Diversity and prevalence of ornithophilic louse flies (Diptera: Hippoboscidae: Ornithomyinae) in Serbia

Tibor REKECKI¹, Draženko Z. RAJKOVIĆ²*
¹Bird Protection and Study Society of Serbia, Novi Sad, Serbia
²Center for Biodiversity Research, Novi Sad, Serbia

Abstract: Ectoparasites are diverse organisms that exploit animal hosts using various strategies. One such group represents the louse flies (Hippoboscidae) from the subfamily Ornithomyinae, which are permanent, highly specialised, hematophagous ectoparasites of poultry and wild birds found worldwide. The main objective of this research is to examine the mean abundance, prevalence, and diversity of ornithophilic louse flies in Serbia. They have been detected through visual inspection or fumigation of caught birds. In total, 79 louse flies belonging to four genera and six species were collected from 62 individuals within 26 host species. A single louse fly infested the bulk of captured birds, while hippoboscid females were statistically dominant within the overall sample. Most detected louse flies belonged to the genus Ornithomya, with Ornithomya avicularia and Ornithomya fringillina being the most abundant at the species level. The prevalence was relatively low, along with the mean intensity and abundance. This study contributes to a better understanding of louse flies and their parasite-host associations in Serbia. It also fills a gap in the knowledge of their diversity and distribution in South-eastern Europe.

Key words: Aves, ectoparasites, hippoboscid fly, infestation, parasitology

1. Introduction
The louse flies or keds (Diptera: Hippoboscidae) are elusive, obligate, blood-sucking ectoparasites that infest birds and mammals (Lloyd, 2002; Reeves and Lloyd, 2019). However, certain species have also occasionally attacked humans (Lloyd, 2002; Mehlhorn and Piekarski, 2002). The louse flies occur worldwide, with the richest fauna reported in the Old-World subtropics and tropics (Hutson, 1984). The family Hippoboscidae is divided into three subfamilies, Ornithomyinae, Hippoboscinae, and Lipopteniinae, with 213 species placed in 21 genera, representing a relatively small family of the order Diptera (Dick, 2018).

Altogether, 51 species of louse flies occur in the Palearctic region (Meißner et al., 2020), while 31 species have been recorded in Europe so far (Pape et al., 2015; Nartshuk et al., 2019). The family includes winged species that can fly, as well as those with vestigial or no wings that are flightless. Some have an extensive host range, while others are typically restricted to a few or even one host species (monoxenous). The louse flies exhibit an interesting form of reproduction called adenotrophic viviparity. Briefly, the larva completes its development in the female uterus, where milk gland secretions feed it. After fully developing, the larva is ejected and pupates in the substrate (Lloyd, 2002; Doszhanov, 2003; Reeves and Lloyd, 2019). Among Hippoboscidae, the subfamily Ornithomyinae represents the largest louse fly group with 16 genera and 171 distinct species (Dick, 2018; Reeves and Lloyd, 2019).

Members of the subfamily Ornithomyinae, also known as bird, feather, or flat flies, are exclusive bird ectoparasites (Lloyd, 2002). Species from this subfamily are winged, usually brown in colour, dorsoventrally flattened with a depressed head, a relatively tough exoskeleton and lengthy, thin legs equipped with claw-like structures well adapted for moving through the bird’s plumage (Lloyd, 2002). Some species serve as vectors of various pathogenic agents (Baker, 1967; Santiago-Alarcon et al., 2012; Bezerra-Santos and Otranto, 2020; Santolíková et al., 2022) as well as disseminators of mites and lice (Hill et al., 1967; Gaud and Atyeo, 1996; Harbison et al., 2008). However, the impact of avian louse flies on their hosts is often negligible. Still, irregularly, a high mortality rate has been reported in nestlings, weak and highly infested individuals (Oosthuiizen and Markus, 1972; Lucius et al., 2018). The European continent is home to 21 species of the subfamily Ornithomyinae (Pape et al., 2015; Nartshuk et al., 2019).
The monophyletic subfamily Ornithomyinae represents relatively poorly studied arthropods, especially outside developed countries. Until now, most publications were related to the taxonomy, host relationship, infestation rate, and reproduction (Hutson, 1984; Dick, 2018; Reeves and Lloyd, 2019). Unfortunately, our knowledge of the fundamental ecological parameters, such as spatial and altitudinal distribution, species diversity, prevalence, and host relations of avian louse flies in the Central Balkan Peninsula (e.g., Serbia) is almost completely lacking. There are only three historical records of two louse fly species that parasite birds in the territory of Serbia. *Ornithoica turdi* (Olivier in Latreille, 1812), along with *Ornithomya avicularia* (Linnaeus, 1758), was recorded in 1911 in the Southeastern Banat, Northern Serbia (Soós, 1955), while in the early 1930s, *Ornithomya avicularia* was accidentally found close to Majdanpek, Eastern Serbia (Živojinović, 1950).

Therefore, the primary aim of this article is to summarise recent records of louse flies associated with birds and provide an initial checklist for Serbia. Additionally, we provide the prevalence rate, mean abundance, and intensity. Although faunistic incomplete, the presented research, and its achievements may serve as a valuable reference and a useful starting point for further, more comprehensive studies of louse fly species in Serbia and adjacent countries.

### 2. Materials and methods

The louse fly specimens were collected during extensive fieldwork, particularly bird ringing, between 2019 and 2022, except for one fly specimen that was accidentally collected in 2008. In search of louse flies, we visited 49 randomly chosen locations in Serbia. At least one individual louse fly was recorded in a total of 27 different locations (see Table). We aimed to cover almost all the main habitat types found in Serbia; thus, we caught wild birds in densely populated areas, wetlands, different forest ecosystems, mosaics, and open terrains. Wild birds were captured randomly with standard ornithological mist nets, mainly during the fall migration (July–October), as flies reached a population peak during this period (Hutson, 1984; Doszhanov, 2003). In addition to mist-netting, a small portion of nestlings was randomly inspected directly on the nest cup. In total, we carefully examined 1442 individuals among 62 distinct bird species. Therefore, records of louse flies are based on accidental and targeted catches of wild birds for scientific purposes (e.g., bird ringing). We detected the presence of louse flies through visual inspection of different body parts (Sorjonen, 1971; Bartos et al., 2020) or with the help of a fumigation chamber method using small cotton balls soaked in ethyl-acetate as a fumigant (Visnak and Dumbacher, 1999; Clayton and Drown, 2001). Almost all discovered louse flies were picked off via forceps or fingertips, stored in 70% ethyl alcohol-filled plastic microvials, and preserved at room temperature for further analyses (e.g., identification). Each microvial is adequately labelled and possesses the following information: date, exact location (toponym) and the host species. In addition to the stored louse flies, we also included the individuals who escaped during the birds’ handling or mist net extraction for prevalence computation. We calculated the prevalence using the following formula: IB / EB × 100, where IB = the number of infested birds and EB = the number of examined birds (Bush et al., 1997). In addition to the prevalence, we calculated mean intensity and mean abundance according to Bush et al., (1997). Photographs were obtained with a SONY ILCE-7RM3 camera and digitally processed using free-of-charge Inkscape (version 1.2.2) software. The collected louse flies were identified and sexed based on their morphological features under a BTC STM7b stereoscopic microscope using dichotomous keys by Büttiker, (1994) and Oboña et al., (2022). For each registered species, in the results chapter, we provide the toponym name, host species, date, number of louse flies, and sex, if possible. The taxonomy of louse flies follows Dick, (2018), while hosts are given according to HBW and BirdLife International¹. We display descriptive statistical parameters as mean ± standard error and percentage share (%). Descriptive statistics were computed in the IMB SPSS software version 23 for Windows². All collected specimens of louse flies are currently deposited in the author’s private collection.

### 3. Results

#### 3.1. Diversity and prevalence

During the study period, 79 ornithophilic louse flies belonging to four genera were registered on 62 different host individuals (4.3% of prevalence). Of these, 53 were collected and stored in microvials, while an additional 26 escaped during handling and examining 20 captured bird individuals of nine different species. Louse flies were collected from 26 host species (including humans) belonging to six orders (Accipitriformes, Strigiformes, Caprimulgiformes, Coraciiformes, Passeriformes, and Primates) and 18 families (Accipitridae, Strigidae, Apodidae, Coraciidae, Hirundinidae, Prunellidae, Turdidae, Sylviidae, Acrocephalidae, Locustellidae, Regulidae, Muscicapidae, ¹ HBW, BirdLife International (2022). Handbook of the Birds of the World and BirdLife International digital checklist of the birds of the world, version 7 [online]. Website http://datazone.birdlife.org/userfiles/file/Species/Taxonomy/HBW-BirdLife_Checklist_v7_Dec22.zip [accessed 22 03 2023].

### Table. Toponyms (locations) of successful sampling of at least one specimen of avian louse flies (Hippoboscidae, Ornithomyinae) with general site characteristics.

<table>
<thead>
<tr>
<th>Toponym</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Elevation (m)</th>
<th>Toponym environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vlasina Rid, Vlasina Lake</td>
<td>42.725539</td>
<td>22.326430</td>
<td>1311</td>
<td>Hotels and weekend houses on the lake shore</td>
</tr>
<tr>
<td>Đonovo polje, Golija Mt.</td>
<td>43.327808</td>
<td>20.290639</td>
<td>1723</td>
<td>Upland coniferous forest edge</td>
</tr>
<tr>
<td>Ludaš Lake, Hajdukovo</td>
<td>46.104299</td>
<td>19.830705</td>
<td>93</td>
<td>Lowland reedbed</td>
</tr>
<tr>
<td>Sopotnica, Jadovnik Mt.</td>
<td>43.305685</td>
<td>19.736348</td>
<td>984</td>
<td>Planted coniferous stand with shrubs</td>
</tr>
<tr>
<td>Savin krs, Jadovnik Mt.</td>
<td>43.274607</td>
<td>19.776613</td>
<td>1428</td>
<td>Rocky habitat with juniper bushes</td>
</tr>
<tr>
<td>Livade, Ostrovo, Novi Bečej</td>
<td>45.610345</td>
<td>20.275780</td>
<td>74</td>
<td>Lowland pastures and reedbeds</td>
</tr>
<tr>
<td>Kamara humka, Novi Kneževac</td>
<td>46.082997</td>
<td>20.152846</td>
<td>73</td>
<td>Forest stand surrounded by agricultural land</td>
</tr>
<tr>
<td>Kozja stena, Tara Mt.</td>
<td>43.942637</td>
<td>19.422816</td>
<td>1007</td>
<td>Mature mixed forest</td>
</tr>
<tr>
<td>Mitrovac na Tari, Tara Mt.</td>
<td>43.924484</td>
<td>19.424817</td>
<td>1101</td>
<td>Weekend resort surrounded by meadows and forest</td>
</tr>
<tr>
<td>Gradski park, Novi Kneževac</td>
<td>46.045389</td>
<td>20.090011</td>
<td>86</td>
<td>City park</td>
</tr>
<tr>
<td>Bara Đurica, Utrine, Labudovo okno</td>
<td>44.836318</td>
<td>21.308519</td>
<td>65</td>
<td>Reedbed leaning on pasture</td>
</tr>
<tr>
<td>Bežanijska kosa, Belgrade</td>
<td>44.815335</td>
<td>20.366697</td>
<td>102</td>
<td>Cemetery with ornamental trees and bushes</td>
</tr>
<tr>
<td>Đurđevo brdo, Zvijezda Mt.</td>
<td>43.904639</td>
<td>19.330613</td>
<td>1365</td>
<td>Meadow in the succession stage</td>
</tr>
<tr>
<td>Sokolina, Tara Mt.</td>
<td>43.922308</td>
<td>19.503151</td>
<td>986</td>
<td>Mixed young forest with shrubs</td>
</tr>
<tr>
<td>Strajkov salaš, Zlatica, Mokrin</td>
<td>45.926138</td>
<td>20.282003</td>
<td>74</td>
<td>Gallery forest</td>
</tr>
<tr>
<td>Mali Jadovnik, Jadovnik</td>
<td>43.30397</td>
<td>19.776804</td>
<td>1500</td>
<td>Coniferous forest edge</td>
</tr>
<tr>
<td>Kašanj, Jadovnik</td>
<td>43.327219</td>
<td>19.779341</td>
<td>1268</td>
<td>Coniferous forest edge</td>
</tr>
<tr>
<td>Bećarac, Novi Sad</td>
<td>45.246133</td>
<td>19.856059</td>
<td>77</td>
<td>Gallery forest</td>
</tr>
<tr>
<td>Novi Kneževac</td>
<td>46.03933</td>
<td>20.091511</td>
<td>84</td>
<td>Private yard with orchard and garden</td>
</tr>
<tr>
<td>Gložan</td>
<td>45.275151</td>
<td>19.562293</td>
<td>78</td>
<td>Private yard with orchard and garden</td>
</tr>
<tr>
<td>Bara Jezero, Prvi rejon, Stanišić</td>
<td>45.988066</td>
<td>19.169472</td>
<td>91</td>
<td>Reedbed surrounded by agricultural land</td>
</tr>
<tr>
<td>Blato, Vlasina Lake</td>
<td>42.677997</td>
<td>22.347797</td>
<td>1215</td>
<td>Peat bog</td>
</tr>
<tr>
<td>Veliko blato, Krnjača, Beograd</td>
<td>44.864022</td>
<td>20.502278</td>
<td>72</td>
<td>Fishpond lake with reed on edges</td>
</tr>
<tr>
<td>Veliko Kopovo, Novi Bečej</td>
<td>45.629848</td>
<td>20.199581</td>
<td>72</td>
<td>Reedbed with bushes and young trees</td>
</tr>
<tr>
<td>Ljote, Vlasina Lake</td>
<td>42.712238</td>
<td>22.328675</td>
<td>1227</td>
<td>Wet meadow with scattered bushes</td>
</tr>
<tr>
<td>Rusanda Lake, Melenci</td>
<td>45.527633</td>
<td>20.299403</td>
<td>72</td>
<td>Lake with reed on edges</td>
</tr>
<tr>
<td>Došini, Ljote, Vlasina Lake</td>
<td>42.71188</td>
<td>22.325674</td>
<td>1258</td>
<td>Settlement</td>
</tr>
</tbody>
</table>
Paridae, Panuridae, Laniidae, Fringillidae, Emberizidae, and Hominidae). Some of the recorded louse fly species are shown in Figure. In 34 (80.9%) cases, hosts were infested by solitary louse flies. Two louse fly individuals on a single host were found in six (14.3%) cases, while three (2.4%) or four (2.4%) specimens of hippoboscid were encountered per one bird individual. As a result, frequencies of infestation between single and multiple infested hosts were statistically significant (chi-square test: $\chi^2 = 71.71$, $p < 0.001$). No host specimen was infected by more than one species of louse fly. In the analysed sample, the louse flies’ females were four times more numerous than males (chi-square test: $\chi^2 = 20.55$, $p < 0.001$). Dominance among the four genera of louse flies is ranked as follows: *Ornithomya* (81.1%), *Crataerina* (13.2%), and *Ornithoica* (5.7%). On the species level, *Ornithomya avicularia* was the most representative species (43.4%), followed by *O. fringillina* (32.1%). In comparison, other species each contributed less than 10%. Finally, the mean intensity was $1.27 \pm 0.01$, and the mean abundance was $0.05 \pm 0.01$.

3.2. Catalogue of the registered species

Phylum: Arthropoda von Siebold, 1848  
Class: Insecta Linnaeus, 1758  
Order: Diptera Linnaeus, 1758  
Family: Hippoboscidae Samouelle, 1819  
Subfamily: Ornithomyinae Bigot, 1853  
Genus: *Ornithomya* Latreille, 1802  
Species: *Ornithomya avicularia* (Linneus, 1758)

Figure. Dorsal view of four louse fly species Hippoboscidae, Ornithomyinae recorded in Serbia. A = *Crataerina hirundinis* (♀), B = *Ornithomya avicularia* (♀), C = *Ornithomya fringillina* (♀), D = *Crataerina pallida* (♂). Length of the scale bar = 2 mm.
Donovo polje, Golija Mt.: Aegolius funereus, 23.10.2019, 1♂; Sopotnica, Jadovnik Mt.: Parus major, 10.08.2020, 1♀; Savin krš, Jadovnik Mt.: Chloris chloris, 13.08.2020, 1♀; Livade, Ostrovo, Novi Bečej: Coracias garrulus, 13.06.2021, 1♂; Kamara humka, Novi Kneževac: Aquila heliaca 02.07.2021, 1♀; Kozja stena, Tara Mt.: human, 20.07.2021, 1♂, 1♂; Mitrovac na Tari, Tara Mt.: Turdus merula, 21.07.2021, 1♂; Ludaš Lake, Hajdukovo: Acrocephalus arundinaceus, 10.08.2021, 1♂; Gradski park, Novi Kneževac: Asio otus, 04.09.2021, 1♀; Bara Đurica, Utrine, Labudovo okno: Asio otus, 11.09.2021, 4♂; Bežanijska kosa, Belgrade: Asio otus, 18.09.2021, 1♀; Đurđev brdo, Zvijezda Mt.: Aegolius funereus, 26.09.2021, 2♂; Sokolina, Tara Mt.: Emberiza cia, 28.09.2021, 1♂; Strajkov salaš, Zlatica, Mokrin: Aquila heliaca, 06.07.2022, 1♀; Sopotnica, Jadovnik Mt.: Fringilla coelebs, 19.07.2022, 1♂; Sopotnica, Jadovnik Mt.: Turdus merula, 18.08.2022, 1♂; Mali Jadovnik, Jadovnik Mt.: Aegolius funereus, 08.10.2022, 1♀; Kašanj, Jadovnik Mt.: Prunella modularis, 10.09.2022, 1♀.

Species: Ornithomya fringillina Curtis, 1836

Species: Ornithomya rupes Hutson, 1981
Genus: Ornithoica Rondani, 1878
Species: Ornithoica turdi (Olivier in Latreille, 1811)
Ludaš Lake, Hajdukovo: Locustella luscinoides, 13.08.2021, 3♀.
Genus: Crataeirina von Olfers, 1816
Species: Crataeirina hirundinis (Linnaeus, 1758)
Mitrovac na Tari, Tara Mt.: Delichon urbicum, 22.07.2021, 1♂, 1♀; Vlasina Rid, Vlasina Lake: Delichon urbicum, 03.06.2022, 1♀; Vlasina Rid, Vlasina Lake: Delichon urbicum, 22.07.2021, 1♂.
Species: Crataeirina pallida (Olivier in Latreille, 1811)
Vlasina Rid, Vlasina Lake: Apus pallidus, 02.06.2022, 2♂; Vlasina Rid, Vlasina Lake: Apus pallidus, 26.07.2022, 1♀.

4. Discussion
Our research provides the first extensive study on ectoparasites’ diversity, distribution, and prevalence in wild birds in Serbia. From a faunistic perspective, the study reveals the discovery of four new louse fly species in Serbia: Ornithomya fringillina, O. rupes, Crataeirina hirundinis, and C. pallida. Moreover, we confirmed the earlier accidental findings of Ornithomya avicularia and Ornithoica turdi dating back to the early XX century. Overall, less than a third of the European fauna of ornithophilic louse flies were recorded in Serbia. Nevertheless, compared to neighbouring countries, the richness of louse fly species from the subfamily Ornithomyinae presented in this article is slightly lower. For instance, eight distinct species are recorded in Bulgaria and Hungary (Beran, 1972; Popov, 1995; Papp, 2001) and nine in Croatia (Trilar and Krčmar, 2005). These minor differences are apparently due to the lack of directed research on specific groups of birds or species that are potential hosts rather than the poorer avifauna in Serbia. For example, louse flies species recorded in neighbouring countries, and related to specific host species or families, like Crataeirina melbae (Rondani, 1879) or Icosta ardeae (Macquart, 1835), are highly likely to exist in Serbia since there are over 11,000 breeding pairs of herons (Ardeidae) and 100 breeding pairs of Tachymarptis melba in the country (Puzović et al., 2015). Otherwise, it should be noted that the high prevalence is typically observed in aggregated and colonial birds (Anderson and May, 1978; Lee and Clayton, 1995); thus, it is only a matter of time and research efforts before these fly species are discovered in Serbia. The T. melba is particularly interesting since, in addition to the increase in abundance, also the range was rapidly expanded to the north, conquering highly populated urban city districts. Therefore, a minimum of twelve pairs are nesting in Belgrade (the capital city), and four pairs occupied Novi Sad (Pannonian plain) in the last few years (Rajković, unpublished data). Such rapid expansion of the host’s spatial distribution can also lead to the possible expansion of the louse flies in other new areas, as demonstrated in some previous studies (Knab, 1916; Reeves and Lloyd, 2019; Mašlankov et al., 2022) and consequently, more accessible sampling by researchers.

Further, our research findings suggest that louse flies were relatively uncommon among wild birds in Serbia during the study period, as evidenced by the captured bird specimens. However, the occurrence and abundance of louse flies can significantly vary depending on various factors, such as the distribution and abundance of the host species, habitat type, period of the year, and climatic conditions (Hutson, 1984; Doszhanov, 2003). Also, the mist nets used to capture birds completely cover their bodies and feathers, making it likely that some louse flies escape unnoticed before they are detected by a researcher. There-
fore, further research with a larger and more diverse sample size is recommended to obtain more accurate data on their prevalence. On the contrary, findings of louse flies on birds from six orders and 18 families indicate a rather broad host range for these parasites in Serbia. The Passeriformes order was the most commonly infested, and the two generalist species from the genus *Ornithomyia* were the most abundant. These findings are consistent with previous studies on louse fly infestation and abundance in birds in other regions of Europe (Trilar and Krčmar, 2005; Oboňa et al., 2019; Bartos et al., 2020; Lehikoinen et al., 2021; Santolíková et al., 2022). Regarding sex ratio, we found a significantly lower proportion of male specimens than females. This significantly skewed ratio is consistent with the results obtained in similar studies (e.g., McClure, 1984; McClure and Krčmar, 2005). This phenomenon is well known and is a consequence of the earlier appearance in the season, shorter life, and greater mobility from host to host of male specimens (Hutson, 1984; McClure, 1984). Only one species of louse fly infested the majority of hosts. However, it is important to note that the mean intensity observed in this study may have been influenced by factors like host age, phenology or sex, and condition in general. Also, the relatively limited number of louse flies collected in this study makes it difficult to draw firm conclusions about interspecific interactions or patterns of host specificity.

In conclusion, our study provides a noteworthy addendum on the abundance, diversity, and distribution of avian louse flies in Serbia and adds to our understanding of the regional diversity and ecology of this group of parasites. The obtained results show that louse flies are present in a rather small percentage of birds in Serbia but have a relatively broad host range and modest species composition. Further studies are needed to explore the factors influencing the prevalence and intensity of louse fly infestations in different avian communities and habitats. Lastly, targeted research of certain host species and families is necessary to obtain an entire picture of the louse fly fauna of Serbia and the central Balkan Peninsula.

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