

Taxonomic Re-evaluation of *Megacollybia* Species in Korea

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Abstract The diversity of the genus *Megacollybia* in Korea was examined based on morphological observation and analysis of molecular data. Currently, the genus is consisted of nine species with a global distribution. However, only *M. platyphylla* has been reported in the floral survey of Korea. During our re-evaluation of the taxonomic diversity of *Megacollybia* in Korea, six *Megacollybia* specimens collected in 2012 were identified based on internal transcribed spacer (ITS) sequences and morphology. Here, we report two *Megacollybia* species, *M. clitocyboidea* and *M. marginata*, as newly recorded species from Korea. The microscopic features of the two species are provided in this study. To our knowledge, this is the first phylogenetic analysis of Korean *Megacollybia* species.

Keywords *Megacollybia clitocyboidea*, *Megacollybia marginata*, *Megacollybia platyphylla*, Morphology, Phylogeny, Unrecorded species

Megacollybia platyphylla (Pers.: Fr.) Kotl. & Pouzar, the type species of *Megacollybia* Kotl. & Pouzar (Marasmiaceae, Agaricales, Basidiomycota), is macroscopically characterized by its radially fibrillose-streaked, gray-brown pileus, its very broad, distant, white lamellae, and white mycelial strands attached to the stipe base [1]. This genus was considered monotypic until early 2007. However, Hughes *et al.* [2] confirmed that this genus is consisted of several other species besides *M. platyphylla*, including: *M. clitocyboidea* R. H. Petersen, Takehashi & Nagas; *M. fusca* J. L. Mata, Aime & T. W. Henkel; *M. marginata* R. H. Petersen, O. V. Morozova & J. L. Mata; *M. rodmanii* R. H. Petersen, K. W. Hughes & Lickey (with f. *murina* R. H. Petersen & K. W. Hughes); and *M. subfurfuracea* R. H. Petersen and *M.*

texensis R. H. Petersen & D. P. Lewis; based on internal transcribed spacer (ITS) region of ribosomal RNA and detailed morphological observations [2, 3]. The distribution of the type species *M. platyphylla* is restricted to Europe [2, 3]. Recently, Coimbra *et al.* [4] described another species of *Megacollybia*, *M. rimosa* V. R. M. Coimbra & F. Wartchow, from Brazil mainly based on morphological characteristics (characterized by the rimose to cracked pileus, pruinose stipe surface, clavate to lageniform pileocystidia and presence of clamp-connections). In Korea, only *M. platyphylla* has been reported [5]. In light of the recent revision of the genus *Megacollybia*, it is necessary to re-evaluate the taxonomic diversity of *Megacollybia* species in Korea with molecular data coupled with morphological observations [2]. During a survey of the diversity of Korean mushrooms in 2012, we collected six specimens of *Megacollybia* (KA12-0113, KA12-0146, KA12-0211, KA12-0459, KA12-0596, and KA12-1329). Here, we report two *Megacollybia* spp. new to Korea, *M. clitocyboidea* and *M. marginata*, based on molecular data and morphological observations.

MATERIALS AND METHODS

Specimens and morphological observations. The collected *Megacollybia* specimens (Table 1) were dried and deposited in the herbarium of Korea National Arboretum (KA) (Fig. 1). Reliable DNA sequence data for other *Megacollybia* spp. and outgroup species were retrieved from Genbank based on the study of Hughes *et al.* [2] for the

Mycobiology 2014 March, 42(1): 22-26
<http://dx.doi.org/10.5941/MYCO.2014.42.1.22>
pISSN 1229-8093 • eISSN 2092-9323
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Received October 31, 2013

Accepted February 15, 2014

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Table 1. A list of *Megaacollybia* specimens and outgroup species in phylogenetic analysis

Species	Specimens No.	Locality	Collection date	GenBank No.
<i>Clitocybula oculus</i>	PBM1156	USA, New Hampshire	-	DQ192178
<i>Gerronema strombodes</i>	TENN60718	USA, Tennessee	-	EU623640
<i>G. subclavatum</i>	DAOM 194858	USA, Florida	-	U66434
<i>Megaacollybia clitocyboidea</i>	TENN60766	Russia, Primorsky Territory	-	EU623653
<i>M. clitocyboidea</i>	TENN62231 T	Japan, Hokkaido	-	EU623664
<i>M. clitocyboidea</i>	TENN62229	China, Jilin Provenca	-	EU623665
<i>M. clitocyboidea</i>	KA12-0211	Korea, Gyeonggi, Gwangneung Forest	12 Jun 2012	KF576320
<i>M. clitocyboidea</i>	KA12-0459	Korea, Gyeonggi, Gwangneung Forest	25 Jun 2012	KF576321
<i>M. clitocyboidea</i>	KA12-0596	Korea, Gyeonggi, Gwangneung Forest	13 Jul 2012	KF576322
<i>M. fallax</i>	MICH 45432 T	USA, Idaho	-	EU623722
<i>M. fallax</i>	TENN59447	USA, Washington	-	EU623723
<i>M. fusca</i>	BRG, isotype LSU	Guyana	-	EU623739
<i>M. fusca</i>	AEFM1012 (NY)	Colombia	-	EU623741
<i>M. marginata</i>	TENN60743	Russia, Primorsky Territory	-	EU623681
<i>M. marginata</i>	TENN60752 T	Russia, Primorsky Territory	-	EU623685
<i>M. marginata</i>	TENN58493	Czech Republic	-	EU623689
<i>M. marginata</i>	KA12-0113	Korea, Gyeonggi, Gwangneung Forest	31 May 2012	KF576323
<i>M. marginata</i>	KA12-0146	Korea, Gangwon, Daegwanryeong Natural Recreation Forest	9 Jun 2012	KF576324
<i>M. marginata</i>	KA12-1329	Korea, Ulleung Island, Nari Basin	5 Sep 2012	KF576325
<i>M. platyphylla</i>	TENN59541	Finland	-	EU623691
<i>M. platyphylla</i>	TENN59308	Austria	-	EU623703
<i>M. platyphylla</i>	TENN57963	Germany	-	EU623704
<i>M. platyphylla</i>	TENN58227	Russia, Leningrad Region	-	EU623707
<i>M. rodmani</i>	FCME13275	Mexico	-	EU623758
<i>M. rodmani</i>	TENN59430 T	USA, Tennessee, GSMNP	-	EU623761
<i>M. rodmani</i>	AEFM1333 (NY)	Costa Rica	-	EU623777
<i>M. subfurfuracea</i>	TENN59558 T	USA, Arkansas	-	EU623744
<i>M. texensis</i>	TENN62059 T	USA, Texas	-	EU623730
<i>M. texensis</i>	TENN59935	USA, Texas, Hardin Co.	-	EU623738

Bold-letters indicated the Korean *Megaacollybia* specimens.

T, type-materials.

phylogenetic analyses (Table 1, Fig. 2). Macro-morphological characteristics were determined based on the field notes and color photos of basidiomata. Micro-morphological characteristics of dried specimens were examined after sectioning and rehydrating in 3% KOH solution. Basidia, cheliocystidia, caulocystidia, and basidiospores were stained with 1% phloxine and 1% Congo-red solutions. Microscopic observations were made using an Olympus BX53 microscope and Jenoptik ProgRes C14 Plus Camera (Jenoptik Corporation, Jena, Germany). Measurements of microscopic characteristics were made using the ProgRes Capture Pro v.2.8.8. software (Jenoptik Corporation).

PCR amplification and sequencing. DNA was isolated from fresh fruit bodies (approximately 0.1 g) using a modified CTAB procedure of Doyle and Doyle [6]. ITS regions of the ribosomal RNA were amplified using ITS5 and ITS4 primers. PCR mixtures contained 0.5 pmol of each primer, 0.25 mM dNTPs, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM of MgCl₂, 2.5 U of Taq DNA polymerase, and 15 ng of template DNA. PCR conditions for ITS were as follows: an initial denaturation step at 94°C for 4 min,

followed by 34 cycles at 94°C for 40 sec, 52°C for 40 sec, and 72°C for 60 sec, and a final elongation step at 72°C for 8 min. The PCR products were purified and sequenced by Macrogen Inc., Seoul, Korea. Sequences generated from the present study were deposited in Genbank (Table 1).

Phylogenetic analyses. We edited raw sequences using PHYDIT 3.2 [6]. For phylogenetic analyses, DNA sequences were aligned using ClustalX 1.81 [7] with manual adjustment using PHYDIT. We excluded ambiguously aligned regions in the phylogenetic analyses. Maximum parsimony (MP) analysis was performed using the heuristic search option in PAUP* 4.0 [8] with the following settings: all characters were equally weighted, gaps were treated as missing characters, starting trees were obtained by random addition with 1,000 replicates, and tree bisection-reconnection (TBR) branch swapping algorithm was used. Maximum likelihood (ML) phylogenetic tree with bootstrap analysis was constructed using the general time-reversible (GTR) evolutionary model and the gamma distribution of rate heterogeneity. Nodal supports for MP and ML analyses were estimated using nonparametric bootstrapping with 1,000 replicates. The

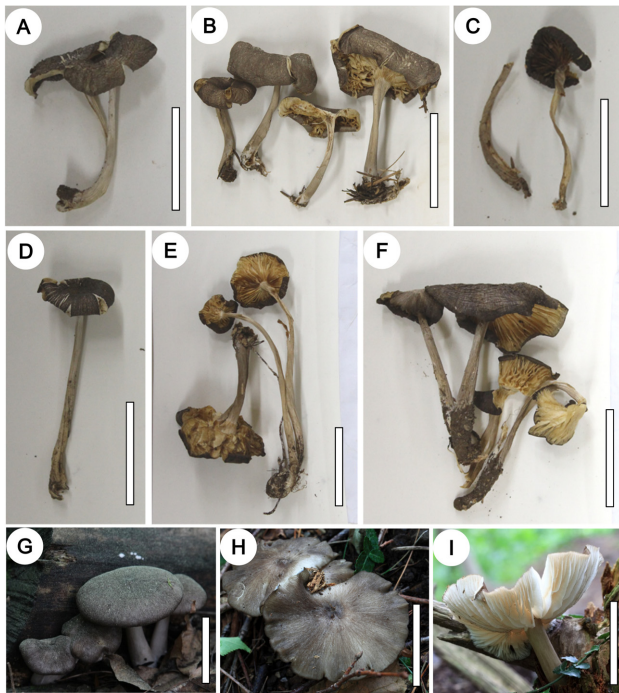


Fig. 1. Korean *Megacollybia* specimens. A~C, G, Fruit-bodies of *M. clitocyboidea* (A, KA12-0211; B, G, KA12-0459; C, KA12-0596); D~F, H, I, Fruit-bodies of *M. marginata* (D, KA12-0113; E, KA12-0146; F, H, I, KA12-1329) (scale bars = ca. 5 cm).

option of each replicate for bootstrapping analyses was chosen as a heuristic search of 100 stepwise random addition replicates and TBR branch swapping for each bootstrap replicate. For Bayesian inference (BI), MrBayes 3.1 [9] was employed to infer the phylogenetic relationships. Posterior probabilities (PP) were approximated using the metropolis-coupled Markov Chain Monte Carlo method. Two parallel runs were conducted with one cold and three heated chains for 5 million generations, respectively, starting with a random tree. The trees were sampled every 100 generations. We interpreted the convergence of two independent runs had converged when the average standard deviation of the split frequencies dropped below 0.01. The trees obtained before the convergence were discarded using the burn-in command, and the remaining trees were used to calculate a 50% majority consensus tree and to estimate PP. PP values below 0.95 were not considered significant. Based on the studies of Moncalvo *et al.* [10] and Hughes *et al.* [2], we selected three species, *Clitocybula oculus* AFTOL-ID 1554, *Gerronema strombodes* TENN60718 and *G. subclavatum* DAOM194858 as outgroups.

RESULTS AND DISCUSSION

Identification of *Megacollybia* species in Korea. The alignment of the ITS regions of ribosomal RNA included 29 taxa and 879 characters, of which 129 were parsimony-

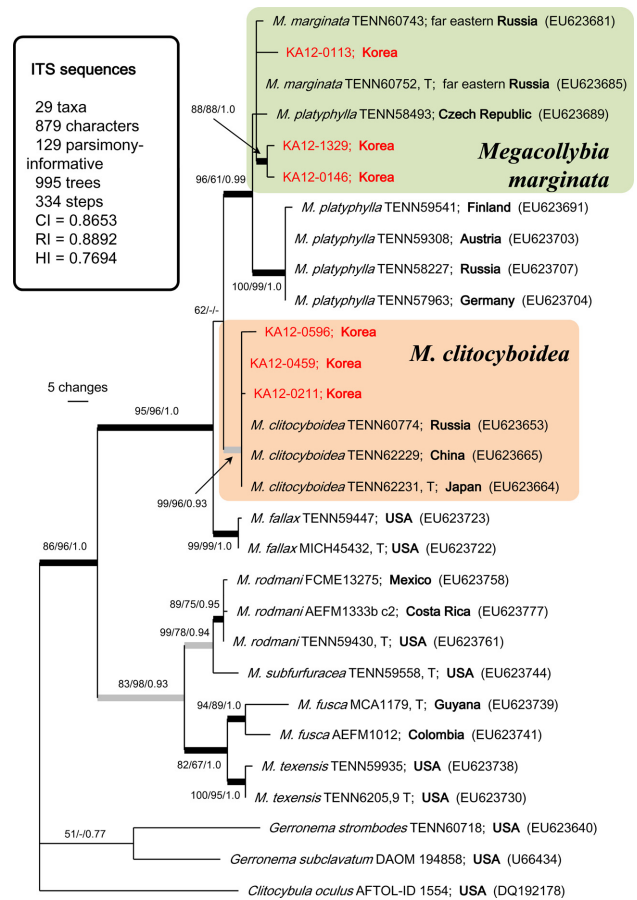


Fig. 2. One of 995 most parsimonious trees from a heuristic analysis of internal transcribed spacer (ITS) sequences. Broad black branches indicate maximum parsimony and maximum likelihood bootstrap support value (MPBS and MLBS) > 70% and Bayesian posterior probabilities (PP) > 0.95 (MPBS/MLBS/PP). Broad gray branches indicate MPBS and MLBS > 70% and 0.89 < PP < 0.95. Only MPBS values > 50% are shown above or below branches. *Clitocybula oculus* AFTOL-ID 1554, *Gerronema strombodes* TENN60718, and *G. subclavatum* DAOM 194858 were used as outgroups.

informative. MP tree was 334 steps long, with a consistency index (CI) of 0.8653, a retention index (RI) of 0.8892, and a homoplasy index (HI) of 0.7694. ML analyses resulted in a tree with a log-likelihood (-ln) of 1553.13. The Bayesian analysis was conducted using GTR + I + G model, and the first 45,000 trees were discarded as burn-in (burninfrac = 0.45) because the ITS dataset of four chains reached convergence after approximately 4,500,000 generations. The phylogenetic inference from MP, ML, and BI analyses was consistent in placing the major clades in phylogenetic relationships. MP tree was presented with MP and ML bootstrap support values and BI-PP for each node (MPBS/MLBS/PP) (Fig. 2).

Six specimens of Korean *Megacollybia* (KA12-0113, KA12-0146, KA12-0211, KA12-0459, KA12-0596, and KA12-1329)

were placed in two separate clades in the present phylogenetic study. Out of these six samples, three *Megacollybia* specimens KA12-12-0211, KA12-0459, and KA12-0596 were placed in the *M. clitocyboidea* clade with high support values (MPBS/MLBS/PP = 99/96/0.93), whereas the remaining three (KA12-0113, KA12-0146, and KA12-1329) were placed in the *M. marginata* clade. Two specimens, KA12-0146 and KA12-1329, formed a group with high support values (MPBS/MLBS/PP = 88/88/1.0) within the *M. marginata* clade that was not strongly supported. Sequence comparisons with *M. marginata* TENN06752 (type material of *M. marginata*) showed that the ITS sequences of KA12-0146 and KA12-1329 were identical to that of *M. marginata* TENN06752, with 99.2% (637/642 bp) and 99.4% (638/642 bp), respectively.

In Korea, species of *Megacollybia* had been identified as *M. platyphylla* because it was considered as a monotypic genus. However, Hughes *et al.* [2] reported that the distribution of *M. platyphylla* may be restricted to Europe and western and central Russia. In our results, none of our six specimens was identified as *M. platyphylla*, which is consistent with the findings of Hughes *et al.* [2]. In the present study, three of our six specimens were identified as *M. clitocyboidea* and the rest of them as *M. marginata*, both of which were previously reported as temperate Asian species [2]. These two species could be morphologically distinguished by the color of pileus disc, cheilocystidia and the characteristics of marginate lamellae [2]. *Megacollybia clitocyboidea* is characterized by the color of pileus disc that is mummy brown to clove brown, later discoloring to grayish brown to yellowish brown. The disc surface is minutely scabrous or furfuraceous and the lamellar edge is not marginate. The stipe is buffy brown to deep olive buff. Its cheilocystidia is not or rarely protruded beyond the basidia and it is conspicuously clamped [2]. On the other hand, *M. marginata* is characterized by the pileus disc usually without scabers or squamules and the lamellar edge is dark brown with distinct margin [2]. Hughes *et al.* [2] reported that a collection of one particular *Megacollybia* species from the Czech Republic (TENN58493, they regarded it as *M. platyphylla*) may represent an intermediate between *M. marginata* and *M. platyphylla*. Although this specimen (TENN58493) lacks marginate gills, it should be considered as *M. marginata* because TENN58493 was placed in the *M. marginata* clade (Fig. 2). To our knowledge, this is the first phylogenetic analysis for the genus *Megacollybia* in Korea. *Megacollybia clitocyboidea* and *M. marginata* are newly recorded species of the genus *Megacollybia* in Korea, and further floral survey is required to comprehensively discuss the distribution of *M. platyphylla* in Korea.

Taxonomic description of two newly recorded *Megacollybia* species in Korea.

Megacollybia clitocyboidea R. H. Petersen, Takehashi & Nagas., Rep. Tottori Mycol. Inst. 45: 17 (2007; [2]) (Figs. 1A~1C, 1G, 3).

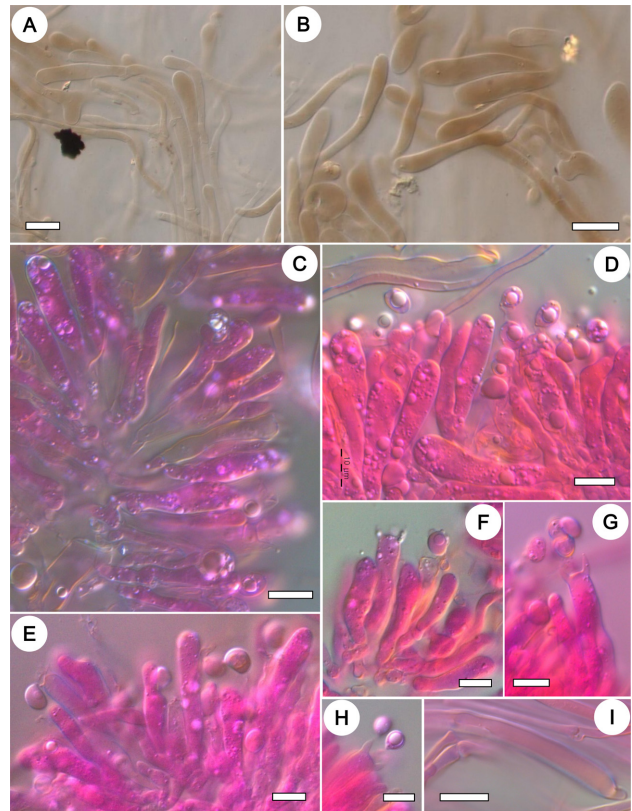


Fig. 3. Microscopic observation of *Megacollybia clitocyboidea*. A, B, Pileipellis terminal cells; C~H, Basidia, cheilocystidia and basidiospores (C, E, Basidia with cheilocystidia; D, F~H, Basidia with basidiospores); I, Caulocystidia (scale bars: A, B, I = 20 μ m; C~H = 10 μ m).

Description: Pileus 30~100 mm diam., surface minutely scabrous or furfuraceous, mummy brown- to clove-colored disc. Lamellae edge not marginate. Stipe 45~110 \times 5~10 mm. Pileipellis slender and radial hyphae; conspicuously clamped; pileipellis terminal cells 30~130 \times 9~25 μ m, digitate, clavate to broadly clavate. Basidia clavate, 25~55 \times 10~15 μ m, conspicuously clamped, 4-spored. Basidiospores broadly ellipsoid to broadly ovate, 6.5~10.0 \times 4.7~7.5 μ m. Cheilocystidia not or rarely protruding, digitate, narrowly or broadly clavate, conspicuously clamped, 24~70 \times 8~20 μ m. Caulocystidia narrowly rounded to subcapitate ends, 28~150 \times 10~17 μ m.

Distribution: Eastern Asia.

Specimens examined: Korea, Gyeonggi, Gwangneung Forest, coll. Han *et al.*, 12 Jun 2012 (KA12-0211), 25 Jun 2012 (KA12-0459), 13 Jul 2012 (KA12-0596).

Megacollybia marginata R. H. Petersen, O. V. Morozova & J. L. Mata, Rep. Tottori Mycol. Inst. 45: 21 (2007; [2]) (Figs. 1D~1F, 1H, 1I, 4).

Description: Pileus 35~90 mm diam., usually without scabers or squamules, dark fuscous brown color disc. Lamellae edge marginate. Stipe 60~110 \times 4~8 mm. Pileipellis terminal cells 32~105 \times 6~25 μ m, clavate to slightly erect; conspicuously clamped. Basidia clavate, thin-walled, 36~45

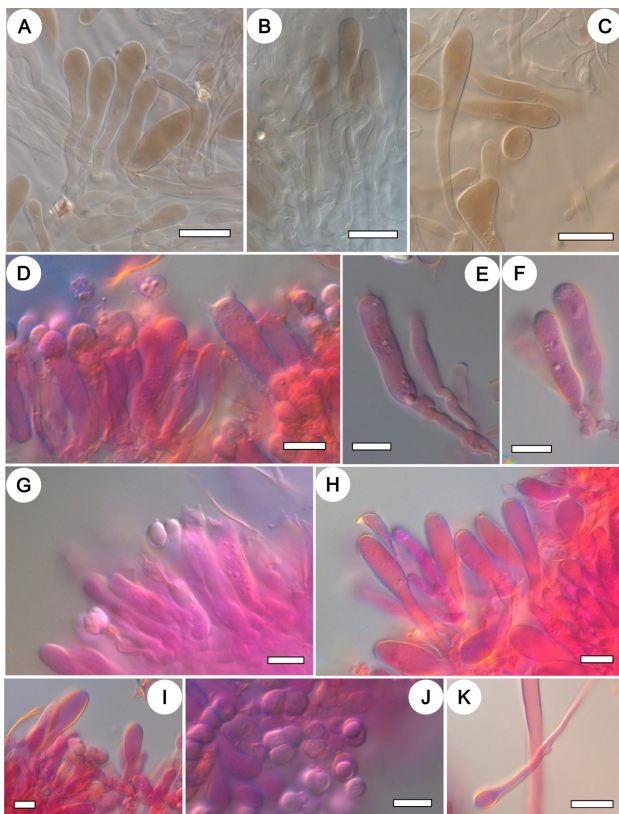


Fig. 4. Microscopic observation of *Megacollybia marginata*. A~C, Pileipellis terminal cells; D~G, Basidia with basidiospores (D, G, Basidia with basidiospores; E, F, Basidia); H, I, Cheilocystidia; J, Basidiospores; K, Caulocystidia (scale bars: A~C, K = 20 μm ; D~J = 10 μm).

$\times 7\text{--}11\ \mu\text{m}$, basal clamped, 4-spored. Basidiospores broadly ellipsoid, elliptical to rarely subglobose, $6.2\text{--}9.6 \times 4\text{--}7.2\ \mu\text{m}$. Cheilocystidia abundant, protruding beyond basidia, clavate to broadly clavate, conspicuously clamped, $30\text{--}70 \times 8\text{--}20\ \mu\text{m}$. Caulocystidia narrowly clavate to subsphaeropedunculate, $35\text{--}85 \times 5\text{--}15\ \mu\text{m}$.

Distribution: Czech Republic, far eastern Russia, Korea.

Specimens examined: Korea, Gyeonggi, Gwangneung Forest, coll. Han *et al.*, 31 May 2012 (KA12-0113); Gangwon, Daegwanryeong Natural Recreation Forest, coll. Han *et al.*, 9 Jun 2012 (KA12-0146); Ulleung Island, Nari

Basin, coll. Han *et al.*, 5 Sep 2012 (KA12-1329).

ACKNOWLEDGEMENTS

This work was supported by the Korea National Arboretum (Project No. KNA 1-1-10) and the research program of agricultural science and technology development (PJ010279) of the National Institute of Horticultural and Herbal Science.

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