Chapter

Chloroplast Genome Characteristics of Plants on the Tibetan Plateau

Ying Liu, Jinping Qin, Zhengsheng Li, Lijun Zhang and Xinyou Wang

Abstract

Located in the interior of Asia, the Qinghai-Tibet Plateau is the largest plateau in China and the highest plateau in the world. It is also known as the "Roof of the world" and the "third pole". The various nature reserves on the Qinghai-Tibet Plateau are a treasure house of natural resources with the strangest ecological environment and the richest biological resources on the roof of the world. They are of high scientific value. This chapter will describe the chloroplast genome characteristics of several plants on the Qinghai-Tibet Plateau, such as *Aster, Asterothamnus centraliasiaticus, Aster altaicus, Corethrodendron multijugum*, *Clematis nannophylla*, and so on.

Keywords: chloroplast genome, *Aster*, *Asterothamnus centraliasiaticus*, *Aster altaicus*, *Corethrodendron multijugum*, *clematis nannophylla*

1. Introduction

Chloroplasts are important organelles in plants that help in photosynthesis [1]. Chloroplasts of higher plants are metabolic centers that sustain life on Earth through photosynthesis, as a semi-autonomous organelle with own DNA that encodes and has an independent genetic system [2]. For several reasons, cp genomes are more commonly used to study plant molecular evolution and phylogeny than mitochondrial genomes. The researchers note that differences in chloroplast genome sequences and their highly differentiated regions between plant species and individual plants not only enable a more comprehensive study of the taxonomy and phylogeny of these species, but also facilitate the identification, breeding, and conservation of valuable biological genetic information among related species [3, 4]. Here, we collected representative plants of alpine meadow, alpine steppe, desert steppe grassland types on the Qinghai-Tibet Plateau and determined their chloroplast genome sequences to understand their genome characteristics and phylogenetic evolution.

2. Species morphological characteristics and distribution information of five *Aster* species

Aster is a perennial herb of the Compositae family. Most *Aster* plants have well-developed roots, rhizoid type, and rapid reproduction [5]. With large root surface area, a large number of nutrients in soil can be absorbed and utilized for ecological restoration, giving full play to their ornamental and ecological value. It is vigorously used in greening and beautifying the environment to give full play to its ecological functions [6].

Aster has a flower head, with ligulate of purple or bluish-purple flowers and tubular of yellow flowers, which is of high ornamental value. *Aster* plants is one of the traditional Chinese herbs, and most *Aster* species contain a large number of important medicinal components such as flavonoids and quercetin [7–9], and the root and stem of *Aster* plants are commonly used as medicines. *Aster* species has a long history of medicinal use, there are many varieties of *Aster* distributed on the Qinghai-Tibet Plateau, and it is used in traditional Tibetan medicine as a medolome to treat seasonal epidemic diseases and clear heat and detoxification [10–12].

China is the main origin and distribution center of Eurasian *Aster* [12, 13], with about 123 species, including 82 endemic species. *Aster* has many species and abundant resources in China, widely distributed in northeast, northwest, southwest, and south China [5]. The Qinghai-Tibet Plateau has a unique geographical location, rich grassland resources, and high species diversity; according to the Flora of China, the Qinghai-Tibet Plateau is also one of the distribution centers of *Aster*. Here, we selected Guoluo Prefecture, the source of the Yellow River in the hinterland of the Qinghai-Tibet Plateau, to conduct a comprehensive survey of *Aster* germplasm resources and investigate the morphological characteristics of *Aster* species and determine the chloroplast genome of *Aster* species. Combined molecular tools with classical morphological information could prove a valuable information for a more accurate classification of Aster species.

2.1 Morphological characteristics of five species of *Aster*

2.1.1 Aster yunnanensis *var.* labrangensis *(Hand. -Mazz.) ling*

A. yunnanensis var. *labrangensis* is usually perennial herbs, and its height is about 20-70 cm (**Figure 1A**); Rhizome; stems erect and upper branched, stems 2–5 branched; both surfaces of leaves hairy and glandular, half clasped; Involucres hemispheric, 4-7 cm in diameter. The Ray florets are bluish-purple, the disk florets are yellow, the sparsely to moderately villous are 2 layers, short outside and long inside. Flowering period is July-August, and fruit period September-October. Generally found in hillside meadows, alpine meadow or thicket, elevation 3300–4300 m.

2.1.2 Aster farreri *W. W. Sm. et J. F. Jeffr*

A. farreri is usually perennial herbs, its height is about 30–60 cm (**Figure 1B**); rhizome long; stems erect, simple, sparsely to moderately villous. The leaves are basal and cauline, and sparsely villous, eglandular, margin entire or sparsely serrulate, villous-ciliate;blade oblanceolate to narrowly oblanceolate, 1.5–22 × 0.7–2.3 cm, leaf base gradually narrow, apex acute; middle cauline leaves linear-lanceolate, 7–13 x 0.7–2 cm, base rounded, subvalvate to valvate, apex acuminate; upper blade linear,

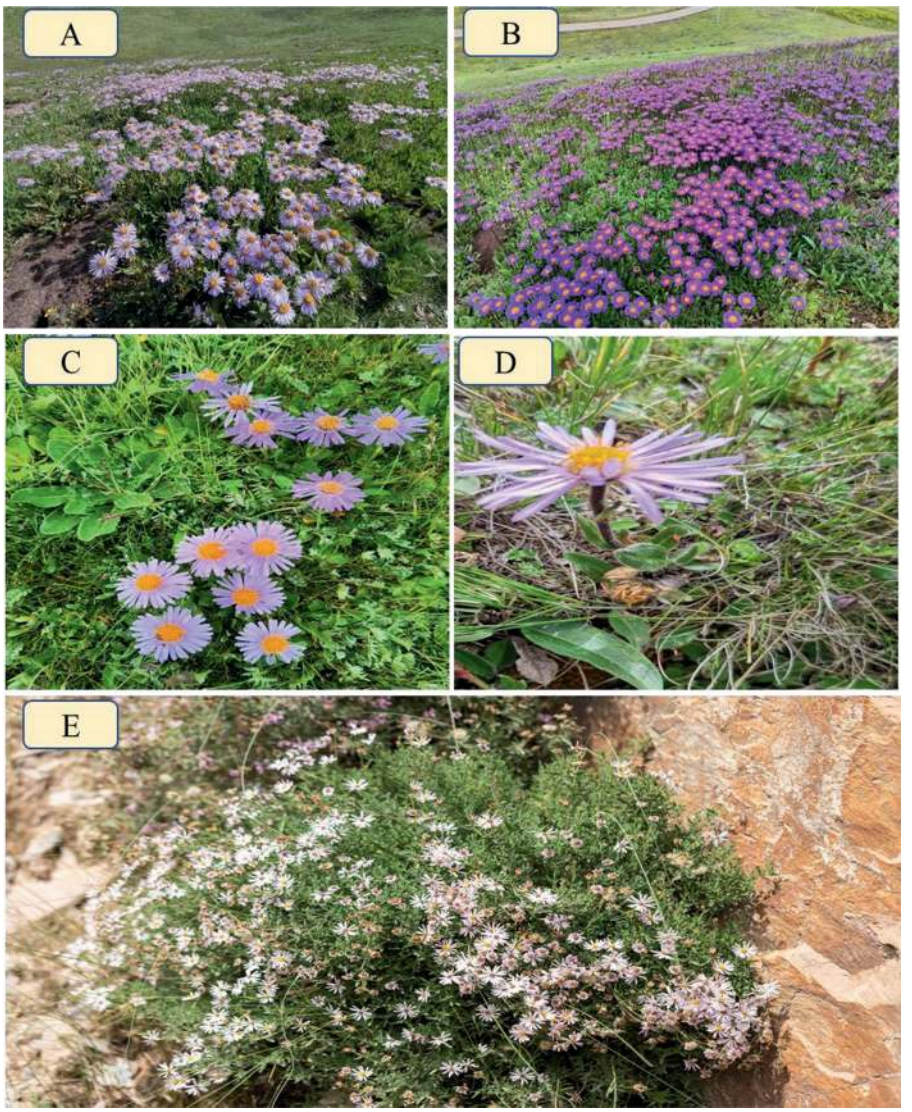


Figure 1.
Morphology and habitat map of five species of Aster. *(A)* Aster yunnanensis *var.* labrangensis*; (B)* Aster farreri*; (C)* Aster souliei*; (D)* Aster asteroids*; (E)* Aster poliothamnus.

about 2 x 0.1 cm, apex sharp. The capitulum is terminal, solitary, 5–8 cm in diameter, and the involucre is hemispherical, 2–2.4 cm in diameter; ray florets are purplish blue or lilac, disk florets are yellow; Pappus 2 layers, white or smudge white, outer layer very short. Flowering period is in July-August, and fruit period is in August-September. Generally distributed in Alpine, subalpine slope grassland, sand and gravel, elevation 2600–4200 m.

2.1.3 Aster souliei *Franch*

A. Souliei is usually perennial herbs, its height is about 2–45 cm (**Figure 1C**), sometimes caespitose; rhizomes robust, woody. Stems solitary, erect. The leaves are

basal and cauline, the leaf surface is smooth to sparsely hairy, the margin is whole, and the basal leaves present at flowering stage are spatulate or obovate to oblanceolate; inflorescence terminal and solitary, 3–6 cm in diameter, involucral hemispherical, 6–8 mm; floret 25–55 radiate, bluish-purple to purple, tube glabrous, lamina 12–25 × 2–3 mm, glabrous, glandular; disk florets yellow,3–5 mm, pappus 1 layer, purplish brown. The flowering period is from July to August, and the fruit period is from August to September. It is generally distributed in alpine thickets, meadow, and hillside meadows, with an altitude of 2700–4500 m.

2.1.4 Aster asteroids *(DC.) O. Kuntze*

A. asteroids is usually perennial herbs, its height is about 2–25 cm (**Figure 1D**); rhizomes short; roots tuberoid, near ground surface, with 2–6 tuberous roots, radish shape, stems erect, solitary, 2–15 cm tall to 30 cm. Basal leaves are dense and live at flowering stage, obovate, or oblong, 1–4 cm long, 0.4–0.8 thin, 1.7 cm wide, leaf base tapered into a short stalk, subentire, with few fine teeth; middle leaf oblong or oblong spatulate, apex obtuse or acuminate, sessile, upper leaf linear. All leaves are with long hairs that are sparse or dense above, glabrous below or hairy only along veins, with long marginal hairs, with three veins off base. The capitula terminal are solitary at the end of the stem and are 2–3.5 cm in diameter. Involucral hemispherical, 0.7–1.5 cm in diameter; involucral bracts 2–3 layers, subequal, linear-lanceolate, 5.5–7 mm long, 1–1.5 mm wide, apical acuminate, abaxially and margin with purplish-brown dense hairs. Ray florets1 layer, about 30–60, tongue bluish-purple, 10–20 mm long, 1–2 mm wide, apical point; disk florets orange-yellow, tube 1 mm long, split 1–2 mm long, with black or colorless glandular hairs; Achenes oblong, up to 3 mm long, white sparsely hairy or silky. The flowering period is from June to July, and the fruit period is from July to August. It is generally distributed in Alpine meadow, thickets, and hillside grassland, with an altitude of 2750–4800 m.

2.1.5 Aster poliothamnus *diels*

A. poliothamnus is usually perennial subshrubs, and its height is about 15–100 cm (**Figure 1E**), sometimes shrublike, caespitose, caudex woody. Stems branched, branches erect, there are dense leaves. Lower leaf withered; middle leaf oblong or linear oblong, about 1–2 cm long and 0.2–0.5 cm wide, entire margin, blade base slightly narrow or sharply narrow, apex blunt or pointed, leaf margin flat or slightly reversed; upper leaf small, elliptic. All leaves are short strigose above, pilose below, glandular on both sides, midvein raised below, lateral vein not visible. Inflorescence densely corymbose or solitary at branch ends; inflorescence peels are thin, 1–2.5 cm long, with sparse bracteoles. The involucre is broad campanulate, 5–7 mm long, 5–7 mm in diameter; involucral bracts 4–5 layers, imbricate arranged, outer oval or oblong-lanceolate, 2–3 mm long, all or upper grassy, apically apical, outer or only along midvein densely puberulent and glandular; inner layer up to 7 mm long, 0.7 mm wide, subleathery, upper grassy and reddish purple, marginal hairs. 10–20 Ray florets, lilac, oblong, 7–10 mm long, 1.2–2 mm wide. The disk florets are yellow, 5–6 mm long and 1.6–2 mm long. The crest is dirty white and about 5 mm long. Achenes oblong, 2–2.5 mm long, often ribbed on one side, covered with white silky hair. The flowering period is from July to August, and the fruit period is from August to September. It is generally distributed in arid slopes, alpine timberline, glades, and so on, with an altitude of 1800–3800 m.

2.2 Chloroplast genome characteristics of *Aster* species

We sequenced the chloroplast genome of five *Aster* leaves collected from Maqin County, Guoluo Prefecture, Qinghai Province, China (36°010 N, 103°450 E, altitude 3970 m). The chloroplast genome was sequenced. And the assembled chloroplast genome and its detailed annotation were submitted to GenBank (*Aster yunnanensis* var. *labrangensis*: OQ569735.1; *Aster diplostephioides*: OQ603807.1; *Aster farreri*: OQ603808.1; *Aster souliei*: NC_073537.1; *Aster asteroides*: NC_073536.1; *Aster poliothamnus*: OQ658689.1).

The complete chloroplast genome sequence lengths varied from 152,549 to 153,087 bp of five species of Aster (**Figure 2**), which has the quadripartite structure typical for most higher plants and highly conserved, respectively, which was divided into a LSC (84218-84,742 bp) and an SSC (18165–18,307 bp) regions separated by a pair of inverted repeats (IR, 24960–25,031 bp). In addition, the overall GC content of the chloroplast genome sequence was 37.3%. Since all the rRNAs were located in the IR

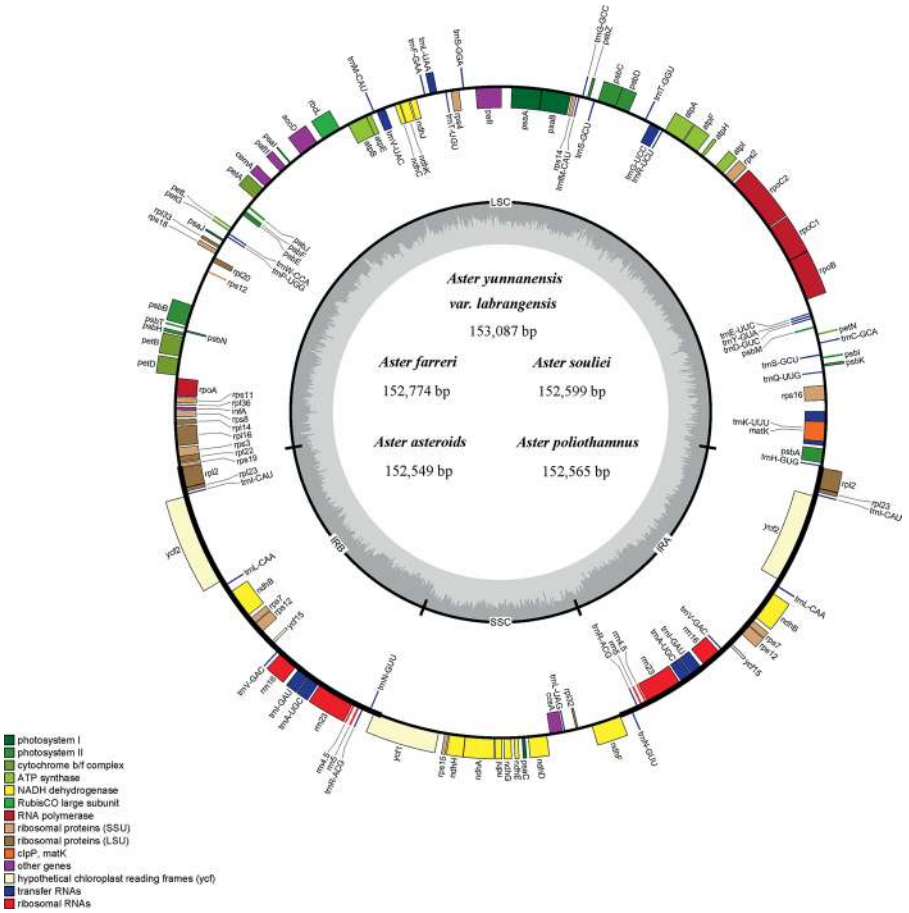


Figure 2.
Chloroplast genome map of five Aster *species. Genes inside the circle are transcribed clockwise, and those outsides are transcribed counter-clockwise. Genes of different functions are color-coded. The darker gray in the inner circle shows the GC content, while the lighter gray shows the AT content. The red and blue lines indicate GC skew, the red means GC skew greater than zero, while the blue means GC skew smaller than zero. Same as the chloroplast genome characterization below.*

regions, the GC content of the IR regions (42.9%) was higher than that of the LSC (35.2%) and SSC (31.2%) regions, respectively. A total of 130 genes were annotated successfully, including 85 protein-coding genes, 37 tRNAs, 8 rRNAs, respectively.

The CDS and tRNA of the five *Aster* species were mainly distributed in the LSC region, rRNA was only distributed in the IR region, and only one tRNA was distributed in the SSC region. Nineteen genes had two copies, which comprised of seven CDS coding genes, seven tRNA genes, and all four rRNA species (rrn16, rrn23, rrn4.5, and rrn5). In the genome, eighteen genes contain introns, among them 17 contains 1 intron and pafI contains 2 introns. The start codon of all CDS is ATG except rps19, where the start codon of rps19 is GTG, respectively.

Similarity in codon usage and amino acid frequencies were observed in five *Aster* species. And the results showed that about twenty-five thousand amino acids were detected in the chloroplast genome of five *Aster* species, in which Leucine was the most abundant, with two thousand and seven hundred codons (10.8%), followed by Isoleucine with two thousand and one hundred codons (8.4%), Serine and Glycine, with about 1960 and 1750 codons (7.7% and 6.9%), and Cysteine was the least abundant. There are 29 codons with RSCU values greater than 1 in five Aster. Methionine and Tryptophan had RSCU values equal to 1, but the most preferred codon was TTA, encoding Leucine (Leu), with an RSCU value of about 1.85.

SSRs are highly polymorphic molecular genetic markers, widely used, especially for population, evolutionary, and conservation genetics studies and forensics [14]. SSRs are composed of one or a few consecutive repeated nucleotides. By analysis of SSR dynamics in chloroplast genomes of five *Aster* species show that the pentanucleotide SSRs was only found in *A. asteroids* and *A. poliothamnus*, while the types and numbers of SSRs varied across species of *Aster*, indicating genetic diversity among species. A6 contains less mononucleotides and trinucleotides than the other four species of *Aster*. In five *Aster* species chloroplast genome, mononucleotide repeats are the most abundant repeats, followed by tetranucleotides, dinucleotides, and trinucleotides repeat, while pentanucleotides and hexanucleotides repeats rarely occur, and all of the mononucleotide repeats consisted of either A or T bases. SSR repeats in LSC region were much higher than SSC region and IR region in five *Aster* species.

The chloroplast genomes are of great significance in the reconstruction of plants phylogenetic relationships and evolutionary history [15]. In our study, we constructed a phylogenetic tree using the sequences of the whole chloroplast genomes of 41 species in the family *Aster*, including 24 (including *Heteropappus* and *Symphyotrichum*) species and using 15 species in *Senecio, Diplostephium*, *Asterothamnus, Artemisia*, and *Erigeron* as outgroups. The alignment of plastomes was generated by MAFFT. The maximum-likelihood (ML) analysis was performed using MEGA11, of which the bootstrap values were calculated using 1000 replicates with the best selected GTR + G model [14]. The result showed that all clades were strongly supported, *A. ageratoides* var. *scaberulus* is sister to a clade formed by *A. yunnanensis* var. *labrangensis*, *A. farreri*, *A. asteroids*, *A. souliei*, and *A. batangensis* according to the current sampling extent, and *A. poliothamnus* was sister to *A. sampsonii*.

2.3 Discussion and conclusion

In this chapter, the architecture of the basic characteristics, codon usage bias, SSRs and phylogenetic relationships of chloroplast genomes of five *Aster* species are studied. Five species of *Aster* have a typical quadripartite structure and 130 functional

genes have been annotated. Moreover, in the coding strand, the bias of T over A existed in all five Aster species, while the bias of G over C differed. These findings, in combination with identified introns, codon usage bias, and SSRs, enrich our knowledge on chloroplast biology and genetic diversity of five *Aster* species and lay a strong foundation for further studies on molecular marker development, phylogenetic analysis, population studies, and chloroplast genome engineering.

3. Morphological characteristics and chloroplast genome analysis of *Aster altaicus* Willd

3.1 Species morphological characteristics and distribution information

Aster altaicus Willd., depicted in **Figure 3** [16], is a perennial herbaceous plant. It was previously classified as *Heteropappus altaicus* when it was part of the *Heteropappus* genus. However, due to its unique feature of ray floret having very short crown hairs, it was often associated with *Aster*, although some other *Aster* species also exhibit this trait. Eventually, it was reclassified solely under the *Aster* category [17]. Typically, these plants grow to be 15–40 cm tall and possess transversely or vertically woody roots. The stems originate at the base and give rise to multiple branches, which can be either erect or obliquely branched. These branches are covered with upwardly curved or spreading hairs and contain glands situated distally. The leaf blade is linear, oblong, or oblanceolate, measuring 3–35 mm in length and 1–7 mm in width. The anterior ends of the leaves can be blunt or acute and are usually complete, featuring short coarse or fine hairs on both upper and lower surfaces, accompanied by glandular dots. At the uppermost parts of the branches, multiple heads grow either individually or in an umbellate fashion. The involucre, approximately 1–1.5 cm in diameter, takes the shape of a hemispherical



Figure 3.
Morphology and habitat map of Aster altaicus. *From Yueliang Bay park in Guide County, Hainan Tibetan autonomous prefecture, Qinghai Province, China (101°43′95“ E, 36°04´61” N), photograph by Ying Liu.*

structure and consists of 2–3 layers of nearly equal-length involucral bracts. These bracts are oblong-lanceolate or linear, approximately 5 mm long and 1.5 mm wide, with tapered pointed tips. They exhibit narrow membranous margins and glandular hairs on the abaxial side. The flower head contains 15–20 ray florets with tubes measuring around 2.5 mm. The ligules of these florets are blue, linear-oblong, reaching a length of 15 mm and a width of approximately 2 mm. The ray florets themselves are yellow, about 5 mm long, featuring five lobes of varying lengths and small external hairs. The achenes are oblong-obovate and hairy, with reddish-brown crown hairs, approximately 4 mm long and thicker. Flowering and fruiting occur from July to October [18].

A. altaicus Willd. thrives in various environments such as grasslands, deserts, sands, and arid mountainous areas at altitudes ranging from 0 to 4000 meters. This plant is widely spread in northern China and is mainly produced in Asia [17]. It is an important medicinal plant, widely used in traditional Chinese medicine and Mongolian medicine [19], and holds some forage value [20].

3.2 Characterization and phylogenetic appreciation of the chloroplast genome of *A. altaicus*

The chloroplast genome of *A. altaicus* leaves, collected from Yueliang Bay Park in Guide County, Hainan Tibetan Autonomous Prefecture, Qinghai Province, China (101°43′95“ E, 36°04′61” N), was sequenced. Subsequently, the assembled chloroplast genome, along with detailed annotations, was submitted to GenBank under accession number NC072176. The analysis revealed that the *A. altaicus* chloroplast genome is 152,473 bp in size and possesses an average GC content of 37.3%. It exhibits a typical four-part structure, including a large single-copy region (LSC) spanning 84,235 bp, a small single-copy region (SSC) spanning 18,218 bp, and two inverted repeat regions (IRs) spanning 25,013 bp (**Figure 4**). A total of 129 genes were successfully annotated, encompassing 85 protein-coding genes, 8 ribosomal RNA genes, and 36 transfer RNA genes. Among these genes, 17 contain one intron, while 3 contain two introns (**Figures 3** and **4**).

In this study, a phylogenetic analysis was conducted using the complete chloroplast genome of *A. altaicus* and 25 other *Aster* species, and two *Medicago* species (*Medicago monspeliaca* and *Medicago monspeliaca*) of Fabaceae (Two *Medicago* species were used as outgroups). The results showed that *A. altaicus* and *Aster altaicus* var. *uchiyamae* have a strong sister relationship.

3.3 Discussion and conclusion

A. altaicus Willd. belongs to the *Aster* genus of the *Asteraceae* family. Previous studies have explored the complete chloroplast genomes of various *Aster* species [21–25]. In this study, we present the first successfully assembled and annotated complete chloroplast genome of *A. altaicus* Willd., collected from the Tibetan Plateau. With a length of 152,473 bp, its sequence exhibits a typical tetramerization structure observed in other *Aster* species. Additionally, for systematic evolutionary tree analysis, we incorporated chloroplast whole genome data from 25 other *Aster* species. The results indicate a strong relationship between *A. altaicus* Willd. and *A. altaicus* from Korea, as well as *A. altaicus var. uchiyamae* and the samples used in this study. This research contributes to valuable genetic resources for future investigations on *A. altaicus* Willd. and proves to be essential in studying the phylogenetic relationships of *A. altaicus* Willd. It is crucial to continue exploring the genetic

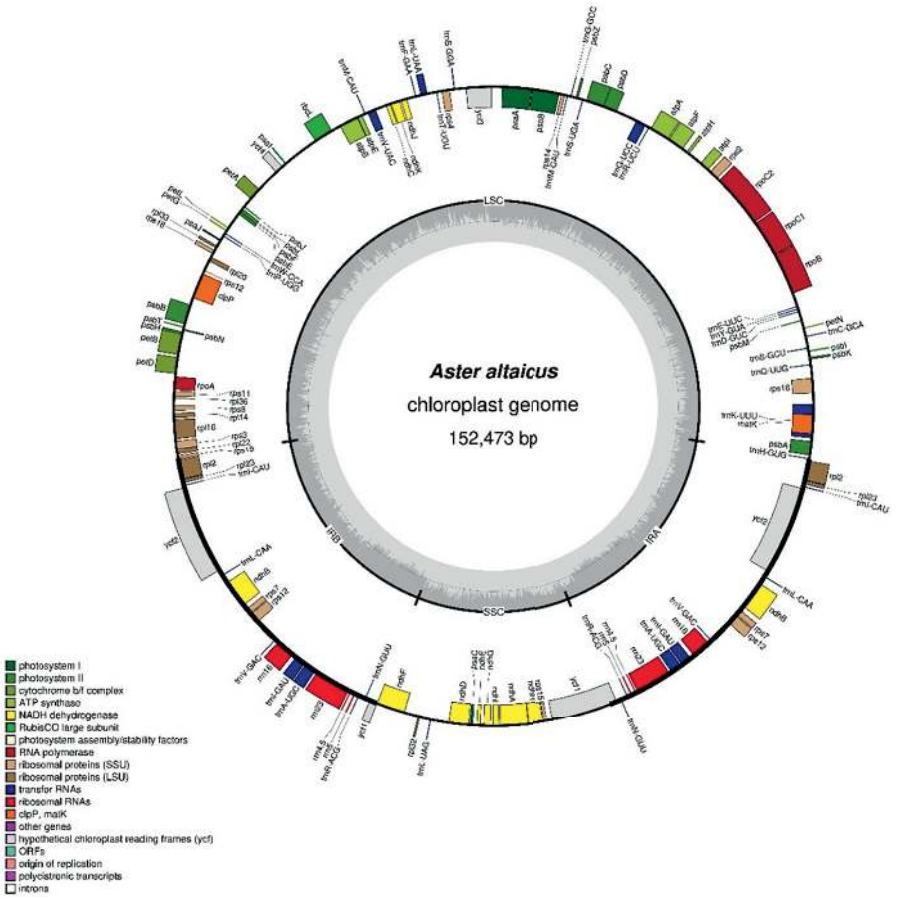


Figure 4.
Chloroplast genome map of Aster altaicus.

information of *Asteraceae* and advancing classification research within the *Asteraceae* family, including the *Aster* genus.

4. Morphological characteristics and chloroplast genome analysis of *Asterothamnus centraliasiaticus*

4.1 Species morphological characteristics and distribution information

Asterothamnus centraliasiaticus Novopokr. [26, 27] (**Figure 1**), also known as Aster centraliasiaticus and Aster alyssoides, is a perennial deciduous half-shrub of the family Asteraceae [28], native to arid and semi-arid areas of the Qinghai-Tibetan Plateau and northwestern Mongolia in China. It is a perennial deciduous half-shrub of the family Asteraceae [28], native to the arid and semi-arid regions of the Qinghai-Tibetan Plateau and northwestern Mongolia in China, and naturally distributed in the areas of 1300–3900 meters above sea level. *A. centraliasiaticus* is an ecologically important species in its range, especially for its role in soil stabilization and as a food resource for livestock and wildlife. In addition, *A. centraliasiaticus* has brightly

colored flowers and can also be used as an ornamental plant. *A. centraliasiaticus* is a much-branched half-shrub, with plants up to 120 cm tall. Its stems are cespitose, branched below, with inflorescence branches above. *A. centraliasiaticus* is a not very tall half-shrub with many branches below, and a root neck buried in the soil, from which branches emanate. Its axial roots are deeply embedded in the soil up to about 1 m. Most of the adventitious roots grow from the branches, forming a rather extended root system with increased space for water and nutrient uptake in dry and early environments, and the width of the root system is usually several times the width of the aboveground crown. Aboveground older branches are highly lignified, grayish-yellow, and stout, and newer branches are slender and grayish-green. Centraliasiaticus is a super-arid desert plant found in deserts and desert grassland zones, preferring to grow on loose, gravelly alluvial and floodplain soils. It often forms communities along dry riverbeds and flowlines, and is also found on stony hills and pre-hill floodplain slopes (**Figure 5**) [26].

4.2 Characterization and phylogenetic appreciation of the chloroplast genome of *A. centraliasiaticus*

We sequenced the chloroplast genome of *A. centraliasiaticus* leaves collected from Yueliang Bay Park in Guide County, Hainan Tibetan Autonomous Prefecture, Qinghai Province, China (101°53′47“ E, 36°09′23” N, 2050 m a.s.l.). The chloroplast genome was sequenced. And the assembled chloroplast genome and its detailed annotation were submitted to GenBank with the accession number OP909739. The



Figure 5.
Morphology and habitat map of Asterothamnus centraliasiaticus*. From Yueliang Bay park in Guide County, Hainan Tibetan autonomous prefecture, Qinghai Province, China, photograph by Zheng-sheng Li.*

results indicate that the complete cp genome of *A. centraliasiaticus* is 152,205 bp in length (**Figure 6**) and comprises a pair of inverted repeats (IR) regions of 25,031 bp each, a large single-copy (LSC) region of 83,956 bp and a small single-copy (SSC) region of 18,187 bp. The GC content of *A. centraliasiaticus* is 37.32%. A total of 130 genes were successfully annotated containing 85 protein-coding genes, 37 transfer RNA genes, and 8 ribosomal RNA genes. Among these genes, 21 genes have one intron each, and two genes contain two introns.

The complete chloroplast sequences of *A. centraliasiaticus,* and other seventeen species in six genera (eight *Aster* species, two *Artemisia* species, one *Pericallis* species, one *Guizotia* species, two *Ambrosia* species, and three *Cynara* species) within *Asteraceae*, were used in the phylogenetic analysis. Two *Oryza* species (Oryza punctata and Oryza minuta) of Poaceae were used as outgroups. The phylogenetic analysis indicated that *A. centraliasiaticus* was close to *Aster hypoleucus* and *Aster lavandulifolius.*

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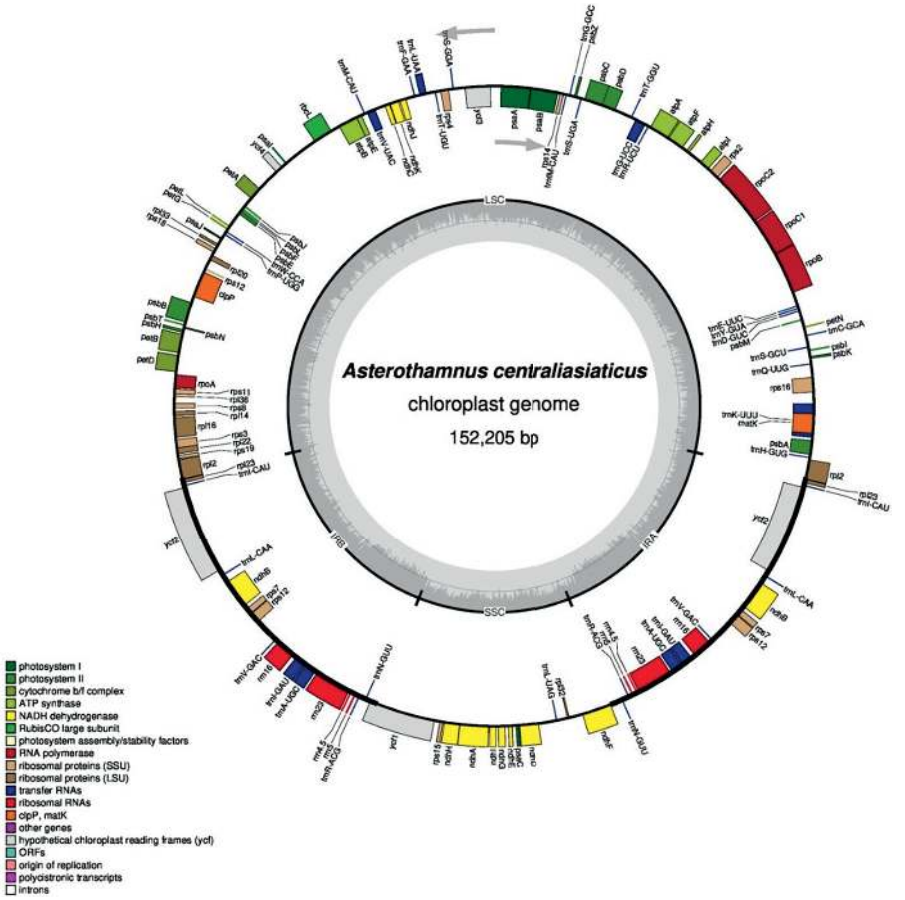


Figure 6.
Chloroplast genome map of Asterothamnus centraliasiaticus.

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4.3 Discussion and conclusion

Sequencing chloroplast genomes from various plants has provided valuable insights into chloroplast biology, biodiversity conservation, and genetic information that can be harnessed for improving agronomic traits or developing high-value agricultural and biomedical products. In this study, we assembled the chloroplast genome of *A. centraliasiaticus* using Illumina HiSeq2500 sequences. Our findings reveal that the complete cp genome of *A. centraliasiaticus* spans a length of 152,205 bp. Consistent with previous studies, it exhibits a standard quadripartite structure, consisting of a pair of inverted repeat (IR) regions of 25,031 bp each, a large single-copy (LSC) region of 83,956 bp, and a small single-copy (SSC) region of 18,187 bp. We successfully annotated a total of 130 genes, including 85 protein-coding genes, 37 transfer RNA genes, and 8 ribosomal RNA genes. Moreover, the chloroplast genome sequence has been widely utilized for determining evolutionary relationships among plants. In this study, the maximum-likelihood (ML) phylogenetic analysis based on the complete chloroplast genome data strongly supported the close relationship between *A. centraliasiaticus* and *A. hersileeoides*. These findings provide valuable genetic information for germplasm protection and informed development strategies. Furthermore, our analysis revealed that some species of the genus Aster are more distantly related at the chloroplast genome level compared to *A. centraliasiaticus*. This discovery challenges the current botanical species classification of *A. centraliasiaticus* and offers new insights and theoretical support for future plant taxonomy research.

5. Ecology, structural characteristics, and chloroplast genomes of *Corethrodendron multijugum* (maxim.) species

5.1 Morphological characteristics and distribution information

Corethrodendron multijugum (Maxim.) [29] (**Figure 7**), formerly known as *Hedysarum multijugum* [30, 31], also known as *Hedysarum multijugum f. albiflorum*. This genus *Hedysarum* was recently revised to the genus *Corethrodendron* based on morphological and molecular evidence of several barcoding regions, including plastid and nuclear regions [29]. Now, this species belongs to the *Corethrodendron* of *Fabaceae* in *Fabales.*

C. multijugum is a semishrub or herb, woody only at the base, 0.3–1 m tall. Roots lignified. Stem erect, much branched, slenderly striate, densely white pilose, longitudinally furrowed. Leaves 6–18 cm long; stipules brown scaly, ovate-lanceolate, 2–5 mm long, base connate, apex free, abaxially pilose; leaf rachis grooved, densely gray-white pubescent; leaflets 15–35, elliptic, ovate, or obovate, 5–12 mm long, 3–6 mm wide, apex obtuse or slightly concave, base subrounded or rounded-cuneate, ventrally glabrous, abaxially densely appressed pubescent; The petiole is very short and hairy. Racemes growing in axils of branches, 20–35 cm long, 9–25 flowers growing sparsely; bracts caducous; pedicels 2–3 mm long, pilose; calyx obliquely campanulate, 5–6 mm long, calyx teeth subulate or acute, 3–4 times shorter than calyx tube, lower calyx teeth slightly longer than or twice as long as upper calyx teeth, usually



Figure 7.
Morphology and habitat map of Corethrodendron multijugum. *From Yueliang Bay park in Guide County, Hainan Tibetan autonomous prefecture, Qinghai Province, China (101°43′95" E, 36°04′61" N), photograph by Ying Liu.*

splitting between upper calyx teeth deeper below middle of calyx tube, sometimes splitting deeper between both calyx teeth and upper calyx, outside covered with Corolla 15–19 mm long, purple-red or rose-red, with yellow spots; flagellum obovate, slightly concave at first end, 14–18 mm long, claw short; wing petals narrow, 6–8 mm long, ca. 1 mm wide, claw half as long as limb, auricles nearly as long as petiole; keel petal slightly shorter than flagellum, anterior lower angle bow-shaped curved. Ovary linear, pubescent. Pods flattened, usually l-3-noded; ovate or semiorbicular nodding pods ca. 5 mm long and 4 mm wide, sparsely pubescent, with reticulate and small spines on sides. Fl. Jun-Aug, fr. Jul-Sep [12, 29–32].

The plant is widely distributed in northwestern, northern, and southwestern of China, including Sichuan, Tibet, Xinjiang, Qinghai, Gansu, Ningxia, Shaanxi, Shanxi, Inner Mongolia, Henan, and Hubei, and grows mainly in gravelly floodplains and riverbanks in desert areas around 1800–3800 m above sea level, on sunny slopes, gullies, embankments, gravelly lands, gravelly slopes in grassland areas and in certain deciduous broad-leaved forest areas on dry mountain phi and gravelly river banks in some deciduous broad-leaved forest areas [12, 29–34]. It is distributed in all states and counties of Qinghai Province [12, 29]. It is distributed abroad in Mongolia and Russia. The type specimens were collected from the western part of the Hexi Corridor in Gansu [29–32]. This plant has deep roots, strong cold, and drought tolerance [35–37]. It is not only a widely used in traditional Chinese medicine, but also an excellent feed, soil, and water conservation plant, which has important medicinal and economic values [38–42].

5.2 Characterization and phylogenetic appreciation of the chloroplast genome of *Corethrodendron multijugum*

The complete chloroplast genome was sequenced using the Illumina Hiseq 2500 platform (Illumina, SanDiego, CA) with paired-end reads of 150 bp by Genesky

Biotechnologies Inc., Shanghai, China. And the complete chloroplast genome structure of *C. multijugum* was a circular DNA molecule (**Figure 8**), with a length of 122,994 bp (GenBank accession no. NC069301). Unlike the typical tetrad structure of most angiosperm chloroplast genomes, the *C. multijugum* chloroplast genome does not have the typical tetrad structure consisting of a large single copy (LSC), a small single copy (SSC), and a pair of inverted repeats (IRs), and similarly in *Hedysarum polybotrys* var. *alaschanicum* results [43]. The overall G + C content of the whole genome is 34.5%, and the genome presented a negative AT-skew (−0.002) on the J-strand. The genome contains 110 genes, including 76 protein-coding genes (PCGs), 30 transfer RNA genes (tRNAs), 4 ribosomal RNA unit genes (rRNAs). There are 17 genes containing one intron (s) and one trans-splicing genes rps12, and two genes (ycf2 and ycf3) contain two introns. A total of 65 simple sequence repeat (SSR) markers ranging from mononucleotide to tetranucleotide repeat motif were identified in *C. multijugum* chloroplast genome, and mononucleotide had the most repeats.

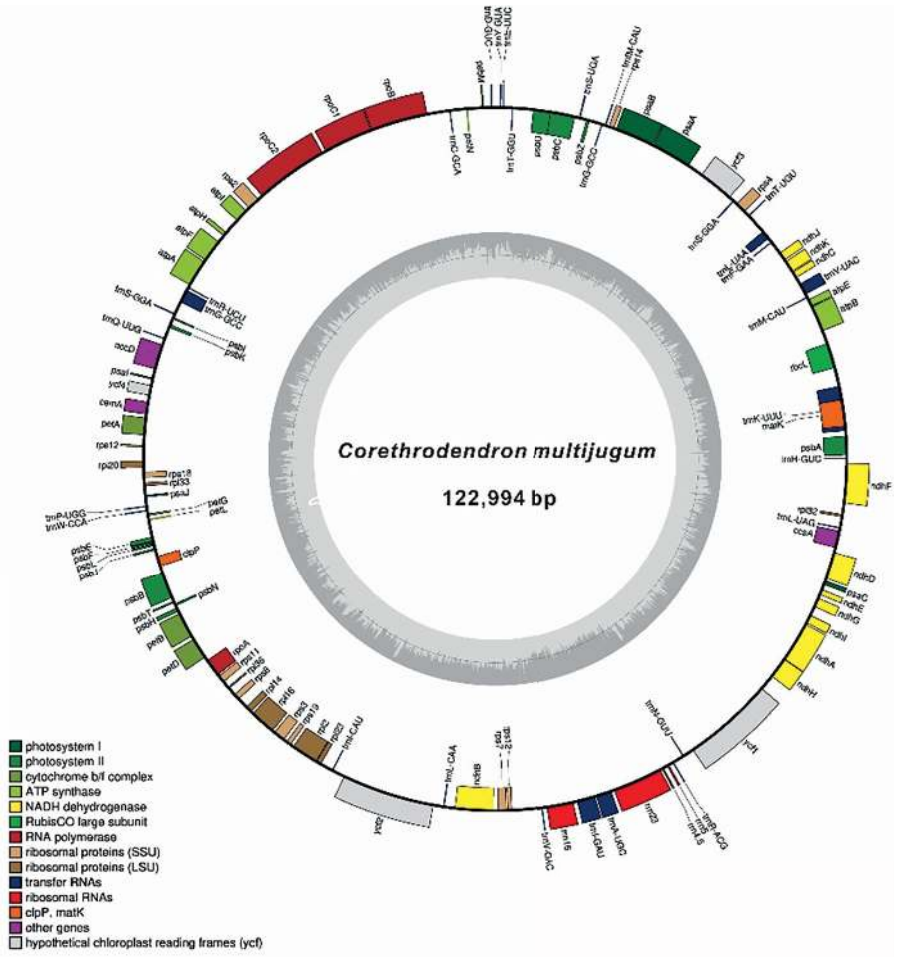


Figure 8.
Chloroplast genome map of Corethrodendron multijugum.

All of the 20 chloroplast genome sequences of Fabaceae were obtained from GenBank and used for phylogenetic analysis, and two chloroplast genome sequences of Rosaceae were used as outgroups. Phylogenetic analysis indicated a strong sister relationship with *C. multijugum* and *Hedysarum petrovii* (**Figure 9**). This study will contribute to a better understanding of the evolutionary pattern of chloroplast genomes in *C. multijugum* and provide more basis for the identification and application of *Corethrodendron* plants.

5.3 Discussion and conclusion

In this study, the chloroplast genome of *C. multijugum* is reported for the first time. We described the sequence structures and annotated genes in the genome. Its sequence length was found to be 122, 994 bp, similar to that of other *Corethrodendron* (*Hedysarum*) species. What's different from other studies is that compared with other previously published chloroplast genomes of *Corethrodendron* (*Hedysarum*) species, the *C. multijugum* chloroplast genome does not have the typical quadripartite structure, and similar genetic structure results have been demonstrated in both genus *Hedysarum* [43] and genus *Astragalus* [44]. What is special about this study is that the phylogenetic tree was reconstructed to confirm the phylogenetic of *C. multijugum* for the first time. The chloroplast genome of *C. multijugum* will contribute to a better understanding of the evolutionary mode of the chloroplast genome and provide more evidence for the identification and application of *Corethrodendron* species.

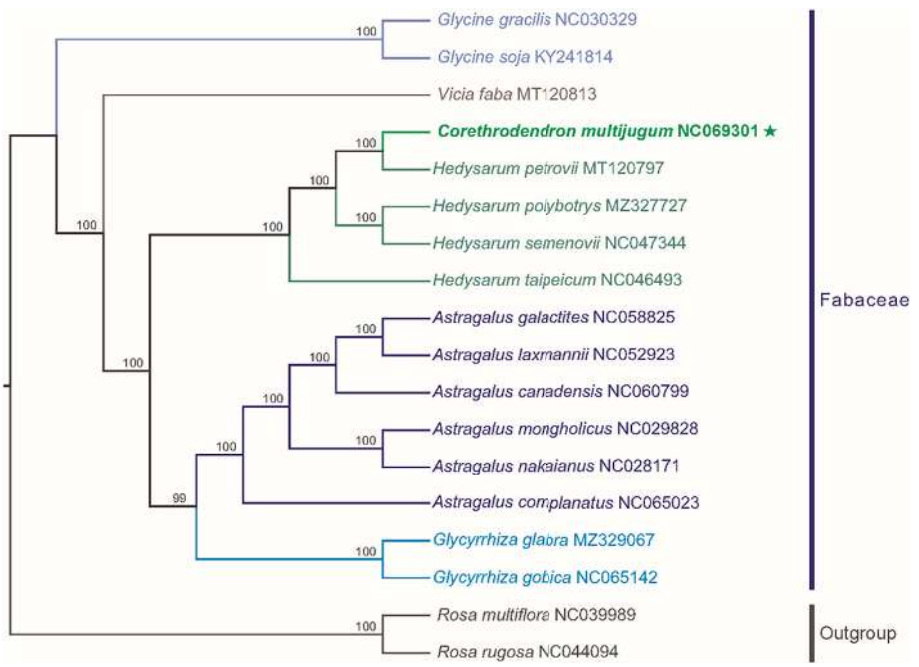


Figure 9.
Chloroplast phylogeny of 16 Fabaceae species based on the complete chloroplast genome sequences. The asterisk represents the assembled plastome sequence in this picture. The clades of species are represented with black lines.

6. Morphological characteristics and chloroplast genome analysis of *Clematis nannophylla* Maxim

6.1 Morphological characteristics and distribution informations

Clematis is belong to widely Ranunculaceae family and distributed worldwide [21, 45]. *Clematis nannophylla* is Small perennial shrub, and has high ornamental, ecological, and medicinal value [22, 45]. *C. nannophylla* is normally found dry or gravelly slopes; 1200–3200 m. Gansu, SW Nei Mongol, Ningxia, Qinghai, Shaanxi (**Figure 10**).

Clematis nannophylla is usually small shrubs, erected 30–100 cm tall. The branches are reddish-brown and ribbed, and the branchlets are densely appressed puberulous and fall off later. Leaves are simple opposite or several fascicled, almost sessile or up to 4 mm long; leaf blade contour subovate, 0.5–1 cm long, 3–8 mm wide, leaf blade pinnately lobed, with lobes 2–3 or 4 in pairs, or lobes subdivided 2–3, lobes or lobules elliptic to broadly obtusely cuneate or lanceolate, 1–4 mm long, with varying 2–3 notched small teeth or entire margin, glabrous or pubescent. Flowers solitary or cyme with 3 flowers; sepals 4, yellow, oblong to obovate, 0.8–1.5 cm long, 5–7 mm wide, pubescent outside, margin densely villous, interior pubescent to nearly glabrous; stamens glabrous, filaments lanceolate, longer than anthers. Achenes oval, about 5 mm long, puberulent. The flowering period is from July to August, and the fruit period is from August to September [22].

6.2 Characterization and phylogenetic appreciation of the chloroplast genome of *C. nannophylla*

The chloroplast genome of *C. nannophylla* was 159,801 bp in length and divided into four distinct regions, such as large single copy region (LSC, 79,526 bp), small single copy region (SSC, 18,185 bp), and a pair of inverted repeat regions (31,045 bp). The genome annotation predicted a total of 133 genes, including 89 protein-coding



Figure 10.
Morphology and habitat map of clematis nannophylla*. From Yueliang Bay park in Guide County, Hainan Tibetan autonomous prefecture, Qinghai Province, China (101°43′95“ E, 36°04´61” N), photograph by Ying Liu.*

genes, 36 tRNA genes, and 8 rRNA genes. Phylogenetic analysis with the reported chloroplast genomes revealed that *C. nannophylla* is nested in Sect. *Fruticella* of family Ranunculaceae and has a close relationship to *C. fruticosa* and *C. songorica* (**Figure 11**).

6.3 Discussion and conclusion

In summary, the complete cp genome sequence of *C. nannophylla* was sequenced and compared with other related species, providing an important reference for the phylogeny of *C. nannophylla*. Although the cp genomes of *C. nannophylla* was identical to each other *Clematis* species in genome structures, gene contents, and GC contents, phylogenetic analysis showed that *C. nannophylla* is closely related to *C. fruticose*, *C. tomentella*, and *C. songarica*, and the well-resolved phylogenetic tree showed monophyletic origin of genus *Clematis* and genus *Aconitum* as its sister genus. The cp genome information in this study provides reference data for molecular marker development, phylogenetic analysis, population study, and cp genome processes, as well as for better exploitation and utilization of *C. nannophylla*. The results can guide more efficient germplasm resource utilization, conservation, and breeding strategy development.

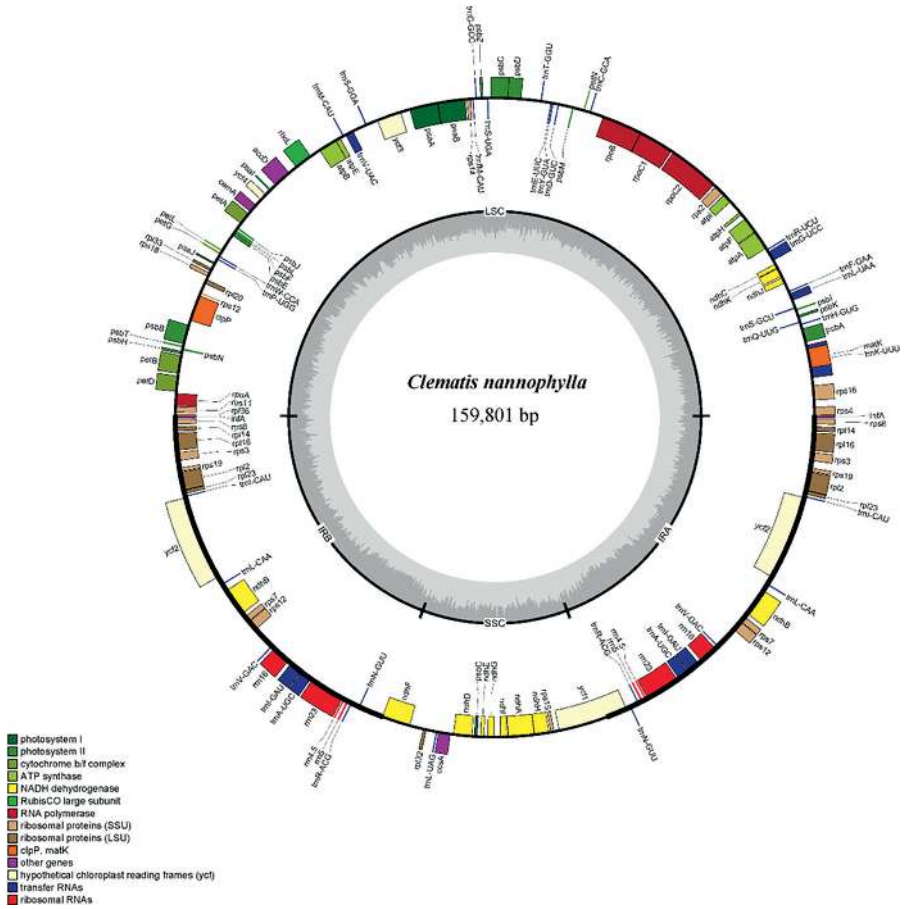


Figure 11.
Chloroplast genome map of clematis nannophylla.

Funding

This work was supported by the Qinghai Science and Technology Department of the senior scientist responsibility system project (2024-SF-101).

Conflict of interest

The authors declare no conflict of interest.

Abbreviations

cp	chloroplast
LSC	large single copy
SSC	small single copy
IR	inverted repeat
SSR	simple sequence repeat
ML	maximum likelihood
RSCU	relative synonymous codon usage
PCGs	protein-coding genes

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