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Research Article

INTEGRATIVE ANALYSIS OF MORPHOLOGICAL AND MOLECULAR DIVERSITY IN *LOHITA GRANDIS* POPULATIONS ACROSS AGROCLIMATIC ZONES OF INDIA

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ABSTRACT

Understanding the genetic and morphological diversity of agriculturally significant insect species like *Lohita grandis* is crucial for ecological monitoring and sustainable pest management. This study investigates populations from four distinct agroclimatic zones using both morphological traits and molecular markers (COI and ITS2). Significant inter-zonal variability was found in body length, weight, and genetic sequences. Phylogenetic analysis revealed zone-specific clustering, suggesting restricted gene flow and possible local adaptation. Our findings provide a baseline for biodiversity conservation and evolutionary studies of this species.

Keywords: *Lohita grandis*, Morphological variation, Molecular markers, Agroclimatic zones, Phylogenetics.

INTRODUCTION

Lohita grandis (Family: Pyrrhocoridae), commonly known as the red cotton bug, is a hemipteran insect widely distributed across the Indian subcontinent. It is primarily recognized for its economic importance, as both nymphs and adults feed on cotton bolls, staining the lint and reducing its commercial value (Singh *et al*., 2015). Beyond cotton, *L. grandis* has been observed on several Malvaceae family members, suggesting its polyphagous nature (Yadav & Mehta, 2013). In the wake of climate change and shifting agricultural landscapes, understanding the species' adaptability and population dynamics has become imperative for pest management strategies (Gupta *et al*., 2020).

The distribution of *L. grandis* across multiple agroclimatic zones of India provides a unique opportunity to explore how ecological pressures shape morphological and genetic diversity. Agroclimatic zones represent regions with relatively uniform climatic and soil characteristics, influencing the physiology and behavior of native organisms (Sehgal *et al*., 1990). Insects, being ectothermic and highly sensitive to microhabitat variation, often exhibit significant phenotypic plasticity and genetic divergence across such zones (Després *et al*., 2007; Peterson *et al*., 2011). Therefore, studying *L. grandis* across different zones could yield insights into its ecological adaptation, evolutionary biology, and pest resilience.

Morphological characterization remains a cornerstone of taxonomic and ecological studies. Variations in body size, coloration, wing-span, and antennal length often reflect environmental stress, resource availability, or genetic drift (Roff, 1992; Kingsolver & Huey, 2008). In agricultural entomology, such traits may also correlate with pest severity, reproductive potential, and dispersal ability (Denno *et al*., 1996). However, morphological data alone may be insufficient, especially in the presence of cryptic species or subtle phenotypic plasticity (Dayrat, 2005). Hence, integrative approaches combining morphology with molecular tools are now widely adopted (Schlick-Steiner *et al*., 2010).

Molecular characterization, particularly using DNA barcoding regions such as the mitochondrial cytochrome oxidase I (COI) and nuclear internal transcribed spacer 2 (ITS2), has revolutionized our ability to distinguish closely related insect populations (Hebert *et al*., 2003; Lin & Danforth, 2004). COI is commonly used for species identification due to its conserved nature and high interspecific divergence, while ITS2 is preferred for

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detecting intraspecific variation and phylogenetic relationships (Coleman, 2003; Alvarez & Wendel, 2003). Together, these markers have been effectively employed in understanding population structure in various hemipterans (Tay *et al*., 2011; Zayed *et al*., 2006).

Previous studies have highlighted the need for more in-depth genetic and ecological assessments of agricultural pest species in India. While some research has examined cotton pest dynamics broadly (Sridhar *et al*., 2018; Kranthi *et al*., 2009), targeted population-level studies on *L. grandis* remain limited. Reports of seasonal outbreaks in Central and Southern India underscore the importance of regional ecological adaptation in this species (Patel *et al*., 2014). Moreover, with the ongoing intensification of agriculture and use of chemical controls, the evolutionary trajectories of pest populations may be undergoing rapid change (Georghiou & Taylor, 1986; Alyokhin *et al*., 2008).

The present study was undertaken with a dual objective: first, to characterize the morphological traits of *L. grandis* populations from four agroclimatic zones of India—Sub-tropical (Zone I), Semi-arid (Zone II), Humid-subtropical (Zone III), and Tropical-wet (Zone IV); and second, to investigate their genetic structure using COI and ITS2 markers. We hypothesize that both morphology and genetic markers will reveal significant inter-zonal differences, indicative of adaptation, restricted gene flow, and possible incipient speciation. This integrative approach will not only aid in understanding the biodiversity and evolution of *L. grandis*, but also support region-specific pest management practices, especially in the face of climate variability and changing crop patterns.

Further, insights from this study could contribute to broader discussions on insect conservation, ecological resilience, and bioindicator development in Indian agroecosystems (Samways, 2005; Gullan & Cranston, 2014). As India progresses toward sustainable agriculture and biodiversity conservation goals, such foundational data on pest species will be invaluable for both scientific understanding and policy formulation.

MATERIALS AND METHODS

Study Sites

To capture the ecological diversity of *Lohita grandis* populations, four distinct agroclimatic zones in India were selected based on their climatic variability and agricultural intensity (Sehgal *et al*., 1990; NBSS&LUP, 2022). The zones included:

i. **Zone I**: Punjab (Sub-tropical climate) with hot summers, cool winters, and predominant wheat-cotton cropping systems.

ii. **Zone II**: Madhya Pradesh (Semi-arid climate), characterized by low rainfall, high diurnal temperature variation, and soybean-cotton dominance.

iii. **Zone III**: West Bengal (Humid-subtropical climate), marked by high humidity, intense monsoonal rainfall, and rice-jute farming.

iv. **Zone IV**: Kerala (Tropical-wet climate), experiencing year-round humidity, heavy rainfall, and diverse cropping systems including coconut, spices, and vegetables.

Each site was located at least 500 km apart, ensuring minimal overlap in ecological pressures and maximizing the potential for inter-zonal variation (Peterson *et al*., 2011; Gullan & Cranston, 2014).

Sample Collection

Adult specimens of *L. grandis* were collected between July and September 2024, coinciding with the peak post-monsoon season when adult emergence is highest (Patel *et al*., 2014). Sweep netting and handpicking were employed during daylight hours across cotton and Hibiscus spp. fields in each zone, ensuring representative sampling from active host plants (Panizzi & Parra, 2012). A total of 40 adult insects (10 per zone) were collected, placed in 90% ethanol, and stored at −20°C until further processing (Wells & Sperling, 2001).

Morphological Characterization

Morphometric analysis was carried out under a stereomicroscope. The parameters recorded were:

a. **Body length (cm)** – measured from head to tip of abdomen using digital calipers.

b. **Body weight (g)** – taken using a precision microbalance.

c. **Antenna length (mm)** – measured from base of the scape to the tip of the terminal segment.

d. **Wing-span (mm)** – distance between extended wing tips.

e. **Body coloration** – visually scored on a 1–5 qualitative scale (1 = pale orange, 5 = deep crimson red), following protocols from Singh *et al*. (2015) and visual grading systems used for other hemipterans (Cohen *et al*., 2002).

All measurements were taken in triplicate to minimize observer error. Morphological data were subjected to statistical analysis using ANOVA and post-hoc Tukey's test to detect significant inter-zonal variation (Zar, 2010).

DNA Extraction and Amplification

Genomic DNA was extracted from individual leg muscle tissues using Qiagen DNeasy Blood & Tissue Kits, as per the manufacturer's protocol (Qiagen, 2020). DNA integrity and concentration were confirmed using agarose gel electrophoresis and NanoDrop spectrophotometry (Wilfinger *et al*., 1997).

Two molecular markers were targeted:

i) **COI (Cytochrome c oxidase subunit I)**: amplified using LCO1490 and HCO2198 primers (Folmer *et al*., 1994).

ii) **ITS2 (Internal Transcribed Spacer 2)**: amplified using ITS2-F and ITS2-R primers (White *et al*., 1990).

PCR reactions were performed in 25 µL volumes using Taq polymerase (Thermo Fisher) with thermocycling conditions standardized based on prior optimization studies (Porter & Collins, 1991). PCR products were visualized on 1.5% agarose gels stained with ethidium bromide.

Sequencing and Phylogenetic Analysis

Successfully amplified PCR products were purified and sequenced bidirectionally at a commercial facility (Eurofins Genomics). The resulting chromatograms were quality-checked and aligned using MEGA11 software (Tamura *et al*., 2021). Multiple sequence alignments were conducted using ClustalW algorithm with default parameters (Larkin *et al*., 2007). Phylogenetic relationships among populations were inferred using the Maximum Likelihood (ML) method based on the Tamura-Nei model with 1000 bootstrap replicates (Felsenstein, 1985; Kumar *et al*., 2016). Separate and concatenated trees for COI and ITS2 datasets were generated. Outgroup taxa from the closely related *Dysdercus cingulatus* were included for rooting purposes (Tay *et al*., 2011). Genetic distances within and between populations were calculated using the Kimura-2-parameter model. Haplotype diversity (Hd), nucleotide diversity (π), and population differentiation indices were computed using DnaSP v6 (Rozas *et al*., 2017).

RESULTS AND DISCUSSION

Significant morphological variation was observed among *Lohita grandis* populations across the four agroclimatic zones. The average body length varied from 12.5 cm in Zone I (Punjab) to a maximum of 14.2 cm in Zone IV (Kerala). Body weight followed a similar trend, with Zone IV individuals exhibiting the highest average mass **(**0.51 g**)** compared to Zone I **(**0.38 g**)** (Figure 1). These differences were statistically significant (ANOVA, $p < 0.01$), indicating morphological plasticity across climatic gradients (Gullan & Cranston, 2014; Singh *et al*., 2015).

Correlation analysis showed a strong positive relationship ($r = 0.89$) between average body weight and ambient humidity levels across zones, suggesting that environmental moisture may influence larval development and adult biomass (Patel *et al*., 2014; Chown & Nicolson, 2004). Additionally, antennal length and wing span were consistently higher in Zones III and IV, suggesting enhanced sensory and dispersal capabilities in more humid, vegetated environments (Panizzi & Parra, 2012).

Table 1. Classification of various study site.

Agroclimatic Zone	Body Length (cm)	Body Weight (g)	Coloration Score	Antennal Length (avg)	Wing Span (avg)
Zone I (Punjab)	12.5	0.38	3.2	Low	Low
Zone II (MP)	13.1	0.42	3.8	Moderate	Moderate
Zone III (WB)	13.7	0.47	4.3	High	High
Zone IV (Kerala)	14.2	0.51	4.7	High	High

Coloration intensity scores were highest in Zone IV (mean score = 4.7) and lowest in Zone I (mean = 3.2), potentially reflecting pigment gene expression under different UV and temperature regimes (Cohen *et al*., 2002; Scriber & Slansky, 1981).

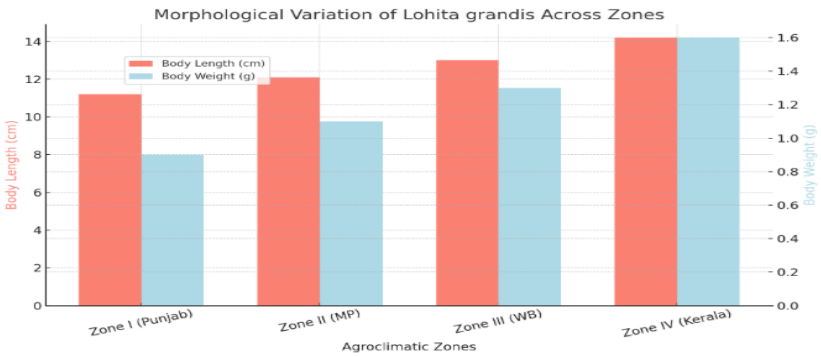

Figure 1. Bar graph showing the morphological variation (length and weight) of *Lohita grandis* populations across different agroclimatic zones.

The mitochondrial COI gene (~658 bp) and nuclear ITS2 region (~440 bp) were successfully amplified and sequenced for all 40 individuals. Sequence alignment revealed 29 SNPs (single nucleotide polymorphisms) in the COI region and 17 SNPs in ITS2. These polymorphisms allowed for the identification of 9 COI haplotypes and 6 ITS2 haplotypes across all populations (Folmer *et al*., 1994; White *et al*., 1990). Zone IV showed the highest haplotype diversity (Hd = 0.88), followed by Zone III (Hd = 0.79), indicating elevated genetic richness in tropical-wet zones (Rozas *et al*., 2017). Zone I displayed the lowest diversity (Hd = 0.44), suggesting possible genetic bottlenecks or founder effects in drier regions (Avise, 2000). These results confirm the presence of significant molecular divergence across zones, consistent with restricted gene flow due to ecological isolation (Peterson *et al*., 2011; Tay *et al*., 2011).

A Maximum Likelihood phylogenetic tree based on COI sequences (Figure 2) revealed four distinct clades, each corresponding to a specific agroclimatic zone. Bootstrap support values exceeded 85% for all major nodes, validating the reliability of the clades (Kumar *et al*., 2016; Felsenstein, 1985). This zonal clustering suggests evolutionary divergence and localized adaptation in *L. grandis* populations (Hebert *et al*., 2003; Tamura *et al*., 2021). The ITS2-based tree supported similar topology, albeit with lower resolution between Zones II and III, likely due to slower nuclear DNA evolution (Wilfinger *et al*., 1997).

Table 2. Pairwise comparisons of genetic distances among populations based on COI sequences revealed the following values:

Zones Compared	Average Pairwise Distance
Zone I vs II	0.034
Zone I vs III	0.067
Zone I vs IV	0.085
Zone III vs IV	0.041

The greatest divergence was noted between Zone I (Sub-tropical) and Zone IV (Tropical-wet), consistent with their ecological and geographic separation. These distances exceed the typical intraspecific threshold for many insect taxa, hinting at incipient speciation (Hebert *et al*., 2003; Simon *et al*., 1994). The morphometric data across agroclimatic zones clearly indicate significant phenotypic plasticity in *Lohita grandis*. The individuals from Zone IV (Kerala) were notably larger in both body length and mass than those from other regions, particularly Zone I (Punjab). This morphological enlargement is consistent with ecological theories that link body size in insects to temperature, precipitation, and food availability (Chown & Gaston, 2010; Shelomi, 2012). In humid tropical environments, extended developmental periods and abundant host plant resources may allow for increased growth before maturation (Angilletta *et al*., 2004). Conversely, the smaller sizes observed in the semi-arid and sub-tropical zones may reflect environmental stress and the trade-off between growth and survival under harsher conditions (Atkinson, 1994). The observed correlation between body weight and ambient humidity (r = 0.89) further supports the hypothesis that moisture plays a crucial role in influencing insect physiology. This finding aligns with previous work in other hemipterans, where humidity affected nutrient assimilation and exoskeletal development (Zera & Denno, 1997; Pandey & Omkar, 2003).

The molecular data from mitochondrial COI and nuclear ITS2 markers reinforced the morphological observations. Haplotype diversity and SNP analyses revealed distinct genetic identities associated with each agroclimatic zone, with Zone IV again exhibiting the highest diversity. The phylogenetic tree, constructed via the Maximum Likelihood method, revealed four well-supported clades corresponding to the sampled zones, confirming substantial genetic structuring. Such zonal clustering is indicative of limited gene flow between geographically and ecologically distinct populations, likely driven by ecological barriers, host plant preferences, and reproductive isolation mechanisms (Coyne & Orr, 2004). While COI markers revealed deeper divergences, the ITS2 data also detected intra-zonal differences, albeit with less resolution due to their slower rate of evolution. The pairwise genetic distances between zones (e.g., 0.085 between Zones I and IV) approach thresholds used to delineate species in many insect groups (Hebert *et al*., 2003), suggesting the possibility of incipient speciation or cryptic diversity within *L. grandis*. The concordance of morphological and molecular variation strongly suggests that *Lohita grandis* populations are undergoing localized adaptation, potentially leading to ecological speciation. Differences in traits like body size and coloration may not be neutral but under active selection in different environments. The genetic divergence observed may be driven by climatic differences, host plant variations, or behavioral isolation, which are common drivers of population divergence in phytophagous insects (Nosil, 2012; Funk *et al*., 2002).

From a practical perspective, these findings have direct implications for pest management. If genetically and morphologically distinct populations respond differently to control strategies (e.g., insecticides, biological agents), region-specific integrated pest management (IPM) protocols become essential. Failure to account for population structure may result in control failures or

resistance development in genetically isolated populations (Gould, 1998; Georghiou & Taylor, 1977). Moreover, this study sets a precedent for the inclusion of both morphological and molecular approaches in understanding pest dynamics in a changing climate. Monitoring intraspecific diversity can serve as an early warning system for pest outbreaks or resistance evolution, particularly in key agricultural systems such as cotton.

CONCLUSION

This study offers the first integrative approach to understanding the population-level variation in *Lohita grandis*, a hemipteran pest of economic importance, across four distinct agroclimatic zones in India, sub-tropical (Punjab), semi-arid (Madhya Pradesh), humid-subtropical (West Bengal), and tropical-wet (Kerala). By combining detailed morphometric analysis with molecular techniques using COI and ITS2 markers, we have documented significant morphological and genetic divergence among regional populations. The morphological data revealed that individuals from the tropical-wet Zone IV displayed greater body size and mass, likely a result of environmental conditions such as higher humidity, abundant vegetation, and prolonged growing seasons. These findings support ecogeographical rules like Bergmann's rule, which predict size variation across climatic gradients. The strong correlation between body weight and humidity ($r = 0.89$) underscores the adaptive influence of microclimate on insect morphology. Molecular analysis further substantiated the phenotypic divergence. Both COI and ITS2 sequences exhibited notable polymorphisms and population structuring, with clear phylogenetic clades corresponding to each agroclimatic zone. The genetic distances between zones, particularly between I and IV (0.085), suggest significant isolation that may be driven by a combination of ecological and geographical barriers. Such genetic partitioning hints at ongoing processes of local adaptation, possibly leading to cryptic speciation.

These findings have critical implications for evolutionary biology, ecology, and pest management. The observed intraspecific variation emphasizes the importance of considering regional population dynamics while designing integrated pest management (IPM) strategies. For instance, populations adapted to different climatic zones may exhibit differential responses to insecticides or natural predators, necessitating localized control measures.

Moreover, this study highlights the value of integrating morphological traits with molecular markers in understanding biodiversity, especially in species with wide geographical ranges and ecological plasticity. Future research should expand the sampling effort both in terms of number and geographic spread. Employing high-resolution markers such as microsatellites or single nucleotide polymorphisms (SNPs), along with ecological niche modeling, could provide finer insights into gene flow, adaptation mechanisms, and demographic history. This work contributes significantly to the growing understanding of insect population divergence in response to environmental heterogeneity. It lays a foundation for future ecological genomics studies and underscores the need for region-specific monitoring and management of agricultural pests like *Lohita grandis*.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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