The complete chloroplast genome of the Jerusalem artichoke (*Helianthus tuberosus* L.) and an adaptive evolutionary analysis of the *ycf2* gene

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Jerusalem artichoke (*Helianthus tuberosus* L.) is widely cultivated in Northwest China which has become an emerging economic crop with rapid development. Because of its elevated inulin content and high resistance, it is widely used in functional food, inulin processing, feed, and ecological management. In this study, Illumina sequencing technology was utilized to assemble and annotate the complete chloroplast genome sequences of Jerusalem artichoke. The total length was 151,431 bp, including four conserved regions: A pair of reverse repeat regions (IRa 24,568 bp and IRb 24,603 bp), a large single-copy region (LSC, 83,981 bp), and a small single-copy region (SSC, 18,279 bp). The genome had a total of 115 genes, with 19 present in the reverse direction in the IR region. 36 simple sequence repeats (SSRs) were identified in the coding and non-coding regions, most of which were biased towards A/T bases. 32 SSRs were distributed in the non-coding regions. Comparative analysis of the chloroplast genome sequence of Jerusalem artichoke and other species of the composite family revealed the chloroplast genome sequences of plants of the composite family to be highly conserved. Differences were observed in 24 gene loci in the coding region, with the degree of differentiation of the *ycf2* gene being the most obvious. Phylogenetic analysis showed *Helianthus petiolaris subsp. fallax* had the closest relationship with Jerusalem artichoke, both members of the *Helianthus* genus. Selective locus detection of the *ycf2* gene in eight species of the composite family was performed to explore adaptive evolution traits of the *ycf2* gene in Jerusalem artichoke. The results show that there are significant and extremely significant positive selection sites at the 1239N and 1518R loci, respectively, indicating that the ycf2 gene has been subject to adaptive evolution and has the potential to be used as a phylogenetic reconstruction locus in the composite family. Insights from our assessment of the complete chloroplast genome

sequences of Jerusalem artichoke will aid in the in-depth study of the evolutionary relationship of the composite family, and provide significant sequencing information for the genetic improvement of Jerusalem artichoke.

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Abstract

Jerusalem artichoke (*Helianthus tuberosus* L.) is widely cultivated in Northwest China which has become an emerging economic crop with rapid development. Because of its elevated inulin content and high resistance, it is widely used in functional food, inulin processing, feed, and ecological management. In this study, Illumina sequencing technology was utilized to assemble and annotate the complete chloroplast genome sequences of Jerusalem artichoke. The total length was 151,431 bp, including four conserved regions: A pair of reverse repeat regions (IRa 24,568 bp and IRb 24,603 bp), a large single-copy region (LSC, 83,981 bp), and a small single-copy region (SSC, 18,279 bp). The genome had a total of 115 genes, with 19 present in the reverse direction in the IR region. 36 simple sequence repeats (SSRs) were identified in the coding and non-coding regions, most of which were biased towards A/T bases. 32 SSRs were distributed in the non-coding regions. Comparative analysis of the chloroplast genome sequence of Jerusalem artichoke and other species of the composite family revealed the chloroplast genome sequences of plants of the composite family to be highly conserved. Differences were observed in 24 gene loci in the coding region, with the degree of differentiation of the *ycf2* gene being the most obvious. Phylogenetic analysis showed *Helianthus petiolaris subsp. fallax* had the closest relationship with Jerusalem artichoke, both members of the *Helianthus* genus. Selective locus detection of the *ycf2* gene in eight species of the composite family was performed to explore adaptive evolution traits of the *ycf2* gene in Jerusalem artichoke. The results show that there are significant and extremely significant positive selection sites at the 1239N and 1518R loci, respectively, indicating that the ycf2 gene has been subject to adaptive evolution and has the potential to be used as a phylogenetic reconstruction locus in the composite family.

Insights from our assessment of the complete chloroplast genome sequences of Jerusalem artichoke will aid in the in-depth study of the evolutionary relationship of the composite family, and provide significant sequencing information for the genetic improvement of Jerusalem artichoke.

Introduction

Jerusalem artichoke (*Helianthus tuberosus* L.) is a species of the composite family native to North America, mainly distributed in the temperate zone of 40-55°C north latitude and the temperate region with the approximate similar latitude in the southern hemisphere. Jerusalem artichoke was brought to China via Europe in the 17th century. It has been grown on a small scale as a pickled vegetable in various regions of China. Jerusalem artichoke is highly resistant and can be grown in saline, alkaline, dry and low temperature conditions. Therefore, it is widely cultivated in various regions of China, especially in the Qinghai plateau in recent years. To date, most research on Jerusalem artichoke has focused on ecological management, feed research and development, and the processing of inulin products. Studies centered on the improvement of saline land in the Songnen Plain have recognized Jerusalem artichoke as an excellent improved crop, which has already been initially grown in saline-alkali grassland(Yan et al. 2008). The overground part of Jerusalem artichoke is tall, making it an easily accessible source of animal feed.

Furthermore, its leaves are especially particularly nutritious compared with other feed ingredients, being rich in lysine and methionine, and having a dry matter content of protein as high as 20%, of which 5% to 6% corresponds to lysine, an essential amino acid (Rawate & Hill 1985). Jerusalem artichoke also utilizes fructan as a source of carbon, instead of starch, as most crops. Fructan can be processed or modified, which providing raw materials for the production of bioethanol, paper, and healthcare products(Saengkanuk et al. 2011; Wang et al. 2015; Wyse et al. 2017).

The composite family is the largest group of dicotyledonous chrysanthemums, encompassing 25,000-30,000 species distributed throughout the world. 52 species and a large number of subspecies have been recognized in the *Helianthus* genus, including Jerusalem artichoke. The morphology of these plants is complex and diverse, leading to difficulties in identification and evolutionary analysis. Jerusalem artichoke is a hexaploid species (2n = 6x = 102), which reproduces mainly through vegetative propagation by tubers(Baldini et al. 2004). The evolutionary assessment of this plant is controversial, with its ancestral species being uncertain. Hybridization experiments between Jerusalem artichoke and *Helianthus annuus L.* have confirmed homologous genes between these species. It is generally believed that the chromosome number of triploid hybrid (AAB) in Jerusalem artichoke is doubled. Moreover, cytogenetic studies have demonstrated two of the three genomes of Jerusalem artichoke are homologous(Atlagić et al. 1993; Kostoff 1934; Kostoff 1939). The diploid (2n = 2x = 34) B genome is provided by the immediate ancestor of *Helianthus annuus L.*, while the autotetraploid (2n = 4x = 68) A genome is provided by the crop in the composite family(Bock et al. 2014; Heiser & Smith 1964; Heiser et al. 1969). *Helianthus hirsutus*is is regarded as the most likely tetraploid ancestor (Bock et al. 2014); while *Helianthus grosseserratus*, and *Helianthus giganteus* are viewed as the most likely diploid ancestors. Sequencing of related species using partial mitochondrial genomes as well as 35 s and 5 s ribosomal DNA has shown the origin of Jerusalem artichoke is very rich and probably linked to the hybridization of tetraploid Hairy *Helianthus annuus* L. and diploid Sawtooth *Helianthus annuus L.* (Bock et al. 2014; Timme et al. 2007). With the development of high-throughput sequencing technology, chloroplast phylogenetic genome evaluation has become a hot topic in the evolutionary research of plants in recent years. Plenty of phylogenetic information is contained in the chloroplast genome, providing a broad data platform for the study of phyletic evolution, and thereby verifying and extending the results of previous studies. The chloroplast genome sequencing of 8 *Helianthus* species has been completed. However, this aspect remains unexplored concerning Jerusalem artichoke.

Thus, in this study, we report the complete chloroplast genome sequencing, assembly and comparative analysis of Jerusalem artichoke. This data will help elucidate the evolutionary history of Jerusalem artichoke and its phylogenetic position in the composite family. In addition, it will lay a foundation for further studies of population genetics and other molecular aspects of Jerusalem artichoke based on chloroplast DNA sequencing.

Materials & Methods

Samples and genome sequencing

Fresh tender leaves of Jerusalem artichoke were obtained from the experimental base of the Qinghai Academy of Agricultural and Forestry Sciences (N36°43′51,, E101°45′24). Chloroplast DNA was extracted through an improved high-throughput chloroplast genome extraction method (Shi et al. 2012). Illumina HiSeq PE150 paired-end sequencing technology was used to establish the library for sequencing. The library was of the DNA small fragment type with 400 bp, 150bp read length with the average depth was 100×.

Chloroplast genome assembly and annotation

FastQC was used for the quality filtering of clean data. SOAPdenovo software was used for pre-assembly (Lee & Lee 1995); while SPAdes v3.6.2 (http://bioinf.spbau.ru/spades) was used for sequence assembly (Bankevich et al. 2012). The sequence of the chloroplast genome of *Helianthus annuus L.* was used as a reference to determine the location of the chloroplast genome. Gapcloser (Luo et al. 2012) and GapFiller (Boetzer & Pirovano 2012) software for repairing gaps; and PrInSeS-G was then used for sequence correction. DOGMA software (http://dogma.ccbb.utexas.edu/) (Wyman et al. 2004) was used for annotation. The gene region and protein coding sequence were manually adjusted according to the initiation codon and termination codon sequences. tRNA was entered into tRNAscan-SE (http://lowelab.ucsc.edu/tRNAscan-SE/) for annotation (Lowe & Chan 2016). rRNA was submitted to the RNAmmer 1.2 Server (http://www.cbs.dtu.dk/services/RNAmmer/) for prediction. The resulting sequence information and annotation results were submitted to Genebank, with the sequence number of MG696658. The Organellar Genome DRAW software (http://ogdraw.mpimp-golm.mpg.de/index.shtml) (Lohse et al. 2013) was used to render a complete circular chloroplast genome map.

Repeats and SSRs analysis

The chloroplast genome was entered into REPuter (Kurtz et al. 2001)to identify forward and reverse repeat sequences. Simple sequence repeats (SSRs) searching was identified by MIcroSAtellite (MISA) software based on perl script (http://pgrc.ipk-gatersleben.de/misa/). The number of repeats from mononucleotide to hexanucleotide was set to 10, 5, 4, 3, 3 and 3.

Comparative analysis of different *Asteraceae* plastomes

The LAGAN model in the mVISTA software (Frazer et al. 2004) was used to perform a comparative analysis of the chloroplast genome of Jerusalem artichoke with *Carthamus tinctorius* (KX822074.1), *Ageratina adenophora* (JF826503.1), *Guizotia abyssinica* (EU549769.1). *Lactuca sativa* (NC_007578.1), *Helianthus argophyllus* (KU314500.1), *Helianthus debilis* (KU312928.1), and *Helianthus petiolaris subsp. fallax* (KU295560.1). After screening for the quality of the original chloroplast genome data of Jerusalem artichoke, the final constructed sequence (the gene sequence extracted from the annotation) and the established chloroplast genome of 15 plant species were compared by Blast++. HomBlocks (Bi et al. 2018) was used to construct a Circos map (http://circos.ca/) to find the reception, relative position and link color of genes. This was then standardized according to the length of all alignment regions. Coloring was performed in accordance with the long, medium, relative short, and short sequence lengths (pink, orange, green, and blue, respectively). COBALT

(https://www.ncbi.nlm.nih.gov/tools/cobalt/cobalt.cgi?CMD=Web) was utilized to compare the differential protein sequence *ycf2*.

Phylogenetic analysis

The following 15 species of the composite family were used for the phylogenetic analysis of Jerusalem artichoke: *Ageratina adenophora* (JF826503.1), *Carthamus tinctorius* (KX822074.1), *Guizotia abyssinica* (NC_010601.1), *Jacobaea vulgaris* (NC_015543.1), *Lactuca sativa* (NC_007578.1), *Helianthus annuus* (NC_007977.1), *Helianthus petiolaris subsp. fallax* (KU295560.1), *Helianthus argophyllus* (KU314500.1), *Helianthus debilis* (KU312928.1), *Helianthus annuus cultivar line HA383* (DQ383815.1), *Helianthus petiolaris* (KU310904.1), *Helianthus praecox* (KU308401.1), *Helianthus annuus subsp. Texanus* (KU306406.1), *Mikania micrantha* (NC_031833.1), and *Taraxacum Mongolicum* (NC_031396.1). MAFFT 7.388 (Katoh et al. 2017) was used to compare 16 chloroplast genome sequences. A phylogenetic tree was constructed with the method of maximum-likelihood and Bayesian, respectively. The GTRGAMMAI model was used in the ML Tree, and RAxML v8.1.24 (Stamatakis 2014) was used to construct the tree. Parameters were set to search for 30 repeats, and the tree with the maximum likelihood value was used. In addition, Bootstrap was set to run 1000 times to detect the credibility of each branch. To build the Bayesian tree, the nucleotide substitution model GTR+I+G in Bayesian analysis was selected according to BIC in the jModelTest 2.1.7 software (Darriba et al. 2012). MrBayes 3.2 (Ronquist et al. 2012) was used for calculations, employing the Markov chain Monte Carlo methodology. Four Markov chains were initialized at the same time. The random tree was marked as the initial tree, and one was saved every 500 trees for a total of 5,000,000 trees. The first 20% of the Burin-in trees was discarded. The remaining trees were used to calculate the posterior possibility of the consistent tree and each branch.

Adaptive evolution traits

The ratio (ω) of the non-synonymous substitution (dN) to the synonymous substitution (dS) of nucleotides is used in most adaptive evolution studies to measure the selection pressure at the nucleic acid or protein level. In addition, the selection pressure is considered to hinder or promote its role in the process of non-synonymous replacement fixation. The positive selection model (M2a, M8) and the control model (M1a, M7, M8a) provided by EasyCodeML software were used to conduct the adaptive evolution analysis in the loci (Gao et al.). The locus model was used to assume that there were different selection pressures at different loci. In other words, the ω values were different, but there was no difference in different branches of the phylogenetic tree. This model was primarily used to detect the existence of positive selection ($\omega > 1$) and negative selection ($\omega < 1$) loci in the *ycf2* gene. Three pairs of comparison models were M1a (near neutral) and M2a (selection), M0 (single ratio) and M3 (discrete), M7 (beta) and M8 (beta & ω) in this study. The former is a zero hypothesis, and the latter is an alternative hypothesis. Models M0 (single ratio) to M3 (discrete) were used to detect different ω values at each point rather than detecting positive selection loci. PAMLx V1.3.1 was used to perform the likelihood ratio test (LRT) in three pairs of models (Yang 1997). Positive selection loci were tested by comparing the significance of the differences between the models. χ^2 distribution was used as the significance test

under the condition of relative degrees of freedom (the difference between the number of two models).

Results

Genome organization and gene features

The chloroplast genome of Jerusalem artichoke had a total length of 151,431 bp. The genome was composed of four parts: A pair of reverse repeat regions, IRa (24,568 bp) and IRb (24,603 bp), separated by a large single-copy region LSC (83981bp) and a small single-copy region SSC (18,279 bp) (Fig. 1). Genes in the coding regions accounted for 55.45% of the genome, including protein-coding genes, tRNA genes and rRNA genes. The chloroplast genome of Jerusalem artichoke had a total guanine-cytosine content (G-C content) of 37.6%; with GC in the IR region corresponding to 43.2%, and GC in the LSC and SSC regions being 35.6% and 31.3%, respectively. The chloroplast genome of Jerusalem artichoke contained 115 genes, including 84 protein-coding genes CDS, 27 tRNA genes and 4 rRNA genes distributed in the IR region. Furthermore, this region encompassed 19 inverse genes, including 8 CDS genes (*ycf2*, *ndhB*, *rps7*, *rps12*, *ycf15*, *ycf1*, *rpl2*, and *rpl23*), 7 tRNA genes, and 4 rRNA genes. The 115 genes contained 60 Protein synthesis and DNA replication genes, 44 Photosynthesis genes, 6 Miscellaneous group genes and 5 pseudogenes of unknown function genes (Table 1). In the chloroplast genome of Jerusalem artichoke, 16 intron-containing genes were annotated, 11 of which were protein-encoding and 5 were tRNA genes. Of the 16 intron genes, the intron sequence in *trnK-UUU* was the longest (2,528 bp), while the intron in the trnL-UAA gene was the smallest (436 bp). There were two introns in the *clpP*, *ycf3* and *rps12* genes, whereas the other genes contained only one intron (Table 2). Since Bock et al. have sequenced the Jerusalem artichoke plastid genome, based on this, we performed a detailed comparison (NCBI Accession: NC023112) and the sequencing results in this study (NCBI Accession: MG696658), which are shown by the results of BRIG (Fig 2), the result of this sequencing is 384 bp more than NC023112, and there are partial base differences in 15 genes: *ccsA*, *atpB*, *clpP*, *ndhB*, *ndhH*, *ndhI*, *petA*, *petD*, *rpl2*, *rpoC1*, *rpoC2*, *rps12*, *rps16*, *ycf1* and *ycf2*, there are multiple differences in *clpP* and *rpoC1* (Table 3).

Repeats and SSRs analysis

Distribution of cpSSR in Jerusalem artichoke was analyzed, revealing 36 different SSR loci in its chloroplast genome. Among them, 32 SSR were composed of A or T, 2 were composed of C, and only 1 was composed of G; indicating the chloroplast genomic SSR of Jerusalem artichoke are biased towards A/T bases (Fig 3). Assessment of SSR distribution found 32 SSR in the non-coding region of the chloroplast genome. The non-coding region mainly includes intergenic spacer (IGS) and introns, accounting for 68% and 20% of the distribution, respectively. In the coding region, there are SSR only in the *rpoC2*, *cemA*, and *ycf1* genes.

241
242 **Comparative analysis of different composite chloroplast**
243 Comparative analysis with the plastomes of other species of the composite family revealed
244 only small differences in plastome size and composition in comparison to that of Jerusalem
245 artichoke (Table 4). There were very few inconsistencies in the types and number of chloroplast
246 genes in several species of the composite family, and the performance was very conserved. The
247 chloroplast genome of Jerusalem art ichoke ranked 5th in the aligned genomes of the 8 chloroplast
248 genomes of the composite family. Length variation in the sequence may be caused by the
249 difference in length between the LSC and IR regions. The chloroplast genome size of 8 crops of
250 the composite family was approximately 150 kb, with a GC content of approximately 37.5%. The
251 number of coding protein genes ranged between 79-89. All of these genomes had 4 rRNA-coding
252 genes and 20-30 tRNA-coding genes. The plastome of Jerusalem artichoke was 327 bp longer than
253 that of *Helianthus petiolaris subsp. fallax* (a crop in the same genus), mainly in the LSC region.
254 In addition, it had 5 more protein-coding genes than that of *Helianthus petiolaris subsp. fallax*,
255 with no difference in the number of rRNA- and tRNA-coding genes.
256 The genomic sequences of 8 composite species were analyzed by the mVISTA software,
257 detecting the variations of the sequences (Fig. 4). Results showed there was less variation between
258 Jerusalem artichoke, *Helianthus petiolaris subsp. fallax* and *Helianthus debilis* and *Helianthus*
259 *argophyllus*. Compared with *Ageratina adenophora*, partial structure was lacking in Jerusalem
260 artichoke.
261 Based on the results of mVISTA, a systematic comparative analysis was performed in a
262 coding region with small variation amplitude (Doorduin et al. 2011). As shown in Fig 5, there were
263 differences among 8 species of the composite family in the following 24 gene loci: *trnN-GUU*,
264 *trnR-ACG*, *trnA-UGU*, *ycf68*, *trnL-GAU*, *trnV-GAC, ycf15*, *rps7*, *ndhB*, *trnL-CAA*, *ycf2*, *trnL-*
265 *CAU*, *rpl23*, *rpl2*, *rps19*, *rps12*, *rpl20*, *rps18, rpl33*, *trnP-UGG*, *petL*, *trnG-UCC*, *trnS-GCU*, and
266 *trnC-GCA*. The discovery of these differential genes provides valuable phylogenetic information
267 for the further evaluation of the composite family.
268 In many studies, the *ycf2* gene has become an alternative choice for the assessment of plant
269 sequence variation and phylogenetic evolution. Our results showed the *ycf2* gene segment had
270 large deletion and inconsistency. The *ycf2* gene of Jerusalem artichoke and seven other composite
271 species was compared. Four species of genus *Helianthus* had 152 amino acid sequence deletions
272 of *ycf2* gene in the segment 308-460(Fig 6). In addition, only *Helianthus petiolaris* had 12 amino
273 acid sequence deletions in the segment 1524-1536 among four *Helianthus* species. There were 12
274 amino acid sequence deletions in the segment 1641-1653 of *Ageratina adenophora* and *Lactuca*
275 *sativa,* as well as in the segment 1641-1664 of *Guizotia abyssinica*. In addition, there were some
276 amino acid site differences. Ultimately, the was greatest similarity was observed between the *ycf2*
277 genes of Jerusalem artichoke and *Helianthus petiolaris subsp. fallax*, except for the presence of 5
278 additional amino acids in the initial site of *ycf2* in the Jerusalem artichoke plastome.
279
280 **Phylogenetic analysis**

To assess the phylogenetic relationships of Jerusalem artichoke, the chloroplast genomes of 15 species of the composite family were compared globally. *Jacobaea vulgaris* was taken as an outgroup, and then RAxML and Bayesian evolutionary trees were constructed respectively. The resulting phylogenetic trees constructed by the two methods shared the same topological structure (Fig 7). All species in the composite family formed three highly supported evolutionary clades: Members of the genus *Helianthus* are included in the first branch, including some *Helianthus annuus L.* species, subspecies and Jerusalem artichoke, as well as *Eupatorieae* and *Millerieae*. On the evolutionary branches of the genus *Helianthus*, Jerusalem artichoke and *Helianthus petiolaris subps. fanax* are in the closest relationship. The common node bootstrap is fully resolved. *Lactuca sativa* and *Taraxacum offcinale* of Crepidinae are contained in the second branch, while *Jacobea vulgaris* is clustered in Senecioninae alone,

Estimation of positive selection loci of the *ycf2* gene in eight species of the composite family

EasyCodeML v1.2 and paml X1.3 were used to calculate the logarithmic likelihood value (InL) and parameter evaluation for the complete sequence data set of the *ycf2* coding region of eight species in the composite family. In the locus model, $\omega > 1$ was allowed in the models M3 (discrete), M2a (selection) and M8 (beta & ω) to assume that the corresponding zero hypothetical models were the M1a (near neutral) model, M0 (one-ratio) model and M7 (beta) model. The M3, M2a and M8 models were significantly superior to their corresponding hypothetical models M0, M1a, M7 and M8a ($P < 0.01$), indicating that there were differences in the selection pressure among the points. After LRT testing, it was found that both M7 vs. M8 and M8a vs. M8 were more consistent with the analyzed data than their original hypothetical models (Table 5), and their original hypothetical models were rejected at a significant level of $P = 0.01$. A consistent positive selection locus, 1239N and 1518R, was found in models M2a and M8, respectively, at 95% and 99% levels calculated by Naïve Empirical Bayes (NEB) (Table 6). There was one positive selection locus 1518R in the M2a model and two positive selection loci 1239N and 1518R in the M8 model according to a Bayes Empirical Bayes analysis. Overall, the posterior probabilities of 1239N and 1518R in the NEB analysis of the M2a and M8 models were greater than 95% and 99%, respectively. Currently, this type of gene has substantial potential for application and diverse functions in the field of plant phylogeny according to the research progress of the chloroplast ycf gene family.

Discussion

The GC content of the Jerusalem artichoke IR region is high. This may be due to the fact that the IR region contained four high-GC rRNA genes (Asaf et al. 2016). High G-C content made conservatism in the IR regions higher than that in the large single-copy (LSC) and small single-copy (SSC) regions (Yang et al. 2014). The sequence and composition of chloroplast genes of Jerusalem artichoke were similar to those of other crops of the composite family (Curci et al. 2015). In addition, we compared the plastid genome and the chloroplast genome of the Jerusalem

artichoke. This comparison revealed that the plastid genome was 384 bp smaller than the chloroplast genome. We further refined the chloroplast genome of the Jerusalem artichoke via comparison with that produced by Bock et al. Fifteen differentially encoded genes were found in the published Jerusalem artichoke genome sequence(Bock et al. 2014). These differences may be due to the differences in sequencing depth and read length between these studies, as accuracy and length of sequences from the Illumina HiSeq 2000 is less than that from the Illumina HiSeq 2500 PE150, which has 100× depth. The 95× is more refined than the genome of the plastid genome, and depth of sequencing affects the number of detected genes, as well as the statistics and expression-related downstream analyses(Desai et al. 2013). A paired-end sequencing approach can also lead to differences in gene detection, as for the same number of reads, paired-end 2×150 bp reads contain more information than do paired-end 2×100 bp reads(Chaisson et al. 2009). In addition, we employed different genome assembly methods than did Bock et al., which may also result in differences in genome sequencing. In conclusion, a 384 bp difference in the conserved chloroplast genome may be a misjudgment as a consequence of the results of late cluster analysis studies, as we found that the overall difference in the chloroplasts of the Composite family ranged between 200 and 400 bp, so the results of this sequencing. These results are beneficial for future chloroplast genome evolution studies, and for research regarding the positive selection of genes. Based on these sequencing results, we were able to comprehensively analyze the characteristics of the Jerusalem artichoke chloroplast genome.

Introns play an important role in selective gene splicing. Because the chloroplast genome was simple, relatively conservative and maternal, chloroplast SSR were highly efficient molecular markers. Moreover, chloroplast simple sequence repeats (cpSSRs) have been widely used previously in crossbreeding, biogeography, and population genetics studies (Bayly et al. 2013). This is consistent with the chloroplast genomes of most angiosperms (Raveendar et al. 2015; Yang et al. 2014). In regards to repeat length, most SSR had 10-20 bp, while fewer had less than 10 bp, indicating the SSR segment of the Jerusalem artichoke chloroplast genome is short. However, the long repeated sequence might promote the rearrangement of the chloroplast genome, causing an increase in population genetic diversity (Qian et al. 2013). This may be related to the vegetative propagation of Jerusalem artichoke, which greatly reduces the probability of genetic variation. The SSR sites distributed in the non-coding region is the majority, while only 3 genes in the coding region have SSR sites, and there are few SSR sites in the coding region of the chloroplast genome, as has been confirmed in Quercus and Saxifragaceae (Liu et al. 2018; Yang et al. 2016). These repetitive structures provide valuable information resources for the future development of molecular markers in the study of the phylogenetic evolution and population genetics of Jerusalem artichoke.

The length variations of the chloroplast genomes of 8 species of the composite family correlated with the lengths of the IR regions, indicating the length of IR region had a significant effect on the length of genome (Guo et al. 2017). Comparative analysis of coding regions in the chloroplast

genome of plants in the composite family showed Jerusalem artichoke and *Helianthus petiolaris subsp. fallax* had the least differences. As a whole, the chloroplast genome of crops in the composite family tends to be conserved. mVISTA analysis showed the coding region was more conserved than the non-coding region, which is consistent with reports on crops in the composite family *such as Cynara cardunculus* (Curci et al. 2015) and *Ageratina adenophora* (Nie et al. 2012). The *ycf2* gene showed the greatest degree of differentiation. In addition, there was a gene deletion in the crops of genus *Helianthus.* At present, many different gene regions are considered potential tools for phylogenetic analysis. These DNA domains will play an important role in the application of molecular phylogeny in this species (Nie et al. 2012). The *ycf2* gene is the largest known plastid gene in angiosperms (Drescher et al. 2000b). Although the *ycf2* gene can be used to predict phylogenetic relationships(Drescher et al. 2000a), its function remains unclear. This suggests the *ycf2* gene is very conserved in the evolution of the species within the composite family. The *ycf2* gene appears to gradually degenerate compared in gramineous crops, with only 734 bp remaining in rice and wheat (Matsuoka et al. 2003). The results of phylogenetic tree analysis using partial angiosperm *ycf2* genes were consistent with those obtained from the whole plastid genome data phylogenetic tree analysis. This provides even more precise details for evolutionary evaluation.(Doorduin et al. 2011)

The composite family is one of the largest families in the plant kingdom, and the chloroplast genome plays an important role in plant classification and phylogenetic analysis. To date, abundant research has evaluated the phylogeny of crops in the composite family. Notably, study of the evolution of the *Aster spathulifolius* chloroplast genome has revealed it bears its closest relationship with *Jacobaea vulgaris* (Choi & Park 2015; HUANG et al. 2010; SOLTIS et al. 2000), which is consistent with previous reports on the uncertainty of the evolution of the Senecioninae tribe (Doorduin et al. 2011). In the group of the composite in which the number of involved species more than or equal to 2, it can be seen that genetic relationship of Jerusalem artichoke is more closely to other species of composite family, genus *Helianthus*. At the same time, Jerusalem artichoke is also the earliest isolated species of the genus *Helianthus*. This provides a theoretical basis for the further study of the relationship between phylogenetic branches of Jerusalem artichoke in the composite family.

The *ycf2* gene fragment is large, and the function of its open reading frame (ORF) fragment is not clear. Compared with other chloroplast coding genes, the nucleotide sequence similarity between *ycf2* of different families is very low, which is less than 50% in bryophytes, pteridophytes and spermatophytes (Wicke et al. 2011). In the increasing number of ycf gene studies, although *ycf2* is highly conserved, the *ycf2* gene shows a wealth of phylogenetic information in Orchidaceae phylogeny. Huang et al. found that the *ycf2* gene has multiple positive selection loci during angiosperm development, and the phylogenetic signal of *ycf2* probably originates from its large sequence length, so that the *ycf2* gene is valuable for future research (Huang et al. 2010). Most chloroplast genes were in a negative selection state in Holcoglossum, but 14 positive selection loci

were detected in the *ycf2* gene (Li et al. 2019). In this study, some positive selection signals were found by establishing evolutionary trees of the adaptive evolution of the *ycf2* gene in the composite family, but the loci were few, which may be related to the number of species. Plants may have a variety of strategies to adapt to the environment, and adaptive modifications to other abiotic stresses of genes in the nucleus are sufficient to maintain the homeostasis of photosynthesis. Therefore, there is no need for adaptive evolution in the chloroplast coding genes (Dolhi et al. 2013; Hirooka et al. 2017; Wang et al. 2019). In this study, research on the *ycf2* gene in the composite family supports the idea of adaptive evolution, but there are currently few studies on adaptive evolution in Compositae crops. Therefore, further studies on the adaptive evolution of chloroplast genes in other species of the composite family are needed in order to explore how to adapt to these changes in environmental migration and climate change.

Conclusions

In this study, the complete chloroplast genome sequence of Jerusalem artichoke was successfully assembled, annotated and analyzed. The chloroplast genome of plants in the composite family is relatively conservative. Variations of the chloroplast genome are scarce between Jerusalem artichoke and plants in the same genus. Compared with composite plants belonging to other genera, we found deletions in the chloroplast genome of Jerusalem artichoke. The identification of repetitive sequences in the chloroplast genome of Jerusalem artichoke, especially SSR, will be helpful for the development of molecular markers, the study of population genetics and the phylogenetic analysis of Jerusalem artichoke. Phylogenetic analysis of plants in the composite family shows Jerusalem artichoke and Helianthus petiolaris subsp. fallax share the closest relationship, both belonging to the composite family, genus Helianthus. The results of this study indicate that the full-length coding region of the ycf2 gene has the potential to be used as a site for the reconstruction of phylogenetic relationships in the composite family, and it is suggested that more extensive investigation and in-depth discussion should be conducted in future studies. Completion of the sequencing of the chloroplast genome will provide key genetic information for further research on Jerusalem artichoke and deepen our understanding on the evolutionary history of the chloroplast genome and phylogenetic position of Jerusalem artichoke. In addition, it may be useful for various molecular biology applications of Jerusalem artichoke in the future.

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Table 1(on next page)

Table 1 List of genes in the chloroplast genome of *Helianthus tuberosus* L.

1 **Table 1 List of genes in the chloroplast genome of *Helianthus tuberosus* L.**

	Groups of genes	***Names of genes***
Protein synthesis and DNA replication	Ribosomal RNAs	*16S r RNA(2×), 23S r RNA(2×), 4.5S r RNA(2×), 5S r RNA(2×)*
	Transfer RNAs	*trnQ-TTG, trnL-TAG, trnD-GTC, trnS-GGA ,trnE-TTC, trnS-GCT, trnY-GTA, trnV-GAC, trnP-TGG, trnH-GTG, trnF-GAA, trnN-GTT, trnT-TGT, trnW-CCA, trnS-TGA, trnV-GAC, trnL-CAA(2×), trnM-CAT(2×), trnC-GCA, trnI-CAT, trnT-GGT, trnI-CAT, trnR-ACG, trnN-GTT, trnR-TCT, trnR-ACG, trnG-GCC*
	Ribosomal protein small subunit	*rps7, rps14,rps12, rps2, rps4, rps12, rps7, rps11, rps16, rps12, rps19(2×), rps3, rps15, rps8, rps19*
	Ribosomal protein large subunit	*rpl14, rpl23, rpl36, rpl2, rpl20, rpl2, rpl32, rpl16, rpl33, rpl23, rpl22*
	Subunit s of RNA polymerase	*rpoB, rpoC(2×), rpoA,*
Photosynthesis	Photosystem I	*psaC, psaA, psaB, psaI, psaJ*
	Photosystem II	*psbZ, psbK, psbB, psbI, psbF, psbN, psbL, psbJ, psbC, psbE, psbM, psbH, psbA, psbD, psbT,*
	Cytochrome b/f complex	*petA, petD, petL, petB, petG, petN*
	ATP synthase	*atpE, atpH, atpA, atpI, atpF, atpB*
	NADH-dehydrogenase	*ndhJ, ndhA, ndhK(2×), ndhG, ndhI, ndhB(2×), ndhH, ndhE, ndhD, ndhC, ndhF,*
	Large subunit Rubisco	*rbcL*
Miscellaneous group	Translation initiation factor IF-1	*infA*
	Acetyl-CoA carboxylase	*accD*
	Cytochrome c biogenesis	*ccsA(2×)*

	Maturase	*matK*
	ATP-dependent protease	*clpP*
	Inner membrane protein	*cemA*
Pseudogenes of unknown function	Conserved hypothetical chloroplast open reading frame	*ycf15(4×), ycf4, ycf3, ycf1(2×), ycf2(2×)*

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Table 2(on next page)

Table 2 Characteristics of genes including introns and exons in the chloroplast genome of *Helianthus tuberosus* L.

Table 2 Characteristics of genes including introns and exons in the chloroplast genome of *Helianthus tuberosus* L.

Gene	Region	Exon I (bp)	Intron I (bp)	Exon II (bp)	Intron II (bp)	Exon III (bp)
*trn*K-UUU	LSC	51	2528	36		
*rps*16	LSC	29	864	226		
*rpo*C1	LSC	431	733	1727		
*atp*F	LSC	144	714	391		
*ycf*3	LSC	152	746	229	700	123
*trn*L-UAA	LSC	36	436	49		
*trn*V-UAC	LSC	36	574	37		
*clp*P	LSC	68	792	290	624	227
*pet*B	LSC	5	775	641		
*pet*D	LSC	8	712	473		
*rpl*2	LSC	392	663	434		
*ndh*B	IR	755	671	776		
*trn*I-GAU	IR	41	776	34		
*trn*A-UGC	IR	37	822	34		
ndhA	SSC	552	1095	538		
rps12	LSC-IR	113		230		29

Table 3(on next page)

Table 3 Comparison of chloroplast and plastid differential genes in *Helianthus tuberosus* L.

1 **Table 3 Comparison of chloroplast and plastid differential genes in *Helianthus tuberosus* L.**

Gene	*NCBI*	*Difference site*				*Difference position and base*
	Accession	*T*	*C*	*A*	*G*	
ccsA	MG696658	36.8	15.6	31.6	16.0	
	NC023112	36.9	15.5	31.6	16.0	579T
atpB	MG696658	36.8	15.6	31.6	16.0	
	NC023112	36.9	15.5	31.6	16.0	348G
clpP	MG696658	28.9	18.0	28.6	24.5	361-363null
	NC023112	29.1	18.1	28.3	24.5	362G/363C/70,361 T
ndhB	MG696658	34.7	19.6	27.6	18.0	
	NC023112	34.8	19.5	27.9	17.8	778-819null
ndhH	MG696658	31.0	15.2	30.9	22.9	
	NC023112	30.9	15.2	30.9	23.0	822G
ndhI	MG696658	34.1	16.2	31.5	18.2	
	NC023112	33.9	16.4	31.5	18.2	433C
petA	MG696658	28.9	19.3	30.8	21.0	
	NC023112	28.9	19.3	30.7	21.1	705G
petD	MG696658	32.9	19.0	27.5	20.5	
	NC023112	32.9	19.0	27.7	20.3	9A
rpl2	MG696658	22.9	18.2	33.5	25.4	
	NC023112	22.9	18.3	33.5	25.3	392-394null
rpoC1	MG696658	30.0	16.9	32.4	20.7	2-22null
	NC023112	30.0	16.9	32.4	20.7	4,5,8,10,11,22A/3,6,9,12,G/7,17,20,C / 2,13,14,15,16,18,19,21T.
rpoC2	MG696658	29.4	17.9	32.5	20.2	
	NC023112	29.4	17.9	32.6	20.2	
rps12	MG696658	23.7	21.3	33.1	21.9	347 null
	NC023112	24.6	21.6	30.8	23.0	346,356A/347,349,351,354G,352T/ 358-376 null
rps16	MG696658	28.5	17.2	33.0	21.3	
	NC023112	28.6	16.5	33.7	21.2	43-54null

ycf1	MG696658	30.6	14.2	39.6	15.6	
	NC023112	30.6	14.2	39.7	15.5	1A. 2-4 null
ycf2	MG696658	31.1	18.5	31.2	19.2	
	NC023112	31.1	18.5	31.2	19.1	4562-4597null

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Table 4(on next page)

Table 4 Comparison of cp genomes among 8 composite species

1 **Table 4 Comparison of cp genomes among 8 composite species**

Species	Size(bp)				G+C(%)	Total number of genes			GeneBank accessions
	Total	LSC	IR	SSC		Protein-coding genes	rRNAs	tRNAs	
Carthamus tinctorius	153675	83606	25407	19156	37.4	89	4	30	KX822074
Ageratina adenophora	150689	84815	23755	18358	37.5	80	4	28	JF826503
Guizotia abyssinica	150689	82855	24777	18277	37.3	79	4	29	HQ234669
Lactuca sativa	152772	84105	25034	18599	37.5	78	4	20	DQ383816
Helianthus tuberosus	151431	83981	24568	18279	37.6	84	4	27	MG696658
Helianthus argophyllus	151862	83845	24588	18149	37.6	80	4	27	KU314500
Helianthus debilis	151678	83799	24502	18121	37.6	82	4	27	KU312928
Helianthus petiolaris subsp. fallax	151104	83530	24633	18308	37.6	79	4	27	KU295560

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Table 5(on next page)

Table 5 Likelihood ratio statistics of positive selection models against their null models (2Δ ln L)

Table 5 Likelihood ratio statistics of positive selection models against their null models (2Δ ln L)

Comparison between models	2Δ ln L	d.f.	P-value
M0 vs. M3	15.2245	4	0.0043 <0.01
M1a vs. M2a	13.5353	2	0.0012 <0.01
M7 vs. M8	15.0177	2	0.0005 <0.01
M8a vs. M8	13.5241	1	0.0002 <0.01

Table 6(on next page)

Table 6 Positive selective amino acid loci and parameter estimation in *ycf2* of 8 species in the compositae family species.

Table 6 Positive selective amino acid loci and parameter estimation in *ycf2* of 8 species in the compositae family species.

Models	*Np*	*lnL*	*Estimates of parameters*	*Positive sites (NEB)*	*Positive sites (BEB)*
M0(one-ratio)	15	-9464.31	ω=0.93903	Not allowed	Not allowed
M3(Discrete)	19	-9456.70	p_0=0.00005, ω_0=0.07668 p_1=0.99613, ω_1=0.86440 p_2=0.00382, ω_2=43.87141	1125W 0.602 1238G 0.779 1239N 0.980* 1476F 0.649 1518R 0.992**	Not allowed
M1a(Near neutral)	16	-9463.47	p_0=0.20671, ω_0=0 p_1=0.79329, ω_1=1	Not allowed	Not allowed
M2a(Selection)	18	-9456.70	p_0=0.98950, ω_0=0.86336 p_1=0.00668, ω_1=1 p_2=0.00382, ω_2=43.84482	1125W 0.602 1238G 0.779 1239N 0.980* 1476F 0.649 1518R 0.992**	331I 0.726 662K 0.727 1125W 0.677 1238G 0.770 1239N 0.940 1476F 0.759 1518R 0.950*
M7(beta)	16	-9464.36	p=0.50360, q=0.00500	Not allowed	Not allowed
M8(beta & ω)	18	-9460.27	p_0=0.66725, p=0.00500 p_1=0.33275, q=1.20677 ω=2.95373	1125W 0.600 1238G 0.778 1239N 0.980* 1476F 0.647 1518R 0.991**	331I 0.882 662K 0.823 1095S 0.526 1125W 0.774 1238G 0.851 1239N 0.965* 1476F 0.844 1518R 0.971*
M8a(beta	17	-9463.50	p_0=0.21119,	Not allowed	Not allowed

& ω =1)	p=3.03780 p_1=0.78881, q=1.57211 ω=1

Positively selected sites (*: P>95%; **: P>99%)

Figure 1

Figure 1 Gene map of the *Helianthus tuberosus* L. chloroplast genome.

Genes drawn outside of the circle are transcribed counter-clockwise, while genes shown on the inside of the circle are transcribed clockwise. Genes belonging to different functional groups are color-coded. The darker gray in the inner circle indicates GC content, while the lighter gray corresponds to AT content.

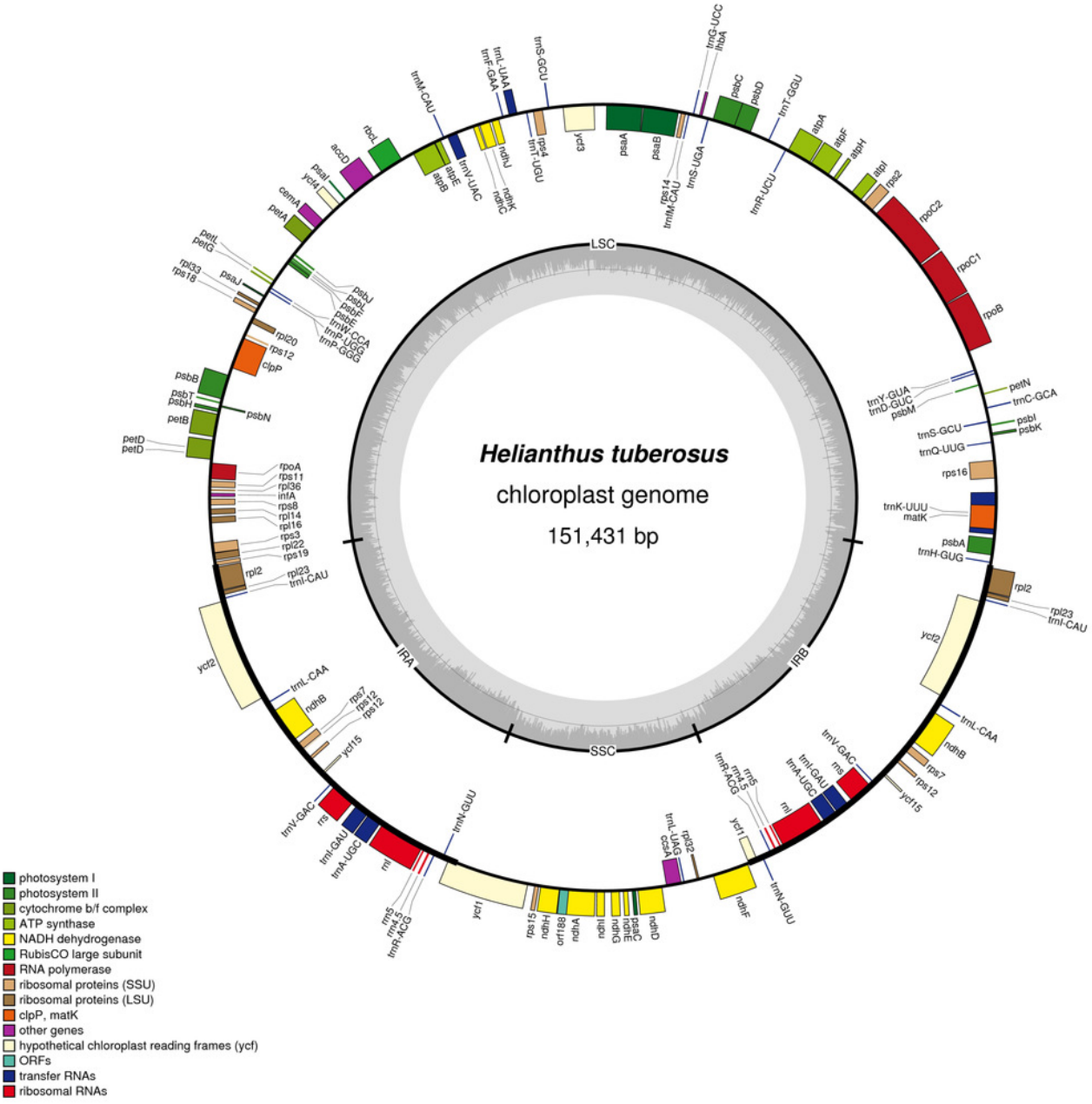
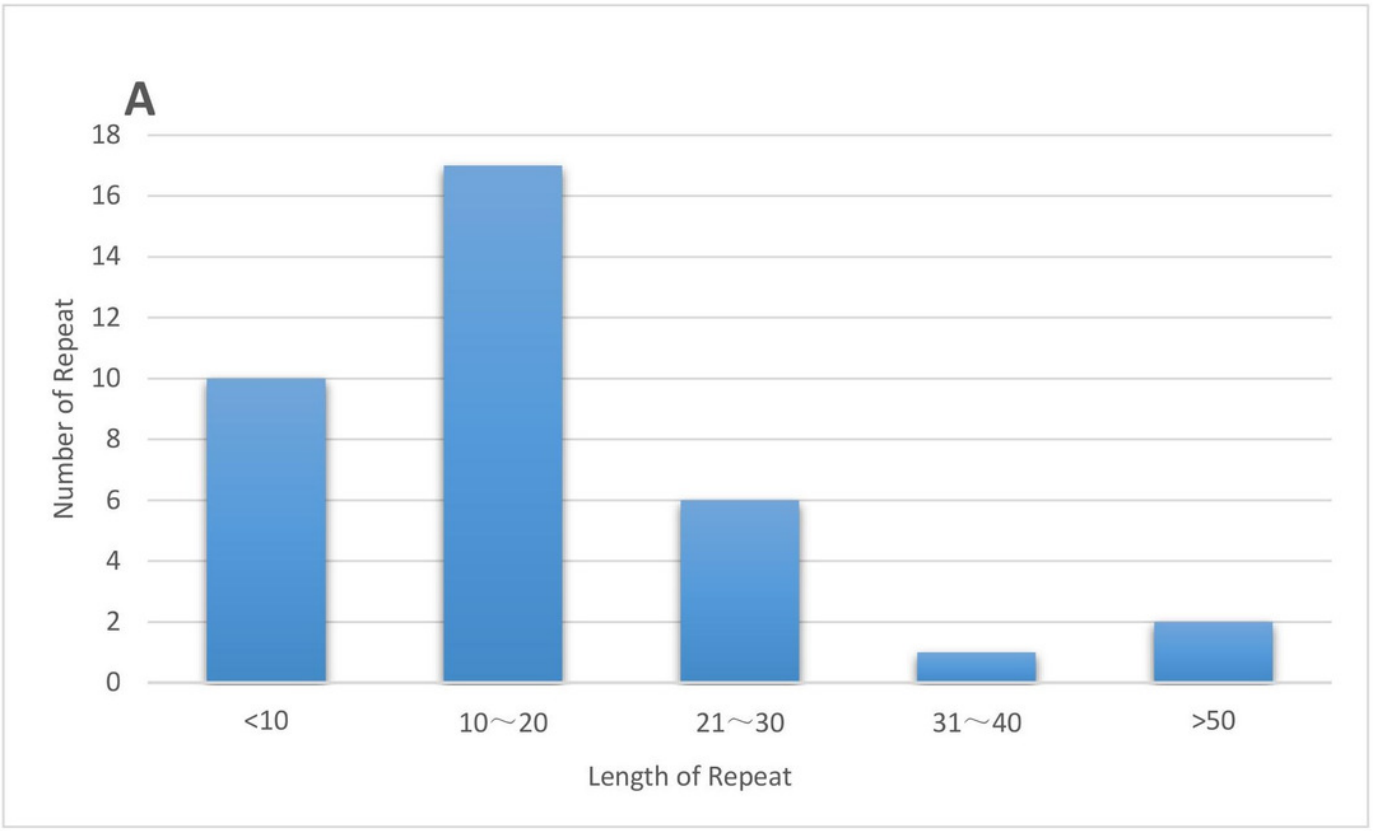

Helianthus tuberosus
chloroplast genome
151,431 bp
LSC
IRA
IRB
SSC
photosystem I
photosystem II
cytochrome b/f complex
ATP synthase
NADH dehydrogenase
RubisCO large subunit
RNA polymerase
ribosomal proteins (SSU)
ribosomal proteins (LSU)
clpP, matK
other genes
hypothetical chloroplast reading frames (ycf)
ORFs
transfer RNAs
ribosomal RNAs

Figure 2

Figure 2 Distribution frequency in *Helianthus tuberosus* L. cp genome.

A: The frequency of repeats, length of repeats; Number of repeats. B: The percentage distribution of gene area.


A
18
16
14
12
10
8
6
4
2
0
Number of Repeat
<10
10～20
21～30
31～40
>50
Length of Repeat

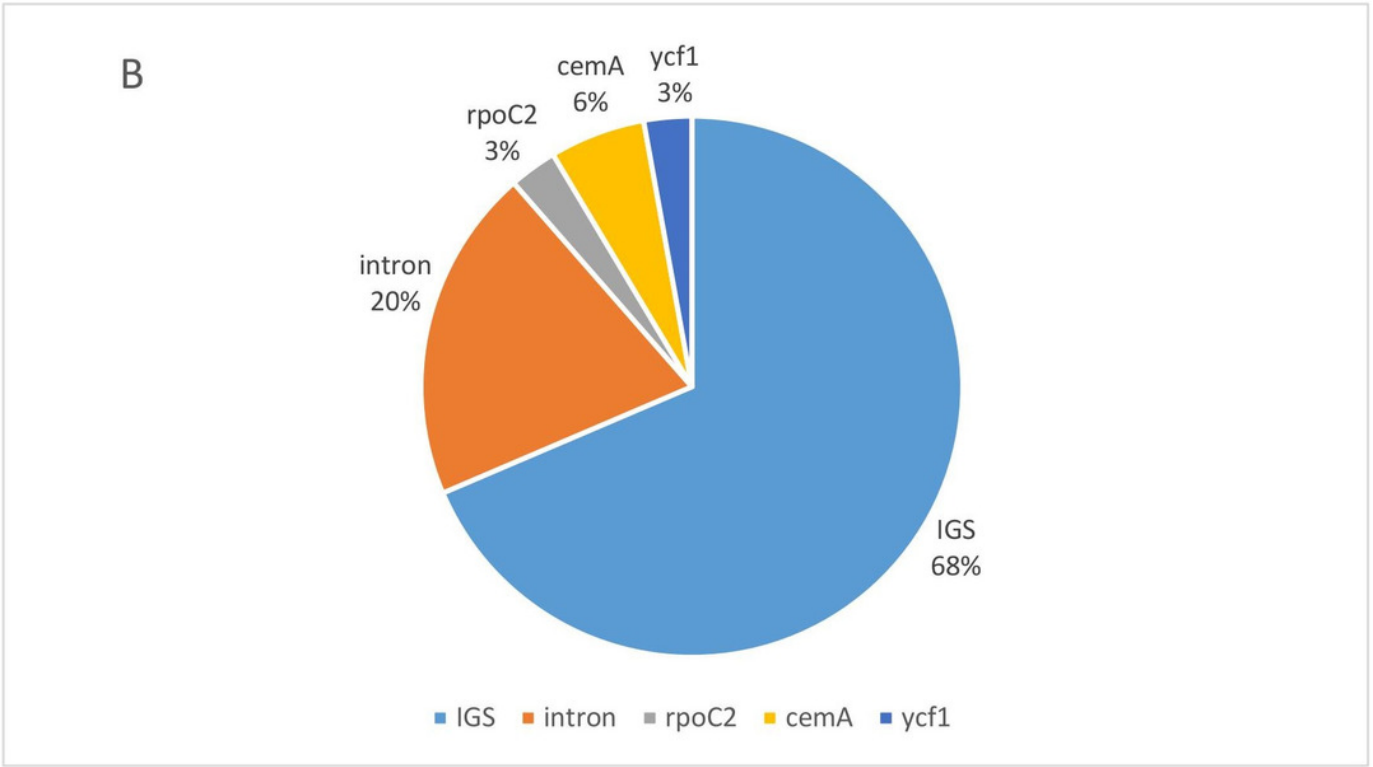

B
cemA
6%
ycf1
3%
rpoC2
3%
intron
20%
IGS
68%
IGS
intron
rpoC2
cemA
ycf1

Figure 3

Figure 3 Compared with Helianthus tuberosus L. chloroplast and plastid genome use BRIG

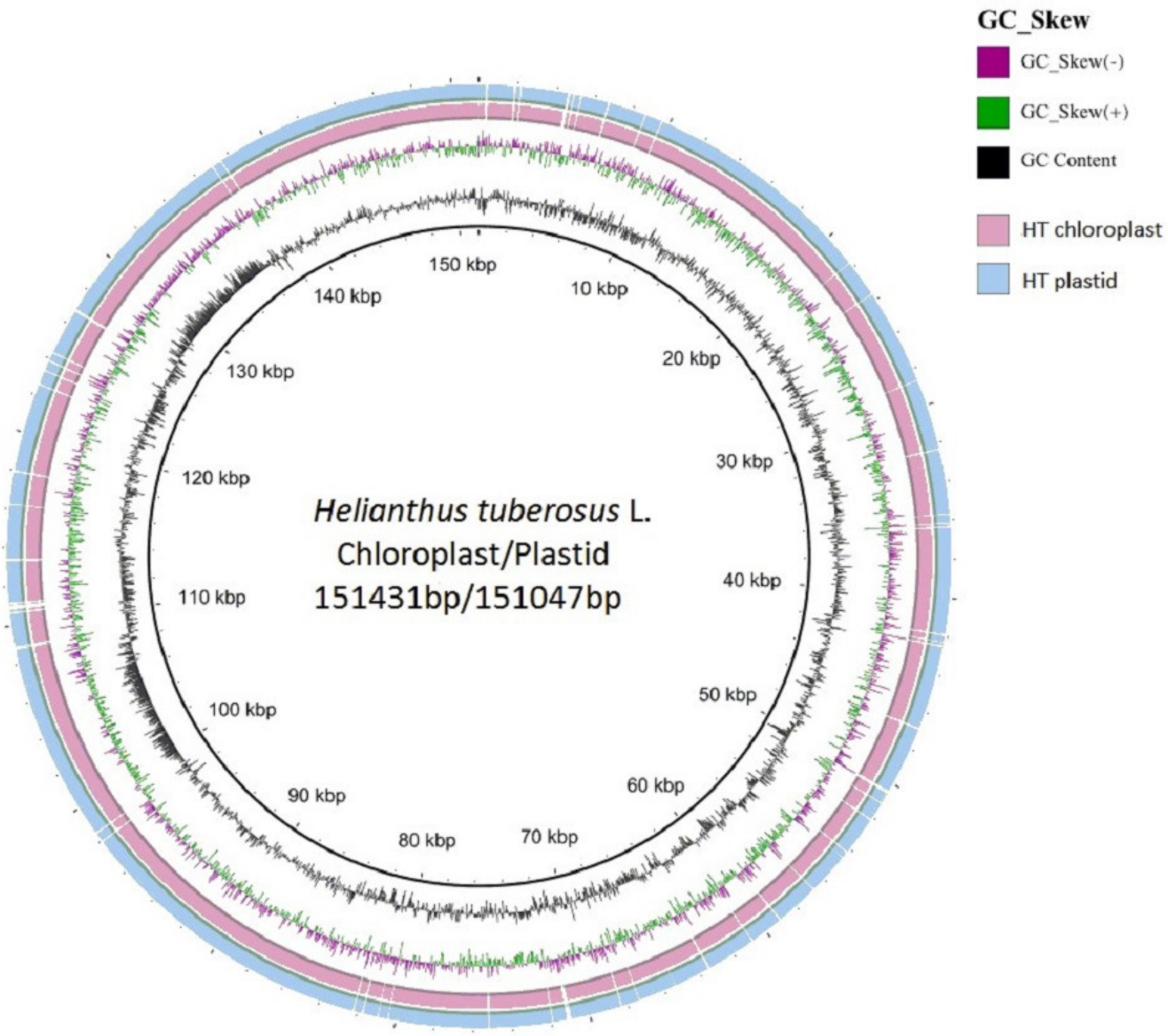


Figure 4

Figure 4 Percent identity plot for the comparison of 8 composite chloroplast genomes.

The whole chloroplast genome was divided into four parts, and the gene names are displayed in sequence on the top line of each part (arrows indicate the transcriptional direction). The sequence similarity of the alignment region of Jerusalem artichoke and seven other species is shown as the filling color in each black stripe. The x-axis indicates the position of the chloroplast genome at a certain site, and the y-axis indicates the average sequence identity percentage (50-100%) with Jerusalem artichoke on the position of a species at a certain position (50-100%). The coding sequences (exons), rRNA, tRNA and the conserved non-coding sequences (CNS) in the genomic region are represented with different colors.

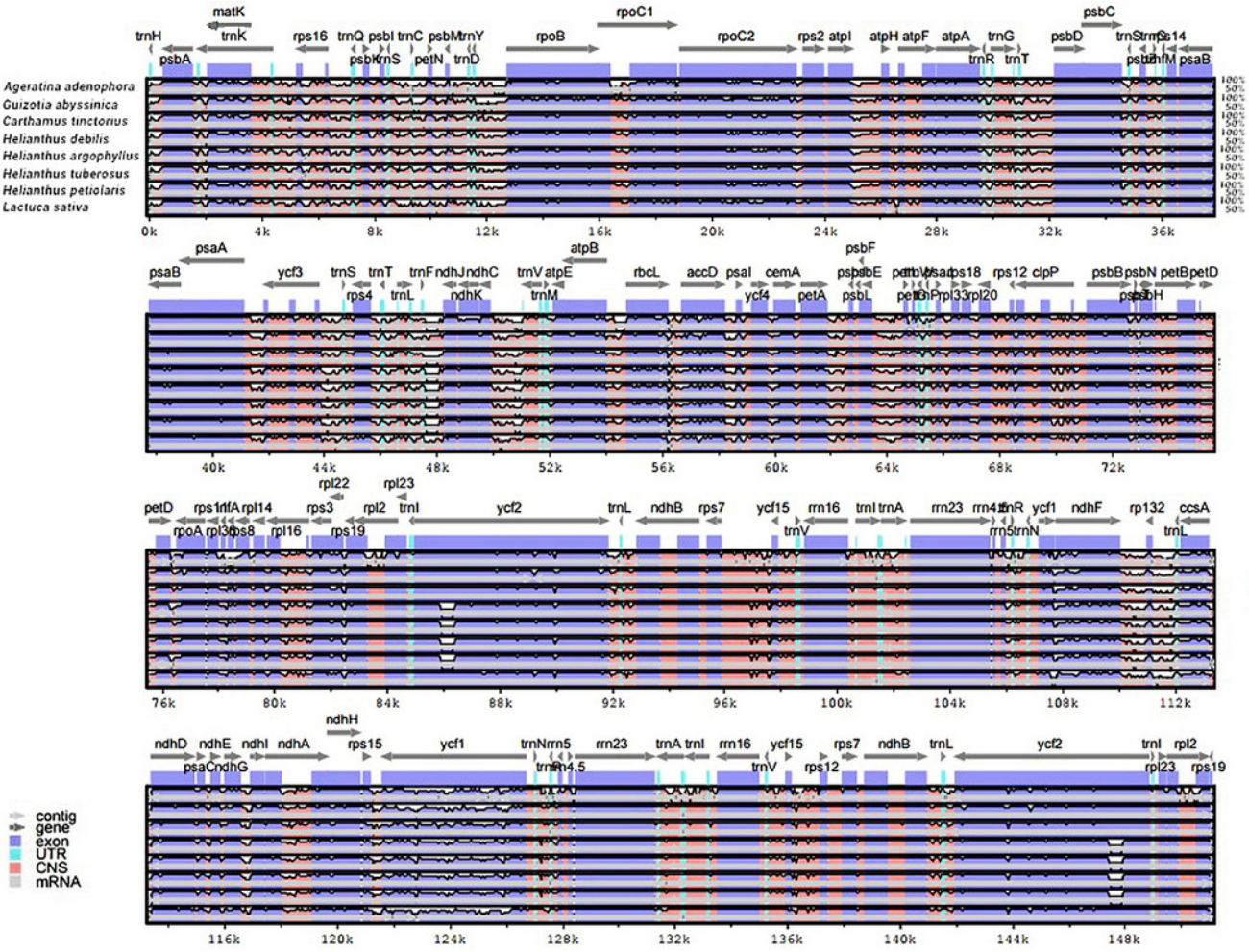

Ageratina adenophora
Guizotia abyssinica
Carthamus tinctorius
Helianthus debilis
Helianthus argophyllus
Helianthus tuberosus
Helianthus petiolaris
Lactuca sativa
contig
gene
exon
UTR
CNS
mRNA

Figure 5

Figure 5. Comparison of the similarity of chloroplast genomes between Jerusalem artichoke and seven other species of crops in the composite family.

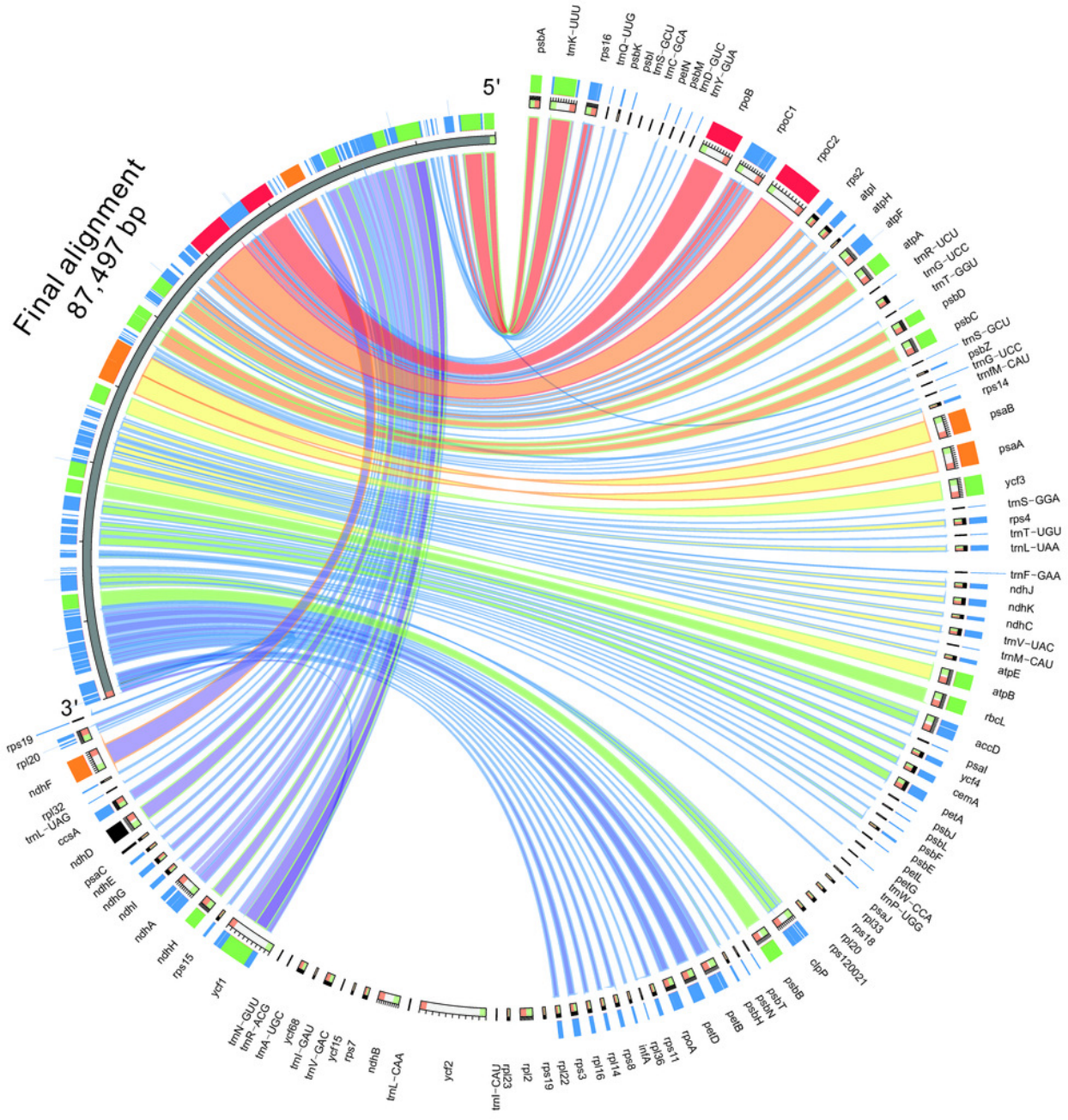


Figure 6

Figure 6. Comparison of the *ycf2* gene sequence in chloroplast genomes between Jerusalem artichoke and seven other species of crops in the composite family.

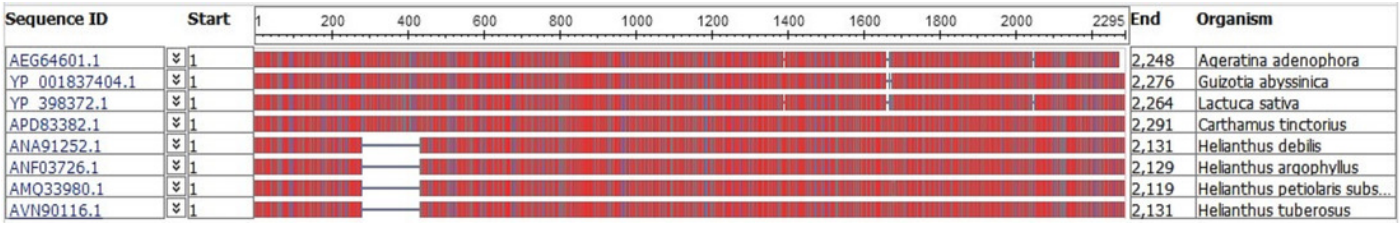


Figure 7

Figure 7. Molecular phylogenetic tree of 16 composite species based on a neighbor joining analysis.

Numbers above and below nodes are bootstrap support values 50%.