Original Article

Prospects and Problems for Identification of Poisonous Plants in China using DNA Barcodes*

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Abstract

Objective Poisonous plants are a deadly threat to public health in China. The traditional clinical diagnosis of the toxic plants is inefficient, fallible, and dependent upon experts. In this study, we tested the performance of DNA barcodes for identification of the most threatening poisonous plants in China.

Methods Seventy-four accessions of 27 toxic plant species in 22 genera and 17 families were sampled and three DNA barcodes (*matK*, *rbcL*, and ITS) were amplified, sequenced and tested. Three methods, Blast, pairwise global alignment (PWG) distance, and Tree-Building were tested for discrimination power.

Results The primer universality of all the three markers was high. Except in the case of ITS for *Hemerocallis minor*, the three barcodes were successfully generated from all the selected species. Among the three methods applied, Blast showed the lowest discrimination rate, whereas PWG Distance and Tree-Building methods were equally effective. The ITS barcode showed highest discrimination rates using the PWG Distance and Tree-Building methods. When the barcodes were combined, discrimination rates were increased for the Blast method.

Conclusion DNA barcoding technique provides us a fast tool for clinical identification of poisonous plants in China. We suggest *matK*, *rbcL*, ITS used in combination as DNA barcodes for authentication of poisonous plants.

Key words: Poisonous plants; DNA barcoding; *matK*; *rbcL*; ITS

Biomed Environ Sci, 2014; 27(10): 794-806 *doi: 10.3967/bes2014.115* *ISSN: 0895-3988*

www.besjournal.com (full text) *CN: 11-2816/Q*

INTRODUCTION

Plants have played an important role in human life for thousands of years[1]. Currently, people in China still use many kinds of natural plants as food and medicine, especially in rural areas. However, many natural and domestic plants potentially are toxic to human beings. Poisonous plants have been major threats to the public health in China for long time. Hundreds of events of accidental intake of toxic plants were reported causing severe poisoning or even death

*This study was supported by the Fundamental Research Funds for the Central Universities (grant no. TD2012-04), the Scientific Research Foundation of the State Human Resource Ministry and the Education Ministry for Returned Chinese Scholars (to L. Xie), the State Key Laboratory Program from the State Key Laboratory of Systematic and Evolutionary Botany (grant no. LSEB2011-07), the Main Direction Program of Knowledge Innovation of the Chinese Academy of Sciences (grant no. KSCX2-EW-Z-1), and the National Natural Science Foundation of China (grant nos.31170201, 81072317, 31110103911).

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every year.

Because different toxic plants bear different chemicals harmful to different target organs and require different treatment when intoxication events happen, the fast and accurate identification of poisonous substances is vital for saving patients' lives. For example *Aconitum* spp. cause arrhythmia, in-properly cooked *Phaseolus vulgaris* is harmful to digestive system, and *Gelsemium elegans* is lethal and can cause paralysis of respiratory muscle. Clinical diagnosis of intoxicated patients is typically based on the morphological analysis of plant fragments and/or based on the symptoms of the patient. These methods are inefficient, fallible and require a considerable amount of training. Usually a variable proportion of plant fragments are unidentifiable, so the plant species identifications can be difficult without distinctive taxonomic characteristics.

In the past decade, DNA barcoding has emerged as a new biological tool to attain accurate, rapid and automatable species identification without morphological inference by using short and standardized DNA regions that can be amplified easily by standard Polymerase Chain Reaction (PCR)[2-4]. These amplified regions possess enough variety to distinguish species from each other by using interspecific diversity and intraspecific congruence. The time required for obtaining barcodes from plant tissues can be condensed to 8-10 hours. Thus, DNA barcoding method will facilitate the work of identifying species rapidly, accurately, and efficiently[5].

The cytochrome C oxidase 1 (CO1) gene in the mitochondrial genome may be an ideal molecular marker for identification of animals[2], but this region is not suitable for plants because of low substitution rates in the plant mitochondrial genome[5-6]. A number of DNA regions, mainly from the plastid genome, have been tested for universality and discriminatory power in barcoding plants[7-11]. The two-marker combination of *rbcL* and *matK* was proposed as the core barcode for land plants[3]. Recently, the internal transcribed spacer (ITS) or ITS2 has been tested as a complimentary plant barcoding region by the China Plant BOL Group[12].

DNA barcoding has been proposed as a useful technique within many disciplines, e.g., conservation biology, forensics, biomedicine, epidemiology, and evolutionary biology etc.[13-17]. Bruni et al.[18] sampled 50 species of poisonous plants and tested for five barcode sequences, among which two were from nuclear genome, including *At103* and *sqd1*, and three were from chloroplast genome, including *matK*, *trnH-psbA*, and *rpoB*. In their study, only one (*matK*) of the three recommended core barcodes for land plants was tested[12]. The results showed that all the selected sequences did not vary when different parts of the plants were used for DNA extraction. They recommended a combination of plastid *matK* and the nuclear maker, *At103*, be used as DNA barcodes.

In the present study, we focused on the most threatening poisonous plants in China and tested three well studied candidate barcodes, *matK*, *rbcL*, and ITS[12]. We evaluated the performance of these three barcoding loci on the identification of poisonous plants, and discuss the prospects and problems of applying DNA barcoding as a rapid and precise tool for identification of toxic plants.

MATERIALS AND METHODS

Toxic Plant Materials

When severe poisoning events happen, local hospitals consult the National Institute of Occupational Health and Poison Control, China Center for Disease Control and Prevention (China CDC) for medical advice, and report the events in related journals. Thus, China CDC generates massive data of poisoning cases. We investigated poisoning reports in medical journals published from 1994-2010 retrieved from the China Hospital Knowledge Database (CHKD). In total, 1060 papers were gathered and poisoning events were recorded by plant taxa. (All the references used in this study are available from the authors upon request.) The top ten poisoning events, number of affected people, and the number of deaths caused by poisonous plants were calculated (Table 1).

Our sampling was based on poisoning events listed in Table 1, with the exception of the gymnosperm, *Ginkgo biloba*. Seventy-four accessions of 27 species in 22 genera and 17 families representing most of the major clades in the angiosperms were included in this study. Multiple individuals were sampled for each species from different populations, and more individuals were sampled from widespread species to fully represent their distribution ranges. All vouchers were deposited in the herbarium of the Beijing Forestry University (BJFC). The materials were collected from the field or from cultivated gardens (Table 2), and were identified by the first author who is working on taxonomy and systematics of Angiosperms.

DNA Extraction, PCR Amplification, and Sequencing

Total genomic DNA was extracted from silica-gel dried leaf material using the CTAB procedure[19]. Polymerase chain reaction (PCR) amplifications for all the barcodes were performed in a 20 μL reaction mixture containing 2 μL 10 × Taq buffer, 1.6 μL (0.4 mmol/L) of dNTPs, 0.5 μL of each primer, 1 U of Taq DNA Polymerase (TaKaRa Biotechnology Co. Ltd., Dalian, China), and 1 μL of genomic DNA (ca. 30 ng). Primers used in this study followed the previous DNA barcoding studies on Angiosperms[3,12]. All the DNA fragments were amplified using 30 cycles at 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 90 s. PCR products were cleaned using PEG8000. Purified PCR products were sequenced using an ABI3730 DNA Sequencer (Applied Biosystems). We sequenced both strands of DNA with overlapping regions to ensure that each base is unambiguous. Electropherograms were assembled; ambiguous bases were corrected; and consensus sequences were generated with Sequencher 4.5 (GeneCodes, Ann Arbor, Michigan, USA). Sequences of the 74 samples generated in this study are deposited in GenBank (Table 2).

Universality

To test the universality of primers, we assembled data on amplification and sequencing success across all selected plant taxa. Different primer sets (1F/724R for *rbcL*; KIM-3F/KIM-1R, 390F/1326R, and XF/5R for *matK*; and ITS1, ITS5/ITS4) were used for barcoding in different taxa as proposed by the CBOL Plant Working Group[3]. The universality of PCR was assessed by the method used by the China Plant BOL Group[12]. A single discrete band on an agarose gel after PCR is considered as successful amplification.

Discrimination Success

We applied Blast, PWG Distance, and Tree-Building to evaluate discrimination success for the single markers and 2 to 3 maker combinations[12]. For the Blast method, all sequences of the three markers were used as query sequences, and the Blast program (v2.2.17) was used to query the reference database with each sample in turn to establish whether the closest hit was a conspecific species and to provide statistics for species discrimination (the query sequence itself was excluded from the list of top hits). Species discrimination was considered successful if all individuals of a species had a top-matching hit of only one conspecific individual[20]. The PWG Distance method (simple pairwise matching for DNA barcoding) recommended by the CBOL Plant Working Group employs distances calculated from pairwise alignments that count unambiguous base substitutions only[3]. We considered discrimination as successful if the minimum uncorrected interspecific p-distance involving a species was larger than its maximum intraspecific distance in closely related species, such as multiple species in *Aconitum*, and *Delphinium*. When using the Tree-Building method, sequences were aligned using MUSCLE v3.6[21], and neighbor-joining trees were constructed with p-distances in PAUP* 4.0b10[22]. The robustness of tree was assessed using bootstrap analyses using 1000 replicates. Species were considered discriminated if all individuals of a species formed a monophyletic group with above 70% bootstrap values[3]. We also evaluated species discrimination for multiple markers of all possible 2- to 3-marker combinations and recording the success of each multi-marker combination.

Table 1. Ranking of Intoxication Accidents Reported in China from 1994-2010

Ranking	Genus (events, %)	Genus (no. of intoxicated, %)	Genus (no. of death, %)
1	*Aconitum* spp. (250, 27.5%)	*Phaseolus vulgaris* (1840, 34.6%)*	*Aconitum* spp. (20, 29.4%)
2	*Coriaria nepalensis* (204, 22.4%)	*Vernicia fordii* (975, 18.3%)*	*Gelsemium elegans* (13, 19.1%)
3	*Datura stramonium* (107, 11.8%)	*Glycine max* (733, 13.8%)*	*Strychnos nux-vomica* (9, 13.2%)
4	*Digitalis purpurea* (79, 8.7%)*	*Solanum tuberosum* (456, 8.6%)*	*Coriaria nepalensis* (7, 10.3%)
5	*Gelsemium elegans* (74, 8.1%)	*Lagenaria siceraria* (302, 5.7%)*	*Tripterygium hypoglaucum* (4, 5.9%)
6	*Phaseolus vulgaris* (60, 6.6%)*	*Aconitum* spp. (286, 5.4%)	*Digitalis purpurea* (4, 5.9%)*
7	*Ginkgo biloba* (38, 4.2%)*	*Coriaria nepalensis* (201, 3.8%)	*Cicuta virosa* (3, 4.4%)
8	*Papaver* spp. (37, 4.1%)	*Illicium* spp. (199, 3.7%)	*Akebia* spp. (3, 4.4%)
9	*Menispermum dahuricum* (31, 3.4%)	*Ricinus communis* (175, 3.3%)	*Papaver* spp. (3, 4.4%)
10	*Strychnos nux-vomica* (29, 3.2%)	*Hemerocallis* spp. (156, 2.9%)*	*Pinellia ternata* (2, 2.9%)

Note. *Domestic plants.

Table 2. Accessions Sampled for Phylogenetic Analyses of This Study, Vouchers are Deposited in BJFC

Family	Taxon	Voucher	Locality	GenBank Accession Number		
				matK	*rbcL*	ITS
Apiaceae	*Cicuta virosa* L.	LX201007091	Qingshan, Inner Mongolia	KF022393	KF022459	KF022331
		LX201007098	Qingshan, Inner Mongolia	KF022394	KF022460	KF022332
Araceae	*Pinellia ternata* (Thunb.) Makino	LX200606026	Beijing	KF022430	KF022498	KF022365
		LX200507034	Beijing	KF022431	KF022499	KF022366
Celastraceae	*Celastrus orbiculatus* Thunb.	LX201008081	Songshan, Beijing	KF022390	KF022456	KF022328
		LX201008041	Jiufeng, Beijing	KF022391	KF022457	KF022329
		LX201007009	Dongling Shan, Beijing	KF022392	KF022458	KF022330
	Tripterygium hypoglaucum (H. Lév.) Hutch.	x2	Erhai, Dali, Yunnan	KF022439	KF022506	KF022374
		LX200905044	Kunming, Yunnan	KF022440	KF022507	KF022375
		LX200909076	Kunming, Yunnan	KF022441	P	P
		LX200906023	Cangshan, Dali, Yunnan	P	KF022508	S
Coriariaceae	*Coriaria nepalensis* Wall.	x6	Binchuan, Yunnan	P	KF022461	KF022333
		LX200905021	Kunming, Yunnan	KF022395	KF022462	KF022334
		x476	Xiangrila, Yunnan	KF022396	P	P
		x543	Erlang Shan, Sichuan	KF022397	KF022463	P
Cucurbitaceae	*Lagenaria siceraria* (Molina) Standl.	LX201209087-1	Beijing	KF022421	KF022488	KF022355
		LX201209087-2	Beijing	KF022422	KF022489	KF022356
Euphorbiaceae	*Ricinus communis* L.	S.K. Shen s.n.	Kunming, Yunnan	KF022432	KF022500	KF022367
		LX200907442	Kunming, Yunnan	KF022433	KF022501	KF022368
	Vernicia fordii (Hemsl.) Airy Shaw	LX200406049	Nanchuan, Chongqing	KF022442	KF022509	KF022376
		LX201004061	Jigong Shan, Henan	KF022443	KF022510	KF022377
		LX201009033	Yichang, Hubei	P	KF022511	KF022378
Fabaceae	*Glycine max* (L.) Merr.	LX201009221-1	Beijing	P	KF022480	KF022349
		LX201009221-2	Beijing	P	KF022481	KF022350
		LX201007006-1	Qingshan, Inner Mongolia	KF022415	KF022482	KF022351
		LX201007006-2	Qingshan, Inner Mongolia	KF022416	KF022483	KF022352
	Phaseolus vulgaris L.	LX201208311-1	Beijing	KF022428	KF022496	KF022363
		LX201208311-2	Beijing	KF022429	KF022497	KF022364
Gelsemiaceae	*Gelsemium elegans* (Gardner et Chapm.) Benth.	C.L.Pan s.n.	Guangxi Medicinal Botanical Garden	KF022413	KF022478	KF022347
		LND 2011007	Guangxi	KF022414	KF022479	KF022348
Lardizabala-ceae	*Akebia trifoliata* (Thunb.) Koidz.	LX200609002	Cult. Beijing Botanical Gard.	KF022387	KF022453	KF022325
		LX201004033	Jigong Shan, Henan	KF022388	KF022454	KF022326
		LX201009076	Yichang, Hubei	KF022389	KF022455	KF022327
Loganiaceae	*Strychnos nux-vomica* L.	C.L.Pan s.n	Guangxi Medicinal Botanical Garden	KF022436	KF022504	KF022371
		LND 2011013	Guangxi	KF022437	KF022505	KF022372
		LND 2011019	Guangxi	KF022438	P	KF022373
Menisperm-aceae	*Menispermum dauricum* DC.	LX201008064	Songshan, Beijing	KF022423	KF022490	KF022357

Continued

Family	Taxon	Voucher	Locality	GenBank Accession Number		
				matK	*rbcL*	ITS
		LX201008037	Jiufeng, Beijing	KF022424	KF022491	KF022358
		LX201106120	Xiaowutai Shan, Hebei	KF022425	KF022492	KF022359
Papaveraceae	*Papaver nudicaule* L.	LX201007119	Dongling Shan, Beijing	KF022426	KF022493	KF022360
		LX201106137	Xiaowutai Shan, Hebei	KF022427	KF022494	KF022361
		LX201205029	Xiaowutai Shan, Hebei	S	KF022495	KF022362
Plantaginaceae	*Digitalis purpurea* L.	LX201009088	Beijing	KF022411	KF022476	KF022345
		LX201009089	Beijing	KF022412	KF022477	KF022346
Ranuncul-aceae	*Aconitum Alboviolaceum* Kom.	LX201007003	Dongling Shan, Beijing	KF022379	KF022444	KF022316
		LX201007014	Dongling Shan, Beijing	KF022380	KF022445	KF022317
		LX201007017	Dongling Shan, Beijing	P	P	P
	Aconitum henryi E. Pritz. ex Diels	LJY201107031	Shennongjia, Hubei	KF022381	KF022446	KF022318
		LJY201107032	Shennongjia, Hubei	KF022382	KF022447	KF022319
	Aconitum rockii H.R. Fletcher et Lauener	x109	Xiangrila, Yunnan	KF022383	KF022448	KF022320
		x112	Xiangrila, Yunnan	KF022384	KF022449	KF022321
	Aconitum sinomontanum Nakai	LX201006075	Dongling Shan, Beijing	KF022385	KF022450	KF022322
		LX201006096	Song Shan, Beijing	KF022386	KF022451	KF022323
		LX201106127	Xiaowutai Shan, Hebei	S	KF022452	KF022324
	Delphinium grandiflorum L.	LX201008014	Dongling Shan, Beijing	KF022402	KF022468	KF022337
		LX201008077	Song Shan, Beijing	KF022403	KF022469	KF022338
		LX201106055	Xiaowutai Shan, Hebei	P	S	P
		LX201208115	Yunmeng shan, Beijing	KF022404	KF022470	S
	Delphinium pachycentrum Hemsl.	x263-1	Kangding, Sichuan	KF022405	KF022471	KF022339
		x263-2	Kangding, Sichuan	KF022406	S	KF022340
		x289	Zheduo Shan, Kangding, Sichuan	KF022407	KF022472	KF022341
	Delphinium tatsienense Franch.	x268-1	LiuBa, Kangding, Sichuan	KF022408	KF022473	KF022342
		x268-2	LiuBa, Kangding, Sichuan	KF022409	KF022474	KF022343
		x276	Shangmuju, Kangding, Sichuan	KF022410	KF022475	KF022344
Schisandr-aceae	*Illicium lanceolatum* A. C. Sm.	LX201004056-1	Jigong Shan, Henan	KF022419	KF022486	KF022353
		LX201004056-2	Jigong Shan, Henan	KF022420	KF022487	KF022354
Solanaceae	*Datura stramonium* L.	LX201208003	Huairou, Beijing	KF022398	KF022464	KF022335
		LX201007074	Qingshan, Inner Mongolia	KF022399	KF022465	KF022336
		LX201205175	Mentougou, Beijing	KF022400	KF022466	P
		LX201106100	Xiaowutai Shan, Hebei	KF022401	KF022467	P
	Solanum tuberosum L.	LX201007004-1	Dongling Shan, Beijing	KF022434	KF022502	KF022369
		LX201007004-2	Dongling Shan, Beijing	KF022435	KF022503	KF022370
Xanthorrh-oeaceae	*Hemerocallis minor* Mill.	LX201007099	Dongling Shan, Beijing	KF022417	KF022484	P
		LX201208049	Huairou, Beijing	KF022418	KF022485	S

Note. P: Missing data by PCR failure; S: Missing data by sequencing failure.

RESULTS

Universality

In the present study, all the poisonous plants were used to test the universality of primer sets. The PCR success levels for *matK*, *rbcL*, and ITS were 91.9%, 94.6%, and 90.5%, respectively. For *matK* region, primer pair 390F/1326R worked better than the other two and generated more successful PCR products. For ITS region, primer pair ITS5/ITS4 worked better than ITS1/ITS4 combination. All the successful PCR products generated clear and single band with certain migration distance on agarose gel. Overall sequencing success rates were 95.6% (*matK*), 97.1% (*rbcL*), 94.0% (ITS), and the total number of barcode sequences generated was, 65 (*matK*), 68 (*rbcL*), and 63 (ITS) (Table 2).

Discriminatory Power

Sequence alignment was successful with no indels in the *rbcL* sequences and with several indels in the *matK* sequences. However, ITS varied greatly and can only be aligned among closely related genera, such as *Aconitum* and *Delphinium*. Thus for ITS sequences, the PWG-Distance and Tree-Building analyses were applied on closely related species and genera whose ITS sequences can be aligned. The ITS sequences of distantly related plant taxa showed unalignable motifs which means they can be discriminated by very significant PWG-Distance values and very long branches in the Tree-Building method. So we treated the situation as success when the ITS sequence were unalignable.

In total, we obtained 196 barcode sequences from all 74 samples of the 27 most threatening poisonous plant species from China. Among the three analytical methods: (i) Blast, (ii) PWG-Distance, and (iii) Tree-Building, Blast often showed the lowest discrimination rates, whereas, PWG distance and Tree-Building methods showed similar discrimination power except *matK*+*rbcL* data (Figure 1). When single markers were considered, *rbcL* generated the lowest score for all the three methods, and the ITS showed highest rates on PWG distance (Figure 1) and Tree-Building methods (Figures 1-5). Combining of the candidate barcodes improved the discrimination rates especially when ITS region was involved. When all these three markers are combined, all the discriminatory methods gave high scores (Figure 1).

It is noteworthy that with the exception of the Blast method for the single markers *matK* (95.5%) or *rbcL* (95.5%), the discrimination rates at genus level of ITS and combined barcodes for all the methods were 100%. For the genera *Aconitum* and *Delphinium*, ITS region showed higher discrimination rate among species within each genus than the plastid regions did when Tree-Building methods were

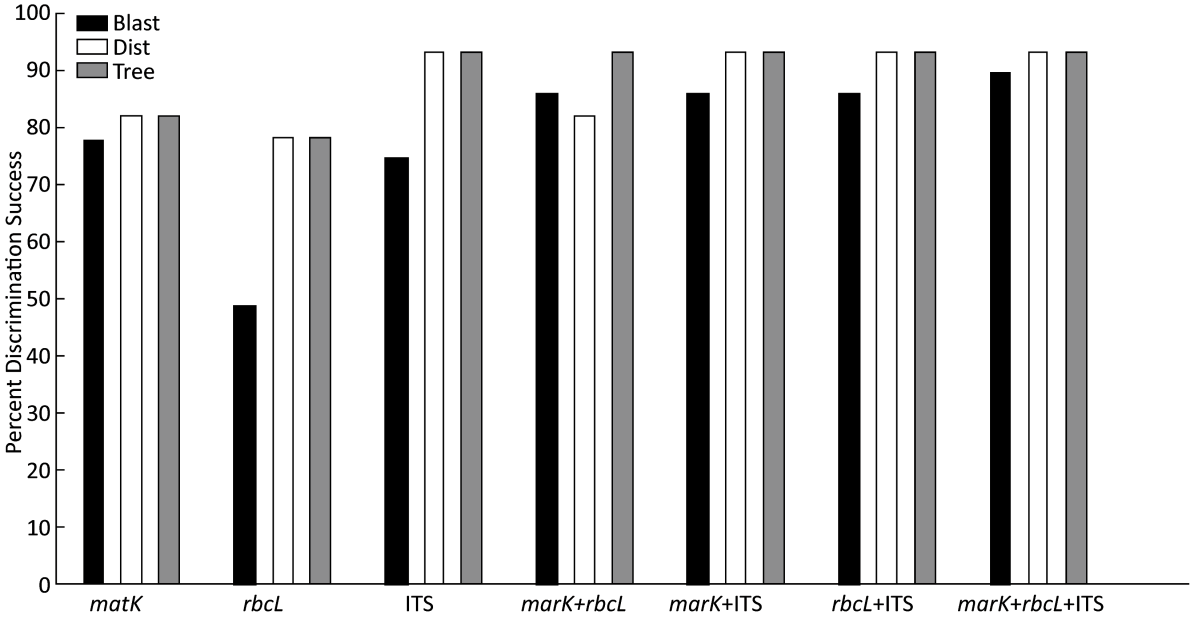

Figure 1. Comparisons of the identification power of candidate barcoding loci for poisonous plants based on different methods.

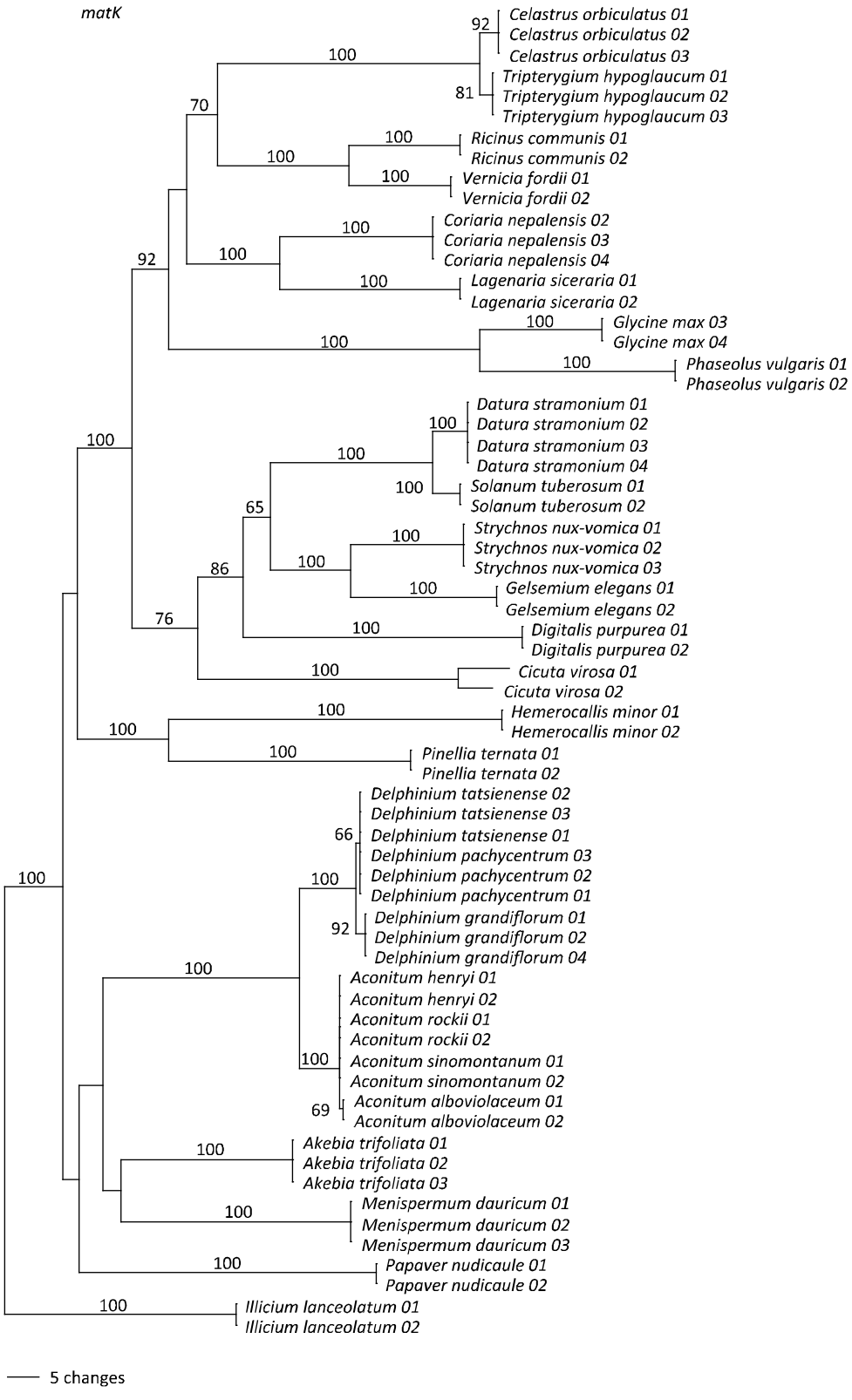

Figure 2. A neighbor-joining tree showing the phylogenetic relationship of the poisonous plant taxa based on *matK*. Bootstrap values (>50%) are marked above the branches. The tree is rooted by *Illicium lanceolatum* according to APG III (2009)[47].

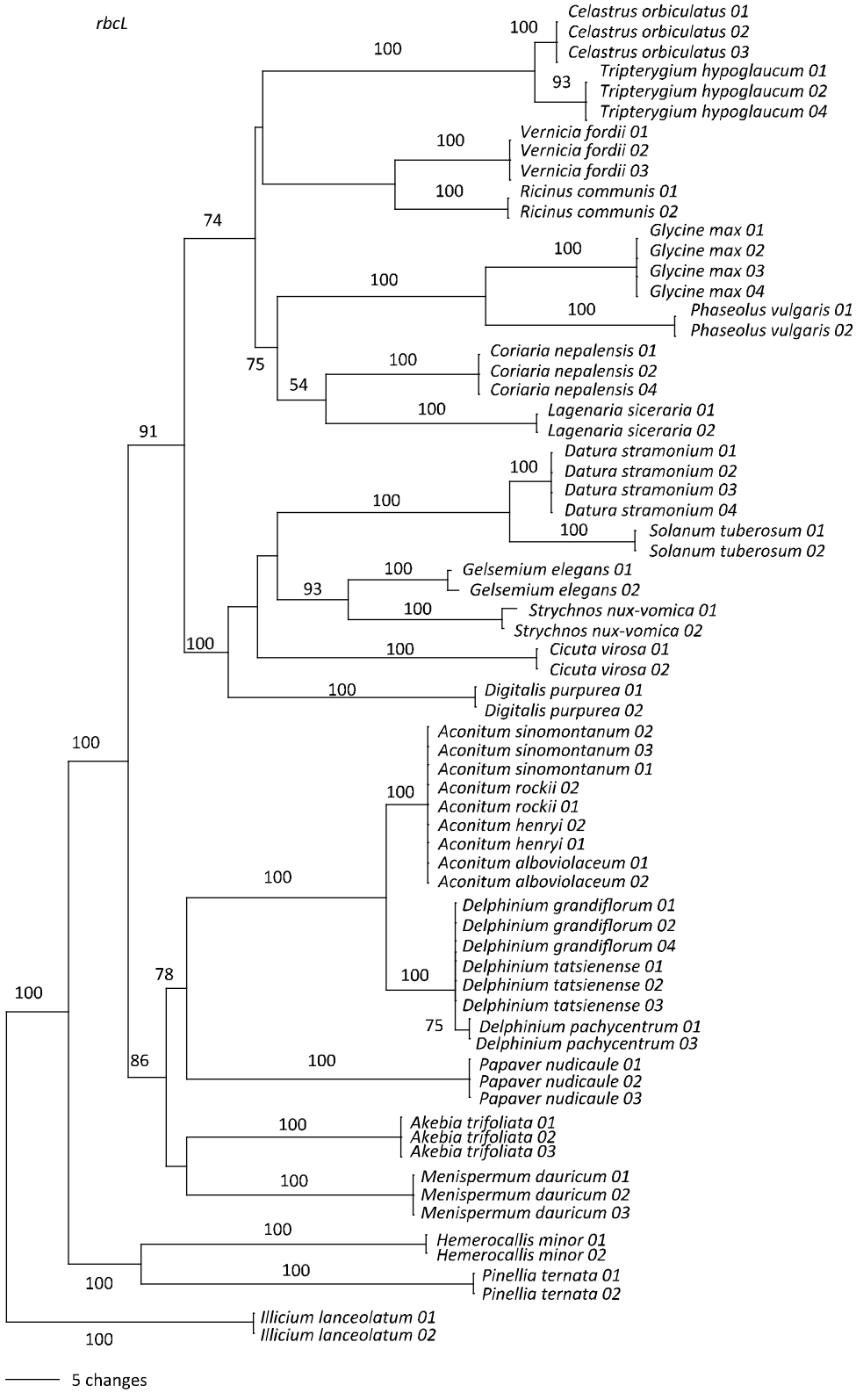

Figure 3. A neighbor-joining tree showing the phylogenetic relationship of the poisonous plant taxa based on *rbcL*. Bootstrap values (>50%) are marked above the branches. The tree is rooted by *Illicium lanceolatum* according to APG III (2009)[47].

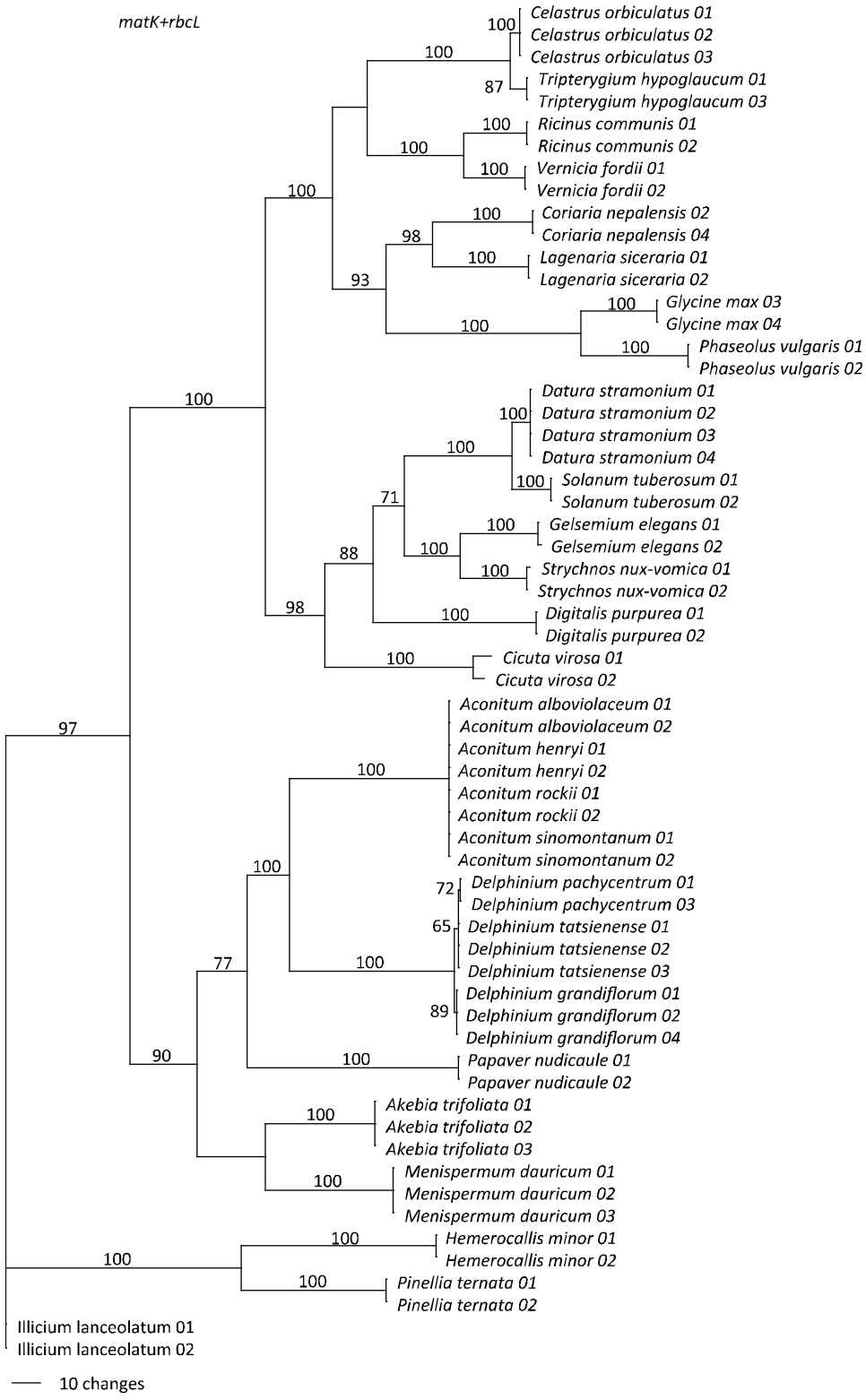

Figure 4. A neighbor-joining tree showing the phylogenetic relationship of the poisonous plant taxa based on *matK+rbcL*. Bootstrap values (>50%) are marked above the branches. The tree is rooted by Illicium lanceolatum according to APG III (2009)[47].

used (Figure 5). All the sampled *Aconitum* species were identified on the tree. But in *Delphinium*, only *D. gradiflorum* can be recognized.

DISCUSSION

From our literature survey and field investigation from China, the following characterizations can be summarized. (1) Not all toxic plants threaten people's health. For example, poisoning events caused by *Antiaris toxicaria*, *Toxicodendron*, or *Urtica*, have never or rarely been reported during the past 20 years. This is because *Antiaris toxicaria* is very rare in China and is too famous to be misused[23-24]. *Toxicodendron* and *Urtica* are common but not deadly[25-26] and rarely reported after poisoning events happened. (2) In the reported events, the toxic plants were vaguely identified especially in large genera. For example, many species in *Aconitum* can cause severe poisoning[27]. However, in the references only 'Wutou' (for an *Aconitum* spp. in Chinese) was reported. Furthermore, in many areas of southwest China, people also refer to plants of *Delphinium* as 'Wutou' or 'Caowu' (also means *Aconitum* spp.), because both genera have the alkaloid aconitine and will cause the same symptoms[28]. This because the two genera are closely related phylogenetically[29]. In this study, although we calculated *Aconitum* as the most dangerous genus, some of the poisoning events may be caused by *Delphinium*. For other poisonous plants, the identification of reports can be trusted at least at the generic level. (3) Large numbers of intoxication events were caused by cultivated or semi-domestic plants, and very few deaths occurred. On the contrary, natural poisonous plants involved fewer people, but the mortality rate was much higher than domestic or semi-domestic plants. *Phaseolus vulgaris* (common bean), *Glycine max* (soybean) and *Solanum tuberosum* (potato) are very popular vegetables in China and can be toxic when they are not properly cooked or poisonous parts are used (e.g., buds of potato) in public canteens. Oil from *Vernicia fordii* is not edible; however, it often is misused carelessly and causes alimentary toxicosis.

Fast identification of poisonous plants using DNA barcodes requires high primer universality for each region. In this study, the universality of the three barcodes tested was similar and the frequencies of successes were high. All the successfully amplified regions generated high quality sequences. Among the three markers, *rbcL* showed the highest universality. This is consistent with the results by China Plant BOL Group[12], who sampled thousands of accessions across angiosperms. At the

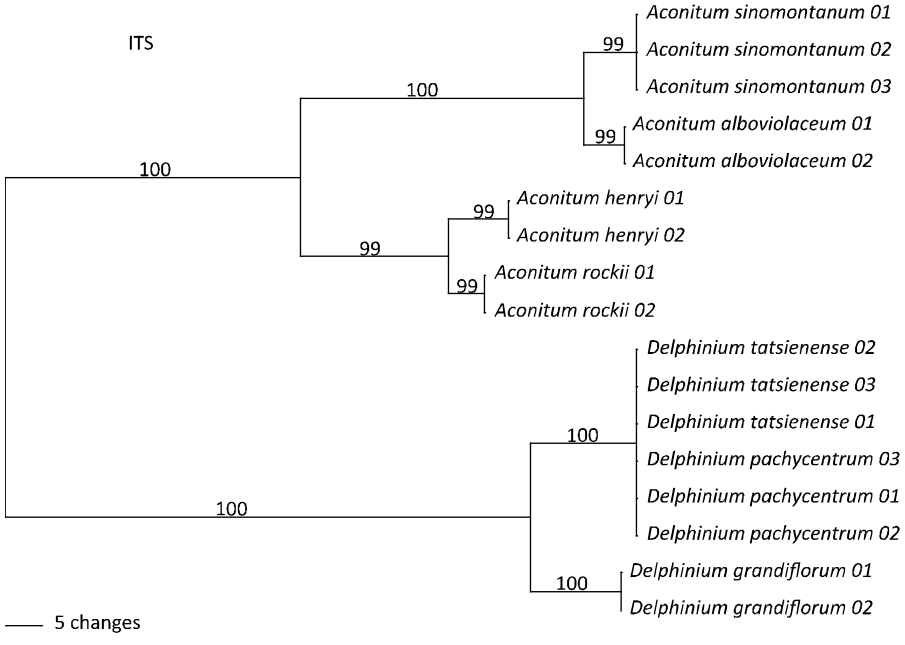

Figure 5. A neighbor-joining tree showing the phylogenetic relationship of *Aconitum* and *Delphinium* based on ITS. Bootstrap values (>50 %) are marked above the branches.

species level, at least one sample of all the selected species successfully generated *matK* and *rbcL* sequences. Only the ITS sequence of *Hemerocallis minor* was not successfully obtained. According to the result, the DNA barcoding technique exhibits a powerful potential for identification of poisonous plants.

The discrimination rates of the Blast method were relatively low. This result is in conflict with the analysis across angiosperms[12]. China Plant BOL Group[12] found that Blast tended to give higher discrimination rates than Distance and Tree-Building methods. This inconsistency was because they used much more intensive sampling at all taxonomic levels in angiosperms. In the present study, we only focused on the reported poisonous plants and the phylogenetic relationships among most of the sampled species and genera are far from close. Thus, when using PWG Distance and Tree-Building methods, the discrimination power showed higher than the Blast method.

Combined analyses increased discrimination power, which is consistent with other studies[3,5,12]. However, species in *Delphinium* were not fully discriminated even when the combined barcodes were used. The genus has more than 300 species and is highly diverse in southwestern China[30-31]. This problem is common in taxonomically complicated and rapidly diversified groups in plants and insects[32-36].

In general, the discrimination power of the barcodes by all the three methods is sufficient for identifying poisonous plants, considering that the generic level identification of toxic plants is very important in the clinical treatment, because toxic plants in same genus tend to have similar toxic chemicals. Considering the primer universality and discrimination power, we suggest that *matK*, *rbcL*, and ITS should be used in combination as a DNA barcode for identifying poisonous plants. Although the Blast method showed low discrimination rate, it is faster and well visualized than the other two methods, and is probably a good method for clinical identification.

Another candidate plastid marker *psbA-trnH* has been tested to have high primer universality and higher discrimination power in many groups of angiosperms, some of which are traditional Chinese pharmacopoeia[3,5,10,12,15,18,24,37-43]. However, this marker also showed some problems. Although the primer universality was high, the sequence quality of *psbA-trnH* was the lower than *matK*, *rbcL*, and ITS detected by China Plant BOL Group[12]. Problems were encountered in assembly of the bidirectional sequences due to ambiguous bases. The sequencing runs of *psbA-trnH* were often unclear downstream of mono-nucleotide repeats. There was also a problem of aligning the *psbA-trnH* sequences across large scale of sampling in angiosperms, especially in poisonous plants[18]. However, *psbA-trnH* showed some potential for discriminating species in Ranunculaceae and other medical or poisonous plants[39,41-43]. Thus this marker should be considered for further investigations on poisonous plants.

There are some problems and challenges for using DNA barcoding as a fast tool for identification poisonous plants in China. The biggest challenge of the application of DNA barcoding technique is that, unlike laboratory conditions (plant materials were dried by silica-gel), the plant material may be cooked or digested by gastric acid of the patient. These will influence the success of total DNA extraction and quality significantly. Further experiments simulating real-world conditions should be conducted. DNA extraction experiments should be designed by pretreatments of the plant materials with acidic conditions, heat, and acid plus heat. Recently, Wood et al.[44] reported that plant DNA can be successfully extracted from coprolites of extinct birds. Also, Hibert et al.[45-46] reported the plant DNA barcodes extracting from herbivores feces. These studies suggest that DNA extraction from digested plant remnants is possible by refining experiment conditions.

The most threatening poisonous plants in China were identified successfully at least on the generic level in this study, showing DNA barcoding has the potential as a useful tool for diagnostic purpose. Although multiple accessions in each species were tested, the sampling of this study was limited in that many genera were represented by only one species and a few individuals. Further studies with more comprehensive and a more balanced sampling scheme should be considered. A workflow of clinic identification of poisonous plants using DNA barcoding should be proposed after more data are obtained by further fundamental studies. We agree with Bruni et al.[18] that establishing a dedicated poisonous plants database with both morphological and molecular information is required to help poisonous centers like China CDC to efficiently identify toxic plants.

ACKNOWLEDGMENTS

The authors thank Dr. Shen SK from Yunnan

University, and Pan CL from Guangxi Medicinal Botanical Garden for kindly providing samples. The lab work was conducted at the Laboratory of Systematic Evolution and Biogeography of Woody Plants of Beijing Forestry University.

DECLARATIONS

The authors declare that there are no conflicts of interest in this study. The authors alone are responsible for the content and writing of the paper.

Received: November 17, 2013;
Accepted: March 22, 2014

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