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DNA barcoding of Euphorbiaceae in Korea

Kyeonghee Kim[1], Ki-Ryong Park[2] and Chae Eun Lim[1,*]

[1]*National Institute of Biological Resources, 42 Hwangyeong-ro, Seo-gu, Incheon 22689, Republic of Korea*
[2]*Department of Environmental and Energy Engineering, Kyungnam University, Changwon 51767, Republic of Korea*

**Correspondent: chaelim@korea.kr*

The Euphorbiaceae family features some of the most economically important plants that are sources of foods, oils, waxes, and medicines. The accurate identification of Euphorbiaceae species is critical in sustainable utilization of plant resources. We examined 234 sequences of nrDNA ITS, cpDNA *rbc*L and *mat*K loci from 20 species in Euphorbiaceae in Korea and three outgroup taxa to develop efficient DNA barcodes. The three barcode loci were successfully amplified and sequenced for all Euphorbiaceae species. nrDNA ITS locus showed the highest mean interspecific K2P distance (0.3034), followed by cpDNA *mat*K (0.0830), and *rbc*L (0.0352) locus. The degree of species resolution for individual barcode loci ranged from 75% (*rbc*L and *mat*K) to 80% (ITS). The degree of species resolution was not enhanced with the different combinations of three barcode loci. The combined data set of the three loci (ITS + *rbc*L + *mat*K) provided 80% of species resolution. These results confirm that ITS locus, as a single barcode, is the best option for barcoding of the Euphorbiaceae in Korea.

Keywords: barcoding, Euphorbiaceae, ITS, *mat*K, plant resources, *rbc*L

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Introduction

Euphorbiaceae is one of the largest groups in the all Malpighiales families, consisting of approximately 6,500 species of four subfamilies (subfm. Euphorbioideae, subfm. Acalyphoideae Beilschm., Cheilosoideae K. Wurdack & Petra Hoffmann, and Crotonoideae Burmeist.) (Govaerts *et al*., 2000; APG IV, 2016). Members of Euphorbieae are monoecious or dioecious trees, shrubs, or herbs with milky latex, highly specialized inflorescence (cyanthium), superior and tri-locular ovary, and axile placentation (Park and Backlund, 2002; Thakur and Patil, 2011). The plants of this family are mainly distributed in the tropical and subtropical regions with some of native and naturalized species in the temperate regions including Asia, Australia, and North America (Heywood, 1985; Webster, 1986; 1994; Savolainen *et al*., 2000; Li *et al*., 2008; APG IV, 2016). A number of Euphorbiaceae species are economically and commercially important as food, ornamental, timber, or raw materials of wax, rubber, and dye (Salatino *et al*., 2007; Bennett, 2010; Kumar *et al*., 2010; Elhassan *et al*., 2015). In particular, many species in the genus *Euphorbia* are used as medicinal plants, although most of the plants in the genus contain toxic chemicals called diterpenoids (Hohmann and Molnár, 2004; Ernst *et al*., 2015). Since ancient times, various indigenous species are utilized as medicines for curing skin diseases, intestinal parasites gonorrhea, warts, and migraines on the basis of traditional folk recipes (Singla and Kamla, 1990; Salatino *et al*., 2007; Bennett, 2010; Kumar *et al*., 2010; Elhassan *et al*., 2015).

For sustainable utilization of Euphorbiaceae as plant resources, accurate species identification and understanding of their phylogenetic relationships are essential steps (Pang *et al*., 2010; Aubriot *et al*., 2013). Although many previous phylogenetic studies were carried on with chloroplast (cp) DNA (*atp*B, *mat*K, *ndh*F, *rbc*L, *trn*L-F) and/or nuclear (nr) DNA (ITS) loci (Wurdack *et al*., 2004; 2005; Tokuoka, 2007; Thakur and Patil, 2011; Yang *et al*., 2012; Aubriot *et al*., 2013; Riina *et al*., 2013), these studies only attempted to resolve higher level (family, genus or subgenus) relationships in Euphorbiaceae.

Meanwhile, Pang *et al*. (2009) largely collected DNA sequences of two cpDNA (*rbc*L, *mat*K) and two nrDNA (ITS1, ITS2) loci of the family Euphorbiaceae from GenBank and evaluated the species identification ability of four barcode loci. Among the four loci, Pang *et al*. (2009) reported the efficiency of the ITS1 and ITS2 loci for discrimination of species in the Euphorbiaceae fam-

ily. However, the sequences from the reference libraries have several limitations; the sequences could be generated from misidentified specimens or they might be obtained from pseudogenes (Wells and Stevens, 2008; Sonet *et al*., 2013). In addition, because of eventual local hybrids or various population structures, species identification could be inaccurate especially when the populations are from geographically distant regions (Stevens *et al*., 2002; Whitworth *et al*., 2007; Wells and Stevens, 2008; Sonet *et al*., 2013). Therefore, the development of DNA barcode systems on the basis of precise species identification is substantial at the regional scale (Kim *et al*., 2014; Choi *et al*., 2018).

As many as 20 species are found in Korea, classified in eight genera representing three subfamilies of Euphorbiaceae, and many species are being used as medicinal and ornamental plants (Park and Park, 2015). However, among those species, taxonomic identities of *Euphorbia maackii* Meinsh. and *E. fauriei* H. Lév. & Vaniot are controversial. *Euphorbia maackii* was first recorded in 1871, and treated as infraspecific taxa of *E. esula* [*E. esula* var. *maackii* (Meinsh.) Hurus.]. In addition, it was placed in synonymy with *E. esula* L. due to overlapping morphological variation (Li *et al*., 2008). *Euphorbia fauriei*, which belongs to *E. pekinensis* complex (Hurusawa, 1940; Park *et al*., 2017), was treated as infraspecific taxa of *E. pekinensis* [(*E. pekinensis* var. *fauriei* (H. Lév. & Vaniot) Hurus., *E. pekinensis* subsp. *fauriei* (H. Lév. & Vaniot) T. Kurosawa & H. Ohasi)] or as a distinct species (Park *et al*., 2002; Chung *et al*., 2003).

In the present study, we examined sequences of the nrDNA ITS locus (partial sequences of ITS1, complete sequences of 5.8S, and partial sequences of ITS2), cpDNA *rbc*L, and *mat*K (partial sequences of each coding loci) of 20 species in Korea to 1) test the universality of three barcode loci in Euphorbiaceae species, 2) assess the potential utility of these three loci for discriminating the Euphorbiaceae species at the local scale, and 3) develop DNA barcode database for Euphorbiaceae in Korea. Additionally, we provide the phylogeny of Korean species in the family Euphorbiaceae.

Materials and Methods

Plant materials, DNA extraction, amplification, and sequencing

A total of 78 samples representing 20 species in eight genera of the family Euphorbiaceae were included in this study (Appendix 1). Most of the species were collected from the natural populations representing 61 populations in all parts of South Korea during the research period. The cultivated species which do not grow wild in Korea were collected from gardens or farmlands; these are *Ricinus communis* L. and *Triadica sebifera* (L.) Small. As outgroups, we selected *Phyllanthus urinaria* L., *P. ussuriensis* Rupr. & Maxim., and *Flueggea suffruticosa* (Pall.) Baill. in Phyllanthaceae based on previous study (Wurdack *et al*., 2004). All of the voucher specimens were deposited in the herbarium of National Institute of Biological Resources (KB) (Appendix 1). Total genomic DNA was extracted using DNeasy plant mini kit (Qiagen, Germany) according to the manufacturer's instructions. Two cpDNA loci (*mat*K and *rbc*L) and a nrDNA locus (ITS) were amplified via polymerase chain reactions (PCR). The ITS locus was amplified and sequenced with the primers ITS1 and ITS4 (White *et al*., 1990), the *rbc*L locus with the primers rbcL1F and rbcL724R (Fay *et al*., 1998), and the *mat*K locus with the primers matK3F and matK1R combination (Table 1). For each PCR, 1 μL of total DNA (10−30 ng) was included in a 20 μL reaction mixture with commercialized PCR premix solution (AccuPower® PCR Premix, Bioneer, Republic of Korea). Polymerase Chain Reaction (PCR) was conducted using the following thermocycler program: initial denaturation at 94°C for 3−5 min, 35 cycles of 94°C for 1 min, 56°C for 1 min, 72°C for 1 min, and final extension at 72°C for 7 min (Table 1). The PCR products were purified with the enzymatic purification method followed by Werle *et al*. (1994). The purified PCR products were sequenced with the ABI Prism BigDye terminator Cycle sequencing kit (Applied Biosystems, USA) and were run on an ABI Prism 3730xl genetic analyzer (Applied Biosystems, USA).

Table 1. PCR/sequencing primers and PCR cycling conditions for the three barcode regions examined in this study. Primer names follow the original publications.

PCR/sequencing primer			PCR cycling condition (35 cycles)				
Region	Forward	Reverse	Pre-denaturation	Denaturation	Annealing	Extension	Final extension
ITS	ITS1*	ITS4*	94°C, 5 min	94°C, 1 min	56°C, 1 min	72°C, 1 min	72°C, 7 min
*rbc*L	rbcL1F†	rbcL724R†	94°C, 3 min	94°C, 1 min	56°C, 1 min	72°C, 1 min	72°C, 7 min
*mat*K	matK390F§	matK1300R§	94°C, 3 min	94°C, 1 min	56°C, 1 min	72°C, 1 min	72°C, 7 min

*White *et al*. (1990)
†Fay *et al*. (1998)
§Ki-Joong Kim (unpublished)

Data analysis

The sequence data of three DNA loci were assembled and edited using Sequencher 5.0 (Gene Codes Co., USA), and aligned using by Clustal W (Thompson *et al*., 1994), and proofread by eye in MEGA X (Kumar *et al*., 2018). Phylogenetic analyses were performed using neighbor-joining (NJ) and maximum parsimony (MP) methods. The NJ and MP analysis were implemented in PAUP* version 4.0a (Swofford, 2003). The Kimura's 2-parameter model (Kimura, 1980) was applied in the NJ analysis. Bootstrap analysis was performed 1,000 times to evaluate the support of each node. To estimate species resolution abilities for each barcode locus and the possible combinations, we conducted a tree-based identification method with the NJ tree. For each set of data, the degree of species resolution was calculated as a percentage of the total number of species correctly identified in the NJ tree divided by the total number of species examined in this study.

In the MP analysis, all characters were equally weighed. Heuristic searches were employed with 1,000 random sequence addition replicates and tree bisection-reconnection (TBR) branch swapping. The MP bootstrap analysis (Felsenstein, 1985) were performed using 1,000 replicates with TBR branch swapping and a random addition sequence. The combined dataset of ITS and the two cpDNA loci were evaluated using incongruence length difference (ILD) test in PAUP*4.0a (Swofford, 2003) under the partition homogeneity test.

Results

Universality of primer sequences

In total, 234 amplified fragments from three barcode loci were obtained from 78 accessions representing 20 species of Euphorbiaceae and three outgroup taxa (Table 2; Appendix 1). In regard of universality of primers, the success rate of PCR was 100% for each of the three loci for 20 Euphorbiaceae species using single primer set (Table 1). The bidirectional sequences with direct Sanger sequencing were successfully generated using the same primer sets used in PCR. All of the 234 sequence data which was newly obtained in this study was submitted to GenBank and WIGIS (Wildlife Genetic Information System, http://species.nibr.go.kr) (Appendix 1).

Characteristics of barcode loci

The sequence characteristics of the three barcode loci (nrDNA ITS and cpDNA *rbc*L, *mat*K) examined in this study are summarized in Table 2. The ITS locus was relatively more variable than two cpDNA loci across the species investigated in this study. The sequence length of ITS was ranged 596–653 bp and there were 455 variable sites (62.7%) when we aligned all sequences into 725 bp aligned sequence dataset (Table 2). The length of *mat*K was ranged 792–798 bp and there were 278 variable sites (34.8%) after we aligned the dataset. In this study, *mat*K had presented one 12 bp-long indel and two 6 bp-long indels within 798 bp of aligned sequence dataset. Another cpDNA locus, *rbc*L sequence was 702 bp for all accessions with 113 variable sites (16.1%). For the combination of two cpDNA data sets, the aligned length was 1500 bp and there were 391 variable sites (27.7%). The final alignment of the three loci combined sequence dataset was 2232 bp in length with 844 variable sites (37.8%) (Table 2).

The ITS locus showed the highest mean interspecific K2P distance (0.3034), followed by *mat*K (0.0830). *rbc*L showed the lowest mean interspecific distance (0.0352). The mean intraspecific K2P distance were 0.0000 for the three barcode loci (Table 2). The barcoding gap, the difference between the intraspecific and interspecific genetic (p-distances), was the largest for the ITS locus with 236 times higher interspecific versus intraspecific K2P distance, followed by *rbc*L (50 times) and *mat*K (40 times) (Fig. 1; Table 2).

Table 2. Statistics for three DNA barcode regions used in this study.

	ITS	*rbc*L	*mat*K	Three regions combined
N accessions	78	85	82	78
Sequence length (bp)	596–653	702	792–798	2092–2153
Aligned length (bp)	725	702	798	2232
G + C ratio (%)	49.8–63.9	41.9–43.4	29.5–33.7	41.1–45.1
N variable characters (%)	455 (62.7)	113 (16.1)	278 (34.8)	844 (37.8)
Intraspecific K2P distance* (mean)	0.0000–0.0077 (0.0000)	0.0000–0.0014 (0.0000)	0.0000–0.0039 (0.0000)	0.0000–0.0038 (0.0000)
Interspecific K2P distance* (mean)	0.0000–0.4014 (0.3034)	0.0000–0.0704 (0.0352)	0.0000–0.1587 (0.0830)	0.0000–0.1654 (0.1270)

*Outgroup taxa excluded.

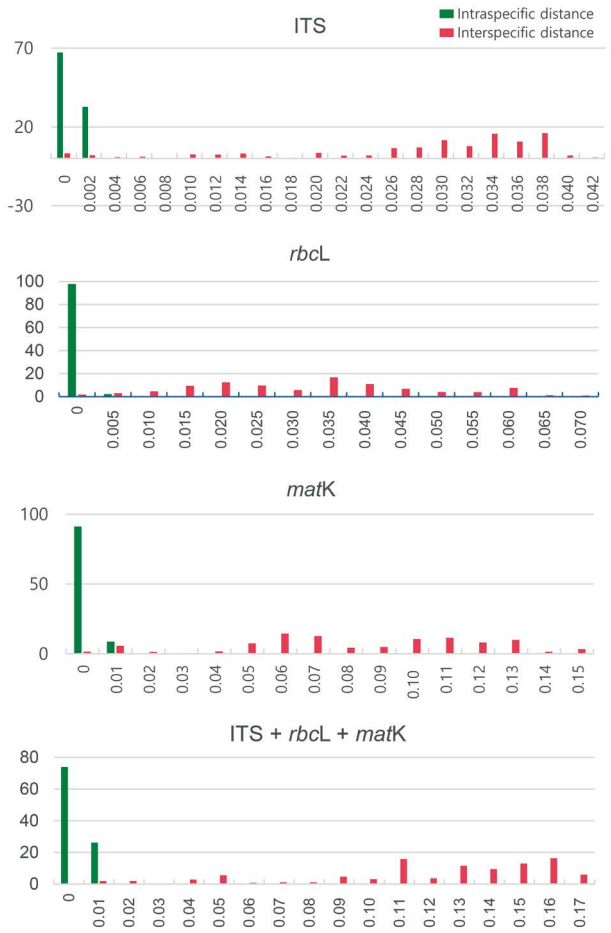

Fig. 1. Relative distributions of Kimura-2-parameter distances across three barcode loci.

Species discrimination success rate

The species discrimination success rate of three barcode loci and their possible combinations were evaluated by tree-based identification of the NJ analysis. The ILD test revealed that the three partitions (nrDNA ITS and cpDNA *rbc*L, *mat*K) were not heterogeneous. Therefore, the combined analysis of three barcode loci was performed with the single-locus analysis. For individual barcode locus, the highest discrimination success rate was 80% in nrDNA ITS, followed by *mat*K and *rbc*L (75% of species discrimination success rate for each locus) (Table 3).

The multi-locus combinations of the three barcode loci did not enhance the species discrimination success rate. The highest species discrimination success rate was 80% in combination of ITS + *mat*K + *rbc*L, followed by combination of *rbc*L + *mat*K (70%) (Table 3). In the combination of three loci which showed the highest species discrimination success rate, all species except for four species (*E. esula* and *E. maackii*, *E. pekinensis* and *E. fauriei*) were resolved (Fig. 2).

Table 3. Species resolving ability of individual barcode regions and their combinations.

Region	N species (N accessions)	Species resolution (%)
ITS	20 (78)	80
*rbc*L	20 (85)	75
*mat*K	20 (82)	75
*rbc*L + *mat*K	20 (78)	75
ITS + *rbc*L + *mat*K	20 (78)	80

Construction of phylogenetic trees

From each single locus sequences and possible combinations, five NJ trees were obtained (Fig. 2). Three of the five data set (*mat*K, *mat*K + *rbc*L, ITS + *rbc*L + *mat*K) showed the same topologies, and the species resolution degree was 75%, 75% and 80%, respectively (Fig. 2; Table 3). Therefore, the NJ tree which is based on the combined data set of the three loci (ITS + *rbc*L + *mat*K) is shown as a representative (Fig. 3). Additionally, a heuristic search of the combined data set of the three loci was performed. A single MP tree with 2,068 steps was found (CI = 0.624, RI = 0.937) and showed the same tree topology with the tree constructed with the NJ analysis (Fig. 3). Bootstrap (BS) values of MP analysis are shown on each branch of NJ tree (Fig. 3).

The NJ and MP trees of combined three locus data set showed that family Euphorbiaceae in Korea was strongly resolved to be monophyletic (BS of NJ/MP = 100/100) (Fig. 3). In Korean Euphorbiaceae, three subfamilies formed strongly supported monophyletic groups; these are subfm. Euphorbioideae, subfm. Acalyphoideae, and subfm. Crotonoideae. The BS values of NJ/MP analysis for each subfamily was 69/80, 98/59, 100/100, respectively (Fig. 3). The three subfamilies are easily distinguished by their type of latex and indumentum, shape of bracts, and presence/absence of petals (Webster, 1994). From the NJ and MP trees, subfm. Acalyphoideae and subfm. Crotonoideae were sisters to each other, and subfm. Euphorbioideae was isolated from the two subfamilies (Figs. 2, 3). Therefore, the combined data set of three barcode loci reflects the phylogenetic relationships of Euphorbiaceae at subfamilial level.

Discussion

In the present study, the success rates of amplification and sequencing success rate of ITS, *rbc*L and *mat*K loci were 100%, which confirm the universality of primers for each locus (Table 1). Among the three barcode loci, the species resolution was highest in ITS (80%) (Table

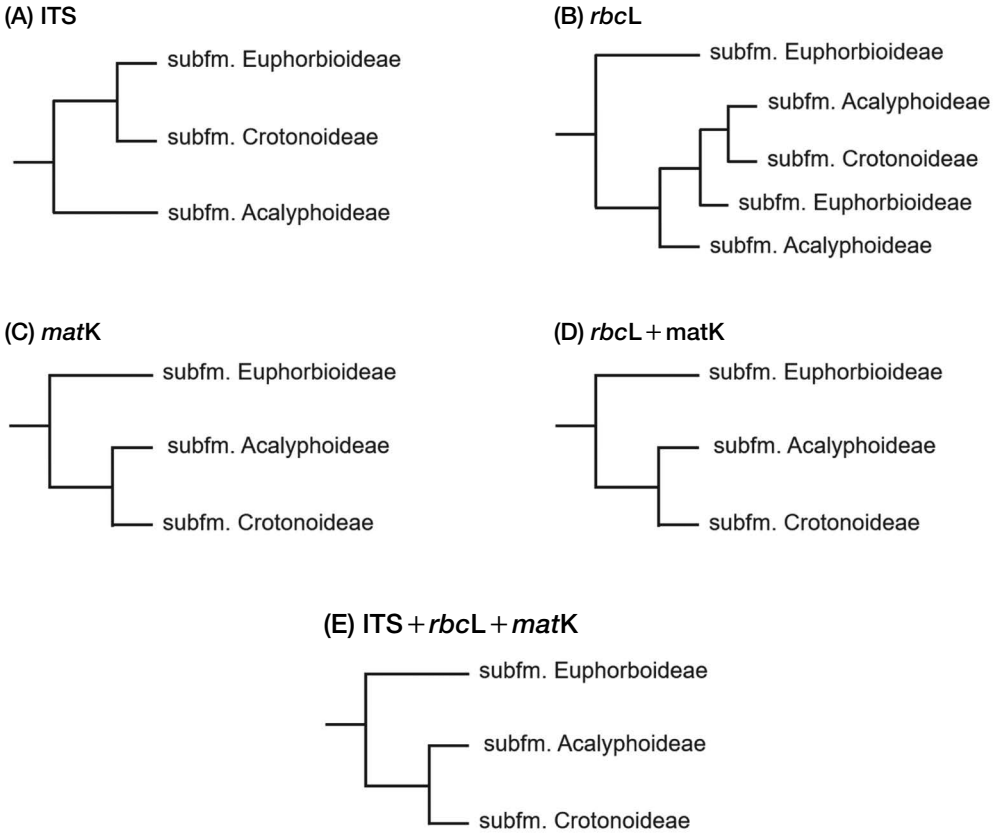

Fig. 2. Summary of contrasting molecular topologies for Euphorbiaceae in Korea.

3), similar to Pang *et al*. (2010) who reported ITS (ITS1/ITS2) locus had the highest species resolution in Euphorbiaceae. Pang *et al*. (2010) reported that the species resolution was from 68% to 100% in ITS1/ITS2 loci in seven genera (*Andrachne* L., *Croton* L., *Euphorbia*, *Glochidion* J.R. Forst. & G. Forst., *Macaranga* Thouars, *Mallotus* Lour., and *Phyllanthus* L.). The other two candidate loci, *rbc*L and *mat*K loci showed lower species resolution in Pang *et al*. (2010). Several previous studies have found that nrDNA ITS showed the higher species identification ability than in cpDNA loci (Petit and Excoffier, 2009; Liu *et al*., 2012; Yuan *et al*., 2015; Genievskaya *et al*., 2017; Kang *et al*., 2017). Since nrDNA is inherited biparentally, the genetic variability is higher than in cpDNA, which is inherited uniparentally (Tsitrone *et al*., 2003; Petit & Excoffier, 2009; Hollingsworth, 2011).

In this study, the combinations of two or more barcode loci showed no significant increase in species discrimination rate (Table 3). The combination of three barcode loci (ITS + *rbc*L + *mat*K) showed 80% of species resolution (Table 3). Meanwhile, previous research revealed the species resolution abilities were increased when nrDNA and cpDNA loci were combined (China Plant BOL Group, 2011; Hollingworth, 2011; Yuan *et al*., 2015; Mishra *et al*., 2017). Despite the combination of *rbc*L + *mat*K being proposed as the 'core barcode' in land plants (Lahaye *et al*., 2008; CBOL Plant Working Group, 2009; Starr *et al*., 2009; Yu *et al*., 2011), it was not the best candidate for Euphorbiaceae species in Korea (Table 3).

We successfully constructed phylogenetic relationships of the species using the combination of ITS + *rbc*L + *mat*K (Fig. 3). From the NJ and MP trees, family Euphorbiaceae in Korea was shown to be monophyletic (Fig. 3). In addition, the three subfamilies in Korean Euphorbiaceae (subfm. Euphorbioideae, subfm. Acalyphoideae, subfm. Corotonoideae) were monophyletic (Fig. 3). However, the subfamilies were supported by relatively low bootstrap values (BS = 69/80 for subfam. Euphorbioideae, 98/59 for subfm. Acalyphoideae, 100/100 for subfam. Crotonoideae) (Fig. 3).

For the genus level, the genus *Euphorbia* of subfm. Euphorbioideae was indicated as a monophyletic group (BS = 100/100) (Fig. 3). Two subgenera below genus *Euphorbia*, subgn. *Esula* and subgn. *Chamaesyce* were also monophyletic and strongly supported in NJ and MP analysis (BS = 100/100 for each node) (Fig. 3). Within subgn. *Esula*, three sections were supported as monophyletic as shown in Riina *et al*. (2013) (BS > 96/100; Fig. 3); these

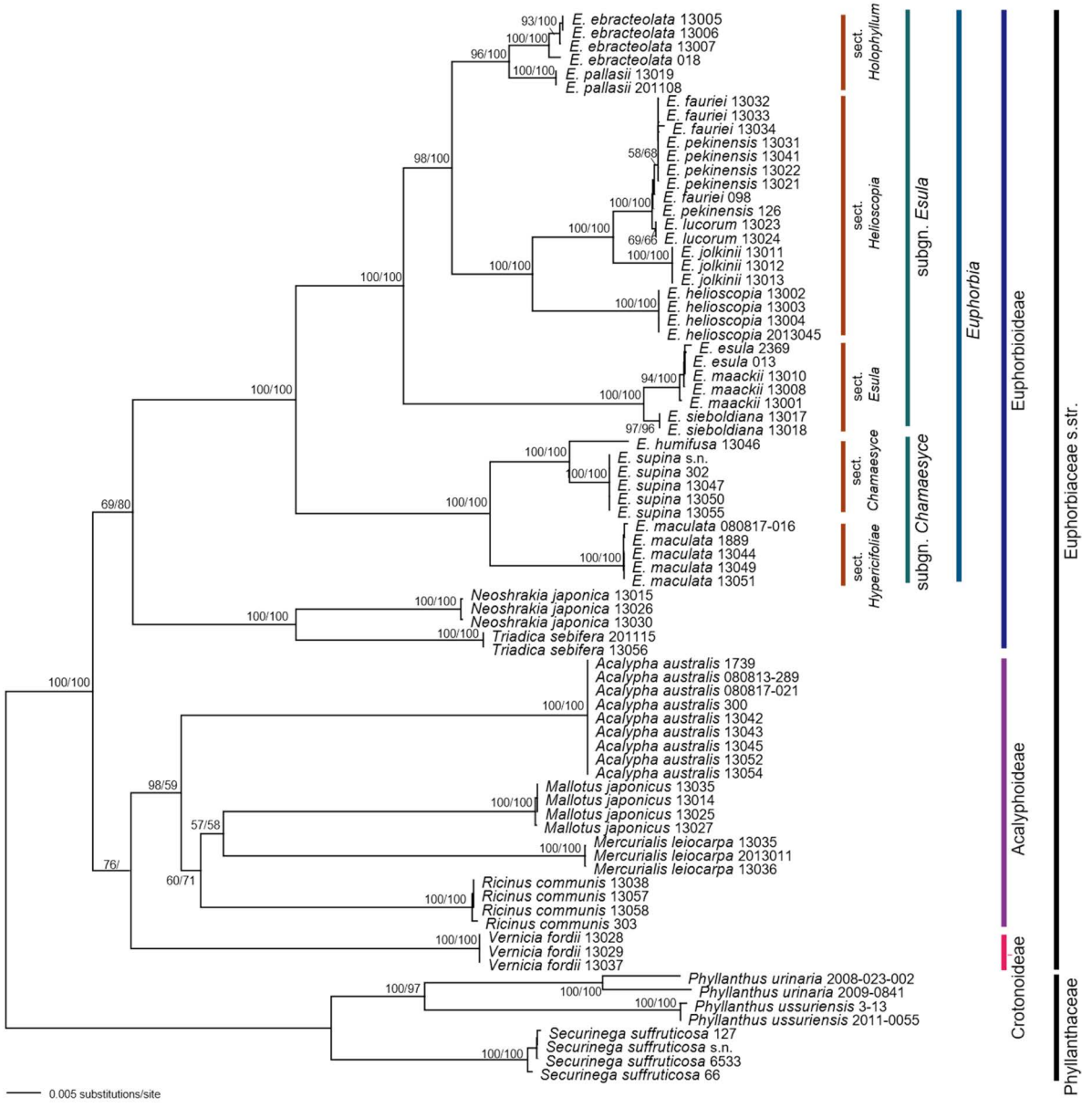

Fig. 3. Neighbour-joining (NJ) tree from the combined analysis of ITS, *rbc*L, and *mat*K loci from Euphorbiaceae in Korea. Numbers above the branches indicate bootstrap values ≥50% in the NJ/ Maximum Parsimony (MP) analysis, respectively.

are sects. *Holophyllum* (*E. ebracteolata* Hayata, *E. pallasii* Turcz. ex Ledeb.), *Helioscopia* (*E. fauriei* H. Lév. & Vaniot, *E. helioscopia* L., *E. jolkinii* Boiss., *E. lucorum*, *E. pekinensis* and *Esula* (*E. esula* L., *E. maackii* Meinsh, *E. sieboldiana* C. Morren & Decne). Within subgn. *Chamaesyce*, two sections were supported as monophyletic (Fig. 3); these are sects. *Chamaesyce* (*E. humifusa* Willd. ex Schlt, *E. supina* Raf.), and *Hypericifoliae* (*E. maculata* L.).

Euphorbia esula and *E. maackii* in *Euphorbia* subgn. *Esula* sect. *Esula* were not discriminated in the NJ tree of combined three locus data set (Fig. 3). Due to the complicated morphological variation, two species were included in '*E. esula* complex' as well as *E. nakaii* Hurus., *E. lunulata* Bunge, and *E. virgata* Waldst. & Kit. (Croizat, 1945). *Euphorbia esula* and *E. maackii* are distinguished by morphological traits, however, the isozyme data did not discriminate the two species (Jung and Park, 2012). Jung and Park (2012) inferred the disagreement of morphological and allozyme data may be associated with the gene flow by introgressive hybridization.

In addition, *E. fauriei* and *E. pekinensis* in subgn. *Esula* sect. *Helioscopia* were not identified by the NJ tree of combined data (Fig. 3). Due to the morphological poly-

morphism, both species were included in '*E. pekinensis* complex' as well as *E. subulatifolia* Hurus., *E. lasiocaula* Boiss., and *E. sinanensis* (Hurus.) T. Kurosawa (Hurusawa, 1940). Park *et al*. (2002) investigated the morphological variation and genetic diversity with isozyme data of the two species. The morphological analysis based on numerical data showed the two species are distinct, however, the isozyme analysis showed the close genetic distance between the two species. Those results suggest that the two species (*E. pekinensis* and *E. fauriei*) may have been derived from a common ancestor recently (Park *et al*., 2002). Therefore, it is more preferred to consider *E. fauriei* as a variety of *E. pekinensis* (*E. pekinensis* var. *fauriei*) than consider *E. fauriei* as a distinct species, following Husurawa (1954). Considering the results of above, the Euphorbiaceae species in Korea would be 100% discriminated by ITS locus.

In conclusion, this study finds that the ITS locus provides the highest degree of species identification, as well as the combination of the three barcode loci (ITS + *rbc*L + *mat*K) in Euphorbiaceae in Korea. In consideration of efficiency, ITS locus is the most suitable for barcoding of the Korean Euphorbiaceae as single locus barcode. The forward and reverse primers, ITS1 and ITS4, were easily applied for amplification and sequencing of Euphorbiaceae in Korea. In addition, a DNA barcode sequence database from three barcode loci is successfully established on the basis of the correctly identified samples. The DNA barcode, as well as the sequence database of Euphorbiaceae in Korea developed in this study, will contribute to the conservation and sustainable utilization of the Euphorbiaceae (spurges) as plant resources.

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Appendix 1. Voucher information and GenBank Accession numbers for the Euphorbiaceae taxa examined in this study. All voucher specimens are deposited at KB.

Taxon	Voucher	GenBank/WIGIS number (ITS, *rbc*L, *mat*K)
Acalypha australis L. 1	Gyeongbuk, Yeongcheon-si, Geumho-eup, *Won 1739* (NIBRVP0000206011)	MT444816, MT447232, MT447311 WBN0358748, WBN0358881, WBN0358962
	Jeju, Jeju-si, Jocheon-eup, *Kwon 080813-289* (NIBRVP0000279160)	MT444817, MT447233, MT447312 WBN0359127, WBN0359126, WBN0359125
	Chungnam, Dangjin-gun, Myeoncheon-myeon, *Han & Kim SK080817-021* (NIBRVP0000279297)	MT444818, MT447234, MT447313 WBN0358749, WBN0358882, WBN0358963
	Incheon, Seo-gu, Gyeongseo-dong, *Ryu celim300* (NIBRVP0000493930)	MT444819, MT447235, MT447314 WBN0358750, WBN0358883, WBN0358964
	Chungnam, Taean-gun, Anmyeon-eup, *Park 13042* (NIBRVP0000464226)	MT444820, MT447236, MT447315 WBN0358751, WBN0358884, WBN0358965
	Chungnam, Taean-gun, Anmyeon-eup, *Park 13043* (NIBRVP0000464227)	MT444821, MT447237, MT447316 WBN0358752, WBN0358885, WBN0358966
	Gyeongbuk, Cheongsong-gun, Jinbo-myeon, *Park 13045* (NIBRVP0000464229)	MT444822, MT447238, MT447317 WBN0358753, WBN0358886, WBN0358967
	Gyeongnam, Changnyeong-gun, Yueo-myeon, *Park 13052* (NIBRVP0000464236)	MT444823, MT447239, MT447318 WBN0358754, WBN0358887, WBN0358968
	Gyeongnam, Changnyeong-gun, Yueo-myeon, *Park 13054* (NIBRVP0000464238)	MT444824, MT447240, MT447319 WBN0358755, WBN0358888, WBN0358969
Euphorbia ebracteolata Hayata	Gyeongnam, Haman-gun, Yeohang-myeon, *Park 13005* (NIBRVP0000464190)	MT444774, MT447190, MT447269 WBN0358756, WBN0358889, WBN0358970
	Gyeongnam, Haman-gun, Yeohang-myeon, *Park 13006* (NIBRVP0000464191)	MT444775, MT447191, MT447270 WBN0358757, WBN0358890, WBN0358971
	Gyeongnam, Changwon-si, Jinbuk-myeon, *Park 13007* (NIBRVP0000464192)	MT444776, MT447192, MT447271 WBN0358758, WBN0358891, WBN0358972
	Incheon, Bukdo-myeon, Is. Janbong-do, *Lim et al. celim018* (NIBRVP0000464252)	MT444777, MT447193, MT447272 WBN0358759, WBN0358892, WBN0358973
E. esula L.	Gyeongbuk, Yeongdeok-gun, Ganggu-myeon, *Won 2369* (NIBRGR0000084952)	MT444778, MT447194, MT447273 WBN0358760, WBN0358893, WBN0358974
	Jeju, Seogwipo-si, Andeok-myeon, *Jeong & Han JJ013* (NIBRGR0000085266)	MT444779, MT447195, MT447274 WBN0358761, WBN0358894, WBN0358975
E. fauriei H. Lév. & Vaniot	Jeju, Seogwipo-si, Mt. Hallasan, *Park 13032* (NIBRVP0000464216)	MT444780, MT447196, MT447275 WBN0358763, WBN0358897, WBN0358978
	Jeju, Seogwipo-si, Mt. Hallasan, *Park 13033* (NIBRVP0000464217)	MT444781, MT447197, MT447276 WBN0358764, WBN0358898, WBN0358979
	Jeju, Seogwipo-si, Mt. Hallasan, *Park 13034* (NIBRVP0000464218)	MT444782, MT447198, MT447277 WBN0358765, WBN0358899, WBN0358980
	Jeju, Seogwipo-si, Mt. Hallasan, *Oh et al. celim098* (NIBRVP0000464304)	MT444783, MT447199, MT447278 WBN0358766, WBN0358900, WBN0358981

Appendix 1. Continued.

Taxon	Voucher	GenBank/WIGIS number (ITS, *rbc*L, *mat*K)
E. helioscopia L.	Gyeongbuk, Yeongdeok-gun, Byeonggok-myeon, *Park 13002* (NIBRVP0000464187)	MT444784, MT447200, MT447279 WBN0358767, WBN0358901, WBN0358982
	Gyeongbuk, Uljin-gun, Jukbyeon-myeon, *Park 13003* (NIBRVP0000464188)	MT444785, MT447201, MT447280 WBN0358768, WBN0358902, WBN0358983
	Gyeongbuk, Yeongdeok-gun, Namjeong-myeon, *Park 13004* (NIBRVP0000464189)	MT444786, MT447202, MT447281 WBN0358769, WBN0358903, WBN0358984
	Jeju, Seogwipo-si, Hangyeong-myeon, *Kwak et al. mk2013045* (NIBRVP0000426689)	MT444787, MT447203, MT447282 WBN0358770, WBN0358904, WBN0358985
E. humifusa Willd. ex Schlt.	Gangwon, Yeongwol-gun, Yeongwol-eup, *Park 13046* (NIBRVP0000464230)	MT44478, MT447204, MT447283 WBN0358771, WBN0358905, WBN0358986
E. jolkinii Boiss.	Jeju, Jeju-si, Gujwa-eup, *Park 13011* (NIBRVP0000464196)	MT444789, MT447205, MT447284 WBN0358772, WBN0358906, WBN0358987
	Jeju, Jeju-si, Gujwa-eup, *Park 13012* (NIBRVP0000464197)	MT444790, MT447206, MT447285 WBN0358773, WBN0358907, WBN0358988
	Jeju, Jeju-si, Gujwa-eup, *Park 13013* (NIBRVP0000464198)	MT444791, MT447207, MT447286 WBN0358774, WBN0358908, WBN0358989
E. lucorum Rupr.	Chungbuk, Danyang-gun, Maepo-eup, *Park 13023* (NIBRVP0000464207)	MT444792, MT447208, MT447287 WBN0358775, WBN0358909, WBN0358990
	Chungbuk, Danyang-gun, Danyang-eup, *Park 13024* (NIBRVP0000464208)	MT444793, MT447209, MT447288 WBN0358776, WBN0358910, WBN0358991
E. maackii Meinsh.	Gyeongbuk, Yeongdeok-gun, Byeonggok-myeon, *Park 13001* (NIBRVP0000464186)	MT444794, MT447210, MT447289 WBN0358777, WBN0358911, WBN0358992
	Jeju, Seogwipo-si, Seongsan-eup, *Park 13008* (NIBRVP0000464193)	MT444795, MT447211, MT447290 WBN0358778, WBN0358912, WBN0358993
	Jeju, Jeju-si, Gujwa-eup, *Park 13010* (NIBRVP0000464195)	MT444796, MT447212, MT447291 WBN0358779, WBN0358913, WBN0358994
E. maculata L.	Gyeongbuk, Gyeongsan-si, Jillyang-eup, *Won et al. 1889* (NIBRVP0000206033)	MT444798, MT447213, MT447293 WBN0358780, WBN0358914, WBN0358995
	Gyeonggi, Gapyeong-gun, Buk-myeon, *Han & Kim SK080817-016* (NIBRVP0000278557)	MT444797, MT447214, MT447292 WBN0358781, WBN0358915, WBN0358996
	Gyeongbuk, Cheongsong-gun, Jinbo-myeon, *Park 13044* (NIBRVP0000464228)	MT444799, MT447215, MT447294 WBN0358782, WBN0358916, WBN0358997
	Daegu, Dong-gu, Bullo-dong, *Park 13049* (NIBRVP0000464233)	MT444800, MT447216, MT447295 WBN0358783, WBN0358917, WBN0358998
	Gyeongnam, Changnyeong-gun, *Park 13051* (NIBRVP0000464235)	MT444801, MT447217, MT447296 WBN0358784, WBN0358918, WBN0358999

Appendix 1. Continued.

Taxon	Voucher	GenBank/WIGIS number (ITS, *rbc*L, *mat*K)
E. pallasii Turcz. ex Ledeb.	China, Jilin (transplanted to Gyeongnam, Changwon-si, Jinbuk-myeon) *Park 13019* (NIBRVP0000464204)	MT444802, MT447218, MT447297 WBN0358785, WBN0358919, WBN0359000
	China, Jilin, *Park 201108* (NIBRVP0000362773)	MT444803, MT447219, MT447298 WBN0358786, WBN0358920, WBN0359001
E. pekinensis Boiss.	Busan, Gijang-gun, Gijang-eup, *Park 13021* (NIBRVP0000464205)	MT444804, MT447220, MT447299 WBN0358787, WBN0358921, WBN0359002
	Ulsan, Ulju-gun, Seosaeng-myeon, *Park 13022* (NIBRVP0000464206)	MT444805, MT447221, MT447300 WBN0358788, WBN0358922, WBN0359003
	Gyeongnam, Uiryeong-gun, Uiryeong-eup, *Park 13031* (NIBRVP0000464215)	MT444806, MT447222, MT447301 WBN0358789, WBN0358923, WBN0359004
	Daegu, Dalseong-gun, Gachang-myeon, Mt. Choejeongsan, *Oh et al. celim126* (NIBRVP0000464321)	MT444807, MT447223, MT447302 WBN0358790, WBN0358924, WBN0359005
	Chungnam, Taean-gun, Anmyeon-eup, *Park 13041* (NIBRVP0000464225)	MT444808, MT447224, MT447303 WBN0358791, WBN0358925, WBN0359006
E. sieboldiana C. Morren & Decne	Jeju, Jeju-si, Bonggae-dong, *Park 13017* (NIBRVP0000464202)	MT444809, MT447225, MT447304 WBN0358792, WBN0358927, WBN0359007
	Jeju, Jeju-si, Yonggang-dong, Halla Eco-Forest, *Park 13018* (NIBRVP0000464203)	MT444810, MT447226, MT447305 WBN0358793, WBN0358928, WBN0359008
E. supina Raf.	Seoul, Gwanak-gu, Mt. Gwanaksan, *Hong et al. s.n.* (NIBRVP0000414160)	MT444811, MT447227, MT447306 WBN0358794, WBN0358930, WBN0359010
	Incheon, Seo-gu, Gyeongseo-dong, *Ryu celim302* (NIBRVP0000493932)	MT444812, MT447228, MT447307 WBN0358795, WBN0358931, WBN0359011
	Gangwon, Yeongwol-gun, Yeongwol-eup, *Park 13047* (NIBRVP0000464231)	MT444813, MT447229, MT447308 WBN0358796, WBN0358932, WBN0359012
	Gyeongnam, Changwon-si, Jinbuk-myeon, *Park 13050* (NIBRVP0000464234)	MT444814, MT447230, MT447309 WBN0358797, WBN0358933, WBN0359013
	Gyeongnam, Changnyeong-gun, *Park 13055* (NIBRVP0000464239)	MT444815, MT447231, MT447310 WBN0358798, WBN0358934, WBN0359014
Mallotus japonicus (L. f.) Mull. Arg.	Jeju, Jeju-si, Seongsan-eup, *Park 13009* (NIBRVP0000464194)	MT444825, MT447241, MT447320 WBN0358799, WBN0358935, WBN0359015
	Jeju, Jeju-si, Gujwa-eup, *Park 13014* (NIBRVP0000464199)	MT444826, MT447242, MT447321 WBN0358800, WBN0358936, WBN0359016
	Jeonnam, Mokpo-si, Jukgyo-dong, *Park 13025* (NIBRVP0000464209)	MT444827, MT447243, MT447322 WBN0358801, WBN0358937, WBN0359017
	Jeonnam, Yeosu-si, Mipyeong-dong, *Park 13027* (NIBRVP0000464211)	MT444828, MT447244, MT447323 WBN0358802, WBN0358938, WBN0359018

Appendix 1. Continued.

Taxon	Voucher	GenBank/WIGIS number (ITS, *rbc*L, *mat*K)
Mercurialis leiocarpa Siebold. & Zucc.	Jeju, Jeju-si, Aewol-eup, *Park 13035* (NIBRVP0000464219)	MT444829, MT447245, MT447324 WBN0358803, WBN0358939, WBN0359019
	Jeju, Jeju-si, Bonggae-dong, *Park 13036* (NIBRVP0000464220)	MT444830, MT447246, MT447325 WBN0358804, WBN0358940, WBN0359020
	Jeju, Jeju-si, Arail-dong, *Kwak et al. mk2013011* (NIBRVP0000428149)	MT444831, MT447247, MT447326 WBN0358805, WBN0358941, WBN0359021
Neoshirakia japonica (Siebold. & Zucc.) Esser	Jeju, Seogwipo-si, Namwon-eup, *Park 13015* (NIBRVP0000464200)	MT444832, MT447248, MT447327 WBN0358806, WBN0358942, WBN0359022
	Jeonnam, Muan-gun, onggye-myeon, *Park 13026* (NIBRVP0000464210)	MT444833, MT447249, MT447328 WBN0358807, WBN0358943, WBN0359023
	Gyeongnam, Hadong-gun, Hwagae-myeon, *Park 13030* (NIBRVP0000464214)	MT444834, MT447250, MT447329 WBN0358808, WBN0358944, WBN0359024
Ricinus communis L.	Gyeongnam, Changwon-si, Jinbuk-myeon, cultivated, *Park 13038* (NIBRVP0000464222)	MT444846, MT447259, MT447341 WBN0358809, WBN0358945, WBN0359025
	Incheon, Seo-gu, Gyeongseo-dong, cultivated, *Ryu celim303* (NIBRVP0000493933)	MT444844, MT447260, MT447339 WBN0358810, WBN0358946, WBN0359026
	Gyeongnam, Changwon-si, Jinbuk-myeon, cultivated, *Park 13057* (NIBRVP0000464241)	MT444845, MT447261, MT447340 WBN0358811, WBN0358947, WBN0359027
	Gyeongnam, Changwon-si, Jinbuk-myeon, cultivated, *Park 13058* (NIBRVP0000464242)	MT444843, MT447262, MT447338 WBN0358812, WBN0358948, WBN0359028
Triadica sebifera (L.) Small	Gyeongnam, Jinju-si, Ibanseong-myeon, Gyeongnam Arboretum, cultivated, *Park 201115* (NIBRVP0000362781)	MT444847, MT447263, MT447342 WBN0358813, WBN0358949, WBN0359029
	Gyeongnam, Jinju-si, Ibanseong-myeon, Gyeongnam Arboretum, cultivated, *Park 13056* (NIBRVP0000464240)	MT444848, MT447264, MT447343 WBN0358814, WBN0358950, WBN0359030
Vernicia fordii (Hemsl.) Airy Shaw	Jeonnam, Yeosu-si, Mipyeong-dong, *Park 13028* (NIBRVP0000464212)	MT444849, MT447265, MT447344 WBN0358815, WBN0358951, WBN0359031
	Jeonnam, Wando-gun, Bogil-myeon, Is. Bogildo, *Park 13029* (NIBRVP0000464213)	MT444850, MT447266, MT447345 WBN0358816, WBN0358952, WBN0359032
	Gyeongnam, Changwon-si, Jinbuk-myeon, *Park 13037* (NIBRVP0000464221)	MT444851, MT447267, MT447346 WBN0358817, WBN0358953, WBN0359033
Phyllanthus urinaria L.	Jeju, Jeju-si, Jocheon-eup, *Kim & Ku YK2008-023-002* (NIBRVP0000206288)	MT444835, MT447251, MT447330 WBN0358818, WBN0358954, WBN0359034
	Cambodia, Mondulkiri Prov., *Kim et al. TCA2009-0841* (NIBRVP0000250961)	MT444836, MT447252, MT447331 WBN0358819, WBN0358955, WBN0359035

Appendix 1. Continued.

Taxon	Voucher	GenBank/WIGIS number (ITS, *rbc*L, *mat*K)
P. ussuriensis Ruprecht & Maximowicz	Jeju, Seogwipo-si, Namwon-eup, *Kim ybku3-13* (NIBRVP0000281601)	MT444837, MT447253, MT447332 WBN0358820, WBN0358957, WBN0359036
	Jeonbuk, Buan-gun, Gyehwa-myeon, *Cheong & Park SNU2011-0055* (NIBRVP0000442869)	MT444838, MT447254, MT447333 WBN0358821, WBN0358958, WBN0359037
Securinega suffruticosa (Pall.) Rehder	Daegu, Dalseong-gun, Gachang-myeon, Mt. Choejeongsan, *Oh et al. celim127* (NIBRVP0000464322)	MT444839, MT447255, MT447334 WBN0358822, WBN0358959, WBN0359038
	Gyeonggi, Namyangju-si, Sudong-myeon, Mt. Chukryeong, *Anonymous sn* (VDWCVP0000001550)	MT444840, MT447256, MT447335 WBN0359209, WBN0359207, WBN035208
	Chungbuk, Danyang-gun, Yeongchun-myeon, Mt. Taehwasan, *Jang 66* (NIBRVP0000139618)	MT444841, MT447257, MT447336 WBN0358823, WBN0358960, WBN0359039
	Jeonnam, Suncheon-si, Woldeung-myeon, Mt. Huiasan, *Lee et al. 6533* (NIBRVP0000269026)	MT444852, MT447258, MT447337 WBN0358824, WBN0358961, WBN0359040