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Molecular Characterization and DNA Barcoding of Black Gram Pod Borers from Andhra Pradesh, India

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ABSTRACT

Background: Four different pod borers species *viz*., Spotted pod borer (*Maruca vitrata*), Tobacco cutworm (*Spodoptera litura*), Gram blue butterfly (*Euchrysops cnejus*) and Banner moth (*Eublemma dimidialis*) were collected from black gram fields of Tirupati district, Andhra Pradesh.

Methods: Molecular characterization of mt*COI* sequences of the four black gram pod borer species were deposited in NCBI and accession numbers were allotted, *M. vitrata* (PP938848), *S. litura* (PP938758), *E. cnejus* (PP938841) and *E. dimidialis* (PP938850) respectively. DNA barcodes were generated and BINs were allotted for four different pod borers and available at BOLD public database.

Result: The phylogenetic analysis of all the pod borers together revealed that the pod borer species were distinctly separated at genus level and were grouped into congeneric clusters. The DNA barcode of *E. dimidialis* (BOLD:AAB6502) was generated for the first time from India and the DNA barcode of *E. cnejus* (BOLD:AAD0845) was generated for the first time from Andhra Pradesh, India.

Key words: Black gram, *Eublemma dimidialis*, *Euchrysops cnejus*, *Maruca vitrata*, Molecular characterization, Pod borers, *Spodoptera litura*.

INTRODUCTION

Pulses are protein rich seeds of legumes and are often referred as poor man's meat due to their high nutritional value and low price (Umbarkar *et al.,* 2010). Black gram [*Vigna mungo* (L.) Hepper] is one of the important pulse crops having considerable importance in food and nutritional security in the world. Black gram productivity is constrained by various biotic and abiotic stresses. Insect pests and diseases ranks first among the biotic stresses that have a significant impact on crop yield and productivity. Among the numerous insect pests, the pod borers are very important causing heavy yield loss. The grain yield loss by *M. vitrata* alone ranges between 2 to 84 per cent and economic loss between 20 to 25 per cent (Naik and Mallapur, 2019). The larvae of *M. vitrata* hides inside the webbings made of flowers and pods and feeds from the inside (Kavitha and Vijayaraghavan, 2023). This hiding behaviour of the pod borer protects the larvae from its natural enemies (Singh and Singh, 2021) and also makes it difficult to manage the insect with insecticides (Sambathkumar *et al.,* 2015).

Changing cropping systems, reduction of interval between harvesting of black gram and sowing of next crop and increased mechanization might have resulted in the variations in species composition of black gram pod borers. The continuous food availability due to growing of multiple pulse crops in the surrounding fields results in severe outbreaks of pod borers across different pulse crops because of their polyphagous nature. Continuous cropping and sowing of black gram in summer months as rice fallow pulse have favoured the survival of pod borers in irrigated areas of Andhra Pradesh.

Advancements in the study of insect population genetics, particularly through molecular markers and phylogeographical approaches, play a crucial role in elucidating the population structure and gene flow which aids in devising effective strategies for pest control (Behura, 2006, Zimmer *et al.,* 2014). The mt*COI* gene sequence, characterized by low variability, is widely used for distinguishing insect species and studying phylogenetic evolution, especially regarding inter-specific and intra-specific population differentiation and the extent of gene flow (Hurst and Jiggins, 2005; Behura, 2006). The genetic diversity within populations offers insights into both historical and contemporary demographic aspects, contributing to the characterization of the demographic history of crop pests (Ouvrard *et al.,* 2016). Additionally,

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DNA barcoding has been used extensively to identify immature life stages of insects. The use of Barcode Index Numbers (BINs) in DNA barcoding offers enhanced taxonomic resolution compared to traditional methods, aiding in the identification of insect specimens up to the species level (Hebert *et al.,* 2003). Understanding the genetic diversity of the pod borer species will aid in the development of effective pest management strategies.

MATERIALS AND METHODS

Sample collection

Larvae and adults of black gram pod borers *viz.,* Spotted pod borer (*Maruca vitrata*), Tobacco cutworm (*Spodoptera litura*), Gram blue butterfly (*Euchrysops cnejus*) and Banner moth (*Eublemma dimidialis*) were collected from black gram fields in Tirupati of Andhra Pradesh during 2023-24 and were identified based on the taxonomic keys. The collected specimens were preserved in 95 per cent ethanol, labelled and kept in deep freezer (-20°C). For each species, genomic DNA was extracted from an individual larva separately except *E. cnejus*, where adult moth legs were used.

Extraction of DNA

DNA was extracted separately from each larva and adult of the four different pod borer species following modified CTAB method (Murray and Thomson, 1980). DNA samples were quantified by Nanodrop® 2000 and quality was checked on 1 per cent (w/v) agarose gel.

PCR amplification and sequencing of *COI*

About 100 ng genomic DNA was used for PCR analysis using universal *COI* primers (LCO1490: 5′-GTCAAC AAATCATAAAGATATTGG-3′ and HCO2198: 5′-TAAACTTCA GGGTGACCAAAAAATCA-3′) (Folmer *et al.,* 1994). PCR reactions were performed in 25 µl volume of mix containing the components of 10X PCR reaction buffer, 2.5 mM of $MgCl_2$, 10 mM of dNTPs, 10 pM of each primer, 2.5 units of Taq DNA polymerase and 100 ng of DNA template. The program of PCR cycles for *COI* was performed as follows: 94°C for initial denaturation for 4 min followed by 35 cycles of 94°C for 30 sec, annealing at 54.2°C, 53.6°C, 55.1°C and 52.4°C for 45 sec for *M. vitrata, S. litura, E. cnejus* and *E. dimidialis*, respectively, extension at 72°C for 1 min followed by a final extension of 72°C for 10 min using Eppendorf thermal cycler (Eppendorf, Germany). Agarose gel electrophoresis of PCR amplified DNA was performed as described by Sambrook and Russell (2001) in one per cent agarose gel (w/v) and the migration pattern of the DNA fragments in the gel was recorded using gel documentation system (Alpha Innotech, USA) in an auto exposure mode. Sequencing was performed at automated DNA sequencing facility (Eurofin Genomics India Pvt. Ltd., Bengaluru).

Data analysis

The sequences of each pod borer species were compared with sequences on GenBank using nBLAST for homologs of the gene, to match with the sequence similarity for correct identification of species based on total score, expected value, maximum identical query coverage and maximum score (Altschul *et al.,* 1990). The trimmed sequences were submitted to NCBI GenBank for acquiring accession numbers. The sequences present in NCBI database were retrieved in FASTA format for comparative genetic study. Sequence assembling, nucleotide alignment and per cent identity matrix were done with Bio Edit 7.0 (Hall, 1999). Phylogenetic trees were constructed using pod borer nucleotide sequences obtained from current study along with the pod borer sequences of same genus and species retrieved from the NCBI and BOLD databases. Additionally diverse pod borer species were included as an outgroup in the construction of the phylogenetic tree. The Maximum Likelihood (ML) tree was constructed with 1000 bootstrap replications under distance model Tamura 3-parameter using Molecular Evolutionary Genetic Analysis 11.0 (MEGA11) (Tamura *et al.,* 2021).

DNA barcodes were generated using mt*COI* sequences of different black gram pod borers *viz., M. vitrata*, *S. litura*, *E. cnejus* and *E. dimidialis* and deposited in Barcode of Life Data Systems (BOLD).

RESULTS AND DISCUSSION

A total of four mt*COI* gene sequences of four different black gram pod borers species identified from black gram fields in Tirupati, Andhra Pradesh *viz.,* Spotted pod borer (*M. vitrata*), Tobacco cutworm (*S. litura*), Gram blue butterfly (*E. cnejus*) and Banner moth (*E. dimidialis*) have been generated.

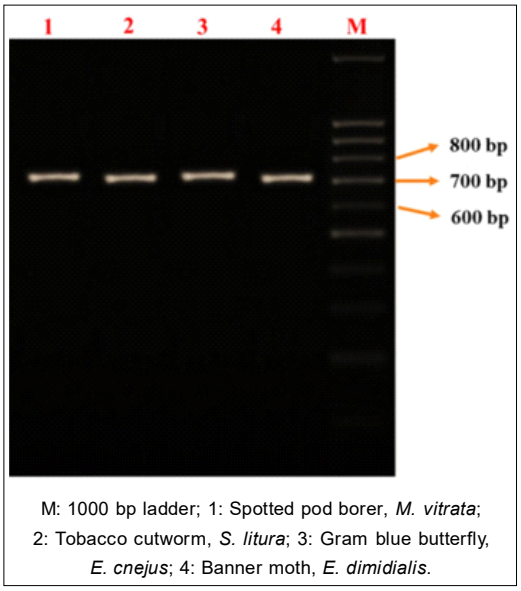
The PCR amplification of mt*COI* gene with universal primers (LCO1490 and HCO2198) resulted in 700 bp amplicons among the four pod borer populations as shown in Fig 1. The trimmed sequences produced using mt*COI* primers were submitted to NCBI and accession numbers were obtained for four black gram pod borers *viz.*, *M. vitrata* (PP938848), *S. litura* (PP938758), *E. cnejus* (PP938841) and *E. dimidialis* (PP938850). The sequences were also compared with available sequences in the data base from India and other parts of the world mined from public databases of NCBI and BOLD.

The homology search of mt*COI* sequences of pod borer species under study using nBLAST analysis showed 97-100% similarity in GenBank. Analysis of the average nucleotide composition of the mt*COI* gene fragments of *M. vitrata*, *S. litura*, *E. cnejus* and *E. dimidialis* showed high A+T content *viz.,* 69.6%, 70.4%, 69.8% and 70.8% respectively and low G+C content *viz.,* 30.4%, 29.6%, 30.2% and 29.2% respectively (Table 1). The *COI* region in

Table 1: Mean ATGC content of different black gram pod borer sequences from Andhra Pradesh, India.

Black gram pod borers	AT (%)	GC (%)
Spotted pod borer, *M. vitrata*	69.6	30.4
Tobacco cutworm, *S. litura*	70.4	29.6
Gram blue butterfly, *E. cnejus*	69.8	30.2
Banner moth, *E. dimidialis*	70.8	29.2

arthropods is frequently characterized by a high content of AT and a low GC composition (Lunt *et al.,* 1996; Navajas *et al.,* 1996; Shashank *et al.,* 2018) which confirms that *COI* gene of pod borers is AT rich in nature as in other insect populations. The multiple sequence alignments of the four pod borer species shared high similarities with their consensus sequences *i.e., M. vitrata* (95%), *S. litura* (99%), *E. cnejus* (100%) and *E. dimidialis* (99%). A total of 29 mutations were predicted in *M. vitrata* with 20 being transitional mutations and 9 transversional mutations, whereas, in *S. litura* only two transitional mutations were predicted. Similarly, 12 mutations were predicted in *E. dimidialis* with ten being transitional mutations and two transversional mutations.



M: 1000 bp ladder; 1: Spotted pod borer, *M. vitrata*; 2: Tobacco cutworm, *S. litura*; 3: Gram blue butterfly, *E. cnejus*; 4: Banner moth, *E. dimidialis*.

Fig 1: PCR amplification of mt*COI* of four different pod borers (700 bp).

Sequence Similarity Identity Matrix (SSIM) analysis of the mt*COI* gene sequence of the pod borer population from Tirupati, Andhra Pradesh along with other pod borer sequences retrieved from NCBI and BOLD databases showed less genetic distances among the species.

Phylogenetic relationships were analysed using four mt*COI* sequences of black gram pod borers obtained from the current study along with 80 other pod borer sequences retrieved from NCBI and BOLD databases (Fig 2). Additionally, *H. armigera* pod borer sequence was included as an outgroup in the construction of the phylogenetic tree. Phylogenetic tree revealed that the four pod borers were distinctly separated at genus level based on the nucleotide divergence among the species. The pod borers, *M. vitrata, S. litura, E. cnejus* and *E. dimidialis* were grouped typically into distinct congeneric clusters and *H. armigera* was highly diverse and distinctly out grouped.

DNA barcoding of black gram pod borers

DNA Barcodes were generated for all the four black gram pod borers (*M. vitrata, S. litura, E. cnejus* and *E. dimidialis*)

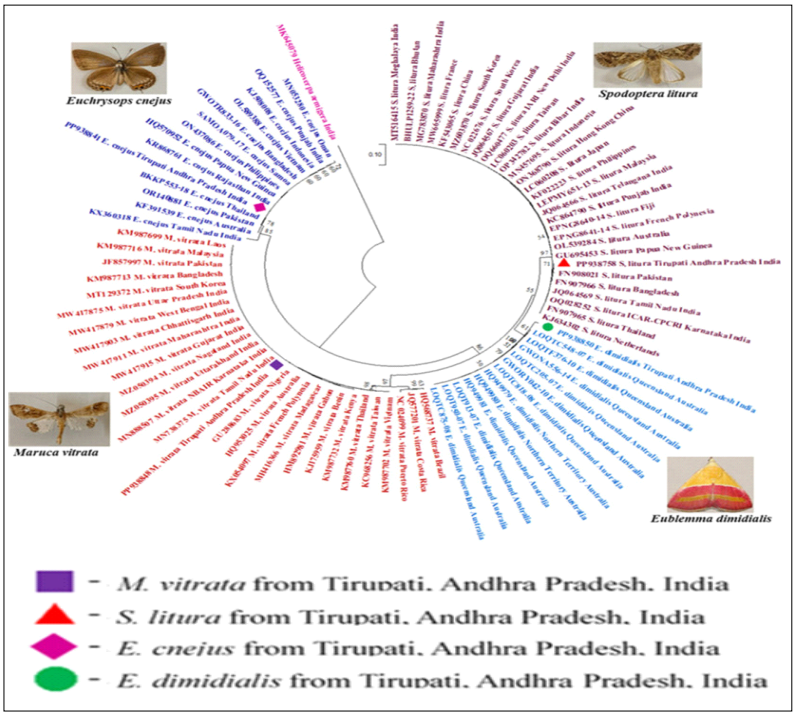


Fig 2: Maximum likelihood phylogenetic tree illustrating the relationships of mt*COI* sequence of black gram pod borers *viz., M. vitrata, S. litura, E. cnejus* and *E. dimidialis* collected from Tirupati, Andhra Pradesh, India in comparison with other black gram pod borer sequences mined from NCBI and BOLD databases using Tamura 3-prameter model and 1000 bootstrap replicates.

a. *Maruca vitrata* from Tirupati Andhra Pradesh, India. Sample code: MVTPT, NCBI Accession no: PP938848, Process ID: MVTPT001-24, BIN: BOLD: AAB2756

b. *Spodoptera litura* from Tirupati Andhra Pradesh, India. Sample code: SLTPT, NCBI Accession no: PP938758, Process ID: SLTPT001-24, BIN: BOLD: AAA8626

c. *Euchrysops cnejus* from Tirupati Andhra Pradesh, India. Sample code: ECTPT, NCBI Accession no: PP938841, Process ID: ECTPT001-24, BIN: BOLD: AAD0845

d. *Eublemma dimidialis* from Tirupati Andhra Pradesh, India. Sample code: EDTPT, NCBI Accession no: PP938850, Process ID: EDTPT001-24, BIN: BOLD: AAB6502

Fig 3: DNA barcodes generated for black gram pod borers from Andhra Pradesh, India.

and were allotted with BINs and Process IDs when submitted to Barcode of Life Data (BOLD). All the four pod borers under study were allotted with BINs *viz., M. vitrata* (BOLD:AAB2756), *S. litura* (BOLD:AAA8626), *E. cnejus* (BOLD:AAD0845) and *E. dimidialis* (BOLD:AAB6502) (Fig 3). The DNA barcode of *E. cnejus* (BOLD:AAD0845) have been generated for the first time from the state of Andhra Pradesh, South India. Similarly, the DNA barcode of *E. dimidialis* (BOLD:AAB6502) have been generated for the first time from India as the second country to report in addition to Australia.

CONCLUSION

This study reports four different pod borer species from black gram in Tirupati, Andhra Pradesh *viz., M. vitrata, S. litura, E. cnejus* and *E. dimidialis*. Phylogenetic analysis of the four pod borer species revealed that the populations have some degree of differentiation. There is a high genetic variation among the pod borer populations grouping them at genus level and low genetic variation between the pod borer populations. The data generated from this study will act as a valuable genomic resource in the population and will also aid in predicting migration pattern of the four pod borer species. However, more taxonomic and molecular studies are required, incorporating whole genome sequence of different pod borers to understand the population dynamics, pest shifts due to environmental changes and subsequent spread of the pod borers in black gram ecosystem that needs to be documented.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

No specific permits were required for the described studies and no specific permissions were required for these locations/activities. We confirm that samples were taken from non-endangered, non-protected species on open, public lands.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or

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REFERENCES

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology.* **215:** 403-410.

Behura, S.K. (2006). Molecular marker systems in insects: Current trends and future avenues. *Molecular Ecology*. **15(11):** 3087-3113.

Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. (1994). DNA primers for amplifcation of mitochondrial *cytochrome c oxidase subunit I* from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology.* **3:** 294-299.

Hall, T. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series.* **41:** 95-98.

Hebert, P.D.N., Cywinska A., Ball, S.L. and Dewaard J.R. (2003). Biological identifications through DNA barcodes. Philosophical Transactions of the Royal Society London. Series B: Biological Science. **270(1512):** 313-321.

Hurst, G.D. and Jiggins, F.M. (2005). Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. Proceedings of the Royal Society B: Biological Sciences. **272(1572):** 1525-1534.

Kavitha, Z. and Vijayaraghavan, C. (2023). Habitat manipulation: An important component of IPM in the management of webbing caterpillar, *Maruca vitrata* (Geyer) in pigeonpea. *Legume Research-An International Journal*. **46(6):** 762-769. doi: 10.18805/LR-4412.

Lunt, D.H., Zhang, D.X., Szymura, J.M. and Hewltt, O.M. (1996). The insect *cytochrome oxidase I* gene: Evolutionary patterns and conserved primers for phylogenetic studies. *Insect Molecular Biology.* **5(3):** 153-165.

Murray, M.G. and Thompson, W.F. (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research.* **8(19):** 4321-4326.

Naik, M.G. and Mallapur, C.P. (2019). Field screening of blackgram genotypes against spotted pod borer, *Maruca vitrata* (Geyer). *Journal of Entomology and Zoology Studies*. **7(3):** 631-634.

Navajas, M., Gutierrez, J., Lagnel, J. and Boursot, P. (1996). Mitochondrial *cytochrome oxidase I* in tetranychid mites: A comparison between molecular phylogeny and changes of morphological and life history traits. *Bulletin of Entomological Research.* **86(4):** 407-417.

Ouvrard, P., Hicks, D.M., Mouland, M., Nicholls, J.A., Baldock, K.C., Goddard, M.A. and Stone, G.N. (2016). Molecular taxonomic analysis of the plant associations of adult pollen beetles (Nitidulidae: Meligethinae) and the population structure of *Brassicogethes aeneus*. *Genome.* **59(12):** 1101-1116.

Sambathkumar, S., Durairaj, C., Ganapathy, N. and Mohankumar, S. (2015). Field evaluation of newer insecticide molecules and botanicals against pod borers of red gram. *Legume Research-An International Journal.* **38(2):** 260-267. doi: 10.5958/0976-0571.2015.00048.X.

Sambrook, J. and Russell, D.W. (2001). Gel Electrophoresis of DNA and Pulsed Field Agarose Gel Electrophoresis. In: Molecular Cloning, A Laboratory Manual, [Sambrook, J. and Russell, D.W (eds.)] third ed, Cold Spring Harbour Laboratory Press, New York, USA. 5.4-5.17.

Shashank, P.R., Twinkle, S., Chandrashehar, K., Meshram, N.M., Suroshe, S.S. and Bajracharya, A.S.R. (2018). Genetic homogeneity in South American tomato pinworm, *Tuta absoluta*: A new invasive pest to oriental region. *3 Biotech.* **8:** 350.

Singh, S.K. and Singh, P.S. (2021). Biochemical factors associated with resistance to spotted pod borer, *Maruca vitrata* (Fabricius) in green gram. *Legume Research-An International Journal.* **44(11):** 1398-1401. doi: 10.18805/LR-4302.

Tamura, K., Stecher, G. and Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*. **38(7):** 3022-3027.

Umbarkar, P.S., Parsana, G.J. and Jethva, D.M. (2010). Seasonal incidence of gram pod borer, *Helicoverpa armigera* (Hübner) on green gram. *Legume Research-An International Journal*. **33(2):** 148-149.

Zimmer, C.T., Maiwald, F., Schorn, C., Bass, C., Ott, M.C. and Nauen, R. (2014). A de novo transcriptome of European pollen beetle populations and its analysis, with special reference to insecticide action and resistance. *Insect Molecular Biology.* **23(4):** 511-526.