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# 基于叶绿体 DNA *trn*L 内含子序列数据的檀香目科间系统发育关系的研究

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**摘要**:应用叶绿体 DNA *trn*L 内含子序列分析檀香目科间的系统发育关系。取样研究的檀香目个体的 *trn*L 内含子序列长度在科间呈现较大差异(从 291 bp 到 587 bp)。最大简约性分析产生的严格一致树与以前已发表的基于其它基因的檀香目的分子系统学研究结果大体一致。香芙木属(铁青树科)是最早分支出的类群;桑寄生科、槲寄生科分别表现为单系类群,檀香科为并系;桑寄生科和槲寄生科并不具密切亲缘关系,槲寄生科从檀香科内衍生出来。本研究表明,具相对高的核苷酸替换率的叶绿体 DNA *trn*L 内含子序列可为高等级类群系统发育关系的研究提供更多的信息位点。

**关键词**:檀香目;桑寄生科;铁青树科;山柑科;檀香科;槲寄生科;*trn*L;系统发育关系
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# Interfamilial Relationships of Santalales as Revealed by Chloroplast *trn*L Intron Sequences

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**Abstract:** Interfamilial relationships of Santalales were investigated using chloroplast intron sequences. The lengths of *trn*L intron regions present considerable variation among the sampled Santalalean families, varying from 291 to 587 bp. The topology of strict consensus tree generated from parsimony analysis is largely congruent with trees previously published based on DNA sequences of other genes, which revealed the basal position of *Schoepfia* (Olacaceae), the monophyly of Loranthaceae and Viscaceae, and the paraphyly of Santalaceae. Loranthaceae are distinct from Viscaceae, the latter is derived from within Santalaceae. Our study also demonstrates the utility of the rapidly evolved chloroplast *trn*L intron for addressing relationships among the component taxa of Santalales.

**Key words:** Santalales; Loranthaceae; Olacaceae; Opiliaceae; Santalaceae; Viscaceae; *trn*L; Phylogenetic relationship

As traditionally defined, the order Santalales consist of seven families: Eremolepidaceae, Loranthaceae, Misodendraceae, Olacaceae, Opiliaceae, Santalaceae and Viscaceae[1], and form a well-supported clade in several broad phylogenetic analyses of angiosperms[2-4]. Santalales represent the most diverse assemblage of life forms among angiosperms as it includes members ranging from nonparasites to hemiparasites, and from root parasites to aerial parasites (mistletoes).

Whereas the monophyly of Santalales is strongly supported, the interfamilial relationships of the order remain unclear. In the past decades phylogenetic relationships of Santalales have been addressed by different authors[5-9] (see review in Nickrent et al.[2]).

Until recently, molecular phylogenetic investiga-

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tions have been attempted to clarify phylogenetic relationships among genera and families of Santalales using nuclear 18S and plastid *rbc*L sequences[2,10]. The most recent study[1] obtained 18S and *rbc*L sequences from 66 of the 155 genera in the order; the strict consensus parsimony tree was generally congruent with trees previously reported[2,10], which indicated the monophyly of Opiliaceae, Loranthaceae, and Viscaceae, and the paraphyly of Santalaceae (including Eremolepidaceae) and Olacaceae. Nevertheless, support for interfamilial relationships was moderate or low, thus greater sampling of taxa and genes is needed to reveal more robust phylogenetic relationships in the order. In the present study we sequenced a more rapidly evolving region, *trn*L intron of chloroplast DNA, and sampled more taxa mainly from China, which were poorly represented in the previous studies, to provide additional phylogenetic signal, as a complement to studies undertaken by Nickrent and his collaborators.

## 1 Materials and methods

**Plant sampling** Of the seven traditionally recognized families[6], five were sampled in the present study, e.g., Loranthaceae (6 genera), Opiliaceae (1 genus), Olacaceae (1 genus), Santalaceae (4 genera), and Viscaceae (2 genera). Overall, 27 accessions of *trn*L intron sequences were acquired, while other 6 accessions were retrieved from GenBank. In previous global analyses of angiosperms using multiple genes[4], Santalales appears (unresolved) at the base of the core eudicots. It is unclear at present which taxon is the sister group to Santalales; in the present study two species of *Alnus* (Betulaceae, Fagales) were designated as outgroups[1].

**Genomic DNA extraction, PCR amplification, and sequencing** Total DNA was extracted from fresh or silica dried leaves, following the method of Doyle and Doyle[11]. The *trn*L intron was amplified by the polymerase chain reaction (PCR) with primers "c" and "d" of Taberlet et al[12]. The PCR products were purified using QIAquick Gel Extraction Kit (QIAGEN). Sequencing reactions were performed using the dye-terminator cycle-sequencing ready-reaction kit following the manufacturer's protocol, and analyzed on an ABI 377 Automated DNA Sequencer (Applied Biosystems).

**Phylogenetic analysis** Sequences of the *trn*L intron were preliminarily aligned with Clustal X[13] and then manually adjusted to accommodate indel events otherwise not properly recognized. Multiple alignments applying different parameters in Clustal X have been explored to investigate their consequence on phylogenetic inference. The lengths of *trn*L intron regions present considerable variation among the sampled families of Santalales: samples of Loranthaceae unexceptionally have shorter sequences, ranging from 291 to 356 bp, while the remaining samples of Santalales have longer ones, ranging from 443 to 587 bp. The alignment was thus difficult due to the great length differences. When introducing indels, Clustal X always placed a long deletion (up to 267 bp) in the end part of alignment matrix for the Loranthaceae samples, which was apparently incorrect. The alignment thus needed careful manual adjustment, and we used the software of Se-Al Sequence Alignment Editor to properly place gaps. In addition to minimizing the number of informative characters (indels and substitutions), other criteria of alignment and mutational interpretation outlined by Oxelman et al.[14] and Simmons and Ochoterena[15] were adopted. Potentially informative and unambiguously assessed indels of *trn*L region were scored as binary characters (1 for insertion, 0 for gap) regardless of their length and added to the data matrix (61 such characters in total).

Maximum parsimony analysis was performed on the data matrix using PAUP* v4.0b8[16]. The analysis used heuristic searches with random addition and TBR branch swapping. Clade robustness was evaluated by bootstrap analysis[17] using 1 000 replicates of heuristic searches, with simple addition sequence and TBR branch swapping.

## 2 Results and discussion

### 2.1 Sequence characteristics of the *trn*L intron

All the newly acquired sequences have been submitted to GenBank (Table 1). The lengths of the

unaligned *trn*L intron fragments range from 291 to 587 bp; the aligned sequences had 766 positions. These data resulted in uncorrected pairwise sequence divergence ranged from 0.6% (between *Cansjera rheedii* 1 and *C. rheedii* 2) to 59.7% (between *Korthalsella complanata* and *Schoepfia jasminodora*) among the ingroup (distance matrix not shown). Within major groups (families), distances ranged from 1.0% to 30.2% in Loranthaceae, from 1.1% to 38.4% in Viscaceae, and from 0.7% to 21.8% in Santalaceae.

While the chloroplast *trn*L intron was widely used to resolve phylogenetic relationships at the species or among closely related genera[12,18-20], in some studies the intron was employed to reveal higher level relationships in some large families or orders (e.g., among orders and families of asterids[21]; among tribes of Asteraceae[22]; among palm tribes [23]; among genera of Acanthaceae [24]; among genera of Leguminosae[25, 26]), although these studies always also included sequences of the adjacent and less conserved

Table 1 Samples used in the phylogenetic analysis of Santalales. GenBank accession numbers marked with '*' were retrieved from the database, and the others were acquired by the present study

| Taxon | Source/voucher | GenBank accession number |
|---|---|---|
| **Loranthaceae** | | |
| *Dendrophthoe pentandra* (L.) Miq. | Yunnan, China; R.-L. Han 1001-L17 | AY191131 |
| *Helixanthera parasitica* Lour. | Guangdong, China; R.-L. Han 10-L47 | AY191145 |
| *H. pierrei* Danser | Yunnan, China; R.-L. Han 005-L23 | AY191135 |
| *Loranthus delavayi* Van Tiegh. 1 | Guangdong, China; R.-L. Han 00-C8 | AY191153 |
| *L. delavayi* Van Tiegh. 2 | Guangdong, China; R.-L. Han 204-L43 | AY191144 |
| *Macrosolen cochinchinensis* (Lour.) Van Tiegh. | Yunnan, China; R.-L. Han 306-L24 | AY191136 |
| *Scurrula chingii* (Cheng) H. S. Kiu | Yunnan, China; R.-L. Han 010-L22 | AY191134 |
| *S. notothixoides* (Hance) Danser | Hainan, China; R.-L. Han 01-C16 | AY191147 |
| *S. parasitica* L. var. *graciliflora* (Wall. ex DC.) H. S. Kiu | Yunnan, China; R.-L. Han 319-L37 | AY191141 |
| *S. parasitica* L. var. *parasitica* | Yunnan, China; R.-L. Han 2013-L26 | AY191138 |
| *S. sootepensis* (Craib) Danser | Yunnan, China; R.-L. Han 321-L38 | AY191139 |
| *S.* sp. | Hainan, China; R.-L. Han 02-C10 | AY191146 |
| *Taxillus chinensis* (DC.) Danser | Guangdong, China; R.-L. Han 4-L41 | AY191143 |
| *T. sutchuenensis* (Lecomte) Danser | Yunnan, China; R.-L. Han 401-L20 | AY191133 |
| **Olacaceae** | | |
| *Schoepfia jasminodora* Sieb. et Zucc. | Guangdong, China; R.-L. Han 065-C | AY191152 |
| **Opiliaceae** | | |
| *Cansjera rheedii* J. F. Gmel. 1 | Hainan, China; R.-L. Han 048-C18 | AY191148 |
| *C. rheedii* J. F. Gmel. 2 | Hainan, China; R.-L. Han 047-C19 | AY191149 |
| **Santalaceae** | | |
| *Dendrotrophe polyneura* (Hu) D. D. Tao | Yunnan, China; R.-L. Han 309-L28 | AY191140 |
| *Osyris wightiana* Wall. ex Wight | Yunnan, China; R.-L. Han 2017-L39 | AY191142 |
| *Pyrularia sinensis* Wu | Yunnan, China; R.-L. Han 305-L25 | AY191137 |
| *Santalum album* L. | Guangdong, China; R.-L. Han 060-C5 | AY191151 |
| *S. papuanum* Summerh. | Guangdong, China; R.-L. Han 061-C4 | AY191150 |
| **Viscaceae** | | |
| *Korthalsella complanata* (Tieghem) Engler | - | AF055688* |
| *K. japonica* (Thunberg) Engler | - | AF055697* |
| *K. lindsayi* (Oliver ex J. D. Hooker) Engler | - | AF055679* |
| *K. papuana* Danser | - | AF055673* |
| *Viscum album* L. | - | AF180540* |
| *V. articulatum* Burm f. | Guangdong, China; R.-L. Han 11-L9 | AY191131 |
| *V. cruciatum* Boissier | - | AF180541* |
| *V. liquidambaricolum* Hayata | Yunnan, China; R.-L. Han 2004-L5 | AY191130 |
| *V. multinerve* (Hayata) Hayata | Guangdong, China; R.-L. Han 201-L4 | AY191129 |
| *V. ovalifolium* DC. | Yunnan, China; R.-L. Han 2001-L1 | AY191127 |
| *V.* sp. | Yunnan, China; R.-L. Han 2003-L3 | AY191128 |
| **Outgroups** | | |
| *Alnus firma* Sieb. et Zucc. | - | AB063524* |
| *A. glutinosa* (L.) Gaertn. | - | AF327573* |

"-" not available

region of *trn*L-F intergeneric spacer. In the study of Leguminosae using the *trn*L intron [25], pairwise distances among the three subfamilies vary from 7% to 9%, while within major groups (subfamilies, tribes, subtribes) distances range from 1.8% to 5.3%. While the patterns of substitution rate variation across organellar gene loci are complex and rate acceleration is not a general evolutionary feature of Santalales[2], the present study demonstrates that in the *trn*L intron of plastid genome, rate acceleration has occurred in the hemiparasitic Santalales relative to autotrophic plants.

## 2.2 Phylogenetic relationships

Heuristic search of parsimony analysis of the *trn*L intron data generates 3 equally most parsimonious trees, with a length of 888 steps, CI = 0.72 (excluding uninformative characters), and RI = 0.87. In the strict consensus tree (Figure 1), *Schoepfia jasminodora* (Olacaceae) is placed at the basal position, sister to the clade containing the remaining taxa of Santalales. The latter clade furthermore consists of two subclades, one for Loranthaceae, the other for Santalaceae/Viscaceae/ Opiliaceae.

The strict consensus (Figure 1) of *trn*L intron

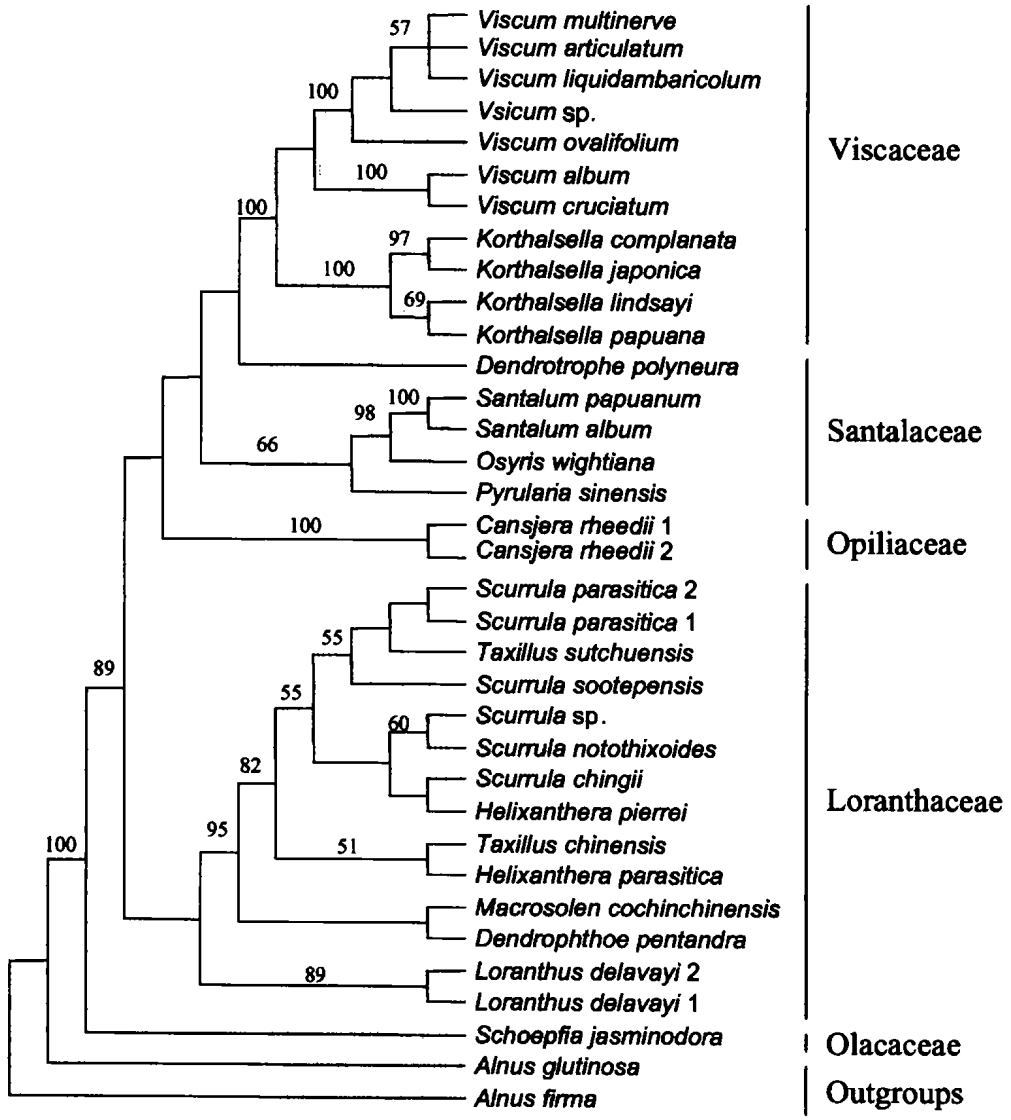


Fig. 1 Strict consensus tree of the three most parsimonious trees from *trn*L intron sequences of Santalales species. Tree length = 888 steps, CI = 0.72, RI = 0.87. Numbers above lines represent bootstrap values in 1000 replicates. Familial classification system follows Kuijt[6]. *Scurrula parasitica* 1, *Scurrula parasitica* var. *parasitica*; *Scurrula parasitica* 2, *Scurrula parasitica* var. *graciliflora*.

sequences is topologically congruent with trees previously published [1,2,10]. Monophyly of Santalales is strongly supported (100%). Santalales minus *Schoepfia* (Olacaceae) are monophyletic with high bootstrap support (89%). In previous studies [1, 2], however, *Schoepfia* is not closely related to Olacaceae; it is sister to the mistletoe genus *Misodendrum* (Misodendraceae), and this clade is in turn sister to Loranthaceae, although these relationships received only moderate support. The limited sampling of Olacaceae in this study prohibited the test of paraphyly of this family, but revealed that *Schoepfia* is the first-branching member of Santalales sampled in this study.

Loranthaceae, in the present study composed of five genera, appears as a monophyletic group, although the support is low (BS < 50%). Loranthaceae minus *Loranthus delavayi* (two accessions) are monophyletic with high BS support (95%). The genera *Helixanthera* and *Taxillus* (with two species sampled each) are scattered in different clades, their monophyly awaits further studies. Contrasting with the hypothesis of Bhandari and Vohra [9], and consistent with that of Kuijt [6,7] and Wiens and Barlow [8], Loranthaceae are distinct from Viscaceae.

Analyses of 18S rDNA and *rbc*L sequences [1,2,10] show that Santalaceae are not monophyletic but a grade that culminates in Viscaceae. The present study obtained a similar evolutionary pattern (Figure 1). *Cansjera rheedii* appeared as the sister to Santalaceae/Viscaceae, although this relationship had low support (BS< 50% ). Samples of Viscaceae (two genera) constitute a clade with full bootstrap support, and were derived from within Santalaceae, with *Dendrotrophe polyneura* (Santalaceae) as its sister, suggesting the paraphyly of Santalaceae.

As with previous studies, the present study fails to resolve a monophyletic Santalaceae. This study also demonstrates the utility of the rapidly evolved chloroplast *trn*L intron for addressing relationships among the component taxa of Santalales. Further studies, particularly using greater sampling of taxa, will be required before the phylogeny and evolution of this group of parasitic plants is resolved.

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