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PLASTOME REPORT

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Characterization of the complete chloroplast genome sequence of *Agave durangensis* (Asparagales: Asparagaceae: Agavoideae)

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ABSTRACT

Agave durangensis commonly known as agave cenizo, is an endemic *Agave* species in Mexico used for mescal production, yet its taxonomic delimitation is still controversial. This study aimed to enhance taxonomic clarity by characterizing its chloroplast genome. Chloroplast DNA was isolated from 2-year-old *A. durangensis* leaves. The complete chloroplast genome size was 156,441 bp, comprising a large single-copy region (LSC), a pair of inverted repeat regions (IR), and a small single-copy region (SSC). Annotation revealed 87 protein-coding genes, 38 tRNAs, and 8 rRNAs, with notable gene inversions. Phylogenetic analysis suggests, *A. durangensis* forms a separate lineage within the *Agave* genus.

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KEYWORDS
Chloroplast genome; *Agave durangensis*; phylogenetic analysis

Introduction

Agaves are emblematic plants of Mexico, where they are endemic and particularly used in the production of distilled beverages commonly known as "mescals". Of the total species employed for beverage production in the country, 66% are extracted from natural populations (Torres et al. 2015). The considerable genetic and morphological variations within this population make it challenging to accurately identify a particular species, leading to significant variability in the mescal quality. This is the case of *Agave durangensis* Gentry (1982), a native plant of Durango, Mexico, used for mescal production. This species belongs to the section *Ditepalae* and its reproduction is mainly by seeds, conferring it an enormous genetic and morphological variability (Almaraz-Abarca et al. 2013). Because of this, the specific delimitation and taxonomic relationship of this species is difficult, considering as a complex instead a taxonomically defined species by several authors. Several studies have attempted to identify morphological, chemical, or genetic markers to delimit and identify this taxon (Gentry 1982; Delgado-Alvarado et al. 2019). An alternative for comparative studies at species, genus and family level is the use of chloroplast genome. Nearly 600 chloroplast genomes are deposited at the NCBI Organelle Genome Resources and only eight belong to *Agave* genus (Firetti et al. 2017). Therefore, the present study aimed to obtain the complete cp genome sequence of *A. durangensis* to improve its identification and taxonomic delimitation and enrich the *Agave* chloroplast genome information.

Methods

A specimen was deposited at the National Herbarium of Mexico (MEXU), National Autonomous University of Mexico (http://datosabiertos.unam.mx/IBUNAM:MEXU:1307193, Lat: 23. 70606° Log: −104.21086°, Gerardo Adolfo Salazar Chavez, gasc@ib.unam.mx) under the voucher number IBUNAM:MEXU:1307193 (Figure 1A). Seeds from the specimen deposited were cultivated and 2-year-old *A. durangensis* seedlings (Figure 1B) were used for chloroplast isolation using an updated sucrose density gradient method (Takamatsu et al. 2018). Isolated and whole chloroplasts were used for cpDNA extraction, using a CTAB-based protocol (Keb-Llanes et al. 2002); then, sent to the University Unit of Massive Sequencing and Bioinformatics at the Institute of Biotechnology, UNAM (Cuernavaca, Mexico) for sequencing by the Illumina MiSeq platform, obtaining 3.5 Gb raw reads.

Methodology

The raw reads were subjected to assembly using Novoplasty (Galaxy Version 4.3.1 + galaxy0), employing the *rbcL* gene and the chloroplast genome of *A. americana* (https://www.ncbi.nlm.nih.gov/nuccore/KX519714.1/) as the seed and

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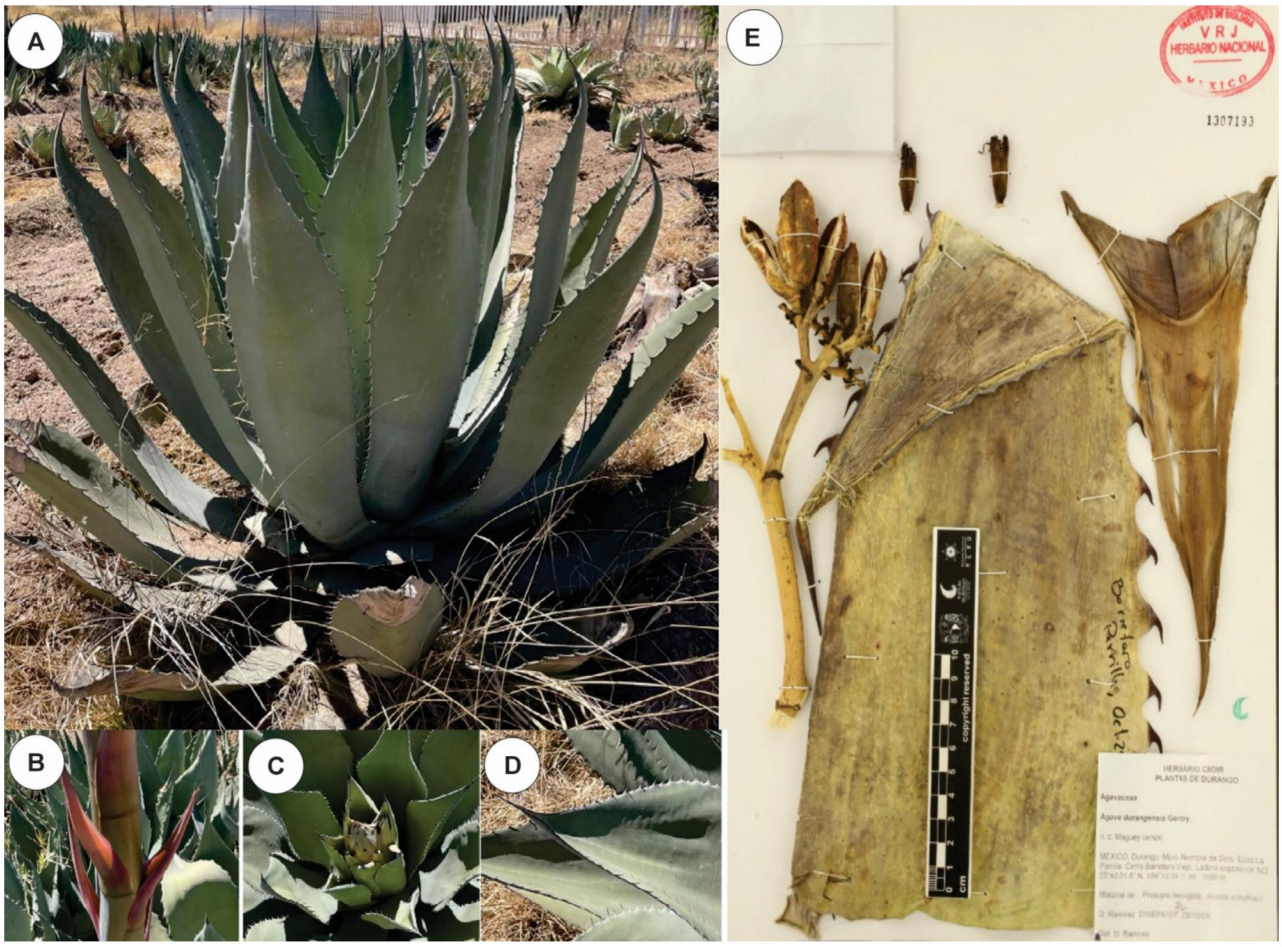

Figure 1. (A) Species reference image of *Agave durangensis* Gentry showing floral stalk (B), rosette (C) and leaves (D). These tissues represent the characteristic morphology of agave cenizo (*A. durangensis*) used mainly for mescal production. The images were obtained from a plant cultivated from seeds of an individual with voucher number IBUNAM:MEXU:1307193 (E). The picture was taken by the first author (ana gonzalez trillo) on july 2023, at an experimental plot in the CIIDIR facilities.

reference sequences, respectively. The assembly was manually reoriented to match them with those of the cp genomes of other agaves. Raw data were deposited to SRA under the accession number PRJNA932938 (Afgan et al. 2018). The genomic map and genome annotation were constructed with the GeSeq tool of Chlorobox Server and Geneious (Kearse et al. 2012; Tillich et al. 2017). Sequencing depth and coverage map of *A. durangensis* was generated following the protocol of Ni et al. (2023). For comparative analysis the cp genomes (whole sequence in fasta format) of *A. durangensis*, nine *Agave* species, and seven related species of the Asparagaceae family (outgroup) were aligned using Geneious Prime (2023.2 version) with a Mafft plugin (Nakamura et al. 2018). The maximum likelihood (ML) phylogenetic tree was constructed with GRT + gamma model and 1000 bootstrap replicates, using RAxML software (Galaxy Version 1.0.0) (The Galaxy Community, 2022).

Results

It was possible to assemble the complete cp genome of *A. durangensis* (Figure 2) (NCBI accession number: OP939491.2) with a size of 156,441 bp and 37.8% of GC content. In the annotation, 87 protein-coding genes, 38 tRNAs, and 8 rRNAs were found. The genomic structural regions correspond to a small single copy region (SSC, 18,271 bp), a large single copy region (LSC 85,344 bp), and a pair of inverted repeat regions (IR, 26,563 bp). The sequencing depth and coverage map of *A. durangensis* showed a minimal depth of 7 X, and an average depth of 234.66 X, indicating that a relatively enough reads for each site in the assembly (Supplementary figure 1). The map of the cp genome showed an inverted region involving the genes *trnE*, *trnY* and *trnD*. comparing with the other cp genomes of agaves. *A. durangensis* shares this peculiarity with *A. attenuata* (KX931447.1) and *A. virginica* (*Manfreda virginica)* (KX931461.1).

The phylogenetic tree reveals that *A. durangensis* forms an independent group, with a bootstrap value (BS) of 100%, separated from a node that consists of two branches (both with 100% BS): one formed by *A. attenuata* and another comprising the remaining *Agave* species. Consequently, a close relationship is observed among the latter group: *A. cantula* (100% BS), *A. fourcroydes* (100% BS), *A. sisalana* (88% BS), *A. americana* (46% BS), *A. amaniensis* (100% BS), *A. hybrid* cultivar cultivar H.11648 (100% BS) and *A. angustifolia* (100% BS). emphasizing a higher level of proximity than others. The

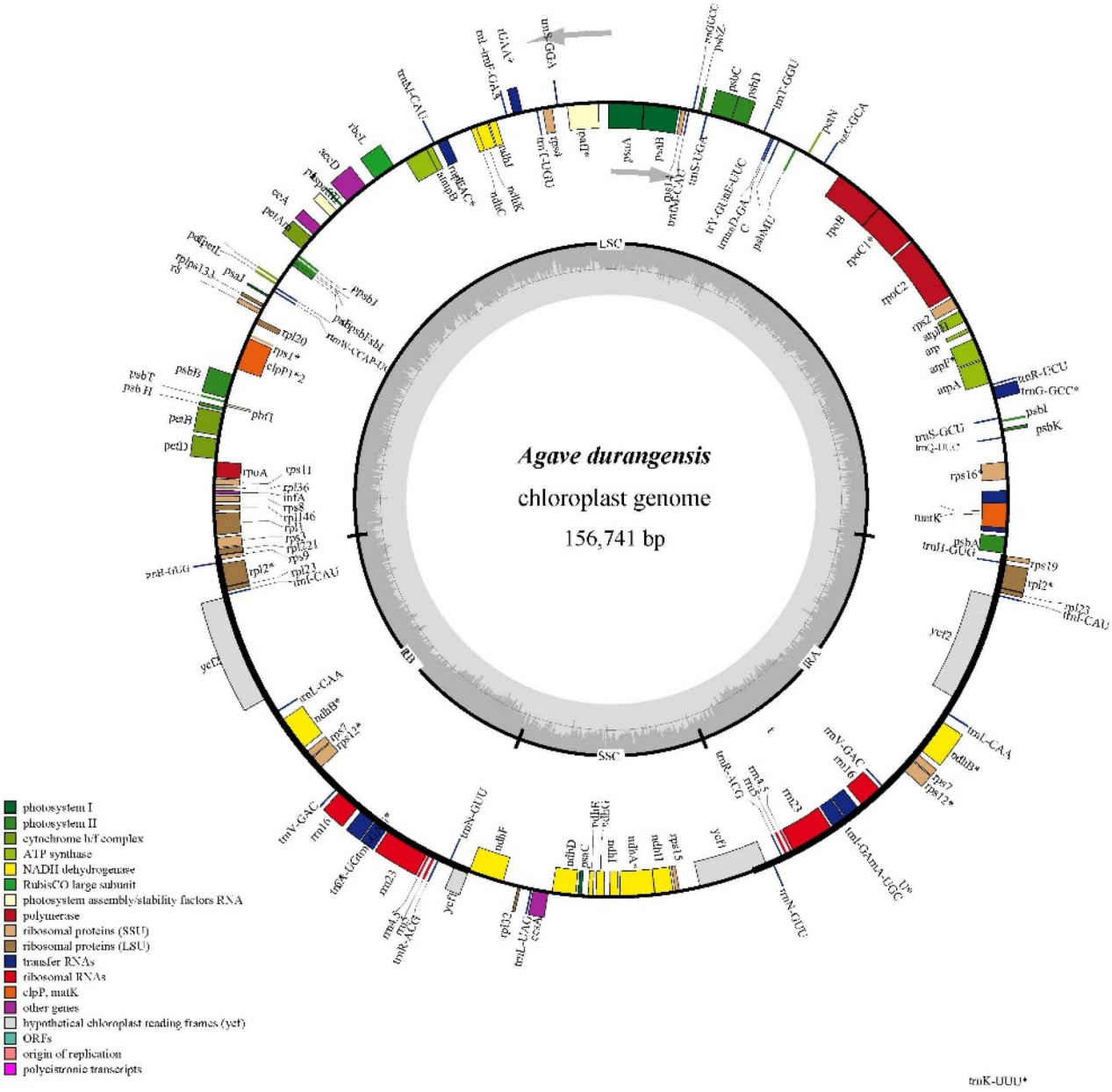

Figure 2. Chloroplast genome map of *Agave durangensis* Gentry (OP939491.2) Genes are indicated in both inside and outside the circle, transcribed in a clockwise and counterclockwise direction, respectively. Different color indicates the functional category of each gene. The black inner circle provides a representation of the chloroplast structure, which is divided into four regions: the small single-copy region (SSC), large single-copy region (LSC), and a pair of inverted repeats (IRa and IRb). The gray ring represents the GC content throughout the genome. Asterisks highlight genes that contain introns. The map was created using GeSeq tool of Chlorobox server.

tree's topology places *A. durangensis* near the node of the other agaves as a sister branch, closely related to *M. virginica* (99% BS) (Figure 3).

Discussion and conclusions

The size of the chloroplast genome of *A. durangensis* is similar to that of other *Agave* species, the map of the cp genome showed an inverted region involving the genes *trnE*, *trnY* and *trnD*. This was found when comparing the cp genomes of agaves, where only *A. attenuate* and *M. virginica* coincide with these inversions. This could be an important source of variation to be considered as a potential genetic marker. The presence of these kinds of arrays is related to evolutionary forces across the species (Silva et al. 2019). Thus, it explains the phylogenetic tree of the current work, in which *A. durangensis* forms a monophyletic group with other species of the genus *Agave*. *A. durangensis* is located at the base of the evolutionary tree of the genus, representing one of the most ancient species. The bootstrap values are sufficiently reliable, allowing for the clear identification of *A. durangensis* as a taxonomically different species, confirming that it is a complex taxon belonging to the *Ditepalae* section (Almaraz-Abarca et al. 2013). The topology of the tree is confirmed also, as it corresponds to the recent work with *Agave* species (Xu et al. 2022) where *A. attenuate* and *M. virginica* formed a separate branch from the other *Agave* species (76% BS). In the present work, the ML tree isolate *M. virginica* (100% BS) to *A. attenuate* by incorporating *A. durangensis* into the analysis, the

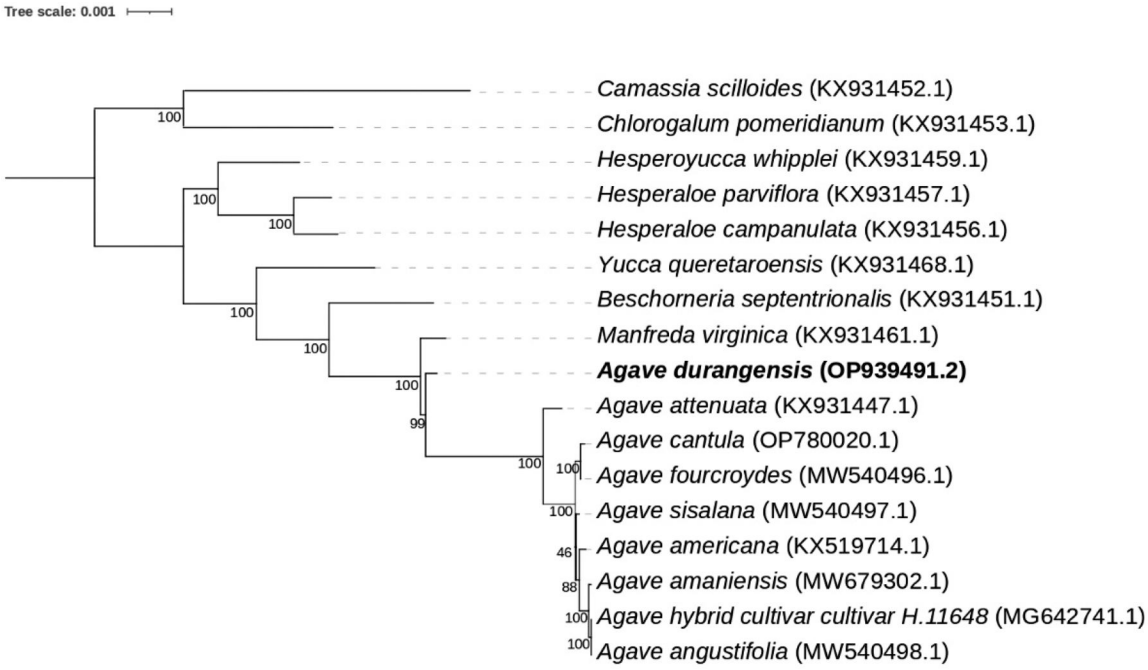

Figure 3. Maximum likelihood tree including the cp genome of *A. durangensis* (OP939491.2), nine *Agave* species and seven species as outgroup belonging to the Asparagaceae family: *Manfreda virginica* KX931461.1 (Yeeun et al. 2018), *Agave attenuata* KX931447.1 (Liu et al. 2022), *Agave cantula* OP780020.1, *Agave fourcroydes* (Qin et al. 2021a), *Agave sisalana* MW540497.1 (Yang et al. 2021), *Agave americana* KX519714.1 (Yang et al. 2021), *Agave amaniensis* MW679302.1 (Bochao et al. 2022), *Agave hybrid* cultivar cultivar H.11648 MG642741.1 (Jin et al. 2020), *Agave angustifolia* MW540498.1 (Qin et al. 2021b), *Camassia scilloides* KX931452.1 (McKain et al. 2016), *Chlorogalum pomedarium* KX931453.1 (McKain et al. 2016), *Hesperoyucca whipplei* KX931459.1 (Li et al. 2019), *Hesperaloe parviflora* KX931457.1 (Zhang et al. 2023), *Hesperaloe campanulata* KX931456.1 (Qin et al. 2021a), *Yucca queretaroensis* KX931468.1 (She et al. 2020) and *Beschorneria septentrionalis* KX931451.1 (Zhang et al. 2023).

support values are strong to resolve the separation of the nodes. This suggests that the use of the information contained in the cp genome of *A. durangensis* will be useful for conservation and evolutionary studies of this and other related species.

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Author contributions

Gonzalez-Trillo developed lab work and bioinformatics analyses, Torres Ricario and Reyes Lopez contributed to bioinformatics analyses and manuscript revision. Barraza Salas, Almaraz Abarca, Gutierrez Velazquez, and Herrera Arrieta contributed to plant identification, chloroplast isolation, and cpDNA isolation. Monreal-García contributed to the manuscript revision and the statistical analyses.

Disclosure statement

No potential conflict of interest has been reported by the author(s)

Statement permission

The current research was carried out using Agave seedlings, the studied species is not endangered or under any control regime; hence, no permission or ethical approval is required.

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Data availability statement

The chloroplast genome data that support the findings of this study are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov/nuccore/op939491 under the accession OP939491 of Bio project: PRJNA932938, SRA: SRS16717948 and Bio Sample: SAMN33215303.

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