Two Particularly Evolutionary Loci of \textit{trnL-ndhJ} of cpDNA of Yulania Baotaina, a New Species (Magnoliaceae) from China

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To cite this article:
Da-Li Fu, Qun Zhang, Min Xu, Dao-Shun Zhou, Yue Qin, Yin-Meng Li. Two Particularly Evolutionary Loci of \textit{trnL-ndhJ} of cpDNA of Yulania Baotaina, a New Species (Magnoliaceae) from China. \textit{American Journal of Agriculture and Forestry}. Special Issue: \textit{The New Evolutionary Theory & Practice}. Vol. 7, No. 5, 2019, pp. 229-233. doi: 10.11648/j.ajaf.20190705.19

Received: August 30, 2019; Accepted: September 16, 2019; Published: September 23, 2019

Abstract: The partial sequence of \textit{trnL-ndhJ} of chloroplast genome of the new species of the genus Yulania Spach of Magnoliaceae, Yulania baotaina D. L. Fu, Q. Zhang et M. Xu, collected from Baotai Mountain of Yongping County of Yunnan province of China, and the similar species, Y. campbellii (Hook. f. et Thoms.) D. L. Fu, collected from Yongping and Gongshan county of Yunnan, and Yadong county of Xizang, were amplified and sequenced respectively, which were compared with 39 samples of the chloroplast complete genomes of Magnoliaceae in the NCBI (National Center for Biotechnology Information, USA) database. The results indicated that Y. baotaina has two PEL (particularly evolutionary loci): \textit{trnL-ndhJ} (...TTTCA TTTCGGAA TTTCCTA TTTTCTT...) not possessed by others, and the oppositely evolutionary loci of transversions \textit{trnL-ndhJ} (...TTTCA TTTCTGAA TTTCATA TTTTCTT...) is possessed by all contrasted experimental materials and most simples of Magnoliaceae in NCBI. So the new species was described and illustrated. Its floral buds cylindrical, ovaries subcordiform, and peduncles of flower and fruits obviously elongate are particular, which can be easily distinguished to the similar species and all other ones in the genus. The new species is distributed in Hengduan Mountain of west Yunnan province and south Xizang Autonomous Region at an altitude of 1900–3000 m, a colloquial name is Mulianhua, just as the similar species Y. campbellii. The designated type of the new species was collected from Baotai Mountain of Yongping County at altitude of 2600 m and kept at the Chinese Academy of Forestry. This paper will provide a new research method that can be easily verified for the evolutionary taxonomy and the new science of evolutionomy.

Keywords: PEL (Particularly Evolutionary Loci), Yulania Baotaina, \textit{trnL-ndhJ}, Evolutionomy, New Species, Magnoliaceae

1. Introduction

As the most primitive taxa of Fructophyta D. L. Fu & H. Fu [1], Yulania Spach [2-10] has a very important position and plays an important role in the evolutionomy of fruit plants. The large and beautiful early spring flowers of Yulania Spach were also deeply impressed and loved by the world's gardeners. For example, “A variable species, spectacular in bloom, native to a wide area of the Himalayan region from E Nepal through Bhutan, N Assam, Upper Burma (Myanmar), SE Tibet (Xizang), into Yunnan and Szechwan (Sichuan) provinces, China”[11], Yulania campbellii (Hook. f. et Thoms.) D. L. Fu (\textit{Magnolia campbellii}) [2-6, 8-14], had attracted admirations from horticulturists in the world, just as that D. J. Callaway had written “This species is often considered by writers and gardeners to be the most spectacular of all magnolias, and the most desirable”[12]. In the investigation of the plant resources of Yulania Spach, a new species, very similar to the most spectacular and most desirable species Y. campbellii (Hook. f. et Thoms.) D. L. Fu,
was found by the authors. In order to determine its evolutionary taxonomic status, the new species and the similar species were collected from Yunnan province and Xizang Autonomous Region of China, and the partial sequences of chloroplast genome, trnL-ndhJ, were amplified and sequenced respectively. The partial sequences were also compared with 39 samples of the chloroplast complete genomes of Magnoliaceae in the NCBI (National Center for Biotechnology Information, USA) database, and the results are as follows.

2. Materials & Methods

2.1. Plant Materials

Seven samples of the leaves of two similar species were collected from China: two of the new species of Yulania baotaina from Mountain Baotai in Yongping County of Yunnan province, five of the similar species Yulania campbellii (Hook. f. et Thoms.) D. L. Fu from Yongping and Gongshan County of Yunnan province and Yadong county of Xizang Autonomous Region respectively (see Table 1).

Table 1. Experimental materials of Magnolia sect. Rytidospermum.

| Species             | Experimental material | Samples | Collected place          |
|---------------------|-----------------------|---------|--------------------------|
| Yulania baotaina    | leaves                | 2       | Yongping, Yunnan, China  |
| Y. campbellii       | leaves                | 1       | Yongping, Yunnan, China  |
| Y. campbellii       | leaves                | 2       | Gongshan, Yunnan, China  |
| Y. campbellii       | leaves                | 2       | Yadong, Xizang, China    |

2.2. PCR Primer Design

A pairs of primers, trnL-ndhJ-Y01-F and trnL-ndhJ-Y01-R were designed using Primer Premier 6, the sequences of primers and the length of amplification and sequencing of cpDNA, see Table 2.

2.3. PCR Amplification

Total genomic DNA was isolated from silica-dried leaves of 7 materials belong to 2 species, 2 samples of Y. baotaina and 5 of Y. campbellii, using a modified CTAB method [15]. The primers of PCR amplification are trnL-ndhJ_Y01 (see Table 2). PCR amplifications were performed in 15µL volume containing 0.2 µL forward primer and 0.2 µL reverse primer, 1µL genomic DNA, 7.5 µL 2x Es Taq MasterMix, 6.1 µL ddH₂O, and with the following cycles: 5 min initial denaturation at 94°C; 10 cycles of 30 s at 94°C, 45 s at 61°C and 2 min at 72°C; 27 cycles of 30 s at 94°C, 45 s at 56°C and 2 min at 72°C; and 5 min final extension at 72°C. All PCR reactions were performed in T-gradient (Biometra). The amplified products of PCR were extracted and purified with the Gel Extraction Kit (OMEGA).
2.4. DNA Sequencing

The purified DNAs were sequenced using ABI 3730 XL. The PCR amplifications were performed in 15 µL volume containing 0.2 µL forward primer and 0.2 µL reverse primer, 1 µL purified DNA, 7.5 µL 2x Es Taq MasterMix, 6.1 µL ddH2O, and with the following cycles: 3 min initial denaturation at 95°C; and 26 cycles of 10 s at 95°C, 10 s at 50°C and 4 min at 60°C.

2.5 DNA Sequence Analysis

The partial correct sequences of absolutely coinident sequencing using the forward primer and reverse primer were analyzed. The particularly evolutionary loci could be easily found out and verified using some software such as Microsoft Word. The contrasted chloroplast complete genomes of Magnoliaceae in the NCBI database see Table 3.

3. Two PEL (Particularly Evolutionary Loci) of cpDNA of Yulania Baotaina

There are 810 bp absolutely coincident sequences of Yulania baotaina, using the forward primer and reverse primer of trnL-ndhJ_Y01 respectively, which can be regarded as the correct partial cpDNA sequence of the new species. The sequence is as follow:

Yulania baotaina, partial sequence of trnL-ndhJ of cpDNA
TTCTCATTCACCTACCTCTTCTTCAAAAATGGGTCCGA
CCATAAATGTTTCTCTTTATCTACAAAGTCTTGTGTA
ATATGATATACTGAAAAATGCCCATATTATGGAAGA
GAAATTCCTATTGAACTATTCACAGTCATATACTTA
CTCCTACACTACAAAGCCTCCTTGTGGAATGC
GAAAATCAGGACCTAGTGAAGATTTTGGAATGCA
TTTGAAGCTCCCTTTAATTGACAGACCAAGTCTCTCT
AGTAGGATAGCAGCTGGAGAATGGTGGATAGCTC
AGCTGCTTAGAAGACAGCTGAAATCTCTGCTGTA
CCAGTTCAAAATCTGTGTTCTCAGCACGGTGGTTAATGTA
TCAAACGGAATACCTACATAAATGATCAGATAGTAC
GGGATCATATAATTTAATAAGCTAATAGTCTAGCCA
TAGACATTAGCTGATACTAGATACATACATTACAT
CTAAGCTATATAAATGGAATCCTCCTTCTTTTGTGAGATGG
GTAAAGAATATATGGAAGTAAAGAAGAAGAAG
GATTCTACTCTCTCTCCTTGTGTTATATGTA
CCTCCTCTGCTCAAAAGGAAATGTATACCTCCTAC
ATACATGTTAGTGGCTGGCTGAAAACCCCA
AAGTCTAGTCTAGGAGTGGAAGATAGGAAAGAA
CAGGATATCATTCCAGATACAATACAAAGAAGAAGA
TTGCACTCCTCTTCTCATTGCAATTTCTCATT
TCTTTTCTATATCTTCTCTTCTTTTTCTTATT.

Compared to its similar species, Y. campbellii, and the other species should be described and illustrated.

4. Yulania Baotaina D. L. Fu, Q. Zhang et M. Xu, sp. Nov., Figure 1

Arbor decidua usque ad 30 m alta. Ramuli crassi anotittii pallide virides vel flavo-virides nitidi sub glabri, lenticellis albis rotundis; rumulis abbreviates dense 3-nodos, crassissis, dense pallide flavo-villosiss. Gemmata foliiferae cylindraceae 1.8–3.6 cm longae et 0.4–0.8 cm diam. virides vel flavor-virides in sicco extus pallide flavo-villosae. Alabastra solitaria terminalia cylindrica 7.0–8.0 cm longa et 1.5–2.0 cm diam. extus pallide flavo-villosae. Folia alterna subcoriacea longe elliptica 15.0–23.0 cm longa et 7.5–11.5 cm lata apice acuminata basi rotundata margine integra supra viridia glabra nitida subtus pallide viridia, dense pallide albo-tomentosa, costis et nervis lateralibus dense villosis, nervis longitudinali-dehiscentibus, connetivis apice cum mucronibus atro-purpureis, thecis lateribus albo-villosis. Folliceta cylindrica 3.2–3.7 cm longa et 1.5 cm lata purpureo-rubra extus basi 3.3–4.1 cm latae et 1.0–1.2 cm diam. glabri vel sparse villosi apice saepe sparse villosi; tepala 12 in quoque flore, petaliformia simplicia, elliptica vel oblonga, 10.0–12.5 cm longa et 4.0–5.0 cm lata purpureo-rubra extus basi atro-purpurea ad purpureo-brunnea, intus pallida in colore; stamina numerosa 2.6–3.2 cm longa, filamentis 0.8–1.2 mm longis atro-purpureo-rubris, thecis lateribus longitudinali-dehiscentibus, connotitis apice cum mucronibus triangularis; gynoecium cylindricum 3.2–3.7 cm longum purpureo-brunneum, pistillis simplicibus numerosis, ovarii subcordiformibus purpureo-brunneis sparse albo-pubescentibus, stylis et stigmatibus purpureo-rubris. Folliceta cylindrica 10.0–14.0 cm longa et 2.0–3.0 cm diam. atro- ruby apice saepe conica curvata; pedicelli fructiferi robusti 3.5–4.5 cm longi et...
1.2–1.3 cm diam. apice sparse villosi.

Typus: The holotype D. L. Fu 2018031201 (Holotypus hic designatus, CAF) was branchlets with flower collected from Mountain Baotai, Yongping county, Yunnan province at alt. 2600 m on Mar. 12, 2017, and the paratype D. L. Fu 2017093001 (Paratypus, CAF) was branchlets with fruit, floral bud and leaves, collected from the same position on Sept. 09, 2017.

The new species is similar to Yulania campbellii (Hook. f. & Thoms.) D. L. Fu, but its foliar buds were yellowish-green when dried, floral buds cylindrical and villous; ovaries subcordiform, purplish-brown and sparse pubescent at the bases, peduncles of flowers and fruits obviously elongate, are different from the latter’s that foliar buds black-brown when dried, floral buds ovoid and long villous; ovaries rectangular, yellowish-green and glabrous, peduncles of flower and fruits not elongate. Floral buds cylindrical, ovaries subcordiform, peduncles of flowers and fruits obviously elongate are also the particular characters of the new species which can be easily distinguished to all other ones in the genus. So the new species is supported by cpDNA evolutionomy and morphological evolutionomy based on the evolutionary continuity principle and the evolutionary particularity principle, which provides a new research method that can be easily verified for the evolutionary taxonomy and the new science of evolutionomy.

5. Conclusion

Yulania baotaina has at least two PEL of cpDNA, which are trnL-ndhJ (...TTTCAATTCCGAATTTCCTATTTTCTT...). Its floral buds cylindrical, ovaries subcordiform, and peduncles of flower and fruits obviously elongate are particular, which can be easily distinguished to the similar species and all other ones in the genus. The new species distributed in Hengduan Mountain of west Yunnan province and south Xizang Autonomous Region at an altitude of 1900~3000 m. Flowering March-April and fruiting September-October. The colloquial name is Mulianhua, just as the similar species Yulania campbellii (Hook. f. & Thoms.) D. L. Fu.

The flowers of the new species are large, purple and colorful, and graceful. The floral buds are large and cylindrical, and the fruit is dark red and has various shapes. It is an excellent ornamental tree species. Floral buds are used in traditional Chinese medicine for "Xinyi" and are also the raw materials for extracting essence.

Acknowledgements

This work was supported by the Fundamental Research Funds for the Central Non-profit Research Institution of CAF (CAFYBB2016MA009). All sequencing was done by Beijing Boyoushun Biotechnology Limited Corporation. We are grateful to Prof. Wencai Wang, the plant taxonomist and the academician of the Chinese Academy of Sciences, for his valuable suggestions and corrections in the Latin description. We thank Professor Junkai Gao of National Forestry and Grassland Administration, Suolang Wangdui of Department of Forestry of Xizang Autonomous Region and Jianbing Yue of Forestry Investigation and Planning Institute of Xizang Autonomous Region, and Engineers Yongxing Wang, Zhaowei Hou et al. of Forest Bureau of Yongping County, Xiaoyang He and Yun Xun of Forest Bureau of Gongshan County of Yunnan province, and Qin Zhao of Forest Bureau of Linzhi City, Pengfei Zhang of Forest Bureau of Motuo County, Laba Ciren et al. of Forest Bureau of Yadong County of Xizang Autonomous Region for the helps in the investigation of the plant resources.

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