



Morphological variation in *Echinorhynchus truttae* Schrank, 1788 and the *E. bothniensis* Zdzitowiecki & Valtonen, 1987 species complex from freshwater fishes of northern Europe

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Abstract

Echinorhynchus truttae and the *E. bothniensis* species complex are common parasites of salmoniform and other fishes in northern Europe. *E. bothniensis* and its sibling species *E. 'bothniensis'* are thought to be closely related to the Nearctic *E. leidyi* Van Cleave, 1924 based on morphological similarity and common usage of a mysid intermediate host. This study provides the first analysis of morphological and meristic variation in *E. truttae* and expands our knowledge of anatomical variability in the *E. bothniensis* group. Morphological variability in *E. truttae* was found to be far greater than previously reported, with part of the variance attributable to sexual dimorphism. *E. truttae*, the two species of the *E. bothniensis* group and *E. leidyi* displayed considerable interspecific overlap in the ranges of all conventional morphological characters. However, [Proboscis profiler](#), a tool for detecting acanthocephalan morphotypes using multivariate analysis of hook morphometrics, successfully separated *E. truttae* from the other taxa. The *E. bothniensis* species group could not be reliably distinguished from *E. leidyi* (or each other), providing further evidence of the affinity of these taxa. Observations on the distribution of *E. truttae* in its definitive host population are also reported.

Keywords

Acanthocephala, *Echinorhynchus truttae*, *Echinorhynchus bothniensis*, *Echinorhynchus leidyi*, cryptic speciation, sibling species, morphology, morphometric, meristic, description, Proboscis Profiler, overdispersion, parasite, salmoniform

Introduction

Echinorhynchus bothniensis Zdzitowiecki & Valtonen, 1987 was originally described from *Osmerus eperlanus* L. from the oligohaline waters of the Bothnian Bay, northern Baltic. In earlier studies (Timola 1980, Valtonen 1980, Valtonen 1983) this acanthocephalan had been determined as *E. gadi* Zoega in Müller, 1776 (see Zdzitowiecki and Valtonen 1987). The first evidence that *E. 'gadi'* in *O. eperlanus* was biologically distinct from *E. gadi* in *Gadus morhua* L. and other marine fish came from amphipod infection experiments; acanthors of *E. gadi* from Baltic *G. morhua* were infective to *Gammarus zaddachi* Sexton, but acanthors of *E. 'gadi'* from *O. eperlanus* were not (Valtonen et al. 1983). Moreover, Valtonen et al. (1983) noted that the mature females of *E. 'gadi'* from *O. eperlanus* were smaller than the mature females of *E. gadi* found in *G. morhua*. A detailed morphological study by Zdzitowiecki and Valtonen (1987) revealed marked differences in egg dimensions between *E. 'gadi'* from *O. eperlanus* and *E. gadi* from *G. morhua*. Furthermore, male *E. 'gadi'* from *O. eperlanus* tended to exhibit one or more pairs of parallel cement glands in contrast to the moniliform pattern displayed by *E. gadi* from *G. morhua*, although there was some interspecific overlap in cement gland arrangement. On the basis of these morphological differences Zdzitowiecki and Valtonen (1987) accorded specific rank to *E. 'gadi'* from *O. eperlanus* by naming it *E. bothniensis*. Other true definitive hosts (*i.e.* hosts in which gravid female worms have been found) of *E. bothniensis* from the Bothnian Bay include *Lampetra fluviatilis* (L.), *Salmo trutta* L., *Lota lota* (L.), *Myoxocephalus quadricornis* (L.) and *Platichthys flesus* (L.) (Valtonen and Crompton 1990). The intermediate hosts belongs to the *Mysis relicta* Lovén (Mysidacea) species group (Valtonen and Crompton 1990). It is important to note that this species group has recently been split, on the basis of molecular and morphological characters (Audzijonytė and Väinölä 2005) into four named taxa: *M. relicta* (*sensu stricto*), *M. salemaai* Audzijonytė & Väinölä, 2005, *M. segerstralei* Audzijonytė & Väinölä, 2005 and *M. diluviana* Audzijonytė & Väinölä, 2005.

Using multilocus enzyme electrophoresis Väinölä et al. (1994) demonstrated that not only is *E. bothniensis* heterospecific to *E. gadi*, but that both taxa represent complexes of sibling species. One species of the *E. bothniensis* group was found in *O. eperlanus* from the Bothnian Bay and in *O. eperlanus* and *M. relicta* (*sensu stricto*) (as *M. relicta* sp. I *sensu* Väinölä 1986) from Lake Keitele, central Finland. Väinölä et al. (1994) referred to this species as *E. bothniensis*, since its range included the type-locality. A second species found in *Coregonus lavaretus* (L.), *Platichthys flesus* (L.), *Salvelinus alpinus* (L.) and *Mysis segerstralei* Audzijonytė & Väinölä, 2005 (as *M. relicta* sp. III *sensu* Väinölä 1986) from

Lake Pulmankijärvi, northern Finland was designated *E. bothniensis*' (Väinölä et al. 1994). Neither of the lacustrine populations of the *E. bothniensis* group have previously been studied morphologically.

E. bothniensis is morphologically very similar to the North American *E. leidy* Van Cleave, 1924 (= *Echinorhynchus salvelini* Linkins in Ward & Whipple, 1918 nec Schrank, 1788), but apparently differs slightly from the latter species in hook formula and cement gland arrangement (Zdzitowiecki and Valtonen 1987). *Mysis relicta* (sensu lato) is reported as the intermediate host of *E. leidy* (Prychitko and Nero 1983, Wolff 1984). More precisely, these intermediate host records for Nearctic *E. leidy* will correspond to *M. segerstralei* and/or *M. diluviana*; *M. relicta* sensu stricto appears to be confined to north European fresh and brackish waters (Audzijonytė and Väinölä 2005). Definitive hosts include salmonid and coregonid fishes. Väinölä et al. (1994) postulated that the common usage of *M. relicta* group species as intermediate hosts defines *E. leidy* and the *E. bothniensis* group as a clade of closely related species, because the known life cycles of all other *Echinorhynchus* spp. involve an amphipod intermediate host. Furthermore, these authors advanced an hypothesis of co-speciation of the acanthocephalans with their mysid hosts.

Both *E. bothniensis* and *E. leidy* have a similar hooks formula and cement gland arrangement to a congener, *E. truttae* Schrank, 1788 found in salmoniform fishes of the Palaearctic. *E. truttae* utilises an amphipod (*Gammarus pulex* (L.)), rather than a mysid, as an intermediate host (Awachie 1966) and so is apparently biologically distinct from the *E. bothniensis* group and *E. leidy*. Zdzitowiecki and Valtonen (1987) reported that *E. bothniensis* could be distinguished from *E. truttae*, because the latter has a longer proboscis and much shorter eggs. However, the diagnostic value of these characters was equivocal, since anatomical variability in *E. truttae* had never been assessed. The means to discriminate between the *E. bothniensis* group and *E. truttae* is of real significance to workers conducting faunistic surveys or other studies on these acanthocephalans. The two taxa share some of the same species of definitive host (e.g. *Salmo trutta*) (see Petrochenko 1956, Valtonen and Crompton 1990) and may well occur in sympatry, since their intermediate hosts have overlapping geographical ranges in northern Europe (see Pinkster 1978, Väinölä et al. 1994).

E. truttae is typically a parasite of salmoniform fishes and has been reported from a variety of species including *S. trutta* (e.g. Awachie 1966), *S. alpinus* (e.g. Dorucu et al. 1995), *Salvelinus leucomaenis* (Pallas) (Nagasawa et al. 1997), *C. lavaretus* (e.g. Petrochenko 1956), *Thymallus thymallus* (L.) (e.g. Petrochenko 1956), *Thymallus arcticus baicalensis* Dybowski (Baldanova and Pronin 1998, Baldanova 2000) and *Oncorhynchus mykiss* (Walbaum) (Dorucu et al. 1995, Holland and Kennedy 1997). *E. truttae* is found throughout Europe (including Ireland and the British Isles) and its range extends across Siberia all the way to the Bering Straits (Petrochenko 1956). Golvan (1994) suggested that *E. truttae* (sensu Zhukov 1960) from the Kurile Islands, northwest Pacific, may be another species.

The principal aims of the present study were: (i) to ascertain whether the two sibling species of the *E. bothniensis* group can be distinguished from each other, and from *E. leidy*, using morphological characters; (ii) to review the taxonomy of *E. bothniensis* and *E.*

'*bothniensis*'; (iii) to quantify morphological variability in *E. truttae*; and (iv) to identify the best characters for discriminating this taxon from the *E. bothniensis* sibling species and *E. leidyi*. Additionally, some observations on the ecology of *E. truttae* are reported.

Material and methods

Material

Table 1 provides a detailed list of all material studied, including accession numbers. A total of 19 specimens (7 males; 12 females) of *Echinorhynchus truttae* were collected from wild brown trout (*Salmo trutta* L.) from two streams (Loch Walton Burn and Loch Coulter Burn) in the River Carron catchment, central Scotland. The fish were caught by electro-fishing and were transported live to the laboratory where they were killed by a blow to the head and examined for acanthocephalan infection within 24 hours. Acanthocephalans found were washed and relaxed in refrigerated distilled water before being fixed in 75% alcohol. These acanthocephalans were identified as *Echinorhynchus truttae* using the keys in Petrochenko (1956). They were judged to be *E. truttae*, rather than members of the morphologically similar *E. bothniensis* group or *E. leidyi*, because the lotic environment they were collected from is unlikely to support populations of the lentic *Mysis relicta*, the intermediate host of the *E. bothniensis* group. Furthermore, the trout sampled were in their first year of life and so were unlikely to have spent any time outside their natal stream where they might potentially have been infected with *E. bothniensis*.

Table 1. Material Studied.						
Species	Host	Locality	Date Collected	Accession Numbers	ID Prefix in Supplementary Files	Number of Specimens
<i>E.truttae</i>	<i>Salmotrutta</i> L.	Drummore, southwest Scotland	NA	BM (NH) 1986.764–793	t1.	74 (45 f, 29 m)
<i>E.truttae</i>	<i>S.trutta</i>	Loch Walton Burn, River Carron catchment, central Scotland (National Grid Reference NS 668 865)	24th June 1996	BM (NH) 2002.2.4.264–275	t2.	11 (4 f, 7 m)
<i>E.truttae</i>	<i>S.trutta</i>	Loch Coulter Burn, River Carron catchment, central Scotland (National Grid Reference NS 761 865)	20th September 1996	BM (NH) 2002.2.4.276–283	t3.	8 (8 f, 0 m)

<i>E.bothniensis</i>	<i>Osmeruseperlanus</i> L.	Bothnian Bay, Baltic Sea	13th July 1985	BM (NH) 1987.1070–1074 (paratypes)	b1.	1 (1 f, 0 m)
<i>E.bothniensis</i>	<i>O.eperlanus</i>	Lake Keitele, central Finland	10th October 1996	BM (NH) 2002.2.4.102–122	b2.	19 (8 f, 0 m)
<i>E.bothniensis</i>	<i>O.eperlanus</i>	Lake Keitele, central Finland	26th October 1989	BM (NH) 1989.1474–1491	b4.	13 (6 f, 7 m)
<i>E. 'bothniensis'</i>	<i>Salvelinusalpinus</i> (L.)	Lake Pulmankijärvi, northern Finland	14th June 1989	BM (NH) 1989.1241–1248	b5.	7 (4 f, 3 m)
<i>E. 'bothniensis'</i>	<i>S.alpinus</i>	Lake Pulmankijärvi, northern Finland	NA	BM (NH) 1989.1439–1468	b6.	2 (2 f, 0 m)
<i>E. 'bothniensis'</i>	<i>Coregonuslavaretus</i> (L.)	Lake Pulmankijärvi, northern Finland	NA	BM (NH) 1989.1259–1270	b7.	16 (8 f, 8 m)
<i>E. 'bothniensis'</i>	<i>C.lavaretus</i>	Lake Pulmankijärvi, northern Finland	14th–16th June 1989	BM (NH) 1989.1406–1420	b8.	5 (3 f, 2 m)
<i>E. 'bothniensis'</i>	<i>Platichthysflesus</i> (L.)	Lake Pulmankijärvi, northern Finland	11th June 1990	NA	b9.	4 (3 f, 1 m)
<i>E.leidy</i>	<i>S.alpinus</i>	Kinguk Lake, Northwest Territories, Canada 64°40'N 75°30'W	27th August 1984	CMNPA 1985–0146	i1.	3 (3 f, 0 m)
<i>E.leidy</i>	<i>C.lavaretus</i>	Southern Indian Lake, Manitoba, Canada 58°45'N 98°55'W	8th June 1982	CMNPA 1985–0138	i2.	5 (0 f, 5 m)
<i>E.leidy</i>	<i>S.alpinus</i>	Unnamed lake, Northwest Territories, Canada 64°26'N 77°45'W	29th August 1984	CMNPA 1985–0149	i3.	5 (0 f, 5 m)

A series of *E. truttae* (74 specimens; 45 females; 29 males) collected by Dr A Pike, University of Aberdeen, from *S. trutta* from Drummore, on the south-west coast of Scotland, held in the spirit collection of the Natural History Museum was also studied. Most of these acanthocephalans had well everted probosces and displayed no tegumental folding, suggesting that they had been relaxed in water before being fixed.

All of the specimens of the *E. bothniensis* group studied were collected between 1985 and 1997 by Professor E. T. Valtonen of the University of Jyväskylä and deposited in the spirit collection of The Natural History Museum. Some of this material had been fully relaxed in water prior to fixation. Most of the *E. bothniensis* material came from one host species, *O. eperlanus*, from the freshwater Lake Keitele, central Finland. This population of *E. bothniensis* is thought to have been isolated from conspecifics in the Bothnian Bay for at

least 6,000 years (Väinölä et al. 1994). Five paratypes of *E. bothniensis* (BM(NH) 1987.1070-1074) from *O. eperlanus* from the Bothnian Bay were also examined, but only one female worm was in a suitable condition for measuring hook morphometrics.

E. 'bothniensis' is known only from Lake Pulmankijärvi in northern Lapland, on the Finnish-Norwegian border. This freshwater lake lies 17 metres above sea level and drains into the Barents Sea. Samples of *E. 'bothniensis'* were obtained from the following hosts: *Salvelinus alpinus* (L.), *Coregonus lavaretus* (L.) and *Platichthys flesus* (L.).

In addition to the northern European material described above, voucher specimens of the Nearctic *E. leidyi* from the Canadian Museum of Nature were also examined. These acanthocephalans were collected by Shostak et al. (1986) during their extensive survey of morphological variability in *E. gadi*, *E. leidyi* and *E. salmonis* Müller, 1784 from northern Canada.

Light microscopy

The specimens of *E. leidyi* from the Canadian Museum of Nature had been fixed in formalin-acetic acid-alcohol (FAA), stained with Semichon's carmine and permanently mounted in Permount (Fisher Scientific). All other acanthocephalans were prepared for light microscopy by dehydration through an alcohol series followed by clearing in lactophenol. Measurements were made with aid of a digitizing tablet (KS 100, Version 3, Carl Zeiss Vision). Hook morphometric data were recorded from one longitudinal row in which all of the hooks were visible in profile using the method described by Wayland (2010). Morphometric and meristic data were collected during a PhD studentship (Wayland 2002).

Morphological data analysis

Statistical analysis and visualization of morphometric and meristic data were performed using the [R language and environment](#) (R Core Team 2012). Boxplots augmented with strip charts were created using the R package [beeswarm](#) (Eklund 2012). [Proboscis profiler](#) (Wayland 2010) was used to analyse both intra and interspecific variation in hook measurements. Proboscis profiler, based on the meristogram of Huffman and Bullock (1975), was developed to detect morphological heterogeneity in collections of superficially similar acanthocephalan worms based on the multivariate statistical analysis of proboscis hook dimensions. For a detailed description of this tool with examples, please refer to Wayland (2010). In brief, the Proboscis profiler algorithm is composed of the following sequential steps:

1. Proboscis profiler requires blade length and base width measurements from each of the hooks in at least one longitudinal row of hooks per specimen. In each longitudinal row hooks are numbered sequentially, starting with the most distal hook.
2. Hook position is standardized. Counted position number of each hook in a given row is multiplied by 100 and divided by $n + 1$, where n = the total number of hooks

in the row and the constant 1 is a corrective factor for centring the data-points in graphs.

3. A moving average (arithmetic mean) routine is applied to the data from each row of hooks and considers a user-defined segment of the percent-position axis for each measurement (length and base). The segment advances through the data from anterior to posterior in 1% increments.
4. Unsupervised pattern recognition using principal component analysis.
5. Hierarchical clustering of the first two principal components from step 4.

Ecological data analysis

For each of the two host populations studied (Loch Walton Burn and Loch Coulter Burn), [Quantitative Parasitology](#) (Rózsa et al. 2000, Reiczigel 2003) was used to calculate an exact confidence interval for the prevalence of infection (using the Sterne method), a bootstrap confidence interval for mean abundance and the aggregation index (variance/mean). The R package [fitdistrplus](#) (Delignette-Muller et al. 2013) was used to determine whether a Poisson or a negative binomial distribution provided the best description of the occurrence of *Echinorhynchus truttae* in its definitive host populations.

Data resources

All data collected for this study are available as supplementary files.

Morphological data

Standard morphometric and meristic data for female and male acanthocephalans can be found in Suppl. materials 1, 2 respectively. Egg and acanthor dimensions are listed in Suppl. material 3. Hook measurement data for female and male acanthocephalans (Suppl. materials 4, 5 respectively) are in a file format suitable as input to the [Proboscis Profiler software](#) (Wayland 2010).

Ecological data

Suppl. materials 6, 7 contain data on the occurrence of *E. truttae* in samples of its definitive host *S. trutta* from Loch Coulter and Loch Walton respectively. For each fish examined, fork length and intensity of infection were recorded.

Results

Variation in conventional morphological characters

Initially an assessment was made of intraspecific and interspecific variation in conventional morphological characters, *i.e.* those characters used by most acanthocephalan taxonomists in the differential diagnosis of *Echinorhynchus* species. Summaries of these

variables for the female and male acanthocephalans examined in this study are provided in Tables 2, 3 respectively. Data for the three *E. truttae* populations (Loch Walton Burn, Loch Coulter Burn and Drummore) have been pooled, because, in the absence of any inter-site morphological variability, these acanthocephalans were assumed to be conspecific. Additionally, for comparative purposes, Tables 2, 3 contain data for *E. bothniensis* from *O. eperlanus* in the Bothnian Bay (original description by Zdzitowiecki and Valtonen 1987) and an extensive collection of *E. leidyi* from various fishes across northern Canadian waters (Shostak et al. 1986). It is important to note that these additional data were recorded from acanthocephalans prepared for light microscopy using methods different from those employed in the current study, although in all studies acanthocephalans were relaxed in fresh water prior to fixation to evert proboscides. Zdzitowiecki and Valtonen (1987) fixed their samples of *E. bothniensis* in alcohol and examined them as wet mounts, similarly to the current study, however they used creosote rather than lactophenol as a clearing agent. By contrast, Shostak et al. (1986) fixed their samples in formalin-acetic acid-alcohol (FAA), stained them with acetocarmine and mounted them in synthetic resin.

Table 2.

Morphometrics of female *Echinorhynchus bothniensis*, *E. 'bothniensis'*, *E. leidyi* and *E. truttae* (range; mean + standard deviation and sample size in parentheses). Data available in Suppl. materials 1, 3.

	<i>E.bothniensis</i> Bothnian Bay (Zdzitowiecki and Valtonen, 1987)	<i>E.bothniensis</i> Lake Keitele (this study)	<i>E. 'bothniensis'</i> Lake Pulmankijärvi (this study)	<i>E.leidyi</i> Northern Canada (Shostak et al., 1986)	<i>E.truttae</i> Scotland (this study)
Body length (mm)	10.5 – 27.1 (—; 38)	10.1 - 25.1 (16.0 ± 4.44; 14)	8.2 – 15.8 (10.9 ± 2.28; 18)	3.9 – 31.6 (16.4 ± 4.36; 476)	9.0 – 18.9 (14.0 ± 2.00; 56)
Body width (mm)	1.12 – 3.13 (—; 38)	1.14 – 2.76 (1.89 ± 0.50; 14)	0.71 – 2.72 (1.32 ± 0.50; 20)	0.60 – 3.0 (1.2 ± 0.26; 478)	0.85 – 2.02 (1.19 ± 0.25; 56)
Body length/ width	—	5.6 – 11.8 (8.6 ± 1.52; 14)	3.8 – 13.8 (9.2 ± 2.34; 18)	4.3 – 27.4 (13.7 ± 3.40; 466)	7.4 – 16.5 (12.1 ± 2.02; 56)
Proboscis length	660 – 940 (846 ± 60; 38)	611 – 787 (717 ± 56.6; 7)	711 – 904 (823 ± 77.3; 5)	733 – 1335 (1037 ± 116.6; 508)	869 – 1188 (1009 ± 59.7; 56)
Proboscis width	230 – 290 (264 ± 15; 38)	248 – 344 (308 ± 33.2; 11)	213 – 334 (285 ± 34.3; 19)	187 – 355 (274 ± 31.0; 508)	249 – 359 (309 ± 22.2; 56)
Proboscis length/width	2.82 – 3.67 (3.21 ± 0.21; 38)	2.03 ± 2.95 (2.47 ± 0.370; 7)	2.61 – 3.77 (3.04 ± 0.500; 5)	2.64 – 5.98 (3.81 ± 0.414; 508)	2.73 – 3.93 (3.28 ± 0.289; 56)
Number of rows of hooks	18 – 22	18 – 21 (19.2 ± 0.98; 14)	18 – 22 (19.5 ± 1.07; 19)	14 – 23 (18.1 ± 1.66; 508)	16 – 22 (19.6 ± 1.44; 57)

Number of hooks per row	11 – 15	11 – 12 (11.9 ± 0.35; 8)	12 – 15 (13.2 ± 1.10; 5)	10 – 17 (14.1 ± 1.11; 508)	12 – 17 (14.6 ± 0.98; 57)
Maximum length of hook blade	57 – 72 (64 ± 3.0; 38)	57 – 66 (61 ± 3.6; 4)	64 – 68 (65 ± 2.1; 3)	52 – 84 (70 ± 4.8; 508)	68 – 91 (78 ± 3.8; 46)
Proboscis receptacle length	1080 – 1850 (1497 ± 176; 38)	1237 – 2195 (1615 ± 249; 14)	668 – 1922 (1284 ± 323; 20)	—	1486 – 2855 (1901 ± 287; 56)
Proboscis receptacle width	300 – 430 (366 ± 33; 38)	336 – 618 (436 ± 77; 14)	167 – 431 (296 ± 63; 20)	—	318 ± 616 (407 ± 77; 56)
Lemniscus length	870 – 1890 (—; 38)	958 – 1963 (1462 ± 323; 14)	510 – 1543 (901 ± 290; 19)	—	935 – 2434 (1670 ± 293; 56)
Lemniscus width	220 – 540 (—; 38)	212 – 616 (361 ± 111; 14)	99 – 441 (266 ± 90; 19)	—	201 – 693 (350 ± 93; 56)
Genital complex length	1480 – 2270 (1846 ± 201; 38)	1575 – 2104 (1912 ± 186; 6)	991 – 1669 (1356 ± 193; 12)	—	1357 – 2761 (1792 ± 289; 25)
Uterine bell length	—	375 – 734 (551 ± 147; 6)	265 – 555 (368 ± 93; 12)	—	429 – 878 (568 ± 93; 25)
Uterus length	—	1060 – 1749 (1314 ± 212; 8)	646 – 1203 (902 ± 158; 13)	—	614 – 1592 (1003 ± 191; 42)
Uterus width	—	110 – 237 (161 ± 44.1; 11)	41 – 157 (71 ± 34.1; 16)	—	56 – 219 (110 ± 30.1; 55)
Vagina length	—	218 – 344 (273 ± 42.9; 14)	183 – 281 (221 ± 25.6; 14)	—	234 – 394 (294 ± 29.7; 56)
Vagina width	—	62 – 144 (103 ± 26.1; 14)	65 – 98 (80 ± 10.3; 14)	—	72 – 149 (109 ± 15.2; 56)
Vaginal sphincter width	—	97 – 208 (142 ± 33.9; 14)	61 – 125 (82 ± 19.3; 15)	—	91 – 182 (126 ± 19.4; 56)
Sphincter width to vagina width ratio	—	1.04 – 1.97 (1.41 ± 0.271; 14)	0.73 – 1.28 (1.02 ± 0.184; 14)	—	0.88 – 2.01 (1.17 ± 0.161; 56)
Egg length	140 – 168 (156 ± 7; 38)	127 – 166 (148 ± 12.6; 15)	121 – 152 (137 ± 11.4; 9)	90 – 135 (115 ± 8.2; 134)	120 – 173 (140 ± 11.0; 117)
Egg width	22 – 29 (25 ± 1; 38)	19 – 31 (23 ± 3.1; 15)	19 – 23 (21 ± 1.2; 9)	—	22 – 34 (27 ± 2.2; 117)

Acanthor length	—	67–80 (73 ± 3.5; 15)	67–78 (74 ± 3.9; 9)	—	70–90 (80 ± 4.4; 117)
Acanthor width	—	14–19 (17 ± 1.5; 15)	14–19 (17 ± 1.5; 9)	—	17–24 (20 ± 1.4; 117)

Table 3.

Morphometrics of male *Echinorhynchus bothniensis*, *E. 'bothniensis'*, *E. leidyi* and *E. truttae* (range; mean + standard deviation and sample size in parentheses). Data available in Suppl. material 2.

	<i>E.bothniensis</i> Bothnian Bay (Zdzitowiecki and Valtonen, 1987)	<i>E.bothniensis</i> Lake Keitele (this study)	<i>E. 'bothniensis'</i> Lake Pulmankijärvi (this study)	<i>E.leidyi</i> Northern Canada (Shostak et al., 1986)	<i>E.truttae</i> Scotland (this study)
Body length (mm)	8.9 – 15.8	7.4 – 15.9 (10.9 ± 2.9; 16)	4.5 – 9.7 (7.3 ± 1.6; 14)	5.1 – 19.7 (10.3 ± 2.51; 360)	7.2 – 10.9 (8.9 ± 1.09; 32)
Body width (mm)	1.13 – 2.39	0.93 – 2.17 (1.47 ± 0.36; 14)	0.58 – 1.78 (1.04 ± 0.37; 14)	0.6 – 1.9 (1.0 ± 0.20; 353)	0.69 – 1.32 (0.90 ± 0.12; 32)
Body length/width	—	5.5 – 10.3 (7.8 ± 1.42; 14)	4.9 – 10.2 (7.4 ± 1.40; 14)	5.6 – 21.0 (10.7 ± 3.03; 352)	6.7 – 12.2 (10.0 ± 1.29; 32)
Reproductive system length (mm)	—	5.1 – 11.0 (7.4 ± 2.17; 13)	3.0 – 6.3 (4.8 ± 1.08; 14)	—	4.0 – 6.6 (5.4 ± 0.69; 32)
Proboscis length	690 – 830 (756 ± 36; 50)	617 – 751 (683 ± 42.8; 13)	—	658 – 1203 (930 ± 93.3; 381)	733 – 1019 (903 ± 59.6; 32)
Proboscis width	220 – 280 (240 ± 13; 50)	204 – 329 (265 ± 37.8; 16)	204 – 287 (256 ± 24.6; 8)	176 – 314 (245 ± 27.6; 381)	205 – 326 (264 ± 29.0; 32)
Proboscis length/width	2.69 – 3.51 (3.16 ± 0.22; 50)	2.00 – 3.16 (2.51 ± 0.327; 13)	—	2.57 – 5.24 (3.83 ± 0.424; 381)	2.67 – 4.07 (3.46 ± 0.381; 32)
Number of rows of hooks	17 – 20	17 – 21 (19.0 ± 1.50; 17)	18 – 22 (19.4 ± 1.26 10)	12 – 22 (17.5 ± 1.77; 381)	16 – 22 (18.7 ± 1.45; 35)
Number of hooks per row	11 – 14	11 – 13 (11.9 ± 0.59; 15)	—	10 – 16 (13.4 ± 0.98; 381)	11 – 15 (14.0 ± 0.95; 35)
Maximum length of hook blade	55 – 71 (62 ± 4; 50)	50 – 61 (57 ± 3.9; 6)	—	45 – 82 (64 ± 4.8; 381)	67 – 84 (75 ± 3.7; 26)

Proboscis receptacle length	1140 – 1800 (1452 ± 137; 50)	1042 – 1982 (1559 ± 231; 17)	913 – 1262 (1086 ± 125; 13)	—	1376 – 2384 (1779 ± 199; 32)
Proboscis receptacle width	240 – 350 (303 ± 27; 50)	141 – 402 (332 ± 67; 17)	154 – 345 (257 ± 62.6; 14)	—	278 – 499 (369 ± 41.9; 32)
Lemniscus length	720 – 1470	756 – 1678 (1219 ± 281; 15)	496 – 977 (717 ± 157; 11)	—	1172 – 1775 (1468 ± 164; 32)
Lemniscus width	150 – 480	173 – 553 (326 ± 106; 15)	107 – 268 (207 ± 54.3; 12)	—	135 – 390 (288 ± 58.3; 32)
Anterior testes length	800 – 1680	761 – 1682 (1172 ± 332; 12)	403 – 934 (649 ± 165; 13)	—	707 – 1249 (1050 ± 126; 28)
Anterior testes width	370 – 670	289 – 831 (476 ± 145; 12)	136 – 447 (312 ± 88.0; 13)	—	394 – 637 (513 ± 70.0; 28)
Posterior testes length	810 – 1700	686 – 1602 (1069 ± 295; 12)	387 – 929 (640 ± 161; 13)	—	694 – 1198 (975 ± 136; 28)
Posterior testes width	300 – 680	306 – 837 (475 ± 158; 12)	197 – 471 (334 ± 84; 13)	—	394 – 591 (506 ± 55; 28)
Cement gland width	—	178 – 954 (356 ± 207; 17)	164 – 404 (282 ± 84; 14)	—	198 – 575 (365 ± 83; 32)
Saeftigen's pouch length	750 – 1050	659 – 1413 (925 ± 227; 17)	500 – 871 (684 ± 117; 13)	—	538 – 854 (733 ± 77; 32)
Saeftigen's pouch width	160 – 270	116 – 336 (227 ± 72; 17)	101 – 237 (165 ± 45; 13)	—	187 – 374 (288 ± 44; 32)
Penis width	85 – 113 (98 ± 7; 50)	50 – 105 (79 ± 16; 16)	45 – 89 (63 ± 12; 9)	—	66 – 110 (85 ± 11; 32)
Bursal sucker diameter	—	137 – 219 (182 ± 23; 11)	135 – 191 (164 ± 16; 10)	—	123 – 197 (152 ± 20; 15)

The extent of intraspecific morphological variability for the taxa studied can be seen in Tables 2, 3. The mean and range of values for each morphometric are very similar for both *E. bothniensis* population, i.e. the Bothnian Bay and Lake Keitele. An analysis of the cause of intraspecific variation in morphological characters was attempted for *E. truttae* only, as sample numbers for the other taxa were considered to be too small for a meaningful statistical analysis. All anatomical characters common to both sexes are larger in females than males (compare data in tables Tables 2, 3 and also see boxplots in Suppl. material 8). Sexual dimorphism is also clearly apparent in a principal components analysis of conventional morphological characters (Fig. 1a). There is considerable separation of

females from males in the first principal component, which accounts for 36% of the variation in the dataset. The variables contributing most to the separation of the two sexes (i.e. those with the highest loadings for principal component one) are: lemniscus length, proboscis receptacle length and width, body length and proboscis length and width (Fig. 1b). Body size is positively correlated (Bonferroni corrected p-value < 0.05) with the size of several anatomical characters of female *E. truttae* (Table 4), namely, body width ($r^2=0.257$), proboscis length ($r^2=0.317$), proboscis receptacle length ($r^2=0.284$), lemniscus length ($r^2=0.364$), lemniscus width ($r^2=0.237$), vagina width ($r^2=0.246$) and vaginal sphincter width ($r^2=0.251$). In male *E. truttae* (Table 5), a significant positive correlation with body length is only demonstrated for the length of the reproductive system ($r^2=0.876$), lemniscus length ($r^2=0.487$) and the length of the testes ($r^2=0.346$ for anterior testis; $r^2=0.469$ for posterior testis). Evidence of morphological variation in *E. truttae* between the three sample sites was not found, even after taking sexual dimorphism into account.

Table 4.

Correlation of morphometric variables with body length in female *Echinorhynchus truttae*.

Correlation measured by Pearson's product-moment correlation coefficient (r). The raw p value is the probability that the sample correlation coefficient could have come from a population with a correlation coefficient of zero. The Bonferroni correction was used to control the family wise error rate across multiple tests of significance. Data available in Suppl. material 1.

Variable	n	r	raw p	Bonferroni p
Body width	56	0.507	0.000066	0.000997
Proboscis length	56	0.563	0.000006	0.000092
Proboscis width	56	0.041	0.763773	1.000000
Proboscis receptacle length	56	0.533	0.000023	0.000346
Proboscis receptacle width	56	0.375	0.004442	0.066630
Lemniscus length	56	0.603	<0.000001	0.000013
Lemniscus width	56	0.487	0.000142	0.002128
Genital complex length	25	0.438	0.028697	0.430462
Uterine bell length	25	0.266	0.198106	1.000000
Uterus length	42	0.376	0.014200	0.212997
Uterus width	55	0.123	0.369147	1.000000
Vagina length	56	0.273	0.041850	0.627757
Vagina width	56	0.496	0.000100	0.001500
Vaginal sphincter width	56	0.501	0.000085	0.001281
Maximum length of hook blade	46	0.267	0.072923	1.000000

Table 5.

Correlation of morphometric variables with body length in male *Echinorhynchus truttae*.

Correlation measured by Pearson's product-moment correlation coefficient (r). The raw p value is the probability that the sample correlation coefficient could have come from a population with a correlation coefficient of zero. The Bonferroni correction was used to control the family wise error rate across multiple tests of significance. Data available in Suppl. material 2.

Variable	n	r	raw p	Bonferroni p
Reproductive system length	32	0.936	<0.000001	<0.000001
Body width	32	0.417	0.017468	0.314424
Proboscis length	32	0.298	0.097440	1.000000
Proboscis width	32	-0.054	0.769724	1.000000
Proboscis receptacle length	32	0.131	0.474205	1.000000
Proboscis receptacle width	32	0.236	0.193402	1.000000
Lemniscus length	32	0.698	0.000009	0.000159
Lemniscus width	32	0.330	0.064692	1.000000
Anterior testis length	28	0.588	0.001008	0.018152
Anterior testis width	28	0.446	0.017358	0.312447
Posterior testis length	28	0.685	0.000059	0.001058
Posterior testis width	28	0.352	0.065541	1.000000
Cement gland width	32	0.296	0.099633	1.000000
Saefftigen's pouch length	32	0.360	0.043181	0.777265
Saefftigen's pouch width	32	0.174	0.339571	1.000000
Penis width	32	0.217	0.232671	1.000000
Bursal sucker diameter	15	0.259	0.350967	1.000000
Maximum length of hook blade	23	0.428	0.041548	0.747868

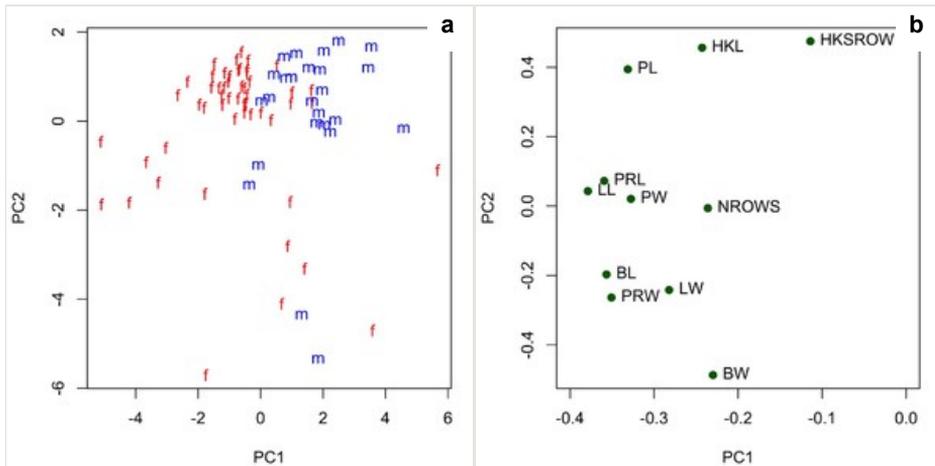


Figure 1.

Sexual dimorphism in *Echinorhynchus truttiae* revealed by principal component analysis of morphometric and meristic variables for 53 females and 25 males. The first (PC1) and second (PC2) principal components account for 36% and 24% of the variation in the data, respectively. Analysis based on data in Suppl. materials 1, 2.

a: Scatterplot of the scores for the first two principal components (PC1 and PC2). Key: f, female; m, male.

b: Scatterplot of the loadings for PC1 and PC2. Key: BL, body length; BW, body width; PL, proboscis length; PW, proboscis width; PRL, proboscis receptacle length; PRW, proboscis receptacle width; LL, lemniscus length; LW, lemniscus width; HKL, maximum hook blade length; NROWS, number of longitudinal rows of hooks; HKSROW, maximum number of hooks per longitudinal row.

Although there are interspecific differences in the means of some of the morphometric variables (e.g. maximum length of hook blade) listed in Tables 2, 3, interspecific overlap in their ranges prevents any single morphometric variable from being used to reliably discriminate any of the species in this study. For a graphical representation of interspecific variation in each conventional morphological character, see boxplots in Suppl. materials 9, 10.

Marked intraspecific, but subtle interspecific anatomic variation was observed in the male reproductive system. Four of 32 male *E. truttiae* had only one testis, which measured 793–1530 × 393–730µm. No monorchid males were found in *E. bothniensis* or *E. 'bothniensis'*. All of the *Echinorhynchus* spp. studied typically displayed six cement glands, but the number of glands was variable in *E. 'bothniensis'* and *E. truttiae*. Of eleven specimens of *E. 'bothniensis'*, nine (82%) exhibited six cement glands, but two (18%) had only five. Cement gland number was recorded from 35 male *E. truttiae*; the numbers displaying 4, 5, 6 and 8 cement glands were 1 (3%), 3 (9%), 30 (86%) and 1 (3%), respectively. Cement gland arrangements of specimens with six glands are summarized in Table 6. It is interesting to note that none of the specimens of *E. truttiae* were found to exhibit the moniliform pattern (chain-like, six singles) and that the majority (96%) had either one or two paired cement

glands. This is in contrast to the other taxa, where a large proportion of the males (21–57%) display the moniliform pattern. In *E. 'bothniensis'* pairs of cement glands consisted of the third and fourth, or fourth and fifth glands from the anterior. In *E. bothniensis* pairs were made up of any two adjacent cement glands (*i.e.* first and second, second and third, third and fourth, fourth and fifth or fifth and sixth).

Table 6.

Cement gland arrangement in males of the *Echinorhynchus bothniensis* group and *E. truttae*

Notation for cement gland pattern from Shostak et al. (1986): B, clumped, three staggered pairs; C, chainlike, two pairs and two singles; D, chainlike, one pair and four singles; E, chainlike, six singles. Only specimens with six cement glands included. Data available in Suppl. material 2.

	B	C	D	E
<i>E.bothniensis</i> (Lake Keitele)	1	4	10	4
	(5.30%)	(21.10%)	(52.60%)	(21.10%)
<i>E. 'bothniensis'</i> (Lake Pulmankijärvi)	0	0	4	5
			(44.40%)	(55.60%)
<i>E.leidyi</i> (Northern Canada, Shostak et al., 1986)	1	36	181	118
	(0.30%)	(10.70%)	(53.90%)	(35.10%)
<i>E.truttae</i> (Scotland)	1	16	13	0
	(3.30%)	(53.30%)	(43.30%)	

Proboscis profiles

Before attempting to use the [Proboscis Profiler](#) to discriminate taxa, potential confounding variables should be considered. Preparation is one such problem (Palaeartic samples fixed in alcohol, then cleared and temporarily mounted in lactophenol vs Nearctic samples fixed in FAA, stained with acetocarmine and permanently mounted in synthetic resin), but cannot be controlled in this analysis. Therefore, it is important to exercise caution when making comparisons between *E. leidyi* and the other taxa. Radial asymmetry of proboscis hooks is another potential problem (Wayland 2010). Unfortunately, the importance of radial asymmetry was not known at the time of data collection and so no record was made of which surface of the proboscis (dorsal, ventral or lateral) the measured hooks were situated. One confounding factor which can be measured and, if necessary, controlled (by profiling females and males separately) is sexual dimorphism. This phenomenon was investigated in *E. truttae*, because hook data from a complete longitudinal row are available (Suppl. materials 4, 5) for a relatively large number of both female (n=46) and male (n=26) acanthocephalans.

Fig. 2 shows hook blade length and base width variables of the 72 *E. truttae* specimens plotted against a standardized position (for definition, see morphological data analysis section of material and methods). Sexual dimorphism is not readily apparent in these two plots. Proboscis profiles were generated with a moving average segment of 11; the minimum sized moving average segment that can be applied to this dataset. Principal

component analysis of these proboscis profiles revealed subtle sexual dimorphism, with some separation of the females from males in principal component one (PC1), which describes 49% of the variation in the dataset (Fig. 3a). A Welch two sample t-test found a significant difference ($p=0.005$) between females and males in the scores for PC1. Base width variables show higher loadings than blade length variables for PC1 (Fig. 3b), suggesting that female *E. truttae* tend to have 'stouter' hooks than males. In view of this strong evidence of sexual dimorphism in proboscis profiles, the two sexes are considered separately in the inter-specific comparisons that follow.

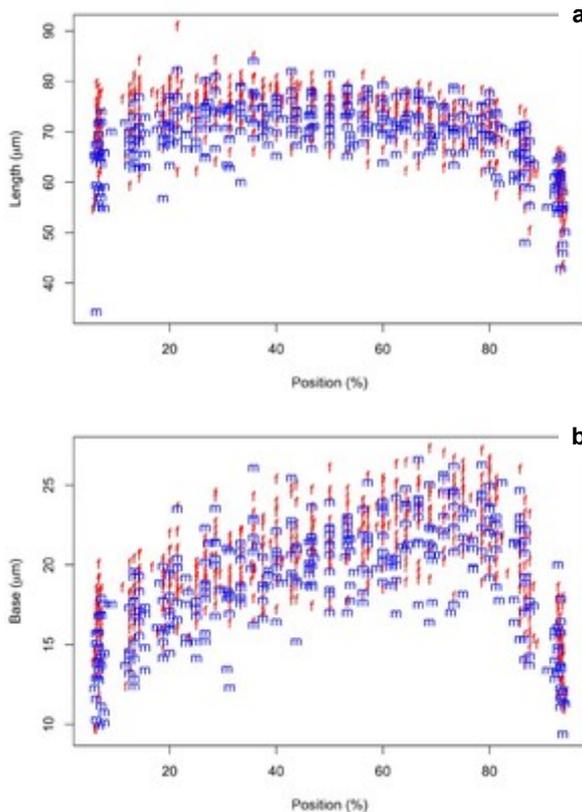


Figure 2.

Positional variation in two hook morphometrics of female and male *Echinorhynchus truttae* (number of individuals are 46 and 26 respectively). Analysis based on data in Suppl. materials 4, 5.

a: Hook blade length plotted against standardized position.

b: Hook base width plotted against standardized position.

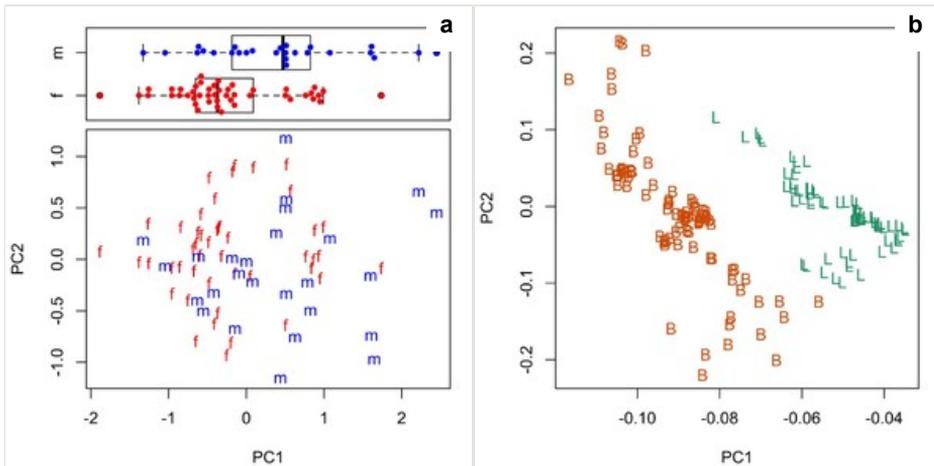


Figure 3.

Principal component analysis of the proboscis profiles of female and male *Echinorhynchus truttae*. The first (PC1) and second (PC2) principal components describe 49% and 15% of the variance in the data. Analysis based on data in Suppl. materials 4, 5.

a: Scatterplot of the scores for the first two principal components (PC1 and PC2). Inset boxplot shows distribution of scores for PC1. Key: f, female; m, male.

b: Scatterplot of the loadings for PC1 and PC2. Key: L, length variables; B, base variables.

Proboscis profiles for 56 female acanthocephalans (5 of *E. bothniensis*, 2 of *E. 'bothniensis'*, 3 of *E. leidy* and 46 of *E. truttae*) were generated using a moving average segment of 10; the minimum sized moving average segment applicable. This dataset of female hook morphometrics (Suppl. material 4) includes data from one of the paratypes of *E. bothniensis* from the Bothnian Bay. Fig. 4 shows positional variation in raw hook morphometrics of female worms; whilst some interspecific variation is apparent, the taxa are indistinguishable. A principal component analysis of the proboscis profiles was performed and a scatterplot of the scores for the first two principal components (Fig. 5a) shows a clear separation of *E. truttae* from the other taxa. The loadings plot for the first two principal components (Fig. 5b) shows that blade length and base width measurements from hooks in the 80.5–95.5% region of the proboscis are driving the separation of *E. truttae* from the other taxa along PC1 (this first principal component accounts for 64% of the variance in the dataset). *E. bothniensis*, *E. 'bothniensis'* and *E. leidy* are not separated from each other in the scores plot for PC1 and PC2. Hierarchical clustering was used to objectively partition the proboscis profiles into morphotypes; a Euclidean distance matrix was calculated from the scores for PC1 and PC2 and a dendrogram was computed using the complete agglomeration method as implemented in the `R` function `hclust` (Fig. 6). The dendrogram shows the presence of two distinct groups: one containing all profiles of *E. truttae* and the other comprising the profiles of the other taxa. The proboscis profile of one specimen of *E. leidy* clustered with the *E. truttae* profiles. The *E. truttae* cluster comprises two subclusters which are not related to geographical location.

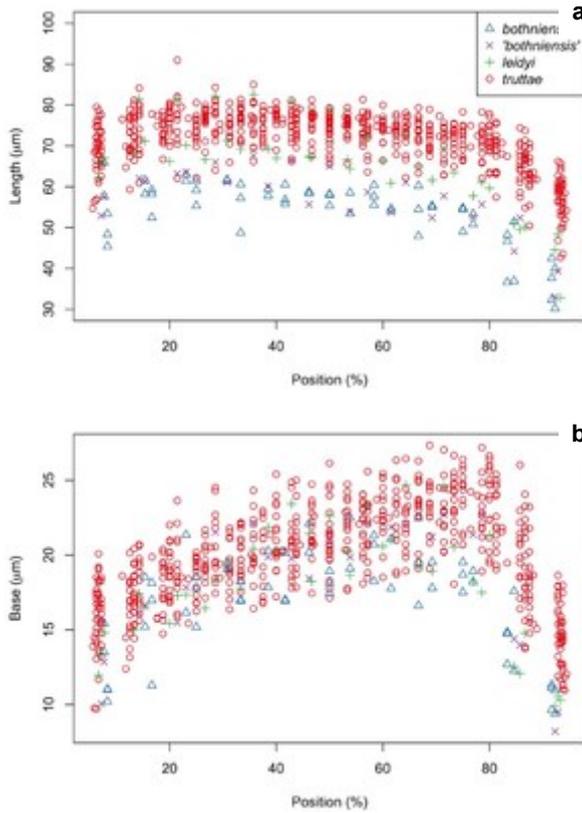


Figure 4.

Positional variation in two hook morphometrics of female *Echinorhynchus bothniensis*, *E. 'bothniensis'*, *E. leidyi* and *E. truttae* (number of individuals were 5, 2, 3 and 46, respectively). Analysis based on data in Suppl. material 4.

a: Hook blade length plotted against standardized position.

b: Hook base width plotted against standardized position.

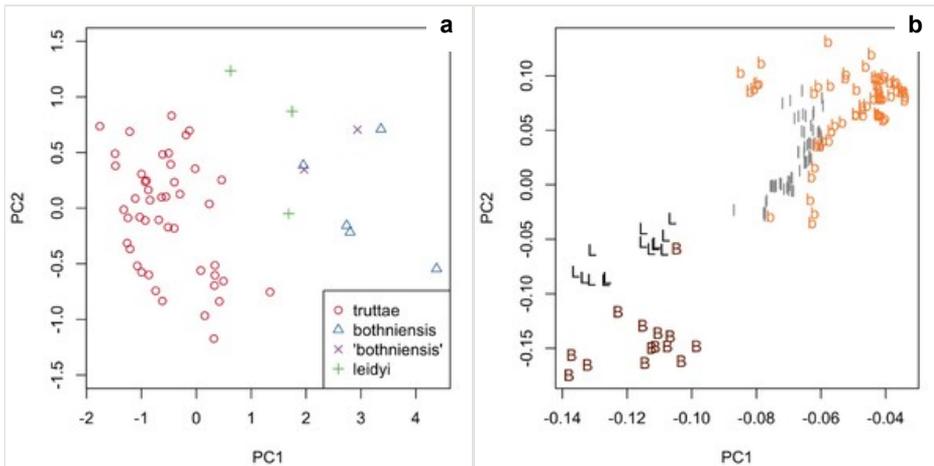


Figure 5.

Principal component analysis of the proboscis profiles of female *Echinorhynchus bothniensis*, *E. 'bothniensis'*, *E. leidy* and *E. truttae*. The first (PC1) and second (PC2) principal components describe 64% and 10% of the variance in the data, respectively. Analysis based on data in Suppl. material 4.

a: Scatterplot of the scores for the first two principal components (PC1 and PC2).

b: Scatterplot of the loadings for the first two principal components. Key: l and b, length and base measurements respectively, from hooks in the 4.5-79.5% region of the proboscis; L and B, length and base measurements respectively, from hooks in the 80.5-95.5% region of the proboscis.

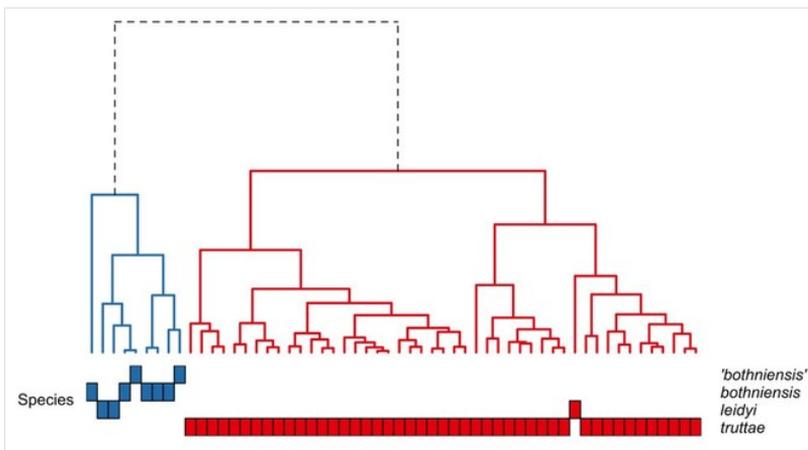


Figure 6.

Dendrogram showing the similarity between the proboscis profiles of female *Echinorhynchus bothniensis*, *E. 'bothniensis'*, *E. leidy* and *E. truttae*. A principal component analysis was applied to the proboscis profile data and the dendrogram was created from hierarchical clustering of the scores for principal components one and two. Analysis based on data in Suppl. material 4.

None of the male specimens of *E. bothniensis* had fully everted proboscides and so hook morphometric data could not be collected from them. Therefore, the analysis of interspecific variation in proboscis profiles for male worms was limited to three species: *E. bothniensis* (n=5), *E. leidy* (n=10) and *E. truttae* (n=26) (data available as Suppl. material 5). Plots of hook morphometrics against standardized position (Fig. 7) show some separation of *E. truttae* from the other taxa; this is most apparent in blade length measurements towards the base of the proboscis (Fig. 7b). Proboscis profiles were generated with a moving average segment of 11, the minimum applicable to the dataset, and then further investigated using principal components analysis. A scores plot for PC1 and PC2 (Fig. 8a) showed a clear separation of *E. truttae* from the other two taxa, and a partial separation of *E. bothniensis* from *E. leidy*. As was found for the female proboscis profiles, blade length and base width measurements from hooks at the base of the proboscis (80–95% region) are driving the separation of *E. truttae* from the other taxa (Fig. 8b). Hierarchical clustering partitioned the male proboscis profiles into three groups corresponding to the three taxa (Fig. 9). However, the proboscis profiles for one of the 10 specimens of *E. leidy* was placed in the *E. bothniensis* cluster. As in the dendrogram for female acanthocephalans, the *E. truttae* branch bifurcates into two subclusters which are not related to sampling locality.

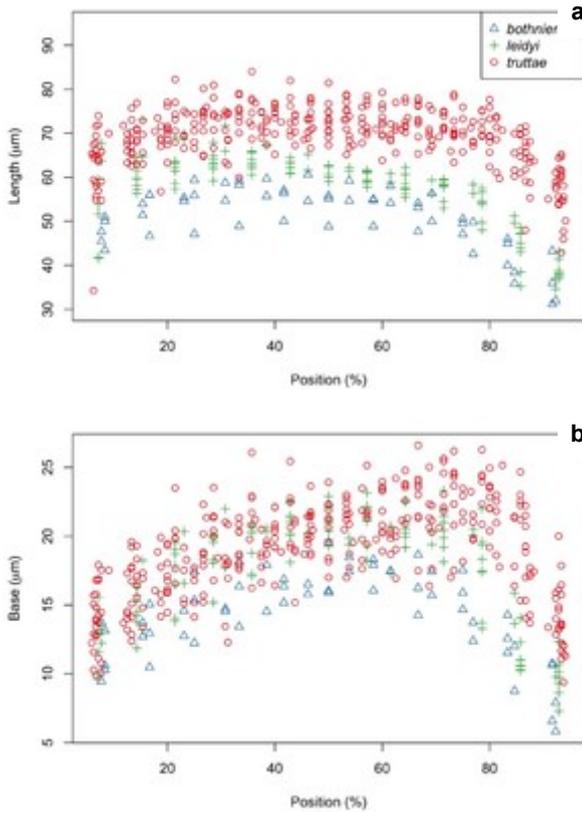


Figure 7.

Positional variation in two hook morphometrics of male *Echinorhynchus bothniensis*, *E. leidyi* and *E. truttae* (number of individuals are 5, 10 and 26 respectively). Analysis based on data in Suppl. material 5.

a: Hook blade length plotted against standardized position.

b: Hook base width plotted against standardized position.

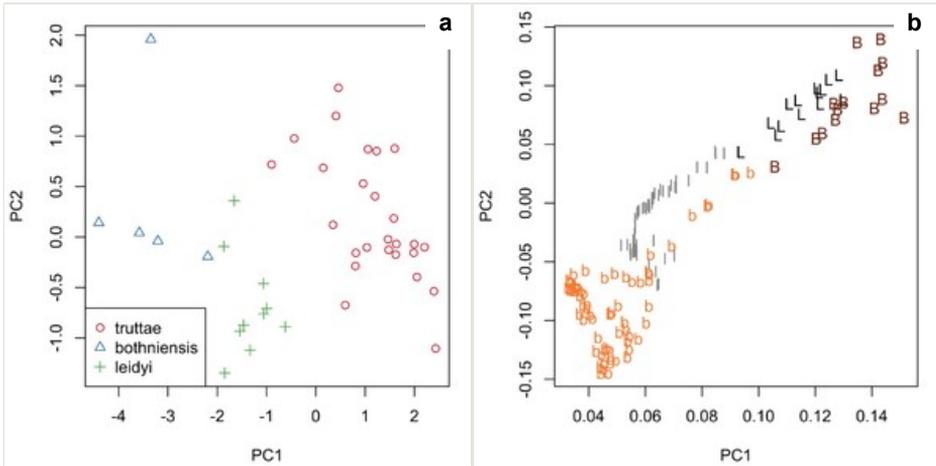


Figure 8.

Principal component analysis of the proboscis profiles of male *Echinorhynchus bothniensis*, *E. leidy* and *E. truttae*. The first (PC1) and second (PC2) principal components describe 70% and 12% of the variance in the data respectively. Analysis based on data in Suppl. material 5.

a: Scatterplot of the scores for the first two principal components (PC1 and PC2).

b: Scatterplot of the loadings for the first two principal components. Key: l and b, length and base measurements respectively, from hooks in the 5-79% region of the proboscis; L and B, length and base measurements respectively, from hooks in the 80-95% region of the proboscis.

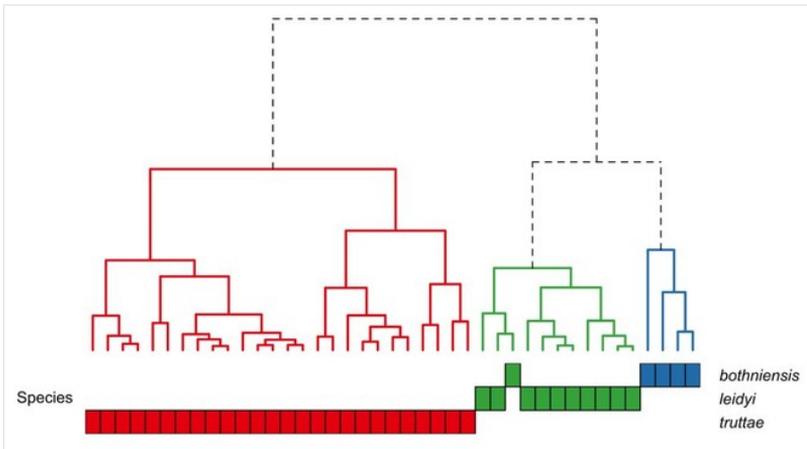


Figure 9.

Dendrogram showing the similarity between the proboscis profiles of male *Echinorhynchus bothniensis*, *E. leidy* and *E. truttae*. A principal component analysis was applied to the proboscis profile data and the dendrogram was created from hierarchical clustering of the scores for principal components one and two. Analysis based on data in Suppl. material 5.

Ecological observations

The frequency distribution of *E. truttae* in its definitive host *Salmo trutta* was recorded for two localities: Loch Walton Burn and Loch Coulter Burn (summary statistics in Table 7; raw data available in Suppl. materials 6, 7). Prevalence of infection was low in both host populations, as were the mean and maximum intensity of infection. Nevertheless, the acanthocephalans were successfully mating, as evident from the presence of gravid females in fish from both localities. The aggregation index was greater than unity in both localities, indicating that the acanthocephalans were overdispersed in their host populations. To further investigate the frequency distribution of the parasite in its host populations, two theoretical distributions were fitted to each dataset (Fig. 10); the Poisson distribution is a good model for a random distribution, while the negative binomial describes overdispersion. A chi-squared test showed that a fitted negative binomial distribution was not significantly different from the observed distribution at both localities (Loch Walton, chi-squared statistic 2.03, p-value 0.155; Loch Coulter, chi-squared statistic 1.81, p-value 0.178). Conversely, the Poisson distribution was a poor fit to the observed data (Loch Walton, chi-squared statistic 13.2, p-value 0.00135; Loch Coulter, chi-squared statistic 6.13, p-value 0.0467).

Table 7.

Frequency distribution of *Echinorhynchus truttae* in definitive host populations. 95% confidence limits (where applicable) in parentheses. Data available in Suppl. materials 6, 7.

	Loch Coulter Burn	Loch Walton Burn
Number of fish examined	42	46
Prevalence (%)	0.119 (0.048 – 0.259)	0.283 (0.171 – 0.434)
Mean intensity of infection	1.4 (1.0 – 1.6)	2 (1.46 – 2.69)
Maximum intensity of infection	2	5
Mean abundance	0.167 (0.0476 – 0.333)	0.565 (0.304 – 0.935)
Overdispersion index (variance/mean)	1.44	2.1

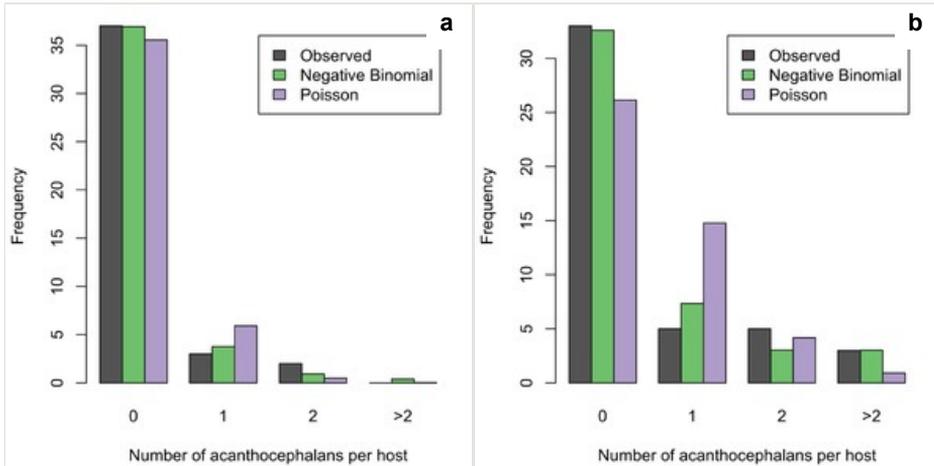


Figure 10.

Observed and fitted distributions of *Echinorhynchus truttae* in two populations of its definitive host *Salmo trutta*. Analysis based on data in Suppl. materials 6, 7.

a: Loch Coulter Burn. Negative binomial distribution has parameters: $\mu=0.167$ and $k=0.261$. Poisson distribution has parameter $\lambda=0.167$. Akaike's information criterion (AIC) for fitted distributions: negative binomial, 43.3; Poisson, 43.9.

b: Loch Walton Burn. Negative binomial distribution has parameters: $\mu=0.565$ and $k=0.375$. Poisson distribution has parameter $\lambda=0.565$. AIC for fitted distributions: negative binomial, 97.5; Poisson, 107.3.

G. pulex, the intermediate host of *E. truttae*, was abundant in both streams. One hundred specimens of this amphipod from Loch Walton Burn were examined by dissection, and while no larval *E. truttae* were found, four cystacanths of *Polymorphus minutus* (Goeze, 1782) (Polymorphida: Polymorphidae) were encountered.

Discussion

Intraspecific morphological variation

This study provides the first detailed account of morphometric and meristic variation in adult *E. truttae*, albeit for populations within a small part of its known geographical range. In the absence of evidence to the contrary, the *E. truttae* samples are assumed to comprise a single biological species. However, given the ubiquity of cryptic speciation in the Acanthocephala (Buron et al. 1986, Väinölä et al. 1994, Steinauer et al. 2007, Martínez-Aquino et al. 2009), this assumption might be unwarranted. The *E. truttae* material examined in the present study conforms well to other published descriptions (Lühe 1911, Meyer 1933, Hoffman 1954) but displays considerably greater morphological variability. The only notable difference between the descriptions provided by different authors concerns the size of the eggs. The wide range of egg dimensions recorded in the present study ($120\text{--}173 \times 22\text{--}34 \mu\text{m}$) encompasses the measurements reported by Hoffman

(1954) ($138 \times 24 \mu\text{m}$), but not the range of dimensions reported by Lühe (1911) ($100\text{--}110 \times 23\text{--}24 \mu\text{m}$) and Meyer (1933) ($100\text{--}110 \times 24 \mu\text{m}$). Discrepancies in egg dimensions between different studies are most likely the result of different fixatives and clearing agents being used to prepare the material for light microscopy, but may also be due to differences in the state of maturity of the acanthors. Shrinkage of eggs following fixation, staining and mounting has been reported by many authors (e.g. Lynch 1936, Cleave and Timmons 1952, Cable and Hopp 1954, Bullock 1962).

E. truttae exhibited sexual dimorphism in all morphometric variables common to both genders. Within each gender, a proportion of the variance in some morphometric variables was explained by body length. Seven morphometric variables (body width, proboscis length, proboscis receptacle length, lemniscus length and width, vagina width and vaginal sphincter width) were found to be positively correlated with body length in female worms, whilst just four (length of reproductive system, lemniscus length, length of both anterior and posterior testis) showed this relationship in males. However, the length range and sample size of male worms was small relative to that of females and this would have made it more difficult to find evidence of any correlation. A positive correlation with body length can be demonstrated for the size of most anatomical structures in palaeacanthocephalans (e.g. Amin and Redlin 1980, Brown 1987). Awachie (1966) found that both female and male *E. truttae* increase in length with time spent in the intestine of their definitive host, *S. trutta*, and that proboscis length increases with body size. Furthermore, body length and time spent in the definitive host intestine were also positively correlated with sexual maturation in female worms.

Proboscis profiler provided tentative evidence for the presence of two distinct morphotypes within *E. truttae* (Figs 6, 9). This variation was not related to geography, as both subgroups contained samples from both the River Carron catchment, central Scotland and Drummore, southwest Scotland. A molecular genetic analysis would be required to test the hypothesis that these two apparent morphotypes represent sibling species.

Small sample sizes prohibited a statistical analysis of intraspecific morphological variation in the other taxa studied. However, comparison of the mean values and ranges of most morphometric variables (Tables 2, 3) suggest that these taxa also display sexual dimorphism. The Bothnian Bay and Lake Keitele populations of *E. bothniensis* are thought to have been reproductively isolated for at least 6000 years (Väinölä et al. 1994); however, this study did not find any obvious morphological divergence between them.

Discrimination of species using morphological characters

The genetic differentiation of *E. bothniensis* and *E. 'bothniensis'* into distinct biological species, as evidenced from allozyme electrophoresis (Väinölä et al. 1994), was not accompanied by obvious divergence in conventional morphological characters. Furthermore, proboscis profiler failed to discriminate these species on the basis of female hook morphometrics. Proboscis profiler could not be used to compare the males of these species, as hook data were not available for male *E. 'bothniensis'*. Proboscis profiler has been used to successfully discriminate two species of the *E. gadi* species group identified

by allozyme electrophoresis (Wayland 2010). However, *E. bothniensis* and *E. 'bothniensis'* probably diverged more recently than the sibling species of the *E. gadi* group (Väinölä et al. 1994) and therefore have had less time to undergo adaptive morphological change. Moreover, if *E. bothniensis* and *E. 'bothniensis'* occur in allopatry, but utilise similar intermediate and definitive hosts, there may be little or no selection pressure to drive morphological divergence. In contrast, the sibling species of *E. gadi* separable by Proboscis profiler occur in sympatry and often in the same host individual. In this case, adaptation to different regions of the definitive host intestine to avoid competition and/or hybridization may have resulted in anatomical changes to the hooks of the proboscis (Wayland et al. 2005).

The anatomically similar *E. leidy* from the Nearctic has not been investigated using molecular markers and so its systematic homogeneity and relationship to *E. bothniensis* and *E. 'bothniensis'* may only be speculated. *E. leidy* could not be discriminated from *E. bothniensis* or *E. 'bothniensis'* using any conventional morphological character or the proboscis profiles of female worms. When applied to male worms, proboscis profiler was quite successful in separating four specimens of *E. bothniensis* from ten specimens of *E. leidy*, however a fifth specimen of *E. bothniensis* was assigned to the *E. leidy* cluster (Fig. 9). Nevertheless, this observation should be interpreted with caution as it is based on a small sample of acanthocephalans and may be an artifact of the different protocols used to prepare samples of the two taxa for light microscopy.

The inability of multivariate statistical analysis to reliably distinguish the Nearctic *E. leidy* from the Palaearctic *E. bothniensis* and *E. 'bothniensis'*, on the basis of morphological characters, is further evidence of the phylogenetic affinity of these taxa. If these acanthocephalans have co-specified with their mysid intermediate hosts, as hypothesised by Väinölä et al. (1994), they will be members of a clade comprising at least four sibling species (Audzijonytė and Väinölä 2005), some of which may occur in sympatry and at least one may have a circumarctic distribution. An extensive sampling effort combined with tandem molecular and morphological analysis was needed to differentiate and characterize the species of the *M. relict* (sensu lato) group; a similar strategy will be required to investigate the diversity in their echinorhynchid parasites.

E. truttae could not be discriminated from *E. leidy* and the *E. bothniensis* species complex on the basis of any single conventional morphological character. However, Proboscis profiler successfully separated *E. truttae* from *E. leidy*, *E. bothniensis* and *E. 'bothniensis'*. The hook morphometric data available here as supplementary files (Suppl. materials 4, 5) serve as a useful reference for *E. truttae*, *E. leidy* and the *E. bothniensis* species group, to which new samples of *Echinorhynchus* spp. from fresh and brackish waters can be compared using Proboscis profiler.

Distribution of acanthocephalans in their definitive host populations

The frequency distribution of macroparasites within their host populations almost invariably shows overdispersion or aggregation; most hosts harbour few or no parasites, and a few hosts harbour large numbers of parasites (Crofton 1971, Pennycuik 1971, Anderson and

May 1978, Anderson and Gordon 1982, Dietz 1982, Dobson 1985, Grenfell et al. 1986, Pacala and Dobson 1988, Guyatt and Bundy 1991, Shaw et al. 1998). Overdispersion is described empirically by the negative binomial distribution (Crofton 1971). In the case of natural infections of Acanthocephala, this distribution has previously been shown to provide an accurate description of the following species in their definitive host populations: *Acanthocephalus clavula* (Dujardin, 1845) in *Gasterosteus aculeatus* L. (see Pennycuik 1971) and *Anguilla anguilla* (L.) (see Shaw et al. 1998); *Acanthocephalus lucii* (Müller, 1776) in *Perca fluviatilis* (L.) (see Shaw et al. 1998); and *Echinorhynchus canyonensis* Huffman & Kliever, 1977 in *Maynea californica* Gilbert (see Huffman and Kliever 1977). In this study the negative binomial provided a good model of the distribution of *E. truttae* in two populations of its definitive host *S. trutta*. However, Hine and Kennedy (1974) found that the negative binomial was a poor fit to the frequency distribution of *Pomphorhynchus laevis* (Müller, 1776) in *Leuciscus leuciscus* (L.), even though the parasite was not randomly distributed in its host population.

The negative binomial distribution has also been used to quantify aggregation of larval acanthocephalans in populations of their intermediate hosts. Hine and Kennedy (1974) found that it was a good fit to the observed frequency distribution of *P. laevis* in a population of *Gammarus pulex* (L.). If there is parasite-induced host mortality, as in the case of natural infections of *G. pulex* by *Polymorphus minutus* (Goeze, 1782), then a truncated negative binomial model is more appropriate (Crofton 1971).

Overdispersion of parasites in their host populations may have various causes, including seasonality in the occurrence of infective stages, spatial aggregation of infective stages, and differences between hosts in behaviour, physiology and immune response to the parasites (e.g. Crofton 1971, Pacala and Dobson 1988, Shaw et al. 1998). *E. truttae* is known to display a seasonal pattern of abundance in its intermediate host, *G. pulex* (see Awachie 1966). However, seasonality should only be a cause of overdispersion in data-sets comprising samples taken throughout the year; in this study the two *E. truttae* data-sets each represented single samples.

Aggregation of cystacanths of *E. truttae* in its amphipod intermediate host *G. pulex*, is a potential cause of the acanthocephalan's overdispersion in its definitive host *S. trutta*. Since cystacanths of *P. minutus* and *P. laevis* have been found to be aggregated in populations of *G. pulex*, then it is plausible that the same phenomenon occurs in *E. truttae*. If the larvae of *E. truttae* were aggregated in their intermediate host population, then, although their fish hosts may have encountered intermediate hosts at random, the worm burden of the intermediate hosts encountered would not be random. This would lead to a heterogeneous distribution of acanthocephalans in the fish population.

It is important to note that overdispersion of acanthocephalans in their definitive hosts can occur in the absence of spatial aggregation of cystacanths. Crompton et al. (1984) found that *Moniliformis moniliformis* (Bremser, 1811) Travassos, 1915 (as *Moniliformis dubius* Meyer, 1932) had an aggregated distribution in groups of rats (*Rattus norvegicus* (Berkenhout)) in which every rat had been fed the same number of cystacanths. Valtonen and Crompton (1990) found that the prevalence and overdispersion of *E. bothniensis*

infections of *O. eperlanus* increased with host size. This observation suggests that overdispersion in this particular host-parasite system is linked to some aspect of the interaction between parasite and definitive host.

Experimental work is necessary to determine the causes of overdispersion of acanthocephalans in their host populations. *Moniliformis moniliformis* in rats serves as a convenient laboratory model for studies on acanthocephalan dispersion in mammalian host populations (Crompton et al. 1984, Stoddart et al. 1991). *E. truttae* in *S. trutta* might represent a useful model for studies of acanthocephalan dispersion in fish populations, since this species has a life cycle which can be completed in the laboratory (Awachie 1966).

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Supplementary materials

Suppl. material 1: Standard morphometric and meristic data from females.

Authors: Matthew T Wayland

Data type: morphological and meristic

Brief description: Comma separated value (csv) file of morphometric data from females. Rows are specimens and columns (column three onwards) are morphometric variables (e.g. proboscis length) or meristic variables (e.g. number of longitudinal rows of hooks). All morphometric measurements are in micrometres. The first column is species and the second column is a unique identifier for the specimen. The unique identifier is composed of two parts: the part before the full stop indicates the sample (please see table 1); the number after the full stop indicates the

specimen number. In the species column, *E. bothniensis* and *E. 'bothniensis'* are listed as bothniensis1 and bothniensis2, respectively.

Filename: female_morphometrics.csv - [Download file](#) (16.65 kb)

Suppl. material 2: Standard morphometric and meristic data from males.

Authors: Matthew T Wayland

Data type: morphological and meristic

Brief description: Comma separated value (csv) file of morphometric data from males. Rows are specimens and columns (column three onwards) are morphometric variables (e.g. proboscis length) or meristic variables (e.g. number of longitudinal rows of hooks). All morphometric measurements are in micrometres. The first column is species and the second column is a unique identifier for the specimen. The unique identifier is composed of two parts: the part before the full stop indicates the sample (please see table 1); the number after the full stop indicates the specimen number. In the species column *E. bothniensis* and *E. 'bothniensis'* are listed as bothniensis1 and bothniensis2, respectively. Notation for cement gland pattern from Shostak *et al.* (1986): B, clumped, three staggered pairs; C, chainlike, two pairs and two singles; D, chainlike, one pair and four singles; E, chainlike, six singles.

Filename: male_morphometrics.csv - [Download file](#) (12.64 kb)

Suppl. material 3: Egg and acanthor dimensions

Authors: Matthew T Wayland

Data type: morphological

Brief description: Comma separated value file with 6 columns: species, specimen, egg length, acanthor length, egg width, acanthor width. All measurements in micrometres. The unique identifier for specimen is composed of two parts: the part before the full stop indicates the sample (please see table 1); the number after the full stop indicates the specimen number. Three eggs were measured from each gravid female. In the species column *E. bothniensis* and *E. 'bothniensis'* are listed as bothniensis1 and bothniensis2, respectively.

Filename: eggs.csv - [Download file](#) (6.21 kb)

Suppl. material 4: Hook measurement data from females

Authors: Matthew T Wayland

Data type: morphological

Brief description: The file is a comma separated value (CSV) format suitable for input to the Acanthocephalan Proboscis Profiler software (<http://acanthocephala.sourceforge.net>). It includes data from one of the paratypes of *E. bothniensis* from the Bothnian Bay, Baltic Sea (specimen: b1.01).

The file has 5 columns: specimen, group, hook, length and base.

specimen - unique identifier for the specimen group - name of group (*E. bothniensis* and *E. 'bothniensis'* are listed as bothniensis1 and bothniensis2, respectively) hook - numerical position

of hook in longitudinal row as counted from the distal end of the probocis length - length of hook blade (micrometres) base - width of hook base (micrometres)

Filename: female_hook_measurements.csv - [Download file](#) (22.38 kb)

Suppl. material 5: Hook measurement data from males

Authors: Matthew T Wayland

Data type: morphological

Brief description: The file is a comma separated value (CSV) format suitable for input to the Acanthocephalan Proboscis Profiler software (<http://acanthocephala.sourceforge.net>).

The file has 5 columns: specimen, group, hook, length and base.

specimen - unique identifier for the specimen

group - name of group (following convention used in other data files, *E. bothniensis* is listed as bothniensis1)

hook - numerical position of hook in longitudinal row as counted from the distal end of the probocis

length - length of hook blade (micrometres)

base - width of hook base (micrometres)

Filename: male_hook_measurements.csv - [Download file](#) (15.41 kb)

Suppl. material 6: Frequency distribution of *Echinorhynchus truttae* in *Salmo trutta* from Loch Coulter Burn

Authors: Matthew T Wayland

Data type: ecological

Brief description: Comma-separated value (CSV) file with two columns: host fork length (mm) and number of worms. Host fish were sampled from Loch Coulter Burn (National Grid Reference NS 761 865) on 20/9/1996. Acanthocephalan voucher specimens: BM(NH) 2002.2.4.276-283.

Filename: truttae_Loch_Coulter_host_distribution.csv - [Download file](#) (278.00 bytes)

Suppl. material 7: Frequency distribution of *Echinorhynchus truttae* in *Salmo trutta* from Loch Walton Burn

Authors: Matthew T Wayland

Data type: ecological

Brief description: Comma-separated value (CSV) file with two columns: host fork length (mm) and number of worms. Host fish were sampled from Loch Walton Burn (National Grid Reference NS 668 865) on 24/6/1996. Acanthocephalan voucher specimens: BM(NH) 2002.2.4.264-275.

Filename: truttae_Loch_Walton_host_distribution.csv - [Download file](#) (278.00 bytes)

Suppl. material 8: Boxplots showing sexual dimorphism in morphometric and meristic characters in *Echinorhynchus truttae*

Authors: Matthew T Wayland

Data type: morphological

Brief description: Boxplots showing sexual dimorphism in morphometric and meristic data for *Echinorhynchus truttae*. For numbers specimens in each plot please see tables 2 and 3.

Filename: truttae_sexual_dimorphism_boxplots.pdf - [Download file](#) (8.43 kb)

Suppl. material 9: Boxplots of morphometric and meristic data from female acanthocephalans.

Authors: Matthew T Wayland

Data type: morphological

Brief description: Boxplots of morphometric and meristic data from female *E. bothniensis* (Lake Keitele), *E. 'bothniensis'* and *E. truttae*.

Filename: female_morphometric_boxplots.pdf - [Download file](#) (12.20 kb)

Suppl. material 10: Boxplots of morphometric and meristic data from male acanthocephalans.

Authors: Matthew T Wayland

Data type: morphological

Brief description: Boxplots of morphometric and meristic data from male *E. bothniensis* (Lake Keitele), *E. 'bothniensis'* and *E. truttae*.

Filename: male_morphometric_boxplots.pdf - [Download file](#) (13.01 kb)