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DNA BARCODING OF *Eudocima cocalus* FROM NORTH KERALA

K.P. PRIYA BHASKARAN[a] AND C.D. SEBASTIAN[b1]

[ab]Molecular Biology Laboratory, Department of Zoology, University of Calicut, Kerala, India

ABSTRACT

***Eudocima cocalus*, the *cocalus* fruit piercing moth, is a moth of the Erebidae family. The adult moths are one of the most severe and economically important agricultural pest among variety of fruits and vegetables. The moths pierce the fruit to suck the juice, thereby damaging the fruit and allowing the ingress of fungal spores and bacteria. Molecular approaches have led to species identification, which allowed rapid detection, discrimination, and identification of cryptic or sibling species based on DNA sequence data. We have developed the phylogenetic reconstruction and analysis of the *Eudocima cocalus* (GenBank Accession No. KX603659) using mitochondrial cytochrome oxidase subunit I (COI) gene. The knowledge of the *lepidopteran* genomic structures will create new method of integrated pest management which can contribute for the sustainable agriculture and maintenance of biodiversity.**

KEYWORDS: *Eudocima cocalus*, Erebidae, Cryptic Species, Sibling Species, Biodiversity.

Lepidoptera is a major order of insects that includes butterflies and moths and is the second most diverse insect pest order outnumbered only by the beetles. About 180,000 species of the Lepidoptera are described, in 126 families and 46 superfamilies [Capinera, 2008], 10% of the total described species of living organisms [Jim, 2007]. As pollinators of many plants, adult moths and butterflies are usually beneficial insects that feed on nectar using their siphoning proboscis. The caterpillars, almost always have chewing mouthparts that are suitable for feeding on various parts of a plant. Most caterpillars are defoliators or miners of succulent plant tissues. Most of the cultivated plants as well as the agricultural crops are attacked by at least one of the lepidopteran pest. Thus, pest insects have adverse and damaging impacts on agricultural crop production. Pest insects may cause problems by damaging crops and food production, parasitising livestock, or being a nuisance and health hazard to humans.

This beautiful looking moth is in fact a great pest, on account of the fact that it likes to make holes in fruits and also suck out the juices. Unfortunately, this can cause crop losses of more than 50% in many crops-by allowing microorganisms to enter-such as Lychee and Carambola.

The female adult moths of this species have forewings that are dark brown with several white spots. The hindwings are yellow with a broad black margin. The male moths have patchy brown forewings with no white spots, but the hindwings are orange with a broad black margin. For both sexes, each forewing has a hooked wingtip, and a concave inner margin. The head and thorax are dark brown, but the abdomen is bright orange. The wingspan is about 6cms.

DNA barcoding is a taxonomic method that uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species [Leong and Kueh, 2011]. Standardisation of a universal and sequenceable locus present in most of the taxa of interest for DNA barcoding that can be amplified with universal PCR primers is the best method to assess a large variation between species yet a relatively small amount of variation within a species [Hebert et.al., 2003]. Molecular phylogenetic analysis using DNA barcoding, especially mitochondrial COI gene sequences were adopted by several workers in various insect orders like Odonata [Jisha Krishnan and Sebastian, 2015], Diptera [Rukhsana et.al., 2014, Bindu and Sebastian, 2014 & Priya Bhaskaran and Sebastian, 2014], Hemiptera [Sreejith and Sebastian, 2014], Hymenoptera [Rukhsana et.al., 2014] and Lepidoptera [Akhilesh and Sebastian, 2014 & Pavana and Sebastian, 2014]. The result of a molecular phylogenetic analysis can be expressed in a phylogenetic tree and is one aspect of molecular systematics, a broader term that also includes the use of molecular data in taxonomy and biogeography.

MATERIALS AND METHODS

Collection and Identification of Specimen

The selected insect specimen *Eudocima cocalus* (Figure 1), was collected from Malappuram, Kerala. A diverse geographical status is spoken about the location, including lowland, highland and marshy areas, and the area exhibits a high diversity of organisms. The specimens were collected from the agricultural fields by employing the sweep net technique. Collected adult specimens were identified morphologically by consulting an expert. The collected specimens were stored at -20°C until the DNA is

[1]Corresponding Author

extracted and voucher specimen is restored.

Figure 1: *Eudocima cocalus*

DNA Extraction, Amplification and Sequencing

DNA was extracted from the tissue of thoracic leg of the specimen, using Origin Kit as per the manufactures guidelines. The DNA isolated was confirmed using 1% agarose gel and 2ng was amplified for COI gene using the appropriate forward (5'- cattggagatgaccaaatttataatg -3') and reverse (5'-tgaaattaatccaaatccaggtaaa-3') primers. The PCR reaction mixture comprised of 2ng of genomic DNA (1µl), 1µl each of forward and reverse primer at a concentration of 5µM, 1µl of dNTPs (2.5mM), 2µl 10X reaction bufer, 0.20µl Taq polymerase (5U/µl) and 13.8µl H_2O. The PCR profile consisted of initial denaturation step of 5min at 95°C followed by 30 cycles of 10 sec at 95°C, 1 min at 50°C and 45 sec at 72°C and ending with a final phase of 72°C for 3 min. The PCR product was resolved on a 2% TAE-agarose gel, stained with ethidium bromide. To remove unincorporated primers and dNTPs the resultant PCR product was column purified using the nucleic acids purification kit of Gene JET of Fermentas Life Science. The purified PCR product was sequenced by Sanger's dideoxy chain termination method using an ABI 3730XL sequence analyser. The sequences were submitted to NCBI GenBank with accession number KX603659.

Alignment and Analyses

Chromatogram was analyzed for annotation with forward and reverse sequences. Annotated sequences were trimmed off primer sequences and any sequence ambiguity was resolved. The final sequence obtained were aligned using ClustalW programme [Rukhsana et.al., 2014].

Phylogenetic Analyses

Nucleotide sequences were analyzed using MEGA6 software [Bindu and Sebastian, 2014]. The matrix of corrected DNA distances was generated using Kimura Two parameter model and a phylogenetic tree was generated using the neighbor- joining algorithm [Priya Bhaskaran and Sebastian, 2014 & Sreejith and Sebastian, 2014].

RESULTS AND DISCUSSION

The PCR amplified sequences of mitochondrial cytochrome oxidase subunit I gene of *Eudocima cocalus* yielded a single product of 585 bp. The sequence has been deposited in the NCBI GenBank with Accession No. (KX603659). The phylogenetic tree plotted using Neighbor-joining method is presented in Figure 2. NJ clustering analysis showed that *Eudocima cocalus* belong to single monophyletic clade without any overlap. The evolutionary history of *Eudocima cocalus* clearly infers both the inter and intra species divergence. The *Eudocima cocalus* (KX603659) collected from Kerala exhibit 96% similarity to *Eudocima phalonia* (HQ949151) from Canada. Inter species divergence is found to be between 5-10%.

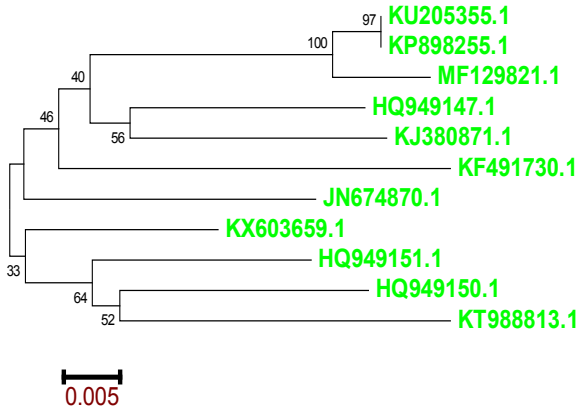

Figure 2: Phylogenetic relationship of *Eudocima cocalus* inferred by Neighbor-Joining method

CONCLUSION

The shortcomings and limitations of the conventional taxonomical identification methods highlighted need for new and simple methods of pest identification. The present study on molecular evolutionary analysis on partial mitochondrial cytochrome subunit I (COI) gene explicates phylogenetic relationships of *Eudocima cocalus*. The study emphasizes that the best phylogenetic studies and inferences can be created through moderately divergent nucleotide data from mitogenomes of which the CO I gene is best suited for deciphering the Lepidopteran taxonomic levels.

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