RESEARCH ARTICLE

Ultrastructural Morphology and Molecular Identification of *Laemobothrion maximum* Lice (Phthiraptera: Laemobothriidae) from Brahminy Kite (*Haliastur indus*) in India

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Abstract

Laemobothrion maximum is one of the largest known avian lice, predominantly parasitizing raptors but detailed morphological and molecular studies from India are scarce. A juvenile Brahminy kite rescued was presented with active lice infestation to VCRI, Orathanadu (TN, India). Lice were collected, morphologically examined and processed for scanning electron microscopy (SEM) and molecular confirmation. Morphological examination identified the specimens as *L. maximum*, measuring 10-11 mm, with a flattened head, sculptured temples, U-shaped sitophore sclerite, cephalic ctenidia and specialized tarsal claws. SEM revealed ultrastructural adaptations including mandibular cuticular projections, diverse trichoid and basiconic sensilla, reticulated abdominal microtrichial patterns. Molecular identification using mitochondrial cytochrome c oxidase subunit-I (*COI)* gene amplification confirmed species identity with high accuracy. This is the first integrated morphological, ultrastructural and molecular description of *L. maximum* infesting Brahminy kite in India. The findings provide insights into its identification features, host specificity and adaptive traits, offering a valuable baseline for future taxonomic, ecological and phylogenetic studies on avian lice.

Key words: Brahminy kite, Cytochrome c oxidase subunit-I (*COI*) gene, Electron microscopy, *Laemobothrion*, Morphometry, Sensilla.

Ind J Vet Sci and Biotech (2026): 10.48165/ijvsbt.22.1.26

Introduction

Birds constitute an integral component of natural ecosystems, contributing significantly to ecological balance in ways comparable to mammals and other fauna. Distributed across forests, villages, wetlands, and urban landscapes, avian species perform diverse ecological roles, including pollination, seed dispersal and scavenging. Among these, raptors such as eagles, kites, vultures and hawks occupy a unique niche as apex predators and scavengers, regulating prey populations and contributing to the natural removal of carrion, thereby maintaining ecological hygiene (Arya *et al.,* 2024). However, in recent decades, populations of many raptors have shown a marked decline, driven by habitat loss, urbanization, exposure to toxic residues and drug metabolites from domestic animals and infectious diseases. Among these threats, parasitic infections remain comparatively underexplored, despite their potential to compromise raptor health and survival (Krone and Cooper, 2002). Parasitic diseases of birds may be caused by protozoa, helminths or ectoparasites, with the latter often exerting indirect but profound impacts.

Ectoparasitic infestations, particularly by lice (order: Phthiraptera), are of notable concern in raptors. Lice are obligate, wingless, dorsoventrally flattened ectoparasites with high host specificity and are broadly classified into chewing lice (Mallophaga) and sucking lice (Anoplura) with the former infesting both birds and mammals and feeding on feathers, skin debris, and secretions (Hatem *et al.,* 2021). Such infestations cause irritation, feather damage, impaired

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How to cite this article: Tamileniyan, E., Jeevitha, M., Latchumikanthan, A., Veeraselvam, M., Arul, V., Velusamy, R., & Swamy, K.K.P. (2026). Ultrastructural Morphology and Molecular Identification of *Laemobothrion maximum* Lice (Phthiraptera: Laemobothriidae) from Brahminy Kite (*Haliastur indus*) in India. Ind J Vet Sci and Biotech, 22(1), 132-138.

Source of support: Nil

Conflict of interest: None

Submitted 02/10/2025 **Accepted** 22/10/2025 **Published** 10/01/2026

thermoregulation, and weight loss (Clayton, 1990). Although not considered primary pathogen vectors, chewing lice may occasionally facilitate the transmission of parasites such as avian infectious etiologies (Martín-Mateo, 2002). Raptors serve as hosts to a variety of chewing lice, among which the genus *Laemobothrion* are particularly important. *Laemobothrion maximum* (Scopoli, 1763) represents one of the largest known avian lice (Jeyathilakan *et al.,* 2021) characterized by its robust morphology and close association with predatory birds. Despite its widespread distribution across raptors, very few studies have focused on its ultra-structural adaptations and molecular characteristics especially in the Indian subcontinent. The present study reports the occurrence of *Laemobothrion maximum* in a rescued Brahminy kite from Tamil Nadu, India, and provides detailed ultra-structural morphology with molecular confirmation. These findings are addition to the knowledge on ectoparasites of raptors and aids in the parasitological investigations in avian conservation biology.

Materials and Methods

Sample Collection and Processing

A juvenile Brahminy kite (*Haliastur indus*) was presented to the Exotic and Special Species Medicine Referral Clinic, Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu, TANUVAS (India). The bird was rescued by the Endangered Wildlife Environment Trust (EWET) from a roadside location and reported to be unable to fly, exhibiting signs of weakness and distress. Upon clinical examination, active lice infestation was observed in the breast region, with the parasites freely moving among the feathers. Lice (n=6) were carefully collected by using forceps and preserved in 70% ethanol and subjected to dehydration through ascending grades of ethanol (50%, 70%, 90% and absolute alcohol) followed by clearing in xylene for 4 h. The cleared specimens were subsequently mounted on glass slides using DPX mount for further examination (Soulsby, 1982).

Scanning Electron Microscopy (SEM)

The collected lice specimens were thoroughly cleaned to remove surface debris and subsequently fixed in 2% buffered glutaraldehyde (pH 7.2) for 12 h. Following fixation, the samples were rinsed three times in phosphate buffer (pH 7.2), followed by washing with double-distilled water. The specimens were then dehydrated through a graded series of acetone and dried using a critical point drier. Dried specimens were mounted onto aluminium stubs using adhesive tapes and subsequently gold-coated using a sputter coater (Polaron SC7620). The prepared samples were examined under a scanning electron microscope (SEM) (FEI Quanta 200, operating at 20 kV) at the Centre for Nanotechnology and Advanced Biomaterials (CeNTAB), Sastra Deemed University, Thanjavur.

Molecular Identification

Genomic DNA was extracted from lice leg segments using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The concentration and purity of the extracted DNA was assessed using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific™). Species-specific identification was performed by PCR amplification of the mitochondrial cytochrome c oxidase subunit-I (*COI*) gene using *Laemobothrion maximum*-specific primers: LAEM-F (5'- TGCTCGCCGGTTTATCAAAA - 3') and LAEM-R (5'-GCTCTACAGGGTCTTCTCGT - 3'). PCR reactions were carried out in a thermal cycler (Genei) under the following conditions: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 56-60 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min. The amplified PCR products were resolved on a 1.5% agarose gel at 75 V using a 100 bp DNA ladder (Gene DireX, Taiwan) as a molecular size marker. Visualization of DNA bands was performed under UV illumination using a gel documentation system (Bio-Rad, USA).

Results and Discussion

Morphological Characteristics of Lice

The lice collected were morphologically identified as *Laemobothrion maximum* through their biometry (Table 1). The adult louse measured 10-11 mm in length marking them to be the largest known bird lice (Fig. 1a, 1b). The head was distinctly flattened, with a very large oral opening extending posteriorly up to the antennal base. Prominent lateral preocular swellings were present in front of the eyes and the temporal angles were narrow. Morphometric analysis showed a head length of 1.97 mm and head width of 1.99 mm. The antennal capsules were bulbous and opened ventrally, while the temples were sculptured with inter-row peg-like projections. The mandibles were the most conspicuous portion of the mouthparts, prominently sclerotized and robust; the labrum, maxillae and labium were comparatively small (Fig. 2a). The sitophore sclerite of the hypopharynx appeared as a large U-shaped structure with two openings. The thorax was well developed and bore three pairs of legs, each ending in two claws (Fig. 2b). The thorax length measured 2.33 mm, supporting the robust structure of the appendages. The abdomen was elongated and broad characterized by pigmented areas along the median line of the abdominal tergites and an irregular arrangement of bristles. Morphometric measurements revealed an abdomen length of 6.50 mm and an abdomen width of 3.14 mm. The posterior end exhibited numerous small and long setae (Fig. 2c). The legs of *Laemobothrion maximum* were firmly attached to the pterothorax and comprised the typical segments: coxa (cx), trochanter (tt), femur (fe), tibia (tb) and tarsus (ts), terminating in a well-developed, sharply pointed claw (cl) (Fig. 3a). The distal end of each leg displayed a robust terminal

claw associated with a prominent aroliar pad (ar), likely aiding in adhesion to the feathers of host (Fig. 3b). The abdominal tergites were characterized by distinct pigmented areas along the median line, interspersed with small fine setae sparsely arranged (Fig. 3c).

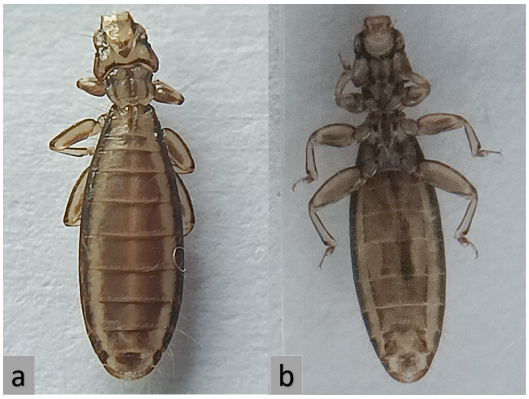

Fig.1: Gross *Laemobothrion maximum*-Dorsal (a) and Ventral (b) view

Table 1: Morphometry of *Laemobothrion maximum* lice (n=6)

Body region / structure	Measurement (mm)
Total body length	10.0–11.0
Head length	1.9–2.0
Head width	1.9–2.0
Thorax length	2.3–2.4
Abdomen length	6.4–6.6
Abdomen width	3.1–3.2
Total leg length	2.7–2.8
Coxa	0.40–0.42
Trochanter	0.20–0.22
Femur	0.61–0.63
Tibia	0.75–0.77
Tarsus	0.52–0.54
Claws	0.17–0.19

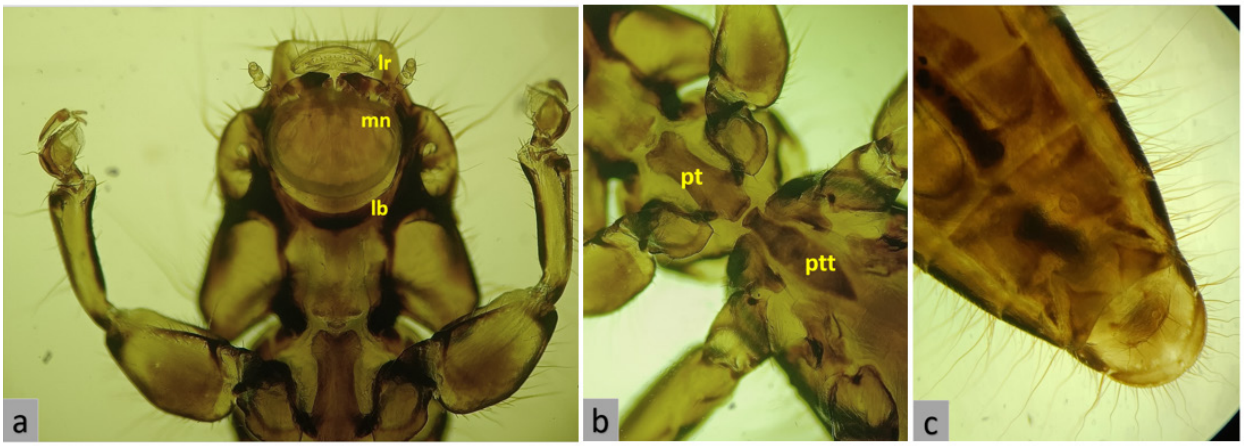

Fig. 2: *Laemobothrion maximum*; Head end showing distinct lateral preocular swellings located anterior to the compound eyes and mouth part distinguished by labrum (lr), mandible (mn) and labium (lb) (a); Thoracic region showing pt: prothorax, the first thoracic segment bearing the anterior pair of legs, ptt: pterothorax formed by the fusion of meso- and metathorax (b); Female - Posterior end showing numerous long and short setae distributed along the terminal segments (c)

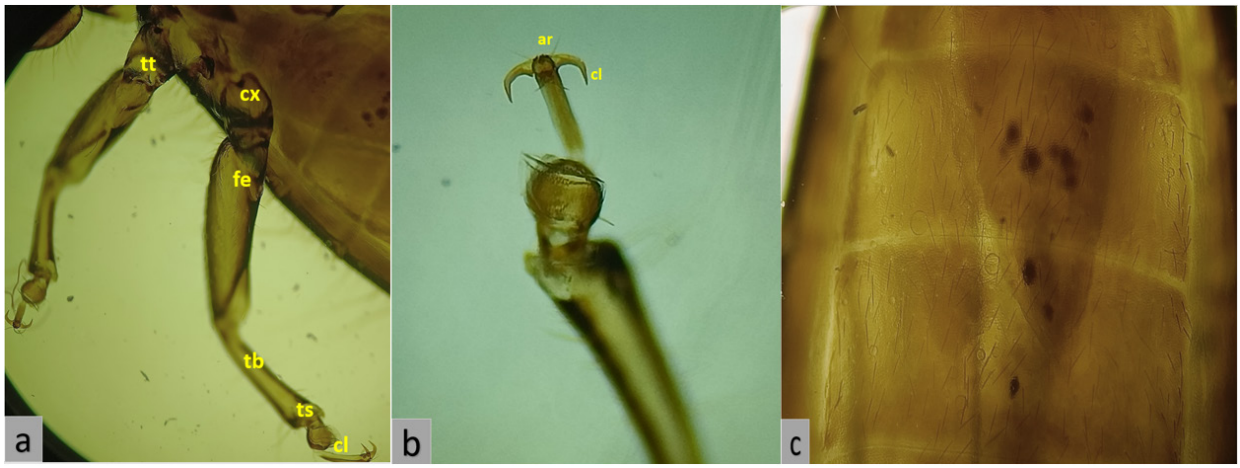

Fig. 3: *Laemobothrion maximum*;Leg showing the attachment of the leg to the pterothorax followed by the coxa (cx), trochanter (tt), femur (fe), tibia (tb), tarsus (ts) and ending with the terminal claw (cl) (a); Distal end of the leg showing the well-developed terminal claw (cl) and associated aroliar pad (ar) (b); Abdomen showing pigmented areas along the median line of the abdominal tergites with irregularly arranged small setae (c)

The present study provides a detailed description of the morphology and ultrastructure of *Laemobothrion maximum*, the largest known bird louse, with adults measuring 10-11 mm in length. These dimensions fall within the upper size range reported for laemobothriids, which typically include the largest representatives of avian lice and the morphometric characteristics observed were consistent with those documented in earlier studies (Mey, 2003; Dik and Ozkayhan, 2007; Clayton *et al.,* 2009; Inci *et al.,* 2010). The strongly flattened head, capacious oral opening extending posteriorly to the antennal bases and narrow temporal angles observed in this study were consistent with earlier morphological accounts of *L. maximum* and related taxa, reflecting adaptations for grasping within the plumage of large raptors (Mey, 2003; Price *et al.,* 2003; Kushwaha, 2015). Similarly, the pigmented median tergal areas and irregular abdominal bristles correspond with features previously noted for this species (Pérez *et al.,* 1995). The hypopharyngeal sitophore sclerite appearing as a large U-shaped structure with two openings is a diagnostic characteristic of Laemobothriidae and has been highlighted in taxonomic redescriptions of *L. maximum* (Pérez *et al.,* 1995; Dalgleish *et al*., 2006; Jeyathilakan *et al.,* 2021).

Ultrastructural Morphology of Lice

Electron microscopy revealed the head of lice displayed prominent anterior subocular setae. The oral opening was very large, extending posteriorly to the antennal base (Fig. 4a). At the base of the mandibles, chitinous modifications were observed, including localized thickening or grooves and sclerotized structural adaptations (Fig. 4b). The base of the labium bears numerous small prolongations, while the mandibular bases exhibited rigid cuticular extensions with variable indentations. The fourth segment of maxillary palp was equipped with several trichoid sensilla and more than 25 apical basiconic sensilla (Fig. 4c). The third antennal segment was particularly prominent, bearing long hairs that projected outward. The antennal pits themselves were lined with trichoid sensilla and the pleural view of the head revealed rows of ~50 µm trichoid sensilla situated near the eyes. Beneath these, rigid spines formed a series of cephalic ctenidia likely serving as protective and anchoring structures in relation to the host integument.

The cuticular surface was densely covered with elongated setae interspersed with fine microstructural ridges (Fig. 5a). The tarsal claws were sharply pointed and slightly curved, exhibiting prominent inner protuberances which arose proximally along the inner margin of the claw and tapered gradually toward the distal tip (Fig. 5b). The overall claw surface was smooth, contrasting with the raised protuberances which formed distinct structural adaptations along the inner edge of the tarsus. The tarsal claws were observed to articulate perpendicularly with the tarsus, with a terminal orifice bordered by basiconic sensilla and claw movements limited by cuticular protuberances. At the tibial apex dense setae were seen with trichoid sensilla. The abdominal segments exhibited a reticulated pattern, with lateral combs of microtrichia located on sternites IV and V (Fig. 6a). The terminal region of the abdomen displayed numerous long and short setae distributed across the posterior segments. The setae varied in length and orientation, with some aligned along the margins while others projected at different angles creating a dense and irregular arrangement along the distal abdominal surface (Fig. 6c).

Ultrastructural observations confirmed a high density of sensory organs, the four-segmented antennae with an enlarged third segment bearing long setae and antennal pits lined with trichoid sensilla, mirror earlier ultrastructural descriptions (Pérez *et al.,* 1995). Leg morphology particularly the robust thoracic appendages terminating in two claws, showed features typical of amblyceran lice. The perpendicular articulation of the tarsal claws, bordered by basiconic sensilla

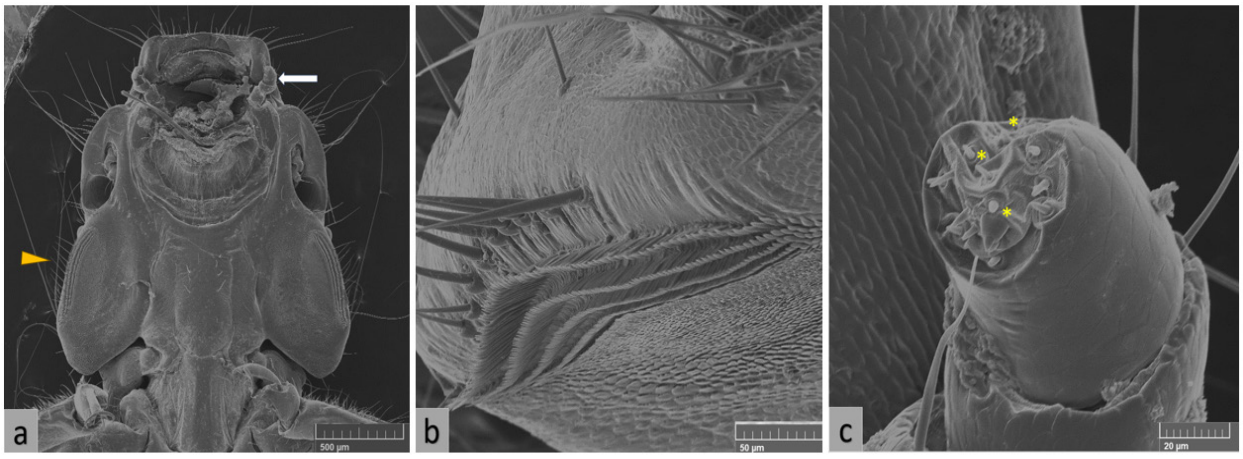

Fig. 4: Ultra structural morphology of *Laemobothrion maximum* - Anterior subocular setae (arrowhead), a very large oral opening extending backward to the antennal base and the maxillary palp (arrow) (a); cuticular modifications at the base of the mandibles, characterized by localized thickening and structural adaptations (b); Distal end of a maxillary palpus with basiconic sensilla (*) appearing as peg-like projections on the cuticular surface (c)

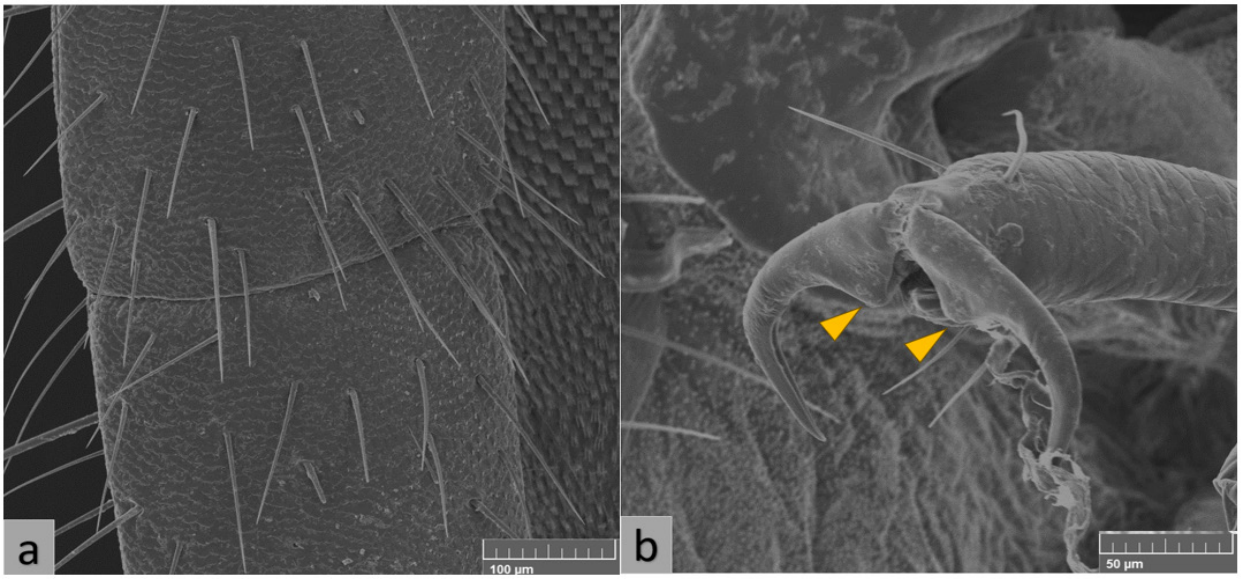

Fig. 5: Ultra structural morphology of *Laemobothrion maximum* - cuticular surface of the , densely covered with elongated setae and microstructural ridges (a); Tarsal claws exhibiting well-defined inner protuberances (arrow head) with a pointed, slightly curved structure; surfaces show smooth cuticular lining and the protuberances arise proximally along the inner margin, tapering towards the distal tip (b)

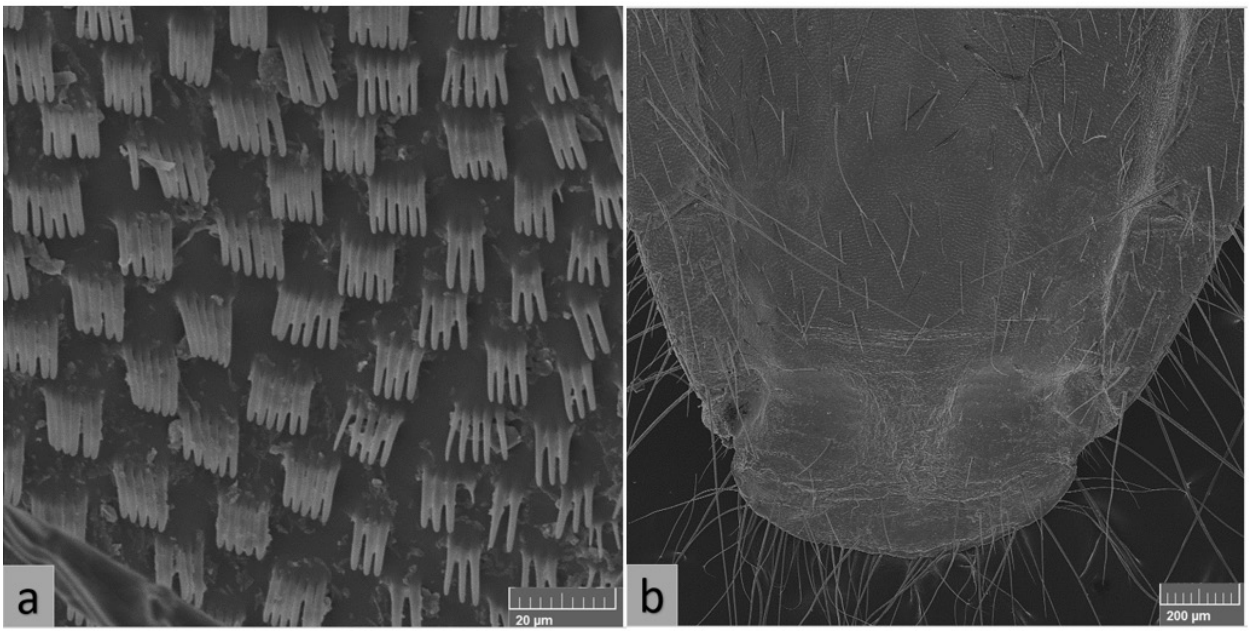

Fig. 6: Microtrichia located laterally on abdominal sternites IV and V, appearing as fine, hair-like cuticular projections distributed along the lateral margins of the sternites (a); Male abdomen tip showing numerous long and short setae distributed across the terminal segments, with setae varying in length and orientation along the distal abdominal margins (b)

and the tibia with trichoid sensilla are in accordance with SEM observations emphasizing locomotory and attachment adaptations (Pérez *et al.,* 1995). These structures likely aid the parasite in clinging to the plumage of large, mobile hosts. Abdominal bristles also showed agreement with prior findings, with long post-spiracular setae on segments II–VIII, reticulated cuticle patterns and combs of microtrichia on sternites IV–V. These microstructures are generally interpreted as enhancing locomotion and frictional resistance on host feathers (Pérez *et al.,* 1995). In females, the post-vulvar region lacked pigmentation and exhibited a specific arrangement of long and short setae, a feature previously recognized as taxonomically relevant within Laemobothriidae (Price *et al.,* 2003; Jeyathilakan *et al.,* 2021).

Molecular Identification and Host Association

In the present study, the parasite was confirmed as *Laemobothrion maximum* using species specific primers. Amplification of parasite DNA from the tissue sample yielded a PCR product of 242 bp (Fig. 7). In the present study, *L. maximum* was identified on its typical avian hosts which aligns closely with earlier Indian reports documenting its

occurrence in the Indian black kite (*Milvus migrans*) (Athira *et al.,* 2019; Lade *et al.,* 2023), greater coucal (*Centropus sinensis*) (Jeyathilakan *et al.,* 2012) and long-billed vulture (*Gyps indicus*) (Kushwaha, 2015). Among these, the reports from black kites and vultures reflect established host-parasite associations, whereas the occurrence in the greater coucal is likely an incidental infestation (Jeyathilakan *et al.,* 2012). Globally, *L. maximum* is predominantly associated with large accipitriform and falconiform raptors, including eagles, buzzards, kites and vultures and its distribution extends across Eurasia and North Africa (Mey, 2003; Price *et al.,* 2003). Studies conducted on long-legged buzzards (*Buteo rufinus*) in Turkey (Dik and Ozkayhan, 2007) and wild birds in the Cappadocia region (Inci *et al.,* 2010) further corroborate the strong preference of the parasites for raptor hosts. The strong host specialization, enabling *L. maximum* to adapt efficiently to the plumage microhabitats and behavioural ecology of large birds of prey, while maintaining relatively limited genetic and morphological divergence across its distribution range. Thus, the findings of the present study strengthen the existing evidence that *L. maximum* exhibits a clear host preference for large raptors, with occasional infestations on non-raptor birds representing phoretic or incidental associations rather than true parasitism. Comparative data from India and abroad demonstrate that despite differences in host species and geographical locations, the morphological, ultrastructural and ecological characteristics of this louse species remain strikingly consistent, highlighting its evolutionary specialization and niche fidelity.

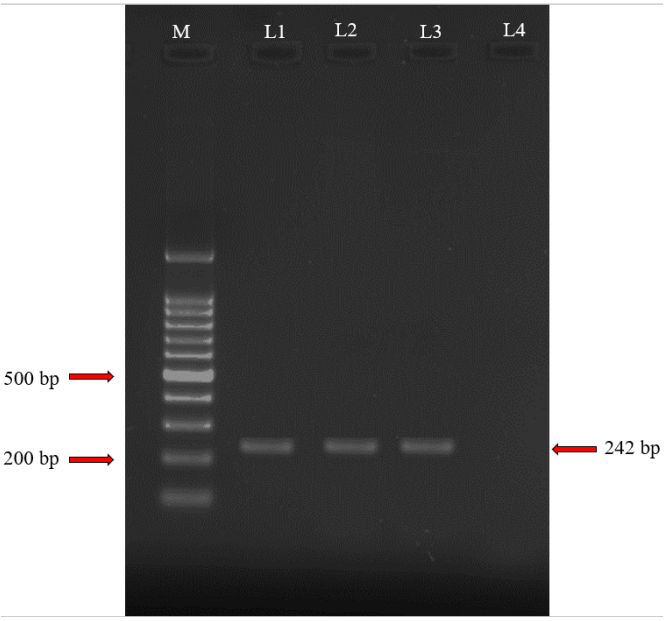

Fig. 7: Agarose gel electrophoresis (1.5%) for PCR products analysis. M: 100 bp DNA ladder; Lane 1 to 3: Amplification of 242 bp fragment of CO I gene of *Laemobothrion maximum;* Lane 4: Negative control

Conclusion

This study represents the first comprehensive report combining morphology, ultrastructure and molecular data on *L. maximum* from *H. indus* in India and highlights its strong host association with accipitriform raptors. The findings enhance the current understanding of the taxonomic placement, ecological adaptation and geographic distribution of this species and provide a valuable baseline for future studies on host–parasite interactions and the epidemiological significance of avian lice in India.

Acknowledgement

The authors are thankful to the Dean, Veterinary College and Research Institute, Orathanadu, Thanjavur for the constant support and facilities provided to carry out this study. We also thankfully acknowledge the Founder and Director, Endangered Wildlife Environment Trust (EWET) for the support. We would like to thankfully acknowledge to the Dean, School of Chemicals and Biotechnology, Sastra Deemed University, Thanjavur for the provision of Scanning Electron Microscopy facility.

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