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MITOGENOME ANNOUNCEMENT

## The chloroplast genome of *Ephedra foeminea* (Ephedraceae, Gnetales), an entomophilous gymnosperm endemic to the Mediterranean area

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### ABSTRACT

This study presents the chloroplast genome of *Ephedra foeminea*, an entomophilous gymnosperm, sister to the remaining (wind-pollinated) species of *Ephedra* (Ephedraceae, Gnetales). Based on the reference-guided assembly, the length of the chloroplast genome was estimated to be 109 584 bp, comprising a large single copy region of 60 027 bp, a small single copy 8079 bp, and inverted repeat regions of 20 739 bp. In total, 118 genes were detected, including 73 protein-coding genes, eight ribosomal RNA genes, and 37 transfer RNA genes. The gene density is 1.076 (genes/kb) and the GC content is 36.7%. The genomic sequence of the entomophilous, Mediterranean species *E. foeminea*, differs from that of the anemophilous, Asian species *E. equisetina* by 1018 point mutations and 1334 indels. The detected variation is useful for future development of new plastid markers for phylogenetic purposes. Our phylogenetic analysis based on 55 protein-coding chloroplast genes resolve *Ephedra* as monophyletic and sister to a *Gnetum-Welwitschia* clade. The Gnetales are sister to Cupressophytes.

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The monogeneric family *Ephedraceae* of the Gnetales comprises 45–55 species that inhabit arid and semiarid regions of the world (Kubitzki, 1990). Morphological and molecular divergence among species of the genus is limited (Huang et al., 2005; Ickert-Bond & Wojciechowski, 2004; Rydin et al., 2010) and the, to date best-sampled, phylogeny of the genus (Rydin & Korall, 2009) is partly poorly supported statistically. So far only the chloroplast genome of a Chinese *Ephedra* species is available (Wu et al., 2009). Here we present the chloroplast genome of a Mediterranean species, *E. foeminea*, which is sister to the remaining species of the genus (Rydin & Korall, 2009). While almost all species of *Ephedra* are wind-pollinated, *E. foeminea* is insect-pollinated and correlates its pollination stage with the lunar cycle (Bolinder et al., 2015; Rydin & Bolinder, 2015). The presented genome provides a basis for the production of new phylogenetic markers and adds to the understanding of molecular variation in the Gnetales.

Total genomic DNA of *E. foeminea* was extracted according to the CTAB method (Doyle, 1991) from the material (S, 15-64199) collected in Asprovalta, Greece, in 2012 (40°46'46.6" N 23°49'09.9" E). A total of 5 450 586 paired-end reads (average length 151 bp) were generated using the Illumina HiSeq 2500 platform (Illumina Inc., San Diego, CA). The genome was assembled using the reference-guided method (reference genome: *E. equisetina*, NC\_011954) in Geneious v. 8.1.3 (Kearse et al., 2012), and annotated using DOGMA (Wyman et al., 2004). Transfer RNA genes were assessed using

tRNAscan-SE v. 1.21 (Schattner et al., 2005). The chloroplast genome of *E. foeminea* (GenBank ID: KT934791) is a circular molecule, estimated to be 109 584 bp in length and comprising a large single copy (LSC) region of 60 027 bp, a small single copy (SSC) region of 8079 bp, and inverted repeat (IRs) regions of 20 739 bp. The overall GC content was 36.7%, and 34.1%, 27.5%, and 42.1% in the LSC, SSC and IRs, respectively. The genome contains 118 unique genes (a gene density of 1.076 genes/kb), comprising 73 protein-coding genes, 37 tRNA genes, and eight rRNA genes. While 10 of the genes (*atpF*, *petB*, *petD*, *rpoC1*, *rpl2*, *rpl16*, *trnA-UGC*, *trnL-UAA*, *trnL-GAU*, and *trnK-UUU*) have only one intron, *ycf3*, contains two introns. The genomic sequence of *E. foeminea* differs from that of *E. equisetina* by 1018 point mutations and 1334 indels (estimated using Geneious variant/SNP finder).

A multiple alignment of 55 protein-coding genes from 14 vascular plants was performed in MAFFT v. 7.017 with the default setting (Kato et al., 2002). A maximum-likelihood analysis was conducted in RaxML v. 7.2.8 (Stamatakis, 2014), using the GTR+GAMMA substitution model (Figure 1). The phylogeny strongly supports the sister relationship between *E. foeminea* and *E. equisetina*, as well as that of *Ephedra* and a *Gnetum-Welwitschia* clade. The Gnetales are sister to Cupressophytes. Seed plant phylogeny is a difficult and unresolved phylogenetic problem (Mathews, 2009) but the here showed “gnecup hypothesis” (Gnetales sister to Cupressophytes) is often retrieved from analyses of chloroplast data.

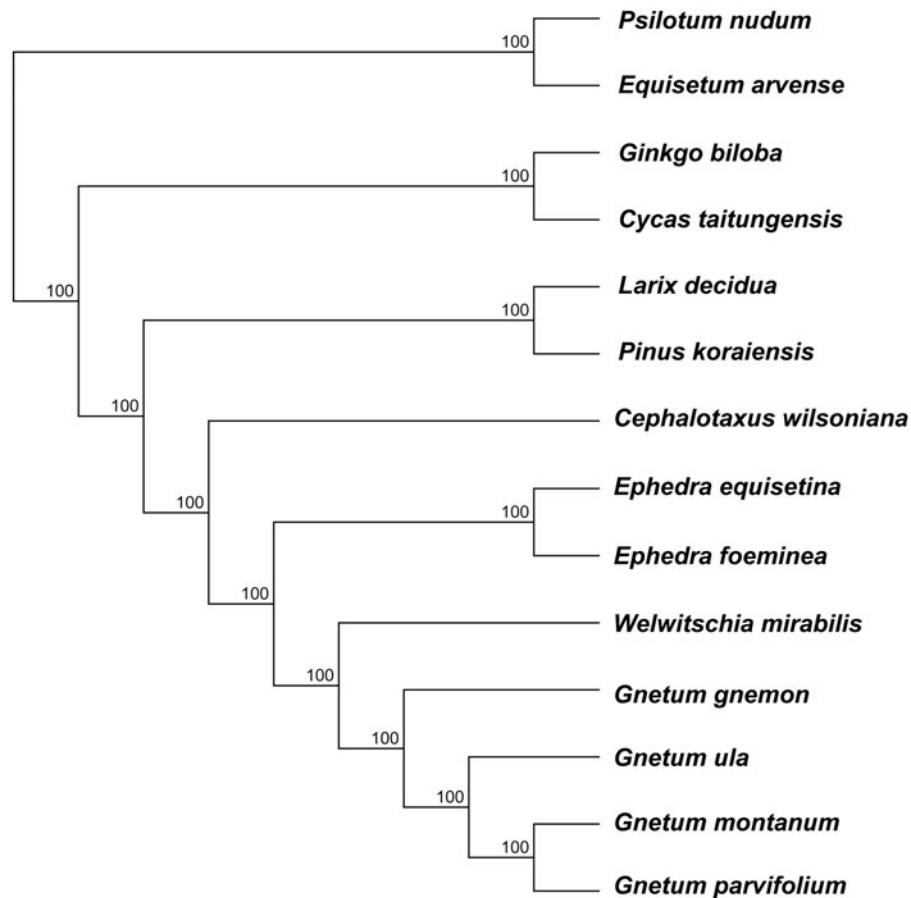


Figure 1. Phylogeny of 14 vascular plants, based on data from 55 protein-coding genes using the maximum-likelihood method and the GTR + GAMMA model. Values at nodes denote rapid bootstrap supports estimated from 1000 replicates. GenBank accession numbers are *Psilotum nudum* NC\_003386 (Monilophyta), *Equisetum arvense* NC\_014699 (Monilophyta), *Ginkgo biloba* AB684440 (Ginkgoales), *Cycas taitungensis* NC\_009618 (Cycadales), *Larix decidua* NC\_016058 (Pinaceae), *Pinus koraiensis* NC\_004677 (Pinaceae), *Cephalotaxus wilsoniana* NC\_016063 (Cupressophyta), *Ephedra equisetina* NC\_011954, *E. foeminea* KT934791 (Ephedraceae), *Welwitschia mirabilis* AP009568 (Welwitschiaceae), *Gnetum gnemon* KP099649, *G. ula* AP014923, *G. montanum* KC427271, and *G. parvifolium* NC\_011942 (Gnetaceae).

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## Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. The study was funded by grants from the Swedish Research Council VR to C. R. and to Birgitta Bremer (The Bergius Botanic Garden, Stockholm).

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