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RESEARCH ARTICLE

First report of the presence of *Pseudoterranova* sp. in the body cavity of salmon (*Salmo salar*) from the Baltic Sea

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Abstract. Despite salmon (*Salmo salar* L.) being an important fish in the Baltic Sea ecosystem and its fisheries, knowledge about the parasite fauna of this top predator is scarce. The Anisakidae nematodes (*Anisakis* spp., *Pseudoterranova* spp., *Contracaecum* sp.) found in Baltic Sea fish species are zoonotic parasites that are hazardous to human health. Due to the marked increase in the numbers of grey seal (*Halichoerus grypus*), the definitive host of *Contracaecum* sp. and *Pseudoterranova* spp., increased dispersion of these parasites has been observed in recent years. The aim of our study was to investigate salmon caught in Polish marine waters for the presence of Anisakidae nematodes. Parasitological inspection of the body cavities of 89 individual salmon was conducted in 2012 and revealed the presence of a *Pseudoterranova* sp. L3 larva. The presence of the parasite is reported here for the first time in the muscle tissue of salmon from the Baltic Sea. The presence of Anisakidae nematodes in commercially important fish species may have implications for human health since these parasites pose a risk of anisakidosis.

Keywords: Anisakidae; Salmon; zoonotic; southern Baltic

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Introduction

Although seafood is part of a healthy diet, in particular cases its consumption may cause health problems. Parasites in wild fish is rather the rule than the exception. Most endoparasites present in visceral fish organs are removed during processing. However, there is a group of endoparasites that is able to migrate to the edible parts of fishes, including muscle tissue and livers. These parasites may cause zoonotic diseases (transmitted from animal species to humans). Zoonotic Anisakidae nematodes of the genera *Anisakis*, *Pseudoterranova*, and *Contracaecum* have been found in a variety of Baltic Sea fish species, including those that are commonly consumed by people.

The life cycles of these nematodes are very similar, with marine mammals playing the role of the definitive hosts with clear host specificity: the definitive hosts for *A. simplex* are cetaceans, which, in the Baltic Sea is represented by the harbor porpoise, *Phocoena phocoena* (Herreras et al. 2004); for *Contracaecum osculatum* – the grey seal, *Halichoerus grypus* (Fagerholm 1990); and for *P. decipiens*, both the harbor seal, *Phoca vitulina* (Aspholm et al. 1995) and the grey seal (Hauksson 2011). In the definitive host, larval stage L4 transforms into the dioecious mature stage that produces fertilized eggs that are

shed to the marine environment. The transformation to larval stages L1 and L2 (or even L3) occurs within the egg (Køie and Fagerholm 1995). Crustaceans, which are intermediate hosts, take up L2 (or L3) larvae and the transformation from L2 to L3 takes place in them. Infected crustaceans are eaten by pelagic fish (e.g., *Sprattus sprattus* (L.), *Clupea harengus* L.), which, in turn, are eaten by predatory fish (e.g., Gadus morhua L., *S. salar*). Marine mammals represent the last link in the trophic chain since they feed on infected fishes and thus become the definitive, i.e. final, hosts and the life cycle is complete (Křie and Fagerholm 1995).

Despite of the fact that salmon (*Salmo salar*) is an important fish in the Baltic Sea ecosystem and its fisheries, knowledge about the parasite fauna of top predatory fish in Polish marine waters and in the Baltic Sea in general is scarce. The aim of our study was to update information on the presence of Anisakidae nematodes in salmon caught in Polish marine waters.

Material and Methods

Salmon were caught in March 2012 in the Polish waters of the southern Baltic Sea (ICES 39G7 and 39G8) during commercial cruises and were gutted on board the fishing vessels. The sex of the fish was determined, and basic measurements (length and gutted weight) were taken. Age was determined by counting growth rings on scales. To evaluate the condition of the fish, Clark's condition factor (K_C) was calculated as follows: $K_C = 100 \times GW \times L^{-3}$, where GW is gutted weight and L is the total length of individual fish. In total, the body cavities of 89 fish were visually inspected for the presence of Anisakidae nematodes. Descriptions of nematode anatomo--morphological features as described in Berland (1961), Berland (1989), and Fagerholm (1982) were used for the preliminary identification of the larva found. Parasitological descriptors were calculated according to definitions in Bush et al. (1997).

The single nematode larva found was also identified using molecular methods (ITS-1 rDNA). Genomic DNA was isolated using the Sherlock AX DNA purification kit (A&A Biotechnology) according to a modified method that employed mechanical lysis (i.e., mincing tissue with a surgical blade). For ITS-1 analysis, a polymerase chain reaction (PCR) was performed using the NC5 (forward) 5'-GTA GGT GAA CCT GCG GAA GGA TCA TT-3' and NC13R (reverse) 5'-GCT GCG TTC TTC ATC GAT 3' primers (Zhu et al., 2000, Zhu et al., 2002). The reaction mixture consisted of 25 µl 2x PCR Master Mix Plus High (A&A Biotechnology), 0.2 µl each primer (concentration 100 µM) and 5 µl DNA template, supplemented with deionized water up to 50 µl. The PCR conditions were as follows: 2 min at 94°C (initial denaturation) followed by 35 cycles of denaturation at 94°C for 30 s, annealing of primers at 58°C for 30 s, strand elongation at 72°C for 45 s, and a final extension step of 5 min at 72°C. PCR products were sequenced directly using standard procedures and the amplification primers. Sequences were analyzed using GeneStudio TM Professional (GeneStudio, Inc., USA) and confirmed by a BLAST search of GenBank. The sequence obtained was deposited in GenBank with the accession number given in the Results.

Results

The salmon investigated in this study were between 43 and 102 cm long, but the majority of the fish (63.92 %) belonged to the length class 70-79 cm. Of the fish analyzed, 66.29% were females (66-96 cm in length) and 33.71% males (43-102 cm in length). The length-weight relationship of the fish was similar for males and females (Fig.1). Clark's condition factor (K_C) was similar for both sexes: females (min = 0.754; max = 1.128; mean = 0.934) and males (min = 0.780; max = 1.131; mean = 0.944). Regarding age, two-year-old fish predominated (71.91%), one-year-old fish constituted 15% of the specimens analyzed, while three- and four-year-olds were 8% and 2% of the sample, respectively.

During visual inspection of the body cavity of a single salmon, one Anisakidae nematode larva was

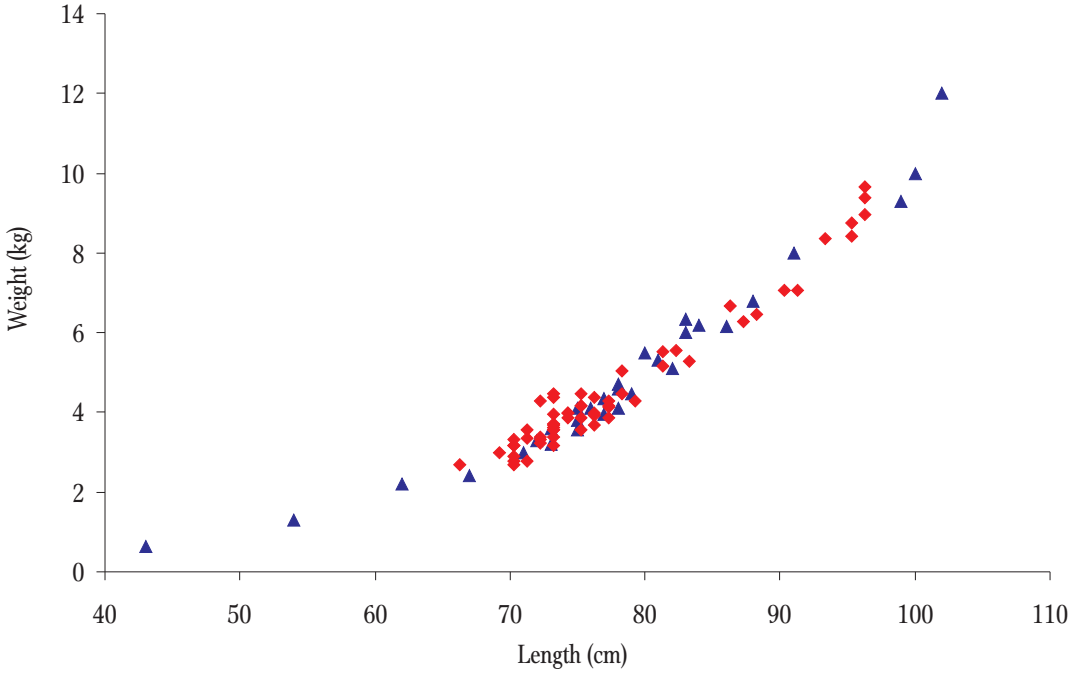

Figure 1. Length-weight relationships of the salmon analyzed (males – blue triangles, females – red dots).

detected attached to muscle tissue. The larva was identified as *Pseudoterranova* sp. based on anatomo-morphological features, and the prevalence of infection was 1.1% (intensity of infection = 1; abundance 1.1%). This parasite of Baltic salmon is presented in Fig. 2.

ITS-1 amplification and sequencing identified the larva as *Pseudoterranova* sp. The sequence was

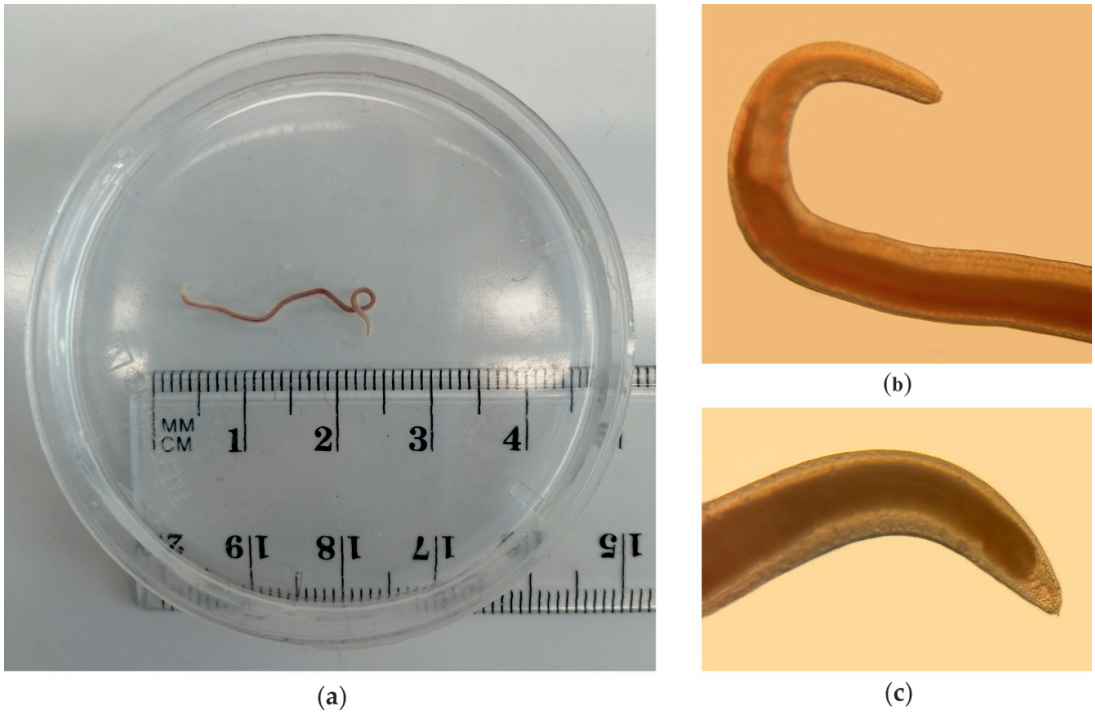

Figure 2. *Pseudoterranova* sp. from Baltic salmon (a) whole parasite; (b) anterior part (head); (c) posterior part (tail).

deposited in GenBank with ID OQ732699. This is the first report of *Pseudoterranova* sp. in the muscle tissue of salmon from the Baltic Sea.

Discussion

The study presents a single case of salmon infected with an Anisakidae larva attached to muscle tissue. Our finding from 2012 it is the first report of *Pseudoterranova* sp. in the body cavity/muscle tissue of a salmon from the Baltic Sea. Although only one larva was found, it can be assumed that results are underestimated due to the limited biological material that was examined (gutted fish) and basic detection method that was applied (visual inspection). It should be emphasized that even the use of basic analytical methods (without examining the viscera or muscle tissue) permitted detecting a zoonotic nematode parasite in salmon.

It is surprising that there are no previous reports on parasitological analyses of this commercially important fish species from the Baltic Sea. This might stem from the fact that detailed parasitological analysis of these large fish are time-consuming, labor-intensive, and expensive due to the value of the fish tested. However, knowledge about the occurrence of these zoonotic nematodes in salmon is crucial because this fish is popular among consumers and muscle tissue is frequently eaten raw (carpaccio, sushi, etc.).

The fish organ specificity of Anisakidae nematodes has been described: *Pseudoterranova* spp. and *Anisakis* spp. larvae migrate to the musculature of the fish, while *Contracaecum* sp. prefers the liver. *Pseudoterranova* spp. that penetrate into the muscle tissue in the body cavity may also indicate post-mortem larval migration. The presence of zoonotic larvae in salmon muscle tissue may have implications for human health as they can cause anisakidosis. A case of human anisakidosis was recently reported in Poland that was caused by the ingestion of viable *A. simplex* present in a meal of raw salmon (Kołodziejczyk et al. 2020). Treatments to kill viable parasites in fishery products intended for human consumption is mandatory in many countries. However, Anisakidae can survive freezing if the procedure is not performed correctly (Podolska et al. 2019).

Salmon is a diadromous fish that spawns in the rivers where it spends the first years of its life. It then migrates to its feeding grounds, which, in the Baltic, are located in the southern part of the sea, east of Bornholm, that is inhabited by seal colonies. The presence of the definitive host (grey seal) is necessary to complete the life cycles of *Contracaecum* sp. and *Pseudoterranova* sp. The percentage of seals infected with these nematodes is as high as 100%, with hundreds of nematodes in a single seal, and this results in the high emission of parasite eggs into the marine environment. In the sea, salmon prey intensively on smaller fish, mostly sprat, *Sprattus sprattus*, and sprat are documented to be infected with *C. osculatum* (Zuo et al. 2016, Nadolna-Ałtyn et al. 2018) and *Pseudoterranova* sp. (Nadolna-Ałtyn et al. 2023a). This is when salmon are exposed to infection with Anisakidae nematodes.

Parasitological inspection of internal organs of twenty-four salmon caught in 2017 in the southern Baltic Sea during feeding migrations indicated the presence of *C. osculatum* (45.8% prevalence; intensity: 1-4) in the livers, but no other Anisakids were detected (Setyawan et al. 2019). An analysis of the livers of 120 salmon caught in Polish marine waters in 2020 revealed that 13% of them were infected with Anisakids, i.e., *C. osculatum* and *Pseudoterranova* sp. (Nadolna-Ałtyn et al. 2023b). This was the first attempt to determine the level of liver infection in salmon caught in Polish marine waters in the southern Baltic Sea (Nadolna-Ałtyn et al. 2023b).

The current study was conducted in 2012, when the dispersion of Anisakidae nematodes in the southern Baltic Sea waters was not as widespread as today. Subsequent research was limited to the detection of parasites in the internal organs of salmon (Setyawan et al. 2019, Nadolna-Ałtyn et al. 2023b). Conducting studies on recently caught salmon that focus on the presence of Anisakidae parasites not only in the body cavity, but also in the muscle tissue, is recommended

and justified, due to the high level of infection observed recently in other fish species caught in the southern Baltic Sea.

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