Canadian Journal of Zoology

Article

A re-evaluation of conflicting taxonomic structures of Eurasian *Triaenophorus* spp. (Cestoda, Bothriocephalidea: Triaenophoridae) based on partial *cox1* mtDNA and 28S rRNA gene sequences

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Abstract

Cestodes of the genus *Triaenophorus* Rudolphi, 1793 are widely distributed parasites of Esocidae, Percidae, Salmonidae, Thimallidae, Cobitidae, Osmeridae, Cyprinidae, Cottiidae, Lotidae, and several others in the Holarctic. The taxonomic arrangements of different authors, based on morphological and ecological–biogeographic characters, suggest the presence of two to five species of this genus in Eurasia. The genetic variation of Eurasian *Triaenophorus* spp. was evaluated using DNA barcoding (*cox1* and 28S gene sequences). This confirmed the validity of five *Triaenophorus* species: *T. amurensis*, *T. crassus*, *T. meridionalis*, *T. nodulosus*, and *T. orientalis*. We demonstrated systematic concordance between traditional meristic criteria and DNA sequence data. Phylogenetic reconstructions support the monophyletic origin of the group of species with a long basal plate of the scolex hook (*T. crassus*, *T. meridionalis*, and *T. orientalis*). *Triaenophorus crassus* is represented by two haplogroups, associated with Siberia and northwestern Russia. Our results show differences between *T. nodulosus*, *T. amurensis*, and *T. crassus* in terms of the haplotype diversity level, which are probably related to the Quaternary history of the development of their ranges, as well as the degree of euryxeny to the second intermediate host.

Key words: *Triaenophorus amurensis*, *Triaenophorus crassus*, *Triaenophorus meridionalis*, *Triaenophorus nodulosus*, *Triaenophorus orientalis*, Cestoda, taxonomy, systematics

Résumé

Les cestodes du genre *Triaenophorus* Rudolphi, 1793 sont des parasites très répandus des ésocidés, percidés, salmonidés, thimallidés, cobitidés, osméridés, cyprinidés, cottidés, lotidés et de plusieurs autres familles de l'Holarctique. Les dispositions taxonomiques de différents auteurs établies à la lumière de caractères morphologiques et écologiques–biogéographiques, indiqueraient la présence de deux à cinq espèces de ce genre en Eurasie. Les variations génétiques des espèces eurasiennes de *Triaenophorus* spp. ont été évaluées par l'entremise de codes-barres d'ADN (séquences des gènes *cox1* et 28S). Elles confirment la validité des cinq espèces de *Triaenophorus* suivantes : *T. amurensis*, *T. crassus*, *T. meridionalis*, *T. nodulosus* et *T. orientalis*. Nous démontrons une concordance systématique entre des critères méristiques traditionnels et les données de séquences d'ADN. Des reconstitutions phylogénétiques appuient l'origine monophylétique du groupe des espèces caractérisées par une longue plaque basale du crochet du scolex (*T. crassus*, *T. meridionalis* et *T. orientalis*). *Triaenophorus crassus* est représenté par deux haplogroupes, associés à la Sibérie et au nord-ouest de la Russie. Nos résultats font ressortir des différences entre *T. nodulosus*,

T. amurensis et *T. crassus* en ce qui concerne le degré de diversité des haplotypes, qui sont probablement reliées à l'histoire quaternaire du développement de leurs aires de répartition, ainsi qu'au degré d'euryxénie avec le deuxième hôte intermédiaire. [Traduit par la Rédaction]

Mots-clés : *Triaenophorus amurensis*, *Triaenophorus crassus*, *Triaenophorus meridionalis*, *Triaenophorus nodulosus*, *Triaenophorus orientalis*, cestodes, taxonomie, systématique

Introduction

The genus *Triaenophorus* Rudolphi, 1793 constitutes cestodes of the order Bothriocephalidea having a scolex with two shallow bothria and four trident-shaped hooks, and strobila devoid of external segmentation (Protasova 1977; Bray et al. 1994; Kuchta et al. 2008). The geographic range of this genus spans northern Eurasia and mainly northern North America. The life cycle of *Triaenophorus* includes copepods as the first intermediate host and various species of omnivorous and planktivorous fishes as the second intermediate host (Kuperman 1973). Adult specimens of the Eurasian species of *Triaenophorus* are specific parasites of esocid fish (Kuperman 1973).

Triaenophorus spp. are one of the most common helminths of freshwater fish in the boreal region. For example, infestation of pike with adult specimens of *Triaenophorus nodulosus* (Pallas, 1781) in the desalinated part of the Gulf of Bothnia may reach 93% (Valtonen et al. 1989). According to Dieterich and Eckmann (2000), plerocercoids of this species occur in more than 80% of perches aged 1 year and older in some waters of Germany. Extensive literature is devoted to the study of various aspects of *Triaenophorus* spp. biology, including pathogenicity for its hosts (Kuperman 1973; Rosen and Dick 1984; Shostak and Dick 1986; Ieshko and Evseeva 1989; Valtonen et al. 1989; Pronin 1990; Evseeva 1994; Pasternak et al. 1999; Izvekova 2001; Rusinek and Kuznedelov 2001; Dezfuli et al. 2014; Schaufler et al. 2014; Izvekova and Solovyev 2012, 2013, 2016; Borvinskaya et al. 2019; Kashinskaya et al. 2021).

The systematics of *Triaenophorus* have undergone a number of fundamental changes over the past 60 years. Until the late 1960s, only two valid species of this genus were recorded in Eurasia: *T. nodulosus* and *Triaenophorus crassus* Forel, 1868. However, a detailed study of *Triaenophorus* undertaken by Kuperman (1968) allowed the description of three other species, *Triaenophorus amurensis* Kuperman, 1968, *Triaenophorus orientalis* Kuperman, 1968, and *Triaenophorus meridionalis* Kuperman, 1968: the first two inhabit the Amur transitional zoogeographic region and the third is typical of the water bodies of southern European Russia. This work was preceded by publications by Dubinina (1964) and Kuperman himself (1965), indicating significant differences in morphology and host specificity (in the phase of the plerocercoid) between *Triaenophorus* from the Amur River basin, Siberia, southeast Europe, and other European water bodies. According to Kuperman (1968, 1973), morphological differences among the five Eurasian species of this genus are primarily related to the size and shape of the scolex hooks.

Dubinina (1987) considered that in Eurasia this genus is represented by only two species, *T. nodulosus* and *T. crassus*, each of which includes two subspecies: *Triaenophorus crassus crassus*, *Triaenophorus crassus orientalis*, *Triaenophorus nodulosus nodulosus*, and *Triaenophorus nodulosus amurensis*. Kuchta et al. (2007) studied the material on *Triaenophorus* spp. from B.I. Kuperman's collection kept at the Zoological Institute of the Russian Academy of Sciences. According to these authors, the ranges of variation of morphological features of *T. amurensis*, *T. orientalis*, and *T. meridionalis* are, in fact, much wider than those indicated by Kuperman (1968, 1973). Kuchta et al. (2007) concluded that reliable identification of the species described by B.I. Kuperman is impossible; therefore, they synonymize *T. amurensis* with *T. nodulosus*, and *T. orientalis* and *T. meridionalis* with *T. crassus*. Hence, these authors have reduced the diversity of the Eurasian group of *Triaenophorus* to only two species. Meanwhile, when describing new species, Kuperman (1968) relied not only on morphological data but also on ecological and biological evidence (the set of second intermediate hosts, embryogenesis, and other biological parameters), which was not taken into account by Kuchta et al. (2007).

As a result, three competing taxonomic models exist in the literature for the description of the Eurasian species of *Triaenophorus*, and classical morphological methods of research do not provide an adequate solution in favor of any one of them. Therefore, we used DNA barcoding to test current taxonomic arrangements and to evaluate the usefulness of *cox1* (mitochondrial cytochrome *c* oxidase subunit 1 gene) and 28S (nuclear large subunit of the rRNA gene) as barcodes for these tapeworms. *cox1* and 28S are two of the most widely used markers for the taxonomy of species of parasitic flatworms (Vilas et al. 2005; Chambrier et al. 2015). The aim of this paper is to identify the species composition of *Triaenophorus* in Eurasia using partial *cox1* and 28S gene sequences obtained from regional fish hosts.

Materials and methods

Study area and sampling

Gravid and subadult specimens or plerocercoids of five species of *Triaenophorus*, *T. amurensis*, *T. crassus*, *T. meridionalis*, *T. nodulosus*, and *T. orientalis*, were collected in the course of a parasitological investigation of fishes caught in water bodies in the European and Asian parts of Russia (Supplement A). Parasites were relaxed in hot water (80–90 °C), fixed in 96% ethanol, and stored at −18 °C.

The species affiliation of the cestodes was annotated in accordance with identification keys using characters described by Kuperman (1968, 1973): the width of the basal plate of scolex hooks, the host, and locality. The scolex hooks were measured on the squashed scolices mounted in Berlese's medium (isolates from the Ob and Yenisei river basins) or the isolated hooks extracted from the bodies using needles followed by treatment with 60 μg/mL proteinase K (all other

isolates). Paragenophores (scolices with four trident-shaped hooks) were deposited in the Museum of Helminthological Collections at the Center of Parasitology of the A.N. Severtsov Institute of Ecology and Evolution (IPEE RAS) in Moscow, Russia (Supplement B).

DNA extraction, amplification, and sequencing

Before DNA extraction, samples fixed in ethanol were washed in water. Total DNA was extracted from single plerocercoids using the DNA-sorb B kit manufacturers' protocols (kit for DNA extraction, Central Research Institute of Epidemiology, Russia). To reconstruct phylogenetic relationships within the genus *Triaenophorus*, partial sequences of *cox1* and 28S were used. PCR amplification of the *cox1* gene fragment was conducted using the Dice1F (ATTAACCCTCACTAAATTWCNTTRGATCATAAG) and Dice11R (TAATACGACTCACTATAGCWGWACHAAATTTHCGATC) primers as previously described (Steenkiste et al. 2015).

Cycling conditions for partial *cox1* were applied according to Steenkiste et al. (2015) with small modifications: 95 °C for 5 min; 3 cycles of 95 °C for 30 s, 51 °C for 40 s, and 72 °C for 1 min; 5 "touchdown" cycles of 95 °C for 30 s, 50–46 °C for 40 s (dropping 1 °C per cycle), and 72 °C for 1 min; 25 cycles of 95 °C for 30 s, 45 °C for 40 s, and 72 °C for 1 min; and a final extension at 72 °C for 5 min.

Partial sequences of the 28S rRNA gene were amplified using LSU5 (TAGGTCGACCCGCTGAAYTTYAGCA) (Littlewood et al. 2000) and 1500R (GCTATCCTGAGGGAAACTTCG) primers (Littlewood et al. 2008). The cycling conditions adopted were the following: 95 °C for 5 min; 34 cycles of 95 °C for 15 s, 57 °C for 30 s, and 72 °C for 80 s; and a final extension at 72 °C for 5 min. Double-stranded DNA was amplified using the BioMaster HS-*Taq* PCR-Color (2×) kit (Novosibirsk, Russia) according to the manufacturer's instructions (http://biolabmix.ru/products/klassicheskaja_pcr/biomaster_hs-taq_pcr-color__2_/). PCRs were 50 μL in volume and contained 25 μL BioMaster HS-*Taq* PCR-Color reaction mix, 0.2 μmol/L of each primer, and 20 μL sterile water, and 3 μL of total DNA was used as template. The PCR products were purified by adsorption on Agencourt Ampure XP (Beckman Coulter, Indianapolis, IN, USA) columns and subjected to Sanger sequencing using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA) with subsequent unincorporated dye removal by 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 the SB RAS Genomics Core Facility (Novosibirsk, Russia). The chromatograms of the amplicon sequences were evaluated based on the sharpness and clear visibility of each peak for each nucleotide. Sites that had more than one peak for the corresponding nucleotide were excluded from the analysis. For the *cox1*, the Dice11R was used as a sequencing primer, whereas for 28S the consensus sequences were obtained by a combination of forward and reverse sequencing. The sequences were manually aligned, edited, and checked for unexpected stop codons in MEGA 7 (Kumar et al. 2016). Newly obtained sequences were deposited into the GenBank database (Supplement A).

Phylogenetic analysis

Analysis of genetic distances was conducted in MEGA 7 (Kumar et al. 2016). The number of haplotypes and levels of DNA polymorphism were calculated using the program DNASP 6 (Rozas et al. 2017). The best model of nucleotide substitutions was determined using MEGA 7. Phylogenetic reconstructions within the genus *Triaenophorus* were performed using the maximum likelihood (ML) and Bayesian inference (BI) approaches. For the ML approach implemented in MEGA 7, the HKY+G model of nucleotide substitutions was used in both cases (*cox1* and 28 rRNA genes). Statistical support to the test of phylogeny was provided using the bootstrap method with 1000 replications. Bayesian analysis was performed with MrBayes v.3.2.1 using the same model as the previous approach. Two simultaneous runs with four Markov chains each were run for 1×10^6 generations and sampled every 500 generations. The first 25% of generations were discarded as burn-in. The sequences of *Dibothriocephalus latus* (Linnaeus, 1758) (Diphyllobothriidae) with accession nos. AB269325.1 (*cox1* gene tree) and DQ925326.1 (28 rRNA gene tree) from the GenBank database (accepted as *Diphyllobotrium latum* in both cases) were included in the phylogenetic analyses as an outgroup. Popart 1.7 software (https://popart.otago.ac.nz) was used to calculate and visualize the median-joining network of phylogenetic relationships among haplotypes (Bandelt et al. 1999).

Results

Species identification

A total of 63 specimens of *Triaenophorus* spp. from different fish species and water bodies of Eurasia were examined (Supplement A). All representatives of *Triaenophorus* were initially assigned to five species based on the width of the basal plate of scolex hooks, the host, and locality: *T. amurensis*, *T. crassus*, *T. meridionalis*, *T. nodulosus*, and *T. orientalis*.

Worms with scolex hooks having a basal plate 63–84 μm wide and parasitizing in the intestines of the Amur pike (*Esox reichertii* Dybowski, 1869) (adult of subadult cestodes) or the liver of cyprinids (plerocercoids) from Sakhalin Island and the Primorsky Region of Russia were referred to as *T. amurensis* (Fig. 1A).

Representatives of *Triaenophorus* with scolex hooks having a basal plate 90–185 μm wide and parasitizing the intestines of northern pike (*Esox lucius* Linnaeus, 1758) (adult of subadult cestodes) and the liver of cottid, lotid, or percid fish (plerocercoids) from Siberia or the European part of Russia were referred to as *T. nodulosus* (Fig. 1B).

Cestodes with scolex hooks having a basal plate 221–411 μm wide and parasitizing the intestines of *E. lucius* (adult of subadult cestodes) and muscles of *Coregonus* spp. (plerocercoids) from Siberia or the European part of Russia were referred to as *T. crassus* (Fig. 2A).

Fig. 1. Scolex hooks of (A) *Triaenophorus amurensis* and (B) *Triaenophorus nodulosus* at the same scale. Scale bar = 10 μm.

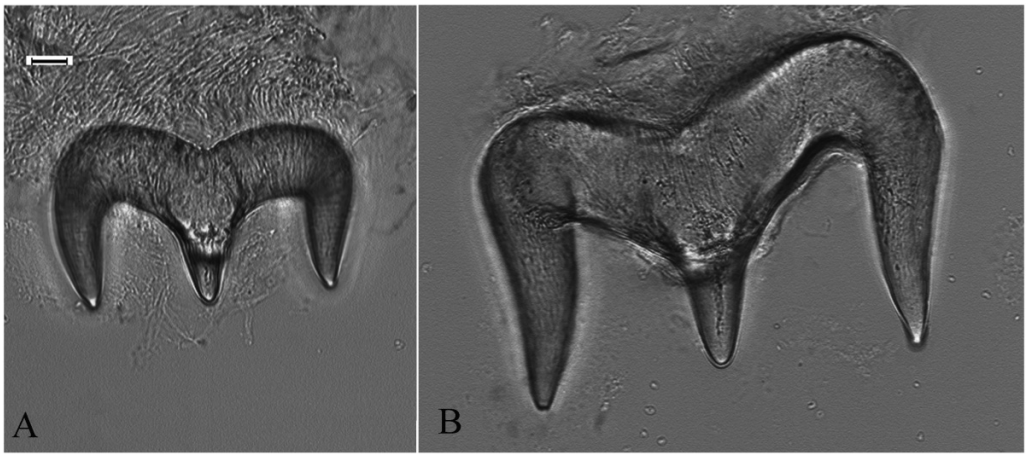
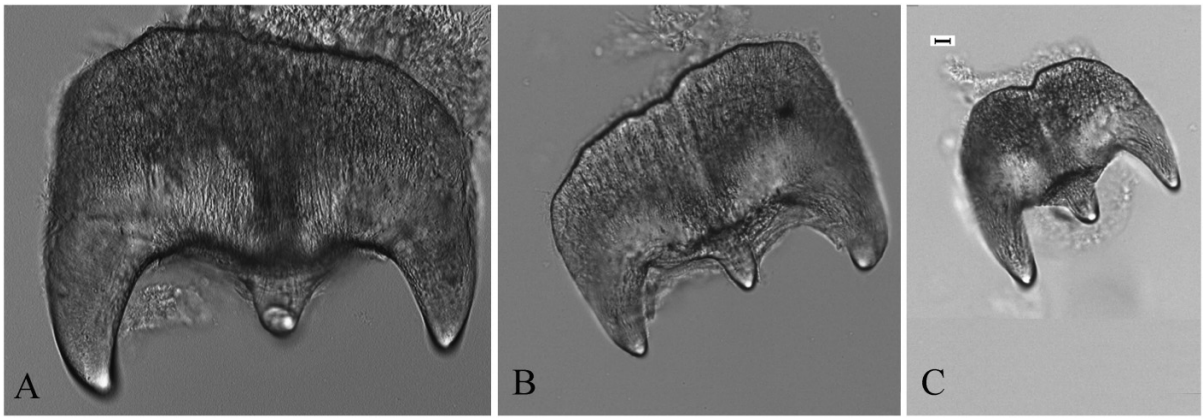


Fig. 2. Scolex hooks of (A) *Triaenophorus crassus*, (B) *Triaenophorus meridionalis*, and (C) *Triaenophorus orientalis* at the same scale. Scale bar = 11 μm.



Specimens with scolex hooks having a basal plate 196–228 μm wide and parasitizing the intestines of *E. lucius* from the Volga delta were referred to as *T. meridionalis* (Fig. 2B).

Worms with scolex hooks having a basal plate 119–133 μm wide and parasitizing the intestines of *E. reichertii* from Sakhalin Island were referred to as *T. orientalis* (Fig. 2C).

Phylogeny and genetic diversity

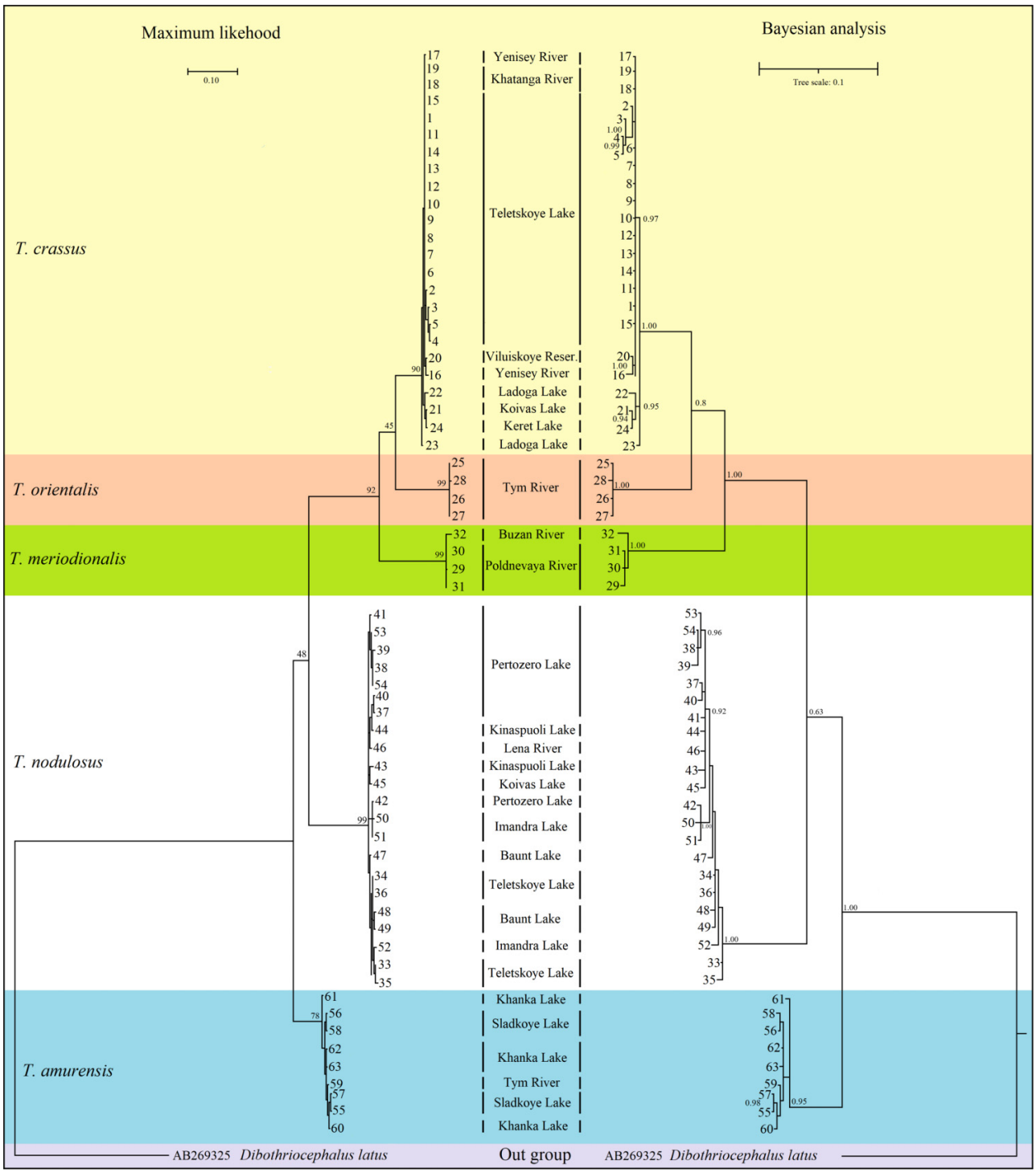
Fragments of the *cox1* gene with a length of 586 bp were amplified and sequenced from 63 specimens of *Triaenophorus* spp. previously identified by traditional taxonomical methods (see above). The studied cestode specimens are distributed among five species-level clades: *T. amurensis*, *T. crassus*, *T. meridionalis*, *T. orientalis*, and *T. nodulosus* (Fig. 3). We did not include the sequence of *Triaenophorus stizostedionis* Miller, 1945 (GenBank accession No. KR780809) in the present analysis because the parts of the *cox1* gene that were sequenced were not homologous loci compared with our data set.

The topologies of the trees constructed by the ML and BI approaches were identical, excluding clustering of the samples within the species-level clades. The posterior probability and bootstrap support (a bootstrap support value of 78% for the *T. amurensis* clade was an exception) values for all species levels were high.

Triaenophorus meridionalis, *T. crassus*, and *T. orientalis* form a highly supported group, in which *T. crassus* and *T. orientalis* appear to be a poorly supported sister species. *Triaenophorus nodulosus* appears as a poorly supported sister taxon to the *T. meridionalis* + (*T. crassus* + *T. orientalis*) group, and *T. amurensis* occupies a sister position to the clade uniting all the mentioned *Triaenophorus* spp.

Only 10 haplotypes with 19 polymorphic sites were identified among 24 specimens of *T. crassus*. The haplotype diversity and nucleotide diversity for this species were 0.667 ± 0.109 and 0.00539, respectively. Twenty haplotypes with 35 polymorphic sites were present in 22 studied specimens of *T. nodulosus*. This species was characterized by the highest levels of haplotype diversity (0.991 ± 0.017) and nucleotide diversity (0.01248). Eight haplotypes with 10 polymorphic sites were found among nine specimens of *T. amurensis*. The haplotype diversity and nucleotide diversity for this species were 0.972 ± 0.064 and 0.00626, respectively. We did not take this parameter into account for *T. meridionalis* and *T. orientalis* due to the low numbers of analyzed specimens.

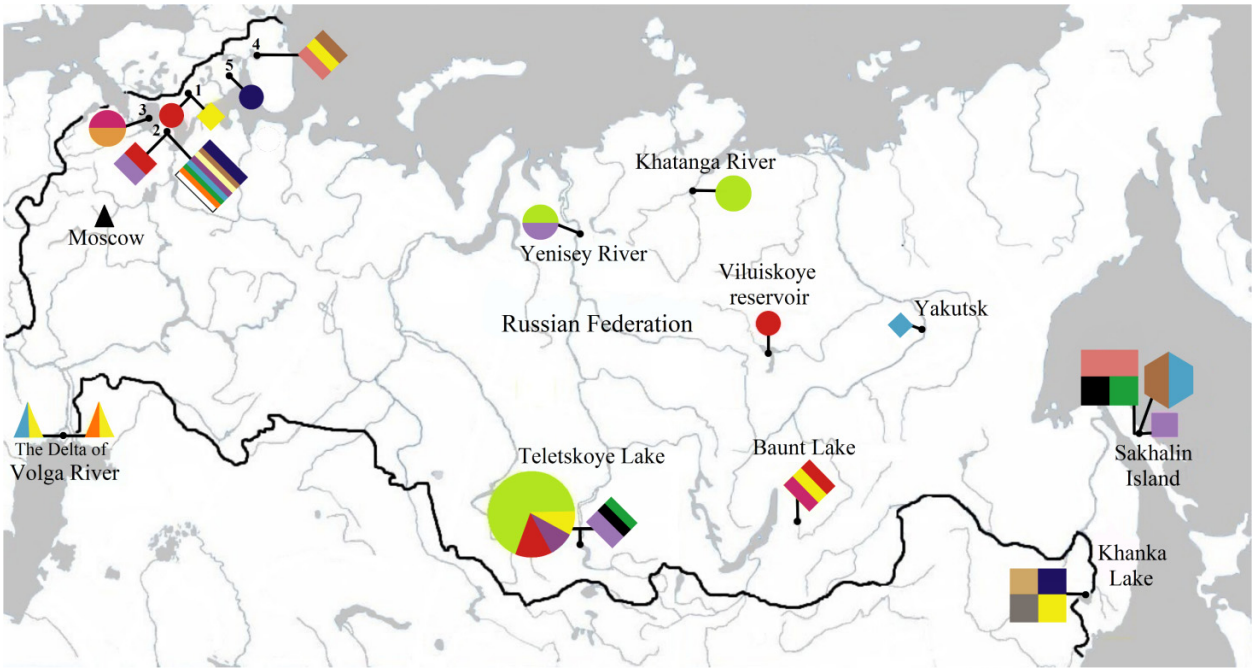
Fig. 3. Phylogenetic relationships of *Triaenophorus* spp. reconstructed by maximum likelihood (ML; left tree) and Bayesian inference (BI; right tree) analyses of *coxI* gene sequences. Sample numbers are displayed at branch tips. Bootstrap values (ML) and posterior probabilities (BI) are displayed at the branch nodes. [Color online.]



In general, the level of intraspecific variability of the portion of the *cox1* genes that were sequenced was much lower than their level of interspecific variability. Within the species-level clades, the mean *p*-distance values for *T. crassus*, *T. orientalis*, *T. meridionalis*, *T. nodulosus*, and *T. amurensis* were 0.54% ± 0.13%, 0.17% ± 0.12%, 0.57% ± 0.22%, 1.25% ± 0.24%, and 0.63% ± 0.20%, respectively. The mean *p*-distance between these clades varied from 10.9% ± 1.3% (*T. crassus* by *T. orientalis*) to 18.0% ± 1.6 (*T. meridionalis* by *T. amurensis*).

The species-level haplogroups are distinctly separated in the haplotype network (Figs. 4 and 5). For some sampling sites, only a single haplotype was identified. The geographically specific haplogroups were found in only one widespread species, *T crassus*. We identified two haplogroups with

Fig. 4. Geographical distribution of *Triaenophorus* spp. haplotypes (*cox1*) across the sampling points (Russian Federation). Circle, *T. crassus*; rhombus, *T. nodulosus*; square, *T. amurensis*; hexagon, *T. orientalis*; triangle, *T. meridionalis*. The single haplotypes are marked by different colors within each pikeworm species symbol. Paint 3D software (available from https://www.microsoft.com/en-us/p/paint-3d/9nblggh5fv99#activetab=pivot:overviewtab) was used to create the figure. The base map was obtained from the open domain plain maps available from https://ru.wikipedia.org/wiki/%D0%A4%D0%B0%D0%B9%D0%BB:Russia_physical_location_map_(Crimea_disputed,_compressed).jpg. [Color online.]



different geographic distributions in this species. One of the mentioned haplogroups of *T. crassus* was recorded in Siberia. The most common haplotype of this haplogroup was found in Lake Teletskoye and the rivers Khatanga and Yenisei (Fig. 5). Another geographically specific haplogroup of this species was found in northwestern Russia. We were unable to identify the differentiation of haplotypes in terms of the frequency of occurrence in this haplogroup due to the small number of samples from northwestern Russia.

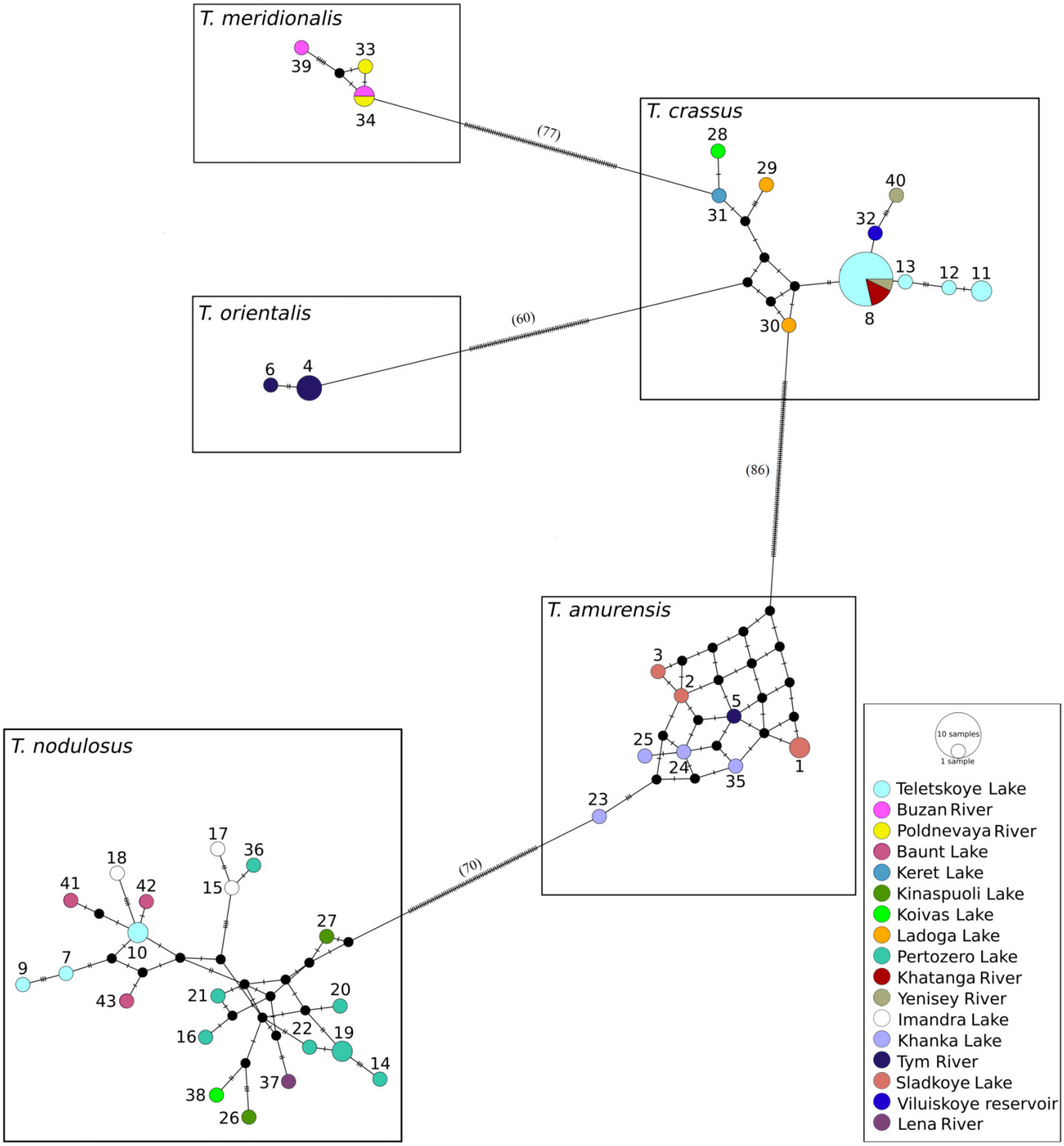
The fragments of the 28S rRNA gene, with a length of 1438 bp, were amplified and sequenced from 26 specimens of *Triaenophorus* spp. previously identified by traditional taxonomical methods (see above). The newly obtained sequences were aligned with those of *T. crassus* (#DQ925334.1, Germany), *T. nodulosus* (#KR780879.1, Great Britain), and *T. stizostedionis* (#KR780900.1, USA) retrieved from the GenBank database. Based on the results of the 28S rRNA gene-based ML and BI analyses, the studied cestode specimens were divided into four lineages: *T. stizostedionis*, *T. amurensis*, *T. nodulosus*, and *T. orientalis* + (*T. crassus* + *T. meridionalis*) (Fig. 6). However, the phylogenetic relationships among these lineages were poorly resolved.

Discussion

This is the first analysis that combines a traditional taxonomic approach together with DNA sequence analyses for species delineation and evolutionary reconstruction of *Triaenophorus* spp. The results of the phylogenetic analyses, based on partial sequences of *cox1* mtDNA and nuclear 28S rRNA genes, confirm the hypothesis of Kuperman (1968, 1973); there are five species within the genus *Triaenophorus* parasitizing fishes of Eurasia. According to Kuchta et al. (2007), the ranges of values of the width of the basal plate of the scolex hooks designated by B.I. Kuperman inadequately describe the variability of this character in each of these five species. Two species from our material, *T. crassus* and *T. nodulosus* (Supplement A), had scolex hooks in which the minimum width of the base was smaller than that indicated by Kuperman (1968, 1973). In turn, the minimum values for the width of the basal plate of scolex hooks in these species overlap (or almost coincide) with the maximum values in *T. meridionalis* and *T. amurensis*, respectively, which is consistent with the data of Kuchta et al. (2007). Nevertheless, primary identification of individual specimens of *T. amurensis*, *T. crassus*, *T. meridionalis*, and *T. nodulosus* became possible on the basis of the composition of the second and/or definitive hosts and biogeographic characteristics.

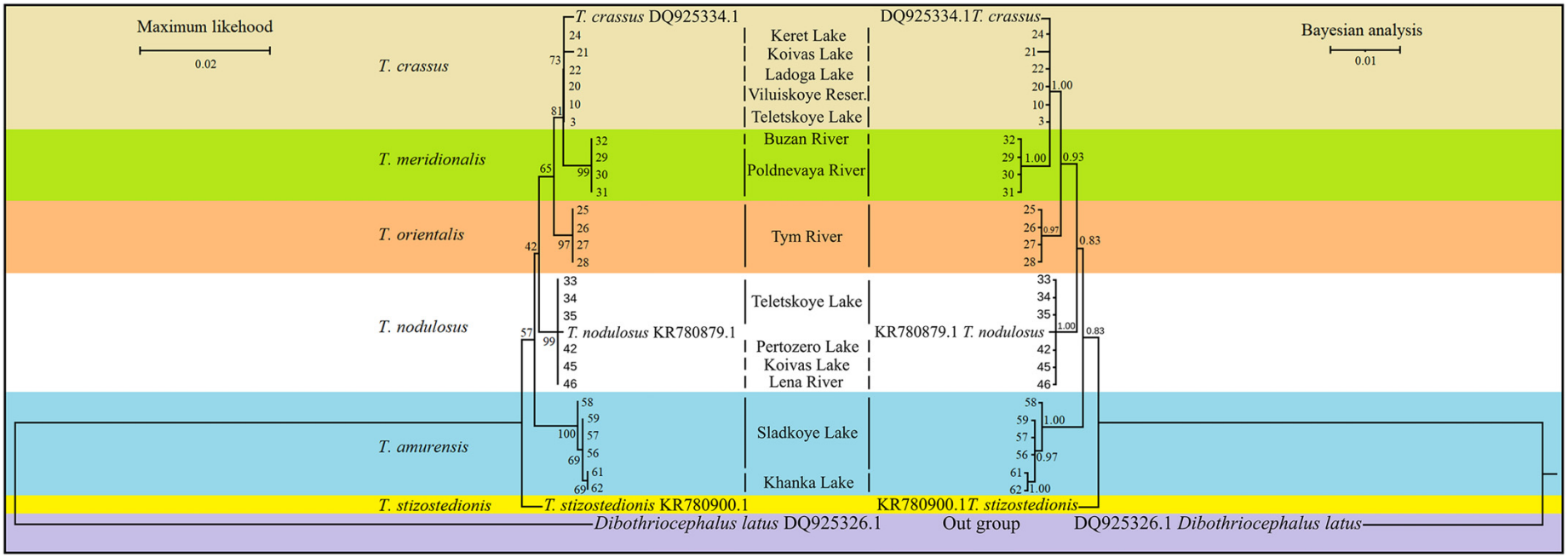
According to Kuperman (1969, 1973), *T. crassus* and *T. nodulosus* are the most ancient species of the genus *Triaenophorus* and share the most recent common ancestor, but *T. nodulosus* is evolutionarily closer to the ancestor than *T. crassus*. All the remaining Eurasian species originated from *T. nodulosus* (*T. amurensis*) and *T. crassus* (*T. orientalis* and *T. meridionalis*), respectively. Meanwhile, Petkevičiūtė and Ieshko (1991)

Fig. 5. Median networks of *Triaenophorus* spp. haplotypes (*cox1*) from studied sample points. Black circle denotes undetected or extinct hypothesized haplotypes. The numbers above circles designate the number of haplotypes. The numbers above connections designate the number of substitutions among the studied cestodes. The diameter of the circles is proportional to the haplotype frequency.



used the plesiomorphic organization of the chromosome set in *T. crassus* to hypothesize a greater closeness of this species to the ancestral form. According to the present results, *T. amurensis* is one of the earliest branching lineages on the trees (based on *cox1* mtDNA and 28S rRNA), and this species is apparently closer to the ancestral form. Our data show that the group of *Triaenophorus* spp. with a long basal plate of the scolex hook (*T. crassus*, *T. meridionalis*, and *T. orientalis*) has a monophyletic origin. However, the reconstruction of the phylogenetic relationships between the *T. crassus*,

Fig. 6. Phylogenetic relationships of *Triaenophorus* spp. reconstructed by maximum likelihood (ML; left tree) and Bayesian inference (BI; right tree) analyses of 28S rRNA gene sequences. Sample numbers are displayed at branch tips. Bootstrap values (ML) and posterior probabilities (BI) are displayed at the branch nodes. [Color online.]



T. meridionalis, and *T. orientalis* clades, obtained using different markers (*cox1* and 28S rRNA genes), yields inconsistent results (Figs. 3 and 6). At the same time, species with a short basal plate of the scolex hook (*T. amurensis*, *T. nodulosus*, and *T. stizostedionis*) form a paraphyletic assemblage (Figs. 3 and 6). It is surprising that *T. stizostedionis* occupied the most basal position compared with other cestodes. Unfortunately, the poor support of the key nodes in our phylograms does not allow us to discuss phylogenetic relationships among *Triaenophorus* spp. in a more global context.

The present results showed that the level of haplotype diversity based on *cox 1* was the lowest for *T. crassus*, higher for *T. amurensis*, and the highest for *T. nodulosus*. The difference in the level of haplotype diversity between *T. amurensis* and *T. nodulosus* was not as obvious as that between *T. amurensis* and *T. crassus*. Both *T. nodulosus* and *T. crassus* use the same fish species as definitive hosts (*E. lucius*) and have a similar holarctic range, whereas *T. amurensis* uses another esocid fish, *E. reichertii*, as a definitive host (Kuperman 1973). This fish species is endemic to the Amur River basin and adjacent rivers that were once part of the paleo-Amur system. Hence, we may assume that the differences found are based on the levels of haplotype diversity between *T. nodulosus* and *T. crassus* as well as between *T. nodulosus*/*T. crassus* and *T. amurensis* via different mechanisms.

It is known that the genetic diversity of a species depends on the effects of demographic processes in populations (Nei 1987). Taking into account the dramatic glacial events of the Quaternary period in northern Eurasia, we may assume that the modern structure of genetic diversity of Eurasian isolates (*T. crassus* and *T. nodulosus*) was formed under the pressure of a genetic bottleneck. At the same time, we may expect different genetic bottleneck pressures for these cestodes due to the different frequencies of dramatic events in various parts of their paleo-areas, different population sizes in the refugia, depletion of some host species in different refugia, etc. All of this could affect both their differences in the level of haplotype diversity and the presence/absence of the geographically specific haplogroups. However, we assume the effect of host specificity as an additional factor that is responsible for the level of haplotype differences between *T. nodulosus* and *T. crassus*. According to Nadler's hypothesis (Nadler 1995; Martinů et al. 2018), parasites with a low degree of host specificity should possess a higher level of genetic diversity than those that are strictly specific. For the studied pair of cestode species, *T. nodulosus* is characterized by a lower degree of host specificity to its second intermediate host, and, consequently, more various fish species are infected by this cestode (Kuperman 1973). The effect of the second intermediate host on the genetic diversity of these species of *Triaenophorus* is clearly seen in the example of the *T. crassus* population from Teletskoye Lake, where this parasite infests sympatric whitefishes, *Coregonus lavaretus pidschian* (Gmelin, 1789) and *Coregonus lavaretus pravdinellus* Dulkeit, 1949 (Kashinskaya et al. 2021). Here, both *T. crassus* and *Coregonus* spp. are characterized by a star-like shape of the network of haplotypes based on mitochondrial DNA data (Fig. 5; Bochkarev et al. 2018). This fact suggests that during some period of time the host and its parasite were subject to similar evolutionary processes.

Triaenophorus amurensis is characterized by the largest difference (among the studied *Trienophorus* spp.) between levels of haplotype and nucleotide diversity. Such differences (relatively high haplotype and low nucleotide diversities) are found in fast-growing populations that were originated from a low number of founders (Avise 2000). The studied specimens of *T. amurensis* were collected from two regions (Supplement A), but mostly from Sakhalin Island. The Amur species of fishes from Sakhalin Island are represented by relict populations that survived the disruption of the paleo-Amur system under the effect of Quaternary transgressions (Lindberg 1972; Bogatov et al. 2006). We hypothesize that the revealed structure of *T. amurensis*'s genetic diversity was determined by the descendants of ancient populations with a small effective size.

Conclusion

This study has provided new data on the evolution of the genus *Triaenophorus* in Eurasia. From this work, it can be concluded that there are significant genetic differences among the five species of the genus *Triaenophorus*, which are taken into account by the taxonomic model of Kuperman (1968): *T. amurensis*, *T. crassus*, *T. meridionalis*, *T. nodulosus*, and *T. orientalis*. Thus, these five previously described species are recognized as valid in accordance with the genetic analyses from this study.

Acknowledgements

We thank M. Shedko (Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of the RAS, Vladivostok, Russia), V. Odnokurtsev (Institute for Biological Problems of Cryolithozone of the Siberian Branch of the RAS, Yakutsk, Russia), S. Novokreshchennykh (Sakhalin Branch of Russian Federal Research Institute of Fisheries and Oceanography, Yuzhno-Sakhalinsk, Russia), and G. Yakovleva and D. Lebedeva (Institute of Biology, Karelian Research Centre RAS, Petrozavodsk) for contributing part of the pikeworm samples. We also thank A. Parshukova (Petrozavodsk) for her technical support. Support for *cox1* sequencing was provided by the Russian Science Foundation (project No. 19-74-10054) and for 28S rRNA sequencing by the Russian Foundation for Basic Research (project No. 19-34-60028). The sample collection in the Karelian and Siberian regions was supported by state orders with project nos. FMEN-2022-0005 and 122011800268-1, respectively.

Article information

History dates

Received: 30 July 2021
Accepted: 16 January 2022
Accepted manuscript online: 16 January 2022

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Supplementary material

Supplement A and Supplement B are available with the article at https://doi.org/10.1139/cjz-2021-0147.

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