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Identification of *BaLanophora japonica* Makino. from Guizhou Province by Morphological Methods and rDNA-ITS Sequence

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Abstract *BaLanophora japonica* Makino. is reported as a newly recorded species in Guizhou Province, it was found in Zhujiashan National Forest Park of Guizhou in Weng'an Xian in Guizhou Province. its main biological characteristics were dissected and observed, and its rDNA-ITS sequences was amplified and sequenced directionally. The blasting of the sequence with *Balanophora japonica* voucher rDNA-ITS showed that there were 100% (671/671) similar between the species and *B.japonica* Makino. combined with its morphology, the species was finally identified as *B. japonica* Makino. which is reported for the first time in Guizhou province. This discovery provides a basis for further research and protection of the biodiversity in the region.

Keywords *BaLanophora japonica* Makino.; BaLanophora; New record; Guizhou Province

BaLanophoraceae is a succulent parasitism herbaceous plant with 120 species of 18 generas distributed in tropical and subtropical regions, and 20 species of 2 genera in China (Qiu and Lin, 1988, pp. 250-279), mainly distributed in Taiwan, Sichuan, Guizhou, Yunnan, Guangdong and other places; the distribution of Balanophora species in Guizhou is recorded as 7 species of 1 genus (Chen, 2004). In 2018, when the author conducted a field plant resource survey in Zhujiashan National Forest Park, Weng'an County, Guizhou Province, author found that there was a distribution of BaLanophoraceae plants. After observing the morphological characteristics of the Balanophora species and molecular identification and relative literature review, the species was confirmed as *B. japonica* Makino.. *B. japonica* Makino. is a species of BaLanophoraceae Plant, which has been recorded mainly in Japan and Guangdong in southern China. It is as newly recorded species specially described and reported in Guizhou Province.

This species found in this survey grow in the evergreen coniferous and broad-leaved mixed arbor forest in the edge of the original forest of Zhujiashan. The environment under the forest is shaded, and the humus soil is usually formed by the accumulation and decay of leaves, and the soil is acidic or slightly acidic. The main tree species in forest area are *Pinus massoniana* and *Symplocaceae*. The species of *symplocaceae* are mainly *Symplocos paniculata*, *Symplocos anomala brand*, *Symplocos sumunta*, *Symplocos grovei* Merr. In this study, it was found that *B. japonica* Makino. parasitizes on fine roots of several species of *symplocaceae* plants in the forest, indicating that the family of *symplocaceae* was the host of the plant. The root system of *symplocaceae* plant is very developed. Its huge root system can absorb the nutrients in the soil well, and provide sufficient nutrients and water for the parasitism of this species. It has been reported that there are many kinds of host plants in the family BaLanophora, which mainly parasitize on legume, mulberry, *Rhododendron* and *Quercus* (Zhang, 1998, Chinese Journal of folk medicine, (2): 27-28). In the investigation of plant resources, it was found that *B. japonica* Makino. distributed in Guizhou, but also found that the *symplocaceae* plant was its host, which not only enriched the species diversity data of BaLanophora in Guizhou, but also enriched the host types of BaLanophora. In terms of species identification of medicinal materials, with the development of molecular biology technology, people began to effectively combine molecular biology technology such as rDNA ITS with traditional morphological identification methods and widely apply them to the authenticity, classification and identification of medicinal plants (Niu et al., 2009; He

and Chai, 2017; Chen et al., 2018). Therefore, on the basis of traditional morphological identification, we use molecular biology technology rDNA ITS to further identify the species of the samples. rDNA ITS is the internal transcribed spacer (ITS) of ribosomal DNA, located between the 18S and 26S rRNA genes, which is divided into two segments (Kress et al, In 2011). ITS sequence has good PCR amplification efficiency and sequencing success rate, with large interspecific variation and small intraspecific variation, becoming an important molecular marker (Chen et al, 2011), which is widely used in the identification and quality evaluation of traditional Chinese medicine, identification of plant germplasm resources, kinship and phylogeny (Gao and Zhao, 2018; Wang, 2018), especially the effective combination of the identification of traditional Chinese medicine and morphological identification, improves the accuracy and stability of the identification of traditional Chinese medicine.

Therefore, in this study, the total genomic DNA of the collected BaLanophora samples was extracted, and the ITS sequence universal primers were used for PCR amplification and two-way sequencing. The ITS sequence of *B. japonica* Makino. Published by GenBank was compared with the BaLanophora samples ITS sequence in this experiment, combined with the morphological identification results, further identified the BaLanophora species distributed in Zhujiashan National Forest Park, Weng'an County, Guizhou Province.

1 Results and Analysis

1.1 Morphological and anatomical observation

B. japonica Makino. was Succulent herb, 5-16 cm. The rhizome, yellowish or hazel. Tuberous root, which was divided into 3-15 breach at the base, and it grown in the fine root of host. Branch is regular subglobose and ellipsoid, its surface has yellowish raised small warts nodule and evident white or yellowish-white stellate lenticels. Inflorescence and stem were subtended by squamous bracts and outward breakthrough for growing from the basal inside of the root. The base of stem was packaged by rhizome sheath. Flower: flesh and cylinder, 2.5-10×0.5-2 cm, the part in yellow. Close to the part of inflorescence is red and the uality of a material is brittle scale bracts stems from the rhizome producing package, alternate, at the bottom of the stem to pale yellow or orange red, ovoid long elliptic, 1.5-3.5×0.5-1.5 cm, concave, apex blunt, and the quality of a material is brittle. Female flowers was red, ellipsoid ovoid to elliptical cylindrical ovoid, ovoid, 2-5.5×1-2.5 cm, with yellowish ellipsoid ovary on the surface and the long filaments than ovary. Addendum was red, inverted triangular or obovate, apex slightly concave, with a short stout stalk, longer than the female flowers, and ovary interspersed flowering period 10 to 12 months. The male flowers have not been found so far (Figure 1).

1.2 rDNA-ITS sequence alignment analysis

In this study, through PCR amplification and sequencing, a total of 1726bp nucleotide ITS sequences of Balanophora species sample was obtained (Sequence ID: query X_126617) (Figure 2). The 18S rRNA ribosomal gene sequences of *B. japonica*, *B.fungosa* and *B. laxiflora*. from GenBank database were downloaded, and the alignment was adjusted by introducing gaps using Clustal X1.83. This resulted in 671 bp as a total alignment length. The similarity of the 671 base pairs is 100% between the species and *B. japonica* Makino *Balanophora japonica* voucher SY014, as shown in the result of the specific sequence alignment (Figure 3); The similarity was 99.85% between the species and *japonica* Su031 SrRNA ribosomal gene ITS sequence , and the similarity alignments were higher than the results of sequence alignments with *B.fungosa* and *B. laxiflora*. (Table 1). According to morphological survey results,the species was similar with *B. japonica* species, the species was finally identified as *B. japonica*.

2 Discussion

In this study, the Balanophora species was assessed using morphological traits and a DNA barcoding approach. We observed the morphology of Balanophora species collected in Zhujiashan National Forest Park, Weng'an County, Guizhou Province, and described the morphological characteristics of the Balanophora species .We surveyed the diagnostic features of all previously known species in Flora of Guizhou and the Flora Reipublicae Popularis Sinicae. There is no related record of the distribution of *B. japonica* Makino in Guizhou from Guizhou Flora. (Chen, 2004, Flora of Guizhou, pp. 1-9) We consulted the records of Balanophoraceae plants in the Flora Reipublicae Popularis Sinicae, the morphological features of Balanophora species sample in Guizhou are

consistent with *B. japonica* Makino. In order to accurately identify the Balanophora species sample is *B. japonica* Makino. We here employed rDNA-ITS sequence molecular technology to conduct molecular identification of Balanophora in Guizhou. PCR amplification and sequencing of the total DNA of Balanophora samples to obtain, A total of 1726 nucleotide sequences were aligned. The blasting of the sequence with *B. japonica* voucher rDNA-ITS showed that there were 100% (671/671) similar between the species and *B. japonica* Makino, combined with the morphological and anatomical observations, the results clearly show that Balanophora specimen is *B. japonica*, and can be readily separated from the other Balanophora.

Figure 1 Morphological characteristics of *Balanophora japonica* Makino.

Note: A: Habitat (Forest land); B: Specimen (Plants and root of host); b₁: Inflorescence; b₂: Scapes; b₃: Scale bract; b₄: Stellate lenticels; b₅: Rhizome; b₆: Root of host; C: Cross section of inflorescence; c₁: Spadicle; c₂: Ovary; c₃: Styles; D: Spadicle; E: Pistil F: Rhizome and flowering shoot; f₁: Rhizome sheath; f₂: Inflorescence; f₃: Scale bract

Figure 2 Electrophoresis of PCR product of rDNA ITS of sample

Note: M: DL2000 bp DNA Marker; 1: PCR product of sample

Query x 1 TGTGTACAAAGGGCAGGGACGTAATCAGCATGAGTTGATGACTCATGCTTACTAGGAATT 60
B. japonica 1614 TGTGTACAAAGGGCAGGGACGTAATCAGCATGAGTTGATGACTCATGCTTACTAGGAATT 1555

Query x 61 CCTCGTTCAAGACCAACAATTGCAATGATCTATCCCCATCACGATGAAAATTCCAAGATT 120
B. japonica 1554 CCTCGTTCAAGACCAACAATTGCAATGATCTATCCCCATCACGATGAAAATTCCAAGATT 1495

Query x 121 ACCCAAGCCTATCGACCAAGGTTATATACTCGTTGAATACATCAGTGTAGCACGCGTGCG 180
B. japonica 1494 ACCCAAGCCTATCGACCAAGGTTATATACTCGTTGAATACATCAGTGTAGCACGCGTGCG 1435

Query x 181 GCCCAAAACATCTAAGGGCATCACAGACCTGTTATTGCCTGAAACTTCCTTGGCCTAATT 240
B. japonica 1434 GCCCAAAACATCTAAGGGCATCACAGACCTGTTATTGCCTGAAACTTCCTTGGCCTAATT 1375

Query x 241 AGCCACAGTCCCTCTAAGAAGCAAACCATGGAAAAAGACTACCATGTAGCTAGTTAATAG 300
B. japonica 1374 AGCCACAGTCCCTCTAAGAAGCAAACCATGGAAAAAGACTACCATGTAGCTAGTTAATAG 1315

Query x 301 GCTAAGGTCTCGTTTCGTTAACGGAATTAACCAGACAAATCACTCCACCAACTAAGAACGG 360
B. japonica 1314 GCTAAGGTCTCGTTTCGTTAACGGAATTAACCAGACAAATCACTCCACCAACTAAGAACGG 1255

Query x 361 CCATGCACCACCACCCATAAAATTATGAAAAAGCTATCAATCTGTCAATCCTTATTATGT 420
B. japonica 1254 CCATGCACCACCACCCATAAAATTATGAAAAAGCTATCAATCTGTCAATCCTTATTATGT 1195

Query x 421 CCGGACCTGGTAAGTTTTCCCGTGTGAGTCAAATTAAGCCGCAGGCTCCACTCCTGGTG 480
B. japonica 1194 CCGGACCTGGTAAGTTTTCCCGTGTGAGTCAAATTAAGCCGCAGGCTCCACTCCTGGTG 1135

Query x 481 GTGCCCTTCCGTCATTCCTTTAAGTTTCAGCCTTGCAGCATACTCCCCCAGAACCCA 540
B. japonica 1134 GTGCCCTTCCGTCATTCCTTTAAGTTTCAGCCTTGCAGCATACTCCCCCAGAACCCA 1075

Query x 541 AATACTTTGATTTCTCATAAGGTACCAGCAAAGTCCTATAAGTAACATCTACTGATCCCT 600
B. japonica 1074 AATACTTTGATTTCTCATAAGGTACCAGCAAAGTCCTATAAGTAACATCTACTGATCCCT 1015

Query x 601 AGTTGGCATCGTTTATGGTTGAGACTAGGACGGTATCTAATCGTCTTCGAGCCCCCAACT 660
B. japonica 1014 AGTTGGCATCGTTTATGGTTGAGACTAGGACGGTATCTAATCGTCTTCGAGCCCCCAACT 955

Query x 661 TTCGTTCTTGA 671
B. japonica 954 TTCGTTCTTGA 944

Figure 3 Sequence alignment of rDNA ITS of *Balanophora japonica* Makino and sample (Query x)

Table 1 Sequence similarity comparison of *Balanophora* Forst. et Forst. f species

Description	Max Score	Total score	Query cover(%)	Per. Identities (%)	Accession
<i>Balanophora japonica</i> voucher SY014	1 240 (671)	1 240	100	100	XC588399.1
<i>Balanophora japonica</i> voucher Su031	1 234	1 234	100	99.85	JN392873.1
<i>Balanophora laxiflora</i> voucher Su027	1 229	1 229	100	99.70	JN392872.1
<i>Balanophora laxiflora</i> voucher Su041	1 229	1 229	100	99.70	JN392869.1
<i>Balanophora fungosa</i> isolate 3CMKP	1 173	1 173	100	98.21	MH094628.1
<i>Balanophora fungosa</i> isolate 3BMKP	1 173	1 173	100	98.21	MH094627.1
<i>Balanophora fungosa</i> isolate 2BMKP	1 173	1 173	100	98.21	MH094626.1
<i>Balanophora fungosa</i> isolate 2A(M)MKP	1 173	1 173	100	98.21	MH094625.1

During the survey of Balanophoraceae in Guizhou, since only female individuals of *B. japonica* Makino can be found locally, the male of *B. japonica* Makino strain have not yet been found in Guizhou. The Flora Reipublicae Popularis Sinicae records that only the female plants were collected from the *B. japonica* Makino, but no male plants were found. Therefore, in the classification system of the Balanophora, *B. japonica* Makino was classified as parthenogenetic. The discovery of the *B. japonica* Makino in Guizhou has enriched the species diversity of the family Balanophoraceae in Guizhou, especially the discovery of the family Symplocaceae as its host, which has enriched the host type of the family Balanophoraceae. This discovery enriches the biodiversity data of Guizhou Province and provides a basis for further research and protection of plant diversity in the region. In addition, Balanophora is a kind of parasitic plant with high medicinal value (Ruan et al., 2006; Tao and Xu, 2017). The karst landforms in Guizhou Province are special, with variable landforms and abundant precipitation. The plant

resources of the Balanophora are widely distributed in Guizhou, and it can be seen in the shaded areas of the valley and the forest. But so far, we have little research on the special life history and habits of Balanophora plants, especially *B. japonica* Makino, and little is known about it. Therefore, in-depth research on its cultivation, living habits, parasitic mechanism is conducive to the rational development and utilization of Balanophora resources. *B. japonica* Makino distribution in Guizhou provides important materials for studying the special living habits and parasitic mechanisms of the parasitic Balanophoraceae plants.

3 Materials and Methods

3.1 Plant materials

Balanophora species sample was collected in Zhujiashan National Forest Park, Weng'an County, Guizhou Province, (Guizhou, PR China), 26°57'~26°58' N, 107°37'~107°42' E, alt. 1 000~1 200 mdec, 2018. Voucher specimens (GBMC) were deposited at the Herbarium located in school of biological sciences, Guizhou Education University.

3.2 Morphological and anatomical observation

In the present study, we collected samples of related Balanophora from Guizhou and confirmed the presence of *B. japonica* in Guizhou based on morphological and molecular data. morphology was examined under light microscope, and reviewed the diagnostic features of all previously known species in Flora of Guizhou and the Flora Reipublicae Popularis Sinicae. Phylogenetic analyses for selected Balanophora species were conducted using nuclear 18s ribosomal DNA and ITS sequences.

3.3 rDNA-ITS sequence analysis experiment

Balanophora species samples collected from Guizhou were sent to Shanghai Shenggong Biological Engineering Co., Ltd. to extract the total genomic DNA, and the extracted DNA as a template to perform PCR amplification of the ITS region was accomplished using the following two primers: 18S -2F: 5'-catatgcttgctcaagat-3'; reverse ITS-3R: 5'-cttctcctctctctaaatg-3' (Liu, 2013). PCR products were purified and sequenced and the edited sequence was aligned using Clustal X1.83. A total of 1726 nucleotide sequences were aligned. ITS of *B. japonica* Makino, *B. laxiflora* and *B. fungosa* were obtained from Genbank, According to results of BLAST tool of sequences, their relevant sequences were retrieved and the sequence similarity compared from NCBI databas.

Authors' contributions

CJF carried out the experimental design and participated in preparation of the manuscript. LTT participated in collection Balanophora species, analysis of experimental results and participated in preparation of the manuscript. MY participated in analysis of experimental results and collection Balanophora species. JCY participated in collection Balanophora species. All authors reviewed the draft of the manuscript. All authors read and approved the final manuscript.

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References

- Chen J.A., Yang L., Li R.Z., Zhang J.M., Hu M., and Liu Y., 2018, Identification of *Panax japonicus* and its related species or adulterants using ITS2 sequence, Zhongcaoyao (Chinese Traditional and Herbal Drugs), 49(15): 3672-3680
- Chen S.L., Pang X.H., Yao H., Han J.P., and Luo K., 2011, Identification system and perspective for DNA barcoding traditional Chinese materia medica, Shijie Kexue Jishu-Zhongyiyao Xiandaihua (World Science and Technology/Modernization of Traditional Chinese Medicine and Materia Medica), 13(5): 747-754
- Gao B.Y., and Zhao Y.M., 2018, Identification of *Magnolia denudata* and its relative species based on ITS sequences, Jiangxi Nongye Xubao (Acta Agriculturae Jiangxi), 30(5): 85-88, 93
- He T.T., and Chai J.H., 2017, Sequence analysis of *Cymbidium* based on ITS gene, Fenzi Zhiwu Yuzhong (Molecular Plant Breeding), 15(1): 52-57
- Kress W.J., and Erickson D.L., 2007, A two-locus global DNA barcode for land plants: the coding rbcL gene complements the non-coding trnH-psbA spacer region, PLoS One, 2(6): e508
<https://doi.org/10.1371/journal.pone.0000508>

- Liu G.D., 2013, Study on horizontal gene transfer and relationships between populations of parasitic herb *Cynomorium songaricum* rupr, Inner Mongolia University, Supervisor: Chen G.L., pp.5
- Niu X.L., Ji K.P., Wu Q., and Lv G.Q., 2009, rDNA ITS sequence molecular marker technology in the study of botany, *Yingyong Xinxixue* (Chian Journal of Bioinformatics), 7(4): 268-271
- Ruan H.L., Zhang Y.H., Pi H.F., Zhao W., and Wu J.Z., 2006, Chemical constituents of *Balanophora japonica* Makino, *Tianran Chanwu Yanjiu Yu Kaifa* (Natural Product Research and Development) 18: 74-75
- Tao R.J., and Xu X.T., 2017, The progress of *Balanophora* plants on chemical constituents and pharmacological Activity, *Zhongguo Minzu Minjian Yiyao* (Chinese Journal of Ethnomedicine and Ethnopharmacy), 4(26): 73-78
- Wang M.M., Wang X.S., Zuo L.Y., and Yu J., 2018, Molecular identification of *Bambusoideae* plants based on ITS sequences, *Xinan Daxue Xuebao* (Journal of Southwest University (Natural Science Edition)), 40(7): 44-50