Parasitic copepods *Caligus lacustris* (Copepoda: Caligidae) on the rainbow trout *Oncorhynchus mykiss* in cage aquaculture: morphology, population demography, and first insights into phylogenetic relationships

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Abstract

Introduction Data on rainbow trout infection with the copepod *Caligus lacustris* in cage aquaculture on Lake Ladoga is presented.

Materials and Methods *Caligus lacustris* (n=127 ex.) were collected from a farm in Lake Ladoga housing cage-reared rainbow trout to describe the size-age and sex structure of the copepod population. Morphological features of the copepods were evaluated according to 10 characters with terminology proposed by Kabata, Gusev (1966). To determinate the phylogenetic position of *C. lacustris* within the genus *Caligus*, fragments of the cytochrome c oxidase subunit 1 mitochondrial gene (COI, 645 bp) and 18S rRNA gene (1617 bp) were sequenced.

Results An increase of parasite prevalence was observed as the lake was warming up from July to September. The morphological features of the crustacean's larval and adult stages, characterized by specific parameters of quantitative traits, are described.

Three COI haplotypes and only one 18S rRNA haplotype of *C. lacustris* were identified among five samples. Based on 18S rRNA analysis (resolution of the COI tree was poor) we can conclude that the clade containing *C. lacustris,* and the aforementioned sister species, appears as an early radiation of the genus *Caligus*.

Conclusions The development of freshwater aquaculture contributes to the transfer of the native parasite *Caligus lacustris* to farmed rainbow trout.

Keywords: Lake Ladoga, 18S rRNA, COI, age structure, sex ratio aquaculture, rainbow trout, parasitic copepods, *Caligus lacustris*

Introduction

Intensive development of the cage aquaculture industry has a wide range of complicated impacts on aquatic ecosystems, including the spread of various pathological agents (viruses, bacteria, fungi, as well as protozoan and metazoan parasites) with fish transported from other regions or farms. These pathogens can be very dangerous for the cage aquaculture industry, with serious ecological and animal welfare consequences [Skov et al 2014; Shinn et al 2015]. The Republic of Karelia (north-east part of Russia) is a very important region for cage aquaculture (mainly salmonids) with annual production around 21,000 t of fish products, where 97% is rainbow trout. The main parasitic diseases in caged trout are ichthyobodosis (costiasis), ichthyophthiriasis (white spot disease), gyrodactylosis, digenean trematode infection, crustacean diseases (e.g. argulosis), among many others [Ieshko et al. 2016]. The incidence of crustaceosis has high epizootic significance and poses a serious problem in fish farming. The issue of parasitic diseases is especially acute in cage farms situated in large lakes where the endemic surrounding habitat has a diverse structure including many ecologic niches and a diverse composition of aquatic organisms, including parasites [Jørgensen et al 2009]. Caligids live in fresh, marine, and brackish waters and are capable of infecting a wide range of hosts [Rahkonen et al 2013]. Only one species of caligids is found in fresh waters within the Palearctic region – *Caligus lacustris* Steenstrup et Lütken, 1861 [Kabata, 1992]. These copepods are localized on the skin and gills, cause anxiety in fish, disrupt the integrity of external surfaces and lead to skin damage, lesions, and bleeding. Overt significant damage to external epithelia can be a source of detrimental alterations to osmotic balance of the entire host organism. In the case of mass infection, injured fish are easily exposed

to bacterial and fungal infections, which can lead to mortalities and economic losses [Pike 1999, Johnson 2004, Costello 2006].

Parasitic copepods have made numerous incursions into freshwater bodies over millennia, with subsequent evolutionary diversification (Boxshall et al. 2000). Caligus as a genus is only sparsely represented in freshwater bodies. However, in ancient lakes (more than 1000,000 years old) where aquatic copepods exist there is a high rate of endemism and species diversity (Gorthner 1994; Boxshall et al., 2000). New analyses have given insight to existing paradigms with potential revisions needed to current taxonomy after the identification of convergent evolution of some physical features (Freeman et al., 2013). This adds more importance for molecular analyses to be combined with morphological characterizations. Further, new species continue to be discovered within the genus Caligus (Oines et al., 2005). The significance of this group of parasites to aquaculture and the recent discoveries of new species and revisions of taxonomies warrants continued phylogenetic analyses, particularly in the case of freshwater species for which a high rate of endemism is already known.

Lake Ladoga is the largest body of freshwater in Europe where 56 species of fish were registered. Over the past 3 years, Lake Ladoga has become a very important place for cage trout farming with an increase in total trout production from 4,756 to 8,123 tonnes (Report of Ministry of Forestry and Fisheries of Karelia Republic). The outbreak of crustaceosis diseases caused by several species of parasitic copepods in trout from Lake Ladoga has lead to high mortality and serious economical losses. One of these parasitic copepods is *C. lacustris* which was first registered in Lake Ladoga in 1934 on whitefish (*Coregonus albula*) [Markewitsch, 1934]. Later on, Rumyantsev (2007) found that *C. lacustris* in the lake may also infect large numbers of various fishes like *Coregonus albula, C. lavaretus, Salmo trutta m. lacustris, S. salar, Osmerus eperlanus, Lota lota, Vimba vimba, Stizostedion lucioperca, Gimnocehalus cernua, Blicca bjoerkna, Abramis ballerus, A. brama,* and *Leuciscus cephalus*. The copepods were recorded primarily on juvenile whitefish in the northern and southern parts of the lake [Barysheva 1949, Barysheva, Bauer 1957, Bauer, Nikolskaya 1957, Rumyantsev et al 2001]. In recent years, there has been an expansion in the habitats of *C. lacustris* and an increase in the infection rate of fish species common in Lake Ladoga: whitefish and smelt [Anikieva et al 2018]. To date, outbreaks of crustaceosis caused by *C. lacustris* have been registered annually on farmed rainbow trout in Lake Ladoga.

Although *C. lacustris* has a great economical impact on the trout cage aquaculture industry, to date, there is only morphological description of larvae and adult stages of *C. lacustris* [Kozikowska, 1958; Gusev, Kabata, 1991] and an absence of molecular genetics analyses.

Hence, the main aim of the present study was to determine the phylogenic position of *C. lacustris*, as a single freshwater species, inside the genus *Caligus* and among other closely related species.

Furthermore, we documented any seasonal variations in the occurrence of *C. lacustris* infections on rainbow trout in fish farms in the lake in order to monitor any annual cycles of activity of this parasite. The morphological descriptions for all developmental stages were included in this work to facilitate a detailed description of the life cycle of *C. lacustris*.

Materials and Methods

Parasitological samples were collected during summer-autumn periods of 2015-2018 from cage-reared rainbow trout from farms in the northern part of Lake Ladoga (60°50′N, 31°33′E). Thirteen samples (the number of fish per sample ranged from 10 to 19) of rainbow trout were examined: 2 samples in 2015 (in July and September), 4 samples in 2016 (in July, August, October, December), 2 samples in 2017 (in June and September), and 5 samples in 2018 (monthly from June to October).

Parasitic copepods were fixed in 70% alcohol and mounted on permanent slides with Faure-Berlese mounting medium. Morphological identification of Caligids was done with reference to a key [Gusev 1987] under a LOMO stereomicroscope (magnification 7-45x). The development of the chalimus stage in the copepod was identified by the presence and number of circles on the frontal filament. Sex was determined for adult copepods (male and female). The morphological evaluation of the copepods included 10 characteristics: body length (BL); carapace length (CL) and carapace width (CW); distance from the edge of frontal plates to eyes (FpeL); genital segment length (GsL) and genital segment width (GsW); abdomen length (AL) and abdomen width (AW); furca length (FL) and furca width (FW). The terminology was given according to Kabata, Gusev (1966). The body length of the copepods was determined excluding chaeta length on caudal ramus; carapace width – in the widest part. The values are presented as a mean ± standard error.

DNA extraction, amplification and sequencing

Total DNA was extracted from five ethanol-preserved individuals of *C. lacustris* using the DNA-sorb B kit (Central Research Institute of Epidemiology, Russia) according to manufacturer's protocol. To determine the phylogenetic position of *C. lacustris* within the genus *Caligus* partial sequences of two genes were used. The fragment of the mitochondrial cytochrome c oxidase subunit 1 gene (COI, 645 bp) was amplified using the primers and PCR conditions as described in Folmer et al. (1994). The fragments of the 18S rRNA gene (1617 bp) were amplified following Øines and Schram (2008). The PCR products were purified by sorption on Agencourt Ampure XP (Beckman Coulter, Indianapolis, IN, USA) and subjected to Sanger sequencing using a BigDye Terminator V.3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA) with subsequent unincorporated dye removal by the Sephadex G-50 gel filtration (GE Healthcare,

Chicago, IL, USA). The Sanger products were analyzed on an ABI 3130XL Genetic Analyzer (Applied Biosystems). The purification and sequencing of PCR products were performed in SB RAS Genomics Core Facility (Novosibirsk, Russia). Sequences were deposited into GenBank (NCBI) under the following accession numbers: AY174158.1, AY174157.1, AY174156.1, AY174155.1, AY174154.1, AY174153.1, AY174152.1, AY174151.1, AY174150.1, AY174149.1, AY174148.1, AY174144.1, AY174145.1, AY174146.1, AY174147.1, AY174139.1, AY174140.1, AY174141.1, AY174142.1, AY174143.1).

Phylogenetic analysis

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Results

Morphological identification

Adult female

Body length is 5.2–6.0 mm, excluding the length of setae on the caudal furca. The shape of the body is caligoid. The cephalothorax is covered with a rounded convex carapace. The carapace margins are terminated with an edge membrane. A frontal plate with well developed lunules is located between the first antennae (Fig. 1, A). There is one eye. The upper part of the genital segment is narrow, gradually expanding in the middle of the segment, the lower part with rounded corners. The abdomen is single-segmented. The caudal furca (Fig. 1, O) is single-segmented, with small plumage on the interior side. The caudal furca has two spinules, three long plumose setae and one short seta with plumage on one side. The length of the egg sacs is up to 4.0–5.5 mm, width 0.3–0.4 mm, in a single row, the number of eggs in the longitudinal row is 30–54. The eggs are oval, in proportions of 2.4 times less long than they are wide.

Antenna I (Fig. 1, B) is two-segmented, typical for the genus *Caligus*.

Antenna II (Fig. 1, C) is three-segmented. The basal segment bears a small claw-shaped two-segmented appendage. The penultimate segment is longer than the basal one. The distal segment is strongly curved in a claw shape. Maxilla I (Fig. 1, D) is in the form of a triangular appendage.

The post-antennary process (Fig. 1, E) has a curving claw-shape. Maxilla II (Fig. 1, F) is two-segmented. The basal segment is shorter and wider than the distal segment. The end part of the distal segment has two outward curved spikes, one of them longer than the other. The lateral interior surface of the distal segment closer to the ending part has a small spike.

Maxilliped (Fig. 1, G) is two-segmented, large. The basal segment is twice as long and wide as the distal segment. The end part of the distal segment is in the form of a claw bending towards the interior. On the interior side of the claw at a distance of two-thirds of a quarter of the base there is a small, pointed spike.

Sternal furca (Fig. 1, H) is an unpaired fork with two parallel diverging rounded branches.

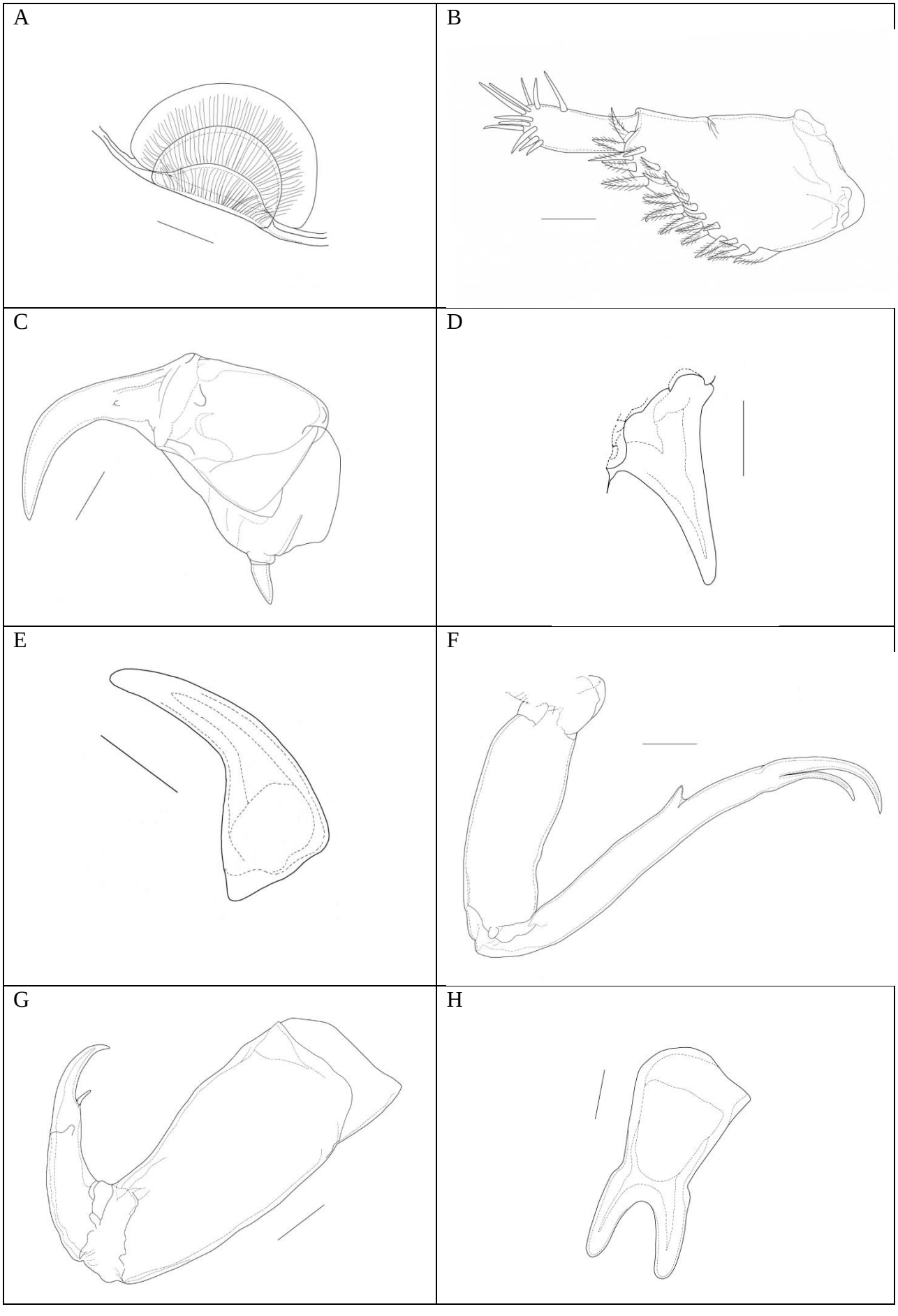
Swimming leg I (Fig. 1, J) is single-branched. The exopodite is three-segmented. The basal segment has two spinules and one process. The penultimate segment is covered with small plumage on the interior side; one small spinule is located in the end part of the segment on the outer side. On the interior side of the distal segment there are three long plumose setae, and on the end part of the segment there are four spinules of equal length.

Swimming leg II (Fig. 1, K) is two-branched. The exopodite of the second leg is tri-segmented. The interior side of the basal segment has small plumage and a long plumose seta. The outer side of the basal segment is without plumage, with a massive claw, extending over the middle segment and two thirds of the distal segment. The interior side of the middle segment of the exopodite is covered with small plumage and has one lengthy plumose seta; on the outer side of the segment there is a small spinule. The distal segment is armed with five setae plumaged on both sides, one seta plumaged on one side, and two small spinules. The endopodite of the second leg is three-segmented. The basal and middle segments are covered with plumage on the outside. There is one long plumaged seta on the inner side of the basal segment, and two on the middle segment. The outer margin of the distal endopodite segment bears six long plumaged setae.

Swimming leg III (Fig. 1, L) is two-branched. The marginal part of the basipodite is covered with plumage. The basipodite has one spinule and a long seta with one side plumaged on the interior side, and a massive exopodite claw is located on the outer margin. The basal segment of the exopodite has one spinule and plumage on the outer side, and one plumaged seta on the interior side. The distal segment of the exopodite has plumage, two small and one medium size spinules, four plumose setae. The endopodite is two-segmented, its basal segment with one long plumose seta. On the outside, the distal segment of the endopodite is covered with plumage and armed with six plumose setae, two of them long, two medium and two small.

Swimming leg IV (Fig. 1, M) is narrow, long, single-branched. Exopodite is three-segmented. The basal and penultimate segments have one spike each on the outside. The distal segment in the end part is armed with one long spike, two middle-sized and one small spinules.

Swimming leg V (Fig. 1, N) is located in the lower part of the genital segment, and has the shape of a flattened plate with two small non-plumaged setae.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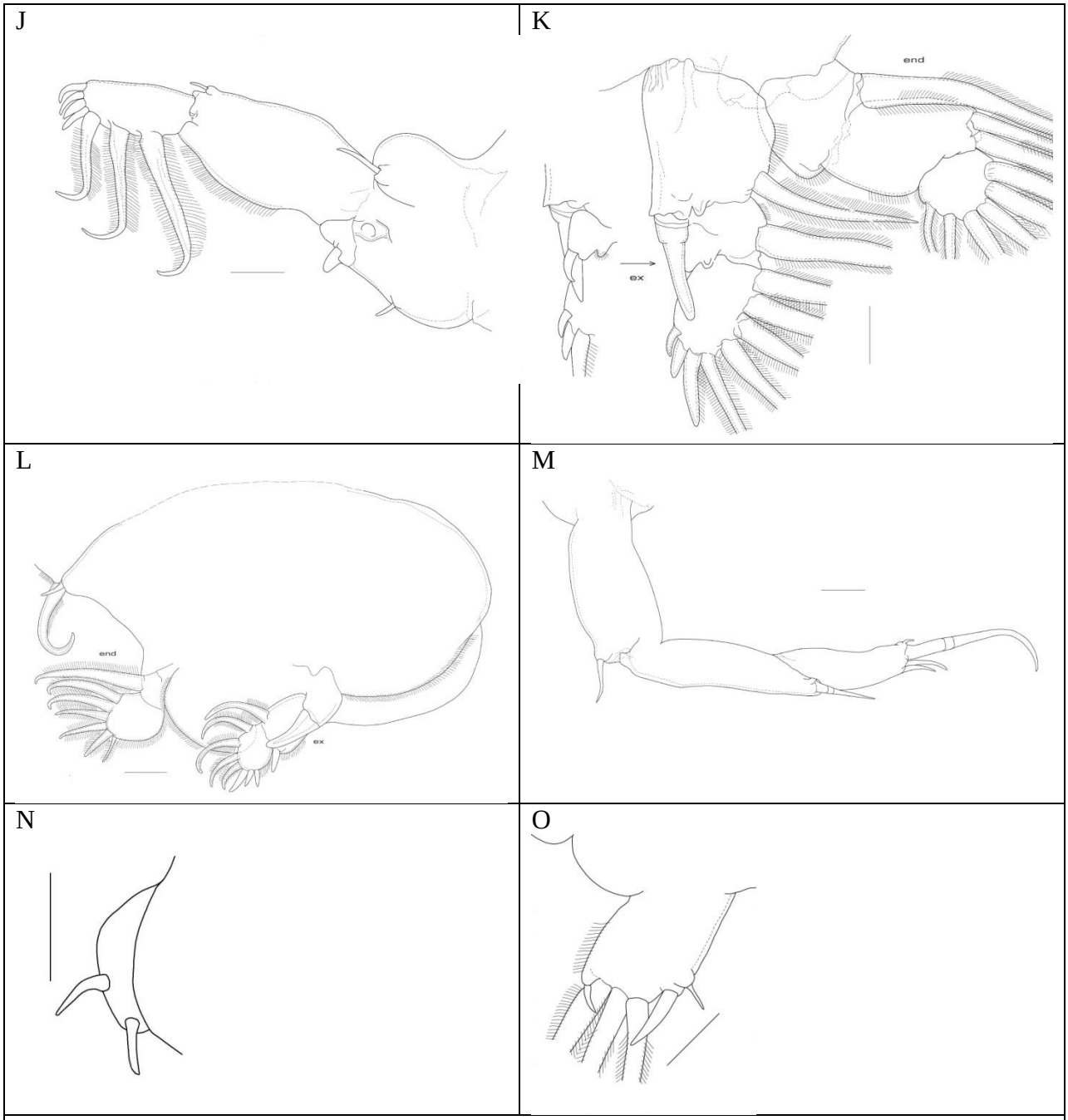

Fig. 1. *Caligus lacustris*, female. A – lunula; B – antenna I; C – antenna II; D – maxilla I; E – postantennary limb; F – maxilla II; G – maxilleped; H – sternal furca; J – leg I; K – leg II; L – leg III; M – leg IV; N – leg V; O – caudal furca. Scale-bars: A–G, J-M, O = 0.1 mm; H, N = 0.05 mm

205

206 **Adult male**

207 The male is similar to the female, but smaller. The body length ranges between 4.0–5.05 mm (sizes
208 are given in the Table 3). There is an additional segment carrying a pair of swimming legs IV
209 between the cephalothorax and the genital segment. The genital segment has rounded lateral edges.
210 The swimming legs V are shaped as small outgrowths with two non-plumaged setae. The width of
211 the genital segment is 1.3 times that of its length. Abdomen is single-segmented, 1.2 times longer
212 than it is wide.

Distinctive characteristics of male vs. female:

Antenna II (Fig. 2, A) is three-segmented. The penultimate segment has a large cushion without spinules. The distal segment is armed with two curved claws, and has a large wide seta on the outer lateral side.

Maxilla I (Fig. 2, B) is with a wide base and a pointed distal part. On the inner side of the base there is a small tubercle and a pointed spike.

The maxilliped (Fig. 2, C) is wide, with a massive claw. The basal segment has a cylindrical cushion on the interior side, covered with grooves and numerous small spinules. The claw is curved on the interior side and has an additional spike.

Sternal furca (Fig. 2, D) with slightly divergent rounded branches. The basal part of the furca has lateral extensions.

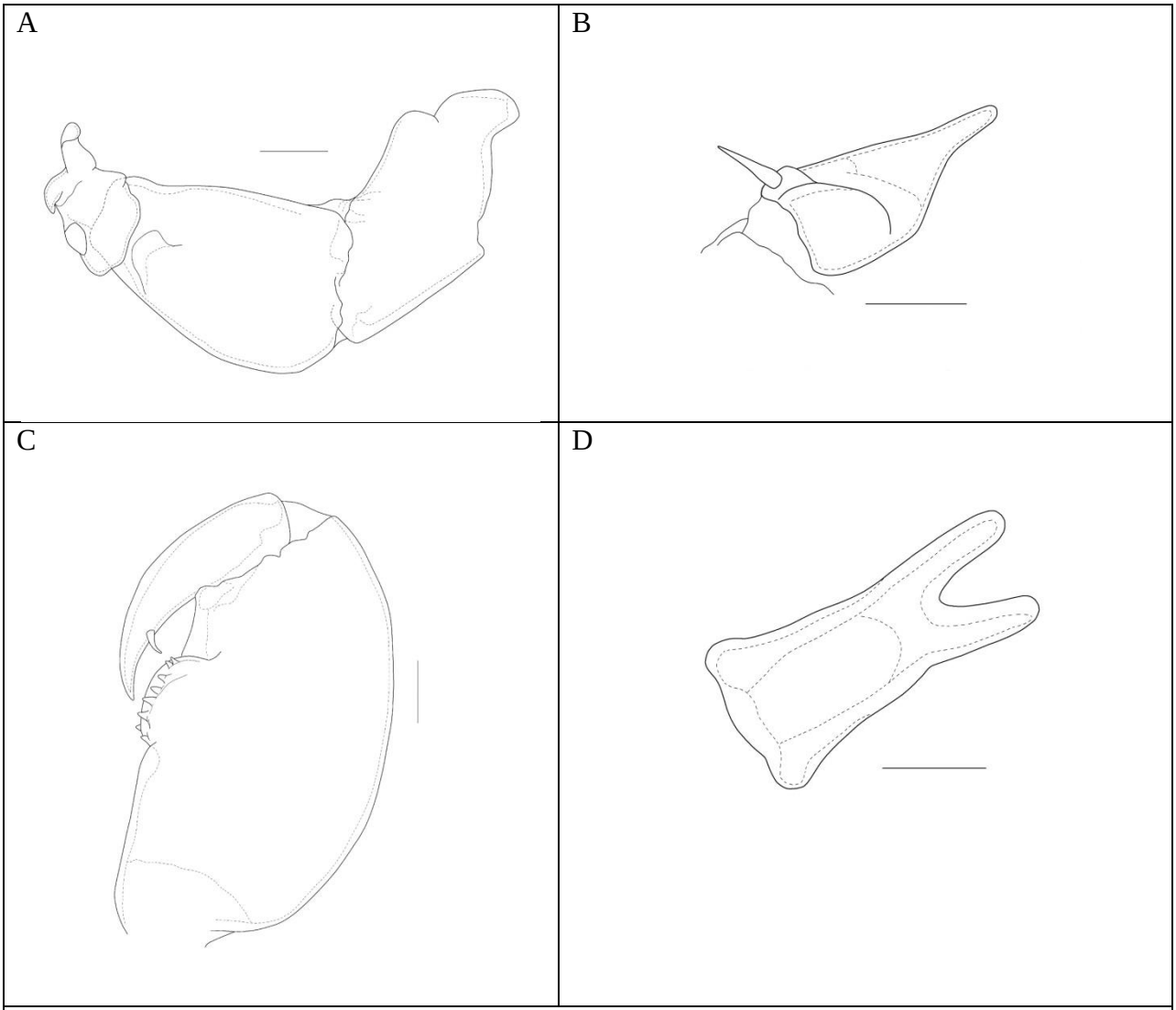

Fig. 2. *Caligus lacustris*, male. A – antenna II; B – maxilla I; C – maxilliped; D – sternal furca. Scale-bars: A–C = 0.1 mm; D = 0.05 mm

C. lacustris possess five pairs of swimming legs (the fifth leg is reduced). This reduced leg looks like a small plate with two unfledged chaeta. The position of spinules and chaeta on the swimming legs (I-IV) are presented in Table 2.

Table 2 Structure of all swimming legs of *C. lacustris*

Leg	Basis	Endopod			Exopod		
		1	2	3	1	2	3
I*	-	-	-	-	0-II	0-I	3,IV
II	-	1-0	2-0	6	1-0-c***	1-I	6,II
III	1**-I-c	1-0	6	-	1-I	4,III	-
IV	-	-	-	-	0-I	0-I	IV
V	2	-	-	-	-	-	-

* I (Roman numerals) – spinules, **1 (Arabic numerals) – chaeta, ***c - claw

Abundance and distribution of *C. lacustris*

Our results show that the prevalence of the *C. lacustris* infection in rainbow trout from cage farms in Lake Ladoga varied among years. The average prevalence and abundance ranged between 9.1 – 62.5% and 0.09 – 5.4 individuals of *C. lacustris* respectively. No mass mortalities of rainbow trout were registered. In June, October and November the fish were free of parasitic copepods (Table 1).

Table 1. The prevalence and intensity of copepod parasites on rainbow trout

	June	July	August	September	October	November	December
2015	ND	15.4/0.85	ND	36/1.02	ND	ND	ND
2016	ND	20/2.6	62.5/5.4	ND	0/0	ND	13.3/2.3
2017	0/0*	ND	ND	9.1/0.09	ND	ND	ND
2018	0/0	0/0	33.3/2.3	0/0	0/0	0/0	ND

* - before slash – prevalence (%), after slash – abundance (ind.), «ND» no data.

The aging structure of *C. lacustris* was also different from month to month. In September of 2015, only chalimus (stage II (9.1%), III (9.1%) and IV♀ (4.5%)) as well as adult females (18.2%) and males (59.1%) were found infecting rainbow trout. In the middle of the next year (July), the chalimus (stages I (14.6%), II (19.5%), III (26.8%) and IV (39%)) was the prevalent stage. In the next month (August), we noted that the proportion of chalimus (stages I (2.9%) and II (5.9%)) dramatically decreased, while the rate of chalimus (stages III (29.4%) and IV (41.2%)) increased. In December, the proportion of adult females was negligible (3 in 2 out of the 15 samples). In August 2018, adult females (60%) and males (40%) were prevalent.

In the second stage larvae (chalimus II) and in adult females of the copepods, the variability of morphological characteristics increased compared to other ontogenetic stages. It is important to note, that the size of stage IV larvae males increased only slightly when the adult stage was reached. Adult females, on the contrary, are significantly larger than the larvae (chalimus IV) (Table 2).

Table 2 Ranges of body length in millimeters of the chalimus stages in *C. lacustris*

Signs	Stages of chalimus development				
	I	II	III	IV ♂	IV ♀
BL, mm	1.19 ± 0.077 0.80–1.47	1.88 ± 0.065 1.62–2.37	2.72 ± 0.036 2.45–3.0	3.57 ± 0.083 3.12–4.37	3.82 ± 0.069 3.25–4.37
CL	0.71 ± 0.058 0.50–0.92	0.96 ± 0.049 0.75–1.30	1.46 ± 0.025 1.30–1.85	1.93 ± 0.047 1.75–2.37	2.04 ± 0.041 1.50–2.25
CW	0.50 ± 0.054 0.27–0.67	0.76 ± 0.035 0.62–1.0	1.16 ± 0.027 1.0–1.50	1.67 ± 0.072 1.50–2.50	1.72 ± 0.030 1.50–2.0
FpeL	0.27 ± 0.027 0.12–0.32	0.38 ± 0.015 0.32–0.47	0.49 ± 0.009 0.42–0.57	0.53 ± 0.014 0.42–0.60	0.59 ± 0.014 0.50–0.75
GsL	0.13 ± 0.015 0.07–0.17	0.19 ± 0.022 0.12–0.30	0.37 ± 0.020 0.25–0.62	0.51 ± 0.024 0.27–0.62	0.58 ± 0.025 0.42–0.87
GsW	0.16 ± 0.017 0.10–0.20	0.28 ± 0.018 0.22–0.42	0.48 ± 0.014 0.37–0.70	0.70 ± 0.028 0.52–0.87	0.74 ± 0.011 0.65–0.87
AL	0.14 ± 0.009 0.12–0.17	0.20 ± 0.017 0.12–0.27	0.48 ± 0.012 0.37–0.62	0.65 ± 0.019 0.55–0.75	0.70 ± 0.015 0.55–0.77
AW	0.16 ± 0.017 0.10–0.22	0.26 ± 0.011 0.17–0.30	0.42 ± 0.010 0.35–0.50	0.49 ± 0.024 0.27–0.62	0.56 ± 0.012 0.50–0.67
FL	0.04 ± 0.005 0.02–0.05	0.06 ± 0.004 0.05–0.08	0.09 ± 0.004 0.07–0.12	0.12 ± 0.002 0.10–0.12	0.12 ± 0.002 0.10–0.15
FW	0.03 ± 0.008 0.01–0.05	0.05 ± 0.000 0.05–0.05	0.07 ± 0.003 0.05–0.10	010 ± 0.004 0.07–0.12	0.10 ± 0.002 0.10–0.12

Phylogenetic relationships

Three COI haplotypes of *C. lacustris* were identified among five samples (two polymorphic sites, haplotype diversity 0.70±0.05, nucleotide diversity 0.00124±0.00047). Only single 18S rRNA haplotype was found. This haplotype was placed to one of the basal clades relative to the other representatives of the genus *Caligus* on the 18S rRNA gene tree obtained (Fig. 3). The other species included in this clade are *Caligus* (formely *Pseudocaligus*) *brevipedis*, *C. centrodonti* and *C. curtus*, where *C. lacustris* is a sister taxon to a group of *C. centrodonti*, *C. brevipedis*, and *C. curtus* (with a high posterior probability support). Similar results, relative to the grouping of *C. lacustris* were obtained in the COI gene tree reconstruction based on the 1st and 2nd codon positions (Fig. 3). This group got a high posterior probability (0.96), but overall resolution of the tree is poor, including branching order within the discussed group and its position relative to other clades of *Caligus* and other Caligidae genera. Thus, accepting the results of 18S rRNA analysis as

more reliable, we can conclude that the clade containing *C. lacustris* and aforementioned sister species is appearing in an early radiation of the genus *Caligus*.

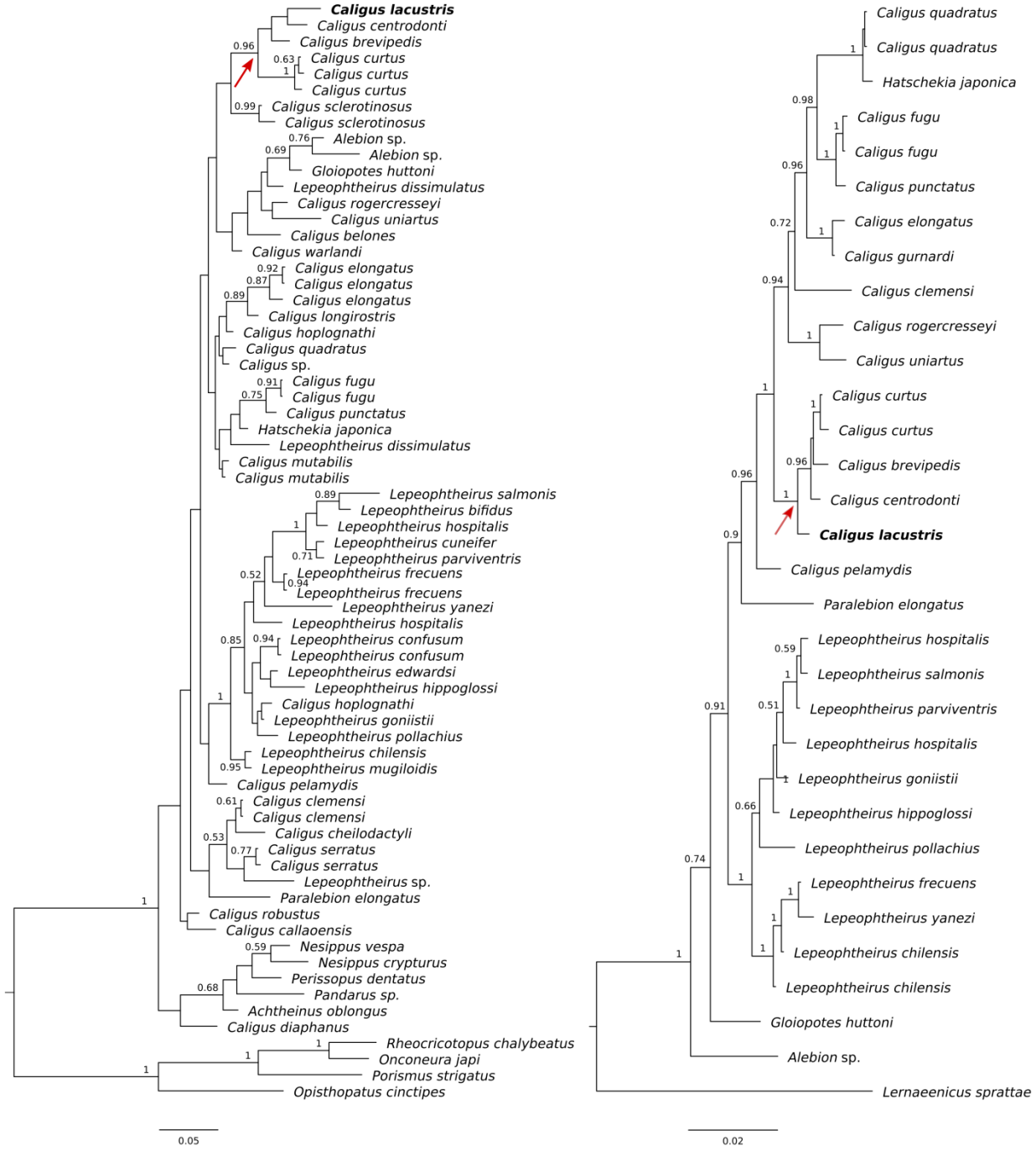

Figure 3. COI gene tree reconstruction based on 1st and 2nd codon positions (A), 18S rRNA gene tree (B)

Discussion

Over the past decades, human impact has severely affected the Lake Ladoga ecosystem [Drabkova 1995, Slepukhina 2000]: the intensive development of caged trout farming in the northwestern region has enriched the lake with nitrogen and phosphorus. In addition to eutrophication, trout farms create areas with high concentrations of fish, generating favorable

conditions for an outbreak of fish disease, including crustaceosis. The latter has high epizootic significance and causes serious problems in fish farming. Caligids live in fresh, marine, and brackish waters infecting a wide range of fish hosts [Rahkonen 2003]. For the marine farming industry, sea lice (Copepoda, Caligidae) have been the most significant pathogenic parasites, causing significant economic loses. In marine aquaculture *Lepeophtheirus salmonis, Caligus rogercresseyi, C. clemensi, C. elongatus, C. orientalis* and *C. teres* can cause deadly infestations or heavy infections on cultured salmonids [Costello, 2006; Øines and Heuch, 2007; Paladini et al., 2017]. *C. lacustris* is the one known freshwater species of a primarily marine genus *Caligus*, common in waterbodies of Northern (in the lake Hjälmaren in Sweden, to the eastern Baltic at Alands for Finland, in the lake Fureso in Denmark), Western (in Germany and in basins which remained from Zuidersee in Netherlands) and Central Europe (Poland), as well as basins of Aral, Black, Caspian, and Baltic seas. This species occurs on the skin and gills of different freshwater and euryhaline fishes [Markewitsch, 1951, 1956; Kaj, 1966; Gusev, 1987] causing disruption of the integrity of external epithelia and leading to skin damage, lesions, and bleeding and may lead to high mortality of various fish i.e. roach (*Rutilus rutilus*), common carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*H. nobilis*), and grass carp (*Ctenopharyngodon idella*) [Osmanov, 1971; Gusev, 1987]. In the case of mass infections, injured fish are easily exposed to bacterial and fungal infections, which can further extend high mortalities and economic losses [Pike, Wadsworth, 1999, Johnson 2004, Costello 2006]. The first case of mass infection caused by *C. lacustris* (prevalence at 100%, intensity at 1–8) on cage-reared rainbow trout was registered in the Bay of Puck, Baltic Sea (Poland) [Rokicki, 1986]. In caged trout farms in Northwest Russia, this species infected whitefish in Lake Uchonoye (Pskov Region) [Voronin, Chernysheva, 1979]. It is well known that the water temperature plays an important role in the development of caligids [Costello 2006]. Thus, in summer months (up to 25°C) in some pond farms of the Caspian and Azov seas, the prevalence and intensity of *C. lacustris* infections in fish (*H. molitrix, H. nobilis, C. carpio*, and *C. idella*) may reach up to 100% and 10 parasites per live fish (50 parasites on the dead fish) respectively.

According to our data, the average intensity of *C. lacustris* was relatively low with range of 0.09–5.4 copepods per trout (up to 30 parasites in some cases). Heavily affected fish were often observed to be jumping out of the water, diving deeper and rising to the surface again. Lake Ladoga is a cold-water lake with an average annual water temperature around 7.1°C. The warmest month is July when the temperature on the water surface may reach 16°C. This fact explains the relatively low average intensity of trout in Lake Ladoga compare to more warm Caspian and Azov seas.

Our study revealed that the most favorable conditions for the mass development of copepods in Lake Ladoga were from July to September, when the water temperature was 13–18°C.

During this period, rainbow trout hosts all ontogenetic stages of the copepod. The absence of the *C. lacustris* on rainbow trout in June, the presence of all larval stages of the copepod and adult individuals in July and August and the occurrence in September of only chalimus IV (juvenile males and females) as well as adult individuals brings us to the conclusion that only one generation develops in the fish farm within a year.

Caligus development also occurs in winter [Denisov 1975]. *C. elongatus*, the causative agent of crustaceosis in *Atlantic salmon* in marine farms, develops from the egg stage to the adult stage in 43 days. Two generations of the copepod can develop within a year. Males die after copulation, females hibernate [Piasecki 1995].

It was shown that the life cycle of *C. lacustris* is represented by seven developmental stages: nauplius, metanauplius, chalimus I, II, III, IV ♀ and ♂, adult stage ♀ and ♂. Sexual dimorphism in caligids at chalimus stages I, II, III is absent, whereas at stage IV it is clearly expressed in the anatomical structure (body size, genital segment, morphological signs in organs of the attachment apparatus) [Kozikowska 1958]. In the present study, we also showed that the sizes and sexual segments of adult copepods on rainbow trout in Lake Ladoga are characterized by a smaller range of variability if compared to *Barbus brachycephalus* from the Aral Sea (Table 3).

Table 3. Variability of morphological characters of *Caligus lacustris* from *Oncorhynchus mykiss* (Lake Ladoga) and *Barbus brachycephalus* (Aral Sea)

Character	This study		Gusev & Kabata, 1991 (copepodid stages of adult females and males from *Barbus brachycephalus*, Aral Sea)	
	Male	Female	Male	Female
BL	4.0–5.05	5.05–6.25	5.50–6.50*	5.10–7.80*
CL	2.25–2.75	2.50–3.50	3.20–3.50	2.80–3.70
CW	1.90–2.60	2.25–2.95	2.10–2.80	2.50–3.40
GsL	0.52–0.80	0.75–2.00	0.70–1.00	1.50–2.40
GsW	0.75–1.10	1.00–1.85	0.70–1.20	1.40–2.40
AL	0.65–0.85	0.75–1.10	1.10–1.40*	1.00–1.50*
AW	0.55–0.75	0.60–0.90	0.60–0.80	0.60–0.80

* – length of body (BL) and length of abdomen (AL) including the length of the caudal furca with external chaeta. All measurements are given in millimeters

Morphological description of swimming legs of *C. lacustris* was first performed by Gusev and Kabata (1991) on a small amount of material: from 1 adult female and 1 preadult male and males at early stages of development. In crustaceans at the stage of halimus III and IV, the complete formation of swimming legs is not complete. Therefore, we add additional data on the

structure of legs from mature (adult) females and males (Table 3). Based on this, our data differ from that of A.V. Gusev and Z. Kabata (1991).

A comparison of adult *C. lacustris* from rainbow trout from Northwest Russia and adult copepods from *B. brachycephalus* [Gusev, Kabata 1991] from Central Asia revealed differences in the variability of morphometric characters. Copepods from rainbow trout had a narrower range of variation in body size, carapace, abdomen and genitals (Table 3). Moreover, the leg I exopodite was three-segmented. On the basal segment has two spinules and one process. On the leg II basal segment of the exopodite there is one long plumose seta and a massive claw, which overlaps over the middle segment and two thirds of the distal segment. On the leg III basipodite there is one spinule, a long seta and massive exopodite claw. The leg IV exopodite is three-segmented. The basal and penultimate segments each have a single spike. The distal segment is armed with four spinules, one of them long.

The phylogenetic position of *C. lacustris* implies that specialization to freshwater habitats had happened once in a group of species containing *C. centrodonti*, *C. curtus*, *C. (P.) brevipedis*, and *C. lacustrus* itself. Notably, Gusev and Kabata (1991) have mentioned *C. curtus* among species to which *C. lacustris* is most similar morphologically (among others are *C. minimus*, *C. mugilis*, and *C. dicentrarchi*, for which no molecular data is available). Phylogenetic relationships within this clade is poorly resolved by mitochondrial COI sequence data, while it is relatively well resolved by nuclear 18S rRNA sequences. According to the 18S gene tree, *C. lacustris* is the earliest diverged species from the most recent common ancestor (MRCA) of the clade. Accepting this scenario, the MRCA of the group gave raise to the two branches, one of them leads to freshwater/brackish water *C. lacustris*, and another to purely marine *C. centrodonti*, *C. curtus*, and *C. brevipedis*. The current range of these marine species is limited to the North-East Atlantic, suggesting a European origin for *C. lacustris*. It is hence likely that evolution of this species might have begun in the waters of the ancient Southern North Sea, which went through several stages of drying during glaciation cycles and regularly received huge flows of freshwater from the Rheine River and water bodies in the territory of the modern Baltic Sea (Cohen et al. 2014; Śliwińska et al. 2019). Though, this hypothesis should be tested in a more explicit biogeographical context with a rigorous sampling across the whole range of *C. lacustris* and other *Caligus* species for which data is currently missing.

Phylogenetic reconstruction of the genera *Caligus*, *Lepeophtheirus* and *Pseudocaligus* sensu lato using COI and 16S mitochondrial markers and nuclear 18S rRNA was previously described and discussed by Øines and Schram (2008). They found the genera to be paraphyletic (polyphyletic) by mitochondrial markers and monophyletic by 18S rRNA. They suggested that the results of the analysis of 18S rRNA should be considered as insufficiently accurate, because of the

low variability of this gene, which thereby provides lower phylogenetic resolution. The validity of genus *Pseudocaligus* was also questioned by these authors, as the member of this genus was nested within *Caligus* by all molecular markers considered. Similar results and conclusions were obtained by Freeman et al. (2013) on an extended set of taxa. In our analyses, we obtained the same results relative to non-monophyly of various Caligidae genera when using mtDNA markers (Fig. 3A). However, the call for the preferable use of mtDNA for the reconstruction of phylogenetic relationships within Caligidae is frivolous, as a substantial level of substitution saturation was detected in the COI dataset. After exclusion of the third codon position, our COI dataset had shrunk to just 415 bp, which is clearly insufficient for robust analysis. On the contrary, the 18S rRNA gave a well resolved tree on which most genera are grouped separately. Further the relation of the only freshwater species in the genus *Caligus* has been presented and provides some insight into the evolutionary origins of this unique member of the genus.

Cage aquaculture is a powerful anthropogenic factor with a cumulative effect, artificially supporting high numbers of parasites due to the formation of a source for the pathogen hosts. Local fish are directly involved in the circulation and spread of the parasite across the surrounding waters of these farms. The high rate of *C. lacustris* infection detected on rainbow trout from a cage farm in Lake Ladoga is evidence of a disease caused by a parasite of native fish species. The materials obtained allow us to conclude that the intensive development of cage trout farming in the northwestern region creates the conditions for epizootics caused by *C. lacustris* in years with high temperatures in summer. Given the prospects for rising temperatures under a predicted regime of climate change this scenario is unlikely to improve over the long term.

Conclusions

A common parasitic copepod species on fish in Lake Ladoga is *Caligus lacustris*, which has a wide distribution and mainly infects salmonid species. Therefore, rainbow trout reared in cage farms can be massively infected with *C. lacustris*. According to our data, the optimal temperature conditions for the development of the copepod are in the range of 13–18 °C. Adult copepods hosted by rainbow trout in Lake Ladoga have a pronounced sexual dimorphism: the length of females was 5.6 ± 0.22 mm, that of males 4.51 ± 0.35 mm. The infection levels detected on rainbow trout during the course of this study are not too high, because the farm was organized quite recently. Based on 18S rRNA analysis (resolution of the COI tree was poor) the clade containing *C. lacustris* and aforementioned sister species is appearing in an early radiation of the genus *Caligus*.

It can be expected that the epizootic situation will continue developing gradually, as the farm continues to function. In parallel with the increase in the infection of trout in cages, native fish will be increasingly infected, thereby elevating the risk of mass epizootics in rainbow trout.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest among them.

Ethical Approval This study protocol was approved by the Research and Ethical Committee of the Institute of Biology, Karelian Research Centre, RAS. International, national, and/or institutional guidelines for the collection of parasites samples from fish were adequately followed.

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Supplementary

Supplementary 1. The deposited sequences from GenBank that were used for phylogenetic reconstructions

GenBank acc. no.	Gene	*Species*
KU317605.1	cytochrome oxidase subunit I (COI) partial	*Caligus cheilodactylus*
EF065619.1	cytochrome oxidase subunit I (COI) partial	*C. quadratus*
EF065616.1	cytochrome oxidase subunit I (COI) partial	*C. diaphanus*
KT209407.1	cytochrome oxidase subunit I (COI) partial	*C. curtus*
KT209384.1	cytochrome oxidase subunit I (COI) partial	*C. elongatus*
KT209299.1	cytochrome oxidase subunit I (COI) partial	*C. elongatus*
KT209233.1	cytochrome oxidase subunit I (COI) partial	*C. curtus*

KT209227.1		*C. elongatus*
	cytochrome oxidase subunit I (COI) partial	
KT209179.1		*C. elongatus*
	cytochrome oxidase subunit I (COI) partial	
KT209134.1		*C. elongatus*
	cytochrome oxidase subunit I (COI) partial	
KT208967.1		*C. elongatus*
	cytochrome oxidase subunit I (COI) partial	
KT208919.1		*C. elongatus*
	cytochrome oxidase subunit I (COI) partial	
KT208896.1		*C. elongatus*
	cytochrome oxidase subunit I (COI) partial	
AY861370.1		*C. centrodonti*
	cytochrome oxidase subunit I (COI) partial	
AY861369.1		*C. gurnardi*
	cytochrome oxidase subunit I (COI) partial	
AY861368.1		*C. belones*
	cytochrome oxidase subunit I (COI) partial	
AY861366.1		*C. curtus*
	cytochrome oxidase subunit I (COI) partial	
AY386272.1		*C. elongatus*
	cytochrome oxidase subunit I (COI) partial	
EF452646.1		*C. warlandi*
	cytochrome oxidase subunit I (COI) partial	
EF452645.1		*C. warlandi*
	cytochrome oxidase subunit I (COI) partial	
EF452644.1		*C. warlandi*
	cytochrome oxidase subunit I (COI) partial	
AM235887.1		*C. clemensi*
	cytochrome oxidase subunit I (COI) partial	

KR049059.1		*C. quadratus*
	cytochrome oxidase subunit I (COI) partial	
KR049058.1		*C. hoplognathi*
	cytochrome oxidase subunit I (COI) partial	
KR049057.1		*C. punctatus*
	cytochrome oxidase subunit I (COI) partial	
KR049056.1		*C. fugu*
	cytochrome oxidase subunit I (COI) partial	
AY386274.1		*Lepeophtheirus salmonis*
	cytochrome oxidase subunit I (COI) partial	
KM896947.1		*Alebion sp.*
	cytochrome oxidase subunit I (COI) partial	
KP681600.1		*C. rogercresseyi*
	18s ribosomal RNA gene, partial sequence	
KP681599.1		*C. rogercresseyi*
	18s ribosomal RNA gene, partial sequence	
KP681598.1		*C. rogercresseyi*
	18s ribosomal RNA gene, partial sequence	
KP681597.1		*C. rogercresseyi*
	18s ribosomal RNA gene, partial sequence	
KP681591.1		*C. rogercresseyi*
	18s ribosomal RNA gene, partial sequence	
KP681590.1		*C. rogercresseyi*
	18s ribosomal RNA gene, partial sequence	
JX845131.1		*C. elongatus*
	18s ribosomal RNA gene, partial sequence	
JX845130.1		*C. elongatus*
	18s ribosomal RNA gene, partial sequence	
JX845129.1		*C. elongatus*
	18s ribosomal RNA gene, partial sequence	

EF088412.1		*C. quadratus*
	18s ribosomal RNA gene, partial sequence	
EF088411.1		*C. pelamydis*
	18s ribosomal RNA gene, partial sequence	
EF088410.1		*C. gurnardi*
	18s ribosomal RNA gene, partial sequence	
EF088409.1		*C. elongatus*
	18s ribosomal RNA gene, partial sequence	
EF088408.1		*C. elongatus*
	18s ribosomal RNA gene, partial sequence	
EF088407.1		*C. curtus*
	18s ribosomal RNA gene, partial sequence	
EF088406.1		*C. centrodonti*
	18s ribosomal RNA gene, partial sequence	
EF088405.1		*C. belones*
	18s ribosomal RNA gene, partial sequence	
DQ123833.1		*C. clemensi*
	18s ribosomal RNA gene, partial sequence	
MF077737.1		*C. curtus*
	18s ribosomal RNA gene, partial sequence	
MF077736.1		*C. elongatus*
	18s ribosomal RNA gene, partial sequence	
KR048778.1	18s ribosomal RNA gene, partial sequence	*C. fugu*
KR048777.1	18s ribosomal RNA gene, partial sequence	*C. punctatus*
KR048776.1	18s ribosomal RNA gene, partial sequence	*C. quadratus*
KJ193735.1	18s ribosomal RNA gene, partial sequence	*C. elongatus*
KJ396107.1	18s ribosomal RNA gene, partial sequence	*C. rogercresseyi*
KC569364.1	18s ribosomal RNA gene, partial sequence	*C. fugu*
KC569363.1	18s ribosomal RNA gene, partial sequence	*C. uniartus*

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