



## Taxonomic resolution of the genus *Bythotrephes* Leydig using molecular markers and re-evaluation of its global distribution

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**Abstract.** *Bythotrephes* Leydig is a predatory, onychopod cladoceran native to Eurasia that typically inhabits oligo- and mesotrophic water bodies of the Palaearctic region. It recently invaded 70 North American lakes, prompting a re-evaluation of the taxonomic status, global distribution, and determinants of local occurrence and abundance. European studies have reported two distinct species, *B. longimanus*, which lacks a kink on the caudal process, and *B. cederstroemi*, which possesses one. We employed sequencing of the mitochondrial gene cytochrome c oxidase subunit I (COI) in addition to Directed Amplification of Minisatellite-region DNA (DAMD) using polymerase chain reactions (PCRs) to assay the genetic nature of these 'species'. These analyses revealed that the two taxa are consistent with a single, common species, *Bythotrephes longimanus* Leydig 1860, thus resolving the nomenclatural issue. Furthermore, a common haplotype was identified between Lake Ontario and Lake Puruvesi, Finland, suggesting a potential invasion corridor via the nearby Baltic Sea. Statistical

analysis revealed that the two forms also occur in similar habitats in Eurasia and North America. Habitat characteristics of water bodies in Northern Europe, where both forms are found and occasionally co-occur, do not differ statistically. Similarly, no significant differences were detected between characteristics of Eurasian habitats that support *longimanus* 'forms' and those of inland lakes in North America that support *cederstroemi* 'forms'. Human activities have had a strong effect on the distribution of *Bythotrephes* in both Europe and North America. Global and local distributions have been affected by ballast water transfer and by boating and fishing activities, respectively. Cultural eutrophication, oligotrophication and acidification alter the suitability of habitats for fish predators, and may indirectly influence *Bythotrephes*' local occurrence and abundance.

**Key words.** Biological invasions, distribution, DAMD, COI, DNA markers, non indigenous species (NIS), fish predation, acidification, eutrophication.

### INTRODUCTION

The spiny water flea *Bythotrephes* Leydig is an onychopod zooplankton whose native range encompasses the Palaearctic region (Ischreyt, 1930;

Flössner, 1972; Grigorovich *et al.*, 1998; Rivier & Grigorovich, 1999). *Bythotrephes* typically inhabits deep, oligotrophic lakes. It has been described as a 'glacial relict' by some but not all researchers (Ischreyt, 1930; Manuilova, 1964; Flössner, 1972).

*Bythotrephes* had a much broader geographical range during glacial and postglacial times, although subsequent climatic changes have restricted its natural distribution (Rylov, 1921; Manuilova, 1964). Human-mediated activities in recent times have largely supplanted natural changes in distribution. For example, Grigorovich *et al.* (1998) and Rivier & Grigorovich (1999) emphasized the importance of human-mediated dispersal in shaping the current range of this genus in the Commonwealth of Independent States (CIS; former USSR). Recent work in North America also has implicated human dispersal vectors to inland lakes from the Great Lakes (Borbely, 2001).

*Bythotrephes* is an important component of many aquatic food webs. It preferentially feeds on small-bodied zooplankters. Also, it feeds on phytoplankton including diatoms, green algae and peridinians (Flössner, 2000). *Bythotrephes* predation can prevent mid-summer increases of some herbivorous, crustacean zooplankton (Patalas, 1954; Bosselmann, 1974; Bürgi & Egli, 1984; Ketelaars & van Breemen, 1993; Yan & Pawson, 1997; Hoffman *et al.*, 2001). In turn, fish predation may affect *Bythotrephes*' abundance and reproductive phenology, particularly in lakes where populations exhibit vertical partitioning (e.g. Plankensteiner, 1992; Yan *et al.*, 2001). In shallow lakes lacking fish, *Bythotrephes* can reach maximum densities of 125 individuals m<sup>-3</sup> (Vekhov, 1987), although densities are typically much lower in systems with fish predators (Svärdson, 1976; Vekhov, 1982). Also, fish predation may influence and limit morphological variation within populations of *Bythotrephes*. For example, variation in the abundance of different forms of *B. longimanus* in Russian waterbodies has been attributed to the presence of fish that selectively feed on *Bythotrephes*, sometimes eliminating it from zooplankton communities (Vekhov, 1987).

*Bythotrephes* was first observed in North America in Lake Ontario in 1982 (Johannsson *et al.*, 1991). This invasion is likely to have resulted from ballast water discharge by a transoceanic ship, the origin of which was likely to be the Baltic Sea near the port of St Petersburg, Russia (Sprules *et al.*, 1990; Berg *et al.*, 2002). It has since spread to all of the other Great Lakes and to at least 64 inland water bodies in Ontario, Michigan, Ohio and Minnesota (Sprules *et al.*, 1990; Johannsson *et al.*, 1991; Yan *et al.*, 1992; MacIsaac *et al.*, 2000;

Borbely, 2001; Appendix 1). *Bythotrephes* also has an extensive invasion history in western and eastern Europe (Ketelaars & van Breemen, 1993; Ketelaars & Gille, 1994; Grigorovich *et al.*, 1998).

Relatively few studies have used abiotic or biotic factors to assess large-scale distributional patterns for *Bythotrephes*. Grigorovich *et al.* (1998) and Rivier & Grigorovich (1999) reported factors that influenced *Bythotrephes* in the CIS, while MacIsaac *et al.* (2000) utilized physical, chemical and biological parameters of European habitats that either supported or lacked *Bythotrephes* to forecast its distribution in Ontario. However, it remains unclear whether dispersal limitation or regional and local factors, including limnological variables or fish predation, is more important in determining its distribution at multiple spatial scales.

One problem in the determination of the global distribution of *Bythotrephes* is uncertainty regarding the number of species contained within the genus. Researchers in Europe have reported at least two morphologically distinct species, *B. longimanus* and *B. cederstroemi* (Mordukhai-Boltovskoi & Rivier, 1987; Grigorovich *et al.*, 1998). The taxonomic status of these 'forms' is confounded by variability in anatomical structures and body dimensions (Mordukhai-Boltovskaya, 1959; Zozulya & Mordukhai-Boltovskoi, 1975, 1977; Zozulya, 1977; Mordukhai-Boltovskoi & Rivier, 1987; Evans, 1988; reviewed in Grigorovich *et al.*, 1998). Berg & Garton (1994) and Berg *et al.* (2002) noted that allozyme variation among sympatric populations of *B. longimanus* and *B. cederstroemi* was often less pronounced than that among allopatric populations of either species, suggesting the existence of only a single genetic species, *B. longimanus*. Also, Weider (1991) found low genetic variability among Great Lakes' populations of *B. cederstroemi* using allozyme analyses. However, allozyme studies are based on protein sequences, and owing to multiple coding patterns, species-based variation in DNA sequences might not be detected. This limitation can be overcome through use of Directed Amplification of Minisatellite-region DNA (DAMD) analysis. This approach uses a variable number of tandem repeats (VNTRs) as primers to amplify fragments of genomic DNA that show little variation within a species but considerable variation between species (e.g. Heath *et al.*, 1993). Recently, Cristescu *et al.* (2001) used mitochondrial gene

sequencing to determine divergence between the confamilial genera *Bythotrephes* and *Cercopagis*. This approach also is suitable for quantifying the divergence, if any, between *longimanus* and *cederstroemi* 'forms'.

Records of *Bythotrephes* are scattered and incomplete for regions of continental Asia and Europe and the British Isles. In this study, we report an updated distribution of *Bythotrephes* and provide possible mechanisms to explain observed distributional patterns. We also assess relationships among abiotic variables and distributions of *longimanus* and *cederstroemi* 'forms' in regions of Europe where they co-occur, and between European and North American populations. Furthermore, we explore the potential influences of biotic variables on the distribution of *Bythotrephes*. In order to determine whether morphological variation within the genus is genetically based, we employ both mitochondrial gene sequencing and DAMD methodology to assess populations of *longimanus* and *cederstroemi*.

## METHODS AND MATERIALS

### Geographical and physicochemical database

A database was created from an extensive literature search for reported occurrences of *Bythotrephes* in Eurasia. Whenever possible, information was obtained for lake name, country of location, lake area and volume. A second search was conducted using lake name and country of location to collect basic physicochemical data including temperature, dissolved oxygen and nutrient (N, P) concentrations, and lake trophic status if these data were not available in the literature that reported *Bythotrephes* occurrence. Lake name, location and citation data are provided in Appendix 1.

In order to test whether *longimanus* and *cederstroemi* 'forms' inhabit different types of waterbodies, we tested abiotic conditions of lakes, classified by the presence of *Bythotrephes*, using nonparametric tests including Kruskal–Wallis ANOVA and Median test. This method was employed to ensure that outlier values inherent in a data set collected from multiple sources and times did not unduly influence statistical outcomes. Nonparametric tests are less sensitive to the impact of single outlier values than are comparable parametric tests (Zar, 1984). Lakes reported to contain

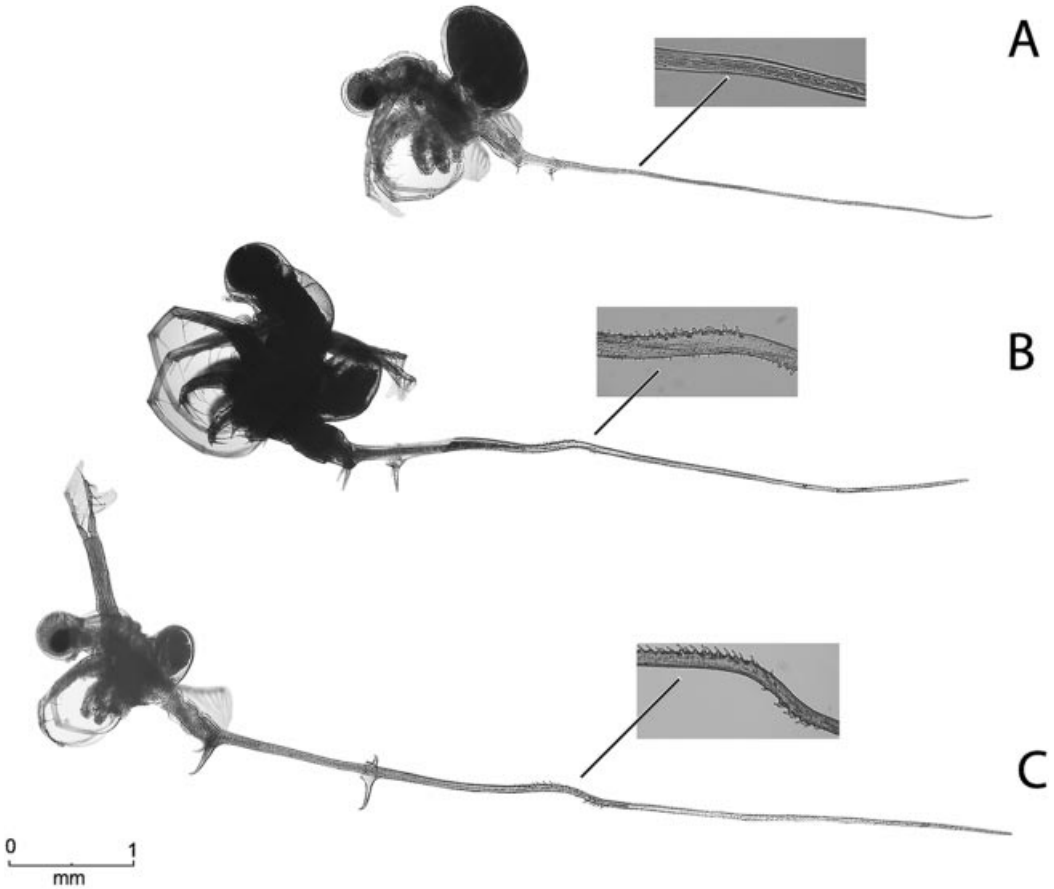
different *Bythotrephes* subspecies were classified at the species level.

### Collection, identification and morphology of *Bythotrephes*

*Bythotrephes* were collected within its native range from Lake Puruvesi, Finland, by vertical hauls of a 450- $\mu$ m plankton net on 2 August 2000 and 2 August 2001. Additional collections were made from locations outside *Bythotrephes*' native range, including the lower Volga River, Russia (27 July 2000), Lake Tyson, Canada (25 July 2000), and the St Clair River, Canada (September 1998). Specimens used for genetic sequencing were collected from Finland (Lake Puruvesi), Canada (Lakes Ontario and Simcoe), the Netherlands (Petrusplaat, near Dordrecht), Germany (Selenter See and Post See), and Russia (Volgograd Reservoir). Collections were made using standard plankton nets (253–450  $\mu$ m mesh) and preserved in 95% ethanol to preserve DNA for genetic analyses. Individual *Bythotrephes* were separated from other plankton using light microscopy.

Measurements of several historically employed structures were made for taxonomic identifications (e.g. caudal process length, distance between articular spines II and III, length of articular spine III, ratio of the distance between articular spines II and III to abdominal length, and ratio of articular spine III length to the diameter of the caudal process) following Grigorovich *et al.* (2000).

Traditional taxonomic classification of *Bythotrephes* depends on a series of instar- and sex-specific characteristics (Manuilova, 1964; Zozulya & Mordukhai-Boltovskoi, 1975; Mordukhai-Boltovskoi & Rivier, 1987). Because instar III parthenogenetic females dominated all samples, we limited our analyses to these individuals. Morphology of the caudal process is the most prominent distinguishing factor in the genus (Martin & Cash-Clark, 1995). However, the taxonomic utility of this structure is limited due to extensive ontogenetic, seasonal and geographical variability (Mordukhai-Boltovskaya, 1959; Zozulya, 1977; Mordukhai-Boltovskoi & Rivier, 1987; Yurista, 1992; Burkhardt, 1994; Ketelaars *et al.*, 1995). We used the ratio of the distance between articular spines II and III to abdominal length, and the ratio of the length of articular spine III



**Fig. 1** Instar II parthenogenetic females of (A) *Bythotrephes longimanus* from Lake Puruvesi, Finland, (B) *Bythotrephes cederstroemi connectens* from the Volga River, Russia, and (C) *Bythotrephes cederstroemi* from Lake Puruvesi, Finland. Within this genus, local 'varieties' have been described (see Rivier, 1998), providing further evidence of polymorphism within *Bythotrephes*.

to the diameter of the caudal process, to further characterize populations. Morphological differences between *longimanus* and *cederstroemi* 'forms' were assessed using either ANOVA or ANCOVA.

The ratio of the length of thoracopod I to total body length, also used previously in assessments of *Bythotrephes* taxonomy (Manuilova, 1964), was variable for instar III parthenogenetic females within each population and therefore did not permit discrimination between 'forms' ( $F_{1,14} = 0.01$ ,  $P = 0.923$ ). An additional index, consisting of the ratio of the caudal process length to total body length, also was incapable of discriminating between 'forms' ( $F_{1,14} = 0.26$ ,  $P = 0.619$ ).

Based upon traditional taxonomic characteristics, we identified the 'forms' *B. longimanus* Leydig 1860, *B. cederstroemi* Schoedler 1863, and *B. cederstroemi connectens* Lilljeborg 1900 (Fig. 1). The *cederstroemi* 'form' was easily distinguished from the *longimanus* 'form' owing to its possession of a longer caudal process with a mid-length kink (Fig. 1A,C). Martin & Cash-Clark (1995) recognized a transitional form, *B. cederstroemi connectens*, which possesses a variably reduced kink (Fig. 1B). Variability in the shape (bent to straight) and amount of serration (two fields of anteriorly pointed spines to individual posteriorly directed spine-like scales) in the kink on the

caudal process of *B. cederstroemi connectens* has been well documented (Mordukhai-Boltovskaya, 1959).

Differences in morphological features were used to confirm the identity of *longimanus* and *cederstroemi* 'forms'. The *cederstroemi* 'form', including *B. cederstroemi connectens*, differed significantly from the *longimanus* 'form' with respect to caudal process length ( $F_{1,14} = 18.23$ ,  $P = 0.001$ ), distance between articular spines II and III ( $F_{1,10} = 8.81$ ,  $P = 0.014$ ), and length of articular spine III ( $F_{1,10} = 9.41$ ,  $P = 0.012$ ); in all cases, these features were longer in the *cederstroemi* 'form' (caudal process length: 4.14 mm vs. 6.90 mm; distance between articular spines II and III: 0.257 mm vs. 0.916 mm; and length of articular spine III: 0.086 mm vs. 0.394 mm). These results did not change when abdominal length and diameter of the caudal process were used as covariates (ANCOVA,  $P < 0.02$ ). Finally, the articular spines on the caudal process were typically bent anteriorly in the *cederstroemi* 'form' but were almost straight or bent posteriorly in the *longimanus* 'form' (Fig. 1).

### Genetic procedures

DNA was extracted from whole individuals of *Bythotrephes* using a modified salt-precipitation protocol (Devlin *et al.*, 1991) or a Wizard Genomic DNA Purification Kit (Promega) based on similar principles. DNA also was extracted from *Cercopagis pengoi* — another invader of the Great Lakes — collected from Lake Ontario, and used as an out-group for genetic analyses. Extracted genomic DNA was used as template DNA in minisatellite repeat primed polymerase chain reactions (PCR), as described in Heath *et al.* (1993). Each reaction used one synthetic oligonucleotide designed from minisatellite repeat core sequence (see Heath *et al.*, 1993) as a primer (NVB-3 = 5'-GGT GAA GCA NAG GTG-3'; or YNZ-22 = 5'-CTC TGG GTG TCG TGC-3'). The balance of the PCR was double-distilled water, manufacture-supplied PCR buffer,  $MgCl_2$ , dNTPs, and Taq DNA polymerase (Heath *et al.*, 1993). Reactions were run using a touchdown approach with the first 5 cycles decreasing 1 °C per cycle from a 51 °C annealing temperature followed by 35 cycles at a 46 °C annealing temperature (60 s). We also used a 72 °C extension cycle (90 s) and a 94 °C denaturing cycle (60 s) in a PTC-225 Programmable

Thermal Controller (MJ Research, Inc.). PCR products were run on 2% agarose gels for 2–4 h at 80–100 V and visualized using UV-transillumination of ethidium bromide-stained gels. Photographs were stored as digitized images for subsequent analyses. Based on the total number of bands analysed (shared or not shared among 'species') a clustering tree was generated using TFPGA software (Miller, 1997). We used Unweighted Pair Group Method based on Arithmetic Means (UPGMA) clustering to measure the similarity between *longimanus* and *cederstroemi* 'forms', with *Cercopagis pengoi* used as an out-group in the analyses.

Phylogenies also were constructed using the mitochondrial gene cytochrome c oxidase subunit I (COI). We used standard DNA amplification and sequencing procedures provided in Cristescu *et al.* (2001) using the primer pair LCO1490 and HCO2198 (Folmer *et al.*, 1994) to amplify a 658 base pair fragment of the COI gene. Bi-directional sequencing was used only when ambiguous sites were encountered. Sequences were aligned using Sequencher (Gene Codes Corporation). UPGMA was used to determine phylogenetic relationships among 'forms'. All sequences obtained in this study have been submitted to GenBank. Accession numbers for *Cercopagis pengoi* are AF 320013 (Black Sea) and AF 320014 (Caspian Sea). Accession numbers for *Bythotrephes* are AF 435122 (Lake Simcoe, Lake Ontario, and Lake Puruvesi), AF 435123 (Lake Puruvesi), AF 435124 (Post See), AF 435125 (Post See), AF 435126 (Selenter See), AF 435127 (Selenter See), AF 435128 (Petrusplaat/Dordrecht), AF 435129 (Volgograd Reservoir), AF 435130 (Volgograd Reservoir), and AF 435131 (Volgograd Reservoir).

## RESULTS

### Geographical distribution of *Bythotrephes*

The contemporary global distribution of *Bythotrephes* extends throughout the Holarctic region and includes the British Isles, mainland and northern Europe, Asia and the Great Lakes region of North America. This distribution includes regions to which it is native and others to which it has been introduced. Recent invasions have been well documented, particularly for North America (MacIsaac *et al.*, 2000; Berg *et al.*, 2002), Belgium and the Netherlands (Ketelaars & van Breemen,



**Fig. 2** Biogeographical distribution of *Bythotrephes* in Eurasia.

1993; Ketelaars & Gille, 1994). However, available historical information from Eurasia is insufficient to discriminate whether these populations are native or introduced.

*Bythotrephes* has an extensive distribution in northern Europe (Fig. 2). In Ireland, it has been observed in at least 21 waterbodies (Appendix 1), including Lough Neagh (Northern Ireland) the largest body of freshwater in the British Isles. Also, *B. longimanus* is a common component of most plankton communities throughout the English Lake District (Goulden, 1964; Harmsworth, 1967; Macan, 1970). In Norway, *B. longimanus* has been reported primarily in the south-western part of the country. Recently, *Bythotrephes* has been identified in at least 385 Norwegian waterbodies (B. Walseng, personal communication). Six of the identified water bodies that currently support *Bythotrephes* are impoundments, suggesting that human activities have created suitable environments for the survival of this species. Additional evidence supports the contention that human activities influence its occurrence in this region. For example, Lake Mjøsa, recently reclaimed from a hypereutrophic state, now supports *Bythotrephes* (Næsje *et al.*, 1987; Rognerud & Kjellberg, 1990). Likewise, it now occurs in Lille Lungegårdsvann, a reclaimed lagoon that has experienced significant limnological change (Lysebo, 1995). In Sweden, *B. longimanus* has been reported from many waterbodies in the south-eastern part of the

country, as well as from those along the western border with Norway. Both 'forms' have been reported to co-occur only in Lake Vänern, the country's largest lake (Svärdson, 1976; Nilsson, 1979). Both forms are, however, widely reported from lakes in Finland (J. Sarvala, pers. comm.) and most lakes reported to support *B. cederstroemi* also contain *B. longimanus*.

The distribution of *Bythotrephes* in continental Europe shows a distinct pattern of zonation, with major belts of occurrence in northern and southern regions of the continent (Fig. 2). For example, *B. longimanus* is found commonly in the lake districts of northern Poland and northern Germany, as well as in Denmark, the Netherlands and Belgium (Appendix 1). In both of the latter countries, it is a non-indigenous species (NIS) that likely was transported from north-eastern locations via commercial ships (Ketelaars & van Breemen, 1993; Ketelaars & Gille, 1994). In southern Europe, *B. longimanus* occurs in lakes throughout the foothills of the Alps in northern Italy, Austria and Switzerland (Appendix 1). It also has been reported farther south and east in Caucasian alpine lakes of Georgia (Kasymov, 1972), Armenia (Behning, 1941; Kasymov, 1972) and Turkey (Mordukhai-Boltovskoi & Rivier, 1987).

The Eurasian distribution of *Bythotrephes* is extensive but patchy, ranging throughout countries of the former Soviet Union and into China (Fig. 2). The greatest concentration of waterbodies

populated by *Bythotrephes* is in north-western Russia, extending from the Finnish border to the Arctic. Both 'forms' have been reported from all longitudes of Russia, including the Anadyr River drainage on the Pacific coast. *Bythotrephes* also occurs in shallow ponds in the Arctic region (Vekhov, 1981, 1982, 1987). In eastern Europe, its occurrence is associated with large river systems (e.g. Dnieper, Don and Volga Rivers) that directly or indirectly connect to the Caspian or Black-Azov Seas. In cool-temperate and subarctic Europe, *Bythotrephes* is located either in the north and associated with large rivers flowing from the south (e.g. Pechora) or with waterbodies located between large river drainages (e.g. Ob and Yenisei). *Bythotrephes* has a patchy north-south distribution in Kazakhstan and south-western Siberia (western Asia) but is frequently associated with river drainages (Tseeb, 1940; Zhadin & Gerd, 1961; Lezin, 1982).

In Eurasia, *Bythotrephes* has been reported from 40°N to 70°N. Its current North American distribution is considerably more confined, ranging from 40°N to 49°N. It is unlikely that its northern distribution in North America will increase appreciably, as human dispersal vectors operate primarily along east-west rather than north-south axes (Borbely, 2001). Natural dispersal vectors such as migrating waterfowl could operate on a north-south axis but are not believed to be as important as human dispersal vectors in determining range expansion for *Bythotrephes*. The rate of new invasions in Ontario is accelerating, a consequence of local diffusive dispersal and long-distance 'jump' dispersal (Borbely, 2001). Recent reports of *Bythotrephes* invasions in Belgium and the Netherlands indicate that range expansion is not confined to North America (e.g. Ketelaars & Gille, 1994).

### ***Bythotrephes*' occurrence in relation to physicochemical properties**

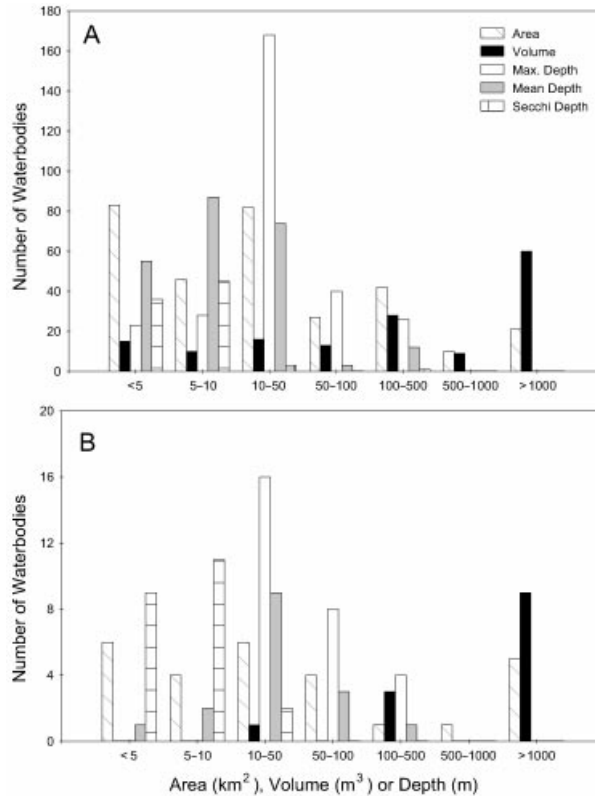
Abiotic conditions of waterbodies that support *B. longimanus* and *B. cederstroemi* were explored to determine whether these 'forms' occur in different types of habitats. Because the former 'form' has been reported from a much larger geographical area than the latter (Fig. 2), we excluded European waterbodies from countries where only one 'form' — typically *longimanus* — has been reported (i.e. we considered only lakes in regions where

'forms' co-occur). Included in our analysis were waterbodies from Estonia, Finland, Kazakhstan, Russia, Sweden and the Ukraine. No significant differences were found between lake types supporting the two 'forms' with respect to lake area ( $\chi^2 = 1.10$ ; d.f. = 1;  $P = 0.294$ ), volume ( $\chi^2 = 0.14$ ; d.f. = 1;  $P = 0.707$ ), or maximum depth ( $\chi^2 = 0.001$ ; d.f. = 1;  $P = 0.925$ ). In addition, there were no significant differences in Secchi depth transparency ( $\chi^2 = 0.96$ ; d.f. = 1;  $P = 0.329$ ) or pH ( $\chi^2 = 1.39$ ; d.f. = 1;  $P = 0.238$ ) between waterbodies that supported *longimanus* and *cederstroemi* 'forms'. However, mean depth differed significantly ( $\chi^2 = 4.17$ ; d.f. = 1;  $P = 0.041$ ), with the *cederstroemi* 'form' occurring in shallower waterbodies than the *longimanus* 'form'.

As the previous analyses did not include waterbodies that supported both 'forms', the analyses were repeated including these systems. In this case, significant differences were noted between waterbodies supporting one 'form' or both 'forms' only with respect to lake volume ( $\chi^2 = 9.03$ ; d.f. = 2;  $P = 0.011$ ). Lakes in which the *cederstroemi* 'form' occurred alone tended to have greater volumes than those in which *longimanus* occurred alone or those in which the 'forms' were sympatric.

It is possible that several variables considered together can resolve differences between habitats utilized by *longimanus* or *cederstroemi* 'forms'. A discriminant function analysis was developed using information on lake area, volume, maximum depth and mean depth to elucidate differences between two co-occurring 'forms' in Europe. There was no significant difference between waterbodies that supported the two 'forms' (Wilks' Lambda = 0.88,  $F_{3,46} = 1.61$ ,  $P = 0.188$ ).

Only the *cederstroemi* 'form' is consistently reported in North America (Sprules *et al.*, 1990; Johannsson *et al.*, 1991; Yan *et al.*, 1992; MacIsaac *et al.*, 2000; Berg *et al.*, 2002). Because physicochemical conditions could influence which 'form' occurs within a geographical region, we compared characteristics of European waterbodies in Austria, Belgium, Britain, Byelorussia, Denmark, Germany, Italy, Latvia, Lithuania, Netherlands, Norway, Poland and Switzerland that support only the *longimanus* 'form' with North American systems (Canada, United States) reported to support the *cederstroemi* 'form'. Significant differences were detected between regions with respect to lake area ( $\chi^2 = 5.64$ ; d.f. = 1;  $P = 0.018$ ), volume



**Fig. 3** Distribution of environmental variables for waterbodies supporting *Bythotrephes* populations in (A) Eurasia and (B) North America.

( $\chi^2 = 4.57$ ; d.f. = 1;  $P = 0.032$ ) and summer bottom water temperature ( $\chi^2 = 7.43$ ; d.f. = 1;  $P = 0.006$ ). However, no differences were detected between European and North American waterbodies for maximum depth ( $\chi^2 = 3.20$ ; d.f. = 1;  $P = 0.074$ ), mean depth ( $\chi^2 = 1.30$ ; d.f. = 1;  $P = 0.255$ ), Secchi disk depth ( $\chi^2 = 1.02$ ; d.f. = 1;  $P = 0.312$ ) or pH ( $\chi^2 = 0.01$ ; d.f. = 1;  $P = 0.918$ ). When each of the five Great Lakes were removed from the analyses, no significant differences were detected between European and North American waterbodies for lake area ( $\chi^2 = 1.93$ ; d.f. = 1;  $P = 0.165$ ), volume ( $\chi^2 = 2.40$ ; d.f. = 1;  $P = 0.122$ ) or summer bottom water temperature ( $\chi^2 = 2.31$ ; d.f. = 1;  $P = 0.129$ ).

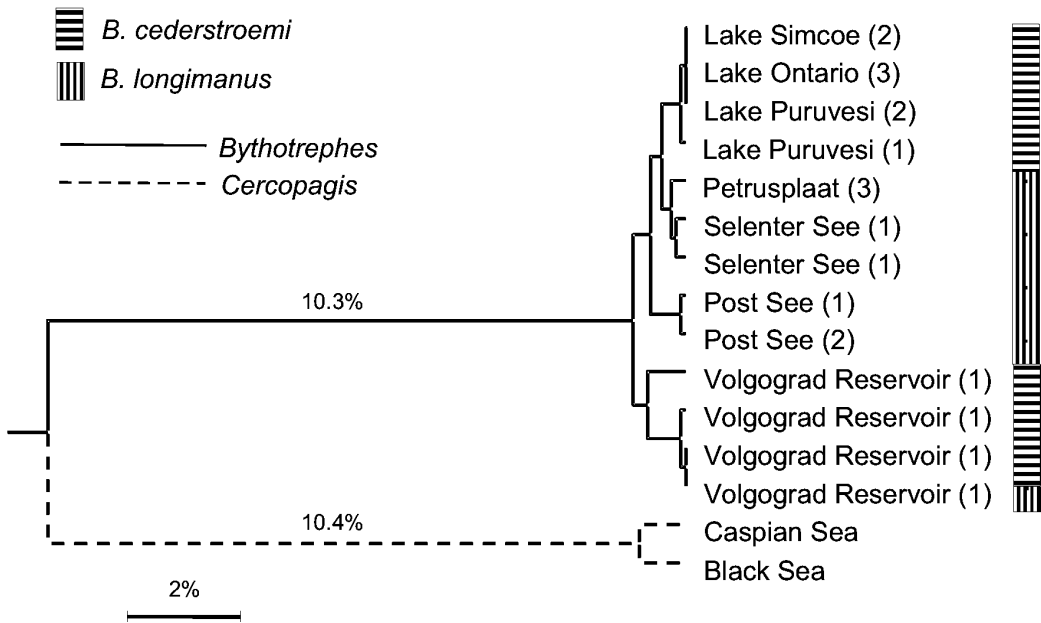
*Bythotrephes* is found in a wide variety of habitats in both Eurasia and North America (Fig. 3). In Eurasia, it is most common in lakes with small to intermediate surface areas, larger volume, intermediate mean and maximum depths, and

shallower Secchi depths (Fig. 3A). A similar trend was apparent in North America (Fig. 3B). However, there are noticeable differences in the relative number of habitats that have larger lake surface area and larger volume, a clear influence of the Great Lakes in the North American analysis (Fig. 3B).

### Genetic analysis of *Bythotrephes*

Sequencing of the mitochondrial gene COI showed that the *longimanus* and *cederstroemi* 'forms' represent a single species, *B. longimanus*. The divergence between allopatric populations of *longimanus* and *cederstroemi* 'forms' was less than 0.6% while divergence between *Bythotrephes* and the confamilial *Cercopagis pengoi* was 26.6% (Cristescu *et al.*, 2001). Furthermore, *longimanus* and *cederstroemi* 'forms' from the Volgograd Reservoir clustered at the same branch of the





**Fig. 4** UPGMA tree based on sequencing of the mitochondrial COI gene showing the divergence between the two 'forms' of *Bythotrephes* (*longimanus* and *cederstroemi*) and *Cercopagis pengoi*, used as an out-group, based on p-distance. Number of individuals sequenced in parentheses.

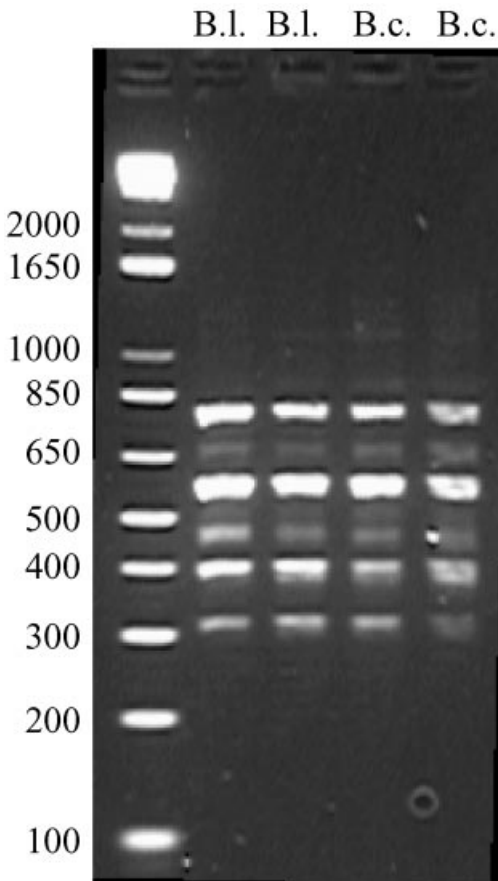
COI generated tree (Fig. 4). Detailed relationships among *longimanus*, *cederstroemi*, and *C. pengoi*, are shown in Fig. 4. The observed DAMD banding patterns indicate that the *longimanus* and *cederstroemi* 'forms' belong to a single species, *B. longimanus*. Using Nei's (1972) distance based on 14 DAMD generated bands, the distance between *longimanus* and *cederstroemi* 'forms' was 0.000 while that between *Bythotrephes* and *C. pengoi* was 0.442. An example of an observed DAMD banding pattern is shown in Fig. 5 (YNZ-22).

## DISCUSSION

Clear differences exist with respect to morphological characteristics of *longimanus* and *cederstroemi* 'forms'. In addition, *Bythotrephes* exhibits morphological variability across both spatial and temporal gradients, variability that has confounded species identity in the genus. However, the genetic analyses reported here indicate that different 'forms' represent a common species, and, according to taxonomic nomenclature rules, the ancestral name of *Bythotrephes longimanus* Leydig, 1860 should

apply. Our findings are consistent with a recent allozyme study that determined allopatric variation within each 'form' exceeded that of sympatric populations of the two 'forms' together (Berg *et al.*, 2002). Moreover, breeding and hatching studies conducted in Europe and North America have produced either instar-based 'forms' or some progeny of the other 'form' (Mordukhai-Boltovskaya, 1959; Zozulya, 1977; Yurista, 1992). Interestingly, a common haplotype is found in Lake Ontario and Lake Puruvesi, Finland, providing additional evidence that *Bythotrephes* introductions to the Great Lakes originated from a population in or adjacent to the Baltic Sea. Previous studies suggested the port of St Petersburg, Russia, as the source of the original Great Lakes population (Sprules *et al.*, 1990; Berg *et al.*, 2002). Also of note, sympatric populations of *longimanus* and *cederstroemi* 'forms' exhibited no differentiation (Volgograd Reservoir, Fig. 4), though greater variability was evident in populations from northern Europe and Russia (Fig. 4).

Yurista (1992) demonstrated that morphological differences exist among *Bythotrephes* individuals



**Fig. 5** Observed banding patterns for both *Bythotrephes* 'forms' using the DAMD primer YNZ-22 for *longimanus* 'form' (B.l.) and the *cederstroemi* 'form' (B.c.). A 1Kb + ladder also is shown. Individuals were collected from Lake Puruvesi, Finland in August 2001.

hatched from resting stages and those produced by parthenogenetic reproduction. Likewise, studies of *Cercopagis* revealed that 'forms' assignable to different morphological subgenera do not differ genetically, but rather appear to be the product of sexual vs. parthenogenetic reproduction (Makarewicz *et al.*, 2001). Seasonality also might be important, as Evans (1988) and Rivier & Grigorovich (1999) demonstrated that the *longimanus* 'form' was found in greater abundances during cold-water months (i.e. during spring and autumn), while the *cederstroemi* 'form' dominated during warm-water months. Consequently, morphological variation observed within the genus

is neither unique nor temporally stable. However, the mechanism responsible for production of different 'forms' has yet to be deciphered.

Characterization of waterbodies supporting the *longimanus* and *cederstroemi* 'forms' indicated a significant difference among lakes only with respect to mean depth. The lack of differentiation among these basins provides further support for the hypothesis that the two 'forms' represent one species. To the extent that variation was revealed, the *cederstroemi* 'form' was associated with shallower waterbodies (median = 6.5 m) than the *longimanus* 'form' (median = 6.9 m). However, it is unclear whether a difference of this magnitude could contribute to distributional differences among these 'forms'. It is more probable that other variables have a greater influence on 'form' distributions (e.g. primary production or predation pressure).

Few differences in physicochemical conditions were observed between Eurasian locations containing the *longimanus* 'form' and North American locations containing the *cederstroemi* 'form'. This finding also provides corroborating evidence that the two 'forms' are variants of the same species. Furthermore, this finding suggests that abiotic conditions are unlikely to limit colonization by *Bythotrephes* in North American waterbodies if appropriate dispersal vectors exist. This finding is consistent with MacIsaac *et al.* (2000), who suggested that many inland lakes in Ontario could support *Bythotrephes* but currently do not owing to a lack of dispersal vectors. However, once *Bythotrephes* has colonized a system, biotic variables including predation could limit its establishment or population size. To date, no study has successfully separated the effects of dispersal limitation and abiotic or biotic limitation on the establishment of new populations of this invasive species.

Morphological variability observed among *Bythotrephes* populations also could be due, in part, to cyclomorphosis. Cyclomorphosis is a defensive mechanism that can result in dramatic morphological changes in cladocerans, including the formation of neckteeth, tailspines, or changes in head shape and length. Cladoceran cyclomorphosis results from abiotic (e.g. water temperature, turbulence and light) or biotic (e.g. presence of predators) factors (e.g. Hebert & Grewe, 1985; Straile & Hälbich, 2000). For example, the phantom midge *Chaoborus* releases kairomone chemicals into the water that trigger

cyclomorphosis in *Daphnia ambigua* (Hebert & Grewe, 1985). It is unclear whether fish predators can trigger cyclomorphosis in *Bythotrephes*, although they clearly influence body size in some European systems (H. Ketelaars, pers. obs.).

*Bythotrephes* in the Great Lakes demonstrate considerable variation in morphology. For example, specimens collected from Lake Superior exhibited small body size and lack of a caudal kink (D. Jensen, pers. comm.), and resembled the *longimanus* 'form' even though only the *cederstroemi* 'form' is consistently reported in North America. Sullivan & Lehman (1998) also reported that *cederstroemi*-type individuals from Lakes Erie, Huron and Michigan displayed considerable differences with respect to body allometry. However, the mechanism(s) responsible is not understood.

Various mechanisms exist to explain the large-scale, global distribution of *Bythotrephes*. As with many cladoceran taxa, *Bythotrephes* can expand its range via natural dispersal of resting eggs by birds (Charalambidou & Ketelaars, unpubl.), fish (Jarnagin *et al.*, 2000), or by passive advection to downstream locations (Herzig, 1985a,b). These resting eggs, stored in sediment egg banks, also allow the continued persistence of *Bythotrephes* in habitats under high predation pressure. In addition, human vectors are responsible for the increased distribution of *Bythotrephes*, including via ships' ballast water, canals or contaminated fishing gear. It now appears that natural dispersal is being dramatically supplanted by human-mediated dispersal. For example, human activities have increased the rate of spread and the geographical range of *Bythotrephes* faster during the latter half of the twentieth century than at any time previously (see MacIsaac *et al.*, 2000). Numerous mechanisms determine *Bythotrephes*' distribution at smaller spatial scales including individual lakes and reservoirs. These factors include fish predation, eutrophication, acidification and reservoir impoundment.

### Fish predation effects

Fish predation is one mechanism that may clearly regulate *Bythotrephes* abundance. Planktivorous fishes selectively consume *Bythotrephes*, especially during summer months when it is most abundant (e.g. Lindström, 1955; Nilsson, 1955; Berg & Grimaldi, 1966; Langeland, 1978; Mills *et al.*, 1992; Coulas *et al.*, 1998). For example,

*Coregonus* spp. in Lago Maggiore, Italy, had a higher preference for *B. longimanus* than for any other crustacean plankton (de Bernardi & Giussani, 1975; de Bernardi *et al.*, 1987). *Bythotrephes* can compose > 90% of total gut contents at times of high predation pressure (Nilsson, 1961, 1965; Fitzmaurice, 1979; Coulas *et al.*, 1998).

A large body of literature has developed illustrating that intense fish predation can be a potent mechanism limiting local occurrence or abundance of *Bythotrephes* (e.g. Huitfeldt-Kaas, 1935; Berg, 1951; Patalas, 1954; Stenson, 1978; Vekhov, 1982). For example, Stenson (1972) and Langeland (1972) showed that *B. longimanus* was eliminated or abundances reduced to non-detectable levels from lakes with high fish predation intensity. Svärdson (1976) reported *Bythotrephes* in 37 of 41 Swedish lakes without fish but in only four of eight lakes with fish. Langeland (1972, 1978) reported that *B. longimanus* had disappeared from three Norwegian lakes between 1896 and 1971, likely due to predation by Arctic char (*Salvelinus alpinus*).

It is important to note that resting eggs may remain in lake sediments for some years, thereby allowing re-establishment of *Bythotrephes* following relaxation of predation pressure or changes in limnological conditions (e.g. recovery from eutrophication or acid stress; see below). Nevertheless, if *Bythotrephes* is found in fish gut contents but is not detectable in water column samples, it is likely to be experiencing intense predation pressure (Stenson, 1978), the magnitude of which might limit its ability to colonize or persist in novel habitats. As evidence, Yan & Pawson (1997) and Yan *et al.* (2001) proposed that *Bythotrephes* gains refuge from planktivorous fish by living in regions of the water column not exploited by fishes. Yan *et al.* (2001) argued that if the hypolimnion is deeper than twice the Secchi disk depth, a warm, dark layer exists wherein hypolimnetic, stenothermal planktivorous fish could not see or prey on overlying *Bythotrephes*. Conversely, if Secchi disk depth exceeds thermocline depth, fish can see and exploit overlying *Bythotrephes* (Yan *et al.*, 2001).

The caudal process affords some protection against gape-limited planktivorous fish, but it is less likely to be effective against larger fishes. In general, larger-bodied planktivores can fully exploit *Bythotrephes*, while smaller individuals may have trouble handling or swallowing this species. Some

smaller fishes can exploit *Bythotrephes*, although preferential selection is best described for old, large age classes (Klemetsen, 1973; Kogan, 1974; de Bernardi *et al.*, 1987). *Bythotrephes* may respond to planktivory through adjustments in clutch size, producing fewer, larger offspring, and by producing longer caudal barb pairs; these defences are apparently most effective against gape-limited planktivorous fishes (Straile & Hälbich, 2000). Thus, strong evidence exists to link fish predation to regulation of occurrence, abundance, life history characteristics and body allometry of *Bythotrephes*.

### Eutrophication and acidification effects

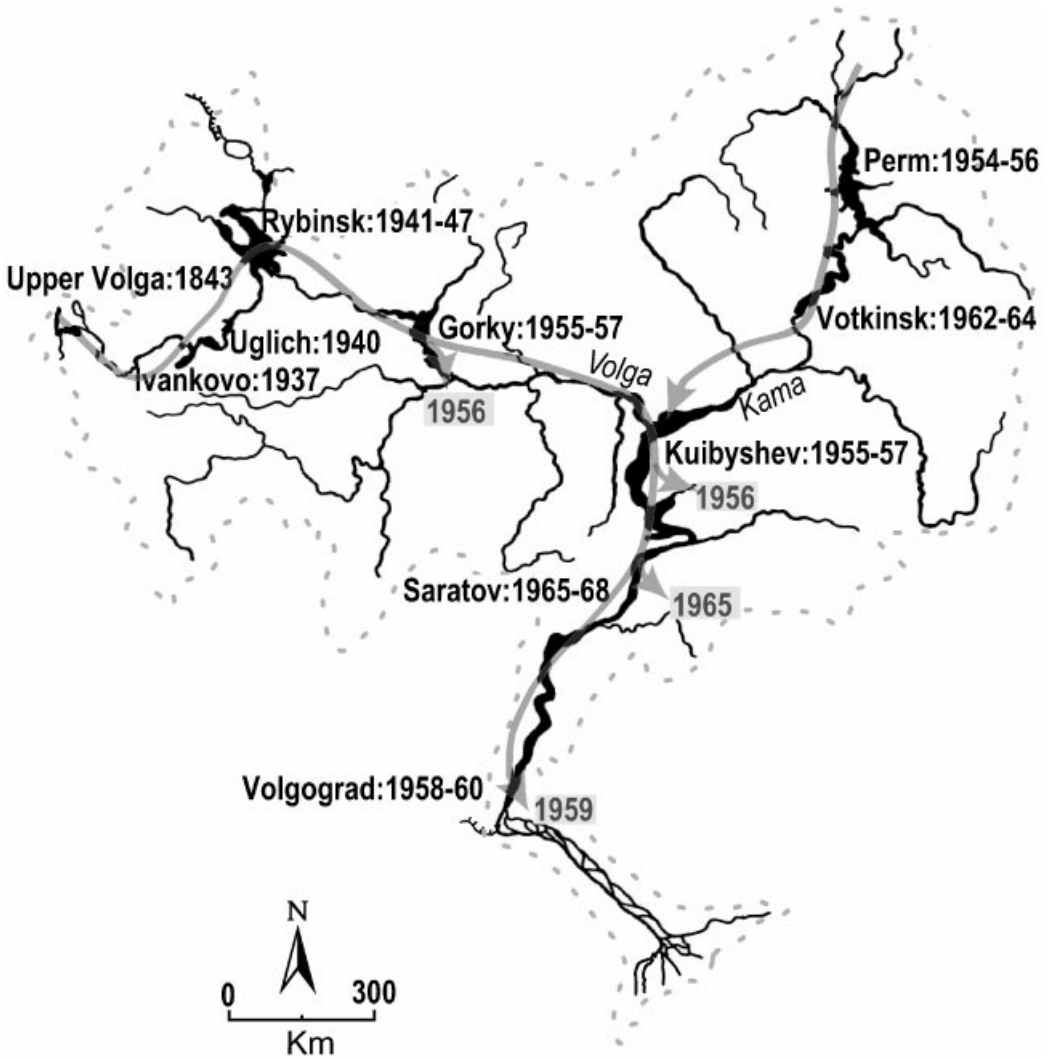
Both cultural eutrophication and anthropogenic acidification operate on regional to local spatial scales. Lake eutrophication strongly affects zooplankton assemblages, including adverse effects on abundance of *Bythotrephes* in most instances. For example, *Bythotrephes* disappeared from the zooplankton community of Lake Ringsjön, Sweden sometime between 1883 and 1972–83 (Bergstrand, 1990) as well as from Lake Võrtsjärvi, Estonia (Haberman, 1998) owing to eutrophication. *Bythotrephes longimanus* is a relatively rare and unimportant member of the plankton community in Lake Tyrifjord, Norway, a system that has been affected by cultural eutrophication since the 1930s (Langeland, 1974). This pattern has been repeated in many other systems across Europe (Goulden, 1964; Harmsworth, 1967; Smyly, 1972; Nilsson & Pejler, 1973; Jeppesen *et al.*, 1996).

The effects of eutrophication on *Bythotrephes* abundance are, however, reversible. In Lake Mondsee, Austria, abundance of *B. longimanus* decreased slightly during a period of rapid eutrophication, but later increased following implementation of pollution control measures (Nauwerck, 1991). These effects were even more pronounced in Lago Maggiore, Italy, where *Bythotrephes* declined in abundance as the lake eutrophied, but subsequently recovered as phosphorus abatement was implemented and the lake experienced oligotrophication (Manca & Ruggiu, 1998). Virtually the same pattern was observed in Lake Lucerne, Switzerland as it experienced eutrophication and then oligotrophication (Mookerji *et al.*, 1998). In Kortowskie Lake, Poland, an intensive restoration program resulted in improvement in water quality and establishment of *B. longimanus* (Widuto,

1977). These observations also are consistent with synoptic surveys conducted throughout the Mazurian lake district of Poland, where *Bythotrephes* occurs most commonly in less eutrophic waterbodies (Gieysztor, 1959; Patalas & Patalas, 1966).

Available evidence indicates that lake eutrophication and the intensity of fish planktivory on *Bythotrephes* are inextricably linked (see MacIsaac *et al.*, 2000). Consequently, the disappearance of *Bythotrephes* from eutrophic lakes may be related more to planktivory than to intolerance of physical or chemical conditions. The best available evidence to explore this concept may be derived from Jeppesen *et al.*'s (1996) study of 30 Danish lakes. In these systems, planktivore biomass was positively correlated with lake trophic condition, while body size of zooplankton was inversely related.

Acid stress is another important factor that affects the distribution of fish and other aquatic taxa. Acid precipitation has affected many lakes in northern Europe, resulting in acid stress and reduced fish population densities. However, lack of fish in mildly acid-stressed or recovering lakes may facilitate establishment or increased population densities of *Bythotrephes*. In Lake Östra Nedsjön, Sweden, for example, *B. longimanus* declined as a limed lake slowly re-acidified, but then increased after a second lime treatment raised lake pH and, more importantly, caused the virtual loss of planktivorous vendace, *C. albula* (Ekström & Hörnström, 1995). *Bythotrephes longimanus* also immigrated to Lake Gårdsjön, Sweden, after liming increased its pH from 4.67 to 7.35 (Stenson & Svensson, 1995). It established in this lake despite the presence of stocked brown trout (*Salmo trutta*) and rainbow trout (*Onchorhynchus mykiss*); however, Stenson & Svensson (1995) argued that planktivory on *Bythotrephes* was likely quite low. Large brown trout preferentially selected juvenile rainbow trout whose diet was heavily dominated (> 99%) by *Daphnia*, thereby allowing *Bythotrephes* to avoid direct predation pressure. Other Swedish studies also have noted increased importance of *B. longimanus* to zooplankton communities in acid lakes with reduced predation pressure (Andersson, 1972; Hörnström *et al.*, 1973). Thus, both lake oligotrophication and acidification may create conditions favourable to establishment and survival of *Bythotrephes*. However, tolerance of *Bythotrephes* to acidification has not been determined.



**Fig. 6** The Volga River drainage showing the reservoir system and year(s) of impoundment for each reservoir. Arrows show the spread of *Bythotrephes* through this system, and the year of establishment for each reservoir successfully invaded is also indicated.

### Reservoir impoundment and other human-mediated dispersal effects

Reservoir impoundment alters the hydrological regime of watersheds. Following impoundment, the water mass of the newly created reservoir assumes the characteristics of a lacustrine environment (i.e. reduced water velocity), while the regulated outflow might have increased or decreased water velocity depending on operations. Zozulya

(1979) reported that *Bythotrephes* abundance was significantly higher near reservoir dams relative to more riverine locations upstream. Its spread through the Volga reservoir system of Russia illustrates the effects human activities have had on the dispersal of this zooplankton in Eurasia (Fig. 6). Originally confined to deep, lacustrine habitats, *Bythotrephes* first appeared in the Upper-Volga Reservoir in the early 1900s (Chugunov, 1922). Despite downstream drift of

planktonic stages or resting eggs, the species failed to establish at downstream locations before the 1930s, likely owing to unfavourable flow conditions (Rivier & Dziuban, 1978). However, with reservoir impoundment on the Upper-Volga River in the 1930s and 1940s, suitable conditions were created for the establishment of new populations (Mordukhai-Boltovskoi & Dziuban, 1976; Rivier & Dziuban, 1978). Rapid colonization by *Bythotrephes* occurred as additional reservoirs were impounded farther downstream (Fig. 6).

Reservoir impoundment also affects fish populations and, indirectly, *Bythotrephes* occurrence. For example, Jensen (1988) reported that size and predation pressure exerted by planktivorous brown trout and Arctic char increased following impoundment of Nesjö Reservoir, Norway, resulting in increased predation on *B. longimanus*. Aass (1969) reported a similar pattern in Aursjøen Reservoir, Norway.

Canal creation is another human-mediated activity that has increased the spread of aquatic species. However, unlike reservoir impoundment that creates favourable conditions for the establishment of new species, canal construction removes barriers to dispersal at regional or larger spatial scales. For example, creation of the Volga-Don Canal in 1952 significantly increased commercial shipping activities, thereby providing a mechanism for the spread of aquatic species in the Ponto-Caspian region (Ricciardi & MacIsaac, 2000; Cristescu *et al.*, 2001). This and other waterways have created 'invasion corridors' that have enhanced dispersal of *Bythotrephes* to the Tsimlyansk Reservoir and Don River Drainage (Mordukhai-Boltovskoi & Rivier, 1987). Shipping activities also have been implicated in the spread of *Bythotrephes* to the Netherlands and Belgium (Ketelaars & van Breemen, 1993; Ketelaars & Gille, 1994). Moreover, Sprules *et al.* (1990) speculated that *Bythotrephes* was likely delivered to the Great Lakes via a ship visiting from the Lake Ladoga–Neva River–Baltic Sea region, a pathway subsequently corroborated by Berg *et al.* (2002) and by this study.

## CONCLUSIONS

Results from the genetic analyses indicate that *longimanus* and *cederstroemi* 'forms' represent a single species, *B. longimanus*. No significant dif-

ferences were detected in key habitat characteristics of waterbodies supporting the two 'forms'. Furthermore, the observed global distribution pattern for *Bythotrephes* (*longimanus* in central and western Europe and *cederstroemi* in North America) provides additional evidence that *Bythotrephes* is a single, polymorphic species. Fish predation, cultural eutrophication, and lake acidification directly or indirectly affect the local distribution, abundance, life history and body size of *Bythotrephes*. Reservoir and canal construction and global shipping activity affect the regional and intercontinental distribution of *Bythotrephes*. Once geographical barriers to intercontinental dispersal have been removed, other human mechanisms disperse the species to novel local and regional habitats.

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## SUPPLEMENTARY MATERIAL

The following material is available from <http://www.blackwell-science.com/products/journals/suppmat/DDI/DDI129/DDI129sm.htm>

**Appendix 1** Eurasia and North America water bodies with reported populations of *Bythotrephes*

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