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CHROMOSOME ANALYSIS OF *PROTEOCEPHALUS OSCULATUS* (CESTODA: PROTEOCEPHALIDEA)

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The cestode order Proteocephalidea Mola, 1928 is dominated by the cosmopolitan genus *Proteocephalus* Weinland, 1858, which includes about 40% of its nominal species. *Proteocephalus* spp. are found in freshwater fishes, amphibians, and reptiles (Freze V.I. 1965: Proteocephalideans – Tapeworm Helminths of Fish, Amphibians and Reptiles. Essentials of Cestodology, Vol V. Publ. House Nauka, Moscow, 540 pp., in Russian; Brooks D.R. 1978: Syst. Zool. 27: 312-323; Brooks D.R., Hoberg E.P., Weekes P.J. 1991: Proc. Biol. Soc. Wash. 104: 651-668). There is presently a lack of agreement on the taxonomic status of many *Proteocephalus* species. Brooks et al. (Brooks et al. 1991, op. cit.) have suggested that various species currently assigned to *Proteocephalus* may be more closely related to a variety of different groups than to each other. The recent comparative studies based on morphological features and molecular systematic analysis have demonstrated a general uniformity among most *Proteocephalus* spp. in Europe (Scholz T., Hanzelová V. 1998: Tapeworms of the Genus *Proteocephalus* Weinland, 1858 (Cestoda: Proteocephalidae), Parasites of Fishes in Europe, Academia, Praha, 118 pp.; Zehnder M.P., Mariaux J. 1999: Int. J. Parasitol. 29: 1841-1852). On the other hand, a high degree of intraspecific morphological variability has recently been found to occur within Palaearctic *Proteocephalus* spp. (Hanzelová V., Špakulová M. 1992: Folia Parasitol. 39: 307-316; Anikieva L.V. 1993: Parazitologiya 27: 260-268; Anikieva L.V. 1995: Parazitologiya 29: 505-510; Hanzelová V., Šnábel V., Král'ová I., Scholz T., D'amelio S. 1999: Can. J. Zool. 77: 1450-1458). Due to the presence of high intraspecific variability and the scarcity of taxonomically useful morphological characters, cytogenetics may be a useful tool to differentiate species and clarify their phylogenetic relationships.

Proteocephalus osculatus (Goeze, 1782) is a specific parasite of the European wels (*Silurus glanis* L.) and is distributed throughout its host's Eurasian range (Freze 1965, op. cit.; Dubinina M.N. 1987: Class Cestoda Rudolphi, 1808. In: O.N. Bauer (Ed.), Key to the Parasites of Freshwater Fishes. Vol 3. Publ. House Nauka, Leningrad, pp. 5-76, in Russian). This study provides the first description of the karyotype of *P. osculatus* based on the analysis of colchicine-treated mitotic chromosomes.

Ten specimens of *P. osculatus* used for karyological exami-nation were recovered from the gut of European wels (*S. glanis*) caught in Curonian Bay near Ventė settlement (Lithuania) in June 1999. Whole living specimens were placed in 0.01% colchicine in physiological solution for 3-4 h at room temperature. Fixation involved three changes (20 min each) of a freshly prepared solution of ethanol-acetic acid (3 : 1). Each slide was made from a single individual using a cellular suspension air-drying technique (Petkevičiūtė R., Ieshko E.P. 1991: Int. J. Parasitol. 21: 11-15). Slides were stained directly with 4% Giemsa in phosphate buffer (pH 6.8) for 40 min.

Metaphase plates, suitable for karyological analysis, were photographed, and photomicrographs were used for construction of karyotypes. Seven well-spread metaphase plates were chosen for karyometric analysis. The classification of chromosomes followed that of Levan et al. (Levan A., Fredga K., Sandberg A. 1964: Hereditas 52: 201-220). When a centromere position was on the borderline between two categories, two chromosome categories were listed.

From 10 specimens of *P. osculatus*, 79 mitotic plates were obtained where the chromosomes were spread well enough to be counted, and of these, 70 contained 18 chromosomes. All other values were lower than 18 and were attributed either to aneuploidies or, more likely, to loss of chromosomes during slide preparation.

The chromosomes of *P. osculatus* were relatively small. Their mean absolute length ranged from 1.02 μm to 3.26 μm (Table 1), and the mean total length of the haploid set (TCL) was 17.49 μm. The first pair of homologues was noticeably larger than the remaining pairs which decreased in size fairly gradually (Fig. 1). According to centromeric index values, chromosomes 1, 7, and 9 were subtelocentric, pairs 2, 5, and 8 submetacentric, pair 3 metacentric, 4 subtelo-submetacentric, and 6 submeta-metacentric.

Within the order Proteocephalidea, chromosome morphology has been described for three *Proteocephalus* spp.: *P. percae*, *P. exiguus*, and *P. macrocephalus* (Špakulová M., Hanzelová V. 1992: Folia Parasitol. 39: 324-326; Petkevičiūtė R. 1993: Biology (Vilnius) 1: 47-48; Hanzelová V., Šnábel V., Špakulová M., Král'ová I., Fagerholm H-P. 1995: Can. J. Zool. 73: 1191-1198; Scholz T., Špakulová M., Šnábel V., Král'ová I., Hanzelová V. 1997: Syst. Parasitol. 37: 1-12). The same chromosome number (2n = 18) has been found, but with some differences in chromosome morphology. A different chromosome number (2n = 14) was reported for another proteocephalidean, *Acanthotaenia multitesticulata*, but without any details of karyotype morphology (Vijayaraghavan S., Subramanyam S. 1980a: Z. Parasitenkd. 63: 65-70). The diploid chromosome numbers in Cestoda range from 6 in *Microsomacanthus* spp. (Hymenolepididae) (Petkevičiūtė R., Regel K.V. 1994: J. Helminthol. 68: 53-55) to 28 in *Nematotaenia dispar* (Nematotaeniidae) (Vijayaraghavan S., Subramanyam S. 1980b: Riv. Parassitol. 41: 371-375). Chro-

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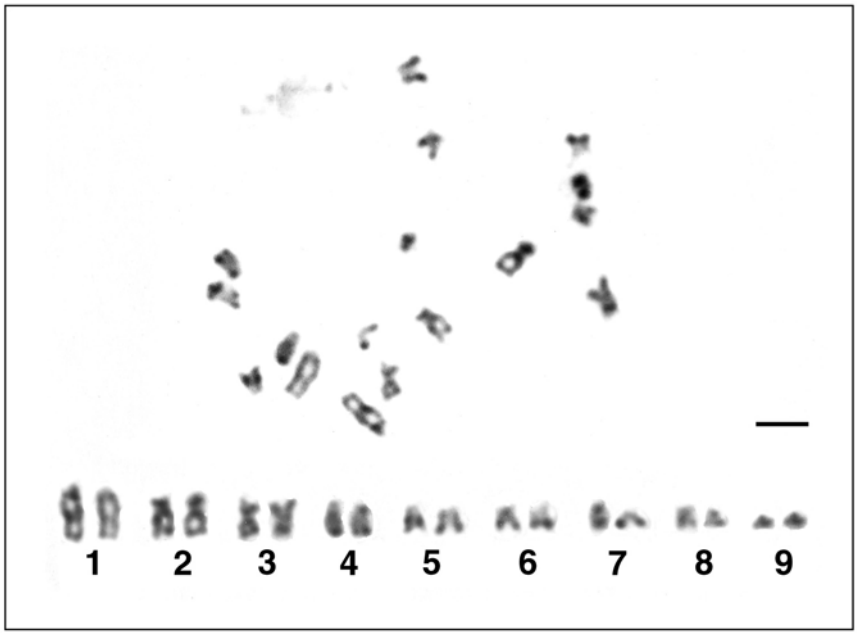

Fig. 1. Mitotic metaphase and karyotype of *Proteocephalus osculatus*. Scale bar = 3 μm.

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

Chromosome number	Absolute length (μm)	Relative length (%)	Centromeric index	Classification*
1	3.26 ± 0.30	18.63 ± 1.19	14.92 ± 0.91	st
2	2.59 ± 0.24	14.80 ± 0.42	29.78 ± 1.21	sm
3	2.35 ± 0.05	13.47 ± 0.83	41.92 ± 1.28	m
4	2.10 ± 0.11	12.06 ± 0.43	22.42 ± 3.55	st-sm
5	1.85 ± 0.20	10.55 ± 0.58	32.05 ± 1.15	sm
6	1.73 ± 0.32	9.83 ± 1.27	34.94 ± 3.13	sm-m
7	1.38 ± 0.07	7.90 ± 0.37	16.47 ± 3.19	st
8	1.21 ± 0.10	6.95 ± 0.62	28.52 ± 4.71	sm
9	1.02 ± 0.10	5.81 ± 0.48	20.95 ± 2.52	st

*m – metacentric, sm – submetacentric, st – subtelocentric chromosomes

mosome numbers generally are quite constant within lower taxonomic categories, such as genus and family. In some families, however, the more species that have been examined, the more variation in chromosome numbers has been recorded. The most frequently reported diploid value in Cestoda is 18, and this has been documented in about 25% of the species investigated belonging to the following five orders – Spathebothriidea, Caryophyllidea, Pseudophyllidea, Proteocephalidea, and Cyclophyllidea. The knowledge of cestode cytogenetics is still too limited to determine whether this number is the one of a hypothetical cestode ancestor or whether its widespread occurrence is the result of independently derived chromosomal rearrangements.

Based on available chromosome data, a close karyological affinity does appear to exist among *Proteocephalus* spp. The karyotype of *P. osculatus*, reported here for the first time, shows the same chromosome number (2n = 18) as the other three species. Measurements of relative lengths of corresponding chromosomes did not show considerable differences among the four species. When the karyotype of *P. osculatus* is compared with that of congeners, it is clear that chromosome rearrangements not affecting the chromosome number but changing the centromere position of corresponding pairs, such as pericentric inversions, must have taken place. Main interspecific differences were observed in the morphology of the chromosome pairs 1 and 9, which are subtelocentric in the karyotype of *P. osculatus* and metacentric in the sets of the other three congeners. The karyotype of *P. osculatus* is generally characterised by a greater number of chromosomes with unequal arms and is thus less symmetrical. Typically, the karyotype of a less advanced species within a given phyletic group includes a higher percentage of uniarmed chromosomes (White M.J.D. 1978: Modes of Speciation. W.H. Freeman & Co., San Francisco,

455 pp.; Birstein V.J. 1987: Cytogenetic and Molecular Aspects of Vertebrate Evolution, Nauka, Moscow, 284 pp., in Russian). The karyotypes of *P. macrocephalus* and *P. exiguus* are fairly symmetrical, including biarmed meta- and submetacentric chromosomes of gradually decreasing size which may be considered characteristic of stable and advanced karyotypes.

The phylogenetic position of the proteocephalideans has been controversial. Freeman (Freeman R.S. 1973: Adv. Parasitol. 11: 481-557) suggested that the Proteocephalidea contained the ancestors of Cyclophyllidea and that an understanding of the former, especially *Proteocephalus*, was a prerequisite for elucidating relationships among the cyclophyllideans. However, the putative relationships between the cyclophyllideans and proteocephalideans, as suggested by Freeman (Freeman R.S. 1973, op. cit.), Jarecka (Jarecka L. 1975: Acta Parasitol. Pol. 23: 93-114), and Brooks et al. (Brooks et al. 1991, op. cit.) have recently been questioned (Mariaux J. 1998: J. Parasitol. 84: 114-124; Hoberg E.P., Jones A., Bray R.A. 1999: Syst. Parasitol. 42: 51-73). Moreover, DNA-based methods of analysis have indicated close relationships among proteocephalidean and tetraphyllidean species (Olson P.D., Caira J.N. 1999: J. Parasitol. 85: 1134-1159). The picture emerging from available karyological data indicates that the different cyclophyllidean families are characterised by the different chromosome numbers. The basic diploid number for the species of Hymenolepididae can be considered to be 2n = 12 (see Petkevičiūtė R., Regel K.V. 1994, op. cit.), while in the Taeniidae and Davaineidae species with 2n = 18 predominate (Rausch V.R., Rausch R.L. 1981: Can. J. Genet. Cytol. 23: 151-154; Mutafova T., Svilenov D. 1985: Khelmintologiya 20: 60-65; Margarian L.G. 1989: Biol. Zh. Arm. 42: 749-752). Karyotype analysis has been performed to date for only one tetraphyllidean species, *Pelichnibothrium speciosum*. Its karyotype, 2n = 16, was characterised as rather primitive, with prevailing acrocentric chromosomes (Petkevičiūtė R., Regel K.V. 1993: Int. J. Parasitol. 23: 17-20). Thus, the possibility of a comparison of karyotypes is rather limited. Before we can assess the karyotype evolution within Cestoda, it will be necessary to gather chromosome information on far more species.

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