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GENOME NOTE

REVISED Plastid genome of *Passiflora tripartita* var. *mollissima*

(poro-poro) from Huánuco, Peru

[version 3; peer review: 2 approved]

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Abstract

Passiflora tripartita var. *mollissima*, known locally as poro-poro, is an important native fruit used in traditional Peruvian medicine with relevant agro-industrial and pharmaceutical potential for its antioxidant capacity for human health. However, to date, only a few genetic data are available, which limits exploring its genetic diversity and developing new genetic studies for its improvement. We report the poro-poro plastid genome to expand the knowledge of its molecular markers, evolutionary studies, molecular pathways, and conservation genetics. The complete chloroplast (cp) genome is 163,451 bp in length with a typical quadripartite structure, containing a large single-copy region of 85,525 bp and a small single-copy region of 13,518 bp, separated by a pair of inverted repeat regions (IR) of 32,204 bp, and the overall GC content was 36.87%. This cp genome contains 128 genes (110 genes were unique and 18 genes were found duplicated in each IR region), including 84 protein-coding genes, 36 transfer RNA-coding genes, eight ribosomal RNA-coding genes, and 13 genes with introns (11 genes with one intron and two genes with two introns). The inverted repeat region boundaries among species were similar in organization, gene order, and content, with a few revisions. The phylogenetic tree reconstructed based on single-copy orthologous genes and maximum likelihood analysis demonstrates poro-poro is most closely related to *Passiflora menispermifolia* and *Passiflora oerstedii*. In summary, our study constitutes a valuable

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resource for studying molecular evolution, phylogenetics, and domestication. It also provides a powerful foundation for conservation genetics research and plant breeding programs. To our knowledge, this is the first report on the plastid genome of *Passiflora tripartita* var. *mollissima* from Peru.

Keywords

Plastid genome, Passifloraceae, *Passiflora tripartita* var. *mollissima*, poro-poro, native fruit, Huánuco, Peru



This article is included in the **Genomics and Genetics** gateway.

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REVISED Amendments from Version 2

We update the circular genome map ensuring a more concise and comprehensible representation.

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Introduction

Passiflora tripartita var. *mollissima* (Kunth) Holms-Niels. & P.M. Jørg (ITIS, 2022) previously known as *Passiflora mollissima* (Kunth) Bailey (Primot *et al.*, 2005), is a semi-perennial fruit plant (Mayorga *et al.*, 2020). It is a diploid species with a small number of chromosomes ($2n = 18$) (Coppens D'Eeckenbrugge, 2001), which is placed in the section Elkea of supersection Tacsonia of subgenus *Passiflora* belonging to the Passifloraceae family (Segura *et al.*, 2005; Ocampo & Coppens d'Eeckenbrugge, 2017). Poro-poro is a native fruit of the Andean region (Ocampo & Coppens d'Eeckenbrugge, 2017). It grows in the Peruvian highlands in the departments of Ancash, Junín, Moquegua, Huanca-velica, and Huánuco at altitudes of 1,000–4,000 m.a.s.l. (Tapia & Fries, 2007; Ríos-García, 2017). It is widely used in traditional medicine (Ríos-García, 2017) and is considered one of the best *Passiflora* species based on its organoleptic characteristics (Primot *et al.*, 2005). This fruit provides a source of vitamins (A, B3, and C) and minerals (magnesium, potassium, phosphorus, sodium, chlorine, iron, calcium, sulfur, zinc, copper, selenium, cobalt, and nickel) (Leterme *et al.*, 2006; Chaparro-Rojas *et al.*, 2014). In addition, it has an elevated antioxidant activity and high content of carotenoids (118.8 mg β -carotene), phenols (460.1 mg gallic acid), and flavonoids (1907.6 mg catechin/100 g) (Leterme *et al.*, 2006; Chaparro-Rojas *et al.*, 2014). Specifically, the high concentration of flavan-3-ols (a group of bioactive compounds) has been associated with beneficial effects on human health, such as cardiovascular protection, neurodegenerative diseases, and as an anti-cancer, anti-microbial, and anti-parasitic agent (Giambanelli *et al.*, 2020; Luo *et al.*, 2022).

Plastome sequences from over 4000 species (Zhou *et al.*, 2021) are small in size with high copy numbers and conserved sequences, enabling a significant understanding of plant molecular evolution, structural variations, and evolutionary relationships of plant diversity (Daniell *et al.*, 2016; Dobrogojski *et al.*, 2020). The plastid genome has a quadripartite structure: a large single-copy (LSC) of 80–90 kilobase pairs (kb), a small single-copy (SSC) of 16–27 kb, and two sets of inverted repeats (IRA and IRB) of 20–28 kb, with 110–130 unique genes, including protein-coding genes, transfer RNA (tRNA), and ribosomal RNA (rRNA) (Ozeki *et al.*, 1989; Wang & Lanfear, 2019). In recent years, declining genome sequencing costs resulted in more than 780 complete plant genomes of different species becoming available (Marks *et al.*, 2021; Sun *et al.*, 2022). Recently, some *Passiflora* plastid genomes such as *Passiflora edulis* (Cauz-Santos *et al.*, 2017), *Passiflora xishuangbannaensis* (Hao & Wu, 2021), *Passiflora caerulea* (Niu *et al.*, 2021), *Passiflora serrulata* (Mou *et al.*, 2021), *Passiflora foetida* (Hopley *et al.*, 2021), and *Passiflora arbelaezii* (Shrestha *et al.*, 2019), became publicly available. However, despite the scarcity of genomic information on underutilized crops (Gioppato *et al.*, 2019), we have only begun to investigate the genomics of plants of great importance for plant breeding programs. The purpose of this research was to obtain the poro-poro plastid genome, which constitutes a valuable resource for studying the molecular evolution, phylogenetics, and domestication of species with beneficial characteristics for human health. In the present study, we report the first plastid genome sequence submitted for an isolate of *Passiflora tripartita* var. *mollissima*, and important native fruit of Peru.

Methods

Plant materials

In November 2022, the fresh leaves of *Passiflora tripartita* var. *mollissima* were collected from Raccha Cedrón locality of Quisqui District, Huánuco Province from Peru (9°53'37"S, 76°26'02"W, altitude 2,945 m.a.s.l.). A herbarium voucher specimen (USM<PER>:MHN331530) was deposited in the Herbario San Marcos (USM) of the Museo de Historia Natural (MHN) at the Universidad Nacional Mayor de San Marcos (UNMSM) (see the *Extended data*, Aliaga *et al.*, 2023a).

DNA extraction

Total genomic DNA was extracted from approximately 100 mg fresh leaves (from voucher number USM<PER>:MHN331530) according to Doyle's (1991) method with slight modifications. The DNA isolation buffer consisted of buffer cetyl-trimethyl ammonium bromide (CTAB) 3% (30g/L CTAB, 100 mM Tris-HCl pH 8.0, 10mM EDTA, 1.4 M NaCl, 0.2% 2-mercaptoethanol), 70% ethanol, chloroform-isoamyl alcohol (24:1), 10 mM ammonium acetate, iso-propanol, TE buffer (10 mM Tris-H, 1 mM EDTA), and RNAase A (10 ug/ml). Genomic DNA quality was assessed using a fluorometry-based Qubit (Thermo Fisher Scientific, USA, catalog number: Q33238) coupled to a Broad Range Assay kit (Thermo Fisher Scientific, USA, catalog number: Q33230). High-quality DNA (230/260 and 260/280 ratios >1.8)

were normalized (20 ng/ μ L) to examine its integrity using 1% (w/v) agarose gel electrophoresis (see the *Extended data*, Aliaga *et al.*, 2023b) with the following equipment: Horizontal gel system (Fisher Scientific, Denmark, catalog number: 11833293, 150mm (length), 100 mm (width)), Transilluminator (Fisher Scientific, Spain, catalog number: 12864008), and digital camera (Canon, Spain, catalog number: 2955C002); Reagents: TAE buffer (40 mM Tris, 20mM NaAc, 1mM EDTA, pH 7.2), loading buffer 6X (Promega, USA, catalog number: G1881, 0.4% orange G, 0.03% bromophenol blue, 0.03% xylene cyanol FF, 15% Ficoll[®] 400, 10mM Tris-HCl pH 7.5 and 50mM EDTA pH 8.0) and Ethidium bromide (Promega, USA, catalog number H5041, 10 mg/ml), and 1 Kb Plus DNA Ladder (ThermoFisher, USA, catalog number: 10787018).

Genome sequencing, assembly, and annotation

Qualified DNA was fragmented, and the TruSeq Nano DNA kit (Illumina, San Diego, CA, USA, catalog number: FC-121-4001) was used to construct an Illumina paired-end (PE) library. PE sequencing (2×150 bp) was performed using the Illumina NovaSeq 6000 platform (Modi *et al.*, 2021) (Illumina, San Diego, Ca, USA, catalog number: 20012850) (Macrogen, Inc., Seoul, Republic of Korea). The quality control of reads was carried out using the FastQC (Wingett & Andrews, 2018) program. All adapters were removed using the Cutadapt (Martin, 2011) program. After that, PE reads (2×150 bp) were evaluated for quality using QUAST (Gurevich *et al.*, 2013) analysis, and subsequent steps used clean data. Then, clean reads obtained were assembled into a circular contig using NOVOPlasty v.4.3 (Dierckxsens *et al.*, 2017), with *P. edulis* (NC_034285) as the reference (Cauz-Santos *et al.*, 2017). Assembled genome was annotated using CpGAVAS2 (Shi *et al.*, 2019), an integrated plastome sequence annotator and GeSeq (Tillich *et al.*, 2017). Transfer RNAs were also checked with ARAGORN v.1.2.38 (Laslett and Canback, 2004), Chloë v.0.1.0 (<https://github.com/ian-small/chloe>) and tRNAscan-SE v2.0 (Chan *et al.*, 2019) incorporated in GeSeq using default settings. A circular genome map was constructed using OGDRAW v.1.3.1 (Greiner *et al.*, 2019). Finally, the completed sequences were submitted to the NCBI GenBank under the accession number OQ910395 (GenBank, 2023).

Phylogenetic analysis

We used 26 complete plastome sequences to infer the phylogenetic relationships among *Passiflora* species, and *Vitis vinifera* was used as an outgroup (see the *Extended data*, Aliaga *et al.*, 2023c). Single-copy orthologous genes were identified using the Orthofinder version 2.2.6 pipeline (Emms & Kelly, 2019). For each gene family, the nucleotide sequences were aligned using the L-INS-i algorithm in MAFFT v7.453 (Katoh & Standley, 2013). A phylogenetic tree based on maximum likelihood (ML) was constructed using RAXML v8.2.12 (Stamatakis, 2014) with the GTRCAT model. A phylogenetic ML tree was reconstructed and edited using MEGA 11 (Tamura *et al.*, 2021) with 1000 replicates.

Results

Plastome of *Passiflora tripartita* var. *mollissima*

The plastid genome sequences of *P. tripartita* var. *mollissima* (poro-poro) (Figure 1) was 163,451 bp in length, with an average coverage depth of 100 $\times$, with a typical quadripartite structure consisting of a large single-copy (LSC) region of 85,525 bp (52.32% in total) and a small single-copy (SSC) region of 13,518 bp (8.27%), separated by a pair of inverted repeat regions (IRs) of 32,204 bp (19.70%). The poro-poro plastome is 12,045 bp longer than that of one of the most economically important species, passion fruit (*P. edulis*) (Cauz-Santos *et al.*, 2017), and is only 7,117 bp longer than that of the longest *Passiflora* plastome reported, i.e., *P. arbelaezii* (Shrestha *et al.*, 2019). The plastome sequence of poro-poro has a similar quadripartite architecture to other plants (Ohyama *et al.*, 1986; Shinozaki *et al.*, 1986; Nguyen *et al.*, 2021). However, the LSC region is 4,150 bp longer than that of *P. xishuangbannaensis* but is 98bp, 195 bp, and 1,927 bp shorter than that of *P. caerulea*, *P. edulis*, and *P. arbelaezii*, respectively. The SSC region is 121 bp, 140 bp, 359 bp, and 754 bp longer than that of *P. caerulea*, *P. edulis*, *P. xishuangbannaensis*, and *P. arbelaezii*, respectively. The IRs regions are 6,024 bp, 6,050 bp, and 11,600 longer than that of *P. caerulea*, *P. edulis*, and *P. xishuangbannaensis*, respectively; however, it is 2,972 bp shorter than that of *P. arbelaezii* (Cauz-Santos *et al.*, 2017; Shrestha *et al.*, 2019; Hao & Wu, 2021; Niu *et al.*, 2021). The plastome structure of the *P. tripartita* var. *mollissima* consisted of A = 30.79%, T(U) = 32.34%, C = 18.67% and G = 18.20%. The overall AT content of the plastid genome was 63.13%, whereas the overall GC content was 36.87% as similar to that of other reported chloroplast genomes from the same family, such as 36.90% in *P. arbelaezii* (Shrestha *et al.*, 2019), 37% in *P. edulis* and *P. serrulata* (Cauz-Santos *et al.*, 2017; Mou *et al.*, 2021), 37.03% in *P. caerulea* (Niu *et al.*, 2021), and 37.1% in *P. xishuangbannaensis* (Hao & Wu, 2021).

Poro-poro plastid genome annotation identified 128 genes, of which 110 were unique, and 18 were duplicated in the inverted repeat (IR) region. The plastome contained 84 protein-coding genes, 36 transfer RNA (tRNA)-coding genes, eight ribosomal RNA (rRNA)-coding genes, and 13 genes with introns (11 genes with one intron and two genes with two introns), as shown in Table 1. The poro-poro plastid genome contained 110 unique genes, of which there were 28 tRNA genes, four rRNA genes, and 78 protein-coding genes. The latter comprised 19 ribosomal subunit genes (nine large subunits and 10 small subunit), four DNA-directed RNA polymerase genes, 46 genes were involved in photosynthesis

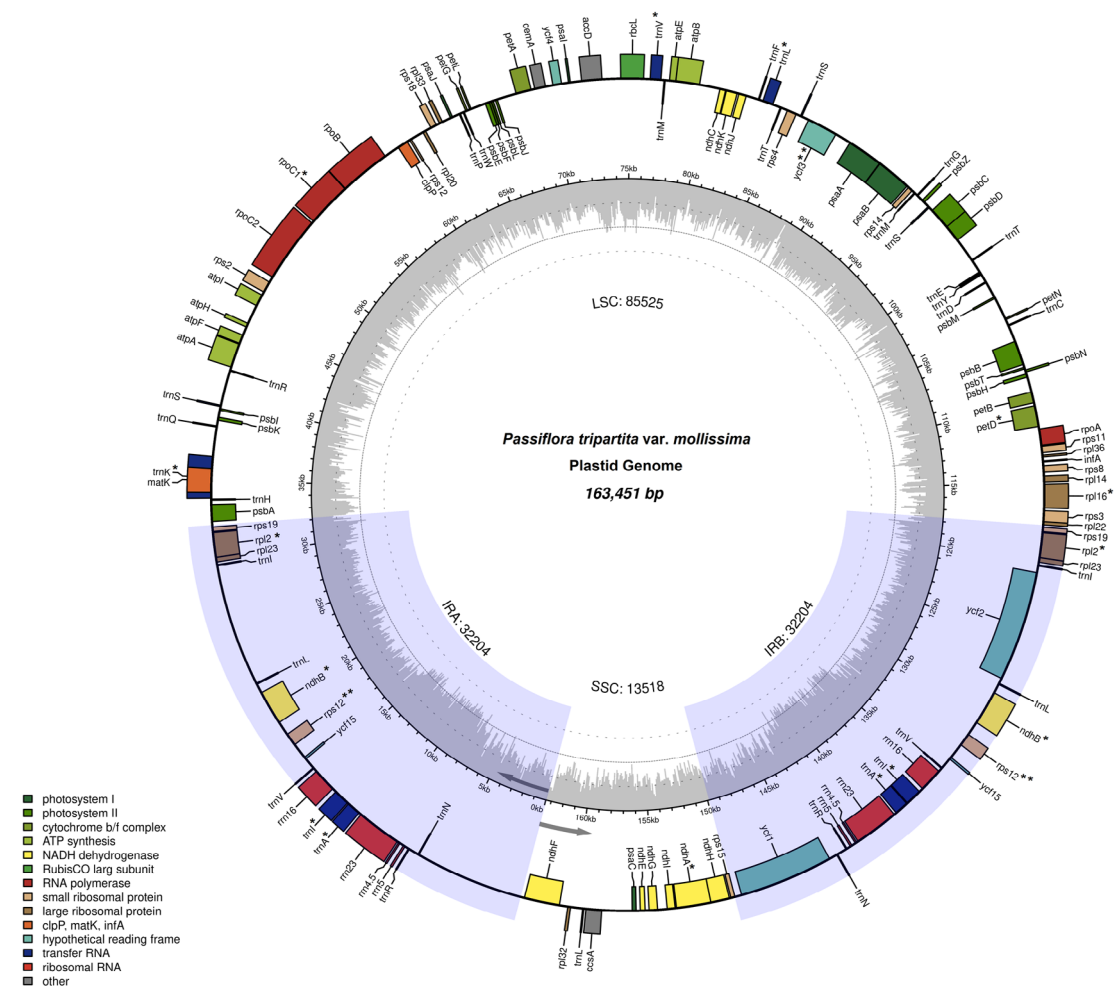


Figure 1. Plastid genome of *Passiflora tripartita* var. *mollissima*. The thick lines indicate the IR1 and IR2 regions, which separate the large single-copy (LSC) and small single-copy (SSC) regions. Genes marked inside the circle are transcribed clockwise, and genes marked outside the circle are transcribed counterclockwise. Genes are color-coded based on their function, shown at the bottom left. The inner circle indicates the inverted boundaries and guanine and cytosine (GC) content.

Table 1. Plastid genome features of the *P. tripartita* var. *mollissima*.

Features	Poro-poro ¹
Genome size (bp)	163,451
^a LSC length (bp)	85,525
^b SSC length (bp)	13,518
^c IR length (bp)	32,204
Total GC content (%)	36.87
^d A content (%)	30.79
^e T(U) content (%)	32.34
^f G content (%)	18.20
^g C content (%)	18.67
Total number of genes	128
Protein-coding genes	84
^h rRNA coding genes	8
ⁱ tRNA coding genes	36
Genes duplicated in IR regions	18

Table 1. Continued

Features	Poroporo ¹
Total introns	13
Single introns (gene)	11
Double introns (gene)	2

¹ Poroporo is the common name of *Passiflora tripartita* var. *mollissima* in Peru.

^aLSC: a large single-copy.

^bSSC: a small single-copy.

^cIR: inverted repeat.

^dA: adenine.

^eT(U): thymine (uracil).

^fG: guanine.

^gC: cytosine.

^hrRNA: ribosomal RNA.

ⁱtRNA: transfer RNA.

(11 encoded subunits of the NADH oxidoreductase, seven for photosystem I, 15 for photosystem II, six for the cytochrome b6/f complex, six for different subunits of ATP synthase, and one for the large chain of ribulose biphosphate carboxylase), eight genes were involved in different functions, and one gene was of unknown function (Table 2).

Table 2. Genes present in the plastid genome of *P. tripartita* var. *mollissima*.

Category	Group of genes	Gene names
Photosynthesis	Subunits of photosystem I	<i>psaA, psaB, psaC, psaI, psaJ, ycf3**</i> , <i>ycf4</i>
	Subunits of photosystem II	<i>psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbM, psbN, psbT, psbZ</i>
	Subunits of cytochrome b/f complex	<i>petA, petB, petD*</i> , <i>petG, petL, petN</i>
	Subunits of ATP synthase	<i>atpA, atpB, atpE, atpF, atpH, atpI</i>
	Subunits of NADH dehydrogenase	<i>ndhA*</i> , <i>ndhB*</i> (X2), <i>ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK</i>
	Large subunit of RUBISCO	<i>rbcl</i>
Self-replication	Large subunits of ribosome	<i>rpl2*</i> (X2), <i>rpl14, rpl16*</i> , <i>rpl20, rpl22, rpl23</i> (X2), <i>rpl32, rpl33, rpl36</i>
	Small subunits of ribosome	<i>rps2, rps3, rps4, rps8, rps11, rps12**</i> (X2) ^a , <i>rps14, rps15, rps18, rps19</i> (X2)
	DNA-dependent RNA polymerase	<i>rpoA, rpoB, rpoC1*</i> , <i>rpoC2</i>
	Ribosomal RNAs	<i>rrn4.5</i> (X2), <i>rrn5</i> (X2), <i>rrn16</i> (X2), <i>rrn23</i> (X2)
	Transfer RNAs	<i>trnA-UGC*</i> (X2), <i>trnC-GCA, trnD-GUC, trnE-UUC, trnF-GAA, trnG-GCC, trnH-GUG, trnI-CAU</i> (X2), <i>trnI-GAU*</i> (X2), <i>trnK-UUU*</i> , <i>trnL-CAA</i> (X2), <i>trnL-UAA*</i> , <i>trnL-UAG, trnM-CAU</i> (X2), <i>trnN-GUU</i> (X2), <i>trnP-UGG, trnQ-UUG, trnR-ACG</i> (X2), <i>trnR-UCU, trnS-GCU, trnS-GGA, trnS-UGA, trnT-GGU, trnT-UGU, trnV-GAC</i> (X2), <i>trnV-UAC*</i> , <i>trnW-CCA, trnY-GUA</i>
Other genes	Maturase	<i>matK</i>
	Protease	<i>clpP</i>
	Envelope membrane protein	<i>cemA</i>
	Acetyl-CoA carboxylase	<i>accD</i>
	C-type cytochrome synthesis gene	<i>ccsA</i>
	Translation initiation factor	<i>InfA</i>
	Component of TIC complex	<i>ycf1, ycf2</i>
Genes of unknown	Proteins of unknown function	<i>ycf15</i> (X2)

*Gene contains one intron.

**Gene contains two introns.

^aGene divided into two independent transcription units. (X2) two gene copies in the IRs.

In the plastid genome, 13 genes contained introns distributed as follows: the LSC, SSC, and IRs regions contained seven genes (*petD*, *rpl16*, *rpoC1*, *trnK-UUU*, *trnL-UAA*, *trnV-UAC*, and *ycf3*), one gene (*ndhA*), and five genes (*ndhB*, *rpl2*, *rps12*, *trnA-UGC*, and *trnI-GAU*) respectively. Similarly, these genes included six protein-coding genes, each with a single intron (*petD*, *ndhA*, *ndhB*, *rpoC1*, *rpl2*, and *rpl16*); five tRNA genes, each with a single intron (*trnA-UGC*, *trnI-GAU*, *trnK-UUU*, *trnL-UAA*, and *trnV-UAC*); and two protein-coding genes with two introns (*ycf3* and *rps12*). Except for 18 genes that were duplicated in the IR region (*ndhB*, *rps19*, *rpl2*, *rpl23*, *rps12*, *ycf15*, *rrn4.5*, *rrn5*, *rrn16*, *rrn23*, *trnA-UGC*, *trnI-GAU*, *trnL-CAA*, *trnM-CAU*, *trnN-GUU*, *trnR-ACG*, and *trnV-GAC*) all genes contained a single copy, as shown in Table 2. The *ycf1* sequence encodes a protein essential for plant viability and a vital component of the translocon on the inner chloroplast membrane (TIC) complex (Kikuchi *et al.*, 2013), and *ycf2* is a component of the ATPase motor protein associated with the TIC complex (Kikuchi *et al.*, 2018).

Contraction and expansion of the IR boundary

In this study, the IR boundary analysis of four *Passiflora* species revealed that the structure and sequences of four junctions, JLB (junction between LSC and IRb), JSB (junction between SSC and IRb), JSA (junction between SSC and IRa), and JLA (junction between LSC and IRa), between the two inverted repeats (IRa and IRb) and the two single-copy regions (LSC and SSC) of *P. tripartita* var. *mollissima*, *P. oerstedii* (147 073 bp; Genbank accession: NC_038124), *P. foetida* (162 266 bp; Genbank accession: NC_043825), and *P. edulis* (151 406 bp; Genbank accession: NC_034285) were similar (Figure 2). The genes of *rps3*, *rps19*, *rpl2*, *rps15*, *ycf1*, *ndhF*, *ndhH*, and *psbA* were located mainly near the IR/LSC and IR/SSC boundaries of the plastome for these four species of *Passiflora*. In the same order that was described, *rps3* is entirely located in the LSC region, at distances of 206 bp, 264 bp, 159 bp, and 206 bp, respectively, from the JLB boundary. For *rps19*, which is in both IR regions, the nucleotide distance from the JLB boundary varies from 128 – 210 bp. In *P. oerstedii*, both copies of the *rps2* gene are in the IR region, and the *ndhH* gene is located in the SSC region.

The *rps15* gene crossed the SSC/IRb boundary, expanding 243 bp and 17 bp in *P. tripartita* var. *mollissima*, respectively. The *rps15* gene is located 182 bp away from the SSC/IRa boundary in *P. foetida* and is located at the end of the SSC region, expanding 81 bp and 20 bp in *P. oerstedii* and *P. edulis*, respectively. In all species compared, the *ndhF* gene is located 234 bp away from the SSC/IRa boundary in *P. tripartita* var. *mollissima*, and is located 29 bp, 14 bp, and 219 bp away from the SSC/IRb boundary in *P. oerstedii*, *P. foetida*, and *P. edulis*. Furthermore, the *ycf1* gene in *P. oerstedii*, *P. foetida*, and *P. edulis* is located 266 – 481 bp away from the SSC/IRa boundary, except for *P. tripartita* var. *mollissima*, which was not present in JSA.

The *infA* gene, which codes for translation initiation factor 1, is present in *P. tripartita* var. *mollissima*, but it is absent from the *P. foetida*, *P. oerstedii*, and *P. edulis* cp genomes. Furthermore, *trnG-UCC* and *ycf68* are unique genes in *P. foetida* and *P. edulis*, respectively. The plastome of *P. tripartita* var. *mollissima* contained seven genes (*ycf1*, *ycf2*,

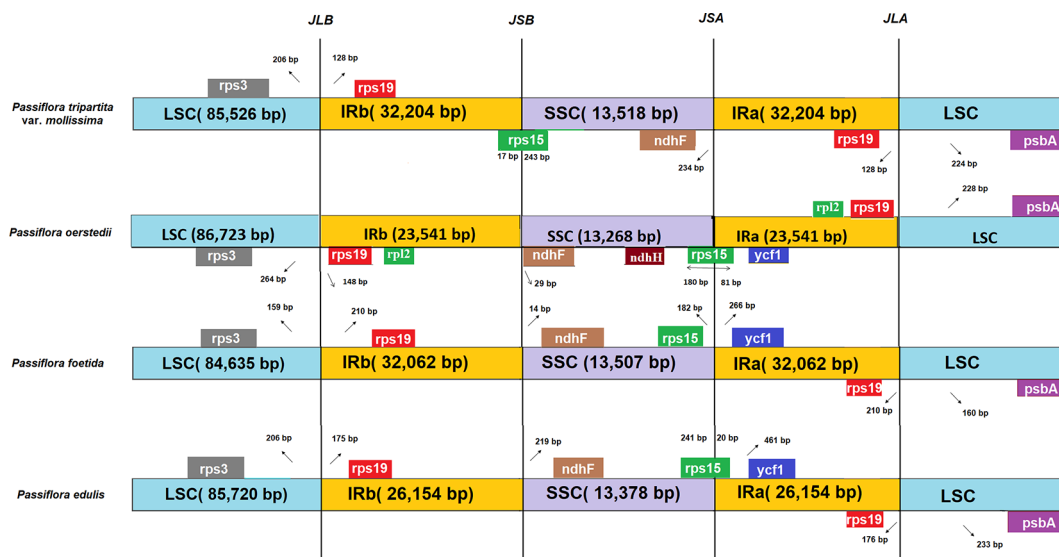


Figure 2. Comparison of IR/SC boundary regions of four *Passiflora* species. Boxes represent the nearby border genes. Gaps between the ends of boundaries and adjacent genes, as well as the sizes of gene segments positioned within a boundary, are depicted. The junction sites of LSC/IRb, IRb/SSC, SSC/IRa, and IRa/LSC are denoted as JLB, JSB, JSA, and JLA, respectively.

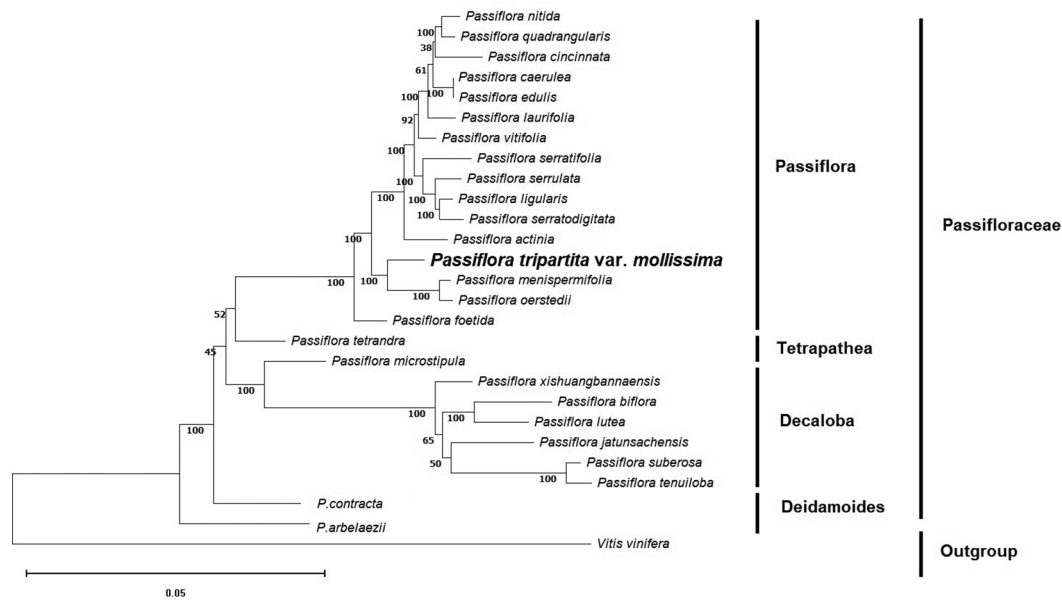


Figure 3. Phylogenetic tree of 27 plastid genomes using maximum likelihood analysis based on single-copy orthologous protein. Bootstrap values on the branches were calculated from 1000 replicates.

ycf15, *rpl20*, *rpl22*, *accD*, *infA*) that were lost or non-functional genes in *P. edulis*; and compared to *P. foetida*, *P. oerstedii*, and *P. edulis*, the *trnM*-CAU gene was not found.

Phylogenetic reconstruction

To identify the evolutionary position of *Passiflora tripartita* var. *mollissima* in the Passifloraceae family, phylogenetic relationships based on the OrthoFinder clustering method were used to avoid erroneous rearrangements in phylogenetic tree reconstruction and provides a more reliable evolutionary analysis (Gabaldón, 2005; Zhang *et al.*, 2012). The phylogenetic tree was constructed based on single-copy orthologous genes (Emms & Kelly, 2019) and maximum likelihood analysis with the complete annotated protein sequences of 27 plastid genomes, of which 26 were from *Passiflora* species. One species, *Vitis vinifera*, was chosen as the outgroup.

Maximum likelihood (ML) bootstrap values ranged from 38%–92% for seven of the 25 nodes. All nodes except the indicated ones (seven nodes) exhibited bootstrap support (BS) values of 100%. These *Passiflora* species were divided into four groups: subgenus *Passiflora* (*P. nitida*, *P. quadrangularis*, *P. cincinnata*, *P. caerulea*, *P. edulis*, *P. laurifolia*, *P. vitifolia*, *P. serratifolia*, *P. serrulata*, *P. ligularis*, *P. serratodigitata*, *P. actinia*, *P. menispermifolia* and *P. oerstedii*), subgenus *Tetrapathea* (*P. tetrandra*), subgenus *Decaloba* (*P. microstipula*, *P. xishuangbannaensis*, *P. biflora*, *P. lutea*, *P. jatunsachensis*, *P. suberosa* and *P. tenuiloba*), and subgenus *Deidamoides* (*P. contracta* and *P. arbelaezii*). The relationships between the four subgenera of *Passiflora* species (*Passiflora*, *Tetrapathea*, *Decaloba*, and *Deidamoides*) were congruent and strongly supported by the same patterns as previously reported (Cauz-Santos *et al.*, 2020; Pacheco *et al.*, 2020). These results resolved *Passiflora tripartita* var. *mollissima* belonging to the subgenus *Passiflora*, which was closely related to *P. menispermifolia* and *P. oerstedii* with 100% BS, and was sister to *P. tetrandra* (subgenus *Tetrapathea*), *P. biflora* (subgenus *Decaloba*), and *P. contracta* (subgenus *Deidamoides*), as shown in the cladogram (Figure 3).

Data availability

Underlying data

Nucleotide: *Passiflora tripartita* var. *mollissima* chloroplast, complete genome. Accession number: OQ910395. <https://identifiers.org/nucleotide:OQ910395> (GenBank, 2023).

Extended data

Figshare: Herbarium specimen voucher of *Passiflora tripartita* var. *mollissima* (Kunth) Holms-Niels. & P.M. Jørg (USM:MHN331530). <https://doi.org/10.6084/m9.figshare.23556654> (Aliaga *et al.*, 2023a).

Figshare: Gel imagen of DNA isolate from poro-poro sample. <https://doi.org/10.6084/m9.figshare.23560755> (Aliaga *et al.*, 2023b).

Figshare: Details of the plastid genome sequences used for phylogenetic analysis. <https://doi.org/10.6084/m9.figshare.23556834> (Aliaga *et al.*, 2023c).

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Acknowledgements

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An earlier version of this article can be found on [Preprints.org](#) (doi: <https://doi.org/10.20944/preprints202306.0463.v2>).

References

- Aliaga F, Zapata-Cruz M, Valverde-Zavaleta SA: Herbarium specimen voucher of *Passiflora tripartita* var. *mollissima* (Kunth) Holms-Niels. & P.M. Jørg (USM<PER>:MHN331530). [Dataset]. *Figshare*. 2023a. [Publisher Full Text](#)
- Aliaga F, Zapata-Cruz M, Valverde-Zavaleta SA: Gel imagen of DNA isolate from poro-poro sample. [Dataset]. *Figshare*. 2023b. [Publisher Full Text](#)
- Aliaga F, Zapata-Cruz M, Valverde-Zavaleta SA: Details of the plastid genome sequences used for phylogenetic analysis. [Dataset]. *Figshare*. 2023c. [Publisher Full Text](#)
- Cauz-Santos LA, da Costa ZP, Callot C, *et al.*: **A Repertory of Rearrangements and the Loss of an Inverted Repeat Region in *Passiflora* Chloroplast Genomes.** *Genome Biol. Evol.* 2020; **12**: 1841–1857. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Cauz-Santos LA, Munhoz CF, Rodde N, *et al.*: **The Chloroplast Genome of *Passiflora edulis* (Passifloraceae) Assembled from Long Sequence Reads: Structural Organization and Phylogenomic Studies in Malpighiales.** *Front. Plant Sci.* 2017; **8**: 334. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chan PP, Lowe TM: **tRNAscan-SE: Searching for tRNA Genes in Genomic Sequences.** *Methods Mol. Biol.* 2019; **1962**: 1–14. [PubMed Abstract](#) | [Publisher Full Text](#)
- Chaparro-Rojas DC, Maldonado ME, Franco-Londoño MC, *et al.*: **Características nutricionales y antioxidantes de la fruta curuba larga (*Passiflora mollissima* Bailey).** *Perspect Nutr. Hum.* 2014; **16**: 203–212. [Publisher Full Text](#)
- Coppens D'Eeckenbrugge G: **Conservación y utilización de recursos genéticos de pasifloras.** COLCIENCIAS Informe final 1999-2001. 2001. (accessed on 01 Jun 2023). [Reference Source](#)
- Daniell H, Lin CS, Yu M, *et al.*: **Chloroplast genomes: diversity, evolution, and applications in genetic engineering.** *Genome Biol.* 2016; **17**: 134. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Dierckxsens N, Mardulyn P, Smits G: **NOVOPlasty: de novo assembly of organelle genomes from whole genome data.** *Nucleic Acids Res.* 2017; **45**: e18. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Dobrogojski J, Adamiec M, Lucinski R: **The chloroplast genome: A review.** *Acta Physiol. Plant.* 2020; **42**: 98. [Publisher Full Text](#)
- Doyle J: **DNA Protocols for Plants.** *Molecular Techniques in Taxonomy.* Hewitt GM, Johnston AWB, Young JPW, editors. Berlin, Germany: Springer; 1991; Volume **57**: pp. 283–293. 978-3-642-83962-7.
- Emms DM, Kelly S: **OrthoFinder: phylogenetic orthology inference for comparative genomics.** *Genome Biol.* 2019; **20**: 238. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Gabaldón T: **Evolution of proteins and proteomes: a phylogenetics approach.** *Evol. Bioinformatics Online.* 2005; **1**: 117693430500100–117693430500161. [Publisher Full Text](#)
- GenBank: Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1982]. [Dataset]. [cited 2023 Jun 21]. [Reference Source](#)
- Giambanelli E, Gómez-Caravaca AM, Ruiz-Torralba A, *et al.*: **New advances in the determination of free and bound phenolic compounds of banana passion fruit pulp (*Passiflora tripartita*, var. *mollissima* (Kunth) L.H. Bailey) and their in vitro antioxidant and hypoglycemic capacities.** *Antioxidants.* 2020; **9**: 628. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Gioppato HA, da Silva MB, Carrara S, *et al.*: **Genomic and transcriptomic approaches to understand *Passiflora* physiology and to contribute to passionfruit breeding.** *Theor. Exp. Plant. Physiol.* 2019; **31**: 173–181. [Publisher Full Text](#)
- Greiner S, Lehwark P, Bock R: **OrganelleGenomeDRAW (OGDRAW) version 1.3.1: expanded toolkit for the graphical visualization of organelle genomes.** *Nucleic Acids Res.* 2019; **47**: W59–W64. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Gurevich A, Saveliev V, Vyahhi N, *et al.*: **QUAST: quality assessment tool for genome assemblies.** *Bioinformatics.* 2013; **29**: 1072–1075. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hao C, Wu F: **The complete chloroplast genome sequence of *Passiflora xishuangbannaensis* (Passifloraceae), a vine endemic to Yunnan, China.** *Mitochondrial DNA B Resour.* 2021; **6**: 1763–1764. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hopley T, Webber BL, Raghu S, *et al.*: **Revealing the Introduction History and Phylogenetic Relationships of *Passiflora foetida* sensu lato in Australia.** *Front. Plant Sci.* 2021; **12**: 651805. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- ITIS: *Passiflora tripartita* var. *mollissima* (Kunth) Holms-Niels. & P.M. Jørg. 2022. (accessed on 01 Jun 2023). [Reference Source](#)
- Katoh K, Standley DM: **MAFFT multiple sequence alignment software version 7: improvements in performance and usability.** *Mol. Biol. Evol.* 2013; **30**: 772–780. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kikuchi S, Asakura Y, Imai M, *et al.*: **Ycf2-FtsH Heteromeric AAA-ATPase Complex Is Required for Chloroplast Protein Import.** *Plant Cell.* 2018; **30**: 2677–2703. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kikuchi S, Bédard J, Hirano M, *et al.*: **Uncovering the protein translocator at the chloroplast inner envelope membrane.** *Science.* 2013; **339**: 571–574. [PubMed Abstract](#) | [Publisher Full Text](#)
- Laslett D, Canback B: **ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences.** *Nucleic Acids Res.* 2004; **32**: 11–16. [PubMed Abstract](#) | [Publisher Full Text](#)
- Leterme P, Buldgen A, Estrada F, *et al.*: **Mineral content of tropical fruits and unconventional foods of the Andes and the rain forest of Colombia.** *Food Chem.* 2006; **95**: 644–652. [Publisher Full Text](#)

- Luo Y, Jian Y, Liu Y, *et al.*: **Flavanols from nature: A phytochemistry and biological activity review.** *Molecules.* 2022; **27**: 719.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Marks RA, Hotaling S, Frandsen PB, *et al.*: **Representation and participation across 20 years of plant genome sequencing.** *Nat. Plants.* 2021; **7**: 1571–1578.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Martin M: **Cutadapt removes adapter sequences from high-throughput sequencing reads.** *EMBnet J.* 2011; **17**: 10–12.
[Publisher Full Text](#)
- Mayorga M, Fischer G, Melgarejo LM, *et al.*: **Growth, development and quality of *Passiflora tripartita* var. *mollissima* fruits under two environmental tropical conditions.** *J. Appl. Bot. Food Qual.* 2020; **93**: 66–75.
[Publisher Full Text](#)
- Modi A, Vai S, Caramelli D, *et al.*: **The illumina sequencing protocol and the novaseq 6000 system.** *Bacterial Pangenomics: Methods and Protocols.* 2nd edn. Mengoni A, Bacci G, Fondi M, editors. New York, USA: Springer; 2021; Volume **2242**: pp. 15–42. 978-1-0716-1099-2.
[Publisher Full Text](#)
- Mou HF, Huang WH, Liu JY, *et al.*: **Complete chloroplast genome sequence of *Passiflora serrulata* Jacq. (Passifloraceae).** *Mitochondrial DNA B Resour.* 2021; **6**: 191–193.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Nguyen HQ, Nguyen TN, Doan TN, *et al.*: **Complete chloroplast genome of novel *Adrinandra megaphylla* Hu species: molecular structure, comparative and phylogenetic analysis.** *Sci. Rep.* 2021; **11**: 11731.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Niu YF, Ni SB, Liu SH, *et al.*: **The complete chloroplast genome of *Passiflora caerulea*, a tropical fruit with a distinctive aroma.** *Mitochondrial DNA B Resour.* 2021; **6**: 488–490.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ocampo J, Coppens d'Eeckenbrugge GC: **Morphological characterization in the genus *passiflora* l.: an approach to understanding its complex variability.** *Plant Syst. Evol.* 2017; **303**: 531–558.
[Publisher Full Text](#)
- Ohyama K, Fukuzawa H, Kohchi T, *et al.*: **Chloroplast gene organization deduced from complete sequence of liverwort *Marchantia polymorpha* chloroplast DNA.** *Nature.* 1986; **322**: 572–574.
[Publisher Full Text](#)
- Ozeki H, Umeson K, Inokuchi H, *et al.*: **The chloroplast genome of plants: a unique origin.** *Genome.* 1989; **31**: 169–174.
[Publisher Full Text](#)
- Pacheco TG, Lopes AS, Welter JF, *et al.*: **Plastome sequences of the subgenus *Passiflora* reveal highly divergent genes and specific evolutionary features.** *Plant Mol. Biol.* 2020; **104**: 21–37.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Primot S, Coppens D'Eeckenbrugge G, Rioux V, *et al.*: **Variación morfológica de tres especies de curubas (*Passiflora tripartita* var. *mollissima*, *P. tarminiana* y *P. mixta*) y sus híbridos en el Valle del Cauca (Colombia).** *Rev. Bras. Frutic.* 2005; **27**: 467–471.
[Publisher Full Text](#)
- Ríos-García G: **Nivel de aceptabilidad del vino de tumbo serrano (*Passiflora mollissima*) elaborado con los parámetros tecnológicos óptimos en la ciudad de Huánuco 2015.** Master thesis, Universidad Nacional Hermilio Valdizán de Huánuco: Huánuco. 2017.
- Segura SD, D'Eeckenbrugge GC, Ocampo CH, *et al.*: **Isozyme variation in *Passiflora* subgenus *tacsonia*: Geographic and interspecific differentiation among the three most common species.** *Genet. Resour. Crop. Evol.* 2005; **52**: 455–463.
[Publisher Full Text](#)
- Shi L, Chen H, Jiang M, *et al.*: **CPGAVAS2, an integrated plastome sequence annotator and analyzer.** *Nucleic Acids Res.* 2019; **47**: W65–W73.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Shinozaki K, Ohme M, Tanaka M, *et al.*: **The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression.** *EMBO J.* 1986; **5**: 2043–2049.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Shrestha B, Weng ML, Theriot EC, *et al.*: **Highly accelerated rates of genomic rearrangements and nucleotide substitutions in plastid genomes of *Passiflora* subgenus *Decaloba*.** *Mol. Phylogenet. Evol.* 2019; **138**: 53–64.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Stamatakis A: **RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies.** *Bioinformatics.* 2014; **30**: 1312–1313.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Sun Y, Shang L, Zhu QH, *et al.*: **Twenty years of plant genome sequencing: achievements and challenges.** *Trends Plant Sci.* 2022; **27**: 391–401.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Tamura K, Stecher G, Kumar S: **MEGA11: Molecular Evolutionary Genetics Analysis Version 11.** *Mol. Biol. Evol.* 2021; **38**: 3022–3027.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Tapia ME, Fries AM: *Guía de campo de los cultivos andinos.* Roma, Italy: FAO; 1st ed. 2007; 22–114.
- Tillich M, Lehwark P, Pellizzer T, *et al.*: **GeSeq - versatile and accurate annotation of organelle genomes.** *Nucleic Acids Res.* 2017; **45**: W6–W11.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Wang W, Lanfear R: **Long-reads reveal that the chloroplast genome exists in two distinct versions in most plants.** *Genome Biol. Evol.* 2019; **11**: 3372–3381.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Wingett SW, Andrews S: **FastQ Screen: A tool for multi-genome mapping and quality control.** *F1000Res.* 2018; **7**: 1338.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Zhang N, Zeng L, Shan H, *et al.*: **Highly conserved low-copy nuclear genes as effective markers for phylogenetic analyses in angiosperms.** *New Phytol.* 2012; **195**: 923–937.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Zhou J, Zhang S, Wang J, *et al.*: **Chloroplast Genomes in *Populus* (Salicaceae): Comparisons from an Intensively Sampled Genus Reveal Dynamic Patterns of Evolution.** *Sci. Rep.* 2021; **11**: 9471.
[PubMed Abstract](#) | [Publisher Full Text](#)

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Version 3

Reviewer Report 22 February 2024

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Rahul G Shelke 

Independent Researcher, Amravati, India

Since the authors attentively considered every recommendation, I have nothing else to add.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Organelle genome sequencing, Transcriptome assembling, Genetic diversity, Barcoding and DNA markers etc

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 14 February 2024

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I have thoroughly reviewed the manuscript and would like to bring to your attention a minor issue in Fig. 1.

Upon careful examination, I have observed that certain genes, including *rrn23*, *rrn23*-fragment, *infA*, *rpoA*, etc., bear double annotations in the figure. I appreciate the authors' efforts in incorporating the suggested changes throughout the manuscript. However, I recommend addressing this specific matter to enhance clarity for readers.

To rectify this, I propose that the extra annotations be removed from the GenBank file before generating the diagram. This correction will streamline the information presented in Fig. 1, ensuring a more concise and comprehensible representation for new readers.

I believe implementing this adjustment will further enhance the overall quality of the manuscript.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Organelle genome sequencing, Transcriptome assembling, Genetic diversity, Barcoding and DNA markers etc

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 14 Feb 2024

Flavio Aliaga

The authors thank Dr. Rahul G. Shelke for taking the time and effort necessary to review the manuscript. We sincerely appreciate all the valuable comments and suggestions that helped us improve the quality of the manuscript.

In the previous version (V.2), we did not update the circular map (Fig. 1). In this new version, all the genes correctly mentioned in Table 2 are observed in Figure 1. Likewise, we clarify that the gene *rps12* is divided into two independent transcription units (Table 2).

We appreciate you greatly for your immense support in reviewing this manuscript.

Competing Interests: No competing interests were disclosed.

Reviewer Report 19 January 2024

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The article can be accepted.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I can assess all aspects of the manuscript and published up to 18 articles in the same field.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 04 September 2023

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Authors need to describe how the current genome will be helpful. I found many species of the genus *Passiflora* in the NCBI. So, authors need clear points about the need for the existing genome. In addition, all the genomes have similar features so the current genome is helpful for the study of evolution and phylogenetics.

The authors mentioned the number of reported genomes up to 800. This statement is not correct and is based on earlier observations. Now the number has increased significantly.

I found authors are not entirely aware of the utilization of some software. For example, authors mention fastqc for trimming while fastqc checks the quality of reads.

The authors used many programs for annotations. I will suggest authors use GeSeq along with Chole, Aragorn and tRNAScan on GeSeq servers for annotation of transfer RNA.

The authors need to give some information on how the current genome is similar to or differs from other reported genomes of *Passiflora*.

Are the rationale for sequencing the genome and the species significance clearly described?

Partly

Are the protocols appropriate and is the work technically sound?

Partly

Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?

Partly

Are the datasets clearly presented in a usable and accessible format, and the assembly and annotation available in an appropriate subject-specific repository?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I can assess all aspects of the manuscript and published up to 18 articles in the same field.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 28 Dec 2023

Flavio Aliaga

Corresponding Author: Flavio Aliaga

Grupo de Investigación en Ecología Evolutiva, Protección de Cultivos, Remediación Ambiental, y Biotecnología (EPROBIO), Universidad Privada del Norte, Trujillo, Peru. Dirección de Investigación, Innovación y Responsabilidad Social, Universidad Privada del Norte, Trujillo, 13011, Peru

1. Authors need to describe how the current genome will be helpful. I found many species of the genus *Passiflora* in the NCBI. So, authors need clear points about the need for the existing genome.

Answer: We would like to thank reviewer 2 for the insightful response. We have considered your comment because the poro-poro plastid genome constitutes a valuable resource for studying the molecular evolution, phylogenetics, and domestication of species with beneficial characteristics for human health.

2. In addition, all the genomes have similar features so the current genome is helpful for

the study of evolution and phylogenetics.

Answer: We are grateful to reviewer 2 for the constructive and valuable comments. Thank you.

3. The authors mentioned the number of reported genomes up to 800. This statement is not correct and is based on earlier observations. Now the number has increased significantly.

Answer. We agree with the reviewer. The manuscript has been corrected:

- " Plastome sequences from over 4000 species..."

4. I found authors are not entirely aware of the utilization of some software. For example, authors mention fastqc for trimming while fastqc checks the quality of reads.

Answer. It has been revised, thank you.

5. The authors used many programs for annotations. I will suggest authors use GeSeq along with Chole, Aragorn and tRNAScan on GeSeq servers for annotation of transfer RNA.

Answer. It has been revised, thank you.

6. The authors need to give some information on how the current genome is similar to or differs from other reported genomes of *Passiflora*

Answer. Thank you for your comment. We have added the figure 2 titled "Comparison of IR/SC boundary regions of four *Passiflora* species".

Competing Interests: No competing interests were disclosed.

Reviewer Report 30 August 2023

<https://doi.org/10.5256/f1000research.151329.r191279>

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Rahul G Shelke

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I extend my appreciation to the authors for their commendable efforts in extensively exploring the plastid genome of *Passiflora tripartita* var. *mollissima*, demonstrating remarkable depth and breadth in their approach. The manuscript substantially enhances our comprehension of this species, emphasizing its plastid genome, genetic diversity, and evolutionary relationships. This

newfound insight carries far-reaching implications, fostering the conservation and sustainable utilization of this species. Consequently, the manuscript's significance is markedly elevated, making a noteworthy contribution to the scientific community's understanding of genetic resource stewardship.

Here is a concise summary of the study's key points:

1. **Implications and Novelty:** The findings of the study hold significant implications. The genetic insights uncovered have the potential to catalyze further investigations in areas such as molecular evolution, conservation genetics, and the development of plant breeding programs. Notably, the manuscript's distinction as the first documentation of the plastid genome of *Passiflora tripartita* var. *mollissima* from Peru underscores its novel contribution to the scientific community.
2. **Methodological Rigor:** The authors' selection of the Illumina Novaseq 6000 platform for DNA sequencing is well-suited to the research objectives. The subsequent analyses and methodologies applied exhibit robustness and alignment with the research goals.

Major comments:

1. While the genes in Table 2 have been organized into categories, it is apparent that certain instances lack gene information or are unclear in their presentation. This calls for a restructuring of the table to ensure a heightened level of clarity and coherence.
2. In Table 2, the authors have indicated the absence of a duplicated copy of the *rrn4.5* gene. It is advised that the authors review the annotation of this gene. Typically, rRNA genes are located within the IR regions and therefore exist in duplicated copies. However, in Figure 1 of the chloroplast genome, the *rrn4.5* gene is depicted in a duplicated copy. I recommend that the authors rectify any discrepancies pertaining to the *rrn4.5* gene both in the table and within the text.
3. The authors have provided comprehensive insights into the expansion and contraction of the LSC, SSC, and IR regions in the compared species. To further enhance reader comprehension, I recommend that the authors consider incorporating the corresponding IRSCOPE figure within the manuscript. This addition would allow readers to access detailed information more effectively and contribute to a clearer understanding of the discussed concepts.
4. Kindly provide information regarding the genes that are missing or subject to gene loss within the compared species.
5. In the abstract, the authors have included the statement: "In summary, our study provides the basis for developing new molecular markers that constitutes a valuable resource for studying molecular evolution and domestication." However, upon reviewing the manuscript, it is evident that the authors have not conducted any SSR, tandem repeat, or DNA diversity analysis, nor have they proposed any markers. Given this situation, I recommend that the authors consider removing the phrase "developing new molecular markers" from the abstract to accurately reflect the scope of their study.

Minor comments:

1. The inclusion of specific methodology details such as "Total genomic DNA was extracted from fresh leaves (herbarium voucher: USM:MHN331531)" and "The DNA was sequenced

using Illumina Novaseq 6000 platform" in the abstract might be better suited for the methodology section rather than the abstract itself. Focusing the abstract on the core findings and implications of the study could enhance its conciseness and clarity.

2. Add the full botanical name of *Passiflora tripartita* in the phylogeny tree to ensure consistency.

3. Gene names should be in italics.

4. It is recommended to include the coverage of the genome assembly within the main text of the manuscript.

The authors adeptly tackle gaps in our understanding of this species' genetics, comparative genomics, and evolutionary relationships. By thoughtfully engaging with reviewer feedback and subsequently refining their manuscript, the authors stand poised to elevate the impact and significance of their research within the realm of plant genomics and beyond. This process will significantly enhance the accuracy and reliability of gene classification and annotation, thereby elevating the overall quality of the manuscript.

Are the rationale for sequencing the genome and the species significance clearly described?

Yes

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?

Yes

Are the datasets clearly presented in a usable and accessible format, and the assembly and annotation available in an appropriate subject-specific repository?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Organelle genome sequencing, Transcriptome assembling, Genetic diversity, Barcoding and DNA markers etc

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 28 Dec 2023

Flavio Aliaga

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The authors thank Dr. Rahul G. Shelke for taking the time and effort necessary to review the manuscript. We sincerely appreciate all the valuable comments and suggestions that helped us improve the quality of the manuscript.

Answers to Reviewer #1

Major comments:

1. While the genes in Table 2 have been organized into categories, it is apparent that certain instances lack gene information or are unclear in their presentation. This calls for a restructuring of the table to ensure a heightened level of clarity and coherence.

Answer: We agree with the reviewer. The manuscript has been revised, thanks.

2. In Table 2, the authors have indicated the absence of a duplicated copy of the *rrn4.5* gene. It is advised that the authors review the annotation of this gene. Typically, rRNA genes are located within the IR regions and therefore exist in duplicated copies. However, in Figure 1 of the chloroplast genome, the *rrn4.5* gene is depicted in a duplicated copy. I recommend that the authors rectify any discrepancies pertaining to the *rrn4.5* gene both in the table and within the text.

Answer: We would like to thank the reviewer for highlighting this important comment. In fact, we checked and updated the duplicated copy of the *rrn4.5* gene. Thank you.

3. The authors have provided comprehensive insights into the expansion and contraction of the LSC, SSC, and IR regions in the compared species. To further enhance reader comprehension, I recommend that the authors consider incorporating the corresponding IRSCOPE figure within the manuscript. This addition would allow readers to access detailed information more effectively and contribute to a clearer understanding of the discussed concepts.

Answer: Thank you for your comment. According to your suggestions, we have added the figure 2 titled "Comparison of IR/SC boundary regions of four *Passiflora* species".

4. Kindly provide information regarding the genes that are missing or subject to gene loss within the compared species.

Answer: Thank you for your reminding. Done.

5. In the abstract, the authors have included the statement: "In summary, our study provides the basis for developing new molecular markers that constitutes a valuable resource for studying molecular evolution and domestication." However, upon reviewing the manuscript, it is evident that the authors have not conducted any SSR, tandem repeat,

or DNA diversity analysis, nor have they proposed any markers. Given this situation, I recommend that the authors consider removing the phrase “developing new molecular markers” from the abstract to accurately reflect the scope of their study.

Answer. We agree with the reviewer. The manuscript has been corrected:

- “In summary, our study constitutes a valuable resource for studying molecular evolution, phylogenetics, and domestication...”

Minor comments:

1. The inclusion of specific methodology details such as "Total genomic DNA was extracted from fresh leaves (herbarium voucher: USM:MHN331531)" and "The DNA was sequenced using Illumina Novaseq 6000 platform" in the abstract might be better suited for the methodology section rather than the abstract itself. Focusing the abstract on the core findings and implications of the study could enhance its conciseness and clarity.

Answer. Yes, we agree with the reviewer. Done.

2. Add the full botanical name of *Passiflora tripartita* in the phylogeny tree to ensure consistency.

Answer. Done.

3. Gene names should be in italics. It is recommended to include the coverage of the genome assembly within the main text of the manuscript.

Answer. Done.

4. The authors adeptly tackle gaps in our understanding of this species' genetics, comparative genomics, and evolutionary relationships. By thoughtfully engaging with reviewer feedback and subsequently refining their manuscript, the authors stand poised to elevate the impact and significance of their research within the realm of plant genomics and beyond. This process will significantly enhance the accuracy and reliability of gene classification and annotation, thereby elevating the overall quality of the manuscript.

Answer: We thank reviewer 1 for all constructive comments that led to substantial improvement of the manuscript. Thank you; it is much appreciated.

Competing Interests: No competing interests were disclosed.

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