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STUDY ON MORPHOLOGY AND GENETICS OF *Scurrula chingii* var. *yunnanensis* H. S. Kiu In C. Y. Wu & H. W. Li

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Abstract: The phylogenetic relationship between two varieties of *Scurrula chingii* has not been clarified. The present study focused on *S. chingii* var. *yunnanensis* to indicate the evolution of this taxa based on both molecular and morphological evidences. The two varieties of *S. chingii* were well supported as non-monophyletic. The results of this study indicated that the two varieties are different in both morphology and genetics and *S. chingii* var. *yunnanensis* could be evolved differently than *S. chingii* var. *chingii*. This study suggests that *S. chingii* var. *yunnanensis* should be redefined to the species rank.

Keywords Loranthaceae, *Scurrula chingii*, *S. chingii* var. *yunnanensis*, non-monophyletic, species level.

1. INTRODUCTION

Scurrula L., the biggest genus of subtribe Scurrulinae, was established in 1753 (Kiu & Gilbert, 2003; Nickrent et al., 2010). *Scurrula* includes ca. 50 species distributed from China to Southeast Asia (Kiu & Gilbert, 2003; Nickrent et al., 2010; Liu et al., 2018). Some species of the genus are used as local medicines in China and Southeast Asia such as: *S. parasitica* L., *S. gracilifolia* (Schult.) Danser. The morphology of *Scurrula* is very close to its sister genus *Taxillus*, however *Scurrula* can be distinguished from *Taxillus* by the following morphological characters: calyx pyriform or turbinate, base attenuate and fruit long attenuate (Le, 2018).

Several taxonomic and phylogenetic studies including *Scurrula* were conducted (Vidal-Russell & Nickrent, 2008a; Nickrent et al., 2010; Liu et al., 2018; Le, 2018). Vidal-Russell & Nickrent (2008a) conducted a phylogenetic study on evolutionary relationship of Loranthaceae. However, this study only sampled three species of *Scurrula* and one species of *Taxillus*. Result of this study indicated that the two genera are closely related with strongly supported by molecular data.

Nickrent et al. (2010) suggested *Scurrula* including about 50 species from China, Southeast Asia and Malaysia. This study also treated the two genera *Scurrula* and *Taxillus*

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in the subtribe Scurrulinae.

Liu et al. (2018) conducted an investigation on the phylogeny and historical biogeography of Loranthaceae, the results of this study supported the monophyly of *Scurrula*. This study also indicated that the genus *Scurrula* is closely relative to *Taxillus* by molecular data.

Le (2018) conducted a extensive research on phylogeny, biogeography and diversification of Santalales based on morphological and molecular data. The study indicated that *Scurrula* is monophyletic group, however the author also suggested that future study is necessary to clarified the phylogenetic relationship within the genus, especial between the varieties of *Scurrula chingii*.

Scurrula chingii (W. C. Cheng) H. S. Kiu was described first time in 1983 by Kiu (Kiu, 1983; Kiu & Gilbert, 2003). This species was distributed in Guangxi, Yunnan and Vietnam. Kiu & Gilbert (2003) recognized two varieties of *Scurrula chingii*: *S. chingii* var. *chingii* and *S. chingii* var. *yunnanensis*. Several studies on phylogeny and taxonomy of Loranthaceae were conducted, however, there are no study focusing on the relationship within *Scurrula* as well as *Scurrula chingii*, thus the relationship between the two varieties was not clarified. The present study aims to (1) reconstruct the phylogeny of the genus *Scurrula*; (2) clarify the phylogenetic relationship and evolution between the two varieties of *S. chingii*.

2. MATERIALS AND METHODS

Taxon sampling, DNA extraction, amplification, sequencing

We sampled the two varieties of *Scurrula chingii* including *S. chingii* var. *chingii* and *S. chingii* var. *yunnanensis*. The two varieties were collected during field trip October 2017 in Guangxi and Yunnan, China respectively. Additionally, we assembled molecular data from Liu et al. (2018) to reconstruct the phylogenetic tree of Loranthaceae that includes the two varieties. Thus, we added sequences of the varieties to the data of Liu et al. (2018). Five molecular makers were used as previous studies including nuclear small-subunit ribosomal DNA (SSU rDNA), large-subunit ribosomal DNA (LSU rDNA), and three chloroplast DNA regions (*rbcL*, *matK* and *trnL-F*) (Vidal-Russell & Nickrent, 2008a; Su et al., 2015; Le et al., 2018; Liu et al., 2018).

Genomic DNA was extracted from silica gel dried tissues using the CTAB procedure (Doyle & Doyle, 1987). Polymerase chain reactions and sequencing were performed using the primers designed by Vidal-Russell & Nickrent (2008a, b) and Taberlet et al. (1991). The primers used for conducting PCR and sequencing were presented in Table 1.

The PCR amplification reactions used MasterMix of the BioMed company. The PCR program consisted of 5 min at 95 °C, 37 cycles of 30 s at 95 °C, 50 s at 52 °C, and 1 min 30 s at 72 °C, with a final extension of 10 min at 72 °C.

PCR products were purified on 1.0% agarose gels. The all PCR products were purified using BioMed multifunctional DNA fragment purification recovery kits then were

sequenced using the amplification primers. The bidirectional sequencing was completed using the ABI 3730 DNA Sequencer (Applied Biosystems, Carlsbad, California, USA). The sequences were aligned either in SeAl (Rambaut, 2007) or Geneious v.8.0.5 (Kearse et al., 2012).

Table 1. *Primers used for PCR and sequencing in this study*

Locus	Primer	Sequence 5'–3'	Reference
Chloroplast			
matK	78F	CAGGAGTATATTTATGCACT	Vidal-Russell & Nickrent, 2008a
	1420R	TCGAAGTATATACTTTATTCG	
rbcL	1F	ATGTCACCACAAACAGARAC	Vidal-Russell & Nickrent, 2008a
	889R	CTATCAATAACTGCATGCAT	
trnL-F	C	CGAAATCGGTAGACGCTACG	Taberlet et al., 1991
	F	ATTTGAACTGGTGACACGAG	
Nuclear			
LSUr DNA	27F	CCCGCTGAGTTTAAGCATA	Vidal-Russell & Nickrent, 2008a
	950F	GCTATCCTGAGGGAAACTTC	
SSUr DNA	12F	TCCTGCCAGTASTCATATGC	Vidal-Russell & Nickrent, 2008a
	1796R	CACCTACGGAAACCTTGTT	

Phylogenetic analyses

Both the maximum likelihood (ML) and Bayesian inference (BI) were carried out for the phylogenetic analyses of Santalaceae s.l. The ML analysis was performed using the program RAxML 8.2.10 (Stamatakis, 2006; Stamatakis et al., 2008) with the GTR + I + G substitution model for each molecular marker and the combined dataset at the Cyper Infrastructure for Phylogenetic Research (CIPRES; www.phylo.org). ML bootstrap analysis was implemented with 1000 replicates. Bayesian inference was conducted in MrBayses 3.1.2 (Ronquist & Huelsenbeck, 2003). The best-fitting models for each marker and the combined data set were determined by the Akaike information Criterion (AIC) as implemented in jModelTest 2.1.6 (Darriba et al., 2012). Bayesian analysis of the combined data set used the GTR + I + G model as determined in jModelTest. The MCMC algorithm was run for 10,000,000 generations with four Markov chain Monte Carlo (MCMC) and trees were sampled every 1000 generations. The program Tracer 1.6 (Rambaut & Drummond, 2007) was used to check that effective sample size (ESS) for all relevant parameters were well above 200 indicating that stationarity probably had been reached. With the first 25% of sampled generations (2500 trees) discarded as burn-in, a 50% majorily-rule consensus tree and posterior probabilities (PP) were obtained using the remaining trees.

Morphological analysis

The morphology of the two varieties of *Scurrula chingii* were carefully checked in the field and herbaria. The morphology of the two varieties was also compared to other specimens in herbaria: PE, HN, KUN, HNU, A, L, P. The herbarium code follows the Index Herbariorum (http://sweetgum.nybg.org/ih/).

3. RESULTS AND DISCUSSION

The study generated 10 new sequences. We added the new sequences to the combined data of Liu et al. (2018) to reconstruct the phylogeny of Loranthaceae as well as the phylogeny of *Scurrula*. The combined data set included 6458 aligned positions for the ingroups and outgroups.

The results from ML and BI trees were highly congruent, the few differences had low support. Thus, we combined the results in ML tree with BS and PP values. The phylogenetic relationships within Loranthaceae are presented in Figure 1.

The combined molecular dataset indicated a strong support for most clades. This result is congruent to previous studies (Vidal-Russell & Nickrent, 2008a; Su et al., 2015; Liu et al., 2018). Loranthaceae was supported as monophyletic, five tribes were recognized within the family. Subtribe Scurrulinae was well supported as monophyletic group and placed into tribe Lorantheae.

The Subtribe Scurrulinae includes two genera *Taxillus* and *Scurrula* (Nickrent et al., 2010). In which, *Scurrula chingii* consists of two varieties, and they were well supported to place within *Scurrula* by molecular data. However, the molecular data strongly supported that the *S. chingii* var. *chingii* and *S. chingii* var. *yunnanensis* are not monophyletic (Figure 1). *S. chingii* var. *chingii* is closely related to *S. buddleioides*, the species distributed from southern China and India (Figure 1). While, *S. chingii* var. *yunnanensis* was supported as sister to a group including *S. parasitica*, *S. pulverulenta*, *S. philippensis*, *S. chingii* var. *chingii* and *S. buddleioides*.

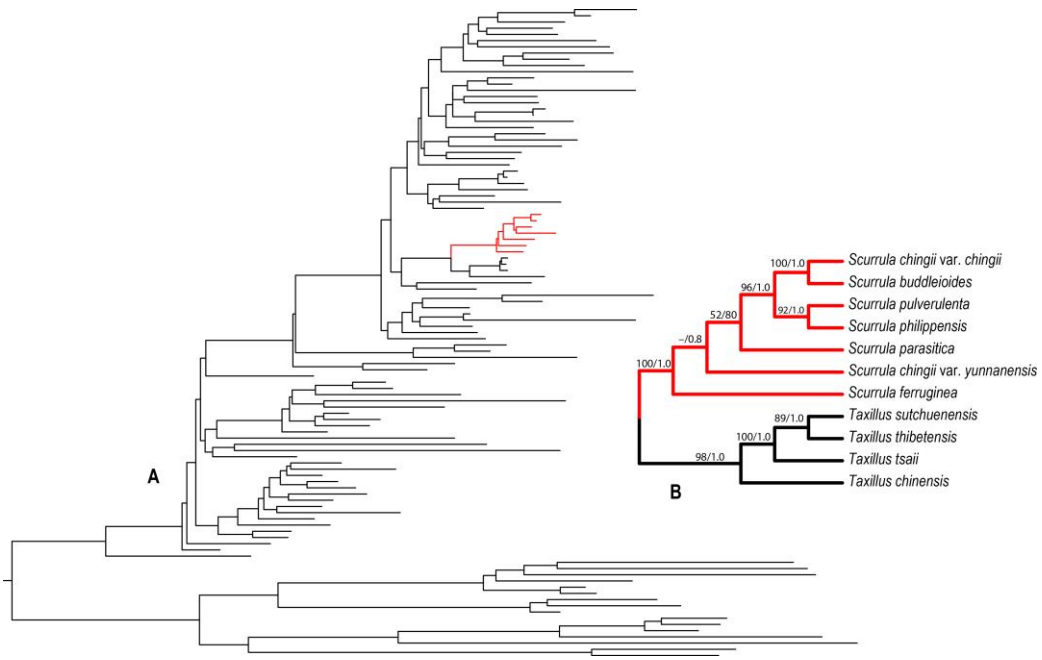

Figure 1. *Maximum likelihood tree showing the phylogenetic relationship of Loranthaceae (A) and the two genera Scurrula and Taxillus (B). Nodal support is given above the branches as ML bootstrap values/Bayesian posterior probabilities (Le C. T., 2018)*

Futhermore, *S. chingii* var. *yunnanensis* is different from *S. chingii* var. *chingii* in both morphology and genetics. We compared the sequences of the two taxa and recognize that sequences of *S. chingii* var. *yunnanensis* have several regions which evolved differently than *S. chingii* var. *chingii* especially in chloroplast regions (Figure 2) (also see in Liu et al., 2018). Those evolutions appear to be an adaptation to the habitat in various regions of southern China and Southeast Asia. *S. chingii* var. *yunnanensis* is endemic to Yunnan (China), while *S. chingii* var. *chingii* is distributed in Guangxi (China), and northern Vietnam (Kiu & Gilbert, 2003; Le, 2018).

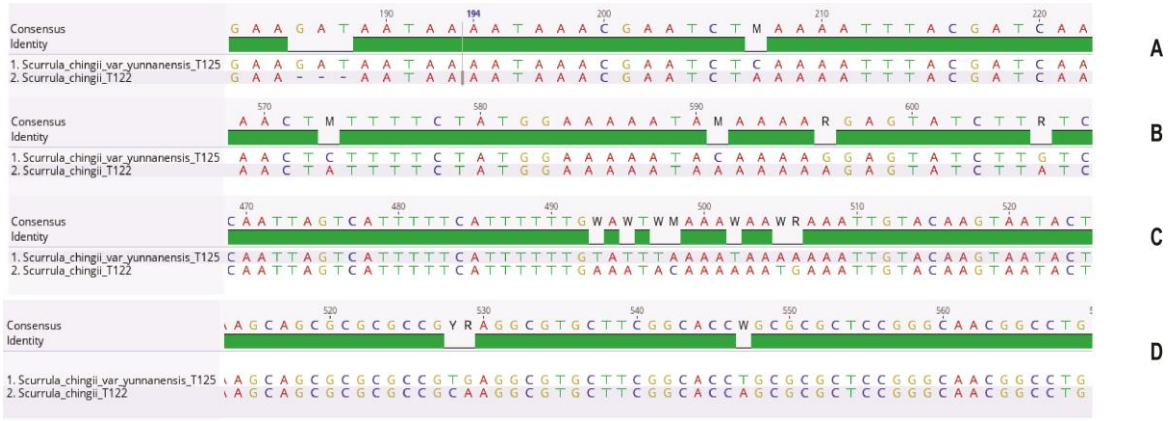

Figure 2. *Differences in sequences genes of Scurrula chingii var. yunnanensis and Scurrula chingii var. chingii. A, B: matK; C: trnL-F; D: LSU rDNA*

Moreover, the morphology of *S. chingii* var. *yunnanensis* can be easily distinguished from *S. chingii* var. *chingii* by the following characters: leaf blade both surfaces glabrous (vs. leaf blade abaxial surface rusty red tomentose or glabrous); peduncle and floral axis less than 10 mm (vs. 10-25 mm); corolla lobes lanceolate (vs. lobes subspatulate) (Figure 3).

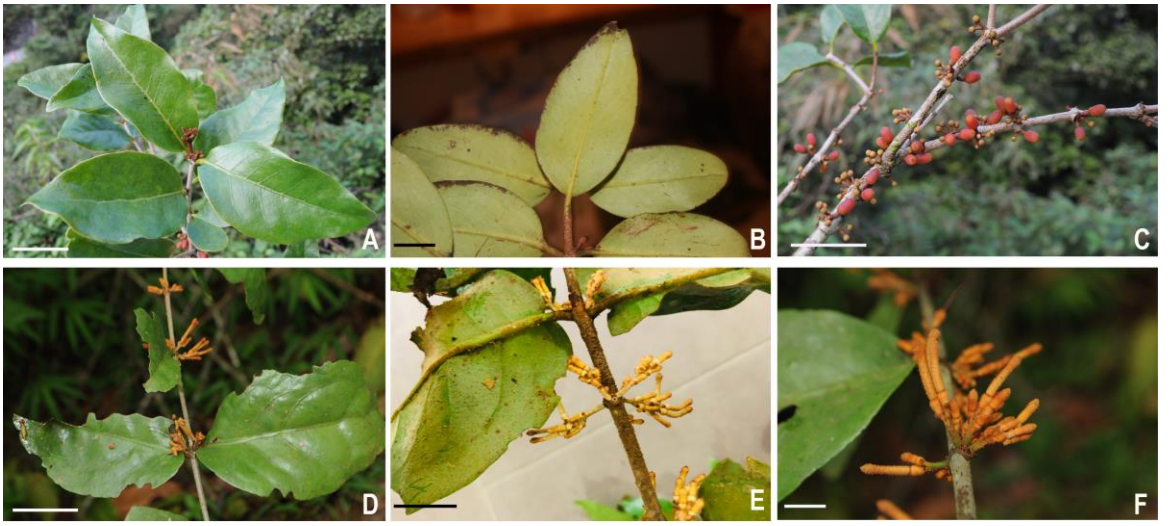

Figure 3. *Morphological comparation between Scurrula chingii var. yunnanensis (A, B, C) and Scurrula chingii var. chingii (D, E, F). Scale bars are 1 cm.*

Based on our results here, we suggest that *S. chingii* var. *yunnanensis* need to redefine to the species rank and this species should be described as *S. yunnanensis*. The taxonomic treatment will be provided in a future study.

4. CONCLUSIONS

The study supported that two varieties of *Scurrula chingii* are not monophyletic group. *S. chingii* var. *yunnanensis* is different from *S. chingii* var. *chingii* in both morphology and genetics. The present study suggests that *S. chingii* var. *yunnanensis* need to redefine to the species rank as *S. yunnanensis*.

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NGHIÊN CỨU HÌNH THÁI VÀ DI TRUYỀN CỦA *Scurrula chingii* var. *yunnanensis* H. S. Kiu In C. Y. Wu & H. W. Li

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Tóm tắt: Cho đến nay mối quan hệ giữa hai thứ của loài *Scurrula chingii* vẫn chưa được nghiên cứu làm rõ. Nghiên cứu hiện tại tập trung vào thứ *S. chingii* var. *yunnanensis* để thể hiện sự tiến hóa khác biệt của nó dựa trên cả bằng chứng phân tử và hình thái. Hai thứ của *S. chingii* được ủng hộ không phải là nhóm đơn phát sinh bằng dự liệu phân tử với chỉ số rất tốt. Kết quả của nghiên cứu chỉ ra rằng hai thứ của *S. chingii* có sự khác biệt nhau cả về hình thái và di truyền, trong đó *S. chingii* var. *yunnanensis* có thể đã tiến hóa khác so với *S. chingii* var. *chingii*. Nghiên cứu này đề xuất *S. chingii* var. *yunnanensis* nên được xem xét lại để sắp xếp ở bậc loài.

Từ khóa: *Scurrula chingii*, *S. chingii* var. *yunnanensis,* Loranthaceae, bậc loài, non-monophyletic.

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