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First DNA barcode and morphological description of *Coptobasis arctalis Guenée*, 1854 (Lepidoptera: Crambidae: Spilomelinae) from Kerala.

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ABSTRACT:

The subfamily Spilomelinae (Lepidoptera: Crambidae) comprises a taxonomically diverse group of moths, many having ecological and agricultural importance. *Coptobasis arctalis* is a poorly characterized moth species within the subfamily Spilomelinae. Despite its presence in tropical Asia, no verified DNA barcode exists in public databases prior to this study. We provide the first DNA barcodes of *C. arctalis* specimens collected from Kerala, India, using the mitochondrial cytochrome c oxidase I (COI) gene. A Neighbor-Joining phylogenetic analysis was conducted using representative Spilomelinae taxa and related species. Both *C. arctalis* specimens (MKP26 and MKP34) formed a well-supported monophyletic clade (100% bootstrap), confirming their species identity and validating their genetic discreteness. Present study contributes the first verified COI sequences for *C. arctalis*, enhancing the molecular reference library for Spilomelinae and supporting integrative taxonomy combining morphological and genetic data.

Keywords: Coptobasis arctalis, Spilomelinae, First DNA barcoding record, Phylogenetics

1.Introduction

The subfamily Spilomelinae covers a diverse group of moths known for their ecological and economic significance. Members of this subfamily act as pollinators as well as agricultural pests. As it comprises morphologically diverse genera, molecular analysis is essential for accurate species-level identification. *Coptobasis arctalis Guenée, 1854.*, has been reported from Indian states such as Kerala, Goa and Maharashtra.

Coptobasis arctalis was originally described by Walker in 1859 as *Coptobasis opisalis* Walker, 1859. Despite its wide distribution, detailed morphological and molecular data about this species remain limited. The application of DNA barcoding using the mitochondrial cytochrome c oxidase I (COI) gene has significantly improved the resolution of Lepidoptera taxonomy. However, existing COI barcodes for *C. arctalis* are not available in the NCBI and this is going to be the first deposit. The present study aims to address the exiting gap by providing a verified DNA barcode of *Coptobasis arctalis* from the specimens collected from India, Kerala, Kuruveli. The study also integrates morphological observations, thereby contributing to a more robust and integrative taxonomic understanding of the genus Coptobasis.

2.Materials and Methods

2.1. Specimen Collection and Preservation

Both specimens with Voucher No. MKP26 and MKP34 were collected on 13 July 2023 and 27 June 2025- from, India, Kerala, Kuruveli using a mercury vapor light trap. The specimen was euthanized using ethyl acetate and later preserved by dry pinning. GPS coordinates were recorded at the collection site (12.1979° N, 75.2581° E). Both specimens were deposited in the Department of Zoology at Government Brennen College, Thalassery, Kerala. India.

2.2. DNA Extraction, Amplification, and Sequencing

Genomic DNA was extracted from leg tissue using the Qiagen DNA Extraction Kit. 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. Chromatogram were carefully analysed using Finch TV 1.4 version and trimmed to remove low quality bases. Sequencing was

performed using NCBI Blast hit analysis tool and assembled using MEGA11 software (Tamura et al., 2021).

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. Eighteen related COI sequences from spilomelinae subfamily were retrieved from GenBank for comparative analysis. *Troides minos* was used as the outgroup. A summary of species used is shown in Table .1.

Sl. No.	Species Name	GenBank Accession No.	Collection Location	Publication details
1	Bradina impressalis	JX970193	Papua New Guinea	Unpublished
2	Bradina nr.	JX017852	unknown	Published online
3	Herpetogramma luctusalis	LC697914	Japan	Published online
4	Mecyna tricolor	LC697931	Japan	Published online
5	Metallarcha beatalis	KF522601	Australia	Published online
6	Omiodes sp.	JQ556375	Costa Rica	Unpublished
7	Oreneia lugubralis	JF860414	Papua New Guinea	Published online
8	Salbia sp.	OM554196	Argentina	Published
9	Udea numeralis	KU497425	Italy	Published online
10	Udea ferrugalis	MW306027	Unknown	Published online
11	Syllepte sp.	KP850201	Papua New Guinea	Published
12	Coptobasis arctalis	PV920630	India, Kerala	This work
13	Coptobasis arctalis	PV920629	India, Kerala	This work
14	Eulepte concordalis	JQ548042	Costa Rica	Unpublished
15	Cadarena pudoraria	MH416059	Madagascar	Published online
16	Synclera jarbusalis	JQ539468	Costa Rica	Published
17	Syllepte vagans	KM987404	Nigeria	Published
18	Troides minos (Outgroup)	KT880663	India	Unpublished

3. Results and Discussion

3.1. Material examined/Source

India: Kerala. 1 Male (Coll. Praveen Kumar M K); Kuruveli, Payyanur (12.1979° N, 75.2581° E) 1 Male (Coll. Praveen Kumar M K); Kuruveli, Payyanur (12.1979° N, 75.2581° E) **3.2. Taxonomic account** Superfamily Pyraloidea Latreille, (1809) Family Crambidae Latreille, (1810) Subfamily Spilomelinae Guenée, (1854) Genus *Coptobasis Lederer*, 1863. Type Species: *Coptobasis arctalis Guenée*, 1854. Type locality: Bombay

3.3. Morphological Diagnosis

Morphological examination was performed using a Magnus stereo zoom microscope. Diagnostic characters were recorded and compared with literature

for confirmation from Walker (1859) Guenée, (1854) and (GBIF). The specimen displayed characteristic wing coloration and venation patterns typical of *Coptobasis arctalis*. (Guenée,1854). Wing span- 40 mm, Triangular forewings with a gently convex outer margin; hindwings taper to a point at the tornus. Forewings exhibit a dark brown to purple-brown hue and glossy. A small white dot or spot at the discal cell on both sides. Zig-zag white lines— median and postmedial—crossing the wings, scalloped in shape. Similar pale markings visible on the hindwings, though generally subtler. Head & thorax match the wing ground colour, occasionally showing a faint sheen. Antennae appear simple and filamentous. Abdomen longitudinally banded, each tergite contrasts subtly with lighter and darker bands, visible in dorsal images (Fig.1.).



Fig .1. Coptobasis arctalis

3.4. DNA Barcoding

COI sequences of 682 bp (MKP26) and 610 bp (MKP34) were confirmed as *Coptobasis arctalis* via BLAST and submitted to GenBank under accession numbers PV920630 and PV920629.

3.5. Phylogenetic Analysis

The Neighbor-joining (NJ) phylogenetic tree (Saitou, N. & Nei, M. 1987) constructed using FASTA sequences delineated clear inter- and intraspecific relationships among the analysed Spilomelinae taxa. Both *Coptobasis arctalis* specimens (MKP26 and MKP34) formed a strongly supported monophyletic clade with 100% bootstrap support and exhibited zero genetic divergence (0.000), indicating conspecificity. This clade was positioned as a sister group to an unidentified *Syllepte* species (KP850201), with a low interspecific divergence of 0.026, suggesting a close evolutionary affinity. All other species grouped into distinct, well supported clades, with interspecific genetic distances ranging from 0.026 to 0.071. The outgroup *Troides minos* (KT880663) was clearly separated from the ingroup taxa, providing a stable root for the tree (Fig .2). These findings validate the species identity of *Coptobasis arctalis* and support the utility of COI barcoding in resolving taxonomic relationships within Spilomelinae. This work, therefore, contributes a new barcode record.

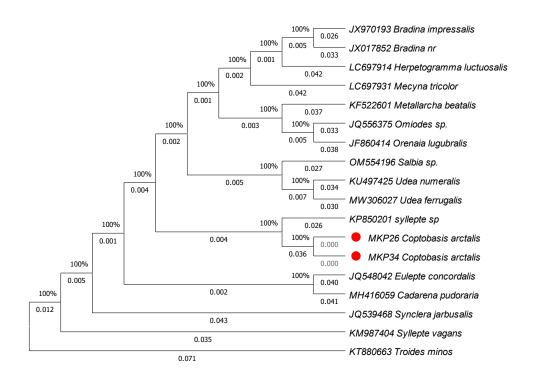


Fig. 2. N- J tree analysis of Coptobasis arctalis

4. Conclusion

The current study presents the first validated COI barcodes for *Coptobasis arctalis*, confirming its genetic distinctiveness and supporting its taxonomic status, contributing to the expanding molecular resources for Spilomelinae improving phylogenetic resolution and species identification.

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Conflict of Interest - None

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