Nematodes and trematodes associated with terrestrial gastropods in Nottingham, England.

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Abstract

A parasitological survey of terrestrial slugs and snails was conducted at popular dog walking locations across the city of Nottingham, with the intensions of finding gastropods infected with medically (or veterinary) important parasites such as lungworm (metastrongyloid nematodes) and trematodes. A total of 800 gastropods were collected from 16 sites over a 225Km^2 area. The extracted nematodes and trematodes were identified by molecular barcoding. Of the 800 gastropods collected, 227 were infected (172 had nematode infections, 37 had trematode infections and 18 had both nematode and trematode infections). Of the nematode infected gastropods sequenced ‘genotyped’ might be a better word, ‘sequenced’ suggests whole genome, seven species were identified, *Agfa flexilis*, *Angiostoma gandavensis*, *Angiostoma margaretae*, *Cosmocerca longicauda*, *Phasmarhabditis hermaphrodita*, *Phasmarhabditis neopapillosa* and an unknown Cosmocercidae species. Of the trematode infected gastropods sequenced, four species were identified, *Brachylaima arcuate*, *Brachylaima fuscata*, *Brachylaima mesostoma* and an unknown Plagiorchioidea species. No lungworm species were found within the city of Nottingham. Another concluding sentence would be good.

Keywords: Lungworms; Nematodes; Trematodes; Parasitology; Gastropods.

Introduction:

Slugs and snails (Class: Gastropoda) comprise approximately 35,000 extant species and can host a diverse range of metazoan parasites (and parasitoids) such as cestodes, trematodes, nematodes, insects and acarids (Barker et al., 2004; Chapman, 2009). There are approximately 25,000 extant species of nematode, 3,500 of which are parasites of invertebrates (Grewal et al., 2003). Of these, 50 metastrongyloid species are medically (or veterinary) important, with notable genera being *Aelurostrongylus*, *Angiostrongylus*, *Crenosoma*, *Elaphostrongylus*, *Muellerius*, *Neostrongylus*, *Oslerus*, *Prostrongylus* and *Troglostrongylus* (Alicata, 1965; Skorping et al., 1980; Campbell et al., 1988; Diez-Baños et al., 1989; Schjetlein et al., 1995; Majoros et al., 2010; Panayotova-Pencheva, 2011; Kim et al., 2014; Patel et al., 2014; Conboy, 2015; Helm et al., 2015; Aziz et al., 2016; Hadi, 2018; Hicklenton et al., 2019; Penagos-Tabares et al., 2020). Nematodes have evolved diverse relationships with gastropods, with some species using them as an intermediate host (e.g. juveniles of lungworm species) while others (Rhabditidae, Mermithidae and Ascarididae) parasitise gastropods and use them as their definitive host; or for other means such as necromeny or transportation (paratenic) (Grewal et al., 2003; Ivanova et al., 2019).

Digenetic trematodes comprise approximately 40,000 extant species, with more than 18,000 described species (Cribb et al., 2001; Kostadinova et al., 2014). Unlike nematodes, digenetic trematodes use invertebrates exclusively as an intermediate host, with a vertebrate (typically a fish, mammal, or bird) being used as their definitive host (Barker, 2004). Notable genera of medical (or veterinary) importance are *Clonorchis*, *Fasciola*, *Fasciolopsis*, *Gastrodiscoides*, *Heterophyes*, *Metagonimus*, *Opisthorchis*, *Paragonimus* and *Schistosoma* (Doughty, 1996; Kostadinova et al., 2014). Trematode species which infect terrestrial gastropods use them in order to infect bird, mammal, or reptile definitive hosts which prey on gastropods (Morley et al., 2008). Most species specialise in infecting one type of definitive host, but some species can infect multiple (Butcher et al., 2005). The lifecycle of these trematodes first involves a gastropod host being infected through the ingestion of faeces contaminated with eggs (excreted by an infected definitive host). After ingestion, it takes one to three months for asexual sporocysts to produce cercariae within the first intermediate gastropod host (Butcher et al., 2003). Gastropods can act as both the first and second intermediate host, as infected snails (first intermediate) shed cercariae in their mucus which can infect other gastropods through bodily contact (or themselves making it a first and second intermediate simultaneously) (Butcher et al., 2005). The successful cercariae develop into mature metacercariae after 4 months and can survive up to another 4 months within the gastropod host. The transmission cycle is completed when the secondary intermediate gastropod host is ingested by a bird, mammal, or reptile definitive host (Morley et al., 2008).

The current understanding of nematodes and trematodes associated with terrestrial gastropods in Europe is based on parasitological surveys conducted in Austria (Penagos-Tabares et al., 2020), Bulgaria and Crimea (Ivanova et al., 2013), The Czech Republic (Heneberg et al., 2016), Denmark (Taubert et al., 2009), France and Germany (Ross et al., 2016; Lange et al., 2018; Gérard et al., 2020), Hungary (Majoros et al., 2010), the Netherlands, Norway and Poland (Filipiak et al., 2020), Sicily (Ivanova et al., 2019), Slovenia (Laznik et al., 2010), Spain (Jefferies et al., 2010) and the United Kingdom (Grewal et al., 2003; Morley et al., 2008; Ross et al., 2010a; Ross et al., 2010b; Patel et al., 2014; Helm et al., 2015; Aziz et al., 2016; Hicklenton et al., 2019).There are more papers you could cite e.g. Belgium (Singh et al. 2019; doi: 10.1017/S0022149X19000105) and this one looked at nematodes and trematodes in Norway • DOI: 10.1016/j.jip.2020.107372 In these studies, there were no medically important nematode or trematode species in terrestrial gastropods in Europe, with only species of veterinary importance and free-living species being reported. Of the nematodes found, lungworm genera such as *Angiostrongylus*, *Crenosoma, Aelurostrongylus* and *Troglostrongylus* are commonly found throughout Europe (references), though the medically important lungworm species *Angiostrongylus cantonensis* was absent. The are seven families of nematodes that have no medical (or veterinary) relevance including Agfidae, Alloionematidae, Angiostomatidae, Cosmocercidae, Diplogasteridae, Mermithidae and Rhabditidae. The most common genera of trematodes found are *Brachylaima*, *Eurytrema*, *Michajlovia*, *Urogonimus* and *Urotocus*. Certain species of *Brachylaima* (Brachylaimiasis) and *Eurytrema* (Eurytrematosis) have been found to cause infection within humans in Australia and Brazil, respectively (Schwertz et al., 2015; Gracenea et al., 2017) though there have as yet been no reports of human infection in Europe. Trematodes associated with terrestrial gastropods in Europe have not been as well studied as nematodes, most probably due to the majority of medically (or veterinary) important species being associated with aquatic snail species.

Lungworm nematode infections have been extensively studied in Europe (Taubert et al., 2009; Patel et al., 2014; Helm et al., 2015; Taylor, 2015; Aziz, 2016; Helm et al., 2017; Lange et al., 2018; Elsheikha et al., 2019; Hicklenton et al., 2019; Fuehrer et al., 2020; Penagos-Tabares et al., 2020). Lungworm infections are fatal to companion animals due to the severe respiratory disease and bleeding disorders caused by the parasite (Taubert et al., 2009). *Angiostrongylus* (*An.*) *vasorum* and *Crenosoma vulpis* are widespread across the United Kingdom, with domesticated dogs and red foxes (*Vulpes vulpes*) acting as their definitive hosts (Helm et al., 2017). Geography is one of the main risk factors for *An. vasorum* infections in dogs, with the most endemic areas of the UK being Southern England and Southern Wales (Patel et al., 2014; Helm et al., 2017; Hicklenton et al., 2019) though

An. vasorum in the UK is spreading northwards, with the parasite already being established in Northern England and Scotland (Aziz et al., 2016; Helm et al., 2015). Reasons for the spread of *An. vasorum* are due to a warmer climate which favours the parasites development and the urbanisation of wild red fox populations acting as a reservoir of infection, with an estimated one in five infected (Helm et al., 2017; Taylor et al., 2015). *Crenosoma vulpis* transmission is the same as *An. vasorum* but is more commonly report in wild canid species than domesticated dogs (Lange et al., 2018). Similarly, *Aelurostrongylus* (*Ae.*) *abstrusus* is globally distributed lungworm species that infects wild and domesticated cat species, with a prevalence of 1.7% in UK house cats (Helm et al., 2017; Elsheikha et al., 2019). Lungworm infections in domesticated cats and dogs are thought to be underreported as some infections can be asymptomatic and milder cases are commonly misdiagnosed to as other disorders like hypersensitivity (Wright, 2009; Penagos-Tabares et al., 2018; Pohly et al., 2022).

The primary aim of this study was to investigate which species of terrestrial gastropods are commonly found at dog walking sites in the city of Nottingham, to determine which nematode and trematode species are associated with these gastropods and to determine infection rates. The secondary aim was to investigate whether lungworm nematode species that cause veterinary disease are found at popular dog walking sites across the city of Nottingham.

Intro is good, loads of info, well written.

Materials and Methods – check whether Journal of Helminthology numbers titles and sub-titles in the guide for authours.

Collection sites and gastropod identification

Slugs and snails were collected from 16 sites across Nottingham from June to November 2020 and June to November 2021. All sites were popular dog walking locations and included recreational grounds, country parks, public gardens, and nature reserves (Figure 1; Table 1). I would state how many slugs and snails you collected here Slugs and snails were collected by hand with 50 specimens collected from each site and with a maximum of ten individuals per species being taken. Specimens were identified morphologically using a Terrestrial Mollusc Key (https://idtools.org/id/mollusc/key.php) (White-McLean, 2011) and the 'Slugs of Britain and Ireland' as an illustrated guide (Rowson et al., 2014).

Figure 1. Map of collection sites (n=16) across the city of Nottingham.

A minor pedantic point which I'm sure you are aware of is tables and figures are not included in the submitted file to a journal and are saved as tiff files for figs and excel files for tables.

Table 1. Collection sites surveyed. I'm not sure what 'search area' brings to the study? You didn't actually walk around e.g. 116,987 km2 so is it worth mentioning?

	Collection site	Code	Search area (Km^2)	Coordinates
1	Basford	BAS	15,288	52.977957, -1.180909
2	Bestwood Country Park	MILL	116,987	53.025337, -1.184712
3	Forest Fields	FOR	5,132	52.96401, -1.159410
4	University Park Campus	UNI	20,506	52.938199, -1.12508
5	Beeston	BEE	1,583	52.922972, -1.214944
6	Toton	TOT	6,469	52.915726, -1.264259
7	Attenborough Nature Reserve	ATEN	33,371	52.909117, -1.221000
8	Kimberley	KIM	5,095	52.997686, -1.268583
9	Clifton South	C-SOU	11,135	52.899179, -1.185660
10	Iremongers Pond	POND	17,958	52.936184, -1.152757
11	Woodthorpe Grange Park	GRAN	143, 670	52.982888, -1.135721
12	Arnot Hill Park	ARNOT	45,220	52.997488, -1.133526
13	Edwalton	EDW	8,181	52.917332, -1.124678
14	Gamston	GAM	24,538	52.928595, -1.108470
15	Carlton	CARL	37,525	52.965511, -1.103516
16	Colwick	COLW	15,920	52.952945, -1.091540

Gastropod Dissection:

Specimens were cryo-euthanised and dissected into four equal pieces within 24-hours of collection and placed into a 50ml falcon tube containing Ash’s digestion solution (0.7% pepsin in 0.5% HCl) for four to eight hours (Ash, 1970)- can this method be commonly used for non-lungworm nematodes? I would be concerned the solution would break apart the cuticle of adult nematodes…. The solution was then placed into 9cm Petri Petri was a person, please capitalisedish and examined under a dissection microscope for the presence of nematodes, or the metacercariae stage of trematodes. Nematodes were categorised as either juvenile or adult worms. When found, nematodes and metacercariae were individually? picked and placed into 0.2ml tubes containing 70% ethanol (adult worms were separated from juveniles) and stored at -20^{O}C. (Table 1).

DNA extraction, PCR amplification and Sequencing

DNA extractions were done on single nematodes or trematodes using a modified CTAB extraction method (Goodacre & Wade, 2001). Extracted samples were resuspended in 100µl of TRIS-HCl, pH 8.0 (10mM) buffer. A list of extracted and sequenced samples for each site can be found in Supplementary tables 1 and 2. Promega GoTaq® G2 Master Mix buffer was used for all PCR reactions: 1µl of DNA template was added to 24µl of 1X Master Mix buffer (1U TAQ, 0.2mM primers, 200µM dNTP, 1.5mM MgCl^2). The nematode DNA samples were identified using the region of the ribosomal RNA spanning the 18S-ITS1-5.8S-ITS2, which was amplified using the universal nematode primer set developed by Nadler et al. (2000) (N93: 5′-TTG AAC CGG GTA AAA GTC G-3′ and N94: 5′-TTA GTT TCT TTT CCT CCG CT-3′). The trematode DNA samples were identified using the 18S rRNA gene, which was amplified using the universal trematode primer set developed by Kim et al. (2019) (LPF: 5′-AGG GAA TGG GTG GAT TTA TT-3′ and LPR: 5′-AGA CAC GAC TGA AAG GTT GC-3′). The PCR conditions used were an initial 2 minutes at 95°C, followed by 35 cycles of 30 seconds at 95°C, 30 secs at 50°C and 2 mins at 72°C, and finally 10 mins at 72°C. PCR products were run and visualised on an ethidium bromide infused 1.5% agarose gel. PCR products were purified and sequenced using Macrogen’s Eco-Seq service. Problematic sequences were re-amplified and sequenced using a higher annealing temperature of 60°C to try and eliminate fungal contaminates amplifying instead of the parasite DNA.

Parasite identification:

Parasite sequences were first grouped together based on similarity, with sequences that were 99% identical being placed together. Next, the NCBI ‘MOLE-BLAST Neighbor Search Tool’ was used to find the closest matching reference sequences on the GenBank database (Altschul et al., 1990; Benson et al., 2013). This tool creates an alignment and a neighbor-joining tree to show the relationship the query sequence has to the reference sequences in the GenBank non-redundant proteins database. Next, a secondary analysis was performed by placing our sequences within an alignment with all of the relevant closest matching GenBank reference sequences. This allowed us to create a maximum likelihood tree to see relationships between our sequences and the references taken from GenBank. The sequences were aligned in Seaview v5.0.5 (Gouy et al., 2021) using the Muscle algorithm, with conserved sites being selected using the Gblocks program (Castresana et al., 2000). The phylogenetic trees were constructed using the Maximum Likelihood method, using a General Time Reversible model incorporating gamma correction (GTR+Γ) in PhyML v3.1 (Guindon et al., 2010), with bootstrap analysis undertaken using 1000 replicates.

Results:

Infection rates:

Of the 800 gastropods collected, 581 were slugs (Agriolimacidae, Arionidae, Boettgerillidae, Limacidae and Milacidae) and 219 were snails (Discidae, Helicidae, Hygromiidae and Oxychilidae). The most common slug species found were *Deroceras invadens* (15%), *Tandonia budapestensis* (13%), *Deroceras reticulatum* (13%), *Arion hortensis* (10%), *Ambigolimax valentianus* (8%), *Limacus maculatus* (7%), *Arion vulgaris* (7%), *Tandonia sowerbyi* (6%), *Arion ater* (6%), *Arion subfuscus* (4%), *Arion rufus* (3%), *Arion silvaticus* (2%), *Limacus flavus* (2%), *Ambigolimax nyctelius* (1%), *Limax maximus* (1%), *Milax gagates* (<1%) and *Boettgerilla pallens* (<1%). The most common snail species found were *Cepaea nemoralis* (28%), *Cornu aspersum* (25%), *Cepaea hortensis* (20%), *Trochulus striolatus* (10%), *Oxychilus alliarius* (7%), *Monacha cantiana* (5%), *Discus rontundas* (3%), *Trochulus hispidus* (1%) and *Arianta arbustorum* (1%).

Overall, 227 specimens were infected (28%) with nematodes or trematodes (or both). Of those, 163 were slugs (28%) and 64 were snails (29%) (Table 2; Figure 2). The only gastropod species without any recorded infections were *A. arbustorum, B. pallens, D. rotundatus and T. hispidus*. Nematodes were found in all other gastropods, with *T. budapestensis*, *D. invadens*, *C. aspersum*, *D. reticulatum*, *A. ater* and *C. nemoralis* accounting for over half of all infections. A total of 533 nematodes were recorded from 190 infected specimens (145 slugs and 45 snails). Of those, only 12 juvenile nematodes were found in 12 hosts (8 slugs and 4 snails). Trematodes were rarer than nematodes, with *A. ater*, *A. hortensis*, *A. nyctelius*, *A. rufus*, *A. silvaticus*, *A. subfuscus*, *A. vulgaris*, *L. flavus*, *L. maximus* and *O. alliarius* having no recorded trematode infections. A total of 242 trematodes were recorded from 55 specimens (30 slugs and 25 snails). Lastly, co-infections of both nematodes and trematodes were even rarer, with only 18 specimens being recorded (13 slugs and 5 snails).

Table 2. Gastropod collection and infections of nematodes and trematodes (metacercariae).

Family	Species	No.	Infected	Nematode	Trematode	Both
Agriolimacidae	*Deroceras invadens*	90	25	15	8	2
	Deroceras reticulatum	75	19	13	3	3
Arionidae	*Arion ater*	33	13	13	0	0
	Arion hortensis	59	11	11	0	0
	Arion rufus	20	5	5	0	0
	Arion silvaticus	14	2	2	0	0
	Arion subfuscus	25	6	6	0	0
	Arion vulgaris	42	8	8	0	0
Boettgerillidae	*Boettgerilla pallens*	2	0	0	0	0
Discidae	*Discus rotundatus*	6	0	0	0	0
Helicidae	*Arianta arbustorum*	2	0	0	0	0
	Cepaea hortensis	44	7	6	1	0
	Cepaea nemoralis	62	14	9	4	1
	Cornu aspersum	54	24	14	7	3
Hygromiidae	*Trochulus hispidus*	3	0	0	0	0
	Trochulus striolatus	22	7	4	3	0
	Monacha cantiana	10	7	1	5	1
Limacidae	*Ambigolimax nyctelius*	5	1	1	0	0
	Ambigolimax valentianus	47	18	8	5	5
	Limacus flavus	10	3	3	0	0
	Limacus maculatus	42	9	8	0	1
	Limax maximus	3	2	2	0	0
Milacidae	*Milax gagates*	2	1	0	0	1
	Tandonia budapestensis	78	31	30	1	0
	Tandonia sowerbyi	34	8	7	0	1
Oxychilidae	*Oxychilus alliarius*	16	6	6	0	0
Total		800	227	172	37	18

Note: Gastropod species with zero infections are greyed out. 'Both' means a co-infection of nematodes and trematodes within a single specimen.

Of the 16 sites surveyed, infection was found at all of them (Table 3). The highest recorded rate of infection was 46% at site 7 (The Attenborough Nature Reserve) and site 13 (Edwalton). The lowest recorded rate of infection was 12% at site 5 (Beeston). Nematode infections were found at all 16 sites, with trematode infections only being found at 13 of the 16 sites (Figure 3). Specimens infected with both nematodes and trematodes were found at 9 of the 16 sites.

Table 3. Infection rate of collected gastropods (n=50) at each site.

	Collection site	Code	Infection rate	Nematode	Trematode
1	Basford	BAS	40%	40%	8%
2	Bestwood Country Park	MILL	16%	8%	8%
3	Forest Fields	FOR	28%	22%	8%
4	University Park Campus	UNI	16%	10%	8%
5	Beeston	BEE	12%	12%	0%
6	Toton	TOT	20%	20%	0%
7	Attenborough Nature Reserve	ATEN	46%	46%	0%
8	Kimberley	KIM	36%	32%	8%
9	Clifton South	C-SOU	28%	26%	2%
10	Iremongers Pond	POND	14%	12%	4%
11	Woodthorpe Grange Park	GRAN	22%	20%	2%
12	Arnot Hill Park	ARNOT	26%	24%	2%
13	Edwalton	EDW	46%	42%	6%
14	Gamston	GAM	40%	20%	28%
15	Carlton	CARL	30%	24%	12%
16	Colwick	COLW	34%	30%	8%

Figure 2. Map of collection sites (n=16) across the city of Nottingham showing infection rates at each collection site. White = uninfected, Grey = nematode infection, Dark grey = trematode infection and Black = nematode/trematode co-infection.

Nematode and trematode identifications:

A total of 35 (23 adults, 12 juveniles) nematodes (Supplementary table 1) and29 trematodes (Supplementary table 2) were 'sequenced' – suggests whole genome sequencing, 'genotyped' might be better . All sequences were grouped together based on similarity (>99%) and those groups were then matched with their closest GenBank references using the BLAST and MOLE-BLAST tool (ranked by lowest E-value). The nematode sequences fitted into seven groups, with all groups except group C2 having a GenBank reference match greater than 99% (Table 4). The trematode sequences fitted into four groups, with all groups except group F1 having a GenBank reference match greater than 99% (Table 4).

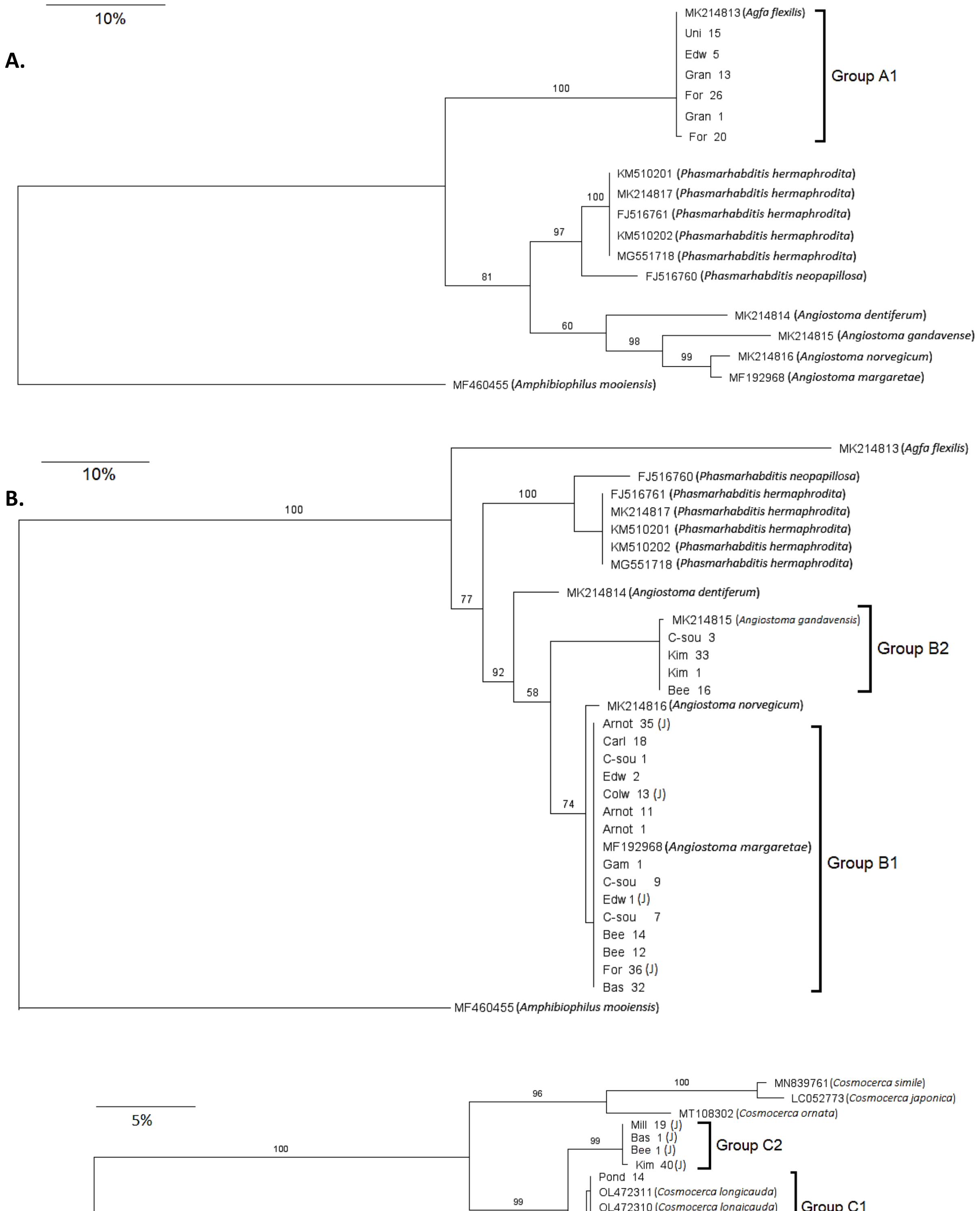
Table 4. BLAST-MOLE results (ranked by E-value) for grouped nematode (groups A-D) and trematode (groups E-F) sequences with their top five closest references.

Nematodes				
Group	Samples	Closest references	Reference name	% Match
A1	EDW 5 FOR 20 FOR 26 GRAN 1 GRAN 13 UNI 15	MK214813	*Agfa flexilis*	99.4
		FJ516760	*Phasmarhabditis neopapillosa*	87
		MF192968	*Angiostoma margaretae*	86
		FJ516761	*Phasmarhabditis hermaphrodita*	85
		MK214815	*Angiostoma gandavensis*	81
B1	ARNOT 1 ARNOT 11 ARNOT 35 (J) BAS 45 BEE 12 BEE 14 CARL 18 COLW 13 (J) C-SOU 1 C-SOU 7 C-SOU 9 EDW 1 (J) EDW 2 FOR 36 (J) GAM 1	MF192968	*Angiostoma margaretae*	99.4
		MK214816	*Angiostoma norvegicum*	92
		MK214815	*Angiostoma gandavensis*	87
		FJ516761	*Phasmarhabditis hermaphrodita*	83
		FJ516760	*Phasmarhabditis neopapillosa*	82
B2	BEE 16 C-SOU 3 KIM 1 KIM 33	MK214815	*Angiostoma gandavensis*	99.7
		MF192968	*Angiostoma margaretae*	86
		MK214816	*Angiostoma norvegicum*	88
		FJ516761	*Phasmarhabditis hermaphrodita*	84
		FJ516760	*Phasmarhabditis neopapillosa*	85
C1	POND 14	OL472311	*Cosmocerca longicauda*	99.9
		LC018444	*Cosmocercoides pulcher*	90
		MH178312	*Cosmocercoides qingtianensis*	90
		AB908161	*Cosmocercoides tonkinensis*	90
		MN839761	*Cosmocerca simile*	96
C2	BAS 1 (J) BEE 1 (J) KIM 40 (J) MILL 19 (J)	OL472311	*Cosmocerca longicauda*	90
		LC018444	*Cosmocercoides pulcher*	88
		MH178312	*Cosmocercoides qingtianensis*	88
		AB908161	*Cosmocercoides tonkinensis*	88
		MN839761	*Cosmocerca simile*	96

Trematodes				
Group	Samples	Closest references	Reference name	% Match
E1	BAS 11 FOR 23 GRAN 8 KIM 3 KIM 10 KIM 37 MILL 4a MILL 4b MILL 31 MILL 32 MILL 35 POND 5 POND 8 UNI 5	KT074950	*Brachylaima arcuata*	99.6%
		KT074955	*Brachylaima mesostoma*	98%
		KT074952	*Brachylaima fuscata*	97%
		AY222085	*Brachylaima thompsoni*	97%
		KP903630	*Urotocus rossitensis*	94%
E2	ARNOT 18 BAS 26 COLW 2 EDW 8 EDW 25 FOR 4 GAM 3 GAM 15 GAM 16 GAM 26 KIM 40	KT074952	*Brachylaima fuscata*	99.8
		AY222085	*Brachylaima thompsoni*	99.4
		KT074955	*Brachylaima mesostoma*	99.2
		KT074950	*Brachylaima arcuata*	98
		KP903638	*Michajlovia migrata*	96
E3	CARL 12 CARL 13 C-SOU 19	KT074955	*Brachylaima mesostoma*	100
		AY222085	*Brachylaima thompsoni*	99.6
		KT074952	*Brachylaima fuscata*	99.2
		KT074950	*Brachylaima arcuata*	99
		KP903638	*Michajlovia migrata*	96
F1	UNI 39	AY222156	*Telorchis assula*	97
		AY222160	*Brachycoelium salamandrae*	96
		AY222159	*Auridistomum chelydrae*	96
		JQ886404	*Mesocoelium lanfrediae*	96
		MZ787582	*Opisthioglyphe ranae*	96

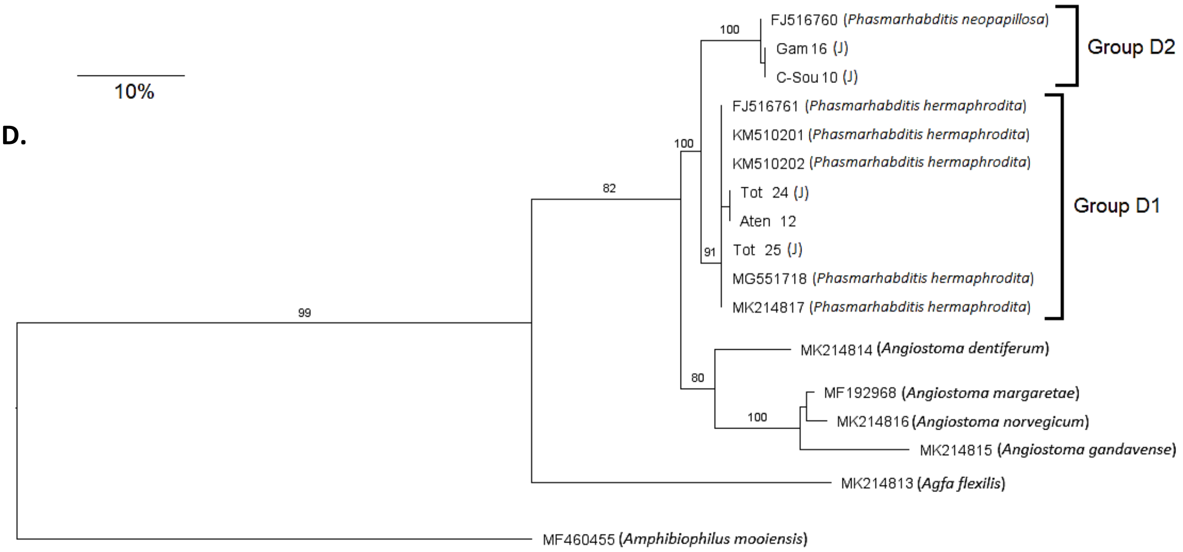
Note: (J) indicates it was a juvenile nematode. Each of the different designated grouping of ITS (nematode) and 18S (trematode) sequences are less than 1% different. Nematode and trematode groups with less than 99% GenBank reference match are coloured grey.

Next, maximum likelihood trees were created for the nematode and trematode sequences by placing each group together with a range of related GenBank references. All trees showed the majority of groups clustered with their closest GenBank reference (Figure 3). Only groups C2 and F1 were not identifiable at the species level. Group C2 was outside of the *Cosmocerca*/*Cosmocercoides* genera (Figure 3C) and group F1 was outside of the *Opisthioglyphe*/ *Macroderoides*/ *Brachycoelium*/ *Mesocoelium*/ *Auridistomum*/ *Telorchis* genera, respectively (Figure 3F).

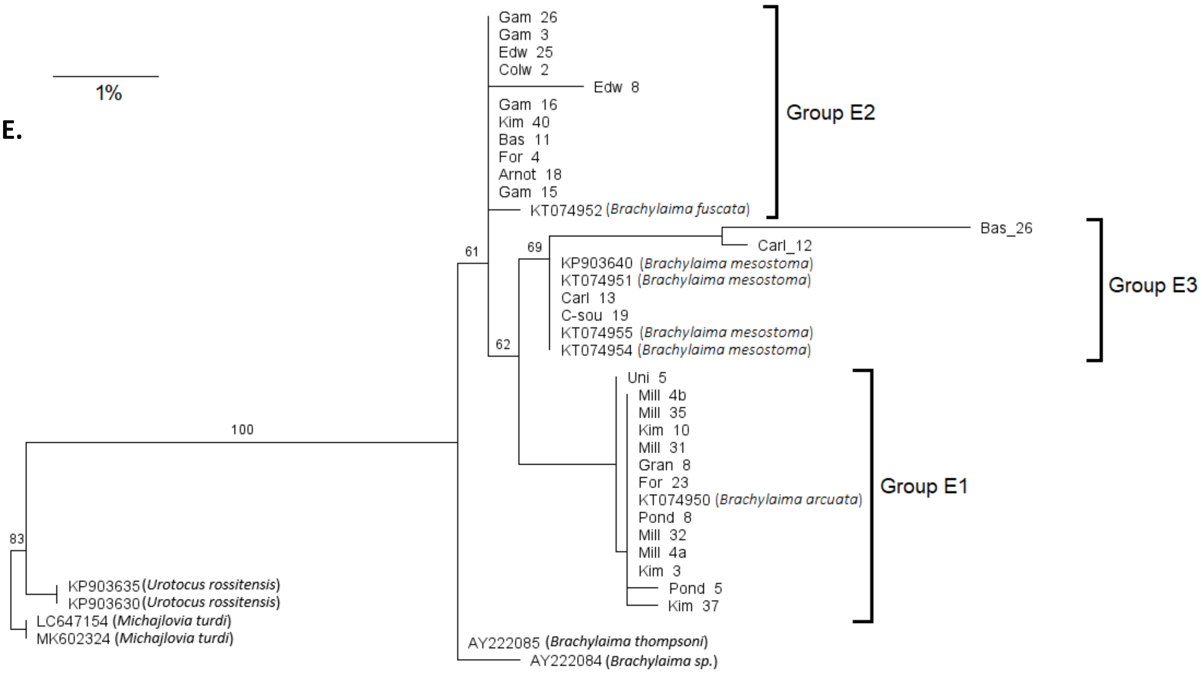


C.

D.


10%
100
FJ516760 (*Phasmarhabditis neopapillosa*)
Gam 16 (J)
C-Sou 10 (J)
Group D2
100
FJ516761 (*Phasmarhabditis hermaphrodita*)
KM510201 (*Phasmarhabditis hermaphrodita*)
KM510202 (*Phasmarhabditis hermaphrodita*)
82
Tot 24 (J)
Aten 12
Group D1
91
Tot 25 (J)
MG551718 (*Phasmarhabditis hermaphrodita*)
99
MK214817 (*Phasmarhabditis hermaphrodita*)
MK214814 (*Angiostoma dentiferum*)
80
MF192968 (*Angiostoma margaretae*)
100
MK214816 (*Angiostoma norvegicum*)
MK214815 (*Angiostoma gandavense*)
MK214813 (*Agfa flexilis*)
MF460455 (*Amphibiophilus mooiensis*)

E.


1%
Gam 26
Gam 3
Edw 25
Colw 2
Edw 8
Gam 16
Kim 40
Bas 11
For 4
Arnot 18
Gam 15
KT074952 (*Brachylaima fuscata*)
Group E2
Bas_26
Carl_12
69
61
KP903640 (*Brachylaima mesostoma*)
KT074951 (*Brachylaima mesostoma*)
Carl 13
C-sou 19
KT074955 (*Brachylaima mesostoma*)
KT074954 (*Brachylaima mesostoma*)
Group E3
62
Uni 5
Mill 4b
Mill 35
Kim 10
Mill 31
Gran 8
100
For 23
KT074950 (*Brachylaima arcuata*)
Group E1
Pond 8
Mill 32
Mill 4a
83
Kim 3
Pond 5
Kim 37
KP903635 (*Urotocus rossitensis*)
KP903630 (*Urotocus rossitensis*)
LC647154 (*Michajlovia turdi*)
MK602324 (*Michajlovia turdi*)
AY222085 (*Brachylaima thompsoni*)
AY222084 (*Brachylaima sp.*)

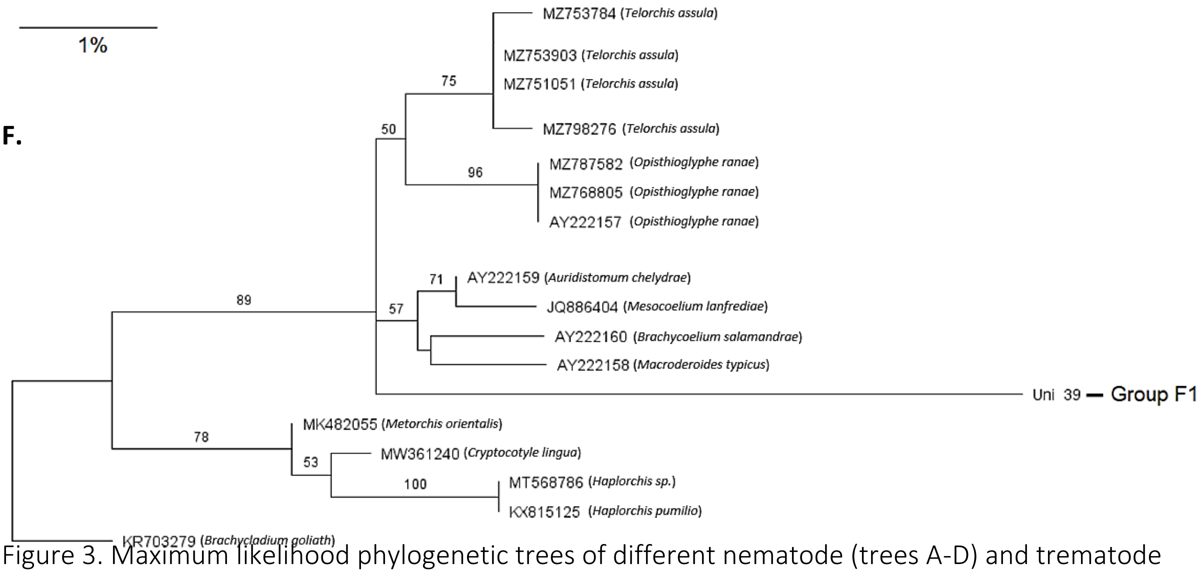


Figure 3. Maximum likelihood phylogenetic trees of different nematode (trees A-D) and trematode (trees E-F) species using the ITS and 18S rRNA gene, respectively. Tree A was created using 325bp of the ITS and is rooted on *Amphibiophilus mooiensis*. Tree B was created using 306bp of the ITS and is rooted on *Amphibiophilus mooiensis*. Tree C was created using 402bp of the ITS and is rooted on *Paraspidodera uncinate*. Tree D was created using 409bp of the ITS and is rooted on *Amphibiophilus mooiensis*. Tree E was created using 450bp of the 18S rRNA and is rooted on *Michajlovia turdi*. Tree F was created using 456bp of the 18S rRNA and is rooted on *Brachycladium goliath*. All trees were generated using PhyML v3.1, the numbers on the branches indicate the bootstrap percentages for 1000 replicates (bootstrap values under 50% are not shown). The scale bar represents percentage sequence divergence. Differing alignment lengths are due to the limited length of GenBank references.

Discussion:

Rate of infection:

The vast majority of gastropods collected and examined were slugs (73%), of which five families were represented (Agriolimacidae, Arionidae, Boettgerillidae, Limacidae and Milacidae). The remaining gastropods were snails, of which four families were represented (Discidae, Helicidae, Hygromiidae and Oxychilidae). The largest families represented were the Arionidae (24%), Agriolimacidae (20%), Helicidae (20%), Milacidae (16%), Limacidae (13%), Hygromiidae (4%), Oxychilidae (2%), Discidae (<1%) and Boettgerillidae (<1%). The overall rate of infections for the gastropods collected was 28%. Both slugs (28%) and snails (29%) had a similar rate of infection. No medically (or veterinary) important lungworm species were found within the city of Nottingham. However, of the 26 gastropod species found, 16 are potential hosts for *Angiostrongylus vasorum*, four are potential hosts for *Aelurostrongylus abstrusus* and four are potential hosts for *Crenosoma vulpis* (Table 5- I wouldn't mention tables or figures in the discussion).

This could be supplementary: Table 5. Gastropod species found at popular dog walking sites in the city of Nottingham and their relevance as intermediate hosts for different lungworm nematode species. Intermediate host status confirmed by Alicata, (1965); Skorping et al., (1980); Campbell et al., (1988); Diez-Baños et al., (1989); Schjetlein et al., (1995); Majoros et al., (2010); Panayotova-Pencheva, (2011); Patel et al., (2014); Helm et al., (2015); Conboy, (2015); Aziz et al., (2016); Hadi, (2018); Lange et al., (2018); Hicklenton et al., (2019); Fuehrer et al., (2020) and Penagos-Tabares et al., (2020).

Family	Species	Intermediate host?
Agriolimacidae	*Deroceras invadens* (Reise, Hutchinson, Schunack & Schlitt, 2011)	Yes [3]
	Deroceras reticulatum (Müller, 1774)	Yes [1, 2, 3, 4, 11]
Arionidae	*Arion ater* (Linnaeus, 1758)	Yes [3]
	Arion hortensis (Férussac, 1819)	Yes [3, 5]
	Arion rufus (Linnaeus, 1758)	Yes [3]
	Arion silvaticus (Lohmander, 1937)	Yes [5]
	Arion subfuscus (O.F. Müller, 1774)	Yes [3, 5]
	Arion vulgaris (Moquin-Tandon, 1855)	Yes [1, 3, 4, 10]
Boettgerillidae	*Boettgerilla pallens* (Simroth, 1912)	No
Discidae	*Discus rotundatus* (Müller, 1774)	Yes [3]
Helicidae	*Arianta arbustorum* (Linnaeus, 1758)	Yes [3, 5]
	Cepaea hortensis (O.F. Müller, 1774)	No
	Cepaea nemoralis (O.F. Müller, 1774)	Yes [3, 6, 7]
	Cornu aspersum (O.F. Müller, 1774)	Yes [1, 3, 4]
Hygromiidae	*Trochulus hispidus* (Linnaeus, 1758)	Yes [5]
	Trochulus striolatus (Pfeiffer, 1828)	No
	Monacha cantiana (Montagu, 1803)	Yes [5, 6, 9]
Limacidae	*Ambigolimax nyctelius* (Bourguignat, 1861)	No
	Ambigolimax valentianus (Férussac, 1821)	No
	Limacus flavus (Linnaeus, 1758)	Yes [2, 3]
	Limacus maculatus (Kaleni-czenko, 1851)	Yes [3]
	Limax maximus (Linnaeus, 1758)	Yes [1, 3, 4, 8, 11]
Milacidae	*Milax gagates* (Draparnaud, 1801)	Yes [3]
	Tandonia budapestensis (Hazay, 1880)	No
	Tandonia sowerbyi (Férussac, 1823)	Yes [3]
Oxychilidae	*Oxychilus alliarius* (Miller, 1822)	Yes [2]

Note: [1]*Aelurostrongylus abstrusus;* [2]*Angiostrongylus cantonensis;* [3]*Angiostrongylus vasorum;* [4]*Crenosoma vulpis;* [5]*Elaphostrongylus rangiferi;* [6]*Muellerius capillaris*; [7]*Neostrongylus linearis*; [8]*Oslerus rostratus*; [9]*Prostrongylus rufescens*; [10]*Troglostrongylus wilsoni*; [11]*Umingmakstrongylus pallikuukensis*.

Nematodes:

A total of 533 nematodes were isolated, with only 12 being juveniles. Juvenile nematodes are a useful indication for the possible presence of lungworm (metastrongyloid) species of veterinary importance like *An. vasorum*. Of those 12 juvenile nematodes, no lungworm species were found. Instead four of them were identified as *Angiostoma margaretae* (Angiostomatidae), a parasite whose definitive host is milacid slug species (Ross et al., 2017) (~~Figure~~). However, we also found it inside of *D. invadens* (Agriolimacidae) and *A. valentianus* (Limacidae) (~~Supplementary table 1~~). The next four were identified as an unknown Cosmocercidae species, a family of parasitic nematodes whose definitive host are reptiles and amphibians (Baker, 1984). The next two were identified as *Phasmarhabditis hermaphrodita* and the final two were identified as *Phasmarhabditis neopapillosa* (Rhabditidae). *Phasmarhabditis* is a genus of facultative parasitic nematodes that can parasitise a large range of gastropod species (Andrus et al., 2019). Of the adult nematodes identified, all belong to one of the seven nematode families that are non-medically (or veterinary) relevant (Agfidae, Angiostomatidae, Cosmocercidae and Rhabditidae).

The interactions these nematode families have with terrestrial gastropods are poorly understood (Wilson et al, 2005). The most understood species is *Phasmarhabditis hermaphrodita*, which has been developed into an effective biological alternative molluscicide (Nemaslug®) that reduces agricultural damage done by gastropod pests (Rae et al., 2007). Unlike chemical molluscicide, Nemaslug has no adverse effects on non-target organisms like beneficial organisms (acarids, annelids, carabids, collembolans, dipterans, isopods and nematodes), or gastropod predators (amphibians, birds, mammals and reptiles) (Iglesias et al., 2003). However, Nemaslug cannot kill every gastropod pest species like chemical molluscicides. This is due to *P. hermaphrodita* only being able to kill smaller gastropod species (e.g., *Deroceras spp*, *Arion hortensis*) and the juveniles of some larger species (*Arion ater*, *Cornu aspersum*) (Rae, 2017), while larger gastropod species (*Ambigolimax spp*, *Cepaea hortensis*, *Limacus spp*, *Limax spp*, *Lissachatina fulica*) are resistant to the fatal effects of *P. hermaphrodita* (Williams et al., 2015; Rae, 2017). Therefore, the investigation into other nematode species (similar to *P. hermaphrodita*) like *Agfa*, *Angiostoma*, *Cosmocerca* nah…these aren't lethal parasites like Phas, I would delete the sentence. or other *Phasmarhabditis* species could lead to the development of better biological molluscicides that are more effective and have a wider range of gastropod hosts than Nemaslug.

Trematodes:

A total of 242 trematodes were counted. Of these 29 were genotyped or identified to species with 14 being identified as *B. arcuata*, 11 being *B. fuscata* and three being *B. mesostoma* (~~Supplementary table 2~~). All these *Brachylaima* species are common gastrointestinal parasites of the bird families Corvidae, Sylviidae and Turdidae (Heneberg et al., 2016). One other trematode sample (belonging to group F1) could not be identified at the species-level. It clustered closely with the genera *Opisthioglyphe*, *Macroderoides*, *Brachycoelium*, *Mesocoelium*, *Auridistomum* and *Telorchis*, placing it within the Plagiorchioidea superfamily (~~Figure 4F~~). Genera of this Plagiorchioidea superfamily are common parasites of amphibians, fishes and reptiles (Tkach et al., 2001).

Brachylaima is a common gastrointestinal parasite of birds, mammals, and reptiles. There are over 60 described species, with *Brachylaima* being found in Africa, the Americas, Asia, Europe, and Oceania (Nasir et al., 1966; Wheeler et al., 1989; Richards et al., 1995; Awharitoma et al., 2003; Butcher et al., 2005; Richardson et al., 2005; Gállego et al., 2014; Gracenea et al., 2017; Nakao et al., 2017; Gérard et al., 2020; Termizi et al. 2021). *Brachylaima cribbi* is the only documented species capable of infecting humans (Butcher et al., 2001) with brachylaimiasis first documented in 1996, with 13 more cases in the subsequent decades after its discovery, all occurring in Australia (Butcher et al., 1996; Gállego et al., 2015). Brachylaimiasis causes diarrhoea, abdominal pain, anorexia, eosinophilia, and weight loss (or decreased weight gain) in infected humans, with a predicted mortality rate of 5-10% in untreated patients (Gállego et al., 2015). Transmission is typically from either the consumption of undercooked land snails (such as *Cornu aspersum*) infected with metacercariae, or the unintentional consumption of infected gastropod slime/faeces/corpse contaminated fruits and vegetables (Butcher et al., 2001).

While the consumption of snails is unpopular in the United Kingdom, on average the world consumes 450,000 tonnes of edible snails every year, of which only 15% come from snail farms (López et al., 2015). Spain, France, Portugal and Belgium are the biggest importers of snails, with approximately 17 million kilograms of snails being imported as a whole from 2020-2021 (United Nations, 2022). Concerns about the rates of *Brachylaima* infection in *Cornu aspersum* at farms and markets has already been raised in France and Spain (Gállego et al., 2015; Gracenea et al., 2017; Gérard et al., 2020). It is unknown what effect non-*Brachylaima cribbi* species have on public health as there are no studies exploring the possibility of brachylaimiasis caused by European *Brachylaima* species. Furthermore, the small size of the *Brachylaima* eggs (<30µm in length) in human faeces can make it difficult to diagnose a case of brachylaimiasis and could lead to frequent misdiagnosis (Gracenea et al., 2017).

I think you need a couple of sentences summing up the whole study and what it means in a wider context.

Conflict of interest

None

Funding

None

References There are a few formatting errors, which I’m sure you will sort ;)

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Supplementary

Supplementary Table 1. Nematode PCR information

Site (Sample no.)	Sequencing result (N93/N94)	Host	Nematode amount
Arnot Hill (ARNOT)	-	-	-
1	*Angiostoma margaretae*	*Tandonia budapestensis*	1
11	*Angiostoma margaretae*	*Tandonia budapestensis*	1
16	Fungal contamination	*Tandonia sowerbyi*	1
18	Fungal contamination	*Trochulus striolatus*	1
21	Fungal contamination	*Arion hortensis*	1
26	Fungal contamination	*Deroceras reticulatum*	1
30	Fungal contamination	*D. reticulatum*	1
35 (J)	*Angiostoma margaretae*	*Tandonia sowerbyi*	1
39	Not extracted	*T. sowerbyi*	1
44	Fungal contamination	*T. striolatus*	1
47	Not extracted	*T. striolatus*	1
50	Fungal contamination	*Cepaea nemoralis*	1
Attenborough (ATEN)	-	-	
1	Fungal contamination	*Arion Ater*	3
2	Not Extracted	*A. Ater*	3
3	Not Extracted	*A. Ater*	1
5	Fungal contamination	*Arion vulgaris*	1
8	Not Extracted	*A. Ater*	2
11	Not Extracted	*A. Ater*	1
12 (J)	*Phasmarhabditis hermaphrodita*	*Arion rufus*	2
13	Not Extracted	*A. rufus*	1
15	Fungal contamination	*A. Ater*	6
16	Not Extracted	*A. Ater*	5
20	Fungal contamination	*C. nemoralis*	1
21	Fungal contamination	*Oxychilus alliarius*	2
24	Fungal contamination	*Cornu aspersum*	4
25	Not Extracted	*A. Ater*	1
29	Not Extracted	*A. Ater*	3
31	Fungal contamination	*A. vulgaris*	1
33	Not Extracted	*A. rufus*	1
34	Not Extracted	*A. rufus*	1
37	Fungal contamination	*C. aspersum*	1
40	Not Extracted	*C. aspersum*	1

43	Not Extracted	*C. aspersum*	1
46	Fungal contamination	*C. nemoralis*	1
50	Not Extracted	*C. nemoralis*	1
Basford (BAS)	-	-	-
1 (J)	*Cosmocercidae spp*	*Cornu aspersum*	27
6	Fungal contamination	*D. reticulatum*	2
11	Fungal contamination	*T. sowerbyi*	3
12	Fungal contamination	*Ambigolimax valentianus*	3
14	Not Extracted	*T. budapestensis*	21
24	Fungal contamination	*A. valentianus*	1
26	Not Extracted	*T. budapestensis*	6
27	Not Extracted	*T. budapestensis*	4
30	Not Extracted	*D. reticulatum*	1
31	Not Extracted	*D. reticulatum*	1
38	Not Extracted	*Milax gagates*	2
39	Not Extracted	*Limacus maculatus*	17
40	Not Extracted	*L. maculatus*	13
41	Not Extracted	*D. reticulatum*	2
42	Not Extracted	*Arion subfuscus*	4
43	Not Extracted	*A. hortensis*	2
45	*Angiostoma margaretae*	*Tandonia budapestensis*	2
47	Not Extracted	*A. hortensis*	4
49	Not Extracted	D. reticulatum	1
50	Not Extracted	D. reticulatum	2
Beeston (BEE)	-	-	-
1 (J)	*Cosmocercidae spp*	*Cornu aspersum*	27
12	*Angiostoma margaretae*	*Tandonia budapestensis*	1
14	*Angiostoma margaretae*	*Tandonia budapestensis*	2
16	*Angiostoma gandavensis*	*Deroceras invadens*	1
25	Fungal contamination	*D. invadens*	1
28	Fungal contamination	*A. ater*	2
Carlton (CARL)	-	-	-
2	Fungal contamination	*C. aspersum*	2
3	Not Extracted	*C. aspersum*	1
8	Fungal contamination	*C. aspersum*	2
13	Fungal contamination	*C. nemoralis*	2
18	*Angiostoma margaretae*	*D. reticulatum*	3
26	Fungal contamination	*T. budapestensis*	1
27	Not Extracted	*T. budapestensis*	1
28	Not Extracted	*T. budapestensis*	2
29	Not Extracted	*T. budapestensis*	3
35	Not Extracted	*T. budapestensis*	3
37	Fungal contamination	*D. invadens*	1
41	Not Extracted	*D. invadens*	5
Colwick (COLW)	-	-	-
2	Fungal contamination	*C. aspersum*	1
7	Fungal contamination	*Limacus flavus*	1
13 (J)	*Angiostoma margaretae*	*Deroceras invadens*	4
18	Fungal contamination	*Arion silvaticus*	1
21	Fungal contamination	*A. valentianus*	1
24	Not Extracted	*A. valentianus*	1
29	Fungal contamination	*C. hortensis*	1
30	Not Extracted	*C. hortensis*	1
31	Not Extracted	*C. hortensis*	1
36	Not Extracted	*C. hortensis*	1
41	Fungal contamination	*A. hortensis*	1

44	Not Extracted	*A. hortensis*	1
46	Not Extracted	*T. budapestensis*	1
47	Not Extracted	*T. budapestensis*	1
49	Not Extracted	*T. budapestensis*	2
Clifton south (C-SOU)	-	-	-
1	*Angiostoma margaretae*	*Deroceras invadens*	3
3	*Angiostoma gandavensis*	*Deroceras reticulum*	3
7	*Angiostoma margaretae*	*Tandonia budapestensis*	1
9	*Angiostoma margaretae*	*Tandonia sowerbyi*	6
10 (J)	*Phasmarhabditis neopapillosa*	*Ambigolimax nyctelius*	4
23	Fungal contamination	*A. vulgaris*	1
26	Not Extracted	*D. invadens*	1
29	Fungal contamination	*D. reticulatum*	1
30	Not Extracted	*D. reticulatum*	1
32	Not Extracted	*T. sowerbyi*	1
36	Not Extracted	*T. budapestensis*	1
42	Fungal contamination	*C. nemoralis*	1
45	Fungal contamination	*A. vulgaris*	1
Edwalton (EDW)	-	-	-
1 (J)	*Angiostoma margaretae*	*Tandonia budapestensis*	31
2	*Angiostoma margaretae*	*Tandonia budapestensis*	5
3	Not Extracted	*T. budapestensis*	11
4	Not Extracted	*T. budapestensis*	2
5	*Agfa flexilis*	*Limacus maculatus*	4
6	Fungal contamination	*A. silvaticus*	2
7	Fungal contamination	*A. hortensis*	1
9	Fungal contamination	*D. invadens*	1
23	Not Extracted	*C. aspersum*	8
27	Not Extracted	*C. aspersum*	22
30	Not Extracted	*T. budapestensis*	1
32	Not Extracted	*T. budapestensis*	1
34	Not Extracted	*C. aspersum*	1
38	Not Extracted	*D. invadens*	1
40	Not Extracted	*D. invadens*	1
41	Not Extracted	*D. invadens*	1
44	Not Extracted	*D. invadens*	1
46	Not Extracted	*T. striolatus*	1
47	Not Extracted	*C. hortensis*	1
50	Not Extracted	*C. hortensis*	1
Forest field (FOR)	-	-	-
18	Not Extracted	*L. maculatus*	1
20	*Agfa flexilis*	*Limacus maculatus*	1
26	*Agfa flexilis*	*Limax maximus*	3
27	Not Extracted	*L. maximus*	4
30	Fungal contamination	*C. aspersum*	5
33	Not Extracted	*L. maculatus*	4
35	Not Extracted	*L. maculatus*	4
36 (J)	*Angiostoma margaretae*	*Ambigolimax valentianus*	1
38	Fungal contamination	*A. hortensis*	1
45	Fungal contamination	*T. budapestensis*	1
46	Not Extracted	*T. budapestensis*	1
Gamston (GAM)	-	-	-
1	*Angiostoma margaretae*	*Deroceras invadens*	2
6	Fungal contamination	*L. maculatus*	1
9	Not Extracted	*L. maculatus*	1
13	Fungal contamination	*A. valentianus*	1

14	Fungal contamination	*A. valentianus*	1
15	Not Extracted	*A. valentianus*	2
16 (J)	*Phasmarhabditis neopapillosa*	*Ambigolimax valentianus*	1
17	Not Extracted	*A. valentianus*	2
27	Fungal contamination	*M. cantiana*	1
Grange Park (GRAN)	-	-	-
1	*Agfa flexilis*	*Tandonia Budapestensis*	1
13	*Agfa flexilis*	*Arion vulgaris*	1
15	Fungal contamination	*A. Vulgaris*	1
23	Fungal contamination	*A. subfuscus*	7
27	Not Extracted	*A. subfuscus*	1
29	Fungal contamination	*A. subfuscus*	2
34	Fungal contamination	*T. budapestensis*	6
37	Fungal contamination	*D. invadens*	2
42	Fungal contamination	*D. reticulatum*	1
48	Fungal contamination	*A. Vulgaris*	1
Kimberley (KIM)	-	-	-
1	*Angiostoma gandavensis*	*Deroceras invadens*	3
9	Not Extracted	*D. invadens*	1
12	Fungal contamination	*O. alliarius*	1
14	Not Extracted	*O. alliarius*	1
18	Not Extracted	*O. alliarius*	1
19	Fungal contamination	*O. alliarius*	1
20	Not Extracted	*O. alliarius*	4
21	Fungal contamination	*A. hortensis*	1
24	Not Extracted	*A. hortensis*	2
32	Not Extracted	*A. hortensis*	2
33	*Angiostoma gandavensis*	*Arion hortensis*	3
37	Not Extracted	*D. reticulatum*	1
40 (J)	*Cosmocercidae spp*	*Cornu aspersum*	32
42	Not Extracted	*C. nemoralis*	1
45	Not Extracted	*C. nemoralis*	1
46	Not Extracted	*C. nemoralis*	1
Mill lake (MILL)	-	-	-
3	Fungal contamination	*C. aspersum*	1
19 (J)	*Cosmocercidae spp*	*Cornu aspersum*	19
21	Fungal contamination	*A. vulgaris*	2
33	Fungal contamination	*M. cantiana*	1
Iremongers pond (POND)	-	-	-
5	Fungal contamination	*D. reticulatum*	1
14	*Cosmocerca longicauda*	*Limax flavus*	7
21	Fungal contamination	*D. reticulatum*	1
25	Fungal contamination	*D. invadens*	1
37	Fungal contamination	*L. flavus*	1
45	Fungal contamination	*A. rufus*	1
Toton (TOT)	-	-	-
11	Fungal contamination	*A. ater*	2
13	Fungal contamination	*T. sowerbyi*	4
21	Fungal contamination	*C. nemoralis*	1
24	*Phasmarhabditis hermaphrodita*	*Cepaea nemoralis*	1
25 (J)	*Phasmarhabditis hermaphrodita*	*Arion subfuscus*	6
26	Fungal contamination	*A. ater*	2
33	Fungal contamination	*A. subfuscus*	1
39	Fungal contamination	*A. ater*	1
47	Fungal contamination	*T. sowerbyi*	1
University Park (UNI)	-	-	-

2	Fungal contamination	*A. valentianus*	1
3	Fungal contamination	*A. valentianus*	1
15	*Agfa flexilis*	*Tandonia budapestensis*	1
17	Fungal contamination	*T. budapestensis*	1
39	Fungal contamination	*A. valentianus*	1

Note: (J) indicates it was a juvenile nematode.

Supplementary Table 2. Trematode identification information

Site (Sample no.)	Sequencing result (LPF/LPR)	Host	Trematode amount
Arnot Hill (ARNOT)	-	-	-
18	*Brachylaima fuscata*	*Trochulus striolatus*	1
Basford (BAS)	-	-	-
11	*Brachylaima arcuata*	*Tandonia sowerbyi*	1
26	*Brachylaima fuscata*	*Cornu aspersum*	2
38	Fungal contamination	*Milax gagates*	4
41	Fungal contamination	*Deroceras reticulatum*	1
Carlton (CARL)	-	-	-
12	*Brachylaima mesostoma*	*Cepaea nemoralis*	4
13	*Brachylaima mesostoma*	*Cepaea nemoralis*	3
19	Fungal contamination	*D. reticulatum*	5
37	Not Extracted	*Deroceras invadens*	4
41	Fungal contamination	*D. invadens*	2
45	Not Extracted	*D. invadens*	3
Colwick (COLW)	-	-	-
2	*Brachylaima fuscata*	*Cornu aspersum*	3
11	Fungal contamination	*D. invadens*	2
21	Fungal contamination	*Ambigolimax valentianus*	2
25	Not Extracted	*A. valentianus*	2
Clifton south (C-SOU)	-	-	-
19	*Brachylaima mesostoma*	*Cepaea nemoralis*	10
Edwalton (EDW)	-	-	-
8	*Brachylaima fuscata*	*Deroceras invadens*	2
20	Fungal contamination	*Trochulus striolatus*	1
23	Fungal contamination	*C. aspersum*	11
25	*Brachylaima fuscata*	*Cornu aspersum*	3
Forest field (FOR)	-	-	-
4	*Brachylaima fuscata*	*Deroceras invadens*	1
18	Fungal contamination	*Limacus maculatus*	1
23	*Brachylaima arcuata*	*Tandonia budapestensis*	2
47	Fungal contamination	*D. invadens*	1
Gamston (GAM)	-	-	-
3	*Brachylaima fuscata*	*Ambigolimax valentianus*	2
13	Not Extracted	*A. valentianus*	9
15	*Brachylaima fuscata*	*Ambigolimax valentianus*	15
16	*Brachylaima fuscata*	*Ambigolimax valentianus*	23
23	Not Extracted	*T. striolatus*	11
26	*Brachylaima fuscata*	*Cepaea hortensis*	2
27	Not Extracted	*Monacha cantiana*	4
28	Not Extracted	*Cornu aspersum*	36
29	Not Extracted	*C. aspersum*	2

30	Not Extracted	*C. aspersum*	3
33	Not Extracted	*C. aspersum*	2
34	Not Extracted	*C. aspersum*	5
37	Fungal contamination	*D. invadens*	1
46	Not Extracted	*C. nemoralis*	2
48	Not Extracted	*C. nemoralis*	2
Grange Park (GRAN)	-	-	-
8	*Brachylaima arcuata*	*Deroceras reticulatum*	4
Kimberley (KIM)	-	-	-
3	*Brachylaima arcuata*	*Deroceras invadens*	3
10	*Brachylaima arcuata*	*Deroceras invadens*	2
37	*Brachylaima arcuata*	*Deroceras reticulatum*	1
40	*Brachylaima fuscata*	*Cornu aspersum*	8
Mill lake (MILL)	-	-	-
4a	*Brachylaima arcuata*	*Monacha cantiana*	14
4b	*Brachylaima arcuata*	*Monacha cantiana*	14
31	*Brachylaima arcuata*	*Monacha cantiana*	3
32	*Brachylaima arcuata*	*Monacha cantiana*	1
35	*Brachylaima arcuata*	*Monacha cantiana*	1
Iremongers pond (POND)	-	-	-
5	*Brachylaima arcuata*	*Deroceras reticulatum*	3
8	*Brachylaima arcuata*	*Deroceras reticulatum*	4
University Park (UNI)	-	-	-
5	*Brachylaima arcuata*	*Ambigolimax valentianus*	2
37	Fungal contamination	A. valentianus	1
39	*Plagiorchioidea spp*	*Ambigolimax valentianus*	8
41	Fungal contamination	A. valentianus	1