J. Parasitol., 90(2), 2004, pp. 425–427

Occurrence of *Proteocephalus tetrastomus* (Rudolphi, 1810) (Cestoda: Proteocephalidea) in Larval Rainbow Smelt (*Osmerus mordax*) in North America: Identification of a Potential Pathogen Confirmed

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ABSTRACT: A morphological evaluation and genetic analysis (sequencing of ITS2 region of rDNA) of proteocephalidean cestodes from rainbow smelt (*Osmerus mordax*) in the Saint Lawrence Estuary, Canada, has shown their conspecificity with *Proteocephalus tetrastomus*, a specific parasite of smelt (Osmeridae), previously known only from northern Europe, Russia, and Japan. The parasite occurs only in larval, but not adult, smelt in the Saint Lawrence Estuary. Prevalence of larval smelt infection was 42% (n = 50), mean intensity 2.1 ± 2.4 and mean abundance 1.1 ± 1.0.

During studies on the population dynamics of parasites of larval rainbow smelt (*Osmerus mordax* Mitchill; Osmeridae) from the Saint Lawrence Estuary, just downstream from Québec City, Canada, heavy infections with small tapeworms, apparently belonging to the Proteocephalidea, were observed. Cestodes were reported previously from the same population of rainbow smelt in the estuary, but they were not identified to species (Courtois and Dodson, 1986; Dauvin and Dodson, 1990; Sirois and Dodson, 2000a, 2000b). Existing data suggest that these cestodes may have a considerable effect on feeding and growth of larval smelt because parasitized smelt larvae have significantly less food in their gut than uninfected ones (Sirois and Dodson, 2000a), and the infected larvae possess lower growth rates than uninfected ones (Sirois and Dodson, 2000b; Bourque, 2003).

A sample of the cestodes from the intestine of fish hosts was used for morphological evaluation. However, their quality was rather poor, therefore the ITS2 region was sequenced to confirm species identification of the parasite. Results of this evaluation are presented in this article.

Smelt larvae were collected on 18 July 2002 in the upstream part of the Saint Lawrence middle estuary, Quebéc, Canada (47°06′40″N, 70°35′00″W; salinity 0–2 psu) with 15-min step-oblique tows from the bottom to the surface using a 500-μm mesh, 1-m plankton net. Rainbow smelt are anadromous, and 2 genetic populations exploit the middle estuary of the Saint Lawrence. The tapeworms studied were found in smelt from the population that spawns in the Saint Lawrence River upstream of Quebec City. After hatching, the larvae are retained in the deep waters of the middle estuary.

Fifty smelt larvae (mean length 21.2 ± 2.3 mm) were collected in 2002 and maintained alive during their transport to the laboratory. Adult smelt were collected near Ile-aux-Coudres (47°25′00″N, 70°20′00″W; salinity 10–15 psu). A total of 300 adults (100 each month) were dissected in June (mean length 125.6 ± 12.3 mm), July (116.9 ± 17.7 mm), and August 2001 (165.6 ± 28.0 mm). Smelt larvae and adults were dissected using a stereomicroscope, and cestodes found in the digestive tract were fixed live in 4% formaldehyde. Parasites (n = 9) were stained with Mayer carmine, dehydrated in an ethanol series, and mounted as permanent preparations. The worms studied have been deposited in the U.S. National Parasite Collection (USNPC nos. 94469) and the helminthological collection of the Institute of Parasitology, AS CR, České Budějovice (IPCAS no. C-297).

Voucher specimens of *Proteocephalus tetrastomus* (Rudolphi, 1810) from *O. eperlanus* (L.) from Europe (Finland, the Netherlands) and *Hypomesus nipponensis* (McAllister) from Japan, studied by Scholz and Hanzelová (1998), were used for comparison with the present material. Measurements of organs and structures are in micrometers.

For sequencing, total deoxyribonucleic acid (DNA) was extracted from a fragment of 1 worm fixed with 70% ethanol using the QIAamp Tissue Kit (Qiagen, Valencia, California). The ITS2 region was amplified by polymerase chain reaction (PCR) using the primers Proteo 1 (5′-CGGTGGATCACTCGGCTC-3′) and Proteo 2 (5′-TCCTCCG CTTATTGATATGC-3′), designed according to the complete sequences of the ITS1-ITS2 genes of *Eubothrium crassum* (Bloch, 1779) and *E. salvelini* (Schrank, 1790) (Král'ová et al., 2001). The following program was used for PCR: first, 15 min at 95 C (HotStarTaq™ DNA polymerase), then 30 cycles of denaturation, 1 min at 94 C for annealing, 1 min at 60 C for extension, 2 min at 72 C, and a final extension of 8 min at 72 C and 15 min at 68 C. The PCR products were cloned using the TOPO™ TA Cloning Kit (Perkin–Elmer, Applied Biosystems, Foster City, California). The products of the sequencing reactions were separated on the Perkin–Elmer ABI PRISM 310 genetic analyser.

The sequence, deposited in the GeneBank under accession number AY 379114, was compared with that of *P. tetrastomus* from *Hypomesus nipponensis* from Suwa Lake, March 1998, Japan (deposited under accession number AY 379113). The sequence similarity was determined using the Martinez–Needleman-Wunsch method implemented in Megalign (DNAstar Inc., Nevada City, California).

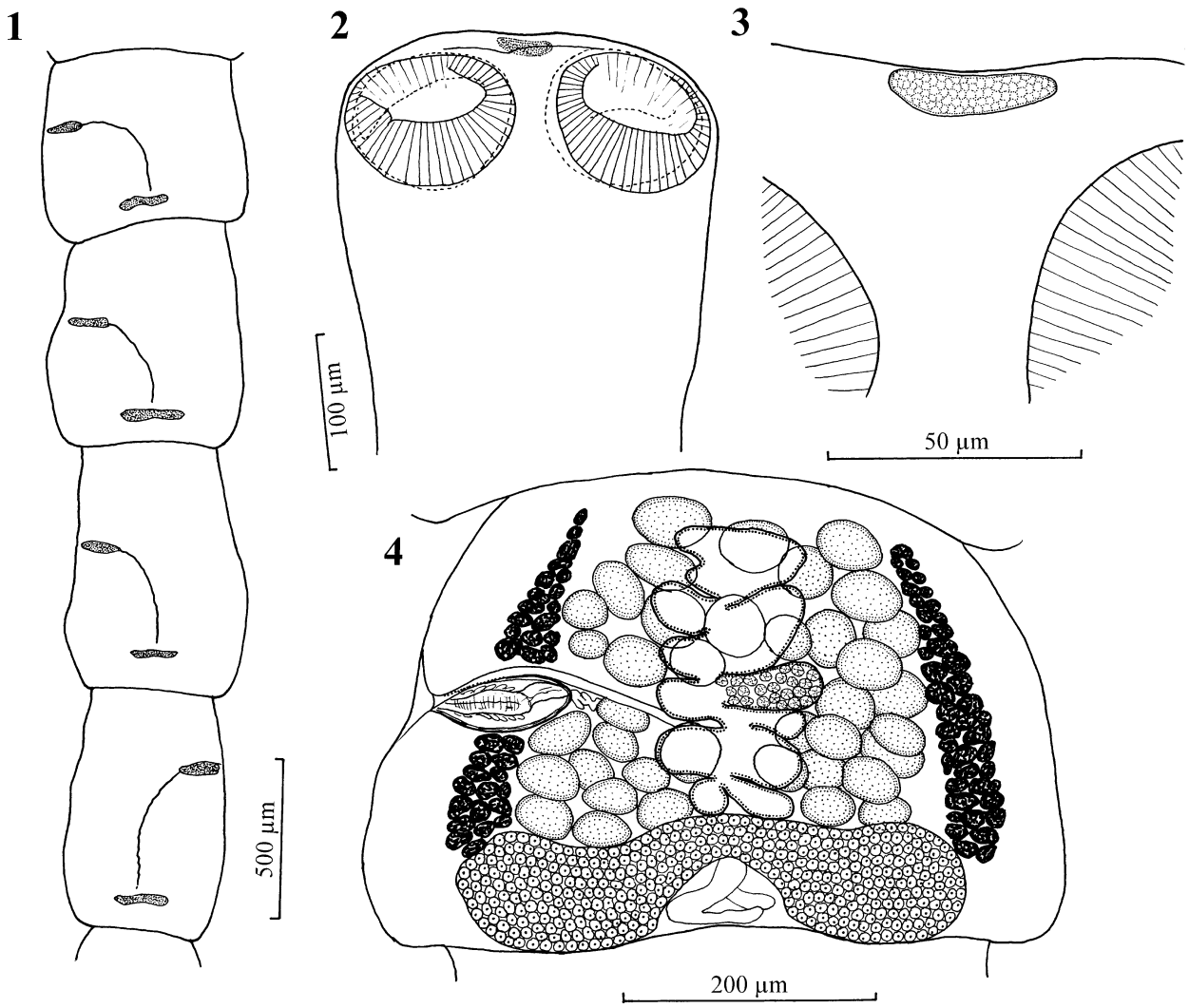
Cestodes were recovered only from larval smelt; no adult was infected. Prevalence of larval smelt infection was 42%, mean intensity 2.1 ± 2.4 and mean abundance 1.1 ± 1.0.

Although the specimens available were in poor condition (contracted or with tissue partly autolyzed), it was possible to observe the following characters specific of the proteocephalidean cestode, *Proteocephalus tetrastomus*, as recently redescribed by Scholz and Hanzelová (1998) on the basis of material from Europe and Japan: strobila short, with trapeziform proglottids, well separated from each other (Fig. 1); scolex semispherical, 230–307 wide, with sublaterally situated suckers (Fig. 2), 99–134 in diameter; apical sucker strongly reduced, represented by an indistinct, flattened mass of cells, 34–36 in diameter, very difficult to see in permanent preparations (Fig. 3); testes in 1 layer, 33–58 in number; cirrus sac relatively short, 106–116 in length, representing 19–23% of proglottid width (496–560); vagina without a well-developed, spherical sphincter (Fig. 4).

The identification of cestodes from rainbow smelt on the basis of morphological characters was confirmed by molecular data. The complete ITS2 region was 535 base pairs long (Fig. 5), and its sequence was identical with that of *Proteocephalus tetrastomus* from *H. nipponensis* (Fig. 5) in all but 1 nucleotide (T instead of G in the position 378). This represents a homology of 99.9%, which has never been found between *Proteocephalus* species considered to be valid taxa (Scholz et al., 2003), and represents strong evidence for the conspecificity of these cestodes.

This is the first report of *P. tetrastomus*, a specific parasite of smelt (Osmeridae), from North America despite the numerous studies undertaken on these potential hosts, some of which involved large sample sizes (see Margolis and Arthur, 1979; Fréchet et al., 1983; McDonald and Margolis, 1995; Hoffman, 1999). The reason may be that *P. tetrastomus* appears to be restricted to larval smelt in North America, and larval fish are usually not surveyed.

This is also the first record of a nominal species of *Proteocephalus* Weinland, 1858 from smelt in this continent, but there are numerous reports of unidentified, usually immature *Proteocephalus* cestodes from smelt in the northeast, including from Lake Erie, Lake Huron, Grand Lake (Labrador), and the Matamek River system (Québec) (Threlfall



FIGURES 1–4. *Proteocephalus tetrastomus* (Rudolphi, 1810) from larval smelt (*Osmerus mordax*). **1.** A schematic illustration of trapeziform proglottids. **2.** Scolex. **3.** Apical part of scolex with strongly reduced apical sucker formed by indistinct mass of cells. **4.** Gravid proglottid.

and Hanek, 1971; Dechtiar, 1972; Hanek and Molnar, 1974; Dechtiar and Nepszy, 1988; Dechtiar et al., 1988; Muzzall and Peebles, 1988; Sirois and Dodson, 2000a).

The distribution of this parasite in smelt in the Saint Lawrence Estuary raises an interesting question: why is this parasite only found in larvae, when conspecifics in Europe and Asia occur in adult smelt (Willemse, 1969; Shimazu, 1990; Scholz and Hanzelová, 1998)?

The life cycle of *P. tetrastomus* is not known but it can be supposed that planktonic copepods serve as intermediate hosts of the parasite, similar to other species of *Proteocephalus* in the Holarctic (Scholz, 1999). The calanoid copepod *Eurytemora affinis* (Temoridae) is the main component of the diet of larval smelt in the Saint Lawrence Estuary (Sirois and Dodson, 2000a) and may serve as the intermediate host of *P. tetrastomus*. Another calanoid copepod, *Epischura baicalensis* (Temoridae), also functions as intermediate host of *Proteocephalus* cestodes in Lake Baikal (Rusinek et al., 1996), whereas all other Palaearctic species use cyclopoid and diaptomid copepods in their life cycles (Scholz, 1999).

As rainbow smelt grow, they feed on larger crustaceans, polychaetes, and even small fish (Scott and Scott, 1988). Thus, adults might only rarely be exposed to infective cestode larvae if *E. affinis* would actually serve as the intermediate host of *P. tetrastomus* in the Saint Lawrence Estuary.

Proteocephalus tetrastomus is the fifth species of *Proteocephalus* with a circumboreal (Holarctic) distribution because *P. filicollis* (Rudolphi, 1802), *P. longicollis* (Zeder, 1800) (syn. *P. exiguus* La Rue, 1911), *P. macrocephalus* (Creplin, 1825), and *P. torulosus* (Batsch, 1786) also occur throughout the Holarctic (La Rue, 1914; Freze, 1965; Scholz and Hanzelová, 1998, 1999; Hanzelová and Scholz, 1999; Hoffman, 1999).

Thanks are due to Václav Hypša for help in sequencing and sequence analyses, and to Martina Borovková, both from the Institute of Parasitology, for technical assistance. Valuable suggestions of 2 anonymous referees are also greatly appreciated. This study has been partly supported by the Grant Agency of the Czech Republic (project no. 524/04/0342) and research project of the Institute of Parasitology, Academy of Sciences of the Czech Republic (Z6 022 909).

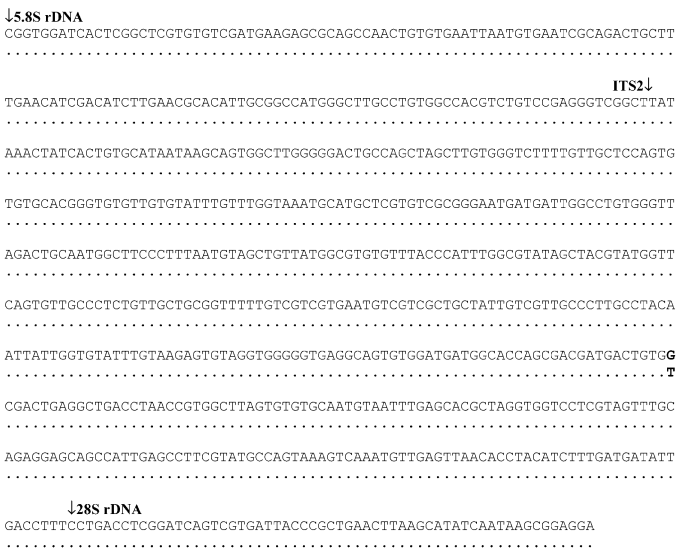


FIGURE 5. ITS2 region sequences of *Proteocephalus tetrastomus* from *Hypomesus nipponensis*, Japan (upper line) and *Osmerus eperlanus* from Canada (lower line).

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