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

Ming-Li Zhang\(^{a,b,*}\), Yun Kang\(^{c}\), Yang Zhong\(^{d}\) and Stewart C. Sanderson\(^{e}\)

\(^{a}\)Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, China; \(^{b}\)Institute of Botany, Chinese Academy of Sciences, Beijing, China; \(^{c}\)Department of Pharmacy, Fudan University, Shanghai, China; \(^{d}\)School of Life Science, Fudan University, Shanghai, China; \(^{e}\)Shrub Sciences Laboratory, Intermountain Research Station, Forest Service, U.S. Department of Agriculture, Utah, USA

(Received 19 August 2011; final version received 4 September 2012)

**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*, *Calophasa*, *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 et al. 2009). The genus comprises about 20 species, mostly endemic to the Qinghai-Tibetan Plateau (QTP) and adjacent regions (e.g. the Hengdian 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 Astragaliinae (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,
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\textsubscript{2} was coupled with the onset of glaciation at the Plio–Pleistocene boundary (ca. 3.4–2.4 Ma; Tripati 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).
Late Cenozoic diversification of *Phyllolobium* (Leguminosae)

These nine *Phyllolobium* species covered most of what was previously recognised as subgenus *Pogonophace* of *Astragalus* (Zhang and Podlech 2006) – see Appendix – and all four sections of *Phyllolobium* (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 *Phyllolobium* 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.
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 MCMCCTREE programs of the PAML package version 4.2 (http://abacus.gene.ucl.ac.uk/software/pamlhtml; Yang 2007). The independent rate model (Rannala and Yang 2007), with the HKY85+Γ5 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).
Late Cenozoic diversification of *Phyllolobium* (Leguminosae)

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.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Node</th>
<th>PL</th>
<th>NPRS</th>
<th>BEAST</th>
<th>PAML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dalbergeria 19.5 constr.</td>
<td>19.50</td>
<td>19.5</td>
<td>19.75 (19.5–20.23)</td>
<td>24.56 (23.26–25.18)</td>
<td></td>
</tr>
<tr>
<td>Pueraria 17.0 constr.</td>
<td>17.00</td>
<td>17.0</td>
<td>17.38 (17.0–18.30)</td>
<td>15.78 (13.16–18.22)</td>
<td></td>
</tr>
<tr>
<td>Swainsona</td>
<td>4.16</td>
<td>5.98</td>
<td>4.51 (1.98–7.62)</td>
<td>4.73 (2.75–7.40)</td>
<td></td>
</tr>
<tr>
<td>sect. <em>Phyllolobium</em> 1</td>
<td>3.62</td>
<td>6.51</td>
<td>3.96 (1.84–6.59)</td>
<td>3.48 (1.91–5.65)</td>
<td></td>
</tr>
<tr>
<td>sect. Trichostylus 3</td>
<td>3.60</td>
<td>5.57</td>
<td>2.98 (1.18–5.10)</td>
<td>2.59 (1.37–4.32)</td>
<td></td>
</tr>
<tr>
<td>sect. Bibracteolati 4</td>
<td>1.95</td>
<td>4.61</td>
<td>2.24 (0.86–3.86)</td>
<td>1.94 (1.00–3.28)</td>
<td></td>
</tr>
<tr>
<td>sect. Oliganthum 5</td>
<td>1.83</td>
<td>3.88</td>
<td>1.53 (0.15–3.45)</td>
<td>1.62 (0.45–3.41)</td>
<td></td>
</tr>
<tr>
<td><em>Phyllolobium</em> tribulifolius</td>
<td>0.63</td>
<td>2.19</td>
<td>0.58 (0.02–1.54)</td>
<td>0.42 (0.07–1.21)</td>
<td></td>
</tr>
</tbody>
</table>

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 Astragaliinae. The Hedysaroid clade (tribe Hedysaraceae comprising *Hedysarum*, *Caragana*, *Calophaca* and *Halimodendron*) and the Astragaliinae 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
During the Early to Mid Pleistocene (ca. 2.24–1.53 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 *Trichostylos* (*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, northwestern 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 ecological 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 × 106 km² (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 ≥ 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 *Pagonophace* (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 \textit{Phyllolobium} 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 \textit{Rhododendron}, \textit{Pedicularis}, \textit{Gentiana}, \textit{Acronea}, \textit{Loxostemom}, \textit{Kobresia}, \textit{Androsace}, \textit{Carex}, and \textit{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, \textit{Phyllolobium} species populations in general occur at the margins of coniferous forest (\textit{Pinus} spp., \textit{Abies} spp., \textit{Tsuga} spp.), shrub forest (mainly \textit{Rhododendron} spp., \textit{Quercus} spp.), and alpine meadow (such as \textit{Koberisa pygmaea}, \textit{K. humilis}, \textit{Stipa} spp., \textit{Carex} spp., and \textit{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. \textit{P. heydei} (= \textit{A. heydei}, = \textit{A. hendersonii}) and \textit{P. tribulifolium}. Given that \textit{P. heydei} occupies a basal position in the phylogenetic trees, whereas \textit{P. tribulifolium} is derived, their distribution patterns perhaps imply that increasing elevation, cold climate, and aridity of the plateau both reduced the range of \textit{Phyllolobium} but also provided opportunities for range expansion of some species.

\textit{Phyllolobium} 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 and western QTP. They generally occur at elevations of (2800) 3500–4000 m, although \textit{P. tribulifolium} can approach ca. 3800–4500 m, and \textit{P. heydei} ca. 4500–5300 m. Morphologically, \textit{P. heydei} is the most prostrate member of the genus with a height of only 1–2 cm. Members of section \textit{Bibracteolati}, that are concentrated in the Hengduan Mountains, have inflated fruits and possess a series of varied flower colours – yellow, white, purple, or purplered – 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 \textit{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; this fundamentally agrees with the hypothesis of intense uplift of QTP 3.6 Ma. However, the diversification of at least some \textit{Phyllolobium} 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 \textit{Phyllolobium} as a recently diversified genus adapted to the cold and dry habitats of the QTP and its environs.

Acknowledgements
We thank Jinhiao Wang and Beijian Zhong (Fudan University, Shanghai) for their help with molecular phylogenetic dating calculation. Thanks to Prof. Richard Abbott, associate editor, and Laszlo Nagy, editor of this journal, and two anonymous reviewers for their valuable and constructive suggestions, and to Peter Comes (Salzburg University, Austria) for his useful and helpful comments and linguistic corrections. This study was financially supported by CAS Important Direction for Knowledge Innovation Project (No. KZCX2-EW-305) to M.L.Z.; Xinjiang Institute of Ecology and Geography to M.L.Z., and CAS, National Natural Science Foundation of China (NSFC 30500035) to Y.K.

Notes on contributors
Ming-Li Zhang is group leader of molecular phylogeny and biogeography; his recent research has been focusing on the QTP and Central Asian biogeography.

Yun Kang’s research interests include legume classification and population genetics.

Yang Zhong is interested in plant phylogeny and computational biology.

Stewart C. Sanderson is interested in plant variation and evolution, recently collaborating with M.L.Z.’s team on molecular biogeography.

References


Podlech (A. balfourianus M.L. Zhang & Podlech (A. camptodontus toides Simps.) FJ236888; camptodontum listed.
A total of 58 species and their GenBank accession numbers are ours, and the remaining were downloaded from GenBank. Phyllolobium Appendices


Appendix
Most samples of Phyllolobium 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.

Phyllolobium balfourianum (N.D. Simpson) M.L. Zhang & Podlech (A. balfourianus Simps.) AF521951; Phyllolobium camptodontum1 (Franch.) M.L. Zhang & Podlech (A. camptodontoid Simps.) FJ236888; Phyllolobium camptodontum2 (Franch.) M.L. Zhang & Podlech (A. camptodontus Franch.) FJ236899; Phyllolobium chinense (Fisch.) M.L. Zhang & Podlech (A. complanatus R. Br. ex Bunge) AF521950; Phyllolobium donianum1 (DC.) M.L. Zhang & Podlech (A. yungensis Ni et P.C. Li) AF521955; Phyllolobium flavovirens1 (K.T. Fu) M.L. Zhang & Podlech (A. angustifoliatulus K.T. Fu) FJ619273; Phyllolobium flavovirens2 (K.T. Fu) M.L. Zhang & Podlech (A. flavovirens K.T. Fu) FJ326898; Phyllolobium heydei (Baker) M.L. Zhang & Podlech (A. hendersonii Baker) AF521957; Phyllolobium milingense (C.C. Ni & P.C. Li) M.L. Zhang & Podlech (A. milingensis Ni et P. C) AF521954; Phyllolobium pastorius (H.T. Tsai & T.T.Yu) M.L. Zhang & Podlech (A. pastorius Tsai et Yu) FJ236892; Phyllolobium tribulifolium1 (Bunge) M.L. Zhang & Podlech (A. tribulifolius Benth. ex Bunge) AF521953; Phyllolobium tribulifolium2 (Bunge) M.L. Zhang & Podlech (A. tanguticus Batalin) AF521956; Phyllolobium tribulifolium3 (Bunge) M.L. Zhang & Podlech (A. tanguticus Batalin) FJ236897; Astragalus adsurgens Pall. AF121674; A. aksuensis (Bunge) AF359753; A. alpinus L. ITS1 L10760+ ITS2 L10767; A. dshimensis Gontsch. AF359755; A. chinensis L. f. AF121681; A. hamosus L. ITS1 L10778+ ITS2 L10779; A. hoactly Franch. AF521952; A. lehmannianus Bunge AF359756; A. lepensis Bunge AF359752; A. membranaceus Bunge AF359749; A. nankaizanensis Sasaki AF121680; A. polycladus Bur. et Franch. AF121676; A. propinquus B. Schischkl. 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; Colutea arborescens L. ITS1 U56009+ ITS2 U56010; Co. istria Mill. ITS1 U69544+ ITS2 U69545; Dalbergia congestiflora Pittier AF068140; D. foiloosa Benth. AF189002; D. sissoo Roxb. AF189023; Hedysarum aculeolatum Michx AY772222; H. carnosulum Greene AY772224; Coronaria sus L. ITS1 L10778; D. foliolosa P. Pittier AF068140; D. foliolosa Pittier AY772224; H. coronarium L. AY772225; H. flexuosum L. AY773512; H. humile L. AY772227; H. membranaceum Coss. et Bal. AY772228; H. pallidium Desf. AY772229; H. spinosiimum L. subsp. capitatum AY772223; Oxytropis besseyi (Rydby) Blank. var. ventosa (Greene) Barneby AF121756; O. lambertii Pursh AF121753; O. multiceps Nutt. AF121760; O. pilosa DC. AF121759; O. sericea Schur AF121757; Puercaria lobata (Willd.) Ohwi (= P. montana var. lobata) AY338215; P. montana (Lour.) Merr. AY338216; P. thomsonii Benth. (= P. montana var. thomsonii) AY338217; Sphaerophysa salaula 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. swainsonoides AF113866.