Original Article

Morphological and Molecular Profiling of the Recently Discovered Ectoparasitic Louse Fly, (*Pseudolynchia canariensis*) (Diptera: Hippoboscidae)

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Abstract

Background: Pseudolynchia canariensis (Diptera: Hippoboscidae) is an obligate blood-feeding ectoparasite of wildlife and a significant ectoparasite of pigeons, responsible for the spread of infections to both birds and humans. **Objective:** This research aimed to detect P. canariensis in adult pigeons and to identify them both physically and molecularly in Jeddah, Saudi Arabia. The prevalence of P. canariensis was 7% among all assessed pigeons. **Methods:** Microscopic examinations revealed that a total of two hundred pigeons were assessed for the presence of P. canariensis from March 2022 to July 2023. **Results:** P. canariensis was identified and documented from pigeons for the first time in Jeddah, Saudi Arabia. P. canariensis is a medium to large-sized organism characterised by a flattened head, thorax, and abdomen, accompanied by short sheathed palpi that are twice as long as they are broad. The wings have feeble posterior veins, comprising five veins posterior to the costal vein. The molecular characterisation of this fly, utilising the COI (GenBank accession No. OR288145), revealed a sequence of 704 bp, exhibiting 99.78% identity with the COI gene of Pseudolynchia sp. **Conclusion:** The study emphasises that pigeons are significant veterinary hosts and may serve as a crucial source of infection for other bird species that share similar parasite fauna; furthermore, sanitation and cleanliness are vital for managing external parasitism.

Keywords: *Pseudolynchia Canariensis*, Morphological Identification, Molecular Identification, First Record, Jeddah City.

Introduction

Ectoparasites are creatures that inhabit the exterior surfaces of their hosts and are crucial to the ecological dynamics of diverse ecosystems. Pseudolynchia canariensis (Diptera: Hippoboscidae), popularly referred to as the pigeon louse fly, is a prominent ectoparasitic insect that significantly affects bird hosts, especially pigeons (Columba livia domestica). The complex link between P. canariensis and its bird hosts has attracted significant interest from researchers aiming to comprehend the morphological, molecular, and ecological dimensions of this parasitic interaction. P. canariensis, first described by Ali *et al.*[1], has been acknowledged as a prevalent ectoparasite of pigeons, infesting their feathers and nesting locations [2]. Its global distribution is intricately linked to the extensive presence of its bird hosts, rendering it a compelling topic for scientific investigation. A crucial element in comprehending the biology of P. canariensis is the thorough analysis of its morphological traits. Numerous research have focused on the morphological and genetic identification of this ectoparasite.[1,3] P. canariensis are non-permanent ectoparasites characterised by a dorsoventrally flattened body measuring between 4 and 12 mm. Both sexes are obligate hematophagous parasites, predominantly residing within the feathers or fur of avian or mammalian hosts during their adult stage.[2] P. canariensis demonstrates viviparity, an adaptation closely linked to its ectoparasitic lifestyle, with the sole free-living stage being the pupae, which are laid in the nests of birds or the burrows of animals.[4] Advancements in molecular biology have transformed parasitology, enabling a more refined comprehension of species identification and genetic variety. Recent investigations utilising molecular techniques, including Polymerase Chain Reaction (PCR) and DNA sequencing, have facilitated the precise identification of P. canariensis and its genetic

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variants.[5] Numerous research have focused on the molecular identification of this ectoparasite, including Metwally *et al*.[3] in Riyadh. Nonetheless, there is no research validating the existence of the bug in the city of Jeddah. The application of molecular technologies has enabled accurate species identification and established a foundation for investigating the genetic links across several populations of P. canariensis.[5] The ecological impact of P. canariensis transcends its function as an ectoparasite, and comprehending the dynamics of its interactions with avian hosts is essential for elucidating the wider ecological ramifications within bird communities.[4] The possible impact of P. canariensis on the health and behaviour of pigeons, along with its involvement in disease transmission, warrants comprehensive examination. Furthermore, investigating the ecological parameters that affect the incidence and distribution of P. canariensis can enhance the understanding of the intricate relationship between parasites and their bird hosts in natural environments. This research report examined the morphological and molecular characterisation of the rare, newly identified louse flies (Pseudolynchia canariensis) and compare them with various strains in the NCBI database.

Methodology

Sample Collection

One hundred pigeons were analysed in the Jeddah governorate from March 2022 to July 2023 over two distinct regions (Al-Khomra and Al-Salama). Pigeons underwent manual external examinations to identify parasites in the body groins (the sparsely feathered areas around the vent, beneath the wings, and between the thighs), as well as in the tail, wings, and body feathers. Flies were collected manually and preserved in 10% ethanol. Collected samples were labelled and dispatched to the Zoology laboratory at the Faculty of Sciences, King Abdulaziz University for further analysis.

Parasitological Examination

The gathered flies were analysed under a stereoscopic microscope to inspect the mouthparts, thorax, legs, and posterior segments of the abdomen, following the methodology outlined by Attia and Salem[5].

DNA Extraction

Genomic DNA was isolated from tissue obtained from preserved fly specimens. The tissue was initially homogenised using a bead homogeniser and subsequently digested overnight at 56 °C. DNA extraction was conducted with the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), adhering to the manufacturer's instructions. The whole DNA was eluted into 200 µL and preserved at −40 °C.

Polymerase Chain Reaction (PCR)

The purity of genomic DNA was assessed using a nanodrop device, yielding a ratio of A260/280 ≥ 1.7. Universal primers LCO1490 (5'GGTCAACAAATCATAAAGATATTGG3') and HCO2198 (5'TAAACTTCAGGGTGACCAAAAAATC - 3') were employed in polymerase chain reaction (PCR) targeting a fragment of 700-800 bp. The conditions for PCR were as follows: Initial denaturation: 5 minutes at 94 °C; denaturation: 1 minute at 94 °C; annealing: 1 minute at 58 °C; extension: 2 minutes at 72 °C; number of cycles: 35; final extension: 10 minutes at 72 °C. To estimate size, 10 µl of molecular weight marker was introduced into the first well. The gel was operated at 127 V for one hour, securely affixed, and linked to the power supply (MOLECULE-ON PS-M-300 V Electrophoresis Power Supply, India). One percent agarose powder was measured and dissolved in 1X TAE buffer by heating the solution in a microwave oven. A final concentration of 0.1 µg/ml ethidium bromide (EtBr) was incorporated into the agarose from a 10 mg/ml stock solution in distilled water. Agarose was subsequently poured into the gel tray, and the comb was positioned at one end. The gel was allowed to set for one hour prior to the removal of the comb. The gel was subsequently positioned in an electrophoresis tank and immersed in 1X TAE buffer, serving as the running buffer. The 5X loading dye was incorporated into the samples at a volume ratio of 5:1, sample to dye. The gel was electrophoresed in a horizontal gel apparatus at 127 volts for 60 to 90 minutes. DNA fragments were visualised using a UV transilluminator and captured with a Viber Lourmat Gel Imaging System. A 700 bp DNA ladder (Promega, USA) served as a marker. The PCR products measured 700 base pairs. Samples underwent sequencing via Sanger methodology at Macrogen, South Korea. A comparison was made between nucleotide sequence data and the NCBI database utilising the Basic Local Alignment Search Tool (BLAST). The aphids collected from the field were identified by matching their sequences with those in the gene bank.

Sequencing

The sequences were manually verified and modified utilising the NCBI database (The National Centre for Biotechnology Information). The gene sequences of the fly were aligned with homologous genes in the GenBank database utilising BLAST (Basic Local Alignment Search Tool).

Results

A total of seven adult male Hippoboscidae specimens were obtained from birds in two distinct districts of Jeddah city for morphological identification. The adult P. canariensis exhibits dorsoventral flattening, enabling the fly to manoeuvre between feathers and conceal itself to evade observation (see Figure 1). The head possesses a prominent compound eye, abbreviated antennae situated in a depression on the skull, and specialised sucking mouthparts designed to pierce the skin of avian hosts and extract their blood. Its mouthpart is orientated anteriorly rather than inferiorly, as depicted in Figure 2 and Figure 3. The

thorax comprises three segments: prothorax, mesothorax, and metathorax, as illustrated in Figure 4. A single pair of well-developed wings is exclusively located on the mesothorax, characterised by their length, which surpasses the body length (see Figure 5). Each thoracic segment is connected to a pair of sturdy legs characterised by robust, expanded femora, indicating its need on the legs when scurrying among the feathers. 4 claws were seen on each of its 6 legs to hook tightly onto the avian host as in Figure 6. The dorsal-ventral abdomen is flat and small, measuring 0.5 cm in length, and features a flexible chitinous covering on the posterior body that allows for expansion during blood feeding or larval development within the female (see Figure 7).

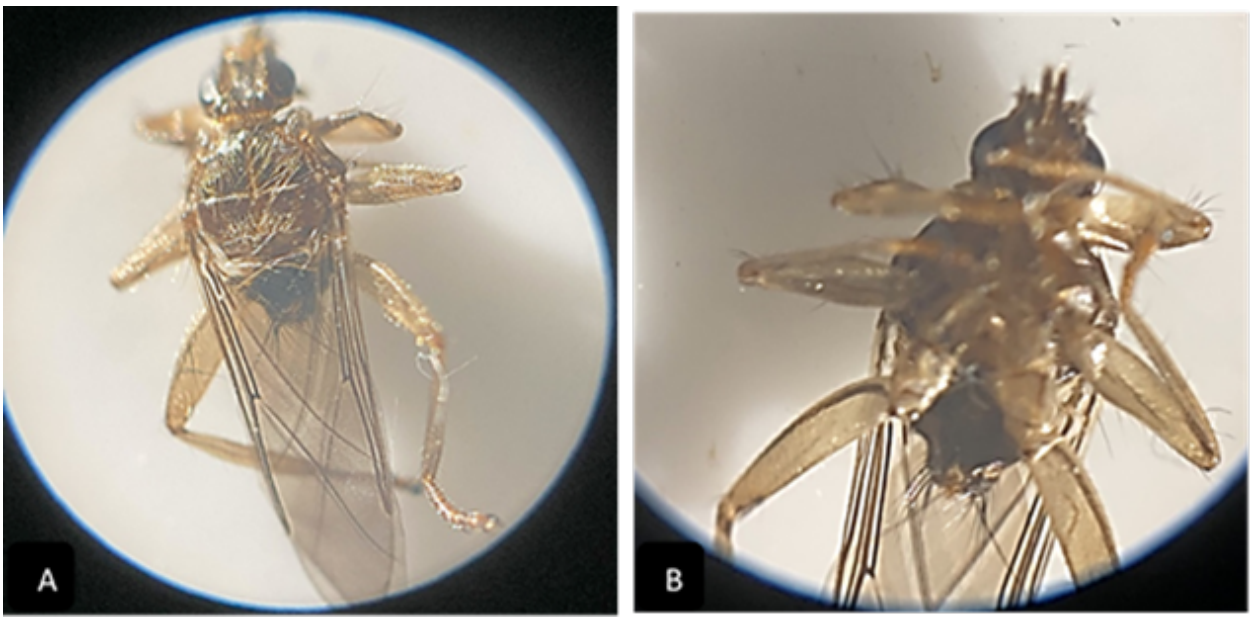

Figure 1: A: Dorsa View of *P. canariensis* adult, B: Figure 2: Ventra View of *P. canariensis* adult.

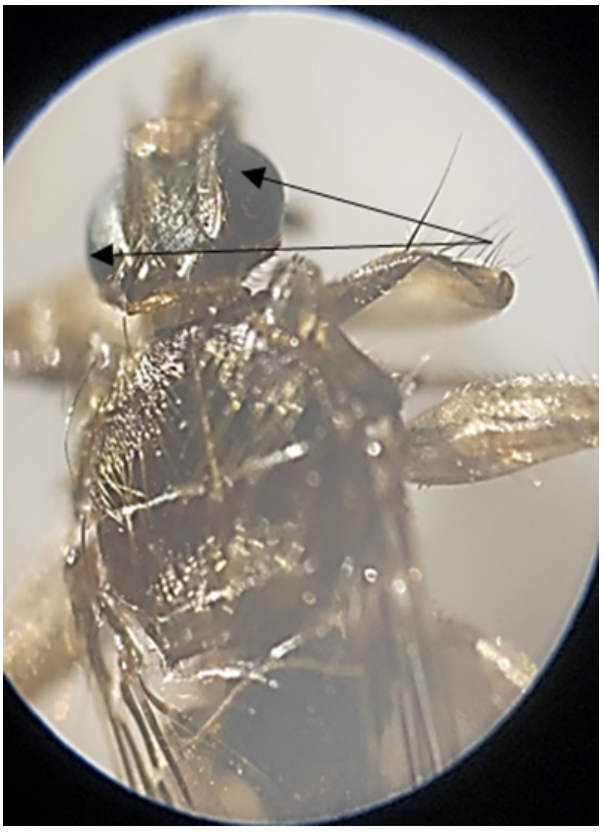

Figure 2: Large Compound Eye.

Figure 3: Pursing Sucking Mouth Parts.

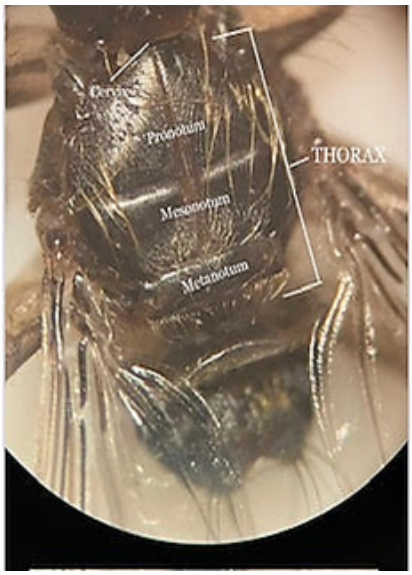

Figure 4: Thorax Segments.

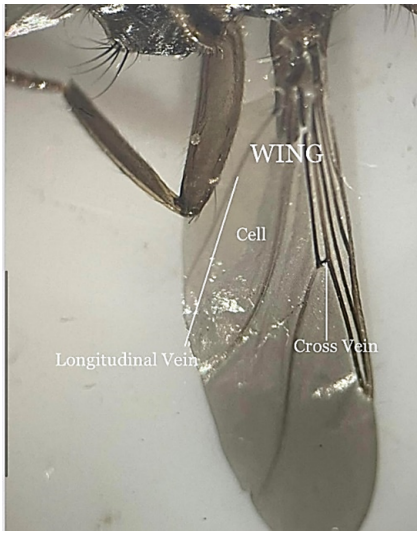

Figure 5: Long Wings.

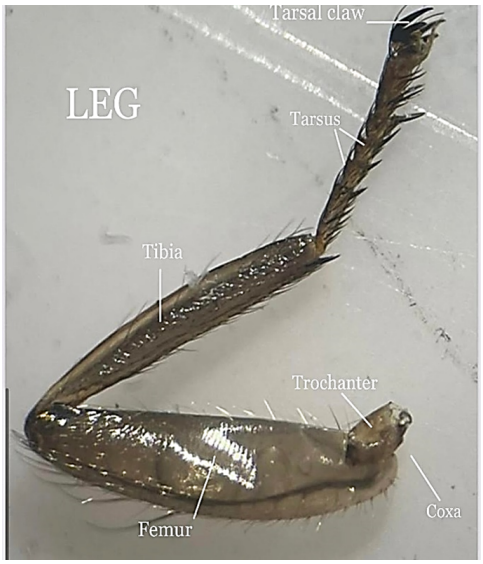

Figure 6: Leg Parts of *P. canariensis*.

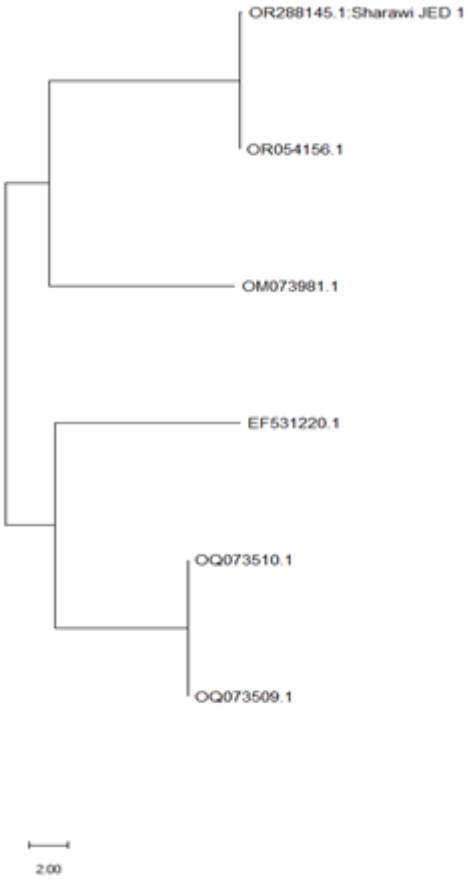

Figure 7: Short Abdomen.

Molecular identification involved phylogenetic analysis utilising sequences from P. canariensis compared with reference sequences from NCBI via BLAST. Our results reveal a notable resemblance of 97%, and the sequences of the novel Deer ked have been deposited in GenBank under accession number OR288145 (Sharawi_JED_1) for the first time in Saudi Arabia, with data recorded and published in the NCBI database (see Figure 8).

Figure 8: Sequences Similarity of *P. canariensis* Compared to GenBank using NCBI Software.

Discussion

A multitude of researchers have examined the morphological identification of P. canariensis across various regions, including Medina[1], Libya[6], Iraq[7], Bangladesh[8], Iran[9], and Brazil[10]. This is the inaugural report of P. canariensis infestation caused by domestic pigeons in Jeddah, Saudi Arabia. Unexpectedly, our investigation revealed that 7% of P. canariensis was identified in the examined pigeons, representing the lowest percentage among the comparable research. Our findings align closely with those of Alkharigy *et al*.[6], who reported that P. canariensis infected 1% of pigeons. A research investigation conducted in Minas Gerais, Brazil, revealed that all free-living pigeons were infested with P. canariensis.[11] Additionally, in Tehran, an infestation of P. canariensis was recorded at 28.57% in pigeon lofts.[12] A research conducted in the Canary Islands revealed that P. canariensis was present in 36% of the examined pigeons.[13] Ghosh Ghosh *et al*.[8] reported that P. canariensis infected 43% of the studied pigeons. Attia and Salem[5] reported a 64% frequency of P. canariensis among the surveyed birds in Egypt. In Madagascar, one species of P. canariensis was documented.[14] The peak infection rate was observed in Medina, where 89% of P. canariensis infected squabs of Harami pigeons.[1] Infection rates of pigeons with P. canariensis have been observed in Bangladesh[15] at 63.33% and in Iran[9] at 63.72%. The heightened occurrence of ectoparasites in warm climates can be ascribed to the necessity of ideal conditions for parasite development and the diminished resistance of avian species to these parasites in elevated temperatures, leading to severe infestations. The declining incidence of P. canariensis infestation in Jeddah City may be attributed to elevated humidity levels. Numerous authors concur with our findings, including Pirali-Kheirabadi *et al*.[16] and Nadeem *et al*.[17]. No research, whether numerous or recent, exist regarding the molecular identification of P. canariensis. A recent study in Egypt found that 99.78% of P. canariensis samples matched the COI gene of Pseudolynchia sp. with accession No. MW853922.[5] Few studies exist, as the majority have focused on the molecular identification of pathogenic organisms associated with the insect rather than the insect itself. Moreover, the dissemination of our data in the NCBI database improves the accessibility and visibility of our discoveries throughout the international scientific community. This portal functions as a data repository and enables collaboration and comparative analyses with other researchers studying related species. Collective knowledge enhances the comprehensive understanding of louse fly genetics and underscores the significance of collaborative endeavours in progressing molecular taxonomy.

In conclusion, our research demonstrates the collaboration between morphological and molecular methodologies in elucidating the genetic identity of P. canariensis. The integration of DNA approaches not only confirmed the physical identification but also offered a refined understanding of the evolutionary relationships within this species. Our research establishes a foundation for future investigations into the molecular ecology and dynamics of P. canariensis, facilitating further exploration of ectoparasitic louse flies, particularly during the Hajj and Umrah season, a period characterised by an influx of pigeons and visitors from various nations.

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