

Gentiana ranae (Gentianaceae), a new species from India revealed by morphology and molecular phylogenetic analyses

Shabir M.^{1*}, Pal A.K.², Dwivedi M.D.^{3*} & J.K. Tiwari⁴

¹Department of Botany, Kargil Campus, University of Ladakh, Saliskote Kargil, Union Territory of Ladakh – 194 105, India

²Plant Diversity, Systematics & Herbarium Division, CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh – 226 001, India

³Botanical Survey of India, Botanic Garden of Indian Republic, Noida, Uttar Pradesh – 201 303, India

⁴Department of Botany and Microbiology, HNB Garhwal University, Srinagar Garhwal, Uttarakhand – 246 174, India

*E-mails: shabir1610@gmail.com; mayank_dwivedi10@yahoo.com

Abstract: A new species of the genus *Gentiana*, *G. ranae* sp. nov., from Rohtang Pass in Himachal Pradesh (India) is described here. The species shows morphological resemblances with *G. glauca* Pall. and *G. venusta* Wall. of sect. *Monopodiae*, but differs in key morphological characters such as a stem with 4–6 fine lines, upper stem leaves more densely enveloping the inflorescence, a light blue corolla with margins of the corolla lobes scabrous to crenulate, horizontally truncate plicae, dentate to erose margins, and ovate to sub-orbicular seeds. Along with the morphological characters the species is supported as a new member of sect. *Monopodiae* in a molecular phylogenetic analyses using the nuclear ribosomal DNA internal transcribed spacers (ITS) and chloroplast *trnL-F* intron–intergenic spacer regions. This new species is described, illustrated and discussed.

Keywords: cpDNA *trnL-F*, *Gentiana glauca*, nrDNA ITS, sect. *Monopodiae*, western Himalaya.

Introduction

The genus *Gentiana* L. (Gentianaceae) belongs to the monophyletic subtribe Gentianinae along with *Crawfordia* Wall., *Kuepferia* Adr.Favre, *Metagentiana* T.N.Ho, *Sinogentiana* Adr.Favre & Y.M.Yuan, and *Tripterosperrum* Blume, consisting of about 362 species (Ho & Liu, 2001; Struwe & Albert, 2002; Favre *et al.*, 2014, 2016). *Gentiana* finds its ecological optimum between 3500–4500 m and is found distributed in the meadows of temperate, sub-alpine and alpine regions around the globe. In India, the

genus comprises *c.* 68 species largely distributed in the Indian Himalayan region (Grag, 1987; Gupta *et al.*, 2012; Shabir *et al.*, 2018).

During a field collection trip to the Rohtang Pass of Himachal Pradesh (India) in October 2017, MS located a population of a species of *Gentiana* growing on the south-facing slope at about 4200–4400 m asl., near the Rohtang temple. Three mature individuals were collected from this population for taxonomic studies. After a critical examination of the specimens, the plants were found to represent a species without any resembling description in the available literature under sect. *Monopodiae*. Therefore, a detailed study was made using molecular and morphological methods in support of its taxonomic status and affinities with *Gentiana*, and the discovered new species is described and illustrated herewith as *Gentiana ranae* sp. nov.

Materials and Methods

Field trips and herbarium visits: Accessions of *Gentiana* specimens were collected from a single population in Rohtang Pass of Himachal Pradesh in western Himalaya. The determination of the collected specimens down to genus level was made after consulting the relevant taxonomic literature (Garg, 1987; Ho & Pringle, 1995; Halda, 1996; Ho & Liu, 2001; Gupta, 2009; Shabir *et al.*, 2017), and consultation of herbarium specimens housed at BSD, CAL, DD, KASH, and LWG and digital images of specimens deposited in BM, E, and K. The holotype (Shabir 308822) was deposited in

LWG. Field photographs were taken using a Nikon Coolpix camera (Tokyo, Japan). Morphological characters were noted and floral parts photographed using a Leica S6 E stereo-zoom microscope (Leica Microsystems, Mumbai, India) and figures and plates were edited using software Adobe Photoshop (Adobe, Inc. California, USA).

Ingroup sampling: In order to determine phylogenetic relationship of the newly sequenced taxon, sequences of 175 *Gentiana* samples of 174 species (out of ca. 362 in the genus) and one variety used in a previous study (Favre *et al.*, 2016) and available on GenBank were included for analysis, and included 6 species out of 16 species currently regarded to belong to sect. *Monopodiae* (Ho & Liu, 2001, Favre *et al.*, 2020). Additionally, three samples were included for the new species (Appendix 1).

Outgroup sampling and rooting: Based on the phylogenetic studies of Favre *et al.* (2016) four species each of the two genera *Crawfordia* and *Kueferia* were chosen as outgroups, and the trees rooted on the *Crawfordia* samples (Yuan *et al.*, 1996; Favre *et al.*, 2010, 2016, 2020) (Appendix 1).

DNA extraction, polymerase chain reaction (PCR) and Sanger sequencing: Total genomic DNA was extracted from silica gel dried leaf material using a DNeasy Plant Mini Kit (Qiagen, Amsterdam, Netherlands). The extracted DNA was evaluated for its quality and quantity by using EPOCH Microplate Spectrophotometer (BioTEK, Winooski, Vermont, USA). DNA amplification and sequencing of the ITS region (ITS1, 5.8S and ITS2) was performed using the primers ITS1 and ITS4 (White *et al.*, 1990). For the chloroplast plastid *trnL* intron and adjacent *trnL-trnF* spacer (*trnL-F* region), we used primers c and f (Taberlet *et al.*, 1991). PCR was performed with standard methods using 25 µl reactions that consisted of 12.5 µl RedDye Master mix (GeNei, Bangalore, India), to which we added 1.5 µl of a 0.3 µM solution of each forward and reverse primer, 2 µl of genomic DNA and 7.5 µl of double distilled water. PCR amplification was performed with an initial denaturation of 1 min at 95° C, 35 cycles of

denaturation for 10 sec at 95°C, primer annealing for 60 sec at 60°C and 1 min of extension at 72°C followed by the last cycle of final extension for 90 sec at 72°C. PCR products were checked for the presence of a single band of appropriate size on a 0.8% agarose gel stained with EtBr. Amplicons were purified using ExoSAP-IT (Fermantas, Burlington, CA, USA), and sequenced at 1st Base Labs., Seri Kembangan 43300, Selangor, Malaysia using Sanger ABI technology. Chromatograms for forward and reverse sequences were assembled and edited using the computer program DNA Baser v.4 (2013, Heracle BioSoft, Lilienthal, Germany) and aligned using Geneious v.6.1.8 (Drummond *et al.*, 2010). All newly acquired sequences have been deposited in GenBank (Appendix 1).

Phylogenetic analyses: The best-fitting partitioning scheme and models for evolution for individual as well as concatenated (ITS + *trnL-F*) regions respectively were selected using PartitionFinder2 (Lanfear *et al.*, 2016). For the ITS region, the data were partitioned into three parts, ITS1, 5.8S and ITS2. For the *trnL-F* region, into the intron and spacer regions. The phylogenetic analyses were performed at the CIPRES Science gateway (Miller *et al.*, 2010). Maximum Likelihood (ML) analyses were performed for the individual nrDNA ITS and cpDNA *trnL-F* regions separately and combined using RAxML-HPC2 on XSEDE v.8.2.12 (Stamatakis, 2014), run under the models determined by Partitionfinder 2 with 20 heuristic searches from distinct random stepwise addition sequence parsimony starting trees, followed by a selection of best scoring tree. RAxML bootstrap (BS) values were calculated using 1,000 replicates. Bayesian Inference (BI) analyses were performed for individual as well as for the combined ITS and *trnL-F* data with MrBayes 3.2.7a (Ronquist *et al.*, 2012) with two runs in parallel with four chains each running for 10 million generations and sampling every 1000th generation. We used Tracer v.1.6.0 (Rambaut *et al.*, 2014) to check for convergence of the chains. Specifically, we visually examined the Tracer plots and further checked that

Table 1. Comparison of morphological characters between *G. ranae*, *G. glauca* and *G. venusta*

Characters	<i>G. ranae</i> M.Shabir & M.D. Dwivedi	<i>G. glauca</i> Pall.	<i>G. venusta</i> Wall.
Habit	Perennial herbs with stoloniferous roots	Perennial herbs, stolons poorly developed	Biennial-perennial with stoloniferous roots
Leaves	Basal leaves rosulate, crowded towards base, cauline leaves mostly obovate, elliptic to spatulate; apex rounded to sub-rounded	Basal leaves crowded, cauline leaves spatulate to obovate-oblong, apex obtuse to rounded	Basal leaves indistinctly rosulate, cauline leaves obovate, spatulate, apex obtuse
Calyx	10–12 mm long, lobes 2–3.5 mm long, unequal, lanceolate, slightly triangular with acute apex, sinus between lobes obtuse to sub-rounded	5–7 mm long, lobes 2–3 mm long, sub-equal, narrowly triangular with apex acute, sinus between lobes acute	8–17 mm long, lobes 3–6 mm long, unequal, narrowly oblong, linear to lanceolate with obtuse-acute apex, sinuses between lobes obtuse
Corolla	Ovate-obovate with acute apex, margins scabrous, crenulate	Elliptic-ovate with apex obtuse, margins entire	Obovate, ovate-oblong with obtuse apex, margins entire
Plicae	Horizontally truncate, margins dentate to erose	Triangular, margins entire	Triangular to truncate, margins entire or 2 to 3 dentate
Stamens	Inserted from the middle of the tube	Inserted at basal part of corolla tube	Inserted at middle of corolla tube
Carpels	5–6 mm long, ovary narrowly ovate to elliptic, style 2–2.5 mm long, stigma lobes orbicular to coiled	10–12 mm long, ovary ellipsoid, style 0.5–1.5 mm long, stigma lobes semi-orbicular	5.0–13 mm long, ovary narrowly lanceolate, style 1.5–3 mm long, stigma lobes coiled
Seeds	0.3–0.4 mm, sub-orbicular, ovate, elliptic, oblong seed coat alveolate, shallow and hexagonal pits	1–1.2 mm, ellipsoid to sub-globose, seed coat with spongy and complex hexagonal pit	0.6–0.8 mm, ellipsoid, glistening, seed coat with rough and complex hexagonal pits

all ESS values were above 200. We also inspected the MrBayes output to make sure that the standard deviation of split frequencies was below 0.01. After exclusion of the first 20% of the sampled trees, we computed a Bayesian 50% majority-rule consensus tree (“allcompat” command in MrBayes) of the remaining 240,000 trees.

Results

Newly acquired sequences: For this study, a total of five sequences (3 ITS and 2 *trnL-F*) for the three samples of the new species were newly generated and have been submitted to GenBank (Appendix 1). The boundaries for the nuclear internal transcribed spacers (ITS1 + ITS2) and the chloroplast *trnL* gene and intron, and the *trnL-F* intergenic spacers for the newly generated sequence was determined using the FEATURES option provided in Appendix 1 for previously submitted

sequences (e.g. Favre *et al.*, 2014, 2016).

Sequence alignments

Nuclear ITS region: The alignment matrix of the ITS region consisted of 667 characters and included 186 sequences. 0.9% of the characters were alignment gaps and the GC content 58%. Out of the 667 aligned characters 458 (72.8%) represented invariable sites, and the pairwise percentage identity was determined to be 93.9% across the alignment matrix including the outgroup samples. It had 523 distinct patterns, with 310 (46%) parsimony-informative sites, 31 (4.6%) autapomorphic sites, and 254 (38%) constant sites.

Plastid *trnL-F* region: The alignment matrix of the *trnL-F* region included 168 sequences (only two *Crawfordia* and one *Kuepferia* sequence were included as outgroups), and was 1,122 characters long. Of these characters, 11.9% were gaps, and

the GC content was 35.8%. Out of the 1,122 aligned characters 459 (64.9%) represented identical sites, and the percentage pairwise identity was 93.1% across all samples included. There was a total of 786 distinct patterns, 267 (23%) parsimony-informative sites, 218 (19.4%) constant, and 67 (5.9%) autapomorphic.

Combined ITS + trnL-F: The combined matrix included 186 samples including the newly generated ones and included 186 ITS and 168 trnL-F sequences downloaded from GenBank, and was 1,789 characters long. Of these, 6.2% were gaps, 47% GC content, 931 (68.4%) sites were identical, and the pairwise percent identity was 93.4%. There were 1,255 distinct patterns, 554 (31%) parsimony-informative sites, and 303 (16.9%) autapomorphic sites. The final alignment has been submitted to TreeBASE (Fig. 1) (<http://purl.org/phylo/treebase/phylo/study/TB2:S28982>) and can also be obtained from the corresponding author on request.

Maximum Likelihood and Bayesian Inference phylogenetic analyses: The ML and BI tree topology retrieved after analysing the combined ITS + trnL-F regions (Fig. 1) had a similar tree topology compared to the tree topologies from previous studies (Yuan *et al.*, 1996; Favre *et al.*, 2010, 2016, 2020). The log-likelihood of the consensus tree was -18,512.409. All the species of *Gentiana* included in the present study originating from a common ancestor and diverged into several single major clades (Fig. 1), representing eleven sections: 1. *Chondrophyllae*, 2. *Pneumonantheae*, 3. *Cruciata*, 4. *Monopodiae*, 5. *Frigidae*, 6. *Microsperma*, 7. *Kudoa*, 8. *Phyllocalyx*, 9. *Calathianae*, 10. *Ciminalis*, 11. *Gentiana*.

The three accessions of the newly discovered species *Gentiana ranae* in one clade (BP: 100%; PP: 1) placed in sect. *Monopodiae* in the phylogeny (BP: 99%; PP: 1) (Fig. 1). The ML and BI trees on individual ITS and trnL-F data supported this placement (data not shown). The new taxon was placed along with other species of the section with the closest being *G. glauca* as sister to the new species (BP: 99%; PP:

0.98). They share key morphological characters such as stem leaves usually in crowded whorl form, and upper leaves enveloping the inflorescence densely. The latter is characterized by stem leaves usually in pairs, upper leaves enveloping the inflorescence less densely. It differs from the new species in key morphological characters such as striate stems, 4–6 lineolate, light blue corolla and scabrous, crenulate lobe margins, plicae horizontally truncate, dentate to erose margins, and the shape and size of seeds (Table 1). Other species in this section clade were *G. davidii*, and a clade consisting of *G. cephalantha*, *G. rigescens* and *G. duclouxii* on a polytomy with *G. ranae* & *G. glauca*, with *G. melandrifolia* on the first branch. Most relationships in this section were not supported (Fig. 1).

Taxonomic Treatment

***Gentiana ranae* M.Shabir & M.D.Dwivedi sp. nov.** Figs. 2 & 3

Type: INDIA, **Himachal Pradesh**, Rohtang Pass, 4000 m, 13.10.2017, *M. Shabir* 308822 (holo LWG; iso GUH).

Perennial herbs, 5–10 cm tall, roots few, weak, stolon forming, presence of nodes and internodes, stem ridged, 4–6 lineolate, prostrate. Radical leaves 5–10 × 2.8–4.5 mm, rosulate, crowded towards the base, present in whorls of 2–3, widely spaced; each whorl contains 8–12 leaves. Cauline leaves 4–15 × 2.5–7 mm, opposite, upper leaves forms whorl around the inflorescence; mostly obovate, elliptic to spatulate with rounded to sub-rounded apex, mid-vein distinct abaxially, margins scabrous, tinged purple. Flowers terminal, 2–4 clustered, sessile. Calyx up to 12 mm long, surrounded by stem leaves; tube broadly tubular, 7.5–9 mm long, longer than the lobes (2/3 of the lobes); lobes unequal 2–3.5 × 0.5–1.15 mm, lanceolate, slightly triangular with acute apex, mid-veins indistinct, margins scabrous, sinus between lobes obtuse to sub-rounded. Corolla light blue, 15–22 mm long, tube much longer than the lobes, lobes short 2–3 × 1.4–1.8 mm, broadly ovate with acute apex,

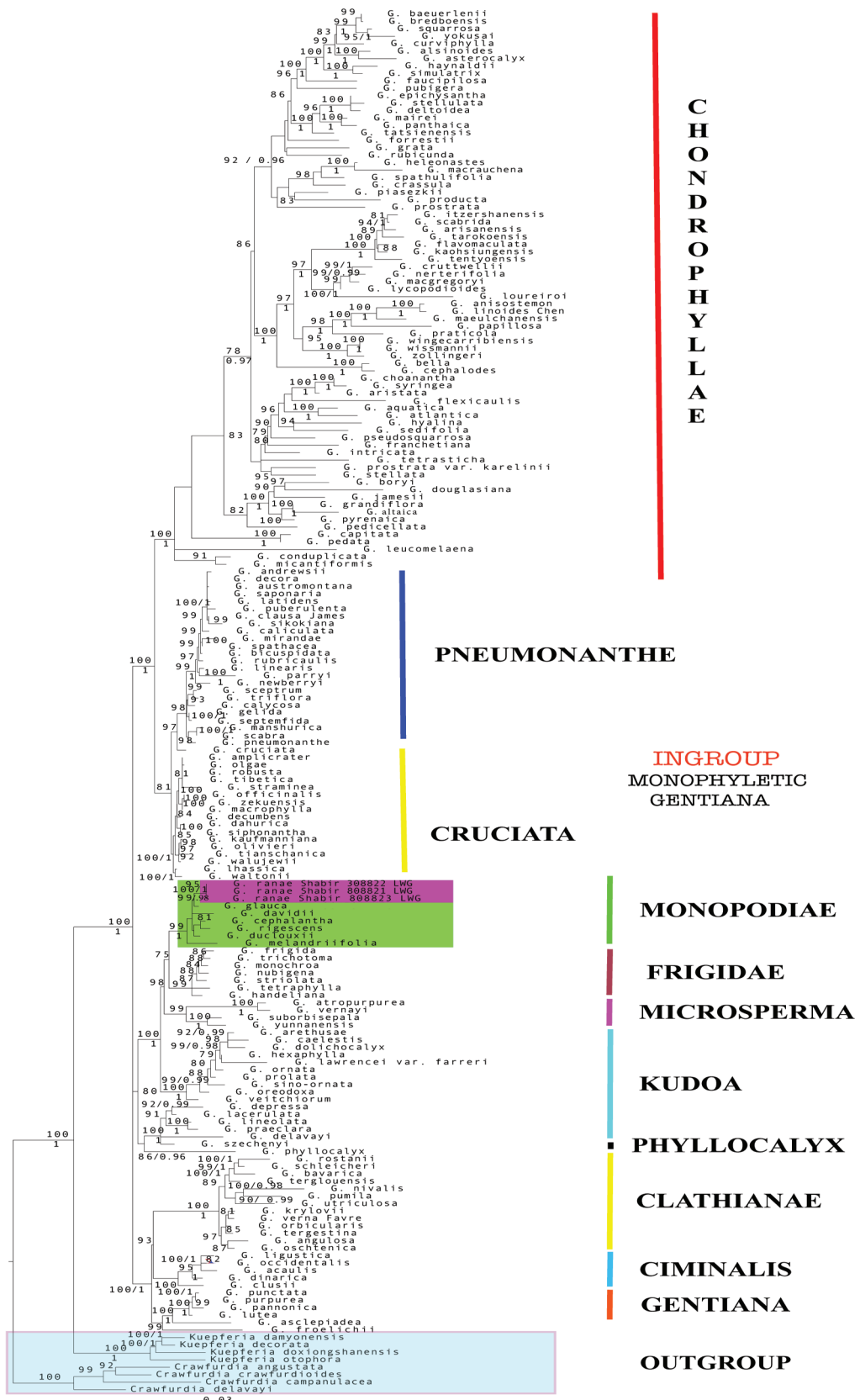


Fig. 1. Best Maximum Likelihood tree retrieved after analysing the combined nrDNA (ITS) and cpDNA (*trnL-F*) sequence data of 186 samples. Only bootstrap values >75% and BI values >0.95 are indicated along the branches. Section names are to the right of the tree.



Fig. 2. *Gentiana ranae* M.Shabir & M.D.Dwivedi: a. Habitat; b. Flowering plant; c. Habit; d. Seeds (Photos by Mohd Shabir).

margins scabrous, crenulate; plicae very short, horizontally truncate, broadly ovate with obtuse to acute apex, margins dentate to erose. Stamens 5, filaments 4–6 mm long, inserted from the middle of the tube, terminating much below the sinus, flattened at base, free part short; anthers 1–1.2 ×

0.25–0.3 mm, ellipsoid, narrow and pointed toward apex. Carpel stipulate, stipe much longer 6–7 mm, ovary narrowly ovate to elliptic 5–6 × 1.6–1.7 mm, style 2–2.5 mm, linear, stigma bifid, lobes orbicular to coiled. Capsule stalked, oblanceolate, 7–15 × 3–6 mm. Seeds 0.3–0.4 × 0.2–0.25 mm, compressed,

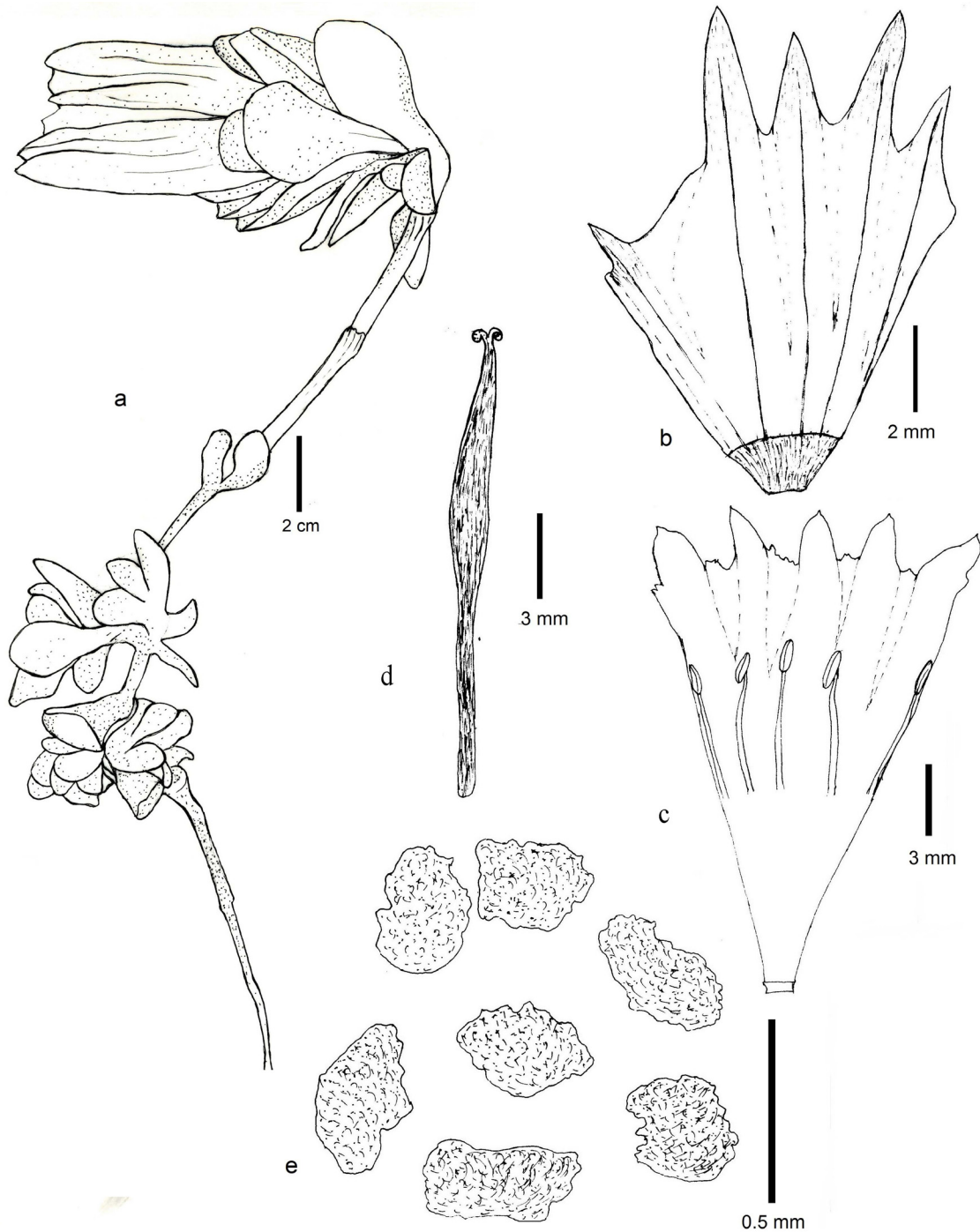


Fig. 3. *Gentiana ranae* M.Shabir & M.D.Dwivedi: a. Habit; b. Calyx; c. Corolla; d. Capsule; e. Seeds (from *M. Shabir* 308832 LWG; drawn by Mohd Shabir).

sub-orbicular, ovate, elliptic, oblong, winged, seed coat alveolate, shallow and hexagonal pits.

Flowering & fruiting: September (flowering), October (fruiting).

Habitat: The species is found distributed in alpine

meadows, grasslands, and on rocky surfaces, on high altitude slopes in the Himalayas, at altitudes between 4000–4300 m. Presently, three individual plants have been identified for this taxon in Rohtang Pass of Himachal Pradesh in western Himalaya from an area of c. 5 km².

Distribution: India, hitherto known only from the type locality.

Etymology: The species is named after Dr. Tikam Singh Rana, Chief Scientist, CSIR–National Botanical Research Institute, Lucknow for his remarkable contribution to the molecular systematics and phylogenetic studies of different plant groups in India.

Discussion

This is the first study to have analysed any Indian species of *Gentiana*. *Gentiana ranae* is morphologically close to the sympatric species *Gentiana glauca* Pall. and *G. venusta* Wall. ex. Griseb. in sect. *Monopodiae*. *Gentiana glauca* was described by Pallas (1789) based on his own collection from Russia. The species is so far known to occur in the United States, Canada, East Russia and Japan (Ho & Liu, 2001), whereas the species *G. venusta* was described by Wallich (1838) from Mt. Emodi, Kumaon in the western Himalaya (India). Later, Grisebach (1838) validated the name. Presently, *G. venusta* is chiefly distributed in the Himalayan region of India, Pakistan, Nepal and Bhutan. According to the natural system of classification of Ho and Liu (1990, 2001), both *G. glauca* and *G. venusta* are treated under ser. *Sikkimenses* of sect. *Isomeria* of genus *Gentiana*. Morphologically the sect. *Isomeria sensu* Ho and Liu (2001) is characterized by their perennial and herbaceous habit, sympodially branched stems, flowering stems arising singly from slender stolons, often creeping and rooting at lower nodes, with terminal inflorescences, solitary flowers or in few-flowered clusters, and usually sessile. The stigma lobes are free, recurved, linear or oblong, and the seed coat alveolate, with simple and shallow or spongy and complex hexagonal pits. The seed testa ornamentation in *Gentiana* can be of high taxonomic value, which provides information to facilitate the identification and delimitation of *Gentiana* at infra-generic levels. Most of the *Gentiana* seed type descriptions are based on single characters, i.e. seed testa ornamentation (Halda, 1996; Ho & Liu, 2001; Davitashvili & Karrer, 2010).

Section *Isomeria sensu* Ho and Liu (2001) is characterized by irregular, strongly raised anticlinal walls without distinct membranous lamellae. Recently, Favre *et al.* (2020) placed ser. *Sikkimenses* of sect. *Isomeria* under sect. *Monopodiae* of *Gentiana*. Series *Sikkimenses* groups with sect. *Monopodiae* in a well-supported lineage distantly related to other sect. *Isomeria* species in his molecular phylogenetic analysis.

Gentiana ranae is characterized by a stem with 4–6 fine lines, upper stem leaves more densely enveloping the inflorescence, light blue corolla and margins of corolla lobes scabrous to crenulate, plicae horizontally truncate and margins dentate to erose. minute seeds ovate to sub-orbicular in shape. The species show morphological resemblance with *G. glauca* in perennial habit, narrowly triangular calyx lobes with acute apex, while the latter differs in having poorly developed stolons, stem leaves usually in pair, upper stem leaves less densely enveloping the inflorescence and stamens inserted at basal part of corolla tube. The species shows morphological resemblance with *G. venusta* in fully developed stolons, leaves obovate to spatulate, upper stem leaves enveloping the base of the inflorescence and stamens inserted from the middle of the corolla tube, but differs from the latter in perennial habit, rosulate basal leaves, absence of papillae on cauline leaves.

The specimen of *G. ranae* was also sent to Dr. J.S. Pringle (an authority of the New World Gentians) for identification, which he identified as a new taxon under sect. *Isomeria* before it was moved under sect. *Monopodiae* by Favre *et al.* (2020). We confirmed this through molecular phylogenetic studies. Our BI and ML phylogenetic analyses on ITS and combined data (Fig. 1; ITS data not shown) indicated that among our sampled taxa, the Indian samples of *Gentiana ranae* sp. nov. (voucher no. *M. Shabir* 308822) were sister to *Gentiana glauca* (reported from the USA, voucher no. *NEU* 394791) with strong support values. The morphological analyses revealed distinctness of *G. ranae* when compared to *G. glauca* (Table 1). In the present study, six (excluding the newly described one) out of a total of 16 species recognised in sect.

Monopodiae were included in the phylogenetic study and the result make a strong case for adding the new species, *G. ranae*, to this section (Fig. 1).

Authors' contribution: MS collected the plants. MS and AKP performed lab work, generated data (morphological + molecular), drafted the manuscript. MDD analysed the molecular data contributed in writing the manuscript with MS. JKT approved the final draft of the manuscript.

Acknowledgments

The authors express their gratitude to Dr. J.S. Pringle for his useful insight into the identification of the species, and to Dr. Adrien Favre for his critical review and comment to improve the manuscript. Dr. Abid Yerimani and Dr. Aniket Ghosh are gratefully acknowledged for their help at the initial stages of the phylogenetic analysis. MS is thankful to UGC, New Delhi for financial assistance as SRF. MDD is thankful to the Director, Botanical Survey of India for support and facilities. The Ministry of Environment, Forest and Climate Change (MoEF & CC), Government of India, New Delhi has provided financial support to MDD as RA (Project 284 GBPNI/NMHS-2017-18/LG-03/570dt.26/02/2018).

Literature Cited

- DAVITASHVILI N. & G. KARRER 2010. Taxonomic importance of seed morphology in *Gentiana* (Gentianaceae). *Botanical Journal of the Linnean Society* 162(1): 101–115. <https://doi.org/10.1111/j.1095-8339.2009.01020.x>
- DRUMMOND A.J., ASHTON B., BUXTON S., CHEUNG M., COOPER A., HELED J., KEARSE M., MOIR R., STONES-HAVAS S., STURROCK S., THIERER T. & A. WILSON 2010. *Geneious*, version 6.1.8. <http://www.geneious.com>
- FAVRE A., YUAN Y.M., KÜPFER P. & N. ALVAREZ 2010. Phylogeny of subtribe Gentianinae (Gentianaceae): Biogeographic inferences despite limitations in temporal calibration points. *Taxon* 59(6): 1701–1711. <https://doi.org/10.1002/tax.596005>
- FAVRE A., MATUSZAK S., SUN H., LIU E., YUAN Y.M. & A.N. MUELLNER-RIEHL 2014. Two new genera of Gentianinae (Gentianaceae): *Sinogentiana* and *Kuepferia* supported by molecular phylogenetic evidence. *Taxon* 63(2): 342–354. <https://doi.org/10.12705/632.5>
- FAVRE A., MICHALAK I., CHEN C.H., WANG J.C., PRINGLE J.S., MATUSZAK H., SUN H., YUAN Y.M., STRUWE L. & A.N. MUELLNER-RIEHL 2016. Out-of-Tibet: the spatio-temporal evolution of *Gentiana* (Gentianaceae). *Journal of Biogeography* 43(10): 1967–1978. <https://doi.org/10.1111/jbi.12840>
- FAVRE A., PRINGLE J.S., HECKENHAUER J., KOZUHAROVA E., GAO Q., LEMMON E.M., LEMMON A.R., SUN H., TKACH N., GEBAUER S., SUN S.S. & P.C. FU 2020. Phylogenetic relationships and sectional delineation within *Gentiana* (Gentianaceae). *Taxon* 69(6): 1221–1238. <https://doi.org/10.1002/tax.12405>
- GARG S. 1987. *Gentianaceae of the Northwest Himalaya (A Revision)*. Today & Tomorrow's Printers and Publishers, New Delhi.
- GRISEBACH A.H.R. 1838. *Genera et Species Gentianearum adjectis observationibus quibusdam phytogeographicis*. Stuttgart and Tübingen.
- GUPTA S. 2009. *Taxonomic studies in Gentianaceae Juss. in India*. Ph.D. Thesis (unpublished), University of Burdwan, Kolkata.
- GUPTA S., MIKHERJEE A. & M. MONDAL 2012. A census of *Gentiana* L. in India, systematics of flowering Plants. In: MAITI G. & S.K. MUKHERJEE (eds.), *Multidisciplinary Approaches in Angiosperm Systematics*, Volume 1, University of Kalyani. Kalyani. pp. 53–58.
- HALDA J.J. 1996. *The genus Gentiana. Dobré: Sen.* University of Tokyo Press, Tokyo.
- HO T.N. & S.W. LIU 1990. The infrageneric classification of *Gentiana* (Gentianaceae). *Bulletin of the British Museum (Natural History) Botany* 20(2): 169–192.
- HO T.N. & J.S. PRINGLE 1995. *Gentiana*. In: WU Z.Y. & P.H. RAVEN (eds.), *Flora of China (Gentianaceae through Boraginaceae)*. Science Press, Beijing and Missouri Botanical Garden Press, St Louis. 16: 15–98.
- HO T.N. & S.W. LIU 2001. *A worldwide monograph of Gentiana*. Science Press, Beijing.
- LANFEAR R., FRANSDEN P. B., WRIGHT M., SENFELD T. & B. CALCOTT 2016. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34(3): 772–773. <https://doi.org/10.1093/molbev/msw260>
- MILLER M.A., PFEIFFER W. & T. SCHWARTZ 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees, *Gateway Computing*

- Environments Workshop (GCE)*, 2010, pp. 1–8, doi: 10.1109/GCE.2010.5676129
- PALLAS P.S. 1789. *Flora Rossica seu stirpium Imperii Rossici par Europam et Asiam indigenarum descriptiones et icones*. Volume 1(2). J.J. Weitbrecht, St. Petersburg. pp.104. t. 93, f. 2.
- RAMBAUT A., SUCHARD M.A., XIE D. & A.J. DRUMMOND 2014. *Tracer* v.1.5. Available from: <http://beast.bio.ed.ac.uk/Tracer>.
- RONQUIST F., TESLENKO M., VAN DER MARK P., AYRES D.L., DARLIN A., HOHNA S. & B. LARGET 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542. <https://doi.org/10.1093/sysbio/sys029>
- SHABIR M., AGNIHOTRI P., HUSAIN D., TIWARI J.K. & T. HUSAIN 2017. On the current status of the genus *Gentiana* L. (Gentianaceae) in India. *Pleione* 11(1): 16–24. <http://pleione.ehsst.org/journals/Pleione111/003%20On%20the%20current%20status%20of%20the%20genus%20Gentiana%20in%20India.pdf>
- SHABIR M., AGNIHOTRI P., TIWARI J.K. & T. HUSAIN 2018. *Gentiana pringlei* (Gentianaceae) – a new species from cold deserts of Ladakh Himalaya, India. *Taiwania* 63(4): 356–359. <https://doi.org/10.6165/tai.2018.63.356>
- STAMATAKIS A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- STRUWE L. & A. ALBERT 2002. *Gentianaceae: Systematics and Natural History*. Cambridge University Press, Cambridge.
- TABERLET P., GIELLY L., PAUTOU G. & J. BOUVET 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109. <https://doi.org/10.1007/BF00037152>
- WALLICH N. 1838. *In: CAT. N. 4389 nom. nud.*, et ex Griseb., *Genera et Species Gentianearum adjectis observationibus quibusdam phytogeographicis*. p. 276.
- WHITE T.J., BRUNS S.L. & J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In: INNIS M., GELFAND D., SNINSKI J. & T.J. WHITE (eds.), PCR Protocols: a guide to methods and applications*. Academic Press, San Diego. pp. 315–322.
- YUAN Y.M., KÜPFER P. & J.J. DOYLE 1996. Infrageneric phylogeny of the genus *Gentiana* (Gentianaceae) inferred from nucleotide sequences of the internal transcribed spacers (ITS) of nuclear ribosomal DNA. *American Journal of Botany* 83(5): 641–652. <https://doi.org/10.2307/2445924>

Appendix 1. Species, collection number, locality, and GenBank accession numbers for the molecular markers used in the present study (nuclear ITS region, and plastid *trnL-F* region; (// indicate no data)

S. No	Gentiana species	Voucher Specimens	Locality	ITS	<i>trnL-F</i>
1	<i>G. acatilis</i> L.	Favre 1003 (LZ) [ITS], G_aca_1 [<i>trnL-F</i>]	Switzerland: Gd-St-Bernhard, Valais [ITS], unknown [<i>trnL-F</i>]	KT907603	JF748586
2	<i>G. alsinoides</i> Franch.	Dickor 14099 (MU)	China: Dejia Mts, Lijiang, Yunnan	KT907605	KT907745
3	<i>G. altaica</i> Laxm.	unknown	unknown	Z71931, Z71932	//
4	<i>G. applicator</i> Burkill	Wu Zhengyi et al. (KUN)	China: Jue En to Zhula, Xizang	KT907606	KT907747
5	<i>G. andrewsii</i> Griseb.	James S. Pringle 2737 (LZ)	United States: Royal Botanical Gardens, Burlington	KT907607	KT907748
6	<i>G. angulosa</i> M.Bieb.	Gutte 33690 (LZ)	Georgia: Caucasus, Kreuzpass unterhalb Mleti	KT907608	KT907749
7	<i>G. anisostemon</i> C. Marquand	Favre 1244 (LZ)	China: Yunnan	KT907609	KT907750
8	<i>G. aquatica</i> L.	Schneeweiß & Tribsch 6697 (WU)	Georgia: Samtskhe-Javakheti	KT907610	//
9	<i>G. arethusa</i> Burkill	Favre & Matuszak 177a (KUN)	China: Baimaxuashan, Yunnan	KT907611	KT907753
10	<i>G. arisanensis</i> Hayata	S.-H. Huang 9632902-02 (CYUT) [ITS], Chen HS, CHC 2162 [<i>trnL-F</i>]	unknown [ITS], Taiwan: Ta-Yu-Ling, Sioulin, Hualien [<i>trnL-F</i>]	JQ890596	KT907754
11	<i>G. aristata</i> Maxim.	Wang Lisong, et al. 07-70 (KUN)	China: Changdu to Wuqishanpass, Xizang [<i>trnL-F</i>]	KT907612	KT907755
12	<i>G. asclepiadea</i> L.	Ge09 [ITS], Yuan 93-20 [<i>trnL-F</i>]	Austria: Prein-Rax [ITS], Bulgaria: Izgorjaloto Gjune reserve [<i>trnL-F</i>]	GU251025	AJ580515
13	<i>G. asterocalyx</i> Diels	Favre & Matuszak 106a	China: Lijiang, Yunnan	KT907613	KT907756
14	<i>G. atlantica</i> Litard. & Maire	Liu1095	unknown	Z71933, Z71934	//
15	<i>G. atropurpurea</i> T.N.Ho	Liu 1095	unknown	AY858678	AY858686
16	<i>G. austromontana</i> J.S.Pringle & Sharp	James S. Pringle 2734 (LZ)	United States: Roan Mountain, North Carolina	KT907615	KT907758
17	<i>G. baeuerlenii</i> L.G.Adams	Adams 4124 (CANBR)	Australia: Orroal Valley	KT907616	KT907759
18	<i>G. bavaria</i> L.	Favre 1002 (LZ)	Switzerland: Sanetsch, Valais	KT907617	KT907760
19	<i>G. bella</i> Franch. ex Hemsl.	HS Chen CHC 2850 (TNM)	China: Chien-hu-Shan, Yunnan	KT907618	KT907761
20	<i>G. bituspidata</i> (G.Don) Briq.	Hernandez & de Hole 947 (HUH)	Mexico: Palo Bendito, Huayacocotla	KT907619	KT907762
21	<i>G. boryi</i> Boiss.	Rivas-Martinez 1959 (L)	Spain: Sierra de Bejar, Candelario, Salamanca	KT907620	KT907763
22	<i>G. bredboensis</i> L.G.Adams	Adams 4150 (CANBR)	Australia: Frogs Hole Creek, New South Wales	KT907622	KT907765
23	<i>G. caelestis</i> (C.Marquand) Harry Sm.	Favre & Matuszak 193a (KUN)	China: Daocheng, Sichuan	KT907624	KT907766

24	<i>G. caliculata</i> Lex. in Lalave & Lex.	Hinton <i>et al.</i> 6819 (HUH)	Mexico: Temascaltepec	KT907707	KT907851
25	<i>G. calycosa</i> Griseb.	Tanya Harvey 2013-01-0062 (LZ)	United States: Jefferson Park	KT907625	KT907767
26	<i>G. capitata</i> Buch.-Ham. ex D.Don	Nusser 723 (B)	Pakistan: Narga Parbat	KT907626	//
27	<i>G. cephalantha</i> Franch. ex Hemsl.	Favre 325 (KUN)	China: Sichuan	KT907627	KT907770
28	<i>G. cephalodes</i> Edgew.	Walter N. Koelz 32729 (L)	India: Assam	KT907628	//
29	<i>G. choanantha</i> C.Marquand	Favre 325	China: Luojishan, Sichuan	KT907629	KT907771
30	<i>G. clausa</i> Raf.	Xizang Expedition, 74-3508 (KUN)	China: Qianning to Daofu, Sichuan	KT907630	KT907773
31	<i>G. chusii</i> Perr. & Songeon	Favre CH13011 (LZ)	Switzerland: Valais	KT907631	KT907774
32	<i>G. con duplicata</i> T.N.Ho	James S. Pringle 2738	United States: Monongahela Nat. Forest, West Virginia	KT907632	//
33	<i>G. crassula</i> Harry Sm.	Favre & Matuszak 205a	China: Yading, Sichuan	KT907633	//
34	<i>G. cruciata</i> L.	Kuepfer 2005-G2 (NEU)	unknown	DQ398635	DQ398713
35	<i>G. cruttwellii</i> Harry Sm.	van Balgooy 76	Papua New Guinea: Mt. Wilhelm	KT907633	KT907779
36	<i>G. curviphylla</i> T.N.Ho	Favre 220a	China: Litang, Sichuan	KT907635	KT907781
37	<i>G. dahurica</i> Fisch.	Favre 319	China: Xiahe, Gansu	KT907636	KT907782
38	<i>G. davidii</i> Franch.	Expedition Team to Northeast 730	China: Manaoko, Zhaorong, Yiliang, Yunnan	KT907637	KT907783
39	<i>G. decora</i> Diels	K. Mathews <i>s.n.</i> (WCUH)	unknown	EU812468	EU834122
40	<i>G. decumbens</i> L.f.	Yuan 2004-83 (IBSC)	unknown	DQ398655	DQ398714
41	<i>G. delavayi</i> Franch.	unknown	unknown	Z48099, Z48080	//
42	<i>G. deltoidea</i> Harry Sm.	Chen HS, CHC 2847	China: Chien-hu-shan, Yunnan	KT907638	//
43	<i>G. depressa</i> D.Don	Favre 1269	Nepal: Kopra Ridge, Annapurna, Pokhara	GU251026	KT907784
44	<i>G. dimarica</i> Beck	Haemmerli 399339 (NEU)	Slovenia: Triggalv Mts, Rzki Podi	KT907639	KT907785
45	<i>G. dolichocalyx</i> T.N.Ho	Boufford <i>et al.</i> 39978	China: Hongyuan, Maiwa Xiang, Sichuan	KT907640	KT907787
46	<i>G. douglasiana</i> Bong.	Calder 35804	Canada: Queen Charlotte Isl., British Columbia	KT907641	KT907786
47	<i>G. dulcousii</i> Franch.	Favre & Matuszak 076	China: Kunming, Yunnan	KT907642	KT907788
48	<i>G. epichysantha</i> Hand. Mazz.	Favre & Matuszak 131a	China: Bashuitai, Yunnan	KT907643	KT907790
49	<i>G. faucipilosa</i> Harry Sm.	Favre & Matuszak 37a	China: Bingzhongluo, Yunnan	KT907644	KT907792
50	<i>G. flavomaculata</i> Hayata	Chen HS, CHC 2364 (TNM)	Taiwan: Kuan-Yun, Sioulin, Hualien	KT907645	KT907793
51	<i>G. flexicaulis</i> Harry Sm.	unknown	unknown	Z71937, Z71938	//
52	<i>G. forrestii</i> C.Marquand	Penghua, Liu Ende <i>et al.</i> 9538 (KUN)	China: Jiaozishan, Luquan County, Yunnan	KT907646	KT907794
53	<i>G. franchetiana</i> Kuhn.	Chen HS, CHC 2860 (TNM)	China: Kan-hai-tzu, Chien-hu-shan, Yunnan	KT907647	//
54	<i>G. frigida</i> Haenke	Schachen 1995 [TTS], K. Gutschke 42 (MJG) [<i>rmL-F</i> as <i>cf. frigida</i>]	unknown	AJ294588, AJ294648	AF102435

55	<i>G. froelichii</i> Jan ex Rchb.	unknown	unknown	unknown	Z71969	X77884
56	<i>G. gelida</i> M.Bieb.	Meierott 07/210 (MU) [ITS], IARC:B 009 [<i>trnL-F</i>]		unknown [ITS], Georgia: Gomborispass, Telavi, Kakheti [<i>trnL-F</i>]	KT907648	AB453087
57	<i>G. glauca</i> Pall.	394791 (NEU)		United States: Eivluk river, Lisburne Ridge, Alaska	KT907649	KT907795
58	<i>G. grandiflora</i> Laxm.	Maximovicz 5084 (MU)		Russia: Sibiria Orientalis	KT907650	KT907796
59	<i>G. grata</i> Harry Sm.	Favre & Matuszak 32a (KUN)		China: Bingzhongluo, Yunnan	KT907651	KT907797
60	<i>G. handeliana</i> Harry Sm.	Liu Ende <i>et al.</i> 1209080 (LZ)		China: Chayu to Jinju, Xizang	KT907652	KT907798
61	<i>G. haynaldii</i> Kanitz	Liu 1242 [ITS], Favre & Matuszak 194a [<i>trnL-F</i>]		unknown [ITS], China: Daocheng, Sichuan [<i>trnL-F</i>]	AY858671	KT907799
62	<i>G. heleomastes</i> Harry Sm.	Favre 206a (KUN)		China: Yading, Sichuan	KT907653	KT907800
63	<i>G. hexaphylla</i> Maxim. ex Kusn.	Boufford <i>et al.</i> 39284 (KUN)		China: Zhangla Cun, Rangtang, Sichuan	KT907654	KT907802
64	<i>G. hyalina</i> T.N.Ho	93-36		unknown	Z71941, Z71942	//
65	<i>G. intricata</i> C.Marquand	W. Yunnan, Jinshajiang Exp., 4522 (KUN)		China: Habaxueshan, Zhongdian, Yunnan	KT907655	KT907803
66	<i>G. itzershanensis</i> T.S.Liu & C.C.Kuo	Chen HS, CHC 2230 (TNM)		Taiwan: Heping Dist., Taichung City	KT907656	//
67	<i>G. jamesii</i> Hemsli.	Toyokuni and Naka, <i>s. n.</i> (LZ)		Japan: Takanagahara Mts, Hokkaido	KT907657	//
68	<i>G. kaohsiungensis</i> Chih H.Chen & J.C.Wang	Chen HS, CHC 1624 (LZ)		Taiwan: Tien-chih, Taoyuan, Kaohsiung County	KT907658	KT907805
69	<i>G. kaufmanniana</i> Regel & Schmalh.	Yuan 2004-98 (IBSC)		unknown	DQ398649	DQ398719
70	<i>G. krylovii</i> Grossh.	Guttre 34959 (LZ)		Kazakhstan: Amary, Nebenthal oberhalb Medeo	KT907660	//
71	<i>G. lacerulata</i> Harry Sm.	Favre 1261 (LZ)		Nepal: Kyanjing Gompa, Lirung Valley, Langtang	KT907661	KT907807
72	<i>G. latidens</i> (House) J.S.Pringle & Weakley	James S. Pringle 2733 (LZ)		United States: Herrin Knob Overlook, Jackson County	KT907662	KT907808
73	<i>G. lawrencei</i> Burkill	XCX 2002006 (KUN) [ITS, var. <i>farrerii</i>], Favre 315 (KUN) [<i>trnL-F</i>]		China: Langmusi, Sichuan [<i>trnL-F</i>]	DQ317492	KT907809
74	<i>G. leucomelaena</i> Maxim.	Boufford <i>et al.</i> 31587 (KUN)		China: Leijilashan, Xizang	KT907663	//
75	<i>G. lhasica</i> Burkill	Yuan 92-62 (NEU) [ITS], Wang Lisong, <i>et al.</i> 07-53 (KUN) [<i>trnL-F</i>]		unknown [ITS], China: Qusong, Budanglashankou, Xizang [<i>trnL-F</i>]	DQ398629	KT907811

76	<i>G. ligustica</i> R.Vilm. & Chopinet	SAC1/2 [ITS], no source [<i>trnL-F</i>]	France: Mont Saccarel [ITS], unknown [<i>trnL-F</i>]	AY773259, AY773260	X77886
77	<i>G. linearis</i> Froel.	James S. Pringle 2735 (LZ)	United States: Whitefield, Coos County, New Hampshire	KT907664	KT907812
78	<i>G. lineolata</i> Franch.	Li Heng, <i>et al.</i> 0651 (KUN)	China: Guanpo, Wuding, Yunnan	KT907665	KT907813
79	<i>G. linoides</i> Franch. ex Hemsl.	Chen HS, CHC 2866 (TNM)	China: Na-Pa-Hai, Yunnan	KT907666	//
80	<i>G. louiretoi</i> (G.Don) Griseb.	Peng hua, Bai bo 199 (KUN)	China: Lijue to Wenbu, Jingdong, Yunnan	KT907667	//
81	<i>G. lutea</i> L.	Favre 1005 (LZ)	Switzerland: Sanetsch, Valais	KT907668	KT907815
82	<i>G. lycopodioides</i> Stapf	Leiden 990.283.911 (L)	Indonesia: Latimojong Mts, Sulawesi	KT907669	//
83	<i>G. macrauchenia</i> C.Marquand	Chen HS, CHC 2853 (TNM)	China: Der-Chin, Yunnan	KT907671	//
84	<i>G. macrophylla</i> Pall.	Xizang Expedition 6419 (IBSC)	China: Weixi, Yunnan	DQ398652	DQ398723
85	<i>G. macgregoryi</i> Hemsl.	Hoogland 7132 (CANBR)	Papua New Guinea	KT907670	KT907817
86	<i>G. maulechuanensis</i> Franch.	Xizang Expedition 6419 (KUN)	China: Yunnan	KT907672	KT907818
87	<i>G. mairei</i> H.Lév.	Wang Hong <i>et al.</i> 01-0062 (KUN)	China: Yunnan	KT907673	KT907819
88	<i>G. manshurica</i> Kitag.	2005-20701D	unknown	GQ864018	GQ864091
89	<i>G. melandriifolia</i> Franch ex. Hemsl.	GXJ2011-054	China	KU512333	//
90	<i>G. micranthiformis</i> Burkill	Xizang Expedition, 7346 (KUN)	China: Xizang	KT907674	KT907820
91	<i>G. mirandae</i> Paray	Hernandez and Trigos, 1188 (HUH)	Mexico	KT907675	KT907821
92	<i>G. monochroa</i> T.N.Ho	Qing Zangbudian, 751342 (KUN)	China: Duoxiongshan, Milin, Paiqu, Xizang,	KT907676	//
93	<i>G. nerterifolia</i> P.Royen	Craig 84 (CANBR)	Papua New Guinea: Sirius Ridge, Telefomin, Sepik district	KT907677	KT907823
94	<i>G. newberryi</i> A.Gray	Tanya Harvey 2013-02 (LZ)	United States: Hand lake, Oregon	KT907678	KT907824
95	<i>G. nivalis</i> L.	A. Jochum 1201 (LZ)	Switzerland: Ferpècle, Valais	KT907679	KT907825
96	<i>G. nubigena</i> Edgew.	Wang Lisong, <i>et al.</i> 07-55 (KUN)	China: Qusong, Budanglashankou, Xizang	KT907680	KT907826
97	<i>G. occidentalis</i> Jakow.	unknown	France: Corbieres [ITS], unknown [<i>trnL-F</i>]	AJ000925, AJ000926	X76229
98	<i>G. officinalis</i> Harry Sm.	Yuan 92-293 (NEU)	unknown	DQ398639	DQ398697
99	<i>G. olgae</i> Regel ex Schmalh.	<i>s. n.</i> (MU)	Germany: Munich Bot. Gard. (Origin: Tianshan)	KT907681	KT907827
100	<i>G. olivieri</i> Griseb.	264 [ITS], anonymous 246 (XJBI) [<i>trnL-F</i>]	unknown	DQ398645	DQ398716
101	<i>G. orbicularis</i> Schur	Favre 1001 (LZ)	Switzerland: Sanetsch Valais	KT907682	KT907828
102	<i>G. oreodoxa</i> Harry Sm.	Liu 1092 (HNWP) [ITS], Wu Zhengyi <i>et al.</i> 5109 (KUN)	unknown [ITS], China: Dongjiulashan pass, Xizang	DQ398657	KT907829
103	<i>G. ornata</i> (D.Don) Wall. ex Griseb.	Favre 1272 (LZ)	Nepal: Khayar Lake, Annapurna, Pokhara	KT907683	KT907830
104	<i>G. oschtenica</i> Woronow	Beck and Lewis (B)	Spain: Canary Islands	KT907684	KT907831
105	<i>G. pannonica</i> Scop.	Favre 1280 (LZ)	Austria: Seehorn, Salzburg	KT907685	KT907833

106	<i>G. panthaica</i> Prain & Burkill	Favre & Matuszak 061a (KUN)	China: Dali, Yunnan	KT907686	KT907834
107	<i>G. papillosa</i> Franch.	Chen HS, CHC 2867 (TNM)	China: Su-Cha-Yun, Yunnan	KT907687	//
108	<i>G. parryi</i> Engelm.	unknown [ITS], Y91-S4 (NEU) [trnL-F]	unknown	Z48096 [ITS1]	DQ398731
109	<i>G. pedata</i> Harry Sm.	Yang Guoping 07-16 (KUN)	China: Puer, Jingdong, Xujijabai, Yunnan	KT907688	KT907835
110	<i>G. pedicellata</i> (D.Don) Griseb.	3rd Bot. Exp. to E Himalaya 10097 (KUN)	Bhutan: Samtengang to Kyebaka	KT907689	KT907836
111	<i>G. phylloclalyx</i> C.B.Clarke	Favre & Matuszak 33a (KUN)	China: Yunnan	KT907690	KT907837
112	<i>G. piasezkii</i> Maxim.	Favre 301 (KUN)	China: Sichuan	KT907691	//
113	<i>G. pneumonanthe</i> L.	Guttre 49389 (LZ)	France: Hautes Alpes	KT907692	X77889 [trnL intron]
114	<i>G. praecleara</i> C.Marquand	Favre 328 (KUN)	China: Yunnan	KT907693	KT907839
115	<i>G. praticola</i> Franch.	Yang Guoping, 07-11 (KUN)	China: Yunnan	KT907694	KT907840
116	<i>G. producta</i> T.N.Ho	unknown	unknown	Z71958 [ITS1]	//
117	<i>G. prolata</i> Balf.f.	Xizang Expedition, 74-2715 (KUN)	China: Yadong, Palidingya, Xizang	KT907695	//
118	<i>G. prostrata</i> Haenke	s. n. (NEU)	United States: Donnelly creek, Alaska	KT907696	KT907841
119	<i>G. prostrata</i> var. <i>karelinii</i> Griseb.	Dickor 4399 (MU)	China: Tangelashan, Geladandong, Xizang	KT907659	KT907806
120	<i>G. pseudo-squarrosa</i> Harry Sm.	J.H. Chen <i>et al.</i> 31614 (KUN)	China: Jianga (Gyamda) to Dege, Xizang	KT907697	KT907842
121	<i>G. puberulenta</i> J.S.Pringle	Pringle 2744 (B)	United States: Tucker Prairie, Callaway County, Missouri	KT907698	KT907843
122	<i>G. pubigera</i> C.Marquand	KIB & Roy. Bot. Gar. Edin. exp. 85-400 (KUN)	China: Lijiang, Yulongshan, Houshan, Yunnan	KT907699	KT907844
123	<i>G. pumila</i> Jacq.	n 98289 (NEU)	Austria: Wiener Schneeberg	KT907700	KT907845
124	<i>G. punctata</i> L.	Triponez 1000 (no voucher)	Switzerland: Nufenenpass, Valais	KT907701	KT907846
125	<i>G. purpurea</i> L.	Triponez 1001 (no voucher)	Switzerland: Val di Blenio, Passo Greina, Ticino	KT907702	KT907847
126	<i>G. pyrenaica</i> L.	NEU n 399315 (NEU)	Turkey	KT907703	KT907848
127	<i>G. ranae</i> M.Shabir & M.D.Dwivedi	M. Shabir 308822 (LWG)	India: Rohrang Pass, Himachal Pradesh	MT645780	//
128	<i>G. ranae</i> M. Shabir & M.D.Dwivedi	M. Shabir 308821 (LWG)	India: Rohrang Pass, Himachal Pradesh	OM971885	OM982650
129	<i>G. ranae</i> M.Shabir & M.D.Dwivedi	M. Shabir 308823 (LWG)	India: Rohrang Pass, Himachal Pradesh	OM971886	OM982651
130	<i>G. rigescens</i> Franch. ex Hemsl.	2005-2704B	unknown	GQ864022	GQ864095
131	<i>G. robusta</i> King ex Hook.f.	Yuan 92-57 (NEU)	unknown	DQ398643	DQ398702
132	<i>G. rostanii</i> Reut. ex Verlot	n 399320 (NEU)	France: Hautes Alpes, Mr Gondran	KT907704	KT907849
133	<i>G. rubicunda</i> Franch.	Wang Hong <i>et al.</i> 03-1098 (KUN)	China: Qiaojia, Qiaoshan, Yunnan	KT907705	AB453058
134	<i>G. rubicanulis</i> Schwein.	J.S. Pringle 847 (LZ)	United States: Pickerel Lake, Dickinson County, Michigan	KT907706	KT907850

135	<i>G. saponaria</i> L.	Bert Pittman 11280501 (MU)	United States: Cedar Creek, Richland, S. Carolina	KT907708	KT907852
136	<i>G. scabra</i> Bunge	Sun, B.Y. 2001-102 (WU)	North Korea: Gangwon-do	KT907709	KT907853
137	<i>G. scabrida</i> Hayata	S.H. Huang 9632902-03 (CYUT) [ITS], Chen HS, CHC 2463 [trnL-F]	Taiwan: Yun-Hai, Alishan, Chiayi [ITS], unknown [trnL-F]	JQ890594	KT907854
138	<i>G. sceptrum</i> Griseb.	Tanja Harvey <i>s. n.</i> (LZ)	United States: Olallie Meadows	KT907710	KT907855
139	<i>G. schleicheri</i> (Vacc.) Kunz.	Favre 1004 (LZ)	Switzerland: Gd-St-Bernard, Valais	KT907711	KT907856
140	<i>G. sedifolia</i> Kunth	unknown [ITS], 60803 (NEU) [trnL-F]	unknown [ITS], Peru: Lac de Soirococha, Camballa [trnL-F]	Z71963, Z71964	KT907857
141	<i>G. septemfida</i> Pall.	n 399316 (NEU) [ITS], unknown [trnL-F]	unknown [ITS], Turkey: Zigana Pass, Trabzon 2156 m [trnL-F]	KT907712	X77896
142	<i>G. sikokiana</i> Maxim.	Tagawa 8771 (MU)	Japan: Oharano, Owase-she, Mie, Honshu	KT907713	KT907858
143	<i>G. simulatrix</i> C. Marquand	Chen HS, CHC 2842 (TNM)	China: Na-Pa-Hai, Yunnan	KT907714	//
144	<i>G. sino-ornata</i> Balf.f.	Favre & Matuszak 224a (KUN)	China: Litang, Sichuan	KT907715	KT907859
145	<i>G. siphonantha</i> Maxim. ex Kusn.	Tian XM <i>et al.</i> QLS-TXM-044 (KUN)	China: Qinghai	KT907716	KT907860
146	<i>G. spathacea</i> Kunth	Troll 2 (MU)	Mexico: Acultzingo	KT907717	KT907861
147	<i>G. spathulifolia</i> Kusn.	Favre 304 (KUN)	China: Songpan, Sichuan	KT907718	//
148	<i>G. squarrosa</i> Ledeb.	Chen HS, CHC 2370 (TNM)	Japan: Tenchaoshan, Honshu	KT907719	KT907863
149	<i>G. stellata</i> Turill	Dickor 5986 (MU)	China: Arun Valley, Everest, Xizang	KT907720	KT907864
150	<i>G. stellulata</i> Harry Sm.	Chen HS, CHC 2865 (TNM)	China: Na-Pa-Hai, Yunnan	KT907721	//
151	<i>G. straminea</i> Maxim.	Favre 313 (KUN)	China: Langmusi, Sichuan	KT907722	KT907866
152	<i>G. striolata</i> T.N.Ho	Favre 221a (KUN)	China: Litang, Sichuan	KT907723	KT907867
153	<i>G. suborbisepala</i> C. Marquand	Chen HS, CHC 2878 (TNM)	China: Da-Bao-Shan, Yunnan	KT907724	//
154	<i>G. syringea</i> T.N.Ho	Favre 318 (KUN)	China: Langmusi, Sichuan	KT907725	//
155	<i>G. szechenyii</i> Kanitz	Liu Ende <i>et al.</i> 1209071 (LZ)	China: Chayu to Jinju Town, Xizang	KT907726	KT907869
156	<i>G. tarokoensis</i> Chih H.Chen & J.C.Wang	Chen HS, CHC 2083 (TNM)	Taiwan: Ching-Shui-Shan, Sioulin, Hualien	KT907727	KT907870
157	<i>G. tatsienensis</i> Franch.	Wang Lisong, <i>et al.</i> 07-13 (KUN)	China: Rannahu, Macun, Ranma, Basu, Xizang	KT907728	KT907871
158	<i>G. tentyensis</i> Masam.	Chen HS, CHC 2697 (TNM)	Taiwan: Tzu-En, Sioulin, Hualien	KT907729	KT907872
159	<i>G. tergestina</i> Beck	Guttre 26.6.1985 (LZ)	Slovenia: Divaca	KT907730	//
160	<i>G. terglouensis</i> Hacq.	NEU n 398280 (NEU)	Austria: Carinthia	KT907731	KT907873
161	<i>G. tetraphylla</i> Maxim. ex Kusn.	<i>s. n.</i> (KUN)	China: Huanglong, Sichuan	KT907732	KT907874
162	<i>G. tetrasticha</i> C. Marquand	unknown	unknown	[ITS2]	
				Z71967, Z71968	//

163	<i>G. tianshanica</i> Rupr. ex Kusun.	<i>s. n.</i> (NEU)	Kazakhstan: Shimkend, Aksu-Jabagly	KT907733	KT907875
164	<i>G. tibetica</i> King ex Hook.f.	Liu 1110 (HNWP)	unknown	DQ398640	DQ398726
165	<i>G. trichotoma</i> Kusun.	Lang Xiecheng <i>et al.</i> 1015 (KUN)	China: Kangding to Xinduqiao, Sichuan	KT907734	KT907877
166	<i>G. triflora</i> Pall.	Yuan CN2K91 (NEU)	unknown	DQ398661	//
167	<i>G. utriculosa</i> L.	<i>s. n.</i> (NEU)	Croatia: Gorski Kotar, Risnjak	KT907735	KT907879
168	<i>G. veitchiorum</i> Hemsl.	Liu 1042	China: Langmusi, Sichuan	AY858677	//
169	<i>G. vernia</i> L.	Favre S3 (LZ)	Switzerland: Mauvoisin, Valais	KT907736	KT907880
170	<i>G. vernayi</i> C. Marquand	Liu 1158 [ITS], Qingzang Team 151454 [rrnL-F]	unknown [ITS], China: Zedang to Song Mountain, Xizang	AY858670	KT907881
171	<i>G. waltonii</i> Burkill	Yuan 92-73 [ITS], Liu Ende <i>et al.</i> 1209035 (LZ) [rrnL-F]	unknown [ITS], China: Laiyi, Jindong Town, Xizang [rrnL-F]	DQ398626	KT907882
172	<i>G. walujewii</i> Regel & Schmalh.	Yuan 2004-91 [ITS], Yuan 2004-93 (IBSC) [rrnL-F]	unknown	DQ398646	DQ398718
173	<i>G. wingecarribiensis</i> L.G. Adams	Adams <i>et al.</i> 2606 (CANBR)	Australia: Wingecarribee swamp, New South Wales	KT907737	KT907883
174	<i>G. wisnmannii</i> J. B. Williams	JMB Smith 33 (CANBR)	Australia: Cathedral Rocks, Ebor, New South Wales	KT907738	KT907884
175	<i>G. yokusai</i> Burkill	Xizang Expedition 12558 (KUN)	China: Yanyuan, Guabiequ, Xiaojinhe, Sichuan	KT907739	KT907885
176	<i>G. yunnanensis</i> Franch.	Favre & Matuszak, 183b (LZ)	China: Between Shangrila and Daocheng	KT907740	KT907886
177	<i>G. zekuenensis</i> T.N.Ho & S.W.Liu	Zhou 1958 (HNWP)	unknown	DQ398656	//
178	<i>G. zollingeri</i> Fawc.	Chen HS CHC 2372 (TNM)	Japan: Iozan, Honshu	KT907741	KT907887
	Gentianinae (Outgroup)				
	Crawfordia Wall.				
179	<i>C. angustata</i> C.B.Clarke	Favre 226 [ITS], Favre 229 [rrnL-F] (KUN)	China: Yunnan	GU251013	KF563978
180	<i>C. campanulacea</i> Wall. & Griff. ex C.B.Clarke	Favre 210	China: Riverside behind Gongshan, NW Yunnan	GU251014	//
181	<i>C. crawfordioides</i> (Marquand) H.Sm.	Wu <i>et al.</i> 4180 (KUN)	China: Yunnan	KJ570863	KJ570877
182	<i>C. delavayi</i> Franch.	Favre 216	China: Mt Cangshan, Yunnan	GU251015	//
	Kuepferia Adr.Favre				
183	<i>K. danymonensis</i> (C.Marquand) Adr.Favre	Exp Team to Qinghai- Tibet Plateau 10232 (KUN)	China: Tibet	KF563951	//
184	<i>K. decorata</i> (Diels) Adr.Favre	Yuan 2003-38 (KUN)	China: Tibet	KF563970	//
185	<i>K. doxiongshanensis</i> (T.N.Ho) Adr.Favre	Yuan 2003-33 (KUN)	China: Tibet	KF563952	//
186	<i>K. otophora</i> (Franch.) Adr.Favre	Favre and Matuszak 056a (KUN)	China: Yunnan	KF563957	//