Fasciolopsis buski*. *J. Chongquin Med. Univ.*, 4: 11. DOI: cnki: ISSN:0253-3626.0.1990-04-004

DORKO E., BARANOVÁ, Z., DUBINSKÝ, P., PISTL, J. (2009): *Bacterial, viral, parasitic and mycotic zoonoses: Viral and parasitic zoonoses.* Equilibria, 367 pp. ISBN 978-80-89284-32-0. (In Slovak)

DREYFUSS , G., RONDELAUD, D. (2008): Biodiversity of flukes. *Parasite,* 15(3): 282 – 285

ERHARDOVÁ-KOTRLÁ, B. (1971). *The occurrence of Fascioloides magna (Bassi, 1875) in Czechoslovakia.* Prague, Czech Republic, Academia, 155 pp.

FLETCHER, H. L., HOEY, E. M., ORR, E. M., TRUDGETT, A., FAIRWEATHER, I., ROBINSON, M. V. (2004): The occurrence and significance of triploidy in the liver fluke, *Fasciola hepatica. Parasitology,* 128(1): 69 – 72. DOI: 10.1017/S003118200300427X

FUKOVÁ, I., NGUYEN, P., MAREC, F. (2005): Codling moth cytogenetics: karyotype, chromosomal location of rDNA, and molecular differentiation of sex chromosomes. *Genome,* 48(6): 1083 – 1092. DOI: 10.1139/G05-063

GAO L. (1985): Observation of meiosis of *Fasciolopsis buski*. *Hereditas (Beijing),* 7 (2): 22 – 23. DOI: cnki: ISSN: 0253-9772.0.1985-02-009

GRESSON, R. A. R. (1965): Spermatogenesis in the hermaphroditic Digenea (Trematoda). *Parasitology,* 55: 117 – 125. DOI: 10.1017/S0031182000068426

ICHIKAWA, M., ITAGAKI, T. (2010): Discrimination of the ITS1 types of *Fasciola* spp. based on a PCR-RFLP method. *Parasitol. Res*., 106(3): 757 – 761. DOI: 10.1007/s00436-010-1724-2

ITAGAKI, T., TSUTSUMI, K. (1998): Triploid form of *Fasciola* in Japan: genetic relationships between *Fasciola hepatica* and *Fasciola gigantica* determined by ITS-2 sequence of nuclear rDNA. *Int. J. Parasitol.* 28(5): 777 – 781. DOI: 10.1016/S0020-7519(98)00037-X

ITAGAKI, T., SAKAGUCHI, K., TERASAKI, K., SASAKI, O., YOSHIHARA, S., VAN DUNG, T. (2009): Occurrence of spermic diploid and aspermic triploid forms of *Fasciola* in Vietnam and their molecular characterization based on nuclear and mitochondrial DNA. *Parasitol. Int.,* 58(1): 81 – 85. DOI: 10.1016/j.parint.2008.11.003

JONES, A. 2005. Family Fasciolidae Railliet, 1895. In: JONES, A., BRAY, R. A., GIBSON, D. I. (Eds) *Keys to the Trematoda.* Volume 2. London, UK: CABI Publishing and The Natural History Museum, pp. 79 – 86.

LEVAN, A., FREDGA, K., SANDBERG, A. (1964): Nomenclature for centromere position on chromosomes. *Hereditas* 52(2): 201 – 220. DOI: 10.1111/j.1601-5223.1964.tb01953.x

LI, J., HE, L. (1988): Analysis of meiosis and karyotype of *Fasciola hepatica*. *J. First Millit. Med. Univ.*, 1: 11. DOI: cnki: ISSN: 1000-2588.0.1988-01-020

LI, G. Q., JIN, J. S., WANG, P. Y. (1988): A study on the chromosomes of *Fasciola hepatica*. *Chin. J. Veter. Sci. Technol*., 6: 11 – 16

LIN, R.Q., DONG, S. J., NIE, K., WANG, C. R., SONG, H. Q., LI, A. X., HUANG, W. Y., ZHU, X. Q. (2007): Sequence analysis of the first internal transcribed spacer of rDNA supports the existence of the intermediate *Fasciola* between *F. hepatica* and *F. gigantica* in mainland China. *Parasitol. Res.,* 101(3): 813 – 817. DOI: 10.1007/s00436-007-0512-0

LO, C. T. (1969): Chromosomes of *Fasciolopsis buski*. Trematoda. Fasciolidae. *Bull. Inst. Zool. Acad. Sinica*, 8(1): 1 – 5

LOTFY, W. M., HILLIER, G. V. (2003): *Fasciola* species in Egypt. *Exp. Pathol. Parasitol.* 6 (11): 9 – 22

LOTFY, W. M., BRANT, S. V., DEJONG, R. J., LE, T. H., DEMIASZKIEWICZ, A., RAJAPAKSE, R. P. V. J., PERERA, V. B. V. P., LAURSEN, J. R., LOKER, E. S. (2008): Evolutionary origins, diversification and biogeography of liver flukes (Digenea, Fasciolidae). *Am. J. Trop. Med. Hyg.,* 79(2): 248 – 255

MAS-COMA, S., BARGUES, M. D., VALERO, M. A. (2005): Fascioliasis and other plant-borne trematode zoonoses. In *Int. J. Parasitol.,* 35(11-12): 1255 – 1278. DOI: 10.1016/j.ijpara.2005.07.010

MORIYAMA, N., TSUJI, M., SETO, T. (1979): Three karyotypes and their phenotypes of Japanese liver flukes (*Fasciola* sp.). *Jpn. J. Parasitol*. 28(1): 23 – 33. (In Japanese with English summary).

MUTAFOVA, T., TSOCHEVA, N., POLYAKOVA-KRSTEVA, O., KRSTEV, L. (1986): Effect of diethylnitrosamin on the chromosome structure of rats infected with *Fasciola hepatica* and on that of the liver fluke. *Khelmithologiya (Sofia)*, 22: 42 – 50

NOVOBILSKÝ, A., HORÁČKOVÁ, E., HIRTOVÁ, L., MODRÝ, D., KOUDELA, B. (2007): The giant liver fluke *Fascioloides magna* (Bassi, 1875) in cervids in the Czech Republic and

potential of its spreading to Germany. *Parasitol. Res.,* 100(3): 549 – 553. DOI: 10.1007/s00436-006-0299-4

OROSOVÁ, M., MAREC, F., OROS, M., XI, B. W., SCHOLZ, T. (2010): A chromosome study and localization of 18S rDNA in *Khawia saurogobii* (Cestoda: Cryophyllidea). *Parasitol. Res*., 106(3): 587 – 593. DOI: 10.1007/s00436-009-1702-8

PENG, M., ICHINOMIYA, M., OHTORI, M., ICHIKAWA, M., SHIBAHARA, T., ITAGAKI, T. (2009): Molecular characterization of *Fasciola hepatica, Fasciola gigantica*, and aspermic *Fasciola* sp. in China based on nuclear and mitochondrial DNA. *Parasitol. Res*., 105(3): 809 – 815. DOI: 10.1007/s00436-009-1459-0

PRASAD, P. K., TANDON, V., BISWAL, D. K., GOSWAMI, L. M., CHATTERJE, A. (2008): Molecular identification of the Indian liver fluke, Fasciola (Trematoda: Fasciolidae) based on the ribosomal internal transcribed spacer regions. *Parasitol. Res.,* 103(6): 1247 – 1255. DOI: 10.1007/s00436-008-1121-2

PYBUS, M. J. (2001): Liver flukes. In SAMUEL, W. M., PYBUS, M. J., KOCAN, A. A. (Eds) *Parasitic diseases of wild mammals*, Ames, Iowa, Iowa State Press, pp. 121 – 149

RHEE, J. K., EUN, G. S., LE, S. B. (1987): Karyotype of *Fasciola* sp. obtained from Korean cattle. *Korean J. Parasitol*., 25(1): 37 – 44. DOI: 10.3347/kjp.1987.25.1.37 (In Korean, abstract in English)

ROMANENKO, L. N., PLESHANOVA, N. M. (1975): Chromosome sets of *Fasciola hepatica* and *Fasciola gigantica*. *Trudy VIGIS (Teoreticheskie problemy veterinarnoy gel'mintologii)*, 22: 137 – 142

SANDERSON, A. R. (1953): Maturation and probable gynogenesis in the liver fluke, *Fasciola hepatica* L. *Nature,* 172(4368): 110 – 112. DOI: 10.1038/172110a0

SAKAGUCHI, Y. (1980): Karyotype and gametogenesis of the common liver fluke, *Fasciola* sp., in Japan. *Jpn. J. Parasitol*., 29: 507 – 513

SAKAGUCHI, Y., NAKAGAWA, C. (1975): A note on the chromosome of the common liver fluke (*Faciola* sp.) from Japan. *Chromosome Inform. Surv*., 19: 25 – 26

SEMYENOVA, S. K., MOROZOVA, E. V., CHRISANFOVA, G. G., GOROKHOV, V. V., ARKHIPOV, I. A., MOSKVIN, A. S., MOVSESSYAN, S. O., RYSKOV, A. P. (2006): Genetic differentiation in eastern European and western Asian populations of the liver fluke, *Fasciola hepatica*, as revealed by mitochondrial *nad*1 and *cox*1 genes. *J. Parasitol*., 92(3): 525 – 530. DOI: 10.1645/GE-673R.1

SRIMUZIPO, P., KOMALAMISRA, C., CHOOCHOTE, W., JIPAKDI, A., VANICHTHANAKOM, P., KEHA, P., RIYONG, D., SUKONTASON, K., KOMALAMISRA, N., SUKONTASON, K., TIPPAWANGKOSOL, P. (2000): Comparative morphometry, morphology of egg and adult surface topography under light and scanning electron microscopies, and metaphase karyotype among three size-races of *Fasciola gigantica* in Thailand. *Southeast Asian J. Trop. Med. Publ. Health,* 31(2): 366 – 373

SUBRAMANYAM, S., VENKAT REDDY P. (1977): The role of chromosomes in the taxonomy of some digenetic trematodes. *The Nucleus*, 20(1 and 2): 128 – 138

ŠPAKULOVÁ, M., KRÁĽOVÁ, I. (1991): Chromosomes of *Fasciola hepatica* (Digenea: Fasciolidae) from western Bohemia (CSFR). *Helminthologia,* 28(4): 197 – 200

ŠPAKULOVÁ, M., RAJSKÝ, D., SOKOL, J., VODŇANSKÝ, M. (2003): *Giant liver fluke (Fascioloides magna), an important liver parasite of ruminants*. Bratislava, Slovak Republic, PaRPRESS, 61 pp.

TERASAKI, K., MORIYAMA-GONDA, N., NODA, Y. (1998): Abnormal spermatogenesis in the common liver fluke (*Fasciola* sp.) from Japan and Korea. *J. Vet. Med. Sci*. 60(12): 1305 – 1309. DOI: 10.1292/jvms.60.1305

TERASAKI, K., NODA, Y., SHIBAHARA, T., ITAGAKI, T. (2000): Morphological comparisons and hypotheses on the origin of polyploids in parthenogenetic *Fasciola* sp. *J. Parasitol*., 86(4): 724 – 729. DOI: 10.1645/0022-3395(2000)086[0724:MCAHOT]2.0.CO;2

VENKAT REDDY, P., SUBRAMANYAM, S. (1973): Chromosome studies in the liver fluke, *Fasciola gigantica* Cobbold, 1856, from Andhra Pradesh. *Curr. Sci*., 42: 288 – 291

YIN, H. Z., YE, B.Y. (1990): Studies on the karyotypes of *Fasciola* spp. *Chin. J. Parasitol. Parasitic Dis.* 8(2): 124 – 126

WHITE, M. J. D. (1973): *Animal Cytology and Evolution*. New York: Cambridge University Press. 961 pp.

RECEIVED JANUARY 8, 2010

ACCEPTED MARCH 11, 2010