

## Intense uplift of the Qinghai-Tibetan Plateau triggered rapid diversification of *Phyllolobium* (Leguminosae) in the Late Cenozoic

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**Background:** *Phyllolobium*, a recently established genus from subgenus *Pogonophace* of *Astragalus*, contains about 20 species and four sections, mostly endemic to the Qinghai-Tibetan Plateau (QTP). The uplift of the QTP undoubtedly affected organismic evolution in the region, but further molecular dating in a phylogenetic context is required to test whether diversification is linked in particular to the intense uplift 3.6 million years ago (Ma).

**Aims:** Based on molecular dating of *Phyllolobium*, we attempted to identify a relationship between diversification and speciation of *Phyllolobium* and the geological event of this intense uplift.

**Methods:** Internal transcribed spacer (ITS) sequence data of *Phyllolobium* were used for the study. Outgroup genera selected were *Astragalus*, *Oxytropis*, *Caragana*, *Calophaca*, *Hamilodendron*, *Hedysarum*, *Sphaerophysa*, *Swainsona* and *Colutea*, as well as *Dalbergia* and *Pueraria*. Both of the latter genera have fossil records, enabling their use in constraint calibrations for dating. Phylogenetic analysis employed maximum parsimony and Bayesian inference, and phylogenetic datings were conducted by using three approaches implemented in the programs r8s, PAML and BEAST.

**Results:** The estimated crown age of *Phyllolobium* was dated to 3.62 Ma in PL-r8s (3.96 in BEAST, 3.48 in PAML, listed similarly in the following), and sections within the genus had ages ranging between 3.60 and 2.55 Ma. These dates coincide with, or follow soon after, the intense uplift of the QTP in the Late Pliocene. By contrast, diversification of sections *Bibracteolati* and *Oliganthum* began at estimated ages of 1.95 Ma (2.24, 1.94) and 1.83 Ma (1.53, 1.62), respectively, and may therefore have been triggered by the ‘Qingzang’ plateau uplift (2.5–1.7 Ma). *P. tribulifolium* had a dated crown age of 0.63 Ma (0.58, 0.42), suggesting that the ‘Kunhuang’ uplift and/or glaciation (occurring between 1.1/0.8 and 0.6 Ma) facilitated diversification.

**Conclusions:** The estimated crown ages of *Phyllolobium* and of sections within it indicate that rapid diversification was likely triggered by consecutive phases of QTP uplift in the Late Pliocene and the Early-to-Mid Pleistocene. However, the diversification of at least some *Phyllolobium* species can be explained by Late Pleistocene glaciation and/or geological events. In sum, these findings characterise *Phyllolobium* as a recently diversified genus adapted to the cold and dry habitats of the QTP and its environs.

**Keywords:** endemism; ITS; *Phyllolobium*; phylogenetic dating; Qinghai-Tibetan Plateau intense uplift

### Introduction

*Phyllolobium* is a recently erected genus from subgenus *Pogonophace* of *Astragalus* (Leguminosae), based on molecular phylogeny and also morphological characteristics of the floral (pollen) brush, which consists of a distinct penicillately hairy stigma (Kang et al. 2003; Kang and Zhang 2004, 2009; Zhang and Podlech 2006; Zhang et al. 2009; Xu et al. 2010). The genus comprises about 20 species, mostly endemic to the Qinghai-Tibetan Plateau (QTP) and adjacent regions (e.g. the Hengduan Mountains which is treated as part of the Sino-Himalayan flora (Wu and Wu 1999)), except for *P. chinense* which occurs in northern China (see Figure 1; Zhang 2000; Zhang and Podlech 2006). *Phyllolobium* is monophyletic, based on previous analyses of nuclear ribosomal DNA sequences of the internal transcribed spacer (ITS) region (Zhang et al. 2009). Four taxonomic sections are recognised,

*Phyllolobium*, *Bibracteolati*, *Trichostylus*, and *Oliganthum* (Zhang et al. 2009). Phylogenetic analysis further shows *Phyllolobium* to be allied to the genera *Sphaerophysa* and *Swainsonia* of subtribe Coluteinae, rather than *Astragalus* of subtribe Astragalinae (Kang et al. 2003; Zhang et al. 2009).

As is well known, the uplift of the QTP during the Late Tertiary had a great influence upon the ecology and evolution of biota in Asia and adjacent regions (e.g. Li et al. 1979; Zhong and Jing 1996; Li and Fang 1998; Shi et al. 1998; Cheng et al. 2000). It is believed that the uplift of the Plateau resulted from the collision of the Indian subcontinent with the Eurasian plate, beginning at ca. 50 million years ago (Ma). Evidence is accumulating for the uplift of some portions of the Plateau to present altitudes as early as 40 Ma (Coleman and Hodges 1995; Spicer et al. 2003; Rowley and Currie 2006; Wang et al. 2008). Consequent desertification on the leeward side,

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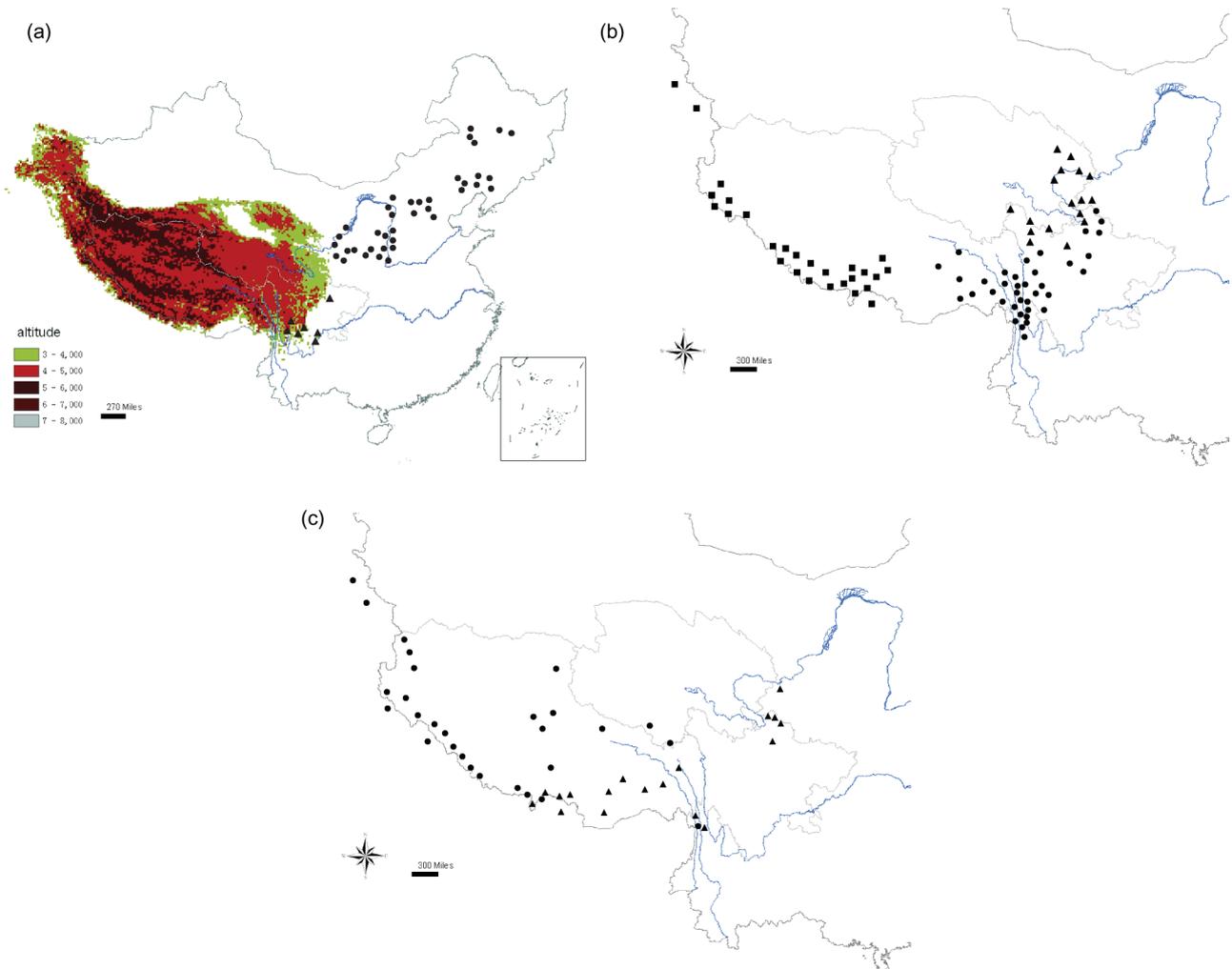


Figure 1. (a) The elevation pattern of the Tibetan Plateau and the distribution of *Phyllolobium chinense* (circles) and *P. eutrichus* (triangles) in China. *P. chinense* is the only member of section *Phyllolobium* that is not endemic to the Tibetan Plateau; (b) distribution of section *Bibracteolati* (filled circles), including, *Astragalus tanguticus* (triangles), and *A. tribulifolius* (squares), both of which have been placed in *P. tribulifolium*; (c) distribution of the sections *Oliganthum* (triangles) and *Trichostylus* (circles).

evidenced by aeolian red clay and loess deposits, is seen as early as 25 Ma, in the Late Oligocene (Guo et al. 2002; Qiang et al. 2011). Loess accumulation has continued over time, but has shown a somewhat geometric increase during the last 5 million years (see Molnar 2005), probably because of a combination of rapid uplift with glaciation. Based on sediments, crust, vegetation, palynology and macrofossils of the Loess Plateau in eastern QTP, Shi et al. (1998, 1999) and Li and Fang (1998) presumed three phases of the QTP uplift, namely, the Qingzang (Qinghai–Xizang) movement, the Kunhuang (Kunlun–Huanghe) movement, and the Gonghe movement, one of which, the Qingzang movement, is the intense Late Pliocene uplift beginning at about 3.6 Ma. This time also marks the beginning of more intense aridity as evidenced by increased loess deposition (An et al. 2001). Soon afterwards, low pCO<sub>2</sub> was coupled with the onset of glaciation at the Plio-Pleistocene boundary (ca. 3.4–2.4 Ma; Tripathi et al. 2009).

Within this historical framework, we can explore relationships that might exist between *Phyllolobium* diversification and the geological process of QTP uplift, as previously done for other genera and species (reviewed in Qiu et al. 2011). Zhang (2003) proposed that the uplift of the QTP affected the evolution of *Phyllolobium* (as *Pogonophace*), but the precise time of its diversification remains to be demonstrated. Here we use molecular phylogenetic dating to examine the evolution of *Phyllolobium* in relation to the timing of QTP uplift over the past few million years.

## Materials and methods

### Taxon sampling and ITS sequence data

ITS sequence data of nine *Phyllolobium* species were generated previously by us for the present study (see Kang et al. 2003; Kang and Zhang 2009; Zhang et al. 2009).

These nine *Phyllobium* species covered most of what was previously recognised as subgenus *Pogonophace* of *Astragalus* (Zhang and Podlech 2006) – see Appendix – and all four sections of *Phyllobium* (Zhang et al. 2009). In particular we paid attention to the section *Bibracteolati*, the largest section in the genus, which includes 11 species and is endemic to the Hengduan Mountains. From this section we examined nine individuals covering five of the 11 species (see Figure 2). Sequences of outgroup genera *Astragalus*, *Oxytropis*, *Caragana*, *Calophaca*, *Hamilodendron*, *Hedysarum*, *Sphaerophysa*, *Swainsona*, *Colutea*, *Dalbergia* and *Pueraria* were obtained from Genbank. *Dalbergia* and *Pueraria* have fossil records, enabling their use in dating calibration. In total, 58 species were included in the analysis (see Appendix).

*Phylogenetic analysis*

ITS sequence data of all 58 species were transferred into BioEdit ver. 5.0.9 (Hall 1999), and alignment was made by using Clustal X 1.83 (Jeanmougin et al. 1998). A dataset of 58 taxa and 639 bps was finally assembled.

*Maximum parsimony analysis*

Parsimony analysis was implemented by employing PAUP ver. 4.0 (Swofford 2002). Multiple tree searches were conducted using heuristic search options, including random addition sequence (100 replicates) holding 10 trees per replicate, and tree-bisection-reconnection (TBR) branch swapping, with retention of multiple parsimonious trees. These parsimonious trees were then used to calculate

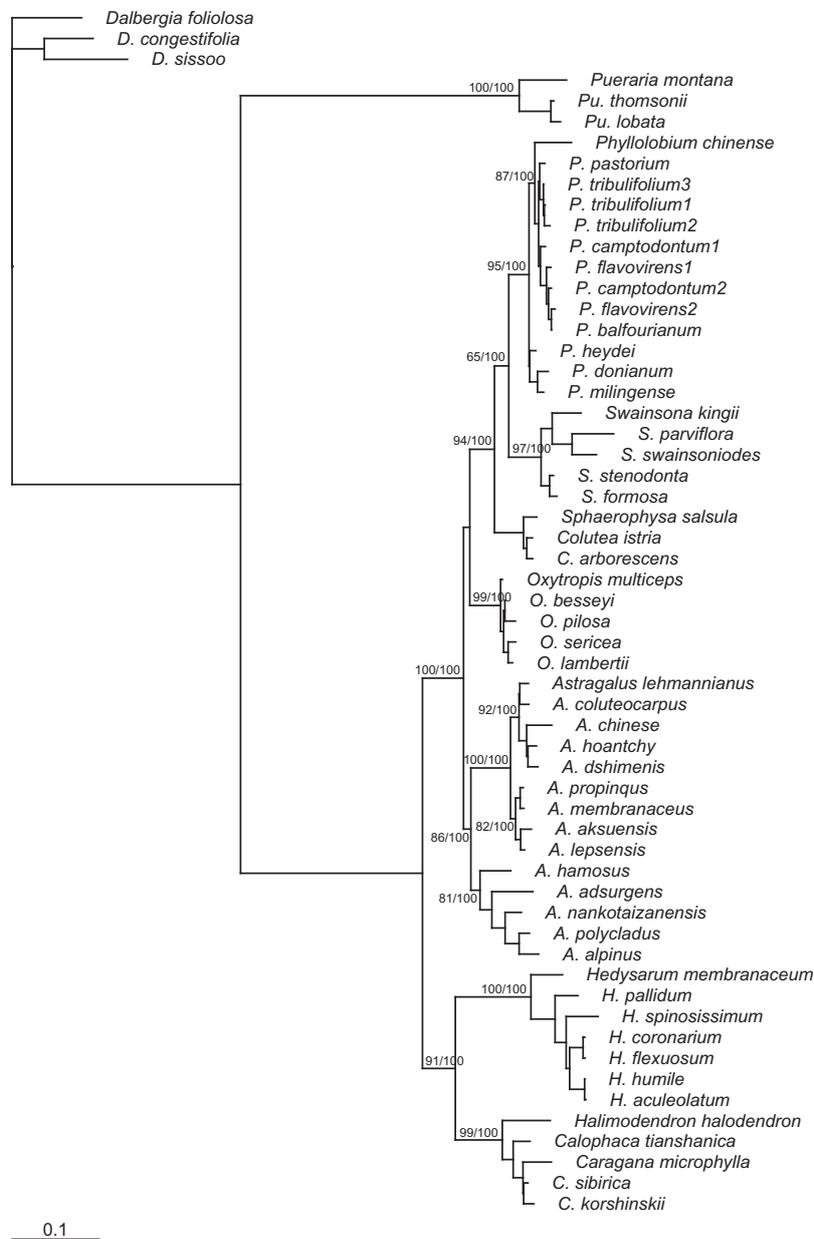


Figure 2. Phylogenetic tree of *Phyllobium* and its outgroups, obtained from a Bayesian analysis. Confidence values were mostly over 80% in bootstrap and 95% in Bayesian posterior probability, as labelled on the figure.

the consensus tree. Bootstrap analyses were conducted to determine clade support. Non-parametric bootstrap resampling proportions (Felsenstein 1985) were estimated from 100 bootstrap replicates, incorporating heuristic parsimony searches, using random addition sequence with 10 replicates and TBR branch swapping options, and setting 'max-trees' to 50,000.

#### Model test

For Bayesian inference, the best fitting DNA substitution models were found using Modeltest 3.5 (Posada and Crandall 1998; Posada and Buckley 2004). The GTR+I+G model was selected by AIC as the most appropriate. Nucleotide frequencies estimated were  $A = 0.2126$ ,  $C = 0.2922$ ,  $G = 0.2561$ , and  $T = 0.2392$ , with a gamma shape parameter of 1.2574 and an assumed proportion of invariable sites of 0.2663.

#### Bayesian inference

Bayesian inference of phylogenetic trees was made by MrBayes version 3.0 (Huelsenbeck and Ronquist 2001), using model parameter values provided by Modeltest (see above). Options in the program were set to 15,000,000 generations of Markov Chain Monte Carlo (MCMC) searches and a sample frequency of 1000. Saturation was reached after a burn-in of 1000 generations. Clade support was assessed using Bayesian posterior probabilities (Huelsenbeck and Ronquist 2001), estimated as the proportion of trees sampled after 'burn-in' that contained each of the observed bipartitions.

#### Dating

*Reference fossils.* In accordance with available fossil evidence (Tao 1992; Tao et al. 2000; Institute of Botany, Nanjing Institute of Geology and Palaeontology (IB and NJGP), Academia Sinica 1978; Guo and Zhou 1992; Li et al. 1995; Wojciechowski 2005; Lavin et al. 2005), two related genera, *Dalbergia* and *Pueraria*, were selected as reference points for dating, using their Miocene fossils from China, North America, and Europe. Maximum fossil ages were used as the generic minimum age of the MRCA (most recent common ancestor), with that for *Pueraria* being 17 Ma (17–5 Ma, fossil record range), and for *Dalbergia* 19.5 Ma (19.5–5 Ma, fossil record range).

*Dating implementation.* Phylogenetic dating was conducted by using three approaches implemented in the programs r8s, PAML and BEAST. In r8s version 1.7 (<http://ginger.ucdavis.edu/r8s/>), two comparable and complementary methods, penalised likelihood (PL) method (semi-parametric rate smoothing, Sanderson 2002) and non-parametric rate smoothing (NPRS; Sanderson 1997) were used on the basis of a maximum likelihood (ML) tree.

The smoothing parameter for the Cross Validation option in r8s was estimated for use in PL and NPRS.

For dating, we used a Bayesian relaxed molecular clock approach as implemented in the BASEML and MCMCTREE programs of the PAML package version 4.2 (<http://abacus.gene.ucl.ac.uk/software/paml.html>; Yang 2007). The independent rate model (Rannala and Yang 2007), with the HKY85+[5 model for nucleotide substitutions, was used. Two separate MCMC analyses were run for 10,000,000 generations (burn-in 10%), with a sample size of 1000. Means and 95% confidence intervals were determined for MRCA nodes.

BEAST v1.46 (<http://beast.bio.ed.ac.uk/>) was also used to estimate divergence times (Drummond and Rambaut 2007). Best-fit models of nucleotide substitution parameters for the priors in BEAST were given by Modeltest. A Yule process speciation prior, an uncorrelated log-normal model of rate variation, and a normal distribution were chosen. Tracer v1.4 (Drummond and Rambaut 2007) was used to measure the effective sample size of parameters, and mean and 95% confidence intervals. Two separate MCMC analyses were run for 10,000,000 generations (burn-in 10%) with a parameter sample size of 1000.

## Results

### Phylogeny

For the maximum parsimony analysis, the ITS sequence dataset consisted of a total of 639 aligned positions, of which 49 were variable and parsimony informative. The 874 most parsimonious trees found had a length of 874, a consistency index (CI) of 0.6362, and a retention index (RI) of 0.8628. Bayesian analysis resulted in 10001 burn-in trees, and a consensus tree (Figure 2) was constructed showing posterior probability support for the clades. Phylogenetic trees from both parsimony and Bayesian analyses had identical topologies, with most clades supported by high bootstrap values (>80%) and posterior probabilities (>0.95).

### Dating

The three dating programs produced roughly congruent estimated ages for the nodes of *Phyllolobium* and its relatives, except for NPRS-r8s (see Table 1). The estimated ages from NPRS-r8s were often higher than those obtained from other methods of dating used in the study (Table 1). For instance, the crown age (i.e. onset of diversification) of *Phyllolobium* (node 1 in Figure 3) was estimated as 3.62 Ma using PL, 3.96 Ma using BEAST, and 3.48 Ma using PAML, but 6.51 Ma using NPRS-r8s. Our results were consistent with previous studies which, in general, showed that estimated ages based on PL-r8s are preferable to those obtained using NPRS-r8s (see also Bremer et al. 2004; Near and Sanderson 2004; Anderson et al. 2005; Bell and Donoghue 2005; Lavin et al. 2005; Wojciechowski 2005).

Table 1. The phylogenetic dating (Ma) results for *Phyllolobium*, using three approaches: r8s (PL, NPRS), BEAST and PAML; in PAML and BEAST the mean and 95% confidence intervals of the nodes are shown. The classification of *Phyllolobium* follows Zhang and Podlech (2006) and Zhang et al. (2009). Node numbers refer to Figure 3.

Taxon	Node	PL	NPRS	BEAST	PAML
<i>Dalbergia</i> 19.5 constr.		19.50	19.5	19.75 (19.5–20.23)	24.56 (23.26–25.18)
<i>Pueraria</i> 17.0 constr.		17.00	17.0	17.38 (17.0–18.30)	15.78 (13.16–18.22)
<i>Astragalus</i> 12.4 constr.		12.40	12.4	12.72 (12.4–13.70)	12.58 (9.92–15.45)
<i>Phyllolobium</i> + <i>Swainsona</i>		7.29	10.28	6.67 (3.62–10.19)	6.96 (4.47–9.95)
<i>Swainsona</i>		4.16	5.98	4.51 (1.98–7.62)	4.73 (2.75–7.40)
<i>Phyllolobium</i>	1	3.62	6.51	3.96 (1.84–6.59)	3.48 (1.91–5.65)
sect. <i>Phyllolobium</i>	2	3.03	5.57	2.98 (1.18–5.10)	2.59 (1.37–4.32)
sect. <i>Trichostylus</i>	3	3.60	6.50	2.78 (0.61–5.18)	2.55 (1.00–4.55)
sect. <i>Bibracteolati</i>	4	1.95	4.61	2.24 (0.86–3.86)	1.94 (1.00–3.28)
sect. <i>Oliganthum</i>	5	1.83	3.88	1.53 (0.15–3.45)	1.62 (0.45–3.41)
<i>Phyllolobium tribulifolius</i>	6	0.63	2.19	0.58 (0.02–1.54)	0.42 (0.07–1.21)

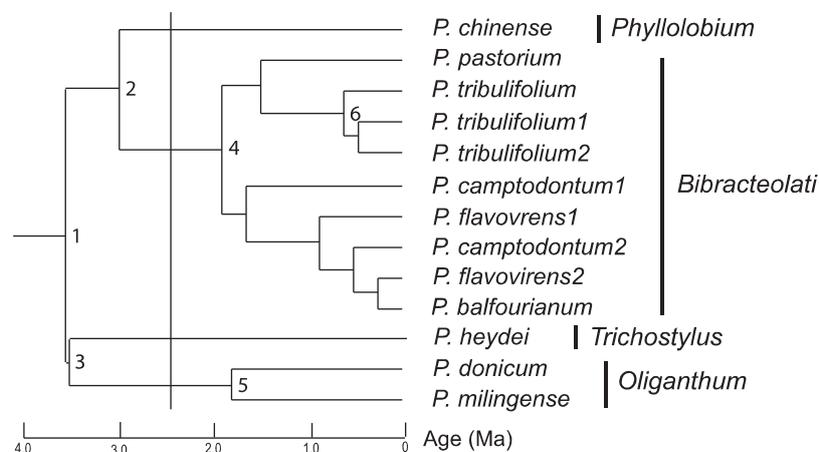


Figure 3. A chronogram produced by PL-r8s dating, illustrating only the taxa of *Phyllolobium*. The *Astragalus* species names in Figure 2 were changed to *Phyllolobium* following Zhang and Podlech (2006) and Zhang et al. (2009). The four sections are labelled by a vertical line, and named on the right of terminal *Phyllolobium* species on the tree. The estimated ages of nodes from r8s, BEAST, and PAML are presented in Table 1.

Due to a basal polytomy, the estimated ages of sections *Trichostylus* (*P. heydei*) and *Oliganthum* were both dated to 3.60 Ma in PL (node 3 in Figure 3) (2.78 Ma in BEAST, and 2.55 Ma in PAML), while the split between the inferred sister sections *Phyllolobium* (*P. chinense*) and *Bibracteolati* was estimated to have occurred at 3.03 (2.98, 2.59) Ma (node 2). The crown age of section *Bibracteolati* was dated to 1.95 (2.24, 1.94) Ma (node 4), which is strikingly similar to that of section *Oliganthum* at 1.83 (1.53, 1.62) Ma (node 5). Overall, the onset of diversification of *Phyllolobium* (3.96–3.48 Ma, depending on the dating method used), and the temporal origin of its four sections (3.60–2.55 Ma) coincides with the Late Pliocene, whereas species proliferation clearly falls within the Pleistocene (<2.4 Ma).

## Discussion

The ITS phylogeny generated in this study (Figure 2) is consistent with other recent legume phylogenies (Kang et al. 2003; Wojciechowski 2003, 2005; Wojciechowski et al. 2004; Lavin et al. 2005; Lewis et al. 2005; Lock

2005; Lock and Schrire 2005). *Phyllolobium* formed a monophyletic group with high support, and was related to *Swainsonia* of subtribe Coluteinae rather than to *Astragalus* of subtribe Astragalinae. The Hedysaroid clade (tribe Hedysareae comprising *Hedysarum*, *Caragana*, *Calophaca* and *Halimodendron*) and the Astragalinae clade including *Astragalus*, were shown to be paraphyletic with *Caragana*, *Calophaca* and *Halimodendron* forming a sister group related to *Hedysarum* rather than *Astragalus*.

## Dating results and their correspondence to QTP uplift

According to our estimated crown age of 3.62 Ma for *Phyllolobium* based on PL analysis (3.96 Ma and 3.48 Ma according to BEAST and PAML analysis, respectively, see Table 1, Figure 3), there is support for the hypothesis of an association between the onset of diversification in the genus and the intense uplift of the QTP 3.6 Ma. Li and Fang (1998) and Shi et al. (1998) divided the uplift since this time into three phases, namely, the Qingzang (Qinghai–Xizang) movement, the Kunhuang (Kunlun–Huanghe) movement, and the Gonghe movement. The Qingzang movement can

be divided further into three stages, A, B and C, whose respective ages are assigned as 3.6 Ma, 2.5 Ma, and 1.7 Ma. The Kunlun–Huanghe movement occurred at the period of 1.1–0.6 Ma, and the Gonghe movement started at 0.15 Ma. These processes and time scales of plateau uplift provide a fundamental reference for the molecular age estimates inferred here.

#### *Comparison of dates of generic and sectional origins and the time of intense uplift*

As stated above, the estimated crown ages of *Phyllolobium* (3.96–3.48 Ma) closely match the beginning of the intense uplift of the QTP during the Late Pliocene (3.6 Ma). Concomitantly, two basal (polytomous) lineages of the genus, i.e. the monotypic section *Trichostylus* (*P. heydei*, central-western Tibet) and the bi-typic section *Oliganthum*, originated at about the same time or shortly thereafter (i.e. 3.60–2.55 Ma). Moreover, the origin of the two remaining sections, i.e. the monotypic section *Phyllolobium* (*P. chinense*, North China), and its species-rich sister, section *Bibracteolati*, still falls within the Late Pliocene (3.04–2.59 Ma). By contrast, considering crown age estimates of both sections *Bibracteolati* and *Oliganthum* together, their diversification started not earlier than the Early-to-Mid Pleistocene (ca. 2.24–1.53 Ma; see below). Overall, the above estimates suggest that the initial diversification of *Phyllolobium*, and the origin of all of its component sections, may have been triggered by the intense QTP uplift in the Late Pliocene (3.6 Ma).

#### *Sectional diversifications during the Qingzang movement (2.5–1.7 Ma) of QTP uplift*

According to Li and Fang (1998) and Shi et al. (1998), the Qingzang movement B–C phase occurred 2.5–1.7 Ma. The two major diversification events in *Phyllolobium* fall into this range. The first involves section *Bibracteolati*, with an estimated crown age of 1.95 (2.24, 1.94) Ma. Most *Phyllolobium* species are included in this section (Zhang and Podlech 2006; Zhang et al. 2009), and all are endemic to the Hengduan Mountains of western Sichuan, north-western Yunnan, and eastern Tibet, hence covering the principal area of the Sino-Himalayan flora (Wu 1988). The second, near-simultaneous diversification event relates to section *Oliganthum*, a new taxon to be extracted from section *Bibracteolati* (Zhang et al. 2009), and comprising *P. donicum* and *P. milingense*. The estimated crown age of this section, i.e. 1.83 (1.53, 1.62) Ma, also falls into the time range of the Qingzang movement B–C phase. Interestingly, this more recent stage in the uplift of the Plateau was likely accompanied by an increase of the Asian monsoon (at ca. 2.0 Ma) to its current condition (Li and Fang 1998). Hence, both geological and climatic factors may have facilitated the diversification of sections *Bibracteolati* and *Oliganthum* especially in the Hengduan Mountains, during the Early-to-Mid Pleistocene (ca. 2.24–1.53 Ma).

#### *Effects of the Kunhuang movement (1.1–0.6 Ma) and glaciation (0.8–0.6 Ma)*

The period 1.1–0.6 Ma corresponds to the Kunhuang movement of plateau uplift (Li et al. 1979; Shi et al. 1995; Li and Fang 1998). At the end of this movement (ca. 0.8–0.6 Ma), a major glaciation occurred (Shi et al. 1995), covering about 20% of the QTP area with an ice sheet of approximately  $5.0 \times 10^5$  km<sup>2</sup> (Zheng et al. 2002). At the same time, the climatic snow line dropped to 1900–1500 m (Li and Fang 1998). In turn, this cold climate caused major vegetation shifts at mid-to-high elevations, i.e. from coniferous–broad leaved mixed forest to coniferous forest, shrub forest, and meadow, and especially to alpine meadow  $\geq 4000$  m elevation (Zhang 1978; Li et al. 1979; Wu 1980). Species populations of *Phyllolobium* generally occur at the margins of these alpine forests, shrublands and meadows. While the Kunhuang movement and/or its associated glaciation may thus have influenced the evolution and distribution of *Phyllolobium* species in general, this is perhaps most clearly seen in *P. tribulifolium* of section *Bibracteolati*. This species occurs in the central-western Himalayas and the central QTP. Morphologically, it differs from other species in *Phyllolobium* in that the stigma is very pubescent, and the pubescence sometimes extends downward to the style. Although previously placed in section *Ebracteolati* (Simp.) K.T. Fu in *Pogonophace* (Zhang 2002), we find it to be entirely nested within section *Bibracteolati*. In general, strong pubescence of the stigma or other organs is supposed to be a cold adaptation in QTP plants (Zhang 2002). The estimated crown age of 0.63 (0.58, 0.42) Ma for this monophyletic species (represented by several accessions; node 6, Figure 3) coincides with the end of the Kunhuang movement/glaciation, which thus may have triggered intraspecific diversification. However, molecular phylogeographic studies at the level of populations are required to address this question in more detail.

#### *Phyllolobium evolution and adaptation*

Concerning the place of origin of *Phyllolobium*, a previous analysis based on morphological characters and distribution patterns (Zhang 2003) suggested northern China (with *P. chinense*) as the ancestral area. Accordingly, the genus may have then radiated south-westward into the Hengduan Mountains and Tibet. However, the genus may have originated instead in the Hengduan Mountains because: (1) most species of *Phyllolobium* are distributed in the Hengduan Mountains and Tibet; (2) diversification of the genus (3.96–3.48 Ma) coincided with the intense QTP uplift; and (3) the Hengduan Mountains are considered to be one of the major centres of origin of northern temperate plant groups in Asia, and an important area from which colonisation occurred westward (into the central QTP/Himalayas) or eastward (via the Qinling Mts.) into northern China (Wu 1988). Thus, *Phyllolobium* may have originated in the Hengduan Mountains, and then spread to and radiated in the central-western Himalayas and northern China.

As with the floristics of the QTP and the Hengduan Mountains, Wu (1987) listed about 20 genera endemic to these regions with *Phyllobium* being part of this group. Due to the high elevation, cold climate, and strong influence of the Pleistocene climate oscillations, the Plateau flora has come to possess endemic groups, particularly of shrubby and herbaceous species, in coniferous forest, shrub forest, and alpine meadow vegetation types (Wu 1980, 1987, 1988). These include numerous taxa of *Rhododendron*, *Pedicularis*, *Gentiana*, *Acronema*, *Loxostemom*, *Kobresia*, *Androsace*, *Carex*, and *Polygonum*.

According to Zhang (1978), the QTP vegetation distribution has characteristics not only vertically along alpine elevations, with a forest–shrubland–meadow series, but also in a horizontal direction, called ‘plateau zonality’, comprising a forest–meadow–grassland–desert series that extends from south-east to north-west across the QTP. In the Hengduan Mountains and eastern Himalayas, *Phyllobium* species populations in general occur at the margins of coniferous forest (*Pinus* spp., *Abies* spp., *Tsuga* spp.), shrub forest (mainly *Rhododendron* spp., *Quercus* spp.), and alpine meadow (such as *Koberisa pygmaea*, *K. humilis*, *Stipa* spp., *Carex* spp., and *Polygonum* spp.) (Zhang 1978; Li et al. 1979; Wu 1980). However, there are only few species that extend in a horizontal direction into the grasslands or deserts of the western and central QTP, i.e. *P. heydei* (= *A. heydei*, = *A. hendersonii*) and *P. tribulifolium*. Given that *P. heydei* occupies a basal position in the phylogenetic trees, whereas *P. tribulifolium* is derived, their distribution patterns perhaps imply that increasing elevation, cold climate, and aridity of the plateau both reduced the range of *Phyllobium* but also provided opportunities for range expansion of some species.

*Phyllobium* species have various adaptive characters in morphology, ecology, and distribution. Those in the Hengduan Mountains generally have a prostrate habit, 6–(15) 20 cm tall, which becomes even more extreme in the central QTP. They generally occur at elevations of (2800) 3500–4000 m, although *P. tribulifolium* can approach ca. 3800–4500 m, and *P. heydei* ca. 4500–5300 m. Morphologically, *P. heydei* is the most prostrate member of the genus with a height of only 1–2 cm. Members of section *Bibracteolati*, that are concentrated in the Hengduan Mountains, have inflated fruits and possess a series of varied flower colours – yellow, white, purple, or purple-red – which may possibly represent adaptations to strong ultraviolet radiation. All of the floristic elements constituting the forest, shrubland, and meadow vegetation types must have responded strongly to intense uplift of the QTP.

## Conclusions

The estimated crown ages of *Phyllobium*, and of sections within it, indicate that rapid diversification was likely triggered by consecutive phases of QTP uplift in the Late Pliocene and the Early-to-Mid Pleistocene; this fundamentally agrees with the hypothesis of intense uplift of

QTP 3.6 Ma. However, the diversification of at least some *Phyllobium* species can be explained by Late Pleistocene glaciation and/or geological events. In sum, as a plant evolutionary case accompanying with the QTP uplift, these findings characterise *Phyllobium* as a recently diversified genus adapted to the cold and dry habitats of the QTP and its environs.

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## References

- An ZS, Kutzbach JE, Prell WL, Porter SC. 2001. Evolution of Asian monsoons and phased uplift of the Himalaya–Tibetan plateau since Late Miocene times. *Nature* 411:62–66.
- Anderson CL, Bremer K, Friis EM. 2005. Dating phylogenetically basal eudicots using *rbcL* sequences and multiple fossil reference points. *American Journal of Botany* 92: 1737–1748.
- Bell CD, Donoghue MJ. 2005. Dating the Dipsacales: comparing models, genes, and evolutionary implications. *American Journal of Botany* 92:284–296.
- Bremer K, Friis EM, Bremer B. 2004. Molecular phylogenetic dating of Asterid flowering plants shows Early Cretaceous diversification. *Systematic Biology* 53:496–505.
- Cheng HB, Powell C, An ZS, Zhou J, Dong GR. 2000. Pliocene uplift of the northern Tibetan Plateau. *Geology* 28:715–718.
- Coleman M, Hodges K. 1995. Evidence for Tibetan Plateau uplift before 14 Myr age from new minimum estimate for east-west extension. *Nature* 374:49–52.
- Drummond AJ, Rambaut A. 2007. ‘BEAST’: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7:214.
- Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791.
- Guo SX, Zhou ZK. 1992. The megafossil legumes from China. In: Herendeen PS, Dilcher DL, editors. *Advances in legume systematics, part 4*. Kew (UK): Royal Botanic Gardens. p. 207–223.

- 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.
- Hall T. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Ser* 41:95–98.
- Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogeny. *Bioinformatics* 17:754–755.
- Institute of Botany, Nanjing Institute of Geology and Palaeontology (IB and NJGP), Academia Sinica. 1978. Chinese plant fossils, Vol. 3, Neogene Floras. Beijing (China): Academic Press.
- Jeanmougin F, Thompson JD, Gibson TJ, Gouy M, Higgins DG. 1998. Multiple sequence alignment with Clustal X. *Trends in Biochemical Sciences* 23:403–405.
- Kang Y, Zhang ML. 2004. Study of pollen brush in selected species of *Astragalus* L. subgenus *Pogonophace* Bunge (Leguminosae). *Plant Systematics and Evolution* 249:1–8.
- Kang Y, Zhang ML. 2009. Pollen brush of *Astragalus* L. subgenus *Pogonophace* Bunge (Leguminosae) and its systematic significance. *Plant Systematics and Evolution* 280:167–174.
- Kang Y, Zhang ML, Chen ZD. 2003. A preliminary phylogenetic study of the subgenus *Pogonophace* (*Astragalus*) in China based on ITS sequence data. *Acta Botanica Sinica* 45:140–145.
- Lavin M, Herendeen PS, Wojciechowski MF. 2005. Evolutionary rates analysis of Leguminosae implicates a rapid diversification of lineages during the Tertiary. *Systematic Biology* 54:575–594.
- Lewis GP, Schrire BD, Mackinder BA, Lock M. 2005. Legumes of the world. Kew (UK): Royal Botanic Gardens.
- Li JJ, Fang XM. 1998. Research on the uplift of the Qinghai-Xizang Plateau and environmental changes. *Chinese Science Bulletin* 43:1569–1574.
- Li JJ, Wen SX, Zhang QS, Wang FB, Zheng BX, Li BY. 1979. A discussion on the period, amplitude and type of the uplift of the Qinghai-Xizang Plateau. *Scientia Sinica* 22:1314–1328.
- Li XX, editor. 1995. Fossil floras of China through the geological ages. Guangzhou (China): Guangdong Scientific and Technology Press.
- Lock JM. 2005. Tribe Hedysareae. In: Lewis G, Shrive B, Mackinder B, Lock M, editors. Legumes of the world. Kew (UK): Royal Botanical Garden. p. 489–495.
- Lock JM, Schrire BD. 2005. Tribe Galegeae. In: Lewis G, Shrive B, Mackinder B, Lock M, editors. Legumes of the world. Kew (UK): Royal Botanical Garden. p. 475–488.
- Molnar P. 2005. Mio-Pliocene growth of the Tibetan Plateau and evolution of East Asian climate. *Palaeontologia Electronica* 8:2A. 23p.
- Near TJ, Sanderson MJ. 2004. Assessing the quality of molecular divergence time estimating by fossil calibration and fossil-based model selection. *Philosophical Transactions of Royal Society London B* 359:1477–1483.
- 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.
- Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14:817–818.
- Qiang XK, An ZS, Song YG, Chang H, Sun YB, Liu WG, Ao H, Dong JB, Fu CF, Wu F, et al. 2011. New eolian red clay sequence on the western Chinese Loess Plateau linked to onset of Asian desertification about 25 Ma ago. *Science China Earth Science* 54:136–144.
- Qiu YX, Fu CX, Comes HP. 2011. Plant molecular phylogeography in China and adjacent regions: tracing the genetic imprints of Quaternary climate and environmental change in the world's most diverse temperate flora. *Molecular Phylogenetics and Evolution* 59:225–244.
- Rannala B, Yang Z. 2007. Inferring speciation times under an episodic molecular clock. *Systematic Biology* 56:453–466.
- Rowley DB, Currie BS. 2006. Palaeo-altimetry of the late Eocene to Miocene Lunpola basin, central Tibet. *Nature* 439:677–681.
- Sanderson MJ. 1997. A nonparametric approach to estimating divergence time in the absence of rate constancy. *Molecular Biology Evolution* 14:1218–1231.
- Sanderson MJ. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology Evolution* 19:101–109.
- Shi YF, Li JJ, Li BY, Yao TD, Wang SM, Li SJ, Cui ZJ, Wang FB, Pan BT, Fang XM, Zhang QS. 1999. Uplift of the Qinghai-Xizang (Tibetan) Plateau and East Asia environmental change during Late Cenozoic. *Acta Geographica Sinica* 54:10–21.
- Shi YF, Tang MC, Ma YZ. 1998. The relation of second rising in Qinghai-Xizang Plateau and Asia Monsoon. *Science in China D* 28:263–271.
- Shi YF, Zheng BX, Li SJ, Ye BS. 1995. Studies on altitude and climatic environment in the middle and east parts of Tibetan Plateau during Quaternary maximum glaciation. *Journal of Glaciology and Geocryology* 17:97–112.
- Spicer RA, Harris NBW, Widdowson M, Herman AB, Guo SX, Valdes PJ, Wolfe JA, Kelly SP. 2003. Constant elevation of southern Tibet over the past 15 million years. *Nature* 421:622–624.
- Swofford DL. 2002. PAUP, phylogenetic analysis using parsimony, version 4. Sunderland (MA): Sinauer Associates.
- Tao JR. 1992. The Tertiary vegetation and flora and floristic regions in China. *Acta Phytotaxonomica Sinica* 31:25–43.
- Tao JR, Zhou ZK, Liu YS. 2000. The evolution of the Late Cretaceous-Cenozoic floras in China. Beijing (China): Science Press.
- Tripathi AK, Roberts CD, Eagle RA. 2009. Coupling of CO<sub>2</sub> and ice sheet stability over major climate transitions of the last 20 million years. *Science* 326:1394–1397.
- Wang CS, Zhao XX, Liu ZF, Lippert PC, Graham SA, Coe RS, Yi HS, Zhu LD, Liu S, Li YL. 2008. Constraints on the early uplift history of the Tibetan Plateau. *PNAS* 105:4987–4982.
- Wojciechowski MF. 2003. Reconstructing the phylogeny of legumes (Fabaceae): an early 21st century perspective. In: Klitgaard BB, Bruneau A, editors. *Advances in legume systematics, part 10*. Kew (UK): Royal Botanical Garden. p. 5–35.
- Wojciechowski MF. 2005. *Astragalus* (Fabaceae): a molecular phylogenetic perspective. *Brittonia* 57:382–396.
- Wojciechowski MF, Lavin M, Sanderson MJ. 2004. A phylogeny of legumes (Leguminosae) based on the analysis of the plastid *matK* gene resolves many well-supported subclades within the family. *American Journal of Botany* 91:1846–1862.
- Wu ZY, editor. 1980. *Vegetation of China*. Beijing (China): Academic Press.
- Wu ZY. 1987. Origin and evolution of Xizang flora. In: Wu ZY, editor. *Flora Xizangica Vol 5*. Beijing (China): Academic Press. p. 874–902.
- Wu ZY. 1988. Hengduan mountain flora and her significance. *Journal of Japan Botany* 63:297–311.
- Wu ZY, Wu SG. 1999. A proposal for new floristic kingdom (realm). In: Zhang AL, Wu SG, editors. *Floristic characteristics and diversity of Eastern Asian plants*. Beijing (China): China Higher Education Press.
- Xu LR, Zhang ML, Podlech D. 2010. *Phyllobium*. In: Wu ZY, Hong DY, Raven P, editors. *Flora of China, vol 10*. Beijing (China): Science Press; St. Louis (MO): Missouri Botanical Garden Press. p. 322–328.

- Yang Z. 2007. PAML 4: Phylogenetic analysis by Maximum Likelihood. *Molecular Biology Evolution* 24: 1586–1591.
- Zhang ML. 2000. Studies on geographical distribution pattern of the subgenus *Pogonophace* (Fabaceae: *Astragalus*) in China using GIS technique. *Acta Botanica Sinica* 42:849–854.
- Zhang ML. 2002. Systematics of *Astragalus* subgenus *Pogonophace* (Leguminosae). *Acta Botanica Yunnanica* 24:543–553.
- Zhang ML. 2003. Biogeography of *Astragalus* subgenus *Pogonophace* (Leguminosae). *Acta Botanica Yunnanica* 25:25–32.
- Zhang ML, Kang Y, Podlech D. 2009. A taxonomic note on the sections of the genus *Phyllobium* (Leguminosae). *Journal of Lanzhou University (Nat. Sci.)* 45:75–78.
- Zhang ML, Podlech D. 2006. Revision of the genus *Phyllobium* Fisch. (Leguminosae-Papilionoideae). *Feddes Repertorium* 117:41–64.
- Zhang XS. 1978. The plateau zonality of vegetation in Xizang. *Acta Botanica Sinica* 20:140–149.
- Zheng BX, Xu QQ, Shen YP. 2002. The relationship between climate change and Quaternary glacial cycles on the Qinghai-Tibetan Plateau: review and speculation. *Quaternary International* 97–98:93–101.
- Zhong DL, Ding L. 1996. The uplifting process and mechanism of Qinghai-Xizang (Tibet) Plateau. *Sciences in China D*, 26:289–295.
- Podlech (*A. angustifoliolatus* K.T. Fu) FJ619273; *Phyllobium flavovirens*2 (K.T. Fu) M.L. Zhang & Podlech (*A. flavovirens* K.T. Fu) FJ236898; *Phyllobium heydei* (Baker) M.L. Zhang & Podlech (*A. hendersonii* Baker) AF521957; *Phyllobium milingense* (C.C. Ni & P.C. Li) M.L. Zhang & Podlech (*A. milingensis* Ni et P.C. Li) AF521954; *Phyllobium pastorium* (H.T. Tsai & T.T. Yu) M.L. Zhang & Podlech (*A. pastorius* Tsai et Yu) FJ236892; *Phyllobium tribulifolium*1 (Bunge) M.L. Zhang & Podlech (*A. tribulifolius* Benth. ex Bunge) AF521953; *Phyllobium tribulifolium*2 (Bunge) M.L. Zhang & Podlech (*A. tanguticus* Batalin) AF521956; *Phyllobium tribulifolium*3 (Bunge) M.L. Zhang & Podlech (*A. tanguticus* Batalin) FJ236897; *Astragalus adsurgens* Pall. AF121674; *A. aksuensis* (Bunge) AF359753; *A. alpinus* L. ITS1 L10760+ ITS2 L10761; *A. dshimensis* Gontsch. AF359755; *A. chinensis* L. f. AF121681; *A. hamosus* L. ITS1 L10778+ ITS2 L10779; *A. hoantchy* Franch. AF521952; *A. lehmannianus* Bunge AF359756; *A. lepsensis* Bunge AF359752; *A. membranaceus* Bunge AF359749; *A. nankotaizanensis* Sasaki AF121680; *A. polycladus* Bur. et Franch. AF121676; *A. propinquus* B. Schischk. AF359751; *Calophaca tianshanica* (B. Fedtsch.) Boris. ITS1 U51220+ ITS2 U51221; *Cararagana korshinskii* Kom. AY626914; *Car. microphylla* Lam. AY626915; *Car. roboroviskyi* Kom. AF521958; *Car. sibirica* Fabr. (= *Car. arborescens* Lam.) AY626912; *Cohutea arborescens* L. ITS1 U56009+ ITS2 U56010; *Co. istria* Mill. ITS1 U69544+ ITS2 U69545; *Dalbergia congestiflora* Pittier AF068140; *D. foliolosa* Benth. AF189002; *D. sissoo* Roxb. AF189023; *Hedysarum aculeolatum* Michx AY772222; *H. carnosulum* Greene AY772224; *H. coronarium* L. AY772225; *H. flexuosum* L. AY775312; *H. humile* L. AY772227; *H. membranaceum* Coss. et Bal. AY772228; *H. pallidum* Desf. AY772229; *H. spinosissimum* L. subsp. *capitatum* AY772223; *Oxytropis besseyi* (Rydb.) Blank. var. *ventosa* (Greene) Barneby AF121756; *O. lamberti* Pursh AF121753; *O. multiceps* Nutt. AF121760; *O. pilosa* DC. AF121759; *O. sericea* Schur AF121757; *Pueraria lobata* (Willd.) Ohwi (= *P. montana* var. *lobata*) AF338215; *P. montana* (Lour.) Merr. AF338216; *P. thomsonii* Benth. (= *P. montana* var. *thomsonii*) AF338217; *Sphaerophysa salsula* DC. ITS1 U560011+ITS2 U560012; *Swainsona formosa* F. Muell. AF113859; *Sw. kingii* F. Muell. AF113860; *Sw. parviflora* Benth. AF113863; *Sw. stenodonta* F. Muell. AF113865; *Sw. swainsonioides* AF113866.

## Appendix

Most samples of *Phyllobium* taxa and their ITS sequence data are ours, and the remaining were downloaded from GenBank. A total of 58 species and their GenBank accession numbers are listed.

*Phyllobium balfourianum* (N.D. Simpson) M.L. Zhang & Podlech (*A. balfourianus* Sims.) AF521951; *Phyllobium camptodontum*1 (Franch.) M.L. Zhang & Podlech (*A. camptonoides* Sims.) FJ236888; *Phyllobium camptodontum*2 (Franch.) M.L. Zhang & Podlech (*A. camptodontus* Franch.) FJ236899; *Phyllobium chinense* (Fisch.) M.L. Zhang & Podlech (*A. complanatus* R. Br. ex Bunge) AF521950; *Phyllobium donianum*1 (DC.) M.L. Zhang & Podlech (*A. yatungensis* Ni et P.C. Li) AF521955; *Phyllobium flavovirens*1 (K.T. Fu) M.L. Zhang &