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Genetic Structure and Phylogenetic Status of Rice Brown Plant Hopper (BPH), *Nilaparvata lugens* Isolated from Kerala, India

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ABSTRACT

Brown plant hopper, *Nilaparvata lugens* is a major pest of paddy, which damages the plants directly by sucking the sap and indirectly by transmission of rice viruses such as ragged stunt virus and grassy stunt virus. Geographically *N. lugens* are distributed throughout South and South East Asia where rice is grown and feed the phloem of plant. Due to the uncontrolled use of insecticides, insecticide resistant plant hoppers were emerged in many geographical locations like Japan, South and Southeast Asia *etc*. In this study we have described the Phylogenetic status of *N. lugens* isolated from Kerala, India. This study revealed that, *N. lugens* isolated from Andhra Pradesh and a few populations of *N. lugens* isolated from China and Japan were genetically similar to *N. lugens* isolated from Kerala. The result of this study also revealed the biasness of COI sequence of *N. lugens* to nucleotides AT.

1. INTRODUCTION

Brown plant hopper (BPH), *Nilaparvata lugens* is one of the most major pest of paddy, which cause huge destruction of rice plants in many rice producing countries [1]. BPH damages plants directly by sucking the sap and indirectly by transmission of rice viruses such as ragged stunt virus and grassy stunt virus [2, 3]. BPH is known to occur in Asia since late forties, it was earlier only a minor pest of rice [4] and in the 1970s and early 1980s the pest poses tremendous problems and was referred to as "a threat to Asian rice production" [5]. *N. lugens* distributed throughout South and South East Asia where rice is grown and they feed the phloem of plant. It infests the rice crop at all stages of plant growth. Both nymph and adult suck the sap from the base of the tillers, plants turn yellow and dry up rapidly. Round yellow patches appear in the paddy field in the early stages of infestation, which soon turn brownish due to the drying up of the plants and this condition is called 'hopperburn'. Severe infestation causes lodging of the crop resulting in yield loss ranging from 10 - 70 % [6]. Plant hoppers have two distinct winged forms: macropterous and brachypterous. Macropterous are long-winged and brachypterous are short-winged and they are ochraceous brown dorsally and brown ventrally. The females lacerate the midrib of the leaf blade or the leaf sheath to lay egg masses in the parenchymatous tissue. The average member of eggs laid varies from 250 - 350. The incubation period is 6 - 9 days and the nymphal period is 10 – 18 days. They undergo four to five molts. The total life-cycle occupies 16 to 27 days [6, 7]. In the chemical control of BPH, carbamates have a generally higher level of ovicidal activity than the organophosphates. Insecticide-induced resurgence of *N. lugens* has been reported from many tropical countries. Destruction of the natural enemies is generally recognized as the primary cause of Insecticide-induced resurgence [7]. Extensive use of chemical insecticides for the control of BPH on rice can cause serious problems including toxicity to natural enemies of BPH such *as Anagrus nilaparvatae* [8], increased production cost, and possible long term agro-ecosystem and human health damage [9,10]. For the control of *N. lugens*, insecticide granules are less effective than sprays or dusts, particularly when applied to older plants with a greater biomass. Insecticide resistant plant hoppers have been most common in Japan where the insecticide use rate on rice is much higher than in other tropical countries. *N. lugens* has also developed resistance to carbofuran in South and Southeast Asia [7].

Different biotypes and sympatric biological species of *N. lugens* have been reported in many studies [11, 12]. Mun *et al*. [13] analyzed the genetic variation among Asian populations of rice plant hoppers, *N. lugens* and *Sogatella furcifera* (Hemiptera: Delphacidae) using mitochondrial DNA sequences. The analyses of isozymes and RAPD-PCR markers indicated that BPH captured from rice plant has high esterase activity compared to BPH captured from *L. hexandra* and they represent two distinct closely

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related sibling species in Malaysia [14, 15]. Latif *et al.* [16] reported that Direct amplified length polymorphism (DALP) also useful for the analysis of genetic polymorphisms in brown plant hopper, *N. lugens* populations. Recently 30 polymorphic microsatellite markers were developed from *N. lugens* genomic libraries using the method of Fast Isolation by AFLP of Sequence Containing Repeats [17]. Zang *et al*. [18] reported complete mitochondrial genome of *N. lugens*. Here we report the genetic variations of brown plant hopper, *N. lugens* isolated from Kerala.

2. MATERIALS AND METHODS

Adult of *N. lugens* were collected from the rice field of Kerala, India and the genomic DNA was isolated using GeNei Ultrapure Mammalian Genomic DNA Prep Kit as per the Manufacturer's instruction. The 5' end of the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using the forward primer with DNA sequence 5' TCGAATTGAATTAGCACAACCAGG 3' and the reverse primer with DNA sequence of 5' AGCTCCTGCTAATACAGGTAAAGAT 3'.

The 25 µl PCR reaction mixture consisted of 2 nanogram of genomic DNA (1 µl), 1 µl each forward and reverse primers at a concentration of 10 µM, 2.5 µl of dNTPs (2 mM), 2.5 µl 10X reaction buffer, 0.20 µl Taq polymerase (5 U/µl) and 16.8 µl H_2O. The PCR profile consisted of an initial denaturation step of 3 min at 95 °C, followed by 30 cycles of 10 sec at 95^0C, 45 sec at 45 °C and 45 sec at 72 °C and ending with a final phase of 72 °C for 3 min. The PCR product was column purified using Mo Bio UltraClean PCR Clean-up Kit (Mo Bio Laboratories, Inc. California) as per the manufacturer's instructions. The purified PCR product was sequenced from both ends using the forward and reverse primers using the Sanger's sequencing method at SciGenom Laboratories Ltd., Cochin.

The forward and reverse sequences were assembled by using ClustalW (http://www.ebi.ac.uk/Tools/msa/clustalw2) after removing the forward and reverse primers and the consensus was taken for the analysis. The phylogenetic analysis was done using MEGA5 software.

3. RESULTS AND DISCUSSION

Brown plant hopper, *N. lugens* under the family Delphacidae is a one of the most destructive rice pest of Kerala and its attack is more common in Kerala. They damage plants by sucking the sap and also transmit viral diseases in plants. The adult brown plant hoppers are dimorphic. They were found in the field as mixed population of adult and nymph. The adult brown plant hoppers are may be winged or half winged. Both adult and nymph cause damage by sucking sap from the plant. The newly hatched nymphs are cottony white and turn to purple brown. The brown hopper populations multiply very fast and can migrate long distance and cause wide spread infestation. In the early stage of infestation yellowish patches were appeared and turns to brownish due to the drying of the plant. In the severely infested field, the rice gives a burned appearance (hopper burn). The partial coding sequence of mitochondrial COI gene of *Nilaparavata lugens* was PCR amplified using the forward primer with DNA sequence of 5' TCGAATTGAATTAGCACAACCAGG 3' and the reverse primer with DNA sequence of 5' AGCTCCTGCTAATACAGGTAAAGAT 3'. The PCR amplification of partial mitochondrial COI gene of *N. lugens* yielded a single product with about 550 bp in size.

Table 1: Percentage of genetic divergence of the *N. lugens* isolated from Kerala and in the related species of order Hemiptera. The GenBank accession numbers are given in parenthesis.

Name of species	% of divergence
Nilaparvata lugens (KC858992)	0.00%
Nilaparvata lugens (KC476395)	0.00%
Nilaparvata lugens (KC476394)	0.00%
Nilaparvata lugens (AB572318)	0.00%
Nilaparvata lugens (AB572314)	0.00%
Nilaparvata lugens (AB572300)	0.00%
Nilaparvata lugens (JN391181)	0.00%
Nilaparvata lugens (AB572309)	0.55%
Nilaparvata lugens (AB572308)	0.55%
Nilaparvata lugens (AB572307)	0.55%
Nilaparvata lugens (AB572306)	0.55%
Nilaparvata lugens (AB572305)	0.55%
Delphacidae sp. (HF968662)	20.45%
Trypetimorpha (KF298420)	20.63%
Trypetimorpha japonica (KF298420)	20.63%
Ommatissus lofouensis (KF298417)	21.02%
Sogatella furcifera (KC476386)	21.08%
Tambinia sp. (JQ410451)	22.67%
Eusarima sp. (HM452253)	22.71%
Kallitaxila sinica (KF298415)	22.93%
Gergithus parallelus (HM452251)	23.35%
Hemisphaerius trilobulus (HM052840)	23.35%
Gergithus iguchii (HM452252)	24.07%
Mongoliana serrata (HM052830)	24.09%
Laodelphax striatellus (HM160143)	25.47%
Nesosydne chambersi (JQ771119)	25.63%
Gergithus sp. (HM052836)	26.49%
Oliarus polyphemus (HF674828)	27.25%
Lycorma delicatula (EU909203)	29.85%
Issidae sp. (HF968665)	31.18%
Catullia subtestacea (KF298414)	31.87%
Nephotettix virescens (HM160144)	42.82%
Nephotettix cincticeps (JN391184)	42.82%

The sequence obtained after removing the primers used for PCR amplification was submitted to GenBank of NCBI (Accession: KF836703). The COI nucleotide sequence analysis revealed the composition of nucleotides in the COI gene of *N. lugens* isolated from Kerala. The COI sequence of *N. lugens* showed bias to nucleotide AT, with following composition of nucleotides **T**=35.10%, **A**= 31.10, **C**= 21.30% and **G**= 12.50%. The *N. lugens* isolated from Kerala showed similarity in the total nucleotide compositions and composition of nucleotides in the each position of codons with that of *N. lugens* isolated from Andhra Pradesh. Some populations of *N. lugens* isolated from China and Japan showed similarity and others showed variations in the total nucleotide composition and composition of nucleotides in the each position of codons with that of *N. lugens* isolated from Kerala. The genetic divergence analysis depicts the degree of divergence of different geographically isolated populations of *N. lugens* with *N. lugens* isolated from Kerala. *N. lugens* isolated from Andhra Pradesh (GenBank Accession No. KC858992) showed 0% divergence with *N. lugens* isolated from Kerala.

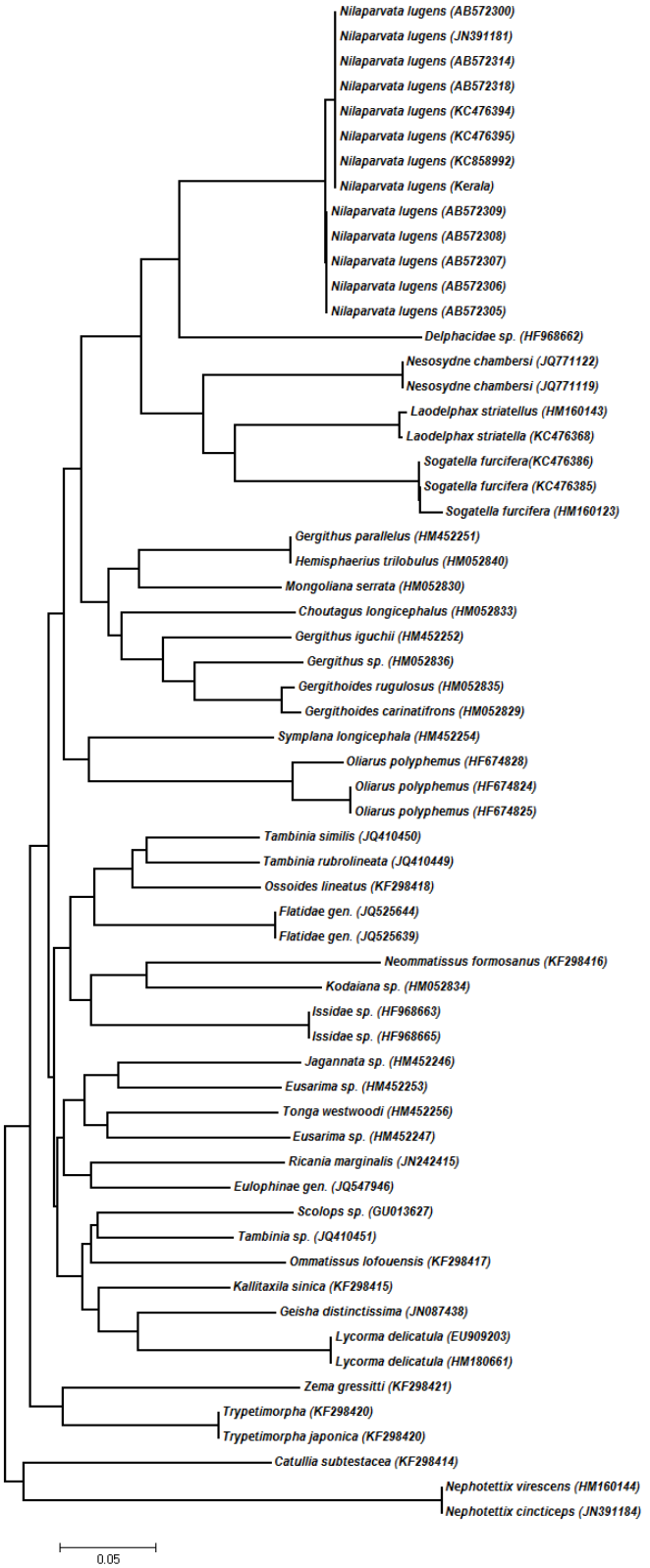

Fig. 1: Phylogenic relationship of *N. lugens* isolated from Kerala inferred by NJ tree method of MEGA5 software. The GenBank accession numbers are given in parenthesis.

Some populations of *N. lugens* isolated from China and Japan (GenBank Accession Nos. AB572318, AB572317, KC476395, KC476394 and B572300) showed 0% divergence and others (GenBank Accession Nos. AB572309, AB572308, AB572307, JX880069 and JN391181) showed 0.55% divergence with *N. lugens* isolated from Kerala (Table - 1). The phylogeny tree generated using Neighbour Joining method revealed the phylogenetic position of *N. lugens* isolated from Kerala (Fig. 1). The *N. lugens* isolated from different geographical location were aligned in single clad and divided in to two separate clad. Phylogeneticaly *N. lugens* isolated from Kerala is the nearest relative of *N. lugens* isolated from Andhra Pradesh and some populations of *N. lugens* isolated China and Japan, they together form a sub-clad.

The composition of nucleotides in the mtDNA J strand of *N. lugens* is **A** = 41.96%, **T** = 34.99%, **G** = 9.43% and **C** = 13.62%. The most of the heteropteran species mitogenomes nucleotide compositions are significantly biased toward **A** and **T**. The mitochondrial genome of hemipteran species has considerable variation in base composition among different hemipteran species [18, 19]. The COI nucleotide sequence analysis of *N. lugens* isolated from Kerala and other geographically isolated population also showed 68% **AT** content in the COI sequence which is varied from the total **AT** content of J strand. The related species of hemiptera also showed the same pattern result. These results indicated that, in the hemiptera the **AT** content of COI gene will be less compared to the total **AT** content of J strand. In the nucleotide triplet code, there is strong compulsion in the nucleotide changes in second position of all codons and first position of many codons. Due to the degenerative character of the triplet code third position of many codons and first position of some codons is less constraint. The variations in the strong constraint positions lead to the variations in the amino acids sequence. But the variations in the less constraint position will not affect (silent) the phenotype and these less constrained codon positions evolved at high rate [20,21]. In the case of COI sequence of *N. lugens* isolated from Kerala showed variation in the composition of nucleotides in the second position of codons with some populations identified from Japan and China (GenBank Accession Nos. AB572309, AB572308, AB572307, JX880069 and JN391181). These varied populations of *N. lugens* may be a different subspecies.The order Hemiptera is the largest group of the hemimetabolous insects [22]. The phylogenetic relationships of this order at the super family and suborder levels are still controversial [23, 24, 25]. The genetic divergence analysis revealed the genetic difference within the species *N. lugens* and between the related species of order Hemiptera. The COI sequence of *N. lugens* showed considerable variations with the related species. Therefore the COI sequence isolated in this study can be used as a barcode to identify this insect species.

4. CONCLUSIONS

The COI sequence of *N. lugens* exhibited considerable variation with other species. Therefore it can be used as DNA barcode to identify this organism. The *N. lugens* isolated from

Andhra Pradesh and a few populations of *N. lugens* isolated from China and Japan were genetically similar to *N. lugens* isolated from Kerala. This study also concluded that the COI sequence of *N. lugens* has biasness to nucleotides AT.

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