CHROMOSOME STUDIES OF *ICHTHYOCOTYLURUS PLATYCEPHALUS* (CREPLIN, 1825) ODENING 1969 WITH DESCRIPTION OF TRIPLOID VARIANT AND COMPARATIVE KARYOLOGY OF THE GENUS *ICHTHYOCOTYLURUS*

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Summary :

This paper reports the first karyological study of trematode *Ichthyocotylurus platycephalus* (Creplin, 1825) Odening 1969. Chromosome number and morphology were studied in somatic cells of parthenites from intermediate host - mollusc *Valvata piscinalis* using an air drying technique and Giemsa staining. The karyotype consisted of 20 chromosomes comprising five pairs of subtelo-acrocentric (1st to 5th), two pairs of submeta-subtelocentric (6th-7th), one submetacentric (9th) and two pairs of metacentric chromosomes (8th, 10th). A supernumerary chromosome variation (presence/absence of B-chromosome) and occurrence of triploidy in one population of *I. platycephalus* are reported. In addition, the karyological analysis of Lithuanian population of *I. variegatus* was carried out. In the light of presented and previously obtained data comparative karyology of genus *Ichthyocotylurus* is discussed.

KEY WORDS : karyotype, Trematoda, *Ichthyocotylurus*, triploidy, B-chromosomes.

Résumé : ÉTUDE CHROMOSOMIQUE DE *ICHTHYOCOTYLURUS PLATYCEPHALUS* (CREPLIN, 1825) ODENING 1969 AVEC LA DESCRIPTION DE LA FORME TRIPLOÏDE ET LA CARYOLOGIE COMPARÉE DU GENRE *ICHTHYOCOTYLURUS*

Cette étude présente la première description du caryotype d'une espèce de Trématode, Ichthyocotylurus platycephalus *(Creplin, 1825) Odening 1969 ainsi que le caryotype de* I. variegatus *non connu auparavant pour les populations lituaniennes. Le nombre et la morphologie des chromosomes ont été étudiés sur les cellules somatiques des formes larvaires obtenues à partir des hôtes intermédiaires, les mollusques* Valvata piscinalis, *par une méthode de suspension cellulaire et coloration au Giemsa. L'analyse effectuée a établi que le nombre diploïde de* I. platycephalus *varie de 20 à 21, à cause de la présence chez certains individus d'un chromosome surnuméraire, ou chromosome B. La garniture chromosomique basique (les chromosomes A) comprend 10 paires dont cinq sont subtélo-acrocentriques (1 à 5), deux subméta-subtélocentriques (6 et 7), une submétacentrique (9) et deux métacentriques (8 et 10). En outre, un cas de triploïde (2N = 3X = 30) a été détecté chez un spécimen de* I. platycephalus. *Les données obtenues ont été comparées et discutées aves celles disponibles pour le genre* Ichthyocotylurus.

MOTS CLÉS : *caryotype, Trématodes,* Ichthyocotylurus, *triploidie, chromosomes surnuméraires.*

INTRODUCTION

The species of the genus *Ichthyocotylurus* for a long time have been considered as belonging to the genus Cotylurus (Szidat, 1928). More recent investigations on the life cycles of species of *Cotylurus* showed that this genus includes two groups of species, differing from each other in the biology, life cycle and morphology of larval stages and the adults. Odening *et. al.* (1969) created within it two subgenera: Cotylurus (set of hosts involved: Pulmonata – snails and leeches – anatine birds) and *Ichthyocotylurus* (Prosobranchia – fishes – piscivorous birds). Niewiadomska (1971) compared differences between these two subgenera with differences existing among the other genera within the family Strigeidae and elevated them to the rank of valid genera.

The taxonomic status of species within the genus *Ichthyocotylurus* has provided considerable discussion. It is difficult to define the set of diagnostic features, which could well separate the species in various stage of development. If validity of *Ichthyocotylurus (Cotylurus) pileatus* (Rudolphi, 1802), *I. (C.) erraticus* (Rudolphi, 1809), *I. (C.) platycephalus* (Creplin, 1825) was undoubtfull for many systematisists (Odening *et. al.*, 1969; Olson, 1970; Sudarikov, 1984), it was otherwise with *I. variegatus*. Dubois (1938) included *I. (C.) variegatus* as synonym of *I. (C.) pileatus*. Odening & Bockhardt (1971) after the elaborating of complicated history and synonymy of metacercaria and adult stated that "*C. variegatus* (= *cumulitestis*) is neither identical with *C. platycephalus* (= *cuculus*), nor with *C. pileatus* just as little as with *I. erraticus*. *I. variegatus* is a species of its own, differing from *I. platycephalus*".

One of the recent efforts to elucidate the specific structure of genus was made by Dubois (1978). On the base

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of original material and museum samples he stated the identity of *I. platycephalus* and *I. variegatus* owing to insufficiency of the differences recorded in cercariae of *I. platycephalus* and *I. variegatus* for good identification of them. On the contrary, Odening (1979) affirmed the species of *I. platycephalus* and *I. variegatus* as valid based oneself on structure of adults, cercariae, metacercariae and localization of adults in the definitive hosts. But it is clear that morphological differences among the various stages of species of *Ichthyocotylurus* are rather vaguely distinctive. In many digenean species the larvae lack distinctive morphological features. Recent studies have emphasised the value of a multidisciplinary approach in resolving taxonomic problems for various group of organisms. Molecular methods can offer a new tool for larval-stage identification (Jousson *et al.*, 1998). The karyotype is one of the basic genetic characteristics of eukaryotic species. In recent years, more than once cytogenetic studies were a good supplement for morphological, biological and other characteristics used for systematic analysis (Baršienė, 1993; Insua *et al.*, 1994). Cytogenetic parameters such as chromosome number and morphology provide investigatory tools to define phylogenetic patterns and genetic structure of species and population (White, 1973).

Until now, chromosomal data were restricted to three species of *Ichthyocotylurus-I. erraticus, I. pileatus* and *I. variegatus* presenting different karyotypes with 2 n = 20 (Baršienė *et al.*, 1990; Bell *et al.*, 1998).

I. platycephalus (Creplin, 1825) is type species of genus *Ichthyocotylurus*. This species as well as *I. variegatus* are fairly common parasites of fish in Lithuanian water bodies (Rauckis, 1988). There are recorded metacercariae of four *Ichthyocotylurus* spp. as parasites of fish in Lithuania. So far there are poorly investigated adult forms, parasiting birds of Lithuania. Only adults of *I. platycephalus* and *I. pileatus* are recorded in Lithuanian water birds (Volskis & Pilipavičiūtė, 1970). Herein, we present the karyotypical data on *I. platycephalus* after conventional staining. A supernumerary chromosome variation and fact of triploidy in population of *I. platycephalus* are reported. In the course of a comparative investigation we report also the karyological analysis of Lithuanian population of *I. variegatus*.

MATERIALS AND METHODS

Cercariae of two species of *Ichthycotylurus* were identified in molluscs of Lithuanian water bodies – *I. platycephalus* (Creplin, 1825) Odening 1969 and *I. variegatus* (Creplin, 1825) Odening 1969. Naturally infected first intermediate hosts – the mollusc *Valvata piscinalis* (Prosobranchia) were collected from May to October on 1994-1998. Parasite identification was confirmed from the morphology of cercariae. Cercariae were examined alive. Four specimens of *V. piscinalis* with parthenites of *I. platycephalus* out of 328 (extensiveness of invasion – 1.22 %) examined in the samples, were collected from river Vilnele and were used as a source of the material for investigation. Also 322 specimens of molluscs *V. piscinalis* were collected from the lake Asveja. Intramolluscan larval stages of the *Ichthyocotylurus* spp. isolated from 22 (extensiveness of invasion – 6.83 %) naturally infected molluscs were used for the cytogenetic analysis. Two species of *Ichthyocotylurus* have been identified here – *I. platycephalus* and *I. variegatus*. 20 specimens were infected with parthenites of *I. platycephalus* (6.21 %) and only two specimens (0.62 %) – with parthenites of *I. variegatus*.

Infected molluscs were treated with 0.01 % colchicine in well water at 22° C for three to 15 hours. Dissected digestive glands, containing sporocysts and developing cercariae were transferred to distilled water for 40-50 minutes for hypotonic treatment, then fixed in freshly prepared and cold solution of ethanol – acetic acid (3:1). Fixation involved three changes (30 minutes, one hour and 24 hours) with storing at 4° C.

Each slide was made from a single snail using an air-drying technique (Kligerman & Bloom, 1977) with some modification. Small pieces of tissue were removed from the fixative on pre-cleaned slides, briefly soaked in some drops of 45 % acetic acid, smeared and flame-dried by holding the slide over a bunsen burner. Then slides were stained with Giemsa (4 %, pH 6.8) for 40 minutes. Phothomicrograps of well spread metaphases were taken with an Amplival photomicroscope under an oil-immersion system. For karyotyping, chromosomes were cut out of the photomicrograps and paired on the basis of their size and centromeric position. Morphometric measurements of chromosomes in the karyotypes of 10 metaphasic cells of each species studied were made. Absolute length in µm with standard deviation were calculated. Then relative length or percent total complement length was expressed as 100 times the absolute chromosome pair length divided by the total length of the haploid complement. The length of the B-chromosome was not included in the total length of haploid complement. Centromeric indexes were calculated by dividing 100 times the length of the short arm by the total chromosome length. Chromosome morphology was defined in accordance with chromosome nomenclature of Levan *et al.* (1964).

RESULTS

Chromosome data were obtained from parthenites of *I. platycephalus* taken from 14 specimens of *V. piscinalis*. The investigated specimens pre-

sented diploid number varying from 2 n = 20 to 21 due to presence of extra small or medium-sized subtelocentric or submetacentric chromosomes considered as supernumeraries (Bs).

The basic karyotype with 2 n = 20 was described from mitotic metaphases of somatic cells of *I. platycephalus* taken from eight specimens of *V. piscinalis* (Fig. 1). A diploid number was scored in 89 somatic cells (89.9 %). Eight cells had an aneuploid number of chromosomes, two cells had a tetraploid set. The total length of the haploid genome – 31.85 µm. Chromosomes range in size from 1.72 to 5.19 (m (Table I). The 1st-5th pairs of comparatively large chromosomes (66-67 % of the total genome complement length) represented the single-armed - subtelo-acrocentric genome units. According to the centromere position, homologues of pair 6th and 7th were observed as submeta-subtelocentric, and those of pair 9th as submetacentric. The pairs 8th and 10th are a clearly recognizable metacentric.

Six snails collected in Asveja were infected with parthenitae of *I. platycephalus* containing 21 chromosomes in the diploid sets of 217 cells out of 285 studied. The morphology of this supernumerary chromosome was different in metaphase plates from various individuals (Fig. 1). The small subtelocentric to submetacentric supernumerary chromosome (Bs) was found in chromosome sets of *I. platycephalus* from four snails. The medium-sized submetacentric supernumerary chromosome was found in examined mitotic metaphases of *I. platycephalus* from two snails. The Bs were clearly distinguishable from the basic chromosomes in the Giemsa staining metaphases (non-homologous to the regular chromosomes). The measurements of chromosomes of basic set and additional chromosomes are given in Table I.

We have observed chromosomal preparation of the intramolluscan stages of *Ichthyocotylurus* prepared from one snail collected in Vilnele in which discovered that many of cells contained 30 instead of the normal 20 chromosomes. Chromosome numbers were determined only from cells in which the entire cell was visible as in Figure 2. Thus, the karyotype was identified as belonging to one cell and not due to an artefact of the preparation. The 30 chromosomes were compared with diploid (2 n = 20) set of *Ichthyocotylurus* – the karyotype was similar to that of *I. platycephalus*. The 30 chromosomes appeared to consist of three sets of haploid (n = 10) *I. platycephalus*. 3 n = 30 was found in 23 (63.89 %) mitotic metaphases and the remaining 13 (36,11 %) being aneuploid – 3 n = 26-29. Parthenites of the triploid forms was morphologically similar to that of diploid.

Chromosomes of 116 mitotic metaphases of somatic cells of *I. variegatus* taken from two specimens of *V. piscinalis* were examined (Fig. 3). Counts of 100 cells (86.21 %) resulted in the diploid number 2 n = 20, 15 cells (12.93 %) resulted as hypoploid – 2 n = 17-19 and only one cell has a hiperploid number of chro-

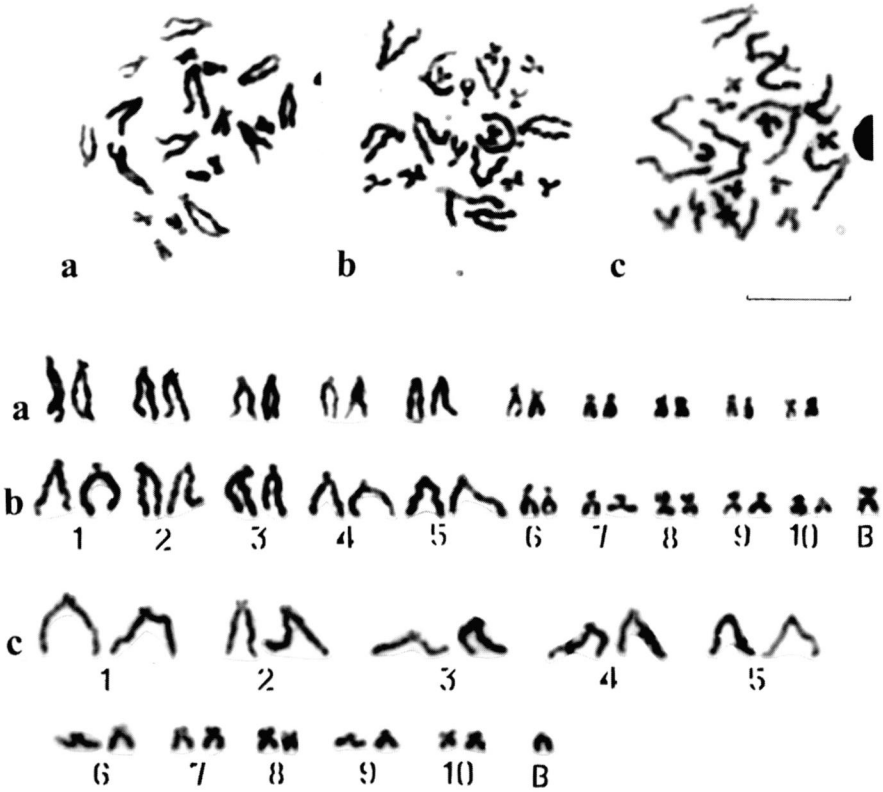


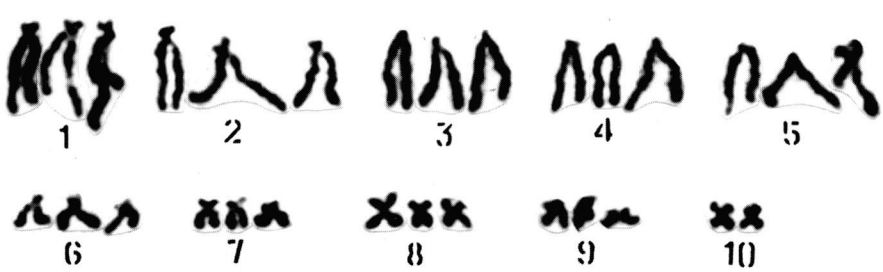
Fig. 1. – Mitotic metaphases and karyotypes of karyomorphs of *Ichthyocotylurus platycephalus* with 2 n = 20 (a) and 2n=21 (b, c). Scale bar = 10 µm.

Chromosome number		Aboslute length (µm)	Relative length (%)	Centrometric indices	Classification
1	A	5.19 ± 1.02	16.22 ± 0.96	13.39 ± 1.71	st-a
	B	5.49 ± 0.84	17.05 ± 0.80	12.03 ± 1.36	st-a
2	A	4.72 ± 0.89	14.79 ± 0.64	13.04 ± 2.08	st-a
	B	4.81 ± 0.81	14.93 ± 1.00	12.39 ± 3.16	st-a
3	A	4.22 ± 0.78	13.22 ± 0.74	12.63 ± 2.56	st-a
		4.20 ± 0.45	13.09 ± 0.52	13.05 ± 1.86	st-a
4	A	3.88 ± 0.70	11.90 ± 0.81	12.55 ± 2.89	st-a
	B	3.86 ± 0.55	12.01 ± 0.44	13.64 ± 2.45	st-a
5	A	3.44 ± 0.79	10.69 ± 0.85	12.79 ± 2.96	st-a
	B	3.56 ± 0.48	11.06 ± 0.42	11.39 ± 2.24	a-st
6	A	2.61 ± 0.42	8.23 ± 0.32	28.52 ± 4.20	sm-st
	B	2.31 ± 0.28	7.21 ± 0.39	26.15 ± 5.52	sm-st
7	A	2.12 ± 0.31	6.73 ± 0.76	28.50 ± 4.03	sm-st
	B	2.10 ± 0.23	6.64 ± 0.47	27.87 ± 3.02	sm-st
8	A	2.02 ± 0.33	6.37 ± 0.40	45.74 ± 1.73	m
	B	1.99 ± 0.17	6.23 ± 0.35	45.01 ± 1.89	m
9	A	1.93 ± 0.23	6.12 ± 0.52	32.23 ± 3.25	sm
	B	1.96 ± 0.16	6.16 ± 0.31	34.77 ± 2.88	sm
10	A	1.72 ± 0.18	5.47 ± 0.61	46.60 ± 2.53	m
	B	1.81 ± 0.19	5.68 ± 0.46	47.41 ± 1.20	m
Bs(b)		2.97 ± 0.18		37.66 ± 5.21	sm-m
Bs(c)		1.91 ± 0.19		23.33 ± 4.17	st-sm

Abbreviations: A, measurements of karyomorph with 2 n = 20; B, measurements of karyomorph with 2 n = 21; Bs(b), Bs(c), variation of supernumerary chromosomes; m, metacentric; sm, submetacentric; st, subtelocentric; a, acrocentric chromosomes.

Table I. – Measurements (means ± SD) and classification of two karyomorhs of *Ichthyocotylurus platycephalus*.

Fig. 2. – Mitotic metaphase and karyotype of triplod form of *Ichthyocotylurus platycephalus* with 3 n = 29. Scale bar = 10 µm.



Chromosome number	Absolute length (µm)	Relative length (%)	Centrometric indices	Classification
1	5.88 ± 1.56	16.23 ± 0.82	47.35 ± 2.09	m
2	5.48 ± 1.50	15.09 ± 0.48	12.56 ± 3.11	st-a
3	4.88 ± 1.32	13.46 ± 0.65	11.90 ± 2.66	a-st
4	4.29 ± 1.10	11.86 ± 0.48	13.66 ± 3.48	st-a
5	3.85 ± 0.88	10.53 ± 0.78	12.91 ± 2.23	st-a
6	2.83 ± 0.75	7.81 ± 0.35	28.46 ± 4.38	sm
7	2.52 ± 0.62	6.95 ± 0.49	26.54 ± 3.21	sm-st
8	2.36 ± 0.61	6.51 ± 0.39	35.96 ± 3.41	sm-m
9	2.14 ± 0.47	5.96 ± 0.45	46.39 ± 2.73	m
10	1.95 ± 0.45	5.42 ± 0.37	45.52 ± 1.89	m

Abbreviations: m, metacentric; sm, submetacentric; st, subtelocentric; a, acrocentric chromosomes

Table II. – Measurements (means ± SD) and classification of chromosomes of *Ichthyocotylurus variegatus*.

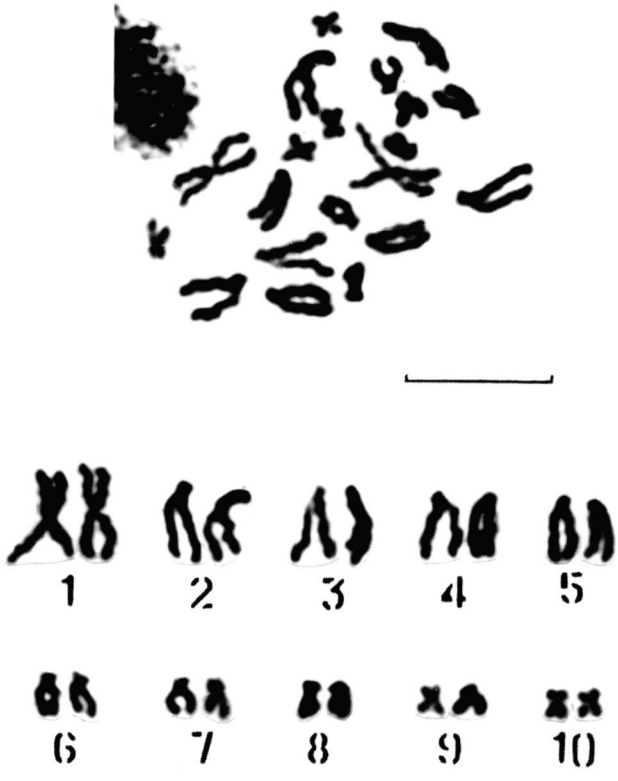


Fig. 3. – Mitotic metaphase and karyotype of Lithuanian population of *Ichthyocotylurus variegatus*. Scale bar = 10 µm.

mosomes. The total length of the haploid genome – 36.18 µm. Chromosomes range in size from 1.95 to 5.88 µm (Table II). The karyotype consisted of one (1st) pair of large and two small (9th and 10th) pairs of metacentric chromosomes; one pair (6th) of submetacentric and (8th) submeta-metacentric chromosomes; four mono-armed (pairs 2-5 represent subtelo-acrocentic units). Chromosomes of 7th pair represented by submeta-subtelocentic chromosomes.

DISCUSSION

Our current knowledge on cytogenetics of various species of family Strigeidae reveals one type of chromosome complement with a clear distinction between five comparatively large and five smaller chromosome pairs (Baršienė, 1993; Petkevičiūtė & Stanevičiūtė, 1999). The same pattern of karyotype is characteristic also for most of karyologically studied representatives of family Diplostomidae (Baršienė & Stanevičiūtė, 1991; Baršienė, 1993; Stanevičiūtė *et al.*, 1998). In recent years karyologically investigated three species of *Ichthyocotylurus* presented the same diploid number 2 n = 20, but showed three different karyotypes (Baršienė *et al.*, 1990; Bell *et al.*, 1998). For the first time reported here karyotype of *I. platycephalus* display a typical strigeid pattern with 20 chromosomes, arranging into two distinct size groups. For the comparative karyology the idiograms of all known karyotypes of genus *Ichthyocotylurus* were constructed (Fig. 4), based on the mean values of relative length and centromeric indices presented in Tables I and II, as well as previously published data (Baršienė *et al.*, 1990; Bell *et al.*, 1998). The subtelocentric type is dominant in the first group of chromosomes. Groups of the small genome units are composed dominantly of submeta- and metacentric chromosomes. Similarity of chromosome relative length among the species showed that this parameter cannot well discriminate inside the genus. In all species studied, a more significant difference was found for centromeric index between corresponding chromosome pairs. The considerable interspecific differences were observed in the morphology of the large elements. The large pericentric inversions occurred in the process of speciation of *Ichthyocotylurus* rather frequently (1st pair of *I. variegatus*, *I. erraticus*, 2nd pair of *I. erraticus*). Such large pericentric inversion in the process of speciation within

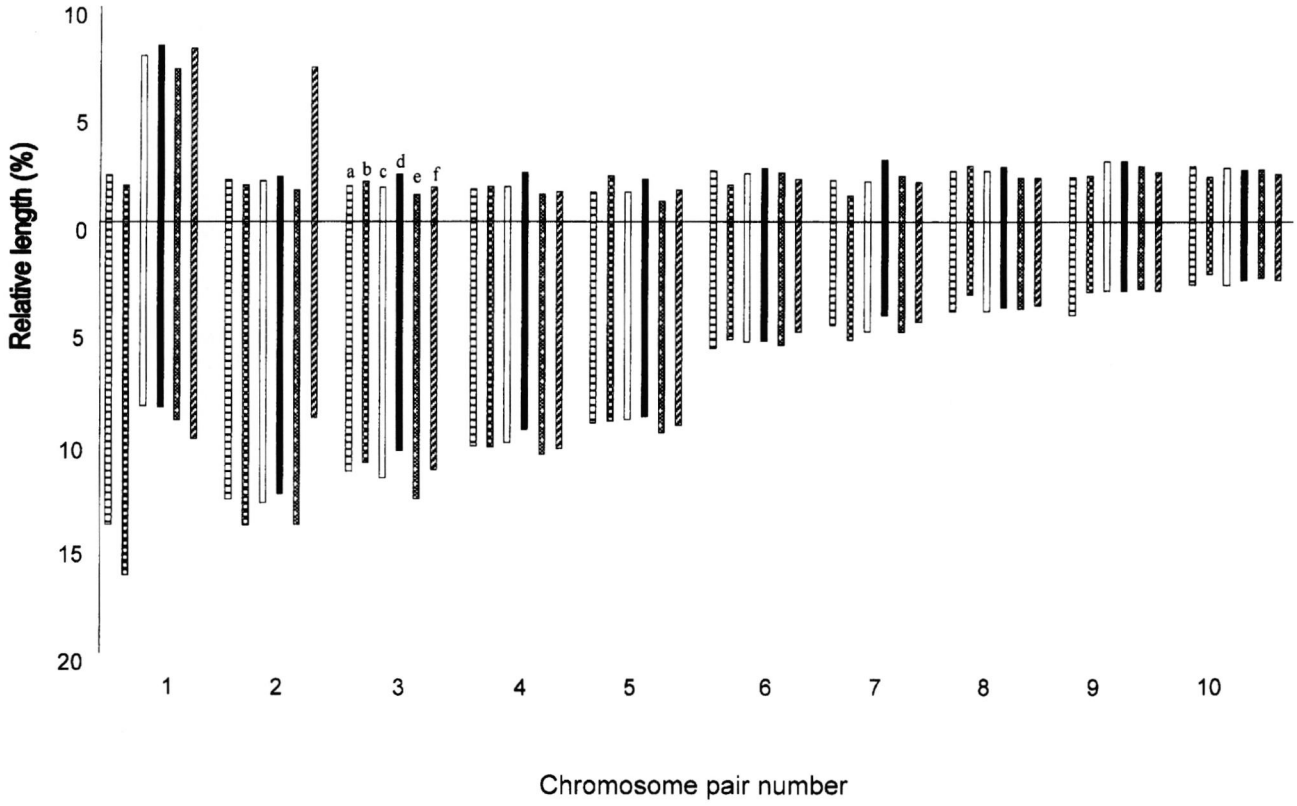


Fig.4. – Idiograms representing the haploid sets of genus *Ichthyocotylurus*: a, *I. platycephalus*; b, *I. pileatus* (data of Baršienė *et al.*, 1990); c, *I. variegatus*; d, *I. variegatus* (data of Bell *et al.*, 1998); e, *I. erraticus* (data of Baršienė *et al.*, 1990); f, *I. erraticus* (data of Bell *et al.*, 1998).

the genus are registered in the families Diplostomidae (*Diplostomum spathaceum*), Strigeidae (*Apatemon gracilis*) (Baršienė & Stanevičiūtė, 1991; Petkevičiūtė & Stanevičiūtė, 1999). Chromosome complements of *I. variegatus* and *I. erraticus* possess a pair of large metacentric chromosomes, at that time *I. platycephalus* and *I. pileatus* have not such pair of chromosomes. So, chromosome sets of *I. variegatus* – *I. erraticus* and *I. platycephalus* – *I. pileatus* seem respectively closer. Species *I. platycephalus* and *I. pileatus* show slight interspecific differences in the relative length. The difference between the length of the longest and the smallest pair in *I. platycephalus* reaches three times, meanwhile in *I. pileatus* this difference reaches four times. The first five pairs of chromosomes of *I. platycephalus* constitute 66-67 %, corresponding chromosomes of *I. pileatus* – 70-71 % of the total complement length. Comparative cytological analysis (by Student's t-test, $P < 0.05$) of these species demonstrated statistically significant differences in the I^C values in chromosome pairs 5, 7 and 9.

Geographical karyotypic variation was found in karyotype of *I. variegatus*. Bell *et al.* (1998) has studied the karyotype of *I. variegatus* population in Scotland. Although no differences were revealed in the measurements of the relative length of chromosomes, the two populations differ in the position of centromere in chromosome pair 7 (I^C = 26.54 according our data and I^C = 39.4 according data from Scotland). Such remarkable difference may be caused by small chromosome rearrangement, most probably pericentric inversion. Slight differences were found in the I^C values of three, four and five pairs of subtelocentric chromosomes, which we associated with the variability of the short arms and with their small dimensions exactly like subjectivity in the metrical evaluation of chromosomal characteristics. Small changes may arise in the centromeric index values due to probable differential condensation of chromosomes. Chromosome condensation is a dynamic process that proceeds at different rates along the length of each chromosome (Francke & Oliver, 1978). The variation in the centromeric index values may be greater in chromosomes with unequal arms. Recorded distinct difference of I^C values of 2nd pair between *I. erraticus* from North-West Chukotka (Baršienė *et al.*, 1990) and Scotland populations was interpreted by authors as interpopulation variation from geographically distant samples or inadequancy and not conspecificity of investigated species (Bell *et al.*, 1998).

Since only recently genus *Ichthyocotylurus* was elevated to the rank of valid genera (Niewiadomska, 1971) it is interesting to compare cytogenetic data of this genus and genus *Cotylurus*. So far, chromosome numbers are reported for four species of genus *Cotylurus* (Petkevičiūtė, 1992; Baršienė, 1993). All species presented the typical strigeid karyotype with diploid chromosome number 2 n = 20. The uniform generic marker has not been singled out in the chromosome set structure among investigated species of genus *Cotylurus* and *Ichthyocotylurus*. The karyotypes of genus *Cotylurus* more frequently are notable for the prevalence of uniarmed chromosomes in both groups of genome elements. With data now available it can be stated that trematodes are karyologically conservative and their karyotypes tend to have the same number and closely related gross chromosome morphology on the genus and even on family taxonomic level (Baršienė, 1993; Stanevičiūtė, 1994).

It should be mentioned an interesting and rather rare fact of triploidy in the metaphase plates from one snail infected with parthenites of *I. platycephalus*. In the Trematoda there are few reports of triploid organism. Triploidy have been reported for *Schistosomatium douthitti* – 3 n = 21 (Short & Menzel, 1959), *Fasciola sp.* – 3 n = 30 (Sakaguchi & Nakagawa, 1975), *Paragonimus westermani* – 3 n = 33 (Sakaguchi & Tada, 1976; Hirai *et al.*, 1985), *Allocreadium fasciatusi* – 3 n = 21 (Ramanjaneyulu & Madhavi, 1984), *Apatemon minor* – 3 n = 30 (Petkevičiūtė, 1992), *Diplostomum* sp. – 3 n = 30 (Stanevičiūtė, 1994), *Schistosoma mansoni* – 2 n = 24 (Hirai & LoVerde, 1989). The presented data suggested that natural triploids might not be so uncommon. The observation of C-banding patterns (Hirai *et al.*, 1985) suggested that the triploid *P. westermani* form was allotriploid, which means composed of two genome set introduced from *P. westermani* (2 n) and another unidentified set. Triploidy in *A. fasciatusi* (Ramanjaneyulu & Madhavi, 1984) was a result of duplication of the chromosome sets rather than fragmentation of the chromosomes. Short & Menzel (1959) suggested that the presence of triploid genomes implied that a diploid complement was present in the egg before fertilization and argued strongly in favour of diploid virgin eggs as a result of meiotic irregularity. In the report of Hirai & LoVerde (1989) triploid *S. mansoni* seems to be determined by either an abnormal egg or sperm resulted from nondisjunction during meiotic division and formed a zygote with either a normal sperm or egg. So the origin of triploidy in most cases it is considered to be the result of fertilization (diploid egg or sperm), then a miracidial embryo with triploid constitution could be produced. Successful infection of a snail with such a miracidium would result in the production through asexual division of thousands of cercarial embryos (Hirai & LoVerde, 1989).

B-chromosomes are elements extra to the normal complement (A chromosomes) which have been found in all major groups of plants and animals and vary in numbers between individuals of a species (Camacho *et al.*, 1997) Beukeboom *et al.*, 1998). It has been estimated that they occur in 10-15 % of eukaryotes (Bell & Burt, 1990). Thus, it is likely that many more species, when analysed with sufficient intensity, will be found to possess Bs. The function and composition of Bs is still a controversial question. Their frequencies in populations are determined by their transmission rates and effects on host fitness. In most cases, they appear to have negative effects on the phenotypic fitness of their host when they occur in high number. There are some models concerning the effects of Bs at low numbers (Beukeboom *et al.*, 1998). Under the heterotic model Bs are considered to have positive fitness effects (White, 1973). The parasitic model assumes that Bs are genomic parasites (Bell & Burt, 1990; Beukeboom *et al.*, 1998). Camacho *et al.* (1997) considers the Bs in the grasshopper *Eyprepocnemis plorans* to be close to the selective neutrality ("near-neutral" model).

Reports on the occurrence of B-chromosomes (Bs) in Trematoda are still scarce. Until now species with occuring Bs are composed aproximately 5 % of all cytogenetically known species of Trematoda. The Bs were recently described in three studied species of the genus *Apatemon* (Baršienė, 1993; Petkevičiūtė & Stanevičiūtė, 1999), also in some species of genera *Notocotylus* (Petkevičiūtė & Baršienė, 1988; Petkevičiūtė *et al.*, 1989a; Baršienė, 1993), *Diplodiscus* (Petkevičiūtė *et al.*, 1989b), *Echinostoma* (Baršienė, 1993). The diploid number variation in species of Trematoda were determined by including more frequently one or two (rare more) Bs of different morphologies and sizes (from rather large metacentric to small acrocentric) in the cells. In most cases Bs were mitotically stable.

In summary, exhibited distinct differences in the chromosome morphology of the investigated *Ichthyocotylurus* spp. showed these as species specific. Species differ mainly by centromeric index between corresponding chromosome pairs and such differences let support the assumption about chromosome rearrangement involved in their karyotypic evolution, i.e. mechanisms not affecting the chromosome number - pericentric inversions, small translocations and deletions. Presented data of cytological investigation of *Ichthyocotylurus* spp. obviously negate the posibility of synonymy *of I. variegatus* and *I. pileatus* as well as *I. variegatus* and *I. platycephalus* and contradict with statement of Dubois concerning identity of them (Dubois, 1938, 1978). At that time these data support the point of view of Odening concerning the validity of these species (Odening & Bockhardt, 1971; Odening, 1979). Our data could serve as a useful start to further inves-

tigations together with molecular, morphological and ecological analyses of genus *Ichthyocotylurus*.

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