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DNA BARCODING OF AGRICULTURALLY IMPORTANT PLUSIINAE (LEPIDOPTERA : NOCTUIDAE)

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ABSTRACT : Accurate and timely identification of insect is an important and challenging task worldwide. The paper provides DNA Barcodes for proper identification of five major pest species of Plusiinae and to show genetic variation among them. Five species belonging to four genera *Thysanoplusia* Ichinose, *Trichoplusia* McDunnough, *Chrysodeixis* Hubner and *Argyrogramma* Hubner of the subfamily Plusiinae were studied. Our study revealed the variation of the Indian population of pest species with rest of world population.

Key words : Plusiinae, pest, barcoding, phylogeny, India.

INTRODUCTION

Plusiinae is a small and taxonomically compact group of moths within family Noctuidae. The main identifying feature of adult is small to medium-sized, robust body and metallic spot present on the forewing. The subfamily is represented by 500 species worldwide (Ronkay *et al*, 2008) and 59 species from India (Shashank and Longjam, 2015). Historical review and classification of Plusiinae were given by Kitching (1984, 1987), Lafontaine and Poole (1991) and Ronkay (2008, 2010). Certain Plusiinae species were represented as major pest of economically important crops belonging to garden vegetables (Cabbage, Cauliflower, Pea plant, Tomato, etc) (Patait *et al*, 2008); Pulses (Chickpea, Pigeon pea (Naresh *et al*, 1986; Mundhe *et al*, 1980; Singh, 1981, 1987); ornamental plants (Rose, Marigold) (Men *et al*, 1997, 1995); fruit plants (Mango, Banana, Indian Blackberry) and few medicinal plants (Alfalfa). Their larva is leaf feeder that feeds the lower leaf surface, leaving the upper surface intact. Most of the species are quite similar in morphology and considered as cryptic species. In this regard, external morphological identification can lead to misidentifications. Example as in case of *C. eriosoma*, *C. acuta* and *A. signata* are almost similar in morphology and considered as sister species. Only morphological data is not enough for accurate identification. Mitochondrial cytochrome oxidase-I (658bp) known as barcode gene widely used for molecular systematics taking advantage of maternally inherited and gives accurate results up to species-level identification. Distribution data from collection records (label data) and previous publications were compiled was taken as a reference (Twinkle *et al*, 2018). During this study, we have generated 25 barcode data of five species to identify species correctly and studied the pattern of species evolution.

MATERIALS AND METHODS

Specimen sampling and morphological identification

Five species belonging to tribe Argyrogrammatini (*Thysanoplusia orichalcea*, *Trichoplusia ni*, *Chrysodeixis eriosoma*, *Chrysodeixis acuta* and *Argyrogramma signata*) were collected from different localities of seven different states of India (Delhi, Rajasthan, Kerala, Punjab, Uttarakhand, Arunachal Pradesh and J&K) with light traps. Moth specimen were processed by pinning, stretching of wings and drying. Leg samples were preserved in 96% ethanol in eppendorf tubes. Species were identified morphologically with available literatures and by genitalia dissection methods. But due to the presence of cryptic species in this subfamily, it is very important to generate barcode and to identify species correctly.

DNA extraction, PCR and sequence alignment

Total genomic DNA was isolated from the leg of a moth by DNeasy Blood and Tissue Kit (Quigen) by the standard manufacturer's protocol. The extracted DNA was used for the subsequent experiments. The universal barcode primer described by Folmer *et al* (1994) (LCO-5'-GGT CAA CAA ATCATA AAG ATA TTG G-3'; HCO-5'-TAA ACT TCA GGGTGA CCA AAA AAT CA-3') specific to mitochondrial cytochrome oxidase I (COI) was used in the present study. The optimized PCR