


The complete chloroplast genome of a rare orchid species *Liparis loeselii* (L.)

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Abstract *Liparis loeselii* is a rare orchid species protected in Europe, in some parts of North America and worldwide in scope of the Washington Convention (CITES). Nowadays, the range of *L. loeselii* is declining, mainly due to loss of its natural habitat. In this study, we reported the complete plastid (cp) genome of *L. loeselii* from Next-Generation Sequencing. The whole genome was 153,687 bp, consisting of a pair of inverted repeats of 25,709 bp, large single copy region and a small single copy region (84,596 and 17,673 bp in length, respectively). The cp genome contained 140 genes, including 81 protein-coding genes, 40 trRNA genes and 8 rRNA genes. The overall GC content of the whole genome was 36.9%. A neighbour-joining phylogenetic analysis demonstrated a close relationship between *L. loeselii* and *Dendrobium officinale*.

Keywords *Liparis loeselii* · Plastid genome · Illumina sequencing · Orchidaceae

The fen orchid, *Liparis loeselii* (L.) Rich. is a species within the family Orchidaceae, distributed in temperate parts of Europe, Asia and North America (Moore 1980). This small perennial plant occurs usually in fens and other wet habitats (Jones 1998; Wheeler 1998). Currently, the range of *L. loeselii* is declining, mainly due to habitat loss (Grootjans et al. 2016; Žalneravičius and Gudžinskas 2016). The fen orchid is protected in Europe (Pillon et al. 2006), in some parts of North America (Rolfsmeier 2007) and worldwide in scope of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Molecular studies of *L. loeselii* revealed that there is no diversity in the species within Europe (UK, Poland, Czech Republic, Hungary, Sweden, Italy, Russia) and North America (Canada) at the level of three plastid loci (*rbcL*, *matK*, *trnL-trnF*) and nuclear ITS (Wiland-Szymańska et al. 2016). Population genetic studies have shown high levels of effective long-distance seed dispersal and low levels of adaptive divergence of the species (Broeck et al. 2014). In the present paper, we report the complete chloroplast genome of *L. loeselii*. The chloroplast genome will contribute to development of conservation strategy for this rare orchid.

A part of fresh leaf from a single individual of *L. loeselii* growing in natural population in Podlachia region of Poland were collected, and total genomic DNA was extracted from about 1 cm² of leaf according to Wiland-Szymańska et al. (2016). We had appropriate permission to collect plant material of *L. loeselii* for molecular studies. Species identity of the individual used for NGS sequencing was further identified on the basis of DNA barcoding. High-throughput sequencing was carried out on the Illumina MiSeq sequencing system following the manufacturer's protocol (Illumina, CA, USA). The plastome assembly was carried out according to the flow chart developed in a

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previous study (Szczecińska et al. 2014). The annotation of cp genome was conducted using the program GENEIOUS R9 (Biomatters Ltd., Auckland, New Zealand) by comparing with the genomes of *Dendrobium officinale* KC771275. The complete and annotated cp genome sequence of *L. loeselii* has been submitted to GenBank with the accession number MF374688. A physical map of the plastome was generated using OGDRAW (<http://ogdraw.mpimp-golm.mpg.de/>) (Lohse et al. 2013).

The complete cpDNA of *L. loeselii* was a circular molecule 153,687 bp in length, comprising a large single copy (LSC) region of 84,596 bp and a small single copy (SSC) region of 17,673 bp, separated by two inverted repeat regions (IRs) of 25,709 bp (Fig. 1). It contained 140 genes, including 81 protein-coding genes, 8 ribosomal RNA genes and 40 tRNA genes. The genome contained 117 unique genes, 22 genes duplicated in the IRs and one gene (*trnM-CAU*) present in the LSC in three

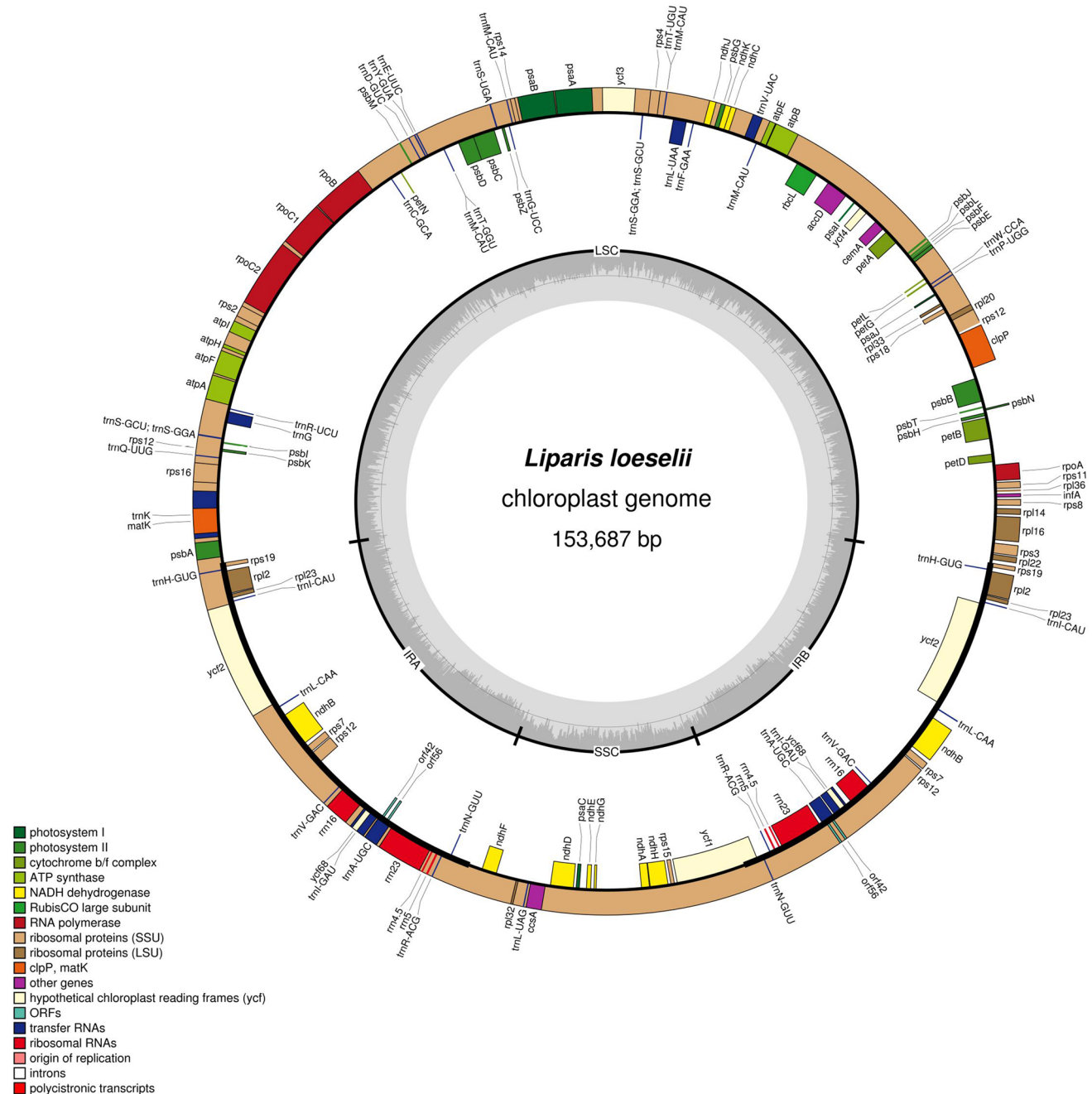
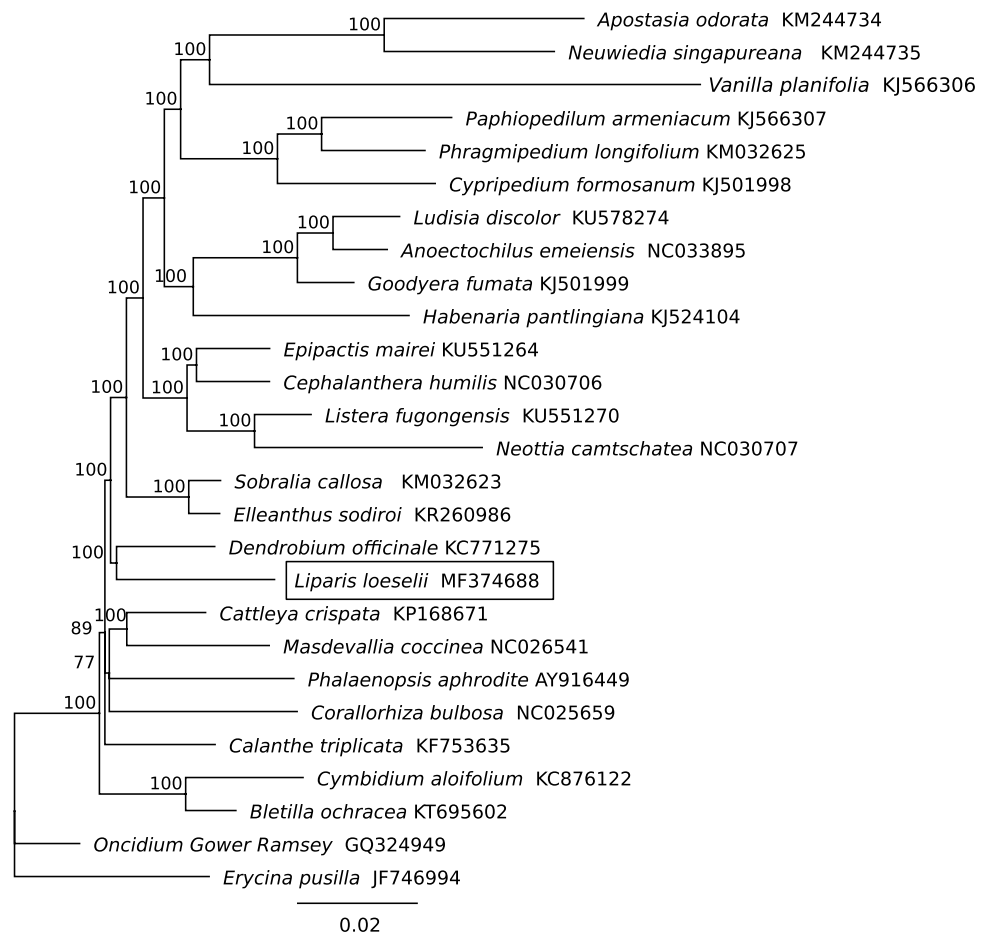


Fig. 1 Gene map of the *L. loeselii* chloroplast genome. Dashed area in the inner circle indicates the GC content

Fig. 2 Phylogenetic position of *L. loeselii* inferred by NJ analysis based on 27 sequences using LSC, SSC and one IR unit. The bootstrap values were based on 500 replicates and are shown next to the nodes. The position of *L. loeselii* is shown in a box



copies. Among annotated genes, nine (*rpl16*, *petB*, *clpP*, *ycf3*, *rpoC1*, *atpF*, *rps16*, *rpl2* and *ndhB*) contained a single intron. One copy of the *rps12* gene harbored two introns and the second, incomplete and probably pseudogene copy had only one intron. The base composition of *L. loeselii* cp genome was uneven (31.9% A, 18.3% C, 18.6% G, 36.9% T) with an overall GC content of 36.9% and the corresponding values of the LSC, SSC and IR regions reaching 34.6, 29.6 and 43.2%, respectively.

To ascertain phylogenetic position of *L. loeselii* within the family Orchidaceae, a neighbor-joining (NJ) phylogenetic tree was constructed with GENEIOUS R9 using LSC, SSC and one IR unit of 27 representatives of Orchidaceae family (Fig. 2). The result indicated that *L. loeselii* is clustered together with *D. officinale* what confirmed close relationship of *Liparis* and *Dendrobium* genera reported in Givnish et al. (2015). The complete plastid genome information reported in this paper provided data useful for population genomic studies, conservation works on *L. loeselii* as well as for phylogenetic studies within Orchidaceae.

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