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Genetic Structure and Phylogeny Status of Rice Blue Beetle *Leptispa pygmaea* (Coleoptera: Chrysomelidae)

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Abstract- Rice blue beetle, *Leptispa pygmaea* is a pest of paddy which feed on paddy leaves by scraping the chlorophyll content. The rice blue beetle has been reported to be a pest of paddy attaining major pest status in many districts of North Kerala. No much work has been reported on this emerging pest. Here we report the partial DNA sequence of cytochrome oxidase subunit I (COI) of *L. pygmaea* (GenBank Accession No. KC427085) and its phylogenetic status. Phylogeneticaly the nearest relative of *L. pygmaea* are *Colasposoma sp. Neolochmea dilatipenni*, *Chelymorpha alternans*.

Index Terms- : cytochrome oxidase subunit I, *Leptispa pygmaea*, phylogeny

I. INTRODUCTION

Rice blue beetle, *Leptispa pygmaea* is a pest of paddy which feeds on the leaves by scraping the chlorophyll matter, resulting in folding and drying of the leaf [1]. Taxonomically *L. pygmaea* comes under the family Chrysomelidae, subfamily Cassidinae and the genus Leptispa. Burlow [2] reported its status as a pest of paddy. Later many researchers reported the infestation of *L. pygmaea* as minor pest of paddy [3-5]. Recently it has been reported that, *L. pygmaea* is a serious pest of paddy and attaining major pest status in Northern Districts (Palakkad, Kannur and Kasaragod) of Kerala, India [6- 7].

Fletcher [8] has illustrated the adult and immature stages of *L. pygmaea*. Various studies has documented the duration of different life stage of *L. pygmaea* as follows, 3-7 days for egg, 6-14 days for larval instars, 2-5 days for pupa and completed its life cycle within 12-24 days [9,10,6,11] . Pratabhan et al. [12] described the natural history and leaf shelter construction of *L. pygmaea*. They have reported that the leaf roll formed by the adult was incomplete and provide limited protection to its egg. But the leaf roll formed by the larvae is complete and provide good protection. Karthikeyan and Jacob [7] reported that, the eggs of *L. pygmaea* are oval in shape and its larval stage consists of five instars. The pupae are attached to the leaf by its posterior end. The adults of *L. pygmaea* are metallic greenish yellow in colour and with longer antennae, narrow thorax and long body (Figure 1).

Genetic data on *L. pygmaea* is not available in spite of its importance as a major pest of paddy. Here we report partial sequence of its mitochondrial cytochrome oxidase subunit I (COI) gene and its phylogenetic status.

Figure 1: *Leptispapygmaea*, rice blue beetle adult

II. MATERIALS AND METHODS

The genomic DNA of *L. pygmaea* was isolated using GeNei Ultrapure Mammalian Genomic DNA Prep Kit (Bangalore GeNei, Bangalore) as per the Manufacturer's instruction. 2 nanogram of genomic DNA was amplified for COI gene using the forward primer with DNA sequence 5'-ATTCAACCAATCATAAAGATATTGG-3'and reverse primer with DNA sequence 5'-CTCCACCAGCAGGATCAAAA-3'. The PCR reaction mixture consisted of 2 nanogram of genomic DNA (1 µl), 1 µl each forward and reverse primers at a concentration of 10 µM, 2 µl of dNTPs (2 mM), 2 µl 10X reaction buffer, 0.20 µl Taq polymerase (5 U/µl) and 12.8 µl H_2O. The PCR profile consisted of an initial denaturation step of 3 min at 95^0C, followed by 35 cycles of 10 sec at 95^0C, 10 sec at 50^0C and 45 sec at 72^0C and ending with a final phase of 72^0C for 3 min. 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 SciGenom Laboratories Ltd., Cochin. The forward and reverse sequences obtained were trimmed off the primer sequences, assembled by using ClustalW and the consensus was taken for the analysis. The nucleotide sequence was searched for its similarity using BLAST programme of NCBI (www.ncbi.nlm.nih.gov). MEGA5 software was used for the phylogeny analysis.

III. RESULTS

The PCR of the COI gene fragment of *L. pygmaea* yielded a single product of 695 bp. The BLAST search using the sequence revealed that the sequence obtained in this study was novel (GenBank Accession No. KC427085). The evolutionary divergence of *L. pygmaea* in Chrysomelidae family is given in table 1.

Table 1: Evolutionary divergence between COI sequences of the species *L. pygmaea* and other species of family Chrysomelidae.

S. No.	Name of species with GenBank Accession No.	% of divergence
1.	*Neolochmea dilatipennis* (AY242519)	14.06%
2.	*Colasposoma sp.* (AY242397)	14.33%
3.	*Monolepta sp.* (AY242414)	15.61%
4.	*Chelymorpha alternans* (AY563962)	15.81%
5.	*Diorhabda sublineata* (JQ782481)	16.67%
6.	*Pyesia sp.* (AY242501)	16.91%
7.	*Paratriarius subimpressa* (AY242461)	17.40%
8.	*Ivalia sp.*(DQ080038)	17.64%
9.	*Aulacophora lewisii* (AY796208)	18.26%
10.	*Aulacophora lewisii* (AY242434)	18.26%
11.	*Cerotoma atrofasciata* (AY533587)	18.36%
12.	*Diabrotica viridula* (FJ039875)	18.83%
13.	*Masurius nr. violaceipennis* (AY242500)	18.83%
14.	*Kytorhinus thermopsis* (HQ177507)	18.93%
15.	*Amphelasma cavum* (AY533590)	19.24%
16.	*Tuberculobruchus albizziarum* (AY625422)	19.37%
17.	*Diabrotica undecimpunctata* (AF278556)	19.50%
18.	*Paranapiacaba significata* (FJ039870)	19.59%
19.	*Cerotoma arcuata* (FJ039866)	19.74%
20.	*Bruchus rufimanus* (AY997313)	20.29%
21.	*Stator limbatus* (AY997329)	20.77%
22.	*Mimosestes ulkei* (AB499964)	20.80%

The composition of nucleotides of *L. pygmaea* in each codone position was analyzed and compared with other species of Chrysomelidae family. The result indicated that the composition of each nucleotide in COI sequence of *L. pygmaea* showed similarity with other species of same family. But the use of nucleotides in each codone position showed difference in the COI sequence of *L. pygmaea* compared to the other species of same family. The average value of nucleotide 'A' used in the first position of codone is 27.80%, but in *L. pygmaea* it showed 33.10%.

The evolutionary history of *L. pygmaea* was inferred using the Neighbor-Joining method (Figure 2). Phylogeneticaly *Colasposoma sp.*, *Neolochmea dilatipenni* and *Chelymorpha alternans* are the nearest relative of *L. pygmaea.*

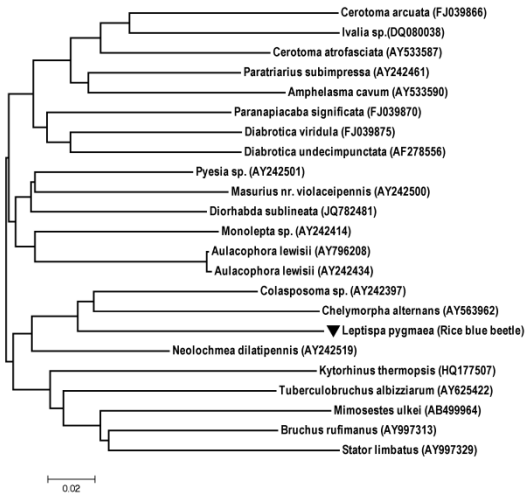

Figure 2: Phylogenetic status of *Leptispa pygmaea* in the family Chrysomelidae. The evolutionary history was inferred using the Neighbor-joining method using COI partial sequence.

IV. DISCUSSION

Variation in the nucleotide sequence is a fundamental property of all living organisms which can be used for their identification and phylogenetic status. DNA barcoding provide rapid and automatable species identification by short standardized DNA fragment as a species tag and its makes the Linnaean classification system more accessible [13].The COI sequence obtained in this study showed variation with other species of the same family. Pointing its use as a DNA barcode to identify the species. The COI sequence can also be used for evolutionary studies and host insect relation studies of *L. pygmaea*. Higher 'AT' content observed in many insect species [14-16]. The *L. pygmaea* and other species of Chrysomelidae family also have higher AT content.

Gurney et al. [17] reported that closely related species have 90% similarity in the standardized DNA sequence and distantly related species have 90% similarity in the same sequence. The *L. pygmaea* in the family Chrysomelidae showed 86-79% similarity with other species of same family. Hence it is clear that the results obtained in this study also justify the above view that, the *L. pygmaea* is coming under the family Chrysomelidae.

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