



Original Article

QR code labeling system for Xueting-related herbs based on DNA barcode

Hong Zhou^a, Shuang-jiao Ma^a, Jing-yuan Song^a, Yu-lin Lin^a, Zheng-jun Wu^b,
Zheng-zhou Han^b, Hui Yao^{a,*}

^a Engineering Research Center of Tradition Chinese Medicine Resource, Ministry of Education, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100193, China

^b China Resources of Sanjiu Medical & Pharmaceutical Co., Ltd., Shenzhen 518110, China

ARTICLE INFO

Article history:

Received 25 November 2017
Revised 11 March 2018
Accepted 17 September 2018
Available online 4 October 2018

Keywords:

DNA labeling system
identification
ITS2
QR code
Xueting-related herbs

ABSTRACT

Objective: Xueting-related herbs include *Spatholobi Caulis* (Jixueteng in Chinese), *Sargentodoxae Caulis* (Daxueteng in Chinese), *Kadsuræ Caulis* (Dianjixueteng in Chinese), and other medicinal plant stems that release reddish-brown juices after being cut. However, similarity in phonetic spelling and sound leads to their misuse in clinic or commercial circulation. Accurate labeling is imperative as well as effective for species identification.

Methods: In this study, the ITS2 sequences of 76 samples of Xueting-related herbs were obtained and analyzed to identify them. And then they were converted into QR codes using the open source PHP QR code. Also, a DNA barcode reference library was established according to these sequences and was used to authenticate the 25 samples of Xueting-related herbs collected from the market.

Results: The lengths of the ITS2 sequences of different Xueting-related herbs ranged from 207 to 235 bp and the GC contents were 57.5%–71.0%. Jixueteng, Daxueteng, and Dianjixueteng were clustered into three clades respectively in the neighbor-joining (NJ) phylogenetic tree, and the efficiency of the BLAST method was 100%. The ITS2 sequences of different Xueting-related herbs were presented vividly and specifically in QR code. Twenty-two of all 25 commercial samples were consistent with the original labels, whereas three samples marked “Dianjixueteng” were authenticated as “Jixueteng”.

Conclusion: QR code labeling system based on DNA barcode is an effective labeling system of Xueting-related herbs for their circulation regulation.

© 2018 Tianjin Press of Chinese Herbal Medicines. Published by Elsevier B.V. All rights reserved.

1. Introduction

Chinese materia medica (CMM) have long historical clinical use and reliable therapeutic efficacy, which attract a global attention and widespread use. The regulations defining their proper use, especially identification of the correct species, are widely recognized, as the use of incorrect species is a threat to consumer safety due to the similar phonetic spelling and sound. For instance, Guangfangji (*Aristolochia fangchi* Y. C. Wu ex L. D. Chow et S. M. Hwang) was misused for Fangji (*Stephania tetrandra* S. Moore), and the toxic Guanmutong (*Aristolochia manshuriensis* Kom.) was mistaken as Mutong (*Akebia quinata* (Houtt.) Decne.), resulting in serious nephropathies (Lord, Tagore, Cook, Gower, & Pusey, 1999; Vanherweghem et al., 1993). Therefore accurate labeling of CMM

is crucial to guarantee their usage, especially for those share similarities in phonetic spelling and sound.

As the labeling system of the traditional Chinese name of CMM emphasizes on the characteristic of CMM, such as their efficiency, origin, appearance feature and medicinal parts, different CMM with similar characteristics may share similar names. Such as *Spatholobi Caulis*, the stem of *Spatholobus suberectus* Dunn releases reddish-brown juices, which like the color of chicken blood after being cut. Therefore, it has the Chinese name of “Jixueteng”. Furthermore, Jixueteng is produced in Yunnan Province, China has the trivial name of “Dianjixueteng”; “Dian” is the abbreviation of Yunnan Province. However, Dianjixueteng in the Chinese Pharmacopoeia refers to another drug *Kadsuræ Caulis*, the stem of *Kadsura interior* A. C. Smith. Moreover, stems of other species that release reddish-brown juices after being cut, such as *Sargentodoxa cuneata* (Oliv.) Rehd. et Wils (Daxueteng in Chinese), *Kadsura heteroclita* (Roxb.) Craib, *Mucuna sempervirens* Hemsl., *Mucuna birdwoodiana* Tutch., *Milletia dielsiana* Harms, and *Milletia tsui* Metc., which are also called “Xueting” in folk (Chen, Xu, Xu, & Jin, 1993a,b; Lin, 2000), leading

* Corresponding author.

E-mail address: hyao@implad.ac.cn (H. Yao).

to confusion in commercial circulation. Although Chinese pharmacopoeia employs both Chinese name and Latin name for individual drug, the nomenclature is too professional to be used by non-professionals.

In order to avoid confusion of Xueting-related herbs (Fig. 1), it is necessary to label them using DNA barcoding before circulation. ITS2 sequence has been used as a universal DNA barcode for plants (Chen et al., 2010; Yao et al., 2010), especially for the sliced, shredded, dried, or simply processed CMM with degraded DNA (Ma et al., 2017; Xin et al., 2015; Zhao et al., 2015). The DNA barcoding identification system for CMM has been established and recorded in Chinese Pharmacopoeia (Pharmacopoeia Committee of P. R. China, 2015; Zhang, Huang, & Yan, 2017). As the 2D barcode is amenable to information storage, recognition, and retrieval, and QR code is the most appropriate symbology for biological genetic information in practical applications (Liu et al., 2012). The labeling system of QR code that consisted of biological genetic information and Latin names can produce genetic ID cards, which reduce nomenclature confusion in CMM market circulation. Here, we established unique genetic ID for Xueting-related herbs using ITS2 barcode and QR code image.

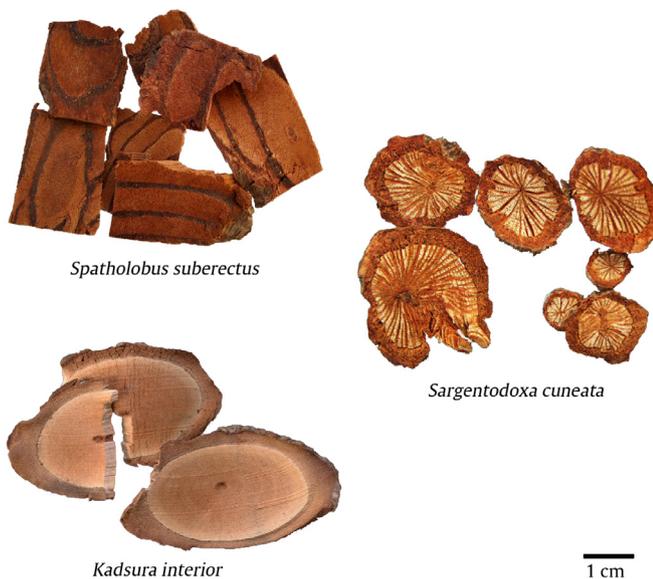


Fig. 1. Morphology of *Spatholobus suberectus* (Jixueteng in Chinese), *Kadsura interior* (Dianjixueteng in Chinese) and *Sargentodoxa cuneata* (Daxueteng in Chinese) recorded in Chinese Pharmacopoeia. They all presented with circular, elliptical or irregularly inclined section, taupe external and red-brown transverse section.

2. Materials and methods

2.1. Sample collection

A total of 67 experimental samples belonging to eight species, namely, *Spatholobus suberectus* Dunn (40 samples), *Sargentodoxa cuneata* (Oliv.) Rehd. et Wils (four samples), *Kadsura interior* A. C. Smith (seven samples), *Kadsura heteroclita* (Roxb.) Craib (two samples), *Mucuna sempervirens* Hemsl. (six samples), *Mucuna birdwoodiana* Tutch. (one sample), *Millettia dielsiana* Harms (six samples), and *Millettia tsui* Metc. (one sample), were used in this study. Sixty-five plant samples were collected from different locations in China and identified according to their morphological characteristics by Professor Yulin Lin from the Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences. Two samples were purchased from the Chinese Food and Drug Inspection Institute. The remaining nine ITS2 sequences were

downloaded from GenBank. In total, 76 sequences were used to establish the DNA barcode library. The haplotypes of the ITS2 regions of these eight species were submitted to GenBank, and the accession numbers were shown in Table 1. All corresponding voucher samples were deposited in the Herbarium of IMPLAD.

2.2. Genomic DNA extraction, amplification, and sequencing

The surfaces of raw drug from market samples were first scraped off and then wiped with 75% ethanol. Genomic DNA was extracted from 25–30 mg of fresh plant samples or 40–45 mg of caulis using the Plant Genomic DNA Kit (Tiangen Biotech Co., Beijing, China). The primers, polymerase chain reaction (PCR) conditions, and sequencing were previously described (Chen et al., 2010; Pharmacopoeia Committee of P. R. China, 2015).

2.3. Sequence analysis and QR code generation

Raw trace files were trimmed to remove the primer and low-quality areas and then assembled. The Hidden Markov model (HMM) was used to remove the 5.8S and 28S sections in order to annotate the ITS2 region (Keller et al., 2009). On the basis of the Kimura-two-parameter (K2P) model, the intra- and inter-specific genetic distances were calculated with MEGA 6.0 (Tamura, Stecher, Peterson, Filipinski, & Kumar, 2013). All the ITS2 sequences were used to calculate genetic distances, whereas the phylogenetic neighbor-joining (NJ) tree was constructed according to the haplotypes of Jixueteng, Daxueteng, Dianjixueteng and other “Xueting” herbs. Bootstrap tests were performed using 1000 replicates to assess the confidence of the phylogenetic relationships via MEGA 6.0.

The QR code images were obtained by encoding the ITS2 sequences of the Xueting-related herbs, as well as their corresponding Latin names, using the open source PHP QR Code. Mobile terminals (such as iPhone and Android devices) can be used as QR code scanners to read the information. Finally, the scanned information can be submitted to DNA barcode identification system (<http://www.tcmbarcodes.cn>) to identify and analyze ITS2 sequences (Chen et al., 2014).

2.4. Species identification of commercial samples

A total of 25 commercial samples, including 16 samples of Jixueteng, six samples of Daxueteng, and three samples of Dianjixueteng, were randomly collected from markets and drug stores, and the ITS2 sequences of these commercial samples were tested by the reference library using BLAST method. Moreover, all 25 sequences and the haplotypes of the vouchers were used to construct the NJ tree.

3. Results

3.1. Analysis of sequence characteristics

Sequence analysis was performed on 76 samples. After annotation, the ITS2 sequence lengths of the 40 Jixueteng samples, 11 Daxueteng samples, and seven Dianjixueteng samples were 207, 234–235, and 231 bp, respectively, and their corresponding GC contents were 69.6%–71.0%, 66.8%–67.7%, and 59.7%–60.2%, respectively. A total of six nucleotide variation sites were found in Jixueteng sample sequences and nine were found in Daxueteng samples. Moreover, only one C-T variation at the 212 bp was found in the ITS2 regions of Dianjixueteng. Based on the variable sites, these three species generated seven, eight, and two haplotypes. Additional details about the sequence characteristics of other Xueting-related herbs were in Table 2.

Table 1
Xueteng-related herbs used in this study.

Names	Species	Families	Haplotype (Number)	Voucher No.	GenBank No.	Sources
<i>Spatholobi Caulis</i> (Jixueteng)	<i>Spatholobus suberectus</i>	Leguminosae	H1 (19)	FDC354, YC0001MT09*, 15, 17, 20, 32, 36–38, 40, 42–44, 46, 48–49, 52, 65, 67	MF416925	Chinese Food and Drug Inspection Institute, Beijing; Guilin, Nanning, Chongzuo, Guangxi; Meizhou, Herbal Garden of Guangzhou University of Chinese Medicine, Guangdong; Vietnam
			H2 (9)	YC0001MT13*–14, 30, 39, 53, 63–64, 66, 68	MF416926	Guangxi Branch Institute of Medicinal Plant Development, Mount Dragon and Tiger, Nanning, Guangxi; Herbal Garden of Guangzhou University of Chinese Medicine, Guangdong
			H3 (7)	YC0001MT07*, 16, 18–19, 29, 45, 51	MF416927	Meizhou, Guangdong
			H4 (2)	YC0001MT08*, 50	MF416928	Meizhou, Guangdong
			H5 (1)	YC0001MT33*	MF416929	Shenzhen, Guangdong
			H6 (1)	YC0001MT31*	MF416930	Jinxiu, Guangxi
			H7 (1)	YC0001MT47*	MF416931	Meizhou, Guangdong
<i>Sargentodoxae Caulis</i> (Daxueteng)	<i>Sargentodoxa cuneata</i>	Lardizabalaceae	A1 (4)	-	EF076045, 49–50, 53	GenBank
			A2 (1)	YC0279MT18*	MF416932	Baoding, Hebei
			A3 (1)	YC0279MT17*	MF416933	Baoding, Hebei
			A4 (1)	-	EF076051	GenBank
			A5 (1)	YC0279MT19*	MF416934	Wuhan, Hubei
			A6 (1)	YC0279MT20*	MF416935	Wuhan, Hubei
			A7 (1)	-	EF076047	GenBank
			A8 (1)	-	EF076054	GenBank
<i>Kadsurae Caulis</i> (Dianjixueteng)	<i>Kadsura interior</i>	Magnoliaceae	B1 (5)	FDC346, YC0699MT01*–02, 04, 06	MF416942	Chinese Food and Drug Inspection Institute, Beijing; Gengma, Yunnan
			B2 (2)	YC0699MT03*, 05	MF416943	Gengma, Yunnan
Other herbal medicine called Xueteng	<i>Kadsura heteroclita</i>	Magnoliaceae	C1 (2)	YC0723MT01*–02	MF416944	South China Botanical Garden, Guangdong
			<i>Mucuna sempervirens</i>	Leguminosae	D1 (5)	YC0724MT01*–04, 06
	D2 (1)	YC0724MT05*	MF416937		Wuhan Botanical Garden, Hubei	
	<i>Mucuna birdwoodiana</i>	Leguminosae	E1 (1)	YC0726MT01*	MF416938	South China Botanical Garden, Guangdong
	<i>Milletia dielsiana</i>		Leguminosae	F1 (4)	YC0725MT02*–05	MF416940
	F2 (1)	YC0725MT01*		MF416941	Kunming, Yunnan	
	F3 (1)	PS0309MT01*		GQ434376	Kunming, Yunnan	
	F4 (1)	-		GQ246022	GenBank	
	F5 (1)	-		GQ434376	GenBank	
	<i>Milletia tsui</i>	Leguminosae	G1 (1)	YC0727MT01*	MF416939	South China Botanical Garden, Guangdong

*ITS2 sequences were submitted to GenBank.

Table 2
ITS2 sequence characteristics of eight Xueteng-related herbs.

Species	Sequence number	Sequence length / bp	GC content / %	Number of variable sites
<i>Spatholobus suberectus</i>	40	207	69.6–71.0	6
<i>Sargentodoxa cuneata</i>	11	234–235	66.8–67.7	9
<i>Kadsura interior</i>	7	231	59.7–60.2	1
<i>Kadsura heteroclita</i>	2	231	61.0	0
<i>Mucuna sempervirens</i>	6	212	69.8–70.3	1
<i>Mucuna birdwoodiana</i>	1	212	68.4	/
<i>Milletia dielsiana</i>	8	219–221	57.5–58.8	6
<i>Milletia tsui</i>	1	233	67.0	/

Note: “/” single sequence cannot be used to analyse intraspecific variations

3.2. Intra- and inter-specific genetic distances and NJ Tree

In this study, all 76 ITS2 sequences were analyzed to calculate genetic distances. Based on the K2P model, the intra-specific genetic distance of Jixueteng varied from 0 to 0.015, with an average of 0.008. Moreover, the maximum intra-specific genetic distances of Daxueteng and Dianjixueteng were 0.017 and 0.004, respectively. Furthermore, the minimum inter-specific K2P genetic distances of Jixueteng, Daxueteng, and Dianjixueteng were 0.271, 0.594, and 0.018, respectively, which were all larger than their maximum intra-specific genetic distances (Table 3). Based on the

NJ tree constructed by the 27 ITS2 sequence haplotypes, Jixueteng, Daxueteng, and Dianjixueteng can be respectively clustered into one clade with a high bootstrap value and be efficiently distinguished from other species (Fig. 2). Thus, the NJ tree results indicated that the ITS2 barcode can correctly identify different kinds of “Xueteng”. Moreover, the inter-specific sequence similarity of ITS2 of *K. interior* (Dianjixueteng in Chinese) and *K. heteroclita* was 97.8%–98.3%, and their inter-specific K2P genetic distance was 0.018–0.022. They formed a single branch with a high bootstrap value in the NJ tree. These results showed that these two species might have a close genetic relationship. However, further evidence is needed as the sequences are scarce.

3.3. Genetic ID card—QR code based on DNA barcode

The ITS2 sequences of Xueteng-related herbs were converted into colored barcode images based on the open source PHP QR code and the coding program (Xin et al., 2015; Chen, 2015). These QR codes included two sections: multicolor barcode in the left and QR code in the right. Different colors represented different bases in the multicolor barcode, and the sequence lengths were numerical (Fig. 3). In this way, the DNA barcode sequences of different kinds of “Xueteng” were presented vividly and specifically in QR code, achieved cross-platform conversion of DNA barcode informa-

Table 3

Intra- and inter-specific genetic distances between *S. suberectus*, *S. cuneata*, *K. interior*, and other Xueting-related herbs.

K2P genetic distances	Relationship	Range of genetic distances (mean)
Intra-specific distances	<i>S. suberectus</i>	0 – 0.015 (0.008)
	<i>S. cuneata</i>	0 – 0.017 (0.008)
	<i>K. interior</i>	0 – 0.004 (0.002)
Inter-specific distances	<i>S. Suberectus</i> and other Xueting	0.271 – 0.697 (0.510)
	<i>S. cuneata</i> and other Xueting	0.594 – 0.809 (0.673)
	<i>K. interior</i> and other Xueting	0.018 – 0.943 (0.696)

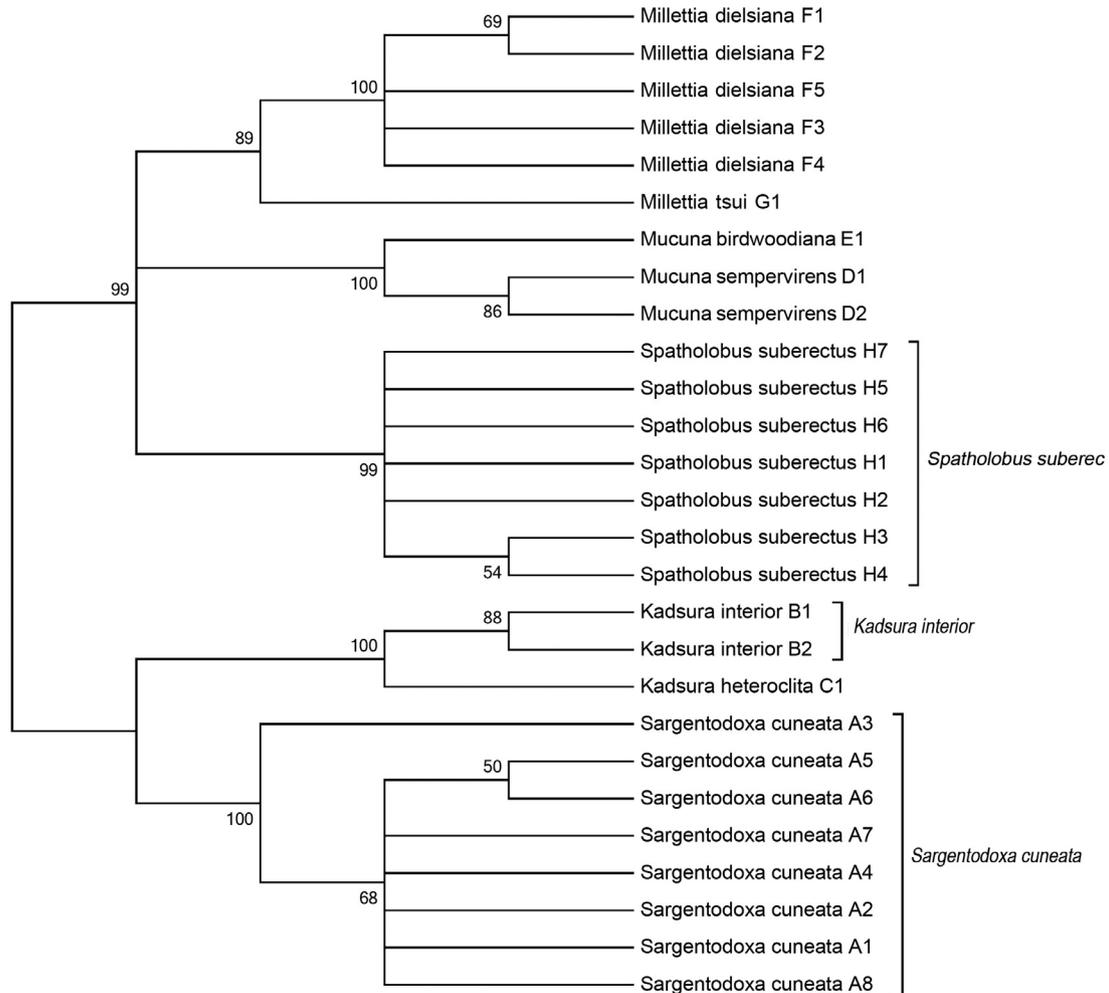


Fig. 2. Phylogenetic NJ tree based on 27 ITS2 sequence haplotypes of eight Xueting-related herbs (Haplotype No. followed Latin name. Bootstrap scores ($\geq 50\%$) were shown for each branch. Different Xueting-related herbs can be efficiently distinguished).

tion and might be applied in resource monitoring, accurate species identification, and quality control of “Xueting” (Fig. 4).

3.4. Authenticity of commercial samples of Xueting-related herbs

To investigate the authenticity of commercially available Xueting-related herbs, we used the BLAST method to identify the species of 25 commercial samples from markets and drug stores. The results in Table 4 showed that 22 (88%) out of the 25 tested samples were identified as the labeled species. The remaining three samples (sample No. CMM023-025) marked “Dianjixueting” (*K. interior*) were authenticated as Jixueting (*S. suberectus*). Moreover, the NJ tree (Fig. 5) showed the same results. A total of 16 samples (sample No. CMM001-016) marked “Jixueting” were clustered into group A, and six samples (sample No. CMM017-022)

marked “Daxueting” were clustered into group B, indicating that these samples were authentic. CMM023, CMM024, and CMM025 were grouped with Jixueting in group A rather than group C, indicating that these three commercial samples marked “Dian Jixueting” were proved to be “Jixueting”.

4. Discussion

4.1. Different nomenclature systems of CMM

The traditional Chinese name of CMM contains extensive and profound thoughts and theories of traditional Chinese medicine (TCM), and it emphasizes on the characteristic of CMM, such as their origin, appearance feature and officinal parts (He, 2014). This nomenclature system has deep cultural connotations but the differences recorded in previous generations of classic herbal works

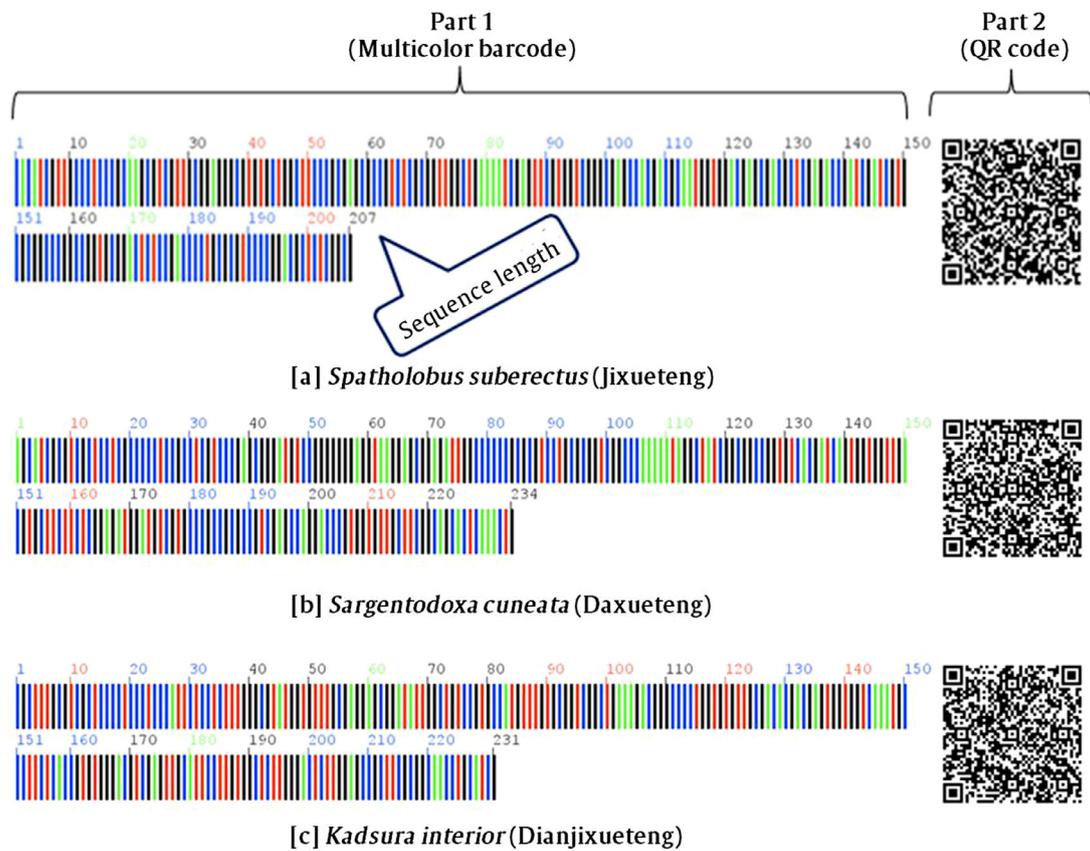


Fig. 3. 2D DNA barcode images of three Xueting-related herbs recorded in Chinese Pharmacopoeia (■A ■T ■C ■G). A labeling included two parts such as multicolor barcode in left and QR code in right. A, T, C and G were marked green, red, blue and black in multicolor barcode, respectively. Sequence lengths were numerical.

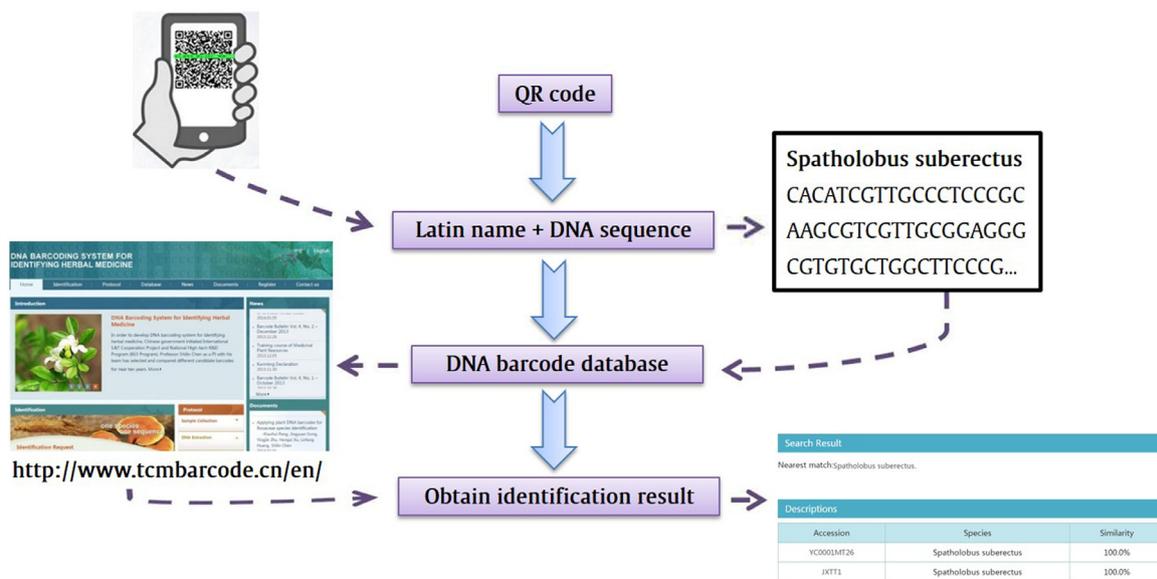


Fig. 4. Usage of 2D DNA barcode in circulation regulation.

and the medication habits of different regions resulted in the phenomenon that some different herbs share the similar phonetic spelling and sound, or one herb has different names, which restrict its usage in international exchanges and market circulation. Since the Binominal nomenclature created by Linnaeus, the scientific name of species is commonly used in plant taxonomy. It is universal in the world and is not limited by the state or lan-

guage. While, the labeling system of scientific name cannot express the characteristics of CMM effectively, as medicinal materials do not equal to the medicinal plants. Therefore, the labeling system of Latin names of crud drugs was formulated and recorded in Chinese Pharmacopoeia. This labeling system combines the Latin name of species and medicinal material characteristics (Chen, 2009). For example, *S. suberectus* is the original plant of Jix-

Table 4

Characteristics and identification of 25 commercial crude drug samples from three herbs recorded in Chinese Pharmacopoeia.

Sample No.	No. of samples	Sources	Market label	Barcode ID
CMM001-003	3	Anguo Medicine Market, Hebei	Jixueteng	<i>S. suberectus</i>
CMM004-005	2	Bozhou Medicine Market, Anhui	Jixueteng	<i>S. suberectus</i>
CMM006	1	Drug Store I, Beijing	Jixueteng	<i>S. suberectus</i>
CMM007-008	2	Drug Store II, Beijing	Jixueteng	<i>S. suberectus</i>
CMM009-011	3	Drug Store III, Guangdong	Jixueteng	<i>S. suberectus</i>
CMM012-014	3	Drug Store IV, Guangdong	Jixueteng	<i>S. suberectus</i>
CMM015-016	2	Drug Store V, Guangdong	Jixueteng	<i>S. suberectus</i>
CMM017-018	2	Anguo Medicine Market, Hebei	Daxueteng	<i>S. cuneata</i>
CMM019-021	3	Drug Store I, Beijing	Daxueteng	<i>S. cuneata</i>
CMM022	1	Chuqimen Medicine Market, Chongqing	Daxueteng	<i>S. cuneata</i>
CMM023-025	3	Drug Store VI, Yunnan	Dianjixueteng	<i>S. suberectus</i>

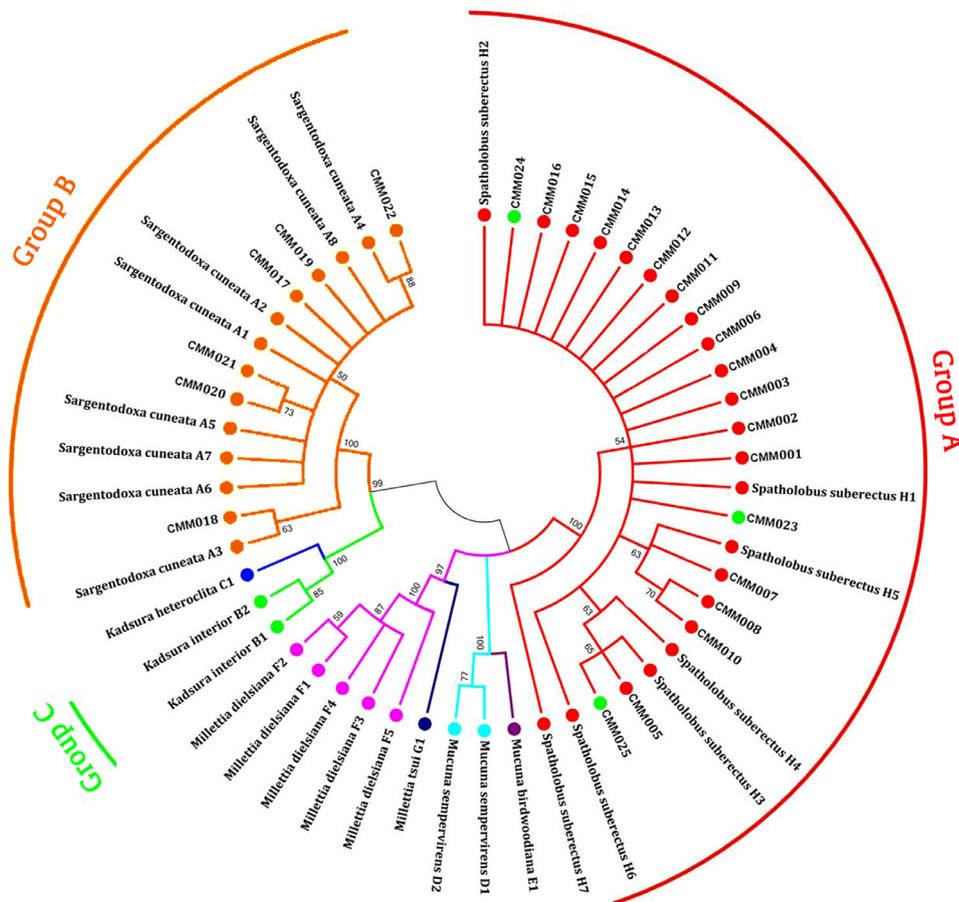


Fig. 5. NJ tree for all haplotypes of database and commercial Xueting samples (Bootstrap scores ($\geq 50\%$) were shown for each branch). Twenty-seven ITS2 sequence haplotypes of eight Xueting-related herbs marked with Latin name and haplotype No. using for database construction, and 25 ITS2 sequences of commercial Xueting samples marked with “CMM”. Jixueteng, Daxueteng and Dianjixueteng were clustered into group A, B and C, respectively. Based on the tree, three commercial samples marked “Dianjixueteng” (CMM023-025) were proved to be “Jixueteng”.

ueteng, and *Spatholobi Caulis* is the Latin name of Jixueteng as its medicinal part is caulis. These two labeling systems are too professional to be used by non-professionals. The QR code labeling system is based on the molecular identification system, which give each CMM a unique genetic ID card. The users can efficiently and quickly get the accurate name of CMM just with a phone. It will not be restricted by nationality, expertise, time, and place. Each of these four labeling systems has their own features, and they are suitable for various situations. And the QR code labeling system may show superiority in commercial circulation and regulation of CMM.

4.2. Application of ITS2 sequence in species identification of Xueting-related herbs

The stability and accuracy of the ITS2 sequence has been verified by many studies (Ma et al., 2017; Song et al., 2012; Xin et al., 2015; Xin et al., 2013; Zhao et al., 2015). In this study, we have successfully amplified and sequenced the ITS2 sequences of 67 samples that included Jixueteng, Daxueteng, Dianjixueteng, and other Xueting-related herbs. Sequencing efficiency of the 25 commercial samples was 100% as well. Huang, Ma, Zhan, and Chen (2015) encountered problems in sequencing and did not obtain qualified

ITS2 sequences after repeated tests. They ascribed the failure to the existence of multiple copies of ITS in the nuclear gene. However, multiple copies actually favor sequence amplification, especially for samples with partially degraded DNAs (Coleman, 2007; Gao et al., 2010). Moreover, we and Yu, Xie, Wu, Tao, and Xu (2016) successfully amplified and sequenced the ITS2 region. We suppose that the overlapping peaks in Huang's research may be caused by the fungal contamination. ITS2 fragments are widely found in plants and fungi (Kress, Wurdack, Zimmer, Welgt, & Janzen, 2005), and the sequencing quality may be negatively affected when the CMM are contaminated with ITS2 sequences from fungi. In our study, we scraped off the surfaces of raw drug market samples and wiped them with 75% ethanol to prevent fungal ITS sequences from interfering with our results. Other compounds, such as polysaccharides and polyphenols, that existed in Xueting-related herbs could co-precipitate with the DNA and form insoluble sticky jelly-like substances, which may seriously affect DNA extraction and PCR amplification. To improve the quantity and quality of DNA, 1% PVP (polyvinylpyrrolidone) and beta-mercaptoethanol were added to the samples before they were crushed, and simultaneously the extraction step by wash buffer were increased 3 times. The water bath time was extended to 8–12 h (Li, Wang, Yu, Wang, & Zhou, 2013).

The ITS2 sequence has already been used to identify some Xueting species (Guo et al., 2017; Yu et al., 2016); however, the sources of the experimental samples in these studies were limited. Yu's study (Yu et al., 2016) merely included six samples of Jixueteng and three samples of Dianjixueteng, which were limited for assessing the intra-specific genetic distance. Moreover, the samples in Guo's (Guo et al., 2017) study only came from Schisandraceae. In contrast, by using 67 experimental samples including eight species, we covered three families in this study. The abundant samples provided robust support to the results, and the ITS2 sequences can effectively distinguish different Xueting species. Additionally, 22 of the commercial samples of Xueting-related herbs were found to be consistent with their labels, whereas three samples marked with "Dianjixueteng" were authenticated as "Jixueteng". Yu's research also found "Jixueteng" mislabeled as "Dianjixueteng" (Yu et al., 2016).

4.3. Power and challenges of genetic ID card—QR code for circulation regulation of CMM

The term "DNA barcode" has been proposed by Hebert 15 years ago (Hebert, Ratnasingham, & deWaard, 2003). But DNA barcode sequences are not amenable to information storage, recognition and retrieve, which limit its practical applications (Liu et al., 2012). Therefore, researchers have given efforts to the practical applications of DNA barcoding technologies, especially in the digital information age. QR code has the largest coding capacity, and it has a relatively high compression ratio, enabling it to display and retrieve DNA barcode information efficiently, thereby making it a new format to represent DNA barcode sequences (Liu et al., 2012). The prevalent use of web servers and mobile terminals (such as iPhone and Android devices) will allow users to capture and analyze DNA sequence information anywhere or anytime. Moreover, combining DNA sequences and QR code will make DNA barcoding applications highly practical. Some studies have been conducted to convert the DNA sequences of medicinal materials into QR code images to monitor the quality of CMM and regulate its circulation (Ma et al., 2017; Xin, Li, Yao, & Chen, 2015). In this study, we applied the QR code technology in Xueting-related herbs and gave each of them a genetic ID card. Other types of metadata such as producing area etc, can be encoded in the QR code. Our study may provide a convenient method for the non-professionals to recognize and purchase appropriate Xueting-related herbs.

Except for the above advantages, the genetic ID card has its limitations in quality supervision of CMM. As it is a DNA-based method, the detection of DNA sequence from medicinal materials does not necessarily indicate the presence of enough bioactive compounds, that is DNA sequence can just distinguish the authenticity of CMM, rather than evaluate their qualities. Besides, the mineral drugs have no DNA, and the DNA of highly process CMM were severely degraded, we could not acquire the genetic information from these CMM. As DNA sequences in all tissues of one species are the same, the consumers were unable to identify whether the DNA sequence comes from the medicinal parts without the morphological information. Thus, DNA barcode need to synergize with traditional methods for species identification. It is a complement rather than substitute to traditional methods.

5. Conclusions

This study gave the genetic ID cards to Xueting-related herbs and established a method for the identification of the original species of the homonymous CMM Xueting. Our results showed that mislabeled Xueting-related herbs are sold in medicine markets, and that the QR code is an effective labeling system for Xueting-related herbs. Furthermore, our method may be used to rapidly check the authenticity of commercial samples of Xueting-related herbs.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We thank Dr. Yan Lu from Department of Pharmacognosy, Fudan University for providing some experimental samples. This research was supported by the CAMS Innovation Fund for Medical Sciences (CIFMS, 2016-I2M-3-016) and the Key Projects of the National Science and Technology Pillar Program (No. 2011BAI07B08).

References

- Chen, D. F., Xu, G. J., Xu, L. S., & Jin, R. Y. (1993a). Macroscopical identification of *Caulis Spatholobi*. *Chinese Materia Medica*, 8(16), 21–24.
- Chen, D. F., Xu, G. J., Xu, L. S., & Jin, R. Y. (1993b). Original plant investigation and merchandise identification of *Caulis Spatholobi*. *Chinese Traditional Herbal Drugs*, 24(1), 34–37.
- Chen, S. L., Pang, X. H., Song, J. Y., Shi, L. C., Yao, H., Han, J. P., et al. (2014). A renaissance in herbal medicine identification: From morphology to DNA. *Biotechnology Advances*, 32(7), 1237–1244.
- Chen, S. L., Yao, H., Han, J. P., Liu, C., Song, J. Y., Shi, L. C., et al. (2010). Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PLoS ONE*, 5(1), E8613.
- Coleman, A. W. (2007). Pan-eukaryote ITS2 homologies revealed by RNA secondary structure. *Nucleic Acids Research*, 35(10), 3322–3329.
- Chen, S. L. (2015). *Standard DNA Barcodes of Chinese Materia Medica in Chinese Pharmacopoeia*. Beijing: Science Press.
- Chen, X. (2009). Discussion on the name of Chinese medicine in Latin. *Chinese Journal of Scientific and Technical Periodicals*, 20(5), 948–949.
- Gao, T., Yao, H., Song, J. Y., Liu, C., Zhu, Y. J., Ma, X. Y., et al. (2010). Identification of medicinal plants in the family Fabaceae using a potential DNA barcode ITS2. *Journal of Ethnopharmacology*, 130(1), 116–121.
- Guo, H. J., Li, X. W., Qi, Y. D., Wei, X. P., Zhang, B. G., & Liu, H. T. (2017). Identification of Dian Ji Xue Teng (*Kadsura interior*) with DNA barcodes. *World Journal of Traditional Chinese Medicine*, 3(1), 11–15.
- Hebert, P. D., Ratnasingham, S., & deWaard, J. R. (2003). Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society B Biological Sciences*, 270(Suppl 1), S96–S99.
- Huang, Q. L., Ma, X. Y., Zhan, R. T., & Chen, W. W. (2015). Comparative analysis and molecular identification of matK Gene from *Spatholobus suberectus* and its adulterants. *Northern Horticulture*, 17, 94–98.
- He, M. J. (2014). Traditional Chinese medicine name and Chinese medical culture. *China Journal of Chinese Medicine*, 29(10), 1482–1484.

- Keller, A., Schleicher, T., Schultz, J., Müller, T., Dandekar, T., & Wolf, M. (2009). 5.8S-28S rRNA interaction and HMM-based ITS2 annotation. *Gene*, 430(1-2), 50–57.
- Kress, W. J., Wurdack, K. J., Zimmer, E. A., Weigt, L. A., & Janzen, D. H. (2005). Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences of the United States of America*, 102(23), 369–8374.
- Lin, S. H. (2000). Discrimination of Xueteng. *Lishizhen Medicine and Materia Medica Research*, 11(4), 362.
- Lord, G. M., Tagore, R., Cook, T., Gower, P., & Pusey, C. D. (1999). Nephropathy caused by Chinese herbs in the UK. *Lancet*, 354(9177), 481–482.
- Liu, C., Shi, L. C., Xu, X. L., Li, H., Xing, H., Liang, D., et al. (2012). DNA barcode goes two-dimensions: DNA QR code web server. *PLoS ONE*, 7(5), E35146.
- Li, J. L., Wang, S., Yu, J., Wang, L., & Zhou, S. L. (2013). A modified CTAB protocol for plant DNA extraction. *Chinese Bulletin of Botany*, 48, 72–78.
- Ma, S. J., Lv, Q. D., Zhou, H., Fang, J., Cheng, W. L., Jiang, C. X., et al. (2017). Identification of Traditional she medicine Shi-Liang tea species and closely related species using the ITS2 barcode. *Applied Sciences*, 7, 195.
- Pharmacopoeia Committee of P. R. China. (2015). *Pharmacopoeia of People's Republic of China, Part I, Part IV*. Beijing: China Medical Science and Technology Press.
- Song, J. Y., Shi, L. C., Li, D. Z., Sun, Y. Z., Niu, Y. Y., Chen, Z. D., et al. (2012). Extensive pyrosequencing reveals frequent intra-genomic variation of internal transcribed spacer regions of nuclear ribosomal DNA. *PLoS ONE*, 7(8), E43971.
- Tamura, K., Stecher, G., Peterson, D., Filipowski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30(12), 2725–2729.
- Vanherweghem, J. L., Depierreux, M., Tielemans, C., Abramowicz, D., Dratwa, M., Jadoul, M., et al. (1993). Rapidly progressive interstitial renal fibrosis in young women: Association with slimming regimen including Chinese herbs. *Lancet*, 341(8842), 387–391.
- Xin, T. Y., Li, X. J., Yao, H., Lin, Y. L., Ma, X. C., Cheng, R. Y., et al. (2015). Survey of commercial *Rhodiola* products revealed species diversity and potential safety issues. *Scientific Reports*, 5, 8337.
- Xin, T. Y., Li, X. W., Yao, H., & Chen, S. L. (2015). A two-dimensional DNA barcode system for circulation regulation of traditional Chinese medicine. *Science China Vitae*, 45(7), 695–702.
- Xin, T. Y., Yao, H., Gao, H. H., Zhou, X. Z., Ma, X. C., Xu, C. Q., et al. (2013). Super food *Lycium barbarum* (Solanaceae) traceability via an internal transcribed spacer 2 barcode. *Food Research International*, 54(2), 1699–1704.
- Yao, H., Song, J. Y., Liu, C., Luo, K., Han, J. P., Li, Y., et al. (2010). Use of ITS2 region as the universal DNA barcode for plants and animals. *PLoS One*, 5(10), E13102.
- Yu, X. X., Xie, Z. Y., Wu, J. W., Tao, J. F., & Xu, X. J. (2016). DNA barcoding identification of *Kadsuriae Caulis* and *Spatholobi Caulis* based on internal transcribed spacer 2 region and secondary structure prediction. *Pharmacognosy Magazine*, 12(Suppl 2), S165–S169.
- Zhang, C. Y., Huang, S. S., & Yan, H. F. (2017). Applications of DNA barcoding in Chinese materia medica identification. *Chinese Traditional and Herbal Drugs*, 48(11), 2306–2312.
- Zhao, S., Chen, X. X., Song, J. Y., Pang, X. H., & Chen, S. L. (2015). Internal transcribed spacer 2 barcode: A good tool for identifying *Acanthopanax Cortex*. *Frontiers in Plant Science*, 6, 840.