Phylogenetic relationships of *Coreanomecon* (Papaveraceae: Papaveroideae), an endemic genus in Korea, using DNA sequences

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(Received 11 November 2018; Revised 24 December 2018; Accepted 28 December 2018)

ABSTRACT: *Coreanomecon* is a monotypic and endemic genus in Korea, distributed mainly in the southern regions. *Coreanomecon* is morphologically similar to *Hylomecon* by producing red latex, easily distinguished from *Chelidonium*, which produces yellow latex. *Coreanomecon* were merged into *Hylomecon* or *Chelidonium* depending on the authors. To understand the phylogenetic relationship of *Coreanomecon*, DNA sequences of chloroplast *rbcL* and *matK* and nuclear Internal Transcribed Spacer (ITS) regions were determined from the species of Papaveroideae (Papaveraceae) in Korea and analyzed with the Maximum Parsimony and Bayesian methods. Phylogenetic analyses of Papaveroideae suggest that *Coreanomecon* is sister to the clade of *Chelidonium* and *Stylophorum* in the ITS data and that it is sister to *Hylomecon* in the chloroplast (cpDNA) data. A constraining analysis using the Shimodaira-Hasegawa test (S-H test) suggested that the ITS data do not reject the sister relationship of *Coreanomecon* and *Hylomecon*. The S-H test also suggested that the cpDNA data is compatible with the placement of *Coreanomecon* as a sister to the clade of *Chelidonium* and *Stylophorum*. Although the conflicting phylogenetic results may stem from insufficient phylogenetic signals, they may also be associated with hybridization between *Hylomecon* and an ancestor of *Stylophorum* and *Chelidonium*. The results of this study suggest that *Coreanomecon* is a distinct lineage as an endemic genus, supporting the morphological data.

Keywords: DNA barcoding, forensic botany, Papaveraceae, species identification

Papaveroideae (Papaveraceae) consists of 23 genera and approximately 240 species, characterized by having milky or colored latex sap, sepals fully enclosing the bud, actinomorphic corollas without spurs, numerous stamens, and a multicarpellate, usually unilocular gynoecium (Kadereit, 1993). Members of Papaveroideae are usually herbs and are distributed mainly in the Northern Hemisphere, extending into Central and South America (Kadereit, 1993). The subfamily was traditionally recognized as a separate family, Papaveraceae (Cronquist, 1988; Takhtajan, 1997; Kim, 2007), but Angiosperm Phylogeny Groups II, III, and IV (The Angiosperm Phylogeny Group, 2003, 2009, 2016) adopted a broad circumscription of Papaveraceae to include Fumariaceae and Pteridophyllaceae (Thorne, 1992; Mabberley, 2008). The more inclusive circumscription of Papaveraceae is supported by recent phylogenetic analyses using molecular and

morphological data (Hoot et al., 1997, 2015; Wang et al., 2009), and it has been used in a broad range of recent literature (Ohwi, 1965; Zhang et al, 2008; Simpson, 2010; Hannan and Clark, 2011).

The diversity of Papaveroideae in Korea is relatively low with eight species in the four genera of *Papaver* L., *Chelidonium* L., *Hylomecon* Maxim., and *Coreanomecon* Nakai (Kim, 2007). The genus *Papaver* consists of approximately 80 species distributed in Central and southwestern Asia, with minor representatives in East Asia, Central and Southern Europe, and North Africa (Kadereit, 1993). *Papaver somniferum* L. is the best known species given its use in the production of opium, the dried latex from the fruits. Opium is the source of many important pharmaceutical drugs, including morphine, heroin, thebaine, codeine, and papaverine. Four species, including cultivated *P. somniferum*, are

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distributed in Korea. Native species of *Papaver* in Korea, *P. coreanum* Nakai and *P. amurense* (N. Busch) N. Busch ex Tolm., are distributed in the northern part of Korea, and their phylogenetic relationships have remained unclear. Owing to the importance in forensic botany to identify *P. somniferum*, the utility of DNA regions, in this case the nuclear Internal Transcribed Spacers (ITS) regions, chloroplast *trnL-trnF* regions and ISSR markers, were evaluated (Lee et al., 2010). These studies, however, did not include any native species of the Korean *Papaver*.

Chelidonium is a monotypic genus distributed in Europe and East Asia, and it is commonly distributed in Korea. *Hylomecon* is also a small genus with two species in East Asia, and *H. vernalis* Maxim. is commonly found in moist places in forests in Korea. *Coreanomecon* is a monotypic and endemic genus in Korea distributed mainly in the southern regions (Son et al., 2012). *Coreanomecon* is morphologically similar to *Hylomecon* by producing red latex, and it is easily distinguished from *Chelidonium*, which produces yellow latex. The taxonomic status of *Coreanomecon* has been controversial. Some authors merged *Coreanomecon* into *Chelidonium* (Ohwi, 1953), and others merged it into *Hylomecon* (Park, 1974; Kadereit, 1993). Palynological studies (Lee and Kim, 1984) and a cladistic analysis of the morphological characters (Kim et al. 1999) have suggested that *Coreanomecon* is distinct from *Chelidonium* and *Hylomecon*. The phylogenetic relationship of *Coreanomecon* according to molecular data have remained unclear.

DNA barcoding is a molecular technique which is used to identify a specimen with DNA sequences of short regions (Hebert et al., 2003; Kress et al., 2005; Xiang et al., 2011). The *rbcL* and *matK* regions in the chloroplast (cpDNA) genome are widely utilized in angiosperms (Hebert et al., 2004; CBOL Plant Working Group, 2009). The utility of other regions of cpDNA, such as *trnH-psbA*, *atpF-atpH*, and *psbK-psbI*, have been shown to be useful for increasing the resolution power (Lahaye et al., 2008; Kim et al., 2014). The ITS regions in nuclear ribosomal DNA have also been used to supplement cpDNA barcodes (Tripathi et al., 2013). DNA barcode regions have been primarily used for identifying unknown specimens at the species level. This type of molecular identification of a species is particular useful when the materials to be identified are fragmentary without having sufficient structures, such as flowers and fruits. Papaveroideae provides an excellent case, as it includes *P. somniferum*, often cultivated in Korea. A regional DNA barcode database for all known species is thus useful for not only applied fields, such as forensic botany, trade control at customs offices, conservation biology, and for the

utilization of plant resources in bio-industries, but also for discovering cryptic species that are difficult to differentiate with morphological features and for reconstructing the phylogenetic relationships of the group of interest.

The objectives of the present study are (1) to infer the phylogenetic relationship of *Coreanomecon*, (2) to examine the implications of the molecular phylogeny on the evolution of the morphological characters of *Coreanomecon*, and (3) to assess the level of differentiation of the species and evaluate the resolution power of DNA barcodes to identify a species of Papaveroideae in Korea, with an emphasis on species of *Papaver*.

Materials and Methods

Taxon sampling

Analyses of Papaveroideae were conducted in three tiers. Analysis 1 includes Korean taxa only to evaluate the level of molecular differentiation among the Korean taxa. All but one species of Papaveroideae distributed in Korea were included in this study, with a total therefore of seven species in four genera (Table 1). *Papaver amurense*, distributed in North Korea and in northeast China, including Inner Mongolia, was not included because samples were not available. Two to six accessions per species were included to represent their distributional range, except for another North Korean species, *P. coreanum* Nakai, in which one sample from Baekdu Mt. was included. All materials for molecular studies were obtained from herbarium specimens deposited at the National Institute of Biological Resources (KB).

Analyses 2 and 3 expanded taxon sampling to infer the phylogenetic relationships of *Coreanomecon* and *P. coreanum* accurately. The chloroplast data from groups closely related to *Coreanomecon*, in this case *Bocconia* L., *Dicranostigma* Hook. f. & Thomson, *Glaucium* Mill., *Eomecon* Hance, *Macleaya* R. Br., *Sanguinaria* L., and *Stylophorum* Nutt., were obtained from GenBank and added to our data set for Analysis 2. Analysis 3 included the nuclear ITS sequences of the tribe Chelidoniae, including *Chelidonium* and *Hylomecon* from foreign populations and those of the *Papaver* clade representing major subclades within the genus (Carolan et al., 2006). Analysis 3 was conducted to infer the placement of *Coreanomecon* and *P. coreanum*, as this has not been accomplished previously.

Gene sampling

Total DNA was extracted from dried leaves of herbarium specimens using a DNeasy Plant Mini Kit (QIAGEN, Hilden,

Table 1. Taxa and voucher information included in this study.

Taxa	Voucher
<i>Chelidonium majus</i> L. var. <i>asiaticum</i> H. Hara	Gyeongsangbuk-do, Yecheon-gun, Hakgasan Mt., 25 May 2015, Kim, Kang, Park & Bae HHU-2015-0297 (KB 542414)
	Gyeongsangnam-do, Hamyang-gun, Gibaeksan Mt., 5 Jun 2015, Ko & Park HNHM-KB-150128 (KB 534818)
	Jeollabuk-do, Sunchang-gun, Gyegwansan Mt., 30 Jul 2015, Im & Park 150905328 (KB 542855)
	Gangwon-do, Yeongwol-gun, Bongraesan Mt., 21 Apr 2012, Yoo & Cheon 453815 (KB 453815)
<i>Coreanomecon hylomeconoides</i> Nakai	Jeollanam-do, Suncheon-si, Jogyesan Mt., 15 Jul 2013, Im & Park Im81911 (KB 494404)
	Jeollanam-do, Gurye-gun, Baekunsan Mt., 15 Jun 2012, Jang 363800 (KB 363800)
	Jeollanam-do, Gurye-gun, Baekunsan Mt., 5 Jun 2013, Kim & Kim seed3048 (KB 433738)
	Chungcheongbuk-do, Jecheon-si, Woraksan Mt., 8 Sep 2007, Kim & Kim 2007-2-012 (KB 531368)
	Jeollanam-do, Suncheon-si, Jogyesan Mt., 13 Oct 2013, Lee 459419 (KB 459419)
<i>Hylomecon vernalis</i> Maxim.	Gangwon-do, Hoengseong-gun, Eoreumgol, 1 May 2014, Kim, Lee & Kim PH140147 (KB 483756)
	Gangwon-do, Taebaek-si, Taebaeksan Mt., 6 Jun 2013, Kim & Kim seed3059 (KB 433749)
	Chungcheongbuk-do, Danyang-gun, Sobaeksan Mt., 9 May 2013, Lim, Nam & Ryu celim061 (KB 464272)
	Jeollabuk-do, Muju-gun, Jeoksangsang Mt., 18 May 2014, Kim & Choi 90080 (KB 512824)
	Chungcheongnam-do, Gongju-si, Geumgyesan Mt., 29 Apr 2015, Kim Geumgye150150 (KB 521098)
	Gangwon-do, Hoengseong-gun, Eoreumgol, 1 May 2014, Kim, Lee & Kim PH140148 (KB 483757)
<i>Papaver dubium</i> L.	Jeju-do, Seogwipo-si, Daejeong-eup, 21 May 2013, Kim & Kim kjs130119 (KB 462723)
	Jeju-do, Jeju-si, Aewol-eup, Gwakji beach, 28 Apr 2016, Kim, An, Hong & Park KimJH16007 (KB 563136)
<i>P. coreanum</i> Nakai	China, Jilin, Antu, Erdaobaihe, Baekdusan Mt., 7 Jul 2012, Hyun NAPI-2012-0582 (KB 412964)
<i>P. rhoeas</i> L.	Gangwon-do, Sokcho-si, Yeongnangho, 28 May 2014, Kim & Kim Youngrangho0259 (KB 479526)
	Gangwon-do, Gangneung-si, Gyeongpoho, 2 Jul 2014, Kim & Kim Gyeongpoho0344 (KB 487035)
	Chungcheongbuk-do, Chungju-si, Sureuisan Mt., 21 Jun 2015, Lee & Lee NAPI-P20150388 (KB 533894)
<i>P. somniferum</i> L.	Gyeonggi-do, Gimpo-si, Yangchon-myeon, 26 May 2012, Kim KIMJH12062 (KB 358615)
	Jeollanam-do, Wando-gun, Cheongsan Island, 28 May 2013, Kim, Kim & Park KIMJH13137 (KB 434554)

Germany). Three DNA barcode regions, specifically chloroplast *rbcl* and *matK* regions and nuclear ribosomal ITS regions, were amplified via polymerase chain reactions (PCR). Primers for the cpDNA barcodes were published in previous studies (Soltis et al., 1992; Sang et al., 1997; Cuénoud et al., 2002; Kress and Erickson, 2007) and are summarized in Kim et al. (2014). The ITS regions were amplified using the primers *its6* and *its9* (Youm et al., 2016). For each PCR reaction, 1 µL of total DNA was included in a 20 µL reaction mixture with Solg EF-Taq DNA polymerase (Solgent, Daejeon, Korea). Amplification of the target regions was conducted with a Veriti thermal cycler (Applied Biosystems, Foster City, CA, USA) under the following conditions: initial denaturation at 95°C for 2 min, 35 cycles each at 95°C for 30 s, at 55°C for 30 s, and at 72°C for 1 min, followed by a final extension at 72°C for 5 min. PCR products were examined on a 1% agarose gel in

1X TBE buffer, purified, and were sent to Solgent (Daejeon, Korea) or Macrogen (Seoul, Korea) for sequencing, where the sequencing reaction was prepared using the same primers used in PCR with BigDye Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems). Sequences were determined using a 3730xl DNA analyzer (Applied Biosystems). Sequences were edited in Sequencher version 5.0 (Gene Codes Corporation, Ann Arbor, MI, USA), aligned manually.

Phylogenetic analysis

The chloroplast DNA barcode data were concatenated and analyzed simultaneously. The ITS data were analyzed separately and in combination with the cpDNA data. *Dicentra* and *Corydalis* were used as outgroups. Phylogenetic analyses were conducted using the maximum parsimony (MP) method in PAUP* (Swofford, 2002). All characters were treated as

unordered and were weighted equally in the MP analyses. Gaps resulting from multiple alignments of indels were treated as missing data. Heuristic searches were used with 100 replicates of random sequence additions with tree bisection-reconnection branch swapping, with all of the best trees saved at each step (MulTrees). Bootstrap analyses (Felsenstein, 1985) of 1,000 pseudoreplicates were conducted with heuristic searches, a simple sequence addition in PAUP* to evaluate the support for each clade.

Bayesian phylogenetic analyses (BI) were performed with the program MrBayes version 3.2.5 (Huelsenbeck and Ronquist, 2001). A Markov chain Monte Carlo (MCMC) algorithm was employed for 2,000,000 generations, sampling trees every 100 generations, with four independent chains running simultaneously. Priors were set based on the Akaike information criterion (AIC) using modeltest (Posada and Crandall 1998). The first 4,000 trees (400,000 generations) were discarded as “burn-in,” and the remaining trees for which the log-likelihood values had reached a plateau were imported in PAUP* to calculate the posterior probability of each clade.

Hypothesis testing

A specific phylogenetic hypothesis was tested using the Shimodaira-Hasegawa (1999; SH) test, as implemented in PAUP*. In this analysis, MP trees in extended ITS data in Analysis 2 were generated while constraining the topology using the heuristic search method described previously. Three constraints were tested: (1) forcing *Coreanomecon* and *Hylomecon* monophyletic, (2) *Coreanomecon* and *Chelidonium* monophyletic, and whether (3) *Coreanomecon* is a sister to the

clade of *Chelidonium* and *Stylophorum*. The topology within the *Papaver/Meconopsis* and *Eomecon/Glaucium* clades was collapsed in the constraint, leaving no effect on the reconstruction of the MP tree in the given data set. The resultant MP trees with the constrained relationships were evaluated with the original MP trees without the constraint. The objective of the constraint analysis was to test whether each data set does not reject the hypothesis that *Coreanomecon* is congeneric to *Hylomecon* or *Chelidonium*. For the SH test, 10,000 bootstrap replicates were re-sampled using the re-estimated log likelihood (RELL) method.

Assessment of utility of DNA barcodes

The resolution ability of the species for each DNA barcode and a combinatory DNA barcode were examined based on the percentage of monophyletic species given the MP and BI trees (Kim et al., 2014; Youm et al., 2016). When only one accession in a species was included, it was treated as monophyletic if the branch leading to the accession was greater than zero.

Results

The final alignment of the cpDNA data (*rbcL* and *matK*) includes 1,371 sites, among which 146 were parsimoniously informative (10.6%) (Table 1). When outgroups were excluded, 113 sites were informative (8.2%). The ITS data consist of 659 sites, of which 221 were parsimoniously informative (33.5%). For the ingroups, only 191 were informative (29.0%). The alignment of the combined data of the Korean taxa included 24 sequences with 2,030 sites, among which 367 were

Table 2. Statistics of DNA barcode regions for Papaveroideae in this study. Numerals in parentheses represent comparisons of in-group taxa only. Species resolutions and inter- and intraspecific distances were calculated in the matrices with the Korean taxa only.

	<i>rbcL</i> + <i>matK</i>	ITS	<i>rbcL</i> + <i>matK</i> + ITS	cpDNA extended	ITS extended
Number of sequences	25	24	24	30	63
Number of sites	1,371	659	2,030	1,358	753
Number of variable sites	232 (137)	303 (230)	535 (367)	285 (204)	442 (430)
Number of parsimony-informative sites	146 (113)	221 (191)	367 (304)	167 (137)	359 (347)
Number of MP trees	1	4	3	1	42
Tree length	282	522	810	387	1,409
CI, excluding autapomorphy	0.8901	0.7746	0.8026	0.7833	0.5130
RI	0.9725	0.9250	0.9374	0.9294	0.8546
Species resolution power, MP, BI (%)	100, 100	100, 100	100, 100	—	—
Intraspecific distance, minimum-maximum	0.0–0.0022	0.0–0.0317	0.0–0.0100	—	—
Interspecific distance, minimum-maximum	0.0769–0.0793	0.3002–0.3199	0.1345–0.1383	—	—

Fig. 1. Phylogenetic analysis of Papaveroideae (Analysis 1). **A.** A single most parsimonious tree from the analysis of *rbcL* and *matK* regions; **B.** One of four maximum parsimony (MP) trees from the analysis of the Internal Transcribed Spacer (ITS) data; **C.** Strict consensus tree of the combined chloroplast DNA (cpDNA) and ITS data; **D.** different topologies in the MP analysis and Bayesian analyses of combined data sets. The bootstrap value and Bayesian posterior probability for each clad are indicated on the branch. Numbers following the species name are DNA accession numbers to distinguish each individual.

Fig. 2. Strict consensus tree from the phylogenetic analysis of the concatenated *rbcL* and *matK* data (Analysis 2). The bootstrap value and Bayesian posterior probability for each clade are indicated on the branch. Numbers following the species name are DNA accession numbers to distinguish each individual. Sequences downloaded from GenBank are indicated with the GenBank accession number following the species.

parsimoniously informative. In the extended data of cpDNA and ITS, which had more taxa of Papaveroideae for Analyses 2 and 3, the alignments include 1,358 and 753 sites, respectively, of which 167 (12.3%) and 359 (47.7%) were

parsimoniously informative (Table 2).

A phylogenetic analysis of the Korean cpDNA data resolved well-supported clades of *Coreanomecon*, *Chelidonium*, *Hylomecon*, and *Papaver* (Fig. 1A). *Coreanomecon* was

Fig. 3. Strict consensus tree from the phylogenetic analysis of the ITS regions (Analysis 3). The bootstrap value and Bayesian posterior probability for each clade are indicated on the branch. Numbers following the species name are DNA accession numbers to distinguish each individual. Sequences downloaded from GenBank are indicated with the GenBank accession number following the species.

supported as a sister to *Hylomecon*. The ITS data also showed that each genus was distinct and well supported (Fig. 1B). However, *Coreanomecon* is sister to *Chelidonium* in the nuclear data (Fig. 1B). A maximum parsimony analysis of combined cpDNA and ITS data produced an unresolved relationship regarding *Coreanomecon* (Fig. 1C). One of the three MP trees placed *Coreanomecon* as a sister to *Hylomecon* and *Chelidonium* and the remaining two trees generate the sister relationship between *Coreanomecon* and *Chelidonium* (Fig. 1D). A Bayesian analysis of the combined data supported the contention that *Coreanomecon* is sister to *Hylomecon* (Fig. 1D). Within the *Papaver* clade, *P. coreanum* is sister to the remaining species, and each species was clearly supported as monophyletic (Fig. 1).

The extended cpDNA data, in which more closely related taxa of *Coreanomecon* and *Papaver* were added to gain a better understanding of the phylogenetic relationship of the endemic genus, are consistent with the cpDNA data with Korean taxa only (Fig. 2). *Stylophorum*, not included in Analysis I (Fig. 1), was sister to *Chelidonium*, and *Coreanomecon* was placed to a sister of *Hylomecon* with strong support. The *Glaucium*/*Dicranostigma* clade was sister to the clade of *Chelidonium*/*Coreanomecon* (Fig. 2). *Eomecon* and *Sanguinaria* formed a strongly supported clade. The phylogenetic relationships among *Macleaya*, the *Eomecon* and *Sanguinaria* clade, and the *Chelidonium*/*Coreanomecon* clade were unresolved. Monophyly of these genera consisting of the tribe Chelidoneae was weakly supported (Bootstrap support of 64% and Bayesian post-probability of 0.6) (Fig. 2). *Papaver* was strongly supported as sister to the tribe Chelidoneae.

Phylogenetic analysis of the extended ITS data showed that *Coreanomecon* is sister to the clade of *Chelidonium* and *Stylophorum* with weak support (Fig. 3). *Stylophorum* was not supported as monophyletic, as *S. diphyllum* Nutt. is more

closely related to *Chelidonium* than it is to *S. lasiocarpus* (Oliv.) Fedde. *Hylomecon* is sister to the clade of *Chelidonium*, *Stylophorum*, and *Coreanomecon*. Other genera in the tribe Chelidoneae, in this case *Glaucium*, *Eomecon*, and *Dicranostigma*, formed a clade with moderate support. *Papaver* and *Meconopsis* together formed a strongly supported monophyletic group (Fig. 3).

Three phylogenetic hypotheses (Fig. 4) were tested using the Shimodaira-Hasegawa test. Enforcing a sister relationship of *Coreanomecon* and *Hylomecon* (Fig. 4A) in the ITS data, both for Korean taxa only and extended samples, required five additional steps (Table 3). The Shimodaira-Hasegawa test did not reject the alternative topology. Enforcement of the sister relationship of *Coreanomecon* and *Chelidonium* (Fig. 4B), as suggested in the ITS data with the Korean taxa only (Fig. 1B), required three additional steps in the cpDNA data with Korean taxa only, and the alternative topology was compatible with the cpDNA data in the S-H test. However, eight additional steps were required to generate the sister relationship of *Coreanomecon* and *Chelidonium* in both cpDNA and ITS data with extended taxon samples, and this relationship was significantly rejected in the S-H test. Placement of *Coreanomecon* as a sister to the clade of *Chelidonium* and *Stylophorum* (Fig. 4C) was not available in the data sets with Korean taxa only because *Stylophorum* is absent in the data. This topology required only three additional steps in the cpDNA data, and the S-H test indicated that it was congruent with the cpDNA data.

The chloroplast *rbcL* and *matK* data and the ITS data clearly resolved all of the species of Papaveroideae in Korea (Fig. 1). MP and Bayesian analyses of separate and combined data suggested that all of the species in Korea, including the four species of *Papaver*, are distinguishable according to their DNA barcodes. The resolution power of the DNA barcodes for the

Fig. 4. Schematic diagram of the topologies used in the constraint analyses. Specific relationships within a triangle, not shown in this figure for brevity, were not constrained in the analysis. **A.** *Coreanomecon* is forced to be a sister to *Hylomecon*; **B.** *Coreanomecon* is made to be a sister to *Chelidonium*; **C.** *Coreanomecon* is enforced to be a sister to the clade of *Stylophorum* and *Chelidonium*.

Table 3. Tree lengths in the MP analysis, log likelihood scores in the ML analysis, and the significance levels in the Shimodaira-Hasegawa (S-H) test for constrained topology. Differences in tree lengths and likelihood scores between the best and constrained trees for each data point are indicated in parenthesis. Schematic diagram of constrained topology is shown in Fig. 4

Data	Statistics	Constrained topology		
		A	B	C
cpDNA (Korean)	Length (MP)	282	285 (+3)	Not available
	-ln (ML)	3,467.365	3,461.414 (+5.95)	
	<i>p</i> -value	Best tree	<i>p</i> > 0.05	
ITS (Korean)	Length (MP)	527 (+5)	522	Not available
	-ln (ML)	5,223.491 (+29.94)	5,193.6543	
	<i>p</i> -value	<i>p</i> > 0.05	Best tree	
cpDNA (extended)	Length (MP)	387	395 (+8)	390 (+3)
	-ln (ML)	4,168.107	4,202.249 (+34.14)	4,176.214 (+8.10)
	<i>p</i> -value	best tree	<i>p</i> < 0.05*	<i>p</i> > 0.05
ITS (extended)	Length (MP)	1,414 (+5)	1,417 (+8)	1,409
	-ln (ML)	7,440.479 (+2.83)	7,557.902 (+20.25)	7,437.650
	<i>p</i> -value	<i>p</i> > 0.05	<i>p</i> < 0.05*	Best tree

Korean Papaveroideae was 100% (Table 2). The minimum value of the interspecific distances among the species in the cpDNA barcode regions were up to 34 times higher than the maximum value of the intraspecific distance in the three species (Table 2). Similarly, the minimum value of the interspecific distances among the species in the ITS barcode were nine times higher than maximum intraspecific value.

Discussion

The chloroplast *rbcL* and *matK* nucleotide sequences and nuclear ITS sequences in a phylogenetic analysis resulted in well-resolved and well-supported phylogenetic trees. Members of Chelidoniaceae, including the Korean endemic genus *Coreanomecon*, are strongly supported as monophyletic (Figs. 1–3). In the phylogenetic trees with the Korean taxa only (Fig. 1), all of the species were resolved as distinct lineages. This suggests that the two cpDNA barcode regions and ITS regions are useful to identify incomplete materials that do not bear adequate characters, such as flowers and fruits.

Our phylogenetic analyses of nuclear and chloroplast data show that the position of *Coreanomecon* is ambiguous. *Coreanomecon* was consistently placed as a sister to *Hylomecon* in the cpDNA data (Figs. 1, 2). The nuclear data indicated that it is sister to the *Stylophorum* and *Chelidonium* clade (Fig. 3). The constraint analysis results suggest that the two different positions of *Coreanomecon* (Fig. 4) are not strongly in conflict with each other. The sister relationship

between *Hylomecon* and *Coreanomecon* represented in the cpDNA data was not rejected in the ITS data by the S-H test (Table 3). Likewise, placement of *Coreanomecon* as a sister to the clade of *Stylophorum* and *Chelidonium* as represented in the extended ITS data was not rejected in the cpDNA data by the S-H test (Table 3).

It may be that phylogenetic signals from the cpDNA and ITS data are not enough to resolve these relationships. In the extended cpDNA data, only three additional steps are needed to place *Coreanomecon* as a sister to the *Stylophorum* and *Chelidonium* clade (Table 3). In the extended ITS data, the bootstrap support value for the placement of *Coreanomecon* as a sister to the *Stylophorum* and *Chelidonium* clade is less than 50%, and the posterior probability is very low (Fig. 3). There are five additional steps are required to enforce *Coreanomecon* as a sister to the *Hylomecon* (Table 3). Concatenation of data does not help to resolve this issue. Our analysis of combined cpDNA and ITS data generated equivocal topologies (Fig. 1C and D).

Alternatively, the weakly conflicting results from the phylogenetic analysis between the cpDNA and the ITS data may have been associated with hybridization. In this scenario, *Coreanomecon* may have derived from hybridization between *Hylomecon* and an ancestor of the *Stylophorum* and *Chelidonium* clade. Plastid DNA is shown to be inherited maternally in *Chelidonium*, *Macleaya*, and *Papaver* in Papaveroideae (Zhang et al., 2003). If we assume the maternal inheritance of cpDNA in *Hylomecon* and *Coreanomecon*,

Hylomecon would have been an ovulate parent for *Coreanomecon*, as the cpDNA trees placed *Coreanomecon* as a sister to *Hylomecon* (Fig. 2). The paternal parent would have been a stem lineage of the *Stylophorum* and *Chelidonium* clade, as the ITS trees suggest that *Coreanomecon* is a sister to the clade (Fig. 3). The branch length leading to *Coreanomecon* is as long as that to *Hylomecon* (Fig. 1A) or to *Chelidonium* (Fig. 1B), suggesting that the hybridization event may be old.

The morphology of *Coreanomecon* is unique in the tribe Chelidoniae. *Coreanomecon* has 12-pericarpate pollen, whereas *Hylomecon*, *Chelidonium*, *Stylophorum lasiocarpum* have the 3-colpate type (Lee and Kim, 1984; Kim et al., 1999). *Stylophorum diphyllum* possesses 12-colpate, rugate, or formaninous pollen (Lee and Kim, 1984). *Coreanomecon* is the only taxon that has scapose inflorescence in the tribe Chelidoniae. Although a mosaic pattern having both of parental characters or an intermediate form is commonly found in hybrids at early generations, the exhibition of novel characters is common in hybrid taxa at high proportions in angiosperms (Rieseberg and Ellstrand, 1993; Rieseberg et al., 2000). More data are needed for further testing of the origin of *Coreanomecon*.

The S-H test indicates that a hypothesis of a sister relationship between *Coreanomecon* and *Chelidonium* significantly conflicts with both the cpDNA and the ITS data when more taxa are included. The S-H test and phylogenetic analyses of DNA sequence data indicate that inclusion of *Coreanomecon* in *Chelidonium*, as was done by Ohwi (1965), is not supported (Table 3). The two genera differ in terms of several morphological characteristics. The leaves of *Coreanomecon* form a rosette, while those of *Chelidonium* are attached onto a branched stem. The inflorescence of *Coreanomecon* is borne from the base of the shoot system and does not have leaves, forming a scape, whereas the inflorescence of *Chelidonium* is an umbel borne at the terminal of the branch or at the axil of the leaves. *Coreanomecon* produces orange to red sap and *Chelidonium* has yellow sap.

Coreanomecon is morphologically similar to *Hylomecon*; both show similar leaf shapes and sap colors, which lead many authors (Lee, 1973; Park, 1974; Kadereit, 1993) to recognize *Coreanomecon* as a part of *Hylomecon*. However, there are significant morphological differences in terms of the inflorescence and pollen type between the taxa, as described above. Our molecular data show that *Coreanomecon* form a separate clade. Therefore, the morphological and molecular data indicate that *Coreanomecon* should be recognized as a genus endemic to Korea (Nakai, 1935; Lee, 1980; Kim, 2007; Chung et al., 2017).

The phylogenetic analysis of the extended ITS data show that *P. coreanum* is sister to *P. anomalum* Fedde, belonging to the clade of sect. *Meconella* (Fig. 3). Members of the sect. *Meconella* are widely distributed at high latitudes in eastern Asia, Greenland, in northern Canada, and in mountainous regions of Europe and the western North America (Rändel, 1974; Kadereit, 1988). The sect. *Meconella* is characterized by filiform filaments, pale anthers, and valvately dehiscent capsules (Carolan, 2006). *Papaver coreanum* match the sectional diagnostic characters well.

The cpDNA and ITS markers provided a high degree of species resolution and resolved all of the species of Papaveroideae in Korea, separately or in combination (Table 2). Adding more taxa (Figs. 2, 3) led to identical results. Considering the economic and forensic importance of Papaveroideae, especially *P. somniferum*, the results of our study are consistent with Lee et al. (2010), showing that DNA barcode regions are highly useful in Papaveroideae.

Acknowledgments

We are grateful to Jung Won Youm, Yun Gyeong Choi, Dong-Hyuk Lee, and Chae Eun Lim for their help at various stages of this study. We thank the curator and other herbarium staff at KB who generously allowed us to obtain plant samples, as well as two anonymous reviewers for their critical reviews of the manuscript. This work was supported by a research grant, NIBR-201714101, from the National Institute of Biological Resources of Korea.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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