

# The complete chloroplast genome sequence of the leafy bladderwort, *Utricularia foliosa* L. (Lentibulariaceae)

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**Abstract** *Utricularia foliosa* L., commonly known as leafy bladderwort, is a widespread aquatic carnivorous plant from the Lentibulariaceae family. The species of the *Utricularia* and *Genlisea* genera are known to possess the smallest nuclear genomes across angiosperms. This study reveals that *U. foliosa* have a typical chloroplast genome of 150,851 bp in length, comprised of a large single-copy region (LSC) of 82,720 bp, a small single-copy region (SSC) of 17,481 bp, and inverted repeat regions (IRs) of 25,325 bp. A total of 139 genes, 88 of which are protein coding genes, 37 tRNA genes, eight rRNA genes and six pseudogenes were identified. All plastid NAD(P)H-dehydrogenase genes are present as intact copies. Phylogenetic analyses indicate that *U. foliosa* is closely related to other suspended or affixed aquatic species belonging to the *Utricularia* sect. *Utricularia*.

**Keywords** Carnivorous plant · Lentibulariaceae · Bladderwort · Plastome · CpDNA

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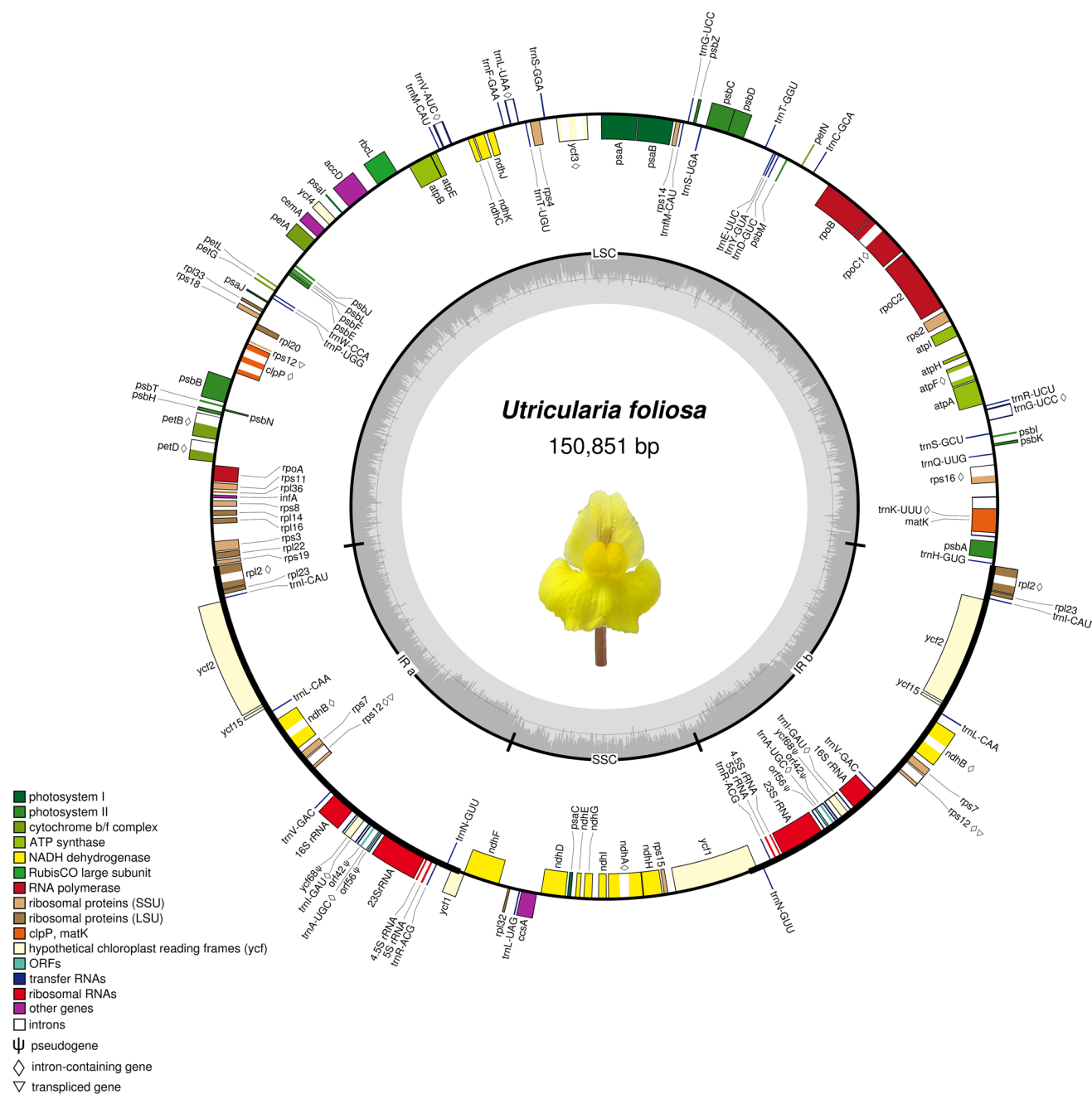
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*Utricularia foliosa* L. (leafy bladderwort, Lentibulariaceae) is a perennial free floating aquatic plant (Taylor 1989) that grows in lakes, ponds and swamps. The genus *Utricularia*, which also includes *Genlisea*, has increasingly gained researchers attention due to the dynamic size of its nuclear DNA content (Albert et al. 2010; Veleba et al. 2014). Despite its widespread distribution in the Americas, Africa and Madagascar, only a few studies of *U. foliosa* have been conducted. These studies have focused mainly on prey composition (Solís-Parra and Críaes-Hernández 2016), bladder respiration and photosynthesis (Adamec 2006). Only a single phylogenetic analysis, with one molecular marker, has been used to determine its phylogenetic position within the *Utricularia* genus (Silva et al. 2016). *U. foliosa* can bloom throughout the year, and is found in slowly flowing, shallow to deep water bodies, such as lakes, marshes and rivers (Taylor 1989). These habitats, where *U. foliosa* is commonly found, are suffering rapid environmental loss due to negative anthropic impacts such as drainage and conversion to urban or agricultural activities.

Herein, we report the first complete chloroplast genome of *U. foliosa*. The annotated *U. foliosa* genome has been deposited in the public database, GenBank, with the accession number: KY025562 (BioProject: PRJNA350159; BioSample: SAMN05933770).

Total genomic DNA was extracted from silica gel dried inflorescences of *Utricularia foliosa*, collected from ponds in the Tietê River in Mogi das Cruzes (São Paulo, Brazil; lat. -23.532294, long. -46.202648, 765 m a.s.l.) using a modified CTAB method (Doyle and Doyle 1987). Herbarium voucher (V.F.O.de Miranda et al. 2070) is deposited at the JABU Herbarium at Universidade Estadual Paulista (UNESP/ FCAV).

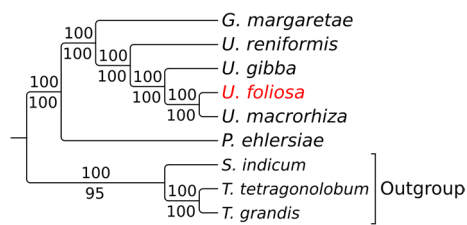
Genomic sequencing was performed on the Illumina MiSeq Platform (Illumina, San Diego, CA), resulting in



**Fig. 1** Gene map of the *Utricularia foliosa* chloroplast genome. Genes belonging to different functional groups are shown in *different colors*. The *open diamond* and *chi* indicate genes with intron(s) and pseudogene, respectively. (Color figure online)

2,933,837 paired-end reads (2×300 bp). The reads were quality trimmed using Platanus\_trim v1.0.7 (Kajitani et al. 2014) and aligned to the reference *Utricularia gibba* chloroplast genome (Genbank accession number: NC\_021449) using Bowtie 2 v.2.2.3 (Langmead and Salzberg 2012). The matched chloroplast reads (135,879 reads) were de novo assembled with SPAdes 3.9 (Bankevich et al. 2012) and ambiguous regions were picked out to extend the length using an iteration method with MITObim v.1.8 (Hahn

et al. 2013). Quality filtered reads were mapped back to the chloroplast genome using Bowtie2 (98.95% overall alignment rate) to confirm assembly accuracy quality and repeat region junctions. The gene annotation was performed using DOGMA (<http://evogen.jgi-psf.org/dogma/>) (Wyman et al. 2004) and confirmed by BLASTn searches on NCBI nucleotide collection database (nt). tRNA genes were annotated using DOGMA and ARAGORN v1.2 (Laslett and Canback 2004). The genome map was generated using OGDRAW



**Fig. 2** Bayesian Inference phylogram based on 53 genes of complete chloroplast genome sequences deposited in public databases. Numbers below the nodes are bootstrap values for maximum likelihood analysis and above the nodes are posterior probability values. Accession numbers: *Genlisea margaretae* NC\_025652, *Pinguicula ehlersiae* NC\_023463, *Sesamum indicum* JN\_637766, *Tanaecium tetragonolobum* NC\_027955, *Tectona grandis* NC\_020098, *Utricularia gibba* NC\_021449, *U. reniformis* NC\_029719, *U. macrorrhiza* NC\_025653

v. 1.2 (<http://ogdraw.mpimp-golm.mpg.de/>) (Lohse et al. 2013) followed by manual modifications.

The phylogenetic position of *U. foliosa* was inferred using 53 genes of the previously published Lentibulariaceae chloroplast genomes, with the *Tectona grandis* (Lamiaceae), *Sesamum indicum* (Pedaliaceae) and *Tanaecium tetragonolobum* (Bignoniaceae) plastomes used as outgroups.

The sequences were aligned using MAFFT v.7 (Katoh and Standley 2013) and probabilistic phylogeny was conducted using Mr. Bayes v.3 for Bayesian inference (Ronquist and Huelsenbeck 2003) with  $5 \times 10^5$  generations, using the default parameters, and RAxML for maximum likelihood (Stamatakis 2014) using the default parameters with bootstrap support of  $10^4$  pseudoreplicates. The evolutionary model used was GTR + G, tested *a priori* using jModelTest (Darriba et al. 2012).

The chloroplast genome of *Utricularia foliosa* is a typical quadripartite structure with a length of 150,851 bp, which contained inverted repeats (IR) of 25,325 bp separated by a large single-copy (LSC) and a small single-copy (SSC) of 82,720 bp and 17,481 bp, respectively. The cpDNA contains 139 genes, comprising 88 protein-coding genes, 8 ribosomal RNA genes, 37 tRNA genes and 6 pseudogenes. Among the annotated genes, 15 of them contain one intron (*atpF*, *petB*, *petD*, *rpl2*, *rpl16*, *rpoC1*, *rps12*, *trnA*-UGC, *trnG*-UCC, *trnI*-GAU, *trnL*-UAA, *trnV*-UAC, *trnK*-UUU, *ndhB*, *ndhA*), and *ycf3* and *clpP* genes contain two introns. The overall GC content of *U. foliosa* cp genome is 37.32%, while it was 43% for IRs, 35.20% for LSC and 30.83% for SSC regions (Fig. 1). Interestingly, all the eleven subunits of the plastid NAD(P)H-dehydrogenase (*ndh*) genes are present as intact copies, corroborating previous studies which suggested that the plastid *ndh* complete gene set may exist only in aquatic forms of carnivorous plants in the Lentibulariaceae family (Silva et al. 2016).

Previous phylogenetic studies have shown that Lentibulariaceae and *Utricularia* are monophyletic groups, supported by molecular and morphological characteristics (Müller et al. 2004; Silva et al. 2016). Our phylogenetic analysis strongly supports the positioning of *U. foliosa* nested within the known related aquatic species *U. gibba* and *U. macrorrhiza* (*Utricularia* sect. *Utricularia*) (Fig. 2).

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