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New chromosome characteristics of the monozoic tapeworm *Caryophyllaeus laticeps* (Cestoda, Caryophyllidea)

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Summary

The karyotype of a caryophyllidean tapeworm *Caryophyllaeus laticeps* (Pallas, 1781) from the freshwater bream *Abramis brama* (L.) caught in the Slovak part of the River Tisa, was described and originally inspected for amount of heterochromatin and its chromosome localization. The chromosome set comprised nine metacentric and one submetacentric (No. 3) pairs (2n = 20). The chromosomes were up to 12.0 ± 2.5 µm long and the mean total length of haploid genome (TLC) reached 80.6 µm that represents one of the highest yet recorded values among tapeworms. C-banding and staining with fluorescent dyes DAPI and YOYO1 revealed a distinct banding pattern explicitly on chromosomes with centromeric bright heterochromatin bands present on all 10 chromosome pairs; no pair showed any interstitial heterochromatin. A complete course of spermatocyte meiosis and dynamics of nucleolus formation and degradation during meiotic division was described.
Keywords: karyotype; meiosis; heterochromatin; *Abramis brama*

Introduction

Cestodes of the order Caryophyllidea are intestine parasites of freshwater cypriniform or siluriform fishes. They use aquatic annelids as intermediate (or sometimes also final) hosts and have several other unique features among Eucestoda as absence of internal proglottidization and external segmentation of the body, and an existence of a single set of reproductive organs (Mackiewicz, 1994).

Karyological studies relating to caryophyllideans are relatively scarce. To date, complete karyotype data of 14 species belonging to the families Caryophyllaeidae and Lytocestidae are known, as summarized by Špakulová *et al*. (2011) and Orosová and Oros (2012). The diploid number varies between 6 and 20, but 2n = 16 or 20 are the most frequent variants. Regarding the morphology, chromosomes of all yet studied caryophyllideans are of mid or large length, however, their shape types (classification) differ significantly; symmetrical, asymmetrical, or rather balanced karyotypes occur randomly (Špakulová *et al*., 2011).

The current paper describes the karyotype of Slovak population of *C. laticeps*, one of the most common caryophyllidean tapeworm species in the Palaearctic Region, using advanced cytogenetic banding methods.

Material and Methods

A total of 16 specimens of *C. laticeps* were isolated from intestines of two freshwater breams *Abramis brama* (L.) caught in a dead arm of the Tisa River near the village Veľké Trakany (south-eastern Slovakia, 48.382956, 22.094082) in April, 2005. The parasites were divided into samples which were studied for morphology, DNA structure, and karyotypes. Using the first two approaches, Bazsaloviscová *et al*. (2014) and Hanzelová *et al.* (2015) found out that these *C. laticeps* individuals (as a part of broader material) belonged to the so called morphotype I out of five intraspecific, phenotypically diverse forms. Four tapeworms used for karyotype analysis belonged to the same variant, as well.

Chromosome preparations

For cytogenetic analyses, four living specimens were incubated in 0.025 % colchicine solution (in saline) for 1 hour, placed into 75 mM KCl for hypotonic treatment for 2 hours and torn at the

testicular body area. The torn worms were placed into freshly prepared cold fixative solution (3:1 methanol:acetic acid) for 1 hour (with at least one replacement of the fixative) and stored at -20°C until further use. Chromosome preparations were made using a spreading method according to Frydrychová and Marec (2002). A defrost tissue sample was transferred into a drop of 60 % acetic acid on a clean slide and torn by tungsten needles. The slide was placed on a heating plate at 45 °C and the drop was slowly drawn along the slide until evaporation. Preparations were then dehydrated in ethanol series (70 %, 80 % and 100 %, 30 s each) and stored at -20°C for further use.

Karyological analysis

Slides were stained with 5 % solution of Giemsa stain (Merck, New Jersey, USA) in phosphate buffer (pH 6.8) for 20 min. In order to obtain the absolute and relative chromosome length and centromeric index, eight complete mitotic Giemsa-stained spreads with clearly distinguishable chromosomes were measured out of 114 evaluated cells from two worms. The centromere position on the chromosomes was classified following Dos Santos Guerra (1986).

C-banding

C-banding was performed according to Sumner (1972) with the aim to analyze the content and distribution of a constitutive heterochromatin. Air-dried C-banded preparations were either stained with 5 % Giemsa solution (pH 6.8) for 30 min, or with 0.5 µg/ml DAPI (4',6-diamino-2-phenylindole; Sigma-Aldrich, Gillingham, UK) for a better resolution of C-bands. The DAPI -stained preparations were mounted in 25 µl of antifade based on DABCO (1,4-diazabicyclo[2.2.2.]octane, Sigma–Aldrich, Gillingham, UK).

YOYO-1 staining

Chromosomal preparations were stained with the DNA and RNA binding dye YOYO-1 (1,1'-[1,3propanediyl-bis[(dimethylimino)-3,1-propanediyl]]bis[4-[(3-methyl-2(3H)- benzoxazolylidene) methyl]]-quinolinum tetraiodide; Molecular Probes, Eugene, OR, USA) to visualize nucleoli. Fluorescent preparations were mounted in antifade based on DABCO (Sigma- Aldrich) (for composition, see Mediouni *et al*. 2004).

Microscopy and image processing

Preparations were observed under a light and fluorescence microscope Leica DM 4000 B equipped with the digital camera DFC 450 C. The micrographs were processed with Adobe Photoshop, version 11.0.

Results

Karyotype

Evaluation of 114 dividing cells disclosed 20 chromosomes in diploid set (2n = 20) (Fig. 1a-c). The chromosomes were relatively large and biarmed, the longest pair measured 11.95 µm and the shortest 2.82 µm (for complete data see Table 1). According to the centromere position, all chromosome pairs were classified as metacentric except for the submetacentric pair No. 3, and the karyotype formula was 2n = 20, n = 9m + 1sm. No distinct secondary constriction was observed in dividing cells (Fig. 1a-c). After C-banding combined with the fluorescent staining with DAPI, clear heterochromatin bands were highlighted at sites of centromeres of all chromosomes but no interstitial heterochromatin bands were detected (Fig. 1b, c).

Table 1. Measurements of chromosomes of *Caryophyllaeus laticeps* (n = 10)

Chromosome pair No.	Absolute length (µm)	Relative length (%)	Centromeric index	Classification
1	11.95 ± 2.52	14.84 ± 0.70	44.01 ± 2.36	m
2	10.72 ± 2.60	13.21 ± 0.66	40.37 ± 1.59	m
3	9.30 ± 1.90	11.53 ± 0.39	36.50 ± 1.71	sm
4	9.14 ± 2.12	11.29 ± 0.36	45.76 ± 1.72	m
5	8.76 ± 0.85	10.90 ± 0.39	48.64 ± 1.45	m
6	7.76 ± 0.77	9.65 ± 0.42	46.28 ± 2.60	m
7	7.51 ± 1.76	9.27 ± 0.44	44.90 ± 2.96	m
8	6.81 ± 1.33	8.47 ± 0.47	43.76 ± 2.96	m
9	5.85 ± 0.96	7.33 ± 0.82	43.92 ± 2.51	m
10	2.82 ± 0.56	3.51 ± 0.36	43.00 ± 4.10	m

Note: m – metacentric, sm – submetacentric chromosome pair

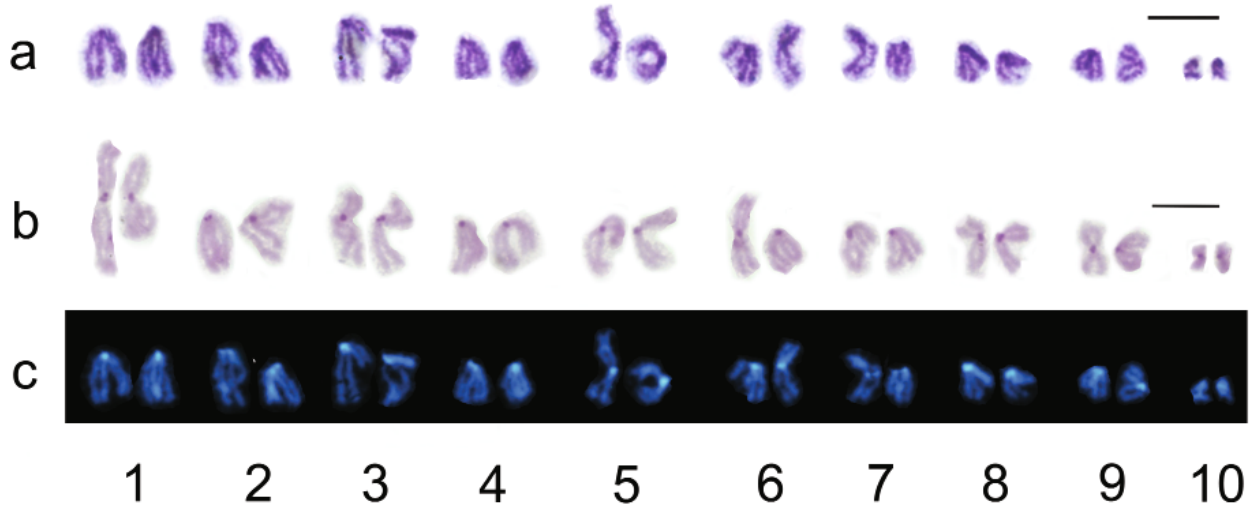

Fig. 1. Chromosome set of *Caryophyllaeus laticeps* derived from spermatogonial mitotic metaphase cells stained using different techniques. a) Giemsa, b) C-banding/Giemsa, c) C-banding/DAPI. Scale bar: 10 µm

A complete course of meiotic division of spermatocytes was documented using the fluorescent staining by YOYO-1, which also allowed highlighting a nucleolus and heterochromatic bands (Fig. 2a–f). In early stages of spermatocyte division, one large nucleolus was observed as a weakly stained mass on the periphery of zygotene nuclei (Fig. 2a), then it gradually degraded until pachytene (Fig. 2b). The nucleolus disappeared during late prophase I before reaching the early diplotene stage (Fig. 2c). Pachytene and early diplotene cells showed clumps of 10 bivalents, with clearly visible heterochromatin blocks present in centromeric region of each bivalent (Fig. 2b–c). Homologue chromosomes of diplotene bivalents also showed clear centromeric heterochomatin signals (Fig 2d). The size assortment of the bivalents and a comparison of centromere position in each pair confirmed the classification of nine pairs as metacentric and the pair No. 3 as submetacentric (Fig. 2d). A subsequent homeotypic division (Fig. 2e, f) revealed usual phases: in meiotic metaphase II, each spermatocyte contained a haploid set of 10 chromosomes (Fig. 2e); subsequently the chromatids of each chromosome separated and two haploid spermatids were formed (Fig. 2f).

Discussion

Caryophyllaeus laticeps has a long history being originally described by Pallas (1781) as *Taenia laticeps,* as the first discovered caryophyllidean tapeworm. It is broadly distributed along the Europe, most of Palaearctic Asia, and northern Africa, utilizing as many as 38 fishes as final hosts (Hanzelová *et al*., 2015).

Recently, thorough molecular and morphological comparison has been done using new extensive collections of *C. laticeps* coming from seven fish host species from a number of localities in seven European countries including Russia (Králová-Hromadová *et al*., 2013; Bazsalovicsová *et al*. 2014; Hanzelová *et al*. 2015). The results showed wide discrepancy between the morphology and genetic structure of the tapeworms; the apparent morphological polymorphism but high degree of genetic uniformity was found. In total, five intraspecific morphotypes (morphotypes I – V) were recognized within *C. laticeps*, largely corresponding to different fish hosts, and representing separate, yet closely related genetic lineages (Bazsalovicsová *et al*. 2014; Hanzelová *et al*. 2015).

The present cytogenetic study characterizes *C. laticeps* belonging to the morphotype I by criteria of latter papers; the work represents a first attempt to deeply analyse karyotypes of individual polymorphic variants. To date, the only other classical description of *C. laticeps* karyotype has been published by Petkevičiūtė and Kuperman (1992) who analysed the parasites isolated from the same fish host *A. brama* from the Rybinsk reservoir on the Volga River, Russia. Both distant populations are similar in standard karyotype characteristics, both having diploid number of ten bi-armed and long pairs (2n = 20), and comparable absolute and relative chromosome lengths as well as the mean total chromosome length of the haploid complements (TCL). For instance, the longest and shortest pairs measured 12.9 and 3.0 µm in Russian samples and 11.9 and 2.8 µm in Slovakian individuals; the corresponding TCL values were 87.8 and 80.6 µm, and the important values of relative length of all the pairs were nearly identical. The only apparent difference was found in the shape of third chromosome pair which was classified as metacentric in the Russian worms but submetacentric in the present chromosome sets. At the moment, it is difficult to explain this inter-population difference because any other information on basic karyology of other populations of the polymorphic species *C. laticeps* is missing.

The above Russian population of *C. laticeps* revealed another interestig feature (Petkevičiūtė & Kuperman 1992). Indeed, among 26 worms analysed by the authors, one triploid specimen was revealed. Moreover, some cells of this tapeworm contained an additional small chromosome. The trisomy of the smallest chromosomes was also detected in another diploid tapeworm individual. It is well known that caryophyllidean taxa may be karyologically unstable. For instance, triploidy was reported in a whole species, a certain population, or solely an individual within the diploid population of four representatives (for review see Špakulová *et al*.,

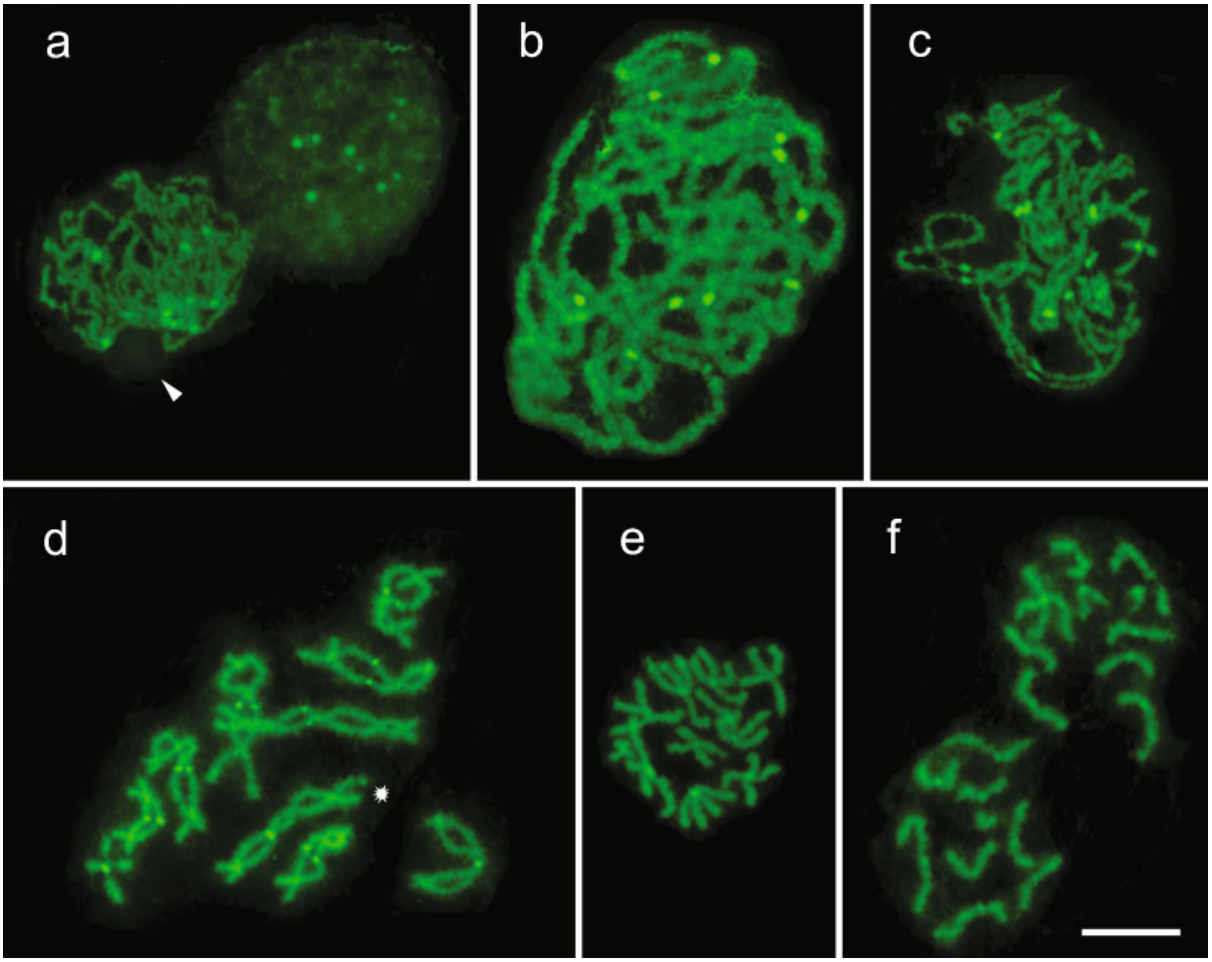

Fig.2. Meiotic division of spermatocytes of *Caryophyllaeus laticeps*. YOYO-1 staining.
a) Interphase nucleus (right) and zygotene stage with nucleolus (arrow) on the periphery. b) Pachytene stage with a clump of ten bivalents, each with a pericentromeric block of heterochromatin. c) Early diplotene stage with early separation of homologues. d) Diplotene stage with clearly visible centromeric heterochromatin. The submetacentric pair No. 3 is marked by asterisk. e) Metaphase II – haploid spermatocyte with 10 chromosomes. f) Early telophase II – two haploid spermatids, each with ten separating chromatids. Scale bar: 10 μm

2011). However, no deviations from the standard diploid number 2n = 20 were recorded within the Slovak population of *C. laticeps*, whether it was examined in this work or in the previous study by Bombarová *et al*. (2009). The latter paper dealt with helminth telomeres reporting also diploid cells of *C. laticeps*. Nevertheless, an occurrence of chromosomal aberrations within the Slovak populations of *C. laticeps* cannot be excluded.

The content and distribution of heterochromatin are considered as important features of genome (Shapiro & von Sternberg, 2005). To date, only limited information exists about patterns of heterochromatin in tapeworms including caryophyllideans. It seems that low amount of heterochromatin is typical for majority of Cestoda and that centromeric or pericentromeric heterochromatin predominates (for review see Špakulová *et al*., 2011). However, some lytocestid caryophyllideans revealed varied patterns: *Khawia saurogobii* Xi et al., 2009 showed centromeric bands at all chromosomes except for homologues No. 1 (Orosová *et al*., 2010a), while *Caryophyllaeides fennica* (Schneider, 1902) lacked the centromeric heterochromatin in the pair No. 2, but additional two interstitial bands were present on short and long arms of No. 7 (Orosová *et al*., 2010b). The chromosomes of triploid *Atractolytocestus huronensis* Anthony, 1958 with (3n = 24) (also Lytocestidae) was characterized by pericentromeric bands only in triplets Nos 2, 4, 6, and 7 (Králová-Hromadová *et al*. 2010). Apparently, current data on *C. laticeps* differ from related lytocestid species, however, their informative phylogenetic value can be evaluated only after the similar analyses will be available for more caryophyllideans.

Distinct C-bands and YOYO1 staining of *C. laticeps* chromosomes confirmed the centromere positions, especially those that are seen in mitotic cells and during the meiotic prophase – diakinesis. During the spermatocyte meiosis, no apparent deviations from the processes known in the majority of invertebrates were found. Similarly, a dynamics of the nucleolus formation and ongoing degradation during cell divisions seemed to be standard, which means that the disintegration of the nucleolus is completed before diakinesis (Wachtler & Stahl, 1993).

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