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Molecular phylogenetic analysis of the *Persea* group (Lauraceae) and its biogeographic implications on the evolution of tropical and subtropical Amphi-Pacific disjunctions[1]

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- *Premise of the study:* The *Persea* group (Lauraceae) has a tropical and subtropical amphi-pacific disjunct distribution with most of its members, and it includes two Macaronesian species. The relationships within the group are still controversial, and its intercontinental disjunction has not been investigated with extensive sampling and precise time dating.
- *Methods:* ITS and *LEAFY* intron II sequences of 78 *Persea* group species and nine other Lauraceae species were analyzed with maximum parsimony and Bayesian inference. Divergence time estimation employed Bayesian Markov chain Monte Carlo method under a relaxed clock.
- *Key results:* Several traditional genera or subgenera within the *Persea* group form well-supported monophyletic groups except *Alseodaphne* and *Dehaasia*. The divergence time of the *Persea* group is estimated as ~55.3 (95% higher posterior densities [HPD] 41.4–69.9) million years ago (mya). Two major divergences within the *Persea* group are estimated as ~51.9 (95% HPD 38.9–63.9) mya and ~48.5 (95% HPD 35.9–59.9) mya.
- *Conclusions: Persea* can be retained as a genus by the inclusion of *Apollonias barbujana* and exclusion a few species that do not fit into the established subgenera. A major revision is recommended for the delimitation between *Alseodaphne*, *Dehaasia*, and *Nothaphoebe*. We suggest that the *Persea* group originated from the Perseeae-Laureae radiation in early Eocene Laurasia. Its amphi-pacific disjunction results from the disruption of boreotropical flora by climatic cooling during the mid- to late Eocene. The American-Macaronesian disjunction may be explained by the long-distance dispersal.

Key words: amphi-pacific; biogeography; disjunction; molecular phylogeny; *Persea* group; Lauraceae.

The *Persea* group, as described by Rohwer et al. (2009), is a subset of the family Lauraceae. It consists of seven currently recognized genera, *Alseodaphne* Nees, *Apollonias* Nees, *Dehaasia* Blume, *Machilus* Rumphius ex Nees, *Nothaphoebe* Blume, *Persea* Mill., and *Phoebe* Nees, including a total of ~400 to 450 species. About 80% of these species are distributed in tropical to subtropical Asia, whereas ~20% are found in warm-temperate to tropical regions of the New World (Fig. 1). Only two species, *Apollonias barbujana* and *Persea indica*, are distributed in the Macaronesian Islands (Canary Islands and Madeira, with *P. indica* also on the Azores). As outlined in more detail in Rohwer et al. (2009), the generic delimitation within the *Persea* group has always been controversial—and somewhat inconsistent. All 80–90 New World species have been placed in a broadly defined genus *Persea* (Kopp, 1966), regardless of characters such as number of pollen sacs per anther, relative size of tepals, or fate of tepals in fruit, whereas these characters have been used to delimit six genera in the Old World (*Alseodaphne* [~50 spp.], *Apollonias* [2 spp.], *Dehaasia* [~35 spp.], *Machilus* [~100 spp.], *Nothaphoebe* [~40 spp.] and *Phoebe* [~100 spp.]; Li et al., 1984, 2008).

This raises several questions: Are the currently recognized genera monophyletic groups? If so, is American *Persea* sister to the Asian genera, or is it nested among them? When did the disjunctions within the group and within the genus *Persea* originate?

Several recent molecular studies confirmed that the *Persea* group is monophyletic (Rohwer, 2000; Chanderbali et al., 2001; Rohwer and Rudolph, 2005; Rohwer et al., 2009), but the relationships within the group are still not well resolved. The most recent molecular study of this group was conducted by

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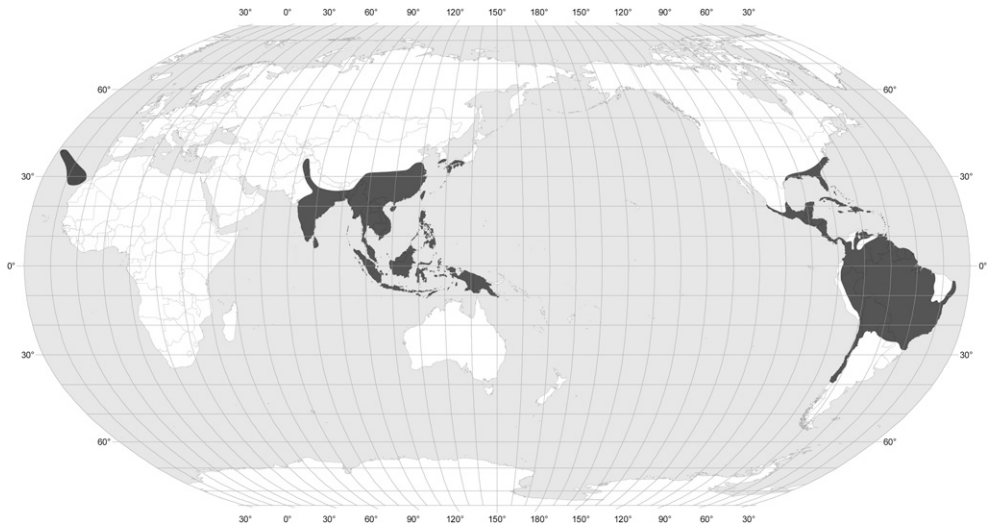

Fig. 1. Geographic distribution of the *Persea* group (Lauraceae) in the Americas, Asia, and Macaronesian Islands. Modification of "World Base Map–Centered on the Pacific", publicly available from Department of Geography (http://www.sjsu.edu/depts/geography/), San Jose State University, San Jose, California, USA.

Rohwer et al. (2009). They analyzed ITS sequences of 61 *Persea* group species and 30 other Lauraceae species and showed that several traditional genera or subgenera formed well-supported groups. All sampled *Machilus* species formed a strongly supported clade. Likewise, the species that had been placed in *Persea* subg. *Eriodaphne* by Kopp (1966), plus a few *Persea* species that were unknown at her time and *Persea indica* from Macaronesia, formed a well-supported clade, while the species attributed to *Persea* subg. *Persea* by Kopp (1966) formed a strongly supported group within another clade including *Alseodaphne*, *Dehaasia*, and *Phoebe*. Within the clade composed of *Alseodaphne*, *Dehaasia*, *Phoebe*, and *Persea* subg. *Persea*, four *Alseodaphne* species and all sampled *Dehaasia* species formed a well-supported group, and species of the genus *Phoebe* were found in two strongly supported clades. Thus, Rohwer et al. (2009) suggested *Machilus* should be treated as a separate genus, as done by Li et al. (1984), not as a subgenus of *Persea* (e.g., Kostermans, 1957). Similarly, *Persea* subg. *Eriodaphne* could, according to their results, be treated as a separate genus, which probably would also include the Macaronesian *Persea indica* (their suggestion that this genus would have to be called *Mutisiopersea* Kosterm., however, was erroneous; the oldest available name for this group is *Farnesia* Heist. ex Fabr.). *Phoebe* was distinct from both *Machilus* and *Persea*. An inclusion of *Dehaasia* in *Alseodaphne*, as foreshadowed in critical comments on their delimitation by, e.g., van der Werff (2001), appeared reasonable. Although the work of Rohwer et al. (2009) already provided important information about the phylogenetic structure of the *Persea* group, the phylogeny was far from complete, due to the limited information from ITS sequences. The relationships between major clades were still unclear, and several clades received unsatisfactory support.

The boreotropical flora was hypothesized to span the northern hemisphere during the climatically warm periods of the Paleocene and have contained many thermophilic tropical and subtropical taxa with diffuse origins (Wolfe, 1975; Tiffney, 1985a). Tiffney (1985a) also noted that most evergreen disjunct taxa (e.g., Magnoliaceae, Lauraceae, and Theaceae) migrated during the early Eocene through both the Bering and North Atlantic land bridges. While disjunctions between eastern Asia and eastern North America have been extensively studied in temperate forest elements (review by Donoghue et al., 2001), tropical amphi-pacific disjunctions have received much less attention. In the cases studied in more detail (van der Hammen and Cleef, 1983; Azuma et al., 2001; Fritsch, 2001; Wang et al., 2004), the authors suggested that the tropical amphi-pacific disjunction mostly resulted from an ancestral boreotropical distribution disrupted by Late Eocene climatic cooling, and subsequent, relatively late (Pliocene) immigration into South America.

For the *Persea* group, Chanderbali et al. (2001) reached the same conclusion. Age estimation suggested that the Asian and American members of the *Persea* group diverged around the Eocene-Oligocene boundary, ca. 32 million years ago (mya). Boreotropical ranges were disrupted by climatic cooling at that time (Wolfe, 1975; Wu, 1983; Tiffney, 1985a, b), and so biotic links between North America and Eurasia were severed. The range of the *Persea* group was divided, and its species receded to warmer paleolatitudes in Asia and the Americas. Due to very small sampling numbers within the *Persea* group and limited information from ITS sequences, this deductive conclusion could still be questionable. Therefore, a much denser sampling within the *Persea* group and additional molecular markers with higher resolution would be necessary to make a more solid resolution.

Finding another appropriate marker, besides the ITS sequences, with higher resolution for the phylogenetic reconstruction of the *Persea* group seemed to be a big challenge in our study. Rohwer et al. (2009) explored the phylogenetic potential of 14 cpDNA markers within this group, and they all showed very little variation. We also tried another five cpDNA markers (*psbA-trnH*, *psbB-psbH*, *trnS-trnG*, *rpoB-trn*C, *trnS-trnfM*), which might have phylogenetic potential within the *Persea* group at the early stage of our study, but the results were still unsatisfactory. Finally, turning our attempts to low-copy nuclear genes seems to be the only promising option.

Low-copy nuclear genes of plants hold great potential to improve the robustness of phylogenetic reconstructions at all

taxonomical levels, especially where universally used cpDNA and nrITS have been unable to generate a strong phylogenetic hypothesis (Sang, 2002). The *LEAFY* gene is a homeotic gene that regulates the floral meristem induction during the early stages of reproductive ontogeny (Schultz and Haughn, 1991; Weigel, 1995; Blázquez, 1997; Blázquez et al., 1997). Frohlich and Meyerowitz (1997) provided universal primers for the second intron and suggested that the second intron of *LEAFY* might have evolved at a high rate and might be useful for phylogenetic reconstructions of closely related species. Several recent molecular studies using the *LEAFY* gene also suggested that it is an excellent candidate as a phylogenetic marker for resolving phylogenetic relationships at lower taxonomic levels due to the generally low copy number in angiosperms and the relatively high level of variation within the introns (Hoot and Taylor, 2001; Oh and Potter, 2003, 2005; Grob et al., 2004; Howarth and Baum, 2005). Therefore, the *LEAFY* second intron (*LEAFY* intron II) was chosen as the molecular marker, along with the ITS sequence, for the phylogenetic reconstruction in our study.

Objectives of our study were to (1) explore the phylogenetic utility of *LEAFY* intron II within the *Persea* group, (2) elucidate the phylogenetic relationships within the *Persea* group, and (3) explain the biogeographic disjunction patterns of the *Persea* group.

MATERIALS AND METHODS

Taxon sampling and DNA extraction—In the present study, a total of 78 species were sampled as representatives of all genera within the *Persea* group (Appendix 1). Also, these samples represented all sections in some large genera such as *Machilus* and *Phoebe* according to the treatment in volume 31 of *Flora Reipublicae Popularis Sinicae* (Li et al., 1984), as well as in *Persea* in the sense of Kopp (1966). Based on recent molecular studies (Rohwer, 2000; Chanderbali et al., 2001; Rohwer et al., 2009), nine species from four closely related genera (*Actinodaphne*, *Lindera*, *Litsea*, *Neolitsea*) were selected as the outgroups (Appendix 1).

Total genomic DNA was extracted from silica-gel dried material or herbarium specimens using the method of Doyle and Doyle (1987) as modified by Li et al. (2004) or using the Invisorb Spin Plant Mini Kit (Invitek GmbH, Berlin, Germany).

PCR amplification and sequencing—The ITS and 5.8S regions were amplified and directly sequenced by using primers of White et al. (1990) and Chanderbali et al. (2001) with minor modifications reported in Li et al. (2004). The PCR program for ITS 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. To reduce problems caused by the secondary structure of the rather GC-rich ITS, 10% DMSO was included in all amplifications (Buckler and Holtsford, 1996; Buckler et al., 1997).

Initial amplification and sequencing of *LEAFY* intron II was carried out with the degenerate primers LFsxl-3 and LFtxr (Frohlich and Meyerowitz, 1997) from a subset of sampled taxa. The sequences obtained were used to design specific primers for the *Persea* group (LFY-F: 5′-GCT TAG ACT ATC TCT TCC ACC TCT ATG AA-3′ and LFY-R: 5′-CCT TCT TCG CAT ACC TGA ACA C-3′). The PCR program for the *LEAFY* intron II amplification was 94°C for 2 min; then 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min; followed by a final extension of 72°C for 10 min. The amplified products were then purified using the QIAquick PCR Purification Kit (Qiagen, Crawley, UK) or Montage PCR Centrifugal Filter Devices (Millipore, Billerica, Massachusetts, USA) before cloning. Cloning was conducted using the pGEM-T Vector Systems (Promega, Madison, Wisconsin, USA) or the TOPO TA cloning Kit (Invitrogen, Carlsbad, California, USA). At least five positive clones from an individual sample were sequenced.

Each fragment was sequenced in both directions using BigDye 3.1 reagents with an ABI 3770 automated sequencer (Applied Biosystems, Carlsbad, California, USA). Sequence chromatogram output files were initially evaluated for base confirmation with Sequencher (Gene Codes Corp., Ann Arbor, Michigan, USA).

Sequence alignment and phylogenetic analysis—Forward and reverse sequences of each fragment were compared to guarantee accuracy and then combined into a single consensus sequence. Both the ITS and *LEAFY* intron II data sets were aligned using the program ClustalX 1.81 (Thompson et al., 1997) and edited manually using BioEdit 7.0.9.0 (Hall, 1999). For the combined ITS + *LEAFY* intron II data set, one of several different *LEAFY* intron II sequences obtained from one individual sample was randomly chosen to be combined with the corresponding ITS sequence obtained from the same sample because different sequences of *LEAFY* intron II from the same individual sample almost invariably formed a single clade (see Results). Gaps were coded as simple indels using the program GapCoder (Young and Healy, 2003). Ambiguous blocks in the alignment as well as indels from those blocks were deleted.

Data analysis employed maximum parsimony (MP) using the program PAUP* 4.0b10 (Swofford, 2003) and Bayesian inference using the program MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). In the MP analysis, a heuristic search was performed with 100 random addition sequence replicates, tree-bisection-reconnection (TBR) swapping, collapse of zero-length branches, MulTrees on, and character state changes equally weighted. Because too many trees were found, trees were limited to 1000 for each random addition sequence replicate. Bootstrap values of the internal nodes were obtained with 100 bootstrap replicates. In each bootstrap replicate, we performed 100 random addition sequence replicates followed by TBR swapping, MulTrees on and keeping no more than 1000 trees per random addition sequence replicate.

In the Bayesian analysis, different sequences were defined as separate data partitions. The evolutionary model for each data set was estimated by the program Modeltest 3.7 (Posada and Crandall, 1998; Posada and Buckley, 2004). The Markov chain Monte Carlo (MCMC) algorithm was run for 1 000 000 generations with four incrementally heated chains, starting from random trees and sampling one out of every 100 generations. Examination of the log-likelihood values in Microsoft (Redmond, Washington, USA) Excel and the observed consistency between runs suggested that the burn-in was reached in about 40 000 generations. Thus, the first 1000 trees (100 000 generations) were discarded to make sure the burn-in period was sufficiently long, and the remaining trees were used to construct the 50%-majority rule consensus tree.

To evaluate the congruence of ITS and *LEAFY* intron II data sets, we conducted a incongruence length difference (ILD) test (Farris et al., 1994) with 100 replicates of the heuristic search, using the same parameters as in the MP analysis, with 100 random addition sequence replicates in PAUP*.

Bayesian dating—We herein employed ITS and *LEAFY* intron II sequences to estimate the divergence time of each clade within the obtained molecular tree, especially the divergence time of the *Persea* group and the time of two major divergences within it. Both ITS and *LEAFY* intron II sequences were used for dating divergence times because higher accuracy and more confidence can be inferred based on multiple sequences (Renner, 2005).

Divergence time estimation employed the Bayesian MCMC method under an uncorrelated lognormal relaxed clock (Drummond et al., 2006) using the program BEAST v1.5.3 (Drummond and Rambaut, 2007). The program BEAUti v1.5.3 (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 analysis of 20 million steps each, with the first 10% 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 v1.5 (Drummond and Rambaut, 2007). The samples from the posterior were summarized on the maximum clade credibility tree using the program TreeAnnotator v1.4.8 (distributed with BEAST) and visualized using the program FigTree v1.3.1. Means and 95% higher posterior densities (HPD) of age estimates were obtained from the combined outputs using Tracer v1.5.

Lauraceae fossil and calibration—Fossil records of Lauraceae include flowers, fruits, inflorescences, leaves, and wood ranging from the early Cretaceous to the late Tertiary (Herendeen, 1991; Eklund, 2000; Frumin et al., 2004). However, there are few reliable fossils that can be unambiguously assigned to an extant group of Lauraceae (Eklund and Kvaček, 1998; Eklund, 2000). *Alseodaphne changchangensis* Jin et Li (Lauraceae), with a perfectly preserved fossil leaf, was found in the coal-bearing series of the Changchang Formation from the Changchang Basin of Hainan Island, China (Li et al., 2009). Based on the features of the sporopollen assemblage, the age of the coal-bearing series of the Changchang Formation is late Early Eocene to early Late Eocene, and this fossil specimen is the earliest occurrence at the lowest latitude for the genus *Alseodaphne* (Li et al., 2009).

Based on the molecular tree obtained from the combined ITS +*LEAFY* intron II data (Fig. 5), the placement of the fossil of *Alseodaphne changchangensis* for age calibration seemed to have several options. Five nodes (A, 1, 2, 3, and 4 in Fig. 5) were considered as candidates for the calibration point. To check the results for their credibility, we calculated what the different calibrations would mean for the age of the *Persea* group, and checked how they compare with the estimates of Chanderbali et al. (2001).

RESULTS

Sequence characters—The length of ITS sequences of the ingroups ranges from 511 to 568 bp, and the GC content is 69.00 to 74.67%. The length of ITS sequences of the outgroups ranges from 522 to 561 bp, and the GC content is 69.16 to 71.66%. The ITS data set has 613 aligned positions, as well as 29 new indel characters (obtained by gapcoding). Of the 642 total characters, 216 are variable (33.64%) and 171 (26.64%) are parsimony-informative.

The length of *LEAFY* intron II sequences of the ingroups ranges from 674 to 740 bp, the GC content is 38.83 to 41.54%. The length of the *LEAFY* intron II sequences of the outgroups ranges from 679 to 724 bp, and the GC content is 39.33 to 40.80%. The *LEAFY* intron II data set has 770 aligned positions, as well as 35 new indel characters (obtained by gapcoding). Of the total 805 characters, 455 are variable (56.52%) and 339 (42.11%) are parsimony-informative.

Results of the ILD test showed significant incongruence between ITS and *LEAFY* data sets ($P = 0.01$). Nevertheless, these two data sets were not only analyzed separately, but also combined and analyzed simultaneously, to get a more resolved and better-supported topology, as in Li et al. (2007). At least in the Bayesian analysis, the combination is not expected to be problematic, as the different partitions were allowed to evolve under different models. The combined ITS + *LEAFY* intron II data set has 1377 aligned positions, as well as 50 new indel characters (obtained by gap-coding). Of the total 1427 characters, 585 are variable (41.00%) and 386 (27.05%) are parsimony-informative. All these numbers differ from the sums of the above data sets because not all sequences present in the *LEAFY* intron II data set are also represented in the combined ITS + *LEAFY* intron II data set.

MP analysis—MP analysis of the ITS data set resulted in 86 004 MP trees, 599 steps long, with a consistency index (CI) of 0.4541 and a retention index (RI) of 0.7716. For the data set of total *LEAFY* intron II sequences, 100 000 MP trees were retained, 810 steps long, with a CI of 0.7296 and a RI of 0.9186. For the combined ITS + *LEAFY* intron II data set, 2507 MP trees were retained, 1234 steps long, with a CI of 0.5810 and a RI of 0.7939.

The consensus trees obtained from both MP and Bayesian analysis, based on the different data sets (ITS, *LEAFY* intron II, and combined ITS + *LEAFY* intron II) respectively, are mostly congruent in their topologies. Their major clades are identical, only with very few differences in some terminal branches. The Bayesian consensus trees always have relatively higher branch supports than the MP consensus trees. Here, only Bayesian consensus trees with both bootstrap support (BS) values and posterior probability support (PPS) values are presented for demonstration.

Bayesian analysis (Figs. 2–4)—The Bayesian consensus tree obtained from the ITS data set (Fig. 2) is largely congruent with the work of Rohwer et al. (2009). The three principal clades within the *Persea* group are (1) the *Persea* clade I, (2) the *Machilus* clade, and (3) a clade including species of *Alseodaphne*, *Dehaasia*, *Nothaphoebe*, *Persea*, and *Phoebe*. Some major clades in the ITS tree, which also appear in the ITS + *LEAFY* intron II tree (Fig. 4), are labeled for comparison. Species showing a conflicting position in the ITS and the ITS + *LEAFY* intron II trees are marked with ♦.

The Bayesian consensus tree based on the data set of total *LEAFY* intron II sequences (Fig. 3) shows that several different sequences obtained from one individual sample form a strongly supported monophyletic clade in most cases. Only a few exceptions occur among very closely related species (mostly in the genus *Machilus*, where the genetic distances are minimal, anyway). As in Fig. 2, some major clades are shown for comparison with the ITS + *LEAFY* intron II tree. Species marked with ♦ show a conflicting position in the *LEAFY* intron II and the ITS + *LEAFY* intron II trees.

Compared with the ITS + *LEAFY* intron II tree, both the ITS and the *LEAFY* intron II trees show less resolution and lower branch supports. Thus, these two trees are not used for the following discussion, and their topologies are not described any further.

The Bayesian consensus tree based on the combined ITS + *LEAFY* intron II data set (Fig. 4) is largely congruent or at least compatible with the *LEAFY* intron II tree (Fig. 3). As in the separate ITS and *LEAFY* intron II trees, the outgroup species are consistently separated from the *Persea* group, and all species (so far investigated) from the *Persea* group form a well-defined monophyletic clade (90% BS and 100% PPS). Within the *Persea* group, there are three principal clades (clade I, II, and III). At the top of the *Persea* group, clade I consists not only of all representatives of *Machilus* and *Dehaasia* included in the present study, but also of most species of *Alseodaphne* (including the type species, *A. semecarpifolia*) and *Nothaphoebe umbelliflora*, the only representative of that genus here. It receives 100% PPS (but no significant BS), and has two principal subclades. The *Machilus* Clade consists of all representatives of *Machilus* included in the present study, plus two *Phoebe* species. It receives both 100% BS and PPS, and shows very little internal resolution, due to extremely low genetic distances among the species of *Machilus*. Sister to the *Machilus* clade is *Dehaasia caesia* with both 100% BS and PPS. The *Alseodaphne-Dehaasia* clade consists of four *Alseodaphne* species and four *Dehaasia* species, as well as *Nothaphoebe umbelliflora* from tropical Asia, which is deeply imbedded among *Alseodaphne* and *Dehaasia* species. The *Alseodaphne-Dehaasia* clade receives 84% BS and 100% PPS, and shows very good internal resolution. Clade II, which is sister to clade I, consists mainly of American *Persea* species, plus *Persea indica* and *Apollonias barbujana* from the Macaronesian Islands. It receives no significant BS but a moderate PPS (92%) and has two principal subclades. The *Persea* clade I, which receives 84% BS and 100% PPS, comprises all species (so far investigated) that had been placed in *Persea* subg. *Eriodaphne* by Kopp (1966), plus a few that had been unknown at her time, and *Persea indica*. The *Persea* clade II, which receives 73% BS and 100% PPS, consists of *Persea americana* and *P. steyermarkii*, two of the three species attributed to *Persea* subg. *Persea* by Kopp (1966), as well as *Apollonias barbujana*. At the bottom of the *Persea* group, clade III consists of 23 species from three genera (*Alseodaphne*, *Persea* and *Phoebe*), and receives 100% PPS but no significant BS. Within clade III, three principal subclades form a trichotomy. The *Phoebe* clade consists of most *Phoebe* species

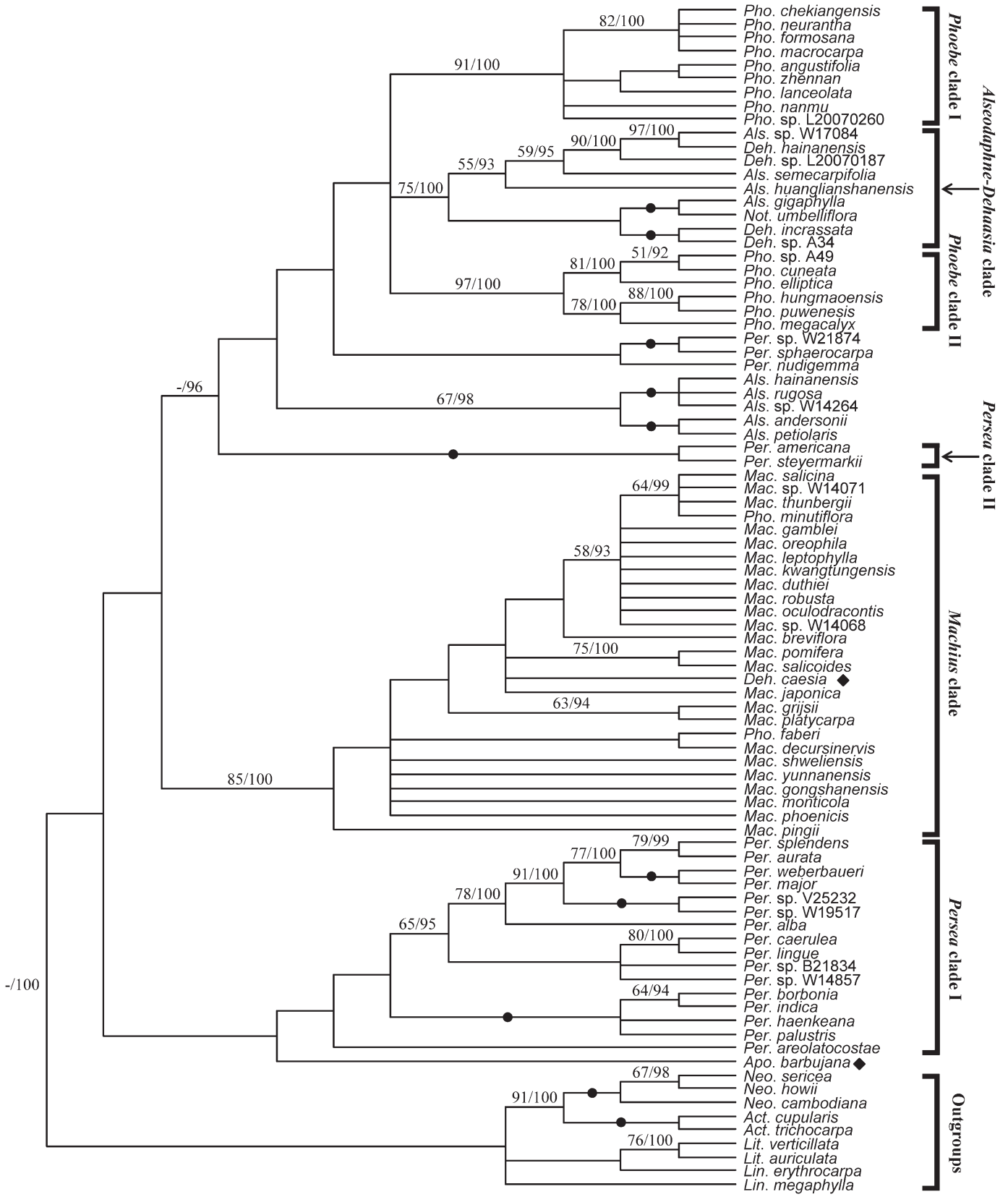

Fig. 2. Bayesian consensus tree based on ITS data. Bootstrap values (≥50%)/Bayesian posterior probabilities (≥90%) are shown above branches. • = both bootstrap value and Bayesian posterior probability 100%. Species marked with ♦ show conflicts of these major clades between ITS and ITS + *LEAFY* intron II trees.

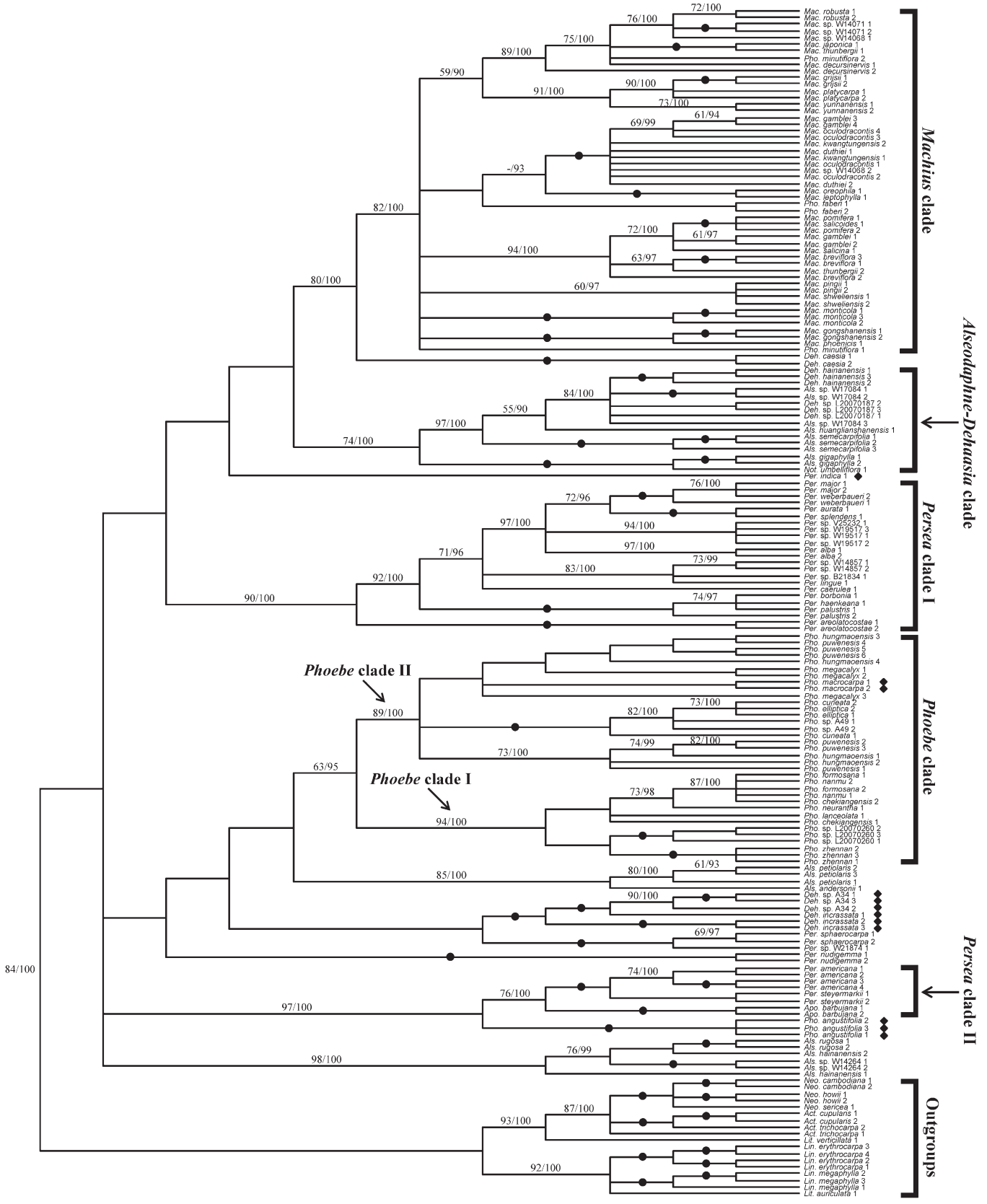

Fig. 3. Bayesian consensus tree based on *LEAFY* intron II data. Bootstrap values (≥50%)/Bayesian posterior probabilities (≥90%) are shown above branches. • = both bootstrap value and Bayesian posterior probability 100%. Species marked with ♦ show conflicts of these major clades between *LEAFY* intron II and ITS + *LEAFY* intron II trees.

included in the present study, and it receives 99% PPS but no significant BS. Within it, two principal subclades are indicated. The *Phoebe* clade I receives 73% BS and 100% PPS, while the *Phoebe* clade II receives 75% BS and 100% PPS. *Persea nudigemma* is weakly supported (only 72% PPS, not shown) as sister species to the *Phoebe* clade. Next to *Persea nudigemma*, three *Alseodaphne* species and two *Persea* species form a small clade that receives weak support (only 69% PPS, not shown). At the bottom of clade III, *Alseodaphne andersoni* and *A. petiolaris* form a small clade that receives both 100% BS and PPS.

Bayesian estimation of divergence times (Fig. 5)—The age estimates for the divergence time of the *Persea* group (node B, Fig. 5) using the different possible calibration points are given in Table 1. Because calibration at nodes 1, 2, 3, and 4 resulted in unlikely old age estimates (see discussion), we finally fixed the age of node A (Fig. 5) at 43 mya with a standard deviation of 3.5 mya, ranging from ~37 to 49 mya and roughly matching the late early Eocene to early late Eocene.

The divergence time of the *Persea* group was estimated as ~55.3 mya (95% higher posterior density [HPD] interval 41.4–69.9 mya, node B, Fig. 5). The first divergence within the *Persea* group was estimated as ~51.9 mya (95% HPD 38.9–63.9 mya, node C, Fig. 5), and the second divergence was obtained as ~48.5 mya (95% HPD 35.9–59.9 mya, node D, Fig. 5).

DISCUSSION

Machilus clade—Just as the results of Rohwer et al. (2009) and Chen et al. (2009) have shown, this genus is by far the most homogeneous group in our molecular analysis. Our results indicate that *Machilus* is monophyletic within the *Persea* group, and its persistent and spreading to reflexed tepals in fruit are important morphological characters for its generic delimitation against the closely related genera *Alseodaphne*, *Dehaasia*, *Nothaphoebe*, and *Persea*. The position of *Phoebe faberi* and *P. minutiflora* within the *Machilus* clade is not surprising. *Phoebe faberi* originally had been described as *Machilus faberi* by Hemsley (in J. Linn. Soc., Bot. 26: 375. 1891), then it was transferred to *Phoebe* by Chun [in Contr. Biol. Laboratory. Sci. Soc. China 1(5): 31–32. 1925]. Its persistent perianth lobes are ovate, leathery, slightly clasping or lax, with extrorse apices when fruiting (Li et al., 2008). *Phoebe minutiflora* has almost the same perianth characters as *M. faberi* when fruiting. Its persistent perianth lobes are slightly thickened, loose, with the apex extrorse or patent (Li et al., 2008). Compared with the perianth characters of *Phoebe* species, where the tepals are leathery to woody, conspicuously thickened and clasping the base of the fruit, these two species are obviously different and more like *Machilus* species. Besides, the fruits of *Phoebe faberi* and *P. minutiflora* are globose, also more like *Machilus* species instead of ellipsoid to elongate, as in *Phoebe* species (Li et al., 2008). Therefore, we think these two species are misplaced in *Phoebe* and should be transferred to *Machilus*. The position of *Dehaasia caesia* as sister to *Machilus* is questionable. Our sample is from a sterile specimen, so that we cannot exclude a misidentification, even though the vegetative characters matched *Dehaasia caesia* (Deby Arifiani, Herbarium Bogoriense, Research Center for Biology-LIPI, Cibinong Science Center, Indonesia, personal communication).

Our study again failed to get a good resolution of the internal structure within the genus *Machilus*, just like the previous works by Rohwer et al. (2009) and Chen et al. (2009). This might be explained by a rather recent species differentiation and/or a strongly decreased substitution rate within this genus (Rohwer et al., 2009). Nevertheless, the presently accepted sections or subsections of *Machilus* as treated by Li et al. (1984) still are questionable according to the limited resolution within the *Machilus* clade. The largest group within the *Machilus* clade receiving a high PPS in the Bayesian analysis, the clade including *Machilus robusta*, *M.* sp. W14068, *M.* sp. W14071, *M. japonica*, *M. decursinervis*, *M. grijsii*, *M. platycarpa*, *M. yunnanensis* and *Phoebe minutiflora*, includes at least one species from *Machilus* sect. *Machilus*, one species from sect. *Glabriflorae* S. Lee, one species from sect. *Megalocarpae* S. Lee, and two species from sect. *Tomentosae* S. Lee. This inconsistency suggests that traditionally used morphological features, such as the presence or absence of hairs on the outside of the tepals in flower, and the shape and size of the fruit are not sufficient as characters for infrageneric delimitation within *Machilus*. However, a much denser sampling of the species and some new molecular markers with higher resolution will be necessary to resolve the relationships within the genus.

Persea clade—Although most of the *Persea* species (so far investigated) form a monophyletic clade with a moderate PPS (92%, Fig. 4), the presence of *Apollonias barbujana* within this clade and the absence of three other *Persea* species (i.e., *Persea nudigemma*, *P. sphaerocarpa* and *P.* sp. W21874) indicate that the genus *Persea*, as presently circumscribed, is polyphyletic. Despite that, the two subgenera (*Persea* subg. *Eriodaphne* and *P.* subg. *Persea*), that were accepted by Kopp (1966), are well supported as monophyletic groups by the molecular data (Fig. 4). *Persea* clade I, which receives 84% BS and 100% PPS, comprises all species (so far investigated) that had been placed in *Persea* subg. *Eriodaphne* by Kopp (1966), plus a few that had been unknown at her time, and the Macaronesian *Persea indica*. Within *Persea* clade II, a small clade receiving 100% BS and PPS consists of *Persea americana* and *P. steyermarkii*, two of the three species that had been attributed to *Persea* subg. *Persea* by Kopp (1966). The third species, *Persea schiedeana* Nees, is not included here, but was strongly supported as sister to these two species in the analysis of Rohwer et al. (2009). In contrast to their study, however, our new results allow retaining *Persea* as a monophyletic genus, if a few species that do not fit morphologically into the established subgenera are excluded (see below), and if *Apollonias barbujana* is included. The genus *Apollonias* has been kept separate mainly because of its disporangiate anthers, but several *Persea* species (albeit in subgen. *Eriodaphne*) have disporangiate anthers as well (Kopp, 1966), and the number of pollen sacs alone is not sufficient as a generic character (Rohwer et al., 2009). The tepals of *A. barbujana* are equal to slightly unequal, and in the fruiting stage they are appressed to the berry, with only their base slightly thickened and their tip sometimes breaking off. They are not too different from those of *Persea americana* (subgen. *Persea*), where they are not appressed but spreading to reflexed, but ultimately their basal part persists (even though this appears insignificantly small, compared to the size of the fruit). Because in our molecular data *A. barbujana* forms a relatively well-supported clade (the *Persea* clade II with 73% BS and 100% PPS) along with *P. americana* and *P. steyermarkii*, we suggest to include *A. barbujana* in *Persea* (rather than to keep *Apollonias* and to create a new genus for *Persea* subgen. *Eriodaphne*, which would require about a hundred new combinations). Unfortunately, our

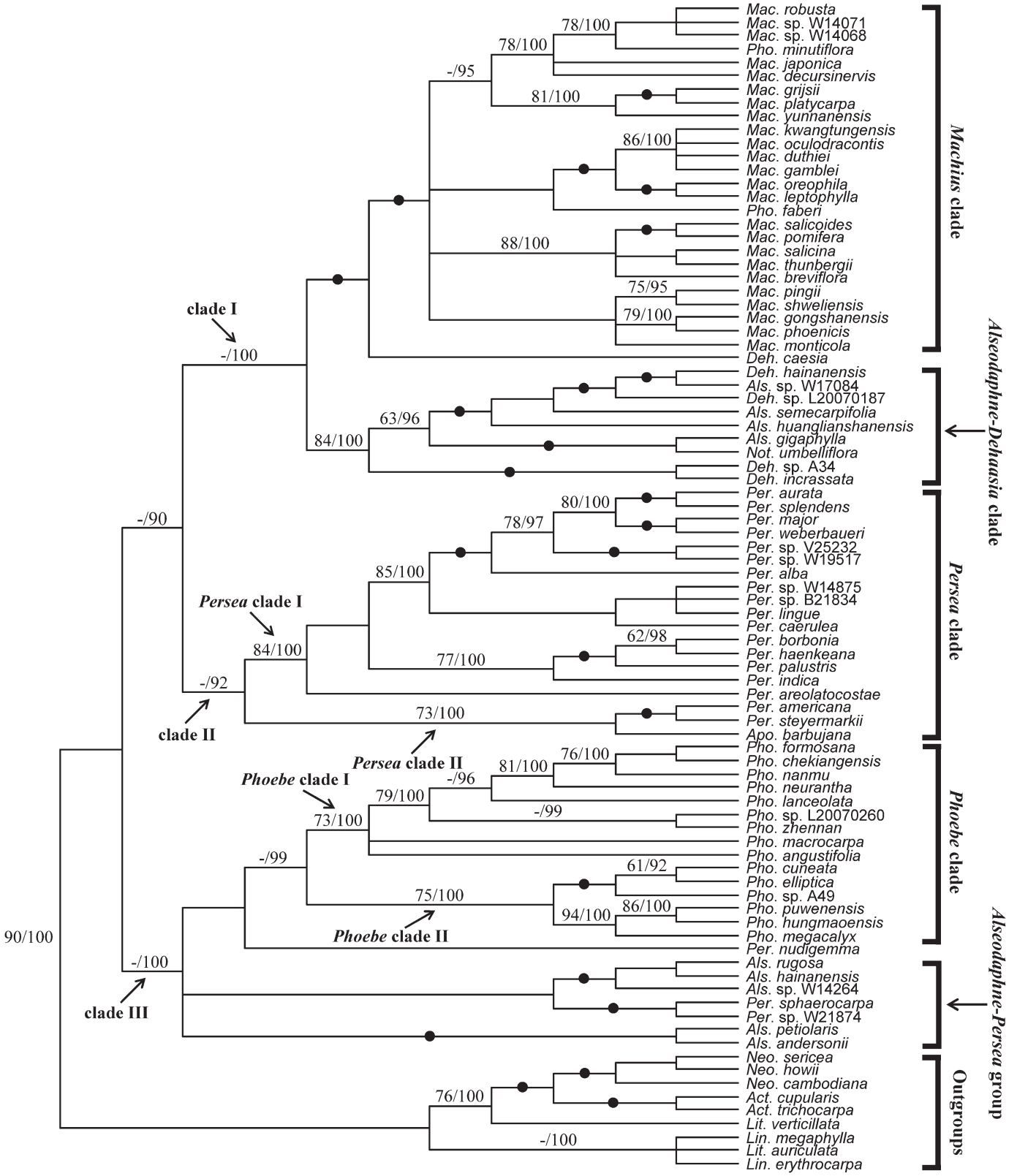

Fig. 4. Bayesian consensus tree based on ITS + *LEAFY* intron II combined data. Bootstrap values (≥50%)/Bayesian posterior probabilities (≥90%) are above branches. • = both bootstrap value and Bayesian posterior probability 100%.

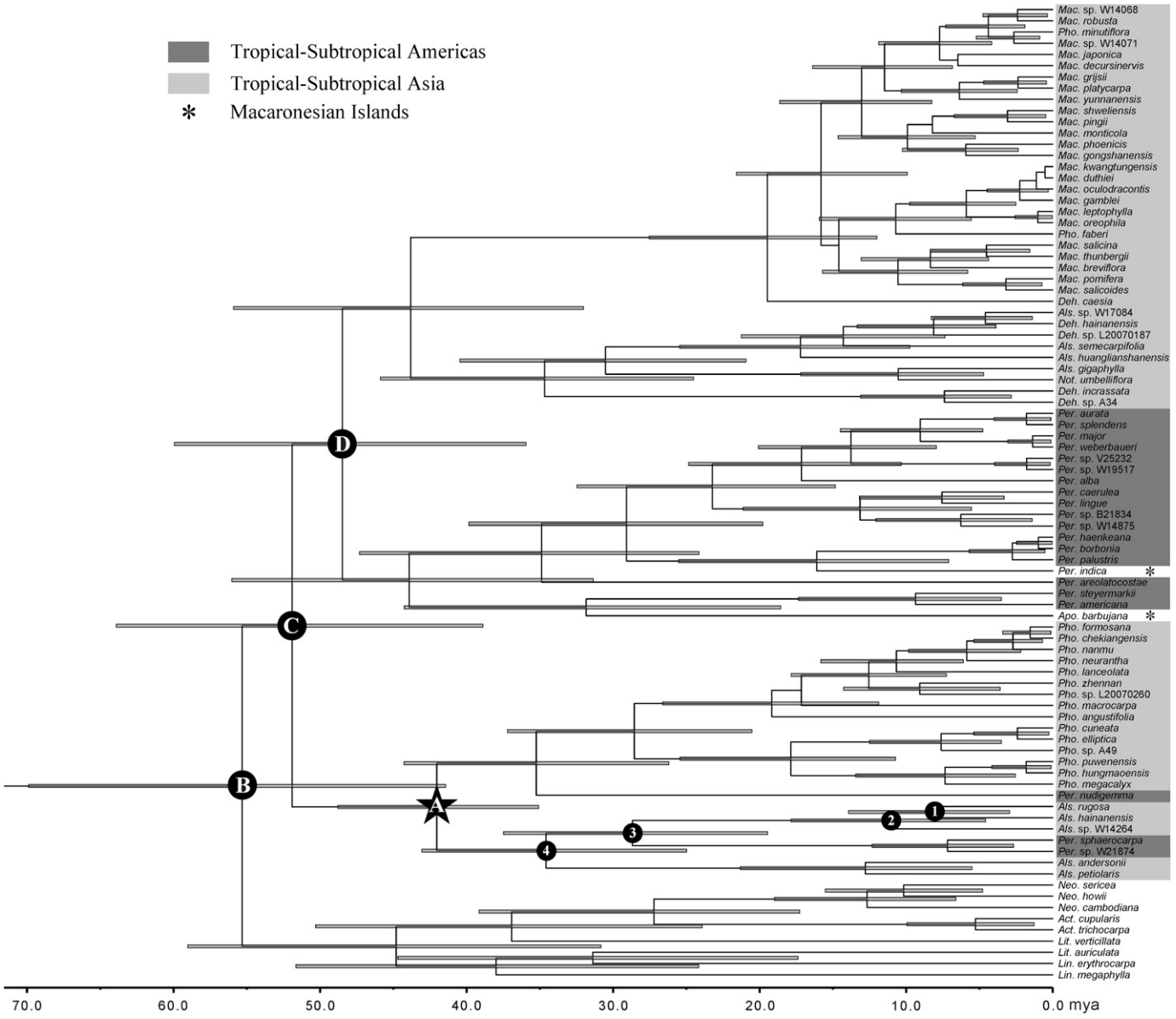

Fig. 5. Chronogram of the *Persea* group and outgroups based on the ITS and *LEAFY* intron II combined data inferred using BEAST software. Calibration point is indicated with black asterisk. Gray bars at the internal nodes represent the 95% high posterior density credibility interval for node ages.

attempts to amplify any DNA fragment from herbarium material of the second species of *Apollonias*, *A. arnottii* Nees from India, have not been successful so far. Rohwer et al. (2009) expected that this species would group with one of the Asian genera (e.g., *Machilus* or *Phoebe*) rather than with *A. barbujana*.

TABLE 1. The age estimates for the divergence time of the *Persea* group (node B, Fig. 5) using different possible calibration points.

Calibration point	Mean of age estimation (mya)	95% HPD (mya)
Node 1	~166.8	86.5–260.3
Node 2	~141.6	63.8–232.9
Node 3	~80.2	51.1–115.4
Node 4	~66.5	46.0–91.0
Node A	~55.3	41.4–69.9

Notes: HPD, high posterior density

As in the results of Rohwer et al. (2009), the ITS sequences and combined data in the present study (Figs. 2, 4) clearly support an American affinity of the Macaronesian *Persea indica*. Although the *LEAFY* intron II tree (Fig. 3) is conflicting in this respect, the branch linking *P. indica* to Asian taxa is very weakly supported (only 54% PPS, not shown). Therefore, we think it is safe to reject the opinion of Rohwer (1993) that this species was possibly misplaced in *Persea*. The tepals of *P. indica* are equal, as in *P. areolatocostae* (also included in *Persea* clade I), although most species of *Persea* subg. *Eriodaphne* have unequal tepals. In the fruiting stage, its tepals are at least initially appressed to the berry (but sometimes spreading in old fruits), with their base slightly more thickened than in *Apollonias* and their tip breaking off more frequently.

Thus, the difference in this character is only gradual. The fruiting pedicel, however, is more thickened toward the fruit than in the other *Persea* species (except *P. americana*, where the fruit is much larger).

Except three species (i.e., *P. nudigemma*, *P. sphaerocarpa* and *P.* sp. W21874), all *Persea* species investigated in the present study as well as *A. barbujana* form the monophyletic *Persea* clade. Although *Persea* subgen. *Eriodaphne* and *Persea* subgen. *Persea* form distinct clades in the molecular tree and (usually) can be recognized by morphological differences, they still belong to one monophyletic group in the present study. Thus, the suggestion of treating *Persea* subg. *Eriodaphne* as a separate genus (Rohwer et al., 2009) seems unnecessary.

Phoebe clade—All species of the genus *Phoebe* investigated based on ITS data by Rohwer et al. (2009) were found in two well-supported clades, part of an unresolved trichotomy with the *Alseodaphne-Dehaasia* clade. After checking the individual trees retrieved in their analysis, they noticed that *Phoebe* might be monophyletic. This assumption is further confirmed in the present study. Except two species misplaced in *Phoebe* (i.e., *P. faberi* and *P. minutiflora*, discussed above), the *Phoebe* clade consists of all *Phoebe* species so far investigated, and it receives 99% PPS (Fig. 4). The complicated taxonomic history of *Nothaphoebe cavaleriei*, treated as *Phoebe* sp. L20070260 here, has already been discussed by Rohwer et al. (2009), and it has been confirmed again that this species really is a *Phoebe*. Therefore, our study indicates that *Phoebe*, as *Machilus*, is monophyletic within the *Persea* group, and its persistent, thickened, leathery to woody tepals, which clasp the base of the fruit, are important characters for its generic delimitation against other genera within the *Persea* group.

Alseodaphne-Dehaasia *clade and Alseodaphne-Persea group*—The *Alseodaphne-Persea* group consists of two small clades, which do not form a clade in the Bayesian consensus tree (Fig. 4). Inspection of the individual trees retrieved in our analysis reveals that the two clades do form a monophyletic group in some of them, but not frequently enough to reach 50% PPS. Just for the purpose of discussion, we put these two small clades together and call them *Alseodaphne-Persea* group.

According to the molecular tree based on the combined ITS + *LEAFY* intron II data (Fig. 4), both *Alseodaphne* and *Dehaasia* are polyphyletic within the *Persea* group. *Alseodaphne* at least has two different origins. Of the nine species investigated, four fall into *Alseodaphne-Dehaasia* clade, whereas five are found in the two clades of the *Alseodaphne-Persea* group. The *Alseodaphne-Dehaasia* clade includes the type species, *A. semecarpifolia*, so that the name *Alseodaphne* will stay with this clade, which represents the traditionally recognized typical *Alseodaphne* species distributed mainly in tropical Asia. The origin of the other five *Alseodaphne* species is apparently different. Most of them (except *A.* sp. W14264) are from tropical and subtropical China, and they may have a single origin, as the branch linking three of them to two species currently placed in *Persea* is not significantly supported. Li et al. (2009) discovered the fossil of *Alseodaphne changchangensis* in Changchang Basin of Hainan Island (China), which is the earliest occurrence of the genus *Alseodaphne* and closest to the living species *A. hainanensis* (within the *Alseodaphne-Persea* group). The fact that *Alseodaphne andersonii* and *A. petiolaris*, which form a separate clade here, differ also morphologically from the traditionally recognized *Alseodaphne* species has already been discussed by Rohwer et al. (2009). In Yunnan (China), at least three further *Alseodaphne* species (*A. sichourensis* H. W. Li, *A. marlipoensis* (H. W. Li) H. W. Li and *A. hokouensis* H. W. Li) are similar to *A. andersonii* and *A. petiolaris*, so that we can expect this group to become larger with increased taxon sampling.

The species of the genus *Dehaasia* are found scattered throughout the *Alseodaphne-Dehaasia* clade (Fig. 4), with the exception of *D. caesia* (see above). Therefore, it will eventually become inevitable to include *Dehaasia* in *Alseodaphne*. It has long been known that the two genera are insufficiently separated (e.g., Kostermans [1973, p. 425]: "*Dehaasia* and *Alseodaphne* are very closely related; the only difference is the number of anther cells, 2 in the former, 4 in the latter… This anther distinction is not correlated with vegetative characters, hence in a sterile state it is not possible (unless the species are known) to distinguish between *Alseodaphne* and *Dehaasia*."; van der Werff [2001, p. 136]: "*Dehaasia* is closely related to *Alseodaphne*, from which it differs only in having 2-locular instead of 4-locular anthers. Vegetatively, it is indistinguishable from *Alseodaphne* and without stamens assigning a specimen to either of these genera is a guess."). Of course, we urge that a careful revision be made before creating dozens of new combinations (although many species have a name in both genera already).

Nothaphoebe umbelliflora, the only species of *Nothaphoebe* in the present study, is nested in the *Alseodaphne-Dehaasia* Clade, as sister to *Alseodaphne gigaphylla* (Fig. 4). Rohwer et al. (2009) found a similarly close relationship between *Nothaphoebe umbelliflora* and *Alseodaphne nigrescens* (Gamble) Kosterm. As the sequences involved were all from sterile specimens from Singapore, they assumed that a misidentification might be involved. However, a similarly close relationship between *N. umbelliflora* and an *Alseodaphne* species was found in the present study, based on another resource of *N. umbelliflora* from Indonesia and different *Alseodaphne* species, so that the assumption made by Rohwer et al. (2009) can be rejected. van der Werff (2001) pointed out that the delimitation between *Alseodaphne* and *Nothaphoebe* was rather vague (only tepals absent vs. persistent when fruiting), and *Nothaphoebe* should be placed in *Alseodaphne*.

According to the results of our study, we think a major revision is needed for the delimitation between *Alseodaphne*, *Dehaasia*, and *Nothaphoebe* due to the weakness of the generic concepts within the *Persea* group. The revision, however, would require a far larger number of species of these three genera to be investigated because the taxon sample is too small in the present study and any conclusion would be premature.

Persea nudigemma, P. sphaerocarpa, *and* P. sp. *W21874*—These three *Persea* species are well separated from most of the *Persea* species investigated in the present study (Fig. 4). *Persea nudigemma* appears to be more closely related with the *Phoebe* clade than with the *Alseodaphne-Persea* group, but this relationship gets only 72% PPS (not shown) and is therefore still uncertain. As described by van der Werff (1994), *Persea nudigemma* appears to belong to a small group of neotropical *Persea* species with equal tepals that are pubescent on both sides and persistent in the fruiting stage (e.g., *P. bernardii* Kopp, *P. rigens* Allen, *P. silvatica* van der Werff, species not investigated in the present study). These species are similar not only to *Persea indica*, but also to *Phoebe*, which may explain the position of *Persea nudigemma* in our analysis. The position of *Persea sphaerocarpa* within the *Alseodaphne-Persea* group in the

analysis is also explainable. van der Werff (2002, p. 575) already noted that this species, together with *Persea albiramea* van der Werff and *P. laevifolia* van der Werff (both collected in Central America), "would very likely be included in *Alseodaphne* had they been collected in tropical Asia." Although *Persea* sp. W21874 is not particularly similar to *P. sphaerocarpa*, its position is not really surprising, either. Its *LEAFY* intron II sequence differs by just three base pairs from that of *Persea sphaerocarpa*. With its stubby twigs with large inflorescence scars and conspicuous bract scars, it is reminiscent not only of *Persea americana*, but also of some species presently placed in *Alseodaphne*, particularly *Alseodaphne petiolaris*, which is found in a closely related clade here.

The variation among the neotropical species presently placed in the genus *Persea* is comparable to the variation among the paleotropical species of the *Persea* group, but while the paleotropical species are divided into several genera that are hard to separate, the neotropical species are placed in one variable genus. Apparently, the origins of *Persea nudigemma*, *P. sphaerocarpa*, and *Persea* sp. W21874 are quite different from most *Persea* species, which belong to the *Persea* clade. Together with the species in this group presently placed in *Alseodaphne* they may perhaps form an additional, hitherto unrecognized genus. However, it is not yet clear how this group can be recognized morphologically—and we certainly do not want another ill-defined genus. The alternative, however, viz. stretching the limits of *Phoebe* so far as to include these species, is not appealing either.

Origin of the* Persea *group—Fixing the calibration point at node 1 or 2 resulted in the estimation of the divergence time of the *Persea* group (node B, Fig. 5) as ~166.8 mya or ~141.6 mya. This age estimation is obviously much too old, older than or about as old as the oldest undisputed angiosperm fossils (~140 mya, Else Marie Friis, Swedish Museum of Natural History, personal communication). Fixing the calibration point at node 3 or 4 resulted in the estimation of the divergence time of the *Persea* group (node B, Fig. 5) as 80.2 mya or 66.5 mya. This would be late Cretaceous, a time from which the Lauraceae are known to have existed—the earliest lauraceous fossils of the extinct genus *Mauldinia* are ~100 million years old (Drinnan et al., 1990, Frumin et al., 2004). However, these estimates appear still too high, considering the fact that the *Persea* group is among the more distal branches in the Lauraceae (Rohwer and Rudolph, 2005). According to the work of Chanderbali et al. (2001), some basal lineages of Lauraceae were established on either Gondwana or Laurasia by the late Cretaceous. *Caryodaphnopsis* Airy Shaw and *Neocinnamomum* H. Liu, with their relatively basal positions in Lauraceae, might be the only extant representatives of the ancient Lauraceae flora documented in mid- to late Cretaceous (Chanderbali et al., 2001). Thus, the fact that no fossil evidence supports the late Cretaceous origin of the *Persea* group makes the placement of the calibration point at node 3 or 4 somewhat questionable. We finally chose node A as the calibration point and obtained a reasonable age estimation (discussed below). It was also reasonable to place the fossil of *Alseodaphne changchangensis* at the beginning of this lineage because it was the earliest occurrence of the genus *Alseodaphne* and related to the extant *A. hainanensis*.

Using this calibration, we estimated the divergence time between the *Persea* group and the Laureae as ~55.3 mya (95% HPD 41.4–69.9 mya, node B, Fig. 5) in the present study. Although this is about 10 million years earlier than the estimates made by Chanderbali et al. (2001) as 44 ± 7 mya and by Nie et al. (2007) as 43.54 ± 1.96 mya, we reach the same general conclusion as Chanderbali et al. (2001, p. 104): "Remaining genera place in a terminal Perseeae-Laureae clade that radiated in early Eocene Laurasia." This estimation of an early Eocene origin of the *Persea* group is supported by the fossil record. The hemispherical cupules of the London Clay Flora (Reid and Chandler, 1933) are restricted to Laureae and Cinnamomeae of the Perseeae-Laureae clade. Well-preserved flowers with the general floral structure of genera in the *Persea* group and Cinnamomeae, but not other members of Lauraceae, have been described from Eocene deposits in North America (Taylor, 1988) and Late Eocene Baltic amber (Conwentz, 1886). Therefore, our results suggest that the *Persea* group originated from the Perseeae-Laureae radiation in early Eocene Laurasia.

The amphi-pacific disjunction pattern of the* Persea *group—According to paleobotanical and geological evidence, the early Eocene (54–50 mya) was the warmest period in the Tertiary, and the boreotropical flora spread circumboreally to high latitudes in the northern hemisphere (Reid and Chandler, 1933; Chandler, 1964; Wolfe, 1978, 1997; Collinson et al., 1981; Miller et al., 1987; Graham, 1999). The paleobotanical evidence indicates that in the late early and early mid-Eocene (50–48 mya) a significant cooling occurred, followed by two steady intervals (46–43 and 37–34 mya) that were separated by a cool interval (42–38 mya) in the mid- to late Eocene (Wolfe, 1978, 1997). Oxygen isotope records also suggest very warm temperatures in the early Eocene, but cooling proceeded during the mid- to late Eocene, with small fluctuations (Miller et al., 1987). The cooling events caused movement of the boreotropical floral elements to lower latitudes, leading to segregation of ancestral lineages of modern tropical plants between North America and Eurasia. Much later, with the closure of the Central American land bridge in the Pliocene, several elements that today show a tropical amphi-pacific disjunction appear in northern South America (van der Hammen and Cleef, 1983; Azuma et al., 2001; Fritsch 2001; Wang et al., 2004).

The molecular phylogenetic analysis indicates that the first divergence within the *Persea* group occurred at ~51.9 mya (95% HPD 38.9–63.9 mya, node C, Fig. 5), within the warmest period in the Tertiary (50–54 mya). The boreotropical flora, including ancestral lineages of modern *Persea* group species, was distributed to high latitudes in the northern hemisphere. Thus, these ancestral lineages could spread between North America and Eurasia through high-latitude land bridges (Beringia or North Atlantic land bridge). The second divergence within the *Persea* group occurred at ~48.5 mya (95% HPD 35.9–59.9 mya, node D, Fig. 5) according to the molecular phylogenetic analysis. This would fall into the cooling period in the late early and early middle Eocene (50–48 mya). As thermophilic tropical elements, ancestral lineages of modern *Persea* group species would quickly respond to the cooling and moved to lower latitudes. The connection between North America and Eurasia was weakened and finally disrupted, so that there was no more exchange between the two continents among these ancestral lineages. This scenario would be consistent with the separation between the bulk of the Asian and American species within the *Persea* group (clade I and II in Fig. 4). Due to further cooling of the global climate, the *Persea* group, along with other boreotropical floral elements, later moved to subtropical and tropical regions of both continents, finally forming the tropical and subtropical amphi-pacific disjunction pattern.

The disjunct distribution between the three neotropical species currently placed in *Persea* (*P. nudigemma*, *P. sphaerocarpa* and *P.* sp. W21874) and the Asian species (*Phoebe* and some *Alseodaphne* species) within clade III (Fig. 4) cannot be explained by the disruption of the boreotropical ranges during the mid- to late Eocene as their divergence time was rather late according to the age estimation (Fig. 5). Their relative late divergence may indicate that a few long-distance dispersal events involving members of the *Persea* group happened after the segregation between North America and Eurasia, although the dispersal mechanism and routes are still unknown. As the distribution center of the modern *Phoebe* and *Alseodaphne* is tropical Asia, the earliest *Alseodaphne* fossil has been found in Hainan (China), and there are two American clades among three Asian clades in clade III (Fig. 4), a long-distance dispersal from Eurasia to North America appears more likely than vice versa. Thus, the present disjunction pattern of the *Persea* group may not only result from the segregation between North America and Eurasia but also from a few long-distance dispersal events (more discussion about long-distance dispersal can be found later, on the American-Macaronesian disjunction). Nevertheless, the relatively weak supports for the affinities between the three neotropical *Persea* species and the Asian species of clade III (*Phoebe* and some *Alseodaphne* species) make our assumption rather preliminary. Therefore, we suggest the neotropical species that are presently placed in *Persea* but fit neither of the established subgenera should be investigated with a much denser sampling and new molecular markers with higher resolution.

The American-Macaronesian disjunction pattern of the* Persea *group—A common origin of Macaronesian *Persea indica*, *Apollonias barbujana*, and neotropical *Persea* species is indicated in the present study (Fig. 4). In the study of Rohwer et al. (2009), two possible hypotheses were proposed to explain this disjunction pattern. One is the relict hypothesis, i.e., that *Persea indica* and *Apollonias barbujana* could be interpreted as relicts from the European-Mediterranean Tertiary laurel forest. This was based on the fact that the Lauraceae have a well-documented fossil record in the Northern Hemisphere, from the early mid-Cretaceous onwards, and they persisted in Europe with considerable diversity at least until the Miocene, in southern Europe even until the Pliocene (Mai, 1971; Taylor, 1988; Drinnan et al., 1990; Crane et al., 1994; Herendeen et al., 1994; Eklund and Kvaček, 1998; Takahashi et al., 1999; Eklund, 2000 and references cited therein). However, the fact that both *Persea indica* and *Apollonias barbujana* are sister to neotropical *Persea* clades from which they diverged at a time much later than the segregation between North America and Eurasia (Fig. 5) rejects the relict hypothesis.

The other possible explanation, viz., the long-distance dispersal hypothesis, was considered less likely by Rohwer et al. (2009). Lauraceae fruits are generally dispersed by birds (Snow, 1981; Moore and Willson, 1982; Wheelwright et al., 1984), but the fruits in the *Persea* group are relatively large and heavy, and birds generally do not fly long distances with such fruits in their beak or their intestines, crossing the Atlantic eastwards from the Americas to Macaronesia against the prevailing trade winds. However, some studies have already indicated that a lot of long-distance dispersal is caused by random incidents (Wilkinson, 1997; Cain et al., 1998; Renner, 2004) and that the dispersal mechanism of the plant itself sometimes is irrelevant for long-distance dispersal (Higgins et al., 2003). Therefore, long-distance dispersal is still a possible explanation of the American–Macaronesian disjunction between the neotropical *Persea* species and the Macaronesian species *Persea indica* and *Apollonias barbujana.*

Examples for an American–Macaronesian disjunction from other plant families are apparently rare. The textbook example of *Bystropogon* L'Hér./*Minthostachys* (Benth.) Spach (Lamiaceae) has recently been shown to be erroneous (Schmidt-Lebuhn, 2008), whereas a close relationship between the Macaronesian *Drusa glandulosa* (Poir.) H. Wolff ex Engl. and the Andean *Bowlesia palmata* Ruiz & Pavon was confirmed by Andersson et al. (2006). These authors, however, did not discuss the disjunction in terms of age, direction, and mechanism of dispersal. Panero et al. (1999) found Macaronesian *Pericallis* Webb (Asteraceae-Senecioneae) to be closely related to the North American genera *Packera* A. Löve & D. Löve, *Dorobaea* Cass, and *Pseudogynoxys* (Greenm.) Cabrera. They also suggested long-distance dispersal from the New World to the Macaronesian islands as the most likely explanation for this disjunction, as the tribe Senecioneae has a relatively recent post-Oligocene origin. However, in the Senecioneae with their small, wind-dispersed fruits it is of course much easier to envisage long-distance dispersal than in the Lauraceae.

Conclusions—Based on the phylogenetic analysis of ITS and *LEAFY* intron II sequences, several traditional genera or subgenera form well-supported monophyletic groups, viz., *Machilus*, *Persea* subg. *Eriodaphne*, *P.* subg. *Persea*, and *Phoebe*. The affinities of the Macaronesian species, *Persea indica* and *Apollonias barbujana*, are clearly American rather than Asian. *Apollonias barbujana* should be transferred to *Persea*, as its most important diagnostic character, viz., disporangiate anthers, occurs in neotropical *Persea* anyway. Both *Alseodaphne* (currently circumscribed as including only species with tetrasporangiate anthers) and *Dehaasia* (separated from *Alseodaphne* by nothing but disporangiate anthers, see van der Werff, 2001) are polyphyletic. While *Alseodaphne* has at least two different origins in different parts of the *Persea* group, the species of *Dehaasia* so far investigated are scattered in one of the two *Alseodaphne* groups. The only species of *Nothaphoebe* so far investigated, *N. umbelliflora*, is likewise nested in *Alseodaphne*, supporting the merging of *Nothaphoebe* in *Alseodaphne* by van der Werff (2001). Still, a major revision of *Alseodaphne*, *Dehaasia*, and *Nothaphoebe* is needed before numerous nomenclatural changes are made in these genera. According to the divergence time estimation, we suggest that the *Persea* group originated from the Perseeae-Laureae radiation in early Eocene Laurasia. The tropical and subtropical amphipacific disjunction pattern of the *Persea* group probably resulted from the disruption of the boreotropical flora by climatic cooling during the mid- to late Eocene. However, a few long-distance dispersal events are likely to have occurred in the *Persea* group after the separation between the American and Eurasian boreotropical forests. The American–Macaronesian disjunction pattern in the *Persea* group is also most likely explained by long-distance dispersal.

LITERATURE CITED

ANDERSSON, L., M. KOCSIS, AND R. ERIKSSON. 2006. Relationships of the genus *Azorella* (Apiaceae) and other hydrocotyloids inferred from sequence variation in three plastid markers. *Taxon* 55: 270–280.

AZUMA, H., J. G. GARCÍA-FRANCO, V. RICO-GRAY, AND L. B. THIEN. 2001. Molecular phylogeny of the Magnoliaceae: The biogeography of tropical and temperate disjunctions. *American Journal of Botany* 88: 2275–2285.

BLÁZQUEZ, M. A. 1997. Illuminating flowers: Constans induces *LEAFY* expression. *BioEssays* 19: 277–279.

BLÁZQUEZ, M. A., L. N. SOOWAL, I. LEE, AND D. WEIGEL. 1997. *LEAFY* expression and flower initiation in *Arabidopsis. Development* 124: 3835–3844.

BUCKLER, E. S., AND T. P. HOLTSFORD. 1996. *Zea* systematics: Ribosomal ITS evidence. *Molecular Biology and Evolution* 13: 612–622.

BUCKLER, E. S., A. IPPOLITO, AND T. P. HOLTSFORD. 1997. The evolution for ribosomal DNA: Divergent paralogues and phylogenetic implications. *Genetics* 145: 821–832.

CAIN, M. L., H. DUMMAN, AND A. MUIR. 1998. Seed dispersal and the Holocene migration of woodland herbs. *Ecological Monographs* 68: 325–347.

CHANDERBALI, A. S., H. VAN DER WERFF, AND S. S. RENNER. 2001. Phylogeny and historical biogeography of Lauraceae: Evidence from the chloroplast and nuclear genomes. *Annals of the Missouri Botanical Garden* 88: 104–134.

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

CHEN, J. Q., L. LI, J. LI, AND H. W. LI. 2009. Bayesian inference of nrDNA ITS sequences from *Machilus* (Lauraceae) and its systematic significance. *Acta Botanica Yunnanica* 31: 117–126 (in Chinese with English abstract).

COLLINSON, M. E., K. FOWLER, AND M. C. BOULTER. 1981. Floristic changes indicate a cooling climate in the Eocene of southern England. *Nature* 291: 315–317.

CONWENTZ, H. 1886. Die Angiosperms des Bernsteins. Die Flora des Bernsteins, vol. 2. Wilhelm Engelmann, Leipzig, Germany.

CRANE, P. R., E. M. FRIIS, AND K. R. PEDERSEN. 1994. Palaeobotanical evidence on the early radiation of magnoliid angiosperms. *Plant Systematics and Evolution* 8: 51–72.

DONOGHUE, M. J., C. D. BELL, AND J. H. LI. 2001. Phylogenetic patterns in Northern Hemisphere plant geography. *International Journal of Plant Sciences* 162: S41–S52.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

DRINNAN, A. N., P. R. CRANE, E. M. FRIIS, AND K. R. PEDERSEN. 1990. Lauraceous flowers from the Potomac Group (mid-Cretaceous) of eastern North America. *Botanical Gazette* 151: 370–384.

DRUMMOND, A. J., AND A. RAMBAUT. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7: 214.

DRUMMOND, A. J., S. Y. W. HO, M. J. PHILLIPS, AND A. RAMBAUT. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biology* 4: 699–710.

EKLUND, H. 2000. Lauraceous flowers from the Late Cretaceous of North Carolina, USA. *Botanical Journal of the Linnean Society* 132: 397–428.

EKLUND, H., AND J. KVAČEK. 1998. Lauraceous inflorescences and flowers from the Cenomanian of Bohemia (Czech Republic, Central Europe). *International Journal of Plant Sciences* 159: 668–686.

FARRIS, J. S., M. KÄLLERSJÖ, A. G. KLUGE, AND C. BULT. 1994. Testing significance of incongruence. *Cladistics* 10: 315–319.

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. *Molecular Phylogenetics and Evolution* 19: 387–408.

FROHLICH, M. W., AND E. M. MEYEROWITZ. 1997. The search for flower homeotic gene homologs in basal angiosperms and Gentales: A potential new source of data on the evolutionary origin of flowers. *International Journal of Plant Sciences* 158: S131–142.

FRUMIN, S., H. EKLUND, AND E. M. FRIIS. 2004. *Mauldinia hirsuta* sp. nov., a new member of the extinct genus *Mauldinia* (Lauraceae) from the Late Cretaceous (Cenomanian-Turonian) of Kazakhstan. *International Journal of Plant Sciences* 165: 883–895.

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

GROB, G. B. J., B. GRAVENDEEL, AND M. C. M. EURLINGS. 2004. Potential phylogenetic utility of the nuclear *FLORICAULA/LEAFY* second intron: Comparison with three chloroplast DNA regions in *Amorphophallus* (Araceae). *Molecular Phylogenetics and Evolution* 30: 13–23.

HALL, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nuclear Acids Symbol Series* 41: 95–98.

HERENDEEN, P. S. 1991. Lauraceous wood from the mid-Cretaceous Potomac Group of eastern North America: *Paraphyllanthoxylon marylandense* sp. nov. *Review of Palaeobotany and Palynology* 69: 277–290.

HERENDEEN, P. S., W. L. CREPET, AND K. C. NIXON. 1994. Fossil flowers and pollen of Lauraceae from the Upper Cretaceous of New Jersey. *Plant Systematics and Evolution* 189: 29–40.

HIGGINS, S. I., S. LAVOREL, AND E. REVILLA. 2003. Estimating plant migration rates under habitat loss and fragmentation. *Oikos* 101: 354–366.

HOOT, S. B., AND W. C. TAYLOR. 2001. The utility of nuclear ITS, a *LEAFY* homolog intron, and chloroplast *atpB-rbcL* spacer region data in phylogenetic analyses and species delimitation in *Isoetes*. *American Fern Journal* 91: 166–177.

HOWARTH, D. G., AND D. A. BAUM. 2005. Genealogical evidence of homoploid hybrid speciation in an adaptive radiation of *Scaevola* (Goodeniaceae) in the Hawaiian Islands. *Evolution; International Journal of Organic Evolution* 59: 948–961.

HUELSENBECK, J. P., AND F. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

KOPP, L. E. 1966. A taxonomic revision of the genus *Persea* in the Western Hemisphere (Perseae-Lauraceae). *Memoirs of the New York Botanical Garden* 14: 1–120.

KOSTERMANS, A. J. G. H. 1957. Lauraceae. *Pengumuman Balai Besar Penjelidikan Kehutanan Indonesia* 57: 1–64.

KOSTERMANS, A. J. G. H. 1973. A synopsis of the genus Dehaasia Bl. (Lauraceae). *Botanische Jahrbucher fur Systematik, Pflanzengeschichte und Pflanzengeographie* 93: 424–480.

LI, H. W., J. LI, P. H. HUANG, F. N. WEI, H. B. CUI, AND H. VAN DER WERFF. 2008. Lauraceae. *In* Z. Y. Wu, P. H. Raven, and D. Y. Hong [eds.], Flora of China, vol. 7, 102–254. Science Press, Beijing, China and Missouri Botanical Garden Press, St. Louis, Missouri, USA.

LI, H. W., P. Y. PAI, S. K. LEE, F. N. WEI, Y. T. WEI, Y. C. YANG, P. H. HUANG, ET AL. 1984. Lauraceae. *In* H. W. Li [eds.], Flora Reipublicae Popularis Sinicae, vol. 31, 1–463. Science Press, Beijing, China.

LI, J., D. C. CHRISTOPHEL, J. G. CONRAN, AND H. W. LI. 2004. Phylogenetic relationships within the 'core' Laureae (*Litsea* complex, Lauraceae) inferred from sequences of the chloroplast gene *mat*K and nuclear ribosomal DNA ITS regions. *Plant Systematics and Evolution* 246: 19–36.

LI, J. Z., J. QIU, W. B. LIAO, AND J. H. JIN. 2009. Eocene fossil *Alseodaphne* from Hainan Island of China and its paleoclimatic implications. *Science in China, series D, Earth Sciences* 52: 1537–1542.

LI, L., J. LI, J. G. CONRAN, AND H. W. LI. 2007. Phylogeny of *Neolitsea* (Lauraceae) inferred from Bayesian analysis of nrDNA ITS and ETS sequences. *Plant Systematics and Evolution* 269: 203–221.

MAI, D. H. 1971. Über fossile Lauraceae und Theaceae in Mitteleuropa. *Feddes Repertorium* 82: 313–341.

MILLER, K. G., R. G. FAIRBANKS, AND G. S. MOUNTAIN. 1987. Tertiary oxygen isotope synthesis, sea level history, and continental margin erosion. *Paleoceanography* 2: 1–19.

MOORE, L. A., AND M. F. WILLSON. 1982. The effect of microhabitat, spatial distribution, and display size on dispersal of *Lindera benzoin* by avian frugivores. *Canadian Journal of Botany* 60: 557–560.

NIE, Z. L., J. WEN, AND H. SUN. 2007. Phylogeny and biogeography of *Sassafras* (Lauraceae) disjunct between eastern Asia and eastern North America. *Plant Systematics and Evolution* 267: 191–203.

OH, S. H., AND D. POTTER. 2003. Phylogenetic utility of the second intron of *LEAFY* in *Neillia* and *Stephanandra* (Rosaceae) and implications for the origin of *Stephanandra*. *Molecular Phylogenetics and Evolution* 29: 203–215.

OH, S. H., AND D. POTTER. 2005. Molecular phylogenetic systematics and biogeography of tribe *Neillieae* (Rosaceae) using DNA sequences of cpDNA, rDNA, and *LEAFY*. *American Journal of Botany* 92: 179–192.

PANERO, J. L., J. FRANCISCO-ORTEGA, R. K. JANSEN, AND A. SANTOS-GUERRA. 1999. Molecular evidence for multiple origins of woodiness and a New World biogeographic connection of the Macaronesian Island endemic *Pericallis* (Asteraceae: Senecioneae). *Proceedings of the National Academy of Sciences, USA* 96: 13886–13891.

POSADA, D., AND T. R. BUCKLEY. 2004. Model selection and model averaging in phylogenetics: Advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology* 53: 793–808.

POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: Testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

REID, E., AND M. CHANDLER. 1933. The Flora of London Clay. British Museum (Natural History), London, UK.

RENNER, S. S. 2004. Tropical trans-Atlantic disjunctions, sea surface currents, and wind patterns. *International Journal of Plant Sciences* 165: S23–33.

RENNER, S. S. 2005. Relaxed molecular clocks for dating historical plant dispersal events. *Trends in Plant Science* 10: 550–558.

ROHWER, J. G. 1993. Lauraceae. *In* K. Kubizki, J. G. Rohwer, and V. Bittrich [eds.], The families and genera of vascular plants, vol. 2, 366–391. Springer-Verlag, Berlin, Germany.

ROHWER, J. G. 2000. Toward a phylogenetic classification of the Lauraceae: Evidence from *mat*K sequences. *Systematic Botany* 25: 60–70.

ROHWER, J. G., J. LI, B. RUDOLPH, S. A. SCHMIDT, H. VAN DER WERFF, AND H. W. LI. 2009. Is *Persea* (Lauraceae) monophyletic? Evidence from nuclear ribosomal ITS sequences. *Taxon* 58: 1153–1167.

ROHWER, J. G., AND B. RUDOLPH. 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., AND J. P. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

SANG, T. 2002. Utility of low-copy nuclear gene sequences in plant phylogenetics. *Critical Reviews in Biochemistry and Molecular Biology* 37: 121–147.

SCHMIDT-LEBUHN, A. N. 2008. Monophyly and phylogenetic relationships of *Minthostachys* (Labiatae, Nepetoideae) examined using morphological and nrITS data. *Plant Systematics and Evolution* 270: 25–38.

SCHULTZ, E. A., AND G. W. HAUGHN. 1991. *LEAFY*, a homeotic gene that regulates inflorescence development in *Arabidopsis*. *Plant Cell* 3: 771–781.

SNOW, D. W. 1981. Tropical frugivorous birds and their food plants: A world survey. *Biotropica* 13: 1–14.

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

TAKAHASHI, M., P. R. CRANE, AND H. ANDO. 1999. Fossil flowers and associated plant fossils from the Kamikitaba Locality (Ashizawa Formation, Futaba Group, Lower Coniacian, Upper Cretaceous) of Northeast Japan. *Journal of Plant Research* 112: 187–206.

TAYLOR, D. 1988. Eocene floral evidence of Lauraceae: Corroboration of the North American megafossil record. *American Journal of Botany* 75: 948–957.

THOMPSON, J. D., T. J. GIBSON, F. PLEWNIAK, F. JEANMOUGIN, AND D. G. HIGGNS. 1997. The Clustal_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876–4882.

TIFFNEY, B. H. 1985a. Perspectives on the origin of the floristic similarities between eastern Asia and eastern North America. *Journal of the Arnold Arboretum* 66: 73–94.

TIFFNEY, B. H. 1985b. The Eocene Atlantic landbridge: Its importance in Tertiary and modern phytogeography of the Northern Hemisphere. *Journal of the Arnold Arboretum* 66: 243–273.

VAN DER HAMMEN, T., AND A. M. CLEEF. 1983. *Trigonobalanus* and the tropical amphi-pacific element in the North Andean forest. *Journal of Biogeography* 10: 437–440.

VAN DER WERFF, H. 1994. Novelties in Neotropical Lauraceae. *Novon* 4: 58–76.

VAN DER WERFF, H. 2001. An annotated key to the genera of Lauraceae in the flora Malesiana region. *Blumea* 46: 125–140.

VAN DER WERFF, H. 2002. A synopsis of *Persea* (Lauraceae) in Central America. *Novon* 12: 575–586.

WANG, Y. G., P. W. FRITSCH, S. H. SHI, F. ALMEDA, B. C. CRUZ, AND L. M. KELLY. 2004. Phylogeny and infrageneric classification of *Symplocos* (Symplocaceae) inferred from DNA sequence data. *American Journal of Botany* 91: 1901–1914.

WEIGEL, D. 1995. The genetics of flower development: From floral induction to ovule morphogenesis. *Annual Review of Genetics* 29: 19–39.

WHEELWRIGHT, N. T., W. A. HABER, K. G. MURRAY, AND C. GUIDON. 1984. Tropical fruit-eating birds and their food plants: A survey of a Costa Rican lower montane forest. *Biotropica* 16: 173–192.

WHITE, T. J., T. D. BRUNS, S. B. LEE, AND J. W. TAYLOR. 1990. Amplification and direct sequencing of ribosomal RNA genes and the internal transcribed spacer in fungi. *In* M. A. Innis, G. H. Gelfand, F. J. Sninsky and T. J. White [eds.], PCR protocols and applications: A laboratory manual, 315–322. Academic Press, Orlando, Florida, USA.

WILKINSON, D. M. 1997. Plant colonization: Are wind dispersed seeds really dispersed by birds at large spatial and temporal scales? *Journal of Biogeography* 24: 61–65.

WOLFE, J. A. 1975. Some aspects of plant geography of the northern hemisphere during the late cretaceous and tertiary. *Annals of the Missouri Botanical Garden* 62: 264–279.

WOLFE, J. A. 1978. A paleobotanical interpretation of Tertiary climates in the Northern Hemisphere. *American Scientist* 66: 694–703.

WOLFE, J. A. 1997. Relations of environmental change to angiosperm evolution during the late Cretaceous and Tertiary. *In* K. Iwatsuki and P. H. Raven [eds.], Evolution and diversification of land plants, 269–290. Springer-Verlag, Tokyo, Japan.

WU, Z. Y. 1983. On the significance of pacific intercontinental discontinuity. *Annals of the Missouri Botanical Garden* 70: 577–590.

YOUNG, N. D., AND J. HEALY. 2003. GapCoder automates the use of indel characters in phylogenetic analysis. *Bioinformatics* 4: 6.

APPENDIX 1. Voucher information and GenBank accessions for ITS and LFY sequences for species examined in this study. Abbreviations of herbaria are as follows: BO, Bogor Botanical Garden; HBG, Hamburg Botanical Garden; HITBC, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences; KUN, Kunming Institute of Botany, Chinese Academy of Sciences; MJG, Miller Japanese Garden; MO, Missouri Botanical Garden; SBG, Singapore Botanic Gardens. GenBank accessions beginning with AF are from Chanderbali et al. (2001), with AY from Li et al. (unpublished), with FJ from Chen et al. (2009), with FM from Rohwer et al. (2009). GenBank accessions beginning with HQ are new sequences.

Taxon	Voucher	Locality	ITS	*LFY*
Ingroups				
Alseodaphne (9)				
Als. andersonii (King ex Hook. f.) Kosterm.	Li J. & Li L. 20070074 (HITBC)	China, Yunnan	FM957793	HQ697002
Als. gigaphylla Kosterm.	Arifiani DA657 (BO)	Indonesia, Java	HQ697181	HQ697003
				HQ697004
Als. hainanensis Merr.	Li L. & Wang Z. H. 20070317 (HITBC)	China, Hainan	FJ755440	HQ697005
				HQ697006
Als. huanglianshanensis H. W. Li & Y. M. Shui	Li L. 20080006 (HITBC)	China, Yunnan	HQ6971812	HQ697007
Als. petiolaris (Meissn.) Hook. f.	Chen J. Q. 07003 (HITBC)	China, Yunnan	FM957796	HQ697008
				HQ697009
				HQ697010
Als. rugosa Merr. & Chun	Li L. & Wang Z. H. 20070369 (HITBC)	China, Hainan	HQ6971813	HQ697011
				HQ697012
Als. semecarpifolia Nees	Arifiani DA658 (BO)	Indonesia, Java	HQ6971814	HQ697013
				HQ697014
				HQ697015
Als. sp. W14264	van der Werff & Nguyen 14264 (MO)	Vietnam, Tuyen Quang	FM957797	HQ697016
				HQ697017
Als. sp. W17084	van der Werff et al., 17084 (MO)	Vietnam, Lang Son	FM957798	HQ697018
				HQ697019
				HQ697020
Apollonias (1)				
Apo. barbujana (Cav.) Bornm.	Rohwer s.n. (HBG)	Spain, Canary Islands	AY934889	HQ697021
				HQ697022
Dehaasia (5)				
Deh. caesia Blume	Arifiani DA493 (BO)	Indonesia, Java	HQ697185	HQ697023
				HQ697024
Deh. hainanensis Kosterm.	Li L. & Wang Z. H. 20070373 (HITBC)	China, Hainan	FJ719308	HQ697025
				HQ697026
				HQ697027
Deh. incrassata (Jack) Kosterm.	Arifiani DA492 (BO)	Indonesia, Java	HQ6971856	HQ697028
				HQ697029
				HQ697030
Deh. sp. A34	Arifiani & van der Werff 34 (MO)	Indonesia, Java	FM957805	HQ697031
				HQ697032
				HQ697033
Deh. sp. L20070187	Li L. 20070187 (HITBC)	China, Yunnan	HQ697187	HQ697034
				HQ697035
				HQ697036
Machilus (24)				
Mac. breviflora (Benth.) Hemsl.	Chen J. Q. et al., 2006013(HITBC)	China, Guangdong	FJ755434	HQ697041
				HQ697042
				HQ697043
Mac. decursinervis Chun	Li J. 2002195 (HITBC)	China, Guangxi	AY934893	HQ697044
				HQ697045
Mac. duthiei King ex Hook. f.	Zhong J. S. 2006094 (HITBC)	China, Yunnan	FJ755425	HQ697054
				HQ697055
Mac. gamblei King ex Hook. f.	Chen J. Q. et al., 2006001 (HITBC)	China, Guangdong	FJ755422	HQ697037
				HQ697038
				HQ697039
				HQ697040
Mac. gongshanensis H. W. Li	Chen J. Q. 07002 (HITBC)	China, Yunnan	FJ755416	HQ697046
				HQ697047
Mac. grijsii Hance	Chen J. Q. et al., 2006028 (HITBC)	China, Guangdong	FJ755420	HQ697048
				HQ697049
Mac. japonica Sieb. & Zucc.	Kim C. K. s.n. (HBG)	Korea, Cheju	AY934891	HQ697050
Mac. kwangtungensis Yang	Chen J. Q. et al., 2006027 (HITBC)	China, Guangdong	FJ755424	HQ697051
				HQ697052
Mac. leptophylla Hand.-Mazz.	Li J. & Li L. 20070190 (HITBC)	China, Zhejiang	FJ755430	HQ697053
Mac. monticola S. Lee	Li L. & Wang Z. H. 20070323 (HITBC)	China, Hainan	FJ755418	HQ697056
				HQ697057
				HQ697058
Mac. oculodracontis Chun	Chen J. Q. et al., 2006037 (HITBC)	China, Guangdong	HQ697188	HQ697059
				HQ697060
				HQ697061
				HQ697062

APPENDIX 1. Continued

Taxon	Voucher	Locality	ITS	*LFY*
Mac. oreophila Hance	Chen J. Q. et al., 2006067 (HITBC)	China, Guangdong	FJ755423	HQ697063
Mac. phoenicis Dunn	Chen J. Q. et al., 2006009 (HITBC)	China, Guangdong	FJ755413	HQ697064
Mac. pingii Cheng ex Yang	Li L. 20070263(HITBC)	China, Sichuan	HQ697189	HQ697065
				HQ697066
Mac. platycarpa Chun	Chen J. Q. et al., 2006073 (HITBC)	China, Guangdong	FJ755421	HQ697067
				HQ697068
Mac. pomifera (Kosterm.) S. Lee	Chen J. Q. et al., 2006064 (HITBC)	China, Guangdong	FJ755432	HQ697069
				HQ697070
Mac. robusta W. W. Sm.	Li J. 2002116 (HITBC)	China, Guangxi	FJ755426	HQ697071
				HQ697072
Mac. salicina Hance	Chen J. Q. et al., 2005001 (HITBC)	China, Yunnan	FJ755428	HQ697073
Mac. salicoides S. Lee	Chen J. Q. et al., 2006090 (HITBC)	China, Guangdong	FJ755433	HQ697074
Mac. shweliensis W. W. Sm.	Li J. 2002087 (HITBC)	China, Guangxi	FJ755414	HQ697075
				HQ697076
Mac. sp. W14068	van der Werff et al., 14068 (MO)	Vietnam, Vinh Phu	FM957812	HQ697077
				HQ697078
Mac. sp. W14071	van der Werff et al., 14071 (MO)	Vietnam, Vinh Phu	FM957813	HQ697079
				HQ697080
Mac. thunbergii Sieb. & Zucc.	Rohwer s.n. (HBG)	Germany, Hamburg	HQ697190	HQ697081
				HQ697082
Mac. yunnanensis Lec .	Zhong J. S. 2006093 (HITBC)	China, Yunnan	FJ755415	HQ697083
				HQ697084
Nothaphoebe (1)				
Not. umbelliflora (Blume) Blume	Arifiani DA495 (BO)	Indonesia, Java	HQ697191	HQ697088
Persea (21)				
Per. alba Nees & Mart.	Borgo & Britez 2165 (MO)	Brazil, Paraná	HQ697192	HQ697089
				HQ697090
Per. americana Mill.	Li J. 2002001 (HITBC)	China, Yunnan	AF272322	HQ697091
				HQ697092
				HQ697093
				HQ697094
Per. areolatocostae (C. K. Allen) van der Werff	Quizhpe 208 (MO)	Ecuador, Zamora-Chinchipe Nangaritza	HQ697193	HQ697095
				HQ697096
Per. aurata Miq.	Folli 4089 (MO)	Brazil, Espirito Santo	HQ697194	HQ697097
Per. borbonia (L.) Spreng.	Kirchner s.n. (HBG)	Germany, Bochum	AY934901	HQ697098
Per. caerulea (Ruiz & Pav.) Mez	Linares 5213 (MO)	Honduras, Depto Francisco Morazán Mipo	FJ755436	HQ697099
Per. haenkeana Mez	Fuentes 7370 (MO)	Bolivia, La Paz Bautista Saavedra	HQ697195	HQ697100
Per. indica (L.) Spreng.	Rohwer s.n. (HBG)	Germany, Hamburg	AY934902	HQ697101
Per. lingue (Ruiz & Pav.) Nees	Aedu 7242 (MO)	Chile, La Araucania	HQ697196	HQ697102
Per. major (Meisn.) Kopp	Araujo 216 (MO)	Brazil, M G Bias Fertes	HQ697197	HQ697103
				HQ697104
Per. nudigemma van der Werff	van der Werff et al., 19209 (MO)	Ecuador, Zamora-Chinchipe	FM957828	HQ697105
				HQ697106
Per. palustris (Raf.) Sarg.	Miller et al., 9018 (MO)	USA, Florida, Leon	FM957831	HQ697107
				HQ697108
Per. sp. B21834	Beck 21834	Bolivia (HBG)	HQ697198	HQ697109
Per. sp. V25232	Vásquez & Ortiz-Gentry 25232 (MO)	Peru, Loreto, Maynas	FM957836	HQ697110
Per. sp. W14857	van der Werff et al., 14857 (MO)	Peru, Amazonas	FM957833	HQ697111
				HQ697112
Per. sp. W19517	van der Werff et al., 19517 (MO)	Ecuador, Zamora-Chinchipe	FM957834	HQ697113
				HQ697114
				HQ697115
Per. sp. W21874	van der Werff 21874 (MO)	Ecuador	FM957835	HQ697116
Per. sphaerocarpa (H. Winkl.) Kosterm.	van der Werff et al., 17889 (MO)	Peru, Pasco, Oxapampa	FM957837	HQ697117
				HQ697118
Per. splendens Meisn.	Fonseca 2568 (MO)	Brazil, Goiás Municipio Campos Belos	HQ697199	HQ697119
Per. steyermarkii C. K. Allen	Monterrosa 408 (MO)	El Salvador, Dept. Santa Ana	HQ697200	HQ697120
				HQ697121
Per. weberbaueri Mez	van der Werff 21626 (MO)	Ecuador	FM957842	HQ697122
				HQ697123
Phoebe (17)				
Pho. angustifolia Meissn.	Li L. 20070058 (HITBC)	China, Yunnan	HQ697201	HQ697124
				HQ697125
				HQ697126
Pho. chekiangensis C. B. Shang	Li J. & Li L. 20070188 (HITBC)	China, Zhejiang	FJ755407	HQ697127
				HQ697128
Pho. cuneata (Blume) Blume	Arifiani 40 (MO)	Indonesia	HQ697202	HQ697129
				HQ697130

APPENDIX 1. Continued

Taxon	Voucher	Locality	ITS	*LFY*
Pho. elliptica (Blume) Blume	Samsuri & Gwee SING2004-28 (SBG)	Singapore	HQ697203	HQ697131
				HQ697132
Pho. faberi (Hemsl.) Chun	Li L. 20070269 (HITBC)	China, Sichuan	HQ697204	HQ697133
				HQ697134
Pho. formosana (Matsum. & Hay.) Hay.	Rohwer 156 (MJG)	Germany, Bonn	HQ697205	HQ697135
				HQ697136
Pho. hungmaoensis S. Lee	Li L. & Wang Z. H. 20070306 (HITBC)	China, Hainan	HQ697206	HQ697137
				HQ697138
				HQ697139
				HQ697140
Pho. lanceolata (Wall. ex Nees) Nees	Chen J. Q. et al., 2006093 (HITBC)	China, Guangdong	FJ755410	HQ697141
Pho. macrocarpa C. Y. Wu	Li J. 2002207 (HITBC)	China, Guangxi	FJ755408	HQ697142
				HQ697143
Pho. megacalyx H. W. Li	Li J. & Li L. 20070026 (HITBC)	China, Yunnan	HQ697207	HQ697144
				HQ697145
				HQ697146
Pho. minutiflora H. W. Li	Chen J. Q. et al., 2005038 (HITBC)	China, Yunnan	HQ697208	HQ697147
				HQ697148
Pho. nanmu (Oliv.) Gamble	Chen J. Q. et al., 2005002 (HITBC)	China, Yunnan	FJ755409	HQ697149
				HQ697150
Pho. neurantha (Hemsl.) Gamble	Li J. & Li L. 20070214 (HITBC)	China, Zhejiang	HQ697209	HQ697151
Pho. puwenensis Cheng	Chen J. Q. et al., 2006065 (HITBC)	China, Guangdong	HQ697210	HQ697152
				HQ697153
				HQ697154
				HQ697155
				HQ697156
				HQ697157
Pho. sp. A49	Arifiani 49 (MO)	Indonesia	HQ697211	HQ697158
				HQ697159
Pho. sp. L20070260 (*Not. cavaleriei* (Lévl.) Yang)	Li L. 20070260 (HITBC)	China, Sichuan	FJ755412	HQ697085
				HQ697086
				HQ697087
Pho. zhennan S. Lee & F. N. Wei	Li L. 20070239 (HITBC)	China, Sichuan	HQ697212	HQ697160
				HQ697161
				HQ697162
Outgroups				
Actinodaphne (2)				
Act. cupularis (Hemsl.) Gamble	Li L. 20070231 (HITBC)	China, Sichuan	HQ697213	HQ697163
				HQ697164
Act. trichocarpa C. K. Allen	Li L. 20070282 (HITBC)	China, Sichuan	HQ697214	HQ697165
				HQ697166
Lindera (2)				
Lin. erythrocarpa Makino	Li J. & Li L. 20070203 (HITBC)	China, Zhejiang	HQ697215	HQ697167
				HQ697168
				HQ697169
				HQ697170
Lin. megaphylla Hemsl.	Li L. 20070236 (HITBC)	China, Sichuan	HQ697216	HQ697171
				HQ697172
				HQ697173
Litsea (2)				
Lit. auriculata Chien et Cheng	Li J. & Li L. 20070195	China, Zhejiang (HITBC)	HQ697217	HQ697174
Lit. verticillata Hance	Li L. & Wang Z. H. 20070337 (HITBC)	China, Hainan	HQ697218	HQ697175
Neolitsea (3)				
Neo. cambodiana Lec.	Li L. & Wang Z. H. 20070327 (HITBC)	China, Hainan	HQ697219	HQ697176
				HQ697177
Neo. howii C. K. Allen	Li L. & Wang Z. H. 20070379 (HITBC)	China, Hainan	HQ697220	HQ697178
				HQ697179
Neo. sericea (Blume) Koidz.	Li J. & Li L. 20070225 (HITBC)	China, Zhejiang	HQ697221	HQ697180