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Research Article

PHYLOGENETIC ANALYSIS AND SEQUENCING OF THE MITOCHONDRIAL CYTOCHROME OXIDASE SUB UNIT I (COI) OF WHITE BACKED PLANT HOPPER, *SOGATELLA FURCIFERA* (HORVATH)

Sreejith K., Sebastian C. D.*
Division of Molecular Biology, Department of Zoology, University of Calicut, Kerala, India
*Corresponding Author Email: drcdsebastian@gmail.com

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ABSTRACT
The family Delphacidae is the largest and most economically important one amongst the hoppers, Fulgoroidea. It has representatives in tropical and subtropical regions that occur in a variety of environments, and its known fossil records dates back to Jurassic period. *Sogatella furcifera,* the white backed plant hopper is a serious pest of rice in many Asian countries. We have performed the phylogenetic reconstruction of the *S. furcifera* using the mitochondrial cytochrome oxidase subunit I (COI) gene. Phylogenetic reconstructions of the COI regions were done using Neighbor joining method. This study gives a clear picture of the mitochondrial genome of the insect and gives the phylogenetics of *S. furcifera* which help us to develop a sustainable and accurate strategy for management of the pest.
Keywords: Delphacidae, mitochondrial gene, COI, *Sogetella furcifera*, Neighbor joining method, Phylogenetics.

INTRODUCTION

The white-backed plant hopper, *Sogetella furcifera* is distributed widely throughout Asia and is considered a major pest of paddy in these regions. It comes under the order Hemiptera, suborder Auchenorrhyncha and family Delphacidae. Both the nymphs and adults sucks plant sap and reduce plant vigor, stunt, yellow leaves, delay tillering, and shrivel grains[1]. The heavy infestation may cause hopper burn which results in complete death of the rice plants[2]. *Sogatella* species are small plant hoppers easily recognized by the possession of a pale yellow or white stripe extending from vertex posteriorly on to the mesonotum (Figure 1). Males have dark frons with clypes and genae. *S. furcifera* can be easily identified from the other *Sogatella* species by structure of the male genitalia. The parameters are strongly dilated at base with relatively small apex which is almost equally bifurcated. The species shows high range of intra specific variations in several characters like intensity and extent of coloration and genital structures within the same population[3]. The female lays 100-350 eggs into stems or along the midribs of leaves after a pre ovi position period of 3-8 days. The hatched nymphs immediately start feeding, preferably at the base of the plant[3]. During the next 12-18 days, they pass through five stages, each resembling the adult a bit more than the previous one. The adult has a lifespan of 4-20 days. The insect has the ability to migrate long distance and cause sudden devastation to rice[4]. The studies on *S. furcifera* have focused mainly on biology[5] its occurrence[6], resistance of the pests and their interactions[7]. The population genetics and genetic diversity of the species have not been well studied, although some of the studies have been conducted in other genera. Knowledge of the genomic structure and analysis of the phylogenetics are essential for the development of rational control strategies. This study investigates the phylogenetic background of the *S. furcifera* from the selected paddy fields of Kerala, India.

MATERIALS AND METHODS

Collection and identification of samples

The area selected for the present study stretches along the paddy fields of northern Kerala, India. The *S. furcifera* were collected from the paddy fields by employing the sweep net technique and aspirator. Collected adult specimens were identified morphologically by consulting publish edtaxonomic keys and related literatures[8,9]. The collected specimens were stored at -20°C until the DNA was extracted.

Preparation of genomic DNA

One of the thoracic legs of the experimental insect, *S. furcifera* was amputated and homogenized using a glass pestle and mortar. The genomic DNA in the homogenate was isolated using Geni Ultrapure genomic DNA prep kit as per the manufacture's instruction.

Sequencing of the genomic DNA

2 ng of genomic DNA was amplified for COI gene using the appropriate forward primer 5′-CACCTGATATAGCTTTCCCCCG-3′ and reverse primer5′-TGCTCCTGCTAAAACTGGCA-3′. The PCR product was resolved on a 2 % TAE- agarose gel, stained with ethidium bromide[10]. To remove unincorporated primers and dNTPs the resultant PCR product was column purified using the nucleic acids purification kit of Gene JET™ of Fermantas Life Science. The purified PCR product was sequenced by Sanger's method using an ABI 3730XL genetic analyzer.

Translation and phylogenetic analysis

The translation of the consensus sequences was done using translation Table 5. The nucleotide sequence and peptide sequence were searched for its similarity using BLAST programme of NCBI. The evolutionary history of *S. furcifera* was inferred using the Neighbor-joining (NJ) method by MEGA6[11].

Table 1: Composition of nucleotides in each position of codon of the COI sequence of *Sogatella furcifera* and in the other related species

Species name	Nucleotide Frequencies in percentage															
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
LC005703|*Sogatella furcifera*	32.6	18.2	30.4	18.8	28	14.8	36.1	21.3	35	25.0	18.3	21.7	35	15.0	36.7	13.3
HM160139.1| *Nilaparvata lugens*	34.8	26.0	27.1	12.2	33	21.3	27.9	18.0	35	36.7	10.0	18.3	37	20.0	43.3	.0
HM160137.1| *Nilaparvata lugens*	34.8	26.0	27.1	12.2	33	21.3	27.9	18.0	35	36.7	10.0	18.3	37	20.0	43.3	.0
HM160136.1| *Nilaparvata lugens*	34.8	26.0	27.1	12.2	33	21.3	27.9	18.0	35	36.7	10.0	18.3	37	20.0	43.3	.0
KF836703.1| *Nilaparvata*	34.8	26.5	26.5	12.2	33	21.3	27.9	18.0	35	36.7	10.0	18.3	37	21.7	41.7	.0
KF836703.1| *Nilaparvata lugens*	34.8	26.5	26.5	12.2	33	21.3	27.9	18.0	35	36.7	10.0	18.3	37	21.7	41.7	.0
JX020555.1| *Typhlocybini* sp	33.1	17.7	33.7	15.5	31	18.0	29.5	21.3	38	30.0	13.3	18.3	30	5.0	58.3	6.7
JX433207.1| *Nesophyla* sp.	29.8	22.7	32.0	15.5	23	23.0	29.5	24.6	37	31.7	13.3	18.3	30	13.3	53.3	3.3
GU447053.1| *Locrisrubra*	30.9	19.9	33.1	16.0	28	19.7	31.1	21.3	38	26.7	15.0	20.0	27	13.3	53.3	6.7
FJ890836.1| *Graphocephala cythura*	35.4	20.4	29.3	14.9	31	18.0	26.2	24.6	40	30.0	13.3	16.7	35	13.3	48.3	3.3
gb|GU447047.1| *Locris maculata*	30.9	20.4	34.3	14.4	26	21.3	31.1	21.3	38	26.7	15.0	20.0	28	13.3	56.7	1.7
gb|KF273399.1| *Halyomorpha halys*	32.6	19.3	32.6	15.5	36	13.1	27.9	23.0	37	30.0	13.3	20.0	25	15.0	56.7	3.3
gb|HM347577.1| *Diaphorina citri*	35.9	21.5	29.8	12.7	33	18.0	31.1	18.0	42	23.3	16.7	18.3	33	23.3	41.7	1.7
gb|KF227085.1| *Linacephalus* sp	39.2	15.3	29.5	15.9	36	13.1	27.9	23.0	39	28.8	13.6	18.6	43	3.6	48.2	5.4
JX020555.1| *Typhlocybini* sp.	33.1	17.7	33.7	15.5	31	18.0	29.5	21.3	38	30.0	13.3	18.3	30	5.0	58.3	6.7
FJ890836.1| *Graphocephala cythura*	35.4	20.4	29.3	14.9	31	18.0	26.2	24.6	40	30.0	13.3	16.7	35	13.3	48.3	3.3
KF227085.1| *Linacephalus* sp	39.2	15.3	29.5	15.9	36	13.1	27.9	23.0	39	28.8	13.6	18.6	43	3.6	48.2	5.4
FJ849062.1| *Sophonia rufofasci*	27.9	17.3	39.7	15.1	20	11.5	41.0	27.9	29	29.3	27.6	13.8	35	11.7	50.0	3.3
Avg.	**33.9**	**21.0**	**30.6**	**14.5**	**31**	**18.1**	**29.7**	**21.4**	**37**	**30.8**	**13.8**	**18.4**	**34**	**14.1**	**48.4**	**3.5**

Table 2: Percentage of genetic divergence of *Sogatella furcifera* isolated from Kerala with related species

Species name	% of divergence
JX433207 *Nesophyla sp.*/USA	0.35
FJ890836 *Graphocephala cythura/ USA*	0.41
FJ890836 *Graphocephala cythura*/California	0.41
GU447053 *Locris rubra*/ USA	0.41
GU447047 *Locrisma culata*	0.45
KF273399 *Halyomorpha halys*	0.45
KF227085 *Linacephalus foveolatus*	0.48
KF227085 *Linacephalus foveolatus*/Australia	0.48
KF836703 *Nilaparvata lugens*/Tamilnadu	0.51
HM160139 *Nilaparvata lugens/ China*	0.51
HM160137 *Nilaparvata lugens/ China*	0.51
HM160136 *Nilaparvata lugens/ China*	0.51
HM347577 *Diaphorina citri/USA*	0.54
FJ849062 *Sophonia_rufofascia*/USA	2.29

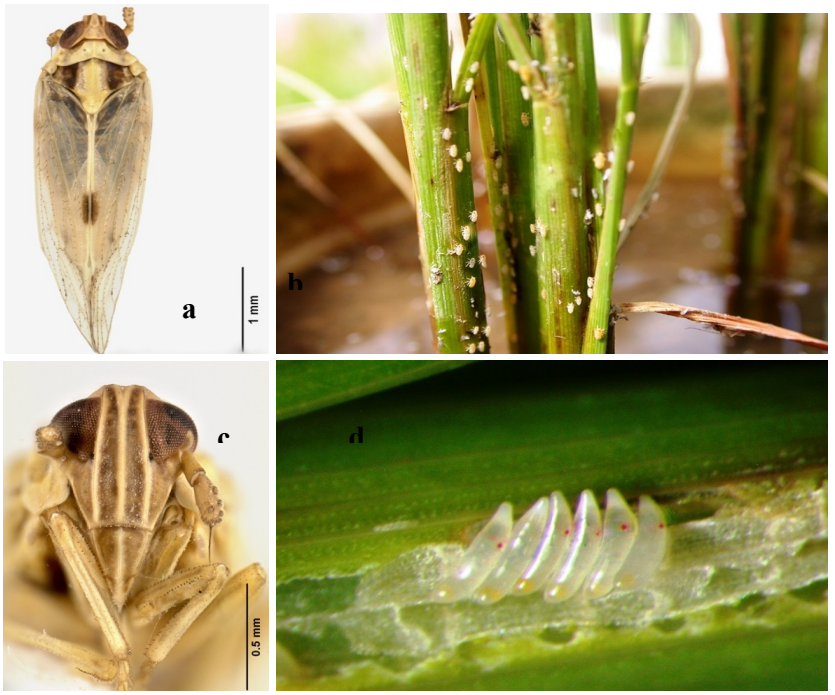

Figure 1: ***Sogatella furcifera.*** **(a) Dorsal view (b) Adults on paddy plant (c) Frontal view (d) Egg mass**

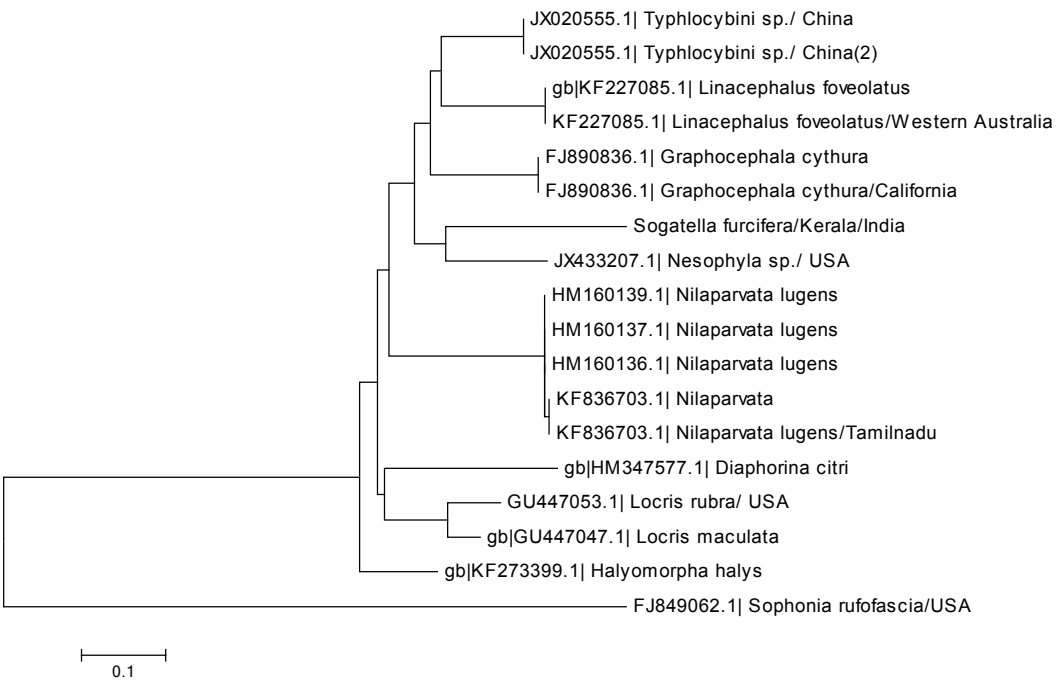

Figure 2: Phylogenic relationship of ***S. furcifera*** **isolated from Kerala, inferred by Neighbor joining method**

RESULT

The PCR product of the mitochondrial cytochrome oxidase I (COI) gene fragments of *S. furcifera* yielded a product of 338bp. The sequence is found to be novel and the same has been deposited in the DNA Bank of Japan (DDBJ) (Accession Number LC005703). The partial COI gene sequence of *S. furcifera* obtained in this study showed 77 % identities with *Nesophyla sp.* isolated from USA. The composition of nucleotides of the family Delphacidae showed clear bias to nucleotide 'AT'. The nucleotide composition analysis revealed the high AT content in the COI gene of *S. furcifera* is (63.0 %). The nucleotides T, C, A and G present in the COI sequence in the following concentrations T = 32.60 %, C = 18.20 %, A = 30.40 % and G = 18.80 % respectively (Table 1). The closer relative *Nesophylla* species shows a nucleotide composition of T = 29.80 %, C = 22.70 %, A = 32.0 % and G = 15.50 % respectively i.e., there is 2.8 % net difference in the concentration of nucleotide 'T'

between these two species. There is 2 % decrease in the concentration of nucleotide 'T' in the second position of the codon of *S. furcifera* compared to *Nesophyla* species which is 37.0 % and 35.0 % respectively. The nucleotide divergence analysis revealed that *S. furcifera* (India) COI sequence showed 0.35 % divergence from the COI sequence of *Nesophyla sp.* isolated from USA (Table 2). The phylogeny analysis using Neighbor-joining tree revealed the sharing of common ancestor. Phylogeny tree (Figure 2) provided by MEGA6 revealed that *S. furcifera* is arranged in one clad with *Nesophylla* sp on the other. The divergence time for the branches was calculated using Rel Time method[11] reveals a divergence of 0.09 times. The equality of evolutionary rate between sequences of *S. furcifera,* Kerala, India against *Nilaparvata lugens* (HM160139), were compared by taking *Nilaparvata lugens* (HM160137) used as an out group in Tajima's relative rate test[12]. Tajima's test results revealed that the configuration of identical sites in all three sequences count 117. The divergent sites in all the three sequences count is 0 and the unique differences in sequences 'A' counts 64. Therefore we can conclude that χ^2 test statistic was 64.00 ($P = 0.00000$ with 1 degree[s] of freedom). *P*-value less than 0.05 are often used to reject the null hypothesis of equal rates between lineages. The analysis involved 3 nucleotide sequences. There were a total of 181 positions in the final dataset[11].

DISCUSSION

The partial COI sequence generated in this study showed considerable variation with other species. The variation in the codons 'A' nucleotide composition in second position of COI sequence of *S. furcifera* (Kerala), India and *Nesophyla* sp. (USA) indicated that it has highest mutation rates. High proportion of 'T' in the second position of codon results in a preference of polar and hydrophobic amino acids in the membrane associated proteins[13]. The average inter specific genetic divergence of related species of *S. furcifera* ranged from 0-0.23 % and 0- 0.12 % respectively[14]. In migratory insect, genetic variation is found in all components of the migratory syndrome, and selection for migration results in a change in the frequency of expression of this components[15]. From a fundamental point of view, since genetic structuring of populations reflects the interaction of genetic drift, mutation, migration and selection. *S. furcifera* attracts their attention because of small size, short lifespan, large population sizes, rapid aerial population dilution and the very long distances over which these insects may fly[16]. Elucidation of genetic structure and evolutionary relationship of the Delphacidae can provide a wealth of information about the nature of ecosystems especially on prey and predator and pest and host interactions. The COI DNA barcode developed in this study can be used for the taxonomy and phylogeny analysis of the *S. furcifera*, for the study of insect host interactions and developing sustainable pest management strategies.

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REFERENCES

1. Khan ZR, Saxena RC. Electronically recorded waveforms associated with the feeding behaviour of *Sogatella furcifera* (Homoptera: Delphacidae) on susceptible and resistant rice varieties. Journal of Economic Entomology 1984; 77(6): 1479 -1482.
2. Pathak MD. Ecology of common pests of rice. Annual Review of Entomology 1968; 13: 257- 296. http://dx.doi.org/10.1146/annurev.en.13.010168.001353
3. Wilson MR, Claridge MF. Handbook for the identification of leafhoppers and plant hoppers of rice. CAB International for International Institute of Entomology, Wallingford, UK; 1991. p. 142.
4. Xian XQ, Zhai BP, Zhang XX, Cheng XN, Wang JQ. Tele connection between ENSO indicates and the early immigration of brown plant hopper: Implication for its medium-and long term forecast. Acta Ecologica Sinica 2007; 27(8): 3144-3154. http://dx.doi.org/10.1016/S1872-2032(07)60069-9
5. Xiao TG, Tang JX. Effects of the susceptibility of rice varieties to *Sogatella furcifera* on nymphal development and reproduction of *Microvelia horvathi* through a food chain. Ecological Entomology 2007; 14 (4): 317-321.
6. Seino H, Shiotsuki Y, Oya S, Hirai Y. Prediction of long distance migration of rice plant hoppers to northern Kyushu considering low-level jet stream. Journal of Agriculture Meteorolgy 1987; 43 (3): 203-208. http://dx.doi.org/10.2480/agrmet.43.203
7. Heinrichs EA, Rapusas HR. Levels of resistance to the White backed plant hopper, *Sogatella furcifera* (Homoptera: Delphacidae) in rice varieties with the same major resistance genes. Environmental Entomology 1985; 14 (1): 83-86.
8. Viraktamath CA. Key to the subfamilies and tribes of leafhoppers (Hemiptera; Cicadellidae) of the Indian Subcontinent. Bionotes 2005a; 7(1): 20 – 24.
9. Viraktamath CA. Key to the subfamilies and tribes of leafhoppers (Hemiptera; Cicadellidae) of the Indian Subcontinent. Bionotes 2005b; 7(2): 44 – 49.
10. Sambrook JF, Russell DW. Molecular cloning: A Laboratory manual. 3rded. New York: Cold Spring Harbor Laboratory Press; 2001. p. 2100.
11. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 2013; 30 (12): 2725-2729. http://dx.doi.org/10.1093/molbev/mst197
12. Tajima F. Simple methods for testing molecular evolutionary clock hypothesis. Genetics 1993; 135 (2): 599-607.
13. Yang L, Ding Y, Chen Y, Zhang S, Huo C, Wang, Y, Yu J, Zhang P, Na H, Zhang H. The proteomics of lipid droplets: structure, dynamics, and functions of the organelle conserved from bacteria to humans. Journal of Lipid Research 2012; 53(7): 1245–1253. http://dx.doi.org/ 10.1194/jlr.R024117
14. Mun JH, Song YH, Heong KL, Roderick GK. Genetic variation among Asian populations of rice plant hoppers, *Nilaparvata lugens* and *Sogatella furcifera* (Hemiptera: Delphacidae): mitochondrial DNA sequences. Bulletin of Entomological Research 1999; 89(3): 245-253. http://dx.doi.org/10.1017/S000748539900036X
15. Roff DA, Fairbairn DJ. The evolution and genetics of migration in insects. Bioscience 2007; 57 (2): 155–164. http://dx.doi.org/10.1641/B570210
16. Loxdale HD, Hardie JIM, Halbert S, Foottit R, Kidd NAC, Carter CI. The relative importance of short and long range movement of flying aphids. Biological Reviews 1993; 68 (2): 291–311. http://dx.doi.org/10.1111/j.1469-185X.1993.tb00998.x

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