

Communication

Characterization of the Complete Chloroplast Genome Sequence of the Socotra Dragon's Blood Tree (*Dracaena cinnabari* Balf.)

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Abstract: The Socotra dragon's blood tree (*Dracaena cinnabari* Balf.) is endemic to the island of Socotra in Yemen. This iconic species plays an essential role in the survival of associated organisms, acting as an umbrella tree. Overexploitation, overgrazing by livestock, global climate change, and insufficient regeneration mean that the populations of this valuable species are declining in the wild. Although there are many studies on the morphology, anatomy, and physiology of *D. cinnabari*, no genomic analysis of this endangered species has been performed so far. Therefore, the main aim of this study was to characterize the complete chloroplast sequence genome of *D. cinnabari* for conservation purposes. The *D. cinnabari* chloroplast genome is 155,371 bp with a total GC content of 37.5%. It has a quadripartite plastid genome structure composed of one large single-copy region of 83,870 bp, one small single-copy region of 18,471 bp, and two inverted repeat regions of 26,515 bp each. One hundred and thirty-two genes were annotated, 86 of which are protein-coding genes, 38 are transfer RNAs, and eight are ribosomal RNAs. Forty simple sequence repeats have also been identified in this chloroplast genome. Comparative analysis of complete sequences of *D. cinnabari* chloroplast genomes with other species of the genus *Dracaena* showed a very high conservativeness of their structure and organization. Phylogenetic inference showed that *D. cinnabari* is much closer to *D. draco*, *D. cochinchinensis*, and *D. cambodiana* than to *D. terniflora*, *D. angustifolia*, *D. hokouensis*, and *D. elliptica*. The results obtained in this study provide new and valuable omics data for further phylogenetic studies of the genus *Dracaena* as well as enable the protection of genetic resources of highly endangered *D. cinnabari*.

Keywords: *Dracaena*; chloroplast genome; conservation genetics; Socotra dragon's blood tree; taxonomy

1. Introduction

Dragon trees [1] of the genus *Dracaena* Vand. ex L. (Asparagaceae, Nolinoideae) are an interesting group of arbors renowned for their red sap [2]. One of them, *Dracaena cinnabari* Balf., was already valued in ancient times as it is today. This species is endemic to the Socotra Island in Yemen, where its sap has been harvested for medical and cultural uses since ancient time [3,4]. Individuals of this iconic species play an important role as umbrella trees, vital to the survival of organisms associated with them [5,6]. Unfortunately,

due to over-exploitation, overgrazing by livestock, global climate change, and insufficient regeneration, the number of trees of this valuable species is currently decreasing [7–10], despite attempts to regenerate it in situ and ex situ [11,12]. The decline in the population of *D. cinnabari* is clearly documented. Adolt and Pavliš [7] predicted the disappearance of the population of *D. cinnabari* on Socotra within 50–144 years. Maděra et al. [13] published an extinction model for *D. cinnabari* with different results; the extinction time of individual sub-populations varies from 31 to 564 years, but the model indicates a population decline of more than 40% in 100 years. Attorre et al. [8] concluded that the current potential habitat of *D. cinnabari* would be reduced by 45% in 2080. The International Union for Conservation of Nature ranked *D. cinnabari* as a vulnerable species [14].

Although representatives of the genus *Dracaena* are quite well described in terms of morphological or anatomical features [15,16], secondary growth in stem and roots [17,18], growth dynamic [10], or chemical diversity of resins [19], there is relatively little genetic and genomic studies [20,21]. According to Madera et al. [2], population genetic analyses are required for assessing contemporary and historical breeding systems and potential bottlenecks to enhance conservation management strategies. Surprisingly for such an iconic species as *D. cinnabari*, no genomic studies have been published to date. Therefore, it is difficult to develop any genetic conservation plans for this species without prior knowledge of its genetic and genomic diversity.

The sequences of chloroplast (cp) genomes obtained as a result of next-generation sequencing (NGS) are a valuable source of data for taxonomy, conservation genetics, and phylogenetics. Their high usefulness in these areas has been repeatedly confirmed, both in the case of representatives of the Asparagaceae family, i.e., *Asparagus officinalis* L. [22], *Asparagus setaceus* (Kunth) Jessop [23], *Convallaria keiskei* Miq. or *Liriope spicata* (Thunb.) Lour. [24] and in the genus *Dracaena*, i.e., *D. cochinchinensis* (Lour.) S.C. Chen, *D. cambodiana* Pierre ex Gagnep., *D. angustifolia* (Medik.) Roxb., *D. terniflora* Roxb., *D. hokouensis* G.Z. Ye and *D. elliptica* Thunb. [25] or recently, *D. draco* (L.) L. [26]. However, no similar study has been published to date on the detailed characterization of the chloroplast genome features for *D. cinnabari*. There is an urgent need to fill this gap and provide missing data.

Therefore, taking into account the lack of genomic studies and the high risk of extinction of *D. cinnabari*, the main objectives of this study are: (1) characterization of the complete genome sequence of *D. cinnabari* chloroplast; (2) comparison of its features with chloroplast genomes of other members of the genus *Dracaena* and (3) determination of the position of *D. cinnabari* within the genus based on the analysis of complete sequences of chloroplast genomes. Our results provide new and valuable omics data for further phylogenetic studies of the genus *Dracaena* and enable the protection of genetic resources of endangered *D. cinnabari*.

2. Materials and Methods

2.1. Plant Material and DNA Isolation

Dracaena cinnabari leaves were obtained from the Botanical Gardens and Arboretum of the Mendel University in Brno (Czech Republic) (49°21' N, 16°61' E) from a specimen cultivated there since 2001 (collection number BZA-7886). The CTAB method [27] was used to extract total DNA. Agarose gel electrophoresis and a NanoDrop spectrophotometer (Thermo Fisher Scientific, Carlsbad, CA, USA) were used to check the quality and purity of the extracted DNA.

2.2. Library Preparing and Sequencing

Ion Xpress Plus Fragment Library Kit (Thermo Fisher Scientific, Waltham, MA, USA) was used to prepare Ion Torrent libraries. Size selection of the obtained DNA fragments was performed using the E-Gel Precast Agarose Electrophoresis System (Thermo Fisher Scientific, Waltham, MA, USA). The resulting Ion Torrent libraries were then sequenced on the Ion Torrent GeneStudio™ S5 System.

2.3. Genome Assembly and Annotation

The filtering of low-quality reads, as well as trimming ends and adapters, were performed with BBDuk Adapter/Quality Trimming V. 35.82 implemented in Geneious Prime 2020.0.49.1.7 [28]. The filtered reads were de novo assembled into contigs using Geneious Assembler with default options.

Contigs were mapped to *Dracaena cambodiana* (NC_039776) using Geneious Mapper with a minimum mapping quality of 30. The obtained reads were used to assemble the complete chloroplast genome of *D. cinnabari* de novo, which was then annotated with CPGAVAS2 [29] and GeSeq [30]. The location of the large single-copy region (LSC) and the small single-copy region (SSC), as well as the calculation of GC content, was done in Geneious Prime 2020.0.49.1.7 [28]. Detection of transfer RNAs was performed with tRNAscan-RE v2.0.3. [31] implemented in GeSeq [30]. The circular gene map of the *D. cinnabari* cp genome was drawn in OGDRAW 1.3.1 [32]. The complete *D. cinnabari* chloroplast genome sequence has been deposited in GenBank under accession number OM961177.

2.4. Genome Comparative Analysis

Genome-wide evolutionary dynamics and events in *Dracaena* species were analyzed using the progressive MAUVE algorithm with default settings and MAUVE [33] plugin v1.1.1 available in Geneious Prime 2020.0.49.1.7 [28]. The chloroplast genome of *Dracaena cinnabari* as a reference sequence was used for comparisons with previously published *Dracaena* species, i.e., *D. angustifolia* [25], *D. draco* [26], and *D. elliptica* [25].

2.5. Identification of Simple Sequence Repeats and Phylogenetic Inference

MIcroSATellite (MISA) [34], with parameters set at ≥ 10 for mononucleotides, ≥ 6 for dinucleotides, and ≥ 5 for tri-, tetra-, penta- and hexanucleotides, respectively, was used to identify simple sequence repeats (SSRs) in the *D. cinnabari* cp genome sequence. Detailed microsatellite characteristics were conducted using the following indices: Total counts, Total Repeat Length (bp), and Mean Length (bp). Total Repeat Length was calculated as SSR repeat motif $\times$ number of repeats (bp).

2.6. Phylogenetic Analysis

Phylogenetic inference was carried out by the maximum likelihood (ML) method using nine complete sequences of chloroplast genomes of various Asparagaceae members, i.e., *Dracaena angustifolia* [MN200193]; *Dracaena cambodiana* [MN200194]; *Dracaena cochinchinensis* [MN200195]; *Dracaena cinnabari* [OM961177]; *Dracaena draco* [MN990038]; *Dracaena elliptica* [MN200196]; *Dracaena hokouensis* [MN200197]; *Dracaena terniflora* [MN200198] and *Asparagus officinalis* [NC_034777] as the outgroup. Complete chloroplast genomes were aligned with MAFFT 7.450 using default settings [35]. A General Time Reversible + Proportion Invariance + Gamma nucleotide substitution model (GTR + I + G) was used as the substitution model for the ML analysis [26]. The ML analysis was performed in RaxML v8.2.11 [36], with 1000 rapid bootstrap replicates along with a search for the best-scoring ML tree in every run and parsimony random seed set to 10.

3. Results

The complete chloroplast genome of *Dracaena cinnabari* is 155,371 bp in length (Figure 1) and exhibits a typical quadripartite structure of large (LSC, 83,870 bp) and small (SSC, 18,471 bp) single-copy regions, which are separated by a pair of inverted regions (IRs, 26,515 bp each). The size of the *D. cinnabari* cp genome, as well as the length of its individual regions, LSC, SSC, and IR, are very similar to those found recently in other members of the genus *Dracaena* (Table 1).

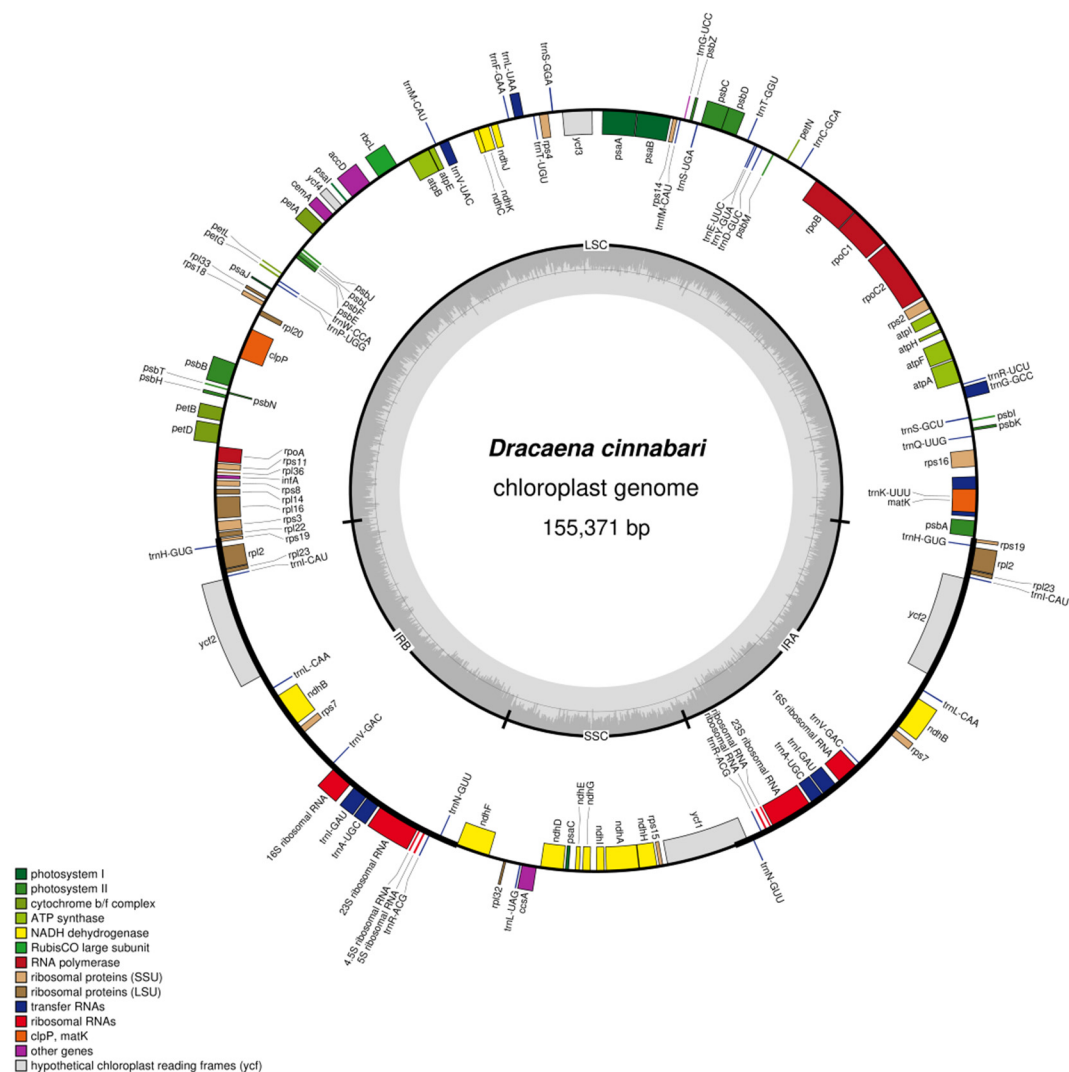


Figure 1. Gene map of the *Dracaena cinnabari* chloroplast genome. The genes inside the circle are transcribed clockwise, while those on the outside are transcribed counterclockwise. Genes belonging to different functional groups are color-coded. The darker gray in the inner circle shows the GC content, while the lighter gray corresponds to the AT content. IRA, IRB, inverted repeats; LSC, large single-copy region; SSC, small single-copy region.

Table 1. Comparison of basic features of chloroplast genomes among four *Dracaena* species.

Species	<i>D. angustifolia</i>	<i>D. cinnabari</i>	<i>D. draco</i>	<i>D. elliptica</i>
Total length (bp)	155,332	155,371	155,422	155,055
IR length (bp)	53,060	53,030	53,004	52,978
LSC length (bp)	83,807	83,870	83,946	83,621
SSC length (bp)	18,465	18,471	18,472	18,456
Total gene number	130	132	132	130
rRNA	8	8	8	8
tRNA	38	38	38	38
GC content (%)	37.5	37.5	37.6	37.5
GenBan accession	MN200193	OM961177	MN990038	MN200196
Reference	[25]	This study	[26]	[25]

The lengths of the LSC and SSC regions of *D. angustifolia*, *D. cinnabari*, *D. draco*, and *D. elliptica* are very similar at approximately 83 kbp and 18.4 kbp, respectively. There were also no major differences between the four *Dracaena* species in the IR regions. The total percentage of GC content in the *D. cinnabari* cp genome is equal to 37.5%.

There are 132 genes (113 unique) described in the *D. cinnabari* chloroplast genome. Of these, eighty-six genes encode proteins, thirty-eight transfer RNA genes, and eight ribosomal RNA genes (Table 1). Eighteen genes found in the *D. cinnabari* genome were duplicated, and one gene exists in three copies. Thirteen genes had one intron, while two genes possessed two introns. The list of genes annotated in the complete cp genome sequence of *D. cinnabari* is presented in Table 2.

Table 2. List of genes present in the *D. cinnabari* chloroplast genome.

No.	Group of Genes	Name of Genes	Number
1	Photosystem I	<i>psaA, psaB, psaC, psaI, psaJ</i>	5
2	Photosystem II	<i>psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbM, psbNpsbT, psbZ</i>	15
3	Cytochrome b/f complex	<i>petA, petB *</i> , <i>petD *</i> , <i>petG, petL, petN</i> ,	6
4	ATP synthase	<i>atpA, atpB, atpE, atpF *</i> , <i>atpH, atpI</i> ,	6
5	NADH dehydrogenase	<i>ndhA *</i> , <i>ndhB *(×2)</i> , <i>ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK</i>	12
6	RubisCO large subunit	<i>rbcL</i>	1
7	RNA polymerase	<i>rpoA, rpoB, rpoC1 *</i> , <i>rpoC2</i>	4
8	Ribosomal proteins –small units (SSU)	<i>rps2, rps3, rps4, rps7(×2), rps8, rps11, rps12 *(×2), rps14, rps15, rps16 *</i> , <i>rps18, rps19(×2)</i> ,	15
9	Ribosomal proteins –large units (LSU)	<i>rpl2 *(×2), rpl14, rpl16 *, rpl20, rpl22, rpl23(×2), rpl32, rpl33, rpl36</i> ,	11
10	Other genes/ Miscellaneous	<i>accD, ccsA, cemA, clpP **, infA, matK</i> ,	6
11	Protein of unknown function	<i>ycf1, ycf2(×2), ycf3 **, ycf4</i>	5
12	Transfer RNAs	<i>trnA-UGC(×2), trnC-GCA, trnD-GUC, trnE-UUC, trnF-GAA, trnG-M-CAU, trnG-GCC, trnH-GUG(×2), trnI-CAU(×2), trnI-GAU *(×2), trnK-UUU *, trnL-CAA(×2), trnL-UAA *, trnL-UAG, trnM-CAU, trnN-GUU(×2), trnP-UGG, trnQ-UUG, trnR-ACG(×2), trnR-UCU, trnS-GCU, trnS-GGA, trnS-UGA, trnT-GGU, trnT-UGU, trnV-GAC(×3), trnV-UAC *, trnW-CCA, trnY-GUA</i>	38
13	Ribosomal RNAs	<i>rrn4.5(×2), rrn5(×2), rrn16(×2), rrn23(×2)</i>	8
Total			132

* Gene contains one intron. ** Gene contains two introns. (×2) indicates that the number of repeat units is 2.

Complete chloroplast genomes alignment of four selected species of *Dracaena*, i.e., *D. angustifolia*, *D. cinnabari*, *D. draco*, and *D. elliptica*, was done using Mauve software (Figure 2). The results obtained in the previous paragraph clearly showed that no major changes in the structural organization of the cp genomes in *Dracaena* could be expected. In fact, genome-wide alignment revealed the presence of only one locally collinear block (LCB) between all the complete chloroplast genomes analyzed. This indicates an extremely high level of similarity in the genome organization between the analyzed *Dracaena* species and the absence of any evolutionary events such as gene loss, duplication, rearrangements, and translocations.

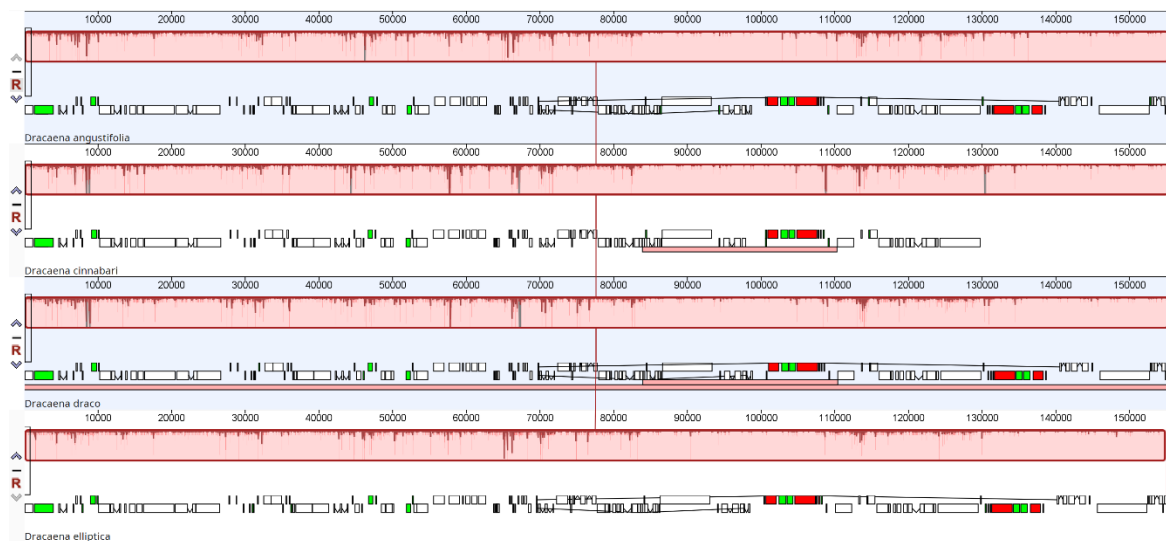


Figure 2. Comparison of four *Dracaena* chloroplast genomes using a progressive MAUVE algorithm.

The obtained results of the whole genomes comparison are also confirmed by the contraction and expansion analysis of the IR regions. In general, the IRb/LSC border is located between *rps19* and *rpl22* genes, and the IRa/LSC border is located between *rps19* and *psbA* genes. The *ycf1* gene spanned the JSA (SSC/IRa) region, and the *ndhF* gene spanned the JSB (IRb/SSC) region in the species studied. Moreover, in the JSB region, the *ycf1* pseudogene is present only in *D. angustifolia* and *D. elliptica*, but not in *D. cinnabari* and *D. draco*. A comparison of the IR/SSC and IR/LSC boundaries for the four *Dracaena* cp genomes is shown in Figure 3.

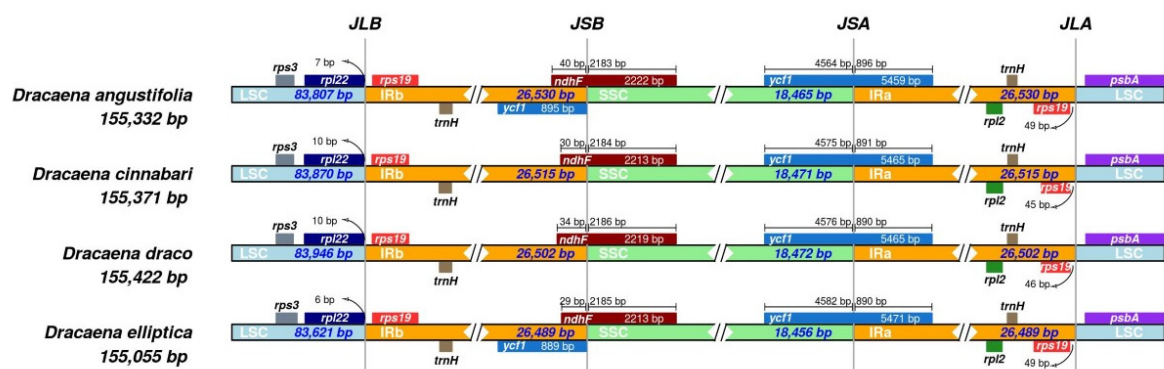


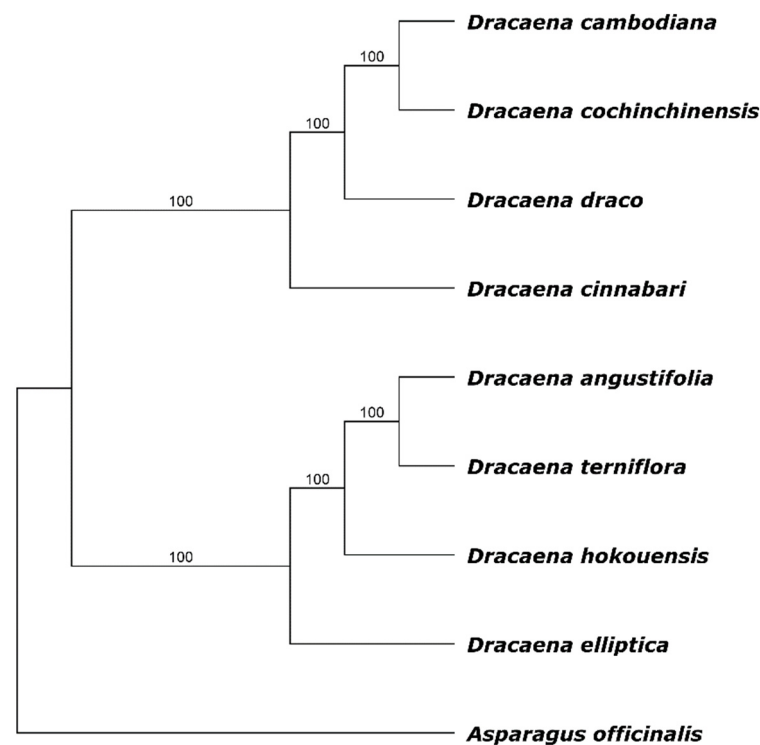
Figure 3. Comparison of the borders of LSC, SSC, and IR regions among the four *Dracaena* chloroplast genomes.

A total of forty simple sequence repeats (SSRs) of at least 10 bp length were identified in the *D. cinnabari* cp genome. The number of SSRs detected in this study is slightly lower than those obtained from other *Dracaena* species, in which the number of SSRs ranged from 42 for *D. angustifolia* and 47 for *D. elliptica* to 60 for *D. draco* using the same methodology (Table 3). Most SSRs identified in this study (Table 3) had a mononucleotide motif (85.0%). The genome sequence of *D. cinnabari* chloroplast compared to three *Dracaena* species was characterized by the shortest total repeat length (363 bp) and lowest mean length (10.68 bp).

Table 3. Characteristics of SSRs present in the four *Dracaena* chloroplast genomes.

Species	Indices	Motif		Total
		Mononucleotide	Dinucleotide	
<i>D. angustifolia</i>	Total counts	38	4	42
	Total Repeat Length (bp)	409	54	
	Mean Length (bp)	10.76	13.5	
<i>D. cinnabari</i>	Total counts	34	6	40
	Total Repeat Length (bp)	363	78	
	Mean Length (bp)	10.68	13	
<i>D. draco</i>	Total counts	56	4	60
	Total Repeat Length (bp)	638	50	
	Mean Length (bp)	11.39	12.5	
<i>D. elliptica</i>	Total counts	44	3	47
	Total Repeat Length (bp)	473	36	
	Mean Length (bp)	10.75	12	

In order to investigate the phylogenetic position of *D. cinnabari* within the *Dracaena* genus, we constructed a phylogenetic tree using the maximum likelihood (ML) algorithm and the complete sequences of cp genomes of nine members of Asparagaceae. As shown in Figure 4, the resulting ML phylogenetic tree clearly indicates that all species of the *Dracaena* genus formed one distinct cluster. This cluster consists of two monophyletic groups. The first solely includes species of the dragon blood's trees: Asian *D. cochinchinensis* and *D. cambodiana*, which create a sister clade, more distant *D. draco* from Morocco and Atlantic islands and the Socotran *D. cinnabari*. The second group includes four Asian species of *Dracaena*, which represent a different ecological group of mesophytic forest plants: *D. hokouensis*, *D. elliptica*, and a sister clade of *D. terniflora* and *D. angustifolia*. *Asparagus officinalis* was outside the two main distinguished *Dracaena* groups.

**Figure 4.** The ML phylogenetic tree of the *Dracaena* genus based on nine complete chloroplast genomes. The numbers near each node are bootstrap support values obtained by ML.

4. Discussion

The main aim of the study was to characterize the complete chloroplast genome sequence of *D. cinnabari*, compare it with the chloroplast genomes of other members of the genus *Dracaena* and determine the position of *D. cinnabari* within the genus based on the analysis of complete sequences of chloroplast genomes. Since *D. cinnabari* is a highly endangered species, it is necessary to undertake all actions that could reduce this undesirable phenomenon. The ideal solution for this purpose seems to be the use of conservation genetics methods and omics data.

Chloroplast genome sequences are commonly used in various areas of biology, i.e., phylogenetics [37,38], DNA barcoding [39,40], taxonomy, evolution, and population genetics [41] of both angiosperms and gymnosperms. In the last five years, many genomic studies of various representatives of the genus *Dracaena* [25,26] and the family Asparagaceae [22,23,42,43] have also been published, but so far, none have included *D. cinnabari*. Taking into account the lack of genomic study on this iconic species, we decided to provide new and valuable omics data which enable the protection of its genetic resources as well as for further phylogenetic studies of the genus *Dracaena*. Especially since Zhang et al. [25] and Takawira-Nyenyanya et al. [44] postulate that the analysis of chloroplast genome and next-generation sequencing should be the preferred method of analyzing the complex evolutionary history of dracenoids.

The length of the complete sequence of the chloroplast genome of *D. cinnabari* (155,371 bp) is very similar to recently published features of chloroplast genomes for other Asparagaceae members, i.e., *Convallaria keiskei* (162,109 bp) or *Liriope spicata* (157,055 bp) [24] and almost identical to other species of *Dracaena*, i.e., *D. angustifolia* (155,332 bp) or *D. draco* (155,422) [25,26]. The differences in the length of the *Dracaena* genomes are really small and do not exceed 400 bp [25]. We also found no major differences in the number of annotated genes in *D. cinnabari* and other *Dracaena* species. The complete chloroplast genome of *D. cinnabari* has a typical quadripartite structure, consisting of a pair of inverted repeats (IRs), separated by a large single-copy region (LSC) and a small single-copy region (SSC) that occurs in many other plant species [45–47], including those of the genus *Dracaena* [25,26] and the family Asparagaceae [23,24,42,43]. Our findings in this study regarding the organization of the *D. cinnabari* cp genome are very similar to those previously published for other members of the genus *Dracaena* [25,26]. On this basis, it can therefore be concluded that the chloroplast genomes in this genus are highly conserved. These observations are also confirmed by the results of whole-genome alignment as well as the analysis of contraction and expansion of the boundaries of the IR regions.

Microsatellites seem to be an ideal DNA marker for conducting population, ecological, and conservation genetics studies. The level of genetic polymorphism detected as well as the wide distribution throughout the genome, make them highly informative molecular markers [48,49]. In our study, we observed some differences between the analyzed *Dracaena* species in the number of microsatellite loci. Recently published data show a higher number of SSR loci in *Dracaena* species (64–71) [25] than those we found for *D. cinnabari* (40) in this study. The differences in numbers, however, result from different parameters of the microsatellite sequence identification and not from changes in the size of the cp genome, and when analyzed under the same conditions, the observed differences between individual species are smaller but still noticeable. The small number of microsatellites found in *D. cinnabari* may mean potential problems with the development of a sufficient number of highly polymorphic microsatellite markers to analyze the genetic diversity of this species population. Nevertheless, the SSRs identified in this study still have some analytical potential that requires testing on a larger number of individuals before being used to characterize *D. cinnabari* genetic resources. Moreover, taking into account the high conservation of the genomes of the genus *Dracaena*, confirmed both in this study and previously by Zhang et al. [25] and Celiński et al. [26], it seems possible to develop one universal set of primers amplifying microsatellite loci in several species. There are many studies showing the usefulness of such universal primers [50,51] as well as the cross-

species amplification [52,53] approach. Understanding genetic resources is the first point in developing conservation plans. Hence, knowing the level of genetic variation is crucial. For some species of Asparagaceae, such development of SSR sets has already been done, incl. for *Maianthemum bicolor* [54]. To the best of the authors' knowledge, there is no such study so far for species of the genus *Dracaena*.

In this study, we paid particular attention to determining the phylogenetic position of *D. cinnabari* within the *Dracaena* genus. Therefore, we constructed a phylogenetic tree using the maximum likelihood algorithm and the complete cp genome sequences of nine members of Asparagaceae. The phylogenetic inference revealed that all species of the *Dracaena* genus formed one separate cluster and that *D. cinnabari* is much closer to *D. draco*, *D. cochinchinensis*, and *D. cambodiana* than to *D. terniflora*, *D. angustifolia*, *D. hokouensis*, and *D. elliptica*. Such grouping of species is consistent with their morphological and physiological characteristics. The results obtained in this study confirm the previously published phylogeny of the *Dracaena* genus reported by Zhang et al. [25] and Celiński et al. [26]. However, the cited studies did not include *D. cinnabari*, and our current research fills this gap perfectly. Moreover, our findings strongly support the conclusions of Takawira-Nyanya et al. [44] about the monophyletic origins of *Dracaena*.

5. Conclusions

This study characterized for the first time the complete chloroplast genome sequence of the *Dracaena cinnabari*. The obtained results broaden our genomic knowledge about this iconic species and help protect its endangered genetic resources. Future work should focus on the development of a microsatellite marker set to characterize the level and distribution of genetic diversity present in the Socotra dragon's blood tree populations. Since the phylogeny of the genus *Dracaena* is still not fully understood, further research in this area should be carried out. These should include both mesophytic and dragon species of *Dracaena* throughout its range, especially from Africa and the Arabian Peninsula.

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