

KNEMIDOKOPTES MITES AND THEIR EFFECTS ON THE GRIPPING POSITION
OF STELLER'S JAY FEET

By

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ABSTRACT

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Steller's jays (*Cyanocitta stelleri*) with swollen legs and feet resembling the signs of scaly leg have been commonly seen around Arcata, California, USA. These signs are thought to be caused by knemidokoptic mites, a group of parasites specialized at exploiting a range of avian hosts. I compared the gripping position in the feet of jays with variable signs of this condition as an index of their ability to perch, confirmed the presence of mites and identified them to the nearest species using genetic analysis, and compared the relatedness of the *cytochrome oxidase subunit I* (COI) gene between mites collected from different host species. Jays with visible signs of mite infestation had greater variability and a reduced degree of contraction in the gripping position of their feet compared to jays without signs, suggesting infestation may have an impact on the host's ability to perch. DNA barcoding of the COI gene (578 base pairs) from mites collected from a Steller's jay was compared to *Knemidokoptes jamaicensis*, *Knemidokoptes derooi*, and to unidentified *Knemidokoptes* sp. collected from different hosts. The mites from a Steller's jay were most closely related to *Knemidokoptes jamaicensis* but had a relatively high sequence divergence of 7.8%, supporting the possibility that the form infesting the Steller's jays may be an undescribed species.

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I'd also like to give a special thanks to Ian Butler and Blair Winnacott for all of their help and patience amplifying DNA from the tiny specimens. Finally, thank you to all of the volunteers who helped capture and sample the Steller's jays; Benjamin Alfaro, Alex Benn, Jayde Blair, Dr. Jeff Black, Dr. Richard Brown, Emily Armstrong Buck, Blake Burris, Brett Burdette, Isaac Henderson, Dylan Henriksen, Marissa Holguin, Brianna Neira, and Kachina Rowland. This project would not have been possible without all of your support.

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INTRODUCTION

The genus *Knemidokoptes* (Acari: Knemidocoptidae) contains 5 species of ectoparasitic mites that are distributed globally and infest birds from a wide range of taxa (Dabert et al. 2011). Female *Knemidokoptes* species mites are viviparous, and offspring undergo two nymphal stages before maturing into adults (Wade 2006). The entire lifecycle takes 3 weeks to complete, and all stages of the mites occur on the host (Wade 2006). Transmission is thought to be direct or through infested nest or roost sites, potentially by actively mobile larvae, nymphs, or adults (Dabert et al. 2013).

Knemidokoptes species mites are responsible for the condition knemidokoptiasis and inhabit several microhabitats on the hosts including the stratum corneum of the face and cere and under the scales of the feet and legs (Dabert et al. 2011). The pathogenesis of knemidokoptiasis in birds resembles that of sarcoptic mange in mammals (Pence 2008).

Knemidokoptes mutans, *Knemidokoptes jamaicensis*, and *Knemidokoptes intermedius* burrow beneath the scales of the legs and feet of birds and are commonly referred to as scaly leg mites (Dabert et al. 2013). Clinical signs of scaly leg include proliferative epidermal overgrowth with crusts and scab formation resulting from massive hyperkeratosis and intense dermal inflammation of the unfeathered part of the legs and feet (Pence 2008). Scaly leg can result in sloughing of claws, loss of digits, or amputation of the entire foot in severe cases (Pence 2008). Swelling caused by inflammation and proliferative skin growth associated with scaly leg is also thought to limit the host's ability to contract its feet and perch (Kaschula 1950, Kirmse 1966, Latta 2003, Pence et

al. 1999). Scaly leg is thought to be a progressive and chronic disease that does not usually resolve itself without treatment (Gaudioso-Levita et al. 2016, Pence et al. 1999). This is supported by evidence that signs are often more prevalent and severe in older individuals (Benkman et al. 2005, Gaudioso-Levita et al. 2016, Mainka et al. 1994).

Cases of scaly leg are commonly associated with *K. mutans* in domestic chickens and with *K. jamaicensis* and *K. intermedius* in wild passerines (Dabert et al. 2011). The list of hosts for *K. jamaicensis* and *K. intermedius* includes 42 passerine species distributed across 6 continents; host records of *K. jamaicensis* and *K. intermedius* are shown in Appendix A. Comparisons of the minimum and maximum morphological measurements show that *K. intermedius* is larger than *K. jamaicensis* (Dabert et al. 2011), but idiosomal measurements of *Knemidokoptes* spp. mites taken from different hosts range along a wide spectrum (Dabert et al. 2013). The species identifications of the mites included in Appendix A. were based on morphological features and show variability in individuals originating from different host species. The broad host specificity and morphological variability within this genus of mites potentially indicates a multispecies complex (Dabert et al. 2013).

A DNA barcoding system based on a region of the *cytochrome oxidase subunit I* (COI) gene has been proposed as a standard procedure for species determination in animals (Herbert et al. 2003). This method of species identification has already been applied to *Knemidokoptes* spp.¹ mites collected from a common chaffinch (*Fringilla*

¹ *Knemidokoptes* spp. refers to multiple species within the genus while *Knemidokoptes* sp. refers to a single or unknown species.

coelebs), pine grosbeak (*Pinicola enucleator*) and golden eagle (*Aquila chrysaetos*) (Dabert et al. 2013, Knowles et al. 2019, Mete et al. 2014). The mites found infesting the feet of Steller's jays have not been previously barcoded and the species identity is unknown.

A population of Steller's jays (*Cyanocitta stelleri*) in northern California show clinical signs of scaly leg caused by an infestation of *Knemidokoptes* sp. mites (Zirpoli et al. 2012). Steller's jays are long-lived corvids that form long-term socially monogamous pair bonds (Overeem et al. 2014). Steller's jays are a member of the perching birds and have anisodactyl feet with opposing digits capable of gripping an object (Kochan 1994). The ability to grip and perch plays an essential role in arboreal locomotion and feeding in many avian taxa (Backus et al. 2015).

My objectives were to examine the effects of scaly leg on the ability of Steller's jays to grip a perch and to obtain COI sequences from their mites for comparison to other sequences available from *K. jamaicensis*, *K. intermedius*, *K. derooi*, and other *Knemidokoptes* sp. collected from different hosts. I created an index of the ability to perch by photographing and measuring the position of Steller's jay feet while gripping a metal dowel. I also sequenced the COI gene of mite specimens collected from three host species, including a Steller's jay, and created a phylogeny comparing these to other sequences available on GenBank.

STUDY AREA

This study was conducted from February 2019 to March 2020 in and near Arcata, California (10T 409056m E 4521987m N) in suburban neighborhoods bordering a second growth coastal redwood (*Sequoia sempervirens*) forest. The Steller's jays included in this study are part of a non-migratory population that are part of an ongoing study (J. M. Black, Humboldt State University), and those sampled included both banded and unbanded individuals. The Steller's jays in this area typically maintain socially monogamous pairs that defend a home territory centered around their nest (Gabriel and Black 2012). This population of jays utilize feeding stations that are spaced throughout the study area and shared by a variety of other birds including Eurasian collared doves (*Streptopelia decaocto*), band-tailed pigeons (*Patagiones fasciata*), dark-eyed juncos (*Junco hyemalis*), white-crowned sparrows (*Zonotrichia leucophrys*), and house sparrows (*Passer domesticus*). None of the other species of birds frequenting the study area have been observed to have obvious signs of scaly leg (J. M. Black, personal communication).

METHODS

This research was conducted under the Humboldt State University Institutional Animal Care and Use Protocol (IACUC No. 18/19.W.54-A). Steller's jays with and without signs of scaly leg were captured using Havahart box traps (Woodstream Corporation, Lititz, PA) and custom-built feeder-traps (25x30x106 cm) outfitted with sliding doors and baited with whole peanuts. Researchers were present during each trapping period to manually trigger the trap and immediately remove any birds that were captured. All of the birds captured in this study were considered adults based on molt wear and gape color (Pyle 1997). Individuals were removed from the trap by hand with a cloth bag and weighed using a 300g spring scale (Pesola, Schindellegi, Switzerland). Tarsus length was measured using 6-inch digital calipers (Harbor Freight, Pittsburgh, PA). The range of motion available in the hallux (D1) and middle (third) digit (D3) was measured by placing the foot of each bird on a 0.5 cm diameter metal perch until it reacted by gripping and contracting the leg muscles. Photos were then taken with a 12-megapixel iPhone camera mounted 13 cm from the end of the perch and oriented laterally towards the bird's contracted foot. A total of four photos were taken from each bird with images taken from each side of both left and right feet. Skin scrapings were collected from birds that showed visible signs of mites using a scalpel and forceps and stored in 95% ethanol. The claws of un-banded birds were marked with a unique combination of nail polish to avoid recapture. Banded birds with visible signs of scaly leg had their bands removed to prevent potential constriction around the band due to swelling.

Gripping Position

The gripping position of the feet was measured by comparing the relative position of features recognizable in the photos. The degree of contraction of the hallux and D3 around the perch was measured by the angles created between the protrusion on the posterior side of the tarsometatarsus (hypotarsus), the center of the perch, and the end of each digit at the base of the claw (Figure 1). Photos from individuals whose foot only contacted the perch at a single point were considered not to have been gripping and were removed from the analysis.

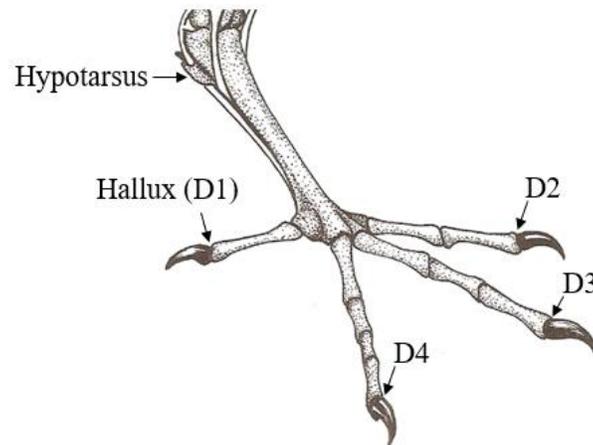


Figure 1. Diagram of an anisodactyl bird foot in an open position. The structure and nomenclature are representative of a Steller's jay foot (modified from Kochan 1994).

Photos were measured using Image-J (National Institutes of Health, Bethesda, MD), and the birds were classified into groups of individuals that either showed visible signs of scaly leg on their feet ($n = 14$) or did not ($n = 15$). Three logistic regressions were used to model the angle of the hallux, D3, tarsus length, and weight against visible signs of scaly leg. The models were compared using Akaike Information Criterion (AIC), and the regression with the best fit was selected (Akaike 1974). Two additional logistic regressions were used to plot the angles of the hallux and third digit (D3) separately against visible signs of scaly leg. The McFadden R-squared value (hereafter referred to as R^2) was used as a pseudo R-squared for logistic models (McFadden 1973). The prevalence of signs of scaly leg in this population was calculated from HSU's ongoing Steller's jay database with data ranging from 1998 to the present (J. M. Black, Humboldt State University, unpublished data). Separate T-tests were used to calculate the differences in the prevalence of signs between hatch-year jays (juveniles), after-hatch-year jays (adults), and between males and females. All statistical analyses were done using RStudio (version 3.4.4, Boston, MA).

Genetic Analysis

All skin scrapings were observed under a dissecting microscope (Olympus, series SZX16, Tokyo, Japan), and any mites detected were photographed, separated from the host tissue, and stored in 95% ethanol. Extraction of total genomic DNA was attempted with skin samples that were expected to contain fragments of mite DNA collected from a

Steller's jay with visible signs of scaly leg, and from between 2 to 4 whole mites depending on the availability of specimens using the DNeasy™ DNA isolation kit (Qiagen Inc., Valencia, CA). The only modification of the standard manufacturer's protocol was an extended incubation time with thermomixing for 72 hours. Whole mite specimens of *Knemidokoptes* spp. collected from a rook (*Corvus frugilegus*), an American robin (*Turdus migratorius*), and a red-winged blackbird (*Agelaius phoeniceus*) were sent by Dr. P. B. Klimov (University of Michigan) for comparison with the DNA from mites collected from Steller's jays (Table 1). These samples were shipped in a cold container while stored in 95% ethanol. The mites taken from the rook were still imbedded in host tissue, and the mites from the American robin and red-winged blackbird had already been separated. All of the specimens included in this table are considered scaly leg mites except *Knemidokoptes derooi*.

Table 1. *Knemidokoptes* spp. used in DNA barcoding analysis with mitochondrial COI.

Host spp.	Mite spp.	Location	Specimens used	Accession #
Steller's jay	<i>Knemidokoptes</i> sp.	Humboldt, CA	2 mites and skin samples (this study)	MW250782
rook	<i>Knemidokoptes</i> sp.	Kazakhstan	4 mites (this study)	MW250784
American robin	<i>K. jamaicensis</i>	San Diego, CA	3 mites (this study)	MW250783
common chaffinch	<i>K. jamaicensis</i>	Romania	Dabert et al. 2013	JQ037816
pine grosbeak	<i>K. jamaicensis</i>	Galena, AK	Knowles et al. 2019	MF043583
golden eagle	<i>K. derooi</i>	Livermore, CA	Metz et al. 2014	KJ787640

The mites were moved from 95% ethanol to 500 μ l Eppendorf tubes with 180 μ l of ATL lysis buffer and 20 μ l of proteinase K (Qiagen Inc., Valencia, CA). The samples were incubated at 56° C with 450 rpm shaking in a thermomixer for 72 hours. DNA isolation was performed using the manufacturer's protocol for purification of total DNA from animal tissues with a final step of eluting 100 μ l buffer AE (instead of 200 μ l) to increase the final DNA concentration. A region of the *cytochrome oxidase subunit I* (COI) was amplified by polymerase chain reactions using the primers bcdF05 and bcdR04 (Dabert et al. 2008) in 25 μ l reaction volumes containing 4.25 μ l water, 0.5 μ l of each primer (1 μ M), 6.25 μ l GoTaq G2 Hot Start Colorless Master Mix (Part#

9PIM743), and 1 μ l of template DNA using a thermocycling profile of one cycle of 3 minutes at 95° C followed by 40 steps of 10 seconds at 95° C, 1 minute at 50° C, 1 minute at 72° C, with a final step of 5 minutes at 72° C. Following amplification, 1 μ l of each PCR product was analyzed by electrophoresis on a 1.5% agarose gel. Samples containing visible bands were sent to MCLAB (San Francisco, CA) with 10 μ l of each primer at 3.2 μ M concentration for sequencing.

Sequences were aligned using Clustal X (version 2.1, University College Dublin, Ireland) and were subsequently trimmed to 578 bp. The sequence from the whole mite specimens collected from a Steller's jay and the sequence from the skin samples expected to contain fragments of mite DNA from another Steller's jay were used to build a consensus sequence. A phylogenetic tree of the COI sequences was constructed in MEGA-X (version 3, Penn State, PA) using the neighbor-joining (NJ) analysis and Kimura 2 parameter (K2P) model (Kimura 1980). The root of the tree was *Myialges trinotoni* mites obtained on GenBank (Acari: Epidermoptidae, accession number KX060553). *Myialges trinotoni* (Epidermoptidae) and *Knemidokoptes* spp. (Knemidocoptidae) are sister families within the superfamily Analgoidea (Dabert and Mirnov 1999).

RESULTS

Thirty-two individual Steller's jays were captured and photographed during this study. Fourteen of those captured showed visible signs of scaly leg, and two intact mite specimens were recovered in the skin scrapings collected from 1 of the 14 jays. Sixteen of the jays captured in this study had been previously banded and 9 of those showed signs of scaly leg.

The broader prevalence of signs of scaly leg in this population of jays was estimated at 27% using the database dedicated to HSU's ongoing Steller's jay study ($n = 550$, J. M. Black, Humboldt State University, unpublished data). Jays that were captured during their hatch-year (juveniles) were less likely to show visible signs of mites than jays captured after-hatch-year (adults) ($t = 3.1$, $df = 544$, $P = 0.002$). Juveniles had a 20% prevalence of signs of scaly leg ($n = 244$) while adults had a 32% prevalence of signs ($n = 306$). There was no significant difference in the prevalence of signs between jays recorded as being male or female ($n = 182$, $t = -0.17$, $df = 179$, $P = 0.87$).

Gripping Position

A total of four photos from each individual were included in the analysis. Several photos were taken from either side of each foot, and the photo from each orientation in which the foot had the greatest degree of contraction around the perch was selected (Figure 2). The angles measured from the hallux and the third digit (D3) on both feet

were each averaged, and individuals received a single score for each digit. Some birds were missing the scale used to mark the distal end of the tarsus, so both tarsi were measured when possible, and the average was used for each individual. Three individuals that did not grip the perch in the photos were removed from the analysis.



Figure 2. Example photos included in the analysis of the gripping position of host feet. Photos in Column A show visible signs of scaly leg, and photos in Column B do not. The white line indicates the shortest distance from the hypotarsus to the center of the perch, the yellow line from the center of the perch to the end of the third digit (D3), and the red line from the center of the perch to the end of the hallux.

Birds with visible signs of mites showed greater variability in the degree of contraction in both the hallux and D3 when compared to birds without signs. Birds with visible signs had 3 times the standard deviation in the angle of the hallux and 1.5 times the standard deviation in the angle of the D3 than birds without signs. The degrees of contraction were calculated as the mean of the angles measured from the hallux and D3 for each bird (\pm the standard deviation; SD). The mean angles measured in birds with signs were $102.1^\circ (\pm 35.4^\circ)$ in the hallux and $67.3^\circ (\pm 14.0^\circ)$ in the D3 ($n = 14$). The mean angles measured in birds without signs were $119.1^\circ (\pm 11.8^\circ)$ in the hallux and $56.7^\circ (\pm 9.1^\circ)$ in the D3 ($n = 15$).

The logistic regression with the best fit included the angle of the hallux, third digit (D3), and the length of the tarsus but did not include weight ($n = 29$, $R^2 = 0.39$). The angles of the hallux and D3 were the best predictors of visible signs of scaly leg ($P = 0.10$ and $P = 0.08$, respectively). Tarsus length was not a significant predictor of scaly leg ($P = 0.89$) but including it in the model produced a better fitted regression (delta $R^2 = 0.09$, delta AIC = 3.3). Two separate logistic regressions using the angle of each digit as the only predictor of signs of scaly leg showed a slight tendency for birds with a smaller angle in the third digit (D3) to show signs ($R^2 = 0.15$, $P = 0.05$, Figure 3) and birds with a larger angle in the hallux to show signs ($R^2 = 0.09$, $P = 0.13$, Figure 4). Because the hallux and third digit (D3) are oriented opposite each other this reflects a reduced degree of contraction towards the perch from both digits in birds with visible signs of scaly leg compared to birds without visible signs.

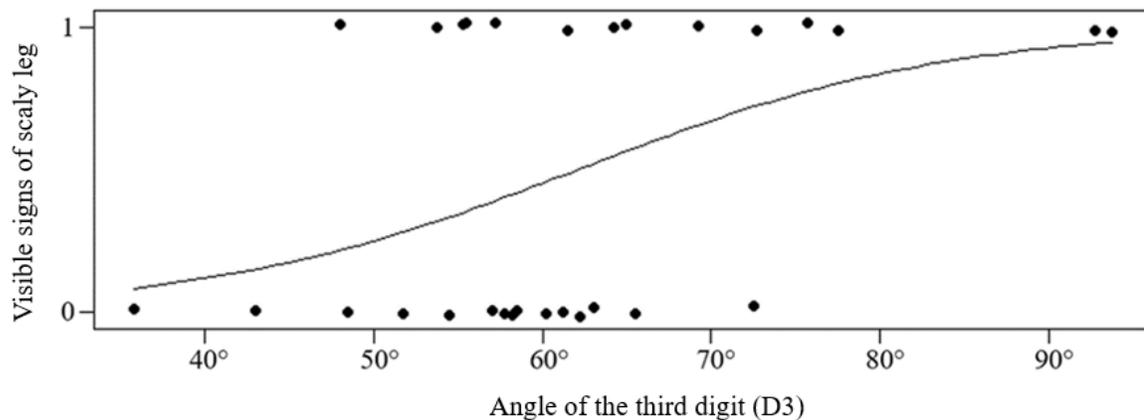


Figure 3. Plot of the degree of closure in the third digit (D3) of birds with (1) and without (0) signs of scaly leg ($n = 29$, $R^2 = 0.15$, $P = 0.05$).

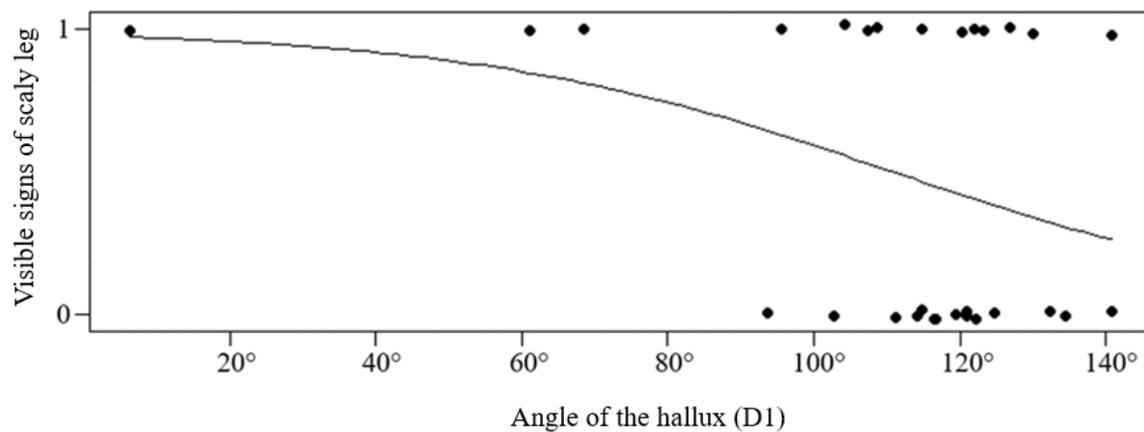


Figure 4. Plot of the degree of closure in the hallux of birds with (1) and without (0) signs of scaly leg ($n = 29$, $R^2 = 0.09$, $P = 0.13$).

Genetic Analysis

Amplification was initially attempted with skin scrapings expected to contain fragments of mite DNA collected from a Steller's jay with visible signs of scaly leg. A second round of amplification was attempted with whole mite specimens pooled together by their respective hosts to increase the amount of tissue available for DNA extraction. Two specimens collected from a different Steller's jay than the initial skin scrapings, 3 specimens collected from an American robin, 4 specimens collected from a rook, and 2 specimens collected from a red-winged blackbird were used. All of the samples were successfully amplified and sequenced except for those collected from a red-winged blackbird. A chromatogram of the sequences obtained from fragments of mite DNA contained in the skin samples collected from a Steller's jay showed a lower signal intensity than the sequences obtained from the whole mite specimens. The chromatograms of the sequences from both mite fragments contained in the skin samples and whole mites collected from Steller's jays were used together to build a consensus sequence. Photographs of specimens collected from the Steller's jay used in the analysis are seen in (Figure 5). These photos were taken while the samples were submerged in 95% ethanol before being lysed for DNA extraction.

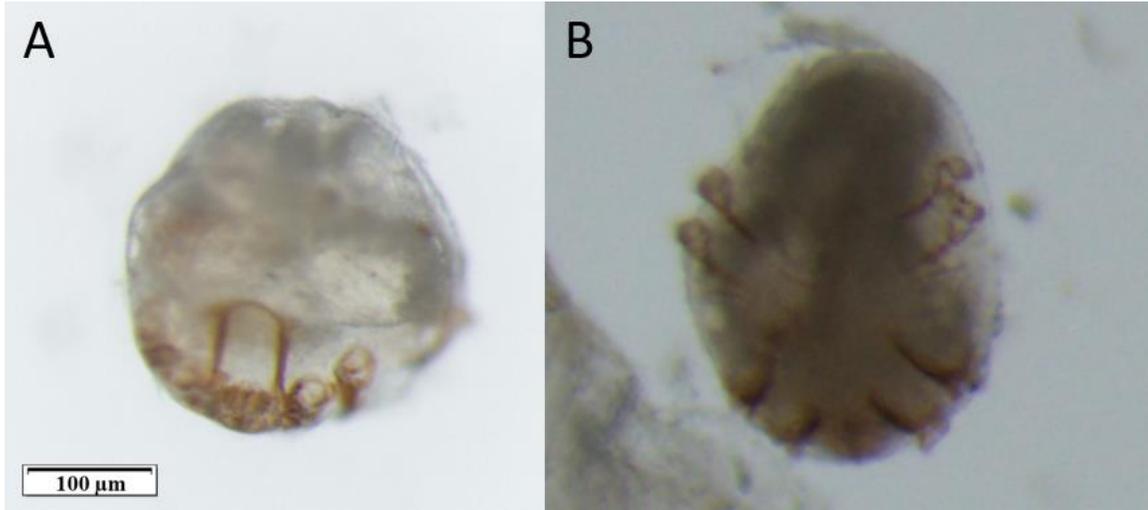


Figure 5. Photos of mites collected from a Steller's jay and used in the genetic analysis. Both specimens appear to be adult females. The specimen in Image A shows the dorsal view and the specimen in Image B shows the ventral view.

Based on a 578 bp sequence of the COI gene, the mites collected from Steller's jays are most closely related to mites collected from a rook and from a pine grosbeak, 7.6% and 7.8% sequence divergence, respectively (Table 2 and Figure 6). Mites taken from the rook were sequenced in this study and identified to genus by P. B. Klimov (University of Michigan, unpublished report). Mites from the pine grosbeak were identified as *K. jamaicensis*, (Knowles et al. 2019, GenBank Accession Number MF043583) making this the closest species match available to the mites collected from a Steller's jay.

The mites collected from the rook and pine grosbeak had the most closely related sequences with 0.5% divergence and are likely from the same species. The largest sequence divergence within the scaly leg mites was 12.5% between mites collected from

a common chaffinch and from a pine grosbeak. Both of these specimens were identified as *K. jamaicensis* based on morphological features (Dabert et al. 2013 and Knowles et al. 2019).

Table 2. Pairwise distances by the number of base substitutions per site from between COI sequences. Analyses were conducted using uncorrected p-distances. There are a total of 578 bp positions.

Host spp.	Mite spp.		1	2	3	4	5	6
Steller's jay	<i>Knemidokoptes</i> sp.	1						
rook	<i>Knemidokoptes</i> sp.	2	0.076					
pine grossbeak	<i>Knemidokoptes jamaicensis</i>	3	0.078	0.005				
American robin	<i>Knemidokoptes jamaicensis</i>	4	0.090	0.111	0.112			
common chaffinch	<i>Knemidokoptes jamaicensis</i>	5	0.095	0.123	0.125	0.048		
golden eagle	<i>Knemidokoptes derooi</i>	6	0.114	0.130	0.131	0.118	0.116	
	<i>Myialges trinotoni</i>	7	0.205	0.231	0.233	0.205	0.217	0.205

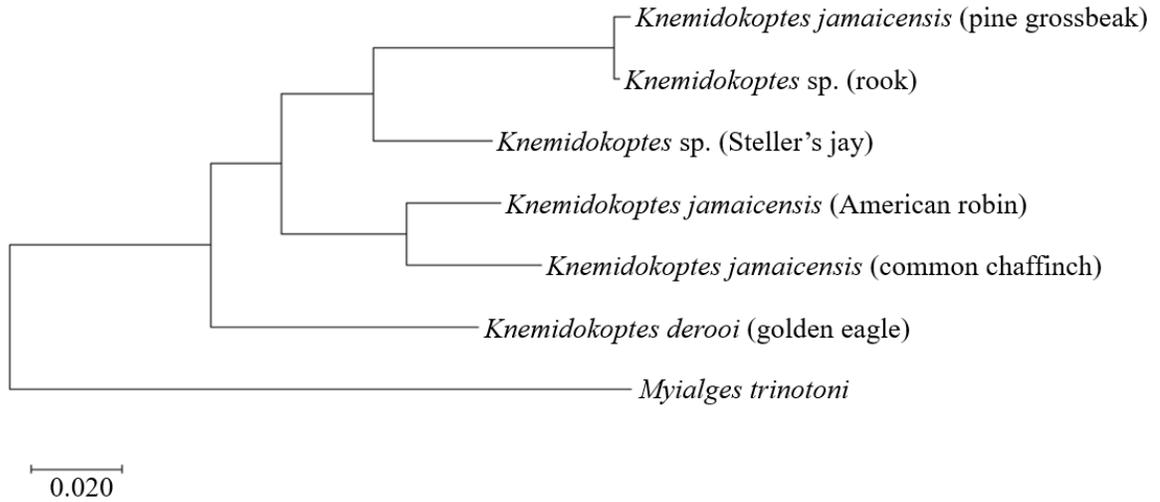


Figure 6. Phylogenetic tree based on the COI gene of mites collected from a Steller's jay, mites from 5 additional *Knemidokoptes* spp., and one *Myialges trinotoni* (Epidermoptidae). The tree was constructed using the Neighbor-Joining method (NJ), and evolutionary distances were computed using the Kimura 2-parameter (K2P) model. The branches were drawn to scale with lengths measured in the number of substitutions per site.

DISCUSSION

Twenty-seven percent of Steller's jays in this population had visible signs of scaly leg. Other reports of the prevalence of *K. jamaicensis* infestations have varied greatly, even within conspecific hosts. Kirmse et al. (1966) reported between 1% and 40% prevalence of *K. jamaicensis* in brown headed cowbirds (*Molothrus ater*) captured at different sites in Ontario between 1963 and 1965. Gaudioso-Levita et al. (2016) reported between 2% and 69% prevalence of *K. jamaicensis* in Hawai'i 'amhakihi (*Hemignathus virens*) across sites with different elevations in Hawai'i between 2007 and 2009. Prevalence of *K. jamaicensis* has also been reported to be as high 80% in a flock of American robins (Pence et al. 1990). This wide range of prevalence may reflect the variability of conditions for transmission within affected populations.

Signs of scaly leg were more common in older birds with a 20% prevalence recorded in jays captured during their hatch-year (juveniles) compared to a 32% prevalence in jays captured after-hatch-year (adults). If birds are exposed to mites while still in the nest, then these results suggest that infestations require time to develop before clinical signs of lesions are evident. Wade (2006) suggested that not all nestlings exposed to mites will become clinically infested and that genetic susceptibility, stressors or a compromised immune system may play a larger role in the clinical manifestation of scaly leg.

In this study mites were only visibly detected in skin scrapings from 1 out of the 14 individuals with signs that were sampled. These scrapings were collected from already

loose scales, and it is likely that the detection probability would be higher with deeper scrapings or by dissecting the legs and feet of deceased birds. A study on the ability to detect subclinical knemidokoptiasis in black sparrowhawks (*Accipiter melanoleucus*) through examination of skin samples from dead birds detected mites in 100% of birds with signs ($n = 3$) and in 0% of birds without signs ($n = 13$, van Velden et al. 2017).

The relatively low P values (ranging from 0.05 to 0.13) and McFadden R-squared values (from 0.09 to 0.39) generated by the models suggest that there is a reduced degree of contraction in the hallux and D3 of birds with visible signs of scaly leg and that the gripping position of Steller's jay feet is highly variable. Tarsus length was included in the full logistic model because it improved the R^2 and AIC values (delta $R^2 = 0.09$, delta AIC = 3.3). Longer tarsi could indicate proportionately longer foot and toe dimensions which may affect the perching position of the feet. Birds that showed visible signs of mites often also had swollen tarsi and missing scales which could have affected the tarsus measurements. The areas most affected by the mites varied between birds and localized swelling on different parts of the legs and feet could have added variability to the perching position. Some of the variability observed in this model could also be explained by birds not consistently gripping the perch in a way that displayed the full contraction available in their feet, low levels of inflammation related to unsuspected knemidokoptiasis, or previous injuries.

The greater variability and reduced degree of contraction in the gripping position observed in feet with visible signs of scaly leg suggests that infestation has an impact on the way birds are able to grip a perch. This could be due to the swelling and

hyperkeratosis around the joints and loss of flexibility, or to birds adapting their grip to avoid discomfort. The effects of an altered perching position on host fitness are not well understood, but poor or uncoordinated perching could result in some energetic cost. Distraction by irritation may reduce vigilance or potentially impact ability to procure food. Distraction, cost of difficult perching, and potentially other issues could lead to a greater likelihood of predation. Debilitated feet may also benefit the mites by encouraging extended resting positions and increased contact with the roost or nest which would be expected to increase the potential of transmission. Dabert et al. (2013) suggested such benefits might result in natural selective pressures that promote mites that cause more signs of disease.

The parasite-mediated handicap hypothesis proposes that disease could be the proximate mechanism regulating the development of energetically costly ornamental traits important in mate selection (Hamilton and Zuk 1982). Zirpoli et al. (2013) tested this hypothesis in the same population of Steller's jays used in this study by measuring the condition of secondary flight feathers between male Steller's jays with ($n = 29$) and without ($n = 34$) visible signs of scaly leg. Occurrence of scaly leg had no effect on feather growth rates ($P = 0.57$) but increased the proportion of ultra-violet wavelengths reflected ($P = 0.013$) (Zirpoli et al. 2013). Feather growth rate is used as an index of the individual bird's nutritional condition during its molting period (Grubb 2006), and the lack of a significant difference in growth rates between birds observed to have variable signs of scaly leg during their molt suggests the impacts of infestation on the ability to procure food are minor. The increased UV reflectance observed in birds with signs of

scaly leg did not support the parasite-mediated handicap hypothesis in this population of jays (Zirpoli et al. 2013).

While knemidokoptiasis can cause debilitation in individual birds and is occasionally responsible for mortality events (Pence 2008), epizootic knemidocoptiasis is unlikely to have long term effects on avian populations (Knowles et al. 2019, Pence 2008). Parasites with a high degree of host specificity, including knemidokoptic mites (Dabert et al. 2013), may tend to be less virulent to avoid dying with their host (Schmid-Hempel 2011). The effects of this condition on mate selection are unclear, but of the 9 banded jays with signs of scaly leg captured in this study, 3 were observed to have a mate the following season (J. M. Black, Humboldt State University, unpublished data).

The development of custom primers with better homology to the binding sites on the COI gene of this group of mites could produce longer sequence reads. However, the 7.8% divergence observed in this study between the mites collected from a Steller's jay and their closest species match to *K. jamaicensis* mites from a pine grosbeak (Knowles et al. 2019) suggest that the mites from a Steller's jay are an undescribed species. The 12.5% divergence between mites collected from a common chaffinch (Dabert et al. 2013) and mites collected from a pine grosbeak (Knowles al. 2019), both identified as *K. jamaicensis* based on morphological features, is evidence of high genetic divergence within this group of mites. The 0.5% divergence observed between the COI sequences of the *Knemidokoptes* sp. specimens collected from a rook (P. B. Klimov, unpublished data) and the *K. jamaicensis* mites collected from a pine grosbeak (Knowles et al. 2019) suggests they could be the same species. The high variability in the divergence of COI

sequences within available *Knemidokoptes* spp. specimens supports the need to reevaluate the taxonomy of this group of mites.

The broad distribution, host specificity, and genetic divergence of scaly leg mites suggests they could be delimited as either a few species with wide trophic specialization or as a series of many cryptic species (Dabert et al. 2013). The lack of reports of scaly leg in other species of birds that frequent the same feeding stations as infested Steller's jays supports the possibility of high host specificity. If *Knemidokopes* transmission is restricted to direct contact or through infested nest and roost sites as reported in the literature (Dabert et al. 2011, 2013; Knowles et al. 2019; Pence 2008), then this could limit interspecific transmission and create fragmented populations of reproductively isolated mites that cospeciate with their hosts. The taxonomic classifications within this genus of mites could be clarified through further genetic evaluation in combination with traditional morphological methods of species identification.

SUMMARY AND RECOMMENDATIONS

Birds with visible signs of scaly leg had an altered gripping position compared to birds without signs, however, the effects of this difference on the host are not clear. More research is needed to understand the modes of transmission of *Knemidokoptes* mites and the effects of infestation on host fitness. This genus of mites shows sufficient genetic divergence between specimens collected from different hosts to merit taxonomic review.

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APPENDIX

Table 3. The host family relationships of *Knemidokoptes intermedius* reported in the literature.

Family	Species	Location	Reference
Artamidae	Pied currawong (<i>Strepera graculina</i>)	Australia	Jaensch et al. 2003
Corvidae	Common raven (<i>Corvus corax</i>)	Romania	Dabert et al. 2011
	Forest raven (<i>Corvus tasmanicus</i>)	Tasmania	Mason and Fain 1988
Menuridae	Superb lyrebird (<i>Menura novaehollandiae</i>)	Australia	Holz et al. 2005
Prunellidae	Rufous-breasted accentor (<i>Prunella strophiata</i>)	Pakistan	Fain and Elsen 1967

Table 4. The host family relationships of *Knemidokoptes jamaicensis* reported in the literature.

Family	Species	Location	Reference
Alaudidae	Eurasian skylark (<i>Alauda arvensis</i>)	Denmark	Poulsen 1964
Corvidae	American crow (<i>Corvus brachyrhynchos</i>)	US (Louisiana)	Pence 1972
Drepanidae	Hawai'i 'amakihi (<i>Hemignathus virens</i>)	US (Hawaii)	Gaudioso-Levita et al. 2016

Family	Species	Location	Reference
Emberizidae	Greater Antillean bullfinch (<i>Loxigilla violacea</i>)	Dominican Republic	Latta and O'connor 2001
	Eastren towhee (<i>Pipilo erythroptalmus</i>)	US (Louisiana)	Pence 1970
Fringillidae	Common linnet (<i>Carduelis cannabina</i>)	Denmark	Poulsen 1964
	Common redpoll (<i>Carduelis flammea</i>)	Denmark	Poulsen 1964
	Twite (<i>Carduelis flavirostris</i>)	Denmark	Poulsen 1964
	Eurasian siskin (<i>Carduelis spinus</i>)	Denmark, Austria	Poulsen 1964, Kutzer 1964
	European goldfinch <i>Chloris chloris</i>	England (Devon)	Macdonald and Gush 1983
	Evening grosbeak (<i>Coccothraustes vespertinus</i>)	US (New Mexico)	R. A. Cole, USGS, unpublished report
	Common chaffinch (<i>Fringilla coelebs</i>)	England, Denmark, Czech Republic	Literák et al. 2005, Macdonald and Gush 1983
	Brambling (<i>Fringilla montifringilla</i>)	England, Denmark	Fain and Elsen 1967, Poulsen 1964
	Red crossbill (<i>Loxia curvirostra</i>)	US (Idaho)	Benkman et al. 2005
Pine grosbeak (<i>Pinicola enucleator</i>)	US (Alaska)	Knowles 2019	

Family	Species	Location	Reference
	Eurasian bullfinch (<i>Pyrrhula pyrrhula</i>)	England, Denmark	Macdonald and Gush 1983
Fringillidae	Atlantic canary (<i>Serinus canaria</i>)	South Africa	Kaschula 1950
Icteridae	Red-winged blackbird (<i>Agelaius phoeniceus</i>)	Canada, US (Arkansas)	Fain and Elsen 1967
	Brewer's blackbird (<i>Euphagus cyanocephalus</i>)	US (Unknown)	Fain and Elsen 1967
	Brown-headed cowbird (<i>Molothrus ater</i>)	Canada (Ontario)	Kirmse 1966
	Common grackle (<i>Quiscalus versicolor</i>)	US, Canada	Fain and Elsen 1967, Kirmse 1966
Motacillidae	Pipits (<i>Anthus sp.</i>)	Sri Lanka	Fain and Elsen 1967
	Cape wagtail (<i>Motacilla capensis</i>)	South Africa	Munday 2006
Mimidae	Gray catbird (<i>Dumetella carolinensis</i>)	US (Washington State)	Fain and Elsen 1967
	Northern mockingbird (<i>Mimus polyglottos</i>)	Dominican Republic	Latta and O'connor 2001
Paridae	Black-capped chickadee (<i>Parus atricapillus</i>)	Canada (Ontario)	Kirmse 1966
Parulidae	Prairie warbler (<i>Dendroica discolor</i>)	Dominican Republic	Latta and O'connor 2001
	Palm warbler (<i>Dendroica palmarum</i>)	Dominican Republic	Latta and O'connor 2001

Family	Species	Location	Reference
	Cape May warbler (<i>Dendroica tigrine</i>)	Dominican Republic	Latta and O'connor 2001
Parulidae	Green-tailed warbler (<i>Microligea palustris</i>)	Dominican Republic	Latta and O'connor 2001
Phaenicophilidae	Black-crowned tanager (<i>Phaenicophilus palmarum</i>)	Dominican Republic	Latta and O'connor 2001
Sittidae	White-breasted nuthatch (<i>Sitta carolinensis umbrosa</i>)	Mexico	Hardy 1965
Turdidae	White-chinned thrush (<i>Turdus aurantius</i>)	Jamaica	Fain and Elsen 1967
	American robin (<i>Turdus migratorius</i>)	US (Oklahoma)	Pence et al. 1999
	Spectacled thrush (<i>Turdus nudigenis</i>)	Trinidad	Fain and Elsen 1967
Tyrannidae	Hispaniolan pewee (<i>Contopus hispaniolensis</i>)	Dominican Republic	Latta and O'connor 2001
	Great crested flycatcher (<i>Myiarchus crinitus</i>)	Canada (Ontario)	Kirmse 1966