



Emergence of the zoonotic biliary trematodes (Opisthorchiidae) in fish of north-western Russia

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Background

- Opisthorchiasis is a parasitic zoonotic disease caused by the trematodes of family Opisthorchiidae. The complex life cycle of these parasites includes a broad range of fish-eating mammals as definitive hosts and two intermediate hosts: freshwater gastropods and cyprinid fish. In Europe, the most harmful fish-borne trematodes for human health are *Opisthorchis felinus*, *Metorchis bilis* and *Pseudamphistomum truncatum*
- From the aquatic environment in the Baltic sea area, *P. truncatum* has been found in seals (1) and in fish from Germany (2) and Denmark (3), and most recently from the sea waters of Russia (4) and Finland (5)

Aim of the study

- to study the occurrence of *Pseudamphistomum truncatum* metacercaria in the cyprinid fish of North-Western Russia

Materials and Methods

- Between 2015 and 2018, 748 specimens of different species of cyprinid fish (roach, ide, rudd, vimba, bream, white bream and bleak) from the Russian part of the Gulf of Finland and 219 specimens from the lakes Ladoga (35), Pskov (25) and Ilmen (159) were received from fishermen
- For detecting the opisthorchiid metacercariae in muscles of fishes light microscopy and digestive method were used. Pieces of trunk muscles were compressed between two glass plates and studied under both a stereomicroscope and a light microscope. Part of the fish was examined for encysted metacercariae by exposing the muscles of pectoral fins together with trunk muscles and skin to 2% pepsin in physiological solution (pH 2-3) for 2 h at 37 °C. Some excysted metacercariae were conserved in cold 70% ethanol for molecular studies and others were fixed in hot physiological solution with fast transfer in 70% ethanol for next measurement and staining in Carmin solution
- Identification of metacercariae from muscles of fish was based on both morphological and molecular methods. Size measurements of cysts and the live excysted metacercariae were made using a calibrated microscope. Molecular identification based on ITS1, ITS2, CO1 and CO3 (5, A. Katokhin, unpublished) was performed from two fish from Vyborg bay and five fish from Lake Ilmen

Host fish and the number of parasites analyzed	Sample origin	Morphology	ITS1	ITS2	CO1	CO3
Roach, <i>Rutilus rutilus</i> , 2x	Gulf of Finland, Vyborg bay	Pt	Pt		Pt	
Roach, <i>Rutilus rutilus</i> , 2x	Gulf of Finland, Vyborg bay	Pt	Pt		Pt	Pt
Roach, <i>Rutilus rutilus</i> , 1x	Lake Ilmen	Mb?		Mb		
Roach, <i>Rutilus rutilus</i> , 1x	Lake Ilmen	Pt		Pt		
Roach, <i>Rutilus rutilus</i> , 3x	Lake Ilmen	Pt	Pt		Pt	Pt
Ide, <i>Leuciscus idus</i> , 1x	Lake Ilmen	Pt?		Pt		
Ide, <i>Leuciscus idus</i> , 1x	Lake Ilmen	Pt				Pt

Table 1. Results of the molecular identification of metacercariae from Vyborg bay and Lake Ilmen. Pt *Pseudamphistomum truncatum*, Mb *Metorchis bilis*

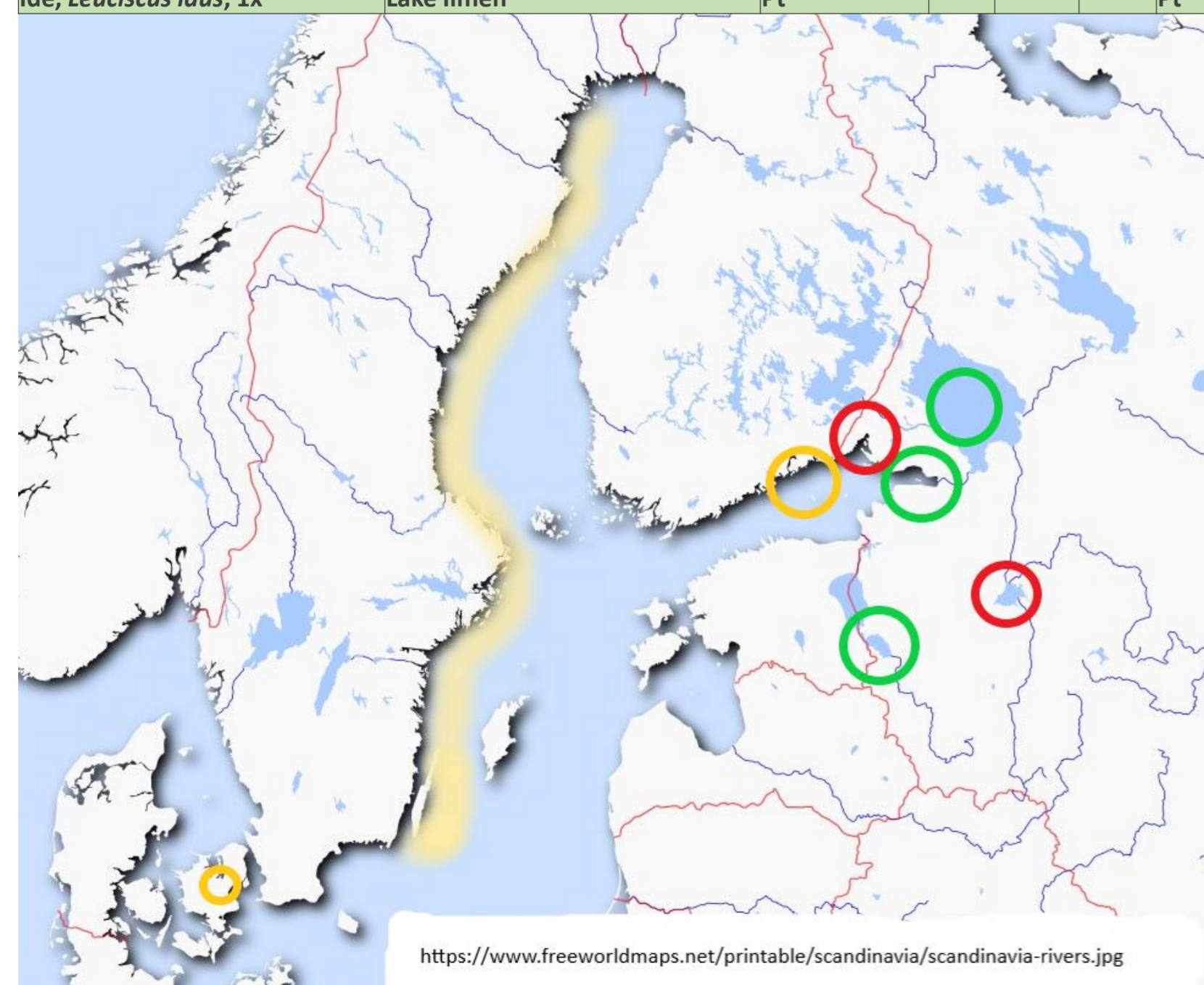


Figure 3. *Pseudamphistomum truncatum* detection in Baltic sea area. Green circles: the parasite not detected Red circles: the parasite detected Orange circles: the parasite detected in earlier studies Yellow-shaded area: the parasite detected in earlier studies in seals

Results

- Opisthorchiid metacercariae that by stereomicroscopy had a large white spot inside were often observed together with metacercariae of *Paracaenogonimus ovatus* (fig. 1a). By the light microscopy the spot was black (fig. 1b). The size of most of the cysts was (375-560 x 290-388)µm and the bodies of the metacercariae (474-980 x 180-193)µm. Sometimes between the cysts and free metacercariae of similar size very small specimens were detected (fig. 2). The molecular investigation was consistent with morphometric data suggesting the presence of *P. truncatum* and *M. bilis* (Table 1)
- Seven species of cyprinid fish (roach, ide, rudd, vimba, bream, white bream and bleak) were found infected with metacercaria of *P. truncatum* in the Gulf of Finland Vyborg Bay and Lake Ilmen. The total level of the infection in the roach was 75.6% in the Vyborg Bay and 54.3% in Lake Ilmen, respectively. Unexpectedly, the bream, which is the main cyprinid fish for human consumption in Russia, had a minimal level of infection in contrast to the other fish species. No metacercaria of *P. truncatum* were found in the lakes Ladoga and Pskov or the area near St.Petersburg in the Gulf of Finland (Fig. 3)

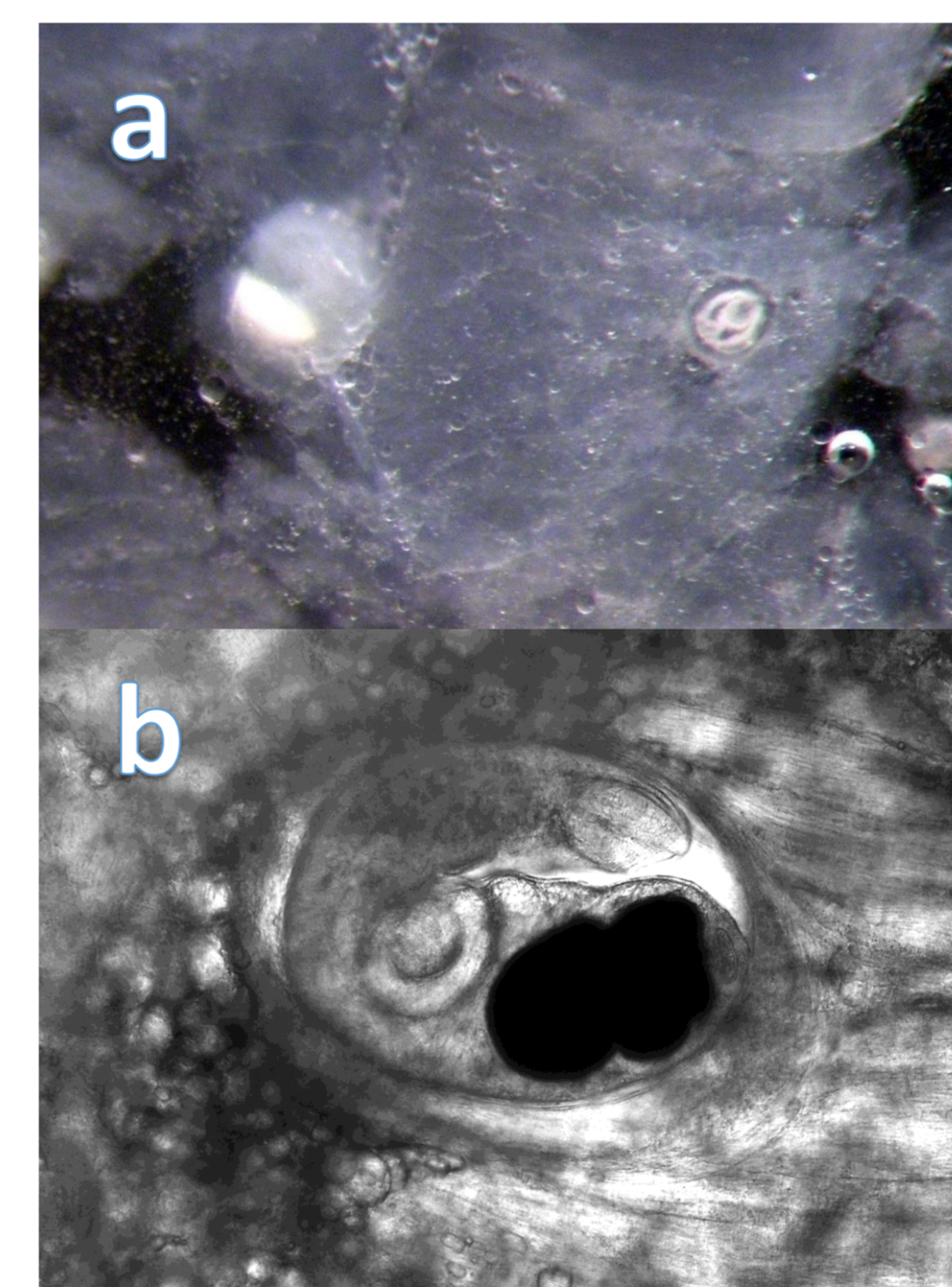


Figure 1. a) a stereomicroscopic view and b) a light microscopic view of muscle tissue with encysted metacercariae of *Pseudamphistomum truncatum*

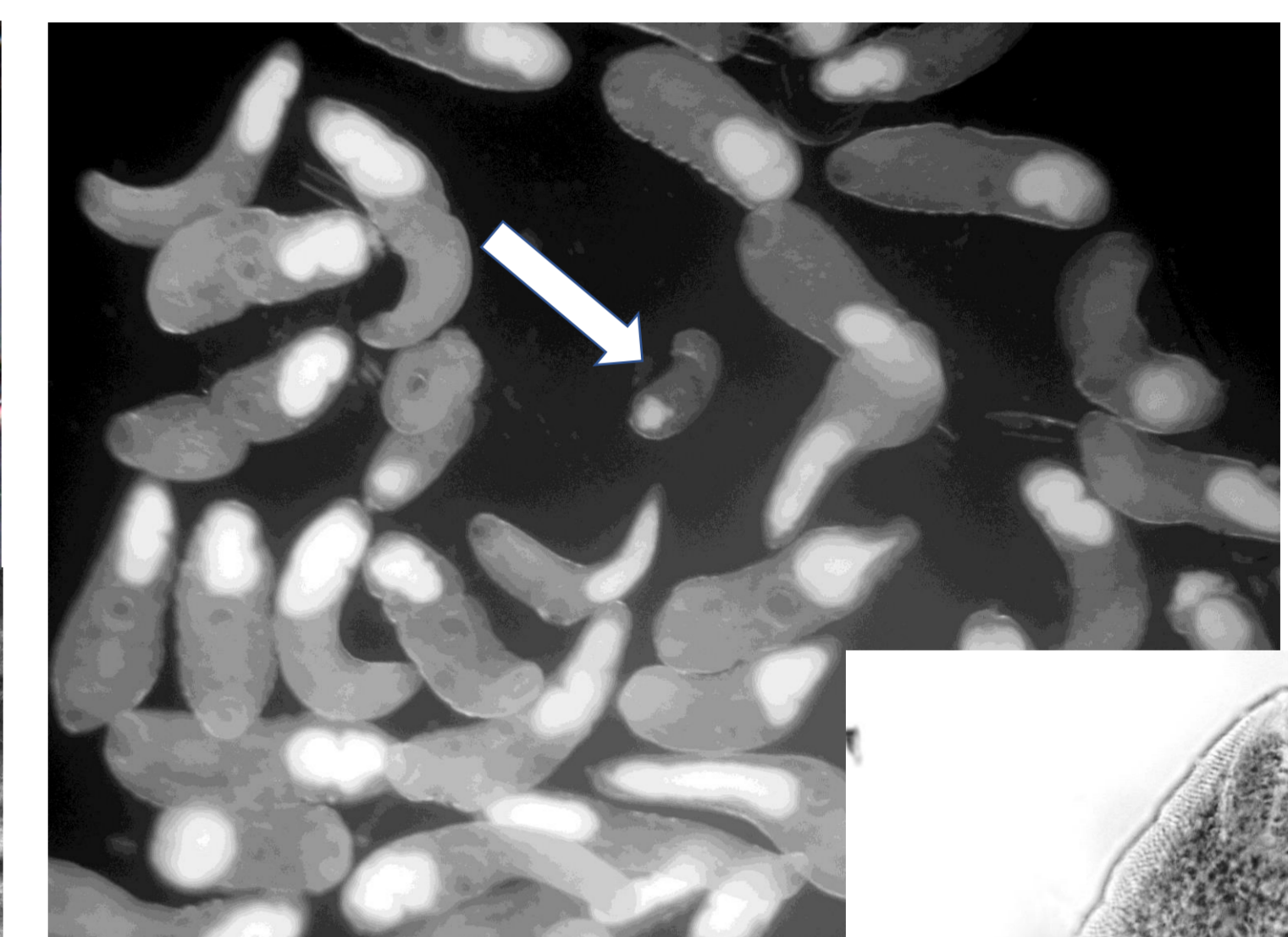


Figure 2. Small metacercarium of *Metorchis bilis* (arrow) between excysted metacercariae of *Pseudamphistomum truncatum*



Figure 4. The short oesophagus of *Pseudamphistomum truncatum* (arrow)

Conclusions

- Metacercariae of three different species of the family Opisthorchiidae (*Opisthorchis felinus*, *Metorchis bilis* and *Pseudamphistomum truncatum*), have very similar morphology, but different size. The largest of them is *P. truncatum*, *M. bilis* is the smallest (6). The size of the cysts and the bodies of metacercariae found in our study were consistent with *P. truncatum*. The same size class (320-456 x 256-376 µm for cysts and 704-1000 x 142-216 µm for bodies) was described earlier in the Danish study (3). Besides the size, the short oesophagus (fig.4) is an important morphological feature for the identification of *P. truncatum* (6). The smaller metacercariae that were sometimes observed were apparently belonging to *Metorchis bilis*, which is also earlier detected in North-Western Russia (4) as well as in Finland (5).
- The results of the limited parasitological investigation of the roach from the Gulf of Finland in the Finnish coastal area were similar to this study (5). The prevalence of this parasite in the Finnish lake area is so far largely unknown, but the results of this study suggest a patchy distribution in lakes. Future research should focus on understanding the life cycle of the parasite under the northern conditions and the overall prevalence, especially because of the zoonotic potential through the human consumption of cyprinid fish.

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