

Mitochondrial DNA Part A

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MITOGENOME ANNOUNCEMENT

Complete chloroplast genome of the orchid *Cattleya crispata* (Orchidaceae:Laeliinae), a Neotropical rupicolous speciesVioleta da Rocha Perini^{1,2}, Bruno Leles³, Carolina Furtado⁴, and Francisco Prosdocimi¹

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Abstract

A partial genome dataset was sequenced for the orchid *Cattleya crispata* using both Illumina and 454 technologies. The chloroplast genome was assembled using iterative runs of MIRA software that yielded a circular molecule with 148,343 bp in length and deposited in GenBank database (Accession Number KP168671). The plastid genome conserved the quadripartite structure present in most Orchidaceae chloroplasts and was composed by 79 protein-coding genes, 39 tRNAs and 8 rRNAs. Genome structure, gene order and orientation were similar to previously described chloroplasts for *Cymbidium* orchids, differing in gene order for petN and psbM genes. Data described here contain the first report of a complete chloroplast for the Neotropical subtribe Laeliinae and may contribute to improve the phylogenetic resolution and allow the development of new molecular markers for population genetic studies of orchids.

Keywords

Espinhaço, next-generation-sequencing, orchid, plastid genome, rock outcrop

History

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Orchidaceae is one of the largest families of flowering plants containing about 850 genera and 20,000 species, also being highly diverse in terms of ecology and morphology (Chase, 2005; Dressler, 1990). The fast and extensive diversification patterns observed in orchids impose a challenge for taxonomists that use plastidial markers such as barcodes to identify and study the clade phylogeny (Cameron et al., 1999; van den Berg et al., 2005, 2009). However, the data representation of chloroplast genomes in orchids (about a dozen) is very small in face of the clade diversity. Here we present the complete chloroplast genome of the rupicolous species *Cattleya crispata*, the first plastid genome of the Neotropical subtribe Laeliinae. This taxon is confined to high-altitude mountains of Espinhaço Range Region in Southeastern Brazil and is considered endangered (Mendonça & Lins, 2007).

A specimen of *C. crispata* was collected in the Iron Quadrangle region. Genomic DNA was extracted from leaf tissue following the protocol described by Doyle & Doyle (1987). Partial genome sequencing of *C. crispata* generated 932 Mb of DNA sequences using Illumina HiSeq and 574 Mb using 454 pyrosequencing. In order to select candidate reads encoding chloroplast sequence, we run a bowtie2 (Langmead & Salzberg, 2012) alignment amongst our dataset into a database created with five complete chloroplast genomes downloaded from NCBI (NC_008591, NC_014056, NC_014874, NC_016471,

NC_018114). Candidate chloroplast reads were input into a *de novo* assembly using MIRA software (Chevreux et al., 1999). MIRA was unable to assemble the whole chloroplast into a single scaffold, thus we merged two scaffolds to produce a candidate sequence that was used as input for a reference-based assembly, allowing the production of the complete sequence described. The complete circularized genome contained 148,343 bp in length and was deposited in GenBank (Accession Number KP168671). The plastid genome conserved the quadripartite structure, composed of two copies of an inverted region (IR), a large single copy region (LSC) and a small single copy (SSC), following the typical pattern observed in photosynthetic orchids (Jheng et al., 2012; Luo et al., 2014). Genome coverage checked using Tablet software (Milne et al., 2013) resulted in ~150× uniformly distributed reads along the genome, with the exception of a single region between the genes *clpP* and *psbB* that showed low coverage. Automatic annotation was performed using DOGMA (Wyman et al., 2004), followed by careful manual curation using Artemis (Carver et al., 2012). Transfer RNA predictions were checked with tRNAscan-SE (Lowe & Eddy, 1997). The gene content composed of 79 protein-coding genes, 39 tRNAs and 8 rRNAs (Figure 1). Genome structure, gene order and orientation were similar to the chloroplast of the orchid genus *Cymbidium*, differing in gene order for two genes (*petN* and *psbM*) (Yang et al., 2013). The complete chloroplast genome of *C. crispata* may benefit further phylogenetic, phylogeographic, population genetic and species delimitation studies, which are essential for the ongoing conservation efforts to manage this endangered species and also contribute for better understanding of Neotropical orchid evolution.

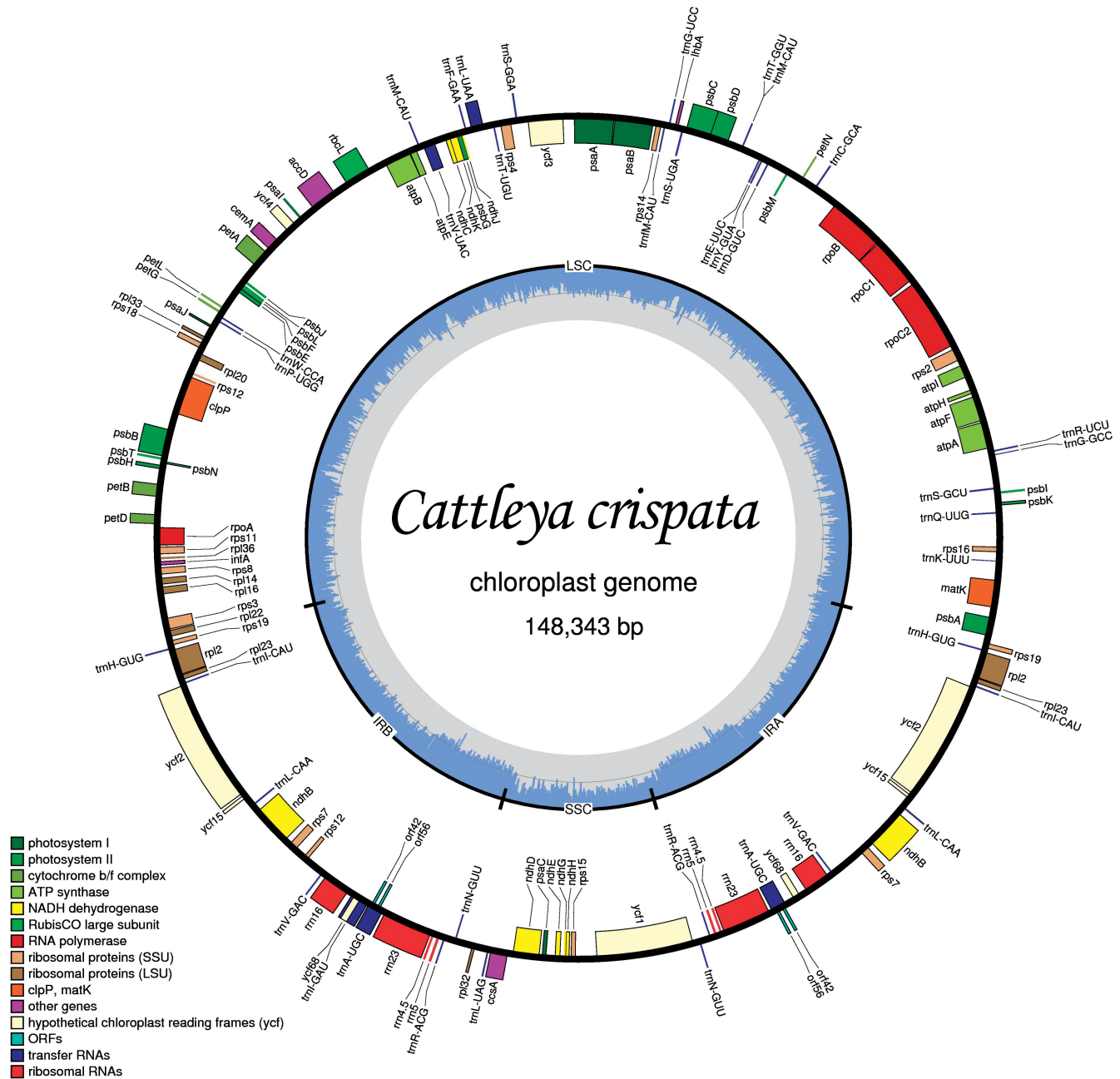


Figure 1. Genome map of the *Cattleya crispata* chloroplast. Genes shown outside of the outer circle are transcribed clockwise, whereas those shown inside are transcribed counterclockwise. Genes belonging to different functional groups are color coded. Area dashed blue in the inner circle indicates GC content while the gray corresponds to AT content of the genome.

Declaration of interest

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