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·快讯 Short Communication·

从 ITS 序列探讨青藏高原特有植物华福花属的亲缘关系

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摘要: 对青藏高原特有的濒危植物华福花(*Sinadoxa corydalifolia* C. Y. Wu, Z. L. Wu et R. F. Huang)的核糖体 DNA 中的内转录间隔区(ITS)序列及 5.8S rRNA 基因的序列进行了测定。同川续断目和五加目有关类群序列的比较及分支分析表明华福花属与五福花属(*Adoxa* L.)近缘, 不支持它可能与五加目或败酱科有亲缘关系的假设。尽管形态上它与五福花属分化十分明显, 但 ITS 碱基分异却较小。引起其系统位置发生争论的外部形态为什么进化得如此之快, 是值得进一步深入探讨的课题。

关键词: 华福花; 内转录间隔区(ITS); 系统学; 进化 亲缘关系, ITS 序列

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The Phylogenetic Relationships of an Endemic Genus *Sinadoxa* in the Qinghai-Xizang Plateau: Evidence from ITS Sequence AnalysisLIU Jian-Quan^{1,2}, CHEN Zhi-Duan², LU An-Ming²

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Key words: *Sinadoxa corydalifolia*; internal transcribed spacers (ITS); systematics; evolution

The monotypic genus, *Sinadoxa*, was recently established by Wu *et al.*^[1]. The only species, *Sinadoxa corydalifolia*, was found in the several localities of the southern Qinghai Province in China during the 1960s and the 1970s. However, in our recent field expeditions from 1995 to 1999, we found that most populations of the species had disappeared. This species has become one of the exceedingly endangered species in the Qinghai-Xizang Plateau. Because of the difficulty of obtaining the materials, *Sinadoxa* was poorly studied. Although it is morphologically different from *Adoxa* and *Tetradoxa* in the characters of rhizome, leaf, calyx and ovary, especially in the highly complicated inflorescence like a spike with several glomerate interrupted clusters, most Chinese researchers accepted *Sinadoxa* as a natural member of the Adoxaceae s. s., to be closely related to *Adoxa*^[2-3]. However, Cronquist^[4] thought that the gynoecium of *Sinadoxa* is very suggestive of the Valeraceae. Donoghue *et al.*^[5] pointed out that "*Sinadoxa* differs in many respects and may be more closely related to Araliales than to *Adoxa*". Although later Donoghue^[6] recognized that it might be more reasonable to treat *Sinadoxa* within the *Adoxa* clade of the Dipsacales, he further declared that this has not

been adequately tested. The karyomorphology of *Sinadoxa* strongly indicated its close relationship with *Adoxa*^[6]. The present paper describes the study of the phylogenetic relationships of *Sinadoxa* using ITS sequence analysis.

1 Materials and Methods

1.1 Plant materials

Leaves of *S. corydalifolia* were collected in Nangchian county of the southern Qinghai (alt. 3 900 m) and dried in silica gel in June, 1999. Voucher specimens (Liu Jianquan 625) were deposited in HNWP. Sequences of *Adoxa*, *Verbunum*, *Sambucus* and other taxa of Dipsacales and Araliales were downloaded from the GenBank (also see Eriksson and Donoghue^[7]). Their accession numbers are U88552-U88557, U88194, U88198, U41381-U41382 and AF077875.

1.2 DNA sequencing

Total DNA was extracted as described by He *et al.*^[8]. PCR reactions were carried out following Zhang *et al.*^[9] and Wang *et al.*^[10] and double-stranded DNA was cycle-sequenced using PCR systems 9 600 kits and analyzed on an ABI 377 automated sequencer from both directions.

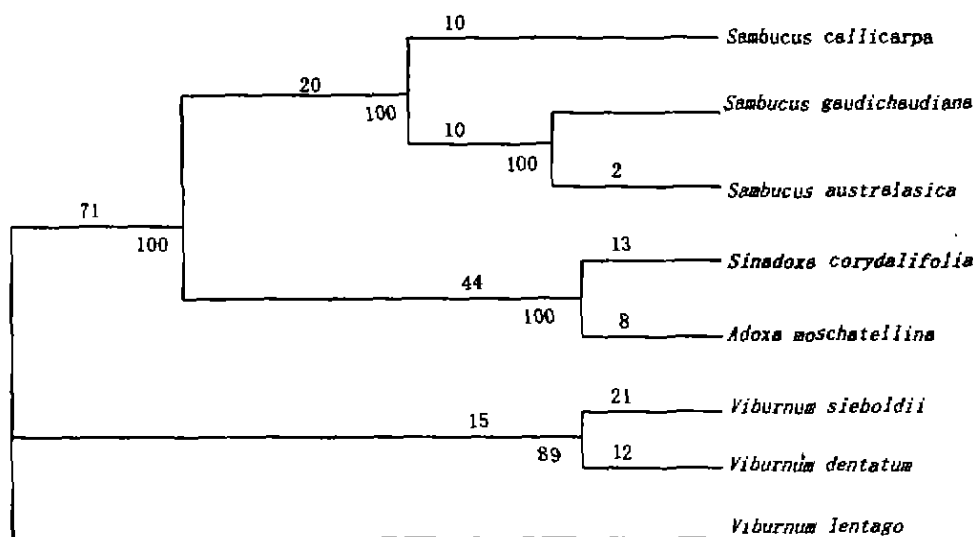
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① Donoghue M J. Phylogeny and phylogenetic taxonomy of Dipsacales. *Amer J Bot.* 1995, 82 (Abstracts): 108.

Table 1 Pairwise genetic distance (below: absolute distances/above: mean distances) of nrDNA ITS and 5.8S rDNA sequences of *Sinadoxa* and other 7 species of *Adoxa*, *Sambucus*, and *Viburnum*

		1	2	3	4	5	6	7	8
1	<i>Sambucus callicarpa</i>	-	0.031	0.034	0.138	0.132	0.242	0.215	0.195
2	<i>Sambucus gaudichaudiana</i>	19	-	0.003	0.142	0.132	0.239	0.220	0.188
3	<i>Sambucus australasica</i>	21	2	-	0.143	0.133	0.242	0.221	0.189
4	<i>Sinadoxa corydalifolia</i>	84	86	87	-	0.034	0.269	0.235	0.231
5	<i>Adoxa moschatellina</i>	80	80	81	21	-	0.259	0.236	0.222
6	<i>Viburnum sieboldii</i>	112	114	115	122	122	132	-	0.084
7	<i>Viburnum lentago</i>	103	99	100	122	117	119	44	-
8	<i>Viburnum dentatum</i>	106	107	108	125	119	122	33	34

**Fig. 1.** Single and most parsimonious tree for *Sinadoxa* and related taxa based on nrDNA ITS and 5.8S rDNA sequences using three *Viburnum* species as the outgroup.

The tree has 239 steps, with CI = 0.908 and RI = 0.908. Base substitutions are indicated above branches. Bootstrap values are indicated below branches.

1.3 Data analysis

The sequences of *S. corydalifolia* and those from Araliales and Dipsacales were aligned and compared using CLUSTALV and the phylogenetic analysis was conducted using PAUP 3.1.1 following Wang *et al.*^[10].

2 Result and Discussion

The sequences of the nuclear ribosomal DNA ITS region and 5.8S rRNA gene of *Sinadoxa corydalifolia* are 611 bp (deposited in the GenBank data base, accession no. AF248611). The length of ITS1 is 225 bp, 5.8S rRNA is 164 bp and ITS2 is 222 bp. We compared the sequences of *S. corydalifolia* with those from Araliales and Dipsacales and found that the sequences of *S. corydalifolia* are similar to those of *Adoxa*, *Viburnum* and *Sambucus*, but distinctly different from other taxa of these two orders. In the first unrooted analysis using all ITS data of these orders, *Sinadoxa* is always combined with the *Adoxa* supported by the bootstrap value of 100%. But in such an analysis, there appeared many unresolved clades

among the representative taxa of the two orders, which might be due to the long branch attraction and the homoplasy evolution of ITS sequences at the higher taxonomic level.

Adoxa, *Viburnum* and *Sambucus* comprise a natural group, and were sometimes treated together as the expanded Adoxaceae^[11,12]. The floral ontogenetical study further indicated that *Adoxa* is closely related to *Sambucus*^[16]. Therefore, *Adoxa* and *Sambucus* were together treated as the Adoxoideae while *Viburnum* comprises the other subfamily of Adoxaceae s.l.^[11-13]. Eriksson and Donoghue^[7] discussed the relationships of *Adoxa* and *Sambucus* using *Viburnum* as the outgroup based on the ITS and morphological data. All *Sambucus* species formed a natural clade, and *Adoxa* comprised the other clade. In the last analysis of ITS data presented here in Table 1 and Fig 1., we mainly used the data of these three genera to be compared to that of *Sinadoxa*. Six species are further selected to represent respectively *Sambucus* clade and *Viburnum* clade.

Using the *Viburnum* as the outgroup, the heuristic, exhaustive and bound-branch searches all resulted in only one tree (Fig. 1). *Sinadoxa* is the sister group of *Adoxa* supported by the bootstrap value of 100% and 46 common base substitutions. The absolute distance between them is only 21 bp and the relative distance is only 0.034, which is the same as that between *Sambucus callicarpa* and *S. australasica*, two representatives from two sections of the genus. The branch length or substitutions from the common node to *Adoxa* is 8, while the length to *Sinadoxa* is 13, only four more steps. This result is surprising and interesting, because two genera are so morphologically different. The habits of two genera are also distinctive. *Sinadoxa* grows on the alpine area at elevations between 3900–4800 m while *Adoxa* grows under woods at altitudes between 2300–3600 m. But why its gross-morphology evolves so fast that causes the dispute of the systematic position of the genus while the sequence divergence is so low, needs a further study. Another pending work is to study the endangering mechanisms and to find the proper protection strategies of *S. corydalifolia*, because the individuals of this species decreased annually. At any rate, the present investigation supports the close relationship of *Sinadoxa* and *Adoxa*, which is consistent with our previous karyomorphological study that *Sinadoxa* has the same basic chromosome number and similar karyotype with *Adoxa*^[6].

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