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High Ectoparasite Loads of Tropical Birds: Chewing Lice on Puerto Rican American Kestrels (*Falco sparverius caribaearum*)

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ABSTRACT: Ectoparasite loads of birds may be governed, in part, by the climatic characteristics of their environment. We surveyed live-trapped American Kestrels (*Falco sparverius caribaearum*) for lice and other ectoparasites on the humid subtropical island of Puerto Rico during March–May 2024. The main goal was to compare the prevalence and abundance of lice on kestrels in Puerto Rico to recently published data on the lice of kestrels 1000 km away in the Bahamas, and to the lice of kestrels in an arid region of the western US (Utah). A brief general inspection of the plumage of 39 captured birds was followed by careful examination of the underside of wing primary feathers under a dissecting microscope. Two species of lice were collected, *Colpocephalum subzerafae* and *Degeeriella carruthi*, neither previously recorded from kestrels in Puerto Rico. The same two species are present on kestrels in the Bahamas. The prevalence and abundance of lice on kestrels in Puerto Rico and the Bahamas were similar, while being much greater than the prevalence and abundance of lice on kestrels in arid Utah, US. We also collected two species of hippoboscid flies (Diptera: Hippoboscidae): *Microlynchia pusilla*, not previously recorded on the American Kestrel, and *Ornithoctona erythrocephala*. These flies, as well as the lice, might affect the health of kestrels both directly, e.g., causing anemia by feeding on blood, and indirectly by vectoring endoparasites.

Key words: Abundance, bird lice, hippoboscid flies, host, humidity, parasite, prevalence.

Ectoparasites may reduce the condition, survival, and reproductive success of birds (Møller et al. 1990; Brown et al. 1995; Clayton et al. 2015). Birds, in turn, combat ectoparasites with a variety of immunological and behavioral defenses (Clayton et al. 2010). Hosts invest a considerable amount of time and energy in these anti-parasite defenses, which are especially important for keeping permanent parasites in check.

Bird lice (Insecta: Phthiraptera) are permanent parasites: They pass all stages of their life cycle on the body of the host (Clayton et al. 2015). They feed on feathers, dead skin, and, in some cases, blood; they have negative effects on host survival and reproduction; and some serve as vectors of other parasites, such as nematode worms (Clayton et al. 2008).

Many bird lice obtain their water directly from the air using a water-vapor uptake system (Rudolph 1983). The efficiency of this system varies with ambient humidity; some species of lice cannot extract enough water to survive when ambient humidity falls below about 35% (Moyer et al. 2002; Malenke et al. 2011). Thus, the prevalence and abundance of lice on birds are potentially influenced both by host defenses and by environmental conditions such as ambient humidity.

A comparison of parasites on different species of Columbiformes (pigeons and doves) among different regions of the world revealed a positive relationship between louse prevalence and ambient humidity. Lice were found on <3% of Columbiformes in the Sonoran Desert of Arizona, whereas 92% and 100% of Columbiformes in Philippine and Peruvian rainforests, respectively, had lice (Clayton et al. 2010). This pattern is consistent with the experimental work that showed that populations of lice on captive pigeons (*Columba livia*) housed at low humidity do not survive well, if at all, compared to lice on pigeons housed at higher humidity (Moyer et al. 2002).

Bush et al. (2023) recently broadened the taxonomic scope of research on the effects of environmental conditions on lice by comparing the louse loads of a nonmigratory subspecies of American Kestrel (*Falco sparverius sparveroides*) in a humid location in San Salvador, Bahamas, to the louse loads of the same species of host living in an arid location, Utah, US. They showed that, as in the case of columbiform lice, the prevalence and abundance of lice on kestrels in the humid Bahamas were far higher than in arid Utah. Our study aimed to collect data on the ectoparasite burdens of another nonmigratory subspecies of

American Kestrel (*F. s. caribaearum*) in a second humid location, Puerto Rico. To accomplish this goal, we captured and quantified the prevalence and abundance of lice and other ectoparasites on American Kestrels in Puerto Rico using the same methods as Bush et al. (2023).

Puerto Rico is located in the Caribbean (17°45′ N to 18°30′ N; 65°45′ W to 67°15′ W), at the eastern edge of the Greater Antilles. The island has a maximum length from east to west of 180 km, and a maximum width from north to south of 64 km. The climate is tropical and predominantly maritime, with a maximum mean temperature of 29.7 C, and a minimum mean temperature of 19.4 C; the mean annual precipitation on the island is 1,687 mm (Daly et al. 2003). The mean annual low humidity in Puerto Rico is 77.8%, similar to the 75.5% of the Bahamas (Weather and Climate 2024a, b).

We used Bal-chatri traps to capture 39 adult kestrels March–May 2024 in Puerto Rico. Upon capture, we fitted each bird with a falconry hood to minimize stress (Madden and Mitchell 2018). We banded each bird with a unique combination of color bands and a metal numbered band for individual identification and to avoid processing recaptured birds, although there were no recaptures. Standard ornithological measurements were taken (e.g., body mass and wing chord) and were reported to the US Geological Survey Bird Banding Laboratory (USGS 2024).

Following Bush et al. (2023), we collected ectoparasite data in two stages, qualitative then quantitative. In the first stage, each bird was trapped and held in one hand while we spent about 5 min searching for adult lice by deflecting feathers of the crown, nape, neck, throat (gulum), breast, belly, and cloacal region (crissum) with a pair of forceps. We did not closely examine feather tracts with tightly packed feathers, such as the back, because deflecting those feathers would risk breaking them. The goal of this first stage was to collect voucher specimens of chewing lice, hippoboscid flies, and any other ectoparasites we encountered for identification. Lice were identified by examination of unmounted specimens with a dissecting scope, use of generic keys (Price et al. 2003), species descriptions (Emerson 1953; Clay 1958; Tendeiro 1988), and comparison with specimens of known identification (University of Utah, Price Institute of Parasite Research). Flies were identified by examination with a dissecting scope, following Maa (1969a, b), Vélez et al. (2020), and Obonõa et al. (2022). Voucher specimens of these ectoparasites were deposited in the Price Institute of Parasite Research, University of Utah, Salt Lake City, US (PIPR050085-050089, accessible via the Symbiota Collections of Arthropods Network at https://scan-bugs.org).

The second stage of data collection involved careful quantification of the eggs of chewing lice. Louse eggs, which are about 0.6 mm long, were observed and counted on the underside of primary feathers 1–6 of the right wing using a dissecting microscope in the field. *Colpocephalum subzerafae* (Fig. 1A), which is the most common louse on kestrels in the Bahamas (Bush et al. 2023), glues its eggs between feather barbs on the underside of flight feathers of the wing, mainly on primaries 1–6 (Fig. 1B). When the egg hatches, its top (operculum) pops off, leaving most of the empty shell attached to the feather (Clayton et al. 2015). Thus, the total number of hatched and unhatched eggs on a feather provides a cumulative record of the number of eggs laid over the life of that feather. This index of louse abundance is meant to provide a coarse estimate of louse infestation. It does not indicate the actual number of lice on the bird at a given time for two reasons. Firstly, the index is based on only one wing, thus it is an underestimate of the total number of eggs on the bird. Secondly, the index provides a cumulative record over many months, which is longer than the life of a louse; thus this index presumably overestimates the actual number of lice on the bird at any point in time.

The annual feather molt for American Kestrels begins in the spring (Smallwood and Bird 2002). Only three birds in our study had started molting primary feathers; thus, the total egg counts (hatched and unhatched) for most birds provided an index of the number of *C. subzerafae* eggs accumulated on each bird since the previous spring. To analyze the data on egg abundance, we used a one-way analysis of variance (ANOVA) after log-transformation of the data, followed by a post hoc Tukey-Kramer test. All

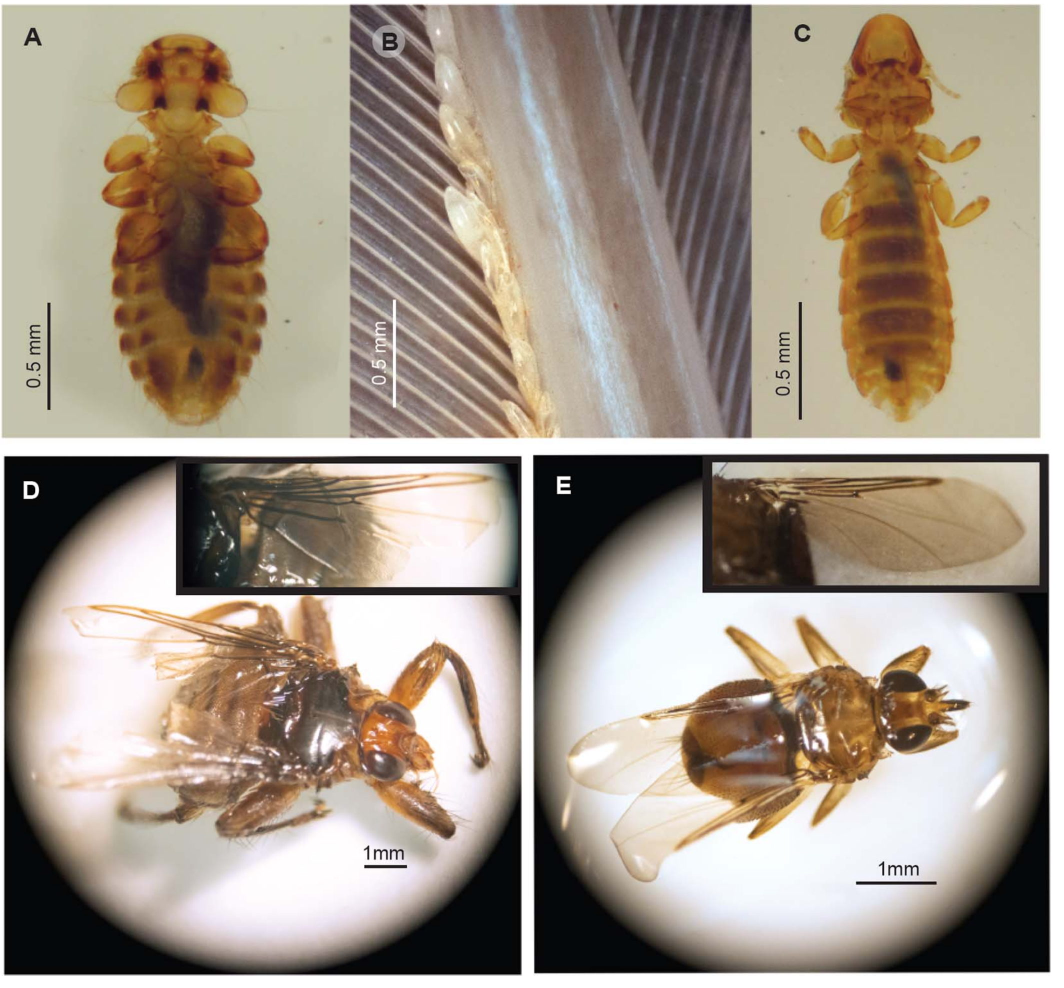

FIGURE 1. Parasitic lice and flies from American Kestrels (*Falco sparverius caribaearum*) examined in Puerto Rico: (A) female *Colpocephalum subzerafae*; (B) *C. subzerafae* eggs (six hatched and four unhatched) glued to the underside of a primary feather; (C) male *Deegeriella carruthi;* (D) *Ornithoctona erythrocephala* with inset close-up showing wing venation; (E) *Microlynchia pusilla*, with inset close-up showing wing venation.

statistical analyses were performed in JMP (ver. 16; SAS Institute, Cary, North Carolina, USA).

We also searched for *Deegeriella carruthi*, a less common species of louse found on kestrels in the Bahamas (Bush et al. 2023). We did not attempt to quantify *D. carruthi* eggs in our study because the attachment site of these eggs remains unknown, and we did not wish to unduly disturb birds by searching through their plumage.

We observed *Colpocephalum subzerafae* (Fig. 1A) crawling on the feathers of 4/39 birds (10%) during the initial examination. During the more detailed microscopic examination of primary feathers, *C. subzerafae* eggs (Fig. 1B) were found on 24/39 (62%) of the 39 birds. The mean (± standard error, SE) abundance of eggs was 28.4 (± 7.6) per bird (range = 0–150). By contrast, *Deegeriella carruthi* (Fig. 1C) was observed on only 1/39 birds (3%).

Louse flies (hippoboscids) belonging to two species were found on three of the birds (7.7%). Two of the flies were identified as *Ornithoctona erythrocephala* (Fig. 1D), which is a large-bodied, sluggish species; both of the specimens we collected were found in the cloacal region of the host beneath the tail. The other fly was identified as *Microlynchia pusilla* (Fig. 1E).

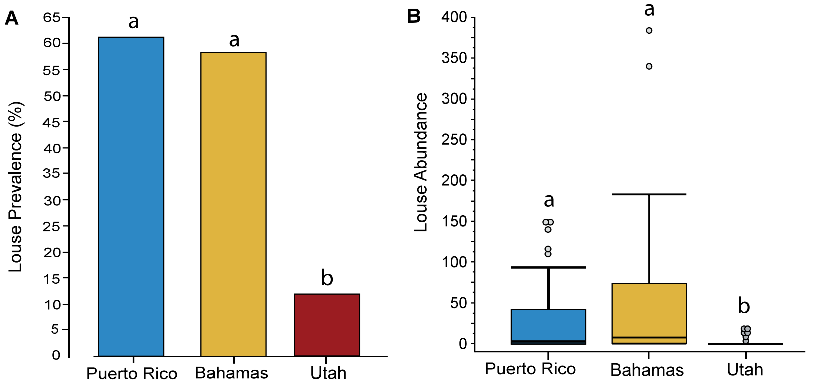

FIGURE 2. Comparison of (A) prevalence and (B) an index of the abundance of lice on 39 American Kestrels (*Falco sparverius caribaearum*) examined in Puerto Rico, 68 American Kestrels examined in the Bahamas, both humid, and 50 American Kestrels examined in arid Utah, USA (Bahama and Utah data from Bush et al. 2023). The index of abundance is the (combined) number of hatched and unhatched eggs glued to the ventral side of primary feathers 1–6 of the right wing. Different letters above bars within each panel indicate statistically significant differences.

This species is a tiny bodied, fast-moving fly that we collected after it abandoned a newly captured kestrel.

The prevalence of lice on kestrels in Puerto Rico (Fig. 2A) did not differ significantly from that on kestrels in the Bahamas (Fisher's exact test n=107, P=0.84). However, as found in the Bahamas in the previous study, the prevalence of lice on kestrels in Puerto Rico was much higher than the prevalence of lice on kestrels in arid Utah (Fig. 2A, Fisher's exact test n=89, P<0.001).

Similarly, the abundance of lice on kestrels in Puerto Rico (Fig. 2B) did not differ significantly from that of lice on kestrels in the Bahamas, despite that the index of abundance for Bahamas birds was based on the accumulation of louse eggs on feathers from molt through November-December (Bush et al. 2023), while our index for Puerto Rican birds was based on the accumulation of eggs from molt through the spring (March–May). The index for Utah birds was based on the accumulation of eggs from molt through November–February (Bush et al. 2023). As in the case of the Bahamas, the abundance of lice on kestrels in Puerto Rico was much greater than the abundance of lice on kestrels in Utah (Fig. 2B, one-way ANOVA, log+1 transformation, $F_{2,156}$ =17.46, P<0.0001; Tukey-Kramer post hoc tests P=0.62 and test P<0.001 for comparisons of louse abundance in Puerto Rico vs. Utah and the Bahamas, respectively).

No previous studies have been done for lice on kestrels in Puerto Rico. We found that *C. subzerafae* was much more common than *D. carruthi* was far less common, found on more than 60% versus on just one bird, respectively. The prevalence and abundance of *C. subzerafae* were relatively easy to quantify because the eggs of this species are easy to locate and count (Bush et al. 2023). In contrast, the location of *D. carruthi* eggs remains undiscovered; hence, the low apparent prevalence of *D. carruthi* documented here may be an underestimate.

The prevalence and abundance of lice on kestrels in Puerto Rico was similar to the study of Bush et al. (2023) on kestrels in the Bahamas. This is not surprising, given that the two localities are both subtropical sites with similar climates. Our results for Puerto Rico are important because they provide independent confirmation that kestrels in humid regions have more lice than birds in arid regions, such as Utah (Bush et al. 2023). In the future, it would be useful to quantify the prevalence and abundance of lice from kestrels in another arid subtropical site. To provide rigorous comparative data, such a study should use the same methodological approach as in Bush et al. (2023) and in the current paper.

Regarding the two hippoboscid flies, *O. erythrocephala* is a widespread parasite found from

Canada to Argentina on dozens of genera of birds in more than two dozen taxonomic families (Maa 1969b; Tossas 2001). It has previously been reported from American Kestrels in Puerto Rico (Wolcott 1936). *Microlynchia pusilla* is also widespread, known from North America (McClure 1984) and South America (da Silva and Pichorim 2013), and from other islands in the Greater Antilles (Maa 1969b) but not previously recorded on birds of Puerto Rico, nor from the American Kestrel throughout its distribution. Like *O. erythrocephala*, this fly is a generalist known from at least 10 different host families (McClure 1984). Adult hippoboscids spend most of their time on the host, feed on blood several times a day, and may cause anemia, emaciation, and slow nestling development. Additionally, they transmit blood parasites, including avian malarial parasites and trypanosomes, that can have negative effects on birds and possibly also transmit viruses (Waite et al. 2012). Further studies would be needed to determine whether the two hippoboscids that we detected significantly affect the health of American Kestrels in Puerto Rico or elsewhere.

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