

Molecular phylogeny of *Solms-laubachia* (Brassicaceae) s.l., based on multiple nuclear and plastid DNA sequences, and its biogeographic implications

^{1,5}Ji-Pei YUE ¹Hang SUN ²David A. BAUM ³Jian-Hua LI ⁴Ihsan A. AL-SHEHBAZ
⁵Richard REE*

¹(Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China)

²(Department of Botany, University of Wisconsin, Madison, WI 53706, USA)

³(Arnold Arboretum and Harvard University Herbaria, 22 Divinity Avenue, MA 02318, USA)

⁴(Missouri Botanical Garden, PO Box 299, St Louis, MO 63166-0299, USA)

⁵(Department of Botany, The Field Museum, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA)

Abstract The Hengduan Mountains region of south-west China is a noted biodiversity hotspot, but the geographic origins and historical assembly of its rich endemic flora, including the sky-island species of *Solms-laubachia* Muschl. (Brassicaceae), have been little studied. Previous molecular studies on the phylogeny of *Solms-laubachia* showed it to be paraphyletic, leading to considerable expansion not only of its taxonomic limits, but also its geographic range, with the inclusion of taxa from outside the Hengduan region. However, these studies provided little resolution of interspecific relationships, preventing inferences about historical biogeography within the clade. In the present study, new sequence data from two nuclear genes (*LEAFY* and *G3pdh*) and two chloroplast intergenic spacers (*petN-psbM* and *psbM-trnD*) were combined with existing markers to increase phylogenetic signals. *Phaeonychium villosum* (Maxim.) Al-Shehbaz was found to be nested within *Solms-laubachia* s.l. In general, phylogenetic relationships appear to be a good predictor of geography, with the Hengduan Mountain endemics embedded in a paraphyletic grade of species from the western Himalayas and central Asia, but they also imply morphological homoplasy. Incongruence was detected between the nuclear and chloroplast gene trees, perhaps resulting from incomplete lineage sorting of ancestral polymorphisms. The crown age of *Solms-laubachia* s.l. was estimated to be approximately 1.42–3.68 mya, using Bayesian relaxed molecular clock analysis. Historical biogeographic analysis using a parametric dispersal–extinction–cladogenesis model inferred central Asia and the western Himalayas as most probable ancestral range of *Solms-laubachia* s.l., and estimated higher rates of eastward expansion than westward during the diversification of descendant lineages. In summary, our results suggest that *Solms-laubachia* s.l. originated during the Pliocene in central Asia, and subsequently migrated eastward into the Hengduan Mountains, colonizing sky-island, alpine scree-slope habitats that may have provided novel ecological opportunity and accelerated speciation, ultimately establishing this region as the present center of diversity of the genus.

Key words biogeography, Brassicaceae, Hengduan Mountains, phylogeny, *Solms-laubachia* s.l.

For the genus *Solms-laubachia* Muschl. (Brassicaceae), recent molecular sequence data have contributed substantially to revision of its phylogenetic relationships. Nine species were traditionally recognized, characterized morphologically by entire leaves, simple or absent trichomes, stigmas that are entire and capitate, and fruits that are latiseptate (flattened parallel to the septum), detach from the pedicel at maturity, and possess a rounded replum concealed by strongly angled valve margins (Lan & Cheo, 1981; Al-Shehbaz

& Yang, 2001). Its closest relative was long thought to be *Parrya* R. Br., with several species originally described under that name. Schulz (1936) placed both genera in the same tribe, Matthioleae, a treatment followed by several taxonomists thereafter (e.g. Lan & Cheo, 1981; Kuan, 1985; Lan, 1987; An, 1995; Huang, 1997). In contrast, Al-Shehbaz (2001) and Al-Shehbaz and Yang (2001) more recently suggested a closer relationship between *Solms-laubachia* and *Desideria* Pamp. and *Leiospora* (C. A. Mey.) F. Dvořák, based primarily on the morphology and detachment of fruits. This hypothesis was subsequently corroborated by evidence from cytology (Yue et al., 2004) and molecular phylogenetics of the family Brassicaceae (Beilstein et al., 2006, 2008; Warwick et al., 2007).

Received: 10 March 2009 Accepted: 14 May 2009

* Author for correspondence. E-mail: <rree@fieldmuseum.org>.

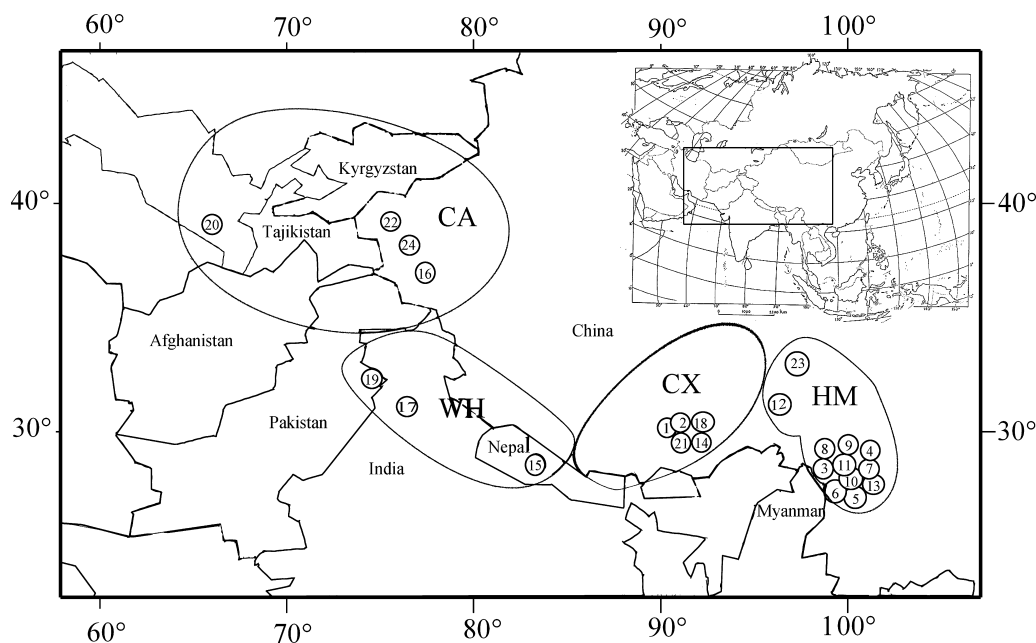


Fig. 1. Approximate distribution range of *Solms-laubachia* s.l. and the sites of species sampled in the study. CA, central Asia; WH, western Himalayas; CX, central Xizang, HM, Hengduan Mountains. Species numbers: 1, *S. platycarpa*; 2, *S. lanata*; 3, *S. linearifolia*; 4, *S. eurycarpa*; 5, *S. pulcherrima*; 6, *S. zhongdianensis*; 7, *S. angustifolia*; 8, *S. xerophyta*; 9, *S. retropilosa*; 10, *S. minor*; 11, *S. grandiflora*; 12, *S. calcicola*; 13, *S. sunhangiana*; 14, *S. baiogoinensis*; 15, *S. himalayensis*; 16, *S. linearis*; 17, *S. stewartii*; 18, *S. prolifera*; 19, *S. pumila*; 20, *S. incana*; 21, *S. jafrii*; 22, *Christolea crassifolia*; 23, *Phaeonychium villosum*; 24, *Leiospora pamirica*.

More intensive taxon sampling of molecular sequence data has since shown that *Solms-laubachia* and *Desideria* are closely related but not reciprocally monophyletic. Phylogenetic analysis of the nuclear gene *Chs* encoding chalcone synthase and the chloroplast gene *matK* revealed that four species of *Desideria* (*D. baiogoinensis* (K. C. Kuan & Z. X. An) Al-Shehbaz, *D. himalayensis* (Camb.) Al-Shehbaz, *D. linearis* (N. Busch) Al-Shehbaz, and *D. stewartii* (T. Anderson) Al-Shehbaz) and one species of *Phaeonychium* (*P. jafrii* Al-Shehbaz) are nested within a well-supported clade of *Solms-laubachia* sensu stricto (Yue et al., 2006). With additional corroborating evidence from the nuclear ribosomal internal transcribed spacer (ITS) region, the chloroplast non-coding *trnL-F* region, and seed epidermal micromorphology, Yue et al. (2008) provided a new synopsis of *Solms-laubachia* that includes 26 species, of which nine were previously recognized, four were described as new, 12 were transferred from *Desideria*, and one was transferred from *Phaeonychium*. *Christolea crassifolia* Cambess., a taxon superficially resembling *Desideria*, was determined as the sister group of the expanded *Solms-laubachia* clade (Yue et al., 2006, 2008). The biogeographic implications of this change are considerable. As traditionally circumscribed, *Solms-laubachia* was endemic to alpine areas of the Hengduan Mountains region of China, encompassing north-

western Yunnan, western Sichuan, and eastern Xizang (Tibet), with the sole exception of *S. platycarpa* (Hook. f. & Thomson) Botsch., whose range extends into Bhutan and Sikkim. The species transferred from *Desideria* are distributed in Kyrgyzstan, Tajikistan, India, Kashmir, Nepal, and western China (Xizang and Xinjiang; Al-Shehbaz, 2001, 2005), and *Phaeonychium jafrii* is endemic to Xizang (Al-Shehbaz, 2000). Under the new circumscription (hereafter *Solms-laubachia* s.l.), the range of the genus is much broader, encompassing central Asia, the western Himalaya, central Xizang, and the Hengduan Mountains region (Fig. 1).

Although Yue et al. (2006, 2008) established the generic boundaries and relationship for *Solms-laubachia*, the low genetic divergence of the markers used did not resolve the phylogeny within the genus. In the present paper, we examine the phylogeny and biogeography of *Solms-laubachia* s.l. in greater detail. To increase phylogenetic resolution, we add data from two nuclear genes (*G3pdh* and *LEAFY*) and two chloroplast non-coding spacer regions (*petN-psbM* and *psbM-trnD*) to published nuclear (*Chs*, ITS) and chloroplast (*matK*, *trnL-F*) data sets, and construct an eight-marker phylogeny. Using a *Chs* matrix with broadly sampled taxa across Brassicaceae, we estimate times of lineage divergence within *Solms-laubachia* s.l. based on relaxed molecular clocks. With these results, we use a

parametric model of geographic range evolution to infer the historical biogeography of *Solms-laubachia* s.l., focusing on the geographic origin of the clade and the relative rates of lineage movement east and west along the Himalayan axis connecting the Hengduan Mountains region to central Asia.

1 Material and methods

1.1 Taxon and gene sampling

Following the synopsis of Yue et al. (2008), 21 species of *Solms-laubachia* s.l. and one each of *Phaeoonychium*, *Christolea*, and *Leiospora* were sampled (Table 1), for which data from a total of eight genes were assembled. New sequences of the nuclear genes *G3pdh* and *LEAFY* and chloroplast non-coding spacers *petN-psbM* and *psbM-trnD* were generated for the present study and combined with existing data sets (*Chs*, ITS, *matK*, and *trnL-F*) obtained from GenBank. We selected *L. pamirica* (Botsch. & Vved.) Botsch. & Pachom. as the outgroup for phylogenetic rooting based on results from Yue et al. (2006, 2008).

1.2 DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from leaf tissues using the cetyltrimethylammonium bromide (CTAB) protocol of Doyle and Doyle (1987). The PCR amplifications of *LEAFY* and *G3pdh* were performed using primers 001F and 832R of Baum et al. (2005), as well as G3PDHX7F and G3PDHX9R of Strand et al. (1997), respectively. A 25 μ L PCR reaction included 20–50 ng DNA, 2.5 μ L of 10 \times *Taq* polymerase buffer, 4 μ L dNTP (2.5 mmol/L), 3 μ L MgCl₂ (25 mmol/L), 1 μ L each primer (10 μ mol/L), 0.3 μ L *Taq* polymerase (5 units/ μ L), and 2 μ L dimethylsulfoxide (DMSO). The PCR program included a 3-min hotstart and then 33 cycles followed by an additional 10 min at 72 °C. Each cycle consisted of 30 s denaturing at 94 °C, 90 s annealing at 50–55 °C, and a 150 s extension at 72 °C. All reactions yielded single dominant bands, which were gel purified using a Qiagen (Valencia, CA, USA) Gel Purification Kit according to the manufacturer's instructions, and then cloned into the pGEM-T cloning vector (Promega, Madison, WI, USA). To detect possible allelic variation, four clones from two independent PCR reactions were sequenced for each sample, using universal vector primers T7 and Sp6.

The primers *petN2* and *psbM2R*, and *psbM2* and *trnD* (Lee & Wen, 2004) were used to amplify the chloroplast non-coding spacers *petN-psbM* and *psbM-trnD*, respectively. The PCR reactions were performed

in 50 μ L, consisting of 50–100 ng DNA, 5 μ L of 10 \times *Taq* polymerase buffer, 4 μ L dNTP (2.5 mmol/L), 4 μ L MgCl₂ (25 mmol/L), 2 μ L each primer (10 μ mol/L), and 0.4 μ L *Taq* polymerase (5 units/ μ L). The PCR program for both regions was the same as described above for *LEAFY*. The PCR products were purified and sequenced following the same procedure described above using the same primers.

Sequences for all genes were analyzed using an automated ABI 3730 DNA Analyser (Applied Biosystems, Foster City, CA, USA), edited using Sequencher (version 4.0; Gene Codes, Ann Arbor, MI, USA), and aligned with Clustal_X version 1.83 (Thompson et al., 1997), followed by manual adjustment.

1.3 Phylogeny reconstruction

Three data matrices of concatenated sequence alignments were constructed. The first included all eight markers (*LEAFY*, *G3pdh*, *Chs*, ITS, *petN-psbM*, *psbM-trnD*, *matK*, *trnL-F*) and all 24 taxa, but 25% of the data was missing (because not all markers were sequenced for every species). The other two matrices contained only either nuclear or chloroplast sequences and each included 17 taxa with complete sequences. For each matrix, phylogenetic analyses were performed using maximum parsimony (MP; Farris et al., 1970; Fitch, 1971). Heuristic parsimony searches were conducted using PAUP 4.0b10 (Swofford, 2002) with the following conditions: 1000 replicates of random sequence addition, holding 10 trees at each step, tree bisection-reconnection (TBR) branch swapping, MULPARS on, and steepest descent off. Nucleotide substitutions were treated as unordered, equally weighted changes, and gaps were treated as missing data. Bootstrap (BS) support values for clades were calculated from 1000 replicates.

Bayesian estimation of phylogeny (Rannala & Yang, 1996; Mau et al., 1999) was conducted using MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003). The best-fitting substitution model for each marker was selected using the Akaike Information Criterion (AIC) as implemented in Modeltest version 3.7 (Posada & Crandall, 1998; Posada & Buckley, 2004). The chosen models were TVM+G (nst = 6, rate = gamma) for *trnL-F*, *petN-psbM* and *psbM-trnD*; TIM+I+G (nst = 6, rate = gamma) for *matK*; GTR+G (nst = 6, rate = gamma) for *Chs* and *G3pdh*; SYM+G (nst = 6, rate = gamma) for ITS; and HKY+G (nst = 2, rate = gamma) for *LEAFY*. For each analysis two simultaneous runs were performed, starting from random trees for 2×10^6 generations, having three heated and one cold chain. Markov chains were sampled every 100 generations. Analyses were run until the average

Table 1 Voucher information and GenBank accession numbers of species used in the present study

Taxon	Voucher	Source	Chloroplast	Accession No.	Nuclear	Accession No.
<i>Solms-laubachia angustifolia</i> J.P. Yue, Al-Shehbaz & H. Sun (狭叶丛菘)	Yue 0250 (KUN)	Yading, Daocheng, Sichuan, China (四川稻城亚丁)	<i>matK</i>	DQ409244	<i>Chs</i>	DQ409220
			<i>trnL-F</i>	DQ523319	ITS	DQ523420
			<i>petN-psbM</i>	DQ523396	<i>G3pdh</i>	DQ523348
<i>Solms-laubachia baioleinensis</i> (K.C. Kuan & C.H. An) J.P. Yue, Al-Shehbaz & H. Sun (藏北丛菘)	Yue 0246 (KUN)	Maizhokunggar, Tibet, China (西藏墨竹工卡)	<i>psbM-trnD</i>	DQ523372	<i>LEAFY</i>	F1026810
			<i>matK</i>	DQ409252	<i>Chs</i>	DQ409228
			<i>trnL-F</i>	DQ523315	ITS	DQ523416
<i>Solms-laubachia calcicola</i> J.P. Yue, Al-Shehbaz & H. Sun (岩生丛菘)	Boufford et al. 31975 (A)	Riwoqé, Tibet, China (西藏类乌齐)	<i>petN-psbM</i>	DQ523392	<i>G3pdh</i>	DQ523344
			<i>psbM-trnD</i>	DQ523368	<i>LEAFY</i>	F1026826
			<i>matK</i>	DQ409260	<i>Chs</i>	DQ409236
<i>Solms-laubachia eurycarpa</i> (Maxim.) Botsch. (宽果丛菘)	Yue 0249 (KUN)	Yading, Daocheng, Sichuan, China (四川稻城亚丁)	<i>trnL-F</i>	DQ523320	ITS	DQ523421
			<i>petN-psbM</i>	DQ523397	<i>G3pdh</i>	DQ523349
			<i>psbM-trnD</i>	DQ523373	<i>LEAFY</i>	F1026816
<i>Solms-laubachia grandiflora</i> J.P. Yue, Al-Shehbaz & H. Sun (大花丛菘)	D. E. Boufford et al. 30727 (KUN)	Xiangcheng, Sichuan, China (四川乡城)	<i>matK</i>	DQ409243	<i>Chs</i>	DQ409219
			<i>trnL-F</i>	DQ523303	ITS	DQ523404
			<i>petN-psbM</i>	DQ523380	<i>G3pdh</i>	DQ523331
<i>Solms-laubachia jaffrii</i> (Al-Shehbaz) J.P. Yue, Al-Shehbaz & H. Sun (杰氏丛菘)	Yue 0233 (KUN)	Lhasa, Tibet, China (西藏拉萨)	<i>psbM-trnD</i>	DQ523356	<i>LEAFY</i>	F1026807
			<i>matK</i>	DQ409258	<i>Chs</i>	DQ409234
			<i>trnL-F</i>	DQ523318	ITS	DQ523419
<i>Solms-laubachia himalayensis</i> (Cambess.) J.P. Yue, Al-Shehbaz & H. Sun (须弥丛菘)	McBenth 1486 (E)	Nepal (尼泊尔)	<i>petN-psbM</i>	DQ523395	<i>G3pdh</i>	DQ523347
			<i>psbM-trnD</i>	DQ523371	<i>LEAFY</i>	F1026817
			<i>matK</i>	DQ409261	<i>Chs</i>	DQ409237
<i>Solms-laubachia lanata</i> Botsch (绵毛丛菘)	Yue 0234 (KUN)	Lhasa, Tibet, China (西藏拉萨)	<i>trnL-F</i>	DQ523321	ITS	DQ523422
			<i>petN-psbM</i>	DQ523398	<i>G3pdh</i>	DQ523350
			<i>psbM-trnD</i>	DQ523374	<i>LEAFY</i>	F1026821
<i>Solms-laubachia linearifolia</i> (W.W. Sm.) O.E. Schulz (线叶丛菘)	Yue 0157 (KUN)	Dèqén, Yunnan, China (云南德钦)	<i>matK</i>	DQ409266	<i>Chs</i>	DQ409242
			<i>trnL-F</i>	DQ523327	ITS1	†AJ628335
			<i>petN-psbM</i>	DQ523390	ITS2	†AJ628336
<i>Solms-laubachia linearis</i> (N. Busch) J.P. Yue, Al-Shehbaz & H. Sun	Bartholomew et al. 9549 (MO)	Xinjiang, China (新疆)	<i>matK</i>	DQ409246	<i>Chs</i>	DQ409222
			<i>trnL-F</i>	DQ523308	ITS	DQ523409
			<i>petN-psbM</i>	DQ523385	<i>G3pdh</i>	DQ523336
<i>Solms-laubachia linearifolia</i> (W.W. Sm.) O.E. Schulz (线叶丛菘)	Yue 0157 (KUN)	Dèqén, Yunnan, China (云南德钦)	<i>psbM-trnD</i>	DQ523361	<i>LEAFY</i>	F1026808
			<i>matK</i>	DQ409249	<i>Chs</i>	DQ409225
			<i>trnL-F</i>	DQ523313	ITS	DQ523414
<i>Solms-laubachia linearis</i> (N. Busch) J.P. Yue, Al-Shehbaz & H. Sun	Bartholomew et al. 9549 (MO)	Xinjiang, China (新疆)	<i>petN-psbM</i>	DQ523390	<i>G3pdh</i>	DQ523342
			<i>psbM-trnD</i>	DQ523366	<i>LEAFY</i>	F1026818
			<i>matK</i>	DQ409254	<i>Chs</i>	DQ409230
<i>Solms-laubachia linearis</i> (N. Busch) J.P. Yue, Al-Shehbaz & H. Sun	Bartholomew et al. 9549 (MO)	Xinjiang, China (新疆)	<i>trnL-F</i>	DQ523316	ITS	DQ523417
			<i>petN-psbM</i>	DQ523393	<i>G3pdh</i>	DQ523345
			<i>psbM-trnD</i>	DQ523369	<i>LEAFY</i>	F1026822

Table 1 Continued

Taxon	Voucher	Source	Markers			
			Chloroplast	Accession No.	Nuclear	
<i>Solms-laubachia minor</i> Hand.-Mazz. (细叶丛藨)	Yue 0379 (KUN)	Yányuan, Sichuan, China (四川盐源)	<i>matK</i> <i>trnL-F</i> <i>petN-psbM</i> <i>psbM-trnD</i>	DQ409257 DQ523317 DQ523394 DQ523370	<i>Cls</i> ITS <i>G3pdh</i> <i>LEAFY</i>	DQ409233 DQ523418 DQ523346 F1026813
<i>Solms-laubachia platycarpa</i> (Hook.f. & Thomson) Botsch. (总状丛藨)	Yue 0239 (KUN)	Damxung, Tibet, China (西藏当雄)	<i>matK</i> <i>trnL-F</i> <i>petN-psbM</i> <i>psbM-trnD</i>	DQ409245 DQ523306 DQ523383 DQ523359	<i>Cls</i> ITS <i>G3pdh</i> <i>LEAFY</i>	DQ409221 DQ523407 DQ523334 F1026819
<i>Solms-laubachia prolifera</i> (Maxim.) J.P. Yue, Al-Shehbaz & H. Sun						†AJ628333
<i>Solms-laubachia pulcherrima</i> Muschl. (丛藨)	Yue 0153 (KUN)	Lijiang, Yunnan China (云南丽江)	<i>matK</i> <i>trnL-F</i> <i>petN-psbM</i> <i>psbM-trnD</i> <i>trnL</i> <i>trnF</i>	DQ409247 DQ523310 DQ523387 DQ523363 DQ479867 DQ518363	<i>Cls</i> ITS <i>G3pdh</i> <i>LEAFY</i> ITS1 ITS2	DQ409223 DQ523411 DQ523338 F1026809
<i>Solms-laubachia pumila</i> (Kurtz) F. Dvořák						
<i>Solms-laubachia retropilosa</i> Botsch. (倒毛丛藨)	Yue 0246 (KUN)	Zogang, Tibet, China (西藏左贡)	<i>matK</i> <i>trnL-F</i> <i>petN-psbM</i> <i>psbM-trnD</i>	DQ409248 DQ523311 DQ523388 DQ523364	<i>Cls</i> ITS <i>G3pdh</i> <i>LEAFY</i>	DQ409224 DQ523412 DQ523339 F1026812
<i>Solms-laubachia stewartii</i> (T. Anderson) J.P. Yue, Al-Shehbaz & H. Sun	McBeath 2105 (E)	India (印度)	<i>matK</i> <i>trnL-F</i>	DQ409265 DQ523328	<i>Cls</i> ITS	DQ409241 EU186027
<i>Solms-laubachia sunhangiana</i> J.P. Yue & Al-Shehbaz (伍须丛藨)	Boufford et al. 33464 (A)	Jiulong, Sichuan, China (四川九龙)				
<i>Solms-laubachia xerophyta</i> (W.W. Sm.) Comber (旱生丛藨)	Yue 0251 (KUN)	Zhongdian, Yunnan, China (云南中甸)	<i>matK</i> <i>trnL-F</i> <i>petN-psbM</i> <i>psbM-trnD</i>	DQ409259 DQ523305 DQ523382 DQ523358	<i>Cls</i> ITS <i>G3pdh</i> <i>LEAFY</i>	DQ409235 DQ523406 DQ523333 F1026811
<i>Solms-laubachia zhongdianensis</i> J.P. Yue, Al-Shehbaz & H. Sun (中甸丛藨)	Yue 0156 (KUN)	Zhongdian, Yunnan, China (云南中甸)	<i>matK</i> <i>trnL-F</i> <i>petN-psbM</i> <i>psbM-trnD</i>	DQ409250 DQ523314 DQ523391 DQ523367	<i>Cls</i> ITS <i>G3pdh</i> <i>LEAFY</i>	DQ409226 DQ523415 DQ523343 F1026820
<i>Phaeonychium villosum</i> (Maxim.) Al-Shehbaz (柔毛藏芥)	Bartholomew et al. 9499 (MO)	Xinjiang, China (新疆)	<i>matK</i> <i>trnL-F</i> <i>petN-psbM</i> <i>psbM-trnD</i>	DQ409256 DQ523322 DQ523399 DQ523375	<i>Cls</i> ITS <i>G3pdh</i> <i>LEAFY</i>	DQ409232 DQ523423 DQ523351 F1026823
<i>Christolea crassifolia</i> Cambess. (高原芥)						
<i>Leiospora pamirica</i> (Botsch. & Vved.) Botsch. & Pachom. (帕米尔光芥)	Bartholomew et al. 9790 (MO)	Xinjiang, China (新疆)	<i>matK</i> <i>trnL-F</i> <i>petN-psbM</i> <i>psbM-trnD</i>	DQ409255 DQ523323 DQ523401 DQ523376	<i>Cls</i> ITS <i>G3pdh</i> <i>LEAFY</i>	DQ409231 DQ523424 DQ523354 F1026824

Note, *Cls*, *matK*, *trnL-F* and internal transcribed spacer (ITS) were available from the GeneBank database, whereas *LEAFY*, *G3pdh*, *petN*, *psbM*, and *psbM-trnD* were sequenced in the present study.

standard deviation of split frequencies approached 0.01, indicating the convergence of two runs. The plot of generation versus log probability was inspected after the run to ensure that stationarity was reached and to determine the burn-in. Typically, approximately 10% of trees were discarded as burn-in. The majority rule consensus tree (not shown) containing posterior probabilities (PP) was built from the remaining sampled trees.

1.4 Estimation of divergence time

Molecular clock analysis of the *Chs* gene was conducted to estimate divergence times within *Solms-laubachia*. This gene was chosen because it has been broadly sampled across Brassicaceae (Koch et al., 2000, 2001), and was used to infer the phylogenetic relationships of *Solms-laubachia* to its presumably related genera *Baimashania*, *Christolea*, *Desideria*, and *Leiospora* etc. (Yue et al., 2006). The *Chs* data matrix in our previous study consisted of 50 sequences representing 30 genera and 50 species within Brassicaceae (Yue et al., 2006); in the present study, we analyzed an alignment of 30 sequences selected from the data set in Yue et al. (2006) using a Bayesian coalescent method implemented in the computer program BEAST version 1.4.1 (Drummond & Rambaut, 2007). The analysis included a relaxed-clock GTR substitution model, with rate variation among sites following a discrete gamma distribution with four rate categories, and rates across branches being uncorrelated and drawn from a log normal distribution. Posterior distributions of parameters were approximated using two independent Markov chain Monte Carlo (MCMC) runs of 2×10^7 steps, discarding a burn-in period of 2×10^4 steps, as determined by convergence analysis using TRACER (Rambaut & Drummond, 2007). Samples from the two chains, which yielded similar results, were combined. To calibrate the *Chs* analysis to absolute time, we used pollen deposits of *Rorippa* (sister of *Cardamine*) from the Pliocene, 2.5–5.0 mya (Mai, 1995), to calibrate the node splitting *Barbarea*–*Rorippa* and *Cardamine*. The distribution of ages at this node was set to follow a normal distribution with a mean of 6 million years (my) and a standard deviation of 0.2 to account for the uncertainty of the fossil age (Fig. 4), which corresponds to the study of Koch et al. (2000).

1.5 Historical biogeography

To infer the geographic history of *Solms-laubachia* s.l., we constructed a dispersal–extinction–cladogenesis (DEC) model of geographic range evolution (Ree & Smith, 2008). The DEC models describe transition rates between geographic ranges as functions of stochastic dispersal events (range expansion) and local extinction

events (range contraction), and enumerate geographic scenarios of lineage divergence at internal nodes on phylogenetic trees. Our model included four component areas arrayed along an east–west axis (i.e. central Asia, the western Himalayas, central Xizang, and the Hengduan Mountains (Fig. 1), which we abbreviate as CA, WH, CX, and HM, respectively). Geographic ranges of all species included in the eight-marker phylogenetic analysis were scored as vectors of presence/absence values for each area. Our model constrained widespread ancestral ranges to occupy a maximum of two spatially adjacent areas (CA+WH, WH+CX, and CX+HM), consistent with observed species ranges, and constrained dispersal events to occur only east or west between adjacent areas.

For a given phylogenetic tree, three rate parameters were estimated, namely the rate of local extinction (e) and rates of eastward and westward dispersal (d_E and d_W , respectively). These rates were first estimated by maximum likelihood without conditioning on any ancestral range values. Then, these “global” rates were used to estimate the most likely ancestral range at the root node of *Solms-laubachia* s.l. (Clade A, Fig. 2). This procedure was repeated across 500 trees randomly sampled from the posterior distribution estimated by Bayesian analysis in order to integrate biogeographic inferences over uncertainty in phylogenetic relationships and branch lengths. All biogeographic analyses were based on the likelihood-based method of Ree et al. (2005) and Ree and Smith (2008) implemented in lagrange2.0.1 (by R. H. Ree and S. A. Smith, available at <http://lagrange.googlecode.com>, accessed July 2008).

2 Results

2.1 Combined eight-gene analysis

Descriptive statistics of all markers are given in Table 2. The eight-marker data set consisted of 8426 characters, of which 7088 were constant and 337 were parsimony informative. The parsimony analysis yielded one most parsimonious tree (MPT) of 1729 steps, which has almost identical topology with consensus trees from the Bayesian analyses (Fig. 2). All species of *Solms-laubachia* s.l. and *Phaeonychium villosum* formed a monophyletic clade (Clade A; Fig. 2) sister to *Christolea crassifolia* with 100% BS and 100% PP support. In Clade A, *S. linearis* and *S. pumila*, together with *S. himalayensis* and *S. stewartii*, formed the weakly supported (62% BS) Clade B (Fig. 2), which was sister to other remaining taxa that formed the weakly supported (59% BS) Clade C (Fig. 2). *Solms-laubachia incana*, which formed the first branch within Clade C, was

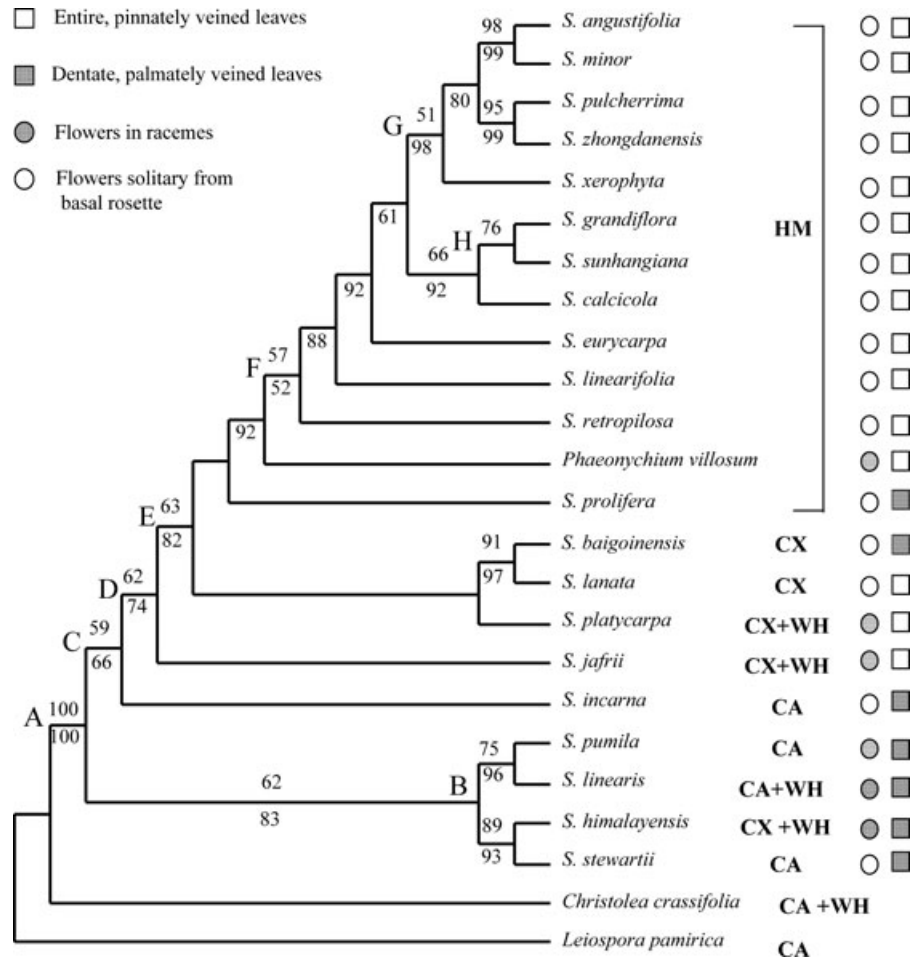


Fig. 2. The single most parsimonious tree generated from analysis of the combined data set for eight markers (*LEAFY*, *G3pdh*, *Chs*, internal transcribed spacer (ITS), *petN-psbM*, *psbM-trnD*, *matK*, and *trnL-F*). Tree length = 1729; consistency index (CI) = 0.85; retention index (RI) = 0.61; rescaled consistency index (RC) = 0.52. Bootstrap (BS; > 50%) and posterior probability (PP) values are shown above and below the branches, respectively. Letters A–H refer to the clades discussed in the text. Species origin: HM, Hengduan Mountains; CX, central Xizang; WH, west Himalayas; CA, central Asia. Note, “*Solms-laubachia*” is abbreviated to “*S.*” throughout.

Table 2 Characteristics of the chloroplast and nuclear data sets for parsimony analysis, as well as pairwise Kimura-2 parameter distances (%) of DNA sequences within ingroup and Hengduan Mountains (HM) species

Dataset	No. aligned sites	No. variable sites (%)	Sequence divergence (ingroup; %)	Sequence divergence (HM; %)	No. MPTs	Tree length	CI	RI	RC
<i>LEAFY</i>	1463	24	0.63–5.4	0.63–2.6	1062	419	0.96	0.78	0.74
<i>G3pdh</i>	693	19.6	0.36–5.0	0.39–2.2	12	93	0.93	0.69	0.64
ITS	622	15.4	0.33–3.4	0.33–2.1	24	125	0.87	0.76	0.66
<i>Chs</i>	1174	12.3	0.35–4.4	0.35–1.5	56	181	0.87	0.71	0.61
Combined nuclear	3952	18.4	0.63–6.7	0.63–1.9	32	897	0.91	0.70	0.63
<i>trnL-F</i>	1074	13.3	0.34–4.2	0.34–3.2	4384	164	0.86	0.79	0.68
<i>matK</i>	1525	9.4	0.23–3.4	0.23–2.34	3	178	0.56	0.61	0.52
<i>psbM-trnD</i>	1100	15.2	0.37–4.6	0.37–3.4	811	222	0.81	0.60	0.49
<i>petN-psbM</i>	773	15.9	0.6–6.8	0.6–3.2	172	164	0.83	0.57	0.47
Combined chloroplast	4474	12.9	0.82–3.8	0.82–2.1	8	757	0.82	0.52	0.43
Combined nuclear and chloroplast	8426	15.9	0.29–8.5	0.75–2.1	1	1729	0.85	0.61	0.52

MPTs, most parsimonious trees; CI, consistency index; RI, retention index; RC, rescaled consistency index.

followed by *S. jafrii* and Clade E (63% BS) to form Clade D (62%). Within Clade E, 11 *Solms-laubachia* s.s. species from Hengduan Mountains formed Clade F (57% BS) and sister to *P. villosum*. Of these 11 species, *S. angustifolia* and *S. minor*, together with *S. pulcherrima* and *S. zhongdianensis*, and *S. xerophyta* formed Clade G (51% BS), and *S. calcicola*, *S. grandiflora*, and *S. sunhangiana* formed Clade H (66% BS). Except for the Clades A–H, the phylogenetic relationships, revealed by the eight markers combined, received less support (BS < 50%).

2.2 Nuclear gene analysis

The concatenated alignment of nuclear genes was 3952 bp in length, of which 3224 sites were invariable and 142 were parsimony informative. The parsimony analysis yielded 32 MPTs of 897 steps (consistency index (CI) = 0.91, retention index (RI) = 0.69, and rescaled consistency index (RC) = 0.63; Fig. 3). Clade A is strongly supported (100% BS, 100% PP). Within Clade A, *S. linearis* is sister to Clade B (100% BS). Species from the Hengduan Mountains form Clade E (91% BS), within which the *S. linearifolia* and *S. retropilosa* clade is sister to the other species that formed Clade F (99% BS).

2.3 Chloroplast gene analysis

The concatenated matrix of chloroplast genes contained 4474 characters, of which 3898 were invariable and 157 were parsimony informative. The parsimony analysis yielded eight MPTs of 757 steps (CI = 0.81, RI = 0.52, and RC = 0.42; Fig. 3). The topologies of these trees are generally, but not entirely, congruent with those from the nuclear gene analysis. In the nuclear tree, *S. lanata* is sister to *S. baiogoinensis* (100% BS, 100% PP), but is weakly supported as sister to *S. calcicola* in the chloroplast tree (53% BS). In the nuclear tree, *S. calcicola* is sister to *S. grandiflora* (97% BS), and *S. platycarpa* is sister to *S. lanata* plus *S. baiogoinensis* in Clade D. In the chloroplast tree, *S. platycarpa* is in Clade E. The chloroplast sister group relationships of *S. angustifolia* and *S. minor*, and *S. pulcherrima* and *S. zhongdianensis* are not resolved in the nuclear tree. Likewise, the sister species *S. linearifolia* and *S. retropilosa* in the nuclear tree are in different clades in the chloroplast tree.

In all analyses, *Solms-laubachia* s.l. species formed a strongly supported monophyletic clade and sister to *C. crassifolia*, and *S. linearis* was always at a relatively early branching (“basal”) position. Owing to differences in taxon sampling, it is difficult to compare phylogenetic relationships resolved by combined nuclear and chloroplast DNA data with those resolved by

separate analyses of nuclear and chloroplast markers directly.

2.4 Estimation of divergence times

The divergence time of *Solms-laubachia* s.l. and *Christolea* from *Leiospora* was estimated to be 5.27 mya with a 95% high posterior density (HPD) interval of 3.0–7.73 mya (Node A; Fig. 4). The crown ages of *Solms-laubachia* s.l. and the Hengduan Mountains clade were estimated at 2.31 mya (95% HPD: 1.42–3.68 mya; Node B; Fig. 4) and 0.87 mya (95% HPD: 0.41–1.42 mya; Node C; Fig. 4), respectively.

2.5 Historical biogeography

Across the 500 trees sampled from the posterior distribution of the eight-marker Bayesian analysis, the ancestral range of *Solms-laubachia* s.l. (excluding outgroups) was reconstructed as spanning central Asia and the western Himalayas 97.8% of the time. Other ancestral ranges inferred were central Asia only (1.6%) and western Himalayas + central Xizang (0.6%). Rates of eastward and westward dispersal, estimated for each tree without conditioning on ancestral ranges at internal nodes, were highly asymmetric in favor of eastward movement (Fig. 5). Parametric inferences of geographic range evolution in *Solms-laubachia* s.l. that incorporated phylogenetic uncertainty thus yielded strong evidence for a western ancestor, with subsequent eastward migration of descendant lineages toward the Hengduan Mountains region.

3 Discussion

As presently delimited, *Solms-laubachia* s.l. consists of 26 species distributed in central Asia (Kyrgyzstan, Afghanistan, Tajikistan, Pakistan, Xinjiang of China), western Himalayas (India, Kashmir, Sikkim, Nepal, Bhutan), central Xizang (Tibet), and the Hengduan Mountains region (south-eastern Xizang, north-western Yunnan, western Sichuan, southern Qinghai, and south-western Gansu). All species occur at altitudes of (3200–) 4000–6200 m, primarily on scree slopes and in rock crevices in the alpine areas of the Himalayan, Karakorum, Pamir, and Hengduan mountain ranges. Previous studies (Yue et al., 2006, 2008) provided little phylogenetic resolution within the genus. In the present study, the addition of nuclear and chloroplast sequence data gives both improved resolution and evidence for the geographic origin of the clade.

The combined nuclear and chloroplast DNA data included 21 of the 26 species of *Solms-laubachia* s.l. Two species, namely *Phaeonychium villosum* and

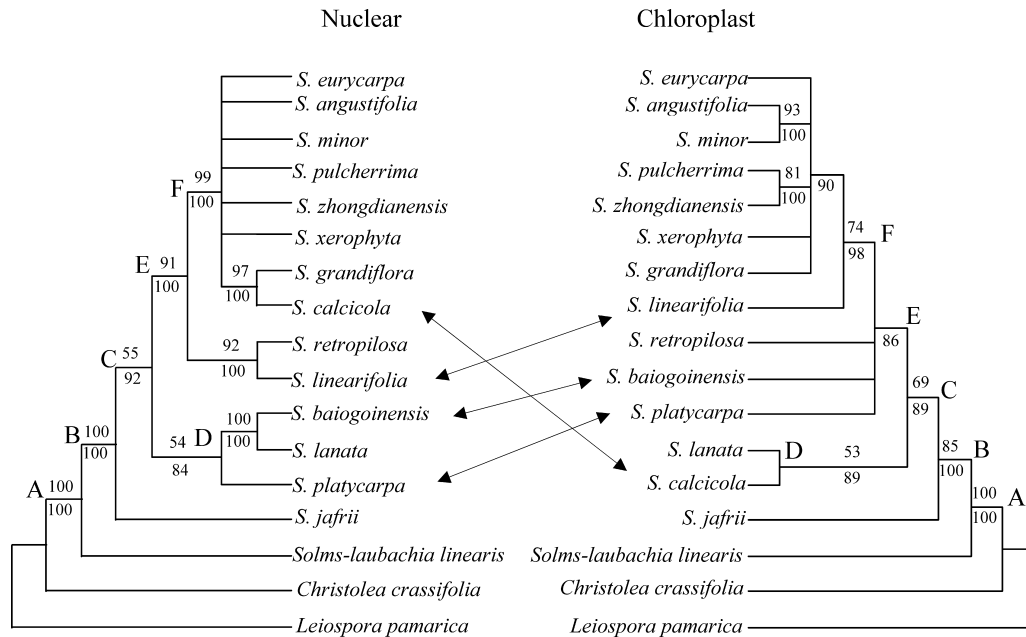


Fig. 3. Comparison of phylogenetic relationships based on combined nuclear and combined chloroplast sequence, respectively. The nuclear tree is the strict consensus tree of 32 most parsimonious trees (MPTs; consistency index (CI) = 0.91, retention index (RI) = 0.69, and rescaled consistency index (RC) = 0.63) and the chloroplast tree is the strict consensus tree of eight MPTs (CI = 0.81, RI = 0.52, and RC = 0.42), both with bootstrap (BS; > 50%) support above and posterior probability (PP) support below the branches. Letters A–H refer to the clades discussed in the text. Arrows point to species that are not in the same position between two phylogenetic trees. Note, “*Solms-laubachia*” is abbreviated to “*S.*” throughout.

S. pumila, are sampled here for the first time. The latter is closely related to *S. linearis*, and *P. villosum* is sister to the Hengduan Mountains clade (Fig. 2). The position of *P. villosum* within *Solms-laubachia* s.l. suggests that it should be transferred to this genus, as was done by Yue et al. (2008) to its previous congener *P. jafrii* (= *S. jafrii*). However, because data from only one marker was available for *P. villosum*, more evidence is needed to make a formal change in taxonomy.

Morphologically, *Solms-laubachia* s.l. is considerably more diverse than traditionally circumscribed due to the nesting of species from other genera within it (Yue et al., 2008). The present study implies even further disparity between closely related species. In the eight-marker phylogeny, *S. baiogoinensis*, which has dentate, palmately veined leaves, is not closely related to species with the same leaf morphology, but to those with entire, pinnately veined leaves. Furthermore, *S. platycarpa*, which has flowers in racemes, is most closely related to species with flowers solitary from a basal rosette (Fig. 2). Both the leaf morphology and flower orientation largely disagree with phylogenetic grouping, suggesting that morphological evolution in *Solms-laubachia* s.l. is very labile.

Compared with these morphological traits, geographic patterns in *Solms-laubachia* s.l. have a clear phylogenetic signal. All species endemic to the Heng-

duan Mountains form one clade nested within a grade of lineages that range progressively eastward from central Asia (Fig. 2). Although the species sampling within this genus was incomplete and the HM clade did not receive strong support, it is unlikely that sampling additional taxa would significantly alter this result. For other clades in Brassicaceae, recent molecular phylogenetic studies similarly suggest that geographic ranges apparently have a stronger phylogenetic signal than morphology (e.g. in Eurasian and North American *Braya* Sternb. & Hoppe (Warwick et al., 2004), American *Draba* L. (Koch & Al-Shehbaz, 2002), American *Noccaea* Moench (Koch & Al-Shehbaz, 2004), and Asian *Yinshania* Ma & Y. Z. Zhao (Koch & Al-Shehbaz, 2000)). These results reinforce the need for caution in using morphological characters alone to construct phylogenetic relationships in Brassicaceae (Al-Shehbaz et al., 2006).

Differences between phylogenetic relationships inferred from the nuclear and chloroplast genomes warrant further investigation. For example, in the nuclear phylogeny, *S. lanata* and *S. baiogoinensis* are sister species (100% BS, 100% PP), a relationship not supported by the chloroplast data (Fig. 3). Discordance of this kind can arise from factors such as reticulate evolution (especially hybridization and introgression), recombination, or lineage sorting (Wendel & Doyle, 1998).

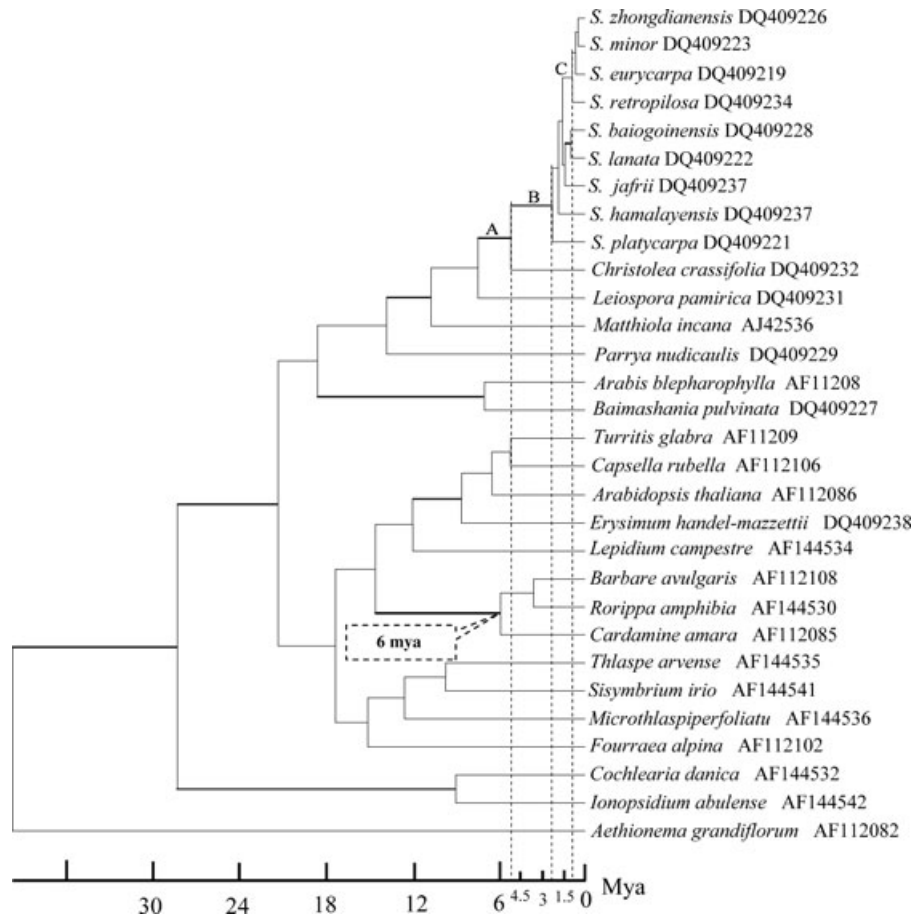


Fig. 4. Chronogram of Brassicaceae generated from BEAST analysis on 30 *Chs* sequences selected from the data matrix in Yue et al. (2006). Clades with posterior probability (PP) support > 95% are indicated by thicker lines. The calibration point was set to 6 mya according to pollen deposit information. The divergence times of three lineages were estimated as follows: A (*Solms-laubachia* s.l. and *Christolea* from *Leiospora*), 5.27 mya with a 95% high posterior density (HPD) interval of 3.0–7.73; B (*Solms-laubachia* s.l. from *Christolea*), 2.31 mya with 95% HPD of 1.42–3.68; and C (Hengduan Mountains *Solms-laubachia* s.l. species), 0.87 mya with 95% HPD of 0.41–1.42. Note, “*Solms-laubachia*” is abbreviated to “*S.*” throughout.

Reticulate evolution caused by polyploidization and hybridization has been reported in several genera of Brassicaceae, such as *Lepidium* L. (Mummenhoff et al., 2001, 2004), *Braya* and *Neotorularia* Hedge & J. Léonard (Warwick et al., 2004), *Yinshania* (Koch & Al-Shehbaz, 2000), and others (see Koch & Lihová, 2006, and references therein). Except for one tetraploid population of *S. retropilosa* ($2n = 28$), all species of *Solms-laubachia* s.l. studied here are known to be diploid with $2n = 14$ (Yue et al., 2003, 2004). Moreover, they are morphologically distinct and allopatrically confined to scree slopes of different mountains. In general, sympatry is very rare in the genus. The most widespread species is *S. eurycarpa*, which co-occurs with *S. linearifolia* and *S. angustifolia* at only one locality each. Surveys of these populations revealed no morphological or molecular evidence for hybridization (Yue, 2006), and no distinct allelic variation was detected among con-

specific clones of the two nuclear genes (*G3pdh* and *LEAFY*) used in the present study. Therefore, although reticulate evolution within *Solms-laubachia* s.l. cannot be ruled out, we suggest that a more likely explanation for incongruence between the nuclear and chloroplast phylogenies is incomplete lineage sorting of ancestral polymorphisms.

With 11 of 26 species endemic to the Hengduan Mountains region, *Solms-laubachia* s.l. is one of many North Temperate plant genera with a center of diversity in this area. Others include *Rhododendron* L., *Primula* L., *Pedicularis* L., *Gentiana* L., *Corydalis* DC., and *Saussurea* DC. (Wu, 1988). This richness and endemism has generally been attributed to two major factors. One is that the region was a refugium during Quaternary fluctuations in climate, preserving lineages from extinction. This idea appears to be gaining support from recent evidence that a number of widely distributed plant species

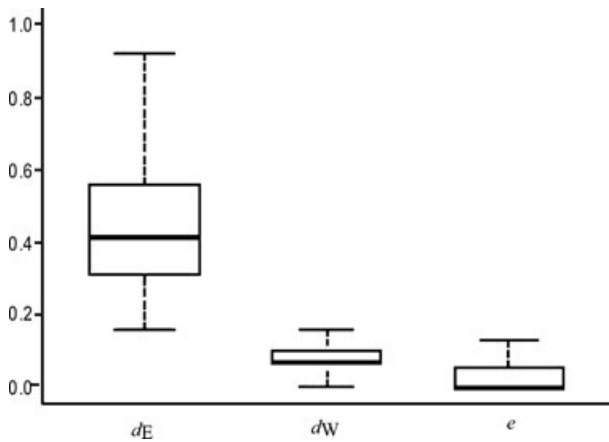


Fig. 5. Box plots of rate parameters for geographic range evolution in *Solms-laubachia* s.l. Each plot shows the median (horizontal line), first quartiles (box), and third quartiles (whiskers) of values estimated by maximum likelihood from 500 phylogenetic trees drawn from the Bayesian posterior density of the combined phylogenetic analysis of eight molecular markers (see text). Parameters are rates of eastward dispersal (d_E), westward dispersal (d_W), and local extinction (e).

exhibit higher population genetic diversity within the Hengduan region than elsewhere in their ranges (e.g. Chen et al., 2008a, 2008b; Yang et al., 2008). The other is that environmental and habitat heterogeneity associated with uplift of the Qinghai-Tibetan Plateau promoted rapid speciation (Axelrod et al., 1998; Liu et al., 2006). This latter hypothesis is somewhat more difficult to test, not least because consensus on the timing and stability of plateau uplift remains elusive (e.g. Shi et al., 1998; Clark et al., 2006; Liu-Zeng et al., 2008). However, phylogenetic inferences about the timing and geographic origins of the plant lineages that assembled the present flora may help provide some clarity.

Within *Solms-laubachia* s.l., sequence divergence between Hengduan endemics is relatively low, with Kimura-2 parameter (K2P) distances ranging from 0.63% to 1.9% for the combined nuclear alignment and from 0.82% to 2.1% for the combined chloroplast alignment (Table 2). This pattern and the poorly resolved phylogenetic relationships within this clade are suggestive of a recent radiation. Here, we estimate the divergence time of *Solms-laubachia* s.l. from its sister group, *Christolea*, to be between 1.42–3.68 mya (Node B; Fig. 4) and the crown age of the Hengduan Mountains clade to be 0.87 mya (Node C; Fig. 4). One may speculate that radiation of this clade was accelerated by post-Miocene uplift of the Qinghai-Tibetan Plateau, a hypothesis that has been raised numerous times in the literature, for example in the context of apparently rapid phylogenetic diversification within *Gentiana* (Yuan & Küpfer, 1997), *Rheum* (Wang et al., 2005), *Crawfordia*

and relatives (Chen et al., 2005b), and *Nannoglottis* (Liu et al., 2002). However, geological evidence for such uplift is mixed at best. Oxygen isotope analyses suggest that current elevations in south-western Tibet have been maintained, or were perhaps even higher than present, since the late Miocene (Saylor et al., 2009). The earliest age at which the plateau reached its present elevation is still contentious: geological and paleontological studies have variously suggested that large-scale uplift (with possible intervening lowering) occurred during at least four different periods: 22, 15–13, 8–7, and 3.5–1.6 mya (Harrison et al., 1992; Li et al., 1995; Shi et al., 1998; An et al., 2001; Guo et al., 2002; Spicer et al., 2003). Phylogenetic divergence time estimates of endemic Hengduan plant clades show similar variation: although our analysis suggests that the radiation of Hengduan endemics in *Solms-laubachia* s.l. may have occurred recently, the greater genetic divergence between species in *Pedicularis* (Yang et al., 2003; Ree, 2005) and *Ligularia* (Liu et al., 2006) points towards diversification coincident with earlier periods of uplift.

The present study uses a quantitative parametric approach to provide strong evidence for a central Asian/western Himalayan origin of *Solms-laubachia* s.l. and a general trend of eastward movement of the lineage into the Hengduan Mountains. A similar geographic pattern is found in *Incarvillea* Juss., a North Temperate, herbaceous clade of 16 species within the primarily tropical and woody family Bignoniaceae, whose phylogeny shows a strongly supported clade of Eastern Himalayan/Hengduan endemics nested within lineages from central Asia (Chen et al., 2005a). Close relationships between central Asian and Hengduan lineages are also apparent in other taxa, such as *Rheum* (Wang et al., 2005) and *Picea* A. Dietr. (Ran et al., 2006). However, as yet there is no evidence for a general pattern or common cause for concerted lineage movement between these two areas. In general, quantitative analyses of Sino-Himalayan historical phytogeography are lacking, despite recent increases in the number of phylogenetic studies being published. By integrating models of paleoclimate and paleoelevation with estimates of the timing of diversification, geographic origin, ecological characteristics etc. of lineages endemic to the Hengduan region, it may be possible to tease apart historical pattern and contingency in the floristic assembly of this biodiversity hotspot.

In summary, we detected morphological homoplasy in species of *Solms-laubachia* s.l. based on their molecular phylogenetic relationships. In contrast, there was a clearer phylogenetic signal in species ranges, with the Hengduan Mountain endemics occupying a derived position relative to species from the western Himalayas

and central Asia. We also detected incongruence between the nuclear and chloroplast gene trees, which we suggest may be explained by incomplete lineage sorting of ancestral polymorphisms. Using Bayesian relaxed molecular clock analysis and pollen deposit calibration, we estimated the divergence time of *Solms-laubachia* s.l. from its sister group to be between 1.42 and 3.85 mya. Results of DEC model analysis infer central Asia and the western Himalayas as the most probable ancestral range of *Solms-laubachia* s.l. and estimate a high rate of eastward movement during the diversification of descendant lineages. We suggest that *Solms-laubachia* s.l. originated during the Pliocene in central Asia and subsequently migrated eastward into the Hengduan Mountains, colonizing sky-island, alpine scree-slope habitats that may have provided novel ecological opportunity and accelerated speciation, ultimately establishing this region as the present center of diversity of the genus.

Acknowledgements This study was supported by the Chinese National Natural Science Foundation (grants no. 30625004, 40771073 to HS), the Yunnan Natural Science Foundation (2008CC013 to HS), the US National Science Foundation (grant no. DEB-0321846, to DEB), the John D. and Catherine T. MacArthur Foundation (grant to JW, RR, and GM), the Innovation Project of the Chinese Academy of Sciences (KSCX2-YW-Z-030) and a Mercer Fellowship from the Arnold Arboretum (to JPY). Fieldwork in Xinjiang (conducted by IAA) was supported by the National Geographic Society (grant no. 7405-03).

References

- Al-Shehbaz IA. 2000. A revision of the genus *Phaeonychium* (Brassicaceae). *Nordic Journal of Botany* 20: 157–163.
- Al-Shehbaz IA. 2001. A review of gamosepaly in the Brassicaceae and a revision of *Desideria*, with a critical evaluation of related genera. *Annals of the Missouri Botanical Garden* 87: 549–563.
- Al-Shehbaz IA. 2005. *Desideria mieheorum* (Brassicaceae), a new species from Tibet. *Novon* 15: 1–3.
- Al-Shehbaz IA, Beilstein MA, Kellogg EA. 2006. Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview. *Plant Systematics and Evolution* 259: 89–120.
- Al-Shehbaz IA, Yang G. 2001. A revision of *Solms-laubachia* (Brassicaceae). *Harvard Paper in Botany* 5: 371–381.
- An ZS, Kutzbaeh JE, Prell WL, Port SC. 2001. Evolution of Asian monsoons and phased uplift of the Himalaya-Tibetan plateau since Late Miocene times. *Nature* 411: 62–66.
- An Z-X (安争夕). 1995. Cruciferae. In: *Flora Xinjiangensis* (新疆植物志). Urumqi: Xinjiang Science & Technology & Hygiene Publishing House. 2(2): 38–229.
- Axelrod DI, Al-Shehbaz IA, Raven PH. 1998. History of the modern flora of China. In: Zhang AL, Wu SG eds. *Floristic characteristics and diversity of East Asian plants*. Beijing: China Higher Education Press. 43–55.
- Baum DA, Yoon HS, Oldham RL. 2005. Molecular evolution of the transcription factor *LEAFY* in Brassicaceae. *Molecular Phylogenetics and Evolution* 37: 1–14.
- Beilstein MA, Al-Shehbaz IA, Kellogg EA. 2006. Brassicaceae phylogeny and trichome evolution. *American Journal of Botany* 93: 607–619.
- Beilstein MA, Al-Shehbaz IA, Mathews S, Kellogg EA. 2008. Brassicaceae phylogeny inferred from Phytochrome A and NDHF sequence data: tribes and trichomes revisited. *American Journal of Botany* 95: 1307–1327.
- Chen JM, Liu F, Gituru WR, Wang QF. 2008a. Chloroplast DNA phylogeography of the Chinese endemic alpine quillwort *Isoetes hypsophila* Hand.-Mazz. (Isoetaceae). *International Journal of Plant Sciences* 169: 792–798.
- Chen ST, Guan KY, Zhou ZK, Olmstead R, Cronk Q. 2005a. Molecular phylogeny of *Incarvillea* (Bignoniaceae) based on ITS and trnL-F sequences. *American Journal of Botany* 92: 625–633.
- Chen SY, Wu GL, Zheng DJ, Gao QB, Duan YZ, Zhang FQ, Chen SL. 2008b. Potential refugium on the Qinghai-Tibet Plateau revealed by the chloroplast DNA phylogeography of the alpine species *Metagentiana striata* (Gentianaceae). *Botanical Journal of the Linnean Society* 157: 125–140.
- Chen SY, Xia T, Wang YJ, Liu JQ, Chen SL. 2005b. Molecular systematics and biogeography of *Crawfordia*, *Metagentiana* and *Tripterospermum* (Gentianaceae) based on nuclear ribosomal and plastid DNA sequences. *Annals of Botany* 96: 413–424.
- Clark MK, Royden LH, Whipple KX, Burchfiel BC, Zhang X, Tang W. 2006. Use of a regional, relict landscape to measure vertical deformation of the eastern Tibetan Plateau. *Journal of Geophysical Research* 111: F03002.
- Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small amounts of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.
- Drummond AJ, Rambaut A. 2007. “BEAST: Bayesian evolutionary analysis by sampling trees.” *BMC Evolutionary Biology* 7: 214 <http://beast.bio.ed.ac.uk/MolecularBiologyandEvolution>.
- Farris JS, Kluge AG, Eckardt MJ. 1970. A numerical approach to phylogenetic systematics. *Systematic Zoology* 19: 172–191.
- Fitch WM. 1971. Toward defining the course of evolution: minimal change for a specific tree topology. *Systematic Zoology* 20: 406–416.
- Guo ZT, Ruddiman WF, Hao QZ, Wu HB, Qiao YS, Zhu RX, Peng SZ, Wei JJ, Yuan BY, Liu TS. 2002. Onset of Asian desertification by 22 Myr ago inferred from loess deposits in China. *Nature* 416: 159–163.
- Harrison TM, Copeland P, Kidd WSF, Yin A. 1992. Raising Tibet. *Science* 255: 1663–1670.
- Huang R-F (黄荣福). 1997. Cruciferae. In: *Flora Qinghaiica* (青海植物志). Xining: Qinghai Peoples's Publishing House. 1: 410–505.
- Koch M, Haubold B, Mitchell-Olds T. 2000. Comparative evolutionary analysis of chalcone synthase and alcohol

- dehydrogenase loci in *Arabidopsis*, *Arabis*, and related genera (Brassicaceae). *Molecular Biology and Evolution* 17: 1483–1498.
- Koch M, Haubold B, Mitchell-Olds T. 2001. Molecular systematics of the Brassicaceae: evidence from coding plastidic *matK* and nuclear *chs* sequences. *American Journal of Botany* 88: 534–544.
- Koch M, Al-Shehbaz IA. 2000. Molecular systematics of the Chinese *Yinshania* (Brassicaceae): evidence from plastid and nuclear ITS DNA sequence data. *Annals of the Missouri Botanical Garden* 87: 246–272.
- Koch M, Al-Shehbaz IA. 2002. Molecular data indicate complex intra- and intercontinental differentiation of American *Draba* (Brassicaceae). *Annals of the Missouri Botanical Garden* 89: 88–109.
- Koch M, Al-Shehbaz IA. 2004. Taxonomic and phylogenetic evaluation of the American “*Thlaspi*” species: identity and relationship to the Eurasian genus *Noccaea* (Brassicaceae). *Systematic Botany* 29: 375–384.
- Koch M, Lihová J. 2006. Polyploidy, hybridization and reticulate evolution: lessons from the Brassicaceae. *Plant Systematics and Evolution* 259: 143–174
- Kuan KC (关克俭). 1985. Cruciferae. In: *Flora Xizangica* (西藏植物志). Beijing: Science Press. 2: 381–384.
- Lan Y-Z (蓝永珍). 1987. *Solms-laubachia*. In: *Flora Reipublicae Popularis Sinicae* (中国植物志). Beijing: Science Press. 33: 326–339.
- Lan Y-Z (蓝永珍), Cheo T-Y (周太炎). 1981. On the Chinese genus *Solms-laubachia* Muschler (Cruciferae). *Acta Phytotaxonomica Sinica* (植物分类学报) 19: 472–480.
- Lee CH, Wen J. 2004. Phylogeny of *Panax* using chloroplast *trnC-trnD* intergenic region and the utility of *trnC-trnD* in interspecific studies of plants. *Molecular Phylogenetics and Evolution* 31: 894–903.
- Li JJ, Shi YF, Li BY. 1995. Uplift of the Qinghai-Xizang (Tibet) Plateau and Global Change. Lanzhou: Lanzhou University Press.
- Liu JQ, Wang YJ, Wang AL, Ohba H, Abbott RJ. 2006. Radiation and diversification within the *Ligularia-Cremanthodium-Parasenecio* complex (Asteraceae) triggered by uplift of the Qinghai-Tibetan Plateau. *Molecular Phylogenetics and Evolution* 38: 31–49.
- Liu JQ, Gao TG, Chen ZD, Lu AM. 2002. Molecular phylogeny and biogeography of the Qinghai-Tibet Plateau endemic *Nannoglottis* (Asteraceae). *Molecular Phylogenetics and Evolution* 23: 307–325.
- Liu-Zeng J, Tapponnier P, Gaudemer Y, Ding L. 2008. Quantifying landscape differences across the Tibetan plateau: Implications for topographic relief evolution. *Journal of Geophysical Research* 113: F04018, doi:10.1029/2007JF000897.
- Mai DH. 1995. Tertiäre Vegetationsgeschichte Europas. G. Fischer, Jena, Stuttgart, New York.
- Mau B, Newton M, Larget B. 1999. Bayesian phylogenetic inference via Markov Chain Monte Carlo methods. *Biometrics* 55: 1–12.
- Mummenhoff K, Brüggemann H, Bowman JL. 2001. Chloroplast DNA phylogeny and biogeography of *Lepidium* (Brassicaceae). *American Journal of Botany* 88: 2051–2063.
- Mummenhoff K, Linder P, Friesen N, Bowman JL, Lee JY, Franzke A. 2004. Molecular evidence for bicontinental hybridogenous genomic constitution in *Lepidium* sensu stricto (Brassicaceae) species from Australia and New Zealand. *American Journal of Botany* 91: 254–261.
- Posada D, Crandall KA. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.
- Posada D, Buckley T. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology* 53: 793–808.
- Rambaut A, Drummond AJ. 2007. Tracer v1.4 [online]. Available from <http://beast.bio.ed.ac.uk/Tracer> [accessed 20 February 2009].
- Ran JH, Wei XX, Wang XQ. 2006. Molecular phylogeny and biogeography of *Picea* (Pinaceae): Implications for phylogeographical studies using cytoplasmic haplotypes. *Molecular Phylogenetics and Evolution* 41: 405–419.
- Rannala B, Yang ZH. 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* 43: 304–311.
- Ree RH. 2005. Phylogeny and the evolution of floral diversity in *Pedicularis* (Orobanchaceae). *International Journal of Plant Sciences* 166: 595–613.
- Ree RH, Moore BR, Webb CO, Donoghue MJ. 2005. A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. *Evolution* 59: 2299–2311.
- Ree RH, Smith SA. 2008. Maximum-likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology* 57(1): 4–14.
- Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- Saylor JE, Quade J, Dettman DL, DeCelles PG, Kapp PA, Ding L. 2009. The late Miocene through present paleoelevation history of southwestern Tibet. *American Journal of Science* 309: 1–42.
- Schulz OE. 1936. Cruciferae. In: Engler A, Harms H eds. *Die natürlichen Pflanzenfamilien*. Leipzig: Verlag von Wilhelm Engelmann 17B: 227–658.
- Shi YF, Li JJ, Li BY. 1998. Uplift and environmental changes of Qinghai-Tibetan Plateau in the Late Cenozoic. Guangzhou: Guangdong Science and Technology Press.
- Spicer RA, Harris NB, Widdowson WM, Herman AB, Guo S, Valdes PJ, Wolfe JA, Kelley SP. 2003. Constant elevation of southern Tibet over the past 15 million years. *Nature* 421: 622–624.
- Strand AE, Leebens-Mack J, Milligan BG. 1997. Nuclear DNA based markers for plant evolutionary biology. *Molecular Ecology* 6: 113–118.
- Swofford DL. 2002. PAUP*: Phylogenetic analysis using parsimony (* and other methods), version 4.0. Sunderland, MA: Sinauer Associates.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876–4882.
- Wang AL, Yang MY, Liu JQ. 2005. Molecular phylogeny, recent radiation and evolution of gross morphology of the Rhubarb genus *Rheum* (Polygonaceae) inferred from chloroplast DNA *trnL-F* sequences. *Annals of Botany* 96: 489–498.

- Warwick SI, Al-Shehbaz IA, Sauder C, Harris JG, Koch M. 2004. Phylogeny of *Braya* and *Neotorularia* (Brassicaceae) based on nuclear ribosomal internal transcribed spacer and chloroplast *trnL* intron sequences. *Canadian Journal of Botany* 82: 376–392.
- Warwick SI, Sauder CA, Al-Shehbaz IA, Jacquemoud F. 2007. Phylogenetic relationships in the tribes Anchonieae, Chorisporeae, Euclidieae, and Hesperideae (Brassicaceae) based on nuclear ribosomal ITS DNA sequences. *Annals of the Missouri Botanical Garden* 94: 56–78.
- Wendel JF, Doyle JJ. 1998. Phylogenetic incongruence: window into genome history and molecular evolution. In: Soltis DE, Soltis PS, Doyle JJ eds. *Molecular systematics of plants II: DNA sequencing*. Boston: Kluwer Academic Publishers. 1–42.
- Wu CY. 1988. Hengduan Mountains flora and its significance. *Journal of Japanese Botany* 63: 297–311.
- Yang FS, Wang XQ, Hong DY. 2003. Unexpected high divergence in nrDNA ITS and extensive parallelism in floral morphology of *Pedicularis* (Orobanchaceae). *Plant Systematics and Evolution* 240: 91–105.
- Yang FS, Li YF, Ding X, Wang XQ. 2008. Extensive population expansion of *Pedicularis longiflora* (Orobanchaceae) on the Qinghai-Tibetan Plateau and its correlation with the Quaternary climate change. *Molecular Ecology* 17: 5135–5145.
- Yuan YM, K pfer P. 1997. The monophyly and rapid evolution of *Gentiana* sect. *Chondrophyllae* Bunge s.l. (Gentianaceae): evidence from the nucleotide sequences of the internal transcribed spacers of nuclear ribosomal DNA. *Botanical Journal of the Linnean Society* 123: 25–43.
- Yue J-P (乐霁培). 2006. Phylogeny of the genus *Solms-Laubachia* (Brassicaceae). Ph. D. Dissertation. Kunming (昆明): Kunming Institute of Botany, Chinese Academy of Sciences.
- Yue JP, Gu ZJ, Al-Shehbaz IA, Sun H. 2004. Cytological studies on the Sino-Himalayan endemic *Solms-Laubachia* Muschler and two related genera (Brassicaceae). *Botanical Journal of the Linnean Society* 145: 77–86.
- Yue JP, Sun H, Al-Shehbaz IA, Gu ZJ. 2003. Cytological studies of five Chinese species of *Solms-laubachia* (Brassicaceae). *Harvard Papers in Botany* 7: 467–473.
- Yue JP, Sun H, Al-Shehbaz IA, Li JH. 2006. Support for an expanded *Solms-laubachia* (Brassicaceae): evidence from sequences of chloroplast and nuclear genes. *Annals of the Missouri Botanical Garden* 93: 402–411.
- Yue JP, Sun H, Li JH, Al-Shehbaz IA. 2008. A synopsis of an expanded *Solms-laubachia* (Brassicaceae), and the description of four new species from western China. *Annals of the Missouri Botanical Garden* 95: 520–538.