单种属弥勒苣苔属系统位置研究：基于分子和细胞学数据 *

谭　英[1,2]，王　智[1,3]，隋学艺[1,4]，胡光万[1]，

MOTLEY Timothy[5,6]，龙春林[1,6]**

（1 中国科学院昆明植物研究所，云南 昆明　650201；2 中国科学院研究生院，北京　100049；3 西南林业大学林学院，

云南 昆明　650224；4 云南农业大学农学院，云南 昆明　650201；5 奥多明尼奥大学生物学部，

美国 维吉尼亚州 诺福克　23529-0266；6 中央民族大学生命环境科学学院，北京　100081）

摘要：弥勒苣苔属是苦苣苔科的单种属，仅分布于中国西南部。为探讨弥勒苣苔在苦苣苔亚科中的系统位置，我们选择了苦苣苔亚科 116 个类群，外类群为苦苣苔亚科以外的 7 个物种。用最大简约法（MP）和贝叶斯分析（BI），对以上类群的核基因 ITS 以及两个叶绿体基因 *trnL-F*、*atpB-rbcL* 数据进行了独立和联合分析。在三个片段联合分析的结果中，弥勒苣苔与马铃苣苔属、后蕊苣苔属、金盏苣苔属、直瓣苣苔属以及川鄂粗筒苣苔构成一个强烈支持的分枝。MP 树中，此分枝为并系，而在 BI 分析中，弥勒苣苔与川鄂粗筒苣苔、直瓣苣苔属互为姐妹类群。同时，第一次报道了弥勒苣苔的染色体数目（$2n=34$）。根据前人报道，马铃苣苔属、后蕊苣苔属、粗筒苣苔属和直瓣苣苔属的染色体数目同为 $2n=34$，这进一步支持我们的分子系统发育分析。

关键词：*atpB-rbcL*；苦苣苔科；ITS；弥勒苣苔属；系统学；*trnL-F*

中图分类号：Q 942，Q 949　　　　　**文献标识码**：A　　　　　**文章编号**：2095-0845（2011）05-465-12

The Systematic Placement of the Monotypic Genus *Paraisometrum* （Gesneriaceae）Based on Molecular and Cytological Data *

TAN Ying[1,2]，WANG Zhi[1,3]，SUI Xue-Yi[1,4]，HU Guang-Wan[1]，

MOTLEY Timothy[5,6]，LONG Chun-Lin[1,6]**

（1 *Kunming Institute of Botany*，*Chinese Academy of Sciences*，Kunming 650201，China；2 *Graduate University of Chinese*

Academy of Sciences，Beijing 100049，China；3 *School of Forestry*，*Southwest Forestry University*，Kunming

650224，China；4 *College of Agronomy*，*Yunnan Agricultural University*，Kunming 650201，China；

5 *Department of Biological Sciences*，*Old Dominion University*，Norfolk，Virginia 23529-0266，USA；

6 *College of Life and Environmental Sciences*，*Minzu University of China*，Beijing 100081，China）

Abstract：*Paraisometrum*，is a monotypic genus endemic to Southwest China，with an uncertain systematic position. To resolve the phylogenetic relationships of *Paraisometrum* within subfamily Cyrtandroideae，we performed phylogenetic analyses of 116 taxa placed in the subfamily Cyrtandroideae and 7 outgroup species selected from other Gesneriaceae subfamilies. The independent and combined analyses were performed on three DNA datasets：the nuclear internal transcribed spacer regions（ITS）and two chloroplast regions（*trnL-F*，*atpB-rbcL*）. Maximum Parsimony（MP）and Bayesian Inference（BI）were used for tree construction. The combined three gene dataset strongly sup-

---

port *Paraisometrum* in a clade containing *Oreocharis*, *Opithandra*, *Isometrum*, *Ancylostemon*, and *Briggsia ros-thomii*. In the MP tree this lineage is an unresolved polytomy, but BI resolves *Paraisometrum* as sister to *B. rosthomii* and the *Ancylostemon* clade. We did the first chromosome counts of *Paraisometrum* (2n = 34). The diploid number 34 has also been reported for *Oreocharis*, *Opithandra*, *Briggsia* and *Ancylostemon*, which lends further support to the placement of *Paraisometrum* in this lineage.

Key words: *atpB-rbcL*; Gesneriaceae; ITS; *Paraisometrum*; Phylogeny; *trnL-F*

Paraisometrum W. T. Wang is a monotypic genus, containing *P. mileense* W. T. Wang, which is endemic to Southwest China (Weitzman *et al.*, 1997) in the Yunnan Province (Shui, 2007a, b) and Guangxi Region (Xu *et al.*, 2009). The holotype of this plant was collected by French missionary Ducloux F. in Mi-le County, Yunnan Province in 1906 and remained unnamed and stored in Paris for nearly 100 years. In 1997, when Weitzman *et al.* wrote an article on Gesneriaceae in the corpus for Flora of China, Prof. W. T. Wang checked Ducloux's specimens in Paris collected from Mi-le County and concluded that it represented a new genus in Gesneriaceae and named it *Paraisometrum mileense* (Weitzman *et al.*, 1997). It was believed extinct until 2006. In 2006, the species was rediscovered in the Shilin County, Yunnan Province by Dr. Yuming Shui, a botanist from the Kunming Institute of Botany, Chinese Academy of Sciences (Shui, 2007a, b). According to the IUCN Species Programme, *Paraisometrum mileense* can be considered an endangered species because of its narrow geographic range and small population size.

The Chinese Gesneriaceae, based on morphological characteristics, have been divided into six tribes: Ramondeae, Didymocarpeae, Trichosporeae, Cyrtandreae, Epithemateae and Titanotricheae (Wang *et al.*, 1992). Wang *et al.* (1998) placed *P. mileense* in the Didymocarpeae, and stated that it is most similar to the genus *Isometrum* Craib because they both have four fertile stamens and a funnelform tube, longer than the lobes. More recently, molecular analyses of subfamily Didymocarpoideae have been conducted by Mayer *et al.* (2003) and Möller *et al.* (2009) based on chloroplast DNA (cpDNA)

or combined ITS and cpDNA sequences, respectively. Neither study included samples of *P. mileense*, which left its phylogenetic position unresolved. The rediscovery of a population of *P. mileense* have made it possible determine the phylogenetic position of this species to test the morphological hypothsis of Wang *et al.* (1998).

To investigate the systematic position of *Paraisometrum*, we used DNA sequences for the nuclear internal transcribed spacer regions (ITS), cpDNA *trnL-F* intron-spacer region (*trnL-F*) and *atpB-rbcL* spacer (*atpB-rbcL*). In addition, we report the chromosome numbers of *Paraisometrum* for the first time and discuss characters among closely related genera.

1 Materials and methods

1.1 Taxon sampling

To produce a reliable phylogeny of the subfamily Cyrtandroideae the ingroup comprised 116 taxa, representing 115 species in 46 genera and included 4 members of Gesnerioid and 3 members of Coronantheroid as outgroups (Appendix). These outgroup taxa were chosen based on results of recent studies of Gesneriaceae (Zimmer *et al.*, 2002; Perret *et al.*, 2003; Möller *et al.*, 2009). Sequences used in this study were from GenBank and supplemented with 17 new sequences (5 individuals of *trnL-F*, 6 *atpB-rbcL*, 6 ITS). For the ingroup we followed the taxonomic classification of Weber (2004). 13 species (5 genera) belonged to Epithematoid Gesneriaceae and 103 species (42 genera) came from Didymocarpoid Gesneriaceae (Appendix).

1.2 Molecular methods

Materials for DNA extraction were from various

forms and diverse sources, including fresh leaves from research collections, silica-dried leaves from our field collections. Genomic DNA was isolated with a modified CTAB protocol (Doyle and Doyle, 1987).

Amplifications of ITS were carried out following the PCR profile described by Möller and Cronk (1997), using forward primer 'ITS 5P' and reverse primer 'ITS 8P'. The *trnL-F* intron-spacer region (*trnL-F*) and *atpB-rbcL* spacer (*atpB-rbcL*) were amplified following Mayer *et al*. (2003) using primers 'c' and 'f' for *trnL-F* (Taberlet *et al*., 1991) and primers 'JF31' and 'JF5' for *atpB-rbcL* (Samuel *et al*., 1997). PCR was performed using a DNAEngine® peltier thermal cycler (Bio-RAD, Inc.). The PCR products were purified using a multifunction DNA purification KIT (BioTeke Inc.). The purified PCR products were cycle-sequenced using an ABI Prism BigDye Terminator Cycle Kit following standard kit protocols (Perkin-Elmer Applied Biosystems) and run on a ABI-PRISM3730 sequencher. Forward and reverse sequences were assembled using SeqMan of DNAStar (DNASTAR, 1999), visually aligned with ClustalX v. 1.83 (Thompson *et al*., 1998) followed by manual correction in BioEdit (Hall, 1999). Sequence alignments and PAUP/Nexus-formatted files for individual regions and combined analyses are available from the authors upon request.

1.3 Phylogenetic analysis

Phylogenetic analyses were performed using Maximum Parsimony (MP) and Bayesian Inference (BI) using the protocols of Rannala and Yang (1996) on the individual ITS, *atpB-rbcL*, *trnL-F*, combined cpDNA and combined three gene datasets respectively. Maximum Parsimony analysis was performed with PAUP* version 4.0b10 (Swofford, 2002). Gaps were treated as missing data, and characters were assumed to be unordered. Heuristic searches were performed employing 1 000 random replicates, holding one tree at each step during stepwise addition, using tree-bisection-reconnection (TBR) branch-swapping algo-

rithm, MulTrees in effect, and steepest descent off. Support for the nodes resolved in the strict consensus of the MP trees was calculated by bootstrap analyses (Felsenstein, 1985) with 1 000 heuristic-search replicates as described above. Bayesian analysis was performed using MrBayes v. 3.1.2 (Huelsenbeck and Ronquist, 2003) for all the datasets. The most appropriate molecular model for each dataset was determined with jModelTest 0.1.1 (Guindon and Gascuel, 2003; Posada, 2008). The model GTR+I+G was selected. The Markov chain Monte Carlo (MC-MC) algorithm was run for 10 000 000 generations with one cold and three heated chains in two independent parallel analyses. Trees were sampled every 100 generations from the chain. The first 20% trees were discarded as burn-in (generations prior to stationary of likelihood values).

1.4 Cytological protocols

Root tips of *Paraisometrum mileense* were pretreated in 0.002 mol·L^{-1} 8-hydroxyquinoline for 4 to 5 h at room temperature, and then fixed in Carnoy's I (3 ethanol : 1 glacial acetic acid) for 30 min at 5℃. After hydrolysis for 1 min in 1 mol·L^{-1} HCl at 60℃, followed by washing through several changes of distilled water, the root tips were dyed in carbol fuchsin and then squashed. The chromosomes were examined using Axioskop 40 made by ZEISS and photographed using JVC TK-C1480BEC color video camera.

2 Results

2.1 Phylogenetic analysis

The main clades found in the parsimony analysies of the individual ITS, *trnL-F*, *atpB-rbcL* and combined cpDNA datasets (not shown) were broadly congruent with, although less well resolved than, the consensus trees from the combined three gene dataset. This reflects the fewer of informative characters in the separate analyses. The results of the combined three gene analyses are presented here.

Of the aligned 2990 characters (ITS：791 characters, *trnL-F*：1189 characters, *atpB-rbcL*：1010

characters）analyzed，1100 were constant，549 un-informative，and 1341 parsimony informative. Maximum parsimony analysis resulted in 4 most parsimonious trees of 7058 steps，with a CI of 0. 44，a RI of 0. 68，and a RC of 0. 30. A strict consensus tree of the combined dataset resolved 92 nodes with bootstrap support（BS）≥50%（Fig. 1）. The phylogram from the Bayesian analysis of the combined dataset resolved 113 nodes，95 of which had posterior probabilities（PP）≥0. 90（Fig. 2）. Support for deep nodes was weak，but the support for the individual major clades was strong.

The MP strict consensus tree had a similar tree topology to that of Bayesian consensus tree. Subfamily Cyrtandroideae is monophyletic and sister to subfamily Gesnerioideae（BS = 97%；PP = 100%）. The tribe Epithemateae is sister to the remaining old world Gesneriaceae with strong support（BS = 100%；PP = 100%）. Genus *Corallodiscus* form a clade（BS = 87%；PP = 100%）and is sister to the remaining taxa（BS = 87%；PP = 100%）. Basal Asiatic genera，*Rhynchotechum*，*Platystemma*，and European genera，*Jancaea*，*Ramonda*，*Haberlea*，constitute a clade（BS = 93%），which is separated from the African and remaining Asiatic genera with moderate support（BS = 87%；PP = 100%）. Among the remaining taxa，*Briggisa*，*Chirita*，and *Streptocarpus* are not monophyletic. *Paraisometrum* is in a well supported clade（BS = 100%，PP = 100%）with *Briggsia rosthornii*，*Isometrum lungshengense*，*Opithandra primuloides*，*Oreocharis auricula* and three species of *Ancylostemon*. The three species of *Ancylostemon* form a monophyletic group with strong support（BS = 100%；PP = 100%）. The BI tree provides more resolution in the *Paraisometrum* clade，*Paraisometrum* is sister to *Ancylostemon* and *Briggsia rosthornii*（PP = 99%）in a polytomy with the other three genera of the lineagewhile the MP tree does not resolve any of the relationships among the genera in the clade.

2. 2 Cytology

Paraisometrum mileense has a diploid number of $2n = 34$，and the chromosome size is small，$<2~\mu m$（Fig. 3）.

3 Discussion

3. 1 Phylogenetic relationships

In our study，Old World Gesneriaceae can be clearly divided into two tribal groups that are congruent with previous studies（Smith *et al.*，1997；Wang and Li，2002；Mayer *et al.*，2003；Möller *et al.*，2009；Wang *et al.*，2010）. Weber（2004）separated subfamily Cyrtandroideae into the small tribe Epithemateae（Epithematoid Gesneriaceae）and the large tribe Didymocarpeae in which the members of Cyrtandreae and Trichosporeae were polyphyletically nested at various places（Didymocarpoid Gesneriaceae）. On the basis of morphological and geographical features，the Didymocarpoid Gesneriaceae can be divided into following groups：Basal Asiatic and European genera，African genera，Advanced Asiatic genera（including genera with twisted capsular fruits，straight capsular fruits，or indehiscent fruits）. The Asiatic and European，and African groups are well defined and supported according to the phylogenetic result made by Möller *et al.*（2009）. In their study，the genus *Briggsia* is also not monophyletic，and *Oreocharis*，one clade of *Briggsia* and *Ancylostemon* compose a clade. Our results show that *Oreocharis*，*Opithandra*，*Isometrum*，*Ancylostemon*，*Briggsia rosthornii* and *Paraisometrum* constitute a clade in the Advanced Asiatic group，and suggest *Paraisometrum* may be mostly related to *Ancylostemon* and *Briggesia rosthornii* than other genera.

3. 2 Morphology

The 5 genera in the clade with *Paraisometrum* have some shared floral characters，supporting their relationship. These genera have campanulate calyx，ad basin 5-sectus，bilabiate corollas，corolla lobes much shorter than the corolla tube，and 4 fertile stamens，stigma number varies，*Isometrum*，*Briggsia*，and *Ancylostemon* have 2 stigmas，whereas *Opithandra* and *Orecharis* can have either 1 or 2 stigmas，and *Paraisometrum* always has a single stigma（Wang *et al.*，1998）.
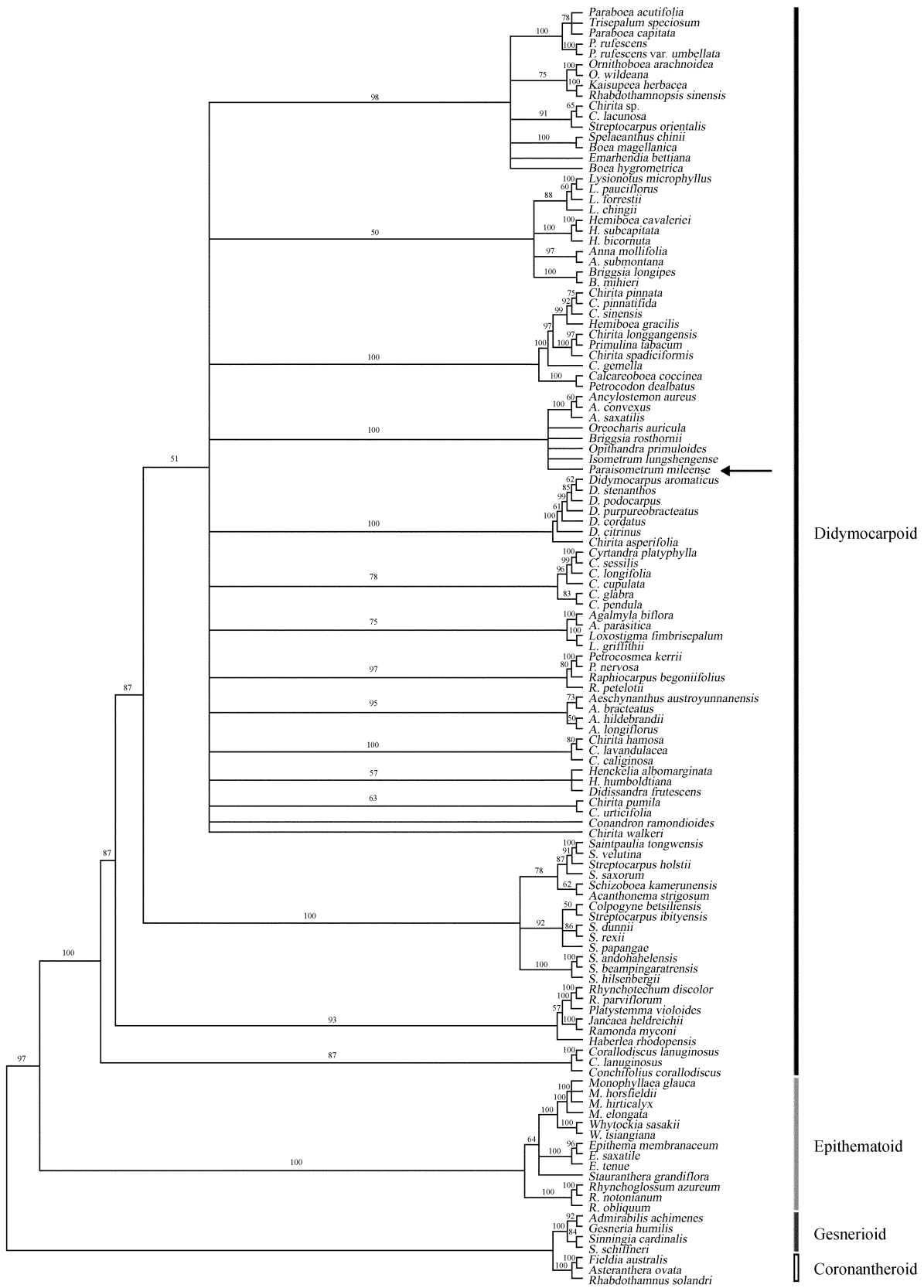
Fig. 1   Strict consensus tree of 4 most parsimonious trees based on the combined ITS, *trnL-F* and *atpB-rbcL* data.

Numbers above branches are bootstrap support values ≥50%. The tribes are labeled with bars

1.00 *Lysionotus microphyllus*
0.98 *L. pauciflorus*
1.00 *L. forrestii*
L. chingii
1.00 *Hemiboea cavaleriei*
0.98 1.00 *H. subcapitata*
H. bicornula
0.92 1.00 *Anna mollifolia*
A. submontana
1.00 *Briggsia longipes*
1.00 *B. mihien*
0.96 1.00 *Petrocosmea kerrii*
1.00 *P. nervosa*
Raphiocarpus begoniifolius
R. petelotii
1.00 *Cyrtandra platyphylla*
1.00 *C. sessilis*
C. longifolia
C. cupulata
1.00 *C. glabra*
1.00 *C. pendula*
1.00 *Chirita pinnata*
1.00 *C. pinnatifida*
C. sinensis
1.00 *Hemiboea gracilis*
1.00 *Chirita longgangensis*
1.00 *Primulina tabacum*
1.00 *Chirita spadiciformis*
C. gemella
1.00 *Calcareoboea coccinea*
Petrocodon dealbatus
0.98 *Ancylostemon aureus*
1.00 *A. convexus*
A. saxatilis
0.99 *Paraisometrum mileense* ←
Briggsia rosthornii
Oreocharis auricula
Isometrum lungshengense
Opithandra primuloides
1.00 *Agalmyla biflora*
0.97 *A. parasitica*
1.00 *Loxostigma fimbrisepalum*
L. griffithii
Henckelia humboldtiana
1.00 *Chirita pumila*
1.00 *C. urticifolia*
C. walkeri
Conandron ramondioides
0.99 0.97 *Didymocarpus aromaticus*
1.00 *D. stenanthos*
D. podocarpus
1.00 *D. purpureobracteatus*
D. cordatus
1.00 *D. citrinus*
Chirita asperifolia
Aeschynanthus austroyunnanensis
A. bracteatus
1.00 *A. longiflorus*
A. hildebrandii
Paraboea capitata
0.95 *Trisepalum speciosum*
1.00 *Paraboea acutifolia*
1.00 *P. rufescens*
P. rufescens var. *umbellata*
Ornithoboea arachnoidea
1.00 *O. wildeana*
Kaisupeea herbacea
Rhabdothamnopsis sinensis
1.00 *Chirita* sp.
1.00 *C. lacunosa*
Streptocarpus orientalis
1.00 *Spelaeanthus chinii*
Boea magellanica
Emarhendia bettiana
Boea hygrometrica
1.00 *Saintpaulia tongwensis*
0.95 *S. velutina*
1.00 *Streptocarpus holstii*
S. saxorum
Schizoboea kamerunensis
0.98 *Acanthonema strigosum*
Streptocarpus andohahelensis
1.00 *S. beampingaratrensis*
S. hilsenbergii
0.96 *Colpogyne betsiliensis*
0.90 *Streptocarpus ibityensis*
1.00 *S. dunnii*
S. rexii
S. papangae
1.00 *Chirita hamosa*
0.99 *C. lavandulacea*
C. caliginosa
0.99 *Henckelia albomarginata*
Didissandra frutescens
1.00 *Rhynchotechum discolor*
1.00 *R. parviflorum*
Platystemma violoides
1.00 *Jancaea heldreichii*
Ramonda myconi
Haberlea rhodopensis
1.00 *Corallodiscus lanuginosus*
1.00 *C. lanuginosus*
C. conchifolius

Didymocarpoid

Monophyllaea glauca
1.00 *M. hirticalyx*
M. horsfieldii
1.00 *M. elongata*
Whytockia sasakii
W. tsiangiana
1.00 *Stauranthera grandiflora*
1.00 *Epithema membranaceum*
E. saxatile
E. tenue

Epithematoid

1.00 *Azureum*
Rhynchoglossum notonianum
R. obliquum

0.99 *Sinningia cardinalis*
1.00 *S. schiffneri*
1.00 *Achimenes admirabilis*
Gesneria humilis

Gesnerioid

1.00 *Fieldia australis*
Asteranthera ovata
Rhabdothamnus solandri
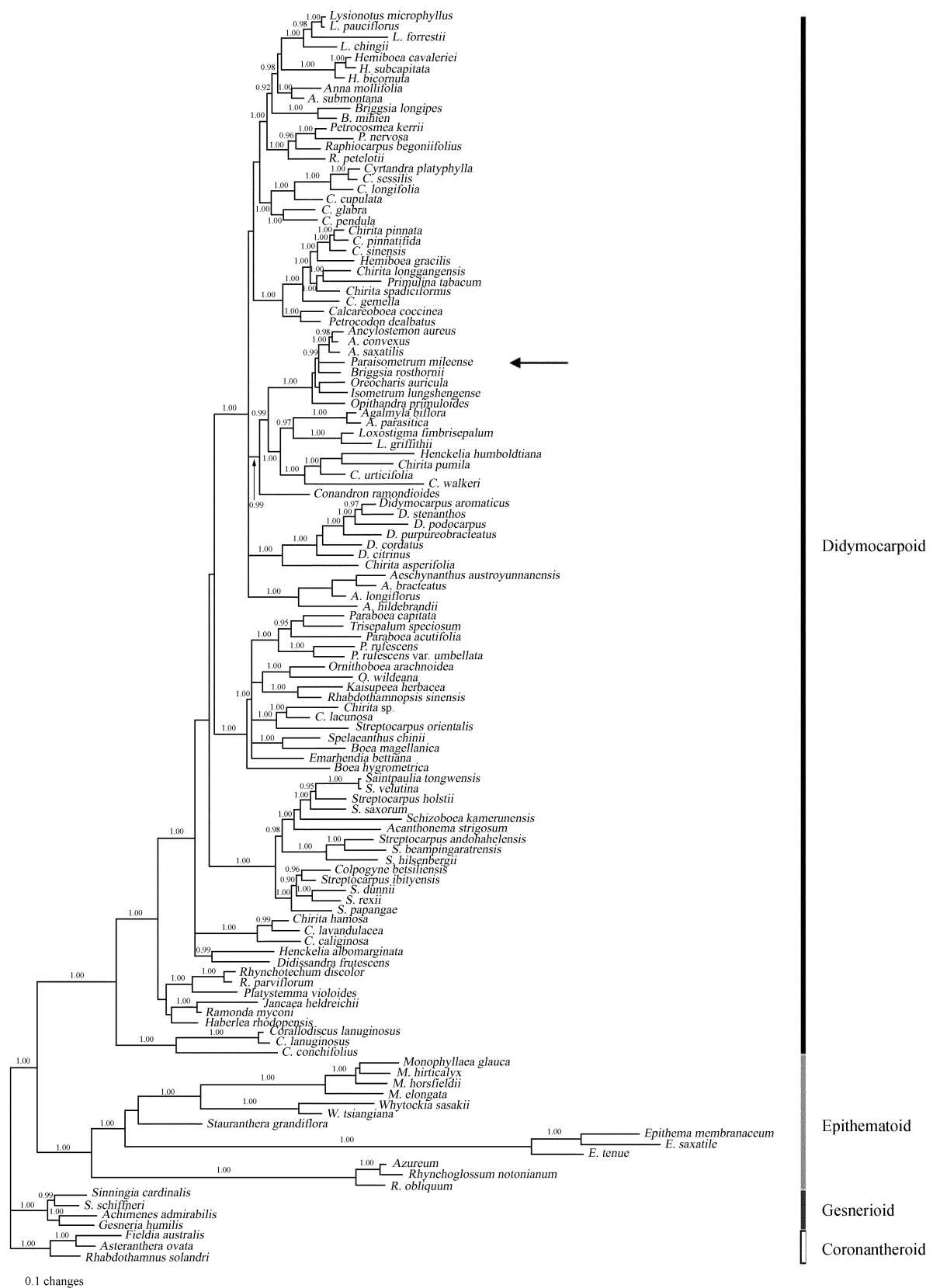
Coronantheroid

0.1 changes

Fig. 2 Bayesian phylogram based on the combined ITS, *trnL-F* and *atpB-rbcL* data. Numbers above branches are posterior probability values (only values ≥0.90 are shown). The tribes are labeled with bars
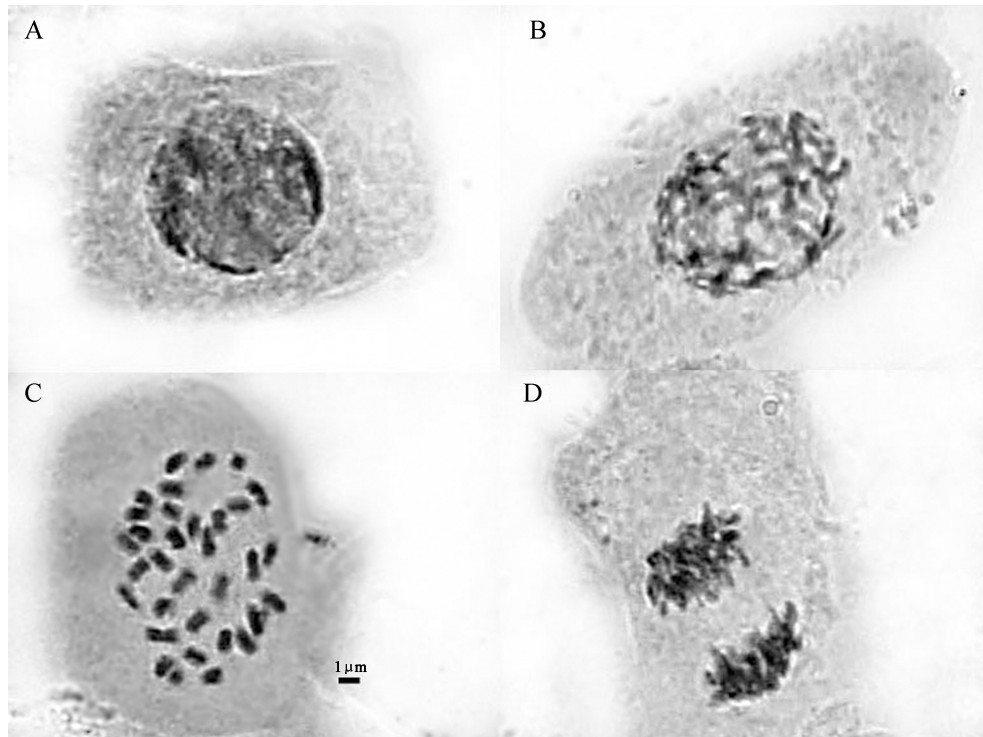
Fig. 3   Karyomorphology of *Paraisometrum mileense*. A. Interphase nucleus（×1000）; B. Mitotic prophase（×1000）;
C. Mitotic metaphase（×1000）; D. Mitotic anaphase（×1000）. The bar is 1 μm long

Burtt（1962）and Pan（1986）held the opinion that based on their observations that *Isometrum* Craib and *Ancylostemon* Craib were closely related, which our results support. Wang *et al.*（1998）hypothesized that *Paraisometrum* was sister to *Isometrum*. Our analysis confirmed that these two genera are closely related in the same lineage, but *Paraisometrum* likely has closer affinities to *Ancylostemon* and *Briggsia rosthornii*. Different from other 3 genera, their abaxial lip is longer than adaxial lip.

3.3   Distribution

Briggsia rosthornii is distributed in Sichuan, Guizhou, and Hubei provinces of Southwest and South-Central China, *Opithandra* is scattered throughout Western China, except for *O. primuloides*, a species restricted to Japan. The distributions of *Oreocharis* and *Ancylostemon* range from Southwestern to Central and Southeastern China（Li, 1996; Wang *et al.*, 1998; Li and Wang, 2004）. *Isometrum* is endemic to China, and centered in Sichuan, where 82% of the species in the genus occur（Pan, 1986）.

3.4   Cytology

Chromosome ploidy levels and base numbers can provide insight into plant evolution, because sister groups often share chromosome numbers or base number（Turner *et al.*, 1961; Ehrendorfer *et al.*, 1968; Walker, 1972; Raven, 1975; Grant, 1982）. However, chromosome counts at the species-level across the family have been made for only about 18% of the taxa in the Gesneriaceae, and the number of genera without chromosome counts is even greater in the subfamily Cyrtandroideae than subfamily Gesnerioideae（Möller and Kiehn, 2004）. In the Epithemateae, the chromosome counts have high variation, with the basic numbers in this group ranging from $n = 8 - 12$,（Ratter, 1975; Skog, 1984; Wang *et al.*, 1998）. It is more complicated in the Didymocarpoid Gesneriaceae, where basic numbers are $n = 4, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18$（Ratter and Prentice, 1967; Ratter, 1975; Kiehn and Weber, 1998; Rashid *et al.*, 2001; Möller and Kiehn, 2004）. In previous studies, the genera

Oreocharis, *Opithandra*, and *Ancylostemon* have the same chromosome number $2n = 34$, *Briggsia* has the chromosome number $2n = 34$ or $2n = 68$ (Ratter, 1963; Ratter and Prentice, 1964; Wang and Gu, 1999; Zhou *et al*., 2004). *Paraisometrum* also has a chromosome complement of $2n = 34$, which is another character uniting members of this clade. The chromosome is too small to do karyotype analysis, or we can do further comparison of these species. Although the data are not yet available, we predict that *Isometrum* to have a karyotype of $2n = 34$.

　　Our molecular analyses place the rare monotypic genus, *Paraisometrum*, among the Didymocarpoid genera, subfamily Cyrtandroideae of Gesneriaceae. *Paraisometrum* belongs in a strongly supported clade with *Oreocharis*, *Opithandra*, *Isometrum*, *Ancylostemon*, and *Briggsia rosthornii* among and nested in an informal group the Advanced Asiatic group. Bayesian analyses support *Paraisometrum*, as sister to *Ancylostemon* and *Briggsia rosthornii*. Because *Briggsia rosthornii* is a single species (and not the type) in a polyphyletic genus further study and taxonomic revision will be required on this clade. So at this time the decision on whether *Briggsia rosthornii* should be considered another monotypic genus or whether it and/or *Paraisometrum mileense* should be combined into an existing genus remain unanswered.

Reference：

Burtt BL, 1962. Studies on the Gesneriaceae of the old world XXIV：tentative keys to the tribes and genera [J]. *Notes from the Royal Botanic Garden Edinburgh*, **24**：205—220

DNASTAR I, 1999. SeqMan II, v. 4.00. *Madison*, *Wisconsin*, *USA*

Doyle JJ, Doyle JL, 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue [J]. *Phytochemmical Bulletin*, **19**：11—15

Ehrendorfer F, Krendl F, Habeler E *et al*., 1968. Chromosome numbers and evolution in primitive angiosperms [J]. *Taxon*, **17**：337—353

Felsenstein J, 1985. Confidence limits on phylogenies：an approach using the bootstrap [J]. *Evolution*, **39**：783—791

Grant V, 1982. Chromosome number patterns in primitive angiosperms [J]. *Botanic Gazette*, **143**：390—394

Guindon S, Gascuel O, 2003. A simple, fast, and accurate method to estimate large phylogenies by Maximum Likelihood [J]. *Systematic Biology*, **52**：696—704

Hall TA, 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT [J]. *Nucleic Acids Symposium Series*, **41**：95—98

Huelsenbeck J, Ronquist F, 2003. MrBayes 3：Bayesian phylogenetic inference under mixed models [J]. *Bioinformatics*, **19**：1572—1574

Kiehn M, Weber A, 1998. Chromosome numbers of Malayan and other paleotropical Gesneriaceae：2. Tribes Trichosporeae, Cyrtandreae and Epithemateae [J]. *Beiträge zur Biologie der Pflanzen*, **70**：445—470

Li ZY, 1996. The geographical distribution of the subfamily Cyrtandroideae Endl. emend. Burtt (Gesneriaceae) [J]. *Acta Phytotaxonomic Sinica*, **34**：341—360

Li ZY, Wang WT, 2004. *Plants of Gesneriaceae in China* [M]. Henan Science and Technology Publication

Möller M, Cronk Q, 1997. Origin and relationships of *Saintpaulia* (Gesneriaceae) based on ribosomal DNA internal transcribed spacer (ITS) sequences [J]. *American Journal of Botany*, **84**：956—965

Möller M, Kiehn M, 2004. A synopsis of cytological studies in Gesneriaceae [J]. *Edinburgh Journal of Botany*, **60**：425—447

Möller M, Pfosser M, Jang CG *et al*., 2009. A preliminary phylogeny of the 'didymocarpoid Gesneriaceae' based on three molecular data sets：Incongruence with available tribal classifications [J]. *American Journal of Botany*, **96**：989—1010

Mayer V, Möller M, Perret M *et al*., 2003. Phylogenetic position and generic differentiation of *Epithemateae* (Gesneriaceae) inferred from plastid DNA sequence data [J]. *American Journal of Botany*, **90**：321—329

Pan KY, 1986. The second revision of the genus *Isometrum* (Gesneriaceae) [J]. *Acta Botanica Yunnanica*, **01**：23—36

Perret M, Chautems A, Spichiger R *et al*., 2003. Systematics and evolution of tribe Sinningieae (Gesneriaceae)：evidence from phylogenetic analyses of six plastid DNA regions and nuclear ncpGS [J]. *American Journal of Botany*, **90**：445—460

Posada D, 2008. jModelTest：phylogenetic model averaging [J]. *Molecular Biology and Evolution*, **25**：1253

Rannala B, Yang Z, 1996. Probability distribution of molecular evolutionary trees：a new method of phylogenetic inference [J]. *Journal of Molecular Evolution*, **43**：304—311

Rashid MH, Jong K, Mendum M, 2001. Cytotaxonomic observations in the genus *Aeschynanthus* (Gesneriaceae) [J]. *Edinburgh*

Journal of Botany, **58**： 31—43

Ratter JA, 1963. Some chromosome numbers in the Gesneriaceae ［J］. *Notes from Royal Botanical Garden of Edinburgh*, **24**： 221—229

Ratter JA, Prentice H, 1964. Chromosome numbers in the Gesneriaceae： II ［J］. *Notes from Royal Botanical Garden Edinburgh*, **25**： 303—307

Ratter JA, 1975. A survey of chromosome numbers in the Gesneriaceae of the Old World ［J］. *Notes from Royal Botanical Garden of Edinburgh*, **33**： 527—543

Ratter JA, Prentice HT, 1967. Chromosome numbers in the Gesneriaceae： III ［J］. *Notes from Royal Botanical Garden of Edinburgh*, **27**： 205—209

Raven P, 1975. The bases of angiosperm phylogeny： cytology ［J］. *Annals of Missouri Botanical Garden*, 724—764

Samuel R, Pinsker W, Kiehn M, 1997. Phylogeny of some species of *Cyrtandra* （Gesneriaceae） inferred from the atpB/rbcL cpDNA intergene region ［J］. *Botanica Acta*, **110**： 503—510

Shui YM, 2007a. *Paraisometrum mileense* re-emerges in Yunnan ［J］. *Science* （*Chinese*）, **1**： 37—38

Shui YM, 2007b. "100-years-lost" plant re-emerges in Yunnan, China ［J］. *Samara*, **12**： 3

Skog LE, 1984. A review of chromosome numbers in the Gesneriaceae ［J］. *Selbyana* （*USA*）, **7**： 252—273

Smith J, Wolfram J, Brown K *et al*., 1997. Tribal relationships in the Gesneriaceae： evidence from DNA sequences of the chloroplast gene ndhF ［J］. *Annals of Missouri Botanical Garden*, 50—66

Swofford D, 2002. PAUP： phylogenetic analysis using parsimony, version 4.0b10 ［J］. *Sunderland, MA： Sinauer Associates*

Taberlet P, Gielly L, Pautou G *et al*., 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA ［J］. *Plant Molecular Biology*, **17**： 1105—1109

Thompson JD, Higgins DG, Gibson TJ, 1998. ClustalX mutiple sequence alignment program （version 1.83） ［J］. *Bethesda, Maryland： National Center for Biotechnology Information*

Turner B, Ellison W, King R, 1961. Chromosome numbers in the Compositae. IV. North American species, with phyletic interpretations ［J］. *American Journal of Botany*, 216—223

Walker J, 1972. Chromosome numbers, phylogeny, phytogeography of the Annonaceae and their bearing on the （original） basic chromosome number of angiosperms ［J］. *Taxon*, **21**： 57—65

Wang WT, Pan KY, Li ZY, 1992. Keys to the Gesneriaceae of China ［J］. *Edinburgh Journal of Botany*, **49**： 5—74

Wang WT, Pan KY, Li ZY *et al*., 1998. Gesneriaceae ［A］. In： Wu ZY, Raven PH ed, *Flora of China* ［M］. Beijing： Science Press, 273—333

Wang YZ, Gu ZJ, 1999. Karyomorphology of four species in *Ancylostemon*, *Briggsiopsis* and *Lysionotus* （Gesneriaceae） ［J］. *Acta Phytotaxonomic Sinica*, **37**： 137—142

Wang YZ, Gu ZJ, Hong DY, 1998. Karyotypes of *Whytockia* （Gesneriaceae） ［J］. *Acta Phytotaxonomic Sinica*, **36**： 28—35

Wang YZ, Li ZY, 2002. Inflorescence development of *Whytockia* （Epithemateae, Gesneriaceae） and phylogenetic implications within Gesneriaceae ［J］. *Plant Systematic Evolution*, **236**： 45—54

Wang YZ, Liang RH, Wang BH *et al*., 2010. Origin and phylogenetic relationships of the Old World Gesneriaceae with actinomorphic flowers inferred from ITS and *trnL-trnF* sequences ［J］. *Taxon*, **59**： 1044—1052

Weber A, 2004. Gesneriaceae ［A］. In： Kubitzki K ed, *The families and genera of vascular plants* ［M］. Springer, Berlin & Heidelberg, Germany, 63—158

Weitzman AL, Skog LE, Wang WT *et al*., 1997. New taxa, new combinations, and notes on Chinese Gesneriaceae ［J］. *Novon*, **7**： 423—435

Xu WB, Pan B, Huang YS *et al*., 2009. *Paraisometrum* W. T. Wang, a newly recorded genus of Gesneriaceae from Guangxi, China ［J］. *Guihaia*, **29**： 581—583

Zhou P, Gu ZJ, Möller M, 2004. New chromosome counts and nuclear characteristics for some members of Gesneriaceae subfamily Cyrtandroideae from China and Vietnam ［J］. *Edinburgh Journal of Botany*, **60**： 449—466

Zimmer E, Roalson E, Skog L *et al*., 2002. Phylogenetic relationships in the Gesnerioideae （Gesneriaceae） based on nrDNA ITS and cpDNA *trnL-F* and *trnE-T* spacer region sequences ［J］. *American Journal of Botany*, **89**： 296—311

Appendix：GenBank accession numbers for the sequences used in the phylogenetic analysis

Taxon	Locality	Voucher	GenBank accession No.		
			atpB-rbcL	*trnL-F*	ITS1/ITS2
Outgroup taxon					
Gesnerioid					
Achimenes admirabilis Wiehler			AJ439982	AJ439827	AY182173
Sinningia cardinalis （Lehm.） H. E. Moore			AJ490931	AJ492318	—
S. schiffneri Fritsch			AJ439900	AJ439745	—
Gesneria humilis L.			AJ439976	AJ439821	AY047051
Coronantheroid					
Asteranthera ovata （Cav.） Hanst			FJ501371	FJ501427	EF445669
Fidldia australis Cunn.			AY423112	AY423130	EF445687
Rhabdothamnux solandri Cunn.			FJ501370	FJ501426	EF445700
Ingroup taxon					
Didymocarpoid					
Acanthonema strigosum Hook. f.			—	FJ501454	FJ501306
Aeschynanthus austroyunnanensis W. T. Wang			FJ501396	FJ501500	AF349218/AF349299
A. bracteatus Wall. ex DC.			—	FJ501501	AF349203/AF349284
A. hildebrandtii Hemsl.			—	AY047099	AY047040
A. longiflorus （Blume） DC.			AJ490920	AJ492307	FJ501333
Agalmyla biflora （Elmer） O. M. Hilliard & B. L. Burtt			FJ501421	FJ501541	FJ501361
A. parasitica （Lam.） Kuntze			FJ501420	FJ501539	—
Ancylostemon aureus （Franch.） B. L. Burtt			FJ501398	FJ501505	FJ501336
A. convexus Craib			—	FJ501506	FJ501337
Anna mollifolia （W. T. Wang） W. T. Wang & K. Y. Pan			—	FJ501543	AF055050/AF055051
A. submontana Pellegr.			FJ501422	FJ501542	FJ501362
Boea hygrometrica （Bunge） R. Br.			—	FJ501476	FJ501319
B. magellanica Lam.			—	FJ501478	FJ501321
Briggsia longipes （Hemsl. Ex Oliv.） Craib			FJ501423	FJ501545	AF055052/AF055053
B. mihieri Craib			—	FJ501544	FJ501363
B. rosthornii （Diels） B. L. Burtt			FJ501425	FJ501547	FJ501365
Calcareoboea coccinea C. Y. Wu ex H. W. Li			FJ501406	FJ501516	FJ501365
Chirita asperifolia （Blume） B. L. Burtt			FJ501419	FJ501538	FJ501359
C. caliginosa C. B. Clarke			FJ501391	FJ501488	FJ501325
C. gemella D. Wood			FJ501408	FJ501523	FJ501345
C. hamosa R. Br.			FJ501392	FJ501489	—
C. lacunosa （Hook f.） B. L. Burtt			FJ501384	FJ501458	FJ501308
C. lavandulacea Stapf.			FJ501390	FJ501497	FJ501324
C. longgangensis W. T. Wang			AJ490903	AJ492290	FJ501347
C. pinnata W. T. Wang			—	FJ501526	FJ501349
C. pinnatifida （Hand.-Mazz.） B. L. Burtt			—	FJ501527	FJ501350
C. pumila D. Don			FJ501393	FJ501491	FJ501327
* *Chirita* sp.	China, Yunnan Prov., Jiaozixueshan Mountain	HGWB-754 （KUN）	HQ327455	HQ327447	HQ327462
C. sinensis Lindl.			FJ501409	FJ501524	FJ501348
C. spadiciformis W. T. Wang			AJ490904	AJ492291	FJ501346
C. urticifolia Buch.-Ham. Ex D. Don			—	FJ501492	FJ501328
C. walkei Gardner			—	FJ501490	FJ501326
Colpogyne betsiliensis B. L. Burtt			—	FJ501445	FJ501302
Conandron ramondioides Sieb. & Zucc.			FJ501405	FJ501515	FJ501340
Corallodiscus conchifolia Batalin			FJ501375	FJ501433	—
C. lanuginosus （Wall. Ex R. Br） B. L. Burtt			FJ501374	FJ501432	—

Continue

Taxon	Locality	Voucher	GenBank accession No.		
			atpB-rbcL	*trnL-F*	ITS1/ITS2
C. lanuginosus （Wall. Ex R. Br.）Burtt	China，Yunnan，Nujiang	WZ2009CM03（KUN）	HQ327458	HQ327450	HQ327465
Cyrtandra cupulata Ridl			FJ50141	FJ501532	AY818826/AY818861
C. glabra Banks ex Gaertn.			AY423119	AY423136	FJ501353
C. longifolia （Wawra）Hillebr. Ex C. B. Clarke			FJ501413	FJ501531	AY818846/AY818881
C. pendula Blume			FJ501412	FJ501530	FJ501354
C. platyphylla A. Gray			FJ501410	FJ591528	—
C. sessilis H. St. JohnKapua *et al*. s. n.			FJ501411	FJ501529	—
Didissandra frutescens （Jack）C. B. Clarke			U91313	FJ501521	—
Didymocarpus aromaticus Wall. ex D. Don			FJ501402	FJ501511	—
D. citrinus Ridul.			AJ490906	AJ492293	DQ912669
D. cordatus Wall. ex DC.			—	AJ492294	DQ912673
D. podocarpus C. B. Clarke			FJ501404	FJ501514	DQ912688
D. purpureobraceatus W. W. Sm			FJ501401	FJ501510	—
D. stenanthos C. B. Clarke			FJ501403	FJ501512	DQ912687
Emarhendia bettiana （M. R. Hend）Kiew			AJ490908	AJ492295	—
Haberlea rhodopensis Friv.			AJ490909	AJ492296	—
Hemiboea bicornuta （Hayata）Ohwi			FJ501416	FJ501534	FJ501356
H. cavaleriei H. Lev.			FJ501415	FJ501533	FJ501355
* *H. gracilis* Franch	China，Hunan，Zhangjiajie	WZ2010ZJJ01（KUN）	HQ327453	HQ327445	HQ327461
H. subcapitata C. B. Clarke			FJ501417	FJ501535	FJ501357
Henckelia albomarginata （Hemsl.）A. Weber			AJ490910	AJ492297	—
H. humboldtiana （Gardner）A. Weber & B. L. Burtt			FJ501389	FJ501485	—
Isometrum lungshengense （W. T. Wang）W. T. Wang & K. Y. Pan			—	GU350690	GU350659
Jancaea heldreichii Boiss.			FJ501378	FJ501439	—
Kaisupeea herbacea （C. B. Clarke）B. L. Burtt			FJ501385	FJ501459	FJ501309
Loxostigma fimbrisepalum K. Y. Pan			FJ501399	FJ501507	—
L. griffithii （Wight）C. B. Clarke			FJ501400	FJ501508	FJ501338
Lysionotus chingii Chun ex W. T. Wang			—	FJ501498	FJ501332
L. forrestii W. W. Sm.			FJ501394	FJ501495	AF349152/AF349233
* *L. microphyllus* var. *microphyllus* W. T. Wang	China，Hunan，Zhangjiajie	WZ2010ZJJ03（KUN）	HQ327456	—	HQ327463
L. pauciflorus Maxim.			FJ501395	FJ501497	FJ501331
Opithandra primuloider （Miq.）B. L. Burtt			FJ501424	FJ501546	FJ501364
Oreocharis auricular （S. Moore）C. B. Clarke			—	FJ501482	FJ501323
Ornithoboea arachnoidea （Diels）Craib			FJ501387	FJ501461	FJ501312
O. wildeana Craib			—	FJ501462	FJ501313
Paraboea acutifolia （Ridl.）B. L. Burtt			—	FJ501464	FJ501314
P. capitata Ridl. var. *capitata*			AJ490911	AJ492298	FJ501315
P. rufescens （Franch.）B. L. Burtt			FJ501388	FJ501469	FJ501316
P. rufescens （Franch.）B. L. Burtt var. *umbellate* （Drake）K. Y. Pan			—	FJ501470	FJ501317
* *Paraisometrum mileense* W. T. Wang	China，Yunnan，Shilin county	TY2009SL01（KUN）	HQ327452	HQ327444	HQ327460
Petrocodon dealbatus Hance			FJ501418	FJ501538	FJ501358
Petrocosmea kerrii Craib			FJ501397	FJ501502	FJ501334

Continue

Taxon	Locality	Voucher	GenBank accession No.		
			atpB-rbcL	*trnL-F*	ITS1/ITS2
P. nervosa Craib			AJ4909012	AJ492299	FJ501335
Platystemma violoides Wall.			FJ501382	FJ501443	—
Primulina tabacum Hance			AJ490913	AJ492300	FJ501352
Ramonda myconi（L.）Rchb.			AJ490914	AJ492301	—
Raphiocarpus begoniifolius（H. Lev.）B. L. Burtt			—	FJ501517	FJ501342
R. petelotii（Pellegr.）B. L. Burtt			—	FJ501518	FJ501343
Rhabdothamnopsis sinensis Hemsl.			AJ490915	AJ492302	FJ501310
Rhynchotechum discolor（Maxim.）B. L. Burtt			FJ501376	FJ501436	—
R. parciflorum Blume			FJ501377	FJ501437	—
Saintpaulia tongwensis B. L. Burtt			—	FJ501446	FJ501303
S. velutina B. L. Burtt			AJ490916	AJ492303	FJ501304
Schizoboea kamerunensis K. Fritsch（B. L. Burtt）			—	FJ501453	FJ501305
Spelaeanthus chinii Kiew			—	FJ501457	FJ501307
Streptocarpus andohahelensis Humbert			—	FJ501449	AF316903
S. beampingaratrensis Humbert var. beampingaratrensis			—	FJ501448	AF316905
S. dunnii Hook. f.			—	FJ501456	AF316951
S. hilsenbergii R. Br.			—	FJ501450	AF316907
S. holstii Engl.			AJ490917	AJ492304	AF316917
S. ibityensis Humbert			—	FJ501455	AF316926
S. orientalis Craib			—	FJ501444	AF316929
S. papangae Humbert			—	FJ501444	AF316929
S. rexii Lindl.			AJ490918	AJ492305	AF316979
S. saxorum Engl.			FJ501383	FJ501447	AF316914
Trisepalum speciosum（Ridl.）B. L. Burtt			AJ490919	AJ492306	—
Epithematoid					
Epithema membranaceum（King）Kiew			AJ490887	AJ492274	—
E. saxatile Blume Weber & Anthonysamy			AJ490888	AJ492275	—
E. taiwanense S. S. Yin			AJ490889	AJ492276	—
E. tenue C. B. Clarke			AJ490890	AJ492277	—
Monophyllaea elongate B. L. Burtt			AJ490892	AJ492279	—
M. glauca C. B. Clarke			AJ490893	AJ492280	—
M. hiricalyx Franch.			AJ490894	AJ492269	—
M. horfieldii R. Br			U91315	AJ492269	—
Rhynchoglossum azureum（Schltdl.）			AJ490895	AJ492282	—
R. notonianum（Wall.）B. L. Burtt			AJ490896	AJ492283	—
R. obliquum Blume			AJ490897	AJ492284	—
Stauranthera grandiflora Benth.			AJ490900	AJ492287	—
Whytockia sasakii（Hayata）B. L. Burtt			AJ490901	AJ492288	—
W. tsiangiana（Hand.-Mazz）A. Weber			AJ490902	AJ492289	—

* Sequences are newly submitted to GenBank. Voucher information of sequences obtained from GenBank is not provided here. Dashed（—）indicate that no sequence was obtained