MUSCLEWORMS, PARELAPHOSTRONGYLUS ANDERSONI (NEMATODA: PROTOSTRONGYLIDAE), DISCOVERED IN COLUMBIA WHITE-TAILED DEER FROM OREGON AND WASHINGTON: IMPLICATIONS FOR BIOGEOGRAPHY AND HOST ASSOCIATIONS

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MUSCLEWORMS, *PARELAPHOSTRONGYLUS ANDERSONI* (NEMATODA: PROTOSTRONGYLIDAE), DISCOVERED IN COLUMBIA WHITE-TAILED DEER FROM OREGON AND WASHINGTON: IMPLICATIONS FOR BIOGEOGRAPHY AND HOST ASSOCIATIONS

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ABSTRACT: *Parelaphostrongylus andersoni* is considered a characteristic nematode infecting white-tailed deer (*Odocoileus virginianus*). Host and geographic distribution for this parasite, however, remain poorly defined in the region of western North America. Fecal samples collected from Columbia white-tailed deer (*O. v. leucurus*) in a restricted range endemic to Oregon and Washington, USA, were examined for dorsal-spined larvae characteristic of many protostrongylid nematodes. Multilocus DNA sequence data (internal transcribed spacer 2 and cytochrome c oxidase subunit 1) established the identity and a new record for *P. andersoni* in a subspecies of white-tailed deer previously unrecognized as hosts. Populations of *P. andersoni* are now recognized along the basin of the lower Columbia River in Oregon and Washington and from south-central Oregon on the North Umpqua River. Current data indicate a potentially broad zone of sympatry for *P. andersoni* and *Parelaphostrongylus odocoilei* in the western region of North America, although these elaphostrongylines seem to be segregated, respectively, in white-tailed deer or in black-tailed and mule deer (*Odocoileus hemionus*) at temperate latitudes. The geographic range for *P. andersoni* in white-tailed deer is extended substantially to the west of the currently defined limit in North America, and we confirm an apparently extensive range for this elpahostrongyline. These observations are explored in the broader context of host and geographic associations for *P. andersoni* and related elaphostrongylines in North American cervids.

Key words: Columbia white-tailed deer, COI, ITS-2, muscleworm, *Odocoileus virginianus leucurus*, *Parelaphostrongylus andersoni*.

INTRODUCTION

The nematode muscleworm, *Parelaphostrongylus andersoni* Prestwood 1972, is an elaphostrongyline parasite occurring in white-tailed deer (*Odocoileus virginianus*), barrenground caribou (*Rangifer tarandus groenlandicus* and *R. t. grantii*), and woodland caribou (*R. t. caribou*) from North America (Prestwood et al., 1974; Anderson and Prestwood, 1981; Lankester, 2001). Although considered a characteristic nematode infecting cervids from the Nearctic, aspects of the host and geographic distribution remain poorly or incompletely defined for this species, as well as congeners including the meningeal worm, *Parelaphostrongylus tenuis* (Dougherty, 1945), and the mule deer muscleworm, *Parelaphostrongylus odocoilei* (Hobmaier and Hobmaier, 1934). Data for *P. andersoni* are particularly sparse for the region of western North America (Lankester, 2001).

Numerous accounts document an apparently disjunct, patchy, but extensive range for *P. andersoni* from the southeastern United States to the Canadian Subarctic and Alaska, USA (Fig. 1). Infected white-tailed deer have been identified from the southeastern United States (Prestwood et al., 1974; Anderson and Prestwood, 1981), New Jersey (Pursglove, 1977), Michigan (Pybus et al., 1990), northeastern Wyoming (Edwards, 1995), and southeastern and south-central British Columbia, Canada (Pybus and Samuel, 1981; Lankester, 2001). This parasite is unknown in natural infections of black-tailed or mule deer (subspecies of *Odocoileus hemionus*), moose (*Alces alces*), or

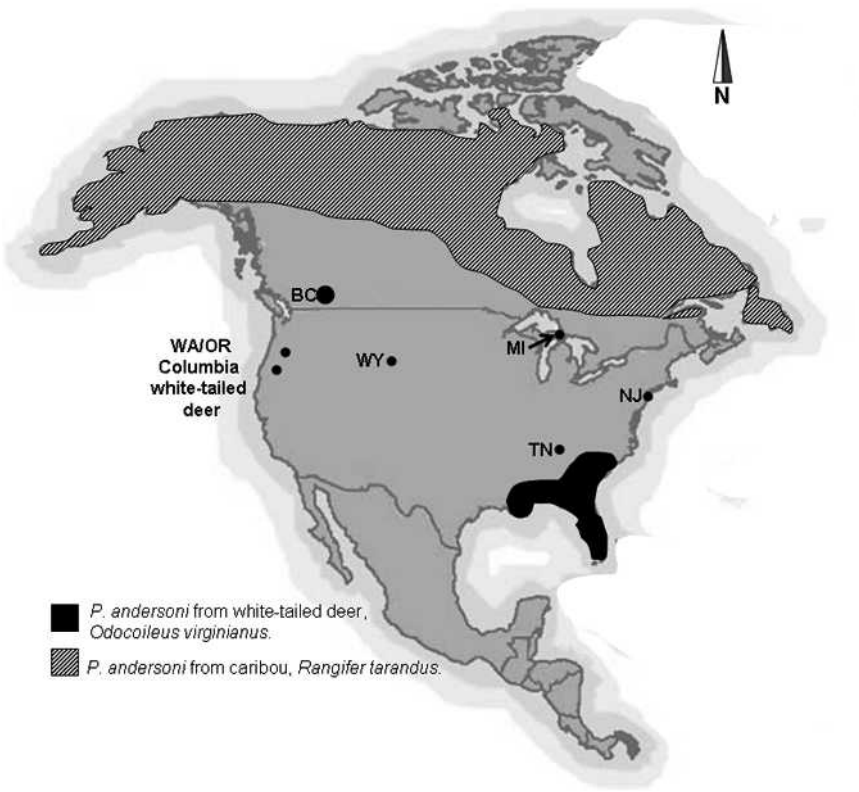

FIGURE 1. Map showing approximation of the known distribution of *Parelaphostrongylus andersoni* (in part based on Lankester, 2001). A prior report of *P. andersoni* in Arkansas as cited by Anderson and Prestwood (1981) and Lankaster (2001) seems to be in error, because Prestwood et al. (1974) to whom this record was attributed did not find this elaphostrongyline in this locality.

potential wild caprine hosts (Kutz et al., 2001, 2007; Jenkins et al., 2005; Mortenson et al., 2006). In both barrenground and woodland caribou, *P. andersoni* is regarded to be geographically widespread, excluding the Arctic islands of Canada, although relatively few records have documented the actual distribution (summarized in Lankester, 2001; Kutz et al., 2007).

Until recently, confirmation of the occurrence and distribution of *P. andersoni* and other species of *Parelaphostrongylus* was dependent on the collection and identification of adult worms recovered from carcasses of various ungulate hosts (Lankester, 2001). The first larval stage of many protostrongylid species is characterized by the presence of a dorsal spine on the tail (Boev, 1975). Such dorsal spined larvae (DSLs) recovered from feces cannot be reliably identified based on morphology, and polymorphism among larvae of some species has been noted (Hoberg et al., 2005). The advent of molecular-based methods has altered our approach to survey and inventory for protostrongylid and other nematodes in ungulates (e.g., Hoberg et al., 2001). Sequence data from individual DSL assessing both nuclear and mitochondrial loci are now used as epidemiologic probes to explore identity, distribution, and phylogeography based on geographically extensive and site intensive sampling (e.g., Jenkins et al., 2005; Mortenson et al., 2006; Kutz et al., 2007; Hoberg et al., unpubl. data). Unequivocal molecular-diagnostic protocols are also available for elaphostrongylines, but they have not yet been extended to all protostrongylids that produce DSLs in North

American ungulates (e.g., Huby-Chilton et al., 2006).

Thirty-eight subspecies of white-tailed deer are widely distributed from North America, across the Panamanian Isthmus, into northwestern South America (Smith, 1991). This suggests the potential for a broad geographic range for *P. andersoni* largely congruent to that of *O. virginianus*. Sampling for protostrongylids in species of *Odocoileus* in the western region of North America has been limited (e.g., Mortenson et al., 2006), and the area encompassing the "doughnut-hole" extending across eastern California, Oregon, Washington, and the Great Basin remains to be explored. Our study serves to continue the exploration of this region by examining elaphostrongyline parasites among populations of Columbia white-tailed deer (*Odocoileus virginianus leucurus*; CWTD) using molecular-based survey.

As the western-most subspecies, CWTD was historically distributed in the lowlands of southwestern Washington and much of Oregon west of the Cascade Mountains (Douglas, 1829; Nash, 1877; Smith, 1985). Currently, CWTD are restricted to two isolated populations, one population in southern Oregon along the North Umpqua River (Douglas County) and the other population along the lower Columbia River, in both Oregon and Washington, the latter now defined by the Julia Butler Hansen Refuge. In the mid-nineteenth century, the range of the CWTD was reduced coincidental with Euro-American settlement of the Pacific Northwest (Livingston, 1987). In the early twentieth century, this subspecies was thought to be extinct (Jewett, 1914; Taylor and Show, 1929; Bailey, 1936; Cowan, 1936). Surveys of CWTD by Scheffer (1940), however, estimated the population to consist of 500–700 animals along the lower Columbia River. These low numbers resulted in their subsequent listing as one of the first endangered mammalian species recognized by the federal government (Gavin, 1979). With recovery through the 1980s (Smith, 1985), populations of CWTD were estimated at 3,000 animals in the north on the Columbia River and 6,000 individuals in southern Oregon when the subspecies was delisted by the Department of Interior in 2003 (FR Doc. 03-17756). Parasitologic information previously available for CWTD has been limited to notes on the occurrence of pulmonary nematodes, with one case identified as *Dictyocaulus viviparus* (Bloch, 1782) (Scheffer, 1940; Gavin et al., 1984); however, this record may be in error because these lungworms in western cervids are attributable to *Dictyocaulus eckerti* Skrjabin, 1931 (Hoberg and Abrams, unpubl. data). During recent precipitous declines in the northern population of CWTD, a health survey was initiated that reported the occurrence of DSLs and a presumptive, but unconfirmed, identification of *P. andersoni* (Creekmore and Glaser, 1999).

In the current study, we address the paucity of definitive information about the host and geographic associations for species of *Parelaphostrongylus* through fecal-based sampling of endemic white-tailed deer in Oregon and Washington. Disjunct populations of CWTD were sampled for DSLs, one population on the lower Columbia River and the other population on the North Umpqua River. We further discuss the biogeography and history of *P. andersoni* and related species in cervid hosts from North America.

MATERIALS AND METHODS

Fecal specimens

Fecal samples were collected from 31 adult CWTD across the range occupied by this subspecies in Oregon and Washington. On the Julia Butler Hansen Refuge at Puget Island, Washington, USA, and on the south bank of the Columbia River in Oregon on 25–26 March 2006, 21 fecal samples were collected in the process of handling animals during a relocation project (Table 1). Activities were covered under the following jurisdiction: 1) Federal Fish and Wildlife permit (TE702631-18, subpermit WNWR-6) was issued by the

TABLE 1. Locality data for fecal samples from Columbia white-tailed deer.

Host collection location (moved to)	No. of deer	% prevalence
Puget Island, Washington, USA	7	43
(Fisher Island)	7	43
South bank of Columbia River, Oregon, USA	15	60
(Lord Island)	9	44
(Crimms Island)	1	0
(Gull Island)	4	100
(not moved)	1	100
Douglas County, Oregon, USA	10	50

US Fish and Wildlife Service; and 2) Oregon Scientific Taking Permit (065-06) was issued by the Oregon Department of Fish and Wildlife for the collection of the deer on the lower Columbia River. Washington state did not require a permit due to involvement of Washington Department of Fish and Wildlife personnel. In Douglas County, along the North Umpqua River, fresh fecal samples were collected from the ground after defecation by 10 identified CWTD, including eight adult females and single male and female fawn; in this area, CWTD are sympatric with Columbia black-tailed deer (CBTD), *Odocoileus. hemionus columbianus*, and thus it was necessary to identify the source of fecal samples.

Fecal specimens were extracted for recovery of first-stage protostrongylid larvae using a modified Beaker-Baermann technique (Forrester and Lankester, 1997; Jenkins et al., 2005); numbers of larvae per gram of feces were not quantified. Dorsal-spined larvae of putative elaphostrongyline nematodes were sorted and individual specimens were transferred by micropipette to single cryo-vials with molecular grade water for further processing. Voucher specimens were preserved in 70% ethanol and deposited in the US National Parasite Collection, USDA, Beltsville, Maryland (USNPC; Table 2).

Comparative specimens

Two isolates of *P. tenius* recovered from fecal samples in white-tailed deer collected on or near Gibson Island, Maryland, USA, were used for comparisons using cytochrome c oxidase subunit 1 (COI; Table 2). Two isolates of *P. odocoilei* from coastal Oregon were collected from CBTD (OR-8244 and OR-8408 in Mortenson et al., 2006), and a third isolate was collected from a Dall's sheep *Ovis dalli* at Katherine Creek, Mackenzie Mountains, Northwest Territories, Canada (Table 2). These three isolates of *P. odocoilei* were used for COI comparison.

DNA extractions and polymerase chain reaction (PCR) amplification

Individual DSLs were assessed via multilocus sequencing of nuclear ribosomal (internal transcribed spacer 2 [ITS-2]) and mitochondrial (COI) DNA. The DNA was extracted from single DSL (two each from 12 CWTD hosts from the lower Columbia River; three each from three CWTD and one from a forth CWTD from Douglas County) using a DNeasy Tissue Kit (QIAGEN, Valencia, California, USA) and eluted twice with 100 µl of AE buffer provided in kit. The PCR amplification (DNA Engine PTC-200, MJ Research, Watertown, Massachusetts, USA) used 4 pmol of each of NC1 and NC2 primers for ITS-2 (Gasser et al., 1993) or primers PtCOI-F (GGTTGGAGAGTTCTAATCATAAAGA) and PtCOI-R (CCCAAACATAGTAGCCAACCA) for COI, 0.2 U of Platinum *Taq* DNA Polymerase High Fidelity (Invitrogen, Carlsbad, California, USA), 4 nmol each of dNTP mix (Sigma-Aldrich, St. Louis, Missouri, USA), and 2 µl of template in 20-µl reaction (94 C for 2 min, 35× [94 C for 20 sec, 50 C for 30 sec, 68 C for 40 sec], 68 C for 7 min).

Cloning and sequencing

Due to difficulty sequencing through a poly-A region, PCR products of ITS-2 were cloned using a TA Cloning Kit (Invitrogen) into One Shot chemically competent cells (Invitrogen). Colonies containing the insert were PCR amplified directly by first transferring cells into 50 µl of molecular grade water and heating to 90 C for 10 min, and then using 2 µl of that as template with M13 forward and reverse primers supplied in the TA Cloning Kit (4 pmol each). The PCR conditions were as described above. PCR products for COI

TABLE 2. Sources of specimens or sequences representing species of *Parelaphostrongylus* used in comparisons and identification.

Host	Location	Coordinates	No. hosts	No. dorsal-spined larvae	GenBank accession no.	US National Parasite Collection voucher
Parelaphostrongylus andersoni						
Odocoileus virginianus leucurus	Puget Island, Washington, USA	46°10′N, 123°21′W	3	6/5/4[a]	EF173707–EF173710, EF173713, EU052282–EU052285	ds[b]
	Columbia River, Oregon, USA	46°08′N, 123°20′W	9	18/17/9	EF173700–EF173706, EF173711, EF173712, EF173714–EF173721, EU052276–EU052281, EU052286–EU052288	98777
	Douglas County, Oregon, USA	43°13′N, 123°20′W	3	7/7/5	EU020128–EU020134, EU029991–EU029995	100038
	Douglas County, Oregon, USA	43°16′N, 123°21′W	2	6/4/4	EU020124–EU020127, EU029987–EU029990	
Parelaphostrongylus tenuis						
Odocoileus virginianus borealis	Gibson Island, Maryland, USA	39°04′N, 76°25′W	2	2/2/2	EF173722–EF173723	ds
Parelaphostrongylus odocoilei						
Odocoileus hemionus columbianus	Oregon, USA	44°23′N, 124°02′W	1	19/9/1	EF173697	95263
	Oregon, USA	44°38′N, 123°27′W	1	20/19/1	EF173698	94882
Ovis dalli dalli	Katherine Creek, Northwest Territories, Canada	65°01′N, 127°35′W	1	4/–/1	EF173699	94891–94894

[a] Number of dorsal-spined larvae extracted/internal transcribed spacer-2 amplified/cytochrome c oxidase subunit amplified.

[b] All materials were destructively sampled (ds) for sequencing, and specific physical vouchers representing individual specimens were not retained.

were sequenced directly. BigDye Terminator v3.1 chemistries and an ABI Prism 3730xl DNA Analyzer were used for sequencing (Applied Biosystems, Foster City, California, USA).

Data analysis

Sequence chromatograms were edited using Sequencher version 4.6 (Gene Codes, Ann Arbor, Michigan, USA). Identification of DSLs based on ITS-2 used a BLAST search, and definitive differentiation was done in reference to fixed nucleotide polymorphism previously documented among the three species of *Parelaphostrongylus* according to Jenkins et al. (2005). Edited COI sequences were aligned in Vector NTI (Invitrogen). The alignment was edited by eye using GeneDoc (Nicholas and Nicholas, 1997) and converted to nexus format in ClustalX (Thompson et al., 1994). Inference of phylogenetic relationship of COI haplotypes was reconstructed in PAUP* 4.0b10 (Swofford, 2001) by means of the neighbor joining uncorrected "P" distance method. All novel sequences from the present study were deposited in GenBank (Table 2).

RESULTS

Specimens of DSLs were found in fecal samples in deer from both the northern and southern populations of CWTD (Table 1). Twelve of the 21 fecal samples from the northern population contained DSLs as follows: three of seven (43%) deer from Puget Island, within the lower Columbia River; and nine of 14 (60%) deer from the south bank of the Columbia River. Five of 10 fecal samples from the southern population contained DSLs, and parasites were demonstrated at each locality within the sampling region; all hosts were adults except for one male fawn.

A 504–511-base pair (bp) fragment of ITS-2 sequence was amplified independently from 33 DSLs in 17 CWTD. Twenty-two DSLs from the northern population of CWTD on the Columbia River were sequenced: two larvae from 10 hosts, and a single larva from two hosts. Eleven DSLs from the southern population of CWTD on the North Umpqua River were sequenced: three each from two hosts, two from two other hosts, and a single DSL from the fifth host. All 33 sequences were identified as being consistent with *P. andersoni* (four extractions failed to yield usable sequence).

After assessment and initial identification based on the ITS-2 data, COI was amplified and sequenced from 22 of the 37 DSLs in CWTD (13 from the northern population and nine from the southern population) and further compared with sequences from *P. tenuis* and *P. odocoilei* (Table 2). The 22 amplicons were 773–863 bp. The 728 bp shared by all isolates of *P. andersoni* represented six haplotypes, none of which differed in pairwise comparisons by more than 8 bp (1%). Additionally, 517–521 bp were aligned for comparison with the other species of *Parelaphostrongylus*. Phylogenetic reconstruction revealed reciprocal monophyly relative to sequences representing other congeneric species.

Multilocus data establish a new record for *P. andersoni* in disjunct populations of a subspecies of *O. virginianus* previously unrecognized as hosts. These new records substantially extend the known geographic range for *P. andersoni* to the basin of the lower Columbia River in Oregon and Washington and to the North Umpqua River in south-central Oregon.

DISCUSSION

Host and geographic range

A new host and geographic record is established for *P. andersoni* in the northern and southern populations of CWTD from Oregon and Washington. Despite a high level of nucleotide conservation within ITS-2 sequences of this genus, *P. odocoilei*, *P. tenuis*, and *P. andersoni* can be differentiated based on several fixed nucleotide polymorphisms (Jenkins et al., 2005); a phylogenetically based comparison of COI data from DSLs in CWTD to sequences from *P. tenuis* and *P. odocoilei* further supports this identification. Thus, we substantially extend the

known geographic distribution of this elaphostrongyline in white-tailed deer to near the Pacific coast of North America (Fig. 1). Furthermore, this confirms the prior presumptive identification of *P. andersoni* in fecal samples from five of 20 CWTD at Tenasillahe Island on the Julia Butler Hansen Refuge (Creekmore and Glaser, 1999).

Discovery of *P. andersoni* in CWTD at disjunct localities in far western North America alters our understanding of geographic range, but it does not yet resolve the issue for continuity versus isolation or heterogenous distributions for parasite populations (Fig. 1). Based on geographically dispersed records for this parasite in *O. virginianus* across North America, occurrence of *P. andersoni* in the Pacific Northwest certainly is not completely unexpected (e.g., Mortenson et al., 2006). Limited records for this parasite may reflect inadequate or incomplete sampling (Lankester, 2001), difficulty in demonstrating the presence of adult parasites during necropsy (Lankester and Hauta, 1989), inability to reliably differentiate DSLs of congeners and other protostrongylids in fecal samples (e.g., Jenkins et al., 2005), or an actual disjunct or heterogenous geographic range driven by historical and environmental factors.

Parelaphostrongylus andersoni has been widely recorded in the southeastern United States (Prestwood et al., 1974). Intensive sampling in this region (121 deer in 11 states) by technically proficient parasitologists may provide an explanation, opposed to being indicative of a higher regional prevalence for the parasite. Lankester (2001) suggested that the apparent disjunct distribution for *P. andersoni* in *O. virginianus* reflected the incomplete nature and difficulty of sampling for this cryptic parasite in the musculature.

Alternatively, a limited distribution may result from competitive interactions with *P. tenuis* in zones of contact for these elaphostrongylines in North America (Lankester and Hauta, 1989; Lankester, 2001). The absence of *P. andersoni* in deer from some eastern localities suggests this elaphostrongyline may be effectively replaced by *P. tenuis* on a cline extending northward in eastern North America, but the western limit for the latter has not been clearly documented outside of southern Canada (Lankester, 2001). It has been postulated that the presence of *P. tenuis*, which is not known to occur in the southeastern United States, suppresses or eliminates infections of *P. andersoni* by cross-immunity or another mechanism (Lankester and Hauta, 1989; Lankester, 2001). Interestingly, the few records documenting natural mixed infections in *O. virginianus* are from the mid- to south Atlantic region of coastal North America. For example, 10 deer examined at the Peaslee Wildlife Management Area in New Jersey revealed both *P. andersoni* and *P. tenuis* (Pursglove, 1977), but it was not clear whether infections were concurrent in single hosts. Additionally, two of 52 white-tailed deer from North Carolina were found to be infected with both species (Prestwood et al., 1974). A concomitant experimental infection has also been demonstrated in a white-tailed deer fawn (Pybus et al., 1990).

Putative isolated or focal populations for *P. andersoni* in Dakota white-tailed deer (*Odocoileus virginianus dakotensis*) from northeastern Wyoming and the northwest white-tailed deer (*Odocoileus virginianus ochrourus*) from southeastern British Columbia are apparently beyond the western limit of the distribution known for *P. tenuis*. Notably *P. tenuis*, but not *P. andersoni*, has been discovered in white-tailed deer (*Odocoileus virginianus truei*) from Costa Rica (Carreno et al., 2001). Additional field data seem to support the contention that sympatry is limited for *P. andersoni* and *P. tenuis*. Necropsy of 42 white-tailed deer failed to find *P. andersoni* in central Maine (Bogaczyn, 1992), where *P. tenuis* is known to occur. In a study involving 10 white-tailed deer in Oklahoma, USA, nine were found

to be infected with *P. tenuis* and none with *P. andersoni* (Pursglove, 1977).

A western distribution for *P. andersoni* would suggest the potential for sympatry with *P. odocoilei* in some habitats and localities (Fig. 1). Throughout the distribution of CWTD, dispersion and habitat use overlapping with CBTD, which range west of the Cascade and Sierra Nevada Mountains, has been well documented (Smith, 1987). The southern population of CWTD is sympatric with black-tailed deer along the North Umpqua River, whereas the northern population along the lower Columbia River is parapatric. *Parelaphostrongylus andersoni* and *P. tenuis* are not known to naturally infect black-tailed deer or mule deer within the host's natural range, and *P. odocoilei* has not been found in white-tailed deer (summarized in Lankester, 2001), despite evidence of hybridization of the two host species (Hughes and Carr, 1993). Preliminary evidence that segregation may be maintained for species of *Parelaphostrongylus* among species of *Odocoileus* is suggested by the occurrence of *P. odocoilei* in coastal populations of CBTD in relative parapatry to CWTD where *P. andersoni* was demonstrated in the current study (Mortenson et al., 2006). Continued sampling, however, is necessary to define the distribution of *Parelaphostrongylus* in the doughnut hole encompassing eastern Oregon and Washington and the Great Basin extending to Wyoming in the east and British Columbia in the north.

Potential limitations on completion of the life cycle for *P. andersoni* due to abiotic environmental factors determining larval development or the distribution of gastropod intermediate hosts could also have an influence on overall geographic distribution. There is a paucity of data, however, for climatologic or biotic factors serving as controls on the potential for transmission for elaphostrongylines and other protostrongylids (Lankester, 2001; Kutz et al., 2002). Known intermediate hosts, including slugs of the genus *Deroceros*, are abundant and widespread in North America, and they indicate the potential for transmission, although such may be influenced by seasonality, microclimate, density of gastropods, and the distribution of cervids (Lankester, 2001; Jenkins et al., 2006). Lankester (2001) had suggested that the broad distribution for *P. andersoni*, which encompasses a range of often extreme habitats from the southeastern United States into the Arctic, was evidence of considerable environmental tolerance for these nematodes.

Collections documented from the northern population in the current study were taken during the translocation of animals within the Julia Butler Hansen Refuge along the Columbia River. Thus, the sources for animals and their history are known, and it is clear that *P. andersoni* has been disseminated with these movements of infected hosts (Table 1). Although the muscleworm is likely to be distributed throughout this refuge in CWTD, the role of host relocation as a determinant of parasite introduction and establishment is clearly demonstrated. Such has been a concern with respect to the distribution of the congener *P. tenuis* and the relationship to emergence of disease attributable to this parasite in new localities and hosts (e.g., Samuel et al., 1992; Lankester, 2001). This serves to emphasize the pervasive nature of anthropogenic events, particularly conservation-based or management-based decisions for translocation that may influence the overall distribution of parasites and pathogens. These observations constitute a strong justification for development of baselines and archival collections for biodiversity to document faunal perturbation (e.g., Hoberg et al., 2001, 2003).

Molecular-based survey

Dorsal-spined larvae of *P. andersoni*, which are easily recovered from fresh feces, are morphologically indistinguishable from those produced by congeners, as well as other protostrongylids such as

Varestrongylus alpenae (Dikmans, 1935) and *Muellerius capillaris* (Mueller, 1889) that occur in North American cervids and caprines (e.g., Boev, 1975; Jenkins et al., 2005; Kutz et al., 2007). Before the advent of molecular markers for these nematodes, adult worms had been required to confirm the identity of species infecting each host. Adult *P. andersoni* are found in or around the blood vessels of the musculature associated with the hindquarters of the host (Lankester, 2001). Therefore, to document an infection, a labor-intensive necropsy was involved, which no doubt has vastly limited the number of geographic records for this and other elaphostrongyline parasites. Molecular analyses of DSLs are necessary to establish the true range of this parasite in white-tailed deer across respective subspecies and their ranges.

Before the application of geographically extensive and site-intensive sampling in conjunction with molecular-based protocols (Jenkins et al., 2005), the ranges described for other elaphostrongylines, such as *P. odocoilei*, were considered to be heterogenous and disjunct (Kutz et al., 2001; Lankester, 2001). Fecal-based surveys in conjunction with population level genetic analysis will be a powerful tool for timely assessment of geographic range; rapidly changing patterns of distribution, such as those anticipated with habitat perturbation and global climate change; and for elucidating the complex coevolutionary history among these parasites and their hosts.

Establishing a broader context for *Parelaphostrongylus* and cervid hosts

Parelaphostrongylus is endemic to the Nearctic and seems to have coevolved with species of *Odocoileus* (Platt, 1984; Carreno and Lankester, 1994). Occurrence of *P. tenuis* in Costa Rica at the northern limits of the Neotropical region is attributed to range expansion for hosts and parasites from North America (Carreno et al., 2001). Furthermore, species of *Parelaphostrongylus* are currently unknown in species of Odocoileini (among *Mazama*, *Ozotoceros*, and *Blastocerus*) or Rangiferini (among *Hippocamelus* and *Pudu*) endemic to South America, potentially consistent with a restricted history for these elaphostrongylines in New World cervids in the Northern Hemisphere (e.g., Carreno and Lankester, 1994).

Historical bottlenecks for deer populations, with local extirpation, may have influenced contemporary patterns for occurrence and population structure relative to past distributions among species of *Parelaphostrongylus*. Such may account in part for heterogenous distributions that are apparent for *P. andersoni*, particularly in the western region of North America, and along the Atlantic coastal plain (Fig. 1). For example, Columbia white-tailed deer underwent a sharp decline in numbers during the 1800s when the Columbia River population was estimated to be approximately 500–700 animals (Scheffer, 1940). The population has since increased to numbers supportable by available habitat, and it is considered stable, although a precipitous decline in the northern population was documented in 1996 (Creekmore and Galser, 1999). Presumably, the northern population has been physically and genetically isolated from the southern population of CWTD in Douglas County, Oregon. Additionally, the Columbia River population seems to have been continuously isolated from other white-tails (*O. v. ochrourus*) in eastern Oregon and Washington by >300 km. Phylogeographic analyses can resolve how this history of isolation has influenced populations of *P. andersoni* in CWTD and how such are related to those populations distributed to the east in both *O. v. ochrourus* and *O. v. dakotensis*.

Results of the current study confirm a geographically extensive range for *P. andersoni* in white-tailed deer. A putative sister-species association with *P. odocoilei* suggests that the distribution of this elaphostrongyline is limited to the Nearctic and that occurrence in caribou (sub-

species of *R. tarandus*) is attributable to host switching during the Pleistocene (Carreno and Lankester, 1994). If caribou were hosts for *P. andersoni* before the last glaciation, genetic partitioning would be predicted between lineages that survived in the eastern Beringian refugium and south of the Laurentide and Cordillera ice sheets. In contrast, host switching from deer may have occurred during the final stage of the Wisconsin south of the Laurentide ice in the precursor of *R. t. caribou*, with secondary geographic expansion and colonization of *R. t. groenlandicus* and *R. t. grantii* during the Holocene. The latter would be consistent with phylogeography for subspecies of *Rangifer* across the Holarctic (Flagstad and Røed, 2003) and represents a framework or testable hypothesis for exploring the history of *P. andersoni* among cervid hosts in the Nearctic.

Whether the current parasite distribution can be explained by a single host-switching event or results from multiple origins from independent colonization events can be tested by examining the partitioning of genetic diversity across the entire range for *P. andersoni* in deer and caribou. Analyses of haplotype diversity can contribute to understanding the biogeography of this species. In addition, a comparison of haplotype diversity of *P. andersoni* among populations of CWTD and other white-tailed deer would be informative about true levels of isolation between these host populations since declines and range retraction by CWTD during the postsettlement period.

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