Contents lists available at ScienceDirect



International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

# Traceback of the *Psoroptes* outbreak in British Columbian bighorn sheep (*Ovis Canadensis*)



IJP

Adam M. Hering<sup>a,\*</sup>, Neil B. Chilton<sup>b</sup>, Tasha Epp<sup>a</sup>, Helen M. Schwantje<sup>c</sup>, Frances Cassirer<sup>d</sup>, Andrew Walker<sup>c</sup>, Craig McLean<sup>c</sup>, Prasobh Raveendran Thampy<sup>b</sup>, Eryn Hanak<sup>g</sup>, Peregrine Wolff<sup>e</sup>, Mark Drew<sup>d</sup>, Katherine D. Bardsley<sup>f</sup>, Murray Woodbury<sup>a</sup>

<sup>a</sup> Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Dr., Saskatoon, Saskatchewan, S7N5B4, Canada

<sup>b</sup> Department of Biology, College of Arts and Science, University of Saskatchewan, 112 Science Place, Saskatoon, Saskatchewan, S7N5E2, Canada

<sup>c</sup> British Columbia's Ministry of Forests, Lands, Natural Resource Operations, and Rural Development, 2080 Labieux Rd, Nanaimo, BC, V9T6J9, Canada

<sup>d</sup> Idaho Department of Fish and Game, 3316 16th Street, Lewiston, ID, 83501, USA

<sup>e</sup> Nevada Department of Wildlife, 6980 Sierra Center Parkway, Suite 120, Reno, NV89511, USA

<sup>f</sup> Department of Veterinary Sciences, Wyoming State Veterinary Laboratory, University of Wyoming, 1174 Snowy Range Road, Laramie, WY, 82070, USA

<sup>g</sup> Pulse Veterinary and Speciality in Edmonton, AB, Canada

ARTICLE INFO

Keywords: Bighorn sheep Oryctolagus cuniculus Ovis canadensis Psoroptes Psoroptes cuniculi Psoroptes ovis Rabbit

## ABSTRACT

*Psoroptes* are a non-burrowing, ectoparasitic, mange-causing mite that has been documented in American bighorn sheep populations throughout the 19th and 20th centuries; however, it was not seen on Canadian bighorn sheep until 2006. The aim of this study was to determine the potential source of the Psoroptes outbreak in Canadian bighorn sheep. Morphological and molecular analyses were used to compare mites recovered from outbreak-associated bighorn sheep, pet rabbits in Canada, and on historically infested bighorn sheep in the USA. The results revealed that *Psoroptes* acquired from the Canadian and outbreak-associated American bighorn sheep were morphologically more similar to those collected from rabbits than mites on historically infested bighorn sheep. Outer opisthosomal setae lengths measured an average of 81.7  $\mu$ m (±7.7  $\mu$ m) in outbreak associated bighorn mites, 88.9 µm (±12.0 µm) in rabbit mites and 151.2 µm (±16.6 µm) in historically infested bighorn mites. The opisthosomal lobe morphology of bighorn mites in the outbreak herds was also more similar to that of rabbit mites, previously described as P. cuniculi, than historically infested bighorn mites, which match previous descriptions of P. ovis. This finding was supported by DNA sequence data of the mitochondrial cytochrome B gene. This is the first report of Psoroptes of the rabbit ecotype on bighorn sheep. The morphological and molecular data therefore support the hypothesis that the source of Psoroptes outbreak in Canadian bighorn sheep represented a disease spillover event from rabbits rather than transmission from infested American bighorn sheep populations.

## 1. Introduction

*Psoroptic* mange, caused by non-burrowing ectoparasitic mites of the genus *Psoroptes*, is a highly pruritic disease known for causing exudative "scabby" lesions and alopecia in numerous mammalian species around the world including domestic and wild sheep, cattle, horses, goats, water buffalo and rabbits (Amer et al., 2015; Pegler et al., 2005; Zahler et al, 1998, 2000). It was once believed that different host species were infested with different species of *Psoroptes* (Sweatman, 1958); however,

taxonomic re-examination has challenged this assumption, prompting consideration of the possibility that transmission between host species could form a route of disease introduction in previously unaffected populations (Pegler et al., 2005).

Prior to European colonization in North America, local First Nations had not observed psoroptic mange in bighorn sheep (Buechner, 1960). It was postulated that *Psoroptes* was brought to North America on infested domestic sheep (*Ovis aries*) in the 1800s and that the parasites were transmitted to wild sheep through contact between the two species

https://doi.org/10.1016/j.ijppaw.2021.02.017

Received 27 November 2020; Received in revised form 24 February 2021; Accepted 24 February 2021 Available online 10 March 2021

<sup>\*</sup> Corresponding author. Dept. of Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, 1540 Shasta place, Victoria, BC, V8S1X9, Canada.

E-mail address: Adamhering@gmail.com (A.M. Hering).

<sup>2213-2244/© 2021</sup> The Authors. Published by Elsevier Ltd on behalf of Australian Society for Parasitology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/hy-nc-nd/4.0/).



**Fig. 1.** Characteristic long segmented peduncle (circle) that differentiates the genus of *Psoroptes* spp. from other psoroptidae that have relatively short unsegmented peduncles. ( $40 \times$  magnification captured on a Zeiss Universal compound light microscope outfitted with a TRke SPOT camera using SPOT image capture software).

(Buechner, 1960; Sweatman, 1958). Following the eradication of

*Psoroptes* from domestic sheep in Canada in 1924 and the USA in 1973 (van den Broek and Huntley, 2003) populations of *Psoroptes* persisted on other host species including pet rabbits (*Oryctolagus cuniculus*) and American populations of bighorn sheep (Boyce et al., 1990).

The disease has been reported in bighorn sheep populations in the United States throughout the late 19th and 20th centuries (Lange et al., 1980); however, the first bighorn sheep with suspicious symptoms were not reported in Canada until 2006 (Stepaniuk, 2006). When infesting bighorn sheep, psoroptic mange generally causes localized hyperkeratotic ear lesions but heavily infested animals can exhibit generalized mange as was the case for the first confirmed case in Canada, a ram that was euthanized in 2011 (Harper et al., 2002; Scott et al., 2013). The outbreak of *Psoroptes* in Canadian bighorn sheep was first identified in the Similkameen region of southern British Columbia (BC) within approximately 50 km of the USA border, and it was later seen in the Sinlahekin population of bighorn sheep in Washington state (WA) that same year (Harris et al., 2018; Scott et al., 2013). Notable population declines have been observed in the affected herds on both sides of the border since detection (Harris et al., 2018; Reid, 2013).

The source of *Psoroptes* in this transborder metapopulation of bighorn sheep was unclear because the population was not known to be physically connected with other USA bighorn herds through any natural migratory routes. They were separated from the closest known *Psoroptes* infested bighorn herds by a distance of over 250 km that includes several large water ways, making natural ram dispersal an unlikely source of introduction (Borg et al., 2017; Cassirer, 2005; Harris et al., 2018).



Fig. 2. Map of the sample origin locations and associated host groups of Psoroptes samples used.

#### Table 1

Herd and host origin of mites inspected, and DNA successfully sequenced. "% mites with both OOS measured" is the proportion of mites for which measurement of the outer opisthosomal setae (OOS) was possible from both the left and right OOS of the mite in which case the longer of the two was included in data analysis.

Host origin	No. of samples used for	morphological and morphor	No of samples (pooled mites) used for molecular analyses					
	# of mites inspected	# hosts represented	% mites with both OOS measured	cytB	cox1			
USA bighorn	67	8	82%	7	7			
Can bighorn	49	6	96%	4	2			
Rabbit	15	3	80%	2	0			
Total	131	21	86%	13	9			

Therefore, it is important to identify the source of the *Psoroptes* introduction in Canadian bighorn sheep to improve understanding of *Psoroptes* transmission, and to identify potential sources of risk for new outbreaks in naïve herds that should be considered by wildlife managers.

The identification of Psoroptes species traditionally relied on host species, body site predilection of the infestation on the host, and the length of the outer opisthosomal setae (OOS) of the adult male mites (Sweatman, 1958). Some cross-species infestation trials have demonstrated mite transmission between host species, as well as, interbreeding of mites previously categorized as heterospecific (Bates, 1999). This suggests that at least some of the recognized species of Psoroptes should be considered conspecific (Bates, 1999). Genetic studies have been conducted on Psoroptes collected from different hosts and geographical regions. The genetic markers used have included microsatellite loci (Evans et al., 2003), the nuclear second internal transcribed region of the ribosomal DNA (Amer et al., 2015; Juan et al., 2015), and the mitochondrial cytochrome *c* oxidase subunit I gene (Juan et al., 2015). The general conclusion of many researchers is that all currently recognized species of Psoroptes, with the possible exception of P. natalensis in water buffalo (Amer et al., 2015), should be unified under a single species, Psoroptes ovis (Evans et al., 2003; Juan et al., 2015; Ochs et al., 1999; OConnor and Klimov, 2015; Pegler et al., 2005; Zahler et al., 1998). Given this, it is possible that Canadian bighorn sheep may have obtained Psoroptes from another competent host species (e.g., rabbits) that occur in the same geographical region. In this study, Psoroptes collected from outbreak-associated bighorn sheep in BC, Canada and WA, USA were compared with those collected from domestic rabbits and from wild bighorn sheep from several historically affected USA bighorn sheep herds using molecular and morphometric data to determine the likely source of the Psoroptes outbreak in BC bighorn sheep.

## 2. Materials and methods

# 2.1. Samples

*Psoroptes* were collected by extracting cerumen and hyperkeratotic skin crusting from the deepest layers of ear lesions and within ear canals of a host (i.e. bighorn sheep or rabbits) using cotton tipped swabs or forceps. Samples were then stored dry or in ethanol until they could be transported for examination. Mites were extricated from the cerumen and crust under a stereoscopic dissecting microscope and identified as *Psoroptes* based on the presence of cone shaped suckers (pulvilli) on the end of their characteristically long jointed pretarsi (peduncles) (Amer et al., 2015; Bates, 1999; Pegler et al., 2005; Sweatman, 1958) (Fig. 1).

*Psoroptes* samples were collected from three different host groups: bighorn sheep in Canada and the USA that are associated with the Canadian outbreak (n = 6), bighorn sheep in the USA not associated with the outbreak (n = 10), and rabbits (n = 3). Mite samples from bighorn sheep believed to be part of the recent outbreak (subsequently referred to as "outbreak associated" *Psoroptes*) came from bighorn sheep in southern BC and northern Washington state (Fig. 2). British Columbia samples (n = 4) were collected in 2017 from naturally infested bighorn sheep captured by helicopter net gunning on the Penticton Indian Band land and surrounding area in BC. Two additional outbreak samples were

collected from animals found dead in the Sinlahekin herd of northern Washington in the USA.

Mite samples collected from USA bighorn sheep not connected to the Canadian outbreak (subsequently referred to as "historic" bighorn *Psoroptes*) came from Idaho (n = 6), Oregon (n = 2), and Nevada (n = 2). Three of the Idaho samples and both of the Oregon samples were obtained from herds that are part of the Hells Canyon metapopulation, a group of bighorn sheep herds at the border of Idaho, Washington and Oregon. The Asotin herd, located in the Washington portion of Hells Canyon, is the nearest infested USA herd to the Sinlahekin and *Psoroptes* infested BC herds. Rabbit samples were collected from three domestic rabbit sources. One from Edmonton, AB, Canada in 2018, a second from Maple Ridge, BC, Canada, in 2019, and an archived *Psoroptes* sample from a rabbit host of unknown origin collected in 1988, obtained from the Wyoming State Veterinary Lab archives (Fig. 2).

# 2.2. Morphological analyses

Subsamples of adult male mites were removed from each sample, placed on a microscope slide, flattened with a cover slide and examined under a computer and camera enabled compound microscope. Micrographs of the opisthosomal lobes and setae were taken under  $40 \times$ magnification using a Zeiss Universal microscope outfitted with a TRke Spot camera. The 4th setae from midline, known as the outer opisthosomal setae (OOS) (Sweatman, 1958), was measured using the free-hand measurement tool, in the SPOT Basic Image Capture software (SPOT Imaging) following calibration using a standardized 0.01 µm stage micrometer. When visible and intact, both the left and right OOS were measured from each mite and the longer of the two measurements was used in data analysis. In cases where one of the OOS was broken or not able to be distinguished from the other opisthosomal setae microscopically, the remaining OOS was used in data analysis. Outer opisthosomal setae lengths were analyzed using a Kruskal-Wallis non-parametric test to compare OOS lengths between individuals and between populations and a Dunn's multiple comparison test was used for post-hoc pairwise comparisons. All statistics were completed in R Studio (Version 1.1.423 - © 2009-2018 RStudio, Inc) using the FSA: Fisheries Stock Analysis R package for the Dunn's test (Ogle et al., 2019).

# 2.3. Molecular analyses

Pooled samples of mites (approximately 5–30 individuals) from a single host were placed in a separate sterile tube containing 60% ethanol. Genomic (g) DNA was extracted from each pooled sample without homogenization using a DNeasy tissue kit<sup>TM</sup> (Qiagen) and the methodology described by Dergousoff and Chilton (2007). Briefly, each pool of mites was transferred into new sterile 1.5 µl plastic tubes to which 180 µl of ATL Buffer and 20 µl of Proteinase K (15 µg/µl) was added. Samples were placed at 55 °C for 12 h, after which 200 µl of AL buffer was added. Samples were vortexed and placed at 70 °C for 10 min. Two hundred microliters of ethanol (100%) was then added to each sample, and the solution was passed through a spin column. Buffers AW1 and AW2 were used to rinse the columns, and the DNA was eluted using 100 µl of AE buffer. The gDNA samples were stored at -70 °C until used for PCR analyses.



Fig. 3. (a) Micrograph of a characteristic opisthosomal lobe of a USA bighorn mites (10× magnification captured on an Olympus C×33 compound light microscope with an Olympus EP50 camera using EP view software). The photographed mite was collected from a bighorn sheep in the Hells Canyon metapopulation. Note the more prominent outer opisthosomal lobe edge (circle) and broad base to the outer opisthosomal setae (arrow), (b) Micrograph of a characteristic opisthosomal lobe of BC bighorn origin mites (10× magnification captured on an Olympus C×33 compound light microscope with an Olympus EP50 camera using EP view software). This mite was collected from a bighorn in the Okanagan region of BC. Note the less distinct outer opisthosomal edge (circle) and the relatively less prominent base of the outer opisthosomal setae (arrow). (c) Micrograph of a characteristic opisthosomal lobe of rabbit origin mites (10× magnification captured on an Olympus C×33 compound light microscope with an Olympus EP50 camera using EP view software). This mite was collected from a rabbit in Maple Ridge, British Columbia. Note the less distinct outer opisthosomal edge (circle) and the relatively less prominent base of the outer opisthosomal setae (arrow).

The primers used to amplify ~660 bp of the mitochondrial gene cytochrome c oxidase subunit I (COI) gene were COF14 (5'- GGTCAA-CAAATCATAAAGATATTGG-3') and COR72 (5'-TAAACTTCAGGGT-GACCAAAAAATC-3') (Wang et 2012). Primers AHNC1 al., (5'-TGTGAGAATAACTCCAATTCTAG -3'), AHNC2 and (5'-GGTGAAAGATACTACCCCACT-3') were designed to amplify ~340 bp of the mitochondrial gene cytochrome B (Cyt B) based on published sequences of this gene (Gu et al., 2014). PCRs of genetic marker were performed in 25 µl volumes containing 200 µM of dNTP (Bio-Rad), 1.5 mM or 3 mM MgCl<sub>2</sub> (for CytB and COI, respectively), 0.75 µM of each

primer, 1.25U DNA polymerase (Phusion HotStart II), 5  $\mu$ l 5X Buffer Phusion Green HF buffer, and 1–2  $\mu$ L of template DNA (for COI and CytB, respectively). Negative control (i.e. no gDNA) samples were included in each set of PCRs. The PCR conditions used for COI were: 95 °C for 5 min, 35 cycles of 95 °C for 1 min (denaturation), 40 °C for 1 min (annealing), and 72 °C for 30s (extension); followed by 72 °C for 5 min (final extension). The PCR conditions used for CytB were 98 °C for 30s, then 35 cycles of 98 °C for 10s, 50 °C for 30s and 72 °C for 1 min, followed by 72 °C for 5 min. Amplicons (5  $\mu$ l) were subjected to 1.5% agarose gel electrophoresis. Amplicons of the expected size were purified (Krakowetz et al., 2014) prior to automated DNA sequencing (Eurofins Genomics) using the same primers used for PCR (i.e., in separate reactions). DNA sequences have been deposited in GenBank under accession numbers MW590279-MW590281 (*COI*) and MW650638-MW650641 (*CytB*). Sequences were manually aligned.

# 3. Results

# 3.1. Morphological analyses

Morphological measurements were performed on a total of 131 mites collected from 17 different host animals (Table 1). The bighorn mites from historically infested herds had opisthosomal lobes containing three prominent opisthosomal setae (2nd or inner, 3rd or middle, and 4th or outer) with a well-defined angle at the outer edge of the opisthosomal lobe (Fig. 3a). Mites from outbreak associated bighorn sheep (Fig. 3b) and rabbit hosts (Fig. 3c) had less prominent OOS, and less distinct outer opisthosomal lobe edges.

The bighorn mites from historically infested herds had OOS with a median length of 150  $\mu$ m (range = 114  $\mu$ m-193 $\mu$ m), outbreak bighorn mite OOS lengths had a median of 81  $\mu$ m (range = 62  $\mu$ m-101 $\mu$ m) and rabbit mite OOS lengths had a median of 85  $\mu$ m (range = 57  $\mu$ m-142 $\mu$ m) (Fig. 4). Outer opisthosomal setae lengths were significantly different between the different host groups (Kruskal-Wallis chi-squared = 96.04, df = 2, P < 0.001). Post-hoc testing using a Dunn's test for pairwise comparison found that outbreak associated bighorn sheep mites were not significantly different from rabbit mites (p = 0.28) but were significantly different from historically infested bighorn mites (p < 0.001). Similarly, rabbit mites were also significantly different from historically infested bighorn mites (p < 0.001).

When blocked by host animal rather than host group, the mite groups were again statistically different (Kruskal-Wallis chi-squared = 104.33, df = 16, P < 0.001). Pairwise comparisons using the Dunn's test revealed no significant differences between the different individuals within any of the three host groups. Of the 15 pairwise comparisons between mites from rabbit hosts and those from outbreak associated bighorn sheep only 1 pair were significantly different, while 43/48 pairwise comparisons of mites from outbreak associated bighorn sheep with the historically infested bighorn hosts were significantly different. The 5/48 comparisons that were not significantly different between the bighorn sheep groups involved the sample from which only one male mite was located for measurement and comparison.

# 3.2. Molecular analyses

DNA sequences were obtained from all but two of the hosts for which morphometric examination was performed. Sequence data was also obtained for three additional samples for which morphological examination was not performed. DNA sequences of *cytB* were obtained for 13 samples (Table 1), encompassing all three host groups. These were compared over an alignment length of 304 positions (Table 2). The four samples from the outbreak associated bighorn samples had *cytB* sequences that were 100% identical to one another. Similarly, there were no differences in *cytB* sequence among the seven historically infested bighorn samples. A comparison of the *cytB* sequences of mites collected from rabbits in Maple Ridge (BC) and Edmonton (AB) revealed one



**Fig. 4.** Distribution of outer opisthosomal setae (OOS) lengths of *Psoroptes* collected from rabbits (labelled Rabbit), USA bighorn sheep (labelled BHS\_USA), and Canada-outbreak associated bighorn sheep (labelled BHS\_CAN). Each is marked with the province or state of host origin. Horizontal lines represent median OOS of each host grouping.

# Table 2

Variable nucleotide positions in the alignment of *cytB* sequences of *Psoroptes* collected from rabbits, Canadian Bighorn sheep (Can BHS) and Bighorn sheep from the United States (GRP USA). A dot indicates the same nucleotide as in the sequence of *Psoroptes* from Can BHS. A ? Represents an unknown nucleotide.

		Ali	Lgr	nm€	∋nt	C E	205	зıt	lor
		6	7	9	1 0 3	1 9 6	2 2 4	2 7 4	2 9 4
CAN BHS		Α	Т	А	А	Т	А	С	Т
RABBIT1	(Edmonton)	?	?	?	?	•	•	•	•
RABBIT2	(Maple Ridge)	•	·	•	•	•	•	·	С
GRP USA		G	G	G	G	C	G	G	•

# Table 3

Variable nucleotide positions in the alignment of *cox1* sequences of *Psoroptes* collected from Canadian bighorn sheep (BHS\_Can1 & BHS\_Can4) and bighorn sheep from the United States (BHS\_USA3 & BHS\_USA4). A dot indicates the same nucleotide as in the sequence of BHS\_CAN1. A ? Represents an unknown nucleotide.

	Alignment position											
	1 1 2	1 7 5	1 7 6	1 9 7	2 6 2	3 1 2	3 1 6	3 2 5	3 3 7	3 4 6	4 1 6	5 4 4
BHS_CAN1 BHS_CAN4 BHS_USA3 BHS_USA4	T C ·	A G G	A G G	T C C	A G G	G ・ C C	A G G	C · T T	C · T T	C · T	A ? G G	T ? C C

nucleotide difference, a pyrimidine transitional mutation at position 291 of the sequence alignment. Although the *cytB* sequence of the rabbit sample from Edmonton was incomplete at the 5' end, it was identical to that of the *cytB* sequences of mites collected from outbreak associated bighorn sheep. In contrast, the mites from outbreak-associated bighorn sheep and from rabbits in Canada differed from mites collected from bighorn sheep in the United States at 7 (2.3%) of the 304 alignment positions (Table 2). These differences in nucleotide sequence consisted of four purine transitions and two transversional mutations.

DNA sequences of *cox1* were obtained from 9 samples (Table 1); two bighorn outbreak samples and 7 historically infested bighorn samples, and these were compared over an alignment length of 638 positions (Table 3). However, no sequences could be obtained for the rabbit samples, and only partial sequences were obtained for many of the bighorn sheep samples. There was one nucleotide difference between

the two samples from Canadian bighorn sheep, consisting of a pyrmidine mutation at position 111 of the sequence alignment (Table 3). Similarly, there was one pyrmidine mutation at position 346 of the sequence alignment of the two samples of bighorn sheep from the United States. In contrast, the *cox1* sequences of mites from Canadian bighorn sheep differed at 10 (1.6%) of the 638 alignment positions from mites collected from Bighorn sheep in the United States (Table 3). These differences in nucleotide sequence consisted of five purine transitions, four pyrimidine transitions and one transversional mutation.

## 4. Discussion

Evidence of two genetically and phenotypically separate groups of *Psoroptes* were apparent based on the morphological and molecular analyses. The distinctly different shape of the opisthosomal lobes (Fig. 3)

and the different lengths of the OOS of the rabbit and the outbreak associated bighorn sheep mites relative to the historically affected bighorn sheep mites shows a clear distinction between these two groups (Fig. 4). These different phenotypes were reported by Boyce et al. (1990) who found that mites collected from bighorn hosts had slightly longer "length of lateral margin of opisthosomal knob" than mites collected from rabbit hosts as well as significantly longer OOS lengths. The mites collected from outbreak associated bighorn sheep in southern BC and northern Washington state are the first example of mites that match the rabbit phenotype causing natural infestation in a wild bighorn sheep population. Despite the small number of rabbit mites that were successfully DNA sequenced, the very high percent similarity found between the outbreak associated bighorn sheep and rabbit mites relative to the historically affected bighorn mites supports these findings. It suggests that these differences are likely to be the result of shared ancestry between the mites of rabbits used in this study and those found on outbreak associated bighorn sheep rather than the possibility of convergent evolution of this phenotype. This indicates that the mite infestation that is present within the outbreak associated bighorn herds may not have originated from nearby infested bighorn populations in the USA, but rather it likely represents a host species jump where the ecotype that is generally found on rabbits began to infest bighorn sheep.

*Psoroptes* samples from wild eastern cotton tail rabbits (*Sylvilagus floridanus*) or white-tailed jackrabbits (*Lepus townsendii*) from BC were solicited for this study but no samples could be located. While *Psoroptes* is known to survive in the environment for a period of 10–14 days, and therefore cross-species transmission events are theoretically possible without direct contact between different host species, the fact that it hadn't been previously documented provoked further inquiry into possible sources of disease introduction. Investigation into the history of bighorn sheep in southern BC revealed a likely possibility in the form of a wildlife park that used to be located on the Penticton Indian Band Reserve in BC called the Okanagan Game Farm. The Okanagan Game Farm was situated in what is now the core area of the Penticton Indian Band bighorn herd. The same herd that provided the majority of *Psoroptes* samples from outbreak associated bighorn sheep used in this study.

In 1999, four years prior to the first record of symptomatic Canadian bighorn sheep, the Okanagan Game Farm closed down (Horton 2007). That facility held a number of exotic and native species, among them a herd of bighorn sheep made up of locally captured as well as imported stock from the USA. The facility also held a domestic rabbit colony bred for feeding the carnivores in the collection. The rabbit colony had chronic lesions of ear mites and heavily infested animals were euthanized for feed. At the time of closure, bighorn sheep were captured, individually inspected by provincial and federal veterinarians and sampled for export to a variety of captive and free-ranging locations in the USA, at which time none showed obvious clinical symptoms of Psoroptes infestation. A game farm employee, as well as private veterinarian who did work for the game farm, however, reported inspecting and attempting to treat bighorn sheep for mange-like symptoms during its operation (C. Lacey, personal communication, January 22, 2016; D. Ward, June 17, 2019). The etiologic cause of those symptoms was not confirmed at that time but it now seems likely that they were infested with Psoroptes.

It is unclear whether the mite infestation originated on the game farm or whether imported animals brought the infestation with them but according to the employee's notes, the mites were first observed on two symptomatic bighorn sheep in 1991, many years after the opening of the park, and continued until the park shut down in 1999 (C. Lacey, personal communication, January 22, 2016). As there are no reports of the rabbit ecotype of *Psoroptes* infesting bighorn sheep elsewhere in North America, it seems unlikely that the infestation was introduced by infested foundation stock of the Okanagan Game Farm bighorn population. This captive situation would have placed captive bighorn sheep in close proximity to infested rabbits facilitating an opportunity for parasite transfer from rabbits to bighorn sheep through fomites, feed, or even direct contact between the animals.

It is reported that during its time in operation bighorn sheep did escape the confines of the game farm to found the "Kruger Hill Subpopulation" (Harper et al., 2002). Thus, it seems highly likely that the mites crossed host species due to captivity at the Okanagan Game Farm, thus explaining why other examples of this host-species jump haven't been documented in natural settings. It is also possible that these different mite strains may exhibit different virulence when infesting bighorn sheep. This may explain why Canadian and Sinlahekin bighorn populations infested with the rabbit variant have experienced population wide declines. Population level effects seem to be less common in most *Psoroptes* infested bighorn populations in the USA, though Boyce et al. (1990) did attribute the functional extirpation of desert bighorn sheep in the San Andres National Wildlife Refuge to *Psoroptes*.

These findings also provide an important timeline for when *Psoroptes* may have first been introduced to Canadian bighorn sheep, which is important for traceback and impact analysis. For example, bighorn sheep were translocated from the Keremeos area of BC (now endemic with *Psoroptes*) to the Okanagan Mountain Park (outside of the current *Psoroptes* affected area) in 2007, before *Psoroptes* was recognized in the Okanagan, but after its introduction (Reid, 2012). The translocated animals were free of clinical symptoms of mange at the time of translocation and *Psoroptes* has not been reported in the Okanagan Mountain Park animals at this time, however further scrutiny of those animals as well as serological testing for exposure in the recipient herd would be worthwhile to ensure that *Psoroptes* was not translocated with any of those animals in 2007.

This study provides a real-world example of the lack of host specificity of the *Psoroptes* mite that has been previously reported in crossinfection trials. It is the first report of a natural infestation of bighorn sheep with mites that are morphologically and genetically associated with domestic rabbits (formerly known as *Psoroptes cuniculi*). It raises questions about the potential difference in the virulence of this strain of *Psoroptes* mites when infesting bighorn sheep. Finally, it suggests that assessment of the *Psoroptes* infestation status of wild or feral rabbits, horses, or other *Psoroptes* susceptible wildlife host species should be considered if wildlife managers want to attempt a *Psoroptes* eradication or management effort in bighorn sheep.

## Declaration of competing interest

None.

# Acknowledgements

The authors would like to acknowledge the numerous biologists and veterinarians that collected the *Psoroptes* samples used in this study. This included Jeff Heinlen who collected *Psoroptes* samples in Washington State, and the Arbutus West animal clinic who provided mites from rabbits. Dr. Mani Virapin of the Canadian Wildlife Health Cooperative is acknowledged for providing instruction to AMH on the morphological identification of *Psoroptes*. We also thank Heather Down, Dr. John Taylor and the University of Victoria for providing lab space and equipment for the mite isolation and morphometric measurement, and to Dr. Ilya Blum for their guidance on the statistical analysis of the morphometric data. The costs for molecular analyses were funded through a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant number 06300-2015 (to NBC).

### References

Amer, S., El Wahab, T.A., El Naby Metwaly, A., Feng, Y., Xiao, L., 2015. Morphologic and genotypic characterization of *Psoroptes* mites from water buffaloes in Egypt. PloS One 10, 1–11.

Bates, P.G., 1999. Inter- and intra-specific variation within the genus *Psoroptes* (Acari: psoroptidae). Vet. Parasitol. 83, 201–217.

#### A.M. Hering et al.

- Borg, N.J., Mitchell, M.S., Lukacs, P.M., Mack, C.M., Waits, L.P., Krausman, P.R., 2017. Behavioral connectivity among bighorn sheep suggests potential for disease spread. J. Wildl. Manag. 81, 38–45.
- Boyce, W., Elliott, L., Clark, R., Jessup, D., 1990. Morphometric analysis of *Psoroptes spp.* mites from bighorn sheep, mule deer, cattle, and rabbits. J. Parasitol. 76, 823–828. van den Broek, A.H., Huntley, J.F., 2003. Sheep scab: the disease, pathogenesis and
- control. J. Comp. Pathol. 128, 79–91. Buechner, H., 1960. The bighorn sheep in the United States, its past, present, and future.
- Wildl. Monogr. 48, 3–174. Cassirer, E.F., 2005. Ecology of Disease in Bighorn Sheep in Hell's Canyon. University of
- British Columbia, USA. Dergousoff, S.J., Chilton, N.B., 2007. Differentiation of three species of ixodid tick,
- Dermacentor andersoni, D. variabilis and D. albipictus, by PCR-based approaches using markers in ribosomal DNA. Mol. Cell. Probes 21, 343–348.
- Evans, L., Dawson, D., Wall, R., Burke, T., Stevens, J., 2003. Isolation of *Psoroptes* scab mite microsatellite markers (Acari: psoroptidae). Mol. Ecol. Notes 3, 420–424.
- Gu, X.-B., Liu, G.-H.H., Song, H.-Q.Q., Liu, T.-Y.Y., Yang, G.-Y.Y., Zhu, X.Q., 2014. The complete mitochondrial genome of the scab mite *Psoroptes cuniculi* (Arthropoda: Arachnida) provides insights into Acari phylogeny. Parasites Vectors 7, 1–10.
- Harper, W.L., Schwantje, H.M., Ethier, T.J., Hatter, I., 2002. Recovery Plan for California Bighorn Sheep in the South Okanagan Valley.
- Harris, R.B., Heinlen, J., Fitkin, S., Oyster, J., Mansfield, K., 2018. Sinlahekin Bighorn Sheep Herd – Update (With Emphasis on *Psoroptes ovis*). Washington Department of Fish and Wildlife.
- Horton, K., 2007 Feb 14. Canada 150: physician and lifelong prankster was a man of many worthy causes. Pentict Her. https://www.pentictonherald.ca/news/article\_0f aabfbc-7264-11e7-8c73-8b6307a32c4d.html.
- Juan, C., Chengcheng, L., Yae, Z., Li, H., 2015. Population identification and divergence threshold in Psoroptidae based on ribosomal ITS2 and mitochondrial COI genes. Parasitol. Res. 114, 3497–3507.

- Krakowetz, C.N., Lindsay, L.R., Chilton, N.B., 2014. Genetic variation in the mitochondrial 16S ribosomal RNA gene of Ixodes scapularis (Acari: Ixodidae). Parasites Vectors 7, 530.
- Lange, R.E., Sandoval, A.V., Meleney, W.P., 1980. Psoroptic scabies in bighorn sheep (*Ovis canadensis mexicana*) in New Mexico. J. Wildl. Dis. 16, 77–82.
- Ochs, H., Mathis, A., Deplazes, P., 1999. Single nucleotide variation in rDNA ITS-2 differentiates *Psoroptes* isolates from sheep and rabbits from the same geographical area. Parasitology 119, 419–424.
- OConnor, B.M., Klimov, P.B., 2015. Review and resolution of some nomenclatural issues regarding the genus (Acari: psoroptidae), scab-mites of domestic and wild mammals. Exp. Appl. Acarol. 66, 337–345.
- Ogle, D.H., Wheeler, P., Dinno, A., 2019. FSA: Fisheries Stock Analysis.
- Pegler, K.R., Evans, L., Stevens, J.R., Wall, R., 2005. Morphological and molecular comparison of host-derived populations of parasitic *Psoroptes* mites. Med. Vet. Entomol. 19, 392–403.
- Reid, A., 2012. Okanagan Mountain Park Bighorn Transplant Monitoring. Penticton, BC.
- Reid, A., 2013. Ashnola/Similkameen Bighorn Inventory March 2013. Penticton, BC.
- Scott, S., Schwantje, H., Bollinger, T., 2013. Psoroptic Mange in California Bighorn Sheep in Southern British Columbia: A Preliminary Review of Occurrence, Risk and Management Options.
- Stepaniuk, D., 2006. Emailed Mortality Report to Helen Schwantje.
- Sweatman, G.K., 1958. On the life history and validity of the species in *Psoroptes*, a genus of mange mite. Can. J. Zool. 36, 905–929.
- Wang, Shuai, Gu, X., Fu, Y., Lai, S., Wang, Shuxian, Peng, X., Yang, G., 2012. Molecular taxonomic relationships of *Psoroptes* and *Chorioptes* mites from China based on COI and 18S rDNA gene sequences. Vet. Parasitol. 184, 392–397.
- Zahler, M., Essig, A., Gothe, R., Rinder, H., 1998. Genetic evidence suggests that *Psoroptes* isolates of different phenotypes, hosts and geographic origins are conspecific. Int. J. Parasitol. 28, 1713–1719.
- Zahler, M., Hendrikx, W.M.L., Essig, A., Rinder, H., Gothe, R., 2000. Species of the genus *Psoroptes* (Acari: psoroptidae): a taxonomic consideration. Exp. Appl. Acarol. 24, 213–225.