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Research Note

Evidence of *Trogloitrema acutum* and *Skrjabingylus* sp. coinfection in a polecat from Lower Austria

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Summary

The trematode *Trogloitrema acutum* and nematodes of the genus *Skrjabingylus* are parasitic helminths infecting nasal sinuses of mustelids. Despite different infection routes of these parasites, their occurrence becomes evident due to their destructive lesions of the bone structure of the head, which appears almost similar in both cases.

This is a report of coinfection of both the trematode and the nematode, in a polecat from Lower Austria, as well as the first attempt to barcode *T. acutum*. The nematode could only be found fragmentally, therefore accurate morphological determination was not possible. DNA barcoding was successful, however, a clear species assignment was not possible as the similarity with published COI sequences of other nematodes was only 87 % or less. The influence of both parasitic helminths on the health condition of the hosts remains elusive and has to be evaluated in separate studies.

Keywords: *Trogloitrema acutum*; *Skrjabingylus*; polecats; COI barcoding primers

Introduction

Trogloitrema acutum is a digenean trematode parasitizing in mustelids, mainly in the polecat *Mustela putorius*. The parasite was described by Leuckart as *Distomum acutum* in 1842. About 50 years later, this trematode was observed in the ferret, *Mustela putorius furo*, which is the domestic form of the polecat, and it was stated as a possible cause of the death of the animals after showing protrusion of the eyes and convulsion of the jaws (Lehmsick, 1942). Controversially, even heavily infected polecats remain in good health condition (Vogel & Voelker, 1978).

The most impressive characteristic of the parasite is the dissolving capacity of the cranium's bone structure. The 2.5 – 4 mm long adult trematodes live in the paranasal sinuses (Lehmsick, 1942; Koubek *et al.*, 2004; Demuth *et al.*, 2009), where they cause bone structure damage by secreting osteolytic substances (Vogel & Voelker, 1978). In substitution of the bone, connective tissue can be found covering the lesions. In minks perforation of this tissue was observed (Vogel & Voelker, 1978). A low number of four to eleven parasite individuals were able to dissolve the bone structure in experimentally infected ferrets (Vogel & Voelker, 1978), whereas in wild polecats numbers of up to 171 individuals were reported (Vogel & Voelker, 1978). The eggs of *T. acutum*, representing characteristic trematode egg shape in sizes

of 65.4 – 101.5 µm x 42.5 – 55.6 µm and an operculum (Vogel & Voelker, 1978), are shed via feces. The miracidia hatch in fresh water and infect the first intermediate hosts – prosobranchiate *Bythinella* sp. snails (Vogel & Voelker, 1978). After a period of up to nine months (probably due to the low temperatures of the snail habitat), cercariae are released (Vogel & Voelker, 1978). These infect anurans, e.g. *Rana temporaria*, the second intermediate hosts, and metacercariae are formed (Vogel & Voelker, 1978; Koubek *et al.*, 2004). Through the ingestion of frogs, polecats and other mustelids become infected (Vogel & Voelker, 1978). The prepatent period is believed to be around 35 to 42 days in ferrets, and adult trematodes show a lifespan of several years (Vogel & Voelker, 1978). Cranial lesions caused by *T. acutum* have so far been reported from the main definitive host, the polecat, but also the ferret. Accidental findings were reported from various mustelids and other carnivores, e.g. the pine marten (*Martes martes*), beech marten (*Martes foina*), badger (*Meles meles*), European otter (*Lutra lutra*), red fox (*Vulpes vulpes*) and mink (*Mustela vison*) (Vogel & Voelker, 1978; Ribas *et al.*, 2012; Torres *et al.*, 2006). Retrospective studies of museum material have shown that caution must be taken with species classification by relying only on lesions of the skulls. Nematodes of the genus *Skrjabingylus* (e.g. *S. nasicola*) are also known to parasitize in polecats and other mustelids, causing similar lesions. The genus



Fig. 1. Lesions of the polecat's head presumably originating from *T. acutum* and/or *Skrjabinigylus* sp. (picture P. Wolf)

Mustela is discussed to be the definite hosts of *S. nasicola* (e.g. Koubek *et al.*, 2004; Torres *et al.*, 2006). However *S. petrowi* (with species of the genus *Martes* as main hosts) was rarely observed in one case within the genus *Mustela* too (Gerard & Barrat, 1986).

Several authors state that, depending on the shape and size of the holes in the skull, a differentiation between *T. acutum* and *Skrjabinigylus* sp. can be made (Vogel & Voelker, 1978; Kierdorf *et al.*, 2006). The lesions made by the nematode should be smaller and not affecting the extended areas of the frontals, whereas the holes originating from the trematode are more severe (Kierdorf *et al.*, 2006). The evaluation of the intensity of infection is not possible (Koubek *et al.*, 2004) and the numbers of parasites and localization might vary in both nematode and trematode. Species confirmation can only be made by the morphological description of the parasites themselves and not by means of lesions in the skull.

Material and Methods

In April 2011 two polecat skulls were obtained from Hainfeld, Lower Austria, indicating the occurrence of nasal parasites. In February 2014 a third polecat was trapped in the same area and the head of the polecat was sent to the Institute of Parasitology, University of Veterinary Medicine Vienna, for investigation of the fresh tissue. First the head was investigated macroscopically. Suspicious bone parts were cut out with a bone scissor and transferred into physiological salt solution. The sediment of the solution was examined with a binocular loupe. The solution was furthermore dropped on slides, covered with cover slips and investigated with a microscope at 100x magnification. Parasites and eggs were determined using morphological keys (Vogel & Voelker, 1978; Koubek *et al.*, 2004).

Parts of the identified parasites were further processed with modified barcode techniques for sequence analysis (Folmer *et al.*, 1994).

Based on complete *COI* sequences retrieved from GenBank® (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>), new primers were de-

signed for amplification and sequencing of sections of the mitochondrial *COI*. For the trematode a primer pair was designed, which allows sequencing a 638 to 668 bp fragment of the *COI* in cestodes, trematodes and monogeneans (summarized in the phylum 'Neodermata'): *COI_Neod_Fw*: 5'-TTTACTTTGGATCAT AAGCG-3' and *COI_Neod_Rv*: 5'-CCAAAAACCAAACATAT GTTGAA-3'. After an initial denaturation of 2 min at 95 °C, denaturation at 95 °C, annealing at 48 °C and elongation at 72 °C were conducted in 1 minute intervals each for 35 cycles, ending up with a final extension of 5 minutes at 72 °C. Another primer pair was specifically designed for amplification and sequencing of a 650 bp section of the *COI* in parasitic nematodes (excluding filaroid taxa): *COI_Nema_fw*: 5'-GAAAGTTCTAATCA TAARGATATTGG-3' and *COI_Nema_rv*: 5'-ACCTCAGGATGA CCAAAAAAYCAA-3. Except for a higher annealing temperature at 50 °C, PCR conditions were the same as for the 'Neodermata' primer pair. PCRs were conducted on the Eppendorf Mastercycler pro S (Eppendorf AG, Hamburg, Germany). The amplicons were purified by Fast-kit (Bio-Rad Laboratories, Vienna, Austria) and sequenced (Microsynth AG, Balgach, Switzerland). Sequences obtained were further processed in GeneDoc (<http://www.psc.edu/biomed/genedoc>) and a BLAST searches were performed in GenBank®. The sequences were submitted to GenBank®.

Results

The two skulls obtained in 2011 indicated the presence of either *T. acutum* and/or *Skrjabinigylus* sp. in polecats in these areas (Fig. 1).

The third polecat was killed via head shot in 2014; therefore, most of the material was not suitable for dissection and preparation. Only a few parts of the skull delivered intact material and were further processed. During section of the fresh material a suspicious fragment of the frontal bone over the right eye, with two lesions of approx. 2 mm diameter, was cut out and washed in physiological salt solution. In the resulting sediment big trematode eggs as well as nematode eggs were observed (Fig. 2a, 2b).

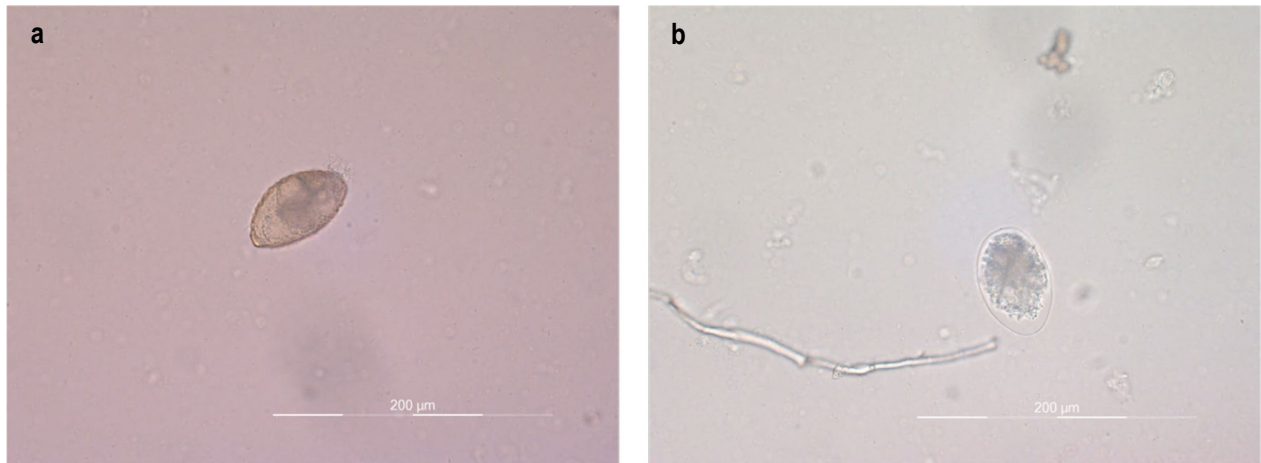


Fig. 2. **a** – Trematode and **b** – Nematode egg found in the sediment during investigation of the head

Furthermore, an adult trematode was detected in the solution (Fig. 3) and was identified as *T. acutum*. On the back side of the suspicious bone lesions six more individuals were isolated by scraping. Four individuals displayed an undamaged body shape and could therefore be measured: length $3.845 \text{ mm} \pm 0.437 \text{ mm}$ (95 % CI), width $2.723 \text{ mm} \pm 0.323 \text{ mm}$ (95 % CI). One individual was analyzed with DNA barcode analysis of the COI (GenBank®: KJ722062), providing the first barcode sequence of this pathogen.



Fig. 3. Adult *T. acutum* found in the nasal sinus of the polecat

Beside these lesion spots in the nasal space, a piece of a nematode was found (Fig. 4). The nematode presumably represents *Skrjabinigylus* sp., but due to lacking of the posterior end exact species classification was not possible. Parts of the nematode were also analyzed with barcode techniques and showed highest sequence similarity (87 % or less) with strongylid nematodes (GenBank®: KM245568). However, species classification was not possible because yet no COI sequences of the genus *Skrjabinigylus* are available on GenBank®.

Discussion

Due to its impressive feeding tracks *T. acutum* is a well-recognized parasite of mustelids, mainly polecats. Regarding the distribution and the life cycle many questions are still waiting for elucidation. Its distribution seems to be restricted to Central

Europe, with uneven abundance over their occurrence range (Koubek *et al.*, 2004). In Austria the first but also last findings of *T. acutum* were documented in 1929 in Styria, Upper and Lower Austria (Schumacher, 1929). Until recently it was believed that the distribution of this parasitic trematode is linked to the occurrence of the freshwater snail *Bythinella* (Vogel & Voelker, 1978). Some other findings near predominating stagnant water bodies suggest that other first intermediate host snails might be involved in the parasite's life cycle (Koubek *et al.*, 2004). The second intermediate hosts, e.g. *Rana temporaria*, *Bombina variegata* and *Bufo bufo* are found in all the habitats (Vogel and Voelker, 1978; Koubek *et al.*, 2004). The ingested anura carry metacercariae, which develop in mustelids to adults parasitizing within the nasal sinus, where they dissolve the bone structure (Vogel & Voelker, 1978). This location can be parasitized by another parasite group, *Skrjabinigylus* spp., which causes very similar lesions in the bones (Koubek *et al.*, 2004; Kierdorf *et al.*, 2006). *Skrjabinigylus* spp. use terrestrial mollusks as intermediate hosts and small mammals are discussed as paratenic hosts (Kierdorf *et al.*, 2006). Coinfections of both *Skrjabinigylus* spp. and *Troglootrema acutum* (as is suggested with this finding) have been observed previously (Kierdorf *et al.*, 2006). In some cases the dissolved bone structure could be linked to *T. acutum* due to the close vicinity of the parasites to the lesions (Kierdorf *et al.*, 2006).



Fig. 4. Pieces of a nematode found in the nasal sinus apart from suspicious lesions

Unfortunately this could not be confirmed within our study due to the destructive force of the bullet killing the polecat. The anterior fragment of the nematode was found free floating in the nasal sinus after removal of the bone structures, therefore an exact localization was impossible.

The impact of the parasites on the polecats is described controversially (Kierdorf *et al.*, 2006). Some authors state impacts on intellectual and locomotory abilities on infected animals (Demuth *et al.*, 2009), obtrusions of eyes and convulsion of the jaws or death (Lehmensick, 1942). Others could not find any influence on the body condition and ability to reach a certain age (Lehmensick, 1942) or an impact on morphological traits of the hosts (Demuth *et al.*, 2009). In these cases, the two shot polecats in 2011 as well as the one caught in 2014 did represent a good shape, when relying on fur appearance, habitus and behavior, which can only be taken as hints for the real health status of the animals. Further analyses are required to measure the clinical impact of these parasites on their hosts.

Similarly the influence on the polecat population has to be evaluated in future studies by investigations of polecats, frogs and snails. In terms of the acquired popularity of keeping ferrets as pets in the recent past, this trematode and nematode have to be considered. Pet owners have to be informed not to feed rodents, frogs and mollusks to their pets.

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