Molecular diversity of *Nilaparvata lugens* (Stål.) (Hemiptera: Delphacidae) from India based on internal transcribed spacer 1 gene

Govindharaj Guru-Pirasanna-Pandi[1,*], Aashish Kumar Anant[1], Jaipal Singh Choudhary[2], Soumya Bharati Babu[1], G. Basana-Gowda[1], M. Annamalai[1], Naveenkumar Patil[1], Totan Adak[1], P. Panneerselvam[1] and Prakash Chandra Rath[1]

[1]Division of Crop Protection, ICAR-National Rice Research Institute, Cuttack 753 006, India
[2]ICAR-RCER Farming System Research Centre for Hill and Plateau Region, Ranchi 834 010, India

Brown planthopper, *Nilaparvata lugens*, is the major pest of rice in India and causes significant yield loss. It causes damage by sucking the plant sap leading to a characteristic symptom called 'hopper burn'. The present study was undertaken to assess the genetic variability of *N. lugens* populations from different rice ecologies in India, to comprehend and assist in planning proper management strategies. We evaluated the molecular diversity in 17 *N. lugens* populations based on internal transcribed spacer 1 (*ITSI*) gene sequences. In all, 53 unique haplotypes were identified and their numbers varied from 1 to 10 in the sampled populations. Genetic diversity indices like nucleotide diversity, haplotype number, haplotype diversity and average number of nucleotide differences revealed low to high levels of genetic diversity among the populations. A highly significant negative relation of Fu's *F* and Tajima's *D* tests with insignificant sum of square deviation (SSD) values indicated possible recent expansion of *N. lugens* in different Indian regions with a population expansion time of 3.9 million years. A non-significant correlation in isolation pattern by distance indicated that geographic barriers present in India are inadequate to bring genetic differentiation among *N. lugens* from different migratory populations. In the present study, the *ITSI* gene sequence was used to analyse genetic structure among *N. lugens* in India.

Keywords: Genetic structure, haplotypes, molecular diversity, *Nilaparvata lugens*, rice.

RICE is the most prominent staple food for millions of people and plays a vital role in India's food security. The country occupies second position both in area and production of rice in the world. Brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) is a serious pest of rice being widely distributed across all the rice-growing ecologies of India and inflicting severe yield loss[1,2]. *N. lugens* is a monophagous phloem-feeder of paddy, causing wilting and hopper burn. The infestation and hopper burn occurrence lead to severe yield loss of up to 70–100% (refs 2, 3). *N. lugens* also acts as vector of grassy stunt and ragged stunt viral diseases of rice plants[4]. It was first reported in India during 1950 in the Madras Providence, and subsequently spread to other parts of the country. At present, *N. lugens* infestation in paddy is reported from almost all major rice-cultivating states of India. BPH infestation and survival prevail throughout the year in countries like Bangladesh, Philippines, Vietnam and Southern India, whereas in middle China, Japan, Korea and northern parts of India, it does not survive in winter months[5–7]. Hence, migration of *N. lugens* from country to country and from other parts of India to its northern part during the next rice-growing season is one of the possible causes of regular BPH infestation.

The present strategies for the management of *N. lugens* mainly depend on chemicals and cultivation of *N. lugens*-resistant rice cultivars[8]. Chemically intensive pest control measures have several drawbacks such as toxicity to non-target organisms like natural enemies, increased cost of production and deterioration in the general health of the agro-ecosystem as well as human beings under long-term exposure[9,10]. Several *N. lugens*-resistant rice cultivars have been released since the 1960s with more than 36 resistant genes and quantitative trait loci[11]. However, developing virulent biotypes against resistant sources is also a major concern for *N. lugens* management. Therefore, one of the critical aspects for successful development of management strategies for this pest is the knowledge of population structure and genetic variability within and among populations present in distinct geographical locations with natural isolation[12–14].

Mitochondrial DNA (mtDNA)-based molecular markers are often used to decipher the intra- and inter-specific relationships, population structure, non-recombinant pattern, gene flow, copy number, pattern of genetic variation, evolution rate, population size and simple maternal inheritance[15–18].

*For correspondence. (e-mail: guru.g@icar.gov.in)

Various mitochondrial and nuclear genes such as cytochrome *c* oxidase subunit I (*COI*), internal transcribed spacers (*ITSI* and *ITSII*) and 12S-16S-18S ribosomal RNA genes have been widely used as genetic markers to differentiate intra- and inter-species population[17–20], and evolution[21]. Knowledge of population structure, gene flow and genetic variation would be helpful to design and execute management strategies programs for migratory insect pests in a large-scale project like area-wide integrated pest management[22].

Despite the economic and ecological impact of *N. lugens* on rice, information on population structure and molecular diversity is lacking in India. Previous reports suggest that adaptation of planthopper populations among inter- and intra-regions differs in response to adaptations in local environmental conditions[12–14]. With this background, the present study was undertaken to understand the population structure and molecular diversity among populations of *N. lugens* in India based on *ITSI* sequences.

Material and methods

Sample collection of N. lugens

Adults of *N. lugens* were collected from 17 locations in India representing different climatic zones, during 2017–19. The sampling sites covered major endemic hot spots of *N. lugens* and rice-cultivating areas of India[1,2,8] (Figure 1). Adult insects were collected from the infested field with the help of an aspirator. Morphological identification was done according to standard methodology[23]. After identification, the adults were stored in 95% ethanol at –20°C till further use.

DNA extraction

DNA from the identified adults of *N. lugens* was extracted using cetyltrimethyl ammonium bromide (CTAB) method[24]. In brief, a sterile micro-pestle was used to grind the preserved specimen of *N. lugens* in 500 µl of CTAB solution (2% w/v) and incubated for 1 h at 65°C in a water bath. Then, 500 µl of 24 : 1 : 1 v/v ratio of phenyl : chloroform : isoamyl alcohol (PCI) was added and centrifuged at 12,000 rpm for 10 min, and the aqueous phase was transferred to fresh microtubes. Pre-chilled isopropanol (450 µl) was added to the aqueous phase to precipitate the DNA and kept at –20°C for 15–30 min, and then centrifuged at 12,000 rpm for 14 min at 4°C. The resultant DNA pellets were washed thrice with ice-cold ethanol (70%) at 12,000 rpm for 10 min at 4°C, dried and resuspended in 100 µl of *Tris*-EDTA (ethylene diamine tetra acetic acid) buffer. The quality and quantity of the extracted genomic DNA were determined using 0.8% agarose gel electrophoresis and a NanoDrop-1000 spectrophotometer (Thermo Fisher Scientific, USA) respectively.

PCR amplification of ITSI gene

Forward (F) and reverse (R) primers with DNA sequence of 5′-GCTAGTCATTACAATGTATGGTGG-3′ and 5′-GCTTGCTGTTAAGTCACAAG-3′ respectively, were used to amplify the partial length (487 bp) of targeted internal transcribed spacers 1 (*ITSI*) gene[25]. The thermal conditions used for PCR amplification of the *ITSI* gene were as follows: initial denaturation for 3 min at 95°C, followed by 35 cycles of denaturation for 30 sec at 94°C, annealing for 30 sec at 58°C and extension for 1 min at 72°C, with a final extension of 10 min at 72°C. Later the PCR product was visualized in 2% (w/v) agarose gel using TAE buffer (40 mM *Tris*-acetate, 1 mM EDTA). The amplicon was freeze-dried and custom-sequenced using the same primers (Agrigenome Labs Limited, India). After manual correction and assembly, the sequences were deposited in GenBank under accession numbers MN887114–MN887162; MN888697–MN888736 and MN956389–MN956498.

Genetic data analysis

Forward and reverse sequencing results were combined and the contigs were prepared using the CAP3 sequence assembly program[26]. ClustalW in MEGA ver. 10.2.4 was used to align the sequences[27]. DnaSP ver. 6.0 was used for unique haplotype identification[28] and ARLEQUIN ver. 3.5 was used for reconfirmation[29]. Further, descriptive statistics details like nucleotide diversity (π), average number of nucleotide difference (k), number of haplotypes (H) and haplotype diversity (Hd) were obtained using DnaSP

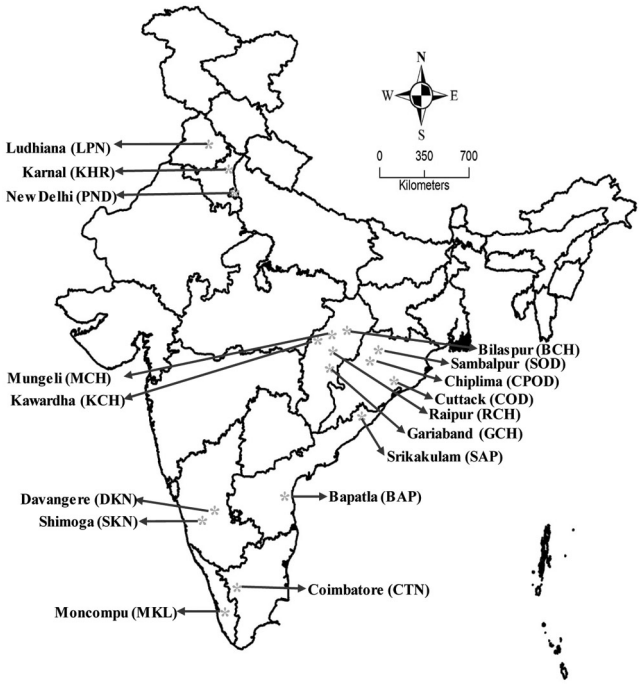

Figure 1. *Nilaparvata lugens* sample collection sites in India.

ver. 6.0. Non-parametric permutation method (5000 permutations) was used to determine statistical significance level of the derived indices with ARLEQUIN ver. 3.5.

Geographical relationship was described among the haplotypes by drawing the median-joining haplotype network using NETWORK ver.10.2 (ref. 30). According to the sampling location, the major clusters of *N. lugens* haplotypes were colour-paletted to depict the migration pattern of BPH across the Indian subcontinent. ARLEQUIN ver. 3.5 was used to partition total variance in its components among and within populations with analysis of molecular variance (AMOVA) hierarchical method.

Tajimas' *D* and Fu's *F* were determined for neutrality[31,32]. DnaSP ver. 6.0 was used to determine the demographic history with the mismatch distribution method. This analysis (mismatch distribution) was also used to test the rapid population expansion model[33]. All parameters were tested against the expected values under the hypothesis of a recent population expansion based on 1000 bootstrap replicates. Kimura's two-parameter method implemented in MEGA software was used to study the genetic distance among population pairs of *N. lugens*. Using the Mantel test, the relationship between geographic distance matrices (km in log scale) and genetic distance (F_{ST}) was estimated to test the isolation-by-distance (IBD) model[34]. Alleles In Space (AIS) software was used for Mantel test analysis with 10,000 randomizations[35]. The geographical distance between each population pair was estimated by Google Maps Distance Calculator (http://www.daftlogic.com/projects-google-maps-distance-calculator.htm).

Results

Nucleotide information

A total of 199 *ITSI* gene sequences from *N. lugens* adults from 17 locations were used for the analysis (Figure 1). The gene sequences of the *ITSI* region from different locations in India alone were used for analysis, as there are no such reports from other countries. The final lengths of the submitted sequences in the NCBI GenBank of *ITSI* genes were of the expected size (484 bp). Sequences were aligned upon display of no insertions and deletions (indels). Nucleotide sequence alignment revealed 74 overall variable sites. The total variable sites included the 28 two-variant and 2 three-variant of total 58 parsimony informative sites. A total of 16 singleton sites were observed; among them, 13 were two-variant and 3 were three-variant.

Intra-population diversity

Genetic diversity analysis of the sequences revealed 53 unique haplotypes from all the populations of *N. lugens* (Table 1). Only 7 out of 53 haplotypes were shared by at least two *N. lugens* populations and the remaining 46 were exclusive to single-population private haplotypes. The most shared/common haplotypes were H7 detected in 62 *N. lugens* individuals, and H11 in 52 individuals, each representing 10 populations. Seven of these populations carried one of the haplotypes, whereas the rest three had both the haplotypes.

Table 2 presents the descriptive analysis results from 17 populations. Genetic diversity indices like haplotype diversity (*Hd*), average number of nucleotide diversity (*K*) and nucleotide diversity (π) varied from 0.182 to 0.955, 0.546 to 14.758 and 0.001 to 0.031 respectively. The results indicated low (COD, RCH and KCH) to high levels (SOD, CPOD, PND and BAP) of genetic diversity among the *N. lugens* populations in India. Among the North Indian states, New Delhi (PND) showed consistently high genetic diversity, whereas some of the southern (Tamil Nadu (Coimbatore, CTN), Andhra Pradesh (Bapatla, BAP) and Kerala (Moncompu, MKL)) and eastern states (Odisha (Sambalpur, SOD), Odisha (Cuttack, COD), Chhattisgarh (Gariyaband, GCH)) also showed high genetic diversity.

Genetic segregation level

AMOVA revealed most of the genetic variations to be intra-population (74.86% at $P < 0.05$) as against inter-population (25.14% at $P < 0.05$; Table 3). All the populations significantly differed in terms of overall fixation index ($F_{ST} = 0.2514$ at $P < 0.05$ level; Table 3). Further, F_{ST} of *N. lugens* was highest for Ludhiana, Punjab (0.315), followed by Cuttack (0.302) and Raipur, Chhattisgarh (0.301), whereas Coimbatore (0.051) exhibited the lowest value. F_{ST} values for the remaining populations ranged from 0.218 to 0.288 (Table 4). After Bonferroni correction, pairwise F_{ST} test among 17 populations exhibited significant differences in 129 out of 153 population pair comparisons.

Low F_{ST} values denote the significance of haplotype divergence in the population structure pattern. These results support the findings of genetic diversity among the Indian populations. For instance, the New Delhi populations had the lowest pairwise F_{ST} value with the Karnal, Haryana and Ludhiana (Punjab) populations, signifying the occurrence of natural breeding between these populations. The eastern and southern (Chhattisgarh, Karnataka and Andhra Pradesh) populations expressed higher pairwise F_{ST} values, and displayed less chance of breeding among populations. However, low pair-wise F_{ST} values were also observed between the North Indian (New Delhi) population and those from eastern (Chhattisgarh (MCH and RCH), and Odisha (SOD) and Southern India (Andhra Pradesh (SAP)). Thus, they represent a large panmictic population from India. Moreover, the Punjab population with no variance was significantly different from all other populations. Non-significant pairwise differences specified the existence of high gene flow among the populations.

Table 1. Genetic diversity and demographic history parameters of *Nilaparvata lugens* in India

H	*Hd*	*K*	π	θ_0	θ_1	τ	Tajima's *D*	Fu's *F*	SSD
53	0.831	3.409	0.007	0.78357	11769.475	3.982	–2.26**	–46.290*	0.050

H, Number of haplotypes; *Hd*, Haplotypes diversity; *K*, Average number of nucleotide difference; π, Nucleotide diversity; θ_0, Effective population size before expansion; θ_1, Effective population size after expansion; τ, Population expansion time (million years); SSD, Sum of square deviation between observed and expected mismatch distribution under a sudden expansion model; *$P < 0.02$; **$P < 0.01$.

Table 2. Genetic diversity indices among *N. lugens* populations from India

Location	Sample no.	*ITS1*			
		H	*Hd*	*K* ± SD	π ± SD
Sambalpur, Odisha (SOD)	10	6	0.844	2.733 ± 1.581	0.006 ± 0.004
Cuttack, Odisha (CPOD)	11	7	0.692	3.133 ± 1.713	0.007 ± 0.004
Chiplima, Odisha (COD)	16	2	0.182	0.546 ± 0.485	0.001 ± 0.001
Raipur, Chhattisgarh (RCH)	10	2	0.200	0.600 ± 0.519	0.001 ± 0.001
Bilaspur, Chhattisgarh (BCH)	10	4	0.644	1.089 ± 0.778	0.002 ± 0.002
Gariaband, Chhattisgarh (GCH)	12	5	0.782	1.346 ± 0.900	0.003 ± 0.002
Kawardha, Chhattisgarh (KCH)	12	2	0.282	0.564 ± 0.490	0.001 ± 0.001
Mungeli, Chhattisgarh (MCH)	10	4	0.733	2.400 ± 1.421	0.005 ± 0.003
New Delhi, Delhi (PND)	14	9	0.835	3.879 ± 2.071	0.008 ± 0.005
Karnal, Haryana (KHR)	10	5	0.756	1.911 ± 1.186	0.004 ± 0.002
Ludhiana, Punjab (LPN)	11	1	n/c	n/c	n/c
Coimbatore, Tamil Nadu (CTN)	12	10	0.955	14.758 ± 7.111	0.031 ± 0.017
Moncompu, Kerala (MKL)	11	5	0.619	2.364 ± 1.393	0.005 ± 0.003
Davangere, Karnataka (DKN)	16	4	0.525	1.125 ± 0.772	0.002 ± 0.002
Shimogga, Karnataka (SKN)	12	3	0.682	1.364 ± 0.904	0.003 ± 0.002
Bapatla, Andhra Pradesh (BAP)	12	7	0.864	3.469 ± 1.903	0.007 ± 0.004
Srikakulam, Andhra Pradesh (SAP)	10	4	0.733	1.489 ± 0.979	0.003 ± 0.002

H, Number of haplotypes; *Hd*, Haplotypes diversity; *K*, Average number of nucleotide difference; π, Nucleotide diversity; SD, Standard deviation; n/c, Not calculated.

Table 3. Summary of AMOVA of different populations of *N. lugens*

Source of variation	*df*	Sum of squares	Variance components	Variation (%)	Fixation index
Among population	16	101.969	0.43460 V_a	25.14	F_{ST} 0.2514*
Within population	182	235.559	1.29428 V_b	74.86	
Total	198	337.528	1.72888		

*$P < 0.05$.

The Mantel test was performed to predict the IBD. Despite distant geographic locations, the present study revealed a non-significant positive correlation between pairwise genetic and geographic distances of the *N. lugens* populations studied ($r = 0.120$ at $P < 0.05$). The non-significant correlation results indicate that geographic barriers present in India are inadequate to bring in genetic differentiation among *N. lugens* from different populations.

Median-joining network among haplotypes

A median-joining (MJ) network of haplotypes was generated to understand the inherited relationship among populations (Figure 2). The MJ network showed that H7 and H11 were the two most common and original/ancestral haplotypes in *N. lugens* populations. Other major haplotypes identified were H1, H14 and H35, which were connected through several mutational steps to the parent haplotypes. Both H7 and H11 haplotypes were shared by 10 *N. lugens* populations. The H7 haplotypes mostly represented the north Indian (New Delhi, Karnal, Ludhiana) and northern Chhattisgarh (Bilaspur, Kawardha, Mungeli) populations, whereas H11 represented populations from Odisha, southern Chhattisgarh (Raipur and Gariaband), Andhra Pradesh, Karnataka and Tamil Nadu. Hence, the present study indicates that Chhattisgarh may be the possible interconnection point among the northern, eastern and southern Indian BPH populations (Figure 2).

Neutrality test and demographic history

To decide any deviation from neutrality, Fu's *F* and Tajima's *D* tests were performed. Tajima's *D* and Fu's *F* values

Table 4. Population-specific F_{ST} and pairwise differences among different population of *N. lugens*

Population	BAP	BCH	COD	CPOD	CTN	DKN	GCH	KCH	KHR	LPN	MCH	MKL	PND	RCH	SAP	SKN	SOD
BAP (0.229)	–																
BCH (0.288)	0.213*	–															
COD (0.302)	0.287*	0.728*	–														
CPOD (0.235)	0.136*	0.345*	0.114*	–													
CTN (0.051)	0.075	0.162*	0.164*	0.129*	–												
DKN (0.287)	0.321*	0.672*	0.115*	0.170*	0.216*	–											
GCH (0.282)	0.344*	0.724*	0.527*	0.356*	0.228*	0.494*	–										
KCH (0.301)	0.384*	0.802*	0.649*	0.398*	0.244*	0.567*	0.226*	–									
KHR (0.269)	0.165*	0.546*	0.274*	0.091	0.124*	0.292*	0.269*	0.299*	–								
LPN (0.315)	0.391*	0.860*	0.786*	0.402*	0.237*	0.622*	0.260*	0.065	0.334*	–							
MCH (0.257)	0.074	0.399*	0.362*	0.089	0.098*	0.372*	0.340*	0.385*	0.055	0.416*	–						
MKL (0.257)	0.198*	0.539*	0.304*	0.203*	0.166*	0.325*	0.166*	0.138*	0.028	0.133	0.171*	–					
PND (0.218)	0.262*	0.543*	0.291*	0.293*	0.196*	0.345*	0.146*	0.073	0.168*	0.096*	0.230*	0.111*	–				
RCH (0.301)	0.249*	0.698*	0.001	0.073	0.143*	0.110	0.514*	0.639*	0.205*	0.779*	0.324*	0.260*	0.299*	–			
SAP (0.279)	0.100	0.463*	0.139*	0.036	0.108*	0.186*	0.461*	0.554*	0.175*	0.622*	0.176*	0.243*	0.301*	0.083	–		
SKN (0.282)	0.179*	0.592*	0.331*	0.177*	0.165*	0.333*	0.261*	0.267*	0.011	0.304*	0.171*	0.026	0.159*	0.279*	0.216*	–	
SOD (0.249)	0.072	0.403*	0.178*	0.039	0.099*	0.221*	0.249*	0.281*	–0.065	0.297*	0.005	0.034	0.171*	0.104*	0.058	0.015	–

Distance values below the diagonal are based on the *ITS1* sequence obtained by a bootstrap procedure (1000 replicates). Values given in parenthesis are population specific F_{ST} based on the respective sequence. *Values (distances) shown are significantly different.

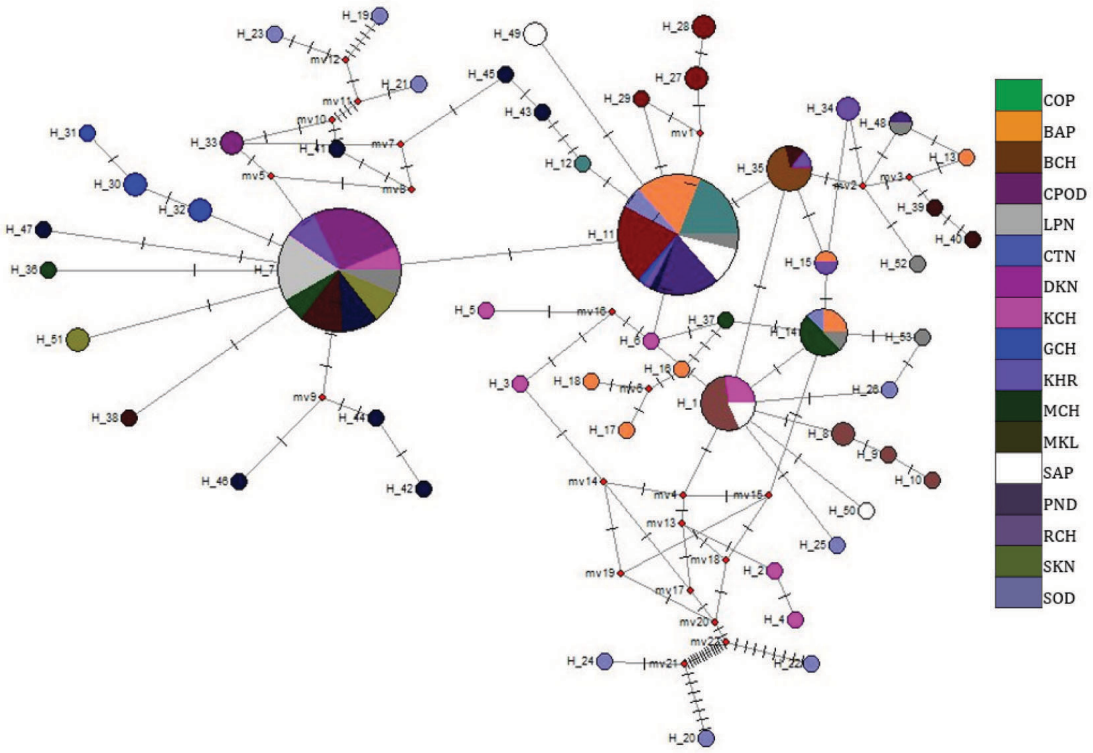

Figure 2. Median-joining network of haplotypes of *N. lugens*. Each circle represents a haplotype and circle diameter is relative to haplotype frequency. Colours represent the geographic origin of specimens and size indicates the proportion of individuals sampled in different populations within the study area. Smaller red squares represent median vectors.

were found to have a highly significant negative relationship with an insignificant sum of square deviation (SSD) values (Table 2), clearly demonstrating that *N. lugens* populations from India do not conform to the theory of neutral evolution, and *N. lugens* would have experienced recent rapid demographic spread and expansion. The mismatch distribution plots were left-skewed unimodel mismatch distributions, suggesting that *N. lugens* is undergoing population expansion. The *ITSI* gene sequence analysis revealed a population expansion time (sec) of 3.982 million years.

Discussion

Knowledge of genetic diversity among insect populations from different regions provides valuable insights into the evolution and understanding of the migration dynamics of an organism. In the present study, the *ITSI* region sequence analysis revealed low to high genetic diversity among Indian populations of *N. lugens*, as the diversity indices like *Hd*, *k* and π may represent a newly colonized site. Specifically, sample size did not influence the above-mentioned three genetic diversity parameters, thereby suggesting that these are valid diversity indices for *N. lugens* populations. It is evident from the present study that the Indian populations of *N. lugens* are not stable and no evidence has been found for neutral evolution in the populations. However, most of the Indian populations could have undergone expansion with stable structure substantiated with low nucleotide ($\pi < 0.001$) and haplotype ($Hd < 0.5$) diversity values[36]. Besides, few Indian populations (Srikakulam, Karnal, Shimoga, Bilaspur and Gariaband) exhibited high *Hd* value (>0.5) and low π value (<0.5%), suggesting that a small proportion of them might have undergone recent population expansion and divergence between geographically subdivided populations. The present study reveals that most of the Indian populations show a high level of genetic diversity which signifies that this pest had established well throughout the country. It is assumed that ancestral populations show significantly more genetic diversity than derivative populations because of the founder effect[37,38]. The high nucleotide diversity in scattered populations of the present study is not conclusive to propose that India is a probable centre of origin for *N. lugens*. The possibility of *N. lugens* having an Indian origin cannot be denied, but required to be confirmed with more number of genes with multi-locations analysis to support the claim. In addition, populations in India's southern and eastern states had higher *K* values for the *ITSI* gene sequences. Therefore, it could be hypothesized that *N. lugens* has adapted well to the southern and eastern Indian conditions due to continuous availability of host plants in a conducive environment. The high variability among *N. lugens* populations was also verified through results of

varietal screening trial of All India Coordinated Rice Improvement Project (AICRIP) to understand the response of *N. lugens* populations to different resistant rice genotypes in the country. The results indicate that Hyderabad (Telengana), Aduthurai (Tamil Nadu), Coimbatore (Tamil Nadu), Gangavati (Karnataka), Ludhiana (Punjab), New Delhi (Delhi), Pantnagar (Uttarakhand), Cuttack (Odisha), Rajendra Nagar (Bihar) and Warangal (Andhra Pradesh) recorded different reactions against *N. lugens* populations for the known gene differentials, viz. ASD 7 (*bph2*), Babawee (*bph4*), Chinsaba (*bph8*), IR 65482-7-216-1-2-B (*Bph18*), Patambi 33 (*bph2* + *Bph3*), Rathu Heenati (*Bph3* + *Bph17*), RP-2068-18-3-5 (*Bph33*), Swarnalatha (*Bph6*), T12 (*Bph7*) and TN1 (susceptible check). Swarnalatha expressed resistant reaction against Hyderabad, Aduthurai and Gangavati populations, but not for other locations. Likewise, Babawee showed resistance against only Gangavati and Warangal populations. Similarly, T12 displayed resistance against only Ludhiana, New Delhi and Warangal populations. Rathu Heenati showed resistance against only Gangavati, Coimbatore and Rajendra Nagar populations. These data suggest the presence of heterogeneous *N. lugens* populations in the country[39,40]. Other than this, migration of *N. lugens* might be happening every year from the southern to the northern Indian states, which needs to be comprehensively studied with inclusion of more locations, especially from North India, where the survival of this pest is difficult in winter due to non-availability of host crop. Moreover, long-distance migration has been previously reported in *N. lugens* from other parts of the world, which assists in fresh colonization and rescue of a species from the status of genetic diversity loss[13]. A similar mechanism was reported earlier in other insect species[41,42].

Previous studies based on sequence analysis of a part of the mitochondrial gene cytochrome oxidase-I (*COI*) suggested that the South Asian *N. lugens* populations exhibited a low level of genetic diversity, whereas the Chinese, Korean and Philippines populations shared a common haplotype[13,24]. In general, due to high selection pressure, migratory species possess low genetic diversity[41] and genetic drift due to small population size in the founder colonies[43]. In the case of *N. lugens*, previous studies have recorded that the Indo-China Peninsula (Bangladesh, Malaysia, Thailand and Vietnam) populations shared identical haplotypes[13,24]. Similarly, the present study reveals less variation within the Indian populations for the *ITSI* gene sequence. Thus, results support that BPH in India could be regarded as a large panmictic population. Our results are supported by an earlier study which indicated no significant polymorphism among Japanese populations[44].

The analysis of molecular variance in the present study showed a high level of genetic differentiation among the populations, even though the observed variance was less compared to within the populations. Similar results have been reported earlier from *N. lugens*[13,24] and *Bactrocera dorsalis*[17], but contrasting results were observed in white-backed planthopper, *Sogatella furcifera*[24]. The high level of genetic differentiation within the populations, random interconnections of geographically isolated populations described through the MJ network and non-significant IBD correlations between genetic and geographic distances indicate weak population genetic structure of *N. lugens* among the Indian populations[45]. Strong mobility in large swarms may surmount the physical and environmental obstacles leading to high rates of gene flow and weakening the genetic structure between populations[24].

In the present study, the network reconstructed exhibited a star-like shape among haplotypes denoting population expansion[46]. We recovered many more haplotypes compared to previous studies with other markers, but geographical clustering of the haplotypes is still not evident. For any given species, the major reasons for gene flow and genetic diversity are ecological suitability of the region, diverse climate adaptability, biotic potential, the own capabilities of the species and natural barriers. However, natural barriers have not played a significant role in genetic differentiation, as indicated by a non-significant correlation in IBD analysis. Similar results were reported in *N. lugens* populations from Vietnam using the *COI* gene sequence, wherein weak genetic structure was observed between the north and south Red River[24]. Similar results were reported in *B. dorsalis* populations from China[46] and India[17]. However, the MJ network analysis did not display any fundamental structure that allowed identification of phylogenetic haplogroups among the Indian populations. Further, this concept is also supported by a non-significant no isolation by distance among populations for *ITSI* gene sequences analysis of Indian populations. As no previous sequence data is available for the *ITSI* gene of BPH populations from other Asian countries, we could not compare our results with the other Asian populations. The sharing of major haplotypes among the Indian populations in the *ITSI* gene sequence analysis indicates recent gene mixing of *N. lugens* of various populations. This is also in line with an interpretation that *N. lugens* is highly migratory and gene flow has occurred in the relatively recent past through migration[47].

Demographic analysis exhibited significant negative Fu's *F* and Tajima's *D* statistical values from the studied populations, which in turn did not fit into a simple model of selective neutrality and bottleneck analysis[48]. The reason for this might be either a recent increase in the size or recent selection pressure experienced by the populations[49]. Our results also suggest the possible expansion of *N. lugens* in terms of more intense populations and colonization in new geographic locations recently in India. This population expansion of *N. lugens* was also supported by unimodal mismatch distribution with non-significant SSD values. The notion of significant population expansion in some species following the MJ network results exhibiting characteristically star-like structure denotes expansion of typical

demographic processes[46]. Besides, the presence of low to high genetic diversity in the region and weak genetic structure of the populations indicated that *N. lugens* colonization may be interpreted as a gradual to fast and range expansion associated with high population number and population growth[45]. However, after the first report of *N. lugens* in the 1950s in India, the spread and diffusion paths are largely unknown. Besides, data from the present study will enhance the *N. lugens* database and be an important source for comprehensive phylogeography studies of *N. lugens* throughout the world.

Conclusion

Genetic diversity and expansion history were studied using the *ITSI* gene sequences among Indian *N. lugens* populations. Results showed invariably low to high level of haplotype diversity, but a weak genetic structure among the studied populations. These populations lacked significant heterogeneity because of observed genetic homogeneity in the *ITSI* gene; hence it does not warrant differential pest management strategies. The findings of this study will be valuable for wide-area management strategies of BPH and region-specific breeding programmes of resistant genotypes in different regions of India.

Conflict of interest: The authors declare no conflict of interest.

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ACKNOWLEDGEMENT. We thank the Director, ICAR-National Rice Research Institute, Cuttack for technical and financial support (in-house project no. 3.6).

Received 10 February 2022; revised accepted 26 March 2022

doi: 10.18520/cs/v122/i12/1392-1400