SYSTEMATICS AND PHYLOGENY

Phylogeny and biogeography of *Caryodaphnopsis* (Lauraceae) inferred from low-copy nuclear gene and ITS sequences

Lang Li,[1] Santiago Madriñán[2] & Jie Li[1]

1 *Plant Phylogenetics and Conservation Group, Centre for Integrative Conservation, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Kunming, Yunnan 650223, P.R. China*

2 *Departamento de Ciencias Biológicas, Universidad de los Andes, CL 18-2 68, Bogotá, D.C. 111711, Colombia*

Author for correspondence: *Jie Li, jieli@xtbg.ac.cn*

ORCID JL, http://orcid.org/0000-0001-8067-749X

DOI http://dx.doi.org/10.12705/653.1

Abstract *Caryodaphnopsis* is a small genus of the Lauraceae. It contains 16 known species with a disjunct tropical amphi-Pacific distribution; 8 species in tropical Asia and 8 species in tropical America. In the present study, *RPB2*, *LEAFY* and ITS sequences of 9 *Caryodaphnopsis* species and 22 other Lauraceae species were analyzed with maximum parsimony and Bayesian inference. Divergence time estimation employed the Bayesian Markov chain Monte Carlo method under a relaxed clock. Ancestral area reconstructions were conducted by using both the statistical dispersal-vicariance analysis and likelihood approach under the dispersal-extinction-cladogenesis model. Based on the results of the phylogenetic analyses, the monophyly of *Caryodaphnopsis* is strongly supported. Asian and American *Caryodaphnopsis* species form two well-supported monophyletic clades, respectively. Independent lineages for *Caryodaphnopsis*, *Neocinnamomum* and *Cassytha* are also suggested. According to the divergence time estimations and ancestral area reconstructions, we suggest that *Caryodaphnopsis* originated in Late Cretaceous Laurasia and its amphi-Pacific disjunction results from the disruption of ancestral boreotropical lineages between Eurasia and North America during the first cooling period of the Eocene.

Keywords amphi-Pacific disjunction; biogeography; *Caryodaphnopsis*; Lauraceae; molecular phylogeny

Supplementary Material Electronic Supplement (Fig. S1A–E) and DNA sequence alignment are available in the Supplementary Data section of the online version of this article at http://ingentaconnect.com/content/iapt/tax

INTRODUCTION

Caryodaphnopsis Airy Shaw is a small genus of the Lauraceae. It contains 16 known species with a disjunct tropical amphi-Pacific distribution (Fig. 1); 8 species in tropical Asia and 8 species in tropical America (Airy Shaw, 1940; Kostermans, 1974; Li & al., 1982 , 2008; Van der Werff & Richter, 1985; Li & Li, 1991; Aymard & Romero-González, 2009; Van der Werff, 2012; Liu & al., 2013). *Caryodaphnopsis* was first described by Airy Shaw (1940), and Kostermans (1957) placed the genus in synonymy with *Persea* Mill., but later reinstated and monographed the genus (Kostermans, 1974). *Caryodaphnopsis* was only known from tropical Asia until Van der Werff & Richter (1985) reported two neotropical species, *C. theobromifolia*(A.H.Gentry) Van der Werff & H.G.Richt. and *C. inaequalis* (A.C.Sm.) Van der Werff & H.G.Richt., based on floral and wood anatomical characters. The genus displays a unique combination of morphological characters within Lauraceae, having opposite (sub-opposite), either trinerved, triplinerved or pinninerved leaves; unequal, deciduous tepals, with the outer three small and the inner three very large; and a large fruit sitting on top of a more or less thickened peduncle lacking a cupule (Rohwer, 1993; Li & al., 2008).

Recent molecular studies suggest that *Caryodaphnopsis* is monophyletic and its phylogenetic position within Lauraceae is between the *Mezilaurus* group+core Lauraceae and the *Cryptocarya* group (Chanderbali & al., 2001; Rohwer & Rudolph, 2005; Wang & al., 2010). Using DNA sequences, a sister relationship between *Caryodaphnopsis* and *Neocinnamomum* H.Liou was suggested in Bayesian analyses, but with only low to moderate support (Rohwer & Rudolph, 2005; Wang & al., 2010), whereas an affinity between *Neocinnamomum* and *Cassytha* L. was suggested by parsimony analyses (Chanderbali & al., 2001; Rohwer & Rudolph, 2005; Wang & al., 2010) and interpreted as an artifact caused by long-branch attraction. Based on the fossil record and molecular clock estimation, a Cretaceous Laurasian origin for *Caryodaphnopsis* has been suggested (Chanderbali & al., 2001), with an estimated Early Cretaceous (Berriasian) divergence time of ~140 Ma. This estimation, however, appears to be much too old, as the earliest unquestionable lauraceous fossil (*Mauldinia* Drinnan & al.) is from the Cenomanian and only ~100 Ma (Drinnan & al., 1990; Frumin & al., 2004). In contrast, Nie & al. (2007)

Received: 2 Aug 2015 | returned for (first) revision: 12 Nov 2015 | (last) revision received: 2 Feb 2016 | accepted: 3 Feb 2016 || publication date(s): online fast track, 1 Jun 2016; in print and online issues, 24 Jun 2016 ||

proposed a Late Cretaceous (Turonian) divergence time of *Caryodaphnopsis* at ~90 Ma, which is more consistent with the relatively basal position of the genus within the family.

The tropical amphi-Pacific disjunct distribution of plants is a well-known and classical biogeographic pattern seen in plants occurring in tropical regions on both sides of the Pacific Basin (Thorne, 1972; Raven, 1988). Studies of plant groups with tropical amphi-Pacific disjunctions such as Araliaceae (Li & Wen, 2013), Fagaceae (Van der Hammen & Cleef, 1983), Lauraceae (Chanderbali & al., 2001; Li & al., 2011), Magnoliaceae (Azuma & al., 2001), Styracaceae (Fritsch, 2001) and Symplocaceae (Wang & al., 2004; Fritsch & al., 2015) suggested that these disjunctions mostly seem to have resulted from the disruption of an ancestral boreotropical distribution by Mid- to Late Eocene climatic cooling, followed by relatively late immigration into Central and South America. Chanderbali & al. (2001) similarly estimated the separation between Asian and American *Caryodaphnopsis* species at ~44 Ma as a result of cooling following the Paleocene-Eocene Thermal Maximum (PETM) event, and Zeng & al. (2014) suggested that the unusual double layered lower leaf epidermis of *Caryodaphnopsis* might be related to climatic cooling and aridification. However, although these studies provided important information about the phylogeny and biogeography of *Caryodaphnopsis*, their results are conflicting, possibly due to a combination of limited taxon sampling and relatively low phylogenetic information seen in ITS and/or cpDNA sequences of some Lauraceae.

Low-copy nuclear genes of plants show potential to improve the robustness of phylogenetic reconstructions at all taxonomic levels, especially where widely used cpDNA and nrITS sequences fail to generate strong phylogenetic signal (Sang, 2002). In particular, recent molecular studies in Lauraceae suggest that low-copy nuclear genes are sufficiently informative to produce well-resolved molecular phylogenies. For example, the *RPB2* gene encodes the second-largest subunit of RNA polymerase II in eukaryotic cells, which is responsible for DNA binding and RNA chain elongation (Woychik & Young, 1990). Partial *RPB2* gene sequences from introns 20–23 were sufficiently variable and informative to produce a well-resolved molecular phylogeny with high statistical support at different taxonomic levels in *Litsea* Lam. and related Lauraceae (Fijridiyanto & Murakami, 2009). Similarly, Li & al. (2011) investigated the phylogeny of the *Persea* group (Lauraceae) and its amphi-Pacific disjunction using the *LEAFY* gene, which regulates floral meristem induction during the early stages of reproductive ontogeny (Schultz & Haughn, 1991; Blazquez, 1997; Blazquez & al., 1997). Their study indicated that the second intron of *LEAFY* is an excellent molecular marker for resolving phylogenetic relationships at lower taxonomic levels in Lauraceae due to its relatively high level of variation. Accordingly, we chose *RPB2* and *LEAFY* along with the universal ITS as molecular markers for phylogenetic reconstruction in the present study.

The objectives of our study were to (1) explore the phylogenetic utility of *RPB2* and *LEAFY* in *Caryodaphnopsis* and related Lauraceae; (2) place *Caryodaphnopsis* phylogenetically within the family; and (3) investigate the biogeographic history of *Caryodaphnopsis* focusing on its tropical amphi-Pacific distribution.

■ MATERIALS AND METHODS

Taxon sampling and DNA extraction. — In the present study, 20 individuals were sampled as representatives of nine *Caryodaphnopsis* species, three from tropical Asia and six from

Fig. 1. The tropical amphi-Pacific disjunct distribution of *Caryodaphnopsis* Airy Shaw (Lauraceae). Stars represent fossils possibly related to *Caryodaphnopsis*, *Caryodaphnopsoxylon richteri* from Germany (Gottwald, 1992) and "Taxon B" from the U.S.A. (Eklund, 2000).

the Neotropics. Based on recent molecular studies (Chanderbali & al., 2001; Rohwer & Rudolph, 2005; Wang & al., 2010), 22 species of *Neocinnamomum*, *Cassytha*, the *Cryptocarya* group and core Lauraceae were selected as the outgroups (Appendix 1). Total genomic DNA was extracted either from field-collected (silica-gel dried) or herbarium leaf material using the Plant Genomic DNA Kit (Tiangen, Beijing, China).

PCR amplification and sequencing. — The whole ITS region was amplified and directly sequenced by using primer combinations LAUR 1-ITS4 and ITS5m-ITS4 (White & al., 1990; Sang & al., 1995; Chanderbali & al., 2001). The PCR protocol for ITS amplification followed the study by Li & al. (2011). Initial amplification of *RPB2* was carried out with universal primers P7F and P11aR (Denton & al., 1998) from a subset of sampled taxa, and the sequences obtained were then used to design specific primers for the amplification of exons 19–23 in *Caryodaphnopsis* (RPB2-CF: 5′-TCCGATCATTATTCTTCC GTTCTTAC-3′ and RPB2-CR: 5′-ATCTCATTCTTACTTTCA CAWACCTCAAC-3′) and the other sampled Lauraceae species (RPB2-F: 5′-GWTCATTATTTTTCCGCTCATACA-3′ and RPB2-R: 5′-ATCTCATTCTTACTTTCACAAATCTCAAC-3′). The PCR program for the *RPB2* amplification was 94°C for 2 min; then 35 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 1 min; followed by a final extension of 72°C for 10 min. The second intron of *LEAFY* was amplified by using the primer combination LFY-F and LFY-R and following the PCR program described in Li & al. (2011). The amplified *RPB2* and *LEAFY* fragments were purified using the E.Z.N.A. Cycle Pure Kit (Omega Bio-Tek, Norcross, Georgia, U.S.A.) before cloning using the pGEM-T Vector Systems Kit (Promega, Madison, Wisconsin, U.S.A.), with at least 5 positive clones from an individual sample sequenced.

Sequence alignment and phylogenetic analysis. — DNA sequences were aligned using ClustalX v.2.1 (Larkin & al., 2007) and then edited manually in BioEdit v.7.2.5 (Hall, 1999) with alignment gaps treated as missing data. A single representative sequence was chosen randomly from multiple clones of each individual, as clone samples from the same individual sample invariably fell into one clade in a preliminary analysis. To avoid ambiguous alignments, five separate datasets were built initially. For the ITS-I and *RPB2*-I datasets, ITS and *RPB2* sequences (with the exclusion of ITS 1 and *RPB2* intron 19 from all sampled taxa) were included. For the ITS-II, *RPB2*-II and *LEAFY*-II datasets, only ITS, *RPB2*, and *LEAFY* sequences from *Caryodaphnopsis* species were included. Because of significant conflicts (bootstrap support, BS ≥ 70%; posterior probability, PP ≥ 0.95) for the position of *Caryodaphnopsis tomentosa* Van der Werff in the ITS and *RPB2* trees (see Results), this species was excluded from the combined datasets. Compared with the ITS and *RPB2* trees, several significant conflicts were found in the *LEAFY* tree (see Results), so the *LEAFY* sequences were excluded from the combined datasets. Thus, the combined dataset I (ITS-I+*RPB2*-I) and combined dataset II (ITS-II+*RPB2*-II) were built using the four separate ITS and *RPB2* datasets, but with the exclusion of *C. tomentosa*.

Data analysis employed maximum parsimony (MP) using the program PAUP* v.4.0b10 (Swofford, 2003) and Bayesian inference (BI) using the program MrBayes v.3.2.2 (Ronquist & Huelsenbeck, 2003). In the MP analysis, a heuristic search was performed with 1000 random addition sequence replicates, tree-bisection-reconnection (TBR) branch swapping, collapse of zero-length branches, MulTrees on, and character state changes equally weighted. Bootstrap values of the internal nodes were obtained with 1000 bootstrap replicates. In each bootstrap replicate, we performed 1000 random addition sequence replicates followed by TBR branch swapping and MulTrees on. In the BI analysis, different sequences were defined as separate data partitions. Based on the Akaike information criterion (AIC; Akaike, 1974), the evolutionary model for each dataset was estimated with jModelTest v.2.1.4 (Posada, 2008; Darriba & al., 2012). The Markov chain Monte Carlo (MCMC) algorithm was run for 2 million generations with four incrementally heated chains, starting from random trees and sampling one out of every 100 generations. The first 25% trees were discarded to make sure the burn-in period was sufficiently long and the remaining trees used to construct the 50% majority-rule consensus tree. Species of the *Cryptocarya* group were used to root the trees that included all sampled taxa. As the Asian and American *Caryodaphnopsis* species formed well-supported clades sister to each other in the trees with the complete sample (see Results), we rooted between these two clades in the trees in which only *Caryodaphnopsis* species were included.

Bayesian dating and fossil calibration. — Based on the combined dataset I, divergence time estimation employed the Bayesian MCMC method under an uncorrelated lognormal relaxed clock using the program BEAST v.1.7.5 (Drummond & al., 2006, 2012). The program BEAUti v.1.7.5 (distributed with BEAST) was used to create the input file to run in BEAST. Different sequences were defined as separate data partitions and model parameters were unlinked across partitions. Posterior distributions of parameters were approximated using two independent MCMC analyses of 20 million steps each, with the first 25% being discarded. The log files were combined to check for convergence on the same distribution and to ensure adequate sample sizes using the program Tracer v.1.5 (Rambaut & Drummond, 2007). The samples from the posteriors were summarized on the maximum clade credibility (MCC) tree using the program TreeAnnotator v.1.7.5 (distributed with BEAST).

Cretaceous reports of Lauraceae include flowers, fruits, inflorescences, leaves and wood recovered from the late Early to Late Cretaceous of Europe, Asia and North America (e.g., Drinnan & al., 1990; Herendeen, 1994; Mickle, 1996; Eklund & Kvaček, 1998; Eklund, 2000; Takahashi & al., 1999). The extinct lauraceous genus *Neusenia* H.Eklund was established by Eklund (2000) to accommodate an excellently preserved flower with tetrasporangiate anthers and psilate pollen grains from the Late Cretaceous, i.e., Santonian/Campanian (~83 Ma) Neuse River locality in North Carolina, U.S.A. Based on the floral and pollen characters of *Neusenia*, especially the sessile, almost triangular staminodes and smooth, spherical pollen grains, it has been considered to be closer to *Neocinnamomum* than to other extant Lauraceae (Eklund, 2000). Atkinson & al. (2015) agreed that *Neusenia* could be assigned to *Neocinnamomum*,

supporting evidence for a Cretaceous origin of the *Neocinnamomum* lineage. Thus, an exponential prior with an offset of 83 Ma and mean of 1.0 was set for node A (see Fig. 4).

The divergence between the *Persea* group and Laureae has been estimated to the Early Eocene (Li & al., 2011), and this is supported by fossils from the Early to Late Eocene of Europe and North America. Cupulate hemispherical Lauraceae fruits reported from the London Clay Flora by Reid & Chandler (1933) are restricted to Laureae and Cinnamomeae, whereas well-preserved flowers with the general floral structure of the *Persea* group were described from the Eocene of North America (Taylor, 1988) and from Late Eocene Baltic amber (Conwentz, 1886). Thus, an exponential prior with offset of 52 Ma and mean of 1.0 was set for node B (see Fig. 4), based on the age of the London Clay Flora. Although the monospecific African *Hypodaphnis* Stapf has been considered to be the first branching extant genus of Lauraceae (Rohwer, 2000; Chanderbali & al., 2001; Rohwer & Rudolph, 2005), it could not be included in the present study. However, the *Cryptocarya* group is closely related to *Hypodaphnis* and their divergence times were regarded as very similar (Rohwer, 2000; Chanderbali & al., 2001; Rohwer & Rudolph, 2005; Nie & al., 2007). According to the work of Nie & al. (2007), a normal prior with mean of 98 Ma and stdev of 1.0 was set for node C (see Fig. 4), such that the root of the molecular tree could not be older than 100 Ma, as the earliest accepted Lauraceae fossils of the extinct genus *Mauldinia* were dated to ~100 Ma (Drinnan & al., 1990; Frumin & al., 2004).

Ancestral area reconstructions. — Ancestral area reconstructions were conducted by using both the statistical dispersal-vicariance analysis (S-DIVA; Yu & al., 2010) and likelihood approach under the dispersal-extinction-cladogenesis (DEC) model (Ree & Smith, 2008). Both analyses were implemented in RASP v.3.1 (Yu & al., 2015). Bayesian trees obtained from BEAST analysis were used as input for the S-DIVA and DEC analyses. The MCC tree produced in the BEAST analysis was used as a condensed tree. According to the distribution range of sampled taxa, two biogeographic areas were defined: (A) tropical and subtropical Asia, (B) tropical America.

■ RESULTS

Phylogenetic analysis. — Character statistics, evolutionary models (AIC) for each dataset and tree statistics of the MP analysis for each dataset are presented in Table 1. As the consensus trees obtained from both the MP and BI analyses are almost identical in their topologies, only the Bayesian consensus trees with BS and PP values are presented here. Molecular trees of the separate datasets (ITS-I, *RPB2*-I, ITS-II, *RPB2*-II, *LEAFY*-II) are presented in the Electronic Supplement (Fig. S1A–E). Phylogenies inferred from the separate ITS and *RPB2* datasets (ITS-I and *RPB2*-I, ITS-II and *RPB2*-II) are mostly congruent in their topologies and the different position of *Caryodaphnopsis tomentosa* is the only significant conflict (BS ≥ 70%; PP ≥ 0.95) between the ITS and *RPB2* (ITS-I and *RPB2*-I, ITS-II and *RPB2*-II) trees. Compared with reports for other Lauraceae genera (e.g., Li & al., 2011), *Caryodaphnopsis* has much shorter and unusual *LEAFY* (intron 2) sequences. The molecular tree inferred from the *LEAFY* dataset (*LEAFY*-II) was poorly resolved and there were several significant conflicts between it and the ITS and *RPB2* (ITS-II and *RPB2*-II) trees. Thus, the phylogenetic utility of *LEAFY* appears to be limited in *Caryodaphnopsis* and the *LEAFY* sequences were excluded from further analyses.

In Lauraceae, combined datasets are often able to generate more resolved and better-supported phylogenies when single molecular marker fails to give good phylogenetic signal (Li & al., 2007; Li & al., 2011). In the present study, the molecular trees from the combined datasets (I and II) are congruent with phylogenies inferred from separate datasets and are more resolved and better supported internally (Figs. 2, 3).

In the molecular tree obtained from combined dataset I (Fig. 2) there are three principal clades. The first clade (BS 100%, PP 1.00) consists of five species of *Beilschmiedia* Nees and *Cryptocarya* R.Br. (both from the *Cryptocarya* group). The monophyletic *Caryodaphnopsis* clade consists of all *Caryodaphnopsis* individuals investigated in the present study and receives BS of 100% and PP of 1.00. Within the *Caryodaphnopsis* clade, the Asian and American species each formed well-supported clades.

Table 1. Character statistics, tree statistics of maximum parsimony analyses, and evolutionary model for each dataset.

	ITS-I	*RPB2*-I	Combined dataset I	ITS-II	*RPB2*-II	Combined dataset II	*LEAFY*-II
Taxa included	31	31	30	9	9	8	9
Aligned length [bp]	430	829	1259	563	971	1534	335
Variable characters (%)	169 (39.3)	442 (53.3)	608 (48.3)	63 (11.2)	102 (10.5)	160 (10.4)	67 (20.0)
Parsimony-informative characters (%)	141 (32.8)	342 (41.3)	482 (38.3)	56 (9.9)	62 (6.4)	117 (7.6)	43 (12.8)
Number of most parsimonious trees	8	160	48	4	8	2	3
Tree length	351	707	1075	74	111	187	75
Consistency index	0.70	0.82	0.77	0.91	0.95	0.89	0.92
Retention index	0.91	0.96	0.95	0.97	0.98	0.96	0.96
Evolutionary Model (AIC)	TVM+I+G	TIM+I+G	TVM+I+G (ITS) TIM+G (*RPB2*)	TrN+I	TrN+G	TrN+I (ITS) TrN+G (*RPB2*)	HKY

Sister to the *Caryodaphnopsis* clade is a large clade consisting of 17 species of *Cassytha*, *Neocinnamomum* and the core Lauraceae with high branch support (BS 96%, PP 1.00) and good internal generic resolution, with *Cassytha filiformis* L. (the only *Cassytha* sampled) sister to the remainder of the clade. The four sampled *Neocinnamomum* species formed a well-supported monophyletic clade (BS 100%, PP 1.00), but with poor internal resolution. The remaining 12 core Lauraceae species of the Cinnamomeae, Laureae and *Persea* group formed clades corresponding to these three groups with moderate to high branch support. The combined dataset II analysis (Fig. 3) produced good resolution within *Caryodaphnopsis* with most branches receiving high statistic support (BS > 80%, PP 1.00). As in the combined dataset I tree (Fig. 2), the Asian and American species formed well-supported clades (BS 100%, PP 1.00) and most multi-sampled species were monophyletic and moderately to highly supported, except for *Caryodaphnopsis tonkinensis* (Lecomte) Airy Shaw and *C. burgeri* N.Zamora & Poveda.

Divergence time estimation. — Divergence times of major nodes inferred from the BEAST analysis were largely consistent with earlier findings (Chanderbali & al., 2001; Nie & al., 2007). *Caryodaphnopsis* originated at ~96.8 Ma (95% high posterior density [HPD] = 93.1–99.8 Ma, node D, Fig. 4) in the Late Cretaceous. The split between the Asian clade and American clade was estimated as ~48.0 Ma (95% HPD = 25.7–74.7 Ma, node E, Fig. 4) during the Mid-Eocene, after the PETM. The American species probably diversified during the Early Oligocene ~31.7 Ma (95% HPD = 14.6–53.3 Ma, node F, Fig. 4), with the Asian species radiating in the Late Oligocene ~23.9 Ma (95% HPD = 7.1–43.9 Ma, node G, Fig. 4).

Ancestral area reconstructions. — Both S-DIVA and DEC analyses suggested Asia and America as the possible ancestral area of *Caryodaphnopsis* (marginal probability 100) implying a Laurasian origin of the genus. Only one vicariance event was detected at ~48.0 Ma (node E, Fig. 4) in the split of *Caryodaphnopsis* into two continental lineages.

■ DISCUSSION

Phylogeny of *Caryodaphnopsis*. — The *RPB2* sequences show strong phylogenetic signal, making them useful for phylogenetic reconstruction in *Caryodaphnopsis*. When combined with ITS sequences, they provide good phylogenetic resolution at both the generic and intragenic level (Figs. 2, 3), especially for the basal Lauraceae lineages. As in several earlier studies

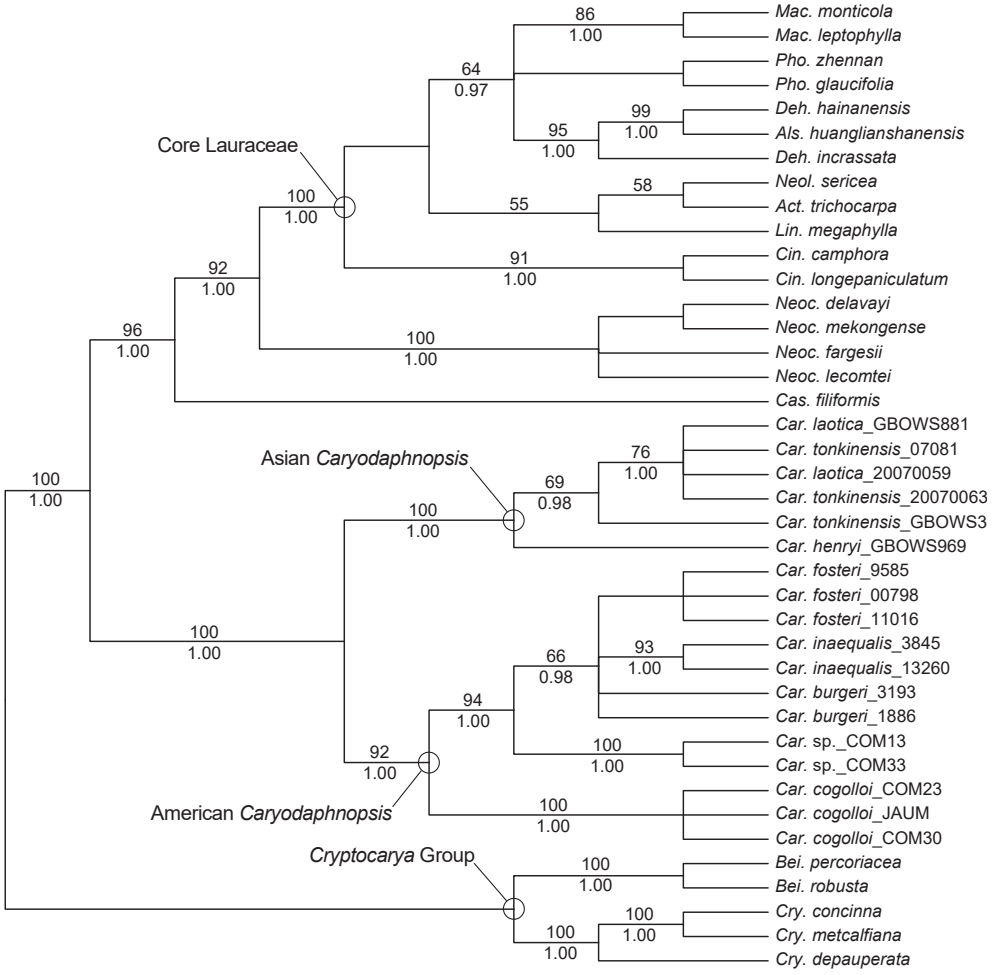

Fig. 2. Bayesian consensus tree of combined dataset I. Bootstrap support (BS ≥ 50%) and Bayesian posterior probability (PP ≥ 0.95) are shown above and below branches. *Act.* = *Actinodaphne*, *Als.* = *Alseodaphne*, *Bei.* = *Beilschmiedia*, *Car.* = *Caryodaphnopsis*, *Cas.* = *Cassytha*, *Cin.* = *Cinnamomum*, *Cry.* = *Cryptocarya*, *Deh.* = *Dehaasia*, *Lin.* = *Lindera*, *Mac.* = *Machilus*, *Neoc.* = *Neocinnamomum*, *Neol.* = *Neolitsea*, *Pho.* = *Phoebe*.

(Chanderbali & al., 2001; Rohwer & Rudolph, 2005; Wang & al., 2010), *Caryodaphnopsis* is monophyletic (Fig. 2), supporting the combination of opposite leaves, strongly unequal tepals, distinctive wood and bark anatomy as generic characters (Van der Werff & Richter, 1996). Similarly, the unique double-layered lower leaf epidermis of *Caryodaphnopsis* is autapomorphic within Lauraceae (Zeng & al., 2014).

The relatively close relationship between *Caryodaphnopsis* and *Neocinnamomum* found by Rohwer & Rudolph (2005) and Wang & al. (2010) was not found in the present study where *Neocinnamomum* and *Cassytha* are positioned in a different clade (Fig. 2). Rohwer & Rudolph (2005) found several general morphological characters common to *Caryodaphnopsis* and *Neocinnamomum*, e.g., triplinerved leaves, usually four-locular anthers with locelli arranged in a shallow arc and fruits sitting almost free on a rather swollen pedicel. Wang & al. (2010) further suggested that the reduced thyrses of *Caryodaphnopsis tonkinensis* were similar to the compound thyrses of *Neocinnamomum caudatum* (Nees) Merr. Considering our results, the use of these relatively widespread lauraceous morphological similarities to infer a close relationship between *Caryodaphnopsis* and *Neocinnamomum* seems questionable.

Caryodaphnopsis has opposite leaves, a deciduous perianth with strongly unequal tepal whorls and lacks a cupule, whereas *Neocinnamomum* has alternate leaves, subequal tepal whorls and a shallow cupule with persistent perianth lobes. Furthermore, two-locular anthers occur in some *Caryodaphnopsis* species and the anther cells of *Neocinnamomum* are arranged in an almost transverse series. Although the reduced thyrses of *Caryodaphnopsis tonkinensis* do somewhat resemble the inflorescences of *Neocinnamomum caudatum*, the subopposite cymes of *C. tonkinensis*, *C. inaequalis*, *C. metallica* Kosterm. and *C. tomentosa* (Kostermans, 1974; Van der Werff & Richter, 1985; Van der Werff, 1991) might also correspond to inflorescence types seen in members of the *Cryptocarya* group (Van der Werff & Richter, 1996).

The molecular tree from the combined dataset II shows good internal resolution of *Caryodaphnopsis* (Fig. 3) with most branches well-supported (BS > 80%, PP 1.00). As with the combined dataset I tree (Fig. 2), only one divergence event between Asian and American species was detected, with each geographic species group forming a well-supported clade. In addition, conspecific individuals all formed species clades, except for those of *C. tonkinensis* and *C. burgeri*. The apparently strongly supported separation of three individuals of *C. tonkinensis* may represent sample misidentifications, or may indicate intraspecific diversity, particularly as *C. tonkinensis* is the most widespread species of Asian

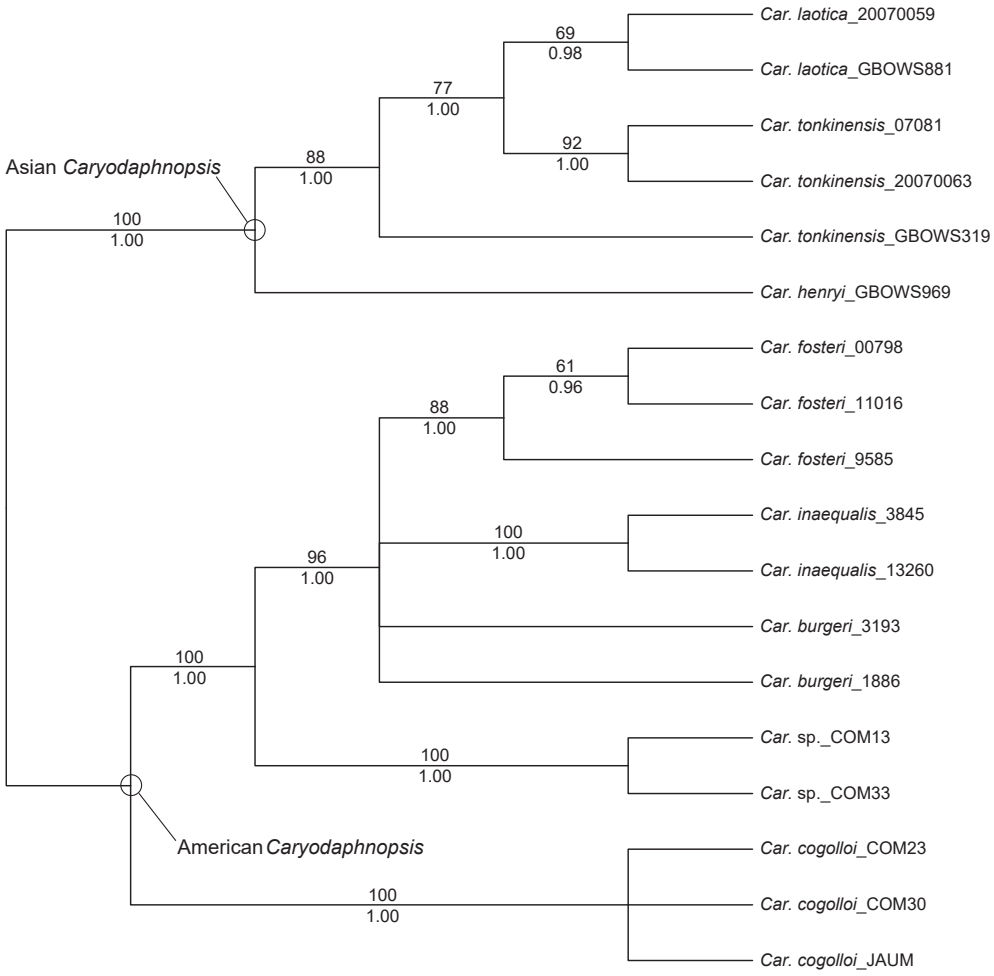

Fig. 3. Bayesian consensus tree of combined dataset II. Bootstrap support (BS ≥ 50%) and Bayesian posterior probability (PP ≥ 0.95) are shown above and below branches.

Caryodaphnopsis, ranging from tropical southwest China and North Vietnam to Malaysia and the Philippines (Li & al., 2008). Accordingly, more populations from across its range should be sampled before the placement of the different samples can be understood.

Although the Neotropical *C. cogolloi* Van der Werff and *C. inaequalis* both have distinctive pinnately veined leaves and avocado-shaped fruits, whereas most *Caryodaphnopsis* species have tripliveined leaves and round fruits (Van der Werff & Richter, 1985; Van der Werff, 1988), they were placed in two different clades (Fig. 3), suggesting that leaf venation and fruit shape characters may not be phylogenetically informative in *Caryodaphnopsis*. Li & al. (2007) similarly found that the penninerved and triplinerved species of *Neolitsea* (Benth. & Hook.f.) Merr. failed to form clades. They noticed that the *Neolitsea* lineages largely correspond with fruit shape (elliptic,

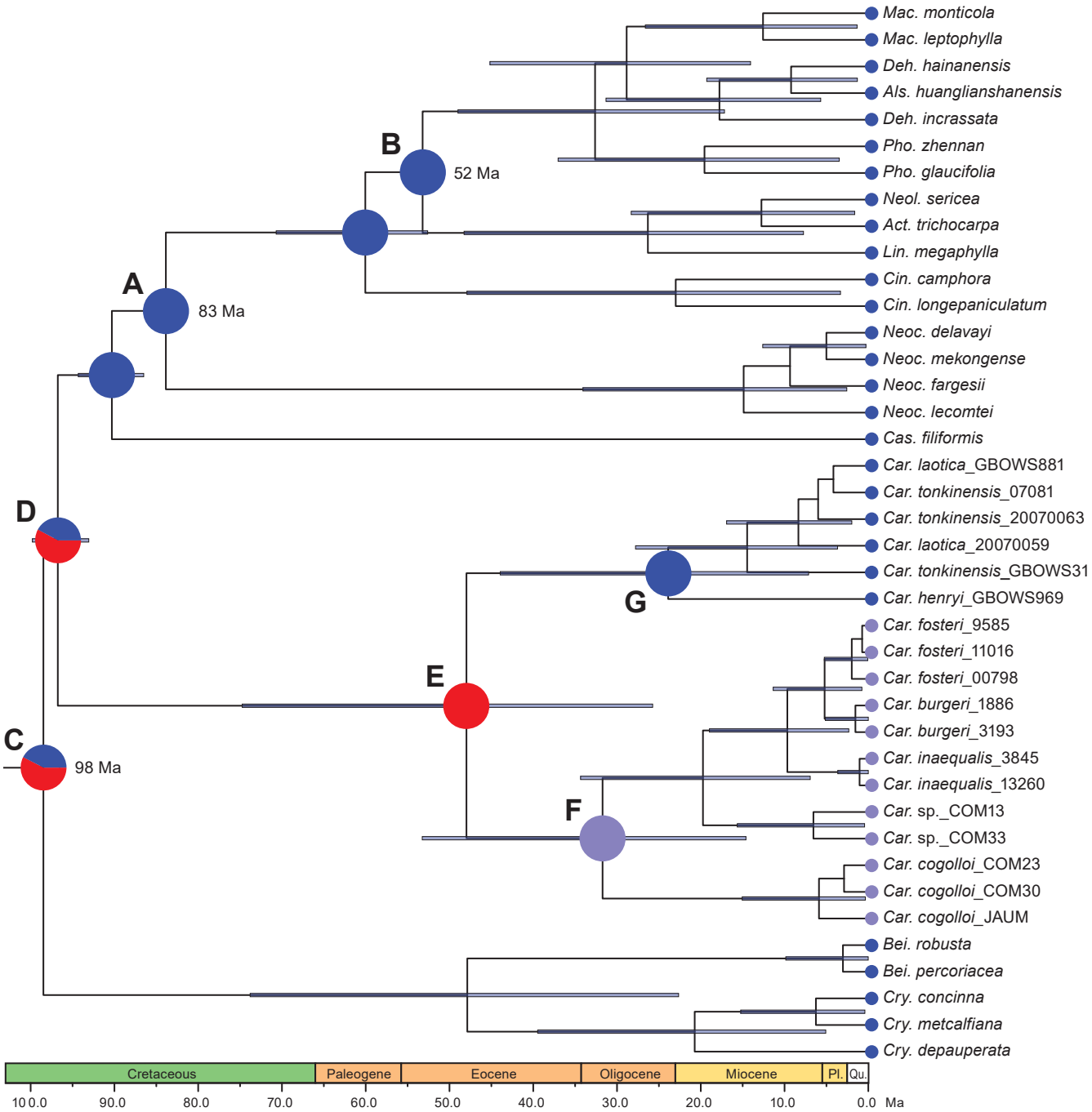

Fig. 4. Molecular dating tree derived from BEAST based on combined dataset I. Gray bars at the internal nodes represent the 95% high posterior density (HPD) credibility interval for node ages. Biogeographic distributions of sampled taxa are shown at tips: blue, tropical and subtropical Asia; purple, tropical America; red, tropical Asia and America. Ancestral distributions of major nodes are shown as pie charts.

ovoid vs. globose), but there were still globose-fruited species within the predominantly elliptic- or ovoid-fruited clade.

Although the present study has produced a well-resolved phylogeny for *Caryodaphnopsis*, it still suffers from relatively limited sampling especially in tropical Asia. The missing species either from Asia or America are expected to fall into the monophyletic *Caryodaphnopsis* clade. It is also possible that more than one divergence between Asian and American *Caryodaphnopsis* species might be uncovered by more extensive taxon sampling. In addition, population-level sampling is also needed in variable and/or widespread taxa to evaluate intraspecific diversity and species delimitation in the genus.

Origin of *Caryodaphnopsis*. — The divergence of *Caryodaphnopsis* from the rest of Lauraceae was estimated as ~96.8 Ma (HPD = 93.1–99.8 Ma) in the present study, similar to the estimated divergence time of Nie & al. (2007). As in the study by Chanderbali & al. (2001), ancestral area reconstructions suggested that both Asia and America were the possible ancestral regions for *Caryodaphnopsis*, implying a Laurasian origin, and this is also supported by the fossil record. The Late Cretaceous North American fossil flowers and fruits of "Taxon B" (Fig. 1) with strongly unequal tepal whorls and a naked fruit on a slightly enlarged pedicel are comparable to only four extant genera: *Caryodaphnopsis*, *Dehaasia* Blume, *Nothaphoebe* Blume and *Persea* (Eklund, 2000). In particular, "Taxon B" has fruits very similar to *Caryodaphnopsis* (Eklund, 2000), and members of the *Persea* group are very unlikely to have a Late Cretaceous origin (Li & al., 2011). Similarly, the Late Eocene fossil wood of *Caryodaphnopsoxylon richteri* H.Gottwald from Germany (Fig. 1) shares a unique xylem anatomy with *Caryodaphnopsis* (Gottwald, 1992), further supporting a Laurasian origin for *Caryodaphnopsis*.

The tropical amphi-Pacific disjunction of *Caryodaphnopsis*. — The boreotropical flora was hypothesized to have spread to high latitudes across the Northern Hemisphere during the Early Eocene around the PETM, which was the warmest period in the Cenozoic, and contained many thermophilic tropical and subtropical taxa (e.g., Reid & Chandler, 1933; Chandler, 1964; Wolfe, 1975, 1978, 1997; Collinson & al., 1981; Miller & al., 1987; Graham, 1999). Tiffney (1985) also noted that many now-disjunct evergreen taxa such as Magnoliaceae, Lauraceae and Theaceae migrated across both the Bering and North Atlantic land bridges during the Early Eocene. Paleobotanical evidence suggests that there was a significant cooling event in the Late Early Eocene (50–48 Ma), followed by two steady intervals in the Mid- to Late Eocene (46–43 and 37–34 Ma) separated by a second cool interval (42–38 Ma; Wolfe, 1978, 1997). These cooling events pushed boreotropical floral elements to lower latitudes, leading to the separation of widespread ancestral lineages between Eurasia and North America. Later, with ongoing high latitude cooling and the development of the Central American land bridge in the Pliocene, several elements that today show a tropical amphi-Pacific disjunction spread to northern South America (e.g., Van der Hammen & Cleef, 1983; Azuma & al., 2001; Fritsch, 2001; Wang & al., 2004).

Several Lauraceae lineages with apparently Laurasian ancestry show amphi-Pacific disjunctions, including *Caryodaphnopsis*, *Cinnamomum* Schaeff., *Lindera* Thunb., *Litsea*, *Sassafras* J.Presl, the *Cinnamomum* group of Cinnamomeae, the *Persea* group and Laureae (Chanderbali & al., 2001). Climatic cooling during the Mid- to Late Eocene restricted tropical ancestral lineages of Lauraceae to lower paleolatitudes, severing prior circumboreal links, while the intercontinental radiation of temperate taxa across northern latitudes would have been possible until much later in the Cenozoic (Chanderbali & al., 2001; Nie & al., 2007; Li & al., 2011).

For example, Li & al. (2011) suggested that the tropical and subtropical amphi-Pacific disjunctions seen in the *Persea* group resulted from disruption of an ancestral connection during the first cooling period of the Eocene (50–48 Ma). In contrast, the estimated ~13–16 Ma divergence time between the Asian and North American species of the temperate genus *Sassafras* is consistent with the opening of the Bering Strait (Chanderbali & al., 2001; Nie & al., 2007), supporting the view of Wolfe & Leopold (1967) that the Mid-Miocene disruption of the Bering land bridge was the most likely explanation for modern northern temperate floristic disjunctions between North America and Asia.

Several recent molecular dating studies of boreotropical plant lineages found that the divergence times between Eurasian and American clades ranged from the Early to Late Eocene, including taxa of Annonaceae (~41.8 Ma, Su & Saunders, 2009), Araliaceae (~41.8 Ma, Li & Wen, 2013), Arecaceae (~48.6 Ma, Baker & Couvreur, 2013), Lauraceae (~48.5 Ma, Li & al., 2011), Magnoliaceae (~42.0 Ma, Azuma & al., 2001) and Symplocaceae (~35.0 Ma, Fritsch & al., 2015). In the present study, the estimated divergence time between Asian and American *Caryodaphnopsis* was ~48.0 Ma (95% HPD = 25.7–74.7 Ma), similar to the estimated time for the split between Asian and American *Persea* group species at ~48.5 Ma (Li & al., 2011).

Accordingly, the disruption of ancestral boreotropical lineages by climate change is the most plausible explanation for the amphi-Pacific disjunction of extant *Caryodaphnopsis* lineages, as the age estimate for their divergence neither supports ancient vicariance due to the split between South America and Africa (followed by migration from Africa to Eurasia) in the Late Cretaceous, nor post-Eocene intercontinental long-distance dispersal. The ancestral lineages of *Caryodaphnopsis* were apparently part of the widespread Early Eocene high-latitude Northern Hemisphere boreotropical flora and the divergence between the Asian and American *Caryodaphnopsis* clades (~48.0 Ma) corresponds to the first major Eocene cooling event (50–48 Ma), as seen for other tropical lineages of Lauraceae shared between Eurasia and North America (Li & al., 2011). Thermophilic tropical elements of ancestral *Caryodaphnopsis* probably responded to significant cooling by retreating from high latitudes, resulting in their segregation into Eurasian and North American lineages. Further global cooling following this segregation would then have restricted these lineages to tropical regions in both continents, with subsequent southward migration into Central and South America resulting in the tropical amphi-Pacific disjunction pattern seen today.

■ ACKNOWLEDGEMENTS

The authors thank all the collectors and herbaria in Appendix 1 for sample collection and providing leaf materials. They also thank X.Q. Ci and J.F. Huang for their assistance in the laboratory. Special thanks go to H. van der Werff for providing leaf material and helpful comments, and to J.G. Conran for assistance with improvement of the manuscript. This study was supported by the National Natural Science Foundation of China (No. 31200167, 31370245), West Light Foundation of CAS (No. Y3XB031K01) and CAS 135 program (XTBG-F04).

■ LITERATURE CITED

Airy-Shaw, H.K. 1940. Notes on two Asiatic genera of Lauraceae. *Kew Bull.* 1940: 74–77. http://dx.doi.org/10.2307/4111844

Akaike, H. 1974. A new look at the statistical model identification. *IEEE Trans. Automatic Control* 19: 716–723. http://dx.doi.org/10.1109/tac.1974.1100705

Atkinson, A., Stockey, R.A., Rothwell, G.W., Mindell, R.A. & Bolton M.J. 2015. Lauraceous flowers from the Eocene of Vancouver Island: *Tinaflora beardiae* gen. et sp. nov. (Lauraceae). *Int. J. Pl. Sci.* 176: 567–585. http://dx.doi.org/10.1086/681586

Aymard, C.G.A. & Romero-González, G.A. 2009. *Caryodaphnopsis fieldii* (Lauraceae), a new species from the coastal Cordillera of Venezuela. *Novon* 19: 7–10. http://dx.doi.org/10.3417/2007043

Azuma, H., Garcia-Franco, J.G., Rico-Gray, V. & Thien, L.B. 2001. Molecular phylogeny of the Magnoliaceae: The biogeography of tropical and temperate disjunctions. *Amer. J. Bot.* 88: 2275–2285. http://dx.doi.org/10.2307/3558389

Baker, W.J. & Couvreur, T.L.P. 2013. Global biogeography and diversification of palms sheds light on the evolution of tropical lineages. I. Historical biogeography. *J. Biogeogr.* 40: 274–285. http://dx.doi.org/10.1111/j.1365-2699.2012.02795.x

Blazquez, M.A. 1997. Illuminating flowers: Constans induces *LEAFY* expression. *BioEssays* 19: 277–279. http://dx.doi.org/10.1002/bies.950190403

Blazquez, M.A., Soowal, L.N., Lee, I. & Weigel, D. 1997. *LEAFY* expression and flower initiation in *Arabidopsis*. *Development* 124: 3835–3844.

Chanderbali, A.S., Van der Werff, H. & Renner, S.S. 2001. Phylogeny and historical biogeography of Lauraceae: Evidence from the chloroplast and nuclear genomes. *Ann. Missouri Bot. Gard.* 88: 104–134. http://dx.doi.org/10.2307/2666133

Chandler, M.E.J. 1964. A summary and survey of findings in the light of recent botanical observations. P. 151 in: Chandler, M.E.J. (ed.), *The lower Tertiary floras of southern England*, vol. 4. London: British Museum (Natural History).

Collinson, M.E., Fowler, K. & Boulter, M.C. 1981. Floristic changes indicate a cooling climate in the Eocene of southern England. *Nature* 291: 315–317. http://dx.doi.org/10.1038/291315a0

Conwentz, H. 1886. *Die Flora des Bernsteins*, vol. 2, *Die Angiospermen des Bernsteins*. Danzig: Engelmann. http://dx.doi.org/10.5962/bhl.title.29891

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. 2012. jModelTest 2: More models, new heuristics and parallel computing. *Nature, Meth.* 9: 772. http://dx.doi.org/10.1038/nmeth.2109

Denton, A.L., McConaughy, B.L. & Hall, B.D. 1998. Usefulness of RNA polymerase II coding sequences for estimation of green plant phylogeny. *Molec. Biol. Evol.* 15: 1082–1085. http://dx.doi.org/10.1093/oxfordjournals.molbev.a026007

Drinnan, A.N., Crane, P.R., Friis, E.M. & Pedersen, K.R. 1990. Lauraceous flowers from the Potomac Group (mid-Cretaceous) of eastern North America. *Bot. Gaz.* 151: 370–384. http://dx.doi.org/10.1086/337838

Drummond, A.J., Ho, S.Y.W., Phillips, M.J. & Rambaut, A. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biol.* 4: e88. http://dx.doi.org/10.1371/journal.pbio.0040088

Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molec. Biol. Evol.* 29: 1969–1973. http://dx.doi.org/10.1093/molbev/mss075

Eklund, H. 2000. Lauraceous flowers from the Late Cretaceous of North Carolina, U.S.A. *Bot. J. Linn. Soc.* 132: 397–428. http://dx.doi.org/10.1006/bojl.1999.0295

Eklund, H. & Kvaček, J. 1998. Lauraceous inflorescences and flowers from the Cenomanian of Bohemia (Czech Republic, Central Europe). *Int. J. Pl. Sci.* 159: 668–686. http://dx.doi.org/10.1086/297585

Fijridiyanto, I.A. & Murakami, N. 2009. Phylogeny of *Litsea* and related genera (Laureae-Lauraceae) based on analysis of *rpb2* gene sequences. *J. Pl. Res.* 122: 283–298. http://dx.doi.org/10.1007/s10265-009-0218-8

Fritsch, P.W. 2001. Phylogeny and biogeography of the flowering plant genus *Styrax* (Styracaceae) based on chloroplast DNA restriction sites and DNA sequences of the internal transcribed spacer region. *Molec. Phylogen. Evol.* 19: 387–408. http://dx.doi.org/10.1006/mpev.2001.0933

Fritsch, P.W., Manchester, S.R., Stone, R.D., Cruz, B.C. & Almeda, F. 2015. Northern Hemisphere origins of the amphi-Pacific tropical plant family Symplocaceae. *J. Biogeogr.* 42: 891–901. http://dx.doi.org/10.1111/jbi.12442

Frumin, S., Eklund, H. & Friis, E.M. 2004. *Mauldinia hirsuta* sp. nov., a new member of the extinct genus *Mauldinia* (Lauraceae) from the Late Cretaceous (Cenomanian-Turonian) of Kazakhstan. *Int. J. Pl. Sci.* 165: 883–895. http://dx.doi.org/10.1086/421479

Gottwald, H. 1992. Hölzer aus marinen Sanden des oberen Eozän von Helmstedt (Niedersachsen). *Palaeontographica, Abt. B, Palaophytol.* 225: 27–103.

Graham, A. 1999. *Late Cretaceous and Cenozoic history of North American vegetation north of Mexico*. New York: Oxford University Press.

Hall, T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.* 41: 95–98.

Herendeen, P.S., Crepet, W.L. & Nixon, K.C. 1994. Fossil flowers and pollen of Lauraceae from the Upper Cretaceous of New Jersey. *Pl. Syst. Evol.* 189: 29–40. http://dx.doi.org/10.1007/bf00937576

Kostermans, A.J.G.H. 1957. Lauraceae. *Pengum. Balai Besar Penjel. Kehut. Indonesia* 57: 1–64.

Kostermans, A.J.G.H. 1974. A monograph of *Caryodaphnopsis* Airy Shaw. *Reinwardtia* 9: 123–137.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J. & Higgins, D.G. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23: 2947–2948. http://dx.doi.org/10.1093/bioinformatics/btm404

Li, H.W., Pai, P.Y., Lee, S.K., Wei, F.N., Wei, Y.T., Yang, Y.C., Huang, P.H., Tsui, H.P., Shia, Z.D. & Li, J.L. 1982. Lauraceae. Pp. 1–463 in: Li, H.W. (ed.), *Flora Reipublicae Popularis Sinicae*, vol. 31. Beijing: Science Press.

Li, H.W., Li, J., Huang, P.H., Wei, F.N., Tsui, H.P. & Van der Werff, H. 2008. Lauraceae. Pp. 102–254 in: Wu, Z.Y., Raven, P.H. & Hong, D.Y. (eds.), *Flora of China*, vol. 7. Beijing: Science Press; St. Louis: Missouri Botanical Garden Press.

Li, L., Li, J., Conran, J.G. & Li, H.W. 2007. Phylogeny of *Neolitsea* (Lauraceae) inferred from Bayesian analysis of nrDNA ITS and ETS sequences. *Pl. Syst. Evol.* 293: 203–221. http://dx.doi.org/10.1007/s00606-007-0580-8

Li, L., Li, J., Rohwer, J.G., Van der Werff, H., Wang, Z.H. & Li, H.W., 2011. Molecular phylogenetic analysis of the *Persea* group (Lauraceae) and its biogeographic implications on the evolution of

tropical and subtropical amphi-Pacific disjunctions. *Amer. J. Bot.* 98: 1520–1536. http://dx.doi.org/10.3732/ajb.1100006

Li, R. & Wen, J. 2013. Phylogeny and biogeography of *Dendropanax* (Araliaceae), an amphi-Pacific disjunct genus between tropical/subtropical Asia and the Neotropics. *Syst. Bot.* 38: 536–551. http://dx.doi.org/10.1600/036364413x666606

Li, X.W. & Li, J. 1991. Notes on the taxonomy and distribution of the genus *Caryodaphnopsis* of Lauraceae and to discuss the characteristics of its area-type. *Acta Bot. Yunnan.* 13: 1–17.

Liu, B., Yang, Y. & Ma, K.P., 2013. A new species of *Caryodaphnopsis* Airy Shaw (Lauraceae) from southeastern Yunnan, China. *Phytotaxa* 118: 1–8. http://dx.doi.org/10.11646/phytotaxa.118.1.1

Mickle, J. E. 1996. *Grexlupus carolinensis*, a new probable lauraceous fruit from the Late Cretaceous of North Carolina. *J. Elisha Mitchell Sci. Soc.* 112: 1–6.

Miller, K.G., Fairbanks, R.G. & Mountain, G.S. 1987. Tertiary oxygen isotope synthesis, sea level history, and continental margin erosion. *Paleoceanography* 2: 1–19. http://dx.doi.org/10.1029/pa002i001p00001

Nie, Z.L., Wen, J. & Sun, H. 2007. Phylogeny and biogeography of *Sassafras* (Lauraceae) disjunct between eastern Asia and eastern North America. *Pl. Syst. Evol.* 267: 191–203. http://dx.doi.org/10.1007/s00606-007-0550-1

Posada, D. 2008. jModelTest: Phylogenetic model averaging. *Molec. Biol. Evol.* 25: 1253–1256. http://dx.doi.org/10.1093/molbev/msn083

Rambaut, A. & Drummond, A. 2007. Tracer, version 1.5. http://beast.bio.ed.ac.uk/Tracer

Raven, P.H. 1988. Tropical floristics tomorrow. *Taxon* 37: 549–560. http://dx.doi.org/10.2307/1221098

Ree, R.H. & Smith, S.A. 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Syst. Biol.* 57: 4–14. http://dx.doi.org/10.1080/10635150701883881

Reid, E.M. & Chandler, M.E.J. 1933. *The London Clay Flora*. London: British Museum (Natural History).

Rohwer, J.G. 1993. Lauraceae. Pp. 366–391 in: Kubitzki, K., Rohwer, J.G. & Bittrich, V. (eds.), *The families and genera of vascular plants*, vol. 2. Berlin: Springer. http://dx.doi.org/10.1007/978-3-662-02899-5_46

Rohwer, J.G. 2000. Toward a phylogenetic classification of the Lauraceae: Evidence from *matK* sequences. *Syst. Bot.* 25: 60–70. http://dx.doi.org/10.2307/2666673

Rohwer, J.G. & Rudolph, B. 2005. Jumping genera: The phylogenetic positions of *Cassytha*, *Hypodaphnis*, and *Neocinnamomum* (Lauraceae) based on different analyses of *trnK* intron sequences. *Ann. Missouri Bot. Gard.* 92: 153–178.

Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. http://dx.doi.org/10.1093/bioinformatics/btg180

Sang, T. 2002. Utility of low-copy nuclear gene sequences in plant phylogenetics. *Crit. Rev. Biochem. Molec. Biol.* 37: 121–147. http://dx.doi.org/10.1080/10409230290771474

Sang, T., Crawford, D.J. & Stuessy, T.F. 1995. Documentation of reticulate evolution in peonies (*Paeonia*) using internal transcribed spacer sequences of nuclear ribosomal DNA: Implications for biogeography and concerted evolution. *Proc. Natl. Acad. Sci. U. S. A.* 92: 6813–6817. http://dx.doi.org/10.1073/pnas.92.15.6813

Schultz, E.A. & Haughn, G.W. 1991. *LEAFY*, a homeotic gene that regulates inflorescence development in *Arabidopsis*. *Pl. Cell* 3: 771–781. http://dx.doi.org/10.2307/3869271

Su, Y.C.F. & Saunders, R.M.K. 2009. Evolutionary divergence times in the Annonaceae: Evidence of a late Miocene origin of *Pseuduvaria* in Sundaland with subsequent diversification in New Guinea. *B. M. C. Evol. Biol.* 9: 153. http://dx.doi.org/10.1186/1471-2148-9-153

Swofford, D.L. 2003. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sunderland, Massachusetts: Sinauer.

Takahashi, M., Crane, P.R. & Ando, H. 1999. Fossil flowers and associated plant fossils from the Kamikitaba locality (Ashizawa formation, Futaba group, Lower Coniacian, Upper Cretaceous) of Northeast Japan. *J. Pl. Res.* 112: 187–206. http://dx.doi.org/10.1007/pl00013872

Taylor, D. 1988. Eocene floral evidence of Lauraceae: Corroboration of the North American megafossil record. *Amer. J. Bot.* 75: 948–957. http://dx.doi.org/10.2307/2443760

Thorne, R.F. 1972. Major disjunctions in the geographic ranges of seed plants. *Quart. Rev. Biol.* 47: 365–411. http://dx.doi.org/10.1086/407399

Tiffney, B.H. 1985. Perspectives on the origin of the floristic similarities between eastern Asia and eastern North America. *J. Arnold. Arbor.* 66: 73–94. http://dx.doi.org/10.5962/bhl.part.13179

Van der Hammen, T. & Cleef, A.M. 1983. *Trigonobalanus* and the tropical amphi-Pacific element in the North Andean forest. *J. Biogeogr.* 10: 437–440. http://dx.doi.org/10.2307/2844750

Van der Werff, H. 1988. Eight new species and one new combination of Neotropical Lauraceae. *Ann. Missouri Bot. Gard.* 75: 402–419. http://dx.doi.org/10.2307/2399431

Van der Werff, H. 1991. New species of Lauraceae from Ecuador and Peru. *Ann. Missouri Bot. Gard.* 78: 409–423. http://dx.doi.org/10.2307/2399570

Van der Werff, H. 2012. A new species of *Caryodaphnopsis* (Lauraceae) from Peru. *Harvard Pap. Bot.* 17: 39–41. http://dx.doi.org/10.3100/025.017.0108

Van der Werff, H. & Richter, H.G. 1985. *Caryodaphnopsis* Airy Shaw (Lauraceae), a genus new to the Neotropics. *Syst. Bot.* 10: 166–173. http://dx.doi.org/10.2307/2418342

Van der Werff, H. & Richter, H.G. 1996. Toward an improved classification of Lauraceae. *Ann. Missouri Bot. Gard.* 83: 409–418. http://dx.doi.org/10.2307/2399870

Wang, Y.G., Fritsch, P.W., Shi, S.H., Almeda, F., Cruz, B.C. & Kelly, L.M. 2004. Phylogeny and infrageneric classification of *Symplocos* (Symplocaceae) inferred from DNA sequence data. *Amer. J. Bot.* 91: 1901–1914. http://dx.doi.org/10.3732/ajb.91.11.1901

Wang, Z.H., Li, J., Conran, J.G. & Li, H.W. 2010. Phylogeny of the Southeast Asian endemic genus *Neocinnamomum* H. Liu (Lauraceae). *Pl. Syst. Evol.* 290: 173–184. http://dx.doi.org/10.1007/s00606-010-0359-1

White, T.H., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 in: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (eds.), *PCR protocols: A guide to methods and amplifications*. San Diego: Academic Press. http://dx.doi.org/10.1016/b978-0-12-372180-8.50042-1

Wolfe, J.A. 1975. Some aspects of plant geography of the Northern Hemisphere during the Late Cretaceous and Tertiary. *Ann. Missouri Bot. Gard.* 62: 264–279. http://dx.doi.org/10.2307/2395198

Wolfe, J.A. 1978. A paleobotanical interpretation of Tertiary climates in the Northern Hemisphere. *Amer. Sci.* 66: 694–703.

Wolfe, J.A. 1997. Relations of environmental change to angiosperm evolution during the late Cretaceous and Tertiary. Pp. 269–290 in: Iwatsuki, K. & Raven, P.H. (eds.), *Evolution and diversification of land plants*. Tokyo: Springer. http://dx.doi.org/10.1007/978-4-431-65918-1_13

Wolfe, J.A. & Leopold, E.B. 1967. Neogene and early Quaternary vegetation of northwestern North America and northeastern Asia. Pp. 193–206 in: Hopkins, D.M. (ed.), *The Bering Land Bridge*. Stanford: Stanford University Press.

Woychik, N.A. & Young, R.A. 1990. RNA polymerase II: Subunit structure and function. *Trends Biochem. Sci.* 15: 347–350. http://dx.doi.org/10.1016/0968-0004(90)90074-L

Yu, Y., Harris, A.J. & He, X.J. 2010. S-DIVA (Statistical Dispersal-Vicariance Analysis): A tool for inferring biogeographic histories. *Molec. Phylogen. Evol.* 56: 848–850. http://dx.doi.org/10.1016/j.ympev.2010.04.011

Yu, Y., Harris, A.J., Blair, C. & He, X.J. 2015. RASP (Reconstruct Ancestral State in Phylogenies): A tool for historical biogeography. *Molec. Phylogen. Evol.* 87: 46–49. http://dx.doi.org/10.1016/j.ympev.2015.03.008

Zeng, G., Liu, B., Van der Werff, H., Ferguson, D.K. & Yang, Y. 2014. Origin and evolution of the unusual leaf epidermis of *Caryodaphnopsis* (Lauraceae). *Perspect. Pl. Ecol. Evol. Syst.* 16: 296–309. http://dx.doi.org/10.1016/j.ppees.2014.07.003

Appendix 1. Species examined in this study (ingroups and outgroups).

Taxon, collection locality, voucher specimen (herbarium), GenBank accession for ITS / *RPB2* / *LEAFY* sequences. Asterisk (*) indicates accession numbers obtained from Wang & al. (2010) and Li & al. (2011); all other sequencens were newly obtained.

Ingroups: ***Caryodaphnopsis burgeri*** N.Zamora & Poveda, Costa Rica, *J.F. Morales 3193* (MO), KT248572 / KT248631, KT248632 / KT248581; ***Caryodaphnopsis burgeri***, Costa Rica, *N. Zamora 1886* (MO), KT248571 / KT248633, KT248634, KT248635, KT248636 / KT248582; ***Caryodaphnopsis cogolloi*** Van der Werff, Colombia, *S. Madriñán COM23* (JAUM), KT248561 / KT248637, KT248638, KT248639, KT248640 / KT248583, KT248584; ***Caryodaphnopsis cogolloi***, Colombia, *S. Madriñán COM30* (JAUM), KT248562 / KT248641, KT248642, KT248643, KT248644, KT248645 / KT248585, KT248586, KT248587, KT248588, KT248589; ***Caryodaphnopsis cogolloi***, Colombia, *S. Madriñán s.n.* (JAUM), KT248563 / KT248646, KT248647, KT248648 / KT248590, KT248591, KT248592; ***Caryodaphnopsis fosteri*** Van der Werff, Peru, *R.B. Foster 9585* (MO), KT248570 / KT248649 / KT248593, KT248594; ***Caryodaphnopsis fosteri***, Peru, *R.B. Foster 11016* (MO), KT248568 / KT248650, KT248651, KT248652 / KT248595, KT248596, KT248597, KT248598; ***Caryodaphnopsis fosteri***, Colombia, *A. Gentry 00798* (MO), KT248569 / KT248653, KT248654, KT248655, KT248656 / KT248599, KT248600, KT248601, KT248602, KT248603; ***Caryodaphnopsis henryi*** Airy Shaw, China, *X.Q. Ci GBOWS969* (HITBC), KT248557 / KT248657, KT248658 / KT248604, KT248605; ***Caryodaphnopsis inaequalis*** (A.C.Sm.) Van der Werff & H.G.Richt., Peru, *Grandez Z. 3845* (MO), KT248574 / KT248659 / KT248606, KT248607, KT248608; ***Caryodaphnopsis inaequalis***, locality unknown, *J. Pipoly & al. 13260* (MO), KT248573 / KT248660, KT248661, KT248662, KT248663 / KT248609, KT248610, KT248611; ***Caryodaphnopsis laotica*** Airy Shaw, China, *X.Q. Ci GBOWS881* (HITBC), KT248560 / KT248664, KT248665, KT248666 / KT248612, KT248613, KT248614; ***Caryodaphnopsis laotica*** Airy Shaw, China, *L. Li 20070059* (HITBC), GU082364* / KT248667, KT248668 / KT248615; ***Caryodaphnopsis* sp.**, Colombia, *S. Madriñán COM13* (JAUM), KT248565 / KT248669, KT248670, KT248671, KT248672, KT248673 / KT248616, KT248617, KT248618; ***Caryodaphnopsis* sp.**, Colombia, *S. Madriñán COM33* (JAUM), KT248564 / KT248674, KT248675, KT248676 / KT248619, KT248620; ***Caryodaphnopsis tomentosa*** Van der Werff, Peru, *R. Vásquez & al. 25239* (MO), KT248567 / KT248677, KT248678, KT248679 / KT248621; ***Caryodaphnopsis tomentosa***, Ecuador, *D. Neill & J. Zuleta 10134* (MO), KT248566 / KT248680, KT248681 / KT248622 / KT248623; ***Caryodaphnopsis tonkinensis*** (Lecomte) Airy Shaw, China, *X.Q. Ci GBOWS319* (HITBC), KT248558 / KT248682, KT248683, KT248684, KT248685 / KT248624, KT248625; ***Caryodaphnopsis tonkinensis***, China, *L. Li 20070063* (HITBC), KT248559 / KT248686, KT248687, KT248688, KT248689, KT248690, KT248691 / KT248626, KT248627; ***Caryodaphnopsis tonkinensis***, China, *Z.H. Wang 07081* (HITBC), GU082365* / KT248692, KT248693, KT248694, KT248695, KT248696 / KT248628, KT248629, KT248630. **Outgroups (ITS / *RPB2* only):** ***Alseodaphne huanglianshanensis*** H.W.Li & Y.M.Shui, China, *L. Li 20080006* (HITBC), HQ697182* / KT248697; ***Actinodaphne trichocarpa*** C.K.Allen, China, *L. Li 20070282* (HITBC), HQ697214* / KT248698, KT248699, KT248700, KT248701, KT248702, KT248703; ***Beilschmiedia percoriacea*** C.K.Allen, China, *Z.H. Wang 08062* (HITBC), KT248575 / KT248704, KT248705; ***Beilschmiedia robusta*** C.K.Allen, China, *Z.H. Wang 08063* (HITBC), GU082363* / KT248706, KT248707; ***Cassytha filiformis*** Linn., China, *J.S. Zhong 08041* (HITBC), GU082366 / KT248708, KT248709; ***Cinnamomum camphora*** (L.) J.Presl, China, *L. Li & J.F. Huang H-DLS08* (HITBC), KT248576 / KT248710, KT248711, KT248712, KT248713; ***Cinnamomum longepaniculatum*** (Gamble) N.Chao ex H.W.Li, China, *J. Li & al. CXQ452* (HITBC), KT248577 / KT248714, KT248715; ***Cryptocarya concinna*** Hance, China, *Z.H. Wang 08066* (HITBC), KT248578 / KT248716; ***Cryptocarya depauperata*** H.W.Li, China, *Z.H. Wang 08065* (HITBC), KT248579 / KT248717, KT248718; ***Cryptocarya metcalfiana*** C.K.Allen, China, *Z.H. Wang 08067* (HITBC), GU117751* / KT248719; ***Dehaasia hainanensis*** Kosterm., China, *L. Li & Z.H. Wang 20070373* (HITBC), FJ719308* / KT248720, KT248721, KT248722, KT248723, KT248724, KT248725; ***Dehaasia incrassata*** (Jack) Kosterm., Indonesia, *D. Arifiani DA492* (BO), HQ697186 / KT248726, KT248727; ***Lindera megaphylla*** Hemsl., China, *L. Li 20070236* (HITBC), HQ697216* / KT248728, KT248729, KT248730, KT248731, KT248732, KT248733; ***Machilus leptophylla*** Hand.-Mazz., China, *J. Li & L. Li 20070190* (HITBC), FJ755430* / KT248734, KT248735, KT248736, KT248737, KT248738, KT248739; ***Machilus monticola*** S.K.Lee, China, *L. Li & Z.H. Wang 20070323* (HITBC), FJ755418* / KT248740, KT248741, KT248742, KT248743, KT248744, KT248745; ***Neocinnamomum delavayi*** (Lecomte) H.Liou, China, *Z.H. Wang 07087* (HITBC), GU082369* / KT248746, KT248747, KT248748; ***Neocinnamomum fargesii*** (Lecomte) Kosterm., China, *L. Li 20070304* (HITBC), GU082370* / KT248749; ***Neocinnamomum lecomtei*** Liou, China, *H. Akiyama & al. 1122* (KUN), GU082371* / KT248750, KT248751; ***Neocinnamomum mekongense*** (Hand.-Mazz.) Kosterm., China, *Z.H. Wang 07094* (HITBC), GU082372* / KT248752; ***Neolitsea sericea*** (Blume) Koidz., China, *J. Li & L. Li 20070225* (HITBC), HQ697221* / KT248753, KT248754, KT248755, KT248756; ***Phoebe glaucifolia*** S.K.Lee & F.N.Wei, China, *J.Q. Chen & al., 2005002* (HITBC), KT248580 / KT248757, KT248758, KT248759, KT248760; ***Phoebe zhennan*** S.K.Lee & F.N.Wei, China, *L. Li 20070239* (HITBC), HQ697212* / KT248761, KT248762, KT248763.