The presence of parasitic mites on small mammals in Algonquin Provincial Park, Ontario, Canada

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Abstract: Here we identify, for the first time, a generalist orange parasitic mite found on multiple small-mammal species in Algonquin Provincial Park, Ontario, Canada. Results from 18S rRNA sequencing suggest that these mites may belong to the family Trombiculidae (Acari: Trombidiformes), although morphological and additional genetic analyses are needed to confirm this identification. The presence of these mites was associated with lower body condition in North American red squirrels (*Tamiasciurus hudsonicus* (Erxleben, 1777)), suggesting that these mites may be a detriment to fitness in small mammals or are more successful when targeting individuals in poorer condition. A lack of sex-biased host infection, emergence dates outside of the host reproductive season, and stability of mite prevalence across study years indicate mite population dynamics may be driven predominantly by factors external from host status, such as climatic conditions or habitat preference. Future research should focus on providing additional reliable identification of these mites, as well as broadening our knowledge on their range, pathogenicity, and impact on small-mammal community dynamics.

Key words: small mammal, body condition, parasitism, mite, red squirrel, *Tamiasciurus hudsonicus*.

Résumé : Nous identifions, pour la première fois, un acarien orange parasite généraliste présent sur plusieurs espèces de petits mammifères dans le parc provincial Algonquin (Ontario, Canada). Les résultats de séquençage d'ARNr 18S donnent à penser que ces acariens appartiennent à la famille des trombiculidés (acariens : trombidiformes), bien qu'une analyse morphologique et d'autres analyses génétiques soient nécessaires pour confirmer cette identification. La présence de ces acariens est associée à un faible embonpoint chez les écureuils roux (*Tamiasciurus hudsonicus* (Erxleben, 1777)), ce qui indiquerait qu'ils pourraient avoir une influence délétère sur l'aptitude de petits mammifères ou qu'ils ont plus de succès quand ils ciblent des individus de plus faible embonpoint. L'absence de biais des taux d'infection selon le sexe de l'hôte, leurs dates d'émergence en dehors de la période de reproduction de l'hôte et la stabilité de la prévalence de ces acariens d'une année d'étude à l'autre indiquent que la dynamique de leurs populations pourrait être principalement contrôlée par des facteurs externes à l'état de l'hôte, comme des conditions climatiques ou des préférences en matière d'habitat. Les travaux futurs devraient mettre l'accent sur d'autres identifications fiables de ces acariens, ainsi que sur l'élargissement des connaissances sur leur aire de répartition, leur pathogénicité et leur incidence sur la dynamique des communautés de petits mammifères. [Traduit par la Rédaction]

Mots-clés : petits mammifères, embonpoint, parasitisme, acarien, écureuil roux, *Tamiasciurus hudsonicus*.

Introduction

Parasites negatively influence host fitness (Khokhlova et al. 2002), making it important to understand how parasites affect host physiology, ecology, and evolution (Devevey and Christe 2009). Organisms possess a finite supply of resources and energy, which is allocated to processes necessary for survival, including growth, maintenance, immune system regulation, reproduction, and foraging (Sheldon and Verhulst 1996; Lochmiller and Deerenberg 2000; Morand et al. 2006; Schwanz 2008; Gooderham and Schulte-Hostedde 2011). Parasites can directly reduce resources available to their host in numerous ways, including the extraction of nutrients from blood and tissue (Khokhlova et al. 2002; Schwanz 2008; Hillegass et al. 2010). Parasites may also affect their host by forcing them to allocate energy away from reproduction, foraging, and (or) growth. These resources may then be increasingly invested into immunity to mitigate the negative effects of the parasite, for example, increasing inflammation

around the infected site to prevent parasites from reaching blood sources in hosts (Lochmiller and Deerenberg 2000; Owen et al. 2009). This reallocation of energy can lead to a reduction in body condition of both the host and their offspring, and may ultimately lead to changes in host population dynamics (Sheldon and Verhulst 1996; Schwanz 2008; Gooderham and Schulte-Hostedde 2011; Patterson et al. 2013).

Parasitic mites are a diverse taxonomic group of arthropods and some mites, such as chigger mites from the family Trombiculidae, damage the skin of their hosts by biting or tunnelling and injecting salivary molecules into open wounds, potentially causing severe dermatitis, necrosis, or pathogen transmission (reviewed in Shatrov and Kudryashova 2006). Mites often exhibit seasonal population cycles, which can be related to climatic conditions (e.g., temperature, precipitation, and humidity) and (or) host reproduction (Jameson and Brennan 1957; Mumcuoglu et al. 1999; Thomas et al. 2002; Podder et al. 2009; Viljoen et al. 2011). Increased parasitism may also occur around the hosts' breeding season, as the

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Published at www.nrcresearchpress.com/cjz on 11 November 2016.

Fig. 1. *Leptotrombidium* mites on the ear of a northern red-backed vole (*Myodes rutilus*). These mites have been observed on the ears and genitals of several species of small mammals in Algonquin Provincial Park, Ontario, Canada (photograph by E. Schmidt). Figure appears in colour on the Web.
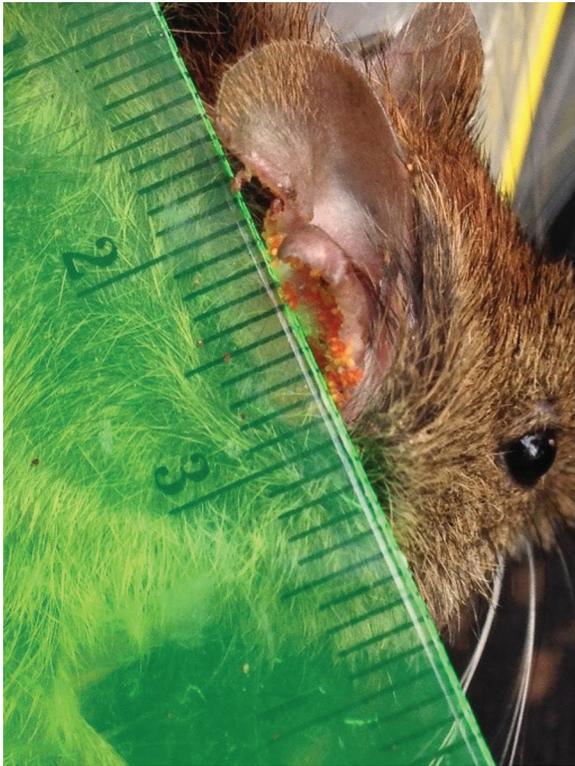
host becomes more vulnerable to parasitism with energy being allocated towards reproduction and parental care rather than immune function and grooming habits (Cattadori et al. 2005; Lucan 2006; Cornell et al. 2008; Viljoen et al. 2011). Male-biased parasitism may appear in species where males are larger, increasing the available resources for the parasite (Moore and Wilson 2002; Krasnov et al. 2005a). Similarly, male hosts may travel to search for mates or compete for territories, increasing the likelihood of contact with mites, and may be less able to immunologically challenge a mite infestation due to a trade-off between testosterone and immunity (Folstad and Karter 1992; Zuk and McKean 1996; Klein 2004; Krasnov et al. 2005a; Viljoen et al. 2011).

Orange mites have been observed on the ears and genitals of numerous species of small mammals in Algonquin Provincial Park, Ontario, Canada, including deer mice (*Peromyscus maniculatus* (Wagner, 1845)), northern red-backed voles (*Myodes rutilus* (Pallas, 1779)), eastern chipmunks (*Tamias striatus* (L., 1758)), woodland jumping mice (*Napaeozapus insignis* (Miller, 1891)), and North American red squirrels (*Tamiasciurus hudsonicus* (Erxleben, 1777)) (A.I. Schulte-Hostedde, personal communication; Fig. 1). Although common, little is known about these mites, and potential impacts on host fitness have not been investigated. Here, we attempt to identify these mites and, within a population of North American red squirrels, examine the relationship between mite infection and a measure of host fitness. We explore the factors contributing to mite population dynamics, such as host sex-biased parasitism. Finally, we examine variation in the anatomical region of infestation on red squirrel hosts to investigate if the location of mite on host body varies between host sexes or between years.

Materials and methods

This study was conducted in Algonquin Provincial Park, Ontario, Canada (45°54′N, 78°26′W), across two field seasons (May–August) in 2014 and 2015. All methods used in this study were reviewed and approved by the Animal Care Committee (ACC) at Laurentian University, Sudbury, Ontario, Canada.

Field methods

Adult red squirrels (>120 g; range 131–218 g) were captured as part of a long-term study, using a ~21 ha grid of 150 Tomahawk live traps (Tomahawk Live Trap Co., Hazelhurst, Wisconsin, USA) (Gorrell and Schulte-Hostedde 2008). Traps were spaced at 20 m intervals and were set overnight (hours of 1830–0530) and in the early morning (hours of 0530–0900) once per week for the duration of the study. Upon first capture, individuals were given unique alphanumeric ear tags (National Brand and Tag Co., Newport, Kentucky, USA) for future identification.

Woodland jumping mice, northern red-backed voles, eastern chipmunks, and deer mice were trapped opportunistically as part of a separate long-term study on small mammals in Algonquin Provincial Park using Sherman and Longworth traps (Rogers Manufacturing Co., Kelowna, British Columbia, Canada; detailed methods in Thompson et al. 2012).

Sample collection

Upon capture, red squirrels were placed into secure handling bags and were thoroughly visually inspected on the entire body for the presence of orange mites. If mites were found to be present, then the location(s) of the mites on the host (body region: ears, anus, genitals, chest, legs, tail, or back) was recorded. As mites were often clumped in small patches, a subsample (~1 g) of mites was collected from the host using sterilized tweezers to pull mites off of the host. Mites were placed into 1.5 mL microcentrifuge tubes, put on ice in the field, and stored in a –20 °C freezer within 2 h.

Morphological measurements of trapped red squirrels were taken at all captures including mass (Pesola scale ±1 g), right hindfoot length (heel to tip of longest nail; ruler ±1 mm), skull length (distance from occipital crest to tip of nose; calipers ±0.1 mm), and skull width (distance from parietal bones; calipers ±0.1 mm) (Gorrell and Schulte-Hostedde 2008).

As mites present on other host species were opportunistically sampled, no morphological or population data were recorded for these hosts. Mite sampling methods for these host species, however, mirrored those of the red squirrels.

Taxonomic identification

Nine individual mites (red squirrel: $n = 3$; northern red-backed vole: $n = 3$; deer mouse: $n = 2$; jumping field mouse: $n = 1$) were sent to the Canadian Centre for DNA Barcoding (CCDB) at the University of Guelph, Guelph, Ontario, Canada, to undergo sequencing to evaluate if mite samples were genetically identical across host species and if they could be taxonomically identified. In brief, a glass-fibre protocol (Ivanova et al. 2006) was used to extract DNA from the mite tissue and the 658 bp target region of the cytochrome *c* oxidase I (COI) gene was amplified using polymerase chain reaction (PCR). The 13.5 µL PCR mixture included 6.25 µL of 10% trehalose, 1.25 µL of 10× PCR buffer, 0.625 µL (2.5 mm) of $MgCl_2$, 0.125 µL (10 µm) of each oligonucleotide primer, 0.625 µL (10 mm) of dNTPs, 0.625 µL of *Taq* polymerase, and 4 µL of H_2O + template DNA (Hajibabaei et al. 2005). PCRs were run under the following cycle conditions: 1 min at 94 °C, followed by five cycles of 30 s at 94 °C, 40 s at 50 °C, and 1 min at 72 °C, followed by 35 cycles of 30 s at 94 °C, 40 s at 55 °C, and 1 min at 72 °C, and finally 10 min at 72 °C. DNA extracts were PCR amplified using the universal forward and reverse primer pair C_LepFolF (5′-ATTCAACCAATCATAAAGATATTGG-3′) and C_LepFolR (5′-TAAACTTCTGGATGTCCAAAAAATC-3′), respectively (Stein et al. 2013).

Fig. 2. Relationship of trombiculid prevalence to North American red squirrel (*Tamiasciurus hudsonicus*) body condition (A) and anatomical variation on host body in 2014 and 2015 (B) in Algonquin Provincial Park, Ontario, Canada.
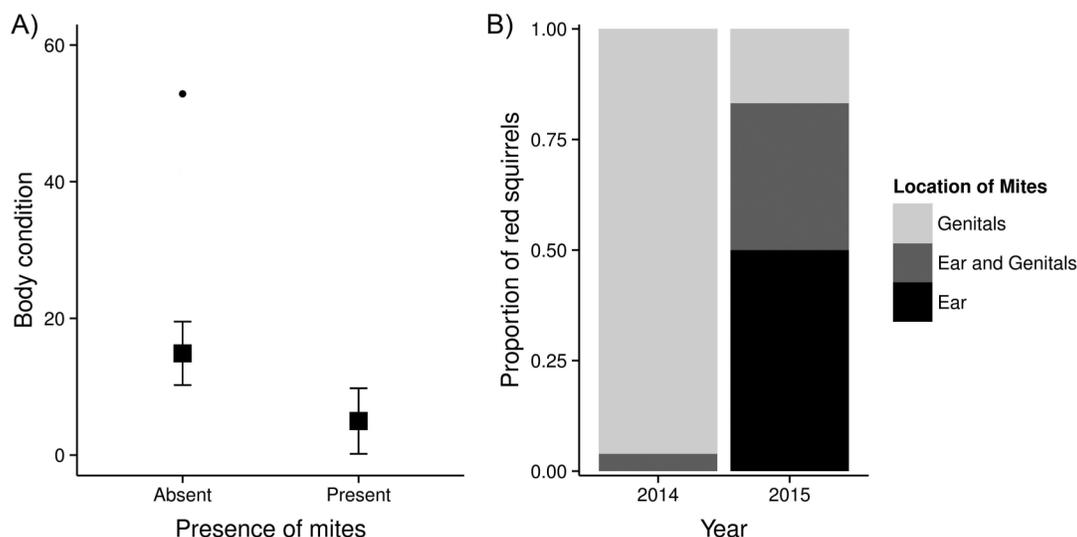
To further attempt to taxonomically identify mites, 18S rRNA sequencing of 12 mites sampled from 12 northern red-backed voles were performed using primers 31F1 and 344R2 (which both broadly target arthropods) as described by Foley et al. (2013). These samples were selected for 18S sampling based on their hosts' close relation to the Amargosa vole (*Microtus californicus scirpensis* Bailey, 1900 = *Microtus californicus* (Peale, 1848)), as previously studied by Foley et al. (2013). Samples from both DNA barcoding and 18S rRNA methods were then sequenced using an ABI 3730 sequencer (Applied Biosystems, Foster City, California, USA, and UC Davis Sequencing, Davis, California, USA, respectively) and evaluated for taxonomic classification by conducting a BLAST search in GenBank (NCBI: http://blast.ncbi.nlm.nih.gov/Blast.cgi).

COI sequences have been submitted to BOLD under the accession numbers ESAP238-15 to ESAP246-15.

Analysis of red squirrel body condition

As individuals were caught more than once over the study period, mean morphological dimensions were calculated. The first component from a principal component analysis (PCA) was used as a proxy for body size from individuals' hind-foot length, skull width, and skull length measurements (Gorrell and Schulte-Hostedde 2008). This first component (eigenvalue 3.37) explained 53.8% of the variance in the data, loaded positively with skull length (0.80), skull width (0.45), and hind-foot length (0.40), and was used as a body-size proxy for the hosts. Body condition was then estimated using residuals from the regression of body mass and body size (Schulte-Hostedde et al. 2005).

Exploratory and statistical analysis

We scored individuals as either infected (yes) or not infected (no) for the duration of the study year, regardless of the date of first infection, allowing us to broadly explore the relationship between long-term host body condition and infection status. This relationship was examined using a binomial general linear mixed effects model (GLMM) with repeated measures in R (R Core Team 2014), including mite infection (yes or no) as the dependent variable, body condition, sex, and year as fixed effects, and individual as a random effect.

To test for differences in anatomical region infestation in red squirrels, a general linear mixed effects model (GLMM) with repeated measures was performed, including location on host body (genitals, ears, or both) as the dependent variable, sex and year as fixed effects, and individual as a random effect. One female was

excluded from this data because the location of the mite infestation was unknown.

Results

Taxonomic identification of mites

DNA barcoding of the COI gene revealed identical sequences (99% similarity) from mites collected across host species, including red squirrels, northern red-backed voles, deer mice, and jumping mice, but could not be reliably classified using the current sequences in GenBank. The 18S rRNA sequencing reliably identified five samples as belonging to the family Trombiculidae. Sequencing from the remaining samples failed to give a reliable identification, likely due to low amplification efficiency and lack of available mite sequences in the database.

Red squirrel population dynamics

In total, 78 squirrels were included in this study (2014: male = 27, female = 28; 2015: male = 18, female = 8), with three squirrels caught in both years. Within years, the number of per-capita recaptures ranged from 2 to 20 (2014) and from 1 to 14 (2015).

In 2014, 24 individuals were infected with mites (44% of the population). Twenty-three individuals had mites present on the genitals and one individual was infected with mites on both the ears and the genitals. In 2015, seven individuals were infected (27% of the population), with mites being found on the genitals of one individual, found on the ears of three individuals, and found on both the ears and the genitals of two individuals.

Mite–host interactions

Initial emergence of orange mites on red squirrels in 2014 and 2015 both occurred in mid-July, with the first sightings on 15 July and 21 July, respectively. Body condition was significantly lower in individuals with mites, regardless of a presence of an outlier in the data set (with outlier: $Z_{[1,75]} = -2.21$, $p = 0.027$; without outlier: $Z_{[1,75]} = -2.139$, $p = 0.032$; Fig. 2A). Mite prevalence did not differ across years ($Z_{[1,75]} = -0.92$, $p = 0.36$) or sexes ($Z_{[1,75]} = -0.159$, $p = 0.87$). The location of mites on the host body changed significantly across years ($Z_{[1,74]} = 2.95$, $p = 0.003$; Fig. 2B), but there were no differences in the location of mites on the bodies of males and females ($Z_{[1,74]} = -0.023$, $p = 0.99$).

Discussion

Taxonomic identification

We used DNA barcoding to assess the identity of mites from four small-mammal species and found that all had identical COI sequences, suggesting that these mites are of the same species and are likely host generalists. The 18S rRNA sequencing revealed that this species may belong to the family Trombiculidae, although a low degree of reliable identification at the genus and species level is preventative to providing a more concrete taxonomic identification. The ambiguity of specific taxonomic classification (e.g., genus and species) between the two sequencing methods supports the possibility that the lack of sequencing data in the BLAST database for trombiculid species is preventing the reliable identification of these mites and morphological identification is necessary to confirm their taxonomy.

The behaviour of mites seen in this study also suggests that these mites may belong to the family Trombiculidae and particularly may be presenting in the larval stages on these small-mammalian hosts. Trombiculid larvae parasitize a variety of terrestrial vertebrates, feeding on the surface of the host epidermis and prefer host body locations such as the head, ears, armpits, and genitalia (reviewed in Shatrov and Kudryashova 2006). Larvae drop off from hosts and eventually become active soil-dwelling predatory adult mites (Takahashi et al. 1988). Because of the behaviour seen in this study, these mites may likely be trombiculid larvae, although morphological identification is necessary to confirm this suggestion.

Parasite–host interactions

We observed a significant negative association between body condition of red squirrels and mite infection. These results are similar to trombiculid mites found on Taiwan field mice (*Apodemus semotus* Thomas, 1908), where abundance of mites was negatively associated with host reproduction and survival (Lin et al. 2014). As a result, there may be direct fitness consequences to the presence of mites on red squirrel hosts, potentially impacting host population dynamics. Alternatively, mites may be more successful when targeting unhealthy individuals. Although not determined in this study, previous work has negatively linked host body condition to parasitic infection and, furthermore, parasite fitness may be highest on immunocompromised hosts (Krasnov et al. 2005*b*).

Three main lines of evidence suggest that mite dynamics in this red squirrel population are perhaps less driven by host condition than other factors affecting mite prevalence. First, we found no difference in mite prevalence between host sexes. Although sex-biased parasitism is complex, our results align with other mite species, such as trombiculid mites found on Taiwan field mice, which display no host sex-bias (Lin et al. 2014). Second, in both 2014 and 2015, the initial emergence of mites on the study population was observed in mid-July, making it unlikely that the mite infestation was associated with the breeding season of the North American red squirrel (April–May). Third, the relative number of infected red squirrel hosts in the population did not change significantly between the two study years, despite large population differences in red squirrels. When combined, these results indicate that mite prevalence may be linked to factors external to host status. Although not directly measured in this study, prevalence of other mite species may be linked to climatic or habitat conditions in other small-mammal hosts, such as gray squirrels (*Sciurus carolinensis* Gmelin, 1788; Durden et al. 2004), Taiwan field mice (Lin et al. 2014), eastern rock sengis (*Elephantulus myurus* Thomas and Schwann, 1906; Fagir et al. 2015), and marsh rice rats (*Oryzomys palustris* (Harlan, 1837); Carmichael et al. 2007).

Mites were found exclusively on the ears and genitals of red squirrel hosts in this population. This is similar to other trombiculid species that have been found on the cochlea and genital or anus regions of other small mammals (Elton and Keay 1936;

Jameson and Brennan 1957; Kharadov 2009; Lin et al. 2014). The location of mites on the host, however, did significantly differ across years because mites were found predominately on the ears (2015) and (or) genitals (2014 and 2015). These results suggest that mite infection, as opposed to the location of mite infection, may have a larger impact on host body condition; however, future studies are needed to determine the significance associated with attachment site.

Future directions

Future research should look to provide a more accurate identification of these mites, possibly through morphological techniques. Additionally, although prevalence among sexes and years was accounted for in this study, future studies should also assess intensity of infection (such as a measure of mite abundance) to provide more comprehensive information about the relationship between mites and their hosts. Lastly, future studies should attempt to confirm the relationship between the abundance of these mites and the various climatic and habitat conditions.

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