

Manuscript title: **Plastome evolution in the sole hemiparasitic genus laurel dodder (*Cassytha*) and insights into the plastid phylogenomics of Lauraceae**

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**Supplementary figures and tables.**

**Supplementary figure 1.** Dot-plot analyses of the plastomes of *Neo. delavayi* and *Cin. camphora* (A), *Cin. camphora* and *Cry. chinensis* (B), and *Cas. filiformis* and *Cin. camphora*.

**Supplementary figure 2.** PCR assays for detection of the two reduced fragments in *Cas. filiformis* and *Cas. pubescens*. Compared to the corresponding region in *Cin. camphora*, reduction of the two examined fragments is due to deletion of the two segments *3'ndhB-ψycf2* and *ndhB-ψycf1* in the plastome of *Cas. filiformis*. As a result, our PCR amplicons with the expected size indicate that both *Cas. filiformis* and *Cas. pubescens* have lost the two segments that are nevertheless, present in the IR of *Cin. camphora*. Green and blue arrows are the primer pairs used for the PCR assays.

**Supplementary figure 3.** Presence, pseudogenization, or absence of genes in the plastomes of *Cas. filiformis*, *Cin. camphora*, *Cry. chinensis*, and *Neo. delavayi*. Genes in bold are the 67 common protein-coding genes.

**Supplementary figure 4.** Box-plots comparing the length of syntenic non-genic loci among the plastomes of *Cas. filiformis*, *Cin. camphora*, *Cry. chinensis*, and *Neo. delavayi*.

**Supplementary figure 5.** Two possible scenarios for IR evolution in Lauraceae.

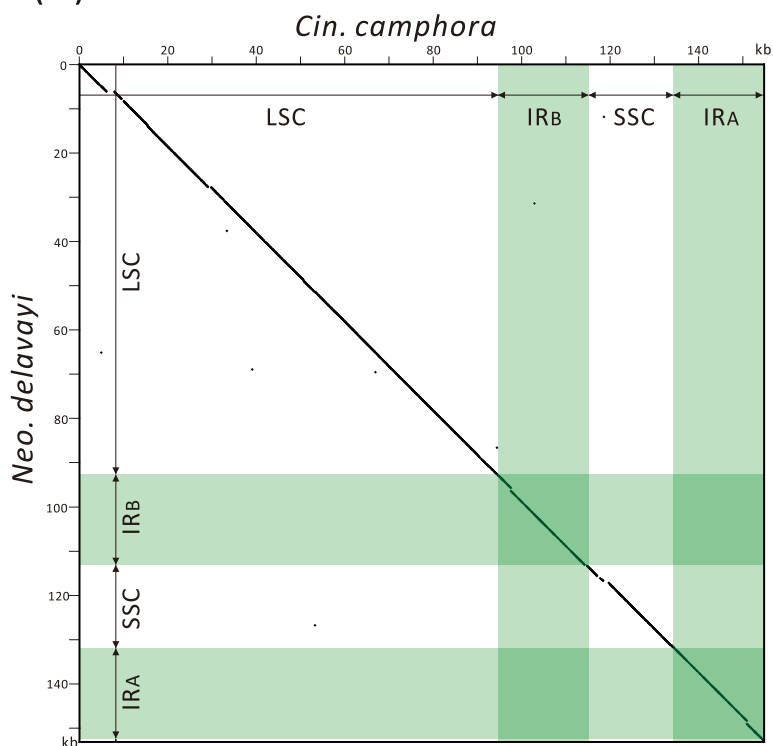
**Supplementary figure 6.** Trees based on non-synonymous ( $d_N$ ) or synonymous ( $d_S$ ) sites in the concatenated house-keeping (HK) or photosynthetic (PS) genes. All tree topologies were constrained as for the ML tree shown in Fig. 2. The species of Lauraceae are in green and the branch leading to *Cas. filiformis* is denoted by “\*”.

**Supplementary figure 7.** Estimated divergence times across 16 magnoliid species. MYA: million years ago.

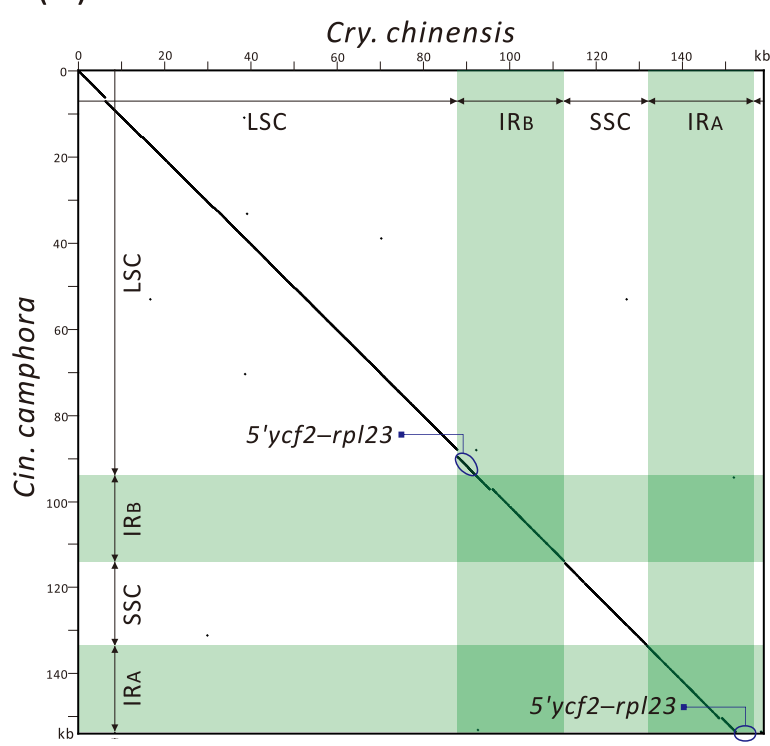
**Supplementary figure 8.** Maximum parsimony tree inferred from the 67 shared plastid genes with *Amb. trichopoda* as the outgroup. Bootstrap support values estimated from 1,000 replicates are shown along branches. The consistency index (CI) is 0.545 and the retention index (RI) is 0.633. The scale bar for branches are indicated.

**Supplementary figure 1.** Dot-plot analyses of the plastomes of *Neo. delavayi* and *Cin. camphora* (A), *Cin. camphora* and *Cry. chinensis* (B), and *Cas. filiformis* and *Cin. camphora*.

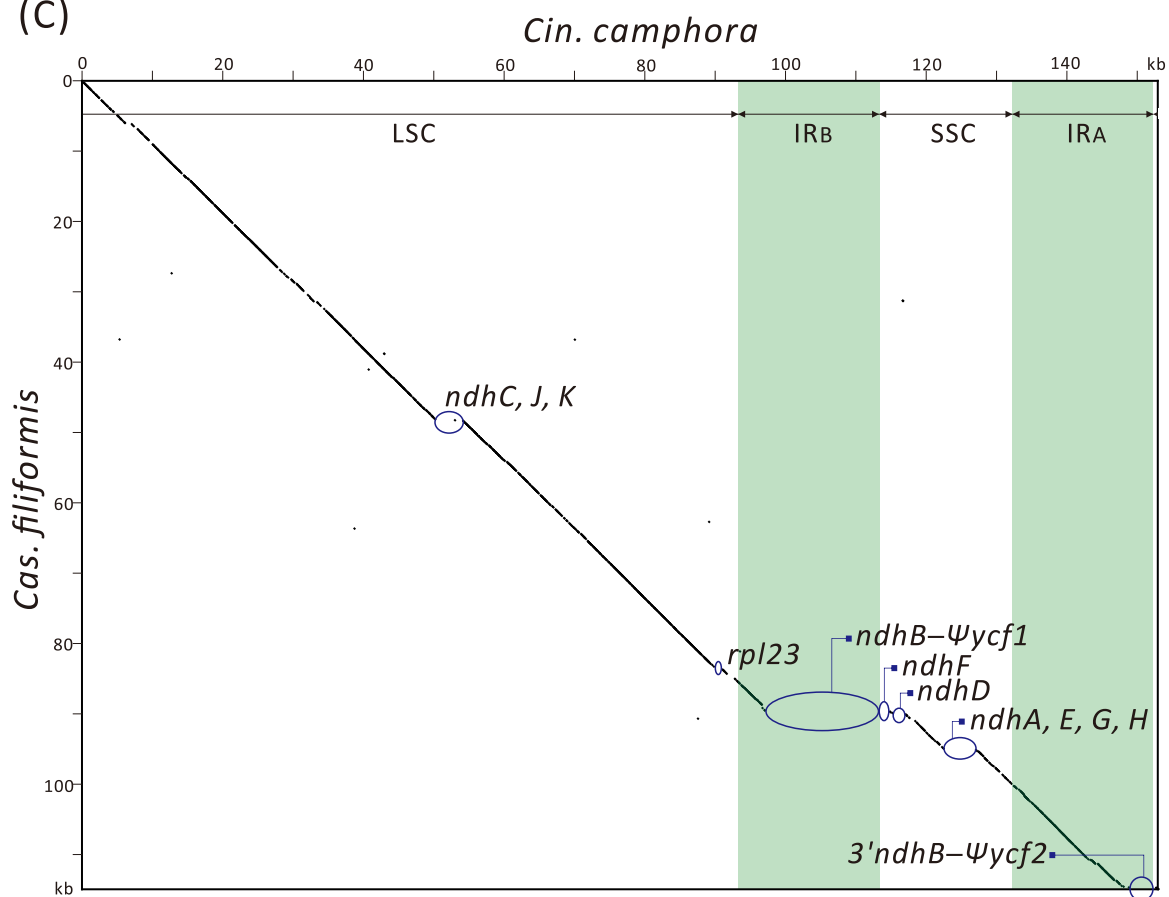
(A)



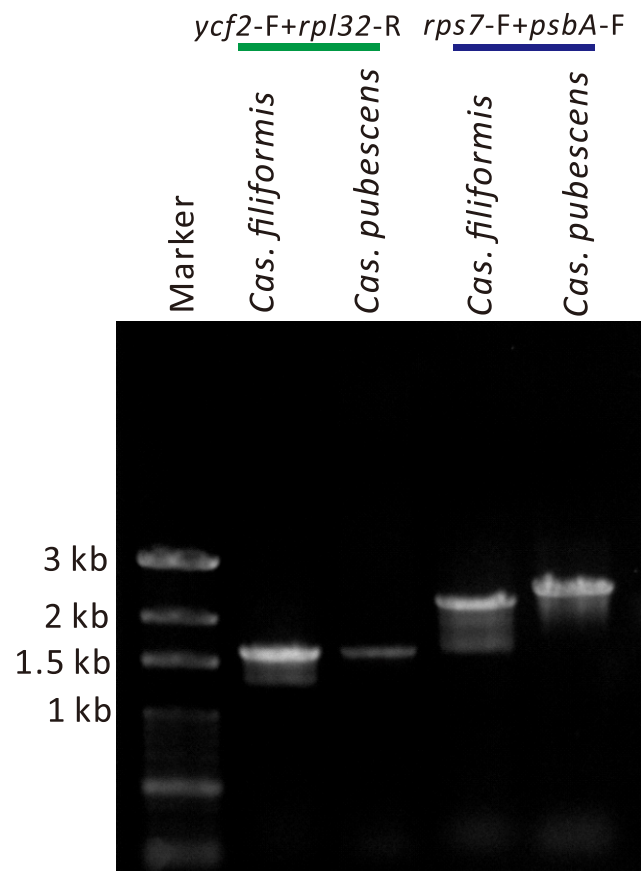
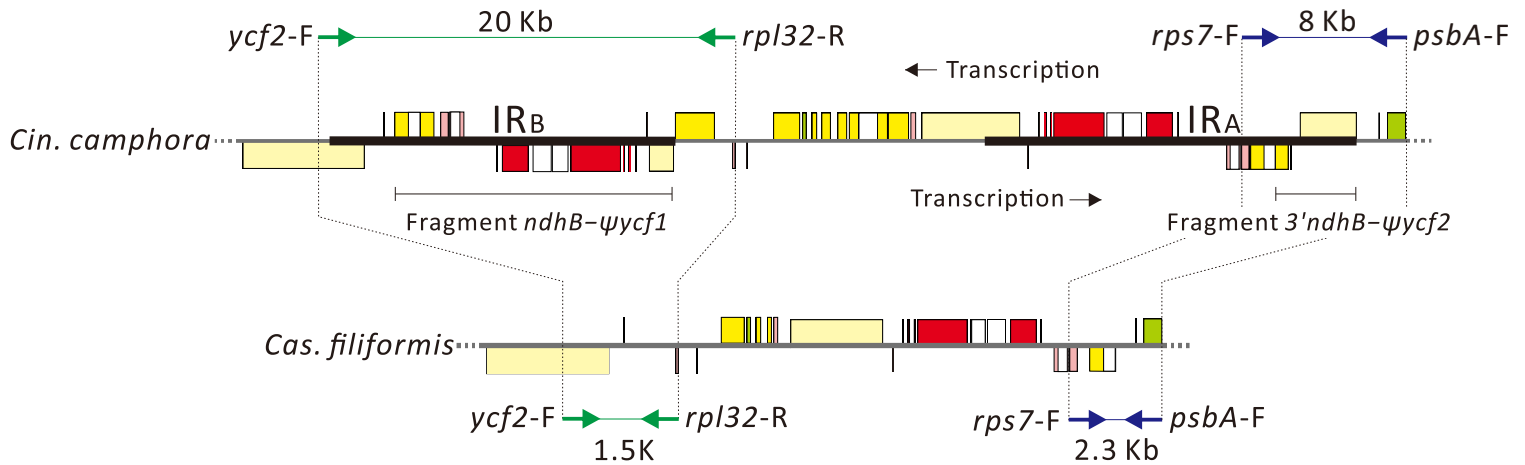
(B)



(C)

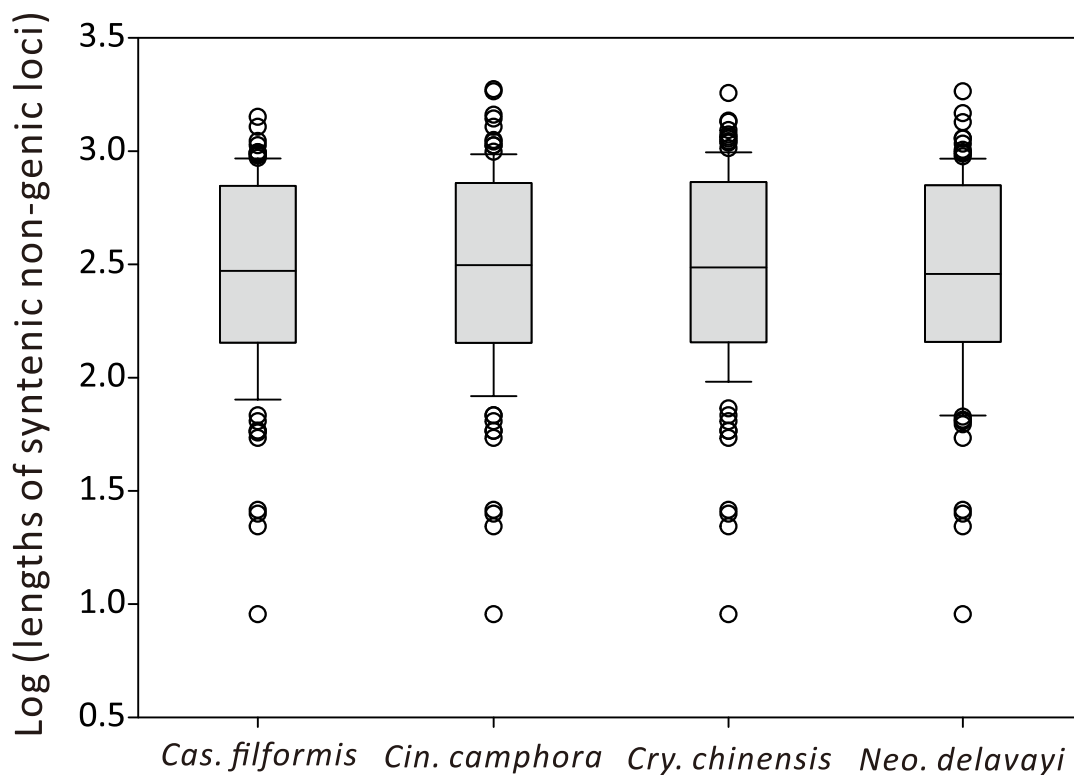


**Supplementary figure 2.** PCR assays for detection of the two reduced fragments in *Cas. filiformis* and *Cas. pubescens*. Compared to the corresponding region in *Cin. camphora*, reduction of the two examined fragments is due to deletion of the two segments 3'*ndhB*- $\Psi$ *ycf2* and *ndhB*- $\Psi$ *ycf1* in the plastome of *Cas. filiformis*. As a result, our PCR amplicons with the expected size indicate that both *Cas. filiformis* and *Cas. pubescens* have lost the two segments that are nevertheless, present in the IR of *Cin. camphora*. Green and blue arrows are the primer pairs used for the PCR assays.





**Supplementary figure 4.** Box-plots comparing the length of syntenic non-genic loci among the plastomes of *Cas. filiformis*, *Cin. camphora*, *Cry. chinensis*, and *Neo. delavayi*.



*P* values for 2-sided Wilcoxon rank-sum tests

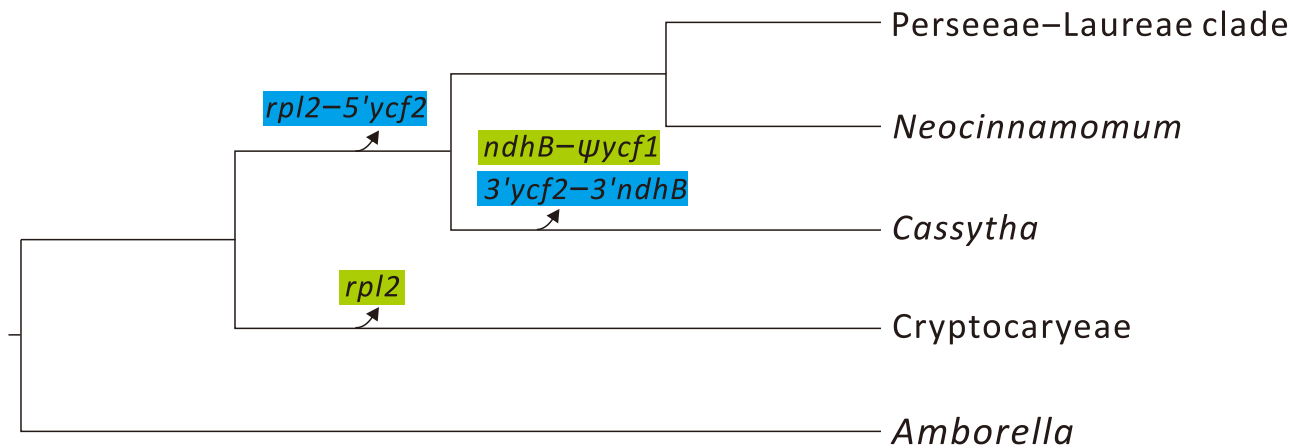
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<i>Cin. camphora</i>	0.8552		
<i>Cry. chinensis</i>	0.6817	0.8291	
<i>Neo. delavayi</i>	0.9521	0.9000	0.7110
	<i>Cas. filiformis</i>	<i>Cin. camphora</i>	<i>Cry. chinensis</i>

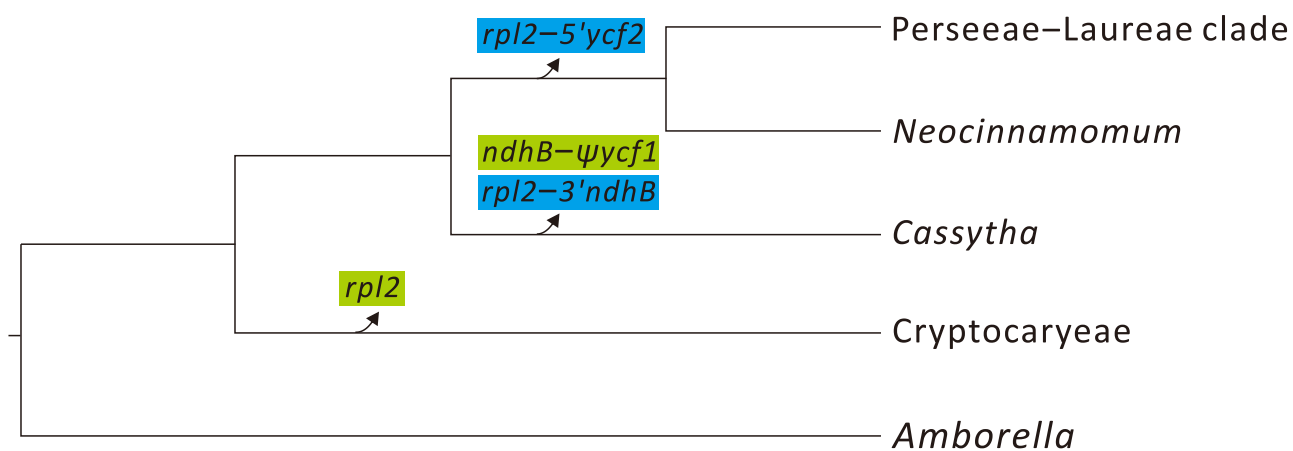
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Supplementary figure 5. Two possible scenarios for the IR evolution in Lauraceae.

A



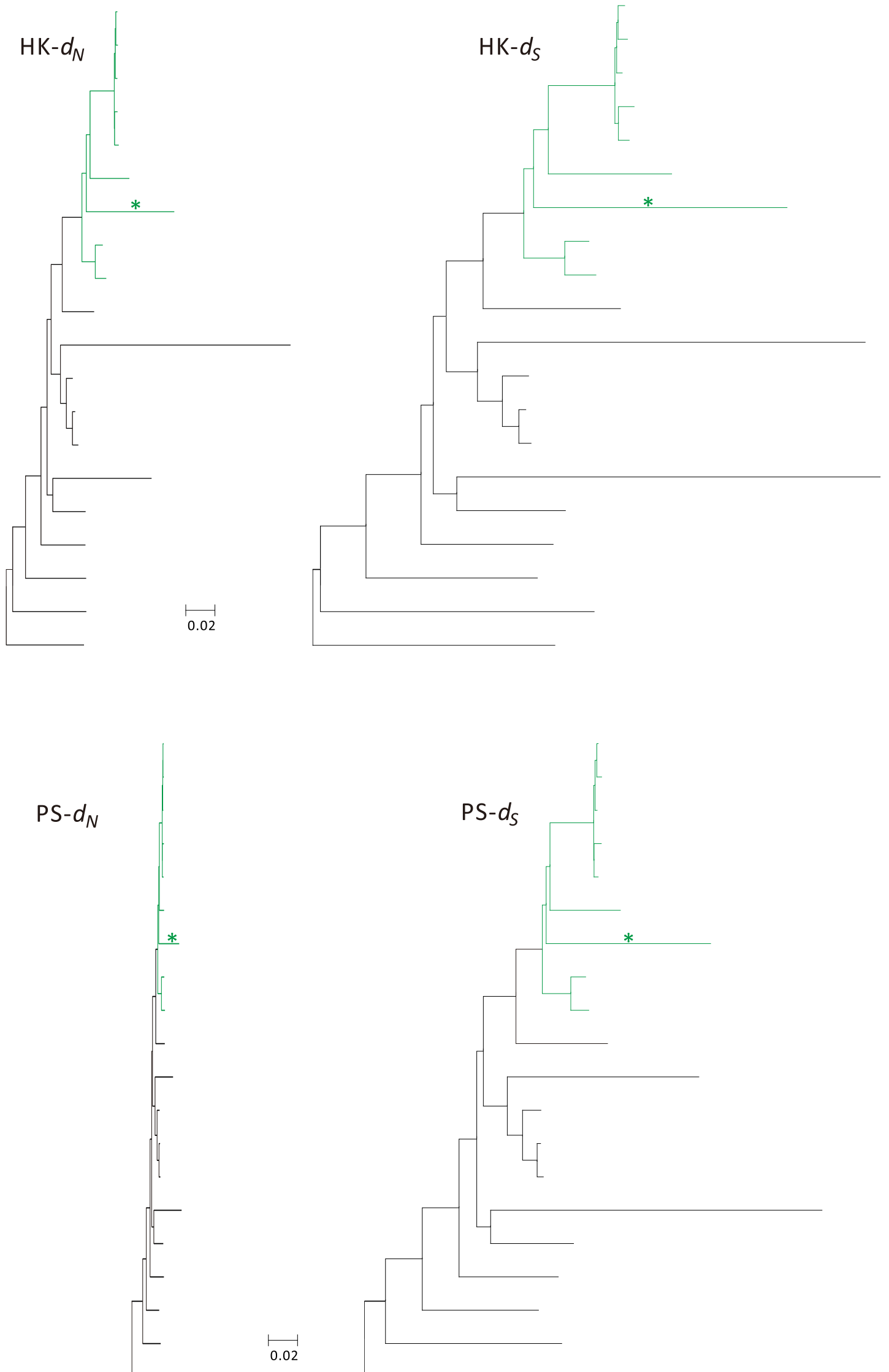
B



■ Loss of fargments or genes due to contraction of the IRA

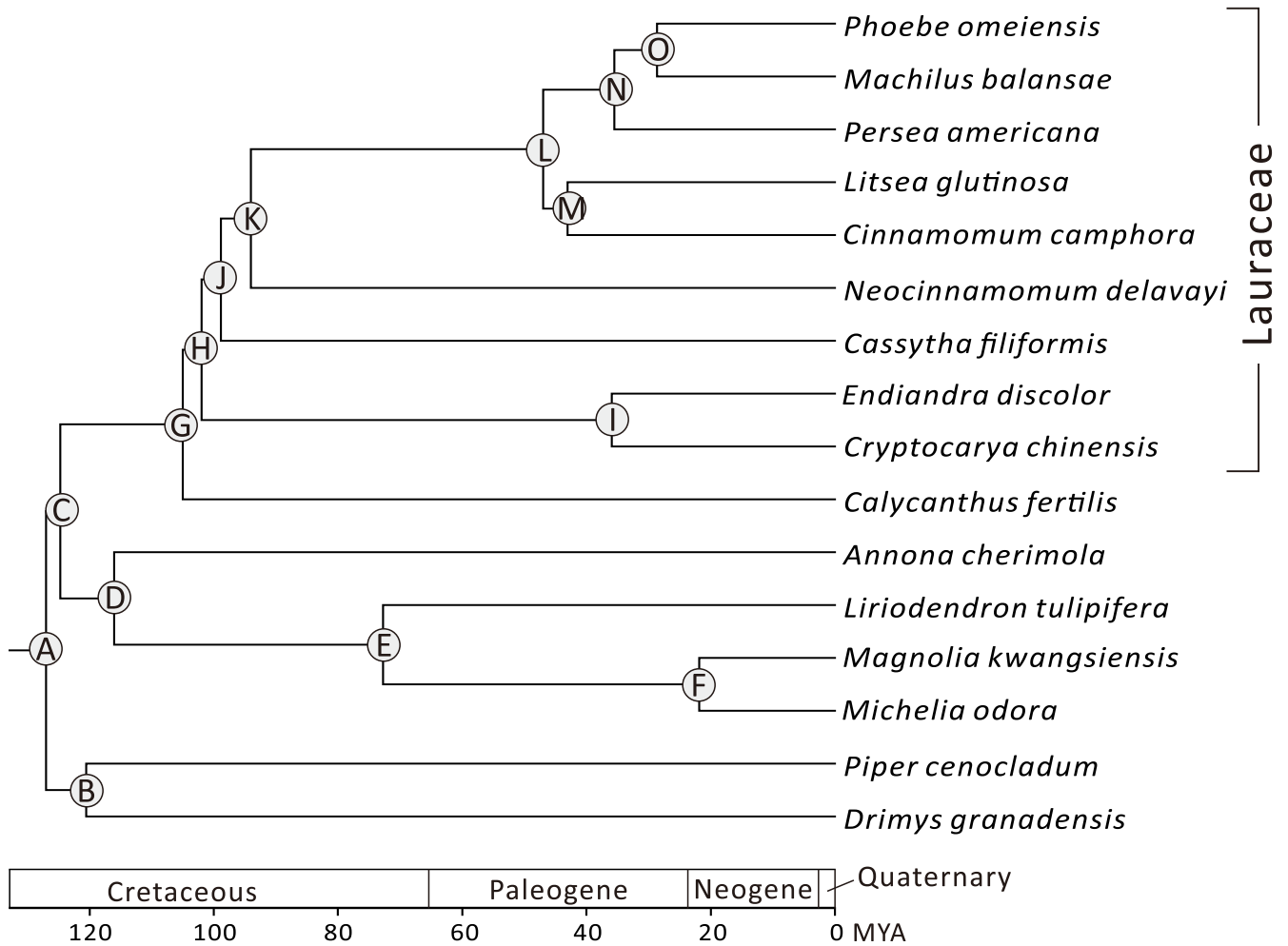
■ Loss of fargments or genes due to contraction of the IRB

**Supplementary figure 6.** Trees based on non-synonymous ( $d_N$ ) or synonymous ( $d_S$ ) sites in the concatenated house-keeping (HK) or photosynthetic (PS) genes. All tree topologies were constrained as for the ML tree shown in Fig. 2. The species of Lauraceae are in green and the branch leading to *Cas. filiformis* is denoted by “\*”.





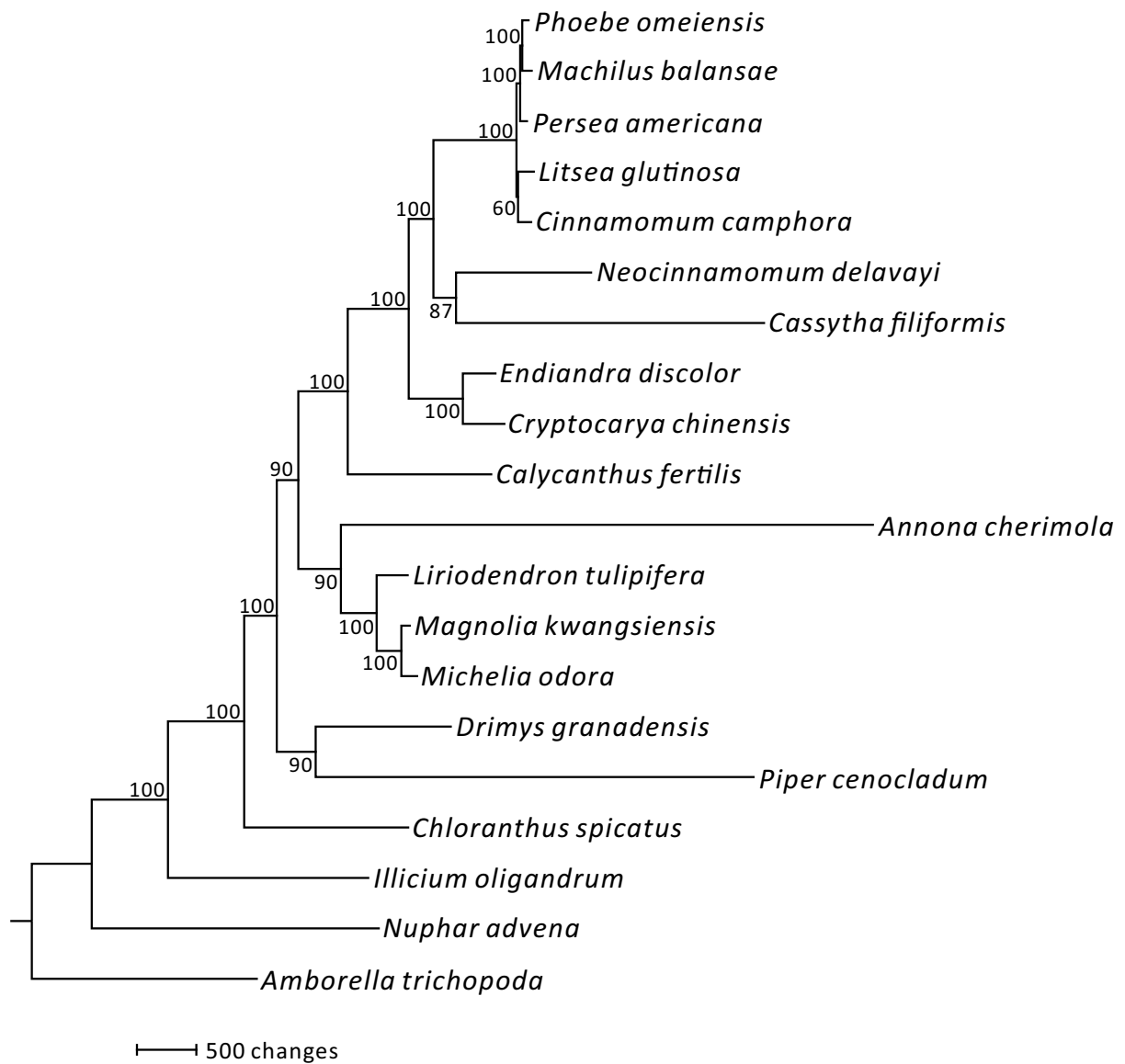
**Supplementary figure 7.** Estimated divergence times across 16 magnoliid species. MYA: million years ago.



Node	Divergence time (MYA)	95% confidence interval
A*	127	—
B	120.495	120.2482–120.7418
C	124.7039	124.6142–124.7936
D*	116	—
E	72.74	71.27456–74.20544
F	21.875	21.21487–22.53513
G*	105	—
H*	102	—
I	35.89455	35.44649–36.3426
J	98.84955	98.73001–98.96908
K	93.9975	93.77836–94.21664
L*	47	—
M	43.05909	42.7554–43.36279
N	35.49955	34.64733–36.35176
O	28.63386	27.70872–29.55901

\*constrained age (Time Tree, Hedges et al. 2015)

**Supplementary figure 8.** Maximum parsimony tree inferred from the 67 shared plastid genes with *Amb. trichopoda* as the outgroup. Bootstrap support values estimated from 1,000 replicates are shown along branches. The consistency index (CI) is 0.545 and the retention index (RI) is 0.633. The scale bar for branches are indicated.



**Supplementary table 1.** Primers used for gap closing

Species	Primer
<i>Cinnamomum camphora</i>	psbA: CAGCTTGGCCTGTAGTAGGTATCTG ycf2: GAATAGAGGAACTCCCAAGCATTTG ndhF: GCAAGTTTCTTTGTAGGAGAAGG trnN: ACCGCTCTACCACTGAGCTACTGAG
<i>Neocinnamomum delavayi</i>	psbJ: TGTAAGTGGTATTCCTGTGATCGGTTTC petA: CTGCGAGATGTTGGGAATTGC psbA: AATCTGTAGTTGACAGTCAAGGTCGTG ycf2: TGATCCAATAGCGTTCCGTTAGATAG ycf1: GTAGTTTTGTTGGTTGGTTAATTGGTC ndhF: CCCATGAATCGGACAATACTATGC
<i>Cassytha filiformis</i>	psbA: AATCTGTAGTTGACAGTCAAGGTCGTG rps7: AGAGACTCATAGAATGGCAGAGGC ycf2: ACGGAACGCTATTGGATCAAATG rpl32: AATGCCCGGTAGAAATGGATTTAG rps18: GATTAATTACTATTGCTATAAAACAAGCTCG rpl20: GGAGACATAGAACAAAATGCGTTC accD: GCTCGATCTATGGTCCGAAACAAC rbcL: CTAGCTGCTGCTTGTGAGGTATGG psbD: CGTTGCATTGGAGGAGCGA trnT: TGGACCTGACCCTTTGAATCATG rps16: CCATTTCAAAGAAGGCGGAGG trnK: TTTCTTTGTTGGTGGAGTCCTAACG
<i>Cryptocarya chinensis</i>	trnN: TCGTAGTGGCTCCGTTCCATG ndhF: CAAAATTGATGGGAATGTAAGAAATCTG psbA: AAGTTATGCATGAACGTAATGCTCAC ycf2-1: TTGTTGAACCCTGTGAATCACGTG rps19: TGGTAGATGCCCCGGGACCAAG ycf2-2: CACCGAATCGAATTTGGTCCATG

**Supplementary Table 2.** The 20 basal angiosperm species and their plastome accession numbers in this study. \*Newly sequenced species.

Classification	Order	Family	Species	Accession
Magnoliids	Magnoliales	Annonaceae	<i>Annona cherimola</i>	NC_030166
		Magnoliaceae	<i>Liriodendron tulipifera</i>	NC_008326
			<i>Magnolia kwangsiensis</i>	NC_015892
			<i>Michelia odora</i>	JX280398
	Lurales	Calycanthaceae	<i>Calycanthus fertilis</i>	NC_004993
		Lauraceae	<i>Cassytha filiformis</i> *	LC210517
			<i>Cinnamomum camphora</i> *	LC228240
			<i>Neocinnamomum delavayi</i> *	LC213014
			<i>Cryptocarya chinensis</i> *	LC212965
			<i>Endiandra discolor</i>	KT588615
			<i>Litsea glutinosa</i>	KU382356
			<i>Machilus balansae</i>	NC_028074
			<i>Persea americana</i>	KX437771
	<i>Phoebe omeiensis</i>	KX437772		
Canellales	Winteraceae	<i>Drimys granadensis</i>	NC_008456	
Piperales	Piperaceae	<i>Piper cenocladum</i>	NC_008457	
Other basal angiosperm	Amborellales	Amborellaceae	<i>Amborella trichopoda</i>	NC_005086
	Nymphaeales	Nymphaeaceae	<i>Nuphar advena</i>	NC_008788
	Austrobaileyales	Schisandraceae	<i>Illicium oligandrum</i>	NC_009600
	Chloranthales	Chloranthaceae	<i>Chloranthus spicatus</i>	NC_009598