Incidence of louse flies on birds from the Russian Far East (Diptera: Hippoboscidae) and investigation of the status of some taxa by DNA barcoding

О заражении птиц кровососками (Diptera: Hippoboscidae) на Дальнем Востоке России и использование ДНК-штрих-кодирования для проверки статуса некоторых таксонов

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ABSTRACT. We report seven species of bird louse flies (Hippoboscidae) collected from 33 bird hosts from Muraviovka Park for Sustainable Land Use (Oblast Amur, Russia). Overall, there is a distinct majority of female flies. Our record of *Icosta holoptera* represents the first observation in Russia. DNA barcoding showed *Ornithomya aobatonis* as a valid species (stat. rev., *Ornithomya avicularia aobatonis*). This species is the one most frequently observed in our investigations. Our records represent a westward expansion of the known distribution ranges of all species, which were previously restricted to an isolated area in the easternmost part of Russia and the neighboring border regions. Our records of *Ornithomya chloropus* suggest a gap closure the western distribution area. Five of the louse flies (5.6%) served as phorents for mites (Acari) and their egg deposits.

РЕЗЮМЕ. В ходе нашего исследования в Муравьёвском парке (Амурская область, Россия) были обнаружены семь видов мух-кровососок (Hippoboscidae) на 33 птиц-хозяевах. Наиболее часто встречалась самка *Icosta holoptera*. Нами была осуществлена ДНК-шифровка, что *Ornithomya aobatonis* является самостоятельным видом (stat. rev., *O. avicularia aobatonis*). Этот вид был наиболее обычен в наших материалах. Исследования показали, что для всех видов, граница ареала которых проходила по самому востоку России и приграничным территориям, отмечена более западные находки. Для *Ornithomya chloropus* наши результаты предполагают отсутствие разрыва между восточной и западной популяциями. Пять из отловленных кровососов (5.6%), оказались переносчиками клещей (Acari) и их яиц.

Introduction

Louse flies are obligate bloodsucking parasites of mammals and birds and have occasionally been known to attack humans [Mehlhorn, Piekarski, 2002; Lucius et al., 2018]. Because of the varied degree of host specificity within these insects, a distinction is made between monoxene, oligoxene, and polyxene species [Büttiker, 1994]. Usually, the effect of louse flies on their hosts is insignificant, but an increased mortality rate has occasionally been observed in juvenile birds [Sellenschlo, 1984; Lucius et al., 2018].

Some species with hosts that live in colonies (e.g. *Stenepteryx hirundinis* on *Delichon urbicum*) or in larger flocks (e.g. *Melophagus ovinus* on *Ovis gmelini*).

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Sustainable Land Use (49°55′N, 127°40′E, Fig. 1) is Makarchenko et al., 2015. Grebennikov et al., 2017; Kirichenko et al., 2019, by only a very limited number of pioneering studies East (as many parts of Northern Asia) has been targeted through DNA barcoding database is a prerequisite for from Eastern Asia or from any part of Russia. A thorough DNA barcoding database is a prerequisite for large-scale biodiversity assessments and metabarcoding studies [Taberlet, 2012], but so far, the Russian Far East (as many parts of Northern Asia) has been targeted by only a very limited number of pioneering studies [Grebennikov et al., 2011, Kirichenko et al., 2018].

While there is some amount of distribution data, only very little genetic data on louse flies are available worldwide. As of 09 April 2020, the Barcode of Life Data Systems database (BOLD) [Ratnasingham, Hebert, 2007] lists 410 published DNA barcode records of Hippoboscidae out of more than 2,000,000 dipteran records. None of the published records of louse flies are from Eastern Asia or from any part of Russia. A thorough DNA barcoding database is a prerequisite for large-scale biodiversity assessments and metabarcoding studies [Taberlet, 2012], but so far, the Russian Far East (as many parts of Northern Asia) has been targeted by only a very limited number of pioneering studies [Grebennikov et al., 2017; Kirichenko et al.; 2019, Makarchenko et al., 2015].

The bird-ringing station at the Muraviovka Park for Sustainable Land Use (49°55′N, 127°40′E, Fig. 1) is located 50 km south of Blagoveschensk at the middle section of the river Amur in the southern Zeya-Bureya plane (Amur Region, Russia). Founded in 1994, this privately owned and operated park covers some 6,500 hectares of wetlands, meadows, fields and small woods. The wetlands meet the IBA criteria (Important Birds Area) and the guidelines of the Ramsar Convention (listed since 1995). Research in the field of bird migration has been conducted there since 2011 [Heim, Smirenski, 2013, 2017].

In 2017, birds caught for ringing were examined for the occurrence of louse flies. The faunistic results of the collection of Hippoboscidae were incorporated into a bachelor’s thesis at the Dresden University of Applied Sciences (Germany) [Meißner, 2019]. The goal of the study was to give a contribution to the louse fly fauna of the Far East of Russia. At the same time, the material was to be compared with that of some Hippoboscidae from Europe on the basis of the DNA barcode in order to biochemically check the respective species or subspecies affiliations, to compare it with the taxonomic findings and to discover cryptic species.

Material and Methods

In 2017, ringers of the bird-ringing station caught the birds by net (250 net meters) and marked them individually with rings of the Bird Ringing Centre of Russia. The objective was to gain insight into the phenomenology, behaviour and population trends of migrating songbirds [Heim 2017, Heim et al., 2018].

We examined a total of 7,222 birds representing 128 species, and collected louse flies during the spring (28 April to 13 July 2017) and autumn season (25 July to 21 September 2017). To preserve the insects, 2.0 ml twist-top vials containing 100% undenatured ethyl alcohol (both BioForm) were used. Some louse flies left the host before collection and had to be caught on the window or walls in the station room and were not assigned to a host specimen.

To identify the louse fly species, we used a stereomicroscope [Nikon] microscope, the VHX-5000 (Keyence) digital microscope and numerous literature resources [Bütikker, 1994; Doszhanov, 2003; Gruin, 1989; Farafonova, 2001; Iwasa, Choi, 2015; Maa, 1962, 1963, 1966a-b, 1967, 1969a-d; Narshuk et al., 2018; Theodor, Oldroyd, 1964]. The images taken with the digital microscope were edited using the free software program GNU Image Manipulation Program [GIMP team, 2019]. All Hippoboscidae examined have been maintained in the collection of the corresponding author. The nomenclature of louse flies follows the current world checklist [Dick, 2006], but in conformance with Doszhanov [2003], i.e. without taking into consideration the names of Ornithomya and Ornithoica subspecies.

The DNA barcoding was carried out in the Zoologische Staatssammlung (Bavarian State Collection of Zoology), Munich. We wanted to explore if we find evidence for cryptic species within our data. For this, we
extracted the mitochondrial gene Cytochrome c oxidase subunit I (COI) of 32 individuals. A sample from the legs of these individuals was obtained for the DNA extraction, which was done using the Phenol-Chloroform extraction protocol [Sambrook et al., 1989]. The COI sequence was amplified using LepF1 and LepR1, purified with magnetic beads, and then sent them for Sanger Sequencing to the Sequencing Service at the Faculty of Biology, division of genetics, at the Ludwig-Maximilians-Universität München (LMU). After obtaining the sequences, we trimmed and made the consensus sequence using the program Geneious v5.5.9. After obtaining the consensus sequence for each sample, we downloaded sequences from Hippoboscidae of the same region of COI as ours from BOLD [Ratnasingham, Hebert, 2007] (Table 1). We aligned them using MAFFT [Katoh et al., 2002], and then uploaded the alignment to CIPRES [Miller et al., 2010] for constructing a phylogenetic tree using RAxML v.8.2.12 with 1,000 bootstrap replicates [Stamatakis, 2014].

To test if there are cryptic species within the data we applied a Generalized Mixed Yule Coalescent (GMYC) method, which is a likelihood method that combines the diversification between species with the genealogical branching within species for a single-locus, so that it can make a better reconstruction of species boundaries given the data [Fujisawa, Barraclough, 2013]. For making the analysis, we required an ultra-metric tree, which was estimated using the previously aligned sequences with BEAST v2 [Bouckaert et al., 2019]. We calculated the parameters using the associated application BEAUti.

We used an GTR model, and we constrained the analysis to form two clades that represent the subfamilies Lipoteniinae and Ornithomyiinae. We uploaded the tree to the GMYC web server (Exelixis Lab, https://species.h-its.org/gmyc/) and analysed the output for single and multiple thresholds.

To corroborate the results obtained from the GMYC, we uploaded the COI sequences to the BOLD database [Ratnasingham, Hebert, 2013], so that we could obtain the BIN (Barcode Index Number; which is equal for the individuals of the same species) for each sequenced individual of our sample. The BIN is a number that acts as an Operational Taxonomic Unit (OTU), in which any individual with the same BIN should have less than 2.2% of divergence with others with the same number. To obtain the BIN, the sequences that do not have the minimum quality are filtered (e.g. have less than 500bp, have 1% approximately of ambiguous bases, have stop codons, or improbable COI peptides), then translated to amino acids, and aligned using a Hidden Markov Model to other sequences in the BOLD database [Ratnasingham, Hebert, 2013]. After the alignment took place, different methods were used for clustering: first a Single Linkage Clustering, in which sequences with less than 2.2% of divergence are clustered together, and then a Markov clustering that collapses to the same BIN those groups that show less than 4.4% divergence [Ratnasingham, Hebert, 2013]. The result is that each sequence becomes associated to a BIN number, either an already existing one or a newly established one, representing a new OTU.

Table 1. Reference samples for DNA-Barcoding.
Таблица 1. Контрольные образцы для ДНК-штрих-кодирования.

| Species                      | Country of origin | Collectors                      |
|------------------------------|-------------------|---------------------------------|
| H2 Ornithomyia avicularia    | Germany           | Doczekal, D. & Voith, J.        |
| H3 Ornithomyia avicularia    | Germany           | Doczekal, D.                    |
| GO-49 Ornithomyia chloropus (chloropus) | Germany       | Michael Hupfer                  |
| GBDP292007 Ornithomyia avicularia | Finland        | BOLD Systems, Mined from GenBank, NCB | |
| GBDP2839619 Ornithomyia fringillina | Canada          | BOLD Systems, Mined from GenBank, NCB |
| GBDP2839519 Ornithomyia fringillina | Canada          | BOLD Systems, Mined from GenBank, NCB |
| GBDP291707 Ornithomyia fringillina | Germany        | BOLD Systems, Mined from GenBank, NCB |
| GB1874615 Ornithophila gestroi | ?                | BOLD Systems, Mined from GenBank, NCB |
| GBDP2700619 Lipoptena forsitosa | Lithuania       | BOLD Systems, Mined from GenBank, NCB |

Parentheses: subspecies concept of Maa, 1967; ? = data not given. Samples from Europe were incorporated into the analyses for taxonomic placement of the DNA barcodes. BOLD data was downloaded from the BOLD database, all other sequences were newly generated in this study.

(В скобках: статус подвида принят по Maa, 1967; ? = данные не указаны. Образцы из Европы были включены в анализ ДНК для сравнения. Данные BOLD были загружены из базы данных BOLD, все остальные последовательности были созданы заново в этом исследовании.)
Results

We collected a total of 100 louse flies of altogether seven species (Table 2) and recorded 91 breeding and migratory birds of 33 species being hosts. Furthermore, seven Hippoboscidae without assignment to a host were collected. In total, twelve times more females than males were caught.

Species identification by DNA Barcoding, faunistic results and hosts per species

The identification keys led us to eight different species and subspecies. Altogether 19 individuals were investigated by DNA barcoding. The samples of *Icosta holoptera* and *Ornithoica unicolor* did not produce any results in the context of the DNA barcoding, leaving 15 individuals with usable sequences. The results are shown in a maximum likelihood tree (Fig. 2). In this way, seven louse fly species and two different females of uncertain species level (cf. status) were identified. The taxonomic status of a further species was changed (stat.rev.) and three other species rsp. subspecies were recognized as synonyms of formerly described ones (see below). Flies of *Ornithomya fringillina* from Canada, Europe and the Russian Far East are located in the same barcoding cluster. In the case of *Ornithomya fringillina* and *Ornithomya chloropus*, records indicate a gap closure between the known eastern and western distribution areas of those species (see Doszhanov, 2003).

Table 2. Species and abundance of louse flies from Muraviovka Park.

| Species and individuals with unknown taxonomic status | n | % |
|------------------------------------------------------|---|---|
| *Icosta holoptera* | 2 | 1.9 |
| *Ornithoica momiyamai* | 5 | 2.8 |
| *Ornithoica unicolor* | 1 | 0.9 |
| *Ornithomya aobatonis* | 81 | 75.7 |
| "Ornithomya A"* | 1 | 0.9 |
| *Ornithomya chloropus* | 7 | 6.5 |
| *Ornithomya fringillina* | 7 | 6.5 |
| "Ornithomya B"* | 1 | 0.9 |
| *Ornithophila metallica* | 2 | 1.9 |
| Total | 107 | 100 |

n = number, * result of DNA-barcoding

Fig. 2: Cytochrome c oxidase subunit I (COI) gene tree for Hippoboscidae. The tree was estimated using maximum likelihood (RAxML). COI sequences used to estimate the tree were obtained from some individuals from Muraviovka park, individuals recollected in Germany, and sequences downloaded from BOLD. Numbers above the branch are bootstrap values.
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Species account*

Icosta holoptera (Lutz, 1915)  
Fig. 3.

Material. 2 ♂, 25.V.2017, host: both on one Gallinago stenura.

Note. New record for Russian Federation. Number of hosts: 1.

Ornithoica montiyamae Kishida, 1932  
Fig. 4.

Material. 30.VII.2017, host: Emberiza spodocephala; 1 ♂, 23.VII.2017, host: Locustella certhiola; 1 ♂, 28.VIII.2017, host: Luscinia calliope; 1 ♂, 26.VII.2017, host: Parus major; 1 ♂, 26.VII.2017, host: unknown.

Note. Number of hosts: 4.

Ornithoica unicolor Speiser, 1900  
Fig. 5.

Material. 9, IX.2017, host: Asio otus.

Note. Number of hosts: 1.

Ornithomya aobatonis (Matsumura, 1905), stat.rev.  
Fig. 6

= Ornithomya avicularia aobatonis Matsumura, 1905

Material. 6 ♂♂, 75 ♀♀, sex ratio: 1 ♂♂: 13 ♀♀.

3 ♂♂, 19.VIII.2017, host: Acipiter gularis; 1 ♂, 12.VII.2017, leg. Korschesky, host: Acrocephalus aedon; 1 ♂, 29.VII.2017, host: Acrocephalus aedon; 1 ♂, 29.VII.2017, host: Acrocephalus aedon; 1 ♂, 29.VII.2017, host: Acrocephalus aedon; 1 ♂, 21.VII.2017, host: Acrocephalus aedon; 1 ♂, 17.VIII.2017, host: Acrocephalus bistriiceps; 1 ♂, 31.VII.2017, host: Acrocephalus orientalis; 1 ♂, 17.VIII.2017, host: Anthus hodgsoni; 1 ♂, 10.IX.2017, host: Anthus hodgsoni; 1 ♂, 16.IX.2017, host: Anthus hodgsoni; 1 ♂, 5.VIII.2017, host: Asio otus; 1 ♂, 09.IX.2017, host: Asio otus; 1 ♂, 9.IX.2017, host: Asio otus; 1 ♂, 13.VIII.2017, host: Dendrocolpus leucota; 1 ♂, 2.VII.2017, host: Dendrocolpus major; 1 ♂, 13.VII.2017, leg. Korschesky, host: Emberiza elegans; 1 ♂, 18.VIII.2017, host: Emberiza rutila; 1 ♂, 30.VI.2017, host: Emberiza spodocephala; 1 ♂, 26.VII.2017, host: Emberiza spodocephala; 1 ♂, 26.VII.2017, host: Emberiza spodocephala; 1 ♂, 28.VII.2017, host: Emberiza spodocephala; 1 ♂, 29.VII.2017, host: Emberiza spodocephala; 1 ♂, 29.VII.2017, host: Emberiza spodocephala; 1 ♂, 31.VII.2017, host: Emberiza spodocephala; 1 ♂, 19.VIII.2017, host: Emberiza spodocephala; 1 ♂, 27.VII.2017, host: Ficedula albicilla; 1 ♂, 28.VII.2017, host: Ficedula albicilla; 1 ♂, 31.VII.2017, host: Ficedula albicilla; 1 ♂, 14.VIII.2017, host: Ficedula albicilla; 1 ♂, 15.VIII.2017, host: Ficedula albicilla; 1 ♂, 29.VII.2017, host: Ficedula zanthopygia; 1 ♂, 28.VII.2017, host: Ficedula zanthopygia; 1 ♂, 12.VII.2017, host: Ficedula zanthopygia; 1 ♂, 14.VIII.2017, host: Ficedula zanthopygia; 1 ♂, 16.VIII.2017, host: Ficedula zanthopygia; 1 ♂, 19.IX.2017, host: Gallinago gallinago; 1 ♂, 19.VIII.2017, host: Gallinago stenura; 1 ♂, 23.VII.2017, host: Locustella certhiola; 1 ♂, 23.VIII.2017, host: Locustella certhiola; 1 ♂, 06.IX.2017, host: Locustella lanceolata; 1 ♂, 14.VIII.2017, host: Luscinia calliope; 1 ♂, 17.VIII.2017, host: Luscinia calliope; 1 ♂, 13.VIII.2017, host: Muscicapidae; 1 ♂, 25.VIII.2017, host: Muscicapidae; 1 ♂, 19.IX.2017, host: Ninox scutulata; 1 ♂, 22.VIII.2017, host: Otus sunia; 1 ♂, 22.VIII.2017, host: Otus

* unless stated otherwise noted, collectors of flies are Hein/Meißner.

NOTE. Number of hosts: 16.

“Ornithoica A”

Material. 24.VII.2017, host: Luscinia calliope.

Note. Number of hosts: 1.

Ornithomya chloropus Bergroth, 1901  
Fig. 7.

Material. 1 ♂, 10.IX.2017, host: Anthus hodgsoni; 1 ♂, 18.IX.2017, host: Anthus hodgsoni; 1 ♂, 18.IX.2017, host: Anthus hodgsoni; 1 ♂, 6.IX.2017, host: Emberiza chrysophrys; 1 ♂, 21.VIII.2017, host: Emberiza spodocephala; 1 ♂, 12.IX.2017, host: Fringilla montifringilla; 1 ♂, 22.VI.2017, host: Passer montanus.

Note. Number of hosts: 5.

Ornithoica fringillina Curtis, 1836

Material. 18.IX.2017, host: Emberiza spodocephala; 1 ♂, 19.IX.2017, host: Anthus hodgsoni; 1 ♂, 10.IX.2017, host: Emberiza pusilla; 1 ♂, 21.IX.2017, host: Emberiza pusilla; 1 ♂, 21.IX.2017, host: Emberiza suppression; 1 ♂, 8.VIII.2017, host: Ficedula zanthopygia.

Note. Number of hosts: 3.

“Ornithoica B”

Material. 26.VII.2017, host: Ficedula zanthopygia.

Note. Number of hosts: 1.

Ornithophila metallica (Schiner, 1864)

Material. 21.VII.2017, host: Acrocephalus aedon; 1 ♂, 23.VI.2017, host: Luscinia calliope.

Note. Number of hosts: 2.

Number of louse flies and louse fly species per host

Usually, one louse fly was detected on each host. In four cases, two parasites of the same species were found on one bird (Icosta holoptera on Gallinago stenura, Ornithoica aobatonis each on two Acrocephalus aedon and one Otus sunia). Three specimens of Ornithoica aobatonis occurred on a single Acipiter gularis. In three cases, louse flies of different species were found each on a single host (Ornithoica aobatonis / Ornithoica monyiamai on Locustella certhiola, Ornithoica aobatonis/Ornithophila metallica on Acrocephalus aedon, Ornithoica aobatonis/Ornithoica unicolor on Asio otus).

New infestation after recapture

After the recapture of two hosts, their new infestation was detected in each case with the same louse fly species: Emberiza spodocephala XR17580, captured
26.VII.17 (1 ♀, *Ornithomya aobatonis*), recaptured 19.VIII.17 (1 ♀, *Ornithomya aobatonis*); *Luscinia calliope* KA13207, captured 14.VIII.17 (1 ♀, *Ornithomya aobatonis*), recaptured 17.VIII.17 (1 ♀, *Ornithomya aobatonis*).

**Phoresy**

We observed phoretic mites (Acari) on five louse flies (5.6% of all collected Hippoboscidae). These were three females of *Ornithomya avicularia* (phoresy ratio 26.VII.17 (1 ♀, *Ornithomya aobatonis*), recaptured 19.VIII.17 (1 ♀, *Ornithomya aobatonis*); *Luscinia calliope* KA13207, captured 14.VIII.17 (1 ♀, *Ornithomya aobatonis*), recaptured 17.VIII.17 (1 ♀, *Ornithomya aobatonis*).

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3.7%) and two females of *Ornithomya chloropus* (phoresy ratio 28.6, %). The birds acting as hosts were two *Anthus hodgsoni*, two *Emberiza spodocephala* and one *Ficedula albicilla*, all of them juvenile birds. The mites (adults, eggs) were attached to the abdomen of the louse flies in all cases (Fig. 8).

![Mites and mite eggs on abdomen of female Ornithomya avicularia, ventral view.](image)

Discussion

With her extensive publications Maa [1962, 1963, 1966a–b, 1969a–b, 1967, 1969 b–d] created the basis for the faunistic research on louse flies of the whole world. She also described numerous subspecies, whose taxonomic differences, however, are hardly recognizable in many cases. In agreement with some relevant Russian authors (e.g. Farafonova, 2001; Doszhanov, 2003; Matyukhin, 2016), we therefore operate at the species level.

Sixteen louse fly species are known from the Russian Far East [Soós, Hürka, 1986; Farafonova, 2001; Doszhanov, 2003; Nartshuk et al., 2018]. In our study we detected six of them, including one species with new taxonomic status, one species as a new record for the region, and two flies with divergent DNA barcodes that were marked as new OTUs (“Ornithomya A”, *Ornithomya B*, Fig. 2). It should be noted, that the capture methodology did not cover the entire possible host range of the region. For this reason, and the fact that no louse flies from mammals were examined, the scope of the louse fly records is limited. In the sex ratio, a distinct majority of females was found, consistent with the observations of other authors (e. g. Theodor, Oldroyd, 1964; Soós, Hürka 1986; Farafonova, 2001; Labitzke et al., 2018; Nartshuk et al., 2018; Knauthe, Jentzsch, 2019). The dominance of females might be explained by the earlier appearance of males in the season, which might cause them to die off sooner [Theodor, Oldroyd, 1964]. According to Bättiker [1994], males are also more mobile and can thus leave the host more quickly (e.g. already when taking the bird out of a net).

Doszhanov [2003] published distribution maps of the louse flies from the Russian Far East. For all species presented here with the exception of the new record *Icosta holoptera* and *Ornithophila metallica* (known from the region, see Nartshuk & Matyukhin [2019]), these maps show a gap between the Eurasian area in the west, up to the Ob River and Novsibirsk, and the Sichote Alin Mountains, eastern Manchuria, Japan and Korea in the east. The present investigations extend the western boundary of this isolated area in the easternmost part, and in the case of *Ornithomya chloropus* our records suggest a gap closure between the eastern and the western distribution areas. *Icosta holoptera* is one of the five species of the genus *Icosta* for Russia (see Doszhanov, 1977, 2003). So far, it has only been found in China (Zhejiang Province), the Malay Peninsula, the Philippines, New Guinea [Maa, 1969b] and Japan [Mogi et al., 2002]. Gruiiformes and Charadriiformes are known as host birds, which is in concordance with our record on a Pintail Snipe (Charadriiformes) serving as host.
Ornithomya avicularia aobatonis is used as a subspecies of *O. avicularia* in various recent publications [Dick, 2006; Sato, Mogi, 2008; Yamauchi, Kuroki, 2009; Suh et al., 2012], while in the annual checklist of *The Catalogue of Life* [2019] *O. aobatonis* (Matsumura, 1905) is listed as a valid species without reference. The current study provides two clearly separated COI barcodes, which supports the hypothesis of two distinct and valid species.

Discovery of cryptic species is one of the goals that can be achieved with DNA barcodes, although this method can only be a decision-making aid. In the case of *Ornithomya chloropus*, there are discrepancies between different lines of evidence. The individuals of the numbers 2, 73 and 94 meet the characteristics of the subspecies *Ornithomya chloropus extensa* from Asia mentioned by Maa [1967: 738] and differ morphologically from *Ornithomya chloropus chloropus* from Europe, which suggests that both subspecies could be two different species. Based on the fact that the specimens of *Ornithomya chloropus extensa* from Muraviovk Park are retrieved as members of the same COI barcode cluster as *Ornithomya chloropus chloropus* GO49 from Europe (Fig. 2), there is no evidence for this hypothesis in the present case. Further investigations must clarify how morphological differences of *Ornithomya chloropus* from Europe and the Far East can be classified despite the common barcode cluster. Until further notice, the classification into two subspecies within the meaning of Maa [1967] continues.

The common barcoding results of *Ornithomya fringillina* from North America, Europe and Muraviovka Park leaves no doubt about the taxonomic classification of the species. But two further louse flies (named “*Ornithomya A*”, “*Ornithomya B*”) have unique COI sequences, each of which differs from the other clusters in the maximum likelihood tree. Morphological differences of the neighbouring species (*Ornithomya avicularia* and *Ornithomya fringillina*), respectively could not be detected. Further research is necessary to clarify how this barcoding finding could be classified.

Winged louseflies reach their hosts by flying. In the case of louseflies that parasitize mammals, this can be observed regularly. In the case of louseflies parasitizing birds, however, this is difficult to prove. Breeding nests are usually mentioned as the point of contact between louse flies and birds [Büttiker 1994; Schöne, Schmäschke, 2015; own observations]. In the present case, two examples of louse flies are given, which had found birds outside of their breeding nests and in one case after a short time period of maximum three days.

Five of the louse flies collected (5.6%) served as phoretic for mites and their egg deposits. The phoresy rate detected in this work is thus clearly above the value of 0.6% found by Matyuikhin [2016] for the European part of Russia. Recent studies from Germany also show obvious differences in the proportion of louse flies used as a transport medium in Saxony, 1.0% [Knauth, Jentzsch, 2019] and Thuringia, 12.0% [Labitzke et al., 2018].

The mites and mallophages are commonly found on the abdomen (Fig. 8) or wing base of the flies [Sellenschloß, 1984; Böttiker, 1994].

A total of 33 bird species were identified as hosts of louseflies and assigned to individual louse fly species (see results). We proved the occurrence of louse flies on rarely caught bird species such as Pintail Snipe, Brown Hawk Owl and Oriental Scops Owl for the very first time.

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