The complete chloroplast genome sequence of *Gentiana lawrencei* var. *farreri* (Gentianaceae) and a comparative analysis with its congeneric species

Peng-Cheng Fu, Yan-Zhao Zhang, Hui-Min Geng, Shi-Long Chen

Background. The chloroplast (cp) genome is useful in plant systematics, genetic diversity, molecular identification and divergence dating. The genus *Gentiana* contains 362 species, but there are only two valuable cp genomes. The purpose of this study is to report the complete cp genome and its characterization of *G. lawrencei* var. *farreri* which is endemic to the Qinghai-Tibetan Plateau (QTP).

Methods. Using high throughput sequencing technology, we got the complete nucleotide sequence of the *G. lawrencei* var. *farreri* cp genome. The gene divergence, simple sequence repeats (SSRs) and phylogenetics were studied. The comparison analysis was performed with its congeneric species *G. straminea*.

Results. The cp genome of *G. lawrencei* var. *farreri* is a circular molecule of 138,750 bp, containing a pair of 24,653 bp inverted repeats, separated by small and large single-copy regions of 11,365 and 78,082 bp, respectively. The cp genome contains 130 known genes, including 85 protein coding genes (PCGs), eight ribosomal RNA genes and 37 tRNA genes. Comparative analyses indicated that *G. lawrencei* var. *farreri* is 10,241 bp shorter than its congeneric species *G. Straminea*. Four large gaps were detected that are responsible for 85% of the total sequence loss. Further detailed analyses revealed that 10 PCGs were included in the four gaps that encode nine NADH dehydrogenase subunits. The cp gene content, order and orientation are similar to those of its congeneric species, but with some variation among the PCGs. Three genes, *ndhB*, *ndhF* and *clpP*, have high nonsynonymous to synonymous values. There are 34 simple sequence repeats (SSRs) in the *G. lawrencei* var. *farreri* cp genome, of which 25 are mononucleotide repeats: no dinucleotide repeats were detected. Comparison with the *G. Straminea* cp genome indicated that five SSRs have length polymorphisms and 23 SSRs are species-specific. The phylogenetic analysis of 48 PCGs from 12 Gentianales taxa cp genomes clearly identified three clades, which indicated the potential of cp genomes in phylogenetics.

Discussion. The “missing” sequence of *G. lawrencei* var. *farreri* mainly consistent of *ndh* genes which could be dispensable under chilling-stressed conditions in QTP. The complete cp genome sequence of *G. lawrencei* var. *farreri* provides intragenic information that will aid its conservation and contribute to genetic and phylogenetic research in the Gentianaceae.

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Introduction

The chloroplast (cp) is the photosynthetic organelle that provides essential energy for plants, and is hypothesized to have arisen from ancient endosymbiotic cyanobacteria (Neuhaus & Emes, 2000). In angiosperms, most cp genomes are typically circular DNA molecules, containing one large single-copy region (LSC), one small single-copy region (SSC) and a pair of inverted repeats (IRs) (Palmer, 1985; Jansen et al., 2005). The sizes of cp genomes in angiosperms range from 120 kb to 160 kb caused by expansion of the IR regions and evolutionary contractions (Palmer, 1985; Wang et al., 2008).

Recently, the number of completely sequenced cp genomes from higher plants has increased significantly. The cp genome is useful in plant systematics research because of its maternal inheritance and highly conserved structures. They are widely used in the study of genetic diversity, molecular identification, phylogenetic classification and divergence dating (Shaw et al., 2007; Nikiforova et al., 2013; Carbonell-Caballero et al., 2015; Williams et al., 2016).

The family Gentianaceae has approximately 700 species (He, 1988) and is the third largest family of the Gentianales order in the Asterids clade. However, only two complete chloroplast genomes have been sequenced in this family so far (Ni et al., 2016). The *Gentiana* is the largest genus in the Gentianaceae, containing 15 sections and about 362 species (Ho & Liu, 2001). *Gentiana* plants have been widely used as traditional Chinese and Tibetan medicines (Ho & Liu 2001) and are edificators in the Qinghai-Tibetan Plateau (QTP) alpine meadow. Although some studies have been carried out on the phylogenetics of the *Gentiana*, they have all been based on

one or several DNA fragments (Yuan & Küpfer, 1997; Yuan, Küpfer & Doyle, 1996; Zhang et al, 2009). Together with their complicated evolutionary history (Yuan & Küpfer, 1997), the phylogenetic relationships of *Gentiana* remain controversial (Ho & Liu, 2001; Favre et al., 2010). At present, there are only two complete cp genomes in the *Gentiana*: *G. straminea* and *G. crassicaulis*, which both belong to the same sect, *Cruciata* Gaudin (Ni et al., 2016). Therefore, it is necessary to develop genomic resources for *Gentiana* to provide intragenic information to help its conservation and to provide valuable information to study their phylogenetic relationships and the evolutionary history of the genus.

Gentiana lawrencei var. *farreri* T. N. Ho is endemic to the QTP and belongs to the sect *Kudoa* (Masamune) Satake & Toyokuni ex Toyokuni. It has very beautiful flowers and has been used in traditional Chinese and Tibetan medicine (Yang et al., 2012). Here, we report the cp genome sequence of *G. lawrencei* var. *farreri* and present a comparative analysis with its congeneric species *G. straminea*. The genome structure, insertions and deletions, repeat sequences and phylogenetics were analyzed. This study provided large amounts of sequence information for phylogenetic and evolutionary studies of the *Gentiana* and the Gentianaceae.

Materials and methods

Sample collection, genome sequencing, and assembly

Based on its morphological characteristics, *G. lawrencei* var. *farreri* was sampled in Qilian Mountain (101°22′33″E, 37°29′53″N, Qinghai, China) from a single plant. Total genomic DNA

was isolated from young leaves using a Dzup plant genomic DNA extraction kit (Sangon, Shanghai, China), following the manufacturer's instructions. After DNA isolation, approximately 5–10 μg of DNA was sheared, followed by adapter ligation and library amplification. Then, a quarter of one flow-cell lane containing the fragmented genomic DNA of *G. lawrencei* var. *farreri* was sequenced using the Illumina HiSeq 4000 platform (Biomarker, Beijing, China), yielding 36.08 million 150-bp paired-end reads from a library of approximately 350-bp DNA fragments. Reads corresponding to plastid DNA were identified using a BLASTN (E-value: 10^{-6}) search against the plastome sequences of two *Gentiana* taxa: *G. straminea* (GenBank accession no. NC_027441) and *G. crassicaulis* (NC_027442). A total of 2,517,802 reads (6.97%) were recovered and assembled using Velvet 1.2.10 (Zerbino & Birney, 2008). Eight contigs, ranging in size from 926 to 47,806 bp, were obtained. All the genomic regions located at the junction between the two contigs were verified by Sanger sequencing. The primers used were designed using PRIMER V5.0 and are provided in supplementary Table S1. The *G. lawrencei* var. *farreri* plastome sequence was deposited in GenBank (accession no. KX096882).

Genome annotation

The protein coding genes (PCGs), tRNAs and rRNAs in the cp genome were predicted and annotated using Dual Organellar GenoMe Annotator (DOGMA) using default parameters (Wyman, Jansen & Boore, 2004). The positions of the start and stop codons, or intron junctions of the PCGs, were verified using BLASTN searches. The cp gene map was drawn using OGDraw v1.2 (Lohse, Drechsel & Bock, 2007). Simple sequence repeats (SSRs) were detected

using MSDB 2.4 (http://msdb.biosv.com) with minimal repeat numbers of 10, 5, 4, 3, 3, and 3 for mono-, di-, tri-, tetra-, penta-, and hexa-nucleotides, respectively.

Comparative analysis with *G. straminea*

The cp genome sequence from *G. straminea* (NC_027441) was obtained from the National Center for Biotechnology Information (NCBI). Genome comparison to identify the differences between *G. lawrencei* var. *farreri* and *G. straminea* was performed using Geneious 5.6 (Kearse et al., 2012). The nucleotide and amino acid diversity was analyzed using BLASTN and BLASTP. Nonsynonymous (Ka) to synonymous (Ks) (Ka/Ks)ratios were calculated using DnaSP v5.10 (Librado & Rozas, 2009).

Phylogenetic analysis

To illustrate the phylogenetic relationships of the *Gentiana* with other major Gentianales clades, the other 12 available complete cp genomes were downloaded from GenBank (Table S2). *Lactuca sativa* from Asteraceae was used as the outgroup. Forty-eight PCGs (*atpA*, *atpB*, *atpE*, *atpH*, *atpI*, *cemA*, *matK*, *ndhD*, *ndhE*, *petA*, *petB*, *petD*, *petG*, *petL*, *petN*, *psaA*, *psaB*, *psaI*, *psaJ*, *psbA*, *psbC*, *psbD*, *psbE*, *psbF*, *psbH*, *psbI*, *psbJ*, *psbK*, *psbL*, *psbM*, *psbN*, *psbT*, *rbcL*, *rpl14*, *rpl16*, *rpl20*, *rpl22*, *rpl33*, *rpl36*, *rpoA*, *rps2*, *rps3*, *rps4*, *rps8*, *rps11*, *rps14*, *rps15* and *rps18*) found in all of the species were extracted from the selected cp genomes. The amino acid sequences of each of the 48 cp PCGs were aligned using MSWAT (http://mswat.ccbb.utexas.edu/) with default settings, and back translated to nucleotide sequences.

92 Phylogenetic analyses were performed using the concatenated nucleotide sequences and
93 PhyML3.1 software (Guindon & Gascuel, 2003) using the maximum likelihood (ML) method.
94 PhyML searches relied on the GTR model of nucleotide substitution. A bootstrap analysis was
95 performed with 100 replications.

96 **Results**

97 **The overall structure and general features of the *G. lawrencei* var. *farreri* cp genome**

98 The cp genome of *G. lawrencei* var. *farreri* is a closed circular molecule of 138,750 bp (Fig. 1),
99 comprising a pair of IR regions (IRa and IRb) of 24,653 bp, one LSC region of 78,082 bp and
100 one SSC region of 11,365 bp. It has an overall typical quadripartite structure that resembles the
101 majority of land plant cp genomes (Shinozaki et al., 1986). The GC contents of the LSC, SSC,
102 and IR regions and the whole cp genome are 35.7, 30.0, 43.6 and 38.0%, respectively, which are
103 similar to the other reported *Gentiana* cp genomes (Ni et al., 2016). The cp genome of *G.*
104 *lawrencei* var. *farreri* contains 130 genes, including 85 PCGs accounting for 66,215 bp, and 37
105 tRNA and eight rRNA genes accounting for 11,781 bp. Among the 130 genes, 18 are located in
106 the IR region. Most genes are present as a single copy, while all the rRNA genes and some of the
107 tRNA and PCGs in the IR occur as double copies. A total of 84 unigene were detected in the cp
108 genome and this category is detailed in Table S3. Four genes each have one intron (*atpF*, *rpoC1*,
109 *ndhB* and *rpl2*) and two PCGs (*clpP* and *ndhF*) and 1 ycf (*ycf3*) have two introns. Like most
110 other land plants, *rps12* is trans-spliced, with its two 3′ end residues separated by an intron in the
111 IR region, and the 5′ end exon is in the LSC region (Fig. 1). The 37 tRNAs contained 30

different tRNA genes and the eight rRNA genes contained four different tRNA genes. Both the number and types of the tRNAs are consistent with those presented in other species of vascular plants (Shinozaki et al., 1986).

Comparison of *G. lawrencei* var. *farreri* and *G. straminea* cp genomes

A comparative analysis between the cp genomes in *Gentiana* revealed that *G. lawrencei* var. *farreri* is 10,241 bp shorter than that of *G. straminea*. As for the four parts of the cp genome, the LSC, SSC and IR of *G. lawrencei* var. *farreri* are 3185 bp, 5720 bp and 680 bp shorter than those of *G. Straminea*, respectively (Table 1). Four big gaps (GapA–D) were detected: GapA (2241 bp) in the LSC, GapB (958 bp) in IRb, GapC (4582 bp) in the SSC and GapD (958 bp) in IRa. The four gaps represent 85% of the "missing" genome. All the gaps were verified by Sanger sequencing with primers designed using PRIMER V5 (Table S1). Compared with *G. straminea*, GapA contains three PCGs (*ndhJ*, *ndhK* and *ndhC*), GapB and GapD contain exon 2 of *ndhB* and GapC contains five PCGs (*ndhE*, *ndhG*, *ndhI*, *ndhA* and parts of *ndhH*). A comparative analysis between *G. lawrencei* var. *farreri* and *G. straminea* cp genomes revealed that the sequence similarities between the *trnH-GUG-psbA*, *trnK-UUU-trnQ-UUG*, *trnS-GCU-trnG-GCC*, *atpH-atpI*, *rpoB-trnC-GCA*, *psbC-trnS-UGA*, *trnT-UGU-trnL-UAA*, *atpB-rbcL*, *ycf1-ndhF*, *rpl32-trnL-UAG* and *trnL-CAA-ycf15* intergenic regions are very low.

Divergence hotspot

The complete cp genomes of *G. lawrencei* var. *farreri* and *G. straminea* were compared using

131 the mVISTA program to determine the level of sequence divergence. The comparison showed
132 that the coding regions of both cp genomes are highly conserved compared with the noncoding
133 regions. However, the intergenic regions showed the greatest divergence between the two cp
134 genomes. More divergence was found in the sequences of *clpP*, *ndhB*, *ndhD*, *ndhE*, *ndhF* and
135 *ndhH*, which are distributed mainly in the SSC regions, compared with other PCGs. The
136 nucleotide and amino acid sequences of the PCGs of *G. lawrencei* var. *farreri* and *G. straminea*
137 are highly similar, with average sequence similarities of 95.0 and 93.0%, respectively. Between
138 the two species, the nucleotide sequence identities of the LSC, SSC, and IR are 88.7, 61.0, and
139 92.9%, respectively. The most conserved genes include all the rRNA genes, the genes from
140 photosystem I, the cytochrome b/f complex genes and the ATP synthesis genes (Table S3).

141 **Divergence of coding gene sequence**

142 Seventy-four PCGs are shared between the two species. Compared with *G. Straminea*, 14 out of
143 the 74 shared PCGs had deletions and six had insertions (Table S4). The average Ks values
144 between the two *Gentiana* species were 0.0551, 0.1133, and 0.0243 in the LSC, SSC, and IR
145 regions, respectively, with a total average Ks of 0.0642 across all regions (Table S4). Although
146 the coding region is highly conserved, we did observe slight variations. Based on the comparison
147 of Ka/Ks values among the regions, higher Ks values were observed for some genes, including
148 *rps8*, *rpl14*, *rpl36*, *rpl32*, *ndhD*, *rpl36* and *ndhH*. The distribution of Ks values indicated that the
149 SSC region is under greater selection pressure than the rest of the cp genome. The Ka/Ks ratio
150 was also calculated, which was >1 for *ndhB* in the IR region, *ndhF* in SSC region and *clpP* from

the LSC region (Fig. 2).

SSR analysis

Thirty-four SSR loci, 394 bp in length, were detected in the *G. lawrencei* var. *farreri* cp genome, and there were 25, three, five, and two mono-, tri-, tetra-, and penta-nucleotide repeats, respectively (Table S5). No dinucleotide repeats were detected in the cp genome. Most of the SSRs are mononucleotide repeats, which is consistent with the study of George et al. (2015). Thirty of the 34 SSRs comprised A and T nucleotides, with a higher AT content (95.9%) in these sequences compared with the rest of the genome. Among the SSRs, 23 were located in intergenic regions and 11 were found in coding genes, including those in the *ccsA*, *rpoC1*, *ndhF*, *atpF*, *rpl32*, *matK*, *rpoA*, *atpB* and *psaB* genes. Compared with *G. Straminea*, six loci were identical, five were polymorphic, 28 were lost and 23 were specific to *G. lawrencei* var. *farreri* (Table S5).

Phylogenetic relationship

An ML phylogenetic tree was constructed using 48 PCGs from 12 Gentianales taxa that clearly indicated three clades (Fig. 3). One contains the three species of Gentianaceae, the second contains the two species of Rubiaceae and the third contains the seven species of Apocynaceae. All the nodes in the tree have high support.

Discussion

Evolution of *G. lawrencei* var. *farreri*

Much of the variation in the sequence complexity of angiosperm cp genomes appears to be the result of rather small length mutations. However, our comparative analysis showed that *G. lawrencei* var. *farreri* is 10,241 bp shorter than *G. straminea*. Although the cp genome size is variable, ranging from 120 kb to 160 kb (Wang et al., 2008), huge genome losses in congeneric taxa are rare. In general, most of the size changes in angiosperm cp genomes can be accounted for by rare deletions and duplications leading to massive changes in the size of the IR region (Palmer, 1985). This is not the case for *G. lawrencei* var. *farreri* and *G. straminea*. The total length variation mainly occurred in the SSC (5720 bp, 55.85%) and LSC (3158 bp, 30.84%) regions rather than the two IR regions (1360 bp, 13.28%). In the SSC region, more than half (50.33%) was lost. Therefore, the cp genome size variation in the two *Gentiana* taxa was not caused by deletions in the IR regions, but by deletions in the SSC and LSC regions. Although the IR region can vary from 10 to 76 kb among angiosperms, in the great majority of species it is a rather constant 22–26 kb in size (Ohnert, Crouse & Schmitt, 1982). The junction between the IR and LSC region is located within the *rps19* gene in *G. lawrencei* var. *farreri*, similar to four diverse dicots and monocots, which suggests some selection is operating to constrain the boundaries of the IR (Palmer, 1985).

The SSC region of *G. lawrencei* var. *farreri* has experienced drastic variation. Compared with *G. straminea*, the SSC region contributes 55.85% of the cp genome sequence length variation and only showed 61.0% nucleotide identity. The SSC region also has a much higher Ks (0.1133) than the LSC (0.0551) and IR (0.0243) regions. It has been suggested that the differences in the

evolutionary rates between the SSC and LSC might reflect differences in the proportion of coding *vs*. noncoding regions in these sequences (Walker, Zanis & Emery, 2014); however, this does not appear to be true in our study. Although the SSC region is a "hotspot" for inversion events (Liu et al., 2013; Walker, 2015), we did not detect inversion in the SSC region of *G. lawrencei* var. *farreri*. The functional genes associated with the variation in the SSC region of *G. lawrencei* var. *farreri* might provide an insight into the reasons for the drastic variation. In the SSC region, the gene loss and divergence focus on the *ndh* genes.

In higher plants, gene loss is an ongoing process. The huge genome loss in *G. lawrencei* var. *farreri* was mainly accounted for by four big gaps, which caused the loss of the entire *ndhJ*, *ndhK*, *ndhC*, *ndhE*, *ndhG*, *ndhI*, and *ndhA* genes and partial loss of *ndhH* and *ndhB*. The protein products of all the lost genes are NADH dehydrogenase (NDH) subunits. The cp DNA of most of the higher plants contains 11 *ndh* genes, which encode protein subunits of the thylakoid NDH complex. The complex is analogous to mitochondrial complex I (EC 1.6.5.3), which catalyzes the transfer of electrons from NADH to plastoquinone (Sazanov, Burrows & Nixon, 1998). The cp *ndh* genes have been retained in most higher plants (Martín & Sabater, 2010), but appear to have been lost frequently in parasitic and epiphytic plants (e.g. Stefanovi & Olmstead, 2005), along with other cp genes, apparently associated with a loss of or reduction in photosynthetic capability (Iles, Smith & Graham, 2013). Although the *ndh* genes could be dispensable under mild non-stressing environments, transgenic plants defective in *ndh* genes showed that the NDH complex is required to optimize photophosphorylation rates and showed impaired photosynthesis

209 rates under stress conditions (Marín & Sabater, 2010). Cyclic photophosphorylation via the NDH
210 pathway might play an important role in regulating CO_2 assimilation under heat-stress conditions,
211 but is less important under chilling-stressed conditions (Wang et al., 2006). Therefore, the
212 absence of NDH in *G. lawrencei* var. *farreri* is understandable when considering the cool
213 conditions in the QTP, which is the natural habitat of *Gentiana* (Ho & Liu, 2001). Meanwhile,
214 the *ndh* loss between two congeneric species might offer a clue to the divergence and evolution
215 of *Gentiana*.

216 Variation in the divergence of the coding region was observed between the two *Gentiana*
217 species. Although the coding region was generally highly conserved, the *rps8*, *rpl14*, and *rpl36*
218 genes of the LSC region and the *rpl32*, *ndhD*, *ndhF*, and *ndhH* genes of the SSC region of *G.*
219 *lawrencei* var. *farreri* showed a higher evolution rate compared with other genes. Based on the
220 sequence identity among the three regions, the IR region is more conserved than the LSC and
221 SSC regions. This agrees with previous studies that hypothesized that the frequent recombinant
222 events occurring in the IR region result in selective constraints on sequence homogeneity,
223 causing them to diverge at a slower rate than the LSC and SSC regions (Qian et al., 2013; Cho et
224 al., 2015). Our data confirm a positive selection pressure and neutral evolution of the protein
225 coding genes. The *ndhB* gene of the IR region, *ndhF* of the SSC region and *clpP* from the LSC
226 region of *G. lawrencei* var. *farreri* presented higher Ka/Ks ratios (>1.0), indicating that they had
227 evolved under positive selection. The *clpP* gene also showed a high Ka/Ks ratio in *Fagopyrum*
228 *tataricum* (Cho et al., 2015). Interestingly, the *ndhB* and *ndhF* genes experienced positive

229 selection pressure. In the absence of nine *ndh* genes in *G. lawrencei* var. *farreri*, the remaining
230 *ndhB* and *ndhF* genes might play an important role in cyclic photophosphorylation, although the
231 functions of *ndhB* and *ndhF* genes are unknown. The *ndhB* and *ndhF* genes are probably
232 transcribed independently as monocistronic mRNAs (Martín & Sabater, 2010). This independent
233 transcription might have contributed to the selection pressure.

234 **Phylogenetic value**

235 The ML phylogenetic tree of Gentianales constructed using 48 PCGs clearly grouped the taxa
236 from the three families into three clades. The phylogenetic relationships were consistent with
237 previous studies that classified the three families as three monophyletic clades and identified the
238 Rubiaceae as the base group in the Gentianales (Backlund, Oxelman & Bremer, 2000). The cp
239 genome has also been used successfully for phylogenetic reconstruction in several studies
240 (Carbonell-Caballero et al., 2015; Williams et al., 2016). However, these studies were all based
241 on one or several DNA fragments, which, together with their complicated evolutionary history,
242 have led to the phylogenetic relationships of *Gentiana* being controversial (Ho & Liu 2001;
243 Favre et al., 2010). For example, the sect *Chondrophy*, which has 10 series and 163 species,
244 experienced rapid evolution (Yuan & Küpfer, 1997), making the phylogenetic relationships of
245 this section difficult to determine. In addition, previous phylogenetic analyses based on internal
246 transcribed spacer regions in the sect *Chondrophy* indicated that the sect should be treated as a
247 new genus or sub-genus (Zhang et al., 2009). Our analysis also showed the massive cp genome
248 variation; therefore, to realize the full potential of the cp genome in phylogenetic analysis, more

249 taxa of different sects should be included in the cp genome comparison analysis.

250 Chloroplast SSRs are new tools for studies in plant ecology and evolution (Provan, Powell &
251 Hollingsworth, 2001). Microsatellites often show high levels of polymorphism and are thus used
252 widely in studies of genetics and evolution. However, SSRs in the nuclear genome are usually
253 species-specific and are thus used mainly for intraspecific genetic studies rather than
254 phylogenetic studies of related species. Unlike nuclear SSRs, chloroplast SSRs are frequently
255 cross-amplified in related species and thus could be used for phylogenetic studies (Provan,
256 Powell & Hollingsworth, 2001). We detected five polymorphic SSRs between *G. lawrencei* var.
257 *farreri* and *G. straminea*, which belong to different sects. SSRs are more polymorphic than cp
258 loci that are amplified by universal primers; therefore, the polymorphic SSRs could offer higher
259 resolution for phylogenetic tree construction in *Gentiana* and intragenus sects.

260

261 **Conclusion**

262 We present the first report of the complete cp genome sequence of *G. lawrencei* var. *farreri* and
263 describe its evolutionary characteristics in comparison with *G. straminea*. The divergence
264 hotspots and SSRs clarified here could be used as molecular markers and will be useful for
265 further studies on population genetics, phylogenetics and evolution of the genus *Gentiana*.

266

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Table 1(on next page)

Comparison of genome contents of *G. lawrencei* var. *farreri* and *G. straminea.*

1

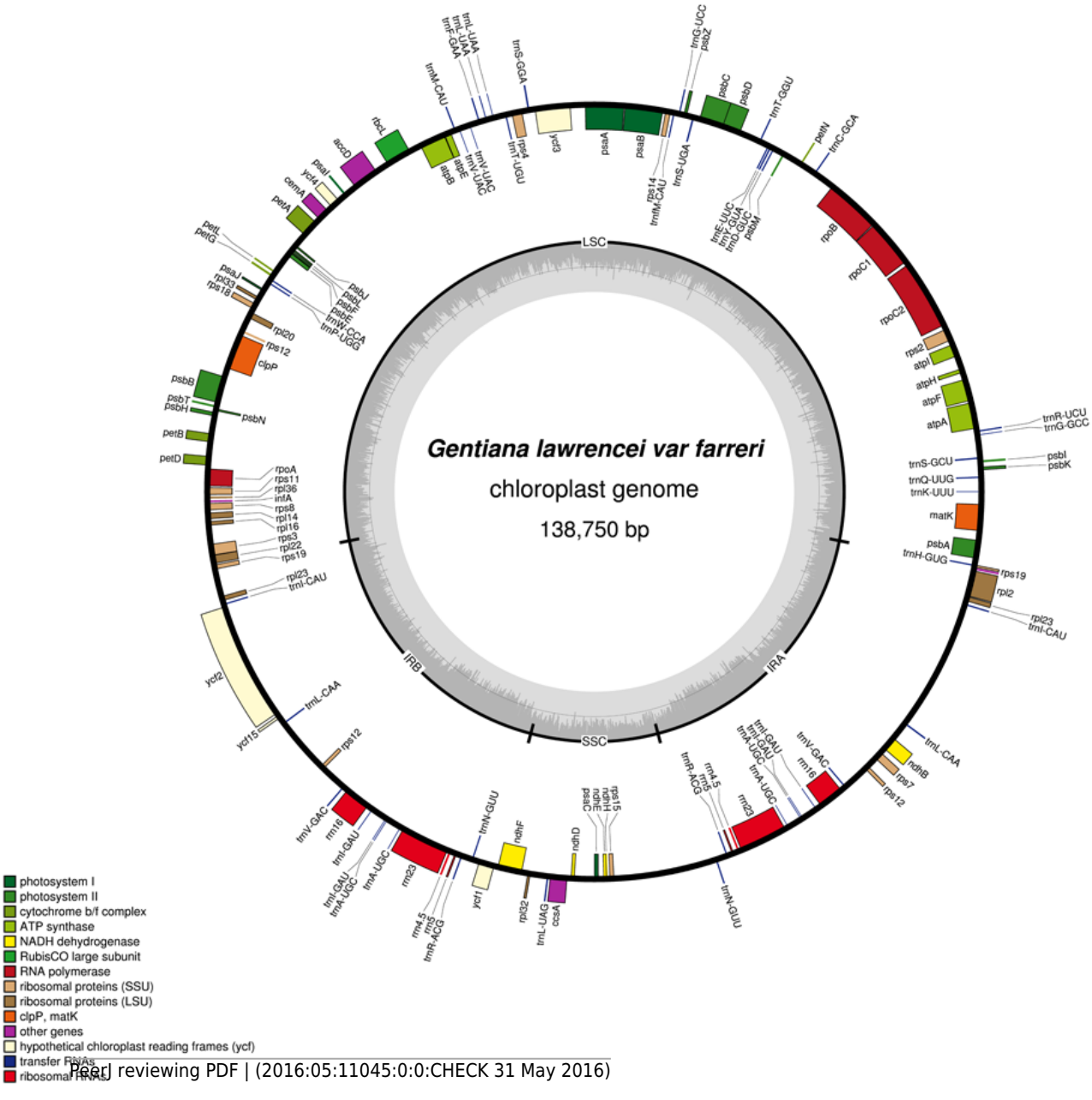
	G. lawrencei var. *farreri*	*G. straminea*
Total Sequence Length (bp)	138,750	148,991
Large Single Copy (bp)	78,082	81,240
Inverted Repeat Region (bp)	24,653	25,333
Small Single Copy (bp)	11,365	17,085
GC Content (%)	38	37.7
Total CDS Bases (bp)	66,215	75,780
Average CDS Length (bp)	779	758
Total RNA Bases (bp)	11,781	11861
Average Intergenic Distance (bp)	467	403

2

1

Map of the chloroplast genome of *G. lawrencei* var. *farreri*.

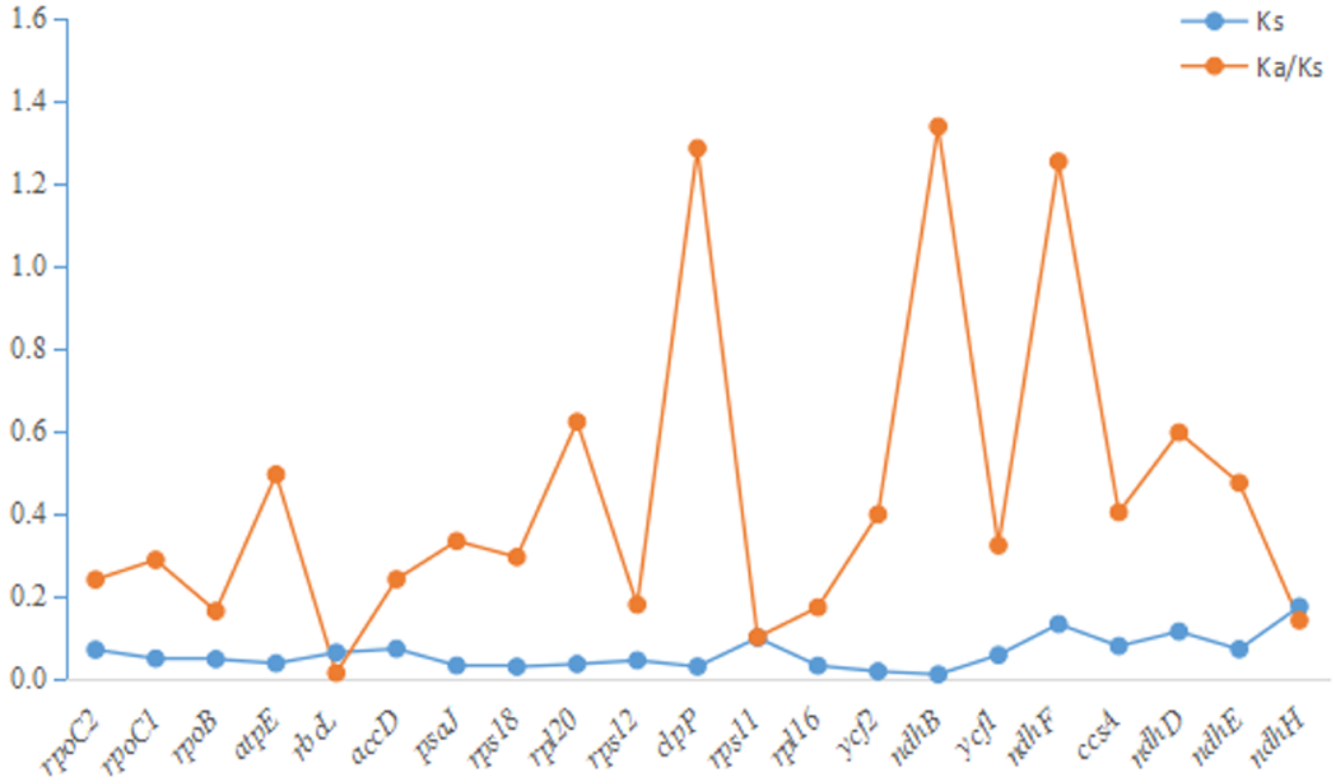
Genes drawn inside the circle are transcribed clockwise, and those outside are transcribed counterclockwise. Genes belonging to different functional groups are shown in different colors.



2

Gene-specific Ks values and Ka/Ks ratios between the chloroplast genomes of two *Gentiana* species (*G. lawrencei* var. *farreri* and *G. straminea*).

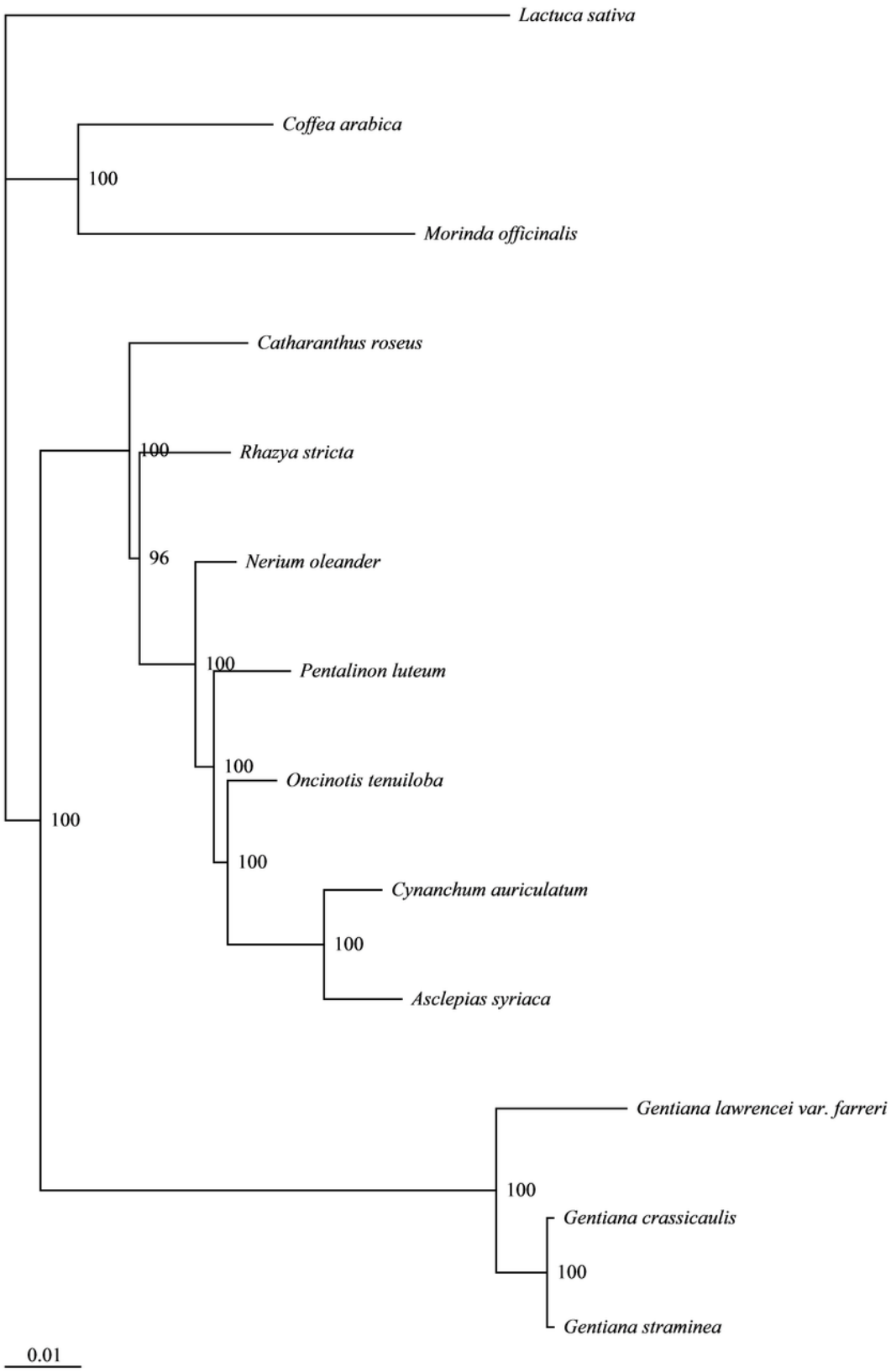
Three genes (*clpP*, *ndhB* and *ndhF*) returned Ka/Ks ratios greater than 1.0, whereas the Ka/Ks ratios of the other genes were less than 1.0.



3

Phylogenetic analysis of 12 Gentianales species using 48 CDS regions of the chloroplast genomes.

Data sources: *Gentiana straminea* (NC_027441); *Gentiana crassicaulis* (NC_027442); *Catharanthus roseus* (NC_021423); *Rhazya stricta* (NC_024292); *Nerium oleander* (NC_025656); *Pentalinon luteum* (NC_025658); *Oncinotis tenuiloba* (NC_025657); *Cynanchum auriculatum* (NC_029460); *Asclepias syriaca* (NC_022432); *Coffea arabica* (NC_008535); *Morinda officinalis* (NC_028009) and *Lactuca sativa* (NC_007578).


Lactuca sativa
Coffea arabica
100
Morinda officinalis
Catharanthus roseus
100
Rhazya stricta
96
Nerium oleander
100
Pentalinon luteum
100
Oncinotis tenuiloba
100
Cynanchum auriculatum
100
Asclepias syriaca
100
Gentiana lawrencei var. farreri
100
Gentiana crassicaulis
100
Gentiana straminea
0.01