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PLASTOME REPORT

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Complete chloroplast genome of Austral king fern *Todea barbara* (L.) Moore (Osmundaceae)

Hyoung Tae Kim[a] and Jung Sung Kim[b]

[a]Department of Ecological and Environmental System, Kyungpook National University, Sangju, Kyungpook, South Korea; [b]Department of Forest Science, Chungbuk National University, Cheongju, Chungbuk, South Korea

ABSTRACT

In this study, we determined the chloroplast genome sequence of the Austral king fern, *Todea barbara* (L.) Moore. The plastome of *T. barbara* is a typical circular form composed of 144,208 bp with two inverted repeats (IRs; 10,442 bp), a large single copy (LSC; 101,059 bp), and a small single copy (SSC; 22,265 bp). The complete sequence comprises 131 genes, namely 85 protein-coding genes, eight ribosomal RNAs, and 38 transfer RNAs. The guanine–cytosine (GC) content of the genome was found to be 39.9%. Additionally, U-to-C RNA editing sites were identified in eight genes: *atpE*, *chlB*, *clpP*, *matK*, *rpl20*, *rpoB*, *rpoC1*, and *rpoC2*. Phylogenetic analysis using 85 coding gene sequences revealed that the genera *Todea* and *Osmunda* form a clade and that the genus *Osmundastrum* is a sister genus to both.

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Introduction

Todea Willd. ex Bernh. is a genus of the family Osmundaceae and consists of only two species: *Todea barbara* (L.) Moore 1857 and *T. papuana* Hennipman 1968 (PPG I 2016). Unlike *T. papuana*, which occurs only in Papua New Guinea, *T. barbara*, known as the Austral king fern, is widely distributed; it is found in New Zealand, eastern and southeastern Australia, and southern Africa (Kato 2007).

T. barbara is an arborescent fern with a single trunk that grows to be massive with vegetative buds and develops a large root mantle. The fronds are large and coriaceous. Sporangia are restricted to the lower pinnules of the primary pinnae, especially in the lower part of the frond, and they are massed along the minor veinlets (Figure 1; Flora of Australia 2023, http://www.ausflora.org.au).

The first chloroplast (cp) genome reported from the family Osmundaceae, obtained from *Osmundatrum cinnamomeum* (L.) C.Presl, showed that the cp genome feature of this early diverging leptosporangiate fern family was similar to that of eusporangiate ferns (Kim et al. 2014). However, only two complete chloroplast (cp) genomes of *Osmunda* species have been sequenced, and there is, to date, no information on the remaining four genera within Osmundaceae (*Claytosmunda*, *Leptopteris*, *Plenasium*, and *Todea*). Accordingly, we sequenced the complete cp genome of *T. barbara* and analyzed the phylogenetic relationships of this species within the family Osmundaceae.

Figure 1. Photographs of *Todea barbara* captured by Ki-Joong Kim at the collection site. The Austral king fern has a barrel-shaped trunk covered in dark brown to black fibrous aerial roots. The stipes are smooth and yellow-brown, with ear-like lobes at the base. The fronds are large and coriaceous.

CONTACT Jung Sung Kim jungsung@cbnu.ac.kr Department of Forest Science, Chungbuk National University, Cheongju, Chungbuk 28644, South Korea

Materials and methods

A specimen of *Todea barbara* was collected from the Royal Botanical Garden Sydney, Sydney, Australia (33° 86′ 41.24″ S, 151° 21′ 67.96″ E) in January 2019 by Prof. Ki-Joong Kim of the Korea University and was dispensed at the Plant DNA Bank in Korea (PDBK, https://pdbk.korea.ac.kr/), located in Seoul, according to the dispensing procedure of the PDBK. In addition, the specimen was deposited at the Korea University Herbarium (https://pdbk.korea.ac.kr/, contact to Ki-Joong Kim, kimkj@korea.ac.kr) under the voucher number TA2019-0031.

High-throughput sequencing of a sample from the collected specimen was performed using an Illumina Hiseq X-ten sequencing platform. In total, 17,563,188 paired-end raw reads (151 bp in length) were generated, and the raw data were trimmed using Trimmomatic 0.39 (Bolger et al. 2014), with the options LEADING:10, TRAILING:10, SLIDINGWINDOWS:4:20, and MINLEN:50. The complete cp genome was assembled into the reference genome of *Osmunda japonica* (MK554796) using 17,539,752 trimmed reads and annotated using the protocol described by Kim and Chase (2017), except for the read-trimming stage. Lastly, the cp genome sequence was constructed with 477.2 coverage depth and submitted to the NCBI database under the accession number OP793887. In addition, a cp genome map was generated using CPGView (Liu et al. 2023; http://www.1kmpg.cn/cpgview/).

To perform a phylogenetic analysis of the family Osmundaceae, the cp genomes of three species (*Osmunda japonica*, MK554796; *Osmunda mildei*, MZ292715; and *Osmundastrum cinnamomeum*, NC024157) were utilized, and three cp genomes from *Angiopteris* (downloaded from GenBank)

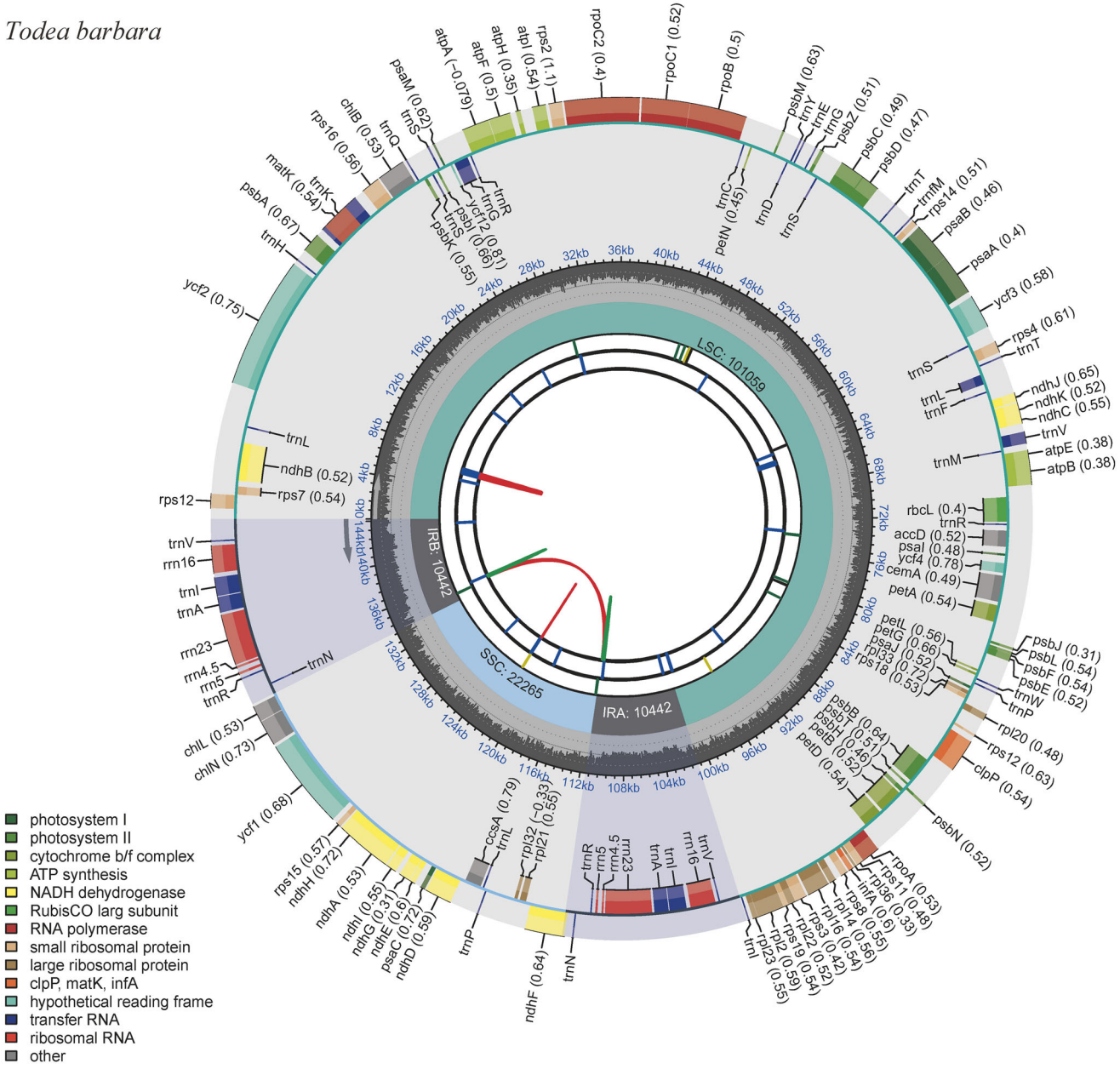

Figure 2. Complete chloroplast (cp) genome map of *Todea barbara*, containing six tracks. From the center, the first track shows the dispersed repeats, which consist of direct (red) and palindromic (green) repeats. The second and third tracks show the long and short tandem repeats, respectively. The regional composition of the genome, LSC, SSC, and IRs, are identified on the fourth track. The GC content along the genome is plotted in the fifth track. The genes are shown on the outer sixth track.

Table 1. Comparison of the complete chloroplast genomes of *Todea* and its related species used in the present study.

Species (Acc. No.)	Total length (bp)	LSC (bp)	SSC (bp)	IR (bp)	GC content (%)
Osmunda japonica (MK554796)	143,220	100,464	22,224	10,266	40.4
Osmunda mildei (MZ292715)	143,682	100,823	22,225	10,317	40.4
Osmundatrum cinnamomeum (NC024157)	142,812	100,294	22,300	10,109	40.2
Todea barbara (OP793887)	144,208	101,059	22,265	10,442	39.9

	atpE		chlB		clpP		matK						rpl20		rpoB				rpoC1		rpoC2	
	217	219	457	459	535	537	475	479	229	231	232	234	523	525	112	114	1648	1650	2857	2859	1648	1650	1060	1062
Osmunda japonica	CGA		CAA		CAA		CAA		CAA		CAG		CGA		CAA		CAA		CAG		CAA		CGA	
Osmunda mildei	CGA		CAA		CAA		CAA		CAA		CAA		CGA		CAA		CAA		CAG		CAA		CGA	
Osmundastrum cinnamomeum	CGA		CAA		CAA		CAA		CAA		CAA		CGA		CAA		CAA		CAG		CAA		CGA	
Todea barbara	TGA		TAA		TAA		TAG		TAA		TAA		TGA		TAA		TAA		TAG		TAA		TGA	

Figure 3. The internal stop codons in eight genes of *Todea barbara*. The numbers above the bases indicate the positions of the gene.

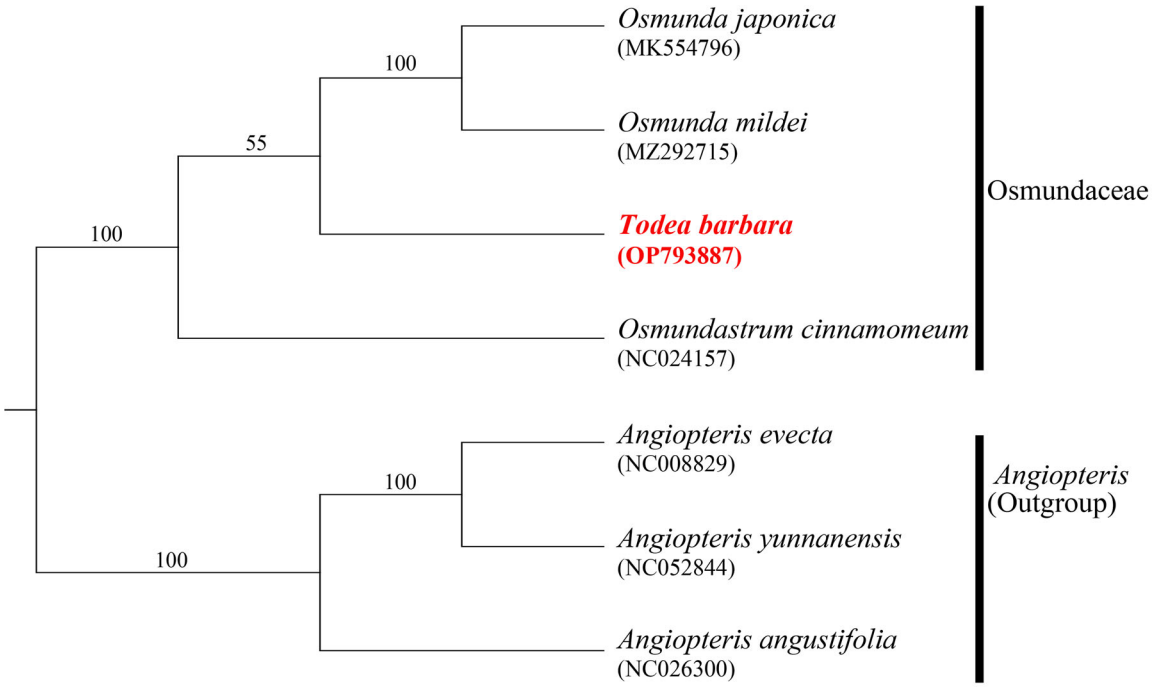

Figure 4. Maximum likelihood tree based on the 85 cp coding genes. Numbers on the nodes represent the bootstrap support values. The outgroups were three *Angiopteris* of *A. evecta* (NC024157; Roper et al. 2007), *A. yunnanensis* (NC052844; Jiang et al. 2019), and *A. angustifolia* (NC026300). Two *Osumnda* species (*O. japonica*; MK554796, Xu et al. 2019 and *O. mildei*; MZ292715) and *Osmundatrum cinnamomeum* (NC024157; Kim et al. 2014) were also included for the molecular phylogenetic analysis of the family Osmundaceae.

were treated as outgroups. Each coding gene was aligned using MAFFT (Katoh et al. 2002). The concatenated alignment using 85 coding genes was 74,123 bp long. Partition models were chosen using ModelFinder with edge-proportional partitions and merge options (Chernomor et al. 2016; Kalyaanamoorthy et al. 2017). A phylogenetic tree based on maximum likelihood analysis was constructed using IQ-TREE 2 (Minh et al. 2020) with 10,000 ultrafast bootstraps (Hoang et al. 2018). The IQ-TREE condition was iqtree2.exe-s Concatenated.phy-p partition_length.nex-m MFP + MERGE-bb 10000.

Results and discussion

The complete cp genome of *Todea barbara* (OP793887, Figure 2) is 144,208 bp in length, with two inverted repeats (IRs; 10,442 bp) between a large single copy (LSC; 101,059 bp) and a small single copy (SSC; 22,265 bp). It is longer than other Osmundaceae cp genomes recorded to date (Table 1) and comprises 131 genes, namely 85 protein-coding genes, eight ribosomal RNAs, and 38 transfer RNAs. The guanine–cytosine (GC) content was 39.9%. Contrary to the other documented cp genomes of the family Osmundaceae (*Osmunda* and *Osmundastrum*), which have been shown to be similar to those of eusporangiate ferns in terms of restricted RNA editing sites (Wolf et al. 2004; Kim et al. 2014; Guo et al. 2015), eight genes in the cp genome of *T. barbara*, namely *atpE*, *chlB*, *clpP*, *matK*, *rpl20*, *rpoB*, *rpoC1*, and *rpoC2*, are likely to have internal stop codons (Figure 3) even it was not experimentally proved. Because these genes are generally conserved in the cp genomes of monilophytes, U-to-C RNA

editing events are thought to occur independently in *T. barbara* within the family. Although C-to-U RNA editing occurs more often in land plants, U-to-C RNA editing has been observed in hornwort and monilophyte organelles (Knie et al. 2016).

A phylogenetic tree of the family Osmundaceae, constructed using 85 coding gene sequences, revealed that the genera *Todea* and *Osmunda* form a clade in the family, and the genus *Osmundastrum* is sister to them (Figure 4). This finding is consistent with the previously reported phylogenetic relationships among genera in the family Osmundaceae, as determined by analyzing seven cp genes (Metzgar et al. 2008). However, the low bootstrap value for the clade composed of *Todea* and *Osmunda* appears to be due to the restricted number of taxa, in particular, the lack of taxa closely related to *Leptoptaris*, which is regarded as a sister to *Todea* (Yatabe et al. 1999; Jud et al. 2008; Carvalho et al. 2013).

Conclusions

The complete cp genome of *Todea Barbara*, investigated for the first time in this study, was found to have a typical circular form composed of 144,208 bp and 131 genes. Based on molecular phylogenetic analysis using 85 coding gene sequences of cp genome, we determined that the genera *Todea* and *Osmunda* form a clade in the family, and the genus *Osmundastrum* is sister to them. In addition, U-to-C RNA editing sites were observed in eight genes: *atpE*, *chlB*, *clpP*, *matK*, *rpl20*, *rpoB*, *rpoC1*, and *rpoC2* in the cp genome of *T. barbara*.

Acknowledgment

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Ethical approval

Todea barbara is not included in the list of Australian threatened plants (https://www.environment.gov.au/cgi-bin/sprat/public/publicthreatenedlist.pl?wanted=flora) and was collected with permission from the Royal Botanical Garden Sydney. Research on this species, including the collection of plant materials, was conducted following the guidelines provided by Korea University and Kyoungpook National University.

Author contributions

Conceiving and designing the study, JSK and HTK; performing and analyzing data, HTK; writing the original draft, JSK and HTK; review and editing, JSK; supervision, JSK. All authors have read and agreed to the published version of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The genome sequence data supporting the findings of this study are available from NCBI GenBank under accession no. OP793887 (https://www.ncbi.nlm.nih.gov/nuccore/OP793887.1/). The associated BioProject, SRA, and Bio-Sample numbers are PRJNA954512, SRR24137031, and SAMN34146952, respectively.

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