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Molecular Phylogenetic Study of *Bactrocera tau* using Mitochondrial Cytochrome Oxidase Subunit I Gene Sequence

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Abstract: ***Diptera, order of winged insects is commonly known as flies. They are one of the most successful groups of organisms on Earth. They are very diverse biologically and occupies virtually every terrestrial niche. Many have co-evolved in association with plants and other invertebrates. Bactrocera tau is one of the most severe and economically important agricultural pest. B.tau, "devastating pest of cucurbits" is a serious pest in many of the Asian countries. The damage is done by the B.tau larvae that feeds in the fruit. We have developed the phylogenetic reconstruction and analysis of the B. tau (Genbank Accession No. KX603660.1) using mitochondrial cytochrome oxidase subunit I (COI) gene. The knowledge of the dipteran genomic structures will create new method of integrated pest management and will contribute for the sustainable agriculture and maintenance of biodiversity.***

Keywords: *B.tau*, COI, biodiversity, pest, dipteran, cucurbits

1. Introduction

Bactrocera (Zeugodacus) tau (Walker), is one of the serious pests of cucurbitaceae. The larvae are phytophagous. Females deposit eggs in living, healthy plant tissue using their telescopic ovipositors. The larvae find their food upon emerging. The larvae develop in leaves, stems, flowers, seeds, fruits, and roots of the host plant, depending on the species.

This species was found to be of regular occurrence and active during late summer. Pest management tools mainly rely on proper identification of arthropod species. However, keeping in mind the shortcomings and limitations of the conventional taxonomical identification methods of pest identification, DNA barcoding is used. A major feature of DNA barcoding is that it allows prompt identification of pest young instars, as well as of the fragmentary cuticular body parts. Partial DNA sequences of the mitochondrial gene such as Cytochrome oxidase I (COI) and other molecular markers have been used to identify and discover new species. The mitochondrial DNA has been extensively analysed [1] and proven to be an important tool in species delimitation as it possesses biological properties making it suitable as a marker for molecular biodiversity [2] and [3]. DNA barcoding has proved to be a versatile tool with a variety of applications, for example, by facilitating the association between different developmental stages in insects.

The molecular phylogenetic analysis using the mitochondrial COI gene sequences were carried out by many workers in varied group of organisms such as *Culex quinquefasciatus*[4], *Armigeres subalbatus*[5], green bottle fly, *Lucilia sericata*[6], cockroaches[7] and odonates[8].

2. Materials and Method

Bactrocera (Zeugodacus) tau (Walker) used in the present study was collected from Malappuram district in Kerala, India (Figure 1).

Figure 1: *Bactrocera tau*

Mitochondrial genomic DNA was extracted from the experimental insect. The tissue was homogenized using a glass pestle and mortar. The genomic DNA in the homogenate was extracted using a GeNei Ultrapure Mammalian Genomic DNA Prep Kit in accordance to the manufacturer's instructions. About 2 ng of genomic DNA was amplified for mitochondrial cytochrome oxidase subunit I (COI) gene using the forward primer with DNA sequence 5'- GGTCAACAAATCATAAAGATATTGG -3' and reverse primer with DNA sequence 5'- TAAACTTCAGGGTGACCAAAAAATCA -3'. The PCR reaction mixture consisted of 2 ng of genomic DNA, 1µl each forward and reverse primers at a concentration of 2.5 µM, 2.5 µl of dNTPs (2mM), 2.5 µl of 10X reaction buffer, 1.20 µl of Taq polymerase (3U/µl) and 11.8 µl H_2O. The PCR profile consisted of an initial denaturation step of 2 minutes at 95°C, followed by 30 cycles of 5s at 95°C, 45s at 50°C and 45s at 72°C and ending with a final phase of 72°C for 3 minutes. The PCR products were resolved on a 1% TAE-agarose gel, stained with Ethidium Bromide and photographed using a gel documentation system. After ascertaining the PCR amplification of the corresponding COI fragment, the remaining portion of the PCR products were column purified using Mo Bio Ultraclean PCR Clean-up Kit (Mo Bio Laboratories, Inc. California) as per the manufacturer's instructions. The purified PCR

products were sequenced from both ends using the forward and reverse primers used for the PCR using Sanger's sequencing method (Sanger, 1975)[9]. The forward and reverse sequences obtained were trimmed for the primer sequences, assembled by using ClustalW and the consensus was taken for the analysis. The nucleotide sequence and peptide sequence were searched for its similarity using BLAST programme of NCBI (www.ncbi.nlm.nih.gov/) and Inter and intra specific genetic diversity were calculated using Kimura 2-parameter model with the pair wise deletion option and the difference in the nucleotide in codon usage partial COI sequence of *Bactrocera tau* using MEGA6 software.

3. Results and Discussion

The PCR of the COI gene fragment of *Bactrocera tau* yielded product size of 598 bp. The BLAST search using the sequences revealed that the sequences obtained in this study was novel. The evolutionarily close relative of *Bactrocera tau* is *Bactrocera cucurbitae* (Genbank Accession No. DQ116246.1) from Newzealand submitted to NCBI.

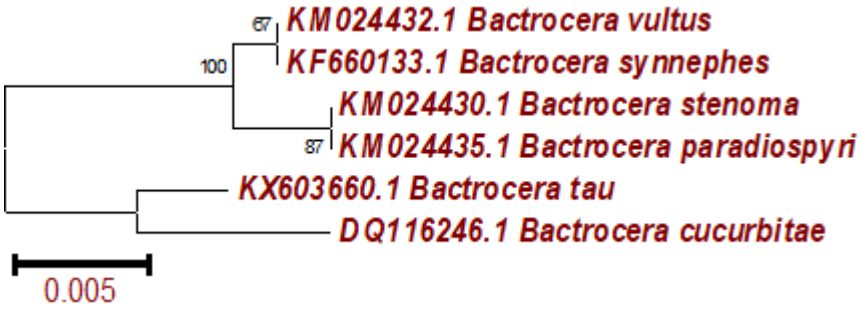

Figure 2: Comparison of phylogenetic status of *Bactrocera tau* using nucleotide

Table 1: The Evolutionary Nucleotide Divergence of *Bactrocera tau* with other related Species

KX603660.1 *Bactrocera tau*	
DQ116246.1 *Bactrocera cucurbitae*	1.1
KM024432.1 *Bactrocera vultus*	3.8
KF660133.1 *Bactrocera synnephes*	3.8
KM024430.1 *Bactrocera stenoma*	4.0
KM024435.1 *Bactrocera paradiospyri*	4.0

The evolutionary history was inferred using the Neighbor-joining method using COI partial sequence. The analysis of the evolutionary history of *Bactrocera tau* was done using the Neighbor- joining method (Figure 2).

4. Conclusions

Variation in the nucleotide is a fundamental property of all living organisms which can be used for the identification and phylogenetic status assessment. The COI sequence obtained in this study showed nucleotide variation between *Bactrocera tau* and *Bactrocera cucurbitae* (Genbank Accession No. DQ116246.1) to be 1.1% (Table. 1). BLAST result concludes that COI gene sequence of *B.tau* was found to be novel. The genetic level identification remains a prospect although COI divergence appear too low to regularity in enabling species diagnosis within the insects. Phylogeny analysis using NJ tree revealed the sharing of common ancestor for various species and is found to be in a diverged clade. The phylogenetically close species of *Bactrocera tau* is *Bactrocera cucurbitae* (Genbank Accession No. DQ116246.1). Inter specific divergence of partial coding fragment of COI gene is very efficient for species identification (Hebert *et al*., 2003)[10].

The present study suggests that the best phylogenetic inferences can be generated through moderately divergent nucleotide database from mitogenomes, among which the COI gene is best studied.

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