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# Phylogenetics Support the Description of a New Sichuanese Species, Susanne's Gentian, *Gentiana susanneae* (Gentianaceae)

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***Abstract***—The region of the Qinghai-Tibet Plateau hosts a remarkable biodiversity, including a plethora of endemics. There, the process of documenting biodiversity is still ongoing, and recently, an unusual plant of *Gentiana* section *Frigida* was discovered at Cuopu Lakes (Sichuan, China). Although *Flora of China* identified these specimens as *G. algida* based upon unmistakable traits (e.g. corolla colour and length, sessile flowers), it was obvious that the Sichuanese plants strongly differed morphologically from *G. algida* specimens from Siberia and North America. In order to clarify their relationships, we used an integrative approach, investigating not only morphological traits, but also phylogenetic relationships (based upon plastome and rDNA-cistron), as well as chloroplast structure. Whereas the rDNA-cistron phylogeny was poorly resolved, the plastome phylogeny was well supported in both Bayesian inference and maximum likelihood analyses. Our findings indicate that the samples of *G. algida* from Cuopu Lakes are morphologically and genetically distinct from those collected elsewhere, supporting the recognition of a new species, ***G. susanneae***. The new species differs from *G. purdomii* (another similar species) by having sessile flowers, and from *G. algida* by being a generally much taller plant with more and longer flowers, and by having a smaller calyx. ***Gentiana susanneae*** clusters with other species from the region of the Qinghai-Tibet Plateau from which it differs morphologically, and is only distantly related to the *G. algida*/*G. frigida* clade, which stems from a dispersal event out of Tibet. Based upon these solid lines of evidence, ***G. susanneae*** is described and illustrated (drawings and photographs). In addition, a conservation assessment and an identification key modified from *Flora of China* are provided.

***Keywords***—Biogeography, *Gentiana* sect. *Frigida*, Qinghai-Tibet Plateau.

概要—笔者在四川省措普湖附近发现了龙胆属高山组 (*Gentiana* section *Frigida*) 的一种植物。虽然《*Flora of China*》将其归为高山龙胆 (*G. algida*), 但其在形态特征上与西伯利亚和北美洲的个体存在明显差异。本研究对其形态特征进行了调查,随后基于叶绿体全基因组序列和完整ITS序列进行系统发育关系分析,并比较了叶绿体基因组的结构变异。结果表明,措普湖的高山龙胆种群与其他地区的样品不在同一遗传支系上,支持将其作为一个新种——巴塘龙胆 (***G. susanneae***), 并在此对其进行了描述。

The region of the Qinghai-Tibet plateau (QTP) hosts a remarkable species richness of plants (e.g. Kier et al. 2005) and several global biodiversity hotspots (Myers et al. 2000; Mittermeier et al. 2011), including the Hengduan Mountains (namely "The Mountains of Southwest China"). This region may host the oldest persisting alpine flora on Earth (Ding et al. 2020), and biogeographically, it has been involved in bidirectional biological interchange with surrounding areas (e.g. Päckert et al. 2020), often acting as source of biodiversity for adjacent regions and beyond (Xing and Ree 2017). In general, colonisation events of the QTP tend to be older (e.g. early establishment of the alpine flora, Eocene; Ebersbach et al. 2017; Ding et al. 2020), whereas the "Out-of-Tibet Hypothesis" seems to be verified at more recent times (ca. 5 Ma; Deng et al. 2011; Wang et al. 2014, 2016). In plants, out of Tibet dispersal has been detected for example in *Hippophaë* L. (Jia et al. 2012), *Tripterospermum* Blume (Matuszak et al. 2016), and *Saxifraga* L. (Ebersbach et al. 2017).

Another genus displaying a pattern of "out of Tibet" dispersal is *Gentiana* L. (Favre et al. 2016). This species-rich (ca. 360 species) and subcosmopolitan genus originated in the region of the QTP, from where it colonised other major mountain systems (e.g. the Andes, the European Alpine System, the Rockies). Hosting about 250 species (Ho and Pringle 1995), the region of the QTP is currently the main center of diversity for *Gentiana*. In the genus, dispersals out of Tibet occurred repeatedly, and although one dispersal event may date back to the Early Miocene, most of these events were concentrated in the Pliocene and Pleistocene (Favre et al. 2016). *Gentiana* is divided into several sections, of which the number has varied among taxonomic treatments based upon morphological evidence (e.g. Kusnezow 1895; Marquand 1937; Pringle 1978; Ho and Pringle 1995; Ho and Liu 1990, 2001). Building upon a series of early molecular studies (Yuan and Küpfer 1995, 1997; Yuan et al. 1996), the delineation of the genus as well as its different sections has been greatly improved in recent years (Favre et al. 2010, 2014, 2020). Six of the 13 currently recognized sections of *Gentiana* contain lineages which have escaped the region of the QTP (*Gentiana* sections *Chondrophyllae* Bunge, *Cruciata* Gaudin, *Frigida* Kusn., *Kudoa* (Masam.) Satake & Toyok. ex Toyok, *Monopodiae* (Harry Sm.) T.N.Ho, and *Pneumonanthe* Gaudin). In one of these sections, *G.* sect. *Frigida*, two sister species appeared to have resulted from a single dispersal event from the QTP region towards eastern Asia and North America (*G. algida* Pallas) and Europe (*G. frigida* Haenke) (Favre et al. 2016).

In 2016 and 2018 unusual plants of *G.* sect. *Frigida* (Figs. 1, 2) were discovered and collected at Cuopu Lakes (Sichuan, China). The determination of these specimens (using *Flora of China*; Ho and Pringle 1995) unequivocally identified them as *G. algida* based upon the colour and length of their corolla (white, > 3.5 cm) and their sessile flowers. Except for this latter trait, the specimens we discovered also resemble

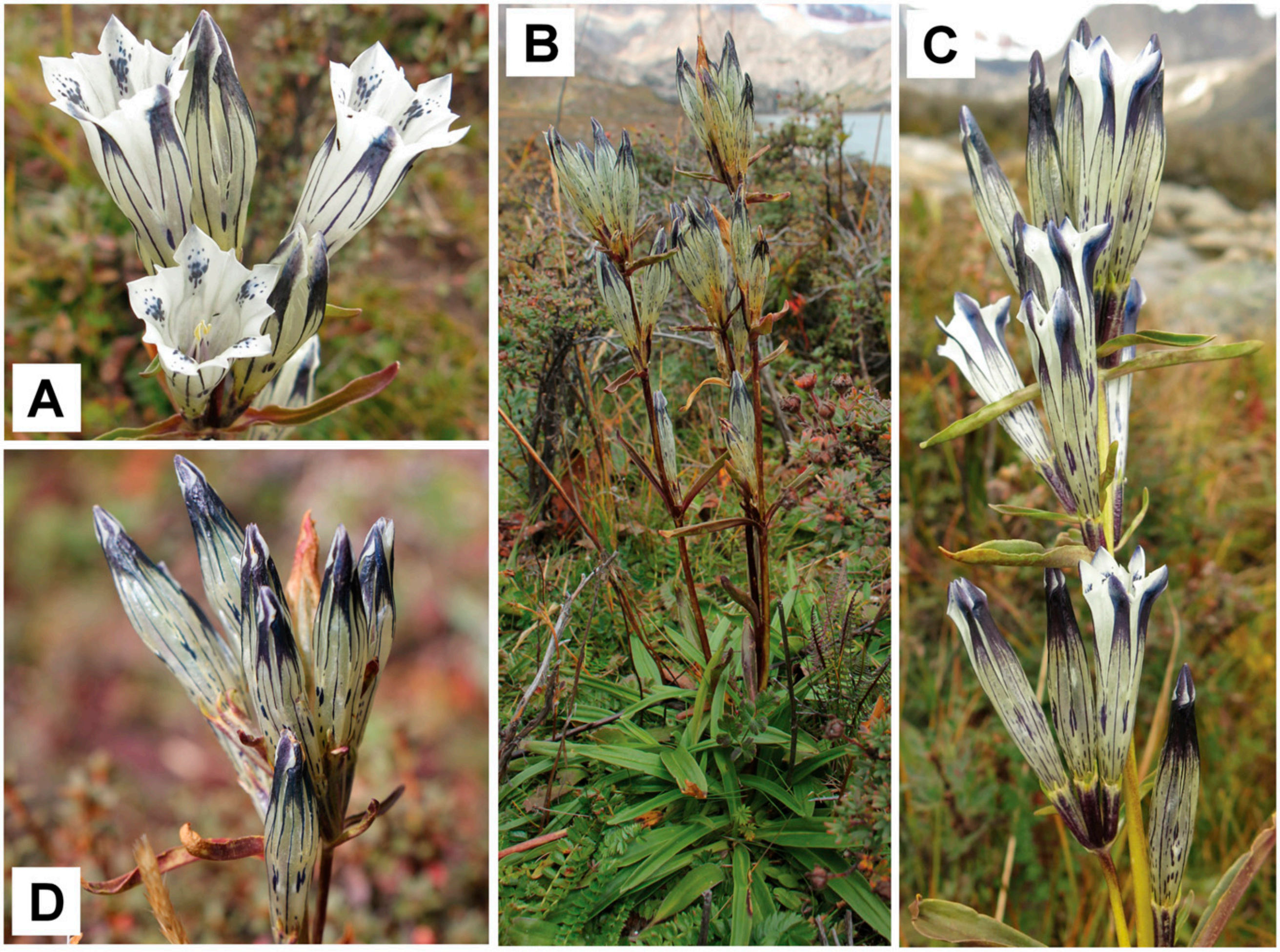

FIG. 1. Pictures of *Gentiana susanneae*. A. Open corolla. B. The entire plant including rosette and stem leaves. C. Inflorescence. D. Closed corollas. Photographs by Adrien Favre and Susanne Fritz. Pictures (A) and (C) are of the holotype, *AFCN18_201d* (FR).

*G. purdomii* C.Marquand. Yet, it appeared obvious that *G. algida* specimens from Sichuan strongly differed morphologically from those collected later in North America in 2019 and from *G. purdomii* (plant size, number and length of flowers, etc.). Here, we will describe the specimens of Cuopu Lakes as a new species, *G. susanneae*, using both genetic (phylogenetic relationships and chloroplast structure) and morphological evidence, and ask whether the new species is part of the lineage that went out of Tibet, as could be suggested by the morphology alone.

## MATERIALS AND METHODS

***Material Collection***—To recover phylogenetic relationships among species of *G.* sect. *Frigida* as accurately as possible, our sampling design included as many species as we could find in this section. Many of these species are endemic to the region of Tibet. Our sampling includes 8 of the currently 20 described species (including *G. susanneae*). Some species were collected in the wild in China, North America, and Austria, and deposited at several herbaria including KUN, FR, and LZ. Freshly collected material was dried and preserved in silica gel. To complete our data set, we retrieved material from herbarium specimens (deposited at FR, M, and W), and verified their identification mainly using *Flora of China* (Ho and Pringle 1995). We included species of other closely related sections of *Gentiana* as outgroup (i.e. belonging to *G.* sect. *Monopodiae* and *G.* sect. *Kudoa*). Information regarding our samples, their geographical origin as well as the herbarium where corresponding vouchers were deposited, is listed in Supplemental Appendix S1 (Favre et al. 2022).

The determination of the specimens collected at Cuopu Lakes (using *Flora of China*) unequivocally identified these plants as *G. algida*. Since their appearance diverged so much from those collected in North America, we attempted to find samples of *G. algida* at the type locality for comparison. The type specimen of the name *G. algida* is at S (accession number G-2816) and was collected by Pallas. Unfortunately, the type (as well as its probable isotype at BM) lacks locality data, and thus, attempting to correlate its location with what is known of Pallas's itineraries was not effective. However, since Pallas is known to have done much of his botanical exploration in eastern Russia (e.g. Altai, Baikal region), it is quite likely that his collection of *G. algida* came from there. Therefore, in order to represent the overall distribution range of *G. algida*, we also included samples from Kamchatka, Eastern Siberia, and the Altai gathered from herbaria (M and W).

***DNA Extraction and Sequencing***—The DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) was used to extract total DNA, following the manufacturer's protocol except for a longer incubation time in the lysis buffer (extended to two hours). Whole genome library preparation (fragment size 350 bp) and sequencing were performed by Novogene (Novogene BioTech, Inc. Beijing, China), using the NovaSeq 6000 PE 150 sequencing system. Two Gb of raw data were generated per sample.

***Chloroplast Characterization***—Raw reads were filtered and trimmed with Trimmomatic v0.32 (Bolger et al. 2014) with default parameters to remove adaptor sequences and low-quality reads and sites, and then checked for quality with FastQC v0.11.2. Plastomes were assembled de novo using GetOrganelle v1.7.1 (Jin et al. 2020). Each plastid genome was annotated with GeSeq (Tillich et al. 2017) and PGA (Qu et al. 2019) using the default parameters. All plastome sequences were saved as GB2sequin

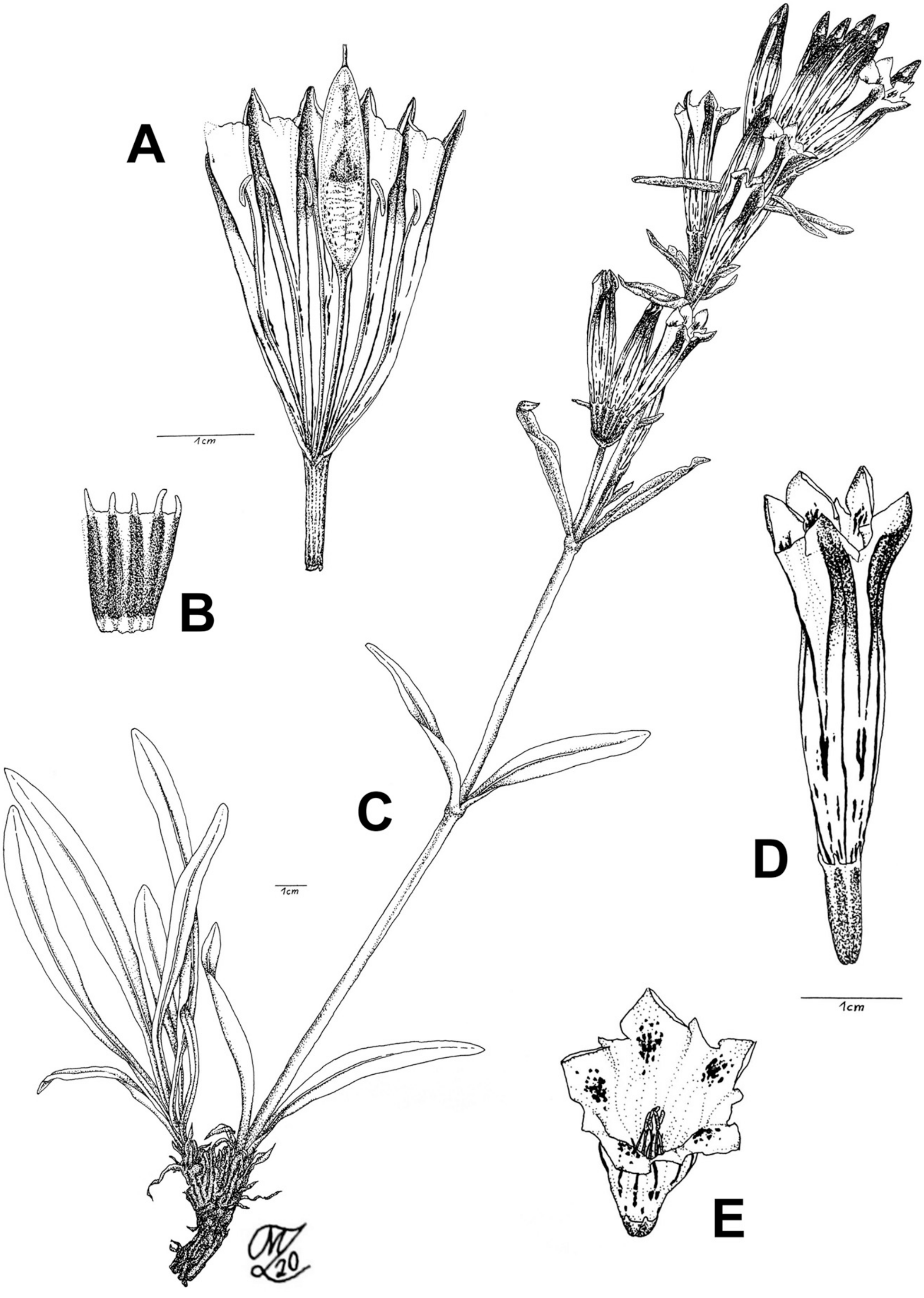

FIG. 2. Drawing representing *Gentiana susanneae*. A. Flower dissected above the calyx. B. Dissected calyx. C. The habit of the species. D–E. Side (D) and top (E) views of a flower. Drawing by Martina Zwanziger from voucher *Favre AFCN18_201d* (FR), the holotype.

files (Lehwark and Greiner 2018) and deposited in GenBank (accession numbers MW218874–MW218877, MZ579508, and MZ579509). Genome comparisons were conducted to identify structural differences among species of *G.* sect. *Frigida*, using mVISTA (Frazer et al. 2004).

***Phylogenetic Analyses***—From the raw genomic data, we extracted the entire plastid genomes and rDNA cistron. We used Geneious Prime® 2019.0.4 (https://www.geneious.com) and mapped the reads to sequences of closely related species already available on GenBank (Fu et al. 2016; Favre et al. 2020). We aligned sequences in Geneious Prime using MAFFT v. 7 (Katoh and Standley 2013), including a few sequences available on GenBank (see Supplemental Appendix S1, Favre et al. 2022). We inspected the resulting alignments and edited them wherever necessary in Geneious Prime® 2019.0.4. Only a few ambiguous positions (of which homology was difficult to ascertain) had to be removed, particularly in non-coding regions. We obtained matrices of 141,794 bp (plastid) and 6948 bp (rDNA cistron), with 7230 and 320 variable sites, respectively. The alignments we used for phylogenetic analysis are available from Dryad (Favre et al. 2022).

Maximum likelihood (ML) and Bayesian inference (BI) were run for both alignments separately (rDNA cistron, and plastid). For ML analyses we used RAXMLGUI 2.0 beta (Edler et al. 2019) for RAxML v. 8 (Stamatakis 2014), with GTR+Γ+I as model of evolution (as recommended by the built-in model testing). Statistical support (BS) was calculated via bootstrap analysis with 1000 replicates (Felsenstein 1985). BI analyses were performed with MrBayes 3.2.6 (Ronquist et al. 2012), with GTR+Γ+I as model of evolution as above. Four runs were started from random trees, with four coupled incrementally heated Monte Carlo Markov chains each (MCMC; one cold and three heated), for 50 million generations sampling every 5000th. We checked that potential scale reduction factor (PSRF) values, effective sample sizes (ESS), and average standard deviations of split frequencies (asdsf) were well within acceptable values. The first quarter (25%) of the sampled trees were discarded as burn-in. With the remaining trees, a majority rule consensus tree and posterior probabilities (PP) of bipartitions were computed. Finally, we used Figtree v. 1.4.0 (Rambaut 2010) to visualize phylogenetic trees corresponding to each analysis and dataset.

## RESULTS

***Phylogenetic relationships***—ML and BI analyses based upon the rDNA-cistron resulted in poorly resolved phylogenies (see Supplemental Appendix S2, Favre et al. 2022). Therefore, we will only present the results derived from chloroplast data (Fig. 3). ML and BI analyses generated identical topologies, in both cases with almost all nodes being fully supported (i.e. 100 BS and 1.00 PP, respectively). *Gentiana* sect. *Frigida* was divided into two main clades, one of these containing all species endemic to the region of the QTP (including *G. susanneae*), whereas the other included *G. frigida* (Europe) and all five accessions of *G. algida* (USA, Russia). Phylogenetic relationships within the QTP clade were fully resolved. The new species *G. susanneae* is most closely related to *G. erectosepala* T.N.Ho, whereas *G. handeliana* Harry Sm. groups with *G. atuntsiensis* W.W.Smith, and *G. purdomii* C.Marquand is sister to *G. nubigena* Edgeworth. Phylogenetic relationships are less well supported within the *G. frigida*/*G. algida* clade. Accessions of *G. algida* originating from America, Kamchatka, and South Siberia clustered together, whereas their phylogenetic position with regard to *G. frigida* (Europe) and an accession of *G. algida* from the Altai remains unclear (1.00 PP, 90 BS).

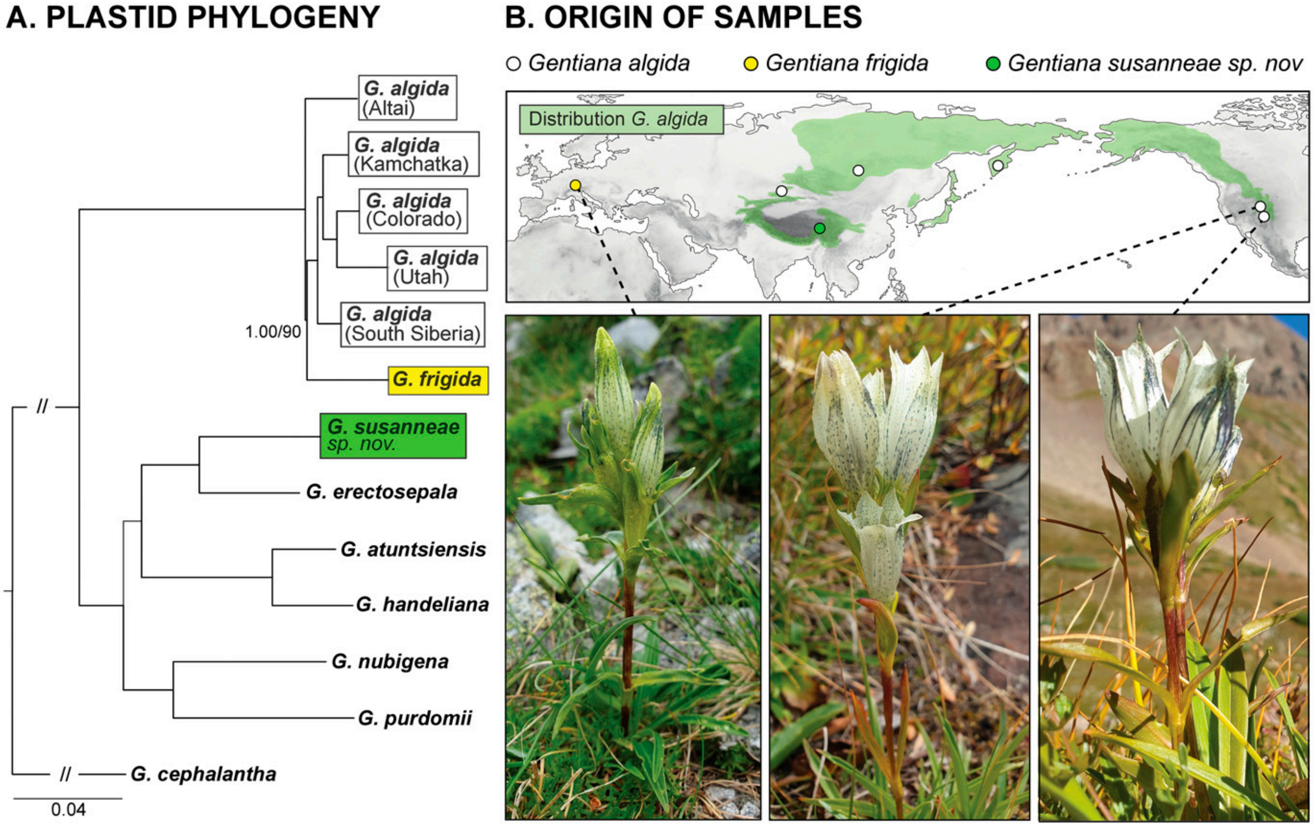

FIG. 3. A. Phylogram of *Gentiana* section *Frigida* derived from entire chloroplast genomes, showing the phylogenetic relationships among *G. susanneae* and closely related species. Phylogenetic support values for both Bayesian inference and maximum likelihood are shown at nodes only when they differ from 1.00 posterior probability and 100% bootstrap support. B. Origin of samples (coloured circles) of *Gentiana algida*, *G. frigida*, and *G. susanneae*, as well as distribution (in green) of *G. algida*. For comparison, pictures of *G. frigida* (Austria, 2019) and *G. algida* (USA, 2019) are provided. The map was downloaded from WORLDCLIM (Hijmans and al. 2005). Photographs by Adrien Favre.

***Chloroplast Characterization*****—**The complete plastome of *Gentiana susanneae* is a circular-mapping molecule with a length of 143,913 bp. The large single copy, the inverted repeat, and the small single copy regions were 77,200, 24,868, and 16,977 bp, respectively. Although substantial length variation occurs among sections within the genus (Sun et al. 2019; Fu et al. 2021), we found that within *G.* sect. *Frigida*, the plastome of *G. susanneae* had the same gene content and order as other species. Only minor size variations were detected among *G. susanneae* and *G. algida*, the samples of the latter (Altai, Kamchatka, and Siberia, respectively) with a length of 143,725, 143,487, and 143,626 bp. Comparison analysis showed that the plastomes of *G. susanneae* and *G. algida* mainly differed at intergenic regions such as *petN-psbM*, *psbE-petL*, *trnR*$^{-ACG}$-*trnN*$^{-GUU}$, *rpl32-trnL*$^{-UAG}$, *trnL*$^{-CAA}$-*ycf15* (Table 1; Supplemental Appendix S3, Favre et al. 2022), in terms of sequence and length, not structure nor order.

## DISCUSSION

The structure and gene order of the chloroplast appears not to vary among species of *G.* sect. *Frigida*, some minor differences occurring predominantly in non-coding areas in terms of length and sequence. Thus, no meaningful patterns of evolution could be derived from our data with this approach. However, our phylogenetic evidence based on plastome sequences (Fig. 3) appears to be solid, and in *Gentiana*, plastomes have been shown to recover almost identical topologies as single copy loci from the nucleus, whereas the rDNA-cistron was always weaker in resolving phylogenetic relationships (Favre et al. 2020). This is a trend we observe in our study, too. Unequivocally, it shows that expanding the circumscription of *G. algida* so as to include the plants of Cuopu Lakes would engulf the whole section into a single species. We therefore describe the plants from Cuopu Lakes, originally determined as *G. algida*, as a new species named *Gentiana susanneae.* This new species is easily distinguishable (e.g. by its height, corolla length and colour) from congeners in the region of the QTP and elsewhere (see Taxonomic Treatment).

All samples gathered outside of the QTP region and representing the species *G. algida* and *G. frigida* appear to cluster together, and *G. susanneae* is nested among congeners that only occur in an area encompassing the border areas of E Tibet, W Sichuan, and NW Yunnan. Therefore, despite its morphological resemblance to *G. algida*, *G. susanneae* is not part of the lineages that dispersed out of Tibet. However, our phylogeny does not include all species of *G.* sect. *Frigida* (8 out of 20). All missing species occur in the Himalayas and the Hengduan Mountains, and all of them morphologically resemble at least one species of the Tibetan clade that is included in our phylogeny (e.g. *G. omeiensis* resembles *G. atuntsiensis*). It is therefore likely that even if more species were included, *G.* sect. *Frigida* would still be composed of two clades, one Tibetan and one of which species derive from a single dispersal out of Tibet.

Whether *G. algida*, as described by Pallas in Siberia, occurs in the region of the QTP at all remains unknown. In fact, according to *Flora of China* (Ho and Pringle 1995), the species *G. purdomii* is morphologically very similar to *G. algida*. *Gentiana purdomii* occurs throughout the eastern part of the region of the QTP, where its distribution range largely overlaps, at least theoretically, with that of *G. algida* (sensu *Flora of China*). The two species differ by only a few morphological traits, the flowers of the former being possibly smaller (3–5 cm) and usually long-pedicellate (pedicels up to 4 cm), whereas flowers of the latter (4–5 cm) are short-pedicellate (pedicels max. 1 cm). If the length of the pedicels is not a stable trait, then it would be easy to see how some smaller and short-pedicellate specimens of *G. purdomii* could have been mistaken for *G. algida* as described by Pallas in Siberia. In fact, our extensive fieldwork and herbarium experience has revealed that *G. algida* and *G. purdomii* (sensu *Flora of China*) are extremely hard to distinguish based on this trait. Indeed, a number of specimens display intermediate length for their corolla and pedicels. In any case, despite their strong morphological resemblance, *G. purdomii* (from China) and *G. algida* (from Siberia and North America) do not cluster together, and neither of them clusters with the new species *G. susanneae*. Therefore, these three species belong to three independent lineages, and *G. algida* as described by Pallas (in Siberia) may not occur in the QTP region. If this is the case, the circumscription of *G. purdomii* should be extended to include short-pedicellate individuals, the description of *G. susanneae* remaining unaffected by this suggestion. Unfortunately, to date, the samples at hand do not suffice to provide a definite answer.

## TAXONOMIC TREATMENT

***Gentiana susanneae*** Adr.Favre, sp. nov. TYPE: CHINA, Sichuan Province, Batang County, surroundings of Cuopu Lakes

TABLE 1. Plastome structure and sequence information for *Gentiana* species included in this study. Columns LSC, IR, and SSC report the length of the large single-copy, inverted repeat, and small single-copy regions, respectively, in base pairs. Newly sequenced plastomes are marked with asterisks next to the GenBank accession numbers. Information regarding the origin of the samples listed in this table are available from Supplemental Appendix S1 (Favre et al. 2022).

| Species | NCBI no. | LSC | IR | SSC | Total |
|---|---|---|---|---|---|
| *Gentiana susanneae* sp. nov. | MW218877* | 77,200 | 24,868 | 16,977 | 143,913 |
| *Gentiana algida* (Altai) | MW218874* | 77,393 | 24,676 | 16,980 | 143,725 |
| *Gentiana algida* (Kamchatka) | MW218875* | 77,442 | 24,593 | 16,998 | 143,626 |
| *Gentiana algida* (Siberia) | MW218876* | 77,556 | 24,652 | 16,627 | 143,487 |
| *Gentiana algida* (Utah) | MZ579508* | 77,502 | 24,621 | 16,971 | 143,715 |
| *Gentiana algida* (Colorado) | MZ579509* | 77,531 | 24,626 | 16,973 | 143,756 |
| *Gentiana frigida* | MW405446* | 77,507 | 24,592 | 16,970 | 143,661 |
| *Gentiana purdomii* | MW405447* | 77,490 | 24,928 | 16,944 | 144,290 |
| *Gentiana atuntsiensis* | MW405445* | 77,277 | 24,980 | 17,001 | 144,238 |
| *Gentiana handeliana* | MN199143 | 77,014 | 24,917 | 16,965 | 143,813 |
| *Gentiana nubigena* | MN199157 | 77,439 | 24,700 | 16,539 | 143,378 |

(Cuoniba), near the road between Batang and Litang, 4490 m a. s. l. [30°18′27.03″N, 99°33′16.11″E], flowering, 21 September 2018 (holotype: *Adrien Favre AFCN18_201d*, FR; isotypes: *Adrien Favre AFCN18_201a,b,c,e*: KUN, M, W; paratype: *Peng-Cheng Fu, Fu2016171*, Haizi pass, road between Batang and Litang, 21 August 2016 [30°16′47″N, 99°33′01″E]: Herbarium of Luoyang Normal University).

The new species is illustrated in Figs. 1 and 2, and is easily recognizable from other white-flowering species of *Gentiana* sect. *Frigida* in China, including *G. algida*, *G. purdomii*, *G. erectosepala*, *G. handeliana,* and *G. phyllopoda* H.Léveillé. First of all, *G. susanneae* is a much taller plant (up to ca. 80 cm), whereas other species are described as reaching a maximum height of 30 cm (*G. phyllopoda*), 25 cm (*G. purdomii*), 20 cm (*G. algida*, *G. erectosepala*), or 15 cm (*G. handeliana*). Moreover, *Gentiana susanneae* has much larger flowers (length: 5–6 cm) than those of *G. erectosepala*, *G. handeliana,* and *G. phyllopoda* (for all three, maximum flower length is 3.5 cm). Its flowers are sessile in contrast to those of *G. erectosepala* and *G. purdomii,* both of which have distinctly pedicellate flowers. Unlike *G. handeliana* and *G. phyllopoda*, *G. susanneae* has erect but neither reflexed nor spreading calyx lobes. Furthermore, the new species differs from *G. algida* by having an obconic and more slender corolla tube, whereas that of *G. algida* is ventricose. Also, the calyx lobes of *G. susanneae* are much smaller than the calyx tube, whereas those of *G. algida,* although unequal in length (Hultén 1968), are about as long as the calyx tube. Finally, *G. algida* is fewer-flowered (flowers solitary or rarely in terminal 2- or 3(–8)-flowered cymes) in comparison to *G. susanneae*, which usually possesses a number of axillary cymes. These morphological traits are strong support for the description of a new species.

***Description***—Perennials 30–60(–80) cm tall. Stems 1 or 2, erect, simple, glabrous. Basal leaves in 1(–2) erect rosettes (usually 6 to 8 leaves concurrently persistent); petiole 1–4 cm; leaf blade not folded, linear-elliptic to lanceolate, 5–13 cm × 5–13 mm, base narrowed, apex obtuse, veins 1 or 3. Stem leaves 1 to 4 pairs, the distal leaves sometimes slightly folded; petiole 3–15(–20) mm; leaf blade narrowly linear-elliptic to lanceolate, 3.5–6 cm × 4–15 mm, apex obtuse, veins 1 or 3. Inflorescences terminal clusters of 3 to 5 flowers, usually with axillary sessile flowers and/or few-flowered clusters on peduncle-like branches. Flowers sessile, rarely lower flowers pedicellate (1–20 mm). Calyx obconic to campanulate, often suffused with red, 7–16 mm in length, tube usually entire, rarely split on the side for 3–5 mm; lobes erect, oblong-triangular, 1–6 mm in length, apex acute to obtuse. Corolla white to pale yellow, with dark blue-purple stripes along the primary veins of the corolla, in short streaks bordering the insertion of the filaments, and in and below the lobes in the distal third of the corolla, and with dark blue-purple spots on lobes, tubular-campanulate to funnelform, length 4.5–6.5 cm; lobes ovate to triangular, 4–7 mm long, apex obtuse; plicae obliquely truncate, margin entire to shallowly erose. Stamens inserted at middle of corolla tube; length of filaments 1.3–1.6 cm; anthers narrowly ellipsoid, whitish or pale yellow, ca. 3.5 mm. Stigma lobes linear. Capsules ovoid-ellipsoid, 2–2.5 cm in length; gynophore ca. 2–2.5 cm long at maturity. Seeds light brown, broadly ellipsoid to subglobose, 1.4–2 mm. Flowers and fruits August to September.

***Distribution and Habitat***—*Gentiana susanneae* is only known from the type locality. The new species was found off the road between Litang and Batang (Sichuan, China), at the Haizi pass and around the Cuopu Lakes (Cuoniba), at an elevation between 4400 and 4600 m. It grows among shrubs (which are sometimes up to ca. 1 m), around riverbeds and on rocky islands among river arms and on adjacent slopes.

***Etymology***—The specific epithet was chosen in honour of Prof. Susanne Fritz, who is a zoologist at the Senckenberg Research Institute Frankfurt and Goethe University Frankfurt, as well as an enthusiastic assistant in field botany. By extension, the new species is named in honour of all individuals named "Susanne" in academia. Susanne was the most common first name for female professors in Germany in 2019.

***Informal Conservation Status***—We estimate that the entire population spreads over at least 8 km$^2$ between the northern shores of Cuopu Lakes and the mountain flank south of Haizi pass. The population is likely to contain at least a few thousand individuals. Minor parts of the area of occupancy may be directly threatened by road construction and constantly increasing tourism. Thus, with an area of occupancy less than 10 km$^2$ (criterion B2a), we hypothesize that if a formal conservation assessment were performed, the new species would probably be regarded as Critically Endangered based on the IUCN (2012) criteria. However, it is possible that the area of occupancy could be larger, since the species' habitat occurs frequently at similar elevations around the range of Mt. Xiashe. If true, then the status of the new species could be downgraded to Endangered.

***Phenology***—Full flowering was observed in August 2016 and September 2018.

### Key to *Gentiana susanneae* and Most Similar Species

Molecular and morphological evidence clearly places *Gentiana susanneae* in *Gentiana* section *Frigida*. We therefore modified the determination key for Chinese species of *G.* sect. *Frigida* as provided in *Flora of China* (Ho and Pringle 1995) in order to include *G. susanneae*. Only the first part of the key needed to be modified, and thus *Flora of China* can be used as usual for species placed under 2b and 1b.

1\. Corolla 3–6 cm . . . . . . . . . . 2
  2\. Corollas almost white, pale yellow, or yellowish green, with dark blue spots and/or stripes . . . . . . . . . . 3
    3\. All or most flowers distinctly pedicellate; basal pedicels often over 4 cm . . . . . . . . . . *G. purdomii*
    3\. Usually only lowermost axillary flowers with true pedicels or all flowers sessile above bracts; pedicels generally less than 1 cm . . . . . . . . . . 4
      4\. Plants 30–50(–80) cm tall; flowers in terminal clusters of 3 to 5 flowers, usually also in upper axis and/or in few-flowered clusters on peduncle-like branches; calyx lobes 1–5(–6) mm, calyx tube 7–16 mm, corolla 4.5–6.5 cm . . . . . . . . . . *G. susanneae*
      4\. Plants 8–20 cm tall; flowers usually solitary, rarely in terminal 2- or 3(–8)-flowered cymes; calyx lobes 5–6 mm, calyx tube 20–22 mm, corolla 4–5 cm . . . . . . . . . . *G. algida*
  2\. Corollas pale to dark blue (basal part of tube may be whitish or yellowish) . . . . . . . . . . *Flora of China* (2b)
1\. Corolla 1.5–3.5 cm . . . . . . . . . . *Flora of China* (1b)

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## Author contributions

AF and PCF collected plant material in the field. AF produced the data and performed phylogenetic analyses. PCF analysed the structure of the chloroplasts. All authors interpreted the results. AF and JSP produced the taxonomic treatment. All authors wrote the manuscript. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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