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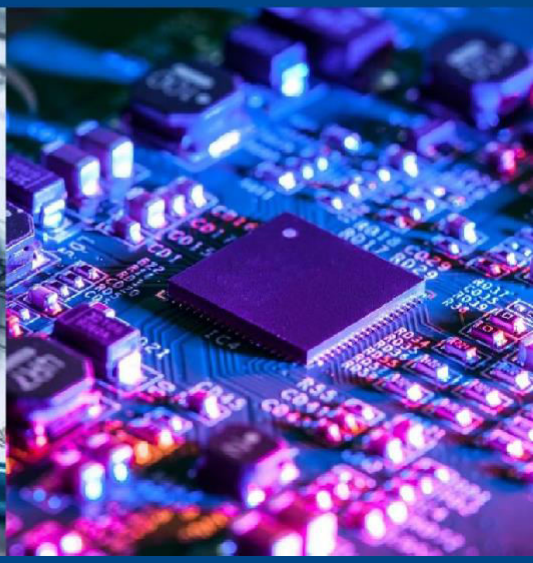

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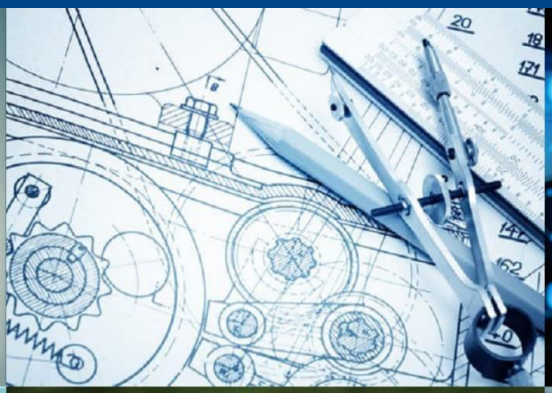

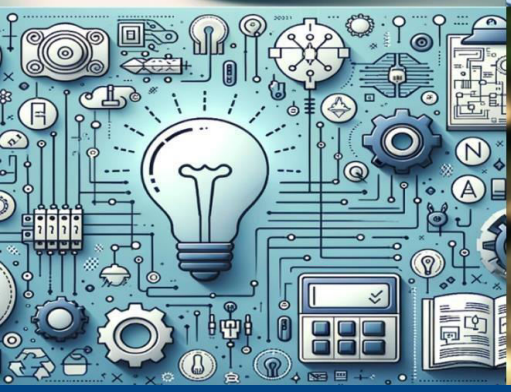

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First DNA Barcode and Morphological Description of *Endocrossis Flavibasalis* Moore, 1867 (Lepidoptera: Crambidae: Spilomelinae) from Kerala, India.

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ABSTRACT: Current study presents the first mitochondrial DNA barcode for the species *Endocrossis flavibasalis*, under the family Crambidae, subfamily Spilomelinae and tribe Margaroniini. It remains underexplored in terms of genetic and morphological data. Species identity confirmed through morphological characteristics. DNA was extracted and the COI gene amplified using universal primers. A Neighbor-Joining (NJ) phylogenetic tree was constructed to compare this species with related taxa, supporting its distinct placement within Spilomelinae. This study backs a valuable reference for future taxonomic and biodiversity research on South Asian Lepidoptera.

KEYWORDS: *Endocrossis flavibasalis*, spilomelinae, DNA barcoding, India.

I. INTRODUCTION

The subfamily spilomelinae covers a various group of moths known for their ecological and economic significance. Members of this subfamily act as a major agricultural pest. As it comprises morphologically diverse genera, molecular analysis is needed for accurate species-level identification.

Endocrossis flavibasalis was originally described by Frederic Moore (1867) as *Botyodes flavibasalis* from "Bengal" in the Proceedings of the Zoological Society of London in 1889. Meyrick erected the genus *Endocrossis* and fixed *Botyodes flavibasalis* as its type species. Hampson subsequently treated *Endocrossis* under *Botyodes* in the Fauna of British India (1896). A geographic race, *interruptalis*, was later named from Guangdong by Caradja & Meyrick, 1933. Modern revisions have revalidated *Endocrossis* as a distinct genus and explicitly reused it for its type species *B. flavibasalis.*

The application of DNA barcoding by using the mitochondrial cytochrome c oxidase I (COI) gene has revolutionized the accuracy of insect taxonomy. This study aims to fill that gap by providing the first DNA barcode of *E. flavibasalis* from its collection locality in Kerala, India. It also complements molecular data with detailed morphological observations, contributing to a more complete taxonomic framework for the genus *Endocrossis.*

II. MATERIALS AND METHODS

2.1. Material examined (♂♂).

MKP18 (♂) (Coll. Unnikrishnan M P): Alakkad, Kerala, India; 13 July 2023; collected at a LED light trap; euthanized with ethyl acetate; preserved dry-pinned; deposited in the Research Collection, Department of Zoology, Govt. Brennen College, Thalassery, Kerala, India.

MKP42 (♂) (Coll. Praveen Kumar M K): Kuruveli, Kerala, India (12.1979° N, 75.2581° E); 8 July 2025; collected at a at a LED light trap; euthanized with ethyl acetate; preserved dry-pinned; deposited in the same collection.

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2.2. DNA Extraction, Amplification, and Sequencing.

Genomic DNA was isolated from the tissues using NucleoSpin® Tissue Kit (Macherey-Nagel) following manufacturer's instructions. The quality of the DNA isolated was checked using agarose gel electrophoresis. The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). The COI gene was amplified using universal primers LCO1490 and HCO2198 (Folmer et al., 1994) in a 25 µl PCR reaction. PCR conditions followed standard protocols, and amplification was confirmed via agarose gel electrophoresis. Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following manufactures protocol. The sequence quality was checked using Sequence Scanner Software v1(Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond et al., 2010). The sequence was submitted to GenBank under accession number PV789701 & PV948961.

2.3. Phylogenetic Analysis.

Neighbor-Joining (NJ) tree was constructed using MEGA11 (Tamura, K., Stecher, G., & Kumar, S, 2021) with the P-distance model. Bootstrap analysis was conducted with 1000 replicates.Twenty related COI sequences from Spilomelinae were retrieved from GenBank for comparative analysis. *Troides minos* was used as the outgroup. Table 1. provides Gen bank details for the mt DNA COI sequences utilized in the construction of the phylogenetic tree.

Sl. No.	Species Name	GenBank Accession No.	Collection Location	Publication details
1	*Prophantis androstigmata*	KY323247	Papua New Guinea	Published
2	*Prophantis androstigmata*	HQ953179	Australia	Unpublished
3	*Pycnarmon lactiferalis*	LC697860	Japan	Published
4	*Pycnarmon cribrata*	HQ953171	Australia	Unpublished
5	*Rehimena leptophaes*	KF389795	Australia	Published
6	*Rehimena surusalis*	HQ952769	Australia	Unpublished
7	*Salbia zena*	JQ571868	Costa Rica	Unpublished
8	*Apogeshna stenialis*	JQ572403	Costa Rica	Unpublished
9	*Apogeshna stenialis*	JQ571339	Costa Rica	Unpublished
10	*Lamprosema victoriae*	HQ572772	USA	Unpublished
11	*Endocrossis flavibasalis*	PV789701	India	This work
12	*Agathodes sp.*	JQ540331	Costa Rica	Unpublished
13	*Cnaphalocrocis sp.*	MH415559	Madagascar	Published
14	*Diaphania sp.*	JQ556077	Costa Rica	Unpublished
15	*Copitarsia decolora*	EU371455	Unknown	Unpublished
16	*Cirrhochrista caconalis*	HQ952985	Australia	Unpublished
17	*Cirrhochrista caconalis*	HQ952986	Australia	Unpublished

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18	*Endocrossis flavibasalis*	KU205353	India	Unpublished
19	*Endocrossis flavibasalis*	PV948961	India	This work
20	*Endocrossis flavibasalis*	PX056891	India	Unpublished
21	*Troides minos* (Outgroup)	KT880663	India	Unpublished

Table1. Gen Bank details for the mt DNA COI sequences utilized in the construction of the phylogenetic tree.

III. RESULTS AND DISCUSSION

3.1. Taxonomic account.

Superfamily: Pyraloidea Latreille, (1809).
Family Crambidae: Latreille, (1810).
Subfamily: Spilomelinae Guenée, (1854).
Tribe: Margaroniini Swinhoe and Charles Cotes (1889).
Genus: *Endocrossis* Meyrick, (1863).
Type Species: *Endocrossis flavibasalis* Moore (1867).
Type locality: Bengal.

3.2. Morphological Identification.

Yellow thorax and abdomen, matching the wing ground colour. Paired black spots. Thorax characterized by seven spots. The forewings, with a broad blackish marginal band extending from the apex to the tornus. The inner margin of the band is irregularly indented. Several small black spots are present along the medial area. A conspicuous pale dot is present near the tornal region. The hindwings yellow, with broad dark marginal band. A fine terminal line and pale fringe are present (Fig.1).

Fig.1: *Endocrossis flavibasalis* Moore (1867).

3.3. DNA Barcoding & Phylogenetic analysis.

The 581 bp COI sequence obtained represents the first barcode record for *E. flavibasalis*. BLAST analysis confirmed species-level identity. The sequence has been archived under GenBank Accession No. PV789701(MKP18) and PV948961(MKP42). The Neighbor-Joining (N-J) (Saitou & Nei ,1987) tree based on COI clearly shows *Endocrossis flavibasalis* as a well-supported monophyletic clade (bootstrap 100%), comprising three concordant accessions

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(PV789701, PV948961 PX056891) (Fig.2). Internal branch lengths within this clade are very shallow (≤0.003), consistent with intraspecific variation rather than deep lineage splits. In contrast, KU205353 labelled as *E. flavibasalis* (Tamil Nadu) is placed far outside the *Endocrossis* clade, near *Cirrhochrista caconalis* and with the outgroup (*Troides minos*). Given the extreme topological displacement and long intervening branches, KU205353 is most plausibly misidentified or contaminated. We therefore exclude KU205353 from species-level comparisons and recommend re-examination of its voucher and raw chromatograms.

Overall, the integrated Phylogeny and diagnostic morphology with 100%-supported COI clade with minimal divergence confirms the identity of our *E. flavibasalis* material. While minor sequence differences among our three barcodes likely reflect routine population-level polymorphism, broader geographic sampling and the addition of nuclear markers (e.g., EF-1α, wingless) will be valuable to test for any fine-scale structure across southern India.

Bootstrap support was high (>100%) at major nodes. The use of *Troides minos* as an outgroup provided a robust phylogenetic context, and the high bootstrap support across internal clades lends credibility to the observed relationships. This work, therefore, contributes not only a new barcode record but also raises important questions about intraspecific genetic variation in *E. flavibasalis*.

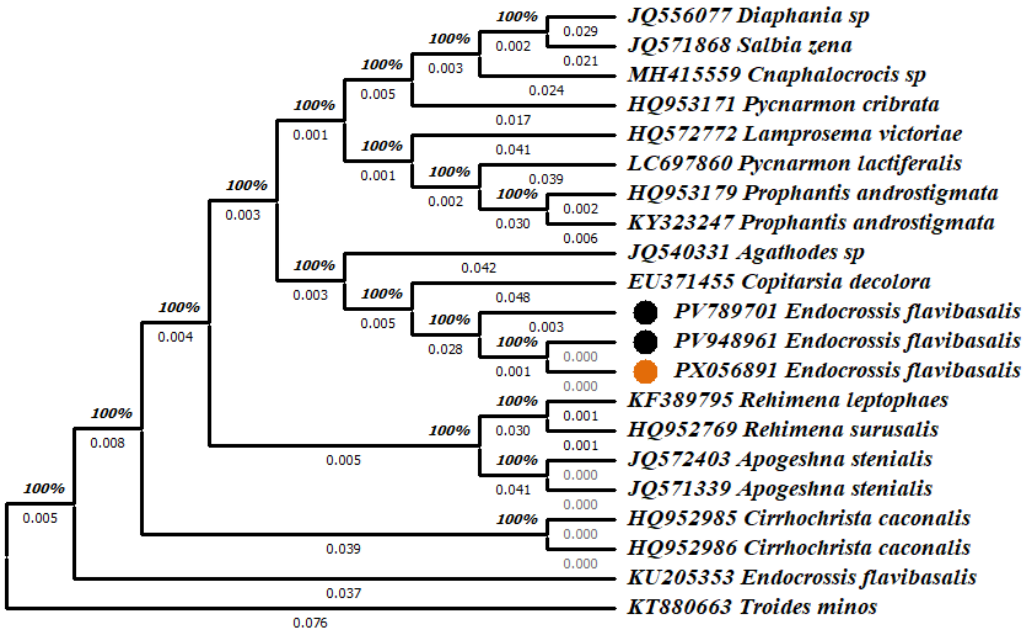

Fig.2: N- J tree analysis of *Endocrossis flavibasalis* COI DNA gene sequences.

IV. CONCLUSION AND FUTURE WORK

This study delivers an integrative taxonomic analysis of *Endocrossis flavibasalis* from Northern Kerala, combining with morphology with COI barcoding. Diagnostic external characters match with historical descriptions**.** Importantly, our sequences constitute the **first COI DNA barcodes** for ***E. flavibasalis*** deposited as reference records, providing a validated baseline for future work.

The COI Neighbor-Joining analysis recovers *E. flavibasalis* as a well-supported monophyletic unit (100% bootstrap) with very shallow intraspecific distances (≤0.003 K2P) across our three barcodes, consistent with routine population-level polymorphism. A publicly available record labelled *E. flavibasalis* (KU205353) falls far outside this clade, indicating likely misidentification or contamination; this underscores the need for careful curation of reference barcodes.

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Together, these results (i) confirm the identity of our material, (ii) provide **the first COI references** from the Western Ghats region, and (iii) establish a baseline for geographic and population comparisons within *E. flavibasalis*. We recommend expanding sampling across the species' Indian range and incorporating nuclear loci (e.g., EF-1α, wingless) and denser population coverage to test for fine-scale structure or cryptic diversity. The combination of rigorous morphology, and multilocus genetics will be key to resolving intraspecific variation and stabilizing identifications within this group.

V. ACKNOWLEDGEMENTS

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Competing interests
Authors have declared that no competing interests exist.

Authors' Contributions
PKMK and JJ designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. SJ managed the analyses of the study. UMP managed the literature searches. VAM helped in the draft preparation. All authors read and approved the final manuscript.

Consent
Not Applicable

Ethical approval
Not Applicable

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