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SHORTER CONTRIBUTIONS

FIRST RECORDED RED-LEGGED GRASSHOPPER-MITE INTERACTION IN SOUTHWEST VIRGINIA

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ABSTRACT

On 8 August 2022, we collected a red-legged grasshopper (*Melanoplus femurrubrum* [De Greer, 1773]) parasitized by a larval mite of the genus *Leptus* in Pulaski County, Virginia. Although red-legged grasshoppers are common in Virginia and the presence of *Leptus* sp. has been documented in Virginia, this specific grasshopper-mite interaction is both a new host record and potentially a new species of mite that infests orthopterans in the state. We discuss the context upon which this interaction was observed and the mite identification via molecular techniques.

Keywords: Appalachia, DNA barcoding, ectoparasitism, *Leptus*, *Melanoplus femurrubrum*.

BACKGROUND

Grasshoppers are an important driver of nutrient cycling in grassland ecosystems: as herbivores (Nitschke et al., 2015; Nakajima & Miyashita, 2021), pests (Olfert et al., 2021), and food sources for secondary consumers, including humans (Belovsky et al., 2011; Leonard et al., 2020). Mites (Acarina) often aid in grasshopper population control (Daugherty, 1963) by altering their ability to allocate energy for reproduction (Branson, 2003) or by directly feeding on grasshopper eggs (Daugherty, 1963; Belovsky et al., 1997; Varenhorst et al., 2019). Larval mites from the genus *Eutrombidium* commonly ectoparasitize *Chorthippus* spp. and *Melanopus* spp. grasshoppers in western United States and in other countries (Table 1). Mites in the genus *Leptus* are ectoparasites of grasshoppers in South Dakota (Varenhorst et al., 2019) and in Iran (Khademi et al., 2015). However, mites in this genus commonly parasitize other arthropods in the U.S. (Townsend et al., 2006) and globally (e.g., Southcott, 1992; Cokendolpher, 1993; Southcott, 1999; Mąkol et al., 2011; Pereira et al., 2012; Pinto et al., 2014; Atwa et al., 2017; Bernard et al., 2019; Cordero-

Table 1. Published mite and Orthoptera host interactions and distributions, as detailed through a literature search and this study.

Parasite/Host	Location	Citation
Charletonia mite, *Charletonia adellae* (Haitlinger, 2007)		
Pyrogomorphidae, *Zonocerus elegans* (L., 1758)	Madagascar	Haitlinger, 2007
Charletonia mite, *Charletonia brunni* (Oudemans, 1910)		
Pyrgomorphidae (Brunner von Wattenwyl, 1873)	Benin	Haitlinger, 2006
Pyrgomorphidae (Brunner von Wattenwyl, 1873)	Ethiopia	Haitlinger, 2006
Pyrgrmorphidae (Brunner von Wattenwyl, 1873)	Ghana	Haitlinger, 2006
Pyrgomorphidae (Brunner von Wattenwyl, 1873)	Nigeria	Haitlinger, 2006
Pyrgomorphidae (Brunner von Wattenwyl, 1873)	Tanzania	Haitlinger, 2006
Charletonia mite, *Charletonia domawiti* (Haitlinger, 2004)		
Lubber grasshopper, Romaleidae (Brunner von Wattenwyl, 1893)	French Guiana	Mayoral & Barranco, 2011b
Gray bird grasshopper, *Schistocerca nitens* (Thunberg, 1815)	French Guiana	Mayoral & Barranco, 2011b
Short-horned grasshopper, *Abracris flavolineata* (De Greer 1773)	French Guiana	Mayoral & Barranco, 2011b
Charletonia mite, *Charletonia keyi* (Southcott, 1983)		
Pyrogromophidae, *Greyacris profundesulcata* (Carl, 1916)	Australia	Key, 1991
Charletonia mite, *Charletonia salazari* (Mayoral & Barranco, 2011)		
Tettigoniidae, *Neoconocephalus triops* (L., 1758)	Costa Rica	Mayoral & Barranco, 2011a
Charletonia mite, *Charletonia* sp. (Oudemans, 1910)		
Variegated grasshopper, *Zonocerus variegatus* (L., 1785)	Southern Cameroon	Kekeunou et al., 2015
Red grasshopper mite, *Eutrombidium trigonum* (Hermann, 1804)		
Bow-winged grasshopper, *Chorthippus biguttulus* (L., 1758)	Poland	Haitlinger, 2004
Lesser field grasshopper, *Chorthippus mollis* (Charpentier, 1825)	Poland	Haitlinger, 2004
Field grasshopper, *Chorthippus brunneus* (Thunberg, 1815)	Poland	Haitlinger, 2004
Common green grasshopper, *Omocestus viridulus* (L., 1758)	Poland	Haitlinger, 2004
Two-striped grasshopper, *Melanoplus bivittatus* (Say, 1825)	South Dakota, USA	Severin, 1944
Sand-bar locust, *Melanoplus foedus fluviatilis* Bruner, 1897	South Dakota, USA	Severin, 1944

Red mite, *Eutrombidium locustarum* (Walsh, 1866)		
Migratory grasshopper, *Melanoplus sanguinipes* (Fabricius, 1798)	Montana, USA	Belovsky et al., 1997
White-whiskered grasshopper, *Ageneotettix deorum* (Scudder, 1876)	Montana, USA	Belovsky et al., 1997
Eutrombidium rostratus (Scopoli, 1763)		
Red-legged grasshopper, *Melanoplus femurrubrum* (De Greer, 1773)	Michigan, USA	Bland, 1976
Eutrombidium djordjevici sp. nov. Verdun, 1909		
Italian locust, *Calliptamus italicus* (L., 1758)	Montenegro	Saboori & Pesic, 2006
Leptus dubius (Paoli, 1937)		
Sharp-tailed grasshopper, *Euchorthippus declivus* (Brisout, 1848)	Italy	Southcott, 1992
Slant-faced grasshopper, *Stauroderus* sp. (Bolívar, 1897)	Italy	Southcott, 1992
Moroccan locust, *Dociostaurus maroccanus* (Thunberg, 1815)	Italy	Southcott, 1992
Red-winged grasshopper, *Oedipoda miniata* (Pallas, 1771)	Italy	Southcott, 1992
Apulian stone grasshopper, *Prionotropis hystrix appula* (Costa, 1836)	Italy	Southcott, 1992
Short-horned grasshopper, *Calliptamus barbarus* (Costa, 1836)	Italy	Southcott, 1992
Leptus multisolenidiae (Mayoral and Barranco, 2011)		
Feldheuschrecken grasshopper, *Episomacris gruneri* (Descamps and Amédégnato, 1970)	French Guiana	Mayoral & Barranco, 2011b
Leptus nikanori (Haitlinger, 2000)		
Horsehead grasshopper, *Pseudoproscopia scabra* (Klug, 1820)	Costa Rica	Mayoral & Barranco, 2011a
Tettigoniidae, *Scopiorinus mucronatus* (Saussure & Pictet, 1898)	Costa Rica	Mayoral & Barranco, 2011a
Tettigoniidae, *Lophaspis scabricula* (Brunner von Wattenwyl, 1895)	Costa Rica	Mayoral & Barranco, 2011a
Tettigoniidae, *Idiarthron hamuliferum* (Beier, 1960)	Costa Rica	Mayoral & Barranco, 2011a
Leptus sp. Latreille, 1796		
Arid lands spur-throat grasshopper, *Melanoplus aridus* (Scudder, 1878)	Arizona, USA	https://bugguide.net/node/view/2171125
Unknown grasshopper	South Dakota, USA	Varenhorst et al., 2019
Unknown grasshopper	Iran	Khademi et al., 2015
Red-legged grasshopper, *Melanoplus femurrubrum* (De Greer, 1773)	Virginia, USA	This study

Rivera et al., 2018; Guimarães et al., 2019; Hakimitabar et al., 2020; Torrico-Bazoberry et al., 2020; Ìlyas & Saboori, 2023), including from the orders Araneae, Coleoptera, Collembola, Diptera, Dermaptera, Hemiptera, Lepidoptera, Opiliones, and Odonata. Despite this widespread record of parasitism, nothing is known about grasshopper-mite interactions in the Appalachian Mountain region. We report on the opportunistic discovery of a larval mite from the genus *Leptus* infesting a red-legged grasshopper (*Melanoplus femurrubrum* [DeGreer, 1773]) in southwest Virginia.

SPECIMEN COLLECTION & IDENTIFICATION

The grasshopper host with the later discovered mite attachment was collected on 8 August 2022 as part of a study comparing insect diversity across southwest Virginia interior and exterior to cemeteries; our secondary objective was to determine whether cemeteries could serve as refugia for insects in an urbanized landscape. Sampling was conducted in a 50 m plot S of the SW edge of Sunrise Burial Park, Fairlawn, Pulaski County, Virginia (37.14763 °N, 80.58911 °W).

All arthropods captured in our 20-m by 1-m sweep net transect were transferred to a 1-gallon freezer bag, placed on ice in the field, and then euthanized by storing them in the freezer at 0 ºC in our lab until identification. Using a stereomicroscope (Stemi 508; Carl Zeiss Microscopy, LLC, White Plains, NY) to identify the arthropods in our sample, we discovered that one larval mite was attached to a male nymph of the red-legged grasshopper, on the ventral side of the terminal abdominal segment (Fig. 1). No other larvae were found on this grasshopper. The grasshopper was pinned and cataloged in the natural history collection in the Department of Biology at Radford University (Catalog No. RU18307). Images of the larval mite attached to the host were made using the microscope's camera (Axiocam 105; Carl Zeiss Microscopy, LLC, White Plains, NY; Fig.1).

Figure 1. Photographic documentation of the red-legged grasshopper (*Melanoplus femurrubrum*, [De Greer, 1773]) and *Leptus* sp. mite interaction: grasshopper host (left; image by Amber Gordon), mite at 20X magnification (middle), and mite at 50X magnification (right). The specimen was collected on 8 August 2022 near the Sunrise Burial Park, Fairlawn, Pulaski County, Virginia.

Following photography, the mite was removed from the grasshopper with fine-tipped forceps and stored in 70% ethanol at -20 °C. A crude DNA extraction was performed by grinding the mite in 100 µl of a 10% Chelex solution. This solution was then incubated at 98 °C for 30 minutes followed by cooling to 4 °C. The sample was spun at 16,000 RPM to pellet the Chelex and cell debris. The supernatant was transferred to a clean tube and stored at -20 °C until polymerase chain reaction (PCR) analysis.

The 658-bp Folmer region of the mitochondrial COI barcode region was amplified using the "universal primers" LCO1490 and HCO2198 (Folmer et al., 1994). The PCR amplifications were conducted in a 50-µl volume including 25 µl 2 x taq master mix containing 1.5 mM MgCl (New England Biolabs, Ipswich, MA), 0.25 µM of each primer, 3 µl of template DNA. We

performed PCR with an initial denaturation step of 94 °C for 3 min followed by 35 cycles at 94 °C for 45 s, 52 °C for 45 s, 68 °C for 1 min and one cycle at 68 °C for 10 min. Successful amplification was verified by electrophoresed through a 1.5% agarose gel in TBE. The sample was then purified and sequenced in both directions using Sanger di-deoxy method (Sanger et al., 1977).

Forward and reverse strands were aligned and checked for stop codons using Codon Code Aligner ® resulting in a 647-bp fragment of high-quality sequence. This sequence was used as a query against the National Center for Biological Information (NCBI) nucleotide database using BLASTN (Zhang et al., 2000) and the International Barcode of Life Database (iBOLD; Ratnasingham & Hebert, 2007) using the Identification Search Tool. An estimate of phylogenetic relationship of our specimen to other taxa in the database was made using the Neighbor-Joining method (Saitou & Nei, 1987)

The highest sequence similarity observed among published sequences was 82% with a specimen in the genus *Leptus*. Phylogenetic analysis using the Neighbor-Joining algorithm (Saitou and Nei, 1987) results in our specimen clustering within the genus *Leptus* with taxa from several countries outside of the U.S., namely Argentina, Brazil, Honduras, Australia, Canada, Mexico, and Russia.

SPECIMEN COLLECTION & IDENTIFICATION

Although red-legged grasshoppers are common in Virginia (iBOL, 2023) and the presence of *Leptus* sp. has been documented in northern (iBOL, 2024) and southeastern (Townsend et al., 2006) Virginia, this parasite-host interaction represents the first record of this type of interaction in southwest Virginia (K. Ivanov, VMNH, personal communication; R. Eckerlin, NOVACC, personal communication). The relatively low sequence similarity of our specimen of *Leptus* when compared to others suggests that this individual represents a species lacking sequence information in this database (Nielsen & Matz, 2006). While we cannot identify the species based on comparison of the COI sequence to other taxa in the DNA database, the clustering of our specimen with other taxa in *Leptus* sp. is consistent with the general biology for these mites as larval ectoparasites on terrestrial arthropods.

Leptus mite-host ecology remains open for continued study (Bernard et al., 2019; Haarder & Mąkol, 2022). Although the hosts have been identified for 83% of the *Leptus* sp., the prevalence of infestation is relatively unknown (Bernard et al., 2019). In one study, Odonate infestations were present for only 7.1% of the 126 dragonflies collected and only at 7.5% of the 57 sites studied (Bernard et al., 2019). In another study, the opilionid infestation ranged from 0.5 to 20.3% of the harvestman depending on collection season (Townsend et al., 2006). The density of *Leptus sp.* on arthropod hosts ranges from one (this study; Townsend et al., 2006; Mayoral & Barranco 2011; Guimarães et al., 2019) to 49, the most seen in the literature (e.g., Bernard et al., 2019).

Leptus mites attach to their hosts in various of locations. Typically, they are attached under the developing or mature wings on the dorsal side of the thorax of hemipterans and coleopteran (Haarder & Mąkol, 2022) and opilionids (Mąkol et al., 2011), legs of orthopterans (Mayoral & Barranco, 2011a, 2011b; Varenhorst et al., 2019) and opilionids (Mąkol et al., 2011), the ventral side of the body in anisopterans (Bernard et al., 2019), antennae of lepidopterans (Southcott, 1992), wings of orthopterans (Mayoral & Barranco, 2011a), the areas of soft cuticle on odonates (Bernard et al., 2019), or the dorsal side of the abdomen of dipterans (Guimarães et al., 2019). However, our single mite specimen was attached ventrally to terminal abdominal segment. This attachment location has not been documented in red-legged grasshoppers but given the wide range of

attachment locations documented in other arthropods, this mite's attachment on the grasshopper's soft cuticle is not unexpected. This location may also be a spot where the grasshopper's grooming behaviors were not effective in removing the mite.

The anecdotal discovery of this new larval mite-grasshopper host interaction sets the stage for future studies of insect-parasite interactions in highly modified urbanized habitats. Urbanization has been known to change grasshopper behavior and dispersal abilities (Waterschoot et al., 2023), and urbanization, as well as other types of human land use, can affect host-parasite interactions and abundances (e.g., Maaz et al., 2018, Benedict, et al. 2021, Kwak et al., 2022; but see Baardsen et al., 2021).

We plan to expand the number urbanized habitats look for more interactions like this one and potentially build upon our knowledge of terrestrial arthropod parasitism in Appalachia, specifically to better understand the role that urbanization plays on mite distribution and their biology. Further, as the iBOLD system expands with the addition of new taxa, we hope there will be specimens with a higher sequence similarity allowing us to be more confident in the taxonomy. Indeed, as we proceed with this study it would be useful to include an expert in mite taxonomy to verify the systematics of specimens as DNA data is entered into the database.

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