Turczaninowia 28, 2: 112–120 (2025)
https://www.doi.org/10.14258/turczaninowia.28.2.11
http://turczaninowia.asu.ru

ISSN 1560–7259 (print edition)
TURCZANINOWIA
ISSN 1560–7267 (online edition)

УДК 582.952.6+575.113

Characterization of the complete chloroplast genome of *Pedicularis flava* (Orobanchaceae)

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Keywords: flora of Mongolia, *Pedicularis*, phylogeny, plastome, whole genome.

Summary. *Pedicularis flava* Pall. is perennial herb and is distributed in China (Inner Mongolia), Mongolia and Russia (Zabaikalye Territory – Chita city, Republic of Tyva). In Mongolia, this species is quite widely distributed across several phytogeographical regions. In this study, we assembled and annotated the complete plastid genome of *P. flava* using the high-through sequencing for the first time. The plastome of *P. flava* was identified as a double-stranded circular DNA molecule 149 087 bp long with 38.4 % GC content. The plastome consists of a large single-copy (LSC) region (83 576 bp) and a small single-copy (SSC) region (14 169 bp) and separated by two inverted repeats (IRs) of 25 671 bp each. The plastome encodes 113 unique genes which is similar to other *Pedicularis* species. This includes 4 rRNA, 30 tRNA, and 79 protein-coding genes, among which 4 rRNA, 7 tRNA, and 7 protein-coding genes were duplicated in the IR regions. Finally, phylogenetic analysis shows that *Pedicularis* species were formed a monophyletic clade and *P. flava* was clustered with *P. resupinata*.

Поступило в редакцию 23.12.2024
Принято к публикации 19.05.2025

Submitted 23.12.2024
Accepted 19.05.2025

Характеристика полного генома хлоропластов *Pedicularis flava* (Orobanchaceae)

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Ключевые слова: геном, пластом, флора Монголии, целый геном, *Pedicularis*.

Аннотация. *Pedicularis flava* Pall. – многолетнее травянистое растение, распространённое в Китае (Внутренняя Монголия), Монголии и России (Забайкальский край (г. Чита), Республика Тыва). В Монголии этот вид довольно широко распространён в нескольких фитогеографических регионах. В этом исследовании мы впервые собрали и аннотировали полный пластидный геном *P. flava* с помощью высокопроникающего секвенирования. Кольцевой геном *P. flava* был идентифицирован как двухцепочечная кольцевая молекула ДНК длиной 149 087 пар с содержанием GC 38,4 %. Геном состоит из однокопийного большого фрагмента (LSC) генома (83 576 п. н.), однокопийного малого фрагмента (SSC) генома (14 169 п. н.), разделённых двумя инвертированными повторами (IRs) по 25 671 п. н. каждый. Пластом кодирует 113 уникальных генов, что сходно с другими видами *Pedicularis*. Он включает 4 гена рРНК, 30 генов тРНК и 79 белок-кодирующих генов, среди которых 4 рРНК, 7 тРНК и 7 белок-кодирующих генов были продублированы в IR-регионах. Филогенетический анализ показал, что виды *Pedicularis* образовали монофилетическую кладу, а *P. flava* сгруппировался с *P. resupinata*.

Introduction

Plastomes (chloroplast genome) play a pivotal role in plant cells, underscored by their widespread application in evolutionary and phylogenetic studies (Gao et al., 2019; Nyamgerel et al., 2023). The chloroplast genome has the characteristics of short sequence (120–160 kb), containing conserved sequence regions (*mat*K, *rbc*L, *trn*H-*psb*A and *trn*L-F), rich, simple repeat sequence sites, easy extraction and purification, and parthenogenetic inheritance (Kusnetsov, 2018). Therefore, the number of plastome studies on vascular plants have been published in recent years (e. g., Li et al., 2021; Oyuntsetseg et al., 2024; Jiang et al., 2025).

Pedicularis L. comprises more than 680 species and is largest genera of the Orobanchaceae family. This genus is widely distributed in subarctic and temperate to tropical mountains (POWO, 2024). Among these, several species have been studied with the plastome analysis (e. g., Li et al., 2021; Wang M. et al., 2024; Wang T. et al., 2024), but the plastome data of *P. flava* is still unknown. *Pedicularis flava* Pall. is perennial herb and is distributed in China (Inner Mongolia), Mongolia and Russia (Zabaikalye Territory – Chita city, Republic of Tyva) (Kosachev, 2010; POWO, 2024). In Mongolia, this species is quite widely distributed across several phytogeographical regions (Baasanmunkh et al., 2022) (Fig. 1). For example, a total of 99 observations of *P. flava* (https://www.inaturalist.org/observations?taxon_id=1051194) observed by the citizen scientist in the "Flora of Mongolia" project in iNaturalist.

In this study, we sequenced the complete plastome of *P. flava* from Mongolia for the first time. In addition, we analyzed the general genome structure, codon usage, repeat sequences, IR boundaries, and phylogenetic position of *P. flava* with closely related species based on NCBI data.

Material and methods

Fresh leaves of *P. flava* were collected from the Terelj National Park, Ulaanbaatar, Mongolia (N49.42286, E107.51102). The voucher specimen was deposited in the Herbaria of the National University of Mongolia (UBU). The detailed photographs of each part were taken during our field expeditions. Total genomic DNA was extracted from silica gel-dried leaf material using the CTAB method (Doyle J. J., Doyle J. L., 1987) with slight modification. A chloroplast genome library was prepared using the

TruSeq DNA Nano Kit along with the NextSeq 500 platform (Illumina, San Diego, CA, USA), following the manufacturers protocol. Trimmomatic v.0.36 (Bolger et al., 2014) was used to remove adapter sequences and low-quality reads to reduce bias. A base quality plot generated using FastQC v.0.11.5 (Antil et al., 2023) was used to check the overall quality of the data and show the range of quality values for each cycle. NOVOplasty v.4.1.0 was used to perform *de novo* assembly using various k-mers (Dierckxsens et al., 2016). The chloroplast genome annotation of *P. flava* was performed using the GeSeq web server (https://chlorobox.mpimp-golm.mpg.de/geseq.html) (Tillich et al., 2017) to predict the location of genes. A circular map was visualized using the CPGView web server (http://www.1kmpg.cn) (Liu S. et al., 2023). The analysis of Relative Synonymous Codon Usage (RSCU) in the protein-coding genes was performed using MEGA v.11.0.10 (Tamura et al., 2021). Simple Sequence Repeats (SSRs) were detected using the Microsatellite Identification Tool (MISA) web server (Beier et al., 2017), with the minimum number of repeat parameters set to 10, 6, 5, 5, 5, and 5 for mono-, di-, tri-, tetra-, penta-, and hexanucleotides, respectively. We used REPuter to identify forward, reverse, palindromic, and complementary repeats with a minimum length of 20 bp, 90 % identity, and a Hamming distance of 3. Long tandem repeats were identified using the Tandem repeats finder with a minimum alignment score of 50 and a maximum period size of 500; the identity of repeats was set to ≥ 100 %. The entire chloroplast genome was aligned using MAFFT v.7.490 (Katoh, 2002) as implemented in Geneious Prime® 2024.0.5 (http://www.geneious.com). To determine phylogenetic position, a maximum likelihood analysis was performed using RaxML v.8.2.11 (Stamatakis, 2014) under the optimal model of GTR+G+I, with 1000 bootstrap replicates. The reconstructed trees were visualized using FigTree v.1.4.2 (Rambaut, 2012).

Results and discussion

The plastome of *P. flava* from Mongolia was sequenced for the first time. The genome sequence data that validate the findings of this work are readily accessible in the GenBank database of the National Center for Biotechnology Information (NCBI) at (https://www.ncbi.nlm.nih.gov/) under accession number PQ759906. A total of 11.2 Gbp paired-end (150 bp) sequencing data including 73 912 088 reads, was obtained. After trimming and normalization, 10 Gbp bases with 65 758 072 reads were retained for assembly. *De novo* assembly generated a single contig with 100 % coverage, and the sequencing depth was 66 417. The plastid genome of *P. flava* was identified as a double-stranded circular DNA molecule 149 087 bp long. The plastome consists of a large single-copy (LSC) region (83 576 bp) and a small single-copy (SSC) region (14 169 bp), and separated by two inverted repeats (IRs) of 25 671 bp each (Fig. 2). The overall GC content was 38.4 %, with regional GC contents of 43.4 % in the IR, 36.4 % in the LSC, and 32.5 % in the SSC. Normally, higher GC content than other plants (Cho et al., 2018; Wang M. et al., 2024; Wang T. et al., 2024). The plastome encodes 113 unique genes which is similar to other *Pedicularis* species. This includes 4 rRNA, 30 tRNA, and 79 protein-coding genes, among which 4 rRNA, 7 tRNA, and 7 protein-coding genes were duplicated in the IR regions (Table 1). The junction regions of the genome revealed that *ycf*1 span in the IRb/SSC and SSC/IRa junctions (Fig. 3). There were 11 cis-splicing genes with one intron, two of which (*ycf*3 and *clpP*1) had two introns (Fig. 4a), and one trans-splicing (*rps*12) gene with three exons (Fig. 4b).

The codon usage study identified total of 25 187 codons, with Leucine (Leu – 2 628), Isoleucine (Ile – 2 119), and Serine (Ser – 1 970) codons being the most prevalent. In addition, the coding rates were highest for the AUU (Ile – 1027), AAA (Lys – 1022), and GAA (Glu – 972) codons, whereas the coding rates were lowest for the UGC (Cys – 66), AGC (Ser – 114), and CGC (Arg – 125). In addition, the highest RSCU values were highest for the UUA (Leu – 1.83), AGA (Arg – 1.77), and GCU (Ala – 1.7) codons, whereas the coding rates were lowest for UAC (Tyr – 0.38), GAC (Asp – 0.38), AGC (Ser – 0.35).

Chloroplast microsatellites can be potentially useful markers for ecological and evolutionary studies because of their non-recombinant, uniparental inherited nature of organelle genomes (Provan et al., 2001). *Pedicularis* plastomes revealed from 37 to 55 SSRs (Wang M. et al., 2024), and *P. flava* includes 50 SSRs; the most abundant motif was mononucleotide (29), followed by di (10), tri (4), tetra (6) and penta (1), mainly in the intergenic spacer region (Fig. 2). The dominance of mononucleotide SSRs was commonly observed in *Pedicularis* (Wang M. et al., 2024; Wang T. et al., 2024). This SSR marker can be used to determine genetic variations in population genetic studies. Furthermore, we found 11 direct and nine palindromic palindromic repeats, and 22 long tandem repeats that were generally from 15 to 19 bp long (Fig. 2).

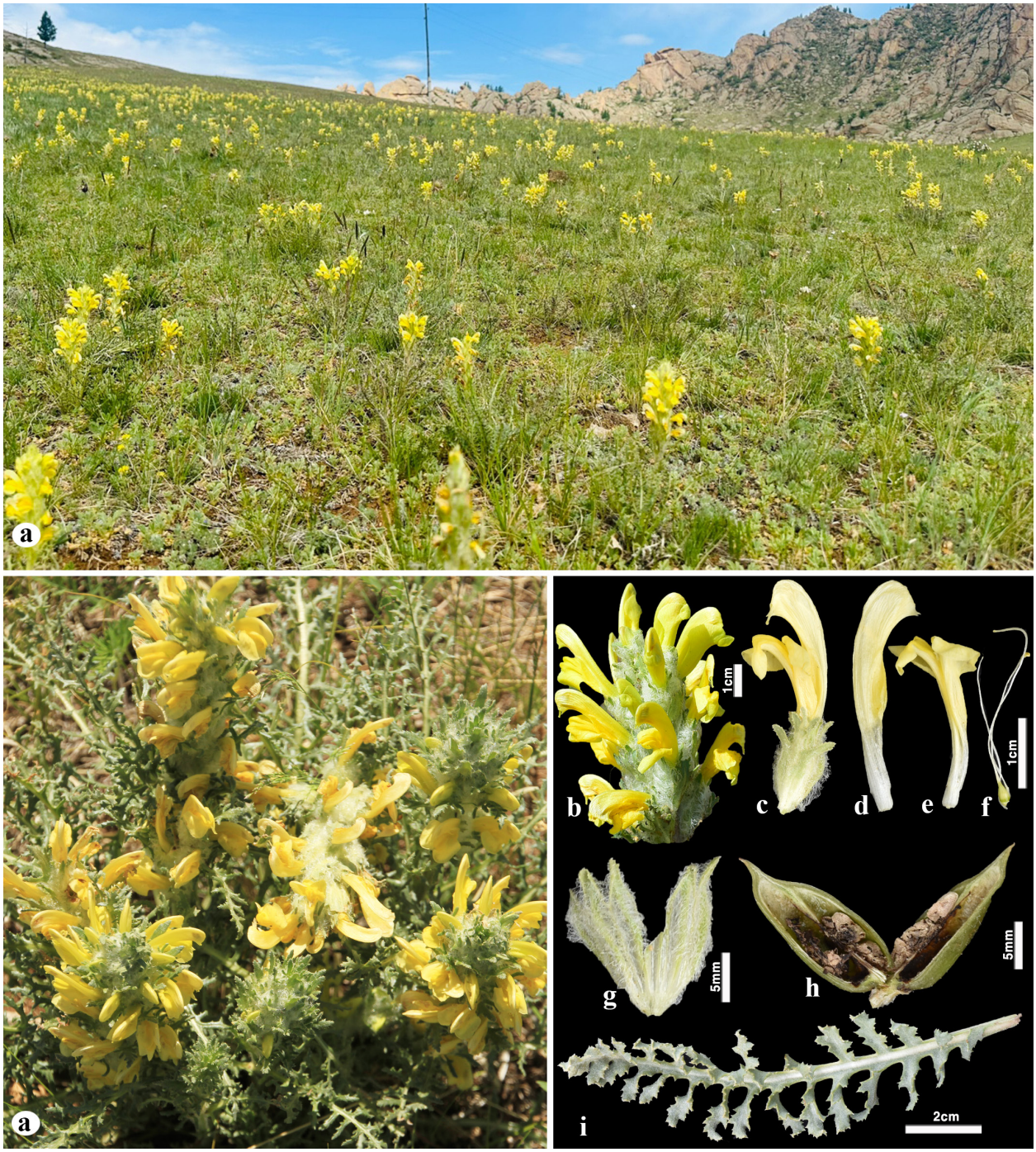

Fig. 1. *Pedicularis flava* in Mongolia: a – general habit; b – raceme; c – flower; d – corolla upper lip; e – corolla lower lip; f – pistil and stamens; g – calyx; h – capsule; i – leaf (Photo credit: S. Baasanmunkh).

To verify the phylogenetic relationships of *P. flava*, we compared whole plastomes of 24 *Pedicularis* species and two outgroups (*Scrophularia dentata* and *S. henryi*), retrieved from the NCBI database (Zhang et al., 2017; Wu et al., 2019; Wang M. et al., 2024, Wang T. et al., 2024; Zhang et al., 2024). The alignment included 183 399 nucleotide sites, of which 22 007 bp (12 %) were variable. Phylogenetic analyses showed *Pedicularis* species formed a monophyletic clade (Fig. 5). In the previous studies used nuclear and plastid DNA markers and protein-coding sequences of plastome that showed *Pedicularis* species were monophyletic within genus (Yu et al., 2011; Liu M.-L. et al., 2013; Wu et al., 2019; Li et al., 2021; Wang M. et al., 2024; Wang T. et al., 2024; Zhang et al., 2024). Our tree topology is similar with previous studies. *P. flava* was most close to *P. resupinata* based on whole chloroplast genome, supported by

a strong bootstrap value. However, according to the phylogenetic tree of N. Tkach et al. (2014), *P. flava* is more closely related to *P. dasystachys* based on ITS and *mat*K markers. The phylogenetic analyses of this study still had some limitations. Future studies need to expand the acquisition of samples and increase the data availability of whole chloroplast genomes of *Pedicularis*.

Table 1. Genes of the plastid genome of *Pedicularis flava*

	Group of genes	Name of genes
RNA genes	Ribosomal RNA	*rrn*4.5[c], *rrn*5[c], *rrn*16[c], *rrn*23[c]
	Transfer RNA	*trn*A-UGC[a,c], *trn*C-GCA, *trn*D-GUC, *trn*E-UUC[a,c], *trn*F-GAA, *trn*fM-CAU, *trn*G-GCC, *trn*G-UCC[a], *trn*H-GUG, *trn*I-CAU[c], *trn*I-GAU[a,c], *trn*K-UUU[a], *trn*L-CAA[c], *trn*L-UAA[a], *trn*L-UAG, *trn*M-CAU[c], *trn*N-GUU[c], *trn*P-UGG, *trn*Q-UUG, *trn*R-ACG[c], *trn*R-UCU, *trn*S-GCU, *trn*S-GGA, *trn*S-UGA, *trn*T-GGU, *trn*T-UGU, *trn*V-GAC[c], *trn*V-UAC[a], *trn*W-CCA, *trn*Y-GUA
Ribosomal proteins	Small subunit	*rps*2, *rps*3, *rps*4, *rps*7[c], *rps*8, *rps*11, *rps*12[c, b, d], *rps*14, *rps*15, *rps*16[a], *rps*18 *rps*19
	Large subunit	*rpl*2[c], *rpl*14, *rpl*16, *rpl*20, *rpl*22, *rpl*23[c], *rpl*32, *rpl*33, *rpl*36,
Transcription	RNA polymerase	*rpo*A, *rpo*B, *rpo*C1[a], *rpo*C2
Protein genes	Photosystem I	*psa*A, *psa*B, *psa*C, *psa*I, *psa*J, *ycf*3[b], *ycf*4
	Photosystem II	*psb*A, *psb*B, *psb*C, *psb*D, *psb*E, *psb*F, *psb*H, *psb*I, *psb*J, *psb*K, *psb*L, *psb*M, *psb*N, *psb*T, *psb*Z
	Cytochrome b6/f	*pet*A, *pet*B[a], *pet*D[a], *pet*G, *pet*L, *pet*N
	ATP synthase	*atp*A, *atp*B, *atp*E, *atp*F[a], *atp*H, *atp*I
	Rubisco	*rbc*L
	NADH dehydrogenase	*ndh*A[a], *ndh*B[a,c], *ndh*C, *ndh*D, *ndh*E, *ndh*F, *ndh*G, *ndh*H, *ndh*I, *ndh*J, *ndh*K
	ATP-dependent protease subunit P	*clp*P[b]
	Chloroplast envelope membrane protein	*cem*A
Transitional initiation factor		*inf*A
Maturase		*mat*K
Subunit acetyl-coA carboxylase		*acc*D
C-type cytochrome synthesis		*ccs*A
Hypothetical proteins		*ycf*1, *ycf*2[c], *ycf*15[c]
Component of TIC complex		*ycf*3[b]

Note: [a]Gene with one intron; [b]Gene with two introns; [c]Gene with copies; [d]Trans-splicing gene.

Conclusion

In this study, we sequenced and analyzed the complete chloroplast genome of *P. flava* from Mongolia for the first time, including the general genome structure, codon usage, repeat sequences, IR boundaries, and phylogenetic position. This genomic data was compared with those of the other plastome data of all available *Pedicularis* species. It was confirmed that the complete plastome feature of *P. flava* was almost congruent and highly conserved, which could be used to understand the plastome evolution and evolutionary relationships of the *Pedicularis* genus.

Acknowledgements

This work was supported by the National Research Foundation of Korea (Grant No. RS-2022-NR068406) and the Korea Basic Science Institute (National Research Facilities and Equipment Center) grant funded by the Ministry of Education (Grant No. 2023R1A6C101B022).

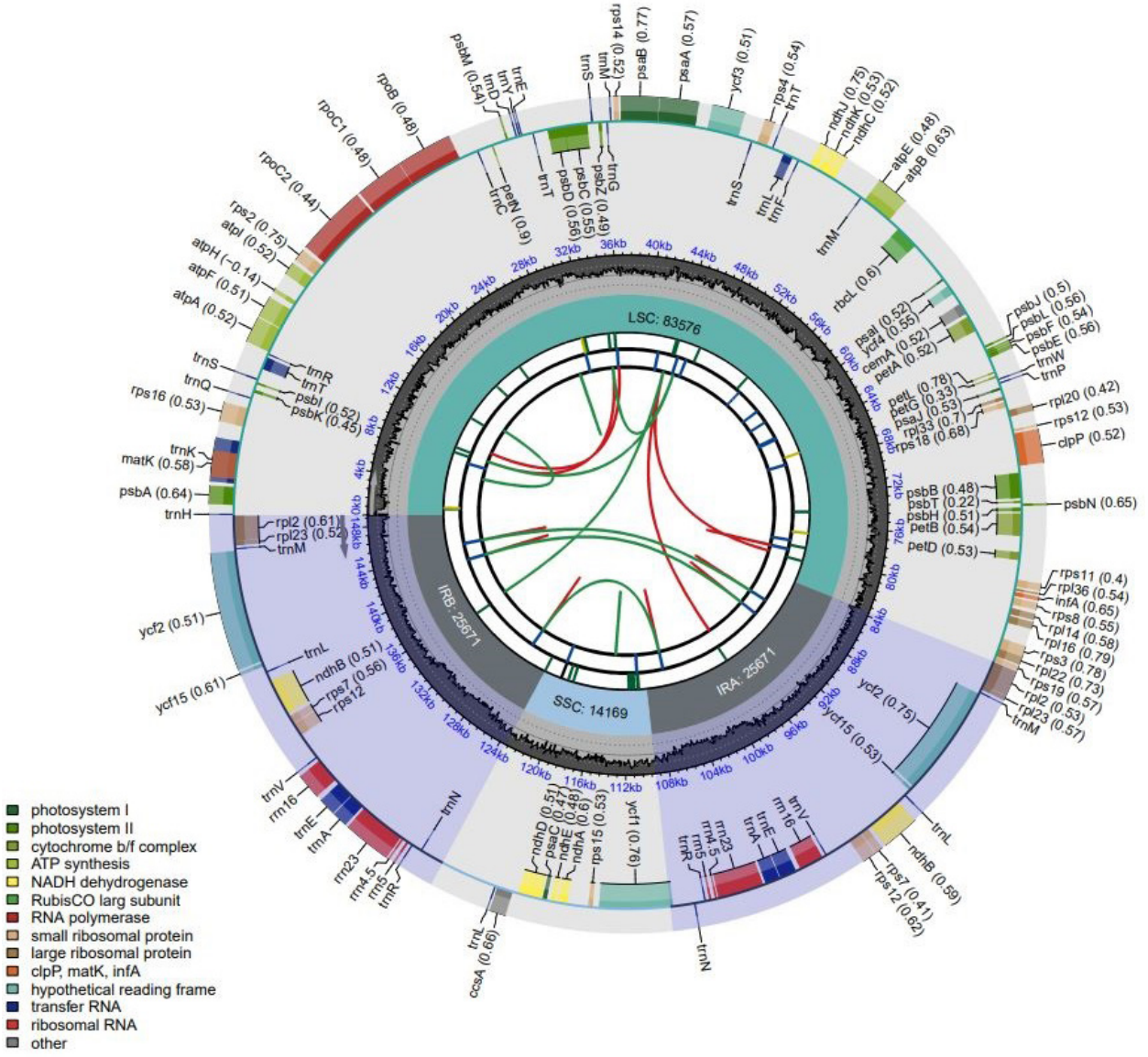

Fig. 2. Circular gene map of the complete chloroplast genome of *Pedicularis flava*. From the center outward, the first track shows the dispersed repeats: direct and palindromic repeats, connected with red and green arcs, respectively. The second track shows the long tandem repeats as short blue bars. The third track shows the microsatellite sequences as different-colored short bars: black, complex repeat; green, mononucleotides; yellow, dinucleotides. The genomic regions are shown on the fourth track. The GC content along the genome is plotted on the fifth track. The genes are shown on the sixth track. The optional codon usage bias is displayed in the parenthesis after the gene name. Genes are color-coded by their functional classification. The transcription directions for the inner and outer genes are clockwise and anticlockwise, respectively.

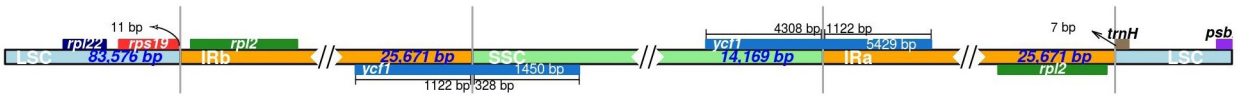

Fig. 3. Comparison of LSC, IR, and SSC junction positions in complete chloroplast genome of *Pedicularis flava*. LSC, large single-copy region; SSC, small single-copy region; IR, inverted repeat region.

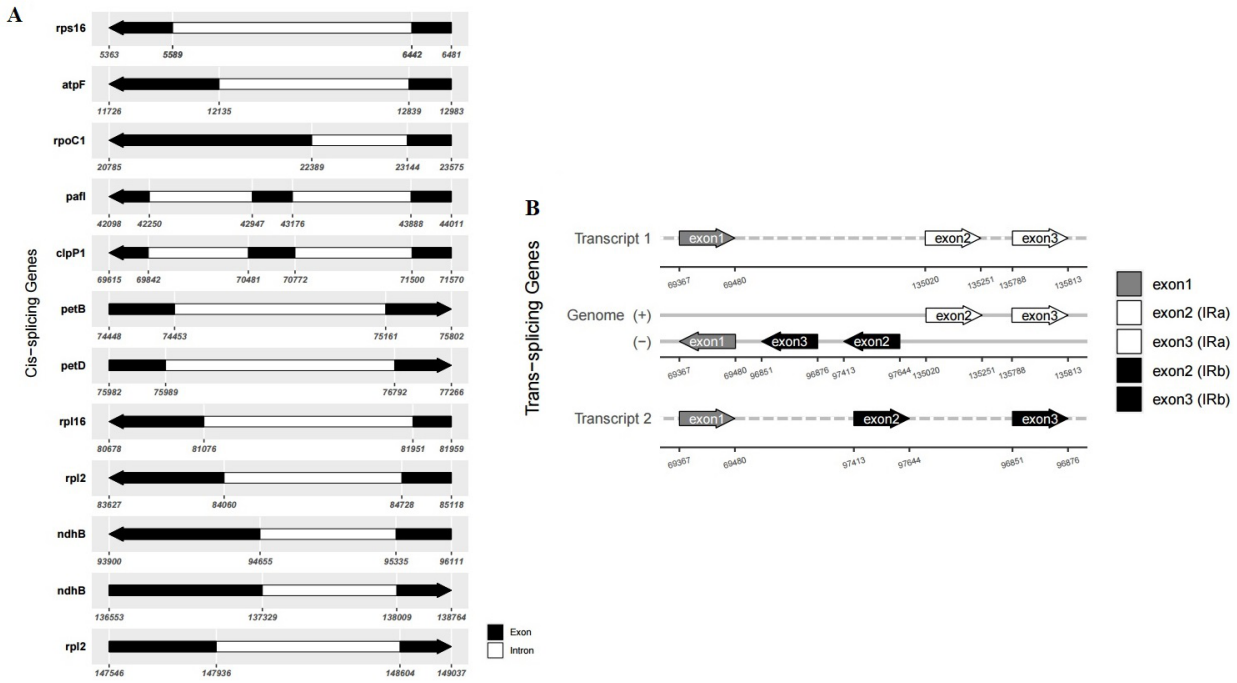

Fig. 4. Schematic map of the cis-splicing genes (A) and trans-splicing gene (B) in complete chloroplast genome of *Pedicularis flava*. The arrow indicates the sense direction of the gene.

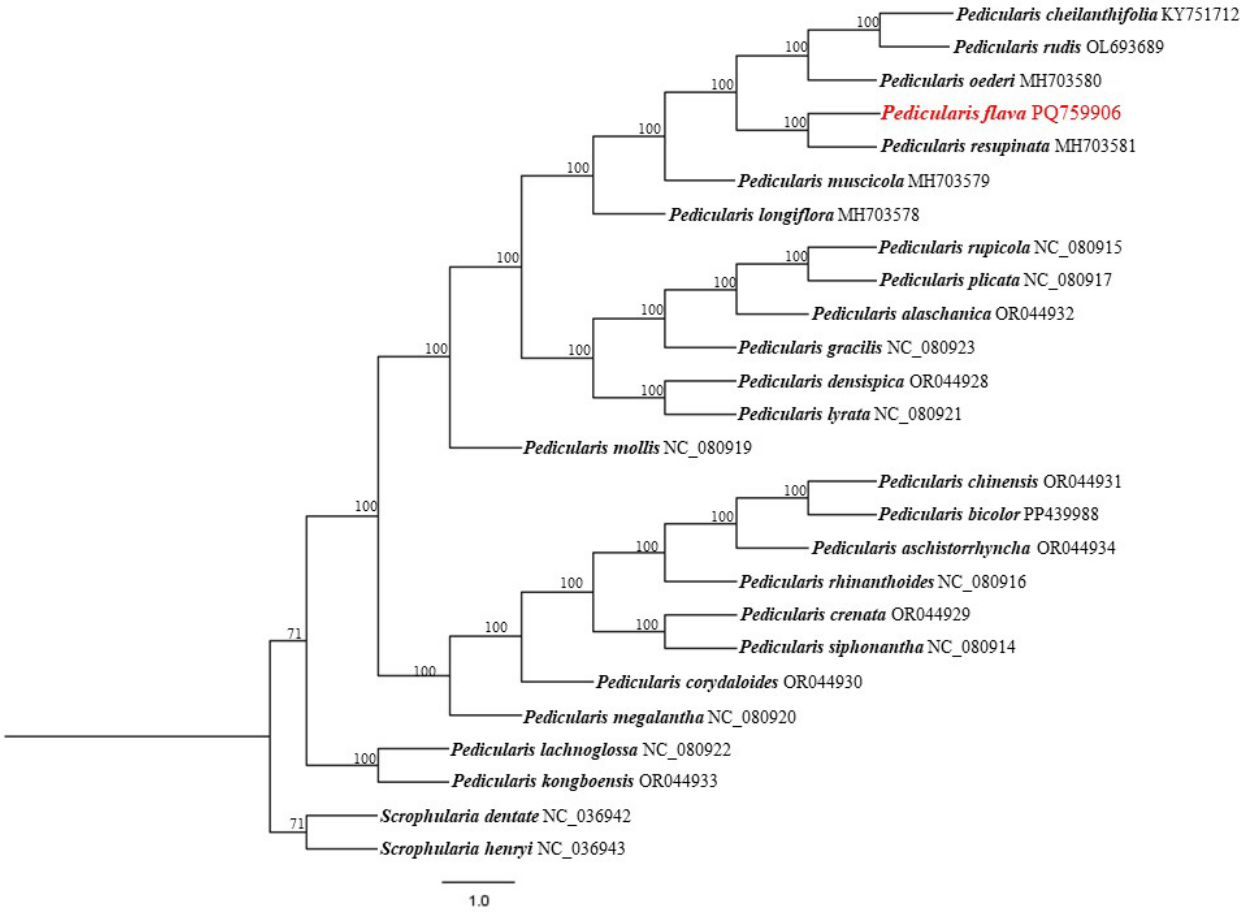

Fig. 5. Phylogenetic tree of the *Pedicularis* species based on the whole chloroplast genome using maximum likelihood. Maximum support values are indicated at branch level.

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