

Rapid Communication**First record of the Asian fish tapeworm *Schyzocotyle (Bothriocephalus) acheilognathi* (Yamaguti, 1934) in Scandinavia**Haakon Hansen^{1,*} and Marta Alarcón^{1,2}¹Norwegian Veterinary Institute, P.O. Box 750 Sentrum, NO-0106 Oslo, Norway.²FishVet Group Norway, P. O. Box 1012, 0218 Oslo, NorwayAuthor e-mails: haakon.hansen@vetinst.no (HH), marta.alarcon@fishvetgroup.com (MA)

*Corresponding author

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Received: 23 July 2018**Accepted:** 21 December 2018**Published:** 23 March 2019**Handling editor:** Markéta Ondračková**Thematic editor:** Kenneth Hayes**Copyright:** © Hansen et al.This is an open access article distributed under terms of the Creative Commons Attribution License ([Attribution 4.0 International - CC BY 4.0](https://creativecommons.org/licenses/by/4.0/)).**OPEN ACCESS****Abstract**

This paper provides the first report of the invasive Asian fish tapeworm, *Schyzocotyle (Bothriocephalus) acheilognathi* (Yamaguti, 1934), from Norway and Scandinavia. The parasite was found during routine post mortem disease examination of an imported koi carp, *Cyprinus carpio*, from an outdoor pond in the North of Norway. The intestine contained live tapeworms and these tapeworms were identified as *S. acheilognathi* by DNA sequencing of partial 18S and 28S ribosomal DNA and comparison of the obtained sequences with sequences in GenBank. The infected fish specimen along with the other fish in the pond were exterminated. The risk of infection from the pond to other fish outside of the pond was thus negligible. However, the finding of *S. acheilognathi* in imported aquarium fish shows that such import poses a risk of introducing pathogens to new areas. Had this fish pond been situated in the south of Norway where the temperatures are higher and where susceptible hosts are readily available, an escape of fish or release of eggs from the pond could potentially have resulted in infection of local fish populations.

Key words: Norway, invasive species, parasite, cestode, ribosomal 18S, ribosomal 28S**Introduction**

Introduction of pathogens via the introduction of fish to new areas is well documented and several pathogenic parasites, such as e.g. the rosette agent, *Sphaerothecum destruens* Arkush, Mendoza, Adkison and Hedrick, 2003, and the monogenean *Gyrodactylus salaris* Malmberg, 1957, have been introduced to non-native areas via this route (Hansen et al. 2003; Ercan et al. 2015). The Asian fish tapeworm (AFT) *Schyzocotyle acheilognathi* (Yamaguti, 1934), previously known as *Bothriocephalus acheilognathi* Yamaguti, 1934, (Brabec et al. 2015), is a famous example of a parasite that has been spread from its native range and established itself in new areas where it has caused mortality in aquaculture and might have a negative impact on local fish populations (for a recent review of AFT, see Kuchta et al. 2018). The parasite is probably native to East Asia and/or Africa and was introduced to other parts of the world via introduction of fish and is

now present on all continents except Antarctica. It was probably spread to Europe via imported grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844) and common carp, *Cyprinus carpio* Linnaeus, 1758, but the parasite shows low host specificity and has now been recorded from 312 freshwater fish species worldwide (Kuchta et al. 2018).

Although present in many European countries, *S. acheilognathi* has so far been found in Scandinavia. Here we present the first finding of this parasite in a koi carp, *C. carpio*, imported to Norway.

Materials and methods

In August 2012, the Norwegian Veterinary Institute (NVI) received one specimen of koi carp for autopsy that originated from a private fish pond in Nordland county, northern Norway. The fish specimen had a length of 16 cm and weighed 70,6 grams. The fish population in the pond consisted of 12 koi carp and one gold fish and were bought in two different aquarium shops in Mo i Rana (66°18'49.3"N; 14°08'31.2"E) and Fauske (67°15'31.8"N; 15°23'30.8"E), Nordland county, during the last 8 years. The one koi carp that was submitted to NVI for autopsy was bought together with five other koi carps. Two of these fish died soon after having been stocked in an indoor aquarium that was used as a temporary holding tank before stocking in the outdoor pond. The owner of the pond had also experienced mortality in other batches of fish purchased for stocking in the same pond, but autopsy was not carried out on any of these fish.

The fish underwent complete autopsy and histological examination where the gills, skin, muscle, kidney, spleen, hepatopancreas, heart, brain and gastrointestinal tract were examined. Tapeworms were isolated from the stomach and intestine and stored in 96% EtOH for further examination and species diagnosis.

DNA from three tapeworms was extracted separately with the DNeasy kit on a QiaCube extraction machine according to the manufacturer's instruction. Three different primer pairs were used to amplify ribosomal 28S and 18S: the first pair, C1/D2 (Hassouna et al. 1984) amplifies approximately 1000 bp of 28S, the second pair, LSU5/1200R (Waeschenbach et al. 2007) amplifies approximately 1300 bp of 28S and the third pair, PBS18F/PBS18R (Cone et al. 2010) amplifies approximately 500 bp of 18S. The PCR reactions were carried out with puRe Taq Ready-to-Go PCR beads (Amersham Biosciences) in a GeneAmp PCR System 9700 (Applied Biosystems). The following protocol was used for PCR: 4 min at 95 °C, followed by 35 cycles of 1 min at 95 °C, 1 min at 55 °C and 2 min at 72 °C. The PCR products were sequenced on an Abi 3700 XL and proofread in Vector NTI ver 11.5 (Invitrogen). After proofreading the sequences were subjected to a BlastN search in Genbank (Zhang et al. 2000) to identify the species.



Figure 1. Autopsy of koi carp showing A: whole fish, B and C: hyperaemia and adhesions of the internal organs, and D: tapeworms removed from the intestine. Photographs by Geir Bornø.

Results and discussion

The autopsy (see Figure 1) revealed the presence of mucus on the tail fin (Figure 1A) and gills and a mixture of fungi (probably genus *Saprolegnia*) and bacteria were seen in direct microscopy. The fish showed hyperemia and adhesions in internal organs (Figure 1). In the stomach and intestine, live, moving tapeworms were found (Figure 1D). The tapeworms were not counted but occupied the whole intestinal lumen. Histological examination showed inflammation and necrosis in the gill and again a mixture of fungi and bacteria, in addition to unidentified parasites interlamellarly. Inflammatory cells were apparent in the lamina propria of the intestine. In addition, changes compatible with autolysis were found in all organs. The number of tapeworms in the intestine and the damage caused by the tapeworms makes it likely that these parasites were contributing to the death of this fish specimen.

Three of the tapeworms found in the intestine were subjected to molecular analysis to identify the species. The PCR and subsequent sequencing yielded a 1381 bp sequence of the 28S and a 493 bp of the 18S. The BlastN searches (as of 23.07.2018) were almost identical to sequences from individuals identified as *S. acheilognathi* (e.g. identity to *S. acheilognathi* KX060604; 18S: 492/493 bp, 28S: 1380/1381 bp). The 28S and 18S sequences representing the Norwegian specimen are deposited under GenBank accession number MH671916 and MH671917. The molecular analysis confirms that this is the first finding of this parasite in Scandinavia.

For a parasite with such a wide host range as shown for *S. acheilognathi* (Kuchta et al. 2018), temperature, and not the presence of particular hosts, might be the limiting factor for its distribution. However, even though the development of eggs ceases below 12 °C, there are parts of almost every water systems, like smaller streams and backwaters, that may be sufficiently warm during summer to allow development (Choudhury and Cole 2012). Thus, there is a potential risk for establishment of this parasite in any water system that contains copepod and fish hosts. Several fish known to be hosts for *S. acheilognathi*, like roach, *Rutilus rutilus* (L., 1758), rudd, *Scardinius erythrophthalmus* (L., 1758), bream, *Abramis brama* (L., 1758), and bleak, *Alburnus alburnus* (L., 1758), listed in the appendices of Kuchta et al. (2018), are common in the southwestern parts of Norway. Also, several copepod hosts like *Acanthocyclops robustus* (Sars, 1863), *Cyclops strenuus* Fischer, 1851, and *Paracyclops poppei* (Rehberg, 1880) are present in Norwegian freshwaters. As areas in the south of Norway are warm during summer with water temperatures well above 12 °C and both potential fish and copepod hosts are available, one cannot rule out that an infection could be established in the wild, if introduced.

All the fish in the pond population from where the examined fish specimen was taken, were exterminated soon after the detection of *S. acheilognathi*. Thus, there was no risk of infection for the local fish populations. The current finding also occurred in an area far to the north with low water temperatures for most of the year and without many potential wild fish hosts for the parasite. These facts combined would probably render it unlikely that the parasite would establish in the area should it have been spread to wild populations. However, the koi carp might have come from an infected stock of fish which might also have been sold to aquarists further south in Norway. There the establishment in the wild is, as discussed above, more likely should the fish or eggs escape to habitats outside an aquarium or pond.

We conclude that the parasites found in the koi carp in the present study represents the first finding of *S. acheilognathi* in Norway and Scandinavia and that this finding again shows the risk that accompanies the import of fish in the aquarium trade.

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