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Faberia pinnatifida (Asteraceae; Cichorieae), a New Species from Sichuan, China

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Abstract—Faberia pinnatifida (Asteraceae; Cichorieae), a species occurring at high altitudes in Muli and Yanyuan Xian, Sichuan, China, is described as new based on morphological, palynological, and molecular phylogenetic data. The ITS data indicate that *F. pinnatifida* is closely related to *F. glaucescens* and *F. thibetica*, but it is easily distinguished from *F. glaucescens* by its scapigerous stem with only one or two leaves, and from *F. thibetica* in its much taller stature and elliptic to oblanceolate leaf blade. Faberia pinnatifida somewhat resembles *F. ceterach* in the scapigerous stem and pinnatifid leaves, but readily differs in its corymbiform synflorescence and nodding capitula bearing ca. 30 florets.

Keywords—Phylogeny, taxonomy.

During field studies on Huolu Shan, Yanyuan Xian, Sichuan, in August 2010, an interesting plant was collected on scree slopes and in meadows around the summit of the mountain. One more population of the same species was discovered in August 2015 at the pass between Muli and Yanyuan in Muli Xian during a botanical expedition for the Pan Himalaya Flora project (FLPH). These plants have latex, lyrately pinnatifid to pinnatipartite leaves, and nodding capitula with purplish blue ligulate florets (Fig. 1), obviously belonging to tribe Cichorieae. At first glance, the plants in question resembled members of Dubyaea Candolle or Faberia Hemsley because of the purplish blue florets and phyllaries in several series gradually longer centripetally (Fig. 1C). The phyllaries of species of Dubyaea usually have stiff, yellowish brown, brownish, or blackish hairs, whereas those of Faberia are glabrous. Our plants have glabrous phyllaries (Fig. 1C) and are thus more similar to Faberia.

Faberia, as most recently circumscribed, includes eight species in central and southwestern China (Shih and Kilian 2011; Liu et al. 2013). Karyological, palynological, and molecular phylogenetic data indicate that Faberia may have originated via hybridization and subsequent polyploidization between a member of Lactucinae with x = 9 and a member of Crepidinae with x = 8, and is well recognized by x = 17 and larger, 4-porate pollen grains (Liu et al. 2013). As recognition of natural groups within Cichorieae is rather difficult due to the lack of clear diagnostic morphological features and extensive parallel evolution (Kilian et al. 2009), we applied palynological and molecular phylogenetic data to test the generic affiliation of these plants. The pollen morphology of these and five additional species of Faberia were examined, and two datasets (nrITS and plastid) published in a previous study of Faberia were adopted to elucidate the phylogenetic relationships. Results are presented below along with the description of this new species, F. pinnatifida.

MATERIALS AND METHODS

Pollen Morphology—Pollen morphology of five species of *Faberia* and *F. pinnatifida* was examined using scanning electron microscopy. The source of the materials is given in Table 1. Pollen removed from mature florets was directly mounted onto stubs. Samples were sputter-coated with gold and then examined with a JSM-6360LV scanning electron microscope.

Taxon Sampling, DNA Extraction, PCR Amplification, and Sequencing—To test the phylogenetic position of *Faberia pinnatifida,* the ITS and cpDNA (*matK, psbA-trnH, rbcL,* and *trnL-F*) datasets from a previous

phylogenetic study of Faberia (Liu et al. 2013) were adopted. The two datasets, exactly paralleling each other, contained 44 published accessions and the newly obtained sequences of F. pinnatifida, with Hieracium umbellatum L. chosen as outgroup. The leaves of F. pinnatifida were collected from Huolu Shan, Yanyuan Xian, Sichuan Province. Total DNA was extracted from silica-gel dried leaves using the modified CTAB procedure (Doyle and Doyle 1987). Nuclear ITS, plastid matK, psbA-trnH, rbcL, and trnL-F were amplified and sequenced for F. pinnatifida using five pairs of primers, namely ITS5 and ITS4 (White et al. 1990), 3F-KIM (CGTACAGTACTTTTGTGTTTACGAG) and 1R-KIM (ACCCAGTCCATCTGGAAATCTTGGTTC) (Ki-Joong Kim, unpubl. data), psbA(F) and trnH(R) (Kim et al. 1999), rbcL-1F and rbcL-724R (Fay et al. 1997), and tab-c and tab-f (Taberlet et al. 1991), following the same procedure described in Liu et al. (2013). GenBank accession numbers for all sequences used are given in Appendix 1, and the data used for phylogenetic analysis is available in Dryad (Liu et al. 2018). Voucher specimens of the new species are deposited in the Herbarium of the Arnold Arboretum (A), the Herbarium of the Kunming Institute of Botany (KUN), and the Herbarium of Sun Yat-sen University (SYS). For generic circumscription and species treatment within Cichorieae, we followed Kilian et al. (2009) and Shih and Kilian (2011).

Phylogenetic Analysis-As the possible hybrid origin of Faberia resulted in strong incongruence between nuclear and chloroplast DNA data (Liu et al. 2013), we analyzed the ITS and combined cpDNA datasets separately. Bayesian inference (BI), maximum parsimony (MP), and maximum likelihood (ML) methods for phylogenetic estimation were conducted using MrBayes v. 3.1.2 (Huelsenbeck and Ronquist 2001), PAUP* v. 4.0b10 (Swofford 2003), and PhyML v. 3.0 (Guindon and Gascuel 2003), respectively. Gaps were treated as missing data in a conservative approach. Datasets were analyzed in MrModeltest v. 2.2 (Nylander 2004) and jModeltest v. 0.1.1 (Guindon and Gascuel 2003; Posada 2008) to select the evolutionary models for BI and ML analyses, respectively. The GTR + G and GTR + I + G models were used for the BI analysis of ITS and cpDNA datasets, respectively. Markov Chain Monte Carlo analyses (MCMC) were performed with four simultaneous chains of 2,000,000 generations sampling one tree every 100 generations. We verified that the average deviation of split frequencies had reached a value below 0.01. Trees that preceded the stabilization of the same likelihood value found in all four chains (the burn-in) were discarded, and the remaining trees were used to construct a 50% majority-rule consensus tree with Bayesian posterior probabilities. For maximum parsimony analyses, heuristic searches were performed using 1000 random additions of taxa, the BEST trees option, and tree-bisection-reconnection (TBR) branch swapping algorithm, with MaxTrees set to 100. The trees were evaluated by bootstrap analysis with 500 replicates of 1000 random additions. For maximum likelihood analyses, the models selected for the ITS data set were SYM + I + G (AIC) and SYM + G (BIC), those selected for the cpDNA dataset were GTR + I + G (AIC) and TPM1uf + I + G (BIC). Models chosen by the two criteria were used separately for both datasets. The BEST tree topology search option was selected, with five random starting trees. Non-parametric bootstrap values were computed with 100 replicates to evaluate the trees.

Results

Pollen Morphology—As shown in Fig. 2, the pollen grains of Faberia cavaleriei H.Lév., F. faberi (Hemsl.) N.



FIG. 1. *Faberia pinnatifida*. A. Habit. B. Top view of capitulum. C. Lateral view of capitulum showing phyllaries. D. Achene, scale bar = 5 mm. All from Sichuan, Muli Xian, Muli-Yanyuan Pass (voucher *FLPH Sichuan Expedition* 151148, PE).

Kilian, Z.H.Wang & J.W.Zhang, *F. nanchuanensis* C. Shih, *F. sinensis* Hemsl., *F. thibetica* (Franch.) Beauverd, and *F. pinnatifida* were all spherical, echinolophate, and 4-porate with four poral, eight abporal, and eight paraporal lacunae.

Phylogenetic Analysis—The aligned matrix contained 605 characters in the ITS dataset and 2963 in the combined cpDNA dataset. Trees generated by the Bayesian, maximum parsimony, and maximum likelihood analyses of the same dataset had almost identical overall topologies. Only the 50%

TABLE 1. Source of materials studied.

Taxon	Locality	Voucher	Figure
Faberia cavaleriei	Suiyang, Guizhou	Tao Deng 090715001 (IBSC, KUN)	1A, B
F. faberi	Qiaojia, Yunnan	Chen Ren & Long-yuan Wang 378 (IBSC)	1C, D
F. nanchuanensis	Nanchuan, Chongqing	Tao Deng 090624001 (IBSC, KUN)	1E, F
F. sinensis	Emei, Sichuan	Ying Liu 2010094 (IBSC)	1G, H
F. thibetica	Kangding, Sichuan	Ying Liu 2010089 (IBSC)	1I, J
F. pinnatifida	Yanyuan, Sichuan	D. E. Boufford et al.42863 (A, KUN, SYS)	1K, L

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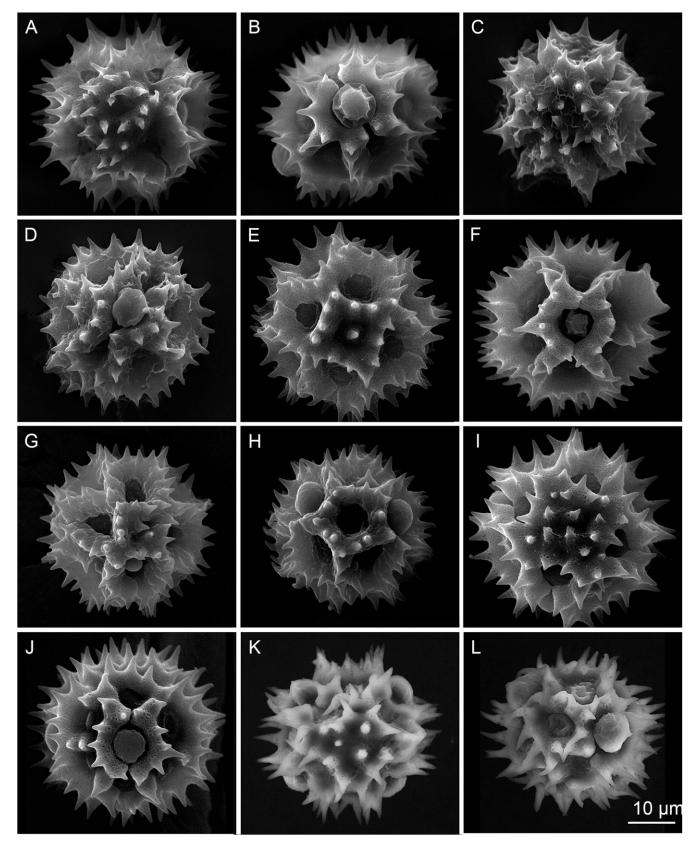


FIG. 2. Scanning electron micrographs of pollen grains of *Faberia*; polar view and equatorial view. A–B. *F. cavaleriei*. C–D. *F. faberi*. E–F. *F. nanchuanensis*. G–H. *F. sinensis*. I–J. *F. thibetica*. K–L. *F. pinnatifida*. Scale bar = 10 μ m.

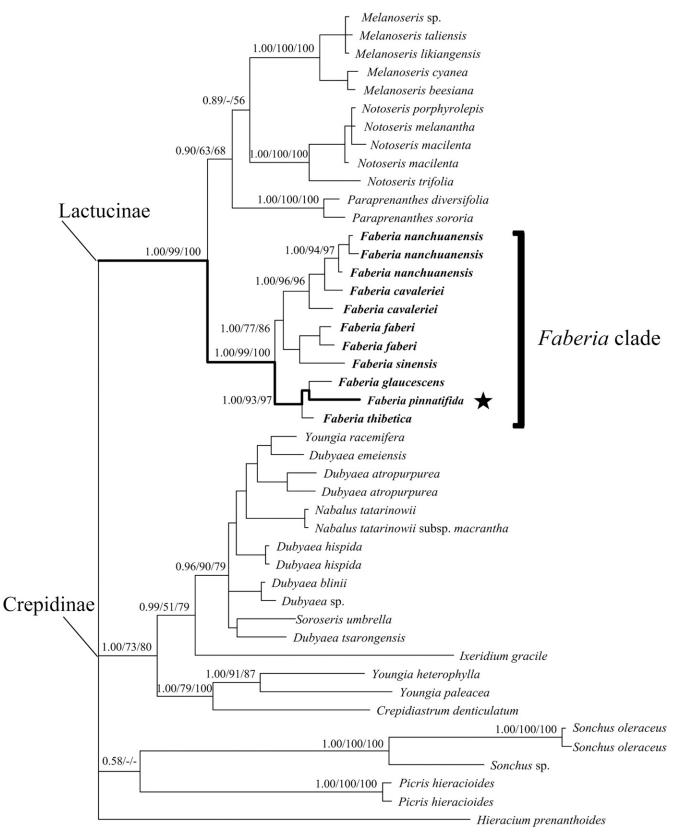


FIG. 3. Phylogram of Bayesian inference analysis of ITS dataset showing phylogenetic position of *Faberia pinnatifida*. Species of *Faberia* are indicated in bold; branches leading to *F. pinnatifida* are indicated by bold line. Numbers on branches are Bayesian posterior probabilities, bootstrap values obtained from maximum parsimony, and maximum likelihood analyses. Bootstrap values below 50% are indicated by '-'.

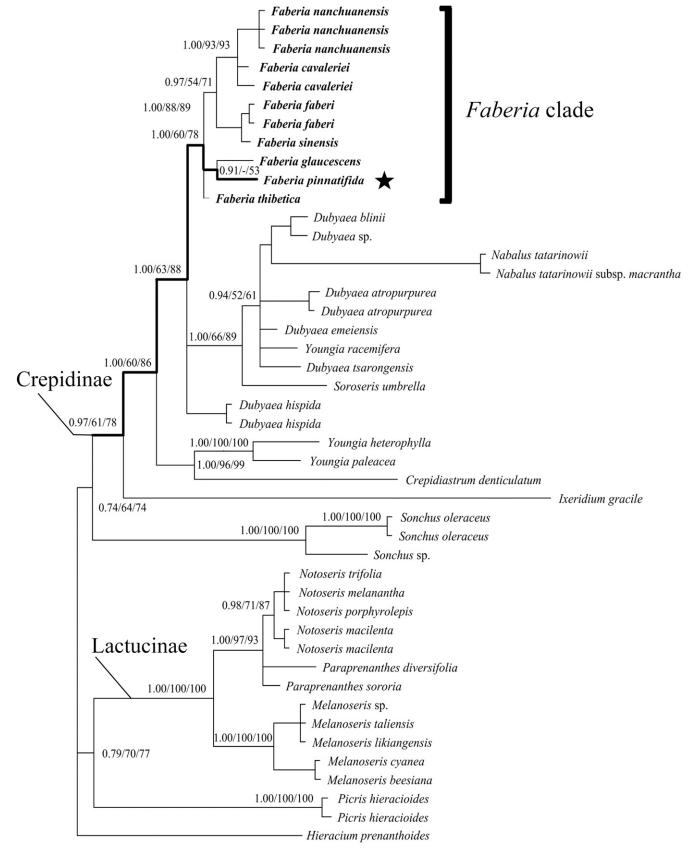


FIG. 4. Phylogram of Bayesian inference analysis of cpDNA dataset showing phylogenetic position of *Faberia pinnatifida*. Species of *Faberi* are indicated in bold; branches leading to *F. pinnatifida* are indicated by bold line. Numbers on branches are Bayesian posterior probabilities, bootstrap values obtained from maximum parsimony, and maximum likelihood analyses. Bootstrap values below 50% are indicated by '-'.

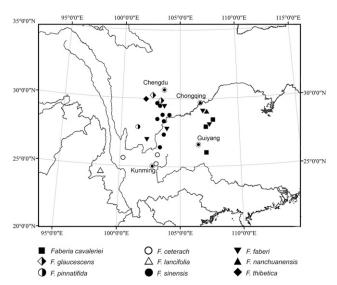


FIG. 5. Distribution of species of Faberia.

majority-rule consensus trees obtained from BI analysis are shown here, with Bayesian posterior probabilities and bootstrap values obtained from MP and ML analyses indicated on the branches (Figs. 3-4). The phylogenies derived from the ITS and chloroplast datasets were identical with that of Liu et al. (2013), showing both congruence and incongruence between datasets. Although the subtribal affiliation of Faberia was strikingly different as indicated by nuclear (subtribe Lactucinae) and plastid data (subtribe Crepidinae), the Faberia clade as a whole was well recognized, with strong support by ITS data (PP = 1.0, BS MP = 99%, BS ML = 100%), and strong to moderate support by chloroplast data (PP = 1.0, BS MP = 60%, BS ML = 78%). Faberia pinnatifida nested firmly in the Faberia clade together with six additional species of Faberia in both trees (Figs. 3-4). In the ITS phylogenetic trees, F. pinnatifida formed a well-supported subclade with F. thibetica and F. glaucescens (Stebbins) Ying Liu, Y.S.Chen & Q.E.Yang (PP = 1.0, BS = 93% and 97%) (Fig. 3), whereas in the plastid phylogenetic trees it clustered with F. glaucescens, but with only weak support (PP = 0.91, BS < 50% and 53%) (Fig. 4).

DISCUSSION

Both phylogenetic analyses and pollen morphology strongly supported the generic affiliation of Faberia pinnatifida within Faberia. Our phylogenetic analyses revealed that F. pinnatifida constantly grouped within the Faberia clade in both ITS (Fig. 3) and plastid (Fig. 4) phylogenetic trees, indicating that it should be treated as a member of Faberia. Observation of pollen morphology also showed that the pollen grains of F. pinnatifida were spherical, echinolophate, and 4-porate (Fig. 2K-L). These pollen features have been found to be shared by all species of Faberia so far examined, including F. cavaleriei, F. faberi, F. nanchuanensis, F. sinensis, F. thibetica (Fig. 2), and also F. glaucescens (Stebbins 1940). Stebbins (1940) pointed out that all species of Cichorieae with 4-porate pollen grains and known chromosome numbers were polyploids. Therefore, F. pinnatifida may also be a polyploid although we failed to determine its chromosome number because of the difficulty in collecting living material.

Faberia pinnatifida also resembles *Faberia* in gross morphology and geographic distribution. Since the transfer of the morphologically distinctive *Prenanthes faberi* Hemsl. to *Faberia* as *F. faberi* (Shih and Kilian 2011), *Faberia* was re-circumscribed

as being more diverse in gross morphology. Nevertheless, most species of *Faberia* are scapigerous or subscapigerous herbs with somewhat leathery leaves, a corymbiform or paniculiform synflorescence, narrowly cylindrical to campanulate involucres, several series of glabrous phyllaries becoming gradually longer toward the apex of the involucre, reddish or bluish purple florets, achenes narrowly ellipsoid with a truncate apex, and brownish pappi. *Faberia pinnatifida* resembles most species in its corymbiform synflorescence, narrowly campanulate involucres, glabrous phyllaries in several series gradually longer centripetally, bluish purple florets, narrowly ellipsoid achenes, and brownish pappi (Fig. 1). Geographically, it is narrowly endemic in Muli and Yanyuan Xian, Sichuan, China, within the range of *Faberia* as a whole (Fig. 5).

The ITS data suggest a closest relationship among Faberia pinnatifida, F. glaucescens, and F. thibetica, while plastid data place F. pinnatifida somewhat near F. glaucescens. In total, we detected 27 to 29 substitutions and two indels between F. pinnatifida and F. glaucescens, and 21 substitutions and three indels between F. pinnatifida and F. thibetica, showing F. pinnatifida to be well differentiated from its potentially closest relatives. All three species occur in Sichuan and resemble each other in their nodding capitula, but F. pinnatifida is easily distinguished from F. glaucescens by its scapigerous stem having only one or two leaves (vs. leafy stem), and from F. *thibetica* by its much larger stature 46–75 cm tall (vs. 15–35 cm), and its elliptic to oblanceolate (vs. triangular-ovate) leaf blade. Faberia pinnatifida somewhat resembles F. ceterach, a species endemic to Yunnan, in its scapigerous stem and pinnatifid leaves, but readily differs in its corymbiform synflorescence (vs. narrowly paniculiform) and nodding capitula (vs. not nodding) having ca. 30 florets (vs. 10–15). Therefore, both molecular data and our herbarium and field observations show that F. pinnatifida does not match well with any of the species of Faberia so far described, leading us to recognize it as a new species. The description of F. pinnatifida and a key to separate it from other species of Faberia are provided below.

TAXONOMIC TREATMENT

Faberia pinnatifida Ying Liu, Yousheng Chen & Boufford, sp. nov. TYPE: CHINA. Sichuan: Yanyuan County, E of Wodi-xiang; around summit of Huolu Shan Peak, scree slopes, meadows, and adjacent primary *Picea* forest over limestone; 27°54'11"N, 101°37'5"E; 4300–4320 m, 13 Aug 2010, D. E. Boufford, L. Y. Chen, X. H. Li, J. R. Shevock & J. P. Yue 42863 (holotype: SYS; isotypes: A, KUN).

Herbs, perennial, 46–75 cm tall, with short rhizomes. Stems erect, glabrous, branched apically. Rosette leaves narrowly elliptic to oblanceolate; petiole 3–8 cm long, blade 6–16 × 2.5–6.7 cm, lyrately pinnatifid to pinnatipartite, base cuneate and narrowly decurrent along petiole, apex acute to acuminate, abaxially pale glaucous, adaxially green and glabrous; lateral lobes 8–14, triangular to broadly ovate, gradually smaller toward leaf base; terminal lobe triangular to lanceolate. Stem leaves usually 1 or 2, only one leaf similar to rosette leaves; upper leaf often linear and bract-like. Synflorescence laxly branched, corymbiform, with 2–7 capitula. Capitula nodding, with ca. 30 florets. Involucre ca. 15 × 6 mm. Phyllaries abaxially dark green, glabrous, apex obtuse or acute, margin and apex shortly whitish fimbriate; outer phyllaries ca. 14, 13–15 × 3–4 mm. Florets

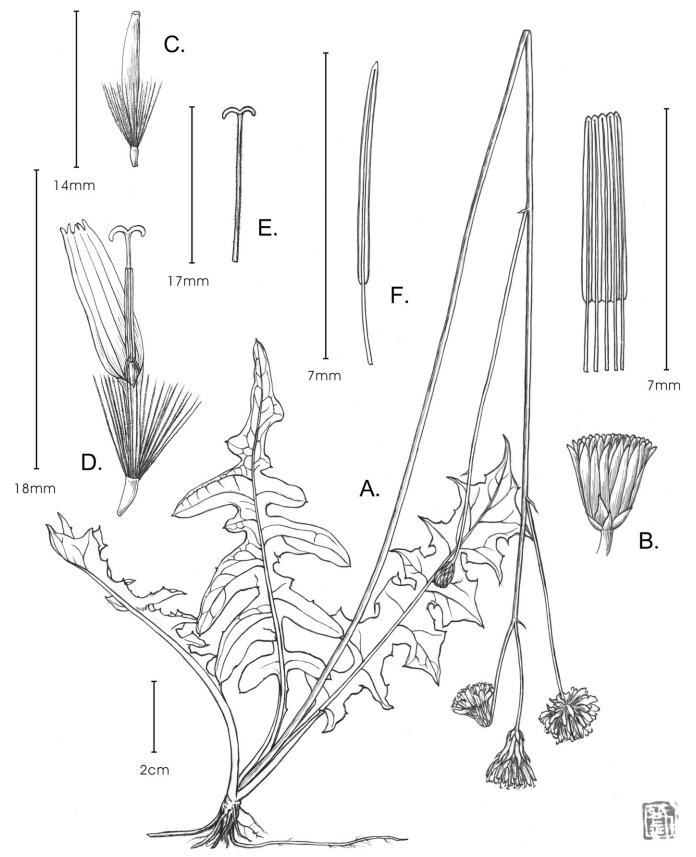


FIG. 6. Faberia pinnatifida. A. Habit. B. Capitulum showing phyllaries. C-D. Young and mature florets. E. Style and stigma. F. Stamen.

purplish blue. Achenes brown, narrowly ellipsoid, 4–5 mm long, apex truncate. Pappus brownish, ca. 6 mm long. Flowering August–September, fruiting September–October. Figure 6.

Distribution and Ecology—Known from northern Yanyuan Xian and southern Muli Xian, Sichuan, China, on scree slopes, in meadows, thickets, and primary *Picea* forests over limestone.

Etymology—The epithet is based on the pinnately lobed to partite leaves.

Conservation Concerns—*Faberia pinnatifida* is known only from a small area around northern Yanyuan and southern Muli xian. Of the two populations known, the one at the Muli-Yanyuan Pass included no more than 50 clumps. The extent of the population on Huolu Shan was not determined.

Additional Specimen Examined—China.—SICHUAN: Muli Xian. Xiamaidixiang, Muli-Yanyuan Pass, 27°44′4.13″N, 101°14′5.71″E, 3000 m, 25 Aug 2015, *FLPH Sichuan Expedition 151148* (PE).

Key

1.	Leaves lyrately pinnatifid to lyrately pinnatisect
1.	Leaf blade not divided or at most coarsely sinuate-dentate
2	(1). Terminal leaf lobe much larger than lateral lobes, to 2/3 of entire leaf <i>F. sinensis</i> .
	(1). Terminal leaf lobe never conspicuously larger than lateral lobes
3	(1). Leaf blade lanceolate to narrowly elliptic or oblanceolate, at least ca. $3 \times as$ long as wide
3	(1). Leaf blade broadly elliptic, ovate, or pentagonal, mostly not more than ca. $2 \times as$ long as wide
4	(2). Synflorescence narrowly paniculiform, usually with 10–20 capitula; involucre 1.1–1.3 cm long; capitula erect, with 10–15 florets F. ceterach.
4	(2). Synflorescence corymbiform, with 2–7 capitula; involucre \geq 1.5 cm long; capitula nodding, with more than 25 florets
5	(3). Capitula many in a leafless narrowly paniculiform synflorescence
5	(3). Capitula 1–3 on short branchlets subtended by upper stem leaves F. nanchuanensis
6	(3). Stem leafy; leaf petiole usually shorter than blade; leaf blade triangular-ovate to pentagonal; capitula with 5 florets; inner phyllaries 5 F. faberi
6	(3). Stem scapigerous or subscapigerous, leaf petiole distinctly longer than blade; leaf blade broadly elliptic to ovate; capitula with more than 10 florets;
	inner phyllaries at least 8
7	(4). Stem leafy, leaf base attenuate to an auriculately clasping base <i>F. glaucescens</i>
7	(4). Stem scapigerous with only 1 or 2 leaves; leaf base cuneate to petiole-like <i>F. pinnatifida</i>
8	(6). Herbs 60–80 cm tall; leaf blade mostly more than 5 cm long; capitula not nodding F. cavaleriei
8	(6). Herbs 15–35 cm tall; leaf blade at most ca. 4 cm long; capitula nodding F. thibetica

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APPENDIX 1. Taxon, GenBank accession numbers (ITS/matK/psbA-trnH/ rbcL/trnL-F)-Crepidiastrum, C. denticulatum (Houtt.) Pak & Kawano, KF154369, KF195984, KF196116, KF196028, KF196072; Dubyaea, D. atropurpurea Stebbins, KF154377, KF195992, KF196124, KF196036, KF196080; D. atropurpurea, KF154381, KF195996, KF196128, KF196040, KF196084; D. blinii (H. Lév.) N. Kilian, KF154374, KF195989, KF196121, KF196033, KF196077; D. emeiensis C.Shih, KF154376, KF195991, KF196123, KF196035, KF196079; D. hispida DC., KF154379, KF195994, KF196126, KF196038, KF196082; D. hispida DC., KF154380, KF195995, KF196127, KF196039, KF196083; D. tsarongensis (W.W.Sm.) Stebbins, KF154378, KF195993, KF196125, KF196037, KF196081; Dubyaea sp., KF154375, KF195990, KF196122, KF196034, KF196078; Faberia, F. cavaleriei H.Lév., KF154395, KF196010, KF196142, KF196054, KF196098; F. cavaleriei, KF154397, KF196012, KF196144, KF196056, KF196100; F. faberi (Hemsl.) N.Kilian, KF154396, KF196011, KF196143, KF196055, KF196099; F. faberi, KF154398, KF196013, KF196145, KF196057, KF196101; F. glaucescens Stebbins, KF154382, KF195997, KF196129, KF196041, KF196085; F. nanchuanensis C. Shih, KF154399, KF196014, KF196146, KF196058, KF196102; F. nanchuanensis, KF154401, KF196016, KF196148, KF196060, KF196104; F. nanchuanensis, KF154400, KF196015, KF196147, KF196059, KF196103; F. pinnatifida, D. E. Boufford, L. Y. Chen, X. H. Li, J. R. Shevock & J. P. Yue 42863 (A, KUN, SYS), China: Sichuan, Yanyuan, MF188171, MF170029, MF170030, MF170031, MF170032; F. sinensis Hemsl., KF154402, KF196017, KF196149, KF196061, KF196105; F. thibetica (Franch.) Beauverd, KF154403, KF196018, KF196150, KF196062, KF196106; Hieracium, H. umbellatum L., KF154360, KF195975, KF196107, KF196019, KF196063; Ixeridium, I. gracile (DC.) Pak & Kawano, KF154366, KF195981, KF196113, KF196025, KF196069; Melanoseris, M. beesiana (Diels) N.Kilian, KF154394, KF196009, KF196141, KF196053, KF196097; M. cyanea (D.Don) Edgew., KF154384, KF195999, KF196131, KF196043, KF196087; M. likiangensis (Franch.) N.Kilian & Z.H. Wang, KF154385, KF196000, KF196132, KF196044, KF196088; M. taliensis (C. Shih) N.Kilian & Z.H.Wang, KF154386, KF196001, KF196133, KF196045, KF196089; Melanoseris sp., KF154383, KF195998, KF196130, KF196042, KF196086; Nabalus, Nab. tatarinowii (Maxim.) Nakai, KF154367, KF195982, KF196114, KF196026, KF196070; Nab. tatarinowii subsp. macrantha (Stebbins) N.Kilian, KF154368, KF195983, KF196115, KF196027,

KF196071; Notoseris, Not. macilenta (Vaniot & H.Lév.) N. Kilian, KF154389,
KF196004, KF196136, KF196048, KF196092; Not. macilenta, KF154390,
KF196005, KF196137, KF196049, KF196093; Not. melanantha (Franch.) C.
Shih, KF154391, KF196006, KF196138, KF196050, KF196094; Not. porphyrolepis C. Shih, KF154388, KF196003, KF196135, KF196094; Not. porphyrolepis C. Shih, KF154388, KF196003, KF196135, KF196047, KF196091; Not. triflora (Hemsl.) C. Shih, KF154387, KF196002, KF196134, KF196096, KF196096, KF196096, KF196097, KF196097, KF196051, KF196055; Par. sororia (Miq.) C.Shih, KF154393, KF196008, KF196140, KF196052, KF196096; Picris, Pic. hieracioides L., KF154362, KF195977, KF196109, KF196021, KF196065;

Pic. hieracioides, KF154361, KF195976, KF196108, KF196020, KF196064;
Sonchus, Son. oleraceus L., KF154364, KF195979, KF196111, KF196023,
KF196067; Son. oleraceus, KF154365, KF195980, KF196112, KF196024,
KF196068; Sonchus sp., KF154363, KF195978, KF196110, KF196022,
KF196066; Soroseris, Sor. umbrella (Franch.) Stebbins, KF154370,
KF195985, KF196117, KF196029, KF196073; Youngia, Y. heterophylla
(Hemsl.) Babc. & Stebbins, KF154372, KF195987, KF196119, KF196031,
KF196075; Y. paleacea (Diels) Babc. & Stebbins, KF154373, KF195988,
KF194371, KF195986, KF196118, KF196030, KF196074.