The Canadian Entomologist (2020), **152**, 342–354
doi:10.4039/tce.2020.26

Published on behalf of the Entomological Society of Canada

ARTICLE

Diptera communities of raptor (Aves) nests in Nova Scotia, Canada

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(Received 3 December 2019; accepted 9 March 2020; first published online 27 April 2020)

Abstract
The identity, richness, and abundance of true flies (Diptera) from the nests of three cavity-nesting raptors (Aves) were investigated in northern Nova Scotia, Canada. After fledging, flies were extracted from the nest material using Berlese funnels within an emergence chamber. Thirty-one species/morphospecies from 14 families were collected, including eight new records for Nova Scotia and two new records for eastern North America.

Introduction

Bird nests are micro-ecosystems with diverse communities of invertebrates, from ectoparasites to commensal species. Most studies of the arthropods in bird nests have focussed on the presence and impact of ectoparasites (Møller *et al.* 1990; Loye and Zuk 1991; Krištofík *et al.* 2001, 2002, 2003, 2007; Fairn *et al.* 2014), including fleas (Siphonaptera) (Phipps and Bennett 1974); mites (Acari) (Wasylik 1971); and nest-associated Diptera in the families Muscidae (Lowenberg-Neto 2008), Calliphoridae (Bennett and Whitworth 1991; Whitworth and Bennett 1992), and Carnidae (Cannings 1986a, 1986b; Dawson and Bortolotti 1997). However, nests also support diverse communities of commensal arthropods, including mites, flies, moths (Lepidoptera), beetles (Coleoptera), and various other Insecta. The first significant publications documenting the general fauna associated with bird nests were by Hicks (1959, 1962, 1971), who started building a checklist of arthropod species occurrence and their associated bird species. Since then, several studies have documented the Arthropoda communities associated with passerine (Passeriformes) birds, but relatively few have treated the communities associated with birds of prey. Some of the raptors for which associated insect communities have been documented include Eurasian griffon vulture (*Gyps fulvus* (Hablitz); Aves: Accipitridae) (Dražina and Špoljar 2009), boreal owl (*Aegolius funereus* (Bonaparte); Aves: Strigidae) (Krištofík *et al.* 2003; Majka *et al.* 2006), lesser spotted eagle (*Aquila pomarina* Brehm; Aves: Accipitridae) (Krištofík *et al.* 2009), American kestrel (*Falco sparverius* Linnaeus; Aves: Falconidae) (Philips and Dindal 1990; Neubig and Smallwood 1999), northern saw-whet owl (*Aegolius acadicus* (Gmelin); Aves: Strigidae) (Philips *et al.* 1983; Majka *et al.* 2006), red-footed falcon (*Falco vespertinus* Linnaeus; Aves: Falconidae) (Soltész *et al.* 2018), saker falcon (*Falco cherrug* Gray; Aves: Falconidae) (Merkl *et al.* 2004), and screech owl (*Megascops asio* (Linnaeus); Aves: Strigidae) (Philips and Dindal 1990).

Subject editor: Rayda Krell

Fig. 1. **A**, Inside the nest box with young chicks, feathers, guano, and prey; **B**, emergence trap with Berlese funnel inside and lamp on the outside.

Here, we document the Diptera species found associated with three species of raptors occurring in Nova Scotia, Canada: American kestrel (*Falco sparverius*), boreal owl (*Aegolius funereus*), and northern saw-whet owl (*Aegolius acadicus*). This follows the confirmation that the boreal owl now has a breeding population in Nova Scotia (Lauff 2009), where it is now known to be sympatric with the northern saw-whet owl. This could lead to competition for available nesting area and resources, so it is important to fully understand the nesting environment. Majka *et al.* (2006) did preliminary work on the Coleoptera communities associated with raptor nests in an earlier stage of this study, while the work reported here considers the Diptera in a larger number of nests. Diptera are diverse and abundant in nests (Rotheray 1991; Iwasa *et al.* 1995; Gatt 2001) and include a large number of commensal species. It is not currently known whether these nest-associated fly communities differ between the nest boxes of different raptor species.

Materials and methods

Following the discovery of boreal owls breeding in Nova Scotia in 2004, nest boxes were installed in habitat suitable for the species to provide additional nesting sites for breeding pairs (Lauff 2009). The nest boxes followed the design of Korpimaki (1985) and were built from rough-cut pine lumber. Wood shavings were placed in the box to a depth of approximately 5 cm. Nest boxes were mounted 3–4 m high on living trees, with the entrance hole typically facing between south and east.

Active nests were periodically visited to verify if the nest was still active, to ascertain when nestlings (Fig. 1A) would be ready to leave the nest and to record some other details including clutch size, abundance, and type of stored prey. Once the young owls had fledged, the nest material was transferred into wooden or plastic totes for transport to the laboratory where it was then placed into Berlese funnels inside emergence chambers; a 60-W incandescent bulb, approximately 50 cm from the funnel and set outside the chamber, was used to drive the funnel (Fig. 1B). The funnels collected arthropods crawling downwards, while insects flying upwards were retained in the emergence traps; collections ceased when a week went by with no further captures. The insects extracted were preserved in 70% isopropyl alcohol except for the specimens from two nests collected in 2018, which were placed in 95% ethanol to allow better DNA extraction. All adult dipterans were dried using a critical-point-dryer prior to mounting on paper points.

In total, 28 nests were collected: two American kestrel, two boreal owl, and 24 northern saw-whet owl, all located in northern Cape Breton Island and the northern mainland of Nova Scotia, Canada (Fig. 2). The fly communities associated with each raptor species were assessed for

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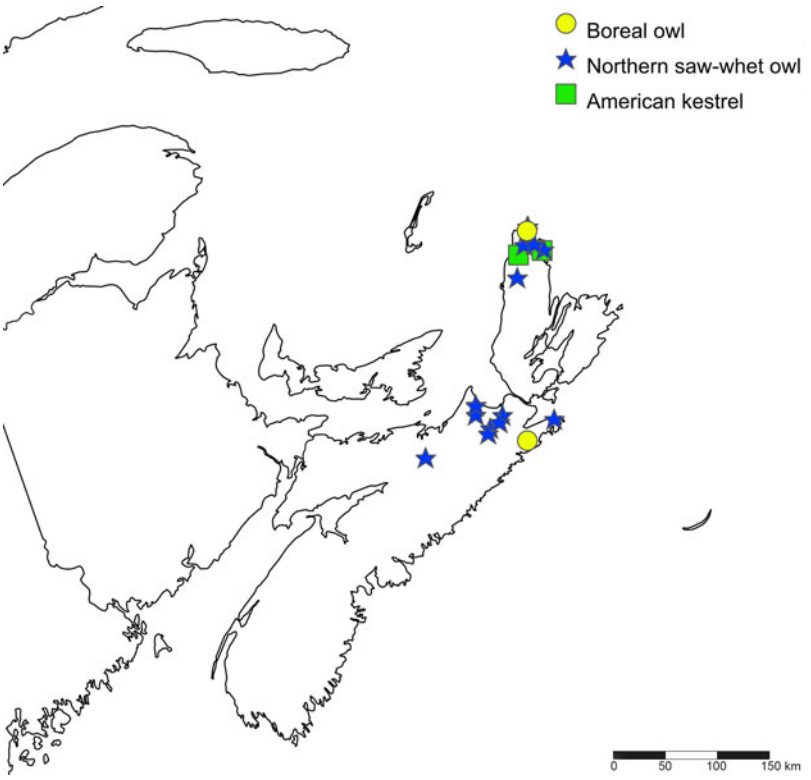

Fig. 2. Location of the 28 nests in Nova Scotia for each bird species.

richness, abundance, and trophic guild. Dominance was calculated as the percentage abundance of each species in comparison to the total number of specimens collected across all nests, regardless of the bird species. Constancy of occurrence was given as the number of nests where the species occurred compared to the total number of nests collected. These measures provided some indication of whether the presence of certain species was expected in the nests or accidental. Measures of richness and abundance of flies were compared to the number of fledglings per nests to see if there was any correlation.

A microplate of 95 specimens, including all apparent morphospecies, was sequenced for cytochrome c oxidase I (COI) prior to detailed examination of the material by specialists. This yielded sequences corresponding to most of the species later recognised by Diptera taxonomists involved with the project. All the sequences were uploaded to the Barcode of Life Database System (BOLD: http://boldsystems.org) (Ratnasingham and Hebert 2007). Most species met the requirements to be successfully assigned to a barcode index number (BIN), which is considered a good proxy for species for most groups (Ratnasingham and Hebert 2013). A maximum likelihood tree was generated with Mega X using general time reversible model with gamma distributed with invariant sites to show the molecular diversity of the nests. For the species not represented among the successful sequences, a public sequence was included for representations when available (except for *Ornithomyia bequaerti* Maa (Diptera: Hippoboscidae) and unidentified morphospecies, *e.g.*, Chironomidae (Diptera) species). All of these are released publicly through BOLD (https://doi.org/10.5883/DS-OWLNS) and GenBank (www.ncbi.nlm.nih.gov/genbank/). All vouchers are split among these institutions: Centre for Biodiversity Genomics (Guelph, Ontario, Canada), Canadian National Collection of Insects, Arachnids, and Nematodes (Ottawa, Ontario, Canada), University of Guelph Insect Collection (Guelph, Ontario, Canada),

https://doi.org/10.4039/tce.2020.26 Published online by Cambridge University Press

Nova Scotia Museum (Halifax, Nova Scotia, Canada), Department of Lands and Forestry, Nova Scotia (Halifax, Nova Scotia, Canada), and St. Francis Xavier University (Antigonish, Nova Scotia, Canada).

Results

Richness

A total of 2830 specimens were extracted from the nests, representing 31 species/morphospecies (excluding the specimens that could not be identified due to specimen quality) from 14 families of flies (Table 1). Most of the abundance and richness were from northern saw-whet owl samples. There was no unique species found in boreal owl nests, but two species were only collected from American kestrel nests (*Ornithomyia bequaerti* and *Phytomyptera* Róndani (Diptera: Tachinidae) species). The number of nests sampled and the number of flies collected varied greatly between years (Fig. 3). The number of fledglings was not strongly correlated with either the abundance of flies (Fig. 4A) or the species richness (Fig. 4B) of flies that emerged from the nest.

Only one known bird ectoparasite, *Ornithomyia bequaerti*, was collected. This single specimen probably fell off the bird into the nest, as adult hippoboscids are usually collected by physically inspecting birds or bats (Mammalia: Chiroptera). One black fly (*Prosimulium* Roubaud; Diptera: Simuliidae) was collected, but members of this genus normally do not feed on birds (Adler *et al.* 2004); the fly was therefore likely in the surrounding area to feed on humans servicing the nest. Most of the flies collected during this study were saprophagous or coprophagous (Table 1), which is not surprising due to the presence of wood shavings, feathers, guano, regurgitated pellets, and others detritus upon which to feed (Fig. 1A).

The dominance and constancy of occurrence did vary according to species and family of flies, with some groups abundant and predictably encountered in bird nests, while others are occasional or accidental visitors (Table 1). Accidental species might have been attracted by the proximity of resources found in the nest or by the other insects already present. For that reason, taxa rarely reported from nests in the literature or those represented only by singletons and doubletons (*i.e.*, species with a dominance < 0.1) are here considered as accidental or incidental nest inhabitants: *Calliphora livida* Hall (Diptera: Calliphoridae) (Hicks 1959), Chironomidae, *Gymnochiromyia concolor* (Malloch) (Diptera: Chyromyidae) (McAlpine *et al.* 1987), *Conicera tibialis* Schmitz (Diptera: Phoridae) (Disney 1994), Hippoboscidae, *Psychoda cinerea* Banks (Diptera: Psychodidae) (Hicks 1959), *Scatopsciara* Edwards (Diptera, Sciaridae) (Hicks 1959, 1962), and *Phytomyptera* (parasitoid of Lepidoptera: Tortricidae (Arnaud 1978)). The more common flies reared from the nests, as expected based on Hicks (1959, 1962, 1971) and other literature, belonged to the families Fanniidae, Heleomzyidae, Milichiidae, Muscidae, and Sphaeroceridae. Those dominant families also included a few species represented only by singletons and doubletons.

Family-level summaries

Fanniidae and Muscidae. Of the three species of Fanniidae, the widespread species *Fannia manicata* (Meigen) has the longest history of documentation from nests of birds and is also found in nests of hornets and wasps (Hymenoptera: Vespidae) (Hicks 1959, 1971; Chillcott 1960; Iwasa *et al.* 1995; Rozkošný *et al.* 1997; Adamska *et al.* 2018; Soltész *et al.* 2018). *Fannia leucosticta* (Meigen) has also been collected from bird nests before and reared from mink (*Mustela* Linnaeus; Mammalia: Mustilidae) and pig (*Sus scrofa domesticus* Erxleben; Mammalia: Suidae) excrement (Chillcott 1960; Hicks 1971). *Fannia subpubescens* Collin was collected in 15 of the 28 nests although there are no previously published records of this species from either bird nests

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Table 1. Diversity of Diptera species per bird species with their trophic guild, dominance across the number of specimens and constancy of occurrence across all 28 nests.

Species	American kestrel	Boreal owl	Northern saw-whet owl	Trophic guild	Dominance (%)	Constancy (number)
Calliphoridae						
Calliphora livida Hall			2	Saprophagous	0.07	1
Chironomidae						
Unidentified species			1	–	0.04	1
Chyromyidae						
Gymnochiromyia concolor (Malloch)*			2	–	0.07	2
Fanniidae						
Fannia leucosticta (Meigen)*			1	Necrophagous/ coprophagous	0.04	1
Fannia manicata (Meigen)		29	35	Saprophagous/ coprophagous	2.26	12
Fannia subpubescens Collin†		96	94	–	6.71	15
Heleomyzidae						
Heleomyza brachypterna (Loew)			12	Saprophagous/ coprophagous	0.42	1
Heteromyza oculata Fallen		18	109	Saprophagous	4.49	9
Neossos atlanticus Gilbert and Wheeler*			1	Saprophagous	0.04	1
Neossos marylandicus Malloch*			63	Saprophagous	2.23	3
Near *Neossos californicus* species 1			12	Saprophagous	0.42	1
Near *Neossos californicus* species 2			1	Saprophagous	0.04	1
Near *Neossos californicus* species 3			2	Saprophagous	0.07	1
Neossos species 4			102	Saprophagous	3.60	7
Neossos species 5	1		106	Saprophagous	3.78	9
Neossos species 6			129	Saprophagous	4.56	7
Undetermined *Neossos* species		1	13	Saprophagous	0.49	7
Hippoboscidae						
Ornithomyia bequaerti Maa*	1			Hematophagous	0.04	1
Milichiidae						
Leptometopa halteralis (Coquillett)			3	Saprophagous	0.11	2
Leptometopa latipes (Meigen)	7		1568	Saprophagous/ coprophagous	55.65	15

(*Continued*)

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Table 1. (*Continued*)

Species	American kestrel	Boreal owl	Northern saw-whet owl	Trophic guild	Dominance (%)	Constancy (number)
Undetermined *Leptometopa* species			42	–	1.48	7
Muscidae						
Hydrotaea basdeni Collin*	2		84	–	3.04	8
Potamia littoralis Robineau-Desvoidy*			135	Saprophagous/ predaceous/ coprophagous	4.77	7
Phoridae						
Conicera tibialis Schmitz			2	Necrophagous/ coprophagous	0.07	1
Psychodidae						
Psychoda cinerea Banks			1	Saprophagous	0.04	1
Sciaridae						
Scatopsciara species			1	Mycetophagous	0.04	1
Simuliidae						
Probably *Prosimulium mixtum* Syme and Davies complex			1	Hematophagous	0.04	1
Sphaeroceridae						
Minilimosina parva (Malloch)			3	Polysaprophagous	0.11	1
Minilimosina parvula (Stenhammar)			73	Polysaprophagous	2.58	3
Minilimosina zeda Marshall†			32	–	1.13	1
Telomerina flavipes (Meigen)			12	Polysaprophagous	0.42	3
Telomerina Roháček species			2	–	0.07	1
Tachinidae						
Phytomyptera species	4			Parasitoid	0.14	1
Undetermined						
Undetermined		7	20	–	0.95	9

*First Nova Scotia record.
†First record from eastern North America.

or eastern North America at all. Many previous studies of bird nests identified *Fannia* Robineau-Desvoidy specimens only to genus, so it is possible that the species was overlooked. Most previous records are from Europe or from northwestern North America (Chillcott 1960). Both species of Muscidae reported here, *Hydrotaea basdeni* Collin (Huckett 1954; Hicks 1959, 1971) and *Potamia littoralis* Robineau-Desvoidy (Iwasa *et al.* 1995; Soltész *et al.* 2018), are known to be common in bird nests but neither had previously been recorded from Nova Scotia.

Heleomyzidae. Heleomyzidae, including *Heleomyza brachypterna* (Loew) (Hicks 1959, 1971; Garnett and Foote 1966) and *Heteromyza oculata* Fallen, are known from bird nests as well as

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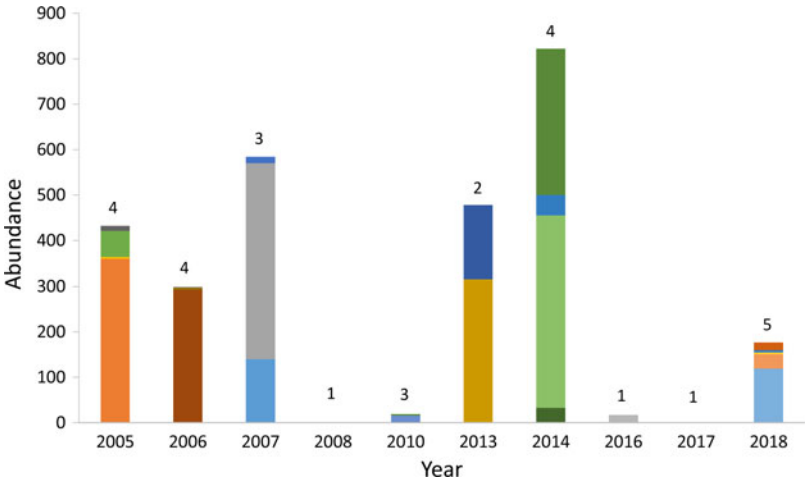

Fig. 3. Yearly fly abundance per nest collected each year, with each nest represented by a unique colour. The number atop each column represents the total number of nests collected that year.

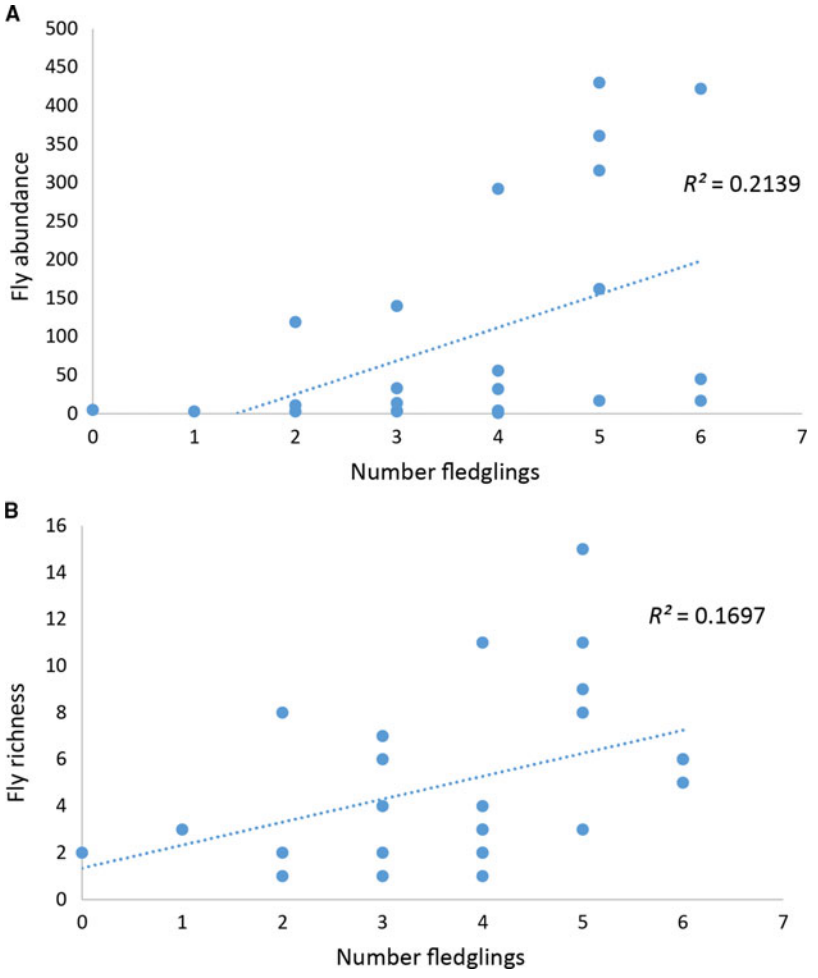

Fig. 4. Correlation to the number of fledglings. **A**, fly abundance; **B**, fly richness.

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burrows of small Mammalia (Gill 1962). *Neossos* Malloch, the most abundant heleomyzid genus in this study, is an apparent nest specialist and is rarely collected outside of bird nests (Gilbert and Wheeler 2007; Solecki and Wheeler 2015). The large number of *Neossos* specimens collected in this study contrasts with other studies of raptor nests mentioned in Gilbert and Wheeler (2007). Some of the specimens collected in the current study have external morphological characteristics of *N. atlanticus* Gilbert and Wheeler and *N. marylandicus* Malloch, while others seem to fall into morphospecies with genitalia structurally similar to the European *N. broersei* (de Meijere). As discussed below, however, sequence data reflect only two species.

Milichiidae. *Leptometopa latipes* (Coquillett) appears to be by far the most common and abundant fly species found in bird nests (Hicks 1959, 1962; Brake 2000; Soltész *et al.* 2018), but molecular data suggest that the *L. latipes* collected in this study is actually two species. These two similar species (one undescribed) together account for over 55% of all flies extracted in this study and were identified from more than half the nests collected. Another *Leptometopa* Becker species, *L. halteralis*, is recorded here from bird nests for the first time. There are other records of "*Leptometopa* sp." from bird nest studies, but no published records explicitly identifying *L. halteralis* from nests (de Mello *et al.* 2007). Other *Leptometopa* species are known from a wide range of habitats such as dung, latrines, bat guano, bird nests, flowers, and Fungi (Brake 2000, de Mello *et al.* 2007), but there is little information about habitat specificity of individual species.

Sphaeroceridae. Although several species of Sphaeroceridae are routinely collected from bird nests, the species involved are usually polysaprophagous and not specifically associated with birds. *Minilimosina parva* (Malloch) and *M. parvula* (Stenhammar), for example, were both abundant in our samples, but both species are widespread and occur in a wide range of habitats. *Minilimosina parva* is usually found on carrion and *M. parvula* is more common on fungi (Marshall 1985). *Minilimosina zeda* Marshall was also abundant in raptor nests in this study but was previously known only from a few specimens associated with dry carrion or carrion traps in northwestern North America (Alberta, Canada to Alaska, United States of America) (Marshall 1985). *Telomerina flavipes* (Meigen), like *M. parva*, is a widespread species most often associated with carrion (Marshall and Roháček 1984). It has been collected from the nests of red-footed falcons (Soltész *et al.* 2018) and in burrows of rabbits, along with *M. parvula* (Roháček 2019). It has also been collected in caves, on fungi, on dung, and various other mammal burrows (Marshall and Roháček 1984).

Molecular

The maximum likelihood tree (Fig. 5) shows the species richness found in these nests. There was a near perfect correspondence of one unique BIN for each species except for the genus *Neossos* (Heleomyzidae) and the species *Leptometopa latipes* (Milichiidae). *Neossos* grouped in two major clusters despite morphological evidence suggesting multiple species. Each cluster on the tree corresponds to a different surstylus shape. The first grouping, including *Neossos atlanticus* and *N. marylandicus*, is characterised by a surstylus shaped like a lobster claw, while the second grouping includes several apparent morphospecies characterised by a paddle-shaped surstylus similar to that of the European species, *N. broersei* (de Meijere). GenBank records from *N. broersei* demonstrate that the species is distinct from those in this study and would create a third cluster. The molecular data thus suggest that *Neossos* includes only two species in our study area (the Nearctic *N. marylandicus* and a new species), instead of the multiple species suggested by external morphology. A thorough reconsideration of the species taxonomy of the genus is necessary to determine whether this disparity between molecular and morphological evidence reflects a failure

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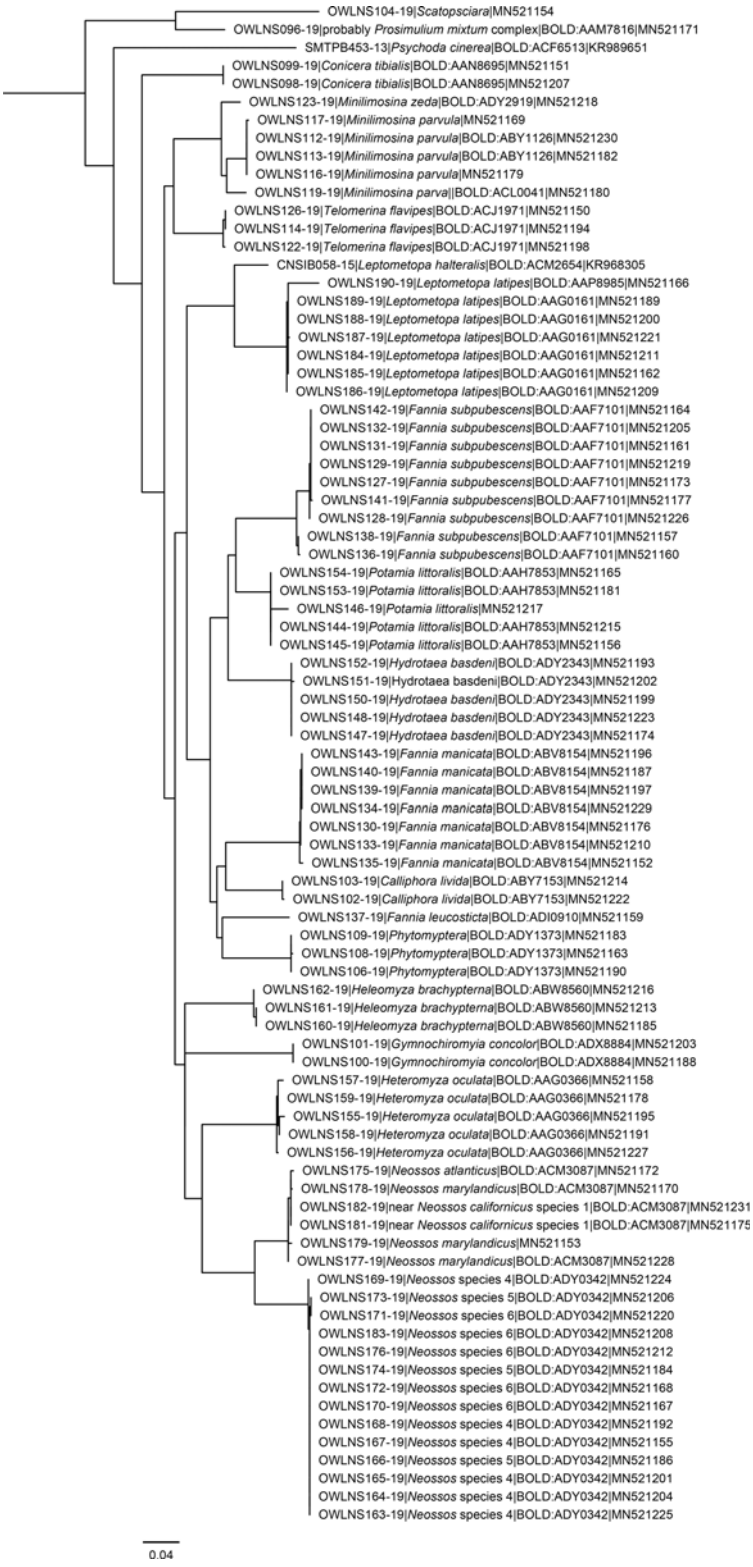

Fig. 5. Maximum likelihood tree of the dipteran richness collected from the 28 nests (BOLD process ID|species|BIN|GenBank accession).

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of COI sequences to distinguish some of the species, or if it results from high infraspecific morphological variation and a resultant oversplitting of the genus. Sequence data for *Leptometopa*, in contrast to that for *Neossos*, suggest that the species diversity is higher than previously recognised. *Leptometopa latipes* divides into two molecular clusters, suggesting that there is an undescribed species superficially similar to *L. latipes*. The distinction between the two clusters is supported by their placement into distinctive BINs with small within BIN variation and a divergence of more than 2.63%. Additional records available through the BOLD public data portal give more support to each BIN. Examination of male genitalia of specimens in each COI cluster confirms that they are different and that *L. latipes* seems to consist of two species, one of which is undescribed. *Leptometopa* is in clear need of revision to determine the definitions, diagnoses, and relationships of these species.

Discussion

Since only two boreal owl nests were studied, in contrast to the many northern saw-whet owl nests, there are insufficient data for a quantitative comparison of fly communities associated with the nests of these two species. However, there does not seem to be any obvious difference.

Most of the flies collected were saprophagous species associated with carrion, fungi, or dung. The carrion-associated species would be expected to show increased numbers in response to the number of fledglings in the nest, which might be correlated with the presence of shed skin, feathers, carrion debris, or even dead fledglings. There was, however, no strong correlation between the number of fledglings and either the abundance or richness of flies. Iwasa *et al.* (1995) suggested that saprophagous and coprophagous species are likely to be affected by the faeces present in the nest, the building materials and habits of the birds, and the habitat where the nest was located. This did not appear to be the case here despite the accumulation of faeces due to the parents not cleaning the nest.

Parasitic species in the families Calliphoridae and Carnidae associated with nesting birds were not collected during this study. A third parasitic family, the Hippoboscidae, was represented only by a single (probably incidental) specimen of *Ornithomyia bequaerti*. *Carnus hemapterus* Nitzsch (Diptera: Carnidae) has been collected from American kestrels and northern saw-whet owl by other workers (Cannings 1986b; Dawson and Bortolotti 1997), and it is commonly encountered in raptor and hole-nesting species across North America (Capelle and Whitworth 1973; Cannings 1986a) but was not recorded in this study. Bird blow flies in the genus *Protocalliphora* Hough (Diptera: Calliphoridae) were also apparently absent from our samples, even though they are commonly associated with cavity-nesting birds and they have previously been collected from different raptor species (Sabrosky *et al.* 1989; Bennett and Whitworth 1992). It is possible that emptying the nest boxes after the owls left diminished ectoparasite populations, as it has been shown to occur in other studies (Mappes *et al.* 1994; Rendell and Verbeek 1996; Dawson and Bortolotti 1997). However, it is also possible that they had already emerged and left the nest as the larvae follow closely the life cycle of the nestlings that they feed upon (Sabrosky *et al.* 1989; Bennett and Whitworth 1991). It would have been interesting to comb through the nest materials to see if any *Protocalliphora* puparia were present and how many nests were infested.

Because of the limited number of boreal owl nests collected, it was not possible to conclude that any fly species was more associated with one raptor species than another. However, the dipteran communities associated with the nests of these raptor species were extensively documented for the first time.

Acknowledgements. The authors thank Sean Prosser for his assistance with the sequencing and Brian Brown (Phoridae), Monty Wood (Simuliidae), and Jim O'Hara (Tachinidae) for assisting in identifications. Sequence analysis was enabled by awards to Paul Hebert from the Canada Foundation for Innovation and from Ann and Chris Evans. The authors also thank Greg

Giffen, Richard Murphy, and Stephen Bushell for access to their nest boxes. The authors also thank many volunteers notably Fritz McEvoy and David Rasmussen for help in the field. This work was supported by grants from Bird Studies Canada, the Nova Scotia Museum of Natural History, the Nova Scotia Bird Society, and the Nova Scotia Department of Natural Resources (Nova Scotia Habitat Conservation Fund – Contributions from Hunters and Trappers).

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Cite this article: Levesque-Beaudin, V., Sinclair, B.J., Marshall, S.A., and Lauff, R.F. 2020. Diptera communities of raptor (Aves) nests in Nova Scotia, Canada. The Canadian Entomologist, **152:** 342–354. https://doi.org/10.4039/tce.2020.26.