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Scolex morphology of *Proteocephalus* tapeworms (Cestoda: Proteocephalidae), parasites of freshwater fish in the Palaearctic Region

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Abstract. The morphology of the scoleces of 11 *Proteocephalus* species, parasites of freshwater fish in the Palaearctic Region, was compared using light and scanning electron microscopy. The following taxa were evaluated: *Proteocephalus ambiguus* (Dujardin, 1845); *P. cernuae* (Gmelin, 1790); *P. exiguus* La Rue, 1911; *P. filicollis* (Rudolphi, 1802); *P. macrocephalus* (Creplin, 1825); *P. osculatus* (Goeze, 1782); *P. percae* (Müller, 1780); *P. pollanicola* Gresson, 1952; *P. sagittus* (Grimm, 1872); *P. thymalli* (Annenkova-Chlopina, 1923); and *P. torulosus* (Batsch, 1786). Some features as overall shape of the scolex, its size, shape and size of an apical sucker were found to be fairly stable and species-specific. The taxa more easily distinguishable from congeners on the basis of their scolex morphology were *P. cernuae*, *P. macrocephalus*, *P. osculatus*, *P. percae* and *P. torulosus*. The taxonomic importance of the scolex is discussed.

The genus *Proteocephalus* Weinland, 1858 accomodates tapeworms parasitizing reptiles and freshwater fish, with numerous taxa occurring in the Palaearctic Region (Freze 1965, Priemer 1982, Schmidt 1986, Chubb et al. 1987, Dubinina 1987, Scholz 1989). The identification of *Proteocephalus* species is often difficult due to a general uniformity in their strobilar morphology (Freze 1965) and, on the contrary, due to a considerable intraspecific variability of morphological features in the strobila previously considered to be of taxonomic importance and used in identification keys (Ieshko and Anikieva 1980, Anikieva et al. 1983, Anikieva 1992, 1993, Hanzelová and Špakulová 1992, Scholz and Hanzelová 1994, Hanzelová et al. 1995a,b).

The morphology of the scolex is one of the most important characteristics used for the classification of proteocephalidean cestodes at generic and family levels (Freze 1965, Schmidt 1986, Rego 1994). However, morphological features of the scolex have only infrequently been used for species differentiation, mainly with respect to the presence or absence of an apical sucker (Freze 1965, Rødland 1983, Dubinina 1987). The only detailed comparative study of scolex morphology of *Proteocephalus* species using both light and electron (scanning – SEM, and transmission – TEM) microscopies has been carried out by Andersen (1979). Comparison of five species (*P. filicollis*, *P. gobiorum*, *P. macrocephalus*, *P. percae* and *Proteocephalus* sp. from trout and char, most probably *P. exiguus*) showed that, despite a high intraspecific variability, some features appear to be suitable for distinguishing individual species.

To test the assumption about the suitability of scolex morphology for taxonomy and to find suitable species–specific markers, a comparative study of the scoleces of *Proteocephalus* species in freshwater fish in the Palaearctic Region was carried out and the results are presented here.

MATERIALS AND METHODS

To prevent artificial effects of different fixation methods on worm morphology and damage of the tegument, specimens fixed with hot fixative as described below were preferably used for this study. Tapeworms were isolated from the host intestine, placed into a small amount of saline and immediately fixed with hot (almost boiling) 4% formaldehyde solution. After fixation, the worms were maintained in 4% formaldehyde solution until staining. Before staining, they were washed in 70% ethanol, stained with iron hydrochloric carmine according to de Chambrier et al. (1992), destained in acid ethanol (100 ml 70% ethanol + 2 ml concentrated HCl), dehydrated, cleared in increasing concentration (10, 50, 90 and 100 %) of clove oil (*oleum caryophylli*) and mounted in Canada balsam. Stained specimens were studied using Nomarski interference contrast in a Leitz Aristoplan microscope. Specimens for scanning electron microscopy (SEM) were

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fixed by the same method, dehydrated, CO_2 critical-point dried, covered with gold and observed under a Jeol JSM 6300 microscope.

Specimens of the following species were used (numbers of specimens used for scanning electron microscopy and biometrical analysis are given in parentheses):

1. *Proteocephalus cernuae* (Gmelin, 1790): 10 specimens (of these, 3 used for SEM and 5 for biometry) from *Gymnocephalus cernuus* (Linnaeus), Laborec River, Slovakia; Štědronín – Orlík water reservoir and Mácha Lake, Czech Republic.

2. *Proteocephalus exiguus* La Rue, 1911: 76 specimens (13 SEM; 13 biometry) from *Coregonus lavaretus maraena* (Bloch), Lac Léman at Versoix and Lac Bienne, Switzerland; 63 specimens (8 SEM; 29 biometry) from *Coregonus autumnalis* (Pallas), Baikal Lake, Russia; 21 specimens (6 SEM) from *Oncorhynchus mykiss* (Walbaum), trout farm Těšenov near H. Cerekev; Východná, Dobšiná water reservoir and Morské Oko lake, Slovakia; 6 specimens (6 SEM) from *Salvelinus fontinalis* (Mitchill), Dobšiná water reservoir, Slovakia.

3. *Proteocephalus filicollis* (Rudolphi, 1802): 10 specimens (2 SEM) from *Gasterosteus aculeatus* (Linnaeus), Airthrey Loch, Stirling University, Scotland, UK.

4. *Proteocephalus macrocephalus* (Creplin, 1825): 84 specimens (28 SEM; 23 biometry) from *Anguilla anguilla* (Linnaeus), Lužnice River at Tábor, Ohře River at Postoloprty, Třeboň, Želivka water reservoir, Štědronín – Orlík water reservoir, all Czech Republic.

5. *Proteocephalus osculatus* (Goeze, 1782): 29 specimens (5 SEM; 10 biometry) from *Silurus glanis* Linnaeus, Štědronín – Orlík water reservoir, Czech Republic.

6. *Proteocephalus percae* (Müller, 1780): 73 specimens (12 SEM; 27 biometry) from *Perca fluviatilis* Linnaeus, Dobšiná water reservoir, Slovakia; Lac Léman at Versoix, Lac de Bienne, Lac Morat and Lac de Neuchâtel, all Switzerland.

7. *Proteocephalus pollanicola* Gresson, 1952: 13 specimens (2 SEM; 11 biometry) from *Coregonus pollan* Thompson, Louth Neagh (Toome Bay), Northern Ireland, UK.

8. *Proteocephalus torulosus* (Batsch, 1786): 27 specimens (16 SEM; 11 biometry) from *Leuciscus cephalus* (Linnaeus), Rokytná River, South Moravia, Czech Republic; 9 specimens (9 SEM) from *Barbus barbus* (Linnaeus), Jihlava River, S. Moravia; 1 specimen (1 SEM) from *Chalcalburnus chalcoides* (Güldenstädt), Mondsee, Austria; 7 specimens (3 SEM) from *Alburnus alburnus* (Linnaeus), Laborec River, East Slovakia.

Since freshly collected material of some other species fixed with hot formaldehyde solution had not been available, specimens fixed by other procedures (cold 4% formaldehyde solution under a slight coverslip pressure or 70% ethanol after relaxation of worms in distilled water or in saline) were also considered for morphological study. Of these specimens, however, only *P. thymalli* was available in sufficient numbers to be included into statistical analysis. These were as follows:

9. *Proteocephalus ambiguus* (Dujardin, 1845): 5 specimens from *Pungitius pungitius* (Linnaeus), Karelia, Russia.

10. *Proteocephalus sagittus* (Grimm, 1872): 8 specimens from *Noemacheilus barbatulus*, Rokytka Brook near Říčany, Czech Republic; and Dobšiná water reservoir, Slovakia.

11. *Proteocephalus thymalli* (Annenkova-Chlopina, 1923): 30 specimens (5 SEM) from *Thymallus baicalensis* Dybowski, Baical Lake, Russia; 23 specimens (2 SEM; 16 biometry) from *T. nigrescens* Dorogostaisky, Khubsugul Lake, River Bayan-Gol, Mongolia; 8 specimens from *T. arcticus grubei* Dybowski, Amur River, Russia.

In addition to the above listed material, reference specimens of *P. exiguus*, *P. macrocephalus*, *P. osculatus*, *P. percae* and *Proteocephalus* sp. from the Natural History Museum, Budapest, Hungary; Museum d'histoire naturelle, Geneva, Switzerland; and the Natural History Museum, London, UK, were evaluated as well.

The following morphological and biometrical features of the scolex were evaluated:

1. **Shape of scolex**: 1.1. Overall shape from dorsoventral and lateral views. 1.2. Maximum width of scolex (WS); in some species (e.g., *P. percae*) with an indistinct neck region, the width of the scolex was measured at the level of the posterior margins of the suckers.

2. **Neck**: 2.1. Presence of a neck in the form of a distinct constriction between the scolex and the anterior part of the strobila, i.e. the neck narrower than the scolex, or the border between the scolex and neck is indistinct when the neck is wider than the scolex; 2.2. Width of the neck (WN).

3. **Suckers**: 3.1. Position of suckers (anterior, anterolateral, sublateral, lateral); "lateral" position used here means that the suckers are aligned two towards the dorsal surface and two towards the ventral surface with apertures directed laterally to the median line of the body; 3.2. Diameter of suckers (DS, i.e. arithmetical mean of diameters of all suckers; in oval suckers the diameter was calculated as a mean of their length and width); 3.3. Ratio of the diameter of suckers (DS) to the width of the scolex (WS), i.e. DS/WS.

4. **Apical sucker**: 4.1. Presence or absence; 4.2. Shape; 4.3. Diameter (DAS) – transverse diameter, i.e. "width"; 4.4. Length of the apical sucker (LAS) – longitudinal diameter; 4.5. Ratio of the diameter of apical sucker to its length (DAS/LAS); 4.6. Ratio of the diameter of the apical sucker to diameter of suckers (DAS/DS); 4.7. Ratio of the diameter of the apical sucker to the width of the scolex (DAS/WS).

Measurements of each feature were statistically evaluated using arithmetical mean (X), standard error (SE) and coefficient of variability (CV), where CV = SD (standard deviation) /X × 100 [%]. Values were compared using one-way ANOVA.

5. **Gland cells**: the presence and distribution of cells of presumably glandular character (see Žďárská and Nebesářová 1995) in the scolex and neck region.

6. **Osmoregulatory system**: course of osmoregulatory canals and their morphology within the scolex.

Fig. 1. A-D – *Proteocephalus ambiguus* from *Pungitius pungitius*, Karelia, Russia; **E-K** – *P. cernuae* from *Gymnocephalus cernuus*, Czech Republic and Slovakia; **L-P** – *P. filicollis* from *Gasterosteus aculeatus*, Scotland. Dorsoventral view (A-I, K-P); subapical view (J). Note osmoregulatory system and numerous gland cells posterior to suckers in K. Tegumental microtriches illustrated only in K. Scale bars = 100 μm.
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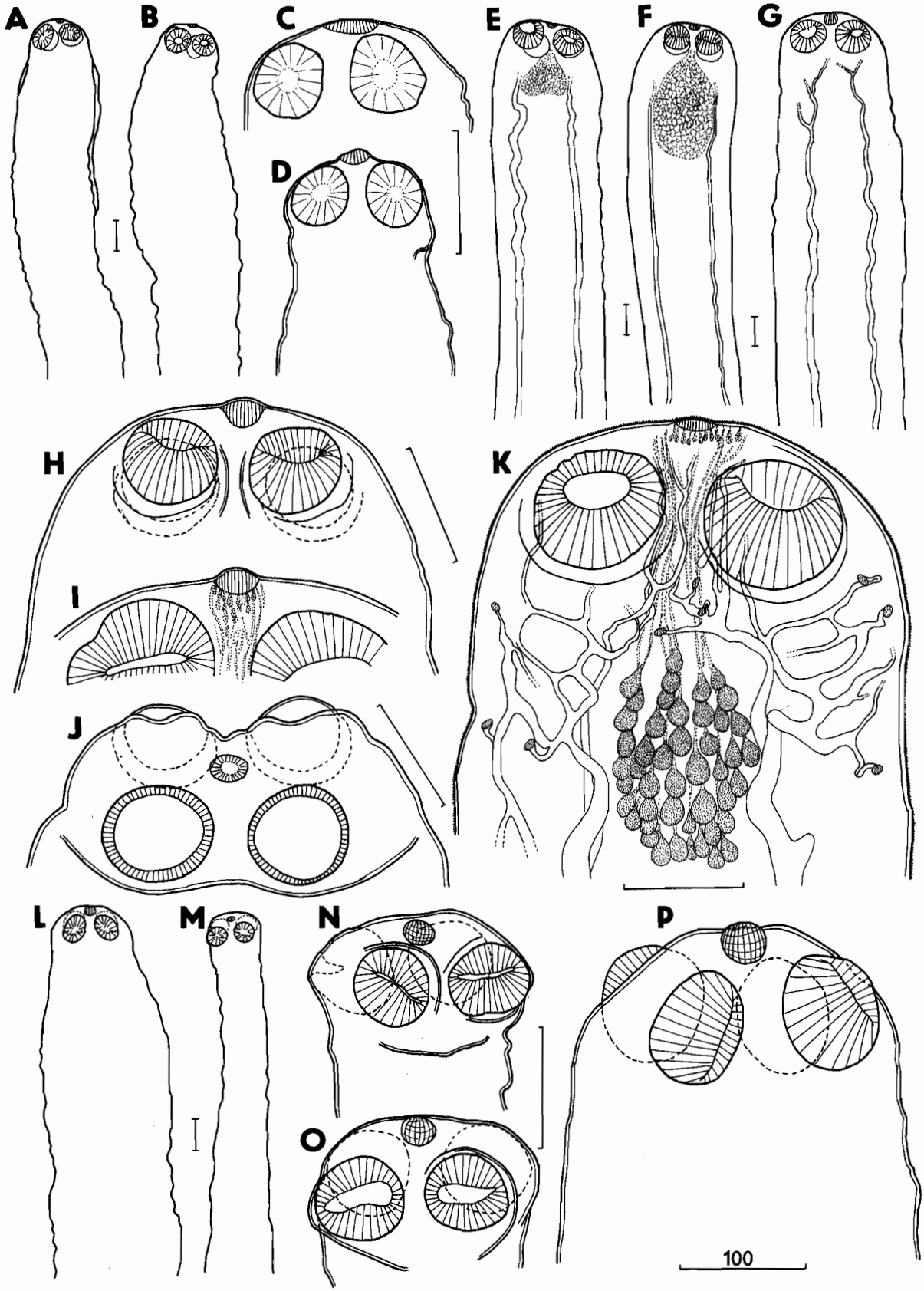
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RESULTS

Descriptions of features evaluated

1. Shape of scolex

1.1. Overall shape. The scoleces were rounded to spherical in dorsoventral view in *P. ambiguus* (Fig. 1 A-B), *P. exiguus* (Fig. 3), *P. filicollis* (Fig. 4A), *P. macrocephalus* (Fig. 5A-C) and *P. pollanicola* (Fig. 7B,C), bluntly ended in *P. cernuae* (Fig. 1E,F), tapering anteriorly in *P. percae* (Fig. 5J,M,N), or club-shaped in *P. thymalli* (Fig. 7D-F) and *P. torulosus* (Figs. 8G-J). In lateral view, the scoleces were dorsoventrally flattened in *P. cernuae* (Fig. 2B,C) and *P. percae* (Fig. 6C,D) and oval in remaining species.

1.2. Width of scolex (WS; Table 1). *Proteocephalus thymalli*, *P. torulosus* and *P. pollanicola* significantly differed one from another and from all other species, the scoleces of the two former species being very large, mostly wider than 400 μm. *Proteocephalus percae* and evaluated populations of *P. exiguus* (see Discussion) had significantly smaller scoleces than other species (Table 1). The coefficient of variability was lower than 15% in all species, except for *P. thymalli*; in three species (*P. cernuae*, *P. exiguus* from *C. lavaretus*, and *P. osculatus*), it was lower than 10% (Table 1).

2. Neck

2.1. Presence of neck. The absence of a distinct constriction between the scolex and the anterior part of the strobila was typical of *P. percae* (Fig. 6A,B); other species had mostly the scolex wider than the neck. The most distinct neck was present in the scoleces of *P. osculatus* (Fig. 5F,G). *Proteocephalus thymalli* (Fig. 7D-F) and *P. torulosus* (Fig. 8G-I) had a very long neck.

2.2. Width of neck (WN; Table 2). This character did not appear to be suitable for distinguishing most species studied despite significant differences between some groups of species, e.g., between that formed by *P. osculatus*, *P. cernuae* and *P. thymalli*, and all other taxa (Table 2). The coefficient of variability was high (11-32%) in all taxa, excluding *P. cernuae* and *P. pollanicola* (7% - Table 2).

3. Suckers

3.1. Position of suckers. Anteriorly directed suckers were found in *P. pollanicola* (Fig. 2I,J) and *P. thymalli* (Fig. 6F,G); laterally situated in *P. macrocephalus* (Fig. 4D,E), *P. osculatus* (Fig. 4H) and *P. percae* (Fig. 6A,B). Other species had mostly sublaterally to anterolaterally situated suckers: *P. cernuae* (Fig. 2C), *P. exiguus* (Fig. 2E), *P. filicollis* (Fig. 4A), *P. sagittus* (Fig. 8C), *P. torulosus* (Fig. 6I).

3.2. Diameter of suckers (DS; Table 3). *Proteocephalus percae* and *P. exiguus* had significantly

Table 1. Width of scolex (WS).

Species	Mean	SE	Range	Cv	Groups
P. percae	169	4	141-214	12	A
P. exiguus[1]	171	3	134-205	10	A
P. exiguus[2]	176	3	160-198	6	A
P. pollanicola	278	4	202-304	11	B
P. macrocephalus	318	10	272-484	15	C
P. cernuae	344	14	288-368	8	CD
P. osculatus	381	9	352-440	7	D
P. torulosus	492	19	416-630	13	E
P. thymalli	546	30	376-780	21	F

[1]from *Coregonus autumnalis*, Baikal Lake, Russia
[2]from *Coregonus lavaretus*, Lac de Bienne, Switzerland

Number of specimens measured: *P. percae* 27; *P. exiguus*[1] 29; *P. exiguus*[2] 13; *P. pollanicola* 11; *P. macrocephalus* 23, *P. cernuae* 5; *P. osculatus* 10; *P. torulosus* 11; *P. thymalli* 16.

Table 2. Width of neck (WN).

Species[1]	Mean	SE	Range	Cv	Groups
P. exiguus[2]	132	5	64-176	20	A
P. exiguus[3]	142	4	118-166	11	A
P. torulosus	184	18	112-296	32	B
P. macrocephalus	203	6	128-272	15	BC
P. pollanicola	224	4	202-246	7	C
P. osculatus	312	14	232-368	14	D
P. cernuae	330	11	304-360	7	DE
P. thymalli	345	17	224-480	19	E

[1]*P. percae*, lacking a distinct neck, is not included
[2]from *Coregonus autumnalis*, Baikal Lake, Russia
[3]from *Coregonus lavaretus*, Lac de Bienne, Switzerland

Table 3. Diameter of suckers (DS).

Species	Mean	SE	Range	Cv	Groups
P. percae	55	3	49-64	14	A
P. exiguus[1]	59	1	46-72	11	A
P. exiguus[2]	65	3	53-76	10	A
P. macrocephalus	86	2	72-116	12	B
P. cernuae	89	8	75-108	15	BC
P. pollanicola	107	7	86-122	12	CD
P. osculatus	123	3	108-139	7	D
P. torulosus	170	7	138-209	13	E
P. thymalli	173	10	147-227	12	E

[1]from *Coregonus autumnalis*, Baikal Lake, Russia
[2]from *Coregonus lavaretus*, Lac de Bienne, Switzerland

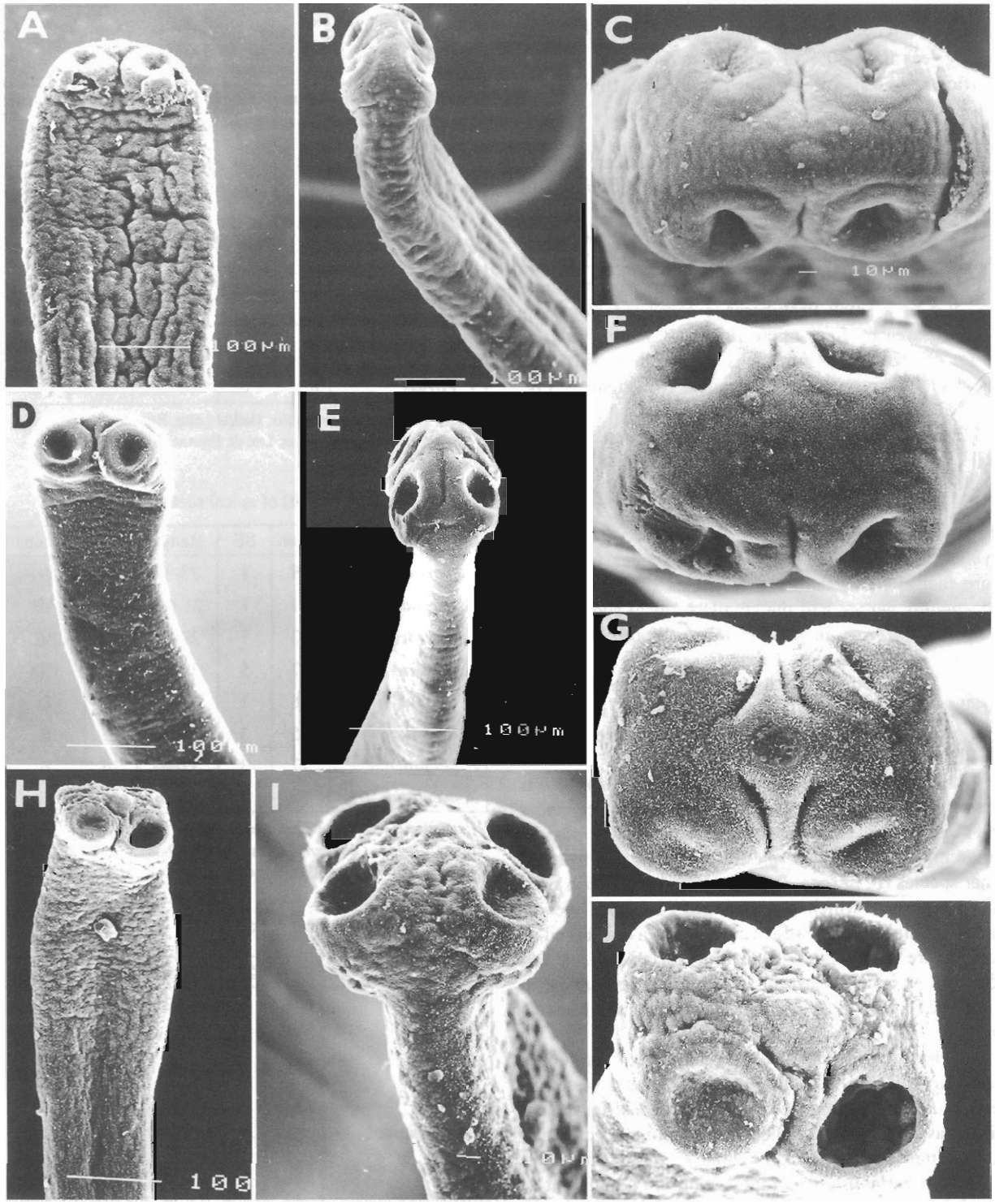

Fig. 2. A-C – *Proteocephalus cernuae* from *Gymnocephalus cernuus*, Czech Republic; **D-G** – *P. exiguus* from *Coregonus autumnalis*, Russia; **H-J** – *P. pollanicola* from *Coregonus pollan*, Northern Ireland, UK. Dorsoventral view (A,D,H); lateral view (B,E,I); apical view (C,F,G,J). Scanning electron microscopy.

smaller suckers (diameter less than 80 µm) than all congeners; *P. torulosus* and *P. thymalli* significantly differed from all other species studied by their large suckers (diameter of suckers larger than 140 µm – Table 3). In general, there was not very high variability in this character in all species (coefficient of variability ranging from 7 to 15%).

3.3. Ratio of sucker diameter to scolex width (DS/WS; Table 4). Only two species, *P. cernuae* and *P. macrocephalus*, significantly differed from other

species in having relatively small lateral suckers, representing less than 30% of scolex width (Table 4). The coefficient of variability ranged from 7 to 16% (Table 4).

4. Apical sucker

4.1. Presence or absence. An apical sucker was absent only in *P. torulosus* and *P. sagittus*, the scoleces of which had numerous gland cells concentrated in their apical regions (Fig. 8C,F,K). In other taxa, the apical sucker was always present. In SEM, the apical sucker could be seen as a small, deep pit (*P. macrocephalus* – Fig. 4G) or a small protuberance separated from surrounding area by finer microtriches (*P. macrocephalus* – Fig. 4F). In some specimens observed in SEM, the apical sucker was not distinguishable (*P. exiguus* – Fig. 2F; *P. filicollis* – Fig. 4C).

4.2. Shape. The apical sucker of *P. osculatus* was well-developed, with a deep cavity (Fig. 4J); other species had a vestigial apical sucker.

4.3. Diameter of apical sucker (DAS; Table 5). Three species, *P. osculatus*, *P. pollanicola* and *P. thymalli*, had much larger apical sucker (diameter more than 50 μm) than other species (Table 5). *Proteocephalus percae* had the smallest apical sucker, significantly differing in this feature from other species, except for *P. exiguus* from *Coregonus autumnalis* (Table 5). Despite these differences, a coefficient of variability lower than 10% was found only in *P. macrocephalus*; in other species, it ranged between 10 to 27% (Table 5).

4.4. Length of apical sucker (LAS – Table 6). In this character, *P. osculatus*, *P. thymalli*, *P. macrocephalus* and *P. pollanicola* significantly differed in their possessing a high apical sucker one from another and from all other species (*P. percae*, *P. cernuae* and *P. exiguus*), the apical sucker of which was flattened (Table 6). Nevertheless, a high intraspecific variability was observed in this feature and the coefficient of variability reached 15% and more in almost all species, with its maximum values (25-30%) in *P. pollanicola*, *P. exiguus* from *C. lavaretus*, and *P. thymalli* (Table 6).

4.5. Ratio of apical sucker diameter to its length (DAS/LAS; Table 7). Only slight differences were observed: *P. macrocephalus* and *P. osculatus* had apical sucker as long as wide whereas remaining species had more flattened apical sucker (Table 7). In this character, quite high variability was found, with the coefficient of variability ranging from 14 to 25% (Table 7).

4.6. Ratio of apical sucker diameter to diameter of suckers (DAS/DS; Table 8). Four groups could be recognized, differing significantly from each other (Table 8). Members of group A (*P. macrocephalus*, *P. thymalli* and *P. cernuae*) had a small apical sucker, representing less than 50% of the diameter of the lateral suckers. Relatively large apical suckers, representing about 40-75% of diameter of lateral suckers, were present in *P. exiguus* from *C. autumnalis*, the only member of group B, and members of group C, *P. percae*, *P. exiguus* from *C. lavaretus* and *P. osculatus*. *Proteocephalus pollanicola*, the only member of group D, significantly differed from all other species in having a large apical sucker, with a diameter up to 90% of that of

Table 4. Ratio of sucker diameter to width of scolex (DS/WS).

Species	Mean	SE	Range	Cv	Groups
P. cernuae	0.25	0.02	0.21-0.31	16	A
P. macrocephalus	0.27	0.01	0.21-0.32	11	A
P. percae	0.31	0.01	0.23-0.39	12	B
P. osculatus	0.33	0.01	0.29-0.38	7	BC
P. thymalli	0.33	0.01	0.24-0.43	15	BC
P. torulosus	0.34	0.01	0.23-0.41	13	BC
P. exiguus[1]	0.34	0.01	0.24-0.40	12	D
P. pollanicola	0.35	0.03	0.31-0.46	12	D
P. exiguus[2]	0.35	0.01	0.31-0.39	8	D

[1]from *Coregonus autumnalis*, Baikal Lake, Russia
[2]from *Coregonus lavaretus*, Lac de Bienne, Switzerland

Table 5. Diameter (width) of apical sucker (DAS).

Species[1]	Mean	SE	Range	Cv	Groups
P. percae	29	1	23-37	14	A
P. exiguus[2]	31	1	21-44	16	AB
P. macrocephalus	33	1	29-42	9	BC
P. cernuae	37	5	28-55	27	CD
P. exiguus[3]	38	1	26-44	11	D
P. thymalli	75	3	58-96	15	E
P. pollanicola	77	3	52-90	14	E
P. osculatus	78	3	64-86	10	E

[1]*P. torulosus* has no apical sucker
[2]from *Coregonus autumnalis*, Baikal Lake, Russia
[3]from *Coregonus lavaretus*, Lac de Bienne, Switzerland

Table 6. Length of apical sucker (LAS).

Species	Mean	SE	Range	Cv	Groups
P. percae	18	1	14-23	15	A
P. cernuae	21	1	18-23	10	AB
P. exiguus[1]	22	1	13-31	18	B
P. exiguus[2]	25	2	17-41	28	B
P. pollanicola	31	3	30	18-44	C
P. macrocephalus	36	1	26-48	15	D
P. thymalli	42	3	26-64	25	E
P. osculatus	70	3	58-90	16	F

[1]from *Coregonus autumnalis*, Baikal Lake, Russia
[2]from *Coregonus lavaretus*, Lac de Bienne, Switzerland

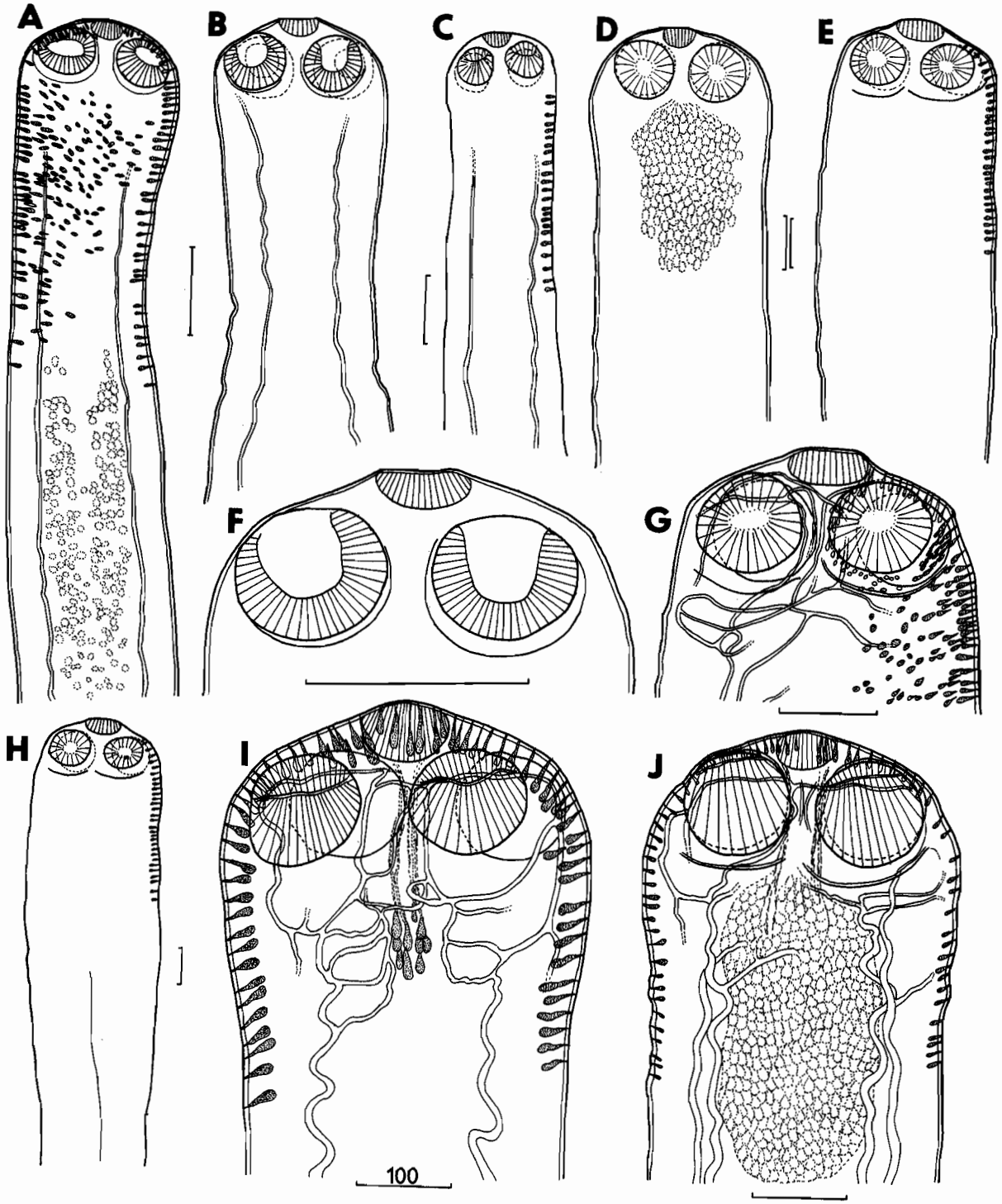

Fig. 3. **A,B,F** – *Proteocephalus exiguus* from *Coregonus autumnalis*, Russia; **C,I** – *P. exiguus* from *C. lavaretus*, Switzerland; **D,E,G,H,J** – *P. exiguus* from *Oncorhynchus mykiss*, Slovakia. Dorsoventral view. Note osmoregulatory canals and gland cells in G,I,J (in G gland cells illustrated only in one side). Scale bars = 100 μm.

the lateral suckers (Table 8). The coefficient of variability was similar in all species, ranging between 10 and 18% (Table 8).

4.7. Ratio of apical sucker diameter to scolex width (DAS/WS; Table 9). *Proteocephalus pollanicola* (group E) significantly differed from all other species, its apical sucker representing more than 1/4 of scolex width. Significant differences were also found between the following groups: A) *P. cernuae* and *P. macrocephalus* with relatively small apical sucker (about 10% of scolex width); B) *P. thymalli*; C) *P. percae* and *P. exiguus* from *C. autumnalis*; and D) *P. osculatus* and *P. exiguus*

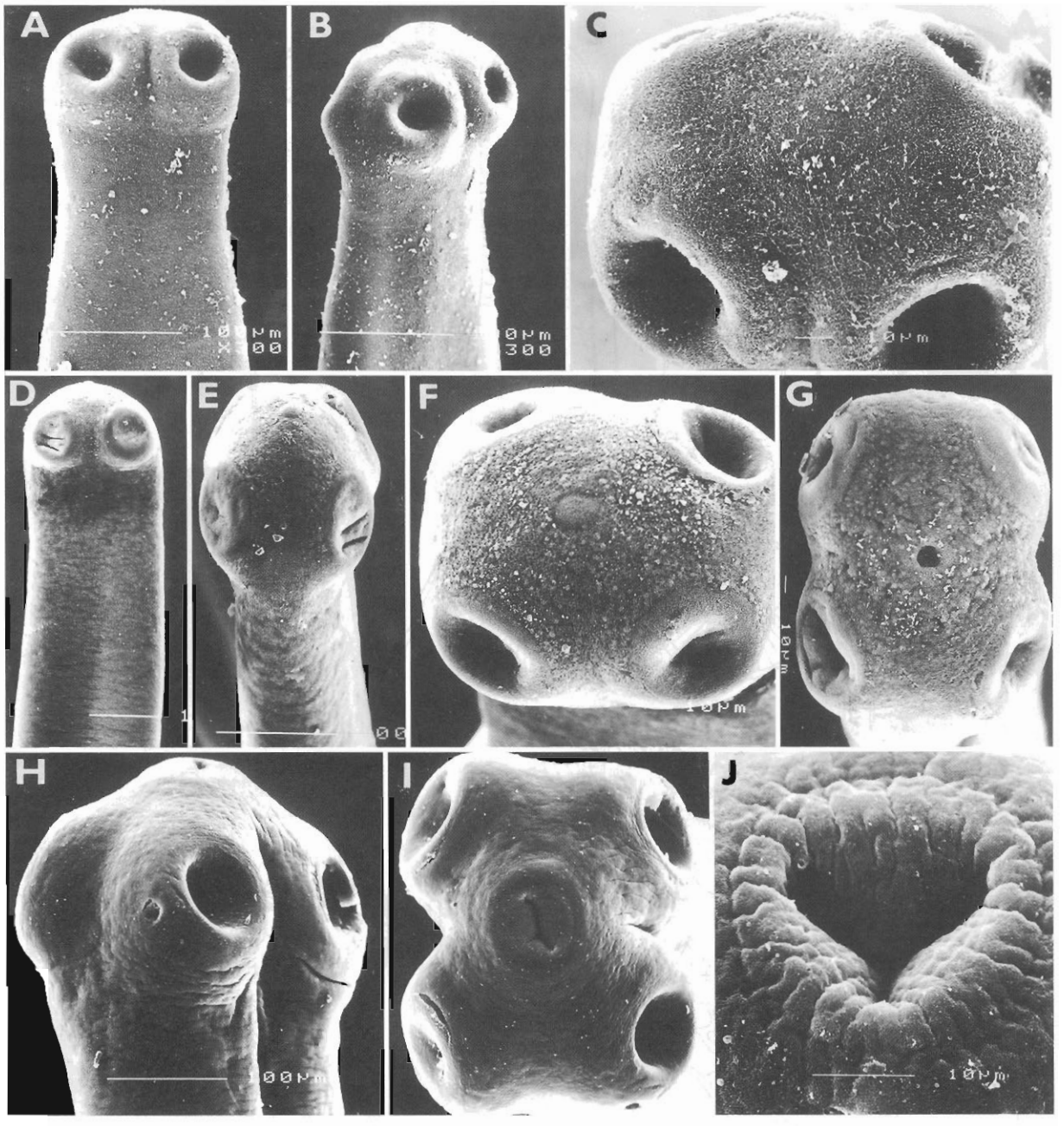

Fig. 4. A-C – *Proteocephalus filicollis* from *Gasterosteus aculeatus*, Scotland, UK; **D-G** – *P. macrocephalus* from *Anguilla anguilla*, Czech Republic; **H-J** – *P. osculatus* from *Silurus glanis*, Czech Republic. Dorsoventral view (A,D); lateral view (B,E,H); apical view (C,F,G,I); apical sucker (J). Note absence of distinct apical sucker in C. Scanning electron microscopy.

from *C. lavaretus* (Table 9). Regarding intraspecific variability, this feature was stable in most taxa (coefficient of variability between 7 and 17%), with the exception of *P. cernuae* (30%).

5. Gland cells

Gland cells within the scoleces and neck regions were found in the following species: *P. cernuae* (Fig. 1E,F,K), *P. exiguus* (Fig. 3A,D,G,I,J), *P.macrocephalus* (Fig. 5E), *P. osculatus* (Fig. 5L), *P. percae* (Fig. 5J,O-Q), *P. sagittus* (Fig. 8C,F) and *P. torulosus* (Fig. 8K). Differences between species in the morphology and distribution of the gland cells are presented in the descriptions of species.

6. Osmoregulatory system

It was possible to study the osmoregulatory system in the scoleces of *P. cernuae*, *P. exiguus*, *P. macrocephalus*, *P. osculatus* and *P. percae*. An identical arrangement of the osmoregulatory system was observed:

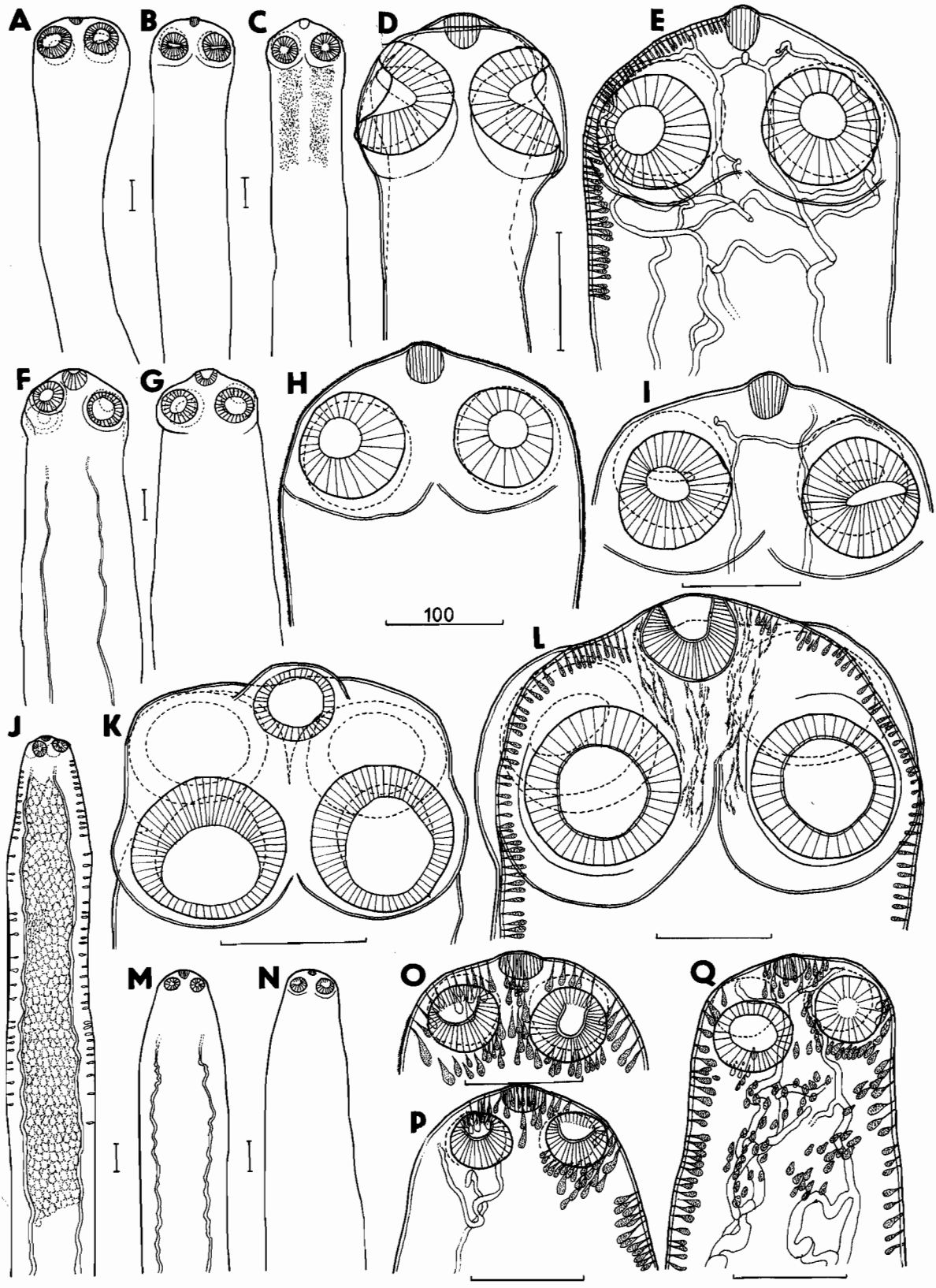

Fig. 5. A-E,H,I – *Proteocephalus macrocephalus* from *Anguilla anguilla*, Czech Republic; **F,G,K,L** – *P. osculatus* from *Silurus glanis*, Czech Republic; **J,M-Q** – *P. percae* from *Perca fluviatilis*, Switzerland. Dorsoventral view (A-C,E-Q); lateral view (D). Note subtegumental gland cells in E,L and O-Q, and osmoregulatory canals in E,I and P-Q. Tegumental microtriches illustrated only in H and gland cells in E and P only in one side. Scale bars = 100 μm.

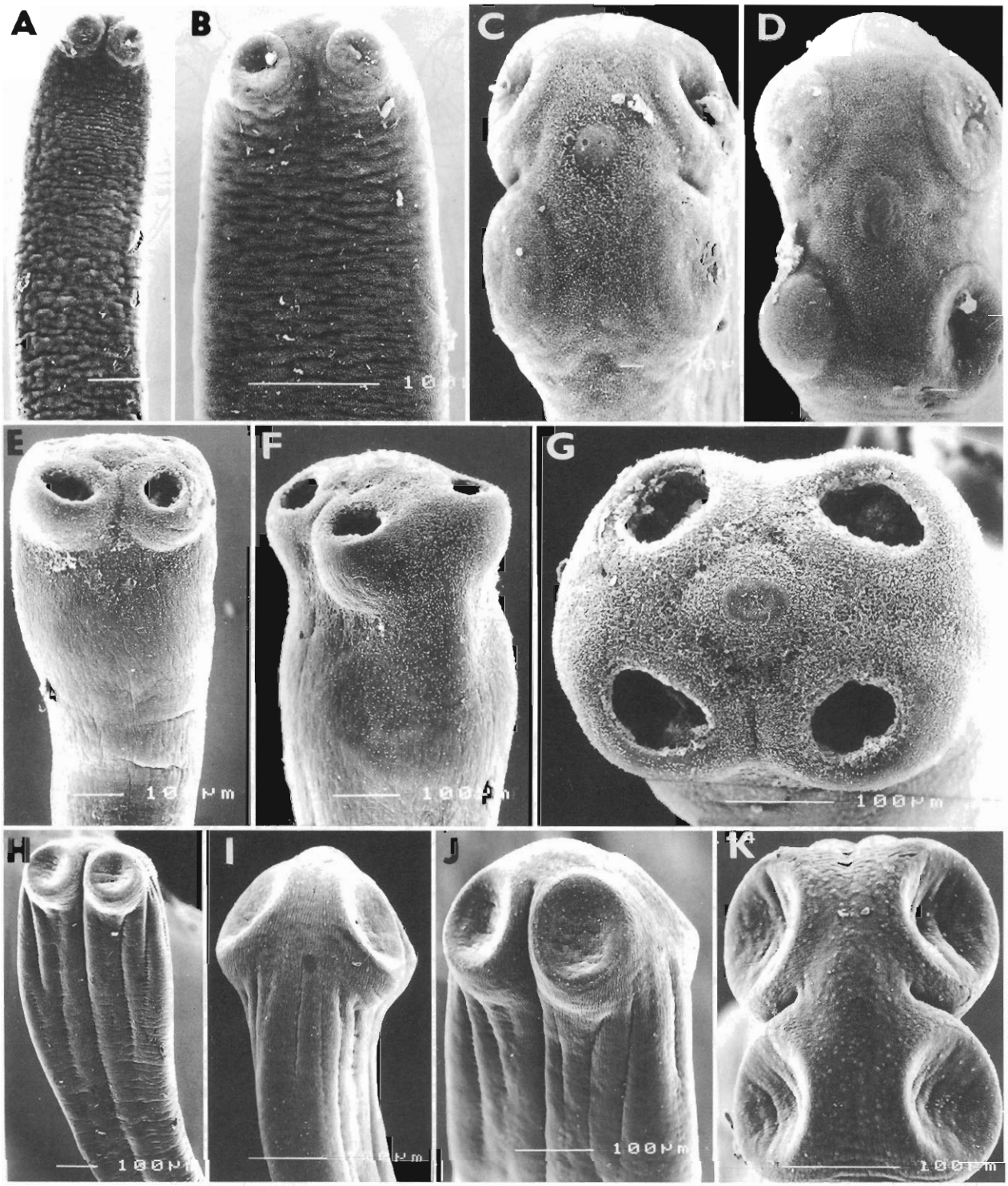

Fig. 6. A-D - *Proteocephalus percae* from *Perca fluviatilis*, Ružín, Slovakia; **E-G** – *P. thymalli* from *Thymallus arcticus nigrescens*, Mongolia; **H-K** - *P. torulosus* from *Leuciscus cephalus*, Czech Republic. Dorsoventral view (A,B,E,H,J); lateral view (F,I); apical view (C,D,G,K). Scanning electron microscopy (E-G – specimens fixed with ethanol).

two pairs of main collecting ducts (ventral ducts wider than dorsal ones) branched in the neck region and posterior to the suckers, forming a dense net of secondary canals, surrounding the lateral suckers and reaching up to the apical sucker. The canals ended blindly, not opening on the surface (*P. cernuae* - Fig. 1K; *P.exiguus*–Fig. 3G,I,J; *P. macrocephalus* – Fig. 5E; *P. percae* – Fig. 5Q).

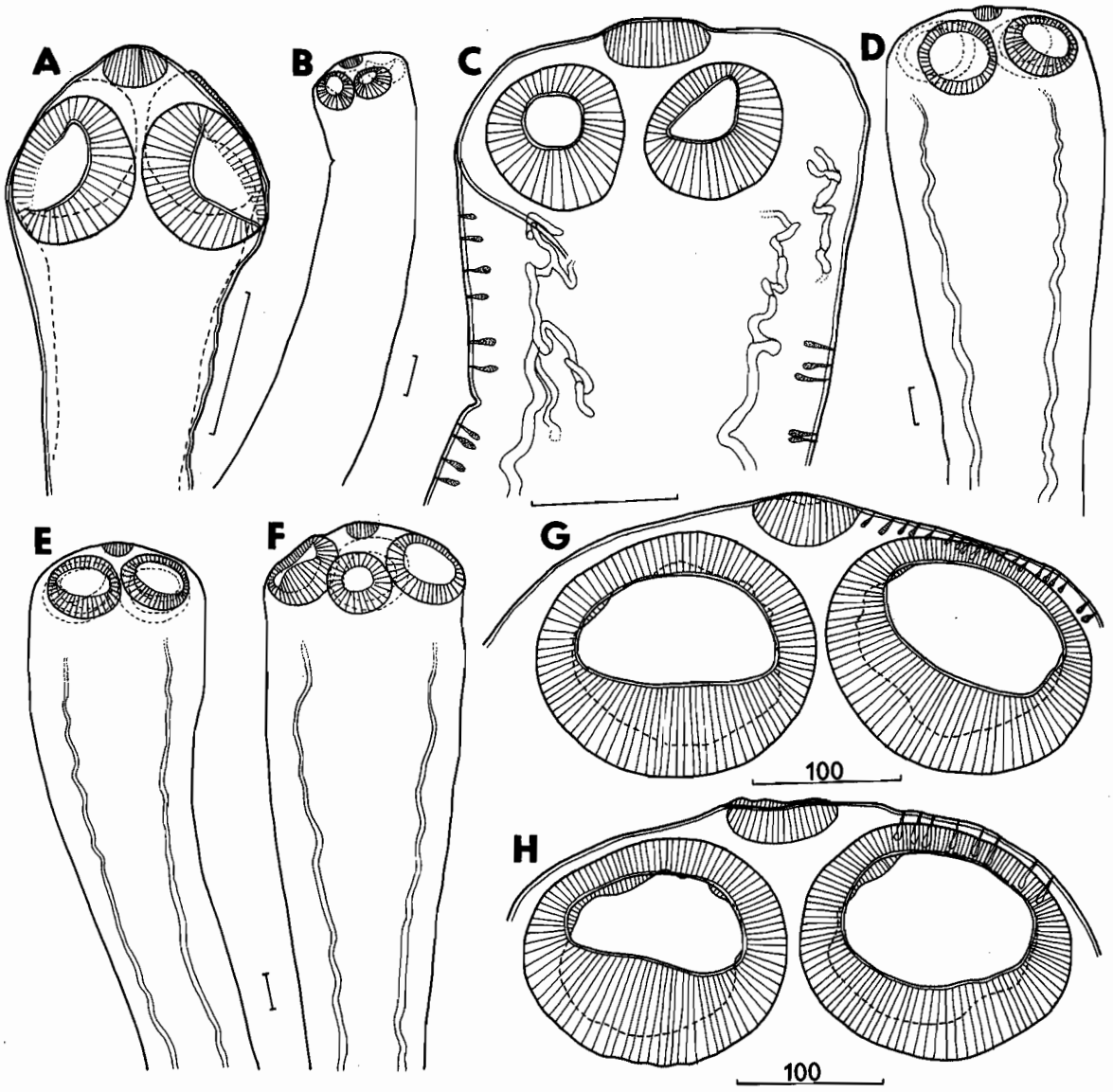

Fig. 7. A-C – *Proteocephalus pollanicola* from *Coregonus pollan*, Northern Ireland, UK; **D-H** – *P. thymalli* from *Thymallus arcticus nigrescens*, Mongolia (D-H). Lateral view (A); dorsoventral view (B-H). Note subtegumental gland cells in C,G and H. Scale bars = 100 µm.

Descriptions of species

On the basis of their morphology, the scoleces of individual species can be characterized as follows:

Proteocephalus ambiguus Fig. 1A-D

Scolex rounded, 157-220 wide; neck mostly indistinct or only slightly narrower than scolex (Fig. 1D) 169-232 wide; suckers sublateral, 52-62 in diameter; apical sucker vestigial, small and flattened (Fig. 1C,D), 20-32 in diameter. Glands cells not observed.

Proteocephalus cernuae Figs. 1E-K, 2A-C

Scolex bluntly ended, dorsoventrally flattened; neck only slightly narrower than scolex; suckers anterolateral, relatively small, representing only about 1/4 of scolex width (Table 4); apical sucker vestigial, relatively small, flattened to slightly elliptical (Fig. 1H,I,K), representing only about 10% of scolex width (Table 9). Numerous gland cells concentrated posterior to suckers, forming oval area in neck region (Fig. 1F,K). Few unicellular glands surrounding apical sucker (Fig. 1I,K).

Proteocephalus exiguus Figs. 2D-G, 3

Scolex small (Table 1; see Discussion), rounded; neck narrow (Table 2), almost always narrower than scolex (Fig. 3); suckers prominent, sublateral (Fig. 2D,E), small in its diameter (Table 3) but large relative to scolex width (Table 4; Fig. 3); apical sucker

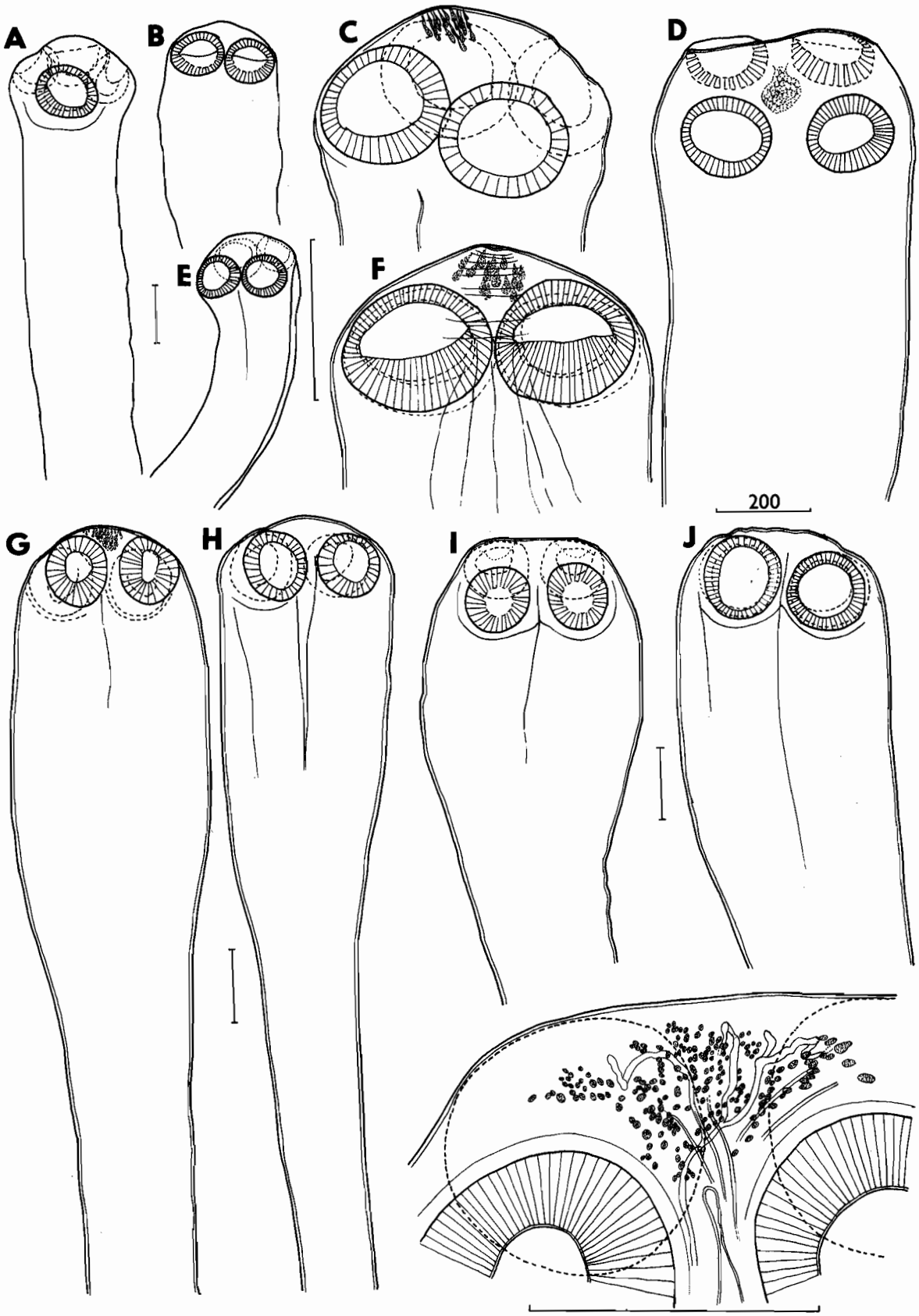

Fig. 8. A-F – *Proteocephalus sagittus* from *Noemacheilus barbatulus*, Dobšiná, Slovakia; fixed with 70% ethanol; **G-K** – *P. torulosus* from *Leuciscus cephalus*, Czech Republic. Dorsoventral view. Note concentration of gland cells opening outside in apical part of scolex (C,F,K) and blindly ended canals of osmoregulatory system (K). Scale bars = 200 μm.

relatively large in relation to scolex width (Table 9), mostly flattened (Fig. 3F,G) to oval (Fig. 3I,J). Numerous oval, unicellar glands concentrated posterior to scolex (Fig. 3A,D,J). Bottle-shaped glands, opening outside, lining margins of scolex, including its apical region (Fig. 3I,J).

The scoleces of almost all specimens from 4 different hosts (*Coregonus autumnalis*, *C. lavaretus*, *Oncorhynchus mykiss* and *Salvelinus fontinalis*) and 3 geographical regions (Russia, Switzerland and Slovakia) were rather similar in their morphological characteristics (Fig. 3). The scoleces of tapeworms from *C. lavaretus* were always larger than those from *C. autumnalis* but statistically significant differences were found only in 3 of 9 biometrical characters which, however, overlap between both populations (Tables 5, 8 and 9).

Proteocephalus filicollis Figs. 1L-P, 4A-C

Scolex rounded (Fig. 1L,M), 114-231 wide; neck narrower than scolex or as wide as scolex, 124-251 wide; suckers sublateral, relatively large, 38-93 in diameter; apical sucker vestigial, small, spherical (Fig. 1N-P), 19-32 in diameter, not always visible in SEM (Fig. 4C). Gland cells not observed.

Proteocephalus macrocephalus Figs.4D-G, 5A-E,H,I

Scolex rounded (Fig. 4D); neck narrower than scolex; suckers lateral (Fig. 4D,E), large in relation to scolex width (Table 4, Fig. 5A-C); apical sucker vestigial, small (Fig. 5H,I), representing less than 50% of lateral suckers and about 10% of scolex width, spherical (Fig. 5D,H) as high as wide (Table 7, Fig. 5E,I). Two types of gland, bottle-shaped cells present: smaller cells betweeen apical sucker and suckers (Fig. 5E), and larger cells posterior to lateral suckers and in neck region (Fig. 5E).

Proteocephalus osculatus Figs. 4H-J, 5F,G,K,L

Scolex short and wide (Table 1), with prominent lateral and apical suckers (Fig. 5F,G); neck conspicuous, narrower than scolex; suckers large, lateral to sublateral (Fig. 4H); apical sucker large and long (Tables 5 and 6), well-developed and strongly muscular with deep cavity (Fig. 4J, 5K,L). Numerous bottle-shaped gland cells lining margin of body from neck region to apical part of scolex; ducts of gland cells form several bundles between suckers, with openings surrounding apical sucker (Fig. 5L).

Proteocephalus percae Figs. 5J,M-Q, 6A-D

Scolex small (Table 1), tapering anteriorly (Figs. 5J, 6A,B), dorsoventrally flattened (Fig. 6C,D); neck always wider than scolex (Figs. 5M,N, 6A,B); suckers small (Table 3), lateral to sublateral (Figs. 5O-Q, 6B,C); apical sucker vestigial, small and flattened (Tables 5 and 6, Fig. 6C,D).

Numerous gland cells concentrated medially posterior to scolex (Fig. 5J). Other bottle-shaped cells

Table 7. Ratio of diameter of apical sucker to its length (DAS/LAS).

Species	Mean	SE	Range	Cv	Groups
P. macrocephalus	0.93	0.03	0.76-1.23	14	A
P. osculatus	1.15	0.08	0.71-1.43	21	A
P. exiguus[1]	1.48	0.07	0.70-2.38	25	B
P. percae	1.67	0.05	1.22-2.43	17	BC
P. exiguus[2]	1.72	0.09	0.95-2.29	19	BC
P. cernuae	1.75	0.17	1.33-2.39	20	BC
P. pollanicola	1.86	0.33	1.82-3.35	21	C
P. thymalli	1.87	0.1	1.24-2.66	21	C

[1]from *Coregonus autumnalis*, Baikal Lake, Russia
[2]from *Coregonus lavaretus*, Lac de Bienne, Switzerland

Table 8. Ratio of diameter of apical sucker to diameter of suckers (DAS/DS).

Species	Mean	SE	Range	Cv	Groups
P. macrocephalus	0.38	0.01	0.29-0.48	11	A
P. thymalli	0.42	0.01	0.35-0.44	10	A
P. cernuae	0.42	0.03	0.33-0.51	16	A
P. exiguus[1]	0.53	0.02	0.36-0.81	18	B
P. percae	0.58	0.02	0.38-0.73	17	C
P. exiguus[2]	0.62	0.03	0.42-0.77	17	C
P. osculatus	0.58	0.02	0.48-0.73	12	C
P. pollanicola	0.76	0.03	0.56-0.90	13	D

[1]from *Coregonus autumnalis*, Baikal Lake, Russia
[2]from *Coregonus lavaretus*, Lac de Bienne, Switzerland

Table 9. Ratio of diameter of apical sucker to diameter of suckers (DAS/DS).

Species	Mean	SE	Range	Cv	Groups
P. macrocephalus	0.11	0	0.07-0.14	17	A
P. thymalli	0.11	0.02	0.08-0.16	30	A
P. cernuae	0.14	0	0.11-0.17	13	B
P. exiguus[1]	0.17	0	0.12-0.22	14	C
P. percae	0.18	0	0.12-0.25	13	C
P. exiguus[2]	0.21	0.01	0.15-0.23	12	D
P. osculatus	0.22	0.01	0.16-0.25	11	D
P. pollanicola	0.29	0.01	0.26-0.31	7	E

[1]from *Coregonus autumnalis*, Baikal Lake, Russia
[2]from *Coregonus lavaretus*, Lac de Bienne, Switzerland

scattered laterally from apical part of scolex, including suckers, to neck region (Fig. 5J,O-Q).

Proteocephalus pollanicola Figs. 2H-J, 7A-C

Scolex similar in shape to that of *P. exiguus* as described above, differing only in anteriorly directed suckers (Fig. 2I,J) and by larger measurements (Tables 1-9), mainly by much larger apical sucker (diameter more than 50 μm, representing more than 25% of scolex width – Tables 5 and 9). Gland cells of similar distribution as in *P. exiguus*.

Proteocephalus sagittus Fig. 8A-F

Scolex spherical, short (? contracted specimens – Fig. 8A,C,E) to long, club-shaped (Fig. 8B); neck narrower than scolex; suckers sublateral; apical sucker absent; bottle-shaped gland cells concentrated within apical end of scolex (Fig. 8C,D,F).

Proteocephalus thymalli Figs. 6E-G, 7D-H

Scolex long and wide (Table 1), club-shaped (? contracted specimens; Figs. 6E,F, 7D-F); neck narrower than scolex, situated far posterior to suckers (Figs. 6E, 7E,F); suckers very large (Table 3), directed anteriorly (? contraction due to fixation) (Fig. 6E-G); apical sucker vestigial, quite large and flattened (Fig. 7G,H; Tables 5-7). Small gland cells lining margins of scolex (Fig. 7G,H).

Proteocephalus torulosus Figs. 6H-K, 8G-K

Scolex very long and wide (Table 1), club-shaped (Figs. 6H, 8G-J), dorsoventrally slightly flattened (Fig. 6J), with longitudinal wrinkles (Fig. 6H-J); neck narrower than scolex, very far posterior to suckers (Fig. 8G,H); suckers sublateral (Fig. 6H-K), large (Table 3); apical sucker absent, numerous gland cells accumulated in apical region (Fig. 8G,K), opening by small ducts around apex of scolex.

DISCUSSION

The present study enabled to characterise 11 *Proteocephalus* species on the basis of their scoleces. Some species, e.g., *P. cernuae*, *P. macrocephalus*, *P. osculatus* and *P. percae*, appeared to be easily distinguishable from congeners on the basis of their scolex morphology.

Biometrical evaluation revealed statistically significant differences between individual taxa studied in some features (Tables 1-9). However, intraspecific variability expressed by the coefficient of variability was low only in a few characters and taxa. The most stable characters appeared to be the width of the scolex (in 3 species coefficient lower than 10%), the diameter of suckers and the diameter of suckers to the width of the scolex. On the other hand, some indices as the ratio of the diameter of the apical sucker to its length were quite variable.

In addition, many metrical features overlapped in their values so that they seem to have limited taxonomic value for distinguishing closely similar taxa. Moreover, the present data are based only on selected populations of individual species and they should be compared only with those based on similarly fixed material, i.e. not that fixed under pressure or with cold fixative.

Regarding intraspecific variability, it was found that the scoleces of *P. osculatus* had the lowest coefficient of variability in most characters measured; similarly, the scoleces of *P. macrocephalus* exhibited rather low variation in their measurements.

No significant differences were observed in the morphology of the scoleces of *P. exiguus* from different hosts (*Coregonus lavaretus maraena*, *C. autumnalis*, *Oncorhynchus mykiss*, *Salvelinus fontinalis*) and geographical regions (Central Europe, Russia) despite the fact that the respective populations considerably differed in their size and strobilar morphology as the length of the strobila, the shape of proglottides, testis number and relative size of the cirrus-sac (unpublished data). It can be assumed that the scolex of *P. exiguus* possesses more stable characters than the strobila, the morphology of which has been proved to be rather variable (Ieshko and Anikieva 1980, Anikieva et al. 1983, Hanzelová and Špakulová 1992, Šnábel et al. 1994, Hanzelová et al. 1995a).

Some species, however, were only slightly different from others so that the following groups of species could be recognized. The first couple was formed by *P. exiguus* and *P. pollanicola*, a rare species described from *Coregonus pollan* (*C. autumnalis pollan* according to some authors) in Northern Ireland (Gresson 1952). The scolex of *P. pollanicola* differed from that of *P. exiguus* mainly by larger measurements as the diameter of an apical sucker, representing more than 50% of suckers' diameter and more than 1/4 of the scolex width. However, it should be emphasized that only two populations of *P. exiguus* were studied herein. Previous studies demonstrated that populations of *P. exiguus* from other hosts, e.g., *Brachymystax lenok* or *Oncorhynchus mykiss*, have much larger scoleces than those of the specimens studied herein (cf. Scholz and Ergens 1990, Šnábel et al. 1994). Similar strobilar morphology of both taxa (see Freze 1965) and host spectrum of *P. exiguus*, comprising a wide variety of salmonoid fish of the genera *Brachymystax*, *Coregonus*, *Salmo*, *Oncorhynchus*, *Salvelinus* in the Holarctic Region (Hanzelová et al. 1995a), indicate doubtful validity of *P. pollanicola*[1].

[1]Morphological and genetic (RAPD) evaluation of a new material of *P. pollanicola* obtained after this paper had been accepted for publication showed that this taxon is a synonym of *P. exiguus* (see Scholz et al. 1998).

The second group included *P. torulosus*, occurring in cyprinid fish from the Palaearctic Region (Freze 1965), and *P. sagittus*, a fairly rare parasite of stone loach (*Noemacheilus barbatulus*) (Grimm 1872). Although the scoleces of *P. sagittus* specimens studied were more frequently spherical than club-shaped as typical of *P. torulosus*, a close similarity between these taxa is evident: both have large, sublaterally situated suckers and no apical sucker, which is replaced by gland cells concentrated in the apical part of the scolex. More spherical shape of *P. sagittus* may be related to the fact that only specimens fixed with ethanol or cold formalin were available, which might resulted in their unnatural contraction. Taking into account this similarity in the scolex morphology and resemblance in the strobilar morphology (see, e.g., Freze 1965, Dubinina 1987, Scholz 1989), the identity of both the taxa seems to be quite probable.

Two species from sticklebacks, *P. ambiguus* and *P. filicollis*, were rather similar in most features of the scolex, except for slightly different shape of an apical sucker. However, it must be emphasized that only a few *P. filicollis* specimens were studied and that *P. ambiguus* tapeworms available for this study were not fixed with hot fixative, which might have influenced the morphology of the scolex, including that of the apical sucker.

The scolex of *P. filicollis* was characterized by Andersen (1979) and Rødland (1983) as being rounded to dorsoventrally flattened, with a distinct apical sucker. Rødland (1983) considered the presence or absence of the apical sucker to be one of the most important discriminative characters between *P. filicollis* and *P. ambiguus*. According to him, *P. filicollis* has the apical organ (= sucker), which is always visible on SEM photomicrographs as a frontal pit, whereas *P. ambiguus* has no apical organ. However, the apical sucker on the scoleces of *P. filicollis* was not always distinguishable in SEM (Fig. 4C) in this study and, on the other hand, the apical sucker was present in all specimens of *P. ambiguus* (Fig. 1A-D).

The scolex of *P. thymalli*, a parasite described from *Thymallus arcticus* and also reported from the salmonid *Brachymystax lenok* (Freze 1965), distinctly differed from congeners in its possession of the swollen (club-shaped), quite wide scolex and a large apical sucker. Nevertheless, it must be pointed out that only ethanol-fixed specimens were available in this study so that the typical appearance of the scolex might be a result of unnatural contraction caused by this fixation. In other features as the diameter of suckers related to the scolex width and in the morphology of the apical sucker, *P. thymalli* resembled *P. exiguus*. It is evident that more detailed comparative study, based on fresh material, is necessary.

Rintamäki and Valtonen (1983) studied, using SEM, the scolex morphology of four *Proteocephalus* species, *P. cernuae*, *P. exiguus*, *P. longicollis* and *P. percae*. Their descriptions of the scoleces correspond more or less to the present data: indistinct neck and a small fifth (= apical) sucker in *P. cernuae*; round, slightly dorsoventrally flattened scolex with anteriorly directed suckers (contraction due to fixation?) and a relatively large apical sucker in *P. exiguus*; and laterally directed suckers and dorsoventral flattening of the scolex of *P. percae*.

Andersen (1979) also found the scolex of *P. percae* to be dorsoventrally flattened, with suckers directed laterally, not anteriorly, and with a small and shallow vestigial apical sucker, which tallies with the present data. A good agreement between the present data and those by Andersen (1979) was also found in the characterisation of the scolex of *P. macrocephalus*. The position and relative size of suckers were already used for differentiation of *P. macrocephalus* from *P. cernuae*, a species with quite similar strobilar morphology (Scholz and Kepr 1988). Scholz et al. (1997) also considered the scolex of *P. macrocephalus* to possess species-specific features.

Andersen (1979) expressed her doubts about usefulness of the size and shape of an apical sucker for differentiating species studied, i.e. *P. filicollis*, *P. gobiorum*, *P. macrocephalus*, *P. percae* and *Proteocephalus* sp. from *Salmo trutta* and *Salvelinus fontinalis* (probably *P. exiguus*). This study, however, demonstrated suitability of the apical sucker for distinguishing some species. The shape and size of this sucker was also used in the synonymization of *P. dubius* La Rue, 1911, a rare parasite of perch, with *P. percae*, commonly occurring in the same fish host (Scholz et al. 1995). Similarly, Hanzelová et al. (1996) used the size and shape of the apical sucker for the identification of *P. exiguus* tapeworms from an atypical host, perch, from a small lake in Slovakia; their identification was confirmed by isoenzyme analysis (Hanzelová et al. 1996).

The present SEM study, in accordance with observations by Andersen (1979), and Rintamäki and Valtonen (1983), revealed that the apical sucker was not always recognizable in SEM photomicrographs even in those species in which the apical sucker was easily to distinguish in permanent mounts (e.g., *P. cernuae*, *P. exiguus*, *P. filicollis*). The only species having the apical sucker always observable in SEM was *P. osculatus*. It seems that caution must be taken when considering the presence or absence of an apical sucker merely on the basis of scanning electron microscopy.

Using Nomarski interference contrast, differences in the distribution, shape and size of gland cells within the scolex and neck region of *P. cernuae*, *P. exiguus*, *P. macrocephalus*, *P. osculatus*, *P. percae*, *P. sagittus* and *P. torulosus* were observed. It seems that there are several types of gland cells, which tallies with observations by Žďárská and Nebesářová (1995), who described two types of subtegumental gland cells in the scolex of *P.*

macrocephalus. However, the suitability of gland cells as an additional taxonomic character requires further studies in other *Proteocephalus* taxa, including transmission electron microscopy (Stoitsova et al. 1995). In contrast to gland cells, the morphology of the osmoregulatory system appeared to be fairly similar among *Proteocephalus* species and no significant differences were found.

The present investigation was mainly based on the material fixed by the same technique because it had been observed that fixation with cold fixatives can cause contraction of worms so that the neck region present in living worms can be absent. Fixation with hot formaldehyde solution made it possible to obtain specimens suitable for morphological evaluation, including scanning electron microscopy, and biometrical analysis.

This comparative study revealed that the scolex represents a relatively stable structure, possessing species-specific characters in some taxa. This tallies with the fact that the scolex is formed early during the development of *Proteocephalus* procercoids within the intermediate host and it is morphologically quite similar to that of adult worms (see, e.g., Wootten 1974, Priemer 1980, 1987, Anikieva et al. 1983, Rusinek 1989, Scholz 1991, 1993, Scholz et al. 1997).

The present observations also stressed the necessity to provide comparable data on the scolex morphology of other *Proteocephalus* species in order to assess their intraspecific variability and to test the suitability of differential criteria found. This was emphasized already by Andersen (1979) who stated: ".. a future aim must be a revision based on comparative studies on material of known origin treated and handled equally so that characters of real value for specific diagnoses can be described".

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