JSE Journal of Systematics and Evolution

doi: 10.1111/jse.12536

Research Article

Plastid phylogenomics improve phylogenetic resolution in the Lauraceae

Yu Song[1,2,3†], Wen-Bin Yu[1,2,3†], Yun-Hong Tan[1,2,3†], Jian-Jun Jin[4], Bo Wang[1,2], Jun-Bo Yang[4*], Bing Liu[5,6*], and Richard T. Corlett[1,2,3*]

[1]Center for Integrative Conservation, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla 666303, Yunnan, China
[2]Center of Conservation Biology, Core Botanical Gardens, Chinese Academy of Sciences, Mengla 666303, Yunnan, China
[3]Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences, Yezin, Nay Pyi Taw 05282, Myanmar
[4]Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China
[5]State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China
[6]Sino-African Joint Research Center, Chinese Academy of Sciences, Wuhan 430074, China
[†]These authors contributed equally to this work.
*Authors for correspondence. Richard T. Corlett. E-mail: corlett@xtbg.org.cn; Bing Liu. E-mail: liubing@ibcas.ac.cn; Jun-Bo Yang. E-mail: jbyang@mail.kib.ac.cn
Received 21 March 2019; Accepted 31 July 2019; Article first published online 24 August 2019

Abstract The family Lauraceae is a major component of tropical and subtropical forests worldwide, and includes some commercially important timber trees and medicinal plants. However, phylogenetic relationships within Lauraceae have long been problematic due to low sequence divergence in commonly used markers, even between morphologically distinct taxa within the family. Here we present phylogenetic analyses of 43 newly generated Lauraceae plastomes together with 77 plastomes obtained from GenBank, representing 24 genera of Lauraceae and 17 related families of angiosperms, plus nine barcodes from 19 additional species in 18 genera of Lauraceae, in order to reconstruct highly supported relationships for the Lauraceae. Our phylogeny supports the relationships: sisterhood of the Lauraceae and a clade containing Hernandiaceae and Monimiaceae, with Atherospermataceae and Gomortegaceae being the next sister groups, followed by Calycanthaceae. Our results highlight a monophyletic Lauraceae, with nine well-supported clades as follows: *Hypodaphnis* clade, *Beilschmiedia–Cryptocarya* clade, *Cassytha* clade, *Neocinnamomum* clade, *Caryodaphnopsis* clade, *Chlorocardium–Mezilaurus* clade, *Machilus–Persea* clade, *Cinnamomum–Ocotea* clade, and *Laurus–Neolitsea* clade. The topology recovered here is consistent with the patterns of plastome structural evolution and morphological synapomorphies reported previously. More specifically, flower sex, living type, inflorescence type, ovary position, anther locus number, leaf arrangement, leaf venation, lateral vein number, tree height, and inflorescence location all represent morphological synapomorphies of different lineages. Our findings have taxonomic implications and two new tribes, Caryodaphnopsideae and Neocinnamomeae, are described, and the composition of four other tribes is updated. The phylogeny recovered here provides a robust phylogenetic framework through which to address the evolutionary history of the Magnoliids, the third-largest group of Mesangiospermae.

Key words: chloroplast, Laurales, magnoliids, phylogenetic relationships.

1 Introduction

The family Lauraceae is distributed in tropical and warm temperate regions worldwide, especially in Southeast Asia and South America. The family includes more than 50 genera (Chanderbali et al., 2001) and 3500 species (Rohwer, 1993). Most of the members are aromatic evergreen trees or shrubs, but the family also includes parasitic vines in the genus *Cassytha* L. (Weber, 1981). In East Asia, the Lauraceae is among the top four woody families (along with the Fagaceae, Magnoliaceae, and Theaceae) in terms of the number of species in tropical seasonal rain forest and subtropical evergreen broad-leaved forest (Fang & Yoda, 1989; Tang, 2015). Many Lauraceae species provide important economic products, including timber, perfume, spices, herbal medicines, and fruit crops (Li et al., 2008a). The Lauraceae have an undifferentiated perianth that represents an intermediate stage before the evolutionary differentiation of sepals and petals (Chanderbali et al., 2009; Poinar, 2017; Song et al., 2018a). However, variation in floral and other morphological traits among Lauraceae species is relatively limited, complicating species delineation and identification, and contributing to the unsettled taxonomic history of the group (Julia et al., 2009; Sajo et al., 2016).

Over the last two decades, molecular sequence data have greatly improved our understanding of relationships within

the Lauraceae, and within the Laurales. For example, Renner (1998) reconstructed a monophyletic Laurales based on sequences of two chloroplast DNA (cpDNA) markers (*rbcL* and *trnL-trnF*) from 54 species of Atherospermataceae, Calycanthaceae, Gomortegaceae, Hernandiaceae, Lauraceae, Monimiaceae, and Siparunaceae. However, a second study based on six cpDNA markers (*rbcL*, *rpl16*, *trnT-trnL*, *trnL-trnF*, *atpB-rbcL*, and *psbA-trnH*) and 40 taxa only recovered a weakly supported Laurales (Renner, 1999).

The first study that aimed at reconstructing phylogenetic relationships within the Lauraceae (Rohwer, 2000) recovered a monophyletic Lauraceae with low support (59%) and seven main clades within the family, using the cpDNA marker *matK* and 48 species. A second study (Chanderbali et al., 2001), based on a higher sampling of cpDNA markers (*trnL-trnF*, *psbA-trnH*, *trnT-trnL*, and *rpl16*) and the nuclear 26S ribosomal DNA (rDNA) for 33 species reconstructed a monophyletic Lauraceae (96%) including six main clades: *Hypodaphnis* clade, Cryptocaryeae, *Cassytha–Neocinnamomum* clade, *Caryodaphnopsis* clade, *Chlorocardium–Mezilaurus* clade, Perseae, and Laureae. A third study (Rohwer & Rudolph, 2005), using the intron of *trnK* with 49 species, constructed a Bayesian tree in order to settle the positions of three "jumping genera," *Cassytha*, *Hypodaphnis* Stapf, and *Neocinnamomum* H. Liou, which appeared in different positions in different previous analyses. Additional plastid markers, including *rpl16* intron, *psbA-trnH*, and *trnL-trnF*, have been used to resolve the relationships among some species in morphologically defined genera, including *Beilschmiedia* Nees, *Cryptocarya* R. Br., *Lindera* Thunb., *Litsea* Lam., *Neocinnamomum*, and *Sassafras* Nees (Nie et al., 2007; Wang et al., 2010; Liu et al., 2013; van der Merwe et al., 2016).

The original research (Chanderbali et al., 2001) using the rDNA marker internal transcribed spacer (ITS) and sampling 94 species confirmed the previous terminal group (Laureae) in the sense of Rohwer (2000), *Persea* group, and *Chlorocardium–Mezilaurus* clade, and showed a dozen subclades within the previous terminal group, albeit most of them without bootstrap support. In addition, ITS, *rpb2*, and *LEAFY* intron II sequences as well as the plastid sequences have been tried to resolve the phylogenetic and species identification problems in *Actinodaphne* Nees, *Alseodaphne* Nees, *Cinnamomum* Schaeff., *Cryptocarya*, *Lindera*, *Litsea*, *Machilus* Rumph. ex Nees, *Neolitsea* (Benth.) Merr., *Persea* Mill., and *Phoebe* Nees (Li et al., 2008b, 2011, 2016; Fijridiyanto & Murakami, 2009; Rohwer et al., 2009; Huang et al., 2016). In all of these studies, however, the reported sequences had limited ability to resolve phylogenetic problems at the species level or within some genera. The extremely low genetic divergence within Lauraceae genera, described by Rohwer (Rohwer, 2000; Rohwer et al., 2009) and Song (Song et al., 2015, 2016, 2017a, 2018b; Zhao et al., 2018), can be explained by recent species differentiation and/or a greatly decreased substitution rate within the genera.

Despite all the efforts to reconstruct the Lauraceae phylogeny, relationships have remained poorly resolved and/or supported due to low sequence divergence of commonly used genetic markers within this plant family. The development of high-throughput sequencing techniques has led to a rapid accumulation of plant genetic resources in public databases, and the relationships among plant species are now clearer than before. Compared with the previous molecular markers with lengths of hundreds of base pairs (bp), next generation sequencing techniques have the advantage of producing sequence contigs with lengths of dozens of kilobase pairs, and the advent of these technologies has facilitated rapid progress in the field of plastid genome research (Daniell et al., 2016). Plastomes have provided resolution for difficult clades such as bamboos, palms, the Rosaceae, and the Theaceae (Ma et al., 2014; Barrett et al., 2016; Yu et al., 2017; Zhang et al., 2017), as well as several genera within the Lauraceae (Song et al., 2016, 2017a, 2018b; Liao et al., 2018; Zhao et al., 2018; Chen et al., 2019; Tian et al., 2019). In this study, we compiled a dataset composed of 113 plastomes, 43 of which were newly generated for this study, to reconstruct the main relationships within the Lauraceae and among seven of the nine families of the Laurales recognized to date.

2 Material and Methods

2.1 Taxon sampling

We sampled 43 taxa representing 18 genera of the Lauraceae. Voucher specimens for the 43 sampled taxa were deposited in the Herbarium of the Xishuangbanna Tropical Botanical Garden (HITBC), Chinese Academy of Sciences (Table 1). In addition, we also obtained sequences from another 77 previously reported plastomes from GenBank (54 plastomes of the Lauraceae and 23 plastomes of 17 related families). A total of 89 taxa from all subfamilies and 24 genera of Lauraceae were included.

To integrate the plastomes uploaded by other research teams, duplicate plastomes were reported for eight taxa, including *Cassytha filiformis* L., *Cryptocarya chinensis* (Hance) Hemsl., *Laurus nobilis* L., *Lindera glauca* (Sieb. & Zucc.) Blume, *Lin. obtusiloba* Blume, *Litsea cubeba* (Lour.) Pers., *Lit. glutinosa* (Lour.) C. B. Rob., and *Phoebe zhennan* S. K. Lee & F. N. Wei. An additional 15 species of magnoliids and eight species of non-magnoliid angiosperms with published plastome data (Table S1) and 19 species of Lauraceae with more than three plastid DNA fragments in GenBank (Table S2) were added in order to better understand the phylogenetic relationships between the sequenced taxa from 24 genera and the representative species from another 18 genera in the Lauraceae.

2.2 Plastome sequencing and data assembly

Genomic DNA was extracted from 3 cm^2 fresh leaves or silica-dried leaf materials using the CTAB method (Doyle & Dickson, 1987). Long-range polymerase chain reaction was carried out following Zhang et al. (2016), with their 15 pairs of universal primers. The 15 purified polymerase chain reaction products were mixed with 0.4 μg for each. A total of 6 μg product was further fragmented into small pieces by Covaris S220 at BGI-Shenzhen in Guangdong. Indexed paired-end libraries were prepared following the manufacturer's manual (Illumina, San Diego, CA, USA). Sequencing was carried out on Illumina Hiseq 2000 at BGI-Shenzhen in Guangdong, and more than 100 Mb of sequence data for each sample was obtained. Paired-end reads were filtered with the GetOrganelle Kit (version 1.4.0) to select clean ones, with the

Table 1 Summary of 44 complete plastomes of Lauraceae

Taxon	Voucher	Geographic origin	Accession No.	Total cpDNA size (bp)	Length of LSC region (bp)	Length of IR region (bp)	Length of SSC region (bp)	Loss event
Actinodaphne cupularis (Hemsl.) Gamble	SY64801	Guizhou, China	LAU00034	152 720	93 821	20 016	18 816	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Actinodaphne pilosa (Lour.) Merr.	SY01420	Guangxi, China	LAU00035	152 772	93 798	20 066	18 842	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Alseodaphne hainanensis Merr.	SY33167	Hainan, China	LAU00036	152 848	93 767	20 093	18 885	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Alseodaphne rugosa Merr. & Chun	SY55701	Hainan, China	LAU00037	152 761	93 724	20 071	18 895	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Alseodaphne yunnanensis Kosterm.	SY32622	Yunnan, China	LAU00038	152 762	93 749	20 032	18 910	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Beilschmiedia turbinata Bing Liu & Y. Yang	SY33250	Yunnan, China	LAU00039	158 414	89 240	25 488	18 196	*rpl2* copy from IRa
Beilschmiedia fasciata H. W. Li	SY33216	Yunnan, China	LAU00040	158 338	89 239	25 450	18 199	*rpl2* copy from IRa
Beilschmiedia robusta C. K. Allen	SY35213	Yunnan, China	LAU00041	158 337	89 273	25 445	18 173	*rpl2* copy from IRa
Beilschmiedia rufohirtella H. W. Li	SY32749	Yunnan, China	LAU00042	158 417	89 275	25 463	18 216	*rpl2* copy from IRa
Caryodaphnopsis tonkinensis (Lecomte) Airy Shaw	SY01520	Yunnan, China	LAU00043	148 829	91 762	19 695	17 677	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Cinnamomum aromaticum Nees	SY01468	Yunnan, China	LAU00044	152 725	93 693	20 066	18 900	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Cinnamomum bodinieri H. Lév.	SY35180	Yunnan, China	LAU00045	152 643	93 588	20 096	18 863	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Cinnamomum burmanni (Nees & T. Nees) Blume	SY01614	Yunnan, China	LAU00046	152 775	93 688	20 092	18 903	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Cinnamomum heyneanum Nees	SY01661	Yunnan, China	LAU00047	152 679	93 712	20 074	18 819	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Cryptocarya wrayi Gamble	SY01502	Sulawesi, Indonesia	LAU00048	157 728	89 247	24 624	19 233	*rpl2* copy from IRa
Cryptocarya chingii W. C. Cheng	SY01426	Yunnan, China	LAU00049	157 132	88 984	24 621	18 906	*rpl2* copy from IRa

Continued

Table 1 Continued

Taxon	Voucher	Geographic origin	Accession No.	Total cpDNA size (bp)	Length of LSC region (bp)	Length of IR region (bp)	Length of SSC region (bp)	Loss event
Cryptocarya densiflora Blume	SY87701	Hainan, China	LAU00050	157 739	89 263	24 621	19 234	*rpl2* copy from IRa
Cryptocarya yunnanensis H. W. Li	SY01436	Yunnan, China	LAU00051	157 162	89 031	24 617	18 897	*rpl2* copy from IRa
Dehaasia incrassata (Jack) Kosterm.	SY60401	Sulawesi, Indonesia	LAU00052	152 723	93 712	20 056	18 899	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Endiandra dolichocarpa S. K. Lee & Y. T. Wei	SY01505	Yunnan, China	LAU00053	158 610	89 317	25 522	18 249	*rpl2* copy from IRa
Endiandra muelleri Meisn.	SY34008	Australia	LAU00054	158 571	89 321	25 507	18 236	*rpl2* copy from IRa
Iteadaphne caudata (Nees) H. W. Li	SY34578	Yunnan, China	LAU00055	152 370	93 455	20 089	18 791	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Laurus nobilis L.	SY83201	Zhejiang, China	LAU00056	152 608	93 516	20 061	18 970	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Lindera obtusiloba Blume	SY34010	Gansu, China	LAU00057	152 716	93 642	20 066	18 942	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Litsea glutinosa (Lour.) C. B. Rob.	SY01341	Yunnan, China	LAU00058	152 721	93 701	20 062	18 896	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Litsea tsinlingensis Y. C. Yang & P. H. Huang	SY34166	Gansu, China	LAU00059	152 424	93 517	20 042	18 823	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Litsea cubeba (Lour.) Pers.	SY34280	Yunnan, China	LAU00060	152 738	93 688	20 064	18 922	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Litsea cubeba (Lour.) Pers.	SY34228	Hainan, China	LAU00061	152 782	93 682	20 064	18 972	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Litsea magnifolia Gillespie	SY34575	Yunnan, China	LAU00062	152 696	93 646	20 066	18 918	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Litsea monopetala (Roxb.) Pers.	SY01491	Yunnan, China	LAU00063	152 652	93 696	20 054	18 848	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Litsea panamanja (Buch.-Ham. ex Nees) Hook. f.	SY01348	Yunnan, China	LAU00064	152 735	93 812	20 042	18 839	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb

Continued

Table 1 Continued

Taxon	Voucher	Geographic origin	Accession No.	Total cpDNA size (bp)	Length of LSC region (bp)	Length of IR region (bp)	Length of SSC region (bp)	Loss event
Litsea pierrei Lecomte	SY33352	Yunnan, China	LAU00065	152 772	93 796	20 066	18 844	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Machilus fasciculata H. W. Li	SY01499	Yunnan, China	LAU00066	152 617	93 664	20 074	18 805	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Machilus pauhoi Kaneh.	SY32193	Hubei, China	LAU00067	152 620	93 670	20 074	18 802	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Machilus thunbergii Siebold & Zucc.	SY33308	Chiba-ken, Japan	LAU00068	152 550	93 650	20 050	18 800	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Machilus minutiloba S. K. Lee	SY31123	Zhejiang, China	LAU00069	152 543	93 687	20 025	18 806	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Neolitsea chui Merr.	SY34830	Yunnan, China	LAU00070	152 727	93 836	20 016	18 808	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Neolitsea oblongifolia Merr. & Chun	SY34201	Hainan, China	LAU00071	152 747	93 843	20 016	18 821	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Nothaphoebe umbelliflora (Blume) Blume	SY60101	Sulawesi, Indonesia	LAU00072	152 832	93 747	20 078	18 870	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Persea americana var. *drymifolia* (Schltdl. & Cham.) S. F. Blake	SY01551	Cuba	LAU00073	152 862	93 845	20 196	18 599	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Persea borbonia (L.) Spreng.	SY34006	Texas, USA	LAU00074	152 394	93 314	20 076	18 928	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Phoebe lanceolata (Wall. ex Nees) Nees	SY01498	Yunnan, China	LAU00075	152 809	93 767	20 073	18 896	*rpl2*, *rpl23*, *trnI*, and a fragment of *ycf2* copy from IRb
Syndiclis anlungensis H. W. Li	SY34295	Guizhou, China	LAU00076	158 573	89 378	25 499	18 197	*rpl2* copy from IRa
Syndiclis marlipoensis H. W. Li	SY33194	Yunnan, China	LAU00077	158 537	89 359	25 491	18 196	*rpl2* copy from IRa

cpDNA, chloroplast DNA; IR, inverted repeat; LSC, large single copy; SSC, small single copy.

parameters as follows: w 103, R 15, K 95 to105, P 300 000 (Jin et al., 2018). High-quality short reads were viewed and edited using Bandage (Wick et al., 2015) to assemble a circular plastome. The genome was adjusted and annotated using Geneious 10.1.3 (Kearse et al., 2012). The annotated plastid genome sequence was submitted to the Lauraceae Chloroplast Genome Database, accession numbers LAU00034 to LAU00077.

2.3 Phylogenetic analyses

Two different data matrices (Table S3) were assembled and analyzed using both maximum likelihood (ML) and Bayesian inference (BI) methods. Matrix I included 120 complete plastid genomes, including members of Amborellales (1 sp.), Nymphaeales (3 spp.), Austrobaileyales (2 spp.), Chloranthales (2 spp.), Canellales (1 sp.), Piperales (2 spp.), Magnoliales (3 spp.), and Laurales (106 spp.) (Table S1). *Amborella trichopoda* Baill. was used as outgroup. Matrix II included 112 Laurales species, including 93 complete plastomes and 19 additional taxa with three to nine loci obtained with Sanger sequencing (i.e., *matK*, *psbA-trnH*, *rbcL*, *rpl16*, *rpoB*, *rpoC1*, *trnL*, *trnL-trnF*, and *trnT-trnL*) (Table S2). *Peumus boldus* Molina, *Gyrocarpus americanus* Jacq., *Illigera grandiflora* W.W. Sm. & Jeffrey, and *I. celebica* Miq. were used as outgroups. To evaluate potential conflict among regions, we divided Matrix I into five subsets: large single copy (LSC) (Matrix III), small single copy (SSC) (Matrix IV), inverted repeat (IR) (Matrix V), coding regions (Matrix VI), and noncoding regions (Matrix VII). These matrixes were analyzed using the BI method.

The alignments were carried out in Mauve 2.4.0 and adjusted manually in Geneious 9.1.7 (Darling et al., 2004; Kearse et al., 2012). Bayesian inference was undertaken using MrBayes 3.2.6 (Ronquist & Huelsenbeck, 2003). The best-fit DNA substitution models selected using jModelTest 2.1.10 were used for phylogeny reconstruction (Guindon & Gascuel, 2003; Darriba et al., 2012). The optimal models for Markov chain Monte Carlo were run in MrBayes for 1 000 000 generations. The BI analysis started with a random tree and sampled every 1000 generations. The first 25% of the trees was discarded as burn-in, and the remaining trees were used to generate a majority-rule consensus tree. Maximum likelihood analysis was carried out using RAxML 7.2.6 (Stamatakis, 2014). The best-fit DNA substitution models for Matrix I and Matrix II were chosen as "GTR+I+G" (freqA = 0.3223, freqC = 0.1764, freqG = 0.1768, freqT = 0.3245, R (a) [AC] = 1.0343, R(b) [AG] = 2.3544, R(c) [AT] = 0.4056, R (d) [CG] = 0.6855, R(e) [CT] = 2.3004, R(f) [GT] = 1.0000, p-inv = 0.1820, gamma shape = 0.9600) and "GTR+G" (freqA = 0.3194, freqC = 0.1835, freqG = 0.1822, freqT = 0.3150, R(a) [AC] = 0.9479, R(b) [AG] = 2.1801, R (c) [AT] = 0.3523, R(d) [CG] = 0.4914, R(e) [CT] = 2.2558, R(f) [GT] = 1.0000, gamma shape = 0.415) to construct the phylogenetic tree, respectively. One thousand bootstrap replicates were undertaken to obtain node support.

2.4 Morphological analyses

Fifteen morphological characters for 108 taxa from 42 genera representing the family Lauraceae were obtained from the *Flora of China* (Li et al., 2008b), herbarium specimens, and available published reports (Table S4). The following morphological characters were coded: (1) habit; (2) living type; (3) leaf arrangement; (4) leaf venation; (5) flower sex; (6) inflorescence type; (7) inflorescence location; (8) tepal number; (9) stamen number; (10) anther locus number; (11) ovary position; (12) tree height; (13) lateral vein number; (14) leaf length; and (15) leaf width (Table 2). A matrix of 15 morphological traits was constructed for the taxa investigated, with five taxa substituting for the closely related *Aspidostemon parvifolium* (Scott-Elliott) van der Werff (*A.* sp.), *Licaria caribaea* Gomez-Laurito & Cascante (*L. chrysophylla* (Meisn.) Kosterm.), *Mezilaurus introrsa* F.M. Alves & van der Werff (*M. triunca* Werff), *Ocotea ambrensis* Werff (*O.* sp.), *Pleurothyrium bilocellatum* Werff (*Pl. cuneifolium* Nees), and *Potameia micrantha* Werff (*Po. microphylla* Kosterm.) (Table S5).

To assess whether the morphological traits were phylogenetically conserved at the level of the whole phylogeny, we calculated Blomberg's *K*-values (Garland et al., 1992) and mean Pagel's lambda (λ) (Pagel, 1999) at the taxa level for each trait, thus obtaining phylogenetic information. Both indices assume the classic Brownian motion (BM) evolutionary model, with values varying from zero to higher than one for *K* or λ. *K*-values close to zero indicate the phylogenetic signal is weaker than expected from the BM model of character evolution (low levels of phylogenetic character conservation). *K*-values close to or higher than one indicate strong phylogenetic signal (Molina-Venegas & Rodríguez, 2017). λ-Values close to zero indicate there is no phylogenetic signal (the traits have evolved independently of phylogeny, and the traits of close relatives are not more similar than those of distant relatives), and λ-values close to or higher than one indicate trait evolution according to BM. The significance of phylogenetic signals was determined by shuffling species' character values (999 times) across the tips of the phylogenetic tree and comparing the resulting *K*-values to those computed from the observed character data (Eichenberg et al., 2015), whereas the statistical significance of λ was assessed based on a comparison with the likelihood of a model that assumes complete phylogenetic independence (Pagel, 1999). The ML tree based on the complete chloroplast genome sequences of 90 species plus nine barcodes from 19 additional species provided the standard tree topology. Phylogenetic signal analyses were carried out using the routines provided in the picante package available for R (Kembel et al., 2010).

3 Results

3.1 Plastome variation and phylogenetic placement of Lauraceae among magnoliids

Complete plastid genomes of 43 Lauraceae taxa were newly determined in the present study. All of these assembled into single circular genomes presenting a typical quadripartite structure, including one LSC with 88 984 (*Cryptocarya chingii* W. C. Cheng) – 93 843 bp (*Neolitsea oblongifolia* Merr. & Chun), one SSC with 17 778 (*Caryodaphnopsis tonkinensis* (Lecomte) Airy Shaw) – 19 234 bp (*Cryptocarya densiflora* Blume), and a pair of IR with 19 695 (*Caryodaphnopsis tonkinensis*) – 25 522 bp (*Endiandra dolichocarpa* S. K. Lee & Y. T. Wei) (Table 1). Across all the 43 taxa of Lauraceae, there was 1.07-fold variation in the sizes of plastomes, ranging from a minimum of 148 829 bp in *Caryodaphnopsis*

Table 2 Trait coding for morphological analysis of Lauraceae

No.	Traits	Trait states				
1	Habit	Evergreen (0)	Deciduous (1)			
2	Living type	Autotrophic tree (0)	Parasitic vine (1)			
3	Leaf arrangement	Leafless (0)	Alternate (1)	Alternate or opposite (2)	Opposite (3)	Subverticillate (4)
4	Leaf venation	Leafless (0)	Trinerved (1)	Triplinerved (2)	Penninerved (3)	
5	Flower sex	Bisexual (0)	Unisexual (1)			
6	Inflorescence type	Pseudo-umbel (1)	Thyrse (2)	Thyrse or fascicle (3)	Raceme rather a spike (4)	Panicle (5)
7	Inflorescence location	Indeterminate (0)	Axillary (1)	Axillary and terminal (2)	Terminal or subterminal (3)	
8	Tepal number	6 (1)	4 (2)	>6 (3)		
9	Stamen number	3× (1)	3/2× (2)	2× (3)		
10	Anther locus number	4 (1)	2 (2)			
11	Ovary position	Superior (1)	Semi-inferior (2)	Inferior (3)		
12	Tree height (m)					
13	Lateral vein number					
14	Leaf length (cm)					
15	Leaf width (cm)					

tonkinensis to a maximum of 158 610 bp in *Endiandra dolichocarpa* (Table 1). The plastome of *Endiandra dolichocarpa* encoded a set of 130 genes, of which 85 are protein-coding genes, 37 are transfer RNA genes, and eight are rRNA genes, and the plastome of *Caryodaphnopsis tonkinensis* encoded a set of 127 genes, of which 83 are protein-coding genes, 36 are transfer RNA genes, and eight are rRNA genes (Fig. 1).

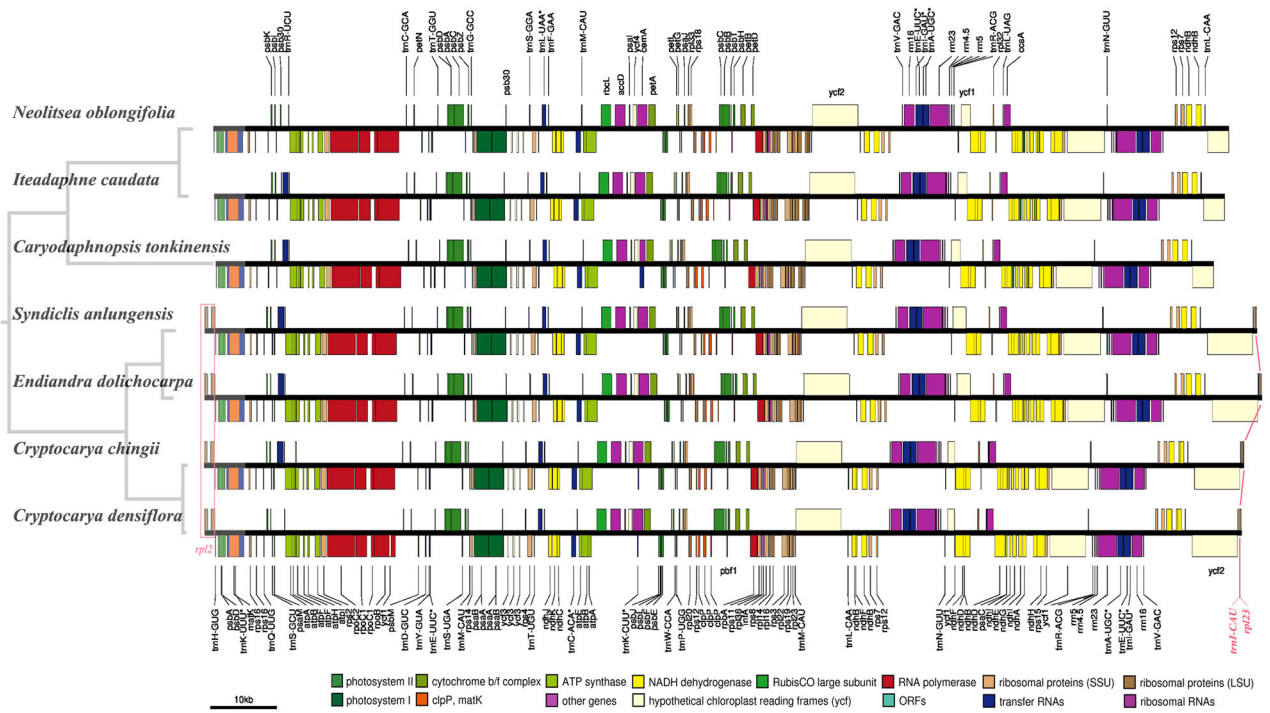

Fig. 1. Gene maps of *Neolitsea oblongifolia*, *Iteadaphne caudata*, *Caryodaphnopsis tonkinensis*, *Syndiclis anlungensis*, *Endiandra dolichocarpa*, *Cryptocarya chingii*, and *Cryptocarya densiflora* plastomes.

Both ML and BI analyses of the complete plastome sequences fully resolved phylogenetic relationships among the major clades and most genera, and most resolved relationships had high internal support (Figs. 2, S1). The BI trees constructed with the five subsets of the genomes were largely consistent with the topologies of the ML analyses of complete plastomes, with differences only in clades that are weakly supported at the species level, such as the locations of *Actinodaphne pilosa* (Lour.) Merr., *Beilschmiedia turbinata* Bing Liu & Y. Yang, *Lin. benzoin* (L.) Blume, and *Persea borbonia* (L.) Spreng. (Fig. S2). In the ML and BI trees of complete plastomes (Figs. 2, S1), the Lauraceae were strongly supported as monophyletic (ML-BS = 100%, BI–posterior probability [PP] = 1.00), sisterhood of Lauraceae (C01) and a clade containing Hernandiaceae (C02) and Monimiaceae (C03) was highly supported (ML-BS = 100%, BI-PP = 1.00), another clade containing Atherospermataceae (C04) and Gomortegaceae (C05) was the next sister group (ML-BS = 100%, BI-PP = 1.00), followed by Calycanthaceae (C06) (ML-BS = 100%, BI-PP = 1.00). All of these groups belong to the order Laurales. Two main clades (ML-BS = 100%, BI-PP = 1.00), corresponding to the orders Laurales (C01-C06) + Magnoliales (C07) and Canellales (C09) + Piperales (C08), made up the magnoliids (Figs. 2, S1). The sisterhood of the Laurales and Magnoliales, with a clade containing Piperales and Canellales being the next sister group, followed by Chloranthales (C10), was highly supported. In the Lauraceae (Figs. 2, S1), the first group (ML-BS = 100%, BI-PP = 1.00) included species of *Beilschmiedia*, *Cryptocarya*, *Endiandra* R. Br., *Eusideroxylon* Teijsm. & Binn., and *Syndiclis* Hook. f. (S02), the second group (ML-BS = 100%, BI-PP = 1.00) included species of *Cassytha* (S03), the third group (ML-BS = 100%, BI-PP = 1.00) included species of *Neocinnamomum* (S04), the fourth group (ML-BS = 100%, BI-PP = 1.00) included species of *Caryodaphnopsis* Airy Shaw (S05), whereas the core group (ML-BS = 100%, BI-PP = 1.00) included three clades with species of *Alseodaphne* Nees, *Dehaasia* Blume, *Machilus*, *Nothaphoebe* Blume, *Persea*, and *Phoebe* (S07) in the first clade (ML-BS = 100%, BI-PP = 1.00), species of *Cinnamomum*, *Nectandra* Rol. ex Rottb., and *Sassafras* (S08) in the second clade (ML-BS = 100%, BI-PP = 1.00), and species of *Actinodaphne*, *Iteadaphne* Blume, *Laurus* L., *Lindera*, *Litsea*, *Neolitsea*, and *Parasassafras* D.G. Long (S09) in the third clade (ML-BS = 100%, BI-PP = 1.00).

3.2 Phylogenetic relationships among the Lauraceae genera

To better understand the phylogenetic relationships between the 89 sequenced taxa from 25 genera and the other genera with reported barcoding data in the Lauraceae, we downloaded available sequences from GenBank, including *matK*, *psbA-trnH*, *rbcL*, *rpl16*, *rpoB*, *rpoC1*, *trnT-trnL*, *trnL*, and *trnL-trnF* of 19 Lauraceae species (Table S2). Lauraceae was divided into nine clades: a *Hypodaphnis* clade (S01), a *Beilschmiedia–Cryptocarya* clade (BI-PP = 1.00, ML-BS = 100%) (S02), a *Cassytha* clade (BI-PP = 1.00, ML-BS = 100%) (S03), a *Neocinnamomum* clade (BI-PP = 1.00, ML-BS = 100%) (S04), a *Caryodaphnopsis* clade (BI-PP = 1.00, ML-BS = 100%) (S05), a *Chlorocardium–Mezilaurus* clade (BI-PP = 1.00, ML-BS = 100%) (S06), a *Machilus–Persea* clade (BI-PP = 1.00, ML-BS = 75%) (S07), a *Cinnamomum–Ocotea* clade (BI-PP = 1.00, ML-BS = 96%) (S08), and a *Laurus–Neolitsea* clade (BI-PP = 1.00, ML-BS = 79%) (S09) (Fig. 3). The *Laurus–Neolitsea* clade was further divided into six main subclades. Subclade I (BI-PP = 0.78, ML-BS = 43%) includes *Lin. communis* Hemsl., *Lin. glauca*, *Lin. megaphylla* Hand.-Mazz., *Lin. nacusua* Nees, plus *Laurus nobilis*, *Lit. glutinosa*, *Lit. magnoliifolia* Yen C. Yang & P.H. Huang, *Lit. monopetala* Roxb., and *Lit. tsilingensis* Y.C. Yang & P.H. Huang. Subclade II (BI-PP = 1.00, ML-BS = 97%) includes *Lin. obtusiloba*, *Lit. cubeba*, *Lit. panamonja* Hook.f., and *Lit. pierrei* Lecomte. Subclade III includes only *Parasassafras confertiflorum* (Meisn.) D.G. Long. Subclade IV (BI-PP = 0.99, ML-BS = 53%) includes *Lin. benzoin* Meisn., *Lin. latifolia* Hook.f., *Lin. metcalfiana* C.K. Allen, and *Lin. robusta* (C.K. Allen) H.P. Tsui. Subclade V (BI-PP = 1.00, ML-BS = 66%) includes *Iteadaphne caudata* (Nees) H.W. Li, *Lin. aggregata* (Sims) Kosterm., *Lin. chunii* Merr., *Lin. limprichtii* H. Winkler, and *Lin. pulcherrima* (Nees) Benth. Subclade VI (BI-PP = 0.98, ML-BS = 43%) includes *Actinodaphne cupularis* Gamble, *Actinodaphne pilosa* (Lour.) Merr., *Actinodaphne trichocarpa* C.K. Allen, *Neolitsea chui* Merr., *Neolitsea oblongifolia*, and *Neolitsea sericea* (Blume) Koidz.

3.3 Influence of plant phylogeny on morphological traits

Of the acquirable 15 morphological traits examined, 10 traits were phylogenetically conservative as indicated by Blomberg's *K*-values and Pagel's λ-values (Table 3), respectively. *K*-values of discrete characters were all significant: inflorescence type ($K = 20.710$), living type ($K = 4.435$), ovary position ($K = 2.889$), flower sex ($K = 1.378$), leaf venation ($K = 0.875$), leaf arrangement ($K = 0.346$), habit ($K = 0.334$), and inflorescence location ($K = 0.296$) showed intermediate phylogenetic signals. λ-Values of discrete characters except habit were all significant and from high to low values were: living type ($\lambda = 8.099$), flower sex ($\lambda = 7.197$), inflorescence type ($\lambda = 1.000$), ovary position ($\lambda = 0.950$), leaf venation ($\lambda = 0.946$), leaf arrangement ($\lambda = 0.942$), and inflorescence location ($\lambda = 0.691$) showed intermediate phylogenetic signals. For quantitative characters, stamen number ($K = 0.765$, $\lambda = 1.000$), lateral vein number ($K = 0.499$, $\lambda = 0.879$), anther locus number ($K = 0.402$, $\lambda = 0.691$), and tree height ($K = 0.315$, $\lambda = 0.728$) showed a relatively strong phylogenetic signal, whereas neither leaf length nor leaf width showed a significant phylogenetical signal in either method.

4 Discussion

This study included 120 plastid genomes for plants from eight orders, namely Amborellales, Austrobaileyales, Canellales, Chloranthales, Laurales, Magnoliales, Nymphaeales, and Piperales. All of these complete plastome sequences of Lauraceae and related families yielded a fully resolved tree, consistent with the Angiosperm Phylogeny Group's most recent phylogeny, APG IV (Byng et al., 2016). In Laurales, six of seven families (lack of sequence data from Siparunaceae), including Atherospermataceae, Calycanthaceae, Gomortegaceae, Hernandiaceae, Lauraceae, and Monimiaceae, were recognized. These relationships differ from those reported by Renner (1999), Zanis et al. (2002), and Massoni et al. (2014). The topology here provides a 100% supported sisterhood between Monimiaceae and Hernandiaceae, but not between

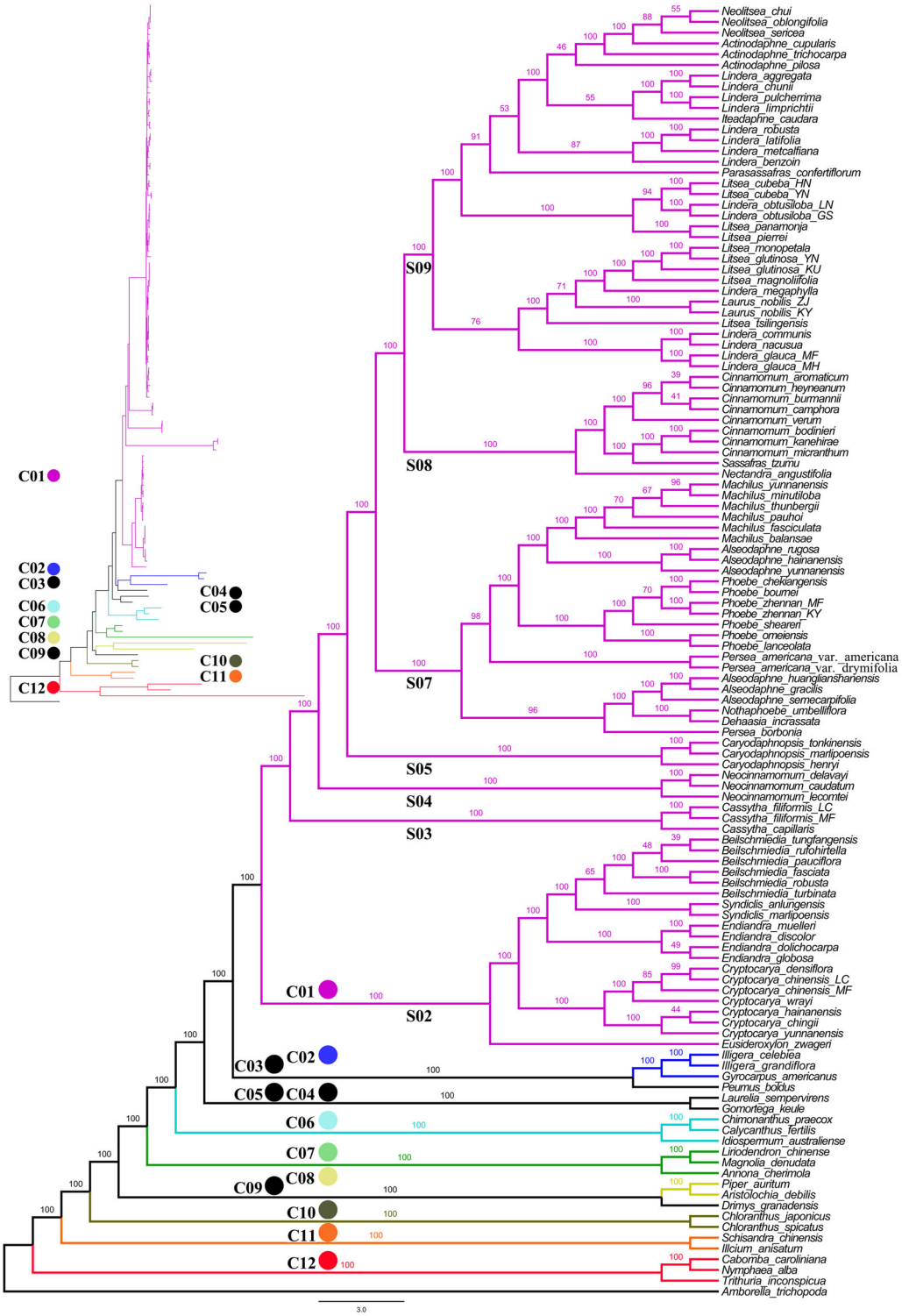

Fig. 2. Molecular phylogenetic tree of 98 taxa of Laurales and 14 taxa of related angiosperms based on plastome sequences using unpartitioned maximum likelihood. Numbers at each node are bootstrap support values. Different branches are marked as C01 (Lauraceae clade), C02 (Hernandiaceae clade), C03 (Monimiaceae clade), C04 (Atherospermataceae clade), C05 (Gomortegaceae clade), C06 (Calycanthaceae clade), C07 (Magnoliales clade), C08 (Piperales clade), C09 (Canellales clade), C10 (Chloranthales clade), C11 (Austrobaileyales clade), C12 (Nymphaeales clade), S02 (*Beilschmiedia–Cryptocarya* subclade), S03 (*Cassytha* subclade), S04 (*Neocinnamomum* subclade), S05 (*Caryodaphnopsis* subclade), S07 (*Machilus–Persea* subclade), S08 (*Cinnamomum–Ocotea* subclade), and S09 (the *Laurus–Neolitsea* subclade). The tree is rooted with the plastome sequence of *Amborella trichopoda*.

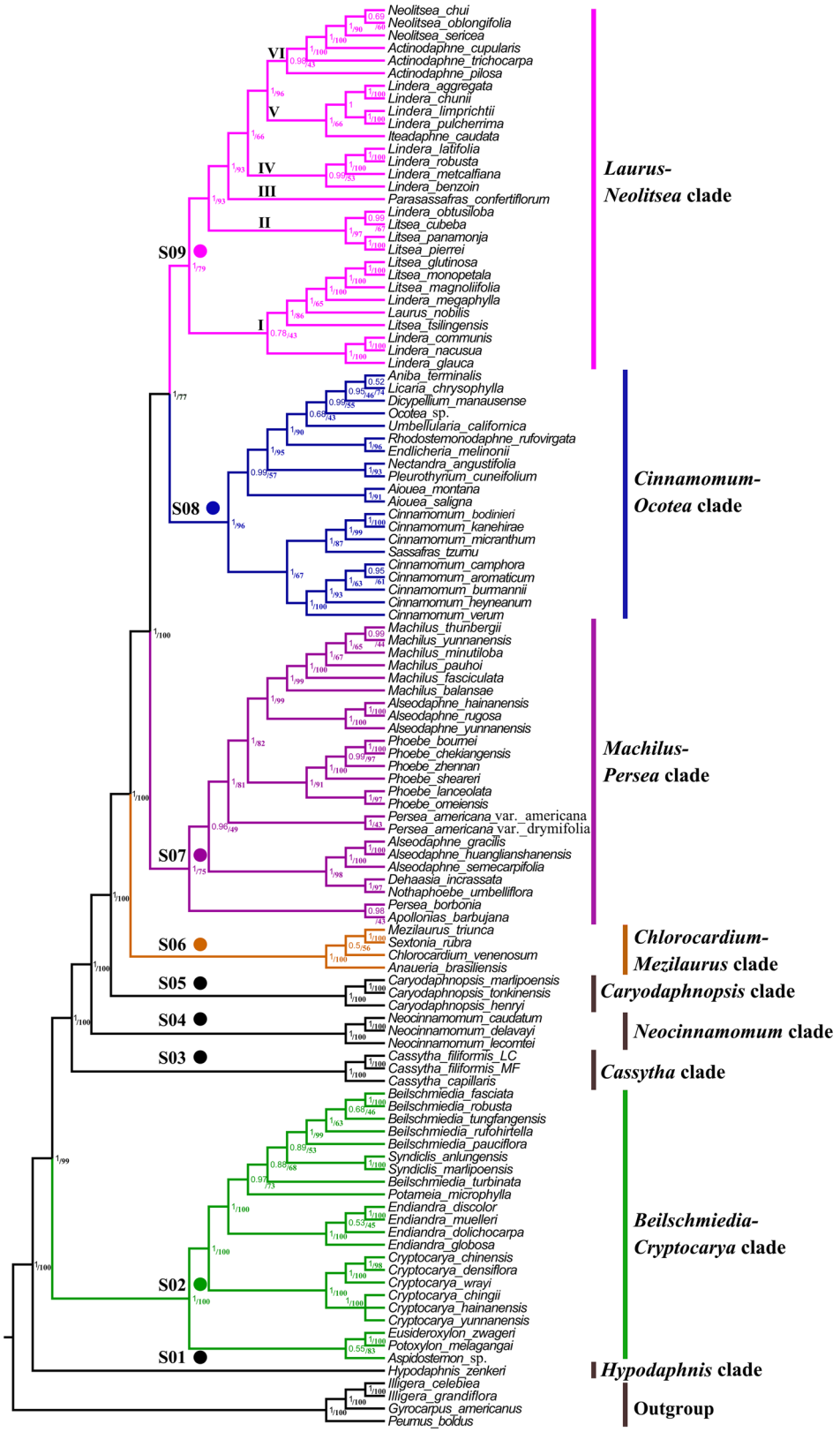

Fig. 3. Continued

Table 3 Eleven of 15 traits of Lauraceae (Nos. 3–6 and 9–15) were congruent with molecular phylogenies

No.	Trait	Blomb (*K*)	Blomb_*P*	Lambda (λ)	Lambda_*P*
1	Habit	0.3336446	0.00049995	2.674714e+00	0.00126
2	Living type	4.4353137	0.00009999	8.098694e+00	0.00009
3	Leaf arrangement	0.3463675	0.00009999	9.417512e−01	0.00000
4	Leaf venation	0.8749521	0.00009999	9.457564e−01	0.00000
5	Flower sex	1.3777338	0.00009999	7.196653e+00	0.00000
6	Inflorescence type	20.7100400	0.00009999	1.000000e+00	0.00000
7	Inflorescence location	0.2958475	0.00009999	6.908090e−01	0.00001
8	Tepal number	0.3188790	0.00039996	1.000000e+00	0.00009
9	Stamen number	0.7652453	0.00009999	1.000000e+00	0.00000
10	Anther locus number	0.4022628	0.00009999	6.908090e−01	0.00000
11	Ovary position	2.8886225	0.00009999	9.504732e−01	0.00000
12	Tree height	0.3151265	0.00009999	7.275807e−01	0.00000
13	Lateral vein number	0.4968533	0.00009999	8.786124e−01	0.00000
14	Leaf length	0.1834140	0.06209379	3.245943e−01	0.06195
15	Leaf width	0.1942557	0.12838716	2.614485e−07	0.50000

Monimiaceae and Lauraceae (Fig. 2), indicating the need for complete plastid genome sequences instead of a small numbers of chloroplast markers.

Our plastid phylogenomic analysis further confirms the monophyly of the Lauraceae, in agreement with previously published phylogenetic studies (Chanderbali et al., 2001; Rohwer & Rudolph, 2005). The topology obtained here for the first time shows that plastomes, with appropriate sampling, can provide robust and significantly supported relationships among deep lineages of Lauraceae. Nine such phylogenetically meaningful clades were identified among the deep lineages of the Lauraceae. These lineages are consistent with a recent structural comparison showing that different clades of Lauraceae have apparently experienced different loss events (Song et al., 2017b). The length difference between the plastomes mainly results from the presence of two copies of *rpl2*, *rpl23*, and *trnI*-CAU in the plastome of the *Beilschmiedia–Cryptocarya* clade, whereas only one copy of each of the three genes is present in the sequenced species of the *Cassytha*, *Neocinnamomum*, *Machilus–Persea*, *Cinnamomum–Ocotea*, and *Laurus–Neolitsea* clades (Fig. 1). The plastomes of *Calycanthus* (Calycanthaceae, Laurales) have lost *rpl2* in the IRb region, but the plastome of *Caryodaphnopsis henryi* Airy Shaw (Lauraceae) remains intact, as do those of the non-Laurales magnoliid genera *Piper* L., *Liriodendron* L., and *Magnolia* L. and the early diverging angiosperm orders Amborellales, Nymphaeales, Austrobaileyales, and Chloranthales (Song et al., 2017b). However, there are no more sequence data available from species of the *Chlorocardium–Mezilaurus* clade or *Hypodaphnis zenkeri* Stapf; complete plastid genomes would be ideal. Including more taxa of the *Chlorocardium–Mezilaurus* clade, and particularly a complete plastid sequence of the earliest divergent genus *Hypodaphnis*, would help integrate our understanding of plastid genome structural evolution in Lauraceae.

The backbones of the phylogenomic topologies obtained here are consistent with previously published phylogenetic relationships (Chanderbali et al., 2001), but problems within several major clades in the Lauraceae were solved. In the *Beilschmiedia–Cryptocarya* clade, previous phylogenetic analyses with plastid sequence *trnK* found that members of *Endiandra* were sister to *Beilschmiedia tawa* Kirk (Rohwer & Rudolph, 2005). However, our phylogenomic analysis shows sisterhood of a group containing six *Beilschmiedia* species and two *Syndiclis* species, and another group containing four *Endiandra* species (Figs. 2, S1), with strong support, as in a previously published phylogenetic tree constructed with a combination of plastid markers *trnL-trnF*, *psbA-trnH*, and *rpl16* and the nuclear barcoding marker ITS (Liu et al., 2013). In the *Cinnamomum–Ocotea* clade, *Sassafras tzumu* (Hemsl.) Hemsl. has previously been retrieved as the sister to the Cinnamomeae group, likewise without significant support in their cpDNA or ITS data (Rohwer & Rudolph, 2005; Nie et al., 2007; Rohde et al., 2017), and to *Cinnamomum bodinieri* H. Lév. plus *C. glanduliferum* (Wall.) Nees (BI-PP = 1.00, ML-BS = 75%) in the cpDNA data (*psbA-trnH* and *trnG-trnS* spacers) of Rohde et al. (2017). Our phylogenomic analysis shows *S. tzumu* is nested among the members of *Cinnamomum* (Figs. 2, 3), which is compatible with the chloroplast data in Rohde et al. (2017). More importantly, we further resolve the backbone of the phylogeny of the *Laurus–Neolitsea* clade (Figs. 2, 3), previously called the core Laureae in the sense of Chanderbali et al (2001), through the sequencing and analysis of plastid genomes. The clade is discussed in further detail below.

Fig. 3. Molecular phylogenetic tree of 108 taxa of Lauraceae and four related taxa of Laurales based on plastome sequences using Bayesian inference and unpartitioned maximum likelihood. Numbers at each node are Bayesian posterior probabilities/maximum likelihood bootstrap support values. Different branches are marked as S01 (*Hypodaphnis* clade), S02 (*Beilschmiedia–Cryptocarya* subclade), S03 (*Cassytha* subclade), S04 (*Neocinnamomum* subclade), S05 (*Caryodaphnopsis* subclade), S06 (*Chlorocardium–Mezilaurus* subclade), S07 (*Machilus–Persea* subclade), S08 (*Cinnamomum–Ocotea* subclade), and S09 (*Laurus–Neolitsea* subclade). The tree is rooted with the plastome sequences of *Illigera celebica*, *Illigera grandiflora*, *Gyrocarpus americanus*, and *Peumus boldus*.

4.1 *Laurus–Neolitsea* clade

Previous Sanger markers provided limited information to resolve the relationships among *Actinodaphne*, *Adenodaphne* S. Moore, *Laurus*, *Lindera*, *Litsea*, *Neolitsea*, *Parasassafras*, and *Sassafras*. The studies attempting to resolve the relationships within the *Laurus–Neolitsea* clade used a maximum of five genetic markers of ITS and external transcribed spacer (ETS), or plastid regions *matK*, *psbA-trnH*, and *trnL-trnF*, with little success (Rohwer, 2000; Chanderbali et al., 2001; Li et al., 2004, 2007, 2008b). Here, we have improved the phylogenetic resolution both near the tips and along the backbone. Within the *Laurus–Neolitsea* clade, our phylogenetic analyses yielded support for four of six subclades (Figs. 2, 3) among these taxa with umbellate inflorescences (or compound inflorescences consisting of umbels) (Tables 2, S5). Four *Litsea* species, *Lit. glutinosa*, *Lit. monopetala*, *Lit. magnoliifolia*, and *Lit. tsilingensis*, as well as *Laurus nobilis*, were located in subclade I with four *Lindera* species, *Lin. communis*, *Lin. glauca*, *Lin. megaphylla*, and *Lin. nacusua*, whereas *Lin. obtusiloba* was located in subclade II with three *Litsea* species, *Lit. cubeba*, *Lit. panamonja*, and *Lit. pierrei*, which is in agreement with a previous phylogenetic result by Fijridiyanto & Murakami (2009), who defined the relationships among seven *Lindera* species and 19 *Litsea* species. In subclade III, the previously reported phylogenetic positions of *Parasassafras confertiflorum* varied from sister to a clade consisting of four taxa, *Adenodaphne uniflora* (Guillaum.) Kosterm., *Iteadaphne* sp., cf. *Lit. elongata* Benth. & Hook.f., and *Lit. coreana* H.Lév. (Chanderbali et al., 2001), to *Lin. erythrocarpa* Makino (Chanderbali et al., 2001), to *Sinosassafras flavinervia* (C.K. Allen) H.W. Li and a clade consisting of *Lin. fruticosa* Hemsl., *Lin. reflexa* Hemsl., and *Lit. umbellata* Thunb, albeit without support (Li et al., 2004), or to *Laurus nobilis* (Nie et al., 2007). However, our phylogenomic analysis retrieves an independent subclade of *P. confertiflorum*, as in a previously published phylogenetic tree constructed with a combination of nuclear barcoding marker ITS and ETS (Li et al., 2008b). Subclade IV contains four *Lindera* species, *Lin. benzoin*, *Lin. latifolia*, *Lin. metcalfiana*, and *Lin. robusta*, with long-pedunculate, racemiform-arranged inflorescences, and well-developed terminal buds on shortened brachyblasts. The other four *Lindera* species and *Iteadaphne caudata* were found to form the strongly supported subclade V. All of these five species share trinerved leaves and well-developed terminal buds on shortened brachyblasts (Tian et al., 2019). In subclade VI, species of *Actinodaphne* and *Neolitsea* and several members of the other clades are all dioecious plants with the fruit seated on a disciform or discoid perianth tube. However, *Actinodaphne* proved to be paraphyletic and the generic delineation between *Actinodaphne* and *Neolitsea* remains unresolved, as in Li et al. (2007).

4.2 *Cinnamomum–Ocotea* clade

Using the nuclear marker ITS, the first molecular phylogenetic analyses indicated that the American genera *Aiouea* Aubl., *Aniba* Aubl., *Dicypellium* Nees & Mart., *Endlicheria* Nees, *Kubitzkia* van der Werff, *Licaria* Aubl., *Mocinnodaphne* Lorea-Hern., *Nectandra*, *Paraia* Rohwer, H.G. Richt. & van der Werff, *Pleurothyrium* Nees, *Rhodostemonodaphne* Rohwer & Kubitzki, *Umbellularia* (Nees) Nutt., and *Urbanodendron* Mez, along with American and African *Ocotea* Aubl. species, formed a weakly supported clade with *Cinnamomum* and *Sassafras* species from Asia and America (Chanderbali et al., 2001). This was further supported by phylogenetic analysis of the matrices of three nuclear regions (Huang et al., 2016), two plastid sequences (Rohde et al., 2017), and ITS plus different plastid markers (Trofimov et al., 2016; Rohde et al., 2017), implying that the *Cinnamomum–Ocotea* clade is monophyletic. In our phylogenetic analysis, this clade is 100% supported in the bootstrap analysis and the PP is 1.00 (Figs. 2, 3). This clade shares the same inflorescence type (Tables 2, S4) as the *Persea–Machilus* clade (below), but the cupules are usually well-developed and enlarged (Fig. S3), and the fruits are sometimes partly enclosed by the bowl- or cup-shaped cupules. Many taxa of the Ocotea complex have shallow cupules. Sometimes they are plate-like (e.g., in *Ocotea floribunda* (Sw.) Mez), and sometimes the berry is sitting free on a swollen pedicel (e.g., in *O. minarum* (Nees & C. Mart) Mez). *Sassafras* is nested within the Asian members of *Cinnamomum*, whereas the American members (*Cinnamomum triplinerve* (Ruiz & Pav.) Kosterm. = *Aiouea montana*) are closely related to *Aiouea* Aubl., to which they have been recently transferred (Rohde et al., 2017). *Cinnamomum aromaticum* Nees, *C. burmanni* Roxb., *C. heyneanum* Nees, and *C. verum* J. Presl belong to *C.* sect. *Cinnamomum*, which is characterized by opposite and triplinerved leaves, whereas *C. bodinieri*, *C. micranthum* (Hayata) Hayata and *C. kanehirae* Hayata belong to *C.* sect. *Camphora*, which has alternate and usually penninerved leaves (Tables 2, S4). It is strange that *Cinnamomum camphora* (L.) J. Presl is placed among the species of sect. *Cinnamomum* here, but it is consistent with the plastid dataset in Rohde et al. (2017). Our results indicate that *C.* sect. *Camphora* is more closely related to *Sassafras*, and that the non-monophyletic genus *Cinnamomum* also needs further studies based on intensive sampling.

4.3 *Machilus–Persea* clade

This clade, previously called the *Persea* group, includes at least eight genera, *Alseodaphne*, *Apollonias* Nees, *Dehaasia*, *Machilus*, *Nothaphoebe*, *Persea*, *Phoebe*, and the new *Alseodaphnopsis* (Mo et al., 2017). To distinguish these species and reconstruct their phylogenetic relationships, five molecular phylogenetic analyses have confirmed the monophyly of the *Machilus–Persea* clade based on two variable regions from nuclear markers ITS and *LEAFY* intron II and several barcodes from plastids mentioned before (Rohwer et al., 2009; Li et al., 2011). In our phylogenomic analyses using complete plastomes, this clade is 100% supported in the bootstrap analysis and the PP is 1.00 (Figs. 2, 3), and it is also supported by shared morphological characters, such as penninerved venation, thyrsoid inflorescences (consisting of cymes whose lateral flowers are opposite (van der Werff & Richter, 1996)), and undeveloped cupules (sometimes pedicels enlarged) (Tables 2, S4; Fig. S3). Our results confirm the recognition of *Alseodaphnopsis* (including *Alseodaphne hainanensis* Merr., *A. rugosa* Merr. & Chun, and *A. yunnanensis* Kosterm.) as a distinct genus and further support a sister relationship between the genera *Alseodaphnopsis* and *Machilus*. Both genera include species with 4-locular stamens, perulate terminal buds, and persistent perianth lobes in young fruit. However, 2-locular stamens are found in all species of *Dehaasia* and some species of *Persea*. *Persea americana* Mill. belongs to *P.* subg.

Persea, whereas *P. borbonia* Spreng. belongs to *P.* subg. *Eriodaphne*. The main difference between the subgenera *Persea* and *Eriodaphne* is that the tepals are subequal in subgen. *Persea*, and only remnants of their base persist in fruit, whereas they are strongly unequal and persistent in fruit in subgen. *Eriodaphne*. Our results also indicate that *P. borbonia*, rather than *P. americana*, is more closely related to *Apollonias*, which is consistent with the result of Rohwer et al. (2009), in contrast to Li et al. (2011), and that the non-monophyletic genus *Persea* needs further studies based on intensive sampling.

4.4 *Chlorocardium–Mezilaurus* clade

As expected, the small group of South American genera *Sextonia* van der Werff, *Mezilaurus* Kuntze ex Taub., *Chlorocardium* Rohwer, H. G. Richt. & van der Werff, and *Anaueria* Kosterm. is further supported by our phylogenetic analysis of the data matrix combining plastid sequences. This clade is 100% supported in the bootstrap analysis and PP is 1.00 (Fig. 3). *Williamodendron* Kubitzki & H.G. Richt. was shown to be included in this clade (Chanderbali et al., 2001), but is absent from our study because of the lack of plastid sequences in GenBank (only two short sequences). It has been reported that the taxa of *Anaueria* and *Chlorocardium* share opposite leaves (Tables 2, S4), whereas *Sextonia*, *Williamodendron*, and *Mezilaurus* share clustered leaves (Chanderbali et al., 2001). In the *Chlorocardium–Mezilaurus* clade there is some kind of a cupule, sometimes small as in *Mezilaurus*, sometimes large as in *Chlorocardium* and *Sextonia*. The cupules are not found in species of the *Machilus–Persea* clade, which supports the independent relationship between the *Chlorocardium–Mezilaurus* and *Machilus–Persea* clades in our results.

4.5 *Cassytha*, *Neocinnamomum*, and *Caryodaphnopsis* clades

For the relationship among *Caryodaphnopsis*, *Cassytha*, and *Neocinnamomum*, previous phylogenetic analysis with different markers reported that the parasitic genus *Cassytha* was sub-basal (Rohwer, 2000), *Cassytha* was sister to the genus *Neocinnamomum* (Chanderbali et al., 2001; Wang et al., 2010), and both *Neocinnamomum* and *Caryodaphnopsis* were located in the same clade (Rohwer & Rudolph, 2005). In our phylogenomic analysis, however, the highly supported relationship was sisterhood of the core group (*Chlorocardium–Mezilaurus* clade, *Machilus–Persea* clade, *Cinnamomum–Ocotea* clade, and *Laurus–Neolitsea* clade) and the genus *Caryodaphnopsis*, with *Neocinnamomum* being the next sister group, followed by *Cassytha* (Figs. 2, 3). This independent relationship among the three genera is entirely different from the study using plastid sequences *psbA-trnH* and *trnK* and nuclear barcoding marker ITS with low support (59%–85%) (Wang et al., 2010), but congruent with the present study using nuclear barcoding markers ITS, *LEAFY*, and *RPB2* with high support (92%–100%) (Li et al., 2016) and the recent comparison based on 47 Lauraceae plastid genomes with strong support (100%) (Song et al., 2017a), indicating that the three monophyletic groups *Caryodaphnopsis*, *Cassytha*, and *Neocinnamomum* should exist, and the evidence from complete plastid genomes is necessary. Morphologically, the distinct characters of the three genera, including the parasitic habit and reduced leaves in *Cassytha*, alternate, triplinerved leaves and enlarged cupules in *Neocinnamomum*, and opposite, triplinerved leaves and unequal tepals in *Caryodaphnopsis* (Tables 2, S4; Fig. S3), support the conclusion that they are isolated, early diverging clades within the Lauraceae.

4.6 *Beilschmiedia–Cryptocarya* clade

The *Beilschmiedia–Cryptocarya* clade was well supported in previously published molecular phylogenetic analyses of plastid *matK* sequences (Rohwer, 2000), concatenated sequences of *trnL-trnF*, *psbA-trnH*, *trnT-trnL*, *rpl16*, and 26S rDNA (Chanderbali et al., 2001), and nuclear ITS sequences (Liu et al., 2017). In our study, the clade uniting eight genera, *Aspidostemon* Rohwer & H.G. Richt., *Potoxylon* Kosterm., *Eusideroxylon*, *Cryptocarya*, *Endiandra*, *Potameia* Thouars, *Syndiclis*, and *Beilschmiedia*, is 100% supported in the bootstrap analysis and had a PP of 1.00 (Figs. 2, S1). Including only species with complete genome sequences, the same clade uniting *Eusideroxylon*, *Cryptocarya*, *Endiandra*, *Syndiclis*, and *Beilschmiedia* is also 100% supported in the bootstrap analysis and the PP is 1.00 (Fig. 3). The close relationship among these genera is supported by morphological similarities, such as paniculate inflorescences (consisting of raceme-like cymes whose lateral flowers are alternate (van der Werff & Richter, 1996)), and drupe-like fruit (Tables 2, S4; Fig. S3). Within the *Beilschmiedia–Cryptocarya* clade, the cupules are small or absent in *Beilschmiedia* and its allies (*Endiandra*, *Potameia*, and *Syndiclis*) but enlarged and enclosing the fruits in the rest of the genera (*Aspidostemon*, *Dahlgrenodendron* J.J.M. van der Merwe & A.E. van Wyk, *Potoxylon*, *Eusideroxylon*, and *Cryptocarya*).

4.7 *Hypodaphnis* clade

Previous molecular phylogenetic analyses based on different datasets, with only one *matK* fragment (Rohwer, 2000), two variable regions from plastid *trnL-trnF* and *psbA-trnH*, and multiple regions from nuclear 26S rDNA and from plastid *trnL-trnF*, *psbA-trnH*, *trnT-trnL*, and *rpl16* (Chanderbali et al., 2001), found that the monotypic African genus *Hypodaphnis* is a "jumping genus," appearing as a sister to all other Lauraceae or to the Cryptocaryeae in different analyses. In our analyses, in agreement with Rohwer & Rudolph (2005), *Hypodaphnis* is located outside the *Beilschmiedia–Cryptocarya* clade and as the basal branch within the Lauraceae by ML and BI methods, respectively (Fig. 3). Careful comparison of its characters with those of other Laurales has shown that there are many morphological and anatomical similarities, including wood structure (Richter, 1981), embryological features (Kimoto & Tobe, 2008), and the stomatal arrangement (Carpenter et al., 2007), between *Hypodaphnis* and the outgroup taxa of Hernandiaceae, rather than other species in the Lauraceae (Rohwer, 2000; Rohwer & Rudolph, 2005). Moreover, *Hypodaphnis* has a true inferior ovary, which is unique among all the members of Lauraceae.

4.8 Taxonomic implications

The most recent and commonly adopted suprageneric classification of Lauraceae was proposed by van der Werff & Richter (1996). Their work divided Lauraceae into two subfamilies (Cassythoideae and Lauroideae) and three tribes (Laureae, Perseeae, and Cryptocaryeae) based on

inflorescence structure and wood and bark anatomy. A quarter of a century later, a great deal of molecular and morphological evidence does not agree with these groupings. Our study represents a phylogeny that offers a well-resolved and strongly supported topology that provides insight into relationships among 44 genera within the family Lauraceae. This phylogenetic framework reported here provides the basis for a revised suprageneric classification, and it is an appropriate opportunity for us to divide Lauraceae into six tribes and to update the suprageneric classification of the Lauraceae.

4.9 Hypodaphnideae Kosterm. ex Reveal

Hypodaphnideae is endemic in West Africa, including a single species *Hypodaphnis zenkeri*. Evergreen tree or shrub. Leaves alternate; leaf blade papery, pinninerved. Inflorescences axillary, umbel-like panicle, bracts caducous, many flowered. Flowers small, bisexual, 3-merous, characterized by having the ovary in an inferior position. Perianth tube short, perianth segments 6, outer 3 slightly larger. Stamens 9, of third whorl, with basal glands, staminodes absent. Filaments finely and minutely pubescent along the sides, slightly longer than the anthers, anthers 4-celled. Fruit ovoid. Cupule encloses fruit.

4.10 Cryptocaryeae Nees

Evergreen trees or shrubs. Leaves opposite, subopposite, or alternate; leaf blade leathery, thickly leathery, or papery, usually pinninerved, rarely triplinerved. Inflorescences axillary, terminal, bracts small, not forming an involucre, characterized by having the panicle inflorescences, flowers usually small, bisexual, rarely unisexual, mostly 3-merous, occasionally 2-merous. Perianth tube short, perianth segments 6, rarely 4. Stamens 9, rarely 4, occasionally 8. Anthers mostly 2-celled, rarely 4-celled. Ovary superior, rarely part-inferior. Cupule enveloped or not enveloped fruit.

This tribe contains at least 13 genera: *Aspidostemon* (ca. 30 spp. in Madagascar), *Dahlgrenodendron* (at least one species in South Africa), *Eusideroxylon* (a single species in the Greater Sunda Islands), *Potoxylon* (a single species in the Greater Sunda Islands), *Cryptocarya* (ca. 350 spp. in tropical and subtropical regions of all continents), *Ravensara* Sonn. (10–20 spp. in Madagascar and Comoro Islands), *Endiandra* (ca. 100 spp. in Southeast Asia, eastern Australia, and the western Pacific Islands), *Triadodaphne Kosterm.* (at least one species in Papua New Guinea), *Potameia* (3–10 spp. in Madagascar), *Beilschmiedia* (ca. 250 spp. in tropical and subtropical regions of all continents), *Yasunia* van der Werff (at least two species in Peru and Ecuador, respectively), *Sinopora* J. Li, N.H. Xia & H.W. Li (a single species in China), and *Syndiclis* (10–20 spp. in India and Southeast Asia).

4.11 Cassytheae Dumortier

Cassytheae contains over 20 species occurring in tropical to subtropical regions of Australia, Africa, Asia, and America. Parasitic herb. Leaves reduced to minute scales. Inflorescence is always axillary, characterized by the spike, rarely a raceme, seldom a panicle. Flowers small, bisexual, 3-merous, inserted in stalked or stalkless scale-like bracts, each with 2 bracteoles adnate to perianth base. Perianth tube turbinate or ovoid, contracted on top after anthesis; perianth segments 6, outer 3 smaller. Stamens 12, staminodes 3, of innermost whorl, anthers 2-celled. Ovary nearly excluded in perianth tube when in flower, semi-inferior. Fruit included in dilated fleshy perianth tube, free; perianth tube with orifice and persistent lobes on top.

4.12 Neocinnamomeae Yu Song, W. B. Yu & Y. H. Tan trib. nov. Type: *Neocinnamomum* H. Liou

Neocinnamomeae includes approximately 7 species endemic to tropical Asia. Evergreen, semi-evergreen or deciduous shrubs and small trees. Leaves alternate; leaf blade papery or sub-leathery, strongly triplinerved. Inflorescences thyrse or fascicle, axillary or terminal or solitary in leaf axils. Bracts minute, rusty sericeous. Flowers small, pedicellate, bisexual, 3-merous. Perianth tube rather shallow; perianth segments 6, sub-equal. Stamens 9, of third whorl, with basal glands, the fourth whorl staminodal, anthers 4-celled. Ovary merging into a slightly shorter style with small peltate stigma, superior. Fruit ellipsoid or globose, seated on the shallow, fleshy, thickened, club-shaped large cup, which merges into a slender pedicel, the tepals enlarged, persistent, erect or patent.

4.13 Caryodaphnopsideae Yu Song, W. B. Yu & Y. H. Tan trib. nov. Type: *Caryodaphnopsis* Airy Shaw

Caryodaphnopsideae includes nearly 20 species with a disjunct tropical amphi-Pacific distribution. Evergreen shrubs or small to medium-sized trees. Leaves opposite or subopposite; leaf blade papery or sub-leathery, either trinerved, triplinerved, or pinninerved. Bracts minute. Inflorescences thyrse, axillary, shorter than the leaves; flowers small, bisexual, 3-merous, bracts and bracteoles minute. Perianth tubes very short or almost absent, perianth lobes 6, deciduous, outer ones sharply small. Fertile stamens 9, anthers 4-celled or occasionally all 2-celled, or 2-celled in first and second whorls and 4-celled in third whorl. Fruit shiny green, large, narrowly ellipsoid-globose or ellipsoid. Fruit stalk slightly thickened, dilated on top.

4.14 Laureae Maout & Decaisne

Trees or shrubs, evergreen or deciduous, dioecious or monoecious. Leaves alternate, subverticillate, rarely opposite; leaf blade leathery, thickly leathery, or papery, pinninerved or triplinerved. Inflorescences thyrse, sometimes pseudo-umbel, usually axillary, sometimes terminal or subterminal. Flowers small, bisexual or unisexual, usually 3-merous, sometimes 2-merous, having the ovary in a superior position. Perianth tube long or short; perianth segments usually 6, sometimes 4. Stamens 3–32, anthers 4-celled or 2-celled. Fruit berry, sometimes with persistent perianth parts at the base.

This tribe contains over 39 genera: *Cinnadenia* Kosterm. (two species in Southeast Asia), *Anaueria* (a single species in South America), *Chlorocardium* (two species in South America), *Mezilaurus* (10–20 spp. occurring from Costa Rica to the southeast of Brazil), *Williamodendron* (three species in South America), *Sextonia* (two species in South America), *Apollonias* (a single species in the Azores, Canary Islands, and Madeira), *Persea* (ca. 100 spp. in America and Macaronesian Islands), *Alseodaphne* (ca. 40 spp. in Asia), *Dehaasia* (ca. 50 spp. in Asia and islands of Borneo, and

New Guinea), *Nothaphoebe* (ca. 40 spp. in Asia), *Phoebe* (ca. 100 spp. in Asia), *Machilus* (ca. 100 spp. in Asia), *Cinnamomum* (ca. 250 spp. in Asia, Oceania, and Australasia), *Sassafras* (two species in Asia and one species in North America), *Aiouea* (60–100 spp. in North and South America), *Nectandra* (150–300 spp. in South America), *Pleurothyrium* (50–100 spp. in South America), *Umbellularia* (a single species in North America), *Rhodostemonodaphne* (ca. 40 spp. in America), *Endlicheria* (60–100 spp. in South America), *Ocotea* (ca. 400 spp. in tropical and subtropical areas of the Americas, Africa, the Caribbean and West Indies, Madagascar, and the Mascarene Islands), *Dicypellium* (two species in South America), *Aniba* (40–60 spp. in America and Caribbean Islands), *Licaria* (ca. 40 spp. in Central America and South America), *Mocinnodaphne* (a single species in North America and Central America), *Paraia* (a single species in southeastern Brazil), *Urbanodendron* (three species in Brazil), *Kubitzkia* (two species in South America), *Povedadaphne* W.C. Burger (a single species in Costa Rica), *Damburneya* Raf. (a single species in America), *Lindera* (ca. 100 spp. in Asia, three species in North America, and one species in eastern Australia), *Litsea* (100–200 spp. in tropical and subtropical areas of both hemispheres), *Laurus* (two species in Europe), *Parasassafras* (a single species in Asia), *Sinosassafras* H.W. Li (a single species in Asia), *Iteadaphne* (20–50 spp. in Asia), *Actinodaphne* (ca. 100 spp. in tropical and subtropical Asia), and *Neolitsea* (ca. 100 spp. in Asia).

5 Conclusions

We present phylogenetic analyses of 120 complete plastid genomes and nine barcodes from 19 additional species, which represent 42 genera of Lauraceae and 17 related families of angiosperms, in combination with a matrix of 15 morphological traits of 108 taxa to reconstruct well-resolved relationships of 70% of genera within the family Lauraceae. This phylogenetic framework strongly supported the nine monotypic clades that offered insight to improve the tribal classification of Lauraceae. Two new tribes Caryodaphnopsideae and Neocinnamomeae are described, and the compositions of four tribes, Cassytheae, Cryptocaryeae, Laureae, and Hypodaphnideae, are updated based on our phylogenetic framework and phylogenetically conservative traits.

Acknowledgements

The authors would like to thank Jing Yang, Juan-Hong Zhang, Chun-Yan Lin, Zheng-Shan He, and Ji-Xiong Yang at the Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy Sciences (CAS), for sequencing technology. We thank the Central Laboratory at Xishuangbanna Tropical Botanical Garden for their assistance in bioinformatics analysis. We sincerely thank Professor Jens G. Rohwer, Professor Shiliang Zhou, and Professor Peter F. Stevens for critical and invaluable comments that greatly improved our manuscript. This work was supported by a grant of the Large-scale Scientific Facilities, CAS (No. 2017-LSF-GBOWS-02), the CAS 135 Program (2017XTBG-T03), the CAS "Light of West China" Program (Y7XB061B01), the National Natural Science Foundation of China (Nos. 31600531 and 31500165), the Southeast Asia Biodiversity Research Institute, CAS (Y4ZK111B01), and CAS International Research and Education Development Program (SAJC201613).

Conflict of Interest

The authors report no conflict of interest.

Data Archiving Statement

The complete plastid genome sequence data of the 43 Lauraceae species have been submitted to the Lauraceae Chloroplast Genome Database (https://lcgdb.wordpress.com).

References

Barrett CF, Baker WJ, Comer JR, Conran JG, Lahmeyer SC, Leebens-Mack JH, Li J, Lim GS, Mayfield-Jones DR, Perez L, Medina J, Pires JC, Santos C, Stevenson DW, Zomlefer WB, Davis JI. 2016. Plastid genomes reveal support for deep phylogenetic relationships and extensive rate variation among palms and other commelinid monocots. *New Phytologist* 209: 855–870.

Byng JW, Chase MW, Christenhusz MJ, Fay MF, Judd WS, Mabberley DJ, Sennikov AN, Soltis DE, Soltis PS, Stevens PF. 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* 181: 1–20.

Carpenter RJ, Jordan GJ, Hill RS. 2007. A toothed Lauraceae leaf from the early Eocene of Tasmania, Australia. *International Journal of Plant Sciences* 168: 1191–1198.

Chanderbali AS, Albert VA, Leebens-Mack J, Altman NS, Soltis DE, Soltis PS. 2009. Transcriptional signatures of ancient floral developmental genetics in avocado (*Persea americana*; Lauraceae). *Proceedings of the National Academy of Sciences USA* 106: 8929–8934.

Chanderbali AS, van der Werff H, Renner SS. 2001. Phylogeny and historical biogeography of Lauraceae: Evidence from the chloroplast and nuclear genomes. *Annals of the Missouri Botanical Garden* 88: 104–134.

Chen HH, Liu C, Han LH, Song Y, Tang LZ. 2019. The plastid genome of an oil plants *Cinnamomum chago* (Lauraceae). *Mitochondrial DNA* 4: 1733–1734.

Daniell H, Lin CS, Yu M, Chang WJ. 2016. Chloroplast genomes: Diversity, evolution, and applications in genetic engineering. *Genome Biology* 17: 134.

Darling ACE, Mau B, Blattner FR, Perna NT. 2004. Mauve: Multiple alignment of conserved genomic sequence with rearrangements. *Genome Research* 14: 1394–1403.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods* 9: 772–772.

Doyle JJ, Dickson EE. 1987. Preservation of plant-samples for DNA restriction endonuclease analysis. *Taxon* 36: 715–722.

Eichenberg D, Purschke O, Ristok C, Wessjohann L, Bruelheide H. 2015. Trade-offs between physical and chemical carbon-based leaf defence: Of intraspecific variation and trait evolution. *Journal of Ecology* 103: 1667–1679.

Fang JY, Yoda K. 1989. Climate and vegetation in China II. Distribution of main vegetation types and thermal climate. *Ecological Research* 4: 71–83.

Fijridiyanto IA, Murakami N. 2009. Phylogeny of *Litsea* and related genera (Laureae–Lauraceae) based on analysis of *rpb2* gene sequences. *Journal of Plant Research* 122: 283–298.

Garland T, Harvey PH, Ives AR. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Systematic Biology* 41: 18–32.

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52: 696–704.

Huang JF, Li L, van der Werff H, Li HW, Rohwer JG, Crayn DM, Meng HH, van der Merwe M, Conran JG, Li J. 2016. Origins and evolution of cinnamon and camphor: A phylogenetic and historical biogeographical analysis of the *Cinnamomum* group (Lauraceae). *Molecular Phylogenetics and Evolution* 96: 33–44.

Jin JJ, Yu WB, Yang JB, Song Y, Yi TS, Li DZ. 2018. GetOrganelle: A simple and fast pipeline for de novo assembly of a complete circular chloroplast genome using genome skimming data. *bioRxiv* 156479. https://doi.org/10.1101/256479

Julia S, Soepadmo E, Yahud W. 2009. Problem in the generic delimitation between *Alseodaphne*, *Dehaasia* and *Nothaphoebe* (Lauraceae) in Borneo. *Blumea* 54: 192–197.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649.

Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26: 1463–1464.

Kimoto Y, Tobe H. 2008. Embryology of Illigera and Sparattanthelium (Hernandiaceae, Laurales): Family characteristics and relationships. *International Journal of Plant Sciences* 169: 391–408.

Li HW, Li J, Huang PH, Wei FN, Cui HB, van der Werff H. 2008a. Lauraceae. In: Wu ZY, Raven PH, Hong DY eds. *Flora of China*. Beijing: Science Press; St. Louis: Missouri Botanical Garden Press. 7: 102–254.

Li J, Christophel DC, Conran JG, Li HW. 2004. Phylogenetic relationships within the 'core' Laureae (*Litsea* complex, Lauraceae) inferred from sequences of the chloroplast gene *matK* and nuclear ribosomal DNA ITS regions. *Plant Systematics and Evolution* 246: 19–34.

Li J, Conran JG, Christophel DC, Li ZM, Li L, Li HW. 2008b. Phylogenetic relationships of the *Litsea* complex and core Laureae (Lauraceae) using ITS and ETS sequences and morphology. *Annals of the Missouri Botanical Garden* 95: 580–599.

Li L, Li J, Conran JG, Li XW, Li HW. 2007. Phylogeny of *Neolitsea* (Lauraceae) inferred from Bayesian analysis of nrDNA ITS and ETS sequences. *Plant Systematics and Evolution* 269: 203–221.

Li L, Li J, Rohwer JG, van der Werff H, Wang ZH, Li HW. 2011. Molecular phylogenetic analysis of the *Persea* group (Lauraceae) and its biogeographic implications on the evolution of tropical and subtropical Amphi-Pacific disjunctions. *American Journal of Botany* 98: 1520–1536.

Li L, Madrinan S, Li J. 2016. Phylogeny and biogeography of *Caryodaphnopsis* (Lauraceae) inferred from low-copy nuclear gene and *ITS* sequences. *Taxon* 65: 433–443.

Liao Q, Ye TH, Song Y. 2018. Complete chloroplast genome sequence of a subtropical tree, *Parasassafras confertiflorum* (Lauranceae). *Mitochondrial DNA* 3: 1216–1217.

Liu ZF, Ci XQ, Li L, Li HW, Conran JG, Li J. 2017. DNA barcoding evaluation and implications for phylogenetic relationships in Lauraceae from China. *PLoS One* 12: e0175788.

Liu B, Yang Y, Xie L, Zeng G, Ma K. 2013. *Beilschmiedia turbinata*: A newly recognized but dying species of Lauraceae from tropical Asia based on morphological and molecular data. *PLoS One* 8: e67636.

Ma PF, Zhang YX, Zeng CX, Guo ZH, Li DZ. 2014. Chloroplast phylogenomic analyses resolve deep-level relationships of an intractable Bamboo tribe Arundinarieae (Poaceae). *Systematic Biology* 63: 933–950.

Massoni J, Forest F, Sauquet H. 2014. Increased sampling of both genes and taxa improves resolution of phylogenetic relationships within Magnoliidae, a large and early-diverging clade of angiosperms. *Molecular Phylogenetics and Evolution* 70: 84–93.

Mo YQ, Li L, Li JW, Rohwer JG, Li HW, Li J. 2017. *Alseodaphnopsis*: A new genus of Lauraceae based on molecular and morphological evidence. *PLoS One* 12: e0186545.

Molina-Venegas R, Rodríguez MÁ. 2017. Revisiting phylogenetic signal; strong or negligible impacts of polytomies and branch length information? *BMC Evolutionary Biology* 17: 113.

Nie ZL, Wen J, Sun H. 2007. Phylogeny and biogeography of *Sassafras* (Lauraceae) disjunct between eastern Asia and eastern North America. *Plant Systematics and Evolution* 267: 191–203.

Pagel M. 1999. Inferring the historical patterns of biological evolution. *Nature* 401: 876–884.

Poinar GO, Jr. 2017. A mid-Cretaceous Lauraceae flower, *Cascolaurus burmitis* gen. et sp. nov., in Myanmar amber. *Cretaceous Research* 71: 96–101.

Renner SS. 1998. Phylogenetic affinities of Monimiaceae based on cpDNA gene and spacer sequences. *Perspectives in Plant Ecology, Evolution and Systematics* 1: 61–77.

Renner SS. 1999. Circumscription and phylogeny of the Laurales: Evidence from molecular and morphological data. *American Journal of Botany* 86: 1301–1315.

Richter H. 1981. Anatomie des sekundaren Xylems und der Rinde der Lauraceae. Sonderb. Naturwiss. *Vereins Hamburg* 5: 1–148.

Rohde R, Rudolph B, Ruthe K, Lorea-Hernández FG, de Moraes PLR, Li J, Rohwer JG. 2017. Neither *Phoebe* nor *Cinnamomum* – the tetrasporangiate species of *Aiouea* (Lauraceae). *Taxon* 66: 1085–1111.

Rohwer JG. 1993. Lauraceae. In: Kubitzki K, Rohwer JG, Bittrich V eds. *The families and genera of vascular plants*. Berlin: Springer-Verlag. 2: 366–391.

Rohwer JG. 2000. Toward a phylogenetic classification of the Lauraceae: Evidence from *matK* sequences. *Systematic Botany* 25: 60–71.

Rohwer JG, Li J, Rudolph B, Schmidt SA, van der Werff H, Li H. 2009. Is *Persea* (Lauraceae) monophyletic? Evidence from nuclear ribosomal ITS sequences. *Taxon* 58: 1153–1167.

Rohwer JG, Rudolph B. 2005. Jumping genera: The phylogenetic positions of *Cassytha*, *Hypodaphnis*, and *Neocinnamomum* (Lauraceae) based on different analyses of *trnK* intron sequences. *Annals of the Missouri Botanical Garden* 92: 153–178.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Sajo MG, Moraes PLR, Assis LCS, Rudall PJ. 2016. Comparative floral anatomy and development in neotropical Lauraceae. *International Journal of Plant Sciences* 177: 579–589.

Song Y, Dong WP, Liu B, Xu C, Yao X, Gao J, Corlett RT. 2015. Comparative analysis of complete chloroplast genome sequences of two tropical trees *Machilus yunnanensis* and *Machilus balansae* in the family Lauraceae. *Frontiers in Plant Science* 6: 662.

Song Y, Gan Y, Liu LY, Corlett RT. 2018a. The floral transcriptome of *Machilus yunnanensis*, a tree in the magnoliid family Lauraceae. *Computational Biology and Chemistry* 77: 456–465.

Song Y, Yao X, Liu B, Tan Y, Corlett RT. 2018b. Complete plastid genome sequences of three tropical *Alseodaphne* trees in the family Lauraceae. *Holzforschung* 72: 337–345.

Song Y, Yao X, Tan Y, Gan Y, Corlett RT. 2016. Complete chloroplast genome sequence of the avocado: Gene organization, comparative analysis, and phylogenetic relationships with other Lauraceae. *Canadian Journal of Forest Research* 46: 1293–1301.

Song Y, Yao X, Tan YH, Gan Y, Yang JB, Corlett RT. 2017a. Comparative analysis of complete chloroplast genome sequences of two subtropical trees, *Phoebe sheareri* and *Phoebe omeiensis* (Lauraceae). *Tree Genetics & Genomes* 13: 120.

Song Y, Yu WB, Tan YH, Liu B, Yao X, Jin JJ, Padmanaba M, Yang JB, Corlett RT. 2017b. Evolutionary comparisons of the chloroplast genome in Lauraceae and insights into loss events in the Magnoliids. *Genome Biology Evolution* 9: 2354–2364.

Stamatakis A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.

Tang CQ. 2015. Evergreen broad-leaved forests. In: Tang CQ ed. *The subtropical vegetation of Southwestern China: Plant distribution, diversity and ecology*. Utrecht: Springer Netherlands. 49–112.

Tian XY, Ye JW, Song Y. 2019. Plastome sequences help to improve the systematic position of trinerved *Lindera* species in the family Lauraceae. *PeerJ* 7: e7662.

Trofimov D, Rudolph B, Rohwer JG. 2016. Phylogenetic study of the genus *Nectandra* (Lauraceae), and reinstatement of *Damburneya*. *Taxon* 65: 980–996.

van der Merwe M, Crayn DM, Ford AJ, Weston PH, Rossetto M. 2016. Evolution of Australian *Cryptocarya* (Lauraceae) based on nuclear and plastid phylogenetic trees: Evidence of recent landscape-level disjunctions. *Australian Systematic Botany* 29: 157–166.

van der Werff H, Richter HG. 1996. Toward an improved classification of Lauraceae. *Annals of the Missouri Botanical Garden* 83: 409–418.

Wang ZH, Li J, Conran JG, Li HW. 2010. Phylogeny of the Southeast Asian endemic genus *Neocinnamomum* H. Liu (Lauraceae). *Plant Systematics and Evolution* 290: 173–184.

Weber JZ. 1981. A taxonomic revision of *Cassytha* (Lauraceae) in Australia. *Journal of the Adelaide Botanic Gardens* 3: 187–262.

Wick RR, Schultz MB, Zobel J, Holt KE. 2015. Bandage: Interactive visualization of de novo genome assemblies. *Bioinformatics* 31: 3350–3352.

Yu XQ, Gao LM, Soltis DE, Soltis PS, Yang JB, Fang L, Yang SX, Li DZ. 2017. Insights into the historical assembly of East Asian subtropical evergreen broadleaved forests revealed by the temporal history of the tea family. *New Phytologist* 215: 1235–1248.

Zanis MJ, Soltis DE, Soltis PS, Mathews S, Donoghue MJ. 2002. The root of the angiosperms revisited. *Proceedings of the National Academy of Sciences USA* 99: 6848–6853.

Zhang SD, Jin JJ, Chen SY, Chase MW, Soltis DE, Li HT, Yang JB, Li DZ, Yi TS. 2017. Diversification of Rosaceae since the Late Cretaceous based on plastid phylogenomics. *New Phytologist* 214: 1355–1367.

Zhang T, Zeng CX, Yang JB, Li HT, Li DZ. 2016. Fifteen novel universal primer pairs for sequencing whole chloroplast genomes and a primer pair for nuclear ribosomal DNAs. *Journal of Systematics and Evolution* 54: 219–227.

Zhao ML, Song Y, Ni J, Yao X, Tan YH, Xu ZF. 2018. Comparative chloroplast genomics and phylogenetics of nine *Lindera* species (Lauraceae). *Scientific Reports* 8: 8844.

Supplementary Material

The following supplementary material is available online for this article at http://onlinelibrary.wiley.com/doi/10.1111/jse.12536/suppinfo:

Table S1. Plant materials and Genbank accession numbers for complete plastome sequenced.

Table S2. Plant materials for two different data matrices.

Table S3. Plant materials and Genbank accession numbers for DNA regions sequenced.

Table S4. References of morphological characters for the taxa in the family Lauraceae.

Table S5. Data matrix of morphological characters in the study.

Fig. S1. Molecular phylogenetic tree of 98 taxa of Laurales and 14 taxa of related angiosperms based on complete plastome sequences using Bayesian inference (BI). Numbers at each node are Bayesian PPs. The tree is rooted with the plastome sequence of *Amborella trichopoda*.

Fig. S2. Molecular phylogenetic tree of 98 taxa of Laurales and 14 taxa of related angiosperms based on LSC regions (A), SSC regions (B), IR regions (C), coding regions (D), and noncoding regions (E) of plastome sequences using Bayesian inference (BI). Numbers at each node are Bayesian PPs. These trees are rooted with the sequence of *Amborella trichopoda*.

Fig. S3. The lignified endocarp of Lauraceae. *Eusideroxylon zwageri* (A), *Cryptocarya yunnanensis* (B), *Syndiclis anlungensis* (C), *Endiandra dolichocarpa* (D), *Beilschmiedia* sp. (E), *Caryodaphnopsis henryi* (F), *Neocinnamomum caudatum* (G), *Alseodaphnopsis andersonii* (H), *Machilus minutiflora* (I), *Litsea magnoliifolia* (J), *Litsea pierre* (K), *Actinodaphne forrestii* (L), *Cinnamomum verum* (M).