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Fauna of Louse Flies (Hippoboscidae) from Scops Owls (*Otus*: Strigidae) of the Nansei Islands, Japan, with Information on Their Phylogenetic Positions and Population Structure

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Louse flies (Hippoboscidae) are ectoparasitic dipterans found on the body of birds and mammals. They have been reported from a wide geographic range, and eight species from four genera have been recorded from the Nansei Islands, a stretch of islands lying between Southwestern Japan and Taiwan. However, due to difficulties in obtaining samples, we are yet to have a complete understanding of the fauna and our knowledge on their distribution remains limited. Here, we collected louse fly samples from two scops owl species (*Otus elegans* and *O. semitorques*) from three islands within the Nansei Islands, and identified them based on their morphological characteristics. Three species, *Ornithoica exilis*, *Ornithomya avicularia* and *Icosta amamiensis*, were reported with different appearance of species on each island. This is the first record of *Om. avicularia* for Okinawajima, and the first record of this family for Haterumajima (*Oc. exilis* and *Om. avicularia*) and Minami-Daitojima (*Om. avicularia*). We also sequenced mitochondrial *COI* gene to estimate their phylogenetic positions, and also to understand the inter-insular population structure of *Oc. exilis* and *Om. avicularia*, which were collected from multiple islands. We found no inter-insular genetic differentiation between the populations, despite the host owls having low dispersal ability, indicating low host specificity and host switch of these species. We provide a complete list of all 422 specimens examined and identified in this study, along with records of phoresy by mites and chewing lice. We also present an updated key to bird-parasitising Hippoboscidae species reported from the Nansei Islands and unreported potential species.

Keywords: Ryukyu Islands, Haplotype network, Cox1, Ectoparasite, Biodiversity

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BACKGROUND

The Hippoboscidae is a dipteran family of ectoparasites specialised in blood-sucking with 21 genera and more than 200 species (Dick 2006). They are distinguished from the other dipteran families by their flattened body, broadly separated coxae, and firmly attached head. The adult fly sucks the blood of birds or mammals, and each female fly bears a pupa. Although some species are known to be specialists in terms of the host species they parasite on (Lehikoinen et al. 2021), many species are indicated to have a broad host range and widespread geographic distribution (Bequaert 1953).

The fundamental study of Hippoboscidae in the East Asia and adjacent region was summarized by Maa (1989, oriental) and Soós and Hůrka (1986, Palaearctic; see also a review by Matyukhin et al. [2017]), and has been further updated in recent years by Doszhanov (2003, Palaearctic), Nartshuk et al. (2024, Russia), Yatsuk et al. (2025, Russia), Wang et al. (2022, China), Yatsuk et al. (2024, Vietnamese *Icosta*), etc. Japanese Hippoboscid fauna has been pursued vigorously by Maa (1967), Mogi et al. (2002), and several other researchers (Sato and Mogi 2008; Ohishi et al. 2009; Sakai et al. 2018). However, even the fauna of hippoboscids has not yet been fully investigated due to the difficulty in obtaining permission to catch host birds to capture louse flies (Mogi 1977; Nakamura 2016).

Within Japan, the Nansei Islands, which lies between Southwestern Japan and Taiwan, is known to be a biodiversity hotspot with unique ecosystems (WWF Japan 2010), making it an attractive region to investigate the louse fly diversity. So far, four genera and eight species of hippoboscids have been reported from the Nansei Islands (Table S1), of which six were recorded from Okinawajima, the biggest island of the Nansei Islands (Fig. 1A). Several species have been reported from Amami-Oshima and Ishigakijima, and a single species reported from Nakanoshima (belonging to the Tokara Islands), Tokunoshima and Iriomotejima. However, the fauna of hippoboscids on other islands, such as Minami-Daitojima and Haterumajima, still remain unknown (Fig. 1A). Understanding parasite diversity and host-parasite relationship on such small islands is important since parasites may have prominent negative effects on their hosts – such as transmission of avian malaria (Atkinson and Samuel 2010) – due to their small population sizes, reduced genetic diversity, and limited dispersal opportunities (Whittaker and Fernández-Palacios 2007).

In this study, we collected louse flies parasitising on two Scops Owl species: Ryukyu Scops Owl (*Otus elegans* [Cassin, 1852]) and Japanese Scops Owl (*Otus semitorques pryeri* [Gurney, 1889]) from Okinawajima (hereafter "Okinawa", including the adjacent Yagajijima [Fig. 1B]), Minami-Daitojima ("Daito"), and Haterumajima ("Hateruma"). Ryukyu Scops Owl is a small nocturnal owl species mainly inhabiting the Nansei Islands, with its distribution ranging from Okinoshima and the Nansei Islands of Japan, to the northern islands of the Philippines (Gill et al. 2024; The Ornithological Society of Japan 2024). Within Japan, two subspecies are recognised, with *O. e. elegans* widely distributed across the Nansei Islands, and *O. e. interpositus* Kuroda, 1923 endemic to Daito, an oceanic island which lies 400 km east to Okinawa. Since they are residential on each island with little inter-insular dispersal (Sawada et al. 2023) genetic differentiation on each island is recognised (Takagi and Saitoh unpub.), with inter-insular differences in traits such as vocal characteristics (Takagi 2011). The other host species, Japanese Scops Owl, is also a resident species but is only found on Okinawa out of the three sampling islands, and this population is classified as subspecies *pryeri*. Louse flies are usually seen moving freely between the feathers of the owls and can sometimes be seen on the surface (Fig. S1).

Here, we report three species of hippoboscids collected from the two Scops Owl host species from the three islands, Okinawa, Daito, and Hateruma. In Daito and Hateruma, they are the first records of this family. Extensive capture of the host species allowed us to collect more quantitative data on the louse fly occurrence. We also sequenced mitochondrial *COI* gene (*COI*) in order to estimate the phylogenetic positions of the obtained samples, and to understand the inter-insular genetic structure of species that were recorded on multiple islands.

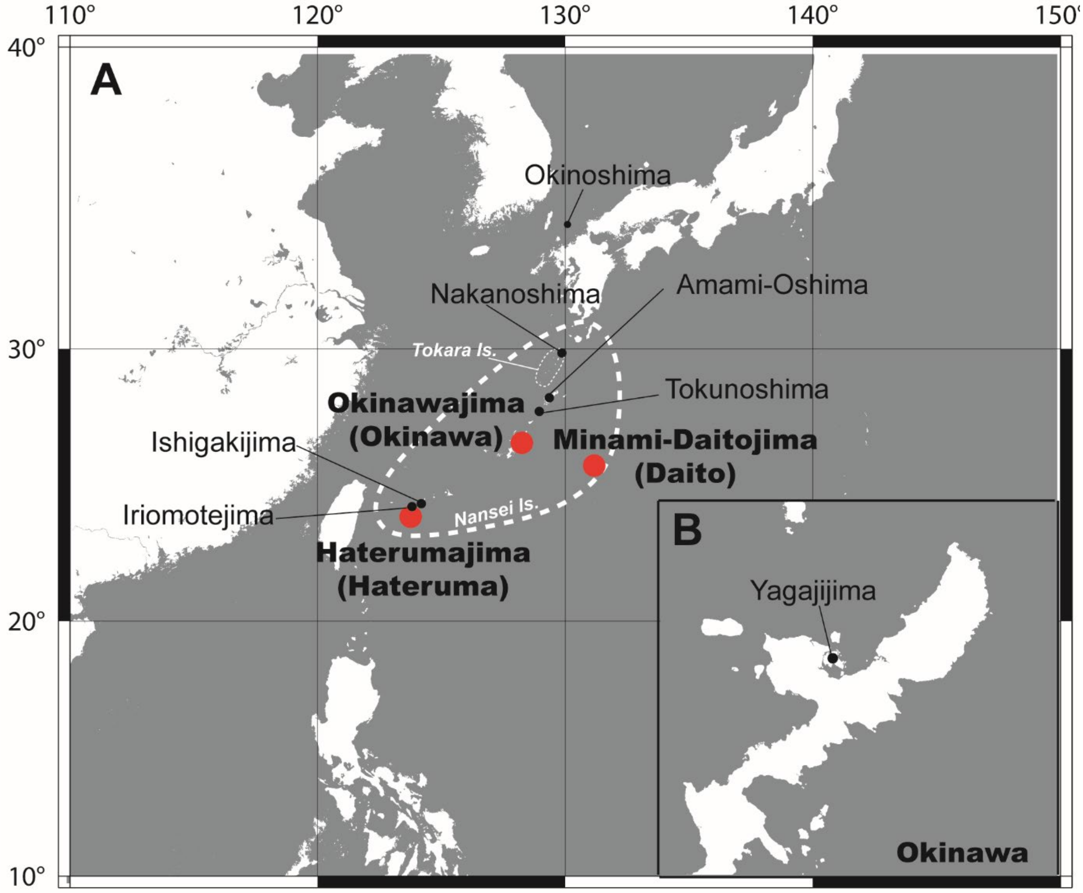

Fig. 1. Map of the Nansei Islands and the three sampling sites.

MATERIALS AND METHODS

Sampling

As a part of ecological studies conducted on Ryukyu Scops Owls and Japanese Scops Owls, we obtained louse flies from three islands in the Nansei Islands, Japan (Fig. 1A, B): Okinawa, Daito, and Hateruma.

From 2009 to 2023, birds were captured using mist nests or directly taken from breeding nestboxes. In the course of the process of ringing, blood sampling and taking measurements of the bird, any louse fly found on the body of the bird was captured by hand and fixed in 80% or 100% ethanol. Once taken back to the laboratory, samples were preserved at −18°C.

Identification

Morphological observations of the specimens were made under a stereomicroscope (Nikon SMZ, Japan). Photos were taken with Leica M205C with Canon eos 6D Mark II and the stacked images were created by the software Zerene Stacker (version 1.04, Zerene Systems LLC, Richland, WA, USA).

We identified specimens following the key provided by Maa (1967) and also referred to: Maa (1963), Mogi et al. (2002), Sato and Mogi (2008), Oboňa et al. (2022), and Matyukhin et al. (2023) for *Ornithomya*; and Maa (1969b) and Mogi (1977) for *Icosta*. The wing length and the combined length of head and thorax were measured following the methods in Bequaert (1952). We described the wing veins following the venation system illustrated by Maa (1967). This system differs slightly from the traditional and alternative venation systems widely used in Diptera (Cumming and Wood 2017).

After observation, the specimens were preserved in 99% ethanol. Out of the 422 samples observed and identified, 60 samples were chosen at random and dissected to remove the right hind leg distal to femur-tibial joint, and their DNA extracted for molecular analysis (Table 1). Most of these detached leg sclerites were also preserved after the DNA extraction.

Table 1. Total number of identified louse fly samples, with number of samples used for molecular analysis shown in brackets

Species	Okinawa	Daito	Hateruma	Total
Ornithoica exilis	24 (7)	0	23 (10)	47 (17)
Ornithomya avicularia	294 (19)	75 (20)	4 (2)	373 (41)
Icosta amamiensis	2 (2)	0	0	2 (2)
Total	320 (28)	75 (20)	27 (12)	422 (60)

Molecular analysis

Total DNA was extracted from the legs of 28, 20, and 12 specimens of three species from the Okinawa, Daito, and Hateruma populations, respectively (Table 1) using NucleoSpin Tissue XS Kit (Macherey-Nagel, Germany) following the manufacturer's protocol. The primers used for PCR amplification and sequencing for COI are LepF1 and LepR1 (Hebert et al. 2004). PCR amplification condition with TaKaRa Ex Taq DNA polymerase (TaKaRa Bio, Japan) or KOD FX Neo (TOYOBO, Japan) was as follows: denature at 94°C for 3 minutes, 5 cycles of 94°C for 30 seconds, 48°C for 40 seconds and 72°C for 1 minute, followed by 30 cycles at 94°C for 30 seconds, 51°C for 30 seconds and 72°C for 1 minute, with a final annealing step of 72°C for 5 minutes. Amplification of the target sequence was visually checked by electrophoresis, and the PCR product was cleaned up using Exo SAP-IT express (Thermo Fisher Scientific, USA). All nucleotide sequences were determined by direct sequencing in both forward and reverse directions with a QuantumDye™ Terminator Cycle Sequencing Kit v3.1 (Tomy Digital Biology, Japan) with the following PCR conditions: denature at 96°C for 3 minutes, 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds and 60°C for 4 minutes. Fragments were collected with ethanol precipitation and 3730 DNA Analyzer (Life Technologies, USA) was used for sequence determination. Forward and reverse sequences were concatenated by using MEGA 11 (Tamura et al. 2021). BLAST (Altschul et al. 1990) was used to search the International Nucleotide Sequence Database (INSD, 2024) for sequences most similar to ours.

Population genetics

COI sequences were determined for 7 and 10 specimens of *Oc. exilis* from Okinawa and Hateruma, 19, 20 and 2 specimens of *Om. avicularia* from Okinawa, Daito, and Hateruma, and 2 specimens of *I. amamiensis* from Okinawa, respectively (Table 1; Table S1 for accession numbers). After alignment by means of Clustal W (Thompson et al. 1994), an integer neighbour-joining (IntNJ) network was constructed with PopART v.1.7 (Leigh and Bryant 2015) at 0.50 reticulation tolerance. Haplotype diversity (h), nucleotide diversity (π), Tajima's *D* (Tajima 1989), and Fu's F_S (Fu 1997) were calculated with DnaSP v.6.12.03 (Rozas et al. 2017) and Fst was calculated with Arlequin ver. 3.5.2.2 (Excoffier and Lischer 2010).

Phylogeny

A part of *COI* sequences used for population genetic analysis was used to analyse phylogeny (Table 1). We reconstructed the phylogenetic relationships within Hippoboscidae and *Om. avicularia*. For Hippoboscidae, we used 68 sequences from 35 louse fly species, 7 sequences from 3 species determined in this study, and 2 outgroup taxa (*Drosophila melanogaster* and *Glossina fuscipes fuscipes*). For *Om. avicularia*, we analysed 44 sequences obtained from the INSD in addition to 41 sequences determined in this study, using *Om. anchineura* and *Om. comosa* as outgroup taxa. Details of the sequences are provided in Table S3 (Hippoboscidae) and Table S4 (*Om. avicularia*). After alignment using Clustal W, the sequences were trimmed to the shortest length of 476 bp for Hippoboscidae and 544 bp for *Om. avicularia*. The optimal substitution models (GTR+F+I+G4 and TPM2u+F+R2) were determined under the corrected AIC (Akaike information criterion) option in PartitionFinder 2.1.1 (Lanfear et al. 2017), using the greedy algorithm (Lanfear et al. 2012). A maximum likelihood (ML) analysis was conducted in IQ-TREE 2.1.2 (Minh et al. 2020), with branch support values obtained by analyses of Shimodaira-Hasegawa approximate likelihood-ratio test (SH-aLRT) and an ultrafast bootstrap analysis of 1,000 pseudoreplicates under the "bnni" option (Hoang et al. 2018). The ML tree was drawn with FigTree v1.4.4 (Rambaut 2018).

RESULTS

Occurrence of Hippoboscidae flies on *Otus* owls from three islands

In total, 320, 75 and 27 hippoboscid specimens were collected between 2009–2023 from Okinawa, Daito and Hateruma, respectively (Table S1). In Okinawa, specimens were found on both species of owl, with a maximum of 15 flies collected from a single host individual. The following three species were collected.

Family Hippoboscidae Samouelle, 1819

Subfamily Hippoboscinae Brues & Melander, 1932

Genus *Ornithoica* Rondani, 1878

***Ornithoica* (*Ornithoica*) *exilis* (Walker, 1861)**

(Fig. 2)

Specimens examined: [Okinawa]: 13 ♂♂, 11 ♀♀; [Hateruma]: 7 ♂♂, 15 ♀♀, 1 gynandromorph. For detailed data, see table S2.

Distribution: JAPAN: Okinawajima (Yamauchi and Ozaki [2007]; Kondo [2010]; this study), Haterumajima (this study), Iriomotejima (Mogi et al. 2002), Ishigakijima (Maa 1966), Honshu; Oriental and Australian region (Maa 1966; Mogi 2014).

Remarks: *Ornithoica exilis* was the dominant hippoboscid on Hateruma but not found on Daito from *O. elegans*. In Okinawa, 2 females were collected from *O. e. elegans*, and 13 males and 8 females from *O. semitorques pryeri*. The host species of one female is unknown, as it had already been detached at the time of collection. In Hateruma, all the specimens were collected from *O. e. elegans.* The gynandromorph of *Oc. exilis* is reported in several papers, for example Maa (1966).

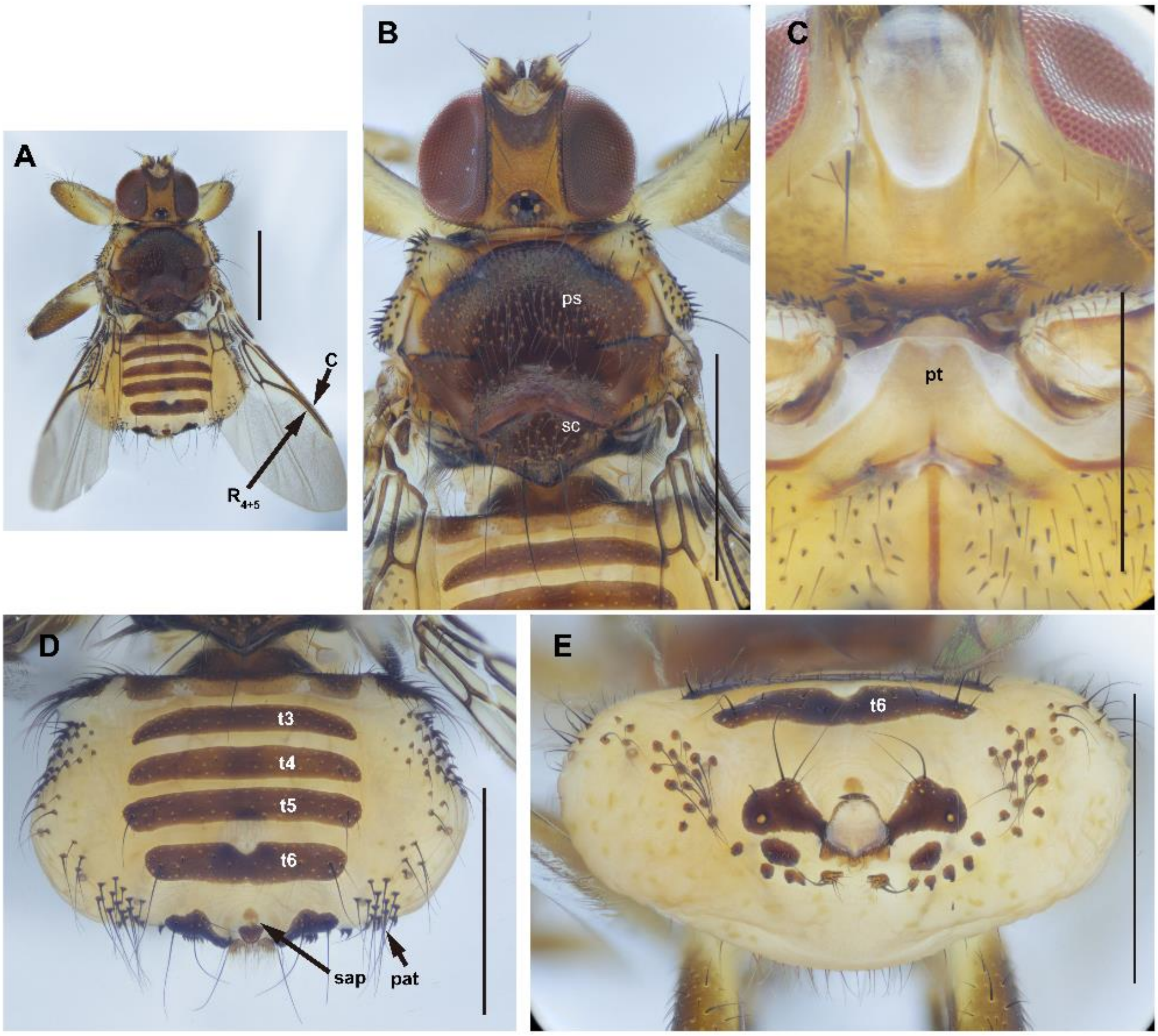

Fig. 2. *Ornithoica exilis* (HTM_HIP21-17). A, Whole body, dorsal view; B, Scutellum, dorsal view; C, Anterior edge of thoracic sterna; D, Female abdomen, dorsal view; E, Female tergite of 6th abdominal segment. Abbreviations: C, costa; R_{4+5}, radial vein 4+5; pat, para-analtuft; ps, prescutum; pt, prosternum; sap, supra-anal plate; sc, scutellum; t3–6, tergite of third to sixth abdominal segments. Scale bars: A, B, D, E = 1 mm; C = 0.5 mm.

Genus *Ornithomya* Latreille, 1802

***Ornithomya avicularia* (Linnaeus, 1758)**

(Fig. 3)

Specimens examined: [Okinawa]: 86 ♂♂, 207 ♀♀; [Daito]: 31 ♂♂, 44 ♀♀; [Hateruma]: 4 ♀♀. For detailed data, see table S2.

Distribution: JAPAN: Amami-Oshima (Mogi [1977], as subspecies *aobatonis*; Mogi et al. [2002]); Okinawajima (this study), Minami-Daitojima (this study), Haterumajima (this study), Hokkaido, Honshu, Shikoku, Kyushu; Palaearctic (records as *Om. a. aobatonis* are limited from East Siberia, Korean Peninsula and Japan [Mogi 2014]).

Morphology: We distinguished *Ornithomya avicularia* from *Om. candida* based on the length ratio of femur 1 to 3 (Sato and Mogi 2008) and distribution of strong bristles on female tergite 6 (Maa [1967]; Fig. 3F, C); from *Om. fringillina* based on the wing length, the combined length of head and thorax, and the number and the arrangement of preapical bristles on the scutellum (compared to fig. 4 of Hill [1962]; Fig. 2D); from *Om. chloropus* by the body and the wing length, and the distribution of the strong bristles on female tergite 6 (Theodor and Oldroyd 1964); from *Om. fuscipennis* by eye width to face width. We also checked the vibrissal spines (Fig. 3C) and the rows of spines of hind tarsomere 1 (Fig. 3B), but these characters are more or less variable as Mogi et al. (2002) mentioned. The posterior row out of the two rows of spines on hind tarsomere 1 is sometimes reduced and difficult to recognise the spines as a row.

Some intraspecific differences from the previous descriptions were found. In males, most specimens lack vibrissal spine. Most of our specimens differed from previous literature in that the second microtrichial stripe of wing cell 1m continues to wing margin (Fig. 3A), posteriormost pair of vibrassal bristle (Fig. 3C) and bristles of posterior margin of laterite 2 are longer, and bristles on tergite 6 are thicker and longer (Fig. 3F, G). This tendency was particularly strong in the individuals on Daito. Wing microtrichial patterns were highly variable: wing cell 3r with or without a median anterior bare area; posteriormost microtrichial stripe of cell 1m ranging from absent to present, occasionally contacting vein M3+4 in the apical two-thirds of the stripe; and cell 2m with or without a microtrichial stripe. These patterns encompassed a broader range of gradual variation than that reported for typical *Om. avicularia* (Maa 1967, fig. 26; Hutson 1984, fig. 1). The condition most divergent from the typical *Om. avicularia* morphology is illustrated in figure 3A. These microtrichial character states were not correlated with *COI* haplotypes.

We finally identified our specimens as this species by the following characters: the ratio of femur 1 to 3 ranged 0.69–0.77, the number of scutellar preapical bristles with shorter bristles at both sides, and the distribution of setae of female tergite 6 (Fig. 3F, G).

Remarks: Here we record *Om. avicularia* for the first time from three islands: Okinawa, Daito and Hateruma. This was the most abundant hippoboscid fly on *O. elegans* from Okinawa. We did not attempt to identify the specimens to a subspecies level since there is still an ongoing discussion on the handling of subspecies in this species (details in DISCUSSION).

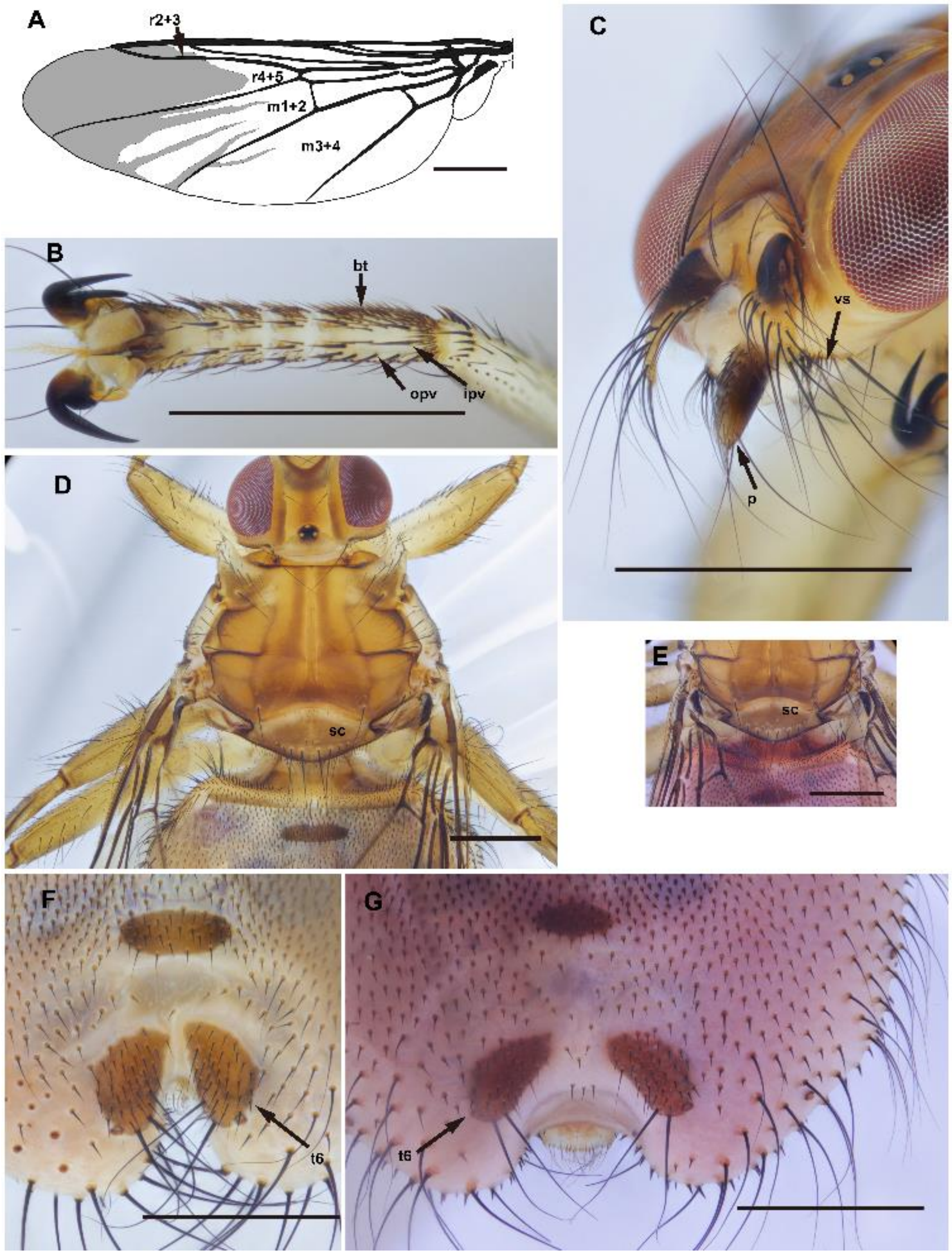

Fig. 3. *Ornithomya avicularia*. A, D, F, HIP20-22 (Daito); B, C, OKN-HIP22-27; E, G, OKN-H22-019. A, Wing venation and distribution of microtrichia; B, Tarsus of left hind leg; C, Head, anterolateral view; D, Thorax, dorsal view; E, Scutellum, dorsal view; F, G, Female abdomen, dorsal views. Abbreviations: bt, basitarsus, first tarsomere, the most proximal segment of tarsus; ipv, inner row of posteroventral bristles; m1+2, wing cell m_{1+2}, the area posterior to the wing vein M1+2; m3+4, wing cell m_{3+4}, the area behind the vein M3+4; opv, outer row of posteroventral bristles; p, palpus; r2+3, wing cell r_{2+3}, area posterior to the vein R2+3; r4+5, wing cell r_{4+5}, area posterior to the vein R4+5; sc, scutellum; t6, tergite of sixth abdominal segment; vs, vibrissal spine. Scale bars: A–G = 1 mm.

Genus *Icosta* Speiser, 1905

***Icosta* (*Icosta*) *amamiensis* Mogi, 1977**

(Fig. 4)

Specimens examined: [Okinawa]: 2♀♀. For detailed data, see table S2.

Distribution: JAPAN: Nakanoshima (Ohishi et al. 2009), Amami-Oshima (Mogi 1977), Okinawajima (Mogi et al. 2002; this study).

Morphology: Following characters suggest that our specimens belong to the subgroup C of *plana* group (Maa 1969b): scutellum with 2 bristles, and bristles aparted more than 2x median length of scutellum (Fig. 4D, sb); crossveins *rm* and *im* present (Fig. 4A); compound eyes more than ½ as wide as face; palpus of mouth more than 2x as long as height; tarsomere 3 and 4 of tarsus 1 with outer lobes, which are longer than inner lobes (Fig. 4F, ol); thickness of wing vein R_{2+3} constant; wing 6.0 mm long (Fig. 4A); frontal process with straight inner margin in dorsal view; wing microtrichia far from reaching vein *2A*, fill cell *im*. We finally identified these specimens to *I. amamiensis* Mogi, 1977 according to his original description, by the wing length, the tarsal spines (Fig. 4F) and the laterite 7 (Fig. 4H, l7).

Remarks: Only two individuals were found from Okinawa, both from *O. s. pryeri*. This is the 4th host record of *I. amamiensis*.

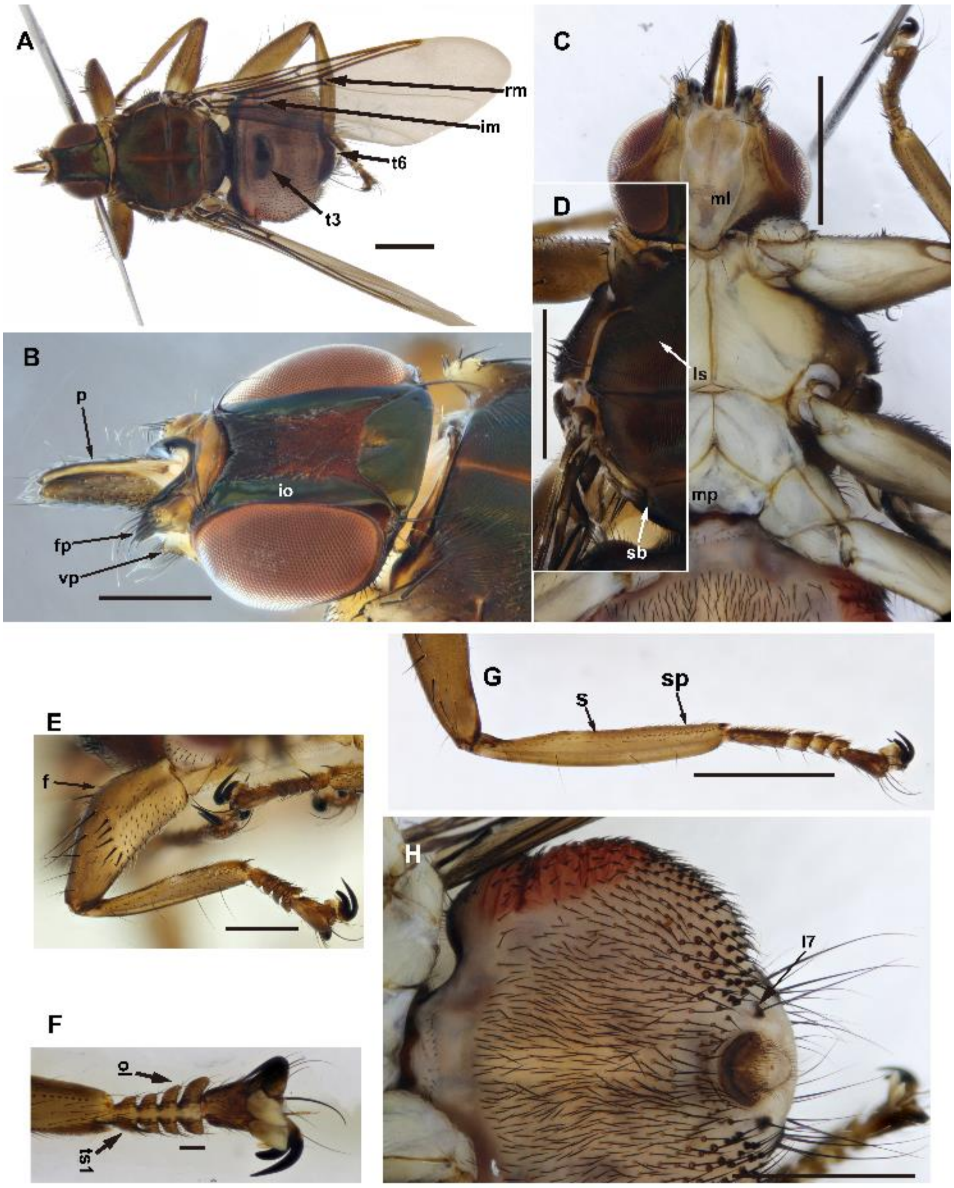

Fig. 4. *Icosta amamiensis*. A, Whole body, dorsal view (OKN-HIP21-34); B, Head, dorsolateral view (OKN-HIP21-34); C, Thorax, ventral view (OKN-HIP21-34); D, Thorax, dorsal view (OKN-HIP21-34); E, Right femur 1 (OKN-HIP21-70); F, Tibia 1 (OKN-HIP21-34); G, Left tibia 3 (OKN-HIP21-70); H, Abdomen, ventral view (OKN-HIP21-34). Abbreviations: f, femur; fp, frontal process; im, wing inter-medial crossvein between M1+2 and M3+4; io, inner orbital area; ls, laterocentral setae; l7, laterite of 7th abdominal segment; ml, lobe of anterior edge of mesothoracic sterna; mp, metasternal process; ol, outer lobe; p, palpi; rm, wing crossvein connecting vein R4+5 to vein M1+2; s, a low of sensillae; sb, scutellar bristle; sp, a low of sensory pores; t3, tergite of third abdominal segment; t6, tergite of sixth abdominal segment; ts1, first segment of tarsus; vp, vibrissal process. Scale bars: A, C, D, G, H = 1 mm; B, E = 0.5 mm; F = 0.1 mm.

Phoresy

Of the 422 specimens observed, 17 (15 *Om. avicularia* from Okinawa and 2 *Oc. exilis* from Hateruma) were parasitised by mites (Fig. S2) and 7 (6 *Om. avicularia* from Okinawa collected from *O. s. pryeri* and 1 from Daito collected from *O. e. interpositus*) had chewing lice Philopteridae gen. sp. (Phthiraptera, Ischnocera) attached (Fig. S3).

Molecular information and genetic distances

For *Oc. exilis*, we determined *COI* sequences for 7 and 10 individuals from Okinawa and Hateruma, respectively (658 bp, encoding 218 amino acids; Tables 1, S2). The mean *p*-distances within populations were 0.21–0.29% and between them was 0.23%. Maximum p-distances within and between populations were both 0.61%. Pairwise F_{ST} value was -0.0490 (p = 0.565, Table 2). The most similar sequence to ours (LC833964) was from "*Ornithoica bistativa*" (OR045886; per. Ident., 95.49%; query cover, 95%).

Table 2. Inter- and intra-population genetic distances (*p*-distance, as percentages) and pairwise F_{ST} for *COI* (658 bp) for *Ornithoica exilis* from two islands in the Nansei Islands. Numbers shaded in grey are values of F_{ST}; numbers in parentheses in the table are mean values of *p*-distances or *p*-values of F_{ST}

	Okinawa	Hateruma
Okinawa	0–0.61 (0.29)	−0.0490 (p = 0.656)
Hateruma	0–0.61 (0.23)	0–0.46 (0.21)

For *Om. avicularia*, we determined *COI* sequences for a total of 41 individuals, 19, 20 and 2 from Okinawa, Daito and Hateruma, respectively (658 bp, encoding 218 amino acids; Tables 1, S2). The mean *p*-distances within populations were 0.30–0.47% and those between them were 0.34–0.45%. Maximum *p*-distances within and between populations were both 1.22%. Pairwise F_{ST} values were 0.0299 (p = 0.0721) between Okinawa and Hateruma, −0.0801 (p = 0.955) between Okinawa and Hateruma, and 0.0428 (p = 0.315) between Daito and Hateruma (Table 3). The most similar sequence to ours (LC833993) was from "*Ornithomya avicularia*" (OR064831; per. Ident., 99.54%; query cover, 100%).

Table 3. Inter- and intra-population genetic distances (*p*-distance, as percentages) and pairwise F_{ST} for *COI* (658 bp) for *Ornithomya avicularia* from three islands in the Nansei Islands. Numbers shaded in grey are values of F_{ST}; numbers in parentheses in the table are mean values of *p*-distances or *p*-values of F_{ST}

	Okinawa	Daito	Hateruma
Okinawa	0–1.22 (0.38)	0.0299 (p = 0.0721)	-0.0801 (p = 0.955)
Daito	0–1.22 (0.45)	0–0.91 (0.47)	0.0428 (p = 0.315)
Hateruma	0–0.76 (0.34)	0–0.76 (0.41)	0.3

For *I. amamiensis*, we determined *COI* sequences for 2 individuals from Okinawa (658 bp, encoding 218 amino acids; LC833967, Tables 1, S2). The two sequences were identical. The most similar sequence to ours was from "*Icosta americana*" (MZ261719; per. ident., 90.38%; query cover 99%).

Genetic diversity and neutrality tests

Seven haplotypes were detected (Fig. 5A) among the 17 *COI* sequences from the two *Oc. exilis* populations (Okinawa, Hateruma). The most common haplotype (H6) was shared by both populations. The haplotype and nucleotide diversities of Okinawa were Hd = 0.810 and Pi = 0.00289 and of Hateruma were Hd = 0.867 and Pi = 0.00203 (Table 4). Except Fu's F_S for Hateruma, -2.97 ($p = 0.00842$), all of Tajima's D and Fu's F_S values were not significant ($p > 0.02$).

Twenty-two haplotypes were detected among 41 *COI* sequences from three *Om. avicularia* populations (Okinawa, Daito, Hateruma, Fig. 5B). The most common haplotype (H11) was shared by all of 3 populations. The haplotype and nucleotide diversities of Okinawa were Hd = 0.953 and Pi = 0.00385, of Daito were Hd = 0.911 and Pi = 0.00468, and of Hateruma were Hd = 1 and Pi = 0.00304, respectively. Tajima's D of overall population was significant ($p < 0.02$; D = -1.80; Table 4) and Fu's F_S values of overall population, Okinawa and Daito were significant (F_S = -15.9, -10.4, -3.77, respectively; Table 4).

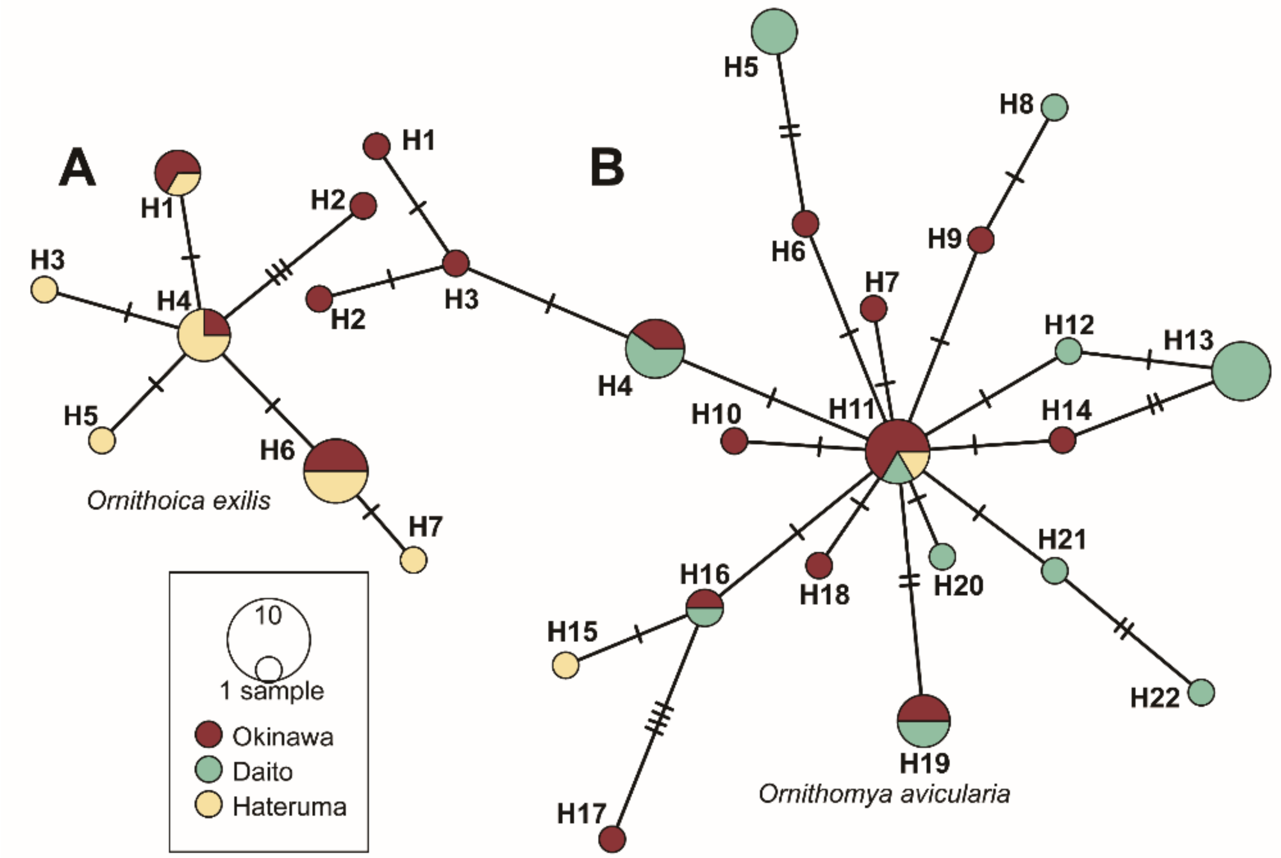

Fig. 5. Haplotype network of *Ornithoica exilis* and *Ornithomya avicularia* taken from Okinawajima, Minami-Daitojima and Haterumajima. A, Haplotype network of *Oc. exilis*; B, haplotype network of *Om. avicularia*.

Table 4. Genetic diversity and results of neutrality tests based on *COI* sequences (658 bp) for *Ornithoica exilis* and *Ornithomya avicularia* from two or three islands in the Nansei Islands

	N	Hd	Pi	Tajima's *D*	Fu's F_S
Ornithoica exilis					
Okinawa	7	0.810	0.00289	-0.330 (p = 0.399)	−0.226 (p = 0.401)
Hateruma	10	0.867	0.00203	-0.985 (p = 0.228)	−2.97* (p = 0.00842)
Overall	17	0.824	0.00232	-1.25 (p = 0.119)	−2.42 (p = 0.0275)
Ornithomya avicularia					
Okinawa	19	0.953	0.00385	-1.68 (p = 0.0250)	-10.4* (p = 0.00)
Daito	20	0.911	0.00468	-1.00 (p = 0.154)	-3.77* (p = 0.0170)
Hateruma	2	1	0.00304	-	-
Overall	41	0.945	0.00433	-1.80* (p = 0.0100)	-15.9* (p = 0.00)

Abbreviations: N, number of individuals; Hd, haplotype diversity; Pi, nucleotide diversity; *, significant at $p < 0.02$.

Phylogeny

In our *COI*-based ML-tree for hippoboscids (Fig 6, Table S3), *Oc. exilis* specimens from two islands formed a highly supported clade (99.7/100%, HS-aLRT/uBS) as sister taxon to *Oc. bistativa* and *Oc. momiyamai*. *Om. avicularia* from three islands formed a highly supported clade (95.2/100%) as sister to *Om. avicularia* from Europe. *I. amamiensis* was a sister to *I. americana* collected from Canada with moderate support (89.5/88%). Except for the genus *Ornithomya*, all genera were recovered as well- or moderate- supported clades.

In a ML-tree for *Om. avicularia*, 41 specimens from the three islands recovered a clade with *Om. avicularia* collected from Far East Russia (OR064831) with high support (99.3/97%). This clade is sister to clade containing specimens from Europe and North America with moderate support (82.4/61%; Fig. 7, Table S4).

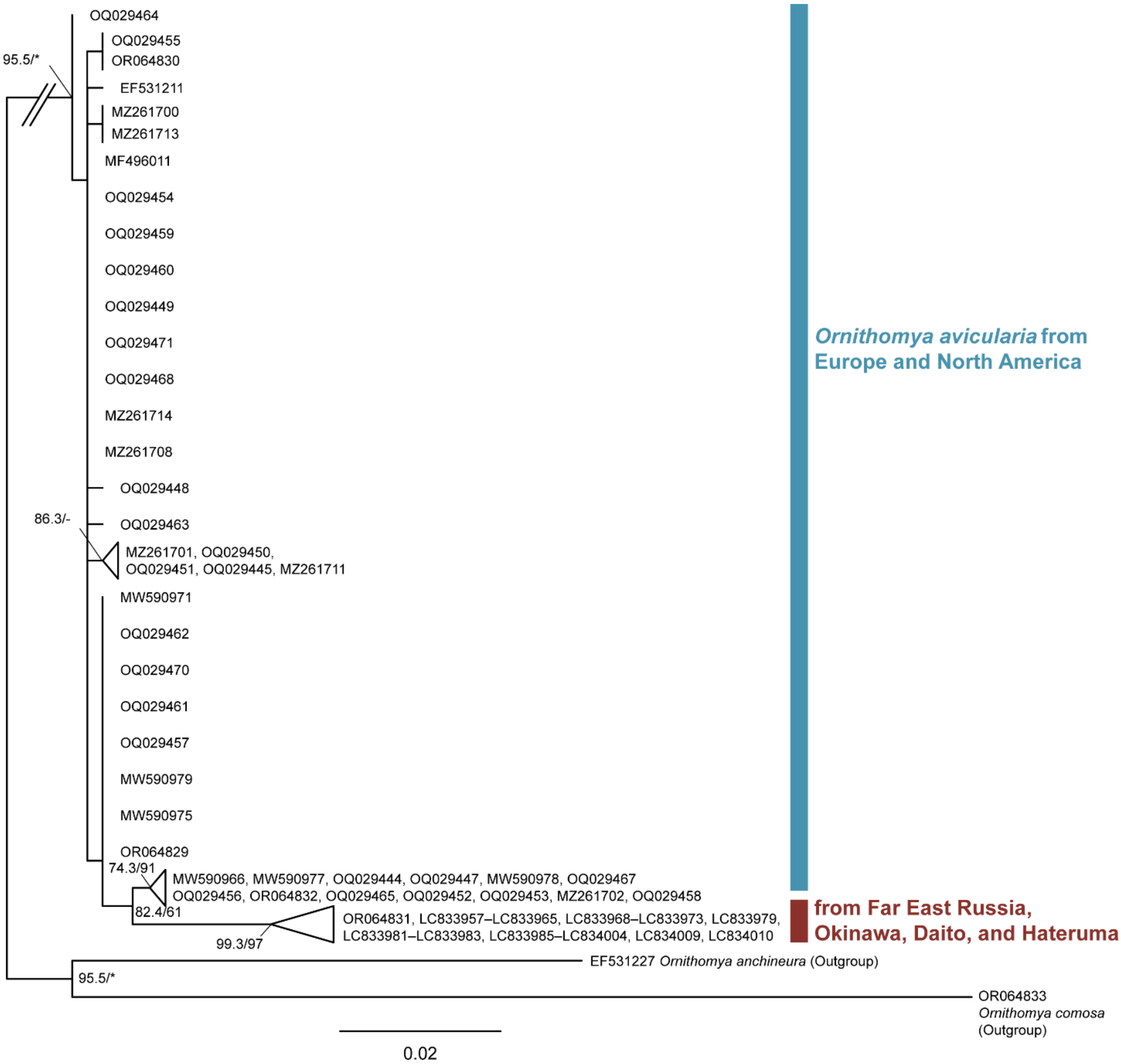

Fig. 7. Maximum-likelihood phylogenetic tree of *Ornithomya avicularia* based on *COI* sequences (544 bp). Numbers next to nodes indicate values from the Shimodaira-Hasegawa approximate likelihood-ratio test (SH-aLRT) and ultrafast bootstrap support (uBS). Asterisks indicate full

support and supports less than 60 are not shown in the tree. The scale at bottom right indicates branch length in substitutions per site.

DISCUSSION

Records of Hippoboscidae from the Nansei Islands

In Okinawa, *Om. avicularia* is recorded for the first time. Though *Oc. exilis* and *I. amamiensis* have been reported from this island, we report new host species, *O. semitorques pryeri* and *O. elegans* (Mogi et al. 2002; Yamauchi and Ozaki 2007; Kondo 2010). In Daito and Hateruma, as there was no record of Hippoboscidae so far, this is the first record of this family.

Icosta amamiensis and *I. holoptera omnisetosa* are the only species of the genus from the Nansei Islands. To date, six *Icosta* species have been recorded from Japan (Mogi 2014; Sakai et al. 2018). Three species have been reported from Honshu and from the Oriental region excluding the Nansei Islands, leaving a distribution gap in the Nansei Islands. This disjunct distribution indicates that the species are likely to inhabit the Nansei Islands but have not yet been reported.

Ornithomya avicularia aobatonis Matsumura, 1905 was originally described as a full species, *Om. aobatonis* from Hokkaido and Honshu, Japan. Maa (1967) redescribed it as a subspecies of *Om. avicularia* and clarified the morphological differences between this subspecies and nominal subspecies. For decades, researchers followed this systematics (*e.g.*, Dick [2006]), but recently there is an opinion that *Om. avicularia aobatonis* should be treated as a nominal species by molecular phylogenetic analyses based on *COI* (Meißner et al. 2020). To resolve this taxonomic problem, Mogi et al. (2023) pointed out that we need fine morphological observations in addition to molecular data. It is worthy of noting that most of our specimens are featured by having the traits of *Om. avicularia aobatonis* sensu Maa (1967) such as (1) distribution of setae and shape of female abdominal sixth tergite, (2) extensive wing microtrichia, and (3) straight shape of palpus. Our specimens recovered a clade with *Om. avicularia* (OR064831) collected from the Far East where Meißner et al. (2020) has also reported "*aobatonis*" (Fig. 7). On the other hand, *Om. avicularia avicularia* like characters were also found in our specimens: palpus narrower and bare area on palpus larger than *Om. avicularia aobatonis* (refer to Fig. 29 in Maa [1967]). In this paper, we refrain from concluding the taxonomic status for them and broadly use the species name *Om. avicularia*, following Nartshuk et al. (2022) and Mogi et al. (2023).

Regarding the distribution of wing microtrichia in *Om. avicularia*, we observed substantial intraspecific variation within the population, including among individuals sharing the same haplotype. Wing microtrichial patterns have sometimes been used to distinguish *Om. avicularia* from other *Ornithomya* species: Hutson (1984) reported a large bare area in cell 3r in *Om. avicularia*, and Nartshuk et al. (2024) described cell 2m as entirely bare in this species. However, in our specimens, some individuals lacked a bare area in cell 3r, lacked a third stripe in cell 1m, and exhibited a microtrichial stripe in cell 2m, despite being identified as *Om. avicularia* based on other morphological characters and molecular evidence. A large variety of wing microtrichial patterns has been reported by Wawman et al. (2025) from another *Ornithomya* species, *Om. fringillina*. This suggests that the reliability of wing microtrichia for species identification warrants careful consideration.

Population genetics and dispersal ability

Given that the louse flies were collected from residential owls, we expected the disjunct population to be genetically isolated between islands. However, our analysis on population genetics on *Oc. exilis* and *Om. avicularia* revealed that the genetic distances between and within the population are small enough (*i.e.*, small p-distance), and there is no genetic gap between islands (*i.e.*, pairwise Fst were less than 0.05 between all populations, and no gap was observed in haplotype networks; Tables 2, 3 and Fig. 5). When Hd = 0.5, Pi = 0.005 were considered as edges (Grant and Bowen 1998), every population of two species has large Hd and small Pi value. This indicates that these populations underwent a bottleneck followed by rapid population growth and accumulation of mutations (Grant and Bowen 1998). As the population of *Om. avicularia* from Okinawa and overall population have negative Tajima's D and Fu's F_S value with significant p-value, recent population expansion are predicted in these regions and species.

Absence of island-specific genetic divergence within *Om. avicularia* is contrary to the genetic differentiation seen in the host species *O. elegans*. This may be explained by a low host-parasite specificity between the louse fly and the owl, since there are records of *Om. avicularia* from birds of other genera such as *Zoothera*, *Emberiza* (Maa 1967) and *Ficedula* (Kano 2000). Although the number of resident bird species tend to be low on islands, the Nansei Islands play an important part of the East Asian-Australasian flyway for migrant birds, sometimes acting as a bottleneck of land for migratory landbirds (Yong et al. 2015 2021). Since louse flies are known to sometimes fly into air from their host bird (Harbison et al. 2008; Nakayama 2010), host switch may occur from the owls to migrant bird species, resulting in the movement and dispersal of the flies between the islands.

Phylogenetic position

We successfully added *Oc. exilis* and *I. amamiensis* in the *COI*-based ML-tree for Hippoboscidae (Fig. 6). Genus *Ornithoica* and *Icosta* each recovered a clade, consistent with our morphological observations and previous study conducted by Yatsuk et al. (2023). However, we could not provide enough verification on the phylogenetic relationship above the genus level (such as subfamilies or tribes) due to the very low support values at the basal branches. It may be desirable to use genetic markers with slower evolutionary rates in order to clarify these relationships.

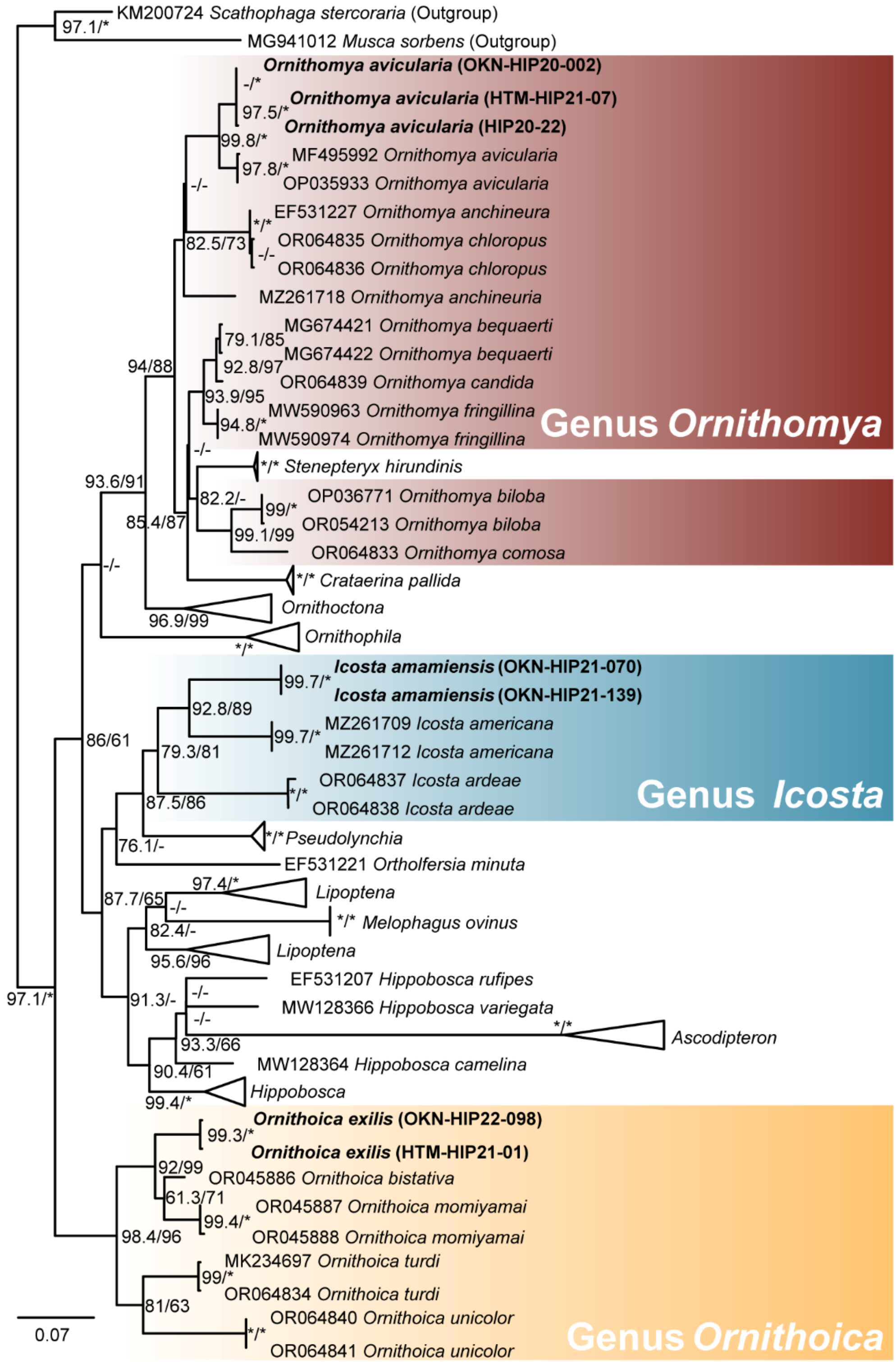

Fig. 6. Maximum-likelihood phylogenetic tree of Hippoboscidae based on *COI* sequences (476 bp). Numbers next to nodes indicate values from the Shimodaira-Hasegawa approximate likelihood-ratio test (SH-aLRT) and ultrafast bootstrap support (uBS). Asterisks indicate full support and supports less than 60 are not shown in the tree. The scale at bottom left indicates branch length in substitutions per site.

Within *Om. avicularia*, we did not detect a genetic differentiation between samples collected from a wide geographic area ranging from Europe and Western Russia to Eastern Canada (Fig. 7). There is likely a large-scale genetic exchange, possibly due to the flies parasitising on bird species with a large and continuous distribution range, or ones that use the East-Atlantic flyway (Stroud et al. 2004). Birds breeding in Europe and Western Russia may encounter those from Northern Canada and Greenland in Western Africa where they winter, which could provide opportunities for the flies to disperse and switch hosts. On the other hand, the Nansei Islands samples used in this study recovered an independent clade with a sample from Far East Russia. These areas overlap with the East Asian-Australasian flyway, thus there may be an independent movement and dispersal of the flies, creating a genetically separate population. Extensive sampling in areas that fall within this flyway and comparison with the host species may give us insight into the connection between population structure within *Om. avicularia*, its distribution and host species.

Brief key to bird host Hippoboscidae of the Nansei Islands

To facilitate research on the Nansei Islands, we compiled a brief key to identify Hippoboscid species. This key covers the species recorded and possibly distributed in the Nansei Islands. This key is modified from the keys by Maa (1963 1967) and Oboňa et al. (2022) (*Ornithomya*), and referred descriptions by Maa (1962 1969c) for *Om. fuscipennis* and Iwasa (2001) for *Crataerina pacifica*.

For more descriptive keys of Japanese *Ornithoctona* and *Ornithoica*, see Maa (1967). For *Ornithoctona*, *Ornithoica* and *Icosta* (except for *I. amamiensis*, which closely resembles *I. chalcolampra*), world keys are available in Maa (1969a), Maa (1966) and Maa (1969b), respectively.

1-1. Wing at least three times as long as wide 15
1-2. Wing functional to fly 2
2-1. Wing cross vein *im* present 3
2-2. Wing cross vein *im* absent (only one cross vein posterior to vein *R*) .... *Pseudolynchia canariensis* (Macquart, 1839)
3-1. Wing cross vein *m-cu* present (three cross vein posterior to vein *R*) 4
3-2. Wing cross vein *m-cu* absent (two cross vein posterior to vein *R*); ocelli often reduced or absent .... genus *Icosta* 10
4-1. Apical half of wing vein R_{4+5} running very close to vein *C*; wing membrane with microtrichia .... genus *Ornithoica* 5
4-2. Wing vein R_{4+5} coalescent with vein *C* at apex of R_{2+3} (see Fig. 2 in Maa [1967] or Fig. 6 in Oboňa et al. [2022]); wing membrane entirely bare .... *Ornithophila metallica* Schiner, 1864

4-3. Wing vein R_{2+3} and R_{4+5} well apart from vein *C*; condition of microtrichia on wing membrane various 6

5-1. wing 2.4–2.7 mm long .... *Ornithoica momiyamai* Kishida, 1932

5-2. wing 2.8–3.8 mm long .... *Ornithoica exilis* (Walker, 1861)

5-3. wing 3.9–4.4 mm long .... *Ornithoica unicolor* Speiser, 1900

6-1. Posterior margin of wing base adjacent to scutellum (axillary cord) with pale setae; wing cross veins *r-m* and *im* very close, at most length of *im*; wing cell m_{1+2} with microtrichia .... genus *Ornithomya* 8

6-2. Posterior margin of wing base adjacent to scutellum (axillary cord, see Fig. 19 in Maa [1967]) with stout black bristles; wing cross vein *im* often located at ca. 2/3 from *m-cu* to *r-m*; wing cell m_1 bare.... genus *Ornithoctona* 7

7-1. Wing < 7.5 mm long .... *Ornithoctona australasiae* (Fabricius, 1805)

7-2. Wing 8–11 mm long .... *Ornithoctona plicata* (von Olfers, 1816)

8-1. Wing membrane marked by microtrichia (see fig. 26 in Maa [1967] or fig. 4 in Nartshuk et al. [2024]); postorbit distinctly narrower than inner orbit; found from various birds 9

8-2. Wing membrane almost entirely covered by microtrichia (for example, see Fig. 2 in Oboňa et al. [2022]); postorbit not narrower than inner orbit; found from swallows (Hirundinidae) .... *Ornithomya comosa* Austen, 1930

9-1. Face broader, 1.5 times broader than eye in front view of head (minimum distance between compound eyes / eye width = 1.5); wing length 5.3–6.5 mm (Maa [1967], specimens from Japan and Korea; in Maa [1962], probably based on specimens from Europe and around Australia: 6.0–6.8 mm in male, 6.5–7.5 mm in female; in Wawman et al. [2025], 4.8−8.7 mm including both sexes) ………………………………………………………….. *Ornithomya avicularia* (Linnaeus, 1758)

9-2. Face narrower, 1.1 times broader than eye in front view of head (minimum distance between compound eyes / eye width = 1.1); wing length of male 6.4–7.8 mm, female 7.3–8.4 mm .... *Ornithomya fuscipennis* Bigot, 1885

10-1. Wing longer than 6.0 mm; palpus in profile more than 2× as long as wide ………………... 11

10-2. Wing not longer than 6.0 mm: palpus length various, about 1.5× to more than 2× of width in profile ………………………………………………………………………………………... 12

11-1. Wing 6.0–6.2 mm long; number of spines on venter of fore tarsus (tarsal spines under foreleg) 1.0.0.0 (basal first tarsomere to distal forth tarsomere); wing cell “*2m+1a*” posteriorly bare ……………………………………………………………... *Icosta (Icosta) amamiensis* Mogi, 1977

11-2. Wing 5.1–5.7 mm long; number of spines on venter of fore tarsus 1.1.1.0; wing cell “*2m+1a*” posteriorly bare ……………………………………... *Icosta (Icosta) chalcolampra* (Speiser, 1904)

11-3. Wing 8.5–9.5 mm long; number of spines on venter of fore tarsus 2.1.1.0 (same number as mid tarsus); wing cell "*2m+1a*" entirely covered with microtrichia ………………………………………………………... *Icosta (Icosta) longipalpis* (Macquart, 1835)

12-1. Palpus mediated proportion, 1.5–2× as long as wide in profile (fig. 2. *Ardeae* in Maa [1969b]) 13

12-2. Palpus narrower, at least 2× as long as wide in profile 14

13-1. Undersurface of cell *2a* entirely setulose, bare stripe on upper surface running narrowly along apical (inner) margin and only about as wide as vein *2A* ……………………………………………….. *Icosta (Ardmoeca) ardeae ardeae* (Macquart, 1835)

13-2. At least apical (inner) 1/3 of cell *2a* bare on both surfaces ("setulae covering entire membrane including alula; upper surface of cell *2a* with very narrow bare stripe along posterior margin"; original description in Maa [1969b: 155]) .... *Icosta* (*Ardmoeca*) *holoptera omnisetosa* Maa, 1969

14-1. Ocelli absent; wing (fig. 48) 3.6–4.2 mm long; female hind tarsus (fig. 146 in Maa [1969b]) usually with 1–2 more or less well-developed minor spines under segment 1 near outer apical corner …………………………………………..... *Icosta* (*Ornithoponus*) *sensilis sensilis* Maa, 1969

14-2. Three ocelli present; wing not more than 6 mm long ……………………………………………….. *Icosta* (*Ornithoponus*) *maquilingensis* (Ferris, 1924)

15-1. Wing narrow, 6–8× as long as wide; wing crossvein *m-cu* present ……………………………………………………………….. *Stenepteryx hirundinis* (Linnaeus, 1758)

15-2. Wing short, and at most 3× as long as wide; wing crossvein *m-cu* absent .... *Crataerina pacifica* Iwasa, 2001

CONCLUSIONS

We report three species of Hippoboscidae from three islands of the Nansei Islands – *Oc.exilis* (Okinawa and Hateruma), *Om. avicularia* (Okinawa, Daito and Hateruma) and *I. amamiensis* (Okinawa). Phylogenetic positions of *Oc. exilis* and *Om. avicularia* are consistent with previous research, and we determined the COI sequence of *Oc. exilis* and *I. amamiensis* for the first time, placing them within the monophyletic clade of each genus. Despite the host species *O. elegans* known to have inter-insular genetic difference, neither *Om. avicularia* nor *Oc. exilis*, collected from several islands, showed genetic differentiation. This is likely due to low host preference, with Hippoboscids switching hosts to migratory bird species, that disperse them between islands. Low host preference may also explain the inter-specific population structure seen in *Om. avicularia*, where the populations may correspond to the areas within the different migratory flyways of birds.

Our records of the Hippoboscidae species on the Nansei Islands extend our knowledge on the louse fly fauna and ecology and build the foundation for further study in the surrounding region.

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Authors' contributions: TH identified the specimens and conducted the taxonomic analysis. AS, NK, and ME collected specimens. ME and MM conducted molecular analyses. All authors contributed to the manuscript.

Competing interests: The authors have no competing interests to disclose.

Availability of data and materials: DNA sequences generated in the study have been deposited in the DNA Data Bank of Japan (DDBJ) database.

Consent for publication: All the authors consent to the publication of this manuscript.

REFERENCES

Atkinson CT, Samuel MD. 2010. Avian malaria *Plasmodium relictum* in native Hawaiian forest birds: epizootiology and demographic impacts on apapane *Himatione sanguinea*. J Avian Biol **41:**357–366. doi:10.1111/j.1600-048X.2009.04915.x.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol **215:**403–410. doi:10.1016/S0022-2836(05)80360-2.

Bequaert JC. 1952. The Hippoboscidae or louse-flies (Diptera) of mammals and birds. Part I. Structure, physiology and natural history. Entomol Am (n ser) **32:**1–209.

Bequaert JC. 1953. The Hippoboscidae or louse-flies (Diptera) of mammals and birds. Part I. Structure, physiology and natural history (continued from volume XXXIII). Entomol Am (n ser) **33:**111–442.

Cumming JM, Wood DM. 2017. Adult morphology and terminology. In: Kirk-Spriggs AH, Sinclair BJ (eds) Manual of Afrotropical Diptera Volume 1. South African National Biodiversity Institute, Pretoria.

Dick CW. 2006 Checklist of World Hippoboscidae (Diptera: Hippoboscoidea). Field Mus Nat Hist, Chicago.

Doszhanov TN. 2003. Mukhi-Krovososki (Diptera, Hippoboscidae) Palearktiki [Louse Flies (Diptera, Hippoboscidae) of the Palearctic Region], Almaty: Nauka.

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour **10:**564–567. doi:10.1111/j.1755-0998.2010.02847.x.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics **147:**915–925. doi:10.1093/genetics/147.2.915

Gill F, Donsker D, Rasmussen. 2024. IOC World Bird List (v15.1). https://www.worldbirdnames.org/new/. Accessed 9 Jan 2024.

Grant W, Bowen BW. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. J Hered **89:**415–426. doi:10.1093/jhered/89.5.415.

Harbison CW, Bush SE, Malenke JR, Clayton DH. 2008. Comparative transmission dynamics of competing parasite species. Ecology **89:**3186–3194. doi:10.1890/07-1745.1.

Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. PNAS **101:**14812–14817. doi:10.1073/pnas.0406166101.

Hill DS. 1962. Revision of the British species of *Ornithomyia* Latreille (Diptera: Hippoboscidae). Proc R entomol Soc London Ser B, Taxonomy **31:**11–18. doi:10.1111/j.1365-3113.1962.tb01163.x.

Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. 2018. UFBoot2: Improving the ultrafast bootstrap approximation. Mol Biol Evol **35:**518–522. doi:10.1093/molbev/msx281

Hutson AM. 1984. Keds, flat-flies and bat-flies. Diptera, Hippoboscidae and Nycteribiidae. Handbooks for the identification of British insects, 10(7). Royal Entomological Society of London, London.

INSD. 2024. International Nucleotide Sequence Database. https://www.insdc.org/. Accessed 18 Aug 2024.

Iwasa M. 2001. A new species of the genus *Crataerina* von Olfers (Diptera, Hippoboscidae) with reduced wings from *Apus pacificus* (Apodiformes, Apodidae) in Japan. Entomol Sci **4:**191–194.

Kano R. 2000. Report of hippoboscid flies collected in the Institute for Nature Study, National Science Museum. Rep Inst Nat Stu **31:**9–11.

Kondo M. 2010. Collection records of two species of louse flies from the Ryukyu Islands. Pulex **89:**553–554.

Lanfear R, Calcott B, Ho SYW, Guindon S. 2012. Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol Biol Evol **29:**1695–1701. doi:10.1093/molbev/mss020.

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2017. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol Biol Evol **34:**772–773. doi:10.1093/molbev/msw260.

Lehikoinen A, Pohjola P, Valkama J, Mutanen M, Pohjoismäki JLO. 2021. Promiscuous specialists: Host specificity patterns among generalist louse flies. PLoS ONE **16:**e0247698. doi:10.1371/journal.pone.0247698.

Leigh JW, Bryant D. 2015. Popart: Full-feature software for haplotype network construction. Methods Ecol Evol **6:**1110–1116. doi:10.1111/2041-210X.12410.

Maa TC. 1962. Notes on the Hippoboscidae (Diptera), 1. Pac Insects **4:**583–614.

Maa TC. 1963. Genera and species of Hippoboscidae (Diptera): types, synonymy, habitats and natural groupings. Pac Insect Monogr **6:**1–186.

Maa TC. 1966. Studies in Hippoboscidae (Diptera). Pac Insect Monogr. **10:**1–148.

Maa TC. 1967. A synopsis of Diptera Pupipara of Japan. Pac Insects **9:**727–760.

Maa TC. 1969a. Synopses of the genera *Ornithophila* and *Ornithoctona* with remarks on their habitat diversification (Diptera: Hippoboscidae). Pac Insects Monogr **20:**1–23.

Maa TC. 1969b. Revision of *Icosta* (= *Lynchia* Auctt.) with erection of a related genus *Phthona*. Pac Insects Monogr **20:**25–203.

Maa TC. 1969c. Notes on the Hippoboscidae (Diptera). II. Pac Insects Monogr **20**:237–260.

Maa TC. 1989. 114. Family Hippoboscidae. *In*: Evenhuis NI (eds) Catalogue of the Diptera of the Australasian and Oceanian Regions. Bishop Museum Press and E. J. Brill, Honolulu.

Matyukhin AV, Artemiev AV, Panov IN. 2017. Parasitological studies of birds: louse-flies (Diptera: Hippoboscidae) in Karelia. Tr. Karel. naučn. centra Ross. akad. nauk, Biogeogr Ser **7:**60–72. doi:10.17076/bg586

Matyukhin AV, Yatsuk AA, Red'kin YA, Smirnov PA, Nartshuk EP. 2023. A new species of the genus *Ornithomya* Latreille (Diptera, Hippoboscidae) from Iturup (Kuril Islands). Entomol Rev **103:**450–454. doi:10.1134/S0013873823040061.

Meißner BR, Rodríguez-Vera F, Hawlitschek O, Heim W, Jentzsch M. 2020. 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 Entomol J **29:**327–335. doi:10.15298/rusentj.29.3.14.

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R, Teeling E. 2020. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. Mol Biol Evol **37:**1530–1534. doi:10.1093/molbev/msaa015.

Mogi M. 1977. Notes on Hippoboscidae (Diptera) from the Ryukyu islands with description of a new *Icosta* species. Kontyû **45:**395–398.

Mogi M. 2014. Family Hippoboscidae. *In*: Nakamura T, Saigusa T, Suwa M (eds) Catalogue of the Insects of Japan Vol. 8 Pt 2. Touka Shobo, Fukuoka.

Mogi M, Kim H-C, Klein TA. 2023. Notes on Diptera Pupipara of the eastern Palaearctic region 1. The taxonomic status of species and subspecies of Hippoboscidae and Nycteribiidae described from temperate Japan and the Republic of Korea. Med Entomol Zool **74:**1–5. doi:10.7601/mez.74.1.

Mogi M, Mano T, Sawada I. 2002. Records of Hippoboscidae, Nycteribiidae and Streblidae (Diptera) from Japan. Med Entomol Zool **53:**141–165. doi:10.7601/mez.53.141.

Nakamura T. 2016. On the publishing of "Catalogue of the Insects of Japan Vol. 8 Diptera" and resolution of Japanese Diptera fauna. Jpn J ent (N S) **19:**22–30. doi:10.20848/kontyu.19.1_22.

Nakayama H. 2010. An aspect in the life history of *Ornithomya avicularia aobatonis* Matsumura (Diptera: Hippoboscidae) observed at Minami Park, Fukuoka, Kyushu, Japan. Med Entomol Zool **61:**125–128. doi:10.7601/mez.61.125.

Nartshuk EP, Matyukhin AV, Shokhrin VP. 2022. Parasitic louse flies (Diptera, Hippoboscidae) and their associations with bird hosts in the south of the Russian Far East. Entomol Rev **102:**367–376. doi:10.1134/S0013873822030083.

Nartshuk EP, Matyukhin AV, Mikhail YM. 2024. Description of a new *Ornithomya* Latreille, 1802 (Diptera: Hippoboscidae) species with a key to all species of this genus. Proc Zool Inst RAS **328:**640–657. doi:10.31610/trudyzin/2024.328.4.640.

Oboňa J, Fogašová K, Fulín M, Greš S, Manko P et al. 2022. Updated taxonomic keys for European Hippoboscidae (Diptera), and expansion in Central Europe of the bird louse fly *Ornithomya comosa* (Austen, 1930) with the first record from Slovakia. Zookeys **1115:**81–101. doi:10.3897/zookeys.1115.80146.

Ohishi H, Kataoka N, Yamazaki H. 2009. *Icosta chalcolampra* (Diptera, Hyppoboscidae) new to Japan. The Dipterist's Club of Japan "HANA ABU" **28:**61–63.

Rambaut A. 2018. FigTree v1.4.4. In: Molecular evolution, phylogenetics and epidemiology. http://tree.bio.ed.ac.uk/software/figtree/. Accessed 18 Aug. 2023.

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P et al. 2017. DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. Mol Biol Evol **34:**3299–3302. doi:10.1093/molbev/msx248.

Sakai J, Kuno K, Hotta M. 2018. The first record of *Icosta (Icosta) longipalpis* (Diptera: Hippoboscidae) from Oriental Honey Buzzerd in Japan. Bird Res **14:**19–21. doi:10.11211/birdresearch.14.S19.

Sato M, Mogi M. 2008. Records of some blood-sucking flies from birds and bats of Japan (Diptera: Hippoboscidae, Nycteribiidae and Streblidae). Rishiri Studies **27**41–48.

Sawada A, Tatematsu K, Kawano T, Takagi M. 2023. First direct evidence of inter-island dispersal of Ryukyu scops owl *Otus elegans*. Ornithol Sci **22:**191–194. doi:10.2326/osj.22.191.

Soós Á, Hůrka K. 1986. Family Hippoboscidae. *In*: Soós Á, Papp L (eds) Catalogue of Palaearctic Diptera 11. Akademiai Kaidó, Budapest, pp. 215–226.

Stroud DA, Davidson NC, West R, Scott DA, Haanstra L, Thorup O, Ganter B, Delany S (compilers) on behalf of the International Wader Study Group. 2004. Status of migratory wader populations in Africa and Western Eurasia in the 1990s. Int Wader Stud **15:**1–259.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics **123:**585–595. doi:10.1093/genetics/123.3.585.

Takagi M. 2011. Vicariance and dispersal in the differentiation of vocalization in the Ryukyu Scops Owl *Otus elegans*. Ibis **153:**779–788. doi:10.1111/j.1474-919X.2011.01163.x.

Tamura K, Stecher G, Kumar S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis version 11. Mol Biol Evol **38:**3022–3027. doi:10.1093/molbev/msab120.

Theodor O, Oldroyd H. 1964. Hippoboscidae. In: Lindner E (ed) Die Fliegen der Palaearktischen Region. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.

The Ornithological Society of Japan. 2024. Check-list of Japanese birds, 8th revised edition. The Ornithological Society of Japan, Tokyo.

Thompson J, Higgins D, Gibson T. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res **22:**4673–4680.doi:10.1093/nar/22.22.4673.

Wang X, Zhou R, Lu L, Wang C, Liu Q. 2022. A new record of *Ornithoica aequisenta* and an updated checklist of Hippoboscidae, Nycteribiidae, and Streblidae in China. J Med Entomol **59:**1071–1075. doi:10.1093/jme/tjac012.

Wawman DC, Bailey AS, Fiddaman SR, Jones BP, Johnson N, Smith AL. 2025. Clarifying the taxonomy of the Finch Louse Fly *Ornithomya fringillina* (Curtis) (Diptera: Hippoboscidae) - an analysis of morphotypes. Acta Parasitol **70:**175. doi:10.1007/s11686-025-01113-z.

Whittaker RJ, Fernández-Palacios JM. 2007. Island biogeography: Ecology, evolution, and conservation. Oxford University Press, London.

WWF Japan. 2010. Nansei Islands Biological Diversity Evaluation Project Report. WWF Japan, Tokyo.

Yamauchi T, Ozaki K. 2007. The louse fly *Ornithoica exilis* (Diptera: Hippoboscidae) collected from the Okinawa Rail *Gallirallus okinawae* (Gruiformes: Rallidae). J Yamashina Inst Ornithol **38:**97–99. doi:10.3312/jyio.38.97.

Yatsuk AA, Triseleva TA, Narchuk EP, Matyukhin AV, Safonkin AF. 2023. Morphology of the wings and attachment apparatus in the evolution of the family Hippoboscidae (Diptera). Integr Zool **0**:1–14. doi:10.1111/1749-4877.12786.

Yatsuk AA, Nartshuk EP, Bushuev A, Kerimov A, Văn Linh N et al. 2024. Description of a new species of *Icosta* Speiser, 1905 (Diptera: Hippoboscidae) from Southern Vietnam with the updated key to the subgenus *Icosta*. Int J Parasitol Parasites Wildl **25:**101026. doi:10.1016/j.ijppaw.2024.101026.

Yatsuk AA, Nartshuk EP, Triseleva T, Shokhrin V, Tolstenkov O et al. 2025. A new species of louse fly, *Ornithomya* Latreille, 1802 (Diptera: Hippoboscidae), from the Russian far East. Int J Parasitol Parasites Wildl **27:**101111. doi:10.1016/j.ijppaw.2025.101111.

Yong DL, Heim W, Chowdhury SU, Choi C-Y, Ktitorov P et al. 2021. The state of migratory landbirds in the East Asian Flyway: Distributions, threats, and conservation needs. Front Ecol Evol **9:**613172. doi:10.3389/fevo.2021.613172.

Yong DLI, Liu Y, Low BW, Española CP, Choi C-Y et al. 2015. Migratory songbirds in the East Asian-Australasian Flyway: a review from a conservation perspective. Bird Conserv Int **25:**1–37. doi:10.1017/S0959270914000276.

Supplementary materials

Fig. S1. Louse fly (presumably *Ornithomya avicularia*) moving freely on the abdomen of *Otus elegans interpositus* in Minami-Daitojima. (download)

Fig. S2. Lice parasitising on *Ornithomya avicularia* collected from *Otus semitorques pryeri* in Okinawajima. (download)

Fig. S3. Chewing lice Philopteridae gen. sp. (Phthiraptera, Ischnocera) collected with OKN-HIP22-005, OKN-HIP22-006 and OKN-HIP22-007 from *Otus semitorques pryeri* in Okinawa. (download)

Table S1. List of Hippoboscidae species previously reported from the Nansei Islands. (download)

Table S2. List of samples examined in this study with information on its sampling location, date, species, sex, presence of phoresy and INSD accession number. “na” in Date column refers to unknown dates. “*Otus elegans elegans / Otus elegans interpositus / Otus semitorques pryeri*” in Host column refers to host species from which the sample was collected. (download)

Table S3. Taxon list and accession number of sequences used for constructing phylogenetic tree of Hippoboscidae. (download)

Table S4. List of taxa and accession numbers used for phylogenetic analysis of *Ornithomya avicularia*, excluding sequences determined in this study. (download)