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PLASTOME REPORT

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Structural characterization and phylogenetic analysis of the chloroplast genome of *Cryptocoryne crispatula* var. balansae (gagnep.) N. Jacobsen 1991

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ABSTRACT

Cryptocoryne is a popular ornamental aquatic plant for aquarists, although only six species are found in China. Destruction of the natural habitats of *Cryptocoryne* for human activities has led to a decline in its numbers. In this report, we sequenced and annotated the *Cryptocoryne crispatula var. balansae* chloroplast genome for the first time. Results showed that the length of the chloroplast genome was 182,935 bp and the GC content was 34%. The chloroplast genome encoded 137 genes, including 92 encoded protein genes, 37 tRNA genes, and 8 rRNA genes. Phylogenetic analysis supported the the monophyletic nature of the genus and indicated that it was the first species to be differentiated in the chloroplast genome of *Cryptocoryne* and formed a separate branch. These findings offer valuable genomic resources for comparative studies in *Cryptocoryne* and Araceae, thereby aiding genetic diversity and phylogenetic analyses.

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Cryptocoryne; chloroplast genome; Araceae; sequence characterization

Introduction

Cryptocoryne crispatula var. balansae is a perennial herb in the family Araceae. It is an amphibious plant that exists in submerged and emergent forms. *Cryptocoryne* plants usually grow in water, with leaves growing from underground rhizomes. In winter, tubular flame buds emerge from the axils of the leaves and appear slightly enlarged near the base. The upper part of the inflorescence axis comprises numerous male flowers, and the female flowers (4–7) are arranged below in 1–2 rounds. A bell-shaped septum covers the male inflorescence and a cup-shaped appendage is attached to the septum to prevent water from entering the inflorescence cavity from the bract tube. *Cryptocoryne* is a wetland vascular plant that is of great concern in China (Zhao et al. 2017). Owing to the changeable shape and color of leaves and spathes, slow growth rate, large landscape plasticity, and strong ornamental value, it is popular with most aquarists, resulting in several varieties being on the verge of extinction. In addition to environmental factors, the breeding methods and biological characteristics of *Cryptocoryne* are important reasons for its endangerment. The ploidy of *Cryptocoryne* sp. is complex, with some being haploids. Thus, obtaining seeds for reproduction through self-fertilization is challenging.

Chloroplasts are organelles that perform photosynthesis, facilitate plant growth and development, and participate in biochemical processes such as pigment and fatty acid production. In addition, chloroplasts have an independent genome that has its own replication, transcription, and translation mechanisms (Hughey et al. 2001; Raubeson and Jansen 2005). The highly conserved characteristics and rapid evolutionary rate of chloroplasts make it an ideal tool for studying evolution and molecular ecology (Verbruggen et al. 2010; Janouškovec et al. 2013); therefore, the chloroplast genome is widely used to explore plant genome-wide structural information, genetic diversity, and species protection (Nie et al. 2012; Han et al. 2016; Xue et al. 2017; Dong et al. 2021). Complete chloroplast genome identification, sequencing, assembly annotation, and phylogenetic analysis would facilitate the study of genetic breeding and cultivation of *C. crispatula* var. balansae.

Currently, *Cryptocoryne* plants are in the early investigation stage (Othman 1997; Ipor et al. 2015); thus, the taxonomy and systematics of the genus have been based on morphological characteristics and geographical distribution. Moreover, inadequate gene bank data are available on *Cryptocoryne* sp., and the Chinese nomenclature is also inconsistent. To provide a better understanding and utilization of *C. crispatula* var. balansae, we first sequenced and analyzed the whole chloroplast genome of this species using second-generation sequencing technology. The abundant chloroplast genome data of *C. crispatula* var. balansae will provide a basis for the identification, protection, and sustainable utilization of *C. crispatula* var. balansae germplasm resources.

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Materials and methods

Fresh leaves of *C. crispatula* var. balansae were collected from Du'an Yao Autonomous County, Hechi City, Guangxi (N108°0'4.7", E23°58'10.7"). The original plant was identified as *C. crispatula* var. balansae by Professor Lin Xiao, and pictures of the original plants were taken by our team (Figure 1). The specimens were preserved in the Laboratory of Department of Ecology and Institute of Hydrobiology, Jinan University (Prof. Lin Xiao, E-mail: xiaolin1980@jnu.edu.cn) under voucher number GX_HC_202305210004F. The leaves were dried and stored with a silica gel desiccant, which was purchased from Sangon Biotech Co., Ltd (Shanghai, China). The samples were sent to Wuhan Tianyi Huiyuan Gene Technology Co., Ltd. (Wuhan, China) for total DNA extraction and second-generation sequencing.

SPAdes v3.14.1 (Bankevich et al. 2012) was used to perform the preliminary splicing of clean data. and the published near-source chloroplast data and protein-coding gene sequences (Sun et al. 2021) were used as a reference. The chloroplast genome was annotated using PGA software (https://githu b.com/quxia ojian/PGA; Qu et al. 2019). CPGview (http://www.1kmpg.cn/cpgview/; Liu et al. 2023) software was used to draw the chloroplast genome map, cis-splicing gene map and trans-splicing gene map of *C. crispatula* var. balansae. The complete chloroplast genome sequence of *C. crispatula* var. balansae was submitted online to the NCBI database under the accession number (PP155471).

Data on 16 species of Araceae were downloaded from GenBank. In the present study, 15 chloroplast genomes were compared with the chloroplast genome of *C. crispatula* var. balansae. PhyloSuite v1.2.1 (Zhang et al. 2020) software was used to extract the common protein-coding genes of all species, and the *rbcL*, *atpB*, and *matK* genes were selected for comparison, optimization, and model selection. These sequences were aligned in batches using MAFFT v7.313. ModelFinder was used to select the best-fit partition model (Edge-linked) using the BIC criterion. IQ-TREE v1.6.12 (Nguyen

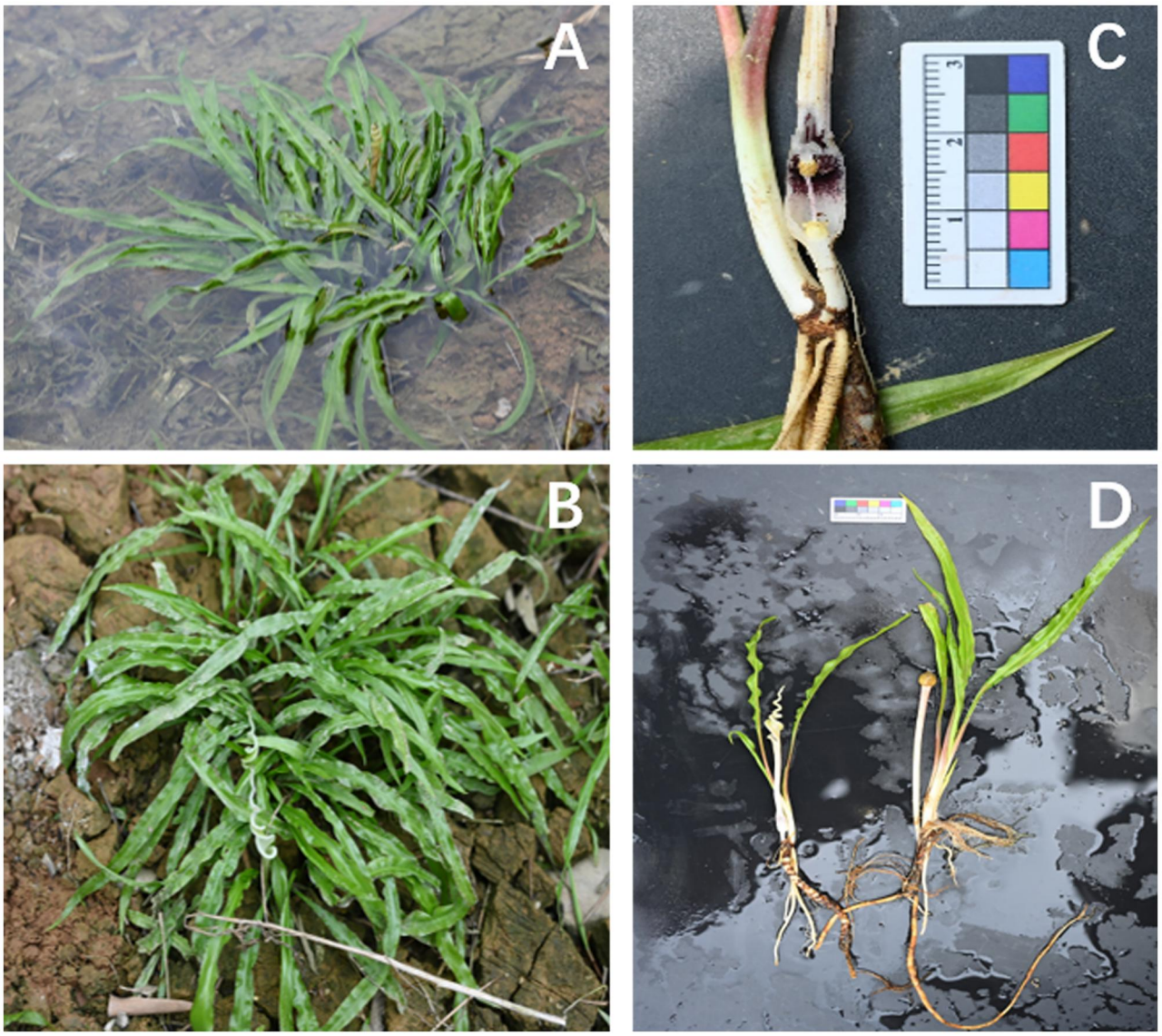

Figure 1. *C. crispatula* var. balansae plants. (A: Submerged form; B: Emerged form; C: Longitudinally dissected kettle; D: Plant habit).—photograph: 20 may 2023 by Prof. Lin Xiao.

et al. 2015) was used to construct trees based on the maximum likelihood (ML) method.

Results

The chloroplast genome sequence of *C. crispatula* var. balansae was 182,935 bp in length, with an average sequencing depth of 228.78X (Figure S1). The chloroplast genome consisted of two inverted repeats (IRA and IRB, both 39,489 bp), a large single-copy region of LSC (95,756 bp), and a small single-copy region of SSC (8,201 bp). The GC content was 34.0%. (Figure 2) The chloroplast genome of *C. crispatula* var. balansae encodes a total of 137 genes, including 92 protein-coding genes, 37 tRNA genes and 8 rRNA genes. Among them, 15 genes (*trnK*-UUU, *rps16*, *trnG*-UCC, *atpF*, *rpoC1*, *trnL*-UAA, *trnV*-UAC, *petB*, *petD*, *rpl16*, *rpl2*, *ndhB*, *trnI*-GAU, *trnA*-UGC, and *ndhA*) contained one intron, two genes (*clpP1* and *pafI*) contained two introns. 11 cis-splicing genes including *rps16*, *atpF*, *rpoC1*, *paf1*, *clpP1*, *petB*, *petD*, *rpl16*, *rpl2*、*ndhA* and *ndhB* (two copies) (Figure S2), and *rps12* with three exons is a trans-spliced gene (Figure S3)

The phylogenetic analysis showed that species in the genera *Lemna*, *Cryptocoryne*, *Pothos*, *Typhonium* and *Amorphophallus* clustered into one branch. *Cryptocoryne* was further divided into two branches, with one including *C. elliptica*, *C. nurii*, *C. longicauda* and *C. striolata*, and the other including *C. crispatula* var. balansae. (Figure 3).

Discussion and conclusions

Cryptocoryne species are widely distributed, with over 500 species found globally, including six found in China (Reumer 1984). As an ornamental aquatic plant, *Cryptocoryne* has a

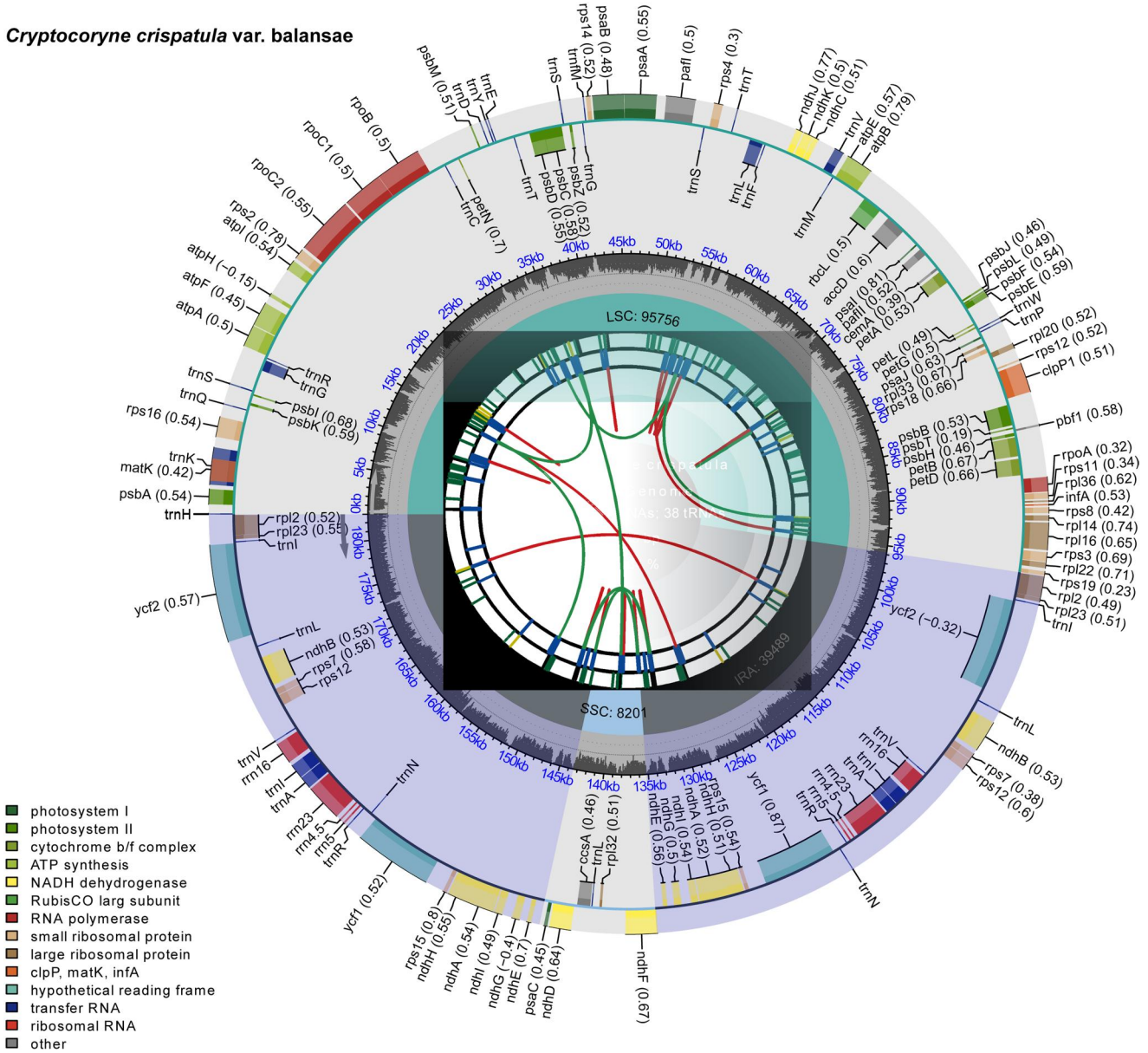

Figure 2. Map of the chloroplast genome of *C. crispatula* var. balansae. Genes lying outside the outer circle are transcribed clockwise, while those inside the circle are transcribed counterclockwise. Genes belonging to different functional groups are color-coded. The innermost darker grey corresponds to GC content, while the quadripartite structure (LSC, SSC, IRA, and IRB) is illustrated on the inner circle accordingly.

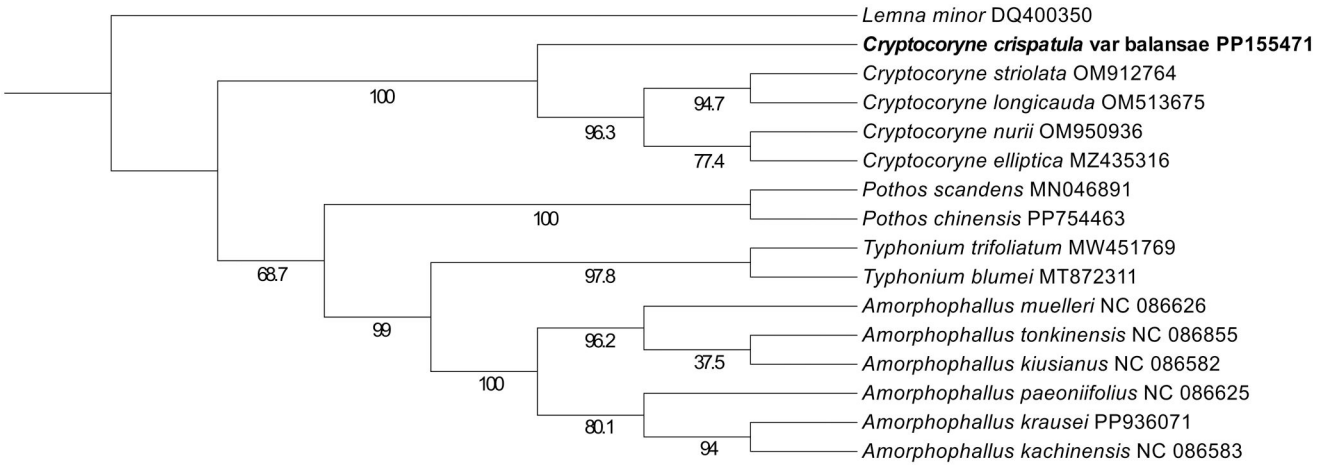

Figure 3. Maximum-likelihood tree of *C. crispatula* var. balansae and its related species. Phylogenetic tree constructed by maximum-likelihood (ML) analysis based on complete chloroplast genome sequences, including *Cryptocoryne elliptica* (MZ435316 (Talkah et al. 2022)), *Lemna minor* (DQ400350 (Mardanov et al. 2008)), *Cryptocoryne nurii* (OM950936), *Cryptocoryne striolata* (OM912764), *Cryptocoryne longicauda* (OM513675), *C. crispatula* var. balansae (PP155471; this study), *Pothos scandens* (MN046891 (Abdullah et al. 2020)), *Pothos chinensis* (PP754463), *Typhonium trifoliatum* (MW451769), *Typhonium blumei* (MT872311), *Amorphophallus krausei* (PP936071), *Amorphophallus tonkinensis* (NC_086855 (Yin et al. 2024)), *Amorphophallus muelleri* (NC_086626), *Amorphophallus paeoniifolius* (NC_086625 (Li et al. 2024)), *Amorphophallus kachinensis* (NC_086583 (Gao and Yin, 2024a)), *Amorphophallus kiusianus* (NC_086582 (Gao and Yin, 2024b)).

high economic value; however, owing to the destruction of its habitat by human activities, its abundance has greatly reduced (Huang et al. 2020). To further explore the biological characteristics of *Cryptocoryne* species, the *C. crispatula* var. balansae chloroplast genome was sequenced, assembled, and annotated for the first time (to the best of our knowledge) using next-generation sequencing. The results revealed that the chloroplast genome of *C. crispatula* var. balansae was 182,935 bp long and possessed a quadripartite structure, consistent with the genome structure of most angiosperm chloroplasts (Jansen et al. 2005). Compared with other species within the genus *Cryptocoryne*, including *C. elliptica*, *C. nurii*, *C. longicauda*, and *C. striolata*, the chloroplast genome of *C. crispatula* var. balansae is longer, indicating significant differences among *Cryptocoryne* species. Phylogenetic analysis of chloroplast genome sequence of *C. crispatula* var. balansae and other *Cryptocoryne* species revealed that *C. crispatula* var. balansae showed the earliest divergence among the published chloroplast genomes of *Cryptocoryne* species, forming a distinct branch.

Numerous studies have described the characteristics of *Cryptocoryne* species (Tanaka et al. 2007; Wongso et al. 2017). However, different interpretations have been provided on the relationships and classification methods among the populations of *Cryptocoryne* plants. Nevertheless, differences in the morphology and color of leaves and spathes have been widely recognized as important characteristics for distinguishing varieties (Jacobsen et al. 2015b). In 2015, Jacobsen et al. conducted an amplified fragment length polymorphism molecular marker analysis on over 400 collected varieties to elucidate the genetic relationships within this population. They found that geographical proximity was more closely related to genetic relationships than morphological similarities (Jacobsen et al. 2015a). Therefore, by analyzing the chloroplast genome structure and phylogenetics of *C. crispatula* var. balansae, the molecular information for this species can be supplemented, thereby enriching the molecular biology resources of the genus. This study also provides a reference for the conservation of germplasm resources and genetic variation research on *Cryptocoryne* ornamental aquatic plants.

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Ethics approval and informed consent

Not required for this study.

Author contributions

Li Yu, Qian He and Lin Xiao conceived and designed the study. Yu-Han Jing and Ying-Wei Huang conducted experimental work. Li Yu and Cheng-He Sun carried out data analysis. Li Yu wrote the manuscript. Li Yu and Cheng-He Sun revised the manuscript. All authors contributed to interpretation of results, read, and approved the final draft.

Disclosure statement

The authors declare that they have no competing interests.

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Data availability statement

The complete chloroplast genome sequence data that support the findings of this study are freely available in the NCBI GenBank (https://www.ncbi.nlm.nih.gov/) under accession number of PP155471. The raw

sequencing data of *C. crispatula* var. balansae have also been deposited into the NCBI Bio-Project and SRA database under accession numbers PRJNA1102495 and SRR28762466, respectively. Information on the plant material was deposited into NCBI Bio-Sample database under accession number SAMN41022772.

References

Abdullah, Henriquez CL, Mehmood F, Carlsen MM, Islam M, Waheed MT, Poczai P, Croat TB, Ahmed I. 2020. Complete chloroplast genomes of *Anthurium huixtlense* and *Pothos scandens* (Pothoideae, Araceae): unique inverted repeat expansion and contraction affect rate of evolution. J Mol Evol. 88(7): 562–574. doi:10.1007/s00239-020-09958-w.

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, et al. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 19(5):455–477. doi:10.1089/cmb.2012.0021.

Dong WP, Xu C, Liu YL, Shi JP, Li WY, Suo ZL. 2021. Chloroplast phylogenomics and divergence times of *Lagerstroemia* (Lythraceae). BMC Genomics. 22(1):434. doi:10.1186/s12864-021-07769-x.

Gao Y, Yin S. 2024. Assembly and analysis of the chloroplast genome of *Amorphophallus kachinensis* Engler & Gehrmann (Araceae) from Southwestern China: implications for conservation and utilization. Mitochondrial DNA B Resour. 9(4):452–456. doi:10.1080/23802359.2024.2338550.

Gao Y, Yin S. 2024. The complete chloroplast genome assembly of *Amorphophallus kiusianus* makino 1913 (Araceae) from Southern China. Mitochondrial DNA B Resour. 9(4):522–526. doi:10.1080/23802359.2024.2342934.

Han LM, Chen C, Wang B, Wang ZZ. 2016. The complete chloroplast genome sequence of medicinal plant *Pinellia ternata*. Mitochondrial DNA A DNA Mapp Seq Anal. 27(4):2921–2922. doi:10.3109/19401736.2015.1060441.

Huang S, Luo Y, Liu S, Cao J. 2020. Research progress on ornamental aquatic plants of *Cryptotaenia*. Guangdong Agric Sci. 47:30–36. doi:10.16768/j.issn.1004-874X.2020.02.005.

Hughey JR, Silva PC, Hommersand MH. 2001. Solving taxonomic and nomenclatural problems in Pacific Gigartinaceae (Rhodophyta) using DNA from type material. J Phycol. 37(6):1091–1109. doi:10.1046/j.1529-8817.2001.01048.x.

Ipor IB, Ørgaard M, Jacobsen N. 2015. *Cryptocoryne* × *batangkayanensis* (Araceae), a new hybrid from Sarawak. Willdenowia. 45(2):183–187. doi:10.3372/wi.45.45204.

Jacobsen N, Bastmeijer J, Christensen C, Idei T, Lange CA, Orabi J, Sookchaloem D, Toneato F, Oergaard M. 2015a. The use of AFLP markers to elucidate relationships within *Cryptocoryne* (Araceae). Aroideana. 38e: 186–193.

Jacobsen N, Bastmeijer JD, Bogner J, Van N, Du QBH, Ørgaard M. 2015b. The identity of *Cryptocoryne crispatula* var. tonkinensis (Araceae). Willdenowia. 45(2):177–182. doi:10.3372/wi.45.45203.

Janouškovec J, Liu SL, Martone PT, Carré W, Leblanc C, Collén J, Keeling PJ. 2013. Evolution of red algal plastid genomes: ancient architectures, introns, horizontal gene transfer, and taxonomic utility of plastid markers. PLoS One. 8(3):e59001. doi:10.1371/journal.pone.0059001.

Jansen RK, Raubeson LA, Boore JL, dePamphilis CW, Chumley TW, Haberle RC, Wyman SK, Alverson AJ, Peery R, Herman SJ, et al. 2005. Methods for obtaining and analyzing whole chloroplast genome sequences. Methods Enzymol. 395:348–384. doi:10.1016/S0076-6879(05)95020-9.

Li L, Yang M, Qi Y, Yu Y, Gao P, Yang S, Zhao Y, Guo J, Liu J, Huang F, et al. 2024. Complete chloroplast genome and phylogenetic analysis of *Amorphophallus paeoniifolius* (Araceae). Mitochondrial DNA B Resour. 9(7):865–870. doi:10.1080/23802359.2024.2378966.

Liu S, Ni Y, Li J, Zhang X, Yang H, Chen H, Liu C. 2023. CPGView: a package for visualizing detailed chloroplast genome structures. Mol Ecol Resour. 23(3):694–704. doi:10.1111/1755-0998.13729.

Mardanov AV, Ravin NV, Kuznetsov BB, Samigullin TH, Antonov AS, Kolganova TV, Skyabin KG. 2008. Complete sequence of the duckweed (*Lemna minor*) chloroplast genome: structural organization and phylogenetic relationships to other angiosperms. J Mol Evol. 66(6):555–564. doi:10.1007/s00239-008-9091-7.

Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and efective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 32(1):268–274. doi:10.1093/molbev/msu300.

Nie XJ, Lv SZ, Zhang YX, Du XH, Wang L, Biradar SS, Tan XF, Wan FH, Weining S. 2012. Complete chloroplast genome sequence of a major invasive species, Crofton weed (*Ageratina adenophora*). PLoS One. 7(5):e36869. doi:10.1371/journal.pone.0036869.

Othman AS. 1997. Molecular systematics of the tropical aquatic plant genus, 'Cryptocoryne' Fischer ex Wydler (Araceae). University of St Andrews.

Qu XJ, Moore MJ, Li DZ, Yi TS. 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.

Raubeson LA, Jansen RK. 2005. Chloroplast genomes of plants. In: Plant diversity and evolution: genotypic and phenotypic variation in higher plants. CABI. p. 45–68. doi:10.1079/9780851999043.0045.

Reumer JWF. 1984. Cytotaxonomy and evolution in *Cryptocoryne* (Araceae). Genetica. 65(2):149–158. doi:10.1007/BF00135279.

Sun Y, Zou P, Jiang N, Fang Y, Liu G. 2021. Comparative analysis of the complete chloroplast genomes of nine *Paphiopedilum* species. Front Genet. 12:772415. doi:10.3389/fgene.2021.772415.

Talkah NSM, Wongso S, Othman AS. 2022. Complete chloroplast genome data for *Cryptocoryne elliptica* (Araceae) from Peninsular Malaysia. Data Brief. 42:108075. doi:10.1016/j.dib.2022.108075.

Tanaka N, Tanaka N, Ohi-Toma T, Murata J. 2007. New or noteworthy plant collections from Myanmar (2) *Aponogeton lakhonensis*, *Cryptocoryne cruddasiana*, *C. crispatula* var. balansae and *Stichoneuron membranaceum*. Journal of Japanese Botany. 82:266.

Verbruggen H, Maggs CA, Saunders GW, Le L, Gall HSY, De O. 2010. Data mining approach identifies research priorities and data requirements for resolving the red algal tree of life. BMC Evol Biol. 10(1):16. doi:10.1186/1471-2148-10-16.

Wongso S, Bastmeijer JD, Budianto H, Ipor IB, Munk KR, Ørgaard M, Jacobsen N. 2017. Six new *Cryptocoryne taxa* (Araceae) from Kalimantan, Borneo. Willdenowia. 47(3):325–339. doi:10.3372/wi.47.47314.

Xue ZQ, Xue JH, Victorovna KM, Ma KP. 2017. The complete chloroplast DNA sequence of *Trapa maximowiczii* Korsh. (Trapaceae), and comparative analysis with other Myrtales species. Aquat Bot. 143:54–62. doi:10.1016/j.aquabot.2017.09.003.

Yin S, Chen H, Wu W, Gao Y. 2024. The complete chloroplast genome assembly of *Amorphophallus tonkinensis* Engler and Gehrmann 1911 from southwestern China. Mitochondrial DNA B Resour. 9(5):592–596. doi:10.1080/23802359.2024.2349771.

Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT. 2020. PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Mol Ecol Resour. 20(1):348–355. doi:10.1111/1755-0998.13096.

Zhao HY, He MX, Liu RH, Jiang Y, Liang SC. 2017. Preliminary study on vascular plants of key protected wetlands in China. Wetland Sci. 15: 532–539. doi:10.13248/j.cnki.wetlandsci.