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Infection patterns and new definitive host records for New Zealand gordiid hairworms (phylum Nematomorpha)

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ABSTRACT

Some parasites modify the phenotype of their host in order to increase transmission to another host or to an environment suitable for reproduction. This phenomenon, known as host manipulation, is found across many parasite taxa. Freshwater hairworms are known for the behavioural changes they cause in their terrestrial arthropod hosts, increasing their likelihood of entering water to exit the host and reproduce. Understanding how infected arthropods move around in the natural environment could help uncover alterations in spatial distribution or movement induced by hairworms in their terrestrial definitive hosts. Moreover, few hairworm-host records exist for New Zealand, so any additional record could help elucidate their true host specificity. Here, we investigated whether infected terrestrial arthropods were more likely to approach streams in two subalpine communities of invertebrates, using a spatial grid of specialised pitfall traps. Although hairworm infection could not explain the movements of arthropod hosts near streams, we found several new host records for hairworms, including the first records for the recently described *Gordionus maori*. We also found some new host-parasite associations for mermithid nematodes. These records show that the host specificity of hairworms is quite low, suggesting that their diversity and distribution may be greater than what is currently known for New Zealand.

1. Introduction

Parasites need to infect a host, either to pursue development or to reproduce. The pressures that accompany this fundamental aspect of parasitic life cycles have resulted, across an evolutionary timescale, in a multitude of adaptive solutions [1]. In fact, certain lineages are capable, through direct or indirect mechanisms, of altering the phenotype of their current host to increase the odds of transmission to a subsequent host or to an environment suitable for reproduction [2,3]. This phenomenon, known as host manipulation, has been reported across numerous host-parasite systems [4], although its true adaptive nature has been debated for decades [5–8]. Phenotypic alterations can include any change in the appearance or behaviour of the host that favours the life cycle of the parasite. For instance, tadpoles infected with the trematode *Ribeiroia ondatrae* develop into frogs with malformed or additional limbs, which increases the odds of these amphibian hosts being eaten by the definitive avian host of the trematode [9]. Other remarkable examples include caterpillars that appear to guard over the parasitic wasps that recently left their body to pupate, thus offering some protection against natural enemies [10].

These striking examples of host manipulation display the wide array of strategies that parasites employ to increase the likelihood of completing their life cycle. Studying host phenotypic alterations in a natural setting can provide strong evidence that host manipulation is indeed adaptive [7,11]. For example, only from a long-term observational study in the field was it possible to quantify the natural effects of a host-manipulating fungal infection in ants [12]. In that study, the authors concluded that the parasite was akin to a chronic infection for ant colonies. Therefore, exploring the effects of host manipulation in natural conditions can help understand its true impact on both host and parasite fitness.

In the current study, we focused on the behavioural manipulation of terrestrial hosts by gordiid or freshwater hairworms (phylum Nematomorpha). These specialised parasites develop and mature within terrestrial arthropods (mainly scavenger or predatory insects) that consume paratenic hosts infected with dormant cysts [13,14]. Paratenic hosts consist of aquatic insect larvae that emerge as terrestrial adults from streams and rivers, thus transporting hairworm cysts from water to land [15]. When mature, hairworms need to exit from their terrestrial host in water to mate. This life cycle trait likely explains why hosts

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infected with mature hairworms appear to move around more erratically [16–18], thus increasing the odds of hosts (and hairworms) entering water. Although hairworm host manipulation has been largely misrepresented in both the popular media and the scientific literature [19], empirical evidence does suggest that hosts infected with hairworms are far more likely to enter water than uninfected conspecifics [20]. How exactly the hairworm accomplishes this considerable change in host behaviour remains poorly understood [19,21,22]. While previous studies, using naturally infected hosts, looked at the movement of hosts in an artificial setting [16–18], there has been no direct test of host movement patterns in a natural environment. This type of data would greatly strengthen our understanding of host behavioural changes and how infection increases the odds of naturally entering water.

In New Zealand, six species of freshwater hairworms have been reported from four genera, five of which are currently described (see Yadav et al. (2018) and references therein). Definitive host records, the last one dating back to 2000, were collated by Poinar [24] and Schmidt-Rhaesa [25] and include three families of orthopterans (Acrididae, Anostostomatidae, and Rhaphidophoridae), one family of ground beetles (Carabidae), and the order of cockroaches (Blattodea). Notably, three hairworm species were observed in various endemic wētā hosts [26]. The genetic diversity of New Zealand hairworms was studied across locations in South Island [27], highlighting the possibility of cryptic species within the same population. In sum, although currently recognised hairworm species are well characterised, little is known of their definitive hosts across New Zealand, a country that includes a rich diversity of endemic insect species [28], many of which having the potential to be infected by hairworms.

The aim of the current study was twofold. Our primary goal was to explore the spatial and temporal infection patterns of hairworms throughout two subalpine communities of terrestrial invertebrates in New Zealand. Here, we tested whether insects infected with mature hairworms were likelier to approach streams than to move away from streams, using a series of specialised pitfall traps set within a spatial grid, and if this pattern correlated with sampling season. We predicted that, if hairworms induce erratic behaviours in their hosts prior to entering water, there would be no obvious pattern in the distribution of infected hosts captured within the spatial grid. Alternatively, if hairworms do induce a directed movement towards water, this would be reflected in the number of infected insects captured per trap. Secondly, as a by-product of this sampling effort, we expected to identify new hairworm-host associations with the invertebrates captured in the traps, given that host records for New Zealand hairworms are relatively poor. Any new definitive host record would provide more information on the host specificity of hairworms, as well as elucidate the potential distribution of hairworms in New Zealand. This study provides novel insights into the hidden infection patterns of this poorly understood group of parasites.

2. Materials and methods

2.1. Field methods

Terrestrial invertebrates were captured in pitfall traps installed near the streams of two locations: Rock and Pillar Conservation Area (45°26′03"S 170°04′32"E; Site A) and Kopuwai Conservation Area (45°20′43"S 169°11′55"E; Site B) in Otago, New Zealand (Department of Conservation Authorisation Number 68065-RES). These sites were chosen because adult hairworms have been reported in the streams of both [23,27]. Also, they are subalpine in elevation (approximately 1320 and 1580 m in altitude, respectively), had similar vegetation cover, and both streams were also relatively similar with respect to general width and velocity. For each site, a 100-m section of stream was selected and three transects were drawn perpendicularly from the stream every 50 m. Along the transects, we installed pitfall traps at 5, 25, and 45 m from the stream, for a total of nine traps per site set within a spatial grid of 3 × 3 (Fig. 1).

We used X-shaped guidance barrier pitfall traps with 0.75-m-long plastic barriers planted into the ground for a height of approximately 0.07 m above ground and an angle of 90° between barriers [29] (Fig. 1). We selected this design because it has been shown to substantially increase the overall effectiveness of pitfall traps with respect to total captures and relative taxonomic composition [30]. For the container, we used a plastic cup (height X width: 0.115 × 0.09 m) filled with 250 mL of propylene glycol to preserve invertebrates. Two out of the four sides of the X-shaped trap were blocked off with strong adhesive tape so that invertebrates could only fall into the container if walking either towards the stream or away from it (Fig. 1). Each container was fitted with a plastic separator to properly identify from which side invertebrates fell into the trap. We covered the traps with metal roofs (0.25 × 0.25 m) to protect against flooding and placed rocks on top to stop the wind from dislodging them. Traps were installed for a seven-day period at the beginning of each month, from November 2020 to March 2021 (for a total of five sampling periods). These were reinstalled in the exact same

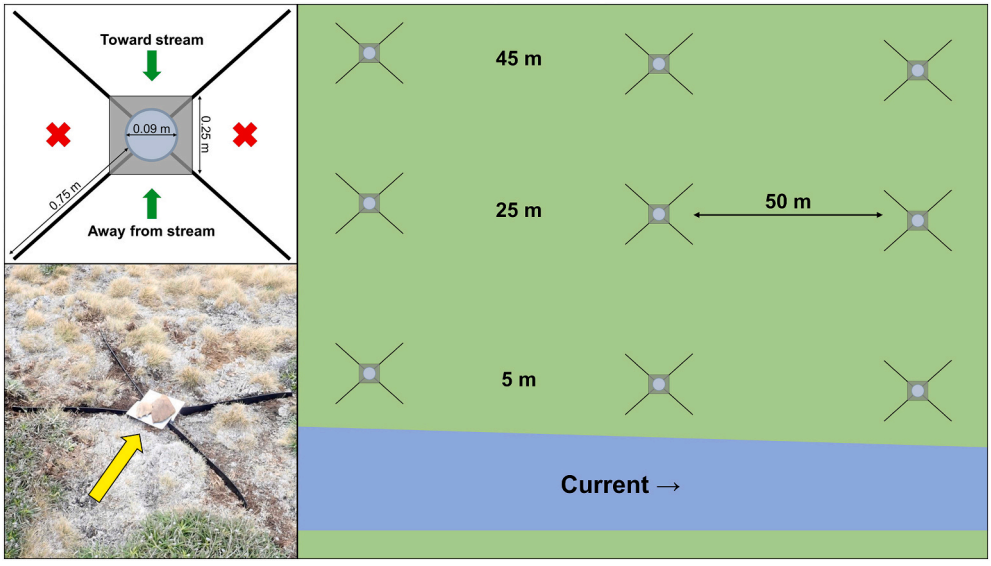

Fig. 1. Pitfall traps and study design. Field study design for investigating the spatial patterns of terrestrial insects infected with hairworms (phylum Nematomorpha) in two subalpine locations in Otago, New Zealand. The top left panel shows the schematic with dimensions (not proportional) of pitfall traps used in the study, with green arrows showing which sides invertebrates could fall into the container (blue circle) and red crosses showing the sides that were blocked off. The four black bars represent the plastic barriers and the grey square represents the metal roof, which is translucent here to see the container beneath. The bottom left panel shows a pitfall trap deployed in the field, with a yellow arrow pointing towards the stream. The right panel shows the spatial grid of nine pitfall traps set alongside the stream, with distances of traps from the stream and between transects. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

locations every month. At the end of each period, traps were disassembled and the containers were sealed and transported back to the laboratory. There, we removed invertebrates from both sides of each trap and stored them separately in 75% ethanol until further processing.

2.2. Laboratory methods

We first identified all the scavenger and predatory arthropods captured in the traps that were likely to be infected with hairworms to the lowest taxonomic level possible using the following taxon-specific keys: Araneae [31,32], Carabidae [33], Dermaptera [34], Orthoptera [35,36], and Scarabaeidae [37]. For these, some individuals were assigned to a family and then separated into morphospecies based on their appearance, as some characteristics were difficult to confirm under the dissecting microscope, e.g., the pedipalp tarsi of some male spiders. All other invertebrates that were unlikely to host hairworms were separated by family, order, or class. To rehydrate tissues and facilitate dissections, we removed the samples from their ethanol solution and submerged them in tap water for at least 24 h. Before dissection, we measured the width of head capsules of the invertebrates that had one using a microscope reticle. We also noted the sex of the individual when possible and looked for any sign of external damage, e.g., a hole in the posterior end, indicating that a hairworm had egressed prior to collection. Afterwards, invertebrates were carefully opened up using fine tweezers and a scalpel or a pair of spring scissors with a 10-mm cutting edge. We started with the abdomen, since hairworms typically develop in that part of the host. If a worm was present and it was intact, we removed it to measure its length to the nearest millimetre by straightening it with tweezers over a ruler without stretching it, and its width at mid-length using the microscope reticle. Regardless of taxon, all invertebrates captured in the pitfall traps were dissected to look for hairworms.

All worms were initially assigned to a genus based on their external morphology and by comparing them to species previously reported in the streams [23,27,38]. However, some individuals could not be identified by morphology alone, i.e., immature hairworms or mermithid nematodes. Therefore, we cut a small section (around 5 mm) from every worm to extract DNA using DNeasy® Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Then, we ran polymerase chain reactions (PCR) to amplify DNA using the nematode primers Nem18SF and Nem18SR targeting partial 18S ribosomal RNA, following the PCR conditions from Wood, Wilmshurst, Rawlence, Bonner, Worthy, Kinsella and Cooper [39], and two sets of New Zealand hairworm primers from Tobias, Yadav, Schmidt-Rhaesa and Poulin [27]: NZHW_CO1_F and NZHW_CO1_R targeting the mitochondrial CO1 gene; HW_Grp5_ITS_F and HW_Grp5_ITS_R targeting a partial region of ribosomal RNA. These two pairs of primers were used under the following PCR conditions: initial denaturing step at 94 °C for 4 min, followed by 40 cycles of 94 °C for 30 s, 48 °C for 30 s, and 72 °C for 60 s, and a final extension at 72 °C for 5 min. The PCR product was then visualised via gel electrophoresis on a 1.5% agarose gel. Amplified DNA was sequenced with Sanger sequencing provided by the University of Otago Genetic Analysis Services. These sequences were then matched with ones uploaded to NCBI using BLAST, which allowed us to confirm the species (or family) of each worm.

2.3. Data analysis

All statistical analyses were performed in R version 4.1.0 [40]. Based on the arthropods captured per side per trap, we tested whether the probability of adult hairworm infection was higher in individuals that walked towards the stream versus those that walked away from it. We also tested whether this probability of infection differed between traps placed at increasing distances from the stream. Due to the small number of hairworms present in all of our samples (see Section 3.1), we decided to use Bayesian multilevel modelling with the *brms* package [41,42]. Since infection status for an individual is strictly discrete with only two possible outcomes (infected or uninfected), we implemented a Bernoulli distribution into the models to account for this. Models were built with priors obtained from the "get_prior" function; the "adapt_delta" function was increased to 0.99 to lower the number of divergent transitions after warmup. Stacking weights were computed with the *loo* package [43] to select the model that best fitted the posterior distribution. The main predictors tested were the walking direction of individuals (two levels; towards or away from the stream, reference level = away) and the distance of the trap (three levels; 5, 25, and 45 m, reference level = 5 m). We also included as a random factor the family to which each individual arthropod belonged, to account for potential stochastic effects brought by the phylogeny of host taxa. Sampling sites, dates, and transects were all pooled together for this analysis, due to the very low number of adult hairworms collected in hosts (see Section 3.1).

3. Results

3.1. General results

A total of 1969 invertebrates were captured in the pitfall traps and were thus dissected to look for hairworms (1163 from Site A and 806 from Site B); no vertebrate was captured. Out of the 41 taxa identified (including morphospecies and higher taxonomic groupings, see Section 2.2), 24 were present in both sites. Notably, a few species of carabid and a species of dermapteran were captured only at Site A. In contrast, some coleopteran species of scarabaeid and scirtid were captured only in Site B. The total number of invertebrates captured per taxon per site varied across sampling dates (Tables A.1 and A.2). From these, 21 worms were found inside 17 invertebrates (15 single infections, one double infection, and one quadruple infection). The BLAST results confirmed that 12 were hairworms and three were mermithid nematodes, of which the latter were found in spiders, i.e., one species of Lycosidae and a morphospecies of Amaurobiidae; six worms could not be identified with the DNA sequences obtained from PCRs (Table 1). For hairworms, eight mature individuals were found either inside the abdominal cavity of their host or egressing from them (Fig. 2) (six from Site A and two from Site B). Apart from these, four worms were identified as immature hairworms that had not yet produced their adult cuticle (Table 1). Immature hairworms were not included in the statistical analysis, since they cannot leave their host until they mature. From the eight arthropod hosts harbouring mature hairworms, four were caught walking towards the stream and four were caught walking away from it, indicating that there was no trend in the direction of host movement. Four species of hairworm were identified (confirmed with BLAST results) and were found in four insect families across four orders and two families of spider (Table 1). The prevalence of each species per host taxa per site varied between 0.4% and 33.3% (the latter estimate was based only on three captured insects).

3.2. Statistical results

The taxonomic composition of insect host taxa walking either towards the stream or away from it, in interaction with the distance of pitfall traps relative to the stream, was somewhat consistent across sampling dates in both sites, except for the traps placed at 5 m in Site B, which captured fewer invertebrates in total (Fig. 3). According to our selection criteria for Bayesian multilevel modelling, the best model was the null model (stacking weight = 1.000); none of the factors tested here added any predictive value to the null model. Therefore, no effect sizes were observed in both direction of movement and distance from the stream.

4. Discussion

Studying the effects of host manipulation in a natural context can

Table 1
Hairworms (phylum Nematomorpha) and mermithids (phylum Nematoda) found within various invertebrates caught in pitfall traps in two subalpine locations in Otago, New Zealand. Where worm identification was possible, the prevalence was calculated per host species per site.

Hairworm	Site	Identification	Age	Length (mm)	Width (mm)	Host species (family)	Prevalence (total captured)
1	A	*Gordionus maori*	adult	113	0.20	*Celatoblatta quinquemaculata* (Blattidae)	4.8% (21)
2	A	*G. maori*	adult	104	0.13	*Megadromus* sp. (Carabidae)	3.7% (27)
3	A	*G. maori*	adult	57	0.20	Labiidae morphospecies	33.3% (3)
4	A	*Gordius paranensis*	adult	78	0.33	*Holcaspis* sp. (Carabidae)	1.4% (72)
5	A	*G. paranensis*	adult	192	0.27	*Mecodema* sp. (Carabidae)	2.7% (37)
6	A	*G. paranensis*	adult	331	0.20	*Megadromus* sp. (Carabidae)	7.4% (27)
7	A	*G. paranensis*	juvenile	NA	0.20	*Megadromus* sp. (Carabidae)	7.4% (27)
8	A	*G. paranensis*	juvenile	NA	0.07	*Anoteropsis* sp. 1 (Lycosidae)	0.4% (229)
9	B	*Euchordodes nigromaculatus*	adult	92	0.13	*C. quinquemaculata* (Blattidae)	0.6% (172)
10	B	*Parachordodes diblastus*	adult	300	0.40	*Hemiandrus* sp. (Anostostomatidae)	2.9% (34)
11	B	*P. diblastus*	juvenile	NA	NA	*Mecodema* sp. (Carabidae)	1.7% (58)
12	B	Nematomorpha sp.	juvenile	45	0.13	Amaurobiidae morphospecies 2[†]	2.6% (39)
Mermithid	Site	Identification	Age	Length (mm)	Width (mm)	Host species (family)	Prevalence (total captured)
1	B	Mermithidae sp. 1	NA	36	0.20	*Anoteropsis* sp. 1 (Lycosidae)[**]	1.1% (175)
2	B	Mermithidae sp. 1	NA	32	0.13	*Anoteropsis* sp. 1 (Lycosidae)[**]	1.1% (175)
3	B	Mermithidae sp. 2	NA	71	0.20	Amaurobiidae morphospecies 1[†]	3.3% (30)
Unidentified worms	Site	Identification	Age	Length (mm)	Width (mm)	Host species (family)	
1	A	Unknown	NA	204	0.20	*Mecodema* sp. (Carabidae)	
2	A	Unknown	NA	NA	NA	*Anoteropsis* sp. 1 (Lycosidae)[*]	
3	A	Unknown	NA	NA	NA	*Anoteropsis* sp. 1 (Lycosidae)[*]	
4	A	Unknown	NA	NA	NA	*Anoteropsis* sp. 1 (Lycosidae)[*]	
5	A	Unknown	NA	NA	NA	*Anoteropsis* sp. 1 (Lycosidae)[*]	
6	A	Unknown	NA	NA	0.07	*Anoteropsis* sp. 1 (Lycosidae)	

[*] Same individual host.
[**] Same individual host.
[†] These morphospecies were impossible to differentiate morphologically from species of the family Desidae.

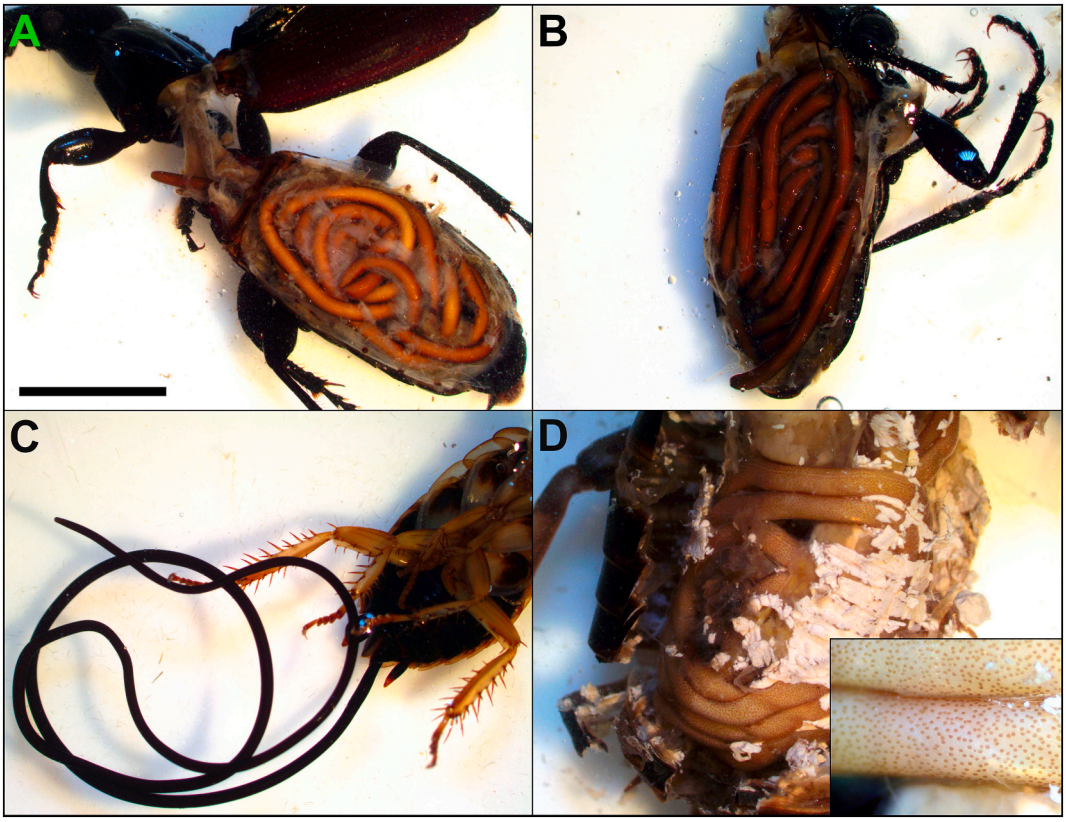

Fig. 2. Mature hairworms found inside terrestrial arthropods. Hairworms (phylum Nematomorpha) found within various terrestrial insect hosts captured in pitfall traps in two subalpine locations in Otago, New Zealand. (A) *Gordius paranensis* found inside *Mecodema* sp. (Coleoptera: Carabidae). (B) *G. paranensis* found inside *Megadromus* sp. (Coleoptera: Carabidae). (C) *Euchordodes nigromaculatus* found egressing from *Celatoblatta quinquemaculata* (Blattodea: Blattidae). (D) *Parachordodes diblastus* found inside *Hemiandrus* sp. (Orthoptera: Anostostomatidae); the bottom right panel shows a closeup of the hairworm cuticle, which highlights the dark superareoles characteristic of this genus.

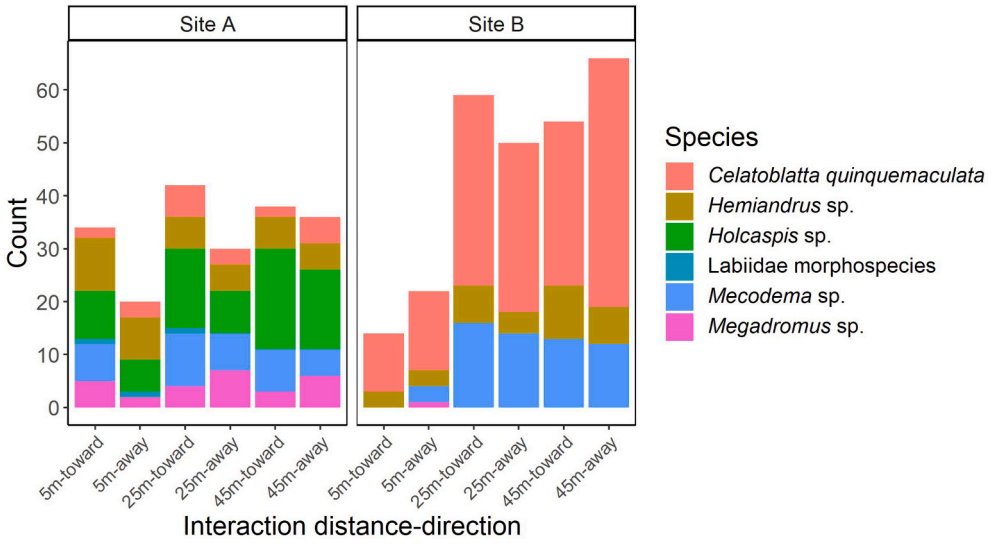

Fig. 3. Infected host taxa captured in pitfall traps. Stacked bar graphs of insects captured in pitfall traps in two sampling locations in Otago, New Zealand, with transects and sampling periods pooled together. The "distance" indicates how far the trap was from the stream, whereas "direction" indicates in which direction the insect was walking (towards or away from the stream). Only insect taxa that had at least one individual infected with a hairworm (phylum Nematomorpha) are shown here.

help understand the true impact that parasites have on their hosts [7]. The primary goal of the current study was to investigate the infection dynamics of gordiid hairworms in their definitive terrestrial hosts across two communities of subalpine invertebrates in New Zealand. Previous studies suggested that hairworms induce erratic movements in their host [16–18], but no direct tests of this hypothesis have been performed to date. Although we discovered multiple new hairworm-host associations in both sampling sites, it was not possible to observe whether mature hairworms affected host behaviour to increase their likelihood of entering water or not. This was due to the low number of infected insects captured throughout the sampling season, which can be explained by the overall low prevalence of hairworms observed here. In order to test such host behavioural patterns, we would need a sample size of mature hairworms with enough statistical power to properly quantify the odds that infected insects either approach streams in a directed movement towards water, or simply move around erratically in their environment, which ultimately increases their chances of encountering a stream and falling into it. Ideally, to remove interspecific differences of host manipulation between hairworms, this type of study should only be done for one species of hairworm. Also, the spatial grid of pitfall traps used here was only active for seven days every month, due to restrictions of the sampling permit. Perhaps if traps were kept open throughout the sampling period and containers were replaced regularly, we would have captured enough infected individuals to properly test our hypotheses in a natural setting.

The four hairworm species collected at our sampling sites have all been previously reported from these locations [23,27]. Here, the six insect species and two spider species infected with hairworms represent new host records in New Zealand. *Euchordodes nigromaculatus*, considered an endemic species of hairworm [26], has only been reported in three species of acridid and anostostomatid orthopterans that are known to inhabit subalpine habitats [35,44]. Therefore, their occurrence in the alpine cockroach *Celatoblatta quinquemaculata*, which is common at higher altitudes in the Otago region [45], is unsurprising. Two species of Blattodea have previously been reported as hosts of unidentified hairworms in New Zealand [46] and may represent an important group for these parasites [47]. Although the acridid *Sigaus australis* is a known host species for *E. nigromaculatus* [44], none were found infected here. The endemic hairworm *Parachordodes diblastus* has only been observed in three wētā hosts, two species in the genus *Hemideina* and one *Hemiandrus* [26], therefore their presence in an undescribed species of the latter genus observed here is unsurprising. However, the ground beetle *Mecodema* sp. was identified as a new host order for *P. diblastus*.

No host record exists for the recently described and endemic *Gordionus maori* [23], so the fact that we found this species across three orders of insects, including the new host order Dermaptera for New Zealand, may signify that they have a relatively low host specificity. *Gordius paranensis*, which has also been reported in South America [48], was the most numerous species collected in this study. Most individuals were found within ground beetles, which is not uncommon for this genus [49]. However, one juvenile was also found within a species of *Anoteropsis*, a type of wolf spider. Schmidt-Rhaesa [25] collated records of hairworms in spiders, but still, few exist and doubts have been raised about their veracity [50]. The prevalence of *G. paranensis* was 0.4% in this species of arachnid and the hairworm was identified as a juvenile, therefore this may be a case of spurious infection in a suboptimal host. Another unidentified hairworm was found inside a morphospecies of spider in the family Amaurobiidae (or Desidae); this parasite was also a juvenile.

Two species of mermithid, confirmed with 18S sequences, were found within two families of spiders. There are some reports of mermithids in New Zealand (e.g., [51,52]), and these arachnid hosts represent new records [53]. Mermithids have been found in spiders in other parts of the world [54,55], which makes these new host-parasite associations unsurprising. With their potential negative impacts on invertebrate populations [56], there is a need to better describe the diversity of mermithids in New Zealand. For instance, mermithids have been found in the Cromwell chafer beetle (Bronwen Presswell, personal communication), a critically endangered species that is limited to a small reserve in Central Otago. Therefore, characterising the diversity and host specificity of these parasites could help better assess the risk of infection in endangered populations.

5. Conclusion

In this study, we reported new host-parasite associations for freshwater hairworms and mermithid nematodes, two parasite taxa that typically develop within terrestrial invertebrates. New Zealand hairworms do not appear to be very host-specific, infecting insects across multiple orders. This appears to be a general trait for this phylum [25]. Although it was not possible to confirm here, how hosts behave in the wild ultimately increases their odds of entering water, which is a crucial step in the life cycle of these parasites. For mermithids, we reported new host records, highlighting the need for more research to better understand their distribution in New Zealand. Because of the unique diversity of animals in New Zealand and its varied topography, there is indeed the potential for further host-parasite associations of hairworms and mermithids to be discovered.

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Authors' contributions

JFD, AF, and RP conceived and designed the study; JFD and AF collected the samples; JFD processed the samples and compiled the data; JFD and AF analysed the data; JFD wrote the paper, with critical input from AF and RP. All authors gave final approval for publication.

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Appendix A

Table A.1
Invertebrates caught in the pitfall traps per sampling period for Site A. The distance and direction of traps, as well as all three transects, are pooled for each period. Note that each date corresponds to the end of the seven-day trapping period.

Class	Order	Family	Genus or species	2020-11-05	2020-12-05	2021-01-10	2021-02-06	2021-03-06
Arachnida	Araneae	Amaurobiidae[1]	Morphospecies 1	1	2	3	10	6
			Morphospecies 2	12	55	5	43	6
		Gnaphosidae	NA	4	5	–	–	–
		Lycosidae	*Anoteropsis* sp. 1	64	64	15	62	24
			Anoteropsis sp. 2	5	7	–	–	–
		NA	spp.	–	–	1	–	–
		Salticidae	Morphospecies 1	–	–	–	1	–
		Stiphidiidae	Morphospecies 1	–	–	–	1	1
	NA	NA	spp.	–	–	1	6	–
	Opiliones	NA	spp.	–	–	2	1	–
Chilopoda	NA	NA	spp.	1	1	1	–	1
Clitellata	Haplotaxida	Lumbricidae	spp.	1	–	5	–	–
Diplopoda	NA	NA	spp.	4	3	–	1	–
Insecta	Blattodea	Blattidae	*Celatoblatta quinquemaculata*	3	6	3	7	2
	Coleoptera	Carabidae	*Cicindela* sp.	1	–	–	–	–
			Holcaspis sp.	33	14	12	8	5
			Mecodema sp.	18	2	8	5	4
			Megadromus sp.	14	3	4	2	4
			Morphospecies 1	–	1	–	–	–
			Notagonum sp.	1	2	–	1	–
		Chrysomelidae	spp.	–	–	–	1	–
		Coccinellidae	spp.	3	3	–	–	–
		Curculionidae	spp.	49	72	64	47	7
		Elateridae	Morphospecies 1	4	–	1	–	–
		NA	spp.	–	–	1	–	–
	Dermaptera	Labiidae	Morphospecies 1	–	2	1	–	–
	Diptera	NA	spp.	–	8	2	3	–
	Hemiptera	NA	spp.	1	2	1	1	–
	Hymenoptera	NA	spp.	–	–	1	4	–
	Lepidoptera	NA	spp.	–	1	–	1	–
	Orthoptera	Acrididae	*Sigaus australis*	34	21	2	128	58
		Anostostomatidae	*Hemiandrus* sp.	6	3	3	7	21
Malacostraca	Amphipoda	Talitridae	Morphospecies 1	2	1	4	1	4

[1] Species in this family are impossible to differentiate morphologically from species of the family Desidae.

Table A.2
Invertebrates caught in the pitfall traps per sampling period for Site B. The distance and direction of traps, as well as all three transects, are pooled for each period. Note that each date corresponds to the end of the seven-day trapping period.

Class	Order	Family	Genus or species	2020-11-06	2020-12-06	2021-01-11	2021-02-07	2021-03-07
Arachnida	Araneae	Amaurobiidae[1]	Morphospecies 1	8	3	6	9	4
			Morphospecies 2	4	4	4	19	8
			Allotrochosina schauinslandi	1	–	–	–	–
		Lycosidae	*Anoteropsis* sp. 1	79	27	12	31	26
			Anoteropsis sp. 2	6	1	–	–	–
		NA	spp.	1	2	–	18	–
		Stiphidiidae	Morphospecies 1	–	–	–	3	4
	NA	NA	spp.	–	–	–	1	–
	Opiliones	NA	spp.	3	4	20	6	5
Chilopoda	NA	NA	spp.	2	2	–	1	3
Clitellata	Haplotaxida	Lumbricidae	spp.	–	–	–	1	–
Diplopoda	NA	NA	spp.	–	–	1	–	–
Entognatha	Entomobryomorpha	NA	Morphospecies 1	–	1	–	–	1

(*continued on next page*)

Table A.2 (*continued*)

Class	Order	Family	Genus or species	2020-11-06	2020-12-06	2021-01-11	2021-02-07	2021-03-07
	Blattodea	Blattidae	*Celatoblatta quinquemaculata*	13	18	57	61	23
			Mecodema sp.	32	4	12	6	4
		Carabidae	*Megadromus* sp.	–	–	–	–	1
			Notagonum sp.	8	4	–	2	1
		Chrysomelidae	spp.	1	3	2	1	–
	Coleoptera	Curculionidae	spp.	6	3	4	3	–
		NA	spp.	1	–	2	–	–
		Scarabaeidae	*Aphodius* sp.	–	1	–	–	–
			Scythrodes sp.	3	1	2	1	1
Insecta		Scirtidae	spp.	1	–	5	1	–
	Diptera	NA	spp.	–	40	33	42	7
		Tipulidae	spp.	–	–	2	–	1
	Hemiptera	NA	spp.	1	–	2	1	–
	Hymenoptera	NA	spp.	–	–	2	5	1
	Lepidoptera	NA	spp.	–	–	5	4	1
	Neuroptera	NA	Morphospecies 1	–	–	1	–	–
	Orthoptera	Acrididae	*Sigaus australis*	–	–	–	1	2
		Anostostomatidae	*Hemiandrus* sp.	2	2	19	5	6
	Plecoptera	NA	spp.	–	1	–	1	–

[1] Species in this family are impossible to differentiate morphologically from species of the family Desidae.

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