

# First Approach Toward Assembly and Annotation of the Complete Mitochondrial Genome of *Colobanthus Quitensis* (Kunth) Bartl. (Caryophyllaceae)

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## 1. INTRODUCTION

The genus *Colobanthus* (Caryophyllaceae) includes 26 species. These plants are primarily found in the southern hemisphere, including the islands of the South Pacific, Australasia, central and southern South America, Antarctica, and the subantarctic islands (West 1991; Sneddon 1999; Gray 1976). The most famous representative of this genus is *Colobanthus quitensis*, which is one of only two flowering plants, along with *Deschampsia antarctica* E. Desv., considered native to Antarctica (Skottsberg 1954). *C. quitensis* has become a model species in the study of plant adaptation to the extremely difficult environmental conditions of the Antarctic, which include not only low temperature but also its rapid changes (cyclical melting and freezing), short vegetation period, strong winds, the phenomenon of polar night and day, and strong UV radiation (Gianoli et al. 2004; Navarrete-Gallegos 2012).

Despite numerous morpho-physiological studies aimed at exploring the process of *C. quitensis* adaptation to polar conditions, genetic data on this issue are very limited. Our previous studies, in which we reported the results of comparative characterization of the complete chloroplast genome sequences of *C. quitensis* (sequence acquired from the GenBank) and six other *Colobanthus* species from areas characterized by a milder climate (sequences of our authorship), did not confirm the unique character of the *C. quitensis* plastome (Androsiuk et al. 2020).

Although mitochondrial DNA is widely used in phylogeography, it has been shown that environmental stressors, nutrient availability, and climate can have a significant impact on mitochondrial genomes, favoring the persistence of particular haplotypes to meet the metabolic demands of the local environment (Noll et al. 2022; Shen et al. 2022).

During the project realization, it is planned to obtain for the first time, a complete sequence and conduct a detailed characterization of the mitochondrial genome of *C. quitensis*. The mitochondrial genome sequence of the *C. quitensis* will provide data necessary for further studies aimed at explaining the role of mitochondria in the development and evolution of mechanisms responsible for adaptation of the species to polar conditions. It will also become the reference sequence, which will facilitate the development of mitochondrial genomes of other *Colobanthus* species.

## 2. RESULTS AND DISCUSSION

Here we present the preliminary results obtained during the realization of our project which was launched in March 2025. Due to the specific nature of the plant mitochondrial genome (size even over 10 Mb, possible multichromosomal structure, relatively small number of genes and large share of non-coding regions and repetitive sequences), the Oxford Nanopore Technology platform generating single reads up to 30 Kb long was used to sequence and assemble the complete mitochondrial genome of *C. quitensis*. The library preparation and long-read DNA sequencing was performed according to procedure described in Krawczyk et al. (2025).

Nanopore sequencing generated 2.9M raw reads up to 450 kb with N50 = 11.4 kb. Base-calling with SUP model and Herro based correction enable mean Q-score at 27.8. Out of obtained read, 4.7% were identified as mitochondria resulted in 33x mean coverage. The *C. quitensis* draft mitochondrial genome was characterized by a complex, multichromosomal structure. The assembly results revealed that the complete *C. quitensis* mitochondrial genome is 2.2 Mbp bp in length with a GC content of 41% and consists of 9 contigs (pseudochromosomes), each could be mapped as a circular molecule due to flanking repeats. The size of these pseudochromosomes range from 133 to 566 kb. However, ca. 5% of mapped reads supports potentially different topology, suggesting presence alternative assembly paths.

Our observations are concordant with previously published data pointing at high variation in size and structure of mitochondrial genomes within Caryophyllales. In this respect the genus *Silene* (Caryophyllaceae) is especially well-studied. The genus *Silene* includes species with simple and slowly evolving mitochondrial genomes, e.g. *S. latifolia* (253 kb) and *S. vulgaris* (427 kb), as well as taxa whose mitogenomes are large, have a multichromosomal structure and a high mutation rate, e.g. *S. noctiflora* (6.7 Mb) and *S. conica* (11.4 Mb) (Sloan et al. 2012).

The gene composition of *C. quitensis* mitogenome is very conservative with obligatory set of core protein genes and 2 genes representing various, facultative ribosomal protein mitochondrial genes. Among these sequences, there were genes for four subunits of ATP synthase (*atp1*, *atp4*, *atp6*, and *atp9*), four genes associated with cytochrome c biogenesis (*ccmB*, *ccmC*, *ccmFc*, and *ccmFn*), a gene for ubiquinol cytochrome c reductase (*cob*), three genes for subunits of cytochrome c oxidase (*cox1–3*), a maturase gene (*matR*), a sequence for transport membrane protein (*mttB*), sequences for nine subunits of NADH dehydrogenase (*nad1–6*, *nad7*, *nad9*, and *nad4L*), sequences for three components of the small subunit of the ribosome (*rps11–13*). Moreover, we annotated full set of sequences for tRNAs and rRNAs genes which were detected in five clusters, scattered along 3 pseudochromosomes. Among protein-coding genes, *rps12*, *rpl5*, *cob* and *ccmFn* were duplicated in one of scaffolds. Assembly of mitochondrial genome enabled identification of three plastid to mitochondrion transfer events, ranging from 1.5 to 4 kbp.

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