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PHYLOGENETIC STATUS OF RICE DARK HEADED STEMBORER, *CHILO POLYCHRYSUS*

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ABSTRACT

Generally, stemborers were considerd as the most serious pest of the paddy. The stemborer larvae enter to the tiller and feed inside, which leads to characteristics damage symptoms of 'dead hearts' or 'white ears. Five pyralid rice stem borer species were reported from India and the phylogenetic relationship of them was not yet studied in detail. In this study we have determined the phylogenetic status of rice dark headed stemborer Chilo polychrysus using Cytochrome oxidase subunit I (COI) partial coding sequence. The COI sequence of C. polychrysus showed 9%, 10 and 13% divergence with stem-borers viz. C. suppressalis, Scirpophaga excerptalis and Scirpophaga innotata respectively. Interestingly it showed only 6-8% divergence with many species of different genus of Lepidotera family. Phylogenetically Lampides boeticus, Maculinea ario, Oleria olerioides, Oleria fumata, Greta andromica and Pseudoscada florula are the nearest relative of C. polychrysus. In the phylogeny tree the C. polychrysus and C. suppressalis were not aligned in a same clad. Therefore, further studies are needed to determine the taxonomic position of C. polychrysus.

KEY WORDS

Cytochrome oxidase subunit I, Phylogeny, Divergence, Chilo polychrysus

INTRODUCTION

The stem borers infest the paddy from the seedling to maturing stage and they are generally considered as the most serious pest of the rice world. About fifty species in three families comes under the stem borer group of the rice. They are family Pyralidae, Noctuidae (Lepidoptera) and Diposidae (Diptera). Among the stem borers the pyralid borers are the most common and destructive [1, 2]. The stem borer infestation results in characteristics symptoms of 'dead hearts' or 'white ears' depending upon the stage of the crop. The stem borer larvae enter the tiller and feed inside, which leads to the damage of dead heart. The stem borer infestation at the grain filling stage leads to the stoppage of the further grain filling and that cause the formation of partially filled grains [3].

The lepidopteran stem borers lay eggs in masses containing 50-80 eggs and a single female of the lepidopteran stem borer can lay up to 100-200 eggs. The freshly hatched larvae are highly negatively geotropic and curl up ward to the tip of the plant. They show strong tendency to disperse and with the help of the silken thread they reach neighbouring plant. Later they curl down and enter the leaf sheath through a common

hole and they start to feed. After one week they bore the stem and feed inside. Before pupating the fully grown up larvae makes an exit hole in the internodes and it is covered with fine web. The emerged adult comes out through this hole. The pupation of the stem borer usually takes place in the straw. During the unfavourable condition, most of the stem borer species can pass through more profound physiological change that helps the stem borer to live for months in dormancy stage called diapauses [1, 2].

It is difficult to control the stem borers using the chemical insecticides, because after hatching, the larvae are present only for few hours on the outer surface of the leaves. Farmers depends on many chemical insecticides to control the stem borers, but many of them are not effective [1, 2].

The study on the genetic structure of various stem borers has been reported recently. Moyal *et al*. [4] studied the systematics and molecular phylogeny of two African stem borer genera using cytochrome oxidase sub unit 1 (COI) partial coding sequence. The origin and taxonomic status of the stem borer *Sesamia nonagrioides* was explained using nuclear and mitochondrial DNA [5].

Five rice stem borer species were reported from India. They are *Scirpophaga incertulas* (yellow stem borer), *Sesamia inferens* (pink stem borer), *Scirpophaga innotata* (white borer), *Chilo polychrysus* (dark headed stem borer) and striped stem borer *Chilo suppressalis* [3]. Among them yellow stem borer, white stem borer and dark headed stem borers were found in the paddy field of Kerala. In this study we have studied the phylogenetic status of dark headed stemborer, *Chilo polychrysus* using partial coding sequence of Cytochrome oxidase Sub-unit I (COI).

MATERIALS AND METHODS

The adult insects were collected from the paddy field of Alappuzha, Kerala, India and the genomic DNA was isolated using GeNei Ultrapure Mammalian Genomic DNA Prep Kit (Bangalore GeNei, Bangalore) as per the Manufacturer's instruction.

The COI gene partial coding sequence was amplified using the forward primer with DNA sequence 5'CATTGGAGATGACCAAATTTATAATG 3' and the reverse primer with DNA sequence of 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 profile consisted of an initial denaturation step of 5 min at 95^0C, followed by 35 cycles of 10s at 95^0C, 10s at 55^0C and 1 min at 72^0C and ending with a final phase of 72^0C for 3 min. The PCR products were resolved on a 1% TAE- agarose gel, stained with EtBr and photographed using a gel documentation system. After ascertaining the PCR amplification of the corresponding COI fragment, the remaining portion of 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 used for the PCR using the Sanger's sequencing method at Xcelris Laboratories Ltd., Ahmedabad. The forward and reverse sequences obtained were trimmed for the primer sequences, assembled by using Clustal W and the consensus was taken for the analysis. The evolutionary history was inferred using the Neighbor-joining method by MEGA5.

RESULTS AND DISCUSSION

Dark headed stem borer *C. polychrysus* under the family Crambidae has seen in many part of Kerala, India and it often attain major pest status in Kerala. The forewing of adult *C. polychrysus* is brownish ochreous with a raised dark metallic spot in the cell, a series of small black dots covered with golden scales (Fig. 1).

The PCR amplification of partial mitochondrial COI gene of *C. polychrysus* yielded a single product with about 600 bp in size. The sequence obtained after removing the primers used for PCR amplification was submitted to GenBank of NCBI (Accession No. KC631647). The COI nucleotide sequence analysis of *C. polychrysus* showed the **AT** bias with following concentration of nucleotides T=41.10, **A=** 30.9, C=15.40 and G= 12.70. It is also noted that the *C. polychrysus* has high **AT** content than the average **AT** content of related species of Order Lepidoptera. The COI nucleotide sequence analysis also revealed that the variation in the overall frequency distribution of the nucleotides in each position of codon of *C. polychrysus* compared to that of other related species of Order Lepidoptera.

Figure. 1. Rice dark headed stemborer, *C. polychrysus*

Table - 1. Percentage of genetic divergence of *C. polychrysus* isolated from Kerala with the related species of Order Lepidoptera. The GenBank accession numbers are given in parenthesis.

Name of Species	% of divergence
Phostria sp. (JQ556864)	6%
Phostria sp. (JQ573111)	6%
Cydalima nr. pfeifferae (JX970251)	7%
Udea delineatalis (JF497043)	7%
Desmia sp. (JQ564908)	7%
Desmia sp. (JQ573562)	7%
Glyphodes perspectalis (JN087377)	7%
Aetholix flavibasalis (JX970157)	7%
Archon apollinus (DQ351031)	8%
Glyphodes perspectalis (AB751247)	8%
Greta andromica (DQ157496)	8%
Herpetogramma salbialis (JQ602435)	8%
Herpetogramma salbialis (JQ538995)	8%
Maculinea arion (HQ918149)	8%
Pseudoscada florula (EU069101)	8%
Archon apollinaris (DQ351033)	8%
Omiodes humeralis (JX017896)	8%
Phostria latiapicalis (JQ533114)	8%
Cydalima sp (HM906190)	8%
Udea languidalis (JF497052)	8%
Chilo suppressalis (HQ860290)	9%
Oleria olerioides ssp. (FN646256)	9%
Oleria fumata (FN651622)	9%
Lampides boeticus (EU919321)	9%
Chilo suppressalis (JF339041)	9%
Chilo suppressalis (AB238202)	9%
Scirpophaga excerptalis (KC306948)	10%
Scirpophaga innotata (AB495264)	13%

The genetic divergence analysis of COI sequence clearly depicts the genetic relationship of the *C. polychrysus* isolated from Kerala with the related species of Order Lepidoptera (Table - 1). The COI sequence of *C.*

polychrysus isolated from Kerala showed 9% divergence with *C. suppressalis*. But it showed only 6-8% divergence with many species of different genus of Lepidotera family. The rice stemborer *C. polychrysus* showed 10 and 13% divergence with the stemborers *Scirpophaga excerptalis* and *Scirpophaga innotata* respectively.

The phylogenetic analysis using NJ tree method revealed the phylogeny of *C. polychrysus* isolated from Kerala (Fig.2). *Lampides boeticus, Maculinea ario, Oleria olerioides, Oleria fumata, Greta andromica* and *Pseudoscada florula* are the nearest genetic relative of *C. polychrysus*. *C. polychrysus* is not arranged in the clad of Stemborers like *C. suppressalis, Scirpophaga excerptalis* and *Scirpophaga innotata.*

The nucleotide composition analysis clearly revealed that the **AT** composition of the *C. polychrysus* is less to the mean observed **AT** composition (77%) of Mt DNA of insects [6]. But it has clear bias to the **AT**. The **AT** composition bias with low **G** in the sense and **C** in the template strand may have arisen through directional mutational pressure [7]. The analysis of nucleotides in each position of codon revealed the high **AT** composition in the third position of codon (95.30%) compared to the first (64.50%) and second position (58%). In many insect species the second position of codon contains high **AT**. The high content of the **T** in the second position of codon may be related to the preference for non-polar and hydrophobic amino acids in the membrane associated proteins [8]. But in the case of *C. polychrysus* which contains less **AT** in the second position of codon.

The *C. polychrysus* showed high variations in the third position of codon compared to first and second position of codon of other related species of Order Lepidoptera. This variation may be due to the compulsion in the nucleotide changes in the different codon positions. 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 [9, 10]. The variation in the first and second positions of codon leads to morphological variation within the family.

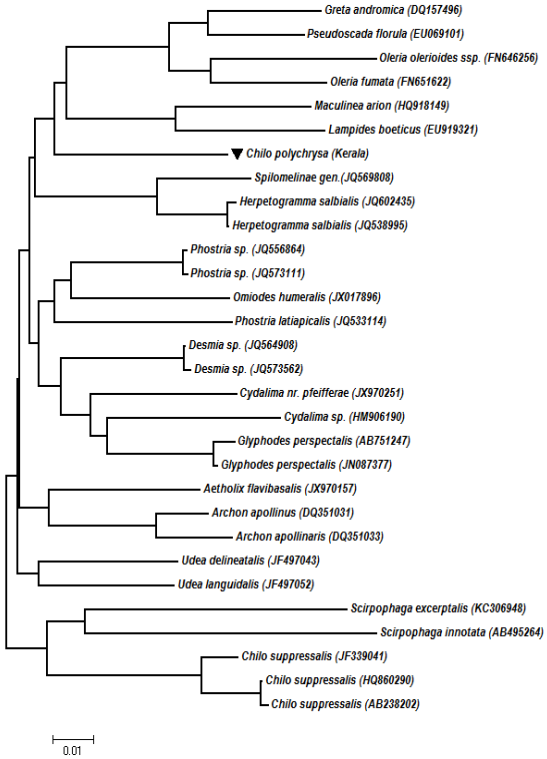

Figure 2. Phylogenic relationship of *C. polychrysus* isolated from Kerala, inferred by NJ tree method of MEGA5 software. The GenBank accession numbers are given in parenthesis.

The COI sequence of *C. suppressalis* contains unusual **CAG** repeats [11]. Compared to *C. suppressalis* the **CAG** repeats in the *C. polychrysus* are found less. In the phylogeny tree the *C. polychrysus* and *C. suppressalis* were aligned in different clad. Usually in the phylogeny tree the related species were aligned as nearest relative in the same clad. Therefore, further studies are needed to determine the taxonomic position of *C. polychrysus*. The COI sequence of *C. polychrysus* showed considerable variation with other species analyzed in this study therefore it can be used as DNA barcode for the accurate identification.

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