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PLASTOME REPORT

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The complete chloroplast genome sequence of leibnitzia anandria (linnaeus) turczaninow

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ABSTRACT

Leibnitzia anandria is a perennial herbaceous plant with medicinal properties, and the entire plant can be used in traditional medicine. Leibnitzia anandria was once classified under the genus Gerbera Cass., but was reclassified under Leibnitzia Cass. recently. In this study, using the GeneLab M sequencing technology of the Genemind platform, we have sequenced, assembled, and analyzed the complete chloroplast genome of Leibnitzia anandria for the first time. The genome is 154168 bp in length, consisting of a large single-copy region(LSC, 80166 bp), a small single-copy region(SSC, 18202 bp), and a pair of inverted repeat sequences(IR, 27900 bp). We have predicted and annotated a total of 133 genes, including 88 protein-coding genes, 37 tRNA-coding genes, and 8 rRNA-coding genes. The results of the phylogenetic analysis indicate that Leibnitzia anandria and Leibnitzia nepalensis, as well as the closely related Gerbera plant, clustered into a separate clade, rather than grouping together with the other plants belonging to the tribe Mutisieae. This study provides new information for the phylogeny research of Leibnitzia anandria, contributing to a better understanding of its taxonomy and evolution.

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Introduction

Leibnitzia anandria (Linnaeus) Turczaninow (Gerbera anandria _ Linnaeus 1758) is a perennial herbaceous plant and the model species of genus Leibnitzia, belonging to the tribe Mutisieae in the subfamily Carduoideae of the family Asteraceae (Wu and Peng 2004). As a traditional medicinal plant, the whole plant of Leibnitzia anandria can be used as medicine and contains components such as coumarin, with functions of clearing heat and detoxifying, diuresis and reducing swelling, as well as relieving cough and arresting bleeding (Gu et al. 1987, Qiu and Du 2005). Leibnitzia anandria was once classified as Gerbera Cass. (Cheng 1996), but later separated into the independent genus of Leibnitzia Cass. The main difference between Leibnitzia and Gerbera L. lies in the former having two reproductive periods in a year and there are also differences in the inflorescence morphology, leaves and plant size (Wu and Peng 2002). In this study, we sequenced and assembled the chloroplast genome of Leibnitzia anandria for the first time using Genemind platform's GeneLab M sequencing technology. Through comprehensive annotation and evolutionary analysis, this study provides a reference for understanding its classification and further study.

Materials and methords

Plant materials and DNA extraction

Samples of Leibnitzia anandria were collected from Wanxian Mountain, Xinxiang City, Henan Province, Chnia (113.6°E, 35.7°N). Total genomic DNA extracted from fresh leaf tissue using the Hipure SF Plant DNA Mini Kit following the manufacturer's instruction (Magen). The specimen was deposited at Shanghai Origingene Biotechnology CO., Ltd. in Shanghai Zizhu International Education Park by author (binghua.ru@origin-gene.com) under voucher Number YS001489 (Figure 1).

Genome sequencing, assembly, and annotation

The chloroplast genome of Leibnitzia anandria was sequenced by the next-generation sequencing (GenoLab M, Genemind sequencing platform). The quality of the sequenceing raw data was evaluated by FastQC (Andrews 2013), and trimmed using the software Cutadapt (Martin 2011) to obtain high-quality clean reads for subsequent analysis. The clean reads were de novo assembled and the complete chloroplast genome was obtained using assembly software NOVOPlast (Nicolas et al. 2017). The assembled genome was annotated by the software PGA (Qu et al. 2019), and manual

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Figure 1. Leibnitzia anandria (linnaeus) turczaninow. The main performance of *leibnitzia anandria* is that the leaves are basal, larger, lotus-like, and are oblong-lanceolate in shape. (A): the photo of *leibnitzia anandria* was taken by the authors in Wanxian Mountain, Xinxiang City, Henan Province, China(coordinates: 113.6E, 35.7N);(B): the leaf of *leibnitzia anandria* from (A).

correction was performed for all annotation results. A circular map of the chloroplast genome was generated by the online tool OGDRAW (Greiner et al. 2019). Relative synonymous codon usage (RSCU) in protein coding sequences of Leibnitzia anandria was determined in CodonW. Simple sequence repeats (SSRs) of Leibnitzia anandria was determined by MISA.

Phylogenetic tree construction

To determine the phylogenetic relationship of Leibnitzia anandria, we used two datasets: the whole cp genome sequences from 14 related species of family Asteraceae, and the chloroplast DNA trnL-rpl32 sequences from these 14 spcies, along with 6 other chloroplast DNA trnL-rpl32 sequences from genus Leibnitzia and Gerbera, respectively. We also selected Codonopsis minima and Echinocodon lobophyllus from the family Campanulaceae as outgroups. The sequences of these species were downloaded from NCBI (http://www.ncbi.nlm.nih.gov/). All sequences were aligned using MAFFT software with default alignment parameters and then edited manually (Katoh et al. 2019). Conserved sequences among different species were identified using Gblock software. A maximum likelihood tree with bootstrap support was built using the RAxML software based on the GTRCATI model. The phylogenetic tree was visualized by software Itol version V1.4.4 (https://iTOL.embl.de/) (Letunic and Bork 2016).

Result

By using next-generation high-throughput sequencing, 49455796 raw reads were obtained, which were filtered to yield 49448954 high-quality reads, with a total length of 7352487736 bp. The chloroplast genome of Leibnitzia anandria was assembled, with a total length of 154168 bp, GC content of 37.66%, and structure that includes a large single-copy region (LSC, 80166 bp), a small single-copy region (SSC, 18202 bp), and a pair of inverted repeat regions (IR, 27900 bp). A total of 133 genes were annotated, including 88 protein-coding genes, 37 tRNA-coding genes, and 8 rRNA-coding genes. Additionally, 26 simple sequence repeat(SSR) sequences were identified in the chloroplast genome of Leibnitzia anandria, among which mononucleotide repeats were the most abundant(12T, 10 A, and 1 G), followed by dinucleotide repeats (2AT), and one trinucleotide repeat (TCC) (Figure 2).

To further investigate the phylogenetic relationship of Leibnitzia anandria, we selected 14 chloroplast genome sequences and 6 chloroplast DNA trnL-rpl32 sequences from family Asteraceae. Codonopsis minima and Echinocodon lobophyllus were chosen as outgroups. The GTRCATI model was determined as the best-fit model. We using the MAFFT method to align the genome dataset and the chloroplast DNA trnL-rpl32 dataset with the relevant data of Leibnitzia anandria, respectively, and constructed phylogenetic trees. The results indicated that the topologies of both datasets trees using ML methods were highly consistent with each other. Therefore, the topological stuctures of consensus phylogenetic trees with two datasets were integrated here with 2 support values on the branch (Figure 3). Single bootstrap value from chloroplast DNA trnL-rpl32 sequences, two bootstrap value from left to right, respectively represented from the complete cp genome and from chloroplast DNA trnL-rpl32 sequences. Additional, The original trees with two datasets were also represented in the Figure S1 and Figure S2.

The phylogenetic trees based on two datasets indicate that Leibnitzia anandria, along with other Leibnitzia species and closely related Gerbera species, form a distinct branch. Species from the Ainsliaea, Myripnois, and Pertya genera, which belong to the Mutisiae tribe (Funk et al. 2016), cluster together as a separate clade. On the other hand, the Nouelia genus, also belonging to the Mutisiae tribe (Funk et al. 2016), forms a separate branch. The results based on the trnL-rpl32 sequence indicate that Leibnitzia anandria is more closely

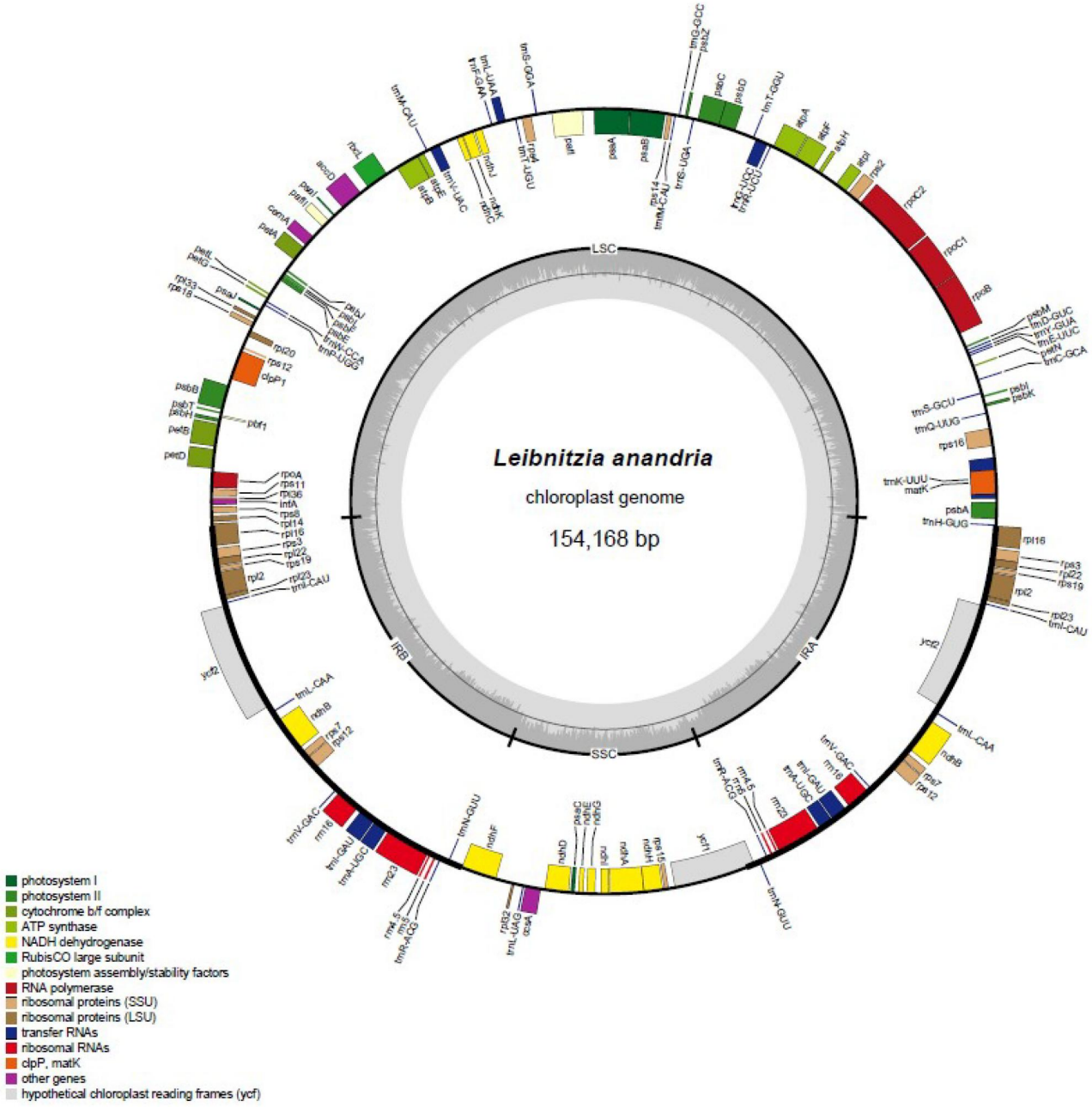

Figure 2. Genomic map of the leibnitzia anandria chloroplast genome generated by the online tool OGDRAW. Genes outside the circle are transcribed in a counterclockwise direction and those inside in a clockwise direction. LSC: large single-copy; SSC: small single-copy; IR: inverted repeat. The inner circle's dashed region represents the GC content of the chloroplast genome of L. anandria. Genes belonging to different functional groups are represented using different colors.

related to L. occimadrensis and L. lyrate than to L. nepalensis. Interestingly, species G. henryi and species within the Leibnitzia genus cluster together instead of with other species from the Gerbera genus.

Conclusion and discussion

In this research, we assembled the chloroplast genome of Leibnitzia anandria for the first time, revealing a 154,168 bp sequence that encodes 133 genes, including both large and small single-copy areas, alongside two inverted repeat regions. Sequencing depth confirmed the assembly's comprehensive accuracy. Phylogenetic analysis showed that L. anandria forms a distinct clade with L. nepalensis and related Gerbera species, diverging from other Mutisieae members. Given the scarcity of complete chloroplast genomes for Leibnitzia and Gerbera, we highlighted the trnL-rpl32 region as a potential DNA barcode for phylogenetic studies (Cui et al. 2020). This study contributes six trnL-rpl32 sequences from Leibnitzia and Gerbera, compared against 15 other species' sequences, including L. anandria's. The results support the chloroplast genome findings and suggest a closer relationship between L. anandria and L. lyrate as well as L. occimadrensis than with L. nepalensis, confirming previous studies (Baird et al. 2010). Interestingly, G. henryi from the Gerbera genus appears more closely related to Leibnitzia than to other Gerbera species, indicating a particular link between Asian Gerbera and Leibnitzia species, in contrast to African Gerbera species (Xu et al. 2018). This study advances

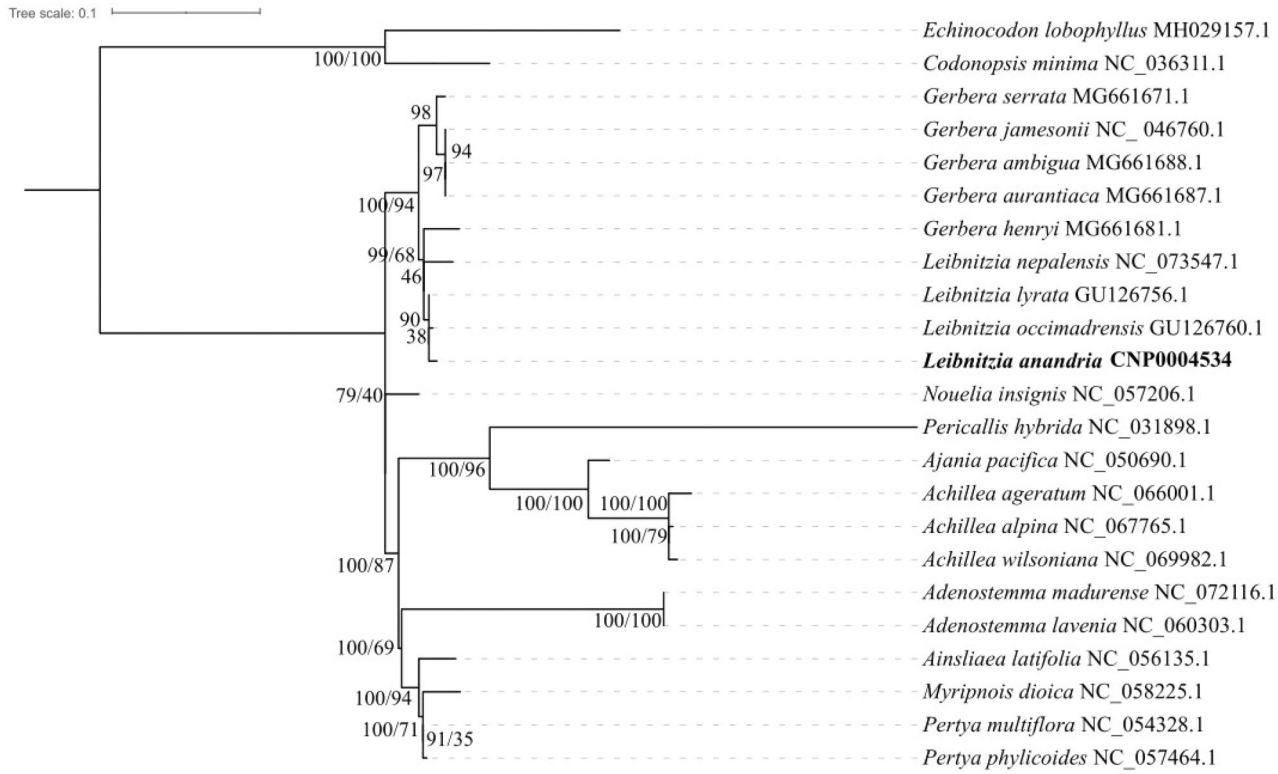

Figure 3. Maximum likelihood phylogenetic tree of leibnitzia anandria, and other 22 asteraceae species constructed using two different datasets. Bootstrap support values are given at the nodes, single bootstrap value form chloroplast DNA trnL-rpl32 sequences, two bootstrap value from left to right, respectively represented from the complete cp genome and from chloroplast DNA trnL-rpl32 sequences. Codonopsis minima and echinocodon lobophyllus were used as outgroups. The accession number of sequence of each plant species is shown after the species names:NC_046760.1(Zhang et al. 2019), NC_058225.1(Chen et al. 2019), NC_057206.1(Tian and Fu 2020), NC_057464.1(Wang et al. 2020), NC_054328.1(Jiang 2020), NC_067765.1(Niu 2021), NC_066001.1(Leonardo et al. 2022), NC_062413.1(Pires Paula 2020), NC_069982.1(Luo and Fu 2022), NC_072116.1(Kim et al. 2023), NC_060303.1(Li 2020), NC_053927.1(Luo and Pan 2019), NC_056135.1(Yin 2020), NC_050690.1(Kim and Kim 2020), NC_031898.1(Wang et al. 2015), NC_073547.1(Chen 2023), GU126760.1(Baird et al. 2010), GU126756.1(Baird et al. 2010), MG661671.1(Xu et al. 2018),MG661681.1(Xu et al. 2018),MG661688.1(Xu et al. 2018),MG661687.1(Xu et al. 2018), NC_036311.1(Cheon et al. 2017), MH029157.1(Wang et al. 2018).

our understanding of L. anandria's phylogeny, aiding in its taxonomic and evolutionary delineation.

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Ethical approval

We confirmed that all the research meets ethical guidelines and adheres to the legal requirements of the study country. This study did not involve any ethical issues, so no ethics committee or relevant permissions were required.

Author contributions

Binghua Ru contributed to the study concept and design; Ting Wang collected the samples and performed the experiments; Yongfeng Liu and Xiaochao Zhao analyzed and interpreted the data; Ming Lei contributed to the research design, data analysis, and overall supervision of the study. all authors read and approved the final manuscript; and that all authors agree to be accountable for all aspects of the work.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availiability statement

The genome sequence data that supported the findings of this study are openly available in GeneBank of NCBI at https://www.ncbi.nlm.nih.gov under the accession No.PP566209. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1081144, SRR28110553 and SAMN40149392 respectively.

References

Andrews S. 2013. Babraham Bioinformatics -FastQC A Quality Control tool for High Throughput Sequence Data.

Baird KE, Funk VA, Wen J, Weeks A. 2010. Molecular phylogenetic analysis of Leibnitzia Cass. (Asteraceae: mutisieae: gerbera-complex), an Asian North American disjunct genus. J Syst Evol. 48(3):161–174. doi: 10.1111/j.1759-6831.2010.00077.x.

Cheng YQ. 1996. Compositae(9), Chinese Academy of Sciences Flora of the People's Republic of China editorial board. In: Flora of the People's Republic of China. Vol. 79, 82–83. Beijing: Science Press.

Cheon K-S, Kim K-A, Yoo K-O. 2017. Direct Submission. Submitted (07-FEB-2017) Biological Sciences, Kangwon National University, 1 Gangwondaehak-gil, Chuncheon-si, Gangwon-do 24341, South Korea.

Chen L, Luo S, Zhang X, Du L, Yang Q, Liu ZL. 2019. The complete chloroplast genome of Myripnois dioica (Asteraceae). Mitochondrial DNA B Resour. 4(1):1911–1912. doi:10.1080/23802359.2019.1616629.

Cui N, Liao BS, Liang CL, Li SF, Zhang H, Xu J, Li XW, Chen SL. 2020. Complete chloroplast genome of Salvia plebeia L. (Lamiaceae): organization, specific barcode and phylogenetic analysis. Chin J Nat Med. 18(8):563–572.

Chen H. 2023. Direct Submission. Submitted (27-FEB-2023) College of Life Sciences, Sichuan Normal University, Chenglong Avenue, chengdu, sichuan 610101, China.

Funk VA, Pasini E, Bonifacino JM, Katinas L. 2016. Home at last: the enigmatic genera Eriachaenium and Adenocaulon (Compositae: mutisioideae;Mutisieae;Adenocaulinae. PhytoKeys. 60(60):1–19. doi:10.3897/phytokeys.60.6795.

Gu LH, Wang SX, Li M, Zhu TR. 1987. Studies on the antibacterial constituents of Gerbera. Acta Pharmaceutica Sinica. 4:272–277.

Greiner S, Lehwark P, Bock R. 2019. Organellar Genome DRAW (OGDRAW) version 1.3.1: expanded toolkit for the graphical visualization of organellar genomes. Nucl Acid Res. 47:59–64.

Jiang M. 2020. Direct Submission. Submitted (21-OCT-2020) Life Sciences, Taizhou University, 1139 Shifu Road, TAIZHOU, ZHEJIANG PROVINCE 318000, China.

Katoh K, Rozewicki J, Yamada KD. 2019. MAFFT online service: multiple sequence alignment interactive sequence choice and visualization. Brief Bioinform. 20(4):1160–1166. doi:10.1093/bib/bbx108.

Kim HT, Kim JS. 2020. The complete chloroplast genome sequence of Ajania pacifica (Nakai) Bremer & Humphries. Mitochondrial DNA B Resour. 5(3):2399–2400. doi:10.1080/23802359.2020.1750981.

Kim JE, Kim S-C, Jang JE, Gil H-Y. 2023. Complete chloroplast genome sequence of Adenostemma madurense (Asteraceae). J Asia Pac Biodivers. 16:710–714.

Leonardo IC, Alberti A, Denoeud F, Barreto Crespo MT, Capelo J, Gaspar FB. 2022. Direct Submission. Submitted (27-MAY-2022) Food Safety and Microbiology, Food & Health Division, iBET - Instituto de Biologia Experimental e Tecnologica,Apartado 12, Oeiras 2781-901, Portugal.

Letunic I, Bork P. 2016. Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Res. 44(W1):W242–5. doi:10.1093/nar/gkw290.

Li Y. 2020. Direct Submission. Submitted (09-FEB-2021) School of Pharmacy, Weifang Medical University, Weifang 261053, China, Fuyan Mountain Campus: no. 7166, Bao Tong West Street, Weifang 261053, China.

Luo J-J, Fu Z-X. 2022. Direct Submission. Submitted (31-MAY-2022) College of Life Sciences, Sichuan Normal University, Sichuan Normal University, Chengdu, Sichuan 610101, China.

Luo ZP, Pan LW. 2019. Characterization of the complete mitochondrial genome of Ageratum Conyzoides. Mitochondrial DNA B Resour. 4(2):3540–3541. doi:10.1080/23802359.2019.1676176.

Martin M. 2011. Cut adapt removes adapter sequences from high-throughput sequencing reads. EMBnet j. 17(1):10–12. doi:10.14806/ej.17.1.200.

Nicolas D, Patrick M, Guillaume S. 2017. Novoplasty: de novo assembly of organelle genomes from whole genome data. Nucleic Acids Res. 45:e18.

Niu Y. 2021. Direct Submission.Submitted (30-NOV-2021) College of Horticulture, Gansu Agricultural University, 1 Yinmen Village, Lanzhou, Gansu 730000, China.

Pires Paula D. 2020. Direct Submission. Submitted (20-MAY-2020) Biological Control, Embrapa, Parque Estacao Biologica, PqEB, Av. W5 Norte (final) Caixa Postal 02372, Brasilia, DF 70770-917, Brazil.

Qiu DW, Du J. 2005. Chinese materia medica: seedling Medicine Volume. Guiyang: Guizhou Science and Technology Press. p. 59.

Qu X-J, Moore MJ, Li D-Z, Yi T-S. 2019. PGA: a software package for rapid, accurate, and flexible batch annotation of plastomes. Plant Methods. 15(1):50. doi:10.1186/s13007-019-0435-7.

Tian X, Fu Z. 2020. Direct Submission. Submitted (27-APR-2020) College of Life Sciences, Zhengzhou University, No. 100 Science Avenue, Zhengzhou, Henan 450001, China.

Wang B, Han L, Wang Z. 2015. Direct Submission. Submitted (15-JUL-2015) Shaanxi Normal University, Western Chang'an Street, Chang'an District, Xi'an, Shaanxi 710119, China.

Wang YC, Zhou YF, Chen C, Mao SL. 2018. The complete chloroplast genome sequence of Echinocodon Lobophyllus (Campanulaceae) revealed by next-generation sequencing and phylogenetic implication. Mitochondrial DNA B Resour. 3(1):450–451. doi:10.1080/23802359.2018.1462117.

Wang B, Zhao Q, Wang XH, Fu ZX. 2020. The complete chloroplast genome of Pertya phylicoides (Asteraceae,Pertyeae): a shurby endemic species from China. Mitochondrial DNA B Resour. 5(1):963–964. doi:10.1080/23802359.2020.1722763.

Wu ZY, Peng H. 2002. Revision and geographical distribution of the genus Polygonum from China. Stud Plants Yunnan. 24(2):137–146.

Wu ZY, Peng H. 2004. Compositae. kunming institute of botany, chinese academy of sciences. In: Flora of Yunnan. Vol. 13. Beijing: Science Press. p. 654–658.

Xu X, Zheng W, Funk VA, Wen J. 2018. Home at Last II: gerbera hieracioides (Kunth) Zardini (Mutisieae, Asteraceae) is really a Chaptalia. PhytoKeys. 95(95):93–106. doi:10.3897/phytokeys.95.22916.

Yin X. 2020. Direct Submission. Submitted (01-DEC-2020) food science college, Tibet agriculture and animal husbandry university, yucaixilu 100, Linzhi, Tibet, China.

Zhang Y-Y, Liu F, Wang X-Q, Shi X-B, Tian N, Lai Z-X, Cheng C-Z. 2019. Characterization of the complete chloroplast genome of Gerbera jamesonii Bolus in China and phylogenetic relationships. Mitochondrial DNA B Resour. 4(2):2706–2707. doi:10.1080/23802359.2019.1644230.