

An annotated list of species of the *Proteocephalus* Weinland, 1858 aggregate *sensu de* Chambrier et al. (2004) (Cestoda: Proteocephalidea), parasites of fishes in the Palaearctic Region, their phylogenetic relationships and a key to their identification

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Abstract A list and key to the identification of valid species of tapeworms of the *Proteocephalus* Weinland, 1858 aggregate *sensu de* Chambrier et al. (2004), i.e. species of the genus occurring in fresh- and brackish-water fishes in the Palaearctic Region, are provided, with data on their hosts and geographical distribution. Instead of 32 taxa listed by Schmidt (1986) and subsequent authors, only the following 14 species are considered to be valid: *P. ambiguus* (Dujardin, 1845) (type-species); *P. cernuae* (Gmelin, 1790); *P. filicollis* (Rudolphi, 1802); *P. fluviatilis* Bangham, 1925; *P. gobiorum* Dogiel & Bychowsky, 1939; *P. longicollis* (Zeder, 1800); *P. macrocephalus* (Creplin, 1825); *P. midoriensis*

Shimazu, 1990; *P. percae* (Müller, 1780); *P. plecoglossi* Yamaguti, 1934; *P. sagittus* (Grimm, 1872); *P. tetrastomus* (Rudolphi, 1810); *P. thymalli* (Annenkova-Chlopina, 1923); and *P. torulosus* (Batsch, 1786). An analysis of sequences of the nuclear genes (ITS2 and V4 region of 18S rDNA) revealed the following phylogenetic relationships for these taxa: *P. torulosus* ((*P. midoriensis*, *P. sagittus*) (*P. fluviatilis* (*P. filicollis*, *P. gobiorum*, *P. macrocephalus*)) (*P. cernuae*, *P. plecoglossi*, *P. tetrastomus* (*P. longicollis*, *P. percae*) (*P. ambiguus*, *P. thymalli*))). *P. pronini* Rusinek, 2001 from grayling *Thymallus arcticus nigrescens* is synonymised with *P. thymalli*. *P. esocis* La Rue, 1911 is apparently invalid but its conspecificity with either *P. percae* or *P. longicollis* could not be confirmed due to the absence of the scolex in the holotype and the unavailability of other material for morphological and molecular studies. *P. osculatus* (Goeze, 1782) has recently been transferred to *Glanitaenia* de Chambrier, Mariaux, Vaucher & Zehnder, 2004. The validity of the genus is supported by the position of *G. osculata* within the Proteocephalidea, based on molecular data, as well as its morphology and nature of the definitive host (the European wels *Silurus glanis*). *P. hemispherous* Rahemo & Al-Niaeemi, 2001, described from *S. glanis* in Iraq, is transferred to *Postgangesia* Akhmerov, 1960 as *Postgangesia hemispherous* (Rahemo & Al-Niaeemi, 2001) n. comb.

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Introduction

Tapeworms of the *Proteocephalus* Weinland, 1858 aggregate *sensu de Chambrier et al.* (2004) are parasitic in freshwater fishes in the Palaearctic Region (see de Chambrier, Zehnder, Vaucher & Mariaux, 2004). Since 1780, when O.F. Müller described *Taenia percae*, the first species currently recognised as a member of *Proteocephalus*, several tens of taxa (73 according to Freze, 1965 and 91 according to Schmidt, 1986) have been described.

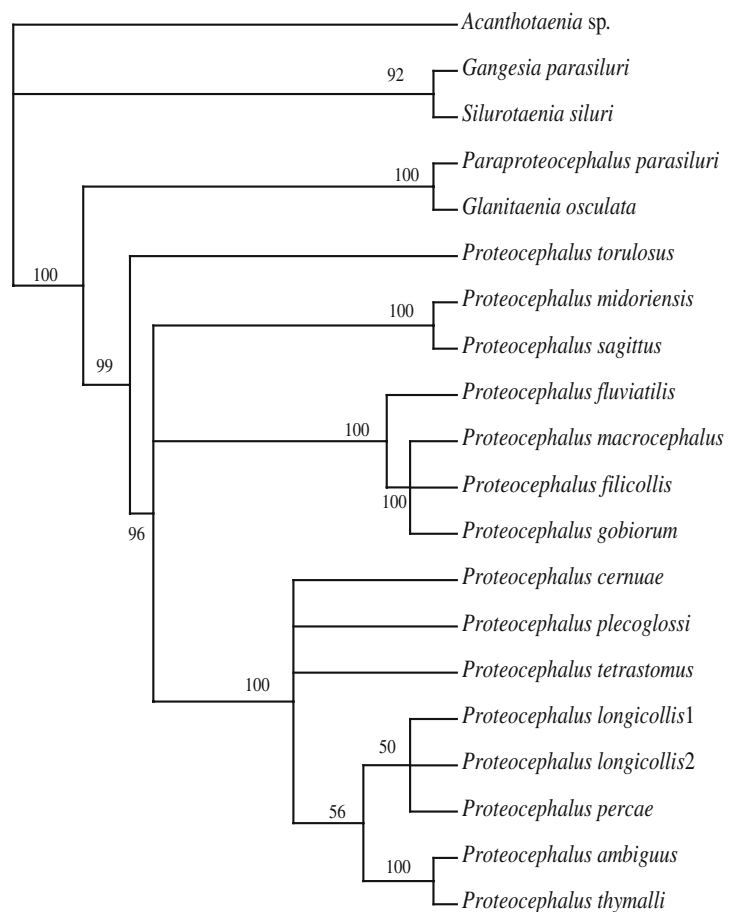
However, recent studies on the systematics of species of the aggregate and several taxa of *Proteocephalus (sensu lato)* from North American freshwater fishes, carried out by the present authors (Scholz & Hanzelová, 1998, 1999; Hanzelová & Scholz, 1999), have demonstrated that the actual number of valid species of *Proteocephalus* is much lower. In a series of papers, numerous species have

been synonymised and all taxa from European fishes were revised and redescribed (Scholz & Hanzelová, 1998). In order to avoid confusions regarding the validity of species of the *Proteocephalus* aggregate, an annotated list of its taxa considered to be valid is presented, with a brief information on their definitive hosts and geographical distribution. Phylogenetic relationships of all valid taxa inferred from an analysis of the ITS2 and V4 regions of 18S rRNA genes are also discussed, based on the majority consensus of 32 trees of maximum parsimony analysis of three matrices (Fig. 1).

Materials and methods

The list is based primarily on the results of multidisciplinary studies of the present authors and their co-workers: Shimazu (1990, 1993), Hanzelová &

Fig. 1 Phylogenetic relationships of *Proteocephalus* species from the Palaearctic Region based on 5.8S + ITS2 and V4 region of 18S rRNA gene sequences: majority consensus tree of 32 consensus trees obtained by maximum parsimony analyses (according to the matrix alignment parameters: TL = 1536–3041; CI = 0.75–0.78). The numbers at the nodes show bootstrap support



Scholz (1992, 1993, 1999), Hanzelová & Špakulová (1992), Špakulová & Hanzelová (1992), Scholz & Hanzelová (1994, 1998, 1999), Šnábel, Hanzelová & Fagerholm (1994), Šnábel, Hanzelová, Mattiucci, D'Amelio & Paggi (1996), Hanzelová, Scholz & Fagerholm (1995), Hanzelová, Šnábel, Špakulová, Fagerholm & Král'ová (1995), Hanzelová, Šnábel & Špakulová (1996), Hanzelová, Šnábel, Král'ová, Scholz & D'Amelio (1999), Scholz, Hanzelová & Šnábel (1995), Scholz, Špakulová, Šnábel, Král'ová & Hanzelová (1997), Scholz, Drábek & Hanzelová (1998), Scholz, Hanzelová, Králová & Griffiths (1998), Scholz, Škeříková, Hanzelová, Koubková & Baruš (2003), Scholz, Marcogliese, Bourque, Škeříková & Dodson (2004), Turčeková & Král'ová (1995), Král'ová (1996), Král'ová & Špakulová (1996), Škeříková, Hypša & Scholz (2001) and Hypša, Škeříková & Scholz (2005). The most detailed information on the morphology of European taxa of *Proteocephalus*, including key to the species, was provided by Scholz & Hanzelová (1998), whereas Japanese taxa were redescribed by Shimazu (1990). Scholz, Drábek & Hanzelová (1998) presented a detailed account of the morphology and measurements of the scoleces of most species of *Proteocephalus* from the Palaearctic Region.

In addition to *Proteocephalus* species studied previously, new material of *P. ambiguus* (Dujardin, 1845) from *Pungitius pungitius* (L.) in Poland and the following species from Japan and Finland was morphologically evaluated: Japan: *P. fluviatilis* Bangham, 1925 from *Micropterus dolomieu* Lacépède, Lake Nojiri, Nagano Prefecture; *P. midoriensis* Shimazu, 1990 from *Lefua echigonia* Jordan & Richardson, Iiyama, Nagano Prefecture and the Yanamunegawa River, Kosei Town, Shiga Prefecture; *P. plecoglossi* Yamaguti, 1934 from *Plecoglossus altivelis altivelis* (Temminck & Schlegel), Lake Biwa, Shiga Prefecture; *P. plecoglossi* juv. from *Gymnogobius isaza* (Tanaka), *Hemibarbus barbus* (Temminck & Schlegel), *Opsariichthys uncirostris* (Temminck & Schlegel) and *Tridentiger brevispinis* Katsuyama, Arai & Nakamura, Lake Biwa, Shiga Prefecture; *P. tetrastomus* (Rudolphi, 1810) from *Hypomesus nipponensis* (McAllister), Lake Suwa, Nagano Prefecture, and Lake Ogawara, Aomori Prefecture; Finland: *P. cernuae* (Gmelin, 1790) from *Gymnocephalus cernuus* (L.); *P. filicollis* (Rudolphi, 1802) from *Gasterosteus aculeatus* L.; *P. longicollis*

(Zeder, 1800) from *Coregonus albula* (L.) and *C. widegreni* Malmgren; *P. percae* (Müller, 1780) from *Perca fluviatilis* L.; *P. tetrastomus* from *Osmerus eperlanus* (L.) – all cestodes from the Bothnian Bay at Kiviniemi near Oulu, Finland.

An assessment of the phylogenetic relationships is primarily based on the methodology and multigene analysis carried out by Hypša et al. (2005), but molecular data provided by Zehnder & Mariaux (1999), Škeříková et al. (2001), de Chambrier et al. (2004) and Scholz et al. (2004) were also considered, if necessary. In addition, six new sequences of the V4 region of 18S rRNA gene and/or 5.8S + ITS-2 of *P. ambiguus* from *Pungitius pungitius* in Poland, *P. longicollis* (Zeder, 1800) from *Oncorhynchus mykiss* (Walbaum) in Scotland, *P. percae* from *Perca fluviatilis* L. in Switzerland and *P. thymalli* from *Thymallus arcticus* (Pallas) in Russia, were obtained and analysed (Table 1).

Cestodes intended for sequencing were fixed with 96% ethanol. Total DNA was extracted from 0.5 cm of strobila using the QIAamp Tissue kit (Qiagen). The V4 region of the 18S rRNA gene was amplified by PCR using the primers Ces1 (5'-CCA GCA GCC GCG GTA ACT CCA-3') and Ces2 (5'-CCC CCG CCT GTC TCT TTT GAT-3') designed according to the complete sequences of the 18S rRNA gene of *Proteocephalus exiguus* La Rue, 1911 (= *Proteocephalus longicollis* (Zeder, 1800); Král'ová et al., 1997). The 5.8S rRNA-ITS2 genes were amplified by PCR using the primers Proteo1 (5'-CGG TGG ATC ACT CGG CTC-3') and Proteo2 (5'-TCC TCC GCT TAT TGA TAT GC-3') designed according to the complete sequences of the ITS1-ITS2 genes of *Eubothrium crassum* (Bloch, 1779) and *E. salvelini* (Schrank, 1790) (Král'ová, Hanzelová, Scholz, Gerdeaux & Špakulová, 2001).

The following schedule was used for the PCR: first, 15 min at 95°C (HotStarTaq™ DNA polymerase); then 30 cycles: denaturation, 1 min at 94°C, annealing 1 min at 60°C, extension, 2 min at 72°C, and at the end the final extension 10 min at 68°C. The PCR products were cloned into pGEM-T Easy System 1 (Promega) and sequenced in both directions using T7 and SP6 primers. DNA sequencing was performed on automated sequencer model 310 ABI PRISM (PE-Biosystems) using Big-Dye DNA sequencing kit (PE-Biosystems). Accession numbers of the six sequences obtained (DQ427096–DQ427101), as well as the 32 sequences

Table 1 The taxa and genes included in the phylogenetic analysis (new sequences in bold and marked with an asterisk)

Sample	ITS-2 + 5.8S	V4-18S rRNA
<i>Acanthotaenia</i> sp. (outgroup)	AY551137	AF267292
<i>Gangesia parasiluri</i> (outgroup)	AY551144	AF267293
<i>Glanitaenia osculata</i>	AY551169	AF335508
<i>Paraproteocephalus parasiluri</i>	AY551157	AY551121
<i>Proteocephalus ambiguus</i>	DQ427096*	DQ427100*
<i>Proteocephalus cernuae</i>	AY551160	AY551125
<i>Proteocephalus filicollis</i>	AY551162	AF335506
<i>Proteocephalus fluviatilis</i>	AY551163	AY551126
<i>Proteocephalus gobiorum</i>	no PCR product	AY551127
<i>Proteoceph. longicollis</i> 1 (Switzerland)	AY551166	X99976
<i>Proteoceph. longicollis</i> 2 (Scotland)	DQ427097*	DQ427101*
<i>Proteocephalus macrocephalus</i>	AY551167	AF335507
<i>Proteocephalus midoriensis</i>	AY551168	AY551130
<i>Proteocephalus percae</i>	DQ427098*	AF335509
<i>Proteocephalus plecoglossi</i>	AY551171	AY551132
<i>Proteocephalus sagittus</i>	AY375548	AY375550
<i>Proteocephalus tetrastomus</i>	AY379113	AF335510
<i>Proteocephalus thymalli</i>	DQ427099*	no PCR product
<i>Proteocephalus torulosus</i>	AY375549	AF335511
<i>Silurotaenia siluri</i> (outgroup)	AY551175	AF267297

retrieved from Gene Bank, are given in Table 1. Three taxa, *Acanthotaenia* sp., *Gangesia parasiluri* Yamaguti, 1934 and *Silurotaenia siluri* (Batsch, 1786), were used as outgroups, based on their position among proteocephalideans (de Chambrier et al., 2004; Hypša et al., 2005).

The new sequences were aligned manually to the three alignments combining ITS2 + V4-18S rRNA (“basic matrices” used by Hypša et al., 2005) using the Bioedit program (Hall, 1999) in respect of their

high similarity to the sequence of *P. longicollis* from *Coregonus lavaretus* in Switzerland (see alignment parameters in Table 2). The alignments are available upon request from the authors.

The phylogenetic analysis and calculation of nodal support (maximum parsimony – MP and bootstrap) were performed using PAUP version 4.0 (Swofford, 2003). The MP analyses were performed under the assumption of Tv/Ts ratio 1:1, 1:2 and 1:3. A bootstrap support (1,000 replicates) was calculated for Tv/Ts 1:2.

Table 2 Alignment parameters

	Matrix 1			Matrix 2			Matrix 3		
Alignment parameters:									
Gap opening penalty	15			1			0.7		
Gap extension penalty	10			0.1			0.7		
Transition weight	20			1			0.7		
Number of characters	1,907			2,129			1,882		
Phylogenetic analyses:									
Tv/Ts ratio	1:1	1:2	1:3	1:1	1:2	1:3	1:1	1:2	1:3
Informative characters	413	440	440	391	409	409	424	448	448
Number of trees	2	4	2	2	4	2	10	4	2

Reference material (types or vouchers) of *P. pamirensis* Dzhililov & Ashurova, 1971 (syn. of *P. sagittus* – see Scholz et al., 2003) and *P. pronini* Rusinek, 2001 has not been available, despite several attempts to contact the authors of the original descriptions or institution where the type-specimens should have been deposited. These two taxa and specimens of *P. esocis* were not available for molecular analysis.

In the present list, information on the type- and other hosts (only reliable host records are considered; for a more complete list of hosts from Europe – see Scholz & Hanzelová, 1998), type-localities and geographical distribution is provided. References in the list of valid species refer only to the most pertinent papers, especially those providing detailed data on the morphology and systematics of the respective taxa.

Molecular data and phylogenetic analyses

New sequences of variable V4 region of 18S rRNA gene and complete ITS2 were obtained for 2 and 4 taxa, respectively (Table 1). The lengths of V4-18S rRNA varied from 454 bp (*P. ambiguus*) to 455 bp (*P. longicollis*), and for ITS2 from 741 bp (*P. ambiguus*) to 745 bp (*P. longicollis*).

The three matrices, combining the ITS2 and V4-18S rRNA gene sequences, provided altogether 1,882–2,129 characters (Table 2). Under MP criterion, the number of parsimony informative characters varied from 391–448 (Table 2). The majority consensus of 32 trees yielded a partly resolved topology of three well-supported monophyletic clusters with *P. torulosus* at their base (Fig. 1).

List of valid species

Proteocephalus ambiguus (Dujardin, 1845) – type-species

Syns *Taenia ambigua* Dujardin, 1845; *Proteocephalus filicollis* (Rudolphi, 1802) auct. in part

Type-host: Nine-spined stickleback *Pungitius pungitius* (L.) (Gasterosteiformes: Gasterosteidae).

Type-locality: Rennes, France.

Distribution: Europe, including Russia.

Reference: Rødland (1983); Scholz & Hanzelová (1998).

Comments: *P. ambiguus* occurs exclusively in the nine-spined stickleback (Willemse, 1968). Many confusions have existed as to the differentiation of this species from morphologically similar species, *P. filicollis*, a parasite of the three-spined stickleback *Gasterosteus aculeatus*, until Willemse (1968) and Rødland (1983) confirmed the validity of both the taxa and distinguished them on the basis of several morphological and biological characteristics. Although Scholz & Hanzelová (1998) questioned some of the differential features provided by Rødland (1983), they considered both the species to be valid. The validity of the species is supported by differences in the sequences of the ITS2 and 18S rRNA from those of *P. filicollis* (67.9% and 98.0%, respectively; see Table 3). Sequences of ITS-2 + 5.8S of *P. ambiguus* and *P. thymalli* were almost identical (similarity of 99.9%), which contrasts with the fact that these taxa are markedly different in their morphology (see Scholz & Hanzelová, 1998 and a key provided below). A high similarity (99.1%) was also found between the sequence of the V4 region of the 18S rRNA gene of *P. ambiguus* and that of one of two samples of *P. longicollis* (Table 3), although the latter species closely resembles *P. thymalli* in its morphology, thus being markedly different from *P. ambiguus*. The reasons for this surprising genetic similarity of morphologically distinct taxa specific to unrelated fish hosts require further study.

Phylogenetic relationships: *P. ambiguus* formed a clade with morphologically distinct *P. thymalli* (Fig. 1) and similarity of their ITS2 sequences was very high (99.9%) (no PCR product of 18S rDNA was obtained from *P. thymalli* sample despite several attempts). Correctness of *P. ambiguus* sequences was confirmed by repeated sequencing, with the result differing only in 2 nucleotides for both *P. ambiguus* samples; species identification of *P. ambiguus* samples sequenced was also confirmed before repeated trials.

Proteocephalus cernuae (Gmelin, 1790)

Syn. *Taenia cernuae* Gmelin, 1790

Type-host: Ruff *Gymnocephalus cernuus* (L.) (Perciformes: Percidae).

Type-locality: Not known, probably Germany.

Distribution: Europe, northern Asia (Russia).

Table 3 Percent similarity of individual sequences

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.
1. <i>Proteocephalus ambiguus</i>	*	99.8	98.0	98.5	98.2	96.7	99.1	97.8	96.7	94.5	99.3	99.3	98.0	99.6	-	96.7
2. <i>Proteocephalus cernuae</i>	95.2	*	98.2	98.7	98.5	96.9	99.3	98.0	96.9	94.3	99.6	99.6	98.2	100	-	96.9
3. <i>Proteocephalus filicollis</i>	67.9	67.6	*	97.8	99.3	95.2	97.6	98.5	95.9	93.2	97.8	97.8	97.1	98.2	-	95.6
4. <i>Proteocephalus fluviatilis</i>	74.4	73.5	74.4	*	98.0	95.9	98.0	97.6	96.1	93.6	98.2	98.2	97.4	98.7	-	96.0
5. <i>Proteocephalus gobiorum</i>	-	-	-	-	*	95.4	97.8	99.1	95.9	93.4	98.0	98.0	97.1	98.5	-	95.8
6. <i>Proteocephalus longicollis</i> 1	97.7	95.6	68.1	74.1	-	*	96.3	95.2	94.2	92.0	96.5	96.5	95.2	96.9	-	93.9
7. <i>Proteocephalus longicollis</i> 2	98.1	95.5	67.1	74.1	-	99.3	*	97.4	96.3	93.7	98.9	98.9	97.6	99.3	-	96.3
8. <i>Proteocephalus macrocephalus</i>	64.2	65.8	83.8	72.0	-	64.6	64.5	*	95.4	93.0	97.6	97.6	96.7	98.0	-	95.4
9. <i>Proteocephalus midoriensis</i>	69.2	68.9	60.6	64.7	-	69.6	69.7	59.9	*	93.3	96.5	96.5	98.3	96.9	-	95.2
10. <i>Proteocephalus osculatus</i> [#]	66.6	69.9	57.1	56.3	-	66.8	67.1	59.6	58.8	*	93.9	93.9	94.3	94.3	-	92.8
11. <i>Proteocephalus percae</i>	97.4	94.4	67.9	73.9	-	97.5	97.6	64.2	69.2	64.7	*	99.1	97.8	99.6	-	96.5
12. <i>Proteocephalus plecoglossi</i>	95.4	93.0	67.9	73.7	-	95.1	95.4	65.7	68.8	66.5	94.3	*	97.8	99.6	-	96.5
13. <i>Proteocephalus sagittus</i>	68.7	67.4	61.9	50.9	-	68.5	69.0	60.4	81.3	67.7	68.4	68.1	*	98.2	-	96.9
14. <i>Proteocephalus tetrastomus</i>	96.0	94.2	67.5	60.7	-	95.9	96.0	63.7	69.2	66.2	94.8	94.9	68.5	*	-	96.9
15. <i>Proteocephalus thymalli</i>	99.9	95.1	67.8	74.2	-	97.6	98.0	64.3	69.2	66.5	97.3	95.2	68.0	95.9	*	-
16. <i>Proteocephalus torulosus</i>	72.2	71.8	64.2	67.0	-	72.0	72.4	63.6	68.2	70.1	71.9	72.0	70.3	72.8	72.2	*

* ITS-2 + 5.8S similarities on the left from the asterisks, V4-18S rRNA similarities on the right

[#] Synonym of *Glanitaenia osculata* (see de Chambrier et al., 2004)

Reference: Scholz & Hanzelová (1998).

Comments: This species seems to be a specific parasite of ruff. Findings in other percids (*Perca fluviatilis* and *Sander lucioperca*) should be confirmed on the basis of new material. The species was redescribed by Scholz and Hanzelová (1998), who provided morphological characters enabling its differentiation from *P. percae* and other taxa of the genus.

Phylogenetic relationships: In a multigene analysis (Hypša et al., 2005), *P. cernuae* formed a clade with *P. longicollis* from salmoniform fish, although the species differ considerably in scolex morphology (Scholz, Drábek, & Hanzelová, 1998) and several strobilar characters (Scholz & Hanzelová, 1998). The adding of new sequences of *P. ambiguus* and *P. thymalli*, however, changed the topology of the tree and *P. cernuae* appeared in a polytomy formed by two species from osmeriform fish, namely *P. plecoglossi* and *P. tetrastomus* (Fig. 1). In all analyses, *P. cernuae* was not closely related to *P. percae*, another species from percid fish, but sequence similarity was relatively high (94.4% for ITS2 and 99.6% for V4 18S rDNA).

Proteocephalus filicollis (Rudolphi, 1802)

Syn. *Taenia filicollis* Rudolphi, 1802

Type-host: Three-spined stickleback *Gasterosteus aculeatus* L. (Gasterosteiformes: Gasterosteidae).

Type-locality: Greifswald, Germany.

Distribution: Circumboreal (Europe, northern Russia, North America).

References: Rødland (1983); Scholz & Hanzelová (1998).

Comments: This cestode is specific to three-spined stickleback in the Holarctic Region (Freze, 1965; Hoffman, 1999).

Phylogenetic relationships: Close relationship of *P. filicollis* with *P. macrocephalus* from eels was found in all analyses based on morphological data, 18S (Škeříková et al., 2001) and 28S rRNA (de Chambrier et al., 2004; Zehnder & Mariaux, 1999). In a multigene analysis (Hypša et al., 2005; present data), these two species formed a clade together with *P. gobiorum* from gobiids. Sequence similarity of these cestodes (Table 2) corresponds neither with relationships of their definitive hosts, stickleback, eels and gobiid fishes, because they are phylogenetically

distant (Nelson, 1994), nor with their morphology, although the “wide” form of *P. filicollis* somewhat resembles in its strobilar morphology that of *P. macrocephalus* (see Scholz & Hanzelová, 1998).

***Proteocephalus fluviatilis* Bangham, 1925**

Syn. *Proteocephalus osburni* Bangham, 1925

Type-host: Smallmouth bass *Micropterus dolomieu* Lacépède (Perciformes: Centrarchidae).

Type-locality: Ohio, USA.

Other hosts: *Ambloplites rupestris* (Rafinesque), *Lepomis auritus* (L.), *Micropterus salmoides* (Lacépède) (Centrarchidae).

Distribution: North America, Japan (introduced).

Reference: Shimazu (1990).

Comments: *Proteocephalus fluviatilis* was described from the smallmouth bass *Micropterus dolomieu* and later reported from other centrarchids in North America (Hoffman, 1999; Freze, 1965; Margolis & Arthur, 1979). Shimazu (1990) found this cestode in the largemouth bass *M. salmoides* from Lake Kizaki, Nagano Prefecture, in central Japan. The present material of *P. fluviatilis* was found in smallmouth bass from Lake Nojiri, Nagano Prefecture. The parasite was introduced into Japan, most probably with centrarchid fishes imported from North America. *Phylogenetic relationships*: In a multigene analysis (Hypša et al., 2005; present study), *P. fluviatilis* formed a sister group to the clade composed of *P. gobiorum*, *P. filicollis* and *P. macrocephalus*. All these taxa can be easily distinguished each other on the basis of their scolex morphology (Scholz, Drábek, & Hanzelová, 1998) and some characteristics of the strobila (Scholz & Hanzelová, 1998; Shimazu, 1990). Sequence similarity of *P. fluviatilis* with two other species from perciform fishes, i.e. *P. cernuae* and *P. percae*, is rather low (73.5–73.9% for ITS2 and 98.2–98.7% for V4).

***Proteocephalus gobiorum* Dogiel & Bychowsky, 1939**

Syn. *Proteocephalus subtilis* Naidenova, 1974

Type-host: Not designated, probably *Benthophilus macrocephalus* (Pallas) (Perciformes: Gobiidae).

Type-locality: Volga River estuary, Caspian Sea, Russia.

Other hosts: Gobies (*Benthophilus*, *Gobius*, *Neogobius*, *Pomatoschistus*).

Distribution: Northern Europe, Russia and Ukraine.

Reference: Scholz & Hanzelová (1998).

Comments: This tapeworm appears to be a specific parasite of gobies (Gobiidae) that have been reported in brackish waters of the Eastern Atlantic (Skagerrak), Baltic, Black and Caspian Seas. Scholz & Hanzelová (1998) did not find any morphological differences between specimens from *Gobius niger* and *Pomatoschistus microps* from Norway and those from a gobiid fish from Russia, probably from the Caspian Sea. However, the material of *P. gobiorum* studied by Scholz & Hanzelová (1998) was limited in number and no specimens were available for a comparative DNA analysis of tapeworms from different fish hosts and geographical regions.

Phylogenetic relationships: Molecular data demonstrate a close relationship of this species with *P. filicollis* and *P. macrocephalus* (Fig. 1), despite the unrelatedness of their fish definitive hosts and marked differences in their morphology, especially the scolex (*P. gobiorum* is devoid of an apical sucker which is present, yet reduced, in *P. filicollis* and *P. macrocephalus*). Despite several attempts, no PCR product for the ITS2 gene of *P. gobiorum* was obtained and thus its close relatedness to *P. filicollis* and *P. macrocephalus* should be confirmed by other gene(s). The absence of ethanol-fixed material of *P. gobiorum* from different seas and gobiid fish also makes it impossible to assess the range of intra-specific variability of this taxon, with its fairly wide host spectrum and distribution.

***Proteocephalus longicollis* (Zeder, 1800)**

Syns *Alyselminthus longicollis* Zeder, 1800; *Taenia salmonisomul* Pallas, 1811; *T. cyclops* Linstow, 1877; *T. salmonisumblae* Zschokke, 1884; *T. salvelini* Linton, 1897 (?); *Ichthyotaenia esocis* Schneider, 1905 (?); *I. agonis* Barbieri, 1909; *Proteocephalus pusillus* Ward, 1910; *P. exiguus* La Rue, 1911; *P. fallax* La Rue, 1911; *P. neglectus* La Rue, 1911; *P. laruei* Faust, 1920; *P. arcticus* Cooper, 1921; *P. coregoni* Wardle, 1932 (?); *P. wickliffi* Hunter & Bangham, 1933; *P. parallacticus* MacLulich, 1943; *P. californicus* Haderlie, 1950; *P. salmonidicola* Alexander, 1951; *P. pollanicola* Gresson, 1952; *P. tumidocollis* Wagner, 1953; *P. albulae* Freze & Kazakov, 1969

Type-host: Trout *Salmo trutta trutta* L. (Salmoniformes: Salmonidae).

Type-locality: Not known, probably Germany.

Other hosts: Salmoniform fishes (Salmonidae: *Oncorhynchus*, *Salmo*, *Salvelinus*; Coregonidae: *Coregonus*) and smelt (Osmeriformes: Osmeridae).

Distribution: Europe, Asia (Armenia, Russia, Mongolia), North America.

References: Hanzelová & Scholz (1993, 1999); Hanzelová, Scholz & Fagerholm (1995); Hanzelová et al. (1996); Scholz, Drábek & Hanzelová (1998), Scholz, Hanzelová et al. (1998); Scholz & Hanzelová (1998).

Comments: This is the most variable and frequent species of *Proteocephalus* that occurs in a wide range of definitive hosts, especially trout and whitefish, in the Holarctic Region (Ieshko & Anikieva, 1980; Hanzelová & Scholz, 1999; Scholz & Hanzelová, 1998). Such a wide host range may explain the fact that sequences of the two isolates of *P. longicollis* from different hosts differed (Table 3), even more than some morphologically well delimited taxa specific to unrelated hosts. However, despite these sequence differences, especially those in the V4 region of 18S rRNA, both isolates are considered to represent one species, because they appeared most closely to each other on the phylogenetic tree, forming a monophyletic group with *P. percae* (Fig. 1), and they share morphological characteristics considered to be species-specific, such as the shape of the scolex and apical sucker, and the morphology of the terminal genitalia formed by a long cirrus-sac and vaginal canal with a well-developed vaginal sphincter (Hanzelová, Šnábel et al., 1995; Scholz & Hanzelová, 1998). Only a detailed molecular study based on multigene analysis of a high number of samples from a much wider spectrum of fish hosts might reveal possible existence of cryptic species (Olson & Tkach, 2005).

Phylogenetic relationships: *P. longicollis* is morphologically similar to *P. percae* from percoid fish (see Šnábel et al. 1994; Hanzelová, Šnábel, Špakulová, et al., 1995; Scholz et al., 1995; Král'ová, 1996). This tallies with results of phylogenetic analyses based on sequences of 16S rDNA (Zehnder & Mariaux, 1999 – fig. 2), 18S rRNA (Škeříková et al. 2001 – fig. 1; this study) and ITS2 (present data – Fig. 1) genes as well as morphological characters (Škeříková et al., 2001 – fig. 2). The sequence similarity of these species, especially that of ITS2, is high (97.5–97.6% – Table 2).

***Proteocephalus macrocephalus* (Creplin, 1825)**

Syns *Taenia macrocephala* Creplin, 1825; *T. hemisphaerica* Molin, 1859; *T. dilatata* Linton, 1889

Type-host: Common eel *Anguilla anguilla* (L.) (Anguilliformes: Anguillidae).

Type-locality: Greifswald, Germany.

Other hosts: Eels (*Anguilla* spp.).

Distribution: Europe, including Russia, North Africa (Morocco), North America.

References: Scholz et al. (1997); Scholz & Hanzelová (1998); Hoffman (1999).

Comments: This cestode is specific to eels and has circumboreal (Holarctic) distribution (Freze, 1965; Scholz & Hanzelová, 1998; Hoffman, 1999).

Phylogenetic relationships: Results of analyses inferred from morphological characters and molecular data (Zehnder & Mariaux, 1999; Škeříková et al., 2001; de Chambrier et al., 2004) are congruent in demonstrating a close relationship of *P. macrocephalus* with *P. filicollis*. These taxa also form a well-supported clade with *P. gobiorum* (Fig. 1), with very high similarity of sequences of the V4 region of 18S rRNA (99.1–99.3%).

***Proteocephalus midoriensis* Shimazu, 1990**

Type-host: *Lefua echigonia* Jordan & Richardson (Cypriniformes: Balitoridae).

Type-locality: Midori, Nagano Prefecture, Japan.

Distribution: Japan.

Reference: Shimazu (1990).

Comments: *Proteocephalus midoriensis* has been found only in the type-host and its distribution area may be restricted to central Japan. Morphologically, it is rather similar to *P. sagittus* (Grimm, 1870), another cestode from a balitorid fish, *Barbatula barbatula* (L.). *P. midoriensis* differs in having a smaller scolex, more testes arranged in at least two layers, a less convoluted seminal vesicle, a smaller cirrus-sac, deeply indented ovarian lobes, a much more weakly developed vaginal sphincter, a longer and more sinuous seminal receptacle, more uterine branches and larger eggs (Shimazu, 1990). Moreover, no worms of *Proteocephalus* have been obtained from *B. barbatulain* Hokkaido, northern Japan (T. Shimazu, unpublished data).

Phylogenetic relationships: Based on molecular data (Hypša et al., 2005; this study), *P. midoriensis* and *P. sagittus* form a well-supported clade. Values of sequence similarity (81.3% and 98.3% for ITS2 and V4, respectively) indicate that both taxa are separate species differing slightly in strobilar morphology, the spectrum of definitive hosts and geographical distribution.

***Proteocephalus percae* (Müller, 1780)**

Syns *Taenia percae* Müller, 1780; *T. ocellata* Rudolphi, 1802; *Ichthyotaenia esocis* Schneider, 1905 (?); *P. dubius* La Rue, 1911

Type-host: Perch *Perca fluviatilis* L. (Perciformes: Percidae).

Type-locality: Denmark.

Other hosts: *Aspro*, *Gymnocephalus*, *Sander* (Percidae).

Distribution: Europe, Asia (Azerbaijan, Kazakhstan, Mongolia, Siberia).

References: Scholz & Hanzelová (1998), Hanzelová et al. (1999).

Comments: *Proteocephalus percae* is a common and widely distributed parasite of perch, which may also occur in other percid fishes (Freze, 1965; Anikieva, 1995a; Scholz & Hanzelová, 1998).

Phylogenetic relationships: Morphological and molecular data (sequences of ITS, 16S and 18S rRNA genes – Zehnder & Mariaux, 1999; Škeříková et al., 2001; present study) demonstrate a close phylogenetic relationship of *P. percae* with *P. longicollis* (Fig. 1). This corresponds well with the morphological similarity of these species (Hanzelová, Šnábel et al., 1995, 1999), but does not reflect phylogenetically unrelatedness of their fish definitive hosts (Nelson, 1994).

Zehnder & Mariaux (1999) found *P. percae* to be closely related to *P. torulosus*, but this conclusion was based on the incorrect partial sequence of 28S rRNA of *P. percae* (M.P. Zehnder – personal communication). de Chambrier et al. (2004) did not include *P. torulosus* in their analysis, but *P. percae* appeared as a basal taxon of the Palaearctic species of *Proteocephalus*, i.e. in the same position as *P. torulosus* in a tree inferred from a multigene analysis of several nuclear genes performed by Hypša et al. (2005). It is probable that the sequence belongs in fact to *P. torulosus*.

***Proteocephalus plecoglossi* Yamaguti, 1934**

Syn. *Proteocephalus neglectus* of Kataoka & Momma (1932)

Type-host: *Plecoglossus altivelis altivelis* (Temminck & Schlegel) (Osmeriformes: Plecoglossidae).

Type-locality: Lake Biwa, Shiga Prefecture, Japan.

Distribution: Japan.

References: Yamaguti (1934); Shimazu (1990, 1993).

Comments: *Proteocephalus plecoglossi* is a specific parasite of *Plecoglossus altivelis altivelis* and occurs only in Lake Biwa, central Japan (Kataoka & Momma, 1932; Yamaguti, 1934; Shimazu, 1990, 1993). This cestode resembles in its morphology *Proteocephalus neglectus* La Rue, 1911 (= *P. longicollis*, according to Scholz & Hanzelová, 1998) and was misidentified as *P. neglectus* by Kataoka & Momma (1932).

Phylogenetic relationships: Molecular data also indicate close relationships of *P. plecoglossi* with *P. longicollis* (Zehnder & Mariaux, 1999; Hypša et al., 2005; this study – Fig. 1, Table 3). However, in an analysis of sequences of ITS and the V4 region of 18S rRNA genes, *P. plecoglossi* appeared in a polytomy composed of *P. tetrastomus* and *P. cernuae* (Fig. 1). *P. tetrastomus* also occurs in osmeriform fish, but it differs markedly from *P. plecoglossi* in its morphology, especially in possessing trapezoid, well-separated proglottids and a rudimentary, almost indistinguishable apical sucker (versus the strobila composed of rectangular or oblong proglottids and a reduced but readily distinguishable apical sucker in *P. plecoglossi*) (Shimazu, 1990, 1993; Scholz & Hanzelová, 1998).

***Proteocephalus sagittus* (Grimm, 1872)**

Syns *Taenia sagitta* Grimm, 1872; *Proteocephalus pamirensis* Dzhililov & Ashurova, 1971

Type-host: Stone loach *Barbatula barbatula* (L.) (Cypriniformes: Balitoridae).

Type-locality: St. Petersburg, Russia.

Other hosts: *Noemacheilus stoliczkai* (Steindachner) [syn. of *Tryplophysa stoliczkae* (Steindachner)] (Balitoridae), *Cobitis taenia* L. (Cypriniformes: Cobitidae).

Distribution: Europe, including Russia and the Ukraine, Tadjikistan.

Reference: Scholz et al. (2003).

Comments: Scholz & Hanzelová (1998) synonymised *P. sagittus* with *P. torulosus* (Batsch, 1786), a parasite of cyprinid fishes in the Holarctic Region. However, Scholz et al. (2003) resurrected the former taxon on the basis of new morphological and molecular data obtained during a study of freshly collected material of *P. sagittus* from the type-host. *P. sagittus* differs from *P. torulosus* in scolex morphology, especially its shape, the position of the vagina in relation to the cirrus-sac, the length of the cirrus-sac, more distinct osmoregulatory canals and sequences of the V4 region and ITS2 (sequence similarity 96.9% and 70.3%, respectively) (Scholz et al., 2003; Table 2). The authors (Scholz et al., 2003) also synonymised *P. pamirensis*, a species described from the Tibetan stone loach *Trypophysa stoliczkae* (Steindachner) in Tadzhikistan (Dzhalilov & Ashurova, 1971), with *P. sagittus*.

Phylogenetic relationships: *P. sagittus* forms a well-supported clade with another *Proteocephalus* species, *P. midoriensis*, from a balitorid fish limited in distribution to central Japan. A combined analysis (Hypša et al., 2005; present data – Fig. 1) has also shown that *P. torulosus* and *P. sagittus* actually represent two separate, relatively unrelated species, thus confirming the taxonomic conclusions of Scholz et al. (2003).

***Proteocephalus tetrastomus* (Rudolphi, 1810)**

Syns *Scolex tetrastomus* Rudolphi, 1810; *Taenia longicollis* of Linstow (1891), Dubinina (1952, 1987) and Freze (1965) [nec Zeder (1800)]

Type-host: Not known.

Type-locality: Not known, probably Germany.

Other hosts: *Hypomesus nipponensis* (McAllister), *Osmerus eperlanus* (L.) (probably type-host), *O. mordax* (Mitchill) (all Osmeriformes: Osmeridae).
Distribution: Europe, including Russia, Japan, Canada.

References: Willemse (1969); Shimazu (1990); Anikieva (1998); Scholz & Hanzelová (1998); Scholz et al. (2004).

Comments: This species had been confused with *P. longicollis* by many authors, e.g. Linstow (1891), Dubinina (1952), Freze (1965), etc., until Willemse (1969) recognised the presence of two, morphologically and ecologically distinct species of

Proteocephalus in *Osmerus eperlanus*, namely *P. tetrastomus* and *P. longicollis*. The former species is a specific parasite of smelts (Osmeridae) recorded from the northern part of Europe, Russia and Japan. The latter taxon, *P. longicollis*, is a very rare parasite of smelt but occurs frequently in numerous salmonid and coregonid fish (Scholz & Hanzelová, 1998; Hanzelová & Scholz, 1999). Recently, *P. tetrastomus* has been found in larval rainbow smelt (*O. mordax*) in the Saint Lawrence estuary, Canada (Scholz et al., 2004), where it is supposed to regulate the early life-history survival of rainbow smelt estuarine populations (Bourque et al., 2006).

Phylogenetic relationships: Morphological (Škeříková et al., 2001) and molecular data (Zehnder & Mariaux, 1999; Hypša et al., 2005; present study – Fig. 1) demonstrate consistently a close phylogenetic relationships of *P. tetrastomus* with *P. plecoglossi* that form a polytomy with *P. cernuae* (Fig. 1). Both the taxa from osmeriform fish, *P. tetrastomus* and *P. plecoglossi*, are easy to distinguish on the basis of their morphology (see Shimazu, 1990 and comments above on *P. plecoglossi*).

***Proteocephalus thymalli* (Annenkova-Chlopina, 1923)**

Syns *Ichthyotaenia thymalli* Annenkova-Chlopina, 1923; *Proteocephalus pronini* Rusinek, 2001 (new synonym)

Type-host: Baikal black grayling *Thymallus arcticus baicalensis* (Dybowski) (Salmoniformes: Salmonidae).
Type-locality: Lake Baikal, Russia.

Other hosts: Grayling (*Thymallus thymallus* (L.)), *T. nigrescens* Dorogostaisky).

Distribution: Europe (Russia, Yugoslavia), Asia (Russia, including Siberia, Mongolia).

Reference: Scholz & Hanzelová (1998).

Comments: *Proteocephalus thymalli* is a specific parasite of graylings and is almost indistinguishable morphologically from *P. longicollis*, which has been reported from a wide spectrum of salmoniform fish, including graylings (Freze, 1965; Dubinina, 1987; Scholz & Hanzelová, 1998). Both taxa differ from each other only in the shape of the scolex, which is club-shaped in *P. thymalli* (versus rounded in *P. longicollis* – Scholz, Drábek & Hanzelová (1998) and in their susceptibility to experimental infection

(see Rusinek, 1987). Scholz & Hanzelová (1998) doubted the validity of *P. thymalli* but retained this species among the valid *Proteocephalus* species because they lacked suitable material for morphological and genetic evaluation.

Rusinek (2001) described a new species, *P. pronini*, from the intestine of *Thymallus nigrescens* in the Mongolian Lake Hovsgol. The description is insufficient and some taxonomically important characters, such as the morphology of the cirrus-sac, the course of the vaginal canal, the presence/absence of a vaginal sphincter, the position of the seminal receptacle, etc., were not described or illustrated. Some other characteristics, such as the length of the neck and the number of proglottids and uterine diverticula, are also difficult to evaluate from the original description (Rusinek, 2001). The new species was differentiated from *P. thymalli* by only a few quantitative characteristics which exhibit high intra-specific and individual variability in the Palaearctic species of *Proteocephalus* (see Scholz & Hanzelová, 1998 and references therein). In addition, measurements of the characters selected for the differentiation of *P. pronini* and *P. thymalli* presented in a comparative table (Rusinek, 2001) are almost identical or they overlap markedly. The most characteristic feature of *P. pronini* appears to be the presence of a club-shaped scolex with sublaterally situated suckers and a small, but distinct, flattened apical sucker (Rusinek, 2001 – Fig. A). However, a scolex of an identical shape is also the only morphological characteristic distinguishing *P. thymalli* from *P. longicollis* (see Hanzelová & Scholz, 1998; Scholz et al., 1998). Therefore, *P. pronini* is considered to be a junior synonym of *P. thymalli*, which occurs in conspecific fish hosts and has been found in the same region as *P. pronini*, i.e. northern Mongolia (Scholz & Ergens, 1990).

Phylogenetic relationships: A comparison of the ITS2 sequences of *P. thymalli* with those of other species of *Proteocephalus* has revealed that it forms, together with *P. ambiguus* from *Pungitius pungitius*, a sister group of the clade composed of *Proteocephalus longicollis* and *P. percae* (Fig. 1). Sequence similarity between *P. thymalli* and *P. longicollis* is high (97.6–98.0% for the ITS2) and indicates that the validity of *P. thymalli* needs to be supported by new molecular data, because no PCR product for the V4 region of the 18S rRNA was obtained in this study.

Rusinek & Kuznedelov (2002) published partial sequences (558 bp) of the 18S rDNA of two populations of *P. thymalli* from Russia and Mongolia, respectively. The sequences, which have not been deposited in GenBank and cover the highly conservative region at the 5' end, were compared with those of other European *Proteocephalus* species to test their similarity and also possible synonymy with *P. longicollis*. The results showed a higher similarity of *P. thymalli* with *P. percae* (98.6%) and *P. tetrastomus* (98.2%) than with *P. longicollis* (96.8%). However, a closer study of the sequences of *P. longicollis* and *P. thymalli* showed inaccuracies at several positions which possibly affected the apparent similarity. Missing or redundant base at some conservative positions in *P. thymalli* could be caused by natural errors of the DNA polymerase. The most serious problem was in the variable region of the sequence of *P. thymalli*, in which non-standard F (?) bases were used (Rusinek & Kuznedelov, 2002), which did not permit more exact conclusions. Because there was no explanation for this symbol in the paper of the Russian authors, these bases were treated as unknown.

***Proteocephalus torulosus* (Batsch, 1786)**

Syns *Taenia torulosa* Batsch, 1786; *Proteocephalus ptychocheilus* Faust, 1920; *P. ruzskyi* Titova, 1946; *P. cobraeformis* Haderlie, 1953

Type-host: Ide *Leuciscus idus* (L.) (Cypriniformes: Cyprinidae).

Type-locality: Not known, probably Germany.

Other hosts: Numerous species of cyprinid fish (e.g. *Abramis*, *Alburnus*, *Barbus*, *Leuciscus*, *Phoxinus*, *Rutilus*), with members of the Leuciscini being the most suitable hosts. Records from balitorid (*Barbatula barbatula*) and cobitid (*Cobitis taenia*) fish are questionable and need to be confirmed.

Distribution: Europe, Asia (Siberia, Far East), western part of North America.

References: Scholz & Hanzelová (1998, 1999).

Comments: Recent taxonomic studies have shown that *P. torulosus* is a circumboreal parasite reported from numerous species of cyprinid fish (Scholz & Hanzelová, 1998, 1999). It possesses some morphological characteristics unique or rare among the species of the *Proteocephalus* aggregate, such as a

long, club-shaped scolex, with longitudinal wrinkles and without an apical sucker, testes arranged in at least two layers, a short cirrus-sac and a feebly developed vaginal sphincter (Scholz & Hanzelová, 1998; Škeříková et al., 2001).

Phylogenetic relationships: A distinct position of *P. torulosus* among the taxa of the *Proteocephalus* aggregate, based on its morphology, is supported by the results of the phylogenetic analyses, in which this cestode forms a separate, most basal clade in almost all of the trees inferred from molecular data (Zehnder & Mariaux, 1999; Škeříková et al., 2001, Hypša et al., 2005; present data – Fig. 1). A separate position of *P. torulosus* is also reflected in the low values of sequence similarity with respect to other species of the *Proteocephalus* aggregate (Table 3).

Proteocephalus esocis* (Schneider, 1905) – *species inquirenda

Syn. *Ichthyotaenia esocis* Schneider, 1905

Type-host: Pike *Esox lucius* L. (Esociformes: Esocidae).

Type-locality: Reval, Estonia.

Other host: *Perca fluviatilis* L. (Perciformes: Percidae).

Distribution: Europe (Estonia, Russia).

References: Anikieva (1995b), Scholz & Hanzelová (1998).

Comments: This species was described by Schneider (1905) from the intestine of pike *Esox lucius* from Estonia and later also reported from *Perca fluviatilis* (Freze, 1965; Dubinina, 1987). Scholz & Hanzelová (1998), who studied the holotype of *P. esocis*, concluded that this species is apparently invalid and morphologically indistinguishable in its strobilar morphology from *P. longicollis*. However, because of the absence of the scolex in the type-specimens and the similarity between *P. longicollis* and *P. percae* in strobilar morphology, they did not synonymise *P. esocis* with either *P. longicollis* or *P. percae*.

Anikieva (1995b) also considered *Proteocephalus* specimens from pike to belong to either *P. exiguus* (syn. of *P. longicollis*) or *P. percae*. The taxonomic status of *P. esocis* can be resolved only when a new material from the type-host and type-locality is available for morphological and genetic studies.

Discussion

The present list includes only 14 species of the *Proteocephalus* aggregate, which are considered to be valid, from fishes of the Palaearctic Region. This number is much lower than that reported in previous lists of proteocephalidean tapeworms. Freze (1965) reported 25 taxa, whereas Schmidt (1986) as many as 29 species. In addition, three other species, namely *P. hemispherous* Rahemo & El-Niaeemi, 2001, *P. midoriensis* Shimazu, 1990 and *P. pronini* Rusinek, 2001, have been described since 1986.

So a marked difference in the number of valid species between individual authors seems to have been caused by several factors: (i) Freze (1965) and Schmidt (1986) included in their lists taxa described in the 19th Century, which had already been invalidated, e.g. by La Rue (1914); (ii) the books of these authors were largely compilative treatises rather than revisional monographs based on a critical evaluation of the type-material and voucher specimens (although Freze, 1965, redescribed some taxa occurring in Russia); (iii) in the 19th and the first half of the 20th Century, most species of *Proteocephalus*, including those of the *Proteocephalus* aggregate, were described on the basis of minor morphological differences of characters which exhibit great intraspecific and individual variability; the influence of many biotic and abiotic factors, especially the type and size of the definitive hosts, the intensity of infection, fixation methods, etc., on the morphology of individual species had not been considered by many authors; and (iv) genetic methods that may provide new diagnostic markers to distinguish morphologically uniform taxa have been applied to the systematics of the *Proteocephalus* aggregate only recently.

A comparison of sequences of the nuclear genes (ITS2 and V4 region of 18S rRNA) enabled us to assess phylogenetic relationships of species of the *Proteocephalus* aggregate and also helped in a consideration of the validity of some morphologically similar taxa or species of doubtful validity. However, it should be pointed out that sequence differences between some taxa were very low, even lower than between two isolates of *P. longicollis* from different hosts and geographical regions (Table 3). Although this species is polymorphic (Hanzelová, Scholz, & Fagerholm, 1995; Hanzelová, Špakulová, Fagerholm, et al., 1995; Scholz & Hanzelová, 1998), more

molecular data are necessary to confirm its validity or to detect the possible existence of cryptic species, as well as to better assess the phylogenetic relationships of some taxa, especially those for which sequences of only one gene, either ITS2 or 18S, were available, i.e. *P. gobiorum* and *P. thymalli*.

Notwithstanding the above-mentioned caution regarding the genetic delimitation of some taxa, the systematics of this group of fish tapeworms is better resolved than that of many other proteocephalidean cestodes as a result of multidisciplinary studies carried out recently (see Scholz & Hanzelová, 1998; Scholz & de Chambrier, 2003; Hypša et al., 2005, for references). This is also valid for presumed congeneric species found in the Nearctic Region. Regarding the number of species of *Proteocephalus* (their assignment to the *Proteocephalus* aggregate requires further systematic studies – Scholz & de Chambrier, 2003) from freshwater fishes from North America (without Neotropical Mexico and Cuba), Freze (1965) and Schmidt (1986) listed as many as 31 and 33 nominotypical taxa, respectively. Taking into account the narrow host-specificity of most *Proteocephalus* species in the Palaearctic Region, i.e. those of the *Proteocephalus* aggregate (Scholz & Hanzelová, 1998), it is questionable that some North American fish, such as largemouth bass *Micropterus salmoides*, can be infected with as many as 10 species of *Proteocephalus* (Freze, 1965; Hoffman, 1999). It is probable that numerous taxa described from North American fishes are synonymous and the actual number of valid species is much lower, as found for the Palaearctic Region.

Indeed, a taxonomic study of Hanzelová & Scholz (1999) has demonstrated that most probably only one species, *P. longicollis*, rather than 11 and 13 as listed by Schmidt (1986) and Hoffman (1999), respectively, occurs in salmonid fishes from North America. Another study of the same authors (Scholz & Hanzelová, 1999) has also revealed that *P. ptychocheilus* Faust, 1920 and *P. cobraeformis* Haderlie, 1953, described from cyprinid fishes in the western part of North America, are conspecific with *P. torulosus*, which has a Holarctic (circumpolar) distribution. Of the species of the *Proteocephalus* aggregate found in the Palaearctic Region, the following taxa have a circumpolar distribution: *P. filicollis*, *P. fluviatilis* (apparently of Nearctic origin, introduced to Japan with large- and smallmouth

bass), *P. longicollis*, *P. macrocephalus*, *P. tetrastomus* and *P. torulosus*.

Another species of *Proteocephalus*, *P. parasiluri* Yamaguti, 1934, was described from *Silurus asotus* L. in Japan (Yamaguti, 1934). It possesses a metascolex and was placed in *Paraproteocephalus* Chen in Dubinina, 1962 by Shimazu (1993). *Proteocephalus* also contained another species considered to be valid by Scholz & Hanzelová (1998), namely *P. osculatus* (Goeze, 1782) (syns *Taenia osculata* Goeze, 1782; *Ichthyotaenia skorikowi* von Linstow, 1904). It is a specific parasite of the European wels *Silurus glanis* L. in Europe, including Russia, and Asia (Azerbaijan, Iraq, Central Asia), and also occurs accidentally in sturgeons (*Acipenser stellatus* Pallas, *Pseudoscaphirhynchus kaufmanni* (Kessler)) in the Caspian Sea (Anikieva & Kharin, 1997; Scholz & Hanzelová, 1998; Skryabina, 1974). de Chambrier et al. (2004) proposed a new genus, *Glanitaenia*, to accommodate *Proteocephalus osculatus*, because in all molecular analyses it formed a clade with *Paraproteocephalus parasiluri* (Yamaguti, 1934) Shimazu, 1993 [nec *P. parasiluri* (Zmееv, 1936) Chen in Dubinina, 1962], a species typically possessing a metascolex (Shimazu, 1993), thus making the Palaearctic species of *Proteocephalus* paraphyletic. *Glanitaenia osculata* is also markedly distinct from *Proteocephalus* species in possessing a well-developed, functional apical sucker and a greater number of testes. Recent data of Hypša et al. (2005) and those of the present study fully support the validity of *Glanitaenia*.

Rahemo and Al-Niaeemi (2001) described *Proteocephalus hemisphaerous* from the intestine of *Silurus glanis* in the River Tigris at Mosul, Iraq. They distinguished it from *P. osculatus* (= *G. osculata*) in possessing a large and well-developed apical organ, nearly square mature proglottids and the shape of the ovary. Conspecific tapeworms were misidentified as *Silurotaenia siluri* (Batsch, 1786) by Ali, Al-Jafery, and Abdul-Ameer (1987), Al-Kalak (1992) and Mohamad (1995), despite the absence of any spines on the apical organ. *P. hemisphaerous* is undoubtedly a member of the Gangesiinae, based on the morphology of the scolex and strobila. It may well be conspecific with *Postgangesia inarmata* de Chambrier, Al-Kalak & Mariaux, 2003, described from the same fish host (*S. glanis*) in Iraq. However, there is a marked difference in the number of testes between these taxa (70–80 in *P. hemisphaerous* versus 115–151 in *Po. inarmata*)

and the apical organ of *P. hemispherous* appears to be much deeper than that of *P. inarmata*, in which it is flattened (de Chambrier et al., 2003; Rahemo & Al-Niaeemi, 2001). Therefore, *P. hemispherous* is transferred to *Postgangesia* as *Postgangesia hemispherous* (Rahemo & Al-Niaeemi, 2001) n. comb. Its possible conspecificity with *P. inarmata* should be verified on the basis of comparison of the type or voucher specimens of both the taxa.

Recent phylogenetic studies (de Chambrier et al., 2004; Hypša et al., 2005; Škeříková et al., 2001; Zehnder & Mariaux, 1999; present data) provide evidence for the monophyly of the Palaearctic species of *Proteocephalus*, provided that *G. osculata* is excluded from *Proteocephalus*, as proposed by de Chambrier et al. (2004). These authors proposed this group to form the *Proteocephalus* aggregate in order to distinguish it from other, apparently unrelated members of the composite genus *Proteocephalus*. Monophyly of the *Proteocephalus* aggregate corresponds well with a rather uniform morphology of its members, compared to the extreme morphological variation of members of *Proteocephalus* (*sensu lato*) occurring in the Neotropical Region (see de Chambrier & Vaucher, 1999; Rego et al., 1998). Regarding evolutionary relationships with their fish hosts, existing data suggest an incongruence between the phylogenies of the species of the *Proteocephalus* aggregate and the fish they infect, thus indicating host-switching events rather than co-evolution, as already claimed by Škeříková et al. (2001).

Based on the position of individual species in the phylogenetic tree, it is obvious that some morphological characters may be homoplastic and may have appeared independently during the evolution of the group. For example, the absence of an apical sucker in *P. gobiorum* is apparently a derived character, because this species appears in the clade containing species with a distinct apical sucker (*P. filicollis*, *P. fluviatilis* and *P. macrocephalus*), whereas other taxa are devoid of an apical sucker (*P. torulosus*, *P. sagittus* and *P. midoriensis*) and belong to unrelated, more basal clades (Fig. 1). On the contrary, *Glanitaenia osculata* (syn. *Proteocephalus osculatus*), which is basal to all species of the *Proteocephalus* aggregate, including *P. torulosus* (see Hypša et al., 2005; this study), possesses a well-developed apical sucker with a deep cavity. However, the least reduced apical sucker is present in *P. longicollis*,

P. thymalli and *P. percae*, which together form the most derived clade in the tree (Fig. 1).

In order to facilitate identification of tapeworms of the *Proteocephalus* aggregate *sensu* de Chambrier et al. (2004) considered to be valid, the following key is provided.

Key to species of the *Proteocephalus* aggregate from fishes of the Palaearctic Region

This key is primarily based on morphological features of the scolex and strobila summarised by Shimazu (1990 – species from Japan) and Scholz and Hanzelová (1998 – species from Europe, including Russia). However, the spectrum of definitive hosts is also used as a differential criterion because some valid taxa occurring in unrelated fish hosts differ only slightly in their morphology. If possible, only one or a few differential characters are provided in the key. More data on the morphology of individual taxa can be found in their redescrptions (Rødland, 1983; Shimazu, 1990; Scholz & Hanzelová, 1998; Scholz, Drábek & Hanzelová, 1998; Scholz et al., 2003).

1. Proglottids of markedly trapeziform shape; immature proglottides short and very wide. Apical sucker reduced, difficult to see. In smelt (*Osmeridae*). *P. tetrastomus*
- Proglottids of rectangular shape 2
2. Scolex with apical sucker 3
- Scolex without apical sucker 11
3. Scolex terminally blunt, with small apical sucker. Cirrus-sac short, only its proximal third crosses vitelline follicles; vaginal sphincter absent. In ruff (*Gymnocephalus*) *P. cernuae*
- Scolex of different shape 4
4. Large tapeworms (up to 200–300 mm in length), with relatively short cirrus-sac, representing about 1/8–1/4 of proglottid width 5
- Small tapeworms or cirrus-sac longer, about 1/3–2/5 of proglottid width 6
5. Apical sucker vestigial, longer than wide. Vaginal canal widened, thick-walled distally; seminal receptacle far anterior to ovarian isthmus. In eels (*Anguilla*) *P. macrocephalus*

- Apical sucker vestigial, widely oval. Vaginal canal without prominent thickening in distal part; seminal receptacle just anterodorsal to ovarian isthmus. In centrarchids (*Micropterus*) *P. fluviatilis*
- 6. Vaginal sphincter well developed; cirrus-sac elongate, long, representing 1/3–2/5 of proglottid width 7
 - Vaginal sphincter absent; cirrus-sac pyriform, short, representing less than 1/3 of proglottid width. In sticklebacks (*Gasterosteidae*) 10
- 7. Scolex tapering anteriorly; neck indistinct, wider than scolex. In perch (*Perca fluviatilis*) and other percid fishes (*Percidae*).....*P. percae*
 - Scolex not tapering anteriorly; neck usually distinct, narrower than scolex 8
- 8. Scolex club-shaped, with neck far posterior to suckers. In grayling (*Thymallidae*)*P. thymalli*
 - Scolex small, rounded; neck close posterior to suckers..... 9
- 9. Apical sucker large, 22–86 µm in diameter. In salmoniform fishes, rarely in smelt (*Osmeridae*) *P. longicollis*
 - Apical sucker small, 4–7 µm in diameter. In ayu (*Plecoglossus*) *P. plecoglossi*
- 10. Strobila <15 mm long, consisting of <35 proglottids; longitudinal muscles feeble, formed by a single layer of muscular fibres; apical sucker vestigial, flattened. In nine-spined stickleback (*Pungitius pungitius*). *P. ambiguus*
 - Strobila up to 60 mm long, consisting of up to 170 proglottids; longitudinal muscles formed by 2–5 layers of muscular fibres; apical sucker vestigial, oval or elongate. In three-spined stickleback (*Gasterosteus aculeatus*) *P. filicollis*
- 11. Scolex large, club-shaped; neck far posterior to suckers; large tapeworms, up to 120 mm long. In cyprinids *P. torulosus*
 - Scolex of different shape; small tapeworms, only 20–40 mm long 12
- 12. Scolex well separated from strobila by neck, with compact, tear-shaped concentration of large, bottle-shaped gland-cells in apical region; <40 testes in proglottid. In gobiids in brackish waters *P. gobiorum*
 - Neck as wide as anterior part of strobila; scolex with small gland-cells scattered in its apical region, not grouped together; >40 testes in proglottid. In balitorid and cobitid fishes 13
- 13. Scolex >0.3 mm in width. Lateral ovarian lobes not branched. In balitorids (*Barbatula*, *Tryplophysa*) and cobitids (*Cobitis*) *P. sagittus*
 - Scolex <0.3 mm in width. Ovarian lobes with several transverse branches. In balitorids (*Lefua*) *P. midoriensis*

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References

- Ali, N. M., Al-Jafery, A. R., & Abdul-Ameer, K. N. (1987). Parasitic fauna of freshwater fishes in Diyala River, Iraq. *Journal of Biological Sciences Research, Baghdad*, 18, 163–181.
- Al-Kalak, S. H. N. (1992). *A comparative study of the nervous system of some cestodes*. MSc Thesis, Department of Biology, College of Science University of Mosul, Mosul, Iraq, 115 pp. (cited after Rahemo & Al-Niaemi, 2001).
- Anikieva, L. V. (1995a). Variability of a perch's parasite *Proteocephalus percae* in the areal of the host. *Parazitologiya*, 29, 279–288. (In Russian).
- Anikieva, L. V. (1995b). Identity of *Proteocephalus* tapeworms from pike (*Esox lucius*) in Karelian lakes. *Parazitologiya*, 29, 510–515. (In Russian).

- Anikieva, L.V. (1998). Cestodes of the genus *Proteocephalus* (Cestoda: Proteocephalidea) from the European smelt *Osmerus eperlanus*. *Parazitologiya*, 32, 134–140. (In Russian).
- Anikieva, L. V., & Kharin, V. N. (1997). Interspecific differences of the cestodes of the genus *Proteocephalus* (Proteocephalidae) in freshwater fishes of the Holarctic. *Parazitologiya*, 31, 72–80. (In Russian).
- Bourque, J.-F., Dodson, J. J., Ryan, D. A. J., & Marcogliese, D. J. (2006). Cestode parasitism as a regulator of early life-history survival in an estuarine population of rainbow smelt *Osmerus mordax*. *Marine Ecology Progress Series*, 314, 295–307.
- de Chambrier, A., Al-Kallak, S. N. H., & Mariaux, J. (2003). A new tapeworm, *Postgangesia inarmata* n. sp. (Eucestoda: Proteocephalidea: Gangesiinae), parasitic in *Silurus glanis* (Siluriformes) from Iraq and some comments on the Gangesiinae Mola, 1929. *Systematic Parasitology*, 55, 199–209.
- de Chambrier, A., & Vaucher, C. (1999). Proteocephalidae et Monticelliidae (Eucestoda: Proteocephalidea) parasites de poissons d'eau douce au Paraguay, avec descriptions d'un genre nouveau et de dix espèces nouvelles. *Revue Suisse de Zoologie*, 106, 165–240.
- de Chambrier, A., Zehnder, M. P., Vaucher, C., & Mariaux, J. (2004). The evolution of the Proteocephalidea (Platyhelminthes, Eucestoda) based on an enlarged molecular phylogeny, with comments on their uterine development. *Systematic Parasitology*, 57, 159–171.
- Dubinina, M. N. (1952). Some remarks on the classification of tapeworm family Proteocephalidae La Rue and their distribution in the USSR. *Parazitologicheskii Sbornik Zoologicheskogo Instituta AN SSSR*, 14, 281–302. (In Russian).
- Dubinina, M. N. (1987). Class Cestoda Rudolphi, 1808. In O. N. Bauer (Ed.), *Key to the parasites of freshwater fishes of the USSR* (Vol. 3, pp. 5–76). Leningrad: Nauka. (In Russian).
- Dzhalilov, U. D., & Ashurova, M. (1971). [A new species of *Proteocephalus* Weinland, 1858 from fishes of the Pamir basin]. *Izvestiya Akademii Nauk Tadzhik SSR, Otdelenie Biologicheskikh Nauk*, 3(44), 110–111. (In Russian).
- Freze, V. I. (1965). [Proteocephalids – tapeworm helminths of fish, amphibians and reptiles.] *Osnovy Tsetodologii* (Vol. 5, 540 pp). Moscow: Nauka, (In Russian).
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analyses program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.
- Hanzelová, V., & Scholz, T. (1992). Redescription of *Proteocephalus neglectus* La Rue, 1911 (Cestoda: Proteocephalidae), a trout parasite, including designation of its lectotype. *Folia Parasitologica*, 39, 317–323.
- Hanzelová, V., & Scholz, T. (1993). Systematic status of *Proteocephalus tumidocollis* (Cestoda: Proteocephalidae), a parasite of salmonid fishes in North America. *Helminthologia*, 30, 157–161.
- Hanzelová, V., & Scholz, T. (1999). Species of *Proteocephalus* Weinland, 1858 (Cestoda: Proteocephalidae), parasites of coregonid and salmonid fishes from North America: taxonomic reappraisal. *Journal of Parasitology*, 85, 94–101.
- Hanzelová, V., Scholz, T., & Fagerholm, H.-P. (1995). Synonymy of *Proteocephalus neglectus* La Rue, 1911 with *P. exiguus* La Rue, 1911, two fish cestodes from the Holarctic Region. *Systematic Parasitology*, 30, 173–185.
- Hanzelová, V., Šnábel, V., Král'ová, I., Scholz, T., & D'Amelio, S. (1999). Genetic and morphological variability in cestodes of the genus *Proteocephalus*: geographical variation in *Proteocephalus percae* populations. *Canadian Journal of Zoology*, 77, 1450–1458.
- Hanzelová, V., Šnábel, V., Špakulová, M., Fagerholm, H.-P., & Král'ová, I. (1995). A comparative study of the fish parasites *Proteocephalus exiguus* and *P. percae* (Cestoda: Proteocephalidae): morphology, isoenzymes, karyotype. *Canadian Journal of Zoology*, 73, 1191–1198.
- Hanzelová, V., Šnábel, V., & Špakulová, M. (1996). On the host specificity of fish tapeworm *Proteocephalus exiguus* La Rue, 1911 (Cestoda). *Parasite*, 3, 253–257.
- Hanzelová, V., & Špakulová, M. (1992). Biometric variability of *Proteocephalus neglectus* (Cestoda: Proteocephalidae) in two different age groups in the rainbow trout from the Dobšiná dam (East Slovakia). *Folia Parasitologica*, 39, 307–316.
- Hoffman, G. L. (1999). *Parasites of North American freshwater fishes* (Second Edit., 539 pp). Ithaca & London: Comstock Publishing Associates, Cornell University Press
- Hypša, V., Škefíková, A., & Scholz, T. (2005). Multigene analysis and secondary structure characters in a reconstruction of phylogeny, evolution and host-parasite relationship of the order Proteocephalidea (Eucestoda). *Parasitology*, 130, 359–371.
- Ieshko, E. P., & Anikieva, L. V. (1980). Polymorphism of *Proteocephalus exiguus* (Cestoidea: Proteocephalidae), a common parasite of coregonid fish. *Parazitologiya*, 14, 422–426. (In Russian).
- Kataoka, N., & Momma, K. (1932). [On a cestode parasitic in *Plecoglossus altivelis*]. *Dobutsugaku Zasshi*, 44, 127–136. (In Japanese).
- Král'ová, I. (1996). A total DNA characterization in *Proteocephalus exiguus* and *P. percae* (Cestoda: Proteocephalidae): RAPD and hybridization techniques. *Parasitology Research*, 82, 668–671.
- Král'ová, I., & Špakulová, M. (1996). Intraspecific variability of *Proteocephalus exiguus* La Rue, 1911 (Cestoda: Proteocephalidae) as studied by the random amplified polymorphic DNA method (RAPD). *Parasitology Research*, 82, 542–545.
- Král'ová, I., van de Peer, Y., Jirků, M., van Ranst, M., Scholz, T., & Lukeš, J. (1997). Phylogenetic analysis of a fish tapeworm, *Proteocephalus exiguus*, based on the small subunit rRNA gene. *Molecular and Biochemical Parasitology*, 84, 263–266.
- Král'ová, I., Hanzelová, V., Scholz, T., Gerdeaux, D., & Špakulová, M. (2001). A comparison of the internal transcribed spacer of the ribosomal DNA for *Eubothrium crassum* and *Eubothrium salvelini* (Cestoda: Pseudophyllidea), parasites of salmonid fish. *International Journal for Parasitology*, 31, 93–96.
- La Rue, G. R. (1914). A revision of the cestode family Proteocephalidae. *Illinois Biological Monographs*, 1, 3–351.
- Linstow, O. (1891). Über den Bau und Entwicklung von *Taenia longicollis* Rud. Ein Beitrag zur Kenntnis der

- Fischtänen. *Jenische Zeitschrift für Naturwissenschaft*, 25, 565–576.
- Margolis, L., & Arthur, J. R. (1979). Synopsis of the parasites of fishes of Canada. *Bulletin of the Fisheries Research Board of Canada*, 199, 269 pp.
- Mohamad, S. A. (1995). *Studies on the cestodes parasitic in some teleost fishes in Tigris River*. PhD Thesis, Department of Biology, College of Science University of Mosul, Mosul, Iraq, 126 pp. (cited after Rahemo & Al-Niaemi, 2001).
- Müller, O. F. (1780). *Von Bandwürmern* (320 pp). Leipzig: Radebeul Neumann Verlag
- Nelson, J. S. (1994). *Fishes of the world*. (3rd Edit., 600 pp). New York: John Wiley & Sons
- Olson, P. D., & Tkach, V. V. (2005). Advances and trends in the molecular systematics of the parasitic Platyhelminthes. *Advances in Parasitology*, 60, 165–243.
- Rahemo, Z. I. F., & Al-Niaemi, B. H. S. (2001). A new cestode species from a freshwater catfish. *Rivista di Parasitologia*, 42, 71–74.
- Rego, A. A., de Chambrier, A., Hanzelová, V., Hoberg, E. P., Scholz, T., Weekes, P., & Zehnder, M. (1998). Preliminary phylogenetic analysis of subfamilies of the Proteocephalidea (Eucestoda). *Systematic Parasitology*, 40, 1–19.
- Rødland, J. T. (1983). A redescription of the cestodes *Proteocephalus filicollis* (Rudolphi) from *Gasterosteus aculeatus* L., and *P. ambiguus* (Dujardin) from *Pungitius pungitius* (L.). *Zoologica Scripta*, 12, 19–23.
- Rusinek, O. T. (1987). Zum Lebenszyklus von *Proteocephalus exiguus* (Cestoda), im Baikalsee. *Angewandte Parasitologie*, 28, 33–36.
- Rusinek, O. T. (2001). Description of *Proteocephalus pronini* sp. n. (Cestoda: Proteocephalidae) – a parasite of the Lake Hovsgol grayling. *Parazitologiya*, 35, 159–161. (In Russian).
- Rusinek, O. T., & Kuznedelov, K. T. (2002). Comparative morphological and gene systematics analysis of the *Proteocephalus thymalli* (Cestoda: Proteocephalidae), a parasite of graylings in lakes Hovsogol and Baikal. *Parazitologiya*, 36, 71–78. (In Russian).
- Schmidt, G. D. (1986). *CRC Handbook of tapeworm identification* (675 pp). Boca Raton, Florida: CRC Press
- Schneider, G. (1905). Die Ichthyotaenien des Finnischen Meerbusens. *Festschrift für Palmén, Helsingfors*, 1(8), 3–32.
- Scholz, T., & de Chambrier, A. (2003). Taxonomy and biology of proteocephalidean cestodes: current state and perspectives. *Helminthologia*, 40, 65–77.
- Scholz, T., Drábek, R., & Hanzelová, V. (1998). Scolex morphology of *Proteocephalus* tapeworms (Cestoda: Proteocephalidae), parasites of freshwater fish in the Palaearctic Region. *Folia Parasitologica*, 45, 27–43.
- Scholz, T., & Ergens, R. (1990). Cestodes of fish from Mongolia. *Acta Societatis Zoologicae Bohemoslovacae*, 54, 287–304.
- Scholz, T., & Hanzelová, V. (1994). Taxonomic study of two *Proteocephalus* species (Cestoda: Proteocephalidae) parasitizing coregonid fishes: the synonymy of *P. fallax* La Rue, 1911 with *P. exiguus* La Rue, 1911. *Systematic Parasitology*, 27, 1–12.
- Scholz, T., & Hanzelová, V. (1998). Tapeworms of the genus *Proteocephalus* Weinland, 1858 (Cestoda: Proteocephalidae), parasites of fishes in Europe. *Studie AV ČR. Prague: Academia*, No. 2/98, 119 pp.
- Scholz, T., & Hanzelová, V. (1999). Species of *Proteocephalus* Weinland, 1858 (Cestoda: Proteocephalidae) from cyprinid fishes in North America. *Journal of Parasitology*, 85, 150–154.
- Scholz, T., Hanzelová, V., Králová, I., & Griffiths, D. (1998). Synonymy of *Proteocephalus pollanicola* Gresson, 1952 (Cestoda: Proteocephalidae), a parasite of pollan *Coregonus autumnalis pollanicola*, with *P. exiguus* La Rue, 1911. *Systematic Parasitology*, 40, 35–41.
- Scholz, T., Hanzelová, V., & Šnábel, V. (1995). The taxonomic status of *Proteocephalus dubius* La Rue, 1911 (Cestoda: Proteocephalidae), a puzzling parasite of perch (*Perca fluviatilis* L.). *Parasite*, 2, 231–234.
- Scholz, T., Marcogliese, D. J., Bourque, J.-F., Škeříková, A., & Dodson, J. J. (2004). Occurrence of *Proteocephalus tetrastomus* (Rudolphi, 1810) (Cestoda: Proteocephalidea) in larval rainbow smelt (*Osmerus mordax*) in North America: identification of an unusual pathogen confirmed. *Journal of Parasitology*, 90, 425–427.
- Scholz, T., Škeříková, A., Hanzelová, V., Koubková, B., & Baruš, V. (2003). Resurrection of *Proteocephalus sagittus* (Grimm, 1872) (Cestoda: Proteocephalidea) based on morphological and molecular data. *Systematic Parasitology*, 56, 173–181.
- Scholz, T., Špakulová, M., Šnábel, V., Králová, I., & Hanzelová, V. (1997). A multidisciplinary approach to the systematics of *Proteocephalus macrocephalus* (Cestoda: Proteocephalidae). *Systematic Parasitology*, 37, 1–12.
- Shimazu, T. (1990). Some species of the genus *Proteocephalus* (Cestoidea: Proteocephalidae) from Japanese freshwater fishes, with a description of a new species. *Japanese Journal of Parasitology*, 39, 612–624.
- Shimazu, T. (1993). Redescription of *Paraproteocephalus parasiluri* (Yamaguti, 1934) n. comb. (Cestoidea: Proteocephalidae), with notes on four species of the genus *Proteocephalus*, from Japanese freshwater fishes. *Journal of Nagano Prefectural College*, 48, 1–9.
- Skryabina, E. S. (1974). [*Helminths of acipenserid fishes (Acipenseridae Bonaparte, 1831)*] (168 pp.). Moscow: Nauka (In Russian).
- Swofford, D. L. (2003). *PAUP 4.0-phylogenetic analysis using parsimony* (Version 4). Sunderland, Mass.: Sinauer
- Škeříková, A., Hypša, V., & Scholz, T. (2001). Phylogenetic analysis of European species of *Proteocephalus* (Cestoda: Proteocephalidea): compatibility of molecular and morphological data, and parasite-host coevolution. *International Journal for Parasitology*, 31, 1121–1128.
- Šnábel, V., Hanzelová, V., & Fagerholm, H.-P. (1994). Morphological and genetic comparison of two *Proteocephalus* species (Cestoda: Proteocephalidae). *Parasitology Research*, 80, 141–146.
- Šnábel, V., Hanzelová, V., Mattiucci, S., D'Amelio, S., & Paggi, L. (1996). Genetic polymorphism in *Proteocephalus exiguus* shown by enzyme electrophoresis. *Journal of Helminthology*, 70, 345–349.

- Špakulová, M., & Hanzelová, V. (1992). The karyotypes of *Proteocephalus percae* (Cestoda: Proteocephalidae). *Folia Parasitologica*, 39, 324–326.
- Turčeková, L., & Král'ová, I. (1995). Characterization of DNA restriction profiles and rRNA gene restriction fragment length polymorphisms of *Proteocephalus exiguus* and *P. neglectus* from geographically distinct regions. *Journal of Helminthology*, 69, 159–163.
- Willemse, J. J. (1968). *Proteocephalus filicollis* (Rudolphi, 1802) and *Proteocephalus ambiguus* (Dujardin, 1845), two hitherto confused species of cestodes. *Journal of Helminthology*, 42, 395–410.
- Willemse, J. J. (1969). The genus *Proteocephalus* in the Netherlands. *Journal of Helminthology*, 43, 207–222.
- Yamaguti, S. (1934). Studies on the helminth fauna of Japan. Part 4. Cestodes of fishes. *Japanese Journal of Zoology*, 6, 1–112.
- Zehnder, M. P., & Mariaux, J. (1999). Molecular systematic analysis of the order Proteocephalidea (Eucestoda) based on mitochondrial and nuclear rDNA sequences. *International Journal for Parasitology*, 29, 1841–1852.
- Zeder, J. G. H. (1800). *Erster Nachtrag zur Naturgeschichte der Eingeweidewürmer von Johann August Ephraim Goetze*. Leipzig, 320 pp. + 6 pl.