**Short communication**

DNA barcode reveals occurrence of *Mythimna loreyi* (Duponchel) in Punjab, India

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Armyworm *Mythimna* spp. is one of the serious pests of crops belonging to Gramineae family viz. sorghum, millets, rice, maize and wheat in different regions of the world. Although different species of *Mythimna* were existing in different parts of the world, but in Punjab, all populations infesting different host plants are considered as *Mythimna separata*. Eight samples from different regions of Punjab viz. six infesting rice from Patiala (2), Muktsar, Gurdaspur (2) and Bathinda; one each from maize and sorghum from Ludhiana and TarnTaran, respectively were analysed. The DNA barcode region (mitochondrial cytochrome oxidase 1) of each sample was cloned and sequenced. Basic Local Alignment Search Tool (BLAST) analysis showed that four samples collected from rice (Patiala-2, Muktsar, Gurdaspur) showed 99% homology with *M. loreyi*. Two samples from rice (Gurdaspur and Bathinda) and one each from sorghum and maize showed a homology of 99-100% with *M. separata*. Our results report the occurrence of *M. loreyi* species in rice ecosystem in Punjab along with *M. separata*. The phylogenetic analysis of population of this study and population from different countries formed two distinct groups of *M. loreyi* and *M. separata*. The maximum genetic distance was 0.008 and 0.004 among the different population of *M. loreyi* and *M. separata*, respectively.

**Keywords:** *Mythimna loreyi*, *Mythimna separata*, genetic diversity, DNA barcoding

Armyworm *Mythimna* spp. is one of the serious crop pests primarily found on leaves of gramineous plants and during severe outbreaks, total defoliation of affected fields may occur. In India incidence of *M. separata*, *M. loreyi* and *M. unipuncta* have been reported on maize, sugarcane, pearl millet, napier and jhons grass in Haryana,2,3. In southern and central India, population dynamics and key mortality factors of *M. separata* were studied. The outbreak of *M. separata* has been recorded in Dharwad, Karnataka (1980-1981), Andhra Pradesh (1977, 1978, 1981) and at Kullu, Himachal Pradesh (1983). The studies on bionomics and predator and parasitoids of *M. separata* were carried out in Punjab during 1970s.4,6. During 1983-84, the presence of *M. separata* and *M. loreyi* was reported from rice growing areas of Punjab.7 DNA barcode has been found to be a promising method and worldwide it is being used to identify species, subspecies, cryptic species etc. of insect pest. Earlier DNA barcoding was used to identify armyworm species prevalent in Punjab from four different locations and all were identified as *M. separata*.8

In this study larval populations were collected from rice, sorghum and maize from 8 different locations of Punjab (Table 1). The larvae were preserved immediately in absolute alcohol and stored at -20°C till further use. The genomic DNA was isolated from single larvae from each location using standard methods.9 PCR primers LepF (ATTCAACCAATC ATAAAGATAT TGG) and LepR (TAAACTTCTGGA TGTCCAA AAAATCA), the specific primers9 LepA were used to amplify mitochondrial cytochrome oxidase 1 (*mtCO1*) gene of the expected size of about 700 bp with PCR conditions. The purified DNA fragment was cloned in a sequencing vector pTZ57R/T using ‘InsT/A clone PCR product cloning kit’ (Fermentas Life Sciences) and transformed into Escherichia coli DH5-alpha host cells as per manufacturer’s protocol. The plasmid DNA was isolated and amplified with specific primers LepA and universal M13 primers to confirm the clones. The samples were sent to M/S Xcelris (Ahmedabad, India) for sequencing both strands with universal primers whose sites are present in the plasmid. The nucleotide sequence was edited using DNA software Chromaslite 201 and CLC Sequence Viewer 6.5.4 (CLC bio A/S) for any misread by the sequencer. The edited mtCO1 sequences for each sample was subjected to BLAST analysis using NCBI (www://blast.ncbi.nlm.nih.gov/Blast) to find its close homology/identity with database available in GenBank. The edited sequences were submitted to "Barcode of Life Database" and GenBank the BOLD IDs and accession numbers were assigned (Table 1).

To identify any mutations among the samples, sequences were subjected to multiple alignments using CLC Sequence Viewer programme. The
nucleotide sequences of *Mythimna loreyi* (15) from Australia, Mauritius, Pakistan, India and *M. separata* (11) from Australia, Pakistan, Japan, Korea and India with more than 500 bp are downloaded from NCBI. All sequences subjected to ClustalW multiple alignments and a phylogenetic tree was constructed using neighbour-joining method using Tamura 3-parameter methods using MEGA7 program. 

PCR amplification resulted in 700 bp amplicon in all the test samples (Fig. 1A). The amplicon was cloned in pTZ57R/T vector and the recombinant clones were confirmed through PCR amplification with specific and universal M13 primer sets with amplicon of 700 bp and 925 bp, respectively (Fig. 1B). BLAST analysis showed that four samples collected from rice (Patiala-Nabha and Bhadson, Muktsar, Gurdaspur) have 99% sequence homology with *Mythimna loreyi*. Two samples from rice (Gurdaspur and Bathinda) and one each from sorghum and maize (Hoshiarpur and Ludhiana) have a homology of 99 to 100% with *M. separata* (Table 1). The multiple alignments of all *M. loreyi* sequences revealed that the population from Patiala (Nabha and Bhadson) and Gurdaspur are genetically similar, however, there is one nucleotide substitution of "T" instead of "A" at position 14th in Muktsar population (Fig. 2). Using DNA barcoding, we report the occurrence of *M. loreyi* species in Punjab agro-ecosystem.

The phylogenetic analysis formed two distinct groups viz. *M. loreyi* and *M. separata*. The genetic distance between the two groups was 7.1% (Fig. 3). The four samples of *M. loreyi* from Punjab formed separate group than all other population from Australia, Pakistan, Mauritius and one from Ludhiana (India). Maximum genetic distance among the *M. loreyi* and *M. separata* populations is 0.8 and 0.4%, respectively. Among the *M. separata* group, three clusters were formed, however, the genetic distance is very less.

We reported the occurrence of *M. loreyi* species in Punjab agroecosystem from India. Earlier, we reported the genetic diversity *M. separata* from different locations of Punjab (Amritsar, Jalandhar, Gurdaspur, Bathinda). DNA barcoding is an established technique to study identify the different insect pests. Joomun and his co-workers studied the *Mythimna* species complex in Mauritius through DNA barcoding and assess the genetic diversity among different species. They identified three species of *Mythimna* viz. *M. insulicola*, *M. phaea* and *M. pseudoloreyi* which are known to occur in Mauritius. DNA barcoding has been used to identify and assess the genetic diversity of many insects viz. *Bemisia tabaci*, *Mythimna separata*, *Helicoverpa armigera*, *Drosicha mangiferae*, *Leucinodes orbonalis*. Genetic divergence of 7.3 to 10.6 was reported among the three species i.e. *M. loreyi*, *M. insulicola* and *M. phaea*. The maximum genetic distance between the *M. loreyi* and *M. separata* populations in this study is 0.8 and 0.4%, respectively. The average sequence divergence of 0.25% for conspecific individuals and 6.5% for congeneric species was observed using mtCOI sequences. Amplified fragment length polymorphism (AFLP) was used to identify and assess the genetic diversity of many insects.
Fig. 2 — Multiple alignments of *Mythimna loreyi* mtCO1 sequences collected from different regions of Punjab.

Fig. 3 — Phylogenetic analysis of mtCOI gene sequences from *M. loreyi* and *M. separata* populations collected from Punjab and compared with other population of world. The tree was constructed using the neighbour-joining method and evolutionary distances were computed using the Tamura 3-parameter model. GenBank accession no is followed by species name and followed by place of collection. There were a total of 510 positions in the final dataset.
study the genetic variations among the wild type natural and one laboratory population of *Mythimna separata* in China. Although high genetic variability was reported among the individuals of natural populations, there was little genetic differentiation among the three geographic populations. Although, the *M. loreyi* has been reported in Haryana and Punjab in 1980’s, after more than 30 years, this study shows that *M. loreyi* is still prevalent in Punjab conditions. Armyworm is considered as a sporadic pest in Punjab, however, due to changing climatic conditions and occurrence of a mixture of two species of *Mythimna* on rice, wheat, sorghum and maize, its pest status may change in coming years. There is a need to study more populations of armyworm from different regions using different markers to map total genetic diversity.

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**References**