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Alexis D. Hilario-Pérez, Ashley P.G. Dowling. Nasal mites from specimens of the brown-headed cowbird (Icteridae: *Molothrus ater*) from Texas and Arkansas, U.S.A.. *Acarologia*, 2018, 58 (2), pp.296-301. 10.24349/acarologia/20184242 . hal-01709996

HAL Id: hal-01709996

<https://hal.science/hal-01709996>

Submitted on 15 Feb 2018

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Subscriptions: Year 2018 (Volume 58): 380 €

<http://www1.montpellier.inra.fr/CBGP/acarologia/subscribe.php>

Previous volumes (2010-2016): 250 € / year (4 issues)

Acarologia, CBGP, CS 30016, 34988 MONTFERRIER-sur-LEZ Cedex, France

The digitalization of Acarologia papers prior to 2000 was supported by Agropolis Fondation under the reference ID 1500-024 through the « Investissements d'avenir » programme (Labex Agro: ANR-10-LABX-0001-01)



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Nasal mites from specimens of the brown-headed cowbird (Icteridae: *Molothrus ater*) from Texas and Arkansas, U.S.A.

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ABSTRACT

Avian nasal mites are obligate parasites that spend their entire life in the respiratory system of birds. In North America, four families in three orders parasitize birds: Rhinonyssidae (Mesostigmata), Ereynetidae (Prostigmata), Cytoditidae (Astigmata) and Turbinoptidae (Astigmata). Mechanisms of nasal mite transmission among hosts is still unclear and brood parasites like the Brown-headed Cowbird (*Molothrus ater* Boddaert, 1783), which utilize multiple bird species as hosts, are interesting for the study of parasites such as nasal mites. We examined 126 cowbirds for nasal mites and found 84 individuals (66.6 %) to be infested. The most common nasal mite species recovered was *Ptilonyssus icteridius* Strandmann and Furman, 1956. The presence of *P. richmondense* George, 1961, in Brown-headed Cowbird represents a new host association, and a potential new species of *Ptilonyssus* was also recovered. The ereynetid species *Boydalia quiscali* Clark, 1960 was found in two Brown-headed Cowbirds, both times a co-infestation with species of *Ptilonyssus*.

Keywords Nasal Mites, *Molothrus ater*, Rhinonyssidae, Ereynetidae, Survey, *Ptilonyssus*, *Boydalia*
Zoobank <http://zoobank.org/DOAB4350-EF5C-49DE-89B5-7290ABE6B6C1>

Introduction

The Brown-headed Cowbird (*Molothrus ater* Boddaert, 1783) is a member of Icteridae, which also includes blackbirds, grackles, meadowlarks, orioles, cowbirds, and bobolinks. Some *M. ater* are migratory, breeding in the northern parts of North America with winter migration into Mexico, whereas other *M. ater* populations are local residents in southern parts of North America (Sibley 2000). Brown-headed Cowbirds are gregarious birds, sometimes forming mixed flocks with blackbirds and starlings in open areas such as fields, pastures, meadows, forest edges, and lawns (Kaufman 2016).

Molothrus ater is the most abundant species of cowbird in the United States and the most studied in the genus (Dufty 1982). Species of the genus *Molothrus* are all obligate brood parasites, which means that cowbird females lay eggs in the nests of other species of birds that will serve as foster parents (hosts). The female *M. ater* will remove one egg from the nest and lay one of her own, where the foster parents will unknowingly rear the young cowbird. Many hosts will accept the *M. ater* egg as if it were its own. A female *Molothrus ater* can lay a least 40 eggs during a breeding season (Sherry *et al.* 1993).

Brood parasites can be divided into specialists and generalists. The common cuckoo (Cuculidae: *Cuculus canorus* Linnaeus, 1758) is a well-known brood parasite in which races or populations specialize on parasitizing a single host species (Vogl *et al.* 2002), whereas the Brown-headed Cowbird is a generalist, parasitizing 220 known hosts, although only 144

Received 23 June 2017
Accepted 22 September 2017
Published 15 February 2018

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Academic editor
Michel Bertrand

DOI
10.24349/acarologia/20184242

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species have been recorded to successfully rear the cowbird young (Dufty 1985, Briskie *et al.* 1990). Hosts range in size from kinglets to meadowlarks. Additionally, cowbirds will lay their eggs in nests of other icterids. Parasitism by *M. ater* is increased in areas of habitat fragmentation because it allows greater access to host and their nests (Al-jabber 2003). This has led to parasitism by Brown-headed Cowbirds negatively impact some host species, such as Kirtland's Warbler (Parulidae: *Setophaga kirtlandii* Baird, 1852), which is an endangered species in the United States (Trail & Baptista 1993).

Parasitic nasal mites of Brown-headed Cowbirds

Birds are ideal hosts for multiple kinds of symbionts, with an array of different ectoparasites and endoparasites feeding on secretions, blood or tissue, and oils on the birds. Among these symbionts, mites are the most diverse group with approximately 40 families and 3000 species associated with birds (Proctor & Owens 2000; Knee *et al.* 2008). Avian nasal mites, comprising four unrelated families, are obligate parasites of bird respiratory systems, typically the nasal passageways. Rhinonyssidae (Mesostigmata) is the most diverse family with 510 known species found parasitizing species across the bird tree of life. Genera of rhinonyssid tend to show affinities to specific groups of birds. The other families, Ereyetidae (Prostigmata), Cytoditidae (Astigmata) and Turbinoptidae (Astigmata), are much less diverse and less commonly reported.

Nasal mites show characteristics typical of endoparasitic species: reduced shielding, reduced setation, and overall, a body type that would have reduced mobility compared to free-living or ectoparasitic relatives. Possibly because of these characteristics, nasal mites are restricted to the respiratory system of their hosts and not found in nests like many of the ectoparasites. The primary mode of transmission for nasal mites has been hypothesized to be from the parents to the young during feeding (Murray 1966), which obviously involves a lot of beak to beak contact. Another hypothesis is that birds can obtain nasal mites when they socialize in groups, by preening, or courtship billing (Amerson 1967). Because cowbirds are reared by foster parents of a different species, we would expect that cowbird young receive mites from their host, and therefore we would expect the nasal mite fauna of cowbirds to be diverse and representative of their hosts.

Currently, the list of nasal mites previously found in cowbirds includes six species, five Rhinonyssidae (*Ptilonyssus agelaii* Fain and Aitken, 1964; *P. japuibensis* Castro, 1948; *P. icteridius* Strandtmann and Furman, 1956; *Sternostoma strandtmanni* Furman, 1957; and *S. tracheacolum* Lawrence, 1948) and one Ereyetidae (*Boydai quiscalis* Clark, 1960) (Pence, 1975; Knee and Galloway, 2016). Of these species, *P. agelaii* and *S. strandtmanni* have only been found in icterid species. The other mite species have all been found in icterids, and also in species of Emberizidae (new world sparrows and allies), Fringillidae (true finches), Parulidae (new world warblers), and Tyrannidae (tyrant flycatchers). Species in these other bird families are potential hosts of Brown-headed Cowbirds, indicating that these mites may have been transmitted from foster parent to young cowbirds. However, the two species restricted to Icteridae may indicate transmission during mixed icterid flocking, but since *M. ater* does sometimes parasitize other icterids, it is unclear what the transmission route is.

In this study we examined more than 100 specimens of *Molothrus ater* for nasal mites with the aim of addressing questions regarding prevalence and identity of mites in *M. ater* and whether the patterns of infestation suggest a predominant mechanism of transmission.

Materials and methods

This study is based on *Molothrus ater* collected at Fort Hood, Texas (31°11'32"N 97°44'31"W), in the years 2014 and 2015. This collection was donated to the laboratory of ornithology of Dr. Than Boves at Arkansas State University (ASU) in Jonesboro. The collection consisted of 120

individual *M. ater*. In addition, 6 cowbirds salvaged from window strikes in Arkansas were also examined for nasal mites.

The *M. ater* were maintained and stored in a -20°C freezer at the ASU laboratory (Texas specimens) or UA laboratory (Arkansas specimens) until processed. The respiratory passages of the birds were flushed using a mixture of warm water with ethanol. A 5cc syringe was used to push water through the nasal passages. Typically, mite collections were made by four consecutive flushes using hard water pressure applied to each nostril, alternating flushes on both sides of the nasal cavities. The flushed liquid was collected in a 9 cm Petri dish, and was examined for nasal mites using a Leica MZ16 stereomicroscope at 20-25x magnification. Nasal mites were collected and stored in vials of 70% ethanol. For the identification of nasal mites, mites were slide-mounted for observation under the compound microscope. Mites were cleared in 85% lactic acid at 70°C for one hour. They were placed on the slide in a drop of Hoyer’s mounting medium and once positioned correctly, a cover slip was put in place. Identification of immature stages and female adult mites was made using keys (Pence 1975; Knee & Proctor 2006). Mite specimens were deposited in the Acarology Collection at the University of Arkansas.

Results

A total of 84 out of 126 *M. ater* were infested with nasal mites (66.6% prevalence). For the Texas specimens, the prevalence was 65% (78 of 120), whereas all Arkansas cowbirds (6 out of 6) had nasal mites (Table 1). The nasal mites found in Brown-headed Cowbirds specimens consisted primarily of four species of *Ptilonyssus* Berlese & Trouessart, 1889 (Mesostigmata: Rhinonyssidae). The order Prostigmata was represented by one species of *Boydai*a (Ereynetidae: Speleognathinae) (Table 2).

Table 1 Prevalence of nasal mite species collected from 120 specimens examined of Brown-headed Cowbirds from Texas and six specimens examined from Arkansas in 2014-2015.

| Nasal mite species | | Number of hosts infested | |
|---------------------|--|--------------------------|----------|
| | | Texas | Arkansas |
| Single Infestations | <i>Ptilonyssus icteridius</i> | 55 | 6 |
| | <i>Ptilonyssus agelaii</i> | 16 | |
| | <i>Ptilonyssus japuibensis</i> | 1 | |
| | <i>Ptilonyssus richmondinae</i> | 1 | |
| | <i>Ptilonyssus</i> sp. | 1 | |
| Double Infestations | <i>Ptilonyssus icteridius</i> + <i>Ptilonyssus agelaii</i> | 2 | |
| | <i>Boydai</i> a quiscali + <i>Ptilonyssus agelaii</i> | 1 | |
| | <i>Boydai</i> a quiscali + <i>Ptilonyssus icteridius</i> | 1 | |
| Total | | 78 | 6 |
| Prevalence= 66.6 % | | | |

Species of *Ptilonyssus* were the most common and abundant nasal mites, infesting 82 *M. ater* individuals. Five *Ptilonyssus* species were recovered from Brown-headed Cowbirds (*P. icteridius*; *P. agelaii*; *P. japuibensis*; *P. richmondinae* George, 1961; and a potentially new species of *Ptilonyssus*). *Boydai*a quiscali was the only Prostigmata species found and was present in only two cowbirds from Texas. *Boydai*a was found co-infesting with *Ptilonyssus* in both instances.

Ptilonyssus icteridius was the most common and abundant nasal mite species, present in 64 of 126 cowbirds (50.8% infestation), including all six Arkansas specimens. The second most common mite was *P. agelaii*, found in 18 *M. ater* from Texas (15%) (Table 2). *Ptilonyssus* nasal mites from one *M. ater* could not be confidently identified to species.

The number of nasal mites varied from 1-15 per bird. Double infestation was also observed in four *M. ater* specimens (Table 2), two with *P. icteridius* + *P. agelaii*, one with *P. icteridius* +

B. quisicali and one with *B. quisicali* + *P. agelaii*. One species, *Ptilonyssus richmondinae*, was found to be a new nasal mite record for *M. ater*.

Table 2 Species of nasal mites found in Brown-headed Cowbirds from Texas and Arkansas.

| Mite family | Subfamily | Species |
|---------------|-----------------|--|
| Rhinonyssidae | Rhinonyssinae | <i>Ptilonyssus icteridius</i> (Strandtmann and Furman, 1956) |
| | | <i>Ptilonyssus agelaii</i> (Fain and Aitken, 1964) |
| | | <i>Ptilonyssus japuibensis</i> (Castro, 1948) |
| | | <i>Ptilonyssus richmondinae</i> (George, 1961)* |
| | | <i>Ptilonyssus</i> sp. |
| Ereynetidae | Speleognathinae | <i>Boydia quisicali</i> (Clark, 1960) |

* This mite species represents new host record for the Brown-headed Cowbird

Discussion

The Brown-headed Cowbirds in this study showed a high rate of infestation by nasal mites. Sampling of nasal mites in cowbirds from Texas and Arkansas yielded a 66.6% prevalence of infestation overall, similar to a previous study on nasal mites of Brown-headed Cowbirds by Clark (1963), which found up to 55% of *M. ater* from Pennsylvania to be infested with nasal mites. That study had a similar total number of birds (188 from Pennsylvania). This prevalence of infestation is greater compared to previous surveys of nasal mites in non-brood parasites, in which infestations of nasal mites range from 15-40% prevalence (Domrow 1969; Pence 1973; Spicer 1987; Knee *et al.* 2008).

In this study, mites from two orders were found, Mesostigmata and Prostigmata. Each order was represented in the sample by only one family, Rhinonyssidae and Ereynetidae, respectively. The genus *Ptilonyssus* (Rhinonyssidae) is the most common nasal mite genus found in Passeriformes (Pence 1975, Knee *et al.* 2008) and was the most common in this study, recovered from all 84 infested *M. ater*. Results from this study are similar to reports in previous studies of cowbirds (Strandtmann & Furman 1956).

Of the five species of *Ptilonyssus* collected, *Ptilonyssus icteridius* was the most common nasal mite. As shown in other studies (Strandtmann & Furman 1956; Pence 1973), *P. icteridius* is commonly found parasitizing members of the family Icteridae. This nasal mite appears to show specificity, largely at the family level, and commonly infests blackbirds, grackles, and orioles (Pence 1975). *Ptilonyssus icteridius* has also been recorded from five other host species from five different families; however, in all cases, the mites have only been found in one or very few individuals (Strandtmann and Furman 1956; Knee *et al.* 2008; Knee and Galloway 2016), which does not provide enough evidence to rule out that these were accidental associations or misidentifications of the mites.

The second most common nasal mite species was *P. agelaii*, which was found in 18 specimens of *M. ater*. This mite was originally described from the Red-winged Blackbird (*Agelaius phoeniceus* Linnaeus, 1766) and has only been recorded in the type host and Brown-headed Cowbird. One individual *M. ater* had three nasal mites identified as *P. japuibensis*, which is a species that is commonly found in a diversity of warblers, sparrows and buntings (Parulidae, Cardinalidae, Emberizidae), all of which are common hosts of *M. ater*. Knee *et al.* (2008) also found *P. japuibensis* in one Brown-headed Cowbird individual, and Knee and Galloway (2016) recently reported finding the mite in 45 individuals of another icterid, the Common Grackle (*Quiscalus quiscula* Linnaeus, 1758). Because typical hosts of *P. japuibensis* are also hosts of Brown-headed Cowbird, this might suggest an association due to transfer from the foster parents to the young *M. ater* in the nest. However, the higher prevalence of *P.*

japuibensis in the Common Grackle might suggest that infested Brown-headed Cowbirds may transfer mites to Common Grackle during flocking.

A lone individual of *P. richmondenae* was found in one Brown-headed Cowbird from Texas. This species represents a new record for *M. ater* as it has only previously been recorded from the Northern Cardinal (*Cardinalis cardinalis* Linnaeus, 1758). The anomalous host record requires further scrutiny. The morphology of *P. richmondenae* is very similar to that *P. agelaii* and *P. japuibensis*. Similarity of some mites from the genus *Ptilonyssus*, such as species in the “*sairae*” complex, to which *P. agelaii* and *P. japuibensis* belongs, suggest additional analyses need to be conducted. Molecular analysis could determine whether the genus *Ptilonyssus* is less diverse than previously considered, or whether commonly encountered species, such as *P. icteridius*, may represent a set of cryptic species as suggested by Morelli and Spicer (2007). In addition to clarifying some species identifications, molecular analyses might also shed light on the diversity of host associations seen in this study and before. Lastly, one *M. ater* had two specimens of an unidentified *Ptilonyssus*, which may represent a new species, although further examination is required.

Two specimens of *Boydaiia quiscali* were collected, representing the only individuals of the order Prostigmata and family Erythetidae (Speleognathinae) found in this study. *Boydaiia quiscali* has been described from black birds, grackles, and *M. ater* and, along with *B. agelaii*, are the only species in the family that parasitize cowbirds and other members of Icteridae (Pence 1975). Curiously, *B. quiscali* was only found in two Brown-headed Cowbirds, and in both cases the birds were also infested with species of *Ptilonyssus*. one co-infestation was with *P. icteridius* and the other was with *P. agelaii*. Only two other double infestations were found, both birds infested by *P. icteridius* and *P. agelaii*. Double infestations in this study were similar to those found in Clark (1963), where he found birds infested by *Boydaiia* and *Ptilonyssus*.

One species of nasal mite reported for Brown-headed Cowbirds in other studies that was not found in this study is *Sternostoma tracheacolum* (Mesostigmata: Rhinonyssidae). The lack of records in this study most likely reflects the sampling method used. This study used flushes of the nasal cavities to extract mites, however, as the specific name suggest, *S. tracheacolum* is a nasal mite that migrates to the lungs of the host species (Lawrence 1948). Dissection of the respiratory tracts would be required to reliably obtain this species.

One of the goals of this study was to examine the trends of the data to determine the means of nasal mite transmission in Brown-headed Cowbirds. Transmission of mites from foster parents is one means suggested, as is lateral transfer when multiple species flock together. The findings of this study suggest that neither mechanism of transmission of nasal mites can be ruled out for the specimens examined, and in fact, the diversity of nasal mites found in *M. ater* may suggest a combination of the two transmission routes in shaping the Brown-headed Cowbirds nasal mite community. Cowbirds are gregarious and regularly flock with other icterids (primarily grackles and other blackbird species), but not with solitary icterids, such as orioles. Therefore, specific records and careful identification of mites are both necessary to determine mechanisms of transmission. According to Strandtmann and Furman (1956), cowbirds seem to not support nasal mites from non-icterid birds; however, we found a new host record for a mite previously known only from the northern cardinal and as already mentioned, *P. japuibensis* is more commonly found in non-icterid species. Also, the unidentified species found in one *M. ater* might represent a new host record or a new species. Furthermore, with the exception of Clark (1963), who examined 188 birds from Pennsylvania, most surveys of nasal mites from cowbirds have previously only been made from a small number of individuals (e.g., 20 birds from Alabama, two individuals from Canada, and opportunistic checks in birds from Indiana, Rhode Island, California, and Texas). A larger sampling effort across North America might yield different results. Also, determination of the resident versus the migratory populations may be important in figuring out patterns of host use and mite infestation. Lastly, sequence data may be necessary to determine cryptic species, which may be impacting our ability to establish the true level of host specificity in these mites.

Acknowledgements

Many special thanks to Dr. Than Boves and Alix Matthews from Arkansas State University in Jonesboro, who granted us access to their collection of birds, which constituted the largest number of birds in this study. Special thanks to Dr. Robert N. Wiedenmann for all the support and mentoring during this project. Thanks to Dr. Kimberly Smith for helping with the bird collection. Thanks to the Department of Entomology at the University of Arkansas and to the University of Arkansas, Division of Agriculture, and the Arkansas Agricultural Experiment Station, for supporting and funding this project.

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