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Source: *Annals of the Missouri Botanical Garden*, Vol. 88, No. 1 (Winter, 2001), pp. 104-134

Published by: [Missouri Botanical Garden Press](#)

Stable URL: <http://www.jstor.org/stable/2666133>

Accessed: 11/09/2011 12:55

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PHYLOGENY AND HISTORICAL BIOGEOGRAPHY OF LAURACEAE: EVIDENCE FROM THE CHLOROPLAST AND NUCLEAR GENOMES¹

André S. Chanderbali,^{2,3} Henk van der
Werff,³ and Susanne S. Renner^{2,3}

ABSTRACT

Phylogenetic relationships among 122 species of Lauraceae representing 44 of the 55 currently recognized genera are inferred from sequence variation in the chloroplast and nuclear genomes. The *trnL-trnF*, *trnT-trnL*, *psbA-trnH*, and *rpl16* regions of cpDNA, and the 5' end of 26S rDNA resolved major lineages, while the ITS/5.8S region of rDNA resolved a large terminal clade. The phylogenetic estimate is used to assess morphology-based views of relationships and, with a temporal dimension added, to reconstruct the biogeographic history of the family. Results suggest Lauraceae radiated when trans-Tethyan migration was relatively easy, and basal lineages are established on either Gondwanan or Laurasian terrains by the Late Cretaceous. Most genera with Gondwanan histories place in Cryptocaryaceae, but a small group of South American genera, the *Chlorocardium-Mezilaurus* clade, represent a separate Gondwanan lineage. *Caryodaphnopsis* and *Neocinnamomum* may be the only extant representatives of the ancient Lauraceae flora documented in Mid- to Late Cretaceous Laurasian strata. Remaining genera place in a terminal Perseeae-Laureae clade that radiated in Early Eocene Laurasia. Therein, non-cupulate genera associate as the *Persea* group, and cupuliferous genera sort to Laureae of most classifications or Cinnamomeae sensu Kostermans. Laureae are Laurasian relicts in Asia. The *Persea* group and *Cinnamomum* group (of Cinnamomeae) show tropical amph-Pacific disjunctions here credited to disruption of boreotropical ranges by Eocene-Oligocene climatic cooling. The *Ocotea* complex accommodates remaining Cinnamomeae and shows a trans-Atlantic disjunction possibly derived from a Madrean-Tethyan ancestral distribution. These findings support Laurasian ancestry for most extant Lauraceae, with their considerable neotropical representation primarily derived from Early Miocene radiation of the *Ocotea* complex upon reaching South America.

Key words: biogeography, boreotropical, chloroplast DNA, Gondwana, Lauraceae, Laurasia, Madrean-Tethyan, molecular clock, phylogeny, ribosomal DNA.

Lauraceae form a large family of woody plants (except the herbaceous parasite *Cassytha*), with about 50 genera and 2500 to 3000 species distributed throughout tropical to subtropical latitudes. They are among the more speciose basal angiosperm families and have a fossil record that reaches back to the Mid-Cretaceous (Drinnan et al., 1990; Eklund & Kvaček, 1998). Current taxonomic diversity is centered in tropical America and Australasia, and although poorly represented in continental Africa, Lauraceae flourish in Madagascar. In the American tropics they list among the most

prominent components of lowland forests and are frequently dominant elements in montane vegetation (Gentry, 1988).

Given their antiquity, widespread distribution, and ecological prominence, Lauraceae provide a model system for investigating angiosperm biogeography. Moreover, the three tribes recognized by van der Werff and Richter (1996) suggest that major divisions in the family draw along geographic lines. Laureae include three genera with North American-Asian disjunctions (*Litsea*, *Lindera*, *Sassafras*), Mediterranean *Laurus*, and Asian endemics (e.g.,

¹ The authors thank anonymous reviewers for insightful comments; K. Ueda for eight unpublished *trnL-trnF* sequences; J. Rohwer for six unpublished ITS sequences; M. Zanis for five at the time unpublished ITS/5.8S/26S sequences; Y-L. Qiu for providing three DNA aliquots; the Harvard Herbaria for providing leaf material of *Eusideroxylon*; Andrea Schwarzbach for guidance in molecular techniques; and innumerable colleagues who helped to collect material of Lauraceae for this study. This study was undertaken as part of ASC's doctoral dissertation research at the University of Missouri-St. Louis, and was supported by grants from the Mallinckrodt Foundation administered by the International Center for Tropical Ecology, University of Missouri-St. Louis, and from the Smithsonian Institution's Biological Diversity of the Guianas Program to ASC; National Geographic Society grants 4631-91, 5635-96, and 6571-99 to HW; and grants from the University of Missouri system and University of Missouri-St. Louis to SSR. This article is Number 45 in the Smithsonian Institution's Biological Diversity of the Guianas Publication series.

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Actinodaphne and *Neolitsea*). Cryptocaryeae include pantropical *Beilschmiedia* and *Cryptocarya*, and other predominantly Southern Hemispheric genera with narrower ranges (e.g., *Endiandra* and *Potameia*). Perseeae are centered in the Neotropics with three widespread genera. *Cinnamomum* is disjunct between the American and Asian (sub)tropics; *Persea* (including *Machilus*) ranges throughout the Neotropics (into the southeastern U.S.), the Canary Islands, and Asia; and *Ocotea* has about 300 neotropical species, one in Macaronesia, a few in Africa, and about 30 more in Madagascar. Apart from a few Asian genera (e.g., *Dehaasia* and *Phoebe*) considered closely related to *Persea*, all other genera of Perseeae are endemic to the Neotropics (e.g., *Aiouea*, *Aniba*, *Endlicheria*, *Licaria*, *Nectandra*, *Pleurothyrium*, and *Rhodostemonodaphne*). The distributions of Laureae and Cryptocaryeae are consistent with Laurasian and Gondwanan histories, respectively, but that of Perseeae is ambiguous in this regard. The trans-Atlantic distribution of *Ocotea* suggests West Gondwanan history, but the tropical amphi-Pacific distributions of *Cinnamomum* and *Persea* suggest Laurasian affinities. Whether or not these ambiguities are artifacts of tribal and/or generic circumscription is unclear.

The systematics of Lauraceae is unsettled. Laureae are recognized in most prior classifications, but other tribal concepts are not widely accepted. Van der Werff and Richter's (1996) Cryptocaryeae and Perseeae are revised concepts supported by Richter's (1981) study of wood and bark anatomy. Some aspects of Cryptocaryeae gain support from embryology (Heo et al., 1998), but characters for further subdivision of the family were not found. A cladistic analysis of molecular data (Rohwer, 2000) also provided support for Cryptocaryeae and united Perseeae and Laureae in a well-supported but unresolved clade. This Perseeae-Laureae clade accommodates most of the family, and its distribution implies a disjunction between the American and Asian tropics with a minor African presence. Such distributions can be credited to extinction of Gondwanan lineages in Africa (e.g., Raven & Axelrod, 1974), but Laurasian ancestry followed by radiation in tropical Asia and America was favored by Rohwer (2000). Tropical Asia is acknowledged as a harbor for Laurasian relicts (e.g., Wolfe, 1975), but Rohwer's hypothesis that the approximately 800 neotropical species of Perseeae are derived from Laurasian immigrants contrasts with the Gondwanan origin credited to most major neotropical plant groups (e.g., Raven & Axelrod, 1974; Gentry, 1982; references in Goldblatt, 1993; Burnham & Graham, 1999). Further, Raven and Axelrod (1974: 563) al-

lowed that *Persea* was an "old Laurasian genus" but suggested that subgenus *Eriodaphne*, like all other members of the family, arrived in South America via Africa. Alternatively, Taylor (1988) suggested that the fossil flower *Androglandula tennesseensis* Taylor, from the Eocene of North America, is comparable to *Cinnamomum*, *Ocotea*, and *Nectandra*, indicating a boreotropical history for this group of genera, with subsequent migration to South America. Raven and Axelrod (1974) and Taylor (1988) allowed that although differing in the direction of migration, the high level of species diversity and generic endemism require a Late Cretaceous-Early Tertiary arrival in South America. In contrast, Rohwer and Kubitzki (1993) preferred a more recent arrival of this group, possibly as late as the Pliocene closure of the Panamanian Isthmus, followed by rapid radiation in South America.

These alternative views are here assessed by adding a temporal dimension to a phylogenetic estimate for Lauraceae reconstructed with molecular characters retrieved from both chloroplast and nuclear genomes.

MATERIALS AND METHODS

TAXON AND MOLECULAR SAMPLING

A total of 131 species, 122 representing 44 of the 55 currently recognized genera of Lauraceae, and 9 representing 3 outgroup families (Gomortegaceae, Hernandiaceae, and Monimiaceae) were included in this study. Seventy-seven in-group species were included in a broad-scale study based on sequence variation in the *trnL-trnF* and *psbA-trnH* intergenic spacers of cpDNA. Guided by the findings of this molecular and taxon sampling a two-tiered approach was adopted to obtain better representation and phylogenetic resolution at the generic level. Thus a subset of the species representing basal lineages in Lauraceae was also sequenced for the *trnT-trnL* spacer, and the *rpl16* intron of cpDNA, as well as the 5' end of 26S rDNA, while 94 species representing Perseeae, Laureae, and their sister group were sampled for the ITS/5.8S region of nrDNA. Table 1 provides GenBank information for all accessions.

DNA EXTRACTION, PCR AMPLIFICATION, AND SEQUENCING

Total DNA was obtained from silica-gel-dried, herbarium, or fresh leaves using Dneasy (QIAGEN) extraction kits. PCR amplification of chloroplast loci was conducted using standard protocols (e.g., White et al., 1990). For nuclear markers, 10%

Table 1. Sources of plant material. †Previously submitted to Genbank. Contributed by: †J. Rohwer, Univ. Hamburg; †K. Ueda, Univ. Osaka; and †M. Zanis, Washington State Univ.

Taxon	Provenance	Voucher	Genbank accession numbers					
			<i>trnL-trnF</i>	<i>psbA-trnH</i>	<i>trnT-trnL</i>	<i>rpl16</i>	ITS/5.8S	
Lauraceae								
<i>Actinodaphne sesquipedalis</i> Meisn.	Malaysia, Kuala Lumpur	Saw Leng Guan s.n. (KEP)	AF268695	AF268787	—	AF268239	—	AF272247
<i>Adenodaphne uniflora</i> (Guill.) Kosterm.	New Caledonia, Province du Sud	van der Werff 15895 (MO)	—	—	—	—	—	AF272248
<i>Aiouea costaricensis</i> (Mez) Kosterm.	Costa Rica, Heredia	Grayum 8241 (HBG) (J. Rohwer sequence)	—	—	—	—	—	AF272249 [†]
<i>Aiouea dubia</i> (HBK) Mez	Ecuador, Loja	Madsen 75433 (AAU) (J. Rohwer sequence)	—	—	—	—	—	AF272250 [†]
<i>Aiouea guianensis</i> Aubl.	Guyana, Demerara, Timorhi	Taylor 12085 (MO)	AF268696	AF268780	—	—	—	AF272251
<i>Aseodaphne semecarpifolia</i> Nees	Sri Lanka, Central Prov, Kandy	Malcomber 2753 (MO)	AF268697	AF268799	—	—	—	AF272252
<i>Anaueria brasiliensis</i> Kosterm.	Peru, Loreto, Iquitos	Vásquez 25228 (MO)	AF268698	AF268800	AF268840	AF268247	—	AF272253
<i>Aniba cinnamomiflora</i> C. K. Allen	Venezuela, Trujillo, Bonón	Cuello 955 (MO)	AF268700	AF268770	AF268823	—	—	AF272254
<i>Aniba excelsa</i> Kosterm.	Guyana, Demerara, Mabarara Hill	Chanderbali 226 (MO)	—	—	—	—	—	AF272255
<i>Aniba hypoglauca</i> Sandwith	Guyana, Essequibo, Iwokrama Reserve	Chanderbali 165 (MO)	AF268699	AF268771	AF268822	—	—	—
<i>Aniba panurensis</i> (Meisn.) Mez	Guyana, Essequibo, Iwokrama Reserve	Chanderbali 248 (MO)	—	—	—	—	—	AF272256
<i>Apollonia barbujana</i> (Cav.) Bornn.	Canary Islands, Tenerife	Bramwell 628 (MO)	—	—	—	—	—	AF272257
<i>Aspidostemon</i> sp.	Madagascar, Tohiara	van der Werff 12737 (MO)	AF268701	AF268819	AF268843	AF268251	—	—
<i>Beilschmiedia brenesii</i> C. K. Allen	Costa Rica, Puntarenas	Yasuda 1310 (MO)	AF268702	AF268809	—	—	—	AF262004 [†]
<i>Beilschmiedia madagascariensis</i> Kosterm.	Madagascar, Tohiara	Lourey 5015 (MO)	—	AF268810	—	—	—	—
<i>Beilschmiedia ovalis</i> (Blake) C. K. Allen	Costa Rica, San Jose	Yasuda 1301 (MO)	AF268703	AF268811	—	—	—	AF270472
<i>Beilschmiedia sary</i> Kosterm.	Madagascar, Toamasina	van der Werff 12800 (MO)	—	AF268812	—	—	—	—

Table 1. Continued.

Taxon	Provenance	Voucher	Genbank accession numbers						
			<i>trnL-trnF</i>	<i>psbA-trnH</i>	<i>trnT-trnL</i>	<i>rpl16</i>	26S	ITS/5.8S	
<i>Beilschmiedia tilaranensis</i> Nishida	Costa Rica, Guanacaste	Yasuda 1313 (MO)	AF129014 ¹	AF129045 ¹	AF129015 ¹	AF127265 ¹	—	—	
<i>Beilschmiedia velutina</i> (Kosterm.) Kosterm.	Madagascar, Antsiranana	Georges 361 (MO)	AF268704	AF268813	—	—	—	—	
<i>Caryodaphnopsis bilocellata</i> van der Werff	Vietnam, Ninh Binh	van der Werff 14195 (MO)	AF23032 ¹	AF261995 ¹	AF233603 ¹	AF232743 ¹	AF262005 ¹	—	
<i>Caryodaphnopsis tomentosa</i> van der Werff	Peru, Loreto, Iquitos	Vásquez 25239 (MO)	AF268705	AF268807	AF268842	AF268248	—	—	
<i>Cassytha filiformis</i> L.	Guyana, Rupununi, Karanambo	Chanderbali 205 (MO)	AF232034 ¹	AF261996 ¹	AF233605 ¹	AF232744 ¹	AF262006 ¹	—	
<i>Cassytha pubescens</i> R. Br.	Australia, Victoria, Geelong	Foreman 1913 (MEL)	AF232033 ¹	—	AF233604 ¹	—	AF262007 ¹	—	
<i>Chlorocardium rodiei</i> (R. H. Schomb.) Rohwer, H. G. Richt. & van der Werff	Guyana, Demerara, Mabarara Hill	Chanderbali 246 (MO)	AF268706	AF268802	—	AF268245	—	AF272258	
<i>Chlorocardium venenosum</i> (Kosterm. & Pinkley.) Rohwer, H. G. Richt. & van der Werff	Peru, Loreto, Iquitos	Vásquez 25236 (MO)	AF268707	AF268801	AF268840	—	—	AF272259	
<i>Cinnamomum camphora</i> (L.) Presl.	Missouri BG	Chanderbali 322 (MO)	AF129020 ²	AF129048 ¹	AF129021 ¹	AF129019 ¹	—	AF272260	
<i>Cinnamomum chavarrinum</i> (Hammel) Kosterm.	Costa Rica	Gomez-Laurito s.n. (J. Rohwer sequence)	—	—	—	—	—	AF272261 ²	
<i>Cinnamomum cinnamomifolium</i> (HBK) Kosterm.	Ecuador	Thomsen 8942 (AAU) (J. Rohwer sequence)	—	—	—	—	—	AF272262 ²	
<i>Cinnamomum japonicum</i> Siebold	Japan, Honshu, Kyoto	Yasuda 1351 (MO)	AF268708 ³	AF268782	—	—	—	AF272263	
<i>Cinnamomum oleifolium</i> (Mez) Kosterm.	Brazil, Minas Gerais	Lorea-Hernandez 5582 (MO)	—	—	—	—	—	AF272264	
<i>Cinnamomum quadrangulum</i> (Meisn.) Kosterm.	Brazil, Minas Gerais	Lorea-Hernandez 5585 (MO)	—	AF268781	—	—	—	AF272265	
<i>Cinnamomum</i> sp.	Vietnam, Lao Cai	van der Werff 14396 (MO)	—	AF268783	—	—	AF270473	AF272266	
<i>Cinnamomum verum</i> Presl.	India, Kumbhave, Dapoli	Godbole 45108 (MO)	AF268709	AF268784	—	—	—	AF272267	
<i>Cryptocarya chinensis</i> (Hance) Hensl.	Asia	K. Ueda sequence	AF268710 ³	—	—	—	—	—	

Table 1. Continued.

Taxon	Provenance	Voucher	Genbank accession numbers						
			trnL-trnF	psbA-trnH	trnT-trnL	rpl16	26S	ITS/5.8S	
<i>Licaria martiniana</i> (Mez) Kosterm.	Guyana, Essequibo, Iwok-rana Reserve	Chanderbali 264 (MO)	—	—	—	—	—	—	AF272279
<i>Licaria triandra</i> (Sw.) Kosterm.	Fairchild Tropical Garden, Fl., U.S.A.	Qiu 90019 (NCU)	AF268724	AF268774	—	—	—	—	AF272282
<i>Lindera benzoin</i> (L.) Blume	Missouri BG	Chanderbali 324 (MO)	AF268725 ^a	AF268788	AF268833	—	—	—	AF272283
<i>Lindera erythrocarpa</i> Makino	Japan, Honshu, Kyoto	Yasuda 1353 (MO)	AF268726 ^b	—	—	—	—	—	AF272284
<i>Lindera umbellata</i> Thunb.	Japan, Honshu, Kyoto	Yasuda 1354 (MO)	AF268727 ^b	AF268789	—	—	—	—	AF272285
<i>Litsea coreana</i> Leveille	Japan, Honshu, Kyoto	Yasuda 1356 (MO)	AF268728 ^b	AF268791	—	—	—	AF262010 ¹	AF272286
cf. <i>Litsea elongata</i> (Nees) Benth. & Hook. f. (Distributed to MO as <i>Phoebe forrestii</i> W. W. Sm.)	China, Yunnan, Kunming BG	Hyland 14912 (MO)	—	—	—	—	—	—	AF272277
<i>Litsea glaucescens</i> HBK	Mexico, Guerrero, Chichihualco	Lorea-Hernandez 5496 (MO)	AF129035 ¹	AF129063 ¹	AF129036 ¹	—	—	—	—
<i>Mezilaurus trianae</i> van der Werff	Peru, Amazonas, Iquitos	Vásquez 25227 (MO)	AF268729	AF268804	AF268837	AF268246	—	—	AF272287
<i>Mocimodaphne cinnamomoides</i> Lorea-Hern.	Mexico, Guerrero, El Moliste	Lorea-Hernandez 5536 (MO)	—	—	—	—	—	—	AF272288
<i>Nectandra amazonum</i> Nees	Guyana, Essequibo, Iwok-rana Reserve	Chanderbali 217 (MO)	—	—	—	—	—	—	AF272289
<i>Nectandra coriacea</i> (Sw.) Griseb.	U.S.A. Florida, Monroe County	Prinzie 125 (MO)	—	—	—	—	—	—	AF272290
<i>Nectandra cuspidata</i> Nees & Mart.	Guyana, Essequibo, Kamarang	Chanderbali 279 (MO)	—	—	—	—	—	—	AF272291
<i>Nectandra membranacea</i> (Sw.) Griseb.	Brazil, Sao Paulo, Serra do Mar	Lorea-Hernandez 5596 (MO)	AF268730	AF268767	AF268825	AF268244	—	—	—
<i>Nectandra psammophila</i> Nees & C. Mart.	Brazil, Sao Paulo, Serra do Mar	Lorea-Hernandez 5595 (MO)	—	—	—	—	—	—	AF272292
<i>Nectandra purpurea</i> (Ruiz & Pav.) Mez	Peru, Cajamarca, San Ignacio nacio	Campos et al. 3165 (MO)	—	—	—	—	—	—	AF272293
<i>Nectandra salicifolia</i> (HBK) Nees	Costa Rica	Gomez-Laurito s.n. (J. Rohwer sequence)	—	—	—	—	—	—	AF272294 ²
<i>Nectandra turbacensis</i> (HBK) Nees	Puerto Rico, Rio Grande, El Verde	Taylor 11746 (MO)	AF012400 ¹	AF268768	AF268826	—	—	—	AF272295

Table 1. Continued.

Taxon	Provenance	Voucher	Genbank accession numbers					
			<i>trnL-trnF</i>	<i>psbA-trnH</i>	<i>trnT-trnL</i>	<i>rpl16</i>	26S	ITS/5.8S
<i>Neocinnamomum mekongense</i> (Hand.-Mazz) Kosterm.	China, Yunnan	<i>Li Heng 8547</i> (MO)	AF268731	AF268806	AF268841	AF268249	AF270477	—
<i>Neolitsea sericea</i> (Blume) Koidz.	Japan, Honshu, Kyoto	<i>Yasuda 1355</i> (MO)	AF268732 ^s	AF268792	—	AF268243	AF270478	AF272296
<i>Ocotea botranthia</i> Rohrer	U. C. Riverside	<i>Scora 99-J</i> (UCR)	AF268733	AF268776	—	—	—	AF272297
<i>Ocotea bullata</i> (Burchell) E. Mey.	South Africa, Natal, Sky- line Arboretum	<i>Abbot 6208</i> (MO)	AF268734	AF267778	—	—	—	AF272298
<i>Ocotea ceanothifolia</i> (Nees) Mez	Guyana, Demerara, Mabu- ra Hill	<i>Chanderbali 244</i> (MO)	—	—	—	—	—	AF272299
<i>Ocotea foetens</i> (Aiton) Baill.	Madeira, Porto Moniz	<i>Maas 8642</i> (MO)	AF268735	—	—	—	—	AF272300
<i>Ocotea grayi</i> van der Werff	Madagascar, Toliara	<i>van der Werff 12732</i> (MO)	AF268736	—	—	AF232747 ¹	AF270479	AF272301
<i>Ocotea guianensis</i> Aubl.	Guyana, Demerara, Mabu- ra Hill	<i>Chanderbali 232</i> (MO)	AF268737	AF268762	—	—	—	AF272302
<i>Ocotea helicterifolia</i> (Meisn.) Hemsl.	Mexico, Oaxaca, Miahu- atlan	<i>Torres 11911</i> (MO)	—	—	—	—	—	AF272303
<i>Ocotea heydeana</i> (Mez & Donn. Sm.) Bernardi	Honduras, Yoro, Pico Pijol	<i>Evans 1760</i> (MO)	—	—	—	—	—	AF272304
<i>Ocotea ikorylope</i> van der Werff	Cameroon, SW Prov., Rumpi Hills	<i>Thomas 10456</i> (MO)	—	—	—	—	—	AF272305
<i>Ocotea insularis</i> (Meisn.) Mez	Costa Rica, Puntarenas, Coco Is	<i>Rojas 3682</i> (MO)	—	—	—	—	—	AF272306
<i>Ocotea leucoxyloa</i> (Sw.) Laness.	Puerto Rico, San Jose	<i>Taylor 11733</i> (MO)	AF12399 ¹	AF268763	—	—	—	—
<i>Ocotea malcomberi</i> van der Werff	Madagascar, Toliara	<i>van der Werff 12756</i> (MO)	—	AF268779	—	—	—	AF272307
<i>Ocotea nigra</i> Benoist	Guyana, Essequibo, Iwok- rama Reserve	<i>Chanderbali 162</i> (MO)	—	—	—	—	—	AF272308
<i>Ocotea odorifera</i> (Vell.) Rohrer	Brazil, Minas Gerais, Parque Ecol. CEMIG (MO)	<i>Lorea-Hernandez 5578</i>	AF268738	AF268762	—	—	—	AF272309
<i>Ocotea pauciflora</i> (Nees) Mez	Guyana, Demerara, Mabu- ra Hill	<i>Chanderbali 219</i> (MO)	—	AF268764	—	—	—	AF272310
<i>Ocotea percoriacea</i> (Mez) Kos- term.	Brazil, Goias, Fazenda Galheiro	<i>Lorea-Hernandez 5584</i> (MO)	AF268739	—	—	—	—	AF272311
<i>Ocotea pulchella</i> Mart.	Brazil, Minas Gerais, Fa- zenda Caiara	<i>Lorea-Hernandez 5575</i> (MO)	AF268740	—	—	—	—	AF262312

Table 1. Continued.

Taxon	Provenance	Voucher	Genbank accession numbers					
			<i>trnL-trnF</i>	<i>psbA-trnH</i>	<i>trnT-trnL</i>	<i>rpl16</i>	26S	ITS/5.8S
<i>Ocotea quixos</i> (Lam.) Kosterm.	Ecuador, Napo, Jatun Sa- cha	Neill 9487 (MO)	AF232037 ¹	AF261999 ¹	AF233608 ¹	—	AF262011 ¹	AF272313
<i>Ocotea rhychnophylla</i> (Meisn.) Mez	Guyana, Demerara, Mabu- ra Hill	Chanderbali 220 (MO)	—	AF268766	—	—	—	AF272314
<i>Ocotea schomburgkiana</i> (Nees) Mez	Guyana, Essequibo, Iwok- rama Reserve	Chanderbali 286 (MO)	—	—	—	—	—	AF272315
<i>Ocotea spixiana</i> (Nees) Mez	Brazil, Minas Gerais, Parque Ecol. Tripui	Lorea-Hernandez 5574 (MO)	—	—	—	—	—	AF272316
<i>Ocotea tomentella</i> Sandwith	Guyana, Essequibo, Ka- marang	Chanderbali 284 (MO)	AF268741	AF268765	—	—	—	AF272317
<i>Ocotea tristic</i> (Nees & Mart.) Mez	Brazil, Minas Gerais, Parque Ecol. CEMIG	Lorea-Hernandez 5577 (MO)	AF268742	—	—	—	—	AF272318
<i>Ocotea veraguensis</i> (Meisn.) Mez	Nicaragua, Chontales	Stevens 24177 (MO)	—	—	—	—	—	AF272319
<i>Parceta bracteata</i> Rohwer, H. G. Richt. & van der Werff	Brazil, Amazonas, Iquitos	Vicentini 1288 (MO)	AF268743	—	—	—	—	AF272320
<i>Parasassafras confertiflora</i> (Meisn.) Long	China, Yunnan, Lishui Co.	Li Heng 10030 (MO)	—	AF268790	—	AF268238	AF270480	AF272321
<i>Persea americana</i> Mill.	Missouri BG	Chanderbali 323 (MO)	AF268744	AF268794	—	—	—	AF272322
<i>Persea caerulea</i> (Ruiz & Pav.) Mez	Peru, Amazonas	van der Werff 14744 (MO)	—	AF268795	—	—	—	AF272323
<i>Persea lingue</i> (Ruiz & Pav.) Nees ex Kopp	Chile	Greissl 640-99 (MJG)	—	AF268796	—	—	—	AF272324
<i>Persea meridensis</i> Kopp	Venezuela, Trujillo, Bo- con6	Cuello 943 (MO)	AF268745	AF267797	AF268836	—	—	AF272325
<i>Persea</i> sp.	Vietnam, Vinh Phuc	van der Werff 14071 (MO)	—	—	—	—	—	AF272326
<i>Persea thunbergii</i> Siebold & Zucc.	Missouri BG	Chanderbali 328 (MO)	AF268746	AF268798	—	—	—	AF272327
<i>Phoebe formosana</i> (Hayata) Hayata	Bonn BG	Rohwer 156 (MJG)	—	—	—	—	—	AF272328 ²
<i>Pleurothyrium cinereum</i> van der Werff	Peru, San Martin, Riojas	van der Werff 15325 (MO)	AF268747	AF268769	—	—	—	AF272329
<i>Pleurothyrium insigne</i> van der Werff	Ecuador, Napo, Jatun Sa- cha	Neill 9033 (MO)	—	—	—	—	—	AF272330

Table 1. Continued.

Taxon	Provenance	Voucher	Genbank accession numbers						
			<i>trnL-trnF</i>	<i>psbA-trnH</i>	<i>trnT-trnL</i>	<i>rpl16</i>	26S	ITS/5.8S	
<i>Potameia micrantha</i> van der Werff	Madagascar, Toamasina	van der Werff 12777 (MO)	AF268749	AF268815	—	—	—	AF270481	—
<i>Potameia microphylla</i> Kosterm.	Madagascar, Fianarantsoa	van der Werff 12655 (MO)	AF268750	AF268816	—	—	—	—	—
<i>Potoxylon melagangai</i> (Symington) Kosterm.	Brunei, Tutong, Kampong Lamumin	Hyland 14969 (MO)	AF268748	AF268821	—	—	—	—	—
<i>Rhodostemonodaphne crenaticupula</i> Madriñán	Guyana, Essequibo, Iwok-rana Reserve	Chanderbali 265 (MO)	AF268751	AF268759	—	—	—	—	AF272331
<i>Rhodostemonodaphne praeclara</i> (Sandwith) Madriñán	Guyana, Essequibo, Iwok-rana Reserve	Chanderbali 256 (MO)	AF268752	AF268760	AF268827	—	—	—	AF272332
<i>Rhodostemonodaphne recurva</i> van der Werff	Brazil, Amazonas, Manaus	Vicentini 653 (MO)	—	—	—	—	—	—	AF272333
<i>Rhodostemonodaphne scandens</i> Madriñán	Guyana, Essequibo, Iwok-rana Reserve	Chanderbali 271 (MO)	—	—	—	—	—	—	AF272334
<i>Sassafras albidum</i> (Nutt.) Nees	Missouri BG	Chanderbali 325 (MO)	AF268753	AF268793	AF268832	AF268242	—	AF264140*	AF272335
<i>Sassafras tzumu</i> (Hemsl.) Hemsf.	China, Hunan, Xining Co.	Luo Lin-bo 1242 (MO)	—	—	—	—	—	—	AF272336
<i>Sextonia pubescens</i> van der Werff	Peru, Loreto, Iquitos	Vásquez 25229 (MO)	AF232038 ¹	AF262000 ¹	AF233609 ¹	AF232748 ¹	—	AF262012 ¹	AF268808
<i>Sextonia rubra</i> (Mez) van der Werff	Brazil, Amazonas, Manaus	Nascimento 574 (MO)	AF268754	AF268805	—	—	—	AF270482	—
<i>Umbellularia californica</i> (Hook. & Arn.) Nutt.	Missouri BG	Chanderbali 326 (MO)	AF268755	AF268777	—	—	—	—	AF272337
<i>Urbanodendron bahiense</i> (Meisn.) Rohrer	Brazil, Rio de Janeiro	Martinelli 10019 (MO)	—	—	—	—	—	—	AF272338
<i>Urbanodendron verrucosum</i> (Nees) Mez	Brazil, Minas Gerais, Faz. Macedônia	Braga s.n. (BHCB) 19385 (MO)	—	—	—	—	—	—	AF272339
Gomortegaceae									
<i>Gomortega nitida</i> Ruiz and Pav.	Chile	Rodriguez 3070 (CONC)	AF012404 ¹	AF129053 ¹	—	—	—	AF127260 ¹	AF262014 ¹
Hernandiaceae									
<i>Cyrtocarpus americanus</i> Jacq.	Sri Lanka, Colombo BG	Chase 317 (NCU)	AF012398 ¹	AF129054 ¹	AF129025 ¹	—	—	AF127261 ¹	AF262001 ¹
<i>Hernandia moerenhoutiana</i> Guillem.	Australia, Brisbane, Mt. Cootha BG	not available	AF052198 ¹	AF129055 ¹	AF129026 ¹	—	—	AF130310 ¹	—

Table 1. Continued.

Taxon	Provenance	Voucher	Genbank accession numbers					
			<i>trnL-trnF</i>	<i>psbA-trnH</i>	<i>trnT-trnL</i>	<i>rpl16</i>	26S	ITS/5.8S
<i>Illigeria luzonensis</i> (Presl) Merr.	Munich BG	not available	AF052199 ¹	AF129057 ¹	AF129030 ¹	AF127264 ¹	AF262002 ¹	—
<i>Sparattanthelium wonotooboense</i> Kosterm.	Munich BG	Munich BG 47/1311	AF053342 ¹	AF129070 ¹	AF129043 ¹	AF127262 ¹	—	—
Monimiaceae								
<i>Hortonia floribunda</i> Wight ex Arn.	Sri Lanka, Colombo BG	not available	AF040683 ¹	AF129071 ¹	AF129028 ¹	AF129027 ¹	AF264143 ⁴	—
<i>Monimia ovalifolia</i> Thouars	La Reunion	<i>Stasberg s.n.</i> (REU)	AF054896 ¹	AF129065 ¹	AF129038 ¹	AF127269 ¹	AF246144 ⁴	—
<i>Palmeria scandens</i> Muell.	Australia, New South Wales	<i>Bradford 878</i> (MO)	AF052200 ¹	AF129067 ¹	AF129040 ¹	AF127270 ¹	AF264142 ⁴	—
<i>Peumus boldus</i> Molina	Edinburgh BG	Edinburgh BG 19870707	AF012403 ¹	AF129068 ¹	AF129041 ¹	AF127454 ¹	AF264141 ⁴	—

DMSO was added and the PCR protocol described by Kuzoff et al. (1998) was followed. The *trnL-trnF* and *trnT-trnL* regions were amplified using primers designed by Taberlet et al. (1991). The *trnL-trnF* spacer sequences begin near the 5' end of the spacer and include 138 bp of the 5' end of the tRNA-Phe (*trnF*) gene. The spacer between tRNA-Leu (*trnL*) and tRNA-Thr (*trnT*) aligns readily with other lauralean *trnT-trnL* sequences produced by Renner (1999), but is difficult to align with the few available *trnT-trnL* sequences in GenBank, the most similar of which are *trnT-trnL* sequences from *Dioscorea* (48% similarity). The *psbA-trnH* spacer and *rpl16* intron were amplified using primers of Sang et al. (1997) and Asmussen (1999), respectively. The *psbA-trnH* sequences obtained for Lauraceae include the entire spacer region and overlap by about 40 base pairs with the 3' end of the *psbA* gene and 5' end of the *trnH* gene of *Helianthus annuus* L. deposited in Genbank (X60428). The 5' region that includes the first two expansion domains of 26S rDNA was amplified using a forward primer (D. Nickrent, pers. comm.) that anneals at ca. 70 nucleotide positions downstream from the 5' end of the gene and the 641 R reverse primer of Kuzoff et al. (1998). To increase efficiency of PCR amplification of ITS, the few lauraceous sequences obtained using angiosperm-specific ITS A and ITS B primers (Blattner, 1999) were used to design a forward primer (5'-ACCACCACCGCAACCA-3') that anneals at about 10 bp downstream of the 3' end of 18S. This primer (hereafter LAUR 1) appears to be specific to a large terminal clade in Lauraceae that includes the tribes Perseeae and Laureae and their sister group. In most cases it was possible to amplify the entire ITS region using LAUR 1 and ITS B, but for some poor-quality templates it was necessary to amplify the region in sections (ITS1 & ITS2) by combining the appropriate universal primers of White et al. (1990) with LAUR 1 and ITS B. The ITS/5.8S sequences produced include all but the first ca. 10 bp of ITS 1 and the entire ITS 2 and 5.8S regions. Rarely, only ITS 1 or ITS 2 was obtained.

PCR products were purified following the protocol provided by QIAquick gel extraction kits (QIAGEN) and sequenced using the dye terminator cycle sequencing protocol (Applied Biosystems). Sequence reactions were analyzed on ABI 373 or ABI 377 automated sequencers (University of Missouri-Columbia DNA Core Sequencing Facility and University of Missouri-St. Louis D. E. Lee and Family Sequencing Facility). Except for the *psbA-trnH* region, which could only be sequenced from the 5' end, both strands of DNA were read and

consensus sequences generated using Sequencher vers. 3.1 (Gene Codes Corp., 1998). Sequences were manually aligned using the sequence editing facilities of Seqppp VERSION 0.6 (D. Gilbert, Indiana University, Bloomington, 1996). Sequence alignment was relatively straightforward in Lauraceae. However, the first 290 bp of the *trnT-trnL* spacer were removed from analysis because of alignment difficulties among basal Lauraceae and outgroup families. Alignment difficulties with the outgroup were also encountered in the 3' half of the *psbA-trnH* spacer. However, unlike *trnT-trnL* sequences, *psbA-trnH* provided several informative characters within Lauraceae and instead of removing it entirely, outgroup sequences were truncated after alignment became ambiguous (ca. position 300).

PHYLOGENETIC ANALYSES

Initial analyses of the individual data sets were conducted as heuristic searches for most parsimonious trees with 10 random taxon additions and TBR branch swapping using PAUP* version 4.0b4 (Swofford, 1998). Both the MULPARS and COLLAPSE options were in effect, but the STEEPEST DESCENT option was not employed. Characters were assumed to be unordered (i.e., Fitch parsimony), equally weighted, and gaps were treated as missing data. Parsimony uninformative characters were excluded. Bootstrap analyses (Felsenstein, 1985) with 500 replications were performed with the above heuristic search settings but with MAXTREES set to 100. Since these initial searches showed no strongly supported conflict, i.e., alternative clades supported by > 70% bootstrap values (Hillis & Bull, 1993), and P-values from partition-homogeneity tests ranged from 1.0 to 0.6 (strongly indicating congruence), three matrices were compiled for further analyses. Matrix I combined 86 species that were sequenced for the *trnL-trnF* and *psbA-trnH* regions. Matrix II combined *trnL-trnF*, *psbA-trnH*, *trnT-trnL*, *rpl16*, and 26S sequences for 42 taxa representing major lineages identified by analyses of matrix I to further investigate basal relationships. Matrix III included 94 ITS sequences from representatives of a large terminal clade that was poorly resolved by sequence variation in matrix I. The only mutation in matrix I that provided generic-level information in this terminal clade, a 16-bp repeat in *trnL-trnF*, was included in matrix III. Analyses of all three matrices were conducted as above with length mutations (insertions and deletions) introduced as binary characters of equal weight. In addition, minimum evolution topologies

were calculated for the ITS data set using log determinant (LogDet) and maximum likelihood estimates of genetic distances. Maximum likelihood distances were calculated using the Hasegawa-Kishino-Yano (1985) model with rate heterogeneity among sites (HKY- Γ).

MOLECULAR CLOCK ANALYSES

In order to add a temporal dimension to the phylogenetic estimate, divergence times for major branches were estimated. Due to the computational demands of maximum likelihood calculations and because it was not necessary to obtain divergence times for all nodes, likelihood scores were obtained from parsimony-based topologies that were reduced to exemplars of major clades. ITS/5.8S sequences were used for terminal, and the cpDNA and 26S sequences for basal lineages.

Likelihood scores were calculated under the Hasegawa-Kishino-Yano (1985) model with rate heterogeneity among sites, and values obtained with and without a clock constraint compared for significance using a χ^2 test [$\chi^2 = 2(\log L_{\text{clock}} - \log L_{\text{nonclock}})$; d.f. = number of taxa - 2]. For each internal node, HKY- Γ distances (branch lengths) to the tip were computed by PAUP* with the clock constraint enforced. These values represent the number of substitutions per site accumulated along each daughter lineage, and were either divided by time to determine substitution rates, or divided by estimated rates to obtain divergence times. Overlap in taxon sample between terminal and basal topologies allowed use of divergence times estimated in one to calibrate substitution rates and calculate divergence times in the other. Standard deviations of divergence times were estimated as follows. First, standard deviations of HKY- Γ distances were calculated using a formula derived from the relationship $S = Np$, where S is the number of nucleotide substitutions, N is the total number of nucleotide positions in a sequence, and p is the proportion of nucleotides substituted (HKY- Γ distance from node to branch tips). Since the standard deviation of S is the square root of $Np(1 - p)$, or $SD(S) = \sqrt{Np(1 - p)}$, the standard deviation of p is $SD(S)$ divided by N, or $SD(p) = \sqrt{p(1 - p)/N}$. This value is calculated for each divergence of interest and divided by substitution rate to obtain the standard deviation of divergence times.

RESULTS

PHYLOGENETIC ANALYSES

Sequence variation in chloroplast markers and partial 26S sequences was almost limited to basal

Table 2. Characteristics and comparisons of phylogenetic information provided by matrices analyzed in this study. Matrix I combines *trnL-trnF* and *psbA-trnH* cpDNA sequences, Matrix II combines *trnL-trnF*, *trnT-trnL*, *psbA-trnH*, and *rpl16* of cpDNA with 26S rDNA sequences; Matrix III is based on ITS rDNA sequences.

Matrices	Aligned length	Number of representatives of:		Parsimony-informative substitutions among:	
		Lauraceae	Perseeae & Laureae	Lauraceae	Perseeae & Laureae
<i>trnL-trnF</i>	510	76	48	103	7
<i>psbA-trnH</i>	616	75	48	135	35
<i>trnT-trnL</i>	530	33	19	105	12
<i>rpl16</i>	1049	24	10	103	9
26S	592	22	8	77	11
Matrix I	1126	77	48	238	42
Matrix II	3297	42	4	470	n/a
Matrix III	780	94	90	n/a	199

branches in Lauraceae with very little and often no variation among members of tribes Perseeae and Laureae (sensu van der Werff & Richter, 1996). The *trnL-trnF* matrix included 510 aligned positions and yielded 103 informative substitutions within Lauraceae, but only seven of these were informative among Perseeae and Laureae. Similarly, of 616 aligned positions in the *psbA-trnH* matrix, 135 substitutions were parsimony informative within Lauraceae and 35 among Perseeae and Laureae. This trend was also found in other chloroplast loci and partial 26S sequences. Only ITS showed substantial variation within Perseeae and Laureae. Characteristics of individual genetic markers and combined data sets are summarized in Table 2.

Combined *trnL-trnF* and *psbA-trnH* matrix (matrix I) included 1126 aligned positions of which 277 were informative. Four indels from *trnL-trnF* were added as binary characters. Three of these supported a clade comprised of *Beilschmiedia*, *Cryptocarya*, *Endiandra*, and *Potameia*, and the fourth is a 16-base pair repeat found in several neotropical genera of Perseeae and *Umbellularia* of Laureae. Parsimony analyses of matrix I surpassed memory limitations in the first addition replicate, and 29,000 equally parsimonious topologies were retained after 24 hours of branch swapping ($L = 796$, $CI = 0.74$, $RI = 0.89$). The strict consensus topology (Fig. 1) shows two well-supported clades in Lauraceae. One of these includes members of Cryptocaryeae (sensu van der Werff & Richter, 1996), with *Hypodaphnis* tenuously placed as its sister group. The second major clade is unequally divided into a small clade of South American genera (hereafter *Chlorocardium-Mezilaurus* clade) and a large terminal clade comprised of representatives of Laureae and Perseeae (sensu van der Werff & Richter, 1996). This Perseeae-Laureae

clade is essentially unresolved but there is some support for a clade including representatives of *Persea* and *Alseodaphne*, a large generic complex centered around *Ocotea*, and strong support for a clade including all Asian members of *Cinnamomum*. Furthermore, a clade uniting most neotropical genera (*Aniba*, *Endlicheria*, *Kubitzkia*, *Licaria*, *Nectandra*, *Pleurothyrium*, and *Rhodostemonodaphne*) with neotropical (but not paleotropical) species of *Ocotea* and Californian *Umbellularia* is recovered in all trees but does not receive strong support. All members of this clade, except *O. leucoxylon* (Sw.) Laness., have the 16-bp repeat mentioned above. *Caryodaphnopsis*, *Cassytha*, and *Neocinnamomum* constitute a clade in all trees but this association does not receive more than 50% bootstrap support, and neither does its position as the sister group of the second major infrafamilial group.

Matrix II included 3304 characters, of which 684 were parsimony-informative substitutions and 7 were binary-coded length mutations. Parsimony analysis converged on a single island of 2646 equal length trees ($L = 2171$, $CI = 0.71$, $RI = 0.72$), the majority rule consensus of which is shown in Figure 2. The topology is mostly congruent with that provided by matrix I, differing mainly in that *Hypodaphnis* occupies a fairly well supported position (found in 81% of the bootstrap replicates) sister to the rest of the family. The placement of *Caryodaphnopsis*, *Cassytha*, and *Neocinnamomum* in the second main clade receives very strong (98%) bootstrap support, and relationships within the *Chlorocardium-Mezilaurus* clade are altered slightly.

Matrix III, based mainly on ITS sequences, included 259 parsimony-informative substitutions and 19 length mutations that could be unambiguously coded. Parsimony analysis found a single is-

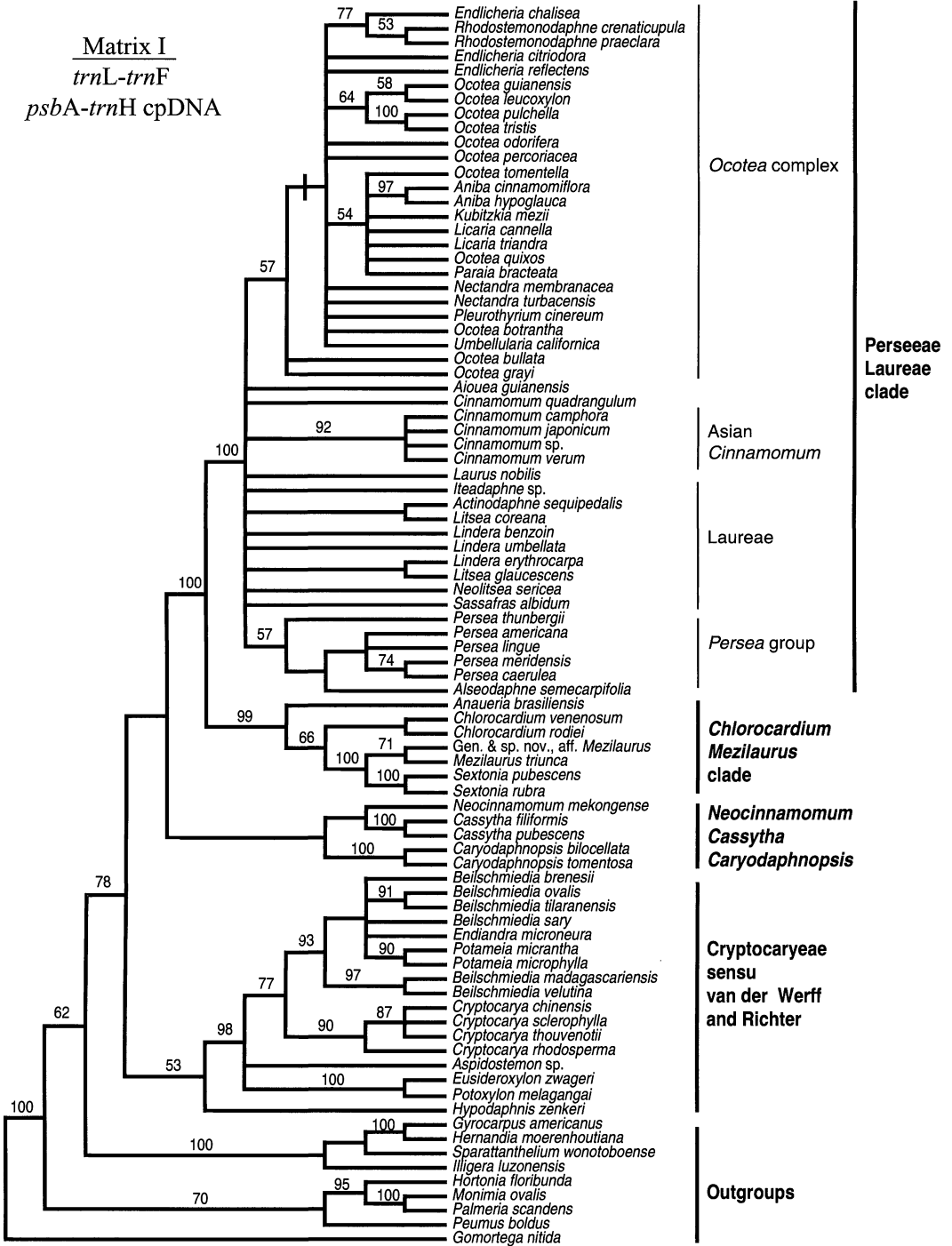


Figure 1. Strict consensus of 29,000 trees retained from parsimony analysis of matrix I (*trnL-trnF* and *psbA-trnH* cpDNA sequences). Numbers above branches indicate bootstrap support. The vertical bar through the branch supporting the terminal clade indicates a 16-bp repeat in the *trnL-trnF* intergenic spacer that unites taxa therein.

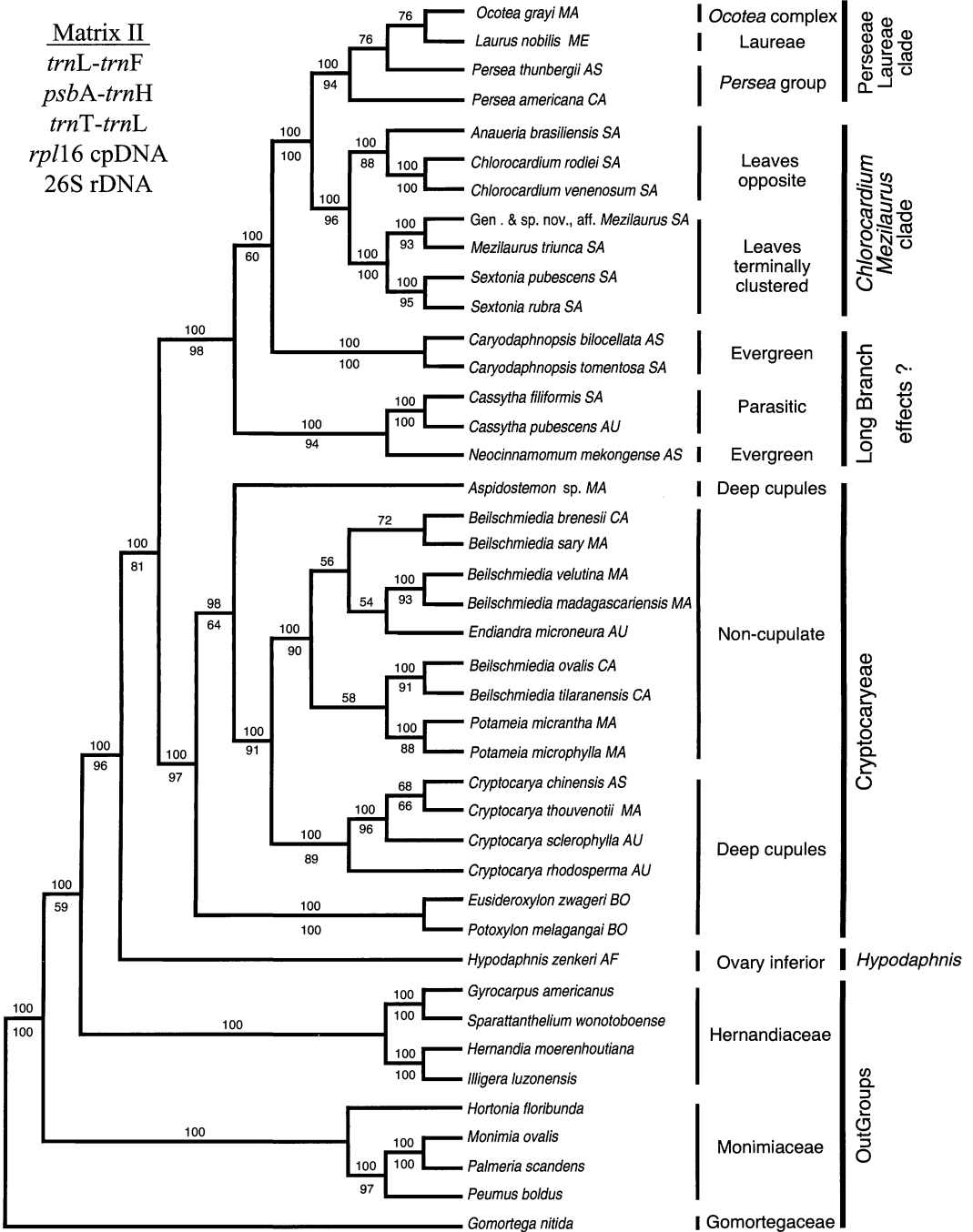


Figure 2. Annotated 50% majority rule tree resulting from parsimony analysis of matrix II (*trnL-trnF*, *psbA-trnH*, *trnT-trnL*, *rpl16*, and 26S sequences). Majority rule percentages are indicated above, and bootstrap values > 50% below, branches. Main clades and significant morphological characters are indicated. Geographic regions represented by each species of Lauraceae are given: AF = Africa, AS = Asia, AU = Australia, BO = Borneo, CA = Central America, MA = Madagascar, ME = Mediterranean, and SA = South America.

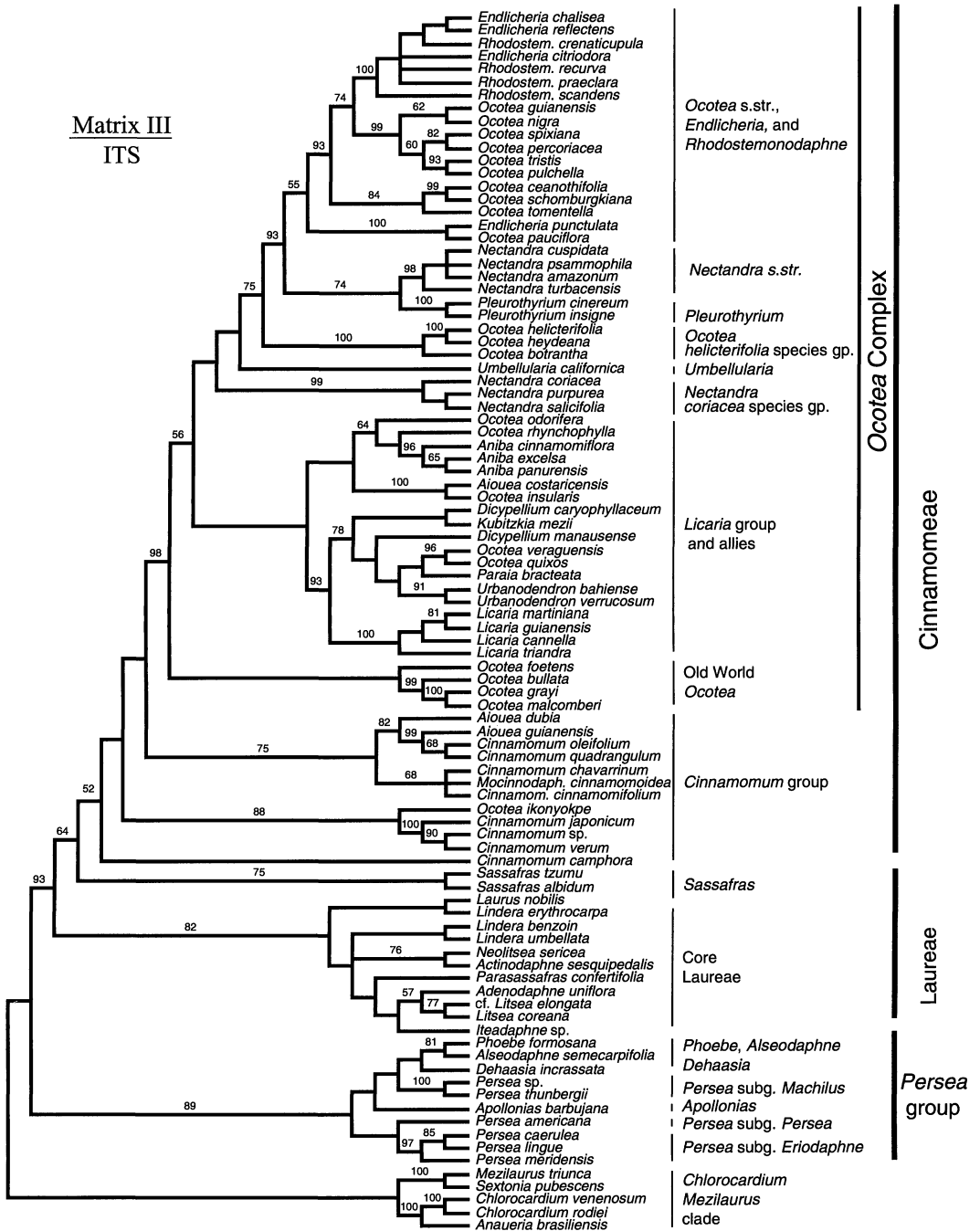


Figure 3. Adams consensus of 567 equally parsimonious trees obtained from unconstrained analysis of matrix III (ITS sequences). Numbers above branches indicate bootstrap support, and vertical bars to the right circumscribe main clades. *Cinnamom.* = *Cinnamomum*, *Mocinnodaph.* = *Mocinnodaphne*, and *Rhodostem.* = *Rhodostemonodaphne*.

land of 567 equal length trees ($L = 1050$, $CI = 0.44$, $RI = 0.75$). The Adams consensus topology (Fig. 3) indicates that Perseeae sensu van der Werff and Richter (1996) are paraphyletic, and that most

of the topological instability exists among members of *Cinnamomum*. *Sassafras* was placed between Laureae and a paraphyletic *Cinnamomum* in all trees. This reconstruction does not receive strong

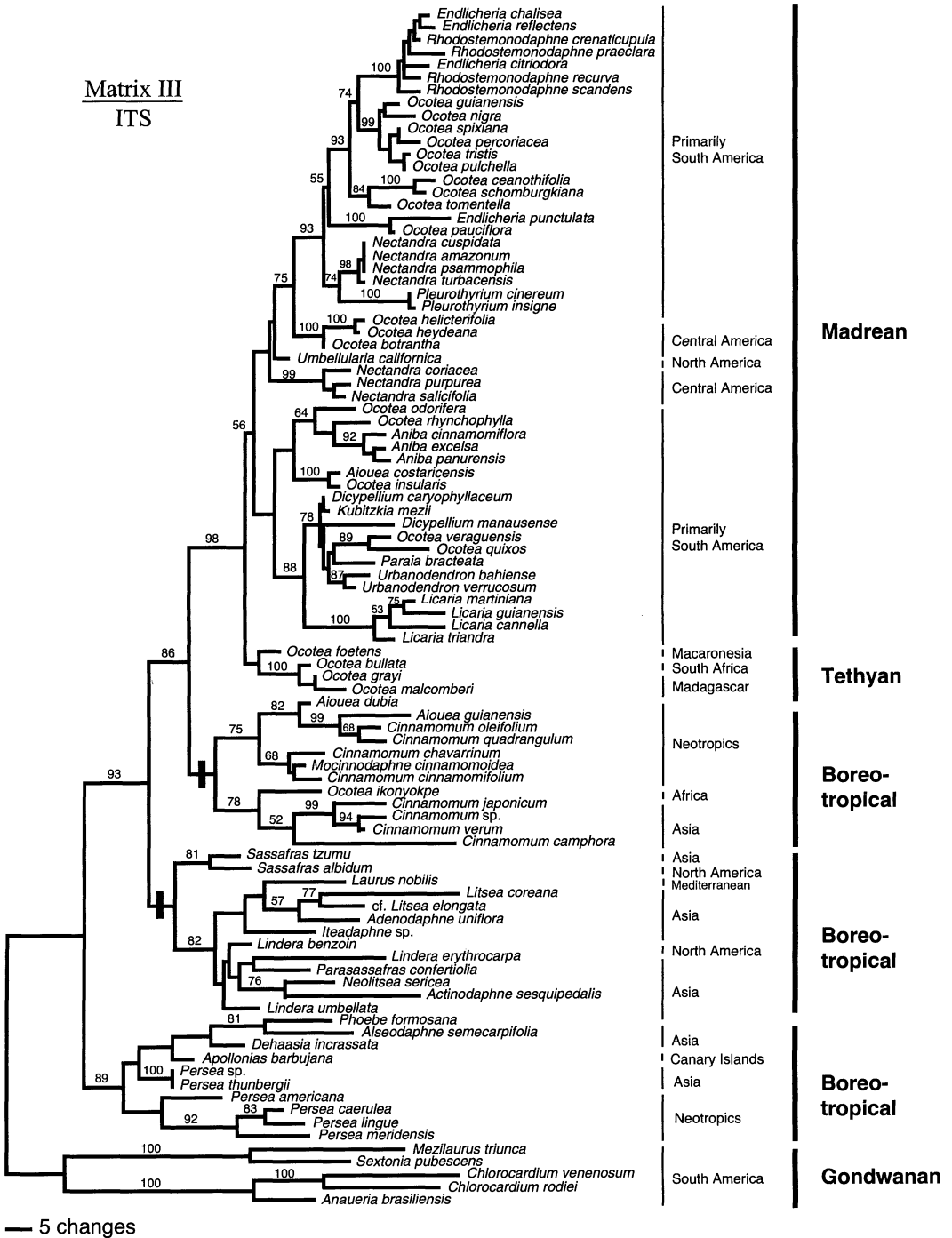


Figure 4. One of 126 equally parsimonious trees obtained with monophyly of the *Cinnamomum* group (*Cinnamomum*, *Aiouea* p.p., *Mocinnodaphne*, and *Ocotea* p.p.) and *Laureae* enforced (thick vertical bars) as topological constraints. Bootstrap support values are indicated above unconstrained branches. Annotated bars to the right indicate the distribution and inferred ancestry of clades.

bootstrap support, and constraining monophyly of a clade comprised of *Cinnamomum* and its allies (*Aiouea* p.p., *Mocinnodaphne*, *Ocotea* p.p.) required only one extra step while constraining monophyly of Laureae to include *Sassafras* added three extra steps to parsimony-based trees. One of 126 equally parsimonious trees ($L = 1054$, $CI = 0.44$, $RI = 0.75$) calculated with both topological constraints enforced is shown in Figure 4. Both minimum-evolution analyses recovered topologies that showed the same main clades found by parsimony analyses. Although *Cinnamomum* was again not monophyletic, minimum evolution placed *Sassafras* sister to remaining Laureae, albeit together with *Cinnamomum camphora* (L.) Presl. Differences between parsimony and minimum evolution reconstructions suggest that some instability in parsimony-based topologies can be attributed to characters shared between *Sassafras* and *C. camphora*. With *C. camphora* removed the number of trees resulting from unconstrained parsimony analyses is greatly reduced (to 36, $L = 1022$, $CI = 0.44$, $RI = 0.76$), but *Sassafras* is still placed between Laureae and paraphyletic *Cinnamomum*.

MOLECULAR CLOCK ANALYSES

A likelihood ratio test on the ITS data set reduced to 25 representatives of main lineages in higher Lauraceae (Fig. 5a) indicated that substitution was approximately clock-like. Log likelihood scores with (-2844.00) and without a clock constraint (-2827.85) were not significantly different ($\chi^2 = 32.30$, d.f. = 23, $P > 0.05$). Of molecular markers used to resolve basal relationships, only *rp16* did not reject the molecular clock [$\chi^2 = 2(2590.76 - 2582.73) = 16.06$; d.f. = 10, $P > 0.05$], provided *Neocinnamomum* and *Cassytha* were removed from analysis (Fig. 5b). Results of two calibrations simulating alternative biogeographic scenarios are summarized in Table 3, and those of our preferred calibration are depicted in Figure 5.

DISCUSSION

The two-tiered taxon and molecular sampling adopted in this study provides a generally well sup-

ported generic-level phylogeny for Lauraceae. Data from chloroplast markers and partial 26S sequences resolve main clades, while ITS provides novel resolution among members of Perseeae and Laureae (sensu van der Werff & Richter, 1996). To provide a basis for subsequent biogeographic considerations, phylogenetic relationships among Lauraceae are first discussed.

PHYLOGENETIC RELATIONSHIPS

Several previously recognized taxonomic groups, albeit in different schemes, compare favorably with clades supported by our molecular data. Among these, Cryptocaryeae as circumscribed by van der Werff and Richter (1996), Laureae of most classifications (e.g., Kostermans, 1957; van der Werff & Richter, 1996; Rohwer, 1993a), and Cinnamomeae in the sense of Kostermans (1957) are the largest. In addition, a generic grouping centered around *Persea*, informally recognized by Rohwer (1993a), herein receives considerable support. A fifth major generic grouping, the *Chlorocardium-Mezilaurus* clade, is comprised of taxa whose taxonomic positions have previously been uncertain. Outside of these main clades the position of a few small genera is unsettled.

Hypodaphnis and Cryptocaryeae. Monotypic *Hypodaphnis*, consisting of *H. zenkeri* (Engl.) Stapf from Central Africa, is the only member of Lauraceae with an inferior ovary, and the two analyses that investigated basal relationships in Lauraceae suggested different positions (compare Figs. 1 and 2). The *trnL-trnF* and *psbA-trnH* data sets analyzed in matrix I weakly support a sister group relationship between *Hypodaphnis* and Cryptocaryeae (Fig. 1). Association with Cryptocaryeae is supported by irregular thyrsoid inflorescences (van der Werff & Richter, 1996) and morphological similarity with *Eusideroxylon* and *Potoxylon*, two monotypic Indonesian genera that consistently place basally in Cryptocaryeae (Figs. 1, 2). Like *Hypodaphnis*, they have stamens with four collaterally arranged locelli, but their ovaries are only semi-inferior. However, the larger molecular sample (matrix II) places *Hypodaphnis* sister to remaining Lauraceae with mod-

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Figure 5. Phylograms showing clock enforced HKY- Γ distances on reduced parsimony-based topologies depicting terminal (5a) and basal (5b) clades in Lauraceae. Divergence times in Figure 5a are based on ITS and those in Figure 5b on *rp16* sequences. The time scale below each phylogram reflects a calibration in which equivalent nodes (indicated by *) in Figure 5a and Figure 5b are fixed at 90 million years. Node labels (a-f, x, z, and A-G) correspond to those listed in Table 3. The geographic distributions of terminal taxa are given: AF = Africa, AS = Asia, BO = Borneo, CA = Central America, CI = Canary Islands, MA = Madagascar, MC = Macaronesia, NA = North America, and SA = South America.

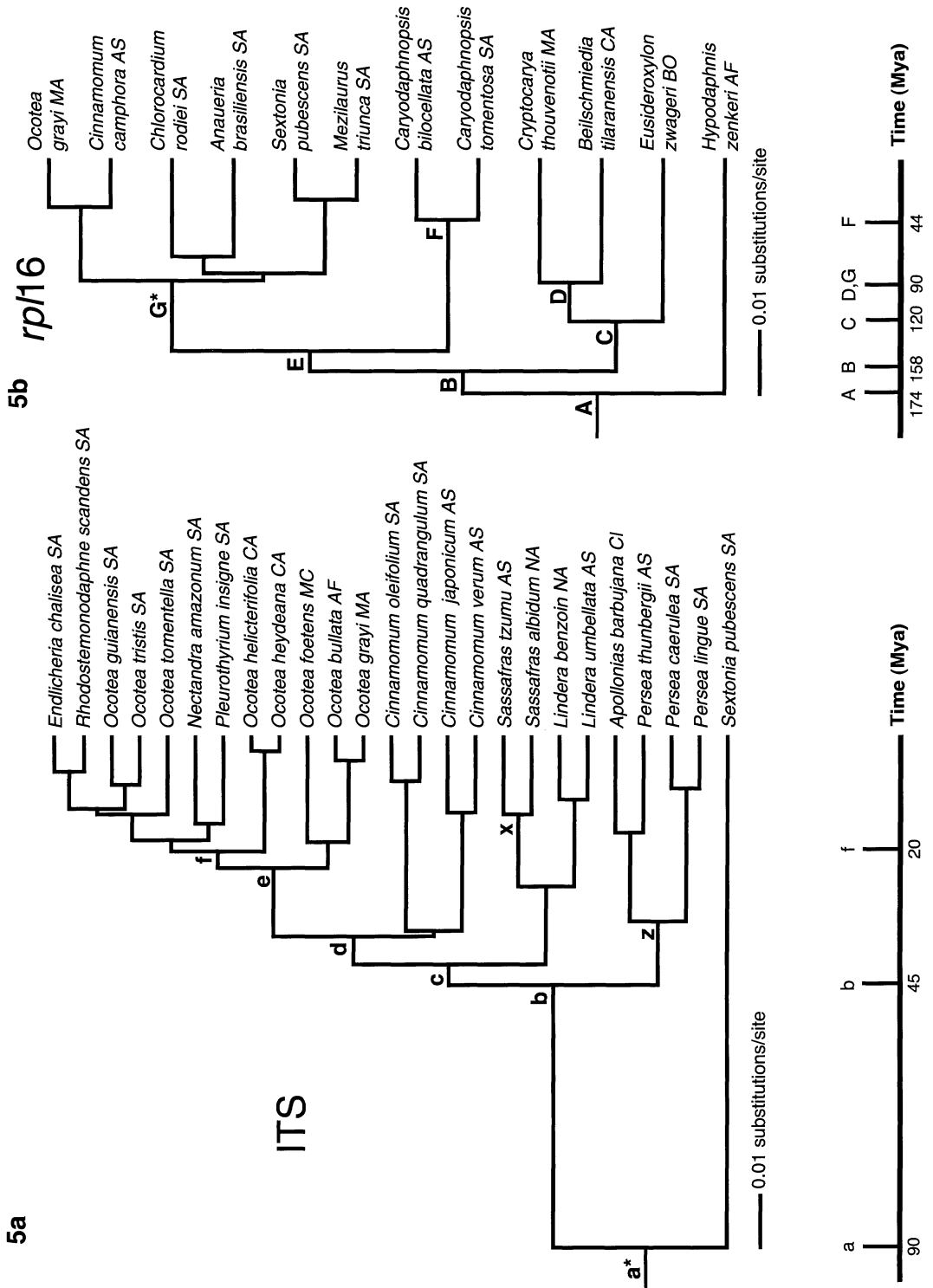


Table 3. Clock enforced HKY- Γ , distances accumulated after divergence events among terminal (Table 3a) and basal (Table 3b) lineages in Lauraceae, and estimates of divergence times simulating two biogeographic scenarios. HKY- Γ , distances in Table 3a are obtained from ITS and those in Table 3b from *rp16* sequences. Calibration 1 simulates West Gondwanan vicariance for the trans-Atlantic disjunction in the *Ocotea* complex; calibration 2 simulates Gondwanan origin for the *Chlorocardium-Mezilaurus* clade. Node labels are equivalent to those in Figure 5, and ages fixed for calibration purposes are underlined. * = Equivalent nodes.

Node	HKY- Γ distance from node to tip \pm SD	Calibration 1 time (Mya) \pm SD	Calibration 2 time (Mya) \pm SD
a. Terminal lineages in Lauraceae.			
a*	0.09740 \pm 0.0106	354 \pm 30	<u>90</u>
b	0.05715 \pm 0.0083	171 \pm 21	44 \pm 7
c	0.04309 \pm 0.0073	156 \pm 20	40 \pm 7
d	0.03793 \pm 0.0068	138 \pm 19	35 \pm 6
e	0.02476 \pm 0.0055	<u>90</u>	23 \pm 5
f	0.02175 \pm 0.0052	79 \pm 14	20 \pm 5
x	0.01437 \pm 0.0042	52 \pm 12	13 \pm 4
z	0.03499 \pm 0.0066	127 \pm 18	32 \pm 6
b. Basal lineages in Lauraceae.			
A	0.03886 \pm 0.0059	682 \pm 105	174 \pm 32
B	0.03513 \pm 0.0056	620 \pm 100	158 \pm 31
C	0.02683 \pm 0.0050	473 \pm 88	120 \pm 27
D	0.02038 \pm 0.0044	360 \pm 77	91 \pm 20
E	0.03162 \pm 0.0054	558 \pm 95	142 \pm 24
F	0.00977 \pm 0.0058	172 \pm 54	44 \pm 14
G*	0.02006 \pm 0.0043	<u>354</u>	<u>90</u>

erately high support (Fig. 2). This peripheral position for *Hypodaphnis* is also indicated by *matK* sequences, albeit with < 50% bootstrap support (Rohwer, 2000), but difficult to support with morphology. Any outgroup comparison is stymied by the unsettled sister family relationship of Lauraceae. Hernandiaceae, with inferior ovaries, are favored over Monimiaceae with 100% bootstrap support by morphology (Doyle & Endress, 2000), but molecular data have been ambiguous (Renner, 1999; Renner & Chanderbali, 2000; Qiu et al., 1999). A Hernandiaceae-Lauraceae clade receives modest support here (Figs. 1, 2), and when *Hypodaphnis* lies sister to remaining Lauraceae (Fig. 2), inferior ovaries are a potential synapomorphy for the two families. However, given the non-inferior state of all other Lauraceae, independent gain, required also by the topology in Figure 1, is equally parsimonious.

Cryptocaryeae sensu stricto. Support for the clade comprised of *Aspidostemon*, *Beilschmiedia*, *Crypto-*

carya, *Endiandra*, *Eusideroxylon*, *Potameia*, and *Potoxylon* is considerable in both analyses investigating basal relationships in Lauraceae (Figs. 1, 2). Anatomical features stress isolation of *Aspidostemon*, *Eusideroxylon*, and *Potoxylon* more than their affinities (Richter, 1981), but close relationship with *Cryptocarya* has been suggested in recent morphology-based systems (e.g., Rohwer, 1993a; van der Werff & Richter, 1996). With *Cryptocarya* they share a deeply urceolate floral hypanthium that develops into deep cupules enclosing the drupe except for a small terminal orifice. Unlike the previous genera, in *Beilschmiedia* and *Endiandra* the hypanthium is shallow and a cupule never develops, while the fruits of *Potameia* are either free or seated in a small discoid structure. Kostermans (1957), stressing the degree of cupule development in his scheme for Lauraceae, placed *Beilschmiedia*, *Endiandra*, and *Potameia* with *Persea* (also non-cupulate, but of the distal Perseeae-Laureae clade). Close relationship with *Cryptocarya* has since gained support from wood and bark anatomy (Richter, 1981), inflorescence morphology (van der Werff & Richter, 1996), embryology (Heo et al., 1998), and molecular data (Rohwer, 2000; herein).

The topology in Cryptocaryeae reveals a trend toward increased ovary exertion, in both flower and fruit. *Eusideroxylon* and *Potoxylon*, with semi-inferior ovaries, lie sister to genera with superior ovaries. *Aspidostemon* branches next (Fig. 2), and *Cryptocarya* lies sister to the non-cupulate clade of *Beilschmiedia*, *Endiandra*, and *Potameia* (Figs. 1, 2). *Endiandra* and *Potameia* have a reduced number of floral parts relative to *Beilschmiedia*, but whether they nest within the latter (Figs. 1, 2) is not well resolved.

Other genera that have been allied to members of Cryptocaryeae but not herein are either monotypic or oligotypic. Their generic status is also controversial (e.g., Rohwer, 1993a). *Dahlgrenodendron*, with only *D. natalensis* (J. H. Ross) J. J. M. van der Merwe & A. E. van Wyk, has distinctive pollen ornamentation (van der Merwe et al., 1988) but is otherwise not different from *Cryptocarya* (Rohwer, 1993a). *Triadodaphne*, with three species, is tentatively distinguished from *Endiandra* by its unequal perianth whorls and deeper hypanthium (Kostermans, 1993). In *Hexapora*, comprised of *H. curtisii* Hook. f., the outer six stamens are extrorse, and the third and fourth whorls staminodial, but otherwise the genus is similar to *Beilschmiedia*. *Brassioidendron*, with only *B. fragrans* C. K. Allen, also has only six fertile stamens, and according to Kostermans (1957) and Hyland (1989) should be included in *Endiandra*.

Cassytha, *Caryodaphnopsis*, and *Neocinnamomum*. These three genera are among the most enigmatic of the family. In our analyses of matrix I, they constitute a statistically uncorroborated clade (with < 50% bootstrap support) that lies sister to the rest of the family, also without support (Fig. 1). This alliance is disbanded by additional molecular characters provided by matrix II, but their position in this part of the tree receives strong support (Fig. 2). Some elements of *Cassytha*'s position may be due to long branch effects in our analyses.

The herbaceous parasitic twiner, *Cassytha*, is the sole exception to the arborescence typical of Lauraceae, and it has usually been placed in a separate subfamily (e.g., Kostermans, 1957; van der Werff & Richter, 1996). Subfamilial position is supported by ab initio cellular endosperm *Cassytha* shares with Hernandiaceae, Monimiaceae, and other Laurales (Heo & Tobe, 1995; Heo et al., 1998). Such endosperm formation is also reported from *Umbellularia* (Bambacioni-Mezzetti, 1941), but nuclear endosperm is found in all other Lauraceae that have been examined, including *Hypodaphnis* (Heo et al., 1998). In Rohwer's *matK* study *Cassytha* was placed between *Hypodaphnis* and the rest of the family, but without strong statistical support. Here, close relationship with *Neocinnamomum* receives very high statistical support from matrix II (Fig. 2), but morphological synapomorphies for these two genera are not known. Instead, *Cassytha* and *Neocinnamomum* have the longest branches in the topology, differing from each other by over 279 mutations (uncorrected "p" distances, uninformative characters included), while the branch uniting them is supported by comparatively few (66) mutations. With *Neocinnamomum* removed from the analysis, *Cassytha* and *Caryodaphnopsis* constitute a clade (cladogram not shown). In both genera the outer whorl of tepals is strongly reduced, but this condition appears elsewhere in Lauraceae (e.g., *Persea*), and other characters to support a *Cassytha*-*Caryodaphnopsis* clade are unknown. However, with both *Neocinnamomum* and *Caryodaphnopsis* removed, *Cassytha* still lies sister to the rest of the family. If long branches cannot attract in their mutual absence (Sidall & Whiting, 1999), this finding would imply that *Cassytha* is correctly placed in this general part of the phylogeny. Alternatively, long branched taxa, such as *Cassytha*, experience multiple substitutions that erode genealogical signal, randomize character states with respect to true relatives, and lead to chance convergence on the molecular states of distant lineages, all qualities

that can mislead phylogenetic estimates (Felsenstein, 1978; Lyons-Weiler & Hoelzer, 1997).

Caryodaphnopsis and *Neocinnamomum* are morphologically similar, sharing triplinerved venation and four-locular anthers with the locelli arranged in a shallow arc (sometimes two-locular in *Caryodaphnopsis*, in a horizontal row in *Neocinnamomum delavayi* (Lecomte) H. Liu). In contrast, *Caryodaphnopsis* has opposite leaves, a perianth of strongly unequal tepals, and lacks a cupule, while *Neocinnamomum* has alternate (spiral) leaves, subequal tepals, and a shallow cupule with persistent tepals. Close relationship between *Neocinnamomum* and *Cinnamomum* (Kostermans, 1974a), and between *Caryodaphnopsis* and *Persea* (Kostermans, 1974b; Rohwer, 1993a), can be ruled out, but the relationships of these two genera are not clearly indicated by our data. They either constitute a clade, albeit with *Cassytha* (Fig. 1), or *Caryodaphnopsis* lies between a *Neocinnamomum*-*Cassytha* clade and the rest of the family (Fig. 2). With *Cassytha* excluded, either *Caryodaphnopsis* or *Neocinnamomum* lies sister to the rest of the family (cladograms not shown). *Neocinnamomum* was not included in Rohwer's *matK* study, and *Caryodaphnopsis* was placed as it is in Figure 2. Anatomical affinities of *Neocinnamomum* and *Caryodaphnopsis* with *Chlorocardium* and Cryptocaryeae, respectively (Richter, 1981), are consistent with their relatively basal position in the family.

Chlorocardium-*Mezilaurus* clade. The clade comprised of *Anaueria*, *Chlorocardium*, *Mezilaurus*, *Sextonia*, and a novel taxon (Gen. & sp. nov.) receives 99% and 96% bootstrap support from matrix I and II, respectively (Fig. 1). It is one of the more intriguing clades in the family. Close relationship between *Anaueria* and *Mezilaurus* has been suggested (Richter, 1981; Rohwer, 1993a), but the possibility that all these taxa constitute a clade of their own has never been considered on morphological grounds. So far, characters uniting them have only been provided by molecular data. A clade comprised of *Chlorocardium* and *Mezilaurus* (plus *Williamodendron*, a small genus of 3 species differing from *Mezilaurus* primarily in the number of locelli; not herein) received moderate support from *matK* sequences, but Rohwer (2000) questioned a close relationship citing anatomical and floral differences. Here, *Chlorocardium* and *Mezilaurus* place in separate subclades that can be characterized by phyllotaxy.

The subclade of Gen. & sp. nov., *Mezilaurus*, and *Sextonia* accommodates taxa with obovate, coriaceous leaves borne in terminal clusters (Fig. 2).

Unlike most other Lauraceae with terminally clustered leaves, in these taxa the clusters are not separated by seasonal growth spurts. Instead, growth is continuous, and apparently quite slow, resulting in a continuous spiral of leaf scars. This growth pattern is rare but not unique to this clade; such leaf clusters are at least also known in *Alseodaphne*, of the *Persea* group (below). In the other subclade, *Anaueria* and *Chlorocardium* share opposite leaves (Fig. 2). This subclade receives considerable molecular support, but both genera find morphological allies within the other subclade. *Anaueria* is anatomically more similar to *Mezilaurus* (Richter, 1981), with which it also shares glandless flowers. *Chlorocardium* is unique with its xylem of coronated vessel elements (Kostermans et al., 1969), and its large rotate flowers with an increased number of stamens (to 20) are unmatched in the clade. However, its papillose, tongue-shaped stamens, all with a pair of small basal glands, are much like those found in the second and third staminal whorls of *Sextonia*.

All South American genera that do not clearly assign to generic groups based on wood and bark anatomy or inflorescence structure (van der Werff & Richter, 1996) place in a *Chlorocardium*–*Mezilaurus* clade. Monotypic Costa Rican *Povedadaphne* (not examined here) was considered close to *Mezilaurus* by Rohwer (1993a), but his *matK* data (Rohwer, 2000) suggested a place with genera here placed in Cinnamomeae (below) where it is morphologically close to the *Ocotea* complex. No other members of the *Chlorocardium*–*Mezilaurus* clade are suspected on morphological grounds.

Monophyletic Groups in the Perseeae–Laureae clade. The large terminal clade that accounts for most of the modern generic and species diversity of Lauraceae includes Laureae of most classification systems and van der Werff and Richter's (1996) Perseeae. The present resolution does not support the dichotomy implied by these tribal concepts (Fig. 3). Instead, five genera of Perseeae (*Persea* group) lie sister to a clade comprised of Laureae and remaining Perseeae. The generic composition of the latter group compares favorably with Kostermans' (1957) Cinnamomeae (Fig. 3). Possible resolution of a Perseeae–Laureae clade into a small *Persea* group and a large Laureae–Cinnamomeae clade was indicated by *matK* sequences (Rohwer, 2000), but topology was unstable and lacked statistical support. Support is stronger here with the *Persea* group and Laureae–Cinnamomeae clade receiving 89% and 93% bootstrap support, respectively (Figs. 3, 4). A close Laureae–Cinnamomeae

relationship has never been formally recognized. Only Kostermans (1957) placed these two tribes in close proximity in his graphical scheme for Lauraceae, conceivably to express his observation that fleshy hemispherical cupules are typical of, and restricted to, genera therein. Elsewhere in Lauraceae hemispherical, but rather woody, cupules also occur in *Chlorocardium* and *Sextonia*, both included in Cinnamomeae by Kostermans (as *Ocotea*), but of the *Chlorocardium*–*Mezilaurus* clade herein.

Persea group. This clade (Fig. 3), including *Alseodaphne*, *Apollonias*, *Dehaasia*, *Persea*, and *Phoebe*, accommodates all non-cupulate genera of van der Werff and Richter's (1996) Perseeae. It is Rohwer's (1993a) *Persea* group, without *Caryodaphnopsis*. *Nothaphoebe* was not investigated herein but is morphologically close to *Alseodaphne*.

Generic delimitation in the *Persea* group has been controversial, and all genera with four-locular anthers have at some point been placed in synonymy under *Persea* (e.g., Bentham, 1880; Kostermans, 1957). *Apollonias* and *Dehaasia* have always been segregated on the basis of their two-locular anthers, but the generic importance of this character and its use to delimit *Apollonias* from *Phoebe* and *Dehaasia* from *Alseodaphne* is questionable (Rohwer et al., 1991; Rohwer, 1993a; van der Werff, in press). Other generic characters, including relative tepal sizes, whether tepals persist in fruit, and if so, manner of persistence, have also been questioned (van der Werff, 1989; Rohwer et al., 1991). Our present sampling does not adequately address these issues. Only *Persea* is represented by more than one species. Asian *Persea* (subg. *Machilus*) places with paleotropical *Alseodaphne*, *Apollonias*, *Dehaasia*, and *Phoebe*, while American *Persea* (subg. *Eriodaphne* and subg. *Persea*) constitutes a separate clade (Fig. 3), but the implication that *Persea* is not monophyletic has little support. Detailed morphological and molecular studies are needed to resolve relationships within this large group of ca. 400 species, most of which are found in tropical Asia.

Laureae. Close relationship among *Actinodaphne*, *Adenodaphne*, *Laurus*, *Lindera*, *Litsea*, *Neolitsea*, *Parasassafras*, and *Sassafras* has been recognized in almost all classifications of Lauraceae. All are dioecious and most have umbellate inflorescences subtended by involucre bracts. In this study, parsimony analyses place *Sassafras* between well-supported core Laureae and remaining genera (Fig. 3). However, membership in Laureae was found by minimum evolution analyses, and parsimony-based trees in which *Sassafras* lies sister to core Laureae

(Fig. 4) are just three steps longer. Introrsely positioned locelli in all staminal whorls support a place for *Sassafras* in Laureae. Elsewhere in Lauraceae introrsely positioned locelli are restricted to the outer two staminal whorls. Another potential synapomorphy is the dioecious breeding system shown by *Sassafras* and core Laureae. In Lauraceae, dioecy is otherwise only found in basally positioned *Hypodaphnis* (Fig. 1) and a distal clade of *Ocotea* s. str., *Endlicheria*, and *Rhodostemonodaphne* (Fig. 3). *Dodecadenia* and *Cinnadenia*, not herein, should also place in Laureae on the basis of dioecy and introrse locelli.

Umbellularia is usually placed in Laureae because of its umbellate involucrate inflorescences (e.g., van der Werff & Richter, 1996), but its flowers are bisexual and locelli of the innermost staminal whorl are extrorse, not introrse. A 16-bp repeat in *trnL-trnF* (Fig. 1) and ITS sequences (Figs. 3, 4) distance *Umbellularia* from Laureae and place it in the *Ocotea* complex (below).

Generic delimitation in Laureae is unsettled. *Litsea* alone accommodates ca. 400 of the approximately 700 species, and most generic limits are probably artificial (Rohwer, 1993a; Li & Christophel, 2000). As with the *Persea* group, detailed systematic studies are needed to resolve natural lineages in Laureae.

Cinnamomeae. All remaining genera were previously placed in Cinnamomeae (sensu Kostermans, 1957), albeit together with *Sassafras*, *Actinodaphne*, and *Neocinnamomum*. With these three genera excluded, Cinnamomeae is van der Werff and Richter's (1996) *Perseeae* without the *Persea* group. Cinnamomeae are thus a sizeable subset of the *Perseeae*-Laureae clade (Fig. 3), accommodating all of its major neotropical genera (e.g., *Aiouea*, *Aniba*, *Endlicheria*, *Licaria*, *Nectandra*, *Pleurothrium*, and *Rhodostemonodaphne*) as well as widespread *Cinnamomum* and *Ocotea*.

Cinnamomeae share hemispherical cupules (rarely poorly developed) with Laureae and retain the thyrsoid non-involucrate inflorescences of the *Persea* group. Thus, uniquely derived features are not obvious. Bootstrap support for Cinnamomeae reaches only 52% in unconstrained parsimony analyses (Fig. 3), but raised to 86% by enforcing monophyly of a generic alliance centered around *Cinnamomum* (Fig. 4).

Cinnamomum group. The delimitation of *Cinnamomum* is based on its nine stamens with four-locular anthers and a fourth androecial whorl of well-developed staminodes provided with sagittate glandular apices. The present data nest two neo-

tropical genera in *Cinnamomum*. Monotypic *Mocinnodaphne* was described to recognize a reduction in number of staminal whorls (Lorea-Hernandez, 1995), and *Aiouea* p.p. [*A. dubia* (HBK) Mez and *A. guianensis* Aubl. herein] differs mainly in locelli number, both characters of traditional generic value. The finding that *Ocotea ikonyokpe* van der Werff, a recently described species from Cameroon, is placed with *Cinnamomum* is surprising. However, a leaf fragment from the holotype sheet (Thomas 10456, MO) was extracted, amplified, and sequenced only with other species of *Ocotea*. Furthermore, ITS1 and ITS2 regions of *O. ikonyokpe* were amplified and sequenced separately. Neither section is identical with accessions of *Cinnamomum*, and both support a place with *Cinnamomum*. In Africa, *O. ikonyokpe* shares (sub)opposite leaves with East African *O. michelsonii* Robyns & Wilczek and *O. usambarensis* Engl. (not herein). All other African *Ocotea* have spirally arranged leaves (van der Werff, 1996). Interestingly, *O. ikonyokpe* associates with Asian *Cinnamomum* (mostly opposite-leaved) instead of mostly alternate-leaved neotropical *Cinnamomum* (Figs. 3, 4). The staminodes, relatively smaller than seen in *Cinnamomum*, and without sagittate apices (although glandular as in *Cinnamomum*), refer this Cameroon species to *Ocotea*, but leaf arrangement is perhaps an overlooked character here.

Neither molecular nor morphological synapomorphies readily appear for the *Cinnamomum* group (*Cinnamomum*, *Aiouea* p.p., *Mocinnodaphne*, and *Ocotea* p.p.), but enforcing monophyly adds only one step to parsimony-based trees (Fig. 4). Still, New and Old World species remain separate subclades in the constrained clade. This New World–Old World dichotomy is also evident in wood and bark anatomy (Richter, 1981), and can be deduced from traditional placement of neotropical *Cinnamomum* in *Phoebe* (of the *Persea* group above) until transferred by Kostermans (1961). With over 350 species distributed from (sub)tropical Asia to the Neotropics, one African member, and a few representatives in Australia and the Pacific Islands (primarily Fiji), the *Cinnamomum* group is speciose and widespread.

Ocotea complex. The remaining genera of Cinnamomeae form a strongly supported clade within which members of *Ocotea* are widely dispersed (Figs. 3, 4). Finding that *Umbellularia* places here, and not in the Laureae, clarifies conflicting indications from floral morphology and inflorescence structure (discussed under Laureae, above). Apart from a few Old World species of *Ocotea*, the com-

plex is restricted to the New World and accounts for most of the generic and species diversity of Lauraceae in the Neotropics.

Clades resolved within the *Ocotea* complex can be circumscribed in geographic, and sometimes morphological, terms. Basal branches in the complex are occupied by Old World species of *Ocotea*, North American *Umbellularia*, and primarily Central American species groups in *Nectandra* and *Ocotea*. Two derived clades are centered in South America. In the more speciose, *Nectandra* s. str. and *Pleurothyrium* lie sister to a dioecious clade comprised of *Endlicheria*, *Ocotea* s. str., and *Rhodostemonodaphne*. In the second and less speciose South America-centered clade, *Aniba*, an assortment of *Ocotea* species, and mostly mono- to oligotypic genera associate with *Licaria*.

Old World Ocotea. Outside of the Neotropics, *Ocotea* is represented by *O. foetens* (Aiton) Baill. in Macaronesia, 7 species in mainland Africa, and about 30 species in Madagascar. In this study, South African *O. bullata* (Burch.) E. Mey. and 2 species from Madagascar, *O. grayi* van der Werff and *O. malcomberi* van der Werff, constitute a strongly supported clade. *Ocotea foetens* is almost indistinguishable from *O. bullata*, but surprisingly its place with Old World *Ocotea* receives < 50% bootstrap support (Figs. 3, 4). Morphological characters distancing Old World *Ocotea* from New World relatives do not readily appear. In the present reconstruction Old World *Ocotea* lie sister to the New World component of the *Ocotea* complex.

Nectandra coriacea species group, *Nectandra* s. str., and *Pleurothyrium*. Representatives of the *Nectandra coriacea* species group, *N. coriacea* (Sw.) Griseb., *N. salicifolia* (HBK) Nees, and *N. purpurea* (Ruiz & Pav.) Mez, place distant from a clade in which a broad morphological representation of *Nectandra* s. str. pairs with *Pleurothyrium* (Figs. 3, 4). Close relationship between *Nectandra* s. str. and *Pleurothyrium* was also indicated by *matK* sequences (Rohwer, 2000). The two share relatively large rotate flowers with heavily papillose tepals and stamens, and relatively poorly developed staminodes. *Nectandra* s. str. and *Pleurothyrium* are centered in South America with ca. 100 and 40 species, respectively. The Central America-centered *Nectandra coriacea* species group (ca. 20 spp.) was perceived as accommodating the most primitive elements of *Nectandra*, retaining *Ocotea*-like non-scalariform venation, tepal and stamen non-papillosity, distinct filaments, and a well-developed fourth androecial whorl of staminodes with glandular apices (Rohwer, 1993b; Rohwer & Ku-

bitzki, 1993). A relatively basal position in the *Ocotea* complex is suggested by ITS sequences (Figs. 3, 4).

Ocotea helicterifolia species group. The clade (Figs. 3, 4) comprised of *Ocotea botrantha* Rohwer, *O. helicterifolia* (Meisn.) Hemsl., and *O. heydeana* (Mez & Donn. Sm.) Bernardi represents a diverse but cohesive assemblage of species in *Ocotea*. Core members are characterized by hirsute leaves and twigs, bisexual flowers with partially papillose tepals, glabrous or weakly papillose anthers with four pollen sacs arranged in two superimposed pairs, and well-developed staminodes (van der Werff, 1999). Close relationship with non-hirsute species with this general floral structure, i.e., the *Ocotea heydeana* species group, and with the *Ocotea sinuata* species group, which differs by more heavily papillose tepals and anthers (here represented by *O. botrantha* Rohwer) was anticipated by Rohwer (1991). With the *Nectandra coriacea* species group, and *Umbellularia*, the *Ocotea helicterifolia* species group shares well-developed staminodes with glandular apices, while their papillose anthers and tepals suggest an affinity with *Nectandra* s. str. and *Pleurothyrium*. Their place in the present phylogeny is consistent with this intermediate morphology. The group is distributed throughout Central America and numbers around 30 species.

Ocotea s. str., *Endlicheria*, and *Rhodostemonodaphne*. A clade comprised of all dioecious *Ocotea* sampled for this study and the only neotropical genera with this breeding system, *Endlicheria* and *Rhodostemonodaphne*, is found in all ITS-based trees. It receives low bootstrap support as a whole (55%), but much better (93%) support within the group above the branch separating *Endlicheria punctulata* (Mez) C. K. Allen and *Ocotea pauciflora* (Nees) Mez from the rest (Fig. 3). As seen in Figure 4, *E. punctulata* and *O. pauciflora* appear to diverge early, shortening the branch supporting the entire clade, but this effect could also be obtained by reversals to ancestral molecular states along the branch uniting these two species.

Taxa placed here include the type species of *Ocotea*, *O. guianensis* Aubl., and represent several species groups recognizable among dioecious *Ocotea* (e.g., Rohwer, 1986). Since representatives of *Endlicheria* and *Rhodostemonodaphne* also sample a wide range of morphological variation within these moderately sized but heterogeneous genera, all approximately 300 dioecious species of the *Ocotea* complex should place here. In this clade generic limits based on locelli arrangement and number are not supported. The *Ocotea* species form a

paraphyletic assemblage within which *Rhodostemonodaphne* and *Endlicheria* are nested (Figs. 3, 4). *Rhodostemonodaphne* has four-locular anthers like *Ocotea*, but the locelli are arranged in a shallow arc or horizontal row rather than superimposed pairs, at least in the outer stamens. *Endlicheria* is two-locular, but with *E. punctulata* paired with *O. pauciflora*, and other species placed with *Rhodostemonodaphne* (Figs. 3, 4), it is at least di-phyletic.

Licaria group. The branch uniting *Dicypellium*, *Kubitzkia*, *Licaria*, *Paraia*, *Urbanodendron*, and two species of *Ocotea* receives 93% bootstrap support (Fig. 3) and seems to be a natural alliance. *Gamanthera* and *Phyllostemonodaphne* (neither herein) associate with the *Licaria* group on morphological grounds. All these genera have cupules with double-rimmed (or more) margins. Given these unusual cupules, a generic alliance centered around *Licaria* has been informally recognized in recent morphological systems (Kostermans, 1957; Rohwer, 1993a). *Ocotea quixos* (Lam.) Kosterm. and *O. veraguensis* (Meisn.) Mez represent the *Ocotea dendrodaphne* species group, a group of 8 species remarkable in *Ocotea* for their ligulate stamens and double-rimmed cupules. A place in the *Licaria* group is thus not surprising. Their distance from the rest of *Ocotea* has been acknowledged by sub-generic (e.g., Mez, 1889; Rohwer, 1986) and even generic status; e.g., *Sassafridium* (Meissner, 1864). Hutchinson (1964) even placed *O. veraguensis* (as *Sassafridium*) in a monotypic tribe because he interpreted the locelli in the third staminal whorl to be introrse, a condition only known in Laureae. In fact the locelli are latrorse-introrse, as also found in *Dicypellium* and *Urbanodendron*, both of the *Licaria* group. Elsewhere in *Ocotea*, double-rimmed cupules are found in a few dioecious species, e.g., *O. cujumary* Mart. and *O. floribunda* (Sw.) Mez. It is unlikely that these species will assign to the *Licaria* group since their morphologically close relatives, *O. guianensis* Aubl. and *O. percoriacea* (Meisn.) Kosterm., respectively, are firmly seated in the dioecious clade discussed above.

Remaining taxa place near the *Licaria* group without strong support (Fig. 3). *Ocotea rhynchophylla* (Meisn.) Mez and *O. odorifera* (Vell.) Rohwer represent species groups that Rohwer (1986) considered intermediate between the *O. dendrodaphne* species group (including *O. quixos* and *O. veraguensis* herein) and the rest of *Ocotea*. Their position near the *Licaria* group is consistent with Rohwer's interpretation. *Aniba*, too, has been associated with *Licaria* (e.g., Kubitzki, 1982), but has simple-rimmed or rarely weakly double-rimmed cupules.

Ocotea insularis (Meisn.) Mez and *Aiouea costaricensis* (Mez) Kosterm. are united with 100% bootstrap support (Figs. 3, 4). The two differ only in the number of locules per anther, again illustrating the weakness of this character in generic delimitation (van der Werff, 1984). Curiously, as noted by van der Werff (1988) and Rohwer et al. (1991), like *A. costaricensis*, other *Aiouea* species with closer ties to *Ocotea* are Central American (e.g., *A. lundelliana* C. K. Allen, not herein), while South American *Aiouea* associate with *Cinnamomum* (e.g., *A. dubia* and *A. guianensis*, Fig. 3).

BIOGEOGRAPHY

Genera and clades in Lauraceae sort into two main geographic groups (Figs. 2, 4). *Hypodaphnis*, the Cryptocaryeae, *Cassytha*, and the *Chlorocardium*–*Mezilaurus* clade as seen in Figure 1 are predominantly or entirely southern hemispheric, while *Caryodaphnopsis*, *Neocinnamomum*, the *Persea* group, the *Cinnamomum* group, and Laureae are either Asian or have amphi-Pacific distributions (Figs. 2, 4). The distributions of these two main groups are consistent with Gondwanan and Laurasian histories, respectively, but the geographic distribution of the *Ocotea* complex is not as easily categorized. This diverse clade is mostly neotropical with a Macaronesia–Afro-Malagasy element added by approximately 40 Old World species of *Ocotea*. Whether ancestors of the *Ocotea* complex migrated into South America via Africa (Raven & Axelrod, 1974) or via North America (Rohwer, 1986; Taylor, 1988; Rohwer & Kubitzki, 1993; Rohwer, 2000) is not evident from the topology alone.

Paleogeographic reconstructions of West Gondwana breakup estimate that direct land connections between Africa and South America were lost by the Upper Mid-Cretaceous, ca. 90 Mya (Sclater et al., 1977; Scotese et al., 1988; Parrish, 1993). Therefore, if the trans-Atlantic disjunction in the *Ocotea* complex can be attributed to West Gondwanan breakup, it would have to be at least 90 Mya old. This biogeographic scenario was simulated on the parsimony-based ITS topology by fixing the New World–Old World divergence (node e in Fig. 5a and Table 3a) at 90 My and using the resulting rate of molecular evolution to estimate divergence times for other nodes. This calibration yields a rate of 0.000275 substitutions per site per million years, a rate that halves the lowest ever reported for ITS (Suh et al., 1993), and which places the divergence of the *Chlorocardium*–*Mezilaurus* clade (Fig. 3; and represented by *Sextonia pubescens* van der Werff in Fig. 5a) from the Perseeae–Laureae clade (includes

the *Ocotea* complex) at 354 Mya (node a in Fig. 5a and Table 3a). This Devonian age precedes earliest undisputed angiosperm fossils. Furthermore, if the equivalent node in the basal topology (node G in Fig. 5b and Table 3b) is fixed at 354 Mya and divergence times for earlier lineages calculated, the radiation of the family is placed in the Pre-Cambrian, ca. 682 Mya. Therefore, the neotropical-African disjunction shown by the *Ocotea* complex is likely to be much younger than West Gondwanan breakup, and some amount of dispersal must have been involved.

How did the *Ocotea* complex, and other members of the family, reach the New World? In both morphological and molecular attributes the *Chlorocardium-Mezilaurus* clade occupies a basal and isolated position in Lauraceae. It is also the only lineage of basal Lauraceae restricted to South America (reaching its northern limit in Costa Rica). These qualities argue for early isolation of the *Chlorocardium-Mezilaurus* clade in South America, presumably initiated by West Gondwana breakup. To assess this biogeographic scenario on other divergence times, substitution rates in the ITS and *rpl16* data sets were re-calibrated using an Upper Mid-Cretaceous (90 Mya) separation of South America from Africa to date the divergence of the *Chlorocardium-Mezilaurus* clade from its sister group (node a in Fig. 5a and Table 3a, node G in Fig. 5b and Table 3b). With this second calibration, radiation of Lauraceae was estimated at 174 ± 32 Mya (node A in Fig. 5b and Table 3b), and radiation of the terminal Perseeae-Laureae clade was placed in the Eocene, 44 ± 7 Mya (node b in Fig. 5a and Table 3a). This estimate for Eocene radiation of the latter group is supported by the fossil record. The hemispherical cupules of the London Clay Flora (Reid & 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). Thus, our second calibration, that which credits South American isolation of the *Chlorocardium-Mezilaurus* clade to West Gondwanan breakup, estimates a realistic age for radiation of the family and complements fossil evidence of radiation of the Perseeae-Laureae group in Early Tertiary Laurasia. This congruence provides confidence that age estimates based on our second calibration are good approximations of actual divergence times.

BASAL LINEAGES

Raven and Axelrod (1974) situated the cradle of the angiosperms in West Gondwana, and its proximity to Laurasia was pivotal to the Mid-Cretaceous presence of angiosperm pollen in the South Laurasia Province (sensu Brenner, 1976). Any explanation of the biogeographic history of Lauraceae also requires early migratory routes between Laurasia and West Gondwana. Southern hemispheric *Hypodaphnis*, Cryptocaryeae, *Cassytha*, and the *Chlorocardium-Mezilaurus* clade indicate Gondwanan history, but *Caryodaphnopsis* and *Neocinnamomum* appear to be Laurasian.

Caryodaphnopsis is disjunct between tropical America and tropical Asia, while *Neocinnamomum* is known only from tropical Asia. They represent the only early lineages in Lauraceae that are present in Asia but are not also known to occur in Africa, Madagascar, and Australia, in contrast to widespread genera in Cryptocaryeae and *Cassytha*. The fossil record suggests that both *Caryodaphnopsis* and *Neocinnamomum* have an ancient Laurasian history. The fossil wood taxon *Caryodaphnopsisoxylon richteri* Gottwald (1992) places the unique xylem anatomy of *Caryodaphnopsis* in Late Eocene Germany. The fossil flower *Neusenienia tetrasporangata* Eklund from Late Cretaceous North America compares favorably with *Neocinnamomum*, and flowers and fruits from the same locality can be compared to *Caryodaphnopsis* (e.g., Eklund, 2000). Although the affinities of the latter fossils cannot be unambiguously assigned, the implied antiquity of *Caryodaphnopsis* and *Neocinnamomum* is consistent with their relatively basal positions in Lauraceae. Most likely, these modern genera are descendants of the Cretaceous Laurasian flora of Lauraceae documented by the fossil genera *Mauldinia* (Drinan et al., 1990; Herendeen et al., 1994, 1999; Eklund & Kvaček, 1998) and *Perseanthus* (Herendeen et al., 1994).

The timing of events that resulted in the modern distribution of *Caryodaphnopsis* can further elucidate its biogeographic history. A relictual presence in South America would imply a continuous paleodistribution from South America to Eurasia. However, such continental configuration also provides direct connections between South America and other Gondwanan terrains (reviewed in Hallam, 1994), and preferential extinction in these Southern landmasses would be necessary to explain the modern disjunction. Alternatively, the *rpl16* data set estimates a relatively recent separation (44 ± 14 Mya) of South American *C. tomentosa* van der Werff from Asian *C. bilocellata* van der Werff (node F in Fig.

5b and Table 3b). This would rule out a relictual presence in South America and is consistent with the view that disjunct distributions between tropical Asia and tropical America are derived from ancestral boreotropical ranges disrupted by Late Eocene climatic cooling (e.g., Wolfe, 1975; Tiffney, 1985a, b; Zhengyi, 1983). Moreover, this calibration implies that isolation of *Caryodaphnopsis* from the rest of the family can be staged in the Early Cretaceous about 140 Mya (node E in Fig. 5b and Table 3b). Increasing separation of Laurasia from Gondwana, a salient feature of Early Cretaceous paleogeography (reviewed in Hallam, 1994), would have disrupted trans-Tethyan ancestral ranges and precipitated the next biogeographic phase in Lauraceae, i.e., radiation on increasingly distant Laurasian and Gondwanan landmasses.

Accordingly, in the Northern Hemisphere, the Mid-Cretaceous fossil taxa, and the direct ancestors of *Caryodaphnopsis* and *Neocinnamomum*, would have spread throughout southern Laurasia until decreasing temperatures and the opening of the north Atlantic constricted their descendants to tropical Asia and America. To the south, truly pantropical genera and clades would have attained their widespread distribution, with seafloor spreading in the South Atlantic and Indian Oceans leading to increased regional endemism. These continental reconfigurations appear to be reflected in the distribution of Southern Hemispheric genera.

Of pantropical genera, *Beilschmiedia* and *Cryptocarya* are the most widespread. The genetic distance-based age estimations indicate that these genera diverged from their most recent common ancestor about 90 ± 20 Mya (node D in Fig. 5b and Table 3b). Variance around these age estimations argues for direct migration throughout Gondwana, and a widespread pre-drift distribution for both *Beilschmiedia* and *Cryptocarya*. The presence of both genera in continental Asia may be due to the rafting of the Indian subcontinent and other Gondwanan fragments to the Asian plate. Later accretions of Gondwanan fragments with the Asian plate and Miocene island hopping across the Indo-Malayan region may also have been involved. The pantropical distribution of *Cassytha* is mostly due to one widespread species, *C. filiformis* L.; all other approximately 20 species are restricted to the Old World and show high regional endemism in Australia (Weber, 1981). Although the Southern Hemispheric distribution centered in the Old World favors a predominantly East Gondwanan history for *Cassytha*, the possibility of a Laurasian history followed by radiation into its associated xeric habitat

cannot be discounted. All other Southern Hemispheric genera have narrower ranges.

Hypodaphnis may be relictual in Central Africa since its ancestors apparently diverged from the rest of the family when direct migration between Gondwana and Laurasia was possible (node A in Fig. 5b and Table 3a). *Eusideroxylon* ranges from Borneo to Sumatra, and *Potoxylon* is endemic to Borneo. With their placement in predominantly Southern Hemispheric Cryptocaryeae, it is possible to regard them as Gondwanan relicts as well. However, their separation from the rest of the tribe is dated at about 120 Mya (node C, Fig. 5b), an age that permits early migration into Laurasia, as envisioned for *Caryodaphnopsis* above. Further, *Trianthera eusideroxylon* Conwentz, an amber-embedded flower from the Eocene-Oligocene boundary of the Baltic area (Conwentz, 1886) compares remarkably well with *Eusideroxylon* and adds to the possibility of a Gondwanan-Laurasian dichotomy in Cryptocaryeae. Upper Cretaceous appearance of the boreotropical *Aquilapollenites* in Borneo (Wolfe, 1975; and references therein), and the composite geological nature of the Indo-Malayan region (Burrett et al., 1991; Michaux, 1991; and references therein), are also consistent with Laurasian history for *Eusideroxylon* and *Potoxylon*.

All other members of Cryptocaryeae and their allies are restricted to, or best represented in, austral parts of the Old World, i.e., East Gondwanan and derived terrains. At the other end of the former southern continent, the *Chlorocardium-Mezilaurus* clade is restricted to South America. Thus, among these Southern Hemispheric genera and clades, more basal groups are either widespread or relictual, and more derived groups are restricted to Eastern or Western Gondwanan fragments, consistent with the progressive dismantling of Gondwana.

THE PERSEAE-LAUREAE CLADE

ROUTES TO LAURASIA

The Perseeae-Laureae clade diverged from its sister group, the *Chlorocardium-Mezilaurus* clade, since the Upper Mid-Cretaceous, but until fossil members appeared in Eocene Laurasia its biogeographic history is a mystery. Three alternative scenarios are conceivable. In a vicariant vein, consider a West Gondwanan common ancestor for the Perseeae-Laureae clade and its sister group, with tectonic activity isolating direct ancestors of the *Chlorocardium-Mezilaurus* clade on South America while stranding those of the Perseeae-Laureae clade on Africa. Ancestors of the Perseeae-Laureae clade then migrate to Laurasia via North Africa.

Alternatively, the northern route of the Perseeae–Laureae clade could have included a South American phase with subsequent migration to North America and Eurasia. Precursors of the Greater Antilles spanned the Panamanian Isthmus as an island chain in the Mid-Cretaceous (Pindell et al., 1988) providing a stepping-stone migratory route out of South America. Yet a third possibility is that the common ancestor of the Perseeae–Laureae and *Chlorocardium–Mezilaurus* clades was Laurasian and shared the former northern continent with *Caryodaphnopsis* and *Neocinnamomum*. This scenario would require that the *Chlorocardium–Mezilaurus* clade migrated into South America via the Mid-Cretaceous stepping-stone route provided by proto-Greater Antilles. Evidence of an early faunal and floristic exchange across this region has accumulated (e.g., Cadle, 1985; Estes & Baez, 1985; Graham, 1995; Burnham & Graham, 1999), but the taxa involved are usually distributed throughout northern Central America and South America, while the *Chlorocardium–Mezilaurus* clade only reaches Costa Rica to the north.

Without an unambiguous Cretaceous fossil record for the Perseeae–Laureae clade, we favor the first or African scenario. North Africa is today part of the largest desert system in the world, but was covered by tropical forest well into the Miocene (Axelrod & Raven, 1978). During the Tertiary, Africa moved progressively northward, and migration to Eurasia across the narrowing Tethys would have become increasingly feasible. The South American scenario invokes a circuitous route to Eurasia and fails to explain why no members of the Perseeae–Laureae clade remain relictual in South America. An early Laurasian history would have to account for the absence of the *Chlorocardium–Mezilaurus* clade in Asia.

ROUTES TO THE NEW WORLD I: *PERSEA* GROUP, *CINNAMOMUM* GROUP, AND LAUREAE

The *Persea* group is most diverse in Asia, with only ca. 80 of its approximately 400 species found in the New World. These occur mainly in montane forests in Central and South America and range from Mexico to Chile, reaching the Atlantic coastal forests in southeastern Brazil. In the Old World, two taxa are present in the Canary Islands. *Apollonias barbujana* (Cav.) Bornm. is placed in an unresolved Asian clade with *Alseodaphne*, *Dehaasia*, *Persea* subg. *Machilus*, and *Phoebe* (Figs. 3, 4). The other Canary Island endemic, *Persea indica* (L.) Spreng., was not sampled here. Pending better resolution within the *Persea* group, current age estimates sug-

gest that its Asian and American members diverged around the Eocene–Oligocene boundary, ca. 32 Mya (node z in Fig. 5a and Table 3a).

The distribution and representation of the *Cinnamomum* group in the Neotropics and Asia is almost identical to that of the *Persea* group. Whether New and Old World clades in the *Cinnamomum* group constitute a monophyletic group is not yet clear. From age estimates obtained by enforcing monophyly (Fig. 5a), the assumed common ancestral gene pool divided around the Eocene–Oligocene boundary, a divergence time coincident with that calculated for the *Persea* group. These similarities in the tropical amph-Pacific disjunctions in the *Cinnamomum* and *Persea* groups suggest commonality. Disruption of boreotropical ranges by climatic cooling around the Eocene–Oligocene boundary (Wolfe, 1975; Tiffney, 1985a, b; Zhengyi, 1983) would be consistent with the present age estimates. Already tenuous biotic links across the North Atlantic and/or Pacific were severed at this time, and divided *Cinnamomum* and *Persea* groups receded to warmer paleolatitudes in Asia and the Americas.

Like the *Cinnamomum* and *Persea* groups, Laureae are most diverse in Asia with a smaller range and representation in the New World. *Lindera*, *Litsea*, and *Sassafras* reach the Americas, but of these only *Litsea* ranges south of temperate North America, to Costa Rica. The unsettled generic delimitation of *Litsea* and *Lindera* cautions against assessment of their disjunctions from morphology, but monophyly of *Sassafras* is well supported here (Figs. 3, 4), providing an opportunity to assess the classic North America–Eastern Asia disjunction first brought into scientific focus by Asa Gray (Boufford & Spongberg, 1983). In *Sassafras*, divergence of Asian *S. tzumu* (Hemsl.) Hemsl. from North American *S. albidum* (Nutt.) Nees was estimated at about 12 Mya (node x in Fig. 5a and Table 3a). This northern temperate disjunction is much younger than the ± 30 Mya old tropical amph-Pacific disjunctions shown by *Cinnamomum* and *Persea* groups. While climatic cooling in the Eocene and Oligocene (Wolfe, 1975; Hallam, 1994) restricted tropical taxa to lower paleolatitudes, for temperate taxa intercontinental connections across northern latitudes would have been possible until much later in the Tertiary. The estimated divergence time separating Asian from North American *Sassafras* coincides well with opening of the Bering Strait, and supports Wolfe and Leopold's (1967) view that Mid-Miocene loss of the Bering land bridge is the most likely cause of north-temperate disjunctions between North America and Asia.

ROUTES TO THE NEW WORLD II: THE *OCOTEA*
COMPLEX

The *Ocotea* complex accommodates most of the taxonomic diversity of neotropical Lauraceae. In the Old World the complex is weakly represented in Macaronesia, Africa, and Madagascar. Any trans-Atlantic disjunction produced by West Gondwanan vicariance was discounted (above) and instead the disjunction dates to around the Oligocene–Miocene boundary, ca. 23 ± 5 Mya (node e in Fig. 5a and Table 3a). The estimated Eocene–Oligocene age (node d in Fig. 5a and Table 3a) of the *Ocotea* complex implies an origin concurrent with the southward movement of megathermal forests (Wolfe, 1975; Hallam, 1994), and its derived position relative to the previous clades indicates boreotropical ancestry. Unlike previous Laurasian taxa, the *Ocotea* complex is absent in Asia. While *Persea* and *Cinnamomum* groups appear as lower montane taxa in the Neotropics, the *Ocotea* complex is especially diverse in the lowlands of South America. Given these differences in distribution and a relatively recent trans-Atlantic disjunction, their biogeographic history may be quite different from that of the other boreotropical lineages. In this regard xeric tolerances shown by African *Ocotea*, Californian *Umbellularia*, and the Central America-centered *Nectandra coriacea* group may be significant. These taxa place basally in the complex, and their sclerophyllous habit, unusual for Lauraceae, adds to taxa that link the Madrean–Tethyan sclerophyllous flora discussed by Axelrod (1975). This broad-leaved flora ranged along the Tethyan coast from North America to southeastern Eurasia and North Africa, and existed relatively continuously since the Late Eocene, only disrupted by increased climatic cooling and drying at the end of the Oligocene (Axelrod, 1975). The 23 ± 5 Mya estimate of the trans-Atlantic disjunction in the *Ocotea* complex is consistent with that expected for taxa with ancestral Madrean–Tethyan ranges (e.g., Fritsch, 1996). Great disparity in species diversity on the two sides of the Atlantic may be attributed to disproportionate opportunities for speciation and differentiation. In the Neogene, continental Africa moves progressively northward into a drier and cooler climate (Hallam, 1994), while tectonic uplift in the Panamanian isthmus (Pindell et al., 1988) provides the Madrean flora of southeastern North America with opportunities for stepping-stone dispersal into South America.

Separation of the Central America-centered *Ocotea helicterifolia* species group from its speciose South America-centered sister group (Fig. 4) ap-

proximately 20 Mya (node f in Fig. 5a and Table 3a) argues for arrival of the *Ocotea* complex in South America prior to Pliocene closure of the Panamanian isthmus. As the timing of the separation coincides with increased uplift of the northern Andes in the early Miocene, it is conceivable that Andean orogeny divided the ancestral range. Further, since lowland genera of the *Ocotea* complex place in either South- or Central America-centered clades (Fig. 4), Andean orogeny appears to maintain generic endemism while allowing lower montane *Cinnamomum* and *Persea* groups to range widely. Exceptionally, South America-centered clades range throughout Central America with widespread species, e.g., *N. cuspidata* Nees of *Nectandra* s. str. (Fig. 4), and vice versa, e.g., *N. purpurea* of the *Nectandra coriacea* species group (Fig. 4). Although these may be secondary range expansions of indigenous South and Central American taxa (e.g., Rohrer & Kubitzki, 1993), they indicate the underlying complexity of biogeographic patterns.

The biogeographic history of Lauraceae outlined here shares much with that proposed by Doyle and Le Thomas (1997) for Annonaceae. As in that diverse magnoliid family, three main phases are recognizable. Early radiation of both families apparently occurred when migration between Gondwana and Laurasia was possible. Next, diversification throughout the Cretaceous produced lauraceous Cryptocaryaceae, perhaps *Cassytha*, and the *Chlorocardium–Mezilaurus* clade on Gondwana, with *Caryodaphnopsis* and its allies on Laurasia. In Annonaceae, *Anaxagorea* appears to be the counterpart of *Caryodaphnopsis*. In both families, renewed contact between Gondwanan and Laurasian fragments in the Early Tertiary resulted in a second radiation on Laurasian terrains. In Lauraceae, this boreotropical phase produced the Perseeae–Laureae clade, but unlike Annonaceae, its descendants did not only recede to the Asian tropics with climatic cooling. Three of the four major lineages of Lauraceae evolved during this period, migrated to the Neotropics, and one of these, the *Ocotea* complex, underwent a major radiation in the New World. This latter radiation has few parallels in neotropical phylogeography. There are indications that some lineages in the Leguminosae (Lavin & Luckow, 1993) and Melastomataceae (Renner & Meyer, in press) are derived from boreotropical ancestors, and Krutzsch (1989) listed possible examples from Bombacaceae, Olacaceae, and Symplocaceae. The emerging prospect of a larger contingent of Laurasian elements in the lowland Neotropics than previously recognized can be assessed when phylogenies of more tropical taxa become available.

OUTLOOK: TOWARD A PHYLOGENETIC
ARRANGEMENT OF LAURACEAE

The utility of morphological classification that expresses evolutionary history and relationships with support from molecular data is obvious. For Lauraceae, appropriate characters to be employed in such a scheme are elusive. Among traditional morphological characters, the number of locules per anther attains generic and even supra-generic importance in early schemes (Nees, 1836; Meisner, 1864; Bentham, 1880; Pax, 1889; Mez, 1889; Kostermans, 1957). Several examples of the weakness of this character have been identified and are confirmed by the present molecular data. Other characters do not fare much better. The use of umbellate involucrate inflorescences to circumscribe Laureae is a salient feature of most classification schemes (e.g., Rohwer, 1993a; van der Werff & Richter, 1996), but this syndrome clearly evolved independently in *Umbellularia*. Similarly, dioecy appears three times on the phylogeny herein, in *Hypodaphnis*, in Laureae, and again in the *Ocotea* complex. Further, Kostermans' (1957) system stresses degree of cupule development, but both extremes are found in Cryptocaryeae, and the non-cupulate condition of *Beilschmiedia* and *Endiandra* therein reappears in the distantly related *Persea* group. Equally problematical, morphological synapomorphies are not readily noted for several clades that receive strong molecular support. In the case of *Cassytha* and *Neocinnamomum* long branch attraction can be held responsible, but on closer examination genera of the *Chlorocardium*-*Mezilaurus* clade can be allied by a mosaic of characters.

Although major clades identified by molecular characters do not yield easily to morphological interpretation, a consensus over major generic groupings in Lauraceae appears to be within reach. Cryptocaryeae as circumscribed by van der Werff and Richter (1996), but probably without *Hypodaphnis*, are now supported by anatomy (Richter, 1981), embryology (Heo et al., 1998), and molecular data (Rohwer, 2000; herein). Considerable molecular support exists for the *Chlorocardium*-*Mezilaurus* clade, a group that is unique from both biogeographic and morphological perspectives. Further consensus concerns a large clade comprised of most remaining genera in Lauraceae that has been found by this and previous molecular data (Rohwer, 2000) and supported by anatomy (Richter, 1981). This group includes all genera placed in the tribes Perseeae and Laureae by van der Werff and Richter (1996), but its subdivision warrants re-examination. Toward this, Perseeae could be more narrowly cir-

cumscribed to accommodate just the *Persea* group, and *Umbellularia* removed from Laureae to Cinnamomeae. The latter also includes all remaining genera of van der Werff and Richter's Perseeae. Several smaller, although morphologically distinctive, taxa are not clearly placed, particularly in the case of *Cassytha*, to a lesser extent for *Caryodaphnopsis* and *Neocinnamomum*, and perhaps *Hypodaphnis*. At a lower taxonomic level, increased sampling is necessary for a thorough reconsideration of current generic concepts among Lauraceae. In this study, only the *Ocotea* complex has been representatively sampled; seemingly natural groups of genera and parts of larger genera have been identified within this complex. Similarly, increased sampling in other main clades identified here will improve our understanding of relationships among Lauraceae.

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