GMR
The Original by FUNPEC-RP

Phylogenetic analyses and species delimitation in the section *Corydalis* (Papaveraceae) based on chloroplast markers

A. Oh

Department of Biology, Chungbuk National University, Cheongju, South Korea

Corresponding author: A. Oh
E-mail: ohamiohami@gmail.com

Genet. Mol. Res. 21 (1): gmr18996
Received November 30, 2021
Accepted December 21, 2021
Published January 31, 2022
DOI http://dx.doi.org/10.4238/gmr18996

ABSTRACT. The genus *Corydalis* (Papaveraceae), which is distributed in temperate regions of the northern hemisphere, has been taxonomically studied mainly on the basis of morphological and molecular genomic information. In the present study, 14 species that belong to the Korean section *Corydalis* were collected in South Korea and phylogenetically analyzed using four chloroplast genomic regions, which include *matK*, *rbcL*, *rpL16* genes and the *trnG* intron. The author tried to include the nuclear Internal Transcribed Spacer (ITS) region in the phylogenetic analysis; however, multiple PCR bands and various band sizes observed led to the conclusion that the ITS region is not suitable for the phylogenetic study of *Corydalis*. When the four chloroplast genomic regions were separately analyzed, different levels of resolution for species delimitation were observed, and in most cases the resolution levels were quite low. When *matK* and *rpL16* were concatenated, the highest resolution for species delimitation was observed. However, when other regions were added to this concatenated region to improve the resolution, the resolution decreased, which was in contrast to the author's expectation and deserves further analysis. At the same time, the author observed inconsistencies between the previously established taxonomy based on morphology and the molecular phylogenies in the present study. This discrepancy needs to be addressed in further detail, so that the

taxonomy of the genus *Corydalis* can fully incorporate both morphology and molecular genomic information. Overall, the present study provides insights into the taxonomy of *Corydalis*, and clearly demonstrates that proper combinations of chloroplast markers can lead to successful discrimination of the species in this genus. Indeed, this study suggests ways to better utilize phylogenetic analyses and species delimitation in this interesting and complicated taxon. Since *Corydalis* is taxonomically challenging and widely used as medicinal plants in Asia, this study can be a valuable source of information on this genus.

Key words: Chloroplast genome; *Corydalis*; Phylogeny; Species delimitation; Taxonomy

INTRODUCTION

The genus *Corydalis* DC., which belongs to the family Papaveraceae, consists of about 440-465 species worldwide (Liden, 1996; Zhang et al., 2008). This genus can be distinguished from other genera within the same family on the basis of various morphological characteristics, including symmetrical flowers, racemose inflorescence, and seeds with elaiosomes. *Corydalis* is distributed in temperate regions of the northern hemisphere, including northeastern Asia and the Himalayas (Oh, 1999; Oh et al., 2004; Ren et al., 2018). Notably, 357 species and 262 endemics belonging to this genus are found in China (Zhang et al., 2008). Liden (1996) reported that Chinese and Tibetan regions are the centers of distribution for this genus based on the fact that all sections of *Corydalis* are present in these regions.

Since De Candolle described the genus *Corydalis* for the first time in 1805, Hooker and Thomson (1855), Fedde (1936), Liden (1996), and other researchers have conducted taxonomic studies on it. *Corydalis* was considered to belong to the family Fumariaceae by some researchers (Lidén, 1996; Oh, 1999; Oh et al., 2004; Oh et al., 2010). However, recently, Papaveraceae is more widely recognized as the family of *Corydalis* (Jiang et al., 2018; Ren et al., 2019; Li et al., 2020; Xu and Wang, 2021). Palibin (1898) first studied Korean *Corydalis* species, and subsequently, Komarov (1903), Nakai (1909, 1914, 1952), and other taxonomists recorded different numbers of species, varieties and formae for this genus. Oh (1986) conducted comprehensive research on Korean *Corydalis* and reported that the genus *Corydalis* consists of four sections: section *Corydalis*, section *Duplotuber*, section *Ramoso-sibiricae*, and section *Sophorocapnos* (Oh, 1999). However, although ample studies have been conducted on the genus *Corydalis*, it is generally recognized that its taxonomy is difficult to elucidate because of its complicated and diverse morphological characters (Ren et al., 2018; Xu and Wang, 2021). Some phylogenetic studies which used different molecular markers were conducted to understand the taxonomy of *Corydalis* (Liden et al., 1995; 1997), but accurate species-level systematics remains incomplete. Species delimitation using the phylogenetic approach is a critical issue in understanding the taxonomy of *Corydalis*, and needs to be further explored.

In phylogenetic studies, some genetic markers are globally used across many different taxa. The Internal Transcribed Spacer (ITS) region of nuclear ribosomal DNA is one of the most popular nuclear markers that is considered to be a useful barcode in plant systematics. It is generally known that ITS can be used to reveal low-level taxonomy, including the relationships between close genera or species within the same genus (Yuan et al., 1996; Alvarez and Wendel, 2003; Mort et al., 2007). Chloroplast genome sequences are also commonly used markers for plant phylogenetic studies (Shaw et al., 2005; Mort et al., 2007; Dong et al., 2012). In addition, recently, complete chloroplast genome sequences are being much investigated as well for the taxonomy and the phylogenetic analyses of the target plants (Wu et al., 2020; Liu et al., 2021). In previous phylogenetic studies on *Corydalis*, ITS and chloroplast markers have been used and their value as DNA barcodes has been assessed (Jiang et al., 2018; Ren et al., 2019).

In the present study, the author provides phylogenetic relationships within the Korean section *Corydalis* using four chloroplast genome sequences, which are *matK*, *rbcL*, *rpL16* genes and the *trnG* intron. By generating phylogenies within section *Corydalis*, the author aimed to enhance the general understanding for the taxonomy of this taxon and to unravel whether the markers used here are useful tools for species delimitation in section *Corydalis*. In addition, the author aimed to explain the inconsistency between the phylogenetic analysis in this study and previously established taxonomy, which is based on morphology. As some markers used in the present study have been previously used in the phylogenetic studies on *Corydalis*, the author expected to effectively complement previous studies on the same taxon as well. Given that chloroplast markers are generally used in phylogenetic studies, this study can address the important and fundamental questions on the utility of chloroplast. Moreover, since *Corydalis* is widely used as medicinal plants in Asian regions, the present study which focuses on species delimitation can greatly contribute to the effective use of this genus.

MATERIAL AND METHODS

Sample collection

All 32 samples used in this study were collected from South Korea (Table 1). Thirteen clearly identified species and one unidentified taxon (*Corydalis* sp.) belonging to the section *Corydalis* were analyzed. Two to three individuals from different locations were used for each species. In the case of *Corydalis alata*, only one individual was used. *Corydalis heterocarpa* and *Corydalis speciosa*, which belong to the section *Sophorocapnos*, were designated as outgroups. The previously established taxonomic classifications used as references were generally based on morphological information.

Genomic DNA was extracted from leaf tissues using the DNeasy Mini Kit (QIAGEN, Seoul, Korea). When a fresh leaf was not available, a dried leaf from a voucher specimen was used instead; 100 mg of fresh leaf tissue and 20 mg of dried leaf

tissue from the voucher specimens were used for the extraction. The protocol provided by the manufacturer was used for DNA extraction.

Table 1. Species used in this study of the section *Corydalis* and the sampling sites in South Korea.

Specie	Site
Corydalis albipetala	Gapyeong county, Nojeok mountaintop
Corydalis albipetala	Pyeongchang county, Woljeong temple
Corydalis albipetala	Pyeongchang county, Woljeong temple entrance
Corydalis turtschaninovii	Yeongju city, Geumgye lake
Corydalis turtschaninovii	Wonju city, Anchang town
Corydalis humilis	Yeongju city, Sobaek mountain, Choam temple
Corydalis humilis	Namyangju city, Chukryeong mountain
Corydalis namdoensis	Yeongcheon city, Bohyeon mountain
Corydalis namdoensis	Kyeongju city, Toham mountain
Corydalis remota	Hapcheon county, Odo mountain
Corydalis remota	Cheongyang county, Chilgap mountain, Chilgap large bridge
Corydalis lineariloba	Taebaek city, Baekdan temple
Corydalis lineariloba	Jeongseon county, Hambaek mountain
Corydalis hallaisanensis	Seoguipo city, 1100 highland
Corydalis hallaisanensis	Jeju city, Sangumburi crater
Corydalis alata	Donghae city, Chorok mountaintop
Corydalis maculata	Namyangju city, Cheonma mountain
Corydalis maculata	Bonghwa county, Cheongryang mountain
Corydalis cornupetala	Kyeongsan city, Kyejeong forest
Corydalis cornupetala	Kyeongsan city, Kyejeong forest 2
Corydalis filistipes	Wooloong county, Hongmun town
Corydalis filistipes	Wooloong county, intake station
Corydalis grandicalyx	Wonju city, Soyongsodong valley
Corydalis grandicalyx	Gapyeong county, Ajaebi hill
Corydalis grandicalyx	Yangpyeong county, Nojeok mountaintop
Corydalis hirtipes	Inje county, Yongdae town, Majang space 1
Corydalis hirtipes	Inje county, Yongdae town, Majang space 2
Corydalis sp.	Gongju city, Gyeryong mountain, Donghak temple
Corydalis sp.	Jangseong county, Naejang mountain, Gain town
Corydalis sp.	Soonchang county, Gangcheon mountain
Corydalis heterocarpa	Gongju city, Gyeryong mountain, Gap temple
Corydalis speciosa	Eumseong county, Gaseop mountain

DNA extraction, PCR and sequencing

To perform PCR amplification of five genomic regions, namely the ITS region in the nucleus and *matK*, *rbcL*, *rpL16*, and *trnG* intron in the chloroplast, five previously reported primer pairs were used. The primer sequences are provided in Table 2. PCR was performed using the ABI ProFlex PCR System (Applied Biosystems, Seoul, Korea) in a 25 μL mixture consisting of 2 μL template DNA, 2.5 μL 10X buffer, 2 μL dNTP, 1 μL primer, 0.25 μL IP-pro-taq, and 17.25 μL DW. The same PCR conditions were used for all five genomic regions. The PCR conditions were as follows: initial denaturation at 94°C for 4 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 53°C for 30 sec, extension at 72°C for 1 min 30 sec, and final extension at 72°C for 10 min.

Sequencing was performed in both directions using the ABI 3730xl Analyzer (Applied Biosystems, Seoul, Korea) and the primers used for sequencing were the same as those used for PCR amplification. The generated sequence data were assembled using SeqMan® (DNASTAR) software. PCR amplification of the nuclear ITS region

did not generate meaningful results that could be further analyzed. Therefore, only the four chloroplast genomic regions were sequenced.

Table 2. Information for primers used in PCR amplification and sequencing of *Corydalis* spp.

Region	Primer	Sequences	References
nrITS	ITS5	GGAAGTAAAAGTCGTAACAAGG	White et al. 1990
	ITS4	TCCTCCGCTTATTGATATGC	
matK	*matK*1166	GGCTTACTAATGGGAT	Perez-Gutierrez et al. 2015
	*matK*192	CGGGTTGCAAMAATAAAGGA	
rpL16	*rpL16*F71	GCTATGCTTAGTGTGTGACTCGTTG	Shaw et al. 2005
	*rpL16*R1516	CCCTTCATTCTTCCTCTATGTTG	
trnG intron	3'*trnG*	GTAGCGGGAATCGAACCCGCATC	Shaw et al. 2005
	5'*trnG*2G	GCGGGTATAGTTTAGTGGTAAAA	
rbcL	*rbcL*-1F	ATGTCACCACAAACAGAAAC	Kress et al., 2005
	rbcL-724R	TCGCATGTACCTGCAGTAGC	

Phylogenetic analysis

The sequences were aligned using MUSCLE (Edgar, 2004) implemented in MEGA7 (Kumar et al., 2016) software. Phylogenetic trees were constructed using MEGA7, the same software. The four chloroplast genomic regions were analyzed separately or in combination. Maximum Likelihood (ML) approach was used for phylogenetic analyses.

RESULTS

When PCR amplification of the nuclear ITS region was performed, more than one band were observed in many cases, and the sizes of these bands were highly variable. Therefore, this region could not be used for phylogenetic analyses. The observation of unexpected PCR band numbers and sizes in the ITS region led to the conclusion that this region is not appropriate for the phylogenetic study of the genus *Corydalis*. When *matK*, *rbcL*, *rpL16*, and *trnG* intron regions were separately used for phylogenetic analyses, many species belonging to the section *Corydalis* did not show monophyletic clustering, indicating that these genomic regions are not suitable for discriminating species when used alone. When the *matK* region was used alone for phylogenetic analysis, *Corydalis turtschaninovii*, *Corydalis hirtipes*, *Corydalis grandicalyx*, *Corydalis albipetala*, and *Corydalis filistipes* showed monophyly each, but other species were not successfully delimited. The *rpL16* region did not have sufficient resolution for discriminating species, except for *C. turtschaninovii*, *C. hirtipes*, *C. filistipes*, and *C. grandicalyx*. In *rbcL* and *trnG* intron, taxonomically meaningful clustering patterns that could identify individual species were not observed except in the case of two or three species. However, when *matK* and *rpL16* were used together, the resolution for species discrimination increased and the highest number of *Corydalis* species, i.e., six species including *C. turtschaninovii*, *C. hirtipes*, *C. grandicalyx*, *C. filistipes*, *C. albipetala*, and *C. cornupetala*, were distinguished (Figure 1). In this case, monophyly of five out of six species was supported by 99% bootstrap value.

Interestingly, when other regions, which are *rbcL* and *trnG* intron, were added to these two concatenated regions, the resolution for species delimitation decreased, resulting in five species being discriminated (Figure 2). In this case, monophyly of four out of five species was supported by 100% bootstrap value.

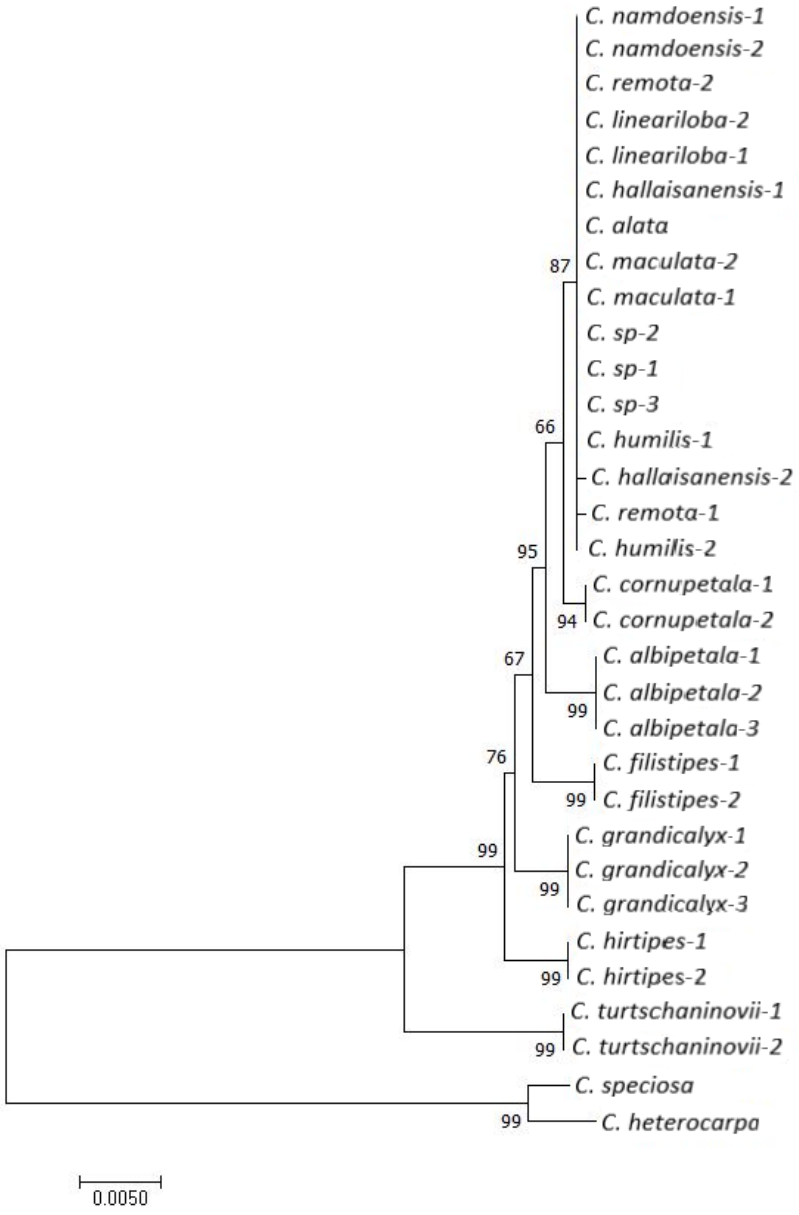

Figure 1. Maximum Likelihood phylogenetic tree of the Korean section *Corydalis* based on concatenated *matK* and *rpL16*.

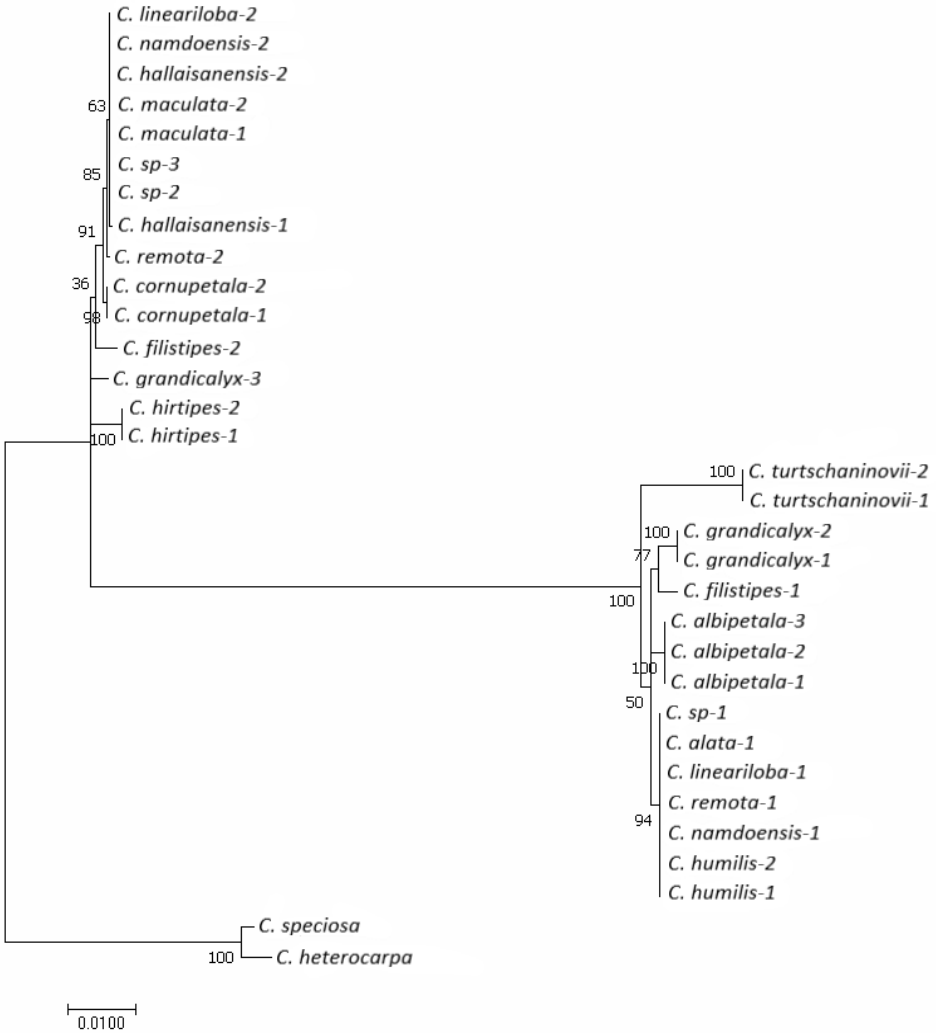

Figure 2. Maximum Likelihood phylogenetic tree of the Korean section *Corydalis* based on concatenated *matK*, *rpL16*, *rbcL* and *trnG* intron.

DISCUSSION

The ITS region of nuclear ribosomal DNA has been often used in phylogenetic studies of the genus *Corydalis* (Liden et al., 1995; Jiang et al., 2018; Ren et al., 2018; Li et al., 2020). While some of these studies supported the general efficacy of nrITS in the taxonomic analysis of *Corydalis*, other doubted about its efficacy. Jiang et al. (2018) reported "the multi-copy nature of ITS region in *Corydalis*" for the first time and suggested that research on *Corydalis* using the ITS region needs to be conducted with caution, even though their study could utilize ITS by conducting cloning. The report of Jiang et al. is consistent with this result, where multiple PCR bands were observed and

further phylogenetic analysis was impossible. One unpublished study previously tried using ITS in phylogenetic study as well, but PCR amplification resulted in multiple bands and various band sizes (data not shown), which is very similar to the result of the present study. Through the author's analysis, the author clarified that the ITS region is not as useful as generally expected, at least in the case of genus *Corydalis*. The findings effectively complemented previous reports on the similar observations.

On performing phylogenetic analyses using four chloroplast genomic regions, the author found that some *Corydalis* species, such as *C. turtschaninovii*, *C. hirtipes*, *C. filistipes*, and *C. grandicalyx*, could be clearly delimited with one or two chloroplast markers. This supports previously established species delimitations based on morphological information. At the same time, the author found that when these chloroplast markers were used alone, the resolution for species discrimination in *Corydalis* was quite low. When different genomic regions were concatenated, the resolution increased to some extent, but not much. When all four regions were used together, the resolution was lower than that when *matK* and *rpL16* were concatenated, which was in contrast to the initial expectation. These indicate that sequence variations in the chloroplast genomic regions used by the author are rather low and that concatenating many regions does not necessarily lead to higher species discrimination resolution. In general, it is known that concatenation of molecular markers results in higher resolution for classification (Bapteste et al., 2008), and many previous studies have used this concatenation approach (Amrine-Madsen et al., 2003; Rokas et al., 2003; Hedges et al., 2004). However, the concatenation of markers can be problematic and may not necessarily lead to the high resolution power the author expect in phylogenetic studies (Kubatko and Degnan, 2007). Specifically, studies have suggested that concatenating many genes in phylogenetic analyses can lead to failure because of the differences in the evolutionary histories of individual genes (Kolaczkowski and Thornton, 2004; Mossel and Vigoda, 2005; Kubatko and Degnan, 2007). Notably, the observation of decreased resolution level deserves further analyses because these analyses can contribute to understanding how genomic markers reflect the actual systematics of target plants and can provide valuable information on how to combine many genomic regions in the phylogenetic study when trying to clearly identify the taxonomy. Ultimately, these analyses may increase the efficiency of phylogenetic studies conducted using various genomic regions.

With the markers used in the present study, the author clearly observed low species delimitation resolution. In many cases, some individuals from different species were clustered together even when the genomic regions were concatenated to improve the resolution. As mentioned above, in the genus *Corydalis*, the currently established taxonomy is mainly based on morphology and not on molecular genetic information (Oh, 1999; Oh et al., 2004; Oh et al., 2010). It is possible that the inconsistency noted between the previously established species delimitation and the phylogenies generated in the present study could be attributed to the fundamental difference between the morphological information and genetic information in their mechanisms for reflecting the inherent taxonomy of the target taxa. Therefore, although the observed discrepancy may lead to the conclusion that the taxonomy based on the phylogenies deviated from

the "actual" taxonomy based on morphology, the author argue that this discrepancy should be more properly addressed and intensely explored to fully incorporate all the different observations available.

As the morphologies of *Corydalis* species are quite complicated, other tools, including phylogenetic analyses, are definitely needed for accurate identification of taxonomy for this genus. Indeed, in the previous studies, some taxonomical conclusions on *Corydalis* were successfully made by using both morphology and genetic analyses (Zhang et al., 2016; Xu and Wang, 2018). The author suggest that other genomic regions should be tested for their utilities in phylogenetic study of this genus or of section *Corydalis*, and that their molecular evolution should be studied in depth as well, so that the taxonomy of *Corydalis* can sufficiently benefit from molecular genetic information. Overall, the present study provides insights into the systematics of the Korean section *Corydalis* and demonstrates that chloroplast regions can be useful in phylogenetic analyses of this taxon when properly concatenated. The present study clearly suggests how to better utilize phylogenetic analyses and species delimitation in this intriguing and complicated taxon.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR201907101).

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Álvarez IJFW. and Wendel JF (2003). Ribosomal ITS sequences and plant phylogenetic inference. *Mol. Phylogenet. Evol.* 29: 417-434.

Amrine-Madsen H, Koepfli KP, Wayne RK, Springer MS (2003). A new phylogenetic marker, apolipoprotein B, provides compelling evidence for eutherian relationships. *Mol. Phylogenet. Evol.* 28: 225-240.

Bapteste E, Susko E, Leigh J, Ruiz-Trillo I, et al. (2008). Alternative methods for concatenation of core genes indicate a lack of resolution in deep nodes of the prokaryotic phylogeny. *Mol. Biol. Evol.* 25: 83-91.

Dong W, Liu J, Yu J, Wang L, et al. (2012). Highly variable chloroplast markers for evaluating plant phylogeny at low taxonomic levels and for DNA barcoding. *PLoS One*. 7: e35071.

Edgar RC (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic. Acids Res.* 32: 1792-1797.

Fedde F (1936). Papaveraceae. *Die Naturlichen Pflanzen-familien*. 17: 5-145.

Hedges SB, Blair JE, Venturi ML, Shoe JL (2004). A molecular time scale of eukaryote evolution and the rise of complex multicellular life. *BMC. Evol. Biol*. 4:2.

Hooker JD and Thomson T (1855). Flora Indica 1: 121-272.

Jiang L, Li M, Zhao F, Chu S, et al. (2018). Molecular identification and taxonomic implication of herbal species in genus *Corydalis* (Papaveraceae). *Molecules*. 23: 1393.

Kolaczkowski B and Thornton JW (2004). Performance of maximum parsimony and likelihood phylogenetics when evolution is heterogeneous. *Nature*. 431: 980-984.

Komarov VL (1903). Florae Manshuriae 2. *Act. Hort. Petrop*. 22: 1-787.

Kress WJ, Wurdack KJ, Zimmer EA, Weigt LA, et al. (2005). Use of DNA barcodes to identify flowering plants. *Proc. Natl. Acad. Sci. U S A*. 102: 8369-8374.

Kubatko LS and Degnan JH (2007). Inconsistency of phylogenetic estimates from concatenated data under coalescence. *Syst. Biol.* 56: 17-24.
Kumar S, Stecher G, Tamura K (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol*. 33: 1870-1874.
Li Q, Guo X, Niu J, Duojie D, et al. (2020). Molecular phylogeography and evolutionary history of the endemic species *Corydalis hendersonii* (Papaveraceae) on the Tibetan Plateau inferred from chloroplast DNA and ITS sequence variation. *Front. Plant. Sci.* 11: 436.
Lidén M, Fukuhara T, Axberg T (1995). Phylogeny of *Corydalis*, ITS and morphology. In *Systematics and Evolution of the Ranunculiflorae* (pp. 183-188). Springer, Vienna.
Lidén M (1996). New taxa of tuberous *Corydalis* (Fumariaceae). *Willdenowia*, 26: 23-35.
Lidén M, Fukuhara T, Rylander J, Oxelman B (1997). Phylogeny and classification of Fumariaceae, with emphasis on Dicentra sl, based on the plastid gene rps 16 intron. *Plant. Syst. Evol.* 206: 411-420.
Liu YY, Kan SL, Wang JL, Cao YN, et al. (2021). Complete chloroplast genome sequences of *Corydalis edulis* and *Corydalis shensiana* (Papaveraceae). *Mitochondrial. DNA. B. Resour*. 6: 257-258.
Mort ME, Archibald JK, Randle CP, Levsen ND, et al. (2007). Inferring phylogeny at low taxonomic levels: utility of rapidly evolving cpDNA and nuclear ITS loci. *Am. J. Bot.* 94: 173-183.
Mossel E and Vigoda E (2005). Phylogenetic MCMC algorithms are misleading on mixtures of trees. *Science*. 309: 2207-2209.
Nakai T (1909). Flora Koreana (I). *J. Coll. Sci. Imp. Univ. Tokyo* 26: 1-304.
Nakai T (1914). Plantæ novæ Japonicæ et Coreanæ 2. *Bot. Mag. (Tokyo)* 28: 28-29.
Nakai T (1952). A synoptical sketch of Korean flora. *Bull. Natl. Sci. Mus., Tokyo* 31: 1-152.
Oh BU (1986). A taxonomic study of genus *Corydalis* in Korea. Ph. D. thesis, Korea Univ. Seoul, Korea.
Oh BU (1999). A taxonomic review of Korean *Corydalis* (Fumariaceae). *Korean J. Pl. Taxon.* 29: 201-230.
Oh BU, Jang CG, Yoon CY (2004). *Corydalis namdoensis* BU Oh et JG Kin: A new species of *Corydalis* sect. *Corydalis* (Fumariaceae) from Korea. *Korean J. Pl. Taxon.* 34: 265-271.
Oh BU, Kim YY, Ji SJ, et al. (2010). *Corydalis alata*: A new species of *Corydalis* sect. *Corydalis* (Fumariaceae) from *Korea. Korean J. Pl. Taxon.* 40: 79-83.
Palibin J (1898). Conspectus flora Koreae, Par I. *Acta. Hort.*
Pérez-Gutiérrez MA, Romero-García AT, Fernández MC, Blanca G, et al. (2015). Evolutionary history of fumitories (subfamily Fumarioideae, Papaveraceae): An old story shaped by the main geological and climatic events in the Northern Hemisphere. *Mol. Phylogenet. Evol.* 88: 75-92.
Ren FM, Wang YW, Xu ZC, Li Y, et al. (2019). DNA Barcoding of *Corydalis*, the Most Taxonomically Complicated Genus of Papaveraceae. *Ecol. Evol.* 9: 1934-1945.
Rokas A, Williams BL, King N, Carroll SB. (2003). Genome scale approaches to resolving incongruence in molecular phylogenies. *Nature.* 425:798-804.
Shaw J, Lickey EB, Beck JT, Farmer SB, et al. (2005). The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *Am. J. Botany*, 92: 142-166.
White TJ, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications*. 18: 315-322.
Wu J, Lin P, Guo Y, Liu M. (2020). The complete chloroplast genome of *Corydalis conspersa*. *Mitochondrial. DNA. B. Resour*. 5: 1977-1978.
Xu X and Wang D. (2018). *Corydalis ternatifolia* belongs to C. sect. *Asterostigmata*, not C. sect. *Incisae* (Papaveraceae): Evidence from morphological and phylogenetic study. *Phytotaxa*, 382: 193-203.
Xu X and Wang D (2021). Comparative chloroplast genomics of *Corydalis* Species (Papaveraceae): evolutionary perspectives on their unusual large scale rearrangements. *Front. Plant. Sci.* 11: 2243.
Yuan YM, Küpfer P, Doyle JJ (1996). Infrageneric phylogeny of the genus *Gentiana* (Gentianaceae) inferred from nucleotide sequences of the internal transcribed spacers (ITS) of nuclear ribosomal DNA. *Am. J. Bot.* 83: 641-652.
Zhang ML, Su ZY, Lidén M (2008). *Corydalis* DC. *Flora of China*, 7: 336-342.
Zhang ZX, Wang D, Yang X (2016). The taxonomic position of *Corydalis parviflora* Su & Lidén (Papaveraceae), a genetically distinct species: Evidence from cpDNA and nDNA sequences. *Biochem. Syst. Ecol.* 67: 134-141.

