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Haplotype Variations and Genetic Structure of Indonesian Green Leafhopper *Nephotettix virescens* (Distant.) (Hemiptera: Cicadellidae) Population based on Cytochrome C Oxidase I and II Genes

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Running title: Haplotype Variations of Indonesian *N. virescens*

Abstract. The green leafhopper (GLH), *Nephotettix virescens* (Hemiptera: Cicadellidae) is an insect vector of the important rice tungro viruses. Currently, information on cytochrome c oxidase (*COI* and *COII*) genetic variation in *N. virescens* in Indonesia is unknown. This study investigated the haplotype variations of *N. virescens* populations that were collected from eight locations in six Indonesian provinces, i.e., Aceh, West Java, Central Java, East Java, Bali, and South Sulawesi. Primers for the *COI* and *COII* genes of *N. virescens* were then designed based on *N. cincticeps* (GenBank KP749836). Two groups of sequences of the mitochondrial DNA gene**, i.e**, *COI* (735 base pairs) and *COII* (612 bp), were used to investigate the green leafhopper haplotype variations. There were 128 sequences of *COI* and *COII* obtained from eight populations. Of the 64 GLH individuals, there were 14 *COI* haplotypes with one common and 13 specific haplotypes, and 10 *COII* haplotypes with two common and eight specific haplotypes. Based on *COI* gene, we found four group of haplotypes from Sumatera, Jawa, Bali and Sulawesi. These haplotype variations are applicable as a DNA marker for Indonesian *N. virescens* identification. Our results have important implications for monitoring and control of this important agriculture insect.

Keywords: *Nephotettix virescens,* Green leafhopper, Haplotype variations, *COI* and *COII gene*

INTRODUCTION

Nephotettix virescens (Hemiptera: Cicadellidae) is an insect vector of tungro viruses that infests rice crops distributed in India, Bangladesh, Pakistan, Burma, Hong Kong, Taiwan, Laos, Malaysia, Indonesia, and the Philippines (Dale 1994). The green leafhopper, *N. virescens* has been widespread in Indonesia and is a dominant species in almost all regions**,** such as Java, Bali, Lombok, Sumatera, Kalimantan, Sulawesi, and Irian Jaya (Siwi 1985). Therefore, the accurate identity of vector insects from

different regions was needed to develop a vector control for *N. virescens* species in integrated rice tungro disease management and monitoring the insect distribution. Information on the distribution pattern of *N. virescens* might indicate a pattern for the spread of tungro virus to other regions. In this study, a molecular approach was used to investigate the genetic diversity of *N. virescens.*

The *COI* and *COII* of mitochondrial DNA have been widely used as molecular markers for studying genetic diversity and intra-species relationships, i.e., the diversity of sugarcane stem borer insects (*Eldana saccharina),* between South African and North African populations (King *et al*. 2002). The *COI* gene showed genetic variations of *N. virescens* from Kerala and Orissa, India (Sreejith & Sebastian 2015). The identification using *COI* gene was successfully performed for the Japanese leafhopper population (Kamitani 2011) and leafhopper *Cofana spectra* from Kerala, India (Hemiptera: Cicadellidae) (Sreejith & Sebastian 2014). However, molecular markers for the Indonesian *N. virescens* GLH population have not been studied. To understand their genetic variations, we examined mitochondrial *COI* and *COII* in *N. virescens* populations from eight locations in six Indonesian provinces.

MATERIALS AND METHODS

Green Leafhopper Collections

A total of 64 *N. virescens* samples (GLHs) were collected from eight locations in six provinces in Indonesia, namely, Aceh, West Java, Central Java, East Java, Bali and South Sulawesi. All GLH samples were collected from the Ciherang rice variety except from Sidrap (TN1 variety) and Bogor and Situbondo (IR64 variety). The insects were collected during the vegetative rice plant stage, except in the Bali area, which was conducted during the generative stage (Table 1). The GLH samples were collected using a sweeping net 34 cm in diameter and were stored in absolute ethyl alcohol.

Primer Design and DNA Extraction

The *COI* and *COII* primers were designed using the Primer3 program (http://bioinfo.ut.ee/primer3-0.4.0/) from the complete genome of *N. cincticeps* mitochondrion (numbers in the parentheses following the primer sequences are the position based on GenBank Acc Number KP749836): COI primers Nvir_COI_F1m: 5'CAGTTTTACTACTTTATTCAGACACC'3 (14775-14800) and Nvir_COI_R1m: 5'AGCTCACCATATATTTACTGTAGG'3 (857-881); primers for COII gene: Nvir_COII_F1m: 5'TATGGCAGATTAGTGCAATGAAC'3 (1563-1583) and Nvir_COII_R2m 5'CTGAACATTGCCCAAAGAATAATC'3 (2263-2283). The total DNA was extracted from the thorax tissues using the genomic DNA mini kit method (Geneaid biotech ltd, Taipei, Taiwan).

Amplification of *COI* and *COII genes*

Reaction volumes of 25 µl were used in the amplification process of each gene. The total volume contained 10x KAPA taq buffer (KAPA Biosystems, Wilmington, USA), 25 mM $MgCl_2$, 2,5 mM dNTP, 5 U/µl KAPA taq polymerase (KAPA Biosytems, Wilmington, USA), 1 µl of DNA template and sterile water. Mitochondrial DNA genes were amplified for 30 cycles, consisting of pre-denaturation at 95ºC for 2 min, denaturation at 95ºC for 1 min, annealing at 45-46ºC for 55 sec of *COI* and 48ºC for 30 sec of *COII*, elongation at 72ºC for 1 min, and post-elongation at 72ºC for 2 min.

The PCR products were resolved by 1.5% agarose gel electrophoresis and stained using DiamondTM Nucleic Acid Dye (Promega, Madison, USA). The visualisation process was carried out to ensure that the amplification process was successful. DNA sequencing was performed for two genes and two primers using sequencing services (First BASE, Selangor, Malaysia).

Data Analysis

To identify general and specific haplotypes, GLH *COI* and *COII* sequences from this study were aligned with those from database entries in GenBank (Table 2) using ClustalX 2 (Larkin *et al*. 2007). The Basic Local Alignment Search Tool-Nucleotide (BLAST-N) program (www.ncbi.nlm.nih. gov/Blast.cgi) was used for the homologous analysis of both mitochondrial DNA genes. The number of substitutions and pairwise distances of *COI* and *COII* nucleotide sequences were analysed using MEGA6 (Tamura *et al*. 2013). The genetic structure parameters measured in this study were haplotype diversity (Hd), nucleotide diversity (Л), and fixation index value (Fst) analysed using DnaSP 5.1 program (Rozas *et al*. 2003). The phylogenetic trees were constructed using the neighbour-joining (NJ) method with 1000x bootstraps implemented in the MEGA6 program (Tamura *et al.* 2013). *Sogatella furcifera* and *Nephotettix cincticeps* were chosen as the outgroups.

RESULTS

Nephotettix virescens* Sequence Variations of *COI* and *COII

Base lengths of approximately 735 bp were successfully amplified by *COI* primers from this study (Table 2). Blast-n analysis showed that the *COI* of the *N. virescens* population was 98% homologous with *N. virescens* from Orissa, India (GenBank accession number KF371523). A total of 14 haplotypes were found in the *COI* sequence with 19 nucleotide variations, consisting of 17 transition and 2 transversion substitutions (Suppl. 1). The *COI* transition frequently occurs than transversion. The transitions were found in all three codon positions, while transversion substitution was only in the third codon position (Figure 1). All of the *COI* haplotypes were obtained and submitted to DDBJ (DNA Data Bank of Japan) under accession number: LC330970 - LC330983 (HT1-HT14) (Suppl. 2).

For the *COII* gene, the base length was approximately 612 bp, was amplified from 64 samples (Table 2) and was 99% homologous with *N. virescens* from Goa, India (GenBank Acc Number KR230205). There are 10 haplotypes found in the *COII* gene with 12 nucleotide variations and only transition substitutions in all three codon

positions (Suppl. 1). The number of substitutions with p-distances corrected by the Tamura-Nei's model in codon positions 1, 2, and 3 are presented in Figure 1. The ten haplotypes of the *COII* gene were named HT1 to HT10 and submitted to DDBJ under accession numbers LC330984 - LC330993 (Suppl. 2).

Genetic Diversity of *COI* and *COII* from *N. virescens*

A total of 14 haplotypes (HT1-HT14) in the *COI* gene were found in 64 individuals of *N. virescens* from this study (Suppl. 1). Three haplotypes (HT1, HT10, and HT12) were found in several sample locations, i.e., HT1 was shared among all locations, one haplotype (HT10) was found in three locations and one haplotype (HT12) was found in two locations. Eleven haplotypes were found in only one location, and these haplotypes were specific (unique) haplotypes (Suppl. 3). Haplotypes 2 and 3 were only found in Sulawesi. Haplotypes 4 and 6 were found in Bali. A total of 6 haplotypes were found in *N. virescens* from Java, i.e., haplotypes 5, 7, 8, 9, 11, and 12. The remaining two haplotypes (haplotypes 13 and 14) were found in *N. virescens* from Sumatera (Suppl. 3). The highest number (haplotypes 1, 10, 12, 13, and 14) and lowest (only one haplotype) of *COI* haplotypes were found in Kutacane and Bogor, respectively (Suppl. 3). The *COI* haplotype distribution from eight locations is shown in Figure 2.

The average value of *COI* haplotype diversity (Hd) was relatively moderate (0.546), and the value ranged from 0 to 0.857. The lowest and highest values were found in Bogor and Kutacane, respectively (Table 3). The average value of *COI* nucleotide diversity (Л) was relatively low (0.00204), ranging from 0 to 0.0043. Genetic differentiation among the *N. virescens* populations was highly significant, ranging from -0.0667 to 0.5517 ($0.001<P<0.01$) (Table 4). The *COI* Fst values for Kutacane, Wonosobo, and Sidrap were different from the five other locations, showing that these three populations have low gene flow than the remaining populations.

The phylogenetic analysis among *COI* haplotypes showed that the *N. virescens* in Indonesian formed a monophyletic clade and divided into two groups. The two specific haplotypes (HT2 and HT3) from Sidrap (South Sulawesi) are clustered in one group, and the remaining 12 haplotypes formed the other group (Figure 3a). We did construct the phylogenetic tree of two concatenated genes of *COI* and *COII* (Figure 3c). The topology of the concatenated gene tree is similar to that of the single *COI* gene, that is the Haplotype 2 and 3 from Sulawesi clustered in a single clade and the other haplotypes from Sumatera, Java and Bali clustered in a single clade.

The *COII* haplotype variations were detected in 10 haplotypes (HT1-HT10) from 64 individuals of *N. virescens*. One haplotype (HT1) was found in all locations, and one haplotype (HT2) was found in seven locations. The remaining eight haplotypes are specific to one location, i.e., haplotypes 3, 4, 5, 6, 7, 8, 9, and 10 (Suppl. 3). Haplotypes 3 and 4 were only found in Sulawesi. A total of five haplotypes (haplotypes 5, 6, 7, 8 and 9) were found from Java, and haplotype 10 was found in *N. virescens* from Sumatera (Suppl. 3). The *COII* haplotype distribution from eight locations is shown in Figure 2.

The highest *COII* haplotypes were found in Sidrap and Wonosobo locations with four haplotype variations. The lowest *COII* haplotypes were found in three locations, namely, Kutacane, Bogor, and Gianyar, with only two haplotypes variations

(Suppl. 3). The analysis of *COII* genetic diversity showed that the mean values of Hd were relatively moderate (0.665 ± 0.046) and that Л was low (0.00208 ± 0.00041) (Table 3). The highest genetic diversities were shown by Wonosobo and Sidrap populations (Hd = 0.750 ± 0.139, Л = 0.0015 ± 0.0004), whereas the lowest was Kutacane (Hd = 0.250 ± 0.180, Л = 0.0004 ± 0.0003) (Table 3). The *COII* Fst values ranged from -0.1428 to 0.53571 (P<0.001)), and the Kutacane and Sidrap populations were significantly different from the other populations (Table 4). The phylogenetic analysis between *COII* haplotypes formed a single monophyletic clade and is not reflected in a geographic distribution (Figure 3b).

DISCUSSION

This study obtained the pattern of haplotype distribution and the first report for haplotype variation of *N. virescens* from Indonesia based on *COI* and *COII* mitochondrial genes. The haplotype sharing among the Indonesian populations indicated that there was a history of gene flow for the *N. virescens* species. The distribution of haplotypes (HT1) in all sample locations suggested that the Indonesian *N. virescens* population lives in a homogeneous group of haplotypes and that there might be panmixia. Panmixia might occur if a population lives in a homogeneous landscape (Freeland *et al*. 2011). An example of panmixia also occurs in the *Hishimonus phycitis* (Hemiptera: Cicadellidae) leafhopper in Iran. The HT1 of *COI* haplotype distribution in Iran *H. phycitis* populations was shared by all the Iran populations, which means that the Iran populations form a homogeneous group of haplotypes (Shabani *et al.* 2013). Additionally, the mtDNA sequence divergences within species of Indonesian *N. virescens* were 0.2% among geographic locations. In other insects, the mtDNA sequence divergence was 1% for rice leafhopper; *Cofana spectra* (Hemiptera: Cicadellidae) (Sreejith & Sebastian 2014); that of the two species of rice planthopper were 0.23% and 0.12% for *Nilaparvata lugens* and *Sogatella furcifera* (Hemiptera: Delphacidae), respectively (Mun *et al*. 1999); the divergence was 0.2% and 1.2% for two species of mushroom fly (Bae *et al*. 2001; and the divergence was 0.9% for the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae) (Li *et al*. 2006).

In addition, some haplotypes are only found in certain locations, where there are four and three specific haplotypes for *COI* and *COII*, respectively. The four specific haplotypes are from Java, Sumatera, Bali, and Sulawesi. The three specific haplotypes are from Java, Sumatera, and Sulawesi. The haplotype variations are applicable as a DNA marker for the identification of *N. virescens* from Indonesia. These genetic variations of *N. virescens* can be used as the DNA barcode to identify *N. virescens* in each region. Thus, the distribution of green leafhoppers from one region to the other region can be determined. The haplotype of *N. virescens* in particular region can be used as monitoring tool for the presence of GLH.

The genetic variation analysis of both mitochondrial DNA genes (*COI* and *COII*) found moderate haplotype diversity (mean Hd *COI* = 0.546 ± 0.075, Hd *COII* = 0.665 ± 0.046, P <0.001) for *N. virescens* in Indonesia. These results suggested that among the *N. virescens* populations, there was a high gene flow. The haplotype diversity of *H.*

phycitis in the Iran and Oman populations was 0.76 and suggested that there was a differentiation and high gene flow among the populations within the geographical units (Shabani *et al*. 2013).

This study found that the three populations, i.e., those from Kutacane of Aceh; Wonosobo of Central Java and Sidrap of South Sulawesi, had low gene flow. This genetic structure might be due to the geographical location of the Wonosobo location in the highlands approximately 500-1000 m above sea level (asl) and surrounded by mountains, with temperatures of approximately 15-26ºC, thus causing a low gene flow rate among other populations. In addition to the case in Wonosobo, the occurrence of high genetic differentiation was also shown in Kutacane and Sidrap populations. This finding might be due to a geographical barrier (the Java Sea and the Sunda strait) and a relatively far distance of those two from Java Island. The main factor limiting gene flow between *Graphocephala antropunctata* (Hemiptera: Cicadellidae) populations in California is the geographical distance (Ballman *et al.* 2011). The genetic diversity of an organism is influenced by many factors, such as geographical boundaries and distance. For example, the genetic structure of *Anopheles punctipennis* (Diptera: Culicidae) has been influenced by climate fluctuation and mountain boundaries (Fairley et al. 2000). The genetic diversity among populations of *Graphocephala atropunctata* (Hemiptera: Cicadellidae) in California was reported to be due to separation by geographic distances (Ballman *et al*. 2011). The geographical barrier and climatic conditions (Persian Gulf and Omanic seas) have influenced the genetic differentiation of *Hishimonus phycitis* (Hemiptera: Cicadellidae) between the Iran population and the Oman population (Shabani *et al.* 2013).

The phylogeny construction showed that the *COI* gene provides more information about genetic variation than the *COII* gene, which forms two clusters (Figure 3). The phylogenetic analysis showed that the branches formed in the *COI* are relatively longer compared with the *COII*. A long branch indicated differences in the nucleotide sequence (Li 1997). The high substitution rates at the first and third codon positions suggested that the *COI* gene evolved faster than the *COII* gene. The *COI* genes also evolved faster than the *COII* gene of the *Sesamia nonagrioides* (Lepidoptera: Noctuidae) population from Greece and Spain caused by a high substitution rate at the first and third codon positions (Kourti 2006). Therefore, the *COI* sequence might be effectively used as a barcode for the identification of *N. virescens* species. The mitochondrial *COI* gene has been used to estimate phylogenetic relationships at different taxonomic levels of Japanese leafhoppers (Kamitani 2011).

The information obtained from the studies of gene flow and population genetic structure of a leafhopper vector can be used to determine spread and migration and to identify invasive individuals (Rollins *et al*. 2006). Because *N. virescens* is a tungro virus vector insect in rice, it is necessary to have strategic control and monitoring the spread of the insect.

CONCLUSION

This study has provided a database of haplotype variation for *N. virescens* based on mitochondrial DNA *COI* and *COII* genes. Our main findings are thirteen and

eight specific haplotypes for *COI* and *COII* genes of GLH, respectively. Those haplotypes have been successfully used for genetic markers of *N. virescens* in Indonesia. The specific haplotypes of the *COI* gene revealed four haplotype groups, i.e., haplotype for Sumatera, Java, Bali and Sulawesi. The DNA barcode of *COI* gene sequences are able support the policy makers in practice management, such as monitoring and control for *N. virescens* species. For further research, the sample size and sampling locations in each island will be augmented. This addition might be able to clarify the species markers on each island.

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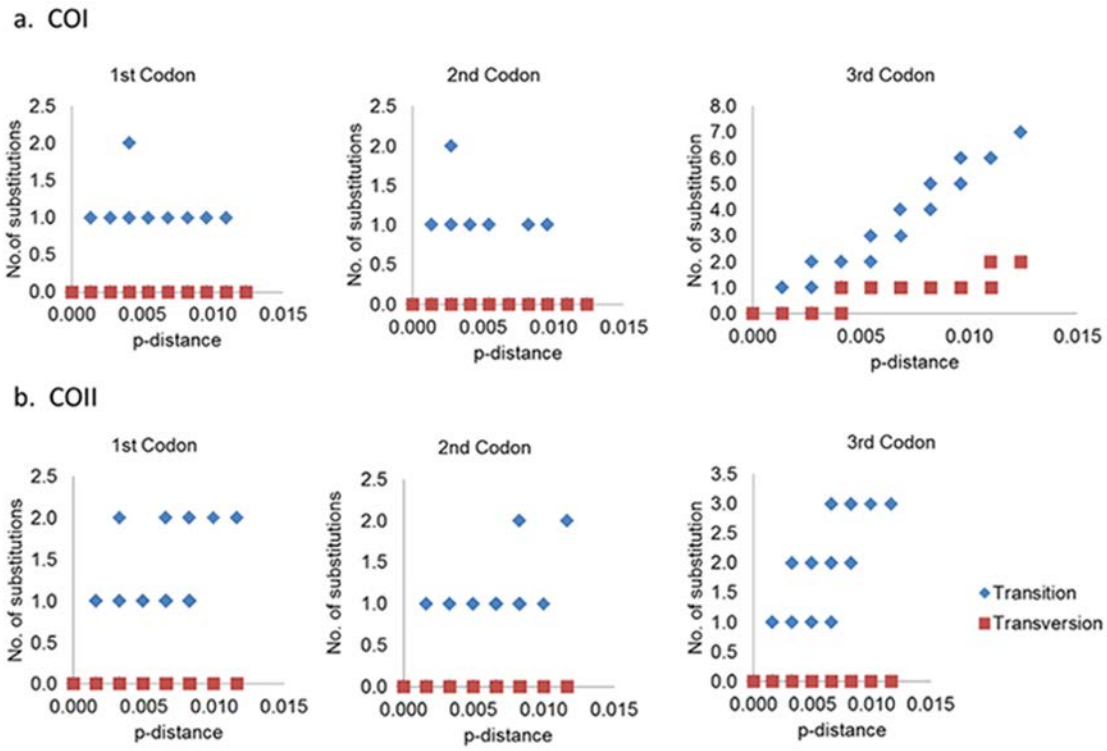

Figure 1: The transition rate is higher than transversion substitution (corrected by Tamura-Nei's model) for COI (a) and *COII* (b) in *N. virescens*.

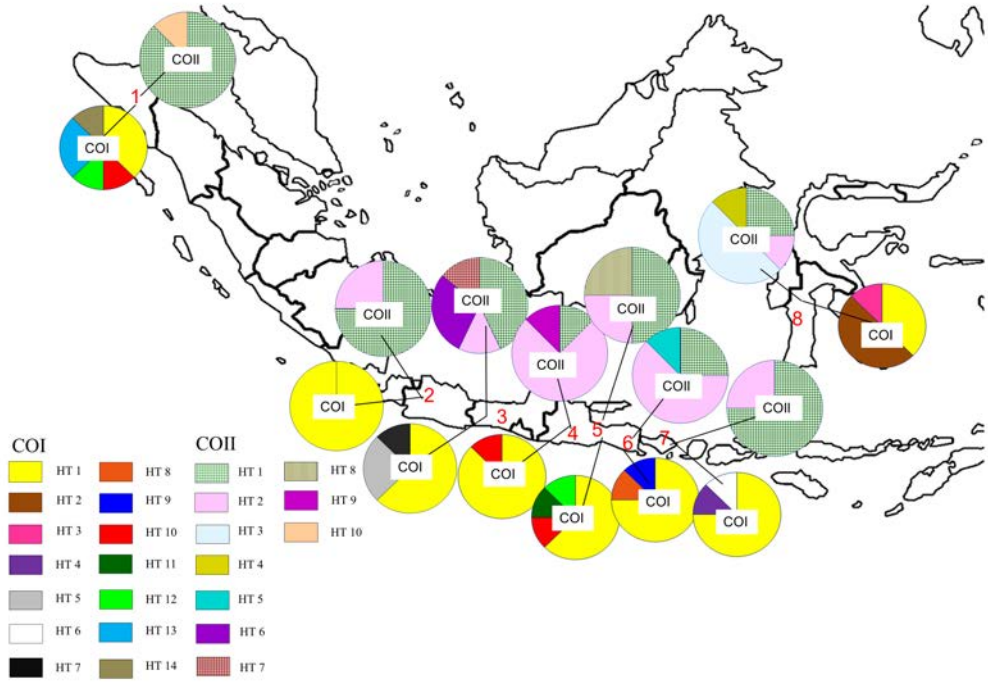

Figure 2: The distribution of haplotypes of *N. virescens* based on *COI* and *COII* genes from eight locations in Indonesia.

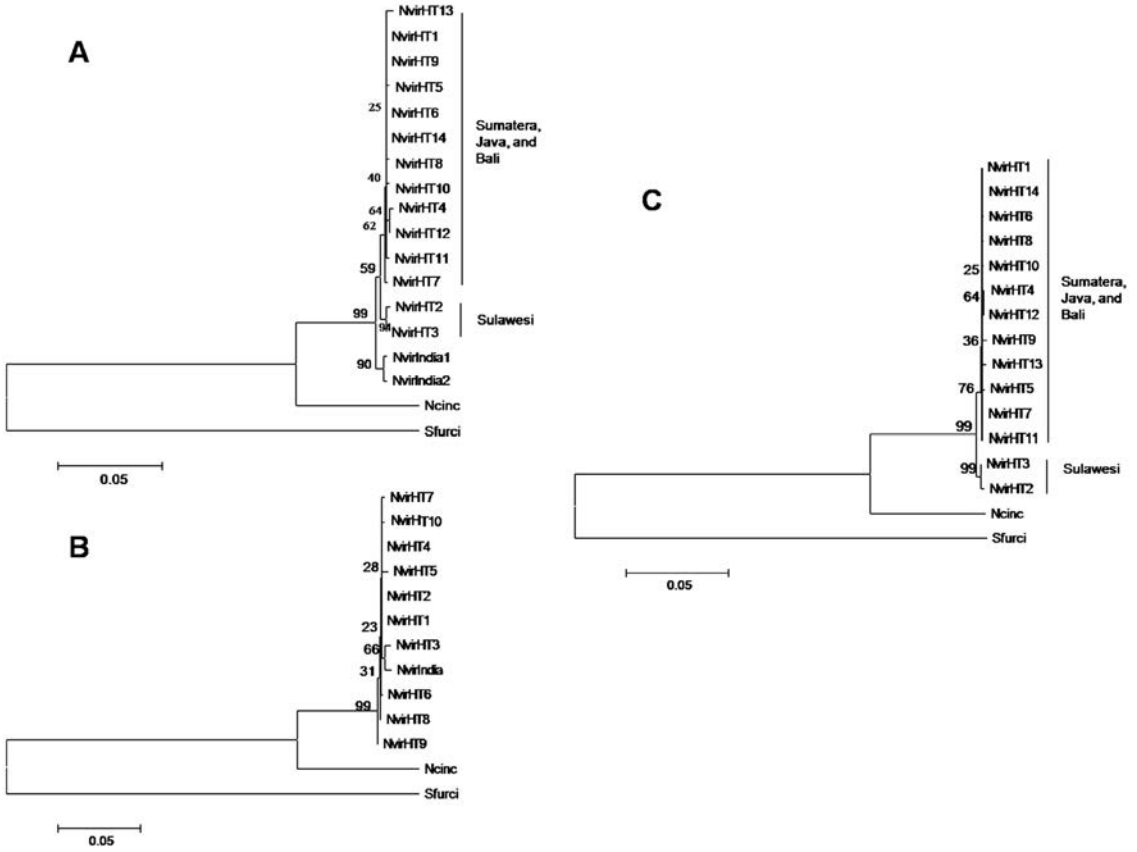

Figure 3: Phylogenetic trees showing relationship among haplotypes of *Nephotettix virescens* population from Indonesia, based on *COI* (A) and *COII* (B) mitochondrial DNA and the concatenated genes (C) which were constructed using Neighbour Joining method. The branches support of the trees was evaluated by bootstrapping with 1000x iterations. Different results revealed from both genes. The *COI* gene identified the geographical distribution of haplotypes by showing a cluster of *N. virescens* from Sulawesi and a cluster of Sumatera, Java and Bali haplotypes. Lack of geographical cluster was showed by using COII gene. The topology of the concatenated genes is in agreement with the *COI* gene. *Sogatella furcifera* and *Nephotettix cincticeps* were used as the outgroup. Abbreviations: Nvir = *Nephotettix virescens*; HT1-14 = haplotypes 1-14, Ncinc = *Nephotettix cincticeps*, Sfurci = *Sogatela furcifera*. Haplotype and populations codes are given in Supplement 1 and Table 2, respectively.

Table 1. Sampling information of different geographical populations of *N. virescens* in Indonesia

No.	Sampling Location	Population Code	Coordinates	Host plant	∑*	Collector**
1.	Kutacane, Aceh	NvirKTN	97º.48'46 E 3º.29'44 S	Ciherang, Vegetative stage	8	RYu
2.	Bogor, West Java	NvirBGR	106º.47' E 6º.37' S	IR 64, Vegetative stage	8	IMZ
3.	Wonosobo, Central Java	NvirWSB	109º.53'43 E 7º.24'6 S	Ciherang, Vegetative stage	8	AAK
4.	Blitar, East Java	NvirBLT	112º.11'46 E 8º.11'14 S	Ciherang, Vegetative stage	8	RYu
5.	Situbondo, East Java	NvirSIT	-	IR 64, Vegetative stage	8	MDS
6.	Banyuwangi East Java	NvirBYW	114º.11'44 E 8º.19'1 S	Ciherang, Vegetative stage	8	RYu
7.	Gianyar, Bali	NvirGIN	115º.18'26 E 8º.36'49 S	Ciherang, Generative stage	8	RYu
8.	Sidrap, South Sulawesi	NvirSDR	119º.49.501' E 3º.50.957' S	TN1, Vegetative stage	8	EKL

* ∑: Total Individuals;

** Collector : RYu: Rafika Yuniawati; IMZ: Ifa Manzila; MDS: I Made Samudra; AAK: Arma Aditya Kartika; EKL: Ema Komalasari

Table 2. Species used in the construction of phylogenetic trees

No	Species	Location	Abbreviations*	Acc Num	Length of Bases		References
					COI	*COII*	
Ingroup							
1.	*N. virescens*	Kutacane	NvirKTN	DDBJ (Suppl.2)	735	612	
2.	*N. virescens*	Bogor	NvirBGR	DDBJ (Suppl.2)	735	612	
3.	*N. virescens*	Wonosobo	NvirWSB	DDBJ (Suppl.2)	735	612	
4.	*N. virescens*	Blitar	NvirBLT	DDBJ (Suppl.2)	735	612	Current research
5.	*N. virescens*	Situbondo	NvirSIT	DDBJ (Suppl.2)	735	612	
6.	*N. virescens*	Banyuwangi	NvirBYW	DDBJ (Suppl.2)	735	612	
7.	*N. virescens*	Gianyar	NvirGIN	DDBJ (Suppl.2)	735	612	
8.	*N. virescens*	Sidrap	NvirSDR	DDBJ (Suppl.2)	735	612	
9.	*N. virescens* voucher LHCT2	Orissa, India	NvirIndia1	GenBank KF371523	658	-	Unpublished
10.	*N. virescens voucher GTB8D1220*	Meghalaya, India	NvirIndia2	GenBank KX351396	575	-	Unpublished
11.	*N. virescens voucher INHS:CHI054*	Goa, India	NvirIndia	GenBank KR230205	-	582	Zahniser & Dietrich 2015

Outgroup							
12.	*N. cincticeps*	China	Ncinc	GenBank KP749836	1539	681	Unpublished
13.	*Sogatella furcifera* isolate WBPHHN	China	Sfurci	GenBank KC512914	1533	664	Zhang *et al.* 2014

*Abbreviations are named referring to the species and location

Table 3. Genetic diversity analysis of Indonesian GLH based on *COI* and *COII* sequences

No	Population	Hd ±SD		Л ± SD	
		COI	*COII*	*COI*	*COII*
1.	NvirKTN	0.857 ± 0.108	0.250 ± 0.180	0.0026 ± 0.0006	0.0004 ± 0.0003
2.	NvirBGR	0.000 ± 0.000	0.429 ± 0.169	0.0000 ± 0.0000	0.0007 ± 0.0003
3.	NvirWSB	0.607 ± 0.164	0.750 ± 0.139	0.0009 ± 0.0003	0.0015 ± 0.0004
4.	NvirBLT	0.250 ± 0.180	0.464 ± 0.200	0.0003 ± 0.0002	0.0011 ± 0.0005
5.	NvirSIT	0.643 ± 0.184	0.714 ± 0.123	0.0010 ± 0.0003	0.0014 ± 0.0003
6.	NvirBYW	0.464 ± 0,200	0.607 ± 0.164	0.0007 ± 0.0003	0.0015 ± 0.0006
7.	NvirGIN	0.464 ± 0.200	0.429 ± 0.169	0.0009 ± 0.0005	0.0007 ± 0.0003
8.	NvirSDR	0.679 ± 0.122	0.750 ± 0.139	0.0043 ± 0.0009	0.0040 ± 0.0008
	Total	0.546 ± 0.075	0.665 ± 0.046	0.00204 ± 0.00047	0.00208 ± 0.00415

Note: Hd = haplotype diversity, л = nucleotide diversity. The details of population abbreviations are given in Table 2.

Table 4. Fixation index value (Fst) of Indonesian GLH populations based on *COI* (above diagonal) and *COII* (below diagonal)

	NvirKTN	NvirBGR	NvirWSB	NvirBLT	NvirSIT	NvirBYW	NvirGIN	NvirSDR
NvirKTN		0.09524**	0.09524**	0.06227**	0.03106**	0.07792**	0.05167**	0.44361**
NvirBGR	0.09524***		0.09524**	0.00000	0.00000	0.00000	0.00000	0.55172**
NvirWSB	0.05714***	0.01299***		0.07143**	0.04762**	0.05714**	0.04762**	0.50893**
NvirBLT	0.53571***	0.26190***	0.32331***		-0.06667	0.00000	0.00000	0.53333**
NvirSIT	0.11429***	-0.02857	0.04762***	0.23214***		0.00000	-0.04348	0.50000**
NvirBYW	0.47619***	0.22449***	0.29252***	-0.07143***	0.20635***		0.00000	0.51613**
NvirGIN	0.09524***	-0.14286	0.01299***	0.26190***	-0.02857	0.22449***		0.50000**
NvirSDR	0.48980***	0.46844***	0.43459***	0.51261***	0.44073***	0.49326***	0.46844***	

Notes: Negative values should be interpreted as no genetic differentiation between two populations. Details on the population code are given in Table 2,*COI* : **0.001<P<0.01; *COII*: ***P <0.001.

APPENDIX

Supplement 1. Variable positions of 14 *COI* and 10 *COII* haplotypes of Indonesian *N. virescens* populations

Haplo Type (HT)	Nucleotide position																														
	COI																			*COII*											
	006	027	045	063	190	198	249	284	286	342	450	465	485	492	570	636	660	720	732	836	892	907	935	938	733	428	440	447	532	556	574
Substitution	ts	ts	tv	ts	ts	ts	ts	ts	ts	ts	ts	ts	ts	ts	tv	ts	ts	ts	ts	ts	ts	ts	ts	ts	ts	ts	ts	ts	ts	ts	ts
HT1	G	T	A	T	G	A	T	C	T	C	C	T	A	T	A	C	C	A	T	T	T	G	T	G	A	C	G	A	G	T	T
HT2	.	.	C	.	.	G	.	.	.	T	.	.	.	C	.	.	T	G	.	.	.	.	.	.	.	.	.	.	G	.	.	.
HT3	.	.	C	.	.	G	.	.	.	.	.	.	.	C	.	.	T	G	.	C	C	.	.	.	.	T	.	.	A	.	.
HT4	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	.	.	A	.	.
HT5	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	G	.	.	C
HT6	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.
HT7	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.
HT8	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.
HT9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	A	.	.	.	.
HT10	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
HT11	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.												
HT12	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.												
HT13	A	.	.	.	.	.	.	.	.	.	.	C	.	.	T	.	.	.	.												
HT14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.												
∑nucleotide variation	19																			12											

Supplement 2. *COI* and *COII* haplotypes of *Nephotettix virescens* in Indonesia available from DDBJ (DNA Data Bank of Japan)

No.		Haplotype code	Base length	Accession Number
COI haplotypes:				
1	Haplotype 1	HT1	870	LC330970
2	Haplotype 2	HT2	840	LC330971
3	Haplotype 3	HT3	795	LC330972
4	Haplotype 4	HT4	786	LC330973
5	Haplotype 5	HT5	870	LC330974
6	Haplotype 6	HT6	879	LC330975
7	Haplotype 7	HT7	840	LC330976
8	Haplotype 8	HT8	795	LC330977
9	Haplotype 9	HT9	849	LC330978
10	Haplotype 10	HT10	768	LC330979
11	Haplotype 11	HT11	849	LC330980
12	Haplotype 12	HT12	789	LC330981
13	Haplotype 13	HT13	813	LC330982
14	Haplotype 14	HT14	819	LC330983
COII haplotypes:				
15	Haplotype 1	HT1	612	LC330984
16	Haplotype 2	HT2	615	LC330985
17	Haplotype 3	HT3	612	LC330986
18	Haplotype 4	HT4	612	LC330987
19	Haplotype 5	HT5	612	LC330988
20	Haplotype 6	HT6	612	LC330989
21	Haplotype 7	HT7	612	LC330990
22	Haplotype 8	HT8	612	LC330991
23	Haplotype 9	HT9	612	LC330992
24	Haplotype 10	HT10	612	LC330993

Supplement 3. The *N. virescens* haplotype frequencies of *COI* and *COII*

No	Location	Gene	N / ∑HT	Haplotypes (HT)													
				1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	KTN	*COI*	8/5	3	-	-	-	-	-	-	-	-	1	-	1	2	1
		COII	8/2	7	-	-	-	-	-	-	-	-	1	-	-	-	-
2	BGR	*COI*	8/1	8	-	-	-	-	-	-	-	-	-	-	-	-	-
		COII	8/2	6	2	-	-	-	-	-	-	-	-	-	-	-	-
3	WSB	*COI*	8/3	5	-	-	-	2	-	1	-	-	-	-	-	-	-
		COII	8/4	3	1	-	-	-	2	1	-	-	-	-	-	-	-
4	BLT	*COI*	8/2	7	-	-	-	-	-	-	-	-	1	-	-	-	-
		COII	8/3	1	6	-	-	-	-	-	-	1	-	-	-	-	-
5	SIT	*COI*	8/4	5	-	-	-	-	-	-	-	-	1	1	1	-	-
		COII	8/3	4	2	-	-	-	-	-	2	-	-	-	-	-	-
6	BYW	*COI*	8/3	6	-	-	-	-	-	-	1	1	-	-	-	-	-
		COII	8/3	2	5	1	-	-	-	-	-	-	-	-	-	-	-
7	GIN	*COI*	8/3	6	-	-	1	-	1	-	-	-	-	-	-	-	-
		COII	8/2	6	2	-	-	-	-	-	-	-	-	-	-	-	-
8	SDR	*COI*	8/3	3	4	1	-	-	-	-	-	-	-	-	-	-	-
		COII	8/4	2	1	4	1	-	-	-	-	-	-	-	-	-	-

Abbrevation : N: Total number of individuals; ΣHT: Number of Haplotype; '-': not found haplotype in the location, Location abbreviation refers to Table 2.