

Diversity and taxonomy of Eurasian *Triaenophorus* spp. (Cestoda, Bothriocephalidea: Triaenophoridae) based on partial *cox1* mtDNA gene sequences

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Cestodes of the genus *Triaenophorus* are one of the most common parasites of esocids, percids, salmonids and fish of a number of other families in the Holarctic. Until the late 1960s, only two valid species of this genus were recorded in Eurasia: *T. nodulosus* and *T. crassus* Forel, 1868. Kuperman (1968) described three other species, *T. amurensis* Kuperman, 1968, *T. orientalis* Kuperman, 1968 and *T. meridionalis* Kuperman, 1968. According to Dubinina (1987) in Eurasia this genus is represented by only two species, *T. nodulosus* and *T. crassus*, each of which includes two subspecies. Kuchta et al. (2007) synonymize *T. amurensis* with *T. nodulosus*, and *T. orientalis* and *T. meridionalis* with *T. crassus*. As a result, three competing taxonomic models exist in the literature for description of the Eurasian species of *Triaenophorus* and classical morphological methods of research do not provide an adequate solution in favor of one of them.



Fig. 3. Geographical distribution of *Triaenophorus* spp. haplotypes 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.

To reconstruct phylogenetic relationships within the genus *Triaenophorus* partial *cox1* gene sequences were used. Analysis of genetic distances was conducted in MEGA 7 (Kumar et al., 2016). Phylogenetic reconstruction was performed using the Maximum Likelihood (ML) and the Bayesian inference (BI) approaches with MEGA 7 MrBayes v.3.2.1 respectively. The median-joining network of haplotypes was calculated and visualized using Popart 1.7 software (<https://popart.otago.ac.nz>). Newly obtained sequences were deposited into the GenBank database.

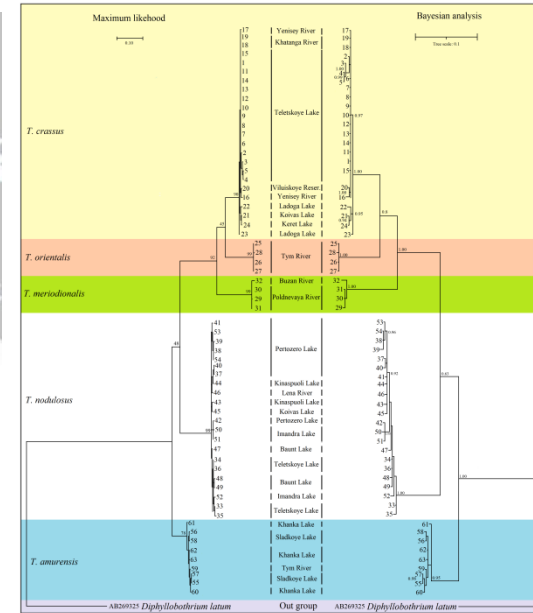


Fig. 3 Phylogenetic relationships of *Triaenophorus* spp. reconstructed by Maximum Likelihood (left tree) and Bayesian Inference (right tree) analyses. Bootstrap values (ML) and posterior probabilities (BA) are displayed at the branch nodes.

Within the species-level clades, the mean p-distance values for *T. crassus*, *T. orientalis*, *T. meridionalis*, *T. nodulosus* and *T. amurensis* were $0.54 \pm 0.13\%$, $0.17 \pm 0.12\%$, $0.57 \pm 0.22\%$, $1.25 \pm 0.24\%$, and $0.63 \pm 0.20\%$ respectively. The mean p-distance between these clades varied in range from $10.9 \pm 1.3\%$ (*T. crassus* by *T. orientalis*) to $18.0 \pm 1.6\%$ (*T. meridionalis* by *T. amurensis*).

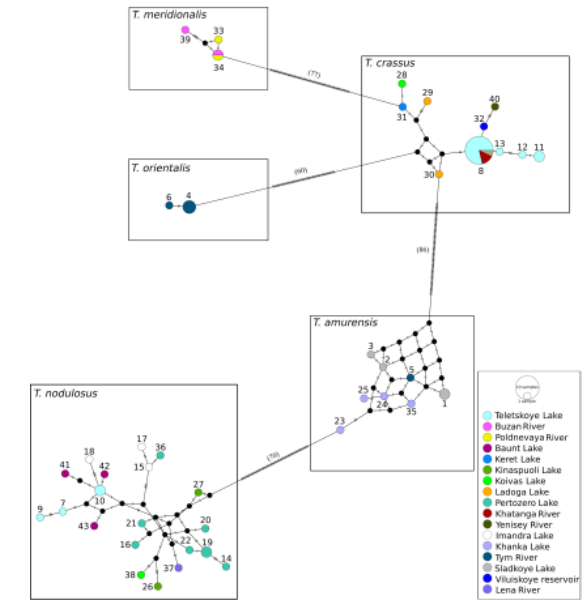


Fig. 5. Median networks of *Triaenophorus* spp. haplotypes from studied sample points. Numbers above connections designate the number of substitutions among studied cestodes. Diameter of circles is proportional to haplotype frequency.

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 species previously described are recognized as valid in accordance with the genetic analyses from this study.

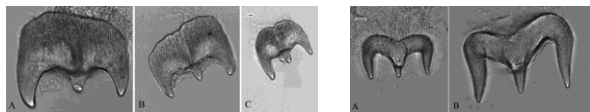


Fig. 1. Scolex hooks of *Triaenophorus crassus* (A), *T. meridionalis* (B) and *T. orientalis* (C) at the same scale. Scale bar 11 µm.

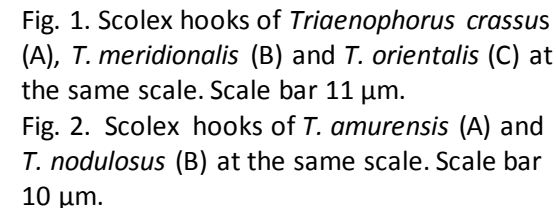


Fig. 2. Scolex hooks of *T. amurensis* (A) and *T. nodulosus* (B) at the same scale. Scale bar 10 µm.