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PLASTOME REPORT

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The complete chloroplast genome sequence and phylogenetic analysis of *Actinidia suberifolia* C.Y. Wu (actinidiaceae)

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ABSTRACT

Actinidia suberifolia C.Y. Wu (Actinidiaceae), an endemic species of Yunnan province in China, exhibits substantial therapeutic importance in daily life. Given its narrow distribution and small population size, complete genome sequence is needed to reveal its phylogenetic position in *Actinidia*. In this study, we assembled and annotated the complete chloroplast genome of *A. suberifolia* and conducted the phylogenetic analysis among the genus *Actinidia*. The results showed that *A. suberifolia* had a typical quadripartite structure, exhibiting a total length of 156,716 bp and consisting of two inverted repeats (IRs) of 23,805 bp separated by a large single-copy (LSC) and a small single-copy (SSC) of 88,437 bp and 20,669 bp. A maximum-likelihood (ML) phylogenetic tree including *A. suberifolia* and 21 related species indicated that it was close to *Actinidia latifolia*. The study will offer valuable genetic resources and improve the phylogenetic resolution of *Actinidia*.

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Actinidia suberifolia; chloroplast genome; phylogenetic analysis

Introduction

The genus *Actinidia*, commonly known as kiwifruit, is an economically and nutritionally important fruit crop known for its significant nutritional value in the worldwide (Testolin et al. 2016). Some key species, such as *Actinidia chinensis* and *A. chinensis* var. *deliciosa*, have attracted considerable attention due to the abundant nutritional qualities like extremely high content of vitamin C, dietary fibers, carotenoids and chlorophylls (Liang et al. 2020). Together with the other two sister genera *Clematoclethra* and *Saurauia*, the genus *Actinidia* belongs to Actinidiaceae and comprises of 75 species according to the recent taxonomic revision (Huang et al. 2013). However, frequent interspecific hybridization and introgression events present substantial challenges to its taxonomic studies. Therefore, it is essential to provide more genomic resources and explore the phylogenetic relationships within the genus *Actinidia*.

Actinidia suberifolia C.Y. Wu, an endemic species of Yunnan province in China, is distributed in counties such as Pingbian and Mengzi in Yunnan Province and growing in mountain forests at altitudes of 600 – 1000 m (Figure 1, Li et al. 2007). According to the investigation conducted by the local people, *A. suberifolia* expresses considerable medicinal benefits in their life. The leaves are broadly used to relieve pain and promote blood circulation, mainly intended for treating muscle damage. Currently, its intrageneric relationships and species discrimination are still uncertain due to a lack of genomic resources. Therefore, it is necessary to explore the complete chloroplast genome sequence of *A. suberifolia*.

In this study, we assembled and annotated the complete chloroplast genome of *A. suberifolia* for the first time, and conducted the phylogenetic analysis among the genus *Actinidia*. The results provide valuable data support, which is conducive to further exploration of the species conservation, molecular evolution, and taxonomic studies in this genus.

Materials and methods

The fresh leaves of *A. suberifolia* were collected from Hekou County, Yunnan Province, China (22.834920°N, 103.651209°E, elevation ca. 645.6 m). The voucher specimen (voucher: HIB0259840; contact person: Guangwan Hu, guangwanhu@wbgcas.cn) has been deposited at the Herbarium of Wuhan Botanical Garden, Chinese Academy of Sciences.

Second-generation sequencing technology was utilized for library construction and sequencing of total DNA extracted from silica-gel dried leaves. The raw sequencing data was processed using fastp v0.23.2 (Chen et al. 2018) to remove low-quality reads and adapter sequences. After quality control, the clean reads were used to assemble the chloroplast genome by using GetOrganelle v1.7.5 with parameter "-R 15 -t 20 -k 21,45,65,85,105 -F embplant_pt" (Jin et al. 2020) with the first-published cp genome of

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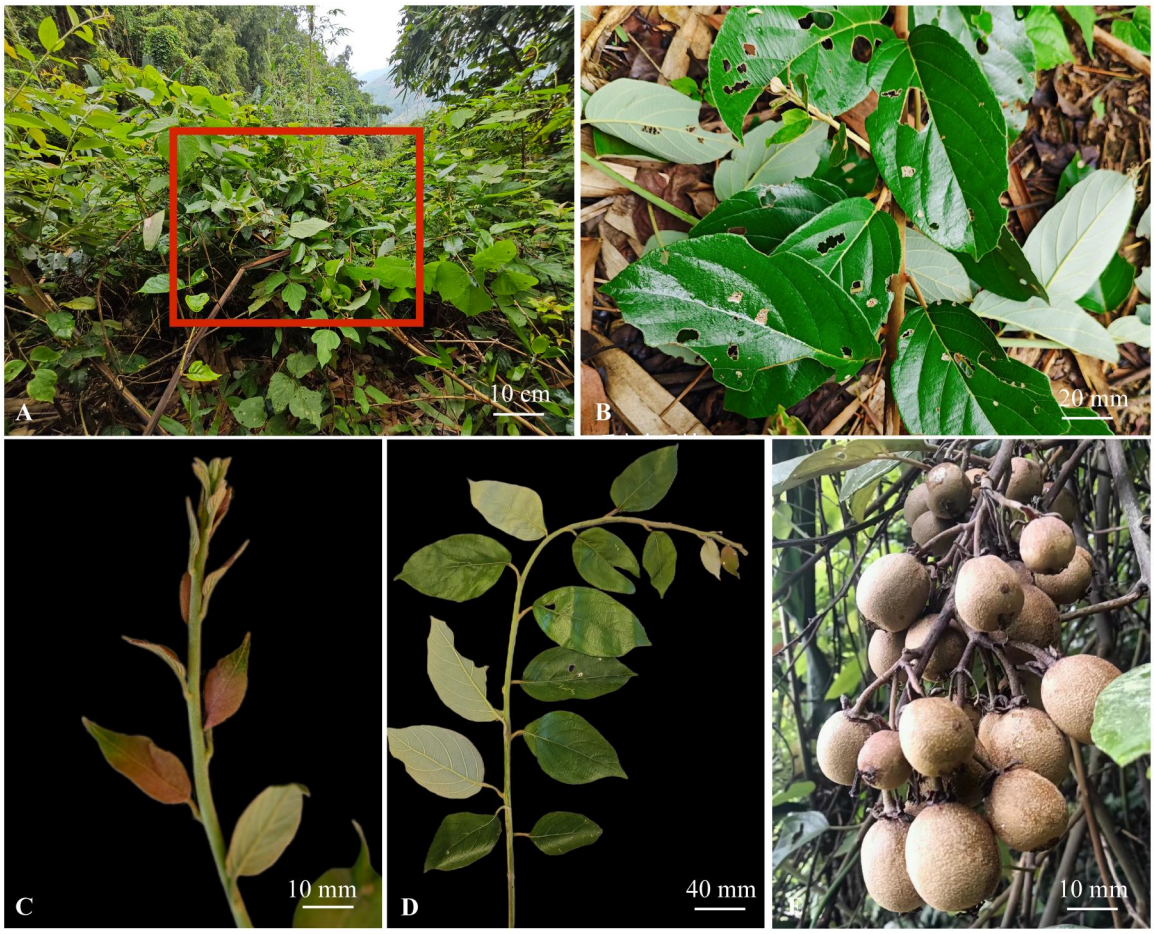

Figure 1. The morphological characteristics of *A. suberifolia*. (A) The natural habitat. The plant grows densely intertwined with surrounding vegetation, which is highlighted by a red box in the figure. (B) The mature leaves. The leaf blade is narrowly elliptical, thick and papery, with the abaxial surface very densely covered with cinnamon-colored hairs, especially along the midrib and lateral veins, while the rest is stellate tomentose. The leaf base is cuneate to rounded, the margin is shallowly and remotely serrulate, and the apex is acute to shortly acuminate. The leaf damage shown was caused by incidental insect feeding, which is not a species-specific trait. (C) The budding leaves. (D) The flattened arrangement of leaves. (E) The fruits are sub-globose and densely covered with rust-colored tomentose hairs. The photos of the species were taken by Quan Jiang.

A. chinensis as reference (Yao et al. 2015). The assembled scaffolds and their connectivity were visualized and adjusted by using Bandage v0.8.1 (Wick et al. 2015). The chloroplast annotation was finished using PGA (Qu et al. 2019) and adjusted manually when necessary, then visualized by CPGview (Liu et al. 2023, http://www.1kmpg.cn/cpgview). The annotated chloroplast genome was deposited to GenBank under the accession number: PQ789263.

To evaluate the evolutionary relationships of *A. suberifolia* within *Actinidia*, we downloaded 20 cp genome sequences of *Actinidia* and one species of *Clematoclethra* from the NCBI database (http://www.ncbi.nlm.nih.gov/). Among them, *Clematoclethra scandens* subsp. *Hemsleyi* was used as an outgroup. Sequence alignment was performed by MAFFT v.7 (Nakamura et al. 2018), and the Maximum-likelihood (ML) tree was reconstructed in IQ-TREE v2.0.3 (Bui et al. 2020) with rapid 1,000 bootstrap replicates. The phylogenic tree was then visualized by the online website Chiplot (Xie et al. 2023).

Results

The complete chloroplast genome of *A. suberifolia* exhibited a typical quadripartite structure, spanning 156,716 bp in total length (Figure 2). It consisted of a large single-copy (LSC) region of 88,437 bp, a small single-copy (SSC) region of 20,669 bp, and a pair of inverted repeat regions (IRa and IRb), each 23,805 bp in length. The overall GC content of *A. suberifolia* was 37.2% and the average coverage depth of the genome assembly was 5474.8× (Figure S1). The annotated genome contained 132 genes in total, including 85 protein-coding genes (CDS), 39 transfer RNA genes (tRNA), and 8 ribosomal RNA genes (rRNA). Among these, 12 genes (*rps*16, *atp*F, *rpo*C1, *ycf*3, *clp*P, *pet*B, *pet*D, *rpl*16, *rpl*2, *ndh*B, *ndh*A, *ndh*B) were cis-splicing genes (Figure S2), and *rps*12 was a trans-splicing gene with three unique exons (Figure S3).

The reconstructed phylogeny based on 22 whole chloroplast genome sequences revealed the phylogenetic position of *A. suberifolia* within *Actinidia* (Figure 3). In the phylogenetic tree, 21 *Actinidia* species can be classified into three main groups, which was largely in accordance with the previous studies (Liu et al. 2017; Wang et al. 2022). Most of the bootstrap values of the tree branches were 100, suggesting its strong phylogenetic resolution. Our results indicated that *A. suberifolia* was closely related to *Actinidia latifolia, Actinidia eriantha, Actinidia styracifolia and Actinidia fulvicoma* and they formed a strongly supported clade.

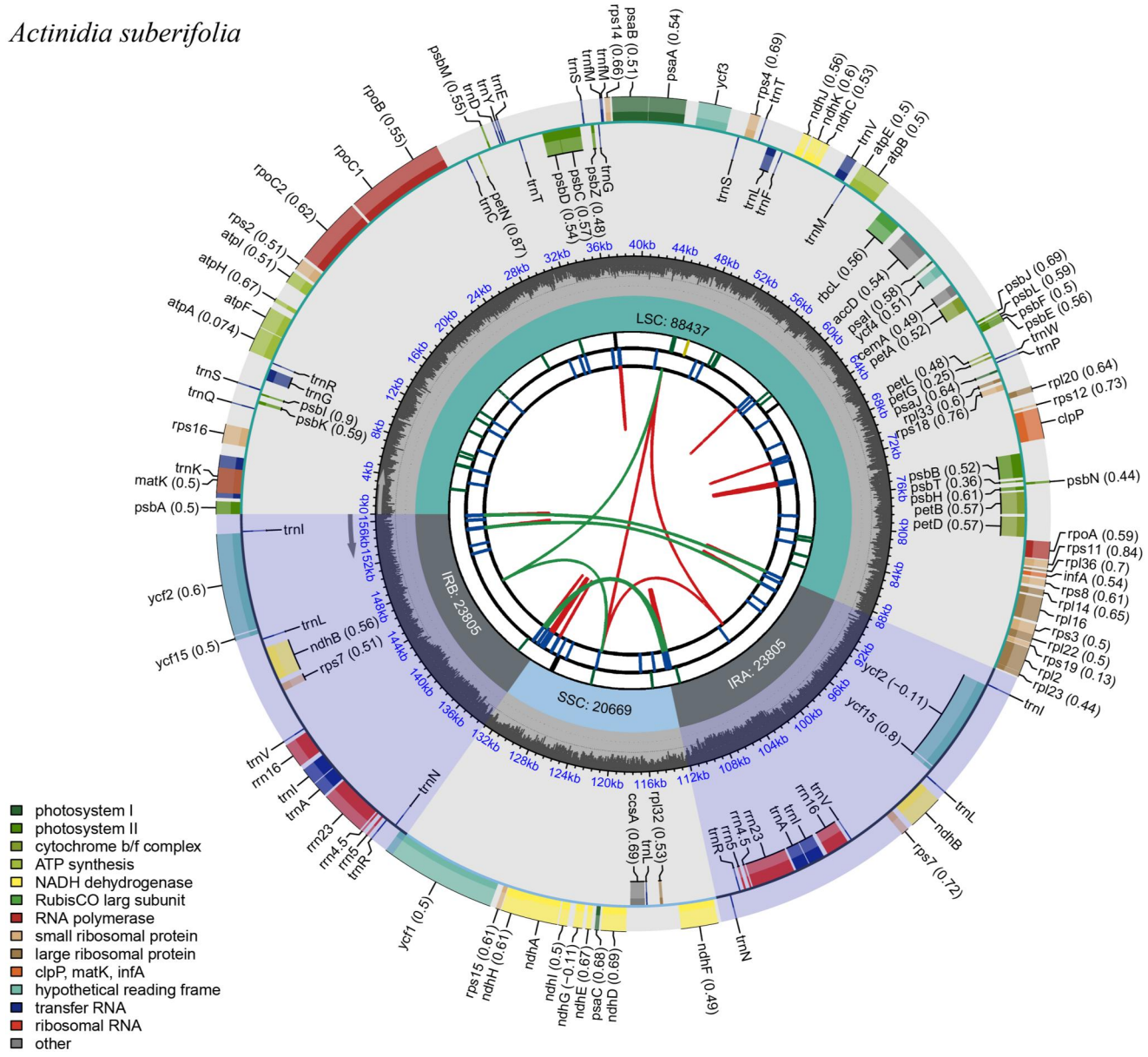

Figure 2. Gene map representing the chloroplast genome of *A. suberifolia*. The map includes six circles: the genes located inside and outside the outermost circle respectively represent the location in plus and minus DNA strand. The grey area in the second circle represents the corresponding GC content at different positions. The third circle displays the locations and length of the LSC, SSC, IRA and IRB regions. The fourth circle shows short tandem repeats or microsatellites color-coded by unit size: black (complex repeats), green (mononucleotide), yellow (dinucleotide). The fifth circle illustrates long tandem repeats using vertical blue bars. The innermost circle displays dispersed repeats, with direct repeats shown as red arcs and palindromic repeats as green arcs. The gene categories are displayed in different colors in the bottom left corner.

Conclusions and discussion

In this study, the complete chloroplast genome sequence of *A. suberifolia* was reported for the first time, exhibiting a total length of 156,716 bp and consisting of two IRs of 23,805 bp separated by an LSC and an SSC of 88,437 bp and 20,669 bp, respectively. The analysis of the genome revealed a typical quadripartite structure and gene content similar to other species of *Actinidia*. Phylogenetic analysis elucidated the phylogenetic position of *A. suberifolia* within the genus, showing that it had a close genetic relationship with *A. latifolia, A. eriantha, A. styracifolia and A. fulvicoma*, and the phylogenetic tree displayed consistent topology with the previous reports. Consistent with the traditional *Actinidia* classification system (four-section and four-series taxonomy), these species clustered within the Ser. Perfectae C. F. Liang of the Sect. Stellatae Li (Chat et al. 2004), as defined by shared morphological traits. This congruence not only confirmed the reliability of morphology-based taxonomy but also established a genomic framework for future taxonomic revisions through integrating molecular and morphological evidence.

This study provided valuable genomic resources for understanding the phylogenetic relationships and taxonomic classifications within the genus *Actinidia*. However, this study solely conducted phylogenetic analysis of *Actinidia* at the chloroplast genome level and included a relatively limited number of species. Therefore, further investigations into the

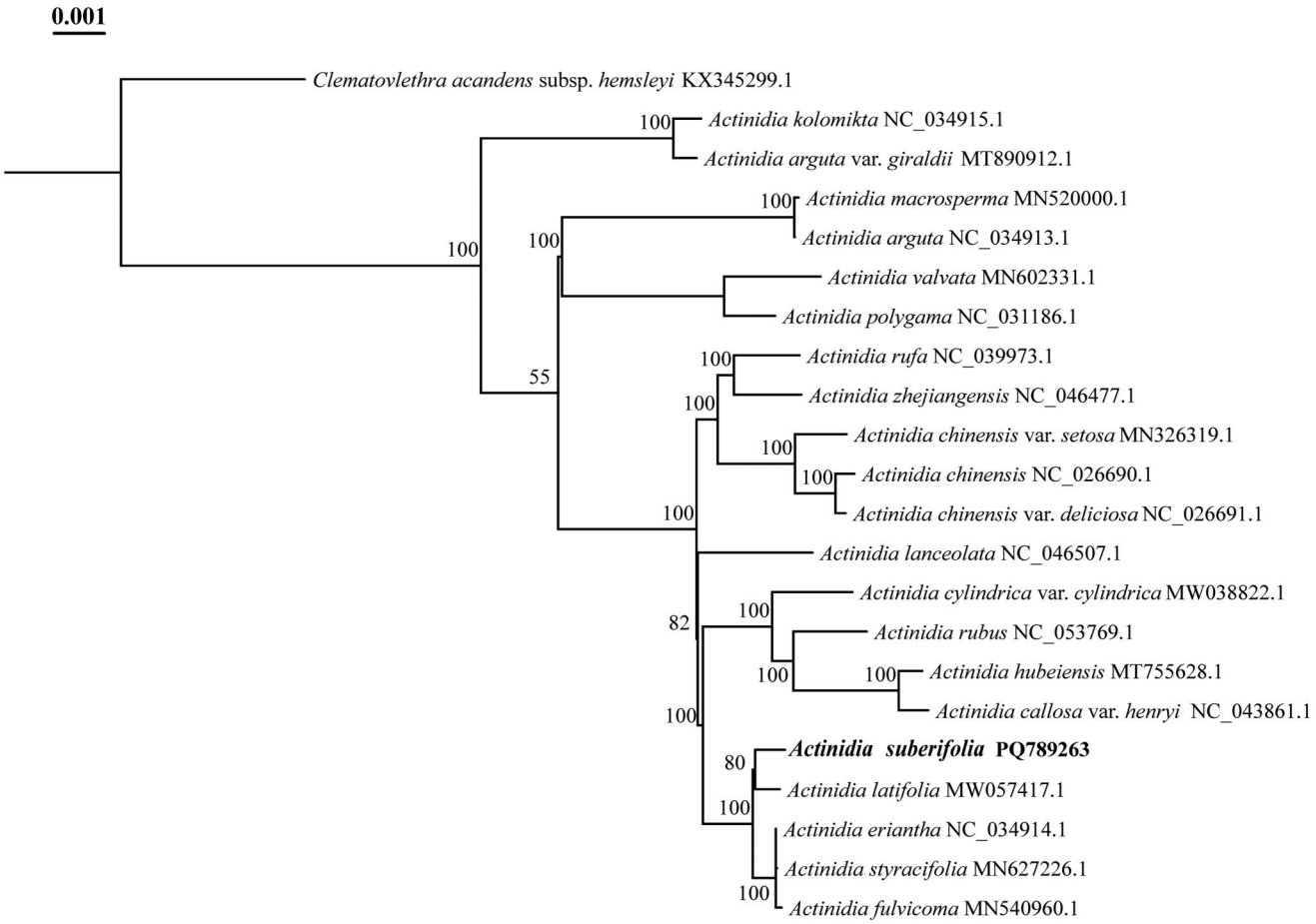

Figure 3. Maximum-likelihood tree based on the chloroplast gene sequences of *A. suberifolia* and 21 other species. The number next to the nodes indicates the bootstrap values. The scale bar in the top left corner of the figure represents the phylogenetic distance of 0.001 nucleotide substitutions per site. The following sequences are used: *A. arguta* NC_034913.1 (Lin et al. 2018), *A. arguta* var. *giraldii* MT890912.1 (Ding et al. 2021), *A. callosa* var. *henryi* NC_043861.1 (Wu et al. 2019), *A. chinensis* NC_026690.1 (Yao et al. 2015), *A. chinensis* var. *deliciosa* NC_026691.1 (Yao et al. 2015), *A. chinensis* var. *setosa* MN326319.1 (Lin et al. 2019), *A. cylindrica* var. *cylindrica* MW038822.1 (Ma & Liu 2019), *A. eriantha* NC_034914.1 (Tang et al. 2019), *A. fulvicoma* MN540960.1 (Zhang et al. 2019), *A. hubeiensis* MT755628.1, *A. kolomikta* NC_034915.1 (Lan et al. 2018), *A. lanceolata* NC_046507.1 (Zhang & Liu 2019), *A. latifolia* MW057417.1 (Yang et al. 2021), *A. macrosperma* MN520000.1 (Chen et al. 2019), *A. polygama* NC_031186.1 (Wang et al. 2016), *A. rubus* NC_053769.1 (Xu et al. 2020), *A. rufa* NC_039973.1 (Kim et al. 2018), *A. styracifolia* MN627226.1 (Yang et al. 2019), *A. suberifolia* PQ789263, *A. valvata* MN602331.1 (Chen et al. 2020), *A. zhejiangensis* NC_046477.1 (Ai & Liu 2019), *Clematoclethra scandens* subsp. *hemsleyi* KX345299.1 (Wang et al. 2016).

phylogenetic relationships within *Actinidia* are highly recommended. In future studies, more extensive sampling and multi-genome level analyses will undoubtedly enhance our comprehension of the evolutionary history of *Actinidia*.

Acknowledgments

Jingwen Huang conceived and designed the study. Qingchao Zhang, Quan Jiang and Lianhai Wu collected the samples and analyzed the data. Qingchao Zhang and Jingwen Huang drafted the manuscript. Xiaohong Yao supervised the project and approved the final version of the manuscript. All authors read and approved the final manuscript before submission.

Author contributions

CRediT: **Qingchao Zhang**: Investigation, Resources, Writing – original draft; **Lianhai Wu**: Investigation, Resources; **Quan Jiang**: Resources; **Jingwen Huang**: Conceptualization, Writing – original draft, Writing – review & editing; **Xiaohong Yao**: Funding acquisition, Writing – review & editing.

Ethical approval

The collecting of *A. suberifolia* did not require specific permission from authorities and conformed to the requirement of international ethics. We only collected some leaf samples and did not cause any damage to the local environment. This study did not involve ethical issues.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The complete chloroplast genome sequence of *Actinidia suberifolia* is available in GenBank of NCBI under the accession number PQ789263. The associated BioProject, Bio-Sample and SRA numbers are PRJNA1202025, SAMN45947641 and SRR31820701, respectively.

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