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NOTE

First record of proliferative kidney disease agent *Tetracapsuloides bryosalmonae* in wild brown trout and European grayling in Finland

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ABSTRACT: The myxozoan endoparasite *Tetracapsuloides bryosalmonae* causes temperature-driven proliferative kidney disease (PKD) in salmonid fishes. Despite the economic and ecological importance of PKD, information about the distribution of the parasite is still scarce. Here, we report for the first time the occurrence of *T. bryosalmonae* in wild brown trout *Salmo trutta* and European grayling *Thymallus thymallus* populations in Finland. We detected *T. bryosalmonae* at high prevalence in both brown trout and European grayling from the transboundary Finnish–Russian River Koutajoki system (Rivers Oulankajoki, Kuusinkijoki, Kitkajoki, Maaninkajoki, and Juumajoki) in north-eastern Finland. In southern Finland, *T. bryosalmonae* was detected in River Siuntionjoki young-of-the-year brown trout collected both in 2015 and 2016 (100% prevalence), while the parasite was not observed in fish from 3 other rivers (Ingarskila, Mustajoki, and Vantaanjoki) flowing to the Gulf of Finland. Our results, together with those from recent studies of Atlantic salmon, indicate that *T. bryosalmonae* is distributed over much higher latitudes in northern Europe than previously appreciated. We expect that increasing water temperatures will likely cause new PKD outbreaks in these more northerly regions in the future.

KEY WORDS: Host · Pathogen · Bryozoa · Freshwater environments · DNA · PCR · *Salmo trutta* · *Thymallus thymallus*

INTRODUCTION

The myxozoan endoparasite *Tetracapsuloides bryosalmonae* (Malacosporea) is the causative agent of widespread proliferative kidney disease (PKD) in salmonid fishes. The parasitic infection can cause severe mortality in both wild and captive salmonid populations (Hedrick et al. 1993, Feist et al. 2002, Wahli et al. 2002, Sterud et al. 2007). Because of temperature-dependent pathology and recently reported outbreaks, PKD has been recognized as an emerging disease (Okamura et al. 2011).

T. bryosalmonae has a complex life cycle that includes freshwater bryozoans, commonly from the genera *Fredericella* and *Plumatella*, as invertebrate hosts (Anderson et al. 1999, Morris & Adams 2006). Salmonid fish are subsequently infected upon contact with waterborne *T. bryosalmonae* spores, which develop in bryozoans (Longshaw et al. 2002). Once *T. bryosalmonae* has entered through the fish gills, the

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parasite propagates in blood, until reaching the kidney where further propagation and differentiation takes place (reviewed by Okamura et al. 2011). The clinical symptoms of PKD in fish include renal swelling, exophthalmia, and anemia (Hedrick et al. 1993). Anemia decreases individual performance of fish by lowering metabolic rate and aerobic scope, also causing a reduction in upper thermal tolerance (Bruneaux et al. 2017). As a result, PKD can lead to high mortalities at elevated water temperatures, while fish show less severe clinical signs of disease and are able to survive the infection when water temperature is low (Bettge et al. 2009, Okamura et al. 2011, Schmidt-Posthaus & Wahli 2015).

Recent work has demonstrated that *T. bryosalmonae* is probably widespread in northern Europe (e.g. Kristmundsson et al. 2010, Dash & Vasemägi 2014, Mo & Jørgensen 2017). For example, *T. bryosalmonae* is widely spread in anadromous brown trout *Salmo trutta* populations in Denmark (Skovgaard & Buchmann 2012) and in Estonia (Dash & Vasemägi 2014). Similarly, Mo & Jørgensen (2017) reported that *T. bryosalmonae* is rather common in Atlantic salmon (in 64 out of 91 rivers) and brown trout populations (in 17 out of 19 rivers) in Norway. However, we still know relatively little about the distribution and prevalence of *T. bryosalmonae* infections in northern latitudes. Moreover, it is not clear if *T. bryosalmonae* infects other salmonid species, such as European grayling *Thymallus thymallus*, in northern Europe.

In this study, we aimed to describe the distribution and prevalence of *T. bryosalmonae* in brown trout and European grayling in a series of rivers in southern and north-eastern Finland. We used a molecular genetic approach (Dash & Vasemägi 2014) to detect the presence of *T. bryosalmonae* infection at 13 sites and morphological examination to further characterize the extent of kidney swelling (renal hyperplasia) in 1 particular river system (River Siuntionjoki).

MATERIALS AND METHODS

We collected brown trout from 4 rivers in southern Finland (Rivers Ingarskila, Mustajoki, Vantaanjoki, Siuntionjoki; altogether 5 sites) flowing to the Gulf of Finland and the Baltic Sea. We also collected brown trout and European grayling from the Finnish–Russian River Koutajoki (River Kovda in Russian) system in north-eastern Finland (Rivers Oulankajoki, Kuusinkijoki, Kitkajoki, Maaninkajoki, and Juumajoki; altogether 8 sites) that flows to the White Sea. All collections were conducted using standard electrofishing procedures (Table 1). All samples from southern Finland consisted of young-of-the-year (0+) anadromous brown trout. The samples from north-eastern Finland originated from resident or lake migrating brown trout and consisted of 0+ and/or older fish. Similarly, both young-of-the-year and older grayling were collected from the River Koutajoki system in north-eastern Finland. The fish were killed by an overdose of buffered tricaine methanesulfonate (MS222, Sigma Aldrich), benzocaine (p-aminobenzoic acid ethyl ester, Sigma Aldrich), or by a sharp blow to the head. Three 0+ trout were found dead after electrofishing at Passilankoski on the River Siuntionjoki in 2016.

Fish were cut with a scalpel along the transverse plane between the front and the end of the dorsal fin, and the whole body section was subsequently stored in 96% ethanol. A small piece (~10 mm^3) of kidney was removed from this body section for identification of *Tetracapsuloides bryosalmonae* DNA using a multiplex PCR approach as in Dash & Vasemägi (2014) with minor modifications. DNA was extracted from the kidney by following the Macherey-Nagel NucleoSpin® Tissue DNA purification protocol. Multiplex PCR was used to amplify a *Salmo* sp. specific fragment (GenBank accession number CA064223, Vasemägi et al. 2010) and 3 *T. bryosalmonae*-specific fragments of the 18s rRNA gene (298, 166, and 756 bp) as in Dash & Vasemägi (2014). Co-amplification of a DNA fragment from a salmonid host served as an internal PCR control, which ensured that a failure of amplification of *T. bryosalmonae*-specific fragments was not misinterpreted as the absence of parasite in that sample (Fig. 1). Two known PKD-positive and 2 PKD-negative brown trout samples were used as positive and negative controls, respectively. Gel electrophoresis was performed using 2% agarose gel (1×TBE) stained with 2 µl of Midori Green Nucleic Acid Staining Solution (Nippon Genetics Europe). Two µl of GeneRuler 100 bp DNA ladder was used as a size standard in every gel. Samples were run for approximately 90 min with 100 V of electric charge. The 95% confidence intervals (CI) for *T. bryosalmonae* prevalence for individual sites were estimated with continuity correction as in Newcombe (1998) using a web-page calculator (www.vassarstats.net).

To complement molecular diagnostic analysis, we obtained quantitative estimates of renal swelling for a small number of brown trout from the River Siuntionjoki (tributary, n = 5; main channel, n = 1). A body section was produced by cutting the fish dorsoven-

Table 1. Prevalence (prev.) of *Tetracapsuloides bryosalmonae* (*Tb*) in studied brown trout *Salmo trutta* and European grayling *Thymallus thymallus* populations in Finland; n: total number of studied fish. 95% confidence intervals for prevalence estimates are presented when n > 1

River	Tributary/site	Coordinates	Date	*S. trutta*				*T. thymallus*			
			(dd.mm.yy)	n	Age	*Tb* prev. (%)	95% CI (%)	n	Age	*Tb* prev. (%)	95% CI (%)
Ingarskila	Ingarskilan Myllykoski (main channel)	60° 05.01′, 24° 02.87′	8.10.2015	10	0+	0	0–35				
Mustajoki	Koskenmäenkoski (main channel)	60° 58.81′, 28° 24.21′	22.9.2015	10	0+	0	0–35				
Vantaanjoki	Longinoja (main channel)	60° 14.56′, 25° 00.09′	22.9.2015	10	0+	0	0–35				
Siuntionjoki	Passilankoski (main channel)	24° 13.81′, 60° 11.35′	24.8.2015	16	0+	100	76–100				
Siuntionjoki	Passilankoski (main channel)	24° 13.81′, 60° 11.35′	19.8.2016	3	0+	100	31–100				
Siuntionjoki	Lempansån (tributary)	24° 09.71′, 60° 13.68′	7.9.2016	5	0+	100	46–100				
Koutajoki	Oulankajoki, Pikkukön-gäs (main channel)	66° 22.22′, 29° 18.27′	1.11.2016	13	0+, ≥1	85	54–97	1	0+	100	
Koutajoki	Oulankajoki, Alakoski (main channel)	66° 21.99′, 29° 19.14′	1.11.2016	16	0+, ≥1	94	68–100				
Koutajoki	Maaninkajoki, lower (tributary)	66° 24.56′, 28° 50.18′	1.11.2016	14	0+, ≥1	21	6–51	6	0+	0	0–48
Koutajoki	Maaninkajoki upper (tributary)	66° 25.58′, 28° 42.63′	1.11.2016	22	0+, ≥1	100	82–100				
Koutajoki	Kuusinkijoki lower (main channel)	66° 14.90′, 29° 40.57′	2.11.2016	30	0+, ≥1	93	76–99	13	0+	100	72–100
Koutajoki	Kuusinkijoki upper (main channel)	66° 11.20′, 29° 34.38′	2.11.2016	1	≥1	100		31	0+, ≥1	100	86–100
Koutajoki	Juumajoki (tributary)	66° 12.76′, 29° 45.17′	2.11.2016	18	≥1	0.83	58–96	10	0+	100	66–100
Koutajoki	Kitkajoki (main channel)	66° 16.24′, 29° 28.83′	3.11.2016	39	0+, ≥1	100	89–100	9	1+	100	63–100

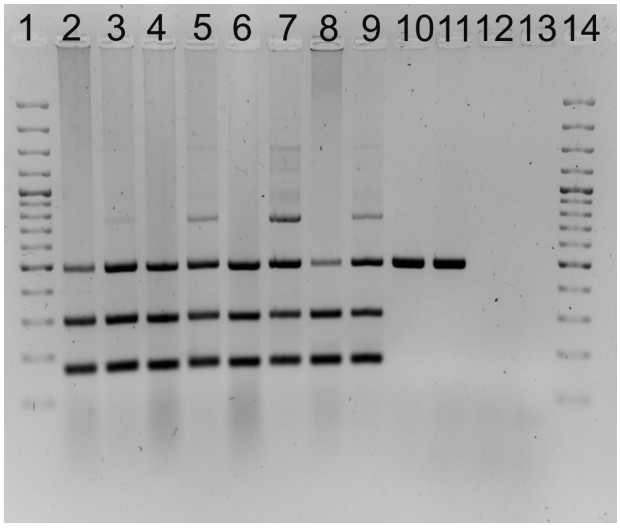

Fig. 1. Multiplex PCR for detection of *Tetracapsuloides bryosalmonae* from brown trout *Salmo trutta* kidney tissue. Image of 2% agarose gel consisting of *T. bryosalmonae*-specific DNA fragments (primary targets of 298 and 166 bp; secondary target of 756 bp) and *Salmo* sp.-specific fragment (~500 bp); Lanes 1 and 14: 100–1500 bp DNA ladder; Lanes 2 to 7: replicated 0+ brown trout kidney samples from Siuntionjoki; Lanes 8, 9: *T. bryosalmonae* positive control; Lanes 10, 11: *T. bryosalmonae* negative control; Lanes 12, 13: no template controls

trally between the anterior and posterior end of the dorsal fin with a scalpel as described above. Before storing the sample in 96% ethanol, the body section was photographed with a digital camera for measurement of the kidney-to-body thickness ratio (K:B ratio, as in Fig. 1a in Bruneaux et al. 2017) to estimate

the PKD-induced hyperplasia of the kidney. We compared the estimated kidney hyperplasia measurements (K:B ratio) to published data on *T. bryosalmonae*-infected 0+ brown trout (n = 77) collected from the River Vainupea, Estonia (Bruneaux et al. 2017).

When testing the multiplex PCR method for *T. bryosalmonae* screening in grayling, we noted that the amplification product of the *Salmo* sp.-specific fragment was very weak or absent. Therefore, for a subset of grayling specimens (n = 6), we used grayling-specific microsatellite primers (Str85INRA, using the slightly modified amplification protocol described by Swatdipong et al. 2009) to test if the lack of amplification of the parasite could be explained by DNA degradation, PCR inhibition, or primer mismatch. As all 6 individuals collected from the River Maaninkajoki (lower site) successfully amplified the grayling-specific microsatellite locus but failed to produce *T. bryosalmonae* amplicons, we conclude that these individuals most likely did not carry *T. bryosalmonae* infections.

RESULTS

Tetracapsuloides bryosalmonae infections were found in 133 of 151 (88%) brown trout and in 64 of 70 (91%) grayling samples collected from the River Koutajoki system in north-eastern Finland (Table 1). At individual sites, *T. bryosalmonae* prevalence in brown trout varied from 21% (95% CI: 6–51, n = 14) to 100% (95% CI: 89–100, n = 22). For grayling, *T. bryosalmonae* prevalence at individual sites varied from 0% (95% CI: 0–48, n = 6) to 100% (95% CI: 72–100, n = 12; and CI: 86–100, n = 31). When present, *T. bryosalmonae* showed high prevalence (>83%) in both species, except for brown trout in the lower site of the River Maaninkajoki (prevalence 21%, 95% CI = 6–51; n = 14). Throughout the River Koutajoki system, both young-of-the year and older fish were found to be infected by *T. bryosalmonae* showing no differences in prevalence (2×2 contingency table, Fisher's exact test, all tests, $p < 0.05$). Similarly, the prevalence estimates for brown trout and grayling did not differ at any studied site (2×2 contingency table, Fisher's exact test, all tests, $p < 0.05$). However, it is important to remember that these estimates may be influenced by the low number of analyzed fish. We also caught 3 *T. bryosalmonae*-infected brown trout with the adipose fin removed (River Oulankajoki, Pikkuköngäs) indicating that these individuals originated from the Kuusamo fish hatchery (Natural Resources Institute Finland). Both wild and hatchery brown trout in the River Koutajoki system are therefore infected by *T. bryosalmonae*.

In southern Finland, *T. bryosalmonae* infections were found in 24 of 54 (44%) brown trout samples. All *T. bryosalmonae* infected samples originated from the River Siuntionjoki main channel (2015: prevalence 100%, 95% CI: 76–100, n = 16; 2016: prevalence 100%, 95% CI: 31–100, n = 3) and its tributary, the River Lempansån (prevalence 100%, 95% CI: 46–100, n = 5). The other 3 southern Finnish brown trout populations were apparently *T. bryosalmonae* free (n = 10 for each population, Table 1).

Comparison of the renal swelling of the 6 samples with the earlier data from anadromous brown trout collected from Estonia (River Vainupea, Bruneaux et al. 2017) indicated that some individuals from the River Siuntionjoki exhibited severe signs of PKD-induced kidney hyperplasia (K:B ratio > 0.3; Fig. 2). Measured water temperatures in the River Siuntionjoki main channel and its tributary the Lempansån further support these findings (Fig. 3), as severe clinical PKD symptoms typically occur when water temperatures reach 15°C and above (Morris et al. 2005, Bettge et al. 2009). The considerably higher water temperatures in the River Siuntionjoki main channel

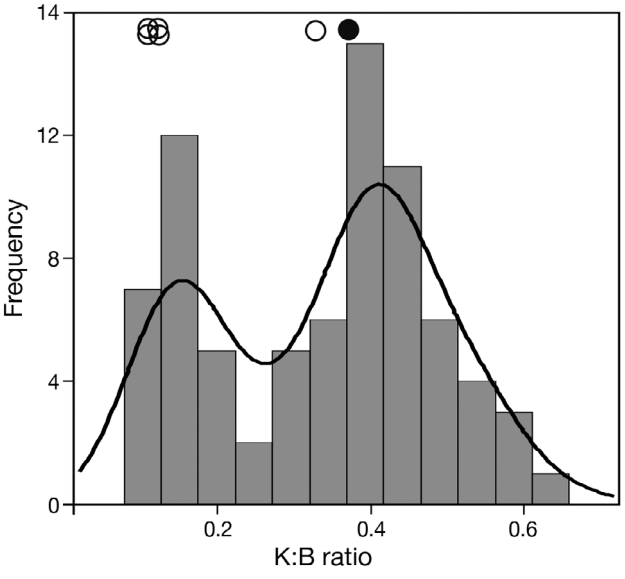

Fig. 2. Bimodal distribution of kidney hyperplasia measurements (kidney to body [K:B] ratio) ranging from normal to extremely swollen kidneys in *Tetracapsuloides bryosalmonae* positive 0+ brown trout *Salmo trutta* (n = 77) collected from the River Vainupea, Estonia (Bruneaux et al. 2017). Solid line represents kernel density estimate for the River Vainupea. Estimated K:B ratios for individual juvenile brown trout caught from the River Siuntionjoki main channel (n = 1) and its tributary, the River Lempansån (n = 5), are marked with filled and open circles, respectively

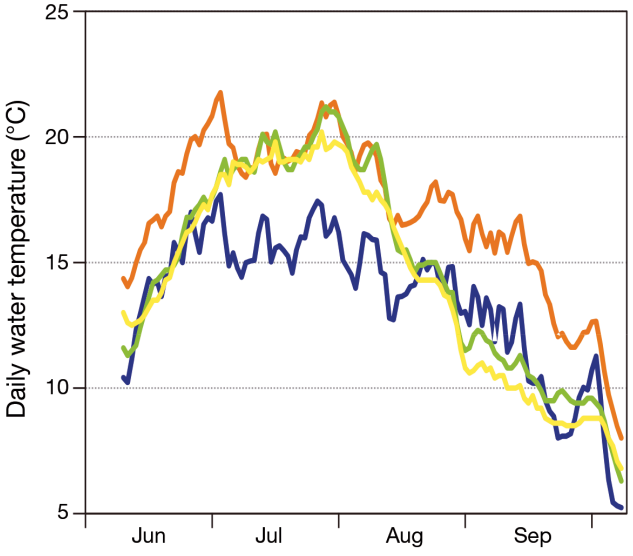

Fig. 3. Daily water temperature in the Rivers Siuntionjoki (orange), Lempansån (blue), Kitkajoki (green), and Oulankajoki (yellow) from June to October 2016

over the 4 mo period from June to September (84 d >16°C, 52 d >18°C) compared to its tributary the Lempansån (23 d >16°C, 0 d >18°C, average difference 3.45°C, Fig. 3) may cause juvenile brown trout in the main channel to suffer more severe PKD symptoms than their conspecifics in the River Lempasån. The Rivers Oulankajoki and Kitkajoki in north-eastern Finland also showed high water temperatures during July and early August in 2016, when the measured daily water temperatures exceeded 18°C (Fig. 1c). High water temperatures in these rivers are likely caused by large lake systems upstream (e.g. Lakes Kitkajärvi, Kallunkijärvi, and Onkamojärvi). As a result, it is likely that PKD can have important negative effects on wild salmonid populations in Finland, especially during warm summers.

DISCUSSION

There have been 2 earlier reports on PKD in Finland according to the Finnish Food Safety Authority EVIRA (www.evira.fi/en/), and in both cases *Tetracapsuloides bryosalmonae* was observed in farmed fish. PKD was reported for the first time in 2006 in a fish farm in the Åland Archipelago among 1 yr old rainbow trout *Oncorhynchus mykiss* imported from Denmark and in 2013, *T. bryosalmonae* was observed in farmed Arctic char *Salvelinus alpinus* in the Lake Inari area of northern Finland (https://www.evira.fi/elaimet/elainten-terveys-ja-elaintaudit/elaintaudit/kalat-ja-ravut/pkd/). The latter case is thought to represent a *T. bryosalmonae* outbreak endemic to the Lake Inari system (A. M. Eriksson-Kallio, EVIRA, pers. comm.). More recently, *T. bryosalmonae* was reported in wild Atlantic salmon from 2 Norwegian/Finnish rivers (River Teno/Tana and Neidenelva/Näätämö) that drain into the Barents Sea (Mo & Jørgensen 2017). Here, we reported the presence of the PKD agent *T. bryosalmonae* for the first time in Finnish wild brown trout and grayling populations. When present, *T. bryosalmonae* infections occurred at high prevalence consistent with earlier reports from Denmark, Estonia, and Norway (Skovgaard & Buchmann 2012, Dash & Vasemägi 2014, Mo & Jørgensen 2017). However, to better understand parasite proliferation and disease dynamics in both species, temporal monitoring of disease symptoms and parasite prevalence is necessary.

Our results, together with recent findings in Atlantic salmon (Mo & Jørgensen 2017), indicate that *T. bryosalmonae* is distributed over much higher latitudes in northern Europe than previously appreciated. Given that the distribution of *T. bryosalmonae* does not seem to be limited by high latitude, we expect that increasing water temperatures due to global warming will likely cause new PKD outbreaks in northern regions in the future. Therefore, future efforts to understand the impact of *T. bryosalmonae* in salmonid fish should focus on parasite abundance and prevalence as well as the physiological impact of infection by quantifying disease symptoms, such as kidney hyperplasia and hematocrit levels (e.g. Bruneaux et al. 2017, Debes et al. 2017). In view of the endangered status of brown trout in Finland (Finnish Red Data Book; Rassi et al. 2010), more information about the occurrence of *T. bryosalmonae*, its bryozoan host, host condition-dependent developmental cycling (Okamura 2016), and the physiological and ecological effects of PKD on wild trout populations is urgently needed.

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