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PLASTOME ANNOUNCEMENT

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Characterization of the complete chloroplast genome of *Elymus alashanicus* (Keng) S. L. Chen, and its phylogenetic analysis

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ABSTRACT

Elymus alashanicus (Keng) S. L. Chen, a herbaceous plant endemic to China, plays a crucial role in the local ecosystems. In this study, we sequenced and characterized the complete chloroplast (cp) genome of *E. alashanicus*, which is 135,072 bp in length and arranged in a circular form. The cp genome includes a pair of inverted repeats (IRa and IRb) of 20,813 bp each, separated by a large single-copy (LSC) region of 80,678 bp and a small single-copy (SSC) region of 12,768 bp. The cp genome contains 130 genes, including 83 protein-coding genes, 39 tRNA genes, and eight rRNA genes. Phylogenetic analysis revealed that *E. alashanicus* is closely related to *Elymus breviaristatus* and *Campeiostachys dahurica var. tangutorum* in current sampling. Our findings provide valuable insights into the cp genome of *E. alashanicus*, which could contribute to further studies on the evolution and conservation of this species.

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Introduction

Elymus alashanicus (Keng) S. L. Chen, a perennial herb of the genus *Roegneria* (Triticeae), was first described in 1963 and is widely distributed in northwest China (Zhang et al. 2009; Lei et al. 2016). This species is known for its excellent genes of disease and stress resistance and high feed value, making it an important natural gene bank for forage breeding and improvement of wheat-related plants (Cai 2002). However,

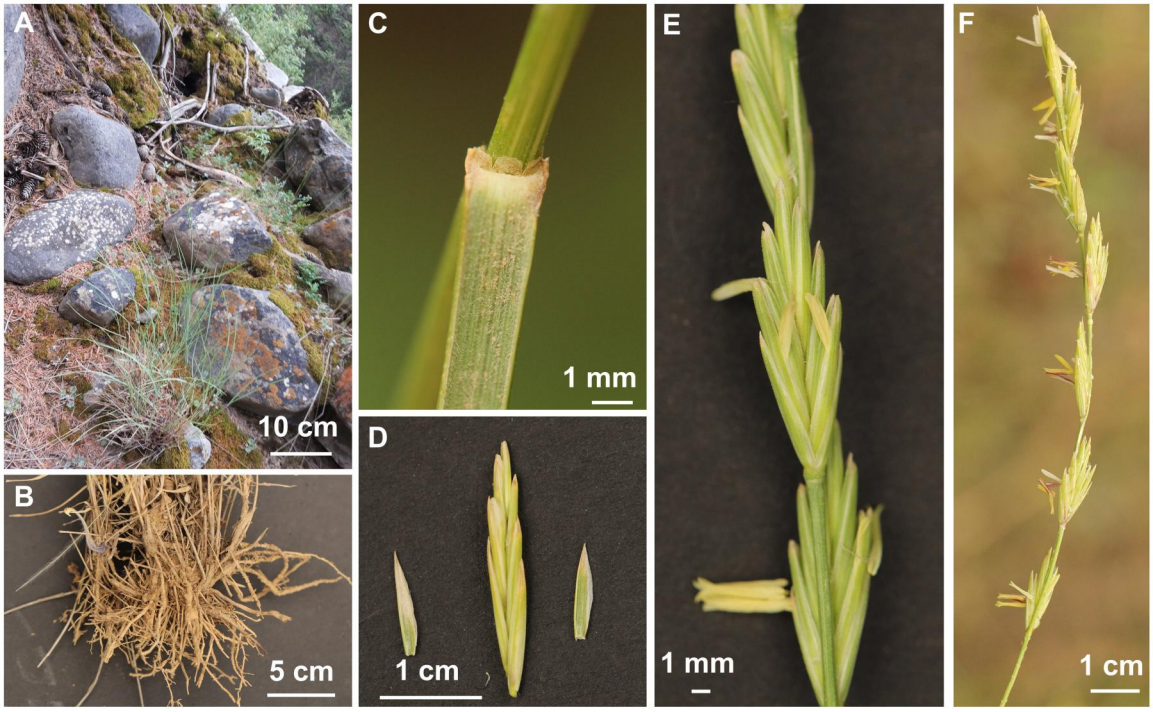

Figure 1. The species reference image for *E. alashanicus*. Morphological characteristics of whole plant (A), root (B), ligule (C), glumes (D), spikelet (E), and inflorescence (F) of *E. alashanicus* (photos taken by author Feng Jin in Helan Mountain, Inner Mongolia, China).

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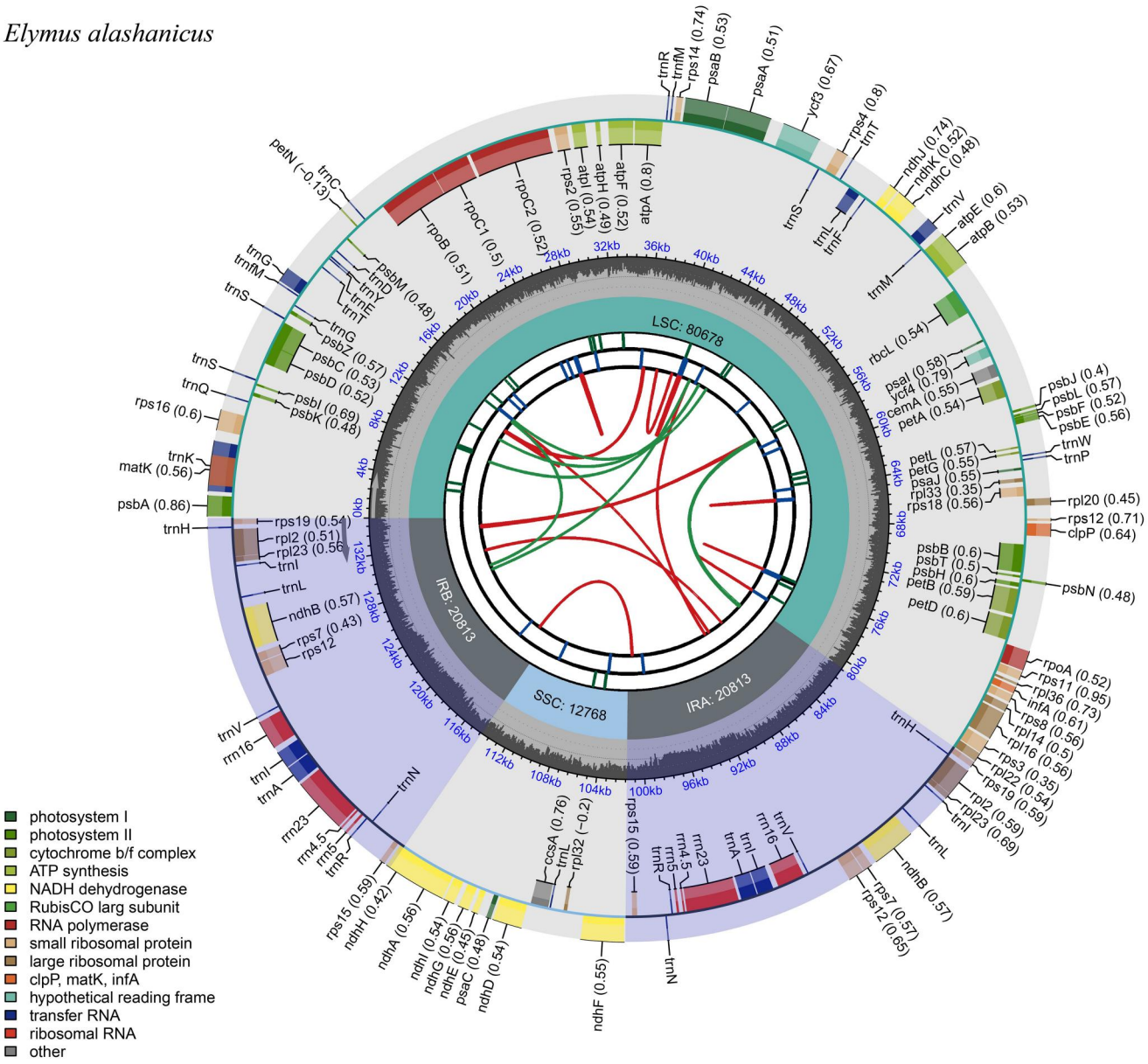

Figure 2. Schematic circular map of overall features of *E. alashanicus* chloroplast genome. Graphic showing features of its plastome was generated using CPGview. The map contains six tracks. From the inner circle, the first track depicts the dispersed repeats connected by red (forward direction) and green (reverse direction) arcs, respectively. The second track shows the long tandem repeats as short blue bars. The third track displays the short tandem repeats or microsatellite sequences as short bars with different colors. The fourth track depicts the sizes of the inverted repeats (IRa and IRb), small single-copy (SSC), and large single-copy (LSC). The fifth track plots the distribution of GC contents along the plastome. The sixth track displays the genes belonging to different functional groups with different colored boxes. The outer and inner genes are transcribed in the clockwise and counterclockwise directions, respectively.

there is limited information on the chloroplast (cp) genomes of *E. alashanicus*. An in-depth analysis of the cp genomics of this genus will provide a foundation for phylogenetic studies and aid taxonomical analyses.

Materials and methods

Sample collection and preservation

Fresh leaves of *E. alashanicus* were collected from Helan Mountain (N38°22′52″, E105°42′53″) in Inner Mongolia, China (Figure 1). A specimen was deposited at herbarium of Inner Mongolia Normal University (NMTC, http://bio.imnu.edu.cn/info/1097/1401.htm, contact person and email: FengJin, jinfeng@imnu.edu.cn) under the voucher number HLS20190828-67.

DNA extraction and sequencing

Total genomic DNA was extracted using the method of Doyle and Doyle (Doyle and Doyle 1987). Short-insert library (insert size, 300 bp) was prepared and then sequenced using the Illumina HiSeq platform in NextOmics (Wuhan, China).

Assembly, annotation, and visualization

The complete genome was de novo assembled using NOVOPlasty (Dierckxsens et al. 2017) and annotated with the online annotation tool GeSeq (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html). The complete plastome of *E. alashanicus* has been deposited in GenBank under accession number OL444890. A circular map of its plastome was visualized

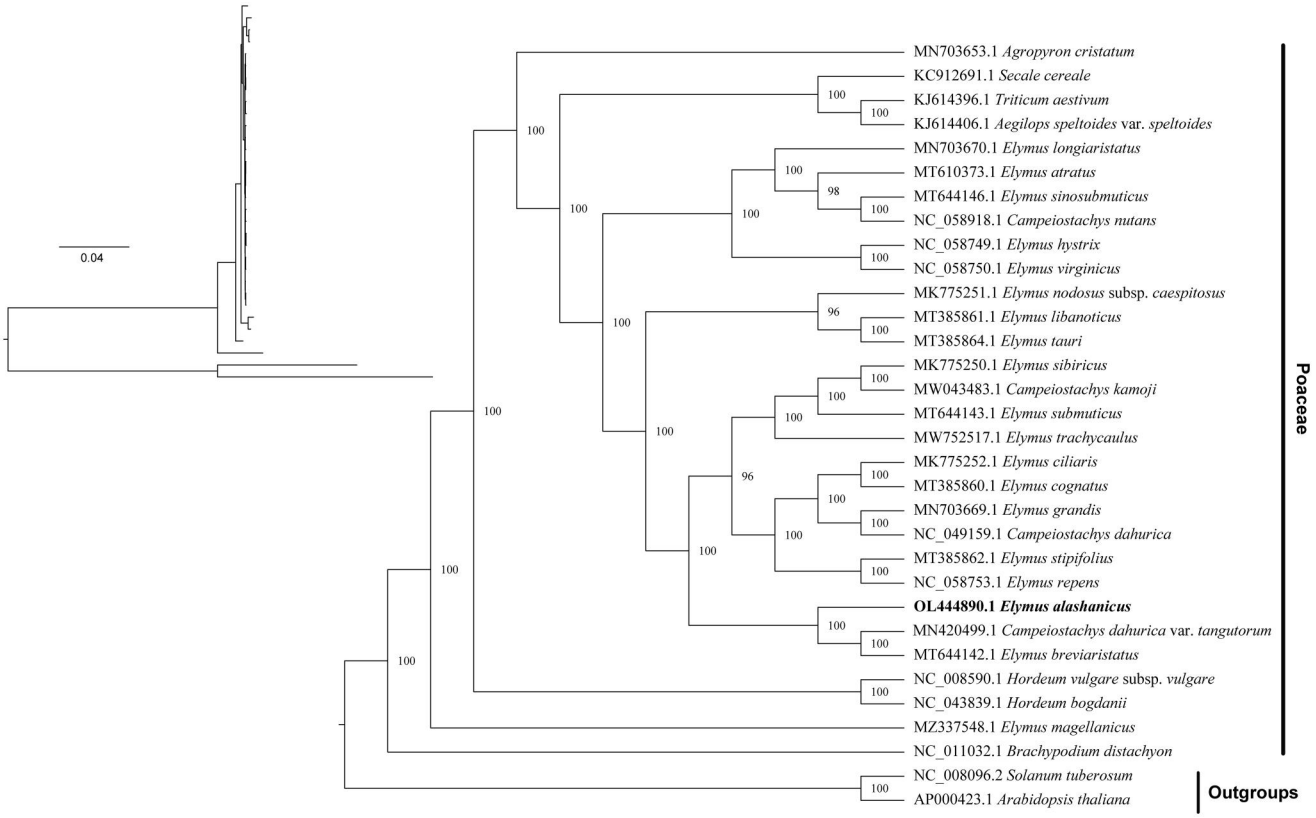

Figure 3. Maximum-likelihood and Bayesian inference phylogenetic trees of 30 *Poaceae* species with *Arabidopsis thaliana* and *Solanum tuberosum* as outgroups. The main phylogenetic tree was presented based on the BI tree, because of highly similarity among the sequences, showed lower branch support of the ML evolutionary tree. Species names are displayed with indicate lines in the right side, and the numbers above branches indicate supportive values of BI phylogenetic trees. GenBank accession numbers of the following sequences were used to construct the phylogenetic tree: *Agropyron cristatum* MN703653.1 (Chen et al. 2018), *Secale cereale* KC912691.1 (Li et al. 2015), *Triticum aestivum* KJ614396.1 (Han et al. 2021), *Aegilops speltoides var. speltoides* KJ614406.1 (Chen et al. 2018), *Elymus longiaristatus* MN703670.1 (Hu et al. 2015), *Elymus atratus* MT610373.1 (Gao et al. 2014), *Elymus sinosubmuticus* MT644146.1 (Tan et al. 2022), *Campeiostachys nutans* NC_058918.1 (Dong et al. 2015), *Elymus hystrix* NC_058749.1 (Dong et al. 2015), *Elymus virginicus* NC_058750.1 (Dong et al. 2015), *Elymus nodosus subsp. caespitosus* MK775251.1 (Chen et al. 2020), *Elymus libanoticus* MT385861.1 (Xia and Liu 2020), *Elymus tauri* MT385864.1 (Liu et al. 2021), *Elymus sibiricus* MK775250.1 (Chen et al. 2022), *Campeiostachys kamoji* MW043483.1 (Liu et al. 2021), *Elymus submuticus* MT644143.1 (Y. NI 2011), *Elymus trachycaulus* MW752517.1 (Wu et al. 2016), *Elymus ciliaris* MK775252.1 (Hu et al. 2013), *Elymus cognatus* MT385860.1 (Hu et al. 2015), *Elymus grandis* MN703669.1 (Yu et al. 2010), *Campeiostachys dahurica* NC_049159.1 (Tan et al. 2021), *Elymus stipifolius* MT385862.1 (Yang et al. 2017), *Elymus repens* NC_058753.1 (Mason-Gamer 2008), *Campeiostachys dahurica var. tangutorum* MN420499.1 (Yang et al. 2015; Jing et al. 2019), *Elymus breviaristatus* MT644142.1 (Tan et al. 2022), *Hordeum vulgare subsp. vulgare* NC_008590.1 (Zhelyazkova et al. 2012), *Hordeum bogdanii* NC_043839.1 (Cui et al. 2021), *Elymus magellanicus* MZ337548.1 (Wu et al. 2022), *Brachypodium distachyon* NC_011032.1 (Sancho et al. 2018), *Solanum tuberosum* NC_008096.2 (Occhialini et al. 2020), and *Arabidopsis thaliana* AP000423.1 (Provan 2000).

using the CPGView online web (http://www.1kmpg.cn/cpgview) (Liu et al. 2023).

Phylogenetic reconstruction

To determine the phylogenetic position of *E. alashanicus*, the complete cp genome sequences of 30 species of *Poaceae* plants, plus *Arabidopsis thaliana* (AP000423.1) and *Solanum tuberosum* (NC_008096.2) as outgroups were obtained from NCBI (https://www.ncbi.nlm.nih.gov/).

Bootstrapped maximum-likelihood (ML) with 1000 replicates was constructed based on GTR + I+GAMMA model in jModelTest v. 2.1.10. Additionally, Bayesian inference (BI) trees were constructed using the Markov chain Monte Carlo (MCMC) method in MrBayes (v3.2.7) (Darriba et al. 2012). Each sequence was aligned using the 'auto' mode in MAFFT (v7.427), then the algorithm was run for 1,000,000 iterations, and samples were taken every 100 iterations (Katoh and Standley 2013). The first 25% of the resulting trees were removed as 'burn-in', and the majority-rule consensus tree was used as the final result.

Results

The cp genome of *E. alashanicus* is 135,072 bp in length, with an average sequencing depth of 466X (Supplementary Figure S1). It exhibited a typical four-stage structure, consisting of a large single-copy (LSC) region of 80,678 bp, a small single-copy (SSC) region of 12,768 bp, and two inverted repeat regions (IRa/IRb), both measuring 20,813 bp (Figure 2). The content of CG in whole cp genome was 38.33%, and a total of 130 genes were encoded, including 83 protein-coding genes, 39 tRNA genes, and eight rRNA genes. Among them, 10 protein-coding genes (*rps16*, *atpF*, *petB*, *petD*, *rpl16*, *rpl2*, *ndhB*, *ndhA*, *ndhB*, and *rpl2*) and eight tRNA genes (*trnK-UUU*, *trnG-UCC*, *trnL-UAA*, *trnV-UAC*, *trnI-GAU*, *trnA-UGC*, *trnA-UGC*, and *trnI-GAU*) containing one intron and three genes (*ycf3*, *rps12*, and *rps12*) had two introns. Furthermore, a trans-spliced gene rps12 and three small-exon genes (*petB*, *petD*, and *rpl16*) were verified and annotated using multiple sequence alignment (Supplementary Figures S2 and S3).

Phylogenetic analysis based on the whole cp genome sequences of 31 reported species and the new data from this

study showed that *E. alashanicus* was clustered with *Elymus breviaristatus* and *Campeiostachys dahurica var. tangutorum* with full supportive values of the BI trees (Figure 3).

Discussion and conclusions

E. alashanicus is a species with excellent production performance and high economic value due to its strong tillering ability and resistance to poverty and drought. This study presented the first complete cp genome of *E. alashanicus* and annotated its structure. The phylogenetic analysis revealed that *E. alashanicus* was closely related to *Elymus breviaristatus* and *Campeiostachys dahurica var. tangutorum*. *E. alashanicus* is also known as *Roegneria alashanicus* (https://www.worldfloraonline.org/taxon/wfo-0000895903), and the classification of plants from these genera has always been inconsistent due to their complex morphology. Here, the complete cp genome sequence of *E. alashanicus* suggests that it is genetically more related to the genus *Elymus*. This study could provide valuable genomic information for further investigation of its taxonomy evolution and population genetics.

Ethical approval

No specific permissions were needed to perform this research as *E. alashanicus* is not a protected plant, and no damage could be caused to its population.

Author contributions

Feng Jin designed the project and performed the sample collection. Bao Sarina and Jinghuan Li analyzed and interpretated the sequencing data and drafted the paper. Feng Jin revised it and approved the final version to be published. Sarina and Feng Jin contributed equally to this work and all authors agree to be accountable for all aspects of the work.

Disclosure statement

No potential conflict of interest was reported by the authors. All authors revised and approved the manuscript.

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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov/ under the accession no. OL444890.1. The associated BioProject, SRA, and BioSample numbers are PRJNA778469, SRR16913047, and SAMN22960735, respectively.

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