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## SHORT COMMUNICATION



# Oribatid mites (Acariformes, Sarcoptiformes: Oribatida) in the gills of *Salmo* spp. (Actinopterygii: Salmonidae) parr – Short communication

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## ABSTRACT

Live oribatid mites of the family Malaconothridae were found on *Salmo* spp. parr caught in the rivers of Northwest Russia. The mites were localised in the gill filaments and enclosed in connective tissue capsules. The encapsulation was accompanied by hyperplasia and displacement of the respiratory epithelium. One mite specimen was an adult female, while all the other specimens were protonymphs. The adult female and one protonymph specimen were identified as *Tyrphonothrus* sp. Other protonymphs could be identified only at the family level. The obtained partial 18S rDNA sequence of one protonymph was 100% identical to that of *Tyrphonothrus maior* (NCBI accession No. KY922215). This is the first report of living malaconothrid mites encapsulated in fish gills, and the phenomenon may assume parasitic behaviour. However, the nature of the relationship between the mites and the fish requires further investigations.

## KEYWORDS

Malaconothridae, *Tyrphonothrus*, fish, protonymphs

Oribatida is one of the largest groups of mites, comprising more than 163 families, 1,300 genera and subgenera and 11,207 species and subspecies (Subías, 2004). Most oribatids are soil-dwelling, but some (less than a hundred species) are associated with water. Oribatids associated with water can be divided into two ecological groups: truly aquatic mites always living in freshwater habitats and amphibiotic oribatids requiring air for reproduction (Schatz and Behan-Pelletier, 2008).

There are some records of oribatid nymphs and adults on freshwater fish (e.g. Olszowski, 1996). The nature of the relationship between these mites and the hosts is imperfectly understood. It is not always clear whether it is phoresy, facultative parasitism or true parasitism. The effect of oribatids on the gill epithelium and skin of the hosts is also poorly studied (Hare and Burt, 1975; Fain and Lambrechts, 1987).

In this paper, we report the occurrence of oribatid mites in the gills of wild parr (0+–3+) of *Salmo salar* L., 1758 and *Salmo trutta* L., 1758 from the rivers of Northwest Russia (Table 1). The fish were examined with the aid of a stereomicroscope under × 28 magnification. The mites collected for the morphological and molecular analyses were extracted from the gill tissue with preparative needles and fixed in 96% ethanol. Their morphology was examined under light microscope AxioLab A1 (Zeiss), using temporary mounts.

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Table 1. Occurrence of malaconothrid mites on *Salmo* spp. parr from rivers in the drainage basins of the Baltic, the White and the Barents Sea

Species and number of examined hosts	Date of collection	Locality	Prevalence, %	Estimated intensity, individuals/fish
<i>Salmo salar</i> (n = 22)	6 October 2018	River Kamennaya (White Sea Basin), 64°30'02.80" N 30°30'49.17" E	13.6	1
<i>Salmo trutta</i> (n = 15)	3 October 2019	River Nuris (White Sea Basin), 66°10'05.48" N 30°31'49.24" E	6.7	1
<i>S. salar</i> (n = 15)	9 October 2019	River Hiitola (Baltic Sea Basin), 61°10'56.08" N 29°51'45.06" E	13.3	2
<i>S. salar</i> (n = 15)	28 September 2019	River Ukhta (White Sea Basin), N65°13'35.08" E31°08'22.23"	6.7	1
<i>S. salar</i> (n = 15)	15 October 2020	River Pecha (Barents Sea Basin), 68°31'37.34" N 31°35'19.45" E	20	1
<i>S. trutta</i> (n = 15)	17 October 2020	River Soigjoki (Barents Sea Basin), 68°30'08.45" N 28°44'41.88" E	13.3	1–2
<i>S. trutta</i> (n = 16)	19 October 2020	River Nautsijoki (Barents Sea Basin), 68°54'57.53" N 28°59'28.37" E	12.5	1
<i>S. salar</i> (n = 11)	16 November 2020	River Gladyshevka (Baltic Sea Basin), 60°23'73.63" N 29°44'33.08" E	18.1	1–2

Genomic DNA was extracted from one protonymph found in gills of *S. salar* parr from River Pecha (Table 1) using the QIAamp DNA Micro Kit (Qiagen), following the manufacturer's protocol with modifications described by Matthews et al. (2018). A partial 18S rDNA fragment (1,686 bp) was amplified in nested PCR assays (one first [1.0] and three second rounds [2.1–2.3]), using Encyclo Plus PCR kit (Evrogen JSC, Russia). For all reactions, the 20 µl PCR mix contained 1 × PCR buffer, 0.2 mM of dNTPs, 0.4 µM forward and reverse primers, 0.75 × Encyclo polymerase Mix, and 0.3 µl gDNA (for round 1.0) or 0.1 µl PCR product of the first round (for rounds 2.1–2.3). For the first round, the following thermocycling profile was used: initial denaturation of 2 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 56 °C, 2 min (+1 s/cycle) at 72 °C, with a final extension of 7 min at 72 °C. For all second rounds, the profile was: initial denaturation of 2 min at 94 °C, followed by 15 cycles of 30 s at 94 °C, 23 s at 57 °C, 1 min 10 s at 72 °C, then 18 cycles of 30 s at 94 °C, 25 s at 63 °C, 1 min 10 s at 72 °C, with a final extension of 7 min at 72 °C. The primers previously described by Klimov et al. (2018) were as follows: For round 1.0, 18S\_1F\_G (TAC CTG GTT GAT CCT GCC AGT AG) and 18S\_8r\_MH (TAA TGA TCC TTC CGC AGG TTC ACC T); for 2.1, 18S\_1F\_sh\_T (TGT AAA ACG ACG GCC AGT CCT GCC AGT AGT CAT ATG CTT G) and 18S\_719R\_T (CAG GAA ACA GCT ATG ACC AAT ATA CGC TAT TGG AGC TGG A); for 2.2, 18S\_521F\_T (TGT AAA ACG ACG GCC AGT ACA TCC AAG GAA GGC AGC AG) and 18S\_1587R\_T (CAG GAA ACA GCT ATG ACC AAC TAA GAA CGG CCA TGC AC); for 2.3, 18S\_1321F\_T (TGT AAA ACG ACG GCC AGT CCC TAG TTC TAA CCA TAA ACG ATG C) and 18S\_8R\_sh\_T (CAG GAA ACA GCT ATG ACC GGT TCA CCT ACG GAA ACC TTG). All second round PCR products were

purified using the Cleanup S-Cap Kit (Evrogen JSC, Russia), and sequenced using the standard primers (M13 forward and reverse tails, TGT AAA ACG ACG GCC AGT and CAG GAA ACA GCT ATG ACC) and an ABI BigDye Terminator v3.1 Cycle Sequencing Kit (ThermoFisher Scientific), on an ABI 3500 Genetic Analyzer.

The obtained DNA sequence of the examined protonymph was deposited in NCBI GenBank (#MZ357964). The DNA sequence alignment including the sequences of other representatives of the Malaconothridae from GenBank was generated with CLUSTALW algorithm (Sievers et al., 2011). The genetic distances were calculated in MEGA 7 (Kumar et al., 2016). Unfortunately, attempts to obtain an amplicon from the other collected specimens were unsuccessful.

For histopathological study, a gill fragment with an encapsulated mite was fixed in 10% neutral buffered formalin. The fixed gill fragment was embedded in paraffin using STP-120 (ThermoFisher Scientific); paraffin blocks were made using EC-350 Embedding Centre (ThermoFisher Scientific), then cut into sections 5–6 µm thick on a microtome HM-440 (ThermoFisher Scientific). The sections were stained with haematoxylin and eosin.

All the mites found on *Salmo* spp. parr were always localised in the gill filaments. They were enclosed in elongate, multilayer capsules formed by connective tissue. The capsule walls, 40–45 µm thick, were composed of compactly and regularly arranged fibrocytes and fibres (Fig. 1). Hyperplasia and displacement of the respiratory epithelium were observed around the capsules. All the mites were alive and showed motility when extracted from the capsules. The prevalence of the mites ranged from 6.7 to 20%, and the estimated intensity of infection was 1–2 individuals per fish (Table 1).



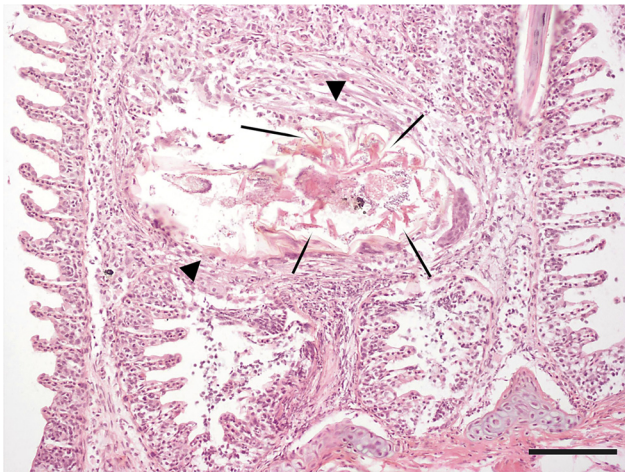


Fig. 1. Histological section of the gill of *Salmo salar* parr (River Kamennaya) with an encapsulated protonymph of *Malaconothridae* gen. sp. The muscle of the mite is indicated by thin arrows and the connective tissue capsule composed of fibrocytes and fibres is indicated by an arrowhead. Scale bar = 100  $\mu$ m

One specimen collected from *S. salar* parr from River Gladyshevka was an adult female, identified based on morphological features as *Tyrphonothrurus* sp. (*Malaconothridae*). Unfortunately, the species of this female could not be identified, because its body setae were damaged during extraction from the capsule. The other specimens were protonymphs. On the basis of morphology, they belong to the family *Malaconothridae*. The obtained 18S rDNA sequence of the protonymph specimen from River Pecha was 100% identical to that of *Tyrphonothrurus maior* (Berlese, 1910) (= *Trimalaconothrus novus* [Sellnick, 1921]) (NCBI accession No. KY922215) collected from an unknown water body in Nizhegorodskaya Oblast of Russia. Thus, this protonymph was identified as *Tyrphonothrurus* sp., since 18S rDNA is not always suitable for the identification of closely related species. On the other hand, the 18S rDNA sequences of *T. maior* (KY922215) and *Tyrphonothrurus* sp. protonymph had a high degree of similarity with those of three *Malaconothrus monodactylus* (Michael, 1888) (= *Malaconothrus gracilis* Hammen, 1952) specimens (JQ000044, KR081621, EF091424), as they differ from *M. monodactylus* 18S rDNA in only a single variable site, representing 0.06% of the difference. All the other protonymphs were classified as *Malaconothridae* gen. sp., since malaconothrid nymphs cannot be reliably identified at the genus and species level on the basis of morphological characters. Our results confirm the findings of Klimov et al. (2018) that a low level of divergence was detectable between the 18S rDNA of two malaconothrid genera, *Tyrphonothrurus* Knülle, 1957 and *Malaconothrus* Berlese, 1904.

Mites of the family *Malaconothridae* have a cosmopolitan distribution and live in moist moss, in wet soil, and sometimes in freshwater habitats (Fain et al., 1990; Norton and Behan-Pelletier, 2009; Colloff and Cameron, 2013). Freshwater malaconothrids are regarded as amphibiotic (Schatz and Behan-Pelletier, 2008). Malaconothrids may

feed on detritus, higher plants, fungi, green algae, and bacteria (Seniczak, 2011), and it is unknown whether they can consume fish tissues.

Here, we report the occurrence of living malaconothrids encapsulated in fish gill tissue. Although the presence of mites of fish has been reported before (Fain et al., 1990; Sokolov and Reshetnikov, 2020), this is the first report that living malaconothrid mites were observed encapsulated in fish gills. Encapsulation in gill tissue of salmonids has been described only for an oribatid mite from the family *Trhypochthoniidae*, *Trhypochthoniellus* sp. (Hare and Burt, 1975).

Proctor et al. (1997) suggest that sarcoptiform mites get onto fish gills accidentally when the fish feed at the bottom. However, our numerous findings of malaconothrid mites on the gills of *Salmo* spp. parr (eight waterbodies of different sea basins, with the prevalence of 6.7–20%) raise doubts about the accidental nature of their presence. Many oribatids disperse with the help of various animals such as insects, amphibians, birds, and rodents (Norton, 1980; Beaty et al., 2013). There are no data on the population dynamics of malaconothrids, but the fact that nearly all the specimens discovered on salmon parr were protonymphs may indicate that they spread by fish as phoretic host, after a reproduction peak of mites in late summer.

At the same time, we cannot be sure that the presence of oribatid mites in salmon parr gills described here may be explained by phoresy, because the mites were found in an encapsulated state. Hare and Burt (1975) argue that encapsulation of oribatids in fish gills does not unequivocally prove them parasitic, since the gill tissue reacts in a similar way to any foreign body. Nonetheless, living malaconothrids can hardly be encapsulated without the stimulation of the proliferative activity of the branchial epithelium. Perhaps, the encapsulation has resulted from the irritation of the epithelium, which occurs when the mites move or feed on mucus. Whatever the case is, the relationships between malaconothrid mites and the fish cannot be explained based on the available data, and their elucidation calls for experimental research.

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