

Preliminary search of intraspecific chloroplast DNA variation of nine evergreen broad leaved plants in East Asia

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ABSTRACT: In order to acquire information on chloroplast DNA markers to evaluate the genetic diversity of evergreen broad leaved plants, we investigated the intraspecific variation of cpDNA in eight non-coding regions of nine species commonly distributed in East Asia. Although no variations were detected in *psbA-trnH*, *rpoB-trnC*, *rpl16* and *atpB-rbcL* regions, a relatively large amount of intraspecific variations was detected in the *psbC-trnS*, *rps16* and *trnL-F* regions. These results suggested that these three cpDNA markers are suitable to assess genetic diversity of the species investigated in this study. In contrast, intraspecific variations were detected in seven taxa except *Hedera rhombea* and *Neolitsea aciculata*. *Neolitsea sericea* and the taxa of *Quercus* had many polymorphic sites.

Keywords: Chloroplast DNA, East Asia, evergreen broad leaved plants, intraspecific variation

Global climatic oscillations during the Quaternary have had a strong influence on species distributions and evolution, and thus the present distribution patterns of plant and animal species are the results of migration and extinction due to climatic changes (Hewitt, 2000, 2003). Recently, our understanding of processes that have influenced the current distributions of populations and species has been greatly enhanced by phylogeography, which can be utilized to infer historical scenarios and account for the spatial arrangements (Avise, 2000). Variations in chloroplast DNA may enable us to trace seed migration and may provide an insight into the historical biogeography and phylogeography of a plant species (Soltis et al., 1997; Soltis and Soltis, 1998). Thus, intraspecific variations of cpDNA have been frequently used in phylogeographic studies (Petit et al., 2002; Rendell and Ennos, 2003; Aoki et al., 2004, 2006; Heuertz et al., 2004; Sugahara et al., 2011).

The phylogeographical structure for the temperate plant taxa distributed in East Asia have been shown to be formed due to the expansion and contraction of distribution through several land-bridges, as lowered sea-levels exposed wide stretches of the continental shelf of the East China Sea during the Pleistocene (Li et al., 2008; Qiu et al., 2009a,b; Bai et al., 2010). However, historical migrations of warm-temperate evergreen tree species in this region are still poorly understood. There have only been a few phylogeographical studies on these

species in East Asia, and most population genetic studies on these species have focused only on localized regions, such as China, Korea, Japan or Taiwan (Chung et al., 2000; Cheng et al., 2005; Wang et al., 2005; Aoki et al., 2006).

Therefore, before evaluating the genetic diversity of East Asian evergreen broad leaved plants, we first need to accumulate information on cpDNA markers of these species. In the present study, we investigated the intraspecific variation of cpDNA in eight non-coding regions of nine evergreen broad leaved plants that are commonly distributed in East Asian warm-temperate regions.

Materials and Methods

Sampling

We sampled 98 individuals from 24 populations; seven from Korea, 12 from Japan, three from China, and two from Taiwan. This dataset included the following nine species of East Asian evergreen broad leaved plants: *Quercus acuta* Thunb., *Quercus salicina* Blume, *Quercus gilva* Blume, *Quercus myrsinifolia* Blume (Fagaceae); *Neolitsea sericea* (Blume) Koidz., *Neolitsea aciculata* (Blume) Koidz., *Machilus thunbergii* Siebold & Zucc. (Lauraceae); *Hedera rhombea* (Miq.) Bean (Araliaceae); *Trachelospermum asiaticum* (Siebold & Zucc.) Nakai (Apocynaceae) (Appendix 1). Voucher specimens for this study were deposited in herbarium at Inha University (IUI).

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DNA extraction and PCR amplification

Genomic DNA was extracted from fresh or silica-gel dried leaves using a G-spin™ IIP Kit for plant (Intron). Eight cpDNA non-coding regions were amplified by polymerase chain reaction (PCR). Universal primers were used to amplify the *trnL*-F, which included the *trnL* intron and *trnL*-*trnF* intergenic spacer (Taberlet et al., 1991), *rps16* intron (Nishizawa and Watano 2000, Shaw et al., 2005), *rpl16* intron (Nishizawa and Watano 2000), *psbA*-*trnH* intergenic spacer (Sang et al., 1997), *psbC*-*trnS* intergenic spacer (Nishizawa and Watano 2000), *rpoB*-*trnC* intergenic spacer (Shaw et al., 2005), *atpB*-*rpoC* intergenic spacer (Chiang et al., 1998) and *petD*-*rpoA* intergenic spacer (Nishizawa and Watano, 2000).

Polymerase chain reactions (PCR) were conducted using a GeneAmp® PCR System 2700 Thermal Cycler (Applied Biosystems). Each reaction mixture contained 200 µM dNTPs (GeneCraft), 1x PCR buffer with 1.5 mM MgCl₂, 1 U of Taq DNA polymerase (TaKaRa), 10 ng of DNA, and an appropriate concentration of primers in a total volume of 50 µL. The PCR conditions included an initial denaturation at 94°C for 2 min; followed by 35 cycles at 94°C for 30s, 52°C for 45s, and 72°C for 1 min; with a final extension at 72°C for 10 min. PCR products were visualized on 2% agarose gels, purified using PCRquick-spin™ (Intron) and sequenced with an ABI 3100 Genetic Analyzer using the ABI BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). All

sequences were aligned manually using the program Clustal X ver. 1.83 (Thompson et al., 1997).

Results and Discussion

The following intraspecific cpDNA variations were detected in four non-coding regions: *psbC*-*trnS*, *rps16*, *trnL*-F, *petD*-*rpoA* regions. The *psbC*-*trnS* region was the most variable (11 polymorphisms detected in five taxa), followed by the *rps16* region containing eight polymorphisms in five taxa, *trnL*-F region containing six polymorphisms in four taxa, and then *petD*-*rpoA* region containing one polymorphism in one taxon. These regions contained indels, which included length polymorphisms (1 to 5 bp), nucleotide substitutions and inversions. The proportion of mutational events (including indel characters) of each region varied depending on the taxa (Table 1). Details of the intraspecific variation of eight non-coding regions examined are represented in Table 1.

In the *psbC*-*trnS* region, the sequence length varied from 239 bp (*H. rhombea*) to 293 bp (*Quercus* sp.), and the proportion of mutational events varied from 0% (*N. aciculata*, *M. thunbergii*, *H. rhombea*, *T. asiaticum*) to 1.02% (*Q. acuta*, *Q. salicina*). In the *rps16* region, complete sequence data on *N. sericea*, *N. aciculata* and *M. thunbergii* of Lauraceae could not be obtained when Shaw et al. (2005)'s primer pair were used, because the sequence includes a long poly T or A. On

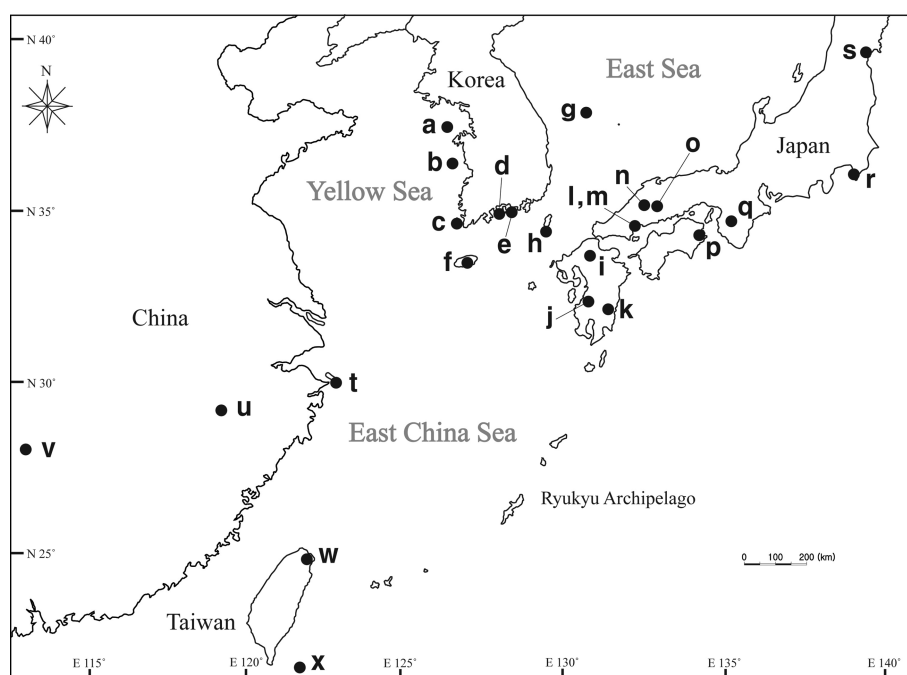


Fig. 1. Locations of 24 populations of warm temperate evergreen broad leaved plants sampled. The symbols of each population are corresponding to those of Table 2.

Table 1. Intraspecific variation of chloroplast DNA for nine East Asian evergreen broad leaved plants.

Species	cpDNA regions										A total number of polymorphic sites
	<i>trnL-F</i>	<i>rps16/1</i>	<i>rps16/2</i>	<i>rps16</i>	<i>rpl16</i>	<i>psbA-trnH</i>	<i>psbC-trnS</i>	<i>rpoB-trnC</i>	<i>atpB-rbcL</i>	<i>petD-rpoA</i>	
<i>Quercus acuta</i>	O (961bp)			2NS, 1SSRs (0.33%, 916bp)	O (329bp)	!	1NI, 2NS (1.02%, 293bp)	!	—	—	6
<i>Quercus salicina</i>	O (961bp)			O (910bp)	O (329bp)	!	1NI, 2NS (1.02%, 293bp)	!	—	—	3
<i>Quercus gilva</i>	1SSRs (0.1%, 961bp)			1NS (0.11%, 910bp)	O (329bp)	!	2NS (0.68%, 293bp)	!	—	—	4
<i>Quercus myrsinifolia</i>	1Indel, 1SSRs (0.21%, 961bp)			1NS (0.11%, 910bp)	O (329bp)	!	1NI, 1NS (0.68%, 293bp)	!	—	—	5
<i>Neolitsea sericea</i>	2Indel (0.22%, 925bp)	!	1SSRs, 1NS (0.75%, 265bp)	!	O (236bp)	!	1NS (0.41%, 241bp)	!	O (861bp)	1NS (0.32%, 314)	6
<i>Neolitsea aciculata</i>	O (920bp)	!	O (263bp)	!	O (236bp)	!	O (241bp)	O (1143bp)	O (861bp)	O (314bp)	0
<i>Machilus thunbergii</i>	1NS (0.11%, 920bp)	!	!	!	O (232bp)	!	O (241bp)	O (1141bp)	O (854bp)	O (314bp)	1
<i>Hedera rhombea</i>	O (968bp)			O (899bp)	O (208bp)	!	O (239bp)	O (1316bp)	O (886bp)	O (292bp)	0
<i>Trachelospermum asiaticum</i>	O (937bp)			1NS (0.12%, 839bp)	!	O (363bp)	O (284bp)	O (1308bp)	O (844bp)	!	1
A total number of polymorphic sites	6			8	0	0	11	0	0	1	

The number of variable site was compared with nine species; NI, Nucleotide inversion; NS, Nucleotide substitution; !, Insufficient data; O, No variation; —, No or poor amplification; SSRs, Simple sequence repeats; ID, Insertion/Deletion.

The proportion of observed mutational events and length of aligned sequence were represented in blankets.

Table 2. Distribution and frequencies of cpDNA haplotypes for nine East Asian evergreen broad leaved species.

Species	Korea			Japan								China			Taiwan		Number of haplotypes	Number of populations	Number of samples								
	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p				q	r	s	t	u	v	w	x
<i>Quercus acuta</i>	1 ^(A)					4 ^(B)	1 ^(C)		2 ^(B)	3 ^(B)	3 ^(B)							1 ^(A)	1 ^(B)						3	8	16
<i>Quercus salicina</i>			1 ^(A)	1 ^(C)		4 ^(C)	2 ^(C)		2 ^(C)	2 ^(C)	2 ^(C)							1 ^(C)					1 ^(C)		2	9	16
<i>Quercus gilva</i>						2 ^(D)				1 ^(E)	3 ^(E)											1 ^(A)			3	4	7
<i>Quercus myrsinifolia</i>			2 ^(F)						1 ^(G)	1 ^(C)	1 ^(F)							1 ^(G)							3	5	6
<i>Neolitsea sericea</i>	1 ^(A)				1 ^(E)	2 ^(A, D)	2 ^(B)		1 ^(A)					2 ^(C, F)	2 ^(I)			1 ^(G)	1 ^(A)						8	9	13
<i>Neolitsea aciculata</i>						1 ^(A)		2 ^(A)				2 ^(A)													1	3	5
<i>Machilus thumbergii</i>	1 ^(A)			1 ^(A)		1 ^(A)	1 ^(A)		1 ^(A)									1 ^(A)	1 ^(A)	1 ^(B)		1 ^(B)	1 ^(A)		2	10	10
<i>Hedera rhombea</i>	1 ^(A)					1 ^(A)	2 ^(A)										1 ^(A)	1 ^(A)	1 ^(A)						1	6	7
<i>Trachelospermum asiaticum</i>	1 ^(A)	1 ^(A)		2 ^(A)		4 ^(A)			2 ^(A)	2 ^(A)						2 ^(A)	3 ^(B)		1 ^(A)						2	9	18

a. Incheon, Deokjeok Archipelago; b. Chungcheongnam-do, Isl. Oeyeon; c. Jeollanam-do, Mt. Cheomchal; d. Jeollanam-do, Isl. Geumo; e. Gyeongsangnam-do, Mijo-ri; f. Jeju-do; g. Gyeongsangbuk-do, Isl. Ulleung; h. Nagasaki Pref., Isl. Tsushima; i. Fukuoka Pref., Mt. Koshoyama; j. Kumamoto Pref., Mt. Siroiwayama; k. Miyazaki Pref., Aya-cho; l. Hiroshima Pref., Isl. Itsukushima; m. Hiroshima Pref., around Temp. Gokurakuji; n. Hiroshima Pref., Miyoshi-shi; o. Hiroshima Pref., Shobara-shi; p. Tokushima Pref., Mt. Bizan; q. Wakayama Pref., Mt. Koya; r. Chiba Pref., Mt. Nokogiri; s. Miyagi Pref., Mt. Aobayama; t. Zhejiang Prov., Isl. Putuo. u. Anhui Prov., Qian kou min zhai; v. Hunan Prov., Nanyue Dist. Mt. Hengshan; w. Shiding-shiang, Tianwang peak; x. Taitung, Isl. Lanyu. The symbols in blanket are haplotypes.

the other hand, Aoki et al. (2003) found that Japanese evergreen broad leaved plants showed the largest amount of intraspecific variation in the *rps16* region. Thus, we used Nishizawa and Watano (2000)'s primer pair in order to obtain partial sequence data from the *rps16* region in Lauraceous species. The sequence length of the *rps16* region varied from 839bp (*T. asiaticum*) to 916bp (*Q. acuta*), and the proportion of mutational events varied from 0% (*Q. salicina*, *N. aciculata*, *H. rhombea*) to 0.75% (*N. sericea*). In the *trnL-F* region, the sequence length varied from 920bp (*N. aciculata*, *M. thunbergii*) to 968bp (*H. rhombea*), and the proportion of mutational events varied from 0% (*Q. acuta*, *Q. salicina*, *Q. gilva*, *N. aciculata*, *H. rhombea*, *T. asiaticum*) to 0.22% (*N. sericea*). The *petD-rpoA* region had only one informative character in *N. sericea*.

Among the four variable regions, the *psbC-trnS* intergenic spacer, *rps16* intron and *trnL-F* (*trnL* intron and *trnL-trnF* intergenic spacer) regions, showed polymorphisms in many species (4 to 5). These three regions have been frequently used to infer the evolutionary history or the past migration of plants in species level (Aoki et al., 2004, 2006; Wu et al., 2006; Ramos et al., 2007; Schonswetter et al., 2007; Li et al., 2008; Qiu et al., 2009b). The remaining four regions (*psbA-trnH*, *atpB-rbcL*, *rpoB-trnC* and *rpl16*) did not contain intraspecific cpDNA variations in the all species examined. In addition, the *rpl16* intron region has often been used to detect genetic variations among populations (Aoki et al., 2004, 2006, Shaw and Small, 2005). In this study, no intraspecific variation was detected in the *rpl16* region. However, complete sequences were obtained for all species except *T. asiaticum*.

Therefore, we suggest that the above three cpDNA markers (*psbC-trnS*, *rps16* and *trnL-F*) are suitable to assess the genetic diversity of East Asian evergreen broad leaved plants investigated in this study.

Based on the polymorphisms found in the present study, the cpDNA haplotypes for each species were determined (Table 3, 4, 5 and 6). The distribution and frequencies of haplotypes among the 24 sampled populations are shown in Table 2. *N. sericea* had the most abundant haplotypes with six polymorphisms among the nine species examined. The regional specific haplotypes of *N. sericea* were found in Korea and Japan, respectively but individuals of East China did not contain any unique haplotypes. In the taxa of *Quercus*, the examined species had two to three haplotypes with three to six polymorphisms. Regional specific haplotypes of *Q. acuta* and *Q. salicina* were found in Korea, and those of *Q. myrsinifolia* were found in Japan. However, all of haplotypes found in *Q. gilva* were regional specific and the haplotypes

Table 3. Polymorphic sites and haplotypes of *N. sericea*.

Haplotype	<i>trnL-F</i>		<i>rps16</i>		<i>psbC-trnS</i>	<i>petD-rpoA</i>
	3	6	0	1	1	0
	4	2	4	4	8	3
	6	5	3	0	7	9
A	.	2	T	T ₁₅	C	G
B	.	.	.	T ₁₄	T	T
C	.	.	.	T ₁₄	.	T
D	.	2	.	.	T	.
E	.	.	.	T ₁₂	.	.
F	1
G
H	1	.	G	.	.	.

Notes :

1. Deletion = AAGGAAGAATCGAATATTCAGTGATCAAATCA-TTCACTCCTCGGATAGATCT;
2. Duplication = TCTTT

Table 4. Polymorphic sites and haplotypes of *Quercus* species.

Haplotype	<i>trnL-F</i>		<i>rps16</i>				<i>psbC-trnS</i>			
	6	6	0	2	5	6	7	1	2	2
	4	6	7	9	5	9	1	8	5	5
	6	2	7	1	6	3	4	3	2	4
A	.	T ₁₁	A	C	C	A	.	.	T	T
B	.	.	.	T	.	T	2	.	A	.
C	3	A	A
D	.	T ₁₀	A	.
E	.	T ₁₀	C	A	.
F	A	.
G	1	T ₁₂	.	.	T	.	.	.	A	.

Notes:

1. Deletion = TTACAAAT; 2. Duplication = TAA₂;
3. Inversion = GGGGGA

Table 5. Polymorphic sites and haplotypes of *M. thunbergii*.

Haplotype	<i>trnL-F</i>										
	4										
	7										
	7										
A	G
B	T

Table 6. Polymorphic sites and haplotypes of *T. asiaticum*.

Haplotype	<i>rps16</i>										
	6										
	3										
	9										
A	G
B	T

were not shared among the regions. *M. thunbergii* and *T. asiaticum* contained two haplotypes with one polymorphism in the *trnL-F* and *rps16* regions, respectively. The rare haplotype of *M. thunbergii* was found in China and Taiwan. The regional specific haplotype of *T. asiaticum* was found in Japan (Table 2). On the other hand, several haplotypes of *Quercus* species were found to be shared between these species. This may be due to introgression, which has been reported for several evergreen *Quercus* species of Japan (Aoki et al., 2003). Although the results presented in this study are preliminary, it appears that the geographic distribution patterns of haplotypes of *Quercus* species are specific in species level.

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- Appendix 1.** Voucher specimens for nine East Asian evergreen broad leaved species used in this study.
- Quercus acuta* Thunb., Korea:** Isl. Nap, Deokjeok-myeon, Ongjin-gun, Incheon, 12 Aug. 2007, *B. H. Choi & J. H. Lee* 68075 (IUI); Mt. Sioreum, Seogwipo-si, Jeju-do, 13 Aug. 2009, *B. H. Choi et al.* 98102, 98138, 98154, 98158 (IUI); Seo-myeon, Ulleung-gun, Gyeongsangbuk-do, 16 Oct. 2010, *J. H. Lee & J. S. Park* 1010037 (IUI); **Japan:** Mt. Koshoyama, Asakura-shi, Fukuoka Pref., 21 Jun. 2010, *J. H. Lee & J. S. Park* K1-1, K1-5 (IUI); Aya-cho, Higashimorokata-gun, Miyazaki Pref., 23 Jun. 2010, *J. H. Lee & J. S. Park* K4-1, K4-3, K4-5 (IUI); Around Temp. Gokurakuji, Hiroshima Pref., 31 Mar. 2010, *J. H. Lee* 103525, 103527, 103533 (IUI); Mt. Nokogiri, Futtsu-shi, Chiba Pref., 14 Jul. 2010, *D. H. Lee* 10714 (IUI); Mt. Aobayama, Sendai-shi, Miyagi Pref., 27 Sep. 2010, *B. H. Choi* 10901 (IUI).
- Quercus salicina* Blume, Korea:** Mt. Cheomchal, Jindo-gun, Jeollanam-do, 9 Jun. 2010, *J. H. Lee & J. S. Park* 16068 (IUI); Isl. Geumo, Nam-myeon, Yeosu-si, Jeollanam-do, 28 Oct. 2009, *J. H. Lee* 910337 (IUI); Around 3 Sallokgyo, Seogwipo-si, Jeju-do, 12 Dec. 2009, *J. H. Lee* 912557, 912551 (IUI); Around 5 Sallokgyo, Seogwipo-si, Jeju-do, 12 Dec. 2009, *J. H. Lee* 912544, 912533 (IUI); Seo-myeon, Ulleung-gun, Gyeongsangbuk-do, 16 Oct. 2010, *J. H. Lee & J. S. Park* 1010027, 1010028 (IUI); **Japan:** Mt. Koshoyama, Asakura-shi, Fukuoka Pref., 21 Jun. 2010, *J. H. Lee & J. S. Park* K1-37, K1-39 (IUI); Aya-cho, Higashimorokata-gun, Miyazaki Pref., 23 Jun. 2010, *J. H. Lee & J. S. Park* K4-19, K4-21 (IUI); Around Temp. Gokurakuji, Hiroshima Pref., 31 Mar. 2010, *J. H. Lee* 103516, 103513 (IUI); Mt. Aobayama, Sendai-shi, Miyagi Pref., 27 Sep. 2010, *B. H. Choi* 10908 (IUI); **Taiwan:** Huangdi temple, Shiding-shiang, New Taipei, 8 Oct. 2010, *B. H. Choi & J. H. Lee* 10872 (IUI).
- Quercus gilva* Blume, Korea:** Around Osulloc Tea Museum, Seogwipo-si, Jeju-do, 10 Dec. 2009, *J. H. Lee & J. S. Park* 912044, 912047 (IUI); **Japan:** Mt. Siroiwayama, Nishiusuki-gun, Kumamoto Pref., 22 Jun. 2010, *J. H. Lee & J. S. Park* K2-31 (IUI); Aya-cho, Higashimorokata-gun, Miyazaki Pref., 23 Jun. 2010, *J. H. Lee & J. S. Park* K4-53, K4-54, K4-55 (IUI); **China:** Mt. Hengshan, Nanyue Dist., Hengyang-shi, Hunan Prov., 7 Jun. 2002, *Z. H. Hu* 275 (PE).
- Quercus myrsinifolia* Blume, Korea:** Mt. Cheomchal, Gogun-myeon, Jindo-gun, Jeollanam-do, 9 Jun. 2010, *J. H. Lee & J. S. Park* 16057, 16054 (IUI); **Japan:** Mt. Siroiwayama, Nishiusuki-gun, Kumamoto Pref., 23 Jun. 2010, *J. H. Lee & J. S. Park* K3-23 (IUI); Aya-cho, Higashimorokata-gun, Miyazaki Pref., 23 Jun. 2010, *J. H. Lee & J. S. Park* K4-65

(IUI); Around Temp. Gokurakuji, Hiroshima Pref., 31 Mar. 2010, *J. H. Lee 103512* (IUI); Mt. Aobayama, Sendai-shi, Miyagi Pref., Sep. 27 2010, *B. H. Choi 109013* (IUI).

***Neolitsea sericea* (Blume) Koidz., Korea:** Isl. Hagwangdae, Deokjeok-myeon, Ongjin-gun, Incheon, Korea, 17 Apr. 2010, *J. H. Lee & J. S. Park 104001* (IUI); Mijo-ri, Mijo-myeon, Namhae-gun, Gyeongsangnam-do, 12 Mar. 2010, *J. H. Lee & J. S. Park 1003004* (IUI); Around 2 Sallokdoro, Seogwipo-si, Jeju-do, 10 Oct. 2009, *B. H. Choi & J. H. Lee 910024, 910023* (IUI); Jeodong-ri, Ulleung-eup, Gyeongsangbuk-do, 28 Jul. 2009, *B. H. Choi 97237, 97235* (IUI); **Japan:** Mt. Koshoyama, Asakura-shi, Fukuoka Pref., 21 Jun. 2010, *J. H. Lee & J. S. Park K1-15* (IUI); Miyoshi-shi, Daejeon-si, Simane Pref., 26 Feb. 2009, *J. H. Lee 90214, 90222* (IUI); Mt. Bizan, Tokushima-shi, Tokushima Pref., 29 Mar. 2010, *J. H. Lee 103305, 103308* (IUI); Mt. Aobayama, Sendai-shi, Miyagi Pref., 27 Sep. 2010, *B. H. Choi 109016* (IUI); **China:** Isl. Putuo, Zhoushan Archipelago, Zhejiang, 31 Dec. 2010, *J. H. Lee 101201* (IUI).

***Neolitsea aciculata* (Blume) Koidz., Korea:** Isl. Geumo, Nam-myeon, Yeosu-si, Jeollanam-do, 28 Oct. 2009, *J. H. Lee LJH910343* (IUI); Mt. Sioreum, Seogwipo-si, Jeju-do, 13 Oct. 2009, *B. H. Choi et al. 98161* (IUI); **Japan:** Isl. Tsushima, Mt. Daterayama, Nagasaki Pref., 16 Sep. 2009, *B. H. Choi 99032* (IUI); Isl. Itsukushima, Hiroshima Pref., 28 Feb. 2009, *J. H. Lee 90266, 90263* (IUI).

***Machilus thunbergii* Siebold & Zucc., Korea:** Isl. Hago, Deokjeok-myeon, Ongjin-gun, Incheon, 31 Aug. 2006, *H. B. Shim & J. H. Lee 68138(1-10)* (IUI); Isl. Geumo, Yeosu-si, Jeollanam-do, 28 Sep. 2009, *J. H. Lee LJH910353* (IUI); Mt. Sioreum, Seogwipo-si, Jeju-do, 4 Jun. 2009, *J. H. Lee et al 96132* (IUI); Jeodong-ri, Ulleung-eup, Gyeongsangbuk-do, 15 Oct. 2010, *J. H. Lee & J. S. Park 1010064* (IUI); **Japan:** Mt. Koshoyama, Asakura-shi, Fukuoka Pref., 21 Jun. 2010, *J. H. Lee & J. S. Park K1-42* (IUI); Mt. Nokogiri, Futtsu-shi, Chiba Pref., 26 Feb. 2009, *B. H. Choi 92012* (IUI); **China:** Isl. Putuo,

Zhoushan Archipelago, Zhejiang, 31 Dec. 2010, *J. H. Lee 101220* (IUI); Qian Kou Min Zhai, Anhui, 22 Jul. 2010, *B. H. Choi & D. H. Lee 10701* (IUI); **Taiwan:** Huangdi temple, New Taipei, 8 Oct. 2010, *B. H. Choi & J. H. Lee 10888* (IUI); Isl. Lanyu, Taitung, 7 Oct. 2010, *B. H. Choi & J. H. Lee 10864* (IUI).

***Hedera rhombea* (Miq.) Bean, Korea:** Isl. Beolseom, Deokjeok-myeon, Ongjin-gun, Incheon, 12 Aug. 2006, *B. H. Choi & J. H. Lee 68106* (IUI); Bongnae Falls, Ulleung-eup, Gyeongsangbuk-do, 15 Oct. 2010, *J. H. Lee & J. S. Park 1010056* (IUI); Taehwaryeong old road, Ulleung-eup, Gyeongsangbuk-do, 16 Oct. 2010, *J. H. Lee & J. S. Park 1010016* (IUI); Suakgyo, Seogwipo-si, Jeju-do, 1 Apr. 2008, *J. H. Lee 804009* (IUI); **Japan:** Shobara-shi, Hiroshima Pref., 25 Feb. 2009, *J. H. Lee 7* (IUI); Mt. Nokogiri, Futtsu-shi, Chiba Pref., 26 Feb. 2009, *B. H. Choi 92011* (IUI); **China:** Isl. Putuo, Zhoushan Archipelago, Zhejiang, 31 Dec. 2010, *J. H. Lee 101210* (IUI).

***Trachelospermum asiaticum* (Siebold & Zucc.) Nakai, Korea:** Isl. Ul, Deokjeok-myeon, Ongjin-gun, Incheon, 18 Apr. 2010, *J. H. Lee & J. S. Park 104059* (IUI); Isl. Oeyeon, Boryeong-si, Chungcheongnam-do, 4 Apr. 2010, *J. H. Lee & J. S. Park 104053* (IUI); Mt. Daebu, Isl. Geumo, Yeosu-si, Jeollanam-do, 25 Oct. 2010, *J. S. Park & I. S. Choi 1010137, 1010138* (IUI); Dongbaekdongsan, Jeju-si, Jeju-do, 10 Dec. 2009, *J. H. Lee & J. S. Park 912601, 912602* (IUI); Andeok Valley, Seogwipo-si, Jeju-do, 11 Dec. 2009, *J. H. Lee & J. S. Park 912627, 912628* (IUI); **Japan:** Mt. Koshoyama, Asakura-shi, Fukuoka Pref., 21 Jun. 2010, *J. H. Lee & J. S. Park K1-21, K1-31* (IUI); Aya-cho, Higashimorokata-gun, Miyazaki Pref., 23 Jun. 2010, *J. H. Lee & J. S. Park K4-72, K4-73* (IUI); Mt. Kanmuri, Yamagata-gun, Hiroshima Pref., 30 Mar. 2010, *J. H. Lee, 103409, 103411* (IUI); Mt. Koya, Wakayama Pref., 29 Mar. 2010, *J. H. Lee 103211, 103212, 103213* (IUI); Mt. Aobayama, Sendai-shi, Miyagi Pref., 26 Feb. 2009, *B. H. Choi 09201* (IUI).