Genetic differences among populations of *Gynura procumbens* (Lour.) Merr.

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Abstract

Twenty-four populations of *Gynura procumbens* from different geographical regions were studied to determine the genetic differences using ITS and *trnL-trnF* sequences. The ITS sequence had an aligned length of 718 bp and a G+C content of 49.8%, with 5 variable sites and 3 parsimonious informative sites. The *trnL-trnF* sequence had an aligned length of 835 bp and a G+C content of 35.0%, with 6 variable sites and 3 parsimonious informative sites. The phylogenetic tree based on *trnL-trnF* sequences showed that all populations of *Gynura procumbens* clustered into one branch, indicating that this sequence has strong identifying abilities in genetic difference studies. Furthermore, the phylogenetic analysis using both ITS and *trnL-trnF* sequences showed similar results. Populations collected from Taiwan and Indonesia were grouped into one clade, while those collected from Guizhou, Guangxi, Fujian, and Guangdong shared a close relationship, corresponding to their geographical distribution pattern.

Introduction

Gynura procumbens (Lour.) Merr. (longevity spinach) is a medicinal and edible plant that belongs to the Compositae family. It has creeping herbaceous stems that are light brown, glabrous, or pubescent when young. The leaves are oval or elliptical, with wavy or entire margins and yellow pubescence on both sides[1–2]. This perennial herb is mainly distributed in Yunnan, Guangxi, Hainan, and other regions in China, and has a sweet, light, and flat taste[3]. Its medicinal effects include dredging meridians and activating collaterals, anti-inflammatory and cough relief, removal of blood stasis, swelling reduction, and promoting blood circulation and muscle generation[4–5]. *G. procumbens* is a medicinal material with high nutritional value, as it contains 12 fatty acids, of which unsaturated fatty acids constitute 68.89% of the total fatty acid content[6].

The propagation techniques of *G. procumbens* mainly include tissue culture[7–8] and cutting propagation[9]. The latest research indicates that longevity spinach is suitable for interplanting under the forest and has ideal economic benefits[10].

Plant nuclear ribosomal internal transcribed spacer (nr DNA ITS) has a fast evolution rate and provides rich information sites, making it widely used in the study of phylogenetic relationships between plant-related genera and species[11–13]. Shen[14] used ITS sequences to identify *Paulownia fortunei*, providing a new method for the study of the phylogenetic and genetic diversity of this species. The *trnL-trnF* fragment in the chloroplast genome DNA is a gene spacer without selection pressure, with moderate length and a greater evolutionary rate than functional coding regions. Therefore, it is often used in phylogenetic studies of intergeneric and subgeneric taxa[15–16]. Feng[17] used *trnL-trnF* fragment sequences to systematically analyze the interspecific relationships of wild peonies in 15 populations.

In this study, the ITS sequence and *trnL-trnF* sequence of 24 different populations of *G. procumbens* were compared and analyzed to discuss genetic differences and relationships between different populations. This research provides a basis for the systematic study and breeding of *G. procumbens*.

Material and Methods

Test materials and DNA extraction

A total of 24 geographical populations of *G. procumbens* were collected from various provinces in China, including Jiangxi, Guangdong, Guangxi, Yunnan, Guizhou, Fujian, Taiwan, as well as from Indonesia and other locations in 2014–2015 (Table 1). Each sample consisted of 5 genotypic provenances, resulting in a total of 120 genotypes. The collected plants were cultivated in the greenhouse of Jiangxi Environmental Engineering Vocational College in Ganzhou City, Jiangxi, China. Fresh young leaves were collected in July 2015, and genomic DNA was extracted using the CTAB method. The purity was assessed using 1% agarose gel electrophoresis, and after determining the concentration by spectrophotometer, the DNA was stored at -20°C for later use.

Target sequence and PCR amplification

The primers used for PCR amplification of the ITS sequence were EC1 (5'-GAGGAAGGAGAAGTCGTAAC-3') and EC2 (5'-GTTCGCTCGCCGTTACTAAG-3'). The primers used for PCR amplification of the *trnL-trnF* sequence were trnL_F (5'-CGAAATCGGTAGACGCTACG-3') and trnL_R (5'-ATTTGAACTGGTGACACGAG-3'). The reaction system consisted of 25μl: 1μl DNA template, 1μl primer, 15.25μl ddH2O, 0.25μl TaKaRa LA Taq, 4μl dNTP Mixture, and 2.5μl 10× LA PCR Buffer (Mg2 + Plus). The PCR amplification procedure was as follows: denaturation at 94°C for 30 s, annealing at 52°C for 45 s, extension at 72°C for 1 min, for 35 cycles; with an initial denaturation at 95°C for 2 min, and a final extension at 72°C for 4 min. The PCR products were detected by 1.2% agarose gel electrophoresis and sent to Beijing Zhongke Xilin Bioengineering Co., Ltd. for sequencing. The ITS and *trnL-trnF* sequences of *Gynura formosana* were obtained from GenBank.

Table 1
Detailed information for the 24 populations of G. procumbens (Lour.) Merr.

Population	Location	Collection time	Origin
1	Ganzhou City, Jiangxi Province	2013.06.15	Cultivated
2	Indonesia	2014.07.08	Cultivated
3	Taiwan Province	2014.8.17	Wild
4	Shanghai City	2014.10.10	Cultivated
5	Wuxi City, Jiangsu province	2014.10.10	Cultivated
6	Institute of Plant Protection, Chinese Academy of Agricultural Sciences	2014.10.10	Cultivated
7	Qiandongnan 1, Guizhou Province	2014.10.10	Wild
8	Qiandongnan 2, Guizhou Province	2014.10.10	Wild
9	Huangshan city, Anhui Province	2014.10.11	Cultivated
10	Yongxing Town, Hainan Province	2015.04.05	Wild
11	Shishan Town 1, Hainan Province	2015.04.05	Wild
12	Shishan Town 2, Hainan Province	2015.04.05	Wild
13	Zuntan Town, Hainan Province	2015.04.05	Wild
14	Jianfengling Forest Park, Hainan Province	2015.04.04	Wild
15	Nanning City, Guangxi Province	2015.04.08	Cultivated
16	Liuzhou City, Guangxi Province	2015.04.10	Cultivated
17	Baise City, Guangxi Province	2015.04.10	Cultivated
18	Fangchenggang City, Guangxi Province	2014.10.10	Wild
19	Zhongshan city, Guangdong province	2015.04.12	Wild
20	Huizhou City, Guangdong Province	2014.08.17	Wild
21	Kunming city, Yunnan province	2014.10.10	Wild
22	Dali city, Yunnan province	2014.07.10	Wild
23	Zouma Town, Chongqing City	2015.04.12	Cultivated
24	Nanping city, Fujian province	2015.04.12	Wild

Data Analysis

The DNA sequence of *G. procumbens* was obtained by sequencing and integrated with the selected outgroup sequence, after removing the primers at both ends. Clustal X software was used to perform multiple sequence alignment, and manual correction was done on individual sites after alignment. MEGA software was utilized to calculate the base composition frequency, conversion/transversion ratio, mutation sites (sites with more than two base types), and the number of parsimony information sites (special mutation sites where there are not only two or more base types at this site, but each base type appears in more than two groups). The Maximum Parsimony analysis was performed to construct a phylogenetic tree. The evolutionary distance between sequences was calculated using the Kimura-2 parameter, and the bootstrap method was used to test and evaluate the phylogenetic tree with 1000 replicates. The bootstrap value was interpreted as follows: <50% indicates no support, 50%-74% indicates weak support, and 75%-100% indicates strong support. The nucleotide diversity and haplotype diversity of *G. procumbens* were analyzed using DnaSP software.

Results

ITS and trn L-trn F sequence characteristics of different populations of G. procumbens

The ITS and *trnL-trnF* sequences were amplified using PCR, sequenced, and compared using BioEdit software. The ITS sequence was 718 bp in length, with a G + C content of 49.8%. It contained 5 variable sites, 3 parsimony informative sites, and 2 single sites. The *trnL-trnF* sequence was 835 bp in length, with a G + C content of 35.0%. It contained 6 variable sites, 3 parsimony informative sites, and 3 single sites. The genetic distance among ITS sequences ranged from 0.000 to 0.035, with an average of 0.002, and some populations had a genetic distance of 0. The genetic distance between *trnL-trnF* sequences ranged from 0.000 to 0.012, with an average genetic distance of 0.007, and the distance between most populations was 0. The overall genetic distance of the ITS sequence was smaller than that of the *trnL-trnF* sequence (Fig. 1).

Phylogenetic analysis of ITS and trn L-trn F sequences of different populations of G. procumbens

Two phylogenetic trees of G. procumbens were constructed using the ITS sequence and *trnL-trnF* sequence, respectively, by the maximum parsimony method (Figs. 1 and 2). Figure 1 shows that the outgroup of *Gynura divaricate* is located at the base of the tree, and all *G. procumbens* are clustered into one branch with a self-supporting rate of 100%. Among them, sequences collected from Taiwan, Indonesia, Hainan, Yunnan, and Beijing were grouped with a self-supporting rate of 60%. The remaining sequences were grouped into another branch, with a self-supporting rate of 79%. Each cluster has multiple parallel branches, such as specimens collected from different locations in Hainan Province forming typical parallel branches. Additionally, the *G. procumbens* species collected in Jiangsu, Jiangxi, Anhui, Chongqing, and Shanghai also formed parallel branches in this phylogenetic tree.

In the phylogenetic tree constructed based on the *trnL-trnF* sequence (Fig. 2), the outgroup *Brassica chinensis* was located at the base of the tree, and all *G. procumbens* were independent, with a bootstrap support rate of 100%. Sequences collected from Taiwan, Indonesia, and Hainan were grouped in one

branch with a self-supporting rate of 66%, while the remaining sequences were grouped into another branch with a self-supporting rate of 76%. In this phylogenetic tree, groups collected from the same province were grouped into one branch, and the geographical populations collected from different provinces also had clear branches. However, the Beijing and Yunnan populations were grouped, which was inconsistent with the geographical pattern, and the support rate was 89%, higher than the phylogenetic tree based on the ITS sequence.

Discussion

Application of ITS and trnL-trnF sequences in genetic diversity analysis of different populations of plants

The ITS sequence exhibits high variation and is often used to distinguish species with close genetic relationships[19]. For instance, Li[20] investigated the phylogenetic relationships of *Torreya* plants using ITS sequences, while Li Yunxia[21] analyzed the phylogenetic relationships of *Oxytropis* using both ITS and *trnL-trnF* sequences and found that it had stronger identification capabilities. However, some studies have suggested that the ITS sequence is not suitable for phylogenetic analysis of species and populations due to its variability, evolutionary history, and analysis methods[22]. This discrepancy may be attributed to the differential identification abilities of ITS and *trnL-trnF* sequences in different groups.

The chloroplast genome is an essential molecular marker for plant molecular phylogeny and molecular geography owing to its unique features such as single-parent inheritance, simple structure, multiple copies, and low recombination[23]. The *trnL-trnF* sequence, which belongs to a non-coding intergenic region, has a high evolutionary rate and can provide valuable information on mutation and variable sites. This sequence is widely used in phylogenetic and phylogeographic studies of mosses, ferns, gymnosperms, and angiosperms, including analyses of interfamily, intergeneric, and interspecific relationships and intraspecific phylogeography[24–26]. For instance, Meng[27] explored the phylogeography of *Hippophae rhamnoides* based on chloroplast DNA *trnL-trnF* sequences, while Zhang Xuemei[28] used the *trnL-trnF* sequence to analyze the phylogenetic and phylogeography of *Taxus wallichiana* var. *mairei*.

Genetic relationship among different geographical populations of G. procumbens

In this study, we constructed a phylogenetic tree of 24 geographical populations of *G. procumbens* using the nuclear gene ITS sequence and chloroplast gene *trnL-trnF* sequence. The phylogenetic tree based on the two sequence fragments grouped the same collections from the same site into one branch, with a high support rate. For example, collections from Jianfengling Forest Park in Hainan Province, Shishan Town, Yongxing Town, and Zuntan Town in Haikou City were clustered together with a 100% support rate. Furthermore, sequences from Taiwan and Indonesia were clustered into one branch and then with the Hainan group in the two phylogenetic trees. The populations from Guizhou, Guangxi, Fujian, and Guangdong were closely related in the phylogenetic tree, consistent with the geographical distribution pattern of *G. procumbens*. However, in the phylogenetic tree based on ITS sequence and *trnL-trnF* sequence, the Yunnan group and Beijing group were clustered into one branch, which was not consistent

with their geographical distribution. The Beijing group in this experiment is cultivated, and its introduction history lacks detailed records. Based on the branch relationship of the phylogenetic tree, it is speculated that it may be introduced from the southern region adjacent to Hainan.

Comparative analysis of the two phylogenetic trees showed that different geographical populations of *G. procumbens* could be clearly clustered in the phylogenetic tree based on the chloroplast *trnL-trnF* sequence, indicating a similar genetic background. However, in the phylogenetic tree based on ITS sequences, the groups collected from Jiangsu, Anhui, Jiangxi, Chongqing, and Shanghai could not be completely clustered into one group, forming typical parallel branches. This result may be due to the higher evolutionary rate of nuclear DNA than that of chloroplast DNA in angiosperms[29].

Conclusion

The phylogenetic tree based on *trnL-trnF* sequences showed that all populations of *Gynura procumbens* clustered into one branch, indicating that this sequence has strong identifying abilities in genetic difference studies. Furthermore, the phylogenetic analysis using both ITS and *trnL-trnF* sequences showed similar results. Populations collected from Taiwan and Indonesia were grouped into one clade, while those collected from Guizhou, Guangxi, Fujian, and Guangdong shared a close relationship, corresponding to their geographical distribution pattern.

Declarations

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Contributions

QP, DH and XT conceived and designed this research. YY, HG YC and CC collected the samples. JY, YC, LC, JW and LW performed the experiments and analyzed the data. JY and PG wrote the manuscript. XG, DH and QZ revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

1. Wei S (2007) Cuttages and medicinal value of *Gynurasegetum*. Chinese Wild Plant Resources 1114:68-69.
2. Li YK (1989) Flora of Guizhou. Sichuan Ethnic Publishing House 223.
3. Qiongxin Zhong (2001) Investigation on medicinal plant resources of *Gynura* in Hainan island. Journal of Hainan Normal University 14: 42-43.
4. Chen L, Wang JJ et al (2009) Advances in studies on active constituents from plants of *Gynura Cass* and their pharmacological activities. Chinese Traditional and Herbal Drugs 40: 666-669.
5. Min LL, Tang YJ (2009) Research progress of *Gynura* plants. Journal of Chinese Medicinal Materials 32:1322-1325.
6. Dong F, Zhu M et al (2012) Analysis of fatty acid composition of *Gynura procumbens*. Journal of Capital Normal University (Natural Science Edition) 33:1-4.
7. Song XQ, Wu XY, Zhu SC et al (2011) The rapid propagation of tissue culture on *Gynura procumbens*. Journal of Guizhou Normal University (Natural Sciences) 29:1-3.
8. Shi TL, Zhao DD, Yu BN et al (2012) Study on optimization of rapid propagation system of *Gynura procumbens*. Journal of Agricultural Sciences 33:69-72.
9. Yu JF, Huang DY et al (2014) Cutting breeding techniques in *Gynura procumbens*. Nonwood Forest Research 32:189-191.
10. Gu P, Huang DY, Yu JF et al (2014) Management technology and economic benefit analysis of interplanting *Gynura procumbens* in *Camellia oleifera* forest. Silviculture & Management 11:28-30.
11. Kress WJ, Wurdaek KJ, Zirnrner EA et al (2005) Use of DNA barcodes to identify flowering Plants. Proceedings of the National Aeademy of Scienees, USA 102:8369-8374.
12. Edwards D, Hom A, Taylor D et al (2008) DNA barcoding of a large genus, *Aspalathus* L. (Fabaeeae). Taxon 57:1317-1327.

13. Takamiya T, Wongsawad P, Tajima N et al (2011) Identification of *Dendrobium* species used for herbal medicines based on ribosomal DNA internal transcribed spacer sequence. Biological & Pharmaceutical Bulletin 34:779-782.
14. Shen XB, Qiao J, Li FD et al (2013) Mensuration of ITS sequences and characteristics analysis of *Paulownia*. Journal of Central South University of Forestry & Technology 33:30-33.
15. Taberlet P, Gielly L, Pautou G et al (1991) Universal primers for amplification of three noncoding regions of chloroplast DNA. Plant Molecular Biology 17:1105-1109.
16. Gong X, Wang HC, Yang Y et al (2013) Molecular characteristics and phylogenetic analysis of Vitaceae based on *Trn*L-F spacer sequences. Journal of Fruit Science 30:28-36.
17. Feng YL, Cheng J, Zang RX et al (2015) Phylogenetic relationships of *Paeonia* Sect. Moutan based on chloroplast *psb*A-*trn*H and *trn*L-F sequences. Journal of Biology 32:55-58.
18. Doyle JJ (1990) Isolation of plant DNA from fresh tissue. Focus 12:13-15.
19. Fukuda T, Song IJ, Ito T et al (2012) Comparing with phylogenetic trees inferred from cpDNA, ITS sequence and RAPD analysis in the genus *Asparagus* (Asparagaceae). Environmental Control in Biology 50:13-18.
20. Li JH, Davis CC, Donoghue MJ et al (2001) Phylogenetic relationships of *Torreya* (Taxaceae) inferred from sequences of nuclear ribosomal DNA ITS region. Harvard Papers in Botany 6:275-281.
21. Li YX, Lan FR, Chang CY et al (2011) Molecular phylogeny of Oxytropis DC. Qinghai-Tibetan plateau by ITS and *trn*L-F sequences. Journal of Northwest A & F University (Nat. Sci. Ed.) 39:188-193.
22. Chen JS, Zheng FC (2007) Application of ITS sequences in fungi classification and identification. Journal of Anhui Afri.Sci 35:3785-3786.
23. Schaal BA, Hayworth DA, Olsen KM et al. Phylogeographic studies in plants: Problems and prospects. Molecular Ecology 7:465-474.
24. Stech M, Quandt D, Frey W (2003) Molecular circumscription of the hornworts (Anthocerotophyta) based on the chloroplast DNA *trn*L-*trn*F region. Journal of Plant Research 116:389-398.
25. Su YJ, Wang T, Zheng B et al (2005) Genetic differentiation of relictual populations of *Alsophila spinulosa* in southern China inferred from cpDNA *trnL-F* noncoding sequences. Molecular Phylogenetics and Evolution 34:323-333.
26. Hao DC, Huang BL, Chen SL et al (2009) Evolution of the chloroplast *trn*L-*trn*F region in the gymnosperm lineages Taxaceae and Cephalotaxaceae. Biochemical Genetics 47:351–369.
27. Meng LH,Yang HL, Wu GL et al (2008) Phylogeography of Hippophae neurocarpa(Elaeagnaceae) inferred from the chloroplast DNA *trn*L-F sequence variation. Journal of Systematics and Evolution (Formerly Acta Phytotaxonomica Sinica) 46:32-40.
28. Zhang XM, Li DZ, Gao LM (2012) Phylogeographical study on *Taxus wallichiana* var. mairei(Lemee & Leveille) L.K.Fu & Nan Li. Acta Bot. Borea 32:1983-1989.
29. Tang P,Peng C (2010) The rate and manner of chloroplast genome evolution. Bulletin of Biology 45:8-10.

Figures

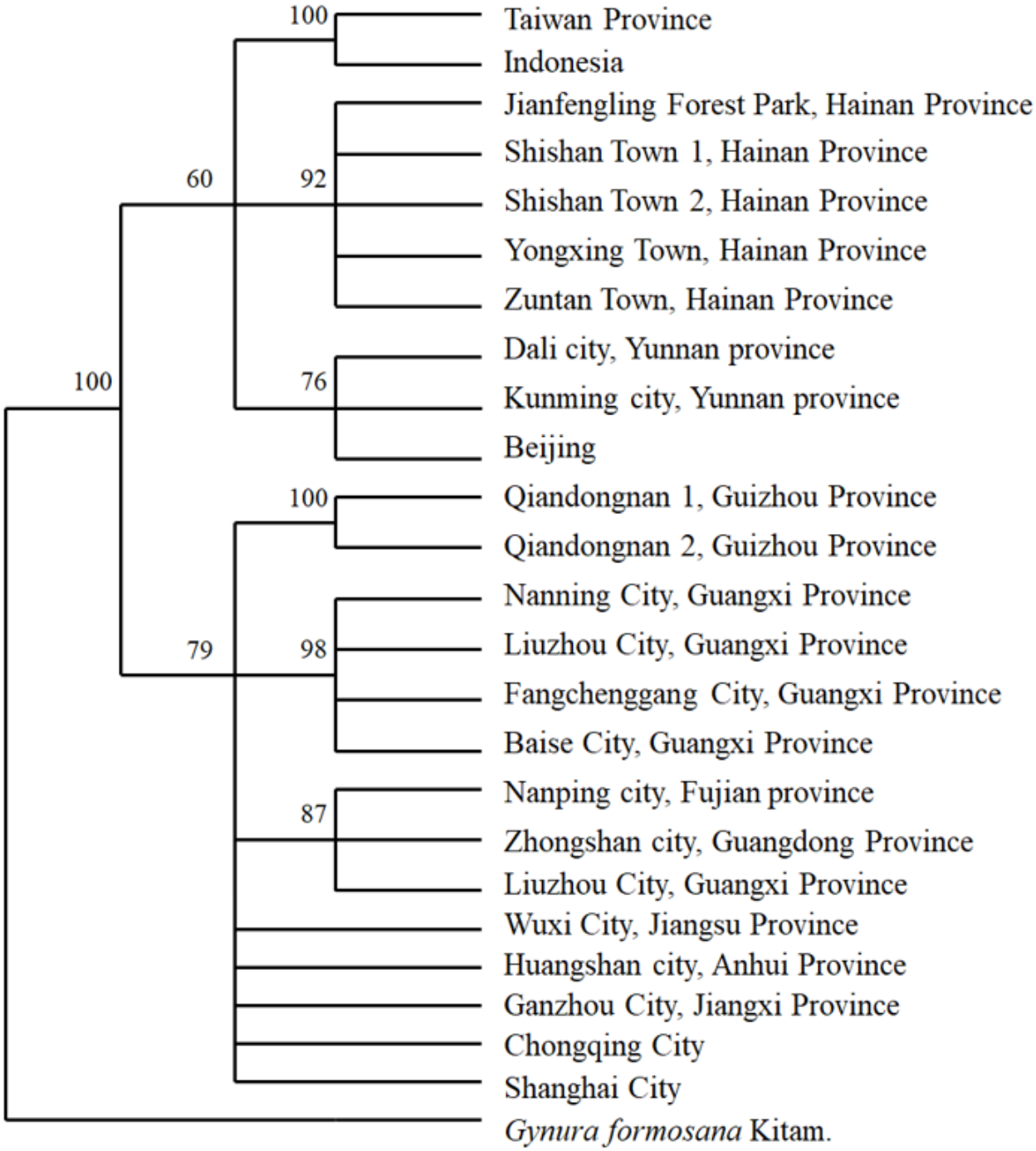

Figure 1

Maximal parsimonious tree of *G. procumbens* based on sequences of ITS.

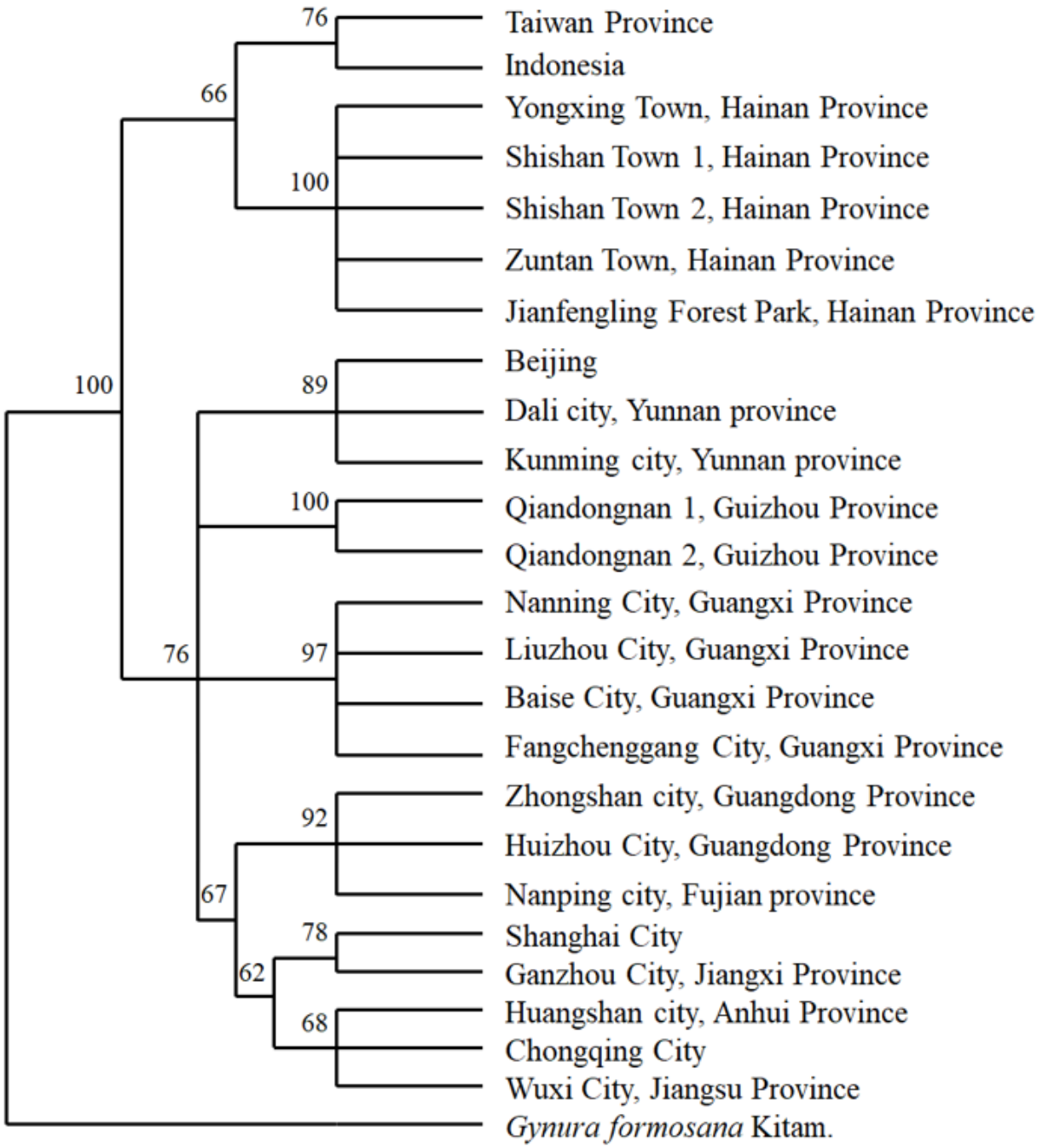

Figure 2

Maximal parsimonious tree of *G. procumbens* based on sequences of *trnL-trnF*.