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The complete chloroplast genome of *Petunia exserta* (Solanaceae: Petunioideae), an endangered ornamental species

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ABSTRACT

Petunia exserta is an ornamental species on the brink of extinction in the wild. We report here the complete chloroplast genome of *P. exserta*, which is 156,598 bp in size consisting of a large single-copy region (87,095 bp), a small single-copy region (18,643 bp), and a pair of inverted repeats (25,430 bp for each). The chloroplast (used 'cp' hereafter) genome contains 132 genes, including 8 rRNA genes, 37 tRNA genes, and 87 protein-coding genes. Phylogenetic analysis demonstrated that *P. exserta* was most closely related to *P. hybrida*, and they together were closer to *Calibrachoa hybrida* than other taxa in the Solanaceae family.

Petunia exserta J.R. Stehm., a recently discovered wild species of the *Petunia* genus in the Solanaceae (nightshade) family, has an erect growth habit and red star-shaped flowers with exserted styles and anthers. This species was only found in a very small area (about 500 km^2) of the Serra do Sudeste region of southern Brazil and is already considered to be near extinction in the wild, with a very limited number of wild plants left in its native habitat (Lorenz-Lemke et al. 2006; Segatto et al. 2014). Different from the other species of the genus that are pollinated either by bees or by moths, *P. exserta* is pollinated by hummingbirds, which provide a good system for the genetic study of pollination syndromes (Klahre et al. 2011). The speciation process and evolutionary history of *P. exserta* is intriguing because it can intercross with other *Petunia* taxa in experimental conditions (Watanabe et al., 2001), and some rare cases of natural hybridization between *P. axillaris* and *P. exserta* was also found (Lorenz-Lemke et al. 2006). Previously, plastid markers were used to analyze the evolutionary relationship of *Petunia* species, which, however, revealed little genetic differentiation among the species (Ando et al. 2005; Kulcheski et al. 2006; Lorenz-Lemke et al. 2010). Recently, the complete chloroplast genome sequence of *P. hybrida,* an artificial hybrid between *P. integrifolia* and *P. axillaris*, was published (Wong et al. 2019). Here we report the complete chloroplast genome of *P. exserta*, which will provide valuable information for evolutionary studies and hybrid identification in *Petunia*.

P.exserta was obtained from the Swammerdam Institute for Life Sciences, University of Amsterdam in the Netherlands (gift from prof. Ronald Koes) and stored in the Germplasm Resource Nursery of Ornamental Plants in Guangzhou (N113°20′25″, E23°13′47″). Total genomic DNA was extracted from fresh leaves using the modified 2 × CTAB method (Doyle and Doyle 1987). The residual whole plant was processed to a voucher specimen (specimen code SYS-Bore-2020-02-10.3, under the charge of Liu Guofeng, 344591851@qq.com) and deposited in Sun Yat-sen University Herbarium. DNA samples were randomly fragmented into ∼400 bp using an ultrasonicator, followed by DNA library construction and paired-end sequencing (2 × 150 bp) on an Illumina Novaseq platform. Approximately 7.58 Gb of raw data was generated and then assembled by GetOrganelle (Jin et al. 2020). Sanger sequencing method was used to verify the LSC, SSC and IR junction region which the primers were designed by a online software Primer3 v. 0.4.0 (Koressaar and Remm 2007; Untergasser et al. 2012) (Table S1). The genome was annotated by using GeSeq (Tillich et al. 2017), followed by manual correction and confirmation.
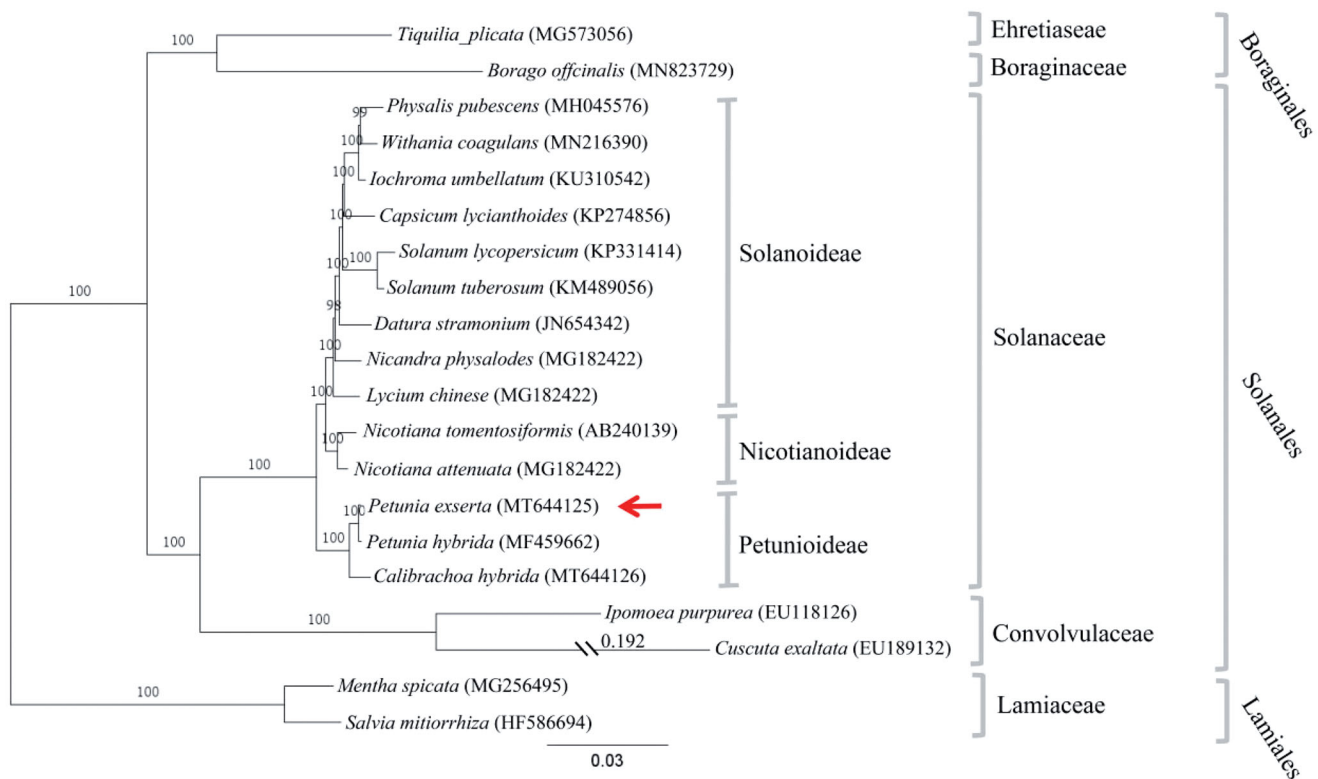
The complete chloroplast genome of *P. exserta* (GenBank accession no. MT644125) was 156,598 bp in length with total GC content of 37.81%. It contains an 87,095 bp large single-copy (LSC) region, a 18,643 bp small single-copy (SSC) region, and two 25,430 bp inverted repeat (IR) regions. A total of 132 genes were predicted, including 8 rRNA genes, 37 tRNA genes, and 87 protein-coding genes, among them all the rRNA genes, 14 tRNA genes, and 12 protein-coding genes locate in the IR regions.

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⬤ Supplemental data for this article can be accessed here.

Figure 1. Phylogenetic tree based on the complete chloroplast genome sequences of 20 species from Solanales, Lamiales, and Boraginales, with *Mentha spicata* and *Salvia mitiorrhiza* as outgroup. The phylogenetic position of *P. exserta* is indicated by red arrow. Distance was shown for truncated branches. Bootstrap values (1000 replicates) are indicated at nodes. Scale bar: substitutions per site.

To clarify the evolutionary position of *P. exserta*, complete chloroplast genomic sequences of 20 species from three orders (Lamiales, Solanales, and Boraginales) were aligned by MAFFT v7.307 (Katoh and Standley 2013), and then a maximum likelihood (ML) tree was constructed by RAxML version 8 with 1000 bootstrap replicates under the GTRGAMMA substitution model (Stamatakis 2014). The phylogenetic analysis showed that *P. exserta* was clustered together with *P. hybrida* first, and then closer to *Calibrachoa hybrida* than other taxa (Figure 1). Based on the phylogenetic tree, *P. exserta*, *P. hybrida*, and *C. hybrida* form the Petunioideae lineage that was emerged evolutionarily earlier than the Nicotianodeae and Solanoideae lineages, which is consistent with the results reported previously (Wong et al. 2019; Liu et al. 2020).

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data that support the findings of this study are openly available in NCBI at https://www.ncbi.nlm.nih.gov/nuccore/MT644125 under the accession no. MT644125. And the associated Bioproject, SRA, Bio-sample numbers are PRJNA675663, SRP291795 and SAMN16711698 respectively.

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