FOLIA PARASITOLOGICA 39: 324–326, 1992.

THE KARYOTYPE OF *PROTEOCEPHALUS PERCAE* (CESTODA: PROTEOCEPHALIDAE)

The tapeworms of the genus *Proteocephalus* Weinland, 1858 are frequent parasites of many freshwater fishes. The taxonomy of *Proteocephalus* species is very complex because of the extraordinary difficult morphological species differentiation (Anikieva L. V. et al. 1983: Ecological analysis of coregonid parasites, Nauka Leningrad, 168 pp., in Russian; Chubb J. C. et al. 1987: J. Fish Biol. 31: 517–543); thus, new marker characters are endeavoured to be found. Karyotypes seem to represent such class of characters, and therefore, the karyotype of. *P. percae* (Müller, 1780) was described. This is the first cytogenetically investigated species within the family Proteocephalidae.

Cestodes were obtained by the dissection of perch (*Perca fluviatilis* L.) from the Ružín reservoir (Eastern Slovakia, CSFR) in January 1991. Five living adult individuals were placed in 0.025% colchicine in hypotonic solution (0.6% sodium citrate) for 16 h at 4 °C. Both immature and mature segments of all the five specimens were used together for the further analysis. They were mechanically disrupted in hypotonic solution of sodium citrate so that most of cells in the reproductive organs were released. The cells have been then fixed in methanol : acetic acid (3 : 1) for 1 hour. The preparations were made by air-dried method (Mutafova T. and Vasilev I. 1982: Khelminthologia, Sofia, 14 : 70–76) and stained with 4% Giemsa in a phosphate buffer pH 6.8 for one hour. Ten metaphase plates were used for karyometric analysis. The classification of chromosomes followed that of Levan A. et al. (1964: Hereditas 52: 201–220).

Thirty five metaphase cells were analyzed. Most of them (88.6%) consist of 18 chromosomes (Fig. 1) in the diploid set, the other were aneuploid (nullisomic, 2n = 16). All chromosomes were contracted, their absolute lengths ranged from 1.0 to 2.7 μm. It is likely that the size of chromosomes could have been influenced by a 16-hour exposure of live worms to colchicine. As a matter of fact, colchicine has been known to bear on the condensation of chromosomes. This procedure resulted from our effort to collect fairly large amount of cells in a metaphase stage, since with a shorter exposure to colchicine these cells were only rarely observed.

Homologues of distinct pairs of chromosomes were not easily identifiable according to their size and morphology. Only the first, largest pair differed significantly in its size from the second one (Table 1). Some of the cells studied (43.6%) displayed moderate dimorphism of the first pair of chromosomes (Fig. 1), with one chromosome longer and fairly submetacentric and the second metacentric. Chromosomes of the pair No. 1 of the other cells were identical (Fig. 2). The length of remaining chromosomes decreased gradually. They were meta- or submetacentric, only the pairs Nos. 5 and 7 were subtelocentric (Fig. 3, Table 1). It was difficult to be sure of the centromere position in some cells because of the contracted state of the chromosomes.

A survey of the available literature revealed that only some of the cestode species from freshwater fishes have been investigated. In the family Proteocephalidae, no chromosome data for any species exist. From the other taxa of this group, diploid numbers of 20 species of the order Caryophyllidea are 6, 12, 14, 16, 18 and 20 and this range is combined with the triploids with a maximum of 30 chromosomes (Mackiewicz J. S. 1982: Parasitology 84: 397–417). Grey and Mackiewicz (1974: Exp. Parasitol. 36 : 159–166) have reported that unlike any other cestodes, the chromosomes of caryophyllids can be studied with relative ease and are of the large size up to 9 μm.

The karyotypes of seven species of the order Pseudophyllidea are known. Three of them, *Diphyllobothrium latum* (L.), *D. osmeri* Linstow and *D. dendriticum* (Nitzsch) have 18 small, biarmed chromosomes (Wikgren B. P. and Gustafsson M. K. S. 1965: Acta Acad. Aboesis, Series B, 25 : 1–12). Petkevičiuté R. et al. (1991 a, b: Int. J. Parasit. 21: 11–15, ibidem: 125–127) found that *Triaenophorus nodulosus* (Pallas) possessed 26, *T. crassus* Forel 18 and *Eubothrium rugosum* (Batsch) 16 mainly biarmed but relatively large chromosomes (maximum length 6.75, 8.50, and 8.80 μm, respectively).

Table 1. Measurements (means ± SD) and classification of chromosomes of *Proteocephalus percae*

Chromosome No.	Relative length (%)	Centromeric index	Classification
1	16.79 ± 1.42	43.27 ± 4.07	m
2	13.89 ± 1.14	45.04 ± 1.41	m
3	12.64 ± 0.73	45.81 ± 3.05	m
4	11.72 ± 0.93	39.73 ± 4.15	m–sm
5	11.22 ± 0.65	15.55 ± 4.74	st–t
6	10.58 ± 1.27	43.80 ± 4.61	m
7	9.24 ± 0.64	19.36 ± 4.68	st
8	7.74 ± 1.44	43.69 ± 1.97	m
9	6.27 ± 0.65	33.87 ± 2.93	sm

Classification of chromosomes: m – metacentric, sm – submetacentric, st – subtelocentric, t – telocentric

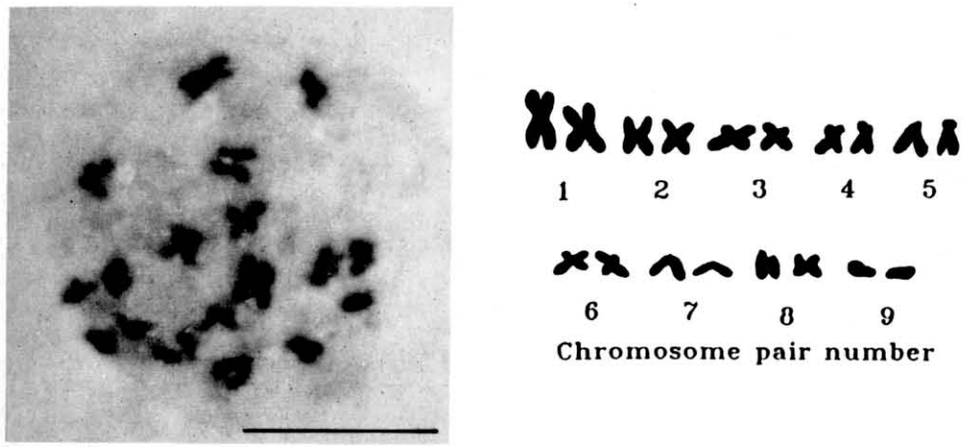

Fig. 1. – Mitotic chromosomes of *Proteocephalus percae*. Scale bar = 10 μm.

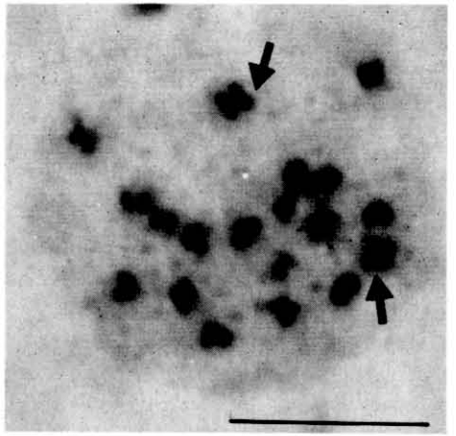

Fig. 2. – Contracted mitotic chromosomes. Scale bar = 10 μm. Arrows show chromosomes No. 1.

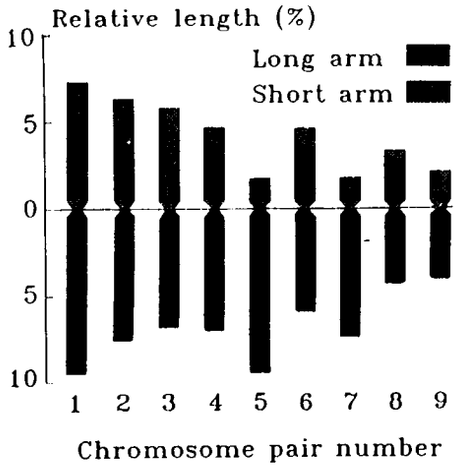

Fig. 3. – Idiogram of *Proteocephalus percae.*

Bothriocephalus acheilognathi Yamaguti has 14 biarmed, small chromosomes (maximum length 2.7 μm), with the size polymorphism of chromosomes of the 4th pair (Nedeva I. and Mutafova T. 1988: Khelminthologia, Sofia, 26: 39–46).

Like the most of cestode karyotypes known, *P. percae* has mainly biarmed chromosomes – 7 pairs of 9 are meta- or submetacentric, and all of them are very small (contracted). The slightly different chromosomes of pair No. 1 observed in some cells of *P. percae* seem to be analogous with similar phenomenon described by Nedeva and Mutafova (op. cit.) in *B. acheilognathi.* Also in the gonochoric cestode *Shipleya inermis* Fuhrmann (family Dioecocestidae), Rausch R. L. and Rausch V. R. (1990: Ann. Parasitol. Hum. Comp. 65: 229–237) have reported that in females the chromosomes of pair 1 were slightly heteromorphic. They suggested that females produced gametes of two types relative to heterochromatic DNA, while males were homogametic, and that sex-determining effects were associated therein. In the case of the hermaphroditic cestodes – bothriocephalids and proteocephalids – this phenomenon cannot be properly explained. It may be supposed that since the largest chromosome of *P. percae* in our material could have been affected by colchicine, it can contract more and have more variation giving the appearance of dimorphism.

M. ŠPAKULOVÁ and V. HANZELOVÁ
Helminthological Institute,
Slovak Academy of Sciences,
Košice, Czechoslovakia

Received 16 January 1992 Accepted 22 April 1992