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

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**A B S T R A C T**

*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 on historically infested bighorn sheep. Outer opisthosomal setae lengths measured an average of 81.7 μm (±7.7 μm) 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. caniculi*, 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.

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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).

![Characteristic long segmented peduncle (circle) that differentiates the genus of Psoroptes spp. from other psoroptidae that have relatively short unsegmented peduncles. (40× magnification captured on a Zeiss Universal compound light microscope outfitted with a TRKe SPOT camera using SPOT image capture software).](image1)

![Map of the sample origin locations and associated host groups of Psoroptes samples used.](image2)
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 specificity, 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; O’Connor 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.

Table 1

| Host origin       | No. of samples used for morphological and morphometric analyses | 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    |

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™ (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.
primer, 1.25U DNA polymerase (Phusion HotStart II), 5 µl 5X Buffer Phusion Green HF buffer, and 1–2 µl 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 µl) 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 µm (range = 114 µm–193µm), outbreak bighorn mite OOS lengths had a median of 81 µm (range = 62 µm–101µm) and rabbit mite OOS lengths had a median of 85 µm (range = 57 µm–142µm) (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. 3. (a) Micrograph of a characteristic opisthosomal lobe of a USA bighorn mite (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 sheep in the Hells Canyon metapopulation. Note the more prominent outer opisthosomal lobe 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).](image-url)
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,14,115(128,546),(429,585) 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 pyrimidine mutation at position 111 of the sequence alignment (Table 3). Similarly, there was one pyrimidine mutation at position 346 of the sequence alignment of the two samples of 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.

### 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 ectotype 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 and northern Washington state revealed a likely possibility in the form of Psoroptes that is generally found on rabbits began to infest 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 cross-infection 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.

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