

Unexpected high divergence in nrDNA ITS and extensive parallelism in floral morphology of *Pedicularis* (Orobanchaceae)

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Abstract. Internal transcribed spacer (ITS) region sequences of nuclear ribosomal DNA (nrDNA) were used in the phylogenetic reconstruction of *Pedicularis*, a genus with strong adaptive radiation. Forty-two species representing 12 greges of the genus were all, except *P. resupinata*, sampled from the Hengduan Mountain region, China. A high level of ITS sequence variation was found among the species distributed in such a small geographical area, which had been rarely reported in other groups. The great ITS divergence in *Pedicularis* could be explained by a relatively ancient origin and diversification of the genus followed by migration of different floristic components into the Hengduan Mountains, or accelerated rates of molecular evolution in parasitic lineages of Orobanchaceae. In the present ITS phylogeny, almost all the main clades are not consistent with the high hierarchical taxa within *Pedicularis*, which implies that significant parallel evolution occurred in floral morphology of the genus, and that undue attention has been paid to corolla characters in the intragenus classifications.

Key words: nrDNA ITS, molecular evolution, floral morphology, adaptive radiation, Hengduan Mountains, *Pedicularis*.

Introduction

The internal transcribed spacer (ITS) region of 18S-26S nuclear ribosomal DNA (nrDNA) has been widely used in assessing phylogenetic relationships at lower taxonomic level because of its high copy number, rapid concerted evolution, and diverse rates of evolution within and among component subunits and spacers. Even within species ITS sequences showed some promise for investigations on relationships among allopatric or disjunct populations (Baldwin et al. 1995). However, some studies showed that ITS sequence has a bad resolution in retrieving the evolutionary history of young clades such as taxa with adaptive radiation (Kita et al. 1995, Yuan et al. 1996, Aïnouche and Bayer 1999, Alice and Campbell 1999), although the relationships among subgenera, sections or series could be revealed sometimes. More studies are obviously needed to investigate the variation pattern of nrDNA ITS in radiated taxa from more territories. Moreover, studies on the relationship between molecular variation and morphological divergence will shed special light on speciation mechanisms.

Pedicularis (Lousewort), with more than 500 species, is a typical group of adaptive radiation considering its wide distribution, diverse habitats, high level of species diversity and extreme variation of the corolla morphology likely unequalled by any other genus of angiosperms (Li 1948, Tsoong 1956a, Yang et al. 1998), and offers us a good paradigm to learn the evolution pattern of nrDNA in local, rapidly radiated taxa. The genus, confined to the Northern Hemisphere, is a member of the arctic-alpine flora and the majority of species occur in the arctic regions and alpine meadows. It is striking that 352 species, more than half of the genus, occur in China (Yang et al. 1998). In particular, 214 species are centralized in the Hengduan Mountains (Yang et al. 1998) (Fig. 1), the eastern Himalayan diversity “hot

spot” (Wilson 1992) and one of the plant diversity centers of the world containing about 9,000 plant species in about 500,000 km². Most species in this area are endemic (Hong 1983, Yang et al. 1998), but few studies have been performed on the speciation mechanism of taxa distributed in this region.

It is suggested that *Pedicularis* is a thoroughly natural group, though flowers of the genus show great diversity (Maximowicz 1888, Li 1948). The evolution patterns of *Pedicularis* are so complex that ten influential but controversial systems of classification (Steven 1823; Bunge 1841, 1846, Bentham 1846, Maximowicz 1888, Prain 1890, Bonati 1910, Limpricht 1924, Hurusawa 1947, 1948a, 1948b, 1948c, 1949a, 1949b; Li 1948, 1949; Tsoong 1955, 1961, 1963) have been proposed by ten

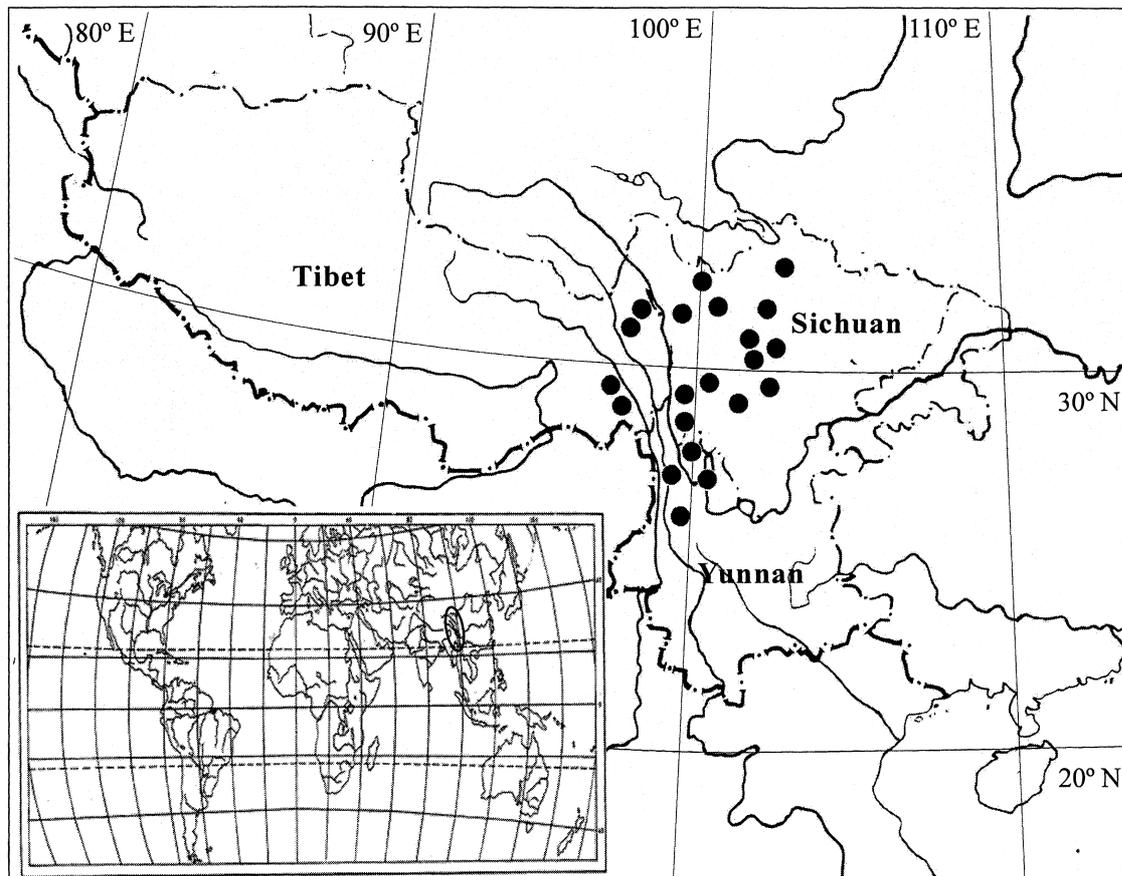


Fig. 1. Collection sites (rotundity) of *Pedicularis* in the study (*P. resupinata* sampled from E. Inner Mongolia was not indicated). Ellipse on the world map indicates the Hengduan Mountains

authors, but a series of questions have been hanging in doubt. Some hot-debated puzzles include: 1) Where and when did the genus originate? 2) Has the divergence of floral morphology taken place under the pressure of ecological selection? 3) Are morphological variations correlated with molecular evolution?

In the present study, we try to investigate the evolutionary history of *Pedicularis* species in the Hengduan Mountains and explore the relationship between morphological radiation and molecular evolution in the genus based on sequence analysis of nrDNA ITS region.

Materials and methods

Plant materials. Forty-two species representing 12 out of 13 greges in Tsoong (1963) were all, except *P. resupinata*, sampled from the Hengduan Mountain region (Fig. 1, Table 1). Identification of specimens and names of taxa were based on Tsoong (1963) and Yang et al. (1998). Vouchers are deposited in PE. Additional sequences used in the present study include nrDNA ITS of *Orobanche minor* (AF437315) and *Castilleja sulphurea* (AF478944) (Beardsley and Olmstead 2002) retrieved from Genbank.

DNA extraction and PCR amplification. Total genomic DNA was isolated from silica gel dried leaves using a modified CTAB method (Rogers and Bendich 1988). The entire ITS region, comprising ITS1, 5.8S and ITS2, was amplified via the polymerase chain reaction (PCR) using primers “ITS1” and “ITS4” designed by White et al. (1990). The reaction volume was 25 μ L, containing 10–50 ng of total DNA, 6 pmol of each primer, 0.2 mM of each dNTP, 2 mM $MgCl_2$ and 1.0 unit of Taq DNA polymerase. Amplification was conducted in a Peltier Thermal cycler (PTC-200, MJ RESEARCH). PCR cycles were as follows: one cycle of 120 s at 70 $^{\circ}C$, 2 cycles of 50 s at 94 $^{\circ}C$, 20 s at 55 $^{\circ}C$ and 50 s at 72 $^{\circ}C$, followed by 30 cycles of 20 s at 94 $^{\circ}C$, 20 s at 55 $^{\circ}C$, and 50 s at 72 $^{\circ}C$, with a final extension step for 6 min at 72 $^{\circ}C$. The PCR products were purified using Genclean (BioDev). Sequencing was done on ABI PRISM 377 DNA Sequencer using the ABI Prism Bigdye Terminator Cycle Sequencing Ready Reaction Kit.

Data analysis. Sequence alignments were made with CLUSTAL X (Thompson et al. 1997) and refined manually. The ITS region boundaries were determined by comparison with various published sequences available in GenBank. Sequence divergence was calculated with MEGA version 2.1 (Kumar et al. 2001) based on the Kimura 2-parameter model (Kimura 1980). Maximum parsimony analysis was performed using PAUP v.4.0 program (Swofford 1998) on the basis of nucleotide substitutions in aligned sequences. Based on the latest circumscription for the families Scrophulariaceae and Orobanchaceae (Reveal et al. 1999), and on recently published studies on molecular systematics of the related families (Olmstead and Reeves 1995, Reeves and Olmstead 1998, Young et al. 1999, Olmstead et al. 2001), *Orobanche minor* and *Castilleja sulphurea* were selected as the outgroups. The HEURISTIC searches used RANDOM stepwise addition of taxa and tree bisection-reconnection (TBR) branch swapping. Gaps were coded as missing data. Characters and character-state changes were weighted equally. In order to examine the robustness of various clades revealed in the most parsimonious trees, the bootstrap analysis was performed with 500 replicates using the “Heuristic” search option.

Results

Length of the entire ITS region ranges from 598 to 606 bp in *Pedicularis* species sampled. The ITS1 (228–238 bp) was slightly longer than ITS2 (220–228 bp). No length variation was found in 5.8S (143bp) region. The G + C content varied from 58.50% to 65.80% (average = 62.80%) in ITS1 and from 60.40% to 68.60% (average = 64.1%) in ITS2. Great divergence of nrDNA ITS was found among the *Pedicularis* species analyzed (Fig. 2). Excluding the outgroups, the pairwise sequence divergence varied from 0 to 14.4% (average = 7.6%) for the entire ITS region, and the extreme divergence reached 23.1% (*P. densispica* vs. *P. integrifolia*) for ITS1 (mean = 9.3%), and 18.6% (*P. confertiflora* vs. *P. sigmoides*, *P. longicaulis* vs. *P. integrifolia*) for ITS2 (mean = 11.4%) respectively. For the five species sampled from Tibet and the 28

Table 1. *Pedicularis* species used in this study

Species	Grex (Tsoong, 1963)	Voucher specimens and their origin	GenBank Accession No.
<i>P. cinerascens</i> *	1. Sceptrum	YANG2014: Kangding, W. Sichuan	AY155302
<i>P. lophotricha</i> *	1. Sceptrum	YANG2050: Daocheng, W. Sichuan	AY155308
<i>P. rhodotricha</i> *	1. Sceptrum	YANG2039: Daocheng, W. Sichuan	AY155304
<i>P. rudis</i>	1. Sceptrum	YANG0001: Lijiang, NW. Yunnan	AY155305
<i>P. trichoglossa</i> ^{#*}	1. Sceptrum	YANG0171: Qamdo, E. Tibet	AY155309
<i>P. lachnoglossa</i> *	2. Neosceptrum	YANG2047: Daocheng, W. Sichuan	AY155303
<i>P. longicaulis</i>	3. Cyclocladus	YANG0213: Lijiang, NW. Yunnan	AY155289
<i>P. axillaris</i> *	4. Dolichomiscus	YANG0024: Zhongdian, NW. Yunnan	AY155300
<i>P. laxiflora</i>	4. Dolichomiscus	YANG0083: Kangding, W. Sichuan	AY155313
<i>P. cyathophylla</i> *	6. Cyathophora	YANG2051: Daocheng, W. Sichuan	AY155310
<i>P. cyathophylloides</i> [#]	6. Cyathophora	YANG0148: Jamda, E. Tibet	AY155312
<i>P. superba</i> *	6. Cyathophora	YANG0051: Zhongdian, NW. Yunnan	AY155311
<i>P. resupinata</i>	7. Pedicularis	YANG0250: E. Inner Mongolia	AY155298
<i>P. anas</i> *	8. Sigmantha	YANG2097: Hongyuan, W. Sichuan	AY155279
<i>P. cheilanthifolia</i>	8. Sigmantha	YANG0100: Garze, W. Sichuan	AY155278
<i>P. globifera</i>	8. Sigmantha	YANG0135: Garze, W. Sichuan	AY155277
<i>P. likiangensis</i> *	8. Sigmantha	YANG0009: Deqen, NW. Yunnan	AY155297
<i>P. lineata</i>	8. Sigmantha	YANG2020: Kangding, W. Sichuan	AY155285
<i>P. microchila</i> *	8. Sigmantha	YANG2121: Xiaojin, N. Sichuan	AY155286
<i>P. roylei</i>	8. Sigmantha	YANG0056: Zhongdian, NW. Yunnan	AY155281
<i>P. rupicola</i> ^{#*}	8. Sigmantha	YANG0196: Markam, E. Tibet	AY155280
<i>P. verticillata</i>	8. Sigmantha	YANG0121: Garze, W. Sichuan	AY155291
<i>P. brevibrabis</i> *	9. Orthosiphonia	YANG0061: Xiangcheng, W. Sichuan	AY155296
<i>P. densispica</i> ^{#*}	9. Orthosiphonia	YANG0184: Zogang, E. Tibet	AY155290
<i>P. duclouxii</i> *	9. Orthosiphonia	YANG0120: Garze, W. Sichuan	AY155299
<i>P. gyrorhyncha</i> *	9. Orthosiphonia	YANG0046: Jianchuan, NW. Yunnan	AY155288
<i>P. oliveriana</i> [#]	9. Orthosiphonia	YANG0180: Qamdo, E. Tibet	AY155306
<i>P. torta</i>	10. Apocladus	YANG2113: Barkam, N. Sichuan	AY155284
<i>P. integrifolia</i> *	11. Brachyphyllum	YANG2043: Daocheng, W. Sichuan	AY155301
<i>P. confertiflora</i>	11. Brachyphyllum	YANG2045: Daocheng, W. Sichuan	AY155282
<i>P. debilis</i> *	11. Brachyphyllum	YANG0008: Deqen, NW. Yunnan	AY155293
<i>P. lutescens</i> *	11. Brachyphyllum	YANG0053: Zhongdian, NW. Yunnan	AY155283
<i>P. pheulpinii</i> *	11. Brachyphyllum	YANG0115: Garze, W. Sichuan	AY155292
<i>P. urceolata</i> *	11. Brachyphyllum	YANG2011: Kangding, W. Sichuan	AY155287
<i>P. tatsienensis</i> *	12. Dolichophyllum	YANG2118: Xiaojin, N. Sichuan	AY155294
<i>P. tatsienensis</i> *	12. Dolichophyllum	YANG0117: Garze, W. Sichuan	AY155295
<i>P. franchetiana</i> *	13. Rhizophyllum	YANG2049: Daocheng, W. Sichuan	AY155314
<i>P. mychophila</i> *	13. Rhizophyllum	YANG0217: Lijiang, NW. Yunnan	AY155318
<i>P. mussotii</i> *	13. Rhizophyllum	YANG2119: Xiaojin, N. Sichuan	AY155317
<i>P. praeceptorum</i> *	13. Rhizophyllum	YANG0011: Deqen, NW. Yunnan	AY155319
<i>P. rhynchodonta</i> *	13. Rhizophyllum	YANG2048: Daocheng, W. Sichuan	AY155307
<i>P. siphonantha</i>	13. Rhizophyllum	YANG2026: Kangding, W. Sichuan	AY155315
<i>P. sigmoides</i> *	13. Rhizophyllum	YANG0052: Zhongdian, NW. Yunnan	AY155316

* Species endemic to the Hengduan Mountains; # Samples collected from Tibet

	11112222	22233333	33333333	44444444	55555666	67777778	88888899	99999000	11222222	22222223	33444455	55566677	77888	88999990	00011889	99999
<i>P. cinerascens</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. rhyrachodonta</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. oliveriana</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. laxiflora</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. lophotricha</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. trichoglossa</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. lachnoglossa</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. rhodotricha</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. rudis</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. duclouxii</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. integrifolia</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. cyathophylla</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. superba</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. cyathophylloides</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. siphonantha</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. mussoii</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. franchetiana</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. paeruptorum</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. signoides</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. mychophila</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. globifera</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. cheilanthifolia</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. anas</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. rupicola</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. roylei</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. gyrorhyncha</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. densispica</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. torta</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. pheulpinii</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. likiangensis</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. urceolata</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. confertiflora</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. lutescens</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. microchila</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. lineata</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. verticillata</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. longicaulis</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. tatsienensis</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. tatsienensis</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. breviflavis</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. debilis</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. resupinata</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
<i>P. axillaris</i>	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T

Fig. 2. The variable sites in the aligned (ITS1 and ITS2) sequences of *Pedicularis*. The numbers on the rows indicate the positions of nucleotides

species endemic to the Hengduan Mountains, the pairwise sequence divergence of the entire ITS region varied from 2.2% to 11.4% (average = 8.2%) and 0 to 14.9% (average = 7.9%) respectively. Sequence alignment required 14 gaps of 1 to 7 bp in ITS1 and 26 gaps of 1 to 4 bp in ITS2. In the aligned sequences of 621 bp, 297 sites are variable, with 188 phylogenetically informative.

Heuristic search employing *Orobanche minor* and *Castilleja sulphurea* as the outgroups generated only one most parsimonious tree with a tree length of 891 steps, a consistency index (CI) of 0.5331 and a retention index (RI) of 0.6228 (Fig. 3). The monophyly of *Pedicularis* was strongly supported (75% bootstrap support) with *P. laxiflora* as the most basal branch. However, the deep branches of the genus had short branch lengths and were weakly supported by the bootstrap test. In particular, neither the five species from Tibet nor the 28 species endemic to the Hengduan Mountains formed a monophyletic group (Fig. 3). In the most parsimonious tree, five major clades were found within *Pedicularis*. Clade I included a single species *P. laxiflora*. In Clade II, all the five species examined in grex 1 (Scepterum) fell into the subclade 2A, together with *P. oliveriana* of grex 9 (Orthosiphonia) and *P. lachnoglossa* of grex 2 (Neoscepterum). Subclade 2B included three species of different greges, i.e., *P. resupinata* of grex 7, *P. axillaris* of grex 4 and *P. rhynchodonta* of grex 13. Clade III was composed of two solidly supported subclades, namely subclade 3A (83% bootstrap support) comprising all examined species of grex 13, except *P. rhynchodonta*, and subclade 3B (100% bootstrap support) including all the three species of grex 6 (Cyathophora) sampled in the present study. Clade IV and clade V are two polyphyletic clades. The former comprised six representatives of three greges, i.e., Orthosiphonia (9), Brachyphyllum (11) and Dolichophyllum (12), while the latter consisted of sixteen species of five greges, namely Cyclocladus (3), Sigmantha (8), Orthosiphonia (9), Apocladus (10) and Brachyphyllum (11). When employing

Orobanche minor and *Castilleja sulphurea* as the outgroup respectively, we also obtained only one most parsimonious tree with the same topology as Fig. 3.

Discussion

Unexpected high divergence of nrDNA ITS and its implications for the origin of *Pedicularis* species in the Hengduan Mountains. The most striking result of our research is the marked variation of the ITS sequences among *Pedicularis* species examined (Fig. 2), which conflicts with the congruent result obtained from a mass of previous studies (Hodges and Arnold 1994a, Baldwin 1997, Baldwin and Sanderson 1998, Richardson et al. 2001) that high morphological diversity is opposed to low genetic variation in taxa with adaptive radiation. For example, in the ITS1 region of 14 *Aquilegia* species sampled from Europe, Asia and North America, there are only 14 variable substitution sites detected (Hodges and Arnold 1994a). Of the nine European *Aconitum* species studied, six species formed a monophyletic clade with no more than six variable sites in the nrDNA ITS region (Utelli et al. 2001). Among island *Echium* species with remarkable morphological diversity, the average Kimura two-parameter distance of nrDNA ITS sequences is only 0.0019 (Böhle et al. 1996). Though *Pittosporum* exhibits great degree of morphological variability among the south Pacific members, the pairwise distance among nine Hawaiian species of the genus is 0 (Gemmill et al. 2002). The nrDNA ITS variation is also very low in the silversword alliance and only a small minority of potentially informative characters in each data set shows variation with possible relevance to understand basal relationships in the group (Baldwin 1997). *Sinadoxa*, a monotypic genus endemic to the Qinghai-Tibet Plateau, is morphologically distinct from its progenitor *Adoxa* by its unique and highly complicated inflorescence like a spike with several glomerate interrupted clusters, whereas the ITS sequence divergence between the two genera is only 3.4% (Liu et al.

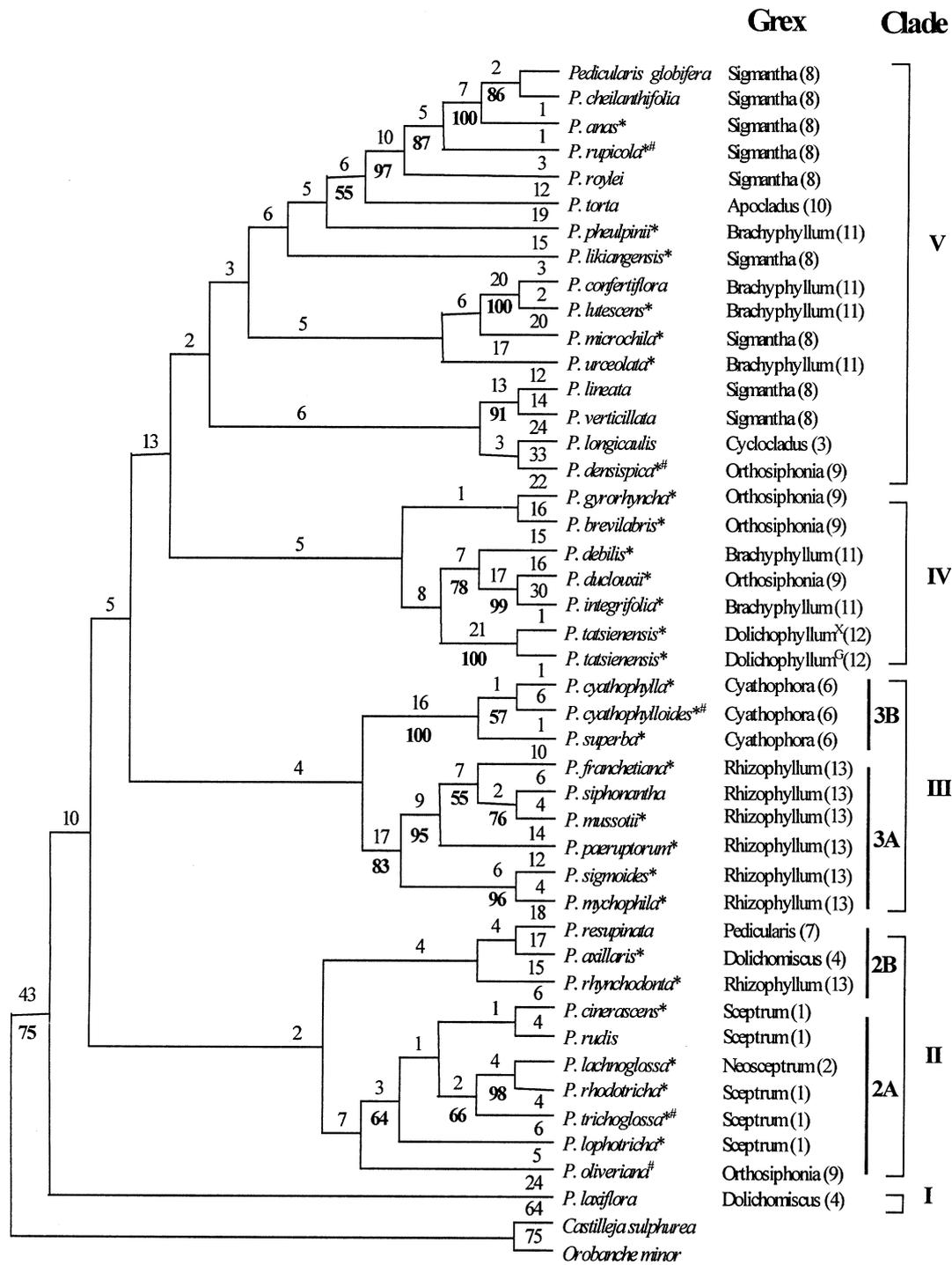


Fig. 3. Single most parsimonious tree of 43 taxa of *Pedicularis* constructed from the sequence analysis of the nrDNA ITS region (ITS1-5.8S-ITS2). Numbers of nucleotide substitutions are shown above branches, and bootstrap values (> 50%) from 500 replicates are indicated below branches. Multiple sequences from the same species are differentiated by locality (G = Garze, W. Sichuan, X = Xiaojin, N. Sichuan). Symbols * and # show species endemic to the Hengduan Mountains and samples from Tibet, respectively

2000). *Lomatogoniopsis*, another genus endemic to the Qinghai-Tibet Plateau, is obviously different from *Lomatogonium* in having protruding glands at the corolla base and non-vascularized scale at the inner lobes, but very low divergence of ITS sequence (less than 2%) was detected between them despite the distinct morphological differences (Liu et al. 2001). In particular, Zhang (2002) examined 25 species of *Aconitum* sampled from the Hengduan Mountains, showing that the nrDNA ITS sequence divergence varied from 0 to 5.3% (average = 1.8%). In contrast, the sequence divergence of nrDNA ITS among *Pedicularis* species from the same region is so high (average = 7.3%) that it is difficult to make an alignment, although the genus is a typical group of adaptive radiation. High divergence in ITS sequences of *Pedicularis* species across such a small geographical region could be explained from two aspects as follows.

Firstly, there could be a relatively ancient origin and diversification of the genus followed by migration of different floristic components into the Hengduan Mountains. Limpricht (1924) suggested that *Pedicularis* came into being at the end of the Cretaceous or at the beginning of the Eocene, but Tsoong (1956a) thought, from biogeographic viewpoint, that the late Miocene or Pliocene was a more reasonable period for the origination of the genus. Rate of rDNA substitution has been proved to be a useful calibration, by comparing with fossil and paleoclimatic data, to evaluate the upper limit age of speciation (Suh et al. 1993, Baldwin and Sanderson 1998, Richardson et al. 2001). Many taxa from recent radiation including Darwin's finches, Hawaiian honeycreepers, Hawaiian silverswords, and African cichlid fishes (Givnish and Sytsma 1997), showed low nucleotide variation. And the phenomenon of low ITS region variation vs. high morphological diversity found in highly diverged and widely distributed taxa, such as *Aconitum* and *Aquilegia*, has also been suggested as the result of recent speciation (Hodges and Arnold 1994a, 1994b; Utelli

et al. 2001, Kita and Ito 2000). Surprisingly, great divergence of ITS sequence vs. high floral morphological variation was found in *Pedicularis*, validating that the genus has a relatively ancient origin. Although it is difficult to presume the absolute time of the origin of *Pedicularis* since no fossil of Orobanchaceae could be used to calibrate nucleotide substitution rate within the genus, the initial immigration of *Pedicularis* into the Hengduan Mountains might be tracked down to the late Miocene, because changes in oxygen composition of soil carbonates in Pakistan about 8–9 Myr ago suggested a change from mixed needle-leaf and broad leaf forests to grassland vegetation along the northeastern margin of the Tibetan plateau about 8.5 Myr ago (An et al. 2001). Meanwhile, the vertical vegetation zone came into being in the Hengduan Mountains by reason of the rapid uplift of the Tibetan plateau and then the onset of East Asian monsoons (Chang 1983, An et al. 2001), which provided favorable natural conditions, such as warm climate and plentiful rainfall, for the development of *Pedicularis*.

Our deduction that the origin of *Pedicularis* was relatively ancient does not conflict with young geological history of the Hengduan Mountains, where nearly half of the *Pedicularis* species are distributed. Just as Prain (1980) and Tsoong (1956a, 1956b) supposed, *Pedicularis* originated in circumpolar region and then migrated southwards along different routes, among which one main line experienced long distance migration and reached ultimately the Himalayas. That is to say, there had been a long evolution history before *Pedicularis* settled down in the Hengduan Mountains. In the ITS phylogeny, all major clades were very weakly supported by the bootstrap test, and each of them possessed only a few synapomorphies. However, most terminals had long branch length, i.e., many apomorphies of each species (Fig. 3). This distribution of molecular characters (nucleotide substitution) supports the point that adaptive radiation could have occurred in the

early diversification of *Pedicularis* (Tsoong 1956b). Meanwhile, *P. resupinata*, a species from Inner Mongolia, was nested within clade II, suggesting the origin of some *Pedicularis* species of the Hengduan Mountains from long distance dispersal. Of course, the fact that about 70% of *Pedicularis* species in the Hengduan Mountains are endemic (Hong 1983, Yang et al. 1998) should imply that the speciation and development of some of them are closely correlated with the uplift of the Himalayas and the correspondingly remarkable changes of environments. The uplifts of the Tibetan plateau since the late Cretaceous, especially the third uplift from the Tertiary to the early Quaternary, directly resulted in the violent, continuous changes of the topography in West China and of climate of East Asia. In other words, some native *Pedicularis* species in the Hengduan Mountains originated from recent radiation. Consequently different floristic components became sympatric, which is also reflected by the polyphyly of the 28 *Pedicularis* species endemic to the Hengduan Mountains (Fig. 3).

Secondly, high nrDNA ITS variation in *Pedicularis* may result from accelerated rates of molecular evolution given that members of the genus are semiparasites. The dramatic losses, alternation or accelerated evolution rate of plastid genes had been observed in some parasitic lineages of angiosperm (dePamphilis 1995, Nickrent et al. 1998), including Orobanchaceae (dePamphilis and Palmer 1990, Wolfe et al. 1992, dePamphilis et al. 1997). Moreover, Nickrent and Starr (1994) reported an accelerated substitution rate from nuclear small subunit rDNA gene of holoparasitic representatives of Balanophoraceae, Rafflesiaceae, Hydnoraceae, and advanced hemiparasitic Viscaceae. Especially, rapid molecular evolution has been reported from nrDNA ITS of hemiparasitic *Arceuthobium* (Viscaceae) (Nickrent et al. 1994). Rates of sequence evolution might be elevated in certain lineages as a result of increased rates of mutation and relaxed selection on functionally conserved sequences (dePamphilis 1995). In fact, ITS-1

and ITS-2 regions of nrDNA are under some evolutionary constraint in structure and sequence, and their transcripts appear to function, at least in part, in the maturation of nrRNAs (Baldwin et al. 1995). The evolutionary constraint on nrDNA ITS was also reflected by a highly conserved motif sequence in ITS-1 of flowering plants (Liu and Schardl 1994). Therefore relaxed functional constraints and enhanced rates of molecular evolution associated with parasitism could give rise to the great nrDNA ITS divergence in *Pedicularis*.

Significant parallel evolution of floral morphology in *Pedicularis*. Homoplasy can distort the inference of phylogenetic relationships, and tie together similar but unrelated taxa (Givnish and Sytsma 1997). The present ITS phylogeny was quite different from those traditional classification systems of *Pedicularis* (Steven 1823; Bunge 1841, 1846; Bentham 1846; Maximowicz 1888; Prain 1890; Bonati 1910; Limpricht 1924; Hurusawa 1947; Li 1948, 1949; Tsoong 1955, 1963) based mainly on variation in leaf arrangement, inflorescence structure and corolla characters. Almost all main clades in the ITS tree are not consistent with the groups at high taxonomic ranks such as gregees, sections, reflecting extensive parallelism in floral morphology of the genus. For example, the ten species of six gregees, i.e. Sceptum (1), Neosceptum (2), Dolichomiscus (4), Pedicularis (7), Orthosiphonia (9) and Rhizophyllum (13), were grouped into clade II, but their corolla morphology is highly diverse, such as thin or quite wide, straight or twisted tubes, normal or dilated helmets, short or quite long, curved or twisted, and toothless or toothed beak. The species in five gregees, namely Cyclocladus (3), Sigmantha (8), Orthosiphonia (9), Apocladus (10) and Brachyphyllum (11), mixed together in clade V, in which corollae were so divergent that toothless, toothed, beaked and long-beaked types were included. In addition, some species of the same series were distributed in different main clades of the ITS tree. For instance, *P. laxiflora* solely forms the basal clade while *P. axillaris* is nested in

clade II. However, in the latest revision (Yang et al. 1998), the two species were grouped into Ser. *Axillares* of grex 4 (Dolichomiscus). According to our further examination of the morphological characters, we found that, in spite of similar corolla morphology, the phyllotaxy and rhizomes of the two species were quite different. *P. axillaris* is characterized by opposite leaves and slender rhizomes with nodes and branches, while leaves of *P. laxiflora* are basically alternate and its rhizomes are stout and short without nodes or branches. It is interesting that *P. lachnoglossa* of grex 2 (Neosceptrum) was nested within subclade 2A comprising all the species of grex 1 (Sceptrum). In particular, the nrDNA ITS sequence of *P. lachnoglossa* was identical to that of *P. rhodotricha* in grex 1 (Sceptrum). In fact, the two species are quite similar in having erect stem, spiral leaves and narrow-lanceolate and pinnatisect laminae. Maximowicz (1888) grouped the two species into Ser. *Tristes*. Li (1948) upgraded *P. lachnoglossa* to Ser. *Lachnoglossae* neighboring to *P. rhodotricha* in Ser. *Trichoglossae*, and both species were grouped into section *Lasioglossa*. The present results consolidated the systematic treatments of Li (1948) and Maximowicz (1888) that the two species are closely related and should not be divided into different groups at high taxonomic ranks.

Moreover, clade III was separated into two strongly supported subclades. Subclade 3A included all the species of the grex 13 (Rhizophyllum) except *P. rhynchodonta* which was nested in subclade 2B. Actually, the long, spicate and centripetal inflorescence of *P. rhynchodonta* clearly distinguished it from the other species of the subclade with short or centrifugal inflorescence. So it may be rational to exclude *P. rhynchodonta* from grex Rhizophyllum based on the present result. The monophyletic subclade 3B included all the three species sampled from grex 6 (Cyathophora), which supports many traditional classification systems (Li 1948; Tsoong 1955, 1963; Yang et al. 1998). Endemic to the Himalayas, this grex was considered as a

natural group (Li 1948; Tsoong 1955, 1963; Yang et al. 1998) characterized by conspicuously perfoliate leaf bases. However, the corolla morphology in this grex, comprising only seven species (Yang et al. 1998), is highly divergent. *P. rex* and *P. thamnophila* have beakless and toothed corolla, while *P. cyathophylloides* represents beakless and toothless one. The corolla of *P. superba* and *P. cyathophylla* is beaked and toothless. In particular, *P. cyathophylla* is characterized by a greatly elongated corolla tube. Besides, the polyphyly of clades IV and V implies that the delimitation among the greges Cyclocladus (3), Dolichopyllum (4), Sigmantha (8), Orthosiphonia (9), Apocladus (10) and Brachyphyllum (11) should be reevaluated. Particularly in clade V, the members of the grex Sigmantha examined in this study do not form a monophyletic clade in the ITS tree, though they all share a series of corolla characters, i.e., decurved tube, toothless galea (toothed sometimes in *P. microchila*), and short beak.

From the above discussions, we may conclude that parallel evolution of morphological characters is very prevalent in *Pedicularis*. The similarities in corolla of distantly related species may be the result of parallel adaptation to pollination (Li 1951; Sprague 1962; Macior 1975, 1983, 1988, 1990, 1993; Kwak 1977; Macior and Sarvesh 1991; Macior and Tang 1997; Macior et al. 2001; Ree 2001; Yang et al. 2002) or adaptation to similar habitats (Li 1951, Tsoong 1961), and each of the main corolla types such as toothed, beaked and long corolla tube might have evolved multiple times among disparate lineages. Unfortunately, for most taxonomic systems, the various characters of corolla such as size, general shape, hairiness, flexure and length of tube, curvature of galea, length and shape of the lower lip and beak, if present, are all considered to be of prime importance, and thus it is very difficult to establish a natural classification system if undue attention is paid to floral characters. Li (1951) and Tsoong (1955) had noticed the phenomena

and cautioned that it was unjustified to circumscribe higher-hierarchical taxa mainly based on differences in corolla. On the contrary, some vegetative characters, such as vegetative form, phyllotaxy, rhizomes characters, petiole characters and inflorescences, are relatively conservative, and thus are more valuable than floral ones in assessing the phylogenetic relationships of major groups in *Pedicularis*.

Although the Hengduan Mountain region is the eastern Himalayan diversity “hot spot” and also one of the plant diversity centers of the world, the present work is the first molecular phylogenetic and evolutionary study on a typical radiation taxon in the region. Though the species included here were almost sampled from the Hengduan Mountains, they represent all major groups of *Pedicularis* in the world (Tsoong 1956a), i.e., most morphological diversity of the genus, since this region is the modern distribution center of *Pedicularis*. Although the results are preliminary, they do shed some light on the phylogeny of *Pedicularis*, and are instructive in guiding future taxonomic revision of the genus. Moreover, the unexpected high divergence of the nrDNA ITS sequence in *Pedicularis* implies that, for different adaptive radiation taxa in the Hengduan Mountains the evolutionary histories are probably highly divergent. To clarify how such an abundant flora is established in the Hengduan Mountains and intensively understand the molecular evolution rule of the adaptive radiation taxa, extensive studies on more representative taxa and using more gene markers are required.

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References

- Aïnouche A. K., Bayer R. J. (1999) Phylogenetic relationships in *Lupinus* (Fabaceae: Papilionoideae) based on internal transcribed spacer sequences (ITS) of nuclear ribosomal DNA. *Amer. J. Bot.* 86: 590–607.
- Alice L. A., Campbell C. S. (1999) Phylogeny of *Rubus* (Rosaceae) based on nuclear ribosomal DNA internal transcribed spacer region sequences. *Amer. J. Bot.* 86: 81–97.
- An Z., Kutzbach J. E., Prell W. L., Porter S. C. (2001) Evolution of Asian monsoons and phased uplift of the Himalaya-Tibetan plateau since Late Miocene times. *Nature* 411: 62–66.
- Baldwin B. G. (1997) Adaptive radiation of the Hawaiian silversword alliance: Congruence and conflict of phylogenetic evidence from molecular and non-molecular investigations. In: Givnish T. J., Sytsma K. J. (eds.) *Molecular evolution and adaptive radiation*. Cambridge University Press, New York, pp. 103–128.
- Baldwin B. G., Sanderson M. J. (1998) Age and rate of diversification of the Hawaiian silversword alliance (Compositae). *Proc. Natl. Acad. Sci. USA* 95: 9402–9406.
- Baldwin B. G., Sanderson M. J., Porter J. M., Wojciechowski M. F., Campbell C. S., Donoghue M. J. (1995) The ITS region of nuclear ribosomal DNA: A valuable source of evidence on angiosperm phylogeny. *Ann. Missouri Bot. Gard.* 82: 247–277.
- Beardsley P. M., Olmstead R. G. (2002) Redefining Phrymaceae: the placement of *Mimulus*, tribe Mimuleae, and *Phryma*. *Amer. J. Bot.* 89: 1093–1102.
- Bentham G. (1846) Scrophulariaceae. In: de Candolle A. (ed.) *Prodromus systematis naturalis regni vegetabilis*. volume 10, pp. 560–582.
- Böhle U. R., Hilger H. H., Martin W. F. (1996) Island colonization and evolution of the insular woody habit in *Echium* L. (Boraginaceae). *Proc. Natl. Acad. Sci. USA* 93: 11740–11745.
- Bonati G. (1910) Contribution à l'étude du genre *Pedicularis*. *Bull. Soc. Bot. France* 57. Mém. 18: 1–35.
- Bunge A. (1841) Ueber eine neue Art der Gattung *Pedicularis*. *Bull. Acad. Sci. St. Petersb.* 8: 241–253.
- Bunge A. (1846) Ueber *Pedicularis comosa* L. und die mit ihr verwandten Arten. *Bull. Phys. Math. Acad. Sci. St. Petersb.* 1: 369–384.

- Chang D. H. S. (1983) The Tibetan Plateau in relation to the vegetation of China. *Ann. Missouri Bot. Gard.* 70: 564–570.
- dePamphilis C. W. (1995) Genes and Genomes. In: Press M. C., Graves J. D. (eds.) *Parasitic plants*. Chapman and Hall, London, pp. 176–205.
- dePamphilis C. W., Palmer J. D. (1990) Loss of photosynthetic and chlororespiratory genes from the plastid genome of a parasitic flowering plant. *Nature* 348: 337–339.
- dePamphilis C. W., Young N. D., Wolfe A. D. (1997) Evolution of plastid gene *rps2* in a lineage of hemiparasitic and holoparasitic plants: Many losses of photosynthesis and complex patterns of rate variation. *Proc. Natl. Acad. Sci. USA* 94: 7367–7372.
- Gemmill C. E. C., Allan G. J., Wagner W. L., Zimmer E. A. (2002) Evolution of insular Pacific *Pittosporum* (Pittosporaceae): Origin of the Hawaiian radiation. *Mol. Phylogenet. Evol.* 22: 31–42.
- Givnish T. J., Sytsma K. J. (1997) Homoplasy in molecular vs. morphological data: the likelihood of correct phylogenetic inference. In: Givnish T. J., Sytsma K. J. (eds.) *Molecular evolution and adaptive radiation*. Cambridge University Press, New York, pp. 55–101.
- Hodges S. A., Arnold M. L. (1994a) Columbines: A geographically widespread species flock. *Proc. Natl. Acad. Sci. USA* 91: 5129–5132.
- Hodges S. A., Arnold M. L. (1994b) Floral and ecological isolation between *Aguilegia formosa* and *Aquilegia pubescens*. *Proc. Natl. Acad. Sci. USA* 91: 2493–2496.
- Hong D. (1983) The distribution of Scrophulariaceae in the Holarctic with special references to the floristic relationships between eastern Asia and eastern North America. *Ann. Missouri Bot. Gard.* 70: 701–712.
- Hurusawa I. (1947) The genus *Pedicularis*. *J. Jap. Bot.* 21: 159–166.
- Hurusawa I. (1948a) The genus *Pedicularis*. *J. Jap. Bot.* 21: 11–16.
- Hurusawa I. (1948b) The genus *Pedicularis*. *J. Jap. Bot.* 22: 70–76.
- Hurusawa I. (1948c) The genus *Pedicularis*. *J. Jap. Bot.* 22: 178–184.
- Hurusawa I. (1949a) The genus *Pedicularis*. *J. Jap. Bot.* 23: 20–24.
- Hurusawa I. (1949b) The genus *Pedicularis*. *J. Jap. Bot.* 23: 106–122.
- Kimura M. (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16: 111–120.
- Kita Y., Ito M. (2000) Nuclear ribosomal ITS sequences and phylogeny in East Asian *Aconitum* subgenus *Aconitum* (Ranunculaceae), with special reference to extensive polymorphism in individual plants. *Plant Syst. Evol.* 225: 1–13.
- Kita Y., Ueda K., Kadota Y. (1995) Molecular phylogeny and evolution of the Asian *Aconitum* subgenus *Aconitum* (Ranunculaceae). *J. Plant Res.* 108: 429–442.
- Kumar S., Tamura K., Jakobsen I. B., Nei M. (2001) *MEGA2*: Molecular Evolutionary Genetics Analysis software. Arizona State University, Tempe, Arizona, USA.
- Kwak M. (1977) Pollination ecology of five hemiparasitic, large-flowered Rhinanthoideae with special reference to the pollination behaviour of nectar-thieving, short-tongued bumblebees. *Acta Bot. Neerl.* 26: 97–108.
- Li H. (1948) A revision of the genus *Pedicularis* in China I. *Proc. Acad. Nat. Sci. Philad.* 100: 205–378.
- Li H. (1949) A revision of the genus *Pedicularis* in China II. *Proc. Acad. Nat. Sci. Philad.* 101: 1–214.
- Li H. (1951) Evolution in the flowers of *Pedicularis*. *Evolution* 5: 158–164.
- Limpricht W. (1924) Studien über die Gattung *Pedicularis*. *Rep. Sp. Nov.* 20: 161–265.
- Liu J. Q., Chen Z. D., Lu A. M. (2000) The phylogenetic relationships of an endemic genus *Sinadoxia* in the Qinghai-Xizang Plateau: Evidence from ITS sequence analysis. *Acta Bot. Sin.* 42: 656–658.
- Liu J. Q., Chen Z. D., Lu A. M. (2001) A preliminary study of the phylogeny of the Swertiinae (Gentianaceae) based on ITS data. *Isr. J. Plant Sci.* 43: 301–308.
- Liu J. S., Schardl C. L. (1994) A conserved sequence in internal transcribed spacer 1 of plant nuclear rRNA genes. *Plant Mol. Biol.* 26: 775–778.
- Macior L. W. (1975) The pollination ecology of *Pedicularis* (Scrophulariaceae) in the Yukong Territory. *Amer. J. Bot.* 62: 1065–1072.
- Macior L. W. (1983) The pollination dynamics of sympatric species of *Pedicularis* (Scrophulariaceae). *Amer. J. Bot.* 70: 844–853.

- Macior L. W. (1988) A preliminary study of the pollination ecology of *Pedicularis* (Scrophulariaceae) in Japan. *Plant Species Biol.* 3: 61–66.
- Macior L. W. (1990) Pollination ecology of *Pedicularis punctata* Decne. (Scrophulariaceae) in the Kashmir Himalaya. *Plant Species Biol.* 5: 215–223.
- Macior L. W. (1993) Pollination ecology of *Pedicularis palustris* L. (Scrophulariaceae) in North America. *Plant Species Biol.* 8: 35–44.
- Macior L. W., Sood K. S. (1991) Pollination ecology of *Pedicularis megalantha* D. Don in the Himachal Himalaya. *Plant Species Biol.* 6: 75–81.
- Macior L. W., Tang Y. (1997) A preliminary study of the pollination ecology of *Pedicularis* in the Chinese Himalaya. *Plant Species Biol.* 12: 1–7.
- Macior L. W., Tang Y., Zhang J. (2001) Reproductive biology of *Pedicularis* (Scrophulariaceae) in the Sichuan Himalaya. *Plant Species Biol.* 16: 83–89.
- Maximowicz C. J. (1888) Diagnoses plantarum novarum Asiaticarum (12). *Bull. Acad. Sci. St. Pétersb.* 32: 477–629.
- Nickrent D. L., Duff R. J., Colwell A. E., Wolfe A. D., Young N. D., Steiner K. E., dePamphilis C. W. (1998) Molecular phylogenetic and evolutionary studies of parasitic plants. In: Soltis D. E., Soltis P. S., Doyle J. J. (eds.) *Molecular systematics of plants II. DNA sequencing*. Kluwer Academic Publishers, Boston, pp. 211–241.
- Nickrent D. L., Schuette K. P., Starr E. M. (1994) A molecular phylogeny of *Arceuthobium* (Viscaceae) based on nuclear ribosomal DNA internal transcribed spacer sequences. *Amer. J. Bot.* 81: 1149–1160.
- Nickrent D. L., Starr E. M. C. (1994) High rates of nucleotide substitution in nuclear small subunit (18S) rDNA from holoparasitic flowering plants. *J. Mol. Evol.* 39: 62–70.
- Olmstead R. G., Reeves P. A. (1995) Evidence for the polyphyly of the Scrophulariaceae based on chloroplast *rbcL* and *ndhF* sequences. *Ann. Missouri Bot. Gard.* 82: 176–193.
- Olmstead R. G., dePamphilis C. W., Wolfe A. D., Young N. D., Elisons W. J., Reeves P. A. (2001) Disintegration of the Scrophulariaceae. *Amer. J. Bot.* 88: 348–361.
- Prain D. (1890) The species of *Pedicularis* of the Indian Empire and its frontiers. *Ann. Roy. Bot. Gard. Calc.* 3: 1–196.
- Ree R. H. (2001) Homoplasy and the phylogeny of *Pedicularis*. PH. D. Dissertation. The Department of Organismic and Evolutionary Biology, Harvard University. Cambridge, Massachusetts.
- Reeves P. A., Olmstead R. G. (1998) Evolution of novel morphological, ecological, and reproductive traits in a clade containing *Antirrhinum*. *Amer. J. Bot.* 85: 1047–1056.
- Reveal J. L., Judd W. S., Olmstead R. G. (1999) Proposal to conserve the name Antirrhinaceae against Plantaginaceae (Magnoliophyta). *Taxon* 48: 182.
- Richardson J. E., Pennington R. T., Pennington T. D., Hollingsworth P. M. (2001) Rapid diversification of a species-rich genus of neotropical rain forest trees. *Science* 293: 2242–2245.
- Rogers S. O., Bendich A. J. (1988) Extraction of DNA from plant tissues. *Plant Mol. Biol. Manual A6*: 1–10.
- Sprague E. F. (1962) Pollination and evolution in *Pedicularis* (Scrophulariaceae). *Aliso* 5: 181–209.
- Steven C. (1823) *Monographia Pedicularis*. *Mém. Soc. Nat. Moscou* 6: 1–60.
- Suh Y., Thien L. B., Reeve H. E., Zimmer E. A. (1993) Molecular evolution and phylogenetic implications of internal transcribed spacer sequences of ribosomal DNA in Winteraceae. *Amer. J. Bot.* 80: 1042–1055.
- Swofford D. L. (1998) PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer, Sunderland, Mass.
- Thompson J. D., Gibson T. J., Plewniak F., Jeanmougin F., Higgins D. G. (1997) CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acids Res.* 25: 4876–4882.
- Tsoong P. C. (1955) A new system for the genus *Pedicularis*. *Acta Phytotax. Sin.* 4: 71–147.
- Tsoong P. C. (1956a) A new system for the genus *Pedicularis*. *Acta Phytotax. Sin.* 5: 205–278.
- Tsoong P. C. (1956b) A new system for the genus *Pedicularis*. *Acta Phytotax. Sin.* 5: 19–73.
- Tsoong P. C. (1961) A new system for the genus *Pedicularis*. *Acta Bot. Sin.* 9: 230–274.
- Tsoong P. C. (1963) Scrophulariaceae (Para II). In: Chien S. S., Chun W. Y. (eds.) *F1. Reip. Pop. Sin* (68). Science Press, Beijing, pp. 1–378.
- Utelli A. B., Roy B. A., Baltisberger M. (2001) Molecular and morphological analyses of European *Aconitum* species (Ranunculaceae). *Plant Syst. Evol.* 224: 195–212.

- White T. J., Burus T. D., Lee S. B., Taylor J. W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M. A., Gelfand D. H., Sninsky J. J., White T. J. (eds.) PCR protocols: A guide to methods and applications. Academic Press, New York, pp. 315–322.
- Wilson E. O. (1992) The diversity of Life. Belknap Press of Harvard University Press, Cambridge, MA.
- Wolfe K. H., Morden C. W., Palmer J. D. (1992) Function and evolution of a minimal plastid genome from a nonphotosynthetic parasitic plant. Proc. Natl. Acad. Sci. 89: 10648–10652.
- Yang C. F., Guo Y. H., Gituru R. W., Sun S. G. (2002) Variation in stigma morphology – How does it contribute to pollination adaption in *Pedicularis* (Orobanchaceae)? Plant Syst. Evol. 236: 89–98.
- Yang H., Holmgren N. H., Mill R. R. (1998) *Pedicularis* L. In: Wu Z. Y., Raven P. H. (eds.) Flora of China (18). Science Press, Beijing, pp. 97–209.
- Young N. D., Steiner K. E., dePamphilis C. W. (1999) The evolution of parasitism in the Scrophulariaceae/Orobanchaceae: plastid gene sequences refute an evolutionary transition series. Ann. Missouri. Bot. Gard. 86: 876–893.
- Yuan Y. M., Küpfer P., Doyle J. J. (1996) Infrageneric phylogeny of the genus *Gentiana* (Gentianaceae) inferred from nucleotide sequences of the internal transcribed spacers (ITS) of nuclear ribosomal DNA. Amer. J. Bot. 83: 641–652.
- Zhang F. M. (2002) A preliminary study on speciation of *Aconitum delavayi* complex (Ranunculaceae) in Hengduan Mountains. PH. D. Dissertation. Institute of Botany, Chinese Academy of Sciences, Beijing, China.

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