

## Host specificity and geographical distribution of *Eubothrium* in European salmonid fish

T. Scholz<sup>1\*</sup>, R. Kuchta<sup>2</sup>, A.P. Shinn<sup>3</sup>, V. Šnábel<sup>4</sup> and V. Hanzelová<sup>4</sup>

<sup>1</sup>Institute of Parasitology, Academy of Sciences of the Czech Republic and

<sup>2</sup>Faculty of Biological Sciences, University of South Bohemia, Branišovská 31, 370 05 České Budějovice, Czech Republic: <sup>3</sup>Institute of Aquaculture, University of Stirling, Stirling, FK9 4LA, Scotland, UK:

<sup>4</sup>Parasitological Institute, Slovak Academy of Sciences, Hlinkova 3, 040 01 Košice, Slovak Republic

### Abstract

The host specificity and distribution of *Eubothrium crassum* (Bloch, 1779) and *Eubothrium salvelini* (Schrank, 1790), morphologically fairly similar pseudophyllidean tapeworms parasitizing salmonid fish, were critically assessed on the basis of morphological and genetic evaluation of extensive material collected from different definitive hosts and geographical regions in Europe. *Eubothrium crassum* occurs in fish of the genera *Salmo*, i.e. salmon (*S. salar* – both freshwater and marine), sea trout (*S. trutta trutta*), brown trout (*S. trutta fario*), and lake trout (*S. trutta lacustris*), and also in Danubian salmon (*Hucho hucho*) and vendace (*Coregonus albula*). *Eubothrium salvelini* parasitizes Arctic char (*Salvelinus alpinus*) and brook trout (*Salvelinus fontinalis*) in Europe, and also whitefish (*Coregonus wartmanni*). Rainbow trout (*Oncorhynchus mykiss*), which is not a native European fish species, was found to be a suitable definitive host for both *Eubothrium* species, which may occur simultaneously in the same fish. Previous records of *E. crassum* in Arctic char and brook trout, and those of *E. salvelini* in fish of the genus *Salmo* were most probably misidentifications. Most studies of *Eubothrium* have involved salmonids from the northern part of Europe, with few records from southern and south-eastern Europe. This study also confirmed the reliability of the morphology of the apical disc for the discrimination of *E. crassum* and *E. salvelini*.

### Introduction

Cestodes of the genus *Eubothrium* Nybelin, 1922 (Pseudophyllidea) represent a unique group of fish helminths because some species occur in the sea, whilst others are exclusively freshwater and one species, *E. crassum* (Bloch, 1779), lives in both environments (Kennedy, 1978a,b; Andersen & Kennedy, 1983). Despite these differences in biology and ecology, *Eubothrium* tapeworms may be difficult to identify due to the uniform morphology and close similarity of most species

(Andersen & Kennedy, 1983). Problems in the identification of two of the most common species, *E. salvelini* (Schrank, 1790) and *E. crassum*, parasitizing salmonid fish in the Holarctic Region (Protasova, 1977; Kennedy, 1978a,b), have contributed to an unsatisfactory knowledge of the spectrum of definitive hosts, host specificity, and geographical distribution of these parasites.

*Eubothrium crassum* is a typical cestode of salmon (*Salmo salar* L.), sea and brown trout (*Salmo trutta trutta* L. and *S. trutta fario* L.) (Kennedy, 1978a,b; Andersen & Kennedy, 1983), but it has also been reported from a wide spectrum of other salmonid fish of the genera *Brachymystax*, *Coregonus*, *Hucho*, *Oncorhynchus*, *Salmo*, *Salvelinus* and *Thymallus* (see table 1 for references). In Europe, *E. salvelini*

\*Fax: 00420 38 53 10 388  
E-mail: [tscholoz@paru.cas.cz](mailto:tscholoz@paru.cas.cz)

occurs most frequently in Arctic char (*Salvelinus alpinus* (L.)) (Kennedy, 1978a), but it has been found in other salmonids, including those serving as the definitive hosts of *E. crassum* (table 1).

In the present study, the host spectrum of *E. crassum* and *E. salvelini* in Europe was critically examined on the basis of an evaluation of extensive material collected from different hosts (both freshwater and marine, wild and cultured) and regions of Europe. Besides numerous recently collected specimens of *Eubothrium*, voucher specimens from museum collections were also examined, with an emphasis on cestodes from 'atypical' or less common fish hosts.

### Materials and methods

Freshly collected cestodes from the following hosts and localities were studied:

1. Salmon (*Salmo salar* L.) – marine (cultured in cages): Loch Fyne near Cairndow; Isle of Arran; Firth of Clyde at Dunoon, all Scotland, UK, collected in March 2001, Tighnabruaich, Scotland, September 2002; marine (wild population): Kursiu Marios, Curonian Bay, Lithuania, October 1999; River Tay and River Forth, both Scotland, September 2002; freshwater (smolt from cage culture): Skogseidvatn Lake, Norway, January 2001; Loch Arkaig, Scotland, UK, March 2001.
2. Sea trout (*Salmo trutta trutta* L.) – Kursiu Marios, Curonian Bay, Lithuania, October 1999; Myrdalsvatnet, Norway, October 1999; Isetfjord, Denmark, January 2001; River Esk, Scotland, September 2002.
3. Brown trout (*Salmo trutta fario* L.) – Vouglans water reservoir, Jura, France, November 1999; Hafravatn, Iceland, November 2000; Loch Leven, Scotland, March 2001; Loch Doyme and Loch Earn, Scotland, September 2002.
4. Lake trout (*Salmo trutta lacustris* L.) – Lake Annecy and Lake Bourget, France; Lake Geneva, France and Switzerland, 1997–2000.
5. Rainbow trout (*Oncorhynchus mykiss* (Walbaum)) – Skoge Lake, Norway, March 2000; Loch Awe and Loch Earn (both cage culture); Loch Leven (wild population), all Scotland, UK, March and April 2001.
6. Arctic char (*Salvelinus alpinus* (L.)) – Røye, Norway, February 1988; Lake Annecy and Lake Bourget, France; Lake Geneva, France and Switzerland, 1997–2000; Hafravatn, Iceland, November 2000; Loch Doyme, Scotland, September 2002.
7. Brook trout (*Salvelinus fontinalis* (Mitchill)) – Fuschlsee, Austria, August 1995.

Specimens for morphological evaluation were fixed in hot 4% formaldehyde solution (Scholz & Hanzelová, 1998; Hanzelová *et al.*, 2002).

Specimens for isoenzyme analysis were frozen in liquid nitrogen, stored at  $-70^{\circ}\text{C}$  and then analysed by the isoelectrofocusing (IEF) technique as described by Šnábel *et al.* (1998). Isoelectric focusing was conducted on an LKB Multiphor 2117 system equipped with an LKB 2197 power supply. Gels were cast using ampholytes with the pH ranges 3.5–9.5 and 4.0–6.5. Three enzyme

systems were used as genetic markers (see Král'ová & Šnábel, 2000): acid phosphatase (ACP, EC 3.1.3.2), phosphoglucosmutase (PGM, EC 5.4.2.2), and glucose-phosphate isomerase (GPI, EC 5.3.1.9). Isoenzymes were designated in order of mobility from the cathode; the number 100 was given to the commonest signal.

Species identification was based on the following characters:

1. The morphology of the apical disc (see Andersen, 1979; Andersen & Kennedy, 1983; Chubb *et al.*, 1987; Hanzelová *et al.*, 2002): only two dorsoventral grooves (incisions) in *E. salvelini* versus at least four grooves, including two on the lateral sides of the disc, in *E. crassum* (fig. 1). Each scolex was observed unmounted and the number of gooves was counted under the light microscope in the *en face* view. Some scoleces were then prepared for scanning electron microscopy (SEM) using standard procedures (see Scholz *et al.*, 1998) and observed with a Jeol JSM 6300.
2. The position of the vitelline follicles (Andersen & Kennedy, 1983): cortical in *E. crassum* and medullary in *E. salvelini* (see fig. 3 in Hanzelová *et al.*, 2002). The position of the follicles was observed in 10  $\mu\text{m}$  paraffin sections stained with Heidenhain's haematoxylin-eosin.
3. The presence of species-specific alleles found at *Acp*, *Pgm* and *Gpi* loci that code for the above-mentioned enzymes (Král'ová & Šnábel, 2000).

The identification of museum specimens was based on the morphology of the apical disc (see above) and strobilar characters described by Hanzelová *et al.* (2002). The possibility of simultaneous infections in individual fish hosts with both cestode species was tested in a sample of 91 *Eubothrium* tapeworms from ten rainbow trout collected in Loch Awe in June 2001. Each specimen was treated separately, the scolex and posterior part of the strobila were fixed for morphological evaluation (morphology of 77 scolices and topology of vitelline follicles in cross sections of 28 specimens were examined), and the middle part of the body was frozen for isoenzyme analysis (88 tapeworms).

In addition to freshly collected tapeworms, the following specimens from museum collections were evaluated:

1. The Natural History Museum, Vienna (Naturhistorisches Museum Wien – NMW), Austria: *E. crassum* from asp *Aspius aspius* (Collection Nos. 3010 and 16153 (7)), eel *Anguilla anguilla* (Nos. 3009a, 3009f and 16153 (20, 35, 37 and 40)), whitefish *Coregonus wartmanni* (Nos. 3040 (35) and 16172), and Danubian salmon *Salmo hucho* (= *Hucho hucho*) (No. 2617), all specimens from Austria.
2. The Natural History Museum, London, UK (BMNH): *E. crassum* from vendace *Coregonus albula* (1981.5.7.29–33), from *Coregonus* sp. (1981.5.7.28), both from Finland, and *S. alpinus* from Spitzbergen, Norway (1924.3.11.86–90); *E. salvelini* from *Salmo trutta* (1986.2.21.16–18); and *Eubothrium* sp. from pike *Esox lucius* (1986.2.21.19; 1989.1.24.52–59), both from Ireland.
3. The Natural History Museum, Geneva (Muséum d'Histoire Naturelle, Genève – MHNG), Switzerland: *E. crassum* from *S. salar* (002/097–9), *S. trutta lacustris* (036/012/4, INVE 17858, 19002, 28077), and *Coregonus* sp.

Table 1. Records of adults of *Eubothrium crassum* and *E. salvelini* from salmonid fish in Europe, including specimens from museum collections studied by the present authors (findings of immature tapeworms in paratenic hosts omitted). Records considered doubtful and requiring confirmation in italics and marked with a question mark.

| Host  | <i>E. crassum</i>  | <i>E. salvelini</i>   |
|---|--|---|
| <i>Acantholingua ohridana</i>                               | Hristovski <i>et al.</i> 1999 (?)  | Hristovski <i>et al.</i> 1999 (?)   |
| <i>Coregonus albula</i>                                     | Andersen & Kennedy 1983; present study   | –   |
| <i>Coregonus fera</i>                                       | –  | Nybelin 1922; Joyeux & Baer 1936  |
| <i>Coregonus lacareus</i>                                   | Zandt 1924; Kritischer 1990 (?)  | Nybelin 1922; Vik 1963  |
| <i>Coregonus wartmanni</i>                                  | BMNH 1981.5:7.28-33; MNHG 036/067  | Kritischer 1990; "E. crassum" (NMW 3040 and 16172); present study   |
| <i>Coregonus</i> sp.  | Holčik <i>et al.</i> 1988; NMW 2617 – present study  | Nybelin 1922 (?)  |
| <i>Hucho hucho</i>  | Kane 1966; Wootten 1972; Ingham & Arne 1973; Otto & Körting 1973; Kennedy 1974; Andersen 1979; Andersen & Kennedy 1983; Kennedy <i>et al.</i> 1991; Engelhardt & Mirle 1993; Buchmann <i>et al.</i> 1995; present study  | Holčik <i>et al.</i> 1988   |
| <i>Oncorhynchus mykiss</i>                                  | Stojanovski <i>et al.</i> 1998   | Otto & Körting 1973 (reported as <i>Eubothrium</i> sp.); present study  |
| <i>Salmo letnica</i>  | Nybelin 1922; Markowski 1933; Joyeux & Baer 1936; Mulcahy & Kennedy 1970; Rokicki 1975; Kennedy 1978b; Dorovskikh 2000; Petkevičiūtė & Bondarenko 2001 (for other records – see Kennedy 1978b); MNHG 002/097-099; present study  | Stojanovski <i>et al.</i> 1998 (?)  |
| <i>Salmo salar</i>  | Nybelin 1922; Chappell & Owen 1969; Wootten 1972; Campbell 1974; Rokicki 1975; Kennedy 1978b; Fahy 1980; Buchmann 1987; Molloy <i>et al.</i> 1993; Byrne <i>et al.</i> 1999, 2002 (for other records – see Kennedy 1978b); "E. salvelini" (BMNH 1986.2.21.16-18); present study  | –   |
| <i>Salmo trutta trutta</i>                                  | Heitz 1918; Rosen 1918; Zandt 1924; Dogiel & Petrushevski 1935; Joyeux & Baer 1936; Wootten 1972; Rokicki 1975; Ozelik & Deufel 1989; Hartvigsen & Kennedy 1993; Kennedy, 1996; Hanzelová <i>et al.</i> 1999; Kral'ová <i>et al.</i> 2001 (for other records – see Kennedy 1978b); MNHG 036/012/4, INVE 17858, 19002, 28077; present study | –   |
| <i>Salmo trutta fario</i> and<br><i>S. trutta lacustris</i> | Zandt 1924 (?); Polyaniski 1966 (?); Mulcahy & Kennedy 1970 (?) (misidentification of <i>E. salvelini</i> according to Kennedy, 1978b)   | Nybelin 1922; Vik 1963; Chappell & Owen 1969; Kennedy 1977, 1978a; Andersen 1979; Conneely & McCarthy 1984; Rydlo 1985; Hoffmann <i>et al.</i> 1986; Giovinazzo 1989; Kritischer 1991; Due & Curtis 1995; Gerdeux <i>et al.</i> 1995; Knudsen <i>et al.</i> 1997; Hanzelová <i>et al.</i> 1999 (for other records – see Kennedy 1978b); "Eubothrium sp." (MNHG INVE 26301, 29413-4); ZMC; present study |
| <i>Salvelinus alpinus</i>                                   | –  | M. Rydlo, unpublished; present study  |
| <i>Salvelinus fontinalis</i>                                | –  | Nybelin 1922 (?)  |
| <i>Thymallus thymallus</i>                                  | Zandt 1924 (?); Kane 1966 (?)  | –   |

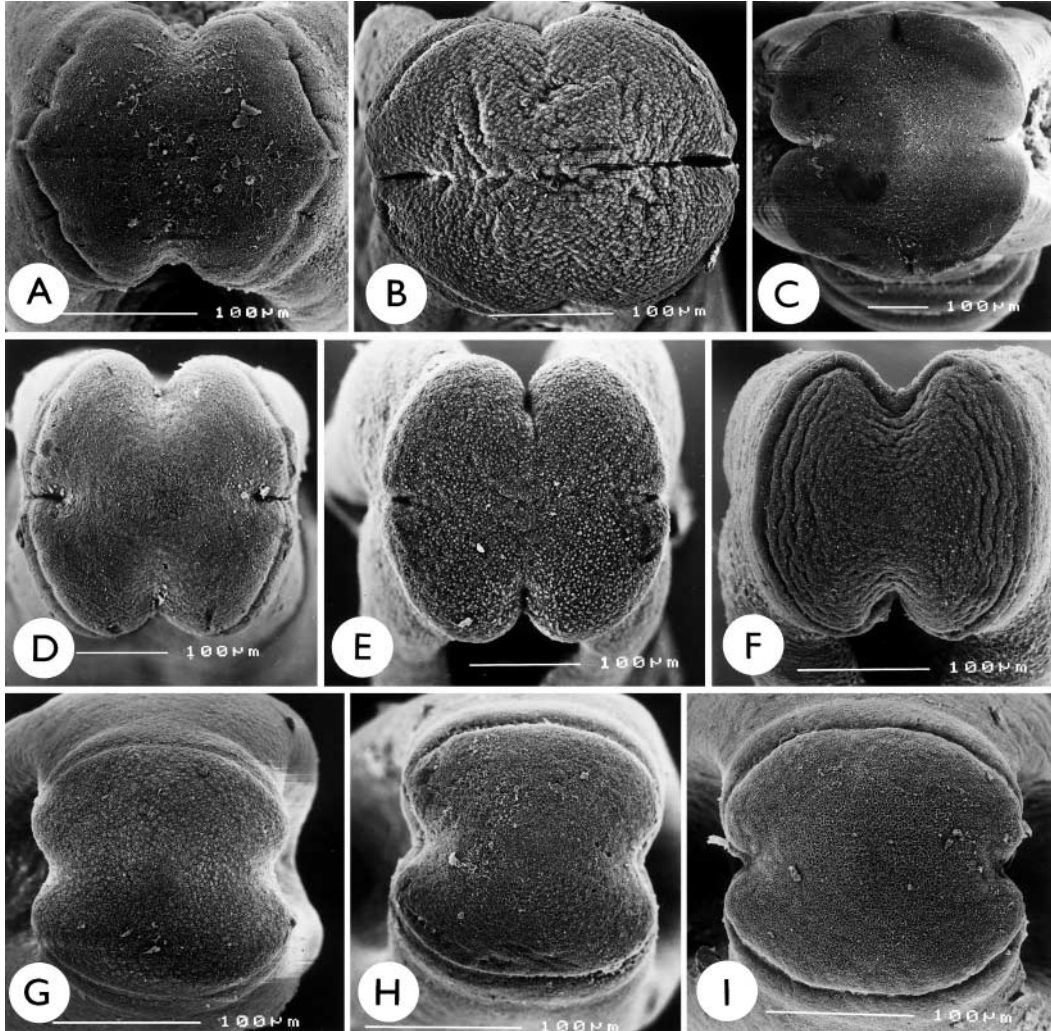


Fig. 1. Scanning electron micrographs of scoleces of *Eubothrium crassum* (A–E) and *E. salvelini* (F–I) from different salmonid hosts: (A) *Salmo salar*, marine, Scotland; (B) *S. salar*, freshwater, Scotland; (C) *Salmo trutta trutta*, Norway; (D) *S. trutta fario*, Scotland; (E) *Oncorhynchus mykiss*, Scotland (Loch Leven); (F) *O. mykiss*, Scotland (Loch Earn); (G) *Salvelinus alpinus*, France; (H) *Salvelinus fontinalis*, Austria; (I) *O. mykiss*, Scotland (Loch Awe).

(036/067); *Eubothrium* sp. from *S. alpinus* (INVE 26301, 29413–4), all from Switzerland.

4. The Zoological Museum, Copenhagen (Zoologisk Museum, København), Denmark (ZMC); *E. salvelini* from *S. alpinus* from several localities in Greenland (Grønland, Denmark).

## Results

### *Principal definitive hosts of E. crassum and E. salvelini*

Salmon (*Salmo salar*), both freshwater and marine from cage culture and wild populations (fig. 1A,B), sea trout (*S. trutta trutta*) (fig. 1C), brown trout (*S. trutta fario*) (fig. 1D), and lake trout (*S. trutta lacustris*) were infected exclusively with *E. crassum*. *Eubothrium salvelini* was found in Arctic char (*Salvelinus alpinus*) (fig. 1G) and brook trout (*S. fontinalis*) (fig. 1H).

### *Eubothrium in rainbow trout (Oncorhynchus mykiss)*

Both cestode species were found in rainbow trout from Scotland. In rainbow trout from Loch Awe, *E. crassum* and *E. salvelini* (fig. 1I) occurred simultaneously, but *E. crassum* predominated. Mixed infections occurred in five fish, four hosts harboured exclusively *E. crassum*, and one rainbow trout was infected with *E. salvelini*. Rainbow trout from Loch Earn were infected with *E. salvelini* only (fig. 1F), whereas rainbow trout from Loch Leven (fig. 1E; both localities in Scotland) and Norway were infected with *E. crassum*. Thus, rainbow trout seems to be a suitable (but not specific) definitive host of *E. crassum* and *E. salvelini*.

### *Other definitive hosts of E. crassum and E. salvelini*

*Eubothrium crassum* has also been found in Danubian salmon (*Hucho hucho*) from Austria and vendace

(*Coregonus albula*) from Finland. *Eubothrium salvelini* was recorded from whitefish (*Coregonus wartmanni*) from Austria.

Examination of other museum specimens has shown several misidentifications (see table 1). Tapeworms identified as '*E. salvelini*' from *Salmo trutta trutta* from Ireland (BMNH 1986.2.21.16–18) actually belong to *E. crassum* and '*E. crassum*' from *C. wartmanni* from Austria (NMW 3040 and 16172) is *E. salvelini*. Identifications of *E. crassum* from *Coregonus* sp. from Finland (BMNH 1981.5.7.28–33), from *Salmo salar* (MNHG 002/097–9), *Salmo trutta lacustris* (MNHG 036/012/4, INVE 17858, 19002, 28077), and *Coregonus* sp. (MNHG 036/067), all from Switzerland, are considered to be correct. '*Eubothrium*' sp. from *Salvelinus alpinus* from Switzerland (MHNG INVE 26301, 29413–4) is *E. salvelini* and '*E. salvelini*' from the same host from Greenland, Denmark (ZMC) was also correctly identified.

'*Eubothrium crassum*' from *Salvelinus alpinus* from Spitzbergen, Norway (BMNH 1924.3.11.86–90) was represented by sections of insufficient quality to identify them. '*Eubothrium* sp.' from *Esox lucius* from Ireland (BMNH 1986.2.21.19; 1989.1.24.52–59), and '*E. crassum*' from *Aspius aspius* (NMW Nos. 3010 and 16153) were juvenile specimens, which prevented their reliable identification. '*E. crassum*' from *Anguilla anguilla* from Austria (NMW 3009a, 3009f and 16153), reported by Kritscher (1988), actually represents *Bothriocephalus claviceps* (Goeze, 1782), a pseudophyllidean tapeworm specific to eels (see Scholz, 1997).

#### Geographical distribution of *E. crassum* and *E. salvelini* in Europe

The present data (asterisk), supplemented by reliable published records (see table 1), indicate the presence of *E. crassum* in the following European countries: Austria\*, Azerbaidzhan, Denmark\*, Estonia, Finland\*, France\*, Germany, Iceland\*, Ireland\*, Lithuania\*, Macedonia, Norway\*, Poland, Russia, Sweden, Switzerland\*, UK\*, Ukraine, and Yugoslavia; *E. salvelini* has been recorded in salmonids in Austria\*, Denmark\* including Greenland\*, France\*, Germany, Iceland\*, Ireland, Italy, Macedonia, Norway\*, Russia, Sweden, Switzerland\*, UK\*, and Yugoslavia. No recent records of the occurrence of either *Eubothrium* species exist from the Iberian Peninsula, the Netherlands, Belgium, Czech Republic, Slovakia, Hungary, most countries of the Balkan Peninsula, and Transcaucasian republics (Armenia, Georgia).

#### Identification of *E. crassum* and *E. salvelini*

This study confirmed the suitability of the morphology of the apical disc observed *en face* (in unmounted specimens) for the differentiation of mature *E. crassum* and *E. salvelini* (see Andersen, 1979; Chubb *et al.*, 1987). Species discrimination was confirmed independently by isoenzyme analyses and examination of sections of respective specimens. It was found, however, that immature worms of *E. crassum* with poorly developed incisions (grooves) on the apical disc may sometimes be difficult to identify.

#### GPI

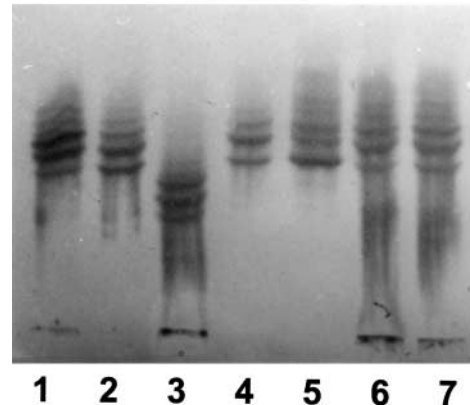


Fig. 2. Profile of glucose-phosphate isomerase obtained using isoelectrofocusing. Lanes 1, 2, 4, 5, 6, 7, *Eubothrium crassum* isolates; lane 3, *E. salvelini* isolate. All screened samples were recovered from rainbow trout in Loch Awe.

#### Electrophoretical comparison of *E. crassum* and *E. salvelini*

Using GPI, PGM and ACP enzyme systems, the two species were readily discernible at the genetic level. With GPI and PGM, alleles attributed to *E. salvelini* migrated more cathodically compared to *E. crassum* alleles. GPI gave either single- or triple-banded phenotypes in *E. salvelini*, while three isoenzymes of different mobility were detected in *E. crassum* (fig. 2). The PGM generated pattern was characterized by two isoenzymes in *E. salvelini* and a more complex pattern displaying three electromorphs in *E. crassum*. ACP produced three patterns (represented by heterozygous and alternative homozygous forms) in *E. salvelini*, unlike the single monomorphic band expressed by *E. crassum* isolates. The mobility values of major bands scored for each enzyme are listed in table 2.

#### Discussion

The results of the present study correspond with the observations of some previous authors (Vik, 1963; Kennedy, 1978b; Hanzelová *et al.*, 1999, 2002) in that *E. crassum* was found in fish of the genus *Salmo* (salmon, sea, brown, and lake trout), whereas *E. salvelini* occurred predominantly in Arctic char (*Salvelinus alpinus*), even in those localities where these fish lived sympatrically. Although extensive *Eubothrium* material from different regions of Europe was evaluated (table 1), no *E. crassum* were found in species of *Salvelinus* and, vice

Table 2. Major isoenzymes detected in three differentiating loci for *Eubothrium crassum* and *E. salvelini*.

| Enzyme | <i>E. crassum</i> | <i>E. salvelini</i> |
|--------|-------------------|---------------------|
| GPI    | 106, 108, 110     | 100, 102, 104       |
| PGM    | 105, 111, 117     | 97, 100             |
| ACP    | 90, 100           | 94                  |

versa, *E. salvelini* never infected any species of *Salmo*. It can be concluded, therefore, that *E. crassum* and *E. salvelini* show a strict specificity to their salmonid fish hosts. In Europe, these fish are infected with only one species of *Eubothrium*, either *E. crassum* or *E. salvelini*.

To date, all but one sample of *Eubothrium* from rainbow trout in Europe were identified as *E. crassum* (see table 1). The present study has shown, however, that rainbow trout, which is not native to Europe (see Froese & Pauly, 2001), may harbour both *Eubothrium* species and become infected simultaneously. In mixed infections (Loch Awe), *E. crassum* was more abundant than *E. salvelini*. In Loch Earn, *E. salvelini* was the only *Eubothrium* species occurring in rainbow trout and its identification was confirmed by DNA-based methods (Král'ová-Hromadová *et al.*, 2003).

The common occurrence of *E. salvelini* in rainbow trout in Scotland raises the question as to whether this species has been overlooked or misidentified during previous surveys of fish parasites in the UK (see Kennedy, 1974; Holland & Kennedy, 1997), or whether it has appeared only recently in British populations of rainbow trout. The latter have been stocked in Loch Earn and Loch Awe for the past 15–20 years and it is possible that *E. salvelini*, previously occurring in resident populations of char, colonized rainbow trout for the first time. In Loch Leven, famous for its unique strain of brown trout, rainbow trout were first stocked as recently as 1994, which may explain the absence of *E. salvelini* from rainbow trout in this locality.

The susceptibility of rainbow trout to simultaneous infection with *E. crassum* and *E. salvelini* may reflect the phylogenetic relationships of this fish with other salmonids, given that members of the genus *Oncorhynchus* form an intermediate clade between those including species of *Salmo* and *Salvelinus* (Phillips & Oakley, 1997). Rainbow trout occurred originally in the Russian Far East and the north-western part of North America, where salmonids of the genus *Oncorhynchus* harbour both *Eubothrium* species (Andersen & Kennedy, 1983).

Results of examining museum deposited specimens have provided evidence that *E. crassum* and *E. salvelini* may occur in other salmonid genera, such as *Hucho* (*E. crassum*) and *Coregonus* (*E. crassum* and *E. salvelini*). A critical evaluation of these specimens has also shown numerous misidentifications of *Eubothrium* and confirmed the suggestions of Kennedy (1978a) and Andersen & Kennedy (1983) about the unreliability of previous records from 'atypical' hosts (see table 1).

Available data demonstrate that *Eubothrium* occur in the northern part of Europe, apparently reflecting the geographical distribution of salmonid fish. The absence of recent records of *E. crassum* and *E. salvelini* in some countries where native salmonids occur (e.g. Balkan Peninsula) may reflect the lack of parasitological surveys rather than the absence of these cestodes. *Eubothrium crassum* seems to be more widely distributed in Europe, which may be related to the wider spectrum of its definitive hosts. In some countries, such as the Czech Republic (see Moravec, 2001), *E. crassum* disappeared at the beginning of the 20th century after the Atlantic salmon stopped upstream migration as a consequence of the construction of barrages and weirs on rivers.

The usefulness of isoenzyme patterns as species-specific markers (see Král'ová & Šnábel, 2000) was demonstrated in a study of *Eubothrium* from a mixed infection of rainbow trout from Loch Awe (fig. 2; table 2). The enzyme systems used appeared to be suitable for species discrimination and results obtained were congruent with those based on morphological characters, i.e. the morphology of the apical disc and the position of vitelline follicles in cross sections.

### Acknowledgements

The authors are indebted to the following persons who kindly provided specimens: Karin Andersen, University of Oslo, Norway; Björn Berland, University of Bergen, Norway; Thomas Bjerre and Kurt Buchmann, Royal Veterinary and Agricultural University, Copenhagen, Denmark; Ivica Král'ová-Hromadová, Parasitological Institute, Slovak Academy of Sciences, Košice, Slovakia; Romualda Petkevičiūtė, Institute of Ecology, Lithuanian Academy of Sciences, Vilnius, Lithuania; and Jim Treasurer, Fort William, Scotland. Thanks are also due to Eileen Harris and David I. Gibson, The Natural History Museum, London, UK; Alain de Chambrier and Claude Vaucher, Natural History Museum, Geneva, Switzerland; Helmut Sattmann, Natural History Museum, Vienna, Austria; and Reinhardt Kristensen, Zoological Museum, Copenhagen, Denmark, for the loan of specimens from museum collections. The stay of T.S. and R.K. at the Institute of Aquaculture, University of Stirling, Scotland, was supported by the Access to Research Infrastructure Action of the Improving Human Potential Programme of the European Community (contract HPRI-CT-1999-00106). The authors are indebted to Christina Sommerville, head of the Laboratory of Parasitology, and Rod Wootten, Deputy Director, Institute of Aquaculture, for their support and providing necessary facilities. This study was partially supported by the Grant Agency of the Czech Republic (project No. 524/01/0314) and the Slovak Grant Agency VEGA (project No. 2/1020/21).

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