

# Phylogenetic significance of the characteristics of simple sequence repeats at the genus level based on the complete chloroplast genome sequences of Cyatheaceae

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March 16, 2021

## Abstract

Recent taxonomic and molecular phylogenetic studies have shown that *Gymnosphaera* should be recognized as an independent taxonomic unit at the genus level under the family Cyatheaceae. In this study, the complete chloroplast genomes of the eight species of Cyatheaceae were sequenced, and their phylogenetic relationships were reconstructed using the maximum likelihood, Bayesian inference, maximum parsimony, and neighbor-joining methods, and the characteristics of their simple sequence repeats (SSRs) were compared and analyzed for the first time. The results showed that when Cyatheaceae was divided into three genera, the number, relative abundance, relative density, and GC content of all SSRs and of SSRs of certain unit lengths in the chloroplast genomes of the eight species of Cyatheaceae were genus specific in the whole chloroplast genomes and in their different regions (large single-copy, small single-copy, inverted repeat, intergenic spacer, intron, rRNA gene, and coding sequence regions). The SSRs overall and the single-nucleotide SSRs had significant differences in number, relative abundance, relative density, and GC content between the chloroplast genomes, their intergenic regions, and large single-copy regions. When Cyatheaceae was divided into two genera, only the difference in GC content was significant. Therefore, our results support the restoration of the hierarchical status of *Gymnosphaera*. This study provides an important basis for the identification of the phylogenetic relationship of Cyatheaceae plants.

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**Abstract** Recent taxonomic and molecular phylogenetic studies have shown that *Gymnosphaera* should be recognized as an independent taxonomic unit at the genus level under the family Cyatheaceae. In this

study, the complete chloroplast genomes of the eight species of Cyatheaceae were sequenced, and their phylogenetic relationships were reconstructed using the maximum likelihood, Bayesian inference, maximum parsimony, and neighbor-joining methods, and the characteristics of their simple sequence repeats (SSRs) were compared and analyzed for the first time. The results showed that when Cyatheaceae was divided into three genera, the number, relative abundance, relative density, and GC content of all SSRs and of SSRs of certain unit lengths in the chloroplast genomes of the eight species of Cyatheaceae were genus specific in the whole chloroplast genomes and in their different regions (large single-copy, small single-copy, inverted repeat, intergenic spacer, intron, rRNA gene, and coding sequence regions). The SSRs overall and the single-nucleotide SSRs had significant differences in number, relative abundance, relative density, and GC content between the chloroplast genomes, their intergenic regions, and large single-copy regions. When Cyatheaceae was divided into two genera, only the difference in GC content was significant. Therefore, our results support the restoration of the hierarchical status of *Gymnosphaera*. This study provides an important basis for the identification of the phylogenetic relationship of Cyatheaceae plants.

**Keywords** Cyatheaceae, Phylogeny, SSR, *Gymnosphaera*

Also known as microsatellites, simple sequence repeats (SSRs) are short tandem repeat sequences with a motif length of 1-6 bp that are characterized by high variability, high repeatability, codominant inheritance, and interspecies generality. SSRs have been widely used in species identification, genetic diversity, and phylogenetic relationships (Chmielewski et al., 2015; Dashnow et al., 2015). SSRs are caused by slipped strand mispairing and subsequent errors during DNA replication, repair, and recombination (Levinson & Gutman, 1987). They are widespread in the genomes of prokaryotes, eukaryotes, and some viruses (Mrazek et al., 2007; Toth et al., 2000; Zhao et al., 2011). SSRs are mainly found in intergenic and noncoding regions, with a few in introns (Toth et al., 2000; Li et al., 2004). Under selection, the number of genomic SSRs is significantly higher than that under random accumulation (Ellegren, 2004). The characteristics of genomic SSRs in different taxa (such as their distribution pattern) accurately reflect their phylogenetic relationships (Srivastava et al., 2019). SSRs are highly mutagenic and are an important source of genetic evolution, and they can play an important role in genome evolution by generating and maintaining quantitative genetic variation (Chaoui et al., 2012; Gemayel et al., 2010; Kashi & King, 2006). Therefore, the identification and characterization of SSRs can contribute to the study of population variation, germline identification, and genetic diversity.

Cyatheaceae belong to the phylum Pteridophyta, the class Polypodiopsida, and the order Cyatheales, with more than 600 species present today. Cyatheaceae account for the vast majority of known tree ferns and are mainly distributed in warm and humid tropical and subtropical regions (Kramer, 1990; Korall et al., 2006; Smith et al., 2006; PPG I, 2016). The classification of the genera in Cyatheaceae has always been a difficult problem. In the early classification system, Christensen (1906) classified Cyatheaceae into *Cyathea* Sm. (with a complete cup-shaped indusium), *Hemitelia* R. Br. (with a partial indusium), and *Alsophila* R. Br. (without an indusium) based on the characteristics of indusium. Holttum (1963) treated Cyatheaceae plants as a genus (*Cyathea*) and divided it into two subgenera, *Cyathea* subg. *Cyathea* (marginate scales) and *Cyathea* subg. *Sphaopteris* (Bernh.) Holttum (conform scales), based on the structural characteristics of the scales at the base of the petiole. Based on the study of the American Cyatheaceae plants, Tryon (1970) delineated three evolutionary paths and six genera, namely, (1) *Sphaopteris* Bernh. (with conform scales); (2) *Alsophila* and *Nephrolepis* R. M. Tryon (with marginate scales and apical setae); and (3) *Trichipteris* C. Presl, *Cyathea* and *Cnemidaria* C. Presl (with marginate scales but without apical setae). Ching (1978) classified the Cyatheaceae plants that grow in China into three genera: *Sphaopteris*, *Gymnosphaera* and *Alsophila*. On this basis, Xia (1989) reduced the genera *Alsophila* and *Alsophila* to subgenera, respectively, and combined them into *Alsophila*.

Recent molecular phylogenetic studies have shown that Cyatheaceae includes four monophyletic groups, namely, *Alsophila*, *Cyathea*, *Gymnosphaera*, and *Sphaopteris* (Dong & Zuo, 2018; Janssen & Rako-tondrainibe, 2008; Korall et al., 2007; Korall & Pryer, 2014). The morphological characteristics of *Gymnosphaera*, such as marginate scales, a slightly black rachis, and the lack of an indusium, and molecu-

lar evidence suggest that it is different from other Cyatheaceae plants, and particularly obvious is its different sporogenesis from *Alsophila* (*Gymnosphaera* produces 64 spores per sac, *Alsophila* produces 16 spores per sac) (Dong & Zuo, 2018). Still, some scholars classify *Gymnosphaera* into the genus *Alsophila* (Xia 1989; Zhang and Nishida, 2013; PPG I, 2016).

In this study, the chloroplast genomes of eight species of Cyatheaceae were sequenced, and the chloroplast genome, the SSRs of different unit lengths in the chloroplast genome, and the number, relative abundance, relative density, and GC content of all SSRs and SSRs of specific unit lengths in the intergenic spacer regions (IGS), intron regions, and protein-coding sequence regions (CDS) of the chloroplast genome were compared and analyzed to reveal the phylogenetic significance of the SSR characteristics of the chloroplast genome of Cyatheaceae plants.

## 1 Materials and methods

### 1.1 Sampling

The leaves of *Alsophila denticulata* Baker and *Alsophila metteniana* Hance were collected from Nankunshan in Huizhou and the botanical garden of South China Agricultural University in Guangzhou, respectively. The leaves of *Alsophila podophylla* Hook and *Alsophila gigantea* Wall. ex Hook were collected from the South China Botanical Garden of the Chinese Academy of Sciences in Guangzhou (Liu et al., 2018; Wang et al., 2019b). The leaves of *Alsophila costularis* Baker, *Sphaeropteris brunoniana* (Hook.) R. M. Tryon, and *Sphaeropteris lepifera* (Hook.) R. M. Tryon were collected from the Fairy Lake Botanical Garden of the Chinese Academy of Sciences in Shenzhen (Wang et al., 2019a; Liu et al., 2020; Zhu et al., 2020). The leaves of *Alsophila spinulosa* (Wall. ex Hook.) R. M. Tryon were collected from the Wuhan Botanical Garden of the Chinese Academy of Sciences in Wuhan (Gao et al., 2009). Fresh young leaves from well-grown plants were collected, wrapped in tin paper, flash-frozen in liquid nitrogen, and then stored at -80 °C before use.

### 1.2 DNA extraction and sequencing

The plant genomic DNA extraction kit (TIANGEN) was used to extract the total DNA of the samples. After the quality of the total DNA samples was confirmed by Shanghai Hanyu Biotechnology Co., Ltd., the samples were subjected to bidirectional sequencing using an Illumina HiSeq 2500, and the raw data obtained were converted into raw reads by CASAVA base-calling analysis. The clean data obtained after removing the adaptor-containing, low-quality sequences were taken for subsequent analysis. Data processing was performed by Trimmomatic v0.32 (Bolger et al., 2014) with the following steps: (1) removal of sequences containing N bases; (2) removal of adaptor sequences in the reads; (3) removal of low-quality bases (Q value < 20) from the reads in the 3' to 5' direction; (4) removal of low-quality bases (Q value < 20) from the reads in the 5' to 3' direction; (5) removal of four bases with an average base quality of less than 20; and (6) removal of the reads and their pairs with a length less than 50 nt. Velvet v1.2.03 (Zerbino & Birney, 2008) was used to assemble the clean data.

### 1.3 Characterization of chloroplast genome

The chloroplast genome of *Alsophila* was used as the reference genome, and Dual Organellar GenoMe Annotator (DOGMA) (Milne et al., 2010) was used to predict the protein-coding genes, rRNA genes, and tRNA genes in other genomes. Geneious Prime (Kearse et al., 2012) was used for manual correction according to the reference genome. The Shuffle-Lagan mode in the online software mVISTA (Frazer et al., 2004) was used for genome-wide comparison. Organellar Genome DRAW (OGDRAW) (Lohse et al., 2007) was used to draw the physical chloroplast genome map, and Sequin software was used for the submission of the chloroplast genome of *Alsophila denticulata*. Microsatellite repeats were predicted using the software MISA (Beier et al., 2017). The threshold repeat number of single-nucleotide units was set to 10, the threshold repeat number of dinucleotide units was set to six, and the threshold repeat number of trinucleotides, tetranucleotides, pentanucleotides, and hexanucleotides was set to three. The minimum distance between two SSRs was set to 0 bp, that is, there was no statistical compound SSR. The distribution characteristics of SSRs of different species in the whole genome and its different regions were compared and analyzed. Among these character-

istics, the relative abundance refers to the number of SSRs in the unit sequence length (kb), and the relative density refers to the length of the SSR (bp) in the unit sequence length (kb).

## 1.4 Phylogenetic analysis

The maximum likelihood (ML), Bayesian inference (BI), maximum parsimony (MP) and neighbor-joining (NJ) methods were used for phylogenetic analysis. MAFFT software (Katoh & Standley, 2013) was used to align the complete chloroplast genome sequences of eight species of Cyatheaceae and one species of *Cibotium*, *Cibotium barometz* (Linn.) J. Sm. A phylogenetic tree was constructed using *Cibotium barometz* (Linn.) J. Sm. as an outgroup. When the ML, MP, and BI trees had been constructed, the whole chloroplast genome was screened in MrModelTest software to obtain the optimal nucleotide substitution model (GTR+I+G) selected based on the Akaike information criterion, and the relevant parameters were estimated. The ML tree was constructed by the software RAxML8.0.20 (Stamatakis, 2014), GTRGAMMAI was selected as the nucleotide substitution model, and the confidence of the branch was completed using the bootstrap analysis in autoMR. The BI tree was constructed by MrBayes v3.2.0 software (Ronquist et al., 2012) and was estimated by running 2,000,000 generations (Nst = 6, rates = invgamma). The MP tree was constructed in PAUP 4.0 software (Swofford, 2002) with the bootstrap value set to 1000. The NJ tree was constructed in MEGA7.0 software (Kumar et al., 2016), and the maximum composite likelihood algorithm was selected with the bootstrap value set to 1000 times. The resulting phylogenetic tree was viewed and edited in Figtree v 1.4.3 software.

## 1.5 Statistical analysis

When *Gymnosphaera* is considered as an independent taxonomic unit at the genus level, the eight species of Cyatheaceae are divided into three genera. That is, *Alsophila denticulata*, *Alsophilapodophylla*, *Alsophila metteniana*, and *Alsophila gigantea* belong to the genus *Gymnosphaera*; *Alsophila spinulosa* and *Alsophila costularis* belong to the genus *Alsophila*; and *Sphaopteris brunoniana* and *Sphaopteris lepifera* belong to the genus *Sphaopteris*. When *Gymnosphaera* is classified into the genus *Alsophila*, Cyatheaceae is divided into two genera. The Kruskal-Wallis H test and Mann-Whitney U test in IBM SPSS v22.0 software (Allen et al., 2014) were used to analyze the significance of the differences between taxa when three genera and two genera were assumed, respectively. The statistical results covered the whole chloroplast genome, the SSRs of different unit lengths in the chloroplast genome, and the number, relative abundance, relative density, and GC content of SSRs and SSRs of different unit lengths in the IGS, LSC, intronic, and CDS regions of the chloroplast genome of the eight species of Cyatheaceae. Photovoltaic (PV) cluster analysis using the ward linkage method in R v3.5.1 (R Core Team, 2013) was performed on the SSRs of the chloroplast genome and its IGS regions, LSC regions and on the number, relative abundance, relative density, and GC content of single-nucleotide SSRs of the chloroplast genome with Euclidean distance as the measurement. The number of repetitions is 10,000.

## 2 Results and analysis

### 2.1 Genome structures and characteristics

The chloroplast genomes of all eight species of Cyatheaceae are double-stranded, closed, circular molecules with a typical tetrad structure (with *Alsophila denticulata* as an example, as shown in Figure 1). The genome length ranges from 154,046 bp (*Alsophila denticulata*) to 166,151 bp (*Alsophila gigantea*). The structure includes a large single-copy region (LSC) (bp 85975–92315), a small single-copy region (SSC) (bp 23245–28874), and an inverted repeat region (IR) (bp 23245–28874), of which IRA and IRB are two inverted repeat regions. The GC content of each region of the chloroplast genome of different species varied little, the total GC content ranging from 40.3% to 41.9% (Table 1). Only LSC, SSC, and one IR were analyzed. The chloroplast genome of each species of Cyatheaceae contained 117 genes, which encoded 85 proteins, four rRNAs, and 28 tRNAs. Pseudogenes (*ycf66*, *trnT-UGU*) were also present in these genomes. Among these genes are 13 genes located in the IR region. The *ndhB* gene spans the LSC and IRA regions, and there is a duplicated exon 2 sequence of the *ndhB* gene near the boundary of the IRB. Twelve genes have one intron, and three genes (*ycf3*, *clpP*, and *rps12*) have two introns.

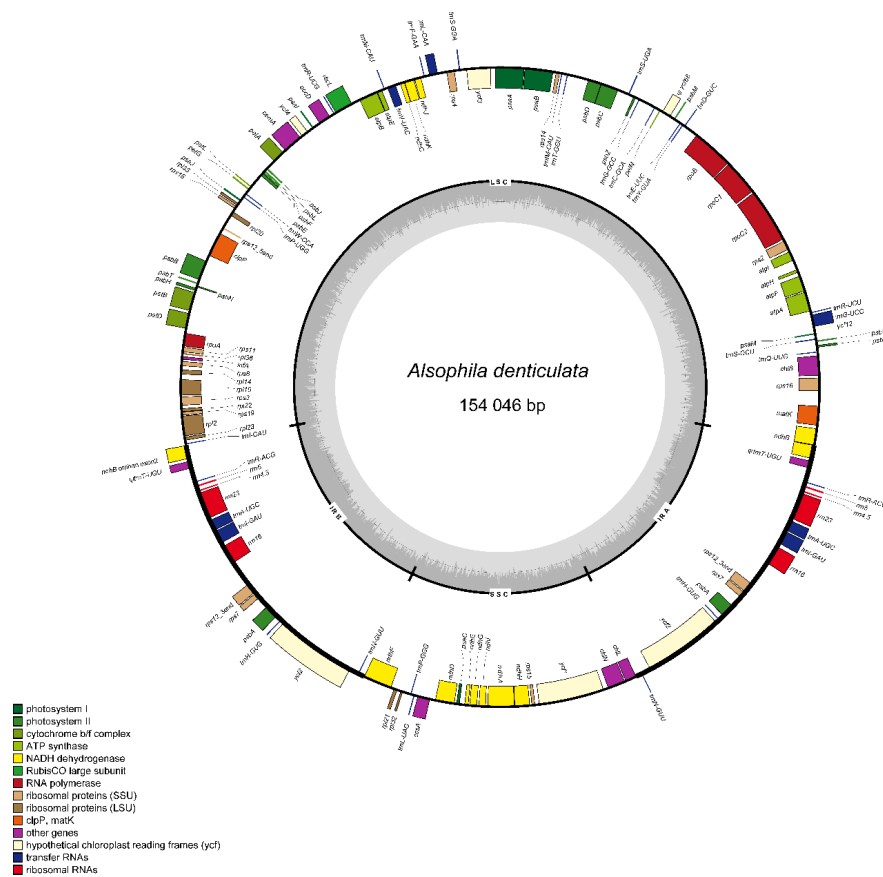


Figure 1 Gene map of the chloroplast genome of *Alsophila denticulata*. Genes located in outside of the outer circle are transcribed in the counter-clockwise direction, whereas those inside are transcribed in the clockwise direction. Color codes represent different functional gene groups. In the middle circle, the GC and AT content variations are indicated by darker gray and lighter gray, respectively.

Table 1 List of eight Cyatheaceae species, GenBank accession numbers, and structural features of chloroplast genomes

| Species               | GenBank No. | LSC         | LSC  | LSC        | IR          | IR   | IR         | SSC         | S    |
|-----------------------|-------------|-------------|------|------------|-------------|------|------------|-------------|------|
|                       |             | Length (bp) | GC%  | Length (%) | Length (bp) | GC%  | Length (%) | Length (bp) | GC%  |
| <i>A. denticulata</i> | MN795320    | 85975       | 40.0 | 55.8       | 23245       | 40.8 | 15.1       | 21581       | 39.5 |
| <i>A. podophylla</i>  | MG282389    | 86762       | 40.0 | 52.2       | 28874       | 46.2 | 17.4       | 21641       | 39.5 |
| <i>A. gigantea</i>    | MH603068    | 92315       | 41.1 | 57.1       | 23831       | 43.3 | 14.7       | 21702       | 39.5 |
| <i>A. metteniana</i>  | MT726940    | 92292       | 41.1 | 57.1       | 23822       | 43.3 | 14.7       | 21666       | 39.5 |
| <i>A. costularis</i>  | MH684489    | 86338       | 39.6 | 55.1       | 24356       | 43.0 | 15.5       | 21625       | 39.5 |
| <i>A. spinulosa</i>   | NC012818    | 86308       | 39.6 | 55.1       | 24365       | 43.0 | 15.6       | 21623       | 39.5 |
| <i>S. brunoniana</i>  | MT543220    | 86196       | 39.2 | 55.0       | 24011       | 43.2 | 15.3       | 22441       | 39.5 |
| <i>S. lepifera</i>    | MN623357    | 86349       | 39.3 | 53.3       | 24067       | 43.2 | 14.8       | 27733       | 40.0 |

LSC: Large Single Copy-Region; IR: Inverted Repeat Region; SSC: Small Single Copy-Region

## 2.2 Analysis of the characteristics of SSRs

The number, relative abundance, relative density, and GC content of SSRs in the chloroplast genomes of all eight species of Cyatheaaceae were systematically compared (Table 2). The number (121–122), relative abundance (0.77–0.78/bp), relative density (9.81–9.82 bp/kb), and GC content (0.18–0.20) of SSRs in the chloroplast genomes of *Alsophila spinulosa* and *Alsophila costularis*; the number (102), relative abundance (0.63–0.65/bp), relative density (6.70–8.18 bp/kb), and GC content (0.08–0.10) of SSRs in the chloroplast genomes of *Sphaeropteris brunoniana* and *Sphaeropteris lepifera*; and the number (61–67), relative abundance (0.40/bp), relative density (4.11–5.06 bp/kb), and GC content (0.22–0.29) of SSRs of *Alsophila denticulata*, *Alsophila podophylla*, *Alsophila metteniana*, and *Alsophila gigantea* had similar values, which were not proportional to the size of the genome. The proportions of GC bases in the chloroplast genomes of the eight species of Cyatheaaceae were much lower than the proportions of AT. SSRs accounted for 75.5–86.2%, 13.7–20.6%, 2.0–3.9%, and 2.0% of the IGS, Intron, CDS, and rRNA gene regions (pseudogenes were treated as IGS). Among them, SSR was only detected in the CDS of the chloroplast genomes of *Sphaeropteris brunoniana*, *Sphaeropteris lepifera*, *Alsophila spinulosa*, and *Alsophila costularis*, and SSRs were detected in the rRNA genes of the chloroplast genomes of *Sphaeropteris brunoniana* and *Sphaeropteris lepifera*. When *Gymnosphaera* was considered as an independent classification unit at the genus level, the eight species of Cyatheaaceae were divided into three genera. That is, *Alsophila denticulata*, *Alsophila podophylla*, *Alsophila metteniana*, and *Alsophila gigantea* belonged to the genus *Gymnosphaera*; *Alsophila spinulosa* and *Alsophila costularis* belonged to the genus *Alsophila*; and *Sphaeropteris brunoniana* and *Sphaeropteris lepifera* belonged to the genus *Sphaeropteris*. The number, relative abundance, relative density, and GC content of SSRs in the chloroplast genomes of the three genera of Cyatheaaceae also had similar values in different regions of the genome (LSC, SSC, and IR; IGS, intron, CDS, and rRNA gene regions), indicating that in the phylogenetic background of the three genera, the characteristics of SSRs are genus specific at the level of the genome and its different regions (Figure 3, Tables 2 and 3, and Supplementary Tables 9 and 10). The distribution of SSRs of different motif types in the three Cyatheaaceae genera also had genus specificity (Figure 2). These results showed that on the phylogenetic background of dividing the eight species of Cyatheaaceae into three genera, different taxa had different patterns of SSR characteristics in the chloroplast genome, namely, the SSR characteristics of the chloroplast genomes of the eight species of Cyatheaaceae were consistent with their phylogenetic relationship.

Table 2 Overview of the eight Cyatheaaceae chloroplast genomes and characteristics of their SSRs

|                             | <i>A. denticulata</i> | <i>A. podophylla</i> | <i>A. gigantea</i> | <i>A. metteniana</i> | <i>A. costularis</i> | <i>A. spinulosa</i> |
|-----------------------------|-----------------------|----------------------|--------------------|----------------------|----------------------|---------------------|
| Sequence analyzed (kb)      | 154.05                | 166.15               | 161.68             | 161.60               | 156.68               | 156.66              |
| No. of SSRs                 | 61                    | 67                   | 64                 | 65                   | 121                  | 122                 |
| Relative abundance (No./bp) | 0.40                  | 0.40                 | 0.40               | 0.40                 | 0.77                 | 0.78                |
| Total length of SSRs (bp)   | 634                   | 802                  | 818                | 801                  | 1539                 | 1538                |
| Relative density (bp/kb)    | 4.11                  | 4.82                 | 5.06               | 4.96                 | 9.82                 | 9.81                |
| GC content                  | 0.24                  | 0.25                 | 0.29               | 0.22                 | 0.20                 | 0.18                |
| Genome content              | 0.004                 | 0.005                | 0.005              | 0.005                | 0.010                | 0.010               |

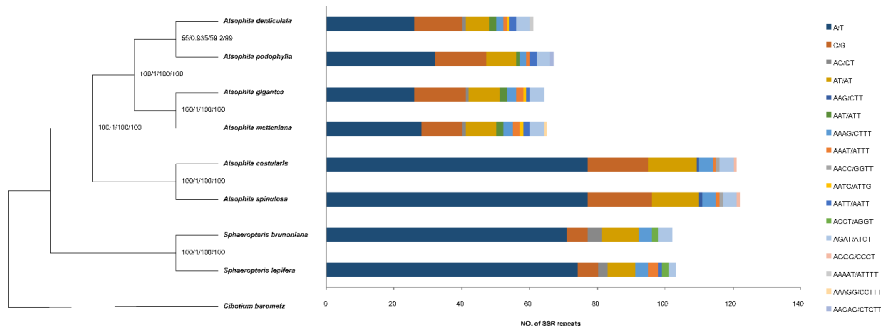


Figure 2 ML, BI, MP, and NJ phylogenetic trees based on eight complete chloroplast genome sequences in Cyatheaaceae and the distribution of different types of SSR motifs. The outgroup is *Cibotium barometz*.

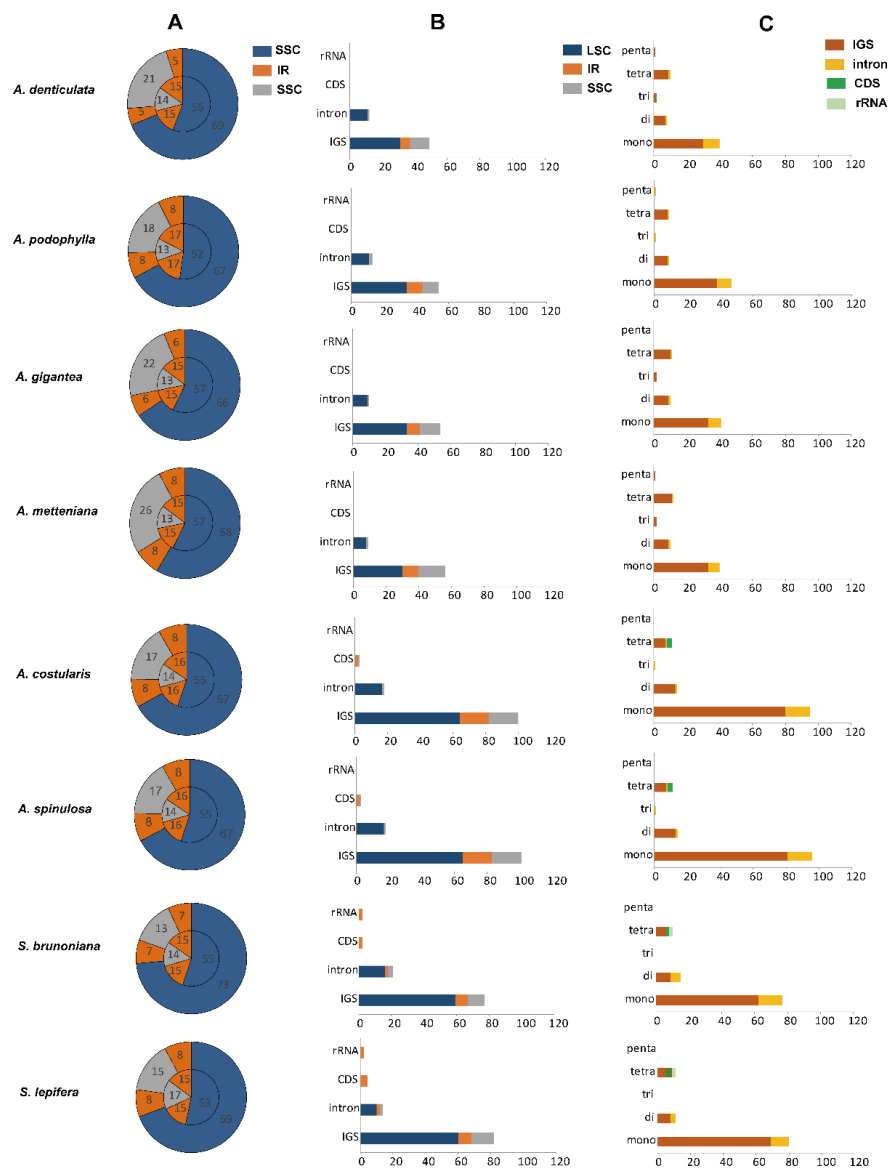


Figure 3 Comparison of microsatellite repeats among the eight Cyatheaaceae chloroplast genomes.

(A) The inner circle is the distribution ratio of the four regions (LSC, SSC, and IRs) in the genome, and the outer circle is the distribution ratio of SSRs between the four regions. (B) Distribution ratio of SSRs in different regions (LSC, SSC, and IRs) of chloroplast the genome. (C) Ratio of mono- to pentanucleotide SSRs in different regions (IGS, intron, CDS, and rRNA gene) of the chloroplast genome. Numbers represent the distribution ratio of SSR numbers. LSC: large single-copy region; SSC: small single-copy region; IR: inverted repeat region; IGS: intergenic spacer region; CDS: coding sequence region.

## 2.3 Analysis of the types and characteristics of SSRs of different nucleotide numbers

The proportions of single-nucleotide, dinucleotide, trinucleotide, tetranucleotide, and pentanucleotide SSRs

in each species were 62.5-78.0%, 10.6-15.6%, 0-3.3%, 9.0-18.5%, and 0-1.5%, respectively. No hexanucleotide SSR was detected. Among single-nucleotide repeats, there were more A/T motifs, and the dinucleotide repeats were dominated by AT/TA motifs. The single-nucleotide, dinucleotide, trinucleotide, tetranucleotide, and pentanucleotide SSRs of the chloroplast genomes of the three genera were similar in number, relative abundance, relative density, and GC content at the level of the genome and in the specific regions of the genome (LSC, SSC, and IRs; IGS, intron, CDS, and rRNA gene regions). This was especially true for single-nucleotide and dinucleotide SSRs (Table 3, Supplementary Tables 10 and 11). The number, relative abundance, relative density, and GC content of SSRs of different unit lengths and SSRs in different regions of the genome had genus specificity in the phylogenetic context of dividing the eight species of Cyatheaceae into three genera. In addition, the number, relative abundance, and relative density of SSRs of different base types in the chloroplast genomes of the three genera of plants also had genus specificity. This was especially true for single- and dinucleotide SSRs (Figure 4, Supplementary Table 9).

Table 3 The number, relative abundance, relative density, and GC content of mono- to pentanucleotide SSRs in the eight chloroplast genomes of Cyatheaceae

| Repeat type     | Characteristics of SSR | <i>A. denticulata</i> | <i>A. podophylla</i> | <i>A. gigantea</i> | <i>A. metteniana</i> | <i>A. costularis</i> |
|-----------------|------------------------|-----------------------|----------------------|--------------------|----------------------|----------------------|
| Mononucleotide  | No. of SSRs            | 40                    | 47                   | 41                 | 40                   | 95                   |
|                 | Abundance (No./kb)     | 0.26                  | 0.28                 | 0.25               | 0.25                 | 0.61                 |
|                 | Density (bp/kb)        | 2.93                  | 3.16                 | 3.13               | 2.88                 | 7.67                 |
|                 | GC                     | 0.36                  | 0.35                 | 0.40               | 0.3                  | 0.22                 |
| Dinucleotide    | No. of SSRs            | 8                     | 9                    | 10                 | 10                   | 14                   |
|                 | Abundance (No./kb)     | 0.05                  | 0.05                 | 0.06               | 0.06                 | 0.09                 |
|                 | Density (bp/kb)        | 0.69                  | 0.83                 | 0.94               | 0.92                 | 1.35                 |
|                 | GC                     | 0.06                  | 0                    | 0.08               | 0.05                 | 0                    |
| Trinucleotide   | No. of SSRs            | 2                     | 1                    | 2                  | 2                    | 1                    |
|                 | Abundance (No./kb)     | 0.01                  | 0.01                 | 0.01               | 0.01                 | 0.01                 |
|                 | Density (bp/kb)        | 0.16                  | 0.07                 | 0.15               | 0.15                 | 0.08                 |
|                 | GC                     | 0                     | 0                    | 0                  | 0                    | 0.33                 |
| Tetranucleotide | No. of SSRs            | 10                    | 9                    | 11                 | 12                   | 11                   |
|                 | Abundance (No./kb)     | 0.80                  | 0.05                 | 0.07               | 0.07                 | 0.07                 |
|                 | Density (bp/kb)        | 0.14                  | 0.67                 | 0.84               | 0.92                 | 0.87                 |
|                 | GC                     | 0.18                  | 0.14                 | 0.18               | 0.16                 | 0.29                 |
| Pentanucleotide | No. of SSRs            | 1                     | 1                    | 0                  | 1                    | 0                    |
|                 | Abundance (No./kb)     | 0.01                  | 0.01                 | 0                  | 0.01                 | 0                    |
|                 | Density (bp/kb)        | 0.10                  | 0.09                 | 0                  | 0.09                 | 0                    |
|                 | GC                     | 0                     | 0.4                  | 0                  | 0.4                  | 0                    |

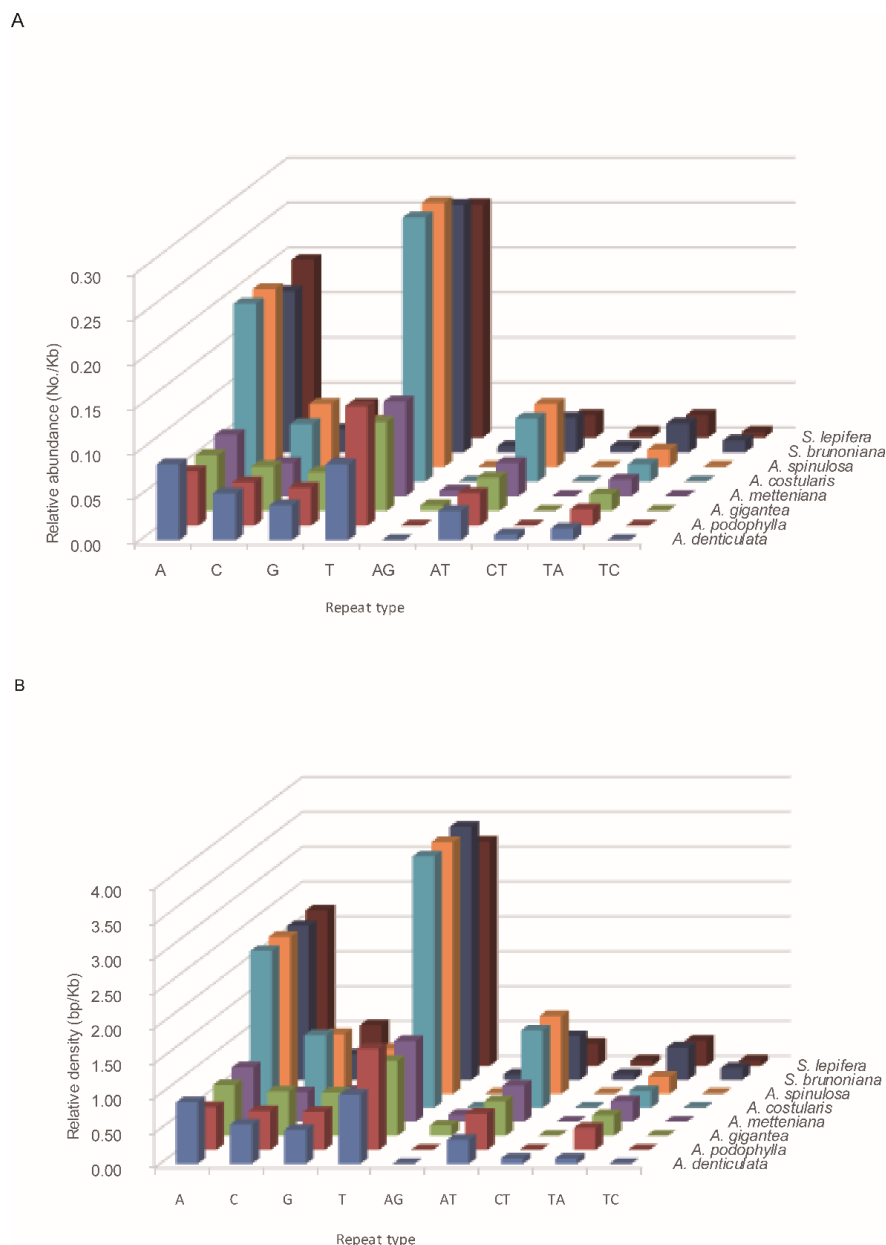


Figure 4 Relative abundance and relative density of single- and dinucleotide SSRs in the eight chloroplast genomes of Cyatheaceae.

## 2.4 Phylogenetic analysis

The chloroplast genomes of the eight species of Chinese Cyatheaceae were compared globally, and the phylogenetic trees were constructed with four methods (ML, BI, MP, and NJ) using *Cibotium barometz* (Linn.) J. Sm. as an outgroup, as shown in Figure 2. The topologies of the four trees were consistent, except that the support rate of the branches of the *Alsophila denticulata* and *Alsophila gigantea* was lower (the bootstrap values of ML, MP, and NJ were 55%, 59.2%, 99%, respectively, and the posterior probability of BI was 0.935). The support rate of the other branches was higher (the bootstrap values of ML, MP, and NJ were all 100%, and the posterior probability of BI was 1.00). Closely related *Sphaeropteris brunoniana* and *Sphaeropteris*

*lepifera* were clustered into one branch, which was located at the base of the phylogenetic tree. That means they were an earlier-diverged group in this family. *Alsophila denticulata* ,*Alsophila podophylla* , *Alsophila gigantea* , and*Alsophila metteniana* were clustered into one branch, which was located inside the branch of *Sphaeropteris brunoniana* and*Sphaeropteris lepifera* and was a sister group of the branch formed by *Alsophila spinulosa* and *Alsophila costularis* .

## 2.5 Statistical analysis of the results

The Kruskal-Wallis H-test and Mann-Whitney U-test values are shown in Supplementary Table 11. In this study, only SSRs in the chloroplast genome in its IGS and LSC regions, as well as single-nucleotide SSRs in the whole chloroplast genome, were considered. There were significant differences in the number, relative abundance, relative density, and GC content of SSRs when the eight species of Cyatheaceae were divided into three genera (Kruskal-Wallis H,  $P < 0.05$ ). When*Gymnosphaera* was included in the genus *Alsophila* , they were divided into two genera, and only the difference in the GC content was significant (Table 4). The number of SSRs in other regions of the chloroplast genome and the number of SSRs of other unit lengths were small, so they are not discussed in this study. The clustering results of the number, relative abundance, relative density, and GC content of the SSRs in the chloroplast genomes and their IGS regions and the single-nucleotide SSRs of the whole chloroplast genome of the eight species of Cyatheaceae (Figure 5) showed that the eight species were divided into two groups. That is, *Sphaeropteris brunoniana* ,*Sphaeropteris lepifera* , *Alsophila spinulosa* and*Alsophila costularis* were in a group, and *Alsophila denticulata* , *Alsophila podophylla* , *Alsophila metteniana* , and *Alsophila gigantea* were in a group.

Table 4 Significance test of the number, relative abundance, relative density, and GC content of SSRs in the whole chloroplast genome, IGS, and LSC and mononucleotide SSRs in the whole chloroplast genome of the eight Cyatheaceae species

| Classification treatment | Genus                | Species               | Characteristics of SSR      | $P$     | $P$     | $P$     | $P$            | Literature cited                                                                                                |
|--------------------------|----------------------|-----------------------|-----------------------------|---------|---------|---------|----------------|-----------------------------------------------------------------------------------------------------------------|
| Three genera             | <i>Gymnosphaera</i>  | <i>A. denticulata</i> | No. of SSRs                 | Genome  | IGS     | LSC     | Mononucleotide | Smith et al., 2000; Korall et al., 2001; Janssen et al., 2002; Korall and Pryer, 2014; Ching, 1978; Dong, 2019. |
|                          |                      | <i>A. podophylla</i>  |                             | 0.048*  | 0.048*  | 0.048*  | 0.048*         |                                                                                                                 |
|                          |                      | <i>A. gigantea</i>    |                             | 0.033*  | 0.050 * | 0.050 * | 0.050 *        |                                                                                                                 |
|                          | <i>Alsophila</i>     | <i>A. metteniana</i>  | Relative abundance (No./kb) | 0.050 * | 0.050 * | 0.050 * | 0.050 *        |                                                                                                                 |
|                          |                      | <i>A. costularis</i>  | Relative density (bp/kb)    | 0.050 * | 0.050 * | 0.050 * | 0.050 *        |                                                                                                                 |
|                          |                      | <i>A. spinulosa</i>   | GC content                  | 0.050 * | 0.050 * | 0.050 * | 0.050 *        |                                                                                                                 |
|                          | <i>Sphaeropteris</i> | <i>S. brunoniana</i>  |                             |         |         |         |                |                                                                                                                 |
|                          |                      | <i>S. lepifera</i>    |                             |         |         |         |                |                                                                                                                 |

| Classification treatment | Genus                | Species                                    | Characteristics of SSR      | <i>P</i> | <i>P</i> | <i>P</i> | <i>P</i> | Literature cited                            |
|--------------------------|----------------------|--------------------------------------------|-----------------------------|----------|----------|----------|----------|---------------------------------------------|
| Two genera               | <i>Alsophila</i>     | <i>A. denticulata</i>                      | No. of SSRs                 | 0.502    | 0.502    | 0.502    | 0.502    | PPG I, 2016; Xu 1989; Zhang & Nishida 2013. |
|                          |                      | <i>A. podophylla</i>                       |                             |          |          |          |          |                                             |
|                          |                      | <i>A. gigantea</i>                         | Relative abundance (No./kb) | 0.478    | 0.505    | 0.505    | 0.505    |                                             |
|                          |                      | <i>A. metteniana</i>                       |                             |          |          |          |          |                                             |
|                          |                      | <i>A. costularis</i>                       | Relative density (bp/kb)    | 0.505    | 0.505    | 0.505    | 0.505    |                                             |
|                          |                      | <i>A. spinulosa</i>                        |                             |          |          |          |          |                                             |
|                          | <i>Sphaeropteris</i> | <i>S. brunoniana</i><br><i>S. lepifera</i> | GC content                  | 0.046*   | 0.046*   | 0.046*   | 0.046*   |                                             |

When Cyatheaceae plants are treated as three genera, the Kruskal-Wallis H test is used; when treated as two genera, the Mann-Whitney U test is used; \*:  $P \leq 0.05$ ; IGS: intergenic spacer; LSC: large single-copy region.

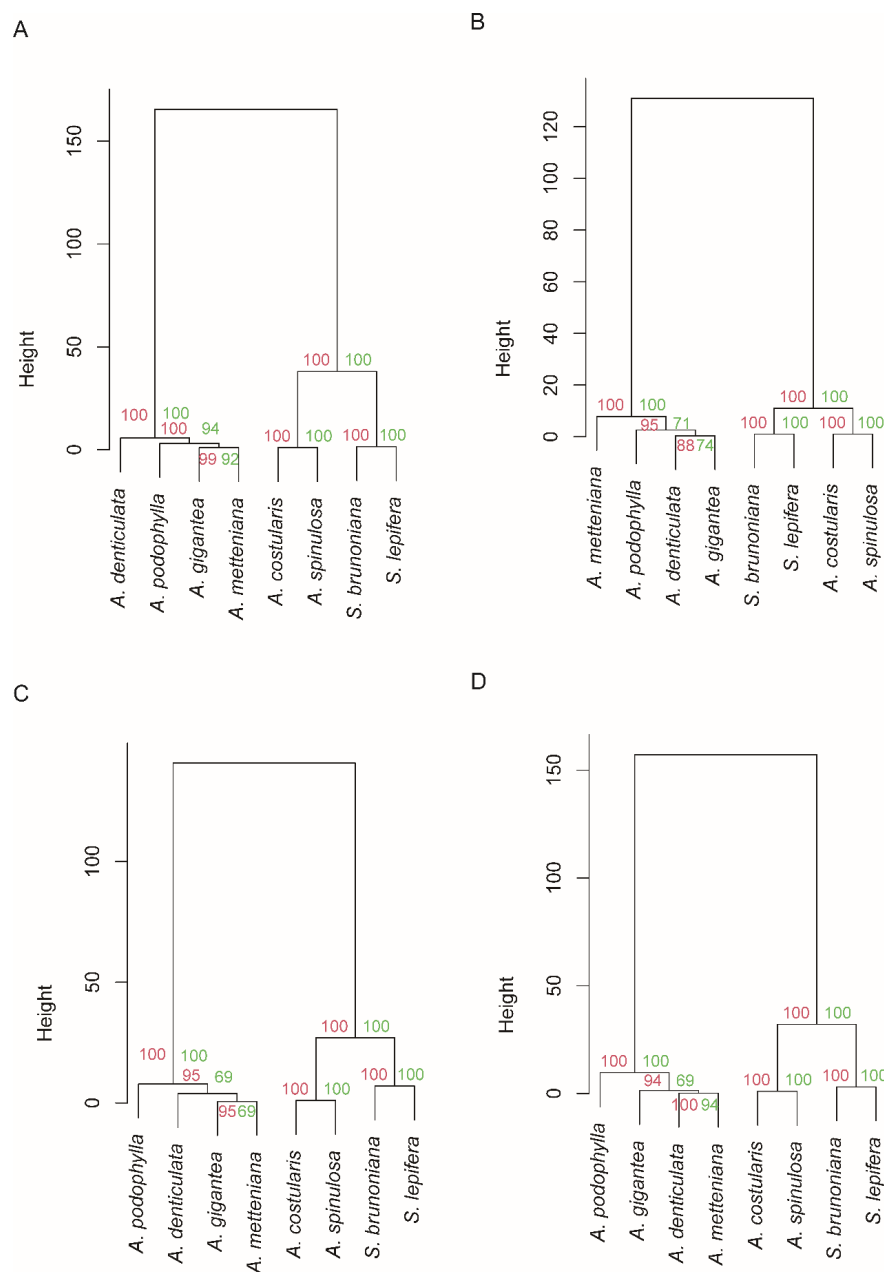


Figure 5 Clustering analysis of 8 Cyatheaceae species based on the number, relative abundance, relative density, and GC content of SSRs across the whole chloroplast genome (A), LSC (B), IGS (C) and mononucleotide (D) SSRs in the chloroplast genome.

### 3 Discussion

#### 3.1 Characteristics of SSRs of chloroplast genomes of eight species of Cyatheaceae

The chloroplast genomes of all eight species of Cyatheaceae are similar in structure and gene content, and the types and order of genes are the same. On the phylogenetic background of dividing the eight species of Cyatheaceae into three genera, the characteristics of the chloroplast genome SSRs have genus specificity. The distribution of SSRs is not random, which has an effect on gene regulation, DNA recombination, DNA

replication, the cell cycle, and DNA mismatch repair, and the presence of repeat motifs and their repeat number can affect DNA recombination (Li et al., 2002). SSR copy number is an important source of genetic variation and can produce large phenotypic variation (Gemayel et al., 2010; Kashi & King, 2006). Single-nucleotide SSRs are the most abundant, which is a characteristic of eukaryotic genomes (Sharma et al., 2007), and A/T motifs are the most common. The number, relative abundance, relative density, and GC content of SSRs of different unit length are also genus specific. This is especially true for single-nucleotide and dinucleotide SSRs, which may be related to the lower content of SSRs of other unit lengths. The distribution of different repeat types (from single-nucleotide to hexanucleotide) of motifs in coding and noncoding regions, introns, and intergenic regions displays a high degree of taxon specificity, which can be partially explained by the interaction of mutation mechanisms and differential selection (Toth et al., 2000). The SSRs in eukaryotic genomes are mainly located in intergenic and noncoding regions, with a few in exons (Toth et al., 2000; Li et al., 2004), and the results of this study are consistent with this. This phenomenon is related to the higher variability (Nie et al., 2012; Wu et al., 2010) and faster evolutionary rates of the intergenic and noncoding regions of the chloroplast genome, so their sequences can be used to effectively classify low taxonomic and closely related groups and subspecies variant plants. The proportion of SSRs in the IR region was 2-3.3 times the proportion of IR sequences out of the whole genome sequence (Figure 3), indicating that there are fewer SSRs in the IR region. Mismatch repair is the key to the stability of SSRs. SSRs have a high mutation rate, which facilitates the study of the effect of environmental factors on the mutation rate of the genome. The lower distribution of SSRs in the IR region may be related to the lower mutation rate in the IR region (Ellegren, 2004; Li et al., 2016). The lower GC content may be associated with the fact that GC-rich regions are prone to mutations toward AT (Ren et al., 2007). The high GC content is significantly associated with the high recombination rate in meiosis (Tortereau et al., 2012), while AT-rich SSRs may be more conducive to maintaining the stability of the genome structure. The number, relative abundance, relative density, and GC content of SSRs are not proportional to the size of genome, indicating that the abundance of SSRs is related to the genetic characteristics of the species (Li et al., 2014).

### 3.2 Phylogenetic significance of SSR characteristics of chloroplast genomes of the eight species of Cyatheaaceae

The focus of the debate between the Holttum and Edwards (1963) system and the Tryon (1970) system, which classify Cyatheaaceae based on morphological characteristics, is the theoretical explanation of the morphological evolution of indusium in this family. In this study, we show that the genus *Sphaeropteris*, lacking the indusium, is a basal group, supporting Tryon's hypothesis that the indusium is derived from the tissues or scales on the abaxial side of the leaf, away from the leaf margin, and that the indusium is a derived trait, which is consistent with a phylogenetic analysis based on the chloroplast *trnL* intron sequence and the *trnL-F* intergenic region sequence (Wang et al., 2003). Dong (2018) pointed out that *Gymnosphaera* and *Alsophila* were significantly differentiated in morphological traits such as petiole color, the presence or absence of degenerated pinnae at the base, and the presence or absence of indusium and sporogenesis, and advocated the restoration of the hierarchical status of the genus *Gymnosphaera* to reflect the divergence mechanisms of this group of plants in molecular phylogeny, morphology, and sporogenesis. In this study, eight species of Cyatheaaceae were divided into three genera or two genera, in which case SSRs were compared by the Kruskal-Wallis H test or the Mann-Whitney U test, respectively, and the results showed that *Gymnosphaera* was an independent genus-level taxon under the Cyatheaaceae family. The results of the PV clustering analysis of SSRs also indicated that *Gymnosphaera* should be independent from the genus *Alsophila*. Phylogenetic trees were constructed using the chloroplast genomes of eight species of Cyatheaaceae, and the topology of the phylogenetic trees obtained by the four methods was consistent. Except for the lower support rate of the branches of the *Alsophila denticulata* and *Alsophila gigantea*, the support rate for the branches was 100%. This result supports the monophyletic nature of *Gymnosphaera*, and its sister group is the genus *Alsophila*. *Gymnosphaera*, *Alsophila*, and *Cyathea* constitute a monophyletic group with a high support rate, while *Sphaeropteris* resolved as the basal group of the Cyatheaaceae family. In this phylogenetic context, the SSR characteristics have genus specificity.

SSRs play a role in genome-wide regulation. Some definite distribution patterns exist in the genomes of

organisms, and the characteristic distribution of SSRs in the genomes of different taxonomic units has a significantly similar pattern (Qi et al., 2015; Wang et al., 2015; Liu et al., 2017; Manee et al., 2019; Srivastava et al., 2019). The SSRs of different groups of genomes have specific distribution patterns, which are related to their common ancestors. Evolutionary trends have been linked to the inclusion of SSRs, which may have been preserved because of their ability to adapt to novel regulatory mechanisms (Srivastava et al., 2019). Unique DNA replication, repair, and recombination mechanisms may play an important role in the evolution of SSRs (Katti et al., 2001). The molecular mechanism of the origin of SSRs is not yet fully understood. The most common mutation mechanism affecting SSRs is slipped replication. Other mechanisms, such as unequal crossing-over, nucleotide substitution, and duplication events, are also responsible for SSR variation (Schlotterer & Tautz, 1992; Hancock, 1999). Codon preference, DNA replication, and mismatch repair systems, as well as the unique structure and function of the genome, may be responsible for the unique SSR distribution pattern in plant genomes. In addition, the length, motif structure, and GC content of genomic SSRs are also factors that influence the evolution of the SSRs (Chakraborty et al., 1997; Anderson et al., 2000; Whittaker et al., 2003). The plants in the three genera of Cyatheaceae have similar phenotypes and specific characteristics, which may be the result of interactions of their common ancestors with similar habitats. The analysis of the characteristics of SSRs provides useful clues for the phylogenetic study of Cyatheaceae and helps to understand the evolution of SSRs in plant genomes.

The chloroplast genome has a simple structure, low molecular weight, and high copy number, and its genes are maternally inherited in ferns with few gene rearrangements, thus facilitating the study of plant phylogeny (Tonti-Filippini et al., 2017). Based on high-throughput sequencing technology, the highly conserved chloroplast genome sequence will make primer design easier. SSR markers in chloroplast genomes can be used for the analysis of multiple chloroplast regions, thereby improving the resolution of phylogenetic studies of target species (Melotto-Passarin et al., 2011). Since the software programs that identify SSRs are limited by their efficiency and parameter settings and may also be affected by the quality of the SSR dataset generated, their accuracy needs to be improved (Ellegren, 2004; Lim et al., 2013). In addition, chloroplast genomes play an important role in dissecting the higher hierarchical phylogenetic relationships of ferns. However, in the process of evolution, plants experience events such as hybridization, polyploidization, introgression, and incomplete lineage sorting, so the evolutionary relationship of plants is essentially reticular. Such reticular relationships are often manifested in the form of gene tree conflicts (Guo & Ge, 2005).

In this study, the chloroplast genomes of eight species of Cyatheaceae were used to construct phylogenetic trees. This analytical method has some limitations. There are still relatively few studies on phylogenetic relationships in ferns that have analyzed the SSRs of the chloroplast genome. This study provides a new basis for the classification of Cyatheaceae at the levels of species and genus, thus advancing the phylogenetic study of Cyatheaceae. In the future, more genomic and transcriptomic data are needed to validate these results.

## Acknowledgments

We would like to thank the Wuhan Botanical Garden, South China Botanical Garden and Fairy Lake Botanical Garden of the Chinese Academy of Sciences for providing samples of Cyatheaceae plants.

## Conflict of interest

The authors declare no competing interests.

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helpful comments.

## Author contributions

MZ conducted the statistics and analysis of the data, and wrote the manuscript. PPF, JYP and JYL analyzed part of the data and provided Suggestions. YJS and TW designed the study and wrote the manuscript.

## Data availability statement

The chloroplast genomes of *Alsophila denticulata* Baker and *Alsophila metteniana* Hance have been submitted to the National Center for Biotechnology Information (NCBI) and have not yet been published. The other 6 chloroplast genomes of Cyatheaceae species can be downloaded from NCBI.

## Funding

This work was funded by the National Natural Science Foundation of China (31670200, 31872670).

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Appendix table 1-8 List of simple sequence repeats(SSRs) in the chloroplast genomes of 8 species in Cyatheaceae.

Appendix table 9 The number, relative abundance, and relative density of SSR motifs in the chloroplast genomes of 8 species in Cyatheaceae.

Appendix table 10 The number, relative abundance, relative density and GC content of different types of motif SSRs in different regions in the chloroplast genomes of 8 species in Cyatheaceae.

Appendix table 11 Significant differences in the number, relative abundance, relative density, and GC content of the chloroplast genomes, Mono- to Pentanucleotide SSRs, IGS, LSC, intron and CDS regions SSRs of chloroplast genomes, and the mono- to pentanucleotide SSRs in the chloroplast genomes of 8 Cyatheaceae species

Appendix table 1 List of simple sequence repeats (SSRs) in the chloroplast genomes of *Alsophila denticulata*

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | star  | end   | Location                   |
|---------|----------|--------|--------|-----------|------|-------|-------|----------------------------|
| 1       | p5       | spacer | LSC    | ATTTT     | 15   | 5331  | 5345  | <i>rps16-chlB</i>          |
| 2       | p1       | spacer | LSC    | G         | 14   | 7040  | 7053  | <i>chlB-trnQ(UGG)</i>      |
| 3       | p1       | spacer | LSC    | C         | 11   | 8373  | 8383  | <i>trnS(GCU)-psaM</i>      |
| 4       | p1       | intron | LSC    | A         | 12   | 9401  | 9412  | <i>trnG(UCC)</i>           |
| 5       | p4       | intron | LSC    | AATT      | 12   | 9648  | 9659  | <i>trnG(UCC)</i>           |
| 6       | p1       | spacer | LSC    | G         | 16   | 13299 | 13314 | <i>atpF-atpH</i>           |
| 7       | p1       | spacer | LSC    | C         | 12   | 14280 | 14291 | <i>atpH-atpI</i>           |
| 8       | p1       | spacer | LSC    | C         | 12   | 15231 | 15242 | <i>atpI-rps2</i>           |
| 9       | p1       | intron | LSC    | C         | 10   | 22475 | 22484 | <i>rpoC1</i>               |
| 10      | p1       | spacer | LSC    | A         | 11   | 23468 | 23478 | <i>rpoC1-rpoB</i>          |
| 11      | p1       | spacer | LSC    | C         | 10   | 26793 | 26802 | <i>rpoB-trnD(GUC)</i>      |
| 12      | p4       | spacer | LSC    | ATAG      | 12   | 27725 | 27736 | <i>rpoB-trnD(GUC)</i>      |
| 13      | p1       | spacer | LSC    | T         | 13   | 28419 | 28431 | <i>trnE(UUC)-psbM</i>      |
| 14      | p4       | spacer | LSC    | TGAT      | 12   | 28511 | 28522 | <i>trnE(UUC)-psbM</i>      |
| 15      | p1       | spacer | LSC    | A         | 10   | 29560 | 29569 | <i>ycf66</i>               |
| 16      | p1       | spacer | LSC    | G         | 14   | 29640 | 29653 | <i>ycf66</i>               |
| 17      | p1       | spacer | LSC    | G         | 10   | 31641 | 31650 | <i>trnC(GCA)-trnG(GCC)</i> |
| 18      | p1       | spacer | LSC    | A         | 10   | 31715 | 31724 | <i>trnC(GCA)-trnG(GCC)</i> |
| 19      | p1       | spacer | LSC    | C         | 10   | 32102 | 32111 | <i>trnG(GCC)-psbZ</i>      |
| 20      | p4       | spacer | LSC    | TATC      | 16   | 33543 | 33558 | <i>trnS(UGA)-psbC</i>      |
| 21      | p4       | spacer | LSC    | ATAG      | 12   | 33562 | 33573 | <i>trnS(UGA)-psbC</i>      |
| 22      | p1       | spacer | LSC    | A         | 10   | 37593 | 37602 | <i>psbD-trnT(GGU)</i>      |
| 23      | p1       | intron | LSC    | A         | 11   | 44104 | 44114 | <i>ycf3</i>                |

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | star   | end    | Location                   |
|---------|----------|--------|--------|-----------|------|--------|--------|----------------------------|
| 24      | p1       | intron | LSC    | T         | 13   | 44123  | 44135  | <i>ycf3</i>                |
| 25      | p1       | intron | LSC    | C         | 11   | 45216  | 45226  | <i>ycf3</i>                |
| 26      | p1       | spacer | LSC    | T         | 10   | 45601  | 45610  | <i>ycf3-trnS(GGA)</i>      |
| 27      | p1       | spacer | LSC    | G         | 10   | 47798  | 47807  | <i>rps4-trnL(CAA)</i>      |
| 28      | p1       | spacer | LSC    | A         | 10   | 51902  | 51911  | <i>trnV(UAC)-trnM(CAU)</i> |
| 29      | p1       | spacer | LSC    | T         | 11   | 56710  | 56720  | <i>trnR(UCG)-accD</i>      |
| 30      | p4       | spacer | LSC    | TATT      | 12   | 57765  | 57776  | <i>accD-psaI</i>           |
| 31      | p2       | spacer | LSC    | AT        | 18   | 58231  | 58248  | <i>accD-psaI</i>           |
| 32      | p2       | spacer | LSC    | TA        | 12   | 62570  | 62581  | <i>petA-psbJ</i>           |
| 33      | p1       | spacer | LSC    | A         | 10   | 68052  | 68061  | <i>rpl20-rps12</i>         |
| 34      | p1       | spacer | LSC    | T         | 11   | 68120  | 68130  | <i>rpl20-rps12</i>         |
| 35      | p4       | spacer | LSC    | GATA      | 12   | 69048  | 69059  | <i>rps12-clpP</i>          |
| 36      | p1       | intron | LSC    | C         | 11   | 69574  | 69584  | <i>chlP</i>                |
| 37      | p1       | intron | LSC    | T         | 11   | 69615  | 69625  | <i>chlP</i>                |
| 38      | p1       | intron | LSC    | T         | 11   | 70413  | 70423  | <i>chlP</i>                |
| 39      | p2       | intron | LSC    | CT        | 12   | 70704  | 70715  | <i>chlP</i>                |
| 40      | p2       | spacer | LSC    | AT        | 12   | 79990  | 80001  | <i>rps8-rpl14</i>          |
| 41      | p1       | intron | LSC    | T         | 12   | 81564  | 81575  | <i>rpl16</i>               |
| 42      | p1       | spacer | LSC    | T         | 10   | 85769  | 85778  | <i>rpl23-trnI(CAU)</i>     |
| 43      | p2       | spacer | IRA    | AT        | 12   | 87653  | 87664  | <i>trnT(UGU)</i>           |
| 44      | p4       | spacer | IRA    | TCTT      | 12   | 96474  | 96485  | <i>rrn16-rps12</i>         |
| 45      | p1       | spacer | IRA    | A         | 12   | 97573  | 97584  | <i>rrn16-rps12</i>         |
| 46      | p1       | spacer | SSC    | A         | 10   | 111684 | 111693 | <i>ndhF-rpl21</i>          |
| 47      | p3       | spacer | SSC    | AAT       | 12   | 112846 | 112857 | <i>rpl32-trnP(GGG)</i>     |
| 48      | p2       | spacer | SSC    | TA        | 12   | 112860 | 112871 | <i>rpl32-trnP(GGG)</i>     |
| 49      | p3       | spacer | SSC    | TAT       | 12   | 112873 | 112884 | <i>rpl32-trnP(GGG)</i>     |
| 50      | p1       | spacer | SSC    | A         | 10   | 113225 | 113234 | <i>trnP(GGG)-trnL(UAG)</i> |
| 51      | p1       | spacer | SSC    | T         | 11   | 113467 | 113477 | <i>trnL(UAG)-ccsA</i>      |
| 52      | p1       | spacer | SSC    | A         | 10   | 116602 | 116611 | <i>ndhD-psaC</i>           |
| 53      | p4       | spacer | SSC    | AATT      | 12   | 116610 | 116621 | <i>ndhD-psaC</i>           |
| 54      | p1       | spacer | SSC    | G         | 11   | 117154 | 117164 | <i>psaC-ndhE</i>           |
| 55      | p1       | spacer | SSC    | T         | 11   | 118927 | 118937 | <i>ndhI-ndhA</i>           |
| 56      | p2       | spacer | SSC    | AT        | 16   | 118966 | 118981 | <i>ndhI-ndhA</i>           |
| 57      | p1       | intron | SSC    | T         | 17   | 120503 | 120519 | <i>ndhA</i>                |
| 58      | p1       | spacer | SSC    | A         | 10   | 122795 | 122804 | <i>rps15-ycf1</i>          |
| 59      | p1       | spacer | IRB    | T         | 12   | 142438 | 142449 | <i>rps12-rrn16</i>         |
| 60      | p4       | spacer | IRB    | AAAG      | 12   | 143536 | 143547 | <i>rps12-rrn16</i>         |
| 61      | p2       | spacer | IRB    | AT        | 12   | 152358 | 152369 | <i>trnT(UGU)</i>           |

Appendix table 2 List of simple sequence repeats (SSRs) in the chloroplast genomes of *Alsophila podophylla*

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | start | end   | Location               |
|---------|----------|--------|--------|-----------|------|-------|-------|------------------------|
| 1       | p1       | spacer | LSC    | G         | 13   | 2113  | 2125  | <i>ndhB-matK</i>       |
| 2       | p1       | intron | LSC    | T         | 10   | 5156  | 5165  | <i>rps16</i>           |
| 3       | p1       | spacer | LSC    | G         | 16   | 7667  | 7682  | <i>chlB-trnQ(UUG)</i>  |
| 4       | p2       | spacer | LSC    | AT        | 14   | 9108  | 9121  | <i>trnS(GUC)-psaM</i>  |
| 5       | p1       | spacer | LSC    | G         | 11   | 9713  | 9723  | <i>ycf12-trnG(UCC)</i> |
| 6       | p4       | intron | LSC    | AATT      | 12   | 10328 | 10339 | <i>trnG(UCC)</i>       |
| 7       | p1       | spacer | LSC    | C         | 14   | 14948 | 14961 | <i>atpH-atpI</i>       |

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | start  | end    | Location                   |
|---------|----------|--------|--------|-----------|------|--------|--------|----------------------------|
| 8       | p1       | spacer | LSC    | C         | 11   | 15898  | 15908  | <i>atpI-rps2</i>           |
| 9       | p1       | spacer | LSC    | C         | 11   | 27484  | 27494  | <i>rpoB-trnD(GUC)</i>      |
| 10      | p4       | spacer | LSC    | ATAG      | 12   | 28409  | 28420  | <i>rpoB-trnD(GUC)</i>      |
| 11      | p1       | spacer | LSC    | T         | 12   | 29103  | 29114  | <i>trnE(UUC)-psbM</i>      |
| 12      | p4       | spacer | LSC    | TGAT      | 12   | 29197  | 29208  | <i>trnE(UUC)-psbM</i>      |
| 13      | p1       | spacer | LSC    | G         | 13   | 30325  | 30337  | <i>ycf66</i>               |
| 14      | p1       | spacer | LSC    | A         | 10   | 32400  | 32409  | <i>trnC(GCA)-trnG(GCC)</i> |
| 15      | p1       | spacer | LSC    | C         | 12   | 32787  | 32798  | <i>trnG(GCC)-psbZ</i>      |
| 16      | p4       | spacer | LSC    | TATC      | 16   | 34223  | 34238  | <i>trnS(UGA)-psbC</i>      |
| 17      | p4       | spacer | LSC    | ATAG      | 12   | 34242  | 34253  | <i>trnS(UGA)-psbC</i>      |
| 18      | p1       | spacer | LSC    | T         | 10   | 37895  | 37904  | <i>psbD-trnT(GGU)</i>      |
| 19      | p5       | intron | LSC    | TTCTC     | 15   | 39317  | 39331  | <i>rps14</i>               |
| 20      | p3       | intron | LSC    | AAT       | 12   | 44793  | 44819  | <i>ycf3</i>                |
| 21      | p1       | intron | LSC    | T         | 10   | 44810  | 44819  | <i>ycf3</i>                |
| 22      | p1       | intron | LSC    | C         | 11   | 45899  | 45909  | <i>ycf3</i>                |
| 23      | p1       | spacer | LSC    | T         | 10   | 46284  | 46293  | <i>ycf3-trnS(GGA)</i>      |
| 24      | p1       | spacer | LSC    | T         | 10   | 46867  | 46876  | <i>trnS(GGA)-rps4</i>      |
| 25      | p1       | spacer | LSC    | G         | 12   | 48604  | 48615  | <i>rps4-trnL(CAA)</i>      |
| 26      | p1       | spacer | LSC    | T         | 10   | 57501  | 57510  | <i>trnR(UCG)-accD</i>      |
| 27      | p4       | spacer | LSC    | TATT      | 12   | 58555  | 58566  | <i>accD-psaI</i>           |
| 28      | p1       | spacer | LSC    | A         | 10   | 58615  | 58624  | <i>accD-psaI</i>           |
| 29      | p2       | spacer | LSC    | AT        | 18   | 59022  | 59039  | <i>accD-psaI</i>           |
| 30      | p2       | spacer | LSC    | TA        | 14   | 63376  | 63389  | <i>petA-psbJ</i>           |
| 31      | p1       | spacer | LSC    | T         | 10   | 66579  | 66588  | <i>trnW(CCA)-trnP(UGG)</i> |
| 32      | p1       | spacer | LSC    | C         | 12   | 67291  | 67302  | <i>psaJ-rpl33</i>          |
| 33      | p1       | spacer | LSC    | A         | 10   | 68892  | 68901  | <i>rpl20-rps12</i>         |
| 34      | p1       | spacer | LSC    | T         | 11   | 68960  | 68970  | <i>rpl20-rps12</i>         |
| 35      | p4       | spacer | LSC    | GATA      | 12   | 69890  | 69901  | <i>rps12-clpP</i>          |
| 36      | p1       | intron | LSC    | T         | 10   | 70443  | 70452  | <i>clpP</i>                |
| 37      | p1       | intron | LSC    | T         | 11   | 71239  | 71249  | <i>clpP</i>                |
| 38      | p1       | intron | LSC    | T         | 11   | 75002  | 75012  | <i>petB</i>                |
| 39      | p1       | spacer | LSC    | T         | 10   | 77981  | 77990  | <i>petD-rpoA</i>           |
| 40      | p1       | spacer | LSC    | A         | 10   | 79921  | 79930  | <i>rpl36-infA</i>          |
| 41      | p1       | spacer | LSC    | T         | 11   | 80751  | 80761  | <i>rps8-rpl14</i>          |
| 42      | p1       | spacer | LSC    | A         | 10   | 80792  | 80801  | <i>rps8-rpl14</i>          |
| 43      | p2       | spacer | LSC    | AT        | 16   | 80801  | 80816  | <i>rps8-rpl14</i>          |
| 44      | p1       | intron | LSC    | T         | 13   | 82378  | 82390  | <i>rpl16</i>               |
| 45      | p1       | intron | LSC    | T         | 10   | 82733  | 82742  | <i>rpl16</i>               |
| 46      | p2       | spacer | IRB    | AT        | 12   | 87789  | 87800  | <i>trnT(UGU)</i>           |
| 47      | p1       | spacer | IRB    | A         | 10   | 94889  | 94898  | <i>trnI(GAU)-rrn16</i>     |
| 48      | p1       | spacer | IRB    | A         | 12   | 104005 | 104016 | <i>rrn16-rps12</i>         |
| 49      | p1       | spacer | IRB    | A         | 10   | 107991 | 108000 | <i>trnH(GUG)-ycf2</i>      |
| 50      | p1       | spacer | IRB    | C         | 10   | 115418 | 115427 | <i>trnN(GUU)-ndhF</i>      |
| 51      | p1       | spacer | SSC    | A         | 10   | 117925 | 117934 | <i>ndhF-rpl21</i>          |
| 52      | p2       | spacer | SSC    | TA        | 22   | 119288 | 119309 | <i>rpl32-trnP(GGG)</i>     |
| 53      | p4       | spacer | SSC    | AAAT      | 12   | 119665 | 119676 | <i>trnP(GGG)-trnL(UAG)</i> |
| 54      | p1       | spacer | SSC    | T         | 13   | 119901 | 119913 | <i>trnL(UAG)-ccsA</i>      |
| 55      | p1       | spacer | SSC    | A         | 10   | 123033 | 123042 | <i>ndhD-psaC</i>           |
| 56      | p4       | spacer | SSC    | AATT      | 12   | 123041 | 123052 | <i>ndhD-psaC</i>           |
| 57      | p1       | spacer | SSC    | G         | 15   | 123587 | 123601 | <i>psaC-ndhE</i>           |

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | start  | end    | Location               |
|---------|----------|--------|--------|-----------|------|--------|--------|------------------------|
| 58      | p1       | spacer | SSC    | T         | 10   | 125374 | 125383 | <i>ndhI-ndhA</i>       |
| 59      | p2       | spacer | SSC    | AT        | 14   | 125418 | 125431 | <i>ndhI-ndhA</i>       |
| 60      | p2       | intron | SSC    | TA        | 16   | 126454 | 126469 | <i>ndhA</i>            |
| 61      | p1       | intron | SSC    | T         | 17   | 126960 | 126976 | <i>ndhA</i>            |
| 62      | p1       | spacer | SSC    | C         | 11   | 134606 | 134616 | <i>ycf1-chlN</i>       |
| 63      | p1       | spacer | IRA    | G         | 10   | 137487 | 137496 | <i>chlL-trnN(GUU)</i>  |
| 64      | p1       | spacer | IRA    | T         | 10   | 144914 | 144923 | <i>ycf2-trnH(GUG)</i>  |
| 65      | p1       | spacer | IRA    | T         | 12   | 148898 | 148909 | <i>rps12-rrn16</i>     |
| 66      | p1       | spacer | IRA    | T         | 10   | 158016 | 158025 | <i>rrn16-trnI(GAU)</i> |
| 67      | p2       | spacer | IRA    | AT        | 12   | 165114 | 165125 | <i>trnT(UGU)</i>       |

Appendix table 3 List of simple sequence repeats (SSRs) in the chloroplast genomes of *Alsophila gigantea*

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | star  | end   | Location                   |
|---------|----------|--------|--------|-----------|------|-------|-------|----------------------------|
| 1       | p1       | spacer | LSC    | G         | 10   | 3677  | 3686  | <i>matK-rps16</i>          |
| 2       | p1       | spacer | LSC    | G         | 11   | 7015  | 7025  | <i>chlB-trnQ(UUG)</i>      |
| 3       | p2       | spacer | LSC    | AT        | 14   | 8451  | 8464  | <i>trnS(GUC)-psaM</i>      |
| 4       | p4       | intron | LSC    | AATT      | 12   | 9622  | 9633  | <i>trnG(UCC)</i>           |
| 5       | p1       | spacer | LSC    | C         | 10   | 14251 | 14260 | <i>atpH-atpI</i>           |
| 6       | p1       | spacer | LSC    | C         | 10   | 15197 | 15206 | <i>atpI-rps2</i>           |
| 7       | p1       | intron | LSC    | C         | 10   | 22460 | 22469 | <i>rpoC1</i>               |
| 8       | p1       | spacer | LSC    | T         | 10   | 27192 | 27201 | <i>rpoB-trnD(GUC)</i>      |
| 9       | p4       | spacer | LSC    | ATAG      | 12   | 27704 | 27715 | <i>rpoB-trnD(GUC)</i>      |
| 10      | p4       | spacer | LSC    | TGAT      | 12   | 28490 | 28501 | <i>trnE(UUC)-psbM</i>      |
| 11      | p1       | spacer | LSC    | G         | 17   | 29622 | 29638 | <i>ycf66</i>               |
| 12      | p1       | spacer | LSC    | G         | 11   | 31628 | 31638 | <i>trnC(GCA)-trnG(GCC)</i> |
| 13      | p1       | spacer | LSC    | C         | 10   | 32088 | 32097 | <i>trnG(GCC)-psbZ</i>      |
| 14      | p4       | spacer | LSC    | TATC      | 12   | 33522 | 33533 | <i>trnS(UGA)-psbC</i>      |
| 15      | p4       | spacer | LSC    | ATAG      | 12   | 33537 | 33548 | <i>trnS(UGA)-psbC</i>      |
| 16      | p1       | spacer | LSC    | T         | 10   | 36642 | 36651 | <i>psbD-trnT(GGU)</i>      |
| 17      | p2       | spacer | LSC    | AG        | 24   | 36747 | 36770 | <i>psbD-trnT(GGU)</i>      |
| 18      | p1       | intron | LSC    | A         | 10   | 44100 | 44109 | <i>ycf3</i>                |
| 19      | p1       | intron | LSC    | T         | 12   | 44122 | 44133 | <i>ycf3</i>                |
| 20      | p1       | spacer | LSC    | T         | 10   | 45598 | 45607 | <i>ycf3-trnS(GGA)</i>      |
| 21      | p1       | spacer | LSC    | G         | 10   | 47441 | 47450 | <i>rps4-trnL(CAA)</i>      |
| 22      | p1       | spacer | LSC    | G         | 10   | 47936 | 47945 | <i>rps4-trnL(CAA)</i>      |
| 23      | p1       | spacer | LSC    | A         | 14   | 48913 | 48926 | <i>trnL(CAA)-trnF(GAA)</i> |
| 24      | p1       | spacer | LSC    | T         | 10   | 49833 | 49842 | <i>ndhJ-ndhK</i>           |
| 25      | p1       | spacer | LSC    | A         | 11   | 52073 | 52083 | <i>trnV(UAC)-trnM(CAU)</i> |
| 26      | p1       | spacer | LSC    | T         | 11   | 54541 | 54551 | <i>atpB-rbcL</i>           |
| 27      | p1       | spacer | LSC    | T         | 10   | 56891 | 56900 | <i>trnR(UCG)-accD</i>      |
| 28      | p4       | spacer | LSC    | TATT      | 12   | 57947 | 57958 | <i>accD-psaI</i>           |
| 29      | p2       | spacer | LSC    | AT        | 14   | 58413 | 58426 | <i>accD-psaI</i>           |
| 30      | p2       | spacer | LSC    | TA        | 16   | 62759 | 62774 | <i>petA-psbJ</i>           |
| 31      | p1       | spacer | LSC    | C         | 11   | 72815 | 72825 | <i>psaJ-rpl33</i>          |
| 32      | p1       | spacer | LSC    | A         | 11   | 74414 | 74424 | <i>rpl20-rps12</i>         |
| 33      | p1       | spacer | LSC    | T         | 10   | 74483 | 74492 | <i>rpl20-rps12</i>         |
| 34      | p4       | spacer | LSC    | GATA      | 12   | 75418 | 75429 | <i>rps12-clpP</i>          |
| 35      | p1       | intron | LSC    | C         | 11   | 75940 | 75950 | <i>clpP</i>                |

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | star   | end    | Location                   |
|---------|----------|--------|--------|-----------|------|--------|--------|----------------------------|
| 36      | p1       | intron | LSC    | T         | 10   | 75981  | 75990  | <i>clpP</i>                |
| 37      | p1       | intron | LSC    | T         | 11   | 81166  | 81176  | <i>petB</i>                |
| 38      | p1       | spacer | LSC    | A         | 11   | 86310  | 86320  | <i>rps8-rpl14</i>          |
| 39      | p2       | spacer | LSC    | AT        | 16   | 86320  | 86335  | <i>rps8-rpl14</i>          |
| 40      | p1       | spacer | LSC    | T         | 11   | 86335  | 86345  | <i>rps8-rpl14</i>          |
| 41      | p1       | intron | LSC    | T         | 12   | 88251  | 88262  | <i>rpl16</i>               |
| 42      | p1       | intron | LSC    | C         | 10   | 91441  | 91450  | <i>rpl2</i>                |
| 43      | p2       | spacer | IRB    | AT        | 12   | 93990  | 94001  | <i>trnT(UGU)</i>           |
| 44      | p1       | spacer | IRB    | A         | 12   | 101110 | 101121 | <i>trnI(GAU)-rrn16</i>     |
| 45      | p4       | spacer | IRB    | TCTT      | 12   | 103414 | 103425 | <i>rrn16-rps12</i>         |
| 46      | p1       | spacer | IRB    | A         | 12   | 104529 | 104540 | <i>rrn16-rps12</i>         |
| 47      | p3       | spacer | SSC    | AAT       | 12   | 119784 | 119795 | <i>rpl32-trnP(GGG)</i>     |
| 48      | p2       | spacer | SSC    | TA        | 18   | 119798 | 119815 | <i>rpl32-trnP(GGG)</i>     |
| 49      | p3       | spacer | SSC    | TAT       | 12   | 119817 | 119828 | <i>rpl32-trnP(GGG)</i>     |
| 50      | p4       | spacer | SSC    | GAAA      | 12   | 120167 | 120178 | <i>trnP(GGG)-trnL(UAG)</i> |
| 51      | p1       | spacer | SSC    | T         | 13   | 120409 | 120421 | <i>trnL(UAG)-ccsA</i>      |
| 52      | p1       | spacer | SSC    | A         | 11   | 121680 | 121690 | <i>ccsA-ndhD</i>           |
| 53      | p1       | spacer | SSC    | A         | 13   | 123545 | 123557 | <i>ndhD-psaC</i>           |
| 54      | p1       | spacer | SSC    | T         | 10   | 123566 | 123575 | <i>ndhD-psaC</i>           |
| 55      | p1       | spacer | SSC    | G         | 31   | 124104 | 124134 | <i>psaC-ndhE</i>           |
| 56      | p1       | spacer | SSC    | A         | 24   | 125949 | 125961 | <i>ndhI-ndhA</i>           |
| 57      | p2       | spacer | SSC    | AT        | 12   | 125961 | 125972 | <i>ndhI-ndhA</i>           |
| 58      | p2       | intron | SSC    | TA        | 14   | 126999 | 127012 | <i>ndhA</i>                |
| 59      | p1       | spacer | SSC    | C         | 31   | 135145 | 135175 | <i>ycf1-chlN</i>           |
| 60      | p4       | spacer | SSC    | AAAT      | 16   | 135394 | 135409 | <i>ycf1-chlN</i>           |
| 61      | p1       | spacer | IRA    | T         | 12   | 149455 | 149466 | <i>rps12-rrn16</i>         |
| 62      | p4       | spacer | IRA    | AAAG      | 12   | 150569 | 150580 | <i>rps12-rrn16</i>         |
| 63      | p1       | spacer | IRA    | T         | 12   | 152874 | 152885 | <i>rrn16-trnI(GAU)</i>     |
| 64      | p2       | spacer | IRA    | AT        | 12   | 159994 | 160005 | <i>trnT(UGU)</i>           |

Appendix table 4 List of simple sequence repeats (SSRs) in the chloroplast genomes of *Alsophila gigantea*

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | star  | end   | Location                   |
|---------|----------|--------|--------|-----------|------|-------|-------|----------------------------|
| 1       | p1       | spacer | LSC    | G         | 12   | 7094  | 7105  | <i>chlB-trnQ(UUG)</i>      |
| 2       | p1       | spacer | LSC    | T         | 10   | 8416  | 8425  | <i>trnS(GUC)-psaM</i>      |
| 3       | p2       | spacer | LSC    | AT        | 14   | 8532  | 8545  | <i>trnS(GUC)-psaM</i>      |
| 4       | p4       | intron | LSC    | AATT      | 12   | 9703  | 9714  | <i>trnG(UCC)</i>           |
| 5       | p1       | intron | LSC    | C         | 10   | 22538 | 22547 | <i>trnG(UCC)</i>           |
| 6       | p1       | spacer | LSC    | T         | 10   | 27270 | 27279 | <i>rpoB-trnD(GUC)</i>      |
| 7       | p4       | spacer | LSC    | ATAG      | 12   | 27782 | 27793 | <i>rpoB-trnD(GUC)</i>      |
| 8       | p4       | spacer | LSC    | TGAT      | 12   | 28568 | 28579 | <i>trnE(UUC)-psbM</i>      |
| 9       | p1       | spacer | LSC    | G         | 14   | 29700 | 29713 | <i>ycf66</i>               |
| 10      | p1       | spacer | LSC    | G         | 11   | 31714 | 31724 | <i>trnC(GCA)-trnG(GCC)</i> |
| 11      | p1       | spacer | LSC    | C         | 12   | 32174 | 32185 | <i>trnG(GCC)-psbZ</i>      |
| 12      | p4       | spacer | LSC    | TATC      | 12   | 33610 | 33621 | <i>trnS(UGA)-psbC</i>      |
| 13      | p4       | spacer | LSC    | ATAG      | 12   | 33625 | 33636 | <i>trnS(UGA)-psbC</i>      |
| 14      | p1       | spacer | LSC    | T         | 10   | 36730 | 36739 | <i>psbD-trnT(GGU)</i>      |
| 15      | p2       | spacer | LSC    | AG        | 16   | 36835 | 36850 | <i>psbD-trnT(GGU)</i>      |
| 16      | p1       | intron | LSC    | T         | 13   | 44201 | 44213 | <i>ycf3</i>                |

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | star   | end    | Location                   |
|---------|----------|--------|--------|-----------|------|--------|--------|----------------------------|
| 17      | p1       | spacer | LSC    | T         | 10   | 45678  | 45687  | <i>ycf3-trnS(GGA)</i>      |
| 18      | p1       | spacer | LSC    | G         | 10   | 47521  | 47530  | <i>rps4-trnL(CAA)</i>      |
| 19      | p1       | spacer | LSC    | G         | 10   | 48027  | 48036  | <i>rps4-trnL(CAA)</i>      |
| 20      | p1       | spacer | LSC    | A         | 13   | 48988  | 49000  | <i>trnL(CAA)-trnF(GAA)</i> |
| 21      | p1       | spacer | LSC    | T         | 10   | 49907  | 49916  | <i>ndhJ-ndhK</i>           |
| 22      | p1       | spacer | LSC    | A         | 10   | 52147  | 52156  | <i>trnV(UAC)-trnM(CAU)</i> |
| 23      | p1       | spacer | LSC    | T         | 11   | 54614  | 54624  | <i>atpB-rbcL</i>           |
| 24      | p1       | spacer | LSC    | T         | 10   | 56964  | 56973  | <i>trnR(UCG)-accD</i>      |
| 25      | p4       | spacer | LSC    | TATT      | 12   | 58020  | 58031  | <i>accD-psaI</i>           |
| 26      | p2       | spacer | LSC    | AT        | 14   | 58486  | 58499  | <i>accD-psaI</i>           |
| 27      | p2       | spacer | LSC    | TA        | 16   | 62833  | 62848  | <i>petA-psbJ</i>           |
| 28      | p5       | spacer | LSC    | CTTTC     | 15   | 71752  | 71766  | <i>petG-trnW(CCA)</i>      |
| 29      | p1       | spacer | LSC    | C         | 12   | 72879  | 72890  | <i>psaJ-rpl33</i>          |
| 30      | p1       | spacer | LSC    | A         | 10   | 74479  | 74488  | <i>rpl20-rps12</i>         |
| 31      | p1       | spacer | LSC    | T         | 10   | 74547  | 74556  | <i>rpl20-rps12</i>         |
| 32      | p4       | spacer | LSC    | GATA      | 12   | 75482  | 75493  | <i>rps12-clpP</i>          |
| 33      | p1       | intron | LSC    | C         | 10   | 76004  | 76013  | <i>clpP</i>                |
| 34      | p1       | intron | LSC    | T         | 14   | 76044  | 76057  | <i>clpP</i>                |
| 35      | p1       | intron | LSC    | T         | 10   | 81233  | 81242  | <i>petB</i>                |
| 36      | p2       | spacer | LSC    | AT        | 18   | 86384  | 86401  | <i>rps8-rpl14</i>          |
| 37      | p1       | intron | LSC    | T         | 11   | 88315  | 88325  | <i>rpl16</i>               |
| 38      | p1       | intron | LSC    | C         | 10   | 91506  | 91515  | <i>rpl2</i>                |
| 39      | p2       | spacer | IRB    | AT        | 12   | 94055  | 94066  | <i>trnT(UGU)</i>           |
| 40      | p1       | spacer | IRB    | A         | 12   | 101175 | 101186 | <i>trnI(GAU)-rrn16</i>     |
| 41      | p4       | spacer | IRB    | TCTT      | 12   | 103479 | 103490 | <i>rrn16-rps12</i>         |
| 42      | p1       | spacer | IRB    | A         | 14   | 104578 | 104591 | <i>rrn16-rps12</i>         |
| 43      | p1       | spacer | IRB    | T         | 10   | 106780 | 106789 | <i>rps7-psbA</i>           |
| 44      | p3       | spacer | SSC    | AAT       | 12   | 119836 | 119847 | <i>rpl32-trnP(GGG)</i>     |
| 45      | p2       | spacer | SSC    | TA        | 18   | 119850 | 119867 | <i>rpl32-trnP(GGG)</i>     |
| 46      | p3       | spacer | SSC    | TAT       | 12   | 119869 | 119880 | <i>rpl32-trnP(GGG)</i>     |
| 47      | p4       | spacer | SSC    | GAAA      | 12   | 120219 | 120230 | <i>trnP(GGG)-trnL(UAG)</i> |
| 48      | p1       | spacer | SSC    | T         | 12   | 120461 | 120472 | <i>trnL(UAG)-ccsA</i>      |
| 49      | p1       | spacer | SSC    | A         | 11   | 121731 | 121741 | <i>ccsA-ndhD</i>           |
| 50      | p1       | spacer | SSC    | A         | 11   | 123596 | 123606 | <i>ndhD-psaC</i>           |
| 51      | p4       | spacer | SSC    | AATT      | 12   | 123605 | 123616 | <i>ndhD-psaC</i>           |
| 52      | p1       | spacer | SSC    | T         | 10   | 123615 | 123624 | <i>ndhD-psaC</i>           |
| 53      | p1       | spacer | SSC    | G         | 15   | 124153 | 124167 | <i>psaC-ndhE</i>           |
| 54      | p1       | spacer | SSC    | A         | 24   | 125982 | 125992 | <i>ndhI-ndhA</i>           |
| 55      | p2       | spacer | SSC    | AT        | 14   | 125992 | 126005 | <i>ndhI-ndhA</i>           |
| 56      | p1       | spacer | SSC    | A         | 13   | 126041 | 126053 | <i>ndhI-ndhA</i>           |
| 57      | p2       | intron | SSC    | TA        | 14   | 127030 | 127043 | <i>ndhA</i>                |
| 58      | p1       | spacer | SSC    | A         | 11   | 129846 | 129856 | <i>rps15-ycf1</i>          |
| 59      | p1       | spacer | SSC    | C         | 14   | 135178 | 135191 | <i>ycf1-chlN</i>           |
| 60      | p4       | spacer | SSC    | AAAT      | 16   | 135410 | 135425 | <i>ycf1-chlN</i>           |
| 61      | p1       | spacer | IRA    | A         | 10   | 147274 | 147283 | <i>psbA-rps7</i>           |
| 62      | p1       | spacer | IRA    | T         | 14   | 149472 | 149485 | <i>rps12-rrn16</i>         |
| 63      | p4       | spacer | IRA    | AAAG      | 12   | 150572 | 150583 | <i>rps12-rrn16</i>         |
| 64      | p1       | spacer | IRA    | T         | 12   | 152877 | 152888 | <i>rrn16-trnI(GAU)</i>     |
| 65      | p2       | spacer | IRA    | AT        | 12   | 159997 | 160008 | <i>trnT(UGU)</i>           |

Appendix table 5 List of simple sequence repeats (SSRs) in the chloroplast genomes of *Alsophila costularis*

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | star  | end   | Location                   |
|---------|----------|--------|--------|-----------|------|-------|-------|----------------------------|
| 1       | p1       | intron | LSC    | T         | 11   | 367   | 374   | <i>ndhB</i>                |
| 2       | p1       | spacer | LSC    | A         | 12   | 3443  | 3454  | <i>matK-rps16</i>          |
| 3       | p1       | spacer | LSC    | T         | 10   | 3538  | 3547  | <i>matK-rps16</i>          |
| 4       | p1       | intron | LSC    | T         | 11   | 4288  | 4298  | <i>rps16</i>               |
| 5       | p1       | spacer | LSC    | G         | 15   | 6798  | 6812  | <i>chlB-trnQ(UUG)</i>      |
| 6       | p1       | spacer | LSC    | A         | 11   | 7517  | 7527  | <i>psbK-psbI</i>           |
| 7       | p2       | spacer | LSC    | TA        | 12   | 7529  | 7540  | <i>psbK-psbI</i>           |
| 8       | p1       | spacer | LSC    | C         | 13   | 8126  | 8138  | <i>trnS(GCU)-psaM</i>      |
| 9       | p2       | spacer | LSC    | AT        | 16   | 8229  | 8244  | <i>trnS(GCU)-psaM</i>      |
| 10      | p1       | spacer | LSC    | T         | 10   | 8244  | 8253  | <i>trnS(GCU)-psaM</i>      |
| 11      | p1       | spacer | LSC    | G         | 11   | 8797  | 8807  | <i>ycf12-trnG(UCC)</i>     |
| 12      | p1       | intron | LSC    | C         | 14   | 9681  | 9694  | <i>trnG(UCC)</i>           |
| 13      | p1       | spacer | LSC    | C         | 19   | 14047 | 14065 | <i>atpH-atpI</i>           |
| 14      | p1       | spacer | LSC    | A         | 11   | 14078 | 14088 | <i>atpH-atpI</i>           |
| 15      | p1       | spacer | LSC    | T         | 13   | 15006 | 15018 | <i>atpI-rps2</i>           |
| 16      | p1       | intron | LSC    | T         | 13   | 22288 | 22300 | <i>rpoC1</i>               |
| 17      | p3       | intron | LSC    | AAG       | 12   | 22479 | 22490 | <i>rpoC1</i>               |
| 18      | p1       | spacer | LSC    | A         | 11   | 23235 | 23245 | <i>rpoC1-rpoB</i>          |
| 19      | p1       | spacer | LSC    | T         | 10   | 27599 | 27608 | <i>rpoB-trnD(GUC)</i>      |
| 20      | p1       | spacer | LSC    | A         | 12   | 28205 | 28216 | <i>trnE(UUC)-psbM</i>      |
| 21      | p1       | spacer | LSC    | A         | 12   | 29369 | 29380 | <i>ycf66</i>               |
| 22      | p2       | spacer | LSC    | AT        | 18   | 30361 | 30378 | <i>petN-trnC(GCA)</i>      |
| 23      | p1       | spacer | LSC    | G         | 12   | 31701 | 31712 | <i>trnC(GCA)-trnG(GCC)</i> |
| 24      | p1       | spacer | LSC    | A         | 13   | 31782 | 31794 | <i>trnC(GCA)-trnG(GCC)</i> |
| 25      | p1       | spacer | LSC    | A         | 12   | 32996 | 33007 | <i>trnS(UGA)-psbC</i>      |
| 26      | p1       | spacer | LSC    | T         | 13   | 33067 | 33079 | <i>trnS(UGA)-psbC</i>      |
| 27      | p4       | spacer | LSC    | ATCT      | 16   | 33600 | 33615 | <i>trnS(UGA)-psbC</i>      |
| 28      | p4       | spacer | LSC    | ATAG      | 12   | 33618 | 33629 | <i>trnS(UGA)-psbC</i>      |
| 29      | p1       | spacer | LSC    | T         | 11   | 36726 | 36736 | <i>psbD-trnT(GGU)</i>      |
| 30      | p1       | spacer | LSC    | A         | 10   | 36854 | 36863 | <i>psbD-trnT(GGU)</i>      |
| 31      | p1       | spacer | LSC    | T         | 19   | 37272 | 37290 | <i>psbD-trnT(GGU)</i>      |
| 32      | p1       | spacer | LSC    | A         | 12   | 37670 | 37681 | <i>psbD-trnT(GGU)</i>      |
| 33      | p1       | intron | LSC    | T         | 11   | 44231 | 44241 | <i>ycf3</i>                |
| 34      | p1       | intron | LSC    | C         | 11   | 45328 | 45338 | <i>ycf3</i>                |
| 35      | p1       | spacer | LSC    | T         | 15   | 45725 | 45739 | <i>ycf3-trnS(GGA)</i>      |
| 36      | p1       | spacer | LSC    | A         | 10   | 45781 | 45790 | <i>ycf3-trnS(GGA)</i>      |
| 37      | p1       | spacer | LSC    | T         | 12   | 45851 | 45862 | <i>ycf3-trnS(GGA)</i>      |
| 38      | p1       | spacer | LSC    | T         | 11   | 46321 | 46331 | <i>trnS(GGA)-rps4</i>      |
| 39      | p1       | spacer | LSC    | G         | 10   | 48024 | 48033 | <i>rps4-trnL(CAA)</i>      |
| 40      | p1       | intron | LSC    | A         | 14   | 48342 | 48355 | <i>trnL(CAA)</i>           |
| 41      | p1       | spacer | LSC    | T         | 14   | 48944 | 48957 | <i>trnL(CAA)-trnF(GAA)</i> |
| 42      | p1       | spacer | LSC    | T         | 10   | 51286 | 51295 | <i>ndhC-trnV(UAC)</i>      |
| 43      | p1       | spacer | LSC    | A         | 11   | 51302 | 51312 | <i>ndhC-trnV(UAC)</i>      |
| 44      | p1       | spacer | LSC    | A         | 10   | 52147 | 52156 | <i>trnV(UAC)-trnM(CAU)</i> |
| 45      | p2       | spacer | LSC    | AT        | 14   | 52242 | 52255 | <i>trnV(UAC)-trnM(CAU)</i> |
| 46      | p1       | spacer | LSC    | T         | 13   | 54712 | 54724 | <i>atpB-rbcL</i>           |
| 47      | p1       | spacer | LSC    | T         | 12   | 56557 | 56568 | <i>rbcL-trnR(UCG)</i>      |
| 48      | p1       | spacer | LSC    | T         | 19   | 56978 | 56996 | <i>trnR(UCG)-accD</i>      |

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | star   | end    | Location                   |
|---------|----------|--------|--------|-----------|------|--------|--------|----------------------------|
| 49      | p4       | spacer | LSC    | TATT      | 12   | 58025  | 58036  | <i>accD-psaI</i>           |
| 50      | p1       | spacer | LSC    | A         | 10   | 58085  | 58094  | <i>accD-psaI</i>           |
| 51      | p1       | spacer | LSC    | T         | 10   | 58508  | 58517  | <i>accD-psaI</i>           |
| 52      | p1       | spacer | LSC    | C         | 11   | 62532  | 62542  | <i>petA-psbJ</i>           |
| 53      | p1       | spacer | LSC    | T         | 13   | 63161  | 63173  | <i>petA-psbJ</i>           |
| 54      | p1       | spacer | LSC    | T         | 11   | 64725  | 64735  | <i>psbE-petL</i>           |
| 55      | p1       | spacer | LSC    | T         | 10   | 65541  | 65550  | <i>petG-trnW(CCA)</i>      |
| 56      | p1       | spacer | LSC    | T         | 10   | 65940  | 65949  | <i>trnW(CCA)-trnP(UGG)</i> |
| 57      | p1       | spacer | LSC    | T         | 18   | 67335  | 67352  | <i>rps18-rpl20</i>         |
| 58      | p1       | spacer | LSC    | T         | 12   | 68310  | 68321  | <i>rpl20-rps12</i>         |
| 59      | p1       | spacer | LSC    | T         | 11   | 68513  | 68523  | <i>rpl20-rps12</i>         |
| 60      | p1       | spacer | LSC    | T         | 11   | 68770  | 68780  | <i>rpl20-rps12</i>         |
| 61      | p4       | spacer | LSC    | TATC      | 12   | 69226  | 69237  | <i>rps12-clpP</i>          |
| 62      | p4       | spacer | LSC    | GATA      | 12   | 69244  | 69255  | <i>rps12-clpP</i>          |
| 63      | p1       | intron | LSC    | C         | 42   | 69761  | 69802  | <i>clpP</i>                |
| 64      | p1       | intron | LSC    | C         | 10   | 70520  | 70529  | <i>clpP</i>                |
| 65      | p2       | intron | LSC    | AT        | 24   | 70655  | 70678  | <i>clpP</i>                |
| 66      | p1       | spacer | LSC    | A         | 11   | 71659  | 71669  | <i>clpP-psbB</i>           |
| 67      | p1       | intron | LSC    | T         | 16   | 74446  | 74461  | <i>petB</i>                |
| 68      | p1       | intron | LSC    | T         | 10   | 76585  | 76594  | <i>petD</i>                |
| 69      | p1       | spacer | LSC    | T         | 13   | 80192  | 80204  | <i>rps8-rpl14</i>          |
| 70      | p2       | spacer | LSC    | AT        | 18   | 80224  | 80241  | <i>rps8-rpl14</i>          |
| 71      | p1       | spacer | LSC    | A         | 14   | 80308  | 80321  | <i>rps8-rpl14</i>          |
| 72      | p2       | spacer | LSC    | TA        | 12   | 80322  | 80333  | <i>rps8-rpl14</i>          |
| 73      | p1       | spacer | LSC    | T         | 12   | 80342  | 80353  | <i>rps8-rpl14</i>          |
| 74      | p1       | intron | LSC    | T         | 12   | 81928  | 81939  | <i>rpl16</i>               |
| 75      | p4       | intron | LSC    | CCTC      | 12   | 82094  | 82105  | <i>rpl16</i>               |
| 76      | p1       | intron | LSC    | T         | 13   | 82267  | 82279  | <i>rpl16</i>               |
| 77      | p1       | spacer | LSC    | T         | 10   | 82493  | 82502  | <i>rpl16-rps3</i>          |
| 78      | p1       | intron | LSC    | A         | 14   | 84696  | 84709  | <i>rpl2</i>                |
| 79      | p1       | spacer | LSC    | A         | 12   | 85684  | 85695  | <i>rpl2-rpl23</i>          |
| 80      | p1       | spacer | LSC    | T         | 13   | 85997  | 86009  | <i>rpl23-trnI(CAU)</i>     |
| 81      | p1       | spacer | LSC    | T         | 11   | 86139  | 86149  | <i>rpl23-trnI(CAU)</i>     |
| 82      | p2       | spacer | IRB    | AT        | 12   | 88021  | 88032  | <i>trnT(UGU)</i>           |
| 83      | p2       | spacer | IRB    | AT        | 12   | 88476  | 88487  | <i>trnT(UGU)-trnR(ACG)</i> |
| 84      | p1       | spacer | IRB    | T         | 13   | 89923  | 89935  | <i>trnR(ACG)-rrn5</i>      |
| 85      | p1       | spacer | IRB    | C         | 13   | 97451  | 97463  | <i>rrn16-rps12</i>         |
| 86      | p4       | spacer | IRB    | TCTT      | 12   | 97984  | 97995  | <i>rrn16-rps12</i>         |
| 87      | p1       | spacer | IRB    | T         | 11   | 99097  | 99107  | <i>rrn16-rps12</i>         |
| 88      | p1       | spacer | IRB    | A         | 12   | 103055 | 103066 | <i>trnH(GUG)-ycf2</i>      |
| 89      | p4       | CDS    | IRB    | AGAA      | 12   | 108231 | 108242 | <i>ycf2</i>                |
| 90      | p1       | spacer | IRB    | A         | 17   | 109641 | 109657 | <i>ycf2-trnN(GUU)</i>      |
| 91      | p1       | spacer | IRB    | G         | 17   | 110427 | 110443 | <i>trnN(GUU)-ndhF</i>      |
| 92      | p1       | spacer | SSC    | T         | 10   | 113600 | 113609 | <i>rpl21-rpl32</i>         |
| 93      | p1       | spacer | SSC    | A         | 13   | 113713 | 113725 | <i>rpl21-rpl32</i>         |
| 94      | p2       | spacer | SSC    | AT        | 16   | 113725 | 113740 | <i>rpl21-rpl32</i>         |
| 95      | p1       | spacer | SSC    | A         | 12   | 114078 | 114089 | <i>rpl32-trnP(GGG)</i>     |
| 96      | p1       | spacer | SSC    | G         | 10   | 114333 | 114342 | <i>rpl32-trnP(GGG)</i>     |
| 97      | p1       | spacer | SSC    | T         | 14   | 114400 | 114413 | <i>rpl32-trnP(GGG)</i>     |
| 98      | p1       | spacer | SSC    | A         | 13   | 114740 | 114752 | <i>trnP(GGG)-trnL(UAG)</i> |

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | star   | end    | Location                   |
|---------|----------|--------|--------|-----------|------|--------|--------|----------------------------|
| 99      | p1       | spacer | SSC    | A         | 11   | 114900 | 114910 | <i>trnL(UAG)-ccsA</i>      |
| 100     | p1       | spacer | SSC    | T         | 12   | 114979 | 114990 | <i>trnL(UAG)-ccsA</i>      |
| 101     | p1       | spacer | SSC    | A         | 11   | 118132 | 118142 | <i>ndhD-psaC</i>           |
| 102     | p1       | spacer | SSC    | T         | 12   | 118147 | 118158 | <i>ndhD-psaC</i>           |
| 103     | p1       | spacer | SSC    | G         | 13   | 118694 | 118706 | <i>psaC-ndhE</i>           |
| 104     | p2       | spacer | SSC    | TA        | 14   | 120509 | 120522 | <i>ndhI-ndhA</i>           |
| 105     | p1       | spacer | SSC    | A         | 13   | 120526 | 120538 | <i>ndhI-ndhA</i>           |
| 106     | p2       | spacer | SSC    | AT        | 20   | 120538 | 120557 | <i>ndhI-ndhA</i>           |
| 107     | p1       | spacer | SSC    | A         | 10   | 120602 | 120611 | <i>ndhI-ndhA</i>           |
| 108     | p1       | intron | SSC    | T         | 10   | 121522 | 121531 | <i>ndhA</i>                |
| 109     | p1       | spacer | SSC    | A         | 14   | 124314 | 124327 | <i>rps15-ycf1</i>          |
| 110     | p4       | CDS    | SSC    | ACCA      | 12   | 129098 | 129109 | <i>ycf1</i>                |
| 111     | p1       | spacer | SSC    | C         | 13   | 129639 | 129651 | <i>ycf1-chlN</i>           |
| 112     | p1       | spacer | IRA    | C         | 17   | 132571 | 132587 | <i>chlL-trnN(GUU)</i>      |
| 113     | p1       | spacer | IRA    | T         | 17   | 133357 | 133373 | <i>trnN(GUU)-ycf2</i>      |
| 114     | p4       | CDS    | IRA    | TTTC      | 12   | 134771 | 134782 | <i>ycf2</i>                |
| 115     | p1       | spacer | IRA    | T         | 12   | 139948 | 139959 | <i>ycf2-trnH(GUG)</i>      |
| 116     | p1       | spacer | IRA    | A         | 11   | 143907 | 143917 | <i>rps12-rrn16</i>         |
| 117     | p4       | spacer | IRA    | AAAG      | 12   | 145018 | 145029 | <i>rps12-rrn16</i>         |
| 118     | p1       | spacer | IRA    | G         | 13   | 145551 | 145563 | <i>rps12-rrn16</i>         |
| 119     | p1       | spacer | IRA    | A         | 13   | 153079 | 153091 | <i>rrn5-trnR(ACG)</i>      |
| 120     | p2       | spacer | IRA    | AT        | 12   | 154527 | 154538 | <i>trnR(ACG)-trnT(UGU)</i> |
| 121     | p2       | spacer | IRA    | AT        | 12   | 154982 | 154993 | <i>trnT(UGU)</i>           |

Appendix table 6 List of simple sequence repeats (SSRs) in the chloroplast genomes of *Alsophila spinulosa*

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | star  | end   | Location               |
|---------|----------|--------|--------|-----------|------|-------|-------|------------------------|
| 1       | p1       | intron | LSC    | T         | 11   | 364   | 374   | <i>ndhB</i>            |
| 2       | p1       | spacer | LSC    | A         | 13   | 3443  | 3455  | <i>matK-rps16</i>      |
| 3       | p1       | spacer | LSC    | T         | 10   | 3539  | 3548  | <i>matK-rps16</i>      |
| 4       | p1       | intron | LSC    | T         | 11   | 4289  | 4299  | <i>rps16</i>           |
| 5       | p1       | spacer | LSC    | G         | 14   | 6799  | 6812  | <i>chlB-trnQ(UUG)</i>  |
| 6       | p1       | spacer | LSC    | A         | 24   | 7517  | 7527  | <i>psbK-psbI</i>       |
| 7       | p2       | spacer | LSC    | TA        | 12   | 7529  | 7540  | <i>psbK-psbI</i>       |
| 8       | p1       | spacer | LSC    | C         | 12   | 8126  | 8137  | <i>trnS(GCU)-psaM</i>  |
| 9       | p2       | spacer | LSC    | AT        | 16   | 8228  | 8243  | <i>trnS(GCU)-psaM</i>  |
| 10      | p1       | spacer | LSC    | T         | 10   | 8243  | 8252  | <i>trnS(GCU)-psaM</i>  |
| 11      | p1       | spacer | LSC    | G         | 11   | 8796  | 8806  | <i>ycf12-trnG(UCC)</i> |
| 12      | p1       | intron | LSC    | C         | 13   | 9680  | 9692  | <i>trnG(UCC)</i>       |
| 13      | p1       | spacer | LSC    | C         | 10   | 14045 | 14054 | <i>atpH-atpI</i>       |
| 14      | p1       | spacer | LSC    | A         | 11   | 14072 | 14082 | <i>atpH-atpI</i>       |
| 15      | p1       | spacer | LSC    | T         | 12   | 15000 | 15011 | <i>atpI-rps2</i>       |
| 16      | p1       | intron | LSC    | T         | 13   | 22281 | 22293 | <i>rpoC1</i>           |
| 17      | p3       | intron | LSC    | AAG       | 12   | 22472 | 22483 | <i>rpoC1</i>           |
| 18      | p1       | spacer | LSC    | A         | 11   | 23228 | 23238 | <i>rpoC1-rpoB</i>      |
| 19      | p1       | spacer | LSC    | T         | 10   | 27592 | 27601 | <i>rpoB-trnD(GUC)</i>  |
| 20      | p1       | spacer | LSC    | A         | 12   | 28198 | 28209 | <i>trnE(UUC)-psbM</i>  |
| 21      | p1       | spacer | LSC    | A         | 12   | 29362 | 29373 | <i>ycf66</i>           |
| 22      | p2       | spacer | LSC    | AT        | 18   | 30354 | 30371 | <i>petN-trnC(GCA)</i>  |

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | star  | end   | Location                   |
|---------|----------|--------|--------|-----------|------|-------|-------|----------------------------|
| 23      | p1       | spacer | LSC    | G         | 12   | 31694 | 31705 | <i>trnC(GCA)-trnG(GCC)</i> |
| 24      | p1       | spacer | LSC    | A         | 13   | 31775 | 31787 | <i>trnC(GCA)-trnG(GCC)</i> |
| 25      | p1       | spacer | LSC    | A         | 12   | 32989 | 33000 | <i>trnS(UGA)-psbC</i>      |
| 26      | p1       | spacer | LSC    | T         | 13   | 33060 | 33072 | <i>trnS(UGA)-psbC</i>      |
| 27      | p4       | spacer | LSC    | ATCT      | 16   | 33593 | 33608 | <i>trnS(UGA)-psbC</i>      |
| 28      | p4       | spacer | LSC    | ATAG      | 12   | 33611 | 33622 | <i>trnS(UGA)-psbC</i>      |
| 29      | p1       | spacer | LSC    | T         | 11   | 36719 | 36729 | <i>psbD-trnT(GGU)</i>      |
| 30      | p1       | spacer | LSC    | A         | 10   | 36847 | 36856 | <i>psbD-trnT(GGU)</i>      |
| 31      | p1       | spacer | LSC    | T         | 19   | 37265 | 37283 | <i>psbD-trnT(GGU)</i>      |
| 32      | p1       | spacer | LSC    | A         | 11   | 37663 | 37673 | <i>psbD-trnT(GGU)</i>      |
| 33      | p1       | intron | LSC    | T         | 12   | 44223 | 44234 | <i>ycf3</i>                |
| 34      | p1       | intron | LSC    | C         | 11   | 45321 | 45331 | <i>ycf3</i>                |
| 35      | p1       | spacer | LSC    | T         | 15   | 45718 | 45732 | <i>ycf3-trnS(GGA)</i>      |
| 36      | p1       | spacer | LSC    | A         | 10   | 45774 | 45783 | <i>ycf3-trnS(GGA)</i>      |
| 37      | p1       | spacer | LSC    | T         | 12   | 45844 | 45855 | <i>ycf3-trnS(GGA)</i>      |
| 38      | p1       | spacer | LSC    | T         | 11   | 46314 | 46324 | <i>trnS(GGA)-rps4</i>      |
| 39      | p1       | spacer | LSC    | G         | 10   | 48017 | 48026 | <i>rps4-trnL(CAA)</i>      |
| 40      | p1       | intron | LSC    | A         | 14   | 48335 | 48348 | <i>trnL(CAA)</i>           |
| 41      | p1       | spacer | LSC    | T         | 13   | 48937 | 48949 | <i>trnL(CAA)-trnF(GAA)</i> |
| 42      | p1       | spacer | LSC    | T         | 10   | 51278 | 51287 | <i>ndhC-trnV(UAC)</i>      |
| 43      | p1       | spacer | LSC    | A         | 11   | 51294 | 51304 | <i>ndhC-trnV(UAC)</i>      |
| 44      | p1       | spacer | LSC    | A         | 10   | 52139 | 52148 | <i>trnV(UAC)-trnM(CAU)</i> |
| 45      | p2       | spacer | LSC    | AT        | 14   | 52234 | 52247 | <i>trnV(UAC)-trnM(CAU)</i> |
| 46      | p1       | spacer | LSC    | T         | 13   | 54704 | 54716 | <i>atpB-rbcL</i>           |
| 47      | p1       | spacer | LSC    | T         | 12   | 56549 | 56560 | <i>rbcL-trnR(UCG)</i>      |
| 48      | p1       | spacer | LSC    | T         | 19   | 56970 | 56988 | <i>trnR(UCG)-accD</i>      |
| 49      | p4       | spacer | LSC    | TATT      | 12   | 58017 | 58028 | <i>accD-psaI</i>           |
| 50      | p1       | spacer | LSC    | A         | 10   | 58077 | 58086 | <i>accD-psaI</i>           |
| 51      | p1       | spacer | LSC    | T         | 11   | 58500 | 58510 | <i>accD-psaI</i>           |
| 52      | p1       | spacer | LSC    | C         | 11   | 62525 | 62535 | <i>petA-psbJ</i>           |
| 53      | p1       | spacer | LSC    | T         | 13   | 63154 | 63166 | <i>petA-psbJ</i>           |
| 54      | p1       | spacer | LSC    | T         | 11   | 64718 | 64728 | <i>psbE-petL</i>           |
| 55      | p1       | spacer | LSC    | T         | 10   | 65534 | 65543 | <i>petG-trnW(CCA)</i>      |
| 56      | p1       | spacer | LSC    | T         | 10   | 65933 | 65942 | <i>trnW(CCA)-trnP(UGG)</i> |
| 57      | p1       | spacer | LSC    | C         | 10   | 66640 | 66649 | <i>psaJ-rpl33</i>          |
| 58      | p1       | spacer | LSC    | T         | 17   | 67329 | 67345 | <i>rps18-rpl20</i>         |
| 59      | p1       | spacer | LSC    | T         | 12   | 68303 | 68314 | <i>rpl20-rps12</i>         |
| 60      | p1       | spacer | LSC    | T         | 11   | 68506 | 68516 | <i>rpl20-rps12</i>         |
| 61      | p1       | spacer | LSC    | T         | 11   | 68763 | 68773 | <i>rpl20-rps12</i>         |
| 62      | p4       | spacer | LSC    | TATC      | 12   | 69219 | 69230 | <i>rps12-clpP</i>          |
| 63      | p4       | spacer | LSC    | GATA      | 12   | 69237 | 69248 | <i>rps12-clpP</i>          |
| 64      | p1       | intron | LSC    | C         | 17   | 69754 | 69770 | <i>clpP</i>                |
| 65      | p1       | intron | LSC    | C         | 11   | 70488 | 70498 | <i>clpP</i>                |
| 66      | p2       | intron | LSC    | AT        | 24   | 70624 | 70647 | <i>clpP</i>                |
| 67      | p1       | spacer | LSC    | A         | 11   | 71628 | 71638 | <i>clpP-psbB</i>           |
| 68      | p1       | intron | LSC    | T         | 16   | 74415 | 74430 | <i>petB</i>                |
| 69      | p1       | intron | LSC    | T         | 10   | 76554 | 76563 | <i>petD</i>                |
| 70      | p1       | spacer | LSC    | T         | 13   | 80161 | 80173 | <i>rps8-rpl14</i>          |
| 71      | p2       | spacer | LSC    | AT        | 18   | 80193 | 80210 | <i>rps8-rpl14</i>          |
| 72      | p1       | spacer | LSC    | A         | 14   | 80277 | 80290 | <i>rps8-rpl14</i>          |

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | star   | end    | Location                   |
|---------|----------|--------|--------|-----------|------|--------|--------|----------------------------|
| 73      | p2       | spacer | LSC    | TA        | 12   | 80291  | 80302  | <i>rps8-rpl14</i>          |
| 74      | p1       | spacer | LSC    | T         | 12   | 80311  | 80322  | <i>rps8-rpl14</i>          |
| 75      | p1       | intron | LSC    | T         | 12   | 81897  | 81908  | <i>rpl16</i>               |
| 76      | p4       | intron | LSC    | CCTC      | 12   | 82063  | 82074  | <i>rpl16</i>               |
| 77      | p1       | intron | LSC    | T         | 13   | 82236  | 82248  | <i>rpl16</i>               |
| 78      | p1       | spacer | LSC    | T         | 10   | 82462  | 82471  | <i>rpl16-rps3</i>          |
| 79      | p1       | intron | LSC    | A         | 15   | 84665  | 84679  | <i>rpl2</i>                |
| 80      | p1       | spacer | LSC    | A         | 12   | 85654  | 85665  | <i>rpl2-rpl23</i>          |
| 81      | p1       | spacer | LSC    | T         | 13   | 85967  | 85979  | <i>rpl23-trnI(CAU)</i>     |
| 82      | p1       | spacer | LSC    | T         | 11   | 86109  | 86119  | <i>rpl23-trnI(CAU)</i>     |
| 83      | p2       | spacer | IRB    | AT        | 12   | 87991  | 88002  | <i>trnT(UGU)</i>           |
| 84      | p2       | spacer | IRB    | AT        | 12   | 88446  | 88457  | <i>trnT(UGU)-trnR(ACG)</i> |
| 85      | p1       | spacer | IRB    | T         | 13   | 89893  | 89905  | <i>trnR(ACG)-rrn5</i>      |
| 86      | p1       | spacer | IRB    | C         | 11   | 97421  | 97431  | <i>rrn16-rps12</i>         |
| 87      | p4       | spacer | IRB    | TCTT      | 12   | 97952  | 97963  | <i>rrn16-rps12</i>         |
| 88      | p1       | spacer | IRB    | T         | 11   | 99065  | 99075  | <i>rrn16-rps12</i>         |
| 89      | p1       | spacer | IRB    | A         | 12   | 103023 | 103034 | <i>trnH(GUG)-ycf2</i>      |
| 90      | p4       | CDS    | IRB    | AGAA      | 12   | 108199 | 108210 | <i>ycf2</i>                |
| 91      | p1       | spacer | IRB    | A         | 23   | 109609 | 109631 | <i>ycf2-trnN(GUU)</i>      |
| 92      | p1       | spacer | IRB    | G         | 15   | 110408 | 110422 | <i>trnN(GUU)-ndhF</i>      |
| 93      | p1       | spacer | SSC    | T         | 10   | 113579 | 113588 | <i>rpl21-rpl32</i>         |
| 94      | p1       | spacer | SSC    | A         | 13   | 113692 | 113704 | <i>rpl21-rpl32</i>         |
| 95      | p2       | spacer | SSC    | AT        | 16   | 113704 | 113719 | <i>rpl21-rpl32</i>         |
| 96      | p1       | spacer | SSC    | A         | 11   | 114057 | 114067 | <i>rpl32-trnP(GGG)</i>     |
| 97      | p1       | spacer | SSC    | G         | 10   | 114311 | 114320 | <i>rpl32-trnP(GGG)</i>     |
| 98      | p1       | spacer | SSC    | T         | 13   | 114378 | 114390 | <i>rpl32-trnP(GGG)</i>     |
| 99      | p1       | spacer | SSC    | A         | 13   | 114717 | 114729 | <i>trnP(GGG)-trnL(UAG)</i> |
| 100     | p1       | spacer | SSC    | A         | 11   | 114877 | 114887 | <i>trnL(UAG)-ccsA</i>      |
| 101     | p1       | spacer | SSC    | T         | 12   | 114956 | 114967 | <i>trnL(UAG)-ccsA</i>      |
| 102     | p1       | spacer | SSC    | A         | 11   | 118109 | 118119 | <i>ndhD-psaC</i>           |
| 103     | p1       | spacer | SSC    | T         | 12   | 118124 | 118135 | <i>ndhD-psaC</i>           |
| 104     | p1       | spacer | SSC    | G         | 13   | 118671 | 118683 | <i>psaC-ndhE</i>           |
| 105     | p2       | spacer | SSC    | TA        | 14   | 120486 | 120499 | <i>ndhI-ndhA</i>           |
| 106     | p1       | spacer | SSC    | A         | 13   | 120503 | 120515 | <i>ndhI-ndhA</i>           |
| 107     | p2       | spacer | SSC    | AT        | 20   | 120515 | 120534 | <i>ndhI-ndhA</i>           |
| 108     | p1       | spacer | SSC    | A         | 10   | 120579 | 120588 | <i>ndhI-ndhA</i>           |
| 109     | p1       | intron | SSC    | T         | 10   | 121499 | 121508 | <i>ndhA</i>                |
| 110     | p1       | spacer | SSC    | A         | 14   | 124291 | 124304 | <i>rps15-ycf1</i>          |
| 111     | p4       | CDS    | SSC    | ACCA      | 12   | 129075 | 129086 | <i>ycf1</i>                |
| 112     | p1       | spacer | SSC    | C         | 13   | 129616 | 129628 | <i>ycf1-chlN</i>           |
| 113     | p1       | spacer | IRA    | C         | 15   | 132548 | 132562 | <i>chlL-trnN(GUU)</i>      |
| 114     | p1       | spacer | IRA    | T         | 23   | 133339 | 133361 | <i>trnN(GUU)-ycf2</i>      |
| 115     | p4       | CDS    | IRA    | TTTC      | 12   | 134759 | 134770 | <i>ycf2</i>                |
| 116     | p1       | spacer | IRA    | T         | 12   | 139936 | 139947 | <i>ycf2-trnH(GUG)</i>      |
| 117     | p1       | spacer | IRA    | A         | 11   | 143895 | 143905 | <i>rps12-rrn16</i>         |
| 118     | p4       | spacer | IRA    | AAAG      | 12   | 145006 | 145017 | <i>rps12-rrn16</i>         |
| 119     | p1       | spacer | IRA    | G         | 11   | 145539 | 145549 | <i>rps12-rrn16</i>         |
| 120     | p1       | spacer | IRA    | A         | 13   | 153065 | 153077 | <i>rrn5-trnR(ACG)</i>      |
| 121     | p2       | spacer | IRA    | AT        | 12   | 154513 | 154524 | <i>trnR(ACG)-trnT(UGU)</i> |

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | star   | end    | Location         |
|---------|----------|--------|--------|-----------|------|--------|--------|------------------|
| 122     | p2       | spacer | IRA    | AT        | 12   | 154968 | 154979 | <i>trnT(UGU)</i> |

Appendix table 7 List of simple sequence repeats (SSRs) in the chloroplast genomes of *Sphaeropteris brunoniana*

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | star  | end   | Location                   |
|---------|----------|--------|--------|-----------|------|-------|-------|----------------------------|
| 1       | p1       | spacer | LSC    | A         | 15   | 3517  | 3531  | <i>matK-rps16</i>          |
| 2       | p1       | spacer | LSC    | T         | 10   | 3614  | 3623  | <i>matK-rps16</i>          |
| 3       | p1       | intron | LSC    | T         | 10   | 4364  | 4373  | <i>rps16</i>               |
| 4       | p1       | intron | LSC    | A         | 11   | 4861  | 4871  | <i>rps16</i>               |
| 5       | p1       | spacer | LSC    | G         | 10   | 6893  | 6902  | <i>chlB-trnQ(UUG)</i>      |
| 6       | p1       | spacer | LSC    | A         | 12   | 7068  | 7619  | <i>psbK-psbI</i>           |
| 7       | p2       | spacer | LSC    | TA        | 12   | 7621  | 7632  | <i>psbK-psbI</i>           |
| 8       | p1       | spacer | LSC    | C         | 17   | 8230  | 8246  | <i>trnS(GCU)-psaM</i>      |
| 9       | p1       | spacer | LSC    | A         | 11   | 8332  | 8342  | <i>trnS(GCU)-psaM</i>      |
| 10      | p1       | spacer | LSC    | T         | 12   | 8359  | 8370  | <i>trnS(GCU)-psaM</i>      |
| 11      | p1       | intron | LSC    | T         | 12   | 9637  | 9648  | <i>trnG(UCC)</i>           |
| 12      | p2       | intron | LSC    | TC        | 14   | 12639 | 12652 | <i>atpF</i>                |
| 13      | p1       | spacer | LSC    | C         | 16   | 13302 | 13317 | <i>atpF-atpH</i>           |
| 14      | p1       | spacer | LSC    | T         | 13   | 15171 | 15183 | <i>atpI-rps2</i>           |
| 15      | p1       | spacer | LSC    | A         | 10   | 23386 | 23395 | <i>rpoC1-rpoB</i>          |
| 16      | p1       | spacer | LSC    | T         | 12   | 27644 | 27655 | <i>rpoB-trnD(GUC)</i>      |
| 17      | p1       | spacer | LSC    | A         | 11   | 28256 | 28266 | <i>trnE(UUC)-psbM</i>      |
| 18      | p1       | spacer | LSC    | T         | 22   | 28291 | 28312 | <i>trnE(UUC)-psbM</i>      |
| 19      | p1       | spacer | LSC    | A         | 14   | 29431 | 29444 | <i>ycf66</i>               |
| 20      | p1       | spacer | LSC    | G         | 14   | 29522 | 29535 | <i>ycf66</i>               |
| 21      | p1       | spacer | LSC    | T         | 13   | 30206 | 30218 | <i>petN-trnC(GCA)</i>      |
| 22      | p1       | spacer | LSC    | T         | 11   | 30804 | 30814 | <i>trnC(GCA)-trnG(GCC)</i> |
| 23      | p4       | spacer | LSC    | TATC      | 16   | 33672 | 33687 | <i>trnS(UGA)-psbC</i>      |
| 24      | p4       | spacer | LSC    | ATAG      | 16   | 33691 | 33706 | <i>trnS(UGA)-psbC</i>      |
| 25      | p1       | spacer | LSC    | T         | 11   | 36882 | 36892 | <i>psbD-trnT(GGU)</i>      |
| 26      | p1       | spacer | LSC    | A         | 10   | 36920 | 36929 | <i>psbD-trnT(GGU)</i>      |
| 27      | p1       | spacer | LSC    | T         | 19   | 37334 | 37352 | <i>psbD-trnT(GGU)</i>      |
| 28      | p1       | spacer | LSC    | T         | 21   | 37588 | 37608 | <i>psbD-trnT(GGU)</i>      |
| 29      | p4       | spacer | LSC    | ATAG      | 12   | 38356 | 38367 | <i>trnfM(CAU)-rps14</i>    |
| 30      | p1       | spacer | LSC    | T         | 10   | 38371 | 38380 | <i>trnfM(CAU)-rps14</i>    |
| 31      | p1       | intron | LSC    | T         | 21   | 44159 | 44179 | <i>ycf3</i>                |
| 32      | p1       | spacer | LSC    | T         | 23   | 45660 | 45682 | <i>ycf3-trnS(GGA)</i>      |
| 33      | p1       | spacer | LSC    | T         | 18   | 45968 | 45985 | <i>ycf3-trnS(GGA)</i>      |
| 34      | p1       | spacer | LSC    | T         | 13   | 46269 | 46281 | <i>trnS(GGA)-rps4</i>      |
| 35      | p1       | intron | LSC    | A         | 11   | 48282 | 48292 | <i>trnL(CAA)</i>           |
| 36      | p2       | spacer | LSC    | TA        | 12   | 48647 | 48658 | <i>trnL(CAA)-trnF(GAA)</i> |
| 37      | p2       | spacer | LSC    | TA        | 24   | 48661 | 48684 | <i>trnL(CAA)-trnF(GAA)</i> |
| 38      | p1       | spacer | LSC    | T         | 12   | 51179 | 51190 | <i>ndhC-trnV(UAC)</i>      |
| 39      | p1       | intron | LSC    | T         | 10   | 51514 | 51523 | <i>trnV(UAC)</i>           |
| 40      | p1       | spacer | LSC    | A         | 16   | 52031 | 52046 | <i>trnV(UAC)-trnM(CAU)</i> |
| 41      | p1       | spacer | LSC    | T         | 10   | 54498 | 54507 | <i>atpB-rbcL</i>           |
| 42      | p1       | spacer | LSC    | T         | 12   | 56441 | 56452 | <i>rbcL-trnR(UCG)</i>      |
| 43      | p1       | spacer | LSC    | T         | 14   | 56859 | 56872 | <i>trnR(UCG)-accD</i>      |

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | star   | end    | Location               |
|---------|----------|--------|--------|-----------|------|--------|--------|------------------------|
| 44      | p1       | spacer | LSC    | T         | 10   | 58386  | 58395  | <i>accD-psaI</i>       |
| 45      | p2       | spacer | LSC    | AT        | 16   | 62683  | 62698  | <i>petA-psbJ</i>       |
| 46      | p1       | spacer | LSC    | T         | 13   | 63021  | 63033  | <i>petA-psbJ</i>       |
| 47      | p1       | spacer | LSC    | T         | 16   | 64669  | 64684  | <i>psbE-petL</i>       |
| 48      | p1       | spacer | LSC    | A         | 12   | 65041  | 65052  | <i>petL-petG</i>       |
| 49      | p1       | spacer | LSC    | C         | 10   | 66553  | 66562  | <i>psaJ-rpl33</i>      |
| 50      | p1       | spacer | LSC    | A         | 13   | 67731  | 67743  | <i>rpl20-rps12</i>     |
| 51      | p1       | spacer | LSC    | T         | 13   | 67974  | 67986  | <i>rpl20-rps12</i>     |
| 52      | p1       | spacer | LSC    | A         | 15   | 68134  | 68148  | <i>rpl20-rps12</i>     |
| 53      | p1       | spacer | LSC    | T         | 11   | 68406  | 68416  | <i>rpl20-rps12</i>     |
| 54      | p4       | spacer | LSC    | TATC      | 12   | 69106  | 69117  | <i>rps12-clpP</i>      |
| 55      | p2       | intron | LSC    | TA        | 12   | 70495  | 70506  | <i>clpP</i>            |
| 56      | p1       | intron | LSC    | C         | 14   | 70536  | 70549  | <i>clpP</i>            |
| 57      | p1       | spacer | LSC    | A         | 12   | 71504  | 71515  | <i>clpP-psbB</i>       |
| 58      | p1       | spacer | LSC    | T         | 11   | 71597  | 71607  | <i>clpP-psbB</i>       |
| 59      | p1       | spacer | LSC    | A         | 18   | 73387  | 73404  | <i>psbB-psbT</i>       |
| 60      | p1       | intron | LSC    | T         | 10   | 74735  | 74744  | <i>petB</i>            |
| 61      | p1       | spacer | LSC    | T         | 10   | 77271  | 77280  | <i>petD-ropA</i>       |
| 62      | p1       | spacer | LSC    | T         | 11   | 77328  | 77338  | <i>petD-ropA</i>       |
| 63      | p1       | spacer | LSC    | T         | 14   | 80050  | 80063  | <i>rps8-rpl14</i>      |
| 64      | p2       | spacer | LSC    | AT        | 14   | 80083  | 80096  | <i>rps8-rpl14</i>      |
| 65      | p2       | spacer | LSC    | AT        | 18   | 80177  | 80194  | <i>rps8-rpl14</i>      |
| 66      | p1       | spacer | LSC    | A         | 13   | 81030  | 81042  | <i>rpl14-rpl16</i>     |
| 67      | p1       | intron | LSC    | T         | 11   | 81765  | 81775  | <i>rpl16</i>           |
| 68      | p1       | intron | LSC    | T         | 11   | 82107  | 82117  | <i>rpl16</i>           |
| 69      | p2       | spacer | LSC    | AT        | 14   | 82324  | 82337  | <i>rpl16-rps3</i>      |
| 70      | p1       | intron | LSC    | A         | 10   | 84458  | 84467  | <i>rpl2</i>            |
| 71      | p1       | intron | LSC    | A         | 10   | 84570  | 84549  | <i>rpl2</i>            |
| 72      | p2       | intron | LSC    | AT        | 12   | 84561  | 84572  | <i>rpl2</i>            |
| 73      | p1       | intron | LSC    | T         | 14   | 84852  | 84865  | <i>rpl2</i>            |
| 74      | p1       | spacer | LSC    | T         | 13   | 85845  | 85857  | <i>rpl23-trnI(CAU)</i> |
| 75      | p1       | spacer | LSC    | T         | 13   | 85991  | 86003  | <i>rpl23-trnI(CAU)</i> |
| 76      | p4       | gene   | IRA    | CTAC      | 12   | 91186  | 91197  | <i>rrn23</i>           |
| 77      | p2       | intron | IRA    | AG        | 12   | 94186  | 94197  | <i>trnI(CAU)</i>       |
| 78      | p4       | spacer | IRA    | TCTT      | 12   | 97474  | 97485  | <i>rrn16-rps12</i>     |
| 79      | p1       | spacer | IRA    | T         | 14   | 98567  | 98580  | <i>rrn16-rps12</i>     |
| 80      | p1       | spacer | IRA    | A         | 12   | 102544 | 102555 | <i>trnH(GUG)-ycf2</i>  |
| 81      | p4       | CDS    | IRA    | AGAA      | 12   | 107744 | 107755 | <i>ycf2</i>            |
| 82      | p1       | spacer | IRA    | A         | 15   | 109158 | 109172 | <i>ycf2-trnN(GUU)</i>  |
| 83      | p1       | spacer | SSC    | T         | 11   | 110207 | 110217 | <i>trnN(GUU)-ndhF</i>  |
| 84      | p1       | spacer | SSC    | A         | 14   | 113545 | 113558 | <i>rpl32-trnP(GGG)</i> |
| 85      | p1       | spacer | SSC    | A         | 11   | 113825 | 113835 | <i>rpl32-trnP(GGG)</i> |
| 86      | p1       | spacer | SSC    | T         | 15   | 113869 | 113883 | <i>rpl32-trnP(GGG)</i> |
| 87      | p1       | spacer | SSC    | A         | 13   | 114374 | 114386 | <i>trnL(UAG)-ccsA</i>  |
| 88      | p2       | spacer | SSC    | AT        | 20   | 114464 | 114483 | <i>trnL(UAG)-ccsA</i>  |
| 89      | p1       | spacer | SSC    | A         | 13   | 115715 | 115727 | <i>ccsA-ndhD</i>       |
| 90      | p1       | spacer | SSC    | A         | 14   | 115972 | 115985 | <i>ccsA-ndhD</i>       |
| 91      | p1       | spacer | SSC    | A         | 14   | 117633 | 117646 | <i>ndhD-psaC</i>       |
| 92      | p2       | spacer | SSC    | CT        | 12   | 119317 | 119328 | <i>ndhG-ndhI</i>       |
| 93      | p2       | intron | SSC    | AT        | 16   | 121036 | 121051 | <i>ndhA</i>            |

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | star   | end    | Location              |
|---------|----------|--------|--------|-----------|------|--------|--------|-----------------------|
| 94      | p1       | intron | SSC    | T         | 11   | 121544 | 121554 | <i>ndhA</i>           |
| 95      | p1       | intron | SSC    | A         | 11   | 123840 | 123850 | <i>rps15-ycf1</i>     |
| 96      | p1       | spacer | IRB    | T         | 15   | 133684 | 133698 | <i>trnN(GUU)-ycf2</i> |
| 97      | p4       | CDS    | IRB    | TTTC      | 12   | 135100 | 135111 | <i>ycf2</i>           |
| 98      | p1       | spacer | IRB    | T         | 12   | 140301 | 140312 | <i>ycf2-trnH(GUG)</i> |
| 99      | p1       | spacer | IRB    | A         | 14   | 144276 | 144289 | <i>rps12-rrn16</i>    |
| 100     | p4       | spacer | IRB    | AAAG      | 12   | 145370 | 145381 | <i>rps12-rrn16</i>    |
| 101     | p2       | intron | IRB    | TC        | 12   | 148658 | 148669 | <i>trnI(GUA)</i>      |
| 102     | p4       | gene   | IRB    | AGGT      | 12   | 151657 | 151668 | <i>rrn23</i>          |

Appendix table 8 List of simple sequence repeats (SSRs) in the chloroplast genomes of *Sphaeropteris lepifera*

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | star  | end   | Location                   |
|---------|----------|--------|--------|-----------|------|-------|-------|----------------------------|
| 1       | p1       | spacer | LSC    | A         | 13   | 3514  | 3526  | <i>matK-rps16</i>          |
| 2       | p1       | spacer | LSC    | T         | 10   | 3613  | 3622  | <i>matK-rps16</i>          |
| 3       | p2       | spacer | LSC    | TA        | 16   | 7615  | 7630  | <i>psbK-psbI</i>           |
| 4       | p1       | spacer | LSC    | C         | 14   | 8229  | 8242  | <i>trnS(GCU)-psaM</i>      |
| 5       | p1       | spacer | LSC    | A         | 22   | 8332  | 8353  | <i>trnS(GCU)-psaM</i>      |
| 6       | p1       | spacer | LSC    | T         | 11   | 8370  | 8380  | <i>trnS(GCU)-psaM</i>      |
| 7       | p1       | intron | LSC    | A         | 10   | 9507  | 9516  | <i>ycf12</i>               |
| 8       | p1       | intron | LSC    | T         | 12   | 9648  | 9659  | <i>ycf12</i>               |
| 9       | p1       | spacer | LSC    | C         | 38   | 13308 | 13345 | <i>atpF-atpH</i>           |
| 10      | p1       | spacer | LSC    | T         | 12   | 15193 | 15204 | <i>atpI-rps2</i>           |
| 11      | p1       | intron | LSC    | C         | 10   | 22421 | 22430 | <i>rpoC1</i>               |
| 12      | p1       | spacer | LSC    | A         | 12   | 23409 | 23420 | <i>rpoC1-rpoB</i>          |
| 13      | p1       | spacer | LSC    | T         | 14   | 27774 | 27787 | <i>rpoB-trnD(GUC)</i>      |
| 14      | p1       | spacer | LSC    | A         | 14   | 28387 | 28400 | <i>trnE(UUC)-psbM</i>      |
| 15      | p1       | spacer | LSC    | T         | 21   | 28425 | 28445 | <i>trnE(UUC)-psbM</i>      |
| 16      | p1       | spacer | LSC    | A         | 12   | 29887 | 29898 | <i>ycf66</i>               |
| 17      | p1       | spacer | LSC    | T         | 10   | 30334 | 30343 | <i>petN-trnC(GCA)</i>      |
| 18      | p1       | spacer | LSC    | A         | 15   | 30525 | 30539 | <i>petN-trnC(GCA)</i>      |
| 19      | p1       | spacer | LSC    | T         | 10   | 30938 | 30947 | <i>trnC(GCA)-trnG(GCC)</i> |
| 20      | p1       | spacer | LSC    | T         | 12   | 32361 | 32372 | <i>trnG(GCC)-psbZ</i>      |
| 21      | p1       | spacer | LSC    | T         | 10   | 33284 | 33293 | <i>trnS(UGA)-psbC</i>      |
| 22      | p4       | spacer | LSC    | TATC      | 16   | 33813 | 33828 | <i>trnS(UGA)-psbC</i>      |
| 23      | p4       | spacer | LSC    | ATAG      | 12   | 33832 | 33843 | <i>trnS(UGA)-psbC</i>      |
| 24      | p1       | spacer | LSC    | T         | 16   | 37027 | 37042 | <i>psbD-trnT(GGU)</i>      |
| 25      | p1       | spacer | LSC    | A         | 13   | 37070 | 37082 | <i>psbD-trnT(GGU)</i>      |
| 26      | p1       | spacer | LSC    | T         | 19   | 37491 | 37509 | <i>psbD-trnT(GGU)</i>      |
| 27      | p1       | spacer | LSC    | T         | 12   | 37753 | 37764 | <i>psbD-trnT(GGU)</i>      |
| 28      | p2       | spacer | LSC    | TA        | 16   | 38517 | 38532 | <i>trnfM-rps14</i>         |
| 29      | p1       | spacer | LSC    | T         | 10   | 38541 | 38550 | <i>trnfM-rps14</i>         |
| 30      | p1       | intron | LSC    | T         | 13   | 44335 | 44347 | <i>ycf3</i>                |
| 31      | p1       | intron | LSC    | C         | 10   | 45444 | 45453 | <i>ycf3</i>                |
| 32      | p1       | spacer | LSC    | T         | 12   | 45838 | 45849 | <i>ycf3-trnS(GGA)</i>      |
| 33      | p1       | spacer | LSC    | T         | 12   | 46134 | 46145 | <i>ycf3-trnS(GGA)</i>      |
| 34      | p1       | spacer | LSC    | T         | 11   | 46434 | 46444 | <i>trnS(GGA)-rps4</i>      |
| 35      | p1       | intron | LSC    | A         | 10   | 48446 | 48455 | <i>trnL(CAA)</i>           |
| 36      | p2       | spacer | LSC    | TA        | 14   | 48820 | 48833 | <i>trnL(CAA)-trnF(GAA)</i> |

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | star   | end    | Location                   |
|---------|----------|--------|--------|-----------|------|--------|--------|----------------------------|
| 37      | p1       | spacer | LSC    | T         | 10   | 51310  | 51319  | <i>ndhC-trnV(UAC)</i>      |
| 38      | p1       | spacer | LSC    | A         | 14   | 52158  | 52171  | <i>trnV(UAC)-trnM(CAU)</i> |
| 39      | p1       | spacer | LSC    | T         | 11   | 54626  | 54636  | <i>atpB-rbcL</i>           |
| 40      | p1       | spacer | LSC    | T         | 13   | 56570  | 56582  | <i>rbcL-trnR(UCG)</i>      |
| 41      | p1       | spacer | LSC    | T         | 13   | 56989  | 57001  | <i>trnR(UCG)-accD</i>      |
| 42      | p1       | spacer | LSC    | A         | 10   | 58098  | 58107  | <i>accD-psaI</i>           |
| 43      | p1       | spacer | LSC    | T         | 12   | 58522  | 58533  | <i>accD-psaI</i>           |
| 44      | p1       | spacer | LSC    | A         | 11   | 59707  | 59717  | <i>ycf4-cemA</i>           |
| 45      | p1       | spacer | LSC    | A         | 10   | 62694  | 62703  | <i>petA-psbJ</i>           |
| 46      | p1       | spacer | LSC    | T         | 14   | 63180  | 63193  | <i>petA-psbJ</i>           |
| 47      | p1       | spacer | LSC    | T         | 10   | 64840  | 64849  | <i>psbE-petL</i>           |
| 48      | p1       | spacer | LSC    | A         | 10   | 65211  | 65220  | <i>petL-petG</i>           |
| 49      | p1       | spacer | LSC    | C         | 12   | 66700  | 66711  | <i>psaJ-rpl33</i>          |
| 50      | p1       | spacer | LSC    | A         | 12   | 67888  | 67899  | <i>rpl20-rps12</i>         |
| 51      | p1       | spacer | LSC    | T         | 13   | 68130  | 68142  | <i>rpl20-rps12</i>         |
| 52      | p1       | spacer | LSC    | A         | 10   | 68290  | 68299  | <i>rpl20-rps12</i>         |
| 53      | p1       | spacer | LSC    | T         | 11   | 68557  | 68567  | <i>rpl20-rps12</i>         |
| 54      | p4       | spacer | LSC    | TTAT      | 12   | 69379  | 69390  | <i>rps12-clpP</i>          |
| 55      | p1       | intron | LSC    | C         | 10   | 70697  | 70706  | <i>clpP</i>                |
| 56      | p1       | spacer | LSC    | A         | 13   | 71660  | 71672  | <i>clpP-psbB</i>           |
| 57      | p1       | spacer | LSC    | T         | 10   | 71754  | 71763  | <i>clpP-psbB</i>           |
| 58      | p1       | spacer | LSC    | A         | 13   | 73543  | 73555  | <i>psbB-psbT</i>           |
| 59      | p1       | spacer | LSC    | A         | 10   | 74268  | 74277  | <i>psbH-petB</i>           |
| 60      | p1       | spacer | LSC    | T         | 13   | 77480  | 77492  | <i>petD-rpoA</i>           |
| 61      | p2       | spacer | LSC    | AT        | 20   | 80238  | 80257  | <i>rps8-rpl14</i>          |
| 62      | p2       | spacer | LSC    | AT        | 16   | 80338  | 80353  | <i>rps8-rpl14</i>          |
| 63      | p1       | spacer | LSC    | A         | 13   | 81186  | 81198  | <i>rpl14-rpl16</i>         |
| 64      | p1       | intron | LSC    | T         | 12   | 81919  | 81930  | <i>rpl16</i>               |
| 65      | p1       | intron | LSC    | T         | 12   | 82262  | 82273  | <i>rpl16</i>               |
| 66      | p1       | spacer | LSC    | T         | 11   | 82483  | 82493  | <i>rpl16-rps3</i>          |
| 67      | p1       | intron | LSC    | T         | 15   | 84995  | 85009  | <i>rpl2</i>                |
| 68      | p1       | spacer | LSC    | A         | 11   | 85683  | 85693  | <i>rpl2-rpl23</i>          |
| 69      | p1       | spacer | LSC    | T         | 12   | 85991  | 86002  | <i>rpl23-trnI(CAU)</i>     |
| 70      | p1       | spacer | LSC    | T         | 16   | 86136  | 86151  | <i>rpl23-trnI(CAU)</i>     |
| 71      | p4       | gene   | IRB    | CTAC      | 12   | 91392  | 91403  | <i>rrn23</i>               |
| 72      | p2       | intron | IRB    | AG        | 14   | 94391  | 94404  | <i>trnI(GAU)</i>           |
| 73      | p4       | spacer | IRB    | TCTT      | 12   | 97681  | 97692  | <i>rrn16-rps12</i>         |
| 74      | p1       | spacer | IRB    | T         | 13   | 98774  | 98786  | <i>rrn16-rps12</i>         |
| 75      | p1       | spacer | IRB    | A         | 12   | 102750 | 102761 | <i>trnH(GUG)-ycf2</i>      |
| 76      | p4       | CDS    | IRB    | TTTA      | 12   | 104706 | 104717 | <i>ycf2</i>                |
| 77      | p4       | CDS    | IRB    | AGAA      | 12   | 107950 | 107961 | <i>ycf2</i>                |
| 78      | p1       | spacer | IRB    | A         | 15   | 109364 | 109378 | <i>ycf2-trnN(GUU)</i>      |
| 79      | p1       | spacer | SSC    | T         | 13   | 110416 | 110428 | <i>ycf2-trnN(GUU)</i>      |
| 80      | p1       | spacer | SSC    | A         | 11   | 112695 | 112705 | <i>ndhF-rpl21</i>          |
| 81      | p2       | spacer | SSC    | AT        | 18   | 113419 | 113436 | <i>rpl21-rpl32</i>         |
| 82      | p1       | spacer | SSC    | A         | 11   | 113761 | 113771 | <i>rpl32-trnP(GGG)</i>     |
| 83      | p1       | spacer | SSC    | T         | 13   | 114080 | 114092 | <i>rpl32-trnP(GGG)</i>     |
| 84      | p1       | spacer | SSC    | T         | 12   | 119615 | 119626 | <i>trnP(GGG)-trnL(UAG)</i> |
| 85      | p1       | spacer | SSC    | A         | 10   | 119831 | 119840 | <i>trnL(UAG)-ccsA</i>      |
| 86      | p2       | spacer | SSC    | AT        | 16   | 119920 | 119935 | <i>trnL(UAG)-ccsA</i>      |

| SSR nr. | SSR type | Locus  | Region | SSR motif | size | star   | end    | Location              |
|---------|----------|--------|--------|-----------|------|--------|--------|-----------------------|
| 87      | p1       | spacer | SSC    | A         | 11   | 121168 | 121178 | <i>ccsA-ndhD</i>      |
| 88      | p1       | spacer | SSC    | A         | 14   | 121427 | 121440 | <i>ccsA-ndhD</i>      |
| 89      | p1       | spacer | SSC    | A         | 28   | 123088 | 123115 | <i>ndhD-psaC</i>      |
| 90      | p2       | spacer | SSC    | CT        | 12   | 124783 | 124794 | <i>ndhG-ndhI</i>      |
| 91      | p2       | intron | SSC    | TA        | 12   | 126492 | 126503 | <i>ndhA</i>           |
| 92      | p1       | intron | SSC    | T         | 13   | 127011 | 127023 | <i>ndhA</i>           |
| 93      | p1       | spacer | SSC    | A         | 10   | 129309 | 129318 | <i>rps15-ycf1</i>     |
| 94      | p1       | spacer | SSC    | A         | 10   | 129333 | 129342 | <i>rps15-ycf1</i>     |
| 95      | p1       | spacer | IRA    | T         | 15   | 139188 | 139202 | <i>trnN(GUU)-ycf2</i> |
| 96      | p4       | CDS    | IRA    | TTTC      | 12   | 140604 | 140615 | <i>ycf2</i>           |
| 97      | p4       | CDS    | IRA    | TAAA      | 12   | 143849 | 143860 | <i>ycf2</i>           |
| 98      | p1       | spacer | IRA    | T         | 12   | 145805 | 145816 | <i>ycf2-trnH(GUG)</i> |
| 99      | p1       | spacer | IRA    | A         | 13   | 149780 | 149792 | <i>rps12-rrn16</i>    |
| 100     | p4       | spacer | IRA    | AAAG      | 12   | 150873 | 150884 | <i>rps12-rrn16</i>    |
| 101     | p2       | intron | IRA    | TC        | 14   | 154161 | 154174 | <i>trnI(GAU)</i>      |
| 102     | p4       | gene   | IRA    | AGGT      | 12   | 157161 | 157172 | <i>rrn23</i>          |

Appendix table 9 The number, relative abundance, relative density, GC content of SSR motifs in the chloroplast genomes of 8 Cyatheaaceae species

| motif type | Characteristics of SSR      | <i>A. denticulata</i> | <i>A. podophylla</i> | <i>A. gigantea</i> | <i>A. metteniana</i> | <i>A. costularis</i> |
|------------|-----------------------------|-----------------------|----------------------|--------------------|----------------------|----------------------|
| A          | No. of SSRs                 | 13                    | 10                   | 10                 | 11                   | 31                   |
|            | Relative abundance (No./Kb) | 0.084                 | 0.060                | 0.062              | 0.068                | 0.198                |
|            | Relative density (bp/Kb)    | 0.883                 | 0.614                | 0.730              | 0.780                | 2.247                |
| C          | No. of SSRs                 | 8                     | 8                    | 8                  | 6                    | 10                   |
|            | Relative abundance (No./Kb) | 0.052                 | 0.048                | 0.049              | 0.037                | 0.064                |
|            | Relative density (bp/Kb)    | 0.565                 | 0.554                | 0.637              | 0.421                | 1.040                |
| G          | No. of SSRs                 | 6                     | 7                    | 7                  | 6                    | 8                    |
|            | Relative abundance (No./Kb) | 0.039                 | 0.042                | 0.043              | 0.037                | 0.051                |
|            | Relative density (bp/Kb)    | 0.487                 | 0.542                | 0.619              | 0.446                | 0.645                |
| T          | No. of SSRs                 | 13                    | 22                   | 16                 | 17                   | 46                   |
|            | Relative abundance (No./Kb) | 0.084                 | 0.132                | 0.099              | 0.105                | 0.294                |
|            | Relative density (bp/Kb)    | 0.993                 | 1.450                | 1.076              | 1.157                | 3.606                |
| AG         | No. of SSRs                 | 0                     | 0                    | 1                  | 1                    | 0                    |
|            | Relative abundance (No./Kb) | 0                     | 0                    | 0.006              | 0.006                | 0                    |
|            | Relative density (bp/Kb)    | 0                     | 0                    | 0.148              | 0.099                | 0                    |
| AT         | No. of SSRs                 | 5                     | 6                    | 6                  | 6                    | 11                   |
|            | Relative abundance (No./Kb) | 0.032                 | 0.036                | 0.037              | 0.037                | 0.070                |
|            | Relative density (bp/Kb)    | 0.344                 | 0.518                | 0.495              | 0.520                | 1.111                |
| CT         | No. of SSRs                 | 1                     | 0                    | 0                  | 0                    | 0                    |
|            | Relative abundance (No./Kb) | 0.006                 | 0                    | 0                  | 0                    | 0                    |
|            | Relative density (bp/Kb)    | 0.078                 | 0                    | 0                  | 0                    | 0                    |
| TA         | No. of SSRs                 | 2                     | 3                    | 3                  | 3                    | 3                    |
|            | Relative abundance (No./Kb) | 0.013                 | 0.018                | 0.019              | 0.019                | 0.019                |
|            | Relative density (bp/Kb)    | 0.078                 | 0.313                | 0.297              | 0.297                | 0.243                |
| TC         | No. of SSRs                 | 0                     | 0                    | 0                  | 0                    | 0                    |
|            | Relative abundance (No./Kb) | 0                     | 0                    | 0                  | 0                    | 0                    |
|            | Relative density (bp/Kb)    | 0                     | 0                    | 0                  | 0                    | 0                    |
| AAG        | No. of SSRs                 | 0                     | 0                    | 0                  | 0                    | 1                    |

| motif type | Characteristics of SSR      | <i>A. denticulata</i> | <i>A. podophylla</i> | <i>A. gigantea</i> | <i>A. metteniana</i> | <i>A. costularis</i> |
|------------|-----------------------------|-----------------------|----------------------|--------------------|----------------------|----------------------|
| AAT        | Relative abundance (No./Kb) | 0                     | 0                    | 0                  | 0                    | 0.006                |
|            | Relative density (bp/Kb)    | 0                     | 0                    | 0                  | 0                    | 0.077                |
|            | No. of SSRs                 | 1                     | 1                    | 1                  | 1                    | 0                    |
| TAT        | Relative abundance (No./Kb) | 0.006                 | 0.006                | 0.006              | 0.006                | 0                    |
|            | Relative density (bp/Kb)    | 0.078                 | 0.072                | 0.074              | 0.074                | 0                    |
|            | No. of SSRs                 | 1                     | 0                    | 1                  | 1                    | 0                    |
| AAAG       | Relative abundance (No./Kb) | 0.006                 | 0                    | 0.006              | 0.006                | 0                    |
|            | Relative density (bp/Kb)    | 0.078                 | 0                    | 0.074              | 0.074                | 0                    |
|            | No. of SSRs                 | 1                     | 0                    | 1                  | 1                    | 1                    |
| AAAT       | Relative abundance (No./Kb) | 0.006                 | 0                    | 0.006              | 0.006                | 0.006                |
|            | Relative density (bp/Kb)    | 0.078                 | 0                    | 0.074              | 0.074                | 0.077                |
|            | No. of SSRs                 | 0.000                 | 1                    | 1                  | 1                    | 0                    |
| AATT       | Relative abundance (No./Kb) | 0.000                 | 0.006                | 0.006              | 0.006                | 0                    |
|            | Relative density (bp/Kb)    | 0.000                 | 0.072                | 0.099              | 0.099                | 0                    |
|            | No. of SSRs                 | 2                     | 2                    | 1                  | 2                    | 0                    |
| ACCA       | Relative abundance (No./Kb) | 0.013                 | 0.012                | 0.006              | 0.012                | 0                    |
|            | Relative density (bp/Kb)    | 0.156                 | 0.144                | 0.074              | 0.149                | 0                    |
|            | No. of SSRs                 | 0                     | 0                    | 0                  | 0                    | 1                    |
| AGAA       | Relative abundance (No./Kb) | 0                     | 0                    | 0                  | 0                    | 0.006                |
|            | Relative density (bp/Kb)    | 0                     | 0                    | 0                  | 0                    | 0.077                |
|            | No. of SSRs                 | 0                     | 0                    | 0                  | 0                    | 1                    |
| AGGT       | Relative abundance (No./Kb) | 0                     | 0                    | 0                  | 0                    | 0.006                |
|            | Relative density (bp/Kb)    | 0                     | 0                    | 0                  | 0                    | 0.077                |
|            | No. of SSRs                 | 0                     | 0                    | 0                  | 0                    | 0                    |
| ATAG       | Relative abundance (No./Kb) | 0                     | 0                    | 0                  | 0                    | 0                    |
|            | Relative density (bp/Kb)    | 0                     | 0                    | 0                  | 0                    | 0                    |
|            | No. of SSRs                 | 2                     | 2                    | 2                  | 2                    | 1                    |
| ATCT       | Relative abundance (No./Kb) | 0.013                 | 0.012                | 0.012              | 0.012                | 0.006                |
|            | Relative density (bp/Kb)    | 0.156                 | 0.144                | 0.148              | 0.149                | 0.077                |
|            | No. of SSRs                 | 0                     | 0                    | 0                  | 0                    | 1                    |
| CCTC       | Relative abundance (No./Kb) | 0                     | 0                    | 0                  | 0                    | 0.006                |
|            | Relative density (bp/Kb)    | 0                     | 0                    | 0                  | 0                    | 0.102                |
|            | No. of SSRs                 | 0                     | 0                    | 0                  | 0                    | 1                    |
| CTAC       | Relative abundance (No./Kb) | 0                     | 0                    | 0                  | 0                    | 0.006                |
|            | Relative density (bp/Kb)    | 0                     | 0                    | 0                  | 0                    | 0.077                |
|            | No. of SSRs                 | 0                     | 0                    | 0                  | 0                    | 0                    |
| GAAA       | Relative abundance (No./Kb) | 0                     | 0                    | 0                  | 0                    | 0                    |
|            | Relative density (bp/Kb)    | 0                     | 0                    | 0                  | 0                    | 0                    |
|            | No. of SSRs                 | 0                     | 0                    | 1                  | 1                    | 0                    |
| GATA       | Relative abundance (No./Kb) | 0                     | 0                    | 0.006              | 0.006                | 0                    |
|            | Relative density (bp/Kb)    | 0                     | 0                    | 0.074              | 0.074                | 0                    |
|            | No. of SSRs                 | 1                     | 1                    | 1                  | 1                    | 1                    |
| TAAA       | Relative abundance (No./Kb) | 0.006                 | 0.006                | 0.006              | 0.006                | 0.006                |
|            | Relative density (bp/Kb)    | 0.078                 | 0.072                | 0.074              | 0.074                | 0.077                |
|            | No. of SSRs                 | 0                     | 0                    | 0                  | 0                    | 0                    |
| TATC       | Relative abundance (No./Kb) | 0                     | 0                    | 0                  | 0                    | 0                    |
|            | Relative density (bp/Kb)    | 0                     | 0                    | 0                  | 0                    | 0                    |
|            | No. of SSRs                 | 1                     | 1                    | 1                  | 1                    | 1                    |
|            | Relative abundance (No./Kb) | 0.006                 | 0.006                | 0.006              | 0.006                | 0.006                |
|            | Relative density (bp/Kb)    | 0.104                 | 0.096                | 0.074              | 0.074                | 0.077                |

| motif type | Characteristics of SSR      | <i>A. denticulata</i> | <i>A. podophylla</i> | <i>A. gigantea</i> | <i>A. metteniana</i> | <i>A. costularis</i> |
|------------|-----------------------------|-----------------------|----------------------|--------------------|----------------------|----------------------|
| TATT       | No. of SSRs                 | 1                     | 1                    | 1                  | 1                    | 1                    |
|            | Relative abundance (No./Kb) | 0.006                 | 0.006                | 0.006              | 0.006                | 0.006                |
|            | Relative density (bp/Kb)    | 0.078                 | 0.072                | 0.074              | 0.074                | 0.077                |
| TCTT       | No. of SSRs                 | 1                     | 0                    | 1                  | 1                    | 1                    |
|            | Relative abundance (No./Kb) | 0.006                 | 0                    | 0.006              | 0.006                | 0.006                |
|            | Relative density (bp/Kb)    | 0.078                 | 0                    | 0.074              | 0.074                | 0.077                |
| TGAT       | No. of SSRs                 | 1                     | 1                    | 1                  | 1                    | 0                    |
|            | Relative abundance (No./Kb) | 0.006                 | 0.006                | 0.006              | 0.006                | 0                    |
|            | Relative density (bp/Kb)    | 0.078                 | 0.072                | 0.074              | 0.074                | 0                    |
| TTAA       | No. of SSRs                 | 0                     | 0                    | 0                  | 0                    | 0                    |
|            | Relative abundance (No./Kb) | 0                     | 0                    | 0                  | 0                    | 0                    |
|            | Relative density (bp/Kb)    | 0                     | 0                    | 0                  | 0                    | 0                    |
| TTAT       | No. of SSRs                 | 0                     | 0                    | 0                  | 0                    | 0                    |
|            | Relative abundance (No./Kb) | 0                     | 0                    | 0                  | 0                    | 0                    |
|            | Relative density (bp/Kb)    | 0                     | 0                    | 0                  | 0                    | 0                    |
| TTTA       | No. of SSRs                 | 0                     | 0                    | 0                  | 0                    | 0                    |
|            | Relative abundance (No./Kb) | 0                     | 0                    | 0                  | 0                    | 0                    |
|            | Relative density (bp/Kb)    | 0                     | 0                    | 0                  | 0                    | 0                    |
| TTTC       | No. of SSRs                 | 0                     | 0                    | 0                  | 0                    | 1                    |
|            | Relative abundance (No./Kb) | 0                     | 0                    | 0                  | 0                    | 0.006                |
|            | Relative density (bp/Kb)    | 0                     | 0                    | 0                  | 0                    | 0.077                |
| ATTTT      | No. of SSRs                 | 1                     | 0                    | 0                  | 0                    | 0                    |
|            | Relative abundance (No./Kb) | 0.006                 | 0                    | 0                  | 0                    | 0                    |
|            | Relative density (bp/Kb)    | 0.097                 | 0                    | 0                  | 0                    | 0                    |
| CTTTC      | No. of SSRs                 | 0                     | 0                    | 0                  | 1                    | 0                    |
|            | Relative abundance (No./Kb) | 0                     | 0                    | 0                  | 0.006                | 0                    |
|            | Relative density (bp/Kb)    | 0                     | 0                    | 0                  | 0.093                | 0                    |
| TTCTC      | No. of SSRs                 | 0                     | 1                    | 0                  | 0                    | 0                    |
|            | Relative abundance (No./Kb) | 0                     | 0.006                | 0                  | 0                    | 0                    |
|            | Relative density (bp/Kb)    | 0                     | 0.090                | 0                  | 0                    | 0                    |

Appendix table 10 The number, relative abundance, relative density and GC content of different types of motif SSRs among different regions in the chloroplast genomes of 8 Cyatheaceae species

| Species               | motif type      | Characteristics of SSR      | IGS  | intron | CDS | gene | LSC  | SSC  | IRA  | IRB  |
|-----------------------|-----------------|-----------------------------|------|--------|-----|------|------|------|------|------|
| <i>A. denticulata</i> | mononucleotide  | No.of SSRs                  | 30   | 10     | 0   | 0    | 30   | 8    | 1    | 1    |
|                       |                 | Relative abundance (No./Kb) | 0.63 | 0.66   | 0   | 0    | 0.35 | 0.37 | 0.04 | 0.04 |
|                       |                 | Relative density (bp/Kb)    | 6.81 | 7.82   | 0   | 0    | 3.92 | 4.17 | 0.52 | 0.52 |
|                       |                 | GC content                  | 0.40 | 0.27   | 0   | 0    | 0.45 | 0.12 | 0    | 0    |
|                       | Dinucleotide    | No.of SSRs                  | 7    | 1      | 0   | 0    | 4    | 2    | 1    | 1    |
|                       |                 | Relative abundance (No./Kb) | 0.15 | 0.07   | 0   | 0    | 0.05 | 0.09 | 0.04 | 0.04 |
|                       |                 | Relative density (bp/Kb)    | 1.35 | 0.79   | 0   | 0    | 0.63 | 1.30 | 0.52 | 0.52 |
|                       |                 | GC content                  | 0    | 0.50   | 0   | 0    | 0.11 | 0    | 0    | 0    |
|                       | Trinucleotide   | No.of SSRs                  | 2    | 0      | 0   | 0    | 0    | 2    | 0    | 0    |
|                       |                 | Relative abundance (No./Kb) | 0.04 | 0      | 0   | 0    | 0    | 0.09 | 0    | 0    |
|                       |                 | Relative density (bp/Kb)    | 0.51 | 0      | 0   | 0    | 0    | 1.11 | 0    | 0    |
|                       |                 | GC content                  | 0    | 0      | 0   | 0    | 0    | 0    | 0    | 0    |
|                       | Tetranucleotide | No.of SSRs                  | 9    | 1      | 0   | 0    | 7    | 1    | 1    | 1    |
|                       |                 | Relative abundance (No./Kb) | 0.19 | 0.07   | 0   | 0    | 0.08 | 0.05 | 0.04 | 0.04 |

| Species              | motif type      | Characteristics of SSR      | IGS  | intron | CDS | gene | LSC  | SSC  | IRA  | IRL  |
|----------------------|-----------------|-----------------------------|------|--------|-----|------|------|------|------|------|
| <i>A. podophylla</i> | Pentanucleotide | Relative density (bp/Kb)    | 2.37 | 0.79   | 0   | 0    | 1.02 | 0.56 | 0.52 | 0.52 |
|                      |                 | GC content                  | 0.20 | 0      | 0   | 0    | 0.18 | 0    | 0.25 | 0.25 |
|                      |                 | No.of SSRs                  | 1    | 0      | 0   | 0    | 1    | 0    | 0    | 0    |
|                      |                 | Relative abundance (No./Kb) | 0.02 | 0      | 0   | 0    | 0.01 | 0    | 0    | 0    |
|                      |                 | Relative density (bp/Kb)    | 0.32 | 0      | 0   | 0    | 0.17 | 0    | 0    | 0    |
|                      | mononucleotide  | GC content                  | 0    | 0      | 0   | 0    | 0    | 0    | 0    | 0    |
|                      |                 | No.of SSRs                  | 38   | 9      | 0   | 0    | 32   | 7    | 4    | 4    |
|                      |                 | Relative abundance (No./Kb) | 0.66 | 0.59   | 0   | 0    | 0.37 | 0.32 | 0.14 | 0.14 |
|                      |                 | Relative density (bp/Kb)    | 7.33 | 6.78   | 0   | 0    | 4.09 | 3.97 | 1.45 | 1.45 |
|                      |                 | GC content                  | 0.41 | 0.11   | 0   | 0    | 0.38 | 0.30 | 0.24 | 0.24 |
|                      | Dinucleotide    | No.of SSRs                  | 8    | 1      | 0   | 0    | 4    | 3    | 1    | 1    |
|                      |                 | Relative abundance (No./Kb) | 0.14 | 0.07   | 0   | 0    | 0.05 | 0.14 | 0.03 | 0.03 |
|                      |                 | Relative density (bp/Kb)    | 2.12 | 1.05   | 0   | 0    | 0.71 | 2.40 | 0.42 | 0.42 |
|                      |                 | GC content                  | 0    | 0      | 0   | 0    | 0    | 0    | 0    | 0    |
|                      |                 | No.of SSRs                  | 0    | 1      | 0   | 0    | 1    | 0    | 0    | 0    |
|                      | Trinucleotide   | Relative abundance (No./Kb) | 0    | 0.07   | 0   | 0    | 0.01 | 0    | 0    | 0    |
|                      |                 | Relative density (bp/Kb)    | 0    | 0.79   | 0   | 0    | 0.14 | 0    | 0    | 0    |
|                      |                 | GC content                  | 0    | 0      | 0   | 0    | 0    | 0    | 0    | 0    |
|                      |                 | No.of SSRs                  | 8    | 1      | 0   | 0    | 7    | 2    | 0    | 0    |
|                      |                 | Relative abundance (No./Kb) | 0.14 | 0.07   | 0   | 0    | 0.08 | 0.09 | 0    | 0    |
|                      | Tetranucleotide | Relative density (bp/Kb)    | 1.74 | 0.79   | 0   | 0    | 1.01 | 1.11 | 0    | 0    |
|                      |                 | GC content                  | 0.16 | 0      | 0   | 0    | 0.18 | 0    | 0    | 0    |
|                      |                 | No.of SSRs                  | 0    | 1      | 0   | 0    | 1    | 0    | 0    | 0    |
|                      |                 | Relative abundance (No./Kb) | 0    | 0.07   | 0   | 0    | 0.01 | 0    | 0    | 0    |
|                      |                 | Relative density (bp/Kb)    | 0    | 0.99   | 0   | 0    | 0.17 | 0    | 0    | 0    |
| <i>A. gigantea</i>   | Pentanucleotide | GC content                  | 0    | 0.40   | 0   | 0    | 0.40 | 0    | 0    | 0    |
|                      |                 | No.of SSRs                  | 33   | 8      | 0   | 0    | 30   | 7    | 2    | 2    |
|                      |                 | Relative abundance (No./Kb) | 0.62 | 0.53   | 0   | 0    | 0.32 | 0.32 | 0.08 | 0.08 |
|                      |                 | Relative density (bp/Kb)    | 7.89 | 5.66   | 0   | 0    | 3.52 | 6.13 | 1.01 | 1.01 |
|                      |                 | GC content                  | 0.41 | 0.36   | 0   | 0    | 0.43 | 0.47 | 0    | 0    |
|                      | Dinucleotide    | No.of SSRs                  | 9    | 1      | 0   | 0    | 5    | 3    | 1    | 1    |
|                      |                 | Relative abundance (No./Kb) | 0.17 | 0.07   | 0   | 0    | 0.05 | 0.14 | 0.04 | 0.04 |
|                      |                 | Relative density (bp/Kb)    | 2.59 | 0.92   | 0   | 0    | 0.91 | 2.03 | 0.50 | 0.50 |
|                      |                 | GC content                  | 0.09 | 0      | 0   | 0    | 0.14 | 0    | 0    | 0    |
|                      |                 | No.of SSRs                  | 2    | 0      | 0   | 0    | 0    | 2    | 0    | 0    |
|                      | Trinucleotide   | Relative abundance (No./Kb) | 0.04 | 0      | 0   | 0    | 0    | 0.09 | 0    | 0    |
|                      |                 | Relative density (bp/Kb)    | 0.45 | 0      | 0   | 0    | 0    | 1.11 | 0    | 0    |
|                      |                 | GC content                  | 0    | 0      | 0   | 0    | 0    | 0    | 0    | 0    |
|                      |                 | No.of SSRs                  | 10   | 1      | 0   | 0    | 7    | 2    | 1    | 1    |
|                      |                 | Relative abundance (No./Kb) | 0.19 | 0.07   | 0   | 0    | 0.08 | 0.09 | 0.04 | 0.04 |
|                      | Tetranucleotide | Relative density (bp/Kb)    | 2.33 | 0.79   | 0   | 0    | 0.91 | 1.29 | 0.50 | 0.50 |
|                      |                 | GC content                  | 0.19 | 0      | 0   | 0    | 0.18 | 0.11 | 0.25 | 0.25 |
|                      |                 | No.of SSRs                  | 0    | 0      | 0   | 0    | 0    | 0    | 0    | 0    |
|                      |                 | Relative abundance (No./Kb) | 0    | 0      | 0   | 0    | 0    | 0    | 0    | 0    |
|                      |                 | Relative density (bp/Kb)    | 0    | 0      | 0   | 0    | 0    | 0    | 0    | 0    |
| <i>A. metteniana</i> | Pentanucleotide | GC content                  | 0    | 0      | 0   | 0    | 0    | 0    | 0    | 0    |
|                      |                 | No.of SSRs                  | 33   | 7      | 0   | 0    | 25   | 9    | 3    | 3    |
|                      |                 | Relative abundance (No./Kb) | 0.62 | 0.46   | 0   | 0    | 0.27 | 0.42 | 0.13 | 0.13 |
|                      |                 | Relative density (bp/Kb)    | 7.29 | 5.14   | 0   | 0    | 2.96 | 5.58 | 1.51 | 1.51 |
|                      |                 | GC content                  | 0.28 | 0.38   | 0   | 0    | 0.41 | 0.24 | 0    | 0    |
|                      | mononucleotide  |                             |      |        |     |      |      |      |      |      |
|                      |                 |                             |      |        |     |      |      |      |      |      |
|                      |                 |                             |      |        |     |      |      |      |      |      |

| Species              | motif type      | Characteristics of SSR      | IGS   | intron | CDS  | gene | LSC  | SSC  | IRA  | IRL  |
|----------------------|-----------------|-----------------------------|-------|--------|------|------|------|------|------|------|
| <i>A. costularis</i> | Dinucleotide    | No.of SSRs                  | 9     | 1      | 0    | 0    | 5    | 3    | 1    | 1    |
|                      |                 | Relative abundance (No./Kb) | 0.17  | 0.07   | 0    | 0    | 0.05 | 0.14 | 0.04 | 0.04 |
|                      |                 | Relative density (bp/Kb)    | 2.52  | 0.92   | 0    | 0    | 0.85 | 2.12 | 0.50 | 0.50 |
|                      |                 | GC content                  | 0.06  | 0      | 0    | 0    | 0.10 | 0    | 0    | 0    |
|                      | Trinucleotide   | No.of SSRs                  | 2     | 0      | 0    | 0    | 0    | 2    | 0    | 0    |
|                      |                 | Relative abundance (No./Kb) | 0.04  | 0      | 0    | 0    | 0    | 0.09 | 0    | 0    |
|                      |                 | Relative density (bp/Kb)    | 0.45  | 0      | 0    | 0    | 0    | 1.11 | 0    | 0    |
|                      |                 | GC content                  | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      | Tetranucleotide | No.of SSRs                  | 11    | 1      | 0    | 0    | 7    | 3    | 1    | 1    |
|                      |                 | Relative abundance (No./Kb) | 0.21  | 0.07   | 0    | 0    | 0.08 | 0.14 | 0.04 | 0.04 |
|                      |                 | Relative density (bp/Kb)    | 2.56  | 0.79   | 0    | 0    | 0.91 | 1.85 | 0.50 | 0.50 |
|                      |                 | GC content                  | 0.15  | 0      | 0    | 0    | 0.18 | 0.08 | 0.25 | 0.25 |
|                      | Pentanucleotide | No.of SSRs                  | 1     | 0      | 0    | 0    | 1    | 0    | 0    | 0    |
|                      |                 | Relative abundance (No./Kb) | 0.02  | 0      | 0    | 0    | 0.01 | 0    | 0    | 0    |
|                      |                 | Relative density (bp/Kb)    | 0.28  | 0      | 0    | 0    | 0.16 | 0    | 0    | 0    |
|                      |                 | GC content                  | 0.4   | 0      | 0    | 0    | 0.4  | 0    | 0    | 0    |
|                      | mononucleotide  | No.of SSRs                  | 80    | 15     | 0    | 0    | 67   | 16   | 6    | 6    |
|                      |                 | Relative abundance (No./Kb) | 1.66  | 0.94   | 0    | 0    | 0.78 | 0.74 | 0.25 | 0.25 |
|                      |                 | Relative density (bp/Kb)    | 20.49 | 13.27  | 0    | 0    | 9.78 | 8.83 | 3.41 | 3.41 |
|                      |                 | GC content                  | 0.19  | 0.36   | 0    | 0    | 0.20 | 0.19 | 0.36 | 0.36 |
|                      | Dinucleotide    | No.of SSRs                  | 13    | 1      | 0    | 0    | 7    | 3    | 2    | 2    |
|                      |                 | Relative abundance (No./Kb) | 0.27  | 0.06   | 0    | 0    | 0.08 | 0.14 | 0.08 | 0.08 |
|                      |                 | Relative density (bp/Kb)    | 3.89  | 1.50   | 0    | 0    | 1.32 | 2.31 | 0.99 | 0.99 |
|                      |                 | GC content                  | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      | Trinucleotide   | No.of SSRs                  | 0     | 1      | 0    | 0    | 1    | 0    | 0    | 0    |
|                      |                 | Relative abundance (No./Kb) | 0     | 0.06   | 0    | 0    | 0.01 | 0    | 0    | 0    |
|                      |                 | Relative density (bp/Kb)    | 0     | 0.75   | 0    | 0    | 0.14 | 0    | 0    | 0    |
|                      |                 | GC content                  | 0     | 0.33   | 0    | 0    | 0.33 | 0    | 0    | 0    |
|                      | Tetranucleotide | No.of SSRs                  | 7     | 1      | 3    | 0    | 6    | 1    | 2    | 2    |
|                      |                 | Relative abundance (No./Kb) | 0.14  | 0.06   | 0.04 | 0    | 0.07 | 0.05 | 0.08 | 0.08 |
|                      |                 | Relative density (bp/Kb)    | 1.82  | 0.75   | 0.45 | 0    | 0.88 | 0.55 | 0.99 | 0.99 |
|                      |                 | GC content                  | 0.22  | 0.75   | 0.33 | 0    | 0.29 | 0.50 | 0.25 | 0.25 |
|                      | Pentanucleotide | No.of SSRs                  | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      |                 | Relative abundance (No./Kb) | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      |                 | Relative density (bp/Kb)    | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      |                 | GC content                  | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
| <i>A. spinulosa</i>  | mononucleotide  | No.of SSRs                  | 81    | 15     | 0    | 0    | 68   | 16   | 6    | 6    |
|                      |                 | Relative abundance (No./Kb) | 1.68  | 0.99   | 0    | 0    | 0.79 | 0.74 | 0.25 | 0.25 |
|                      |                 | Relative density (bp/Kb)    | 20.73 | 12.45  | 0    | 0    | 9.63 | 8.74 | 3.49 | 3.49 |
|                      |                 | GC content                  | 0.18  | 0.28   | 0    | 0    | 0.17 | 0.19 | 0.31 | 0.31 |
|                      | Dinucleotide    | No.of SSRs                  | 13    | 1      | 0    | 0    | 7    | 3    | 2    | 2    |
|                      |                 | Relative abundance (No./Kb) | 0.27  | 0.07   | 0    | 0    | 0.08 | 0.14 | 0.08 | 0.08 |
|                      |                 | Relative density (bp/Kb)    | 3.89  | 1.58   | 0    | 0    | 1.32 | 2.31 | 0.99 | 0.99 |
|                      |                 | GC content                  | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      | Trinucleotide   | No.of SSRs                  | 0     | 1      | 0    | 0    | 1    | 0    | 0    | 0    |
|                      |                 | Relative abundance (No./Kb) | 0     | 0.07   | 0    | 0    | 0.01 | 0    | 0    | 0    |
|                      |                 | Relative density (bp/Kb)    | 0     | 0.79   | 0    | 0    | 0.14 | 0    | 0    | 0    |
|                      |                 | GC content                  | 0     | 0.33   | 0    | 0    | 0.33 | 0    | 0    | 0    |
|                      | Tetranucleotide | No.of SSRs                  | 7     | 1      | 3    | 0    | 6    | 1    | 2    | 2    |
|                      |                 | Relative abundance (No./Kb) | 0.14  | 0.07   | 0.04 | 0    | 0.07 | 0.05 | 0.08 | 0.08 |

| Species              | motif type      | Characteristics of SSR      | IGS   | intron | CDS  | gene | LSC  | SSC  | IRA  | IRB  |
|----------------------|-----------------|-----------------------------|-------|--------|------|------|------|------|------|------|
| <i>S. brunoniana</i> | Pentanucleotide | Relative density (bp/Kb)    | 1.57  | 0.79   | 0.45 | 0    | 0.74 | 0.55 | 0.99 | 0.99 |
|                      |                 | GC content                  | 0.25  | 0.75   | 0.33 | 0    | 0.34 | 0.50 | 0.25 | 0.25 |
|                      |                 | No.of SSRs                  | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      |                 | Relative abundance (No./Kb) | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      | mononucleotide  | Relative density (bp/Kb)    | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      |                 | GC content                  | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      |                 | No.of SSRs                  | 62    | 15     | 0    | 0    | 61   | 10   | 3    | 3    |
|                      |                 | Relative abundance (No./Kb) | 1.29  | 1.04   | 0    | 0    | 0.71 | 0.45 | 0.12 | 0.12 |
|                      | Dinucleotide    | Relative density (bp/Kb)    | 17.20 | 12.25  | 0    | 0    | 9.22 | 5.66 | 1.71 | 1.71 |
|                      |                 | GC content                  | 0.08  | 0.08   | 0    | 0    | 0.10 | 0    | 0    | 0    |
|                      |                 | No.of SSRs                  | 9     | 6      | 0    | 0    | 10   | 3    | 1    | 1    |
|                      |                 | Relative abundance (No./Kb) | 0.19  | 0.42   | 0    | 0    | 0.12 | 0.13 | 0.04 | 0.04 |
|                      | Trinucleotide   | Relative density (bp/Kb)    | 2.95  | 5.40   | 0    | 0    | 1.72 | 2.14 | 0.50 | 0.50 |
|                      |                 | GC content                  | 0.04  | 0.24   | 0    | 0    | 0.05 | 0.13 | 0.50 | 0.50 |
|                      |                 | No.of SSRs                  | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      |                 | Relative abundance (No./Kb) | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      | Tetranucleotide | Relative density (bp/Kb)    | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      |                 | GC content                  | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      |                 | No.of SSRs                  | 6     | 0      | 2    | 2    | 4    | 0    | 3    | 3    |
|                      |                 | Relative abundance (No./Kb) | 0.12  | 0      | 0.02 | 0.22 | 0.05 | 0    | 0.12 | 0.12 |
|                      | Pentanucleotide | Relative density (bp/Kb)    | 1.66  | 0      | 0.30 | 2.64 | 0.65 | 0    | 1.50 | 1.50 |
|                      |                 | GC content                  | 0.25  | 0      | 0.25 | 0.5  | 0.25 | 0    | 0.33 | 0.33 |
|                      |                 | No.of SSRs                  | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      |                 | Relative abundance (No./Kb) | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
| <i>S. lepifera</i>   | mononucleotide  | Relative density (bp/Kb)    | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      |                 | GC content                  | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      |                 | No.of SSRs                  | 69    | 11     | 0    | 0    | 62   | 12   | 3    | 3    |
|                      |                 | Relative abundance (No./Kb) | 1.29  | 0.75   | 0    | 0    | 0.72 | 0.43 | 0.12 | 0.12 |
|                      | Dinucleotide    | Relative density (bp/Kb)    | 16.69 | 8.63   | 0    | 0    | 9.11 | 5.63 | 1.66 | 1.66 |
|                      |                 | GC content                  | 0.07  | 0.24   | 0    | 0    | 0.12 | 0    | 0    | 0    |
|                      |                 | No.of SSRs                  | 8     | 3      | 0    | 0    | 5    | 4    | 1    | 1    |
|                      |                 | Relative abundance (No./Kb) | 0.15  | 0.20   | 0    | 0    | 0.06 | 0.14 | 0.04 | 0.04 |
|                      | Trinucleotide   | Relative density (bp/Kb)    | 2.38  | 2.72   | 0    | 0    | 0.95 | 2.09 | 0.58 | 0.58 |
|                      |                 | GC content                  | 0.05  | 0.35   | 0    | 0    | 0    | 0.10 | 0.50 | 0.50 |
|                      |                 | No.of SSRs                  | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      |                 | Relative abundance (No./Kb) | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      | Tetranucleotide | Relative density (bp/Kb)    | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      |                 | GC content                  | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      |                 | No.of SSRs                  | 5     | 0      | 4    | 2    | 3    | 0    | 4    | 4    |
|                      |                 | Relative abundance (No./Kb) | 0.09  | 0      | 0.05 | 0.22 | 0.03 | 0    | 0.17 | 0.17 |
|                      | Pentanucleotide | Relative density (bp/Kb)    | 1.19  | 0      | 0.60 | 2.64 | 0.46 | 0    | 1.99 | 1.99 |
|                      |                 | GC content                  | 0.20  | 0      | 0.13 | 0.50 | 0.18 | 0    | 0.25 | 0.25 |
|                      |                 | No.of SSRs                  | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      |                 | Relative abundance (No./Kb) | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      |                 | Relative density (bp/Kb)    | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |
|                      |                 | GC content                  | 0     | 0      | 0    | 0    | 0    | 0    | 0    | 0    |

Appendix table 11 Significant differences in the number, relative abundance, relative density, and GC content of the chloroplast genomes, Mono- to Pentanucleotide SSRs, IGS, LSC, intron and CDS regions SSRs of

chloroplast genomes, and the mono- to pentanucleotide SSRs in the chloroplast genomes of 8 Cyatheaceae species

| Classification treatment | Characteristics of SSR      | P         | P               | P         | P         | P    |
|--------------------------|-----------------------------|-----------|-----------------|-----------|-----------|------|
|                          |                             | Genome    | Mono-           | Di-       | Tri-      | Tet- |
| 3 genera                 | No.of SSRs                  | 0.048*    | 0.048*          | 0.065     | 0.057     | 0.70 |
|                          | Relative abundance (No./Kb) | 0.033*    | 0.050*          | 0.069     | 0.103     | 0.40 |
|                          | Relative density            | 0.050*    | 0.050*          | 0.069     | 0.103     | 0.40 |
|                          | GC content                  | 0.050*    | 0.050*          | 0.068     | 0.030*    | 0.00 |
| 2 genera                 | No.of SSRs                  | 0.502     | 0.502           | 0.177     | 0.034*    | 0.72 |
|                          | Relative abundance (No./Kb) | 0.478     | 0.505           | 0.182     | 0.044*    | 0.18 |
|                          | Relative density            | 0.505     | 0.505           | 0.182     | 0.044*    | 0.50 |
|                          | GC content                  | 0.046*    | 0.046*          | 0.040*    | 0.378     | 0.17 |
| P                        | P                           | P         | P               | P         | P         | P    |
| IGS-Mono                 | intron-Mono                 | CDS-Mono  | rRNA gene-Mono  | LSC-Mono  | SSC-Mono  | IRA  |
| 0.048                    | 0.056                       | 1         | 1               | 0.048     | 0.046*    | 0.13 |
| 0.05                     | 0.069                       | 1         | 1               | 0.050*    | 0.050*    | 0.13 |
| 0.05                     | 0.050*                      | 1         | 1               | 0.050*    | 0.105     | 0.03 |
| 0.05                     | 0.223                       | 1         | 1               | 0.050*    | 0.103     | 0.00 |
| 0.502                    | 0.306                       | 1         | 1               | 0.502     | 0.500     | 0.73 |
| 0.505                    | 0.182                       | 1         | 1               | 0.505     | 0.505     | 0.50 |
| 0.505                    | 0.505                       | 1         | 1               | 0.505     | 1         | 0.50 |
| 0.046                    | 0.096                       | 1         | 1               | 0.046*    | 0.044*    | 0.23 |
| P                        | P                           | P         | P               | P         | P         | P    |
| IGS-Tri                  | intron-Tri                  | CDS-Tri   | rRNA gene-Tri   | LSC-Tri   | SSC-Tri   | IRA  |
| 0.122                    | 0.122                       | 1         | 1               | 0.122     | 0.122     | 1    |
| 0.14                     | 0.140                       | 1         | 1               | 0.067     | 0.14      | 1    |
| 0.14                     | 0.140                       | 1         | 1               | 0.067     | 0.14      | 1    |
| 1                        | 0.030*                      | 1         | 1               | 0.030*    | 1         | 1    |
| 0.237                    | 0.237                       | 1         | 1               | 0.237     | 0.237     | 1    |
| 0.252                    | 0.252                       | 1         | 1               | 0.252     | 0.252     | 1    |
| 0.252                    | 0.252                       | 1         | 1               | 0.252     | 0.252     | 1    |
| 1                        | 0.378                       | 1         | 1               | 0.378     | 1         | 1    |
| P                        | P                           | P         | P               | P         | P         | P    |
| IGS-Penta                | intron-Penta                | CDS-Penta | rRNA gene-Penta | LSC-Penta | SSC-Penta | IRA  |
| 0.311                    | 0.607                       | 1         | 1               | 0.122     | 1         | 1    |
| 0.319                    | 0.607                       | 1         | 1               | 0.140     | 1         | 1    |
| 0.319                    | 0.607                       | 1         | 1               | 0.140     | 1         | 1    |
| 0.607                    | 0.607                       | 1         | 1               | 0.311     | 1         | 1    |
| 0.378                    | 0.564                       | 1         | 1               | 0.237     | 1         | 1    |
| 0.383                    | 0.564                       | 1         | 1               | 0.252     | 1         | 1    |
| 0.383                    | 0.564                       | 1         | 1               | 0.252     | 1         | 1    |
| 0.564                    | 0.564                       | 1         | 1               | 0.378     | 1         | 1    |

When 8 Cyatheaceae plants are treated as three genera, Kruskal-Wallis H test is used; when treated as two generas, Mann-Whitney U test is used; \*:  $P$  [?]0.05; IGS: Intergenic Spacer-Region; LSC: Large Single Copy-Region; CDS: Coding Sequence-Region