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Genetic structure and phylogenetic status of rice black bug, scotinophara bispinosa (Hemiptera: Pentatomidae)

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Abstract The sap feeding rice black bug, Scotinophara bispinosa is a one of the major pest of paddy infesting its seedlings to flowering stage which causes severe damages to the wet-season Kharif crop of Kuttanadu, Kerala, India. The adults of S. bispinosa are black in colour and its nymphs and adults suck the sap from the rice stem. The identification of true bugs is relatively difficult using conventional taxonomic methods. Here we report the partial sequence of cytochrome oxidase sub unit I gene (COI) of rice black bug (GenBank accession No. JX469139) and its phylogenetic relationship. The COI gene sequence of S. bispinosa showed considerable variation with other true bugs. The COI DNA barcode developed in this study can be used for the accurate identification and for the study of insect host interaction.

Keywords: cytochrome oxidase subunit I, Scotinophara bispinosa, molecular phylogeny

Introduction

The rice black bug is a rice sap feeding insect belongs to the genus Scotinophara (Hemiptera: Pentatomidae). Miyamoto *et al*. (1983) reported 42 species of sap feeding black bugs. Among the six species of Scotinophara reported from India, Scotinophara bispinosa is the only species reported from Kuttanadu, Kerala, India (Narayanasamy, 2007) which causes severe damages (Fig. 1) to the wet-season Kharif crop (Ambikadevi, 1998, Sosamma *et al*. 1998).

Wongisiri (1975) made a detailed revision on the genus Scotinophara. Cytogenetic and isozyme polymorphism of the rice black bug was studied by Genil (2007). Torres (2008) reported the difference among the different rice black bug populations of Philippines. Torres *et al*. (2010) studied the systematic

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relationship of the rice black bug Scotinophara species using nonmetric multidimensional scaling techniques and parsimony analysis. Cruz *et al*. (2011) adopted geometric morphometrics to investigate the variability in the rice black bug pest.

Fig. 1. Rice black bug S. bispinosa infested paddy field; The brown patches is due to the S. bispinosa infestation.

Torres (2008) reported that some features of Philippines rice black bug were absent in Malaysian samples. He observed that the rice black bug from Philippine is comprised of many cryptic species and suspected that host insect interaction facilitated its evolutionary diversification. A critical analysis of the scientific literature by Krishnaiah *et al*. (2007) revealed the inadequacy of identifying rice black bug specimens up to species level. They also stressed that the taxonomic aspect should be the forerunner of research efforts on rice black bugs. The successful control of any pest is based on correct identification and ability to recognize distinct insect populations. Therefore further studies are essential to understand the population structure of this pest.

DNA barcoding using short nucleotide sequences are widely used for identification of various groups of organisms for their accurate identification. DNA barcoding also provide detailed information about genetic variation within the species. Short mitochondrial nucleotide sequence of genes like COI, COII, cytochrome oxidase subunit B are widely used for many insect species identification and evolutionary studies (Hebert *et al*., 2004; Lee *et al*., 2010). Molecular barcoding and phylogeny analysis of species of genus Scotinophara are not studied in detail despite its economic importance and hence this study.

Materials and methods

The adults of S. bispinosa were collected from the paddy field of Kuttanadu of Southern Kerala. The genomic DNA was extracted using GeNei Ultrapure Mammalian Genomic DNA Prep Kit (Bangalore GeNei, Bangalore) as per the Manufacturer's instruction. The partial gene sequence of COI of S. bispinosa was PCR amplified using the forward primer with DNA sequence 5'CATTGGAGATGACCAAATTTATAATG3' and the reverse primer with DNA sequence 5' TAAACTTCAGGGTGACCAAAAAATCA 3'. The PCR reaction mixture consisted of 2 nanogram of genomic DNA in 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 temperature profile consisted of 95^0C/3 minutes as initial denaturation and followed by 45 cycles of 95^0C/10 seconds, 50^0C/45 seconds, 72^0C/45 seconds and with a final extension of 72^0C for 3 minutes. The PCR amplified 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 product was sequenced with forward and reverse primers using the Sanger's sequencing method at SciGenom Labs, Cochin. The forward and reverse sequence was aligned and the consensus sequence was used for analysis. The phylogeny analysis was done using the MEGA5 software.

Results

The adults of S. bispinosa are black in colour. Both nymphs and adults suck the sap from the rice stem and infest rice plant from seedling to flowering stage. Usually eggs are laid during the night on the upper surface of the rice leaf blade and when the infestation is severe, they are laid on the stem and other parts. They are laid in two parallel rows, 14 to 24 eggs per egg mass. When eggs mature, they turn deep orange red (Fig. 2).

The partial COI sequence of S. bispinosa (GenBank accession No. JX469139) obtained in this study showed 85% similarity with Scotinophara. scotti isolated from Korea. The composition of nucleotides in each codon position showed clear bias to nucleotide 'AT' (84%) in the second position of codon of both species of S. bispinosa and S. scotti. There is 11% decrease in the concentration of nucleotide 'A' in second position of codon in S. bispinosa compared to S. scotti which is 47.4% and 58.5% respectively.

The nucleotide divergence analysis revealed that, the S. bispinosa COI sequence showed 16.71% divergence from the COI sequence of S. scotti. The phylogeny analysis using NJ tree revealed the sharing of common ancestor of

these two species (Fig. 3). Among the COI sequences of true bugs used in this study, Carbula inocia and Halyomorpho haly was the nearest relative of genus Scotinophara. The branch length of S. bispinosa was less compared to the S. scotti indicating less divergence of S. bispinosa from their ancestor.

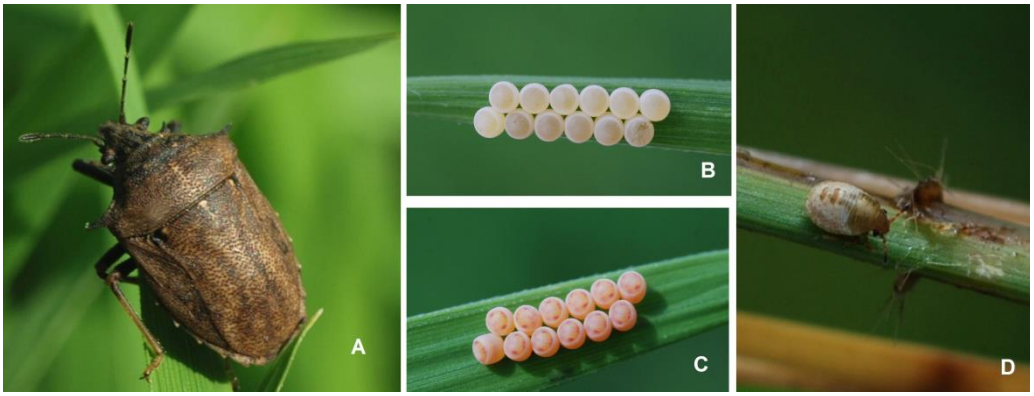

Fig. 2. The different life stages of the Rice black bug S. bispinosa A: Adult; B: Egg ; C: Mature Egg; D: Nymph.

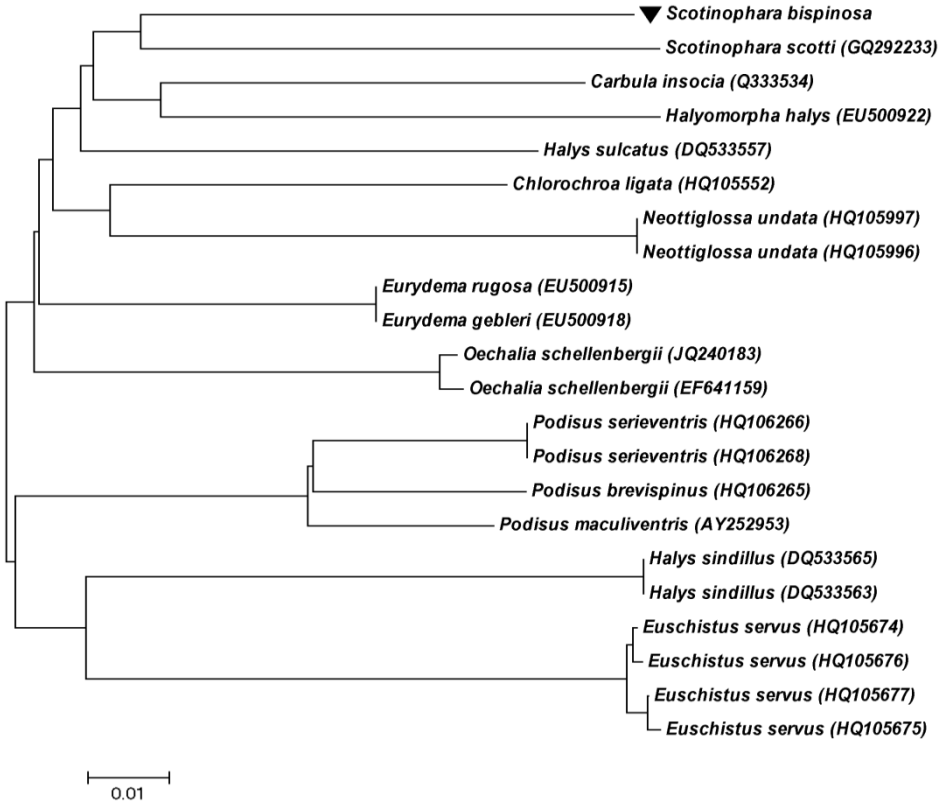

Fig. 3. Phylogenic relationship of S. bispinosa inferred by NJ tree method

Discussions

Partial coding sequence of COI was proved as a powerful tool for the identification of organisms (Hebert *et al*., 2004a). 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. bispinosa and S. scotti 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 membrane associated proteins (Yang *et al*., 2012). Recent reports showed the interspecific divergence of true bugs is 16 times higher to that of intraspecific genetic divergence. The average interspecific and intraspecific genetic divergence of related species of true bugs of Korea and adjacent countries are reported as 6.30% and 0.40% respectively (Jung *et al*., 2011). The high interspecific distance observed between S. bispinosa and S. scotti may be due to the geographical isolation of these species.

The heteropterans play a major role in the ecosystems and in agriculture as a pray or predator or pest or as a biocontrol agent (Schuh and Slater, 1995, Lattin, 1999). The identification of true bugs was tedious without the help of the taxonomic specialist (Jung *et al*., 2011). Elucidation of genetic structure and evolutionary relationship of the heteropteran organisms 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. bispinosa and for the study of insect host interactions.

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