Arch. Biol. Sci., Belgrade, 68(1), 67-79, 2016 DOI:10.2298/ABS150505010L

MORPHOLOGICAL DIVERSITY AND WIDESPREAD HYBRIDIZATION IN THE GENUS *BYTHOTREPHES* LEYDIG, 1860 (BRANCHIOPODA, ONYCHOPODA, CERCOPAGIDAE)

Larisa F. Litvinchuk[1] and Spartak N. Litvinchuk[2,*]

[1] *Zoological Institute, Russian Academy of Sciences*, Universitetskaya emb. 1, St. Petersburg 199034, Russia

[2] *Institute of Cytology, Russian Academy of Sciences,* Tikhoretsky pr. 4, St. Petersburg 194064, Russia

***Corresponding author**: slitvinchuk@yahoo.com

Received: May 5, 2015; **Revised**: November 12, 2015; **Accepted**: November 24, 2015; **Published online:** January 12, 2016

Abstract: Morphological variation in the genus *Bythotrephes* was studied. We analyzed samples from 29 locations through the generic range. Based on the core body length and the total length, as well as several other morphological characters, differences were found that are consistent across five groups of populations previously described as a separate species (*B. cederstroemii*, *B. crassicaudus*, *B. transcaucasicus*, *B. brevimanus*, and *B. longimanus*). Individuals with intermediate morphological characteristics were detected where *B. cederstroemii*, *B. brevimanus* and *B. crassicaudus* have geographic overlap. Based on allozyme analysis, hybrids of *B. cederstroemii* and *B. brevimanus* were revealed in the zone of contact of these two species in the Volga River drainage and northwestern Russia.

Key words: planktonic crustaceans; Bythotrephes; diversity; hybridization

INTRODUCTION

Bythotrephes spp. are predaceous planktonic crustaceans widely distributed across northern Eurasia, including the British Isles, the Scandinavian Peninsula, Denmark, northern Germany and Poland, the Baltic countries, the Alps, the Caucasus, Turkey, European Russia, Siberia, northern Kazakhstan and northwestern China (see maps in Therriault et al., 2002; Litvinchuk, 2007). In the 1980s, the genus invaded the northeastern part of North America (MacIsaac et al., 2000).

The study of morphological variations of the genus *Bythotrephes* has a long history (review in Litvinchuk, 2005). Originally, *B. longimanus* was described from the Bodensee (Lake Constance) in Germany (Leydig, 1860). Later, an additional three species were described, namely *B. cederstroemii*, *B. crassicaudus* (originally *B. crassicauda*) and *B. borealis* (Schödler, 1877; Sars, 1890). Lilljeborg (1901) presented a taxonomic revision of the genus and proposed to distinguish only two species: *B. longimanus* with a straight postabdominal spine and *B. cederstroemii* with a curve in the postabdominal spine. Moreover, he split *B. longimanus* into three varieties: *longimanus*, *arcticus* (new name for *crassicaudus*) and *brevimanus*, and *B. cederstroemii* into *cederstroemii*, *robustus* (new name for *borealis*) and *connectens*. For a long time, the taxonomic structure of the genus with two species was widely accepted (Manuylova, 1964; Scourfield and Harding, 1966; Nilsson, 1979).

The next taxonomic revision was made by Ischreyt (1930, 1935, 1936, 1939). The genus *Bythotrephes* was divided into five species: *B. cederstroemii* from northwestern Europe, *B. longimanus* from the western and southern parts of the Alps, *B. styriacus* from the eastern part of the Alps, *B. balticus* (junior synonym of *B. brevimanus*) from the Baltic Sea region, and *B. crassicaudus* from the Arctic (Ischreyt, 1939). Later, Agnesotti (1935) and Behning (1941) described two new subspecies, *B. longimanus lariano* and *B. l. transcaucasicus*, from Como Lake in northern Italy and the Caucasus, respectively. Additionally, Ischreyt

(1939) recognized a variety *B. longimanus* v. *carnica* from Millstätter See in Austria.

Some authors found that *Bythotrephes* populations from the Scandinavian Peninsula and northwestern Russia may consist of individuals with both curved and straight postabdominal spines (Lilljeborg, 1901; Mordukhai-Boltovskaya, 1959; Manuylova, 1964; Nilsson, 1979). Moreover, Mordukhai-Boltovskaya (1959) cultured *Bythotrephes* from Rybinskoe Reservoir (Russia), which is inhabited by such a population. This author found that parthenogenetic females with curved postabdominal spines could release neonates without the curve and *vice versa*. Based on these results and cyclic changes of abundance of individuals with curved and straight postabdominal spines in this water body, Zozulya and Mordukhai-Boltovskoy (1977) considered the presence or the absence of a curve in the postabdominal spine a result of cyclomorphosis in a single polymorphic species, *B. longimanus*. This point of view was widely accepted by some researchers (e.g., Martin and Cash-Clark, 1995; Grigorovich et al., 1998; Rivier, 1998; Hessen et al., 2011; Yan et al., 2011).

The studies of variable genetic characters (allozymes and microsatellites) revealed at least five different geographic groups of *Bythotrephes*: i) from the southern part of the Alps (Lake Maggiore), ii) from the northwestern part of the Alps (Lake Lucerne), iii) from the western part of the Baltic Sea (Stocksee, Selenter and Vänern lakes), iv) from the eastern part of the Baltic Sea (Päijänne, Vuohijärvi, Kivijärvi, and Ladoga lakes) plus North America, and v) from Lake Kuolimo in eastern Finland (Berg and Garton, 1994; Berg et al., 2002; Therriault et al., 2002; Colautti et al., 2005). Of the first three geographic groups, all individuals have straight postabdominal spines, but the two last groups comprise individuals with straight, curved, or both straight and curved postabdominal spines.

Recently, Litvinchuk (2002, 2007) studied morphological and allozyme variation in the genus *Bythotrephes* with focus on western Russia. It was found that the genus consists of several geographical groups, which could be separated by morphological and genetic characters. Based on these results, the author proposed to distinguish several distinct species in the genus. This point of view was recently supported by several authors (Kotov et al., 2013; Korovchinsky, 2015).

The aim of our work was to study morphological variation in the genus, to test for differences between the majority of previously described forms, and to study variation of morphological and genetic characters in the zones of contact of these forms to reveal their taxonomic status.

MATERIALS AND METHODS

Specimens

Bythotrephes from 29 localities (Fig. 1) were studied and contained five previously described species (apart from *B. styriacus*). This material includes presumed type specimens of *B. transcaucasicus* (Chaldyr Lake; ZISP.1635-1636), and samples which were collected in localities close to type territories of *B. longimanus* (Geneva and Vierwaldstättersee lakes; ZISP.1638, 6855) and *B. crassicaudus* (Verkhnekildinskoe and Pityevoe lakes; ZISP.6850, 6852). ZISP is the Zoological Institute of Russian Academy of Sciences, St. Petersburg, Russia. The locality data (name, coordinates and the numbers of specimens collected) are listed in Table 1.

In total, the morphology of 494 mature adult females and 126 mature adult males was studied. Additionally, the shape of the caudal spine was studied in 177 animals used for allozyme analysis. Mature adult females (with as minimum two pairs of caudal barbs) were determined by the presence of embryos of the last developmental stages or resting eggs in brood pouches. Mature adult males were recognized by the presence of the pair of copulative organs. A female of the first generation (see details in Yurista, 1992; Litvinchuk, 2007) from Lake Pit'evoe that emerged from a dormant egg (possessing four pairs of caudal barbs and a short and straight caudal postabdominal spine) was excluded from the analysis. To avoid observer bias, the first author took all measurements in laterally oriented animals in 16x binoculars with an ocular micrometer.

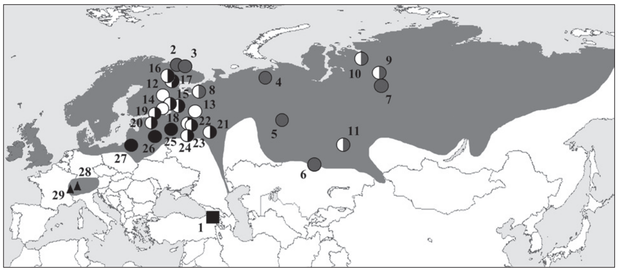

Fig. 1. The native range of *Bythotrephes* (gray area) with geographic location for the samples of studied. *B. transcaucasicus* (black square) – locality 1; *B. crassicaudus* (grey circles) – localities 2-7; samples from the contact zone of *B. crassicaudus* and *B. cederstroemii* (semi-gray circles) – 8-11; *B. cederstroemii* (white circles) – 12-14; samples from the contact zone of *B. cederstroemii* and *B. brevimanus* (semi-black circles) – 15-24; *B. brevimanus* (black circles) – 25-27; and *B. longimanus* (black triangles) – 28-29.

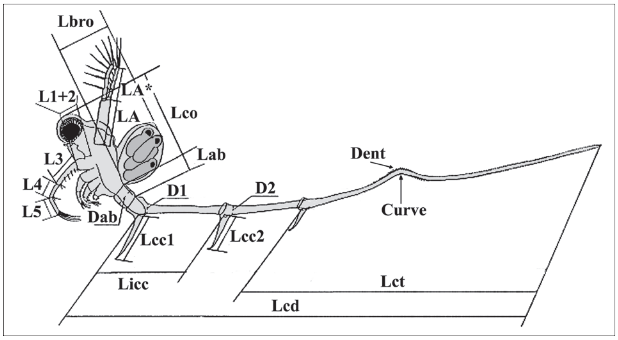

Fig. 2. The scheme of morphological measurements of *Bythotrephes*. *Lco* – core body length; *Lab* – abdomen length; *Lbro* – brood pouch length (only females); *Lcd* – postabdominal spine length; *Lcc1* and *Lcc2* – lengths of the I and II pairs of caudal barbs, respectively; *Licc* – distances between the I and II caudal barbs pairs; *D1* and *D2* – postabdominal spine diameters after the barbs I and II, respectively; *Dab* – abdomen diameter; *Lct* – distance between the basis of barbs of the last (i.e. second or third) pair and the tip of the postabdominal spine; *LA* – length of the antenna II base; *LA** – length of the antenna II branch; *L1+2, L3, L4* and *L5* – lengths of the first two, third, fourth, and fifth limbs segments (respectively) on the first pair of legs. Curve is a curve in the postabdominal spine. Dent is the postabdominal denticulation.

Morphological measurements

We used morphological measurements and indexes described previously by Ischreyt (1930, 1935, 1939) and Litvinchuk (2007). In total, 17 characters were measured for each specimen. The scheme of measurements is given in Fig. 2. We calculated core body length (*Lc*), as the sum of *Lco* and *Lab*, and total length (*TL*), as a sum of *Lc* and *Lcd*. *L1+3* is the total length of the first three limbs segments and *L4+5* the total length of the IV and V limbs segments. *LA* is the total lengths of the antenna II base and ramus.

Allozyme analysis

To reveal presumed hybridization, we used allozyme analysis. For this, we selected populations with the typical morphological characters of *B. crassicaudus* (Lake Pit'evoe; all individuals with straight postabdominal spines), *B. brevimanus* (Lake Vyshtinets; all individuals with straight postabdominal spines), *B. cederstroemii* (Lake Lexozero; all individuals with curved postabdominal spines), and eight "mixed" populations that consisted of individuals with curved and straight postabdominal spines (a presumed geographical contact zone between *B. cederstroemii* and *B. brevimanus*). Specimens from a presumed geographical contact zone between *B. crassicaudus* and *B. cederstroemii* were not included in the analysis because we lacked fresh material. A total of 177 individuals from 11 localities were studied. The location of sampling sites and sample details are shown in Table 1.

Vertical polyacrylamide gel electrophoresis

The collected animals were stored in dry ice in the field and subsequently in a freezer at -80°C in the laboratory. Vertical polyacrylamide gel (6-8%) electrophoresis was performed to analyze the genetic variation of eight putative loci of allozymes and water-soluble proteins: alkaline phosphatase (*Alp*; EC 3.1.3.1.), esterase (*Est-1*; EC 3.1.1.-), esterase D (*EstD-1* and *EstD-2*; EC 3.1.1.-), carbonic anhydrase (*Ca*; EC 4.2.1.1.), NADP-dependent malate dehydrogenase (*sMdhp*; EC 1.1.1.38.), NAD-dependent malate dehydrogenase (*sMdh*; EC 1.1.1.37), and water-soluble protein (*Prot*). Tris-boric-EDTA (pH 8.3) and Tris-citric (pH 8.0) buffers were used for the analysis. The proteins were visualized by standard techniques (Shaw and Prasad, 1970).

Table 1. Geographic locations for the samples of studied *Bythotrephes.*

	Samples	Country	Latitude	Longitude	Fema	Male	Allo	Curv (%)	Dent	Dire	Ba-f	Ba-m
1	Chaldyr Lake	Turkey	41.0504° N	43.2435° E	14	0	0	0	A	B	2.0	–
2	Verkhnekildinskoe Lake	Russia	69.3455° N	34.0766° E	5	4	0	0	A	B	3.0	2.0
3	Pit'evoe Lake	Russia	69.1311° N	36.0623° E	18	10	15	0	A	B	3.0	2.0
4	Pechora River	Russia	67.5328° N	52.5804° E	0	4	0	0	A	B	–	2.0
5	Severnaya Sosva River	Russia	63.9375° N	65.0663° E	1	0	0	0	A	B	3.0	–
6	Sabanty-Kul Lake	Kazakhstan	50.8829° N	70.5605° E	6	0	0	0	A	B	2.0	–
7	Enisey River	Russia	65.7753° N	88.0339° E	4	5	0	0	A	B	2.0	–
8	Severnaya Dvina River	Russia	64.6509° N	39.9228° E	11	4	0	7	P	B	2.6	2.0
9	Podkovka Lake	Russia	68.0667° N	87.5833° E	6	2	0	25	PA	F	2.0	2.0
10	Maloe Sovetskoe Lake	Russia	67.0866° N	83.6800° E	2	0	0	50	PA	FB	2.5	–
11	Malye Chany Lake	Russia	54.5496° N	77.9708° E	59	1	0	43	PA	FB	2.5	2.0
12	Lexozero Lake	Russia	63.8109° N	30.9014° E	33	4	13	100	P	F	3.0	2.0
13	Vozhe Lake	Russia	60.7647° N	38.9331° E	1	0	0	100	P	F	3.0	–
14	Ladoga Lake	Russia	61.3437° N	30.9561° E	29	4	0	100	P	F	3.0	2.0
15	Onega Lake	Russia	61.7939° N	34.4735° E	1	0	6	86	PA	FB	3.0	–
16	Chuna Lake	Russia	67.6376° N	32.6295° E	0	2	0	0	A	B	–	2.0
17	Bol'shoe Cherlivoe Lake	Russia	66.3000° N	33.5000° E	2	1	0	50	P	B	3.0	2.0
18	Suojarvi Lake	Russia	62.1569° N	32.3525° E	1	0	2	0	A	B	3.0	–
19	Gulf of Finland	Russia	60.3778° N	28.5810° E	52	10	36	47	PA	FB	3.0	2.0
20	Chudskoe Lake	Russia	58.5737° N	27.8280° E	30	1	8	56	PA	FB	2.7	2.0
21	Gor'kovkoe Reservoir	Russia	56.9885° N	43.2126° E	21	23	14	69	PA	FB	2.9	2.0
22	Khotavets Lake	Russia	58.5670° N	37.6021° E	45	1	5	100	P	F	3.0	2.0
23	Rybinskoe Reservoir	Russia	58.3339° N	38.1573° E	87	47	46	48	PA	FB	2.8	2.0
24	Uglichskoe Reservoir	Russia	56.7421° N	37.1555° E	6	2	15	13	PA	FB	3.0	2.0
25	Seliger Lake	Russia	57.4872° N	33.0315° E	2	0	0	0	A	B	3.0	–
26	Anninskoe Lake	Russia	56.2000° N	28.7000° E	6	1	0	0	A	B	3.0	2.0
27	Vyshtinets Lake	Russia	54.4312° N	22.7103° E	30	0	17	0	A	B	3.0	–
28	Vierwaldstättersee Lake	Switzerland	47.0185° N	08.3696° E	8	0	0	0	A	B	2.0	–
29	Lake Geneva	Switzerland	46.4390° N	06.5071° E	15	0	0	0	A	B	2.0	–

Curv (%) is the percentage of specimens with a curve in the postabdominal spine; for specimens used in both morphological and allozyme analyses), the presence of postabdominal denticulation (Dent: P – present, A – absent, and PA – both states), the direction of the posterior pair of caudal barbs (Dire: F – forward, B – backward, and FB – both states), the mean number of caudal barbs in mature adult females (Ba-f) and males (Ba-m), the number of mature adult females (Fema) and males (Male) used in the analysis of morphometric characters, and number of specimens used for allozyme analysis (Allo). *B. transcaucasicus* – locality 1; *B. crassicaudus* – 2-7; samples from the contact zone of *B. crassicaudus* and *B. cederstroemii* – 8-11; *B. cederstroemii* – 12-14; samples from the contact zone of *B. cederstroemii* and *B. brevimanus* – 15-24; *B. brevimanus* – 25-27; and *B. longimanus* – 28-29.

Data analysis

Estimates of allele frequencies and genetic variability, i.e., observed and expected heterozygosity expressed in percent, and Nei's (1978) unbiased genetic distances (D_{Nei}) were obtained using the software BIOSYS-2 (Swofford and Selander, 1999). The Hardy-Weinberg equilibrium was evaluated for each locus by exact testing as implemented in the computer software TFPGA (Miller, 1997). Population frequency data for *Bythotrephes* samples were ordered using a multiple correspondence analysis (MCA; an analog of PCA for nominal categorical data). This analysis was performed with the computer software STATISTICA, version 6.0.

We studied "mixed" populations from the presumed geographical contact zone of *B. cederstroemii* and *B. brevimanus* by estimation of the proportion of membership of each population in *B. cederstroemii* and *B. brevimanus* clusters with STRUCTURE, version 2.3 (Pritchard et al., 2000; Falush et al., 2003), a model-based clustering method for inferring population structure using genotype data. We chose the "independent allele frequency" admixture model and ran five pseudoreplicates with 10^5 iterations after a burn-in of 5×10^4 steps. The assignment test from NEW HYBRIDS (10^6 iterations; Anderson and Thompson, 2002), which estimates the posterior probability that genetically sampled individuals fall into each of a set of user-defined hybrid categories, was used to identify *B. cederstroemii*, *B. brevimanus* and their hybrids. Additionally, we used HYBRIDLAB, version 1 (Nielsen et al., 2006) to simulate "known" hybrid individuals based on the data from parental genotypes, and then we ran NEW HYBRIDS with the simulated data in order to assess whether the real data have the necessary power to support the main inference. The criteria used to classify individuals as belonging to the parental species or hybrids (a pooled backcross B_1 and hybrids F_1 and F_2) were $P>0.9$, and individuals were considered "intermediate" when they had a score of $P\leq0.9$.

The nonparametric Kolmogorov-Smirnov test was used to test for differences between the mean values of the morphometric traits of population samples. Principal component analysis (PCA) was used to convert the dataset of measurements of possibly correlated characters into a set of values of linearly uncorrelated variables (principal components). The weight for each principal component was estimated by the loading values. Only populations with the typical morphological characters of *B. transcaucasicus* (locality 1), *B. crassicaudus* (2-7), *B. cederstroemii* (12-14), *B. brevimanus* (25-27), and *B. longimanus* (28-29) were selected for these analyses. Additionally, we used a discriminant function analysis to determine the percentage of successful pairwise identifications. Canonical discriminant analysis (CDA) was used to estimate position in the space of canonical discriminant axes of samples from presumed hybrid zones. The centroids of each sample were plotted on the first two axes. The log-transformed dataset was subjected to PCA and CDA. All statistical analyses were performed with the software STATISTICA, version 6.0. Due to the unbalanced female/male ratio, we excluded males from morphometric analyses.

RESULTS

Most populations (48%) studied were characterized by a straight postabdominal spine (Table 1). Only 14% of populations had a curved postabdominal spine and 38% consisted of individuals with both states of the character. As a rule, specimens with a curved postabdominal spine also had caudal denticulation and a forward-directed posterior pair of caudal barbs, and specimens with straight postabdominal spines had no caudal denticulation and possessed a backward-directed posterior pair of caudal barbs (Table 1). In all mature adult males, we observed two pairs of caudal barbs, but in females we found variation depending on geographic origin (Table 1). In the Alp (localities 28-29) and the Caucasus (1), females had only two pairs of barbs, and the other populations had two or three pairs.

In the PCA (morphometrical characteristics), the first two principal components explained 88% of the total female variability. Regarding the first principal component, most characteristics (*Lco*, *Lab*, *Lcd*, *Lcc1*, *Lcc2*, *D1*, *D2*, *Dab*, *Lct*, *L1+3*, *L4*, *L5*, and *LA*) had the highest loading values, and regarding the second principal component, only *Licc* had a highest loading value. The analysis allowed us to separate five groups. The samples with a curve in the postabdominal spine (*B. cederstroemii* from Lexozero, Vozhe and Ladoga lakes) formed a separate group without any overlap with others (Fig. 3). Among the samples with a

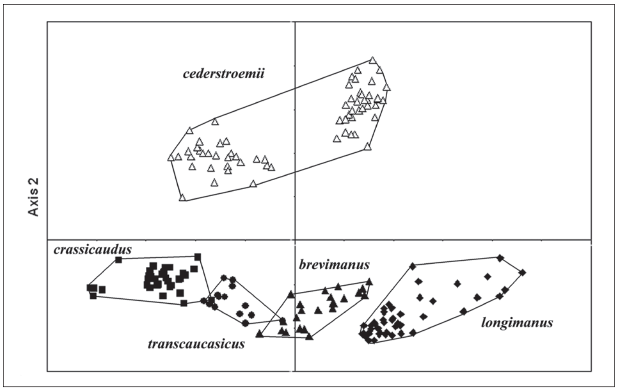

Fig. 3. Plot for females of *Bythotrephes* with (white triangles) and without (black circles, triangles, squares, and rhombs) a curve in the postabdominal spine in the space of the first and second principal component axes (analysis based on morphometric characters).

Table 2. Differences between females of various species of *Bythotrephes* (Kolmogorov-Smirnov test; significant values are in bold face).

Character	ced/bre	ced/lon	ced/tra	ced/cra	bre/lon	bre/tra	bre/cra	lon/tra	lon/cra	tra/cra
Lc	**<0.001**	>0.05	**<0.001**	**<0.001**	**<0.01**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	>0.05
TL	<0.001	<0.001	<0.05	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Lcd/Lc	**<0.001**	**<0.001**	**<0.001**	**<0.001**	>0.05	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Lco/Lab	**<0.001**	**<0.05**	**<0.001**	>0.05	**<0.001**	**<0.001**	**<0.001**	**<0.001**	>0.05	**<0.001**
Lco/Lcc1	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	>0.05	**<0.001**	**<0.001**
Lco/Licc	<0.001	<0.001	**<0.001**	**<0.001**	**<0.001**	**<0.001**	>0.05	**<0.05**	**<0.05**	**<0.001**
Lco/L4+5	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**< 0.01**	>0.05	**<0.001**	**<0.001**	**<0.001**
Lco/LA	>0.05	>0.05	**<0.001**	>0.05	>0.05	**<0.001**	>0.05	**<0.001**	>0.05	**<0.001**
Lab/Dab	**<0.01**	>0.05	**<0.001**	>0.05	**<0.01**	**<0.001**	**<0.05**	**<0.001**	>0.05	<0.001
Lcc1/D1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001	<0.01
Licc/D1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	>0.05	>0.05	>0.05

Lco – core body length; *Lab* – abdomen length; *Lcd* – postabdominal spine length; *Lcc1* – length of the I pair of caudal barbs; *Licc* – distance between the I and II caudal barbs pairs; *D1* – postabdominal spine diameter after the barbs I; *Dab* – abdomen diameter; *Lc* – body length; *TL* – total length; *L4+5* – total lengths of IV and V limbs segments on the first pair of appendages; *LA* – total lengths of the antenna II base and branch; *ced*, *B. cederstroemii* (localities 12–14); *bre*, *B. brevimanus* (25–27); *lon*, *B. longimanus* (28–29); *tra*, *B. transcaucasicus* (1); and *cra*, *B. crassicaudus* (2–7).*ced*, *B. cederstroemii* (localities 12–14); *bre*, *B. brevimanus* (25–27); *lon*, *B. longimanus* (28–29); *tra*, *B. transcaucasicus* (1); and *cra*, *B. crassicaudus* (2–7).

straight postabdominal spine, four groups could be separated. The first (*B. crassicaudus*) inhabits Verkhnekildinskoe and Pit'evoe lakes in the European Arctics, Severnaya Sosva and Enisey rivers in Siberia and Lake Sabanty-Kul in northern Kazakhstan. The second (*B. longimanus*) was found in Vierwaldstättersee and Geneva lakes in the northwestern part of the Alps. The third (*B. brevimanus*) was revealed in Vyshtinets and Anninskoe lakes in the Baltic Sea basin and in Lake Seliger in the Volga River basin. The fourth (*B. transcaucasicus*) was revealed in Lake Chaldyr in the Turkish Caucasus. In the PCA, this group has a small overlap with the first and third groups (Fig. 3).

Bythotrephes crassicaudus significantly differed from other taxa by largest total length and indexes *Lcd/Lc*, *Lco/Lcc1* and *Lcc1/D1* (Table 2); *B. transcaucasicus* by *TL*, *Lcd/Lc*, *Lco/Lab, Lco/Licc, Lco/L4+5, Lco/LA, Lab/Dab,* and *Lcc1/D1*; *B. brevimanus* by *Lc, TL, Lco/Lab, Lab/Dab, Lcc1/D1,* and *Licc1/D1*; *B. longimanus* by *TL*, *Lco/Licc, Lco/L4+5,* and *Lcc1/D1*; and *B. cederstroemii* by *TL*, *Lcd/Lc, Lco/Lcc1, Lco/Licc, Lco/L4+5, Lcc1/D1,* and *Licc/D1*.

The discriminant function analysis showed 100% success of identification of all five groups revealed in PCA. In the CDA, the first two canonical axes obtained 67% of the total discrimination. The values of the standardized coefficients indicated that the greatest contributions to discrimination on the first canonical axis were made by *Lcd* and *L5*, and on the second axis, by *Lcc1*. Among the "mixed" populations, such as in the Khotavets and Onega lakes, most individuals had a curved postabdominal spine (86-100%) and were grouped with *B. cederstroemii*. Specimens from Uglichskoe Reservoir, with both curved and straight postabdominal spines (13% with a curve), were close to *B. brevimanus*. The Severnaya Dvina River sample (7% with a curve) grouped with *B. crassicaudus*. Samples from the Gulf of Finland, Bol'shoe Cherlivoe and Chudskoe lakes, Gor'kovkoe and Rybinskoe reservoirs, with both curved and straight postabdominal spines (47-69% with a curve), as well as from Chuna and Suojarvi lakes with straight postabdominal spines, were in an intermediate position between *B. cederstroemii* and *B. brevimanus* (Fig. 4). The Siberian populations (Malye Chany, Maloe Sovetskoe and Podkovka lakes), with both curved and straight postabdominal spines (25-50% with a curve), were in an intermediate position between *B. cederstroemii* and *B. crassicaudus*.

Among eight putative loci studied, only *EstD-2* was found to be monomorphic in all samples. The allele frequencies at variable loci are shown in Table 3. The loci studied had 1-3 alleles in *B. cederstroemii*

Table 3. Allele frequencies for variable loci surveyed in *Bythotrephes* (*cra*, *B. crassicaudus*; *ced*, *B. cederstroemii*; *bre*, *B. brevimanus*; and contact zone, the geographical contact zone of *B. cederstroemii* and *B. brevimanus*).

Locus	**Allele**	***cra***	***ced***	**contact zone**								***bre***
		3	12	15	18	19	20	21	22	23	24	27
Alp	a							0.036		0.076	0.133	
	b											0.294
	c	0.467										
	d		0.038		0.250	0.147						
	e		0.423	0.333		0.015		0.143		0.141	0.067	
	f					0.235	0.563			0.141	0.233	
	g	0.533	0.538	0.667	0.750	0.603	0.438	0.821	1.000	0.641	0.567	0.706
Ca	a		1.000	1.000		0.333	0.200	1.000		0.200		
	b	0.200			1.000	0.667	0.800		1.000	0.800	1.000	1.000
	c	0.800										
Est-1	**a**	1.000	1.000	0.750		0.136	0.188	0.231		0.144	0.179	
	b			0.250	1.000	0.773	0.813	0.769	0.875	0.844	0.821	1.000
	c					0.091			0.125	0.011		
EstD-1	**a**	1.000	1.000	0.667		0.065	0.250	0.167		0.163	0.100	
	b			0.333	0.750	0.806	0.625	0.833	0.750	0.814	0.900	0.964
	c				0.250	0.129	0.125		0.250	0.023		0.036
sMdh	**a**	0.500	1.000	0.500	0.500	0.579	0.833	0.727	0.750	0.568	0.500	0.600
	b	0.500		0.500	0.500	0.421	0.167	0.273	0.250	0.432	0.500	0.400
sMdhp	**a**	0.450	0.500	1.000		0.278	0.800	0.550	0.500	0.477	1.000	0.550
	b	0.550	0.500		1.000	0.722	0.200	0.450	0.500	0.523		0.450
Prot-1	a		0.778	0.875		0.052	0.125	0.167		0.213	0.136	
	b	0.250	0.222	0.125	1.000	0.948	0.875	0.833	1.000	0.788	0.864	1.000
	c	0.750										
n		15	13	6	2	36	8	14	5	46	15	17
A		1.6	1.5	1.6	1.4	2.4	2.0	1.9	1.5	2.4	1.9	1.5
P		62.5	37.5	62.5	37.5	87.5	87.5	75.0	50.0	87.5	62.5	37.5
H_{obs}		0.34	0.09	0.24	0.25	0.28	0.30	0.28	0.22	0.27	0.22	0.22
H_{exp}		0.29	0.20	0.30	0.25	0.35	0.33	0.28	0.22	0.35	0.24	0.19
Cur		0	1.000	0.500	0	0.361	0.375	0.857	1.000	0.783	0.067	0
Str		–	0.984	0.856	0.025	0.166	0.248	0.427	0.055	0.303	0.200	0.018
Ced		–	0.92	0.17	0	0	0	0	0	0	0	0
Bre		–	0	0	1.00	0.78	0.63	0.29	0.80	0.59	0.53	1.00
Hyb		–	0	0.17	0	0.03	0.25	0.36	0	0.15	0.07	0
Int		–	0.08	0.67	0	0.19	0.13	0.36	0.20	0.26	0.40	0

Sample size (n), observed (H_{obs}) and expected (H_{exp}) heterozygosity expressed in percent, the percentage of polymorphic loci (*P*) and the average number of alleles per locus (*A*), proportions of specimens with a curve in the postabdominal spine (Cur), the *B. cederstroemii* membership based of STRUCTURE analysis (Str), and the percentage of hybrid classes based of NEW HYBRIDS analysis: *Ced* – pure parental *B. cederstroemii*; *Bre* – pure parental *B. brevimanus*; *Hyb* and *Int* – hybrids and intermediate individuals between *B. cederstroemii* and *B. brevimanus* (respectively).Locality numbers as in Table

and 1-2 alleles in *B. crassicaudus* and *B. brevimanus*. The values of observed heterozygosity in *B. crassicaudus* were higher than those in *B. cederstroemii* and *B. brevimanus*, i.e. 0.34 *vs.* 0.09 and 0.22 (Table 3). In populations of these species, no deviations from the expected Hardy-Weinberg equilibrium were observed.

Only one locus (*Ca*) was diagnostic for the pair *B. cederstroemii* (Lake Lexozero) and *B. crassicaudus* (Lake Pit'evoe), two loci (*Est-1* and *EstD-1*) for the pair *B. crassicaudus* and *B. brevimanus* (Lake Vyshtinets), and three loci (*Ca*, *Est-1* and *EstD-1*) for the pair *B. cederstroemii* and *B. brevimanus*. The mean genetic

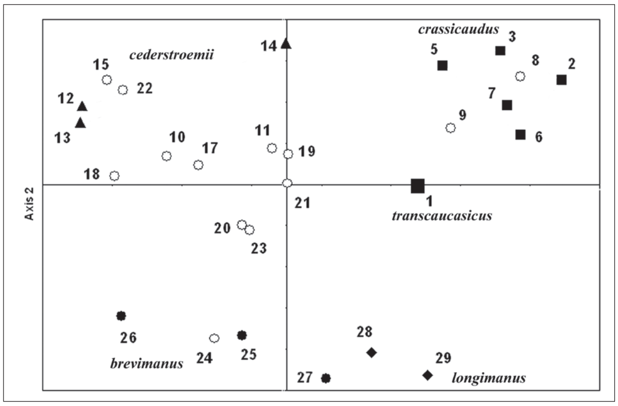

Fig. 4. Plot of centroids for females of *Bythotrephes cederstroemii* (black triangles), *B. crassicaudus* (small black squares), *B. transcaucasicus* (large black squares), *B. longimanus* (black diamonds), *B. brevimanus* (black circles), and "mixed" populations (white circles) in the space of the first and second canonical discriminant axes (analysis based on morphometric characters). Numbers represent localities, which are given in Table 1.

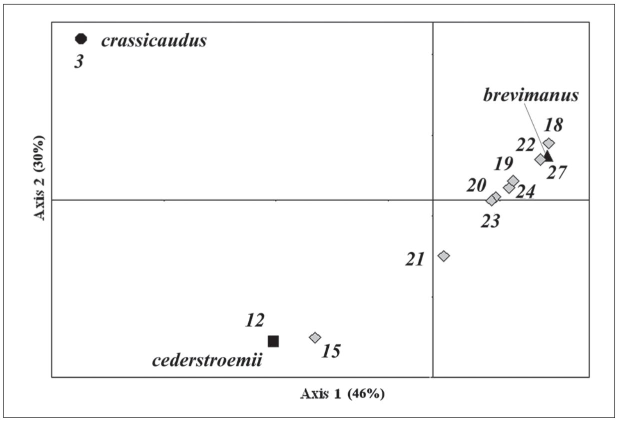

Fig. 5. Multiple correspondence analysis among *Bythotrephes* populations based on allozyme data. *B. crassicaudus* (black circle), *B. cederstroemii* (black square), *B. brevimanus* (black triangle), and populations from the contact zone of *B. cederstroemii* and *B. brevimanus* (gray diamonds). Numbers represent localities, which are given in Table 1.

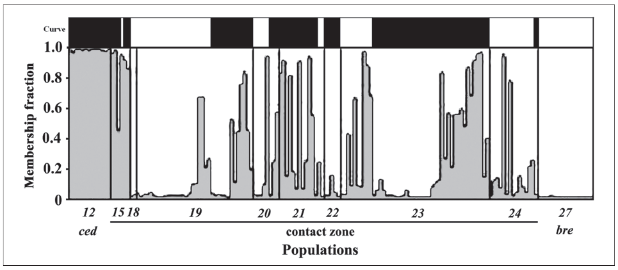

Fig. 6. Bayesian clustering results of the STRUCTURE analysis based on allozyme data. Each individual is represented as vertical line partitioned into colored segments: gray bar to *Bythotrephes cederstroemii* and white bar to *B. brevimanus*, whose length is proportional to the individual's estimated membership fraction in the K=2 cluster. Black lines separate individuals of different populations. The top incretion (Curve) shows the presence (black) or absence (white) of a curve in the postabdominal spine for each specimen studied. Populations of *B. cederstroemii* (ced), *B. brevimanus* (bre) and from "mixed" populations from the contact zone of *B. cederstroemii* and *B. brevimanus* are numbered as in Table 1.

distance (D_{Nei}) between *B. crassicaudus* and *B. cederstroemii* was 0.326, between *B. crassicaudus* and *B. brevimanus* 0.760, and between *B. cederstroemii* and *B. brevimanus* 0.867.

In the MCA analysis, the first two coordinate dimensions explained 46% and 30% (respectively) of the total genetic variance in *Bythotrephes*. Along these coordinate dimensions, populations from Lexozero (*B. cederstroemii*), Pit'evoe (*B. crassicaudus*), and Vyshtinets (*B. brevimanus*) lakes were clearly separated (Fig. 5). The majority of populations with intermediate morphological characters were intermediate in the MCA analysis as well. Only populations from Suojarvi and Khotavets lakes were close to *B. brevimanus*.

STRUCTURE analysis distinguished *B. cederstroemii* from Lake Lexozero and *B. brevimanus* from Lake Vyshtinets as distinct genetic clusters (Fig. 6). The six populations with intermediate morphological characters (the Gulf of Finland, Suojarvi, Khotavets and Chudskoe lakes, Rybinskoe and Uglichskoe reservoirs) had 70-97% of genetic contribution of *B. brevimanus*, whereas a sample from Lake Onega had the majority (86%) of *B. cederstroemii* membership (Fig. 7; Table 3). A population from Gor'kovskoe Reservoir provided a nearly equal genetic contribution of *B. cederstroemii* (43%) and *B. brevimanus* (57%). We found no strong correlation between STRUCTURE results and the presence or absence of a curve in postabdominal spines in individuals from the "mixed" populations originating from the geographical contact zone of *B. cederstroemii* and *B. brevimanus* (Fig. 6 and Table 3).

NEW HYBRIDS analysis found no samples containing both parental *B. brevimanus* and *B. cederstroemii* together (Table 3). We revealed hybrids between

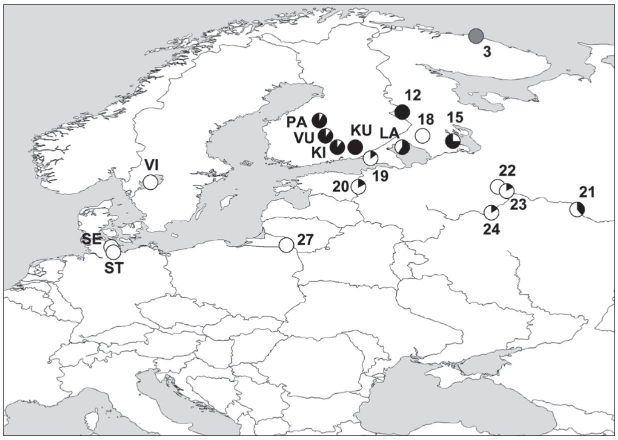

Fig. 7. Map showing distribution of alleles of *B. crassicaudus* (gray), *B. cederstroemii* (black) and *B. brevimanus* (light) based on previously published data (Berg and Garton 1994; Berg et al. 2002) and our data. Our localities are numbered as in Table 1. SE – Selenter, ST – Stocksee, VI – Winem, PA – Paijanne, VU – Vuohijarvi, KI – Kivijarvi, KU – Kuolimo, and LA – Ladoga lakes.

B. brevimanus and *B. cederstroemii* in six populations from the contact zone. The most abundant hybrids were in Gor'kovkoe Reservoir and Lake Chudskoe (36 and 25%, respectively). In Lake Onega, hybrids composed 17% of the population, in Rybinskoe Reservoir 15%, in Uglichskoe Reservoir 7% and in the Gulf of Finland 3% (Table 3).

DISCUSSION

Our morphological study clearly revealed the division of the genus *Bythotrephes* into five groups, corresponding to *B. longimanus*, *B. crassicaudus*, *B. brevimanus*, *B. transcaucasicus*, and *B. cederstroemii*. The obvious differences between three of them (*B. crassicaudus*, *B. brevimanus* and *B. cederstroemii*) were also found in our allozyme analysis. Widespread hybridization was detected where *B. cederstroemii* and *B. brevimanus* have geographic overlaps. Populations from the contact zone, which are usually characterized by intermediate morphological characters, consisted of hybrids (as well as back-crossers) and individuals of one parental species. Hybridization between closely related species is known in some other planktonic cladocerans (Spaak et al., 2004). The relatively low number of hybrids (0-36%) in the contact zone of *B. brevimanus* and *B. cederstroemii* might reflect its reduced fertility and could be a sign of strong selection against hybrids. In the light of these data, despite hybridization, the two lineages *B. brevimanus* and *B. cederstroemii* appear to maintain their independence as distinct evolutionary species (de Queiroz, 2005).

The average genetic difference between *B. crassicaudus* and *B. brevimanus* was nearly the same (D_{Nei} = 0.76) as between *B. cederstroemii* and *B. brevimanus* (0.87). Therefore, we propose to consider *B. crassicaudus* as a separate species as well. Additionally, our morphological study confirmed a distinct position of the West-Alpine (*B. longimanus*) and Caucasian (*B. transcaucasicus*) populations. These forms are significantly different from each other and from the other three species studied, and have allopatric distribution. Therefore, we support the taxonomic status of these forms as separate species.

We uncovered discordance between our data and the results obtained by Therriault et al. (2002), which sequenced a part of the mitochondrial gene COI. The latter authors did not find any significant differences between populations with straight and curved postabdominal spines. The dissimilarity of results obtained in studies of nuclear and mitochondrial markers could be explained by the weak variation of marker used or by the influence of historical or recent asymmetric introgression of mitochondrial DNA, which, in some cases, caused a partial or total mitochondrial DNA replacement (Petit and Excoffier, 2009; Zieliński et al., 2013). In any case, this situation needs further research.

Important information about the distribution of *B. brevimanus* ("*B. longimanus*") and *B. cederstroemii* in the Baltic Sea region provided the previously published allozyme data (Berg and Garton, 1994; Berg et al., 2002). According to them, the Pep-1 locus seems to show diagnostic differences between *B. brevimanus* and *B. cederstroemii*. Populations from Germany and Sweden possess 99-100% of the F allele, which is characteristic for *B. brevimanus*. Other populations are characterized by a predominance of the S allele (Lake Ladoga – 61%, Finland lakes – 86-100%, and

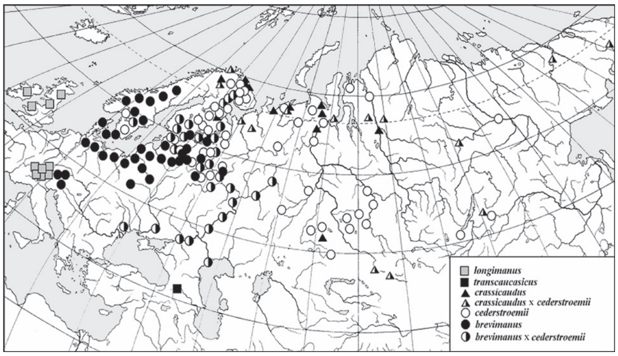

Fig. 8. Map of presumed distribution of five *Bythotrephes* species (including "mixed" populations) based on our results and literature data. References are mentioned in Discussion.

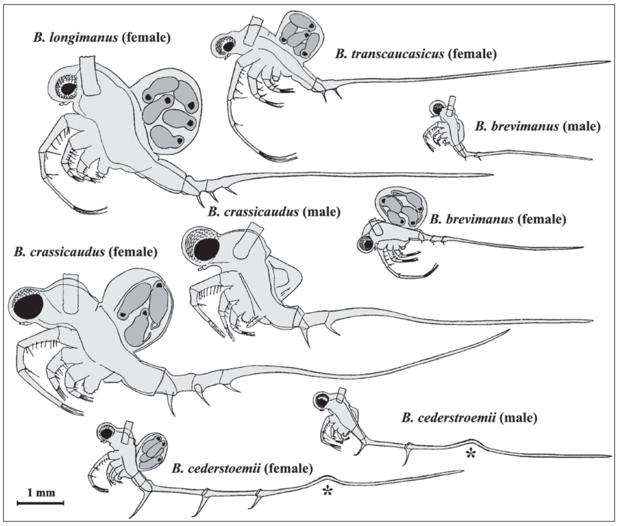

Fig. 9. The lateral view of mature adult parthenogenetic females and males. Antenna II has been removed for clarity. All drawings are in the same scale. * is a curve in the postabdominal spine. All drawings are original.

the Great Lakes of North America – 96%), which is characteristic for *B. cederstroemii*.

Based on the results of our study and literature data hereinafter mentioned (Fig. 8), we can outline the presumed distribution and specify diagnostic characters of the species studied. *Bythotrephes crassicaudus* are characterized by a straight and short postabdominal spine (Fig. 9), backward-directed posterior pair of caudal barbs, large core body length (2.8-6.0 mm in mature females and 2.5-3.4 mm in mature males), 2-3 pairs of caudal barbs in mature females, and low values of indexes *Lcd/Lc* (range 1.7-2.4), *Lco/Lcc1* (3.3-5.3) and *Lcc1/D1* (1.1-2.2) in mature females. This species inhabits small tundra waterbodies in the Arctic part of European Russia (from the Kola Peninsula to the Ural), Siberia (from the Ural to Yakutia Republic) and steppe Sabanty-Kul Lake in northern Kazakhstan (Levander, 1901; Sars, 1903; Sokolova, 1972; Vekhov, 1987). In large river deltas and lakes (i.e., Severnaya Dvina River delta, Podkovka, Maloe Sovetskoe and Malye Chany lakes), the distributions of *B. crassicaudus* and *B. cederstroemii* can overlap. These waterbodies could be inhabited by individuals with intermediate morphological characters. Similar individuals were previously described from small waterbodies near the villages of Muoniovara and Karesuando in the northernmost part of Sweden and adjacent regions of Norway (Sars, 1890; Lilljeborg, 1901), Nyakshingda, Bol'shoe Eravnoe, Burylakh lakes, Yamal Peninsula, Vilyuyskoe Reservoir and Anadyr' River in Siberia (Voronkov, 1911; Kozhov, 1950; Sokolova, 1972; Streletskaya, 1975; Shevelyova, 1981).

Bythotrephes transcaucasicus has a very small range (Fig. 8). It was known from four alpine lakes: Lake Chaldyr (= Çıldır) in northeastern Turkey, Lake Arpa (= Arpilich Reservoir) in Armenia, and Tabatskuri and Toporovan lakes in Georgia (Shikleev, 1929; Behning, 1941). However, the lack of recent observations of the species allows us to consider that this species is extinct. This species differed from others by its straight postabdominal spine (Fig. 9), backward-directed posterior pair of caudal barbs, large core body length (3.1-3.8 mm in mature females), two pairs of caudal barbs in mature females, low values of indexes *Lcd/Lc* (1.4-2.0) and *Lco/Lab* (2.5-3.7) and high values of indexes *Lco/LA* (1.3-1.6) and *Lab/Dab* (1.4-2.4) in mature females.

Bythotrephes longimanus inhabits alpine lakes in Switzerland, and adjacent western Austria, southern Germany and eastern France (Pellose, 1934; Ischreit, 1939; Korovchinsky, 2015). Further studies are needed to clarify the taxonomic status of populations from northern Italy (e.g. Maggiore and Como lakes), western Austria and the British Islands. *Bythotrephes longimanus* is characterized by a straight postabdominal spine, backward-directed posterior pair of caudal barbs, moderate core body length (1.7-2.8 mm in mature females), two pairs of caudal barbs in mature females, and low values of the index *Lco/L4+5* (0.9-1.3) in mature females.

Bythotrephes brevimanus is usually characterized by a straight postabdominal spine (Fig. 9), small body length (1.0-1.8 mm in mature females), very short and backward-directed caudal barbs (of which there are three pairs in mature females), low values of the index *Lcc1/D1* (0.6-1.3) and high values of indexes *Lco/Lab* (4.6-9.7), *Lco/Lcc1* (9.5-26.4) and *Licc/D1* (1.7-4.7) in mature females. This species inhabits northern Germany and Poland (Flößner, 1972), Denmark (Müller, 1867; Ischreit, 1936), Sweden (Lilljeborg, 1901; Nilsson, 1979), the Baltic Republics (Ischreit, 1930), Byelorussia (Rylov, 1915; Petrovich, 1956; Cheremisova, 1960), Shatskie lakes in northwestern Ukraine (Yalynskaya, 1949), and the western part of European Russia (Gryoze, 1933; Salazkin, 1971). Populations from western Austria (*B. styriacus*) are morphologically close to *B. brevimanus* as well (Ischreyt, 1939).

Bythotrephes cederstroemii is distributed in some lakes of Sweden (Lilljeborg, 1901), Estonia (Myaemets, 1966; Haberman, 1977) and in the forest zone of the northwestern part of European Russia (Korovchinsky, 2015), the Ural Mountains (Ulomsky, 1964), northern Kazakhstan (Tseeb, 1940; Malinovskaya, 1961; Smirnova and Ibrasheva, 1979) and Siberia with the Lena River basin as the easternmost limit of the range (Rivier and Grigorovich, 1999; Korovchinsky, 2015). This species has a long and curved postabdominal spine (Fig. 9), small or moderate body length (1.3-2.8 mm in mature females and 1.1-2.4 mm in mature males), very long and forward-directed caudal barbs, and three pairs of caudal barbs in mature females. In Sweden, Finland, and the western part of European Russia this species forms a contact zone with *B. brevimanus* (Volga River reservoirs, and some lakes in the basin of the Baltic and White seas), which usually inhabits both hybrids and a parental species (Fig. 8). These populations are usually characterized by intermediate morphological characters.

Thus, our data clearly show that the genus *Bythotrephes* is represented by at least five closely related species having allo- and parapatric ranges. In the cases of parapatry, species can form wide hybrid zones where hybrids usually coexist with one of the parental species.

Acknowledgments: The authors would like to thank Prof. I.K. Rivier for his great help and support, L.F. Antipova, N.G. Bayanov, P.I. Krylov, D. Kulakov, V.I. Lazareva, M.M. Mel'nik, V.E. Panov, N.V. Polyakova, L.A. Stepanova, A.B. Vasil'eva, and V.Yu. Zakharov for providing data about the distribution of *Bythotrephes* species.

Authors' contributions: The first author was responsible for all morphological and biochemical studies, and the second for statistical analyses and writing the paper.

Conflict of interest disclosure: The authors have no conflicts to report.

REFERENCES

Agnesotti, A. (1935). I bitotrefi del Lario. *Atti Soc. Ital. Sci. nat. Mus. Civ. Stor. Natur. Milano* **74**, 157-172.

Anderson, E.C. and *E.A. Thompson* (2002). A model-based method for identifying species hybrids using multilocus genetic data. *Genetics.* **160**, 1217-1229.

Behning, A.L. (1941). *Cladocera of the Caucasus*. Gruzmedgiz, Tbilisi.

Berg, D.J. and *D.W. Garton* (1994). Genetic differentiation in North American and European populations of the cladoceran *Bythotrephes*. *Limnol. Oceanogr.* **39**, 1503-1516.

Berg, D.J., Garton, D.W., MacIsaac, H.J., Panov, V.E. and *I.V. Telesh* (2002). Changes in genetic structure of North American *Bythotrephes* populations following invasion from Lake Ladoga, Russia. *Freshwater Biol.* **47**, 275-282.

Cheremisova, K.A. (1960). Observations of biology of *Bythotrephes longimanus* Leydig and *Leptodora Kindti* (Focke). *Trudy Belorusskogo NII Rybnogo Khozyaystva, Minsk.* **3**, 125-130.

Colautti, R.I., Manca, M., Viljanen, M., Ketelaars, H.A.M., Bürgi, H., MacIsaac, H.J. and *D.D. Heath* (2005). Invasion genetics of the Eurasian spiny waterflea: evidence for bottlenecks and gene flow using microsatellites. *Mol. Ecol.* **14**, 1869-1879.

de Queiroz, K. (2005). Ernst Mayr and the modern concept of species. *Proc. Natl. Acad. Sci. USA* **102**, 6600-6607.

Falush, D., Stephens, M. and *J.K. Pritchard* (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics.* **164**, 1567-1587.

Flößner, D. (1972). Über familie Polyphemoidea (Onychopoda), In: *Kiemen-und Blattfüßer, Branchiopoda; Fischläuse,* *Branchiura. Die Tierwelt Deutschlands*, 60, 386-407. G. Fischer Verlag, Jena.

Grigorovich, I.A., Pashkova, O.V., Gromova, Y.F. and *C.D.A. Van Overdijk* (1998). *Bythotrephes longimanus* in the Commonwealth of Independent States: variability, distribution and ecology. *Hydrobiologia.* **379**, 183-198.

Gryoze, B.S. (1933). Limnological issue of Valday Lakes and their preliminary fish-economical estimation. *Izvestiya VNIORKh, Leningrad.* **16**, 66-128.

Haberman, J. (1977). Seasonal dynamics of pelagic cladocerans of lakes Pepsi-Pinkva and Vortsjarv. *Izvestia AS Eston. SSR.* **26** (Biology), 2, 108-119.

Hessen, D.O., Bakkestuen, V. and *B. Walseng* (2011). The ecological niches of *Bythotrephes* and *Leptodora*: lessons for predicting long-term effects of invasion. *Biol. Invas.* **13**, 2561-2572.

Ischreyt, G. (1930). Über Korperbau und Lebensweise des *Bythotrephes longimanus* Leydig. *Arch. Hydrobiol.* **21**, 241-323.

Ischreyt, G. (1935 [1934]). Über *Bythotrephes cederstroemi* Schödler. *Int. Revue ges.* Hydrobiol. Hydrogr. **31**, 181-202.

Ischreyt, G. (1936 [1935]). Über die Variabilitat von *Bythotrephes*. *Arch. Hydrobiol.* **29**, 130-136.

Ischreyt, G. (1939). Über den *Bythotrephes* subalpiner Seen. *Arch. Hydrobiol.* **34**, 105-129.

Korovchinsky, N.M. (2015). Redescription of *Bythotrephes longimanus* Leydig, 1860 and *B. cederströmii* Schödler, 1877 (Crustacea: Cladocera: Onychopoda), with notes on the morphology and systematics of the genus *Bythotrephes* Leydig, 1860. *Zootaxa.* **3955**(1), 1-44.

Kotov, A., Forró, L., Korovchinsky, N.M. and *A. Petrusek*. World checklist of freshwater Cladocera species. (2013). http://fada.biodiversity.be/group/show/17

Kozhov, M.M. (1950). *Presnye Vody Vostochnoy Sibiri (Basseyn Baykala, Angary, Vitima, Verkhnego Techeniya Leny i Nizhney Tunguzki) [Fresh waters of Eastern Siberia (bassins of Baikal, Angara, Vitim, upper parts of Lena and Nizhnyaya Tunguzka rivers)]*. Irkutskoe Oblastnoe Gosudarstvennoe Izdatel'stvo, Irkutsk.

Leydig, F. (1860). *Naturgeschichte der Daphniden*. H. Laupp'sche Buchhandlung, Tubingen.

Levander, K.M. (1901). Beitrage zur Fauna and Algenflora der sussen Gewasser an der Murmankuste. *Acta Soc. Fauna Flora Fenn. Helsingfors.* **20**(8), 1-35.

Lilljeborg, W. (1901). Cladocera Sueciae. *Nova* Acta Reg. *Soc. Sci.* Upsaliensis. **19**, 1-701.

Litvinchuk, L.F. (2002). *Systematics and distribution of representatives of the family Cercopagidae (Crustacea, Cladocera) in Northwest Russia*. Ph.D. Thesis. Zoological Institute of Russian Academy of Sciences, St. Petersburg.

Litvinchuk, L.F. (2005). On history of systematics and distribution of representatives of the genus *Bythotrephes* (Polyphemoidea, Cladocera) on the territory of Russia and adjacent countries, In: *Biologicheskie Resursy Vnutrennikh Vod: Bespozvonochnye* (Ed. I.K. Rivier), 224-240. Rybinskiy dom pechati, Rybinsk.

Litvinchuk, L.F. (2007). Systematics and biology of the genus *Bythotrephes*, In: *Vetvistousye Rakkobraznye: Sistematika i Biologia* (Eds. N.N. Smirnov, N.M. Korovchinsky, and A.V. Krylov), 173-198. Vektor TiS, Nizhniy Novgorod.

MacIsaac, H.J., Ketelaars, H.A.M., Grigorovich, I.A., Ramcharan, C.W. and *N.D. Yan* (2000). Modelling *Bythotrephes longimanus* invasions in the Great Lakes basin on its European distribution. *Arch. Hydrobiol.* **149**, 1-21.

Malinovskaya, A.S. (1961). The influence of the salinity factor on the distribution of Cladocera in North Kazakhstan lakes, In: *Trudy Konferentsii po Rybnomu Khozyaysyvu Respublik Sredney Azii i Kazakhstana*, 195-201. Frunze.

Manuylova, E.F. (1964). *Vetvistousye Rachki (Cladocera) Fauny SSSR [Cladoceran Fauna of USSR]*. Moscow-Leningrad.

Martin, J.W. and *C.E. Cash-Clark* (1995). The external morphology of the onychopod cladoceran genus *Bythotrephes* (Crustacea, Branchiopoda, Onychopoda, Cercopagididae), with notes on the morphology and phylogeny of the order Onychopoda. *Zool. Scripta.* **24**, 61-90.

Miller, M.P. (1997). Tools for Population Genetic Analyses (TFPGA) 1.3. http://www.marksgeneticsoftware.net/

Mordukhai-Boltovskaya, E.D. (1959). On the systematics of the genus *Bythotrephes* Leydig (Cladocera). *Byul. Inst. Biol. Vodokhranilishch AN SSSR.* **4**, 29-32.

Müller, P.E. (1867). Denmarks Cladocera. *Schiödtes Naturhist. Tidsskrift.* **3**, 53-240.

Myaemets, A.Kh. (1966). Summer zooplankton of Pskov-Chudskoe Lake. In: *Hydrobiol. Stud.*, 4, 80-96. Tallin.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Proc. Natl. Acad. Sci. USA.* **70**, 3321-3323.

Nielsen, E.E., Bach, L.A. and *P. Kotlicki* (2006). Hybridlab (version 1.0): a program for generating simulated hybrids from population samples. *Mol. Ecol. Notes.* **6**, 971-973.

Nilsson, N-A. (1979). Food and habitat of the fish community of the offshore region of Lake Vänern, Sweden. *Inst. Freshwater Res. Drottningholm Lund.* **58**, 26-139.

Pellose, J. (1934). *Etude sur la faunedes Cladoceres et des Copepodes de la region moyenne des Alpes francaises*. Lion.

Petit, R.J. and *L. Excoffier* (2009). Gene flow and species delimitation. *Trends Ecol. Evol.* **24**, 386-393.

Petrovich, P.G. (1956). Species composition of zooplankton of fish-economic lakes of the western provinces of Byelorussia SSR. *Uchyonye Zapiski Belorusskogo Gosudarstvennogo Universiteta Seriya Biologicheskaya, Minsk.* **26**, 3-39.

Pritchard, J.K., Stephens, M. and *P. Donnelly* (2000). Inference of population structure using multilocus genotype data. *Genetics.* **155**, 945-959.

Rivier, I.K. (1998). *The Predatory Cladocera (Onychopoda: Podonidae, Polyphemidae, Cercopagidae) and Leptodoridae of the World. Guides to the Identification of the Microinvertebrates of the Continental Waters of the World,* 13. Backhuys Publishers, Leiden.

Rivier, I.K. and *I.A. Grigorovich* (1999). Biology of *Bythotrephes* Leydig (Crustacea, Cladocera, Onychopoda): outcomes of study. *Gidrobiol. Zh. Kiev.* **35**(5), 13-40.

Rylov, V.M. (1915). On plankton of some lakes of Vitebsk Province. Fauna of Copepoda and Cladocera. *Trudy Petrogradskogo Obschestva Estestvoispytateley, Petrograd.* **44**(4), 111-162.

Salazkin, A.A. (1971). Zooplankton in oligotrophic lakes of humid zone of USSR. *Gidrobiol. Zh. Kiev* **7**(3), 31-38.

Sars, G.O. (1890). Oversigt af Norges Crustaceer med forelobige Bemaerkninger over de nye eller mindre bekjendte Arter. II (Branchiopoda - Ostracoda - Cirripedia). *Forhandl Vidensks-Selskab Christiania.* **1**, 1-80.

Sars, G.O. (1903). On the crustacean fauna of Central Asia. Ann. Mus. Zool. Acad. Imp. Sci. St.-Pétersbourg. **8**, 157-187.

Schödler, J.E. (1877). Mittheilungen zur Diagnose einiger Cladoceren. *Sitzungs-berichte Ges. Naturf. Freunde Berlin*, 232-233.

Scourfield, D.J. and *J.P. Harding* (1966). *A Key to the British Freshwater Cladocera: with Notes on Their Ecology. Third edition.* Freshwater Biological Association Scientific Publication, 5.

Shaw, C.R. and *R. Prasad* (1970). Starch gel electrophoresis of enzyme: a compilation of recipes. *Bioch. Genet.* **4**, 297-320.

Shevelyova, N.G. (1981). Zooplankton. In: *Ozyora Severo-Zapada Sibirskoy Platformy*, 123-135. Nauka, Novosibirsk.

Shikleev, S.M. (1929). Materials on fauna of Cladocera of the Kavkazskiy State Nature Reserve and the Black Sea coast. *Trudy Severo-Kavkazskoy Assotsiatsii Nauchno-Issledovatel'skikh Institutov, Rostov-na-Donu.* **69**(13), 5-46.

Smirnova, V.A. and *S.I. Ibrasheva* (1979). Faunistic complexes of Cladocera in Kazakhstan. *Izv. AN Kazakh. SSR Ser. Biol. Alma-Ata.* **5**, 26-30.

Sokolova, V.A. (1972). Zooplankton of lakes of Kolyma-Indigirka Lowland. In: *Rybokhozyaystvennoe Osvoenie Ozyor Basseyna Sredney Kolymy*, 87-108. Yakutsk.

Spaak, P., Denk, A., Boersma, M. and *L.J. Weider* (2004). Spatial and temporal patterns of sexual reproduction in a hybrid *Daphnia* species complex. *J.Plankton Res.* **26**, 625-635.

Streletskaya, E.A. (1975). List of Rotifera, Cladocera and Copepoda in waterbodies of Kolyma and Anadyr' rivers basin. In: *Gidrobiologicheskie Issledovaniya Vnutrennikh Vodoyomov Severo-Vostoka SSSR*, 32-60. Vladivostok.

Swofford, D.L. and *R.B. Selander* (1999). *BIOSYS-2: a computer program for the analysis of allelic variation in population genetics and biochemical systematics, Release 2.0.* University of Illinois, Urbana.

Therriault, T.W., Grigorovich, I.A., Cristescu, M.E., Ketelaars, H.A.M., Viljanen, M., Health, D.D. and *H.J. MacIsaac* (2002). Taxonomic resolution of the genus Bythotrephes Leydig using molecular markers and re-evaluation of its global distribution. *Div. Distr.* **8**, 67-84.

Tseeb, Ya.Ya. (1940). On study of fauna of lakes of Northern Kazakhstan in relation to their fish-economic value. *Uchyonye Zapiski Orlovskogo Pedagogicheskogo Instituta, Ser Estestvoznanie i Khimiya, Orel.* **1**, 37-125.

Ulomsky, S.N. (1964). Plankton of Turgoyak Lake. *Trudy Ural'skogo Otdeleniya GosNIORKh, Sverdlovsk.* **6**, 83-91.

Vekhov, N.V. (1987). Peculiarities of distribution, biology and morphological variability of *Bythotrephes longimanus* (Leydig) s. lat. in European Subarctics. *Biol. Nauki Moscow.* **2**, 27-35.

Voronkov, N. (1911). Plankton of waterbodies of the Yamal Peninsula. Rotifera and general characteristics of plankton. Ann. Mus. Zool. Acad. Imp. Sci. St.-Pétersbourg. **16**, 180-214.

Yalynskaya, N.S. (1949). Hydrobiological issue of Shatsk group of lakes in Volyn' Province (preliminary report). *Trudy NII Prudovogo i Ozyorno-Rechnogo Khozyaystva, Kiev.* **6**, 133-151.

Yan, N.D., Leung, B., Lewis, M.A. and *S.D. Peacor* (2011). The spread, establishment and impacts of the spiny water flea, *Bythotrephes longimanus*, in temperate North America: a synopsis of the special issue. *Biol. Inv.* **13**, 2423-2432.

Yurista, P.M. (1992). Embryonic and postembryonic development in *Bythotrephes cederstroemii*. *Can. J. Fish. Aquat. Sci.* **49**, 1118-1125.

Zieliński, P., Nadachowska-Brzyska, K., Wielstra, B., Szkotar, R., Covaciu-Marcov, S.D., Coğalniceanu, D. and *W. Babik* (2013). No evidence for nuclear introgression despite complete mtDNA replacement in the Carpathian newt (*Lissotriton montandoni*). *Mol. Ecol.* **22**, 1884-1903.

Zozulya, S.S. and *F.D. Mordukhai-Boltovskoy* (1977). Seasonal variability of *Bythotrephes longimanus* Leydig (Crustacea, Cladocera). *Doklady AN SSSR.* **232**, 493-495.