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PLASTOME REPORT

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Characterization of the complete chloroplast genome sequence of *Artemisia sylvatica* Maximowicz 1859 (Asteraceae)

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ABSTRACT

Artemisia sylvatica Maximowicz 1859 is one of the medicinal herbs in *Artemisia*. This study presents the complete chloroplast genome of *A. sylvatica*, sequenced using the Illumina NovaSeq platform. The genome is 151,161 bp in length, featuring a GC content of 38%. It consists of a large single-copy (LSC) region of 82,892 bp, a small single-copy (SSC) region of 18,353 bp, and two inverted repeat (IR) regions of 24,958 bp each. In total, the genome contains 132 genes, including 87 protein-coding genes, 37 tRNA genes, and 8 rRNA genes. Phylogenetic analysis positions *A. sylvatica* within the subgenus *Artemisia*, highlighting its evolutionary relationships within this diverse genus. The first chloroplast genome of *A. sylvatica* was reported in this work contributes to the enrichment of genomic data for the genus *Artemisia*.

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Artemisia sylvatica; chloroplast genome; phylogenetic analyses

Artemisia is one of the largest genera in the Asteraceae family, comprising more than 500 species worldwide. Many of these species are well-known medicinal herbs, such as *Artemisia abrotanum, Artemisia absinthium, Artemisia annua, Artemisia dracunculus, and Artemisia vulgaris* (Ekiert et al. 2022; Nurlybekova et al. 2022). *Artemisia sylvatica* Maximowicz 1859 has been reported as an herbal medicine for the treatment of inflammation (Jin et al. 2004; Lee et al. 1998), cancer (Choi and Kim 2013), and the use of anticomplement (Moon et al. 2012). With the development of *Artemisia* species research, increasing numbers of complete chloroplast genomes of *Artemisia* species have been reported (Lee et al. 2016; Lim et al. 2018). However, the complete chloroplast genome of *Artemisia sylvatica* has not yet been sequenced.

In this study, we characterized the chloroplast genome sequence of *A. sylvatica* thoroughly to provide insights into the genome characterization and evolution of this important species. This research contributes to molecular studies within the *Artemisia* genus and the broader Asteraceae family.

Materials and methods

Plant material collection and DNA extraction

Plant samples of *A. sylvatica* were collected from Xinyang, China (longitude 114°04′ E, latitude 32°07′ N) and authenticated by Dr. ZeLong Yu, an expert in plant identification at Xinyang Agricultural and Forestry University (XYAFU). Voucher specimens were deposited at the Dabie Mountain Biodiversity Herbarium (contact person: Wei Zhou, 634858289@qq.com) under accession number ZW20230810025 (Figure 1). Fresh leaves were packaged in thin foil and then frozen by liquid nitrogen for high throughput sequencing. Genomic DNA was extracted using a DNA easy Plant Mini Kit (Qiagen Co., Hilden, Germany) following the manufacturers' instructions. NanoDrop2000C spectrophotometry and electrophoresis in 1% (w/v) agarose gel were used to detect the concentration and integration of the total DNA, respectively.

Sequencing, assembly, and annotation

Extracted DNA was fragmented to an average size of approximately 400 bp using CovarisM220 (Gene Company Limited, China) for paired-end library construction. Paired-end library was constructed using NEXTFLEX® Rapid DNA-Seq (BiooScientific, Austin, TX, USA). Adapters containing the full complement of sequencing primer hybridization sites were ligated to the blunt end of fragments. The paired-end sequencing was performed on the Illumina NovaSeq platform (Illumina Inc., San Diego, CA, USA) at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Raw reads were quality controlled with Trimmomatic and Fast QC software (https://www.bioinformatics.babraham.ac.

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Figure 1. A Specimen of the *A. sylvatica,* (A)a whole plant without the root, exhibited in the Dabie Mountain Biodiversity Herbarium. (B) One of the flowers. (C) The phyllaries of one flower, (D) Bisexual florets inside one flower. The image a was taken by Qiaoyu Zhang. The images B, C and D were taken by Yuan Xu.

uk/projects/fastqc). The assembly strategy of cp genome referred to Zhou's (Zhou et al. 2017). Cp-like reads were extracted by mapping clean reads against the collection of cp genomes retrieved from the NCBI nucleotide database based on their coverage and similarity. Cp contigs were assembled based on cp-like reads using SOAPdenovo2 (Luo et al. 2015), then scaffolded by SSPACE (Boetzer et al. 2011). Finally, gaps were filled with clean reads using the Gap Filler package (Nadalin et al. 2012). The annotation of the *A. sylvatica* chloroplast genome was performed using CPGAVAS2 (http://www.cpgavas2) (Shi et al. 2019), concerning the annotation of *Artemisia princeps* isolate PRPS03 (OP359063.1), and manually curated using Apollo (Misra and Harris 2005). The overall features of the *A. sylvatica* chloroplast genome were visualized using CPGview (Liu et al. 2023).

Phylogenetic tree construction

A total of 45 whole chloroplast genomes from various *Artemisia* species were retrieved from the NCBI database for phylogenetic analysis, including *A. absinthium* (NC_066024.1), *A. annua* (KY085890.1), *A. japonica* (MG951491.1), *A. ordosica* (MN932370.1), *A. selengensis* (MG951497.1), *A. transiliensis* (NC_070210.1) and so on (Supplemental Table 1). These genomes were aligned using MAFFT (Katoh et al. 2002), and the phylogenetic tree was constructed using RaxML-ng after determining the best-fit model for phylogenetic inference (Minh et al. 2020). The resulting tree was visualized using iTOL (https://itol.embl.de/).

Results

A total of 18,450,012 paired reads were assembled to the complete plastid genome of *A. sylvatica*, revealing the average coverage depth was 1292X (Supplemental Figure 1). The complete chloroplast genome of *A. sylvatica* has been submitted to GenBank under the accession number PQ009835. The genome exhibited a typical quadripartite structure (Figure 2) spanning 151,161 bp in total length. The large single copy (LSC) region comprised 82,892 bp, the small single copy (SSC) region comprised 18,353 bp, and a pair of inverted repeat (IR) regions separated the LSC and SSC, each region spanning 24,958 bp. The chloroplast genome encoded 132 complete genes, including 87 protein-coding genes, 37 tRNA genes, and 8 rRNA genes (Supplemental Tables 2, 3, and 4). A total of 96 genes were unique, including 73 protein-coding and 23 tRNA genes, and all the rRNA genes were duplicated in IR regions. The average GC content was 38%. The schematic map of the cis-splicing genes in *A. sylvatica* chloroplast genome was shown in Supplemental Figure 2. Trans-splicing gene *rps12* had three unique exons. Two of them were duplicated as they were located in the IR regions (Supplemental Figure 3).

Annotation of the chloroplast genome identified a total of 40 SSRs, predominantly consisting of mono-nucleotide

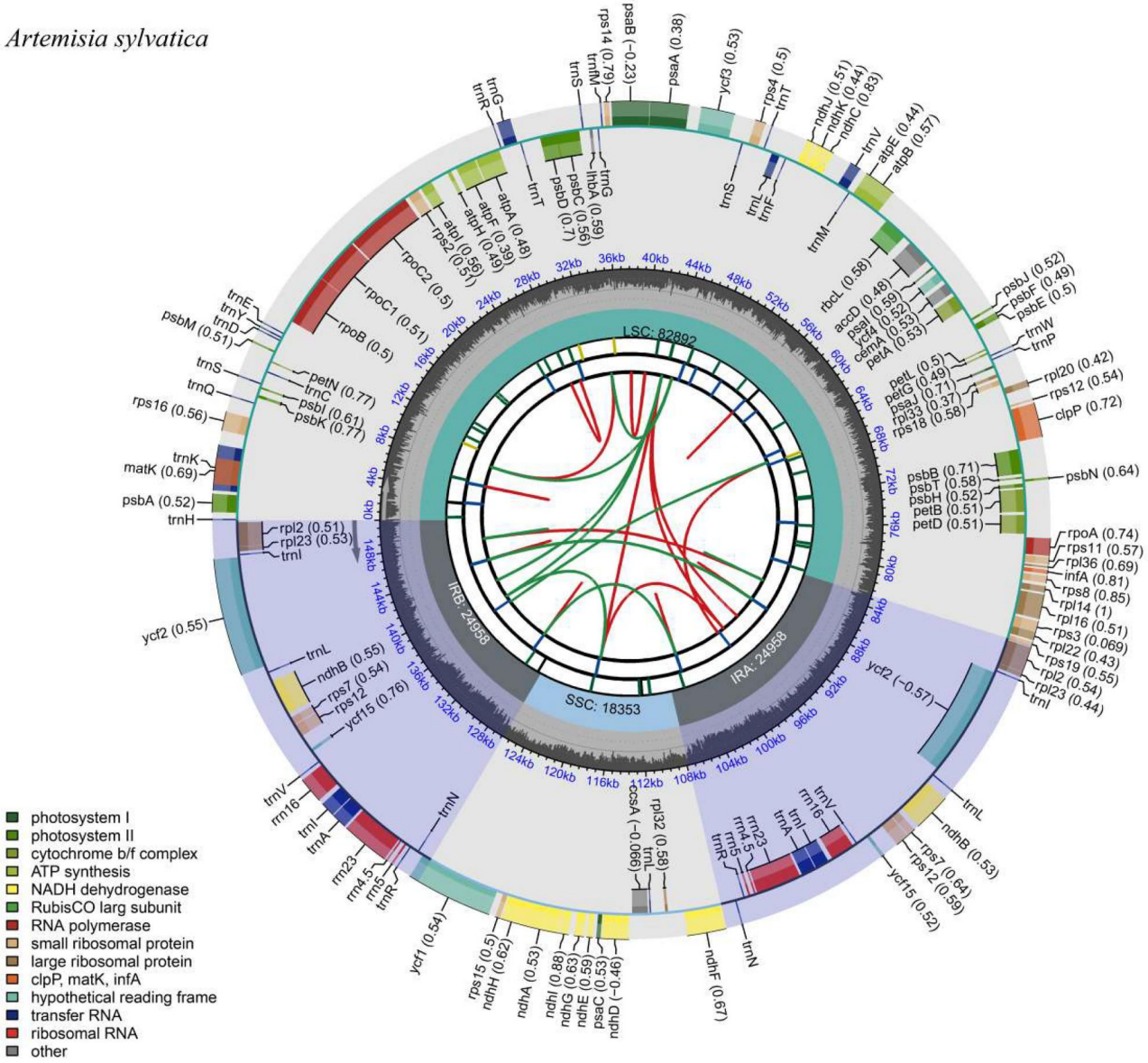

Figure 2. The complete plastome map of *A. sylvatica,* which was generated by CPGview. LSC, SSC, and IRs (IRa and IRb) with their length are represented on the first circle. The second circle showed the GC ratio in dark gray. The outermost circle indicated gene names color-coded by their functional classification. The transcription directions for the inner and outer genes were clockwise and anticlockwise, respectively. The functional classification of the genes was shown in the bottom left corner. The optional codon usage bias was displayed in the parenthesis after the gene name.

repeats (33; 82.5%), followed by di-nucleotide repeats (4; 10%) and tri-nucleotide repeats (3; 7.5%) (Supplemental Table 5 and Supplemental Figure 4).

For further exploration of the phylogenetic relationships of *A. sylvatica* within the *Artemisia* genus, a maximum likelihood tree was constructed using 43 *Artemisia* species and two outgroup species (*Helianthus annuus*-NC_007977.1 and *Helianthus tuberosus*-MG696658.1). The analysis placed the cp genome of *A. sylvatica* in Clade 8 (Jiao et al. 2023), named subgenus *Artemisia* (Figure 3A). The phylogenetic tree (Figure 3B) from the complete chloroplast genome sequences revealed that *A. sylvatica* was closely related to *A.lactiflora* (MW411453.1), additionally, it clustered with nine other species: *A. princeps* (MF034021.1), *A. montana* (KF887960.1), *A. indica* (NC080507.1), *A. tangutica* (MT701043.1), *A. rubripes* (MG951496.1), *A. argyi* (KM386991.1), *A. argyrophylla* (MF034022.1), *A. stolonifera* (MG951500.1), and *A. lancea* (NC 071926.1), all of which were placed within the subgenus *Artemisia*.

Discussion and conclusions

This study reported the first complete chloroplast genome sequence of *A. sylvatica*. The genus *Artemisia* is known for its morphological diversity, characterized by morphological traits like pollen type and floret arrangement within the capitula (Bremer and Humphries 1993; Vallès et al. 2011; Watson et al. 2002). However, molecular sequencing has revealed discrepancies between molecular phylogeny and infrageneric taxonomy of *Artemisia,* challenging the reliability of traditional morphological classifications (Garcia et al. 2011b; Riggins and Seigler 2012; Shultz and Flora of North America Editorial Committee 2006).

Jiao's et al. (2023) research is currently the most comprehensive and authoritative study on *Artemisia* classification by

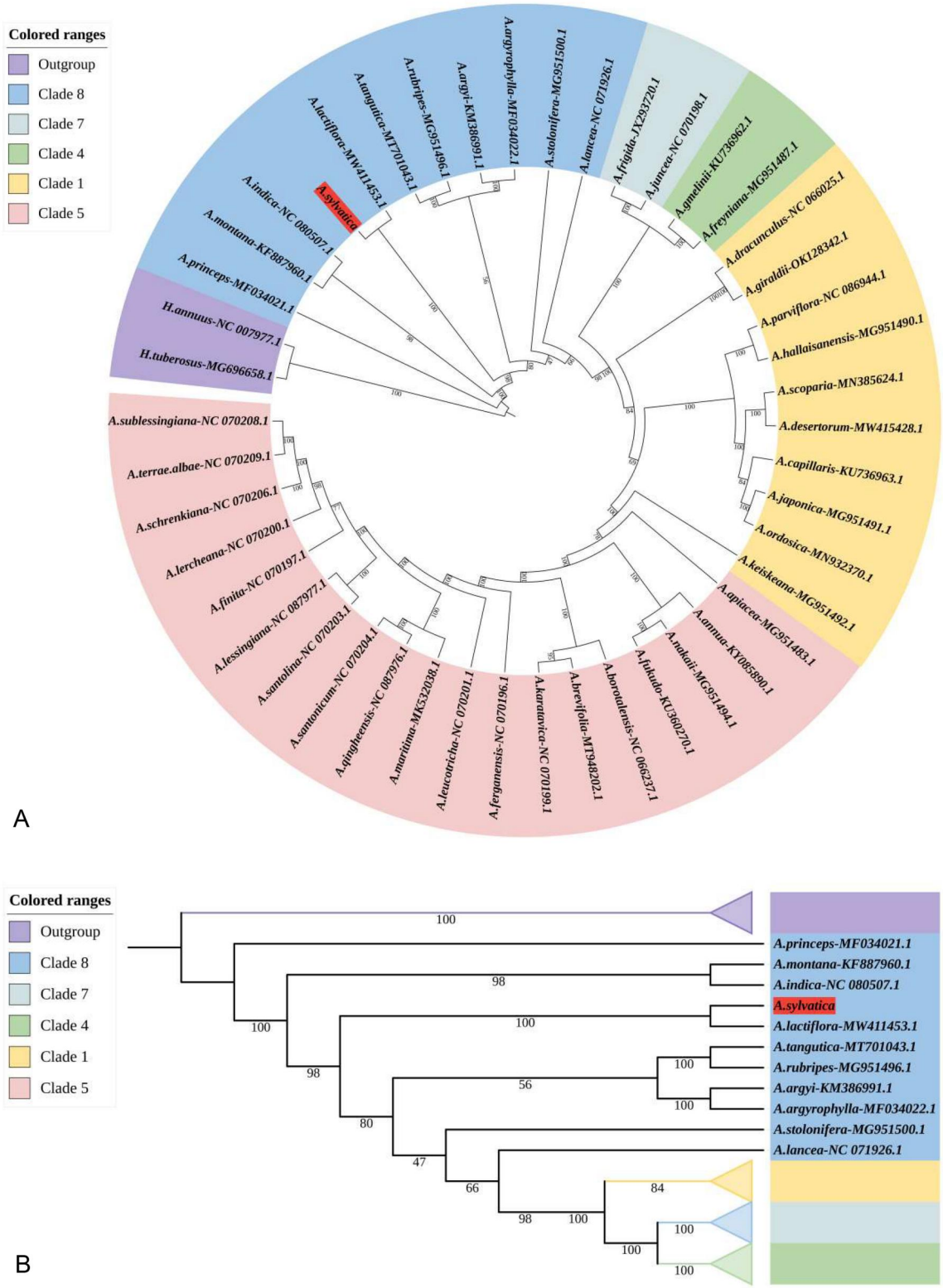

Figure 3. A maximum-likelihood (ML) based phylogenetic tree of *A. sylvatica* and related *Artemisia* species. The numbers on each node indicated the bootstrap support values. (A) The overall phylogeny of all the species and outgroups. A detailed phylogenetic relationship of those species was illustrated in Supplemental Table 1. *Artemisia* was classified into five major clades (Clade 8, Clade 7, Clade 4, Clade 1, and Clade 5 shown by different colors, respectively) (Jiao et al. 2023). (B) A clear relationship of *A. sylvatica* with the other 10 species in the same clade named Clade 8. The following sequences were used: *Helianthus tuberosus* (MG696658.1) (Zhong et al. 2019), *Helianthus.annuus* (NC 007977.1) (Timme et al. 2006), *A.princeps* (MF034021.1) (Min et al. 2019), *A.montana* (KF887960.1) (Cao et al. 2020), *A.indica* (NC 080507.1), *A.lactiflora* (MW411453.1) (Lan et al. 2022), *A.tangutica* (MT701043.1) (Yu et al. 2022), *A.rubripes* (MG951496.1) (Kim et al. 2020), *A.argyi* (KM386991.1) (Kang et al. 2016), *A.argyrophylla* (MF034022.1) (Kim et al. 2020), *A.frigida* (JX293720.1) (Jin et al. 2023a), *A.juncea* (NC 070198.1) (Jin et al. 2023b), *A.gmelinii* (KU736962.1) (Lee et al. 2016), *A.freyniana* (MG951487.1) (Kim et al. 2020), *A.dracunculus* (NC 066025.1), *A.giraldii* (OK128342.1) (Yue et al. 2022), *A.parviflora* (NC 086944.1), *A.hallaisanensis* (MG951490.1) (Lim et al. 2018), *A.scoparia* (MN385624.1) (Iram et al. 2019), *A.desertorum* (MW415428.1), *A.capillaris* (KU736963.1) (Lee et al. 2016), *A.japonica* (MG951491.1) (Kim et al. 2020), *A.ordosica* (MN932370.1) (Lu et al. 2020), *A.keiskeana* (MG951492.1) (Kim et al. 2020), *A.apiacea* (MG951483.1), *A.annua* (KY085890.1) (Xinqiang Guo et al. 2024), *A.nakaii* (MG951494.1) (Kim et al. 2020), *A.fukudo* (KU360270.1) (Kim et al. 2020), *A.borotalensis* (NC 066237.1), *A.brevifolia* (MT948202.1), A.karatavica (NC 070199.1), A.ferganensis (NC 070196.1), A.leucotricha (NC 070201.1), *A.maritima* (MK532038.1) (Jin et al. 2023c), *A.qingheensis* (NC 087976.1), *A.santonicum* (NC 070204.1), *A.santolina* (NC 070203.1), *A.lessingiana* (NC 087977.1), *A.finita* (NC 070197.1), *A.lercheana* (NC 070200.1), *A.schrenkiana* (NC 070206.1), *A.terrae.albae* (NC 070209.1), *A.sublessingiana* (NC 070208.1).

molecular sequencing, sampling 228 species (258 samples) from both fresh and herbarium collections across all subgenera and key geographical areas. He conducted a phylogenomic analysis using nuclear SNPs from genome skimming data, proposing eight subgenera. Our study, based on whole chloroplast genomes, supports this classification and confirms the placement of *A. sylvatica* and ten other species within the subgenus *Artemisia*.

However, our results did not fully align with Jiao's et al. (2023) findings. In our study, *A. sylvatica* and *A. lactiflora* clustered together (Figure 3B), while Jiao reported *A. sylvatica* and *A. princeps* forming a cluster in Clade 8c. This discrepancy likely stem from the differing genetic data used: Jiao's analysis based on nuclear genes and genome skimming, whereas our phylogenetic tree is based on whole chloroplast genomes. Since *Artemisia* plants are wind-pollinated and susceptible to hybridization, phylogenetic trees derived from nuclear genomes and maternally inherited chloroplast genomes may differ. Further experimental verification is necessary to clarify these differences.

Author contributions

Q.Z. and Q.C. conceived the project, Z.Y., C.W., Y. Z., B. M. and Y.X. collected the samples and analyzed the data, Q.Z. and Y.X. drafted this work, Q.C. reviewed the work. All authors agreed to be accountable for all aspects of this work.

Ethical approval

The sample of *A. sylvatica* was collected with permission from the Dongjiahe Botanical Farm, Xinyang City, Henan Province, China, and strictly complied with local and Chinese regulations. No ethical approval is required in this study.

Disclosure statement

No potential conflict of interest was reported by the authors.

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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 number PQ009835. The associated BioProject, BioSample, and SRA numbers are PRJNA1136178, SAMN42506235, and SRR29869130, respectively.

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