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ORIGINAL PAPER

The invasive cestode parasite *Ligula* from salmonids and bullies on the South Island, New Zealand

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Abstract

Freshwater ecosystems are often impacted by biological invasions, including the introduction of exotic parasites capable of infecting native species. Here, we report the occurrence of the introduced tapeworm *Ligula* sp. from common bully, *Gobiomorphus cotidianus*, and quinnat salmon, *Oncorhynchus tshawytscha*, in Lake Hawea, South Island, New Zealand. This parasite has a complex life cycle, reaching its adult stage in fish-eating birds. Worms recovered from the body cavity of fish hosts reached huge sizes (60–300 mm long); however, their low prevalence in fish populations suggests that infections are rare or localised. Molecular analysis (internal transcribed spacer (ITS)1 and ITS2 sequences) confirms that these specimens belong to the genus *Ligula* and suggests tentative routes of invasion into New Zealand. Monitoring the spread of this parasite is important, as it can impact fish populations and also, when infection levels are high, those of piscivorous birds.

Keywords Diphyllobothriidae · *Ligula intestinalis* · Planktonic copepods · Plerocercoids · Salmonids

Introduction

Biological invasions have been reported with increasing frequency as a result of the globalisation of modern transport systems and various environmental changes (Ruiz et al. 2000; Perrings et al. 2005). Introductions of exotic species threaten the integrity and functioning of native ecosystems and represent a major cause of native biodiversity loss (Grosholz 2002; Molnar et al. 2008). Biological invasions can also cause the emergence of new parasitic diseases or affect the incidence or severity of existing ones (Prenter et al. 2004; Dunn 2009). For instance, the introduction of exotic animals that are suitable hosts to native parasites can change the local dynamics of transmission and infection, with negative consequences for native species (Poulin et al. 2011). Of greater concern is the introduction of exotic 'generalist' parasites capable of infecting native animals (Taraschewski 2006; Dunn 2009). For example, although other factors are involved, populations of the native European eel *Anguilla anguilla* Linnaeus have declined markedly since the introduction from Asia of the anguillid-specific nematode *Anguillicoloides crassus*. This parasite has little health impact on Japanese eel (*Anguilla japonica*), its original host, but is highly pathogenic to European eels (i.e. increased mortality, decreased body condition, failed spawning migration; Taraschewski 2006). Invasive parasites, therefore, have the potential to severely impact native host species.

Here, we report the presence of the non-native tapeworm *Ligula* sp. (Cestoda: Diphyllobothriidae) in Lake Hawea, on the South Island of New Zealand. Although many reports of this parasite refer to it as *Ligula intestinalis* Linnaeus, genetic evidence suggests that more than one species may be lumped under this name across its geographical range (Bouzid et al. 2008); therefore, here, we refer to it by its genus name. Species of the genus *Ligula* have a three-host life cycle (Dubinina 1966). Adult worms live in piscivorous birds, with the eggs they produce passing out in the birds' faeces. Eggs hatch after a few days in water, to release free-swimming coracidia. These are ingested by copepods, the first intermediate hosts, in which the parasites develop to the procercoid stage. Fish act as second intermediate hosts; they acquire the parasite by consuming infected copepods. The parasite achieves most of its growth inside the body cavity of the fish

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host, reaching the plerocercoid stage and awaiting transfer by predation to a bird-definitive host. In a small fish, the parasite mass can equal, or even surpass, that of the fish host (e.g., Barus and Prokes 1994). At least at the genus level, *Ligula* cestodes are generalist parasites, having been reported from over 50 freshwater fish species, mostly Cyprinidae (but also Catostomidae, Percidae, etc.) and over 70 bird species (Dubinina 1966; Hoole et al. 2010). They have a broad geographical distribution extending across Europe, Asia and North America (Bouzid et al. 2008; Hoole et al. 2010). In addition, *Ligula* cestodes also occur in Africa (Cowx et al. 2008; Britton et al. 2009) and Australia (Morgan 2003; Chapman et al. 2006). They have been reported once from New Zealand's North Island, by Weekes and Penlington (1986), who found what they identified as *L. intestinalis* in common bully, *Gobiomorphus cotidianus* McDowall, 1975, and rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), from Lake Ototoa, north-west of Auckland. The same authors also mention its occurrence in rainbow trout from Lake Lyndon, Canterbury.

Here, 30 years after Weekes and Penlington (1986), we provide only the second record of this non-native parasite in New Zealand, from a new locality and a new fish host species. We confirm the generic identity of the parasite using molecular data and discuss its likely life cycle in New Zealand and its potential impact on native species.

Methods

Samples

Three fish samples were obtained from Lake Hawea, Central Otago, South Island. Firstly, two common bullies, *G. cotidianus*, recovered from the gut of a brown trout, *Salmo trutta* L., caught in September 2016, and one landlocked quinnat salmon, *Oncorhynchus tshawytscha* (Walbaum, 1792), caught in November 2015, were obtained from a recreational angler. These fish were caught in the south-west portion of the lake. The two bullies and the salmon were obviously infected, and it is their finding that prompted further sampling.

Second, salmonid fish were obtained from participants in the Lake Hawea fishing competition, in November 2016. The body cavity of each fish was inspected for the presence of *Ligula*, and the digestive tract was removed and frozen. Later, it was dissected and all gut contents were identified and sorted into broad taxonomic categories, to assess potential exposure to cestode infection via ingestion of copepods. Third, common bullies were sampled from the western shore of the lake, just south of The Neck, in November 2016, using a combination of minnow traps set overnight and seining. Fish were kept on ice and later dissected to check for *Ligula* infection and determine gut contents.

All fish were measured (fork length) and weighed. The only *Ligula* plerocercoids recovered were from the fish caught by the recreational angler; they were not immediately preserved appropriately and were only much later placed in ethanol after 2 cycles of freezing and thawing. They were individually measured to the nearest millimetre, but because of inadequate preservation, finer details of their morphology could not be characterised. Also, DNA could only be successfully extracted and amplified from three specimens. These specimens had small pieces removed from mid-strobila for DNA extraction, and their remains were preserved as hologenophore vouchers (Pleijel et al. 2008). Voucher specimens were submitted to the Otago Museum (accession numbers IV85282–IV85284).

Genetic analysis

Two isolates of *Ligula* specimens from *Onchorhynchus tshawytscha* and one from *Gobiomorphus cotidianus* were characterised molecularly. Genomic DNA was extracted from isolates in 200 μL of a 5% suspension of Chelex® in deionised water and containing 0.1 mg/ml proteinase K followed by incubation at 56 °C for 5 h, boiling at 90 °C for 8 min, and centrifugation at 14,000×*g* for 10 min.

Partial internal transcribed spacer (ITS)1 and ITS2 regions of the rDNA array were amplified using primers ITS5 (forward) and ITS4 (reverse) (Olson et al. 2002) and Flo1 (forward) and ITS2R (reverse) (Logan et al. 2004). These markers were chosen because multiple ITS1 and ITS2 sequences from *Ligula* and its related genus *Diagramma* (Luo et al. 2003) are available on GenBank, and our purpose was just to confirm the generic identity of the plerocercoids. Polymerase chain reaction (PCR) amplifications were performed in a total volume of 25 μl, comprising 5 μl of MyTaq™ Red reaction buffer (Bioline (Aust) Pty Ltd., Alexandria, New South Wales, Australia), primers at 0.5 mM each, MyTaq™ Red DNA polymerase (Bioline) at 0.025 units/ml and 5 μl of DNA template. The PCR reactions consisted of 38 iterations of the following cycle: 1 min at 96 °C, 1 min at 54 °C and 2 min at 72 °C, beginning with an additional denaturation step of 3 min at 96 °C and ending with a final extension at 72 °C for 7 min. PCR amplicons were purified prior to sequencing using exonuclease I and shrimp alkaline phosphatase enzymes (Werle et al. 1994). The purified PCR products were sequenced using Big Dye Terminator technology (BigDye v. 3.1) on a 3730XL DNA analyser (Applied Biosystems, Foster City, California, USA). Sequencing resulted in a 1096-bp data set comprising a 570-bp fragment of ITS1 and a 526-bp fragment of ITS2.

Newly generated sequences were aligned using ClustalW implemented in MEGA v6 (Tamura et al. 2013). The ITS1 and

ITS2 fragments were concatenated manually. The extremes of the alignments were trimmed to match the shortest sequence prior to phylogenetic analyses. These analyses served to confirm that our specimens nested among other *Ligula* sequences and not to infer actual phylogenetic relationships. The data set included 12 representative sequences of the family Ligulidae and 5 sequences belonging to the Diphyllobothriidae retrieved from GenBank. Phylogenetic analysis was conducted in MEGA6 (Tamura et al. 2013) and inferred using the maximum likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (− 2285.89) was retained, with branch lengths measured in the number of substitutions per site and bootstrap support based on 1000 iterations. All positions containing gaps and missing data were eliminated, and there were a total of 928 positions in the final data set. A representative sequence has been submitted to GenBank (accession numbers KY609970).

Results

Fish infection and diets

In the initial sample, the two common bullies, *G. cotidianus* (fork lengths 67 and 62 mm; weights 4.5 and 3.7 g), recovered from the gut of a brown trout, each harboured a single plerocercoid (92 and 98 mm long, respectively, each over 1 g in weight), with the worm reaching a very large size relative to the bully host (Fig. 1). The quinnat salmon, *O. tshawytscha* (fork length 400 mm; weight 702 g) harboured 50 plerocercoids (mean length, 150 mm; range, 60–300 mm; combined total weight, 80 g), which occupied a large volume within the body cavity (Fig. 2).

Fig. 1 Plerocercoid of the cestode *Ligula* sp. next to the common bully, *Gobiomorphus cotidianus*, from which it was dissected. Scale bar = 1 cm

Fig. 2 Plerocercoids of the cestode *Ligula* sp. spilling out of the body cavity of a quinnat salmon, *Oncorhynchus tshawytscha*. Scale bar = 5 cm

The second sample, obtained from the Lake Hawea fishing competition, yielded 79 salmonids (see Table 1 for details): 24 quinnat salmon (*Oncorhynchus tshawytscha*), 35 rainbow trout (*Oncorhynchus mykiss*) and 20 brown trout (*Salmo trutta*). Quinnat salmon in the lake are landlocked and remain typically small compared to sea-going individuals; rainbow and brown trout were larger and of similar size (Table 1). Sex ratio was roughly 1:1 for all three species. None of the 79 salmonids examined harboured cestode plerocercoids. Our final sample produced 38 common bullies (*Gobiomorphus cotidianus*) large enough to be infected by *Ligula* (Table 1). Their sex ratio was slightly biased towards males, most likely due to size selection towards larger fish during our sampling. No plerocercoid was found in any of the bullies.

Diet analysis showed that all fish species sampled fed on planktonic crustaceans and may thus be exposed to the parasite through their diet. However, close examination revealed that exposure risks vary greatly among fish species (Table 1). For example, *S. trutta* feeds mainly on benthic prey (chironomids, caddisflies, gastropods, small bullies) and has limited exposure to the parasite. In contrast, *O. tshawytscha* is a pelagic feeder more exposed to infection by *Ligula* through its diet (Table 1). Interestingly, while the diets of *S. trutta* and *O. tshawytscha* clearly diverged, that of *O. mykiss* overlapped with the other salmonid species (unpublished data). The diet of common bullies also contained a non-negligible proportion of planktonic crustaceans, including copepods (Table 1).

Genetic analyses

DNA sequences of worms from the salmon and bully hosts proved them to be identical. In a preliminary phylogenetic hypothesis of relationships using partial ITS1 and ITS2 sequences (see Supplementary Material), the specimens are (i) most closely related to *Ligula* samples from cyprinids originating from Northern Ireland, to the exclusion of samples from Iran, Wales and China, and (ii) probably belong to *Ligula intestinalis*.

It is notable, however, that the uncorrected p distances between the *Ligula/Diagramma* branches are highly variable (between 0.10 and 3.62), supporting the findings of previous studies

Table 1 Number (*N*) of individuals per fish species dissected during our study with their mean (± SE) fork length (FL), weight (W), occurrence of plankton in stomachs and number of copepods per stomach

Fish species	*N*	FL (mm ± SE)	*W* (g ± SE)	Occurrence of plankton (% of stomachs)	No. of copepods per fish stomach (mean ± SE (range))
Quinnat salmon, *Oncorhynchus tshawytscha*	24	361 ± 7	536.7 ± 28.2	83.3	3.5 ± 0.8 (0–13)
Rainbow trout, *Oncorhynchus mykiss*	35	481 ± 16	1425.5 ± 124.1	51.4	4.3 ± 1.9 (0–50)
Brown trout, *Salmo trutta*	20	501 ± 12	1335.4 ± 56.5	5.0	0
Common bully, *Gobiomorphus cotidianus*	38	38 ± 1	0.7 ± 0.1	65.8	0.6 ± 0.2 (0–5)

that there is considerable genetic structure among ligulid species, which is currently unsupported by morphological taxonomy (Olson et al. 2002; Štefka et al. 2007; Bouzid et al. 2008).

Discussion

Thirty years after the initial finding by Weekes and Penlington (1986), we provide a further report of *Ligula* infections in New Zealand fish, this time further south and from an additional host species, the quinnat salmon *O. tshawytscha*. Our data indicate lower prevalence among Lake Hawea fish than those reported by Weekes and Penlington (1986) from Lake Ototoa on the North Island. However, the cestode plerocercoids recovered in our study are much larger in size: Weekes and Penlington (1986) report an average worm length of 19 mm in salmonid hosts, compared to the value of 150 mm that we found. The discrepancy is unlikely to be due to seasonal effects, as Weekes and Penlington's (1986) size measurements are based on worms from fish sampled throughout the year. Perhaps, the *Ligula* parasites studied by Weekes and Penlington (1986) are from a different strain from that occurring in Lake Hawea. Our genetic data suggest a general genetic affinity of our specimens with specimens from Northern Ireland and suggest that they probably belong to *Ligula intestinalis*. In addition, a short fragment (400 bp) of ITS2 from an Australian specimen (*Ligula* sp. GenBank EU241125 (Bouzid et al. 2008)), which could not be included in the tree in Supplementary Material because of its much shorter length, is identical to the New Zealand specimens except for a two-base insertion in a GT-repeated segment. This suggests that New Zealand freshwater systems may have been invaded by a *Ligula* lineage of European origin, possibly in a stepping stone manner via Australia and through migratory birds. However, further genetic data on other gene markers and from additional samples in New Zealand, Australia and Eurasia would be necessary to confirm this scenario.

Much remains to be determined regarding the epidemiology and ecological significance of *Ligula* in Lake Hawea. The high number of worms in a single salmon and their absence in other fish suggest a very patchy distribution, perhaps with foci of high infection risk in restricted areas of the lake highly frequented by avian-definitive hosts. Data on the spatial and temporal variations in the parasite's abundance will be essential to assess its potential impact.

Cyclopoid copepods, which are present in Lake Hawea and along with other zooplankton forming part of the diet of common bullies and salmonids, are likely first intermediate hosts for *Ligula* cestodes. Introduced salmonids and common bullies appear to be suitable second intermediate hosts, as would probably other members of the bully genus *Gobiomorphus* and presumably galaxiid fishes too given that Australian galaxiids are readily infected (Morgan 2003; Chapman et al. 2006). Because *Ligula* has not been found in well-studied bully populations elsewhere in New Zealand, the definitive host may be a piscivorous bird with a restricted distribution. For this reason, the Australasian crested grebe (*Podiceps cristatus australis* Linnaeus, 1758) is the prime suspect, as on the South Island, it occurs in large numbers mostly in the Central Otago region where our fish were collected (Jensen and Snoyink 2005; O'Donnell 2013), with pairs nesting on the shores of Lake Hawea (personal observation). Of course, other piscivorous birds (e.g. shags, *Phalacrocorax* spp.) cannot be ruled out as potential definitive hosts.

The tapeworms achieve very little growth and live for only a few days in their bird-definitive hosts (Arme 1997). Bird hosts serve mostly to disperse parasite eggs. *Ligula* infection generally has no impact on bird health (Dubinina 1966), except possibly in some cases of very severe infections (Hare 1945). However, because of the dramatic growth rates and large sizes *Ligula* plerocercoids achieve in their fish intermediate hosts, the latter incur substantial deleterious effects. *Ligula* infection can inhibit fish reproduction through endocrine disruption (Arme 1968, 1997; Carter et al. 2005; Cowx et al. 2008; Hoole et al. 2010). As a consequence of energy being diverted from reproduction to somatic growth and/or through changes in foraging activity, infected fish sometimes attain larger sizes than their uninfected conspecifics (Loot et al. 2002). *Ligula* infection also results in behavioural changes in fish hosts, presumably as an adaptive strategy of the parasite to increase transmission to its next host through enhanced predation on fish (Loot et al. 2001; Britton et al. 2009). Importantly, reduced reproductive output and enhanced predation risk can have population-level consequences. Indeed, Kennedy et al. (2001) have conducted a long-term study in a small English lake demonstrating

population cycles in which rising *Ligula* prevalence caused a crash in the host fish population, leading to a subsequent drop in parasite prevalence followed by a recovery of the fish population, with the cycle then repeating itself.

Cestodes of the genus *Ligula* are not the only parasites threatening to invade New Zealand freshwater ecosystems. The Asian fish tapeworm, *Schyzocotyle acheilognathi* (formerly *Bothriocephalus acheilognathi*) is a highly successful global invader, pathogenic to many fish species, and is now well-established in Australia (Dove and Fletcher 2000; Pérez-Ponce de León et al. 2017). Although it does not have a bird-definitive host and can only be introduced through the trade of fish like carp, its proximity to New Zealand is a concern.

The apparent low prevalence of *Ligula* in Lake Hawea bullies and salmon suggests that the parasite may have arrived in the lake only recently and presently only occurs at low abundance. The immediate consequences are probably minimal. For recreational fishing, the parasite is a rare but unsightly occurrence with no risk for human health; its high visibility makes its accidental consumption unlikely, and it is destroyed by cooking anyway. However, the long-term sustainability of both native fish species and introduced salmonids could be at some risk if *Ligula* increases its local abundance and/or if it spreads to other localities. Efforts are therefore necessary to identify the avian-definitive host, i.e. the agent of parasite dispersal, and to monitor changes in *Ligula* prevalence in the bully populations of Lake Hawea and nearby lakes, such as Lake Wanaka, in order to anticipate and possibly mitigate any future impacts.

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