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Characterization of the complete plastome of *Ophrys aveyronensis*, a Euro-Mediterranean orchid with an intriguing disjunct geographic distribution

Joris A. M. Bertrand[a], Anaïs Gibert[b], Christel Llauro[a] and Olivier Panaud[a]

[a]Laboratoire Génome & Développement des Plantes (UMR 5096 UPVD/CNRS), University of Perpignan Via Domitia, Perpignan, France; [b]PSL Université Paris, EPHE-UPVD-CNRS, USR 3278 CRIOBE, Université de Perpignan, Perpignan, France

ABSTRACT

Ophrys aveyronensis is an orchid with disjunct geographic distribution. For biogeographic and conservation purpose, we sequenced its complete plastome using Illumina data. The complete plastome is 146,816 bp in length, consisting of a pair of inverted repeats (IRs) of 25,006 bp, a large single-copy (LSC) region and a small single-copy region (SSC) of 80,495 and 16,309 bp, respectively. It was found to contain 133 genes, including 86 protein-coding genes, up to 39 trRNA genes and 8 rRNA genes. The overall GC content of the plastid genome is 36.9%. Phylogenetic inference confirms that *O. aveyronensis* is very close to *O. sphegodes*.

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Ophrys sphegodes Mill. subsp. *aveyronensis* (Wood 1983) or *Ophrys aveyronensis* (Delforge 1984) is an orchid species that was first described as endemic to a geographically restricted area in the 'Grands Causses' region (Southern France). Ever since, phenotypically similar populations were discovered sporadically, in North-Eastern Spain (Hermosilla and Soca 1999). These Iberian populations are considered as a distinct taxon by some authors (*i.e. O. vitorica*, Kreutz 2007; Delforge 2016). However, the level of genetic differentiation between the two allopatric groups has never been assessed.

We used a CTAB 2X protocol to extract genomic DNA from an individual of *Ophrys aveyronensis* collected near Lapanouse-de-Cernon, France (N 43.98945° E 3.09045°) with appropriate permit and deposited in the collection of the University of Perpignan Via Domitia under accession number 18-GS-049. Whole genomic libraries were prepared and sequenced in paired-end mode (2 × 150 bp) using Illumina technology by Novogene Co., Ltd (HK). We used NOVOPlasty v.2.7.2 (Dierckxsens et al. 2017) to reconstruct chloroplast genome, the web-based interface of GeSeq v.167 (Tillich et al. 2017) to carry on gene annotation, as well as the viewing and editing features of Geneious v.11.0.5 (https://www.geneious.com). The sequence is available from GenBank (Accession no.: MN120441).

The plastid genome of *O. aveyronensis* is a circular molecule of 146,816 bp in length, comprising a large single-copy (LSC) region and a small single-copy region (SSC) of 80,495 and 16,309 bp, respectively, separated by two inverted repeat regions (IR) of 25,006 bp (Figure 1). We annotated 110 distinct genes, including 79 protein-coding genes, 4 ribosomal RNA genes (all located in the IR) and (up to) 27 distinct tRNA genes. The genome contained 90 unique genes, 19 genes duplicated in the IRs and 1 (*trn*M-CAU) triplicated in the LSC. Among annotated genes, 6 contained one intron (*atp*F, *ndh*A, *ndh*B, *rpl*2, *rpo*C1, *rps*12, *rps*16) and two contained two introns (*clp*P and *ycf*3). The overall GC content of 39.6% was of 34.3, 29.4 and 43.5% in the LSC, SSC and IR regions respectively (see Supplementary materials for more details).

The *Ophrys aveyronensis* plastid genome was comparable in size and structure to other published plastomes of photosynthetic orchids. We used MAFFT v7.3.88 (Katoh et al. 2002; Katoh and Standley 2013) to align the plastome of *O. aveyronensis* with a set of other previously published plastid genomes and reconstructed a phylogenetic tree to verify its systematics placement with RAxML v.8.2.11 (Stamatakis 2014). The phylogenetic hypothesis we generated confirms the strong similarity of *Ophrys aveyronensis* plastid genome with the one of *Ophrys sphegodes* (99.8%) and to a lesser extent to the one of *Ophrys fusca iricolor* (96.2%) recently published by Roma et al. (2018).

CONTACT Joris A. M. Bertrand joris.bertrand@univ-perp.fr Laboratoire Génome & Développement des Plantes (UMR 5096 UPVD/CNRS), Université de Perpignan Via Domitia, 58 avenue Paul Alduy, Bâtiment T, F-66860 Perpignan, France

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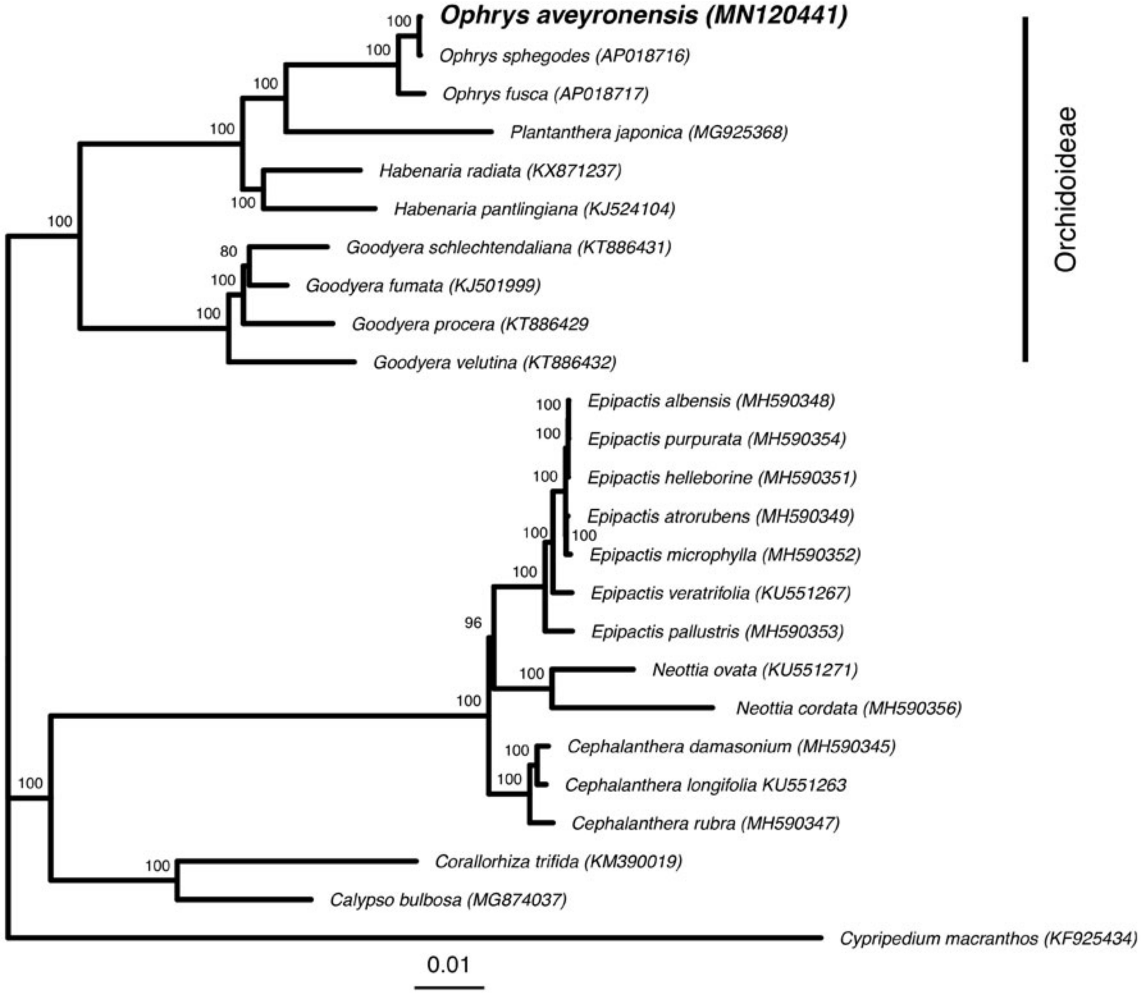

Figure 1. Phylogenetic position of *Ophrys aveyronensis* inferred by Maximum Likelihood method based on 25 whole-plastome orchid sequences for which genera occur in the Euro-Mediterranean region. Node supports correspond to bootstrap values.

Disclosure statement

The authors report no conflict of interest.

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ORCID

Joris A. M. Bertrand http://orcid.org/0000-0002-3379-1019
Anaïs Gibert http://orcid.org/0000-0003-2924-2380
Olivier Panaud http://orcid.org/0000-0002-9292-503X

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