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Research Article

The Complete Chloroplast Genome Sequence of *Actinidia arguta var. purpurea*

Maria Gladysheva-Azgari [1, 2], Natalia Slobodova [1, 2, 3], Eugenia Boulygina [2], Svetlana Tsygankova [1, 2, *], Fedor Sharko [2, 4], Irina Mitrofanova [1]

1. Main Botanical Garden named after N.V. Tsitsin of the Russian Academy of Sciences, Moscow 127276, Russia; E-Mails: marglader@gmail.com; nv.slobodova@gmail.com; svetlana.tsygankova@gmail.com; irimitrofanova@yandex.ru
2. National Research Center "Kurchatov Institute", Moscow 123182, Russia; E-Mails: eugenia.bulygina@gmail.com; fedosic@gmail.com
3. Faculty of Biology and Biotechnology, HSE University, Moscow 101000, Russia
4. Research Center of Biotechnology of the Russian Academy of Sciences, Moscow 119071, Russia

* **Correspondence:** Svetlana Tsygankova; E-Mail: svetlana.tsygankova@gmail.com

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Abstract

In this study, we report the complete chloroplast genome of *Actinidia arguta var. purpurea.* The chloroplast genome is 157,369 bp long as the circular (GC ratio is 37.22%). It has four subregions: a large single-copy (LSC) region of 88,609 bp, a small single-copy (SSC) region of 20,470 bp, and two inverted repeat regions (IRs) of 24,145 bp in each. The chloroplast genome of *A. arguta var. purpurea* contains a total of 113 unique genes, which are 79 protein-coding genes, 4 rRNA genes, and 30 tRNA genes. The phylogenetic analysis revealed that *A. arguta var. purpurea* has the most genetic similarity to *A. kolomikta*. These findings can be used to identify *Actinidia* species.

Keywords
Actinidia arguta var. purpurea; chloroplast genome; phylogenetic analysis

1. Introduction

Data from complete chloroplast genomes expand our understanding of plant diversity and contribute to our understanding of evolutionary relationships among species. Due to its small size (relative to the whole *Actinidia* genome) and the presence of conservative and variable regions, the chloroplast genome is often used to resolve controversial issues of plant phylogeny. *A. arguta var. purpura* was previously distinguished as a separate species based on several morpho-physiological characteristics, and the systematic position of this species is still controversial [1]. And the presence of fruits rich in anthocyanins distinguishes this species from other representatives of kiwiberry and makes it a valuable agricultural crop.

Actinidia arguta var. purpurea was first collected and described by E.H. Wilson in 1908, China, in the province of Sichuan [2]. *Actinidia arguta var. purpurea* is a late-ripening and medium-hardy kiwifruit species that produces elongated, oval-shaped barrel-like fruits with a blunt base and tip [1]. Fruit weight varies from 9.5 g to a maximum of 14.5 g. *A. arguta var. purpurea* is light plum to purple (Figure 1), a key trait involving its unique regulation of anthocyanin metabolism. The taste is sour-sweet, with a slight aroma. It is closely related to *A. arguta*, which has larger, bright green leaves with more serrated leaves and greenish yellow fruits. While most studies have focused on nuclear genomic aspects, the chloroplast genome of *A. arguta var. purpurea* remains less explored. Understanding the chloroplast genome's organization and genetic makeup in *A. arguta var. purpurea* is essential for comprehending the species' evolutionary history, taxonomy, and chloroplast-related traits.

Figure 1 *A. arguta var. purpurea* fruits. The photos were taken by Natalia Slobodova at the Main Botanical Garden, Moscow, Russia, without any copyright issues.

2. Materials and Methods

Fresh leaves of *A. arguta var. purpurea* were obtained from the collection of Far Eastern Species of the Main Botanical Garden named after N.V. Tsitsin of the Russian Academy of Sciences (55.8443° N, 37.5899° E) by Natalia Slobodova. A specimen was deposited at the Main Botanical Garden

(55.8443° N, 37.5899° E) (Natalia Slobodova, nv.slobodova@gmail.com) under the voucher number XY0IP00121. The Lo Piccolo DNA extraction protocol used in this study was slightly modified and adapted from [3]. For sequencing on a GridION device, an additional purification step was applied using Genomic Tip 20/G columns (Qiagen, Germany) following the manufacturer's instructions [4]. The concentration and quality of the extracted DNA was assessed spectrophotometrically on a Nanodrop 1000 device (Thermo Scientific, USA) and using a Qubit fluorometer (Invitrogen, USA) using the Qubit™ dsDNA BR Assay Kit. To create DNA libraries, the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England BioLabs, USA) was used according to the manufacturer's protocol. The libraries generated were sequenced using the advanced NovaSeq 6000 platform (Illumina, USA). For the hybrid assembly, sequencing was conducted on a GridION device (Oxford Nanopore Technologies, UK) using the Ligation Sequencing Kit following the manufacturer's guidelines [5]. The fastp [6] program was used to remove adapters and filter out low-quality reads for Illumina reads. A total of 24,508,833 paired short reads and 836,174 long reads with an average length of 5591.97 bp were used for the hybrid assembly of the chloroplast genome by using the de novo assembler SPAdesv3.15.0 [7] with a coverage of 3331.9× (Figure S1). The OGDRAW platform was used for the chloroplast genome annotation and map [8]. Then, the complete chloroplast genome of *A. arguta var. purpurea* was submitted to GenBank (Accession number: OR538546). Twenty-one complete chloroplast genomes, including 18 *Actinidia* species and three *Vaccinium* species as an outgroup, were used to construct a maximum likelihood (ML) phylogenetic tree. For this analysis, the resulting sequences were aligned using might v7.245 [9] using the iterative method (G-INS-i) and the default parameter settings. A maximum likelihood (ML) phylogenetic tree was then constructed using RAxML v8.2.12 [10], and all nodes were inferred from 1000 bootstrap values. Finally, the visualization was done in the iTOL service [11]. The border regions of the *A. arguta var. purpurea* chloroplast genome and two other closely related species (*A. arguta: NC_034913.1 and A. kolomikta: NC_034915.1*) were identified and displayed using IRscope [12], a tool for visualizing the inverted repeat (IR) regions.

A. arguta var. purpurea is not included in the list of rare and endangered species in the Russian Federation (https://www.mnr.gov.ru/activity/red_book/) and was collected with the permission of the Main Botanical Garden named after N.V. Tsitsin of the Russian Academy of Sciences. No ethical approval was required for this study.

3. Results

The chloroplast genome of *A. arguta var. purpurea* is 157,369 bp in length (GC ratio: 37.22%). The genome contains a large single-copy (LSC) region of 88,609 bp, a small single-copy (SSC) region of 20,470 bp, and a pair of inverted repeat regions (IRA and IRB) of 24,145 bp. 113 unique genes were found and annotated, including 79 protein-coding genes, 30 tRNA genes, and 4 rRNA genes (Figure 2). Among the genes, 15 genes were duplicated in the IR regions, including 3 PCGs genes (*ndhB, rps7* and *ycf2*), 8 tRNA genes (*trnA-UGC, trnH-GUG, trnI-CAU, trnI-GAU, trnL-CAA, trnN-GUU, trnR-ACG and trnV-GAC*) and 4 rRNA genes (*rRNA4.5, rRNA5, rRNA16,* and *rRNA23*). The introns were contained in 13 protein-coding genes and 6 tRNA genes, while *pafI* and *rps12B* contained 2 introns.

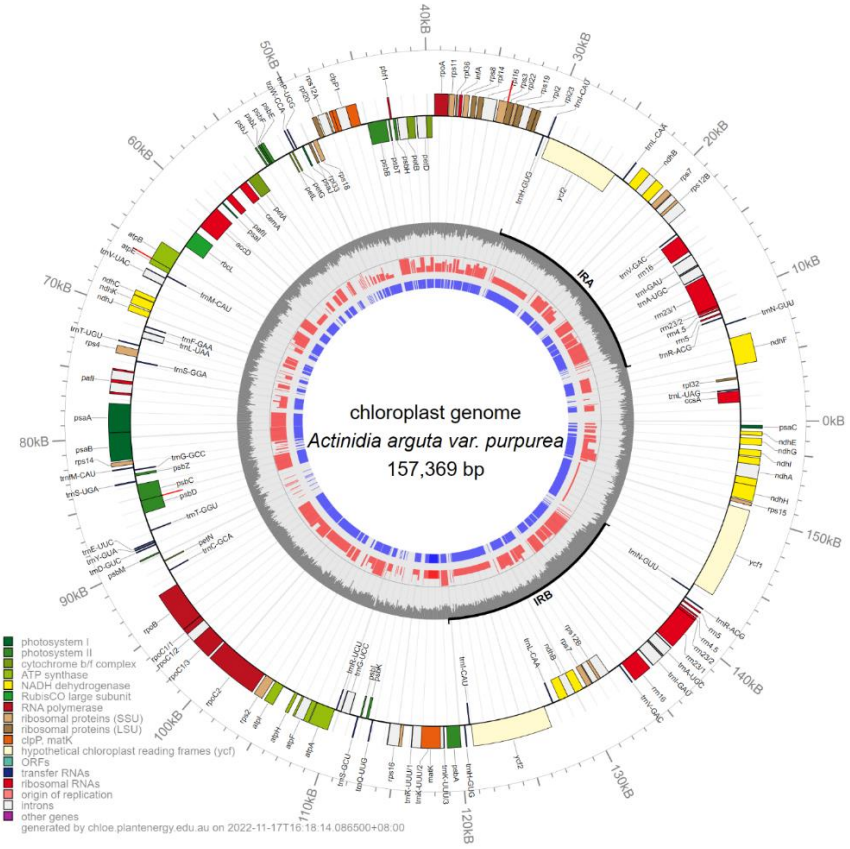

Figure 2 Complete chloroplast (cp) genome map of *A. arguta var. purpurea*. Color coding of genes is based on functional groups they belong to. Dark gray color of inner circle indicates GC content.

Phylogenetic analysis has shown that *A. arguta var. purpurea* is genetically very close to *A. kolomikta* (Maxim. et Rupr.) Maxim (Figure 3).

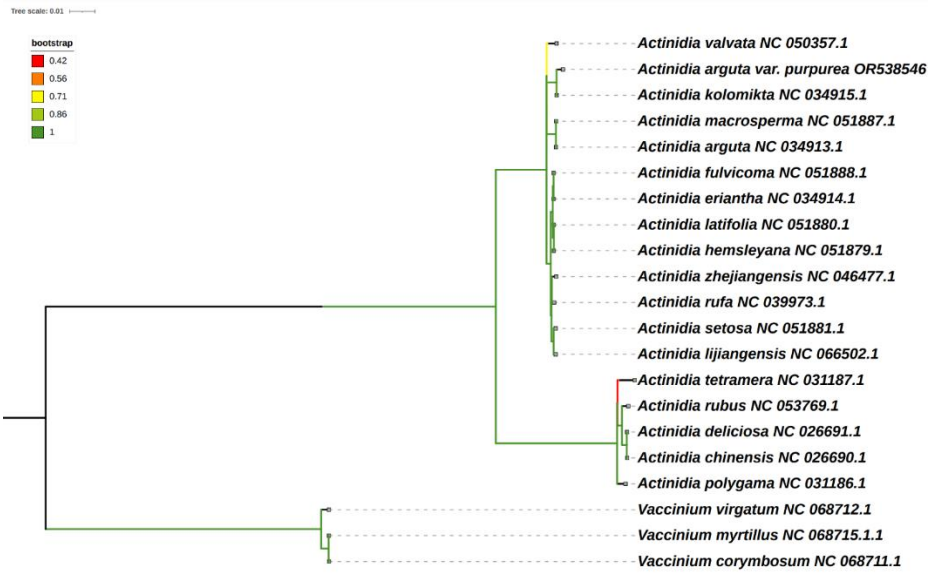

Figure 3 The ML phylogenetic tree, constructed using 21 complete chloroplast genomes, revealed the phylogenetic position of *A. arguta var. purpurea*. Bootstrap support values are shown as a color gradient.

When comparing the chloroplast genome of *A. arguta var. purpurea* with the chloroplast genomes of the related species *A. arguta* and *A. kolomikta*, we did not find significant differences in the structure and order of genes. While a recent study showed that there can be large differences in gene order within the same angiosperm species [13], we do not see large differences when comparing the border regions (LSC, SSC, Ira and IRb) of chloroplast genomes (Figure 4). The only peculiarity is the location of the psbA gene at the LSC/IRb boundary in the genome of *A. arguta var. purpurea*, which is not observed in other genomes.

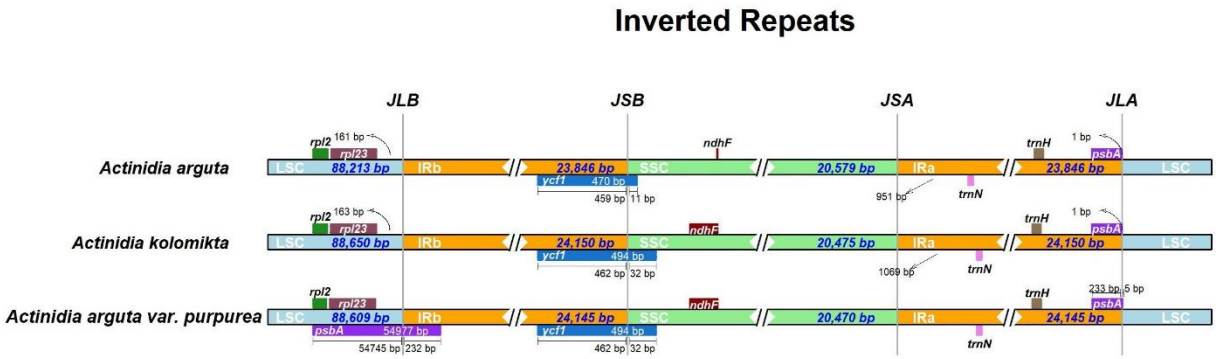

Figure 4 Comparison of border regions of the chloroplast genomes of *A. arguta*, *A. kolomikta* and the de novo assembled *A. arguta var. purpurea* genome. Genes were depicted as rectangles, and the distances between genes and region junctions were represented by the number of bases.

4. Discussion and Conclusion

This study presents the complete assembly and annotation of the chloroplast genome sequence of *A. arguta var. purpurea* for the first time, providing valuable insights into its genetic structure. The findings of this study enhance our understanding of the evolutionary connections within the *Actinidiaceae* family and deepen our knowledge of the phylogenetic placement of *A. purpurea*. In recent times, consumers have been interested in fruits with elevated levels of anthocyanins, which are responsible for the red to purple pigmentation. This is primarily due to these compounds' antioxidant properties and potential health benefits [14]. Foods rich in flavonoids and anthocyanins are believed to significantly prevent diseases [15]. *Actinidia arguta var. purpurea* fruits possess various biological functionalities, including antioxidant, cardioprotective, and anti-inflammatory properties, making them suitable for nutritional supplements.

Author Contributions

ST and IM designed the research study and obtained the funding. MG, NS and EB performed sample collection, DNA extraction, library construction, and sequencing. FS performed bioinformatics analyses. FS wrote and revised the manuscript, and all authors reviewed it.

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Competing Interests

The authors have declared that no competing interests exist.

Additional Materials

1. Figure S1: Coverage depth map and statistics of the chloroplast genome sequence of *Actinidia purpurea* Rehder. The figure was generated with BAM2Plot using mapping results from Bowtie2. The blue graph indicates the coverage depth at each sequence location.

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