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MOLECULAR CHARACTERIZATION OF *SPODOPTERA LITURA* (FABRICIUS) INFESTING COTTON USING MITOCHONDRIAL CYTOCHROME C OXIDASE (COI) GENE

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ABSTRACT

The present study assessed the morphometric and genetic diversity of *Spodoptera litura* populations infesting cotton across eight agro-climatic regions of Gujarat. Field-collected specimens were reared and analyzed using morphometric and molecular approaches. Morphometric data revealed consistent sexual dimorphism, with Jamnagar populations exhibiting slightly larger traits, suggesting possible local adaptation. Measurement error remained below 5%. For molecular analysis, genomic DNA was extracted using the CTAB method. The mitochondrial genes *COXI* (*Cytochrome c oxidase subunit I*) of ~900 bp and *COX II* (*Cytochrome c oxidase subunit II*) of ~850 bp, along with the nuclear gene *TPI* (*Triose phosphate isomerase*) of ~450 bp, were successfully amplified using gene-specific primers. Sanger sequencing yielded 32 high-quality sequences, all confirmed as *S. litura* via BLAST. While mitochondrial genes showed low inter-population divergence (pairwise distance <1.0), TPI revealed higher polymorphism (distance >1.0), indicating greater nuclear variation. Phylogenetic analysis showed close clustering of most populations, with slight divergence in Jamnagar. Sixteen DNA barcodes were submitted to BOLD. Overall, the findings indicate low genetic divergence and strong gene flow across regions, with minor localized variation. The combined morphometric and molecular approach offers valuable insights for monitoring and managing *S. litura* in Gujarat's cotton agroecosystem.

Key words : *Spodoptera litura*, DNA barcoding, Morphometrics, *COXI*, *COXII* & *TPI*.

Introduction

Cotton (*Gossypium hirsutum* L.) is one of the most important commercial fiber crops globally, cultivated in over 80 countries across tropical and subtropical regions (Delhom *et al*., 2024). In India, cotton has held cultural and economic significance since ancient times, contributing substantially to the textile industry and rural livelihoods. With more than 96 lakh hectares under cultivation, India has the largest area under cotton, accounting for nearly 25% of global acreage and 16% of world production (Khadi *et al.*, 2010). Major cotton-producing states like Gujarat, Maharashtra, and Madhya Pradesh form the central cotton zone, contributing about 95% of national output (Singh and Kairon, 2001). Despite its economic value, cotton productivity is threatened by various biotic and abiotic stresses. Among insect pests, *S. litura* (Fabricius), commonly known as the cotton leafworm, is a highly destructive, polyphagous pest infesting over 120 host plants, including cotton, soybean, groundnut, and vegetables (Selvaraj *et al.*, 2010). In India, *S. litura* infestation can lead to 10–30% yield losses depending on environmental conditions and pest management efficacy (Rajendran *et al.*, 2018).

S. litura is a nocturnal moth of the family Noctuidae with high fecundity, pesticide resistance, and dispersal capacity, making it a serious threat to cotton production (Ahmad *et al.*, 2013). Accurate identification of this pest is critical but often limited by traditional morphological methods, particularly in immature stages or degraded specimens (Pogue, 2002). Hence, the integration of molecular approaches, including morphometric and DNA-based tools, has become essential for species identification (Shashank *et al.*, 2022). DNA barcoding using mitochondrial genes, especially the cytochrome c oxidase subunits I and II (*COXI* and *COXII*), has proven effective for species-level identification due to high interspecific divergence and low intraspecific variability (Hebert *et al.*, 2003a; Hebert *et al.*, 2003b). These genes are maternally inherited, lack recombination, and are present in high copy numbers, enhancing amplification and sequencing success (Folmer *et al.*, 1994). The 52 region of *COXI* (~648 bp) is the standard DNA barcode for animals, including Lepidoptera (Hebert *et al.*, 2003a). *COXII* is also used for resolving phylogenetic relationships and population genetics in insects due to its evolutionary utility (Lunt *et al.*, 1996; Simon *et al.*, 1994; Shashank *et al.*, 2015).

In addition to mitochondrial markers, the nuclear gene *TPI* (triose phosphate isomerase) has emerged as a valuable marker for genetic diversity and population structure analysis due to its biparental inheritance and moderate sequence conservation (Wu *et al.*, 2019; Zhou *et al.*, 2021). Although, extensively studied in *S. frugiperda*, *TPI* has shown potential in *S. litura* for identifying host-associated populations and understanding ecological adaptation (Nagoshi, 2010; Nagoshi, 2012; Nagoshi *et al.*, 2017). Mitochondrial DNA has gained prominence in evolutionary biology due to its rapid mutation rate, maternal inheritance and effectiveness in phylogeographic and population genetic studies (Souza *et al.*, 2016). Genetic variation in mtDNA, such as *COXI* and *COXII*, offers insights into population structure and evolutionary relationships among geographically isolated pest populations (Gandhi and Patil, 2017; Chandrayudu *et al.*, 2017).

Despite its economic impact, molecular data on *S. litura* from Gujarat remain scarce. The present study aims to characterize the genetic and morphometric diversity of *S. litura* populations collected from eight distinct cotton-growing locations representing different agroclimatic zones of Gujarat, using *COXI*, *COXII* and *TPI* gene sequences. These insights are expected to contribute to more accurate pest identification, population monitoring, and the development of sustainable management strategies.

Materials and Methods

Sample collection and preservation

During 2024–2025, *S. litura* specimens were collected from eight cotton-growing locations across different agro-climatic zones of Gujarat. The locations selected for the study are MCRS, NAU, Surat; Cotton farm, NAU, Navsari; Agricultural Research Station, Bharuch; Cotton farm, Anand Agricultural University, Anand; Agricultural Research Station, Achhalia; Agricultural Research Station, Hansot; Farmer field, Jamnagar and Cotton Farm, Junagadh Agricultural University, Junagadh. Adult moths were trapped using funnel-type pheromone and light traps placed near infested cotton fields and monitored at dusk and early morning. Additionally, larvae and adults were manually collected from cotton plants in the same fields. All specimens were transported to the Entomology Laboratory, NMCA, NAU, Navsari, and reared in wooden and wire mesh cages (0.3 m^2) for short-term maintenance (Ahmad and Mehmood, 2015).

For molecular analysis, representative specimens were preserved in 70% ethanol and stored at –20°C until DNA extraction. Molecular work was carried out at the Department of Plant Molecular Biology and Biotechnology, NMCA, NAU, Navsari, to investigate intra-species genetic variation across different agro-climatic zones.

Morphometric measurements

Five healthy adults (3 males and 2 females) per location were selected for morphometric analysis. Specimens were immobilized by chilling or killed in hot water (60 °C), then dried and placed on millimetre graph paper. The following seven traits were manually measured: body length and breadth (with and without wing expansion), forewing length, hindwing length, and antennae length. Measurements were repeated five times per individual and the average was used (Ahmad and Mehmood, 2015).

Genomic DNA isolation

Genomic DNA from *S. litura* collected across eight locations was extracted using a modified CTAB method with Proteinase K and RNase treatments, followed by chloroform: isoamyl alcohol purification and isopropanol precipitation. DNA integrity was confirmed by 0.8%

agarose gel electrophoresis, and purity (A260/A280) was assessed using NanoDrop spectrophotometry. Samples were stored at –20°C for further molecular analysis.

PCR Amplification and Purification

Mitochondrial COXI, COXII and nuclear TPI regions were amplified using gene-specific primers (Table 1) following protocols by Nagoshi *et al.* (2017) and Babu *et al.* (2021). Each 20 µl PCR mixture contained master mix, primers, DNA template, and nuclease-free water. The cycling program consisted of initial denaturation at 94°C for 1 min, 35 cycles of 94 °C for 30 s, 58°C for 45 s and 72 °C for 45 s, followed by a final extension at 72 °C for 3 min. Amplicons were confirmed on 2% agarose gels stained with ethidium bromide. PCR products were purified via ethanol–sodium acetate precipitation, resuspended in Hi-Di™ formamide and sequenced by the Sanger method. High-quality reads were trimmed, aligned, and used for molecular analysis.

DNA Sequence analysis

COXI, COXII and TPI reads were assembled into consensus sequences using Sequencher v5.3, screened in MEGA 12 and cleaned in BioEdit v7. Validated sequences were submitted to NCBI GenBank with accession numbers assigned, and identities confirmed via BLASTn (≥98% similarity). Barcode gap, nucleotide composition, and genetic divergence were analyzed from ClustalW alignments. Phylogenetic relationships were inferred using Maximum Likelihood (MEGA 12, 1000 bootstraps) and divergence visualized through pairwise distance heat maps.

Submission to the Barcode of Life Data system (BOLD)

COXI and COXII sequences, along with metadata, images and trace files, were submitted to the Barcode of Life Data System (BOLD; Ratnasingham and Hebert, 2007) to enable global data sharing and accurate species identification through DNA barcoding.

Results and Discussion

Morphometric variation in *S. litura* from Different Agro-Climatic Zones of Gujarat

Morphometric analysis of adult *S. litura* from eight agro-climatic zones of Gujarat revealed significant sexual dimorphism and regional variation in body size, wing dimensions and antennae length. Females were consistently larger than males across all traits, with Jamnagar populations showing the highest values and Anand the lowest. Body length with wings ranged from 23.51 ± 0.02 mm (females, Jamnagar) to 19.45 ± 0.02 mm (males, Navsari), while without wings it ranged from 17.79 ± 0.02 mm (females, Jamnagar) to 16.37 ± 0.02 mm (males). Wing lengths were greater in females, indicating higher dispersal potential, and antennae lengths were longer in females, with maxima in Jamnagar (9.52 ± 0.02 mm) compared to males (7.66 ± 0.02 mm). These trends suggest ecological and host plant influences on morphometric variation rather than genetic divergence, consistent with Ganguly (2020) and Tuan *et al.* (2015). Summary statistics are presented in Tables 1 and 2, and regional male morphometric trends are illustrated in Fig. 1.

Molecular characterization of *S. litura* Populations using *COXI*, *COXII*, and *TPI* genes

Intra-species genetic diversity in *S. litura* was assessed using COXI, COXII and TPI gene markers from specimens collected across eight cotton-growing zones of Gujarat.

Survey, Sample collection and Genomic DNA extraction

Field surveys were conducted across eight major cotton-growing locations in Gujarat—MCRS (NAU, Surat), NAU Cotton Farm (Navsari), ARS (Bharuch, Achhalia, Hansot), AAU Cotton Farm (Anand), JAU (Junagadh) and a farmer's field (Jamnagar)—to collect *S. litura* larvae and adults. Specimens were preserved in 70% ethanol and stored at –20°C for molecular analysis. Pooled samples were used for genomic DNA extraction using a modified CTAB protocol (Zinzuvadiya, 2017; Ganguly, 2020), optimized for yield and integrity. Agarose gel electrophoresis confirmed intact, high-molecular-weight DNA, and NanoDrop readings (A260/280 = 1.78–1.86) indicated high purity, suitable for PCR amplification, DNA barcoding, and sequencing.

PCR amplification

Mitochondrial COXI (~900 bp), COXII (~850 bp), and nuclear TPI (~450 bp) regions were successfully amplified from *S. litura* collected across eight Gujarat locations using primers COI101F/COI911R, COI891F/COI1472R and TPI282F/TPI850R (Nagoshi *et al.*, 2017; Babu *et al.*, 2021). Clear, distinct bands on 2% agarose gels (Fig. 3) confirmed primer specificity and PCR efficiency, consistent with *S. frugiperda* studies (Nagoshi *et al.*, 2017). Sequencing with gene-specific primers and the BDT v3.1 Cycle Sequencing Kit on an ABI 3730xl Genetic Analyzer yielded high-quality bidirectional reads for all loci, providing a robust basis for genetic diversity and phylogenetic analyses (Kalaria *et al.*, 2023) (Fig. 2).

DNA Sequence assembly

Consensus sequences, assembled in Sequencher v5.3

Table 1 : Morphometric characteristics of *S. litura* males collected from different agro-climatic zone of Gujarat.

S. no.	Location	Body length with wings expansion (mean ± SD)	Body breadth with wings expansion (mean ± SD)	Body length without wings expansion (mean ± SD)	Body breadth without wings expansion (mean ± SD)	Forewing length (mean ± SD)	Hindwing length (mean ± SD)	Antennae length (mean ± SD)
1	Navsari	19.45 ± 0.02	35.36 ± 0.03	16.54 ± 0.01	5.16 ± 0.01	17.24 ± 0.02	12.08 ± 0.01	7.23 ± 0.02
2	Bharuch	20.13 ± 0.02	35.96 ± 0.03	16.82 ± 0.01	5.54 ± 0.01	17.47 ± 0.02	12.24 ± 0.02	7.46 ± 0.03
3	Surat	19.53 ± 0.01	35.45 ± 0.02	16.44 ± 0.01	5.23 ± 0.02	17.14 ± 0.02	12.14 ± 0.01	7.14 ± 0.02
4	Anand	20.06 ± 0.02	34.77 ± 0.06	16.37 ± 0.02	5.35 ± 0.03	17.15 ± 0.02	12.06 ± 0.03	7.25 ± 0.02
5	Junagadh	20.34 ± 0.02	35.77 ± 0.02	16.88 ± 0.01	5.72 ± 0.01	17.65 ± 0.02	12.27 ± 0.01	7.45 ± 0.02
6	Hansot	20.22 ± 0.01	35.61 ± 0.04	16.62 ± 0.01	5.26 ± 0.03	17.35 ± 0.01	12.24 ± 0.01	7.37 ± 0.02
7	Jamnagar	20.47 ± 0.02	35.92 ± 0.02	16.97 ± 0.01	5.74 ± 0.01	17.87 ± 0.02	12.34 ± 0.02	7.66 ± 0.02
8	Achhalia	20.25 ± 0.01	35.45 ± 0.03	16.77 ± 0.02	5.57 ± 0.02	17.66 ± 0.01	12.25 ± 0.02	7.45 ± 0.03

Table 2 : Morphometric characteristics of *S. litura* females collected from different agro-climatic zone of Gujarat.

S. no.	Location	Body length with wings (mean ± SD)	Body breadth with wings (mean ± SD)	Body length without wing expansion (mean ± SD)	Body breadth without wing expansion (mean ± SD)	Forewing length (mean ± SD)	Hindwing length (mean ± SD)	Antennae length (mean ± SD)
1	Navsari	22.50 ± 0.02	38.40 ± 0.02	17.21 ± 0.01	7.79 ± 0.02	19.60 ± 0.02	14.29 ± 0.02	9.21 ± 0.02
2	Bharuch	23.00 ± 0.02	37.91 ± 0.02	17.50 ± 0.02	8.00 ± 0.02	19.91 ± 0.02	14.49 ± 0.03	9.41 ± 0.02
3	Surat	22.50 ± 0.03	38.39 ± 0.03	17.21 ± 0.02	7.80 ± 0.02	19.60 ± 0.02	14.30 ± 0.03	9.20 ± 0.02
4	Anand	21.80 ± 0.02	36.81 ± 0.03	16.90 ± 0.01	7.60 ± 0.02	19.20 ± 0.02	14.12 ± 0.02	9.00 ± 0.02
5	Junagadh	22.70 ± 0.02	37.20 ± 0.02	17.00 ± 0.01	7.81 ± 0.01	19.52 ± 0.02	14.29 ± 0.02	9.20 ± 0.02
6	Hansot	22.20 ± 0.01	37.51 ± 0.02	17.10 ± 0.01	7.71 ± 0.02	19.40 ± 0.01	14.20 ± 0.02	9.11 ± 0.02
7	Jamnagar	23.51 ± 0.02	38.99 ± 0.03	17.79 ± 0.02	8.20 ± 0.01	20.02 ± 0.02	14.70 ± 0.02	9.52 ± 0.02
8	Achhalia	22.91 ± 0.02	38.08 ± 0.03	17.31 ± 0.02	7.91 ± 0.01	19.79 ± 0.02	14.40 ± 0.02	9.30 ± 0.02

*Mean of 5 individuals SD = Standard Deviation

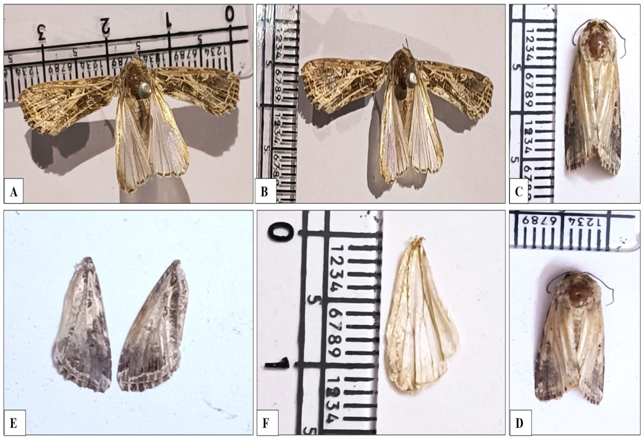

Fig. 1 : Morphometric parameters of *S. litura*. A) and B) Body length and breadth with wings expansion, C) and D) Body length and breadth without wings expansion, E) Forewing length, F) Hindwing length.

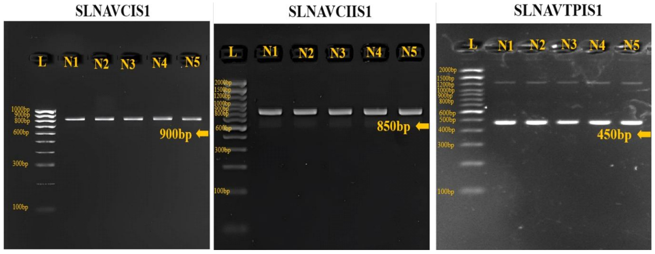

Fig. 2 : PCR amplification of COXI, COXII, and TPI genes of *S. litura* collected from Navsari. Representative sample shown: Ladder (L: 100 bp); Navsari (N1 to N5).

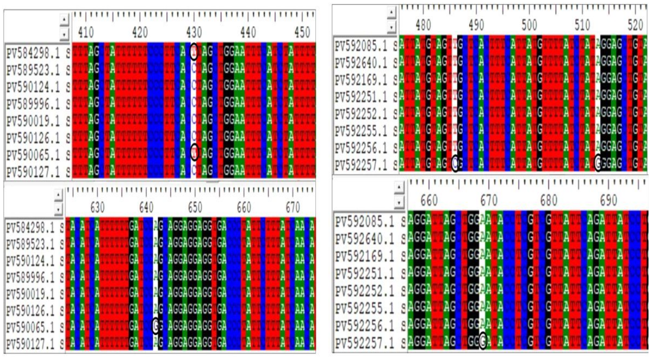

Fig. 3 : SNP variation for Barcode gap analysis of *COXI* and *COXII* sequence of *S. litura* individuals collected from different agro-climatic zone of Gujarat.

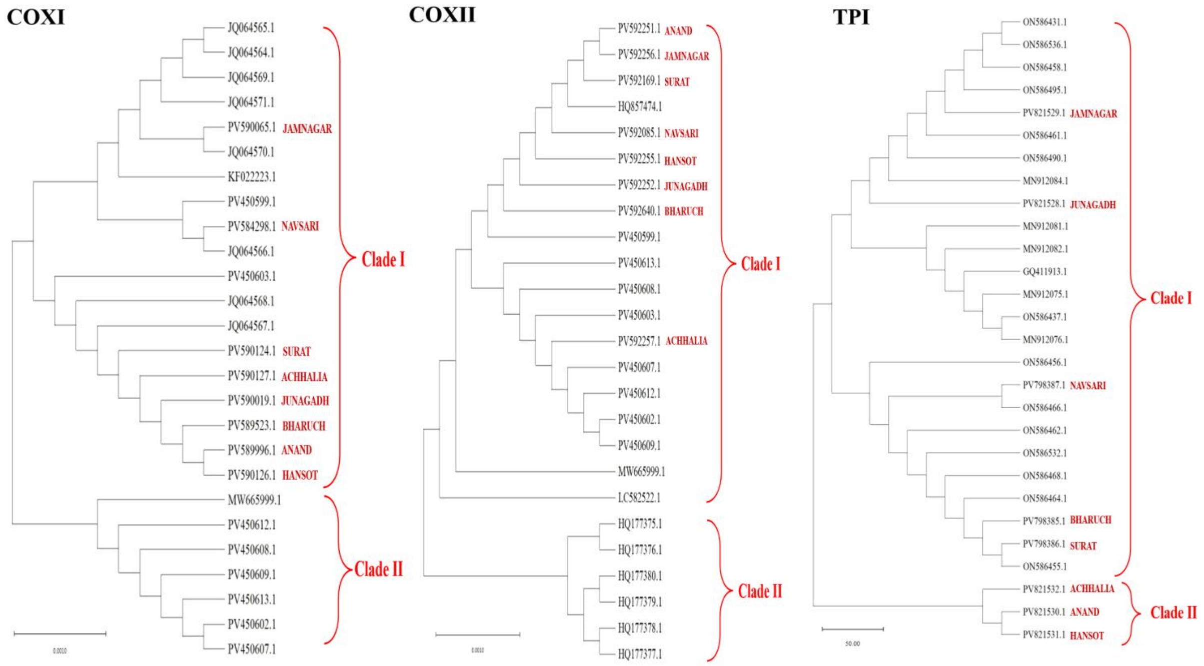

Fig. 4 : Rooted Neighbor-Joining (NJ) tree illustrating the *Phylogenetic* clustering of *S. litura* populations based on partial mitochondrial *COXI*, *COXI* and *TPI* gene sequences using Kimura-2-Parameter (K2P) distances.

(Gene Codes Corp, 2013), were BLASTn-verified (Altschul *et al.*, 1990) showing 98–99% similarity of COXI and COXII with *S. litura* and up to 100% similarity of TPI with *S. frugiperda* (Junagadh 96%). Quality checks in BioEdit v7 (Hall, 1999) confirmed intact ORFs, aligning with morphological identification (Ganguly, 2020). The validated gene regions—especially COXI and TPI—proved effective for species identification, consistent with prior reports (Hebert *et al.*, 2003b). All sequences were submitted to GenBank. Nucleotide composition revealed AT-rich mitochondrial genes (70.56–75.09%) and moderately AT-rich TPI (50.64–55.78%), in agreement with patterns reported in Lepidoptera (Pongen, 2018) (Fig. 3 and Table 3).

Phylogenetic analysis and Genetic distance

Phylogenetic relationships among *S. litura* populations from eight agro-climatic zones of Gujarat were inferred from COXI, COXII and TPI sequences using Maximum Likelihood in MEGA12 (Kumar *et al.*, 2024) under the Kimura 2-parameter model (Kimura, 1980), validated by Neighbor-Joining (Saitou and Nei, 1987) with 1000 bootstraps (Nei and Kumar, 2000). COXI and COXII each formed two clades: Clade I grouped most Gujarat populations with minimal divergence (e.g. Surat, Achhalia, Junagadh, Bharuch, Anand, Hansot), while Clade II contained divergent references (MW665999.1, PV450612.1; HQ857474.1) suggesting ancestral or distinct origins (Wang *et al.*, 2020; Ganguly, 2020); minor sub-structuring was evident in Jamnagar, Navsari (COXI) and Achhalia (COXII).

TPI showed clearer structuring, with Clade I (Jamnagar, Junagadh, Navsari, Bharuch, Surat) being genetically similar, and Clade II (Achhalia, Anand, Hansot) more divergent, some sequences grouping with *S. frugiperda* (Nagoshi, 2010) (Fig. 4). Pairwise distances were lowest for COXI (0.000–0.012) and COXII (0.000–0.008), indicating high mitochondrial homogeneity, while TPI (0.000–1.43) revealed moderate intraspecific divergence—highest in Anand, Hansot, Achhalia (~1.29)—and clear species-level separation from *S. frugiperda* (~1.43) (Figs. 5 and 6, Tables 4, 5 and 6).

Genetic Distance Heatmap visualization

Lower triangular heatmaps of genetic

Table 3 : Percentage composition of AT and GC nucleotides in partial *COXI*, *COXII* and *TPI* gene sequences of *S. litura*.

S. no.	Accession number	Percentage composition of different nucleotide bases					
		T	C	A	G	A+T	G+C
1	PV584298.1	40.12	14.36	30.55	14.97	70.67	29.33
2	PV589522.1	39.58	14.55	31.07	14.8	70.65	29.35
3	PV589523.1	39.49	14.87	31.15	14.49	70.64	29.36
4	PV589528.1	39.83	14.32	30.93	14.92	70.76	29.24
5	PV589529.1	39.77	14.2	31.02	15.00	70.8	29.2
6	PV590124.1	39.83	14.32	30.93	14.92	70.76	29.24
7	PV589996.1	40.32	15.25	30.24	14.19	70.56	29.44
8	PV589999.1	39.67	14.52	31.04	14.77	70.71	29.29
9	PV590123.1	39.67	14.52	31.04	14.77	70.71	29.29
10	PV590019.1	39.59	14.72	30.96	14.72	70.56	29.44
11	PV590126.1	39.49	14.87	31.15	14.49	70.64	29.36
12	PV590125.1	39.67	14.52	31.04	14.77	70.71	29.29
13	PV590064.1	39.60	14.50	31.02	14.88	70.62	29.38
14	PV590065.1	39.87	14.68	30.91	14.55	70.78	29.22
15	PV590127.1	39.59	14.72	30.96	14.72	70.56	29.44
16	PV590128.1	39.67	14.52	31.04	14.77	70.71	29.29
17	PV592085.1	43.23	12.53	31.83	12.41	75.06	24.94
18	PV592640.1	43.23	12.53	31.83	12.41	75.06	24.94
19	PV592169.1	43.66	12.55	31.37	12.42	75.03	24.97
20	PV592251.1	43.70	12.47	31.36	12.47	75.06	24.94
21	PV592252.1	42.69	13.09	31.84	12.38	74.53	25.47
22	PV592255.1	43.34	12.58	31.76	12.33	75.09	24.91
23	PV592256.1	43.72	12.56	31.28	12.44	75.00	25.00
24	PV592257.1	42.13	13.25	30.71	13.91	72.83	27.17
25	PV798387.1	27.84	19.95	27.61	24.59	55.45	44.55
26	PV798385.1	27.89	19.5	27.89	24.72	55.78	44.22
27	PV798386.1	27.65	19.82	27.65	24.88	55.30	44.70
28	PV821530.1	26.08	19.86	28.23	25.84	54.31	45.69
29	PV821528.1	24.46	21.71	26.91	26.91	51.38	48.62
30	PV821531.1	25.99	20.95	27.06	25.99	53.05	46.95
31	PV821529.1	24.36	22.12	26.28	27.24	50.64	49.36
32	PV821532.1	27.18	19.74	27.95	25.13	55.13	44.87

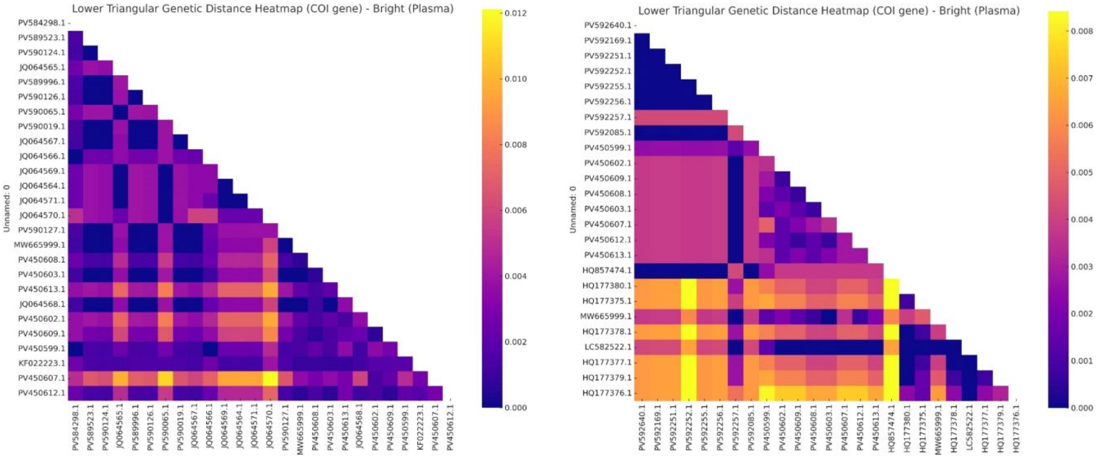

Fig. 5 : Heatmap of pairwise genetic distances among *S. litura* samples based on partial mitochondrial cytochrome oxidase (*COXI* and *COXII*) gene sequences.

Table 4 : Genetic distance matrix of different *S. litura* individuals based on partial mitochondrial (*COXI*) gene sequences.

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**	**U**	**V**	**W**	**X**	**Y**	**Z**
A																										
B	0.001																									
C	0.001	**0.000**																								
D	0.003	0.004	0.004																							
E	0.001	0.000	0.000	0.004																						
F	0.001	0.000	0.000	0.004	0.000																					
G	0.003	0.004	0.004	0.000	0.004	0.004																				
H	0.001	0.000	0.000	0.004	0.000	0.000	0.004																			
I	0.001	0.000	0.000	0.004	0.000	0.000	0.004	0.000																		
J	0.000	0.003	0.002	0.004	0.003	0.003	0.004	0.003	0.002																	
K	0.002	0.004	0.004	0.000	0.004	0.004	0.000	0.004	0.003	0.003																
L	0.002	0.004	0.004	0.000	0.004	0.004	0.000	0.004	0.003	0.003	0.000															
M	0.002	0.004	0.004	0.000	0.004	0.004	0.000	0.004	0.003	0.002	0.000	0.000														
N	0.005	0.004	0.004	0.002	0.004	0.004	0.000	0.004	0.006	0.006	0.002	0.002	0.002													
O	0.001	0.000	0.000	0.004	0.000	0.000	0.004	0.000	0.000	0.003	0.004	0.004	0.004	0.004												
P	0.001	0.000	0.000	0.004	0.000	0.000	0.004	0.000	0.000	0.002	0.003	0.003	0.003	0.006	0.000											
Q	0.002	0.001	0.001	0.005	0.001	0.001	0.005	0.001	0.001	0.002	0.005	0.005	0.005	0.007	0.001	0.001										
R	0.001	0.000	0.000	0.004	0.000	0.000	0.004	0.000	0.000	0.001	0.004	0.004	0.004	0.006	0.000	0.000	0.001									
S	0.004	0.004	0.004	0.007	0.004	0.004	0.008	0.004	0.004	0.005	0.007	0.007	0.007	0.010	0.004	0.002	0.001	0.002								
T	0.001	0.000	0.000	0.004	0.000	0.000	0.004	0.000	0.000	0.002	0.003	0.003	0.003	0.006	0.000	0.000	0.001	0.000	0.004							
U	0.004	0.004	0.004	0.007	0.004	0.004	0.008	0.004	0.004	0.005	0.007	0.007	0.007	0.010	0.004	0.002	0.001	0.002	0.001	0.004						
V	0.004	0.003	0.003	0.006	0.003	0.003	0.007	0.003	0.002	0.004	0.006	0.006	0.006	0.008	0.003	0.001	0.001	0.001	0.002	0.002	0.001					
W	0.000	0.001	0.001	0.002	0.001	0.001	0.003	0.001	0.001	0.000	0.002	0.002	0.002	0.005	0.001	0.001	0.002	0.001	0.004	0.001	0.003	0.003				
X	0.003	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.003	0.001	0.001	0.001	0.004	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.002	0.002			
Y	0.005	0.007	0.006	0.010	0.007	0.007	0.011	0.007	0.006	0.007	0.009	0.010	0.010	**0.012**	0.007	0.004	0.003	0.004	0.003	0.006	0.001	0.002	0.005	0.003		
Z	0.002	0.001	0.001	0.005	0.001	0.001	0.005	0.001	0.001	0.002	0.005	0.005	0.005	0.007	0.001	0.001	0.001	0.001	0.003	0.001	0.001	0.001	0.002	0.002	0.003	

A = PV584298.1, B = PV589523.1, C = PV590124.1, D = JQ064565.1, E = PV589996.1, F = PV590126.1, G = PV590065.1, H = PV590019.1, I = JQ064567.1, J = JQ064566.1, K = JQ064569.1, L = JQ064564.1, M = JQ064571.1, N = JQ064570.1, O = PV590127.1, P = MW665999.1, Q = PV450608.1, R = PV450603.1, S = PV450613.1, T = JQ064568.1, U = PV450602.1, V = PV450609.1, W = PV450599.1, X = KF022223.1, Y = PV450607.1, and Z = PV450612.1

Continued...

Table 5 : Genetic distance matrix of different *S. litura* individuals based on partial mitochondrial (*COXII*) gene sequences.

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**	**U**	**V**	**W**	**X**	**Y**	**Z**
A																										
B	0.000																									
C	0.000	0.000																								
D	0.000	0.000	0.000																							
E	0.000	0.000	0.000	0.000																						
F	0.000	0.000	0.000	0.000	0.000																					
G	0.004	0.004	0.004	0.004	0.004	0.004																				
H	0.000	0.000	0.000	0.000	0.000	0.000	0.004																			
I	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.003																		
J	0.004	0.004	0.004	0.004	0.004	0.004	0.000	0.004	0.004																	
K	0.004	0.004	0.004	0.004	0.004	0.004	0.000	0.004	0.003	0.001																
L	0.004	0.004	0.004	0.004	0.004	0.004	0.000	0.004	0.002	0.001	0.001															
M	0.004	0.004	0.004	0.004	0.004	0.004	0.000	0.004	0.001	0.002	0.001	0.001														
N	0.004	0.004	0.004	0.004	0.004	0.004	0.000	0.004	0.005	0.001	0.002	0.003	0.004													
O	0.004	0.004	0.004	0.004	0.004	0.004	0.000	0.004	0.002	0.001	0.001	0.001	0.001	0.003												
P	0.004	0.004	0.004	0.004	0.004	0.004	0.000	0.004	0.004	0.001	0.002	0.001	0.002	0.003	0.003											
Q	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.003	0.004	0.004	0.004	0.004	0.004	0.004	0.004										
R	0.006	0.006	0.006	0.008	0.006	0.006	0.003	0.006	0.006	0.005	0.005	0.004	0.004	0.005	0.005	0.004	0.008									
S	0.006	0.006	0.006	0.008	0.006	0.006	0.004	0.006	0.007	0.006	0.006	0.005	0.005	0.006	0.006	0.005	0.008	0.001								
T	0.004	0.004	0.004	0.006	0.004	0.004	0.000	0.004	0.001	0.002	0.001	0.001	0.000	0.004	0.001	0.002	0.006	0.004	0.005							
U	0.006	0.006	0.006	0.008	0.006	0.006	0.003	0.006	0.006	0.005	0.005	0.004	0.004	0.005	0.005	0.004	0.008	0.000	0.001	0.004						
V	0.004	0.004	0.004	0.007	0.004	0.004	0.000	0.004	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000					
W	0.006	0.006	0.006	0.008	0.006	0.006	0.003	0.006	0.006	0.005	0.005	0.004	0.004	0.005	0.005	0.004	0.008	0.000	0.001	0.004	0.000	0.000				
X	0.006	0.006	0.006	0.008	0.006	0.006	0.003	0.006	0.007	0.006	0.006	0.005	0.005	0.006	0.006	0.005	0.008	0.000	0.002	0.005	0.001	0.000	0.001			
Y	0.006	0.006	0.006	0.008	0.006	0.006	0.005	0.006	0.008	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.008	0.002	0.002	0.006	0.002	0.000	0.002	0.003		

A = PV592640.1, B = PV592169.1, C = PV592251.1, D = PV592252.1, E = PV592255.1, F = PV592256.1, G = PV592257.1, H = PV592085.1, I = PV450599.1, J = PV450602.1, K = PV450609.1, L = PV450608.1, M = PV450603.1, N = PV450607.1, O = PV450612.1, P = PV450613.1, Q = HQ857474.1, R = HQ177380.1, S = HQ177375.1, T = MW665999.1, U = HQ177378.1, V = LC582522.1, W = HQ177377.1, X = HQ177379.1, and Y = HQ177376.1

Contdinued...

Table 6 : Genetic distance matrix of different *S. litura* individuals based on partial nuclear (*TPI*) gene sequences.

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**	**U**	**V**	**W**	**X**	**Y**	**Z**	**AA**	**AB**
A																												
B	0.00																											
C	0.00	0.00																										
D	1.28	1.29	1.27																									
E	0.01	0.01	0.01	1.20																								
F	1.25	1.25	1.24	0.00	1.17																							
G	0.01	0.01	0.01	1.16	0.00	1.14																						
H	1.18	1.18	1.20	0.06	1.18	0.04	1.14																					
I	0.00	0.00	0.00	1.15	0.01	1.13	0.01	1.13																				
J	0.00	0.00	0.00	1.15	0.01	1.13	0.01	1.13	0.00																			
K	0.00	0.00	0.00	1.15	0.01	1.13	0.01	1.13	0.00	0.00																		
L	0.00	0.00	0.00	1.13	0.00	1.11	0.00	1.11	0.00	0.00	0.00																	
M	0.00	0.00	0.00	1.15	0.01	1.13	0.01	1.13	0.00	0.00	0.00	0.00																
N	0.00	0.00	0.00	1.15	0.01	1.13	0.01	1.13	0.00	0.00	0.00	0.00	0.00															
O	0.00	0.00	0.00	1.14	0.00	1.12	0.00	1.12	0.00	0.00	0.00	0.00	0.00	0.00														
P	0.00	0.00	0.00	1.15	0.01	1.13	0.01	1.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00													
Q	0.01	0.01	0.01	1.15	0.00	1.13	0.00	1.13	0.01	0.01	0.01	0.00	0.01	0.01	0.00	0.01												
R	0.01	0.01	0.01	1.15	0.00	1.13	0.00	1.13	0.01	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.00											
S	0.01	0.01	0.01	1.15	0.00	1.13	0.00	1.13	0.01	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.00	0.00										
T	0.01	0.01	0.01	1.15	0.00	1.13	0.00	1.13	0.01	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.00	0.00	0.00									
U	0.01	0.01	0.01	1.15	0.00	1.13	0.00	1.13	0.01	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.00								
V	0.01	0.01	0.01	1.15	0.00	1.13	0.00	1.13	0.01	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00							
W	0.00	0.00	0.00	1.19	0.01	1.20	0.01	1.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01						
X	0.19	0.18	0.18	1.43	0.01	1.32	0.01	1.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.00					
Y	0.18	0.18	0.17	1.42	0.01	1.32	0.01	1.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01				
Z	0.18	0.18	0.17	1.43	0.01	1.32	0.01	1.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.01			
AA	0.19	0.18	0.18	1.40	0.00	1.33	0.00	1.25	0.01	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.02	0.02		
AB	0.18	0.18	0.17	1.43	0.01	1.32	0.01	1.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.01	0.00	0.02	

A = PV798385.1, B = PV798386.1, C = PV798387.1, D = PV821530.1, E = PV821528.1, F = PV821531.1, G = PV821529.1, H = PV821532.1, I = ON586468.1, J = ON586464.1, K = ON586532.1, L = ON586437.1, M = ON586455.1, N = ON586456.1, O = ON586466.1, P = ON586462.1, Q = ON586431.1, R = ON586458.1, S = ON586536.1, T = ON586490.1, U = ON586461.1, V = ON586495.1, W = GQ411913.1, X = MN912076.1, Y = MN912081.1, Z = MN912075.1, AA = MN912084.1, and AB = MN912082.1

Table 7: List of accession numbers and process ID generated for the sequences deposited in NCBI and BOLD, respectively.

S. no.	Sample ID	Gene	Accession number(NCBI)	Process ID(BOLD)
1	SLNAVCIS1	*COXI*	PV584298.1	SDP1222001-25
2	SLBHCIS2	*COXI*	PV589523.1	SDP1222001-26
3	SLSTCIS3	*COXI*	PV590124.1	SDP1222001-27
4	SLANNDCIS4	*COXI*	PV589996.1	SDP1222001-28
5	SLJNDCIS5	*COXI*	PV590019.1	SDP1222001-29
6	SLHNSCIS6	*COXI*	PV590126.1	SDP1222001-30
7	SLJAMCIS7	*COXI*	PV590065.1	SDP1222001-31
8	SLACHCIS8	*COXI*	PV590127.1	SDP1222001-32
9	SLNAVCIIS1	*COXII*	PV592085.1	SDP1222001-33
10	SLBHCCIIS2	*COXII*	PV592640.1	SDP1222001-34
11	SLSTCIIS3	*COXII*	PV592169.1	SDP1222001-35
12	SLANNDCIIS4	*COXII*	PV592251.1	SDP1222001-36
13	SLJNDCIIS5	*COXII*	PV592252.1	SDP1222001-37
14	SLHNSCIIS6	*COXII*	PV592255.1	SDP1222001-38
15	SLJAMCIIS7	*COXII*	PV592256.1	SDP1222001-39
16	SLACHCIIS8	*COXII*	PV592257.1	SDP1222001-40

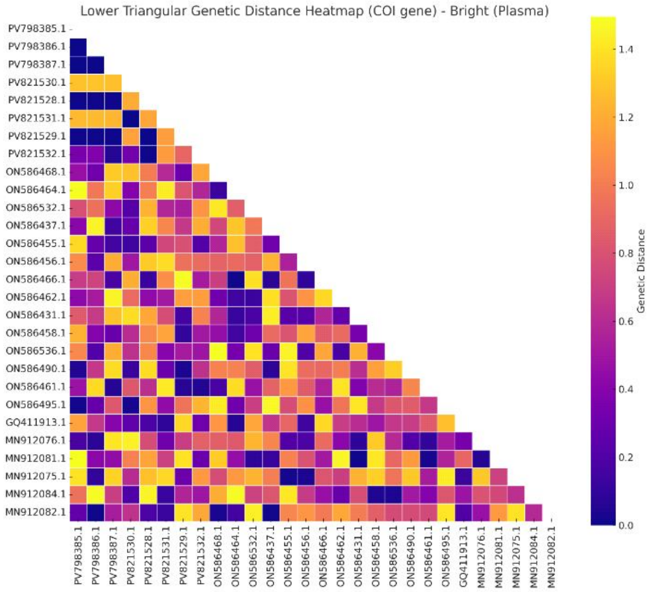

Fig. 6 : Heatmap of pairwise genetic distances among *S. litura* samples based on partial nuclear Triose phosphate isomerase (*TPI*) gene sequences.

distances were generated for COXI, COXII and TPI sequences to assess intra- and inter-population variation among *S. litura* populations across eight agro-climatic zones of Gujarat. COXI (Plasma colormap) and COXII (Viridis colormap) heatmaps showed low divergence among Gujarat populations, indicating high genetic homogeneity and recent common ancestry, with slightly higher divergence in a few reference sequences (e.g., KF022223.1, HQ857474.1). In contrast, the TPI heatmap revealed greater intraspecific variation, particularly in Anand, Hansot, and Achhalia populations, suggesting localized structuring. *S. frugiperda* references were clearly distinct, confirming interspecific resolution. These visualizations corroborate earlier barcoding studies (Rodrigues *et al.*, 2017; Srikaocha and Pompanom, 2025) and highlight strong genetic cohesion in *S. litura* with isolated divergence events (Figs. 5 and 6).

DNA Barcode Submission to BOLD

Specimen-level metadata, including taxonomy, sequences, trace files and images of *S. litura* from eight Gujarat locations, were submitted to the Barcode of Life Data System (BOLD). Unique process IDs were assigned, and COXI and COXII barcodes generated for reliable identification and comparative analysis (Table 7). This submission, consistent with earlier barcoding efforts in thrips and mites (Namitha, 2020; Kadu, 2019; Arunima, 2017), enhances global accessibility and supports future studies on genetic diversity, biogeography, and pest diagnostics.

Conclusion

This study integrated morphometric and molecular approaches to characterize *S. litura* populations from eight cotton-growing regions of Gujarat. Morphometric traits such as head capsule width, body length and wing dimensions showed significant variation, reflecting phenotypic plasticity under regional agro-climatic influences. Molecular analyses of COXI, COXII and TPI sequences confirmed species identity with low intra-specific divergence, while phylogenetic clustering with global references indicated genetic homogeneity and strong gene flow across regions. A total of 32 sequences

(16 COXI, 8 COXII, 8 TPI) were deposited in GenBank and 16 barcodes (8 COXI, 8 COXII) in BOLD (IDs SDP1222001-25 to SDP1222001-40), contributing to global reference libraries. The AT-rich composition and high sequence similarity validated the markers for species identification and population-level studies. Overall, these findings highlight limited regional isolation and support the application of DNA barcoding for accurate identification, pest monitoring, and integrated management of *S. litura* in cotton-based agroecosystems.

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