Ecotoxicology and Environmental Safety 208 (2021) 111691

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Progress in the use of DNA barcodes in the identification and classification of medicinal plants

Jie Yu [a,*], Xi Wu [b], Chang Liu [b], Steve Newmaster [c], Subramanyam Ragupathy [c], W. John Kress [d,*]

[a] College of Horticulture and Landscape Architecture, Southwest University, Chongqing 400716, China
[b] Herbal Medicine from Ministry of Education, Engineering Research Center of Chinese Medicine Resources from Ministry of Education, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100193, China
[c] Centre for Biodiversity Genomics, Biodiversity Institute of Ontario (BIO), University of Guelph, Guelph, Ontario N1G2W1, Canada
[d] Department of Botany, MRC-166, National Museum of Natural History, Smithsonian Institution, P. O. Box 37012, Washington, DC 20013-7012, United States

ARTICLE INFO

Keywords:
DNA barcodes
Medicinal plants
Bar-HRM
Metabarcoding
Chloroplast genome

ABSTRACT

DNA barcoding is an emerging molecular identification and classification technology that has been applied to medicinal plants since 2008. The application of this technique has greatly ensured the safety and effectiveness of medicinal materials. In this paper, we review the application of DNA barcoding and some related technologies over the past 10 years with respect to improving our knowledge of medicinal plant identification and authentication. From single locus-based DNA barcodes to combined markers to genome-scale levels, DNA barcodes contribute more and more genetic information. At the same time, other technologies, such as high-resolution melting (HRM), have been combined with DNA barcoding. With the development of next-generation sequencing (NGS), metabarcoding technology has also been shown to identify species in mixed samples successfully. As a widely used and effective tool, DNA barcoding will become more useful over time in the field of medicinal plants.

1. Introduction

Medicinal plants and herbal supplements have played an important role in the health of human populations for thousands of years. Even today, the global market in these plant species and their products continues to grow. Overtime and especially in the world's contemporary consumer markers the authentication and verification of correct plant product identification has become an increasingly important component of the use and sale of medicinal plants and herbal supplements. In the past 10 years, DNA barcode technologies have evolved from single genes, to combined genes, to genomes, and most recently metabarcoding, all of which are now routinely applied to tracking the use, commercialization, and authentication of medicinal plants across the globe. DNA Barcode technologies have been especially useful in the science and ethnobotany of herbal medicines, which has been crucial for human health.

The current manuscript constitutes a review of the application of DNA barcoding to the field of medicinal plants and pharmacopeias, and combines salient points and recommendations on the future directions of DNA barcoding in both basic and applied fields of medicinal plants and herbal medicines. To begin with, a brief background on DNA barcoding technology and a review of medicinal plants were introduced. Secondly, we introduce the common types and newly-developing techniques of DNA barcoding used in the identification of medicinal plants. Finally, insights on the future of plant DNA barcoding in the field were concluded and prospected. Our manuscript is the first comprehensive review of the application of DNA barcodes in ethnobotany and herbal medicines.

2. Background of DNA barcoding and medicinal plant

2.1. DNA barcoding

2.1.1. Basic concepts

In 2003, the technology of DNA barcoding was proposed (Hebert et al., 2003) for accurate identification of species. In this technology, a standard, short DNA sequence is used as a marker called DNA barcode, for rapid, accurate, and automatic species identification (Hebert and Gregory, 2005). Since then, DNA barcoding technology has been widely used in many applications and recognized as a renaissance for taxonomy

* Corresponding authors.
E-mail addresses: Yujie1982@swu.edu.cn (J. Yu), KRESSJ@si.edu (W.J. Kress).

https://doi.org/10.1016/j.ecoenv.2020.111691
Received 7 July 2020; Received in revised form 3 November 2020; Accepted 17 November 2020
Available online 28 November 2020

(Chen et al., 2014; Kress, 2017).

2.1.2. Methodology

2.1.2.1. Experimental procedure. DNA barcoding technology identification process includes: (1) sample collection, including fresh samples, dry samples, mixed samples and processed products; (2) DNA extraction, (eg, CTAB method, SDS method, PVP method, Phenol-chloroform method, etc); (3) PCR amplification, there are three committed steps for a cycle: denaturation, annealing and extension; (4) DNA sequencing, include the earliest sanger dideoxy DNA sequencing, and then to next-generation sequencing by synthesis and 3rd-generation sequencing by single-molecule; (5) similarity-based database search (using software tool such as BLAST); and (6) species identification.

2.1.2.2. Bioinformatic analysis. Sequence analysis is the most important part of DNA barcoding technology. DNA barcodes contain a variety of analytical methods, such as: Genetic distance method, Genetic similarity analysis, Phylogenetic analysis. Many molecular software tools such as ABGD (Puillandre et al., 2012), Taxon DNA (Meier et al., 2006), MEGA 7.0 (Kumar et al., 2016) were used.

2.1.2.3. Database. Nowadays, there are several DNA barcode databases widely used. In 2003, the first DNA barcode research center was established in Canada (CCDB. http://dnabarcoding.ca/). The Barcode of Life Data System (BOLD, http://boldsystems.org/) contains 253,089 species and 2569,647 barcodes. Chinese herbal medicine DNA barcode identification system: http://www.tcmbarcode.cn/china/.

2.1.3. History

In 2003, the 1st international barcode of life conference was held in Cold Spring Harbor (USA), and the idea of using DNA barcoding technology to identify species was proposed. The *mat*K, *rbc*L, *trn*H-*psb*A, and ITS2 sequences were proposed as the core DNA barcode loci for plants at the 3rd barcode conference in 2009 (Kress et al., 2005; Kress and Erickson, 2007). The 8th world DNA barcode conference was held in Trondheim (Norway) in 2019 where new Next Generation Sequencing technologies in DNA barcoding were widely discussed. DNA barcoding technology continues to be expanded to a wide variety of scientific fields (Kress, 2017).

2.2. Medicinal plants

2.2.1. Basic concepts

The term “medicinal plants” refers to all the plants that can be used as medicines or food supplements, playing important roles in human health. Traditional medicines, teas, and herbal supplements together are an important and large component of the commercial market in biodiversity.

2.2.2. Source and classification of medicinal plants

According to Chen et al. (2014), the total number of medicinal plants in the national pharmacopeia of China is about (~650) from which 78, 847 DNA barcode sequences have been obtained. The definitions of “medicinal plants” in different countries are different. In order to find out about the variety of medicinal plants, we analyzed the types of medicinal plants in five pharmacopoeias of the world. Information for a total of 1133 medicinal plant species has been collected, covering 184 families and 656 genera. The Pharmacopoeia of China (2015) contains 610 species, belonging to 381 genera and 136 families, Pharmacopoeia of India contains 396 species, belonging to 325 genera and 119 families, The Japanese Pharmacopoeia contains 222 species, belonging to 151 genera and 74 families, Korean Pharmacopoeia contains 219 species, belonging to 141 genera and 72 families, and United States Pharmacopoeia contains 141 species, belonging to 103 genera and 47 families. Only 4 species (*Curcuma longa*, *Glycyrrhiza glabra*, *Illicium verum*, *Zingiber officinale*) are common to all five Pharmacopoeias. Further analysis showed that among 1133 medicinal plants, the top 10 families were Asteraceae, Fabaceae, Lamiaceae, Ranunculaceae, Rosaceae, Apiaceae, Apocynaceae, Euphorbiaceae, Rutaceae and Solanaceae (Fig. 1A), while the top 10 genera were *Prunus*, *Clematis*, *Euphorbia*, *Solanum*, *Artemisia*, *Dioscorea*, *Acacia*, *Citrus*, *Ficus*, *Aconitum* (Fig. 1B).

The use of traditional Chinese medicine has a very long history in China. Overall, 2711 kinds of Chinese medicinal materials were recorded in the first edition of The Chinese Pharmacopoeia 2020. For the sake of the content and length of this article, we have only listed 43 species from 29 families, which have distinct Chinese characteristics and important medicinal value (Table S1).

2.2.3. Medicinal plants that have been used in DNA barcoding studies

The 420 angiosperm plant families were recognized by APG 4. Medicinal plants have been recorded in 219 different families (52.1%). Of those 219 families with medicinal plants 142 families including 832 genera have had DNA barcodes applied (64.8%). The remaining 77 families of medicinal plants have no published record of DNA barcode sequence data. Within the major angiosperm evolutionary lineages (Basal Angiosperms, Monocots, Basal Eudicots, Rosids and Asterids) the percent of families with records of medicinal plants (Table 1) is surprisingly uniform with 43.4% in Monocots to 55.6% in Basal Angiosperms. However, the percent of families with medicinal plants to which DNA barcodes have been applied range from 33.3% in Monocots to 78.1% in Basal Eudicots, which are significantly different.

3. Types of DNA barcode markers employed for the identification of medicinal plants

3.1. Single-locus DNA barcode markers

In 2009 at the 3rd World DNA Barcode Conference it was announced that the *mat*K and *rbc*L markers are the core sequences of plant DNA barcodes, with ITS and *trn*H-*psb*A as complementary sequences (Group, 2009). After extensive experiments and verification, Chen and colleagues proposed the ITS2 region as the primary DNA barcode and *trn*H-*psb*A as a complementary sequence for the identification of medicinal plant species (Chen et al., 2010). Since then, many plant scientists have used other markers to evaluate the efficiency of ITS2 and *trn*H-*psb*A by identifying the species in different families or genera: *atp*F-*atp*H (Ran et al., 2010), *rpo*B (Al-Qurainy et al., 2011), *atp*B-*rbc*L, *trn*H-*psb*A, *trn*L-F, *trn*S-G, *atp*F-H, *rbc*L, *mat*K, *rpo*B, *rpo*C1, *nad*1 (Quan and Zhou, 2011), *rbc*L, *mat*K, *psb*A-*trn*H, ITS2, ITS, *trn*L intron, and *trn*L-F (Sun et al., 2011), *trn*L and *rpo*C1 (Madesis et al., 2012), *rpo*C1 (L-Qurainy et al., 2014), *ndh*J (He et al., 2014), *mat*K, *rbc*L, *atp*H-*atp*I, *rpl*32-*trn*L(UAG), *rps*18-*cl*pp, *trn*L-*trn*F, *trn*L-*ndh*J, *trn*S-*trn*fM (Mao et al., 2014), *rbc*L and *trn*L (Buddhachat et al., 2015), *rbc*L, *psb*A-*trn*H and *pet*A-*psb*J (Deng et al., 2015), *mat*K, *rbc*L, *trn*H-*psb*A, ITS, *trn*L-F, 5S-rRNA and 18S-rRNA(Mishra et al., 2016), *rps*16, and *trn*T-F (Mishra et al., 2016), *trn*L (Suesatpanit et al., 2017). The ITS2 secondary structures have also been used to identify the species in different genera, such as *Akebia* (Zhang et al., 2015), *Glehnia* (Zhu et al., 2015), *Physalis* (Feng et al., 2016), and *Smithia* (Umdale et al., 2017).

3.2. Multiple-locus DNA barcode markers

As single-locus marker sequence cannot always provide enough information for low level identification, some scientists used a combination of markers to identify medicinal plants. The most common combinations of DNA markers exist between *mat*K, *rbc*L, *trn*H-*psb*A, and ITS sequences (Newmaster et al., 2013; Fu et al., 2011; Purushothaman et al., 2014). Some other combinations, such as *atp*B-*rbc*L+*trn*L-F and *atp*B-*rbc*L+ -*atp*F-H, have been used in identifying the species of *Prunus* L., which can resolve all five species (Quan and Zhou, 2011). Parveen

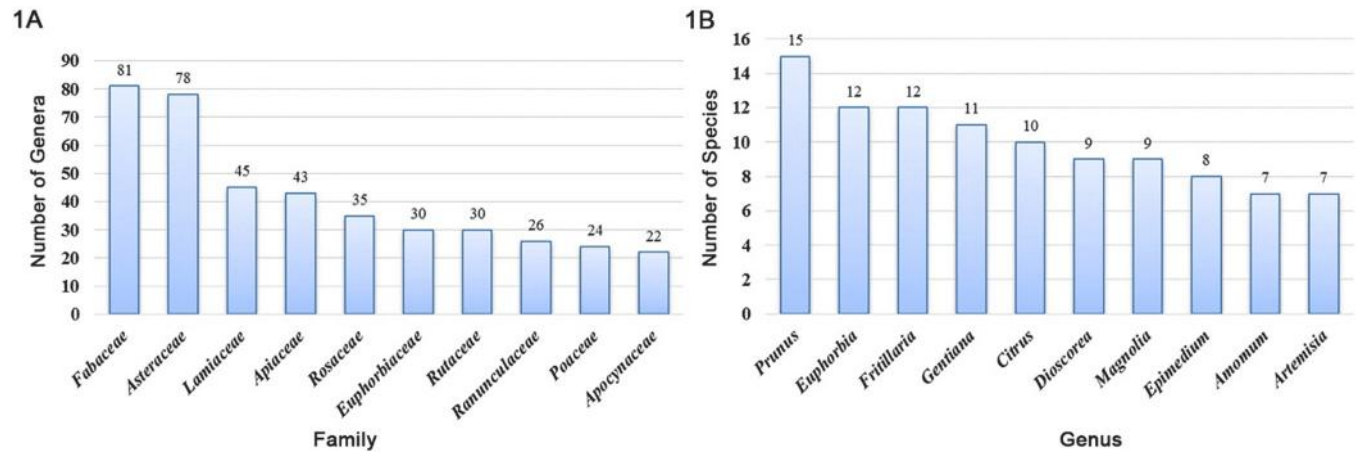

Fig. 1. Top-10 families (1A) and Top-10 genera (1B) recorded in the Pharmacopoeia throughout the world. The Y-axis represents the number of genera in that family or the number of species in that genus recorded in the Pharmacopoeia. We can intuitively see that some important families or genera have made great contributions to the medicinal plants used by human populations.

Table 1
The numbers and proportion of angiosperm families by major evolutionary lineages with reported medicinal plants and medicinal plants with DNA barcode data.

Types	Total number of families	Number of families with medicinal plants	% families with medicinal plants	Number of families with medicinal plants and DNA barcodes	% families with medicinal plants and DNA barcodes
Basal angiosperms	27	15	55.6%	9	60.0%
Basal eudicots	77	38	49.3%	29	76.3%
Monocots	17	8	47.1%	3	37.5%
Rosids	152	87	57.2%	57	65.5%
Asterids	147	71	48.3%	44	62.0%
Total	420	219	52.1%	142	64.8%

has compared the combinations of *rbc*L, *rpo*B, *rpo*C1, *mat*K, and ITS in Orchidaceae, with the maximum species resolution provided by ITS+*mat*K (Parveen et al., 2017).

3.3. Genome-based DNA barcode markers

The chloroplast genome contains all the DNA sequences in a plastid, which contains more genetic information for species identification than any commonly used single-locus marker. By 27 October 2019, the chloroplast genome of 3452 plants has been published on NCBI. It is an important development direction of DNA barcodes using chloroplast genomes to identify and distinguish plants. (https://www.ncbi.nlm.nih.gov/genome/browse#!/organelles/). Govindaraghavan and Li proposed that the entire plastid genome should to be used in the field of DNA barcoding (Govindaraghavan et al., 2012; Li et al., 2015). Sucher suggested the genomic fingerprinting can differentiate between individuals, species and populations, and is useful for the detection of the homogeneity of the samples and presence of adulterants in herbal supplements (Sucher and Carles, 2008). Yang used the chloroplast genome to identify the species of *Datura stramonium* (Yang et al., 2014), He sequenced and analyzed the complete chloroplast genome of *Lonicera japonica*. This study identified unique characteristics of the L. *japonica* cp genome and provided valuable information for the phylogenetic classification and specific barcoding of this medicinal plant (He et al., 2017). Zhou et al. analyzed the molecular structures, and phylogenetic relationship by using complete chloroplast genomes of *Papaver rhoeas* and *Papaver orientale*, and came to the conclusion that the chloroplast genome could be used as a powerful tool to resolve the phylogenetic positions and relationships of Papaveraceae, an important family of medicinal plants (Zhou et al., 2018).

3.4. Summary

In the last decade, many scientists have employed single or multiple locus of DNA barcode markers, and even the entire chloroplast genomes as DNA barcodes to identify species in various families and genera of medicinal plants. However, only recently a consensus started to emerge on the best markers and technologies to use for medicinal plants.

As a general rule, DNA barcoding must take into account cost, efficiency, and convenience. Single-locus DNA barcode markers have advantages in cost, while a combined locus can greatly improve the efficiency of identification. However, for taxa at the intraspecific level (e.g., different ecotypes), sequences containing large-scale information sites, such as the complete chloroplast genomes, are required as super barcodes for resolution enhancement. This is often not necessary for the identification of most medicinal plants with their substitutes/adulterants. As sequencing technologies become mass-produced and cheaper, specific DNA barcodes of medicinal plants targeted at specific taxa are more efficiently selected (e.g., chloroplast genome-based identification of variation "hotspots") instead of using a universal barcode, which will provide new impetus to the development of DNA barcoding.

4. DNA barcoding technology for medicinal plant

4.1. DNA barcoding combined with other technologies

After more than 10 years of development, DNA barcoding using standardized genetic markers has made tremendous progress. Today, barcode markers can be combined with other biotechnologies, such as molecular, chromatographic, and spectrum technologies to obtain better identification results.

These other molecular technologies include SNP (Single Nucleotide Polymorphism, SNP), HRM (High Resolution Melting: HRM), and RFLP

(Restriction Fragment Length Polymorphism: RFLP). The species of *Panax ginseng*, *Amomum villosum*, *Lonicerae japonicae* and its substitutes/adulterants can be identified by the combining SNP and DNA Barcoding (Chen et al., 2013; Huang et al., 2014; Gao et al., 2017). The identification rates of *Equisetum arvense* and *Pulsatilla chinensis* have been compared by HRM and RFLP with DNA barcoding (Saslis-Lagoudakis et al., 2015; Shi et al., 2017).

Chromatography technologies include LC-MS (Liquid Chromatography -Mass Spectrometry: LC-MS), HPLC (High Performance Liquid Chromatography: HPLC), TLC (Thin Layer chromatography). Xiao demonstrated that the chemical profiles determined by LC-MS and DNA profiles in ITS spacer domains could serve as barcode markers for quality control of *Radix Astragali* (Xiao et al., 2011). Zhang showed that DNA barcoding is more powerful than HPLC fingerprint for the identification of *Phellodendri Cortex* and its related species (Zhang et al., 2016). Tests in identifying *Equisetum arvense* by TLC and DNA barcoding demonstrated that the TLC-test is more cost- and time-efficient, but DNA barcoding is more powerful in determining the identity of adulterant species (Saslis-Lagoudakis et al., 2015).

NMR (Nuclear Magnetic Resonance: NMR) is one of the spectrum technologies. Urumarudappa and colleagues provided the first attempt to assess the extent of adulteration of *Saraca asoca* using DNA barcoding and NMR. They found most of the samples were spurious (Urumarudappa et al., 2016).

4.2. The Bar-HRM technology

A new technology that has been used for genotyping medicinal plants in recent years is High Resolution Melting technology (HRM). The melting curve of PCR amplicons depends on the DNA base sequence (e.g., length of DNA sequence, GC content, and difference in base complementarity, etc.). Like many fluorescent PCR technologies, HRM monitors the changes of nucleic acid melting curves in real time by adding specific saturated dyes (such as LC Green and Eva Green), thus achieving genotyping or classification of test populations based on different shapes of melting curves. This technique has many positive characteristics: high throughput, high sensitivity, good specificity, good repeatability, easy operation, and low cost. In addition, closed tube operation greatly reduces the risk of contamination.

In recent years, HRM technology has been gradually applied to the genotyping of medicinal plants. The combination of HRM with standard DNA barcode markers is called Bar-HRM technology. With its high temperature uniformity and temperature resolution, bar-HRM technology enables the resolution accuracy to distinguish the difference of a single base, thus greatly improving the resolution of medicinal plant identification. From 2011 to 2019, at least 24 papers have been published using Bar-HRM technology (Table S2). Among these papers are two review articles (Sun et al., 2016; Mezzasalma et al., 2017), while others are basic research articles. Bar-HRM technology does not require the use of sequence-specific probes and sequencing, which greatly improve the speed of identification. Many barcode sequences like *mat*K, *rbc*L, *trn*H-*psb*A, ITS, *rpo*C, *trn*L, etc. have been combined with HRM technology to identify and distinguish medicinal plants. This method was proved to be accurate, reliable and rapid to authenticate market samples of raw materials in practical aspect and it is feasible to apply for high throughput assay (Buddhachat et al., 2015). Species authentication through Bar-HRM could be used to promote consumer trust, as well as raising the quality of herbal products (Osathanunkul et al., 2015).

4.3. Metabarcoding

With the development of DNA high-throughput sequencing technology, DNA barcodes have been transformed into DNA metabarcoding, which can simultaneously acquire DNA barcode sequences for mixed multi-species samples. This technology uses high-throughput sequencing technology to obtain the barcoded amplicon sequences and uses bioinformatic methods to identify species diversity and composition within a sample. This technology has now been applied to the field of medicinal plants. Dietary analyses by metabarcoding was proposed at the 6th International Barcode of Life Conference (Adamowicz, 2015). De Boer used nrITS1 and nrITS2 DNA metabarcoding to identify orchid and other plant species present in 55 commercial products (De Boer et al., 2017). Arulanhu developments a multi-locus DNA metabarcoding method to identify endangered plant (including *Echinocactus*, *Euphorbia*, *Aloe variegate*, *Dendrobium*, *Cycas revolute*, *Lactuca sativa*) and animal species in complex samples (Arulandhu et al., 2017). Omelchenko improved protocols of ITS1-based metabarcoding and analyzed 39 plant-containing products (Omelchenko et al., 2019). Raclariu uses DNA metabarcoding coupled with chromatography technologies to authenticate 16 *Veronica officinalis* herbal products (Raclariu et al., 2017a), 78 *Hypericum perforatum* herbal products (Raclariu et al., 2017b), and 53 *Echinacea* herbal products (Raclariu et al., 2018a, 2018b). DNA metabarcoding combines other sequencing techniques can determine both prescribed and contaminated species in Traditional Chinese Medicine (TCM) preparations, such as Liuwei Dihuang Wan (Cheng et al., 2014), Jiuwei Qianghuo Wan (Xin et al., 2018).

Raclariu compared the benefits and limitations of DNA Barcoding versus Metabarcoding in herbal product authentication and concluded that both techniques have potential in the context of quality control of both well- and poorly-regulated supply systems (Raclariu et al., 2018a, 2018b). However, accurate determination of species or herbal products by DNA metabarcoding is dependent on a comprehensive and accurate reference library of DNA sequences of a standard genetic marker.

Bell developed a database containing *rbc*L sequences from 38,409 seed plant species, which will assist with identification of plant species using *rbc*L, and increase the resolution and accuracy of identification (Bell et al., 2017). Palme updates a Metaxa2 database (http://microbiology.se/software/metaxa2/), which has led to the adoption of a range of genetic markers for DNA metabarcoding (Palme et al., 2018).

4.4. PacBio-based method for barcoding patent Chinese medicine

The 3rd-generation sequencing technology represented by PacBio sequencing platform is becoming more and more mature in the field of DNA sequencing. It is well known for its high throughput, low cost and short cycle. Which is widely used in the field of large-segment structural variation detection, genome difference analysis of related species. Xiang et al. (2016) analyzed the entire cp genome of *Swertia mussotii* and two other species in Gentianaceae by PacBio platform. Zheng et al. (2017) established a comprehensive quality evaluation system for complex herbal medicine, by using PacBio sequencing and PCR-denaturing gradient gel electrophoresis and chemical approaches to achieve the authentication and quality connotation of the samples.

4.5. Summary

The Bar-HRM technology with high resolution in mutation scanning, showing great potential in medicinal plants. The advantages of Bar-HRM are simple operation, fast and accurate, and closed tube operation to avoid contamination of samples, which are suitable for rapid detection in large quantities. By contrast, metabarcoding performed well in the analysis of mixed samples and processed products of medicinal plants. As for the third generation sequencing technology, it is more used for organelle and nuclear genomes sequencing. For different research purposes, scientists all over the world use DNA barcoding technology as the core technology in combination with other genetic markers with other biotechnologies to make it a more rapid and precise tool (Kress, 2017).

5. Applications of DNA barcoding method in medicinal plants

5.1. DNA barcoding used in the identification of substitutes/adulterants

Due to either shortage of resources or economic benefits, some important herbals have many substitutes or adulterants. The phenomenon of adulteration in the herbal market has become increasingly common. Substitutes/adulterants may cause serious consequences such as poisoning or even death. In order to effectively monitor herbal market protects to ensure their safety for consumers, scientists used the DNA barcoding technology to distinguish authentic products and their counterfeits. These investigations are mainly divided into 2 types, one type is the identification of a specific herbal and their counterfeits. The other type is the identification of conventional Chinese herbal medicines and their processed products such as extracts or proprietary Chinese medicines. Table S3 Listed 20 cases of experimental identification of existing substitutes/adulterants for important plants. Combining the 43 important Chinese medicinal materials and the specific application of DNA barcodes in plant identification described above (Table S1), the results show that DNA barcodes are an important species identification technology.

5.2. DNA barcoding used for the regulation of the medicinal market

National governments have applied DNA barcoding technology in the field of the identification of herbal medicines. Asase in Ghana (Asase and Oppong-Mensah, 2009), Mati in Iraq (Mati and de Boer, 2011), Newmaster in Canada (Newmaster et al., 2013), Zhang in China (Zhang et al., 2014), Mishra in India (Mishra et al., 2016) all used DNA barcoding technology to detect the medicinal herbs market in their country or region. The World Health Organization recommended DNA barcode identification associated with chemical analyses to guarantee medicinal plants quality (Palhares et al., 2015). Subedi used DNA barcodes to protect the wild orchids in Nepal (Subedi et al., 2013). *Crocus sativus* and its adulterants from Chinese markets were identified by using the DNA barcoding technology (Huang et al., 2015). Williamson exposed the illegal trade in *Cycad* species at two traditional medicine markets in South Africa using DNA barcoding technology (Williamson et al., 2016). From these examples we can see that DNA barcoding technology is playing an increasingly important role in the regulation of the medicine markets.

6. Conclusion and future prospects

In this review, we briefly review the development of DNA barcoding in medicinal plants and summarize the common types of DNA barcoding and some common and emerging technologies. As a widely accepted technology, DNA barcoding has played an important role in the classification of medicinal plants, the identification of substitutes/adulterants, and the regulation of the pharmaceutical market. The existing technical means can effectively identify and utilize most plant raw materials and rough-wrought products in the medicinal materials market. But for some fine processing products, (eg. tablets, pills, oral liquids and injections, etc), there is still lack of effective, rapid, and standardized identification methods up to now, especially for the complex botanical components of the Chinese patent medicine, which is challenging for most researchers. Besides, the identification of specific DNA barcodes of medicinal plants is inseparable from the development of genome sequencing. Although the next and 3rd generation sequencing technology has been sophisticated, the research of chloroplast (or plastid) genomics of medical plant is still inadequate. Many of the chloroplast genomes of medicinal plants have not yet been sequenced, which limits our ability to develop new specific DNA barcodes.

The development of DNA barcoding technology in the past has been primarily focused on the selection of markers and the construction of sequence databases. Although universal plant DNA barcodes have not been agreed to by all the scientists, most applications employ the standard four markers *rbc*L, *mat*K, *trn*H-*psb*A and ITS. The international DNA barcode database is maintained by BOLD in Canada (http://www.boldsystems.org/). China has its own DNA barcode database (http://www.tcmbarcode.cn/), which has one million sequences to compare (Bell et al., 2017). With the continuous improvement and the full application of technologies, the super-barcode and metabarcoding joined into the DNA barcode extended family. The research and application of plant DNA barcoding technology in the field of traditional Chinese medicine identification will be more extensive and in-depth. In short, DNA barcoding technology has a broad application prospect in the field of medicinal plants, and it will certainly help the modernization of traditional medicines in countries around the world.

CRediT authorship contribution statement

Jie Yu: Methodology, Writing - original draft. **Xi Wu:** Visualization, Investigation. **Chang Liu:** Software, Validation. **Steve Newmaster** and **Subramanyam Ragupathy:** Data curation, Supervision. **W. John Kress:** Conceptualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to the support from the National Natural Science Foundation of China (31772260), Chongqing Study Abroad Innovation Project (cx2019052), and the National Training Program of Innovation and Entrepreneurship for Undergraduates (X202010635443).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2020.111691.

References

Adamowicz, S.J., 2015. International barcode of life: evolution of a global research community. Genome 58, 51–62.

Al-Qurainy, F., Khan, S., Tarroum, M., Al-Hemaid, F.M., Ali, M.A., 2011. Molecular authentication of the medicinal herb Ruta graveolens (Rutaceae) and an adulterant using nuclear and chloroplast DNA markers. Genet. Mol. Res. 10, 2806–2816.

Arulandhu, A.J., Staats, M., Hagelaar, R., Vooorhuijzen, M.M., Prins, T.W., Scholtens, I. M.J., Costessi, A., Duijsings, D., Rechenmann, F., Gaspar, F., Crespo, M.T.B., Holst-Jensen, A., Birck, M., Burns, M., Haynes, E., Hochegger, R., Klingl, A., Lundberg, L., Natale, C., Niekamp, H., Perri, E., Barbante, A., Rosec, J.P., Seyfarth, R., Sovova, T., Moorleghem, C.V., Ruth, S.V., Peelen, T., Kok, E.J., 2017. Development and validation of a multi-locus DNA metabarcoding method to identify endangered species in complex samples. GigaScience 6, 1–18.

Asase, A., Oppong-Mensah, G., 2009. Traditional antimalarial phytotherapy remedies in herbal markets in southern Ghana. J. Ethnopharmacol. 126, 492–499.

Bell, K.L., Loeffler, V.M., Brosi, B.J., 2017. An RBCL reference library to aid in the identification of plant species mixtures by DNA metabarcoding. Appl. Plant Sci. 5, 1600110.

Buddhachat, K., Osathanunkul, M., Madesis, P., Chomdej, S., Ongchai, S., 2015. Authenticity analyses of Phyllanthus amarus using barcoding coupled with HRM analysis to control its quality for medicinal plant product. Gene 573, 84–90.

Chen, S.L., Yao, H., Han, J.P., Liu, C., Song, J.Y., Shi, L.C., Zhu, Y.G., Ma, X.Y., Gao, T., Pang, X.H., Luo, K., Li, Y., Li, X.W., Jia, X.C., Lin, Y.L., Leon, C., 2010. Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. PLoS One 5, e8613.

Chen, S.L., Pang, X.H., Song, J.Y., Shi, L.C., Yao, H., Han, J.P., Leon, C., 2014. A renaissance in herbal medicine identification: from morphology to DNA. Biotechnol. Adv. 32, 1237–1244.

Chen, X.C., Liao, B.S., Song, J.Y., Pang, X.H., Han, J.P., Chen, S.L., 2013. A fast SNP identification and analysis of intraspecific variation in the medicinal Panax species based on DNA barcoding. Gene 530, 39–43.

Cheng, X., Su, X., Chen, X., Zhao, H.X., Bo, C.P., Xu, J., Bai, H., Ning, K., 2014. Biological ingredient analysis of traditional Chinese medicine preparation based on high-throughput sequencing: the story for Liuwei Dihuang Wan. Sci. Rep. 4, 5147.
De Boer, H.J., Ghorbani, A., Manzanilla, V., Raclariu, A.C., Kreziou, A., Ounjai, S., Osathanunkul, M., Gravendeel, B., 2017. DNA metabarcoding of orchid-derived products reveals widespread illegal orchid trade. Proc. R. Soc. B 284, 20171182.
Deng, J.B., Liu, J., Ahmad, K.S., Ding, C.B., Zhang, L., Zhou, Y.H., Yang, R.W., 2015. Relationships evaluation on six herbal species (*Curcuma*) by DNA barcoding. Pak. J. Bot. 47, 1103–1109.
Feng, S., Jiang, M., Shi, Y.J., Jiao, K., Shen, C., Lu, J., Ying, Q., Wang, H., 2016. Application of the ribosomal DNA ITS2 region of Physalis (Solanaceae): DNA barcoding and phylogenetic study. Front. Plant Sci. 7, 7.
Fu, Y.M., Jiang, W.M., Fu, C.X., 2011. Identification of species within Tetrastigma (Miq.) Planch. (Vitaceae) based on DNA barcoding techniques. J. Syst. Evol. 49, 237–245.
Gao, Z.T., Liu, Y., Wang, X.Y., Song, J.Y., Chen, S.L., Ragupathy, S., Han, J.P., Newmaster, S.G., 2017. Derivative technology of DNA barcoding (nucleotide signature and SNP double peak methods) detects adulterants and substitution in Chinese patent medicines. Sci. Rep. 7, 5858.
Govindaraghavan, S., Hennell, J.R., Sucher, N.J., 2012. From classical taxonomy to genome and metabolome: towards comprehensive quality standards for medicinal herb raw materials and extracts. Fitoterapia 83, 979–988.
Group, C.P.W., 2009. A DNA barcode for land plants. Proc. Natl Acad. Sci. USA 106, 12794–12797.
He, L., Qian, J., Li, X.W., Sun, Z.Y., Xu, X.L., Chen, S., 2017. Complete chloroplast genome of medicinal plant Lonicera japonica: genome rearrangement, intron gain and loss, and implications for phylogenetic studies. Molecules 22, 249.
He, Y., Wan, F., Xiong, L., Li, D.M., Peng, C., 2014. Identification of two chemotypes of Pogostemon cablin (Blanco) Benth. through DNA barcodes. Z. Fur Naturforsch. Sect. C. J. Biosci. 69, 253–258.
Hebert, P.D.N., Gregory, T.R., 2005. The promise of DNA barcoding for taxonomy. Syst. Biol. 54, 852–859.
Hebert, P.D.N., Cywinska, A., Ball, S.L., Dewaard, J.R., 2003. Biological identifications through DNA barcodes. Proc. R. Soc. B Biol. Sci. 270, 313–321.
Huang, Q.L., Duan, Z.G., Yang, J.F., Ma, X.Y., Zhan, R.T., Xu, H., Chen, W., 2014. SNP typing for germplasm identification of Amomum villosum Lour. Based on DNA barcoding markers. PLoS One 9, e114940.
Huang, W.J., Li, F.F., Liu, Y.J., Long, C.L., 2015. Identification of Crocus sativus and its adulterants from Chinese markets by using DNA barcoding technique. Iran. J. Biotechnol. 13, 36–42.
Kress, W.J., 2017. Plant DNA barcodes: applications today and in the future. J. Syst. Evol. 55, 291–307.
Kress, W.J., Erickson, D.L., 2007. A two-locus global DNA barcode for land plants: the coding rbcL gene complements the non-coding trnH-psbA spacer region. PLoS One. 6, e508.
Kress, W.J., Wurdack, J.K., Zimmer A, A.E., Weight, A.L., Janzen H, H.D., 2005. Use of DNA barcodes to identify flowering plants. Proc. Natl. Acad. Sci. USA 102 (23), 8369–8374.
Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.
Li, X.W., Yang, Y., Henry, R.J., Rossetto, M., Wang, Y.T., Chen, S.L., 2015. Plant DNA barcoding: from gene to genome. Biol. Rev. 90, 157–166.
L-Qurainy, F., Khan, S., Nadeem, M., Tarroum, M., Al-Ameri, A., 2014. Selection of DNA barcoding loci and phylogenetic study of a medicinal and endemic plant, *Plectranthus asirensis* JRI Wood from Saudi Arabia. Genet. Mol. Res. 13, 6184–6190.
Madesis, P., Ganopoulos, I., Ralli, P., Tsaftaris, A., 2012. Barcoding the major Mediterranean leguminous crops by combining universal chloroplast and nuclear DNA sequence targets. Genet. Mol. Res. 11, 2548–2558.
Mao, Y.R., Zhang, Y.H., Nakamura, K., Guan, B.C., Qiu, Y.X., 2014. Developing DNA barcodes for species identification in Podophylloideae (Berberidaceae). J. Syst. Evol. 52, 487–499.
Mati, E., De Boer, H., 2011. Ethnobotany and trade of medicinal plants in the Qaysari Market, Kurdish Autonomous Region, Iraq. J. Ethnopharmacol. 133, 490–510.
Meier, R., Shiyang, K., Vaidya, G., Ng, V.P.K.L., 2006. DNA barcoding and taxonomy in Diptera: a tale of high in traspecific variability and low identification success. Syst. Biol. 55, 715–728.
Mezzasalma, V., Ganopoulos, I., Galimberti, A., Cornara, L., Ferri, E., Labra, M., 2017. Poisonous or non-poisonous plants? DNA-based tools and applications for accurate identification. Int. J. Leg. Med. 131, 1–19.
Mishra, P., Kumar, A., Nagireddy, A., Mani, D.N., Shukla, A.K., Tiwari, R., Velusamy, S., 2016. DNA barcoding: an efficient tool to overcome authentication challenges in the herbal market. Plant Biotechnol. J. 14, 8–21.
Newmaster, S.G., Grguric, M., Shanmughanandhan, D., Ramalingam, S., Ragupathy, S., 2013. DNA barcoding detects contamination and substitution in North American herbal products. BMC Med. 11, 222.
Omelchenko, D.O., Speranskaya, A.S., Ayginin, A.A., Khafizov, K., Krinitsina, A.A., Fedotova, A.V., Pozdyshev, D.V., Shtratnikova, V.Y., Kupriyanova, E.V., Shipulin, G. A., Logacheva, M.D., 2019. Improved protocols of ITS1-based metabarcoding and their application in the analysis of plant-containing products. Genes 10, 122.
Osathanunkul, M., Madesis, P., De Boer, H., 2015. Bar-HRM for authentication of plant-based medicines: evaluation of three medicinal products derived from Acanthaceae species. PLoS One 10, e0128476.
Palhares, R.M., Drummond, M.G., Brasil, B.D.A.F., Cosenza, G.P., Brandao, M.D.L., Brandao, M.L., Oliveira, G., 2015. Medicinal plants recommended by the World Health Organization: DNA barcode identification associated with chemical analyses guarantees their quality. PLoS One 10, e0127866.
Palme, J.B., Richardson, R.T., Meola, M., Wurzbacher, C., Tremblay, E.D., Thorell, K., Kanger, K., Eriksson, K.M., Bilodeau, G.J., Johnson, R.M., Hartmann, M., Nilsson, R. H., 2018. Metaxa2 database builder: enabling taxonomic identification from metagenomics or metabarcoding data using any genetic marker. Toxins 10, 17–26.
Parveen, I., Singh, H.K., Malik, S., Raghuvanshi, S., Babbar, S.B., 2017. Evaluating five different loci (rbcL, rpoB, rpoC1, matK, and ITS) for DNA barcoding of Indian orchids. Genome 60, 665–671.
Puillandre, N., Lambert, A., Brouillet, S., Achaz, G., 2012. ABGD, automatic barcode gap discovery of primary species delimitation. Mol. Ecol. 21, 1864–1877.
Purushothaman, N., Newmaster, S.G., Ragupathy, S., Stalin, N., Suresh, D., Arunraj, D.R., Gnanasekaran, G., Vassou, S.L., Narasimhan, D., Parani, M., 2014. A tiered barcode authentication tool to differentiate medicinal Cassia species in India. Genet. Mol. Res. 13, 2959–2968.
Quan, X., Zhou, S.L., 2011. Molecular identification of species in Prunus sect. Persica (Rosaceae), with emphasis on evaluation of candidate barcodes for plants. J. Syst. Evol. 49, 138–145.
Raclariu, A.C., Heinrich, M., Ichim, M.C., De Boer, H.J., 2018a. Benefits and limitations of DNA barcoding and metabarcoding in Herbal product. Phytochem. Anal. 29, 123–128.
Raclariu, A.C., Mocan, A., Popa, M.O., Vlase, L., Ichim, M.C., Crisan, G., Brysting, A.K., De Boer, H., 2017a. Veronica officinalis product authentication using DNA metabarcoding and HPLC-MS reveals widespread adulteration with Veronica chamaedrys. Front. Pharmacol. 8, 8.
Raclariu, A.C., Paltinean, R., Vlase, L., Labarre, A., Manzanilla, V., Ichim, M.C., Crisan, G., Brysting, A.K., De Boer, H., 2017b. Comparative authentication of Hypericum perforatum herbal products using DNA metabarcoding, TLC and HPLC-MS. Sci. Rep. 7, 1291.
Raclariu, A.C., Tebrencu, C.E., Ichim, M.C., Teodora, C.O., Brysting, A.K., De Boer, H., 2018b. What's in the box? Authentication of Echinacea herbal products using DNA metabarcoding and HPTLC. Phytomedicine 44, 32–38.
Ran, J.H., Wang, P.P., Zhao, H.J., Wang, X.Q., 2010. A test of seven candidate barcode regions from the plastome in Picea (Pinaceae). J. Integr. Plant Biol. 52, 1109–1126.
Saslis-Lagoudakis, C.H., Bruun-Lund, S., Iwanycki, N.E., Seberg, O., Petersen, G., Jager, A.K., Ronsted, N., 2015. Identification of common horsetail (Equisetum arvense L.; Equisetaceae) using thin layer chromatography versus DNA barcoding. Sci. Rep. 5, 11942.
Shi, Y.H., Zhao, M.M., Yao, H., Yang, P., Xin, T.Y., Li, B., Sun, W., Chen, S.L., 2017. Rapidly discriminate commercial medicinal Pulsatilla chinensis (Bge.) regel from its adulterants using ITS2 barcoding and specific PCR-RFLP assay. Sci. Rep. 7, 40000.
Subedi, A., Kunwar, B., Choi, Y., Dai, Y.T., Van Andel, T., Chaudhary, R.P., De Boer, H.J., Gravendeel, B., 2013. Collection and trade of wild-harvested orchids in Nepal. J. Ethnobiol. Ethnomed. 9, 64.
Sucher, N.J., Carles, M.C., 2008. Genome-based approaches to the authentication of medicinal plants. Planta Med. 74, 603–623.
Suesatpanit, T., Osathanunkul, K., Madesis, P., Osathanunkul, M., 2017. Should DNA sequence be incorporated with other taxonomical data for routine identifying of plant species? BMC Complement. Altern. Med. 17, 437.
Sun, W., Li, J.J., Xiong, C., Zhao, B., Chen, S.L., 2016. The potential power of Bar-HRM technology in herbal medicine identification. Front. Plant Sci. 7, 7.
Sun, Z.Y., Gao, T., Yao, H., Shi, L.C., Zhu, Y.J., Chen, S.L., 2011. Identification of Lonicera japonica and its related species using the DNA barcoding method. Planta Med. 77, 301–306.
Umdale, S.D., Kshirsagar, P.R., Lekhak, M.M., Gaikwaid, N.B., 2017. Molecular authentication of the traditional medicinal plant "Lakshman Booti" (Smithia conferta Sm.) and its adulterants through DNA barcoding. Pharmacogn. Mag. 13, S224–S229.
Urumarudappa, S.K.J., Gogna, N., Newmaster, S.G., Venkatarangaiah, K., Subramanyam, R., Saroja, S.G., Gudasalamani, R., Dorai, K., Ramanan, U.S., 2016. DNA barcoding and NMR spectroscopy-based assessment of species adulteration in the raw herbal trade of *Saraca asoca* (Roxb.) Willd, an important medicinal plant. Int. J. Leg. Med. 130, 1457–1470.
Williamson, J., Maurin, O., Shiba, S.N.S., Van Der Bank, H., Pfab, M., Pilusa, M., Kabongo, R.M., An Der Bank, M., 2016. Exposing the illegal trade in cycad species (Cycadophyta: Encephalartos) at two traditional medicine markets in South Africa using DNA barcoding. Genome 59, 771–781.
Xiang, B., Li, X., Qian, J., Wang, L.Z., Ma, L., Tian, X.X., Wang, Y., 2016. The complete chloroplast genome sequence of the medicinal plant Swertia mussotii using the PacBio RS II platform. Molecules 21, 1029–1042.
Xiao, W.L., Motley, T.J., Unachukwu, U.J., Lau, C.B.S., Jiang, B., Hong, F., Leung, P.C., Wang, Q.F., Livingston, P.O., Cassileth, B.R., Kennelly, E.J., 2011. Chemical and genetic assessment of variability in commercial Radix Astragali (Astragalus spp.) by ion trap LC-MS and nuclear ribosomal DNA barcoding sequence analyses. J. Agric. Food Chem. 59, 1548–1556.
Xin, T., Xu, Z., Jia, J., Leon, C., Hu, S., Lin, Y.L., Ragupathy, S., Song, J.Y., Newmaster, S. G., 2018. Biomonitoring for traditional herbal medicinal products using DNA metabarcoding and single molecule, real-time sequencing. Acta Pharm. Sin. B 8, 488–497.
Yang, Y., Dang, Y.Y., Li, Q., Lu, J.J., Li, X.W., Wang, Y.T., 2014. Complete chloroplast genome sequence of poisonous and medicinal plant Datura stramonium: organizations and implications for genetic engineering. PLoS One 9, e110656.
Zhang, D.Q., Yang, Y.S., Jiang, B., Duan, L.Z., Zhou, N., 2014. How to correctly identify herbal materials in market: a case study based on DNA barcodes. Afr. J. Tradit. Complement. Altern. Med. 11, 66–76.
Zhang, W., Yuan, Y., Yang, S., Huang, J.J., Huang, L.Q., 2015. ITS2 secondary structure improves discrimination between medicinal "Mu Tong" species when using DNA barcoding. PLoS One 10, e0131185.

Zhang, Z.P., Zhang, Y., Zhang, Z., Yao, H., Liu, H.T., Zhang, B.G., Liao, Y.H., 2016. Comparative analysis of DNA barcoding and HPLC fingerprint to trace species of Phellodendri Cortex, an important traditional chinese medicine from multiple sources. Biol. Pharm. Bull. 39, 1325–1330.
Zheng, X.S., Zhang, P., Liao, B.S., Li, J., Liu, X.Y., Shi, Y.H., Cheng, J.L., Lai, Z.T., Xu, J., Chen, S.L., 2017. A comprehensive quality evaluation system for complex herbal medicine using PacBio sequencing, PCR-denaturing gradient gel electrophoresis, and several chemical approaches. Front. Plant Sci. 8, 1–11.
Zhou, J., Cui, Y., Chen, X., Li, Y., Xu, Z., Duan, B., Li, Y., Song, J., Yao, H., 2018. Complete chloroplast genomes of Papaver rhoeas and Papaver orientale: molecular structures, comparative analysis, and phylogenetic analysis. Molecules 23, 437.
Zhu, X.Z., Zhang, Y.X., Liu, X., Hou, D.Y., Gao, T., 2015. Authentication of commercial processed Glehniae Radix (Beishashen) by DNA barcodes. Chin. Med. 10, 35.