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PLASTOME REPORT

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Complete chloroplast genome and phylogenetic analysis of *Richardia brasiliensis* (Rubiaceae, Rubioideae, Spermacoceae)

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ABSTRACT

We determined the chloroplast genome of *Richardia brasiliensis*. The genome was 154,573 bp with a typical quadripartite structure: a large single-copy region (84,388 bp), a small single-copy region (17,411 bp), and two inverted repeat regions (26,387 bp each). It contained 128 genes: 83 protein-coding genes (PCGs), 37 transfer RNA, and eight ribosomal RNA genes. For phylogenetic inference, 77 shared PCGs were concatenated from the chloroplast genomes of 52 taxa, revealing that *R. brasiliensis* is the most closely related to *Oldenlandia corymbosa*. This chloroplast genome provides a valuable resource for Spermacoceae and facilitates future studies to resolve intergeneric relationships.

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Introduction

Richardia brasiliensis Gomes, 1801 belongs to the tribe Spermacoceae (Rubiaceae), the largest herbaceous lineage in the subfamily Rubioideae, predominantly distributed in tropical regions (Groeninckx et al., 2009; Kårehed et al., 2008; Nuñez-Florentin et al., 2023; Razafimandimbison & Rydin, 2024). Phylogenetic studies have broadened the circumscription of Spermacoceae by incorporating taxa formerly treated as Manettieae and the Hedyotis–Oldenlandia group, resulting in a large tribe of ca. 76 genera and over 1,000 taxa (Groeninckx et al., 2009; Nuñez-Florentin et al., 2023). Nevertheless, intergeneric relationships remain poorly resolved, partly because chloroplast genomes in the tribe are relatively conserved and commonly used plastid markers (e.g. *atpB* and *rbcL*) provide limited variation and low generic-level resolution, prompting efforts to identify more variable regions, including non-coding loci (Groeninckx et al., 2009; Kårehed et al., 2008; Razafimandimbison & Rydin, 2024).

Whole-chloroplast genome data generated by next-generation sequencing provide abundant phylogenetically informative variation, enabling more precise inference of relationships among taxa (Walker et al., 2014). Comparative genomic analyses allows identifying highly variable regions for developing species-specific markers and improving phylogenetic resolution at lower taxonomic levels (Liu et al., 2020; Tang et al., 2022). These approaches are useful for resolving taxonomic issues within Spermacoceae, where intergeneric relationships remain unclear.

Despite the diversity of Spermacoceae, reported chloroplast genomes remain limited and do not cover all genera. To achieve a clearer understanding of the tribal phylogenetic framework, additional chloroplast genome sequences from underrepresented taxa are essential. Accordingly, we aimed to determine the complete chloroplast genome sequence of *R. brasiliensis*, within Spermacoceae, for which no chloroplast genome has yet been reported. The genomic information obtained in this study is expected to provide molecular evidence for distinguishing genera within Spermacoceae, including *R. brasiliensis*.

Material and methods

Sampling and DNA extraction

Richardia brasiliensis was identified and collected by ES Kang on August 10, 2024, in Susan-ri, Jeju-si, Republic of Korea (N 33.44090, E 126.82319) (Figure 1). Fresh leaves were immediately dried and preserved in silica gel after collection, and the voucher specimen (KHB1663521) was deposited in the herbarium of the Korea National Arboretum (KH) (http://www.nature.go.kr; contact: Dong Chan Son, sdclym@korea.kr).

DNA was extracted from the dried leaf material using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. The quality of the extracted DNA was assessed using 2% agarose gel electrophoresis, and high-quality genomic DNA was obtained. Sequencing was

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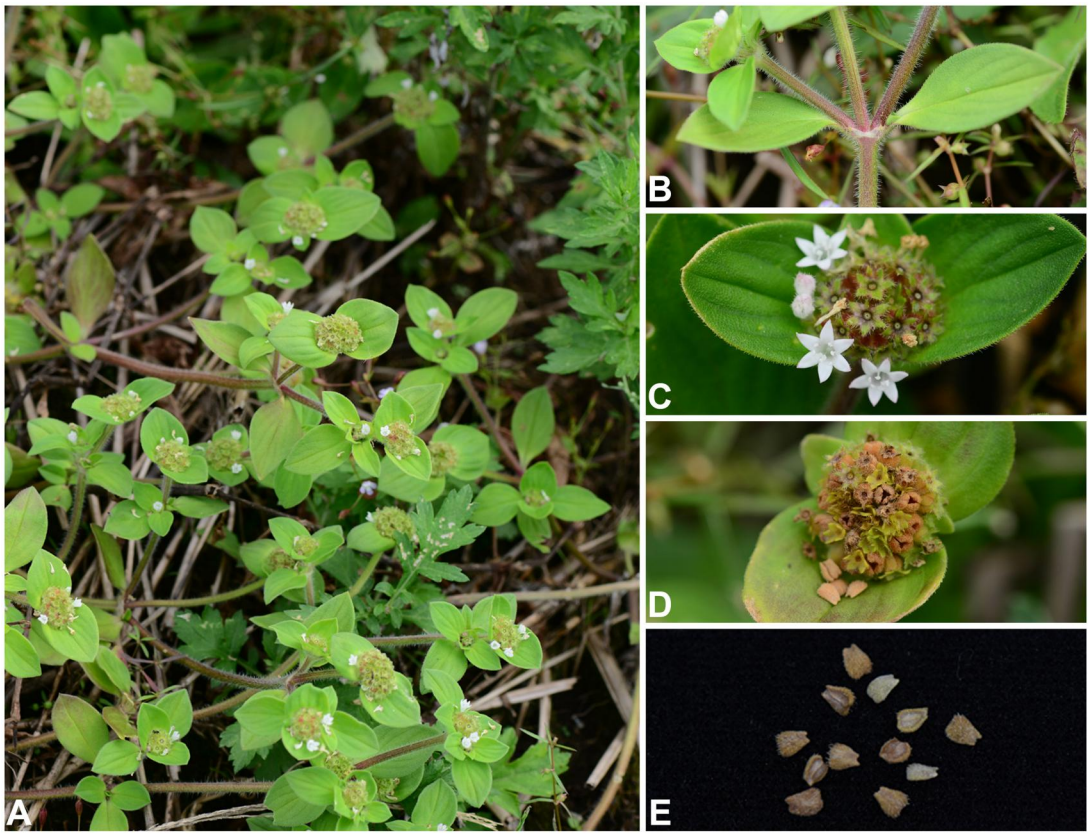

Figure 1. *Richardia brasiliensis*, photographed by Eun Su kang in Susan-Ri, Jeju-Si, Republic of Korea. A. Native to South America, the species commonly grows as a weed along roadsides and in cultivated fields. B. The leaves are opposite, and the petioles are fused with those of the opposite leaves to form a sheath, within which stipules are present. C. The capitate inflorescence bears more than 20 flowers; the flowers are white with a pale purple margin. The calyx and corolla are tubular, and the corolla is 6-lobed. D. At the fruiting stage, the tubular calyx is shed, and the light brown schizocarp splits into three mericarps. E. The broadly obovate mericarp is slightly constricted at both ends, with tubercles and stiff hairs on the dorsal surface, whereas the ventral surface is concave with a keel-like ridge along the center.

performed on an Illumina MiSeq platform to generate 301 bp paired-end reads, yielding 7,074,510 reads.

Assembly and annotation of the chloroplast genome

Chloroplast genome assembly was performed using NOVOPlasty v4.3.5, with the *atpB* sequences of *Diodia virginiana* (KX910957) retrieved from the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/) as a seed sequence. The sequencing coverage depth across the assembled plastome was then assessed following the procedure described by Ni et al. (2023) using the Python script Draw_SequencingDepth.py. Genome annotation was initially conducted using GeSeq (Tillich et al., 2017), and gene order was subsequently verified by comparison with the chloroplast genome of *Scleromitrion diffusum* (accession no. PP078674) retrieved from NCBI. Circular genome maps and schematic representations of cis- and trans-splicing events were visualized using CPGView (http://www.1 kmpg.cn/cpgview).

Phylogenetic analysis

To investigate the phylogenetic relationships of *R. brasiliensis* with closely related taxa, complete chloroplast genome sequences of 52 taxa were collected: 47 taxa in Rubioideae available from NCBI (including the target species) and five taxa from three tribes (Cinchonoideae, Ixoreae, and Gardenieae) used as outgroups. After removing redundant data, 77 protein-coding genes (PCGs) shared among all taxa were extracted and concatenated. A sequence alignment was generated using MAFFT implemented in PhyloSuite v1.2.2 (Zhang et al., 2020), and the best-fit nucleotide substitution model was selected using ModelFinder v2.2.0 (Kalyaanamoorthy et al., 2017). The maximum likelihood (ML) phylogenetic tree was inferred using IQ-TREE v1.2.3 (Nguyen et al., 2015) under the TVM+F + R3 model with 1,000 bootstrap replicates. Bayesian inference (BI) was performed using MrBayes v3.2.7 under the GTR+F + I + G4 model for 2,000,000 generations with a burn-in of 25%. Posterior probabilities (PP) were used to assess branch support. The resulting phylogenetic tree was visualized using FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree).

Results

Chloroplast genome

The chloroplast genome of *R. brasiliensis* was assembled with an average coverage of 302.93× (Figure S1). The complete genome was 154,573 bp, with an overall GC content of

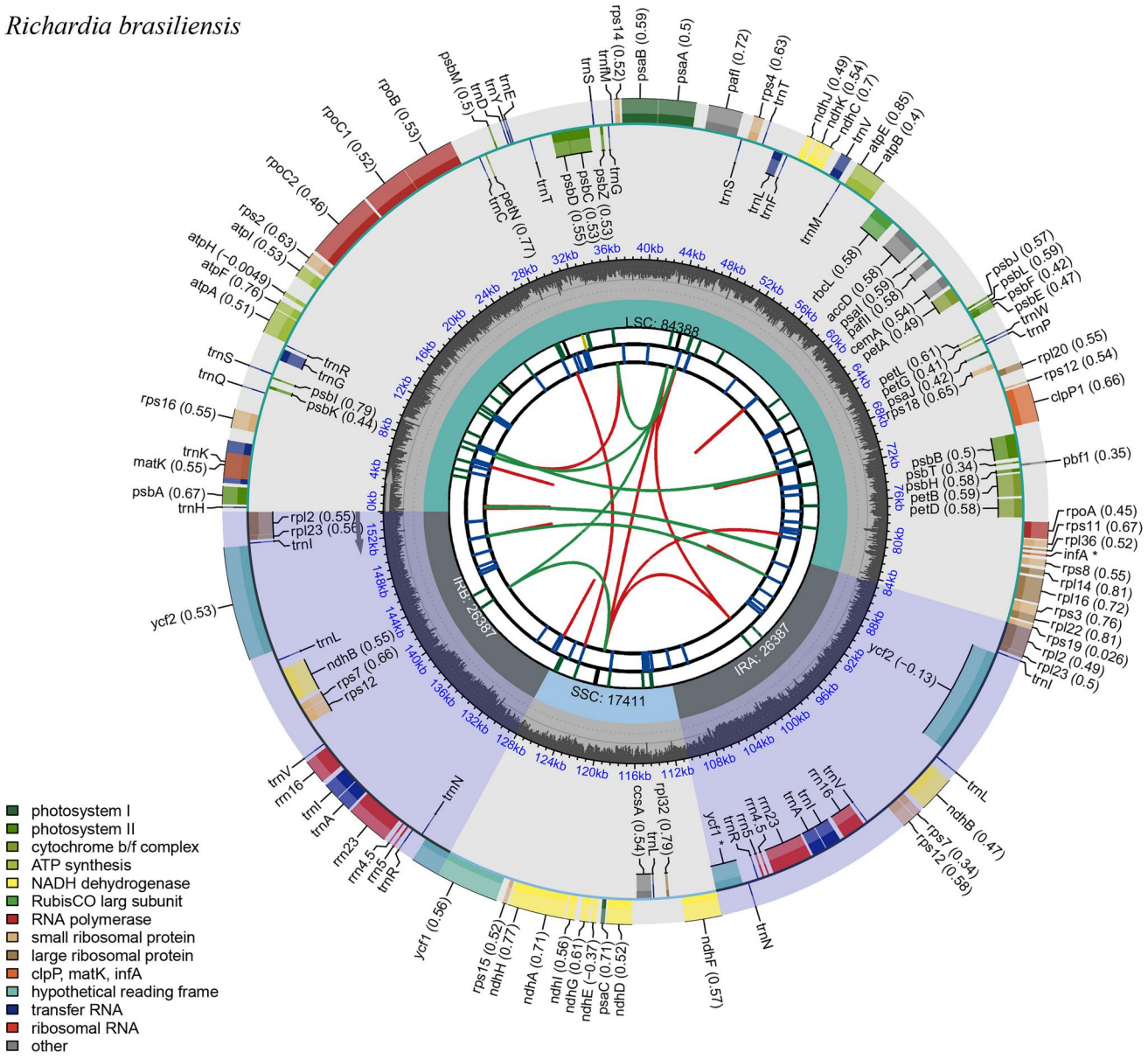

Figure 2. Chloroplast genome map of *Richardia brasiliensis*. The map comprises six distinct tracks. From the center outward, the innermost track shows dispersed repeats connected by red and green arcs, indicating the forward and reverse directions, respectively. The next track highlights long tandem repeats as blue bands, followed by a track marking short tandem repeats (microsatellites), represented as green bands. Another track depicts the GC content across the plastome. The outermost track displays genes as colored boxes, with the inner boxes indicating clockwise transcription and the outer boxes indicating counterclockwise transcription. Numbers in parentheses following gene names represent optional codon usage bias.

36.7%. It exhibited a typical quadripartite structure consisting of two inverted repeat (IR) regions separating a large single-copy (LSC) and small single-copy (SSC) regions (Figure 2). The LSC was 84,388 bp; the SSC, 17,411 bp; and each IR, 26,387 bp. The finalized chloroplast genome has been deposited in NCBI under accession number PX999661.

The chloroplast genome contained 128 genes: 83 PCGs, 37 transfer RNA (tRNA), and 8 ribosomal RNA (rRNA) genes. Among these, six PCGs (*ndhB*, *rpl2*, *rpl23*, *rps7*, *rps12*, and *ycf2*), seven tRNAs (*trnA-UGC*, *trnI-CAU*, *trnI-GAU*, *trnL-CAA*, *trnN-GUU*, *trnR-ACG*, and *trnV-GAC*), and four rRNAs (*rrn4.5*, *rrn5*, *rrn16*, and *rrn23*) were duplicated in the IR region.

Among the PCGs, those containing introns include eight cis-splicing genes with a single intron (*ndhA*, *ndhB*, *petB*, *petD*, *rpl2*, *rpl16*, *rpoC1*, and *rps16*) and two genes with two introns (*clpP1* and *pafI*) (Figure S2A). The 3′ end of the trans-splicing gene *rps12* was located in the LSC region, and its 5′ end was duplicated in the IR regions (Figure S2B).

Phylogenetic analysis

The ML and BI analyses yielded congruent topologies. Phylogenetic analyses showed that taxa belonging to Spermacoceae formed a distinct clade within Rubioideae and diverged earlier than taxa in other tribes, including Anthospermeae, Paederieae, and Rubieae (Figure 3). Within Spermacoceae, the *Hedyotis* group diverged first, followed by *Richardia*, *Oldenlandia*, and *Scleromitrion*. Among the taxa included in this study, *R. brasiliensis* was the most closely related to *Oldenlandia corymbosa*.

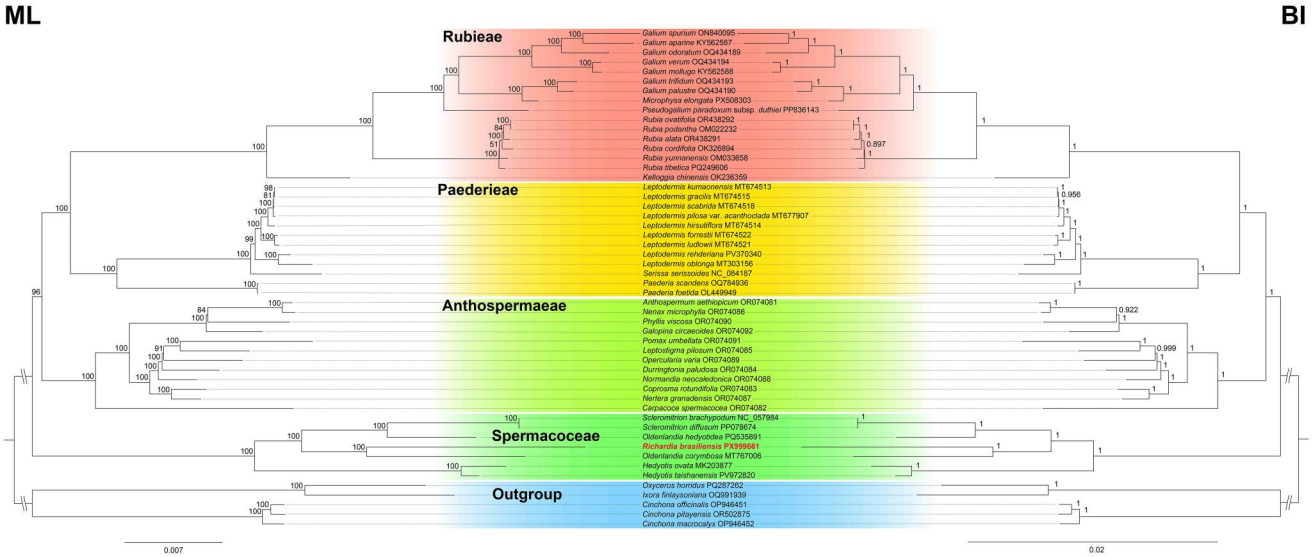

Figure 3. Maximum likelihood (left) and Bayesian (right) phylogenetic tree inferred from 77 concatenated chloroplast protein-coding genes (PCGs) from 52 taxa, including 47 Rubioideae taxa (with *Richardia brasiliensis*) and five outgroup taxa representing three tribes (Cinchonoideae, Ixoreae, and Gardenieae). The chloroplast genome references shown in the figure are as follows (from top to bottom): *Galium spurium* ON840095 (Yin et al., 2023); *Galium aparine* KY562587 (Dann et al., 2017); *Galium odoratum* OQ434189 (Ciborowski et al., 2024); *Galium verum* OQ434194; *Galium mollugo* KY562588 (Dann et al., 2017); *Galium trifidum* OQ434193 (Ciborowski et al., 2024); *Galium palustre* OQ434190 (Ciborowski et al., 2024); *Microphysa elongate* PX508303; *Pseudogalium paradoxum* PP836143 (Yu et al., 2025); *Rubia ovatifolia* OR438292 (Yu et al., 2025); *Rubia podantha* OM022232 (Zhao et al., 2023); *Rubia alata* OR438291 (Yu et al., 2025); *Rubia cordifolia* OK326894 (Chen et al., 2023); *Rubia yunnanensis* OM033658 (Zhao et al., 2022); *Rubia tibetica* PQ249606 (Li et al., 2025); *Kelloggia chinensis* OK236359 (Yang et al., 2022); *Leptodermis kumaonensis* MT674513 (Zhang et al., 2021); *Leptodermis gracilis* MT674515 (Zhang et al., 2021); *Leptodermis scabrida* MT674518 (Zhang et al., 2021); *Leptodermis pilosa* var. *acanthoclada* MT677907 (Zhang et al., 2021); *Leptodermis hirsutiflora* MT674514 (Zhang et al., 2021); *Leptodermis forrestii* MT674522 (Zhang et al., 2021); *Leptodermis ludlowii* MT674521 (Zhang et al., 2021); *Leptodermis rehderiana* PV370340; *Leptodermis oblonga* MT303156 (Guo et al., 2021); *Serissa serissoides* NC_084187; *Paederia scandens* OQ784936 (Wu et al., 2024); *Paederia foetida* OL449949 (Wang et al., 2022); *Anthospermum aethiopicum* OR074081 (Thureborn et al., 2024); *Nenax microphylla* OR074086 (Thureborn et al., 2024); *Phyllis viscosa* OR074090 (Thureborn et al., 2024); *Galopina circaeoides* OR074092 (Thureborn et al., 2024); *Pomax umbellate* OR074091 (Thureborn et al., 2024); *Leptostigma pilosum* OR074085 (Thureborn et al., 2024); *Opercularia varia* OR074089 (Thureborn et al., 2024); *Durringtonia paludosa* OR074084 (Thureborn et al., 2024); *Normandia neocaledonica* OR074088 (Thureborn et al., 2024); *Coprosma rotundifolia* OR074083 (Thureborn et al., 2024); *Nertera granadensis* OR074087 (Thureborn et al., 2024); *Carpacoce spermacocea* OR074082 (Thureborn et al., 2024); *Scleromitrion brachypodum* NC_057984; *Scleromitrion diffusum* PP078674; *Oldenlandia hedyotidea* PQ535891; *Richardia brasiliensis* PX999661 (this study); *Oldenlandia corymbose* MT767006 (Yik et al., 2021); *Hedyotis ovata* MK203877 (Zhang et al., 2019); *Hedyotis taishanensis* PV972820 (Li, 2025); *Oxyceros horridus* PQ287262 (Thi Huynh et al., 2024); *Ixora finlaysoniana* OQ991939 (Nguyen et al., 2026); *Cinchona pitayensis* OR502875; *C. officinalis* OP946451; *C. macrocalyx* OP946452.

Discussion and conclusion

The chloroplast genome length of *R. brasiliensis* was within the range reported for other Spermacoceae taxa (152,327–154,676 bp) (Li, 2025; Yik et al., 2021; Zhang et al., 2019), with no notable differences in gene order compared to that of closely related taxa. Although some regions exhibited relatively low coverage, no gaps were detected, thereby indicating a reliable assembly (Figure S1).

Both ML and BI analyses indicated that Spermacoceae forms a well-supported monophyletic clade, within which *Hedyotis* represents an early-diverging lineage, followed by a clade comprising *Richardia* and *Oldenlandia*. Within this clade, *R. brasiliensis* exhibited the closest phylogenetic relationship with *O. corymbosa*. These results are consistent with those of previous studies (Groeninckx et al., 2009; Kårehed et al., 2008; Nuñez-Florentin et al., 2023) showing close relationships among Spermacoceae taxa despite their historically variable taxonomic treatments (Robbrecht, 1988). Meanwhile, the placement of *Richardia* as a sister to *Oldenlandia* may reflect differences in inferred relationships with closely related taxa, potentially influenced by taxon sampling and plastome-scale data.

However, despite the high diversity within Spermacoceae, the present analysis included taxa representing only four genera, which limited the resolution of intergeneric and interspecific relationships within the tribe. Furthermore, insufficient taxon sampling may have constrained the identification of highly variable genomic regions. A clearer understanding of these relationships will require additional phylogenetic analyses incorporating a broader range of taxa in future studies.

Nevertheless, our results provide a valuable foundation for phylogenetic studies of Spermacoceae. Future analyses with broader taxon sampling are expected to further clarify the complex intergeneric relationships within the tribe.

Ethical approval

The materials used in this study are not listed in the IUCN Red List, and the collection area is not designated as a protected site. This study complied with the Act on the Creation and Furtherance of Arboretums and Gardens.

Author contributions

CRediT: **Eun Su Kang**: Conceptualization, Resources, Writing – original draft; **Hye Been Kim**: Data curation, Investigation, Visualization – review & editing; **Ju Eun Jang**: Data curation, Investigation, Visualization – review & editing; **Dong Chan Son**: Formal analysis, Funding acquisition, Project administration, Writing – review & editing.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The complete chloroplast genome sequence data for *Richardia brasiliensis* is available in GenBank of NCBI (https://www.ncbi.nlm.nih.gov) under accession number PX999661. The associated BioProject, Bio-Sample, and SRA numbers are PRJNA1423427, SAMN55345201, and SRR37227059, respectively.

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