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Biometric variability of *Proteocephalus neglectus* (Cestoda: Proteocephalidae) in two different age groups of the rainbow trout from the Dobšiná water reservoir (East Slovakia)

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Abstract. The variability of 24 morphological characteristics in *Proteocephalus neglectus* La Rue, 1911, a parasite of the rainbow trout (*Oncorhynchus mykiss* (Walbaum)) was studied. Specimens of two morphologically clearly distinguishable types formed the metapopulation (hostophenote) of *P. neglectus*. The cestodes from type A (isolated from smaller, younger fish) were mostly no longer than 50 mm, the mature and gravid segments in almost all of them were more long than wide, contained small number of larger testes and an ovary with wide elliptic lobes. The cestodes from type B (isolated from larger, older fish) tended to be more than 50 mm long: the typical segment was extended in width, contained a large number of smaller testes and an ovary with narrow elongate lobes. The variability of morphological characteristics in *P. neglectus* was found to be high; 35% of the characteristics in helminths of group A and 55% of the characteristics in helminths of group B showed a coefficient of variation > 20%. The most stable characteristics in *P. neglectus* were the number and diameter of testes, length and width of cirrus sac, length of ovarian lobe, diameter of vaginal sphincter and the number of uterine branches. The coefficient of variation for *P. neglectus* egg also was rather low.

The existing systematic classification of the genus *Proteocephalus* Weinland, 1858 is based on the traditional morphological concept of monotypic species. Freze (1965) stressed that the position of some species within the genus, the differential diagnosis of related species and taxonomical importance of some characteristics are not clear. A considerable morphological interspecific similarity (Priemer and Goltz 1986, Scholz and Ergens 1990) and high intraspecific variability of characteristics (Andersen 1979, Chubb et al. 1987) is generally associated with this group of cestodes. Despite the fact that morphology has so far been considered as the basis for the systematics of the genus *Proteocephalus*, only the morphological characteristics of two species, *P. exiguus* La Rue, 1911 and *P. pollanicola* Gresson, 1952, have been intensely investigated and evaluated by statistical methods (Ieshko and Anikieva 1980, Anikieva et al. 1983, Anikieva 1991).

The aim of this study was to provide data on the unexplored variability of morphological characteristics of the tapeworm *Proteocephalus neglectus*, a common parasite of the rainbow trout. The same model has been simultaneously used for biochemical, cytogenetic and DNA analyses (Šnábel et al. 1991).

MATERIALS AND METHODS

Specimens of *Proteocephalus neglectus* were isolated from the intestinal pyloric appendices of the rainbow trout (*Oncorhynchus mykiss* (Walbaum)) in two age categories; fish under 1 year of age (0^+; group A) and fish older than 1 year (1^+; group B). All the fish came from a cage culture placed in the small, eutrophic water reservoir Dobšiná in eastern Slovakia. Most of them were caught in late autumn and in winter. Intensities were rather low, ranging between 3 and 20 tapeworms per infected host.

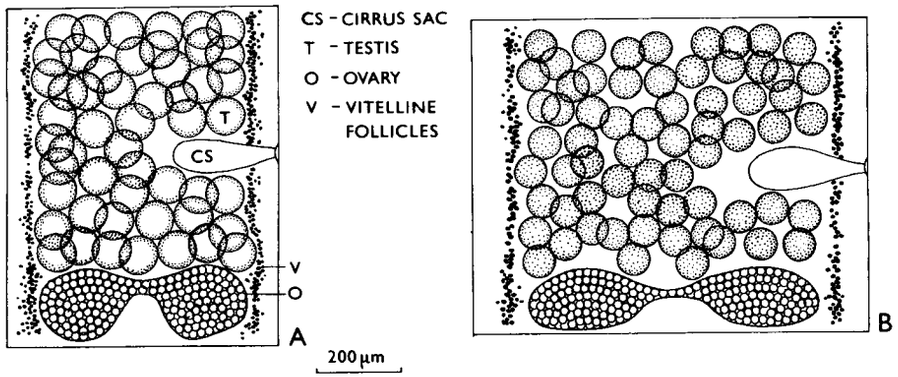

Fig. 1. *Proteocephalus neglectus* – schematic picture of the mature segments. A – type A (smaller, younger fish), B – type B (larger, older fish)

The isolated helminths were placed in tap water to relax, fixed without pressure in 70 % alcohol and stained in iron acetocarmine according to Georgiev et al. (1986). Only fully mature worms with strobila terminated by gravid segments were used for the morphological study. Measurements were taken of: a) 5 morphological characteristics of the scolex and neck of the strobila, b) 12 characteristics in a maximum of ten mature segments of each strobila, beginning with the first egg-bearing segment behind the scolex; c) 3 characteristics in the final five gravid segments excluding the last atypical segment of each strobila (see Table 1). Four other indices were used as indicators, namely the ratio of sucker diameter to scolex width, the ratio of apical organ diameter to sucker diameter, the ratio of cirrus sac length to mature segment width and the ratio of cirrus sac width to length. Four parameters were measured from 100 eggs of *P. neglectus* from group B (we did not succeed in getting the fresh material of cestodes from group A), immediately on their release into water: the diameter of external and internal envelopes, the diameter of oncosphere and the length of embryonal hooks.

Measurements were made from a random sample of 30 individuals in the *P. neglectus* metapopulation (hostophenote) removed from 0^+ and 1^+ host age groups. The terms metapopulation, infrapopulation, hostophenote and organophenote are defined according to Macko (1979), Margolis et al. (1982) and Riggs and Esch (1987). The variability of characteristics in *P. neglectus* was evaluated by the coefficient of variation $Cv = SD/\bar{x} \cdot 100$ (%), whereas in the evaluation of the degree of variability, we considered the value, $Cv = 20$ %, as a threshold in the cestode material studied. The differences in morphometric characteristics in groups A and B were compared by *t*-test and linear regression. All measurements of characteristics are given in µm.

1 – Scolex and neck
2 – Mature segment
3 – Gravid segment
4 – Indices
5 – Egg

Indices
DS/WS – Diameter of sucker/Width of scolex
DAO/DS – Diameter of apical organ/Diameter of sucker
LCS/WMS – Length of cirrus sac/Width of mature segment
WCS/LCS – Width of cirrus sac/Length of cirrus sac

Table 1. Biometrical characteristics of *Proteocephalus neglectus* from rainbow trout recorded in Dobšiná water reservoir

	Characteristics	Group A					Group B					*t* – test
		N	Mean	± SD	Range	Cv	N	Mean	± SD	Range	Cv	
1	Width of scolex	6	231	83.2	159–392	36.0	11	294	55.7	226–411	18.9	
	Diameter of sucker	6	80	14.6	59–103	18.2	11	102	21.8	61–128	21.4	$P < 0.05$
	Width of sucker musculature	6	28	9.5	17–41	33.8	10	29	6.3	20–41	21.8	
	Diameter of apical organ	6	38	4.5	32–44	11.7	10	47	13.2	33–73	28.0	
	Width of neck	6	216	87.8	135–372	40.7	11	259	51.7	186–336	20.0	
2	Length of mature segment	127	800	208.2	381–1492	26.0	147	736	192.1	304–1355	26.1	
	Width of mature segment	127	639	80.0	354–832	12.5	147	928	204.0	502–1408	22.0	$P < 0.001$
	Number of testes	85	51	10.2	29–91	19.9	116	63	10.1	32–91	16.1	$P < 0.01$
	Diameter of testes	127	86	12.8	52–116	14.9	147	78	11.7	53–101	15.0	
	Length of cirrus sac	123	245	30.3	148–310	12.4	144	275	41.1	174–372	15.0	$P < 0.05$
	Width of cirrus sac	120	88	10.6	60–116	12.0	142	97	14.4	56–130	14.8	
	Width of ovary	126	479	66.4	262–680	13.9	101	672	147.1	325–928	21.9	$P < 0.001$
	Length of ovarian lobe	126	202	28.9	93–283	14.3	147	261	50.3	112–424	19.3	$P < 0.001$
	Width of ovarian lobe	126	170	50.4	97–327	29.6	147	147	35.9	72–267	24.4	
	Isthmus of ovary	101	31	8.3	13–72	28.8	132	37	9.0	15–104	24.2	
	Diameter of vaginal sphincter	110	36	4.3	18–55	12.0	124	31	3.8	18–68	12.1	$P < 0.001$
	Width of genital atrium	124	44	4.3	24–76	9.7	145	72	19.5	27–145	27.1	$P < 0.001$
3	Length of gravid segment	56	939	251.3	387–1591	26.8	78	772	264.9	220–1595	34.3	
	Width of gravid segment	56	718	122.6	524–1001	17.1	78	1031	224.5	680–1375	21.8	$P < 0.001$
	Number of uterine branches	9	11	0.6	8–14	5.0	24	11	1.1	9–12	10.4	
4	DS/WS		0.37	0.10	0.22–0.48	27.8		0.35	0.07	0.22–0.44	20.0	
	DAO/DS		0.48	0.08	0.39–0.59	16.8		0.49	0.17	0.33–0.75	35.3	
	LCS/WMS		0.39	0.07	0.27–0.52	18.0		0.30	0.04	0.23–0.38	13.3	$P < 0.001$
	WCS/LCS		0.36	0.04	0.30–0.43	9.9		0.36	0.05	0.26–0.47	15.4	
5	Diameter of external envelope						100	73	8.4	56–95	11.5	
	Diameter of internal envelope						100	41	3.5	34–53	8.6	
	Length of oncosphere						100	24	2.0	21–35	8.1	
	Length of embryonal hook						100	13	1.1	11–15	8.6	

RESULTS

The results of the biometric study of morphological characteristics in *P. neglectus* – including values of average, standard deviation, range (minimum–maximum) and coefficient of variation – are given in Table 1. Regressions of selected characters are shown in Figs. 2–6.

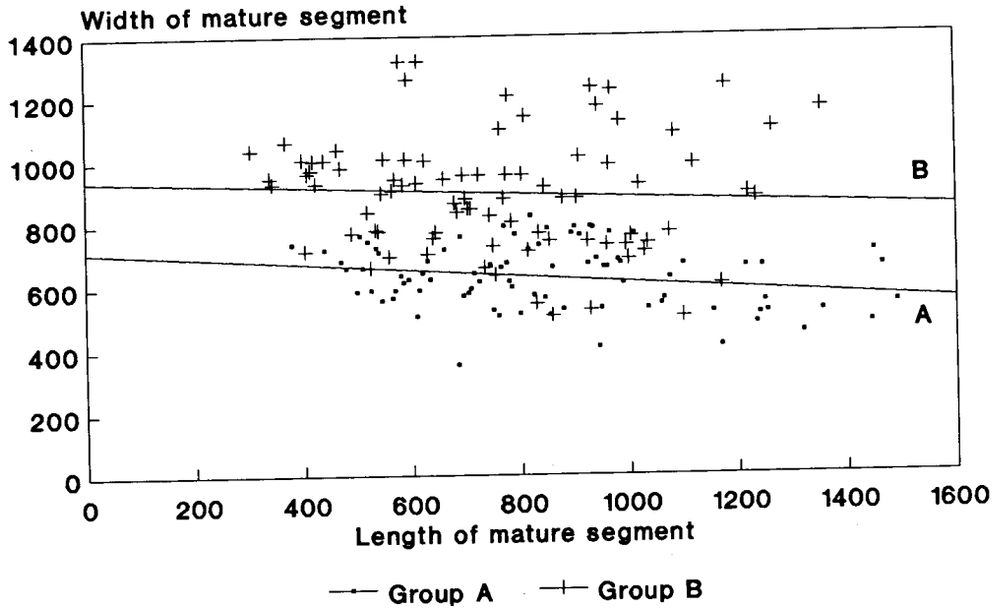

Fig. 2. Scatter diagram and regression lines showing the relationship between the length and width of mature segments in a *P. neglectus* metapopulation.

The metapopulation (hostophenote) of *P. neglectus* from the rainbow trout consisted of two, morphologically distinct types. The cestodes from group A (isolated from 0^+ fish) were < 50 mm. Mature and gravid segments were in almost all cases more long than wide, possessed a small number of larger testes, and an ovary with wide, elliptic lobes. The cestodes from group B (from 1^+ fish) were mostly > 50 mm. A typical segment was extended in width, had numerous smaller testes and an ovary with narrow, elongate lobes (Fig. 1). With the exception of three, all morphological characteristics from the mature segments in group A were smaller than those in worms from group B. The inversion in the size of characteristic, i.e. when smaller helminths from group A showed higher numerical values of characteristics than helminths from group B, was only detected in the size of testes, the width of the ovarian lobe and in the width of the vaginal sphincter.

The variability of morphological characteristics in *P. neglectus* was found to be considerably higher in both groups; 35 % of the characteristics in helminths from group A and 55 % of those from group B showed a coefficient of variation > 20 %. Only seven of 20 characteristics in *P. neglectus* strobila showed < 20 % variation

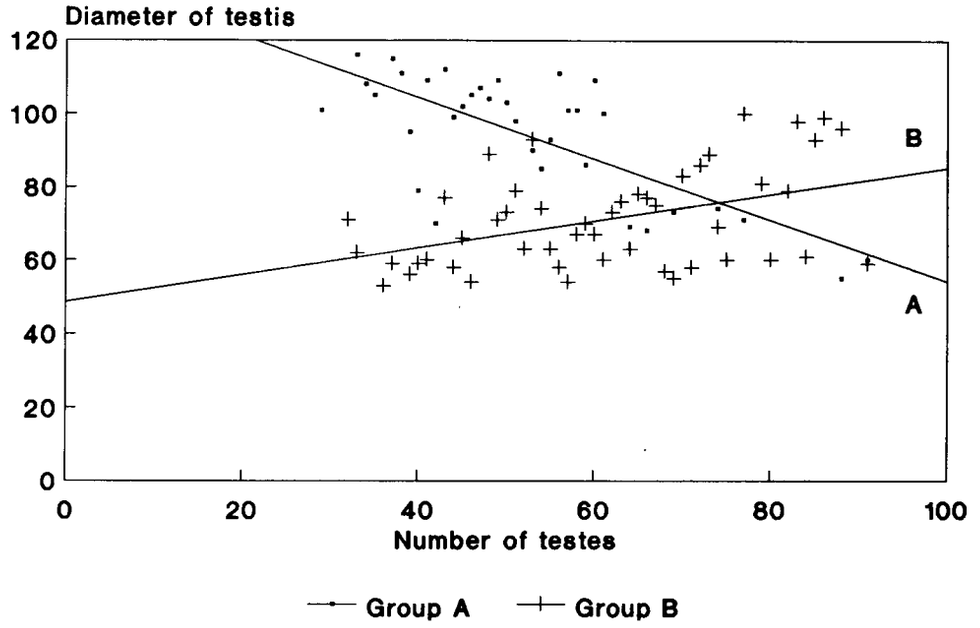

Fig. 3. Scatter diagram and regression lines showing the relationship between the number and diameter of testes in a *P. neglectus* metapopulation.

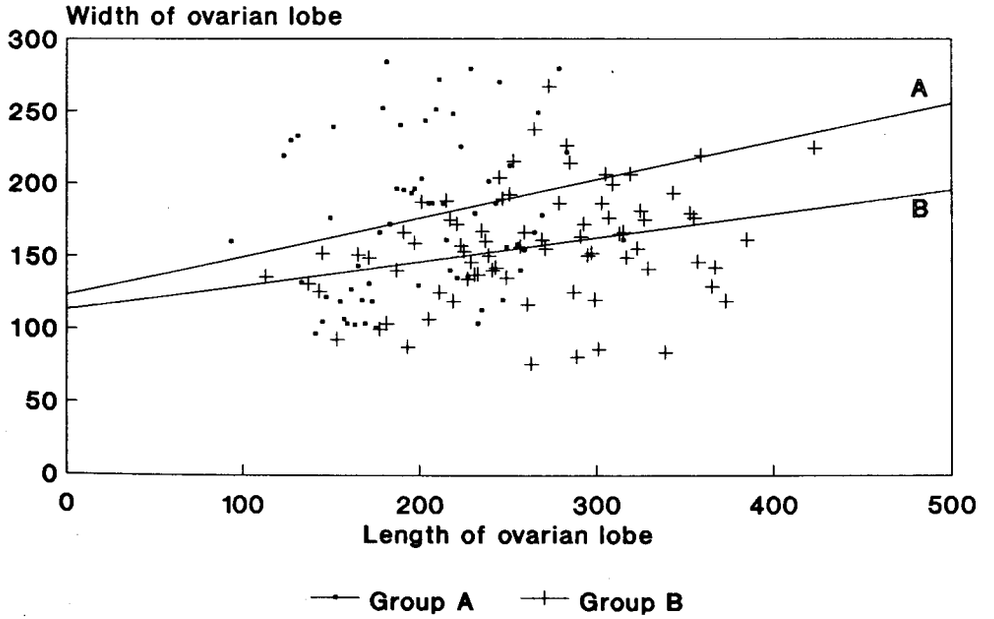

Fig. 4. Scatter diagram and regression lines showing the relationship between the length and width of the ovarian lobe in a *P. neglectus* metapopulation.

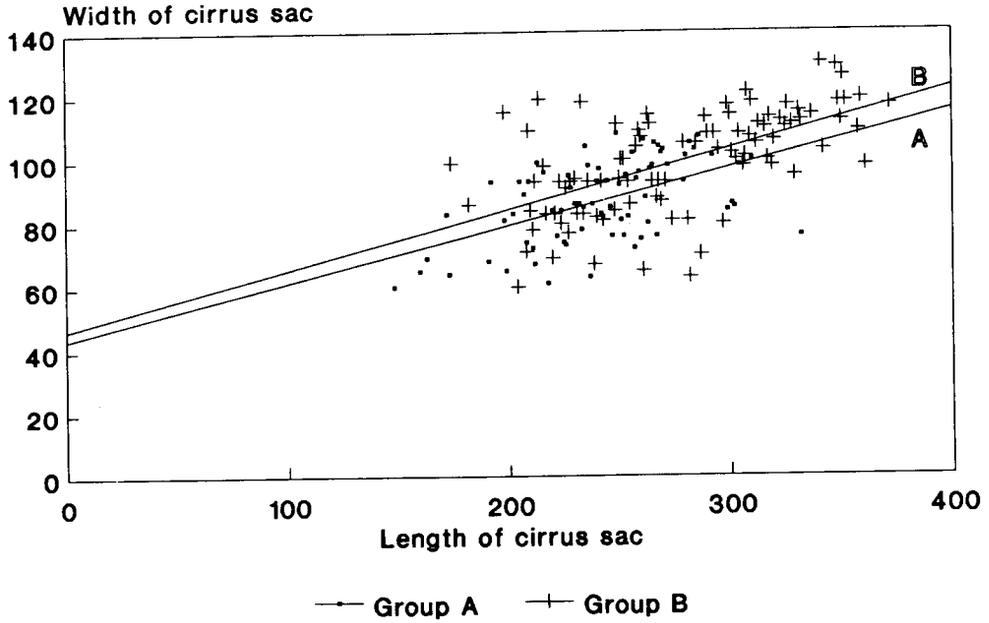

Fig. 5. Scatter diagram and regression lines showing the relationship between the length and width of the cirrus sac in a *P. neglectus* metapopulation.

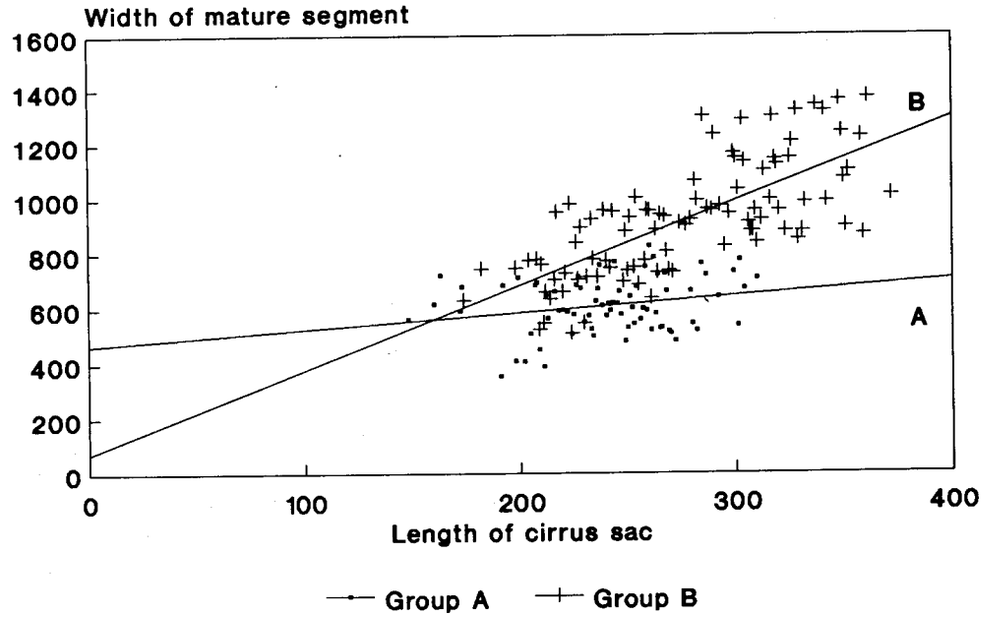

Fig. 6. Scatter diagram and regression lines showing the relationship between the length of cirrus sac and the width of mature segment in a *P. neglectus* metapopulation.

in both the groups. These included the number and diameter of testes, length and width of cirrus sac, length of ovarian lobe, diameter of vaginal sphincter and the number of uterine branches. On the other hand, the most variable characteristics (Cv = 20–34 %) were the width of the sucker musculature, the length of mature and gravid segments, width of the ovarian lobe and width of the ovarian isthmus.

The parameters of *P. neglectus* eggs showed relatively low coefficients of variation (Cv = 8.1–11.5 %). Eggs could have a greater variability, especially in their external hyaline envelopes, which after some time, swell in water and change shape. In the present study, the parameters of *P. neglectus* eggs, however, were measured immediately after their release into water and before their size could change.

DISCUSSION

The study demonstrated the presence of two, morphologically distinct types of *P. neglectus* within a single metapopulation from two age groups of hosts. Since the rainbow trout of both age groups came from the same locality, the same culture, were caught in the same season and their infection was almost uniform, the occurrence of two morphologically dissimilar types of *P. neglectus* is most likely attributed host-induced effects. In cestodes coming from the infrapopulations (organophenotes) of about 20 specimens, no crowding effects on the morphometric characteristics were observed. The variability of characteristics in such cestodes did not significantly differ from those cestodes coming from less numerous infrapopulations. A positive correlation between age and size of the host and the size of the helminth also was reported by Davydov (1978) in the fish cestode *Bothriocephalus gowkongensis (= B. acheilognathi)*. The same phenomenon was reported for *Proteocephalus exiguus* as well (Anikieva and Malakhova 1975).

The occurrence of different morphological forms among proteocephalans has been reported previously. Anikieva et al. (1983) described two morphological forms of *P. exiguus* from *Coregonus albula* and *C. lavaretus*. The two morphs differed from each other, mainly in the size of strobila, the shape of segments, the shapes of the ovaries, cirrus sacs and in the disposition of testes. The morphology of the first group of *P. exiguus* described by these authors corresponds phenotypically to the helminths from group B of *P. neglectus*, and the second group resembles the *P. neglectus* strobila from group A.

The existence of similar morphological forms of *P. neglectus* and *P. exiguus*, the overall phenotypical similarity of these species, the occurrence of both species in salmonid hosts (La Rue 1911, 1914, Anikieva et al. 1983, Priemer and Goltz 1986, Priemer 1987) as well as the observation that existing morphological criteria do not permit the two species to be separated, challenge their taxonomic validity.

The morphology of scolex, number of testes, size of cirrus sac and its ratio to the width of mature segment, as well as number of uterine branches in gravid proglottids are considered by many authors to be the primary criteria by which pro-

teocephalan species are identified (Andersen 1979, Priemer 1982, Chubb et al. 1987, Dubinina 1987).

Contrary to Anikieva et al. (1983), who found no differences between two morphological forms of *P. exiguus* with respect to the number of testes and ratio of cirrus sac length to segment width or the dimensions of scolex and suckers, our findings show significant differences between groups A and B of *P. neglectus* in the size of suckers, number of testes, length of cirrus sac and in the ratio of cirrus sac length to mature segment width (Table 1, Figs. 3, 5, 6). Highly significant differences were also detected in other characteristics whose taxonomic importance is not considered to be very important; these included the width of ovary, length of ovarian lobe, diameter of vaginal sphincter and width of genital atrium. Such differences of the taxonomic characteristics observed within a single metapopulation suggest the need for taxonomic revision. Thus, for example, the characteristics defining the scolex and neck region of *P. neglectus* varied to such a degree that the numeric values of these characteristics could not be used as appropriate taxonomic criteria. The only stable taxonomic criterion detected on the scolex of *P. neglectus* was the ratio of apical organ to the diameter of sucker, a parameter almost identical in both the compared groups (0.48 and 0.49).

Anikieva (1979, 1987) and Ieshko and Anikieva (1980) have stated that the scolex of *P. exiguus* and *P. torulosus* is the most variable region of these cestodes. On the other hand, Andersen (1979) has reported that despite the size and form of the apical sucker not being a reliable character for identification purposes, the scoleces of different species seem to reveal species typical characters which, if used together with strobilar character, might well be suited for species discrimination.

The most consistent structure of the mature segment in both the compared groups of *P. neglectus* was the cirrus sac. Its variability expressed by the coefficient of variation was low (12.4 % and 12.0 % or 15.0 % and 14.8 %) and the ratio of the width and length of the cirrus sac was identical in cestodes of both the types (0.36). On the other hand, the ratio of the length of cirrus sac to the width of mature segment (the parameter used as one of the main diagnostic criteria in proteocephalans) proved to be a rather variable parameter for *P. neglectus*. While in helminths from group A the cirrus sac ran in some cases up to half of the segment, it did not even reach one third of its width (indices 0.39 and 0.30) in helminths from group B. The more suitable relative parameter seems to reveal the ratio of the distal part of the cirrus sac length (distal part means the length of cirrus sac from the genital atrium to the external margin of vitelline follicles) to its total length.

It was found that the larger segments of *P. neglectus* tended to have a greater number of small testes while small segments possessed fewer testes of larger size. However, until the more extensive material of *P. neglectus* and of other proteocephalan species has been processed we will not be in a position to definitively characterize the validity of this dependence.

Finally, the number of uterine branches in a gravid segment is a characteristic

that is almost always used as a principal taxonomic criterion in proteocephalans. We found this characteristic to be very stable in our material (Cv = 4.8 and 10.0 %). As is generally known, many proteocephalan species have between 5 and 12 uterine branches (Chubb et al. 1987, Dubinina 1987). This implies that this characteristic may be useful as a differential criterion only in those species found to have a considerably different number of the uterine branches.

Many taxonomic problems of cestodes in the genus *Proteocephalus* remain. Our commentary regarding the main taxonomic criteria is based on a detailed study of one population of *P. neglectus* as well as on previous experience with other proteocephalans. To confirm their broader application, it will be necessary to use the same methods on other populations of *P. neglectus* as well as on populations of other proteocephalans.

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