Biochemical Systematics and Ecology 67 (2016) 134–141

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

The taxonomic position of *Corydalis parviflora* Su & Lidén (Papaveraceae), a genetically distinct species: Evidence from cpDNA and nDNA sequences

Zhong-Xin Zhang [a, b], Dong Wang [a, *], Xue Yang [a]

[a] College of Life Sciences, Central China Normal University, Key Laboratory for Geographical Process Analysis & Simulation, Hubei Province, Wuhan, 430079, China
[b] College of Life Sciences, Anqing Normal University, The Province Key Laboratory of the Biodiversity Study and Ecology Conservation in Southwest Anhui, Anhui Province, Anqing, 246011, China

ARTICLE INFO

Article history:
Received 11 March 2016
Received in revised form 30 May 2016
Accepted 4 June 2016
Available online 22 June 2016

Keywords:
Corydalis
Corydalis parviflora
cpDNA
nDNA
Taxonomic position

ABSTRACT

The taxonomic position of *Corydalis parviflora* Su & Lidén, an endemic Chinese herb that grows in limestone crevices, has been uncertain. It was presumed to be a hybrid and had been placed in *Corydalis* section *Thalictrifoliae* or section *Sophorocapnos*. To test whether *C. parviflora* represents a hybrid of *Corydalis saxicola* Bunting (section *Thalictrifoliae*) with *Corydalis balansae* Prain (section *Sophorocapnos*) and/or *Corydalis racemosa* Pers. (section *Cheilanthifoliae*), we compared cpDNA (*rbc*L, *psb*A-*trn*H, and the *trn*G intron) and nDNA (NADPH-ferrihemoprotein oxidoreductase) sequences of these taxa and reconstructed phylogenetic trees from 18 *Corydalis* species and two outgroup species. *Corydalis parviflora* differs from its putative parents in both cpDNA and nDNA sequences and represented a separate lineage within section *Sophorocapnos*. These findings demonstrate that *C. parviflora* is not a hybrid and is more closely related to species in section *Sophorocapnos* than in section *Thalictrifoliae*. They also demonstrate that although hybridization is considered a relatively common phenomenon in vascular plants, assumptions of interspecific hybridization that are based on morphological similarities need to be tested through genetic analyses.

1. Introduction

Corydalis DC., the largest genus in Papaveraceae, is primarily distributed in the north temperate region (Wu et al., 1996; Zhang et al., 2008). Approximately 465 species have been described, with three subgenera and 357 species that are divided into 32–45 sections reported from China (Lidén, 1996; Zhang et al., 2008). This genus demonstrates high levels of reticulate evolution and intensive differentiation, and most species display intermediate morphological characters (Wu et al., 1996; Zhang et al., 2008). Many taxonomic studies have relied on morphology, and relatively few molecular phylogenetic studies have been conducted (e.g., Lidén et al., 1995, 1997).

Corydalis parviflora Su & Lidén is a Chinese endemic that grows in limestone crevices. It is a well-defined species that can be clearly distinguished from other *Corydalis* based on its morphology, including a triangular biternate lamina, cleistogamous

* Corresponding author. College of Life Sciences, Central China Normal University, 152 Luoyu Road, Hongshan District, Wuhan, 430079, China.
E-mail addresses: 395351998@qq.com, dongwang.cn@gmail.com (D. Wang).

http://dx.doi.org/10.1016/j.bse.2016.06.003
0305-1978/

flowers that lack a spur, and a horizontal stigma with one large papilla. Based on its chasmophytic habitat, a capsule that is not constricted between the seeds, and the assumption that *C. parviflora* might be a natural hybrid of *Corydalis saxicola* Bunting and *Corydalis balansae* Prain and/or *Corydalis racemosa* (Thunb.) Pers., Wu et al. (1999) treated *C. parviflora* as a member of section *Thalictrifoliae* (Fedde) Lidén. However, because *C. parviflora* has a horizontal stigma and its seeds are flattened with a broad membranous elaiosome, Su and Lidén (1997) and Zhang et al. (2008) assigned it to section *Sophorocapnos* (Turcz.) Fukuhara & Lidén. Therefore, further study was needed to assess its status as a putative hybrid and clarify its taxonomic position.

Nuclear and chloroplast molecular markers are widely used in molecular phylogenies and hybridization studies (Rieseberg et al., 1993; Lidén et al., 1995; Hoot et al., 2015). Maternally inherited chloroplast DNA (cpDNA) is nonrecombining in most angiosperms (Palmer et al., 1983). Therefore, cpDNA sequences of a hybrid species should be identical or very similar to its putative maternal parent. In contrast, biparentally inherited nuclear DNA (nDNA) sequences should reflect both parental genotypes. Hybridization events can generally be inferred more readily from nDNA, as it tends to accumulate nucleotide substitutions more rapidly than cpDNA (Wolfe et al., 1987; Sang, 2002). Therefore, molecular data derived from cpDNA and nDNA can be effectively combined to test hypotheses of hybridization (Ge et al., 1999) and study relationships.

Molecular data are commonly used to address taxonomic and phylogenetic problems. Several species of *Corydalis* were included in molecular phylogenetic studies of Papaveraceae (e.g., Hoot et al., 2015) or Fumarioideae (e.g., Pérez-Gutiérrez et al., 2012, 2015; Sauquet et al., 2015). However, these studies focused on the relationships among tribes or genera in Papaveraceae. So far, relatively few molecular studies have addressed phylogenetic relationships within *Corydalis* (Lidén et al., 1995, 1997). In addition, these studies could not present fine-scale molecular phylogenies owing to limited sampling (<25% of species).

We compared a nuclear gene (NADPH-ferrihemoprotein oxidoreductase) and chloroplast loci (*rbc*L, *psb*A-*trn*H, and *trn*G intron) from *C. parviflora* and its putative parents, namely *C. saxicola*, *C. balansae*, and *C. racemosa*, to test its putative hybrid origin. Additionally, we investigated phylogenetic relationships of four species from *Corydalis* section *Sophorocapnos* and representatives of closely related sections (Zhang et al., 2008) with maximum parsimony (MP) and Bayesian inference (BI). The specimens included five species from section *Cheilanthifoliae*, four species from section *Sophorocapnos*, three species from section *Thalictrifoliae*, two species each from sections *Asterostigma* and *Incisae*, and one species each from sections *Aulacostigma* and *Strictae*. Our aims were to (1) assess whether *C. parviflora* represents a hybrid between its putative parents, and (2) clarify the taxonomic position of *C. parviflora*.

2. Materials and methods

2.1. Plant materials

We sampled 27 accessions, representing 20 species, including *C. parviflora* and its putative parents *C. saxicola*, *C. balansae*, and *C. racemosa*, and ten closely related species: *Corydalis adunca* Maxim. (subgenus *Cremnocapnos*, section *Strictae*); *Corydalis edulis* Maxim. (subgenus *Sophorocapnos*, section *Aulacostigma*); *Corydalis foetida* C. Y. Wu & Z. Y. Su and *Corydalis speciosa* Maxim. (subgenus *Sophorocapnos*, section *Sophorocapnos*); *Corydalis cheilanthifolia* C. Y. Wu & Z. Y. Su, *Corydalis giraldii* Fedde, *Corydalis moupinensis* Franch., and *Corydalis ophiocarpa* Hook. f. & Thoms.(subgenus *Sophorocapnos*, section *Cheilanthifoliae*); *Corydalis tomentella* Franch. and *Corydalis wilsonii* N. E. Brown (subgenus *Sophorocapnos*, section *Thalictrifoliae*); *Corydalis hemsleyana* Franch.ex Prain and *Corydalis incisa* (Thunb.) Pers. (subgenus *Corydalis*, section *Incisae*); and *Corydalis temulifolia* Franch. and *Corydalis ternatifolia* C. Y. Wu (subgenus *Corydalis*, section *Asterostigma*). *Dactylicapnos torulosa* Hutch. and *Hylomecon japonica* Prantl & Kündig were chosen as outgroup taxa. Specimens were collected from their natural habitats in China (Table 1). Plants were identified following the treatment in *Flora of China* (Zhang et al., 2008). Voucher specimens were deposited in the herbarium of Central China Normal University (CCNU), Wuhan, China.

2.2. DNA extraction, PCR amplification, and sequencing

Total DNA was extracted using the modified CTAB method (Doyle, 1987). Three cpDNA fragments, *rbc*L (Hoot et al., 1997), the *psb*A-*trn*H intergenic spacer (Tate and Simpson, 2003), and the *trn*G intron (Demesure et al., 1995) were amplified to study cpDNA variation. A single-copy nuclear gene that encodes an NADPH-ferrihemoprotein oxidoreductase was amplified from the specimens, using the primers and PCR protocol described in a previous paper (Zhang et al., 2015).

PCR reactions were carried out in a volume of 25 μL, containing 40–100 ng of template DNA, 0.8 mM dNTPs, 2.5 μL 10 × PCR buffer [100 mM Tris HCl (pH 8.3), 15 mM $MgCl_2$, and 500 mM KCl](Shanghai Biocolor BioScience &Technology Co., Ltd., Shanghai, China), 0.5 μL 10 mM of each primer, and 1.25 U Taq polymerase (Shanghai Biocolor BioScience & Technology). PCR conditions for chloroplast regions were as follows: 5 min at 95 °C followed by 35 cycles of 60 s at 95 °C, 60 s at 56 °C, 60 s at 72 °C, and a final extension of 10 min at 72 °C. PCR conditions for NADPH were as follows: 5 min at 95 °C followed by 40 cycles of 60 s at 95 °C, 60 s at 59 °C, 90 s at 72 °C, and a final extension of 10 min at 72 °C. The PCR products were sequenced with an ABI 3730 automated sequencer (Applied Biosystems, Waltham, MA, USA) after agarose gel purification using the Gel Band Purification Kit (Wuhan Tianyi Huiyuan Bioscience and Technology Inc., Wuhan, China).

All sequences were deposited in GenBank (see Table 1 for accession numbers).