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Trichinella pseudospiralis in a wolverine *(Gulo gulo)* from the Canadian North

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ABSTRACT

Species of *Trichinella* are a globally distributed assemblage of nematodes, often with distinct host ranges, which include people, domestic, and wild animals. *Trichinella* spp. are important in northern Canada, where dietary habits of people and methods of meat preparation (drying, smoking, fermenting as well as raw) increase the risk posed by these foodborne zoonotic parasites. Outbreaks in the arctic and subarctic regions of Canada and the United States are generally attributed to *T. nativa* (T2) or the T6 genotype, when genetic characterization is performed. We report the discovery of *Trichinella pseudospiralis* (T4), a non-encapsulated species, in a wolverine (*Gulo gulo*) from the Northwest Territories of Canada. This parasite has been previously reported elsewhere from both mammals and carnivorous birds, but our findings represent new host and geographic records for *T. pseudospiralis*. Multiplex PCR and sequencing of fragments of Cytochrome Oxidase Subunit I (COI) and D3 rDNA confirmed the identification. Phylogenetically, Canadian isolates linked with each other and others derived from Palearctic or Neotropical regions, but not elsewhere in the Nearctic (continental USA). We suggest that migratory birds might have played a role in the dispersal of this pathogen 1000's of km to northwestern Canada. Wolverines are not typically consumed by humans, and thus should not pose a direct food safety risk for trichinellosis. However, the current finding suggests that they may serve as an indicator of a broader distribution for *T. pseudospiralis*. Along with infection risk already recognized for *T. nativa* and *Trichinella* T6, our observations emphasize the need for further studies using molecular diagnostics and alternative methods to clarify if this is a solitary case, or if *T. pseudospiralis* and other freeze susceptible species of *Trichinella* (such as *T. spiralis*) circulate more broadly in wildlife in Canada, and elsewhere.

1. Introduction

Among the 24 most significant foodborne parasitic diseases listed by the World Health Organization/United Nations Food and Agriculture Organization, *Trichinella spiralis* globally ranks seventh, with other *Trichinella* spp. ranked as 16th (FAO/WHO, 2014). In a systematic review, 65 818 human cases of trichinellosis were reported worldwide from 1986 to 2009 (Murrell and Pozio, 2011). From a public health perspective, species of *Trichinella* (largely *T. nativa* and *Trichinella* T6) were ranked third among nine zoonotic parasites in northern North America based on an evidence-based qualitative risk analysis (Jenkins et al., 2013).

People or other mammals and birds contract *Trichinella* spp. infection after consuming meat infected with larvae of these parasites (Gottstein et al., 2009; McIntyre et al., 2007; Serhir et al., 2001). Upon gastric digestion of infected meat in an exposed host, the first stage larvae are released, which penetrate intestinal mucosa, and undergo four molts before developing into adult worms within a few days of infection. Males and females copulate and after 5 days post infection, females start releasing newborn larvae, which travel via blood to predilection sites in the skeletal musculature. For encapsulated species of *Trichinella* such as *T. spiralis*, larvae encyst inside muscle cells, whereas

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in other species such as *T. pseudospiralis*, larvae remain unencapsulated. Clinical manifestations are rarely recognized in animals, but human patients may display symptoms including headache, fever, abdominal pain, diarrhea, myalgia, eyelid/facial edema, and even mortality due to cardiac manifestations, depending on infective dose and immune status (Gottstein et al., 2009). *Trichinella pseudospiralis* causes clinical manifestations similar to those caused by *T. spiralis* except with a more prolonged myopathy (Jongwutiwes et al., 1998). Diagnosing which species of *Trichinella* is responsible for human outbreaks is desirable, but rarely performed as it necessitates muscle biopsy and comparative molecular analyses. A broad understanding of the species/genotypes of *Trichinella* circulating in domestic and wild animals can aid understanding of transmission pathways, routes of exposure and developing possible management goals, because species differ in important characteristics, such as host affinities and tolerance to freezing.

All species and genotypes of *Trichinella* have been reported in mammals, whereas *T. papuae* and *T. zimbabwensis* also infect reptilian hosts. *Trichinella pseudospiralis* is the only species in the genus reported in both carnivorous birds and mammals, but the number of reports in mammals exceeds those in avian hosts (Pozio et al., 2009; Zamora et al., 2015). Worldwide, *T. pseudospiralis* has a cosmopolitan distribution and has been reported in 18 mammalian and eight avian species (Pozio, 2016). In Canada, *T. spiralis* has been eradicated from commercially raised pigs, and has only rarely been reported from wildlife (Gajadhar and Forbes, 2010). In contrast to the domestic cycle, a number of sylvatic species of *Trichinella* exist in Canadian wildlife, and include *T. nativa* (T2), *T. murrelli* (T5), *Trichinella* T6, and *T. pseudospiralis* (Jenkins et al., 2013). *Trichinella nativa* and *Trichinella* T6 are freeze-tolerant, and are the most common species found in wildlife hosts in the arctic and sub-arctic zones of Canada. Both species are commonly found in wolves (*Canis lupus*), wolverines (*Gulo gulo*), bears (*Ursus* spp.), arctic foxes (*Vulpes lagopus*), walruses (*Odobenus rosmarus*), and other carnivorous mammals (Gajadhar and Forbes, 2010; Jenkins et al., 2013; Sharma et al., 2018). Recent outbreaks of trichinellosis in Canada have been almost exclusively linked to consumption of game meat (black bear, grizzly bear, walrus) infected with *T. nativa* (Dalcin et al., 2017; Houze et al., 2009; McIntyre et al., 2007; Schellenberg et al., 2003; Serhir et al., 2001).

The public health importance of trichinellosis in the Canadian north necessitates continuing surveillance for species of *Trichinella* in a diverse assemblage of largely mammalian wildlife species. Wolverines are not commonly consumed, but are commonly harvested for fur, and have high prevalence ($\geq$80%) of this parasite due to their high trophic position and scavenging lifestyle (Reichard et al., 2008; Sharma et al., 2018). Therefore, they make excellent sentinels for this and other food borne parasites. Here, we report the discovery of *T. pseudospiralis* in a wolverine from northern Canada, and discuss the epidemiological/phylogenetic associations of this isolate of *T. pseudospiralis*.

2. Material and methods

2.1. Sample collection and transport

Wolverine carcasses were submitted to the Department of Environment and Natural Resources, Government of the Northwest Territories as part of the fur harvest during 2005–2012 in Northwest Territories, and included 131 animals. Tongues and diaphragms were collected from wolverine carcasses and were stored at −20 °C before shipping to the Department of Veterinary Microbiology, Western College of Veterinary Medicine, Saskatoon, Saskatchewan, Canada.

2.2. Sample preparation, digestion and recovery of larvae

Fat and connective tissue were removed from the tongue (or diaphragm, if tongue was not available) of each wolverine, and muscle was cut into 0.5–1.0 cm cubes, mixed and a portion randomly selected to make up to 10 g. Muscle tissues were processed by the pepsin-HCl double separatory funnel digestion method (Forbes and Gajadhar, 1999). *Trichinella* first-stage larvae (L1) were identified based on morphology observed under the stereo-microscope, and counted. The burden of infection was estimated as larvae per gram (LPG). Larval motility was assessed by incubating a petri plate containing the larvae at 37°C for 30 min. Five individual larvae and a pool of ten larvae were collected in six 0.6 ml tubes containing 1X PCR buffer [10 mM Tris-HCl, pH 8.3, 50 mM HCl, 1.5 mM MgCl2, 0.01% (w/v) gelatin] and stored at −20 °C until used for molecular analysis. Compression of small tongue samples using a glass compressorium was also performed to determine if larvae in-situ were encapsulated.

2.3. Molecular identification and sequencing

Parasite genomic DNA was extracted from 5 individual larvae as well as a pool of 10 larvae using a Proteinase K extraction method (Scandrett et al., 2018). To identify species or genotype, primers amplifying internal transcribed spacer regions (ITS 1 and 2) as well as the expansion segment V (ESV) of the large subunit ribosomal DNA (Zarlenga et al., 1999) were used in a multiplex PCR assay. Positive controls of six recognized species of *Trichinella* (*T. spiralis*, *T. nativa*, *T. britovi*, *T pseudospiralis, T murrelli* and *Trichinella* T6), passaged in mice, were provided by the Centre for Food-borne and Animal Parasitology, Canadian Food Inspection Agency (CFIA), Saskatoon. The *T. pseudospiralis* isolate provided was originally recovered from a mountain lion (*Puma concolor couguar*) on Vancouver Island (British Columbia, Canada). This is the only other isolate of *T. pseudospiralis* reported from Canada and had not yet been characterized using the sequencing methods employed in this study. Samples were identified based on the banding patterns of amplified products on the 2.5% agarose gel stained with Red Safe (FroggaBio Inc, ON, Canada) and photographed using a Gel Doc system (Alpha Innotech AlphaImager digital imaging system).

To confirm and compare the sequence from the current *T. pseudospiralis* isolate with other isolates of different geographical origin (Eurasia, Australia, Canada and USA), the D3 domain of nuclear ribosomal DNA (D3 rDNA) and the mitochondrial cytochrome C oxidase subunit I (COI) gene were amplified by PCR using the primer pair 5-ACCCGTCTTGAAACACGGA-3 and 5-GATTAGTCTTTCGCCCCTA-3 and the primer pair 5-GTTTTTTGGGCATCCAGAAGTT-3 and 5-GAAGAAG GTCTAAGGAAGCATTTGA-3, respectively (Gasser et al., 2004; Krivokapich et al., 2015). Amplified segments of 400 bp of the D3 rDNA and 345 bp of the COI gene were purified using ExoSAP-IT as per manufacturer's instructions and sent to Macrogen Korea for sequencing (Macrogen Inc., Seoul, Korea). Consensus sequences for each locus were generated in Geneious 11.1.5 (Biomatters, Ltd., New Zealand) based on forward and reverse Sanger sequences. BLAST searches of the non-redundant nucleotide database at NCBI GenBank were used to confirm the *Trichinella* species diagnosis from the multiplex, and to obtain the nucleotide identity with other isolates. The nucleotide sequences of D3 rDNA and COI were deposited into the GenBank database under Accession Nos. MK333397, MK703809, MK333398 and MK713937. Multiple sequence alignments were carried out using Muscle 3.8.425 multi alignment program (Edgar, 2004) followed by manual optimization and comparison against the COI and D3 rDNA sequences of *T. pseudospiralis* from different geographical locations available in GenBank (Table 1). Phylogenetic analysis was performed in Geneious 11.1.5 using the neighbor-joining algorithm reconstructed from distances calculated using the HKY model of nucleotide substitution with 1000 bootstrap replicates. Maximum likelihood trees were generated in PhyML using the GTR + I + gamma substitution model (Guindon et al., 2010). Sequences from encapsulated *Trichinella spiralis* and *Trichinella nelsoni* were used to root the trees.

As animals were harvested for purposes other than research, this animal use was considered a Level A Category of Invasiveness, as per the Canadian Council on Animal Care (CCAC) recommendations, and

Table 1
Comparison of percentage nucleotide identity of current *T. pseudospiralis* isolate with other isolates from different geographical origins.

DNA sequence	Isolate Code	Host	Accession number	Geographic origin	Identity (%)	Reference
COI		Mountain Lion (*Puma concolor couguar*)	MK713937	Canada (Vancouver Island)	100	Current study
	ISS13	Raccoon dog (*Nyctereutes procyonoides*)	KM357408	Russia (Krasnodar)	100	(Mohandas et al., 2014)
	ISS588	Brown rat (*Rattus norvegicus*)	KM357409	Russia (Kamchatka)	100	(Mohandas et al., 2014)
	ISS176	Tawny eagle (*Aquila rapax*)	KM357410	Kazakhstan	100	(Mohandas et al., 2014)
		Domestic pig (*Sus scrofa)*	KM063187	South America (Argentina)	100	Krivokapich et al. (2015)
	ISS141	Tiger cat (*Dasyurus maculatus*)	EF601545	Australia (Tasmania)	97.6	(Wu et al., 2007)
	ISS1132	Wild pig (*Sus scrofa*)	EF601544	USA (Texas)	94.9	(Wu et al., 2007)
	ISS470	Black vulture (*Coragypus atratus*)	KM357411	USA (Alabama)	94.6	(Mohandas et al., 2014)
D3 rDNA		Mountain Lion (*Puma concolor couguar*)	MK703809	Canada (Vancouver Island)	100	Current study
	ISS13	Raccoon dog (*Nyctereutes procyonoides*)	AJ633056	Russia (Krasnodar)	100	Gasser et al. (2004)
		Domestic pig (*Sus scrofa)*	KM063188	South America (Argentina)	100	Krivokapich et al. (2015)
	ISS470	Black vulture (*Coragypus atratus*)	AJ633058	USA (Alabama)	99.3	Gasser et al. (2004)
	ISS141	Tiger cat (*Dasyurus maculatus*)	AJ633057	Australia (Tasmania)	97.5	Gasser et al. (2004)

thus exempt from animal research ethics review at the University of Saskatchewan. We worked closely with the Government of the Northwest Territories for wildlife research and export permits.

3. Results

3.1. Microscopic examination

The positive animal was a one-year-old male, which had been killed by hunters or trappers in the South Slave Region (Latitude 60.83300 N, Longitude −117.20 W) in 2006. Digestion of tongue and diaphragm resulted in 12 (all comma-shaped, dead) and 44 (3 tightly coiled and 41 comma-shaped, dead) larvae, respectively. When repeated, digestion of tongue muscle resulted in 17 comma-shaped (dead) larvae. Mean LPG in diaphragm (4.4, 44 larvae/10 g) was more than that in tongue (1.45, 29 larvae/20 g). The overall mean larval burden was 2.4 LPG (tongue and diaphragm combined). None of the larvae were motile. We detected only two non-encapsulated *Trichinella* spp. larvae in 40 compressed tongue muscle samples (Fig. 1).

3.2. Multiplex PCR and DNA sequencing

Multiplex PCR revealed amplicons of approximately 310 bp, which corresponded to *T. pseudospiralis*. In order to confirm the species diagnosis and determine whether this isolate was closely related to any particular previously published isolates, identity among nucleotide sequences was considered and phylogenetic analyses were completed.

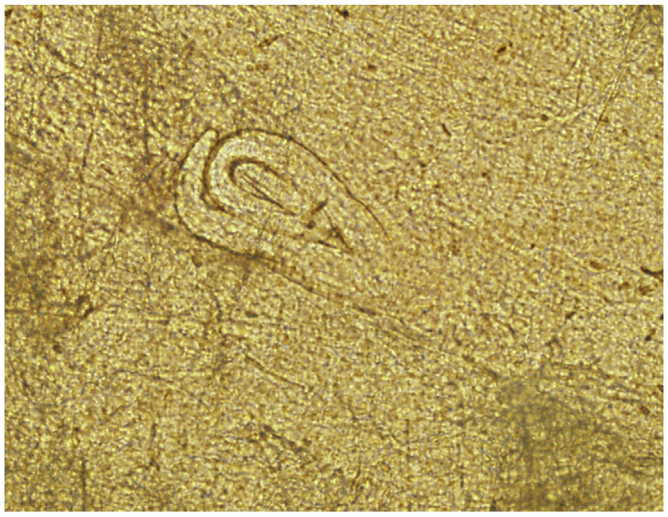

Fig. 1. Photomicrograph of *Trichinella pseudospiralis* larva in compressed tongue muscle of a wolverine (*Gulo gulo*).

High quality sequence for 339 base pairs of COI DNA and 400 base pairs of D3 rDNA was generated from the newly reported wolverine isolate. COI sequence showed 100% nucleotide identity with *T. pseudospiralis* isolates from Russia (Krasnodar and Kamchatka), Kazakhstan and Argentina, compared with 97.6% and 94.6–94.9% with *T. pseudospiralis* isolates from Australia and USA, respectively (Table 1). Similarly, the D3 rDNA sequence was identical to those of isolates from Russia, Argentina and Canada, and 99.3% and 97.5% with those of isolates from USA and Australia, respectively (Table 1). The mitochondrial and nuclear sequences from the described isolate shared no more than 89% identity with any other *Trichinella* species. Using *T. spiralis* (an encapsulated *Trichinella* species) as an outgroup, neighbor-joining and maximum likelihood phylogenetic trees for both mitochondrial and nuclear genes placed the new isolate within a strongly supported clade containing only *T. pseudospiralis* isolates (Fig. 2). Furthermore, the wolverine isolate clustered closely with the Canadian mountain lion isolate and with those of *T. pseudospiralis* previously documented from Russia and Argentina (Fig. 2) rather than isolates from the southern United States. Topologies and branch support were consistent between neighbor-joining and maximum likelihood estimated trees.

4. Discussion

Globally, *T. pseudospiralis* has been reported in several wild animals, for example, raccoon dog (*Nyctereutes procyonoides*), lynx (*Felis lynx*), red fox (*Vulpes vulpes*), Florida panther (*Puma concolor coryi*), mountain lion etc (Airas et al., 2010; Gajadhar and Forbes, 2010; Reichard et al., 2015). We document the first report of *T. pseudospiralis* in wolverine and only the third report in any mustelid host; previous reports include badger (*Meles meles*) and American mink (*Neovison vison*) (Pozio, 2016). In North America, the first three reports were based on histology in a Coopers Hawk (*Accipiter cooperi*) from California (Wheeldon et al., 1982, 1983) and muscle digests from a Great Horned Owl (*Bubo virginianus)* from Iowa, and a Pomarine Jaeger (*Stercorarius pomarinus*) from Alaska (Rausch et al., 1956; Zimmermann and Hubbard, 1969), but were identified prior to the advent of molecular diagnosis. The first verified North American isolate based on DNA hybridization using a species-specific probe was in a black vulture (*Coragypus atratus*) from Alabama (Lindsay et al., 1995). Based on the presence of unencapsulated, freeze susceptible larvae, the multiplex PCR results, and sequencing of both mitochondrial and nuclear DNA loci, we have confirmed that the isolate obtained from this wolverine is consistent with *T. pseudospiralis*. In addition to a new host record for *T. pseudospiralis*, this is the first documentation of the parasite on the mainland of Canada, the second report of *T. pseudospiralis* infecting a wild animal from Canada, and the northernmost observation of the parasite from North America (Table 2, Fig. 3). The only previous report of *T. pseudospiralis* in Canada was in a mountain lion from Vancouver Island in the southwestern region of the country in 2003 (Gajadhar and Forbes,

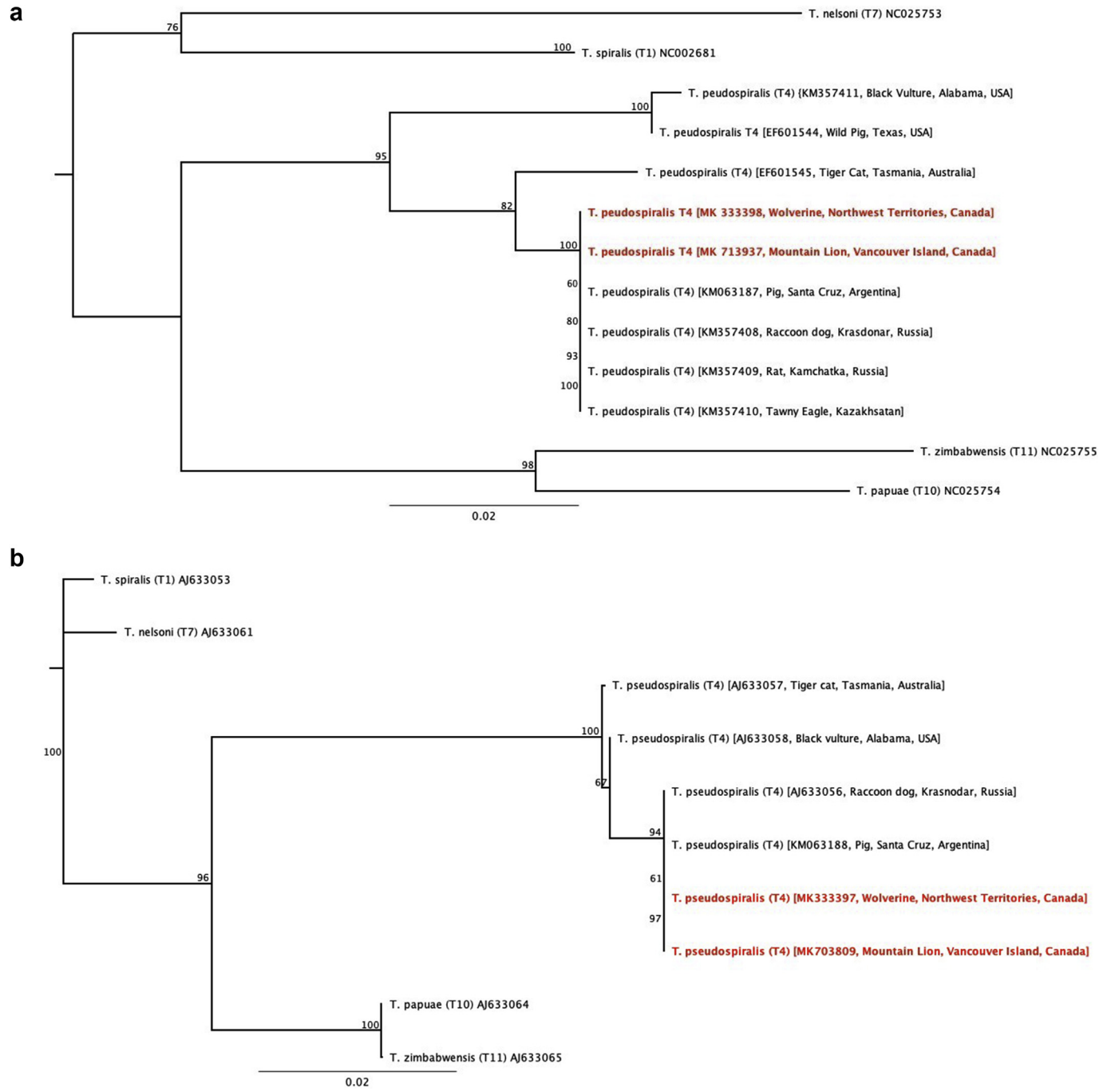

Fig. 2. Neighbor-joining gene trees showing the relationship of the *Trichinella pseudospiralis* isolate reported here with previously described isolates. Sequence from (a.) the cytochrome oxidase 1 mitochondrial gene (COI) and (b.) the D3 domain of nuclear ribosomal DNA (D3 rDNA) placed the newly discovered isolate within a strongly supported clade containing isolates from Russia, Argentina, and Canada. This clade clearly delineated these isolates as belonging to *T. pseudospiralis,* but were notably distinct from conspecific isolates from Alabama and Texas in the continental United States. Tree topology was conserved between neighbor-joining, maximum likelihood, and Bayesian analyses with strong support for all interspecific nodes.

2010). Wolverines in North America have varied home ranges from 100 to 900 km^2 but are known for long-range dispersal (Banci, 1994; Mulders, 2001). Assuming maximum dispersal of 1000 km of the animal under study, this wolverine likely originated within northwestern Canada.

We hypothesize that the arrival of *T. pseudospiralis* in this region could be mediated through migratory birds or mammals. Dietary habits of wolverines vary with season, availability and distribution of prey species and geographical locations. Wolverines are opportunistic foragers, primarily depending on carcasses of caribou (*Rangifer tarandus*), and other carrion and prey in the winter, shifting to vegetation and preying on small mammals, and birds in the summer (Pasitschniak-Arts and Larivière, 1995). Wild birds, especially raptors and birds such as jaegers that typically feed on small to medium sized mammals (e.g., rodents, shrews, lagomorphs), could spread *T. pseudospiralis* over great distances leading to the potential for establishment of new foci of infections in locations previously considered at no or low risk for this parasite (Zamora et al., 2015). Nucleotide sequence analysis placed the isolate discovered in wolverine among only other Canadian isolate previously documented from Canada (100% identity with isolate from a

Table 2
North American and global reports of *T. pseudospiralis* in wild mammals and birds, respectively.

North American reports				
Host	Geographic origin	Year	Number positive	Reference
Black vulture (*Coragypus atratus*)	Alabama, USA	1995	1	Lindsay et al. (1995)
Wild boar	Newcastle, Texas, USA	2000	1	Gamble et al. (2005)
Mountain lion (*Puma concolor couguar*)	Vancouver Island, Canada	2003	1	Gajadhar and Forbes (2010)
Florida panther (*Puma concolor coryi*)	Florida, USA	2012	18	Reichard et al. (2015)
Mountain lion (*Puma concolor couguar*)	Colorado, USA	2014	3	Reichard et al. (2017)
Wolverine (*Gulo gulo*)	Northwest Territories, Canada	2006	1	Current study
Global reports				
Rook (*Corvus frugilegus*)	Chimkent region, Kazakhstan	1975	2	Shaikenov (1980)
Tawny eagle (*Aquila rapax*)	Kazakhstan	1980	1	(Pozio et al., 1992)
Western marsh harrier (*Circus aeroginosus*)	Tasmania, Australia	1990	1	(Obendorf and Clarke, 1992)
Australian masked owl (*Tyto novaehollandiae*)	Tasmania, Australia	1990	1	(Obendorf and Clarke, 1992)
Black vulture (*Coragypus atratus*)	Alabama, USA	1995	1	Lindsay et al. (1995)
Tawny owl (*Strix aluco*)	Stockholm, Sweden	1997	1	(Hurníková et al., 2014)
Tawny owl (*Strix aluco*)	Uppsala, Sweden	2011	1	(Hurníková et al., 2014)
Tawny owl (*Strix aluco*)	Marche, Italy	1998	1	(Pozio et al., 1999)
Little owl (*Athene noctua*)	Marche, Italy	1998	1	(Pozio et al., 1999)
Common buzzard (*Buteo buteo*)	Krasnodar region, Russia	2006	1	Pozio (2016)

mountain lion from Vancouver Island), as well as those from distant and geographically disjunct localities in the Palearctic (Russia and Kazakhstan) and Neotropical regions (Argentina). In contrast, the wolverine-isolate was divergent from other geographically proximate locations in the Nearctic (continental USA). The closest match, sharing 100% nucleotide identity, was with Canadian, Russian, Kazakhstani

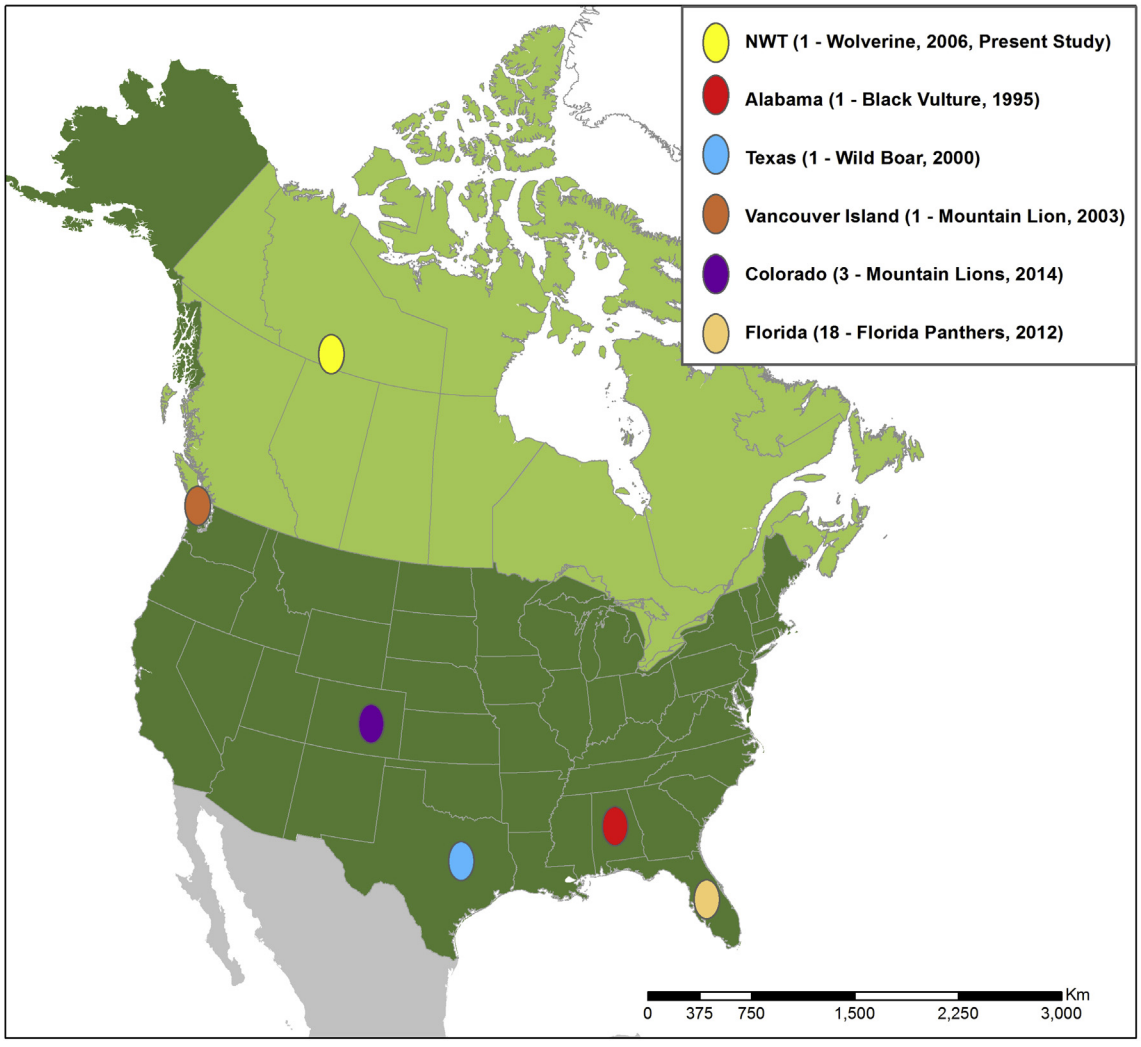

Fig. 3. Geographic locations of this and previously published reports of *Trichinella pseudospiralis* confirmed by multiplex PCR in North America (Lindsay et al., 1995., Gamble et al., 2005; Gajadhar and Forbes, 2010; Reichard et al., 2015, 2017).

and Argentinian isolates, raising the hypothesis of transmission pathways for this isolate linking Eurasia or the Neotropical region to Canada via migratory birds, especially birds of prey or via migratory carnivores within Canada.

Natural infections of *T. pseudospiralis* have been reported in eight species of birds, primarily raptors as well as corvids such as rook (*Corvus frugilegus*) from Russia and Kazakhstan (Table 2). The first natural infection in an avian species was reported among rooks, a large passerine (Corvidae- *Corvus frugilegus*), from the Chimkent region, Kazakhstan in 1975 (Shaikenov, 1980). *Trichinella pseudospiralis* has been reported in mammals more frequently than in birds, but there have been only a limited number of investigations in avian species (Pozio 2005). Additionally, the sensitivity of the digestion assay may be limited when applied to the generally smaller muscle samples (Gottstein et al., 2009) obtainable from birds. Of 23 previous reports, only one was from a bird in North America (Lindsay et al., 1995). Another possibility could be that this parasite was introduced to the region through a migratory terrestrial mammal covering the considerable distance required to reach this region. As the positive wolverine was located close to small human communities (Fort Providence, Fort Resolution, Hay River, and Enterprise), the wolverine may have acquired its infection by scavenging on discarded meat brought in by people from abroad; however, these are not major hubs for international travel or immigration. Based on this single finding of *T. pseudospiralis*, we cannot assume that it is actively circulating in northern Canadian wildlife; further sampling of wild mammals and birds from Northwest Territories and British Columbia and more broadly across North America seems warranted.

The higher burden of *T. pseudospiralis* larvae in this wolverine's diaphragm versus tongue is consistent with a report in foxes experimentally infected with other species of *Trichinella* (Kapel et al., 2005). In contrast, larvae of *T. nativa* and *Trichinella* T6 had a higher intensity of infection in tongue vs diaphragm in wolverine (Sharma et al., 2018). Although less sensitive than digestion (Forbes et al., 2003), the examination of tongue by compressorium was performed to attempt detection of in-situ larvae, particularly those with capsules. However, we did not detect any encapsulated larvae from this wolverine by this method, nor were any other species of *Trichinella* (such as T2 or T6) detected on multiplex PCR, but *T. pseudospiralis*.

In spite of the fact that we used a highly sensitive digestion method for recovery of *Trichinella* spp (Forbes et al., 2003), we found only one positive wolverine (of 131) with *T. pseudospiralis*, whereas prevalence of freeze-resistant species of *Trichinella* (*T. nativa* and *Trichinella* T6) was much higher (62%, 81/131) (Sharma et al., unpublished). The digestion assay is validated for the detection of live larvae in fresh samples; therefore using frozen samples (as in the situation here) may have reduced overall sensitivity especially for freeze susceptible species of *Trichinella* such as *T. pseudospiralis*. The tongue and diaphragm of wolverines tested were kept frozen at −20 °C prior to processing, and underwent two cycles of freeze-thaw, which might have killed larvae of *T. pseudospiralis*, and thus artificially decreased prevalence and intensity of *T. pseudospiralis*. Monitoring studies for *Trichinella* based on freshly harvested wildlife carcasses might reveal a higher prevalence of freeze-susceptible species such as *T. pseudospiralis* and *T. spiralis* than previously suspected. As well, a sequential sieving method could offer higher sensitivity than the standard artificial digestion method for larvae of freeze susceptible *Trichinella* species when sampling frozen tissue (Franssen et al., 2014), frequently the only option for wildlife.

Reports of *T. pseudospiralis* in harvested wild animals from North America indicate that this zoonotic species is circulating and poses a potential risk to public health. Human outbreaks in North America have not been attributed to *T. pseudospiralis*; however, definitive identification is rarely performed on human isolates (Dalcin et al., 2017; Gamble et al., 2005; Houze et al., 2009; McIntyre et al., 2007; Reichard et al., 2017; Serhir et al., 2001). Wolverines are not harvested for food but rather for fur; thus, the presence of *T. pseudospiralis* in this carnivore does not raise immediate public health concerns. As well, *T. pseudospiralis* is susceptible to freezing, lessening the likelihood of successful establishment in the Arctic, even if introduced with some regularity by migratory birds. Future regional surveillance efforts for *Trichinella* spp. could focus on wolverine (as a sentinel species), as well as other carnivorous or omnivorous wildlife, including game animals (such as bear and wild boar), rodents, and birds.

5. Conclusion

We report new host and geographic records for *T. pseudospiralis* in a wolverine, the first report of this parasite on the mainland of Canada. This isolate was most closely related to those from an island in western Canada, Russia and Argentina, but not to populations of parasites at geographically proximate localities from the continental USA. Distribution of *T. pseudospiralis* in subarctic Canada may emphasize the potential role of migratory birds in long distance dispersal and potential introduction of non-native pathogens into remote regions. Additional field sampling is needed to elucidate whether *T. pseudospiralis* is established in the Canadian North or has been introduced transiently to the area via carnivorous migratory birds or terrestrial mammals. Our discovery, linked to broader explorations of parasite diversity, emphasizes the importance of genetic identification of *Trichinella* using sequencing as well as the utility of monitoring for *Trichinella* spp. and other important food-borne parasites in wildlife, especially high trophic level carnivores and scavengers as an upstream measure of human risk.

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