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Genetic Relations related to Chemical Containing and the Efficient Barcodes by *psb*A-*trn*H Spacer and its Combinations with *rbc*L and *mat*K on *Gardenia* Species

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Abstract
Gardenia species have been used worldwide for ornamentation and for traditional medicines. The Thailand species observed include eight native species, *G. carinata*, *G. collinsae*, *G. elata*, *G. jasminoides*, *G. obtusifolia*, *G. saxatilis*, *G. sootepensis* and *G. thailandica*, and three introduced species, *G. gjellerupii*, *G. taitensis*, and *G. volkensii*. The dendrogram constructed from RAPD banding patterns shows a monophyletic group for each species. Genetic similarity (S) values range from 0.916 to 0.997 for intraspecific level and from 0.651 to 0.816 for interspecific level. The S values are in accordance with similar phenotypic characteristics and a common ancestry of the species, genera, and major groups of chemicals contained within them, such as terpenoids and flavonoids. The standard regions in the genus that could be efficiently used as barcode markers are the *trn*H-*psb*A spacer (and combinations of itself), the *rbc*L, and the *mat*K genes. Genetic distance (D) values of *trn*H-*psb*A spacer sequences are 0.005 to 0.481 in intraspecific level, and 0.014 to 0.572 for the interspecific level. The combined sequences give the intraspecific and interspecific D values of 0.004 to 0.102 and 0.008 to 0.178.
Keyword: DNA barcoding, Genetic relationships, *Gardenia* species.

INTRODUCTION

Gardenia is a large genus, ca. 250 species, centered in the old world tropics, in the family Rubiaceae [1,2]. Thirteen species remains were recorded to be native to Thailand [3] when two species, *G. hygrophila* (a synonym of *Kailarsenia hygrophila*) and *G. sessilitlora* (a synonym of *Ceriscodes sessilitlora*), were excluded. They grow in diverse regions such as low-land evergreen forests, montane, and deciduous forests with other various types of vegetation. Because of their large, showy and fragrant flowers, *Gardenia* species are often cultivated for ornamentation. Therefore, Thais have used *Gardenia* flowers in various ways, such as in worshiping Buddha, making garlands for worship, pinning them to women's bun, and using them for treating diseases in Thai traditional medicines. Nowadays, there is one additional usage; the *Gardenia* flowers are popularly accepted in Thai spas because of their intensely sweet fragrance and their beautiful sort of flowers. Many species have been reported for their medicinal properties including for their anti-cancer property [4,5], anti-HIV property [5], and inhibitory activities against *Plasmodium falciparum* [6], *Staphylococus aureus*, *S. epidermidis*, *Escherichia coli* and *Candida albicans* [7]. The anti-cancer and anti-HIV properties are derived from a dihydroxy-pentamethoxy flavone which has been found in *G. obtusifolia* [5]. In addition, four new flavones isolated from *G. carinata* have shown DNA topoisomerase IIα inhibitory and anti-HIV-1 activities [8].

Many *Gardenia* species native to Thailand have been reported to possess chemicals with properties that have been found to be effective in treating a variety of human illnesses. The chemical constituents of three species including one native, *G. elata*, and two introduced, *G. gjellerupii* and *G. volkensii* were examined by using hexane crude leaf extracts that had been subjected to gas chromatography-mass spectrometry (GC-MS). *Gardenia elata* contained monoterpenes α-pinene, terpenenes cyclofenchene, γ-terpenene, β-pinene, and β-myrcene. *Gardenia gjellerupii* was characterized by diterpenes, including neophytadiene, terpenenes palmitic acid, and squalene. Other organic chemical compounds included linolenyl alcohol and (4E,8E,12E,16E)-4,8,13,17,21-pentamethyldocosa-4,8,12,16,20-pentaenal. *Gardenia volkensii* included diterpenes neophytadiene, terpenenes, palmitic acid, methyl (7E,10E,13E)-hexadeca-7,10,13-trienoate, and squalene. Additional chemical constituents were linolenyl alcohol, and (4E,8E,12E,16E)-4,8,13,17,21-pentamethyldocosa-4,8,12,16,20-pentaenal [9].

The properties of *Gardenia* can be assumed depending upon the kinds of compounds they contain. Genetic relations should be studied to support the prediction of species, chemicals, and activities, as well as to discover other related information [10]. In studies performed on genomes, DNA fingerprints based upon inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) methods have been used to effectively determine genetic relationships as revealed by many researches [11-14]. The banding patterns reveal genetic variation and relationships as depicted by a constructed cladogram. The concept critical to cladistics is homology which can be defined as a similarity/distance resulting from common ancestry. These markers for genetic variation are generally independent of environmental factors and are more numerous than phenotypic characters. As a result, they provide a clearer indication of the underlying variations in the genome, as well as depict evolutionary relatedness [15] thus solving the suspected problems.

Currently, creating barcodes for plants is very interesting especially for creating molecular markers of a species in vegetables, foods, and medicines that have been created after plant identification. It can be a species-specific marker and is essential for further identifying plant parts, such as with leaves, short trunks, pods, and chopped or powdered plants. This is especially true in traditional medicines, processed foods, fossilized remains, or herbarium specimens which possess highly degraded DNA or a short reliable region of DNA [16]. According to previous reports, the effective regions for barcoding plants are ITS2, *mat*K, *psb*A-*trn*H spacer regions, *rbc*L, *rpo*B, *rpo*C1, *trn*L-*trn*F [17-22]. Accordingly, based upon our earlier studies [23-28], the most interesting regions that are believed to be effective barcodes for the *Gardenia* species are *rbc*L, *mat*K, and *psb*A-*trn*H spacer regions which have been applied in the present research.

Because all *Gardenia* species have been widely used throughout the generations in Thailand, these flowers have become related to human beings. As a result, they have been cultivated in traditional varieties and cultivars which have led to genetic variations within the species and within the genus. Therefore, the genetic relations of the wild-native species, cultivars and commercial types should be evaluated by using DNA fingerprinting and barcoding by using *rbc*L, *mat*K, and *psb*A-*trn*H spacer regions.

MATERIALS AND METHODS

All species belonging to the genus *Gardenia* were explored and collected in many provinces throughout Thailand during 2011-2012. All specimens were identified followed Chalermglin [29], Low et al. [30], Steyn [31], Zhi et al. [2] and Wong et al. [32]. Voucher specimens were kept at Department of Biology, Faculty of Science, Khon Kaen University. Young leaves were collected and at -20°C for DNA extraction. All collected samples were undergone DNA extraction, DNA fingerprinting and DNA barcoding, respectively. *Ixora finlaysoniana* and *I. chinensis* were included as outgroup for phylogenetic analysis.

Genomic DNA was extracted from all collected samples using the Plant Genomic DNA Extraction Kit (RBC Bioscience). Extracted DNA was examined by subjecting it to 0.8% agarose gel electrophoresis in Tris-acetate-EDTA (TAE) buffer (40 mM Tris acetate, 1 mM EDTA, pH 8.0) stained with ethidium bromide.

Amplifications were carried out on all samples studied using the polymerase chain reaction (PCR). The samples were each examined in 25 μl reactions consisting of GoTaq Green Master Mix (Promega), 0.5 μM primer, and a 20 ng DNA template. Fifty RAPD primers were screened. The 14 primers that successfully amplified clear bands are as follows: (5′-3′) CAGGCCCTTC, AGTCAGCCAC, AATCGGGCTG, GAAACGGGTG, GGGTAACGCC, CAGCACCCAC, TCTGTGCTGG, AGGTGACCGT, GTTGCGATCC, GTTTCGCTCC, TGCTCTGCCC, GGTGACGCAG, GTCCACACGG, and CTGCTGGGAC. The reaction mixture was predenatured at 94°C for 3 min and the amplification was performed with the following 35 thermal cycles of denaturation for 30 sec at 94°C, annealing for 2 min at 40°C, extension for 2 min at 72°C, and then final extension for 7 min at 72°C using a Swift Maxi Thermal Cycler (Esco Micro Pte., Ltd.). Amplification products were detected by 1.2% agarose gel electrophoresis in a TAE buffer and visualized using ethidium bromide staining. The resulting RAPD bands were documented as diallelic characters (present = 1, absent = 0). These resultant data were used to construct a dendrogram using the NTSYS pc 2.1 software [33]. Genetics relations were compared using the results from the constructed dendrogram.

DNA barcoding construction was performed in all studied samples. PCR was performed using the following primer pairs: (5′-3′) ATGTCACCACAAACAGAGACTA AAGC and GTAAAATCAAGTCCACCRCG of the *rbc*L gene region; ATCCATCTGGAAATCTTAGTTC and GTT CTAGCACAAGAAAGTCG of the *mat*K gene region; GTTATGCATGAACGTAATGCTC and CGCGCATGGT GGATTCACAATCC of the *trn*H-*psb*A spacer region (http://www.kew.org/barcoding/update.html; 28 January 2009). The reaction mixture was done in 30 μl consisting of GoTaq Green Master Mix (Promega), 0.25 μM of each primer, and a 10 ng DNA template. The reaction mixture was incubated at 94°C for 1 min and the amplification was performed with the following 35 thermal cycles: denaturation for 30 s at 94°C, annealing for 40 s at 53°C, extension for 40 s at 72°C, and then a final extension for 5 min at 72°C. The amplified products were detected by 1.2% agarose gel electrophoresis in a TAE buffer and visualized with ethidium bromide staining. The amplified specific fragments of the studied samples were sequenced. The sequences of each region and the combination regions were aligned) using MEGA6 [34] indicating genetic distances (nucleotide variations) for a species specific marker for further plant identification. The resulted sequences were submitted to the GenBank.

RESULTS AND DISCUSSION

The native species were found in the forests and the ornamental cultivars were found in flower markets. The collected specimens consisted of eight native Thai species namely *G. carinata*, *G. collinsae*, *G. elata*, *G. jasminoides*, *G. obtusifolia*, *G. saxatilis*, *G. sootepensis*, and *G. thailandica* and three introduced species including *G. gjellerupii*, *G. taitensis*, and *G. volkensii*.

For RAPD fingerprinting from the 14 successful primers produced a total of 2,035 bands, as samples shown in Figure 1, which show as many as 468 characters. The constructed dendrogram (Figure 2) shows a monophyletic group for each ingroup *Gardenia* species and the outgroup, *I. finlaysoniana* and *I. chinensis* which were separated out. Also, there are genetic similarity (S) levels, an output taken concurrently with the dendrogram as shown in the Table 1. The S level of intraspecific species range from 0.916 in *G. sootepensis* to 0.997 in *G. jasminoides*, and that of the interspecific species range from 0.651 between *G. jasminoides* 1 and *G. obtusifolia* 1 to 0.816 between *G. thailandica* 2 and *G. sootepensis* 2. The S-values of intergeneric species between *I. finlaysoniana* and *G. carinata* are 0.630 to 0.738 which are lower in order than the interspecific and intraspecific species.

The successfully amplified fragments of *rbc*L, *mat*K, and *psb*A-*trn*H spacer regions size range approximately 600, 900, and 300-400 bp, respectively (Figure 3). The genetic distances from the sequence variations are shown with a region and with combinations of regions in the Tables 2-8. The genetic distance (D) values evaluated from the *rbc*L sequences (Table 2) of intraspecific and interspecific species are 0.000-0.056 and 0.000-0.061, respectively. The D values evaluated from the *mat*K sequences (Table 3) of intraspecific and interspecific

species are 0.000-0.054 and 0.000-0.186, respectively. The D values evaluated from the *trn*H-*psb*A spacer sequences (Table 4) of intraspecific and interspecific species are 0.005-0.481 and 0.014-0.572, respectively. The D values evaluated from combination sequences of the *rbc*L and the *mat*K genes (Table 5) of intraspecific and interspecific species range from 0.000-0.089 and 0.000-0.088, respectively. The D values evaluated from combination sequences of the *rbc*L gene and the *trn*H-*psb*A spacer (Table 6) of intraspecific and interspecific species range from 0.000-0.045 and 0.000-0.056, respectively. The D values evaluated from combination sequences of the *mat*K gene and the *trn*H-*psb*A spacer (Table 7) of intraspecific and interspecific species range from 0.000-0.142 and 0.000-0.202, respectively. The D values evaluated from combination sequences of all three regions (Table 8) of intraspecific and interspecific species are 0.004-0.102 and 0.008-0.178, respectively. The D values evaluated from sequences of each region and each combination were summarized in Table 9. The resulted sequences deposited in GenBank as according accession numbers shown in Table 10.

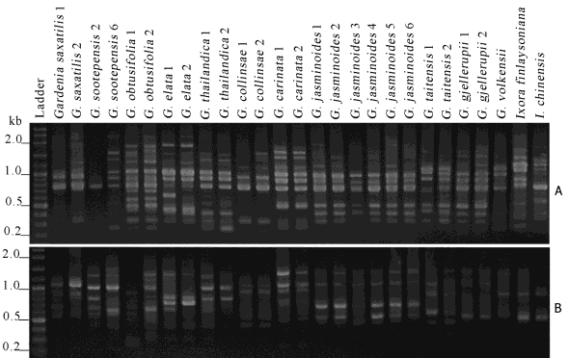

Figure 1. The examples of RAPD fingerprint by primers CAGGCCCTTC (A) and AGTCAGCCAC (B).

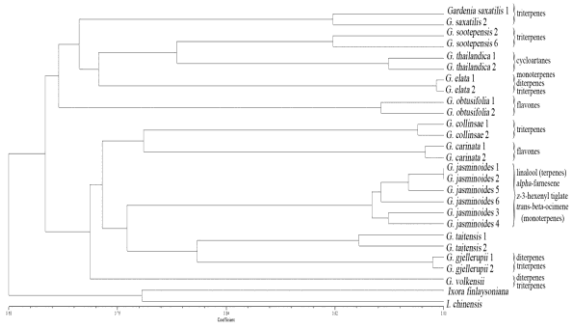

Figure 2. Dendrogram constructed from RAPD bands of the 11 species

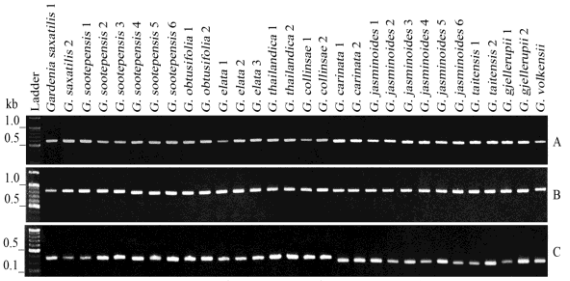

Figure 3. DNA barcode fragments from *rbc*L (A), *mat*K (B) and *trn*H-*psb*A spacer regions (C) of the *Gardenia* species

During the period of one year exploration, 11 species were collected. Whereas some species, namely *G. griffithii*, *G. magnifica*, *G. philasteri*, *G. coronaria*, and *G. tubifera*, which were listed by Smitinand [3] were not discovered. Alternatively, these species may not exist in Thailand or it may have gradually disappeared for two reasons: 1) the species became unpopular as a cultivated plant and/or 2) the species was affected by climate change.

Some samples, which were commonly seen and gathered, are really wild species, but they are becoming mainly economic species used for cultivation and ornamentation. These are especially included within the species collected. The easiest species to find and collect is *G. jasminoides* which has been used extensively for ornamentation because of its aromatic properties in Thai spas, and its use in other Thai customs. In the introduced species ingroup, the one individual, that is difficult to find and collect, is *G. volkensii*. Beside ornamental usages, many species have been used for medicinal properties in Thailand. These include *G. saxatilis*, *G. sootepensis*, *G. obtusifolia*, *G. thailandica*, *G. collinsae*, *G. carinata*, and *G. jasminoides*. Ten triterpenes isolated from the twigs of *G. saxatilis* has been used against the parasite *Plasmodium falciparum* [6]. *Gardenia sootepensis* containing terpene compounds from leaves showed cytotoxic activity against human cancer cell lines [4]. Dihydroxy-pentamexoxy flavone from leaves of *G. obtusifolia* showed activities that suppress proliferation and promotes apoptosis of tumor cells through modulation of multiple cell signaling pathways [5]. Leaves and twigs of *G. thailandica* contain anti-HIV cycloartanes [35]. Dammarane triterpenes from apical buds of *G. collinsae* have shown cytotoxic effects on five human tumor cell lines [37]. Flavones from leaves and twigs of *G. carinata* have shown activities of DNA topoisomerase IIα inhibitory and anti-HIV-1 [8]. The last recorded species, *G. jasminoides*, contains volatile constituents from flowers with anti-microbial properties that act upon *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, and *Candida albicans* [7]. Additionally, the authors studied chemicals in *G. elata*, *G. gjellerupii*, and *G. volkensii* by gas-chromatography mass spectrometry (GC-MS) and discovered that they contain monoterpenes, diterpenes, and triterpenes; diterpenes and triterpenes; and diterpenes and triterpenes, respectively [9]. The species and chemicals contained therein are shown in the dendrogram (Figure 2). The major chemical is and flavone which is found in only *G. obtusifolia* and *G. carinata*. Therefore, the pharmacological activities could be assumed. However, the most important information, that also has an effect on their activities, is the quantity of each of the chemicals. Additionally, the groups of each (the monoterpenes, diterpenes, triterpenes, and the flavones) are comprised of many more substances which may lead to more and different activities than the aforementioned treatments.

The S values (Table 1) of intergeneric, interspecific, and intraspecific species are higher in order starting from 0.630-0.738 in pairs of *I. finlaysoniana* and *G. carinata*, and *I. chinensis* and *G. saxatilis*; 0.651-0.816 in pairs of *G. jasminoides* and *G. obtusifolia*, and *G. thailandica* and *G. sootepensis*; and 0.916-0.997 in individuals of *G. sootepensis*, *G. jasminoides*, respectively. These ordered S-levels are in accordance with similar phenotypic characters in the categories of the genera, species, and individuals. This means that the genome variation is not as great as expected even though these plants are currently experiencing the effects of climate change and/or bleeding for cultivation by human.

Table 1. Genetic similarities of *Gardenia* samples derived from RAPD fingerprinting data calculated by NTSYSpc-2.10p

	G. saxatilis 1	*G. saxatilis* 2	*G. sootepensis* 2	*G. sootepensis* 6	*G. obtusifolia* 1	*G. obtusifolia* 2	*G. elata* 1	*G. elata* 2	*G. thailandica* 1	*G. thailandica* 2	*G. collinsae* 1	*G. collinsae* 2	*G. carinata* 1	*G. carinata* 2	*G. jasminoides* 1	*G. jasminoides* 2	*G. jasminoides* 3	*G. jasminoides* 4	*G. jasminoides* 5	*G. jasminoides* 6	*G. taitensis* 1	*G. taitensis* 2	*G. gjellerupii* 1	*G. gjellerupii* 2	*G. volkensii*	*I. finlaysoniana*	*I. chinensis*
Gardenia saxatilis 1	1.000																										
G. saxatilis 2	0.916	1.000																									
G. sootepensis 2	0.749	0.735	1.000																								
G. sootepensis 6	0.719	0.722	0.916	1.000																							
G. obtusifolia 1	0.686	0.695	0.743	0.751	1.000																						
G. obtusifolia 2	0.670	0.673	0.727	0.741	0.951	1.000																					
G. elata 1	0.727	0.714	0.741	0.711	0.732	0.716	1.000																				
G. elata 2	0.735	0.722	0.743	0.714	0.730	0.714	0.992	1.000																			
G. thailandica 1	0.727	0.719	0.800	0.797	0.711	0.695	0.746	0.754	1.000																		
G. thailandica 2	0.749	0.741	0.816	0.792	0.738	0.711	0.768	0.776	0.957	1.000																	
G. collinsae 1	0.741	0.754	0.716	0.697	0.670	0.665	0.705	0.714	0.738	0.754	1.000																
G. collinsae 2	0.730	0.754	0.705	0.692	0.670	0.665	0.695	0.703	0.727	0.738	0.978	1.000															
G. carinata 1	0.722	0.724	0.735	0.705	0.668	0.678	0.686	0.695	0.746	0.741	0.776	0.770	1.000														
G. carinata 2	0.722	0.735	0.741	0.700	0.668	0.668	0.697	0.705	0.741	0.751	0.786	0.776	0.984	1.000													
G. jasminoides 1	0.727	0.692	0.681	0.646	0.651	0.651	0.692	0.695	0.719	0.719	0.716	0.716	0.757	0.757	1.000												
G. jasminoides 2	0.724	0.695	0.684	0.649	0.654	0.654	0.695	0.697	0.722	0.722	0.719	0.719	0.759	0.759	0.997	1.000											
G. jasminoides 3	0.743	0.724	0.708	0.673	0.673	0.662	0.724	0.727	0.741	0.757	0.738	0.738	0.778	0.784	0.946	0.949	1.000										
G. jasminoides 4	0.727	0.697	0.697	0.662	0.662	0.662	0.703	0.705	0.730	0.735	0.711	0.711	0.762	0.762	0.957	0.959	0.957	1.000									
G. jasminoides 5	0.730	0.700	0.684	0.649	0.659	0.654	0.689	0.692	0.716	0.722	0.714	0.714	0.749	0.749	0.970	0.973	0.938	0.949	1.000								
G. jasminoides 6	0.732	0.703	0.703	0.657	0.673	0.657	0.686	0.689	0.724	0.730	0.722	0.716	0.751	0.751	0.941	0.943	0.930	0.930	0.970	1.000							
G. taitensis 1	0.735	0.705	0.695	0.654	0.681	0.676	0.657	0.665	0.727	0.749	0.719	0.708	0.738	0.743	0.732	0.730	0.765	0.738	0.751	0.759	1.000						
G. taitensis 2	0.757	0.732	0.711	0.676	0.692	0.681	0.689	0.697	0.743	0.770	0.746	0.735	0.759	0.765	0.743	0.741	0.765	0.749	0.762	0.776	0.935	1.000					
G. gjellerupii 1	0.724	0.727	0.722	0.681	0.692	0.692	0.689	0.697	0.743	0.770	0.757	0.746	0.797	0.803	0.759	0.762	0.786	0.781	0.778	0.792	0.805	0.832	1.000				
G. gjellerupii 2	0.724	0.727	0.722	0.686	0.697	0.697	0.695	0.703	0.738	0.765	0.751	0.746	0.797	0.803	0.765	0.768	0.792	0.786	0.784	0.792	0.800	0.827	0.989	1.000			
G. volkensii	0.732	0.714	0.730	0.684	0.711	0.695	0.697	0.700	0.719	0.741	0.716	0.705	0.741	0.757	0.724	0.727	0.741	0.714	0.722	0.741	0.743	0.776	0.759	0.759	1.000		
Ixora finlaysoniana	0.692	0.689	0.684	0.643	0.638	0.632	0.657	0.654	0.651	0.684	0.654	0.643	0.630	0.646	0.657	0.654	0.684	0.646	0.654	0.662	0.659	0.665	0.654	0.649	0.695	1.000	
I. chinensis	0.738	0.724	0.724	0.673	0.651	0.641	0.665	0.673	0.703	0.730	0.705	0.695	0.686	0.703	0.681	0.684	0.708	0.686	0.695	0.703	0.695	0.711	0.716	0.711	0.724	0.776	1.000

Table 2. Genetic distance values from *rbc*L gene of the genus *Gardenia* in Thailand

	G. saxatilis 1	*G. saxatilis* 2	*G. sootepensis* 1	*G. sootepensis* 2	*G. sootepensis* 3	*G. sootepensis* 4	*G. sootepensis* 5	*G. sootepensis* 6	*G. obtusifolia* 1	*G. obtusifolia* 2	*G. elata* 1	*G. elata* 2	*G. elata* 3	*G. thailandica* 1	*G. thailandica* 2	*G. collinsae* 1	*G. collinsae* 2	*G. carinata* 1	*G. carinata* 2	*G. jasminoides* 1	*G. jasminoides* 2	*G. jasminoides* 3	*G. jasminoides* 4	*G. jasminoides* 5	*G. jasminoides* 6	*G. taitensis* 1	*G. taitensis* 2	*G. gjellerupii* 1	*G. gjellerupii* 2	*G. volkensii*
*Gardenia saxatilis*1	0.000																													
G. saxatilis 2	0.030	0.000																												
G. sootepensis 1	0.020	0.040	0.000																											
G. sootepensis 2	0.025	0.035	0.015	0.000																										
G. sootepensis 3	0.056	0.051	0.040	0.035	0.000																									
G. sootepensis 4	0.045	0.051	0.035	0.025	0.030	0.000																								
G. sootepensis 5	0.051	0.035	0.040	0.030	0.040	0.030	0.000																							
G. sootepensis 6	0.030	0.051	0.030	0.025	0.056	0.045	0.051	0.000																						
G. obtusifolia 1	0.030	0.040	0.035	0.020	0.051	0.040	0.040	0.040	0.000																					
G. obtusifolia 2	0.020	0.030	0.030	0.015	0.045	0.035	0.040	0.030	0.010	0.000																				
G. elata 1	0.025	0.035	0.020	0.015	0.045	0.035	0.035	0.035	0.025	0.025	0.000																			
G. elata 2	0.020	0.030	0.020	0.005	0.035	0.025	0.030	0.020	0.020	0.010	0.015	0.000																		
G. elata 3	0.030	0.035	0.025	0.010	0.035	0.025	0.030	0.030	0.025	0.020	0.020	0.010	0.000																	
G. thailandica 1	0.025	0.035	0.015	0.000	0.035	0.025	0.030	0.025	0.020	0.015	0.015	0.005	0.010	0.000																
G. thailandica 2	0.030	0.040	0.030	0.015	0.045	0.035	0.040	0.030	0.030	0.020	0.025	0.010	0.020	0.015	0.000															
G. collinsae 1	0.020	0.030	0.025	0.010	0.035	0.025	0.035	0.030	0.015	0.010	0.020	0.010	0.010	0.010	0.020	0.000														
G. collinsae 2	0.035	0.020	0.035	0.020	0.051	0.045	0.030	0.045	0.030	0.025	0.035	0.025	0.025	0.020	0.035	0.020	0.000													
G. carinata 1	0.025	0.025	0.030	0.015	0.035	0.030	0.035	0.035	0.015	0.015	0.020	0.015	0.020	0.015	0.025	0.010	0.025	0.000												
G. carinata 2	0.020	0.030	0.025	0.010	0.040	0.030	0.030	0.030	0.010	0.010	0.015	0.010	0.015	0.010	0.020	0.005	0.020	0.005	0.000											
G. jasminoides 1	0.020	0.030	0.020	0.005	0.035	0.025	0.030	0.020	0.020	0.010	0.015	0.000	0.010	0.005	0.010	0.010	0.025	0.015	0.010	0.000										
G. jasminoides 2	0.025	0.035	0.020	0.005	0.030	0.020	0.030	0.025	0.020	0.015	0.015	0.005	0.005	0.005	0.015	0.005	0.025	0.015	0.010	0.005	0.000									
G. jasminoides 3	0.040	0.051	0.035	0.020	0.045	0.035	0.045	0.040	0.035	0.030	0.030	0.020	0.020	0.020	0.030	0.020	0.040	0.030	0.025	0.020	0.015	0.000								
G. jasminoides 4	0.025	0.035	0.020	0.005	0.035	0.025	0.025	0.025	0.015	0.015	0.010	0.005	0.010	0.005	0.015	0.010	0.025	0.010	0.005	0.005	0.005	0.020	0.000							
G. jasminoides 5	0.025	0.035	0.020	0.005	0.030	0.020	0.030	0.025	0.020	0.015	0.015	0.005	0.005	0.005	0.015	0.005	0.025	0.015	0.010	0.005	0.000	0.015	0.005	0.000						
G. jasminoides 6	0.020	0.030	0.020	0.005	0.035	0.025	0.030	0.020	0.020	0.010	0.015	0.000	0.010	0.005	0.010	0.010	0.025	0.015	0.010	0.000	0.005	0.020	0.005	0.005	0.000					
G. taitensis 1	0.025	0.045	0.035	0.020	0.056	0.045	0.051	0.045	0.030	0.025	0.035	0.025	0.030	0.020	0.035	0.020	0.030	0.025	0.020	0.025	0.025	0.040	0.025	0.025	0.025	0.000				
G. taitensis 2	0.020	0.025	0.030	0.015	0.040	0.025	0.040	0.030	0.020	0.010	0.025	0.010	0.020	0.015	0.020	0.010	0.025	0.010	0.010	0.010	0.015	0.030	0.015	0.015	0.010	0.025	0.000			
G. gjellerupii 1	0.045	0.045	0.035	0.020	0.045	0.040	0.051	0.045	0.040	0.035	0.035	0.025	0.030	0.020	0.015	0.030	0.040	0.025	0.030	0.025	0.025	0.040	0.025	0.025	0.025	0.040	0.030	0.000		
G. gjellerupii 2	0.025	0.035	0.020	0.005	0.035	0.025	0.025	0.025	0.015	0.015	0.010	0.005	0.010	0.005	0.015	0.010	0.025	0.010	0.005	0.005	0.005	0.020	0.000	0.005	0.005	0.025	0.015	0.025	0.000	
G. volkensii	0.040	0.051	0.040	0.025	0.061	0.051	0.056	0.040	0.025	0.020	0.040	0.030	0.035	0.025	0.040	0.025	0.035	0.030	0.025	0.030	0.030	0.045	0.030	0.030	0.030	0.035	0.030	0.045	0.030	0.000

Table 3. Genetic distance values from the *mat*K region of the genus *Gardenia* in Thailand

	G. saxatilis 1	*G. saxatilis* 2	*G. sootepensis*1	*G. sootepensis* 2	*G. sootepensis*3	*G. sootepensis*4	*G. sootepensis*5	*G. sootepensis*6	*G. obtusifolia* 1	*G. obtusifolia* 2	*G. elata* 1	*G. elata*2	*G. elata*3	*G. thailandica*1	*G. thailandica*2	*G. collinsae* 1	*G. collinsae*2	*G. carinata* 1	*G. carinata* 2	*G. jasminoides*1	*G. jasminoides*2	*G. jasminoides*3	*G. jasminoides*4	*G. jasminoides*5	*G. jasminoides* 6	*G. taitensis*1	*G. taitensis* 2	*G. gjellerupii*1	*G. gjellerupii*2	*G. volkensii*
*Gardenia saxatilis*1	0.000																													
*G. saxatilis*2	0.024	0.000																												
G. sootepensis 1	0.012	0.012	0.000																											
*G. sootepensis*2	0.012	0.012	0.000	0.000																										
*G. sootepensis*3	0.024	0.024	0.012	0.012	0.000																									
*G. sootepensis*4	0.012	0.012	0.000	0.000	0.012	0.000																								
*G. sootepensis*5	0.012	0.012	0.000	0.000	0.012	0.000	0.000																							
*G. sootepensis*6	0.018	0.018	0.006	0.006	0.018	0.006	0.006	0.000																						
G. obtusifolia 1	0.018	0.018	0.006	0.006	0.018	0.006	0.006	0.012	0.000																					
G. obtusifolia 2	0.060	0.060	0.048	0.048	0.060	0.048	0.048	0.054	0.054	0.000																				
*G. elata*1	0.018	0.018	0.006	0.006	0.012	0.006	0.006	0.012	0.012	0.054	0.000																			
*G. elata*2	0.156	0.156	0.144	0.144	0.156	0.144	0.144	0.150	0.150	0.186	0.150	0.000																		
*G. elata*3	0.018	0.018	0.006	0.006	0.012	0.006	0.006	0.012	0.012	0.054	0.000	0.150	0.000																	
*G. thailandica*1	0.012	0.012	0.000	0.000	0.012	0.000	0.000	0.006	0.006	0.048	0.006	0.144	0.006	0.000																
*G. thailandica*2	0.012	0.012	0.000	0.000	0.012	0.000	0.000	0.006	0.006	0.048	0.006	0.144	0.006	0.000	0.000															
*G. collinsae*1	0.018	0.018	0.006	0.006	0.018	0.006	0.006	0.012	0.012	0.054	0.012	0.150	0.012	0.006	0.006	0.000														
*G. collinsae*2	0.018	0.018	0.006	0.006	0.018	0.006	0.006	0.012	0.012	0.054	0.012	0.150	0.012	0.006	0.006	0.000	0.000													
G. carinata 1	0.018	0.018	0.006	0.006	0.018	0.006	0.006	0.012	0.000	0.054	0.012	0.150	0.012	0.006	0.006	0.012	0.012	0.000												
G. carinata 2	0.156	0.156	0.144	0.144	0.156	0.144	0.144	0.150	0.150	0.186	0.150	0.000	0.150	0.144	0.144	0.150	0.150	0.150	0.000											
*G. jasminoides*1	0.024	0.024	0.012	0.012	0.006	0.012	0.012	0.018	0.018	0.060	0.006	0.156	0.006	0.012	0.012	0.018	0.018	0.018	0.156	0.000										
*G. jasminoides*2	0.024	0.024	0.012	0.012	0.012	0.012	0.012	0.018	0.018	0.060	0.006	0.156	0.006	0.012	0.012	0.018	0.018	0.018	0.156	0.006	0.000									
*G. jasminoides*3	0.030	0.030	0.018	0.018	0.018	0.018	0.018	0.024	0.024	0.066	0.012	0.162	0.012	0.018	0.018	0.024	0.024	0.024	0.162	0.012	0.006	0.000								
*G. jasminoides*4	0.012	0.012	0.000	0.000	0.012	0.000	0.000	0.006	0.006	0.048	0.006	0.144	0.006	0.000	0.000	0.006	0.006	0.006	0.144	0.012	0.012	0.018	0.000							
*G. jasminoides*5	0.018	0.018	0.006	0.006	0.012	0.006	0.006	0.012	0.012	0.054	0.000	0.150	0.000	0.006	0.006	0.012	0.012	0.012	0.150	0.006	0.006	0.012	0.006	0.000						
*G. jasminoides*6	0.018	0.018	0.006	0.006	0.012	0.006	0.006	0.012	0.012	0.054	0.000	0.150	0.000	0.006	0.006	0.012	0.012	0.012	0.150	0.006	0.006	0.012	0.006	0.000	0.000					
*G. taitensis*1	0.024	0.024	0.012	0.012	0.012	0.012	0.012	0.018	0.018	0.060	0.006	0.156	0.006	0.012	0.012	0.018	0.018	0.018	0.156	0.006	0.000	0.006	0.012	0.006	0.006	0.000				
*G. taitensis*2	0.012	0.012	0.000	0.000	0.012	0.000	0.000	0.006	0.006	0.048	0.006	0.144	0.006	0.000	0.000	0.006	0.006	0.006	0.144	0.012	0.012	0.018	0.000	0.006	0.006	0.012	0.000			
*G. gjellerupii*1	0.018	0.018	0.006	0.006	0.012	0.006	0.006	0.012	0.012	0.054	0.000	0.150	0.000	0.006	0.006	0.012	0.012	0.012	0.150	0.006	0.006	0.012	0.006	0.000	0.000	0.006	0.006	0.000		
*G. gjellerupii*2	0.012	0.012	0.000	0.000	0.012	0.000	0.000	0.006	0.006	0.048	0.006	0.144	0.006	0.000	0.000	0.006	0.006	0.006	0.144	0.012	0.012	0.018	0.000	0.006	0.006	0.012	0.000	0.006	0.000	
G. volkensii	0.012	0.012	0.000	0.000	0.012	0.000	0.000	0.006	0.006	0.048	0.006	0.144	0.006	0.000	0.000	0.006	0.006	0.006	0.144	0.012	0.012	0.018	0.000	0.006	0.006	0.012	0.000	0.006	0.000	0.000

Table 4. Genetic distance values from *trn*H-*psb*A spacer of the genus *Gardenia* in Thailand

	G. saxatilis 1	*G. saxatilis* 2	*G. sootepensis* 1	*G. sootepensis* 2	*G. sootepensis* 3	*G. sootepensis* 4	*G. sootepensis* 5	*G. sootepensis* 6	*G. obtusifolia* 1	*G. obtusifolia* 2	*G. elata* 1	*G. elata* 2	*G. elata* 3	*G. thailandica* 1	*G. thailandica* 2	*G. collinsae* 1	*G. collinsae* 2	*G. carinata* 1	*G. carinata* 2	*G. jasminoides* 1	*G. jasminoides* 2	*G. jasminoides* 3	*G. jasminoides* 4	*G. jasminoides* 5	*G. jasminoides* 6	*G. taitensis* 1	*G. taitensis* 2	*G. gjellerupii* 1	*G. gjellerupii* 2	*G. volkensii*
*Gardenia saxatilis*1	0.000																													
G. saxatilis 2	0.063	0.000																												
G. sootepensis 1	0.029	0.077	0.000																											
G. sootepensis 2	0.043	0.087	0.034	0.000																										
G. sootepensis 3	0.067	0.034	0.058	0.067	0.000																									
G. sootepensis 4	0.063	0.087	0.053	0.058	0.063	0.000																								
G. sootepensis 5	0.101	0.063	0.091	0.082	0.058	0.091	0.000																							
G. sootepensis 6	0.082	0.106	0.072	0.082	0.096	0.091	0.120	0.000																						
G. obtusifolia 1	0.149	0.183	0.144	0.125	0.168	0.159	0.163	0.178	0.000																					
G. obtusifolia 2	0.313	0.303	0.298	0.288	0.298	0.313	0.298	0.313	0.240	0.000																				
G. elata 1	0.135	0.139	0.120	0.125	0.115	0.144	0.149	0.139	0.226	0.337	0.000																			
G. elata 2	0.072	0.029	0.067	0.072	0.029	0.082	0.063	0.101	0.178	0.293	0.120	0.000																		
G. elata 3	0.034	0.077	0.029	0.024	0.067	0.063	0.101	0.082	0.135	0.298	0.115	0.058	0.000																	
G. thailandica 1	0.024	0.072	0.014	0.019	0.053	0.048	0.087	0.067	0.130	0.298	0.111	0.063	0.014	0.000																
G. thailandica 2	0.082	0.111	0.072	0.072	0.091	0.091	0.125	0.014	0.168	0.313	0.135	0.106	0.072	0.058	0.000															
G. collinsae 1	0.010	0.063	0.029	0.034	0.067	0.063	0.101	0.082	0.139	0.303	0.125	0.072	0.024	0.014	0.072	0.000														
G. collinsae 2	0.072	0.029	0.082	0.096	0.043	0.082	0.077	0.101	0.183	0.308	0.144	0.038	0.082	0.077	0.106	0.072	0.000													
G. carinata 1	0.139	0.168	0.130	0.120	0.154	0.149	0.154	0.154	0.192	0.337	0.212	0.168	0.139	0.125	0.159	0.139	0.183	0.000												
G. carinata 2	0.130	0.135	0.111	0.125	0.120	0.135	0.144	0.159	0.236	0.250	0.154	0.120	0.120	0.115	0.149	0.125	0.144	0.188	0.000											
G. jasminoides 1	0.034	0.072	0.034	0.043	0.053	0.067	0.091	0.082	0.154	0.308	0.120	0.063	0.038	0.029	0.082	0.034	0.082	0.135	0.120	0.000										
G. jasminoides 2	0.019	0.063	0.019	0.034	0.048	0.053	0.082	0.072	0.144	0.303	0.115	0.053	0.024	0.014	0.072	0.019	0.067	0.130	0.111	0.014	0.000									
G. jasminoides 3	0.024	0.063	0.024	0.029	0.048	0.058	0.082	0.072	0.149	0.298	0.115	0.048	0.029	0.019	0.072	0.024	0.072	0.130	0.106	0.014	0.005	0.000								
G. jasminoides 4	0.163	0.149	0.154	0.168	0.144	0.159	0.168	0.192	0.279	0.288	0.207	0.130	0.168	0.159	0.197	0.163	0.159	0.255	0.106	0.149	0.144	0.139	0.000							
G. jasminoides 5	0.197	0.183	0.178	0.192	0.178	0.183	0.212	0.212	0.293	0.293	0.221	0.163	0.188	0.183	0.212	0.192	0.183	0.279	0.130	0.188	0.178	0.173	0.101	0.000						
G. jasminoides 6	0.197	0.183	0.173	0.188	0.168	0.168	0.202	0.216	0.303	0.303	0.216	0.163	0.192	0.178	0.216	0.192	0.183	0.279	0.115	0.188	0.178	0.173	0.082	0.053	0.000					
G. taitensis 1	0.043	0.091	0.034	0.019	0.072	0.058	0.072	0.087	0.120	0.298	0.139	0.082	0.034	0.029	0.087	0.043	0.096	0.120	0.139	0.048	0.034	0.038	0.178	0.207	0.202	0.000				
G. taitensis 2	0.130	0.096	0.120	0.106	0.077	0.096	0.067	0.149	0.192	0.308	0.173	0.077	0.130	0.115	0.154	0.130	0.091	0.168	0.154	0.120	0.111	0.106	0.178	0.197	0.183	0.096	0.000			
G. gjellerupii 1	0.529	0.519	0.505	0.510	0.505	0.510	0.500	0.538	0.529	0.563	0.538	0.510	0.519	0.510	0.534	0.524	0.529	0.529	0.476	0.505	0.510	0.510	0.490	0.495	0.490	0.514	0.529	0.000		
G. gjellerupii 2	0.178	0.178	0.163	0.173	0.173	0.173	0.197	0.207	0.288	0.313	0.221	0.159	0.168	0.163	0.207	0.173	0.178	0.255	0.111	0.168	0.159	0.154	0.111	0.063	0.072	0.188	0.173	0.481	0.000	
G. volkensii	0.548	0.543	0.524	0.529	0.529	0.543	0.519	0.563	0.519	0.591	0.572	0.543	0.538	0.529	0.558	0.543	0.553	0.558	0.538	0.529	0.534	0.534	0.553	0.567	0.567	0.519	0.548	0.144	0.553	0.000

Table 5. Genetic distance values combined from *rbc*L and *mat*K regions of the genus *Gardenia* species in Thailand

	G. saxatilis 1	*G. saxatilis* 2	*G. sootepensis*1	*G. sootepensis* 2	*G. sootepensis*3	*G. sootepensis*4	*G. sootepensis*5	*G. sootepensis*6	*G. obtusifolia* 1	*G. obtusifolia* 2	*G. elata* 1	*G. elata*2	*G. elata*3	*G. thailandica*1	*G. thailandica*2	*G. collinsae* 1	*G. collinsae*2	*G. carinata* 1	*G. carinata* 2	*G. jasminoides*1	*G. jasminoides*2	*G. jasminoides*3	*G. jasminoides*4	*G. jasminoides*5	*G. jasminoides*6	*G. taitensis*1	*G. taitensis* 2	*G. gjellerupii*1	*G. gjellerupii*2	*G. volkensii*
*Gardenia saxatilis*1	0.000																													
*G. saxatilis*2	0.028	0.000																												
G. sootepensis 1	0.014	0.033	0.000																											
*G. sootepensis*2	0.019	0.028	0.014	0.000																										
*G. sootepensis*3	0.047	0.042	0.033	0.028	0.000																									
*G. sootepensis*4	0.037	0.042	0.028	0.019	0.028	0.000																								
*G. sootepensis*5	0.042	0.028	0.033	0.023	0.033	0.023	0.000																							
*G. sootepensis*6	0.074	0.084	0.070	0.056	0.084	0.074	0.079	0.000																						
G. obtusifolia 1	0.023	0.033	0.028	0.014	0.042	0.033	0.037	0.070	0.000																					
G. obtusifolia 2	0.019	0.028	0.023	0.009	0.037	0.028	0.033	0.065	0.005	0.000																				
*G. elata*1	0.060	0.070	0.056	0.051	0.079	0.070	0.074	0.079	0.065	0.060	0.000																			
*G. elata*2	0.019	0.028	0.014	0.000	0.028	0.019	0.023	0.056	0.014	0.009	0.051	0.000																		
*G. elata*3	0.023	0.028	0.019	0.005	0.033	0.023	0.023	0.060	0.019	0.014	0.056	0.005	0.000																	
*G. thailandica*1	0.019	0.028	0.014	0.000	0.028	0.019	0.023	0.056	0.014	0.009	0.051	0.000	0.005	0.000																
*G. thailandica*2	0.028	0.037	0.023	0.009	0.037	0.028	0.033	0.065	0.023	0.019	0.060	0.009	0.014	0.009	0.000															
*G. collinsae*1	0.014	0.023	0.019	0.005	0.033	0.023	0.028	0.060	0.009	0.005	0.056	0.005	0.009	0.005	0.014	0.000														
*G. collinsae*2	0.028	0.014	0.033	0.019	0.042	0.037	0.023	0.074	0.023	0.019	0.070	0.019	0.019	0.019	0.028	0.014	0.000													
G. carinata 1	0.019	0.019	0.023	0.009	0.028	0.023	0.033	0.065	0.014	0.009	0.060	0.009	0.014	0.009	0.019	0.005	0.019	0.000												
G. carinata 2	0.014	0.023	0.019	0.005	0.033	0.023	0.028	0.060	0.009	0.005	0.056	0.005	0.009	0.005	0.014	0.000	0.014	0.005	0.000											
*G. jasminoides*1	0.019	0.028	0.014	0.000	0.028	0.019	0.023	0.056	0.014	0.009	0.051	0.000	0.005	0.000	0.009	0.005	0.019	0.009	0.005	0.000										
*G. jasminoides*2	0.019	0.028	0.014	0.000	0.028	0.019	0.023	0.056	0.014	0.009	0.051	0.000	0.005	0.000	0.009	0.005	0.019	0.009	0.005	0.000	0.000									
*G. jasminoides*3	0.065	0.074	0.060	0.047	0.074	0.065	0.070	0.088	0.060	0.056	0.070	0.047	0.051	0.047	0.056	0.051	0.065	0.056	0.051	0.047	0.047	0.000								
*G. jasminoides*4	0.019	0.028	0.014	0.000	0.028	0.019	0.023	0.056	0.014	0.009	0.051	0.000	0.005	0.000	0.009	0.005	0.019	0.009	0.005	0.000	0.000	0.047	0.000							
*G. jasminoides*5	0.019	0.028	0.014	0.000	0.028	0.019	0.023	0.056	0.014	0.009	0.051	0.000	0.005	0.000	0.009	0.005	0.019	0.009	0.005	0.000	0.000	0.047	0.000	0.000						
*G. jasminoides*6	0.019	0.028	0.014	0.000	0.028	0.019	0.023	0.056	0.014	0.009	0.051	0.000	0.005	0.000	0.009	0.005	0.019	0.009	0.005	0.000	0.000	0.047	0.000	0.000	0.000					
*G. taitensis*1	0.019	0.037	0.033	0.019	0.047	0.037	0.042	0.074	0.023	0.019	0.070	0.019	0.023	0.019	0.028	0.014	0.028	0.019	0.014	0.019	0.019	0.065	0.019	0.019	0.019	0.000				
*G. taitensis*2	0.019	0.023	0.023	0.009	0.033	0.019	0.033	0.065	0.014	0.009	0.060	0.009	0.014	0.009	0.019	0.005	0.019	0.005	0.005	0.009	0.009	0.056	0.009	0.009	0.009	0.019	0.000			
*G. gjellerupii*1	0.037	0.037	0.033	0.019	0.037	0.033	0.042	0.074	0.033	0.028	0.070	0.019	0.023	0.019	0.009	0.023	0.037	0.019	0.023	0.019	0.019	0.065	0.019	0.019	0.019	0.037	0.023	0.000		
*G. gjellerupii*2	0.019	0.028	0.014	0.000	0.028	0.019	0.023	0.056	0.014	0.009	0.051	0.000	0.005	0.000	0.009	0.005	0.019	0.009	0.005	0.000	0.000	0.047	0.000	0.000	0.000	0.019	0.009	0.019	0.000	
G. volkensii	0.033	0.042	0.037	0.023	0.051	0.042	0.047	0.070	0.019	0.014	0.074	0.023	0.028	0.023	0.033	0.019	0.033	0.023	0.019	0.023	0.023	0.070	0.023	0.023	0.023	0.033	0.023	0.042	0.023	0.000

Table 6. Genetic distance values combined from *rbc*L and *trn*H-*psb*A spacer regions of the genus *Gardenia* species in Thailand

	G. saxatilis 1	*G. saxatilis* 2	*G. sootepensis*1	*G. sootepensis* 2	*G. sootepensis*3	*G. sootepensis*4	*G. sootepensis*5	*G. sootepensis*6	*G. obtusifolia* 1	*G. obtusifolia* 2	*G. elata* 1	*G. elata*2	*G. elata*3	*G. thailandica*1	*G. thailandica*2	*G. collinsae* 1	*G. collinsae*2	*G. carinata* 1	*G. carinata* 2	*G. jasminoides*1	*G. jasminoides*2	*G. jasminoides*3	*G. jasminoides*4	*G. jasminoides*5	*G. jasminoides*6	*G. taitensis*1	*G. taitensis* 2	*G. gjellerupii*1	*G. gjellerupii*2	*G. volkensii*
*Gardenia saxatilis*1	0.000																													
*G. saxatilis*2	0.030	0.000																												
G. sootepensis 1	0.015	0.035	0.000																											
*G. sootepensis*2	0.020	0.030	0.015	0.000																										
*G. sootepensis*3	0.051	0.045	0.035	0.030	0.000																									
*G. sootepensis*4	0.040	0.045	0.030	0.020	0.030	0.000																								
*G. sootepensis*5	0.045	0.030	0.035	0.025	0.035	0.025	0.000																							
*G. sootepensis*6	0.030	0.051	0.025	0.020	0.051	0.040	0.045	0.000																						
G. obtusifolia 1	0.025	0.035	0.030	0.015	0.045	0.035	0.040	0.035	0.000																					
G. obtusifolia 2	0.020	0.030	0.025	0.010	0.040	0.030	0.035	0.030	0.005	0.000																				
*G. elata*1	0.020	0.030	0.015	0.010	0.040	0.030	0.035	0.030	0.025	0.020	0.000																			
*G. elata*2	0.020	0.030	0.015	0.000	0.030	0.020	0.025	0.020	0.015	0.010	0.010	0.000																		
*G. elata*3	0.025	0.030	0.020	0.005	0.035	0.025	0.025	0.025	0.020	0.015	0.015	0.005	0.000																	
*G. thailandica*1	0.020	0.030	0.015	0.000	0.030	0.020	0.025	0.020	0.015	0.010	0.010	0.000	0.005	0.000																
*G. thailandica*2	0.030	0.040	0.025	0.010	0.040	0.030	0.035	0.030	0.025	0.020	0.020	0.010	0.015	0.010	0.000															
*G. collinsae*1	0.015	0.025	0.020	0.005	0.035	0.025	0.030	0.025	0.010	0.005	0.015	0.005	0.010	0.005	0.015	0.000														
*G. collinsae*2	0.030	0.015	0.035	0.020	0.045	0.040	0.025	0.040	0.025	0.020	0.030	0.020	0.020	0.020	0.030	0.015	0.000													
G. carinata 1	0.020	0.020	0.025	0.010	0.030	0.025	0.035	0.030	0.015	0.010	0.020	0.010	0.015	0.010	0.020	0.005	0.020	0.000												
G. carinata 2	0.015	0.025	0.020	0.005	0.035	0.025	0.030	0.025	0.010	0.005	0.015	0.005	0.010	0.005	0.015	0.000	0.015	0.005	0.000											
*G. jasminoides*1	0.020	0.030	0.015	0.000	0.030	0.020	0.025	0.020	0.015	0.010	0.010	0.000	0.005	0.000	0.010	0.005	0.020	0.010	0.005	0.000										
*G. jasminoides*2	0.020	0.030	0.015	0.000	0.030	0.020	0.025	0.020	0.015	0.010	0.010	0.000	0.005	0.000	0.010	0.005	0.020	0.010	0.005	0.000	0.000									
*G. jasminoides*3	0.035	0.045	0.030	0.015	0.045	0.035	0.040	0.035	0.030	0.025	0.025	0.015	0.020	0.015	0.025	0.020	0.035	0.025	0.020	0.015	0.015	0.000								
*G. jasminoides*4	0.020	0.030	0.015	0.000	0.030	0.020	0.025	0.020	0.015	0.010	0.010	0.000	0.005	0.000	0.010	0.005	0.020	0.010	0.005	0.000	0.000	0.015	0.000							
*G. jasminoides*5	0.020	0.030	0.015	0.000	0.030	0.020	0.025	0.020	0.015	0.010	0.010	0.000	0.005	0.000	0.010	0.005	0.020	0.010	0.005	0.000	0.000	0.015	0.000	0.000						
*G. jasminoides*6	0.020	0.030	0.015	0.000	0.030	0.020	0.025	0.020	0.015	0.010	0.010	0.000	0.005	0.000	0.010	0.005	0.020	0.010	0.005	0.000	0.000	0.015	0.000	0.000	0.000					
*G. taitensis*1	0.020	0.040	0.035	0.020	0.051	0.040	0.045	0.040	0.025	0.020	0.030	0.020	0.025	0.020	0.030	0.015	0.030	0.020	0.015	0.020	0.020	0.035	0.020	0.020	0.020	0.000				
*G. taitensis*2	0.020	0.025	0.025	0.010	0.035	0.020	0.035	0.030	0.015	0.010	0.020	0.010	0.015	0.010	0.020	0.005	0.020	0.005	0.005	0.010	0.010	0.025	0.010	0.010	0.010	0.020	0.000			
*G. gjellerupii*1	0.040	0.040	0.035	0.020	0.040	0.035	0.045	0.040	0.035	0.030	0.030	0.020	0.025	0.020	0.010	0.025	0.040	0.020	0.025	0.020	0.020	0.035	0.020	0.020	0.020	0.040	0.025	0.000		
*G. gjellerupii*2	0.020	0.030	0.015	0.000	0.030	0.020	0.025	0.020	0.015	0.010	0.010	0.000	0.005	0.000	0.010	0.005	0.020	0.010	0.005	0.000	0.000	0.015	0.000	0.000	0.000	0.020	0.010	0.020	0.000	
G. volkensii	0.035	0.045	0.040	0.025	0.056	0.045	0.051	0.035	0.020	0.015	0.035	0.025	0.030	0.025	0.035	0.020	0.035	0.025	0.020	0.025	0.025	0.040	0.025	0.025	0.025	0.035	0.025	0.045	0.025	0.000

Table 7. Genetic distance values combined from *mat*K and *trn*H- *psb*A regions of the genus *Gardenia* species in Thailand

	G. saxatilis 1	*G. saxatilis* 2	*G. sootepensis*1	*G. sootepensis* 2	*G. sootepensis*3	*G. sootepensis*4	*G. sootepensis*5	*G. sootepensis*6	*G. obtusifolia* 1	*G. obtusifolia* 2	*G. elata* 1	*G. elata*2	*G. elata*3	*G. thailandica*1	*G. thailandica*2	*G. collinsae* 1	*G. collinsae*2	*G. carinata* 1	*G. carinata* 2	*G. jasminoides*1	*G. jasminoides*2	*G. jasminoides*3	*G. jasminoides*4	*G. jasminoides*5	*G. jasminoides*6	*G. taitensis*1	*G. taitensis* 2	*G. gjellerupii*1	*G. gjellerupii*2	*G. volkensii*
*Gardenia saxatilis*1	0.000																													
*G. saxatilis*2	0.066	0.000																												
G. sootepensis 1	0.060	0.071	0.000																											
*G. sootepensis*2	0.060	0.071	0.000	0.000																										
*G. sootepensis*3	0.071	0.082	0.011	0.011	0.000																									
*G. sootepensis*4	0.060	0.071	0.000	0.000	0.011	0.000																								
*G. sootepensis*5	0.055	0.066	0.005	0.005	0.016	0.005	0.000																							
*G. sootepensis*6	0.066	0.077	0.005	0.005	0.016	0.005	0.011	0.000																						
G. obtusifolia 1	0.066	0.077	0.005	0.005	0.016	0.005	0.011	0.011	0.000																					
G. obtusifolia 2	0.104	0.115	0.044	0.044	0.055	0.044	0.049	0.049	0.049	0.000																				
*G. elata*1	0.066	0.077	0.005	0.005	0.011	0.005	0.011	0.011	0.011	0.049	0.000																			
*G. elata*2	0.191	0.202	0.137	0.137	0.148	0.137	0.142	0.142	0.142	0.175	0.142	0.000																		
*G. elata*3	0.066	0.077	0.011	0.011	0.016	0.011	0.016	0.016	0.016	0.055	0.005	0.137	0.000																	
*G. thailandica*1	0.038	0.055	0.022	0.022	0.033	0.022	0.027	0.027	0.027	0.066	0.027	0.153	0.027	0.000																
*G. thailandica*2	0.060	0.071	0.005	0.005	0.016	0.005	0.011	0.011	0.011	0.049	0.011	0.131	0.005	0.022	0.000															
*G. collinsae*1	0.066	0.077	0.005	0.005	0.016	0.005	0.011	0.011	0.011	0.049	0.011	0.142	0.016	0.027	0.011	0.000														
*G. collinsae*2	0.066	0.077	0.005	0.005	0.016	0.005	0.011	0.011	0.011	0.049	0.011	0.142	0.016	0.027	0.011	0.000	0.000													
G. carinata 1	0.066	0.077	0.011	0.011	0.022	0.011	0.016	0.016	0.005	0.055	0.016	0.137	0.011	0.027	0.005	0.016	0.016	0.000												
G. carinata 2	0.191	0.202	0.137	0.137	0.148	0.137	0.142	0.142	0.142	0.175	0.142	0.000	0.137	0.153	0.131	0.142	0.142	0.137	0.000											
*G. jasminoides*1	0.071	0.082	0.011	0.011	0.005	0.011	0.016	0.016	0.016	0.055	0.005	0.148	0.011	0.033	0.016	0.016	0.016	0.022	0.148	0.000										
*G. jasminoides*2	0.071	0.082	0.011	0.011	0.011	0.011	0.016	0.016	0.016	0.055	0.005	0.148	0.011	0.033	0.016	0.016	0.016	0.022	0.148	0.005	0.000									
*G. jasminoides*3	0.077	0.087	0.016	0.016	0.016	0.016	0.022	0.022	0.022	0.060	0.011	0.153	0.016	0.038	0.022	0.022	0.022	0.027	0.153	0.011	0.005	0.000								
*G. jasminoides*4	0.060	0.071	0.005	0.005	0.016	0.005	0.011	0.011	0.011	0.049	0.011	0.131	0.005	0.022	0.000	0.011	0.011	0.005	0.131	0.016	0.016	0.022	0.000							
*G. jasminoides*5	0.066	0.077	0.011	0.011	0.016	0.011	0.016	0.016	0.016	0.055	0.005	0.137	0.000	0.027	0.005	0.016	0.016	0.011	0.137	0.011	0.011	0.016	0.005	0.000						
*G. jasminoides*6	0.066	0.077	0.011	0.011	0.016	0.011	0.016	0.016	0.016	0.055	0.005	0.137	0.000	0.027	0.005	0.016	0.016	0.011	0.137	0.011	0.011	0.016	0.005	0.000	0.000					
*G. taitensis*1	0.071	0.082	0.011	0.011	0.011	0.011	0.016	0.016	0.016	0.055	0.005	0.148	0.011	0.033	0.016	0.016	0.016	0.022	0.148	0.005	0.000	0.005	0.016	0.011	0.011	0.000				
*G. taitensis*2	0.060	0.071	0.000	0.000	0.011	0.000	0.005	0.005	0.005	0.044	0.005	0.137	0.011	0.022	0.005	0.005	0.005	0.011	0.137	0.011	0.011	0.016	0.005	0.011	0.011	0.011	0.000			
*G. gjellerupii*1	0.066	0.077	0.011	0.011	0.016	0.011	0.016	0.016	0.016	0.055	0.005	0.137	0.000	0.027	0.005	0.016	0.016	0.011	0.137	0.011	0.011	0.016	0.005	0.000	0.000	0.011	0.011	0.000		
*G. gjellerupii*2	0.060	0.071	0.005	0.005	0.016	0.005	0.011	0.011	0.011	0.049	0.011	0.131	0.005	0.022	0.000	0.011	0.011	0.005	0.131	0.016	0.016	0.022	0.000	0.005	0.005	0.016	0.005	0.005	0.000	
G. volkensii	0.060	0.071	0.005	0.005	0.016	0.005	0.011	0.011	0.011	0.049	0.011	0.131	0.005	0.022	0.000	0.011	0.011	0.005	0.131	0.016	0.016	0.022	0.000	0.005	0.005	0.016	0.005	0.005	0.000	0.000

Table 8. Genetic distance values combined from *rbc*L, *mat*K and *trn*H- *psb*A spacer of genus *Gardenia* species in Thailand.

	G. saxatilis 1	*G. saxatilis* 2	*G. sootepensis* 1	*G. sootepensis* 2	*G. sootepensis* 3	*G. sootepensis* 4	*G. sootepensis* 5	*G. sootepensis* 6	*G. obtusifolia* 1	*G. obtusifolia* 2	*G. elata* 1	*G. elata* 2	*G. elata* 3	*G. thailandica* 1	*G. thailandica* 2	*G. collinsae* 1	*G. collinsae* 2	*G. carinata* 1	*G. carinata* 2	*G. jasminoides* 1	*G. jasminoides* 2	*G. jasminoides* 3	*G. jasminoides* 4	*G. jasminoides* 5	*G. jasminoides* 6	*G. taitensis* 1	*G. taitensis* 2	*G. gjellerupii* 1	*G. gjellerupii* 2	*G. volkensii*
*Gardenia saxatilis*1	0.000																													
G. saxatilis 2	0.032	0.000																												
G. sootepensis 1	0.049	0.066	0.000																											
G. sootepensis 2	0.046	0.059	0.011	0.000																										
G. sootepensis 3	0.070	0.066	0.034	0.027	0.000																									
G. sootepensis 4	0.065	0.074	0.032	0.023	0.030	0.000																								
G. sootepensis 5	0.070	0.063	0.040	0.030	0.036	0.032	0.000																							
G. sootepensis 6	0.074	0.083	0.044	0.036	0.059	0.057	0.061	0.000																						
G. obtusifolia 1	0.059	0.072	0.034	0.023	0.046	0.042	0.042	0.055	0.000																					
G. obtusifolia 2	0.087	0.089	0.065	0.053	0.066	0.068	0.066	0.078	0.053	0.000																				
G. elata 1	0.093	0.102	0.055	0.055	0.070	0.063	0.066	0.080	0.072	0.097	0.000																			
G. elata 2	0.101	0.099	0.074	0.063	0.072	0.076	0.074	0.091	0.082	0.097	0.102	0.000																		
G. elata 3	0.055	0.066	0.023	0.011	0.028	0.027	0.036	0.046	0.030	0.063	0.053	0.061	0.000																	
G. thailandica 1	0.017	0.030	0.040	0.028	0.055	0.049	0.057	0.065	0.051	0.082	0.082	0.087	0.040	0.000																
G. thailandica 2	0.061	0.068	0.030	0.019	0.040	0.040	0.046	0.038	0.038	0.063	0.068	0.072	0.025	0.047	0.000															
G. collinsae 1	0.047	0.061	0.023	0.011	0.030	0.027	0.034	0.046	0.025	0.057	0.061	0.068	0.015	0.040	0.028	0.000														
G. collinsae 2	0.063	0.051	0.034	0.023	0.032	0.036	0.030	0.055	0.036	0.055	0.068	0.066	0.028	0.051	0.040	0.021	0.000													
G. carinata 1	0.063	0.074	0.036	0.025	0.042	0.040	0.042	0.059	0.030	0.066	0.066	0.078	0.027	0.053	0.038	0.023	0.040	0.000												
G. carinata 2	0.102	0.110	0.080	0.068	0.080	0.080	0.078	0.099	0.078	0.097	0.108	0.019	0.070	0.093	0.076	0.070	0.076	0.074	0.000											
G. jasminoides 1	0.049	0.063	0.019	0.008	0.023	0.025	0.034	0.040	0.027	0.057	0.053	0.063	0.009	0.036	0.023	0.013	0.027	0.027	0.070	0.000										
G. jasminoides 2	0.051	0.065	0.019	0.008	0.023	0.023	0.034	0.042	0.027	0.059	0.053	0.065	0.008	0.036	0.025	0.011	0.027	0.027	0.070	0.004	0.000									
G. jasminoides 3	0.072	0.085	0.040	0.028	0.044	0.044	0.055	0.057	0.047	0.080	0.065	0.085	0.028	0.057	0.046	0.032	0.047	0.047	0.091	0.025	0.021	0.000								
G. jasminoides 4	0.061	0.065	0.034	0.023	0.042	0.044	0.042	0.047	0.038	0.059	0.072	0.072	0.028	0.047	0.025	0.032	0.038	0.038	0.070	0.028	0.028	0.049	0.000							
G. jasminoides 5	0.076	0.074	0.046	0.034	0.044	0.042	0.049	0.061	0.047	0.057	0.078	0.076	0.034	0.063	0.042	0.040	0.036	0.053	0.083	0.036	0.034	0.055	0.042	0.000						
G. jasminoides 6	0.068	0.068	0.040	0.028	0.036	0.032	0.038	0.055	0.044	0.055	0.065	0.065	0.027	0.057	0.036	0.034	0.028	0.036	0.065	0.025	0.027	0.047	0.028	0.027	0.000					
G. taitensis 1	0.051	0.068	0.025	0.013	0.032	0.032	0.042	0.049	0.030	0.063	0.061	0.072	0.017	0.042	0.032	0.017	0.028	0.030	0.074	0.011	0.009	0.030	0.036	0.044	0.034	0.000				
G. taitensis 2	0.061	0.061	0.038	0.027	0.032	0.021	0.030	0.055	0.038	0.055	0.059	0.066	0.032	0.053	0.040	0.028	0.025	0.036	0.072	0.027	0.028	0.049	0.038	0.040	0.023	0.032	0.000			
G. gjellerupii 1	0.169	0.173	0.131	0.125	0.139	0.140	0.142	0.157	0.142	0.144	0.150	0.176	0.129	0.154	0.129	0.137	0.139	0.133	0.175	0.129	0.129	0.150	0.131	0.133	0.127	0.135	0.137	0.000		
G. gjellerupii 2	0.059	0.065	0.027	0.015	0.036	0.028	0.034	0.044	0.034	0.055	0.065	0.065	0.021	0.044	0.025	0.025	0.027	0.032	0.070	0.021	0.021	0.042	0.019	0.027	0.021	0.028	0.028	0.127	0.000	
G. volkensii	0.167	0.175	0.133	0.127	0.146	0.144	0.144	0.156	0.137	0.137	0.156	0.178	0.135	0.156	0.139	0.135	0.137	0.135	0.173	0.135	0.135	0.156	0.133	0.135	0.133	0.137	0.137	0.030	0.129	0.000

Table 9. Summarized of genetic distance (D) values evaluated from sequences of each region and each combination

Region	Range of intraspecific D values	Range of interspecific D values
*rbc*L gene	0.000-0.056	0.000-0.061
*mat*K gene	0.000-0.054	0.000-0.186
*trn*H-*psb*A spacer	0.005-0.481	0.014-0.572
*rbc*L + *mat*K genes	0.000-0.089	0.000-0.088
*rbc*L gene + *trn*H-*psb*A spacer	0.000-0.045	0.000-0.056
*mat*K gene + *trn*H-*psb*A spacer	0.000-0.142	0.000-0.202
*rbc*L gene + *mat*K gene + *trn*H-*psb*A spacer	0.004-0.102	0.008-0.178

Table 10. GenBank accession numbers of three barcoding regions of the *Gardenia* species

No.	Species	GenBank accession number		
		*rbc*L	*mat*K	*psb*A-*trn*H spacer region
1	*G. saxatilis*	JX312196	KC545027	JX312208
		JX436163	KC545028	JX436171
2	*G. sootepensis*	JX312197	KC545029	JX312209
		JX312198	KC545030	JX312210
		JX436164	KC576966	JX436172
		JX436165	KC699237	JX436173
		JX436166	KC699238	JX436174
		JX675225	KC699246	JX675230
3	*G. obtusifolia*	JX312199	KC545031	JX312211
		KC576960	KC736936	KC699252
4	*G. elata*	JX436167	KC699239	JX459576
		JX436168	KC699241	JX436175
		JX312200	KC545032	JX312212
5	*G. thailandica*	JX312201	KC545033	JX312213
		JX459573	KC699242	JX675226
6	*G. collinsae*	JX312202	KC545034	JX312214
		JX436169	KC545035	JX436176
7	*G. carinata*	JX312203	KC545036	JX312215
		KC545037	KC699247	KC699253
8	*G. jasminoides*	JX312204	KC576962	JX312216
		JX312206	KC576964	JX312218
		JX312207	KC576965	JX312219
		KC545038	KC699248	KC699254
		KC545039	KC699250	KC699256
		KC576961	KC699249	KC699255
9	*G. taitensis*	JX312205	KC576963	JX312217
		JX436170	KC699240	JX436177
10	*G. gjellerupii*	JX459575	KC699244	JX675228
		KC545040	KC699251	KC699257
11	*G. volkensii*	JX675224	KC699245	JX675229

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