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Original Article

Identification of Dian Ji Xue Teng (*Kadsura interior*) with DNA barcodes

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ABSTRACT

Objective: To identify *Kadsura interior* (Dian Ji Xue Teng, Schisandraceae) by using DNA barcoding.
Methods: We analyzed five DNA barcodes (ITS, ITS2, *psb*A-*trn*H, *mat*K and *rbc*L) using DNA barcoding in terms of distance-based, tree-based and character-based identification to distinguish *Kadsura interior* and its adulterants.
Results: In distance-based and tree-based identification, *K. interior* could be distinguished easily from the species of *Schisandra* and *K. coccinea*. In character-based identification, there are two single nucleotide polymorphisms (SNPs) in ITS and one SNP in *psb*A-*trn*H which can be used to distinguish *K. interior* from *K. heteroclita* and *K. longipedunculata*.
Conclusion: The results indicate that DNA barcoding can be used to identify *K. interior*. ITS and *psb*A-*trn*H sequence can be the most ideal DNA barcode for discriminating *K. interior* and its adulterants by the combination analysis of distance-based, tree-based and character-based identification (SNPs).
Key words: DNA barcoding, Schisandraceae, SNP, *Kadsura interior*
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Introduction

Kadsura interior A. C. Smith, a species of *Kadsura* (Schisandraceae), is mainly distributed in the southwest of Yunnan Province[1]. Before being recorded in Chinese Pharmacopoeia in 2010, the lianas of *K. interior* had been used as fork herb since two hundred years ago, called Dian Ji Xue Teng, for treating menstrual irregularities, blood deficiencies, and other feminine disorders[2-4]. Modern studies also show that some dibenzocyclooctadiene lignans in *K. interior* have the efficacy of antitumor and anti-HIV[5-7]. Morphologically, it is difficult to distinguish this species from another species *K. heteroclita*, medicinal named Di Xue Xiang, which is usually used for the treatment of rheumatism, punch injury, dysmenorrhea and gastroenteritis[8,9]. Although Law[1] considered they were separate species by distinguishing flower characters, Saunders Richard[10] and Lin[11] believed that there were continuous variation on the size of perianth and the numbers of carpels, so they should be combined into one species[10]. In order to identify the medicine herbs "Xue Teng" by molecular sequences, Zhou et al. chose *psb*A-*trn*H for distinguishing eight species[12]. Although they found a stabilized single nucleotide polymorphism (SNP), SNPs as potential tool to distinguish *K. interior* from *K. heteroclita* could not be further analyzed because of poor samples of *K. heteroclita*. In addition, Zhang et al. investigated the discriminatory power of four commonly used DNA barcodes (ITS, *psb*A-*trn*H, *mat*K, and *rbc*L) for Chinese medicinal plants of the family Schisandraceae and exposed *K. heteroclita* and *K. longipedunculata* could not be discriminated by four commonly used DNA barcodes[13].

DNA barcoding identification technology, a method using relatively short DNA to identify species, is an effective supplement to the traditional identification methods because of its repeatability, convenience and less necessary professional experience in identifying practice[14-19]. However, previous studies of DNA barcoding have not effectively resolved the problem of identifying *K. interior*. In this study, we surveyed 21 populations representing seven Schisandraceae species and obtained the four DNA barcodes (ITS, *mat*K, *psb*A-*trn*H and *rbc*L). Through DNA barcoding analysis, we try to establish a standard identification method for *K. interior*.

Materials and methods

1 Plant materials

64 samples of seven Schisandraceae species were collected from Sichuan, Chongqing, Guizhou, etc. (Table 1). The specimens were collected from the wild, taxonomically identified using Flora Republicae Popularis Sinicae and verified by Zhang Benggang who was a professor in Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College. Leaf material of each sample was dried in silica gel for DNA extraction. *Illicium micranthum*, a member of *Illicium* (a sister group of *Schisandra* and *Kadsura*) was selected as an outgroup for tree-based analyses. The conducted research is not related to either human or animals use.

Table 1. The collection information of the Schisandraceae samples.

Species	Collection number	Tites	Date
K. longipedunculata	2015082801, 2015082903,	Nanchuan, Chongqin	2015.8.29
	2015083102	Emei, Sichuan	2015.8.31
	2015090602, 2015090604	Guiyang, Guizhou	
	2015090801, 2015090802, 2015090803, 2015090804, 2015090805, 2015090806, 2015090807, 2015090808, 2015090809, 2015091001, 2015091002, 2015091101, 2015091102, 2015091103	Leishan, Guizhou	2015.9.8
	2015091801, 2015091802	Baojing, Hunan	2015.9.18
	2015092301, 2015092302, 2015092303, 2015092306, 2015092307, 2015092309, 2015092310	Xingan, Guangxi	2015.9.23
K. heteroclita	2015091201	Jianhe, Guizhou	2015.9.12
	2015091803, 2015091804	Baojing, Hunan	2015.9.18
	2015092104, 2015092105, 2015092106	Jinxiu, Guangxi	2015.9.21
	2015082902	Nanchuan, Chongqin	
	2015090202, 2015090203, 2015090204A, 2015090204B, 2015090208	Emei, Sichuan	
K. coccinea	2015082901	Nanchuan, Chongqin	2015.8.29
	2015083101	Emei, Sichuan	2015.8.31
	2015090502	Guiyang, Guizhou	2015.9.5
	2015091601	Huaihua, Hunan	2015.9.16
	2015092304	Xingan, Guangxi	2015.9.23
K. interior	2015121202, FQ001, FQ2016080601, FQ2016080602, FQ2016080603, FQ2016080604, FQ2016080605, FQ2016080606, FQ2016080607, FQ2016080608	Fengqin, Yunnan	2014-2016
S. rubriflora	2015090102, 2015090103, 2015090104, 2015090201	Emei, Sichuan	2015.9.1
S. propinqua	2015083103, 2015083105	Emei, Sichuan	2015.8.31
S. henryi	2015090209	Emei, Sichuan	2015.9.2
	2015090601, 2015090603	Guiyang, Guizhou	2015.9.5
I. micranthum	2015090207	Emei, Sichuan	2015.9.2

2 DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from dry leaves stored in silica gel and from herbal medicines using the Plant Genomic DNA Kit (Tiangen, Beijing, China) according to the manufacturer protocol. Polymerase chain reaction (PCR) amplification of the targeted DNA regions was performed using 2×Taq PCR MasterMix (Aidlab Biotechnologies Co. Ltd., Beijing, China), which contained 0.05 u/μL of Taq DNA Polymerase, 4 mM $MgCl_2$, 0.4 mM of dNTP and reaction buffer. The PCR mix included 12.5 μL 2×Taq PCR MasterMix, 1 μL each primer (5 μM), 2 μL template DNA and 8.5 μL distilled or deionized water to give a final volume of 25 μL. The primer information and optimal PCR conditions were obtained from previous studies[19,20]. PCR products were examined by electrophoresis using 1% agarose gels and sequenced in both directions by sequencing company.

3 Data analysis

The quality estimation and assembly for the newly generated sequences were performed with Codon Code Aligner 5.1.5 (CodonCode Corp., Dedham, MA, USA). All the newly acquired sequences were confirmed via BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and stored in Genkbank with accession numbers (Table S1). The lianas of some *Schisandra* species are also used as herbal medicines and neither *Schisandra* nor *Kadsura* is monophyletic[21]. So the four DNA barcodes (ITS, *mat*K, *psb*A-*trn*H and *rbc*L) of the *Schisandra* liana herbs were filtered and downloaded from the database of Genbank (Table 2). None of *Kadsura* sequences was downloaded from the database of Genbank because we had enough samples of *Kadsura* and problems of identification might exist in *Kadsura* sequences of Genbank due to taxonomic debate[22]. The sequence alignment for each locus was initially performed using ClustalW. Genetic distances and maximum-likelihood (ML) phylogenetic tree for each DNA region were calculated using MEGA v6.06[23]. The software BioEdit was used to analyze single nucleotide polymorphism (SNP)[24].

Results and analysis

1 Sequence information and distance-based analysis

Totally, we obtained 79 ITS, 79 ITS2, 74 *mat*K, 74 *rbc*L, 76 *psb*A-*trn*H and 63 I-K-A-L (the combination of ITS, matK, *psb*A-*trn*H and rbcL) sequences and analyzed the intra- and inter-specific distances for *K. interior* (Table 3). The lengths of ITS, ITS2, *mat*K, *psb*A-*trn*H and *rbc*L were 671, 228, 729, 455 and 525 bp after sequence alignment, respectively. The GC average contents of ITS, ITS2, *mat*K, *psb*A-*trn*H and *rbc*L were 55.49%, 60.50%, 34.92%, 34.07% and 44.45%, respectively. The intra- and inter-specific distances for *K. interior* were calculated by MEGA v5.05 based on the p-distance model (Table 3). The intra- specific distances for *K. interior* was (0.000±0.000) among all of the DNA barcodes except *psb*A-*trn*H (0.0030 ±0.0030). The inter-specific distances of *rbc*L had no significant

Table 2. Accession numbers of GenBank database.

Barcode	Species	Accession number
ITS	*S. bicolor*	DQ342255, KP689681, KP689682
	S. propinqua	JF978530, JF978531
	S. sphenanthera	KP689644, KP689645, KP689646, KP689647, KP689648
	S. viridis	AF163703, AF263438, JF978539, JF978540, KP689643
*mat*K	*S. bicolor*	GQ248198, KP689790, KP689791
	S. propinqua	JF956216, JF956217
	S. sphenanthera	KP689752, KP689753, KP689754, KP689755, KP689756
*psb*A-*trn*H	*S. bicolor*	KP690009, KP690010
	S. propinqua	JN047088
	S. sphenanthera	KP689971, KP689972, KP689973, KP689974, KP689975
	S. viridis	JN047097, JN047098
*rbc*L	*S. bicolor*	KP689899, KP689900
	S. propinqua	JF944186, JF944187
	S. sphenanthera	KP689861, KP689862, KP689863, KP689864, KP689865

difference. Among ITS2, ITS, *mat*K, *psb*A-*trn*H and I-K-A-L, the inter-specific distance between *K. interior* and *K. heteroclita* was significantly lower than other groups. The same pattern also showed in the inter-specific distances between *K. interior* and *K. longipedunculata*. Based on the intra- and inter-specific distances, the close relationships between *K. interior*, *K. heteroclita* and *K. longipedunculata* can be infered.

2 Tree-based identification

Maximum-likelihood (ML) phylogenetic tree was established to analyze the genetic relationships among species. The ML tree of the combination of ITS, *psb*A-*trn*H, *mat*K, and *rbc*L was presented in Fig. 1 and all the other phylogenetic trees were shown in Fig S1. Samples of *K. interior*, *K. heteroclita* and *K. longipedunculata* were so many that only haplotypes were used for tree-based identification. There were four major clusters (Cluster 1, Cluster 2, Cluster 3 and Cluster 4) in the combination ML tree. Cluster 1 contained *K. interior*, *K. heteroclita* and *K. longipedunculata*. Cluster 2 and Cluster 3 corresponded to *K. coccinea* and *S. propinqua*, respectively. Cluster 4 contained some species of *Schisandra*. The same pattern also showed in the ML trees obtained from other single markers except *rbc*L, in which *S. propinqua* nested in Cluster 1. A monophyletic cluster with 94% bootstrap values was combined with *K. interior* in Cluster 1. Among five commonly used barcodes, the monophyletic cluster of *K. interior* was strongly supported with maximum bootstrap values (96%) in ITS ML tree. In comparison, samples of *K. interior* were not monophyletic in *mat*K and *rbc*L ML tree.

3 Character-based identification

For species identification of Schisandraceae, character-based identification has been evaluated by Zhang et al[13]. According to the ML tree (Fig. 1) and the between group distances for *K. interior* (Table 3), we found that *K. interior* could be distinguished easily from *Schisandra* and *K. coccinea*. In contrast, *K. interior* had very close relationship with *K. heteroclita* and *K. longipedunculata*. Therefore, 10 samples of *K. interior*, 12 samples of *K. heteroclita* and 28 samples of *K. longipedunculata* were used for character-based identification. One transition at position 651 and 2 bp indels at position 227 were detected in ITS and one transition was found in *psb*A-*trn*H at position 218. These SNPs in *psb*A-*trn*H and ITS of *K. interior*, *K. heteroclita* and *K. longipedunculata* clearly separated these individuals into two sequence types, Type I contained individuals of *K. interior* and Type II contained individuals of *K. heteroclita* and *K. longipedunculata* (Table 4). Consequently, SNPs of ITS and *psb*A-*trn*H were capable to distinguish *K. interior* from *K. heteroclita* and *K. longipedunculata*.

Discussion

The lianas of *K. interior* are well-known traditional medicine accepted by Chinese Pharmacopoeia 2015[2]. The liana herbs were traditionally identified by morphological and microscopic methods, but subjective experience of user and fragmentary samples might influenced the efficiency and accuracy of

Table 3. The intra- and inter-specific distances for *K. interior*.

Species 1	Species 2	ITS2	ITS	*mat*K	*psb*A-*trn*H	*rbc*L	I-K-A-L
K. interior	*K. interior*	0.000±0.000	0.000±0.000	0.000±0.000	0.003±0.003	0.000±0.000	0.000±0.000
K. interior	*K. heteroclita*	0.011±0.005	0.006±0.002	0.000±0.000	0.007±0.005	0.006±0.003	0.003±0.001
K. interior	*K. longipedunculata*	0.009±0.005	0.005±0.002	0.000±0.000	0.007±0.005	0.006±0.003	0.003±0.001
K. interior	*K. coccinea*	0.034±0.012	0.031±0.007	0.015±0.004	0.102±0.019	0.006±0.003	0.027±0.004
K. interior	*S. henryi*	0.044±0.013	0.032±0.006	0.016±0.005	0.025±0.010	0.008±0.004	0.020±0.003
K. interior	*S. propinqua*	0.026±0.010	0.029±0.006	0.010±0.004	0.038±0.012	0.004±0.003	0.018±0.002
K. interior	*S. rubriflora*	0.049±0.014	0.036±0.007	0.011±0.004	0.025±0.010	0.008±0.004	0.020±0.003
K. interior	*S. bicolor*	0.035±0.012	0.030±0.006	0.014±0.004	0.020±0.009	0.008±0.004	
K. interior	*S. sphenanthera*	0.050±0.013	0.036±0.007	0.010±0.004	0.025±0.010	0.008±0.004	
K. interior	*S. viridis*	0.050±0.014	0.037±0.007		0.025±0.010		

I-K-A-L: the combination of ITS, *mat*K, *psb*A-*trn*H and *rbc*L. Blank space indicated that the sequences of species 2 was absent or the sequences downloaded from Genbank can not be sure whether extracted from same sample.

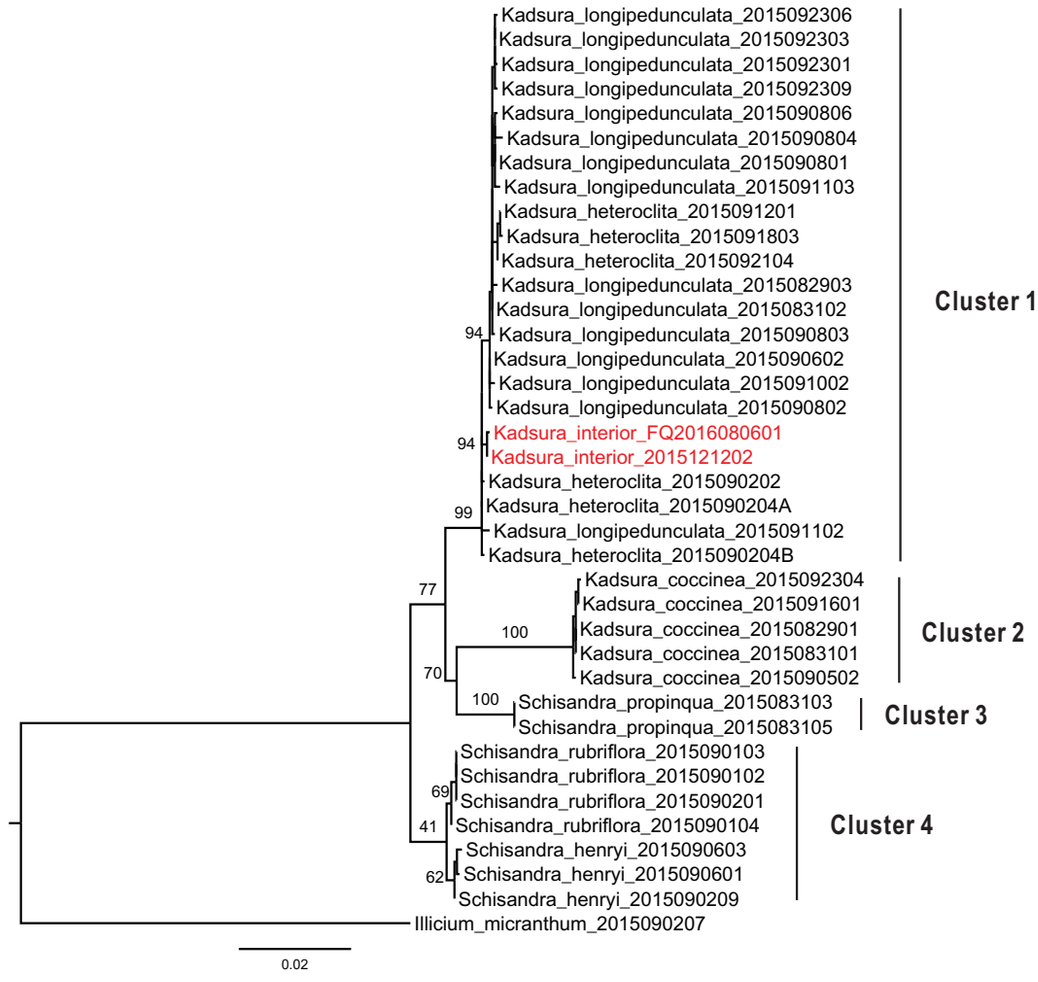

Figure 1. ML phylogenetic tree based on the combination of ITS, *psb*A-*trn*H, *mat*K and *rbc*L. The tree includes seven species from *Schisandra* and *Kadsura*. *Illicium micranthum* is the outgroup. *K. interior* is labeled by red.

authentication[25]. DNA barcoding is a new molecular marker technology, which is a mature technology with easy and universal operation, and has been widely used in the study and application, for instance, this technology has been better used in the authentication of liana herbs[15,16,26]. In this study, we trialed five DNA barcodes by using distance-based, tree-based and character-based identification for distinguishing *K. interior* from the others.

Previous DNA barcoding studies revealed that *K. interior*, *K. heteroclita* and *K. longipedunculata* were hard to be distinguished although *K. interior* could be easily distinguished from other species of Schisandraceae[12,13]. In particular, *K. interior* and *K. heteroclite* were grouped into one species in Flora of China[27]. However, there are still some morphological differences between *K. longipedunculata*, *K. interior* and *K. heteroclite*. *K. longipedunculata* is easily distinguished from *K. interior* and *K. heteroclite* by having spherical androecium while *K. interior* and *K. heteroclite* have ellipsoidal androecium. *K. interior* is rare in China, only distributed at high elevations in the southwest of Yunnan Province. This species can be distinguished from *K. heteroclite* by having obvious filaments, petioles wings and dry leaf with same color on both sides[1]. Moreover, we found that the fruits of *K. interior* were significantly bigger than that of *K. heteroclita* through our field observations. Distinctiveness of *K. interior* was also supported by SNP analysis in this study (Table 4). Three SNPs were found to distinguish *K. interior* from *K. heteroclita* and *K. longipedunculata* in *psb*A-*trn*H, and ITS. 50 individuals of *K. interior*, *K. heteroclita* and *K. longipedunculata* were separated into two types via SNPs. *K. interior* belonged to Type I while *K. heteroclita* and *K. longipedunculata* were grouped into Type II.

In this study, the results supported that the combination of ITS, *psb*A-*trn*H, *mat*K and *rbc*L could be the most ideal DNA barcode for discriminating the plants of *Schisandra* and *Kadsura* at genera level as Zhang et al. reported[13]. However, the best DNA barcode for the species discrimination at the

Table 4. single nucleotide polymorphisms (SNPs) in *psb*A-*trn*H and ITS for *K. interior*, *K. heteroclita* and *K. longipedunculata*.

Type	Taxa (n)	*psb*A-*trn*H		ITS	
		218	227	228	651
Type I	*K. interior* (10)	A	C	C	T
Type II	*K. heteroclita* (12)	C	-	-	C
	K. longipedunculata (28)	C	-	-	C

'-' indicates indels. The number of individuals is shown in the parentheses.

genus level might not always be the most suitable for any species of the genus. For *K. interior*, *K. heteroclita* and *K. longipedunculata*, the combination of ITS, *psb*A-*trn*H, *mat*K and *rbc*L showed lower resolution (Table 3, Fig. 1). Herein, we proposed ITS and *psb*A-*trn*H as the most ideal DNA barcodes for discriminating *K. interior* and its adulterants by the combination distance-based, tree-based and character-based identification. Besides, most authors believed that *K. interior* had very close relationship with *K. heteroclita*. Some studies also suggested that *K. heteroclita* and *K. interior* should be combined into one species by morphological characters[10,11,27]. *K. interior* is mainly distributed in the southwest of Yunnan Province while *K. heteroclita* is widely distributed in south of China, e.g. Guizhou, Guangxi, Hubei, etc. Although the species combination might extend the sources of Dian Ji Xue Teng, it might lead to misuse in clinical practice because of ignorance of the difference between *K. heteroclita* and *K. interior*. This study implied that genetic variation existed between the closely related species *K. interior* and *K. heteroclita*. Therefore, more studies should be required for comparing *K. heteroclita* and *K. interior* in pharmacological and chemical aspects.

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Conflict of interest

All authors declare no conflict of interest.

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Original Article

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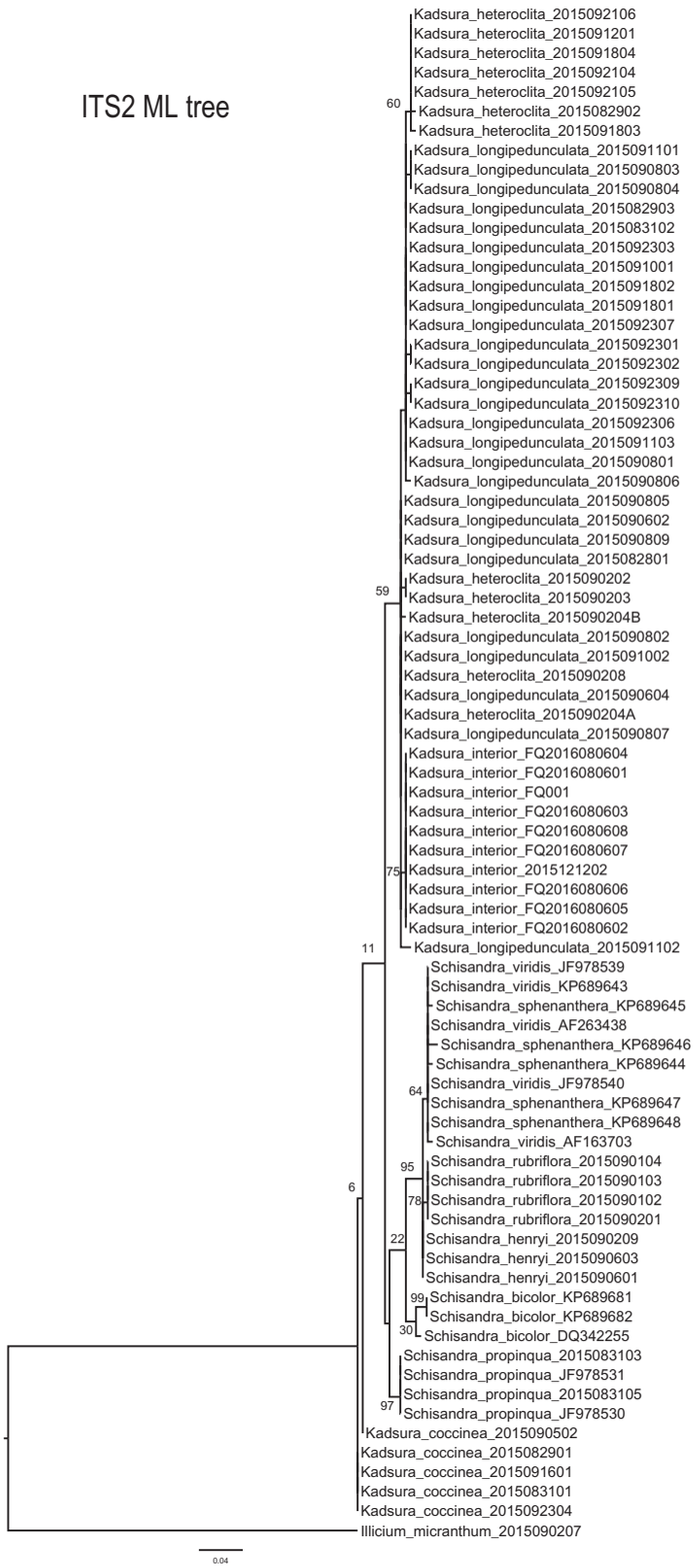
ABSTRACT

Objective: To identify *Kadsura interior* (Dian Ji Xue Teng , Schisandraceae) by using DNA barcoding.
Methods: We analyzed five DNA barcodes (ITS, ITS2, *psb*A-*trn*H, *mat*K and *rbc*L) using DNA barcoding in terms of distance-based, tree-based and character-based identification to distinguish *Kadsura interior* and its adulterants.
Results: In distance-based and tree-based identification, *K. interior* could be distinguished easily from the species of *Schisandra* and *K. coccinea*. In character-based identification, there are two single nucleotide polymorphisms (SNPs) in ITS and one SNP in *psb*A-*trn*H which can be used to distinguish *K. interior* from *K. heteroclita* and *K. longipedunculata*.
Conclusion: The results indicate that DNA barcoding can be used to identify the *K. interior*. The ITS and *psb*A-*trn*H sequence can be the most ideal DNA barcode for discriminating *K. interior* and its adulterants by the combination analysis of distance-based, tree-based and character-based identification (SNPs).
Key words: DNA barcoding, Schisandraceae, SNP, *Kadsura interior*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Kadsura_heteroclita_2015091803
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Kadsura_longipedunculata_2015090804
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Kadsura_longipedunculata_2015090806
Kadsura_longipedunculata_2015090805
Kadsura_longipedunculata_2015090602
Kadsura_longipedunculata_2015090809
Kadsura_longipedunculata_2015082801
Kadsura_heteroclita_2015090202
59
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Kadsura_interior_FQ2016080603
Kadsura_interior_FQ2016080608
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Kadsura_interior_FQ2016080605
Kadsura_interior_FQ2016080602
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Schisandra_sphenanthera_KP689646
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Schisandra_sphenanthera_KP689647
Schisandra_sphenanthera_KP689648
Schisandra_viridis_AF163703
95
Schisandra_rubriflora_2015090104
6
Schisandra_rubriflora_2015090103
78
Schisandra_rubriflora_2015090102
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Schisandra_bicolor_KP689682
30
Schisandra_bicolor_DQ342255
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Schisandra_propinqua_JF978531
Schisandra_propinqua_2015083105
97
Schisandra_propinqua_JF978530
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Kadsura_coccinea_2015082901
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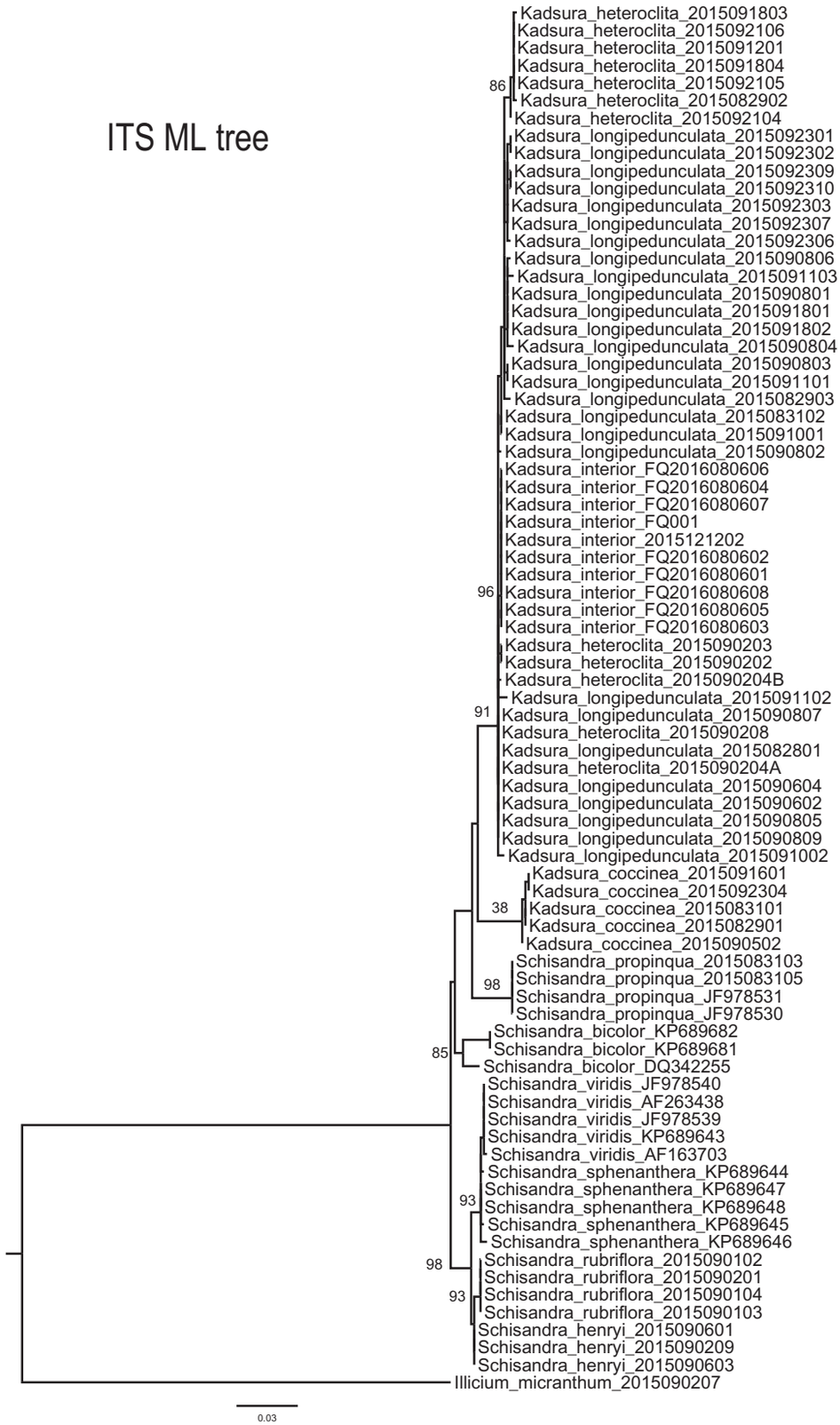
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Kadsura_heteroclita_2015092104
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Kadsura_longipedunculata_2015092302
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Kadsura_longipedunculata_2015091101
Kadsura_longipedunculata_2015082903
Kadsura_longipedunculata_2015083102
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Kadsura_interior_FQ2016080601
96
Kadsura_interior_FQ2016080608
Kadsura_interior_FQ2016080605
Kadsura_interior_FQ2016080603
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Kadsura_heteroclita_2015090202
Kadsura_heteroclita_2015090204B
Kadsura_longipedunculata_2015091102
91
Kadsura_longipedunculata_2015090807
Kadsura_heteroclita_2015090208
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Kadsura_longipedunculata_2015090805
Kadsura_longipedunculata_2015090809
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Kadsura_coccinea_2015092304
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Kadsura_coccinea_2015083101
Kadsura_coccinea_2015082901
Kadsura_coccinea_2015090502
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Schisandra_propinqua_JF978530
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93
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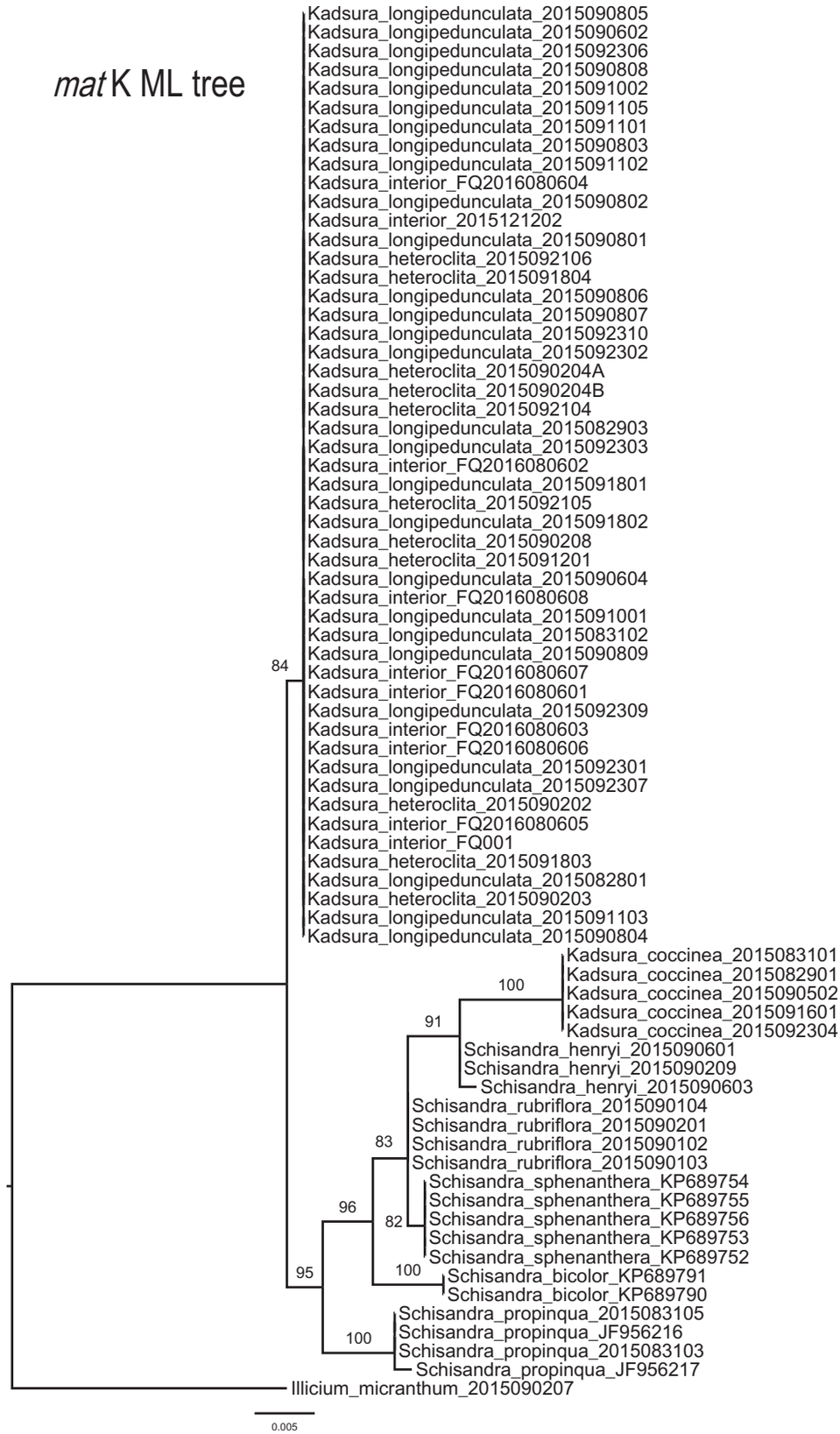
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84
100
91
83
96
82
95
100
100
0.005


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Kadsura_coccinea_2015090502
98
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Schisandra_rubriflora_2015090103
Schisandra_rubriflora_2015090102
Schisandra_henryi_2015090603
Schisandra_rubriflora_2015090201
Schisandra_henryi_2015090209
Schisandra_rubriflora_2015090104
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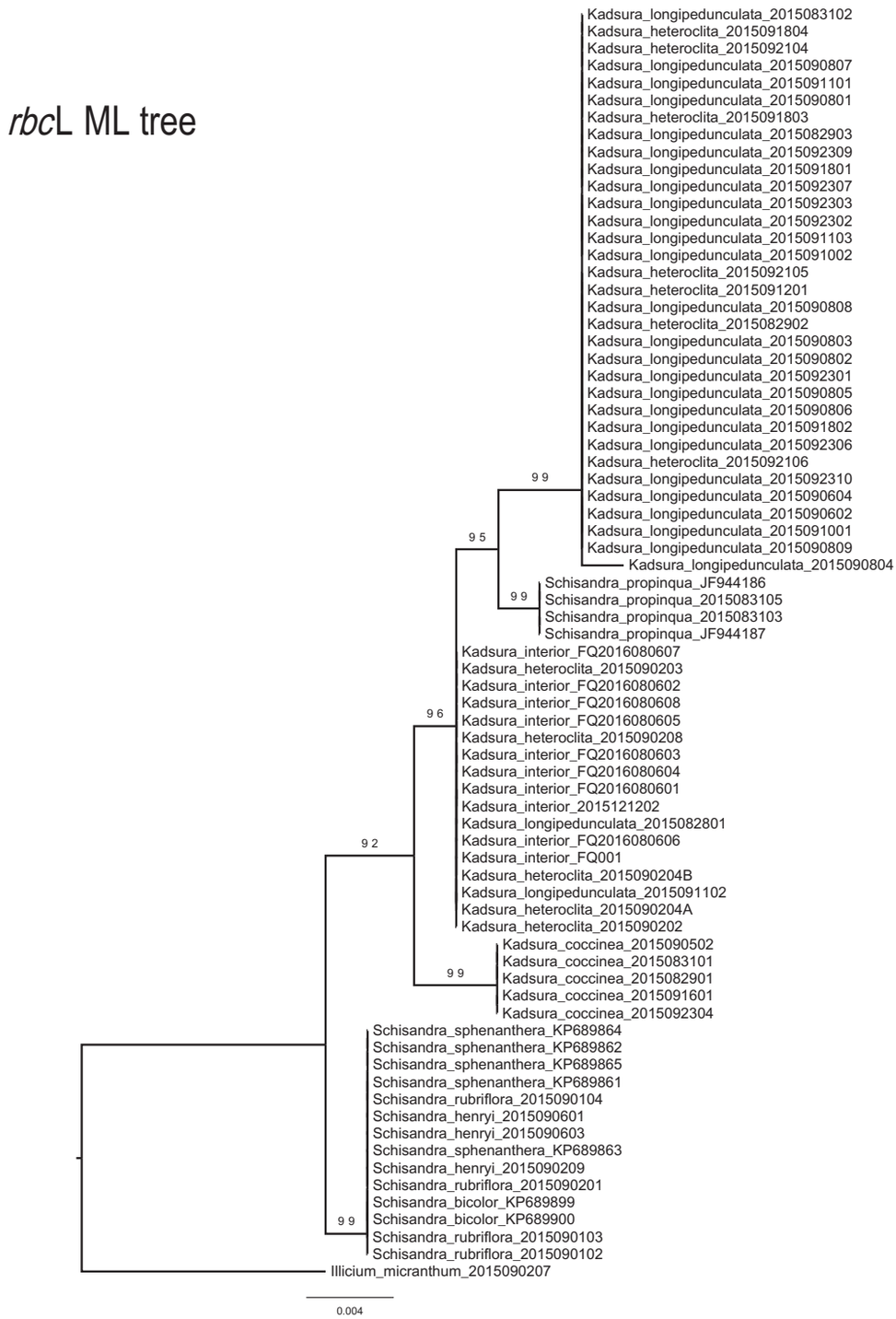
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Kadsura_longipedunculata_2015091002
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Schisandra_henryi_2015090603
Schisandra_sphenanthera_KP689863
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Schisandra_rubriflora_2015090201
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Illicium_micranthum_2015090207
99
95
99
96
92
99
99
0.004

Table S1. Accession numbers of four DNA barcodes sequenced in this study.

Species name	Collection number	Accession number			
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K. coccinea	2015083101	KY884725	KY884790	KY884855	KY884920
K. coccinea	2015090502	KY884726	KY884791	KY884856	KY884921
K. coccinea	2015091601	KY884727	KY884792	KY884857	KY884922
K. coccinea	2015092304	KY884728	KY884793	KY884858	KY884923
K. heteroclita	2015082902	KY884729	–	KY884859	KY884924
K. heteroclita	2015090202	KY884730	KY884813	KY884860	KY884925
K. heteroclita	2015090203	KY884731	KY884814	KY884861	KY884926
K. heteroclita	2015090204A	KY884732	KY884815	KY884862	KY884927
K. heteroclita	2015090204B	KY884733	KY884816	KY884863	KY884928
K. heteroclita	2015090208	KY884734	KY884817	KY884864	KY884929
K. heteroclita	2015091201	KY884735	KY884794	KY884865	KY884930
K. heteroclita	2015091803	KY884736	KY884795	KY884866	KY884931
K. heteroclita	2015091804	KY884737	KY884796	KY884867	KY884932
K. heteroclita	2015092104	KY884738	KY884797	KY884868	KY884933
K. heteroclita	2015092105	KY884739	KY884798	KY884869	KY884934
K. heteroclita	2015092106	KY884740	KY884799	KY884870	KY884935
K. interior	2015121202	KY884742	KY884800	KY884871	KY884936
K. interior	FQ001	KY884743	KY884801	KY884872	KY884937
K. interior	FQ2016080601	KY884744	KY884802	KY884873	KY884938
K. interior	FQ2016080602	KY884745	KY884803	KY884874	KY884939
K. interior	FQ2016080603	KY884746	KY884804	KY884875	KY884940
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K. interior	FQ2016080605	KY884748	KY884806	KY884877	KY884942
K. interior	FQ2016080606	KY884749	KY884807	KY884878	KY884943
K. interior	FQ2016080607	KY884750	KY884808	KY884879	KY884944
K. interior	FQ2016080608	KY884751	KY884809	KY884880	KY884945
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K. longipedunculata	2015090801	KY884757	KY884820	KY884886	KY884951
K. longipedunculata	2015090802	KY884758	KY884821	KY884887	KY884952
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K. longipedunculata	2015090804	KY884760	KY884823	KY884889	KY884954
K. longipedunculata	2015090805	KY884761	KY884824	KY884890	KY884955
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K. longipedunculata	2015091101	KY884767	KY884831	KY884897	KY884962
K. longipedunculata	2015091102	KY884768	KY884832	KY884898	KY884963
K. longipedunculata	2015091103	KY884769	KY884833	KY884899	KY884964
K. longipedunculata	2015091801	KY884770	KY884835	KY884900	KY884965
K. longipedunculata	2015091802	KY884771	KY884836	KY884901	KY884966
K. longipedunculata	2015092301	KY884772	KY884837	KY884902	KY884967
K. longipedunculata	2015092302	KY884773	KY884838	KY884903	KY884968
K. longipedunculata	2015092303	KY884774	KY884839	KY884904	KY884969
K. longipedunculata	2015092306	KY884775	KY884840	KY884905	KY884970
K. longipedunculata	2015092307	KY884776	KY884841	KY884906	KY884971
K. longipedunculata	2015092309	KY884777	KY884842	KY884907	KY884972
K. longipedunculata	2015092310	KY884778	KY884843	KY884908	KY884973
S. henryi	2015090209	KY884779	KY884844	KY884909	KY884974
S. henryi	2015090601	KY884780	KY884845	KY884910	KY884975
S. henryi	2015090603	KY884781	KY884846	KY884911	KY884976
S. propinqua	2015083103	KY884782	KY884847	KY884912	KY884977
S. propinqua	2015083105	KY884783	KY884848	KY884913	KY884978
S. rubriflora	2015090102	KY884784	KY884849	KY884914	KY884979
S. rubriflora	2015090103	KY884785	KY884850	KY884915	KY884980
S. rubriflora	2015090104	KY884786	KY884851	KY884916	KY884981
S. rubriflora	2015090201	KY884787	KY884852	KY884917	KY884982