We report the complete chloroplast genome (plastome) sequences of *Pedicularis cephalantha* (147,087 bp) and *P. nigra* (145,726 bp), endemic to southwestern China. Both plastomes have typical quadripartite structures with one large-single copy region, one small-single copy region, and two inverted repeat regions. Both plastome sequences contained 37 tRNA genes and eight rRNA genes, but they differed in the numbers of protein-coding genes: *P. cephalantha* had 76 functional genes and 12 pseudogenes while *P. nigra* had 74 functional genes and 13 pseudogenes. Phylogenetic analysis shows that *P. cephalantha* and *P. nigra* are closely related, then sister to *P. oederi* in the family Orobanchaceae.
and 132 in *P. nigra*. Both plastomes had 37 tRNA genes and eight rRNA genes, but the numbers of protein-coding genes differed: *P. cephalantha* had 76 functional genes and 12 pseudogenes while *P. nigra* had 74 functional genes and 13 pseudogenes.

To clarify the phylogenetic positions of these two species, we downloaded an additional 29 published plastomes of 22 *Pedicularis* species from GenBank. These complete plastome sequences with one IR region were aligned using MAFFT (Katoh and Standley 2013). The plastome of *Phtheirospermum japonicum* (Thunb.) Kanitz (Orobanchaceae) was selected as the outgroup. A maximum-likelihood tree was reconstructed using raxmlGUI (Edler et al. 2021) with GTRGAMMAI model by running 1000 bootstrap replicates. Our phylogenetic analyses showed that *P. cephalantha* and *P. nigra* formed a clade with 100% bootstrap values (Figure 1), representing Clade 3 as in the previous study (Yu et al. 2015). Our new clade is sister to *P. oederi* Vahl in the Clade 7. The complete chloroplast genomes and phylogenetic results in this study provide new insights into the phylogenetic backbone and evolutionary biology of *Pedicularis*.

**Ethics statement**

No specific permissions were required for these location and activities during fieldwork, and neither of the species studied are listed as endangered or protected in China’s Catalogue of National Protected Key Wild Plants.

**Author contributions**

Hong Wang, Wen-Bin Yu, and Wei-Jia Wang designed and modified the experimental design. Wei-Jia Wang, Rong Liu, You Wu, and Wen-Bin Yu collected and analyzed the data. Wei-Jia Wang, Hong Wang, and Wen-Bin Yu interpreted the results and wrote the manuscript.

**Disclosure statement**

No potential conflict of interest is reported by the authors.
Funding

This study was supported by grants from the National Natural Science Foundation of China [Nos. 31870196, 32071670], the Large-Scale Scientific Facilities of the Chinese Academy of Sciences [No. 2017-LSFGBOWS-02], and the Open Research Project of “Cross-Cooperative Team” of the Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences, the CAS 13S Program [No. 2017XTBG-T03].

ORCID

Wen-Bin Yu http://orcid.org/0000-0002-7643-2112

Data availability statement

The data were collected without violation of the protection of human subjects, or other valid ethical, privacy, or security concerns. Two complete plastome sequences were deposited in GenBank with accession numbers OL606628 and OL544940 and are also available at Figshare (https://doi.org/10.6084/m9.figshare.17097098.v2). The associated BioProject, SRA, and Bio-Sample numbers are PRJNA780958, SRR16960915–SRR16962222, and SAMN23224351–SAMN23224352, respectively.

References

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemistry. 19(1):971–15.

Edler D, Klein J, Antonelli A, Silvestro D. 2021. RaxmlGUI 2.0: a graphical interface and toolkit for phylogenetic analyses using RAxML. Methods Ecol Evol. 12(2):373–377.

Jin J-J, Yu W-B, Yang J-B, Song Y, dePamphilis CW, Yi T-S, Li D-Z. 2020. GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. Genome Biol. 21(1):241.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 30(4):772–780.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, et al. 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 28(12):1647–1649.

Li X, Yang J-B, Wang H, Song Y, Corlett RT, Yao X, Li D-Z, Yu W-B. 2021. Plastid NDH pseudogenization and gene loss in a recently derived lineage from the largest hemiparasitic plant genus Pedicularis (Orobanchaceae). Plant Cell Physiol. 62(6):971–984.

Ree RH. 2005. Phylogeny and the evolution of floral diversity in Pedicularis (Orobanchaceae). Int J Plant Sci. 166(4):595–613.

Robart BW, Gladys C, Frank T, Kilpatrick S. 2015. Phylogeny and biogeography of North American and Asian Pedicularis (Orobanchaceae). Syst Bot. 40(1):229–258.

Tillich M, Lehward P, Pellizzer T, Ulbricht-Jones ES, Fischer A, Bock R, Greiner S. 2017. GeSeq – versatile and accurate annotation of organellar genomes. Nucleic Acids Res. 45(W1):W6–W11.

Yang F-S, Wang X-Q. 2007. Extensive length variation in the cpDNA trnT-trnF region of hemiparasitic Pedicularis and its phylogenetic implications. Plant Syst Evol. 264(3–4):251–264.

Yang H-B, Holmgren NH, Mill RR. 1998. Pedicularis Linn. In: Wu Z-Y, Raven PH, editors. Flora of China. St. Louis, Beijing: Missouri Botanical Garden Press & Science Press; p. 97–209.

Yu W-B, Liu M-L, Wang H, Mill RR, Ree RH, Yang J-B, Li D-Z. 2015. Towards a comprehensive phylogeny of the large temperate genus Pedicularis (Orobanchaceae), with an emphasis on species from the Himalaya-Hengduan Mountains. BMC Plant Biol. 15(1):176.

Zeng C-X, Hollingsworth PM, Yang J, He Z-S, Zhang Z-R, Li D-Z, Yang J-B. 2018. Genome skimming herbarium specimens for DNA barcoding and phylogenomics. Plant Methods. 14(1):43.