

**Universidade de São Paulo  
Escola Superior de Agricultura “Luiz de Queiroz”**

**Mitochondrial genome analysis in *Passiflora***

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Dissertation presented to obtain the degree of Master in  
Science. Area: Genetics and Plant Breeding

**Piracicaba  
2023**

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## RESUMO

### **Análise de genomas mitocondriais em *Passiflora***

O gênero *Passiflora* compreende aproximadamente 525 espécies que apresentam uma grande diversidade morfológica a qual serve de base para a classificação taxonômica em subgêneros e seções. Porém, estudos genômicos ainda são incipientes. Foram desenvolvidas algumas iniciativas para desvendar a estrutura do genoma nuclear e organelar de parte das espécies de *Passiflora*. Pode-se dizer que, até agora, somente os genomas plastidiais (cpDNA) estão mais bem documentados. No entanto, existe uma falta de informação sobre o genoma mitocondrial (mtDNA), o que deve permitir complementar os estudos evolutivos sobre o gênero. O mtDNA das plantas apresenta uma variabilidade notável em termos de tamanho e estrutura do genoma, atribuída a sequências repetitivas de DNA envolvidas na recombinação homóloga e na transferência intracelular de genes (IGT). No entanto, apesar da sua variabilidade, o mtDNA das plantas não contém um grande repertório de genes. Em vez disso, mantém genes essenciais relacionados à fosforilação oxidativa onde ocorre a cadeia de transporte de elétrons. O nosso grupo de investigação documentou inicialmente o mtDNA de *P. organensis* (subgênero *Decaloba*). No presente estudo, foi empregada uma abordagem de montagem híbrida usando leituras curtas e longas geradas a partir das tecnologias Illumina e Oxford Nanopore para desvendar o mtDNA de três espécies de *Passiflora*: *P. haematostigma* (subgênero *Astrophea*), *P. alata* e *P. edulis* (subgênero *Passiflora*). O nosso objetivo foi produzir novos recursos genômicos e gerar conhecimento que possa ser útil em estudos evolutivos sobre o gênero. Apesar dos nossos esforços, não foi possível capturar uma única molécula circular que representasse o "círculo mestre". Para *P. haematostigma*, foram identificadas três sequências circulares com comprimentos de 275.820, 162.684 e 110.306 pb. Em contraste, para *P. edulis* e *P. alata*, uma única sequência com comprimento de 737.209 e 693.479 pb, respectivamente, foram identificadas. Estes genomas de mtDNA contêm um conjunto conservado de 32 genes codificadores de proteínas e 3 genes de rRNA nas três espécies, acompanhados por um número variável de genes de tRNA, entre 25 e 34. Curiosamente, foram observados sítios de inserção de DNA plastidial em *P. organensis* e nas três espécies de *Passiflora*, variando de ~3 a ~5%, destacando a influência dos eventos IGT na variação do tamanho do mtDNA. Estes resultados notáveis sobre o arranjo estrutural e funcional dos mtDNAs de *Astrophea*, *Passiflora* e *Decaloba* sugerem que novos estudos sejam conduzidos visando entender a possível relação entre a organização dos genomas mitocondriais e os padrões evolutivos do gênero.

**Palavras-chave:** Malpighiales; Montagem híbrida; Conteúdo do genoma; Estrutura do genoma; Transferência de genes.

# ABSTRACT

## Mitochondrial genome analysis in *Passiflora*

The genus *Passiflora* comprises approximately 525 species that represent a vast morphological diversity which serves as the basis for taxonomic classification into subgenera and sections. However, genomic studies are still emerging. Some initiatives have been developed to unravel the nuclear and organellar genome structure of some *Passiflora* species. So far, it can be said that plastid genomes (cpDNA) are better documented. Nevertheless, there is a lack of information on the mitochondrial genome (mtDNA), which should allow complementing evolutionary studies on the genus. Plant mtDNA shows remarkable variability in terms of genome size and structure, attributed to repetitive DNA sequences involved in homologous recombination and intracellular gene transfer (IGT). Regardless, despite its variability plant, mtDNA does not contain a large repertoire of genes. Instead, it maintains essential genes related to oxidative phosphorylation where the electron transport chain occurs. Our research group initially documented the mtDNA of *P. Organensis* (subgenus *Decaloba*). In the present study, a hybrid assembly approach using short and long reads generated from Illumina and Oxford Nanopore technologies was employed to unravel the mtDNA of three *Passiflora* species: *P. haematostigma* (subgenus *Astropheia*), *P. alata*, and *P. edulis* (subgenus *Passiflora*). Our aim was to produce new genomic resources and generate knowledge that may be useful in evolutionary approaches to the genus. Despite our efforts, we were unable to capture a single circular molecule representing the “master circle”. For *P. haematostigma*, three circular sequences with lengths of 275,820, 162,684, and 110,306 bp were identified. In contrast with *P. edulis* and *P. alata*, a single sequence with lengths of 737,209 and 693,479 bp, were respectively identified. These mtDNA genomes contain a conserved set of 32 protein-coding genes and 3 rRNA genes in the three species, accompanied by a variable number of tRNA genes ranging from 25 to 34. Interestingly, plastidial DNA insertion sites were observed in *P. organensis* and all three *Passiflora* species, ranging from ~3 to ~5%, highlighting the influence of IGT events on mtDNA size variation. These remarkable results on the structural and functional arrangement of *Astropheia*, *Passiflora*, and *Decaloba* mtDNAs suggest that further studies should be conducted to understand the possible relationship between the organization of mitochondrial genomes and the evolutionary patterns of the genus.

**Keywords:** Malpighiales; Hybrid assembly; Genome content; Genome structure; Gene transfer.

## INTRODUCTION

*Passiflora* is a member of Passifloraceae and is regarded as the largest representative genus within the family that belongs to the Malpighiales order (Judd et al., 2008). The genus includes approximately 525 species distributed in the tropical and subtropical regions of America, Asia, and Oceania (Ulmer & MacDougal, 2004). *Passiflora* species are commonly herbaceous and climbing (vines) plants, eventually trees (see Hansen et al., 2006). Due to the flower morphology diversity, wide geographic range and economic value has been the goal of several studies (see Zerbini et al., 2009; Vieira et al., 2022).

Some initiatives have been developed to unveil the *Passiflora* nuclear genome structure (Costa et al., 2021; Santos et al., 2014; Xia et al., 2021). So far, plastid genomes (cpDNA) are well-documented (Cauz-Santos et al., 2020; Rabah et al., 2019; Shrestha et al., 2019). However, there is a shortage of information on the mitochondrial genome (mtDNA) (Costa et al., 2021; Yang & Wang, 2020).

Plant mtDNA displays remarkable variability in terms of genome sizes. In animals, for instance, it varies from 16 to 17 kbp (Boore, 1999; Ladoukakis & Zouros, 2017), while in yeast, it varies from 75 to 85 kbp (Foury et al., 1998). In plants, the size can vary between species ranging from 66 Kbp in *Viscum scurruloideum* (Skippingtona et al., 2015) to *Silene conica* 11.3 Mbp and between individuals of the same species as in *Silene latifolia* (0.25 Mbp) and *S. conica* (11.3 Mbp) (Allen et al., 2007; Kubo & Newton, 2008; Sloan et al., 2012). In the angiosperm's evolution, intracellular gene transfer (IGT) and horizontal gene transfer (HGT) are well-documented phenomena. The transfer occurs from the chloroplast DNA (cpDNA) and mtDNA to the nucleus. From cpDNA to mtDNA and from the nucleus to mtDNA (Bock, 2010; Keeling & Palmer, 2008; Richardson & Palmer, 2006). Plant mitochondrial genomes are commonly illustrated as circular molecules called a "Master circle" (Gualberto & Newton, 2017; Palmer & Herbon, 1988). However, some studies represent the genome as a complex mixture of molecules, whose branch points may represent active recombination sites (Jackman et al., 2020; Kozik et al., 2019; Nielsen, 2017; Sloan, 2013). The main feature of mitogenomes is the abundance of repetitive sequences both in number and size (Alverson et al., 2011). Large (>500bp) repeats, usually involved in homologous recombination give rise to the formation of sub-genomic molecules, hampering the assembly of these genomes (Gualberto et al., 2014; Gualberto & Newton, 2017; Lonsdale et al., 1984).

The number of available plant mitochondrial sequences has increased rapidly in recent years. In May 2023, a total of 116 land plant sequences are publicly available in the GenBank

database (<https://www.ncbi.nlm.nih.gov/genome/organelle/>). The prevailing representation of deposited plant mtDNA as circular molecules is widely accepted. However, it inaccurately reflects the complex structure. The evolution of the mtDNA structure has been subjected to many investigations. The application of next-generation sequencing (NGS) in recent years has led to unprecedented growth in genomics projects. Combining the advantages of second and third-generation sequencing technologies, hybrid assembly using short and long reads has been adopted. This new approach has proven useful for improving the quality with low error rates, including mt-genome assembly. Thus, the main objective of the present work was to describe the structure and content organization of the mtDNAs of at least one species of the three subgenera: *Astrophea* (*P. haematostigma*), *Decaloba* (*P. organensis*), and *Passiflora* (*P. edulis* and *P. alata*) to make comparative analysis between these species with the available data from *P. organensis* (Costa et al., 2021).

## CONCLUSION

In the present work, we were able to provide new genomic resources concerning the mitochondrial genome of three species: *P. haematostigma* (subgenus *Astrophea*), *P. alata*, and *P. edulis* (subgenus *Passiflora*), and we have expanded the knowledge regarding the previously documented mtDNA of *P. organensis*. Based on the graph analysis, we demonstrated that the mitochondrial genome structure is dynamic and complex, characterized by the presence of repeats, and could exist in various subgenomic isoforms, such as circular, linear, and branched structures. We also identified three circular molecules of the mtDNA for *P. haematostigma* and a single molecule for *P. alata* and *P. edulis*. In the four species, we observed that at least a set of 32 protein-coding genes and three rRNA genes, accompanied by a variable number of tRNA genes. Finally, we observed instances of possible plastidial DNA insertion occupying ~3-5% of total mtDNA genomes, highlighting the influence of IGT events on mtDNA size variation, and small mitovirus-RdRp fragments highlighting the influence of HGT events within the mtDNA. These remarkable findings not only enhance our understanding on the dynamics of mtDNA within the *Astrophea*, *Passiflora*, and *Decaloba* subgenera, but also provide information for taxonomic and evolutionary studies that can benefit from the insights provided herein.

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