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The monophyly and rapid evolution of *Gentiana* sect. *Chondrophyllae* Bunge s.l. (Gentianaceae): evidence from the nucleotide sequences of the internal transcribed spacers of nuclear ribosomal DNA

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The nucleotide sequences of the internal transcribed spacers (ITS) of nuclear ribosomal DNA of 24 representative species of sect. *Chondrophyllae s.l.* have been determined and analysed phylogenetically, together with some species of other sections of the genus *Gentiana*. The ITS sequences strongly support the monophyly of the sect. *Chondrophyllae s.l.* as a whole complex including various different dysploid cytotypes. Species, such as *G. boryi* and *G. pyrenaica*, that had been split into distinct genera by some cytotaxonomists have been proven to be closely related. However, the ITS sequences do not provide sufficient information to make a robust estimation of the phylogenetic relationships among the closely related species and dysploid cytotypes of the complex, beyond recognizing their monophyly and rapid evolution.

ADDITIONAL KEY WORDS: — chromosome number – dysploidy – evolution – molecular systematics – phylogeny – polyploidy – sect. *Dolichocarpa*.

CONTENTS

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INTRODUCTION

The largest and the most widely distributed section of the genus *Gentiana* (*Gentianaceae*), sect. *Chondrophyllae* Bunge *s.l.*, comprises in its broad sense, as circumscribed by Smith (Nilsson, 1967) and Pringle (1978), about 170 mostly biennial species. There are only three species (*G. prostrata* Haenke, *G. pyrenaica* L. and *G. boryi* Boiss.) of the complex in Europe; one species (*G. atlantica* Litard. & Maire) in Africa (North Morocco); three species (*G. aquatica* L., *G. douglasiana* Bong., *G. prostrata*, in North America; three species (*G. pumilio* Standley & Steyerm., *G. prostrata* and *G. sedifolia* Kunth) in Central America; three species (*G. podocarpa* Griseb., *G. prostrata* and *G. sedifolia*) in South America; and one species (*G. quadrifaria* Blume) in Australia. All other species of the complex are found in Asia, highly concentrated in the mountain regions of southwestern China and the nearby Qinghai-Tibet Plateau and the Himalayas. While Ho (1985) and Ho & Liu (1990) treated this complex as three independent sections, sect. *Chondrophyllae* Bunge *s.s.*, sect. *Dolichocarpa* T. N. Ho and sect. *Fimbricorona* T. N. Ho, based mainly on the shape of capsule and floral appendages, Laínz (1976), and Löve & Löve (1975, 1978, 1986) have established three independent genera based only on the three European species of the complex. The segregated 'new genera' are *Chondrophylla* A. Nelson, *Holubogentia* Löve & Löve and *Kuepferella* Laínz, based on *G. prostrata* ($2n = 36$), *G. pyrenaica* ($2n = 26$) and *G. boryi* ($2n = 20$) respectively. The discriminating characters of the segregates were their different basic chromosome numbers. Omer (1989) segregated another new genus, *Qaisera* Omer, from this complex, based on *G. carinata* Griseb., which corresponds to the new section *Fimbricorona* of Ho & Liu (1990). The discriminating character was its fimbrate corolla throat. Taxonomically, a decision as to whether this complex should be treated as a single section, as three different sections, or as four or perhaps more different genera is needed as is an assessment of the monophyly of the complex and its segregates.

To evaluate the validity of the segregates based on different basic chromosome numbers, the extent of chromosome number variation among the taxa and the karyological evolution of the complex must be determined. More extensive karyological investigations on Chinese species, together with previous studies on species from other regions, revealed a wide range of chromosomal number variation among the species of the complex. Different chromosome numbers, including $2n = 12, 14, 16, 18, 20, 22, 24, 26, 30, 32, 36, 38, 40, 44, 48, 60$, and 96, have been documented for the complex (Küpfer & Yuan, 1996; Yuan, Küpfer & Zeltner, unpublished). The wide range of chromosome number variation suggested the polybasic nature and a chromosome evolution through the combination of dysploidization and polyploidization for the complex. The number $2n = 20$ was suggested as the ancestral number and the polarity of the dysploidization was thought to be predominantly descending for this complex (Yuan *et al.*, unpublished). The karyological conclusions do not favour the splitting of the complex either as different sections or as different genera. However, karyological investigations cannot supply robust estimates of monophyly and chromosome evolution because of the high homoplasy of karyological data in this complex (Yuan *et al.*, unpublished). They need to be confirmed by other lines of evidence.

DNA data, particularly DNA sequences, can contribute significantly to the cases in which morphological and karyological data are inconsistent, inconclusive, deficient or poorly analysed (Patterson, Williams & Humphries, 1993). This is

particularly true for plants as the sect. *Chondrophyllae s.l.* complex where high morphological and karyological homoplasy is involved and therefore morphological and karyological conclusions conflict (Yuan *et al.*, unpublished). The internal transcribed spacers (ITS) of nuclear ribosomal DNA has been proven to be useful sources of characters for phylogenetic studies. ITS sequences can provide a valuable set of characters for addressing lower-level phylogenetic questions, and were suggested to play an especially useful role in angiosperm studies by offering independent assessment of lower-level phylogenetic hypotheses based on morphology, karyology or chloroplast DNA (cpDNA) evidence (Baldwin *et al.*, 1995). Baldwin (1992, 1993) first extensively demonstrated the utility of the ITS region for phylogenetic reconstruction in Asteraceae. Some other examples of studies using ITS sequences are: Wojciechowski *et al.* (1993) on *Astragalus* (Fabaceae); Soltis & Kuzoff (1993, 1995) on *Heuchera* and *Lomatium* (Saxifragaceae); Baum, Sytsma & Hoch (1994) on *Epilobium* (Onagraceae); Hodges & Arnold (1994) on *Aquilegia* (Ranunculaceae); Kim & Jansen (1994) on *Krigia* (Asteraceae); Campbell *et al.* (1995) on some Rosaceae; Oxelman & Lidén (1995) on some Caryophyllaceae; Wendel, Schnabel & Seelanan (1995a,b) on *Gossypium* (Malvaceae); Downie & Katz-Downie (1996) on the subfamily *Apioideae* (Apiaceae). The inferred phylogenies of ITS sequences from previous studies were principally concordant with or often better resolved than relationships inferred from cpDNA (e.g. Hodges & Arnold, 1994; Sang *et al.*, 1995), morphological data (e.g. Wojciechowski *et al.*, 1993; Campbell *et al.*, 1995), karyological and cytogenetic data (e.g. Baldwin, 1993; Wojciechowski *et al.*, 1993; Hsiao *et al.*, 1995a,b). A few cases revealed incongruence between ITS-based and cpDNA-based or morphology based phylogenies. Kim & Jansen (1994) showed in *Krigia* (Asteraceae) that ITS trees were partially congruent with morphology and cpDNA trees, but about 22.5% incongruence occurred among their data sets. They concluded that ITS sequence data may have limited utility in interspecific studies or comparisons among closely related genera, especially in the groups which exhibit high levels of sequence divergence. Soltis & Kuzoff (1995) also revealed important discordance between ITS and cpDNA trees. However, the preponderance of data, including morphology, chemistry and allozymes of the same taxa, favour the ITS trees over the cpDNA trees, and the discordance was attributed to hybridization and introgression. Among these examples, ITS phylogenies have provided useful interpretations or confirmations on karyological evolution of some dysploid species groups as in *Astragalus* (Wojciechowski *et al.*, 1993), *Epilobium* (Baum *et al.*, 1994), *Calycadenia* (Baldwin, 1993), and the *Pooideae* (Hsiao *et al.*, 1995a,b).

Our previous studies at generic level of *Gentianinae* (Yuan & Küpfer, 1995) and at sectional level of *Gentiana* (Yuan, Küpfer & Doyle, 1996) have also proven the phylogenetic utility of ITS sequences. The ITS phylogenetic trees generated by parsimony analyses were principally congruent with morphological considerations, and improved or clarified some morphological misinterpretations and conflicts. Five species of the sect. *Chondrophyllae s.l.* have been sampled. They formed a highly supported monophyletic clade (Yuan *et al.*, 1996). Optimization of chromosome numbers on the ITS phylogeny suggested that $2n = 26$ is a plesiomorphic state for a clade comprising sections *Frigidae s.l., Cruciata, Pneumonanthe*, and *Chondrophyllae s.l.*, and $2n = 20$ or $2n = 26$ equivocally as the plesiomorphic condition for sect. *Chondrophyllae s.l.* However, this investigation sampled only a limited number of species (only five out of 170) and did not include all documented cytotypes of the complex, and thus the conclusions are not necessarily applicable over the entire

complex. It is obviously necessary to do a further and more extensive study for such a big and diversified complex.

The present study is concentrated on the sect. *Chondrophyllae s.l.* complex with the following questions to be addressed in particular: (1) is the complex a monophyletic group? (2) does ITS phylogeny support the splitting of the complex into different sections (as by Ho & Liu, 1990) or different genera (as by Löve & Löve, 1975, 1978, 1986, and the others), and (3) what is the phylogenetic utility of ITS sequences for this group of morphologically similar species?

MATERIAL AND METHODS

Plant species and material

ITS sequences were obtained from single individual, or 2–3 pooled individuals in the cases of small plants of a further 19 species representing most of the documented cytotypes of the complex sect. *Chondrophyllae s.l.* The species analysed, the origins of the material and the chromosome numbers of the species are given in Table 1. The chromosome numbers shown in the table were mostly observed directly from the vouchers used for DNA extraction, except for a few cases, as indicated in the table, when the numbers were obtained from literature. Leaves were collected directly from the field using the silica gel method (Chase & Hills, 1991) or were taken from conventionally prepared and recently collected herbarium sheets. All the voucher specimens are deposited in the herbarium of the University of Neuchâtel (NEU). Regrettably, the species of the small section *Fimbricorona* (4 species) remain unavailable to us. To assess the monophyly of the sect. *Chondrophyllae s.l.* complex, previous ITS sequences of five species of the complex and ten representatives of other sections of *Gentiana* were also included. Two species of other genera, *Crawfurdia tibetica* Franch. and *Gentianella campestris* (L.) Börner, were used as outgroups.

Collection of ITS sequence data

Total DNA was extracted from about 2 g of fresh or 100 to 300 mg of dried leaves pulverized in liquid nitrogen or directly in hot 2 × CTAB buffer according to the protocol of Doyle & Doyle (1987). A standard double-strand polymerase chain reaction (PCR) was used to amplify the entire ITS region, using the primers and protocols described previously (Yuan & Küpfer, 1995; Yuan *et al.*, 1996). The amplified ITS fragments were purified by electrophoresis in a 1.6% agarose gel and subsequently using the QIAEX gel extraction kit (QIAGEN AG, Basel). Both strands of purified ITS fragments were directly sequenced using primers 5' end-labelled with digoxigenin (MWG-Biotech, Germany), the DIG-TAQ cycle sequencing kit (Boehringer Mannheim GmbH) and the GATC-1500 Direct Blotting Electrophoresis DNA Sequencer (Constantz, Germany). The sequencing bands were detected by anti-digoxigenin using DIG Nucleic Acid Detection Kit (Boehringer Mannheim GmbH).

Sequence alignment and phylogenetic analysis

The sequence boundaries between the two spacers and the three coding regions (18S, 5.8S and 25S genes) of nrDNA were determined by comparison with published sequences from *Daucus carota* and *Vicia faba* (Yokota *et al.*, 1989) and with our previous data (Yuan & Küpfer, 1995; Yuan *et al.*, 1996).

The pooled ITS1 and ITS2 sequences were aligned using the progressive multiple alignment program Clustal W (version 1.5) for Power Macintosh (Thompson,

TABLE 1. The species and the origin of materials analysed. Voucher: G, Y=Y.-M. Yuan; K=P. Küpfer; Z=L. Zeltner; A=E. Anchisi. The sequences of the species marked with '*' were previously released (Yuan & Küpfer, 1995; Yuan *et al.*, 1996). **The chromosome numbers were observed directly from the vouchers used for DNA study except for those numbers shown in bracts which were obtained from literature

Taxon	Vocher	Origin	$2n$**
sect. *Chondrophyllae* Bunge *s. str.*			
G. altaica Pall.	Y95–49	Hanasi, Xingjiang, China; 3100 m	(26)
G. aristata Maxim.*	Y92–328	Maqü, Gansu, China; 3500 m	14
G. atlantica Litard. & Maire	Z93–S2	Atlas Mt., Morocco; 2200 m?	(48)
G. boryi Boiss.*	Z93–S1	Sierra Nevada, Spain; 2300 m	(20)
G. crassuloides Bureau & Franch.	Y92–265	Mt. Taibai, Shaanxi, China; 2900 m	38
G. flexicaulis H. Sm. ex Marquand	Y92–264	Mt. Taibai, Shaanxi, China; 3400 m	14
G. heleonastes H. Sm. ex Marquand	G032	Maqü, Gansu, China; 3650 m	12
G. intricata Marquand	Y92–198	Lijiang, Yunnan, China; 2700 m	20
G. pantheica Prain Burk.	Y92–248	Dali, Yunnan, China; 3200 m	20
G. piasezkii Maxim.	Y92–272	Mingxian, Gansu, China; 2900 m	36
G. pseudoaquatica Kusn.	Y92–326	Maqü, Gansu, China; 3800 m	20
G. pyrenaica L.*	Y93–14	Mt. Rila, Borovetz, Bulgaria; 2600 m	(26)
G. squarrosa Ledeb.	G046	Xiahe, Gansu, China; 3000 m	38
sect. *Dolichocarpa*			
G. crenulato-truncata (Marquand) T. N. Ho*	Y92–310	Maqü, Gansu, China; 4200 m	18
G. haynaldii Kanitz*	Y92–201	Zhongdian, Yunnan, China; 3400 m	20
G. hyalina T. N. Ho	Y93–36	Maduo, Qinghai, China; 4300 m	12
G. hyalina T. N. Ho aff.	Y92–89	Dingri, Tibet, China; 4500 m	30
G. ludlowii Marquand	Y92–35	Mt. Xiangpi, Qinghai, China; 3800 m	16
G. ludlowii Marquand	Y92–33	Heimahe, Qinghai, China; 3400 m	32
G. ludlowii Marquand aff.	Y92–99	Nyalamu, Tibet, China; 4000 m	22
G. producta T. N. Ho	Y93–79	Ganzi, Sichuan, China; 4000 m	?
G. pudica Maxim.	G178	Maqü, Gansu, China; 3700 m	20
G. sedifolia Kunth	A94–S3	Chacaltaya de Bo Paz, Bolivia; 4800 m	(40)
G. tetrasticha Marquand	Y92–128	Dangxiong, Tibet, China; 4500 m	24
other sections			
G. bavarica L.*	Y93–11	Grand Chavalard, Switzerland; 2200 m	(30)
G. clusii Perr. & Song.*	Y93–13	Grand Chavalard, Switzerland; 2100 m	(36)
G. lutea L.*	Y91–S5	La Tourne, NE, Switzerland; 1200	(40)
G. algida Pall.*	Y91–S10	Trail Ridge, Rocky Mt., USA; 3100 m	(24)
G. callistantha Diels & Gilg*	Y92–298	Luqü, Gansu, China; 3500 m	26
G. urnula H. Sm.*	Y92–71	Langkazi, Tibet, China; 5200 m	26
G. delavayi Franch.*	Y92–229	Lijiang, Yunnan, China; 2900 m	26
G. affinis Griseb.*	Y91–S1	Boulder Colorado, USA; 2300 m	(26)
G. straminea Maxim.*	Y92–313	Maqü, Gansu, China; 3500 m	52
G. rhodantha Franch. ex Hemsel.*	Y93–124	Yingjing, Sichuan, China; 1100 m	46
outgroups			
C. tibetica Franch.*	Y93–121	Mt. Gongga, Sichuan, China; 2700 m	?
Gl. campestris (L.) Börner*	K93–G1	Col du Pt. St. Bernard, Italy; 2150 m	(36)

Higgins & Gibson, 1994). Two passes were conducted to get the basic alignment, i.e. the aligned sequences of the first alignment were fed back again for a second round of alignment process, using the default parameters with opening gap penalty at 10, extension gap penalty at 5 and nucleotide transitions weighted. This alignment was then used as the basic data matrix for phylogenetic analysis. To examine the alignment ambiguity and its impact on phylogenetic analyses, the alignment processes were conducted for further eight rounds in the same way with the same alignment parameters. Each time the generated alignment was subjected to phylogenetic analysis and the aligned sequences were also loaded for a next round of alignment.

Sites from 476 to 492 (Appendix) were excluded from our basic phylogenetic analysis because of the high alignment ambiguity. Phylogenetic trees were reconstructed with gaps coded as missing, using Fitch parsimony, i.e. equal weights and unordered character states, as implemented in PAUP 3.1.1 (Swofford, 1993). Heuristic searches of 100 replicates of random addition of sequences, in combination with ACCTRAN character optimization and TBR + MULPARS branch-swapping options, were conducted to maximize the probability of identifying the most parsimonious trees and to discover multiple islands of trees (Maddison, 1991). Bootstrap values (Felsenstein, 1985) were calculated from 100 replicates of heuristic searches using Fitch parsimony.

Character-state weighted parsimonious analyses were used to test the effects of the more homoplasious character changes (transitions), and other phylogenetic inference methods including neighbour-joining and maximum likelihood were also used. No important difference in tree topologies was found, and thus the results are not shown.

RESULTS

Variation in repeat unit

No evidence of multiple repeat types was found in either PCR or sequencing. Each PCR product was always resolved as a single band on 1.6% agarose gel. Some sequences contained ambiguous sites that could represent polymorphisms, but these were present at a low frequency. With the exception of the regions around site 18, ambiguous sites were scattered throughout the sequences.

Sequence analysis

The basic alignment of the sequences of ITS1 (from site 1 through site 261) and ITS2 (site 262 through site 515) are provided in the Appendix. All the sequences were deposited in the EMBL Nucleotide Database. The length, G + C content, and accession number of EMBL Nucleotide Database of each sequence are given in Table 2 and the Appendix. The length of ITS1 of the sampled sect. *Chondrophyllae s.l.* species ranged from 215 bp (*G. heleonastes* H. Sm. ex Marquand to 233 bp (*G. flexicaulis* H. Sm. ex Marquand), and the length of ITS2 ranged from 221 bp (the accession 92-89 of *G. hyalina* T. N. Ho aff. with $2n = 30$, and *G. producta* T. N. Ho) to 235 bp (*G. flexicaulis*). The multiple alignment of the sequences of sect. *Chondrophyllae s.l.*,

together with the sequences of other sections and the outgroup species, assessed 261 sites for ITS1 and 254 sites for IST2. The G + C content of the complex varied from 52.8% (*G. aristata* Maxim.) to 60.9% (*G. piasezkii* Maxim.) in ITS1 and from 55.6% (the accession 92-89 of *G. hyalina* aff. with $2n = 30$) to 60.5% (*G. pseudoaquatica* Kusn.) in ITS2.

The alignment of the sequences encountered some ambiguity. Several alignment processes have been conducted to estimate impact on the phylogenetic reconstruction. The first pass of the multiple alignment of the 36 species, using the software Clustal W for Power Macintosh (Thompson *et al.*, 1994) with its default settings (transitions weighted, gap opening penalty set at 10, and gap extension penalty set at 5), generated 504 sites for pooled ITS1 and ITS2 sequences. The generated alignment was directly loaded again for the second round of alignment using the same parameters. In the same way, ten rounds of alignments were conducted. The resulting alignment of each round was also directly used as the data matrix for a

TABLE 2. The length and G+C content of the nucleotide sequences of ITS1, ITS2 and ITS1+ITS2 of sect. *Chondrophyllae* s. 1. and the other related species studied

	ITS1		ITS2		ITS1+ITS2	
Taxon	Length (bp)	G+C (%)	Length (bp)	G+C (%)	Length (bp)	G+C (%)
G. altaica	218	57.3	225	58.3	443	57.8
G. aristata	229	52.8	234	58.5	463	55.7
G. atlantica	231	57.2	232	57.8	463	57.4
G. boryi	230	59.1	231	58.4	461	58.8
G. crassuloides	225	57.7	233	60.1	458	58.9
G. flexicaulis	233	56.7	235	55.7	468	56.2
G. heleonastes	215	54.4	227	59.4	442	57.0
G. intricata	223	56.5	228	55.7	451	56.1
G. pantheica	228	57.4	229	56.8	457	57.1
G. piasezkii	220	60.9	226	59.8	446	59.9
G. psedoaquatica	227	59.9	228	60.5	455	60.2
G. pyrenaica	229	60.2	231	58.9	460	59.6
G. squarrosa	221	55.7	223	58.7	444	57.3
G. crenulato-truncata	219	58.2	231	60.1	450	59.0
G. haynaldii	228	59.9	232	59.4	460	59.8
*G. hyalina*12	226	55.7	228	57.9	454	56.8
*G. hyalina*30	224	54.9	221	55.6	445	55.3
*G. ludlowii*16	225	57.8	224	60.3	449	59.0
*G. ludlowii*32	221	57.0	228	56.1	449	56.6
*G. ludlowii*22	225	55.6	228	57.9	453	56.8
G. produncta	226	58.0	221	57.4	447	57.7
G. pudica	219	57.1	223	59.2	442	58.2
G. sedifolia	231	58.8	231	59.3	462	59.1
G. tetrasticha	222	58.6	227	58.6	449	58.6
G. bavarica	230	61.3	231	59.7	461	60.5
G. clusii	232	61.2	231	60.6	463	60.9
G. lutea	221	61.1	231	62.8	452	61.9
G. algida	230	61.3	231	60.6	461	61.0
G. callistantha	227	58.4	230	58.5	457	58.4
G. urnula	232	58.2	233	58.0	465	58.1
G. delavayi	228	56.1	230	57.2	458	56.6
G. affinis	229	59.8	230	59.1	459	59.4
G. straminea	227	60.1	216	56.4	443	58.9
G. rhodantha	228	56.2	232	58.7	460	57.4
C. tibetica	228	53.5	229	57.2	457	55.4
Gl. campestris	232	58.6	230	63.5	462	61.0

TABLE 3. A comparison of the parsimonious trees generated from different alignments of ITS sequences of sect. *Chondrophyllae s. l.* and other species studied. L: length of the shortest trees; CI: consistency index; RI: retention index

Matrix*	Sequence length	Number of trees	L	CI	RI	Support for the clade** A	B	C	D	E	F	G	H	I	J
Basic	498	27	870	0.481	0.477	+	+	+	+	+	+	+	+	+	+
1	504	8	1091	0.459	0.454	+	−	−	+	+	−	−	+	+	+
2	515	2	975	0.467	0.460	+	+	−	+	+	−	+	+	+	+
3	531	6	957	0.469	0.458	+	+	−	+	+	+	+	+	+	+
4	538	32	904	0.477	0.467	+	+	−	−	+	−	+	+	+	+
5	547	32	887	0.484	0.469	+	+	−	+	+	−	+	+	+	+
6	547	296	867	0.490	0.473	+	−	−	−	+	−	+	+	+	+
7	552	26	858	0.479	0.463	+	+	−	+	+	−	+	+	+	+
8	552	6	856	0.481	0.470	+	+	−	+	+	+	+	+	+	+
9	556	8	837	0.484	0.474	+	+	−	−	+	+	+	+	+	+
10	553	952	834	0.492	0.478	+	+	−	−	+	−	+	+	+	+

*Basic corresponds the basic data matrix which was based on the alignment round two with sites from 476 to 492 excluded from parsimonious analyses. The numbers 1 through 10 refer to the data matrices directly from the ten successive alignments by CLUSTAL W (see text).

**The clades A, B, C, D, E, F, G. H. I, and J represent the corresponded clades shown in Fig. 1; +supported; −not supported.

heuristic search of phylogenetic trees using the software PAUP (version 3.1.1; Swofford, 1993). The consensus length of the alignments and some information of the phylogenetic trees generated are shown in Table 3. The alignment generated from the second round was selected as our basic alignment of the data matrix for further phylogenetic searches because the later rounds of the alignments tend to produce congruent estimates of trees and the resolution of these trees becomes reasonably constant, although the alignments differ slightly. The alignment of the second round generated 515 sites (Appendix). In this alignment, 217 sites involved gaps of one to eight base pairs in individual sequences. The 17 sites from 476 to 492 shown in the Appendix were omitted from further phylogenetic analyses because of alignment ambiguity. The high proportion of gaps indicates a rapid sequence evolution among the closely related species of the complex.

The pairwise sequence divergence values were calculated from the basic alignments for all possible combinations of pooled ITS1 and ITS2 sequences, using the DNADIST program of PHYLIP package (version 3.5c; Fensenstein, 1994) with Kimura's two-parameter method (Kimura, 1980). The mean sequence divergence between pairs of species of the sect. *Chondrophyllae s.l.* complex ranged from 2.62% (*G. hyalina* with $2n = 12$ [93-36] versus *G. ludlowii* Marquand aff. with $2n = 22$ [92-99]) to 28.3% (*G. aristata* versus *G. producta* T. N. Ho) for the combined ITS1 and ITS2 data (divergence data matrix not shown). The high sequence divergence values also indicated a rapid evolution of the ITS sequences of the complex.

Phylogenetic analyses

To estimate the impact of the slight alignment ambiguity, parsimony analyses were conducted on different data matrices generated from a series of alignments. Some information including number of the shortest trees, the length, the consistency index and retention index of the trees generated and the congruence of their

resolution of some clades is given in Table 3. While most species of the sect. *Chondrophyllae s.l.* complex remained unresolved and a few species were resolved differently among the different matrices, a few clades remained constant. The matrix generated by the first pass of the alignment program produced eight trees of which the strict consensus is topologically different from all others. However, the second through the tenth gave consistent resolution for a few clades (Table 3). The other species remained unresolved or were resolved differently. The second alignment was chosen as the basic alignment used as the data matrix for further analyses.

The equally weighted parsimony analysis was conducted on the potentially informative characters of the second alignment and 17 ambiguously aligned sites (476 through 492) were excluded. Heuristic tree search strategy was employed. Alternating the starting tree led to different numbers of the most parsimonious trees being retained, suggesting the existence of multiple tree islands. Therefore, a heuristic search with 100 replicates of random addition of sequences and tree-bisection-reconnection (TBR) branch swapping was conducted to maximize the probability of identifying the tree islands. 27 equally parsimonious trees of 870 steps, belonging to four tree islands (two islands of three trees, one island of six trees and one island of 15 trees), were found. Their consistency (CI) index is 0.481 and retention index (RI) is 0.477. The strict consensus of these trees is poorly resolved for the species of sect. *Chondrophyllae s.l.* (Fig. 1). Only a few species were resolved constantly: *G. altaica* Pall. from the Mt. Altai, *G. pyrenaica* from Bulgaria and *G. boryi* from Spain form a relatively stable clade; the Chinese species *G. hyalina* with $2n = 30$, *G. producta, G. ludlowii* with $2n = 16$ and *G. pudica* Maxim. formed a well supported clade in every analysis we attempted; *G. hyalina* with $2n = 12$ always nested together with a cytotype of $2n = 22$ similar to *G. ludlowii*; and *G. crassuloides* Bureau et Franch. ($2n = 40$) often nested with *G. haynaldii* Kanitz ($2n = 20$). Many other species or cytotypes were resolved differently among the most parsimonious trees. None of the 27 equally parsimonious trees supports the monophyly of the segregates, sect. *Chondrophyllae s.s.* and sect. *Dolichocarpa*. Nevertheless, the monophyly of the sect. *Chondrophyllae s.l.* as a whole complex was strongly supported by all the 27 trees (92% bootstrap; Fig 1).

When the parsimony analysis was enforced on the monophyly of the sect. *Dolichocarpa* by topological constraint, one tree of 891 steps was generated which was 21 steps less parsimonious than the maximum parsimonious trees.

DISCUSSION

The monophyly of the sect. Chondrophyllae s.l. *complex*

As mentioned in the introduction, the sect. *Chondrophyllae s.l.* complex has been considered either as a single section, or as three different sections, or as several different genera. The ITS phylogenies do not favour these segregates. None of the ITS trees from any of the analyses supports the monophyly of the sect. *Chondrophyllae s.s.* and sect. *Dolichocarpa*. Despite of the poor resolution of the ITS gene trees on the relationships of the species and cytotypes, the monophyly of the sect. *Chondrophyllae s.l.* as a whole complex is always strongly supported regardless of methods of analyses (Fig. 1; Table 3). The sections *Cruciata* and *Pneumonanthe* were always shown to be the sister group of the complex. Particularly, *G. boryi, G. pyrenaica* and *G. altaica* grouped

together as a relatively highly supported clade. Therefore, the splitting of Laínz (1976) and Löve & Löve (1975, 1978, 1986) on these species should be rejected. The different basic chromosome numbers are not indications of phylogenetic isolation of these species. The ITS phylogenies do not suggest the splitting of sect. *Dolichocarpa* of Ho (1985) either, because no monophyletic clade of either sect. *Chondrophyllae s.s.* or sect. *Dolichocarpa* was obtained. One well supported clade of the ITS tree, shown as clade 'G' in Figure 1, encompasses species of both sect. *Chondrophyllae s.s.* (*G.*

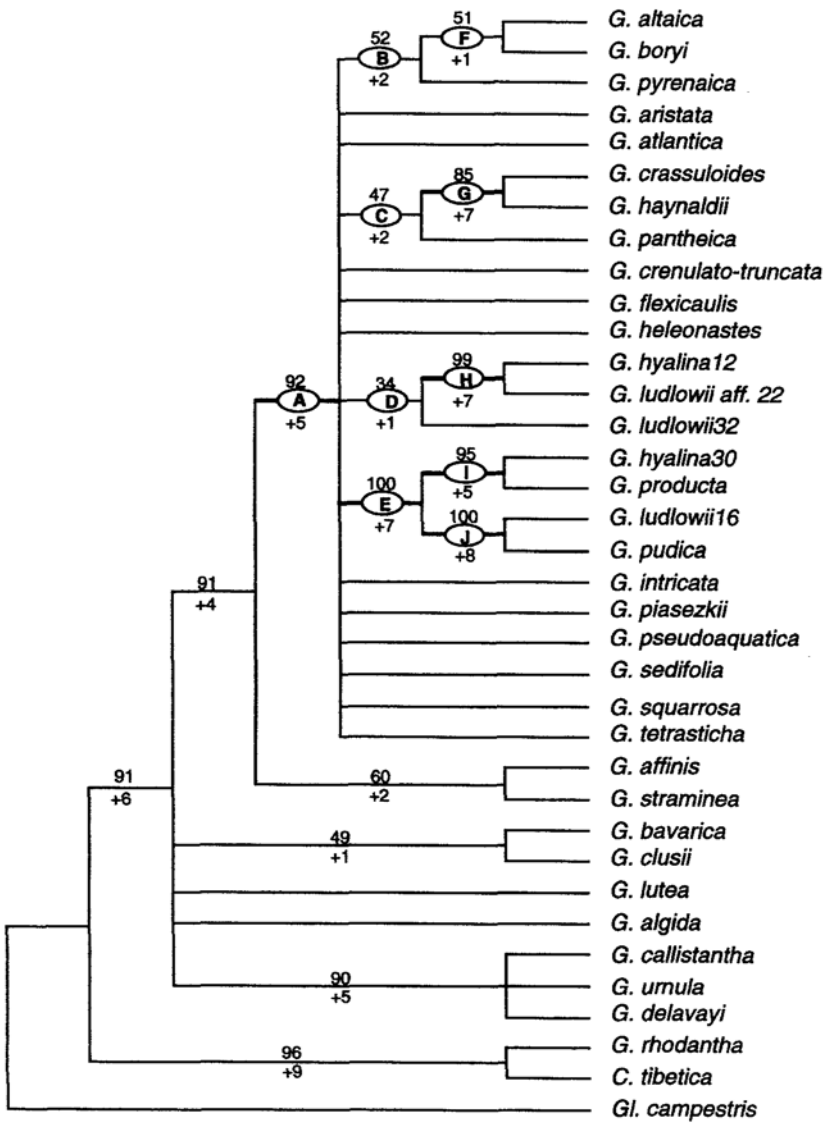

Figure 1. The strict consensus tree of the 27 equally parsimonious trees generated from PAUP heuristic searches of 100 replicates random addition sequence on the combined ITS1 and ITS2 sequences. Tree length = 870, CI = 0.481, RI = 0.477. The numbers above the internal branches are bootstrap values calculated from 100 replicates of heuristic searches. The numbers under the internal branches are the decay indices, i.e. the numbers of extra steps needed to destroy the corresponded branches. The letters A through J in the circles are the clades compared for different alignments (see text and Table 3). The thicker internal branches are supported by all analyses conducted.

crassuloides) and sect. *Dolichocarpa* (*G. haynaldii*). Regrettably, no species of sect. *Fimbricorona* or genus *Qaisera* was available for our ITS investigations.

Although ITS phylogenies cannot provide direct verification of the relationships among different dysploid cytotypes and therefore cannot verify the polarity of the dysploidization, ITS phylogenies do confirm the monophyly of the different dysploid cytotypes. Besides the clade consisting of *G. altaica* ($2n = 26$), *G. boryi* ($2n = 20$) and *G. pyrenaica* ($2n = 26$), the clade consisting of the $2n = 12$ cytotype (93-36) of *G. hyalina* and $2n = 22$ cytotype of *G. ludlowii* aff. (92-99), and the clade consisting of the cytotype $2n = 30$ of *G. hyalina*, the cytotype $2n = 16$ of *G. ludlowii*, the cytotype $2n = 20$ of *G. pudica* and *G. producta* for which the chromosome number is unknown, are also well supported examples of monophyletic dysploid complex.

It is necessary to point out here that the determination of the species names of this complex is by no means an easy task, due to the high morphological variability of the small biennial plants of the complex. Taxonomic circumscription of a certain species and the subsequent determinations based on a few morphological diagnostic characters is not guaranteed to recognize the right phylogenetic lineage and include it within a scope of definite species. A morphologically defined species may include several cytotypes belonging to more than one phylogenetic lineage. Meanwhile, different dysploid cytotypes of a specific phylogenetic lineage might be misleadingly placed into different species. Both situations, together with the existence of unrecognized cryptic species, complicated the interpretation and understanding of phylogenetic relationships among the cytotypes of the complex. The cytotype of the accession No. 92-89 ($2n = 30$) assessed as *G. hyalina* had a distinct chromosome number from the accession No. 93-36 ($2n = 12$); the cytotype of the accession No. 92-99 ($2n = 22$) assessed as *G. ludlowii* had a different number from the accession No. 92-35 ($2n = 16$) and accession No. 92-33 ($2n = 32$). If the taxonomic affiliation of the cytotypes sampled is correct, then ITS phylogenies indicated that these two species are polyphyletic. A close morphological and cytogenetic examination is needed to solve this problem. However, the nesting of different cytotypes in the same highly supported clade, such as the clade consisting of the cytotype $2n = 30$ of *G. hyalina*, the cytotype $2n = 16$ of *G. ludlowii*, the cytotype $2n = 20$ of *G. pudica* and *G. producta* of which chromosome number is unknown, does indicate that different basic chromosome numbers of this complex cannot be used as discriminating taxonomic characters.

Rapid evolution of sect. Chondrophyllae s.l. *complex*

Although the sequence divergence value among the species of the sect. *Chondrophyllae s.l.* complex ranged from 2.62% to 28.3%, the resolution of the trees was rather poor. Closer inspection of data matrix indicated a high proportion of autapomorphic mutations. Both the parsimonious trees and distance trees are characteristic in having rather short internal branches in contrast with the very long terminal branches. Such a situation was also observed in sect. *Calathianae* from chloroplast DNA sequences and was attributed to a star phylogeny, i.e. simultaneous speciation of many species (Gielly & Taberlet, 1996). This could be also the case for sect. *Chondrophyllae s.l.* Most species of the complex shared few synapomorphic characters, which suggested most of the species were derived simultaneously or within a very short period of time followed by subsequent rapid radiation. Therefore,

most mutations were autapomorphic. With regard to the high sequence divergence, wide subcosmopolitan distribution, high morphological diversity, and the high variation of basic chromosome numbers, rapid evolution has probably occurred in this complex, at the molecular, chromosomal and morphological levels. Many species of this complex from the mountain regions of south-west China, centre of diversity for the complex, have very small population sizes and rather restricted distributions. Patches of species are often found on different hills of a small range, which suggested that mutations were fixed rather rapidly in different local places. However, very little is known about the reproductive and population biology of the complex. A further study of these aspects would be useful to verify any evolutionary patterns.

The rapid evolution of the complex might be attributed to the biennial and herbaceous life form of its species. Some biennial species, such as *G. aristata, G. crenulato-truncata* T. N. Ho, *G. crassuloides, G. heleonastes* etc., have accumulated more autapomorphic mutations and therefore had longer terminal branches than some other perennial species such as *G. altaica, G. pyrenaica*, and *G. piasezkii* etc. in both parsimonious and distance trees (not shown). It has been suggested that ITS sequences evolve faster in herbaceous, primarily annual groups of comparatively recent origin than in some ancient woody groups (Baldwin *et al.*, 1995). Similar correlations between plant life form and evolutionary rate have also been noted from chloroplast DNA data (Gaut *et al.*, 1992; Gaut, Muse & Clegg, 1993; Wilson, Gaut & Clegg, 1990). Generation time might be a reason for this situation in sect. *Chondrophyllae s.l.* complex, in which the biennial herbs had shorter generation time resulted in faster accumulation of mutations than perennial ones.

Limit of phylogenetic inference of ITS sequences

It is generally considered that ITS can provide valuable characters for addressing lower level phylogenetic questions (Baldwin *et al.*, 1995). In the case of the genus *Gentiana* and its allied genera, the statement remains appropriate as far as closely related genera and different sections within a genus are concerned (Yuan & Küpfer, 1995; Yuan *et al.*, 1996). In a closely related group of species such as sect. *Chondrophyllae s.l.*, ITS sequences failed to draw a robust conclusion of the species phylogeny, although the sequence divergence remained high. This is particularly obvious when a fast evolving group is examined. The weakness of the phylogenetic resolution was contributed in part by sequence alignment ambiguity, and in another part by insufficient synapomorphic mutations which were further underscored by frequent homoplasious substitutions.

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G. altaica          CCGTCGT-GC AAA----CAA CCAACCCACC C--GCAGAAA C-GCGCCAAG GAAAACGT--
G. aristata         TA.....-.. C..A--C... .......CG. .--CA.A... .-C.C..... ......C.TA
G. atlantica        T...T..-.. ...-----... .......CGG G-C..... .-........ ......A.--
G. boryi            .......-.. ...-----... .......CGG G-C.T.A... .-A..... ......-GT-
G. crassuloides     T.....-.. ...-----... .......CGG GGGA..AG.. --C..GGCCA .G.....--
G. flexicaulis      T.....-.. ...-----... .A.....CGG G-C..... A-..A..... ......CGT-
G. heleonastes      ---.GA-NN. ...-----... .......CGG --GT.GC.G. --AACN.... .......--
G. intricata        T.....-.. ...-----... .......CGG G--CG..... .-..---... ......-GT-
G. pantheica        T.....C.T ...-----... .......GG- ---..... .-........ ......T.--
G. piasezkii        T.....-.. G..-----... .......CG- ---..... .-........ ........--
G. pseudoaquatica   T.....-.. ...-----... .......CG- --C..... .-........ ......TA--
G. pyrenaica        .......-.. ...-----... .......CGG G-C..... .-........ ........--
G. squarrosa        T.....-C. ...-----... .......CG- --G..GC.G. --AACG.... A.......--
G. crenulato-       T.....-.. ...-----..C .A.C...CGG G-G.A..G.. A--..G.... ....C...--
G. haynaldii        T.....-.. ...-----... .......CGG G-C..... .-........ ........--
G. hyalina12        T.....-.. ...-----... .......CG. ---..... .-........ ......T.--
G. hyalina30        T.....-.. G..-----... .......GG- ----G.A... AN-G.....N .....AANN-
G. ludlowii16       T.....-.. G..-----... .......CG. ---..... .-........ ......A.--
G. ludlowii32       T....A.-.T ...-----... .......CG- --C..... .-........ ......T.--
G. ludlowii22       T.....-.. ...-----... .......CN- ---..... .-........ ......T.--
G. producta         T.....-.. G..-----... .......-GG G-C..... .-........ ......A.--
G. pudica           T-.C...-.N G..-----... .......CG- ---..... .-........ ......A.--
G. sedifolia        T.....-.. ...-----... .......CGG G-C..... .-.T...... ........--
G. tetrasticha      T.....-.. ...-----... .......CG- ---.G..... .-........ ......T.--
G. bavarica         T.....-.. .C.-----... .......CGG G-C..... .-........ .......A--
G. clusii           T.....CT. ...-----... .......CGG G-C..... .T.T...... ...T...A--
G. lutea            T.TC...G-. ...-----... .......CGG ----..T... .-........ .......A--
G. algida           T.....-.. ...-----... .......CGG G-C..... .G-....... .......---
G. callistantha     T.....-.. ...-----... .......CGG G-C...T... .-........ .......A--
G. urnula           T.....-.. ...-----... .......CGG G-C...T... .GA..... .......---
G. delavayi         T.....-.A ...----A.. ...-...CGG G-C..... .-........ .......A--
G. affinis          T.....-.. ...-----... .......CGG G-C..... .---..... .......A--
G. straminea        T.....-.T .TG-AAA... .......CGG ----..... .-........ .......A--
G. rhodantha        .......-.. T..-----... .A.....CGG G-C.TC.... AG-..... .....TT.--
C. tibetica         AA.....-.. T..-----..T .A.....CGG G-C...TT.. -G-T..... ..........
Gl. campestris      T.....-.. .C.-----... A......CGG G-C...T... AG-..... ......AA--

G. altaica          -AAATAAGGA TT-GTCTGCC CCCC-GTCGT GT-CGTA--- -TGGTGCG-C ACGGGAGGAT
G. aristata         -...A.G.A. ..-TC..C.. ....-..... .C-....A-T GCC..A..-. .........C
G. atlantica        -...A..... ..-...T.. ....C..... .C-.....--T GC.....-G. .........C
G. boryi            -...A.G.AT .--..... ....-..... ..-.....--T GC.....-. ..........
G. crassuloides     --.....TT C.--.-.... TA..C..... .C-..A.-TT TC.TATT--T CAC..GT.-.
G. flexicaulis      --..A..... ..-..... ...T-..... .C-.....--T GC.....-T .........C
G. heleonastes      --..A..... ..GTC-...T T...-..A.. TC-.....--T AC.---T.-. GAC.......
G. intricata        -.....G.A. .--..... ....-..... .C-.....--T GC.TGCTCT. .........C
G. pantheica        --..A..... ..-..... ....-..... .C-.....--T GC.....-. ..........
G. piasezkii        --..A..... ..-..... ....-..... .C-.....--T GC.....-. ..........
G. pseudoaquatica   --..A..... ..GTC..... T...--C.A.. .C-.....--T GC.....-. ..........
G. pyrenaica        -...C..... ..-..... ....-..... .C-.....--T GC.....-G. ..........
G. squarrosa        --..AT.... ..GTC-.... T..G-TC... .C-.....--T GC...CGA-. ---.....-
G. crenulato-       -...-....T ..-CCT.C.- ....-..GC. AC-..-.--T TC.TC.---- ....--A...
G. haynaldii        -...A.G.AT ..-..... ....-..... .C-.....--T TC...T..C- ..........
G. hyalina12        --..A..... ..-..... ..T.-..... .C-.....--T GC.....-. .........C
G. hyalina30        --..A..... ..-..... ....-..... .C-...T--T GC.....-. .T........
G. ludlowii16       --..A..... C.-..... T...-N.... .C-.....--T GC.....T-. ..........
G. ludlowii32       --..A..... ..-..... ....-..... .C-.....--T -C.....-. .........C
G. ludlowii22       --..A..... ..-..... ..T.-..... .C-.....--A TC....N.-. .........C
G. producta         --..A..... ..-..... T...-..... .AAT..T--T GC...A..-- .T........
G. pudica           --..A..... ..-..... T...-..... .C-.....--T GC.....TG. ..........
G. sedifolia        -...-..... C.-..... T...-..... .C-.....--T GC....TCG. .........C
G. tetrasticha      --..A..... C.-..... ....-..... .C-.....--A TC...T..-. ..........
G. bavarica         --..A.-... .G-.C..... T..T-T..A. .C-.....--C GC....T.-A GGC.....G
G. clusii           --..A..... .G-.C..... T...-....C CC-.....--- CGC...T.-. ..A.A....C
G. lutea            --..A..... .G-.C..... T...-....C .C-.....--- CC.....-T G.T.......
G. algida           -...A..... .G-.C..... T...-..A.. .CCGA..C-G C-....T.CG .........TA
G. callistantha     --..A..... C.-.C..... T...-..... .C-.....--C GC....T.-. .T........
G. urnula           A...A..... ..-.C..T.. T..-..... .CCG-..C-G CA....T.C- .T........
G. delavayi         --..A..... ..-.C..... T...-..... .C-.....--T GC....T.-. .T........
G. affinis          -...-..... .G-.C....- T...-..... .C-.....--C GC....T-G. ....-.....
G. straminea        --..A..... .G-.C.N.-- T...-.G... .C-.....--C GC....T.-. ..........
G. rhodantha        -..GA.G.AT .G-......T TG.T-.A.AC ..C.-GTT-G G-C.CTT.TT C-..T.....
C. tibetica         -G..A..... .G-.CN.... TA..--A.A. TCCG-..C-G C-....T.TT TT..T.....
Gl. campestris      -G..A.G... .G-.CT.... T.T.-.A..C TCCG-.CC-G C-..A.T.CA C-.A....GC
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                                                >>ITS2
G. altaica          CACAGACGCC AAAAGAAACA -ATCGCGTCG CCCCC--AAC A-CCGTGCAT GAAACATT--
G. aristata         ...GT..... T......... -..GT..... .....C-... .-........ ....A...--
G. atlantica        ........-. T.......A. A......... .....CC... .A........ ....A.--TT
G. boryi            ......T... T......... -......... .....CC... .-........ ........GC
G. crassuloides     ...G-...-. T......... -......... .....CC... .C.T...... ........GC
G. flexicaulis      .......... T......... -......... .....CC... .-........ ....A.A.TG
G. heleonastes      ...G..GA.. T......... -......... .-...CC... .-........ ........--
G. intricata        ........T. -......... -......... .....C-... .-.....T.. ........--
G. pantheica        T..G...... T..T...... -......... .....C-... .-........ ........-G
G. piasezkii        ...G...... T......... -..-....... .AA..C-... .-........ ....T...C-
G. pseudoaquatica   ...G...... C......... -..GT..... .....C-... .-........ ........--
G. pyrenaica        ........-. T......... -......... .....CC... .-........ .......--TT
G. squarrosa        ...G...... T......... -......... ..-..C-... .-........ -........--
G. crenulato-       A..G-...-. T......... -......... .....CC... .-........ .......--CT
G. haynaldii        ...G....-. T..-...... -......... .....CC.-A CA.....T.. ........GT
G. hyalina12        .......... T......... -......... .T...CC... .-........ ......C.--
G. hyalina30        ...G..T... T......... -......... .....C-..A .-........ ........--
G. ludlowii16       ...G...T.. T......... -......... .....CC... .-........ ....T...--
G. ludlowii32       ...N...... T......... -......... .....C-... .-........ ......C.--
G. ludlowii22       .......... T......... -......... .T...CC... .-........ ......C.--
G. producta         ...G....-. T......... -......... .....CC.-. .--..... ...-------
G. pudica           ...G...... T......... -......... .....CC... .-........ ....T...--
G. sedifolia        ...G....T. T.......T. -......... .....CC... .-........ .......--CT
G. tetrasticha      ...G...... T......... -......... .....C-... .-........ ........--
G. bavarica         ...G.G.... T....G.... -......... .....CC... .-........ ...G.CA.--
G. clusii           ...G.G.... T.......AC A......... .....CC... .-........ ....TCG.--
G. lutea            ...G.G.... TG.......G -..T...... .....CAC.. --........ ....TCA.--
G. algida           ...G.G.... T......... -......... .....CC... .-........ ..T.TCA.--
G. callistantha     ...G.G.... T......... -......... .....CC... .-........ ...TTCA.--
G. urnula           ...G.G...- T......... -......... .....CC... .-........ ....TCA.--
G. delavayi         ...G.G.... T......... -......... .....C-... .-........ ....TCA.--
G. affinis          ...G.G.... T......... -....TAAGA .....CC... .-........ ........--TT
G. straminea        ...G.G.... T......... -....TT..- ----.CC... .-.....T.. N.......--
G. rhodantha        ...G.G.A.. T......... A...CT.... .....-A... --....CG.C ..CGTCG.--
C. tibetica         ..TG.G.A.. T......... A......... .....CC... C-.....TG. T..CTCG.--
Gl. campestris      A....G.A.. TG........ A......... .....CC... C-.....TG. T..CTCG.--


G. altaica          GCCGGTTGTC GGAGGGG-CG GATATTGGCT TCCCGTG--- -CTTCGG-TG C-GGC-TGGC
G. aristata         .T........ .......-.. ....A..... .......GTC GT.....C.. .-...-....
G. atlantica        .......... TC.....-.. .......... .........- -.....-.. .-...-....
G. boryi            AT--..... .TC....-.. .......... ......A--- -.....-.. .-...-....
G. crassuloides     C.A...C..T .......-.. ..G....... ......T--- -.....-.. .-...-....
G. flexicaulis      A......T.T ......A-.. .......... .........- -.....-.. TG-..A....
G. heleonastes      ......C..A .......-.. ..C....... ......T--- -.....-.. .-...-....
G. intricata        .........T .......--.. .......... .........- -.....-.. .-...-..-.
G. pantheica        C.A...CT.A T.......--.. ..A....... .........- -....T.-.. .-...-.A..
G. piasezkii        ......CT.T .......-.. ..C....... .N........- -.....-.. .-...-....
G. pseudoaquatica   ......C..A .......-.. ..C....... -.........- -.....-.. .-...-....
G. pyrenaica        .......... .......-.. .......... .........- -.....-.. .--.GC....
G. squarrosa        .......... .......-.. .......... .........- -.....-.. .-...-....
G. crenulato-       ......-... .......-.. .......... .........- -.C-..T-.. .-...-....
G. haynaldii        C.G.T.C... .......-.. ..G....... .........- -.....-.. .-...-....
G. hyalina12        .T........ ......A-.. .......... .........- -.....-.. .-...-....
G. hyalina30        .......... .-....A-.. ..C....-.. .........- -.....T-.. .-...-..-.
G. ludlowii16       .......... .-GA...-.. ..C....... .........- -...T..-C. .-...-....
G. ludlowii32       .......... ......T-.. .......... .........- -...T...-. T-...-....
G. ludlowii22       .T........ .-....A-.. .......... .........- -.....-.. .-...-....
G. producta         -.AT.C...G C.....--.. ACA-.G---C .........- -......TC. .-A.GC....
G. pudica           ..G.TC.T.. .-GA...-.. ..C....... .........- -.....-CA .-...-....
G. sedifolia        .......... ......A-.. .......... .........- -.....-.. .-...-....
G. tetrasticha      .....AA... .-.....-.. .......... .........- -.....T-.. .-...-....
G. bavarica         .T....G..A T.....-.. .......... .........- -..C..A-C. .-...-....
G. clusii           ......C... .......-.. .......... .........- -..C...-.. .G-..-.A..
G. lutea            ......C... .......-.. .......... .........- -.....-.. .-...-....
G. algida           ......C... .......-.. .......... .........- -.....-.. .-...-....
G. callistantha     ......C... .......-.. .......... .........- -.....-.. .-...-....
G. urnula           ......C... ......T-GA ....C..... .........- -.....-A .-...-....
G. producta         A.....CA.T .......-.. .......... .........- -....A.-.. .-...-....
G. affinis          .......... .......-.. .......... .........- -.....-.. .-...-....
G. straminea        .......... .......-.. .......... .........- -.....-.. .-...-....
G. rhodantha        ......CAGT C.GA...G.. .......... .........- -.....-.. .G-..-....
C. tibetica         ..T..A.... A.....-.. .......... ......A--- -.....-.. .G-.T-....
Gl. campestris      A.G...GACA T......G.. ..A.C..... .........- -...G.C-C. .-...-....
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G. altaica          CTAAATGCAA -GTCCTTC-C G-GCGGACAC CACGA-CAAG TGGTGGTTGA TTACCTCAAC
G. aristata         .......... -....C.TG. .-A..... G....-.... .......... ..........
G. atlantica        ......T... GT--.C.TG. .CAA..... G....-..-. .......... ..........
G. boryi            .......... -....C.TG. .-..... G....-.... .......... ..........
G. crassuloides     ........G. GT-..C.TG. .-AT..... G....-.... .......... ..G.......
G. flexicaulis      .......... -....C.TG. .-A..... G....-.... .......... ..........
G. heleonastes      ........G. -....C.TG. .-A..... G....-.... .......... ..T.......
G. intricata        .......... -....C.TG. .-A..... G....-.... .......... .........A
G. pantheica        .......TG. -....C.TGT .-A..... G....-.... .......... .AT.....G.
G. piasezkii        ........G. -....C.TG. .-A..... T....-.... .......... ..TN......
G. pseudoaquatica   ......TTG. -....C.TGT .-A..... G....-.... .......... ..G...N...
G. pyrenaica        .......A.. -....C.TC. .-A..... G....-.... .......... ..........
G. squarrosa        .......... -....C.TG. .-A..... G....-.... .......... ..........
G. crenulato-       .......... GTG..C.TT. .TA..... G....-.... .......... ..........
G. haynaldii        ......A.G. GT-..C.TG. .-A..... G....-.... .......... ..........
G. hyalina12        .......... C....C.TG. .-A..... G....-.... .......... ..........
G. hyalina30        A......TG. -....C.TGT .-A..... G....-.... .......... ..T.T.....
G. ludlowii16       A.......G. -....C.TG. .-A..... G....-.... .........G ..T-T.....
G. ludlowii32       ........-. -....C.TG. .-A..... G....-.... .......... ..........
G. ludlowii22       .......... -....C.TG. .-A..... G....-.... .......... ..........
G. producta         A......TG. GT-..C.TGT .-A..... G....A.... .......... ..T.T.....
G. pudica           A.......G. -....C.TG. .-A...--. G....-.... .........G ..T-T.....
G. sedifolia        .......T.. GT-..C.TG. .-A..... G....-.... .......... ..G.T.....
G. tetrasticha      .......... -..GGG.TG. .-A..... G....-.... .......... ..........
G. bavarica         .....C.... -....C.TG. .-A..... G....-.... .......... ..TA..T.G.
G. clusii           .......... -....C.TA. .-A..... G....-.... .......... ..T.......
G. lutea            .......G.. -....C.TG. .-.....G.. G....-.... .......... ..G....G..
G. algida           .....C.... -....C.TG. .-A..... G....-.... .......... -.TTT.....
G. callistantha     ......T... -....C.TG. .-A..... G....-.... .......... A.TA......
G. urnula           ......T... -....C.TG. .-A...CG.. G....-.... .......... A.TA......
G. delavayi         ......T... -....C.TG. .-A..... G....-.... .......... A.TT......
G. affinis          .......... GT-..C.TG. .-A..... G....-..-. .........- ..G.......
G. straminea        .......... -....C.TN- .-A..... G....-..-. .........- ..G.......
G. rhodantha        ......-... -...TC.TGT .-A..... G....-.... .......... ..G.......
C. tibetica         .......... -...TC.TCT .-AT..CG.. G....-.... .......... ..G.......
Gl. campestris      ........G. -....C.TG. .-A.....G. G....-.... .......... .AGT.G....
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G. altaica          TCAGT-T--- TGT-CGCACG TTGA-CCCGT C-GGACGAGG AGACTTCCTC GACCCTA-AT
G. aristata         ....G-.G-C ...-T.G... ....-..... .-...T.... .........T .......-..
G. atlantica        .....-.G-C ...-..G... ....-..... .--.GT.... ....G.A.CT AGA..C.A..
G. boryi            .....-.G-C ...-..T... ....-..... .-...T.... .......... .......-..
G. crassuloides     .A..G-.GC- ...-..TG.. ....C..-.. .-...T.... .........CT .......-..
G. flexicaulis      ...AAA.GC- ...-..G... C...-.A... .-...T.... .........T .......-A..
G. heleonastes      .A..G-NG-- ...-...T.. C...-..... .-...T.... .....A...T .......-T.N
G. intricata        C.TCA-AG-T ...-...... ....-..... .-...TA..A .........T .....AT-..
G. pantheica        .A..G-.G-- ...-...G.. ....-..... .-...TT... .........CT .......C.C
G. piasezkii        .A..G-.G-- ...-...G.. C...-..... .-......... .........CT .....---TA
G. pseudoaquatica   .A..G-.GC- ...CGCGT.. C...-..... .-...T.... .....C...G AC..TA.CCG
G. pyrenaica        .....-.GC- ...-...... ....-..... .-......... .......... .......-A..
G. squarrosa        .....-GC-- ..N-..-... ....-..T.. .-...T.... T.........T .......-T.G
G. crenulato-       ....G-.GC- ..G-..A... ..A.A..... .-...T.... .........T .......TG..
G. haynaldii        .A..G-.GC- ...-..TG.. ....C..-.. .-...T.... ........-CT .......-A..
G. hyalina12        .....-.G-C ..-.TCG... ....-..... .-...TC... .........T .....---TA
G. hyalina30        .A..G-.G-- ...-...T.. .CTG-..T.. .-N..T.... .........AT .....---TC
G. ludlowii16       .A..G-.GC- ...-..TT.. .C..-....- .-.C.T.... .........AT .G....-T.G
G. ludlowii32       .....-.G-C ...-..TTT. ....-..... .-...T...C ....-...GT .NN.T..ATA
G. ludlowii22       .....-.GC- ...-..G... ....-..... .-...T.... .........T .......-T.A
G. producta         .A..G-.GC- ...-...T.. .C..C..T.. T-T..T.... .........AT .......A.C
G. pudica           .A..G-.GC- ...-..TT.. .C..-..... .-...T.... .........AT .......-T.G
G. sedifolia        ....G-.GC- ...-..G... ....-..... .-.T.T.... .........T .......-A..
G. tetrasticha      .....---TT- ...-...T.. ....-..... .-...T.... .......... .......-ATA
G. bavarica         .A..G-.GT- ...-...G.. C..C-..T.. .-......... ......A..T .......-T..
G. clusii           .A..G-.GC- ...-...G.. C..C-..... .-.......T. .....AA..T .......G-..
G. lutea            .A..G-.CG- ...-...G.. C..C-..... .-...T.... ......A..T .......GT.C
G. algida           .A..GG.GC- ...CG-.... ...CC..-.. .-...T.... .........T .......-A.C
G. callistantha     .A..G-.G-C ...-...G.. ....-..... .-...T..CC .......T.T .......-.C
G. urnula           .A..G-.GC- ...CG-.G.. ...CC..T.. .A...T.... .......T.T .......-A.C
G. delavayi         .AG.G-.GC- ...-...G.. ...C-..... .-...T.... ......TT.T .......-.C
G. affinis          .A..G-.GC- ...A...T.. ...C-..... .--C.TT... .........AT .......-A..
G. straminea        .AG.--.GC- ...-...GTA. ...C-..--. .-.-.T.... .........T ....---T..
G. rhodantha        .A..G-.GC- ...CGT.G.. C..CC..-A. .-.A...GA. .....CTG.A .......-G..
C. tibetica         .A..G-.GC- ...CG-TG.. ...CA.T-.. .-.A....A. G....C.G.A .......-G..
Gl. campestris      .A..G-.GC- ...CG-.G.. AC.C-..-.. .-.A.TTT.. .....C.-C. .......-G..
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					EMBL accession # ITS1	ITS2	Voucher
G. altaica	GCATGA--TC	T-TCACGACG	AAT-GCCACG	ACCGC	Z71931	Z71932	Y95-49
G. aristata	...ACG--..	G-.....	CC.-.....	.....	Z48100	Z48116	Y92-328
G. atlantica	.....CGACG	--A..TC...	CC.GCG....	...A.	Z71933	Z71934	Z93-S2
G. boryi	.....C-A..	G-.....G.	CG.-.....	.....	Z48111	Z48118	Z93-S1
G. crassuloides	....-C-G..	G-.....	CC.GC-....	.....	Z71935	Z71936	Y92-265
G. flexicaulis	....-C-G..	G-.....	CC.T.....	.....	Z71937	Z71938	Y92-264
G. heleonastes	-ATGAC-G..	.-.....	CC.-.....	.....	Z71939	Z71940	G032
G. intricata	AG....C-..	G-...N....	CC.-.....	.....	Z71945	Z71946	Y92-198
G. pantheica	.ATCA--G..	G-...T....	C..-.....	.....	Z71953	Z71954	Y92-248
G. piasezkii	A..C.T--G.	G-.....	C..-.....	.....	Z71955	Z71956	Y92-272
G. pseudoaquatica	A.T----TG.	G-.....	CC.-.....	.....	Z71959	Z71960	Y92-326
G. pyrenaica	.....-G..	.-.....	C..GC-....	.....	Z48068	Z48087	Y93-14
G. squarrosa	ATCGA---N.	G-.....	CC.-.....	.....	Z71965	Z71966	G046
G. crenulato-	....--CGC.	G-.....	CG.GC-....	.....	Z48098	Z48079	Y92-310
G. haynaldii	.....C-G..	G-.....	CG.GC-....	.....	Z48065	Z48085	Y92-201
G. hyalina12	--....NG..	A-.....	CC.-.....	.....	Z71941	Z71942	Y93-36
G. hyalina30	A--.ACCAC-	--.T.....	CC.-.....	.....	Z71943	Z71944	Y92-89
G. ludlowii16	-ATGAN-GC-	--G.....	CC.-.....	.....	Z71947	Z71948	Y92-35
G. ludlowii32	...A--TG.G	C-G.....T.	C..-.....	.....	Z71951	Z71952	Y92-33
G. ludlowii22	-TCGA-NG..	A-.....	CC.-.....	.....	Z71949	Z71950	Y92-99
G. producta	....-C-G..	--.T.....	CC.GC-....	.....	Z71957	Z71958	Y93-79
G. pudica	-.CAN-GTC-	--A.....	CC.-.....	.....	Z71961	Z71962	G178
G. sedifolia	.....-CG..	G-.....	CC.GC-....	.....	Z71963	Z71964	A94-S3
G. tetrasticha	.A....CG..	G-.....	CC.-.....	.....	Z71967	Z71968	Y92-128
G. bavarica	....AC-G..	G-.....	TC.-.....	.....	Z48094	Z48075	Y93-11
G. clusii	....AC-G..	G-.....	TC.-.....	.....	Z48097	Z48077	Y93-13
G. lutea	T.GA.-CG..	G-.....	CC.-.....	.....	Z48122	Z48119	Y91-S5
G. algida	...A.C-G..	G-.....	TC.-.....	.....	Z48142	Z48117	Y91-S10
G. callistantha	....-TG..	G-.....	T..-.....	.....	Z48095	Z48078	Y92-298
G. urnula	.....-TG..	G-.....	T..-.....	.....	Z48071	Z48090	Y92-71
G. delavayi	.....CTG..	G-...T.-..	T..-.....	.....	Z48099	Z48080	Y92-229
G. affinis	...C.C-G.T	G-.....	CC.GC-....	.....	Z48061	Z48074	Y91-S1
G. straminea	...~~~-GCA	.-GT.....	T-.-.....	.....	Z48070	Z48091	Y92-313
G. rhodantha	...A.C-A..	GA.....	CT.-..T...	.....	Z48069	Z48089	Y93-124
C. tibetica	.....C-G..	G-...T..T.	CC.-..T...	..T..	Z48145	Z48123	Y93-121
Gl. campestris	...C.C-A..	G-.....	CT.-..T...	.....	Z48104	Z48128	K83-G1