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Plastome Phylogeny and Taxonomy of *Cinnamomum guizhouense* (Lauraceae)

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Abstract: Taxonomy of the genus *Cinnamomum* Schaeff. (Lauraceae) is difficult because of parallel evolution of morphology. Recent phylogenomic and taxonomic studies have clarified the problem and subdivided the Asian *Cinnamomum* into two genera, i.e., *Camphora* Fabr. and *Cinnamomum* sensu stricto. Here we sequenced and characterized the plastome of a recently described species *Cinnamomum guizhouense* C.Y. Deng, Zhi Yang et Y. Yang, performed a phylogenomic analysis, and also conducted a comparative analysis. The plastome of *Cinnamomum guizhouense* is 152,739 bp long and quadri-parted with a pair of inverted repeat regions (IR: 20,132 bp) divided by a small single copy region (SSC: 18,852 bp) and a large single copy region (LSC: 93,623 bp). The plastome possesses a total of 128 genes including 82 protein-coding genes, 36 tRNA genes, and eight rRNA genes, which is similar to most published plastomes of the core Lauraceae group. The plastome of *Cinnamomum guizhouense* displays higher similarity to *Camphora* than *Cinnamomum*. Our phylogenomic result suggests that *Cinnamomum guizhouense* belongs to the *Camphora* clade. As a result, we propose a new combination, i.e. *Camphora guizhouensis* (C.Y. Deng, Zhi Yang et Y. Yang) Zhi Yang et Y.Yang, comb. nov.

Keywords: *Cinnamomum guizhouense*; Lauraceae; phylogenomics; plastome; taxonomy

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1. Introduction

The family Lauraceae, a mostly woody family of primitive angiosperms, contains over 3000 species and has a wide distribution range in the tropics with Tropical America and Tropical Asia as the diversity centers [1]. Taxonomy of the family has been notorious for its difficulty because of paucity of characters and parallel evolution of morphology [2–4]. Recent plastome phylogeny has provided high resolution for nine major clades in the family including Hypodaphnideae, Cryptocaryeae, Cassytheae, Neocinnamomeae, Caryodaphnopsideae, the *Mesilaurus* clade, Perseae, Cinnamomeae, and Laureae [5,6]. However, many generic complexes exist within these clades, e.g. the *Beilschmiedia* group in the Cryptocaryeae [7], the *Alseodaphne* group in the Perseae [8–10], the *Cinnamomum* group in the Cinnamomeae [4,11], and the *Litsea* group and the *Ocotea* group in the Laureae [12–15], how to identify the generic clades and classify them in combination with morphological characters remain problematic. Recent taxonomic studies have proposed new classifications of some of the above-mentioned generic complexes based on phylogenetic studies [4,9,11,14,15]. Taxonomic problems remain due to inadequate species sampling. One way to resolve these taxonomic problems is to include species in new phylogenetic trees and re-consider their taxonomy within a phylogenetic context.

Cinnamomum Schaeff. belongs to the *Cinnamomum-Ocotea* clade or the tribe Cinnamomeae [5,6]. Plants of the genus are economically important and have been used for a long time because of their timber, cinnamon and camphor [16]. However, the taxonomy of the genus has been ambiguous for a long time.

Traditional classification based on morphology treated the genus *Cinnamomum* in its broad sense. Meissner [17] restricted the genus *Cinnamomum* to tropical and subtropical Asia, and subdivided the genus into two sections, i.e., sect. *Malabathrum* (≡sect. *Cinnamomum*) possessing non-perulate buds, opposite and tripliveined leaves lacking domatia in the axils of lateral veins, and sect. *Camphora* Meisn. having perulate buds, alternate and pinnately-veined leaves, and domatia in the axils of lateral veins. Kostermans [16] included American species formerly treated as *Phoebe* Nees in the genus *Cinnamomum* s.l.; but he accepted Meissner's idea and classified the genus into two sections. Rohwer [1] and van der Werff [18] followed the treatment of Kostermans; Rohwer [1] estimated that the genus had up to 350 species including 60 American species.

Molecular phylogenetic studies revealed that the genus *Cinnamomum* s.l. is polyphyletic and should be reclassified. Based on nrITS and a few plastid markers, Chanderbali et al. [19] conducted a phylogeny of the Lauraceae and suggested that the genus *Cinnamomum* is polyphyletic though they sampled only eight species. This result was confirmed and corroborated by Huang et al. [20] with a better species sampling strategy. Rhode et al. [11] excluded the American species from *Cinnamomum* and transferred them to *Aiouea* Aubl.. Zeng et al. [21] found that the upper leaf epidermal cells of *Cinnamomum* include two types: leaf epidermal cells regular and periclinal walls non-reticulate (sect. *Camphora*), leaf epidermal cells irregular and periclinal walls reticulate (sect. *Cinnamomum*) with a few exceptions in the clade of sect. *Cinnamomum*. Using a combination of morphology, anatomy and molecular phylogeny, Yang et al. [22] finally separated the Asian *Cinnamomum* into two genera, i.e. *Camphora* Fabr. and *Cinnamomum* s.s.

Cinnamomum guizhouense C.Y. Deng, Zhi Yang et Y. Yang is a recently described endangered species only represented by two mature trees in Guizhou, southwestern China [22], established before the taxonomic treatment of *Cinnamomum* [4]. The species possesses pinnately veined and alternate leaves and perulate buds [22]. Considering parallel evolution of morphological characters [4,22], it remains unclear whether *Cinnamomum guizhouense* belongs to the genus *Cinnamomum* or not. Molecular phylogeny is an effective approach to determine the systematic position of species [23], and plastomes have been widely used to solve the phylogenetic and taxonomic problems of Lauraceae [10,13,24,25]. Here, we sequenced the plastome of *Cinnamomum guizhouense*, conducted a phylogenomic study, and made a taxonomic treatment of the species in combination with morphology and phylogeny.

2. Materials and Methods

2.1. Plant Materials and Plastome Sequencing

Chloroplast genomes of two samples (C.Y. Deng et Q.M. Ban 2021001 and 2021002) were newly sequenced from silica-gel dried leaf materials (Table 1); one of the samples C.Y. Deng et Q.M. Ban 2021001 belongs to the type collection of *Cinnamomum guizhouense*. To determine the systematic position of *Cinnamomum guizhouense*, plastomes of Cinnamomeae and Laureae were chosen as ingroups, four plastomes of Perseeae were selected as the outgroup. Totally, 31 plastomes were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/, accessed on 9 October 2022) with details listed in Table 2.

Total genomic DNA was extracted from the leaf using a DNA extraction kit (D115-100, Gene Better, Beijing, China). Whole genome sequencing was conducted by Illumina Novo Seq6000 (Novogene, Beijing, China). A total of ~2 Gb of 150 bp paired-end reads were obtained from each sample.

Table 1. Vouchers of species used in this study.

Taxon	Collection	Locality	Latitude	Longitude	Collection Date	Herbarium
Cinnamomum guizhouense C.Y. Deng et al.	C.Y. Deng and Q.M. Ban 2021001	Guizhou: Wangmo	25°21′8″	106°17′44″	20210220	NF
Cinnamomum guizhouense C.Y. Deng et al.	C.Y. Deng and Q.M. Ban 2021002	Guizhou: Wangmo	25°21′8″	106°17′44″	20210220	NF

Table 2. Sequences obtained from GenBank.

Taxon	Accession
Cinnamomeae	
Camphora bodinieri (H. Lév.) Y. Yang et al.	MH394418
Camphora glandulifera (Wall.) Nees	OL943973
Camphora officinarum Nees	MF421523
Camphora parthenoxylon (Jack) Nees	MT621587
Cinnamomum aromaticum Nees	NC_046019
Cinnamomum burmanni (Nees et T.Nees) Blume	MT621613
Cinnamomum guizhouense C.Y. Deng et al. S2021001	OP818854
Cinnamomum guizhouense C.Y. Deng et al. S2021002	OP818855
Cinnamomum chekiangense Nakai	MT621639
Cinnamomum pittosporoides Hand.-Mazz.	NC_048978
Cinnamomum verum J. Presl	NC_046019
Ocotea aciphylla (Nees et Mart.) Mez	OM135246
Ocotea daphnifolia (Meisn.) Mez	OM135247
Ocotea foetens (Aiton) Baill.	OM135248
Sassafras tzumu (Hemsl.) Hemsl.	NC_045268
Laureae	
Actinodaphne obovata (Nees) Blume	NC_50360
Actinodaphne trichocarpa C.K. Allen	MF939342
Laurus azorica (Seub.) Franco	MK041220
Lindera aggregata (Sims) Kosterm.	NC_045252
Lindera communis Hemsl.	NC_045255
Lindera glauca (Siebold et Zucc.) Blume	NC_035953
Lindera robusta (C.K. Allen) H.B. Cui	MH220738
Litsea coreana H. Lév.	NC_045251
Litsea elongata (Nees) Hook. f.	NC_050364
Litsea pungens Hemsl.	NC_050368
Nectandra angustifolia (Schrad.) Nees et Mart.	MF939340
Neolitsea pallens (D. Don) Momiy. et H. Hara	NC_050370
Neolitsea sericea (Blume) Koidz.	MF939341
Parasassafras confertiflorum (Meisn.) D.G. Long	NC_042696
outgroup	
Alseodaphne semecarpifolia Nees	NC_37491
Machilus thunbergii Siebold et Zucc.	NC_038204
Persea americana Mill.	NC_031198
Phoebe sheareri (Hemsl.) Gamble	NC_031191

2.2. Plastome Assembly and Annotation

Circle plastomes were assembled with GetOrganelle (Version 1.7.5.0, Kunming, China) [26] using de novo strategy. Annotation was conducted with GeSeq (https://chlorobox.mpimp-golm.mpg.de/geseq.html, accessed on 29 October 2022, Munich, Germany) [27], then adjusted manually in Geneious Prime (Version 2020.0.5, Auckland, New Zealand). All sequences downloaded from NCBI were re-annotated to avoid potential annotation errors, and ambiguous genes were double-checked by CpGAVAS2 (http://47.96.249.172:16019/analyzer/home, accessed on 29 October 2022, Beijing, China) [28]. The gene map of the plastome was generated by CHLOROPLOT software (https://irscope.shinyapps.io/Chloroplot/, accessed on 30 October 2022, Helsinki, Finland) [29].

2.3. Repeats Analysis

We conducted repeat sequence analyses of the two plastomes with CpGAVAS2. Long repeats (including direct and palindromic repeats) were detected by Vmatch (Version 2.2.1, http://www.vmatch.de/, accessed on 29 October 2022, Hamburg, Germany). Totally, hamming distance of three and repeats no less than 30 bp were searched. Long tandem repeats (LTR, size of repeat unit $\geq$ 7) were identified with the Tandem Repeats Finder (TRF, Version 3.01, https://tandem.bu.edu/trf/trf.html, accessed on 29 October 2022, Boston, MA, USA) [30]. The weights for match, mismatch, indels were set at 2, 7and 7, respectively. The detection parameters of match and indel was set at 80 and 10, respectively. The minimum alignment score was set at 50. The maximum period size was limited to 500 bp. We identified simple sequence repeats (SSRs) with MIcroSAtellite identification (MISA, Version 2.1, https://webblast.ipk-gatersleben.de/misa/, accessed on 29 October 2022, Seeland, Germany) [31] with a set of minimum repeat times of mononucleotides, dinucleotides, trinucleotides, tetranucleotides, pentanucleotides and hexanucleotides set at 10, 6, 5, 5, 5, and 5, respectively.

2.4. Phylogenetic Analysis

Complete chloroplast sequences were used to infer the phylogenetic position of *Cinnamomum guizhouense*. Sequences were aligned with MAFFT (Version 7.480, Tokyo, Japan) [32], followed by a manual check using BioEdit (Version 7.5.5, Wooster, OH, USA) [33]. Gap sites of sequences were removed with Gblocks (Version 0.91b, Barcelona, Spain) [34].

For phylogeny, both Maximum likelihood (ML) and Bayesian inference (BI) were conducted. ModelFinder [35] was used to determine the best-fit model according to the best Bayesian Information Criterion (BIC) score. ML phylogeny was inferred by IQ-TREE (Version 2.1.2, Vienna, Austria) [36] with 5,000 ultrafast bootstraps [37] under K3Pu+F+I+G4 model. BI phylogeny was conducted using MrBayes (Version 3.2.6, Stockholm, Sweden) [38] with the following designations: GTR+F+I+G4 model, number of generations 1,000,000, sampling frequency 1,000; the initial 25% of sampled data were discarded as burn-in. Phylogenetic trees were browsed and adjusted with iTOL (Version 6.6, https://itol.embl.de/, accessed on 30 October 2022, Heidelberg, Germany) [39].

2.5. Comparative Genome Analysis

The pairwise sequence similarity between *Cinnamomum guizhouense* and query sequences of *Cinnamomum* (*Cinnamomum aromaticum*, *Cinnamomum burmanni*, *Cinnamomum chekiangense*, *Cinnamomum pittosporoides* and *Cinnamomum verum*) and *Camphora* (*Camphora bodinieri*, *Camphora glandulifera*, *Camphora parthenoxylon* and *Camphora officinarum*) were computed in R using the "simplot" function of "ggmsa" package [40]. The sequence differences between *Cinnamomum guizhouense* and sampled species of *Camphora* were detected using "seqdiff" function. The sliding window and step size were set at 200 bp and 20 bp, respectively. The similarity and sequence difference plots were illustrated by the "ggplot2" package [41]. IR expansion and contraction plot of plastomes were drawn manually in Adobe Illustrator (Version 2020, San Jose, California, CA, USA).

3. Results

3.1. General Characters of the Plastome

4,247,464 and 4,439,324 pair-end reads were used for de novo assembly of the two plastomes of *Cinnamomum guizhouense* and the mean sequencing coverage of them was 320× and 142×, respectively. The two plastomes of *Cinnamomum guizhouense* were largely congruent, showing no differences in length, gene organization, structure and repeats, and possessing eight variable nucleotide sites. The plastome of *Cinnamomum guizhouense* consists of a total of 128 genes including 82 protein-coding genes, 36 tRNA genes, and eight rRNA genes (Figure 1). The plastome was 152,739 bp in length and consisted of a pair of inverted repeat regions (IR: 20,132 bp), which were divided by a small single copy region (SSC: 18,852 bp) and a large single copy region (LSC: 93,623 bp). The guanine-cytosine

(G-C) content was differentiated in the complete plastome, LSC, SSC, and IRs, which were 39.1%, 37.9%, 33.8% and 44.4%, respectively.

Direct, palindromic and tandem repeats with lengths ranging from 9 bp to 72 bp were detected in the plastome (Figure 2a). Totally, 74 repeats were found, which included 25 direct repeats, 23 palindromic repeats and 26 tandem repeats. Tandem repeats were shorter than other two repeats with lengths almost less than 30 bp. The longest repeat with a length of 72 bp belonged to a direct repeat and was distributed in LSC.

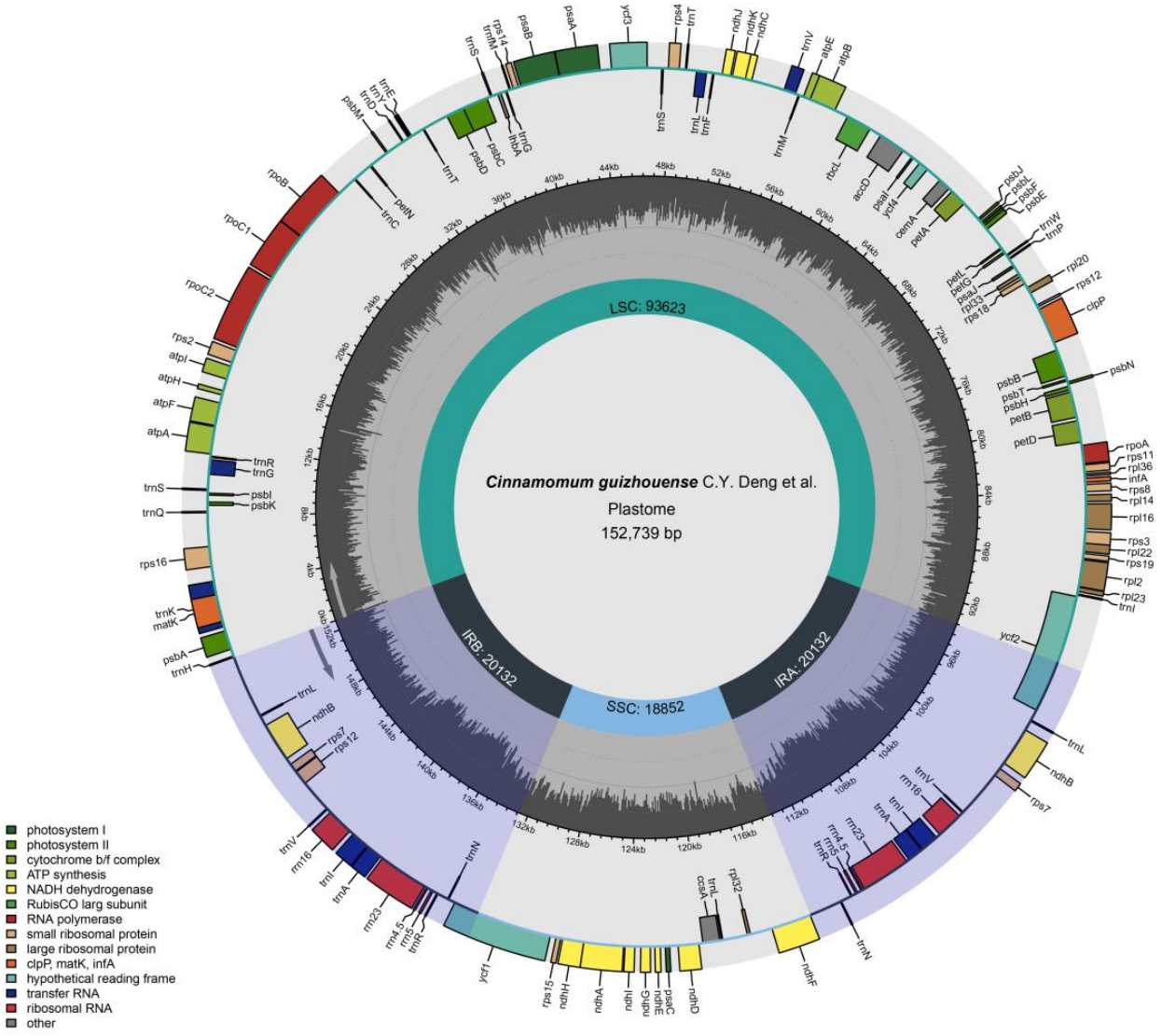

Figure 1. Gene map of *Cinnamomum guizhouense* C.Y. Deng et al.

A total of 73 SSRs was detected in the plastome, 78.1% of which were found in LSC (Figure 2b). Among SSRs, only mononucleotide and dinucleotide units were found. The mononucleotide repeat was the most common SSR representing 93.2%, all of which belonged to A or T monomers. Two AG/CT repeats were found in LSC, while two of three AT/AT were found in LSC and one in SSC, separately.

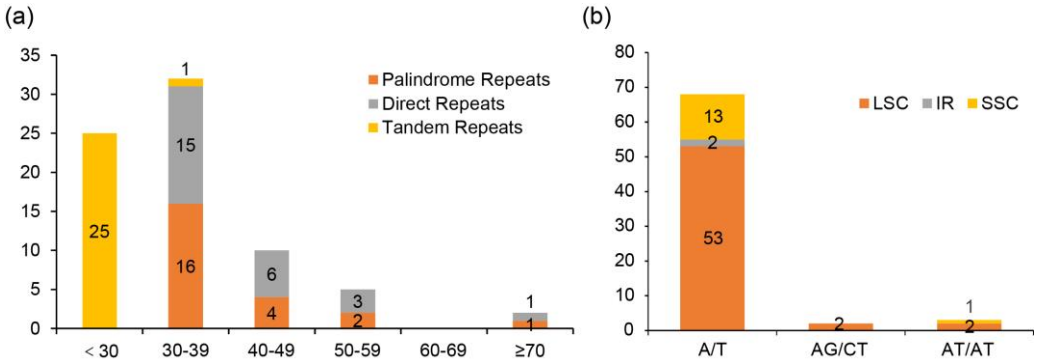

Figure 2. Repeats analysis of the plastome of *Cinnamomum guizhouense* C.Y. Deng et al. (**a**) Number and length of repeats; (**b**) Number and distribution of SSRs.

3.2. Phylogenetic Position of Cinnamomum guizhouense

The topologies using ML and BI methods based on plastomes were congruent and highly supported (Figure 3a). *Camphora* was sister to *Sassafras* (ML: 100%, BI: 1.00). The genus *Cinnamomum* s.s. was sister to a clade including *Camphora* and *Sassafras* (ML: 100%, BI: 1.00). Two samples of *Cinnamomum guizhouense* belonged to the *Camphora* clade. *Cinnamomum guizhouense* was sister to *Camphora bodinieri* (ML: 71%, BI: 1.00), they formed a small clade sister to *Camphora officinarum* (ML: 63%, BI: 0.99). *Camphora glandulifera* is sister to *Camphora parthenoxylon*, they together formed a small clade (ML: 100%, BI: 1.00) which was sister to the small clade including other sampled *Camphora* species (ML: 100%, BI: 1.00).

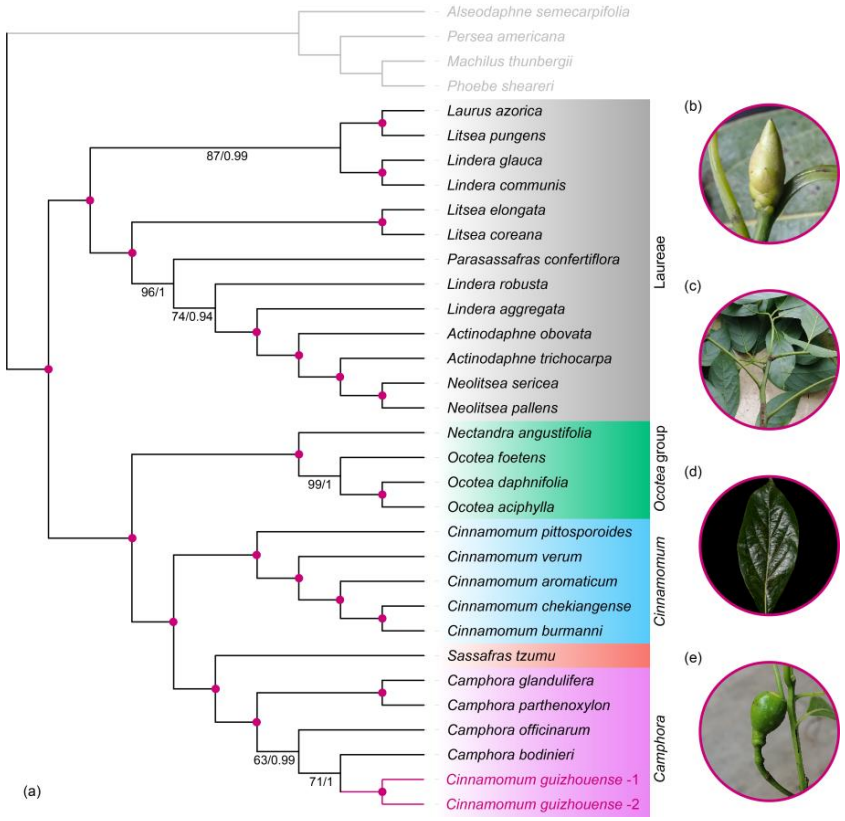

Figure 3. Phylogenetic tree displaying the phylogenetic position of *Cinnamomum guizhouense* C.Y. Deng et al. (**a**) Phylogenetic tree inferred from Bayesian inference and maximum likelihood analysis based on complete plastomes. Ultrafast bootstrap support values (<100%) and Bayesian posterior probabilities (<1) are shown below the branches; (**b**–**e**) Morphology of *Cinnamomum guizhouense*; (**b**) Large perulate terminal buds; (**c**) Alternate leaves; (**d**) Pinnately-veined leaf; (**e**) Deep fruit cupule.

3.3. Comparative Analysis of the Plastomes

Plastome differences between *Cinnamomum guizhouense* and the four species of *Camphora* and the five species of *Cinnamomum* included in this study were displayed in Figure 4. Generally, the plastome of *Cinnamomum guizhouense* showed a higher similarity to those of *Camphora* than to those of *Cinnamomum*. Hotspot regions were discovered near IR boundaries. IRs were more conserved than LSC and SSC, and non-coding regions were more variable than coding regions. In a comparison between *Cinnamomum guizhouense* and sampled species of *Cinnamomum*, nine highly variable regions with a similarity lower than 90% were detected (Figure 4a), included seven non-coding regions (*rps16_trnQ-UUG*, *psbM_trnD-GUC*, *trnF-GAA_ndhJ*, *atpB_rbcL*, *petA_psbJ*, *trnN-GUU_ndhF*, *ndhF_rpl32*) and two coding genes (*ndhF* and *ycf1*). Only five highly variable regions with a similarity lower than 90% were identified between *Cinnamomum guizhouense* and sampled species of *Camphora* (Figures 4b and S1), included *trnN-GUU_ndhF*, *ycf1* and *ycf2* × 3. At the locus *ycf2* in the IR regions, *Cinnamomum guizhouense* showed no difference from *Camphora* species excepting *Camphora bodinieri* (Figure S1).

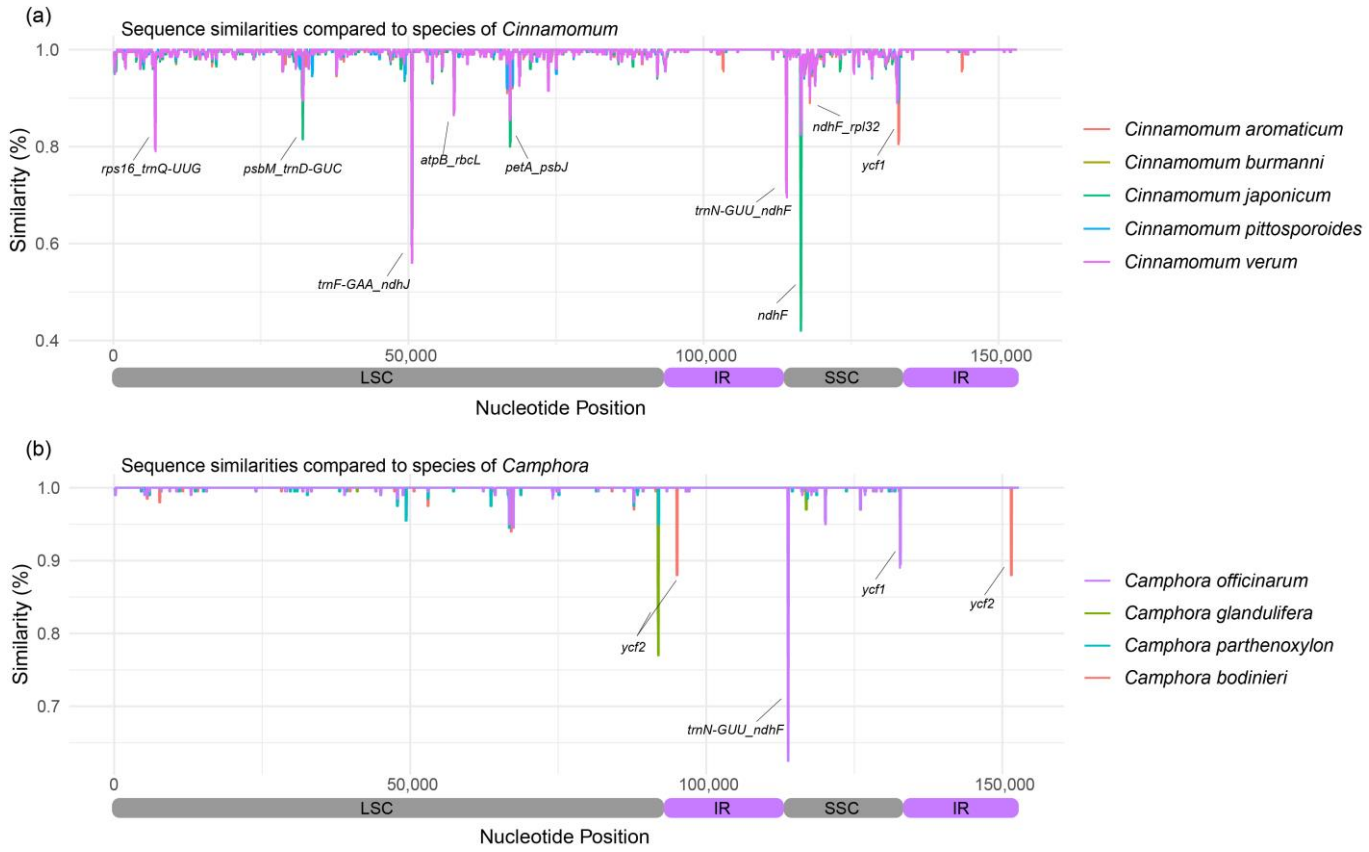

Figure 4. Similarity analysis of the plastome of *Cinnamomum guizhouense* C.Y. Deng et al. (**a**) The similarity plot of the plastome of *Cinnamomum guizhouense* in comparison with the sampled species of *Cinnamomum* Schaeff. (**b**) The similarity plot of the plastome of *Cinnamomum guizhouense* in comparison with the sampled species of *Camphora* Fabr. Regions with similarity lower than 90% are indicated.

The plastome of *Cinnamomum guizhouense* showed no obvious contraction and expansion of IRs in comparison with the four sampled plastomes of *Camphora*, only one nucleotide less between *ycf2* and *trnL-CAA* (Figure 5). *Camphora officinarum* was distinct from other sampled *Camphora* species in missing 21 nucleotides between *ycf1* and *ndhF* and gaining eight nucleotides between *ycf2* and *trnH-GUG*.

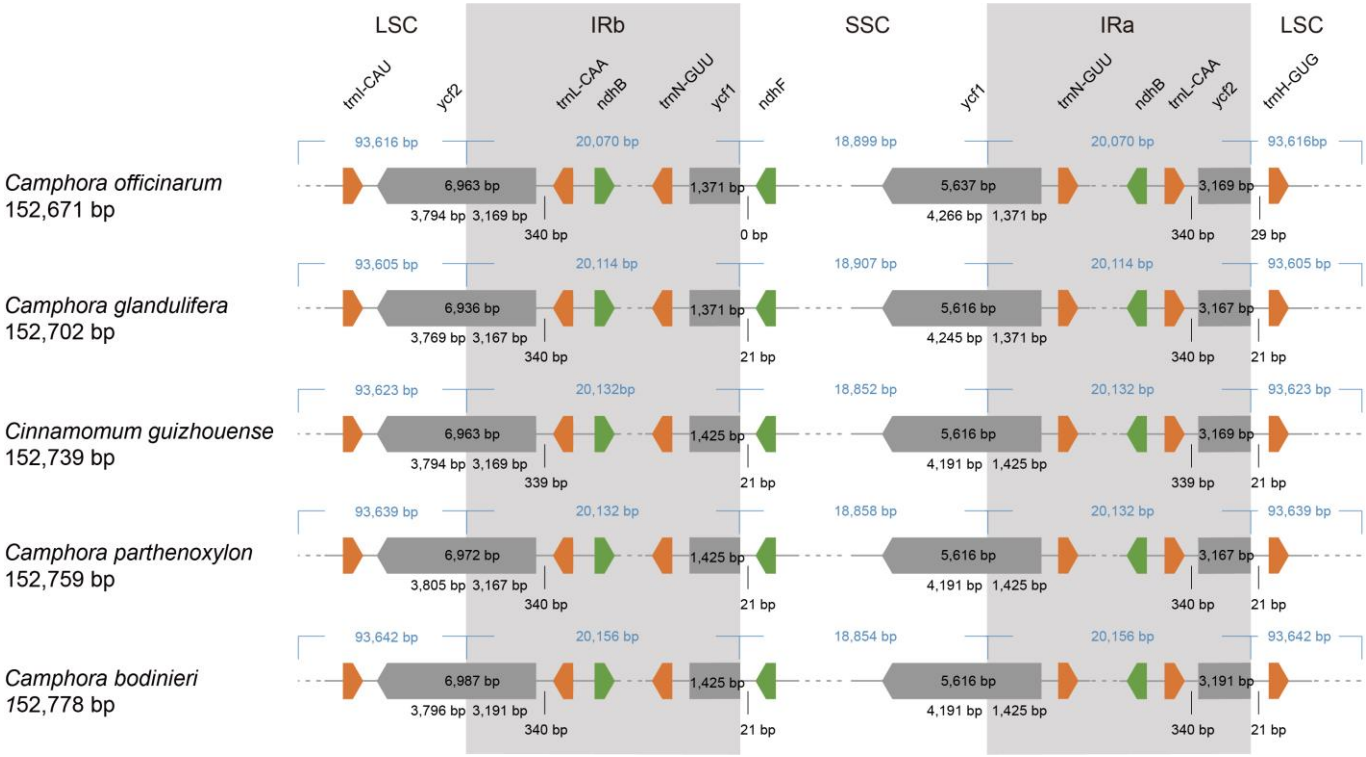

Figure 5. Contraction and expansion of IRs in the plastome of *Cinnamomum guizhouense* C.Y. Deng et al. compared with four plastomes of *Camphora* Fabr.. Genes and their relative positions were not drawn to scale.

4. Discussion

4.1. Phylogenomics and Its Systematic Significance

Yang et al. [4] divided the Asian *Cinnamomum* into two genera, i.e. *Camphora* and *Cinnamomum* according to multi-disciplinary evidence including morphology, anatomy and molecular phylogeny. *Camphora* and *Cinnamomum* were monophyletic in both nuclear and plastome phylogenies. The genus *Cinnamomum* possesses irregular cell shape, sinuous anticlinal walls, and reticulate periclinal walls of upper leaf epidermis, inconspicuous, non-perulate, terminal buds and usually tripliveined leaves; there are exceptions in this clade, *Cinnamomum chago* B.S. Sun & H.L. Zhao, *Cinnamomum saxatile* H.W. Li and *Cinnamomum longipetiolatum* H.W. Li have pinnately-veined leaves. The genus *Camphora* possesses regular cell shape, straight anticlinal walls, and non-reticulate periclinal walls of upper leaf epidermis, prominent perulate terminal buds and pinnately-veined leaves [22].

In this study, both ML and BI trees based on plastomes show congruent topologies with previous studies that *Cinnamomum* is sister to a clade consisting of *Camphora* and *Sassafras* (Figure 3a) [5,10,22]. The two samples of *Cinnamomum guizhouense* constitute a monophyletic group which falls within the *Camphora* clade with robust support and shows a close relationship to *Camphora bodinieri* (Figure 3a). This result is also corroborated by the macromorphology of the species. *Cinnamomum guizhouense* possesses large perulate buds, pinnately-veined and alternate leaves and is similar to *Camphora* but markedly different from *Cinnamomum* (Figure 1b–e) [22]. Considering both morphology and molecular phylogeny, we transfer *Cinnamommum guizhouense* to *Camphora*.

4.2. Plastome Evolution

Plastome organization of *Cinnamomum guizhouense* shows similarity to the sampled plastomes of the core Lauraceae group in lacking re-arrangement, gain and loss of genes (Figure 2). *Cinnamomum guizhouense* shows higher plastome sequence similarity to *Camphora* than to *Cinnamomum* (Figure 4), which supports the taxonomic transfer of *Cinnamomum*

guizhouense to *Camphora*. The five sampled plastomes of *Camphora* showed rather low variability and contained only 31 parsimony-informative characters among the entire plastomes. The low divergence among the *Camphora* plastomes may have been caused by recent diversification of the genus as suggested in the study of Xiao and Ge [10]. Further studies with extensive species sampling of the genus are necessary to verify this hypothesis.

4.3. Taxonomic Treatment

Camphora guizhouensis (C.Y. Deng, Zhi Yang et Y. Yang) Zhi Yang et Y. Yang, comb. nov.

Basionym. *Cinnamomum guizhouense* C.Y. Deng, Zhi Yang et Y. Yang, PhytoKeys 202: 37, figs. 1-3 (2022).

Holotype: CHINA. Guizhou, Wangmo Co., 25°21′8"N, 106°17′44"E, elev. 1081 m, 20 Feb 2021, C.Y. Deng & Q.M. Ban 2021001 (holotype: NF; isotypes: NF, NAS, XIN).

5. Conclusions

Here we sequenced and characterized the plastome of a recently reported species of Lauraceae: *Cinnamomum guizhouense*. The plastome is similar to the plastomes of the core Lauraceae group in gene number and organization, four-parted with a total of 128 genes including 82 protein-coding genes, 36 tRNA genes, and eight rRNA genes. Our phylogenomic result suggests that *Cinnamomum guizhouense* belongs to the *Camphora* clade, as the plastome of the species has a greater similarity to *Camphora* than *Cinnamomum*. A new combination is proposed: *Camphora guizhouense* (C.Y. Deng, Zhi Yang et Y. Yang) Zhi Yang et Y. Yang, comb. nov.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14020310/s1, Figure S1: The sequence differences of the plastome of *Cinnamomum guizhouense* C.Y. Deng et al. compared with four plastomes of *Camphora* Fabr., e.g. (**a**) *Camphora officinarum*, (**b**) *Camphora glandulifera*, (**c**) *Camphora parthenoxylon*, (**d**) *Camphora bodinieri*. The top panels are bar charts showing the number of variants over the whole sequence. The bottom panel shows the gene organization of *Cinnamomum guizhouense*.

Author Contributions: Conceptualization, Y.Y.; methodology, Z.Y.; software, Z.Y.; formal analysis, Z.Y.; data curation, Z.Y.; writing—original draft preparation, Y.Y. and Z.Y.; writing—review and editing, Y.Y., Z.Y. and D.K.F.; visualization, Z.Y.; funding acquisition, Y.Y. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Not applicable.

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Conflicts of Interest: The authors declare no conflict of interest.

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