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Research Article

Avian Afterlives: Integrative Taxonomy of Hippoboscid Flies (Diptera) From Citizen-Reported Bird Carcasses Reveals a New Species and Host–Parasite Diversity in Singapore

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Biodiversity science depends on rigorous characterization of species diversity and ecological roles, yet the ecology of many invertebrate species remains virtually unknown. One pragmatic way to change this is to make use of deceased animals and their companions, such as by salvaging and examining carcasses reported by citizen scientists. Here, we demonstrate how bird carcasses reported by citizen scientists can help generate functional biodiversity data on parasites. We reveal 44 hippoboscid flies (Diptera: Hippoboscidae) found on 36 of 148 salvaged bird carcasses (~24% success rate). Using the principles of large-scale integrative taxonomy (LIT), we first delimited 10 hippoboscid species. One of these, *Pseudolynchia maai* Lee & Oboňa, sp. nov., was new to science and is here formally described. We also provide a pictorial key, image-based diagnostics, and molecular diagnostics for all 10 species. We report 25 species interactions between flies and birds. This study illustrates how combining the efforts of citizen scientists and biologists can support species discovery and generate functional parasite–host data.

1. Introduction

Understanding which species exist and what roles they play in ecosystems is fundamental to biodiversity science and conservation [1, 2]. Yet for most taxa, especially invertebrates, these biodiversity baselines remain poorly documented [3, 4]. A key challenge is that many species are difficult to sample, identify, or observe directly, due to limited spatial and temporal coverage in biodiversity surveys [5, 6] and a shortage of taxonomic expertise.

One simple yet often-overlooked approach to jointly assessing species diversity and ecological function involves collecting specimens associated with parasites, predators, or scavengers, which naturally provide ecological context. These interactions, where one species is found on or inside another (dead or alive), can be recorded as metadata during routine fieldwork, with additional observations coming from examining material in natural history collections and contributions from members of the public. Translating such observations into meaningful biodiversity and ecological datasets requires collaboration

between scientists and the public: the public often documents and reports interactions, while scientists can help with identifying the specimens and developing user-friendly identification tools, such as pictorial keys and annotated photographs showing diagnostic features. All this can support broader participation in ecology and evolutionary biology.

Hippoboscid flies (Diptera: Hippoboscidae) are obligate blood-feeding ectoparasites of birds and mammals, which makes them a model taxon for generating taxonomic and biodiversity data. They are also epidemiologically important because they act as vectors of bloodborne pathogens such as *Haemoproteus*, *Plasmodium*, *Anaplasma*, *Rickettsia*, and *Trypanosoma* spp. in birds [7, 8, 9]. When sampled directly from avian hosts (salvaged or alive), they offer a rare opportunity: each fly is a data point that links the fly species with the host species. Despite their importance in ecological and epidemiological research, it is surprising how little is known about them in Singapore. While 54 species across 12 genera have been documented from Southeast Asia (Supporting Information 4: File S1), only four species have so far been recorded in Singapore, which suggests that much of the diversity remains undocumented. Conceivably, the main impediment is the lack of accessible and up-to-date taxonomic descriptions and keys needed for reliable species-level identification.

Integrative taxonomy addresses this challenge by combining multiple types of data and methods to delimit and describe taxa [10, 11, 12]. In our study, we use molecular data [13] (DNA barcodes) together with morphology and ecology to delimit comprehensive species boundaries. The inclusion of morphology is particularly important, as it ensures that identifications remain accessible to researchers and citizen scientists who may not have access to molecular laboratories, while still enabling the discovery of new species and improving hippoboscid fly taxonomy. Recent advances have made the use of integrative taxonomy involving molecular data scalable: megabarcoding enables high-throughput specimen sorting [14, 15, 16, 17] while large-scale integrative taxonomy (LIT) [18] provides a workflow for validating barcode-based clusters by selecting specific specimens for morphological examination. Lastly, UITOTO [19] provides diagnostic tools for species identification. When applied to specimens associated with other specimens (e.g., via phoresy or parasitism), integrative taxonomy thus yields data on biodiversity and ecological processes. While integrative taxonomy is often conducted by specialist taxonomists, the resulting tools, such as pictorial keys and morphology-labeled photographs, are also important to other scientists (e.g., ecologists, epidemiologists, and evolutionary biologists) and interested members of the public. As such, non-specialists and amateurs with access to widely available tools, such as microscopes, smartphones fitted with clip-on macro lenses or digital cameras (e.g., DSLR or compact cameras), can readily identify and document a broad array of organisms, enabling accurate ecological data and broadening participation in biodiversity research.

Here, we report new data collected from 63 hippoboscid flies, of which 44 were collected from 148 salvaged bird carcasses reported by citizen scientists. Another 11 were collected from nests, five from the wet collection of the Lee Kong Chian Natural History Museum, two from opportunistic active collecting, and one from a Malaise trap (Supporting Information 1: Table S1). We then used integrative taxonomy to reveal 10 species based on DNA barcodes and morphological analysis. This included one species new to science. Our taxonomic and species interaction data were uploaded to the Biodiversity of Singapore (BOS) website (https://singapore.biodiversity.online/), allowing users to identify hippoboscid species and explore host associations. We also provide preliminary insights into hippoboscid–host interaction patterns in Singapore. Our study shows how parasitized carcasses, when studied with integrative taxonomy, can both refine species boundaries and yield functional biodiversity data for ecological and epidemiological research.

2. Materials and Methods

2.1. Sampling. We started by collecting hippoboscid fly specimens from salvaged bird carcasses originally reported by citizen scientists in Singapore [20], as detailed in Lee et al. [21]. We then supplemented our collection with specimens obtained through opportunistic sampling (hand-collecting from the environment and sampling live birds), passive sampling (Malaise traps), and existing museum specimens (Supporting Information 1: Table S1). The flies collected from bird carcasses were preserved in 95% ethanol at −20°C, while all other specimens were preserved in 70% ethanol at room temperature.

2.2. Megabarcoding. We extracted genomic DNA from each fly using two types of DNA extraction methods, QuickExtract (QE; Lucigen, USA) and HotSHOT [22]. For the QE method, we submerged individual specimens/dissected legs in 10 μL of QE and heated them for 18 min at 65 °C and 2 min at 95 °C. For the HotSHOT protocol, we submerged individual specimens/dissected legs into 15 μL of lysis buffer and heated them for 18 min at 65 °C and 2 min at 95 °C [15]. We then added 15 μL of neutralizing buffer.

Subsequently, we amplified the 313-bp fragment of the cytochrome oxidase 1 (*CO1*) gene using the modified primers, m1COlintF: 5′-GGWACWGGWTGAACWGTWTAYCCY CC-3′ [23] and modified jgHCO2198: 5′-TANACYTCNGGRT GNCCRAARAAYCA-3′ [24]. Each PCR reaction contained 7 μL of 2x CWBio Mastermix (Beijing, China) or 2x Rapid Taq Mastermix P222 (Nanjing Vazyme Biotech Co., Ltd, Nanjing, China), 1 μL of 1 mg/mL BSA, 1 μL of 10 μM of each primer, and 4 μL of DNA extract. The PCR conditions are initial denaturation (95 °C for 5 min), followed by 35 cycles of denaturation (94 °C for 30 s), annealing (45 °C for 1 min), extension (72 °C for 1 min), and a final extension (72 °C for 5 min).

For amplicons obtained before 2024, the forward and reverse primers were tagged with a 9-bp oligonucleotide tag at the 5′-end. We cleaned the amplicon pools using a 1:1 ratio of AMPure XP beads (Beckman Coulter Life Sciences, Indiana, USA) and sent the pools to the Genome Institute of Singapore for sequencing on a partial Illumina HiSeq2500 Rapid Run lane (251-bp paired end). The reads were demultiplexed using the same protocol described in Lee et al. [21].

For amplicons obtained in 2024, the forward and reverse primers were tagged with a 13-bp oligonucleotide tag at the 5′-end. We cleaned the amplicon pools using a 1:1.2 ratio of VAHTS DNA Clean Beads N411 (Nanjing Vazyme Biotech Co., Ltd, Nanjing, China) and prepared the sequencing library using Oxford Nanopore Technologies (ONT)'s Ligation Sequencing Kit V14 (SQK-LSK114) and Flongle Sequencing Expansion Kit (EXP-FSE002) as per the manufacturer's protocol version ACDE_9163_v114_revS_29Jun2022. We then sequenced the library on a Flongle FLO-FLG114 flowcell using the MinION Mk1C and MinKNOW v24.02.16 (Ubuntu) (ONT, Oxford, UK). We basecalled the resulting POD5 files using Dorado v0.7.2 with the DNA high-accuracy model (dna_r10.4.1_e8.2_400bps_hac@v4.3.0) and used the ONT-Barcoder 2.2 software [15] to obtain demultiplexed sequences.

All demultiplexed sequences were blasted to the NCBI GenBank database, and any matches that were ≥97% to a nonhippoboscid sequence were removed from the dataset. We then aligned the resulting sequences using Mafft V7 [25] and clustered them into molecular operational taxonomic units (mOTUs) at a range of distance thresholds from haplotype to 5% using Objective Clustering (part of "TaxonDNA" [26]). The 313-bp *COI* barcodes were deposited in the NCBI GenBank database (Accession Numbers: PQ247060−PQ247119). Two barcodes, GenBank accession numbers MT762413 and MT762414, have already been deposited as part of Lee et al. [21].

2.3. Verification of Molecular Clusters. Using the LIT workflow [18], we generated the maximum pairwise distance (p-dist) and instability index for each 3% mOTU (Supporting Information 2: Table S2). We applied a 3% threshold following Herbert et al. [27] to generate clusters that served as preliminary species hypotheses. Note, however, that the results under LIT are not tied to a particular clustering threshold, since species delimitation relies on evaluating alternative molecular hypotheses with a second data source. Choosing a very high or low threshold, thus, does not change the final outcome, but slows down morphological examination. In this study, we flagged clusters with a maximum p-distance ≥ 1.5 or an instability index < 1 as requiring more extensive morphological study. This meant that we picked specimens from the main haplotype and the most distant haplotypes for flagged clusters, while we only examined specimens from the most distant haplotypes for the remaining clusters. This examination was accomplished using a Leica M205C stereomicroscope.

2.4. Morphological Identification of Hippoboscid Flies. We identified the hippoboscid fly specimens using the following papers: Maa [28, 29, 30, 31, 32], and Yatsuk et al. [33], and imaged the specimens using a Leica FLEXACAM C1 (12MP) camera fitted onto a Leica M205 stereomicroscope. We then focus-stacked the images using the Leica LAS X software and prepared the images for publication using Adobe Photoshop 2024. We used these images to create an annotated pictorial key (Supporting Information 5: File S2). We also annotated images that contain diagnostic characteristics based on the earlier-mentioned monographs by Maa [28, 29, 30, 31, 32] and uploaded them onto the BOS website (https://singapore.biodiversity.online/taxon/A-Arth-Hexa-Dipt-Hippoboscidae). All the specimens are deposited in the Zoological Reference Collection (ZRC) at the Lee Kong Chian Natural History Museum in Singapore.

2.5. Molecular Diagnosis. We provided a molecular diagnosis for each species using the software tool UITOTO [20]. For each species, we generated five diagnostic molecular combinations (DMCs) by varying two parameters: minimum length (3–8) and minimum number of exclusive states (2–4), while keeping the number of iterations and refinement strength constant at 50,000 and 0.25, respectively. To test the DMCs generated, we used an independent testing dataset containing Hippoboscidae *COI* sequences downloaded from NCBI GenBank. We aligned them to our 313 bp sequences using Mafft V7 [26] and truncated the GenBank sequences to 313 bp. Any sequences that did not overlap with the 313 bp fragment at all were removed, resulting in 429 GenBank sequences for the independent testing dataset. We then used the default settings for the ALnID2 function in UITOTO [20] to check if the species identifications from GenBank are congruent with our species identifications based on our DMCs only. We selected the DMCs with the greatest discriminatory power based on five criteria (precision, recall, specificity, accuracy, and $F1$ score; [20]; Supporting Information 3: Table S3). We then used the Visualizer function in UITOTO to highlight the diagnostic bases in the holotype sequence for *Pseudolynchia maai* sp. nov. and a random sequence from the largest haplotype for the remaining described species.

2.6. Nomenclatural Acts. The new name contained in this article is available under the International Code of Zoological Nomenclature. This work and the nomenclatural acts contents have been registered in ZooBank. ZooBank Life Science Identifier (LSID) for this publication is: urn:lsid:zoobank.org:pub:5622F756-7760-4068-97DC-F808952BA6A0. The LSID registration and any associated information can be viewed in a web browser by adding the LSID to the prefix http://zoobank.org/.

2.7. Species Accumulation Curves and Interaction Networks. To access our sampling effort, we used the R package "Vegan" [34] to plot species accumulation curves, plotting hippoboscid fly species diversity against the number of bird specimens sampled. To evaluate whether we had fully sampled flies from the best-sampled host group and put our total sampling effort into perspective, we isolated the hippoboscid fly collection data on pigeon hosts (Aves: Columbidae), the most commonly sampled carcasses in our dataset. We also generated bipartite networks for bird hosts and hippoboscid flies using the R package "bipartiteD3" [35], focusing on the host–parasite interactions of the most sampled hippoboscid fly species. The R script and input files are available at https://github.com/leshonlee/hippoboscidae.git.

3. Results

3.1. Megabarcoding and Morphological Identification. We collected a total of 63 hippoboscid flies (Supporting Information 1: Table S1). 44 were obtained from 36 out of 148 salvaged bird carcasses reported by citizen scientists (~24% success rate), 11 were collected through active nest sampling, five were sourced from the wet collection of the Lee Kong Chian Natural

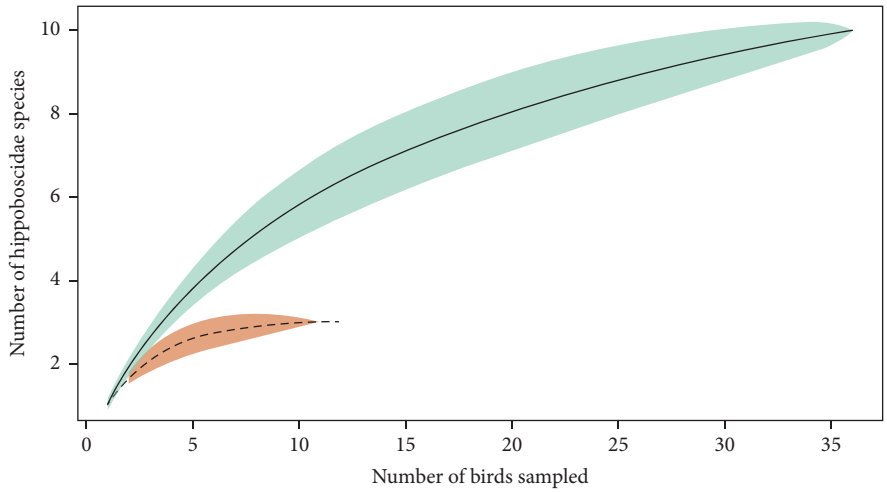


FIGURE 1: Species accumulation curves for all sampled birds (solid line; 95% CI in green) and Columbid hosts (dashed line; 95% CI in orange).

History Museum, two were obtained via opportunistic active sampling, and one was collected using a Malaise trap (Supporting Information 1: Table S1). We then barcoded all 63 hippoboscid flies, which clustered into nine mOTUs at 3% distance threshold (Supporting Information 7: File S4). Using LIT, we found four potentially incongruent (PI) clusters and five non-PI clusters. After verifying the clusters using morphology, one PI cluster remained incongruent with morphological evidence and contained two species, *Pseudolynchia garzettae* and *P. maai* sp. nov. This raised the total number of species to 10.

3.2. Species Accumulation Curve. To assess our sampling effort, we plotted species accumulation curves for all collected birds ($n = 36$) and for Columbid hosts ($n = 12$), the bird family with the highest sample size (Figure 1). The overall curve (Figure 1) has not reached an asymptote, suggesting that additional hippoboscid fly species remain undiscovered in Singapore. In contrast, the "Columbid" curve (Figure 1) shows signs of plateauing, suggesting that most hippoboscid fly species associated with Columbidae hosts in Singapore may already have been detected, although this inference should be treated with caution given the small sample size of 12 birds.

3.3. New Species Description. P. maai Lee & Oboňa sp. nov..

Morphological diagnosis: (♂/♀) Prescutum with long pale fine setae only (Figure 1A,C). Apical scutellar margin is straight (Figure 1A,C). Microtrichia on the wing do not reach vein 2A (Figure 1D). (♀) Pregenital plate is distinctively sclerotized on the sides, leaving a pale strip in the middle (Figure 1B,F). (♂) Basal ventral part of the 1st segment of mid tarsus with peg-like modified spines (Figure 1E). Annotated images of diagnostic features are in https://singapore.biodiversity.online/species/A-Arth-Hexa-Diptera-002954 and in Supporting Information 6: File S3.

Molecular diagnosis: (34: G, 124: C, 145: C, 154: T, 163: C, 169: T, 181: T, 205: G) mapped onto barcode for ZRCBDP0298068: cctttcatcaactattgctcacagaggagcttcGgttgatatagctatttttttcactccatttagcaggaatttcatctattctaggagcagtaaattttattactactgtaattaatatacgCtcaacaggaatttcatttgaCcgaataccTttatttgtCtgatcTgtagtaattacTgcacttttattattattatcattGcctgtattagctggagcaattactatactattaacagatcgaaatttttaatacatcatttttttgacccagccggaggagggggatcctattttatatcaacatttattt.

Remarks: *P. maai* sp. nov. was keyed to *Pseudolynchia canariensis* using the keys provided by Maa [28, 31]. The males of *P. maai* sp. nov. and *P. canariensis* are morphologically similar, with the primary difference being found in females, where the sclerotization of the pregenital plate is different. In *P. maai* sp. nov., the pregenital plate is distinctly sclerotized on the sides, leaving a pale strip in the middle (Figure 2F). In contrast, *P. canariensis* has a uniformly colored pregenital plate, with any slight sclerotisations appearing as gradual color changes without a distinct boundary (Figure 3E). Additionally, DNA barcoding shows a consistent 3.19% difference between the two species (Supporting Information 7: File S4). Geographically, *P. canariensis COI* sequences from GenBank and BOLD cluster together with our *P. canariensis* sequences, confirming its cosmopolitan distribution. In contrast, *P. maai* sp. nov. does not have any related sequence on GenBank or BOLD, indicating that this species is currently only found in Singapore.

Holotype GenBank accession number: PQ247117.

Distribution: Singapore.

Hosts in Singapore: *Spilopelia chinensis* (Scopoli, 1786), *Treron vernans* (Linnaeus, 1771).

Etymology: This specific epithet honors the late Maa Tsing-chao for his tremendous contributions to the family Hippoboscidae. His monographs have been widely used and cited by all who work on Hippoboscidae.

Holotype: ♀, Singapore: Clementi, Ulu Pandan Road (1.3190663, 103.7732965), 28 March 2021, bird host: *S. chinensis* (Scopoli, 1786) (ZRCBDP0298068).

Paratypes: 1♀, Singapore: Clementi, Ulu Pandan Road (1.3190663, 103.7732965), 03 June 2020, bird host: *T. vernans* (Linnaeus, 1771) (ZRCBDP0298058). 1♂, Singapore: Clementi, Ulu Pandan Road (1.3190663, 103.7732965), 25 February 2021, bird host: *S. chinensis* (Scopoli, 1786) (ZRCBDP0298064). 1♀, Singapore: Clementi, Ulu Pandan Road (1.3190663, 103.7732965), 25 February 2021, bird host: *S. chinensis* (Scopoli, 1786) (ZRCBDP0298066). 1♀, same as holotype (ZRCBDP0298069).

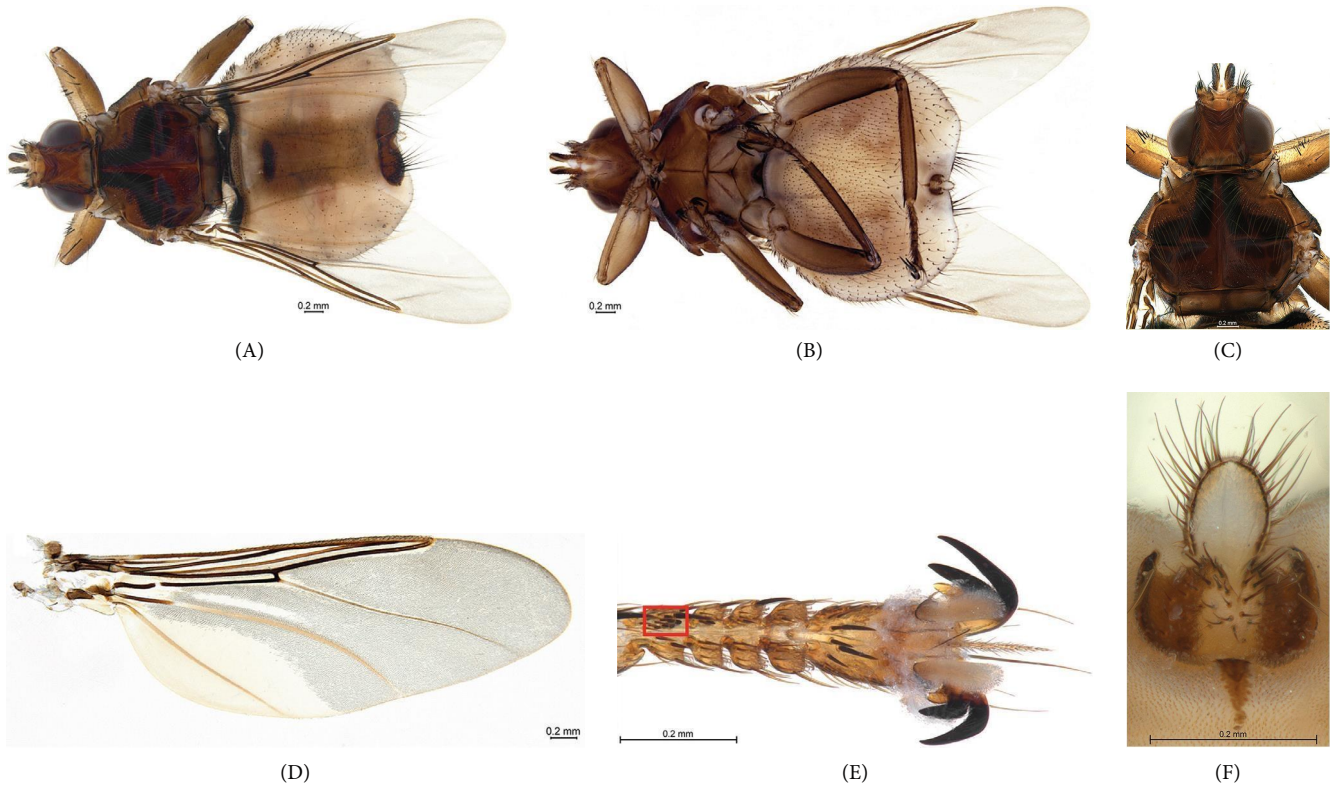


FIGURE 2: *Pseudolynchia maai* sp. nov.. (A) Dorsal habitus (♀, ZRCBDP0298068). (B) Ventral habitus (♀, ZRCBDP0298068). (C) Dorsal view of head and thorax (♀, ZRCBDP0298068). (D) Right wing (♀, ZRCBDP0298068). (E) Ventral view of mid tarsus (♂, ZRCDBP0298051). Boxed in red are the peg-like modified spines on the basal ventral part of the first segment of mid tarsus. (F) Female terminalia (♀, ZRCBDP0298068).

3.4. Species Information and Diagnosis. Subfamily Ornithomyinae

Icosta

(Key: Maa [32]: p. 34)

Icosta (*Ornithoponus*) *maquilingensis* (Ferris, 1924)

(Figure 4; Maa [29]: p. 66)

Morphological diagnosis: https://singapore.biodiversity.online/species/A-Arth-Hexa-Diptera-003087 (Supporting Information 6: File S3).

Molecular diagnosis: (16: C, 62: A, 133: T, 139: T, 146: A, 147: A, 296: C, 298: T) mapped onto barcode for ZRCBDP0432266: tttatcatcaactatCgcccatagaggatcatcagtagatttagctatttttttcccttcatAtagctggaatttcttcaatcctaggggctgtaaattttattactactgtaattaatatacgatcaacaggTatttcTtttgacAAaatacccttatttgtttgatccgtagtaattacagccttattacttcttttatcattacctgttttagcaggggctattactatattattgactgatcgaaattttaatacttcatttttttgatccagctgggggaggagatcctattCtTtatcaacatttattt.

Materials examined: 1♀, Singapore: Kranji Marshes (1.420236,103.728852), 16 May 2018, Malaise Trap KM02 (ZRCBDP0265523). 1♀, Singapore: Sin Ming Housing Estate, 20 April 2022, bird host: *Gallus gallus* (Linnaeus, 1758) (ZRCBDP0432265). 1♀, Singapore: Sin Ming Housing Estate, 20 April 2022, bird host: *G. gallus* (Linnaeus, 1758) (ZRCBDP0432266).

Distribution: Laos, Malaysia (Borneo), Myanmar, Philippines, Taiwan, Thailand, Vietnam [29, 32].

Hosts in Singapore: *G. gallus* (Linnaeus, 1758).

Known host families: Ardeidae, Bucerotidae, Capitonidae, Cuculidae, Phasianidae, Psittacidae, Rallidae [32, 36].

Ecology: The average infestation rate was 28%, and the average number of hippoboscid flies per infested bird was two [29]. Maa [32] has recorded phoretic relationships with unidentified avian lice (Phthiraptera).

Taxonomy and Nomenclature: Maa [32], synonym: *Ornithophila maquilingensis* Ferr., 1924.

Icosta (*Ardmoeca*) *omnisetosa* Maa, 1969

(Figure 5; Maa [32]: p. 152; Nartshuk et al. [37]).

Morphological diagnosis: https://singapore.biodiversity.online/species/A-Arth-Hexa-Diptera-002943 (Supporting Information 6: File S3).

Molecular diagnosis: (13: A, 28: T, 62: C, 85: T, 88: T, 128: T, 173: A, 286: T) mapped onto barcode for ZRCBDP0085805: ntntcatcaacAattgctcatagaggTgcatcagtagatatagctatcttttctcttcatCtagctggaatttcttcaattctTggTgcagtaaattttattactacagtaattaatatacgatctTcaggaatttcatttgatcgaataccattatttgtatgatcagtaAtaattacagcattattattactattatcattaccagtattagctggagcaattactatactattaactgatcgaaattttaatacatcannnttngatccagctggaggaggTgatcctattttatatcaacatttattt.

Materials examined: 1♀, Singapore: Block 565, Choa Chu Kang Street 52 (1.3952778, 103.7455556), 02 April 2017, bird host: *Lewinia striata* (Linnaeus, 1776) (ZRCBDP0085805). 1♀, Singapore: Jurong Bird Park (1.3200634, 103.7033733), 02 December 2021, bird host: *Rallina fasciata* (Raffles, 1822) (ZRCBDP0298059).

FIGURE 3: *Pseudolynchia canariensis*. (A) Female dorsal habitus (ZRCBDP0243305). (B) Female ventral habitus (ZRCBDP0243305). (C) Male dorsal habitus (ZRCBDP0273045). (D) Male ventral habitus (ZRCBDP0273045). (E) Female terminalia (ZRCBDP0298068). (F) Female right wing (ZRCBDP0273048).

Distribution: Known from Oriental Region (New Guinea, Philippines, Malaysia, China), and the Russian Federation (Far East) [28, 32, 37, 38].

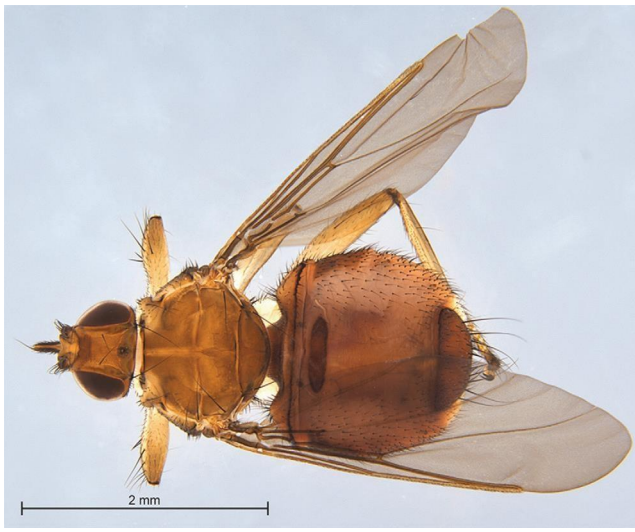
Hosts *in Singapore*: *L. striata* (Linnaeus, 1766), *R. fasciata* (Raffles, 1822).

Known host families: Rallidae, Charadriidae (Possible stray record), Laniidae (Possible stray record) [32, 36].

Ecology: Known to host fungus and mites [32].

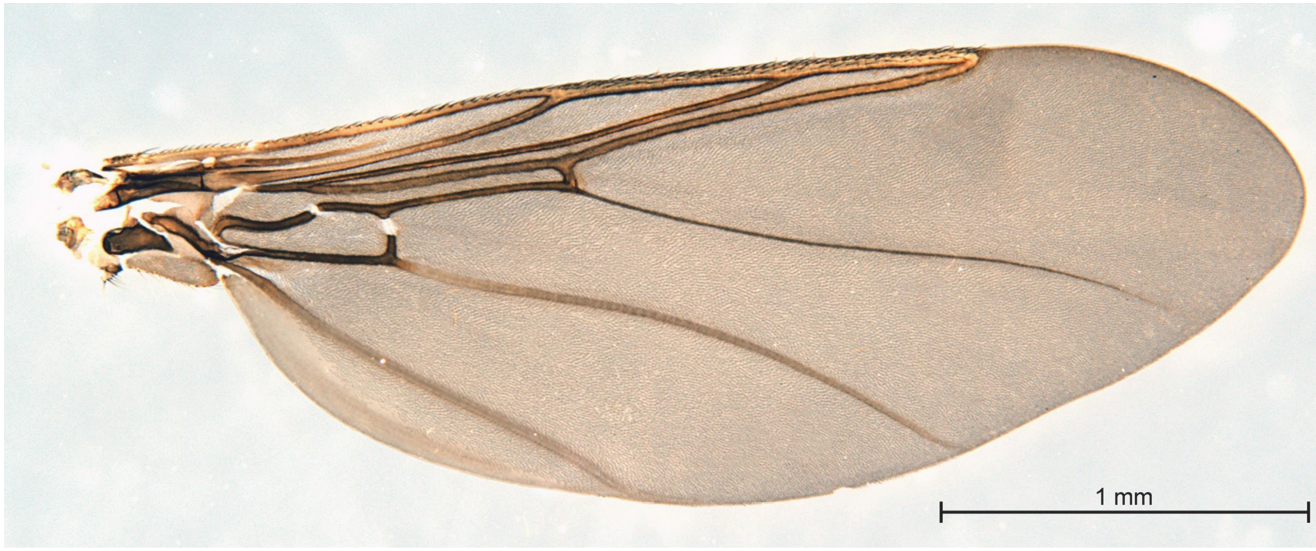
Taxonomy and nomenclature: *I. omnisetosa* was first suggested as a species by Maa [29] but no descriptions were written. Maa [32] subsequently described it as a subspecies, *I. holoptera omnisetosa* Maa, 1969. Nartshuk et al. [37] then elevated it back to species status.

Icosta (*Icosta*) *plana* (Walker, 1861)



(A)

(B)



(C)

Figure 4: *Icosta maquilingensis* (♀, ZRCBDP0432265). (A) Dorsal habitus. (B) Ventral habitus. (C) Right wing.

(Figure 6; Maa [32]: p. 107)

Morphological diagnosis: https://singapore.biodiversity.online/species/A-Arth-Hexa-Diptera-003088 (Supporting Information 6: File S3).

Molecular diagnosis: (13: C, 29: T, 109: C, 197: C, 199: T, 208: G, 262: C, 274: T) mapped onto barcode for ZRCBDP0071948: ctttcatcaacCattgctcacagaggaTcatcagttgatatagctatttttttcattacatttagctggaatttcatcaattttaggagcagtaaattttattactacCgtaattaatatacgatcaacaggaatttcatttgatcgaataccattatttgtttgatctgtagtaattacagcattactactacttCtTtcactaccGgtattagcaggagcaattactatactattaacagatcgaaatttttaatacatcCttttttgatccTgctggaggaggagacccaattttatatcaacatttattt.

Materials examined: 1♀, Singapore: Westside 33 Condominium (1.2943754, 103.7667509), 16 April 2017, bird host: *Chalcophaps indica* (Linnaeus, 1758) (ZRCBDP0071948). 1♀, Singapore, 20 November 2018, bird host: *C. indica* (Linnaeus, 1758) (ZRCBDP0298035).

Distribution: New Guinea [32].

Hosts in Singapore: *C. indica* (Linnaeus, 1758).

Known host families: Paradisaeidae, Cracticidae, Ptilonorhynchidae [32].

Ecology: The highest number of hippoboscid flies per infested bird was three flies [32].

Icosta (*Ornithoponus*) *sensilis* Maa, 1969

(Figure 7; Maa [31]: p. 74)

Morphological diagnosis: https://singapore.biodiversity.online/species/A-Arth-Hexa-Diptera-002942 (Supporting Information 6: File S3).

Molecular diagnosis: (55: T, 137: A, 139: C, 173: T, 187: T, 193: T, 220: T, 229: A) mapped onto barcode for ZRCBDP0273054: cctttcatctactattgcccatagaggagcatcagtagatatagctatttttttcTcttcatttagctggaatttcttcaattttaggagcagtaaattttattactactgtaattaatatacgatcaactggaattAcCtttgaccgaatacctttatttgtatgatcagtaTtaattacagctctTttactTttactatctttaccagttttagctggTgctattacAa

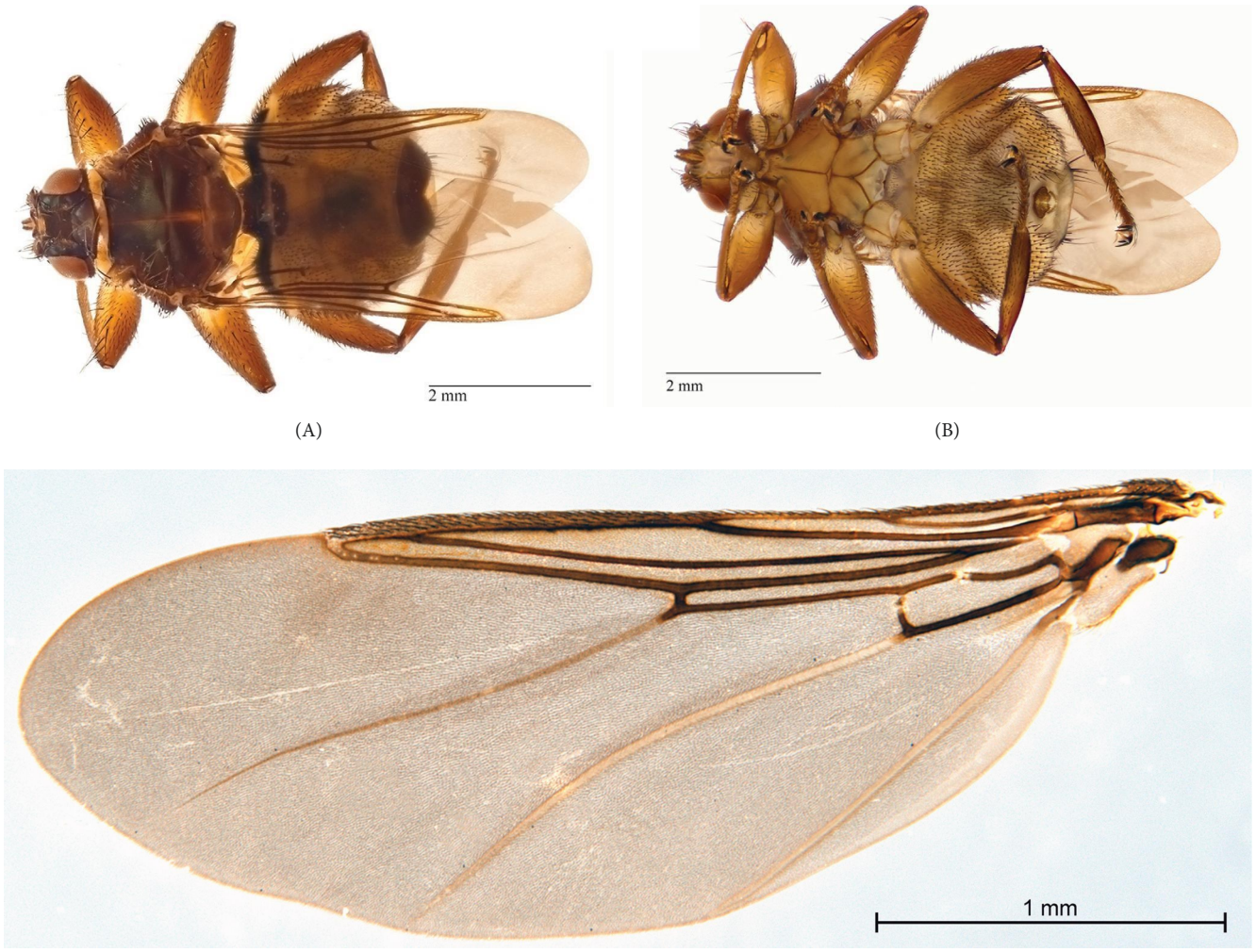


Figure 5: *Icosta omnisetosa*. (A) Dorsal habitus (♀, ZRCBDP0085805). (B) Ventral habitus (♀, ZRCBDP0085805). (C) Left wing (♀, ZRCBDP0298059).

tattattaactgatcgaaattttaatacttcatttttgatccagctggaggaggagatccaa ttttatatcaacatttattt.

Materials examined: 1♂, Singapore: Jalan Mempurong (1.4590975, 103.841095), 21 October 2018, bird host: *Lonchura punctulata* (Linnaeus, 1758) (ZRCBDP0273053). 1♀, Singapore: Jalan Mempurong (1.4590975, 103.841095), 21 October 2018, bird host: *L. punctulata* (Linnaeus, 1758) (ZRCBDP0273054).

Distribution: Oriental region [28, 32].

Hosts in Singapore: *L. punctulata* (Linnaeus, 1758).

Known host families: Cuculidae, Hirundinidae, Laniidae, Motacillidae, Muscicapidae, Ploceidae, Pycnonotidae [32, 36].

Ecology: Highest number of hippoboscid flies per infested bird was three flies [32]. Maa [32] has recorded phoretic relationships with unidentified avian lice (Phthiraptera).

Ornithoica

(Key: Maa [30]: p. 26)

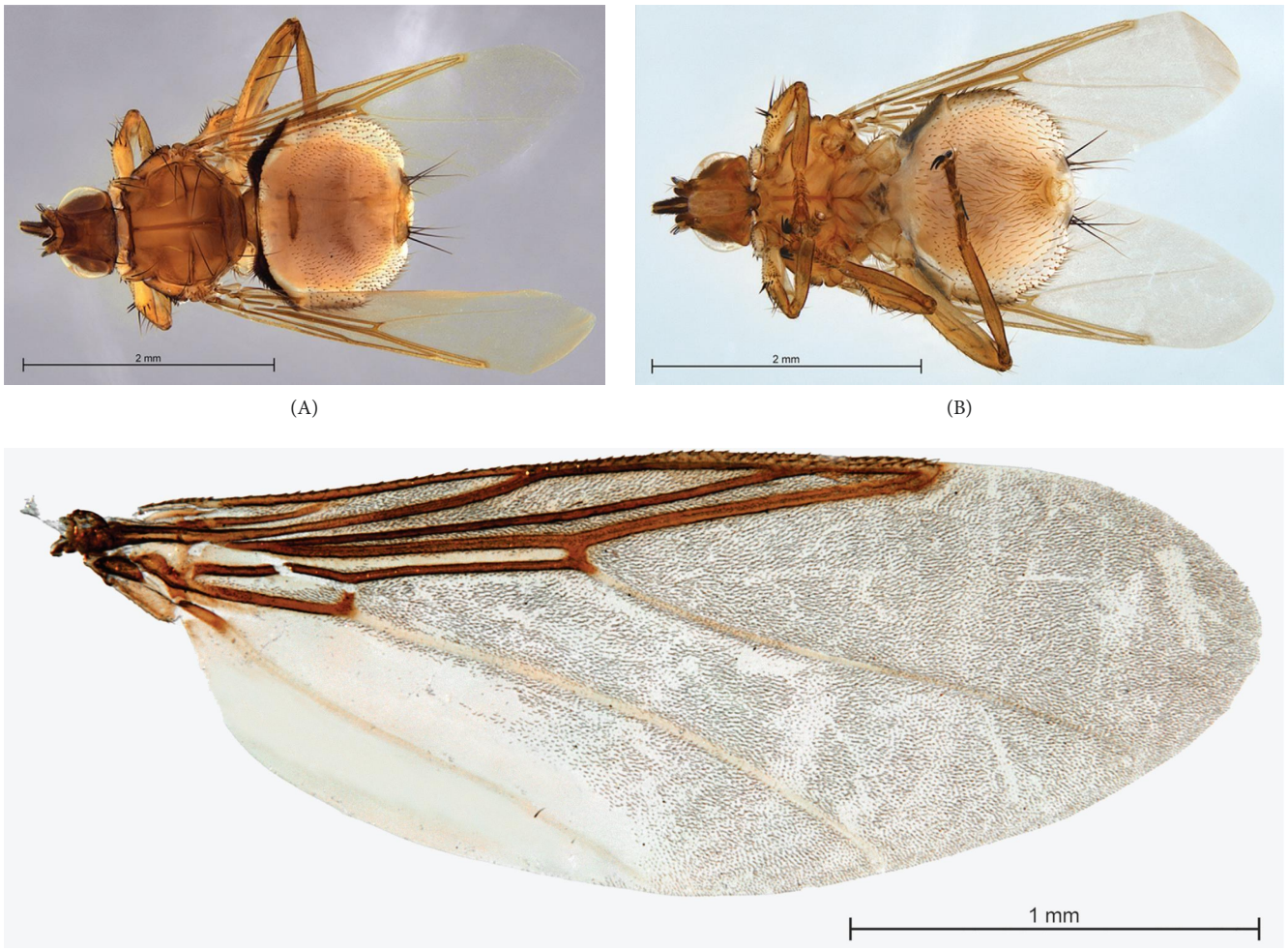
Ornithoica bistativa Maa, 1966

(Figure 8; Maa [30]: p. 52)

Morphological diagnosis: https://singapore.biodiversity.online/species/A-Arth-Hexa-Diptera-002941 (Supporting Information 6: File S3).

Molecular diagnosis: (23: G, 46: A, 54: G, 88: G, 106: A, 172: T, 175: T, 274: T) mapped onto barcode for ZRCBDP0273055: cctatcatctaatattgcccatGgaggagcatcagtagatttagcAatttttaGcctaca tttagctggaatttcatcaattttaggGgctgtaaattttattacAacagtaattaatatacga tcaactggaatttcatttgatcgaatacctttatttgtttgatcagtTgtTattacagctttatta ttattattatctttacctgtattagcaggagcaattactatattattaacagatcgaaatttaaata cttctttttttgacccTgcgggaggaggagacccaattttataccaacatttattt.

Materials examined: 1♂, Singapore, 14 July 2020, bird host: *Oriolus chinensis* Linnaeus, 1766 (ZRCBDP0243299). 1♀, Singapore: 6 Walmer Drive (1.3641362, 103.8650515), 15 October 2018, bird host: *Ceyx erithaca* (Linnaeus, 1758) (ZRCBDP0265522). 1♂, Singapore: 611 Elias Road (1.3751382, 103.940263), 20 January 2019, bird host: *O. chinensis* Linnaeus, 1766 (ZRCBDP0273052). 1♀, Singapore: Devonshire Road (1.298634,103.837396), 13 November 2018, bird host: *Pitta moluccensis* (Müller, 1776) (ZRCBDP0273055). 1♂, Singapore: Devonshire Road (1.298634,103.837396), 13 November 2018, bird host: *P. moluccensis* (Müller, 1776) (ZRCBDP0273056). 1♀, Singapore: Devonshire Road (1.298634,103.837396), 13 November 2018, bird host: *P. moluccensis* (Müller, 1776) (ZRCBDP0273057).



(A) (B)

(C)

Figure 6: *Icosta plana* (♀, ZRCBDP0071948). (A) Dorsal habitus. (B) Ventral habitus. (C) Right wing.

Distribution: Borneo, Thailand, Malaysia, and Japan [30, 39].

Hosts in Singapore: *C. erithaca* (Linnaeus, 1758), *O. chinensis* Linnaeus, 1766, *P. moluccensis* (Müller, 1776).

Known host families: Alcedinidae, Columbidae, Corvidae, Cuculidae, Dicruridae, Muscicapidae, Picidae, Pittidae, Ploceidae, Prionopidae, Pycnonotidae, Sturnidae [30].

Taxonomy and nomenclature: The taxonomy of this species group, consisting of *O. bistativa*, *O. philippinensis*, *O. tridens*, and *O. momiyamai*, is unresolved, and a future detailed revision is necessary. Maa [40] treated *O. bistativa*, *O. philippinensis*, and *O. tridens* as allopatric species of *O. stipituri* (note in Mogi et al. [41]).

Ecology: Lee et al. [21] have mistakenly identified this species as *O. momiyamai*. We here correct this and state that *O. bistativa* is in a phoretic relationship with the louse species *Guimaraesiella* sp. [21].

Ornithoica submicans Maa, 1963

(Figure 9; Maa [30]: p. 99)

Morphological diagnosis: https://singapore.biodiversity.online/species/A-Arth-Hexa-Diptera-002939 (Supporting Information 6: File S3).

Molecular diagnosis: (28: G, 188: C, 190: T, 202: C, 203: C, 205: T, 217: C, 283: G) mapped onto barcode for ZRCBDP0071947: ctatcatcaaatattgctcatggaggGgcttcagtagatttagctatttttagccttcatttagctggaatttcatcaattttaggagctgtaaattttattactacagtaattaatatacgatcaactggaatttcatttgatcgaataccttttatttgtatgatcagtagtaattactgcactaCtTctacttctatcCCtTccagtattagcCggagcaattactatattattaactgatcgaaatttaaatacttctttttttgatccagcaggaggGggggatccaattttataccaacatttattt.

Materials examined: 1♂, Singapore: Block 14 Toh Yi Drive (1.338580,103.775300), 20 March 2015, bird host: *Lophospiza trivirgata* (Temminck, 1824) (ZRCBDP0071947).

Distribution: Philippines [30, 36].

Hosts in Singapore: *L. trivirgata* (Temminck, 1824).

Known host families: Bucerotidae [30, 36].

Ecology: Maa [30] found that the highest number of hippoboscid flies per infested bird was three.

Taxonomy and nomenclature: Maa [30], synonym: *Ornithoica unicolor* Ferr. (nec Speis.) 1927.

Ornithophila.

Ornithophila metallica (Schiner, 1864).

(Figure 10; Schiner 1864 in Theodor and Oldroyd [42]: p. 34).

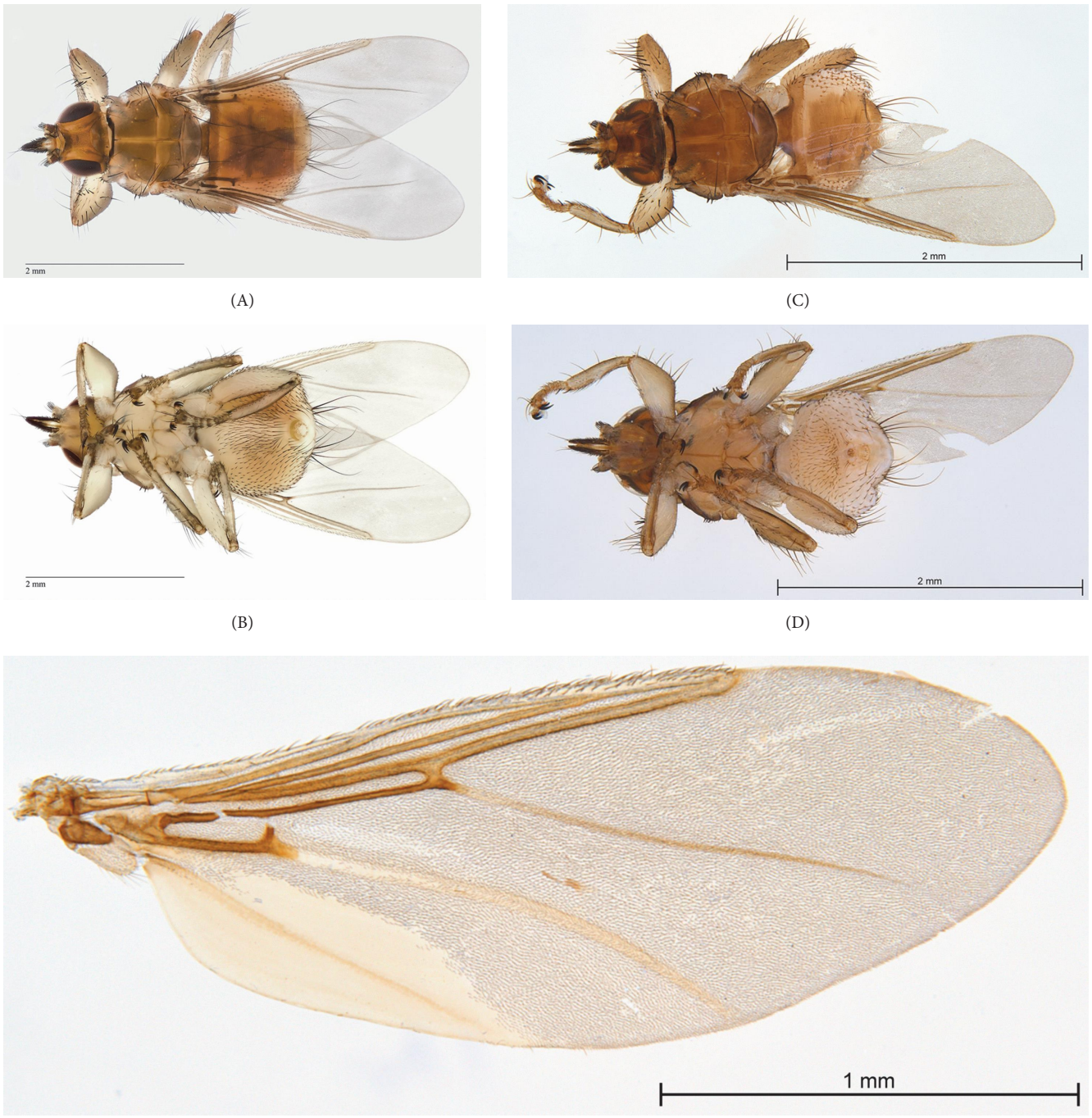


FIGURE 7: *Icosta sensilis*. (A) Female dorsal habitus (ZRCBDP0273054). (B) Female ventral habitus (ZRCBDP0273054). (C) Male dorsal habitus (ZRCBDP0273053). (D) Male ventral habitus (ZRCBDP0273053). (E) Female right wing (ZRCBDP0273054).

Morphological diagnosis: https://singapore.biodiversity.online/species/A-Arth-Hexa-Diptera-002940 (Supporting Information 6: File S3).

Molecular diagnosis: (7: T, 24: T, 29: A, 110: A, 112: T, 212: C, 214: T, 283: T) mapped onto barcode for ZRCBDP0213054: tttatcTtctaatattgctcataTaggaActtccgttgatttagctatttttttcattacatttagctggaatttcttctattttaggagcagtaaattttattactacaAtTattaatatacgatcaacaggaatttcatttgatcgaataccttttatttgtatgatcagtagctattaccgcactattattacttttaagtttacctgttCtTgctggagcaattactatattattaacagatcgaaattttaatacatcatttttttgatccagctggaggTggagacccaattttataccaacatttattt.

Materials examined: 1♀, Singapore: 401 Choa Chu Kang Avenue 3 (1.3784066, 103.7366966), 19 June 2015, bird host: *Aplonis panayensis* (Scopoli, 1786) (ZRCBDP0018462). 1♀, Singapore: National University of Singapore, King Edward

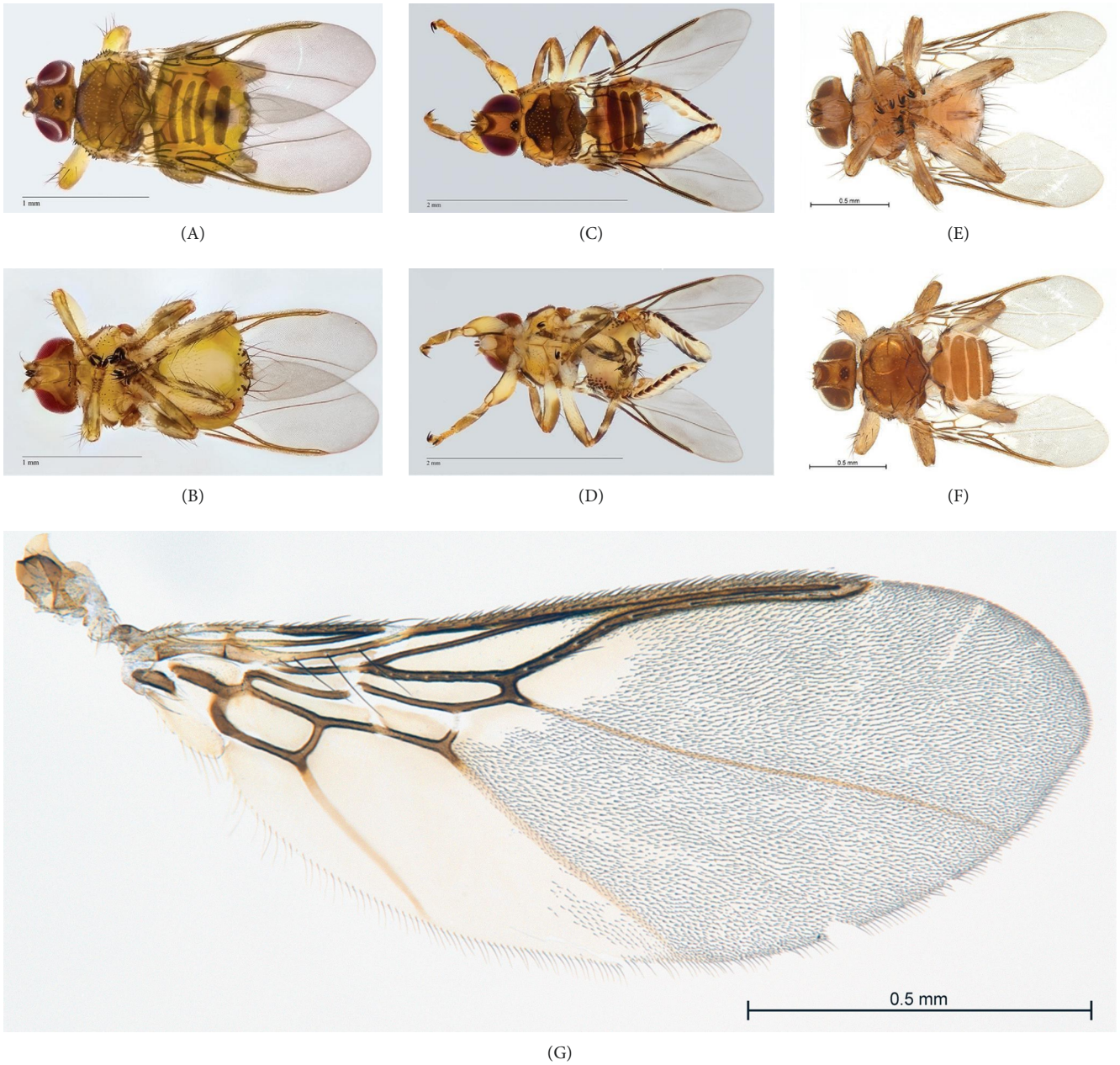


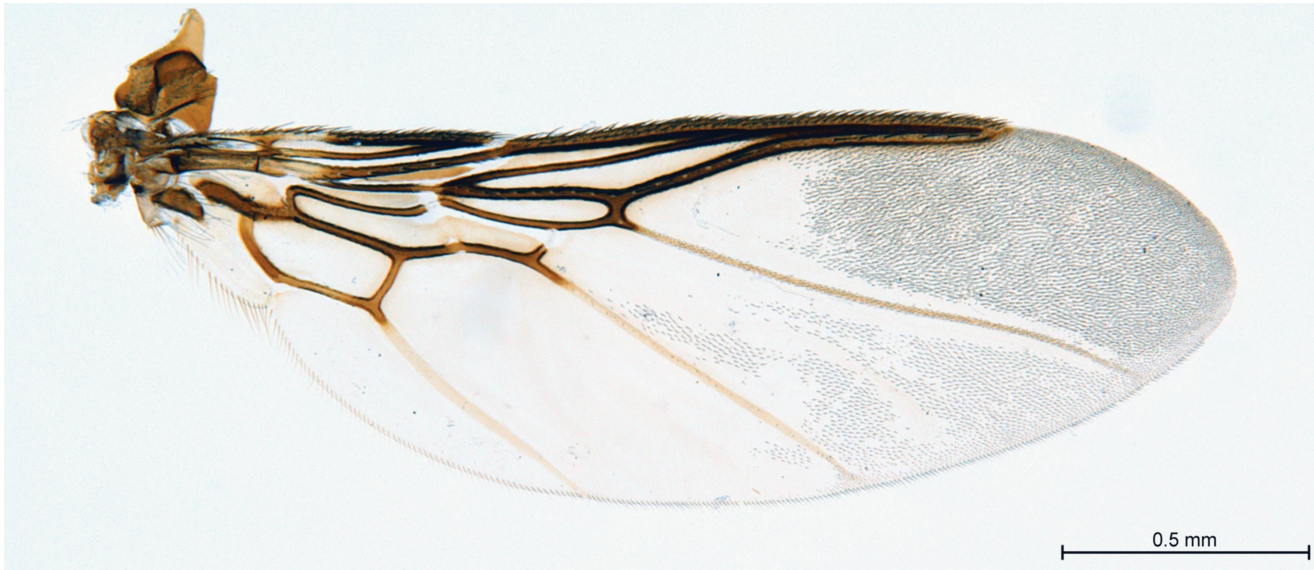
Figure 8: *Ornithoica bistativa*. (A) Female dorsal habitus (ZRCBDP0273055). (B) Female ventral habitus (ZRCBDP0273055). (C) Gynandromorph dorsal habitus (ZRCBDP0273056). (D) Gynandromorph ventral habitus (ZRCBDP0273056). (E) Male dorsal habitus (ZRCBDP0243299). (F) Male ventral habitus (ZRCBDP0243299). (G) Female right wing (ZRCBDP0273057).

VII Hall (1.292132, 103.781308), 09 September 2014, bird host: *A. panayensis* (Scopoli, 1786) (ZRCBDP0213053). 1♂, Singapore: National University of Singapore, King Edward VII Hall (1.292132, 103.781308), 09 September 2014, bird host: *A. panayensis* (Scopoli, 1786) (ZRCBDP0213054). 1♂, Singapore: National University of Singapore, King Edward VII Hall (1.292132, 103.781308), 09 September 2014, bird host: *A. panayensis* (Scopoli, 1786) (ZRCBDP0213055). 1♀, Singapore, 14 July 2020, bird host: *O. chinensis* Linnaeus, 1766 (ZRCBDP0298066). 1♀, Singapore, bird host: *Corvus splendens* Vieillot, 1817 (ZRCBDP0243300). 1♀, Singapore: 89 Gardenia Road (1.354371, 103.8253519), 06 February 2018, bird host: *P. moluccensis* (Müller, 1776) (ZRCBDP0273046). 1♀, Singapore: Depot Road (1.2815816, 103.8076418), 27 March 2015, bird host: *O. chinensis* Linnaeus, 1766 (ZRCBDP0273050). 1♀, Singapore, 02 February 2018, bird host: *P. moluccensis* (Müller, 1776) (ZRCBDP0298034). 1♀, Singapore: National University of Singapore, Kent Ridge Campus, 05 February 2018, bird host: *Lonchura maja* (Linnaeus, 1766) (ZRCBDP0298036). 1♀, Singapore: 9 Tan Boon Chong Avenue (1.315254, 103.788179), 26 May 2019, bird host: *Eurystomus orientalis* (Linnaeus, 1766) (ZRCBDP0353766).

Distribution: Palearctic, Afrotropical, Oriental, and Australasian regions [36].

(A)

(B)



(C)

Figure 9: *Ornithoica submicans* (♂,ZRCBDP0071947). (A) Dorsal habitus. (B) Ventral habitus. (C) Right wing.

Hosts in Singapore: *A. panayensis* (Scopoli, 1786), *C. splendens* Vieillot, 1817, *L. maja* (Linnaeus, 1766), *O. chinensis* Linnaeus, 1766, *P. moluccensis* (Müller, 1776).

Known host families: Accipitridae, Alaudidae, Alcedinidae, Apodidae, Ardeidae, Artamidae, Campephagidae, Capitonidae, Charadriidae, Coliidae, Columbidae, Coraciidae, Corvidae, Cracticidae, Cuculidae, Dicruridae, Eurylaimidae, Falconidae, Fringillidae, Hirundinidae, Irenidae, Laniidae, Megapodiidae, Meliphagidae, Meropidae, Motacillidae, Muscicapidae, Musophagidae, Oriolidae, Paradisaeidae, Pariae, Phasianidae, Picidae, Pittidae, Ploceidae, Prionopidae, Psittacidae, Ptilonorhynchidae, Pycnonotidae, Strigidae, Sturnidae, Trogonidae, Upupidae [36].

Ecology: *O. metallica* is known to be in a phoretic relationship with *Sturnidoecus pastoris* [43], *Brueelia*-complex sp. [44], and *Guimaraesiella* sp. [21].

Taxonomy and nomenclature: Theodor and Oldroyd [42], synonym: *Ornithophila pallipes* Speiser, 1904.

Pseudolynchia

(Keys: Maa [28]: p. 126; Maa [31]: p. 255)

P. canariensis (Macquart in Webb and Berthelot, 1839)

(Figure 3; Maa [28]: p. 128)

Morphological diagnosis: https://singapore.biodiversity.online/species/A-Arth-Hexa-Diptera-002281 (Supporting Information 6: File S3).

Molecular diagnosis: (22: C, 67: A, 83: T, 88: A, 97: T, 203: T, 205: A, 226: C) mapped onto barcode for ZRCBDP0243295: tctttcatcaactattgctcaCagaggagcttcagttgatatagctatttttttcactccatttagcAggaatttcatctattTtaggAgcagtaaaTtttattactactgtaattaatatacgttcaacaggaatttcatttgatcgaatacctttatttgtttgatccgtagtaattactgcacttttattattatcaTtAcctgtattagctggagcaatCactatactattaacagatcgaaattttaatacatcatttttgatccagccggaggaggagatcctattttatatcaacatttattt.

FIGURE 10: *Ornithophila metallica*. (A) Female dorsal habitus (ZRCBDP0018462). (B) Female ventral habitus (ZRCBDP0018462). (C) Male dorsal habitus (ZRCBDP0213053). (D) Male ventral habitus (ZRCBDP0213053). (E) Female right wing (ZRCBDP0273050).

Materials examined: 1♀, Singapore, 29 July 2020, bird host: unknown (ZRCBDP0243294). 1♀, Singapore, 22 April 2020, bird host: *Falco peregrinus Tunstall*, *1771* (ZRCBDP0243295). 1♀, Singapore, 2019, bird host: *Egretta garzetta* (Linnaeus, 1776) (ZRCBDP0243296). 1♂, Singapore, 24 March 2021, bird host: unknown (ZRCBDP0243302). 1♀, Singapore, 2019, Bird Host: *Amaurornis phoenicurus* (Pennant, 1769) (ZRCBDP0243305). 1♀, Singapore, 08 August 2018, bird host: *T. vernans* (Linnaeus, 1771) (ZRCBDP0265521). 1♂, Singapore, 06 October 2017, Bird Host: *Eudynamys scolopaceus* (Linnaeus, 1758) (ZRCBDP0273045). 1♀, Singapore, 16 March 2015, bird host: *Columba livia* Gmelin, 1789 (ZRCBDP0273048). 1♂, Singapore, bird host: unknown (ZRCBDP0298046). 1♂, Singapore, bird host: unknown (ZRCBDP0298047). 1♂, Singapore, bird host: Unknown (ZRCBDP0298048). 1♂, Singapore, bird host: unknown (ZRCBDP0298049). 1♂, Singapore, bird host: unknown (ZRCBDP0298050).1♂, Singapore: 989A Jurong West Street 93 (1.3370723, 103.6916327), 28 January 2022, (ZRCBDP0298060).1♀, Singapore: 989A Jurong West Street 93 (1.3370723, 103.6916327), 08 March 2021 (ZRCBDP0298061). 1♂, Singapore: Wildlife Reserves Singapore (1.3190663, 103.7732965), 19 March 2020, bird host: *Ninox japonica* (Temminck & Schlegel, 1845) (ZRCBDP0351958). 1♂, Singapore: 244 Tanjong Katong Road (1.3062918, 103.8958113), 20 February 2020, bird host: *C. livia* Gmelin, 1789 (ZRCBDP0353756). 1♂, Singapore: 244 Tanjong Katong Road (1.3062918, 103.8958113), 20 February 2020, bird host: *C. livia* Gmelin, 1789 (ZRCBDP0353757). 1♂, Singapore, 16 March 2015, bird host: *C. livia* Gmelin, 1789 (ZRCENT00002034).

Distribution: Cosmopolitan species. It was originally found in the Old World tropics and subtropics but is currently known to be in Afrotropical, Mediterranean, Oriental, Palearctic, Nearctic, and Neotropical regions [28, 36].

Hosts in Singapore: *A. phoenicurus* (Pennant, 1769), *C. livia* Gmelin, 1789, *E. scolopaceus* (Linnaeus, 1758), *F. peregrinus* Tunstall, 1771, *T. vernans* (Linnaeus, 1771).

Known host families: Accipitridae, Alaudidae, Ciconiidae, Columbidae, Coraciidae, Cuculidae, Falconidae, Muscicapidae, Musophagidae, Phasianidae, Pteroclidae, Strigidae, Sturnidae [28, 36].

Ecology: *P. canariensis* may exhibit a preference for specific host species over other avian species from the same family within the same locality [28]. Maa and Kuo [45] examined 19 *Treron sieboldii* (Temminck, 1835), 30 *Spilopelia orientalis* (Latham, 1790), and 56 *S. chinensis* and found that their infestation rates of *P. canariensis* were 0%, 97%, and 96%, respectively, with the highest catch from a single host being 10 flies (average two flies per infested bird (rounded down)).

Bequaert [46, 47] reported that the rate of mite-infested *P. canariensis* ex *C. livia* was about 12.5% for males and 18% for females in the most heavily parasitized host. The number of mites per infested fly varied from one to 10. In nine out of 21 cases, mites were found on two or more different sites on the same individual flies. In 10 of the 21 cases, there were egg clusters as well. *P. canariensis* is also known to have a phoretic relationship with *Columbicola columbae* (Linnaeus, 1758) [48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58].

Taxonomy and nomenclature: Maa [28], synonyms: *Olfersia testacea* Mcq. 1843, *Olfersia rufipes* Mcq. 1847, *Olfersia falcinelli* Rndn. 1879, *Olfersia maura* Bigot, 1885, *Olfersia lividicolor*, 1885, *Olfersia capensis* Bigot, 1885, *Olfersia exornata* Speis. 1900, *Lynchia simillima* Speis. 1904.

Disease associations: *Haemoproteus columbae* Kruse, 1890 [59], *Haemoproteus* [60], *Haemoproteus* and *Plasmodium* [61].

P. garzettae (Rondani, 1879)

(Figure 11; Maa [27]: p. 132)

Morphological diagnosis: https://singapore.biodiversity.online/species/A-Arth-Hexa-Diptera-003086 (Supporting Information 6: File S3).

Molecular diagnosis: (1: T, 40: C, 67: A, 124: C, 154: A, 199: G, 203: C, 277: T) mapped onto barcode for ZRCBDP0243303: TctttcatcaactattgctcacagaggagcttcagttgaCatagctatttttttcactccatttagcAggaatttcatctattttaggagcagtaaactttattactactgtaattaatatacgCtcaacaggaatttcatttgatcgaataccAttatttgtttgatctgtagtaattactgcactttattattattGtcaCtacctgtattagctggagcaattactatactattaacagatcgaaattttaatacatcatttttttgatccagcTggaggaggagatcctattttatatcaacatttattt.

Materials examined: 1♀, Singapore, bird host: *Caprimulgus macrurus* Horsfield, 1821 (ZRCBDP0243303).

Distribution: Africa (Afrotropical region: Kenya, Sudan, Mozambique), Cyprus, France, Switzerland, Thailand, Taiwan, Philippines [28, 36].

Hosts in Singapore: *C. macrurus* Horsfield, 1821.

Known host families: Accipitridae, Apodidae, Burhinidae, Caprimulgidae, Corvidae, Cuculidae, Strigidae [28, 36].

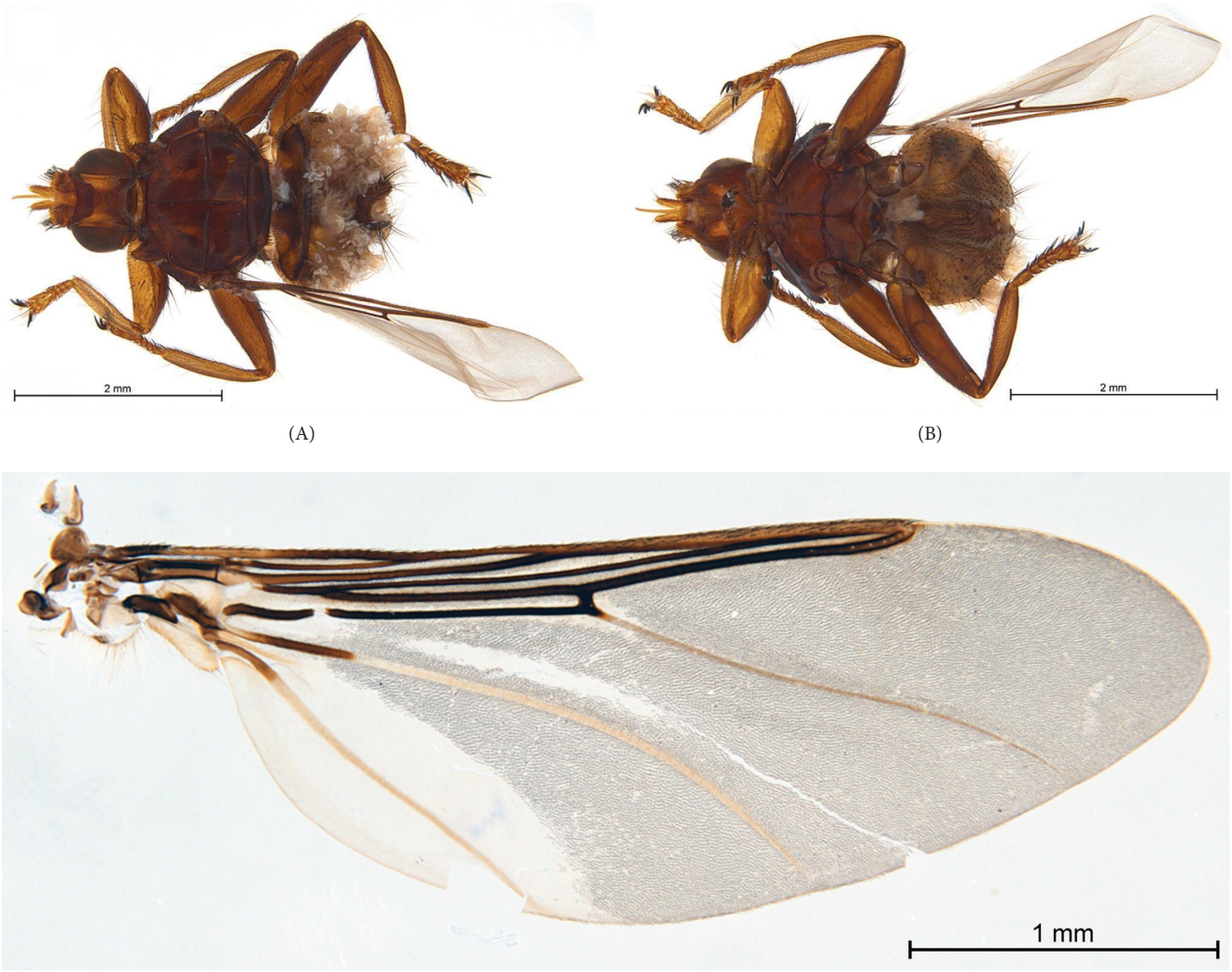
Ecology: Maa & Kuo [45] found that the infestation rate of *P. garzettae* was 44%, and highest catch per infested bird was four flies (average of about two flies (rounded down)).

Taxonomy and nomenclature: Maa [28], synonyms: *Pseudolynchia fradeorum* Tendeiro 1951, *Pseudolynchia rufipes* auctt, nee Mcq.

3.5. Species Interactions. We first plotted a bipartite network for the entire dataset (Supporting Information 8: File S5), revealing 25 species interactions between hippoboscid flies and bird hosts. However, 6 out of 10 hippoboscid fly species were only represented by one or two specimens, often from a single host, limiting the interpretability of the overall network. We thus replotted the bipartite network for the three most abundant hippoboscid species in our dataset (*P. canariensis* ($n = 11$), *O. metallica* ($n = 11$), and *P. maai* sp. nov. ($n = 14$); Figure 12). *P. canariensis* and *O. metallica* were found on seven and six bird species, respectively, each spanning six bird families. In contrast, *P. maai* sp. nov. ($n = 14$) was only found on two bird species from the family Columbidae.

4. Discussion

Hippoboscid flies remain poorly documented in tropical Asia, although they may be important for transmitting disease between bird species. Here, we demonstrate how sampling avian carcasses and active nest sites can be an efficient, low-effort way to obtain hippoboscid fly specimens while obtaining valuable species interaction information. To support accurate identifications of Singapore hippoboscid fly species for



(A) (B)

(C)

Figure 11: *Pseudolynchia garzettae* (♀, ZRCBDP0243303). (A) Dorsal habitus. (B) Ventral habitus. (C) Right wing.

scientists and citizen scientists alike, we provide two identification tools: an easy-to-use pictorial key with annotated images, and molecular diagnoses composed of DMCs based on DNA barcodes. At a minimum, genus-level identification can now be done using good-quality photographs taken with handphones or a digital camera by members of the public by consulting the images on BOS or the pictorial key. Species-level identification, however, may require specimen examination with higher magnification or DNA barcodes, as it often requires information on subtle morphological characters only visible when the specimen is correctly oriented.

As a quick test, we reviewed all 14 hippoboscid observations from Singapore on iNaturalist. Four observations could not be identified because the photos provided insufficient detail to discern important features. For the remaining 10 observations, we used our pictorial key and labeled images on the BOS website to identify eight records to genus (~57%) and two records to species (~14%). The eight records that were identified to genus were from the genera *Pseudolynchia* and *Icosta*. Species identification for *Pseudolynchia* is particularly challenging due to the reliance on the female terminalia as the diagnostic character to differentiate *P. canariensis* from *P. maai* sp. nov., which requires ventral views in the iNaturalist images. Similarly, species identification for *Icosta* depends on lateral views of the head and ventral views of the legs, which were often missing in the available images. Thus, while many iNaturalist images are sufficient for genus-level identification, species-level identification requires a more detailed examination of the specimen or better-quality images, particularly those showing the ventral and lateral aspects.

Prior to this study, only two species recorded for Singapore (*P. canariensis* and *O. metallica*) had DNA barcodes in GenBank or BOLD. Here, we added barcodes for eight additional species, all reliably identified using morphology. By expanding reference data and streamlining identification, our resources lay the foundation for more inclusive biodiversity monitoring, ecological research, and disease transmission studies.

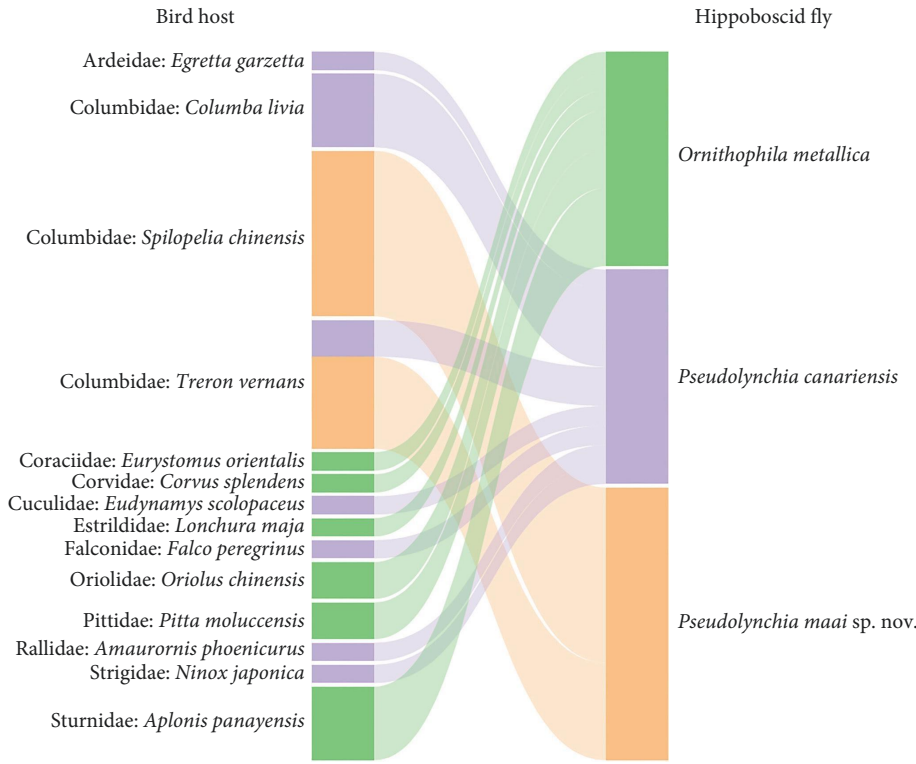


Figure 12: Bipartite species interaction networks between bird host species and the top three most collected hippoboscid fly species in our dataset. The size of the rectangles corresponds to the number of specimens for each species.

4.1. Carcass and Nest Sampling Far Outperform Passive Trapping for Hippoboscid Flies. Direct observation of species interaction yielded 55 of the 58 hippoboscid specimens (94.8%), far outperforming passive methods. Carcass salvage, facilitated by citizen science reports, contributed 44 specimens (~75.8%), while active nest sampling contributed 11 specimens (~19.0%). The relative scarcity of free-flying hippoboscids likely explains the low number of flies in Malaise traps, although other passive methods not used here, such as carbon dioxide traps [62] or blue pan traps [63], may prove more effective. However, a key drawback of passive traps is their inability to retain host–parasite interaction data, which is readily preserved in carcass or nest-based sampling. Recovering such information from passively trapped specimens would require additional methods, such as gut content or fly feces iDNA sequencing [64].

Overall, the sampling of bird carcasses was unexpectedly productive, with hippoboscids recovered from 24% of all carcasses examined (36 out of 148). This is surprising given that hippoboscid flies are thought to rapidly abandon their hosts upon death due to sensitivity to temperature changes [46]. This suggests that carcass salvage, especially in the tropics, may be more effective than previously thought, especially when carcasses are discovered and collected quickly. This bodes well for collecting large amounts of data because Loss et al. [65] estimate that between 365 and 988 million birds die annually from window collisions in the United States, while Grilo et al. [66] estimated 194 million annual bird roadkills in Europe alone. In Singapore, Tan et al. [21] documented 224 fatal bird-building collisions between 2013 and 2020. With such high volumes of bird carcasses being generated and such a relatively high success rate of recovering hippoboscids from the dead birds, carcass sampling could yield significant numbers of specimens. However, we note that the time elapsed between death and collection was unknown for our study, as public reports typically concern the carcass itself rather than the death event. Future work could incorporate autopsy-based estimates of time since death to construct carcass-collection schemes.

In contrast, active nest sampling contributed fewer specimens but remains promising because only two nests yielded 11 flies. Bird ringing initiatives would thus represent an important source of hippoboscid flies. Since the inception of bird ringing, over 100 million birds have been ringed in Europe (EURING databank; www.euring.org) and more than 70 million in North America (North American Bird Banding Laboratory, BBL; www.pwrc.usgs.gov/bbl) [67]. Numerous ringing initiatives have successfully yielded hippoboscid flies directly from mist-netted or banded birds [9, 68, 69, 70], demonstrating the value of this approach. Taken together, these methods (carcass salvage, nest sampling, and bird ringing) are scalable strategies for expanding parasite biodiversity inventories and deepening our understanding of host–parasite interactions.

4.2. Integrative Taxonomy and the Accessibility of Taxonomic Information and Diagnostics. Integrative taxonomy, as originally outlined by Dayrat [10], emphasizes the use of multiple, independent lines of evidence to achieve comprehensive species hypotheses. Our study applies a particular operational

variant of this approach LIT [18], which combines DNA barcoding with targeted morphological examination to provide an efficient and rigorous framework for species delimitation. Using LIT, we successfully delimited a total of 10 species, of which nine were identified, and one new species to science was described, *P. maai* sp. nov.. By combining DNA barcoding and morphological analyses, we were able to resolve complex species delimitation issues that may be confounded when relying on a single line of evidence. For example, in the case of *P. maai* sp. nov., morphology alone might have led to it being considered conspecific with *P. canariensis* due to their subtle morphological differences. However, a 3.15% *COI* sequence divergence between the two suggested the need for detailed morphological examination and revealed the presence of a new species, which has consistent differences in female pregenital sclerotization. This congruence between molecular and morphological characters justified the recognition of *P. maai* sp. nov. as a distinct species.

Beyond species delimitation, integrative taxonomy involving morphological data allows scientists and citizen scientists to participate in biodiversity research as long as practical and openly accessible diagnostic tools are provided. We here uploaded high-resolution annotated images of hippoboscid specimens to BOS (https://singapore.biodiversity.online/taxon/A-Arth-Hexa-Dipt-Hippoboscidae), allowing anyone, anywhere, to explore and identify these flies without physical access to museum collections. To further encourage citizen scientists, we developed an easy-to-use pictorial dichotomous key (Supporting Information 5: File S2), enabling anyone to confidently identify hippoboscid species. Our goal is to democratize biodiversity science, bridging expertise and enthusiasm through practical tools that inspire broader involvement and facilitate discovery.

To facilitate molecular identification, we provided DNA barcodes and a unique combination of nucleotides at specific positions that can diagnose the species in our study with such DMCs. This approach goes beyond simply presenting consensus barcodes (e.g., Sharkey et al. [71]), as specifying a unique combination of DMCs makes the molecular diagnosis contrastive and state-specific, which aligns with the proposal for molecular species diagnosis defined by the International Commission on Zoological Nomenclature (ICZN) [72].

The accuracy of DMCs depends heavily on the quality and geographic scope of the reference dataset, particularly for widespread species with high levels of intraspecific molecular variability. For example, we tested the DMCs generated from our Singapore *P. canariensis* specimens against 30 publicly available sequences on GenBank (EF531220, KF453425, MW853922, OM073981, OQ073507–OQ073525, OR054156, OR288145, OR393872) and BOLD (BIOUG45590-F07, BIOUG46125-A04, BIOUG47846-H05, BIOUG48633-E11). Of these, 10 sequences (from Egypt [$n = 1$], India [$n = 2$], South Africa [$n = 4$], the United States [$n = 1$], Saudi Arabia [$n = 1$], and one sequence without locality information) matched the DMC after allowing for one mismatch. The maximum pairwise distance was 0.69%. In contrast, 20 other sequences (from Russia and Saudi Arabia) had a maximum pairwise distance of 2.17%. This raised the question of whether the identification of the specimens or the DMC was incorrect. Gharsan and Alghamdi [73] provided images for the specimens from Saudi Arabia, which allowed us to confirm that the species was correctly identified. This suggests that the DMC proposed for the widespread *P. canariensis*, based on a geographically limited sample from Singapore, underestimated the intraspecific sequence diversity. This highlights that all diagnoses, morphological or molecular, are hypotheses that are subject to revision as additional sampling reveals additional variation.

4.3. Species Interactions. The bipartite network analysis of the three most sampled hippoboscid fly species in our dataset, *P. canariensis*, *O. metallica*, and *P. maai* sp. nov., revealed distinct patterns of host association (Figure 12). *P. canariensis* and *O. metallica* were each found on hosts from six different bird families, indicating low host specificity. This broad host range likely contributes to their cosmopolitan distributions, allowing them to exploit a wide variety of avian hosts across different geographical regions. In contrast, *P. maai* sp. nov. was only found on two bird species within the family Columbidae. No sequences related to *P. maai* sp. nov. were found on GenBank or BOLD, indicating that this species is currently only known from Singapore. However, more sampling is likely to reveal additional localities since the host species are widespread across Southeast Asia [74, 75], with *Streptopelia chinensis* occurring from East Asia to Australia. To further support public engagement, host associations for all recovered hippoboscid species were also made publicly accessible via the BOS website (https://singapore.biodiversity.online/taxon/A-Arth-Hexa-Dipt-Hippoboscidae). This allows everyone the chance to not only identify their hippoboscid fly species but also explore the parasite–host interactions.

5. Conclusion

We leveraged interested citizen scientists and integrative taxonomy to collect, delimit, and identify 10 hippoboscid species, which led to the description of a new species *P. maai* sp. nov., from specimens collected through species interaction observations. Our approach not only improved our understanding of hippoboscid taxonomy but also yielded valuable interaction data, showcasing how simple methods like bird carcass collection can provide crucial biodiversity insights. Our findings emphasize the importance of making all species easily identifiable through molecular and morphological diagnostics, as well as the need to provide user-friendly tools like annotated specimen images and pictorial keys. This ensures that both researchers and interested members of the public can readily access and use the data for future research. While we suggest that cosmopolitan hippoboscid species tend to infest avian hosts from diverse families, further data is needed to confirm this trend and explore its implications for disease transmission. Overall, we highlight the value of harvesting low-hanging fruit (readily accessible species interaction observations) together with integrative taxonomy to enhance biodiversity understanding, disease dynamics, and ecological relationships, paving the way for future ecological and evolutionary research.

Data Availability Statement

The sequence data used in this study are all available via GenBank (PQ247060–PQ247119, MT762413, and MT762414). All other data are incorporated into the article and its online Supporting Information.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Leshon Lee: conceptualization, methodology, software, formal analysis, investigation, resources, data curation, writing – original draft, writing – review and editing, visualization, project administration. **Jozef Oboňa**: validation, formal analysis, investigation, writing – original draft, writing – review and editing. **Yu Xuan Lee**: validation, formal analysis, investigation, writing – review and editing. **Karyn T. Lee**: validation, formal analysis, investigation, writing – review and editing, visualization. **Jayanthi Puniamoorthy**: formal analysis, investigation, data curation, writing – review and editing, funding acquisition. **Leo Y. K. Tan**: validation, formal analysis, investigation, writing – review and editing, visualization. **Ruirong Choo**: validation, formal analysis, investigation, writing – review and editing. **David J. X. Tan**: methodology, investigation, writing – review and editing. **Mackenzie Kwak**: investigation, resources, writing – review and editing. **Yuchen Ang**: methodology, resources, writing – review and editing, project administration, funding acquisition. **Rudolf Meier**: conceptualization, methodology, resources, writing – review and editing, supervision, project administration, funding acquisition.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting Information 1. Table S1: Singapore hippoboscid fly collection information.

Supporting Information 2. Table S2: Maximum *p*-distance and instability values for each 3% mOTU.

Supporting Information 3. Table S3: Precision, recall, specificity, accuracy, and *F*1 score values for each species DMC.

Supporting Information 4. File S1: Checklist of hippoboscid flies from Southeast Asia.

Supporting Information 5. File S2: Annotated pictorial key to all 10 hippoboscid fly species in Singapore.

Supporting Information 6. File S3: List of diagnostic morphological characters with annotated images for all 10 hippoboscid fly species in Singapore

Supporting Information 7. File S4: 313 bp *COI* dendrogram of Singapore hippoboscid fly.

Supporting Information 8. File S5: Interactive bipartite network in HTML format.

References

[1] J. P. Jones, B. Collen, G. Atkinson, et al., "The Why, What, and How of Global Biodiversity Indicators Beyond the 2010 Target," *Conservation Biology* 25, no. 3 (2011): 450–457.

[2] Royal Society Working Group on Measuring Biodiversity for Conservation, "Measuring Biodiversity for Conservation," (The Royal Society, 2003, https://royalsociety.org/-/media/policy/publications/2003/4294967955.pdf.

[3] L. McRae, S. Deinet, R. Freeman, and H. Doi, "The Diversity-Weighted Living Planet Index: Controlling for Taxonomic Bias in a Global Biodiversity Indicator," *PLoS ONE* 12, no. 1 (2017): e0169156.

[4] A. Srivathsan, Y. Ang, J. M. Heraty, et al., "Convergence of Dominance and Neglect in Flying Insect Diversity," *Nature Ecology and Evolution* 7, no. 7 (2023): 1012–1021.

[5] A. C. Hughes, M. C. Orr, K. Ma, et al., "Sampling Biases Shape Our View of the Natural World," *Ecography* 44, no. 9 (2021): 1259–1269.

[6] R. Ladle and J. Hortal, "Mapping Species Distributions: Living With Uncertainty," *Frontiers of Biogeography* 5, no. 1 (2013): 8-9.

[7] O. Sychra, A. Halajian, D. Engelbrecht, et al., "Louse-Flies (Diptera: Hippoboscidae) of Birds From South Africa: Prevalence and Diversity," *African Entomology* 28, no. 2 (2020): 249–261.

[8] M. M. Attia and H. M. Salem, "Morphological and Molecular Characterization of *Pseudolynchia canariensis* (Diptera: Hippoboscidae) Infesting Domestic Pigeons," *International Journal of Tropical Insect Science* 42, no. 1 (2022): 733–740.

[9] E. Čisovská Bazsalovicsová, B. Víchová, J. Oboňa, A. Radačovská, V. Blažeková, and I. Králová-Hromadová, "Bird Louse Flies *Ornithomya* spp. (Diptera: Hippoboscidae) as Potential Vectors of Mammalian *Babesia* and Other Pathogens," *Vector-Borne and Zoonotic Diseases* 23, no. 5 (2023): 275–283.

[10] B. Dayrat, "Towards Integrative Taxonomy," *Biological Journal of the Linnean Society* 85, no. 3 (2005): 407–417.

[11] K. W. Will, B. D. Mishler, Q. D. Wheeler, and V. Savolainen, "The Perils of DNA Barcoding and the Need for Integrative Taxonomy," *Systematic Biology* 54, no. 5 (2005): 844–851.

[12] E. Pante, C. Schoelinck, and N. Puillandre, "From Integrative Taxonomy to Species Description: One Step Beyond," *Systematic Biology* 64, no. 1 (2015): 152–160.

[13] R. Meier, M. K. N. Lawniczak, and A. Srivathsan, "Illuminating Entomological Dark Matter With DNA Barcodes in an Era of Insect Decline, Deep Learning, and Genomics," *Annual Review of Entomology* 70, no. 1 (2025): 185–204.

[14] R. Meier, W. Wong, A. Srivathsan, and M. Foo, "$1 DNA Barcodes for Reconstructing Complex Phenomes and Finding Rare Species in Specimen-Rich Samples," *Cladistics* 32, no. 1 (2016): 100–110.
[15] A. Srivathsan, L. Lee, K. Katoh, et al., "ONTbarcoder and MinION Barcodes Aid Biodiversity Discovery and Identification by Everyone, for Everyone," *BMC Biology* 19, no. 1 (2021): 1–21.
[16] A. Srivathsan, V. Feng, D. Suárez, B. Emerson, and R. Meier, "ONTbarcoder 2.0: Rapid Species Discovery and Identification With Real-Time Barcoding Facilitated by Oxford Nanopore R10.4," *Cladistics* 40, no. 2 (2024): 192–203.
[17] W. Y. Wang, A. Srivathsan, M. Foo, S. K. Yamane, and R. Meier, "Sorting Specimen-Rich Invertebrate Samples With Cost-Effective NGS Barcodes: Validating a Reverse Workflow for Specimen Processing," *Molecular Ecology Resources* 18, no. 3 (2018): 490–501.
[18] E. Hartop, A. Srivathsan, F. Ronquist, R. Meier, and B. Dayrat, "Towards Large-Scale Integrative Taxonomy (LIT): Resolving the Data Conundrum for Dark Taxa," *Systematic Biology* 71, no. 6 (2022): 1404–1422.
[19] A. Torres, L. Lee, A. Srivathsan, and R. Meier, "UITOTO: A Software for Generating Molecular Diagnoses for Species Descriptions," (bioRxiv(2025).
[20] D. J. X. Tan, N. A. Freymueller, K. M. Teo, W. S. Symes, S. K. Y. Lum, and F. E. Rheindt, "Disentangling the Biotic and Abiotic Drivers of Bird–building Collisions in a Tropical Asian City With Ecological Niche Modeling," *Conservation Biology* 38, no. 4 (2024): e14255.
[21] L. Lee, D. J. X. Tan, J. Oboňa, D. R. Gustafsson, Y. Ang, and R. Meier, "Hitchhiking into the Future on a Fly: Toward a Better Understanding of Phoresy and Avian Louse Evolution (Phthiraptera) by Screening Bird Carcasses for Phoretic Lice on Hippoboscid Flies (Diptera)," *Systematic Entomology* 47, no. 3 (2022): 420–429.
[22] G. E. Truett, P. Heeger, R. L. Mynatt, A. A. Truett, J. A. Walker, and M. L. Warman, "Preparation of PCR-Quality Mouse Genomic DNA With Hot Sodium Hydroxide and Tris (HotSHOT)," *BioTechniques* 29, no. 1 (2000): 52–54.
[23] M. Leray, J. Y. Yang, C. P. Meyer, et al., "A New Versatile Primer Set Targeting a Short Fragment of the Mitochondrial *COI* Region for Metabarcoding Metazoan Diversity: Application for Characterizing Coral Reef Fish Gut Contents," *Frontiers in Zoology* 10, no. 1 (2013): 34.
[24] J. Geller, C. Meyer, M. Parker, and H. Hawk, "Redesign of PCR Primers for Mitochondrial Cytochrome c Oxidase Subunit I for Marine Invertebrates and Application in All-Taxa Biotic Surveys," *Molecular Ecology Resources* 13, no. 5 (2013): 851–861.
[25] K. Katoh and D. M. Standley, "MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability," *Molecular Biology and Evolution* 30, no. 4 (2013): 772–780.
[26] R. Meier, K. Shiyang, G. Vaidya, P. K. L. Ng, and M. Hedin, "DNA Barcoding and Taxonomy in Diptera: A Tale of High Intraspecific Variability and Low Identification Success," *Systematic Biology* 55, no. 5 (2006): 715–728.
[27] P. D. N. Hebert, A. Cywinska, S. L. Ball, and J. R. deWaard, "Biological Identifications Through DNA Barcodes," *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270, no. 1512 (2003): 313–321.
[28] T. C. Maa, "On the Genus *Pseudolynchia* Bequaert," *Pacific Insects Monograph* 10 (1966): 125–138.
[29] T. C. Maa, "Insects of Micronesia Diptera: Hippoboscidae; Streblidae," *Insects of Micronesia* 14, no. 7 (1996): 251–274.
[30] T. C. Maa, "The Genus, *Ornithoica*, Rondani," *Pacific Insects Monograph* 10 (1966): 10–124.
[31] T. C. Maa, "Notes on the Hippoboscidae (Diptera). II," *Pacific Insects Monograph* 20 (1969): 237–260.
[32] T. C. Maa, "Revision of *Icosta* (=*Lynchia*, Auctt.) With Erection of a Related Genus *Phthona* (Diptera: Hippoboscidae)," *Pacific Insects Monograph* 20 (1969): 25–203.
[33] A. A. Yatsuk, A. V. Matyukhin, V. I. Anisimova, M. Y. Markovets, and E. P. Nartshuk, "A New *Ornithophila* (Diptera: Hippoboscidae) Species From Baikal State Nature Reserve (Russia)," *Nature Conservation Research* 9, no. 2 (2024): 100–104.
[34] J. Oksanen, G. Simpson, F. Blanchet, et al., "Vegan: Community Ecology Package," 2024, https://CRAN.R-project.org/package=vegan R package version 2.6-6.1.
[35] C. Terry, "BipartiteD3: Interactive Bipartite Graphs," 2024, R package version 0.3.1 https://CRAN.R-project.org/package=bipartiteD3.
[36] T. C. Maa, "A Revised Checklist and Concise Host Index of Hippoboscidae (Diptera)," *Pacific Insects Monograph* 20 (1969): 261–299.
[37] E. P. Nartshuk, A. A. Yatsuk, P. S. Tomkovich, and A. V. Matyukhin, "Taxonomic Status of *Icosta omnisetosa*, Maa, 1969, stat. n. (Diptera: Hippoboscidae) Is Upgraded to Species Rank," *Far Eastern Entomologist* 508, no. Diptera (2024): 27–32, 2024.
[38] B. R. Meysner, F. Rodriges-Vera, O. Hawlitschek, W. Heim, and M. Jentzsch, "Incidence of Louse Flies on Birds From the Russian Far East (Diptera: Hippoboscidae) and Investigation of the Status of Some Taxa by DNA Barcoding," *Russian Entomological Journal* 29, no. 1 (2020): 327–335.
[39] T. Yoshino, K. Kawakami, H. Sasaki, K. Miyamoto, and M. Asakawa, "A Parasitological Survey of Hwamei *Garrulax canorus* and Red-Billed Leiothrix *Leiothrix lutea* (Passeriforms: Terimiidae)," *Japanese Journal of Ornithology* 52, no. 1 (2003): 39–42.
[40] T. C. Maa, "Family Hippoboscidae," in *A Catalog of the Diptera of the Oriental Region*, ed. M. D. DelÞnado and D. E. Hardy, 3, (The University Press of Hawaii, 1977): 407–418.
[41] M. Mogi, H.-C. Kim, and T. A. Klein, "Notes on Diptera Pupipara of the Eastern Palaearctic Region. 2. Identity of *Ornithoica momiyamai* (Hippoboscidae)," *Medical Entomology and Zoology* 74, no. 1 (2023): 7–12.
[42] O. Theodor and H. Oldroyd, "Hippoboscidae," in *Die Fliegen Der Palaearktischen Region*, ed. E. Lindner, 12, (E. Schweizerbart'sche Verlagsbuchhandlung, 1964): 1–70.
[43] W. Eichler, "Deutsche lausfliegen, ihre lebenweise und ihre hygienische bedentung," *Zeitschrift für hygienische Zoologie und Schädlingsbekämpfung* 31 (1939): 210–226.
[44] G. B. Thompson, "Some Additional Records of an Association Between Hippoboscidae and Mallophaga, Together With a Bibliography of the Previous Records," *Annals and Magazine of Natural History* 18, no. 104 (2009): 309–312.
[45] T. C. Maa and J. S. Kuo, "A Field Survey of Arthropod Parasites of Birds in Taiwan1," *Journal of Medical Entomology* 1, no. 4 (1965): 395–401.
[46] J. C. Bequaert, "The Hippoboscidae or Louse-Flies (Diptera) of Mammals and Birds. Part I. Structure, Physiology and Natural History," *Entomologica Americana* 33 (1953): 211–442.
[47] J. C. Bequaert, "The Hippoboscidae or Louse-Flies (Diptera) of Mammals and Birds. Part II: Taxonomy, Evolution and

Revision of American Genera and Species," *Entomologica Americana* 34 (1955): 1–416.
[48] H. Adie, "The Sporogony of *Haemoproteus columbae*," *Indian Journal of Medical Research* 44 (1915): 671–680.
[49] E. Adly, M. Nasser, D. Soliman, et al., "Analysis of Phoretic Relation Between Chewing Lice and Hippoboscid Flies of *Columba livia*," *Veterinary Parasitology: Regional Studies and Reports* 22 (2020): 100496.
[50] M. A. R. Ansari, "Associations Between the Mallophaga and the Hippoboscidae Infesting Birds," *Journal of the Bombay Natural History Society* 46 (1947): 509–516.
[51] H. L. da Cunha Amaral, F. B. Bergmann, T. Silveira, P. R. S. dos Santos, and R. F. Krüger, "*Pseudolynchia canariensis* (Diptera: Hippoboscidae): Distribution Pattern and Phoretic Association With Skin Mites and Chewing Lice of *Columba livia* (Aves: Columbidae)," *Journal of Natural History* 47 (2013): 2927–2936.
[52] C. R. Hathaway, "Associação entre Mallophaga e Hippoboscidae," *Memórias do Instituto Oswaldo Cruz* 38, no. 3 (1943): 413–417.
[53] L. S. Hiregaudar and F. S. Khambata, "A Record of Mallophagan Phoresy by *Pseudolynchia Maura* (Diptera, Hippoboscidae)," *Current Science* 23 (1954): 335.
[54] J. A. Iannacone, "Registro de un caso de phoresis: Columbicola columbae (L) (Phthiraptera: Insecta) por Pseudolynchia canariensis (Diptera: Insecta) en la zona de Lima, Peru," *Boletin de Lima* 84 (1992): 17-18.
[55] F. Macchioni, M. Magi, F. Mancianti, and S. Perrucci, "Phoretic Association of Mites and Mallophaga With the Pigeon Fly *Pseudolynchia canariensis*," *Parasite* 12, no. 3 (2005): 277–279.
[56] M. Martin, "Life History and Habits of the Pigeon Louse (*Columbicola columbae* [Linnaeus])," *The Canadian Entomologist* 66, no. 1 (1934): 6–16.
[57] A. V. Matyukhin, "ФОРЕЗИЯ ПУХОЕДОВ (Foreziya pukoyedov) (Mallophaga) НА МУХАХ КРОВОСОСКАХ (Na mukhakh krovososkakh) (Hippoboscidae)," *Russian Journal of Parasitology* 38 (2016): 471–474.
[58] R. A. Ward, "Additional Record of Phoresy of Mallophaga on Hippoboscidae," *Bulletin of the Brooklyn Entomological Society* 48 (1953): 128.
[59] T. R. Klei, "Seasonal Occurrence of, *Haemoproteus columbae*, Kruse and Its Vector, *Pseudolynchia canariensis* Bequaert," *Journal of Wildlife Diseases* 11, no. 1 (1975): 130–135.
[60] G. Aydın, "The First Record of Pigeon Fly *Pseudolynchia canariensis* (Macquart, 1839)(Diptera: Hippoboscidae) Vector of Haemoproteus Disease in Van," *Yüzüncü Yıl University Journal of Agricultural Sciences (Turkey)* 19, no. 1 (2009): 49–55.
[61] C. Dranzoa, M. Ocaido, and P. Katete, "The Ecto-, Gastro-Intestinal and Haemo-Parasites of Live Pigeons (*Columba livia*) in Kampala, Uganda," *Avian Pathology* 28, no. 2 (1999): 119–124.
[62] M. A. González, I. Ruiz-Arrondo, S. Magallanes, J. Oboňa, M. J. Ruiz-López, and J. Figuerola, "Molecular and Morphological Analysis Revealed a New *Lipoptena* Species (Diptera: Hippoboscidae) in Southern Spain Harbouring *Coxiella burnetii* and Bacterial Endosymbionts," *Veterinary Parasitology* 332 (2024): 110300.
[63] A. Andreani, M. C. Rosi, R. Guidi, et al., "Colour Preference of the Deer Ked *Lipoptena fortisetosa* (Diptera: Hippoboscidae)," *Insects* 12, no. 9 (2021): 845.
[64] A. Srivathsan, R. K. Loh, E. J. Ong, et al., "Network Analysis With Either Illumina or MinION Reveals that Detecting Vertebrate Species Requires Metabarcoding of iDNA From a Diverse Fly Community," *Molecular Ecology* 32, no. 23 (2023): 6418–6435.
[65] S. R. Loss, T. Will, and P. P. Marra, "Direct Mortality of Birds from Anthropogenic Causes," *Annual Review of Ecology, Evolution, and Systematics* 46, no. 1 (2015): 99–120.
[66] C. Grilo, E. Koroleva, R. Andrášik, B. Michal, and M. González-Suárez, "Roadkill Risk and Population Vulnerability in European Birds and Mammals," *Frontiers in Ecology and the Environment* 18, no. 6 (2020): 323–328.
[67] K. Thorup, F. Korner-Nievergelt, E. B. Cohen, and S. R. Baillie, "Large-Scale Spatial Analysis of Ringing and Re-Encounter Data to Infer Movement Patterns: A Review including Methodological Perspectives," *Methods in Ecology and Evolution* 5, no. 12 (2014): 483–1350.
[68] A. K. Davis, "The Incidence of Hippoboscid Flies on Nova Scotia Landbirds," *Northeastern Naturalist* 5, no. 1 (1998): 83–88.
[69] A. Gonzalez, J. M. Chase, and M. I. O.'Connor, "A Framework for the Detection and Attribution of Biodiversity Change," *Philosophical Transactions of the Royal Society B* 378, no. 1881 (2023): 20220182.
[70] V. Levesque-Beaudin and B. Sinclair, "Louse Fly (Diptera: Hippoboscidae) Associations With Raptors in Southern Canada, With New North American and European Records," *International Journal for Parasitology: Parasites and Wildlife* 16 (2021): 168–174.
[71] M. J. Sharkey, D. H. Janzen, W. Hallwachs, et al., "Minimalist Revision and Description of 403 New Species in 11 subfamilies of Costa Rican Braconid Parasitoid Wasps, including Host Records for 219 Species," *ZooKeys* 1013 (2021): 1–665.
[72] F. E. Rheindt, P. Bouchard, R. L. Pyle, et al., "Tightening the Requirements for Species Diagnoses Would Help Integrate DNA-Based Descriptions in Taxonomic Practice," *PLOS Biology* 21, no. 8 (2023): e3002251.
[73] F. N. Gharsan and S. Q. Alghamdi, "First Report of the Morphological and Molecular Characterization of *Pseudolynchia canariensis* (Diptera: Hippoboscidae) From Al-Baha Region, Saudi Arabia," *Veterinary Parasitology: Regional Studies and Reports* 49 (2024): 100999.
[74] A. Davidson-Onsgard, L. F. Baptista, P. W. Trail, et al., "Pink-Necked Green-Pigeon (*Treron vernans*), Version 2.0," in *Birds of the World*, ed. N. D. Sly, (Cornell Lab of Ornithology, 2023).
[75] K. L. Garrett and R. L. Walker, "Spotted Dove (*Spilopelia chinensis*), Version 1.2," in *Birds of the World*, ed. S. M. Billerman and N. D. Sly, (Cornell Lab of Ornithology, 2023).