Genotype classification and fluorescence visual identification of the Rhizoma Paridis of *Paris polyphylla* var. *yunnanensis* based on the SNPs of ITS sequence

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Original article

Keywords: Paris, polyphylla, var.yunnanensis, rDNA, ITS, Single Nucleotide Polymorphism, Genotypes, Fluorescent visual identification, Adulterant

Posted Date: August 10th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-37342/v2

Abstract

A fluorescent visual identification system of *Paris polyphylla* var. *yunnanensis* was established based on internal transcribed spacer barcoding. It is proposed for the first time that *P. polyphylla* var. *yunnanensis* should be divided into two types of genotypes: YN-I and YN-II according to single nucleotide polymorphism of internal transcribed spacer. In order to avoid false-negative results, two pairs of specific primers for YN-I and YN-II were designed, respectively, and specific visual fluorescent identification systems was established by SYBR Green I fluorescent dye was directly introduced into the PCR system which can be observed directly with the naked eye for the green fluorescent color of PCR system. Therefrom, it has realized the rapid and directly visual identification of two genotypes of *P. polyphylla* var. *yunnanensis* from its common nine adulterants. This study proposed for the first time the existence of different genotypes on the legal basis of Rhizoma Paridis, and provided a model for the accurate identification of different genotypes.

Introduction

Paris L. is a genus in the generalized family Liliaceae of the order Liliflorae, with 28 species and more than 10 varieties in the world, and 24 species and 7 endemic species in China (Gao et al. 2019; Huang et al. 2019). It is mainly distributed in southwestern China, Vietnam, and Uttarakhand and Manipur of India, Nepal, and Europe (Yang et al. 2015). The dried rhizomes of *Paris* are widely used in various countries and regions as a medicinal material. Rhizoma Paridis is one of the traditional bulk medicinal materials for nearly 2,000 years in China, which was first recorded in “Sheng Nong's herbal classic” (Chen et al. 2015). Modern pharmacological studies have shown that it has such pharmacological effects as uterine contraction, analgesia, and hemostasis, antitussive and antiasthmatic, immune regulation (Liu et al. 2016; Guan et al. 2019; Zhang et al. 2018). It is mainly used for the treatment of malignant boils, sore throat, snake and insect bite, fall and flutter pain, convulsions, and other symptoms. The herb is an important raw material for famous proprietary Chinese medicines such as “Yunnan Baiyao” and “Kangbingdu granules”(Lin et al. 2019). In the 2020 edition of “Chinese pharmacopeia”, *P. polyphylla* var. *chinensis* (PPC) and *P. polyphylla* Smith var. *yunnanensis* (Franch.) Hand.-Mazz (PPY) are recorded as the authentic species of Rhizoma Paridis.(Committee Chinese Pharmacopoeia 2020). However, in addition to the pharmacopeia varieties, other species in the *Paris*, such as *Paris vaniotii* H. Lév. (*P. vaniotii*), *Paris fargesii* var. *fargesii* (*P. fargesii*var) and so on have been also widely used as Rhizoma Paridis in 28 ethnic groups such as Zhuang, Dai, Miao, and Tibetan (Wang et al. 2018; Liu et al. 2019). Modern phytochemistry studies indicate that the dried rhizomes of *Paris* species mainly contain steroidal saponins, flavonoids, triterpenoid saponins, ecdysis hormone, and other chemical components. The main active ingredient is steroidal saponins are widely distributed in species of the Genus *Paris*, and there are significant differences in the types, contents, and effects of steroidal saponins among different species (Huang 2018; Kang et al. 2017).

According to Li heng's classification system, PPY belongs to Sect. *Euthyra* Franch. of the Subgenus *Daiswa* (Rafinesque-Schmaltz) H. Li, which is mainly distributed in southwest China (Li 1998; Huang et al.

2019; Luo et al. 2017). Due to the lower natural propagation rate of PPY, the slow natural growth and the longer production cycle of Rhizoma Paridis, the annual demand increases at a rate of 20%, the wild PPY has been plundered for a long time and the resources are on the verge of exhaustion (Duan et al. 2018). After the investigation of the artificial planting base of Rhizoma Paridis in Sichuan and Yunnan province of China, it was found that the cultivation of Rhizoma Paridis was in the primary stage from wild breeding to artificial cultivation, the production and trade of medical material were lack of an industrial standard. And the mixture of certified species recorded in China Pharmacopoeia(CP) and other varieties of *Paris* L. was common in the cultivation, production, circulation, and so on links because of economic interests. According to the national random inspection on the commodities in recent years, the pass rate of multiple batches of Rhizoma Paridis is less than 30% (Ju et al. 2019). It is difficult to distinguish PPY with the adulterants according to their phenotypic trait, microscopic characteristics, and chemical composition because the morphological characteristics of the rhizome of *Paris* species were similar, and the saponin types and contents of the species were widely overlapped. Also, the chemical constituents of the same species varied dynamically in different growth years and different parts of the rhizome (Wang et al. 2015; Chen et al. 2017). This brings great hidden trouble to clinical medication safety. Therefore, it is of great significance for the quality control and drug safety to establish a rapid and accurate method for the identification of PPY with its adulterants in the production and trade of medicinal materials.

Internal transcribed spacer (ITS) is one of the core regions of plant DNA barcoding (Li et al. 2011). In recent years, it has been found that the ITS sequence can provide abundant mutation sites and information sites due to ITS rapid mutation and has become an important molecular marker in the phylogenetic and classification studies of angiosperms with lower taxonomic order (Liu et al. 2014). Guo et, al. (2018) used ITS sequences to identify the origin of cultivated and wild samples of Paridis Rhizoma, and compared the differences between them. It was concluded that the diversity of the varieties in market circulation was caused by the confusion of the origin rather than the artificial domestication. Fang et al. (2016) used ITS barcodes to identify the seeds and seedlings of official and un-official Paridis Rhizoma. Zhu et al. (2010) successfully amplified and sequenced the ITS2 sequences of 11 species of Paridis Rhizoma, and found that the identification success rate of ITS2 for the genus *Paris* was 100%, which was much higher than the other 5 chloroplast sequences. In the study, universal primers were used to amplify and sequence the ITS sequences of PPY and its common related species. By analyzing single nucleotide polymorphism (SNP) variation rule, it is found that PPY should be divided into two types of genotypes, YN-I and YN-II. When using ITS barcodes to identify PPY and its related species with only one pair of specific primers, one genotype may be detected, but another may be missed. In order to avoid false-negative results, two genotype-specific primer pairs of PPY were designed and two genotype fluorescence visualization identification methods were established respectively. The system through the macroscopic observation of fluorescent color can realize the rapid and directly visual identification of PPY from its common adulterant products. It can be provided a new idea and way to solve the problem of authenticating the authenticity of the medicines of the same species and related species. Also, the exploration of common problems in the quality evaluation of Chinese medicine that the origin comes from multiple species of the same genus has a significant demonstration significance.

Materials And Methods

Plant Materials

One hundred and eighty-four samples of ten *Paris* species and subspecies in this experiments, including 34 samples of PPC; 65 samples of PPY; 17 samples of *P. polyphylla*;10 samples of *P. stenophylla*; 8 samples of *P. veitnamensis*; 5 samples of *Paris axialis* H. Li (*P. axialis*); 7 samples of *P. vaniotii*; 22 samples of *Paris fargesii* var. *fargesii* (*P. fargesii*); 13 samples of *P. thibetica*; 3 samples of *Paris forrestii* (Takht.) H. Li (*P. forrestii*) were collected from different localities in China (Table 1). All the samples were stored in the College of Ethnomedicine, Chengdu University of Traditional Chinese Medicine, China.

DNA extraction

The leaves of the *Paris* species were dried with allochroic silica gel, a 30 mg was weighed, and the total DNA of the samples was extracted according to the extraction steps of the plant group DNA isolation kit (Foregene Co., Ltd.). The rhizomes were ground into a powder, then was passed through No.3 sieve. In the study, the extraction steps of the DNA isolation kit in the plant group were improved, which a 20 mg sample of the material was suspended in cell lysis buffer (20μL 75% α-amylase with protease) and stored at -4℃.

DNA amplification and sequencing

DNA extracts were amplified by ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS-L (5'-TCGTAACAAGGTTTCCGTAGGTC-3') primers of ITS sequence (Jiang et al. 2013). Te following polymerase chain reaction (PCR) steps were conducted: 5 μL of 10×*EasyTaq* buffer, 200 μM of deoxy-ribonucleoside triphosphate (dNTPs); 0.25μM of each primer, 2.5U of *EasyTaq* polymerase, 1μL (30ng) of template, and makes up 50μL with distilled water. The mixture was predenatured at 94℃ for 5min and was denatured at 94℃ for 1min, then it underwent 32 cycles of 1 min at 94°C, 1 min at 56.4°C, 1 min at 72°C, and then a final extension for 10 min at 72°C. The PCR products (5μL) were detected by using the agarose gel (3%) electrophoresis method with Nanodrop for quality of DNA and were finally photographed under UV light exposure. The amplified products were directly methods Bi-directional DNA sequencing (Tsing Ke Biotechnology Co. Ltd., Chengdu, China).

Construction of phylogenetic tree (NJ)

The sequenced ITS region fragments were compared and aligned using MEGA 6.0 and removed unreliable and incomplete base sequences at both ends of the phylogenetic tree of ITS sequences for all samples were constructed by Neighbor-joining (N-J) and check the reliability of topological structure of N-J tree used the bootstrap analysis (BS) with tests of 1000 replicates.

Design and screening of PPY specific primers

The ITS sequences of PPY related cultivated species: *P. polyphylla* , *P. stenophylla*, *P. vietnamensis*, *P. axialis*, *P. vaniotii*, *P. fargesii*, *P. thibetica*, *P. forrestii* were compared using Megalign software in DNASTAR to find out SNP sites with a difference of stability. Then designed specific primers in the unique SNP site area of PPY by using Primer Premier 5.0 software and amplified the DNA template of *Paris* samples by PCR with the designed primers. The primer pairs were screened out which Tm difference value less than 5 and secondary structure and low mismatch rate (△G<7) (all primers in Table 2). Finally, the specific primer pairs were screened by the above PCR reaction system, in which the objective band is clear and strong specificity and could effectively identify PPY and other common related species.

Establishment of fluorescence visualization identification system

Primer concentration, annealing temperature, deoxy-ribonucleoside triphosphate (dNTP) concentration, Taq enzyme dosage, cycle number, and template volume were optimized by the single factor method.In the 25 μL reaction system, the parameters of each component were set as follows: Specific primer pairs (μM): 0.3, 0.25, 0.2, 0.15, 0.1; Annealing temperature (℃): 58, 60.6, 62, 63.7, 66.2, 68; dNTP concentration (μM): 80, 120, 200, 280; Taq enzyme dosage (U): 1, 1.5, 2.5, 3.5; Number of cycles: 26, 28, 30, 32, 34;Template volume (ng): 10, 30, 60, 90.PCR reaction steps: 2.5μL of 10×*EasyTaq* buffer, 200 μM of dNTPs; 0.25 μM of each primer, 2.5U of *EasyTaq* polymerase, 1μL (30ng) of template, and makes up 25μL with distilled water. The mixture was predenatured at 94℃ for 5 min and was denatured at 94℃ for 30s, then it underwent 30 cycles of 30s at 64°C, 30s at 72°C, and then a final extension for 5 min at 72°C. The PCR products were stored at 12℃. 5μL of the PCR reaction product mixed with 6 × Loading buffer, then detected on a 3% agarose gel electrophoresis stained with Goldview I. and then performed electrophoresis at 220 V for 7 min and observed under the gel imaging system.20μL of the PCR reaction product mixed 1μL of 1000 × SYBR Green Type I fluorescent dye and observed under ultraviolet light at 365 nm.

Methodological validation

A total of 25 samples as experimental materials including 10 batches of *Paris* samples from 7 traditional Chinese medicine (TCM) manufacturers with three parallel copies of each batch (except for individuals with less than 3 independent pieces in the batch). The universal DNA extraction method and ITS universal primers were used for DNA extraction and ITS amplification and sequencing, and the ITS sequences were compared using BLAST to determine the primitive plants of the experimental materials.

Results

Amplification and purity detection of ITS region

A total of 184 plant samples were used for the amplification of ITS regional fragments. About 700 bp bands were obtained when the PCR products were detected by using the agarose gel electrophoresis method and successfully sequenced and the purity was 1.75-2.11 by Nanodrop test.

SNP site analysis and primer screening of ITS sequence of PPY

When analyzing the ITS sequences of 65 samples from PPY of different habitats, it was found that two genotypes could be classified according to the SNP sites. The diversity of the two genotypes in SNP sites was expressed as 40 sites, which were numbered as YN-I and YN-II respectively. The specific classification criteria of genotypes were shown in Table 3. Among them, there were 35 samples of YN-I genotypes and 30 samples of YN- II genotypes. There were two specific primer pairs that were designed for the two genotypes to avoid the omission of one of the genotypes and produced false-negative results of the PPY test. After sifting the primers in Table 2, YN-IF2/YN-IR2 and YN-IIF3/YN-IIR21 were determined to be the specific primers pairs for the identification of PPY genotype I and II, respectively.

Construction and analysis of phylogenetic tree (N-J tree)

The phylogenetic tree was shown in Figure 1. Phylogenetic tree results showed that the YN-II genotype of PPY was alone clustered into a large branch, indicating that the intraspecific difference of the ITS sequence of YN-II genotype of PPY is smaller than the interspecific differences, and it can be distinguished with its related species. The YN-I genotype of PPY was clustered into a small branch, and it was clustered into a large branch with *P. forrestii* and part of *P. stenophylla* and *P. polyphylla*, indicating that YN-I genotype of PPY has a close relationship with *P. forrestii*, *P. stenophylla,* and *P. polyphylla*. Also, it would provide more molecular evidence for PPY and *P. stenophylla* are classified as *Paris polyphylla* Smith. All PPC were clustered into one big branch, but some of them were interspersed with a few *P. stenophylla* and *P. polyphylla*, and *P. stenophylla* and *P. polyphylla* were clustered into several branches respectively, indicating that as the same subvarieties, *P. stenophylla* and *P. polyphylla* had a transitional period of gene differentiation and a large intrarespecific genetic diversity. *P. fargesii*, *P. thibetica*, and *P. vaniotii* were clustered into one branch respectively, indicating that intraspecific genetic distance of ITS of those 3 species sequences is less than interspecific genetic distance and can be distinguished with other related species. *P. axialis* and *P. vietnamensis* were gathered together into a small branch, and then together into a large branch, indicating that the closer relationship between them.

Fluorescence visualization identification system

The optimized inspection results of the six parameters in the fluorescence visualization identification system of two genotypes of PPY were shown in Fig.2-Fig.7 (YN-Ⅰ genotype), Fig.8-Fig.13 (YN-II genotype). According to the experimental results, the parameters were selected which the obvious specific band brightness and the condition of avoiding nonspecific band amplification and saving experimental materials were considered synthetically. The PCR reaction system and procedures for the two genotypes of PPY were finally determined as follows:

The PCR steps for the identification of YN-Ⅰ genotype of PPY as follows: 2.5μL of 10×*EasyTaq* buffer, 280μM of dNTPs, 0.25μM of YN-IF2, 0.25μM of YN-IR2, 2.5U of *EasyTaq* polymerase, 1μL (30ng) of template, and makes up 25μL with distilled water. The mixture was predenatured at 94℃ for 5 min and

was denatured at 94℃ for 30s, then it underwent 30 cycles of 30s at 64°C, 30s at 72°C, and then a final extension for 5 min at 72°C.

The PCR steps for the identification of YN-II genotype of PPY follows 2.5μL of 10×*EasyTaq* buffer, 280μM of dNTPs, 0.2 μM of YN-IIF3; 0.2μM of YN-IIR21, 1.5U of *EasyTaq* polymerase, 1μL (30ng) of template, and make up 25μL with distilled water. The mixture was predenatured at 94℃ for 5 min and was denatured at 94℃ for 30s, then it underwent 30 cycles of 30s at 64°C, 30s at 72℃, and then a final extension for 5 min at 72°C.

Fluorescent visualization identification of PPY with its common related species

The fluorescence visualization identification results of PPY with its common related species were shown in Figure 14. In the site-specific PCR identification system, there were only DNA amplification products of PPY with obvious bands in agarose gel, while the common related species had no bands. In the fluorescence visualization identification system after the addition of fluorescent substances, only the DNA amplification products of PPY could emit bright green fluorescence, while other common related species could not emit fluorescence. The results showed that the species-specific primers and PCR amplification system designed for PPY had good specificity and could clearly distinguish PPY and its common relatives.

Results of method applicability verification

There are three samples (No. 10, 11, and 22) from 25 samples of *Paris* commercial crude drugs that failed to extract the DNA, and ITS bands still were not amplified after the reextraction. Therefore, the DNA of the three samples was not verified using the specific PCR fluorescence identification system. Analysis and comparison of 22 ITS sequences were shown that the main source of commercial medicinal materials was dried rhizomes of *Paris* and *Trillium*. There are 6 samples that were identified as PPY, including 2 samples of YN-Ⅰ genotype (No. 20-21) and 4 samples of YN-II genotype (No. 1-4). The rest samples were identified as *P. forrestii*, *P. polyphylla*, and species of *Trillium*. The specific information of commercial medicinal materials of *Paris* was shown in Table 4. As shown in Fig.15, the two samples of the YN-I genotype of PPY (No. 20-21) had obvious bands at 248 bp in the YN-I site-specific PCR system, while the other samples had no bands. In the YN-I fluorescence visualization identification system, it has bright green fluorescence, which is distinguished from other non-fluorescent samples. Four samples of the YN-II genotype of PPY (No. 1-4) had obvious bands at 149 bp in the YN-II site-specific PCR system, while the other samples had no bands. In the YN-II fluorescence visualization identification system, there was an obvious bright green fluorescence, which was clearly distinguished from other non-fluorescent samples. The results were consistent with the sequencing results, indicating that the method is accurate, reliable, and specific.

Discussion

DNA barcoding technology, as a new molecular identification technology that has been continuously developed in recent years. It has become an important supplement to traditional Chinese medicine identification technology because of its advantages of rapid, accurate, efficient, objective, and free from the change of individual morphological characteristics and the development of biological individual characteristics (Shi et al. 2016). It is widely used in the identification of authentic Chinese medicines, the identification of genuine products and substitutes, the identification of origin, the identification of multi-origin sources and genetic diversity, the identification of age, and so on. It plays an important role in ensuring the safety and effectiveness of traditional Chinese medicine, protecting the genetic diversity of medicinal plants, and finding or expanding new drug sources (Cai et al. 2017; Srivastava et al. 2016). Among them, ITS (ITS1/ITS2) sequence was recommended to be included in the core barcode of seed plants by China Plant Barcode Of Life Group (China Plant BOL Group) in 2011. After a large number of studies, the ITS sequence had been proved to be able to accurately identify the origin of the bulk of Chinese herbal medicines such as Ginseng, Honeysuckle, Notopterygium incisum, Chinese wolfberry, and Rhizoma ligustici (Zhang et al. 2017).

Indeed, DNA barcode technology also has certain limitations. Standard DNA barcodes have better resolution efficiency between genus, but the resolution within the genus is lower, especially the identification of related species of the same genus in the large genera with high rates of adaptive radiation, which is not satisfactory. The identification efficiency of a single DNA barcode is often not ideal, and improperly designed primers can also lead to erroneous results (Hollingsworth et al. 2016; Ballin et al. 2019). For deeply processed proprietary Chinese medicines, the DNA is highly degraded and content is extremely low, so it is very difficult to extract enough DNA template (Xiong et al. 2015; Fang et al. 2018; Raclariu et al. 2018). Besides, for accurate molecular identification of species, it is necessary to establish a complete reference sequence database that can fully reflect the intra-species variation and inter-species differentiation, which is a huge workload and difficult for large genera (Zhang et al. 2019). In the face of the above problems, the application of DNA barcode technology in the identification of traditional Chinese medicine still needs to be continuously improved and developed.

There has been a great deal of controversy in the taxonomy of the *Paris* genus and its phylogenic relationship has been in a state of ambiguity. In addition to the continuous changes of global climate and geology since the middle ages, the continuous hybridization and geographical migration between related species of *Paris* genus have resulted in radial expansion of the genetic diversity of species of *Paris*, which is proved by the continuous discovery of new species in recent years (Ji et al. 2019; Huang et al. 2016; Xu et al. 2019). Rhizoma Paridis is a medicine material that the origin comes from multiple species of the same genus, the mixture of similar species of the same genus has always been an important factor affecting the quality of TCM. And the present situation has undoubtedly added great difficulties to the identification of medicinal plants. With the unremitting efforts of predecessors such as CDDP marker Zhou, et al. (2019), chloroplasts genome coding gene Song et al. (2017), ITS2 molecular regions coupled with high resolution melting analysis Duan et al. (2018), new techniques and methods for the identification of *Paris* species have been developed continuously. However, current DNA barcoding methods generally require gel electrophoresis, or sequencing and homology analysis (constructed the N-J

tree) to get results, which was not so intuitive in the actual test work. In this study, the site-specific PCR identification system and fluorescence visualization identification system of PPY were established for the first time. This method significantly reduces the time and costs of electrophoretic detection and DNA introduction. Also, the intuitive results effectively improve the speed and ease of use of the method.

According to previous research such as Tang ling et al. (2013) analyzed 15 phenotypic traits of PPY from 20 populations, Chen Zhong-Su Zhi et al. (2017) used SSR molecular markers to analyze the genetic diversity of PPY in 5 different populations, the results showed that PPY is a complex because of phenotypic diversity. Its performance traits are affected by both environmental factors and genes. SNP refers to an intraspecific or interspecific variation of a single nucleotide base in a genome caused by mutations such as transformation, inversion, insertion, deletion, etc. It has become the third generation molecular marker and is widely used in variety identification and genetic diversity analysis, because of its wide distribution, strong genetic stability, high throughput, and fast detection (Mao et al. 2018). In this study, it was found that there are 40 SNP site variations in PPY from the different populations when sequencing the ITS sequences of 65 samples of PPY. This was consistent with Chen Shilin's statement that the comparison of four reference sequences of ITS2 of PPY with a length of 231 bp showed that there were 27 mutation sites (Chen, et al. 2011). Also, the level of genetic diversity of PPY was relatively high was further verified from SNP analysis. Therefore, this study proposed for the first time that PPY should be divided into two genotypes: YN-I and YN-II according to SNP analysis. In order to give consideration to the identification of PPY with two genotypes and avoid false-negative results, specific primers were designed and fluorescence identification methods were established for PPY with two genotypes (Tang et al. 2012).

The efficiency of extracting DNA from commercial Chinese herbal medicines has always been a major problem in molecular identification. Thus, the methods of DNA extraction still need further study. At the same time, the Chinese herbal medicine industry chain was reminded to strengthen the management of bacteriostasis and the management of storage and logistics. (Wu 2016; Fang, et al. 2013; Mishra, et al. 2016). According to specific fluorescence detection and sequencing analysis of commercial medicinal materials of Rhizoma Paridis, the sources of Rhizoma Paridis on the market were complex, not only illegally related species of *Paris* but also other species of *Trillium*. It showed that the *Trillium* plants have flooded the medicinal material market as adulterant products of Rhizoma Paridis, which is a new phenomenon of adulteration of medicinal materials due to the shortage of resources and the rise of prices in recent years. The entry of a large number of adulterant products into the market will seriously affect the quality and clinical efficacy of Rhizoma Paridis. This result also shows the necessity of establishing an accurate, reliable, and practical authentication system for Rhizoma Paridis.

Abbreviations

PPC: *P. polyphylla* var. *chinensis*; **PPY**: *P. polyphylla* Smith var. *yunnanensis* (Franch.) Hand.-Mazz; **ITS**: internal transcribed spacer; **TCM**: traditional Chinese medicine; **PCR**: polymerase chain reaction; **NJ**:

neighbor-joining; **GC**: guanine and cytosine; **dNTP**: deoxy-ribonucleoside triphosphate; **BS**: the bootstrap analysis; **SNP**: single nucleotide polymorphism.

Declarations

Authors' contributions

KZ, RC and HY were associated designing the research. CG, KZ, QW, LZ collected samples and conducted molecular biology experiments. GC and KZ analyzed data. CG, KZ, HY, RC wrote the manuscript. HY are corresponding authors. All authors read and approved the final manuscript.

Acknowledgments

We sincerely appreciate Tsing Ke Biotechnology Co. Ltd., Chengdu, China for providing the service of DNA sequencing technology.

Competing interests

The authors declare that no one have competing interests.

Availability of data and materials

All data and conclusions are freely available to non-profit colleges and research institutions.

Consent for publication

All authors consent for publication.

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Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

This study was funded by the National Sciences Foundation of China Grant 81573545, and the technology innovation and research and development project on Chengdu Science and Technology Bureau Grant 2019-YF05-00346-SN.

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Tables

Table 1: Samples used in this study and the corresponding localities

Sample code	Latin name	Locality	Collected parts
H1	*Paris polyphylla* var. *chinensis*	Dayi, Sichuan	leaf
H2	*P. polyphylla* var. *chinensis*	Shimian, Sichuan	leaf
H3	*P. polyphylla* var. *chinensis*	Pingwu Xiangyan, Sichuan	leaf
H4	*P. polyphylla* var. *chinensis*	Pingwu Mupi, Sichuan	leaf
H5	*P. polyphylla* var. *chinensis*	Pengzhou Bailu, Sichuan	leaf
H6	*P. polyphylla* var. *chinensis*	Pengzhou Bailu, Sichuan	leaf
H7	*P. polyphylla* var. *chinensis*	Pengzhou Bailu, Sichuan	leaf
H8	*P. polyphylla* var. *chinensis*	Ya'an Tianquan, Sichuan	leaf
H9	*P. polyphylla* var. *chinensis*	Xingshan Shuiyuesi, Hubei	leaf
H10	*P. polyphylla* var. *chinensis*	PengzhouGexianshan, Sichuan	leaf
H11	*P. polyphylla* var. *chinensis*	PengzhouGexianshan,Sichuan	leaf
H12	*P. polyphylla* var. *chinensis*	PengzhouGexianshan,Sichuan	leaf
H13	*P. polyphylla* var. *chinensis*	PengzhouGexianshan,Sichuan	leaf
H14	*P. polyphylla* var. *chinensis*	Dayi Qingxia,Sichuan	leaf
H15	*P. polyphylla* var. *chinensis*	Fengcheng,Jiangxi	leaf
H16	*P. polyphylla* var. *chinensis*	Emeishan Zhongfeng,Sichuan	leaf
H17	*P. polyphylla* var. *chinensis*	Emeishan Zhongfeng,Sichuan	leaf
H18	*P. polyphylla* var. *chinensis*	Ya'an Yingjing,Sichuan	rhizome
H19	*P. polyphylla* var. *chinensis*	Ya'an Yanqiao,Sichuan	rhizome
H20	*P. polyphylla* var. *chinensis*	Ya'an Yanchang,Sichuan	rhizome
H21	*P. polyphylla* var. *chinensis*	Ya'an Yanchang,Sichuan	rhizome
H22	*P. polyphylla* var. *chinensis*	Xingshan Shuiyuesi,Hubei	rhizome
H23	*P. polyphylla* var. *chinensis*	Dujiangyan,Sichuan	rhizome
H24	*P. polyphylla* var. *chinensis*	Luzhou,Sichuan	rhizome
H25	*P. polyphylla* var. *chinensis*	Luzhou,Sichuan	rhizome
H26	*P. polyphylla* var. *chinensis*	Chongzhou Jiguanshan,Sichuan	leaf
H27	*P. polyphylla* var. *chinensis*	Nianyang Anxian,Sichuan	leaf

H28	*P. polyphylla* var. *chinensis*	Nianyang Anxian,Sichuan	leaf
H29	*P. polyphylla* var. *chinensis*	Beichuan Xiaoba,Sichuan	leaf
H30	*P. polyphylla* var. *chinensis*	Beichuan Xiaoba,Sichuan	leaf
H31	*P. polyphylla* var. *chinensis*	Chongzhou Huanyuan,Sichuan	leaf
H32	*P. polyphylla* var. *chinensis*	Chongzhou Huanyuan,Sichuan	leaf
H33	*P. polyphylla* var. *chinensis*	Liping Long'e Guizhou	leaf
H34	*P. polyphylla* var. *chinensis*	Liping Long'e Guizhou	leaf
YN1	*P. polyphylla* var. *yunnanensis*	Dafang Shuijing,Sichuan	leaf
YN2	*P. polyphylla* var. *yunnanensis*	Dafang Shuijing,Sichuan	leaf
YN3	*P. polyphylla* var. *yunnanensis*	Bijie Tianbaqiao,Guizhou	leaf
YN4	*P. polyphylla* var. *yunnanensis*	Bijie Tianbaqiao,Guizhou	leaf
YN5	*P. polyphylla* var. *yunnanensis*	Bijie Qixingguan,Guizhou	leaf
YN6	*P. polyphylla* var. *yunnanensis*	Bijie Qixingguan,Guizhou	leaf
*YN7	*P. polyphylla* var. *yunnanensis*	Dafang,Guizhou	leaf
*YN8	*P. polyphylla* var. *yunnanensis*	Dafang,Guizhou	leaf
*YN9	*P. polyphylla* var. *yunnanensis*	Dafang,Guizhou	leaf
*YN10	*P. polyphylla* var. *yunnanensis*	Dafang,Guizhou	leaf
*YN11	*P. polyphylla* var. *yunnanensis*	Dafang,Guizhou	leaf
*YN12	*P. polyphylla* var. *yunnanensis*	Pu'er,Yunnan	leaf
*YN13	*P. polyphylla* var. *yunnanensis*	Pu'er,Yunnan	leaf
YN14	*P. polyphylla* var. *yunnanensis*	Pu'er,Yunnan	leaf
YN15	*P. polyphylla* var. *yunnanensis*	Pu'er,Yunnan	leaf
*YN16	*P. polyphylla* var. *yunnanensis*	Longli,Guizhou	leaf
*YN17	*P. polyphylla* var. *yunnanensis*	Longli,Guizhou	leaf
*YN18	*P. polyphylla* var. *yunnanensis*	Longli,Guizhou	leaf
YN19	*P. polyphylla* var. *yunnanensis*	Xuanwei longchang,Yunnan	leaf
YN20	*P. polyphylla* var. *yunnanensis*	Xuanwei longchang,Yunnan	leaf
YN21	*P. polyphylla* var. *yunnanensis*	Xuanwei longchang,Yunnan	leaf
YN22	*P. polyphylla* var. *yunnanensis*	Xuanwei longchang,Yunnan	leaf

YN23	*P. polyphylla* var. *yunnanensis*	Xuanwei Xize,Yunnan	leaf
YN24	*P. polyphylla* var. *yunnanensis*	Xuanwei Xize,Yunnan	leaf
YN25	*P. polyphylla* var. *yunnanensis*	Xuanwei Xize,Yunnan	leaf
YN26	*P. polyphylla* var. *yunnanensis*	Xuanwei Xize,yunnan	leaf
YN27	*P. polyphylla* var. *yunnanensis*	Xingyi,Guizhou	leaf
YN28	*P. polyphylla* var. *yunnanensis*	Changshun Guangshun,Guizhou	leaf
YN29	*P. polyphylla* var. *yunnanensis*	Changshun Guangshun,Guizhou	leaf
YN30	*P. polyphylla* var. *yunnanensis*	Shimian Tianwan,Sichuan	leaf
YN31	*P. polyphylla* var. *yunnanensis*	Shimian Tianwan,Sichuan	leaf
YN32	*P. polyphylla* var. *yunnanensis*	Shimian Tianwan,Sichuan	leaf
YN33	*P. polyphylla* var. *yunnanensis*	Shimian Tianwan,Sichuan	leaf
*YN34	*P. polyphylla* var. *yunnanensis*	Gengma,Yunnan	leaf
*YN35	*P. polyphylla* var. *yunnanensis*	Liuzhi,Guizhou	leaf
YN36	*P. polyphylla* var. *yunnanensis*	Huili Baiji,Sichuan	leaf
*YN37	*P. polyphylla* var. *yunnanensis*	Changshun Guangshun,Guizhou	leaf
*YN38	*P. polyphylla* var. *yunnanensis*	Changshun Guangshun,Guizhou	leaf
*YN39	*P. polyphylla* var. *yunnanensis*	Jinping,Yunnan	leaf
*YN40	*P. polyphylla* var. *yunnanensis*	Dayi Huashuiwan,Sichuan	leaf
*YN41	*P. polyphylla* var. *yunnanensis*	Dayi Huashuiwan,Sichuan	leaf
*YN42	*P. polyphylla* var. *yunnanensis*	Dayi Huashuiwan,Sichuan	leaf
*YN43	*P. polyphylla* var. *yunnanensis*	Anshun,Guizhou	leaf
*YN44	*P. polyphylla* var. *yunnanensis*	Anshun,Guizhou	leaf
*YN45	*P. polyphylla* var. *yunnanensis*	Gengma,Yunnan	leaf
*YN46	*P. polyphylla* var. *yunnanensis*	Liuzhi,Guizhou	leaf
*YN47	*P. polyphylla* var. *yunnanensis*	Liuzhi Langdai,Guizhou	leaf
*YN48	*P. polyphylla* var. *yunnanensis*	Liuzhi Langdai,Guizhou	leaf
*YN49	*P. polyphylla* var. *yunnanensis*	Liuzhi Langdai,Guizhou	leaf
*YN50	*P. polyphylla* var. *yunnanensis*	Liuzhi,Guizhou	leaf
*YN51	*P. polyphylla* var. *yunnanensis*	Liuzhi,Guizhou	leaf

*YN52	*P. polyphylla* var. *yunnanensis*	Pengzhou,Sichuan	leaf
*YN53	*P. polyphylla* var. *yunnanensis*	Pengzhou,Sichuan	leaf
*YN54	*P. polyphylla* var. *yunnanensis*	Pengzhou,Sichuan	leaf
YN55	*P. polyphylla* var. *yunnanensis*	Pengzhou,Sichuan	leaf
YN56	*P. polyphylla* var. *yunnanensis*	Pengzhou,Sichuan	leaf
YN57	*P. polyphylla* var. *yunnanensis*	Pengzhou,Sichuan	leaf
YN58	*P. polyphylla* var. *yunnanensis*	Tengchong Mingguang,Yunnan	leaf
YN59	*P. polyphylla* var. *yunnanensis*	Tengchong Mingguang,Yunnan	leaf
YN60	*P. polyphylla* var. *yunnanensis*	Tengchong Jietou,Yunnan	leaf
YN61	*P. polyphylla* var. *yunnanensis*	Tengchong Jietou,Yunnan	leaf
YN62	*P. polyphylla* var. *yunnanensis*	Yanyuan Yantang,Sichuan	leaf
YN63	*P. polyphylla* var. *yunnanensis*	Yanyuan Yantang,Sichuan	leaf
YN64	*P. polyphylla* var. *yunnanensis*	Yanbian Gesala,Sichuan	leaf
YN65	*P. polyphylla* var. *yunnanensis*	Yanbian Gesala,Sichuan	leaf
DY1	*P. polyphylla* var. *polyphylla*	Chongzhou Goujia,Sichuan	leaf
DY2	*P. polyphylla* var. *polyphylla*	Ya'an hanyuan,Sichuan	leaf
DY3	*P. polyphylla* var. *polyphylla*	Liangshan Puge,Sichuan	leaf
DY4	*P. polyphylla* var. *polyphylla*	Pingwu Xiangyan,Sichuan	leaf
DY5	*P. polyphylla* var. *polyphylla*	Wenxian Tielou,Gansu	leaf
DY6	*P. polyphylla* var. *polyphylla*	Beichuan Xiaoba,Sichuan	leaf
DY7	*P. polyphylla* var. *polyphylla*	Beichuan Xiaoba,sichuan	leaf
DY8	*P. polyphylla* var. *polyphylla*	Beichuan Xiaoba,Sichuan	leaf
DY9	*P. polyphylla* var. *polyphylla*	Beichuan Xiaoba,Sichuan	leaf
DY10	*P. polyphylla* var. *polyphylla*	Beichuan Xiaoba,Sichuan	leaf
DY11	*P. polyphylla* var. *polyphylla*	Pengzhou Bailu,Sichuan	leaf
DY12	*P. polyphylla* var. *polyphylla*	Pengzhou Bailu,Sichuan	leaf
DY13	*P. polyphylla* var. *polyphylla*	Pengzhou Bailu,Sichuan	leaf
DY14	*P. polyphylla* var. *polyphylla*	Pengzhou Bailu,Sichuan	leaf
DY15	*P. polyphylla* var. *polyphylla*	Pengzhou Bailu,Sichuan	leaf

DY16	*P. polyphylla* var. *polyphylla*	Pengzhou Bailu,Sichuan	leaf
DY17	*P. polyphylla* var. *polyphylla*	Xingshan Shuiyuesi,Hubei	rhizome
XY1	*P. polyphylla* var. *stenophylla*	Pengzhou Bailu,Sichuan	leaf
XY2	*P. polyphylla* var. *stenophylla*	Dayi Xiling,Sichuan	leaf
XY3	*P. polyphylla* var. *stenophylla*	Chongzhou,Sichuan	leaf
XY4	*P. polyphylla* var. *stenophylla*	Gulin,Sichuan	rhizome
XY5	*P. polyphylla* var. *stenophylla*	Ya'an,Sichuan	rhizome
XY6	*P. polyphylla* var. *stenophylla*	Chongzhou Jiguanshan,Sichuan	leaf
XY7	*P. polyphylla* var. *stenophylla*	Chongzhou Jiguanshan,Sichuan	leaf
XY8	*P. polyphylla* var. *stenophylla*	Liupanshui Shuicheng,Guizhou	leaf
XY9	*P. polyphylla* var. *stenophylla*	Liupanshui Shuicheng,Guizhou	leaf
XY10	*P. polyphylla* var. *stenophylla*	Chongzhou Jiguanshan,Sichuan	leaf
N1	*Paris vietnamensis* (Takht.) H. Li	Jinping,Yunnan	leaf
N2	*P. veitnamensis*	Jinping,Yunnan	leaf
N3	*P. veitnamensis*	Jinping,Yunnan	leaf
N4	*P. veitnamensis*	Jinping,Yunnan	leaf
N5	*P. veitnamensis*	Lao Cai,Vietnam	leaf
N6	*P. veitnamensis*	Lao Cai,Vietnam	leaf
N7	*P. veitnamensis*	Phôngsali,Laos	leaf
N8	*P. veitnamensis*	Phôngsali,Laos	leaf
WZL1	*Paris axialis* H. Li	Beichuan Xiaoba,Sichuan	leaf
WZL2	*P. axialis*	Beichuan Xiaoba,Sichuan	leaf
WZL3	*P. axialis*	Chongzhoiu Jiguanshan,Sichuan	leaf
WZL4	*P. axialis*	Chongzhoiu Jiguanshan,Sichuan	leaf
WZL5	*P. axialis*	Ya'an Yingjing,Sichuan	leaf
PF1	*Paris vaniotii* H. Lév.	Chongzhou Goujia,Sichuan	leaf
PF2	*P. vaniotii*	Dayi Huashuiwan,Sichuan	leaf
PF3	*P. vaniotii*	Shimian,Sichuan	leaf
PF4	*P. vaniotii*	Chongzhou Bailuzhen,Sichuan	leaf

PF5	*P. vaniotii*	Chongzhou Bailuzhen,Sichuan	leaf
PF6	*P. vaniotii*	Chongzhoiu Jiguanshan,Sichuan	leaf
PF7	*P. vaniotii*	Chongzhoiu Jiguanshan,Sichuan	leaf
Q1	*Paris fargesii* var. *fargesii*	Chongzhoiu Yulang,Sichuan	leaf
Q2	*P. fargesii* var. *fargesii*	Chongzhou Goujia,Sichuan	leaf
Q3	*P. fargesii* var. *fargesii*	Pingwu Mupi,Sichuan	leaf
Q4	*P. fargesii* var. *fargesii*	Dayi Huashuiwan,Sichuan	leaf
Q5	*P. fargesii* var. *fargesii*	Pengzhou Bailu,sichuan	leaf
Q6	*P. fargesii* var. *fargesii*	Pengzhou Bailu,sichuan	leaf
Q7	*P. fargesii* var. *fargesii*	Pengzhou Bailu,sichuan	leaf
Q8	*P. fargesii* var. *fargesii*	Laifeng,Hubei	rhizome
Q9	*P. fargesii* var. *fargesii*	Jinggangshan Huangyangjie,Jiangxi	leaf
Q10	*P. fargesii* var. *fargesii*	Chongzhou,Sichuan	leaf
Q11	*P. fargesii* var. *fargesii*	Chongzhou,Sichuan	leaf
Q12	*P. fargesii* var. *fargesii*	Pengzhou Bailu,sichuan	leaf
Q13	*P. fargesii* var. *fargesii*	Pengzhou Bailu,sichuan	leaf
Q14	*P. fargesii* var. *fargesii*	Pengzhou Bailu,sichuan	leaf
Q15	*P. fargesii* var. *fargesii*	Pengzhou Bailu,sichuan	leaf
Q16	*P. fargesii* var. *fargesii*	Shuangpai,Hunan	leaf
Q17	*P. fargesii* var. *fargesii*	Shuangpai,Hunan	leaf
Q18	*P. fargesii* var. *fargesii*	Chongzhou,Sichuan	leaf
Q19	*P. fargesii* var. *fargesii*	Chongzhou,Sichuan	leaf
Q20	*P. fargesii* var. *fargesii*	Laifeng,Hubei	leaf
Q21	*P. fargesii* var. *fargesii*	Laifeng,Hubei	leaf
Q22	*P. fargesii* var. *fargesii*	Shuicheng,Guizhou	leaf
HZ1	*P. thibetica*var. *thibetica*	Dayi Xiling,Sichuan	leaf
HZ2	*P. thibetica*var. *thibetica*	Pingwu Mupi,Sichuan	leaf
HZ3	*P. thibetica*var. *thibetica*	Wenxian Tielou,Gansu	leaf
HZ4	*P. thibetica*var. *thibetica*	Anxian Gaochuan,Sichuan	leaf

HZ5	*P. thibetica*var. *thibetica*	Anxian Gaochuan,Sichuan	leaf
HZ6	*P. thibetica*var. *thibetica*	Beichuan Xiaoba,Sichuan	leaf
HZ7	*P. thibetica*var. *thibetica*	Beichuan Xiaoba,Sichuan	leaf
HZ8	*P. thibetica*var. *thibetica*	Pengzhou Bailu,sichuan	leaf
HZ9	*P. thibetica*var. *thibetica*	Pengzhou Bailu,sichuan	leaf
HZ10	*P. thibetica*var. *thibetica*	Chongzhou Jiguanshan,Sichuan	leaf
HZ11	*P. thibetica*var. *thibetica*	Chongzhou Jiguanshan,Sichuan	leaf
HZ12	*P. thibetica*var. *thibetica*	Emeishan,Sichuan	leaf
HZ13	*P. thibetica*var. *thibetica*	Emeishan,Sichuan	leaf
CZ1	*Paris forrestii* (Takht.) H. Li	Tengchong,Yunnan	leaf
CZ2	*P. forrestii*	Tengchong,Yunnan	rhizome
CZ3	*P. forrestii*	Tengchong,Yunnan	leaf

Table 2: PPY primer sequence

Primers category	name	sequence（5’-3’）	dsDNA	TM	GC%
Former primer YN-I	YN-IF1	CACTCGCCGTGTCCGTCTA	19	60	63.2
	YN-IF11	CCACTCGCCGTGTCCGTCTA	20	64	65
	YN-IF2	ACTTTTGACCCACGGGCA	18	59.1	55.6
	YN-IF21	AACACTTTTGACCCACGAGCA	21	60.5	47.6
	YN-IF22	AACACTTTTGACCAACAGGCA	21	61	45.5
	YN-IF23	CAACACTTTTGACCCACAGGCA	22	63.2	50
	YN-IF24	CACTTTTGACCCACGGGCA	19	62.2	57.9
	YN-IF3	CACTTTTGACCCACGGGCA	19	61.9	55
	YN-IF31	GTCGCAATGCTAGGCAACA	19	60.2	57.9
	YN-IF4	GTCGCAATGCTAGGCACCA	18	64	61.1
	YN-IF5	GCGTGCCCTTTGCCGAAT	22	63.5	45.5
After the primer YN-I	YN-IR1	AAACATAAGGCGACGGCATCAT	19	59.6	57.9
	YN-IR11	CAACTATGCCCGTGGGTCA	20	59	55
	YN-IR2	CCAACTATGCTCGTGGGTCA	23	59.9	47.8
	YN-IR21	CTGTCATTAGATGATGCAGTGGC	23	59.5	47.8
	YN-IR22	CTGTCATTAGATGAAGCAGACGC	24	61	45.8
	YN-IR23	ACTGTCATTAGATGATGCAGTGGC	25	61.8	48
	YN-IR24	CTCTCTGATTAGATGATGTAGCGGC	25	63.6	48
	YN-IR3	CACTGTCATTAGATGATGCAGTGGC	20	59.6	55
	YN-IR31	GAAGAGTGGGATGCCAACGT	21	61.7	52.4
	YN-IR4	TGAAGAGTGGGATGCCAAGGT	24	60.7	41.7
	YN-IR5	GTAGCGAAATGCGATACTTGATGT	21	62.6	54.5
	YN-IR6	GCGTCGCTCTGTGCCTACTAT	23	63.2	43.5
Former primer YN-II	YN-IIF1	AACGGATGACGATTATGGTGGAA	20	63	60

	YN-IIF2	CGCACAAGAACACGGGAGGT	21	60.9	47.6
	YN-IIF21	GCATCGGCTAATGACAATGGA	21	60.3	47.6
	YN-IIF22	GCATCGGCTAATGACAAAGGA	23	60.6	43.5
	YN-IIF3	CTGCATCGGCTAATGACAATGTA	20	62.4	60
	YN-IIF31	CACTCGCCGTGTTGGTCTCA	20	59.2	60
	YN-IIF4	CACTCGCCGTACTGGTCTCA	18	63.7	66.7
	YN-IIF41	CCTACCGCGCGCACAAGA	18	62.5	66.7
After the primer YN-II	YN-IIR1	CCTACCGCCCGCACAAGA	20	60.4	55
	YN-IIR2	AAGCCAAAGCCCCTACACCT	21	62.6	57.1
	YN-IIR21	GCCTGGGCGTCACGTCTTATA	23	62.9	52.2
	YN-IIR22	CTGCCTGTGCGACACGTCTTATA	23	59.8	47.8
	YN-IIR23	CTGCCTTCGTGACACAACTTCTA	25	60.1	44
	YN-IIR24	GCCTACATGGTCGTAACGTCTTATA	21	63.7	57.1
	YN-IIR25	GCCTGGGGGTCACGACTTTTA	21	63.1	61.9
	YN-IIR26	GCCTGGGGGTCACGACTTCTA	20	60.4	60
	YN-IIR3	CCTGGGGGTCACGACTTGTA	19	61.2	57.9

Table 3: SNP sites of two genotypes in PPY

Genotypes	Variation sites(bp)										
	9	10	34	36	42	55	62	93	127	131	180
YN-I	T	G	C	T	C	T	A	C	G	A	T
YN-II	C	T	T	C	T	C	G	T	A	T	C
	183	193	197	201	211	212	223	395	411	412	413
YN-I	G	A	C	C	A	T	C	T	G	C	G
YN-II	A	G	T	T	C	C	T	C	A	T	A
	439	443	462	471	476	482	500	520	521	526	527
YN-I	G	A	T	G	T	C	C	G	T	G	G
YN-II	A	G	C	A	C	T	T	T	G	C	A
	594	596	599	604	612	615	622				
YN-I	A	G	A	A	G	C	T				
YN-II	G	C	G	G	T	T	C				

Table 4: Information table of commercial medicinal materials of Paris

Sample number	Merchant-batch	Latin name	Locality
1	KY-1	*P. polyphylla* var. *yunnanensis*	Yun'nan
2	KY-1	*P. polyphylla* var. *yunnanensis*	Yun'nan
3	KY-2	*P. polyphylla* var. *yunnanensis*	Yun'nan
4	DH-1	*P. polyphylla* var. *yunnanensis*	Yun'nan
5	DH-2	*Trillium camschatcense*	Yun'nan
6	DH-2	*Trillium camschatcense*	Yun'nan
7	DH-2	*Trillium camschatcense*	Yun'nan
8	FJ-1	*Paris forrestii*	Yun'nan
9	FJ-1	*Paris forrestii*	Yun'nan
10	FJ-1	--	Yun'nan
11	FJ-2	--	Yun'nan
12	FJ-2	*Trillium govanianum*	Yun'nan
13	FJ-2	*P. forrestii*	Yun'nan
14	JYK	*Trillium govanianum*	Yun'nan
15	JYK	*Trillium undulatum*	Yun'nan
16	JYK	*Trillium govanianum*	Yun'nan
17	SST	*Trillium camschatcense*	Yun'nan
18	SST	*Trillium camschatcense*	Yun'nan
19	SST	*Trillium camschatcense*	Yun'nan
20	YQJ	*P. polyphylla* var. *yunnanensis*	Sichuan
21	YQJ	*P. polyphylla* var. *yunnanensis*	Sichuan
22	YQJ	--	Sichuan
23	WJ	*P. polyphylla*	Sichuan
24	WJ	*P. polyphylla*	Sichuan
25	WJ	*P. polyphylla*	Sichuan

Figures

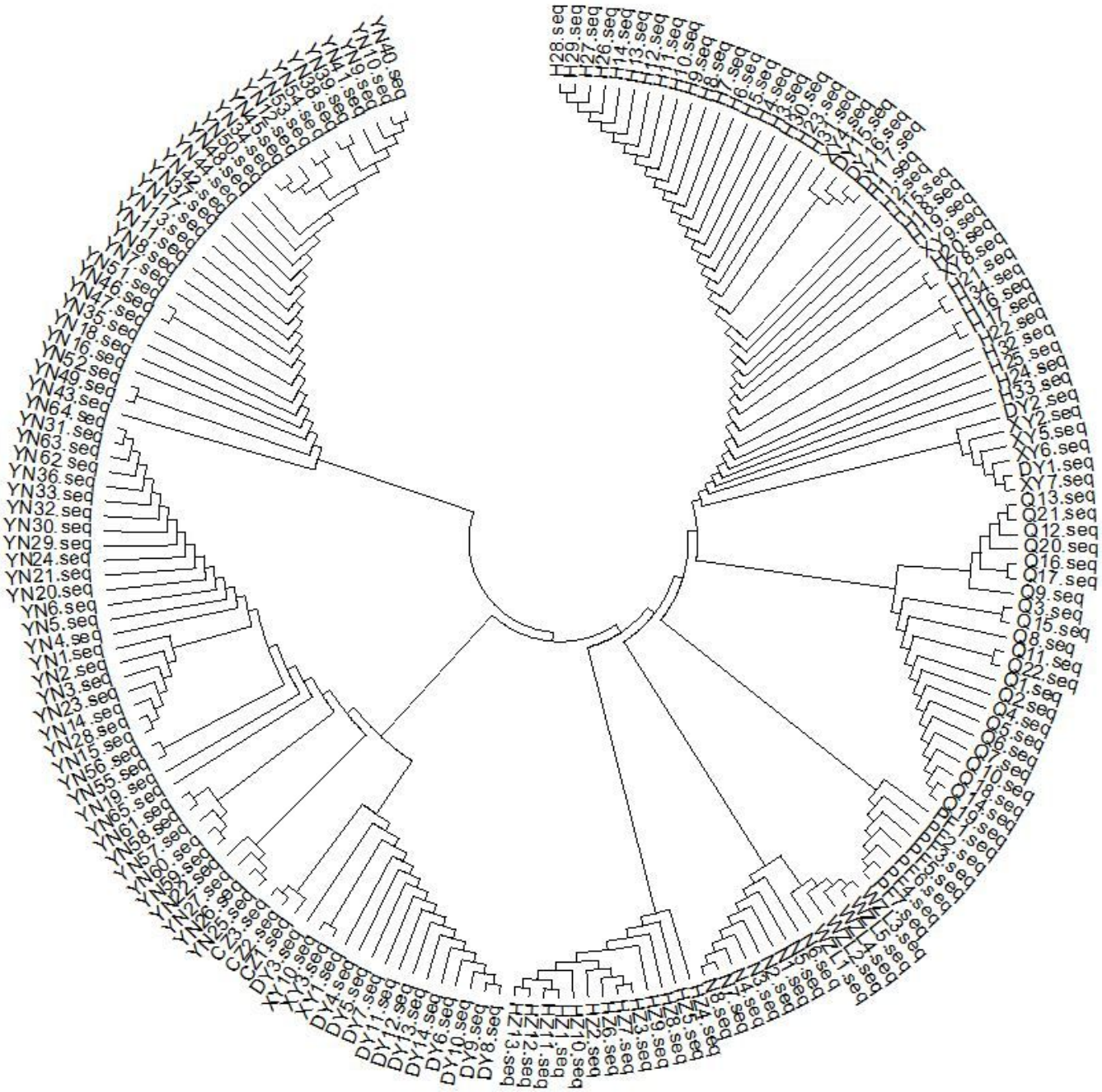

Figure 1

Phylogenetic tree of the Paris genusconstructed based on ITS.

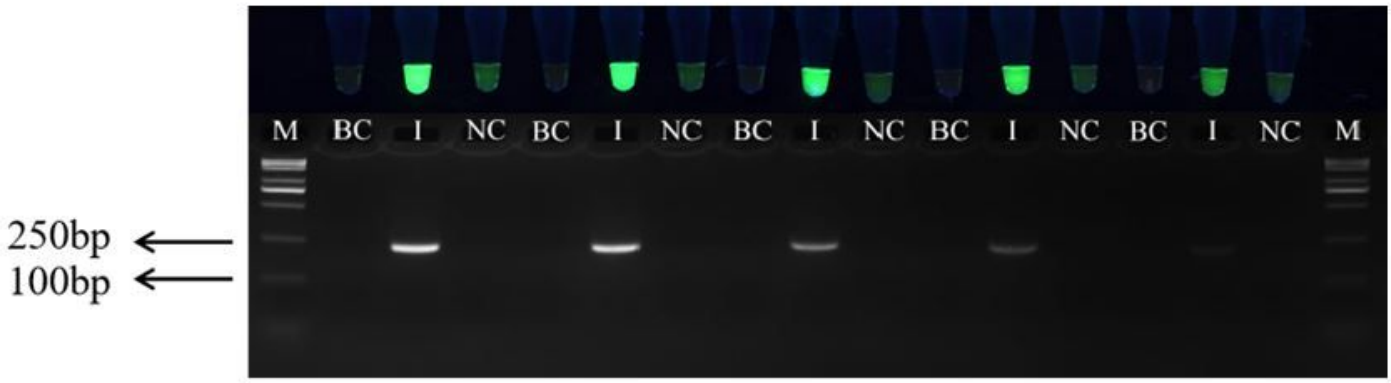

Figure 2

Investigate into primer concentration of specific PCR systemin YN-I.M: Trans2K Plus DNA Marker. BC: blank control. I: PPY (YN-I). NC: P. polyphylla, the same as below in this chapter. In the figure, the specific primer pairs were 0.3μM, 0.25μM, 0.2μM, 0.15μM, and 0.1μM, respectively.

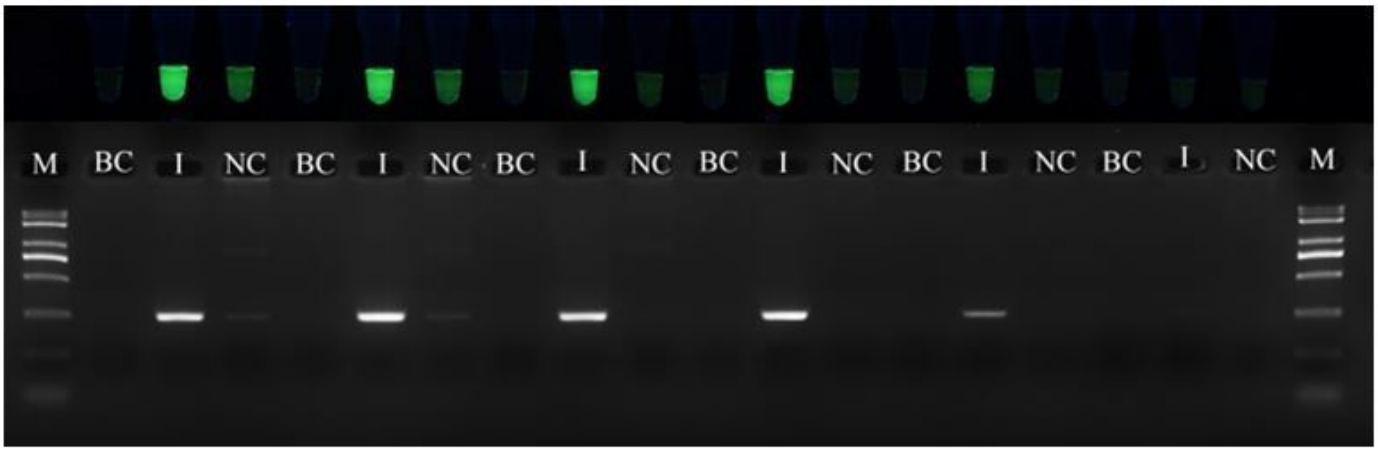

Figure 3

Investigate into annealing temperature of specific PCR systemin YN-I. The annealing temperatures were 58℃, 60.6℃, 62℃, 63.7℃, 66.2℃, and 68℃, respectively.

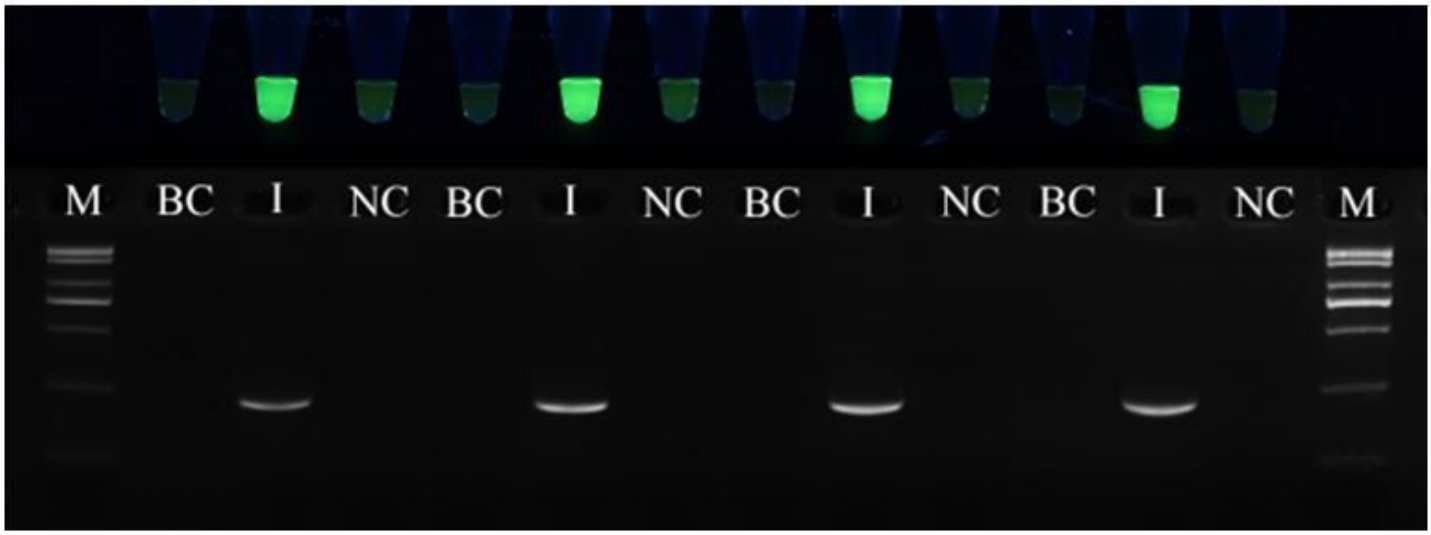

Figure 4

Investigate into dNTP concentration of specific PCR systemin YN-I. dNTP concentration was 80μM, 120μM, 200μM, and 280μM, respectively.

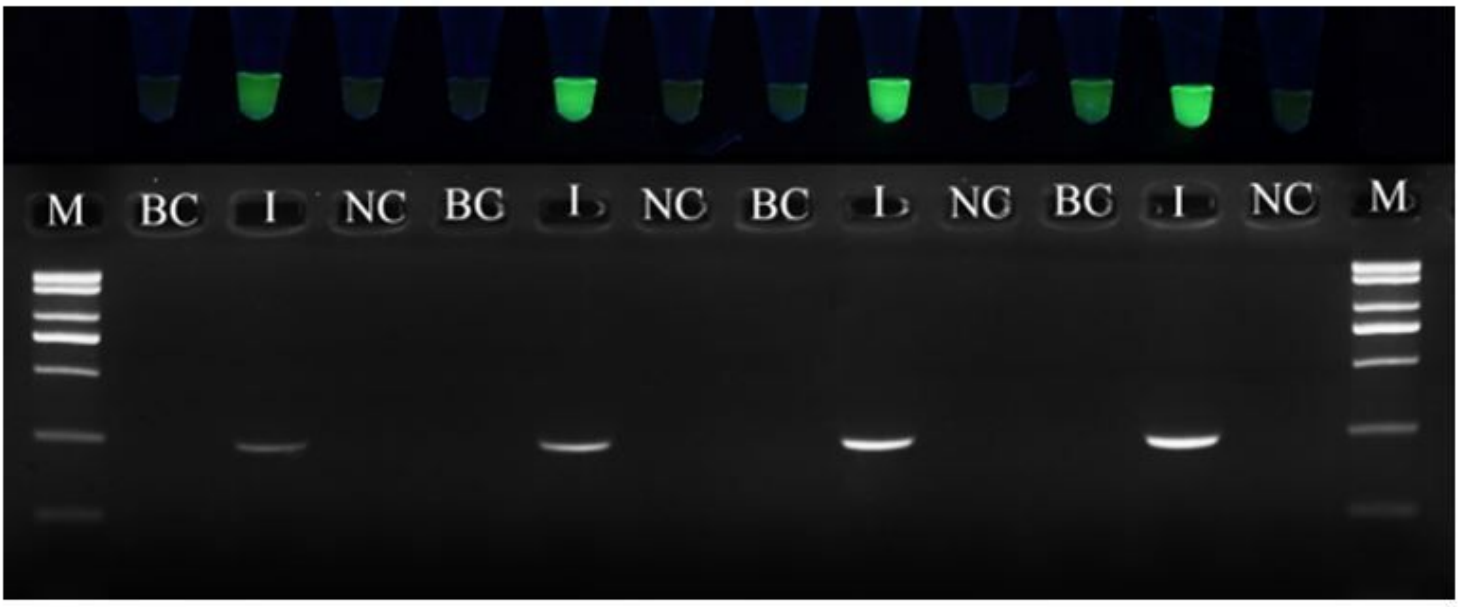

Figure 5

Investigate into aq enzyme dosage of specific PCR systemin YN-I. The Taq enzyme dosage was 1U, 1.5U, 2.5U, and 3.5U, respectively.

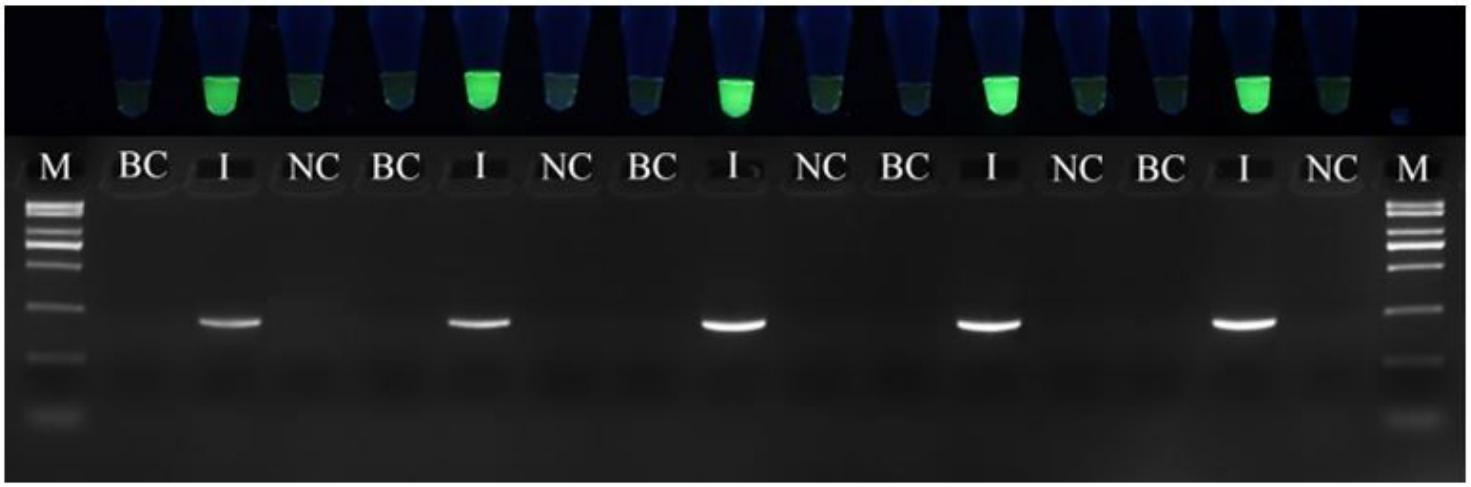

Figure 6

Investigate into the number of cycles of specific PCR systemin YN-I. The number of cycles was 26, 28, 30, 32, 34, respectively.

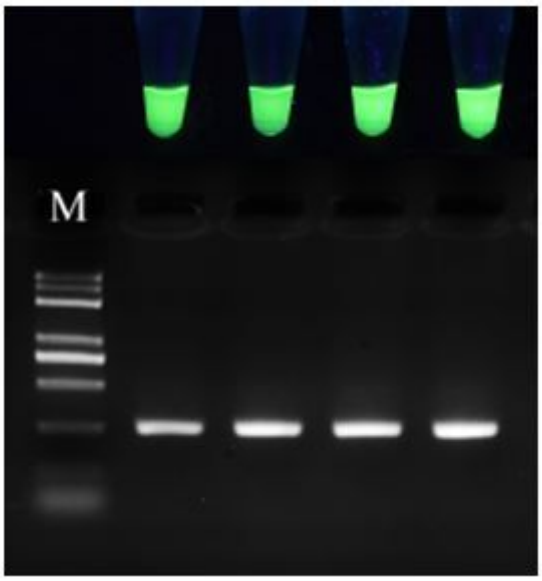

Figure 7

Investigate into the template quantities of specific PCR systemin YN-I. The template quantities were 10ng, 30ng, 60ng, and 90ng, respectively.

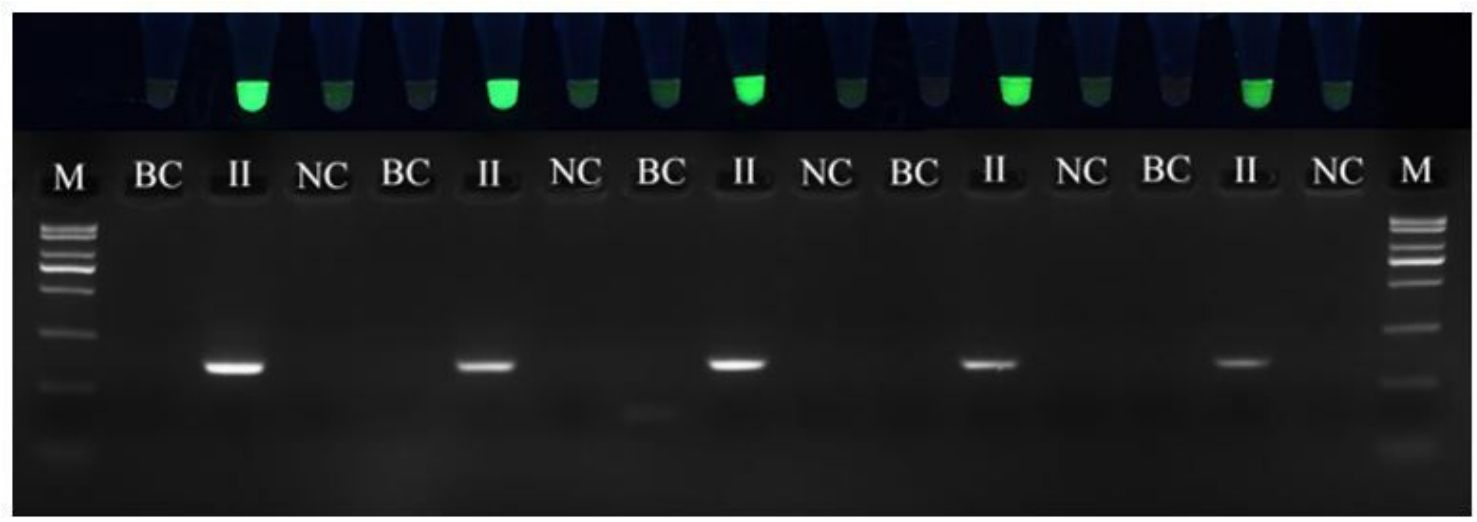

Figure 8

Investigate into primer concentration of specific PCR systemin YN-II. The specific primer pairs were 0.3μM, 0.25μM, 0.2μM, 0.15μM, and 0.1μM, respectively.

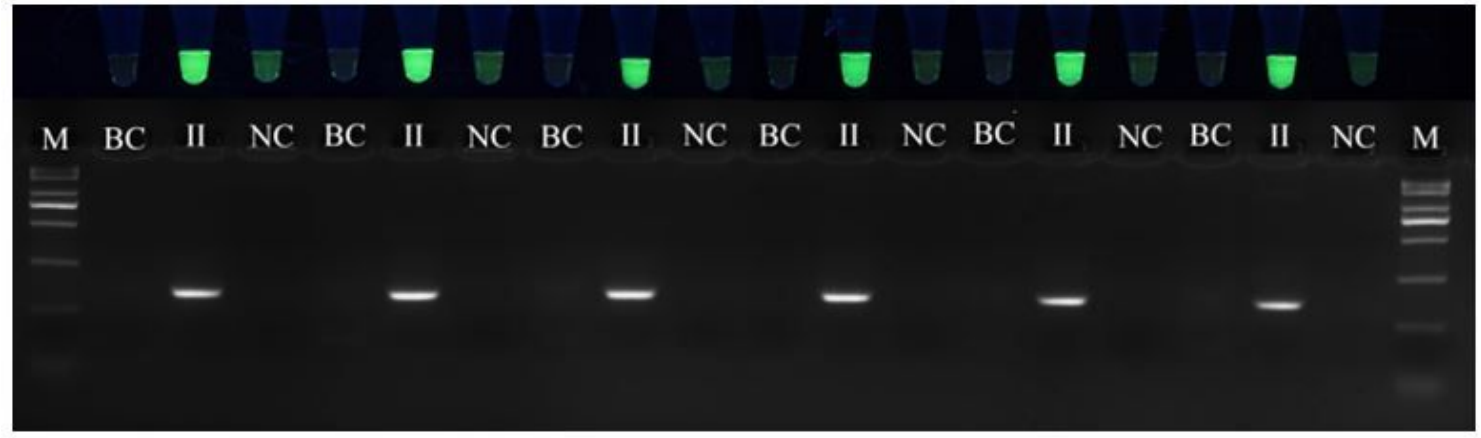

Figure 9

Investigate into annealing temperature of specific PCR systemin YN-II. The annealing temperatures were 58℃, 60.6℃, 62℃, 63.7℃, 66.2℃, and 68℃, respectively.

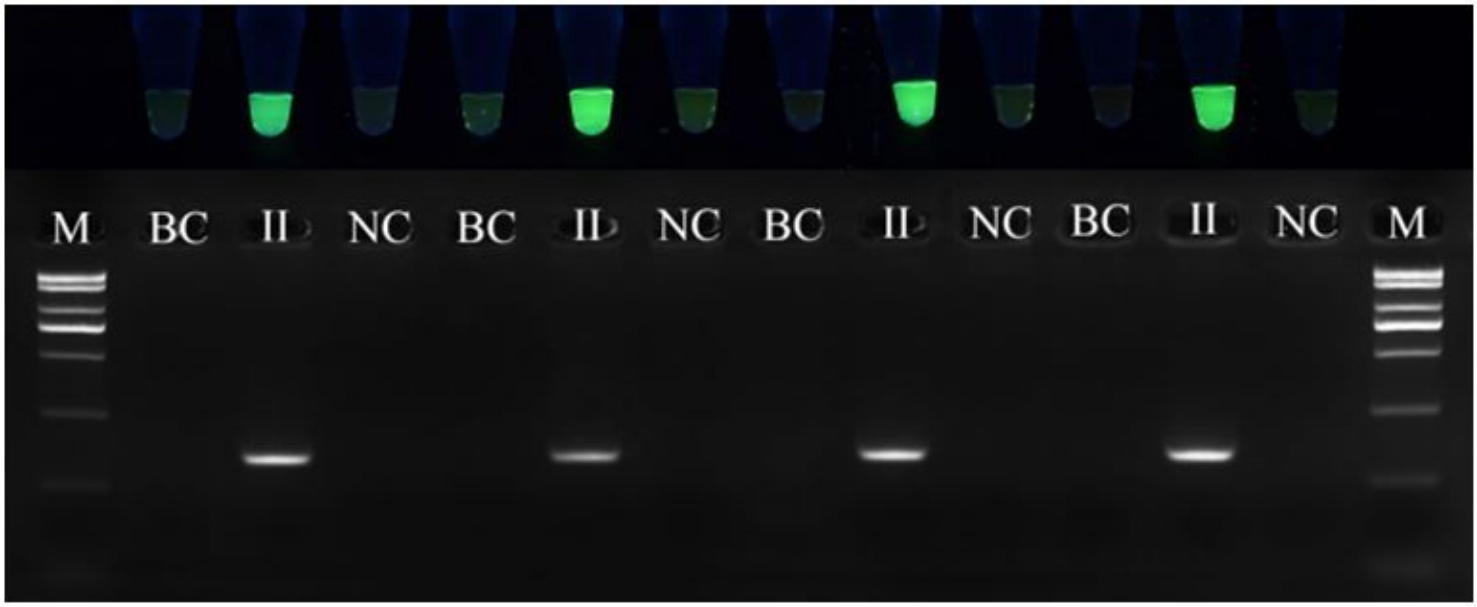

Figure 10

Investigate into dNTP concentration of specific PCR systemin YN-II. dNTP concentration was 80μM, 120μM, 200μM, and 280μM, respectively.

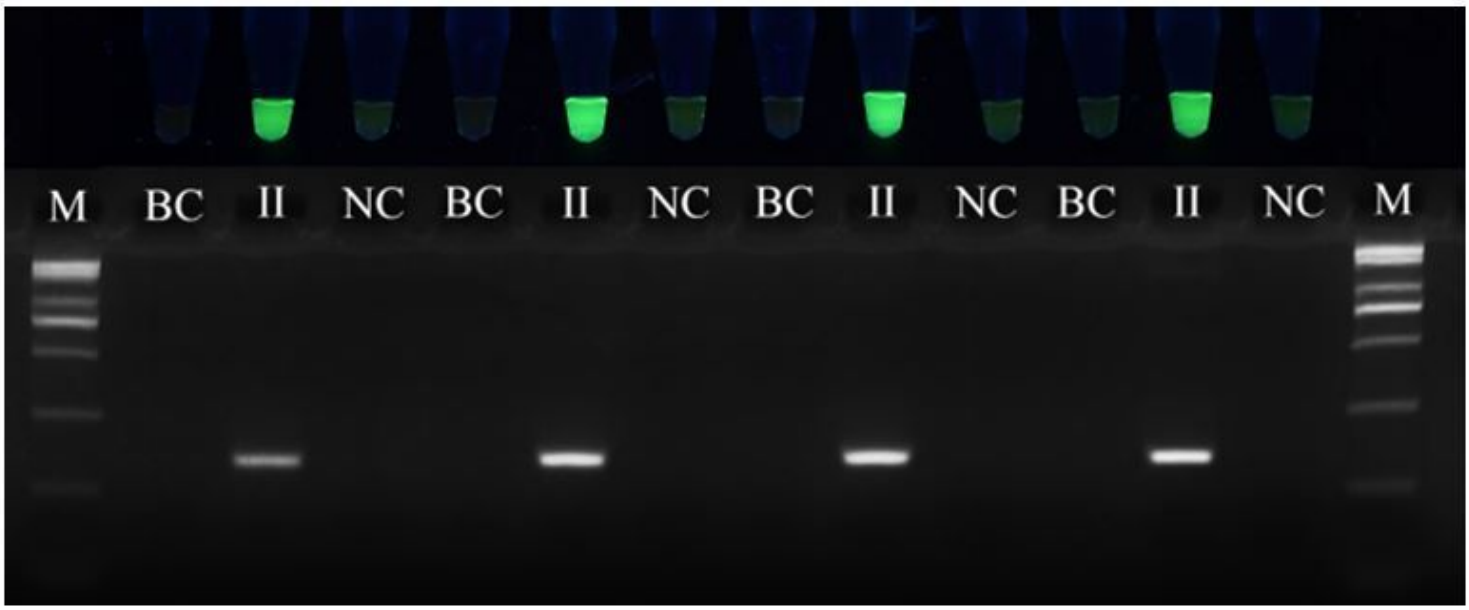

Figure 11

Investigate into aq enzyme dosage of specific PCR systemin YN-II. The Taq enzyme dosage was 1U, 1.5U, 2.5U, and 3.5U, respectively.

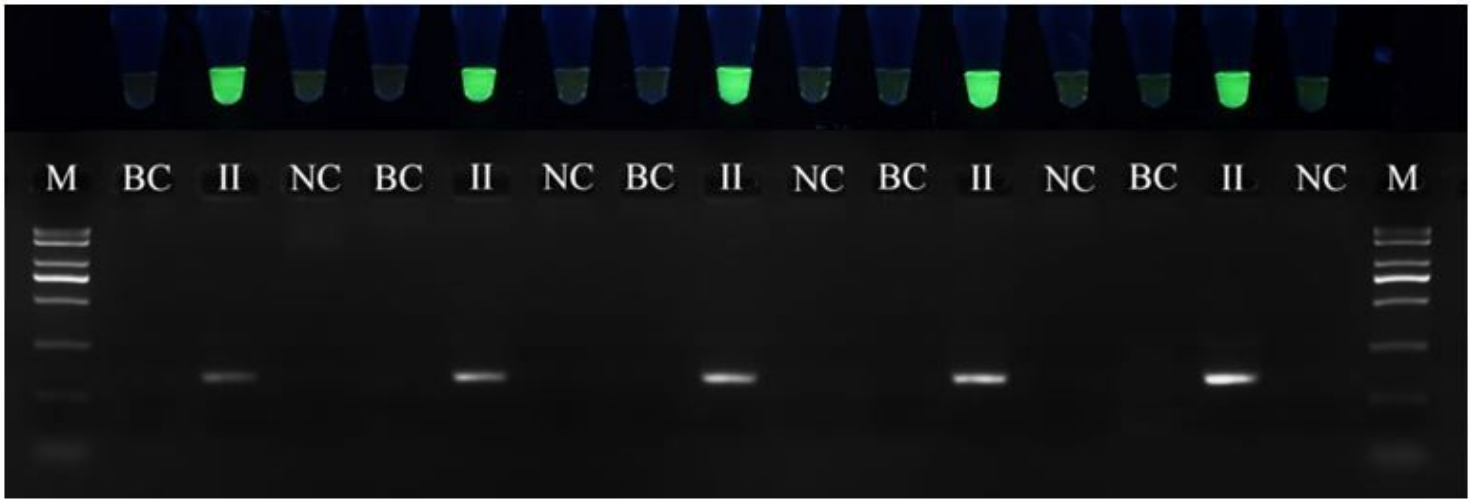

Figure 12

Investigate into the number of cycles of specific PCR systemin YN-II. The number of cycles was 26, 28, 30, 32, 34, respectively.

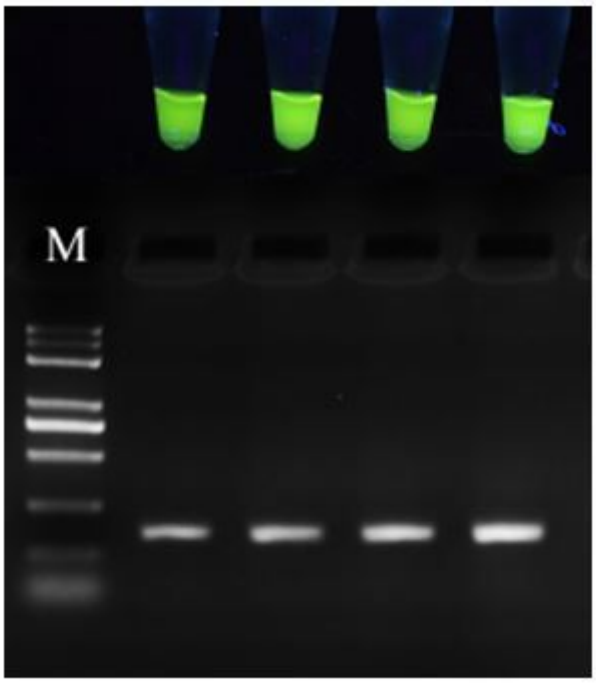

Figure 13

Investigate into the template quantities of specific PCR systemin YN-II. The template quantities were 10 ng, 30 ng, 60 ng, and 90 ng, respectively.

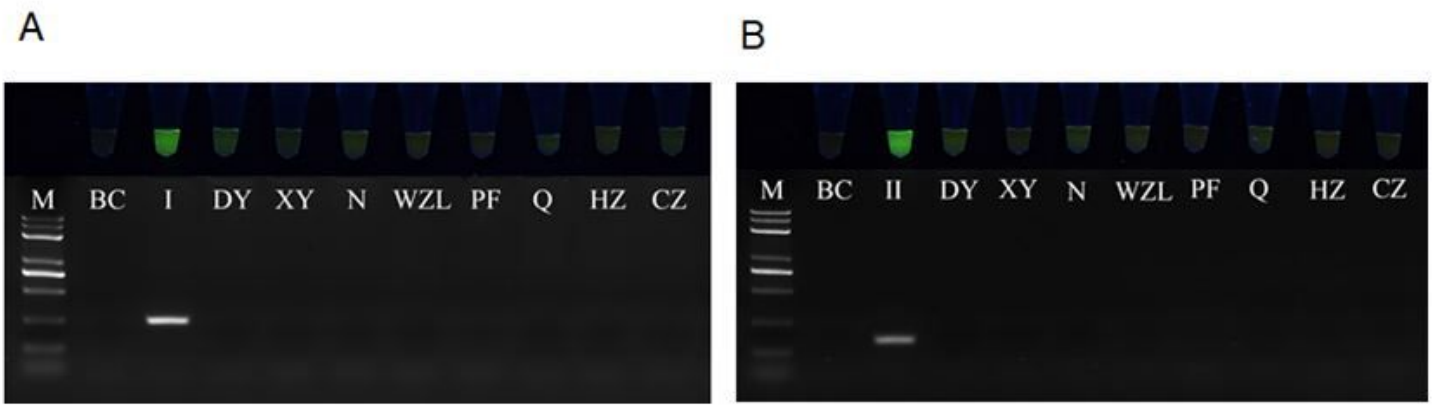

Figure 14

Fluorescent visualization identification of PPY with its common related species. (A) Specific identification of the YN-I system. (B) Specific identification of the YN-II system. M: Trans2K Plus DNA Marker. BC: blank control. I: PPY (YN-I). II: PPY (YN-II). DY: P. polyphylla. XY: P. stenophylla. N: P. vietnamensis. WZL: P. axialis. PF: P. vaniotii. Q:P. fargesii. HZ: P. thibetica. CZ:P. forrestii

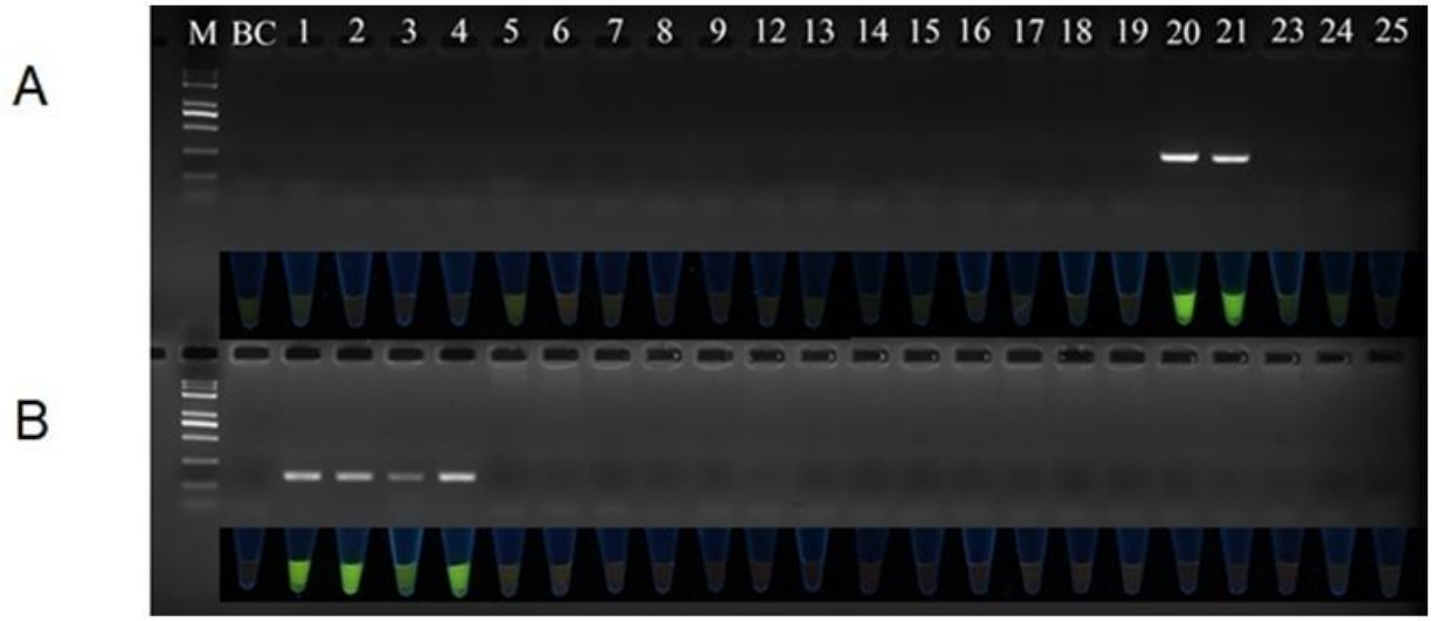

Figure 15

The specific identification of commercial medicinal materials of Paris. (A) The site-specific PCR system and fluorescence visual identification system of commercial medicinal materials of YN-I. (B) The site-specific PCR system and fluorescence visual identification system of commercial medicinal materials of YN-II.

Supplementary Files

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- SupplementaryMaterial.docx