

Research Article

Phylogenetic reconstruction of *Chirita* and allies (Gesneriaceae) with taxonomic treatments

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Abstract *Chirita* D. Don, a large genus in the subfamily Cyrtandroideae of Gesneriaceae, has been the subject of much debate whether it is a natural group or not. In addition, the highly heterogeneous *Chirita* has also been very problematic with regard to delimitation and subdivision. Here we used the nrDNA internal transcribed spacer and cpDNA *trnL-F* for molecular phylogenetic analysis, combined with morphological data. Our results suggest that *Chirita* is an artificial, polyphyletic genus. The most important character that defines *Chirita*, the dorso-ventrally oblique and bilamellar stigma, has evolved convergently in different clades of diandrous Cyrtandroideae. *Chirita* sensu stricto only includes the species of *Chirita* sect. *Chirita*, whereas *Chirita* sect. *Microchirita* is an independent clade located at the basal node of the phylogenetic tree. *Chirita* sect. *Liebigia* is closely related to *Didymocarpus* with an entire stigma unlike other species of *Chirita*. The species of *Chirita* sect. *Gibbosaccus*, *Chiritopsis*, *Primulina*, and *Wentsaiboea* form a monophyletic group that is sister to a strongly supported clade comprising four monotypic genera *Paralagarosolen*, *Calcareaoboea*, *Petrocodon*, and *Tengia*. We further analyzed the morphological evolution of *Chirita* and identified a series of morphological synapomorphies for the monophyletic groups revealed herein, and thereby provide a taxonomic treatment in this study.

Key words *Chirita* D. Don, morphological evolution, phylogenetic reconstruction, polyphyly, taxonomic treatment.

As currently delimited, *Chirita* is a large genus in the subfamily Cyrtandroideae of Gesneriaceae with approximately 140 species distributed widely from the western Himalayas and southern and southwestern China to Southern India, Sri Lanka, and western Malaysia. *Chirita* was established by Don (1822) for three Himalayan herbs, *Chirita urticifolia* Buch.-Ham. ex D. Don, *C. bifolia* D. Don, and *C. pumila* D. Don. The genus was distinguished from *Streptocarpus* by having a straight capsule and from *Didymocarpus* by having a bilobed stigma. An early revision for *Chirita* was carried out by Clarke (1883) who recognized 33 species. Clarke divided *Chirita* into five sections, namely: (i) *Chirita* sect. *Euchirita* Clarke (21 species); (ii) *Chirita* sect. *Liebigia* (Endl.) Clarke (six species); (iii) *Chirita* sect. *Bilabium* (Miq.) Clarke (one species); (iv) *Chirita* sect. *Microchirita* Clarke (four species); and (v) *Chirita* sect. *Gibbosaccus* Clarke

(one species). In 1974, D. Wood provided a comprehensive revision of 77 species for *Chirita* with *Chirita* sect. *Liebigia* and *Chirita* sect. *Bilabium* combined into *Chirita* sect. *Chirita*. In his revision, *Chirita* sect. *Gibbosaccus* Clarke was characterized as perennial acaulescent herbs with leaves often somewhat fleshy, calyces usually divided to the base, and anthers fused face to face. *Chirita* sect. *Chirita* was usually caulescent perennial or annual herbs, calyces often tubular, and anthers fused face to face (*C. asperifolia* (Blume) B. L. Burt has a coarse woody habit remarkably distinctive from other species of *Chirita* sect. *Chirita*). *Chirita* sect. *Microchirita* Clarke was characterized by caulescent annual herbs with epiphyllous inflorescences, calyces appressed to fruits, and anthers joined by apical ligatures. Because of its great practical value, Wood's classification has been widely followed by later authors (Weber, 1975, 2004; Burt, 1977; Wang, 1985a, 1985b; Wang et al., 1990, 1992, 1998; Li & Wang, 2004). Since the 1970s, a large number of new species has been discovered in southern and southwestern China, where most species belong to *Chirita* sect. *Gibbosaccus* under the definition of Wood (1974). Based on the revision

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of Wood (1974), Wang (1985a, 1985b, 1990) further divided *Chirita* sect. *Gibbosaccus* (64 species) into three subsections, *Sinenses*, *Spinulosae*, and *Cicatricosae*, and seven series under subsect. *Sinenses*. *Chirita* sect. *Chirita* was also divided into three subsections, namely *Briggsiodes*, *Urticifoliae*, and *Fasciculiflorae*, and two series under subsect. *Urticifoliae* (Wang, 1985a, 1985b, 1990). Nevertheless, *Chirita* sect. *Liebigia* (Endl.) Clarke was recently revived by Hilliard (2004) to accommodate *C. asperifolia* and allies, distinguished from *Chirita* sect. *Chirita* by their coarse woody or shrubby habits. Hilliard (2004) further remarks that the reinstatement of *Liebigia* at this time is undesirable when the decisive characters of *Chirita* itself are uncertain.

The most important characters that define *Chirita* have been the bilobed stigma (the dorso-ventrally oblique, thin and bilamellar stigma), along with geniculate filaments and divergent anther-thecae. Meanwhile, it has long been questioned whether *Chirita* is natural under this definition (Chun, 1946; Wood, 1974; Weber, 2004). These characters have also been observed in both tetrandrous and diandrous plants outside of *Chirita* in subfamily Cyrtandroideae, including species with the combination of the three characters, such as *Chiritopsis*, *Primulina*, *Wentasiboaea*, and the tetrandrous *Raphiocarpus* (Wang et al., 1990, 1992, 1998; Li & Wang, 2004; Weber, 2004). In his revision of *Chirita*, Wood (1974) pointed out that if the syndrome of bilobed stigmas and geniculate filaments originated several times independently, *Chirita* may be polyphyletic. Weber (2004) remarked that the highly heterogeneous *Chirita* is very problematic with regard to delimitation and subdivision, except that *Chirita* sect. *Microchirita* seems to be a monophyletic alliance.

Recent molecular phylogenetic approaches have helped resolve many longstanding controversies and nurtured a better understanding of the evolutionary processes that have shaped the evolution of close allies (Bräuchler et al., 2004). In Mayer et al. (2003), with respect to the molecular phylogeny of Epithemateae, two species of *Chirita* included in their analysis do not form a clade. Recent molecular phylogenies in Gesneriaceae with increasing numbers of sampled *Chirita* species have further indicated the polyphyly of the genus *Chirita* (Möller et al., 2009; Wang et al., 2010). Li & Wang (2007) were the first to focus on the molecular phylogeny among *Chirita* species and their allies, that is, the molecular phylogeny among species of *Chiritopsis* and *Chirita* sect. *Gibbosaccus* with nrDNA internal transcribed spacer (ITS) and cpDNA *trnL-F* sequence data. Their results show that several parallel clades contain species of both *Chiritopsis* and *Chirita* sect. *Gibbosaccus*, indicating the polyphyly of both taxa

and the close connection between species of *Chirita* sect. *Gibbosaccus* and *Chiritopsis* (Li & Wang, 2007). However, the full resolution of phylogenetic questions regarding the highly heterogeneous species of *Chirita* and the delimitations of genera in this group is far from complete.

With greater sampling, we have carried out a comprehensive investigation on *Chirita* and putatively close allies based on DNA sequence data combined with morphological characters. The goal of this research was to: (i) examine the phylogenetic relations of *Chirita* and allies and find monophyletic clades; (ii) evaluate the evolution of the morphological characters used to circumscribe related taxa; and thereby (iii) provide a systematic treatment at the generic level in *Chirita* and related taxa.

1 Material and methods

1.1 Taxon sampling

We sampled 59 species and varieties in the four sections of *Chirita*, eight species of *Chiritopsis*, 10 species of *Didymocarpus*, and six species from the six monotypic genera, *Calcareoboaea*, *Paralagarosolen*, *Petrocodon*, *Primulina*, *Tengia*, and *Wentsaiboaea*. With the exception of the ITS and *trnL-F* DNA sequences retrieved from GenBank, all sampled materials were collected in the field. The information on all sampled taxa and GenBank accession numbers can be found in the online supplemental data (Table S1). Voucher specimens of all newly collected materials were deposited in the Herbarium of the Institute of Botany, Chinese Academy of Sciences (PE).

1.2 DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from silica gel dried or fresh leaf tissue using the CTAB method of Rogers & Bendich (1988) and used as the template in the polymerase chain reaction. The entire nrDNA ITS region, including ITS1, 5.8S subunit, and ITS2, and the chloroplast DNA region *trnL-F* were chosen for the phylogenetic analysis. These regions were amplified using the ITS primers ITS1 and ITS4 (Wendel et al., 1995) and the *trnL-F* primers c and f (Taberlet et al., 1991), respectively. Amplification products were purified with a Uniq-10 PCR Purification kit (Sangon, Shanghai, China). All ITS and *trnL-F* sequences were obtained directly using a MegaBACE 1000 automatic sequencer (Amersham Biosciences, Sunnyvale, CA, USA) following the manufacturer's protocol. The *trnL-F* was sequenced in both directions using the same primer pairs as for amplification. The ITS1 and ITS4 primers were

used to sequence the ITS region in both directions, with additional sequences from internal primers CITS2 (5'-GCATTCGCTACGTTCTTCA-3') and CITS3 (5'-CCATCGAGTCTTTG AACGCA-3') when sequences from ITS1 and ITS4 primers did not provide sufficient overlap.

1.3 Sequence alignment and phylogenetic analysis

The sequences were aligned using ClustalX (Thompson et al., 1997) and adjusted manually to maximize sequence homology using BioEdit 5.0.9 (Hall, 1999).

Parsimony analysis for each matrix was carried out using maximum parsimony (MP) methods in PAUP*4.0b10 (Swofford, 2003). Characters and character-state changes were weighted equally and gaps were treated as missing data. Heuristic searches were carried out with 1000 replicates of random addition, one tree held at each step during stepwise addition, tree-bisection-reconnection (TBR) branch swapping, MulTrees in effect, and steepest descent off. To examine the robustness of various clades, we ran a bootstrap analysis (Felsenstein, 1985) with 1000 replicates of bootstrapping using a heuristic search with 1000 replicates of random sequence addition and TBR branch swapping.

Bayesian inference analyses were carried out using MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003). Modeltest 3.06 (Posada & Crandall, 1998) was used to select an appropriate model of sequence evolution for each DNA dataset from a comparison of 56 models. Four chains of Markov chain Monte Carlo were each run for 10 000 000 generations, and were sampled every 10 000 generations, starting with a random tree. For each run, the first 20% of sampled trees were excluded as burn in (burn-in = 200). In the majority rule consensus from Bayesian analysis, posterior probability (PP) was used to estimate robustness.

For combined sequence data, the incongruence length difference test (Farris et al., 1994) as implemented in PAUP*4.0b10 (Swofford, 2003) was carried out to assess character congruence between ITS and *trnL-F*, with 1000 replicates, each with 100 random additions with TBR branch swapping. The resulting *p* value was used to determine whether the two datasets contained significant incongruence. Both MP and Bayesian inference analyses for the combined dataset were carried out using the same methods as those used for ITS and *trnL-F*. The two species *Ornithoboea wildeana* and *Paraboea rufescens* were chosen as outgroups for the above analyses.

2 Results

2.1 Internal transcribed spacer analysis

The ITS matrix had aligned sequences of 851 bp, of which 279 (32.78%) were constant, 141 (16.57%) were variable but uninformative, and 431 (50.65%) were parsimony informative. Modeltest indicated GTR + I + G as the best-fit model for the ITS sequence data. The strict consensus of 12 trees yielded by MP analysis (MP length [L] = 2128; consistency index [CI] = 0.494; retention index [RI] = 0.749) was generally congruent with the majority rule consensus Bayesian tree in the topology (Fig. 1).

The MP tree consists of five major clades labeled A–E (Fig. 1). Clades A–D, each well supported, are in turn sister to the remaining groups with moderate to maximum support (bootstrap [BS] values = 79–100%; posterior probabilities [PP] = 100%). Clade A includes all six representatives of *Chirita* sect. *Microchirita* with maximum support (BS = 100%; PP = 100%). The eight species of *Chirita* sect. *Chirita* also receive strong support (BS = 85%; PP = 100%) as a monophyletic clade (B). The species of *Didymocarpus* form a monophyletic lineage with maximum support (BS = 100%; PP = 100%) that is strongly supported as sister to *C. asperifolia*, the representative of *Chirita* sect. *Liebigia*, in clade C (BS = 100%; PP = 100%). The three monotypic genera, *Calcareaoboea*, *Petrocodon*, and *Tengia*, are grouped together as a strongly supported clade (D) (BS = 99%; PP = 100%) that is sister to the remaining groups (clade E) with maximum support (BS = 100%; PP = 100%).

Clade E is a monophyletic branch with strong support (BS = 96%; PP = 100%) and includes all species from *Chirita* sect. *Gibbosaccus*, *Chiritopsis*, and two monotypic genera *Primulina* and *Wentsaiboea* and is further divided into three lineages. The first lineage with low support (BS = 57%; PP = 96%) includes two pairs of sister species, *C. gemella/C. laxiflora* and *C. heterotricha/C. pteropoda*. The second and third lineages are grouped together with strong support (BS = 95%; PP = 100%). The second lineage (BS = 98%; PP = 100%) is a polytomy and includes seven branches with four species of *Chiritopsis* scattered over different branches of *Chirita* sect. *Gibbosaccus*. The third lineage, a poorly supported clade (BS = 60%; PP = 100%), comprises seven branches, in which two species of *Chiritopsis*, *Wentsaiboea* with *Chiritopsis mollifolia*, and *Primulina* are brought into three groups, respectively, together with species of *Chirita* sect. *Gibbosaccus* with high supports (BS = 98–100%; PP = 100%).

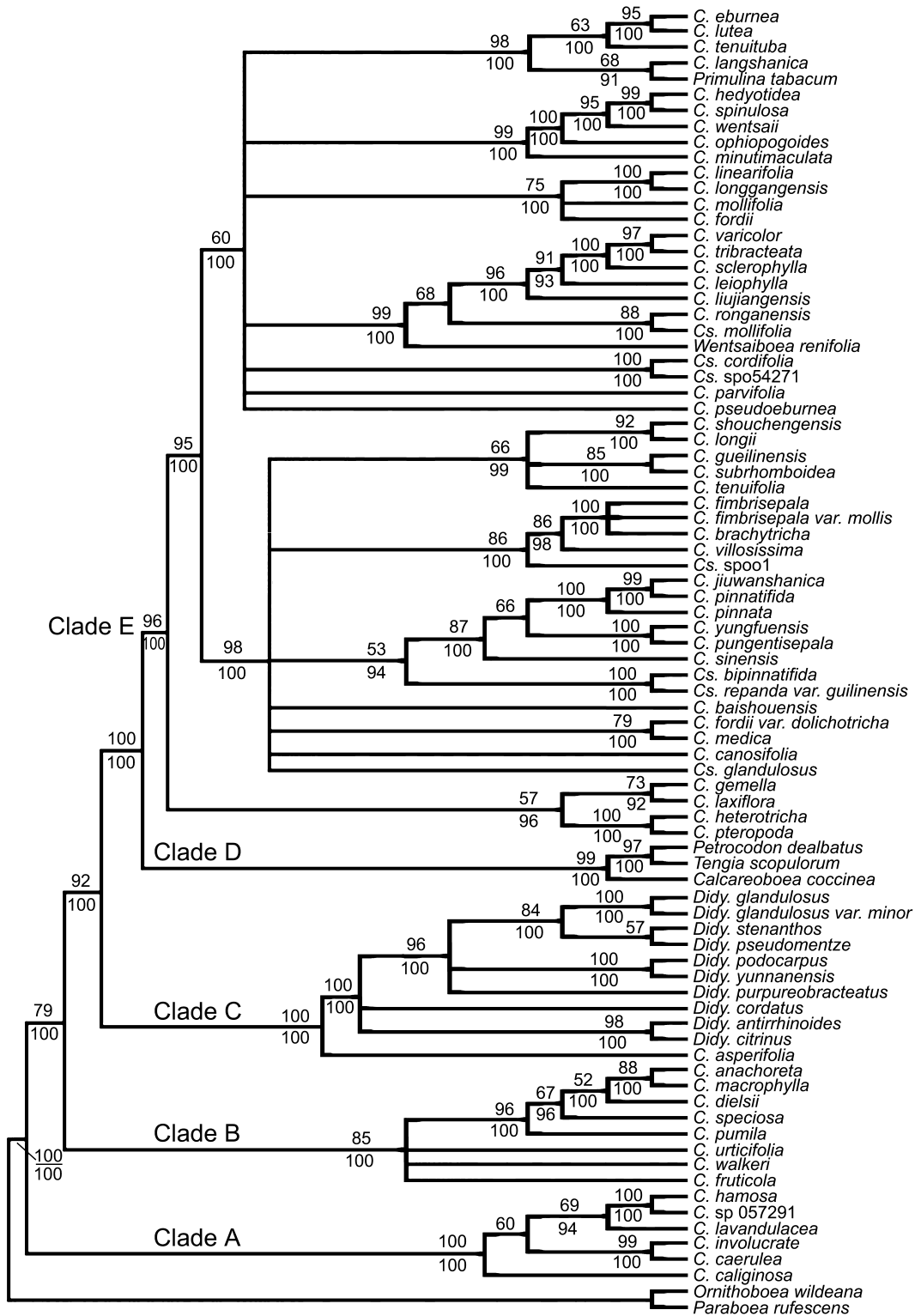


Fig. 1. Strict consensus of 12 most parsimonious trees generated from the internal transcribed spacer data, which is generally congruent with the majority rule consensus from the Bayesian analysis. The bootstrap values are above the branches and the Bayesian posterior probabilities are below the branches. C, *Chirita*; Cs, *Chiritopsis*; Didy, *Didymocarpus*.

2.2 Analysis of *trnL-F*

The *trnL-F* matrix included the same taxa as in the ITS analysis with the exception that *C. involucrate* and *C. caerulea*, included in the ITS matrix, were absent in the *trnL-F* matrix with *Chiritopsis repanda* and *Paralagarosolen fangianum* lacking in the ITS matrix and added to the *trnL-F* matrix (Figs. 1, 2). The aligned *trnL-F* region encompassed 944 bp, of which 703 (74.47%) were constant, 124 (13.14%) were variable but uninformative, and 117 (12.39%) parsimony informative characters. Modeltest indicated TUM + I as the best-fit model for *trnL-F*. The strict consensus of 6645 trees yielded by MP analysis ($L = 321$; $CI = 0.850$; $RI = 0.929$) was congruent in topology with the majority rule consensus Bayesian tree for *trnL-F*.

The topology of the *trnL-F* MP tree (Fig. 2) was congruent with the ITS topology for major clades (Fig. 1). Clades A–D, each as a monophyletic group, are in turn sister to the remaining branches with strong supports ($BS = 90$ – 99% , $PP = 100\%$), identical to those in the ITS tree (Fig. 1). The differences between the cpDNA and the nrDNA trees lie in some minor and terminal branches between some individual species (Figs. 1, 2). For example, the species *C. macrophylla* is grouped with *C. anachoreta* in the ITS tree, whereas it is sister to *C. urticifolia* with maximum support in the *trnL-F* tree (Figs. 1, 2). *Wentsaiboea* is sister to *C. pseudoeburnea* in an isolated branch in the *trnL-F* tree, but grouped with seven other species from *Chirita* sect. *Gibbosaccus* and *Chiritopsis* in a strongly supported clade in the ITS tree (Figs. 1, 2). Other species whose placement is incongruent between the ITS and *trnL-F* trees are all within clade E, especially within *Chirita* sect. *Gibbosaccus*, such as *C. lutea*, *C. mollifolia*, *C. tribracteata*, *C. villosissima* and *C. fordii* var. *dolichotricha*. Clade E is a polytomy with four lineages, including the species from *Chirita* sect. *Gibbosaccus*, *Chiritopsis*, and the monotypic *Primulina* and *Wentsaiboea*, similar to those in the ITS tree except that the first two lineages are grouped together with low support in the ITS tree (Fig. 1).

2.3 Analysis of combined ITS and *trnL-F* data

Ten species whose phylogenetic placement was incongruent between the ITS and *trnL-F* trees were deleted in the combined ITS and *trnL-F* matrix with 69 ingroup species remaining in the analysis. The incongruence length difference test gave a value of $P = 0.154$ with these 10 species removed, indicating that the data from the two distinct marker regions did not contain significant incongruences. Modeltest suggested that the GTR + I + G model best fits the combined data. The combined datasets consisted of 1803 bp, 261

(14.48%) of which were variable and 529 (29.34%) parsimony informative sites. Parsimony analyses resulted in seven trees of equal length ($L = 2276$; $CI = 0.561$; $RI = 0.768$). The strict consensus of seven MP trees was generally congruent with the majority rule consensus from the Bayesian analysis except that *Wentsaiboea* is grouped into two parallel branches in the MP tree and Bayesian tree (Fig. 3). The MP tree from the combined ITS and *trnL-F* data was congruent with the ITS MP tree and *trnL-F* MP tree for the major clades (Figs. 1–3).

The MP tree also comprises five main clades as in the ITS and *trnL-F* trees labeled A–E (Fig. 3). The first clade (A) consists of the representative species of *Chirita* sect. *Microchirita* ($BS = 100\%$; $PP = 100\%$). Clade B, including all species of *Chirita* sect. *Chirita*, gets higher support than the one in the ITS and *trnL-F* trees (Figs. 1–3). In clade C, the species of *Didymocarpus* form a monophyletic lineage of their own with maximum support ($BS = 100\%$; $PP = 100\%$) and are perfectly resolved as sister to *C. asperifolia*, a representative of *Chirita* sect. *Liebigia*. The three monotypic genera *Calcareoboea*, *Petrocodon*, and *Tengia* form clade D with maximum support ($BS = 100\%$; $PP = 100\%$) that is sister to the remaining group, clade E ($BS = 100\%$; $PP = 100\%$). Similar to that in the ITS tree, the monophyletic clade E ($BS = 98\%$; $PP = 100\%$) consists of three lineages that contain all species from *Chirita* sect. *Gibbosaccus*, *Chiritopsis* and the monotypic *Primulina* and *Wentsaiboea*, in which the three lineages get stronger support than in the ITS and *trnL-F* trees (Figs. 1–3).

3 Discussion

3.1 Phylogenetic analyses

The polyphyly of the genus *Chirita* has been gradually manifested with increased sampling of *Chirita* species in different molecular phylogenies in Gesneriaceae (Smith, 1996; Mayer et al., 2003; Li & Wang, 2007; Möller et al., 2009; Wang et al., 2010). The broadened sampling herein with 59 *Chirita* species and 24 allied taxa enables us to draw a phylogenetic framework based on nrDNA ITS and cpDNA *trnL-F* data. All species from *Chirita* sect. *Microchirita* form a monophyletic clade that is sister to the remainder sampled herein. Its position in the trees is in perfect accordance with its morphological uniformity with a monocarpic-annual habit, unusual crested inflorescences with the peduncle fused to the petiole, and anthers fused apically (Wood, 1974; Wang, 1985a, 1985b). The present results are congruent with previous works (Möller et al., 2009; Wang et al., 2010) with respect to *Microchirita* species

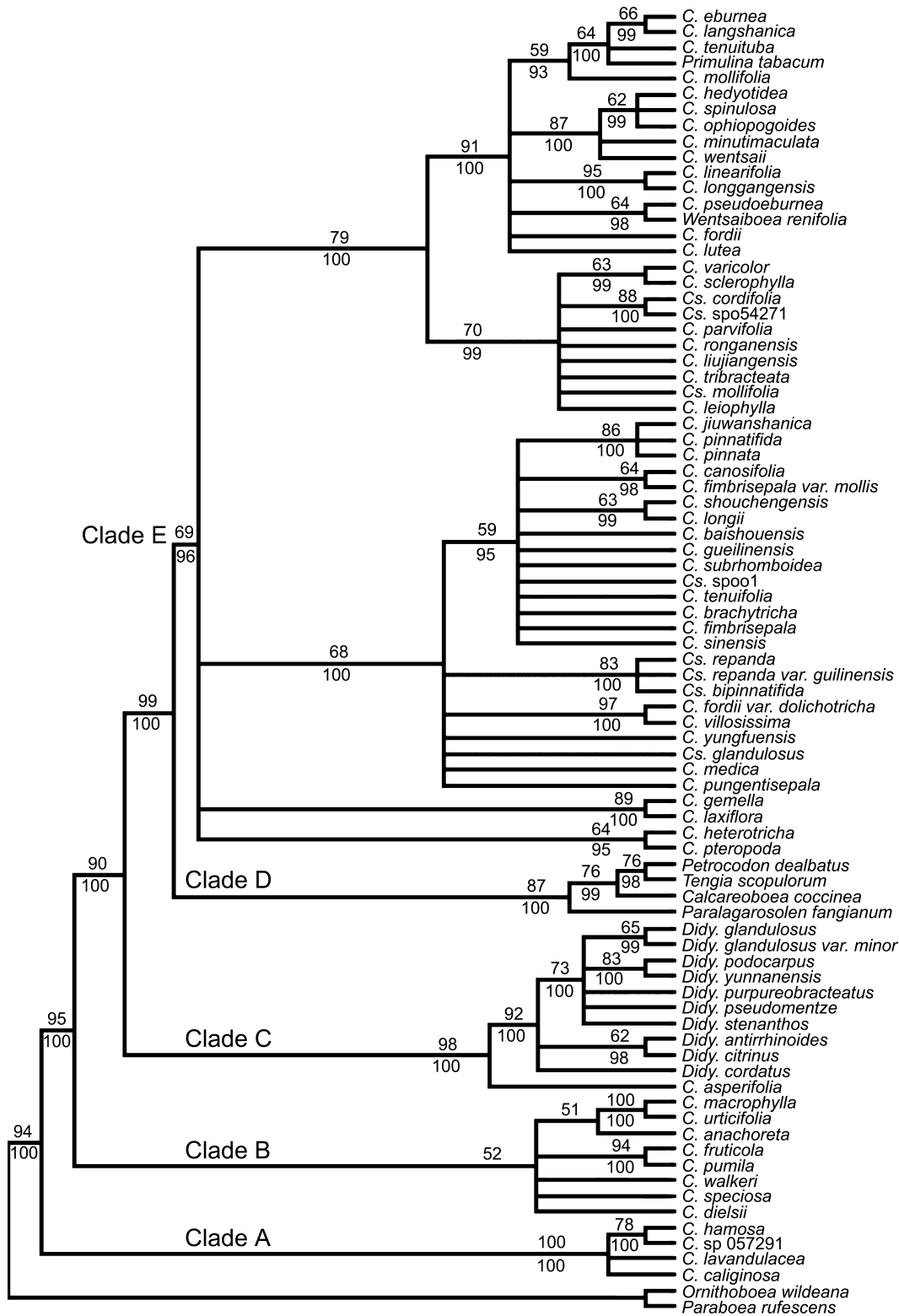


Fig. 2. Strict consensus of 6645 most parsimonious trees generated from the *trnL-F* data, which is congruent with the majority rule consensus from the Bayesian analysis. The bootstrap values are above the branches, and the Bayesian posterior probabilities are below the branches. C, *Chirita*; Cs, *Chiritopsis*; Didy, *Didymocarpus*.

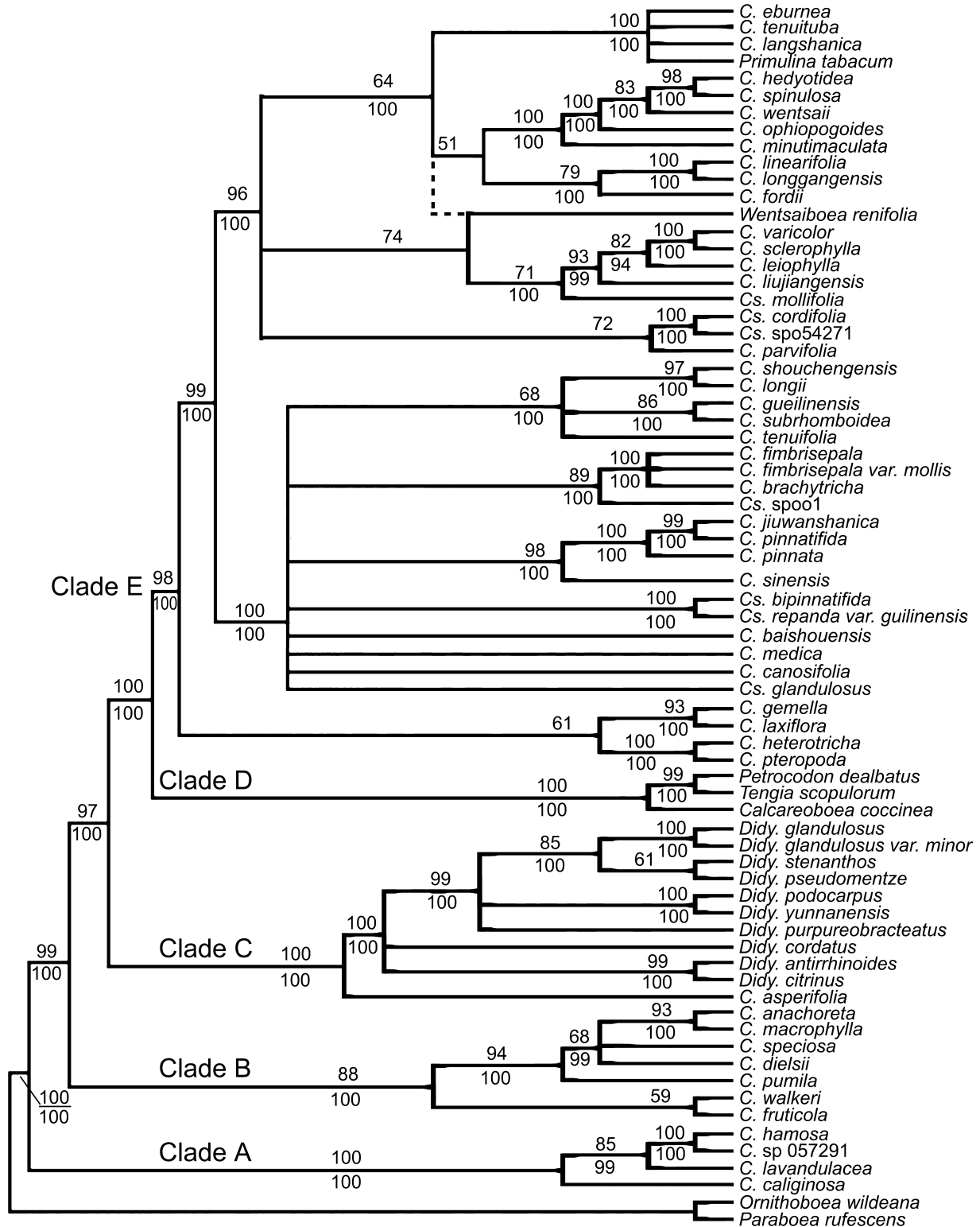


Fig. 3. One of seven most parsimonious trees generated from analysis of combined internal transcribed spacer and *trnL-F* data, which is generally congruent with the majority rule consensus from the Bayesian analysis. The bootstrap values are above the branches and the Bayesian posterior probabilities are below the branches. Branches marked by the broken line indicate the topological discordance between maximum parsimony and Bayesian trees. *C.*, *Chirita*; *Cs.*, *Chiritopsis*; *Didy.*, *Didymocarpus*.

where they form a monophyletic clade located at the basal node of both nrDNA and cpDNA trees parallel to some groups with two or four fertile stamens, such as *Paraboea*, *Corollodiscus*, and *Haberlea*. Even though these plants have highly specialized floral characters, species of *Chirita* sect. *Microchirita* are likely an ancient diandrous clade that might have been directly derived from the basal tetrandrous groups in the subfamily Cyrtandroideae in Gesneriaceae.

Chirita sect. *Chirita* is an independent clade distinguished from *Chirita* sect. *Gibbosaccus* by their caulescent herbs, tubular calyx, and long and relatively narrow corolla tube. They differ from the genus *Didymocarpus* mainly by their bilobed stigma, that is, dorso-ventrally oblique, thin, and bifid stigmatic lamina. In the early classification of *Chirita*, species in sect. *Chirita* were the core members of *Chirita*, in which three of them, *C. urticifolia*, *C. bifolia*, and *C. pumila*, were the first described species of *Chirita* when Don (1822) established the genus with *C. urticifolia* later selected as its Lectotype (Clarke, 1883; Burt, 1954, 1965; Wood, 1974; Wang, 1985a, 1985b). Plants of *Chirita* sect. *Chirita* are widely distributed from the western Himalayas through Burma, southwestern and southern China to Indo-China and Thailand. In addition to the bilobed stigma, this group might have phylogenetically and adaptively descended from a perennial ancestor to become annual herbs or even short-lived annual herbs under heterogeneous and variable ecological environments of the limestone areas, especially the sharply contrasting dry and rainy season under a monsoon climate.

The species of *Didymocarpus* form a monophyletic branch with strong support both in ITS and *trnL-F* trees. Correlatively, *Didymocarpus* is remarkably morphologically distinctive from other groups herein in its entire stigma (style ending in a dorso-ventrally equally developed stigma). However, *C. asperifolia*, the representative of *Chirita* sect. *Liebigia*, is strongly supported as the sister to *Didymocarpus*. *Liebigia* was originally established by Endlicher (1841) and then reduced to *Chirita* sect. *Liebigia* by Clarke (1883) that contained only four species clustered around the type species *C. asperifolia* (*C. blumei*). Wood (1974) further reduced all these species together with the single species of *Chirita* sect. *Bilabium* Clarke to *C. asperifolia* because they were so similar with only minor continuous variation among them and combined it with *Chirita* sect. *Chirita*. *Chirita* sect. *Liebigia* was recently revived by Hilliard (2004) to accommodate *C. asperifolia* and allies. Their high and coarse woody and shrubby habits are characteristic of this group as well as their geographic distribution limited to Sumatra and Java. These traits are remark-

ably different from other *Chirita* species, especially *Chirita* sect. *Chirita*. Apparently, this group shares a common ancestor with *Didymocarpus*, in which the former might have proceeded toward dorso-ventral differentiation of the stigma with abortion or great reduction of the upper lip and enlargement of the lower lip with more or less bifid in appearance similar to that of other *Chirita*.

The four monospecific genera, *Paralagarosolen*, *Calcareoboea*, *Petrocodon*, and *Tengia*, constitute a strongly supported clade. Their flowers are remarkably different from each other which is why they have been placed in distantly related groups in the traditional classification of the Cyrtandroideae (Wang et al., 1990, 1992, 1998; Li & Wang, 2004; Weber, 2004). *Tengia* has been called a “natural peloria” (Donoghue et al., 1998) because it exhibits an almost perfect actinomorphic flower from whorl one to whorl three (Li & Wang, 2004). However, it is deeply nested within the zygomorphic groups with diandrous flowers in Clade D, in which it is sister to *Petrocodon*, and further constitutes a monophyletic group with *Calcareoboea* and *Paralagarosolen*. *Paralagarosolen* described by Wei (2004) is characterized by strongly zygomorphic flowers with bilabiate corollas consisting of a bilobed upper lip and trilobed lower lip and two fertile ventral stamens plus three staminodes and two stigmatic laminae dorso-ventrally equally developed (Li & Wang, 2004; Wei, 2004). *Calcareoboea* is similar to *Paralagarosolen* in its androecium, but it has a specialized bilabiate corolla with upper (dorsal) lip of four short teeth and lower (ventral) lip of a tongue-like single patent lobe (Li & Wang, 2004; Weber, 2004; Wang et al., 2010). Wang et al. (2010) suggest that the short teeth emerging from the top of the highly fused corolla tube is the synapomorphy shared among the three genera, *Tengia*, *Petrocodon*, and *Calcareoboea*. *Petrocodon* further exhibits a morphologically transitional form between *Tengia* and *Calcareoboea*, in which its corolla is almost actinomorphic, similar to that of *Tengia*, whereas its androecium consists of two fertile stamens at the ventral position and three staminodes at the lateral and dorsal positions as in the androecium of *Calcareoboea* and *Paralagarosolen* (Li & Wang, 2004; Wang et al., 2010). Studying morphological characters in light of the molecular phylogeny can enhance our understanding of morphological diversity in relation to the evolutionary history of these clades (Wang et al., 2010). The phylogenetic lineage of the four genera might reflect the shift in floral form as the result of selection by pollinators (Diggle, 1992; Wang et al., 2010). The plants of *Paralagarosolen* have an open corolla mouth with stigma and anthers located almost at the same level below or at the corolla mouth.

In contrast, in *Tengia* the nearly closed corolla with a keyhole opening from which the stigma is far exerted, with all five stamens completely included within the corolla, makes the stigma and anthers completely separated spatially (Wang et al., 2010). In this configuration, pollinators may contact only one set of sex organs while visiting the flower, effectively avoiding self-pollination. This combination of characters in *Tengia* might be related to new pollinators, such as small-sized insects, for cross-pollination in the moist and shady habitats that plants of *Tengia* prefer (Wang et al., 2010). Meanwhile, the morphological specialization of the *Calcareo-boa* flowers might be related to another pollination syndrome corresponding to the long-tongued flies or bees, indicated by its long and curved corolla tube (Lunau, 2004; Reynold et al., 2009; Wang et al., 2010). Apparently, the variation of their floral structure demonstrates a phylogenetic transition and evolutionary shift of floral symmetry from *Paralagarosolen* through *Calcareo-boa* and *Petrocodon* and finally to *Tengia* relating to pollination syndrome and reproductive strategies. This morphological shift in stamen number and corolla shape might be due to the expression differentiation of *CYC*-like genes that have been known to function in patterning floral symmetry (Pang et al., 2010; Yang et al., 2010). In addition, the two equal stigmatic laminae with broadly ovoid capsules in *Paralagarosolen* and the entire stigma with triangular or tooth-like corolla lobes in the other three genera make them sharply distinguished from other groups sampled herein with regard to morphology.

The remaining taxa are strongly supported as a monophyletic group that contains all species from *Chirita* sect. *Gibbosaccus* and *Chiritopsis* as well as two monotypic genera *Primulina* and *Wentsaiboea*. The polyphyly of *Chiritopsis* first revealed by Li & Wang (2007) is further confirmed by the present results. According to Li & Wang (2007), the seemingly morphological uniformity of small plants and minor flowers with ovoid ovaries in *Chiritopsis* is in fact the result of morphologically convergent evolution adaptive to the heterogeneous and variable ecological environments, especially the sharply contrasting dry and rainy season in the subtropical monsoon limestone areas. Although different in flower size, *Chirita* sect. *Gibbosaccus* and *Chiritopsis* share identical proportions of corolla width and length, as well as the ratios of width between the base and mouth of corolla tubes (Li & Wang, 2007). The monotypic *Primulina* was established by Hance (1883). It is a perennial herb with diandrous flowers similar to *Primula* in appearance. Its two ventrally fertile stamens, dorso-ventrally obliquely developed and bifid stigma, and straight capsules are also characteristic of *Chirita*,

especially *Chirita* sect. *Gibbosaccus*. *Wentsaiboea* was recently described (Fang & Qin, 2004) and is diagnosed by small plants with minor flowers, and reniform leaves that are very similar to those of some *Chiritopsis* plants. This species perfectly conforms to the vegetative and floral characters of *Chirita* sect. *Gibbosaccus*, such as much developed rhizomes, broad and relatively short corolla tubes, two ventrally fertile stamens, and the dorso-ventrally obliquely developed and bifid stigma. Morphologically, as mentioned above, the species of this clade are all acaulescent perennial herbs with more or less well-developed rhizomes and somewhat fleshy leaves (Li & Wang, 2004; Li & Wang, 2007). Their flowers usually have broad and relatively short corolla tubes with calyces divided to the bases and stigmas dorso-ventrally obliquely developed that are more or less bifid (Li & Wang, 2007). The combination of the above vegetative and floral characters as a combined synapomorphy is characteristic of this group that is remarkably distinctive from others herein sampled. However, this group contains over 100 species, in which most of them have only minor and more or less continuous variation between or among related species. A large amount of field work at the population level, combined with detailed examination of herbarium specimens, as well as observations in light of population concept would be necessary to fully understand their variation patterns relating to speciation and species circumscription. In addition, a comprehensive investigation into their morphology, anatomy, and floral development, undertaken in the hope of revealing new characters of high taxonomic value, would shed more light on identifying further morphological and anatomical synapomorphies for their subdivision and phylogenetic relationships. On the basis of these studies, further broadened sampling with more DNA regions, including DNA barcoding genes, would be helpful to get this group well resolved in their systematic subdivision and species relationships.

3.2 Taxonomic treatments

Key to redefined genera and allies

1. Stigma entire, depressed-globose to disc-like or truncate, corolla tube cylindrical or funnel-form-tubular, usually straight and not swollen, perennial herbs, rarely subshrubs. **Didymocarpus**
1. Stigma divided, with two stigmatic laminae dorso-ventrally equally developed or one dorso-ventrally oblique and bifid lamina, or stigma entire only when the corolla lobes are triangular or short and tooth-like, corolla tube broadly cylindrical or long funnel-formed, usually pouched ventrally or swollen above middle, shrubby, perennial or annual.

2. Tall and coarse woody herbs or subshrubs, calyx tubular, often inflated, funnel-shaped or balloon-like, corolla tubes ventricose on ventral side 2. **Liebigia**
2. Small caulescent or acaulescent herbs, perennial or annual, calyx divided to the base or tubular but rarely inflated, corolla tube straight or curved, usually swollen above the middle or pouched ventrally.
3. Cymose crested inflorescences with peduncle fused to the petiole, calyx divided to the base, anthers fused apically by projections on their connectives 3. **Microchirita**
3. Cymose inflorescences with peduncle free from the petiole, calyx divided to the base or tubular, anthers fused face to face by their entire adaxial surfaces.
4. Caulescent perennial or annual herbs, sometimes the aerial stem much condensed or creeping, calyx tubular with tooth-like lobes, corolla tube usually swollen above the middle, straight or ventrally curved 1. **Chirita**
4. Acaulescent perennial herbs with more or less well-developed rhizomes, calyx divided to the base with lanceolate lobes.
5. Stigma divided with two stigmatic laminae dorso-ventrally equally developed with oblong corolla lobes and broadly ovoid capsules or stigma entire with triangular or short and tooth-like corolla lobes and linear capsules, corolla tubes narrowly cylindrical, funnellform, urceolate or suburceolate, leaves more or less coriaceous 4. **Petrocodon**
5. Stigma with one dorso-ventrally oblique and bifid lamina with linear capsules, corolla tube usually broad and relatively short, leaves often fleshy 5. **Primulina**

1. Chirita Buch.-Ham. ex D. Don in Edinb. Phil. Journ. 7: 83. 1822.

Lectotype: *Chirita urticifolia* Buch.-Ham. ex D. Don chosen by B. L. Burtt. in Notes R. Bot. Gard. Edinb. 21: 195. 1954.

Syn: *Babactes* DC. ex Meisn., Pl. Vasc. Gen. 1: 302; 2: 211. 1840.

Type: *Babactes oblongifolia* (Roxb.) DC. ex Meisn. (= *Chirita oblongifolia* (Roxb.) Sinclair).

Gonatostemon Regel, Gartenflora 15: 353. 1866.

Type: *Gonatostemon boucheanum* Regel (= *Chirita urticifolia* Buch.-Ham. ex D. Don)

Damrongia Kerr ex Craib in Bull. Misc. Inform. Kew 1918 (10): 364. 1918.

Type: *Damrongia purpureolineata* Kerr ex Craib (= *Chirita purpureolineata* Kerr ex Craib)

Ceraloscyphus Chun in Sunyatsenia 6: 276. 1946.

Type: *Ceraloscyphus coeruleus* Chun (= *Chirita corniculata* Pellegr.)

Chirita sect. *Euchirita* C. B. Clarke, op cit. P. III, (1883) (excl. *C. caliginosa*).

Chirita sect. *Chirita* D. Wood in Notes R. Bot. Gard. Edinb. 33: 123–205. 1974 (excl. *C. asperifolia* (Blume) B. L. Burtt).

Caulescent perennial or annual herbs, sometimes the aerial stem much condensed or creeping, calyx tubular with tooth-like lobes, corolla tube long and relatively narrow, often swollen above the middle, straight or ventrally curved, two ventrally fertile stamens with three staminodes, anthers fused face to face, stigma with a dorso-ventrally oblique and bifid lamina, lamina relatively large, capsules linear.

Widely distributed from the western Himalayas through Burma, southwestern and southern China to Indo-China and Thailand.

2. Liebigia Endl. in Gen. Pl. Suppl. 1407. Feb.–Mar. 1841.

Type: *Liebigia speciosa* (Blume) Endl. (= *Chirita asperifolia* (Blume) B. L. Burtt)

Syn: *Chirita* sect. *Liebigia* (Endl.) C. B. Clarke in A. DC., Monogr. Phan. 5: 122. 1883.

Tromsdorffia Blume, Bijdr. Fl. Ned. Ind. 762. 1826, non Bernhard (1800).

Type: *Tromsdorffia speciosa* Blume (= *Chirita asperifolia* (Blume) B. L. Burtt).

Morstdorffia Steud., Nom. ed. 2, 2: 161. Mar. 1841.

Type: *Morstdorffia speciosa* (Blume) Steud. (= *Chirita asperifolia* (Blume) B. L. Burtt)

Bilabium Miq., Fl. Ind. Bat. 2: 730. 1858.

Type: *Bilabium limans* Miq. (= *Chirita limans* (Miq.) B. L. Burtt)

High and coarse woody herbs or subshrubs, calyx tubular, often inflated, funnel-shaped or balloon-like, corolla tubes ventricose on ventral side, two ventrally fertile stamens with three staminodes, stigma with a dorso-ventrally oblique and bifid lamina.

Limited geographically to Sumatra and Java.

3. Microchirita (C. B. Clarke) Y. Z. Wang **stat. nov.**

Lectotype: *Microchirita hamosa* (*Chirita hamosa* R. Br.) chosen by B. L. Burtt. in Notes R. Bot. Gard. Edinb. 21: 196. 1954.

Syn: *Roettlera* sect. *Microchirita* (C. B. Clarke) K. Fritsch in Pflanzenfam. IV (3B): 148. 1895.

Didymocarpus sect. *Microchirita* (C. B. Clarke) Chun in Sunyatsenia 6: 290. 1946.

Chirita sect. *Microchirita* C. B. Clarke in A. DC., Monogr. Phan. 5: 127. 1883.

Monocarpic and annual herbs, rarely perennials, crested inflorescences with peduncle fused to the petiole, calyces divided to the base, two ventrally fertile

stamens plus three staminodes, anthers fused apically by projections on their connectives, stigma with a dorso-ventrally oblique and bifid lamina.

Distributed from Indonesia, Malaysia, Thailand to Vietnam and southwestern China.

4. Petrocodon Hance in Journ. Bot. Lond. 21: 167. 1883.

Type: *Petrocodon dealbatus* Hance

Syn: *Tengia* Chun in Sunyatsenia 6 (3–4): 279. 1946. **syn. nov.**

Calcareoboea C. Y. Wu ex H. W. Li in Acta Bot. Yunnan. 4 (3): 241. 1982. **syn. nov.**

Paralagarosolen Y. G. Wei in Acta Phytotax. Sin. 42 (6): 528. 2004. **syn. nov.**

Acaulescent perennial herbs with more or less developed rhizomes, leaves more or less coriaceous, calyces divided to the bases, corolla zygomorphic or actinomorphic, tubes narrowly cylindric, funnellform, urceolate or suburceolate, lobes bilabiate or equal, two ventral fertile stamens plus three staminodes or five fertile and equal stamens, stigma with two stigmatic laminae dorso-ventrally equally developed with oblong corolla lobes and broadly ovoid capsules or stigma entire, globose, disc-like or truncate when the corolla lobes are triangular or short and tooth-like, capsules broadly ovoid or linear.

Distributed in southwestern China.

5. Primulina Hance in Journ. Bot. Lond. 21: 169. 1883.

Type: *Primulina tabacum* Hance

Syn: *Chirita* sect. *Gibbosaccus* C. B. Clarke in A. DC., Monogr. Phan. 5: 130. 1883.

Roettlera sect. *Gibbosaccus* (C. B. Cl.) K. Fritsch in Pflanzenfam. IV (3B): 148. 1895.

Wentsaiboea D. Fang et D. H. Qin in Acta Phytotax. Sin. 42 (6): 533. 2004. **syn. nov.**

Acaulescent perennial herbs with more or less well-developed rhizomes and somewhat fleshy leaves, calyces divided to the bases, corolla tubes often broad, two ventrally fertile stamens with three staminodes, anthers fused face to face, stigma with a dorso-ventrally oblique and bifid lamina, lamina relatively small, capsules linear.

Distributed in southern and southwestern China and Vietnam.

6. Synonyms of related species in this study

Microchirita (C. B. Clarke) Y. Z. Wang 勾序苣苔属

Microchirita caerulea (R. Br.) Y. Z. Wang, **comb. nov.** Basionym: *Chirita caerulea* R. Br. in Bennett &

Brown, Pl. Jav. Bar. v: 117. 1840. C. B. Clarke in A. DC. Monogr. Phan. 5: 127. 1883. D. Wood in Notes Roy. Bot. Gard. Edinb. 33 (1): 200. 1974.

Microchirita caliginosa (C. B. Cl.) Y. Z. Wang, **comb. nov.** Basionym: *Chirita caliginosa* C. B. Cl. in A. DC., Monogr. Phan. 5: 122. 1883. D. Wood in Notes Roy. Bot. Gard. Edinb. 33 (1): 186. 1974.

Microchirita hamosa (R. Br.) Y. Z. Wang, **comb. nov.** Basionym: *Chirita hamosa* R. Br. in Benn. et Br. Pl. Jav. Rar. 117. 1840. C. B. Clarke in A. DC. Monogr. Phan. 5: 117. 1883. D. Wood in Notes Roy. Bot. Gard. Edinb. 33 (1): 191. 1974. 勾序苣苔

Microchirita involucrate (Craib) Y. Z. Wang, **comb. nov.** Basionym: *Chirita involucrate* Craib in Gard. Chron. Ser. 3, 83: 140, 25ii, 1928. D. Wood in Notes Roy. Bot. Gard. Edinb. 33 (1): 199. 1974.

Microchirita lavandulacea (Stapf) Y. Z. Wang, **comb. nov.** Basionym: *Chirita lavandulacea* Stapf in Curtis's Bot. Mag. T. 9047. 1925. D. Wood in Notes Roy. Bot. Gard. Edinb. 33 (1): 198. 1974.

Petrocodon Hance 石山苣苔属

Petrocodon coccineus (C. Y. Wu ex H. W. Li) Y. Z. Wang, **comb. nov.** Basionym: *Calcareoboea coccinea* C. Y. Wu ex H. W. Li in Acta Bot. Yunnan. 4 (3): 243, fig. 1. 1982. 朱红苣苔

Petrocodon fangianus (Y. G. Wei) J. M. Li et Y. Z. Wang, **comb. nov.** Basionym: *Paralagarosolen fangianus* Y. G. Wei in Acta Phytotax. Sin. 42 (6): 529, fig. 1. 2004. 方鼎苣苔

Petrocodon scopulorus (Chun) Y. Z. Wang, **comb. nov.** Basionym: *Tengia scopulorum* Chun in Sunyatsenia 6 (3–4): 281, pl. 46. 1946. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 250. 1998. — *Tengia potifolia* S. Z. He in J. China Pharm. Univ. 23 (5): 269. 1992. — *Tengia scopulorum* Chun var. *potiflora* (S. Z. He) W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 250. 1998. **syn. nov.** 世纬苣苔

Primulina Hance 报春苣苔属

Primulina baishouensis (Y. G. Wei, H. Q. Wen et S. H. Zhong) Y. Z. Wang, **comb. nov.** Basionym: *Chirita baishouensis* Y. G. Wei, H. Q. Wen et S. H. Zhong in Acta Phytotax. Sin. 38 (3): 299, fig. 1. 2000. 百寿苣苔

Primulina bipinnatifida (W. T. Wang) Y. Z. Wang et J. M. Li, **comb. nov.** Basionym: *Chiritopsis bipinnatifida* W. T. Wang in Bull. Bot. Res. 1 (3): 26, pl. 1, fig. 9–11, pl. 4, fig. 1. 1981. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 347. 1998. 羽裂小花苣苔

Primulina brachytricha (W. T. Wang et D. Y. Chen) R. B. Mao et Y. Z. Wang, **comb. nov.** Basionym: *Chirita brachytricha* W. T. Wang et D. Y. Chen in Bull. Bot. Res. 5 (3): 54, pl. 3, fig. 4–6. 1985. 短毛苣苔

Primulina carnosifolia (C. Y. Wu et H. W. Li) Y. Z. Wang, **comb. nov.** Basionym: *Chirita carnosifolia* C. Y.

- Wu et H. W. Li in Bull. Bot. Res. 3 (2): 36, photo. 17. 1983. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 329. 1998. 肉叶苣苔
- Primulina cordifolia* (D. Fang et W. T. Wang) Y. Z. Wang, **comb. nov.** Basionym: *Chiritopsis cordifolia* D. Fang et W. T. Wang in Bull. Bot. Res. 2 (4): 54. 1982. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 347. 1998. 心叶小花苣苔
- Primulina eburnea* (Hance) Y. Z. Wang, **comb. nov.** Basionym: *Chirita eburnea* Hance in Journ. Bot. 21: 168. 1883. Clarke in A. DC. Monogr. Phan. 5: 288. 1883; Wood in Notes Roy. Bot. Gard. Edinb. 33 (1): 138. 1974. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 323. 1998. 牛耳朵
- Primulina fimbriepala* (Hand.-Mazz.) Y. Z. Wang, **comb. nov.** Basionym: *Chirita fimbriepala* Hand.-Mazz. in Anz. Akad. Wiss. Wien. Math. -Nat. Kl. 62: 65. 1925. Wood in Notes Roy. Bot. Gard. Edinb. 33 (1): 144. 1974. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 327. 1998. 蚂蟥七
- Primulina fordii* (Hemsl.) Y. Z. Wang, **comb. nov.** Basionym: *Didymocarpus fordii* Hemsl. in Journ. Linn. Soc. Bot. 26: 229. 1890. *Chirita fordii* (Hemsl.) Wood in Notes Roy. Bot. Gard. Edinb. 31: 371, 1972 et 33 (1): 142. 1974. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 329. 1998. 桂粤报春苣苔
- Primulina gemella* (D. Wood) Y. Z. Wang, **comb. nov.** Basionym: *Chirita gemella* D. Wood in Notes Roy. Bot. Gard. 31: 370, iv. 1972. Wood in Notes Roy. Bot. Gard. Edinb. 33 (1): 141. 1974.
- Primulina glandulosa* (D. Fang, L. Zeng et D. H. Qin) Y. Z. Wang, **comb. nov.** Basionym: *Chiritopsis glandulosa* D. Fang, L. Zeng et D. H. Qin in Acta Phytotax. Sin. 31 (5): 470, fig. 1: 5–8. 1993. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 348. 1998. 紫腺小花苣苔
- Primulina gueilinensis* (W. T. Wang) Y. Z. Wang et Yan Liu, **comb. nov.** Basionym: *Chirita gueilinensis* W. T. Wang in Bull. Bot. Res. 1 (4): 43, photo. 2. 1981; et 5 (3): 48. 1985. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 328. 1998. 桂林报春苣苔
- Primulina hedyotideia* (Chun) Y. Z. Wang, **comb. nov.** Basionym: *Didymocarpus hedyotideus* Chun in Sunyatsenia 6: 290. 1946. *Chirita hedyotideia* (Chun) W. T. Wang in Bull. Bot. Res. 1 (4): 65. 1981; et 5 (3): 65. 1985. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 336. 1998. 肥牛草
- Primulina heterotricha* (Merr.) Y. Dong et Y. Z. Wang, **comb. nov.** Basionym: *Chirita heterotricha* Merr. in Lingnan Sci. Journ. 13: 71. 1934. Wood in Notes Roy. Bot. Gard. Edinb. 33 (1): 137. 1974. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 329. 1998. 烟叶苣苔
- Primulina jiuwanshanica* (W. T. Wang) Y. Z. Wang, **comb. nov.** Basionym: *Chirita jiuwanshanica* W. T. Wang in Guihaia 12 (4): 294, fig. 1: 4–6. 1992. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 325. 1998. 九万山报春苣苔
- Primulina langshanica* (W. T. Wang) Y. Z. Wang, **comb. nov.** Basionym: *Chirita langshanica* W. T. Wang in Guihaia 12 (4): 293, fig. 2: 1–3. 1992. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 327. 1998. 葭山苣苔
- Primulina laxiflora* (W. T. Wang) Y. Z. Wang, **comb. nov.** Basionym: *Chirita laxiflora* W. T. Wang in Bull. Bot. Res. 4 (1): 21, pl. 3: 1–2. 1984. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 322. 1998. 疏花苣苔
- Primulina leiophylla* (W. T. Wang) Y. Z. Wang, **comb. nov.** Basionym: *Chirita leiophylla* W. T. Wang in Guihaia 6 (3): 159, fig. 1(4–7). 1986. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 326. 1998. 光叶报春苣苔
- Primulina linearifolia* (W. T. Wang) Y. Z. Wang, **comb. nov.** Basionym: *Chirita linearifolia* W. T. Wang in Bull. Bot. Res. 2 (2): 136, photo. 3. 1983; et 5 (3): 60. 1985; W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 334. 1998. 线叶报春苣苔
- Primulina liujiangensis* (D. Fang et D. H. Qin) Yan Liu, **comb. nov.** Basionym: *Chirita liujiangensis* D. Fang et D. H. Qin in Acta Phytotax. Sin. 32 (6): 568, fig. 2: 1–3. 1994. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 339. 1998. 柳江苣苔
- Primulina longgangensis* (W. T. Wang) Yan Liu et Y. Z. Wang, **comb. nov.** Basionym: *Chirita longgangensis* W. T. Wang in Guihaia 2 (4): 171. 1982. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 333. 1998. 弄岗苣苔
- Primulina longii* (Z. Y. Li) Z. Y. Li, **comb. nov.** Basionym: *Chirita longii* Z. Y. Li in Novon 12 (4): 492, fig. 1. 2002. 龙氏苣苔
- Primulina medica* (D. Fang et W. T. Wang) Y. Z. Wang, **comb. nov.** Basionym: *Chirita medica* D. Fang W. T. Wang in Bull. Bot. Res. 1 (4): 64, fig. 3–5. 1981; et 5 (3): 42. 1985. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 322. 1998. 药用报春苣苔
- Primulina minutimaculata* (D. Fang et W. T. Wang) Y. Z. Wang, **comb. nov.** Basionym: *Chirita minutimaculata* D. Fang et W. T. Wang in Bull. Bot. Res. 1 (4): 55, photo. 8. 1981; et 5 (3): 45. 1985. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 325. 1998. 微斑苣苔
- Primulina mollifolia* (D. Fang et W. T. Wang) J. M. Li et Y. Z. Wang, **comb. nov.** Basionym: *Chiritopsis mollifolia* D. Fang et W. T. Wang in Guihaia 6 (1–2): 6, pl. 3, fig. 1–4. 1986. W. T. Wang et al., in Z. Y.

Wu et P. H. Raven, Fl. China 18: 346. 1998. 密毛小花苣苔

Primulina ophiopogoides (D. Fang et W. T. Wang) Y. Z. Wang, **comb. nov.** Basionym: *Chirita ophiopogoides* D. Fang et W. T. Wang in Bull. Bot. Res. 1 (4): 68, photo. 13. 1981. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 336. 1998. 条叶报春苣苔

Primulina parvifolia (W. T. Wang) Y. Z. Wang et J. M. Li, **comb. nov.** Basionym: *Chirita parvifolia* W. T. Wang in Bull. Bot. Res. 1 (4): 50, fig. 6–8. 1981; et 5 (3): 59. 1985. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 332. 1998. 小叶报春苣苔

Primulina pinnata (W. T. Wang) Y. Z. Wang, **comb. nov.** Basionym: *Chirita pinnata* W. T. Wang in Bull. Bot. Res. 4 (1): 25, pl. 1, fig. 10–13. 1984. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 334. 1998. 复叶苣苔

Primulina pinnatifida (Hand.-Mazz.) Y. Z. Wang, **comb. nov.** Basionym: *Didymocarpus pinnatifidus* Hand.-Mazz. in Sinensia 5: 8. 1934. *Chirita pinnatifida* (Hand.-Mazz.) Burt in Notes Roy. Bot. Gard. Edinb. 23: 99. 1960. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 334. 1998. — *Didymocarpus quercifolia* Wood in Notes Roy. Bot. Gard. Edinb. 31 (3): 369. 1972; et 33 (1): 144. 1974. 羽裂苣苔

Primulina pteropoda (W. T. Wang) Yan Liu, **comb. nov.** Basionym: *Chirita pteropoda* W. T. Wang in Bull. Bot. Res. 5 (3): 51, pl. 1, fig. 6–10. 1985. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 330. 1998. 翅柄苣苔

Primulina renifolia (D. Fang et D. H. Qin) J. M. Li et Y. Z. Wang, **comb. nov.** Basionym: *Wentsaiboea renifolia* D. Fang et D. H. Qin in Acta Phytotax. Sin. 42 (6): 534, fig. 1. 2004. 文采苣苔

Primulina repanda (W. T. Wang) Y. Z. Wang, **comb. nov.** Basionym: *Chiritopsis repanda* W. T. Wang in Bull. Bot. Res. 1 (3): 23, pl. 1, fig. 1–5, 12–13, pl. 3, fig. 1. 1981. 小花苣苔

Primulina sclerophylla (W. T. Wang) Yan Liu, **comb. nov.** Basionym: *Chirita sclerophylla* W. T. Wang in Bull. Bot. Res. 1 (4): 46, photo. 4. 1981. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 331. 1998. 硬叶报春苣苔

Primulina shouchengensis (Z. Y. Li) Z. Y. Li, **comb. nov.** Basionym: *Chirita shouchengensis* Z. Y. Li in Novon 7 (4): 426. 1997. W. T. Wang et al., in Z. Y. Wu et P. H. Raven, Fl. China 18: 338. 1998. 寿城苣苔

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Supplementary Material

Additional Supporting Information may be found in the online version of this article:

Table S1. Species, voucher with collection locality,

and GenBank accession numbers for taxa included in this study.

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