



## Article

### Nine new combinations and one new name of *Primulina* (Gesneriaceae) from South China

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#### Abstract

As a consequence of molecular phylogenetic studies, the monotypic genus *Primulina* has recently been recircumscribed and expanded to include *Chirita* sect. *Gibbosaccus*, *Chiritopsis*, and *Wentsaiboea*. Based on phylogenetic analyses of ITS sequences and apparent morphological similarity, we make further transfers of six species of *Chirita* (*C. leprosa*, *C. lijiangensis*, *C. ningmingensis*, *C. luochengensis*, *C. rongshuiensis*, and *C. tiandengensis*) and four of *Chiritopsis* (*Ch. danxiaensis*, *Ch. hezhouensis*, *Ch. jingxiensis*, and *Ch. longzhouensis*) that were neglected or published around or slightly after these recent taxonomic treatments. The proposed nomenclatural changes include one new name, *P. pseudolinearifolia*, and nine new combinations, *P. danxiaensis*, *P. hezhouensis*, *P. jingxiensis*, *P. leprosa*, *P. lijiangensis*, *P. longzhouensis*, *P. ningmingensis*, *P. rongshuiensis*, and *P. tiandengensis*. The addition of these ten species increases the number of the genus to ca. 150 species, making *Primulina* one of the largest and morphologically most diverse genera of the Old World Gesneriaceae.

**Key words:** *Chirita*, *Chiritopsis*, limestone karsts, molecular taxonomy, Old World Didymocarpoid Gesneriaceae

#### Introduction

The phylogenetic insights brought by molecular data have had profound impacts on the taxonomy of the Old World Gesneriaceae over the past few years (Möller *et al.* 2009, 2011a, 2011b, Wang *et al.* 2011, Weber *et al.* 2011a, 2011b, 2011c). One of the most drastic changes involved the remodeling of *Chirita* Buch.-Ham. ex Don (1825: 89) and associated genera (Wang *et al.* 2011; Weber *et al.* 2011c), resulting in the abandonment of *Chirita* (ca. 150 species) and resurrection and/or new demarcation of *Damrongia* Kerr ex Craib (1918: 364), *Henckelia* Sprengel (1817: 402), *Liebigia* Endlicher (1841: 1407), *Microchirita* (Clarke 1883: 127) Wang in Wang *et al.* (2011: 59) and *Primulina* Hance (1883: 169). Among these genera, the recircumscribed *Primulina* (Wang *et al.* 2011; Weber *et al.* 2011c), previously a monotypic genus including only the highly endangered *P. tabacum* Hance (1882: 169) from southern China (Ren *et al.* 2010), has been expanded to include all species of *Chirita* sect. *Gibbosaccus* Clarke (1883: 130), *Chiritopsis* Wang (1981a: 21), and *Wentsaiboea* Fang & Qin (2004: 533) [but excluding *Wentsaiboea tiandengensis* Liu & Pan in Liu *et al.* (2010: 739)]. Currently the redefined *Primulina* comprises ca. 140 mostly narrowly endemic species (Liu *et al.* 2011, Wang *et al.* 2011, Weber *et al.* 2011c, Hong *et al.* 2012, Huang *et al.* 2012, Li *et al.* 2012, Wen *et al.* 2012a, 2012b, 2012c, Wu *et al.* 2012a, 2012b, Xu *et al.* 2012) found primarily on various landforms of limestone karsts of the Sino-Vietnamese bordering regions (Xu *et al.* 2012), with the center of diversity in Guangxi, South China (Li & Wang 2004, Hou *et al.* 2010, Wei 2010).

In this article, we make further transfers of six species of *Chirita* and four of *Chiritopsis* from South China to *Primulina*, adding 10 species to this incredibly diverse genus. These species include one [*C. jingxiensis* Liu *et al.* in Xu *et al.* (2009: 559)] neglected by and nine others published around or slightly after the treatments of Wang *et al.* (2011) and Weber *et al.* (2011c). To further assure generic placements of these 10 species in *Primulina*, ITS sequences of eight species were amplified and included for phylogenetic analyses.

## Material and Methods

For molecular methods, the experimental protocols outlined in Xu *et al.* (2012), including DNA extraction, PCR amplifications, cycle-sequencing etc., were followed. Of the ten species treated in this study, ITS sequences were collected successfully for eight species (Appendix 1). In total, ITS sequences of 31 species of *Primulina*, including the eight newly acquired, four collected in Xu *et al.* (2012) and 24 downloaded from GenBank, were sampled (Appendix 1). *Petrocodon dealbatus* Hance (1883: 167), *Petrocodon scopulorum* (Chun 1946: 281) Wang (in Wang *et al.* 2011: 60), and *Didymocarpus podocarpus* Clarke (1883: 76) were chosen as outgroups based on recent phylogenetic analyses (Möller *et al.* 2011a; Weber *et al.* 2011c). DNA sequences were aligned using the program MUSCLE implemented in the software MEGA5 (Tamura *et al.* 2011) with minor manual adjustments.

Phylogenetic trees were reconstructed based on maximum parsimony (MP) and maximum likelihood (ML) criteria implemented in MEGA5 (Tamura *et al.* 2011) and Bayesian inference (BI) using MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). MP trees were searched using min-mini heuristic search option. Clade supports (PB) were calculated based on 100 bootstrap resamplings. For ML analysis, MEGA5 selected the Kimura 2-parameter model with a discrete gamma distribution rate among site (K2+G) and 5 discrete gamma categories as the best nucleotide substitution model. A heuristic tree searching option using a close-neighbor-interchange (CNI) inference method was chosen with all site used and initial tree automatically selected. Clade supports (LB) were evaluated based on 100 bootstrap resamplings. Prior to the BI analysis, mrModeltest (Nylander 2004) selected the symmetrical model with gamma distribution (SYM+G) based on the Akaike Information Criterion (AIC). Four runs of Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analyses were performed, with a random starting tree and four chains for each run (one 3 cold and three heated). The MCMCMC length was two million generations, and the chain was sampled once every 100th generation. Bayesian clade posterior probabilities (PP) and average branch lengths were calculated based on the sampled trees after the first 10% of the sampled trees discarded as burn-in.

## Results and Discussion

The ITS matrix consisted of 684 aligned characters, of which 204 (29.8%) are parsimoniously informative. Topologies revealed by MP, ML, and BI analyses and branch supports are summarized in Figure 1. Maximum parsimony analysis resulted in 46 equally most parsimonious trees with a tree length of 749 steps (CI=0.668, RI= 0.612, RCI=0.409). Maximum likelihood analysis uncovered a single tree with a log likelihood (lnL) of -4855.32. Results of all three phylogenetic methods were highly concordant, differing mainly in the relationships that had low supports (Fig. 1).

Rooted by *Pet. dealbatus*, *Pet. scopulorum*, and *D. podocarpus*, *Primulina* is recovered as a moderately (PB=67) to highly supported (LB=97; PP=1.00) clade. As expected based on previous taxonomic accounts, the species of the eight newly acquired ITS sequences are all placed within *Primulina* (Fig. 1), justifying their inclusion into the genus. The inclusion of these eight species further expands the size as well as the morphological diversity of *Primulina*, the most notable one being *Ch. hezhouensis* Wu & Xu in Wu *et al.* (2011a: 132) that possesses one of the smallest flowers (ca. 3–5 mm long) in Gesneriaceae (Wu *et al.* 2011a). Within *Primulina*, the basic relationships are largely congruent with previous studies (Wang *et al.* 2011, Xu *et al.* 2012), with *P. gemella* (Wood 1972: 370) Wang in Wang *et al.* (2011: 61), *P. heterotricha* (Merrill 1934:

71) Wang in Wang *et al.* (2011: 61), and *P. pteropoda* (Wang 1985: 51) Liu in Wang *et al.* (2011: 62) clustered in a subclade (PB=54; LB=53; PP=0.82) sister to the clade (PB=67; LB=57; PP=1.00) composed of the rest of the genus (Fig. 1). Within the latter clade, species are grouped into five highly supported subclades except for *Ch. hezhouensis*, *P. cordifolia* [Fang & Wang in Wang (1982: 54)] Wang in Wang *et al.* (2011: 61), *P. spadiciformis* (Wang 1985: 49) Möller & Weber in Weber *et al.* (2011: 784), and *C. leprosa* Liu & Xu in Xu *et al.* (2010: 705) whose phylogenetic relationships remain inadequately resolved.

Although our current sampling (31 species) comprises only ca. 20% of the known species of *Primulina* and relationships within the genus remain poorly resolved, some phylogenetic signals have emerged with the addition of the newly acquired sequences (Fig. 1). For example, “*Chiritopsis*” is even more polyphyletic than previously suggested (Li & Wang, 2007), indicating the highly homoplasious nature of the “small” flowers as a diagnostic feature. Additionally, species distributed in geographical proximities also appear to be phylogenetically closely related. Specifically, *Chirita lijiangensis* Pan & Xu in Xu *et al.* (2011a: 188) is grouped within the clade mainly composed of species found in northeastern Guangxi [i.e., *P. bipinnatifida* (Wang 1981a: 26) Wang & Li in Wang *et al.* (2011: 60), *P. glandulosa* (Fang *et al.* 1993: 470) Wang in Wang *et al.* (2011: 61), *P. rependa* var. *guilinensis* (Wang 1992: 299) Möller & Weber in Weber *et al.* (2011: 784), and *P. multifida* Pan & Chung in Xu *et al.* (2012: 170)]. The clade to which *Ch. longzhouensis* Pan & Wu in Pan *et al.* (2010: 370), *C. ningmingensis* Liu & Wu in Wu *et al.* (2011b: 422), and *Ch. jingxiensis* belong is mainly composed of species distributed in southern Guangxi. *Chirita rongshuiensis* Liu & Huang in Huang *et al.* (2011: 54) is grouped with species that are all narrowly distributed endemics of northern Guangxi (i.e., *P. mollifolia* [Fang & Wang in Wang 1986: 6] Li & Wang in Wang *et al.* (2011: 61), *P. pseudomollifolia* Xu & Liu in Xu *et al.* (2012: 167), *P. luochengensis* [Liu & Xu in Liu *et al.* (2010: 743)] Möller & Weber in Weber *et al.* (2011: 783), and *P. renifolia* (Fang & Qin 2004: 534) Li & Wang in Wang *et al.* (2011: 62)). *Chiritopsis danxiaensis* Liao *et al.* in Shen *et al.* (2010: 729), a species found only in fissures and crevices on the Danxia landform of Mt. Dangxia of northern Guangdong Province (Shen *et al.* 2010), is most closely related to *P. tabacum*, the type species of the genus known only from a handful of localities centering in the karst regions of northern Guangdong and adjacent Hunan (Ni *et al.* 2006) and Guangxi (Wu *et al.* 2010) Provinces. This biogeographic pattern suggests that geography might have played a crucial role in generating species diversity in *Primulina*. Further studies are currently under way to investigate the speciation mechanisms of this intriguing genus.

## Formal treatment

### *Primulina danxiaensis* (W.B.Liao, S.S.Lin & R.J.Shen) W.B.Liao & K.F.Chung, comb. nov.

Basionym:—*Chiritopsis danxiaensis* W.B.Liao *et al.* in Shen *et al.* (2010: 729).

Type:—CHINA. Guangdong: Renhua County, Mt Danxiashan World Geography Park, Jinshi Stone, growing in stone fissures of local Danxia stone, in depressions surrounded by mature subtropical broad-leaf evergreen forests, 170 m, 12 May 2008, Liao 20080072 (holotype SYS!, isotype IBSC!).

### *Primulina hezhouensis* (W.H.Wu & W.B.Xu) W.B.Xu & K.F.Chung, comb. nov.

Basionym:—*Chiritopsis hezhouensis* W.H.Wu & W.B.Xu in Wu *et al.* (2011a: 132).

Type:—CHINA. Guangxi: Hezhou City. Etang Town, Lugang Village, at the entrance of karst caves, 12 July 2009, Wu & Xu 091748 (holotype IBK!, isotype PE!).

### *Primulina jingxiensis* (YanLiu, W.B.Xu & H.S.Gao) W.B.Xu & K.F.Chung, comb. nov.

Basionym:—*Chiritopsis jingxiensis* YanLiu *et al.* in Xu *et al.* (2009: 559).

Type:—CHINA. Guangxi: Jingxi County, on moist limestone rock face in karst cave, 17 August 2005, Liu *et al.* L1260 (holotype IBK!, isotype PE!).

***Primulina leprosa* (YanLiu & W.B.Xu) W.B.Xu & K.F.Chung, comb. nov.**

Basionym:—*Chirita leprosa* YanLiu & W.B.Xu in Xu *et al.* (2010: 705).

Type:—CHINA. Guangxi: cultivated in Guilin Botanical Garden, introduced from Mashan County, Guling Town, Leping Village, on moist rock face at the entrance of karst caves, 250 m, 10 October 2009, Xu & Pan 091681 (holotype IBK!).

***Primulina lijiangensis* (B.Pan & W.B.Xu) W.B.Xu & K.F.Chung, comb. nov.**

Basionym:—*Chirita lijiangensis* B.Pan & W.B.Xu in Xu *et al.* (2011a: 188).

Type:—CHINA. Guangxi: Guilin City, Yangshuo County, Xingping Town, on moist rock face, 145 m, 5 July 2009, Pan & Xu 09690 (holotype IBK!, isotype PE!).

***Primulina longzhouensis* (B.Pan & W.H.Wu) W.B.Xu & K.F.Chung, comb. nov.**

Basionym:—*Chiritopsis longzhouensis* B.Pan & W.H.Wu in Pan *et al.* (2010: 370).

Type:—CHINA. Guangxi: cultivated in Guilin Botanical Garden, introduced from Shuikou Town, Longzhou County, in the crevice of limestone hills, 4 December 2009, Pan & Wu 091751 (holotype IBK!, isotype PE!).

***Primulina ningmingensis* (YanLiu & W.H.Wu) W.B.Xu & K.F.Chung, comb. nov.**

Basionym:—*Chirita ningmingensis* YanLiu & W.H.Wu in Wu *et al.* (2011b: 422).

Type:—CHINA. Guangxi: Ningming County, Longgang National Nature Reserve, at entrance of a limestone cave, 250 m, 18 August 2002, Liu L0718 (holotype IBK!, isotype PE!).

***Primulina pseudolinearifolia* W.B.Xu & K.F.Chung, nom. nov.**

Basionym:—*Chirita luochengensis* YanLiu & W.B.Xu in Xu *et al.* (2011b: 314) [*non Wentsaiboea luochengensis* YanLiu & W.B.Xu in Liu *et al.* (2010: 743) = *Primulina luochengensis* (YanLiu & W.B.Xu) Mich.Möller & A.Weber in Weber *et al.* (2011c: 783)].

Type:—CHINA. Guangxi: Luocheng County, Huaiqun Town, 350 m, 19 April 2009, Xu & Liu 09383 (holotype IBK!, isotype PE!).

Notes:—Although no molecular data are available for the species, *Chirita luochengensis* is clearly a member of *Chirita* sect. *Gibbosaccus* and very similar (Xu *et al.* 2011b) to *Chirita linearifolia* Wang in Wang & Pan (1982: 136) that has been transferred to *Primulina* [i.e. *P. linearifolia* (Wang) Wang in Wang *et al.* (2011: 61)] on the basis of molecular analysis (Wang *et al.* 2011). Nevertheless further molecular confirmation is needed. The recent transfer of *Wentsaiboea luochengensis* Liu & Xu to *Primulina* [i.e. *P. luochengensis* (Liu & Xu) Möller & A.Weber] blocks the direct transfer of *C. luochengensis* to *Primulina*. The new name *Primulina pseudolinearifolia* is proposed to highlight its morphological similarity to *P. linearifolia*.

***Primulina rongshuiensis* (YanLiu & Y.S.Huang) W.B.Xu & K.F.Chung, comb. nov.**

Basionym:—*Chirita rongshuiensis* YanLiu & Y.S.Huang in Huang *et al.* (2011: 54).

Type:—CHINA. Guangxi: Liuzhou City, Rongshui County, on moist rock face at the entrance of karst cave, 120 m, 02 July 2009, Xu & Huang 09685 (holotype IBK!, isotype PE!).

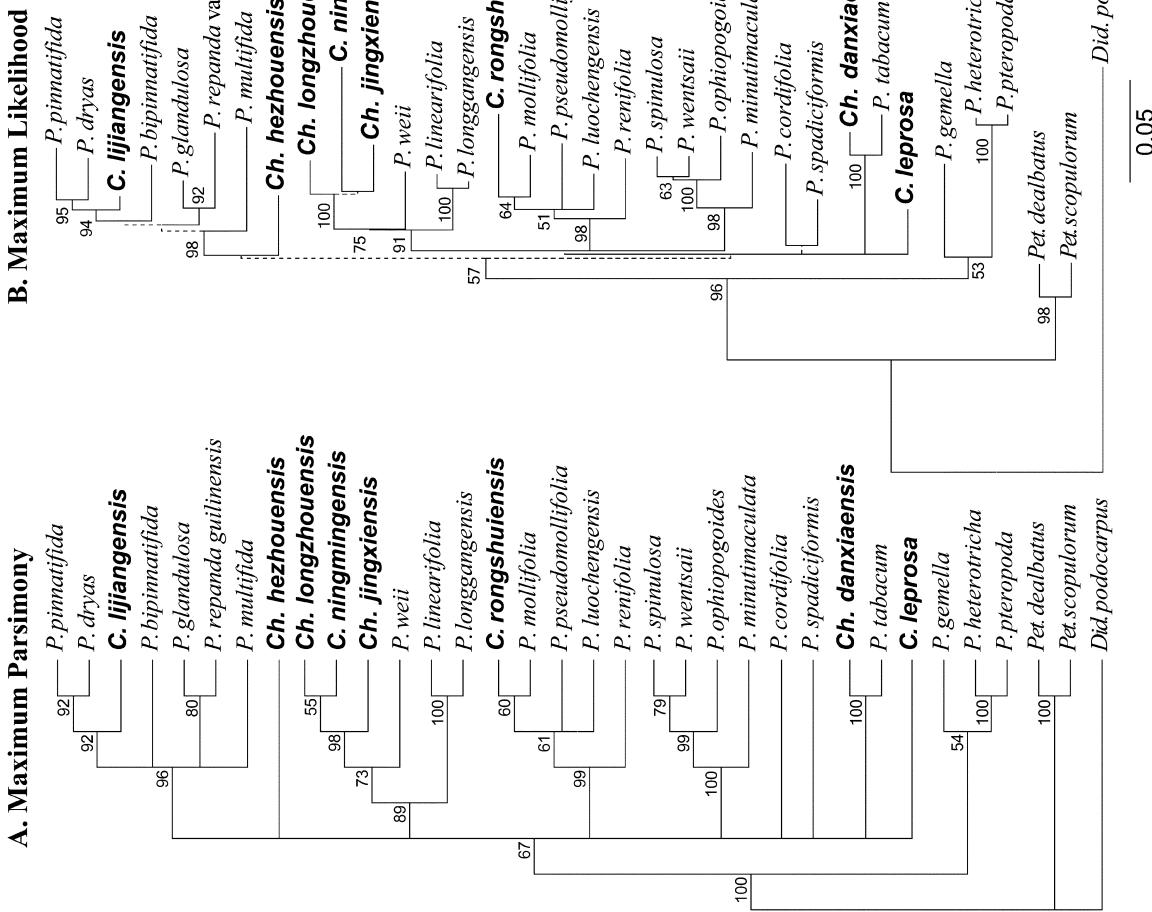
***Primulina tiandengensis* (F.Wen & H.Tang) F.Wen & K.F.Chung, comb. nov.**

Basionym:—*Chirita tiandengensis* F.Wen & H.Tang in Tang & Wen (2011: 233).

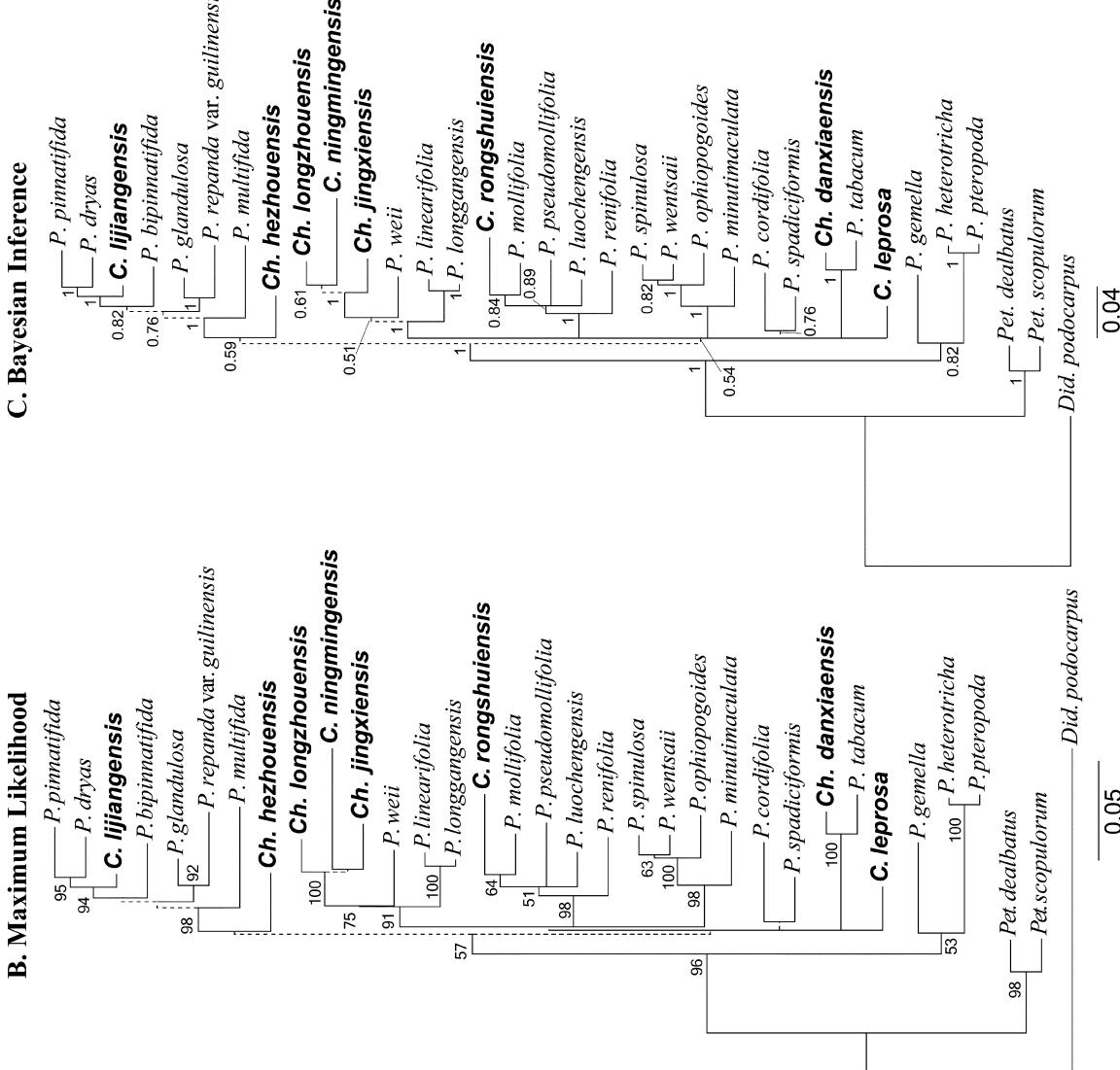
Type:—CHINA. Guangxi: Tiandeng County, Longming Town. Growing on some crevices in a piece of cliff of a limestone hill, under evergreen bushes, 458 m, 16 April 2008, Tang & Wen C08041601, C08062001 (holotype IBK!, isotypes IBK!).

Notes:—Although no molecular data are available for this species, *Chirita tiandengensis* is similar to *C. napoensis* Li in Weitzmann *et al.* (1997: 424), *C. lunglinensis* Wang (1981b: 53), and *C. obtusidentata* Wang (1981b: 57) that have all been transferred to *Primulina* (Weber *et al.* 2011c). Nevertheless further molecular confirmation is needed.

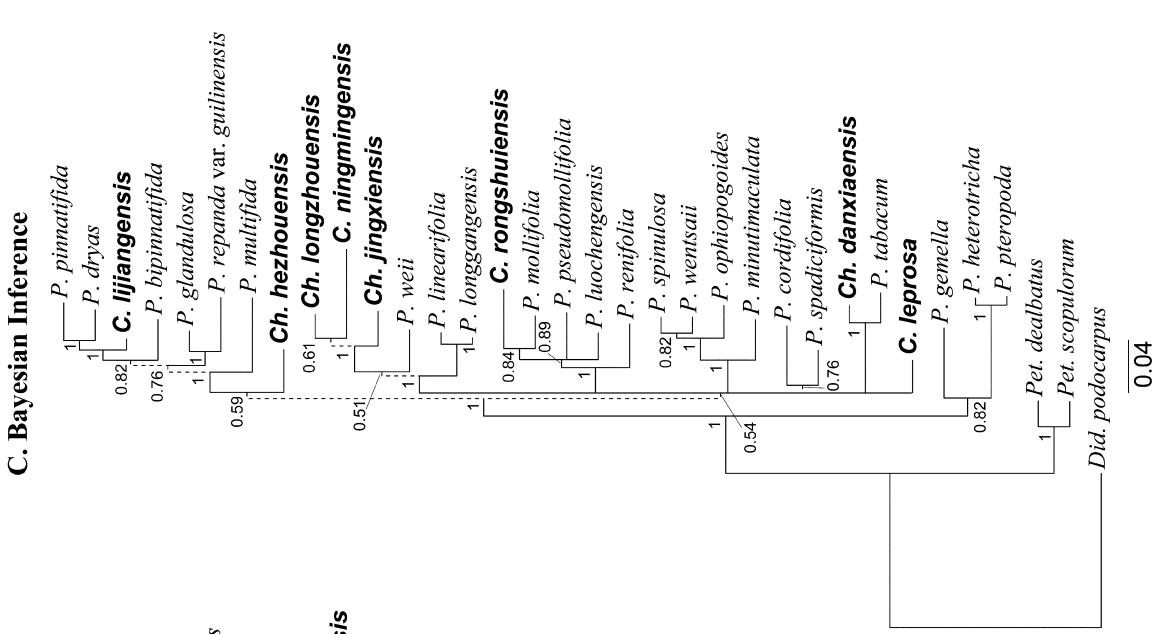
### A. Maximum Parsimony



### B. Maximum Likelihood



### C. Bayesian Inference



**FIGURE 1.** Phylogenetic trees of *Primulina*: A, the strict consensus topology of 46 equally maximum parsimony (MP) trees; B, the single maximum likelihood (ML) tree; C, Bayesian consensus cladogram (BI) with average branch lengths. Numbers adjacent to nodes are bootstrap supports (MP and ML) and BI posterior clade probabilities (>50). Dash lines in ML and BI trees denote clades collapsed in MP tree. Species in **bold italic** show the eight species transferred into *Primulina* in this study.

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**APPENDIX 1.** Species (GenBank accession number). Plants of all newly acquired sequences were collected from their type locality.

*Chirita leprosa* YanLiu & W.B.Xu (JQ713829); *Chirita lijiangensis* B.Pan & W.B.Xu (JQ713835); *Chirita ningmingensis* YanLiu & W.H.Wu (JQ713834); *Chirita rongshuiensis* YanLiu & Y.S.Huang (JQ713836); *Chiritopsis danxiaensis* W.B.Liao, S.S.Lin & R.J.Shen (JQ713830); *Chiritopsis hezhouensis* W.H.Wu & W.B.Xu (JQ713831); *Chiritopsis jingxiensis* YanLiu, W.B.Xu & H.S.Gao (JQ713832); *Chiritopsis longzhouensis* B.Pan & W.H.Wu (JQ713833); *Didymocarpus podocarpus* C.B.Clarke (DQ912688); *Petrocodon dealbatus* Hance (FJ501358); *Petrocodon scopulorum* (Chun) YinZ.Wang (= *Tengia scopulorum* Chun; GU350637); *Primulina bipinnatifida* (W.T.Wang) YinZ.Wang (= *Chiritopsis bipinnatifida* W.T.Wang; DQ872842); *Primulina cordifolia* (D.Fang & W.T.Wang) YinZ.Wang (= *Chiritopsis cordifolia* D.Fang & W.T.Wang; DQ872845); *Primulina dryas* (Dunn) Mich.Möller & A.Weber (= *Chirita sinensis* Lindl.; FJ501348); *Primulina gemella* (D.Wood) YinZ.Wang (= *Chirita gemella* D.Wood; FJ501345); *Primulina glandulosa* (D.Fang, L.Zeng & D.H.Qin) YinZ.Wang (= *Chiritopsis glandulosa* D.Fang, L.Zeng & D.H.Qin; DQ872841); *Primulina heterotricha* (Merr.) YinZ.Wang (= *Chirita heterotricha* Merr.; DQ872826); *Primulina linearifolia* (W.T.Wang) YinZ.Wang (= *Chirita linearifolia* W.T.Wang; DQ872834); *Primulina longgangensis* (W.T.Wang) YinZ.Wang (= *Chirita longgangensis* W.T.Wang; FJ501347); *Primulina luochengensis* (YanLiu & W.B.Xu) Mich.Möller & A.Weber (= *Wentsaiboea luochengensis* YanLiu & W.B.Xu; HQ633046); *Primulina minutimaculata* (D.Fang & W.T.Wang) YinZ.Wang (= *Chirita minutimaculata* D.Fang & W.T.Wang; DQ872828); *Primulina mollifolia* (D.Fang & W.T.Wang) J.M.Li & YinZ.Wang (= *Chiritopsis mollifolia* D.Fang & W.T.Wang; JQ713839); *Primulina multifida* B.Pan & K.F.Chung (JQ713838); *Primulina ophiopogoides* (D.Fang & W.T.Wang) YinZ.Wang (= *Chirita ophiopogoides* D.Fang & W.T.Wang; DQ872829); *Primulina pinnatifida* (Hand.-Mazz.) YinZ.Wang (= *Chirita pinnatifida* (Hand.-Mazz.) B.L.Burtt; FJ501350); *Primulina pseudomollifolia* W.B.Xu & YanLiu (JQ713837); *Primulina pteropoda* (W.T.Wang) YanLiu (= *Chirita pteropoda* W.T.Wang; DQ872827); *Primulina renifolia* (D.Fang & D.H.Qin) J.M.Li & YinZ.Wang (JQ713840); *Primulina repanda* var. *guilinensis* (W.T.Wang) Mich.Möller & A.Weber (= *Chiritopsis repanda* var. *guilinensis* W.T.Wang; DQ872846); *Primulina spadiciformis* (W.T.Wang) Mich.Möller & A.Weber (= *Chirita spadiciformis* W.T.Wang; FJ501346); *Primulina spinulosa* (D.Fang & W.T.Wang) YinZ.Wang (= *Chirita spinulosa* D.Fang & W.T.Wang; DQ872830); *Primulina tabacum* Hance (FJ501352); *Primulina weii* Mich.Möller & A.Weber (= *Chirita mollifolia* D.Fang, Y.G.Wei & J.Murata; DQ872832); *Primulina wentsaii* (D.Fang & L.Zeng) YinZ.Wang (= *Chirita wentsaii* D.Fang & L.Zeng; DQ872831).