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Source: Journal of Parasitology, 110(5) : 516-520

Published By: American Society of Parasitologists

URL: https://doi.org/10.1645/24-80

Journal of Parasitology 2024 110(5) 516–520

Published 30 October 2024

DOI: 10.1645/24-80

Contents and archives available through www.bioone.org or www.jstor.org

Journal of Parasitology

journal homepage: www.journalofparasitology.org

LOUSY ASSOCIATIONS: SUCKING LICE (PHTHIRAPTERA: ANOPLURA) PARASITIZING RODENTS AND LAGOMORPHS IN THE DESERT NATIONAL WILDLIFE REFUGE, NEVADA

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KEY WORDS

Phthiraptera
Anoplura
Nevada
Rodentia
Lagomorpha
Intermountain West

ABSTRACT

Seventy of 190 (37%) mammals, representing 14 rodent and 2 lagomorph species examined in the Desert National Wildlife Refuge in southern Nevada, were parasitized by sucking lice (Phthiraptera: Anoplura). Twelve species of sucking lice (5 species of Hoplopleruridae, 7 species of Polyplacidae) were recorded. Nine of these louse species (*Hoplopleura difficilis*, *Hoplopleura ferrisi*, *Hoplopleura onychomydis*, *Hoplopleura reithrodontomyis*, *Fahrenholzia reducta*, *Haemodipsus setoni*, *Neohaematopinus citellinus*, *Neohaematopinus neotomae*, and *Polyplax auricularis*) are reported from Nevada for the first time, and *Po. auricularis* is recorded from *Peromyscus eremicus* (cactus deermouse) for the first time. Infestation prevalences, mean intensities, sex ratios, host associations, and host specificity are presented and discussed for each louse-host interaction.

Sucking lice are obligate hematophagous ectoparasites that are globally distributed on 12 of the 29 orders of mammals (Durden and Musser, 1994). These insects spend their entire lives on their host, starting as eggs cemented onto hairs, before proceeding through 3 nymphal instars and molting into adults. Adult lice attach to the pelage with specialized tibio-tarsal claws and feed from blood vessels using highly derived mouthparts (Kim, 2006). Sucking lice vector numerous pathogens, and heavy infestations can cause anemia and weight loss in some hosts (Kim, 2006). More than 500 species of sucking lice have been described; however, existing records likely underestimate species diversity and host associations (Durden and Musser, 1994). Even in North America where parasites are relatively better studied (Carlson et al., 2020), louse associations remain poorly documented in many regions, including Nevada. For example, the identification guide to North American sucking lice includes 76 species of lice but records only 6 species from Nevada (Kim et al., 1986). Since 1986, only 4 additional sucking lice have been recorded from the state: a new species described from the pygmy rabbit, *Brachylagus idahoensis*, in 2007 (Durden and Rausch, 2007), as well as 3 new species records (Kucera et al., 2007; Bell et al., 2015, 2021). Although 10 species of sucking lice have been recorded from native mammals in Nevada, the number of actual species in the state should be appreciably higher.

In this paper, we document sucking lice recovered from rodents and lagomorphs in the Desert National Wildlife Refuge in southern Nevada, north of Las Vegas. In addition to documenting new species records of sucking lice from Nevada, we include data on infestation parameters, host preferences, and sex ratios for these lice.

MATERIALS AND METHODS

Small mammals were collected from a variety of habitats across the Desert National Wildlife Refuge, in Clark County and Lincoln County, Nevada, between 15 April and 4 May 2014 (Fig. 1). All small mammals were captured using Sherman traps, except 1 *Lepus californicu*s collected using a 0.410 caliber shotgun. We identified animals in the field based on external morphology and confirmed identifications using skull morphology and, for some difficult-to-identify *Peromyscus* species, by amplifying sections of the mitochondrial cytochrome b gene. For these hosts, frozen liver or muscle was extracted using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, California) and then amplified with the MVZ05 and H15915 primers (Irwin et al., 1991). PCR products were purified with ExoSAP-IT and then sequenced on an ABI Prism 3130 automated sequencer (Applied Biosystems, Waltham, Massachusetts). Forward and reverse sequences were edited and aligned using Geneious 7.1 (Biomatters, Aukland, New Zealand) and then compared to known *Peromyscus* spp. sequences in GenBank.

We collected up to 5 individuals per host species at each locality, euthanizing animals in the field with isoflurane. To collect ectoparasites, we combed each individual with a flea comb before brushing with a stiff toothbrush and conducting a visual inspection to remove attached ticks. Ectoparasites were saved together

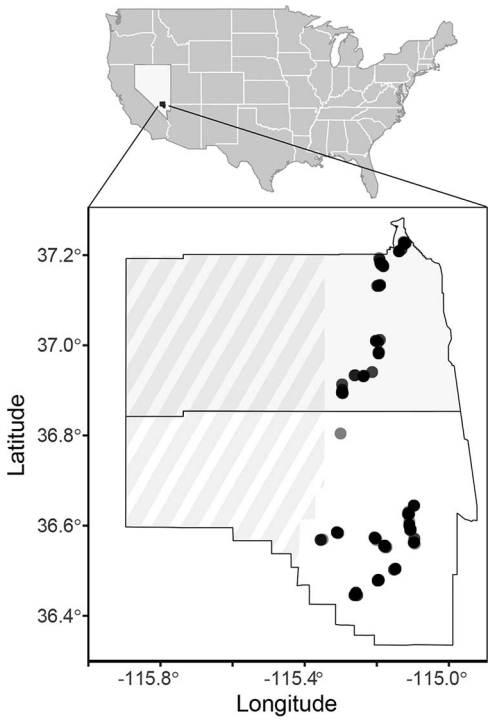

Figure 1. Trapping locations of small mammals sampled for ectoparasites at Desert National Wildlife Refuge in 2014. Each point represents a sampled individual. All trapping occurred in the eastern part of the refuge as the Nevada Test and Training Range (marked with gray lines) prohibits access to the western side. Refuge boundaries from Konzek (2024).

in 95% ethanol and then sorted into major taxonomic groups (i.e., lice, mites, ticks, fleas). We screened 194 small mammals for ectoparasites; however, samples from 4 individuals were lost, and these hosts were excluded from analyses.

Adult lice were identified using Kim et al. (1986). Nymphal lice were identified using Cook and Beer (1959) and Kim (1987) and by comparing specimens with identified slide-mounted nymphs in the collection of L. A. Durden. Most lice could be identified in ethanol, but some specimens were cleared and slide-mounted following the preparation techniques detailed in Kim et al. (1986). Cleared, slide-mounted specimens of 3 uncommon species of lice recorded during this study were digitally photographed using a differential interference contrast Nikon Eclipse Ni-U microscope fitted with a Nikon DS-13 camera (Nikon Inc., Melville, New York). Louse specimens from this study are deposited in the General Ectoparasite Collection at Georgia Southern University (accession numbers L3867–L3940). Host mammals are deposited in the California Academy of Sciences, San Francisco (catalog numbers 30938, 30940–66, 30999–31027, 31032–56, 31058–76, 31083–87, 31089–101, 31103–15, 31120, 31121, 31123–34, 31136–43, 31145–49, 31151–67, 31169–71, 31173–85, 31190–95).

We calculated prevalence (percentage of hosts parasitized) and mean intensity (mean number per infested host) for each host species/louse species partnership following Bush et al. (1997). Louse sex ratios were also calculated if both adult male and female lice were collected from the same host species. Mammal names follow Wilson and Reeder (2005). Figures 1 and 3 were constructed in R v4.2.2 using the ggplot2, maps, rnaturalearth, and bipartite packages (Dorman et al., 2008; Wickham, 2016; Brownrigg, 2023; Massicotte and South, 2024).

RESULTS

Sucking lice were recorded from 70 (37%) of 190 examined small mammals. We found 12 species of lice on the 14 rodent and 2 lagomorph species. Figure 2 shows cleared, male specimens of 3 representative species of lice recorded during this project. Lice were recorded from all but 1 of these mammal species, the exception being the black-tailed jackrabbit, *Lepus californicus* (n = 1). Although most host species were associated with only 1 species of louse, multiple louse species were found on *Peromyscus crinitus*, *Peromyscus eremicus*, *Peromyscus truei*, and *Onychomys torridus*. Similarly, most louse species were associated with only 1 host species; however, *Fahrenholzia pinnata*, *Polyplax auricularis*, *Hoplopleura hesperomydis*, and *Neohaematopinus neotomae* were each collected from multiple host species (Fig. 3; Table I).

Nine species of sucking lice, including 4 hoplopleurids (*Hoplopleura difficilis*, *Hoplopleura ferrisi*, *Hoplopleura onychomydis*, and *Hoplopleura reithrodontomydis*) and 5 polyplacids (*Fahrenholzia reducta*, *Haemodipsus setoni*, *Neohaematopinus citellinus*, *Neohaematopinus neotomae*, and *Po. auricularis*), are reported from Nevada for the first time.

DISCUSSION

Before this study, 10 species of sucking lice had been reported from native mammals in Nevada. Several of these records do not include host data, including records of the enderleinellid *Enderleinellus suturalis* from Virginia Valley, the hoplopleurids *Hoplopleura acanthopus*, and *Ho. hesperomydis* from Douglas County, and the polyplacids *F. pinnata* and *Linognathoides laeviusculus* from the Pine Forest Mountains, Elko County, and *Polyplax alaskensis* without locality data (Kim et al., 1986). Another polyplacid, *Haemodipsus brachylagi* was described from the pygmy rabbit, *B. idahoensis*, from Elko County (Durden and Rausch, 2007). *Fahrenholzia pinnata* has been reported from *Dipodomys deserti* in Clark County, *Dipodomys ordii* in Nye County, and *Dipodomys microps* and *Perognathus longimembris* in Lyon County, Nevada (Light and Hafner, 2007). *Hoplopleura hesperomydis* has been recorded from *Peromyscus truei* in Clark County and *Peromyscus maniculatus* in Elko County and *Po. auricularis* has been recorded from *Peromyscus truei* in Clark County and *Peromyscus maniculatus* in Washoe County (Kucera et al., 2007). *Hoplopleura arboricola* and *Neohaematopinus pacificus* have been recorded from chipmunks (*Tamias* spp.) in several Nevada localities (Bell et al., 2015, 2021). Additionally, there are 3 slide-mounted, previously undocumented, Nevada specimens (1 male, 2 females) of *Ho. onychomydis* from *O. torridus* that were collected in Fish Lake Valley, Esmarelda County, on 29 September 1966 by I. M. Taylor and B. E. Horner, which are now in the Anoplura Collection of L. A. Durden. Combining these records with the new records from the Desert Wildlife Refuge, a total of 19 species of sucking lice have now been recorded from native mammals in Nevada. The actual number is likely about twice that, and future surveys in Nevada should add more species to the state list.

In this study, infestation prevalences ranged from 5% to 86%, and mean intensities ranged from 1.0 to 20.0 for the different

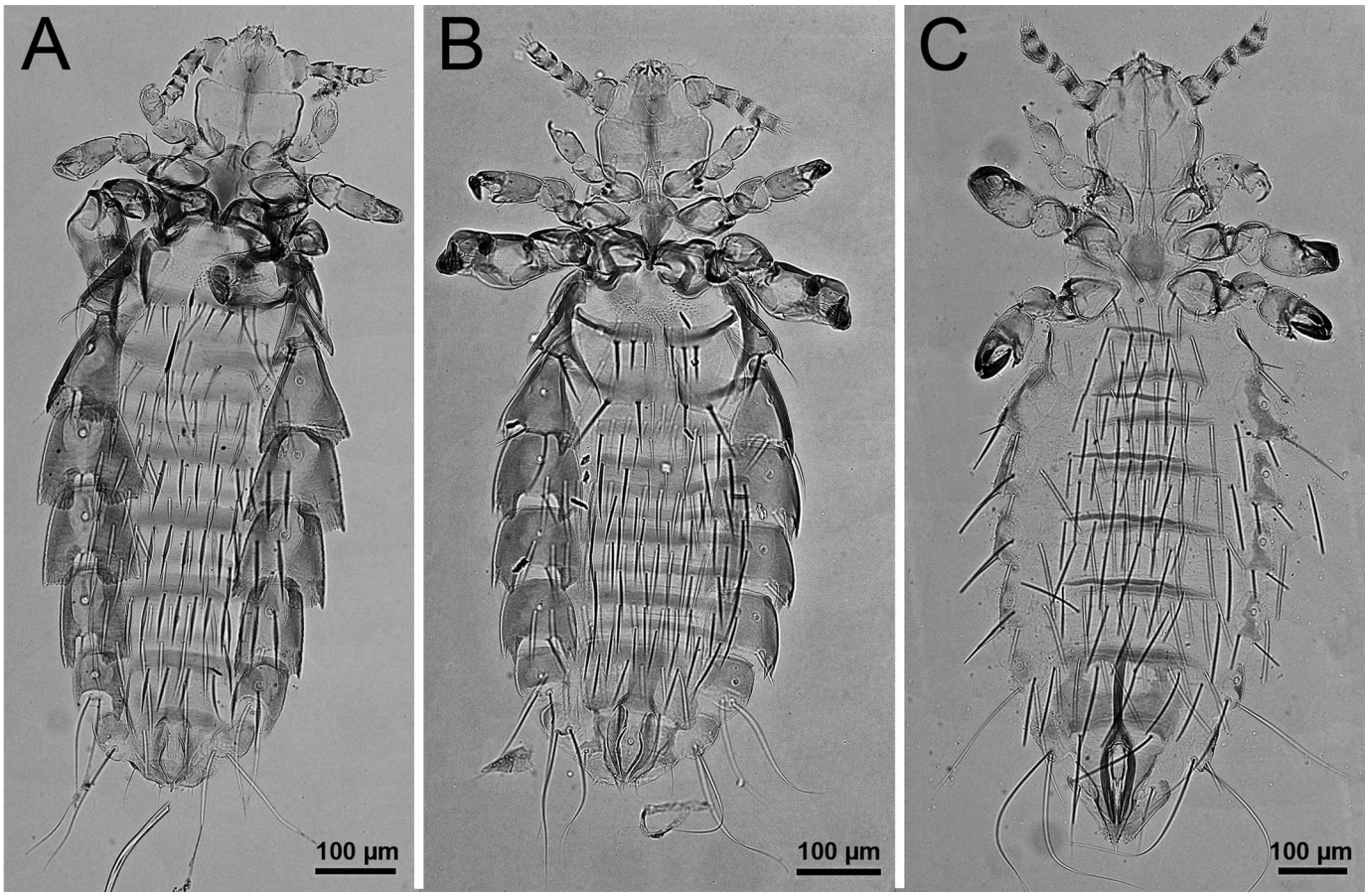

Figure 2. Cleared, slide-mounted male specimens of 3 species of sucking lice recorded during this project: (**A**) *Hoplopleura difficilis*; (**B**) *Hoplopleura onychomydis*; (**C**) *Neohaematopinus neotomae*.

host/louse associations (Table I), which is in line with infestation parameters reported for previous studies in other regions including the adjoining state of Utah (Ignoffo, 1959). In our study, 2 mammals, *Ammospermophilus leucurus* and *D. deserti*, had the highest louse infestation prevalences at 83% and 86%, respectively (Table I). However, 3 different rodent species, *Peromyscus crinitus*, *D. merriami*, and *Perognathus longimembris*, were hosts to lice with the highest mean intensities, at 20.0, 17.6, and 17.0, respectively (Table I). All calculated sex ratios for adult lice (nymphs cannot be determined to sex morphologically) were female-biased. Female-biased sex ratios are widespread for ectoparasites, including sucking lice (Marshall, 1981a), sometimes for undetermined reasons. Adult male lice are typically more mobile than females as they move through the host pelage in search of females and, consequently, may be exposed to more life-ending events such as host grooming or becoming detached from the host.

Host specificity has been stated to be very high for sucking lice (Kim, 1985, 2006). Although it is high, it does not seem to be as high as previously believed, with several species parasitizing 2 or more closely related, usually congeneric, hosts (Durden and Musser, 1994). Conversely, very rarely, a louse species may occur on the "wrong" host for various reasons, and these lice are often referred to as stragglers (Marshall, 1981b). Collected lice in this

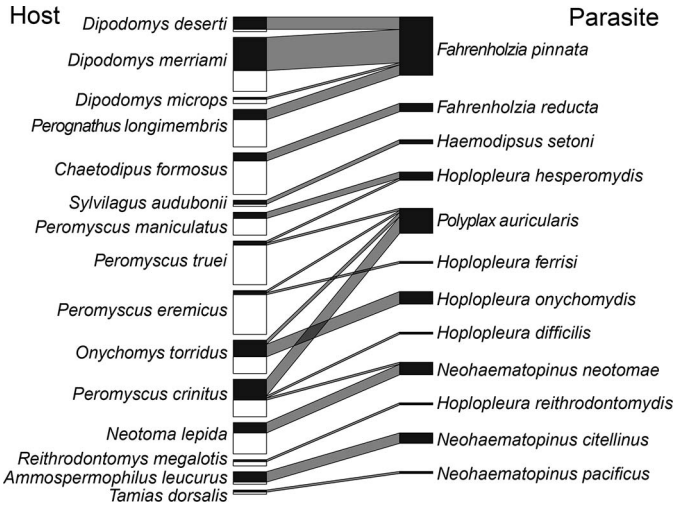

Figure 3. Small mammal-louse associations observed at Desert National Wildlife Refuge in Nevada. Mammalian host species are listed on the left. For hosts, bar size is proportional to the number of sampled individuals and the dark area within each bar corresponds to the infestation prevalence of connected louse species, listed on the right. For parasites, the bar size is proportional to their abundance in the data set.

Table I. Host associations, infestation parameters and sex ratios of sucking lice parasitizing rodents and lagomorphs in the Desert National Wildlife Refuge, Nevada, 2014.

Host species (sample size)/Louse species (stages collected*)	Prevalence (%)	Mean Intensity	Sex Ratio†
Sylvilagus audubonii (n = 3)/*Haemodipsus setoni* (1M,3F)	67	2.0	1:3.0
Ammospermophilus leucurus (n = 6)/*Neohaematopinus citellinus* (3M,11F,10N)	83	4.8	1:3.7
Tamias dorsalis (n = 2)/*Neohaematopinus pacificus* (1N)	50	1.0	NA‡
Dipodomys deserti (n = 7)/*Fahrenholzia pinnata* (5F,7N)	86	2.0	NA
Dipodomys merriami (n =26)/*Fahrenholzia pinnata* (38M,71F,173N)	62	17.6	1:1.9
Dipodomys microps (n = 3)/*Fahrenholzia pinnata* (1F,9N)	33	10.0	NA
Chaetodipus formosus (n = 20)/*Fahrenholzia reducta* (1M,3F,7N)	20	2.8	1:3.0
Perognathus longimembris (n = 18)/*Fahrenholzia pinnata* (1M,11F,73N)	28	17.0	1:11.0
Neotoma lepida (n = 15)/*Neohaematopinus neotomae* (6M,14F,18N)	33	7.6	1:2.3
Onychomys torridus (n = 16)/*Hoplopleura onychomydis* (10M,29F,23N)	38	10.3	1:2.9
Onychomys torridus (n = 16)/*Polyplax auricularis* (2M,4F,7N)	13	6.5	1:2.0
Peromyscus crinitus (n = 17)/*Hoplopleura difficilis* (3M,17F)	6	20.0	1:5.7
Peromyscus crinitus (n = 17)/*Neohaematopinus neotomae* (1F,3N)	6	4.0	NA
Peromyscus crinitus (n = 17)/*Polyplax auricularis* (3M,10F,7N)	47	2.5	1:3.3
Peromyscus eremicus (n = 21)/*Hoplopleura ferrisi* (1N)	5	1.0	NA
Peromyscus eremicus (n = 21)/*Polyplax auricularis* (1M)	5	1.0	NA
Peromyscus maniculatus (n = 11)/*Hoplopleura hesperomydis* (8F,11N)	27	6.3	NA
Peromyscus truei (n = 21)/*Hoplopleura hesperomydis* (1M)	5	1.0	NA
Peromyscus truei (n = 21)/*Polyplax auricularis* (1M)	5	1.0	NA
Reithrodontomys megalotis (n = 3)/*Hoplopleura reithrodontomydis* (1N)	33	1.0	NA

* M, Male(s); F, Female(s); N, Nymph(s).
† Males:Females.
‡ NA, Not Available (both males and females were not recorded for these louse/host associations).

study exhibited the entire spectrum of host specificity, ranging from strict host specificity to parasitism by a single louse species on multiple, closely related hosts to straggling. Strict host specificity was exhibited by *Ho. difficilis* on *Peromyscus crinitus* whereas straggling was recorded for *Neohaematopinus neotomae* (a true parasite of *Neotoma* spp. woodrats) on *Peromyscus crinitus*. Between these 2 extremes, we recorded *F. pinnata* from 4 closely related species of heteromyid rodents, *Ho. hesperomydis* from 2 species of *Peromyscus* and *Po. auricularis* from 4 species of sigmodontine cricetid rodents (3 species of *Peromyscus*, 1 species of *Onychomys*; Fig. 3; Table I). Although we recorded other species of lice (*Ho. ferrisi*, *Ho. onychomydis*, *Ho. reithrodontomyis*, *Ha. setoni*, *Neohaematopinus citellinus*, and *Neohaematopinus pacificus*) from a single host species in this survey, these lice are known to parasitize other closely related hosts in other regions (Kim et al., 1986; Durden and Musser, 1994).

Four species of rodents were parasitized by multiple louse species in this study. *Onychomys torridus* was parasitized by both *Ho. onychomydis* and *Po. auricularis; Peromyscus crinitus* was parasitized by *Ho. difficilis*, *Po. auricularis*, and stragglers of *Neohaematopinus neotomae; Peromyscus eremicus* was parasitized by *Ho. ferrisi* and *Po. auricularis*; and *Peromyscus truei* was parasitized by *Po. auricularis* and *Ho. hesperomydis* (Table I). The collection of *Po. auricularis* from *Peromyscus eremicus* represents the first time this louse has been reported from this rodent. One individual of *Peromyscus crinitus* was parasitized by both *Ho. difficilis* and *Po. auricularis*. Otherwise, these lice did not co-parasitize the same host individuals. However, other ectoparasites, such as fleas, ticks, laelapid mites, and listrophorid mites did co-parasitize some of these hosts.

We provide important new data on sucking lice parasitizing 14 species of rodents and 1 species of lagomorph in Nevada. Nevertheless, future surveys should be conducted to collect additional data on these parasites in the western United States.

ACKNOWLEDGMENTS

We thank Maureen Flannery, Laura Wilkinson, and Galen Rathbun for their substantial contributions to trapping and mammal identification. We thank Stephen E. Greiman (Georgia Southern University, Statesboro) for preparing images of lice. We thank Amy Sprunger, Sarah Bullock, Kaylene Keller, and Orien Richmond of USFWS for funding and logistical support within the Desert National Wildlife Refuge, and we thank Lois Alexander (College of Southern Nevada) and Christy Klinger (Nevada Department of Wildlife) for their expertise and assistance with fieldwork and permits. The authors assert all applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Animal use was approved by the California Academy of Science IACUC (no. 2012-03 "General Collecting of Birds and Mammals") and conducted under Nevada Department of Wildlife collecting permit number S37293.

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