Epimedium tianmenshanensis (Berberidaceae), a new species from Hunan, China

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Epimedium tianmenshanensis (Berberidaceae), a new species from Hunan, China

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Abstract

A distinctive, previously undescribed species of Epimedium (Berberidaceae), which we propose naming E. tianmenshanensis, was found during a floristic survey in Hunan, China. It is similar to E. baojingense and E. franchetii, but has smaller flowers and petals with a short, blunt spur. We determined the phylogenetic position of the new species using DNA sequences of nuclear ITS and two chloroplast regions (atpB-rbcL intergenic spacer and matK). Molecular evidence indicates that E. tianmenshanensis occupies a distinct group related to E. baojingense.

Keywords: Berberidaceae, Epimedium tianmenshanensis, phylogeny

Introduction

Epimedium Linnaeus (1753: 117; barrenwort) is the largest and most morphologically diverse genus of the herbaceous Berberidaceae (Ying et al. 2011). Members of the genus occur in woodland or scrub in temperate hilly or mountain areas, from the Mediterranean region, through western Asia into China and Japan (Ying 2002, Zhang et al. 2007). As currently circumscribed, the genus comprises more than 60 species, of which 52 taxa are endemic to China (Guo et al. 2008, Ying 2001, 2002). Furthermore, accounts of several new species from China have been published in recent years (Guo et al. 2007, He & Xu 2003, Zhang & Li 2009, He et al. 2010, Sheng & Tian 2011). Plants of Epimedium are used in traditional Chinese medicine and are reported to be effective in strengthening the kidneys, curing rheumatism and treating osteoporosis, hypertension, and coronary heart disease (Guo & Xiao 1999, Sun et al. 2005), as well as strengthening immunity and preventing dementia (Sheng & Tian 2011).

Species delimitation in Epimedium has been problematic because of high levels of morphological variation (Sun et al. 2005). With the recent publications of additional species, determining the infrageneric relationships in Epimedium has become a great challenge (Sun et al. 2005). Floral characters, such as petal type, the form and relative size of the petals and inner sepals, and flower dimension are important for the delimitation of species in Epimedium.

Based on the presence/absence of cauline leaves, the genus has been divided into two subgenera, namely Epimedium subg. Epimedium and E. subg. Rhizophyllum (Fischer & Meyer) Stearn (2002: 164); the former is divided into four sections (E. sects. Diphyllon (Komarov) Stearn (2002: 48), Epimedium, Macroceras C. Morren & Decaisne (1834: 349) and Polysphyllum Stearn (2002: 154)) with geographical determinants (Stearn 2002). Species native to China have been grouped within E. subg. Epimedium sect. Diphyllon, characterized by having two opposite cauline leaves usually with three leaflets.

During field investigations and herbarium studies of the “Wuling Mountains National Nature Reserve Group”, we found an unusual isolated population of Epimedium from Tianshenshan National Geological Park, Zhangjiajie City in northwestern Hunan. Subsequent taxonomic studies revealed that plants of this population could be morphologically assigned to E. subg. Epimedium sect. Diphyllon, but differed substantially from all known species of Epimedium. Based on a review of the taxonomic literature, including the Flora of China (Ying et al. 2011), as well as comparisons
with herbarium materials and phylogenetic analysis using data from the nuclear ribosomal internal transcribed spacer (nrITS) and two chloroplast DNA (cpDNA) sequences of this potential new species and related species of the genus, we conclude that this plant represents a new species, which we describe as *Epimedium tianmenshanensis* T. Deng, D.G. Zhang & H. Sun.

**Materials and Methods**

**Morphological analysis**—Plants of an unknown species of *Epimedium* were collected in Hunan, China. Voucher specimens are deposited at KUN and JIU. We compared them with descriptions in the literature and with herbarium specimens (including types) of *Epimedium* in IBSC, KUN, and PE, especially *E. franchetii* Stearn (1996: 396) and *E. baojingense* Q.L. Shen & B.M. Yang (1982: 482), which appeared to be most similar to the plants from Hunan. As diagnostic morphological characters, we examined leaf type, flower dimension, relative size of petals and inner sepals, and the shape of the petal spur. All these traits were examined because of their potential for identification of *Epimedium* species (Ying et al. 2011).

**Molecular analysis**—Nine accessions of the new taxon and two *Epimedium* species (*E. franchetii* and *E. baojingense*) were sequenced for the entire nrITS region (ITS1-5.8S-ITS2) and two cpDNA regions (*atpB-rbcL* intergenic spacer region and *matK*) as described in Sun et al. (2005) and Zhang et al. (2007). Taxa from *E. subg. Epimedium* (31 species) and *E. subg. Rhizophyllum* (two species) with sequences of these regions stored in GenBank were added to form a matrix of 43 accessions in total, including three species of *Vancouveria* Morren & Decaisne (1834: 351) as outgroups based on previous molecular phylogenetic relationships (Sun et al. 2005, Zhang et al. 2007). Voucher information and GenBank accession numbers of all species sampled for DNA are listed in Appendix 1. Consensus sequences were assembled for each individual using DNA Baser v.2.75 (http://www.DnaBaser.com). Multiple-sequence alignment was undertaken using Clustal X v.1.81 (Thompson et al. 1997) with the default alignment parameters and then manually adjusted. The incongruence length difference (ILD) test was conducted to assess data congruency (Farris et al. 1995); this was performed using PAUP* v.4.0b10 (Swofford 2002) with 1000 heuristic search replications.

Phylogenetic analyses were performed using maximum parsimony (MP). Each data set was analyzed separately and then a combined analysis was performed including all regions. The MP analyses were carried out in PAUP* v.4.0b10. Gaps were treated as missing data. All characters and character states were weighted equally and unordered. Searches were conducted over 100 random taxon addition replicates with tree bisection-reconnection (TBR), branch swapping, and MulTrees in effect. Bootstrap analyses (BS, 1000 pseudoreplicates) were conducted to examine the relative level of support for clades on the cladograms (Felsenstein, 1985). The consistency index (CI) and retention index (RI) were calculated to measure the level of homoplasy in the data set.

Phylogenetic analyses of nrITS, cpDNA, and combined datasets were also conducted under Bayesian Markov Chain Monte Carlo (MCMC) inference (BI; Yang & Rannala 1997) using MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003). The models determined were K80 + I (nrITS), GTR + I (cpDNA), and GTR + I + G (combined nrITS and cpDNA) and were based on the Akaike information criterion (AIC; Akaike 1974) as implemented in MODELTEST v.3.1 (Posada & Crandall 1998). Four Markov Chain Monte Carlo simulations were run simultaneously and sampled every 100 generations for a total of 5,000,000 generations. The first 12,500 (25%) of the sample trees from each run were discarded (they represented the burn-in) as determined by Tracer v.1.4 (Rambaut & Drummond 2007). A Bayesian consensus tree was constructed from the remaining trees, yielding the posterior probability (PP) values for each clade.

**Results and Discussion**

**Morphology**—The morphological characters of our newly described species (*E. tianmenshanensis*), *E. baojingense*, and *E. franchetii* are presented in Table 1. The flower size, petal shape and the absence/presence of a spur are distinguishing characters in *Epimedium* species (Stearn 2002). The flower characteristics of our new taxon were found to be unique when compared to related species, *E. baojingense* and *E. franchetii*. The petals of *E. tianmenshanensis* were of a similar size to the inner sepals, whereas those of *E. baojingense* and *E. franchetii* were much longer than the inner sepals. The petal spur of *E. tianmenshanensis* was very short (ca. 5 mm) and blunt in shape, whereas those of *E. baojingense* and *E. franchetii* were well developed (ca. 2 cm) and subulate.
TABLE 1. Taxonomic comparisons between *Epimedium tianmenshanensis* and related species.

<table>
<thead>
<tr>
<th>Characters</th>
<th><em>E. tianmenshanensis</em></th>
<th><em>E. baojingense</em></th>
<th><em>E. franchetii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf type</td>
<td>Trifoliate</td>
<td>Simple</td>
<td>Trifoliate</td>
</tr>
<tr>
<td>Flower length</td>
<td>2–4 mm</td>
<td>Ca. 4.5 cm</td>
<td>Ca. 4.5 cm</td>
</tr>
<tr>
<td>Relative size of the petals and</td>
<td>Petals as long as</td>
<td>Petals much</td>
<td>Petals much</td>
</tr>
<tr>
<td>inner sepals</td>
<td>inner sepals or a</td>
<td>longer than</td>
<td>longer than</td>
</tr>
<tr>
<td></td>
<td>little shorter than</td>
<td>inner sepals</td>
<td>inner sepals</td>
</tr>
<tr>
<td>Spur</td>
<td>Ca. 0.5 cm, slightly</td>
<td>Ca. 2 cm, much</td>
<td>Ca. 2 cm, much</td>
</tr>
<tr>
<td></td>
<td>incurred or not</td>
<td>curved</td>
<td>curved</td>
</tr>
</tbody>
</table>

**Molecular evidence**—The nrITS alignment, comprising 43 accessions from 34 *Epimedium* species and three outgroups, contained 637 aligned nucleotides, 73 (11.5%) of which were variable. The combined cpDNA data matrix consisted of 2379 aligned nucleotides, 114 (4.8%) of which were variable. The ILD test indicated that nrITS and cpDNA sequence data partitions were not significantly incongruent (*P* = 0.051). The combined data set had 3016 aligned positions, with 187 (6.2%) variable sites, 94 (3.1%) of which are parsimoniously informative (Appendix 2).

Maximum parsimony analysis performed on the combined dataset for the nrITS and two cpDNA regions resulted in >10,000 equally parsimonious trees (tree length = 227, CI = 0.75, RI = 0.83). The BI phylogram was identical in topology to the strict consensus tree sampled by the MP analysis, except that the species *E. elatum* C. Morren & Decaisne (1834: 356) was sister to the clade including *E. alpinum* Linnaeus (1753: 117), *E. diphyllum* Loddiges ex Graham (Hooker 1835: t. 3448), *E. grandiflorum* C. Morren (1834: 141, t. 35, fig. A), *E. perralderianum* Cosson (1862: 167), and *E. pinnatum* Fischer ex Candolle (Candolle 1821: 29) (Fig. 4). Six accessions of *E. tianmenshanensis* formed a cluster (BS = 100%, PP = 1.00) and sister to *E. baojingense* (BS = 81%, PP = 0.82; Fig. 4). These two species share some features, such as habitat, inflorescence, and the color of flowers. However, *E. tianmenshanensis* is distinguished from *E. baojingense* by leaf type: *E. tianmenshanensis* typically has trifoliate leaves, whereas *E. baojingense* is one of the few species in the genus with simple leaves (Ying *et al.* 2011, Ying 2001).

Our morphological and molecular evidence indicates that *E. tianmenshanensis* is distinctive for identification as a separate species from the other *Epimedium* species. The following is a description of the previously unnamed species of *Epimedium*.

**Taxonomic treatment**

*Epimedium tianmenshanensis* T. Deng, D. G. Zhang & H. Sun, *sp. nov.* (Figs. 1–3)

**Holotype:**—CHINA. NW Hunan, Zhangjiajie City, Tianmenshan National Geological Park (29°34′N, 110°28′E), wet slopes under or on the edge of mixed forests, alt. 1457 m, 16 May 2007, T. Deng, D. G. Zhang & H. Sun 544 (KUN) (Figs. 1–3).

Herbs, perennial, 28–45 cm tall; stems erect, terete, fistulose, smooth, 4–8 mm diam. Leaves basal and cauline, trifoliate; leaflets thickly leathery, ovate to narrowly ovate, 4.0–9.3 × 2.1–3.9 cm, bases deeply cordate, adaxially smooth, abaxially glabrate, the terminal leaflet with equal, rounded lobes, the lateral leaflets oblique with outer lobes larger and rounded, inner lobes also rounded at base, margins finely spinose-serrate, apex acuminate. Fertile stems with 2 trifoliate, opposite leaves; raceme 13- to 19-flowered, densely glandular hairy; pedicels 0.7–2.0 cm. Flowers small, yellow, 0.2–0.4 cm diam.; outer sepals 4, ovate, caduceous; inner sepals ovoid to widely ovoid, occasionally narrowly so, white, occasionally light mulberry-purple, 3.9–4.6 × 2.1–2.3 mm, margin corrugated; petals incurving, as long as inner sepals or a little shorter than the latter, spurred, but the calcar or spur very short, elongate, slightly incurved or not, ca. 5.0 mm, yellow; stamens yellow, ca. 3.0 mm, slightly exposed from the petals; filaments of stamens erect, ca. 0.7 mm, yellow; anthers valvate, revolute. Capsule ca. 1.2 cm; persistent style ca. 2.5 mm; seeds 2, long ellipsoid to cylindrical, ca. 2.0 × 1.6 mm.

*EPIMEDIUM TIANMENSHANENSIS* SP. NOV. FROM HUNAN Phytotaxa 222 (1) © 2015 Magnolia Press • 35


**Etymology.** The epithet *tianmenshanensis* is derived from the name of the mountain, *Tianmenshan*, where *E. tianmenshanensis* was collected.

**Distribution and Habitat.** Based on present collections, *Epimedium tianmenshanensis* is a rare species. It occurs only in limited areas on Mt. Tianmen, Tianmenshan National Geological Park, Yongding County, Zhangjiajie, Hunan, China, on wet slopes under or on the edge of mixed forests (Fig. 4). During botanical expeditions to Hunan, Hubei, Guizhou and Chongqing provinces in 2009 and 2010, we found no additional populations. Additional collections may help to clarify its full distribution.

**Phenology.** Flowering and fruiting: April–June.

**Proposed IUCN conservation status.** *Epimedium tianmenshanensis* is restricted to Mt. Tianmen and is, based on our observations, uncommon in the area. Further exploration may result in the discovery of additional localities for the new taxon. Because the only known population occupies an area of less than 50 km², *E. tianmenshanensis* should be classified as CE—critically endangered—following the IUCN guidelines (IUCN 2008), at least until other populations are found. Additional ecological and biological studies are needed to determine effective conservation measures.

**Additional Specimen Examined.** CHINA. NW Hunan, Zhangjiajie City, Tianmenshan, wet slopes under the edge of mixed forests, alt. 1450 m, 10 May 2012, *T. Deng et al. 46* (KUN); alt. 1480 m, 2 May 2013, *D.G. Zhang et al. 1358* (JIU).
FIGURE 2. Images of living plants of *Epimedium tianmenshanensis* T. Deng, D. G. Zhang & H. Sun. A, habitat; B, habit; C, inflorescence; D, flower; E, inner sepal; F, petal; G, capsule; H, seed.
FIGURE 4. A. Strict consensus of 100,000 most parsimonious trees (tree length = 227, CI = 0.75, RI = 0.83) from parsimony analysis of the combined nrITS and two cpDNA (atpB-rbcL and matK) sequence data. The numbers above the branches indicate support values (≥75%) for MP analysis. B. Bayesian consensus phylogram obtained using the TreeAnnotator from the Bayesian analyses of the combined data set. Numbers near nodes indicate support values (Bayesian posterior probability [PP]). Six accessions of the new species, E. tianmenshanensis, are highlighted in grey. The classification of subgenera and sections follows Stearn (2002).
Acknowledgements

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http://dx.doi.org/10.3417/2007161
Subg. Epimedium – Sect. Diphylon: *E. acuminatum* Franch., China, Guizhou, DQ851457/DQ851418/JN010358; *E. baojingense* Q.L. Shen & B.M. Yang, China, Hunan, Zhang et al., 4424 [KUN], KJ669689/KJ669673/KJ669681; *E. brachyrrhizum* Stearn, China, DQ851456/DQ851417/JN010363; *E. brevicolorum* Maxim., China, Shaanxi, KJ669667/DQ851427/JN010362; *E. campanulatum* O. Gigu, China, Sichuan, DQ851452/DQ851413/JN010366; *E. dolicostemon* Stearn, China, DQ851487/DQ851489/JN010332; *E. ecalcaratum* G.Y. Zhong, China, Sichuan, DQ851454/DQ851415/JN010342; *E. elongatum* Kom., China, Sichuan, DQ851457/DQ851428/JN010327; *E. epsteinii* Stearn, China, DQ851468/DQ851429/JN010322; *E. fungii* Stearn, China, DQ851488/DQ851490/JN010354; *E. fargesii* Franch., China, DQ851462/DQ851423/JN010343; *E. flavum* Stearn, China, DQ851465/DQ851406/JN010357; *E. franchetii* 476, China, Hubei, Zhang et al., 4400 [KUN], KJ669687/KJ669672/KJ669680; *E. latisepalum* Stearn, China, DQ851469/DQ851430/JN010364; *E. leptorrhizum* Stearn, China, Guizhou, DQ851472/DQ851433/JN010350; *E. membranaceum* K.I. Meyer, China, DQ851458/DQ851419/JN010347; *E. mikiniiorii* Stearn, China, DQ851461/DQ851422/JN010340; *E. myrianthum* Stearn, China, Guizhou, DQ851455/DQ851416/JN010331; *E. ogisui* Stearn, China, Sichuan, DQ851448/DQ851409/JN010324; *E. pauciflorum* K.C. Yen, China, Sichuan, DQ851450/DQ851411/JN010351; *E. platypetalum* K.I. Meyer, China, Sichuan, DQ851451/DQ851412/JN010323; *E. pubescens* Maxim., China, Sichuan, DQ851449/DQ851410/JN010321; *E. rhizomatosum* Stearn, China, DQ851459/DQ851420/JN010348; *E. sagittatum* Maxim., China, Shaanxi, DQ851460/DQ851421/JN010319; *E. simplicifolium* T.S. Ying, China, Guizhou, DQ851473/JN975841/JN010367; *E. stellulatum* Stearn, China, DQ851464/DQ851425/JN010325; *E. tianmenshanensis* T. Deng & al. 1, China, Hunan, Deng et al., 046-1 [KUN], KJ669682/KJ669666/KJ669674; *E. tianmenshanensis* 2, China, Hunan, Deng et al., 046-2 [KUN], KJ669683/KJ669667/KJ669675; *E. tianmenshanensis* 3, China, Hunan, Deng et al., 046-3 [KUN], KJ669684/KJ669668/KJ669676; *E. tianmenshanensis* 4, China, Hunan, Deng et al., 046-4 [KUN], KJ669685/KJ669669/KJ669677; *E. tianmenshanensis* 46, China, Hunan, Zhang et al., 1358 [JIU], KJ669686/KJ669670/KJ669678; *E. tianmenshanensis* 437, China, Hunan, Deng et al., 544 [KUN], KJ669687/KJ669671/KJ669679; *E. wushanense* T.S. Ying, China, DQ851471/DQ851432/JN010360. **Sect.** Epimedium: *E. alpinum* L., Europe, DQ851480/DQ851441/AB069838. – **Sect.** Macroceras: *E. diphylum* Lodd., Japan, DQ851477/DQ851438/JN010369; *E. grandiflorum* C. Morren, Japan, DQ851479/DQ851440/JN010335. – **Sect.** Polypyllon: *E. elatum* C. Morren & Decne., Kashmir, JN010275/DQ851436/JN010368. Subg. Rhizophyllum: *E. perualderianum* Cosss., N Africa, DQ851483/DQ851444/JN010356; *E. pinnatum* Fisch. ex DC., Iran, DQ851482/DQ851443/JN010336. **Outgroups:** *Vancouveria chrysantha* Greene, USA, California, DQ851484/DQ851445/JN010353; *V. hexandra* C. Morren & Decne, USA, California, DQ851485/DQ851446/JN010355.
Appendix 2. Sequence characteristics and information about the most-parsimonious tree (MPT) obtained from parsimony analyses.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>nrITS</th>
<th>cpDNA</th>
<th>Combined</th>
<th>Combined</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>atpB-rbcL</td>
<td>matK</td>
<td>nrITS and cpDNA</td>
</tr>
<tr>
<td>Number of accessions (ingroups/outgroups)</td>
<td>40 (3)</td>
<td>40 (3)</td>
<td>40 (3)</td>
<td>40 (3)</td>
</tr>
<tr>
<td>Total characters</td>
<td>637</td>
<td>708</td>
<td>1671</td>
<td>2379</td>
</tr>
<tr>
<td>Number of variable characters (%)</td>
<td>73 (11.5)</td>
<td>46 (6.5)</td>
<td>68 (4.1)</td>
<td>114 (4.8)</td>
</tr>
<tr>
<td>Number of parsimony-informative characters (%)</td>
<td>38 (6.0)</td>
<td>20 (2.8)</td>
<td>36 (2.2)</td>
<td>56 (2.4)</td>
</tr>
<tr>
<td>Tree length</td>
<td>93</td>
<td>48</td>
<td>73</td>
<td>123</td>
</tr>
<tr>
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<td>120</td>
<td>&gt;10,000</td>
<td>26</td>
<td>&gt;10,000</td>
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<tr>
<td>Consistency index (CI)(a)</td>
<td>0.72</td>
<td>0.91</td>
<td>0.93</td>
<td>0.89</td>
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<tr>
<td>Retention index (RI)</td>
<td>0.82</td>
<td>0.95</td>
<td>0.96</td>
<td>0.94</td>
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<tr>
<td>Model selected (AIC)</td>
<td>K80 + I</td>
<td>F81</td>
<td>GTR + I</td>
<td>GTR + I</td>
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</tbody>
</table>

\(a\)The consistency and retention indexes were calculated excluding uninformative characters.