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Molecular phylogeny of some fruit-piercing moths from Marathwada region of Maharashtra, India

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Abstract
Marathwada region has a great diversity of fruit crops and fruit-piercing moths are one of the major pests of fruit crops. The present study describes the occurrence of fruit-piercing moths from September 2022 to November 2023. four FPMS species were identified *Eudocima materna, Eudocima phalonia*, *Eudocima homaena,* and *Sphingomorpha cholera* they were collected and identified using reference identification keys. In the genus *Eudocima, E. Materna* & *E. Phalonia* cause extensive damage to fruits by piercing ripening fruits while *E. Homaena* has less. To construct a phylogenetic tree, we investigate molecular taxonomy among FPMS with COI molecular markers.

Keywords: Eudocima, Proboscis, COI, Molecular Taxonomy, fruit piercing moths

Introduction
FPMS are serious pests of ripening fruits. Both males and females have specialized proboscis for sucking diverse fruit fluids Kayande *et al*., (2023) [7]., Their proboscis has a sharp tip with tearing hooks which leads to premature fruit fall and higher economic losses Bhumannavar & Viraktamath, (2012) [1]. They have been studied worldwide but unfortunately, very few researchers have worked in some regions of Marathwada such as R. F. Pathre in Jalna and E. S. Shendge in *Othreis*. R. F. Pathre registered five species from Jalna district Pathre *et al*, (2020).
So, we were trying to focus on this major problem of this area, thousands of hectares of land were occupied by various fruit crops. As we surveyed many farmers were not ready to cultivate fruit crops and many of them destroyed fruit crops because of the fruit-piercing moths (Local farmers called them DAS) and water drought, they thought they were the big mosquitos and they all were the same.
Genus *Eudocima* is a major fruit-piercing group worldwide. It is easily identified due to its bright orange color, attractive hind wing patterns, and 48 species. Dharmayanthi *et al*., (2021) [3] genus *Eudocima* now becomes a part of Erebidae, initially among lepidopterists call them as genus *Ophiders* or *Othreis*. similarly same species also have more than one name such as *Eudocima Phalonia* / *Fullonia* / *Fullonica* etc. so current research reveals the study taxonomy and phylogeny of some FPMS from this region.

Material and Methods
1. Study Area
Marathwada is a diverse region with eight districts and 76 talukas. The temperature ranges between 7.8°C (winter) to 42.8°C (summer) as per season (Indian Meteorological Department, regional office Pune, India). The area receives rain from Southeast monsoons. This area has a tropical climate, especially a tropical wet and dry climate with dryness of seven months and rainfall from June to September Ravindra Pathre *et al*., (2020) [4]
Sampling stations were decided from the selective region of Marathwada fruit crops such as citrus, pomegranate, guava, papaya, tomato, and grapes from the Marathwada region mainly from Jalna, Aurangabad, and boundaries of Beed districts. During the ripening season fruits field were visited weekly in hours of darkness from 7.00 pm to 11 pm. FPMs were collected by light trap, Bait trap & hand-picking method from sampling stations such as Paithan, Pachod, Khultabad, Jatwada, Shiradhonwadi, Shahpur Dadhegaon, Bachegaon, Ekrukha, & Dudhapuri etc.

Collection Methods of fruit-piercing moths
A. Light Trap method
We used a 12volt 14amp DC battery for the light trap method as a power supply for white and blue DC LED strips They were focused on white cotton curton to settle down moths and collected by plastic jars. But only a few fruit-piercing moths were attracted to the light trap, that's why we collected them while the piercing fruits LED strip was focusing on moths, they were located by reflecting their eyes. Moths were directly collected in plastic jars.

B. Bait traps
Ripen bananas and Guava were placed in hanging positions in orchards, moths were more attracted to bananas than guava they were collected in plastic vials. (Kamala Jayanthi *et al*., 2015) [6] same day collected moths were killed by chloroform and brought to the laboratory JES College Jalna for further process. Some moths were kept alive for dissecting gut, and Fpms Leg clips were kept in 70 % ethanol for further molecular processes. After mounting moths were kept in the oven at 100 °C for 2 hrs. Fruit-piercing moth Species were identified by their morphological Characters using references and leg clips were sent to the progenome laboratory for molecular processes.

Extractions of DNA from Leg clips of fruit-piercing moths
The DNA was extracted from the leg clip of FPMs by TAKARA NucleoSpin® Tissue Genomic DNA Purification Kit and the quality of DNA was checked on 1% agarose gel electrophoresis. Gel was visualized on a UV Transilluminator (Himedia). Then fragments of gene COI were amplified by LCO_1490F and HCO_2198R primers. The Cycle sequencing is followed by sequencing cleanup by

ethanol precipitation followed by dissolving template in HiDi formamide and bidirectionally sequenced in ABI 3730 Genetic analyzer.

A single discrete PCR amplicon band of approximately 650 bp was observed when resolved on 1.2 % Agarose gel electrophoresis. The PCR amplicon was purified to remove contaminants. Forward and reverse DNA sequencing reaction done of PCR amplicon using LCO_1490F and HCO_2198R primers in BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer, Consensus sequence of COI gene was generated from forward and reverse sequence data using Bioedit software.

Sequence alignment and assembly

PCR products were processed for bi-directional sequencing using ABI PRISM 3730 × 1 Genetic Analyzer (Applied Biosystems, USA). The resulting DNA sequences were aligned using CLUSTALW in MEGA 11, manually trimmed, and edited to obtain complete sequences. The species confirmation depends on the sequence similarity rate. Species Homology checks using the BLASTn program against the NCBI GenBank database and the BOLD database. The neighbor Joining phylogenetic tree was constructed using MEGA 11 with all positions containing gaps missing and aligned data for further molecular analysis. Clade supports were calculated based on 1,000 bootstrap resampling.

Results

1. Identification of fruit-piercing moths

These FPMS were identified by comparing their morphological key characteristics with reference data of Hampson vol II; Hollway 1989; Billberg 1820; E. Hargraves, Mike Hill, A. Zilli & Hogenes and Sachin Gurule. Identified moths are as follows. Moths Photos were clicked in Canon 750 D basic lens and edited in remove. bg.

This study clarifies the taxonomy of FPMS, especially genus *Eudocima* species and their related FPMS using COI gene sequence. A total of 4 species are found in this region from the Erebidae family three of them under the Calpinae subfamily and one from Erebinae. seven samples of COI Sequences were already deposited in the BOLD System with BIN BOLD: AAC5182, BOLD: AAC6829, BOLD: ABA0112, BOLD: ADH0797, under the project molecular phylogeny of some fruit piercing moths from the Marathwada region of Maharashtra and the project code was MSS.

Molecular Analysis

These seven samples of COI nucleotide sequences had no insertion, deletion, contamination, or problematic flags in nucleotide sequence alignments in four species samples,

Table 1: Collection and Sampling Data

Sr. No	Sampling ID	Processing- ID	Sampling Station	Identification of Species
1	SHD1	MSS001-24	Shiradhonwadi	Eudocima Materna
2	SHD2	MSS002-24	Shiradhonwadi	Eudocima Materna
3	SHD3	MSS003-24	Shiradhonwadi	Eudocima Phalonia
4	SHD4	MSS004-24	Shiradhonwadi	Eudocima Phalonia
5	DPA1	MSS005-24	Dudhapuri	Sphingomorpha Chlorea
6	PCS1	MSS006-24	Paithan	Eudocima Homaena
7	PCS2	MSS007-24	Paithan	Eudocima Homaena

Table 1 shows that the samples were collected from which sampling station, and each specimen assigned with unique sample ID and Processing ID. Specimen samples were processed and stored in a BOLD system for sequence storing and molecular analysis.

Table 2: Sequence Composition Analysis

Sample ID	A %	C %	G %	T %	GC %	GC % Codon Pos 1	GC % Codon Pos 2	GC % Codon Pos 3
DPA1	29.66361	15.44343	14.06728	40.82569	29.5107	42.20183	41.74312	4.587156
SHD4	30.27523	15.44343	14.06728	40.21407	29.5107	40.36697	42.66055	5.504587
PCS1	29.70904	16.99847	14.08882	39.20368	31.08729	44.0367	42.12963	7.305936
PCS2	29.74203	16.38847	13.96055	39.90895	30.34901	43.18182	41.09589	6.818182
SHD2	30.19727	15.62974	14.41578	39.75721	30.04552	40.45455	40.63927	9.090909
SHD3	29.59029	15.02276	14.56753	40.81942	29.59029	41.81818	41.55251	5.454545
SHD1	31.60377	16.3522	15.25157	36.79245	31.60377	42.45283	43.39623	8.962264

Table 2 shows the sequence composition analysis of seven samples with their nucleotide proportions all specimens have A &T nucleotide percentages greater than G & C percentages.

Table 3: Distance Summary Table

Label	N	Taxa	Comparisons	Min Dist (%)	Mean Dist (%)	Max Dist (%)	SE Dist (%)
Within Species	6	3	3	0.79	1.58	3.16	0.37
Within Genus	6	1	12	5.59	6.73	8.30	0.07
Within Family	7	1	6	12.11	13.15	15.32	0.18

Table 3 summarizes that the distribution of sequence divergence at each taxonomic level is by the Distance model Kimura two-parameter and pairwise deletion method.

Phylogenetic analysis

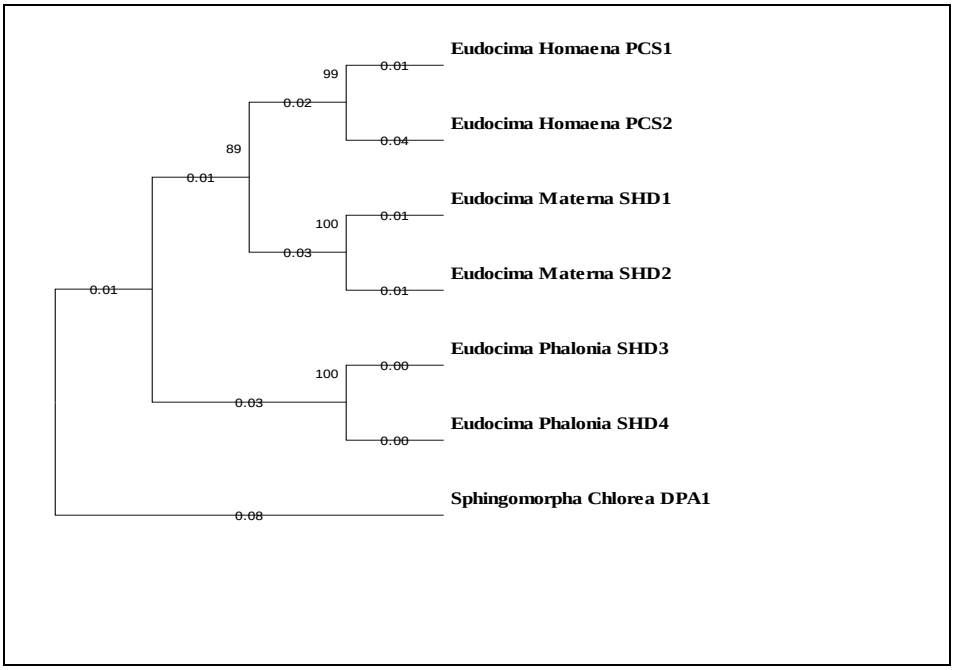
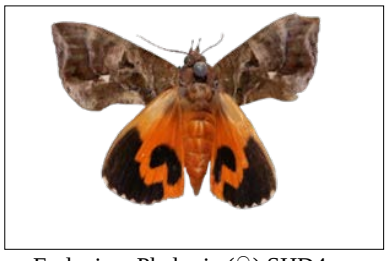


Fig 1

Figures (Moth Plates)



Eudocima Phalonia (♀) SHD4

Eudocima Homaena (♀) PCS1

Eudocima Materna (♀) SHD2

Eudocima Phalonia (♂) SHD3

Eudocima Homaena (♂) PCS2

Eudocima Materna (♂) SHD1

Sphingomorpha Chlorea DPA1

We conducted a preliminary analysis of 7 samples of fruit piercing moths mostly they are belonging to the genus Eudocima based on an approximately 650 bp sequence of (COI) cytochrome oxidase I to construct appropriate molecular phylogeny

A phylogeny tree was constructed using the Neighbour-Joining method and evolutionary distances were calculated using the p-distance method. This phylogeny analysis involved seven nucleotide sequences; Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There was a total of 661 positions in the final dataset. Phylogeny analysis was conducted in MEGA11 Tamura K., (2021) [13]

Discussion

Fruit-piercing moths were first investigated by Carls Linnaeus in the eighteenth century and they were kept in the Noctuidae family in the tribe Calpinae Zaspel & Branham, (2008) [14] while G. F. Hampson describes this genus known as Ophiders. Hampson G. F. to Zilli & W. Hogenes considers Genus Eudocima to belong to Noctuidae,

Catocalinae. Zilli & Hogenes, (2002) [15]. Mike Hill describes Eudocima Materna Belongs to the Noctuidae family. (Mike, n.d.) but the noctuidae family split into distinct families and the erebidae family one of them, In the nineteen century to avoid the complexity that's why eudocima now belong to the erebidae family, so new records of eudocima belong to the family Erebidae and subfamily calpinae. Singh *et al*., (2019) [12]

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