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Reassessment of *Chirita umbrophila* (Gesneriaceae) Based on Molecular and Morphological Evidence

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Abstract—*Chirita umbrophila* is a species endemic to Yunnan, China, and its exact systematic position remains a mystery since it was treated as a species with uncertain generic affiliation in the taxonomic revision of *Chirita* in 1985. In the present study, the phylogenetic relationships between *C. umbrophila* and its allied species were inferred using two nuclear ribosomal DNA regions (ETS and ITS) and three chloroplast regions (*rpl16*, *rps16*, and *trnL-F*). Additionally, the type locality of *C. umbrophila* was revisited and flowering specimens were collected and examined. Our phylogenetic analyses showed that *Chirita umbrophila* is imbedded in three accessions of *Loxostigma kurzii* with strong support. Morphological observations revealed that *C. umbrophila* can be characterized as perennial herbs with somewhat tufted leaves at the top of the aerial stem, large ventricose corollas with yellow-brown spotted interior surfaces, four anthers cohering in pairs, and two equal or subequal stigmas. Detailed morphological analyses suggested that *C. umbrophila* is indistinguishable from *Loxostigma kurzii* (\equiv *Didymocarpus? kurzii*). Therefore, both molecular phylogenetic results and morphological evidence support that *Chirita umbrophila* is conspecific with *Loxostigma kurzii*, and a taxonomic treatment is provided herein.

Keywords—Briggsia, *Loxostigma*, phylogeny, taxonomic treatment.

During the past two decades, field investigations by both botanists and plant hobbyists discovered numerous new gesneriads in China, providing surprising insights into species diversity of Gesneriaceae. The number of species in Gesneriaceae in China has had a marked increase from 1990 to now, evident in the 413 species recorded in the *Flora Reipublicae Popularis Sinicae* (Wang et al. 1990) and the ca. 690 species compiled in the latest list of Chinese Gesneriaceae (Wen et al. 2021). There are more than 60 authors who have recently contributed to the species discovery in China (Möller 2019). However, compared to the strong enthusiasm of searching and describing new species, few people focus their interest on older and problematic species (but see Hong et al. 2020; Lu et al. 2020; Yang et al. 2020). In fact, several species of Gesneriaceae from China described earlier need to be reassessed due to a lack of key morphological characters. For example, *Oreocharis rhytidophylla* C.Y.Wu ex H.W.Li was a doubtful species described on the basis of a single collection with only fruiting material, and its specific status was not confirmed until the rediscovery of flowering plants in the type locality recently (Zhang et al. 2019). An accurate estimate of biological species is critical for fully understanding biodiversity. Therefore, in the present study, we focus on another unknown species, i.e. *Chirita umbrophila* C.Y.Wu ex H.W.Li.

Chirita umbrophila was originally described by Wu (in Li 1983) based on one collection (*Ping-Hua Yu* 1189; Fig. 1A) without flowers. The type locality of *C. umbrophila* is Zhenxiong county, Yunnan province, China (Fig. 2E), and up until now no other specimens were collected after the gathering of the type specimens. Li (1983) placed *C. umbrophila* in *Chirita* sect. *Chirita* (most species in this section had been transferred to the genus *Henckelia* Spreng. by Weber et al. (2011)), and allied it to *Chirita pumila* D.Don [\equiv *Henckelia pumila* (D.Don)

A.Dietr.] and *Chirita forrestii* J.Anthony [\equiv *Henckelia forrestii* (J.Anthony) D.J.Middleton & Mich.Möller]. The recognition of *Chirita umbrophila* as a species belonging to *Chirita* Buch.-Ham. ex Don was mainly based on its taller stem, green-spotted leaves, smaller sepals, and stubby capsules compared to its allies (Li 1983). But the most important diagnostic feature of *Chirita* (now a synonym of *Henckelia* Spreng.) was the presence of the chiritoid stigmas (Brown 1840; Burtt 1965; Wood 1974; Wang 1985a) characterized by a barely developed upper stigma and enlarged lower stigma with usually emarginate to bifid apex (see Results regarding the description of stigma; Weber et al. 2020). However, it was not possible to examine the chiritoid stigmas of *Chirita umbrophila* in the fruiting type specimens. Therefore, Wang (1985b) treated *C. umbrophila* as a species with uncertain generic affiliation. The generic affiliation of *C. umbrophila* was not addressed in several following important publications, including the *Flora Reipublicae Popularis Sinicae* (Wang et al. 1990), *Flora of China* (Wang et al. 1998), and *Plants of Gesneriaceae in China* (Li and Wang 2004). Then, *Chirita* was shown to be highly polyphyletic and disintegrated based on both molecular and morphological evidence (Wang et al. 2011; Weber et al. 2011), and Weber et al. (2011) refrained from placing the species into *Henckelia* and cited "*Chirita umbrophila* C.Y.Wu ex H.W.Li: generic placement uncertain (see Wang et al. 1998)." Up to now, *Chirita umbrophila* remains a species with unknown generic affiliation.

Here we examine the phylogenetic relationships of *C. umbrophila* compared to morphologically similar species and establish species boundaries based on monophyletic groups that can be characterized with phenotypic characters. The aims of this research were to: 1) investigate the floral morphology of *Chirita umbrophila*, especially the stigma; and



FIG. 1. Type specimens of *Chirita umbrophila* (A) and *Loxostigma kurzii* (B–D). A. Holotype of *Chirita umbrophila* (China, Yunnan, Zhenxiang, P.H. Yu 1189, KUN [barcode] KUN1219130). B. Holotype of *Didymocarpus kurzii* (India, Sikkim, Kurz s.n., CAL [barcode] CAL0000019236). C. Lectotype of *Didissandra amabilis* (China, Yunnan, Lijiang, G. Forrest 2689, E [barcode] E00387561). D. Lectotype of *Briggsia amabilis* var. *taliensis* (China, Yunnan, Dali, G. Forrest 4385, E [barcode] E00387582). Note: B–D, copyright © The director of CAL and E, respectively. Used with permission.

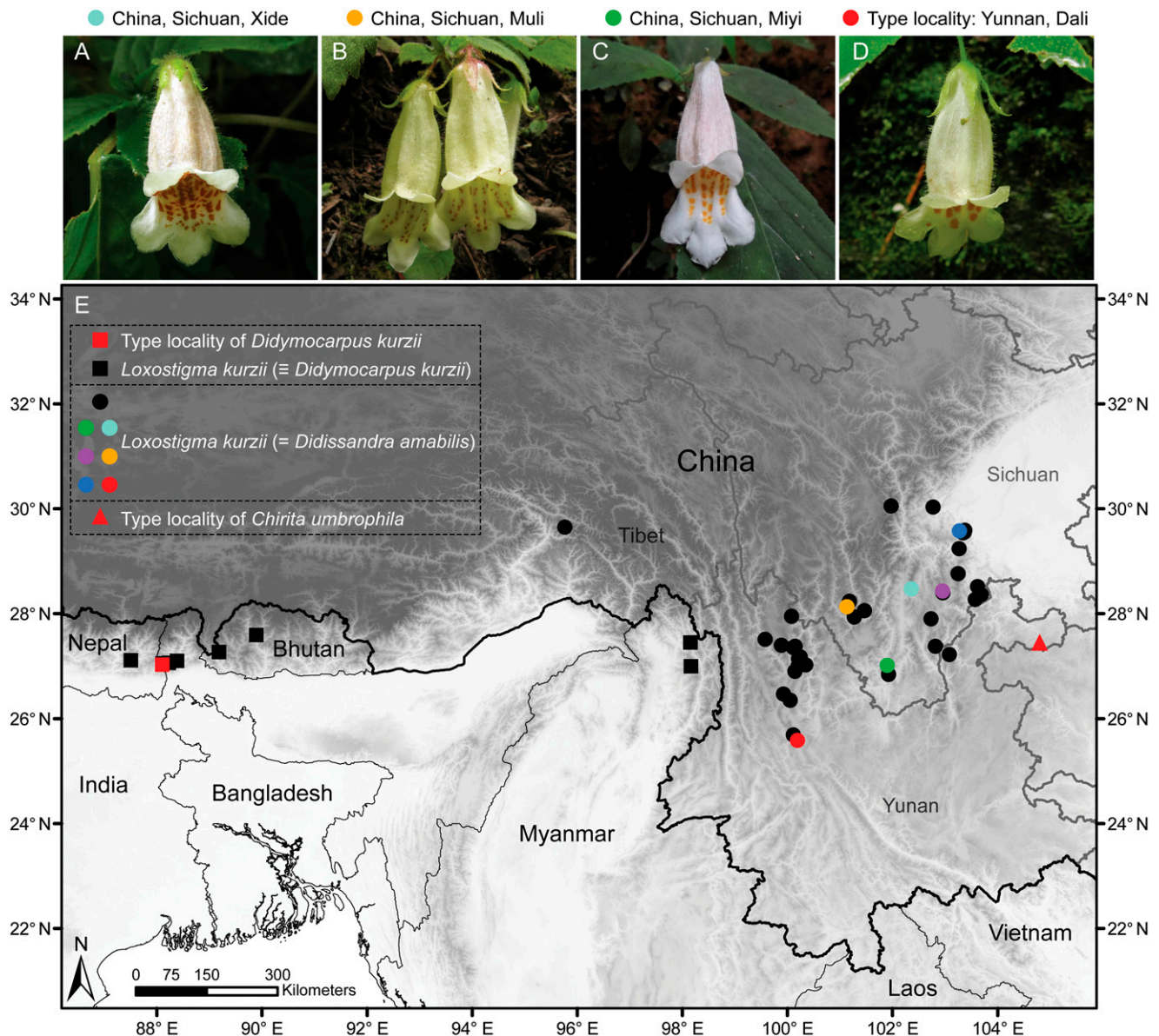


FIG. 2. A–D. Variation of corolla color of *Loxostigma kurzii*. E. The known geographical distribution of *L. kurzii* and *Chirita umbrophila*. The type localities and the samples included in the phylogenetic analysis (Fig. 3) are labeled on the map.

to 2) provide a taxonomic treatment based on the results of molecular phylogenetic reconstruction and comparative morphology.

MATERIALS AND METHODS

Sampling for Phylogenetic Study, Amplification, and Sequencing—

To infer the possible phylogenetic position of *Chirita umbrophila*, we included 61 accessions representing 19 out of 35 genera of subtribe Didymocarpaceae (GRC 2021). We included three individuals of *Loxostigma kurzii* (C.B. Clarke) B.L. Burtt, which has a wide distribution from eastern Nepal to southwestern China (Fig. 2E; Wang et al. 1998; Sun and Zhou 2002; Möller et al. 2014; Sinha and Datta 2016). Two species of *Microchirita* (C.B. Clarke) Yin Z. Wang were chosen as outgroups according to the phylogeny inferred by Möller et al. (2011a). In addition, we selected representative samples of the straight-fruited advanced Asiatic and Malesian genera (Möller et al. 2009) as ingroups based on main clades recovered in Möller et al. (2011a). Sequences of approximately half of the included samples were retrieved from several preceding phylogenetic studies (Möller et al. 2009, 2011a; Qiu et al. 2015; Xu et al. 2021) and 141 sequences of the remaining samples were newly acquired. The full list of sampled

species and corresponding NCBI accession numbers are provided in Appendix 1.

Total genomic DNA was extracted from silica-dried leaves using a modified CTAB protocol (Doyle and Doyle 1987) and used as the template in the polymerase chain reaction (PCR). The nuclear ribosomal external transcribed spacer (ETS), internal transcribed spacer (ITS, including ITS1, 5.8S subunit, and ITS2), and three chloroplast DNA regions (*rpl16*, *rps16*, *trnL-F*) were amplified using primers ETS-B/18S-IGS (Beardsley and Olmstead 2002), ITS-p5/u4 (Cheng et al. 2016), rpl16-F71/R1661 (Jordan et al. 1996; Kelchner and Clark 1997), rps16-2F/R3 (Bremer et al. 2002), and *trnL-F-c/f* (Taberlet et al. 1991), respectively. The PCR conditions for all DNA regions included initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 54°C for 30 s, and 72°C for 50 s, with a final extension at 72°C for 5 min. The PCR products were purified using Tian quick Midi Purification Kit (Tiangen Biotech, Beijing, China) following the manufacturer's protocol, and then were directly sequenced with the same primers used for PCR amplification. All the sequences generated in the present study have been deposited in GenBank (Appendix 1).

Sequence Alignment and Phylogenetic Analyses—Editing and contig assembly were performed in Geneious v. 7.1.8 (Kearse et al. 2012). The newly obtained sequences together with those retrieved from GenBank were aligned with Clustal W (Larkin et al. 2007), followed by manual

adjustment in Geneious v. 7.1.8. Three data matrices, i.e. nuclear (concatenated ETS and ITS), chloroplast (concatenated *rpl16*, *rps16*, and *trnL-F*), and the combined dataset (concatenated nuclear and chloroplast datasets), were generated and used for downstream phylogenetic analyses. The combinability of nuclear and chloroplast was determined by the incongruence length difference (ILD) test (Farris et al. 1994, 1995) implemented in PAUP* 4.0b10 (Swofford 2003). Because the systematic positions for five species, i.e. *Didymocarpus glandulosus* var. *minor* (W.T.Wang) W.T.Wang, *Allostigma guangxiense* W.T.Wang, *Allocheilos guangxiensis* H.Q.Wen, Y.G.Wei & S.H.Zhong, *Petrocosmea minor* Hemsl., and *Didymostigma obtusum* (C.B.Clarke) W.T.Wang, were strongly conflicting between the nuclear and chloroplast phylogenies (see Results), we removed these species in the combined dataset. Both maximum likelihood (ML) and Bayesian inference (BI) were selected to infer the phylogenetic relationships. The ML analysis was performed in IQtree v. 1.7 (Nguyen et al. 2015), and the best-fit model for each gene was automatically determined using the implementation of ModelFinder (Kalyaanamoorthy et al. 2017). Branch support was assessed with 1000 standard nonparametric bootstrap (BS) replicates. Bayesian inference analyses were conducted using MrBayes v. 3.2.7 (Ronquist et al. 2012). For the BI analyses, we determined the best-fit model of evolution for each gene using MrModeltest v. 2.3 (Nylander 2004) according to the Akaike information criterion (AIC; Akaike 1974). The BI analyses consisted of two independent Markov chain Monte Carlo (MCMC) runs each with one cold chain and three heated chains for 10,000,000 generations. For all Bayesian analyses, we ensured that the standard deviation of split frequencies was less than 0.001 and the potential scale reduction factor approached 1 for all parameters. The MrBayes outputs were checked in Tracer v. 1.7 (Rambaut et al. 2018) to confirm that all effective sample size (ESS) values were more than 200. The first 25% of posterior trees were discarded as burn-in and the 50% majority rule consensus trees were generated from the remaining trees. The obtained trees were visualized in FigTree v. 1.4.4 (A. Rambaut, University of Edinburgh, <http://tree.bio.ed.ac.uk/software/figtree>) and modified in Adobe Illustrator (Adobe Systems, Inc. San Jose, California).

Morphological Observations—Molecular phylogenetic analyses recovered *Chirita umbrophila* imbedded in the three accessions of *Loxostigma kurzii* (see results). Therefore, firstly, we consulted publications related to *L. kurzii* and *C. umbrophila* (Clarke 1874, 1876, 1883; Kuntze 1891; Diels 1912; Craib 1919; Evans 1928; Burt 1975; Li 1983; Wang et al. 1990, 1998; Vitek et al. 1997, 2000; Li and Wang 2004; Weber et al. 2011; Möller et al. 2014). Then, we studied herbarium specimens of *C. umbrophila* and its allies, especially *L. kurzii* (\equiv *Didymocarpus kurzii* C.B.Clarke = *Didissandra amabilis* Diels), in CAL, CUH, IBSC, KUN, and PE. We also examined high-resolution digital images and photographs of specimens through the online resources of Chinese Virtual Herbarium (<https://www.cvh.ac.cn/>), herbarium of Royal Botanic Garden Edinburgh (<https://data.rbge.org.uk/search/herbarium/>), GBIF (<https://www.gbif.org/>), and JSTOR (<https://plants.jstor.org/>). Additionally, living materials of *C. umbrophila* were observed in the type locality (Yunnan province, Zhenxiong county) and *L. kurzii* was observed in Sichuan (Xide, Muli, and Miyi counties) and Yunnan (Dali city) provinces. Voucher specimens of *C. umbrophila* were collected in the type locality and deposited in IBSC for future reference. Herbarium acronyms follow Thiers (2021).

RESULTS AND DISCUSSION

Phylogenetic Analyses—The general statistics of all datasets were summarized in Table 1. The alignment length of nuclear dataset was 1353 base pairs (bp), of which 660 bp

(48.78%) were parsimony-informative. The chloroplast alignment contained 2894 bp, including 10.57% parsimony-informative characters (306 bp). The total alignment length of the combined dataset was 4228 bp, of which 922 bp (21.81%) were parsimony-informative. Results of MrModeltest show that the best fit models were GTR+I+G for ETS, ITS, *rpl16*, and *rps16* data matrices and GTR +G for *trnL-F* dataset according to the AIC (Table 1). The ILD test gave a value of $p = 0.11$ after removing the five conflicting taxa, indicating that the remaining data did not contain significant incongruence.

Phylogenetic results inferred from different sequence matrices (nuclear, chloroplast, and combined datasets) and methods (ML and BI) recovered a strongly supported clade consisting of *C. umbrophila* and three samples of *L. kurzii* (Fig. 3; Figs. S1–S5, Li et al. 2021). *Henckelia*, which is sister to all the other ingroup taxa with maximum support, is phylogenetically isolated from the aforementioned clade (Fig. 3). The phylogenies inferred from both nuclear and combined datasets clearly revealed that *C. umbrophila* is clustered with one accession of *L. kurzii* (LPW2012029) with moderate support and the other two accessions of *L. kurzii* (MMO08–1249 and SCMG03) are grouped together with strong support (Fig. 3; Figs. S1–S2, S5). However, the chloroplast dataset failed to recover a bifurcating phylogeny for these four accessions (Figs. S3–S4) likely due to insufficient parsimony-informative characters. Supplemental figures (Figs. S1–S5) are available from the Dryad Digital Repository (Li et al. 2021).

Morphological Observations—The type locality of *Chirita umbrophila* can be unambiguously traced according to the record (China: Yunnan province, Zhenxiong county, Moduo town [now Changba town, Guochang village, Moduo branch], Bojiwan) of type specimens (Fig. 1A). Therefore, we successfully rediscovered *C. umbrophila* in the type locality in April 2021, but unfortunately, the aerial part of the plants was just emerging from the slender underground rhizome (Fig. 4H). Our second visit to the type locality of *C. umbrophila* was carried out in late August 2021, and *C. umbrophila* entered its flowering stage then. We found that the dense hairs on the stem of the young plants (Fig. 4A–B) gradually disappeared on mature plants, and the more or less unequal herbaceous opposite leaves usually cluster at the top of the stem (Fig. 4C). Most importantly, we observed the floral characters of *C. umbrophila* for the first time, and our detailed observations revealed that the corolla of this species is white with yellow-brown spots on the interior, the tube is distinctly ventricose on the lower side, four fertile stamens are coherent in pairs, the thecae are divaricate with confluent apex, and the two stigmas are equal or subequal (Fig. 4D–G).

TABLE 1. Summary of the alignments employed in the phylogenetic analysis.

Data matrices	Number of accessions	Alignment length (base pairs)	Constant sites (base pairs)	Parsimony-uninformative variable sites (base pairs)	Parsimony-informative sites (base pairs)	Model by AIC
ETS	34	525	174	77	274	GTR+I+G
ITS	60	828	312	130	386	GTR+I+G
<i>rpl16</i>	29	1053	878	95	80	GTR+I+G
<i>rps16</i>	40	937	710	108	119	GTR+I+G
<i>trnL-F</i>	60	904	685	112	107	GTR+G
nuclear	61	1353	486	207	660	—
chloroplast	61	2894	2273	315	306	—
combined	56	4228	2816	490	922	—

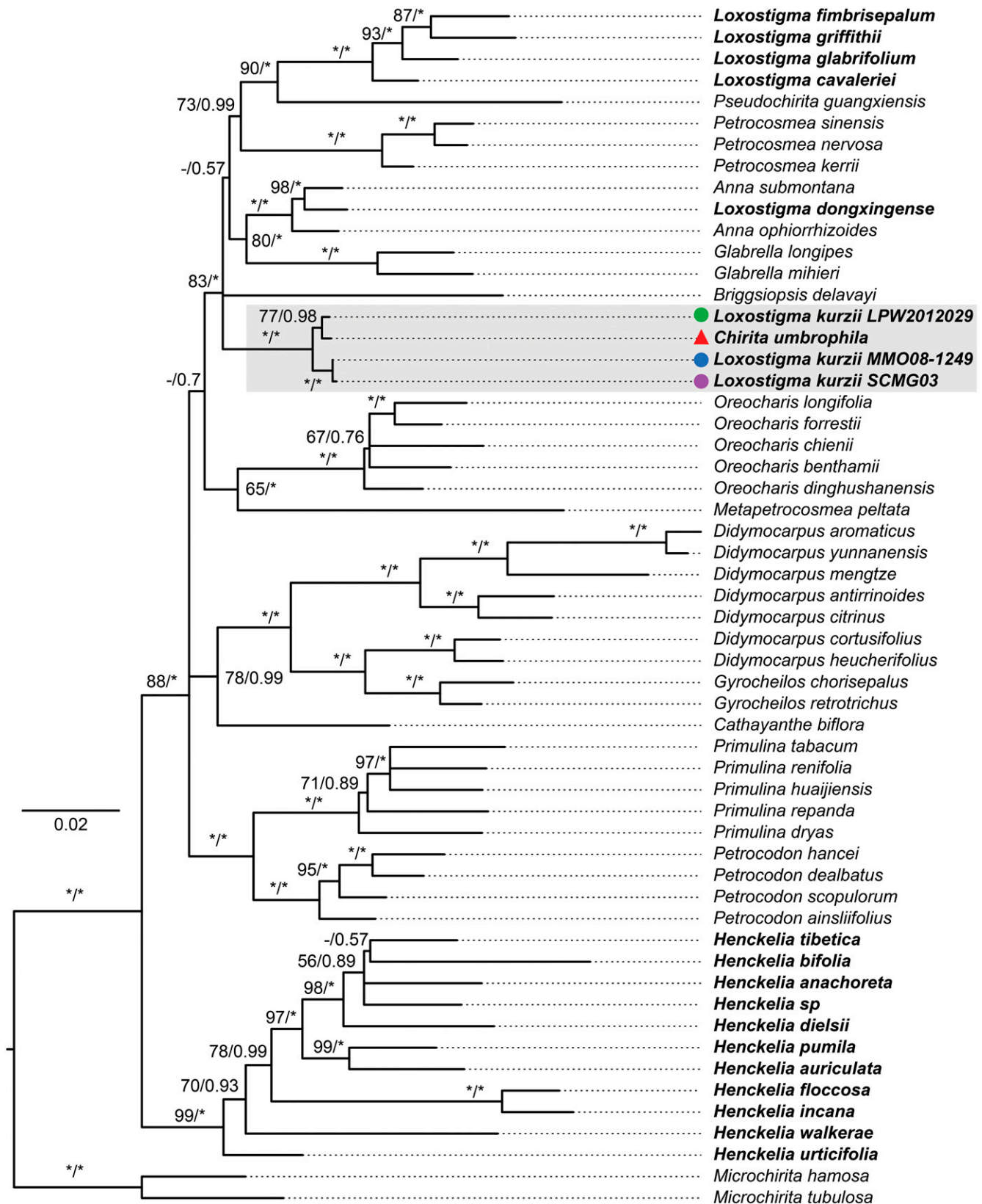


FIG. 3. Maximum likelihood (ML) tree inferred from combined nuclear (ETS, ITS) and chloroplast (*rpl16*, *rps16*, *trnL-F*) datasets. ML bootstrap values and Bayesian inference (BI) posterior probabilities are indicated on the left and right sides of the slashes, respectively. Asterisks indicate maximum support, and dashes represent no support. Colored dots and red triangle correspond to those in Fig. 2.

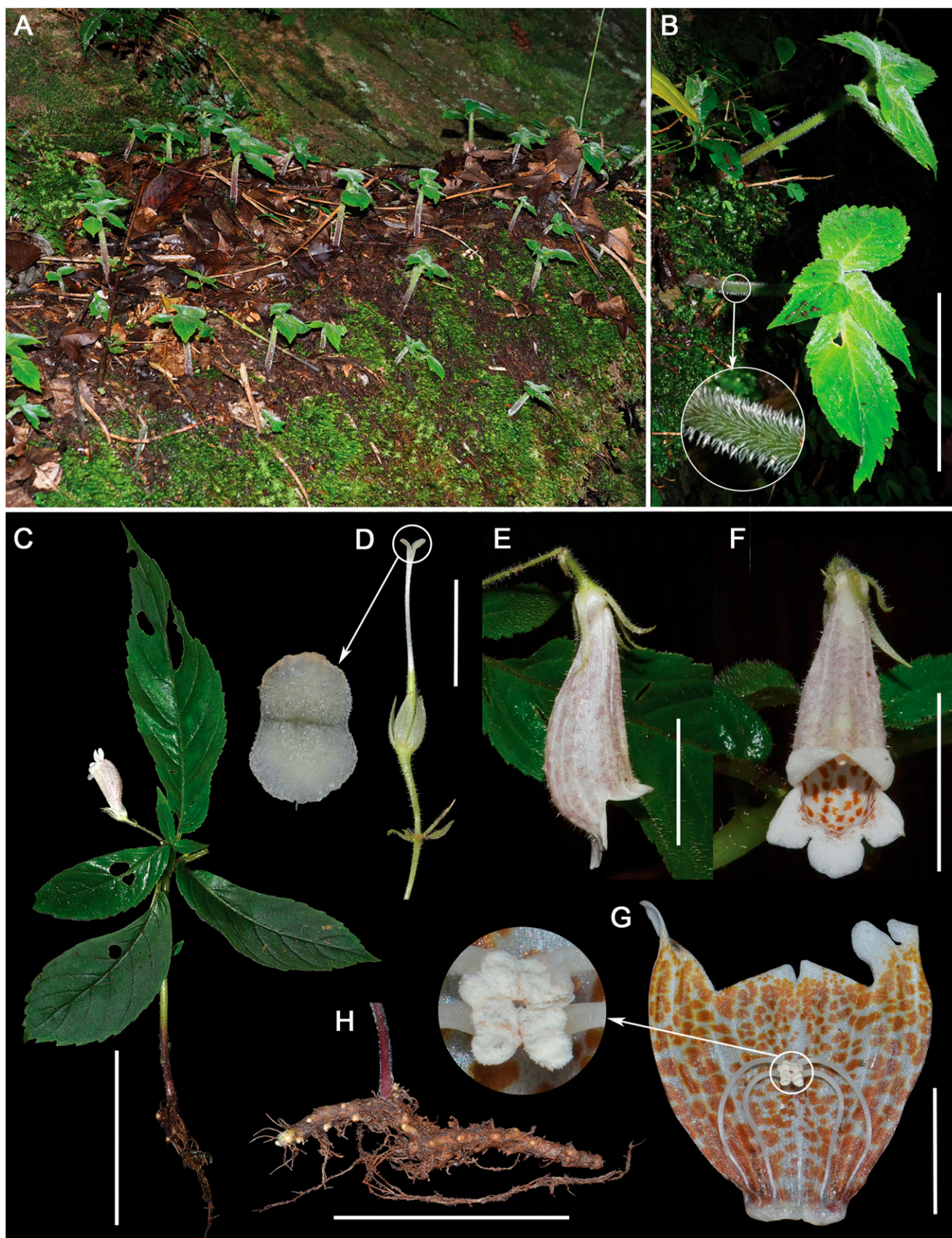


FIG. 4. Morphological characters of *Chirita umbrophila*. A. Elongated shoots arising from the underground rhizomes. B. Young shoots, showing dense eglandular hairs on the stems. C. Habit, indicating the leaves are mainly arranged at the top of stem. D. Pistil, showing the calyx divided to base and the two sub-equal stigmas. E. Side view of the white and distinctly ventricose corolla. F. Front view of corolla. G. Dissection of the corolla, showing the yellow-brown spots and four fertile stamens with each pair coherent and thecae confluent. H. Slender underground rhizome. Scale bars: B, C, H = 10 cm; D–G = 2 cm.

The description of the stigma as “bilobed” in Gesneriaceae is often blurred as the two stigma lobes may either represent the lobes of the upper and lower carpel or fused carpel halves (left- and right-position of stigma lobes) (Burt 1954; Weber et al. 2020). Since the two ovary carpels may each produce a stigma (Wang et al. 1998), we would like to treat the upper and lower “stigma lobes” as two stigmas in the present study to avoid confusion with the left- and right-position of stigma lobes frequently seen in species of *Oreocharis* Benth. and *Petrocodon* Hance as well as many Gesnerioideae (Weber et al. 2020). Our morphological observation found that the two well-developed stigmas of *C. umbrophila* (Fig. 4D) clearly demonstrate that it is not a member of the genus *Chirita*, which possess chiritoid stigmas (Wang et al. 2011; Weber et al. 2011, 2020). *Chirita umbrophila* is characterized by a broad ventricose white corolla with yellow-brown spots on the interior and four anthers cohering in pairs as well as two equally or sub-equally developed lamellate stigmas, which fit well into the concept of the genus *Loxostigma* (sensu Möller et al. 2014). In addition, its slender underground rhizomes, cylindrical stems, short internodes, often entire sepals, straight capsules, and ellipsoid seeds without appendages indicate its affinity with *L. kurzii* (Fig. 1B–D).

The main morphological difference between *C. umbrophila* and *L. kurzii* is that the former has a white corolla while the latter usually has a yellow to orange one (Wang et al. 1998). Actually, we didn't observe the orange corollas of *L. kurzii* possibly due to our insufficient field investigations. However, both white and pale-yellow corollas of *L. kurzii* could be easily found in the field (Fig. 2A–D), and populations with white corollas are rather common across the distribution range of *L. kurzii* and can be observed in Yunnan [Lijiang: *Yu-Zhang Zhao* 21414 (KUN); Xianggelila: *Zhen-Dong Fang et al.* DJDC-688 (SABG)] and Sichuan [Emeishan: *Xing-Lin Jiang & Xiu-Shi Zhang* 31823 (PE); Meigu: *Anonymous* 1930 (SM); Miyi: *Peng-Wei Li* LPW2012029 (PE); Zhaojue: *Zhaojue Expedition* 0909 (SM)] provinces. Besides, records in the Plant Photo Bank of China (PPBC; <http://ppbc.iplant.cn/>) showed that individuals with white or light-yellow flowers even occur in the same population at Emeishan, Sichuan province. Clearly, *Chirita umbrophila* lies within the morphological range of variation for *Loxostigma kurzii*.

Our molecular phylogenetic analyses showed that *Chirita umbrophila* is imbedded in a strongly supported clade comprising otherwise three accessions of *Loxostigma kurzii*, and the comparative morphological analysis revealed that the two species are indistinguishable. Therefore, *Chirita umbrophila* should be reduced to synonymy of *Loxostigma kurzii*.

TAXONOMIC TREATMENT

- LOXOSTIGMA KURZII (C.B. Clarke) B.L. Burt, Notes Roy. Bot. Gard. Edinburgh 34 (1): 104. 1975. *Didymocarpus? kurzii* C.B. Clarke, Commelyn. Cyrtandr. Bengal. 96, t. 66. 1874. *Chirita kurzii* (C.B. Clarke) C.B. Clarke, J. Linn. Soc., Bot. 15: 145. 1876. *Roettlera kurzii* (C.B. Clarke) Kuntze, Revis. Gen. Pl. 2: 476. 1891. *Briggsia kurzii* (C.B. Clarke) W.E. Evans, Notes Roy. Bot. Gard. Edinburgh 16 (77): 133. 1928. TYPE: INDIA. Sikkim (now West Bengal), prope Kursiong (now Kurseong), *Kurz s.n.* (holotype: CAL! [CAL0000019236]).
- Didissandra amabilis* Diels, Notes Roy. Bot. Gard. Edinburgh 5 (25): 224. 1912. *Briggsia amabilis* (Diels) Craib, Notes Roy.

Bot. Gard. Edinburgh 11 (55): 263. 1919. TYPE: CHINA. Yunnan, eastern flank of the Lichiang Range [now Yulong Snow Mountain], Lat. 27°25'N, alt. 10,000–11,000 ft., fl. Aug. 1906, *G. Forrest* 2689 (lectotype: E image! [E00387561], first-step lectotype designated by Vitek et al. (1997: 278); second-step lectotype designated by Möller et al. (2014: 200); isolectotypes: E image! [E00387559, E00387560]).

Briggsia amabilis var. *taliensis* Craib, Notes Roy. Bot. Gard. Edinburgh 11 (55): 263. 1919. TYPE: CHINA. Yunnan, eastern flank of the Tali Range [now Cangshan], Lat. 25°40'N, alt. 9000–10,000 ft., fl. Jul.–Aug. 1906, *G. Forrest* 4385 (lectotype: E image! [E00387582], designated by Möller et al. (2014: 200); isolectotypes: BM image! [BM000041702], IBSC [IBSC0004824], P image! [P03511189]); *ibid.*, fl. Jul. 1910, *G. Forrest* 6893 (syntypes: BM image! [BM000041705], E image! [E00387593], PE! [00140242]).

Chirita umbrophila C.Y. Wu ex H.W. Li, Bull. Bot. Res., Harbin 3 (2): 38. 1983, syn. nov. TYPE: CHINA. Yunnan, Zhenxiang, Bojiwan, alt. 1650 m, 3 Oct. 1957, *P.H. Yu* 1189 (holotype: KUN! [KUN1219130]; isotypes: KUN! [KUN1219133], PE! [00030725]).

Perennial herbs with slender underground rhizomes and long aerial stems. **Stems** 12–30(–50) cm, densely white villous when young, sparsely pubescent or glabrous when flowering and fruiting. **Leaves** opposite, usually somewhat crowded at the upper part of stem; petioles 0.5–8 cm, sparsely pubescent to puberulent; leaf blade oblanceolate to obovate or ovate, 4–17 × 2–7 cm, base oblique, narrowly cuneate, to rounded, margin serrate, apex acuminate, adaxially sparsely appressed pubescent, abaxially nearly glabrous, sparsely pubescent along veins; lateral veins 5–8 on each side of midrib, conspicuous. **Inflorescences** axillary cymes, 1–4-flowered. Peduncles 1–8 cm long, pubescent, intermixed with glandular hairs. Bracts 2, lanceolate to linear, 2–5 × 1–2 mm, margin entire, sparsely pubescent outside, glabrous inside. Pedicels 4–15 mm long, pubescent and glandular pubescent. Calyx lobes lanceolate to narrowly triangular, 1–1.8 × 0.2–0.3 cm, margin entire to sparsely denticulate, sparsely tomentose outside, glabrous inside. **Corolla** yellow, orange, or white, ventricose, 3–5 cm long, pubescent outside, glabrous inside, densely yellow-brown spotted; tube 2.8–4 cm long, slightly constricted above base; upper lip 0.8–1.2 cm long, lobes semi-orbicular, 6–8 × 7–10 mm, apex rounded; lower lip 1–1.5 cm long, 3-lobed, lateral lobes broadly ovate, ventral lobe suborbicular to semi-orbicular, apex obtuse to rounded. **Stamens** 4, inserted at ca. 6 mm above the corolla base; filaments 2–3 cm long, glabrous; anthers narrowly globose, coherent in pairs, thecae confluent; dorsal staminode ca. 2 mm, adnate to corolla ca. 5 mm above base. **Gynoeceum** pubescent and glandular pubescent; ovary linear, 1–1.5 cm long; style 1–2 cm long; stigmas 2, lamellar, equal or subequal. **Capsules** linear, 3.5–4.5 cm long, glabrescent, style persistent. Seeds ca. 0.5 mm long, acute at both ends.

Distribution and Habitat—*Loxostigma kurzii* is distributed in Nepal, India (West Bengal), Bhutan, Myanmar, and China (southern Sichuan, northern Yunnan, and southeastern Tibet) (Fig. 2E), and up to this point field investigations to southeastern Tibet have been scarce and much work is needed to recover more comprehensive distribution data for *L. kurzii*. This species usually grows on rocks covered with soil and moss at altitudes 1100–3600 m above sea level. Its slender rhizome serves the survival of periods with adverse conditions, such as a cold winter.

Phenology—Flowering from June to September and fruiting from August to October.

Notes—*Loxostigma kurzii* has a complicated taxonomic history. There are seven synonyms under the name *L. kurzii*, including four homotypic and three heterotypic synonyms. The basionym of *L. kurzii* is *Didymocarpus? kurzii* C.B. Clarke, which was described in 1874 based on a fruiting specimen deposited at CAL (Clarke 1874). Clarke (1876) further observed flowering material of this species, and then transferred it to the genus *Chirita*. Although Clarke (1883) had restricted the genus *Chirita* to a group of species with only two fertile stamens in his monograph of Cyrandreae, he still placed the four fertile stamens species (*Chirita kurzii*) in this genus without any comment. A few years later, this species was transferred into *Roettlera* Vahl by Kuntze (1891) in his monograph (*Revisio Generum Plantarum*). Evans (1928) realized the similarity between *Chirita kurzii* and *Briggsia amabilis*, and thus he placed the former in the genus *Briggsia*, and gave a new combination, i.e. *Briggsia kurzii* (C.B. Clarke) W.E. Evans.

Didissandra amabilis Diels is one heterotypic synonym of *Loxostigma kurzii* published in 1912 based on two collections (Fig. 1C–D) from Yunnan, China. Craib (1919) redefined *Didissandra* C.B. Clarke as perennial herbs with thick woody rhizomes, rosette-form leaves, medium-sized or small cylindrical corollas, densely hairy inner surfaces of corolla tubes, and coiled filaments. Therefore, *Didissandra amabilis* characterized by perennial herbs with long stems, large ventricose corollas, glabrous inner surfaces of corolla tubes, and slightly curved filaments is incompatible with *Didissandra* in the strict sense. To accommodate the new definition of *Didissandra*, Craib (1919) placed *D. amabilis* in the genus *Briggsia* and established a variety of *Briggsia amabilis* based on slight differences of flowers. Evans (1928) proposed that *B. amabilis* was probably a synonym of *Briggsia kurzii*, a viewpoint also shared by Burt (1975). Now, it is widely accepted that *B. amabilis* is a synonym of *B. kurzii* (Wang et al. 1998; Li and Wang 2004) since both species are similar in all points, including seeds without appendages (Evans 1928).

The genus *Loxostigma* Clarke differs from caulescent *Briggsia* species mainly by its appendaged seeds (Wang et al. 1990, 1998). Burt (1975) expanded *Loxostigma* to include *Briggsia kurzii*, a species with tail-less seeds, and this taxonomic treatment was followed by Vitek et al. (2000). Recently, molecular phylogenetic studies showed the genus *Briggsia* is highly polyphyletic, and it was dismantled with the type species (*Briggsia longifolia* Craib) transferred to the genus *Oreocharis* Benth. (Möller et al. 2011b). Therefore, Möller et al. (2014) revived the name *Loxostigma kurzii* and also transferred three other truly caulescent *Briggsia* species to *Loxostigma* to accommodate their nomenclaturally unacceptable position. However, it is worth noting that the placement of all these truly caulescent *Briggsia* species in *Loxostigma* is not supported by the present and other phylogenetic studies (Wang et al. 2010; Möller et al. 2011a). Nevertheless, we cannot make reasonable taxonomic treatments regarding other *Loxostigma* species in the absence of a robust phylogenetic framework. Therefore, we temporarily treat *Chirita umbrophila* as a synonym of *Loxostigma kurzii*.

Additional Specimens Examined—**Bhutan**. Falu fa, alt. 8000 ft, 29 Aug 1915, R.E. Cooper 4689 (E [E00394337] image!); Seeds collected in Ha Dzong, alt. 10500 ft, cultivated in Royal Botanic Garden, Edinburgh, 1954, L. Shenriff 17555 (E [E00628128] image!). **China**. —SICHUAN: alt. 2500 m, 1 Sep 1938, T.P. Wang 9436 (PE [00140230]); ibid., 1952, W.J. Zheng 6529 (PE [00140260]); ibid., 1930, T. Tang 23399 (PE [00140232]); ibid., 30 Jul 1978, *Anonymous* 547 (SM [SM717900134] image!); Ebian, Shaping, Lugouqiao, 21 Aug 1938, Z.W.

Yao 2819 (NAS [NAS00242662] image!, PE [00140237]); Emeishan, 1952, J.H. Xiong, X.S. Zhang & X.L. Jiang 32295 (IBK [IBK00054328]!, IBSC [0548670]); Emeishan, Qilipo, 29 Jul 1952, X.L. Jiang & X.S. Zhang 31823 (PE [00140238]); Emeishan, Jieyindian, alt. 2500 m, 12 Aug 1957, G.H. Yang 56604 (IBSC [0548666]); KUN [0206448]!, NAS [NAS00242664] image!, PE [00140239]; Emeishan, Jiulaodong, alt. 1800 m, 18 Aug 1957, G.H. Yang 56719 (IBSC [0548667]); NAS [NAS00242661] image!, PE [00140240]; Leibo, alt. 2900 m, 16 Aug 1934, T.T. Yu 3804 (PE [00140233]); Leibo, Xining, Ahegou, alt. 2400 m, 12 Aug 1972, 236 Team 0738 (PE [01805638]); ibid., alt. 1600 m, 11 Aug 1972, 236 Team 0652 (PE [01805636]); Meigu, Houbonaituo, 22 Aug 1959, *Sichuan Econ. Pl. Exped.* 10930 (PE [00140234]); Meigu, Houbonaituo to Yisuoqie, 22 Aug 1959, *Anonymous* 1930 (SM [SM717900136] image!); Meigu, Shuwo, alt. 2200 m, 18 Jul 1959, *Sichuan Econ. Pl. Exped.* 1343 (PE [00140236]); Miyi, Baipo, Youfang, alt. 2200 m, 14 Sep 2012, P.W. Li LPW2012029 (PE [01909901]); Miyi, Puwei, Xifan, R.J. Wang & S. Zhou 1742 (IBSC); Muli, alt. 3333–3666 m, 1 Sep 1922, G. Forrest 22460 (E [E00628110] image!, PE [00140259]); ibid., alt. 3400 m, Aug 1928, J.F. Rock 16920 (E [E00087224] image!), US [00077983] image!); Muli, Baidiao, alt. 3750 m, 1 Sep 1978, Q.S. Zhao et al. 8449 (CDBI [CDBI0130076] image!); Muli, Yaju, alt. 3100–3000 m, 20 Aug 1983, *Qinghai-Xizang Exped.* 13140 (KUN [0206360]), PE [01173175]); Muli, Abidian, alt. 3328m, 8 Sep 2017, L.H. Yang et al. SCML06 (IBSC!); Tatisienlu (now Kangding), 1938, *Mclaren's Collectors AC252* (E [E00628115] image!), P [P03511263] image!); Tianquan, alt. 2500 m, 23 Aug 1978, *Tianquan Exped.* 78–724 (SM [SM717900130] image!); Zhaojue, Hejue, alt. 2320 m, 21 Aug 1979, *Zhaojue Exped.* 0909 (SM [SM717900137] image!). —YUNNAN: alt. 2500 m, 24 Aug 1933, Y. Tsiang 11655 (KUN!, IBSC [0548662]); Oct 1922, G. Forrest 22593 (E [E00087222] image!); G. Forrest 30604 (E [E00628112] image!); alt. 3666 m, 1 Jul 1929, G. Forrest 27936 (E [E00628114] image!, IBSC [0548661]); Chien-chuan (now Jianchuan), Sep 1922, G. Forrest 22282 (E [E00087223] image!), P [P03511190] image!, US [00077982] image!); ibid., Aug 1922, G. Forrest 23210 (E [E00628113] image!), P [P03511191] image!, US [00077981] image!); Dali, Aug 1941, H.C. Wang 1330 (PE [00140257]); Dali, Cangshan, alt. 2500 m, 24 Aug 1933, Y. Tsiang 11655 (IBSC [0548662]); ibid., alt. 2600 m, 11 Sep 2009, Z.J. Yin 1998 (KUN [1220208]); ibid., alt. 3167 m, 11 Aug 1940, L.H. Yang et al. YLH683 (IBSC!); Heqing, Maershan, alt. 2800–2900 m, 23 Aug 1940, R.C. Ching 23950 (KUN [0206379]), PE [00140253]); Lijiang, Mujiashai, 1 Sep 1939, R.C. Ching 21414 (KUN [0206383]); ibid., 1 Jun 1939, Y.Z. Zhao 21414 (KUN [0206386]); Lijiang, Laomeiluo, 28 Aug 1940, R.C. Ching 30984 (KUN [0206375]), PE [00140244]); ibid., 18 Sep 1939, R.C. Ching 30671 (KUN [0206385]), PE [00140258]); Lijiang, Xuesong, alt. 2800 m, 1 Sep 1937, T.T. Yu 15512 (E [E00628109] image!, KUN [0206377]), PE [00140250]); Lijiang, Luomeiluo, alt. 2800–3000 m, 10 Aug 1942, K.M. Feng 8905 (KUN [0206374]), PE [00140247]); Lijiang, Yulong snow mountain, alt. 1100 m, J.F. Rock 24879 (E [E00628111] image!, KUN [0206380]), US [00077984] image!); ibid., alt. 2867 m, 21 Jul 2020, L.H. Yang et al. YLH1061 (IBSC!); Qiaojia, Yaoshan, alt. 3200 m, 16 Aug 1974, *Anonymous* s.n. (HITBC [069204] image!); Xianggelila, Luoji, Niru, alt. 2624 m, 15 Aug 2011, T. Zhang et al. 11CS3126 (KUN [1385187]); Xianggelila, Haba, Longwanbian, alt. 3500 m, 29 Aug 1962, *Zhongdian Exped.* 1645 (PE [00140256]), KUN [0206388]); ibid., alt. 3500 m, 29 Aug 1962, *Zhongdian Exped.* 1617 (KUN [0206381]); Xianggelila, Shangjiang, Mugao, alt. 2985 m, 30 Aug 2011, Z.D. Fang et al. DJDC-688 (SABG [SABG001220] image!); Zhenxiang, Changba, alt. 1800 m, 22 Aug 2021, L.H. Yang & P.W. Li YLH1210 (IBSC!); Zhongdian, Haba, alt. 3110 m, 1962, *Anonymous* s.n. (HITBC [069234] image!). **Tibet**. Medog, Yalu Tsangpo valley, alt. 2000 m, s.d., *Qinghai-Xizang Exped.* 83–11867 (PE, not seen). **India**. —SIKKIM (now West Bengal): Darjeeling, 8000 ft, 12 Aug 1913, R. Lepcha 1225 (E [E00394340] image!); Karkouang, alt. 7000–8000 ft, 4 Jul 1910, W.W. Smith 3039 (E [E00394339] image!); Regio temp, 7 Oct 1857, J.D. Hooker s.n. (K [k000858101] image!); Tonglo, 1 Oct 1857, J.D. Hooker s.n. (K [k000831973] image!); ibid. 9000 ft, 1913, *Ribu & Rhomoo* 6293 (E [E00394338] image!). **Myanmar**. alt. 8000 ft, 1 Aug 1919, F. Kingdon-Ward 3451 (E [E00087225] image!); alt. 10000 ft, 21 Aug 1919, R. Farrer 1244 (E [E00096871] image!); 10000 ft, Sep 1924, G. Forrest 24927 (E [E00096872] image!). **Nepal**. Chauki, 17 Aug 1972, J.F. Dobremez 1539 (E [E00622433] image!).

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AUTHOR CONTRIBUTIONS

LHY and PWL conceived of the presented idea. PWL performed the field work, the lab work, and the analysis, and wrote the manuscript. LHY performed the field work and wrote the manuscript. JFS and DM

revised the manuscript. DM contributed his experience of working more than two decades in Eastern Himalaya and NE states of India. XZS conducted the field work.

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- (Franch.) Mich.Möller & W.H.Chen, P.W. Li LPW2014102 (PE), China, Guizhou, Yanhe, OL689742*, OL753129*, OL689771*, OL689801*, OL689830*, *Gyrocheilos chorisepalus* W.T.Wang, R.B. Mao MRB200963 (PE), China, Guangdong, Fengkai, OL689733*, OL753119*, OL689762*, OL689791*, OL689821*, *Gyrocheilos retrotrichus* W.T.Wang, J.M. Li LJM2003069 (PE), China, Guangxi, Luocheng, OL689734*, OL753120*, OL689763*, OL689792*, OL689822*, *Henckelia anachoreta* (Hance) D.J.Middleton & Mich.Möller, P.W. Li LPW2013143 (PE), China, Yunnan, Maguan, MN637027, MN627938, —, MN637310, MN637589; *Henckelia auriculata* (J.M.Li & S.X.Zhu) D.J.Middleton & Mich.Möller, J.M. Li 08816 (HEAC), China, Yunnan, Hekou, —, FJ796197, —, FJ796196; *Henckelia bifolia* (D.Don) A.Dietr., *Bhaskar Adhikari L2B6* (E), Nepal, Chyalding, —, JF912549, —, JF912522; *Henckelia dielsii* (Borza) D.J.Middleton & Mich.Möller, M. Möller MMO 08-1211 (E), China, Yunnan, Jingdong, —, HQ632967, —, HQ632871; *Henckelia floccosa* (Thwaites) A.Weber & B.L.Burt, C.G. Jang s.n. [G 157] (WU), Sri Lanka, —, HQ632964, —, FJ501486; *Henckelia incana* (Vahl) Spreng., *S.Vogel SVG s.n.* (E), India, Nilgiri mts., —, HQ632965, —, HQ632869; *Henckelia pumila* (D.Don) A.Dietr., R.B. Mao MRB201076 (PE), China, Guizhou, Zhenfeng, —, MN627941, —, MN637592; *Henckelia* sp., J.M. Li LJM200488 (KUN), China, Cultivated in Kunming Institute of Botany, —, MN627939, —, MN637590; *Henckelia tibetica* (Franch.) D.J.Middleton & Mich.Möller, P.W. Li LPW2012025 (PE), China, Sichuan, Miyi, MN637026, MN627937, —, MN637309, MN637588; *Henckelia urticifolia* (Buch.-Ham. ex D.Don) A.Dietr., J.M. Li 05851 (PE), China, Yunnan, —, DQ872835, —, DQ872821; *Henckelia walkerae* (Gardner) D.J.Middleton & Mich.Möller, Skog 7736 (US), cult. Smithsonian 94–250, origin: Sri Lanka; leg. in US 11.03.1996, —, FJ501326, —, FJ501490; *Loxostigma cavalieri* (H.Lév. & Vaniot) B.L.Burt, M.T. Liu LMT2011004 (PE), China, Yunnan, Xichou, OL689735*, OL753121*, OL689764*, OL689793*, OL689823*, *Loxostigma dongxingense* (Chun ex K.Y.Pan) Mich.Möller & H.J. Atkins, R.H. Liang LRH00M9 (PE), China, Guangxi, Dongxing, OL689739*, OL753126*, —, OL689798*, OL689828*, *Loxostigma fimbriosepalum* K.Y.Pan, M.T. Liu LMT2011006 (PE), China, Yunnan, Malipo, OL689736*, OL753122*, OL689765*, OL689794*, OL689824*, *Loxostigma glabrifolium* D.Fang & K.Y.Pan, Y.G. Wei 709 (IBK), China, Guangxi, Napo, —, HQ633006, —, HQ632910; *Loxostigma griffithii* (Wight) C.B. Clarke, C.Y. Feng FCY2013021 (PE), China, Yunnan, Zhaotong, OL689738*, OL753124*, OL689767*, OL689796*, OL689826*, *Loxostigma kurzii* (C.B. Clarke) B.L.Burt, M. Möller MMO 08-1249 (E), China, Sichuan, EmeiShan, —, HQ632970, —, HQ632874; *Loxostigma kurzii* (C.B. Clarke) B.L.Burt, M. Kang et al. SCMG03 (IBSC), China, Sichuan, Meigu, —, MK747109, —, MK746234; *Loxostigma kurzii* (C.B. Clarke) B.L.Burt, P.W. Li LPW2012029 (PE), China, Sichuan, Miyi, OL689740*, OL753127*, OL689769*, OL689799*, OL689829*, *Metapetrocosmea peltata* (Merr. & Chun) W.T.Wang, *Anonymous Li19* (PE), China, Hainan, —, OL753125*, OL689768*, OL689797*, OL689827*, *Oreocharis benthamii* C.B. Clarke, M. Möller MMO08-1317 (E), China, Guangdong, Zhaoqing, —, JF697572, —, JF697584; *Oreocharis chienii* (Chun) Mich.Möller & A.Weber, R.B. Mao MRB201093 (PE), China, Zhejiang, Lishui, OL689725*, OL753111*, OL689754*, OL689783*, OL689813*, *Oreocharis dinghushanensis* (W.T.Wang) Mich.Möller & A.Weber, B.L. Liu LBL2009001 (PE), China, Guangdong, Zhaoqing, OL689751*, OL753137*, OL689780*, OL689810*, OL689839*, *Oreocharis forrestii* Skan, F.P. Liu LFP2016013 (PE), China, Yunnan, Lijiang, OL689724*, OL753110*, OL689753*, OL689782*, OL689812*, *Oreocharis longifolia* (Craib) Mich.Möller & A.Weber, J.M. Li LJM103515 (PE), China, Yunnan, OL689726*, OL753112*, OL689755*, OL689784*, OL689814*, *Petrocodon ainsliifolius* W.H.Chen & Y.M.Shui, P.W. Li LPW2013089 (PE), China, Guangxi, Napo, —, MN627898, —, MN637268, MN637548; *Petrocodon dealbatum* Hance, M.T. Liu LMT2012017 (PE), China, Guizhou, Shibing, MN636999, MN627910, —, MN637282, MN637561; *Petrocodon hancei* (Hemsl.) A.Weber & Mich.Möller, M.T. Liu LMT2012005 (PE), China, Hunan, Xinning, MN637002, MN627913, —, MN637285, MN637564; *Petrocodon scopulorum* (Chun) Yin Z.Wang, P.W. Li LPW2018003 (PE), China, Guizhou, Pingba, MN637000, MN627911, —, MN637283, MN637562; *Petrocosmea kerrii* Craib, *Anonymous 04603* (KUN), China, Yunnan, —, JN092441, —, JN092474; *Petrocosmea minor* Hemsl., Z.J. Qiu QZJ200854 (PE), China, Yunnan, Mengzi, OL689749*, OL753135*, OL689778*, OL689808*, OL689837*, *Petrocosmea nervosa* Craib, Z.J. Qiu QZJ-2008-45 (PE), China, Sichuan, —, JN092446, —, KR006523, JN092479; *Petrocosmea sinensis* Oliv., Z.J. Qiu QZJ200841 (PE), China, Sichuan, Leshan, OL689750*, OL753136*, OL689779*, OL689809*, OL689838*, *Primulina dryas* (Dunn) Mich.Möller & A.Weber, M. Kang et al. GDSZ01 (IBSC), China, Guangdong, Shenzhen, —, KF498055, —, KF498164; *Primulina huaijiensis* Z.L.Ning & J.Wang, M. Kang et al. GDHJ02 (IBSC), China, Guangdong, Huaiji, MN636988, MN627838, MF472012,

APPENDIX 1. Taxa included in the phylogenetic analyses, voucher information, localities, and GenBank accession numbers for ETS, ITS, *rpl16*, *rps16*, and *trnL-F*, respectively. Asterisks indicate sequences generated in this study; dashes indicate data not available.

Ingroup: *Allocheilos guangxiensis* H.Q.Wen, Y.G.Wei & S.H.Zhong, R.B. Mao MRB200934 (PE), China, Cultivated in Guangxi Institute of Botany, OL689727*, OL753113*, OL689756*, OL689785*, OL689815*; *Allostigma guangxiense* W.T.Wang, J.M. Li LJM2003098 (PE), China, Guangxi, Longzhou, OL689728*, OL753114*, OL689757*, OL689786*, OL689816*; *Anna ophiorrhizoides* (Hemsl.) B.L.Burt & R.A.Davidson, R.B. Mao MRB201085 (PE), China, Sichuan, Leshan, OL689730*, OL753116*, OL689759*, OL689788*, OL689818*, *Anna submontana* Pellegr., M.T. Liu LMT2011001 (PE), China, Yunnan, Hekou, OL689729*, OL753115*, OL689758*, OL689787*, OL689817*, *Briggsiopsis delavayi* (Franch.) K.Y.Pan, R.B. Mao MRB201087 (PE), China, Sichuan, Leibo, OL689731*, OL753117*, OL689760*, OL689789*, OL689819*, *Cathayanthus biflora* Chun, M. Möller MMO 08-1327 (E), China, Hainan, Tongshi, —, HQ632996, —, HQ632899; *Chirita umbrophila* C.Y.Wu ex H.W.Li, L.H. Yang & P.W. Li YLH1210 (IBSC), China, Yunnan, Zhenxiong, OL689752*, OK298972*, OL689781*, OL689811*, OK318003*, *Didymocarpus antirrhinoides* A.Weber, Jong 9009 (RBGE accession no. 19650167), Malaysia, —, MN843189, —, MN842994; *Didymocarpus aromaticus* Wall. ex D.Don, *Tibet Expedition4290* (PE), China, Tibet, OL689743*, OL753130*, OL689772*, OL689802*, OL689831*, *Didymocarpus citrinus* Ridl., *Anonymous 1983-0510* (E), Malaysia, Perlis, Kedat Peak, —, DQ912669, —, AJ492293; *Didymocarpus cortusifolius* H.Lév., M.T. Liu LMT2012010 (PE), China, Zhejiang, Leqing, OL689744*, OL753131*, OL689773*, OL689803*, OL689832*, *Didymocarpus glandulosus* var. *minor* (W.T.Wang) W.T.Wang, M.T. Liu LMT2011020 (PE), China, Guangxi, Tianlin, OL689745*, —, OL689774*, OL689804*, OL689833*; *Didymocarpus heucherifolius* Hand.-Mazz., M.T. Liu LMT2012008 (PE), China, Jiangxi, Leping, OL689746*, OL753132*, OL689775*, OL689805*, OL689834*; *Didymocarpus mengtze* W.W.Sm., J.M. Li LJM2003026 (PE), China, Yunnan, OL689747*, OL753133*, OL689776*, OL689806*, OL689835*; *Didymocarpus yunnanensis* (Franch.) W.W.Sm., Z.J. Qiu QZJ0944 (PE), China, Yunnan, Binchuan, OL689748*, OL753134*, OL689777*, OL689807*, OL689836*; *Didymostigma obtusum* (C.B. Clarke) W.T.Wang, R.B. Mao MRB200964 (PE), China, Guangdong, Fengkai, OL689732*, OL753118*, OL689761*, OL689790*, OL689820*, *Glabrella longipes* (Hemsl.) Mich.Möller & W.H.Chen, Z.J. Qiu QZJ0978 (PE), China, Yunnan, Xichou, OL689741*, OL753128*, OL689770*, OL689800*, —; *Glabrella mihieri*

MN637208, MN637488; *Primulina renifolia* (D.Fang & D.H.Qin) J.M.Li & Yin Z.Wang, P.W. Li LPW2015013 (PE), China, Guangxi, Du'an, —, MZ352171, —, MZ355743, MZ355760; *Primulina repanda* (W.T.Wang) Yin Z.Wang, P.W. Li LPW2015011 (PE), China, Guangxi, Tian'e, —, MN627869, —, MN637239, MN637519; *Primulina tabacum* Hance, P.W. Li LPW2014013 (PE), China, Hunan, Dong'an, —, MZ352169, —, MZ355746, MZ355759; *Pseudochirita guangxiensis* (S.Z.Huang) W.T.Wang, M. Möller

MMO 06-798 (E), China, Guangxi, Mashan, —, HQ633003, —, —, HQ632908.

Outgroup: *Microchirita hamosa* (R. Br.) Yin Z.Wang, M. Möller MMO 05-753 (E), China, —, JF912551, —, —, JF912524; *Microchirita tubulosa* (Craib) A.Weber & D.J.Middleton, D. Middleton et al. 4809 (E), Thailand, —, JF912558, —, —, JF912531.