Morphometric discrimination of two allozymically diagnosed sibling species of the *Echinorhynchus gadi* Zoega in Müller complex (Acanthocephala) in the North Sea

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Abstract

Allozyme electrophoresis was used to detect biological species of the *E. gadi* complex from gadids from the northern North Sea. A fixed difference at one of nine enzyme loci surveyed confirmed the existence of two reproductively isolated, sympatric species. Mixed infections of two *E. gadi* spp. (termed A and B) were observed in *Gadus morhua* and *Pollachius virens*. *E. gadi* sp. B was also found in *Melanogrammus aeglefinus* and *Merlangius merlangus*. The presence of gravid females of *E. gadi* spp. A and B in the same host species, *P. virens*, and sometimes in the same host individual, indicates that neither differential host-specificity nor seasonal differences in mating time are responsible for their reproductive isolation. Morphological study of probosces from electrophoretically identified specimens demonstrated that the two species can be discriminated in graphical and cluster analyses of hook morphometrics. *E. gadi* sp. I (of Väinölä et al., 1994) and *E. gadi* sp. A are probably conspecific.

Introduction

Echinorhynchus gadi Zoega in Müller, 1776 is the most commonly reported acanthocephalan from marine fish of the North Atlantic and North Pacific Oceans. At least 10 species of amphipod may serve as intermediate hosts for this parasite (Marcogliese, 1994). *E. gadi* is of significance to fisheries because it is a common parasite of a number of economically important food-fishes, including cod *Gadus morhua* L., haddock *Melanogrammus aeglefinus* (L.), whiting *Merlangius merlangus* (L.) and saithe *Pollachius virens* (L.). Although *E. gadi* is a relatively benign parasite in wild fish, it is a potential pathogen of cod in mariculture due to the increased population density under these conditions. Conversely, this parasite may be useful as a biological tag for discriminating stocks of cod off Newfoundland, Canada (Khan & Tuck, 1995) and for tracing seasonal migrations of cod in northern Norway (Hemmingsen, Lombardo & MacKenzie, 1991). *E. gadi* has also received the attention of workers interested in using parasites as biological indicators of pollution (*e.g.* Khan & Payne, 1997).

Väinölä, Valtonen & Gibson (1994) used allozyme electrophoresis to demonstrate that *E. gadi* in *G. morhua* from the Northeast Atlantic comprises three, partly sympatric, sibling species (designated spp. I–III). *E. gadi* sp. I was found in *G. morhua* from the North Sea, Norwegian Sea and Baltic Sea, and in *M. merlangus* from the North Sea. *E. gadi* spp. II and III were found in *G. morhua* from the North Sea and Norwegian Sea, respectively. An additional survey revealed that *E. gadi* spp. I and III also occur in *G. morhua* from the Gulf of Kandalaksha, White Sea (Dr R. Väinölä, personal communication). Väinölä et al. (1994) did not study their material in detail morphologically, so it was not known whether these species could be identified on the basis of morphological or meristic characters. Furthermore, it was possible that one or more of these species were in fact taxa already recognised from gadiforms from the Northeast Atlantic (i.e. *E. armoricanus* Golvan, 1969 and *E. calloti* Golvan, 1969). Although *E. gadi* spp. I–III were

all found in *G. morhua*, no evidence was available to confirm that all three species were actually reproducing in this host. Acanthocephalans are frequently recorded from hosts in which they cannot attain sexual maturity (Golvan, 1957).

The present study focused on the *E. gadi* group from *G. morhua* and other members of the Gadinae (Gadidae) from the North Sea and used allozyme electrophoresis in conjunction with morphological analyses. The principal aims of this study were: (1) to confirm that at least two of the reported species of the *E. gadi* group commonly occur in gadids from the North Sea; (2) to ascertain whether or not these species can be distinguished morphologically; and (3) to determine if these species are viable in the same species of fish host, since the use of different definitive hosts is of both ecological and evolutionary significance. In addition, the electrophoretic study was extended to include a sample of the recently described *E. brayi* Wayland, Sommerville & Gibson, 1999, a parasite of the rarely encountered deep-sea fish *Pachycara crassiceps* (Roule) (Zoarcidae) from the Porcupine Seabight, Northeast Atlantic. The inclusion of *E. brayi* allowed an estimate to be made of the genetic divergence between this deep-sea species and the shallow-water *E. gadi* group. Furthermore, the genetic interpretation of the electromorph patterns from the *E. gadi* samples was made easier by the availability of *E. brayi* electromorphs for comparison.

Table 1. Allele frequencies in *Echinorhynchus gadi* sp. A, *E. gadi* sp. B and *E. brayi.*

Locus	Allele	*gadi* sp. A	*gadi* sp. B	*brayi*
GPI	100	0.79	0.82	0.00
	95	0.00	0.00	1.00
	90	0.21	0.18	0.00
	(*n*)	(31)	(52)	(13)
LDH	100	1.00	1.00	0.00
	90	0.00	0.00	1.00
	(*n*)	(31)	(57)	(13)
MDH-1	120	0.00	0.00	1.00
	100	1.00	1.00	0.00
	(*n*)	(31)	(57)	(5)
MDH-2	120	1.00	0.00	0.00
	105	0.00	0.00	1.00
	100	0.00	1.00	0.00
	(*n*)	(31)	(57)	(5)
PEP-1	115	0.00	0.00	1.00
	100	1.00	1.00	0.00
	(*n*)	(31)	(54)	(13)
PEP-2	100	1.00	1.00	0.00
	80	0.00	0.00	1.00
	(*n*)	(31)	(54)	(13)
PEP-3	120	0.00	0.00	1.00
	100	1.00	1.00	0.00
	(*n*)	(31)	(54)	(13)
PGM-1	100	1.00	1.00	N.A.
	(*n*)	(29)	(48)	–
PGM-2	115	0.00	0.02	N.A.
	100	1.00	0.98	
	(*n*)	(29)	(48)	–

n, number of individuals studied.
N.A., not sufficient activity.

Materials and methods

Samples

The *Echinorhynchus gadi* samples were collected from gadids (*Gadus morhua*, *Pollachius virens*, *Melanogrammus aeglefinus* and *Merlangius merlangus*) trawled from the northern North Sea (57.5°–61.5°N) between the 16th and 24th August, 1997 (Figure 1). Live acanthocephalans were collected from the intestines of fish, washed and relaxed in refrigerated tap-water, then stored in liquid nitrogen until analysed. On thawing both the anterior and posterior extremities of the worms were removed and fixed in 80% alcohol for morphological study, while the remainder was used for allozyme electrophoresis. The anterior portion of the acanthocephalan included the proboscis, which yielded morphometric data, while the posterior portion of the worm (containing part of the reproductive tract) was used to identify the sex. Often worms 'shattered' into several pieces when removed from liquid nitrogen and this sometimes resulted in loss of or damage to the proboscis. Unique accession numbers were given to each worm so that the results from the electrophoretic and morphological studies on every individual could be linked. The *E. brayi* sample comprised 13 worms collected from the same specimen of *Pachycara crassiceps* (Roule) as the type-material (see Wayland, Sommerville & Gibson, 1999). This host was caught in the Porcupine Seabight (49°49.9′ N, 13°08.2′ W, depth 2,444 metres) on 13th August, 1997. The acanthocephalans were washed in saline and frozen whole in liquid nitrogen.

Electrophoretic study

Cellulose acetate gel was selected as the support medium for electrophoresis. Advantages over other gel media for protein electrophoresis (i.e. starch, poly-

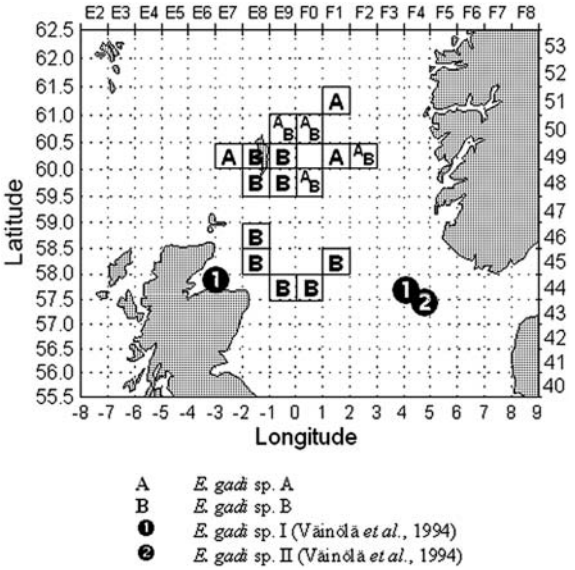

Figure 1. Recorded distribution of *Echinorhynchus gadi* spp. in the North Sea.

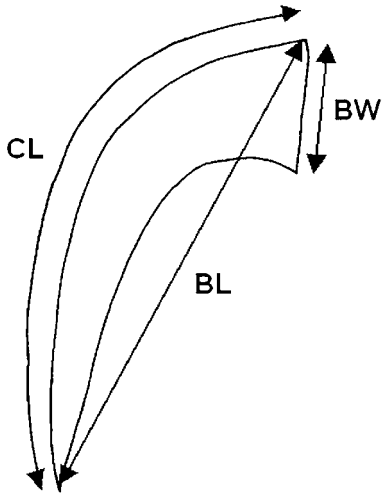

Figure 2. Profile of an acanthocephalan proboscis hook illustrating the method of measuring the characters: blade length (BL); base width (BW); and length of the curvature of the blade (CL). Hook area was defined as the cross-sectional area of a hook viewed in profile.

acrylamide & agarose) include: (i) electrophoresis can be performed on very small quantities of tissue homogenate; (ii) running times are fast, allowing isoenzymes to be screened rapidly; and (iii) less stain is required, reducing costs (Murphy, Sites, Buth & Haufler, 1990).

Individual worms were homogenised in 15–35μl grinding buffer (0.1M Tris HCl, pH 8.0, with 10mg/ml NADP and 10mg/ml sucrose). Homogenates were subjected to electrophoresis through cellulose acetate plates (Titan III, 94 $\times$ 76mm, Helena Laboratories, Beaumont, Texas) for 30 minutes at 12mA, following the methods described by Hebert & Beaton (1989). Seven enzymatic systems known to be useful for distinguishing species pairs within the *E. gadi* complex (Väinölä et al., 1994) were surveyed in this study; these were: aspartate aminotransferase EC 2.6.1.1 (AAT), glucose-6-phosphate isomerase EC 5.3.1.9 (GPI), leucine aminopeptidase EC 3.4.11.1 (LAP), lactate dehydrogenase EC 1.1.1.27 (LDH), malate dehydrogenase EC 1.1.137 (MDH), dipeptidase EC 3.4.-.-, with glycyl-L-leucine as substrate in the staining mixture (PEP-) and phosphoglucomutase EC 2.7.5.1 (PGM). In addition, peptidase EC 3.4.-.-, with L-leucyl alanine as substrate in the staining mixture (PEP-) was also tested. AAT was not detected in any gel and LAP could not be consistently resolved. Staining procedures are those described by Hebert & Beaton (1989) and Richardson, Baverstock & Adams (1986). All enzymes migrated towards the anode. Two continuous buffer systems were used routinely in the electrophoresis: Tris-citrate-EDTA, pH 7.1 (Ayala et al., 1974) for GPI, MDH, LDH and PGM; and *Electra HR* (Helena Laboratories, Beaumont, Texas) with 10% sucrose for the peptidases. The buffers Phosphate pH 7.0 and Tris-maleate pH 7.8 (Richardson, Baverstock & Adams, 1986) were also tested, but did not discriminate additional alleles at any locus and failed to provide adequate resolution of electromorphs. Stained cellulose acetate gels were fixed in 5% acetic acid, air-dried and stored for future reference.

Allelic variation was scored from nine presumptive loci: *GPI*, *LDH*, *MDH-1*, *MDH-2*, *PEP-1*, *PEP-2*, *PEP-3*, *PGM-1* and *PGM-2*. The products of *PEP-1* and *PEP-2* were detected when glycyl-L-leucine was used as substrate in the staining mixture; products of *PEP-1*, *PEP-2* and of a third locus, *PEP-3*, were observed when leucyl alanine was used as substrate. Allozyme nomenclature follows that of Shaklee, Allendorf, Morizot &Whitt (1990). Numbering of multiple loci begins with one for the isozyme closest to the anode and proceeds towards the cathode. At each locus, the most common allele in the *E. gadi* sample is denoted $*100$ and others are designated with their relative allozyme mobility (%) to this reference. Interspecific genetic divergence was measured with Nei's unbiased indices of genetic identity I (similarity measured on a scale of zero to one) and standard genetic distance D (Nei, 1987). D, which can take values be-

tween zero and infinity, is interpreted as an estimate of the average number of electrophoretically observable gene substitutions per locus (Nei, 1987). Interspecific and intraspecific differences in allele frequencies were analysed using the chi-squared test of homogeneity (see Richardson et al., 1986). All analyses of genetic data were performed using SYSTAT 7.0 (SPSS Inc., 444 N. Michigan Avenue, Chicago, IL, 60611).

Morphological study

The extremities of each worm were dehydrated in an alcohol series, then cleared and mounted in lactophenol for light microscopy. Worms were sexed according to the terminal genitalia. The presence of mature shelled acanthors (with polar elongations of the thickened shell membrane) in females was used as a criterion for sexual maturity (see Chubb, 1965). The following data were collected from the acanthocephalans: proboscis length (PL), proboscis width (PW), number of rows of hooks (R), number of hooks per row (H), egg length (EL), acanthor length (AL), egg width (EW), acanthor width (AW) and a series of hook morphometrics outlined below. One additional derived variable was proboscis shape (PL/PW). Hook measurements (Figure 2) were recorded from one longitudinal row in which all hooks were visible in profile. Data recorded from each hook were: position number, starting at one for the most anterior hook; blade length (BL); base width (BW); length of the curvature of the blade (CL); and cross-sectional area (AR). All measurements were made in micrometres using a digitising tablet (KS 100, Version 3, Carl Zeiss Vision). Due to specimen damage during processing, some morphometrics could not be recorded from every specimen.

Morphometric data from hooks were examined for interspecific differences. As a first step, hook morphometrics were plotted against position on the proboscis. Since the number of hooks per longitudinal row can vary between different probosces, position number was converted to a standardised scale using the 'percent-position' notation of Huffman & Bullock (1975). The standardised position of each hook was calculated by multiplying its counted position number by 100 and dividing the result by $n + 1$, where $n =$ number of hooks in the longitudinal row.

Cluster analysis provided an alternative, more objective approach to the study of interspecific variation in hook morphometrics. Clustering sorted the acanthocephalans into groups of morphologically similar specimens, whose membership could be compared with that of groups identified by allozyme electrophoresis. For the purposes of this analysis, hooks were partitioned into 10 groups, each group corresponding to a 10% position segment of the proboscis, to provide 10 data points for each of the four morphometrics (BL, CL, BW and AR). In cases where two hooks occurred in the same 10% segment, mean values for each morphometric were calculated. Hooks from the anterior 0–10% segment of the proboscis were excluded from the analysis, because of missing data for some specimens (hooks were damaged or missing). Before applying clustering algorithms, data were standardised to a common scale by transforming the values of each variable to *z*-scores. In all clustering procedures the normalised Euclidean metric was used as a distance measure.

Both *k* means and hierarchical clustering procedures were used. The *k* means method splits a set of specimens into a specified number of groups by maximising between cluster variation relative to within cluster variation. The hierarchical methods form groups through a process of agglomeration and the results are displayed as a dendrogram. Hierarchical analyses were performed using six different linkage (agglomeration) methods: (1) single (nearest neighbour), (2) complete (furthest neighbour), (3) centroid, (4) average, (5) median and (6) Ward (minimum variance). Initially all 36 hook morphometrics were used to generate clusters. Subsequent analyses were performed on variables which graphs had shown to be useful for discriminating species. The host of each acanthocephalan specimen was mapped onto dendrograms produced by hierarchical clustering in a preliminary analysis of host-induced morphometric variation. All analyses of morphological data were conducted using SYSTAT 7.0.

Additional material

Dr R. Väinölä, University of Helsinki, provided 12 entire, alcohol-fixed specimens (1 male and 11 gravid females) of *E. gadi* sp. I from *G. morhua* caught off Tvärminne, Hanko, northern Baltic Sea on the 21st October, 1992. This material served as a useful morphological reference for *E. gadi* sp. I, to which species detected in the present study could be compared. Although the Baltic Sea specimens were not individually identified using allozyme electrophoresis, over 60 other worms from the same sample had been electrophoretically analysed by Dr Väinölä, and all were identified as *E. gadi* sp. I. These acantho-

cephalans were processed for light microscopy and measured in the manner described above.

Results

Electrophoretic study

Table 1 gives allele frequency estimates at each locus in the species studied. The *E. gadi* sample contained two biological species ($I = 0.8844$; $D = 0.1228$) distinguished by alternative homozygous genotypes at the *MDH-2** locus. The absence of heterozygotes *MDH-2*100/85* among the 88 individuals screened at this locus is strong evidence of complete reproductive isolation. Here, the species with the *MDH-2*100/100* genotype is designated *E. gadi* sp. A and the species with the *MDH-2*85/85* genotype is designated *E. gadi* sp. B. Both species were found in *Gadus morhua* and *Pollachius virens*, sometimes in concurrent infections (see Table 2). *E. gadi* sp. B was also found in *Melanogrammus aeglefinus* and *Merlangius merlangus*. The known distribution of the two species is shown in Figure 1.

E. gadi sp. A and *E. gadi* sp. B shared alleles at all eight of the other loci studied, seven of which were monomorphic. *GPI** was the only polymorphic locus (*i.e.* the frequency of the commonest allele was <0.95). Both species exhibited very similar allele frequencies at this locus; a chi-squared homogeneity test did not detect a significant interspecific difference ($\chi^2 = 0.18$, $p = 0.67$). Allelic variation in *E. brayi* could only be scored at seven loci, because no PGM activity was detected (Table 1). *E. brayi* shared no allozymes with *E. gadi* sp. A and *E. gadi* sp. B ($I =$ zero; $D = \infty$). All seven loci were monomorphic.

Morphological study

Of the 88 electrophoretically identified specimens, only 38 (2 males and 11 females of species A; 10 males and 15 females of species B) were in a satisfactory condition for morphological analysis. Furthermore, due to inadequate clearing and/or specimen damage during processing, some morphometrics could not be recorded from every specimen.

Morphometric and meristic measurements on the probosces of *E. gadi* spp. A and B (Table 3) conform to published descriptions of *E. gadi* (e.g. Petrochenko, 1956; Shostak et al., 1986). A correlation matrix demonstrated a strong association between all hook morphometrics ($R^2 > 0.75$). Furthermore, hook size showed strong positive correlation with proboscis size. Almost 70% of the variation in maximum blade length was related to proboscis length. Sexual dimorphism, with females tending to display larger morphometric measurements than males, was observed in both species, and so data from each sex were analysed separately. *E. gadi* spp. A and B could not be distinguished on the basis of proboscis dimensions, hook counts, egg dimensions or acanthor dimensions (Table 3). However, with the exception of one specimen of *E. gadi* sp. A, the two species were clearly separated in graphs of hook morphometrics (Figures 3, 4).

The outlier of the *E. gadi* sp. A sample, an immature female worm, displayed hook morphometrics which fell into the middle of the range of variation exhibited by female *E. gadi* sp. B (see Figure 4). The proboscis of this worm was unusual in that it tapered from base to apex instead of displaying the cylindrical shape characteristic of *E. gadi* spp. and had only 17 longitudinal rows of hooks (*vs* 18–22 in conspecifics and 18–23 in *E. gadi* sp. B). These peculiarities in proboscis morphology indicate that the specimen is not representative of *E. gadi* sp. A and so measurements on this worm have been listed separately (Table 3).

Morphometric and meristic data for *E. gadi* sp. I of Väinölä et al. (1994) from the Baltic Sea are presented in Figures 3–4 and Table 3 to serve as a morphological reference for this species. In the absence of molecular data, *E. gadi* sp. A and *E. gadi* sp. I might be considered to be conspecific, due to their morphological similarity. The only conspicuous difference between the two populations was observed in the hook morphometrics BW and AR in the 50–70% region of the proboscis of female worms. The hook morphometrics BL, CL and AR effectively discriminate *E. gadi* sp. B from the *E. gadi* sp. A/sp. I morphotype. Differences between the hook morphometrics of these two groups are especially pronounced within the 10–20% segment of hook positions. Data for BL, CL and AR from this region of the proboscis are given in Table 3. The hook measurement BW failed to separate *E. gadi* sp. B from the *E. gadi* sp. A/sp. I morphotype at any position on the proboscis.

Cluster analyses identified two morphologically distinct forms, one corresponding to *E. gadi* spp. A and I and the other to *E. gadi* sp. B. However, in all analyses the *E. gadi* sp. A outlier was grouped among specimens of *E. gadi* sp. B. The cluster analyses were performed on nine males (two *E. gadi* sp. A, six *E. gadi* sp. B and one *E. gadi* sp. I) and 20 females (five *E. gadi* sp. A, nine *E. gadi* sp. B and six *E. gadi* sp.

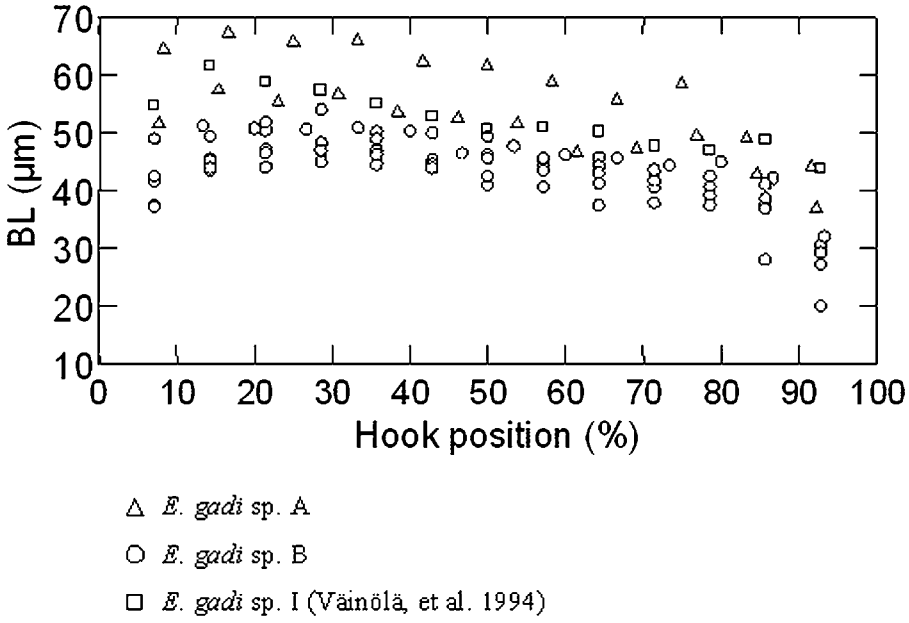

Figure 3. Positional variation in hook blade length (BL) of male *Echinorhynchus gadi* spp. A, B and I (number of individuals studied are 2, 6 and 1, respectively).

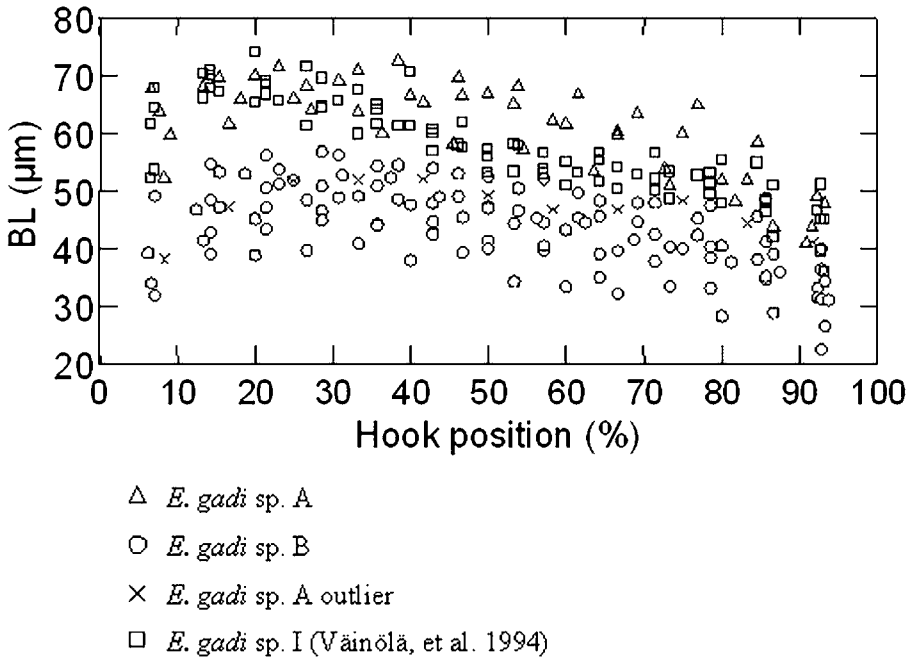

Figure 4. Positional variation in hook blade length (BL) of female *Echinorhynchus gadi* spp. A, B and I (number of individuals studied are 5, 9 and 6, respectively).

Table 2. Host and locality records for *Echinorhynchus gadi* sp. A and *E. gadi* sp. B from the northern North Sea.

Parasite	Host	ICES stat. square(s)
E. gadi A	*Gadus morhua*†	49F1; 49F2; 50E9; 51F1
	Pollachius virens†	48F0; 49E7; 50F0
E. gadi B	*Gadus morhua*	45F1; 48E9; 48F0; 50E9
	Pollachius virens†	48F0; 49E9; 49F2; 50F0
	Melanogrammus aeglefinus†	44E9; 44F0; 45E8; 46E8
	Merlangius merlangus	48E8; 49E8

†Hosts in which gravid female acanthocephalans were found.

I) using the full set of 36 morphometrics and single morphometrics (BL, CL and AR) from the 10–20% region of the proboscis. The latter three variables were selected because they clearly discriminated *E. gadi* spp. A and I from *E. gadi* sp. B in plots of hook morphometrics (see above).

The same clusters were obtained from the *k* means method whether the full data-set or single morphometrics (BL, CL and AR from the 10–20% region of the proboscis) were used. When the males were divided into two groups by *k* means clustering, all specimens of *E. gadi* spp. A and I were placed in one group and every specimen of *E. gadi* sp. B was placed in the other group. The same analysis partitioned the female acanthocephalans into one group, comprising every specimen of *E. gadi* sp. B plus the *E. gadi* sp. A outlier and a second group consisting of every specimen of *E. gadi* sp. I plus the remainder of the *E. gadi* sp. A sample. Clustering of males or females into three groups by the *k* means method did not separate *E. gadi* sp. A from *E. gadi* sp. I as might have been expected, since the two samples represent different populations if not different species, but instead resulted in subdivision of the *E. gadi* sp. B group.

Hierarchical cluster analysis based on the full set of 36 hook morphometrics, using complete, average or Ward linkage methods, separated *E. gadi* spp. A and I from *E. gadi* sp. B at the two cluster level (Figures 5, 6). If hierarchical cluster analyses were based on BL, CL or AR from the 10–20% region of the proboscis, *E. gadi* sp. A and I were discriminated from *E. gadi* sp. B at the two cluster level using any of the linkage methods available (i.e. single, complete, centroid, average, median and Ward). None of the analyses resulted in the segregation of *E. gadi* spp. A and I or grouped the *E. gadi* sp. A outlier with conspecifics. Mapping of hosts onto dendrograms (Figures 5, 6) provided

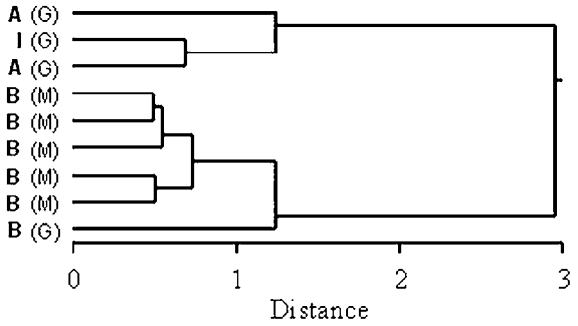

Figure 5. Dendrogram obtained from a complete (furthest neighbour) linkage, hierarchical cluster analysis on 36 hook morphometrics of male *Echinorhynchus gadi* spp. A, B and I (see text for details of data and methods used). The host of each acanthocephalan specimen is shown in parentheses (G, *Gadus morhua*; M, *Melanogrammus aeglefinus*).

little evidence of morphological differences between conspecific acanthocephalans inhabiting different host species. Although the five male specimens of *E. gadi* sp. B from *M. aeglefinus* clustered to the exclusion of the specimen from *G. morhua* (Figure 5), no such pattern was observed among the females of this species (Figure 6).

The presence of gravid females, containing mature shelled acanthors, of *E. gadi* sp. A in *G. morhua* and *P. virens* (Table 2) confirmed that the acanthocephalan can reproduce in these hosts. *E. gadi* sp. B was found to be viable in *P. virens* and *M. aeglefinus*. Gravid females of *E. gadi* sp. A and B were found in the same host individual of *P. virens*. All eleven females of *E. gadi* sp. I from the Baltic Sea contained mature shelled acanthors.

Table 3. Morphometrics (in micrometres as the means ± standard deviation; with the range and sample size in parentheses) of *Echinorhynchus gadi* spp. A, B and I.

	Males			Females			
	E. gadi sp. A	*E. gadi* sp. B	*E. gadi* sp. I	*E. gadi* sp. A	*E. gadi* sp. A variant form	*E. gadi* sp. B	*E. gadi* sp. I
PL	712 ± 6.5	636 ± 85.1	742	751 ± 81.9	588	688 ± 81.4	718 ± 36.6
	(707–717; 2)	(520–749; 10)	(742; 1)	(603–864; 10)	(588; 1)	(582–853; 15)	(637–760; 11)
PW	281 ± 14.5	233 ± 29.0	226	301 ± 27.1	278	267 ± 38.0	276 ± 20.4
	(271–291; 2)	(186–288; 10)	(226; 1)	(266–350; 10)	(278; 1)	(187–331; 15)	(229–298; 11)
PL/PW	2.54 ± 0.108	2.74 ± 0.29	3.28	2.50 ± 0.302	2.12	2.60 ± 0.306	2.62 ± 0.286
	(2.46–2.61; 2)	(2.25–3.07; 10)	(3.28; 1)	(2.00–3.00; 10)	(2.12; 1)	(2.14–3.12; 15)	(2.23–3.26; 11)
R	19.5 ± 0.71	18.8 ± 0.79	18	19.5 ± 1.27	17	19.9 ± 1.25	20.4 ± 1.50
	(19–20; 2)	(18–20; 10)	(18; 1)	(18–22; 10)	(17; 1)	(18–23; 15)	(18–22; 11)
H	12	13.1 ± 0.74	13	12.7 ± 1.34	12	12.9 ± 1.03	13.2 ± 0.75
	(12; 2)	(12–14; 10)	(13; 1)	(11–15; 10)	(12; 1)	(11–15; 15)	(12–14; 11)
BL (10–20%)	62 ± 7.0	46 ± 3.1	62	67 ± 3.7	47	46 ± 5.8	69 ± 2.5
	(57–67; 2)	(43–51; 6)	(62; 1)	(62–70; 4)	(47; 1)	(39–55; 9)	(66–72; 6)
CL (10–20%)	71 ± 5.5	53 ± 4.3	70	79 ± 2.3	55	53 ± 6.4	81 ± 2.5
	(67–75; 2)	(50–60; 6)	(70; 1)	(77–82; 4)	(55; 1)	(46–63; 9)	(79–85; 6)
AR (10–20%)	513 ± 52	327 ± 50	542	657 ± 56	344	342 ± 79	686 ± 64
	(476–549; 2)	(267–385; 6)	(542; 1)	(592–727; 4)	(344; 1)	(252–449; 9)	(593–766)
EL	N/A	N/A	N/A	98 ± 6.6	N/A	98 ± 9.8	96 ± 7.1
				(87–118; 60)		(80–118; 22)	(84–113; 33)
AL	N/A	N/A	N/A	53 ± 3.9	52 ± 3.6	N/A	59 ± 3.3
				(46–64; 60)	(45–58; 22)		(50–64; 33)

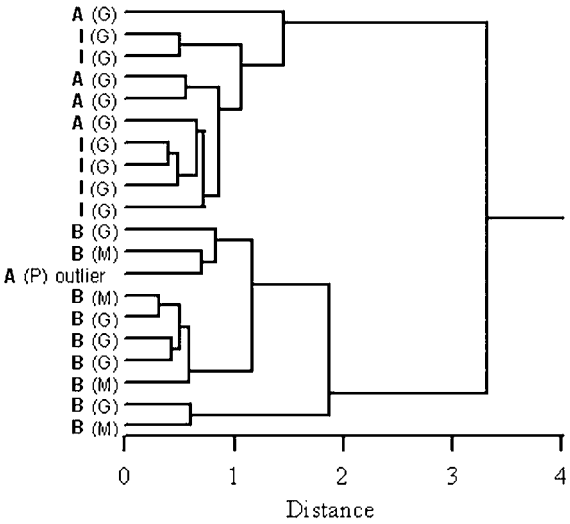

Figure 6. Dendrogram obtained from a complete (furthest neighbour) linkage, hierarchical cluster analysis on 36 hook morphometrics of female *Echinorhynchus gadi* spp. A, B and I (see text for details of data and methods used). The host of each acanthocephalan specimen is shown in parentheses (G, *Gadus morhua*; M, *Melanogrammus aeglefinus*; P, *Pollachius virens*). Exclusion of the *E. gadi* sp. A outlier from the analysis does not alter the topology of the tree.

Discussion

Systematics

The two species of the *Echinorhynchus gadi* complex detected in this survey are likely equivalent to two of the three species found in northeast Atlantic cod by Väinölä et al. (1994). In particular, the hooks of *E. gadi* sp. A are morphologically indistinguishable from those of *E. gadi* sp. I from the Baltic Sea, suggesting that the two are in all probability conspecific. Subtle differences in hook morphology between these samples might be attributable to intraspecific geographical variation, since the Baltic and North Sea populations of *E. gadi* sp. I are reported to be quite genetically divergent (Väinölä et al., 1994). However, since examples of *E. gadi* spp. I-III were not analysed in the present electrophoretic study, it is impossible to say with absolute certainty which, if any, of these species correspond to *E. gadi* sp. A or B.

The preliminary morphological analysis demonstrates that *E. gadi* sp. A and B can be distinguished on the basis of hook morphometrics. However, more

data on intraspecific variation are needed to identify the best morphometric or combination of morphometric parameters for discriminating the two species. The principal difference between the two species is size. The existence of morphological variants (outliers) means that the unequivocal identification of acanthocephalans using hook measurements alone is not possible. Nevertheless, such outliers are probably relatively rare (only one was detected in the sample of 38 specimens studied electrophoretically and morphologically), and so they should not severely compromise a morphological approach to species identification.

The average value of I for interspecific comparisons between *E. gadi* spp. I–III was 0.49 (Väinölä et al., 1994), whereas *E. gadi* sp. A and B appeared much closer with an I value of 0.88. Väinölä et al. (1994) also documented greater intraspecific gene diversity within their *E. gadi* spp. than has been observed in either *E. gadi* sp. A or B. The proportion of polymorphic loci in *E. gadi* spp. I, II and III was 38% (6 of 16), 42% (5 of 12) and 14% (2 of 14), respectively. In *E. gadi* A and B just one (11%) of the nine loci surveyed was found to be polymorphic. These observations suggest that if *E. gadi* sp. A and B do correspond to *E. gadi* spp. identified by Väinölä *et al.* (1994), then the electrophoretic procedures employed in the present study have failed to uncover the full extent of allelic variation at many of the loci examined. This is not entirely surprising, since the support medium used for electrophoresis in this study, cellulose acetate gel, is not as effective at discriminating allozymes as the starch gel used by Väinölä et al. (1994). Cellulose acetate gel electrophoresis (CAGE) separates proteins on the basis of net charge alone, whereas starch gel electrophoresis (SGE) can also separate proteins on the basis of size (shape), because it has smaller pores (Murphy, Sites, Buth & Haufler, 1990). Furthermore, as only small numbers of parasites were available for use in the development of techniques for this study, it is likely that electrophoretic conditions were not optimised for isoenzyme separation. In contrast, Brattey (1995) found that CAGE was equally as effective as SGE at separating allozymes of ascaridoid nematodes.

The genetic divergence of the deep-sea *E. brayi* from the shallow-water *E. gadi* spp. (I = zero) is greater than the maximum divergence reported between species pairs in the majority of animal genera ($I \sim 0.2$–0.3; Thorpe, 1983), but is not exceptional for congeneric helminths (Nadler, 1990). Väinölä et al. (1994) observed a similarly strong differentiation between the *E. gadi* species group and *E. salmonis* Müller, 1784, leading them to the conclusion that *Echinorhynchus* (*sensu* Meyer, 1933; Amin, 1985) represents 'an evolutionary unit deeper and wider than genera in most other animal groups'.

Reproductive isolation of E. gadi *spp. A and B*

Many examples of congeneric acanthocephalans co-occurring in the same definitive host can be found in the literature (e.g. Zdzitowiecki, 1984; Valtonen & Crompton, 1990; Aho, Mulvey, Jacobson & Esch, 1992), yet mechanisms responsible for the reproductive isolation of these species have not been established. Reproductive isolating barriers are classified as prezygotic or postzygotic (Dobzhansky, 1940). Postzygotic barriers are not selectable directly and include: F1 inviability, F1 sterility and hybrid breakdown. Prezygotic barriers, such as habitat isolation, may evolve to prevent gamete wastage and lowered fertility due to hybridisation. The operation of postzygotic barriers cannot be assessed using field data, but the existence of certain prezygotic barriers can be examined.

The presence of gravid females (containing mature shelled acanthors) of both *E. gadi* spp. A and B in the same host species, *P. virens*, and sometimes in the same host individual, shows that differential host-specificity is not responsible for their reproductive isolation. Temporal isolation, through seasonal differences in mating time, can probably also be discounted, because shelled acanthors have been found simultaneously in females of both species. Copulation in acanthocephalans is probably initiated by males (Parshad & Crompton, 1981; Crompton, 1985), but mechanisms that would enable male acanthocephalans to distinguish between conspecific and heterospecific females have yet to be identified, if they exist at all. Marked interspecific differences in the size and shape of copulatory organs apparently provide a mechanical obstacle to heterospecific matings in some monogenean genera (Rohde & Hobbs, 1986). However, no such barrier has been observed among the Acanthocephala. Moreover, the copulatory organs of *Echinorhynchus* spp. appear remarkably homogenous across the entire genus (M. Wayland, unpublished). Perhaps heterospecific matings do take place, but eggs are not fertilised, because of gamete incompatibility.

It is unfortunate that no data were collected on the distribution pattern of *E. gadi* spp. A and B in the host intestine, since this might have revealed microhabi-

tat segregation. Valtonen & Crompton (1990) reported the spatial separation of *E. bothniensis* Zdzitowiecki & Valtonen, 1987 and *E. salmonis* in concurrent infections of *Osmerus eperlanus* (L.) from the Bothnian Bay, Baltic Sea. *E. bothniensis* was found predominantly in the anterior third of the intestine, while *E. salmonis* occupied the posterior third of the intestine. A similar example is provided by Zdzitowiecki (1984) for the distribution of the closely related *Corynosoma hamanni* (Linstow, 1892) and *C. pseudohammanni* Zdzitowiecki, 1984 in the digestive tract of Antarctic seals. *C. hammani* inhabits the pyloric part of the stomach, the duodenum and the anterior region of the jejunum, while *C. pseudohamanni* occupies the median and posterior regions of the small intestine. An acanthocephalan species distribution within its host intestine can be influenced by the presence of congeneric species. Guillen-Hernandez & Whitfield (2001) investigated the gut microhabitat usage of two strains of *Pomphorhynchus laevis* (Müller, 1776) in their host, the flounder *Platichthys flesus*. They found that in single strain infections in individual fish, freshwater and marine/estuarine worms had distinct, but overlapping distributions. However, in mixed strain infections, niche contraction resulted in segregated distributions. It is conceivable that the observed differences in hook size between *E. gadi* sp. A and B might represent adaptations to different regions of the gadid intestine. If this differentiation was in response to competition or the risk of hybridisation, then it represents an example of character displacement (Brown & Wilson, 1956). The interaction of syntopic species is an obvious topic for future ecological research on the *E. gadi* complex.

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