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MOLECULAR AND MORPHOLOGICAL DESCRIPTION OF *DIPLOSTOMUM SPATHACEUM* METACERCARIAE FROM *ABRAMIS BRAMA* L. OF LAKE SYAMOZERO (NORTH-WEST RUSSIA)

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Trematodes *Diplostomum* von Nordmann, 1832 are widely distributed parasites with complex life cycles involving freshwater snails as the first intermediate hosts, various fish species as the second intermediate hosts, and fish-eating birds as definitive hosts. Metacercariae of *Diplostomum* spp. are important fish pathogens with problematic morphological identification. The present research of *Diplostomum* larvae in the eyes of the freshwater bream *Abramis brama* was carried out within the framework of the long-term parasitological monitoring of fish in Lake Syamozero. The study has provided molecular and morphological characterization of *Diplostomum spathaceum* metacercariae from the lens of the freshwater bream. The partial cox1 mtDNA sequences used for molecular identification of the isolates were identical with those obtained previously, but morphological features of the metacercariae matched the literature data only in part. Comparison of different dimensions of parasites by discriminant analysis suggests that *Diplostomum rutili* is a junior synonym of *Diplostomum spathaceum*. New and archival data on diplostomids from bream's eyes demonstrate a long-term increase in the infection prevalence.

Keywords: trematoda, cox1, freshwater bream, eye lens, Karelia

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Lake Syamozero (Baltic Sea drainage basin), located in South Karelia, has long been a unique water body for studying the dynamics of ecosystem eutrophication. Lake Syamozero is the third largest water body in South Karelia (Lake Onego drainage basin, Shuya River catchment). Its area including islands is 270.3 km^2. The lake is mesotrophic. It is a relatively shallow reservoir with a slight decrease in oxygen concentration in the bottom layers. The lake has abundant zooplankton, and the benthic fauna is relatively rich, with a predominance of chironomids. The fauna of molluscs and insect larvae is also diverse. The ichthyofauna of the lake includes 24 species of fish, among which Cyprinids prevail (Novokhatskaya, 2008).

Various aspects of the lake ecosystem functioning, such as the fish fauna, hydrological parameters, fish parasites, have been studied by staff of the Karelian Research Centre RAS since the 1950s. Long-term multidisciplinary studies have shown that increased anthropogenic pressure in the 1970s–1980s has significantly altered the Syamozero ecosystem (Reshetnikov et al., 1982). In the 1990s, the lake has partly recovered ecologically as the anthropogenic pressure decreased (Sterligova et al., 2016).

Research on fish parasites in Syamozero has been underway for more than 50 years (Shulman, 1962; Shulman et al., 1974; Malakhova, Ieshko, 1977; Ieshko, Malakhova, 1982; Novokhatskaya, 2008; Novokhatskaya et al., 2008; etc.). The variations observed in the diversity and prevalence of parasites indicate the extreme lability of parasitic systems, which are responsive to any changes in the hydrological and hydrobiological conditions (Rumyantsev, 1996).

However, for an accurate overview of the changes in the parasite fauna of fish it is necessary to accurately identify these parasites, which is not always easy. For example, larvae of the genus *Diplostomum* von Nordmann, 1832 are difficult to identify morphologically. This is a large genus of widely distributed trematodes. Their life cycles involve freshwater snails as the first intermediate hosts, various fish species as the second intermediate hosts, and fish-eating birds as definitive hosts (Shigin, 1986).

Since the advance of the molecular methods, the systematics of many parasite species, including *Diplostomum* spp., has been revisited, and molecular analysis is now essential for the identification of metacercariae (Astrin et al., 2013; Faltýnková et al., 2014, 2022; Lebedeva et al., 2022; Sokolov et al., 2023; etc.).

The objective of our study is to provide a morphological and molecular description of *Diplostomum* metacercariae from the eyes of the freshwater bream *Abramis brama* L., 1758 in Lake Syamozero as an integral part of investigating the parasite composition in the bream and long-term monitoring of changes in its parasite fauna.

MATERIALS AND METHODS

Sample collection

The work is based on the parasitological studies of bream from the south-western part of Lake Syamozero (Syargilahta Bay). Syargilahta Bay (Fig. 1) is a sheltered bay with small sparse aquatic vegetation and shares all of the abovementioned features with the lake.

Breams *Abramis brama* (20 specimens) were examined by complete parasitological dissection according to the method of Bykhovskaya-Pavlovskaya (1985). Fish aged from 4+ to 9+ years were caught with 30-60 mm mesh nets in June 2024. *Diplostomum* metacercariae were found in the eyes, removed under a preparation stereomicroscope, washed in distilled water and counted. Some larvae were immediately fixed in 96% ethanol and some stained with acetic acid carmine. We used definitions for vouchers in a molecular context proposed by Astrin et al. (2013). Metacercaria paragenophores (morphological voucher) stained with acetic acid carmine were mounted in Canadian balsam for the morphological investigation (Sudarikov, Shigin, 1965). Ten hologenophores (molecular voucher), each from an individual host, fixed in ethanol were used for the molecular studies. They are metacercariae with different body shapes corresponding to those of paragenophores.

The ecological metrics of fish infection, prevalence (E) and mean abundance (M), were calculated according to Bush et al. (1997).

Figure 1. Sampling location: Lake Syamozero. Syargilahta Bay is marked with a black circle.

Morphological examination

Photomicrographs of paragenophores of metacercariae were made with a Levenhuk C1400 NG digital camera attached to Olympus BX-53 microscope using LevenhukToupView image analysis software (V 3.5) at the Core Facility of the Karelian Research Centre of the Russian Academy of Sciences, Petrozavodsk, Russia. The morphology of 40 metacercariae from the eye lens of *A. brama* was investigated. Thirteen morphological characteristics according to Shigin (1986) were scored (in μm): body length (BL), body width (BW), oral sucker length (OSL), oral sucker width (OSW), ventral sucker length (VSL), ventral sucker width (VSW), holdfast organ length (HL), holdfast organ width (HW), pseudosucker length (PSL), pharynx length (PHL), pharynx width (PHW), distance from center of ventral sucker to anterior end of body (O), and number of excretory bodies. Six indices of the relative values of these parameters were used: BW × BL/HW × HL, BW × BL/VSW × VSL, OSW × OSL/VSW × VSL, HW × HL/ VSW × VSL, BW/BL (%), O/BL (%).

Morphological characteristics of the parasites were assessed by means of discriminant analysis in PAST v. 4.05 (Hammer et al., 2001). Values of ten morphometric parameters of metacercariae of different *Diplostomum* species were chosen for the canonical discriminant analysis (BL, BW, OSL, OSW, VSL, VSW, HOL, HOW). The choice was based on the availability of published data and their relevance for our study. We used average measurements of *D. spathaceum* metacercariae from *Abramis brama* (Shigin, 1986), *Gasterosteus aculeatus* Linnaeus, 1758, *Salvelinus alpinus* Linnaeus, 1758 (Faltýnková et al., 2014), *Cyprinus carpio* Linnaeus, 1758 (Niewiadomska, 2010; Pérez-del-Olmo et al., 2014), multiple hosts: *Acipenser ruthenus* Linnaeus, 1758; *Abramis brama* Linnaeus, 1758; *Blicca bjoerkna* Linnaeus, 1758; *Chondrostoma nasus* Linnaeus, 1758; *Leuciscus aspius* Linnaeus, 1758; *Rutilus pigus* (Lacépède, 1804); *Rutilus rutilus* Linnaeus, 1758; *Vimba vimba*

Linnaeus, 1758; *Silurus glanis* Linnaeus, 1758 (Kudlai et al., 2017); and average measurements of *D. rutili* Razmashkin, 1969 (Shigin, 1986) metacercaria from the lens of *Hypophthalmichthys molitrix* (Valenciennes, 1844) to determine their ordination in the canonical axes.

The taxonomy and nomenclature of *Diplostomum* spp. followed the latest studies by Schwelm et al. (2021), Achatz et al. (2022), Faltýnková et al. (2022) and Sokolov et al. (2023).

Slides of paragenophore specimens (DspAb1-DspAb6) of the parasites are deposited in the Helminthological Collections of the Karelian Research Centre, Russian Academy of Sciences (Petrozavodsk, Karelia, Russia).

Molecular and phylogenetic analysis

Genomic DNA was isolated from 10 ethanol-fixed specimens using the DNA-Extran kits (Synthol, Moscow, Russia).

For each of these larvae, we amplified a fragment of the mtDNA cox1 gene using the primers Cox1_schist_5' (5'-TCTTTR GAT CAT AAG CG-3') and Cox1_schist_3' (5'-TAA TGC ATM GGA AAA AAA CA-3') of Lockyer et al. (2003). The PCR assay was carried in 20 µl of reaction mixture containing 5XScreenMix (Evrogen, Moscow Russia), 1.5 pmol of each primer, an 2 µl of DNA; an annealing temperature of 50^{0}C was used for the amplification. PCR products were purified using the Cleanup Standard Extraction Kit (Evrogen, Moscow, Russia) following the manufacturer's instructions and then sequenced directly with the automatic sequencing system ABI PRISM 3100-Avant (Applied Biosystems Inc., Foster City, CA, USA). Consensus sequences were assembled within MEGA v. 10 (Kumar et al., 2018) to the 1170 bp length and deposited in GenBank under the accession numbers PQ461127 – PQ461136.

The identity of the newly generated sequences was checked with the Basic Local Alignment Search Tool (BLASTn) (www.ncbi.nih.gov/BLAST/, accessed on 10 October 2024). In total, the novel sequences were aligned with those of 32 representatives of the genus *Diplostomum* in MEGA v. 10 and trimmed to the shortest length, 384 nt. Blasting of the sequences showed a more than 99% match with a large number of sequences of *Diplostomum spathaceum*. However, we used only 15 of them for the phylogenetic analysis. In this case, we used specimens of the species *D. spathaceum*, for which both molecular and morphological data are available, especially from bream, as well as specimens of this species in different development phases from different geographical locations (Georgieva et al., 2013; Blasco-Costa et al., 2014; Faltýnková et al., 2014; Pérez-del-Olmo et al., 2014; Locke et al., 2015; Kudlai et al., 2017; Achatz et al., 2022; Lebedeva et al., 2023; Diaz-Suarez et al., 2024). We also applied some sequences of *Diplostomum* spp. of "non-lens" localization (Achatz et al., 2022).

The cox1 sequence JX986909 of *Tylodelphys clavata* (Nordmann, 1832) was used as an outgroup.

To assess the phylogenetic relationships of the newly found *Diplostomum* metacercariae, we applied Bayesian inference analysis (BI) and Maximum Likelihood (ML). ML analyses of HKY+G+I parameter distances were carried out using MEGA v10; nodal support was estimated using 1000 bootstrap resamplings. The best-fitting model for BI was identified as TVM + G + I with jModelTest v2.1.2 (Darriba et al., 2012). Bayesian inference analyses were conducted using MrBayes (v3.2.3) (Ronquist et al., 2012). Markov chain Monte Carlo (MCMC) simulations were run for 3,000,000 generations, log-likelihood scores were plotted, and only the final 75% of trees were used to produce the consensus tree. Posterior probability was calculated to estimate nodal support. FigTree v1.4 (Rambaut, 2012) was used to visualize the tree.

Distance matrices (*p*-distance) were calculated with MEGA v. 10 (Kumar et al., 2018). The *cox*1 haplotypes of *Diplostomum* spp. from the lens of the freshwater bream, *Abramis brama*, collected in the present study and previous study in Slovakia (Kudlai et al., 2017) countries were identified with DnaSP v. 6 (Rozas et al., 2017). The haplotype network was reconstructed using the Median-Joining method in PopART software v 1.7 (Population Analysis with Reticulate Trees, http://popart.otago.ac.nz).

RESULTS

We investigated different eye tissues of bream, and *Diplostomum* metacercariae were found only in the lens. Prevalence was 100%, mean abundance 25.3 specimens per fish, intensity from 1 to 51 specimens.

Phylogenetic analysis included ten metacercariae taken from the eyes of different bream specimens fish and indicated that their partial sequences of the cox 1 gene were identical to the published ones of the species *D. spathaceum* (Rudolphi, 1819) Olsson, 1876 (Fig. 2).

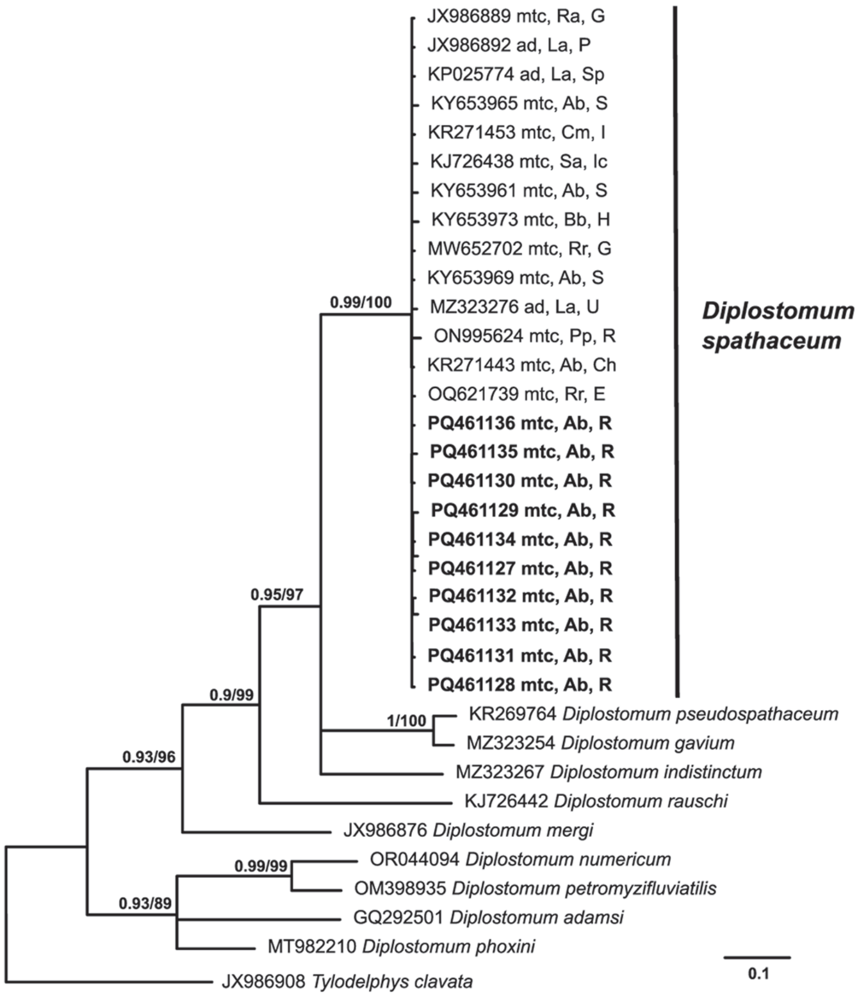

Figure 2. Bayesian inference (BI) and maximum likelihood (ML) analyses tree for *Diplostomum* spp. based on the partial *cox*1 mtDNA sequences (384 bp). Nodal supports from both analyses are indicated as BI/ML. The scale bar indicates the expected number of substitutions per site. Only significant values of the posterior probabilities (≥ 0.5) are indicated. Newly obtained sequences are in bold. Abbreviations: ad – adult, mtc – metacercariae, Ab – *Abramis brama,* Bb – *Blicca bjoerkna*, Cm – *Cyprinion macrostomum*, La – *Larus argentatus*, Pp – *Pungitius pungitius*, Rr – *Rutilus rutilus*, Sa – *Salvelinus alpinus*, Ch – China, E – Estonia, G – Germany, H – Hungary, I – Iraq, Ic – Iceland, R – Russia, S – Slovakia, Sp – Spain.

The metacercariae we studied joined in a clade with representatives of *D. spathaceum* at different developmental stages and from different countries, confirming a wide distribution and occurrence in different host species worldwide (Fig. 2). The *p*-distance between ten investigated parasites from Syamozero was 0.08 – 1%. And *p*-distances among samples of *D. spathaceum* were 0.03 – 1.5%.

There were seven haplotypes (Fig. 3) among the larvae from bream, with haplotype diversity at 0.93. Four of the sequences each had its own haplotype (PQ461127, PQ461129, PQ461130, PQ461134), and three more haplotypes were represented by two specimens each (PQ461132 and PQ461133; PQ461135 and PQ461136; PQ461128 and PQ461131, respectively).

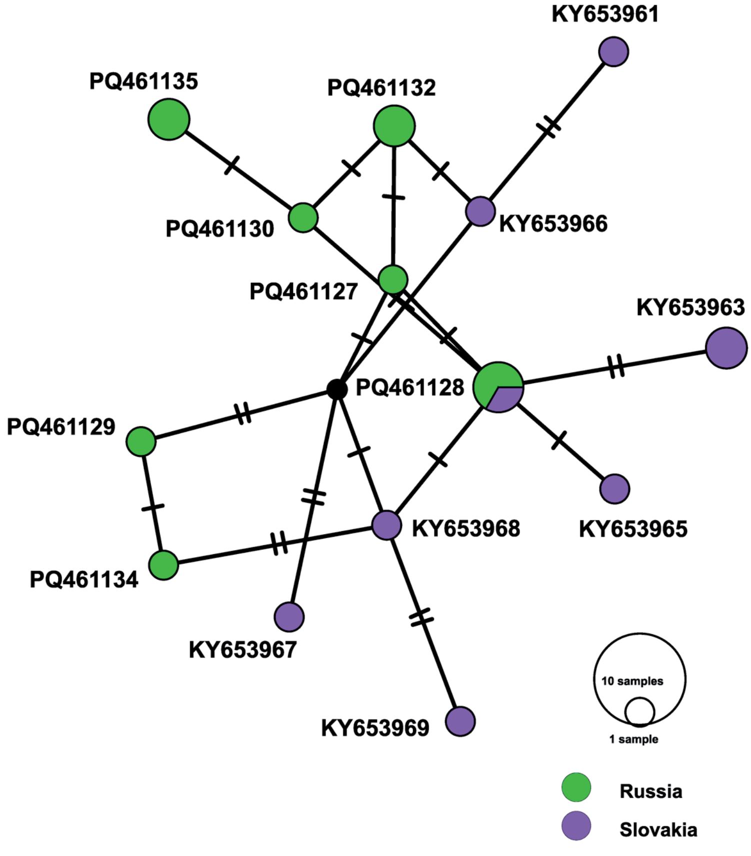

Figure 3. Haplotype network for *Diplostomum spathaceum* based on novel and published partial *cox*1 mtDNA sequences of Kudlai et al. (2017). Unsampled intermediate haplotype is represented by a short intersecting line; each branch corresponds to a single mutational difference and connective lines represent one mutational step. Circle size is proportional to the number of isolates sharing a haplotype; black circles indicate transitive haplotypes that have not been found.

Comparison against the only available cox1 mtDNA sequences of *D. spathaceum* metacercariae from breams from Slovakia (Kudlai et al., 2017) showed that only one haplotype was shared between two Karelian metacercariae (PQ461128, PQ461131) and one Slovakian metacercaria (KY653964).

Along with the phylogenetic analysis, paragenophores (taken from the same host individuals as the larvae taken for the molecular studies) were examined morphologically (Fig. 4).

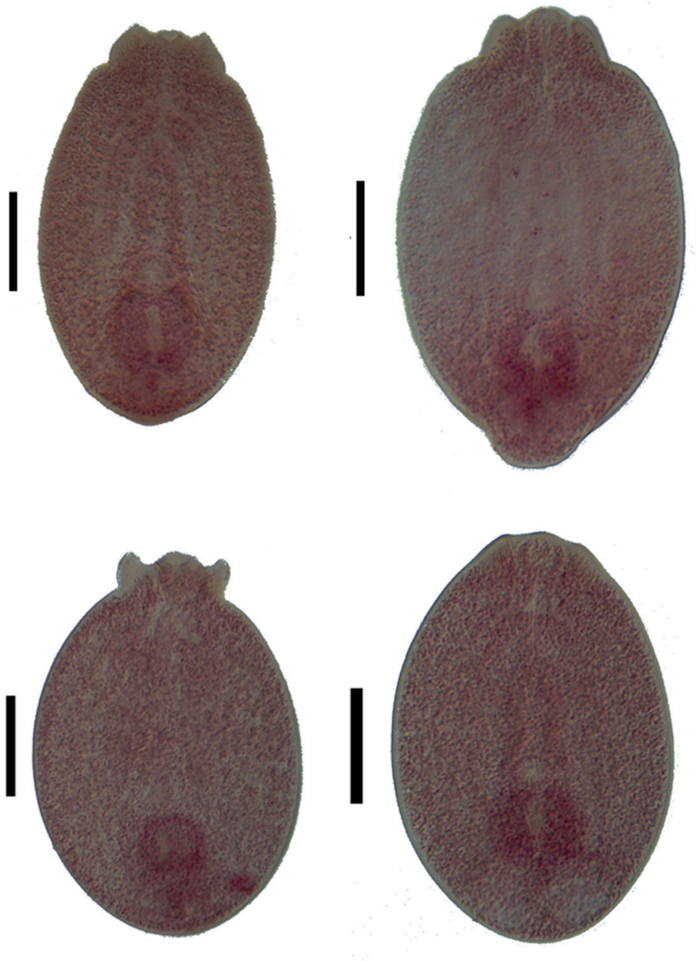

Figure 4. Metacercariae of *Diplostomum spathaceum* with different shape of body from the lens of *Abramis brama* in Lake Syamozero. Scales: 100 μm.

Thirteen morphological attributes of 40 newly found *D. spathaceum* metacercariae and six ratios of attributes were obtained.

Body elongate-oval, 296–499 × 194–313 (403 × 243), with maximum width just anterior to ventral sucker. Oral sucker elongate-oval, 33–70 × 36–61 (54 × 46). Pseudosuckers strongly muscular, mostly prominent, ear-like, 40 – 75 (58) long. Pharynx elongate-oval, 25–39 × 18–28 (34 × 20); oesophagus short, bifurcates closely posterior to pharynx; caeca long, narrow, reaching posterior to holdfast organ. Ventral sucker transversely oval, 32–59 × 40–75 (47 × 53), smaller or larger than oral sucker [sucker width ratio 1:0.92 0.96 (1:1.02)], slightly posterior to mid-body length. Distance from anterior extremity of body to ventral sucker 154 294 (242). Holdfast organ relatively small, transversely oval, bipartite, contiguous with ventral sucker, 57–119 × 61–146 (78 × 82). Excretory vesicle small, V-shaped; reserve excretory system of diplostomid type; excretory concretions small, 177–380 (259) in number. Ratios are BW × BL/HW × HL 9–16 (15); BW × BL/VSW ×VSL 35–44 (40); OSW × OSL/VSW ×VSL 0.92–0.96 (1); HW × HL/VSW ×VSL 2.6–3.9 (2.5); BW/BL (%) 60–66 (63); O/BL (%) 52–60 (59).

Metacercariae from different host specimens did not significantly differ in the number of excretory granules ($p > 0.05$). The parasites' dimensions also were not significantly different ($p > 0.05$). The morphological attributes of metacercariae were also similar, except for the shape of body and pseudosucker (Fig. 4). The body shape of metacercariae from bream's eye lens varied from rounded to elongated oval. A specific feature of the larvae was the morphology of pseudosuckers, which in a majority of metacercariae protruded forward beyond the anterior part of the body and oral suckers.

Discriminant analysis of the newly obtained and previously published data on the mean dimensions of *Diplostomum* spp. metacercariae parasitizing the lens was conducted (Fig. 5). The metacercariae of *D. spathaceum* from Lake Syamozero fell within a single cohort with a 95% confidence interval (large ellipse in Fig. 5).

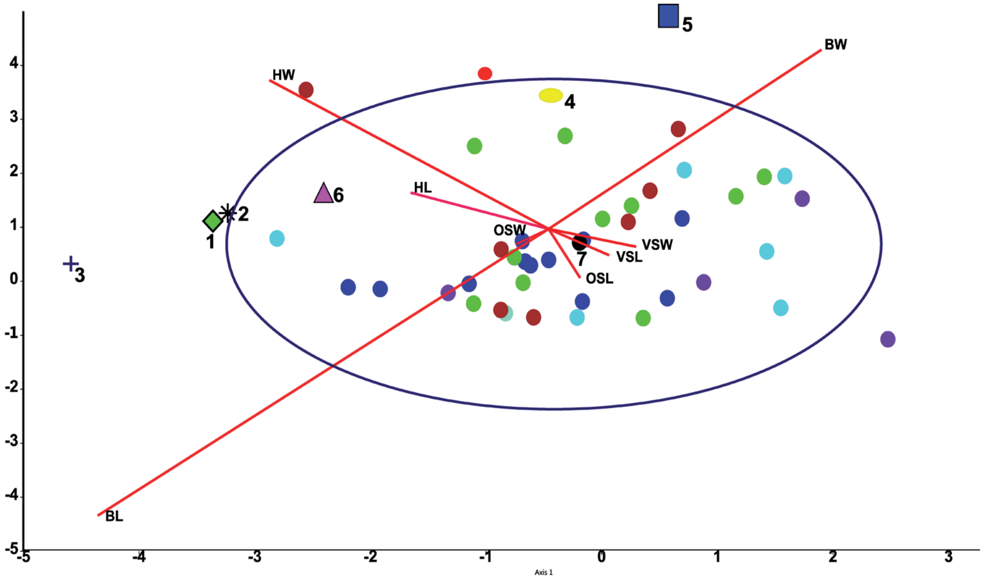

Figure 5. Ordination of the traits of *Diplostomum spathaceum* and *D. rutili* metacercariae by discriminant analysis. The mean values of nine traits, including morphometry, from the present study and the literature were involved: 1 – green rhombus: *D. spathaceum* (Shigin, 1986); 2 – black asterisk: *D. spathaceum* (Niewiadomska, 2010); 3 – blue cross: *D. spathaceum* (Faltýnková et al., 2014); 4 – yellow oval: *D. spathaceum* (Pérez-del-Olmo et al., 2014); 5 – navy square: *D. spathaceum* (Kudlai et al., 2017); 6 – pink triangle: *D. rutile* (Shigin, 1986); 7 – black circle: means of dimensions of *D. spathaceum* from Lake Syamozero (the coloured points – red, light blue, dark blue, green, brown, purple – represent the specimens taken from different host individuals).

Only three individuals from different hosts did not fit into the size group. The most significant contribution to the divergence of the species included in the analysis was from the metacercariae body length and width (BL and BW) on both axes. The length and width of the holdfast organ (HL and HW) were the most significant on the first canonical axis, while OSL, VSL and VSW were the most significant on the second canonical axis. The confidence ellipse includes both data on the dimensions of *D. spathaceum* from the present study and from *Cyprinus carpio* (Pérez-del-Olmo et al., 2014) as well as on *D. rutili* from *Hypophthalmichthys molitrix* (Shigin, 1986). Metacercariae of *D. spathaceum* from *Abramis brama* of Shigin (1986) were the most similar in mean dimensions to the larvae found in *Cyprinus carpio* (Niewiadomska, 2010). While neither of these samples fit within the confidence zone of the Lake Syamozero sample, they proved to be very close to it. Average dimensions of *D. spathaceum* from *G. aculeatus* and *S. alpinus* (Faltýnková et al., 2014) and from multiple hosts (Kudlai et al., 2017) were far both from the confidence ellipse of the Lake Syamozero sample and from each other (Fig. 5).

DISCUSSION

The data we obtained demonstrate that the species *D. spathaceum* parasitizes the lens of freshwater breams in Lake Syamozero. Previously, this species was recorded by researchers either as *Diplostomum* sp. (Shulman, 1962; Ieshko, Malakhova, 1982) or as *D. chromatophorum* (Brown, 1931) Shigin, 1986 (Novokhatskaya et al., 2008). This identification is solely due to the alteration in the systematics of the genus, when different authors tried to distinguish species only on the basis of morphological data and host specificity (Sudarikov et al., 2002; Niewiadomska, 2010). However, owing to the use of molecular methods, the situation with identification and description of diplostomids tends to improve. The partial cox1 mtDNA sequences of the newly found parasites matched numerous previously published sequences for the species *D. spathaceum*, noted not only at the metacercariae stage in different fish species (Faltýnková et al., 2014; Kudlai et al., 2017), but also at the adult stage (Pérez-del-Olmo et al., 2014).

We identified seven haplotypes in ten metacercariae retrieved from bream from Lake Syamozero. Among the 14 cyprinid species in Danube River in the study by Kudlai et al. (2017), *A. brama* had the largest haplotype diversity (8 haplotypes). In total, our data and the above-mentioned report by Kudlai et al. (2017) revealed 14 haplotypes in *Diplostomum spathaceum* metacercariae from bream, with haplotype diversity (Hd) of 0.96.

Our integrated approach (molecular and morphological features) also enabled a detailed analysis of the morphological attributes of *D. spathaceum* larvae (Fig. 5).

The morphology of *D. spathaceum* metacercariae in our study (Fig. 5) is not in full agreement with the descriptions by other authors, i.e. Faltýnková et al. (2014) and Kudlai et al. (2017). The newly investigated specimens differ from those of Faltýnková et al. (2014) in having a shorter and wider body, smaller oral and ventral sucker, holdfast organ. Metacercariae from the host mix of Kudlai et al. (2017) had a smaller length of the body and oral sucker, but larger dimensions of the holdfast organ compared to the parasites from Syamozero. While situated in the very periphery of the confidence ellipse, the larvae reported by Pérez-del-Olmo et al. (2014) had dimension characteristic of the species. Such variability of parasites is probably due to different sample collection and fixation methods, as well as different host species.

The most interesting finding of our analysis is that the mean dimensions of the species *D. rutili* described from the lens of *Hypophthalmichthys molitrix* by Shigin (1986) fall within the confidence ellipse of the newly studied metacercariae of *D. spathaceum*. In addition, Figure 5 demonstrates that the mean dimension values of the species fall within the range between those of the newly described metacercariae and larvae of *D. spathaceum* from Shigin (1986) and Niewiadomska (2010). Furthermore, such a specific feature of the larvae as pseudosuckers protruding forward beyond the anterior part of the body and oral suckers was described as a species-specific trait only for the metacercariae of *D. rutili* Razmashkin, 1969 (Shigin, 1986). Thus, the matching sizes, the presence of the characteristic ear-shaped protruding pseudosuckers, and the similar range of Cyprinid hosts leads us to the assumption that the species *D. rutili* Razmashkin, 1969 may be conspecific to *D. spathaceum* (Rudolphi, 1819) Olsson, 1876.

The joint analysis of our own and published data shows that the rates of *Diplostomum* metacercariae infection in bream of Lake Syamozero have have increased significantly since 1980 (Fig. 6).

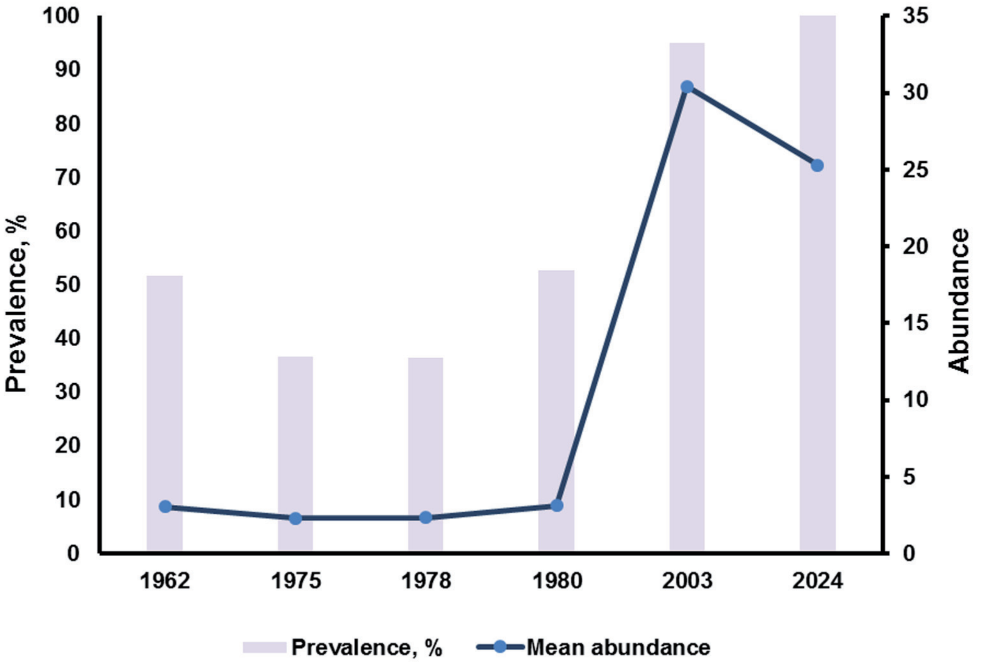

Figure 6. Long-term changes of *Diplostomum* sp. metacercariae infection parameters in eyes of the bream *Abramis brama* in Lake Syamozero.

A similar trend was previously noted in many other fish species in Syamozero (Ieshko, Novokhatskaya, 2008; Novokhatskaya et al., 2005; Novokhatskaya, 2008). It is likely that intensive vegetation growth in the littoral zone induced by eutrophication has created favourable conditions for gastropods, the intermediate hosts of diplostomids (Novokhatskaya, 2008). In addition, the concentration of juveniles of many fish species in shallow waters (Sterligova et al., 2016) and the growing number of gulls in the lake area (Sazonov, 2004) also promote the high abundance of diplostomids.

CONCLUSIONS

Thus, our study has established that the species *D. spathaceum* parasitizes the eye lens of breams in Lake Syamozero. The mtDNA sequences of the species match those obtained earlier, but their morphological features only partially correspond to those described in the literature. Furthermore, discriminant analysis of the size characteristics in combination with the presence of a well-defined three-lobed anterior end of the body suggests that *D. rutili* is a junior synonym of *D. spathaceum*. This study once again confirms the importance of using an integrated approach to diplostomid larvae identification. In addition, this approach opens great opportunities for investigating the species morphological variability within populations.

Long-term data demonstrate a considerable increase in bream infection with *D. spathaceum* and reflect changes occurring both in the host population and in the ecosystem as a whole.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Research Fishing is under Permit of North-West Territorial Administration of the Federal Agency for Fishery (7820240317689) from 14 May 2024.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

REFERENCES

Achatz T.J., Martens J.R., Kostadinova A., Pulis E.E., Orlofske S.A., Bell J.A., Fecchio A., Oyarzún-Ruiz P., Syrota Y.Y., Tkach V.V. 2022. Molecular phylogeny of *Diplostomum*, *Tylodelphys*, *Austrodiplostomum* and *Paralaria* (Digenea: Diplostomidae) necessitates systematic changes and reveals a history of evolutionary host switching events. International Journal for Parasitology 52: 47–63. doi:10.1016/j.ijpara.2021.06.002

Astrin J.J., Zhou X., Misof B. 2013. The importance of biobanking in molecular taxonomy, with proposed definitions for vouchers in a molecular context. Zookeys 30 (365): 67–70. doi: 10.3897/zookeys.365.5875

Blasco-Costa I., Faltýnková A., Georgieva S., Skírnisson K., Scholz T., Kostadinova A. 2014. Fish pathogens near the Arctic Circle: molecular, morphological and ecological evidence for unexpected diversity of *Diplostomum* (Digenea: Diplostomidae) in Iceland. International Journal for Parasitology 44:703–715, https://doi.org/10.1016/j.ijpara.2014.04.009

Bush A.O., Lafferty K.D., Lotz J.M., Shostak A.W. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology 83: 575–583. https://doi.org/10.2307/3284227

Bykhovskaya-Pavlovskaya I.E. 1985. Fish parasites. Guide to the study. Leningrad, Nauka, 121 pp. (In Russian).

Darriba D., Taboada G.L., Doallo R., Posada D. 2012. jModelTest 2: More models, new heuristics and parallel computing. Natural Methods 9: 772–772. https://doi.org/10.1038/nmeth.2109

Diaz-Suarez A., Noreikiene K., Kahar S., Ozerov M.Y., Gross R., Kisand V., Vasemägi A. 2024. DNA metabarcoding reveals spatial and temporal variation of fish eye fluke communities in lake ecosystems. International Journal for Parasitology 54: 33–46. doi: 10.1016/j.ijpara.2023.07.005

Faltýnková A., Georgieva S., Kostadinova A., Blasco-Costa I., Scholz T., Skírnisson K. 2014. *Diplostomum* von Nordmann, 1832 (Digenea: Diplostomidae) in the sub-Arctic: descriptions of the larval stages of six species discovered recently in Iceland. Systematic Parasitology 89: 195–213. https://doi.org/10.1007/s11230-014-9517-0

Faltýnková A., Kudlai O., Pantoja C., Yakovleva G., Lebedeva D. 2022. Another plea for 'best practice' in molecular approaches to trematode systematics: *Diplostomum* sp. clade Q identified as *Diplostomum baeri* Dubois, 1937 in Europe. Parasitology 149: 503–18. doi:10.1017/S0031182021002092

Georgieva S., Soldánová M., Pérez-Del-Olmo A., Dangel D.R., Sitko J., Sures B., Kostadinova A. 2013. Molecular prospecting for European *Diplostomum* (Digenea: Diplostomidae) reveals cryptic diversity. International Journal for Parasitology 43: 57–72. doi: 10.1016/j.ijpara.2012.10.019

Hammer Ø., Harper D.A.T., Ryan P.D. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4: 9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm

Ieshko E.P., Malakhova R.P. 1982. Parasitological characterization of fish infestation as an indicator of ecological changes in a water body. In: Reshetnikov Y.S. (ed.). Changes in the structure of fish population in eutrophic water bodies. Moscow, Nauka, 161–176. (In Russian).

Ieshko E.P., Novokhatskaya O.V. 2008. Trends in succession of parasitofauna of fish of eutrophied water bodies. Journal of Ichthyology 48 (8): 665–670. doi 10.1134/S0032945208080134

Kudlai O., Oros M., Kostadinova A., Georgieva S. 2017. Exploring the diversity of *Diplostomum* (Digenea: Diplostomidae) in fishes from the river Danube using DNA mitochondrial barcodes. Parasites & Vectors 10, 592. doi:10.1186/s13071-017-2518-5

Kumar S., Stecher G., Li M., Knyaz C., Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Molecular Biology and Evolution 35: 1547–1549. https://doi.org/10.1093/molbev/msy096

Lebedeva D.I., Popov I.Y., Yakovleva G.A., Zaicev D.O., Bugmyrin S.V., Makhrov A.A. 2022. No strict host specificity: Brain metacercariae *Diplostomum petromyzifluviatilis* Müller (Diesing, 1850) are conspecific with *Diplostomum* sp. Lineage 4 of Blasco-Costa et al. (2014). Parasitology International 91, 102654. https://doi.org/10.1016/j.parint.2022.10265

Lebedeva D.I., Zaitsev D.O., Alekseeva J.I., Makhrov A.A. 2023. Metazoan parasites of two stickleback species at the Solovetsky Archipelago (White Sea). Marine Biological Journal 8: 33–46. https://doi.org/10.21072/mbj.2023.08.3.03

Locke S.A., Al-Nasiri F.S., Caffara M., Drago F., Kalbe M., Lapierre A.R., McLaughlin J.D., Nie P., Overstreet R.M., Souza G.T.R., Takemoto R.M., Marcogliese D.J. 2015. Diversity, specificity and speciation in larval Diplostomidae (Platyhelminthes: Digenea) in the eyes of freshwater fish, as revealed by DNA barcodes. International Journal for Parasitology 45: 841–855. https://doi.org/10.1016/j.ijpara.2015.07.001

Lockyer A.E., Olson P.D., Stergaard P., Rollinson D., Johnston D.A., Attwood S.W., Southgate V.R., Horak P., Snyder S.D., Le T.H., Agatsuma T., McManus D.P., Carmichael A.C., Naem S., Littlewood D.T.J. 2003. The phylogeny of the Schistosomatidae based on three genes with emphasis on the interrelationships of *Schistosoma*Weinland, 1858. Parasitology 126 (3): 203–224. https://doi.org/ 10.1017/S0031182002002792

Malakhova R.P., Ieshko E.P. 1977. Changes in the parasite fauna of fish of Syamozero for the last 20 years. In: Potapova O.I., Sokolova V.A. (eds). Syamozero and perspectives of its fishery utilization. Petrozavodsk: Karelsky Nauchny Centr, 185–199. (In Russian).

Niewiadomska K. 2010. Przywry (Trematoda).Część ogólna; Część systematyczna – Aspidogastrea, Digenea: Strigeida. Poland, Łódź, Wydawnictwo Uniwersytetu Łódzkiego, 388 pp. (in Polish).

Novokhatskaya O.V. 2008. Parasitofauna of fish of eutrophic lakes (on the example of Syamozero). Theses for the PhD. Zoological Institute of the Russian Academy of Sciences. St. Petersburg, 164 pp. (In Russian).

Novokhatskaya O.V., Ieshko E.P., Lebedeva D.I. 2005. Long-term changes in the parasitofauna of whitefish (Coregonidae) of Syamozero (South Karelia). Salmonid fishes of Eastern Fennoscandia. Petrozavodsk, KRC RAS, C. 97–102. (In Russian).

Novokhatskaya O.V., Ieshko E.P., Sterligova O.P. 2008. Character of long-term changes in the parasitofauna of bream *Abramis brama* L. in an eutrophic water body. Parasitologiya 42 (4): 308–317. (In Russian)

Pérez-del-Olmo A., Georgieva S., Pula H.J., Kostadinova A. 2014. Molecular and morphological evidence for three species of *Diplostomum* (Digenea: Diplostomidae), parasites of fishes and fish-eating birds in Spain. Parasites & Vectors 7: 502. doi:10.1186/s13071-014-0502-x

Rambaut A. 2012. FigTree v1. 4. Molecular evolution, phylogenetics and epidemiology. Available at https://github.com/rambaut/figtree/releases (accessed 1 October 2024)

Reshetnikov Y.S., Popova O.A., Sterligova O.P., Titova V.F., Bushman L.G., Ieshko E.P., Makarova N.P., Malakhova R.P., Pomazovskaya I.V., Smirnov Yu. 1982. Changes in the structure of the fish population of an eutrophying water body. Moscow, Nauka, 248 pp. (In Russian).

Ronquist F., Teslenko M., Van Der Mark P., Ayres D.L., Darling A., Höhna S., Huelsenbeck J.P. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. doi:10.1093/sysbio/sys029

Rozas J., Ferrer-Mata A., Sánchez-DelBarrio J.C., Guirao-Rico S., Librado P., Ramos-Onsins S.E, Sánchez-Gracia A. 2017. DnaSP 6: DNA Sequence Polymorphism Analysis of Large Datasets. Molecular Biology and Evolution 34: 3299–3302. https://doi.org/10.1093/molbev/msx248

Rumyantsev E.A. 1996. Evolution of fish parasite fauna in lakes. Petrozavodsk, KRC RAS. 188 pp. (In Russian).

Sazonov S.V. 2004. Ornithofauna of the taiga of Eastern Fennoscandia. Historical and zonal-landscape factors of formation. Moscow, Nauka, 390 pp. (In Russian).

Schwelm J., Georgieva S., Grabner D., Kostadinova, A., Sures B. 2021. Molecular and morphological characterisation of *Diplostomum phoxini* (Faust, 1918) with a revised classification and an updated nomenclature of the species-level lineages of *Diplostomum* (Digenea: Diplostomidae) sequenced worldwide. Parasitology 148 (13): 1648–1664, https://doi.org/10.1017/S0031182021001372

Shigin A.A. 1986. Trematodes in the fauna of the USSSR: the genus *Diplostomum*. Metacercariae. Moscow, Nauka, 254 pp. (In Russian).

Shulman S.S. 1962. Parasite fauna of fish of Syamozero lakes group. Proceedings of Syamozero complex expedition 2: 173–244. (In Russian).

Shulman S.S., Malakhova R.P., Rybak V.F. 1974. Comparative-ecological analysis of fish parasites of lakes of Karelia. Leningrad, Nauka. 108 pp. (In Russian).

Sokolov S.G., Ieshko E.P., Lebedeva D.I. 2023. Resurrection of *Diplostomum numericum* Niewiadomska, 1988 (Digenea, Diplostomatoidea: Diplostomidae) based on novel molecular data from the type-host. Diversity 15 (7): 840. doi 10.3390/d15070840.

Sterligova O.P., Ilmast N.V., Savosin D.S. 2016. Cyclostomata and fishes of the fresh waters of Karelia. Petrozavodsk, KRC RAS, 223 pp. (In Russian).

Sudarikov V.E., Shigin A.A. 1965. On the methods for working with metacercariae of the Strigeidida. Trudy Gel'mintologicheskoi Lab. Akad. Nauk SSSR 15: 158–166. (In Russian).

Sudarikov V.E., Shigin A.A., Kurochkin Yu.V., Stenko R.P., Yurlova N.I. 2002. Metacercariae of trematodes – parasites of hydrobionts of Russia. V. 1. Moscow, Nauka, 298 pp. (In Russian).

МОЛЕКУЛЯРНАЯ И МОРФОЛОГИЧЕСКАЯ ХАРАКТЕРИСТИКА МЕТАЦЕРКАРИЙ *DIPLOSTOMUM SPATHACEUM* ИЗ *ABRAMIS BRAMA* L. ОЗЕРА СЯМОЗЕРО (СЕВЕРО-ЗАПАД РОССИИ)

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Ключевые слова: трематоды, cox1, лещ, хрусталик глаза, Карелия

РЕЗЮМЕ

Трематоды *Diplostomum* von Nordmann, 1832 – широко распространенные паразиты со сложным жизненным циклом, включающим пресноводных моллюсков в качестве первых промежуточных хозяев, различные виды рыб в качестве вторых промежуточных хозяев и рыбоядных птиц как окончательных хозяев. Метацеркарии *Diplostomum* spp. – опасные патогены рыб с затрудненной морфологической идентификацией. Настоящее исследование метацеркарий *Diplostomum* из хрусталика глаза *Abramis brama* с использованием молекулярных и морфологических методов было проведено в рамках долгосрочного паразитологического мониторинга рыб озера Сямозеро. В результате получены молекулярная и морфологическая характеристики метацеркарий *Diplostomum spathaceum* из хрусталика леща. Частичные последовательности cox1, использованные для молекулярной идентификации изолятов, совпадают с ранее отмеченными для вида. Морфологические признаки метацеркарий лишь частично совпадают с литературными данными. Дискриминантный анализ, проведенный для сравнения размеров паразитов, позволяет предположить, что вид *Diplostomum rutili* является младшим синонимом *Diplostomum spathaceum*. Согласно результатам исследования многолетних изменений встречаемости диплостомид в хрусталиках глаз лещей озера Сямозеро, наблюдается рост зараженности рыб в популяции водоема в течение длительного времени.