

Serendipitous discovery of a novel protostrongylid (Nematoda: Metastrongyloidea) in caribou, muskoxen, and moose from high latitudes of North America based on DNA sequence comparisons

Susan J. Kutz, Ingrid Asmundsson, Eric P. Hoberg, Greg D. Appleyard, Emily J. Jenkins, Kimberlee Beckmen, Marsha Branigan, Lem Butler, Neil B. Chilton, Dorothy Cooley, Brett Elkin, Florence Huby-Chilton, Deborah Johnson, Abdurakhim Kuchboev, John Nagy, Michelle Oakley, Lydden Polley, Richard Popko, Aedes Scheer, Manon Simard, and Alasdair Veitch

Abstract: Fecal samples are often the only feasible means to assess diversity of parasites in wildlife; however, definitive identification of egg or larval stages in feces by morphology is rarely possible. We determined partial sequences from the second internal transcribed spacer region (ITS-2) of nuclear ribosomal DNA for first-stage, dorsal-spined larvae (DSL) in feces from caribou (*Rangifer tarandus tarandus* (L., 1758), *Rangifer tarandus caribou* (Gmelin, 1788), *Rangifer tarandus grantii* (Allen, 1902)), muskoxen (*Ovibos moschatus moschatus* (Zimmermann, 1780), *Ovibos moschatus wardi* Lydekker, 1900), moose (*Alces alces gigas* Miller, 1899 and *Alces alces andersoni* Peterson, 1952), and from the tissue of one slug (*Deroceras laeve* (Müller, 1774)) in Arctic–Subarctic North America. A previously uncharacterized, genetically distinct species was recognized based on sequences of 37 DSL from 19 ungulate hosts and the slug. Sequence similarity among individuals of this novel species was 91%–100%. For many individual DSL, paralogues of ITS-2 were detected. ITS-2 sequences from the novel species were 72%–77% similar to those of *Varestrongylus alpenae* (Dikmans, 1935) and 51%–61% similar to those of other protostrongylids known in North American and some Eurasian ungulates. Results indicate a discrete lineage of an undescribed protostrongylid infecting muskoxen, caribou, and moose from Alaska to Labrador. Symptomatic infections with *Parelaphostrongylus andersoni* Prestwood, 1972 were found in three caribou herds.

Received 17 February 2007. Accepted 21 August 2007. Published on the NRC Research Press Web site at cjz.nrc.ca on 4 December 2007.

S.J. Kutz.¹ Faculty of Veterinary Medicine, University of Calgary, Calgary, AB T2N 4N1, Canada.

I. Asmundsson and E.P. Hoberg. USDA, Agricultural Research Service, US National Parasite Collection and Animal Parasitic Disease Laboratory, BARC East 1180, 10300 Baltimore Avenue, Beltsville, MD 20705, USA.

G.D. Appleyard. Alberta Provincial Laboratory for Public Health (Microbiology), 3030 Hospital Drive, Calgary, AB T2N 4W4, Canada.

E.J. Jenkins. Environment Canada, Canadian Wildlife Service, 115 Perimeter Rd, Saskatoon, SK S7N 0X4, Canada.

K. Beckmen. Alaska Department of Fish and Game, Division of Wildlife Conservation, 1300 College Road, Fairbanks, AK 99701, USA.

M. Branigan and J. Nagy. Department of Environment and Natural Resources, Government of the Northwest Territories, Inuvik, NT X0E 0T0, Canada.

L. Butler. Alaska Department of Fish and Game, Division of Wildlife Conservation, Main Street, King Salmon, AK 99613-0037, USA.

N.B. Chilton. Department of Biology, University of Saskatchewan, Saskatoon, SK S7N 5E2, Canada.

D. Cooley. Department of Natural Resources, Government of Yukon, Dawson City, YT Y0B 1G0, Canada.

B. Elkin. Department of Environment and Natural Resources, Government of the Northwest Territories, Yellowknife, NT X1A 2L9, Canada.

F. Huby-Chilton. Centre for Foodborne and Animal Parasitology, Canadian Food Inspection Agency, Saskatoon, SK S7N 2R3, Canada.

D. Johnson. Department of Environment and Natural Resources, Government of the Northwest Territories, Fort Smith, NT X0E 0P0, Canada.

A. Kuchboev. Institute of Zoology, Uzbek Academy of Sciences, Tashkent, Uzbekistan.

M. Oakley. Department of Natural Resources, Government of Yukon, Haines Junction, YT Y0B 1L0, Canada.

L. Polley. Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK S7N 5B4, Canada.

R. Popko and A. Veitch. Department of Environment and Natural Resources, Government of the Northwest Territories, Norman Wells, NT X0E 0V0, Canada.

A. Scheer. 4488 Wheeler Rd., Prince George, BC V2N 5H7, Canada.

M. Simard. Makivik Corporation, Kuujuuaq, QC J0M 1C0, Canada.

¹Corresponding author (e-mail: skutz@ucalgary.ca).

Résumé : L'échantillonnage des fèces est souvent la seule méthode praticable pour évaluer la diversité des parasites de la faune sauvage; il est, cependant, rarement possible d'identifier de façon sûre les oeufs et les stades larvaires d'après leur morphologie. Nous avons déterminé des séquences partielles de la seconde région de l'espaceur interne transcrit (ITS-2) de l'ADN nucléaire ribosomique des larves à épines dorsales (DSL) de premier stade dans les fèces de caribous (*Rangifer tarandus tarandus* (L., 1758), *Rangifer tarandus caribou* (Gmelin, 1788) et *Rangifer tarandus grantii* (Allen, 1902)), de boeufs musqués (*Ovibos moschatus moschatus* (Zimmermann, 1780) et *Ovibos moschatus wardi* Lydekker, 1900) et d'originaux (*Alces alces gigas* Miller, 1899 et *Alces alces andersoni* Peterson, 1952), ainsi que dans les tissus d'une limace (*Deroceras laeve* (Müller, 1744)) provenant de l'Amérique du Nord Arctique et Subarctique. Nous avons découvert une espèce distincte non encore caractérisée génétiquement d'après les séquences de 37 DSL provenant de 19 hôtes ongulés et de la limace. La similarité des séquences parmi les individus de cette nouvelle espèce est de 91%–100%. Chez plusieurs DSL individuelles, on trouve des paralogues d'ITS-2. Les séquences d'ITS-2 de la nouvelle espèce ont une similarité de 72%–77% avec celles de *Varestrongylus alpenae* (Dikmans, 1935) et de 51%–61% avec celles des autres protostrongylidés connus chez les ongulés d'Amérique du Nord et chez certains ongulés d'Eurasie. Nos résultats indiquent donc l'existence d'une lignée séparée d'un protostrongylidé inédit qui infecte les boeufs musqués, les caribous et les originaux de l'Alaska au Labrador. Il y a des infections sympatriques de cette espèce et de *Parelaphostrongylus andersoni* Prestwood, 1972 dans trois troupeaux de caribous.

[Traduit par la Rédaction]

Introduction

Protostrongylid nematodes are important and often pathogenic parasites of ungulates (Kutz et al. 1999; Lankester 2001; Jenkins et al. 2005b). These parasites have indirect life cycles, requiring gastropod intermediate hosts for development. First-stage larvae among species of the genera *Parelaphostrongylus* Boev and Schulz 1950, *Elaphostrongylus* Cameron 1931, *Umingmakstrongylus* Hoberg, Polley Gunn and Nishi 1995, *Cystocaulus* Schulz, Orlov and Kutass 1933, *Varestrongylus* Bhalerao 1932, and *Muellerius* Cameron 1927 are morphologically similar, bearing a dorsal spine on the tail (Boev 1975; Kontrimavichus et al. 1976). In some cases, genera can be differentiated based on total length of larvae (Gray et al. 1985) or subtle differences in tail morphology (S.J. Kutz, unpublished data for *Umingmakstrongylus pallikuukensis* Hoberg, Polley, Gunn and Nishi 1995). However, substantial variation in tail structure among conspecifics of *Parelaphostrongylus* has been documented (e.g., Hoberg et al. 2005). Consequently, definitive identification of species typically has been based on recovery of adult parasites or application of molecular techniques and DNA sequencing to identify larvae. The latter requires validation by comparisons with sequences from adult parasites that have been identified based on morphology (Jenkins et al. 2005a; Huby-Chilton et al. 2006). Sequence data of the ITS-2 are currently available for all seven species of protostrongylids in North America known to produce dorsal-spined larvae (DSL) (Jenkins et al. 2005a) (Table 1).

Host and geographic ranges for protostrongylids, including elaphostrongyline, muellerine, and protostrongyline at high latitudes of North America, remain poorly described (Hoberg et al. 1995; Kutz et al. 2001b; Lankester 2001). With the exception of the island of Newfoundland, where *Elaphostrongylus rangiferi* Mitskevich, 1960 is thought to have been introduced along with reindeer (*Rangifer tarandus tarandus* (L., 1758)) from the Old World, DSL in either woodland (*Rangifer tarandus caribou* (Gmelin, 1788)) or barren-ground (*Rangifer tarandus groenlandicus* (Borowski, 1780) and *Rangifer tarandus grantii* (Allen, 1902)) caribou native to North America have been assumed to be *Parela-*

phostrongylus andersoni Prestwood, 1972 or *Parelaphostrongylus odocoilei* (Hobmaier and Hobmaier, 1934) (e.g., Lankester 2001). In the Canadian north, the elaphostrongyline *P. andersoni* and *P. odocoilei* have been confirmed previously in woodland caribou and *P. andersoni* in barren-ground caribou by postmortem recovery and morphological examination of adult nematodes (Lankester and Hauta 1989) or by molecular analyses of larvae (Jenkins et al. 2005a; Chilton et al. 2005, Huby-Chilton et al. 2006). Unidentified DSL were found previously in woodland caribou from the Mealy Mountains, Labrador (Lankester and Hauta 1989), from northeastern Alberta (Gray and Samuel 1986), and from Manitoba and Ontario (Lankester et al. 1976). Until the present study, DSL identified in muskoxen (*Ovibos moschatus moschatus* (Zimmermann, 1780)) from North America were attributed to *U. pallikuukensis*, and there were no records of DSL in *Ovibos moschatus wardi* Lydekker, 1900. The few anecdotal reports of DSL in moose (*Alces alces gigas* Miller, 1899) from Alaska had not been confirmed (E.P. Hoberg, unpublished observations).

The aim of the current study was to better define the biodiversity, host range, and geographic distribution for species of Protostrongylidae among ungulates in the Nearctic. The ITS-2 was sequenced for DSL recovered from fecal samples in muskoxen, caribou, and moose across an extensive region from Labrador, through Quebec, Manitoba, Nunavut, and the Northwest Territories, Canada, into Alaska, USA. The results have demonstrated the widespread occurrence of a previously unknown species of Protostrongylidae.

Materials and methods

Parasite recovery

Fecal samples were collected by northern collaborators from muskoxen (*O. m. moschatus* and *O. m. wardi*), barren-ground caribou, woodland caribou, and moose (Tables 2 and 3, Fig. 1). Fecal samples were collected from the ground or from captured animals and were kept frozen until examination. DSL were isolated from fecal samples using a modified beaker Baermann technique; (Forrester and

Table 1. Sources of Protostrongylidae used for comparisons of sequences of the second internal transcribed spacer region (ITS-2).

Species	Host	Geographic locality	GenBank ^a	Vouchers ^b
Muelleriinae				
<i>Cystocaulus ocreatus</i> ^c	<i>Ovis aries</i>	Ferghana Valley, Uzbekistan	EU018481	95144, 97531
<i>Umingakstrongylus pallikuukensis</i>	<i>Ovibos moschatus</i>	Nunavut, Canada	AY648409	94884
<i>Muellerius capillaris</i> ^d	<i>Ovis aries</i>	Ontario, Canada	AY679527	94888, 94889
Elaphostrongylinae				
<i>Elaphostrongylus alces</i> ^e	<i>Alces alces</i>	Sweden	AF504034	NA ^f
<i>Elaphostrongylus cervi</i> ^e	<i>Cervus elaphus</i>	New Zealand	AF504026	NA ^f
<i>Elaphostrongylus rangiferi</i> ^e	<i>Rangifer tarandus tarandus</i>	Pallasjärvi, Finland	EU018482	94877–94879
<i>Elaphostrongylus rangiferi</i> ^e	<i>Rangifer tarandus caribou</i>	Newfoundland, Canada	AF504033	NA ^f
<i>Parelaphostrongylus andersoni</i> ^d	<i>Rangifer tarandus groenlandicus</i>	Northwest Territories, Canada	AY504030	94890
<i>Parelaphostrongylus odocoilei</i> ^d	<i>Ovis dalli dalli</i>	Northwest Territories, Canada	AY504031	94329–94334
<i>Parelaphostrongylus tenuis</i> ^e	<i>Odocoileus virginianus</i>	Minnesota, USA	AF504029	NA ^f
Protostrongylinae				
<i>Protostrongylus rufescens</i> ^c	<i>Ovis aries</i>	Namangan region, Uzbekistan	EU018485	97527
<i>Protostrongylus stilesi</i> ^c	<i>Ovis dalli dalli</i>	Northwest Territories, Canada	EU018484	96821, 96822
Neostrongylinae				
<i>Orthostrongylus macrotis</i> ^c	<i>Odocoileus hemionus</i>	Saskatchewan, Canada	EU018483	96786, 96787
Varestrongylinae				
<i>Varestrongylus alpenae</i> ^d	<i>Odocoileus virginianus</i>	Manitoba, Canada	AY648407	94204

^aGenBank accession numbers for ITS-2 sequences from individual first larval stage (L1) or adults of representative species.

^bVoucher specimens with definitive identifications and accession numbers archived in the US National Parasite Collection.

^cSequence information reported here for the first time.

^dSequence previously in GenBank as reported by Jenkins et al. (2005a).

^eSequence previously in GenBank as reported by Gajadhar et al. (2000).

^fSequences not accompanied by physical voucher specimens held in museum archival collections at time of original report.

Lankester 1997; Jenkins et al. 2005a) (Tables 2 and 3). Larvae were either immediately processed for molecular analyses or frozen at -80°C in tap water. Materials from muskoxen in Quebec were preserved in 70% ethanol and refrigerated prior to molecular analyses. Data for numbers of DSL sequenced and their distribution among host species and geographic localities are summarized in Table 2. Data for the distribution of additional unsequenced DSL in caribou, muskoxen, and moose are summarized in Table 3.

A total of 165 potential slug intermediate hosts (*Dero-ceras laeve* (Müller, 1774)) were examined for the presence of protostrongylid larvae by digestion using pepsin-HCl (Hoberg et al. 1995) (Table 2). Larvae recovered from digests were refrigerated at 4°C in water prior to further analyses.

DNA extraction and amplification

Individual larvae from respective samples were transferred by micropipette to single tubes prior to extraction of DNA. Successful transfer was confirmed by microscopic examination at high power under a binocular dissecting scope. Genomic DNA (gDNA) was extracted from individual larvae either by using a DNeasy Tissue Kit (Qiagen) or by heating to 90°C for 10 min in 10 μL of water and cooling on ice for 25 min. Extraction buffer (20 μL , composed of 0.5 mg/ml of proteinase K, 10 \times PCR buffer, and 2.5% 2-mercaptoethanol) was added and the mixture was incubated at 65°C for 2 h, followed by heating to 90°C for 10 min. A PCR modification from Gajadhar et al. (2000) was performed using the primers NC1 (5'-ACGTCTGGTTCAGGGTTGTT-3') and NC2 (5'-TTAGTTTCTTTTCCCTCCGCT-3'). Each 50 μL PCR

reaction contained 34 μL of water, 5 μL of 10 \times PCR buffer, 4 μL of 25 mmol MgCl_2 , 0.5 μL of 25 mmol dNTPs, 2 μL (40 pmol) of each primer, 0.25 μL of *Taq* DNA polymerase, and 2 μL of sample DNA overlaid with one drop of mineral oil. The amplification conditions used were an initial 3 min denaturation at 94°C , followed by 35 cycles of 94°C for 60 s, 60°C for 60 s, and 72°C for 60 s. A final extension phase of 72°C for 10 min was followed by cooling to 4°C . Reagent-only (i.e., no gDNA) reactions were used as negative controls to detect potential contamination.

PCR products were sequenced directly using NC1 and NC2 primers or cloned using the Topo TA cloning kit (Invitrogen) and sequenced in both directions using M13 forward and reverse primers. Twelve colonies each were sequenced from cloned PCR products of two individual first larval stages (L1s) from moose (Alaska) and mountain woodland caribou (Northwest Territories). Fluorescent sequencing reactions incorporated BigDye Terminator v.3.1 (Applied Biosystems) and were analysed on a 3730xl capillary sequencer (Applied Biosystems).

Sequence analysis

Sequences from DSL were edited using Sequencher 4.6 (Gene Codes) or Seqman and Megalign (DNA Star) and aligned with those from representative protostrongylids using Vector NTI Advance 9 AlignX (Invitrogen). Taxa included species of all genera of protostrongylids occurring in North America and additional species from the Palearctic that produce DSL (Table 1). Sequences at the ITS-2 locus were compared with the following eight genera and 13 spe-

Table 2. Collection data for dorsal-spined larvae sequenced from caribou, muskoxen, and moose across the northern Nearctic.

Map reference	Host species	Place name or herd ID	Coordinates (decimal degrees)	Date	% prevalence (n)	L1 (host)	Novel sp.	<i>Parelaphostrongylus andersoni</i>	GenBank accession No.	Collector(s)
Caribou										
1a	<i>Rangifer tarandus grantii</i>	North Alaska Peninsula	57.60517, -156.51867; 59.63842, -157.36818	Aug. 2005 – June 2006	78 (36)	43 (9)	3	40	EU018478	L. Butler
1b	<i>R. t. grantii</i>	Mulchatna	59.38.305, 157.22.091	June 2006	60 (5)	3 (1)	0	3		B. Dale
2	<i>R. t. grantii</i>	Chisana	61.65167, -140.84528	May 2003	29 (24)	1 (1)	0	1		M. Oakley
3	<i>R. t. grantii</i>	Porcupine	66.5, -136.7	Feb. 2003	47 (17)	4 (4)	1	3	EU018474	A. Scheer
4	<i>R. t. groenlandicus</i>	Cape Bathurst	69.033936, 133.761606	Nov. 2002	61 (44)	4 (4)	2	2	EU018472, EU018476	J. Nagy
5	<i>R. t. caribou</i>	Tsiigehtchic	67.09378, 132.911501	Mar. 2003	35 (17)	1 (1)	0	1		J. Nagy
6	<i>R. t. groenlandicus</i>	Bluenose West		June 2003	15 (26)	1 (1)	0	1		J. Nagy
7	<i>R. t. groenlandicus</i>	Bluenose East	65.02842, -123.47882	Mar. 2002	35 (20)	2 (1)	1	0	EU018467, EU018475	B. Olson
8	<i>R. t. caribou</i>	Godlin Lakes, N.T.	63.8, -128.76667	Mar. 2001	20 (10)	1 (1)	2	0	EU018473	A. Zimmer
9	<i>R. t. groenlandicus</i>	Beverly	61.98333, -102.46667	Apr. 2000	35 (25)	11 (1)	8	3	EU018461	B. Elkin
10	<i>R. t. caribou</i>	Mealy Mountains, Labrador	53, -60	Nov. 2002 – Apr. 2006	32 (25)	100 (5)	3	97	EU018463, EU018466	R. Otto
Muskox										
11	<i>Ovibos moschatus wardi</i>	Aklavik, N.T.	68.5274, -136.26615	Jan. 2000	40 (5)	2 (2)	2	0	EU018469, EU018477	M. Branigan
12	<i>O. m. wardi</i>	Firth River, Yukon	69.3, -139.49806	July 2000	100 (9)	2 (2)	2	0	EU018470, EU018471	D. Cooley, M. Kienzler
13	<i>O. m. moschatus</i>	Thelon, Nunavut	62.43, -109.1	Mar. 2003	50 (2)	1 (1)	1	0	EU018480	D. Johnson, B. Elkin
14	<i>O. m. wardi</i>	Nunavik, Quebec	58.65, -68.95	Dec. 2005	100 (5)	3 (3)	3	0	EU018464, EU018465, EU018479	M. Simard, S. Kutz
Moose										
15	<i>Alces alces gigas</i>	Tlikakila River, Lake Clark, Alaska	60.45133, -153.7955	Apr. 2004	16 (6)	10 (1)	10	0	EU018468	M. Szepanski
Slug										
16	<i>Deroceras laeve</i>	Palmer Lake, N.T.	64.28, -129.37	July 2003	1 (165)	2 (2)	1	0	EU018462	E. Jenkins

Table 3. Prevalence of dorsal-spined larvae in caribou, muskoxen, and moose from Yukon, Northwest Territories, and Nunavut, based on general fecal surveillance 1993–2004.

ID	Host species	Location	Date collected	% prevalence (n)	Collector(s)
04, 09, 13	<i>Rangifer tarandus pearyi</i>	Banks Island, N.T.	Nov. 2000 – Dec. 2002	0 (131)	J. Nagy
99	<i>R. t. pearyi</i>	Victoria Island, N.T.	Aug. 2003	0 (10)	J. Nagy
44, 48, 49, 118, 121	<i>R. t. caribou</i>	Sahtu, N.T.	Jan. 2002 – Mar. 2004	30–90 (112)	R. Popko, A. Zimmer
130	<i>R. t. caribou</i>	South Slave, N.T.	Mar. 2003	10 (31)	D. Johnson
94	<i>R. t. groenlandicus</i>	North Baffin region, Nunavut	Apr. 1993	23 (56)	B. Elkin
141, 120	<i>R. t. groenlandicus</i>	Bluenose East herd, Sahtu, N.T.	Mar. 2004	20–24 (27)	C. Yukon, B. Kenny, A. Zimmer
24, 125	<i>R. t. granti</i>	Chisana herd, SW Yukon	Mar. 2001, May 2004	43, 60 (27)	R. Farnell, M. Oakley
108	<i>R. t. groenlandicus</i>	Bluenose West, Sahtu, N.T.	Mar. 2001	20 (10)	R. Popko
45, 50, 60	<i>Alces alces andersoni</i>	Sahtu, N.T.	Mar. 2002 – May 2002	0 (15)	R. Popko, B. Benn
53, 86, 127	<i>A. a. gigas</i>	Yukon	2002	0 (26)	R. Ward, M. Oakley
135	<i>Ovibos moschatus wardi</i>	Banks Island, N.T.	Aug. 2004	0 (72)	J. Lucas, J. Nagy

Note: Larvae from these collections were not identified by DNA sequencing.

cies: *Parelaphostrongylus tenuis* (Dougherty, 1945), *P. odo-coilei*, *P. andersoni*, *Elaphostrongylus alces* Steen, Chabaud and Reh binder 1989, *E. rangiferi*, *Elaphostrongylus cervi* Cameron, 1931, *Cystocaulus ocreatus* (Railliet et Henry 1908), *U. pallikuukensis*, *Muellerius capillaris* (Mueller, 1889), *Varestrongylus alpenae* (Dikmans, 1935), *Protostrongylus rufescens* (Leuckart, 1865), *Protostrongylus stilesi* Dikmans, 1931, and *Orthostrongylus macrotis* (Dikmans, 1931). Sequences of the ITS-2 for *C. ocreatus*, *P. rufescens*, and *O. macrotis* are reported here for the first time.

Sequence alignments were edited by eye in GeneDoc v. 2.6.002 (Nicholas and Nicholas 1997). A statistics report for this alignment, showing calculations for exact matches and aligned with gaps as a percent value, was generated using GeneDoc. The relationship of these novel sequences to those from other protostrongylids was inferred using PAUP* v. 4.0b10 (Swofford 2002) to construct a neighbour-joining tree based on both uncorrected (P) and HKY85 distances. The best fit model of DNA substitution was determined by Modeltest3.7 (Posada and Crandall 1998).

Morphological comparisons

Based on sequence data, muskoxen from Nunavik and Aklavik had monospecific infections with the undescribed species, and therefore, representative DSL from these hosts were used to describe meristic and morphological characteristics (Table 4, Fig. 2). Specimens were examined using high magnification light microscopy and differential interference contrast. Standard measurements were derived from examination of 10 specimens preserved in ethanol from Nunavik and 20 specimens that were heat-killed in water from Aklavik. Meristic data were then compared for DSL representing species known to occur in North American ungulates (Boev 1975; Kontrimavichus et al. 1976; Lankester 2001).

Results

Prevalence of DSL in fecal samples from ungulates

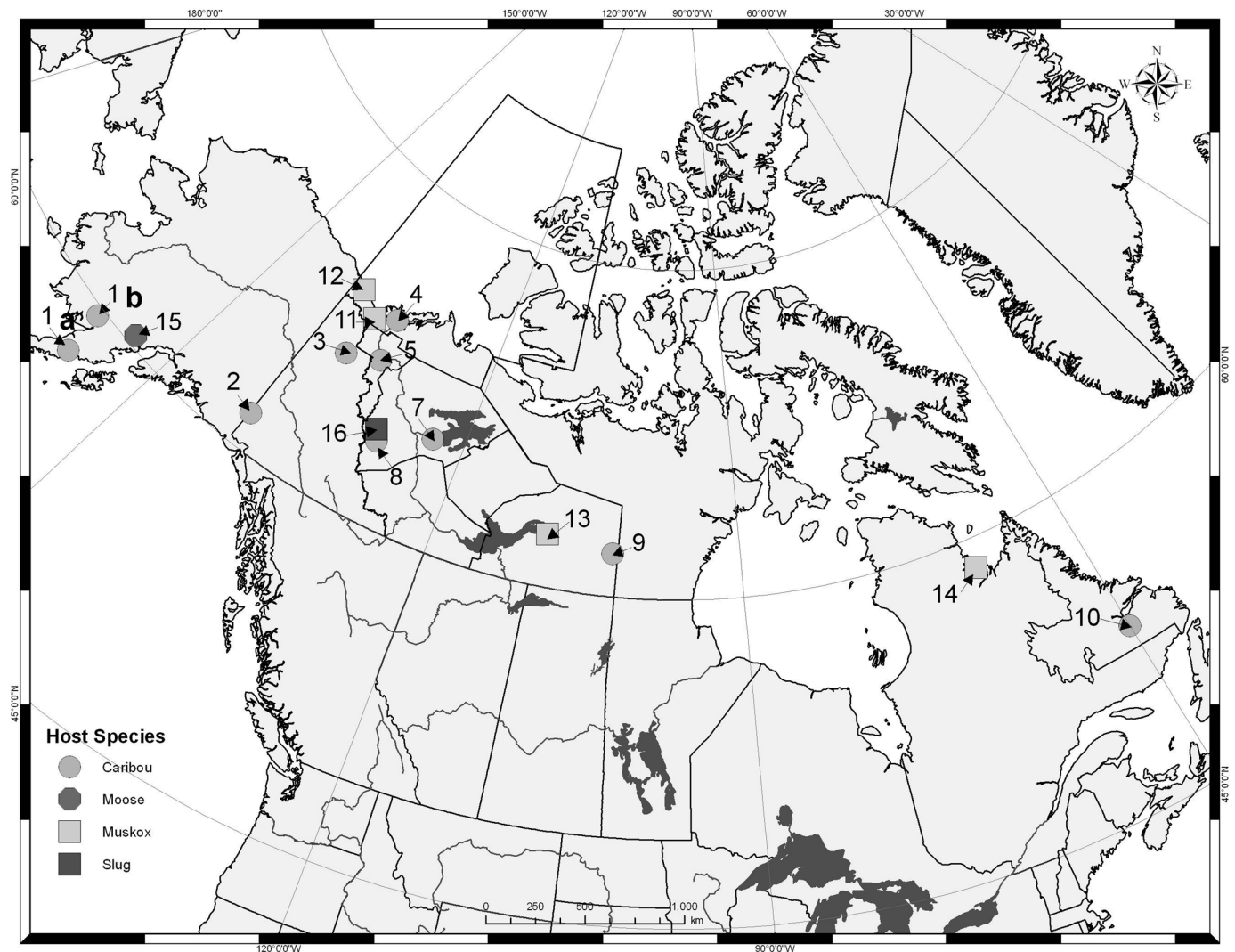
DSL were common in mainland caribou and muskoxen in a range extending from Alaska to Labrador and were present in one moose from Alaska (Tables 2 and 3). The range of prevalence for DSL was 15%–78% (barren ground caribou), 0%–90% (woodland caribou), and 50%–100% (mainland muskoxen). No DSL were found in muskoxen ($n = 72$) or caribou ($n = 141$) on Banks or Victoria islands or from moose (41) in Canada.

Sequence comparisons

91 DSL were sequenced from 36 ungulate hosts, and two third-stage larvae were sequenced from two slugs. Based on direct comparisons of the ITS-2 sequence from individual larvae, a novel species of protostrongylid was identified based on 37 sequences in 19 of the ungulate hosts (six barren ground caribou, four mountain woodland caribou, eight muskoxen, and one moose) (Table 2). *Parelaphostrongylus andersoni* was also identified in caribou, but no other protostrongylids were found in muskoxen or moose.

Twelve individual colonies from cloned PCR products of the novel larvae from one woodland caribou and one moose were sequenced, revealing multiple sequence types of ITS-2 present in single DSL. These appear to represent two major

Fig. 1. Map of Alaska and Canada indicating the sampling sites for larvae that were sequenced. Numbers correspond to identification numbers in Table 2.



sequence types (Table 5), and some nucleotide variation within each group may be a result of the cloning process. Owing to the high level of similarity between these two groups, the exclusion of sequences representing either of these groups does not affect determination of relative similarity based on pairwise comparisons between the unknown and other protostrongylids (Table 6).

The alignment of 38 novel sequences (37 from L1s in ungulates; one from an L3 in a slug) and those for eight genera and 13 species of protostrongylids consists of 459 nucleotides including gaps; individual sequences range from 304–401 bases. A reconstruction of the relationship of the novel sequences to the other protostrongylids using the criterion of minimum evolution and the neighbour-joining algorithm is shown in Fig. 3; the reconstruction shown includes single sequences for each host at each locality for a total of 20. DNA distances employed, HYK85 plus gamma (shape = 1.1459) rates for variable sites, were selected as the model that closest fitted the data as defined by ModelTest using the hierarchical likelihood ratio tests. This relationship was independent of the type of DNA distances used to construct

the tree. Relationships demonstrate reciprocal monophyly (sister groups) with respect to the putative novel species and *V. alpenae*.

Pairwise comparisons among the 38 novel sequences showed a mean of 96%; 91%–100% of residues are an exact match across the entire alignment, less than 2% of the residues aligned with gaps. The mean pairwise comparison among the novel sequences and *V. alpenae*, the most similar of the protostrongylids, is 75% (72%–77%) exact match, with 12% (10%–13%) aligning with gaps. Similarity to 12 other protostrongylids, including any of the elaphostrongylines, was minimal, with ranges between 51%–62% for an exact match and 16%–26% of residues aligned with gaps (Table 6).

In addition to the novel species, sequences of DSL in barren-ground ($n = 54$ DSL in 17 hosts) and woodland caribou ($n = 1$ DSL in 1 host) matched that of *P. andersoni*. Both *P. andersoni* and the novel species were found in the Porcupine, Cape Bathurst, Beverly, North Alaska Peninsula, and Mealy Mountain herds; concurrent infections in individual caribou were demonstrated in the Beverly herd, the Mealy

Table 4. Morphometrics (range (μm) with mean in parentheses) of first-stage larvae (L1) of the putative novel Protostrongylidae in muskoxen from Nunavik, Quebec, and Aklavik, Northwest Territories, with comparisons to species with dorsal-spined larvae occurring in North America (excluding *Elaphostrongylus rangiferi*).

	Protostrongylidae (Nunavik) ^a	Protostrongylidae (Northwest Territories) ^b	<i>Muellerius capillaris</i> ^c	<i>Parelaphostrongylus andersoni</i> ^d	<i>Parelaphostrongylus odocoilei</i> ^e	<i>Parelaphostrongylus tenuis</i> ^f	<i>Umingmakstrongylus pallikuukensis</i> ^g	<i>Varestrongylus alpenae</i> ^h
Body length	281–374 (329)	348–400 (377)	250–320	308–382 (351)	334–428 (387)	310–380 (348)	396–435 (411)	310–380
Body width ⁱ	16–23 (19.5)	17–20 (18)	12–15	17–18 (17)	15–20 (18)	16–19 (18)	18–24 (22)	15–17
Nerve ring ^j	—	78–107 (97)	—	66–109 (94)	89–117 (104)	80–112 (94)	91–120 (108)	85–93
Excretory pore ^j	71–105 (84.5)	92–107 (102)	75–90	66–109 (94)	92–117 (105)	80–112 (94)	103–115 (109)	85–93
Esophagus length ^j	88–155 (128)	151–180 (168)	120–160	163–183 (175)	160–206 (181)	132–181 (165)	189–212 (199)	155–180
% body length ^j	28–46 (38)	43–46 (45)	48–50	41–54 (50)	44–50 (47)	43–48	46–51	47–50
Esophagus width	8–11.5 (10)	9–15 (12)	—	—	—	—	12–16 (14)	—
Genital primordium ^j	173–224 (206)	218–273 (244)	—	216–249 (234)	219–285 (256)	210–246 (224)	257–280 (269)	195–242
% body length ^j	62–64 (63)	61–68 (65)	—	60–70 (66)	64–73 (66)	65–67	—	63–64
Tail length (from anus)	31–42 (35)	32–41 (38)	30–40	27–36 (32)	33–46 (39)	29–41 (32)	38–50 (45)	—
Tail extension (entire) ^k	8–11 (9)	6–12 (9)	—	—	9–10	—	11–16 (14)	—
Tail spike (tip) ^l	1.6–3.0 (2)	—	—	—	2–3	—	2.5–5 (3.5)	—
Dorsal spine length	1.6–3.0 (2)	—	—	—	2–3	—	2–3 (3)	—

^aMeasurements of unknown L1 in muskoxen from Nunavik, Quebec; determined from 10 specimens, preserved in 70% ethanol, at 1600 \times magnification in differential interference contrast.

^bMeasurements of unknown L1 in muskoxen from Aklavik, Northwest Territories; determined from 20 specimens, heat-killed in water, at 400 \times magnification.

^cIn domestic sheep (Boev 1975).

^dIn white-tailed deer from southeastern North America (Prestwood 1972).

^eIn Dall's sheep from the Mackenzie Mountains, Northwest Territories (Kutz et al. 2001b); measurements were based on typical L1 (see Hoberg et al. 2005).

^fIn white-tailed deer (Anderson 1963).

^gIn muskoxen from the Central Canadian Arctic (Hoberg et al. 1995).

^hIn white-tailed deer from New York, USA (data from Cheatum 1948, cited in Boev 1975).

ⁱDetermined at base of esophagus.

^jMeasured from cephalic extremity.

^kMeasured from constriction in tail (anterior tail fold or basal joint) at level of dorsal spine, posteriad to caudal extremity.

^lMeasured from distal tail fold or joint in tail extension, posteriad to caudal extremity.

Fig. 2. Dorsal-spined larva of an unknown species of Protostrongylidae in *Ovibos moschatus wardi* from Nunavik, Quebec. Photomicrograph in differential interference contrast. Organ systems are noted as follows: exp, excretory pore; eij, esophageal–intestinal junction (base of esophagus); gp, genital primordium; an, anus. Note that the dorsal spine is narrow at its base and that the tail extension (the region posteriad from the level of the dorsal spine) has three tail folds (tf) or prominent joints.

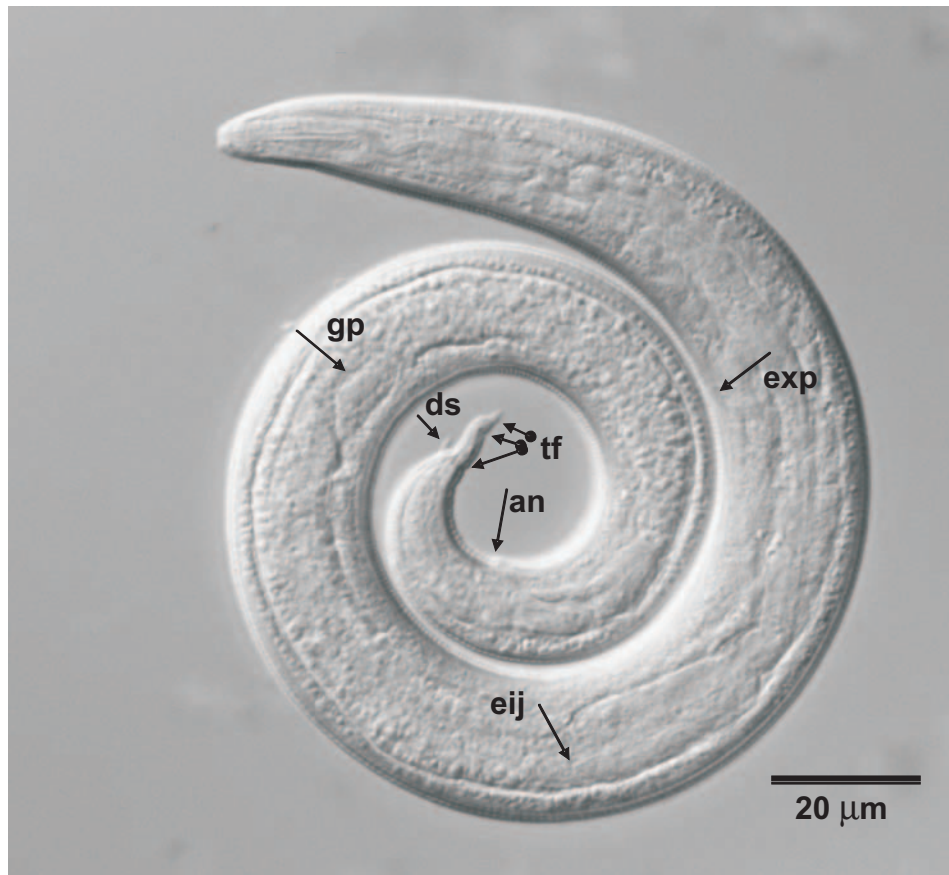


Table 5. Data for the two sequence types of ITS-2 of the putative novel species of Protostrongylidae.

Sequence	Position in ITS-2							
	25	289	325	338	351	407	424	429
1	A	A	GCG	AA	G	AAACAATGCA	T	A
2	G	G	A—	TT	A	—	A	C

Note: Position numbers show distribution of conserved base differences between sequences and position from end of 5.8S rDNA. The numbers apply to sequence 1 after position 325.

Mountain herd, and in Alaska. Sequences from single L3s recovered from two slugs from the Palmer Lake region of the Mackenzie Mountains, Northwest Territories, were identified as *P. odocoilei* and the novel species, respectively. This indicates sympatry for these protostrongylid species in this region.

Morphological comparisons

The DSL from muskoxen in Nunavik and Aklavik were similar in appearance and overall dimensions to DSL from other protostrongylids of the Nearctic (i.e., *M. capillaris*, *P. odocoilei*, *P. andersoni*, *P. tenuis*, *U. pallikuukensis*, and *V. alpenae*) (Table 4, Fig. 2) (Lankester and Hauta 1989; Lankester 2001). Specimens from Aklavik (heat-killed)

were consistently larger than those examined from Nunavik. The latter specimens of DSL had been preserved in 70% ethanol, and as a consequence, meristic data may not be entirely comparable with that reported for other species, particularly where measurements were derived from heat-killed DSL in water.

Discussion

Our results indicate the presence of a previously unrecognized species of protostrongylid circulating in muskoxen, caribou, and moose across an extensive region of the North American Arctic and Subarctic (Tables 2 and 6; Fig. 3). The two sequence types of the undescribed species demonstrated

Fig. 3. Unrooted neighbor-joining (NJ) tree based on HKY85 distances showing relative similarity among a presumed unknown species and other Protostrongylidae. Note reciprocal monophyly for 18 (among 29) representative dorsal-spined larvae (DSL) in barrenground and woodland caribou, muskoxen, and moose and one L3 in a gastropod host relative to *Varestrongylus alpenae* in white-tailed deer with which the unknown shares 75% similarity. Numerical labelling of terminal branches for this unknown protostrongylid is linked directly to data in Table 2 and geographic localities indicated on the map depicting the distribution of sampling (Fig. 1); multiple hosts are represented at some localities. Sequences from single larvae from each recognized host were used to generate the NJ tree. The unknown is further distinct relative to other protostrongylids, including (i) DSL of elaphostrongyline and species of *Elaphostrongylus* (*E. cervi*, *E. alces*, *E. rangiferi*) and *Parelaphostrongylus* (*P. andersoni*, *P. odocoilei*, and *P. tenuis*); (ii) DSL of muellerines, including *Umingmakstrongylus pallikuukensis*, *Muellerius capillaris*, and *Cystocaulus ocreatus*; and (iii) spike-tailed L1 of protostrongyline, including *Protostrongylus rufescens* and *P. stilesi*; and the neostrongyline *Orthostrongylus macrotis*.

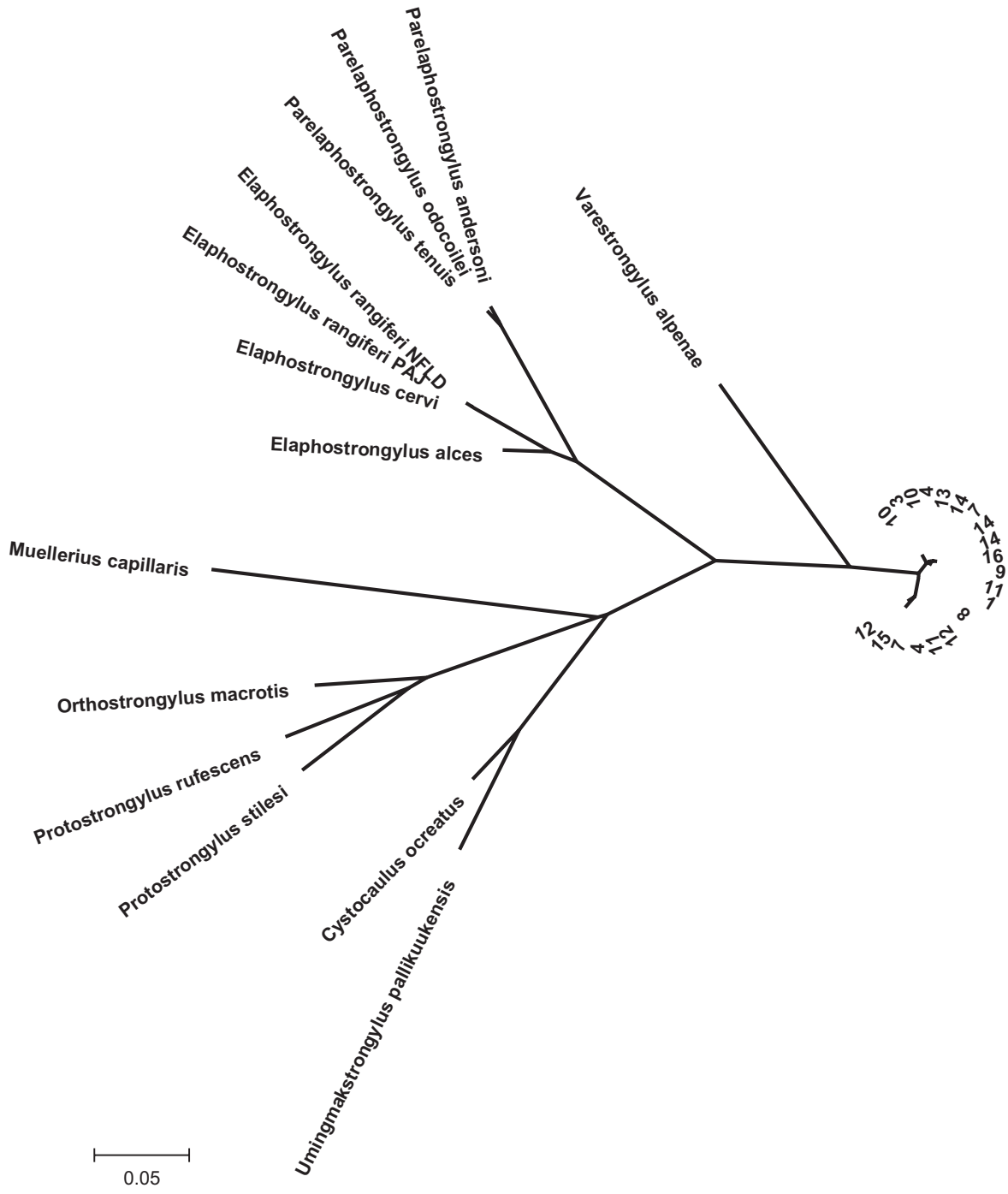


Table 6. Pairwise comparisons for genetic distance in the ITS-2 region among the putative novel species (HKY85 distance matrix, mean with minimum–maximum range in parentheses; below the diagonal), and representative taxa of the Protostrongylidae showing percentage of residues that match exactly across the alignment (percent that align with a gap in parentheses; above the diagonal).

Novel species	V.a.	U.p.	P.r.	P.t.	P.o.	P.a.	O.m.	M.c.	E.r.	E.a.	E.c.	C.o.
Novel species	75 (12)	59 (18)	58 (18)	59 (20)	59 (21)	59 (21)	59 (17)	52 (25)	61 (21)	58 (25)	61 (21)	58 (24)
<i>Varestrongylus alpenae</i> (V.a.)	—	53 (21)	53 (20)	53 (24)	53 (25)	53 (25)	54 (21)	49 (27)	55 (23)	52 (31)	55 (23)	52 (27)
<i>Umingmakstrongylus pallikuukensis</i> (U.p.)	0.4270	—	61 (15)	47 (28)	46 (29)	47 (27)	65 (12)	56 (25)	49 (28)	46 (33)	49 (28)	77 (12)
<i>Protostrongylus rufescens</i> (P.r.)	0.4361	0.3394	—	53 (26)	52 (27)	53 (27)	84 (4)	52 (24)	51 (28)	48 (33)	51 (28)	56 (21)
<i>Parelaphostrongylus tenuis</i> (P.t.)	0.3912	0.4745	0.3585	—	96 (1)	96 (2)	54 (26)	48 (25)	76 (12)	74 (14)	76 (12)	45 (33)
<i>Parelaphostrongylus odocoilei</i> (P.o.)	0.3865	0.4764	0.3653	0.0196	—	95 (3)	53 (27)	48 (25)	77 (10)	75 (13)	77 (10)	44 (34)
<i>Parelaphostrongylus andersoni</i> (P.a.)	0.3812	0.4738	0.3416	0.0141	0.0086	—	54 (27)	48 (26)	76 (12)	76 (13)	76 (12)	45 (33)
<i>Orthostrongylus macrotis</i> (O.m.)	0.4087	0.3035	0.1245	0.3398	0.3406	0.3267	—	56 (23)	53 (27)	50 (32)	53 (27)	58 (20)
<i>Muellertius capillaris</i> (M.c.)	0.4221	0.2942	0.3872	0.4887	0.4918	0.4670	0.3377	—	49 (26)	46 (32)	49 (26)	52 (28)
<i>Elaphostrongylus rangiferi</i> (E.r.)	0.3607	0.4152	0.3730	0.1440	0.1561	0.1390	0.3484	0.4843	—	80 (13)	99 (0)	48 (32)
<i>Elaphostrongylus alces</i> (E.a.)	0.2435	0.3955	0.3593	0.1443	0.1552	0.1353	0.3411	0.4348	0.0729	—	80 (13)	45 (38)
<i>Elaphostrongylus cervi</i> (E.c.)	0.2667	0.4108	0.3687	0.1440	0.1561	0.1393	0.3443	0.4541	0.0000	0.0728	—	48 (32)
<i>Cystocaulus ocreatus</i> (C.o.)	0.2668	0.1235	0.3442	0.4370	0.4387	0.4346	0.3184	0.3348	0.3771	0.3412	0.3729	—

considerable homogeneity and were distinct from all other Protostrongylidae in North America and from *Cystocaulus ocreatus* in caprines and *Elaphostrongylus cervi*, *E. rangiferi*, and *E. alces* in cervids from the Palearctic (Fig. 3). Although comparisons of larval morphology and nucleotide sequences with other protostrongylid species in Eurasia, including *Neostongylus linearis* (Marotel, 1913) and *Pneumocaulus kadenazii* Schulz and Andreeva 1948, are required, it is highly unlikely that this currently undescribed nematode from North America is either of these protostrongylids (Boev 1975; Carreno and Hoberg 1999). DSL of *N. linearis*, which occurs in Caprinae from central Asia and the western Palearctic, have a distinct caudal structure that differs from that seen in the new species (Boev 1975); *P. kadenazii* is a parasite so far only known in musk deer, *Moschus moschiferus* L., 1758, from the Altai region of central Asia, and the detailed structure of the DSL is poorly known (Boev 1975).

In distance-based analyses, the sequences of the unknown consistently grouped with *Varestrongylus alpenae*, a pulmonary parasite of white-tailed deer (*Odocoileus virginianus* (Zimmermann, 1780)) from central and eastern North America (Gray et al. 1985). Reciprocal monophyly is clearly demonstrated (Fig. 3), although sequence similarity is only 72%–77% in a pairwise comparison with *V. alpenae* (Table 6). This may suggest that a search for adult parasites should involve examination of the pulmonary system in ungulate hosts. The unknown is clearly not an elaphostrongyline and thus may not be predicted to occur either in the central nervous system or in the musculature of ungulate hosts.

Prior to the discovery of these novel larvae, we examined lungs from over 50 caribou from the Bluenose East herd, two muskoxen from the Aklavik population, and close to 100 muskoxen sympatric with the Bluenose East caribou herd from the Central Canadian Arctic. *Dictyocaulus eckerti* Skrjabin, 1931 was isolated from both host species and *U. pallikuukensis* and *Protostrongylus stilesi* from muskoxen, but no other nematodes were found in the lungs (S. Kutz, E. Hoberg, B. Elkin and J. Nishi, unpublished data; Hoberg et al. 2002). However, adults of *Varestrongylus* are small, measuring 13–24 mm in length and 0.02–0.10 mm in width (for *V. alpenae*), cause subtle pathology easily missed on gross examination, and may be extremely difficult to detect in the lungs (Gray et al. 1985). Likewise, if the undescribed protostrongylid is associated with an extrapulmonary site, such parasites can be particularly cryptic and obscure (Lankester and Hauta 1989).

Comparative morphology

Although there is extensive overlap in most meristic characters of the new species and other protostrongylids in the Nearctic, specimens of the new species may be relatively smaller (except *M. capillaris*), and the esophagus may represent a lower percentage of overall body length. The DSL that we examined were slightly smaller than those documented by Lankester and Hauta (1989) in woodland caribou from the Mealy Mountains, Labrador, but are in the range of larvae in woodland caribou from Reed Lake, Manitoba (Lankester et al. 1976). Tail structure is similar where three joints or tail folds are typical for the tail extension in L1 of the new species (Fig. 2) and *Umingmakstrongylus* and *Parelaphostrongylus* (Hoberg et al. 1995, 2005); details of fine

structure for the tail extension in *Varestrongylus* and *Muelerius* require further study. In specimens of the new species, however, the basal portion of the tail extension is relatively longer than the midsection or the distal tip, in contrast with *Parelaphostrongylus* or *Umingmakstrongylus*. Additionally, the base of the dorsal spine is relatively narrow in the new species as compared with specimens of any species of *Parelaphostrongylus*. Notably, DSL from the new species resemble those reported and described as a presumptive elaphostrongyline in woodland caribou from Ontario and Manitoba, particularly in the form of the thin dorsal spine (Lankester et al. 1976).

Host and geographic range

We identified the novel ITS-2 sequences in larvae from three ungulate host species, including three subspecies of caribou, and one slug intermediate host, from locations extending from Alaska, USA, to Labrador, Canada (Fig. 1; Table 2). This indicates that the parasite has a broad host and geographic range; however, the prevalence and intensity of infection remain unknown, as our study was not designed to specifically address these factors. Larvae with the novel sequences were not found in some caribou herds, such as the Bluenose West and Chisana, or in woodland caribou from the northwestern Northwest Territories, but this likely reflects insufficient sampling rather than true absence (Table 3). Detection of a third-stage larva in a slug supports the recognition of a typical protostrongylid lifecycle, with gastropods as essential intermediate hosts.

In Alaska, *P. andersoni* had previously been the only protostrongylid reported in barren-ground caribou, and the extent of its distribution remains undocumented (Lankester 2001). We identified DSL of the unknown species in the Porcupine caribou herd and in the North Alaska Peninsula herd, which is sympatric with an infected moose in the area near Lake Clark at the northeastern terminus of the North Alaska Peninsula (Table 2).

Our results suggest that the previously unknown DSL reported by Lankester and Hauta (1989) in woodland caribou from the Mealy Mountains may be the same as the novel protostrongylid that we describe here. However, DNA sequencing is required to determine the identity of unknown protostrongylids in woodland caribou from Alberta, Ontario, and Manitoba (Lankester et al. 1976; Gray and Samuel 1986). DNA sequencing of the ITS-2 from DSL from those and other populations and examination of adult worms remains necessary to determine the complete geographic range of the unknown and other known protostrongylids in North America.

A number of reports of unidentified protostrongylids in North America cervids were summarized by Lankester et al. (1976). In each case, DSL were presumed to be species of *Parelaphostrongylus*, but adult nematodes could not be demonstrated in extrapulmonary sites. Of potential importance here are the reports of DSL similar to *P. tenuis* in moose from Isle Royale, Michigan (Karns and Jordan 1969), in white-tailed deer from Saskatchewan (Bindernagel and Anderson 1972), and in mule deer from Alberta (Samuel and Holmes 1974). In central and eastern Saskatchewan, some populations of DSL were later determined to be *V. alpenae* (Gray et al. 1985). The identity of other populations was never determined, and Lankester et al. (1976) had suggested

that an unknown protostrongylid was circulating among deer and woodland caribou in zones of contact. Thus, these and other unidentified DSL, presumed to be elaphostrongyline (e.g., in woodland caribou from northeastern Alberta and from Manitoba and Ontario) may be the new species identified in this study, or alternatively, a single widespread species of protostrongylid remaining to be characterized in detail.

We suggest experimental studies together with a systematic search incorporating necropsies of appropriate, free-living ungulate species for recovery of adult specimens from pulmonary and extrapulmonary tissues and continued fecal surveys to resolve the host and geographic distribution of this apparently novel parasite. Concurrently, this will serve to provide a more comprehensive knowledge of the distribution for species of *Parelaphostrongylus* and *V. alpenae* in the Nearctic. Another possible northern ungulate host for the novel protostrongylid is Dall's sheep (*Ovis dalli* Nelson, 1884). Although both *P. odocoilei* and *P. stilesi* are geographically widespread in Dall's sheep, and numerous fecal and lung samples have been analyzed, none have revealed the occurrence of other protostrongylids in Alaska, British Columbia, Yukon, and Northwest Territories (Jenkins et al. 2005a; E.P. Hoberg, K. Beckmen, unpublished data). Feces from sympatric and parapatric populations of white-tailed deer, Sitka black-tailed deer (*Odocoileus hemionus sitkensis* Merriam, 1898), mule deer (*Odocoileus hemionus* (Rafinesque, 1817)), and elk (*Cervus elaphus* L., 1758) in potential contact with northern ungulates have yet to be examined extensively.

Currently, documented cervid hosts are all ungulates of great vagility and have a capacity to disperse over large distances, compatible with a complex history for geographic and perhaps host colonization for the unknown species. A common denominator in the distribution of this novel parasite may be barren-ground or woodland caribou, cervids with extensive geographic ranges across the north. It is unlikely that the less vagile caprine bovids (Dall's sheep or musk-oxen) are involved in the dispersal of the parasite. Patterns of sympatry for a diverse assemblage of ungulate species and alternating episodes of biotic expansion and isolation for host and parasite assemblages would have served as drivers for the distribution of a geographically widespread protostrongylid fauna (e.g., Guthrie 1982, 1984; Hoberg et al. 1995; Hoberg 2005); distributions may further be modified by translocation, ecological perturbation, and host switching during the past century (e.g., Hoberg et al. 2002; Kutz et al. 2004).

Moose are late Pleistocene immigrants to North America, only about 10 ka, with ranges determined by geographic expansion from Beringia during the Holocene (Lister 2004). Protostrongylids are essentially unknown in North American moose, other than sporadic fatal infections attributable to *P. tenuis* at temperate latitudes and in zones of contact with white-tailed deer (Lankester 2001). Additionally, although fecal samples ($n = 41$) from moose have been examined, DSL have not been detected across the Yukon and Northwest Territories (Table 3). In contrast, caribou are identified as Beringian endemics with a relatively long history in the Nearctic over the past 2 million years and ranges that extended south of the Laurentide and Cordilleran ice masses during the Pleistocene (Guthrie and Matthews 1971; Kurtén and Andersen 1980; Webb 2000; Flagstad and Røed 2003).

Although elaphostrongylinae are characteristic in caribou and reindeer, no other protostrongylids are known (Boev 1975; Lankester 2001), and in North America, *P. andersoni* appears to be a colonizer from white-tailed deer (Carreno and Lankester 1994). The geographically broad range now demonstrated for this previously undescribed protostrongylid suggests that it may have existed in caribou in refugia in eastern Beringia or south of the Laurentide–Cordillera during the Pleistocene (e.g., Flagstad and Røed 2003); additionally this may suggest affinities to a Holarctic–Palearctic fauna during the Pleistocene (Hoberg 2005). In as much as we have shown that the unknown may not be an elaphostrongyline, any history that has been defined for *Parelaphostrongylus* (e.g., Platt 1984; Carreno and Lankester 1994) may not be revealing with respect to this protostrongylid.

The history, geographic range, and recent surveys of muskoxen for protostrongylids suggest that this host may acquire the novel protostrongylid when sympatric with caribou. Surveys for *U. pallikuukensis* in muskoxen have demonstrated that protostrongylid lungworms appear to have a climatically limited distribution and are absent in the Arctic islands of Canada (Hoberg et al. 1995; Kutz et al. 2001a; 2004). Thus, it is highly improbable that muskoxen (*O. m. wardi*) introduced to northern Quebec from Ellesmere Island were infected with the unknown protostrongylid at the time of translocation. Infections documented in the current study are likely a result of host-switching from barren-ground caribou of the sympatric George River herd. The other two populations of infected muskoxen, northern Yukon (introduced *O. m. wardi*) and Thelon Sanctuary, Nunavut (endemic *O. m. moschatus*) are also sympatric with large migratory herds of caribou (Porcupine; Bathurst–Beverly–Qaminuriak), the most likely source of the novel protostrongylid. Phylogeographic studies of hosts and parasites and molecular-based surveys are requisite in resolving the history and geographic associations for this parasite in the north (Jenkins et al. 2005a).

Our current understanding of the geographic distribution for northern protostrongylids with DSL suggests that four species (the new species, *P. odocoilei*, *P. andersoni*, and *U. pallikuukensis*) could be sympatric and may occur as concomitant infections, depending on the array of ungulates that are present (Lankester and Hauta 1989; Hoberg et al. 1995; Kutz et al. 2001a, 2001b; Lankester 2001; Jenkins et al. 2005a). In the current study, we clearly demonstrate the presence of *P. andersoni* and the unknown species in the same caribou populations, with concurrent infections recognized in the Beverly and Mealy Mountain herds and in Alaska (Table 2). Other species with DSL, including *M. capillaris*, *P. tenuis*, *V. alpenae*, and the introduced *E. rangiferi* are currently unknown at Subarctic latitudes (Kutz 2000; Lankester 2001, Kutz et al. 2001a). Determination of geographic and host range and detection of mixed or sequential infections is confounded by additional variables, including (i) infections of short temporal duration in young hosts and where larval passage is circumscribed in time (e.g., moose and caribou in Newfoundland infected briefly with *P. andersoni* and later with *E. rangiferi*) and (ii) a degree of cross-immunity where initial infection with one protostrongylid may inhibit development and limit sympatry with related species (e.g., *P. andersoni* and *P. tenuis*

in white-tailed deer in the southeastern USA) (M.W. Lankester, unpublished data).

The possibility of sympatry among an assemblage of protostrongylids highlights the importance of applying efficient diagnostic techniques that will identify mixed infections where the abundance for respective species may differ substantially (Huby-Chilton et al. 2006). The greater abundance of DSL of one species versus another in feces may tend to mask the presence of multiple infections in single hosts, particularly when DNA sequencing of individual larvae is the only method for definitive identification. For example, in previous surveys for *Varestrongylus alpenae* in *Odocoileus virginianus* from southern Manitoba, Canada, a single adult male specimen was recovered from pulmonary tissue (1 of 50 deer at Riding Mountain, Manitoba), but sequencing of individual DSL in feces from this host and other white-tailed deer at this site detected only *P. tenuis* (S. Kutz, G. Appleyard, and E. Hoberg, unpublished data). Techniques such as single strand conformation polymorphism (SSCP) are more efficient for species identification in mixed infections and for discovery of previously undescribed species (Huby-Chilton et al. 2006). However, different life-history patterns among protostrongylid species may still necessitate intensive collections across age and sex classes and seasons as well as the examination of relatively large numbers of larval specimens to determine or exclude the occurrence of mixed infections. Documenting concurrent infections is important in understanding pathogenesis, disease, and synergistic effects where multiple species of protostrongylids occur in single hosts or host populations (Kutz et al. 2001b, 2004; Jenkins et al. 2006, 2007).

We have shown that the diversity of the parasite fauna in northern ungulates continues to offer surprises. One of the greatest challenges in collecting and assessing data about populations of parasites and other pathogens in remote regions is access to suitable samples for analyses. Through a network of collaborators, including scientists, wildlife managers, and community members, we have been able to obtain fecal samples from a variety of wildlife species and geographic locations. As demonstrated in this study, the advent of molecular methodologies for definitive identification in conjunction with basic comparative morphology for screening and development and application of archival museum collections have now made it possible to detect hidden parasite biodiversity for protostrongylids from fecal-based surveys (Gajadhar et al. 2000; Hoberg et al. 2001; Jenkins et al. 2005a; Mortenson et al. 2006; Huby-Chilton et al. 2006). Clearly, recovery of adult parasites remains essential for description of this novel species; however, without molecular technologies it is likely that this parasite would have remained unknown for a long time to come. Many of these approaches are still time-consuming and expensive, and new techniques (e.g., SSCP) may lead to a rapid, efficient, and economic basis to distinguish among an array of different species with DSL (Huby-Chilton et al. 2006). It is important to note, however, that for studies of genetic diversity and phylogeography, sequencing of appropriate loci is still necessary (Mortenson et al. 2006).

Acknowledgements

We thank Murray Lankester and two anonymous re-

viewers for extremely helpful comments on earlier versions of the manuscript. We thank Ariel Brunn, Andrew Gajadhar, Elena Garde, Farhad Ghandi, Mathew Herperger, Rebecca Jeffry, Ben Olsen, Robert Otto, Tim Sallow, Jenny Skific, Brent Wagner, and Arianna Zimmer for their technical assistance in the lab and field. We thank Mike Bly and James Auld, Government of the Northwest Territories for producing the map. We also thank the Tetlit Gwich'in Renewable Resources Council, Fort McPherson, Northwest Territories; Wildlife Management Advisory Council (Northwest Territories); Porcupine Caribou Technical Committee; Porcupine Caribou Management Board; Dawson District Renewable Resources Council, Dawson City, Yukon, for their support and advice. This project was supported by Environment and Natural Resources, Government of the Northwest Territories; Yukon Territorial Government; Western Northwest Territories Biophysical Fund, Government of the Northwest Territories; Climate Change Action Fund, Natural Resources Canada; Parks Canada; Faculty of Veterinary Medicine, University of Calgary; Western College of Veterinary Medicine, University of Saskatchewan; Sahtu Renewable Resources Board; Northern Research Institute, Yukon College; Tanner Family Fund; Yukon Foundation; the Yukon Territorial Government; and the Alaska Department of Fish and Game. Some field studies in Alaska are a contribution of the Beringian Coevolution Project supported by the National Science Foundation through DEB 0196095 and 0415668. Field collections in Uzbekistan were supported through the Office of International Research Programs, US Department of Agriculture, Agricultural Research Service, and the Science and Technology Center Ukraine Partner under Partner Project 114.

References

- Anderson, R.C. 1963. The incidence, development and experimental transmission of *Pneumostrongylus tenuis* of the white-tailed deer (*Odocoileus virginianus borealis*) in Ontario. *Can. J. Zool.* **41**: 775–792.
- Bindernagel, J.A., and Anderson, R.C. 1972. Distribution of meningeal worm (*Pneumostrongylus tenuis*) in white tailed deer in Canada. *J. Wildl. Manage.* **36**: 1350–1353.
- Boev, S.N. 1975. Protostrongylids. *Osnovy Nematologii* 25. Akademiia Nauk SSSR, Gel'mintologicheskaiia Laboratoriia, Moskva. [English translation, 1984, US Department of Agriculture, Amerind Publishing Company, New Delhi, India.]
- Carreno, R., and Hoberg, E.P. 1999. Evolutionary relationships among the Protostrongylidae (Nematoda: Metastrongyloidea) as inferred from morphological characters, with consideration of host–parasite evolution. *J. Parasitol.* **85**: 638–648. doi:10.2307/3285736. PMID:10461943.
- Carreno, R., and Lankester, M.W. 1994. A re-evaluation of the phylogeny of *Parelaphostrongylus* Boev and Schulz, 1950 (Nematoda: Protostrongylidae). *Syst. Parasitol.* **28**: 145–151. doi:10.1007/BF00009592.
- Chilton, N.B., Huby-Chilton, F., Lankester, M.W., and Gajadhar, A.A. 2005. A method for extracting genomic DNA from individual elaphostrongyline (Nematoda: Protostrongylidae) larvae and differentiation of *Elaphostrongylus* spp. from *Parelaphostrongylus* spp. by PCR assay. *J. Vet. Diagn. Invest.* **17**: 585–588.
- Flagstad, Ø., and Røed, K.H. 2003. Refugial origins of reindeer (*Rangifer tarandus* L.) inferred from mitochondrial DNA sequences. *Evolution*, **57**: 658–670. PMID:12703955.
- Forrester, S.G., and Lankester, M.W. 1997. Extracting protostrongylid nematode larvae from ungulate feces. *J. Wildl. Dis.* **33**: 511–516. PMID:9249697.
- Gajadhar, A.A., Steeves-Gurnsey, T., Kendall, J., Lankester, M., and Steen, M. 2000. Differentiation of dorsal-spined elaphostrongyline larvae by polymerase chain reaction amplification of ITS-2 of rDNA. *J. Wildl. Dis.* **36**: 713–722. PMID:11085433.
- Gray, J.B., and Samuel, W.M. 1986. *Parelaphostrongylus odocoilei* (Nematoda: Protostrongylidae) and a protostrongylid nematode in woodland caribou (*Rangifer tarandus caribou*) of Alberta. *J. Wildl. Dis.* **22**: 48–50.
- Gray, J.B., Samuel, W.M., Shostak, A.W., and Pybus, M.J. 1985. *Varestrongylus alpenae* (Nematoda: Metastrongyloidea) in white-tailed deer (*Odocoileus virginianus*) of Saskatchewan. *Can. J. Zool.* **63**: 1449–1454.
- Guthrie, R.D. 1982. Mammals of the mammoth steppe as paleoenvironmental indicators. In *Paleoecology of Beringia*. Edited by D.M. Hopkins, J.V. Matthews, Jr., C.E. Schweger, and S.B. Young. Academic Press, New York. pp. 307–326.
- Guthrie, R.D. 1984. Mosaics, allelochemics and nutrients: an ecological theory of late Pleistocene megafaunal extinctions. In *Quaternary extinctions: a prehistoric revolution*. Edited by P.S. Martin and R.G. Klein. University of Arizona Press, Tuscon. pp. 259–298.
- Guthrie, R.D., and Matthews, J.V., Jr. 1971. The Cape Deceit fauna — early Pleistocene mammalian assemblage from the Alaskan Arctic. *Quat. Res.* **1**: 474–510. doi:10.1016/0033-5894(71)90060-3.
- Hoberg, E.P. 2005. Coevolution and biogeography among Nematodirinae (Nematoda: Trichostrongylina) Lagomorpha and Artiodactyla (Mammalia): exploring determinants of history and structure for the northern fauna across the Holarctic. *J. Parasitol.* **91**: 358–369. doi:10.1645/GE-3466. PMID:15986612.
- Hoberg, E.P., Polley, L., Gunn, A., and Nishi, J.S. 1995. *Umingmakstrongylus pallikuukensis* gen. nov. et sp. nov. (Nematoda: Protostrongylidae) from muskoxen, *Ovibos moschatus*, in the central Canadian Arctic, with comments on biology and biogeography. *Can. J. Zool.* **73**: 2266–2282.
- Hoberg, E.P., Kocan, A.A., and Rickard, L.G. 2001. Gastrointestinal strongyles in wild ruminants. In *Parasitic diseases of wild mammals*. Edited by W.M. Samuel, M. Pybus, and A. Kocan. Iowa State University Press, Ames, Iowa. pp. 193–227.
- Hoberg, E.P., Kutz, S.J., Nagy, J., Jenkins, E., Elkin, B., Branigan, M., and Cooley, D. 2002. *Protostrongylus stilesi* (Nematoda: Protostrongylidae): ecological isolation and putative host switching between Dalls' sheep and muskoxen in a contact zone. *Comp. Parasitol.* **69**: 1–9. doi:10.1654/1525-2647(2002)069[0001:PSNPEI]2.0.CO;2.
- Hoberg, E.P., Jenkins, E.J., Rosenthal, B., Wong, M., Erbe, E.F., Kutz, S.J., and Polley, L. 2005. Caudal polymorphism and cephalic morphology among first-stage larvae of *Parelaphostrongylus odocoilei* (Protostrongylidae: Elaphostrongylineae) in Dall's sheep from the Mackenzie Mountains, Canada. *J. Parasitol.* **91**: 1318–1325. doi:10.1645/GE-3503.1. PMID:16539011.
- Huby-Chilton, F., Chilton, N.B., Lankester, M.W., and Gajadhar, A.A. 2006. Single-strand confirmation polymorphism (SSCP) analysis as a new diagnostic tool to distinguish dorsal-spined larvae of the Elaphostrongylineae (Nematoda: Protostrongylidae) from cervids. *Vet. Parasitol.* **135**: 153–162. doi:10.1016/j.vetpar.2005.08.001. PMID:16185812.
- Jenkins, E.J., Appleyard, G.D., Hoberg, E.P., Rosenthal, B.M., Kutz, S.J., Veitch, A.M., Schwantje, H.M., Elkin, B.T., and Polley, L. 2005a. Geographic distribution of the muscle-dwelling nematode *Parelaphostrongylus odocoilei* in North America,

- using molecular identification of first-stage larvae. *J. Parasitol.* **91**: 574–584. doi:10.1645/GE-413R. PMID:16108550.
- Jenkins, E.J., Hoberg, E.P., and Polley, L. 2005b. Development and pathogenesis of *Parelaphostrongylus odocoilei* (Nematoda: Protostrongylidae) in experimentally infected thinhorn sheep (*Ovis dalli*). *J. Wildl. Dis.* **41**: 669–682. PMID:16456155.
- Jenkins, E.J., Veitch, A.M., Kutz, S.J., Hoberg, E.P., and Polley, L. 2006. Climate change and the epidemiology of protostrongylid nematodes in northern ecosystems: *Parelaphostrongylus odocoilei* and *Protostrongylus stilesi* in Dall's sheep (*Ovis d. dalli*). *Parasitology*, **132**: 387–401. doi:10.1017/S0031182005009145. PMID:16332289.
- Jenkins, E.J., Veitch, A.M., Kutz, S.J., Bollinger, T., Chirino-Trejo, M., Elkin, B.T., West, K., Hoberg, E.P., and Polley, L. 2007. Protostrongylid parasites and pneumonia in captive and wild thinhorn sheep (*Ovis dalli*). *J. Wildl. Dis.* **43**: 189–205. PMID:17495303.
- Karns, P.D., and Jordan, P.A. 1969. *Pneumostrongylus tenuis* in moose on a deer-free island. *J. Wildl. Manage.* **33**: 431–433. doi:10.2307/3799850.
- Kontrimavichus, V.L., Deliamure, S.L., and Boev, S.N. 1976. Metastrongyloids of domestic and wild animals. *Osnovy Nematologii* 26. Akademiia Nauk SSSR, Gel'mintologicheskaiia Laboratoriia, Dal'nevostochnyi Nauchnoyi Tsent. Nauka Publishers, Moskva. [English translation, 1985, US Department of Agriculture, Amerind Publishing Company, New Delhi, India.]
- Kurtén, B., and Andersen, E. 1980. Pleistocene mammals of North America. Columbia University Press, New York.
- Kutz, S.J. 2000. The biology of *Umingmakstrongylus pallikuukensis*, a lung nematode of muskoxen in the Canadian arctic: field and laboratory studies. Ph.D. thesis, Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon.
- Kutz, S.J., Hoberg, E.P., and Polley, L. 1999. Experimental infections of muskoxen (*Ovibos moschatus*) and domestic sheep with *Umingmakstrongylus pallikuukensis* (Nematoda: Protostrongylidae): parasite development, population structure, and pathology. *Can. J. Zool.* **77**: 1562–1572. doi:10.1139/cjz-77-10-1562.
- Kutz, S.J., Hoberg, E.P., and Polley, L. 2001a. A new lungworm in muskoxen: an exploration in Arctic parasitology. *Trends Parasitol.* **17**: 276–280. doi:10.1016/S1471-4922(01)01882-7. PMID:11378034.
- Kutz, S.J., Veitch, A.M., Hoberg, E.P., Elkin, B.T., Jenkins, E.J., and Polley, L. 2001b. New host and geographic records for two protostrongylids in Dall's sheep. *J. Wildl. Dis.* **37**: 761–774. PMID:11763740.
- Kutz, S.J., Hoberg, E.P., Nagy, J., Polley, L., and Elkin, B. 2004. "Emerging" parasitic infections in Arctic ungulates. *Integr. Comp. Biol.* **44**: 109–118. doi:10.1093/icb/44.2.109.
- Lankester, M.W. 2001. Extrapulmonary lungworms of cervids., *In* Parasitic diseases of wild mammals. Edited by W.M. Samuel, M.J. Pybus, and A.A. Kocan. Iowa State University Press, Ames, Iowa. pp. 228–278.
- Lankester, M.W., and Hauta, P.L. 1989. *Parelaphostrongylus andersoni* (Nematoda: Protostrongylidae) in caribou (*Rangifer tarandus*) of northern and central Canada. *Can. J. Zool.* **67**: 1966–1975.
- Lankester, M.W., Crichton, V.J., and Timmermann, H.R. 1976. A protostrongylid nematode (Strongylidae: Protostrongylidae) in woodland caribou (*Rangifer tarandus caribou*). *Can. J. Zool.* **54**: 680–684. PMID:7349.
- Lister, A.M. 2004. The impact of Quaternary ice ages on mammalian evolution. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **359**: 221–241. doi:10.1098/rstb.2003.1436. PMID:15101579.
- Mortenson, J.A., Abrams, A., Rosenthal, B., Dunhams, D., Hoberg, E.P., Bildfell, R.J., and Green, R.L. 2006. *Parelaphostrongylus odocoilei* in Columbian black-tailed deer from Oregon. *J. Wildl. Dis.* **42**: 527–535. PMID:17092883.
- Nicholas, K.B., and Nicholas, H.B. 1997. GeneDoc: a tool for editing and annotating multiple sequence alignments. Distributed by the author at www.psc.edu/biomed/genedoc.
- Platt, T.R. 1984. Evolution of Elaphostrongylinae (Nematoda: Metastrongyloidea: Protostrongylidae) parasites of cervids (Mammalia). *Proc. Helminthol. Soc. Wash.* **51**: 196–204.
- Posada, D., and Crandall, K.A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics*, **14**: 817–818. doi:10.1093/bioinformatics/14.9.817. PMID:9918953.
- Prestwood, A.K. 1972. *Parelaphostrongylus andersoni* sp. n. (Metastrongyloidea: Protostrongylidae) from the musculature of white-tailed deer (*Odocoileus virginianus*). *J. Parasitol.* **58**: 897–902. doi:10.2307/3286582. PMID:5078596.
- Samuel, W.M., and Holmes, J. 1974. Search for elaphostrongyline parasites in cervids from Alberta. *Can. J. Zool.* **52**: 401–403. PMID:4819478.
- Swofford, D.S. 2002. PAUP* version 4.0b.10 phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland, Mass.
- Webb, S.D. 2000. Evolutionary history of the New World cervidae. *In* Antelopes, deer and relatives: fossil record, behavioral ecology, systematics and conservation. Edited by E.S. Vrba and G. Schaller. Yale University Press, New Haven, Conn. pp. 38–64.

Copyright of *Canadian Journal of Zoology* is the property of NRC Research Press and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.