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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

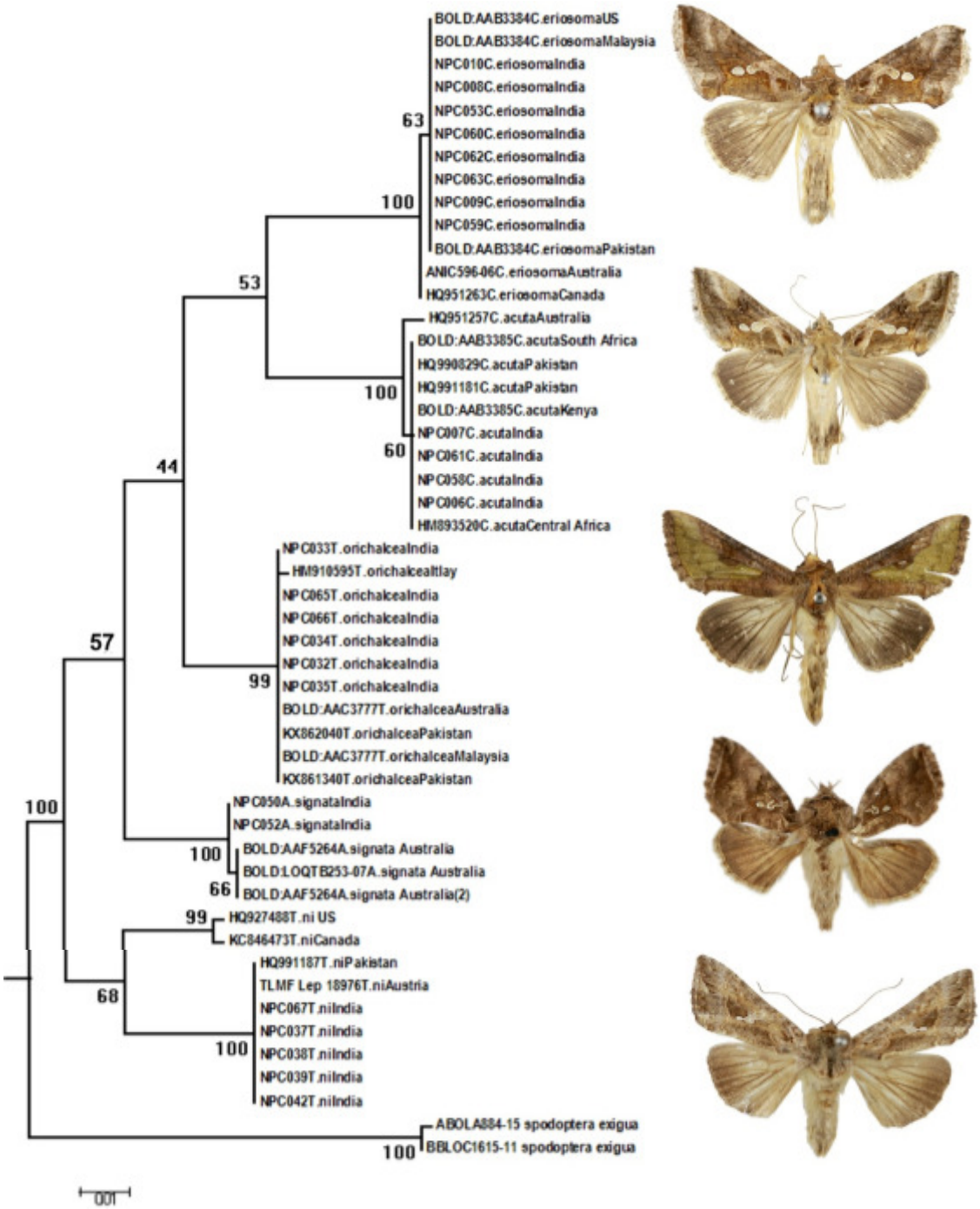

Fig. 1 : Maximum likelihood tree of five species in this study based on partial sequences of the mtDNA CO1 gene.

mixture (25µL) includes; 12.5 µL of Thermo Scientific maxima hot star PCR master mix, 8.5µL molecular water, 1 µL forward primer, 1 µL reverse primer and 2µL of genomic DNA. Thermal cycler with 96 wells (Applied Biosystem) was used for amplification of samples and PCR conditions were as follows: the initial denaturation for 4 min at 94°C followed by 35 cycles of denaturing for 30 s at 94°C, annealing for 1 min at 47°C, an extension time of 50 s at 72°C, with a final extension for 8 min at 72°C. The amplified PCR products were visualized in gel documentation system (AlphaView Version 3.2.2.0) on agarose gel after electrophoresis. Products were sent for purification and sequencing in AgriGenome, Kerala, India. Received sample sequences were first checked through sequence assessment using NCBI nucleotide BLAST.

Phylogenetic analysis

A total of 25 sequences were generated of five pest species during this study. Five species COI sequences in FASTA format were processed and submitted to

Table 1 : List of species DNA Barcode generated during this study with their Sequence ID and BOLD BIN no. along with some downloaded Genbank records outside India.

S. No.	Voucher ID	BOLD Process ID	Species	BIN/Accession no	Location/Country
1.	NPC050	INPLU045-18	*Argyrogramma signata*	BOLD:AAF5264	A.P (India) *
2.	NPC052	INPLU047-18	*Argyrogramma signata*	BOLD:AAF5264	A.P (India) *
3.	-	-	*Argyrogramma signata*	BOLD:AAF5264	Australia
4.	-	-	*Argyrogramma signata*	BOLD:LOQTB253-07	Australia
5.	-	-	*Argyrogramma signata*	BOLD:AAF5264	Australia
6.	NPC007	INPLU037-18	*Chrysodeixis acuta*	BOLD:AAB3385	Delhi(India) *
7.	NPC061	INPLU055-18	*Chrysodeixis acuta*	BOLD:AAB3385	Rajasthan(India)*
8.	NPC058	INPLU052-18	*Chrysodeixis acuta*	BOLD:AAB3385	Kerala (India) *
9.	NPC006	INPLU008-18	*Chrysodeixis acuta*	BOLD:AAB3385	Punjab (India) *
10.	-	-	*Chrysodeixis acuta*	HM893520	Australia
11.	-	-	*Chrysodeixis acuta*	BOLD:AAB3385	South Africa
12.	-	-	*Chrysodeixis acuta*	HQ990829	Pakistan
13.	-	-	*Chrysodeixis acuta*	HQ991181	Pakistan
14.	-	-	*Chrysodeixis acuta*	BOLD:AAB3385	Kenya
15.	-	-	*Chrysodeixis acuta*		Gabon, Central Africa
16.	NPC059	INPLU053-18	*Chrysodeixis eriosoma*	BOLD:AAB3384	Kerala (India) *
17.	NPC009	INPLU010-18	*Chrysodeixis eriosoma*	BOLD:AAB3384	Delhi (India) *
18.	NPC063	INPLU057-18	*Chrysodeixis eriosoma*	BOLD:AAB3384	U.K(India) *
19.	NPC062	INPLU056-18	*Chrysodeixis eriosoma*	BOLD:AAB3384	U.K(India) *
20.	NPC060	INPLU054-18	*Chrysodeixis eriosoma*	BOLD:AAB3384	Rajasthan (India) *
21.	NPC053	INPLU048-18	*Chrysodeixis eriosoma*	BOLD:AAB3384	A.P (India) *
22.	NPC008	INPLU009-18	*Chrysodeixis eriosoma*	BOLD:AAB3384	Delhi (India) *
23.	NPC010	INPLU042-18	*Chrysodeixis eriosoma*	BOLD:AAB3384	H.P (India) *
24.	-	-	*Chrysodeixis eriosoma*	ANIC596-06	Australia
25.	-	-	*Chrysodeixis eriosoma*	BOLD:AAB3384	US
26.	-	-	*Chrysodeixis eriosoma*	BOLD:AAB3384	Australia
27.	-	-	*Chrysodeixis eriosoma*	BOLD:AAB3384	Malaysia
28.	-	-	*Chrysodeixis eriosoma*	BOLD:AAB3384	Pakistan KX862937
29.	-	-	*Chrysodeixis eriosoma*	HQ951263	Canada
30.	NPC033	INPLU038-18	*Thysanoplusia orichalcea*	BOLD:AAC3777	Delhi (India) *
31.	NPC065	INPLU059-18	*Thysanoplusia orichalcea*	BOLD:AAC3777	J&K(India) *
32.	NPC066	INPLU060-18	*Thysanoplusia orichalcea*	BOLD:AAC3777	H.P (India) *
33.	NPC034	INPLU004-18	*Thysanoplusia orichalcea*	BOLD:AAC3777	U.K(India) *
34.	NPC032	INPLU003-18	*Thysanoplusia orichalcea*	BOLD:AAC3777	U.K(India) *
35.	NPC035	INPLU039-18	*Thysanoplusia orichalcea*	BOLD:AAC3777	Delhi (India) *
36.	-	-	*Thysanoplusia orichalcea*	BOLD:AAC3777	Australia
37.	-	-	*Thysanoplusia orichalcea*	KX862040	Pakistan
38.	-	-	*Thysanoplusia orichalcea*	BOLD:AAC3777	Malaysia
39.	-	-	*Thysanoplusia orichalcea*	HM910595	Itlay
40.	-	-	*Thysanoplusia orichalcea*	KX861340	Pakistan
41.	NPC067	INPLU030-18	*Trichoplusia ni*	BOLD:AAC3410	H.P (India) *
42.	NPC037	INPLU026-18	*Trichoplusia ni*	BOLD:AAC3410	Rajasthan (India) *
43.	NPC038	INPLU027-18	*Trichoplusia ni*	BOLD:AAC3410	Rajasthan (India) *
44.	NPC039	INPLU028-18	*Trichoplusia ni*	BOLD:AAC3410	Delhi (India) *
45.	NPC042	INPLU029-18	*Trichoplusia ni*	BOLD:AAC3410	Delhi (India) *
46.	-	-	*Trichoplusia ni*	HQ991187	Pakistan
47.	AWC-07014	*Trichoplusia ni*	HQ927488	Yuma,US	
48.	DH010922	-	*Trichoplusia ni*	KC846473	Canada
49.	TLMF Lep 18976	-	*Trichoplusia ni*	BOLD:AAC3410	Austria
50.	Outgroup	-	Spodoptera exigua	ABOLA884-15	India
51.	Outgroup	-	Spodoptera exigua	BBLOC1615-11	India

* Mark denoted sequences obtained from this study.

GenBank and BOLD database under the project "Indian Plusiinae" for acquiring accession no. and BOLD-IDs. Some of the sequences of five species from different countries were download from BOLD and NCBI database were included for analysis (Table 1). All 51 sequences of Plusiines were aligned using Clustal W with a default setting of gap penalty 15. All positions containing gaps and missing data were eliminated from the dataset. We employed Kimura 2-Parameter distance model (Kimura, 1980) to calculate intraspecific and interspecific pairwise genetic and it is computationally very fast and most widely used method and graphically displayed in an ML (Maximum likelihood) tree by the program MEGA 6.00 software (Tamura *et al*, 2013) (Fig. 1). Tree robustness was evaluated by bootstrapping with 2,000 replicates with *Spodoptera litura* COI sequence as outliers.

RESULTS AND DISCUSSION

The maximum likelihood tree of 5 species represents 5 major clades (Fig. 1) with associated subclades of species. *C. acuta* population of India, Pakistan, Africa, Kenya represented by same clade and population of *C. acuta* from Australia cluster separately. *C. eriosoma* of India, Malaysia, US and Pakistan represented by same clade and population from Australia and Canada cluster separately from the remaining population. There is no genetic difference showed in *T. orichalcea* sequences and population of India, Australia, Malaysia and Pakistan represents same cluster. *A. signata* of Indian population cluster separately with Australian population and *T. ni* of Indian and Pakistan Population make cluster separately as compared to population of US and Canada. The overall average of all sequences is 0.058. Average intraspecific genetic divergence of *A. signata* is 0.001, *C. acuta* (0.001), *C. eriosoma* (0.0005), *T. orichalcea* (0.0003) and *T. ni* is 0.0179. *T.ni* showed more variability of Indian population and population of US above 1%. The distance showed that degree of genetic divergence increased in taxonomic level of taxa. Interspecific evolutionary distance of species as follows: *A. signata* and *C. acuta* is 0.073; *C. acuta* and *C. eriosoma* is 0.062; *C. eriosoma* and *T. orichalcea* is 0.064; *T.orichalcea* and *T. ni* is 0.061.

Total 25 DNA barcode sequences generated during this study. It is a first attempt from India to determine and interrelate species pest of Plusiinae of India with rest of world population. There were few molecular data available for this species from India. DNA barcodes provided in this study will help in the accurate diagnosis of species from India. From a geographical perspective, rates of migration between Indian populations and other countries suggest that dispersal of gene flow over considerable distances is a major factor in the development of genetic variability in the species. Hence, this approach can play important role in formulating viable pest management strategies.

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Disclosure statement

The authors declare that they have no competing interests.

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