Louse fly (Diptera, Hippoboscidae) associations with raptors in southern Canada, with new North American and European records

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ABSTRACT

Louse flies (Diptera, Hippoboscidae) are ectoparasites often found on birds. As they spend most of their life on their host, they are not often collected or studied. Hence, little is known about their species richness and prevalence on raptors in Canada. In this study, louse flies were collected from 184 out of 1467 raptors examined during the 2020 fall migration in southern Ontario, Canada, giving an overall prevalence of 12.5%. In total, 256 louse fly specimens were collected (mean intensity = 1.41) representing four species (identified morphologically, with support of DNA barcoding): *Icosta americana* (91.9%), *Ornithomya anchineuria* (0.3%), *O. avicularia* (7.3%) and *O. bequaerti* (0.3%). Mite clusters were found on 42% of *O. avicularia*. This study also presents the first North American record for *O. avicularia* and the presence of *O. bequaerti* in Europe was confirmed for the first time. Based on the different parameters recorded during banding, it appears that the host species and the month play a part in the presence of louse flies on the host. Further study of louse flies is needed to better understand their prevalence across different bird groups and geographic distribution.

1. Introduction

Louse flies (Diptera: Hippoboscidae) are a small family of flies, with only 213 species worldwide (Dick, 2006) and 31 Nearctic species (Maa and Peterson, 1987). They are ectoparasites feeding on the blood of birds and mammals (Maa and Peterson, 1987). Their entire life cycle depends on that resource acquired from their hosts. To avoid competing for resources and to optimize larval survival, the female rears one larva at a time in its uterus, fed on "milk" that it produces. Once developed, the female will release a prepuparium into the nest (Fig. 1), or surrounding substrate, where the emerging adult can more easily find a host. Being highly specialized, they are rarely encountered elsewhere than on their host, making them a difficult subject to study.

Most large and detailed studies date from the 1950s and 1960s, with the work of Bequaert (1952, 1953, 1954, 1955, 1956) and Maa (1963, 1964, 1965, 1965, 1969a, 1969b, 1969c, 1969d, 1969e). There has been a recent surge of research investigating host specificity (Lehikoinen et al., 2021), microbiomes (McCabe et al., 2020) and their prevalence (Meißner et al., 2020; Sychra et al., 2020). However, these recent studies are often limited to one host in a specific region; e.g., Osprey (*Pandion haliaetus* (Linnaeus)) from Baja California, Mexico (Rodríguez-Estrella and Rivera-Rodríguez, 2019); Great Horned Owls (*Bubo virginianus* (Gmelin)) from Baja California, Mexico (Bolaños-García et al., 2018); Eleonora's falcon (*Falco eleonorae* Gene) from Canary Islands (Gangoso et al., 2019). Few of these recent studies have been located in North America. In fact, only McCabe et al. (2016, 2020) have looked at louse flies on birds in the United States, while a few more have looked at keds (Hippoboscidae: Lipopteninae) on mammals (Evans et al., 2021; Poh et al., 2020; Skvarla et al., 2020; Skvarla and Machtinger, 2019), but none were located in Canada. Most studies in Canada date from before the 2000s, often focusing on one site and with a limited number of hosts. With such a limited scope, the prevalence recorded varied greatly across the different studies: 1.4–16% (Bennett, 1961; Davies, 1958; Davis, 1998; Dick, 1981; Savard and Ibarzabal, 1996; Wheeler and Threlfall, 1986), but present a good record of species found in Canada.

Even today, little is known about prevalence of louse flies by host, geographic distribution, or what makes their host vulnerable to their infestation. Our main objective is to assess the presence of louse flies associated with raptors, here defined as hawks and falcons, and their prevalence on migrating adult birds in southern Ontario, Canada. The second objective is to obtain DNA barcodes for each species of ectoparasite to contribute to the Barcode of Life Data System. The third

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Fig. 1. Female *Icosta americana* with a prepuparium protruding from the end of its abdomen.

objective is to test all factors measured during the banding process (i.e. host species, weight, wing chord, age and sex), including month and banding stations, for correlation with the presence or absence of ectoparasites.

2. Materials and methods

Fieldwork was conducted during the 2020 fall migration, from 1 September to 30 November at the Hawk Cliff banding station (coordinate: 42.6645, −81.1701) in Union, Ontario, Canada. The station is composed of five banding cabins along a transect extending from the shores of Lake Erie inland, managed by one bird bander each. Raptors were banded every day during their peak travel time: morning to mid-afternoon. Live birds of different sizes were used to attract different species of raptors toward the station; e.g. pigeons for large hawks and European starlings for small falcons. A range of mist nets were placed around the decoy to catch them. Once removed from the net, the bird was brought inside the cabin and stored in a size-specific container to minimize stress (e.g. Pringles can), from which they were banded, identified to species, weight and wing chord measured, aged and sexed where possible. The following additional information was recorded: date, time, station number, bander and how each bird was sexed or aged. While handling the bird, all feathers were ruffled and blown upon to inspect and collect louse flies. When present, louse flies were collected by hand and transferred to a vial with 95% ethanol and labelled with a unique number. That number was recorded on the banding data entry sheet to associate the host information to the louse fly specimens. Flies which flew from the bird while in the cabin were caught and transferred to a vial. It is likely that some flies escaped from the time that the bird became entangled in the net through the banding process. The efficiency was not measured; hence the results and prevalence are a close approximation. Once the data process was completed, the bird was released. All bird handling was done by certified bird banders. Although 1486 birds were banded, 19 had missing data and were removed from the dataset, bringing the total to 1467.

All louse fly specimens were dried using a porous ceramic plate, then pinned and labelled. All were identified to species using a combination of morphological dichotomous keys (Hutson, 1984; Maa, 1969c, 1969d; Maa and Peterson, 1987). DNA barcoding and neighbor-joining analysis were used as additional identification tools, especially helpful with the morphologically variable genus, *Ornithomya* Latreille. They were also compared to authoritatively identified specimens housed in the Canadian National Collection of Insects, Arachnids and Nematodes (CNC) in Ottawa. Specimens are deposited in the Centre for Biodiversity Genomics collection (BIOUG) at the University of Guelph (Canada).

Representatives of each species were sampled for sequencing of cytochrome *c* oxidase I (COI) following the standard protocol used at the

Canadian Centre for DNA barcoding (CCDB: http://ccdb.ca/resources/). Mites were found on some prepared and mounted louse flies and were sequenced to learn their identity. As mites were not identified below class, the sequences were matched against the BOLD ID Engine and GenBank records. All sequences were uploaded to the Barcode of Life Database System (BOLD: http://boldsystems.org) (Ratnasingham and Hebert, 2007) and assigned a Barcode Index Number (BIN) as species proxy (Ratnasingham and Hebert, 2013). All of these are released publicly through BOLD (dx.doi.org/10.5883/DS-LOFLY) and GenBank (www.ncbi.nlm.nih.gov/genbank/, Accession: MZ2611700-MZ2617 20). A neighbor-joining tree of the *Ornithomya* species was built using maximum likelihood model in MEGA X (Kumar et al., 2018). Due to the difficulties to confidently identify morphologically variable specimens of *Ornithomya*, GenBank records and public records from BOLD of species present in this study and closely allied species, were added to the tree to assess the identifications from recent literature. All sequences included were longer than 550 bp. This tree is intended to visualize the molecular divergence between the taxa and is not intended to represent an actual phylogeny.

A Generalized Linear Model (GLM), family binomial, with logit link function was set up to test the influence of the factors measured during banding (i.e., host species, month, weight, wing chord, station, age and sex) as categorical variables on louse fly presence/absence (yes/no). As weight and wing chord are likely correlated with each other, their interaction was included in the models to test separately and together. All factors were ordered based on their likeliness of being impactful on the presence of louse flies to create the first model. Five models were tested by removing one factor at a time and comparing their AICc, ΔAICc and AICc weights (ω) (Table 1) as a metric to see which model was a better fit. All statistical analyses and visualizations were conducted in R version 4.0.3 (R Core Team, 2020) with the packages ggplot2 (Wickham, 2016) and AICcmodavg (Mazerolle, 2020). All prevalence and confidence intervals were calculated with Quantitative Parasitology (QPweb) (Reiczigel et al., 2019). QPweb was also used to compare prevalence with the Fisher's exact test.

3. Results

In total, 1467 raptors with complete host data were banded during the 2020 fall migration (Sep–Nov) representing the following 10 species: Accipitriformes, Accipitridae (7 species): *Accipiter cooperii* (Bonaparte) (COHA; Cooper's Hawk), *Accipiter striatus* Vieillot (SSHA; Sharp-shinned Hawk), *Buteo jamaicensis* (Gmelin) (RTHA; Red-tailed Hawk), *Buteo lagopus* (Pontoppidan) (RLHA; Rough-legged Hawk), *Buteo lineatus* (Gmelin) (RSHA; Red-shouldered Hawk), *Buteo platypterus* (Vieillot) (BWHA; Broad-winged Hawk), *Circus hudsonius* (Linnaeus) (NOHA; Northern Harrier); and Falconiformes, Falconidae (3 species): *Falco columbarius* Linnaeus (MERL; Merlin), *Falco peregrinus* Tunstall (PEFA; Peregrine Falcon), *Falco sparverius* Linnaeus (AMKE; American Kestrel) (Table 2). More than half of the birds caught were *A. striatus* (54%), while *B. lagopus*, *B. lineatus* and *B. platypterus* were rarely encountered (n < 4). Hatch year birds are more likely caught, which may be due to lack of experience or being more easily affected by wind drift (Evans et al., 2012; Farmer et al., 2007; McCabe et al., 2016; Mueller et al.,

Table 1

The set of candidate models (simplified formula) to investigate the effects of host species, month, weight, wing chord, station, age and sex on the presence of louse flies ("p").

Model #	Model description
1	ln(p/1-p) = Species + Month + Weight * Wing + Station + Age + Sex
2	ln(p/1-p) = Species + Month + Weight * Wing + Station + Age
3	ln(p/1-p) = Species + Month + Weight * Wing + Station
4	ln(p/1-p) = Species + Month + Weight * Wing
5	ln(p/1-p) = Species + Month + Weight

Table 2
All raptor species banded during 2020 fall migration with their abundance, mean intensity and prevalence of louse flies.

| Host species	No. banded			Total	Louse flies					
	Sep	Oct	Nov		Presence	Mean abundance	Mean intensity (range)	Prevalence (%)	Lower 95% CI (%)	Upper 95% CI (%)
Accipitriformes: Accipitridae										
Accipiter cooperii	24	44	34	102	22	0.27	1.27 (1–3)	21.6	14.6	30.8
Accipiter striatus	409	364	24	797	105	0.19	1.42 (1–5)	13.2	11.0	15.7
Buteo jamaicensis	22	153	107	282	49	0.26	1.51 (1–4)	17.4	13.3	22.3
Buteo lagopus	0	0	2	2	0	0.00	–	0.0	–	–
Buteo lineatus	2	2	0	4	0	0.00	–	0.0	–	–
Buteo platypterus	1	0	0	1	1	1.00	1.00 (1)	100.0	5.0	100.0
Circus hudsonius	33	51	13	97	4	0.04	1.00 (1)	4.1	1.4	10.2
Falconiformes: Falconidae										
Falco columbarius	22	7	4	33	2	0.06	1.00 (1)	6.1	1.1	19.4
Falco peregrinus	6	7	0	13	0	0.00	–	0.0		
Falco sparverius	107	29	0	136	1	0.01	1.00 (1)	0.7	0.0	3.9
Total	626	657	184	1467	184	0.18	1.41 (1–5)	12.5	10.9	14.3

1981); they represented 88.6% of all banded birds. Species banded varied greatly in size and weight (Fig. 2A). These variables are used to determine sex in most species, except for *Buteo* spp. (*B. jamaicensis*, *B. lagopus*, *B. lineatus* and *B. platypterus*), where sex cannot be reliably identified in live birds, hence the sex was recorded as undetermined (Fig. 2B). The sex ratio was skewed toward females (48.0%) compared to

males (32.4%) and unknown (19.6%). Most of the raptors were banded early in the fall migration, with 42.7% in September, 44.8% in October and only 12.5% in November (Table 2). The exception was *B. jamaicensis*, which was more abundant later in the season (54.3% in October and 37.9% in November) and they accounted for 58.2% of all birds banded in November.

Louse flies were found on 184 individual birds representing seven host species for an approximate prevalence of 12.5% (CI: 10.9–14.3%) (Table 2). The majority were found on *A. striatus* (57.5%), but once the abundance was corrected, the mean abundance and the prevalence were higher for *A. cooperii* (21.6%) and *B. jamaicensis* (17.4%). This is excluding *B. platypterus* which had only one individual banded (100%). The difference in prevalence between *A. striatus* and *A. cooperii* was statistically significant (p < 0.05), but the difference between *A. striatus* and *B. jamaicensis* was not (p = 0.11). Some birds had multiple louse flies on them, ranging from 1 to 5 individuals (mean intensity = 1.41), which lead to 259 specimens being collected, representing four species. *Icosta* (*Ornithoponus*) *americana* (Leach) was the most prevalent species of louse fly collected, accounting for 91.9% (n = 238) of all specimens (Fig. 3). This species is quite large with long wings (7–8.5 mm) and with syntergite 1 + 2 laterally with 4–6 heavy spines (Maa, 1969d). The remaining species belonged to the genus *Ornithomya*: *O. avicularia* (Linnaeus) with 19 specimens, and *O. anchineuria* Speiser and *O. bequaerti* Maa as singletons. *Ornithomya avicularia* is the largest of the three based on wing length (5.5–7 mm) and distinguished by 8–10 scutellar setae (Hutson, 1984). *Ornithomya anchineuria* and *O. bequaerti* are quite similar in wing length (4.4–5.0 mm) and presence of four scutellar setae. They can be distinguished by the side piece of female tergite 6 which is elongate in *O. bequaerti* and shortened in *O. anchineuria* (Maa, 1969c). In addition, the wing microtrichia is more extensive in cells m_1 and r_{4+5} in *O. bequaerti* and without a bare strip along the margin of vein M_1 compared to *O. anchineuria* (Maa, 1969c). The louse fly temporal abundance followed a similar pattern to their hosts, with 45.1% collected in September, 45.1% in October and only 9.8% in November (Fig. 3). All species were also distinct, based on their COI sequences and assigned to a unique BIN (see Fig. 4 for *Ornithomya*).

Additional observations include: eight specimens of *I. americana* were found with a prepuparium at the tip of the abdomen (Fig. 1). Most of these (75%) were found late during the fall migration, from late October to November. Clusters of mites with eggs were found on 42% of *O. avicularia* (n = 8), with the majority being collected in September (87.5%). These clusters were located toward the tip of the abdomen as observed by Goater et al. (2018). Singletons of two other fly species were collected during the search for louse flies and were considered transient. One of these transients was the Chloropidae, *Liohippelates pallipes* Loew, collected off *A. striatus* along with two specimens of *I. americana*. This chloropid genus is attracted to fluid secreted from their host's eyes, often referred to as eye gnats (Sabrosky, 1941). The other transient fly was a
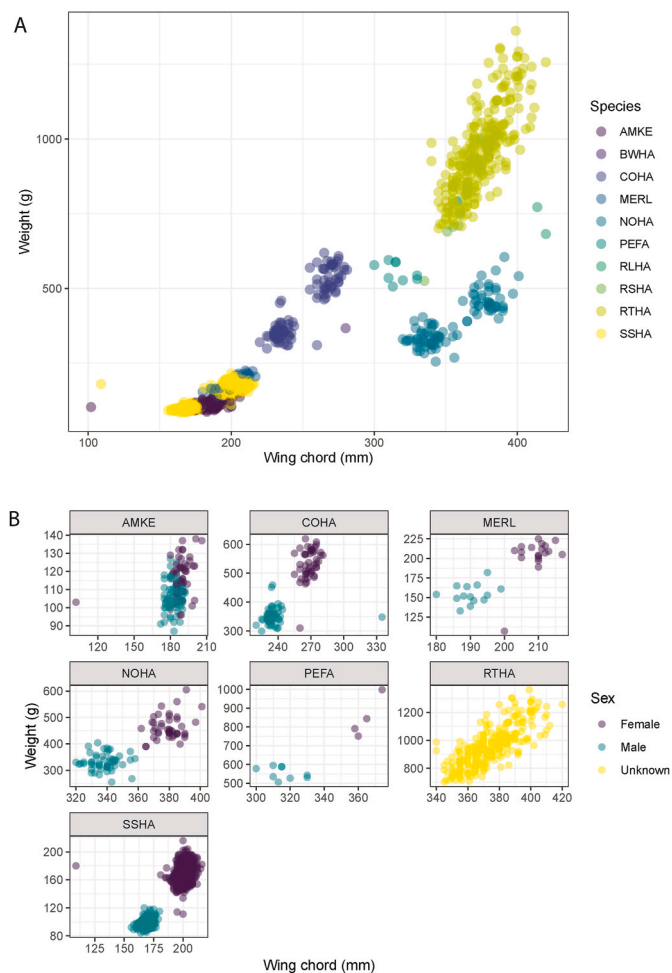
Fig. 2. A) Size representation of all birds banded (n = 1467) and **B)** size representation of the seven most abundant species (n > 5) and their sex by wing chord (mm) and weight (g). Four-letter alpha code: AMKE = *F. sparverius*, BWHA = *B. platypterus*, COHA = *A. cooperii*, MERL = *F. columbarius*, NOHA = *C. hudsonius*, PEFA = *F. peregrinus*, RLHA = *B. lagopus*, RSHA = *B. lineatus*, RTHA = *B. jamaicensis*, SSHA = *A. striatus*.
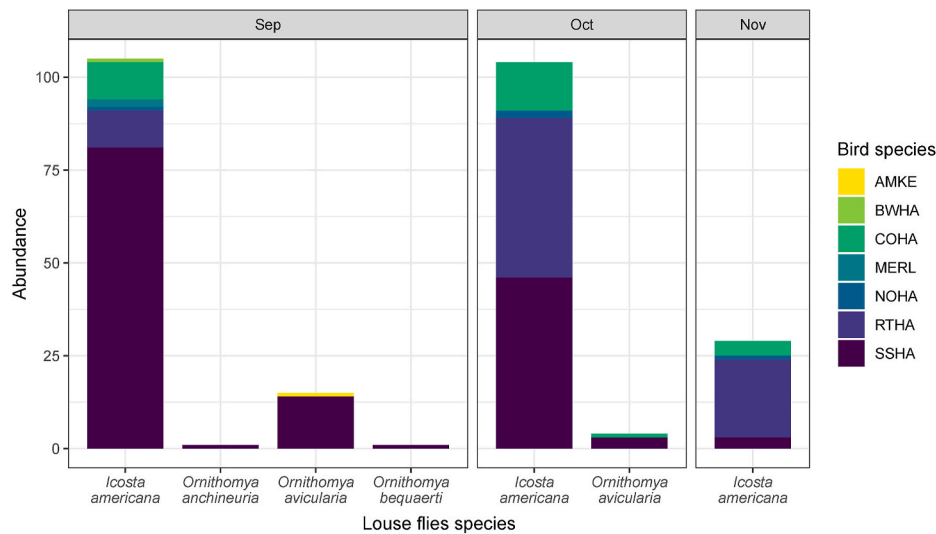
Fig. 3. Abundance of louse fly species by bird and by month. See Fig. 1 for the four-letter alpha code correspondence.
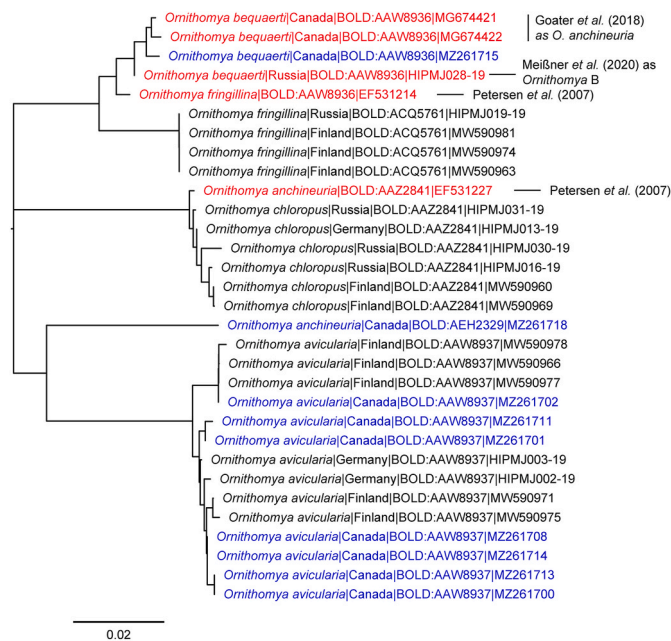

Fig. 4. Neighbor-joining tree with representation of the three species of Ornithomya collected in this study, including GenBank records and BOLD public records of the same and related species (blue colour = specimens from this study; red = suspected or misidentification). Includes BIN and GenBank accession number (or BOLD process ID when not on GenBank). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

species of Heleomyzidae, *Lutomyia spurca* Aldrich, found on *A. cooperii*. The genus is associated with chipmunk burrows (Sabrosky, 1949) and might have transferred from the prey to the bird.

One logistic generalized linear model was a better fit based on its high weight and low AICc compared to the other models and was selected: model 3 (AICc = 973.96, ω = 0.87; Table 3). It contained host species, month, weight, wing chord and station, while omitting age and sex. Only *A. striatus* was significantly more likely to have louse flies (p < 0.01), when compared to the base category, *F. sparverius* (Table 4). Surprisingly *A. cooperii* and *B. jamaicensis* were not significantly more likely to be infested than *F. sparverius*, despite both having a prevalence higher than *A. striatus*. The strong presence of louse flies in September

Table 3

Results of ordinal ranking based on ΔAICc for the logistic generalized linear models of presence of louse flies via categorical predictor variables. K represents the number of estimable parameters, AICc for the second-order variant of Akaike's information criterion, ΔAICc for AICc difference, ω for Akaike weight. Models are ordered from high to low weight.

Model #	K	AICc	ΔAICc	ω
3	20	975.25	0	0.87
2	24	979.34	4.09	0.11
1	26	982.85	7.6	0.02
5	13	1041.84	66.59	0
4	15	1044.02	68.77	0

and October was also significantly higher (p < 0.05) compared to November (Table 4). The remaining variables (i.e. weight, wing chord and station) included in the model were not significantly associated with the presence of parasites. However, their inclusion did make the model a better fit: when removed from the model its Akaike weight became null and its AICc a lot higher, reducing its fit to the data.

4. Discussion

Prevalence of louse flies reported in the literature on birds varies greatly. It is hard to interpret the different studies as the sample size, host species, locality and method for catching birds vary widely. Prevalence is higher in studies in which nestlings were examined than in those in which live birds were caught in mist nets. Nestlings are an easy target as they are limited to the nest, which could lead to a higher number of louse flies completing their entire life cycle inside the nest, while juvenile and adult raptors are always on the move during migration and less likely to aggregate with conspecifics. Previous studies report prevalence from 26 to 65%, with Great Horned Owl nestlings at the lower end (Bolaños-García et al., 2018) and Osprey nestlings at the upper end (Rodríguez-Estrella and Rivera-Rodríguez, 2019), both from Mexico. Pied Flycatcher (*Ficedula hypoleuca* (Pallas)) nests from Fennoscandia had also high prevalence with 59% having *Ornithomya chloropus* Bergroth and 20% with *O. avicularia* (Eeva et al., 2015). While birds caught during migration appear to have lower prevalence than at other times of the year, varying from 1 to 7% (Lehikoinen et al., 2021; Liébana et al., 2011; Meißner et al., 2020; Sychra et al., 2020). One exception was a study done across 84 species of bird in Algonquin Park, Canada, which found an overall prevalence of 16% (Bennett, 1961). The high prevalence was driven by a few species: between 25 and 33% on

Table 4
The logistic generalized linear model with the highest weight (ω = 0.87) describing the link between the presence of louse flies, host species, month, weight, wing chord and station. Species AMKE, Month Nov and Station 1 were used as base categories for the coding and are not displayed here. See Fig. 1 for the four-letter alpha code correspondence.

Variable	Estimate	SE	Lower 95% CI	Upper 95% CI	z-value	p-value	
(Intercept)	−24.40	657.90	−1313.91	1265.12	−0.037	0.970	
SpeciesBWHA	22.22	6523.00	−12761.92	12806.36	0.003	0.997	
SpeciesCOHA	2.15	1.21	−0.23	4.52	1.770	0.077	
SpeciesMERL	1.79	1.26	−0.68	4.25	1.420	0.156	
SpeciesNOHA	0.10	1.56	−2.96	3.15	0.062	0.951	
SpeciesPEFA	−16.52	1730.00	−3407.88	3374.83	−0.010	0.992	
SpeciesRLHA	−16.31	4608.00	−9048.30	9015.69	−0.004	0.997	
SpeciesRSHA	−16.46	3244.00	−6373.79	6340.88	−0.005	0.996	
SpeciesRTHA	−0.15	1.49	−3.06	2.77	−0.098	0.922	
SpeciesSSHA	3.02	1.02	1.02	5.02	2.963	0.003	**
MonthOct	0.61	0.31	0.00	1.22	1.965	0.049	*
MonthSep	0.85	0.34	0.18	1.52	2.492	0.013	*
Weight	0.01	0.00	0.00	0.02	1.493	0.135	
Wing	0.01	0.01	−0.01	0.02	0.861	0.389	
Station3	16.43	657.90	−1273.07	1305.94	0.025	0.980	
Station4	16.84	657.90	−1272.67	1306.34	0.026	0.980	
Station5	16.73	657.90	−1272.78	1306.23	0.025	0.980	
Station6	17.32	657.90	−1272.18	1306.83	0.026	0.979	
Station7	0.10	866.80	−1698.88	1699.09	0.000	1.000	
Weight:Wing	0.00	0.00	0.00	0.00	−0.874	0.382	

Significances: *p < 0.05, **p < 0.01, ***p < 0.001.

young sparrows and thrushes, and 50% on young starlings (Bennett, 1961). Another exception was a study done on raptors in the United States, which had 14% prevalence in 2004 and 53% in 2015 (McCabe et al., 2016). Even with accounting for the small sample size in 2015 (n = 43) by adding the confidence interval on the prevalence (38.3–67.9%), the numbers remain very high. For both sampling years, collecting was spread from September to November, but it was a lot shorter in 2015 (6.7 days vs 17.7 days in 2004), which may have skewed the results. Most of the louse flies were collected in September in 2015 (60%), making it likely that most of the sampling was done around that period. This would match the peak season observed in our study. If sampling could have been more spread out through the season, it would likely have balanced out the prevalence. However, their 2004 results are very similar to our overall 12.5% prevalence. Similarly, Sychra et al. (2020) found 20.8% prevalence on Accipitriformes. It does appear that raptors are more prone to louse flies than are other orders of birds.

The high prevalence of louse flies found on raptors could be partially explained by the presence of one species commonly found on them: *Icosta americana*. This species was dominant on raptors in studies from the United States (McCabe et al., 2020), Mexico (Bolaños-García et al., 2018), and, despite the small sample size, the only louse fly species found on American Kestrels in Argentina (Liébana et al., 2011), and on hawks and owls in Toronto, Canada (Bennett, 1961). It was also dominant in this study, which is not surprising since this louse fly is common in the southern Nearctic Region, reaching as far north as Ontario and Quebec in Canada, and less abundant in the Neotropics (Bequaert, 1955; Maa, 1969d). Most species in the *Icosta* subgenus *Ornithoponus* Aldrich (which includes *I. americana*) are polyxenous with a low host specificity and a lengthy list of hosts (Maa, 1969d). However, *I. americana* does have a preference for Accipitridae and Strigidae, among others (Bequaert, 1955; Bennett, 1961; Maa, 1969d). The genus *Icosta* was also dominant on raptors in South Africa, but represented by different species (Sychra et al., 2020).

Three species of *Ornithomya* were present on raptors in our study. Prior to this study, only two species have been reported from the Nearctic Region: *O. anchineuria* and *O. bequaerti* (Maa, 1969c). This is the first time that *O. avicularia* has been recorded from North America. The sequences obtained from those specimens were a perfect match to European sequences of *O. avicularia* present on BOLD and GenBank (Fig. 4), which confirmed our identification. As multiple specimens were collected (n = 19) through one season, the species is likely well

established in the region. The sequences of the mites found on *O. avicularia* matched the GenBank records (Accession: KT988971, KT988972) identified as *Myialges* cf. *borealis* from mites collected on *O. anchineuria* in Alberta, Canada (Goater et al., 2018). Interestingly, the association of mites with *Ornithomya* has been reported in other studies and with other species: *O. anchineuria* in USA (Main and Anderson, 1970); *O. avicularia* in England (Hill et al., 1967) and in Russia (Meißner et al., 2020); *O. chloropus* in Russia (Meißner et al., 2020); *O. fringillina* Curtis in England (Evans et al., 1963; Hill et al., 1967) and in Portugal (Tomás et al., 2021); and *O. rupes* Hutson in Gibraltar (British territory) and Switzerland (Hutson, 1981). A more detailed examination into *Ornithomya* and its mites is needed to better understand their association and the diversity of those mites in Canada.

Our sequences from *O. anchineuria* and *O. bequaerti* were more puzzling. Sequences of *O. anchineuria* did not match those from Goater et al. (2018) (Fig. 4). In fact, the closest match was over 6% divergent on both BOLD and GenBank, while our *O. bequaerti* was a match for *O. anchineuria* from Goater et al. (2018) and one exemplar of *Ornithomyia* sp. B from Russia (Meißner et al., 2020). The similarity and confusion between *O. anchineuria*, *O. bequaerti*, *O. chloropus* and *O. fringillina* were already apparent to Maa (1969c) and remains today, leading to frequent misidentifications of these species. Specimen quality and preservation can also add to the challenge by obscuring the visibility of certain distinctive characters, e.g. wing microtrichia and side piece of female tergite 6. After communication with the original authors and re-examination of some of the associated exemplar specimens from both Goater et al. (2018) and Meißner et al. (2020), we were able to confirm the identification as *O. bequaerti*. This re-identification results in the first Palaearctic record for *O. bequaerti*, and recognition of an example of Holarctic distribution in this family. Species have seldom been examined across both Nearctic and Palaearctic regions, hence it is not surprising to find two species in this study with an unknown Holarctic distribution. This is increasingly common with the use of DNA barcoding assisting with these discoveries (Landry et al., 2013; Pentinsaari et al., 2019), like here with *O. bequaerti*. Unfortunately, the two specimens from Petersen et al. (2007) included on the tree (Fig. 4) could not be re-examined to confirm their identification.

We identified four species of louse flies on raptors in southern Ontario with a prevalence of 12.5%. The specimens sequenced facilitated the curation of some GenBank and public records, enhancing the BOLD ID Engine. This study has also contributed new BINs to the

Canadian louse fly fauna, which was deemed in need of additional work by Savage et al. (2019). We also found that host species and month were correlated with the presence of louse flies. As seen in previous studies, host species appears to play a large role with louse flies, e.g. Sychra et al. (2020). Seasonality is important, from multi-month periods (Lehikoinen et al., 2021) and even across the short months of fall migration as seen here. However, most variables tested here were not significant, leading us to believe that more factors are likely at play regarding the presence of louse flies. More parameters would need to be measured to improve the model and to have a more complete picture of what is influencing the presence of louse flies. Further study of louse flies in Canada is needed to better understand their prevalence across different bird groups and distribution range.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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