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GENOME NOTE

REVISED Complete chloroplast genomes of endemic *Astragalus* and *Oxytropis* species from Uzbekistan

[version 4; peer review: 2 approved]

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Abstract

Chloroplast genomes provide important insights into plant phylogeny, genome evolution, and molecular marker development. In this study, we sequenced, assembled, and analyzed the complete chloroplast genomes of two endemic species from Uzbekistan, *Astragalus nuratensis* and *Oxytropis pseudorosea*. Genome skimming produced high-quality paired-end reads, yielding mean chloroplast sequencing depths of 638.13× for *Astragalus nuratensis* and 1,725.34× for *Oxytropis pseudorosea*, respectively. The chloroplast genomes were 122,316 bp in *A. nuratensis* and 122,708 bp in *O. pseudorosea*. Both genomes encoded 110 unique genes, including 76 protein-coding genes, 30 transfer RNA genes, and 4 ribosomal RNA genes. Consistent with members of the inverted repeat-lacking clade of Fabaceae, both species lacked the typical inverted repeat regions, resulting in a single-copy genome structure. Phylogenetic analysis based on 119 complete chloroplast genomes resolved major lineages within *Astragalus* and related genera with strong support. *Astragalus nuratensis* was placed within the Phaca clade, while *Oxytropis*

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pseudorosea formed part of a distinct *Oxytropis* lineage. These results provide new genomic resources for understanding evolutionary relationships and plastome evolution in Central Asian legumes.

Any reports and responses or comments on the article can be found at the end of the article.

Keywords

plastome; Fabaceae; IRLC; phylogeny; genome evolution; Central Asia



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This article is included in the **Genomics and Genetics** gateway.

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REVISED Amendments from Version 3

We have added text clarifying that *Oxytropis pseudorosea* (sect. *Eumorpha*) resolves as a sister taxon to *O. ikhbogdicus*, despite the latter's strong morphological affinity to section *Mesogaea*. This revision was implemented to explicitly address a key taxonomic incongruence, highlighting that traditional morphological classifications within *Oxytropis* likely reflect convergent evolution rather than true genetic relationships.

Any further responses from the reviewers can be found at the end of the article

Introduction

Chloroplast (cp) genomes of angiosperms have been widely used in studies of phylogeny, sequence variation, genome evolution, and the development of molecular markers (Dong et al., 2013). In most angiosperms, the cp genome exhibits a conserved quadripartite structure consisting of a large single-copy (LSC) region, a small single-copy (SSC) region, and two inverted repeat (IR) regions (Sugiura, 1992). Nevertheless, structural modifications, including gene rearrangements, inversions, expansion or loss of IR regions, gene loss, and pseudogene formation, have been documented in several lineages, particularly in Fabaceae (Cai et al., 2008; Son & Choi, 2022).

Fabaceae is one of the largest families of flowering plants and includes many species of considerable ecological and agricultural importance. According to recent classifications, the family is divided into six subfamilies: Caesalpinioideae, Cercidoideae, Detarioideae, Dialioideae, Duparquetioideae, and Faboideae (Papilionoideae) (Azani et al., 2017). Within Faboideae, the inverted repeat-lacking clade (IRLC) is characterized by the loss of one copy of the IR region (approximately 25 kb) in the chloroplast genome. This clade comprises about 52 genera and more than 4,000 species, and plastomes of IRLC taxa frequently show gene loss or pseudogenization (e.g., *rps16*, *rpl22*, *infA*, *accD*, and *ycf4*), intron loss (*clpP*, *atpF*, and *rpoC1*), inversions, and occasional transfer of genes to the nuclear genome (Son & Choi, 2022).

Within the IRLC, the genera *Astragalus* L. (3,095 species) and *Oxytropis* DC. (608 species) represent highly diverse and taxonomically complex groups (POWO, 2026). In recent years, complete chloroplast genome sequencing has been increasingly applied to resolve phylogenetic relationships and species boundaries within the genera *Astragalus* and *Oxytropis*. Several chloroplast genomes of species belonging to these genera have been published (Su et al., 2021; Li et al., 2025; Bobur et al., 2026). Despite these advances, chloroplast genome data remain limited for many narrowly distributed endemic species from Uzbekistan. In the present study, we sequenced, assembled, and analyzed the cp genomes of *Oxytropis pseudorosea* Filim. and *Astragalus nuratensis* Popov, two narrowly distributed endemic species from the Nuratau Mountains of Uzbekistan, Central Asia.

Methods

Leaf samples of *Oxytropis pseudorosea* and *Astragalus nuratensis* were collected in 2024 from the Nuratau Mountains, Uzbekistan, by Diyorjon Hamrayev. *Astragalus nuratensis* was identified by Beshko Natalya and its voucher specimen was deposited in the National Herbarium of Uzbekistan (TASH) under accession number TASH-2024-AN-001, whereas *Oxytropis pseudorosea* was identified by Doston Turdiyev and its voucher specimen was deposited in TASH under accession number TASH-2024-OP-002.

Genomic DNA was isolated from leaf tissue using the Tiangen DP305 Plant Genomic DNA Kit (Beijing, China). Libraries were prepared with the NEBNext® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA; Cat. E7370L) following the manufacturer's protocol, with index codes added during preparation. DNA was sonicated to ~350 bp and fragments were end-repaired, A-tailed, and ligated to Illumina adapters, followed by PCR amplification. PCR products were purified using AMPure XP beads (Beverly, USA). Library quality was assessed on an Agilent 5400 system, and concentrations were quantified by qPCR (1.5 nM). Qualified libraries were pooled and sequenced on Illumina platforms using the PE150 strategy at Novogene (Beijing, China).

Raw reads were assembled *de novo* using NOVOPlasty v4.3.5 (Dierckxsens et al., 2017), with the plastomes of *Astragalus agrestis* Douglas ex G. Don and *Oxytropis neimongolica* C.W. Chang & Y.Z. Zhao serving as seed references. The assembly generated a single circular chloroplast genome for each species without structural ambiguities. To assess assembly accuracy and sequencing depth, clean paired-end reads were aligned to the assembled plastomes using BWA-MEM v0.7.17 (Li, 2013). Alignments were subsequently sorted and indexed with SAMtools v1.19.2 (Li et al., 2009). Genome annotation was conducted in Geneious v9.0.2 (Kearse et al., 2012) using closely related reference plastomes. Protein-coding genes, transfer RNAs, and ribosomal RNAs were annotated, and gene boundaries were manually curated to ensure accurate start/stop codons and intron–exon junctions. Furthermore, the cp map was generated using Chloroplot (<https://irscope.shinyapps.io/chloroplot/>) (Zheng et al., 2020).

For several taxa included in the phylogenetic analysis, complete chloroplast genome sequences were not available in GenBank. Only raw sequencing data (SRA accessions; SRR numbers listed in Table 1) were available. Therefore, the chloroplast genomes of these taxa were assembled *de novo* using NOVOPlasty v4.3.5 (Dierckxsens et al., 2017). A total of 119 complete chloroplast genome sequences were included in the phylogenetic analysis. Of these, two plastomes were newly sequenced in this study, while the remaining 117 sequences were downloaded from the NCBI GenBank database (Table 1). Complete chloroplast genome sequences were aligned using MAFFT (Katoh & Standley, 2013). The alignment was manually inspected and used to reconstruct phylogenetic relationships under the maximum likelihood criterion in IQ-TREE 2 (Minh et al., 2020). The best-fit substitution model was selected using ModelFinder (Kalyaanamoorthy et al., 2017), and branch support was assessed with 1,000 ultrafast bootstrap replicates (Hoang et al., 2018).

Table 1. List of taxa used in the phylogenetic analysis, including their respective GenBank accession numbers. For taxa lacking published chloroplast genome accessions, plastomes were assembled in this study from raw reads downloaded from the NCBI Sequence Read Archive (SRR accessions provided).

Species	Accession	Species	Accession
<i>Astragalus camptodontus</i>	SRR12136586	<i>A. aksuensis</i>	SRR12136588
<i>A. confertus</i>	SRR12136589	<i>A. massanderanus</i>	SRR12136591
<i>A. handelii</i>	SRR12136594	<i>A. zacharensis</i>	SRR12136595
<i>A. uliginosus</i>	SRR12136597	<i>A. koburensis</i>	SRR12136600
<i>A. beketowii</i>	SRR12136611	<i>A. jessenii</i>	SRR12136613
<i>A. tibetanus</i>	SRR12136615	<i>A. dendroides</i>	SRR12136619
<i>A. candissimus</i>	SRR12136622	<i>A. platyphyllus</i>	SRR12136623
<i>A. himalayanus</i>	SRR12136624	<i>A. roseus</i>	SRR12136625
<i>A. stalinskii</i>	SRR12136626	<i>A. orbicularifolius</i>	SRR12136627
<i>A. hamosus</i>	SRR12136635	<i>A. heinsensis</i>	SRR12136637
<i>A. hoantchy</i>	SRR12136647	<i>A. saccolacys</i>	SRR12136648
<i>A. neomondelphus</i>	SRR12136653	<i>A. schmalhauseni</i>	SRR12136654
<i>A. zingeri</i>	SRR12136656	<i>A. eximius</i>	SRR12136657
<i>A. xanthomeloides</i>	SRR12136658	<i>A. pileocladus</i>	SRR12136659
<i>A. parrowianus</i>	SRR12136660	<i>A. coluteocarpus</i>	SRR12136661
<i>A. thurberi</i>	SRR12136664	<i>A. americanus</i>	SRR12136665
<i>A. peduncularis</i>	SRR12136666	<i>A. dipelta</i>	SRR12136669
<i>A. acaulis</i>	PV802397	<i>A. austrotristis</i>	NC_063479
<i>A. adsurgens</i>	NC_085710	<i>A. angustidens</i>	ON550413
<i>A. agrestis</i>	PP328800	<i>A. alpinus</i>	NC_063482
<i>A. ampullarius</i>	PV870668	<i>A. arbuscula</i>	ON550410
<i>A. arpilobus</i>	NC_063483	<i>A. arrectus</i>	NC_047381
<i>A. bhotanensis</i>	NC_063484	<i>A. biristatus</i>	MZ923743
<i>A. calycosus</i> var. <i>calycosus</i>	MZ725323	<i>A. canadensis</i> var. <i>brevidens</i>	PV870770
<i>A. chinensis</i>	NC_063486	<i>A. clevelandii</i>	ON550409
<i>A. cognatus</i>	MZ127832	<i>A. chinense</i>	ON550407
<i>A. dilutus</i>	PV870843	<i>A. effusus</i>	MZ901207
<i>A. flexuosus</i> var. <i>flexuosus</i>	ON550403	<i>A. flexus</i>	NC_083391
<i>A. floridus</i>	NC_058825	<i>A. galactites</i>	OY754317
<i>A. glycyphyllos</i>	MY746310	<i>A. gummifer</i>	NC_063487
<i>A. gypsodes</i>	LC764834	<i>A. iranicus</i>	NC_077545
<i>A. juanquensis</i>	PV798172	<i>A. khasianus</i>	ON550401
<i>A. laksamanni</i>	NC_085734	<i>A. leucocephalus</i>	ON550399

Table 1. Continued

Species	Accession	Species	Accession
<i>A. lithophilus</i>	PV870772	<i>A. lonchocarpus</i>	LC764835
<i>A. macropelmatus</i>	OP723863	<i>A. melilotoides</i> var. <i>tenuis</i>	OP723862
<i>A. membranaceus</i>	PV761671	<i>A. membranaceus</i> var. <i>membranaceus</i>	KX255662
<i>A. moseleios</i>	LC764836	<i>A. mongholicus</i>	OR712437
<i>A. mongholicus</i> var. <i>dahuricus</i>	MW719856	<i>A. mongholicus</i> var. <i>nakaianus</i>	KR296789
<i>A. muliensis</i>	NC_083909	<i>A. neglectus</i>	NC_063488
<i>A. nuratensis</i>	PX928918	<i>A. obscurus</i>	NC_063489
<i>A. odoratus</i>	LC764837	<i>A. oxylottis</i>	NC_077543
<i>A. pallasi</i>	NC_077542	<i>A. polysladus</i>	PV910879
<i>A. psilosepalus</i>	NC_083910	<i>A. purpurinus</i>	OR652287
<i>A. rumpens</i>	PX711949	<i>A. scaberrimus</i>	MW654102
<i>A. sereno</i> var. <i>sereno</i>	MZ923754	<i>A. sieversianus</i>	NC_077540
<i>A. sinicus</i>	OM287552	<i>A. stipulatus</i>	OR491698
<i>A. strictus</i>	MT120746	<i>A. toanus</i> var. <i>toanus</i>	MZ923756
<i>A. tongolensis</i>	PX754582	<i>A. tumabtsica</i>	NC_083912
<i>A. vulpinus</i>	ON550388	<i>A. wootonii</i> var. <i>wootonii</i>	MZ923757
<i>A. yunnanensis</i> subsp. <i>incanus</i>	PV156653	<i>A. zerabulaki</i>	PX711948
<i>Oxytropis chiliophylla</i>	PV694277	<i>O. filiformis</i>	PV684033
<i>O. latibracteata</i>	PV694278	<i>Oplonia microphylla</i>	PV684029
<i>O. neimongolica</i>	PV684030	<i>O. proboscidea</i>	PV694280
<i>O. pseudorozea</i>	PX512474	<i>O. sp.</i> TT4869-1	PX652196
<i>Tibetia liangshanensis</i>	MF193597	<i>Alhagi sparsifolia</i>	MW349013
<i>Caragana korshinskii</i>	KX289923	<i>Hedysarum taipeicum</i>	MK426698
<i>Caragana microphylla</i>	KX289922	<i>Corethroedendron multijugum</i>	OP748243
<i>Carmichaelia australis</i>	PV870797	<i>Phyllobium chinense</i>	MZ221114
<i>Phyllobium camptodontum</i>	SRR12136586	<i>Lessertia frutescens</i>	MF286764
<i>Sphaerophysa salsula</i>	PV870675		

Results

Genome skimming generated a total of 39,668,027 paired-end reads for *A. nuratensis* and 16,998,801 paired-end reads for *O. pseudorozea*. After quality filtering, 39,666,696 and 16,994,332 reads were retained as high-quality reads for *A. nuratensis* and *O. pseudorozea*, respectively. Mapping of the filtered reads to the assembled chloroplast reference genomes showed that 540,829 reads (1.36%) in *A. nuratensis* and 1,421,542 reads (8.36%) in *O. pseudorozea* were successfully aligned. Properly paired reads accounted for 1.33% and 8.27% of the total reads, respectively. The chloroplast genome of *Astragalus nuratensis* was recovered with an average sequencing depth of 638.13×, with coverage ranging from 9× to 3273×. In comparison, the chloroplast genome of *Oxytropis pseudorozea* exhibited a substantially higher mean sequencing depth of 1,725.34×, with coverage ranging from 12× to 2146× (Figure 1).

The complete chloroplast genome of *O. pseudorozea* was 122,708 bp in length, whereas that of *A. nuratensis* was 122,316 bp. Both genomes encoded a total of 110 unique genes, including 77 protein-coding genes (CDS), 30 transfer RNA (tRNA) genes, and 4 ribosomal RNA (rRNA) genes (Figure 2). In both *Astragalus nuratensis* and *Oxytropis pseudorozea*, the genes *rpl16*, *rpl2*, *rpoC1*, *ndhA*, *ndhB*, *petB*, *petD*, *atpF*, *clpP*, *trnA-UGC*, *trnG-UCC*, *trnI-GAU*, *trnK-UUU*, *trnL-UAA*, and *trnV-UAC* each contain one intron, while *ycf3* contains two introns. In addition, *rps12* is a trans-spliced gene with two introns (one cis-spliced and one trans-spliced). Consistent with other members of the inverted repeat-lacking clade (IRLC) of legumes, the typical inverted repeat regions were absent in both species, resulting in a single-copy chloroplast genome structure, a feature commonly reported in plastome studies of *Astragalus* and related taxa (Moghaddam et al., 2023; Ma et al., 2025; Li et al., 2025).

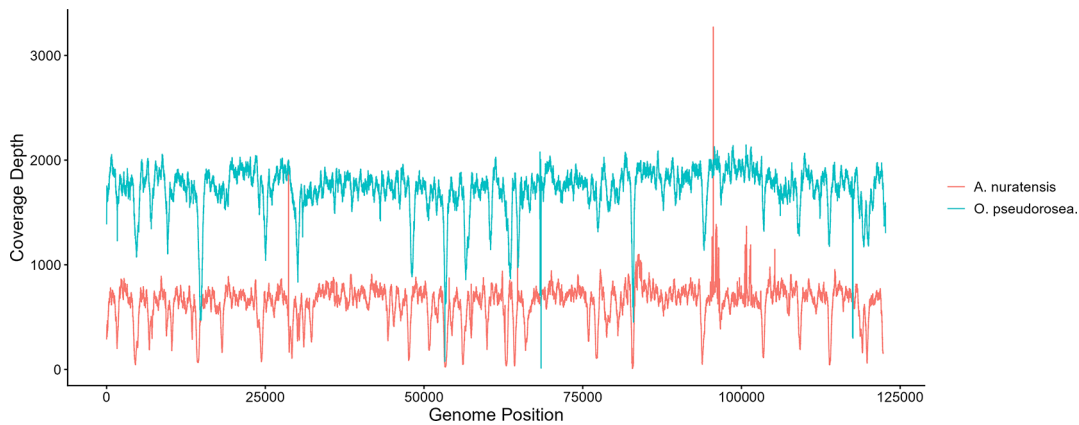


Figure 1. Coverage depth across the chloroplast genomes of *Astragalus nuratensis* and *Oxytropis pseudorosea*.

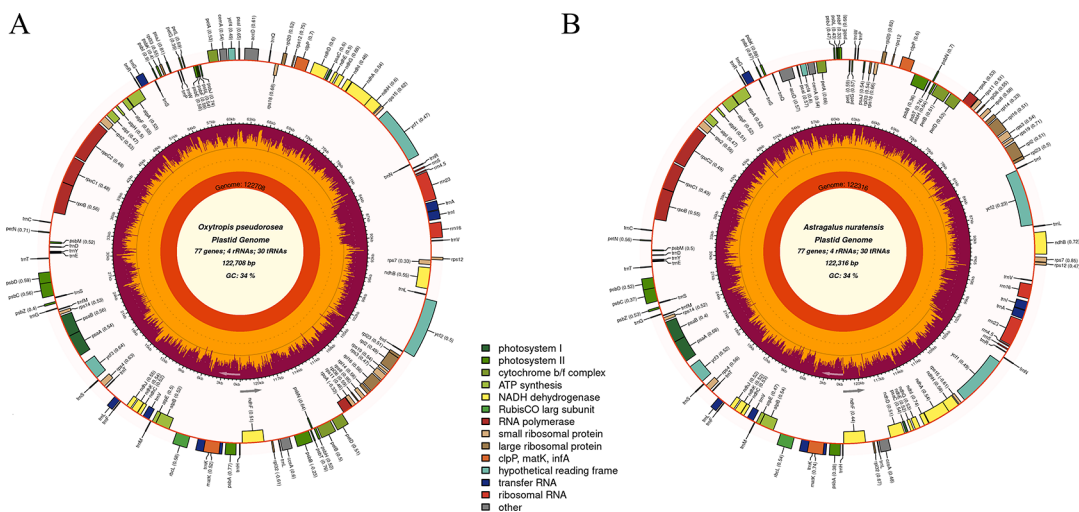


Figure 2. The circular maps of the chloroplast genomes of *Oxytropis pseudorosea* and *Astragalus nuratensis* were drawn using Chloroplot. The innermost circle represents the lengths of chloroplast genomes. The second concentric ring illustrates nucleotide composition, where the orange segment indicates GC content and the yellow segment corresponds to AT content. The outermost circle displays the distribution of genes, which are color-coded according to their functional classification. The transcriptional orientation is indicated such that genes on the inner circle are transcribed clockwise, whereas those on the outer circle are transcribed anticlockwise.

Maximum likelihood analysis based on complete chloroplast genome sequences resolved the sampled taxa into several well-supported clades corresponding to recognized infrageneric groups within *Astragalus* and related genera (Figure 3). Most backbone nodes received strong bootstrap support (BS ≥ 95), indicating a stable phylogenetic structure. The overall topology was consistent with previous phylogenomic studies of *Astragalus*, which also recovered major lineages with strong support using plastome and target-enrichment data (Su et al., 2021; Buono et al., 2025).

Species of *Astragalus* were distributed among the major lineages corresponding to the Hypoglottis, Neo-Astragalus, Diholcos, Astracantha, Contortuplicata, Hamosa, Trimeniaeus, and Phaca clades. Among these, the Phaca clade represented one of the largest lineages and included *A. nuratensis*, which grouped with other members of this clade with strong bootstrap support. In the phylogenetic tree, *A. zerbulaki*, another endemic species, was resolved within the Hypoglottis clade and formed a well-supported sister relationship with *A. rumpens*, indicating close evolutionary relationships among members of this lineage.

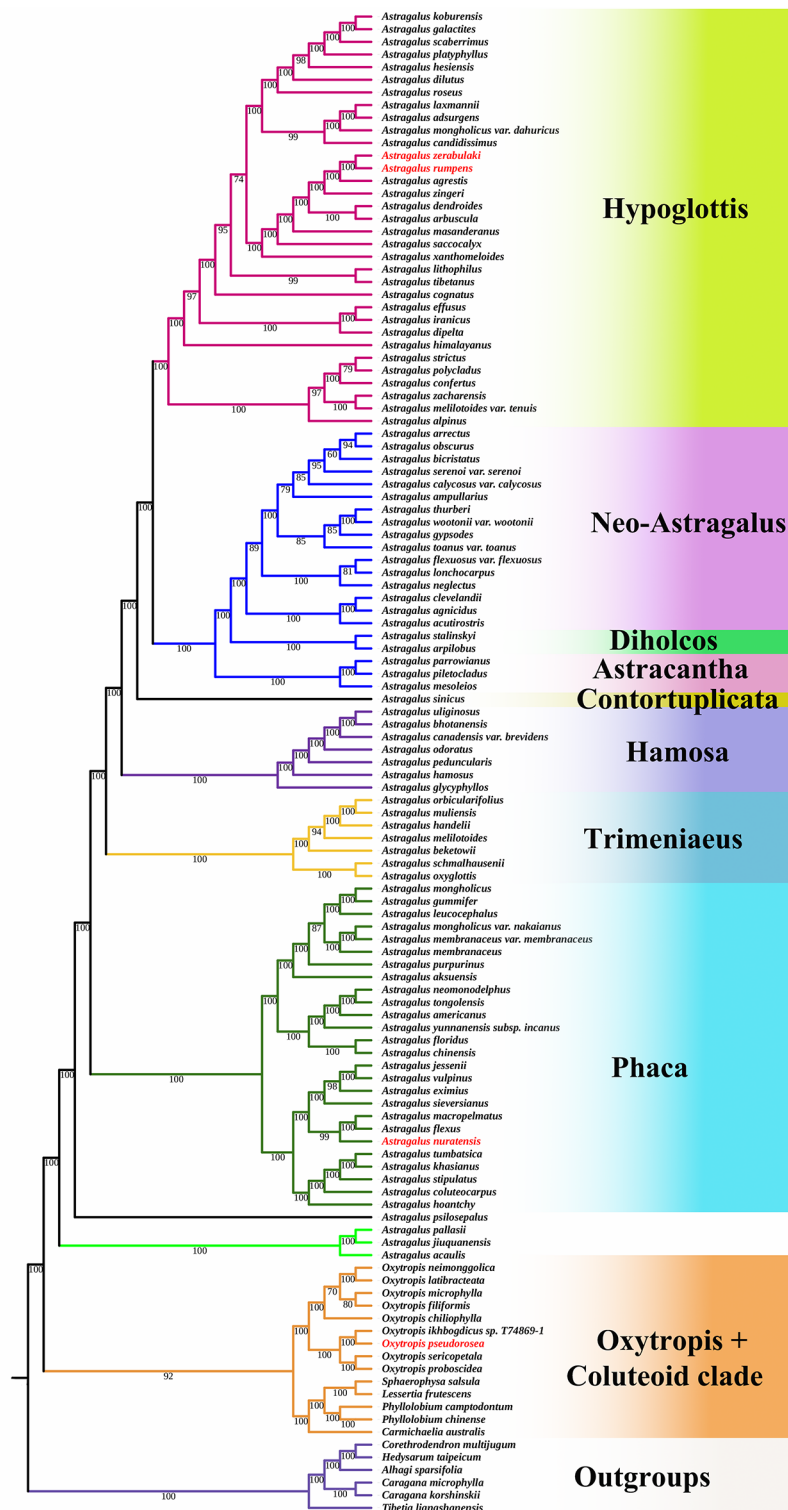


Figure 3. Maximum likelihood phylogenetic tree inferred from complete chloroplast genome sequences showing the relationships of *Astragalus nuratensis*, *Oxytropis pseudorosea*, and other members of the inverted repeat-lacking clade (IRLC).

Species of *Oxytropis*, including *O. pseudorosea*, formed a distinct and well-supported lineage corresponding to the *Oxytropis* + Coluteoid clade, clearly separated from the main *Astragalus* lineages, in agreement with previous plastome-based phylogenies (Buono et al., 2025). *O. pseudorosea* (sect. *Eumorpha*) as sister to *O. ikhbogdicus*, despite the latter's morphological affinity to section *Mesogaea*. This suggests that current morphological classifications in *Oxytropis* may reflect convergent evolution rather than true genetic relationships (Munkhtulga et al., 2026).

Data availability

NCBI BioProject: Raw sequencing data for *Astragalus nuratensis* and *Oxytropis pseudorosea*. Accession number PRJNA1425239. <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1425239> (Karimov, 2026a).

NCBI Sequence Read Archive (SRA): Raw sequencing data of *Astragalus nuratensis* and *Oxytropis pseudorosea*. Accession numbers SRR37271903 and SRR37271902; <https://www.ncbi.nlm.nih.gov/sra/?term=SRR37271903>; <https://www.ncbi.nlm.nih.gov/sra/?term=SRR37271902> (Karimov, 2026b).

NCBI GenBank: Chloroplast genomes of *Astragalus nuratensis* and *Oxytropis pseudorosea*. Accession numbers PX928918 and PX512474; <https://www.ncbi.nlm.nih.gov/nuccore/PX928918.1>; <https://www.ncbi.nlm.nih.gov/nuccore/PX512474.1> (Karimov, 2026c).

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Hoang Dang Khoa Do 

Nguyen Tat Thanh University, Ho Chi Minh City, Ho Chi Minh, Vietnam

The authors revised the manuscript based on the reviewer's comments.
The revised manuscript is now suitable for indexing.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Plant Chloroplast genomes

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 3

Reviewer Report 03 June 2026

<https://doi.org/10.5256/f1000research.202456.r489947>

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Hoang Dang Khoa Do 

¹ Nguyen Tat Thanh University, Ho Chi Minh City, Ho Chi Minh, Vietnam

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Dear Authors,
In the Introduction section, there are two sentences.

"This clade comprises about 52 genera and more than 4,000 species, and plastomes of IRLC taxa frequently show gene loss or pseudogenization (e.g., *rps16*, *rpl22*, *infA*, *accD*, and *ycf4*), intron loss (*clpP*, *atpF*, and *rpoC1*), inversions, and occasional transfer of genes to the nuclear genome (Son & Choi, 2022)."

"Within the IRLC, the genera *Astragalus* L. (3,095 species) and *Oxytropis* DC. (608 species) represent highly diverse and taxonomically complex groups (POWO, 2026)."

I am not sure the reason why the gene names and genus names were not italic in the above two sentences although the authors responded that they fixed it.

Please check and revise.

Thank you.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Plant Chloroplast genomes

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 04 Jun 2026

Bobur Karimov

Thank you for your comment.

This issue was corrected in the revised manuscript submitted by the authors. The genus names and the gene names cited in these sentences were formatted in italics. The current proof appears to have lost this formatting during copy editing or typesetting.

We kindly ask the production team to restore the appropriate italic formatting

Thank you.

Competing Interests: No competing interests were disclosed.

Author Response 12 Jun 2026

Bobur Karimov

The formatting has now been carefully corrected throughout the manuscript, including the two sentences highlighted.

Competing Interests: No competing interests were disclosed.

Version 2

Reviewer Report 22 May 2026

<https://doi.org/10.5256/f1000research.200633.r484854>

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Hoang Dang Khoa Do

¹ Nguyen Tat Thanh University, Ho Chi Minh City, Ho Chi Minh, Vietnam

² Nguyen Tat Thanh University, Ho Chi Minh City, Ho Chi Minh, Vietnam

³ Nguyen Tat Thanh University, Ho Chi Minh City, Ho Chi Minh, Vietnam

Dear Authors,

Thank you very much for revising the manuscript based on the reviewer's comments.

Most of my concerns were resolved.

However, there is still a minor issue about the italic font for gene names (*rps16*, *rpl22*, *infA*, *accD*, and *ycf4*; *clpP*, *atpF*, and *rpoC1*) and genus name (*Oxytropis*) in the Introduction.

Thank you.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Plant Chloroplast genomes

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 25 May 2026

Bobur Karimov

Dear Reviewer,

Thank you very much for your careful review and for your positive comments on our revised manuscript.

We appreciate your additional observation regarding the formatting issue. We have carefully checked the manuscript and corrected the italic formatting for all gene names (*rps16*, *rpl22*, *infA*, *accD*, *ycf4*, *clpP*, *atpF*, and *rpoC1*) as well as the genus name *Oxytropis* in the Introduction.

These corrections have been incorporated into the revised manuscript.

Thank you again for your valuable comments and suggestions.

Sincerely,
The Authors

Competing Interests: No competing interests were disclosed.

Reviewer Report 20 May 2026

<https://doi.org/10.5256/f1000research.200633.r484855>

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Yoshinori Fukasawa 

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Dear Authors,

Thank you for addressing the comments. The revised manuscript has been substantially improved. I am satisfied that the concerns raised in my initial review have been adequately addressed and am happy to approve this article.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Plant genomics and bioinformatics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 27 April 2026

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Yoshinori Fukasawa 

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Dear Authors,

Thank you for reporting the complete chloroplast genomes of *Astragalus nuratensis* and *Oxytropis pseudorosea*. The addition of new plastome resources for *Astragalus* and *Oxytropis*—two of the largest and most taxonomically challenging genera in the inverted repeat-lacking clade (IRLC)—is

a welcome contribution, particularly given the ongoing interest in plastome evolution within this clade. Below I offer several suggestions that I believe would strengthen the manuscript.

1. Citation for the conserved quadripartite structure of chloroplast genomes

In the Introduction, the conserved quadripartite structure of angiosperm chloroplast genomes is cited as (Karimov et al. 2025). However, this is a well-established and broadly recognized feature of plastome architecture that has been described in numerous foundational works. The cited reference reports chloroplast genomes of Cousinia (Asteraceae) species and, while a valuable study in its own right, does not serve as a primary or representative source for this general statement. I would suggest replacing it with one or more widely cited references that originally established this structural model.

2. Chloroplast genome map of *Oxytropis pseudorosea*

Figure 1 presents the chloroplast genome map of *A. nuratensis* only. Since the manuscript reports two newly sequenced plastomes, I would recommend including a corresponding genome map of *O. pseudorosea* as well, either as an additional panel in Figure 1 or as a separate figure. This would provide a more balanced presentation of the two genomes.

3. Discrepancy in gene counts between Figure 1 and the text

The main text states that both genomes encode 76 protein-coding genes, 30 tRNA genes, and 4 rRNA genes (110 unique genes total). However, the annotation within Figure 1 indicates "77 genes; 4 rRNAs; 30 tRNAs" for *A. nuratensis*. Please verify and reconcile these numbers to ensure consistency between the figure and the text.

4. Assembly validation metrics

The authors report mapping clean reads back to the assembled plastomes using BWA-MEM, which is an appropriate approach. However, the current manuscript reports only the mean sequencing depth. To further support the completeness and accuracy of the assemblies, I would suggest briefly reporting additional validation metrics, such as: (i) whether any gaps or regions of zero coverage were observed, (ii) the minimum and maximum coverage across the genome, and/or (iii) a coverage depth plot provided as supplementary material. These additions would allow readers to better assess the quality of the assembled sequences.

5. Reporting gene loss, pseudogenization, and intron loss in the IRLC context

The Introduction appropriately highlights that IRLC plastomes frequently exhibit gene loss or pseudogenization (e.g., *rps16*, *rpl22*, *infA*, *accD*, *ycf4*) and intron loss (e.g., in *clpP*, *atpF*, *rpoC1*). However, the Results section does not describe which of these events, if any, were observed in the two newly sequenced plastomes. Even a brief statement confirming or contrasting the gene/intron content of *A. nuratensis* and *O. pseudorosea* with that of other IRLC members would add informative context to the genome descriptions and enhance the value of this report as a genomic resource.

Summary

This manuscript provides new plastome data for two IRLC species from Central Asia and meets the essential criteria for a Genome Note. The assembly and analytical approaches are technically appropriate.

Are the rationale for sequencing the genome and the species significance clearly described?

Yes

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?

Partly

Are the datasets clearly presented in a usable and accessible format, and the assembly and annotation available in an appropriate subject-specific repository?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Plant genomics and bioinformatics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 29 Apr 2026

Bobur Karimov

Reviewer 2

We sincerely thank the reviewer for the careful evaluation of our manuscript and for the constructive comments, which have helped improve the clarity and quality of the study. Below we provide point-by-point responses.

1. Citation for the conserved quadripartite structure of chloroplast genomes

In the Introduction, the conserved quadripartite structure of angiosperm chloroplast genomes is cited as (Karimov et al. 2025). However, this is a well-established and broadly recognized feature of plastome architecture that has been described in numerous foundational works. The cited reference reports chloroplast genomes of Cousinia (Asteraceae) species and, while a valuable study in its own right, does not serve as a primary or representative source for this general statement. I would suggest replacing it with one or more widely cited references that originally established this structural model.

Response: A more appropriate and widely accepted reference has been added to support the conserved quadripartite structure of angiosperm chloroplast genomes.

2. Chloroplast genome map of Oxytropis pseudorosea

Figure 1 presents the chloroplast genome map of *A. nuratensis* only. Since the manuscript reports two newly sequenced plastomes, I would recommend including a corresponding genome map of *O. pseudorosea* as well, either as an additional panel in Figure 1 or as a separate figure. This would provide a more balanced presentation of the two genomes.

Response: The chloroplast genome map of *Oxytropis pseudorosea* has been added accordingly to provide balanced visualization of both plastomes.

3. Discrepancy in gene counts between Figure 1 and the text

The main text states that both genomes encode 76 protein-coding genes, 30 tRNA genes, and 4 rRNA genes (110 unique genes total). However, the annotation within Figure 1 indicates "77 genes; 4 rRNAs; 30 tRNAs" for *A. nuratensis*. Please verify and reconcile these numbers to ensure consistency between the figure and the text.

Response: The gene annotation has been carefully checked, and inconsistencies between the figure and the text have been corrected to ensure full consistency.

4. Assembly validation metrics

The authors report mapping clean reads back to the assembled plastomes using BWA-MEM, which is an appropriate approach. However, the current manuscript reports only the mean sequencing depth. To further support the completeness and accuracy of the assemblies, I would suggest briefly reporting additional validation metrics, such as: (i) whether any gaps or regions of zero coverage were observed, (ii) the minimum and maximum coverage across the genome, and/or (iii) a coverage depth plot provided as supplementary material. These additions would allow readers to better assess the quality of the assembled sequences.

Response: Additional assembly validation metrics have been included, including minimum and maximum coverage values, assessment of zero-coverage regions, and a coverage depth plot provided as Figure 1.

5. Reporting gene loss, pseudogenization, and intron loss in the IRLC context

The Introduction appropriately highlights that IRLC plastomes frequently exhibit gene loss or pseudogenization (e.g., *rps16*, *rpl22*, *infA*, *accD*, *ycf4*) and intron loss (e.g., in *clpP*, *atpF*, *rpoC1*). However, the Results section does not describe which of these events, if any, were observed in the two newly sequenced plastomes. Even a brief statement confirming or contrasting the gene/intron content of *A. nuratensis* and *O. pseudorosea* with that of other IRLC members would add informative context to the genome descriptions and enhance the value of this report as a genomic resource.

Response: A brief description of gene content and intron structure has been added to the Results comparison with typical IRLC characteristics.

Competing Interests: No competing interests were disclosed.

Reviewer Report 03 April 2026

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Dear Authors,

Thank you very much for adding new genomic data about *Astragalus* and *Oxytropis* species. The content of the manuscript is suitable for Genome Note of F1000 Research.

Please check my comment below.

1/ Please update the role of authors because most authors are for writing the manuscript. Who did the sampling, experiment, and analyzing the data?

2/ "Chloroplast (cp) genomes of angiosperms have been widely used in studies of phylogeny, sequence variation, genome evolution, and the development of molecular markers." Please add references for this statement.

3/ "In most angiosperms, the cp genome exhibits a conserved quadripartite structure consisting of a large single-copy (LSC) region, a small single-copy (SSC) region, and two inverted repeat (IR) regions (Karimov et al., 2025)." the current reference is not suitable for the statement because Karimov et al 2025 only reported complete chloroplast genomes of six Cousinia (Asteraceae) species which are not representative for most angiosperms.

4/ Please use italic font for gene names in the Introduction part.

5/ It would be better if the authors add introduction about the published chloroplast genomes of *Astragalus* and *Oxytropis*, especially *Astragalus zerabulaki* which was mentioned at the end of the manuscript.

6/ "Voucher specimens were deposited in the National Herbarium of Uzbekistan (TASH) under the accession numbers TASH-2024-AN-001 and TASH-2024-OP-002."; "Genome skimming generated high-quality paired-end reads, enabling the recovery of complete plastomes with mean sequencing depths of 638× and 1,725×, respectively." please revise these sentences to clarify which feature is of *Astragalus nuratensis* and which information belongs to *Oxytropis pseudorosea*.

7/ "High-quality reads were assembled de novo using NOVOPlasty v4.3.5" how to get high-quality reads? Please add.

8/ "For several taxa included in the phylogenetic analysis, complete chloroplast genome sequences were not available in GenBank. Only raw sequencing data (SRA accessions; SRR numbers listed in Table 1) were available. Therefore, the chloroplast genomes of these taxa were assembled." How to complete chloroplast genomes from SRA data? Please add.

9/ Because the manuscript reports two chloroplast genome, it would be better if the authors add the chloroplast genome map of *O. pseudorosea* in Figure 1.

10/ "Table 1. List of taxa included in the phylogenetic analysis, accession numbers." Please revise Table 1 caption.

11/ Please correct accession number of *O. pseudorosea* which is not PV684035 from GenBank database in Table 1.

Thank you.

Are the rationale for sequencing the genome and the species significance clearly described?

Yes

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?

Partly

Are the datasets clearly presented in a usable and accessible format, and the assembly and annotation available in an appropriate subject-specific repository?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Plant Chloroplast genomes

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 29 Apr 2026

Bobur Karimov

Reviewer 1

We sincerely thank the reviewer for the careful evaluation of our manuscript and for the valuable comments, which helped improve the quality of the work. Below we provide point-by-point responses.

Comment 1/ Please update the role of authors because most authors are for writing the manuscript. Who did the sampling, experiment, and analyzing the data?

Response: The system did not provide an option to edit and resubmit the updated version with revised author contributions.

Comment 2/ "Chloroplast (cp) genomes of angiosperms have been widely used in studies of phylogeny, sequence variation, genome evolution, and the development of molecular markers." Please add references for this statement.

Response: Appropriate references have been added to support this statement in the revised manuscript.

Comment 3/ "In most angiosperms, the cp genome exhibits a conserved quadripartite structure consisting of a large single-copy (LSC) region, a small single-copy (SSC) region, and two inverted repeat (IR) regions (Karimov et al., 2025)." the current reference is not suitable for the statement because Karimov et al 2025 only reported complete chloroplast genomes

of six Cousinia (Asteraceae) species which are not representative for most angiosperms.

Response: A more appropriate and widely accepted reference has been added to support this statement.

Comment 4/ Please use italic font for gene names in the Introduction part.

Response: Gene names in the Introduction have been corrected and formatted in italics.

Comment 5/ It would be better if the authors add introduction about the published chloroplast genomes of *Astragalus* and *Oxytropis*, especially *Astragalus zerabulaki* which was mentioned at the end of the manuscript.

Response: A new introductory paragraph has been added covering published chloroplast genomes of *Astragalus* and *Oxytropis*

Comment 6/ "Voucher specimens were deposited in the National Herbarium of Uzbekistan (TASH) under the accession numbers TASH-2024-AN-001 and TASH-2024-OP-002."; "Genome skimming generated high-quality paired-end reads, enabling the recovery of complete plastomes with mean sequencing depths of 638× and 1,725×, respectively." please revise these sentences to clarify which feature is of *Astragalus nuratensis* and which information belongs to *Oxytropis pseudorosea*.

Response: The sentences have been revised to clearly distinguish species-specific voucher information and sequencing depth values.

Comment 7/ "High-quality reads were assembled de novo using NOVOPlasty v4.3.5" how to get high-quality reads? Please add.

Response: Raw sequencing reads were directly used, and NOVOPlasty performs internal quality filtering during the assembly process.

Comment 8/ "For several taxa included in the phylogenetic analysis, complete chloroplast genome sequences were not available in GenBank. Only raw sequencing data (SRA accessions; SRR numbers listed in Table 1) were available. Therefore, the chloroplast genomes of these taxa were assembled." How to complete chloroplast genomes from SRA data? Please add.from SRA to novoplasty

Response: Raw sequencing data were downloaded from SRA and assembled into chloroplast genomes using NOVOPlasty following the same assembly workflow.

Comment 9/ Because the manuscript reports two chloroplast genome, it would be better if the authors add the chloroplast genome map of *O. pseudorosea* in Figure 1.

Response: The chloroplast genome map of *Oxytropis pseudorosea* has been added to Figure 1.

Comment 10/ "Table 1. List of taxa included in the phylogenetic analysis, accession numbers." Please revise Table 1 caption.

Response: The caption of Table 1 has been revised for clarity and consistency.

Comment 11/ Please correct accession number of *O. pseudorosea* which is not PV684035 from GenBank database in Table 1.

Response: The accession number has been corrected according to the GenBank database.

Competing Interests: No competing interests were disclosed.

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