



## The complete chloroplast genome sequence of *Haplopteris flexuosa* (Fée) E.H. Crane

Hyoung Tae Kim<sup>a</sup>  and Jung Sung Kim<sup>b</sup> 

<sup>a</sup>Institute of Agriculture Science and Technology, Chungbuk National University, Cheongju, Chungbuk, Republic of Korea; <sup>b</sup>Department of Forest Science, Chungbuk National University, Cheongju, Chungbuk, Republic of Korea

### ABSTRACT

The complete chloroplast genome sequence of *Haplopteris flexuosa*, a member of Vittarioideae (family Pteridaceae), was determined. The chloroplast genome of *H. flexuosa* was 165,664 bp in length with two inverted repeats (32,556 bp) between a large single copy (79,996 bp) and a small single copy (20,556 bp). The GC content was higher than that of related taxa *H. elongata*, and it was caused by high GC content of expanded regions by insertion of mobile open reading frames in fern organelles (MORFFO) found in the family Pteridaceae. And also, we found that *rrn5-rps12* enlarged region of *H. flexuosa* was similar to MORFFOs of other Pteridaceae.

### ARTICLE HISTORY

Received 23 December 2020  
Accepted 18 March 2021

### KEYWORDS

*Haplopteris flexuosa*;  
chloroplast genome;  
MORFFO; GC content



Subfamily Vittarioideae, which belongs to Pteridaceae, is comprised of 12 genera and 345 species (PPG I 2016). Among them, *Haplopteris* C. Presl is characterized by bilateral monolete spores, paraphyses with infundibular apical cells, wide leaves (> 4 mm), bilateral rhizome, and paleotropical distribution (Crane 1997).

*Haplopteris flexuosa* (Fée) E.H. Crane is widely distributed at Bhutan, Cambodia, China, India, Japan, Korea, Laos, Myanmar, Nepal, Thailand, and Vietnam (Wu et al. 2013). As a huge range of habitat, this species has various morphological forms depending on its ecological conditions but constants in its scale, paraphyses with infundibular apical cells, and margin rolled backward to partly cover sori (Wu et al. 2013). The monophyly of these morphological forms is also well-supported by maximum likelihood analysis using chloroplast (cp) DNA genes (Chen et al. 2017).

In this paper, we completely sequenced the cp genome of *H. flexuosa* which was collected from Youngcheon-dong, Seogwipo-si, Jeju island, Korea (33°18'02.83"N, 126°34'46.07"E, alt. 292 m) and the voucher specimen (CBNU2018-0146) was deposited at the Herbarium of Chungbuk National University (CBNU). From a leaf dehydrated using silica-gel, total genomic DNA was extracted by following the manufacturer's protocol for the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and was sequenced using HiSeq X Ten of Illumina (Illumina, San Diego, USA). Totally 2,545,669,740 paired-end reads (151 bp in length) were obtained and they were trimmed by trimmomatic 0.39 (Bolger et al. 2014) with the option LEADING:10, TRAILING:10, SLIDINGWINDOW:4:20, MINLEN:50. And then 2,207,178,626 reads remained and they were assembled to the reference

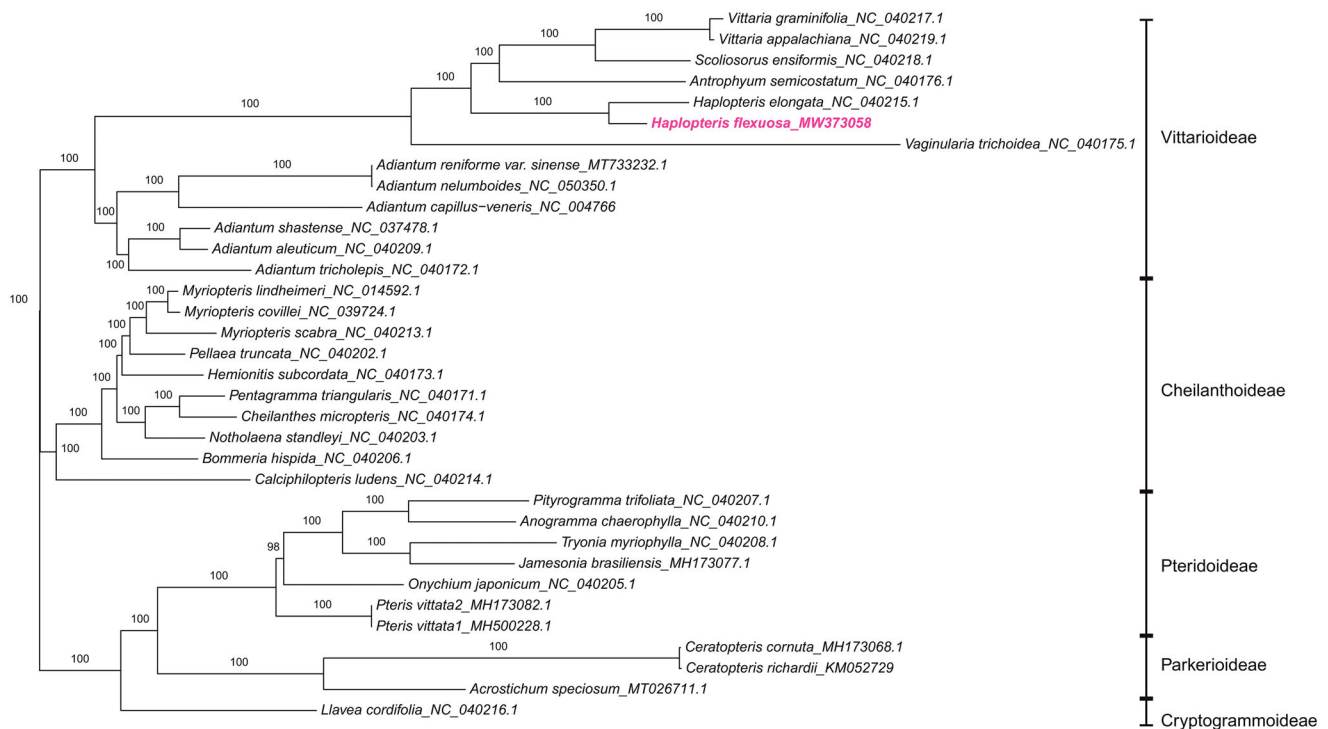
plastome of *H. elongata* (NC 040175) following Kim and Chase (2017). Finally, a circular chloroplast genome sequence was constructed with 219.8 coverage depth. All genes were annotated based on existing genes in other fern chloroplast genomes using Geneious 10.2.6 (Kearse et al. 2012). Each coding gene was aligned using MAFFT (Katoh et al. 2002). The concatenated alignment using 84 coding genes was 74,116 bp in length. Partition models were selected using ModelFinder with edge-proportional partition and merge options (Chernomor et al. 2016; Kalyaanamoorthy et al. 2017). Maximum likelihood analysis was constructed using IQ-Tree 2 (Minh et al. 2020) with 1,000 ultrafast bootstraps (Hoang et al. 2018).

The cp genome of *H. flexuosa* (MW 373058) was 165,664 bp in length with two inverted repeats (IRs; 32,556 bp) between a large single copy (LSC; 79,996 bp) and a small single copy (SSC; 20,556 bp). It had 128 genes consisting of 87 protein-coding genes, eight ribosomal RNAs, 31 transfer RNAs, and two pseudogenes of *rps16* and *trnV-UAC*. The GC content was 41.2%. As a result, the cp genome of *H. flexuosa* was 9,662 bp longer in length and 1.1% higher in GC content compared with the cp genome of related taxa *H. elongata* (NC 040215). It was caused by two expanded regions. The enlarged region between *rps7* and *psbA* was 5,127 bp in length with 51.1% GC content which was first expanded region by the insertion of mobile open reading frames in fern organelles (MORFFO) (Robison et al. 2018) in Pteridaceae. This enlarged region was similar to *rrn5-rps12* enlarged region of *H. elongata*. However, *rrn5-rps12* enlarged region of *H. flexuosa* which was 5,413 bp in length with 48.0% GC content did not correspond to any cp regions of *H.*

**CONTACT** Jung Sung Kim  [jungsung@chungbuk.ac.kr](mailto:jungsung@chungbuk.ac.kr)  Department of Forest Science, Chungbuk National University, Cheongju, Chungbuk, Republic of Korea

© 2021 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.



**Figure 1.** Phylogenetic relationships of *Haplopteris flexuosa* and its related taxa based on the complete chloroplast genome sequences..

*elongata*. Based on the blast search, *rrn5-rps12* region of *H. flexuosa* was similar to MORFFOs of other Pteridaceae.

From the result of molecular phylogenetic analysis, the clade of the genus *Haplopteris* was strongly supported and was sister to the clade of *Vittaria* + *Scoliosorus*+ *Antrophyum* (Figure 1).

## Disclosure statement

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

## Funding

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education [2018R1D1A3B07048213, 2020R1I1A2053517].

## ORCID

Hyoung Tae Kim  <http://orcid.org/0000-0003-1823-4369>

Jung Sung Kim  <http://orcid.org/0000-0002-7658-6330>

## Data availability statement

The genome sequence data that support the findings of this study is available in GenBank of NCBI at (<https://www.ncbi.nlm.nih.gov/>) under the accession no. MW373058.

## References

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 30(15):2114–2120.

Chen C-W, Lindsay S, Kuo L-Y, Fraser-Jenkins CR, Ebihara A, Luu HT, Park CW, Chao Y-S, Huang Y-M, Chiou W-L. 2017. A systematic study of East Asian vittarioid ferns (Pteridaceae: Vittarioideae). *Bot J Linn Soc*. 183(4):545–560.

Chernomor O, von Haeseler A, Minh BQ. 2016. Terrace aware data structure for phylogenomic inference from supermatrices. *Syst Biol*. 65(6): 997–1008.

Crane EH. 1997. A revised circumscription of the genera of the fern family Vittariaceae. *Systematic Botany*. 22(3):509–517 (English).

Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. 2018. UFBoot2: improving the ultrafast bootstrap approximation. *Mol Biol Evol*. 35(2):518–522.

PPG I. 2016. A community-derived classification for extant lycophytes and ferns. *J Syst Evol*. 54(6):563–603.

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermin LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat Methods*. 14(6):587–589.

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res*. 30(14):3059–3066.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*. 28(12): 1647–1649.

Kim HT, Chase MW. 2017. Independent degradation in genes of the plastid *ndh* gene family in species of the orchid genus *Cymbidium* (Orchidaceae; Epidendroideae). *PLOS One*. 12(11):e0187318.

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A, Lanfear R. 2020. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Mol Biol Evol*. 37(5):1530–1534.

Robison TA, Grusz AL, Wolf PG, Mower JP, Fauskee BD, Sosa K, Schuettpelz E. 2018. Mobile elements shape plastome evolution in ferns. *Genome Biol Evol*. 10(10):2558–2571.

Wu Z, Raven P, Hong D. 2013. *Flora of China*. Vol. 2–3 (Pteridophytes). Beijing: Science Press, St. Louis: Missouri Botanical Garden Press.