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Illumina based whole mitochondrial genome of *Junonia iphita* reveals minor intraspecific variation

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ABSTRACT

In the present study, the near complete mitochondrial genome (mitogenome) of *Junonia iphita* (Lepidoptera: Nymphalidae: Nymphalinae) was determined to be 14,892 bp. The gene order and orientation are identical to those in other butterfly species. The phylogenetic tree constructed from the whole mitogenomes using the 13 protein coding genes (PCGs) defines the genetic relatedness of the two *J. iphita* species collected from two different regions. All the *Junonia* species clustered together, and were further subdivided into clade one consisting of *J. almana* and *J. orithya* and clade two comprising of the two *J. iphita* which were collected from Indo and Indochinese subregions separated by river barrier. Comparison between the two *J. iphita* sequences revealed minor variations and Single Nucleotide Polymorphisms were identified at 51 sites amounting to 0.4% of the entire mitochondrial genome.

1. Introduction

The Indomalayan region (mainland tropical Asia) is divided into Indian and Indochinese sub-regions and earlier studies reported that the species from both the regions have independent radiation [1,4,6,8] as well as hybridization [5]. The river Brahmaputra passing through Bangladesh and Northeast India forms the boundaries of Indian and Indochinese subregions, respectively. Therefore, it would be interesting to determine if intraspecific sequence variation occurs in species of Indian and Indochinese subregions in the case of butterflies.

Mitochondrial DNA has become popular in phylogenetic, comparative and evolutionary genomics, population genetics and molecular evolution studies among various animal taxa [3]. In particular, the full-length mitogenome sequences have phylogenetic utility within several insect lineages including Lepidoptera [7]. Many biological studies like ecology, conservation, evolutionary and developmental studies have been done in the lepidopteran family as they are present all over the world [2]. The Nymphalidae butterfly family are the most diverse with 12 subfamilies, 559 genera and 6152 species [14] and for the present study, *Junonia iphita*, a well-known group of brush-footed butterflies was selected. We examined the presence of intraspecific variation within *J. iphita* collected from the Indian and Indochinese sub-regions i.e. North and South of Brahmaputra River by comparing the mitochondrial genomes.

2. Materials and methods

Adult butterfly *J. iphita* (JI_AZ) was collected from Rangpo, Sikkim, Northeast India from the North of Brahmaputra river. DNA was extracted from the leg of butterflies based on the extraction method [15]. The leg was washed with distilled water, dried and macerated with the help of scissors in 1.5 ml eppendorf tube and homogenized with pestle and mortar and 250 μl of extraction buffer (50 mM TrisHCl, 25 mM NaCl, 25 mM EDTA, 1% SDS) was added and mixed gently. Two μl of proteinase *k* (20 mg/ml) was added and incubated in an oven at 56 °C for 30 min. 250 μl of Phenol/Chloroform (1:1) was added, mixed gently and centrifuged at 13,000 rpm for 5 min. The Supernatant was carefully pipetted out and collected in a new eppendorf tube. 450 μl of absolute ice cold ethanol was added to the supernatant and mixed gently by inverting the tube several times and kept in 20 °C for 30 min. The sample was centrifuged at 13,000 rpm for 5 min. at 4 °C. Ethanol was poured off without dislodging the pellet, and 200 μl of 70% ethanol was added, flash spin at 6000 rpm for 1 min. The ethanol was poured off and the pellet was dried in an oven for 5 min. 30 μl of distilled water was added to the tube; the pellet was re-suspended by gently flicking the tube and was stored at 20 °C for further use.

The near complete mitochondrial genome sequence of *J. iphita* (Sikkim, India) was obtained through Illumina MiSeq platform (Scigenome, Cochin, India). The raw data obtained was subjected to quality checking based on base quality score distribution, average base content per read and GC distribution. Based on the quality score, the fastq files were trimmed before performing assembly. Reads contaminated with Illumina adapter were also removed using Cutadapt [9].

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Genome annotation was done for *J. iphita* from north Brahmaputra river and compared with the already published *J. iphita* (JI_Mz) from South Brahmaputra [13] to check for any intraspecific variations. Nucleotide compositions were analyzed with MEGA 5 [12].

3. Phylogenetic analysis

Two *J. iphita* species from North and South of Brahmaputra river were used to build the phylogenetic tree along with sixteen other Nymphalidae mitogenomes which were downloaded from NCBI. *Protantigius supernan* (NC_016016.1 GI: 347600337) was used as out-group. Nucleic acid sequences of 13 concatenated protein-coding genes (PCGs) from all the species were used in phylogenetic analysis. The nucleic acid sequences of 13 mitochondrial PCGs were aligned with Clustal W using MEGA 5 [12]. Maximum parsimony (MP) trees were obtained using PAUP* [11] by heuristic search option with tree-bisection-reconnection (TBR) branch-swapping. The number of bootstrap replicates was set at 1000. Starting tree was obtained via stepwise addition and the number of trees held at each step during stepwise addition equals 1. All characters were equally weighted, and zero length branches were collapsed to polytomies. Multistate taxa were interpreted as uncertainty, topological constraint was not enforced and the generated 50% consensus trees were saved as tree file.

4. Results and discussion

The near complete mitogenome of *J. iphita* is a circular molecule of 14,892 bp in length (NCBI GenBank accession number SRP053322). The gene order and orientations are similar to other nymphalids. The nucleotide compositions are significantly biased toward AT (80%) which is similar to other work on nymphalids *J. orithya* 80.4% [10] and *J.iphita* 80.5% [13]. The mitogenome nucleotide skewness (AT-skew = −0.002, GC-skew = −0.213) indicate a slight AT skew and moderate GC skew.

The phylogenetic tree was constructed for the whole mitochondrial genome using MP method (Fig. 1). In order to study the phylogenetic relationship of the two *J. iphita* samples in relation to other butterfly mitogenomes, a dataset containing concatenated 13 protein coding genes was generated. The final alignment contains 13,269 sites in the matrix with 18 in-group and one out-group taxa. Of these sites, 7547 conserved, 5713 variables and 3963 parsimony informative sites were found. In MP analysis, two heliconiinae species clustered together and limentidinae form the basal clade. Within Nymphalinae subfamily, the four *Junonia* species clustered together with high bootstrap support, which further subdivided into two clade, one clade consist of the *J. almana* and *J. orithya*; the other clade comprising the two *J. iphita* individuals. The phylogenetic tree constructed for the whole mitogenomes using the 13 PCGs region defines the genetic relatedness of the two *J. iphita* species.

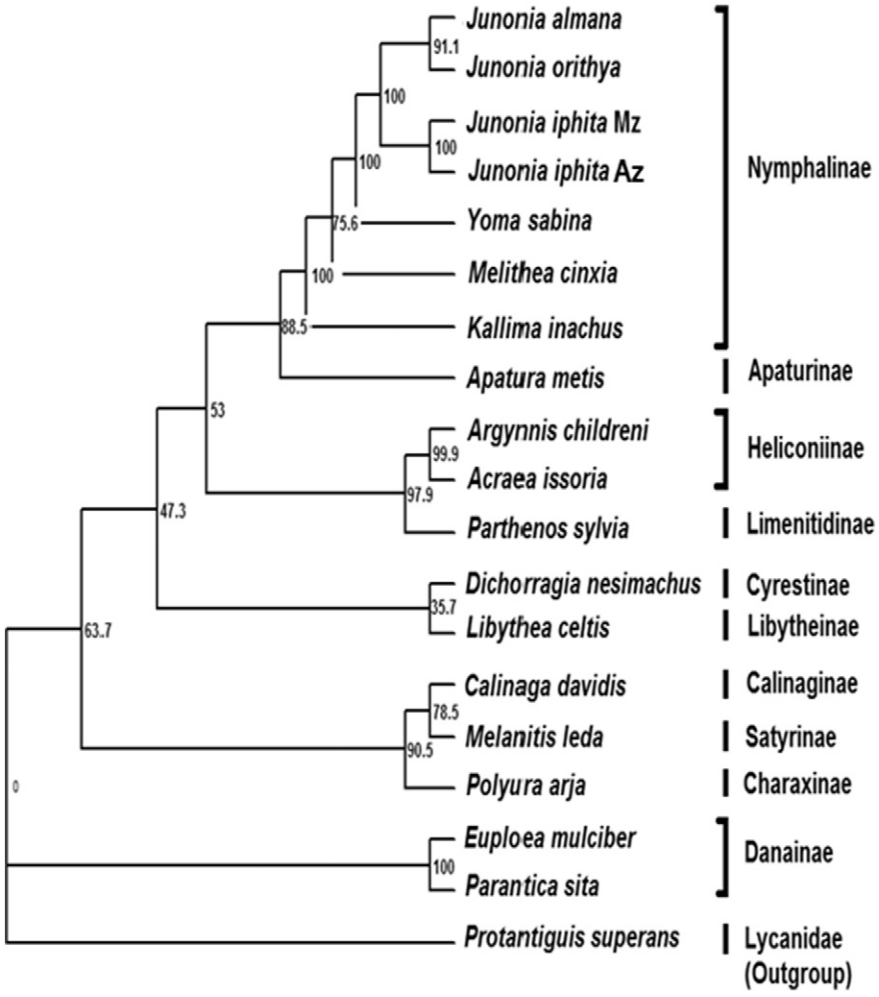

Fig. 1. Maximum Parsimony tree of whole mitochondrial genome using PAUP Software. Number of bootstrap replicates = 1000 Starting tree(s) obtained via stepwise random addition, number of trees held at each step during stepwise addition, branch-swapping algorithm: tree-bisection-reconnection (TBR).

The two *J. iphita* from the north and south Brahmaputra river were compared to study the presence of any intraspecific variations between them and comparison between these two sequences revealed minor variations at 51 sites along the length of the genome. This amounted to 0.4% of the entire mitochondrial genome. These SNP sites were inferred by correlating with the number of variable sites. We observed that the nad1 region of JI_Az ended with a single T while that of JI_Mz ends with TA. In the case of nad5, JI_Az ends with an incomplete stop codon T and JI_ Mz ends with a complete stop codon TAA. Analysis of the tRNA structure revealed similar structures except for the tRNA of Glycine. Here, it was observed that JI_ Mz has an A–A mismatch in its anti-codon stem. This mismatch was absent in the case of JI_Az. Minor variations were observed in the number of nucleotide residues (A, T, G, C), in the number of intergenic spacers and also in the number of base pairs of nad5, cytB, rrnaL and rrnaS. Variations in terms of base pair numbers were also observed in tRNAs.

Although, minor variations were observed within *J. iphita* collected from two different regions, their genome shows 99% similar which might be because of hybridization event. The draft mitogenome information reported here provides opportunity for further research on intraspecific variation within species of Indian and Indochinese subregions.

Competing interests

The authors declare that there are no competing interests.

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