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**Complete Chloroplast Genome Sequence of the Avocado: Gene
Organization, Comparative Analysis, and Phylogenetic Relationships
with Other Lauraceae**

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Abstract

The complete chloroplast sequence of the avocado (*Persea americana* Mill.), a popular tropical fruit tree, was determined in this study. The plastome consists of 152,723 bp, with a typical circular structure including inverted repeats (IRs) 40,104 bp in length separated by a large single-copy region (LSC) and a small single-copy region (SSC) of 93,795 bp and 18,824 bp, respectively. Potential mutation sites including 61 repeats and 679 SSRs were accurately located in the avocado plastome. Compared with the plastomes of five other related Lauraceae, 25 highly variable regions and six micro-inversions were identified and the contraction event of the IR regions was observed. However, the plastomes of Lauraceae are extremely evolutionarily conservative with only 0.32% sequence divergence, which is surprisingly low for angiosperms. Further phylogenetic analyses were conducted using eight complete plastomes of Lauraceae and seven barcoding sequences from 23 *Persea* species. These data support a close relationship between the genera *Persea* and *Machilus*, and suggest that *Persea* is polyphyletic.

Keywords: chloroplast; genome; *Persea*; Lauraceae; Phylogenetic relationship

Introduction

The genus *Persea* in the family Lauraceae includes nearly 100 tree species mainly distributed in tropical South America (Rohwer, et al. 2009). The avocado (*Persea americana* Mill.) was probably domesticated in Mexico and is now widely planted as a popular fruit tree in tropical regions of the world (Chen, et al. 2009). As a member of a basal angiosperm family, the flowers of *Persea* species have an undifferentiated perianth which represents an intermediate stage before the evolutionary differentiation of sepals and petals (Chanderbali, et al. 2009). However, floral and other morphological traits differ relatively little between *Persea* species. So, molecular

methods are needed for species delineation and the resolution of phylogenetic relationships.

In the first molecular study, using the chloroplast marker *matK*, *P. americana*, *P. indica* and *P. lingue*, along with *Phoebe formosana*, formed a weakly supported clade (the ‘*Persea* group’) with *Apollonias barbuiana*, *Dehaasia cuneata*, and *Alseodaphne perakensis* (Rohwer 2000). The second, larger, study reported phylogenetic trees for the Lauraceae constructed with different combinations of chloroplast sequences *trnL-trnF*, *psbA-trnH*, *trnT-trnL*, and *rpl16* and nuclear barcoding markers 26S rDNA and ITS rDNA, and found a well-supported *Persea* group including *P. americana*, *P. lingue*, *P. meridensis*, and *P. caerulea*, along with *Alseodaphne semecarpifolia*, *Dehaasia incrassata*, *Apollonias barbuiana*, *Phoebe formosana* and *Machilus thunbergii* (as *P. thunbergii*) (Chanderbali, et al. 2001). The intron of *trnK* was then used to construct a Bayesian tree with a clade comprising *P. americana*, *P. indica* and other species, such as *Alseodaphne perakensis*, *D. cuneata*, *Apollonias barbuiana* and *M. grijsii* (as *P. grijsii*) (Rohwer and Rudolph 2005).

In order to resolve the relationships among the species in the morphologically defined genus *Persea* and species placed in related genera, *ITS* was used in a study including 61 *Persea* species and 30 other Lauraceae (Rohwer, et al. 2009). The results showed that *Persea* species were divided between three clades, indicating that they were not a natural group. Another study, using two nuclear markers, *ITS* and *LEAFY* intron, confirmed that ‘*Persea*’ was polyphyletic (Li, et al. 2011). These studies showed that additional genome sequences and more variable markers are needed to resolve the phylogenetic problems at the lower taxonomic level.

Here, we report the complete chloroplast (cp) genome sequence and the plastomic features of avocado (*P. americana*), which is the first published complete genome sequence of a member of the genus *Persea*. The phylogenetic relationships among eight Lauraceae species and 23 *Persea* species are then reconstructed, suggesting that

74 *Persea* is polyphyletic.

75

76 **Materials and Methods**

77 **DNA Extraction and Sequencing**

78 Young leaves of avocado (*Persea americana* Mill.) (**Supplementary Figure S1**) were
79 collected from the Xishuangbanna Tropical Botanical Garden (21.9° N, 101.3° E; 549
80 m above sea level) for genomic DNA extraction using the modified CTAB method
81 (Doyle and Dickson 1987). The whole cp genome was sequenced following Yang et al.
82 (2014), and their nine universal primer pairs were used to perform long-range PCR for
83 next-generation sequencing (Yang, et al. 2014). Following purification, PCR products
84 mixed with six microgram were fragmented into small pieces. According to the
85 manufacturer's manual (Illumina), the cleaved DNA fragments were used to construct
86 500 bp short-insert libraries. DNA from avocado was indexed by tags and pooled in
87 one lane of a Genome Analyzer (Illumina HiSeq 2500) for sequencing at the
88 Germplasm Bank of Wild Species, Kunming Institute of Botany (KIB) in Kunming,
89 China.

90

91 **Chloroplast Genome Assembly and Annotation**

92 Using the cp genome of the related Lauraceae species *Machilus balansae* (GenBank
93 accession No. KT348517) as a reference (Song, et al. 2015), the Illumina sequence
94 reads were proofread and assembled with trial version of CLC v8
95 (<https://www.qiagenbioinformatics.com>). All of the genes encoding proteins, transfer
96 RNAs (tRNA), and ribosomal RNAs (rRNAs) (Figure 1) were annotated on the
97 avocado cp genome using the DOGMA software (Wyman, et al. 2004). The complete
98 avocado cp genome sequence was submitted to GenBank (deposited in GenBank:
99 KX437771).

100

101 **Repeat Elements, SSRs, and Micro-inversions Detection**

102 REPuter (Kurtz and Schleiermacher 1999) and Tandem Repeats Finder (TRF)
103 programs (Benson 1999) were used to visualize forward, palindrome, reverse,

complement and tandem sequences in the plastome of avocado, with a minimum repeat size of 18 bp (for REPuter) and 12 bp (for TRF) and a sequence identity greater than 90%. The simple sequence repeats (SSRs) in the plastome of avocado were identified using Phobos v. 3.3.12 (<http://www.ruhr-uni-bochum.de>). Repeats were $\geq$ 10 bp and three repeat units for mono-, di-, tri-, tetra-, penta-, hexa-, 7-, 8-, 9-, 10-nucleotides. The structures of hairpin loops related to micro-inversions were drawn with RNA structure 5.6.9 (<http://rna.urmc.rochester.edu/RNAstructureWeb/Servers/Predict2/Predict2.html>)

112

113 Sliding Window Analysis of the Plastomes

MAFFT version 7 software was used to align the six plastome sequences of avocado (deposited in GenBank: KX437771), *Phoebe omeiensis* (accession No. KX437772), *P. shearereri* (accession No. KX437773), *Machilus balansae* (accession No. KT348517), *M. yunnanensis* (accession No. KT348516), and *Cinnamomum micranthum* (accession No. KR014245) (Kato and Standley 2013). After manual adjustment with BioEdit software (<http://www.mbio.ncsu.edu/bioedit/bioedit.html>), we further conducted a sliding window analysis to calculate the nucleotide variability values (Pi) and the pairwise nucleotide divergence values all over the plastomes in DnaSP version 5 (Librado and Rozas 2009). We defined the window length as 600 base pairs and the step size as 200 base pairs. The values were plotted in the plastomes using an R program.

125

126 Phylogenetic Analyses

The plastome sequences of *Phoebe omeiensis* (accession No. KX437772), *P. shearereri* (accession No. KX437773), *Machilus balansae* (accession No. KT348517), *M. yunnanensis* (accession No. KT348516), *Cinnamomum micranthum* (accession No. KR014245), *Endiandra discolor* (accession No. KT588615), *E. globosa* (accession No. KT588614) and *Calycanthus fertilis* (accession No. NC004993) were collected from GenBank of NCBI. After alignment using MAFFT software with the plastome sequences of avocado, the nine sequences were manually adjusted with BioEdit

software. The nucleotide substitution model was calculated by the jModeltest 2.0 program and the optimal model of “GTR+I+G” was selected (Darriba, et al. 2012). Phylogenetic relationships were reconstructed using a maximum-likelihood (ML) method in MEGA version 5.0 program (Tamura, et al. 2011). One thousand bootstrap replicates were performed in each analysis to obtain the confidence support. The plastome of *C. micranthum* was used as an out-group.

The DNA sequences of *rbcL*, *matK*, *trnL-trnF*, *psbA-trnH*, *ndhF*, *ITS*, and the intron of *LEAFY* of *Persea* species and *Apollonias barbujana* were collected from GenBank of NCBI (Supplementary Table S1). All of these sequences were proofread and aligned with MAFFT software. Incongruous sequences of the same species were deleted. The seven sequence matrixes were then manually adjusted and the same sequences with different accession numbers were merged with Sequencher 4.10 (<http://www.genecodes.com>). The joint matrix including the sequences of *rbcL*, *matK*, *trnL-trnF*, *psbA-trnH*, *ndhF*, *ITS*, and the intron of *LEAFY* was constructed with Sequence Matrix 1.7.8 program (Vaidya, et al. 2011), and then used to calculate the nucleotide substitution model with the jModeltest 2.0 program and the optimal model of “GTR+I+G” was selected. Phylogenetic relationships were reconstructed using Bayesian analysis in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), and the combined analysis was run for 1,000,000 generations, and a burn-in of 25% was used for the analysis.

Results

1. General features of the avocado chloroplast genome

The plastome of avocado (deposited in Genbank: KX437771), with a length of 152,723 bp, was 132 bp and 153 bp smaller than that of *Phoebe omeiensis* (152,855 bp, GenBank accession No. KX437772) and *Phoebe sheareri* (152,876 bp, GenBank accession No. KX437773), and 2 bp, 23 bp and 101 bp larger than that of the *Machilus balansae* (152,721 bp, GenBank accession No. KT348517), *Cinnamomum micranthum* (152,700 bp, GenBank accession No. KR014245), and *M. yunnanensis*

(152,622 bp, GenBank accession No. KT348516), respectively (Song, et al. 2015; Wu, et al. 2015). The A+T contents of the six plastomes are all 61%, the lengths of the inverted repeats (IRs), small single-copy region (SSC) and large single-copy region (LSC) in the plastome of avocado are different from the other five species, and were 20,052 bp, 18,824 bp, and 93,795 bp, respectively (**Figure 1**). The plastome of avocado consists of 79 unique protein-coding genes, 30 transfer RNA (tRNA) genes, and four different ribosome (rRNA) genes. Among all 79 protein coding genes, ten genes, including *atpF*, *petB*, *petD*, *ndhA*, *ndhB*, *rpoC1*, *rps12*, *rps16*, *rpl2*, and *rpl16*, contain one intron, and two genes, *ycf3* and *clpP*, contain two introns (**Table 1**). There are 15 genes duplicated in the IR regions and 1,374 bp of 3'-*ycf1* are truncated at the IRs' boundaries (**Figure 1**).

2. Repeat element and SSR Analysis

As potential trace of genome rearrangement, repeat motifs play an important role in inducing mutation including substitutions and indels (Cavalier-Smith 2002; Yi, et al. 2013). In the plastome of avocado, 66 repeats, not including the IR region, were detected with REPuter and Tandem Repeats Finder programs. These repeats include 17 forward repeats of 18-33 bp, 16 palindromic repeats of 18-48 bp, 14 reverse repeats of 18-25 bp, 12 tandem repeats of 12-24 bp and two complement repeats of 18 and 20 bp (**Table 2**). Most of these repeats (68.9%) were distributed in the LSC region, whereas 18.0% were in the IR regions, and 13.1% in the SSC region. Short tandem repeats, also called simple sequence repeats (SSR), were further identified using the Phobos version 3.3.12 program. A total of 679 potential SSRs were detected in the plastome of avocado, of which 226 are mononucleotides, 38 are dinucleotides, 70 are trinucleotides, 84 are tetranucleotides, 83 are pentanucleotides, 134 are hexanucleotides, 25 are 7-nucleotides, 11 are 8-nucleotides, 6 are 9-nucleotides, and 2 are 10-nucleotides (**Figure 2**). Most of these potential SSRs (66.0%) were distributed in the LSC region, whereas 19.4% were in the IR regions, and 14.6% in the SSC region (**Supplementary Table S2**). All of these SSR loci are potentially useful in population studies of *Persea* species.

194

195 **3. Comparative analysis of the avocado chloroplast genome**

196 To investigate levels of sequence divergence, we calculated the genetic divergence
197 among the plastomes of avocado, *Phoebe omeiensis*, *Phoebe sheareri*, *M. balansae*,
198 *M. yunnanensis* and *C. micranthum* with DnaSP 5.0 software. The nucleotide
199 variability values within 600 bp in the six plastomes varied from 0 to 0.02111 with a
200 mean of 0.00322. The pairwise nucleotide divergence values between two of the six
201 plastomes varied from 0.001 to 0.005 (**Table 3**). Both results indicate high sequence
202 similarity across the six plastomes, suggesting that the chloroplast genomes of
203 Lauraceae species are highly conserved. The whole aligned sequences reveal
204 surprising low divergences with only 3 regions displaying high variation. The three
205 most dissimilar regions of the five plastomes were *ndhF-rpl32*, *rpl32-trnL* and *ycf1*
206 (**Figure 3**). *ndhF-rpl32* and *rpl32-trnL* are located in the SSC region, while *ycf1* is in
207 the IR region. All three loci with nucleotide variability more than 0.015 have been
208 reported before as highly variable regions in seed plants (Dong, et al. 2012; Dong, et
209 al. 2015; Korotkova, et al. 2014; Sarkinen and George 2013; Song, et al. 2015). In
210 addition, another 22 loci with nucleotide variability values more than 0.008 were
211 located (**Figure 3**).

212

213 **4. Micro-inversion mutations**

214 Micro-inversions, forming stable single-stranded hairpin structures, often entail gene
215 duplications and genome rearrangement that can lead to genetic novelty and
216 adaptation (Calvete, et al. 2012; Catalano, et al. 2009). Among the six plastomes of
217 Lauraceae species, we manually identified six micro-inversion events in the regions of
218 *ndhA* intron, *ccsA-ndhD*, *petA-psbJ*, *rps4-trnT*, *rps16-trnQ* and *psbA-trnH* (**Figure**
219 **4A-F**). The micro-inversion in the *ccsA-ndhD* intergenic region has been reported
220 before in two *Machilus* species while three micro-inversions in the *rps4-trnT*,
221 *ycf4-cemA* and *ccsA-ndhD* intergenic regions have been reported before in two
222 *Phoebe* species (Song, et al. 2015). In *Persea* species, two additional micro-inversions
223 with five and 18 bases were detected in the *psbA-trnH* region (**Figure 4E and 4G**).

The loop lengths of these seven micro-inversions varied from 4 bp to 16 bp while their stem length ranged from 9 bp to 25 bp. Further comparison of the stem sequences indicated that they are actually short palindromic repeats in the flanks of the micro-inversions (**Figure 4**).

5. Phylogeny of Sequenced Lauraceae Plastomes

To determine the phylogenetic location of avocado with respect to the other Lauraceae with fully sequenced chloroplast genomes, the complete avocado plastome was used to reconstruct the phylogenetic relationships. With the plastome of *Calycanthus fertilis* (Calycanthaceae, Laurales; GenBank accession No. NC004993) as an outgroup, nine chloroplast genome sequences of Lauraceae, including avocado, *Phoebe shearereri*, *Phoebe omeiensis*, *M. balansae*, *M. yunnanensis*, *C. micranthum*, *Endiandra discolor* (accession No. KT588615), and *E. globosa* (accession No. KT588614), were aligned by the MAFFT program. A maximum likelihood analysis based on the GTR+I+G model was performed with MEGA 5.0 using 1000 bootstrap replicates (**Figure 5**). The phylogenetic tree reveals that avocado is most closely related to *Machilus* and *Phoebe*, which is in agreement with previous reports on the relationships among the three genera (Li, et al. 2011; Rohwer, et al. 2009).

6. Phylogenetic Analysis of the *Persea* species

To determine the phylogenetic relationship of avocado with the other *Persea* species with reported barcoding data, the available sequences, including *rbcL*, *matK*, *trnL-trnF*, *psbA-trnH*, *ndhF*, *ITS*, and intron of *LEAFY*, were collected (**Supplementary Table S1**). The homologous sequences of *Apollonias barbujana*, *M. yunnanensis*, *Phoebe shearereri*, and *C. micranthum* were also collected from NCBI and used as out group. A total of 26 samples including 3 outgroup species were including in the analysis of the data matrix combining nucleotide sequences from the seven cpDNA and nuclear markers. The results of the Bayesian analysis showed that the *Persea* species can be divided into two clades, indicating that *Persea* is paraphyletic: a clade including *P. americana*, *P. floccosa*, *P. gigantea*, *P. nubigena*, *P.*

254 *steyermarkii*, *P. tolimanensis*, *P. parvifolia*, *P. schiedeana* and *Apollonias barbujana*,
255 and a clade comprising *P. longipes*, *P. lingue*, *P. chamissonis*, *P. caerulea*, *P. indica*, *P.*
256 *palustris*, *P. haenkeana*, *P. humilis*, *P. borbonia*, *P. major*, *P. meridensis*, *P.*
257 *weberbaueri*, *P. splendens*, *P. aurata* and *P. alba* (**Figure 6**). In clade I, the avocado,
258 together with another seven *Persea* species considered as the possible ancestors of
259 avocado, is sister to *Apollonias barbujana*. The clade consisting of all the species of
260 *Persea* and *Apollonias* forms a sister group to *M. yunnanensis*.

261

262 **Discussion**

263

264 **1. The contraction of the IR region**

265 This study revealed the whole chloroplast genome for an important tropical fruit crop:
266 avocado of *Persea* genus in the family Lauraceae. The plastome of avocado with the
267 length of 152,723 bp was smaller than the published plastomes of genus *Phoebe* and
268 larger than those of *Machilus* (Song et al., 2015). However, the length of the IR
269 region with 20,052 bp was the smallest among the published plastomes of the
270 Lauraceae. In the published plastomes, the boundaries between IR regions and LSC or
271 SSC are located in two gene sequences, *ycf1* and *ycf2*, which showed incomplete
272 duplication. Unlike *ycf2*, the lengths of the truncated *ycf1* genes were different among
273 the avocado, *Phoebe sheareri*, *Phoebe omeiensis*, *M. balansae*, *M. yunnanensis*, and
274 *C. micranthum*. The length of the truncated *ycf1* gene with 1377 bp was also the
275 smallest among published plastomes of the Lauraceae. The gene *ycf1* crossed the
276 SSC/IRb region, and the truncated *ycf1* gene was located in the IRa region. The length
277 changes in the truncated *ycf1* gene directly drove the contraction of the IR regions in
278 the plastome of avocado.

279

280 **2. Low sequence divergence among Lauraceae**

281 Although the length of the truncated *ycf1* gene varied and the divergence of the
282 unbroken *ycf1* gene was high among the avocado, *Phoebe sheareri*, *Phoebe omeiensis*,
283 *M. balansae*, *M. yunnanensis*, and *C. micranthum*, the nucleotide variability values of

the whole plastomes among the species from the four genera were only 0.32%, which approximates the nucleotide variability of two *Panax* species (0.40%) (Dong, et al. 2014), three *Veroniceae* species (0.4%) (Choi, et al. 2016), and nine *Gossypium* species (0.62%) (Xu, et al. 2012) and was much lower than the sequence divergence among five *Epimedium* species (3.97%) (Zhang, et al. 2016) and six *Cymbidium* species (3.70%) (Yang, et al. 2013). At the molecular level, the reported chloroplast *matK*, *trnK* intron, *trnL-trnF*, *psbA-trnH*, *trnT-trnL* and *rpl16* and nuclear *ITS* and *LEAF* intron II sequences only partially resolved the phylogenetic and species identification problems in the family Lauraceae (Li et al., 2011). As Rohwer first reported, the genetic divergence within the family is surprisingly low (Rohwer 2000).

3. Repeat, micro-inversion, and rearrangement mutations

Although genetic divergence is low overall, there are dozens of divergence hotspots and various mutants, including micro-inversions, were detected in the plastome sequences of species in different genera or the same genus (**Figure 4**) in Lauraceae. Among the plastomes of avocado, *Phoebe sheareri*, *Phoebe omeiensis*, *M. balansae*, *M. yunnanensis*, and *C. micranthum*, six micro-inversion events in the regions of the intron of *ndhA*, *ccsA-ndhD*, *petA-psbJ*, *rps4-trnT*, *rps16-trnQ* and *psbA-trnH* were identified. Among 23 species of *Persea*, two micro-inversion events in the region of *psbA-trnH* were detected. In the flanks of all these seven micro-inversions, palindromic repeats in pairs with lengths of 9 bp to 25 bp were identified. At the single-stranded hairpin formed in the genome rearrangement process, micro-inversion and palindromic repeat sequences constitute the loop and stem. The affinities of the palindromic repeats and micro-inversions in the plastomes of Lauraceae support the suggestion that repeats are crucial in inducing mutants (Yi, et al. 2013).

4. Phylogeny of *Persea* species and sequenced Lauraceae plastomes

Based on two variable regions from nuclear *ITS* and *LEAFY* intron II, previous molecular phylogenetic analyses indicated that the genus *Persea* is polyphyletic, which further supported by our phylogenetic analysis of the matrix of five chloroplast

regions and two nuclear sequences, implying *Persea* is polyphyletic and there are close relationships between avocado and *P. floccosa*, *P. gigantea*, *P. nubigena*, *P. steyermarkii*, *P. tolimanensis*, *P. parvifolia*, *P. schiedeana*. With species from five genera of Lauraceae, our phylogenomic analysis based on published chloroplast genomes supported a monophyletic *Persea* group comprising species of *Phoebe*, *Machilus*, and *Persea* genera. Species of *Endiandra* formed the basal group in the phylogeny, and *Cinnamomum* is separate from both the *Endiandra* group and the *Persea* group as in previously published phylogenetic trees (Chanderbali, et al. 2001; Rohwer 2000; Rohwer and Rudolph 2005).

323

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331 Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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Table 1. Genes encoded by avocado plastomes

Category for genes	Group of gene	Name of gene
Photosynthesis related genes	Rubisco	<i>rbcL</i>
	Photosystem I	<i>psaA, psaB, psaC, psaI, psaJ</i>
	Assembly/stability of photosystem I	<i>**ycf3, ycf4</i>

Transcription and translation related genes	Photosystem II	<i>psbA,psbB,psbC,psbD,psbE,psbF,psbH,psbI,psbJ,psbK,psbL,psbN,psbT,psbZ</i>
	ATP synthase	<i>atpA, atpB, atpE, *atpF, atpH, atpI</i>
	cytochrome b/f complex	<i>petA, *petB, *petD, petG, petL, petN</i>
	cytochrome c synthesis	<i>ccsA</i>
	NADPH dehydrogenase	<i>*ndhA, *ndhB, ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK</i>
	transcription	<i>rpoA, rpoB, *rpoC1, rpoC2</i>
	ribosomal proteins	<i>rps2, rps3, rps4, rps7, rps8, rps11, *rps12, rps14,rps15, *rps16, rps18, rps19,*rpl2, rpl14, *rpl16, rpl20, rpl22, rpl23, rpl32, rpl33,rpl36</i>
	translation initiation factor	<i>infA</i>
	ribosomal RNA	<i>rrn5, rrn4.5, rrn16, rrn23</i>
	transfer RNA	<i>*trnA-UGC, trnC-GCA, trnD-GUC, trnE-UUC, trnF-GAA,trnG-GCC, *trnG-UCC, trnH-GUG, trnI-CAU, *trnI-GAU,*trnK-UUU, trnL-CAA, *trnL-UAA, trnL-UAG, trnM-CAU,trnM-CAU, trnN-GUU, trnP-UGG, trnQ-UUG,trnR-ACG, trnR-UCU, trnS-GCU, trnS-GGA, trnS-UGA, trnT-GGU,trnT-UGU, trnV-GAC, *trnV-UAC, trnW-CCA, trnY-GUA</i>
Other genes	RNA processing	<i>matK</i>
	carbon metabolism	<i>cemA</i>
	fatty acid synthesis	<i>accD</i>
	proteolysis	<i>**clpP</i>
Genes of unknown function	conserved reading frames	<i>ycf1, ycf2</i>
Pseudogenes		<i>ycf15</i>

Asterisks before gene names indicate intron containing genes (*), and double asterisks indicate two introns in the gene.

429 Table 2. Locations of repeats in the avocado plastome.

	Repeat A	Start	Region	Repeat B	Start	Region	Length	Type
	Location			Location				
1	<i>trnH-psbA</i>	215	LSC	<i>clpP intron1</i>	74263	LSC	18	P
2	<i>trnH-psbA</i>	215	LSC	<i>rpl2-rpl23</i>	89466	LSC	18	C
3	<i>trnH-psbA</i>	216	LSC	<i>ndhF-rpl32</i>	116177	SSC	18	F

4	<i>trnH-psbA</i>	220	LSC	<i>rps16 intron</i>	5597	LSC	18	F
5	<i>trnH-psbA</i>	220	LSC	<i>atpA-atpF</i>	13004	LSC	18	R
6	<i>trnH-psbA</i>	222	LSC	<i>trnG-UCC</i>	10963	LSC	18	P
7	<i>trnK-UUU</i>	1892	LSC	<i>rpoB-trnC</i>	28529	LSC	20	P
8	<i>trnK-rps16</i>	4702	LSC	<i>trnK-rps16</i>	4702	LSC	26	P
9	<i>trnK-rps17</i>	5180	LSC	<i>psbM-trnD</i>	31914	LSC	18	P
10	<i>trnK-rps18</i>	5180	LSC	<i>petA-psbJ</i>	66643	LSC	18	F
11	<i>trnK-rps19</i>	5180	LSC	<i>rps19-rpl2</i>	87884	LSC	18	F
12	<i>rps16 intron</i>	5594	LSC	<i>rps16 intron</i>	5594	LSC	18	R
13	<i>rps16</i>	5596	LSC	<i>atpA-atpF</i>	13004	LSC	19	R
14	<i>trnS-GCU</i>	9502	LSC	<i>trnS-UGA</i>	37928	LSC	18	F
15	<i>rps2-rpoC2</i>	17386	LSC	<i>rps15-ycf1</i>	128110	SSC	19	F
16	<i>rpoC2</i>	18825	LSC	<i>rpoC2</i>	18825	LSC	19	R
17	<i>rpoB</i>	25027	LSC	<i>rpoB</i>	25027	LSC	19	R
18	<i>psbM-trnD</i>	31914	LSC	<i>petA-psbJ</i>	66638	LSC	23	P
19	<i>psbM-trnD</i>	31914	LSC	<i>rps19-rpl2</i>	87883	LSC	19	P
20	<i>trnE-trnT</i>	33285	LSC	<i>rps14</i>	39396	LSC	26	F
21	<i>trnS-UGA</i>	37858	LSC	<i>trnS-GGA</i>	47579	LSC	21	P
22	<i>lhbA-trnG</i>	38669	LSC	<i>lhbA-trnG</i>	38669	LSC	28	P
23	<i>trnG-trnfM</i>	38932	LSC	<i>ycf1</i>	129626	SSC	20	C
24	<i>trnfM-CAU</i>	38964	LSC	<i>trnP-UGG</i>	70393	LSC	21	F
25	<i>trnT-trnL</i>	49332	LSC	<i>trnT-trnL</i>	49332	LSC	19	R
26	<i>trnT-trnL</i>	49335	LSC	<i>ndhF-rpl32</i>	116166	SSC	19	R
27	<i>ndhC</i>	52463	LSC	<i>ndhC</i>	52463	LSC	20	R
28	<i>ndhC-trnV</i>	53121	LSC	<i>ndhC-trnV</i>	53121	LSC	25	R
29	<i>ndhC-trnV</i>	53121	LSC	<i>rps3-rps19</i>	87040	LSC	19	F
30	<i>ndhC-trnV</i>	53127	LSC	<i>rps3-rps19</i>	87038	LSC	21	R
31	<i>petA-psbJ</i>	66642	LSC	<i>rps19-rpl2</i>	87883	SSC	20	F
32	<i>rpl20-rps12</i>	72846	LSC	<i>clpP intron2</i>	74944	LSC	20	F
33	<i>clpP intron1</i>	74263	LSC	<i>rpl2-rpl23</i>	89465	LSC	19	R
34	<i>psbT-psbN</i>	77964	LSC	<i>psbT-psbN</i>	77964	LSC	48	P
35	<i>rps19-rpl2</i>	87914	LSC	<i>ycf2</i>	93829	IRa	22	F
36	<i>rps19-rpl2</i>	87914	LSC	<i>ycf2</i>	152667	IRb	22	P
37	<i>rpl2-rpl23</i>	89458	LSC	<i>rpl2-rpl23</i>	89458	LSC	25	R
38	<i>ycf2</i>	95123	IRa	<i>ycf2</i>	95147	IRa	33	F
39	<i>ycf2</i>	95123	IRa	<i>ycf2</i>	151338	IRb	33	P
40	<i>ycf2</i>	95147	IRa	<i>ycf2</i>	151362	IRb	33	P
41	<i>trnN-ycf1</i>	112248	IRa	<i>trnN-ycf1</i>	112267	IRa	21	F
42	<i>trnN-ycf1</i>	112248	IRa	<i>trnN-ycf1</i>	134230	IRb	21	P
43	<i>trnN-ycf1</i>	112267	IRa	<i>trnN-ycf1</i>	134249	IRb	21	P
44	<i>ccsA-ndhD</i>	119975	SSC	<i>ccsA-ndhD</i>	120005	LSC	25	P
45	<i>ndhA intron</i>	125655	SSC	<i>ndhA intron</i>	125655	SSC	19	R
46	<i>ycf1</i>	129875	SSC	<i>ycf1</i>	129875	SSC	19	R
47	<i>ycf1</i>	132638	SSC	<i>ycf1</i>	132656	IRb	30	F

48	<i>ycf1-trnN</i>	134230	IRb	<i>ycf1-trnN</i>	134249	IRb	21	F
49	<i>ycf2</i>	151338	IRb	<i>ycf2</i>	151362	IRb	33	F
50	<i>trnK intron</i>	3906	LSC	<i>trnK intron</i>	3930	LSC	12	T
51	<i>trnE-trnT</i>	33579	LSC	<i>trnE-trnT</i>	33617	LSC	15	T
52	<i>trnT-psbD</i>	34987	LSC	<i>trnT-psbD</i>	35019	LSC	17	T
53	<i>rps4-trnT</i>	48574	LSC	<i>rps4-trnT</i>	48605	LSC	13	T
54	<i>trnT-trnL</i>	48942	LSC	<i>trnT-trnL</i>	48969	LSC	13	T
55	<i>trnF-ndhJ</i>	50724	LSC	<i>trnF-ndhJ</i>	50759	LSC	18	T
56	<i>accD-psaI</i>	62178	LSC	<i>accD-psaI</i>	62223	LSC	15	T
57	<i>psbE-petL</i>	69155	LSC	<i>psbE-petL</i>	69197	LSC	21	T
58	<i>rpl2-rpl23</i>	89457	LSC	<i>rpl2-rpl23</i>	89483	LSC	13	T
59	<i>rpl2-rpl23</i>	89459	LSC	<i>rpl2-rpl23</i>	89485	LSC	14	T
60	<i>ycf2</i>	92333	LSC	<i>ycf2</i>	92383	LSC	21	T
61	<i>rpl32-trnL</i>	118212	SSC	<i>rpl32-trnL</i>	118244	SSC	16	T

F: forward repeats; P: palindromic repeats; R: reverse repeats; C: complement repeats; T: tandem repeats.

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430 Table 3. Pairwise nucleotide divergences of the six plastomes of Lauraceae.

	<i>Persea americana</i>	<i>Machilus balansa</i>	<i>Machilus yunnanensis</i>	<i>Phoebe omeiensis</i>	<i>Phoebe sheareri</i>	<i>Cinnamomum micranthum</i>
<i>Persea americana</i>	—	0.0032	0.0033	0.0028	0.0028	0.0044
<i>Machilus balansa</i>	0.0032	—	0.0015	0.0027	0.0027	0.0049
<i>Machilus yunnanensis</i>	0.0033	0.0015	—	0.0029	0.0029	0.0050
<i>Phoebe omeiensis</i>	0.0028	0.0027	0.0029	—	0.0010	0.0045
<i>Phoebe sheareri</i>	0.0028	0.0027	0.0029	0.0010	—	0.0044
<i>Cinnamomum micranthum</i>	0.0044	0.0049	0.0050	0.0045	0.0044	—

431

432

433 **Figure 1. Gene map of the avocado plastome.** The annotation of the genome was
434 performed using DOGMA. The genes that are drawn outside of the circle are
435 transcribed clockwise, while those inside are counterclockwise. Small single copy
436 (SSC), large single copy (LSC), and inverted repeats (IRa, IRb) are indicated.

437

438 **Figure 2. Number and type of simple sequence repeats of the avocado plastome.**

439 The SSRs was identified using Phobos version 3.3.12 program. SSRs were classified
440 as the number of repeat unit length.

441

442 **Figure 3. Comparison of the nucleotide variability (Pi) values of the six**

443 **plastomes of Lauraceae.** The genetic divergence among the plastomes of avocado,
444 *Phoebe omeiensis*, *Phoebe sheareri*, *Machilus balansae*, *M. yunnanensis* and
445 *Cinnamomum micranthum* was calculated with DnaSP 5.0 software. (window length:
446 600bp, step size: 200bp). X-axis: position of the midpoint of a window, Y-axis:
447 nucleotide diversity of each window.

448

449 **Figure 4. The predicted hairpin loops of inversions in the six plastomes of**

450 **Lauraceae.** The inversions among the plastomes of avocado, *Phoebe omeiensis*,
451 *Phoebe sheareri*, *Machilus balansae*, *M. yunnanensis* and *Cinnamomum micranthum*
452 were manual identified. The structures of hairpin loops were drawn with
453 RNAstructure 5.6. The arrows in the figure indicate the break points in inversion
454 events.

455

456 **Figure 5. Molecular phylogenetic tree of eight species of Lauraceae based on**

457 **complete plastome sequences.** The phylogenetic tree was constructed from available
458 chloroplast genome sequences of eight species in the family Lauraceae by maximum
459 likelihood (ML) method with bootstrapping analysis (1000 reiterations) in MEGA
460 version 5.0 program. The tree is rooted with the plastome sequence of *Calycanthus*
461 *fertilis*.

462

463 **Figure 6. Phylogenetic tree reconstruction of 18 taxa using Bayesian inference**
464 **based on concatenated sequences of seven sequences.** The confederate matrix
465 including *rbcL*, *matK*, *trnL-trnF*, *psbA-trnH*, *ndhF*, *ITS*, and intron of *LEAFY*
466 sequences of *Persea* species, *Apollonias barbujana*, *Machilus yunnanensis*, *Phoebe*
467 *sheareri* and *Cinnamomum micranthum* was used to reconstruct the phylogenetic
468 relationship of *Persea* species using Bayesian Inference (BI) method in MrBayes
469 3.1.2. The combined analysis was run for 1,000,000 generations, and a burn-in of 25%
470 was used for the analysis. The tree is rooted with the sequences of *Phoebe sheareri*.

471

472

473 **Supplementary Material**

474 **Figure S1.** The fruiting branches of avocado.

475 **Table S1.** Genbank accession numbers for DNA sequences of *rbcL*, *matK*, *trnL-trnF*,
476 *psbA-trnH*, *ndhF*, *ITS*, and intron of *LEAFY* sequences of *Persea* species, *Apollonias*
477 *barbujana*, *Machilus yunnanensis*, *Phoebe sheareri* and *Cinnamomum micranthum*.

478 **Table S2.** Distribution of simple sequence repeats in the avocado plastome.

479