
PHYLOGENETIC RELATIONSHIPS OF THE *LITSEA* COMPLEX AND CORE LAUREAE (LAURACEAE) USING ITS AND ETS SEQUENCES AND MORPHOLOGY¹

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ABSTRACT

Nuclear DNA ITS and ETS sequences of 71 representatives from nine genera and 11 sections of the core Laureae were combined with a matrix of morphological characters, analyzed using maximum parsimony with both equally and successively weighted characters, and analyzed for Bayesian inference, minimum evolution by neighbor joining, and maximum likelihood inference for molecular data alone. The large genera *Actinodaphne* Nees, *Lindera* Thunb., and *Litsea* Lam. were polyphyletic, as were *Lindera* sect. *Aperula* (Blume) Benth. and *Litsea* sections *Conodaphne* (Blume) Benth. & Hook. f., *Cylicodaphne* (Nees) Hook. f., and *Tomingodaphne* (Blume) Hook. f. In contrast, *Neolitsea* (Benth.) Merr. was monophyletic and terminal in a larger monophyletic lineage above an *Actinodaphne* grade. A major disparity exists between these molecular results and traditional morphology-based classifications within Lauraceae. These results suggest that the use of two- versus four-celled anthers for Laureae generic delimitation has resulted in polyphyletic or paraphyletic genera, and the character of dimerous versus trimerous flowers is of only limited phylogenetic value. Several of the major lineages in Laureae are supported by inflorescence morphology and ontogeny, with Laureae defined by short shoots with a vegetative terminal bud, splitting into thyrsoid (*Actinodaphne* and *Neolitsea*) versus racemose (*Laurus*, *Litsea* s. str., and *Lindera* s. str. and *Lindera* sect. *Aperula*), although there appear to be at least two different pathways to form the Laureae pseudo-umbel. Similarly, imbricate, early deciduous inflorescence basal involucre bracts defined an *Actinodaphne*–*Neolitsea*–*Parasassafras*–*Sinosassafras* clade, although within it, *Neolitsea* was defined in part by decussate, persistent bracts. Accordingly, our study indicates the need for caution in the use of morphology for assessing affinity in Laureae, as virtually all traditional morphological characteristics show high levels of homoplasy and/or reversal, but future research may help to resolve whether this indicates problems of homology or ontogenetic convergence.

Key words: character evolution, inflorescence structure, Lauraceae, Laureae, phylogenetic classification.

The core Laureae comprise eight genera with approximately 500 species, mainly from tropical and subtropical Asia, making this region a center of generic and infrageneric diversification and distribution for the Laureae (Li, 1995). The group is defined morphologically by its dioecious breeding system, mostly pseudo-umbellate inflorescences subtended by involucre bracts, and introrse anther cells in the third whorl (Li & Christophel, 2000; Li et al., 2004). However, a large portion of what we now regard as the Laureae, including *Actinodaphne* Nees, *Dodecadenia* Nees, *Iteadaphne* Blume, *Laurus* L., *Lindera* Thunb.,

Litsea Lam., *Neolitsea* (Benth.) Merr., *Parasassafras* D. G. Long, and *Sinosassafras* H. W. Li, were only placed together relatively recently (Rohwer, 1993; van der Werff & Richter, 1996). Although half of the genera in the Laureae are monotypic or oligotypic, *Actinodaphne*, *Lindera*, *Litsea*, and *Neolitsea* each possess over 100 species.

Hooker (1890) and Li et al. (1984) divided *Litsea* into four sections, but these divisions were not explicitly phylogenetic. Section *Litsea* is evergreen with alternate, penninerved leaves, a racemiform inflorescence, and non-enlarged perianth tubes with

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reduced or absent perianth lobes. Section *Conodaphne* (Blume) Benth. & Hook. f. is evergreen with alternate or opposite penninerved leaves and non-enlarged or slightly enlarged perianth tubes, whereas section *Cylicodaphne* (Nees) Hook. f. represents evergreen taxa with alternate, penninerved leaves, an enlarged perianth tube, and cup-shaped fruiting cupule. In contrast, section *Tomingodaphne* (Blume) Hook. f. has deciduous, alternate, penninerved leaves and non-enlarged, six-lobed perianth tubes.

Tsui (1987) divided *Lindera* into eight sections, mainly following Hooker's (1890) criteria for *Litsea*, but also incorporating Li's (1985) concept of the "shortened brachyblast," which had been proposed to explain inflorescence evolutionary trends in *Laureae*. Section *Lindera* has taxa with deciduous, penninerved leaves and well-developed terminal buds on shortened brachyblasts; section *Sphaerocarpace* H. P. Tsui possesses deciduous, triplinerved or trinerved leaves and well-developed terminal buds on shortened brachyblasts; and section *Palminerviae* Meisn. was created for deciduous taxa with lobed, trinerved leaves and well-developed terminal buds on shortened brachyblasts. Section *Aperula* (Blume) Benth. has evergreen, penninerved leaves, well-developed terminal buds on shortened brachyblasts, and long-pedunculate, racemiform-arranged inflorescences; section *Cupuliformes* H. P. Tsui possesses evergreen, penninerved leaves, funnel-shaped glands on the third anther whorl, and enlarged perianth tubes forming cup-shaped fruiting cupules. Section *Daphnidium* (Nees) Benth. has evergreen, trinerved or triplinerved leaves, and non-developing terminal buds on shortened brachyblasts; section *Polyadenia* (Nees) Benth. is evergreen with penninerved leaves and ill-developed terminal buds on the axillary short shoots, whereas section *Uniumbellatae* H. P. Tsui has evergreen, trinerved leaves, and the long-pedunculate pseudo-umbel is solitary and borne in the axil of a normally developed leaf.

Generic delimitation in *Laureae* is problematic (Kostermans, 1957; Hutchinson, 1964; Richter, 1981), the major point being the significance of two-versus four-celled anthers as generic descriptors, although recent *Lauraceae* classifications tend to downplay this feature (e.g., Rohwer et al., 1991; Rohwer, 1993; van der Werff & Richter, 1996).

The most recent hypothesis about evolution within the *Laureae* was constructed under the assumption of maximum parsimony (MP) using nucleotide data from chloroplast DNA (cpDNA) *matK* and nuclear DNA (nrDNA) ITS (Li et al., 2004). This study included 22 species and one outgroup taxon, and shed light on major lineages, as well as on the relationships of some previously problematic genera. That study suggested that *Litsea* and *Lindera* are polyphyletic, with no

support for characters such as two- versus four-celled anthers, and identified novel clades that appeared to reflect inflorescence structure and ontogeny. Nevertheless, although the molecular analyses provided some phylogenetic insights of the core *Laureae*, generic boundaries and relationships were still uncertain due to limited sampling and the fact that morphology was not included explicitly in the analyses. In addition, the level of *matK* divergence was particularly low in *Laureae*, resulting in poorly supported groupings.

To reconstruct a more reliable phylogeny of the core *Laureae*, it was necessary to sample additional taxa, as well as to utilize more informative DNA regions. The most widely used sources of molecular data for plant phylogenetic studies at the specific and generic levels are the ITS-1 and ITS-2 regions in nrDNA (Baldwin et al., 1995). However, for *Lauraceae* (Chanderbali et al., 2001) and *Laureae* (Li et al., 2004), these two spacers were too invariant or uninformative to provide robust node inferences by themselves. In contrast, although generally less easy to sequence than ITS, the nrDNA ETS has been shown to be useful for supplementing ITS data in plant phylogenetic studies (e.g., Baldwin & Markos, 1998; Clevinger & Panero, 2000; Markos & Baldwin, 2002; Plovanich & Panero, 2004), giving it the potential to provide additional characters in the *Laureae*.

In addition, because most recent molecular studies in *Lauraceae* have not explicitly included morphology as part of the analysis, preferring instead to discuss character patterns post hoc in relation to the molecular trees, there is a need for a combined analysis that includes both data sets in order to assess the impact, if any, of morphological data on the results.

Accordingly, we have expanded the ITS study of Li et al. (2004) to include 71 taxa from the *Laureae*, three outgroups, and partial sequences from the ETS region, as well as information from morphological characters considered to be useful or definitive above the species level within the *Laureae*. The main goals were to assess the robustness of previously defined clades that had shown weak bootstrap support, as well as to see if support for features such as inflorescence structure was maintained with more extensive sampling and followed the explicit inclusion of morphology into the analyses, rather than just post hoc by character mapping. In particular, the hypotheses being tested were (1) *Laureae* genera based on two-versus four-celled anthers are not phylogenetically supported; (2) *Litsea*, *Lindera*, and *Actinodaphne* are not monophyletic; (3) *Neolitsea* is monophyletic; and (4) inflorescence ontogeny is phylogenetically important in the *Laureae*.

METHODS

TAXON SAMPLING AND DNA EXTRACTION

In addition to 21 samples used previously by Li et al. (2004), except for *Lindera tienchuanensis* W. P. Fang & H. S. Kung (sect. *Uniumbellatae*) and *L. thomsonii* C. K. Allen (sect. *Daphnidium*) for which there was no more available material, an additional 50 species were included to cover the sections within *Lindera* and *Litsea* variously recognized by Li et al. (1984), Benthham (1880), Hooker (1890), Kostermans (1957), and Tsui (1987), as well as to sample more extensively within *Neolitsea* (Table 1). Outgroup sampling included one species each from *Cinnamomum* Schaeff., *Sassafras* J. Presl, and *Umbellularia* (Nees) Nutt., as these genera were sister to the core Laureae in Chanderbali et al. (2001) and Li et al. (2004).

Total genomic DNA was isolated from silica gel-dried material or herbarium specimens following Doyle and Doyle (1987), as modified by Li et al. (2004). Because of suspected fungal contamination in the previous ITS sequences of *Sinosassafras flavinervia* (C. K. Allen) H. W. Li and *Neolitsea confertifolia* (Hemsl.) Merr. (Li et al., 2004), both were re-extracted.

AMPLIFICATION, SEQUENCING, AND SEQUENCE ALIGNMENT

The ITS and 5.8S regions were amplified following the general methodology of White et al. (1990) and polymerase chain reaction (PCR) primers of Chanderbali et al. (2001), using the minor modifications reported in Li et al. (2004). The complete ETS intergenic spacer (IGS) was amplified with the 18S-IGS and 26S-F primers following the protocols of Baldwin and Markos (1998) and Starr et al. (2003) using *Actinodaphne trichocarpa* C. K. Allen, *Lindera latifolia* Hook. f., *Lindera megaphylla* Hemsl., *Litsea cubeba* (Lour.) Pers., and *Neolitsea chunii* Merr. This procedure yielded a product between 2 and 3 kb, of which ca. 650 bases were sequenced using the 18S-IGS primer. A conserved region located about 450 bases from the ETS/18S boundary was selected to design a primer, ETS-1 (CCA GAA CTC GCA CTT GCT GAG CTT), and this region was amplified for all taxa using ETS-1 and 18S-IGS as primers. PCR amplification followed the short-distance PCR protocol of Baldwin and Markos (1998), with an annealing temperature of 55°C, and TaKaRa ExTaq (TaKaRa Biotechnology, Dalian, China). All PCR amplifications included negative controls to detect contamination.

Although most PCR amplifications resulted in a single band, some individuals produced two size classes of PCR product. To isolate each of the two

bands, PCR products were purified and blunt-end ligated into the EcoRV sites of the pMD18-T Simple Vector (TaKaRa) using the Original TA Cloning Kit (available from TaKaRa), and the fragments obtained following the manufacturer's protocol. PCR products were purified using the QIAquick PCR purification Kit (Qiagen, Tokyo, Japan) following protocols provided by the manufacturer, and sequenced in both directions. Sequencing reactions used the same primers as amplification and were conducted on an Applied Biosystems (Foster City, California, U.S.A.) 3100 DNA automated sequencer.

Sequences were checked against GenBank for the taxa of closest matching sequences by means of the BLAST search. Sequence chromatogram output files were aligned initially and edited base by base with the program SeqMan II (Lasergene software package; DNASTAR Inc., Madison, Wisconsin, U.S.A.). The edited sequences were then realigned using ClustalX version 1.8 (Thompson et al., 1997), and additional manual adjustments were made, if necessary, using BioEdit (Hall, 1999). All sequences analyzed in this study were deposited in GenBank (Table 1).

MORPHOLOGY

Morphological data were collected from personal observation of live plants, herbarium specimens, and the available literature. Nineteen morphological characters (Table 2) were chosen for character evolution and mapping because they have been regarded as important in delimiting genera within the *Litsea* complex, and/or between sections within *Lindera* or *Litsea* (Benthham, 1880; Hooker, 1890; Kostermans, 1957; Li et al., 1984; Li, 1985; Tsui, 1987; Rohwer, 1993; van der Werff & Richter, 1996; Li & Christophel, 2000; van der Werff, 2001; Li et al., 2004). A matrix of 19 discrete morphological characters was constructed for all of the taxa investigated (Table 3).

PHYLOGENETIC ANALYSES

We used several methods to reconstruct phylogenies and assess branch support for clades: MP with both equally and successively weighted characters, bootstrapping, Bayesian inference, minimum evolution (ME) by neighbor joining, and maximum likelihood (ML) inference. In this way, clades with a strong, consistent phylogenetic signal should be recovered regardless of the methodology.

MP analyses were performed using PAUP* version 4.01b10 (Swofford, 1999). Congruence of the ITS and ETS data sets was assessed using the partition homogeneity test (PHT; Farris et al., 1995) as

Table 1. List of samples, voucher collection information, and GenBank accession numbers.

Taxon	Voucher	Source	ETS	ITS
<i>Actinodaphne</i> Nees				
<i>A. cupularis</i> (Hemsl.) Gamble	Wuyishan Exped. 1693 (KUN 0601976)	Guizhou, China	AY817123	AY817113
<i>A. Forrestii</i> (C. K. Allen) Kosterm.	Li H.-W. 2 (HITBC)	Yunnan, China	AY934881	AY265399
<i>A. henryi</i> Gamble	Li J. 2002032 (HITBC)	Yunnan, China	AY817130	AY817120
<i>A. kweichowensis</i> Yen C. Yang & P. H. Huang	Li H.-Q. 40091 (KUN 0106643)	Guangxi, China	AY817124	AY817114
<i>A. lecomtei</i> C. K. Allen	Li C.-Q. 3979 (IBK 00003410)	Guangxi, China	AY817122	AY817112
<i>A. obovata</i> (Nees) Blume	Li H.-W. 1 (HITBC)	Yunnan, China	AY934880	AY265398
<i>A. omeiensis</i> (H. Liu) C. K. Allen	Yang G.-H. 55824 (KUN 0047252)	Yunnan, China	AY817127	AY817117
<i>A. paotingensis</i> Yen C. Yang & P. H. Huang	Hainan Exped. 962 (IBK 00003425)	Hannan, China	AY817128	AY817118
<i>A. pilosa</i> (Lour.) Merr.	Xie L.-S. 613 (KUN 0047277)	Guangxi, China	AY817125	AY817115
<i>A. trichocarpa</i> C. K. Allen	Sun B.-X. 0757 (KUN 0047286)	Yunnan, China	AY817126	AY817116
<i>A. tsaii</i> Hu	Feng G.-M. 22638 (KUN 0047322)	Yunnan, China	AY817129	AY817119
<i>Dodecadenia</i> Nees				
<i>D. grandiflora</i> Nees	Wu C.-Y. et al. 75-1048 (KUN 0049206)	Tibet, China	DQ120552	AY265397
<i>Iteadaphne</i> Blume				
<i>I. caudata</i> (Nees) H. W. Li	Li H.-W. 27 (HITBC)	Yunnan, China	DQ120551	AY265396
<i>Laurus</i> L.				
<i>L. nobilis</i> L.	Li H.-W. 16 (HITBC)	Yunnan, China	DQ120553	AY265392
<i>Lindera</i> Thunb.				
Sect. <i>Aperula</i> (Blume) Benth.				
<i>L. latifolia</i> Hook. f.	Li J. 2002076 (HITBC)	Yunnan, China	DQ120541	DQ124264
<i>L. longipedunculata</i> C. K. Allen	Li H. 11114 (KUN 0690203)	Yunnan, China	DQ120542	DQ124265
<i>L. metcalfiana</i> C. K. Allen	Li H.-W. 8 (HITBC)	Yunnan, China	DQ120543	AY265408
Sect. <i>Cupuliformes</i> H. P. Tsui				
<i>L. megaphylla</i> Hemsl.	Li H.-W. 7 (HITBC)	Yunnan, China	AY934882	AY265406
Sect. <i>Daphnidium</i> (Nees) Benth.				
<i>L. chunii</i> Merr.	Li J. 2002134 (HITBC)	Guangxi, China	DQ120547	DQ124266
<i>L. pulcherrima</i> (Nees) Hook. f.	Li J. 2002202 (HITBC)	Guangxi, China	DQ120548	DQ124267
<i>L. villipes</i> H. P. Tsui	Li H. et al. 11876 (KUN 0762162)	Yunnan, China	DQ120550	DQ124268
Sect. <i>Lindera</i>				
<i>L. kariensis</i> W. W. Sm.	Li H. et al. 15305 (KUN 0789698)	Yunnan, China	DQ120539	DQ124263
<i>L. reflexa</i> Hemsl.	Nei M.-X. & Lai S.-K. 3768 (KUN 0100201)	Jiangxi, China	DQ120540	AY265407
Sect. <i>Palminerviae</i> Meisn.				
<i>L. obtusiloba</i> Blume	Sino-Amer. Exped. 1308 (KUN 0151469)	Hubei, China	DQ120546	AY265411
Sect. <i>Polyadenia</i> (Nees) Benth.				
<i>L. communis</i> Hemsl.	Li H.-W. 4 (HITBC)	Yunnan, China	DQ120544	AY265409
Sect. <i>Sphaerocarpace</i> H. P. Tsui				
<i>L. fruticosa</i> Hemsl. (= <i>L. neesiana</i> (Wall. ex Nees) Kurz)	Li G. F. 63966 (KUN 0104915)	Sichuan, China	DQ120545	AY265410
<i>Litsea</i> Lam.				
Sect. <i>Conodaphne</i> (Blume) Benth. & Hook. f.				
<i>L. monopetala</i> (Roxb.) Pers.	Li J. 2002108 (HITBC)	Guangxi, China	DQ120527	DQ120602
<i>L. umbellata</i> (Lour.) Merr.	Li H.-W. 24 (HITBC)	Yunnan, China	DQ120528	AY265404
<i>L. variabilis</i> Hemsl. var. <i>variabilis</i>	Li J. 2002142 (HITBC)	Guangxi, China	DQ120529	DQ120603
<i>L. variabilis</i> var. <i>oblonga</i> Lecomte	Li J. 2002115 (HITBC)	Guangxi, China	DQ120530	DQ120604
Sect. <i>Cylicodaphne</i> (Nees) Hook. f.				

Table 1. Continued.

Taxon	Voucher	Source	ETS	ITS
<i>L. acutivena</i> Hayata	<i>Li J. 2002199</i> (HITBC)	Guangxi, China	DQ120531	DQ120605
<i>L. dilleniifolia</i> P. Y. Pai & P. H. Huang	<i>Li H.-W. 19</i> (HITBC)	Yunnan, China	DQ120532	AY265405
<i>L. elongata</i> (Nees) Hook. f.	<i>Li J. 2002146</i> (HITBC)	Guangxi, China	DQ120533	DQ120606
<i>L. garrettii</i> Gamble	<i>Xia Y.-M. s.n.</i> (HITBC)	Yunnan, China	DQ120534	DQ120607
<i>L. liyuyingii</i> H. Liu	<i>Xia Y.-M. s.n.</i> (HITBC)	Yunnan, China	DQ120535	DQ120608
<i>L. longistaminata</i> (H. Liu) Kosterm.	<i>Xia Y.-M. s.n.</i> (HITBC)	Yunnan, China	DQ120536	DQ120609
<i>L. panamanja</i> (Nees) Hook. f.	<i>Li J. 2002028</i> (HITBC)	Yunnan, China	DQ120537	DQ120610
<i>L. yaoshanensis</i> Yen C. Yang & P. H. Huang	<i>Li J. 2002198</i> (HITBC)	Guangxi, China	DQ120538	DQ120611
Sect. <i>Litsea</i>				
<i>L. glutinosa</i> (Lour.) C. B. Rob.	<i>Li H.-W. 21</i> (HITBC)	Yunnan, China	AY934883	AY265403
Sect. <i>Tomingodaphne</i> (Blume) Hook. f.				
<i>L. cubeba</i> (Lour.) Pers.	<i>Li H.-W. 28</i> (HITBC)	Yunnan, China	DQ120523	AY265402
<i>L. kingii</i> Hook. f.	<i>Li J. 2002170</i> (HITBC)	Guangxi, China	DQ120524	DQ120599
<i>L. rubescens</i> Lecomte	<i>Li J. 2002094</i> (HITBC)	Yunnan, China	DQ120525	DQ120600
<i>L. sericea</i> (Wall. ex Nees) Hook. f.	<i>Li H. et al. 15299</i> (KUN 0789681)	Yunnan, China	DQ120526	DQ120601
<i>Neolitsea</i> (Benth.) Merr.				
<i>N. aurata</i> (Hayata) Koidz. var. <i>aurata</i>	<i>Li J. 2002181</i> (HITBC)	Guangxi, China	DQ120557	DQ124270
<i>N. aurata</i> var. <i>chekiangensis</i> (Nakai) Yen C. Yang & P. H. Huang	<i>Zhang S.-Y. 5482</i> (KUN 0162041)	Zhejiang, China	DQ120558	DQ124271
<i>N. brassii</i> C. K. Allen	<i>Gray, B. 03911</i> (KUN 0793628)	Queensland, Australia	DQ120559	DQ124272
<i>N. cambodiana</i> Lecomte var. <i>glabra</i> C. K. Allen	<i>Li X.-G. 202474</i> (IBK 00009945)	Guangdong, China	DQ120560	DQ124273
<i>N. chrysotricha</i> H. W. Li	<i>Wu S.-G. 7095</i> (KUN 0106438)	Yunnan, China	DQ120561	DQ124274
<i>N. chunii</i> Merr.	<i>Li J. 2002063</i> (HITBC)	Yunnan, China	DQ120562	DQ124275
<i>N. confertifolia</i> (Hemsl.) Merr.	<i>Xi X.-Y. 414</i> (PE 1272040)	Hunan, China	DQ120563	DQ124276
<i>N. dealbata</i> (R. Br.) Merr.	<i>Gray, B. 03993</i> (KUN 0793630)	Queensland, Australia	DQ120564	DQ124277
<i>N. homilantha</i> C. K. Allen	<i>Li J. 2002071</i> (HITBC)	Yunnan, China	DQ120565	DQ124278
<i>N. kuangsiensis</i> H. Liu	<i>Wu S.-J. 3419</i> (IBK 00010186)	Hong Kong, China	DQ120566	DQ124279
<i>N. levinei</i> Merr.	<i>Li H.-W. 29</i> (HITBC)	Yunnan, China	AY934884	AY265401
<i>N. lunglingensis</i> H. W. Li	<i>Li J. 2002058</i> (HITBC)	Yunnan, China	DQ120567	DQ124280
<i>N. ovatifolia</i> Yen C. Yang & P. H. Huang var. <i>ovatifolia</i>	<i>Wu S.-J. 3246</i> (IBK 00010360)	Hong Kong, China	DQ120568	DQ124281
<i>N. ovatifolia</i> var. <i>puberula</i> Yen C. Yang & P. H. Huang	<i>Mao P.-Y. 03875</i> (KUN 0108307)	Yunnan, China	DQ120569	DQ124282
<i>N. pallens</i> (D. Don) Momiy. & H. Hara	<i>Qinhai-Tibet Exped. 5972</i> (KUN 0108358)	Tibet, China	DQ120570	DQ124283
<i>N. phanerophlebia</i> Merr.	<i>Deng L. 7511</i> (KUN 0108338)	Guangdong, China	DQ120571	DQ124284
<i>N. pingbienensis</i> Yen C. Yang & P. H. Huang	<i>Mao P.-Y. 04139</i> (KUN 0108220)	Yunnan, China	DQ120572	DQ124285
<i>N. pinninervis</i> Yen C. Yang & P. H. Huang	<i>Li J. 2002187</i> (HITBC)	Guangxi, China	DQ120573	DQ124286
<i>N. polycarpa</i> H. Liu	<i>Zhou Z.-K. et al. EXLS-0252</i> (KUN 0695675)	Yunnan, China	DQ120574	DQ124287
<i>N. pulchella</i> (Meisn.) Merr.	<i>Li J. 2002166</i> (HITBC)	Guangxi, China	DQ120575	DQ124288
<i>N. sericea</i> (Blume) Koidz.	<i>K. Midorikawa 2180</i> (KUN 0108215)	Honshu, Japan	DQ120576	DQ124289
<i>Neolitsea</i> sp.	<i>Li J. 2002070</i> (HITBC)	Yunnan, China	DQ120581	DQ124294
<i>N. sutchuanensis</i> Y. C. Yang var. <i>sutchuanensis</i>	<i>Zhao Z.-X. 64</i> (KUN 0108178)	Sichuan, China	DQ120577	DQ124290
<i>N. sutchuanensis</i> var. <i>gongshanensis</i> H. W. Li	<i>Feng G.-M. 6987</i> (KUN 0108134)	Yunnan, China	DQ120578	DQ124291
<i>N. undulatifolia</i> (H. Lév.) C. K. Allen	<i>Li J. 2002203</i> (HITBC)	Guangxi, China	DQ120579	DQ124292

Table 1. Continued.

Taxon	Voucher	Source	ETS	ITS
<i>N. wushanica</i> var. <i>pubens</i> Yen C. Yang & P. H. Huang	<i>Liu L.-H. 15149</i> (KUN 0162057)	Hunan, China	DQ120580	DQ124293
<i>Parasassafras</i> D. G. Long				
<i>P. confertiflora</i> (Meisn.) D. G. Long	<i>Qian Y.-Y. 682</i> (KUN 0104558)	Yunnan, China	AY934885	AY265395
<i>Sinosassafras</i> H. W. Li				
<i>S. flavinervia</i> (C. K. Allen) H. W. Li	<i>Liu Y.-H. s.n.</i> (HITBC)	Yunnan, China	AY934886	AY940451
Outgroup taxa				
<i>Cinnamomum pittosporoides</i> Hand.-Mazz.	<i>Li H. 5252</i> (KUN 0108156)	Yunnan, China	DQ120554	DQ124269
<i>Sassafras tzumu</i> (Hemsl.) Hemsl.	<i>Li H.-W. 15</i> (HITBC)	Yunnan, China	DQ120555	AY265391
<i>Umbellularia californica</i> (Hook. & Arn.) Nutt.	<i>van der Werff s.n.</i> (MO)	North America	DQ120556	AY265393

implemented in PAUP*, and because the PHT was non-significant, the data sets were combined for all subsequent analyses. Most parsimonious trees were obtained from 10,000 replicates of random taxon addition using equally weighted (EW) characters (Fitch, 1971) and tree bisection-reconnection (TBR) branch swapping (MULPARS off), followed by swapping on the shortest trees from this analysis with MULPARS on. These trees were then used to re-weight the characters according to the best fit of their rescaled consistency indices (Farris, 1989). New searches were performed with 1000 replicates using successive weighting (SW) until equilibrium was reached (Farris, 1969). Clade support was estimated using bootstrap resampling (Felsenstein, 1985), with 1000 replicates, TBR, EW, and MP optimality criteria performed on the combined weighted ITS and ETS

data with resampling using all characters equally, regardless of weight.

The 19 morphological characters were analyzed using unweighted parsimony, with all characters treated as unordered. Tree search was performed with 1000 replicates of random taxon addition and TBR branch swapping (MULPARS on) in PAUP* version 4.01b10 (Swofford, 1999). The morphological data were then combined with the ITS and ETS data and analyzed using the same settings that were used in the morphological data set. Clade support was estimated with bootstrap resampling (Felsenstein, 1985) for 1000 replicates with TBR, EW, and MP optimality. Morphological character state changes were then plotted on one of the resulting most parsimonious combined analysis trees using MacClade (Maddison & Maddison, 2000).

Table 2. Morphological characters and character states traditionally considered to be taxonomically important at the generic and sectional level in *Laureae*.

1. Habit: evergreen (0), deciduous (1)
2. Leaf arrangement: alternate along stems (0), alternate and crowded at branchlet apices (1), verticillate or subverticillate (2)
3. Leaf venation: pinninerved (0), triplinerved (1), trinerved (2)
4. Inflorescence type: thyrsoid, without vegetative terminal bud in the main axis (0), short shoot (brachyblast) with vegetative terminal bud in the main axis (1)
5. Inflorescence: terminal or subterminal (0), axillary (1)
6. Inflorescence arrangement: panicle (0), raceme (1), fasciculate clustered (2)
7. Inflorescence: sessile (0), stipitate (1)
8. Flower number per inflorescence: >1(0), 1(1)
9. Involucres: absent (0), present (1)
10. Involucres: large (0), minute (1)
11. Involucres: imbricate (0), decussate (1)
12. Involucres: early deciduous (0), persistent (1)
13. Flower sex: bisexual (0), unisexual (1)
14. Basic floral number: dimerous (0), trimerous (1)
15. Perianth segment: present, perfect (0), imperfect, absent or early deciduous (1)
16. Anthers: two-locular (0), four-locular (1)
17. Pollen sacs of the third whorl: latrorse (0), introrse (1), extrorse (2)
18. Fruit shape: globular or oblate (0), ovoid or ellipsoid (1)
19. Fruit cupule shape: flat or discoid (0), cup-shaped (1)

Bayesian phylogeny reconstruction of the combined data was performed with MrBayes 3.0b4 (Huelsenbeck & Ronquist, 2001; Huelsenbeck et al., 2002). The program Modeltest (Posada & Crandall, 1998) was used to test which of the 56 predicted models of DNA substitution best fit the data. Modeltest indicated that the TrN + I + Γ model with rate heterogeneity and among-site rate variation from Tamura and Nei (1993) best fit the two nrDNA regions, and the ML parameters in MrBayes (MB) were “1st nst = 6” and “rates = invgamma.” The Markov chain Monte Carlo process was set so that four chains ran simultaneously for 500,000 generations, with trees sampled every 100 generations, giving a total of 5000 trees in the initial sample. Likelihood value plots for the four chains showed that stationarity had occurred by the 600th tree. Therefore, the first 600 trees were discarded as “burn in,” and the posterior probabilities of the phylogeny and its branches were determined from the remaining 4400 trees.

ME analysis was also performed using neighbor joining on the unweighted data set using MEGA 3.1 (Kumar et al., 2004), with bootstrap support calculated on 10,000 replicates.

ML analysis of the combined, unweighted data was performed using the DNAML option in DAMBE version 4.13 (Xia, 2000; Xia & Xie, 2001).

RESULTS

SEQUENCE CHARACTERISTICS

ITS regions are quite variable between distantly related taxa, so only regions that could be aligned unequivocally were used in this analysis, making our phylogenetic estimates conservative. For the ingroup taxa, the length of the ITS regions, including the 5.8S region, ranged from 568 to 627 bp, and the alignment generated a data set of 689 characters of which 156 (22.64%) were parsimony informative. G plus C content ranged from 64.61% to 74.19%.

Compared to ITS, the approximately 400 bp of the 3' end of the ETS between the 18S subunit and the ETS-1 internal primer were relatively easy to amplify and sequence. For the ingroup taxa, the ETS region varied in length from 350 to 393 bp, and alignment resulted in a matrix of 393 characters of which 99 (25.19%) were parsimony informative, with G plus C content ranging from 46.56% to 55.23%. All sequences were submitted to GenBank (Table 1), and the data matrices for both sequenced regions are available from the primary author upon request.

PHYLOGENETIC ANALYSIS

SW parsimony. Results of phylogenetic analyses of the ITS and ETS regions performed separately

showed no hard incongruences (i.e., there were no contradictory clades supported by bootstrap greater than 60%; data not shown). The PHT (Farris et al., 1995) indicated that the two regions were congruent ($P < 0.01$; see discussions in Sullivan, 1996; Cunningham, 1997; Farris et al., 2000). Because of this and because the ETS and ITS regions occur within the same transcriptional unit and show evidence of a similar and interdependent role in the maturation of ribosomal RNAs (Good et al., 1997), we will only present results for the combined analyses.

The EW analysis produced 30,600 trees (length [L] = 1171, consistency index [CI] = 0.306, retention index [RI] = 0.589), but because all main and virtually all minor branches collapsed under strict consensus, SW was used to try to stabilize the tree topology. SW reduced this to 12 trees (L = 211.5, CI = 0.576, RI = 0.834), producing an almost fully resolved strict consensus tree (Fig. 1).

The SW analysis of the nrDNA data produced a series of clades within a monophyletic core Laureae (93% bootstrap support). These clades are referred to informally as the *Neolitsea*–*Actinodaphne*, and *Litsea*, *Lindera*, and *Aperula* clades. These sat above a basal grade of *Lindera obtusiloba* Blume (sect. *Palminerviae*) and then *Lindera communis* Hemsl. (sect. *Polyadenia*). The *Aperula* clade contained the three sampled species of *Lindera* sect. *Aperula* (*L. latifolia*, *L. longipedunculata* C. K. Allen, and *L. metcalfiana* C. K. Allen) grouped in a terminal pairing with *Litsea cubeba* and *L. kingii* Hook. f. (both section *Tomingodaphne*) above a subclade of *Lindera megaphylla* (sect. *Cupuliformes*) and *Actinodaphne forrestii* (C. K. Allen) Kosterm. Although the *Aperula* clade as a whole was unsupported (< 50% bootstrap), all the branches within it had moderate (> 70%) to strong support (> 90%).

Sitting above *Sinosassafras* and *Parasassafras* was the *Lindera* clade (75% bootstrap support), with two subclades. The first of these represented *Lindera* sect. *Lindera* (2 spp.), plus *Litsea* species from sections *Tomingodaphne* and *Conodaphne* (part). Sister to this was a subclade consisting of *L. fruticosa* Hemsl. (sect. *Sphaerocarpaceae*), *Itadaphne caudata* (Nees) H. W. Li, and the three species of *Lindera* sect. *Daphnidium*, again with most terminal branches moderately supported.

The *Litsea* clade consisted of a terminal lineage representing members of sections *Litsea*, *Conodaphne* (part), and *Cylicodaphne*, but also including *Dodecadenia grandiflora* Nees, all sitting above *Laurus nobilis* L. and *Actinodaphne lecomtei* C. K. Allen. The clade included within it three separate lineages. Section *Litsea* and *Litsea monopetala* (Roxb.) Pers. (sect. *Conodaphne*) formed a strongly supported pair

(99%), sister to a subclade to three species of *Litsea* sect. *Cylicodaphne*: *L. dillenifolia* P. Y. Pai & P. H. Huang, *L. garrettii* Gamble, and *L. panamanja* (Nees) Hook. f. (here called *Cylicodaphne* I), and then to an *L. variabilis* Hemsl. var. *variabilis* and var. *oblonga* Lecomte (sect. *Conodaphne*) subclade, all with moderate to strong bootstrap support. The other branch in the *Litsea* clade was the *Cylicodaphne* II subclade, representing the remainder of *Litsea* sect. *Cylicodaphne* but including an embedded *Dodecadenia grandiflora*, again with most branches showing at least moderate support.

Within the *Neolitsea*–*Actinodaphne* clade, *Actinodaphne* (except *A. forrestii* and *A. lecomtei*) formed a basal grade to a well-supported *Neolitsea* (89%), with the latter divided into two subclades (*Neolitsea* I and *Neolitsea* II) above *N. chrysotricha* H. W. Li and *N. pallens* (D. Don) Momiy. & H. Hara. *Neolitsea* I showed little clear support for the internal branches, whereas all the branches in *Neolitsea* II showed bootstrap support > 50%.

Bayesian analysis. Bayesian analysis of the unweighted Laureae ITS + ETS showed moderate relationship resolution (Fig. 2), with the Bayesian tree corresponding well with much of the SW tree in terms of recovered major lineages. Although not as well resolved as the SW topology, terminal SW clades with high bootstrap support were also present in the Bayesian topology with strong posterior probability support, and both analyses included the *Neolitsea*–*Actinodaphne* clade and many of the major SW subclades.

Nevertheless, there were differences between the results for the two approaches. In the Bayesian tree, the *Lindera* clade was not recovered, with section *Daphnidium* falling instead as part of a polytomy separate from the remainder. Similarly, *Litsea glutinosa* (Lour.) C. B. Rob. (sect. *Litsea* and the type species for the genus) and *L. monopetala* (sect. *Conodaphne*) were separated from the rest of the *Litsea* clade seen in the SW analysis.

ME and ML analyses. The ME (neighbor joining) tree (Fig. 3) was well resolved and similar in major clade structure to the SW and Bayesian cladograms. *Neolitsea* I and II (albeit the latter reduced) were again terminal above an *Actinodaphne* grade, although here *A. paotingensis* Yen C. Yang & P. H. Huang was embedded in a basal *Neolitsea* subclade with *N. brassii* C. K. Allen, *N. wushanica* var. *pubens* Yen C. Yang & P. H. Huang, and *N. sutchuanensis* Y. C. Yang. *Laurus* was basal to the *Actinodaphne*–*Neolitsea* clade, and sister to this group was a clade consisting of part of the MP and MB *Aperula* clade and an expanded *Litsea* clade including the *Litsea* and

Cylicodaphne I and II subclades, as well as *Dodecadenia* and *Iteadaphne*. Below this was a lineage containing the *Lindera*, *L. fruticosa*, and *Daphnidium* subclades, and then a polytomy consisting of *L. communis* paired with *Sinosassafras*, and an “*Aperula* II” clade of *Tomingodaphne*, *Cupuliformes*, and *Actinodaphne forrestii*. *Lindera obtusiloba* and then *Parasassafras* were basal to the remainder of Laureae.

Unlike most of the other analyses, in the ML tree (Fig. 4), *Lindera obtusiloba* and *L. communis* were paired and sister to the *Aperula* clade, with this lineage placed above *Laurus* and below the *Actinodaphne*–*Neolitsea* clade. *Neolitsea* I and II and the *Actinodaphne* grade were all present, although *A. paotingensis* was placed inside *Neolitsea* I, similar to the ME tree, and a subclade of *N. aurata* (Hayata) Koidz. and *N. kwangsiensis* H. Liu was basal to *Neolitsea* as a whole. Below all of these was a major lineage consisting of a *Lindera* clade, *L. fruticosa*, and the *Daphnidium* clade (with *Iteadaphne* basal) and a *Litsea* clade (including *Litsea* and the *Cylicodaphne* I and II subclades). *Actinodaphne lecomtei* was placed well inside the *Actinodaphne* grade, whereas *A. forrestii* was still sister to *Lindera megaphylla*, but this latter pair was placed between *Sinosassafras* and *Parasassafras* at the base of the Laureae.

Combined molecular and morphological analyses. Analysis of the morphological matrix by itself resulted in 317 equally parsimonious trees (L = 72, CI = 0.3056, RI = 0.8214), but these collapsed completely to an unresolved polytomy under strict consensus. When the morphological and molecular data were combined, two trees of 1271 steps (CI = 0.3021, RI = 0.6086) resulted, one of which is shown in Figure 5. This tree also recovered the *Neolitsea*–*Actinodaphne* clade, *Lindera* clade, and *Litsea* clade, which were seen in the molecular analysis, but the *Aperula* clade was now split into two separate entities, with *Aperula* I representing *Lindera* sect. *Aperula* s. str. and *Aperula* II representing *Litsea cubeba* and *L. kingii* (sect. *Tomingodaphne*), *Lindera megaphylla* (sect. *Cupuliformes*), and *Actinodaphne forrestii*. The inclusion of the morphological data changed the bootstrap support for most clades, generally lowering it from the SW analysis, although there was still reasonable bootstrap support for the terminal branches and still little or no deep-branch support.

When the morphological character state changes are plotted on the combined analysis tree (Fig. 5), the unique synapomorphy for the Laureae is the presence of inflorescences with short shoots (brachyblasts) with a vegetative terminal bud on the main axis. Within the Laureae, the *Actinodaphne*–*Neolitsea*–*Parasassafras*–

Table 3. Data matrix of important morphological characters in Laureae for species used in the molecular analyses.

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
<i>Actinodaphne cupularis</i>	0	2	0	0	1	2	0	0	1	0	0	0	1	1	0	1	1	1	1
<i>Actinodaphne forrestii</i>	0	2	0	1	1	2	0	0	1	0	0	0	1	1	0	1	1	1	1
<i>Actinodaphne henryi</i>	0	2	0	0	1	1	1	0	1	0	0	0	1	1	0	1	1	0	1
<i>Actinodaphne kweichowensis</i>	0	2	0	0	1	2	0	0	1	0	0	0	1	1	0	1	1	0	1
<i>Actinodaphne lecomtei</i>	0	2	0	0	1	2	0	0	1	0	0	0	1	1	0	1	1	1	1
<i>Actinodaphne obovata</i>	0	2	1	0	1	1	1	0	1	0	0	0	1	1	0	1	1	1	0
<i>Actinodaphne omeiensis</i>	0	2	0	0	1	2	0	0	1	0	0	0	1	1	0	1	1	0	0
<i>Actinodaphne paotingensis</i>	0	2	0	0	1	2	0	0	1	0	0	0	1	1	0	1	1	?	1
<i>Actinodaphne pilosa</i>	0	2	0	0	1	0	1	0	1	0	0	0	1	1	0	1	1	0	0
<i>Actinodaphne trichocarpa</i>	0	2	0	0	1	2	0	0	1	0	0	0	1	1	0	1	1	0	0
<i>Actinodaphne tsaii</i>	0	2	0	0	1	2	0	0	1	0	0	0	1	1	0	1	1	1	1
<i>Cinnamomum pittosporoides</i>	0	0	1	0	0	0	1	0	0	—	—	—	0	1	0	1	2	0	1
<i>Dodecadenia grandiflora</i>	0	0	0	1	1	2	0	1	1	0	1	1	1	1	0	1	0	1	0
<i>Iteadaphne caudata</i>	0	0	1	1	1	1	0	1	1	0	1	1	1	1	0	0	0	0	0
<i>Laurus nobilis</i>	0	0	0	1	1	1	1	0	1	0	1	1	1	0	0	0	1	1	0
<i>Lindera chunii</i>	0	0	2	1	1	2	1	0	1	0	1	1	1	1	0	0	1	1	0
<i>Lindera communis</i>	0	0	0	1	1	2	0	0	1	0	1	1	1	1	0	0	1	1	0
<i>Lindera fruticosa</i>	1	0	1	1	1	1	1	0	1	0	1	1	1	1	0	0	1	0	0
<i>Lindera kariensis</i>	1	0	0	1	1	2	0	0	1	0	1	1	1	1	0	0	1	1	0
<i>Lindera latifolia</i>	0	0	0	1	1	1	1	0	1	0	1	1	1	1	0	0	1	0	0
<i>Lindera longipedunculata</i>	0	0	0	1	1	1	1	0	1	0	1	1	1	1	0	0	1	0	0
<i>Lindera megaphylla</i>	0	1	0	1	1	1	1	0	1	0	1	1	1	1	0	0	1	1	1
<i>Lindera metcalfiana</i>	0	0	0	1	1	1	1	0	1	0	1	1	1	1	0	0	1	0	0
<i>Lindera obtusiloba</i>	1	0	2	1	1	2	1	0	1	0	1	1	1	1	0	0	1	1	0
<i>Lindera pulcherrima</i>	0	0	2	1	1	2	0	0	1	0	1	1	1	1	0	0	1	1	0
<i>Lindera reflexa</i>	1	0	0	1	1	2	1	0	1	0	1	1	1	1	0	0	1	0	0
<i>Lindera villipes</i>	0	0	2	1	1	2	0	0	1	0	1	1	1	1	0	0	1	1	0
<i>Litsea acutivena</i>	0	1	0	1	1	2	1	0	1	0	1	1	1	1	0	1	1	1	1
<i>Litsea cubeba</i>	1	0	0	1	1	2	1	0	1	0	1	1	1	1	0	1	1	0	0
<i>Litsea dilleniifolia</i>	0	0	0	1	1	1	1	0	1	0	1	1	1	1	0	1	1	0	1
<i>Litsea elongata</i>	0	0	0	1	1	2	1	0	1	0	1	1	1	1	0	1	1	1	1
<i>Litsea garrettii</i>	0	0	0	1	1	1	1	0	1	0	1	1	1	1	0	1	1	1	1
<i>Litsea glutinosa</i>	0	0	0	1	1	1	1	0	1	0	1	1	1	1	1	1	1	0	0
<i>Litsea kingii</i>	1	0	0	1	1	2	1	0	1	0	1	1	1	1	0	1	1	0	0
<i>Litsea liuyingii</i>	0	0	0	1	1	1	1	0	1	0	1	1	1	1	0	1	1	?	?
<i>Litsea longistaminata</i>	0	0	0	1	1	1	1	0	1	0	1	1	1	1	0	1	1	1	0
<i>Litsea monopetala</i>	0	0	0	1	1	2	1	0	1	0	1	1	1	1	1	1	1	1	0
<i>Litsea panamanja</i>	0	0	0	1	1	1	1	0	1	0	1	1	1	1	0	1	1	1	1
<i>Litsea rubescens</i>	1	0	0	1	1	2	1	0	1	0	1	1	1	1	0	1	1	0	0
<i>Litsea sericea</i>	1	0	0	1	1	2	1	0	1	0	1	1	1	1	0	1	1	0	0
<i>Litsea umbellata</i>	0	0	0	1	1	2	1	0	1	0	1	1	1	1	0	1	1	0	1
<i>Litsea variabilis</i> var. <i>variabilis</i>	0	0	0	1	1	2	1	0	1	0	1	1	1	1	0	1	1	0	0
<i>Litsea variabilis</i> var. <i>oblonga</i>	0	0	0	1	1	2	1	0	1	0	1	1	1	1	0	1	1	0	0
<i>Litsea yaoshanensis</i>	0	0	0	1	1	2	1	0	1	0	1	1	1	1	0	1	1	1	1
<i>Neolitsea aurata</i> var. <i>aurata</i>	0	1	1	0	1	2	1	0	1	0	1	1	1	0	0	1	1	1	0
<i>Neolitsea aurata</i> var. <i>chekiangensis</i>	0	1	1	0	1	2	1	0	1	0	1	1	1	0	0	1	1	1	0
<i>Neolitsea brassii</i>	0	2	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
<i>Neolitsea cambodiana</i> var. <i>glabra</i>	0	2	0	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
<i>Neolitsea chrysotricha</i>	0	0	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	1	0
<i>Neolitsea chunii</i>	0	1	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	1	0
<i>Neolitsea confertifolia</i>	0	2	0	0	1	2	0	0	1	0	1	1	1	0	0	1	1	1	0
<i>Neolitsea dealbata</i>	0	2	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
<i>Neolitsea homilantha</i>	0	1	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	1	0
<i>Neolitsea kwangsiensis</i>	0	1	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
<i>Neolitsea levinei</i>	0	2	1	0	1	2	1	0	1	0	1	1	1	0	0	1	1	0	0
<i>Neolitsea lunglingensis</i>	0	0	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	1	0

Table 3. Continued.

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
<i>Neolitsea ovatifolia</i> var. <i>ovatifolia</i>	0	1	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
<i>Neolitsea ovatifolia</i> var. <i>puberula</i>	0	1	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
<i>Neolitsea pallens</i>	0	0	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
<i>Neolitsea phanerophlebia</i>	0	2	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
<i>Neolitsea pingbienensis</i>	0	1	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	1	0
<i>Neolitsea pinninervis</i>	0	1	0	0	1	2	1	0	1	0	1	1	1	0	0	1	1	0	0
<i>Neolitsea polycarpa</i>	0	1	1	0	1	2	1	0	1	0	1	1	1	0	0	1	1	1	0
<i>Neolitsea pulchella</i>	0	1	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
<i>Neolitsea sericea</i>	0	0	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
<i>Neolitsea</i> sp.	0	1	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	1	0
<i>Neolitsea sutchuanensis</i> var. <i>sutchuanensis</i>	0	0	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	1	0
<i>Neolitsea sutchuanensis</i> var. <i>gongshanensis</i>	0	0	1	0	1	2	0	0	1	0	1	1	1	0	0	1	1	1	0
<i>Neolitsea undulatifolia</i>	0	2	0	0	1	2	0	0	1	0	1	1	1	0	0	1	1	1	1
<i>Neolitsea wushanica</i> var. <i>pubens</i>	0	0	0	0	1	2	0	0	1	0	1	1	1	0	0	1	1	0	0
<i>Parasassafras confertiflora</i>	0	0	1	1	1	1	1	0	1	1	0	0	1	1	0	1	0	0	0
<i>Sassafras tzumu</i>	1	1	0	0	0	1	1	0	1	0	0	1	1	1	0	1	1	0	0
<i>Sinosassafras flavinervia</i>	0	0	1	1	1	2	1	0	1	1	0	0	1	1	0	0	0	0	0
<i>Umbellularia californica</i>	0	0	0	0	1	1	1	0	1	0	0	0	0	1	0	1	2	1	0

Sinosassafras clade is united by the presence of imbricate, early deciduous involucre bracts, although *Neolitsea* itself has decussate persistent bracts as one of its synapomorphies. *Parasassafras* and *Sinosassafras* are supported as a pair by the possession of triplinerved leaves, minute involucre bracts, and latrorse pollen sacs for the third whorl, whereas the monophyly of the *Neolitsea*–*Actinodaphne* clade is supported by the synapomorphies of verticillate or subverticillate leaves and thyrsoid inflorescences that lack a terminal bud. Although there are few characters supporting the main branches within the *Actinodaphne* grade, *Neolitsea* is well supported, with triplinerved, alternate, crowded leaves at branch apices, decussate persistent bracts, and dimerous flowers. Within *Neolitsea*, there were no morphological synapomorphies supporting clades I and II, but within I, the species above *N. ovatifolia* Yen C. Yang & P. H. Huang var. *ovatifolia* (Fig. 51a) have verticillate or subverticillate leaves as a synapomorphy (albeit with some reversals), whereas in clade II there are two lineages: IIa, with ovoid to ellipsoid fruits, and IIb, defined by alternate leaves (again with a reversal in *N. brassii*).

In contrast, there were relatively no morphological synapomorphies supporting the *Lindera* or *Aperula* I or II clades, although the *Laurus*–*Litsea* clade had racemose inflorescences, and the *Litsea* clade was defined by four-locular anthers and the *L. glutinosa*–*L. monopetala* pair by the possession of imperfect, absent or early deciduous perianth segments. Within the *Litsea* clade, the *Cylicodaphne* II subclade was

characterized by cup-shaped cupules and united with *Dodecadenia* on fasciculate, clustered inflorescences and ovoid/ellipsoid fruits. Similarly, within the *Lindera* clade, section *Daphnidium* was united by trinerved leaves and ovoid-ellipsoid fruits.

DISCUSSION

PHYLOGENETIC UTILITY OF ETS IN THE LAUREAE

Several studies have shown that greater resolution and support for phylogenetic estimation are achieved by increasing character number and/or taxon representation (Graybeal, 1998; Hillis, 1998; Soltis et al., 1998; Bremer et al., 1999), and our study supports the importance of adding data from both more taxa and more sequence regions to help resolve issues in Laureae. The usefulness of the ETS region in molecular systematics has been suggested previously (Baldwin & Markos, 1998; Bena et al., 1998; Clevinger & Panero, 2000; Linder et al., 2000), and although the sequenced ETS segment is much shorter than the segment in the ITS (393 bp vs. 689 aligned bp) in Laureae, it nevertheless produces a slightly higher percentage of informative sites (25.19%) than that in the ITS (22.64%). The PHT for the two data sets showed congruence, and the combined analysis of the ITS and ETS sequences provided greater resolution and increased support for the relationships than either sequence by itself (trees not shown). This is consistent with the results of other combined ITS/ETS investigations (e.g., Li et al., 2002; Becerra, 2003; Lee et al., 2003; Morgan, 2003; Saar et al., 2003; Urbatsch

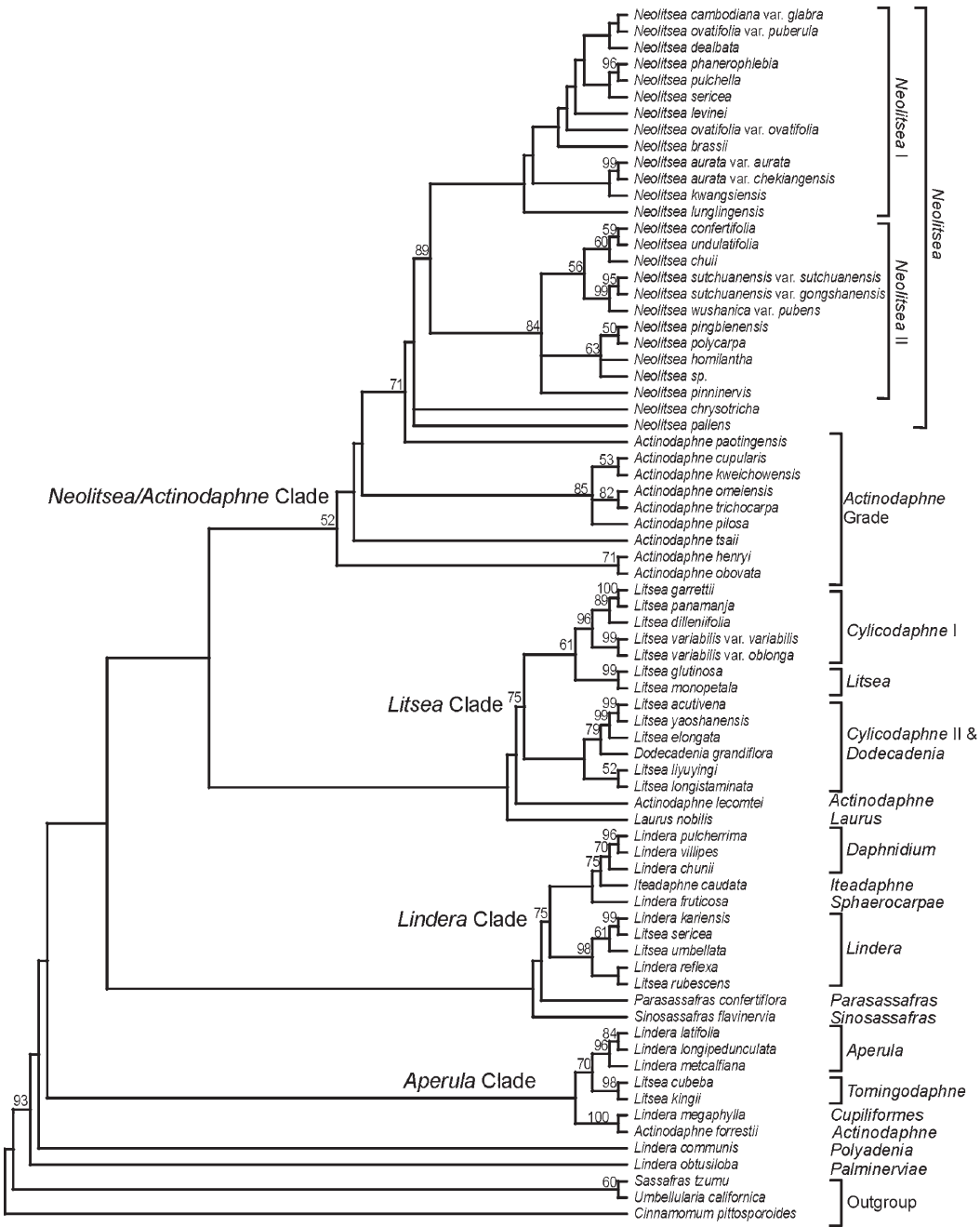


Figure 1. Strict consensus from 12 trees resulting for Laureae after successive weighting of the combined ITS and ETS sequence data. Bootstrap values greater than 50% are indicated on branches.

et al., 2003; Plovanich & Panero, 2004; Roalson & Friar, 2004).

RELATIONSHIPS AMONG MAJOR CLADES

Laureae are a well-supported monophyletic group based on our molecular data, and they share a

morphological synapomorphy of pseudo-umbellate, rarely racemose or paniculate involucre inflorescences. The results of our analyses are, in general, similar to the ITS + *matK* phylogeny of Li et al. (2004), and the four clades of Laureae and other subgroups recognized in that study more or less correspond to clades recovered here. Increased taxon

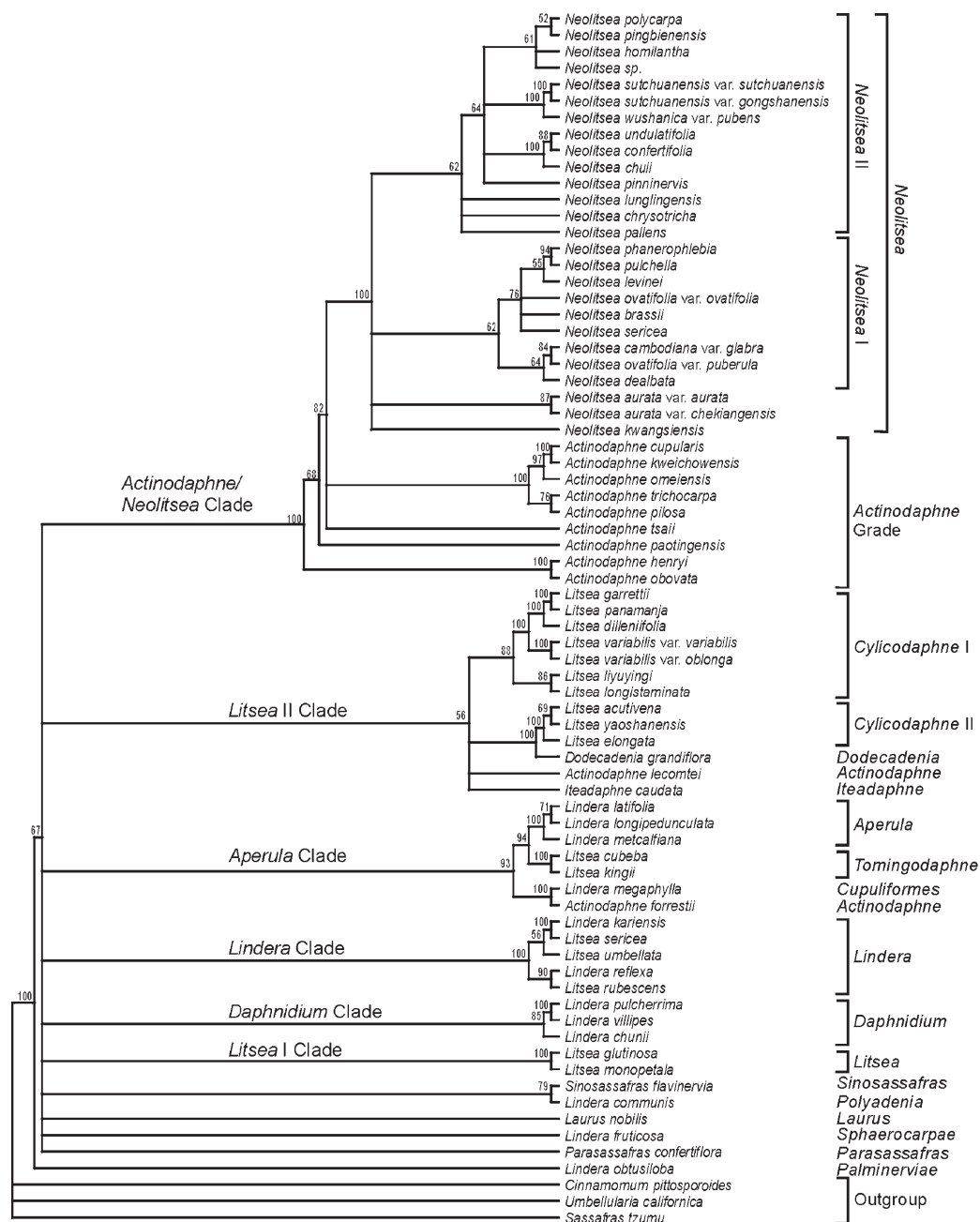
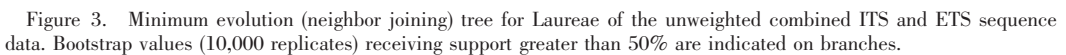


Figure 2. Phylogenetic majority rule tree for Laureae from Bayesian inference analysis of the unweighted combined ITS and ETS sequence data showing posterior probabilities on branches.

sampling as well as the additional sequence data from the ETS region and morphology have largely improved bootstrap, but mainly in more derived regions, with deep branch relationships still unresolved. This suggests that although main lineages are being identified and supported, the positions of many of these clades are unstable under different analysis

procedures (e.g., *Lindera obtusiloba* basal to the Laureae; the isolated, generally basal position of *L. communis*; and the clades *Lindera* and *Aperula* less derived than the *Litsea*, *Laurus*, and *Actinodaphne-Neolitsea* clades). The precise relationships between major clades are, thus, still uncertain, suggesting that the sequence regions are evolving too rapidly to provide



of Li et al. (2004) that inflorescence features and ontogeny are important for helping to understand evolution and improve classification within the Laureae.

The moderately supported association between *Sinosassafras flavinervia* and *Lindera communis* (sect. *Polyadenia*) in the MB and ME analyses also suggests

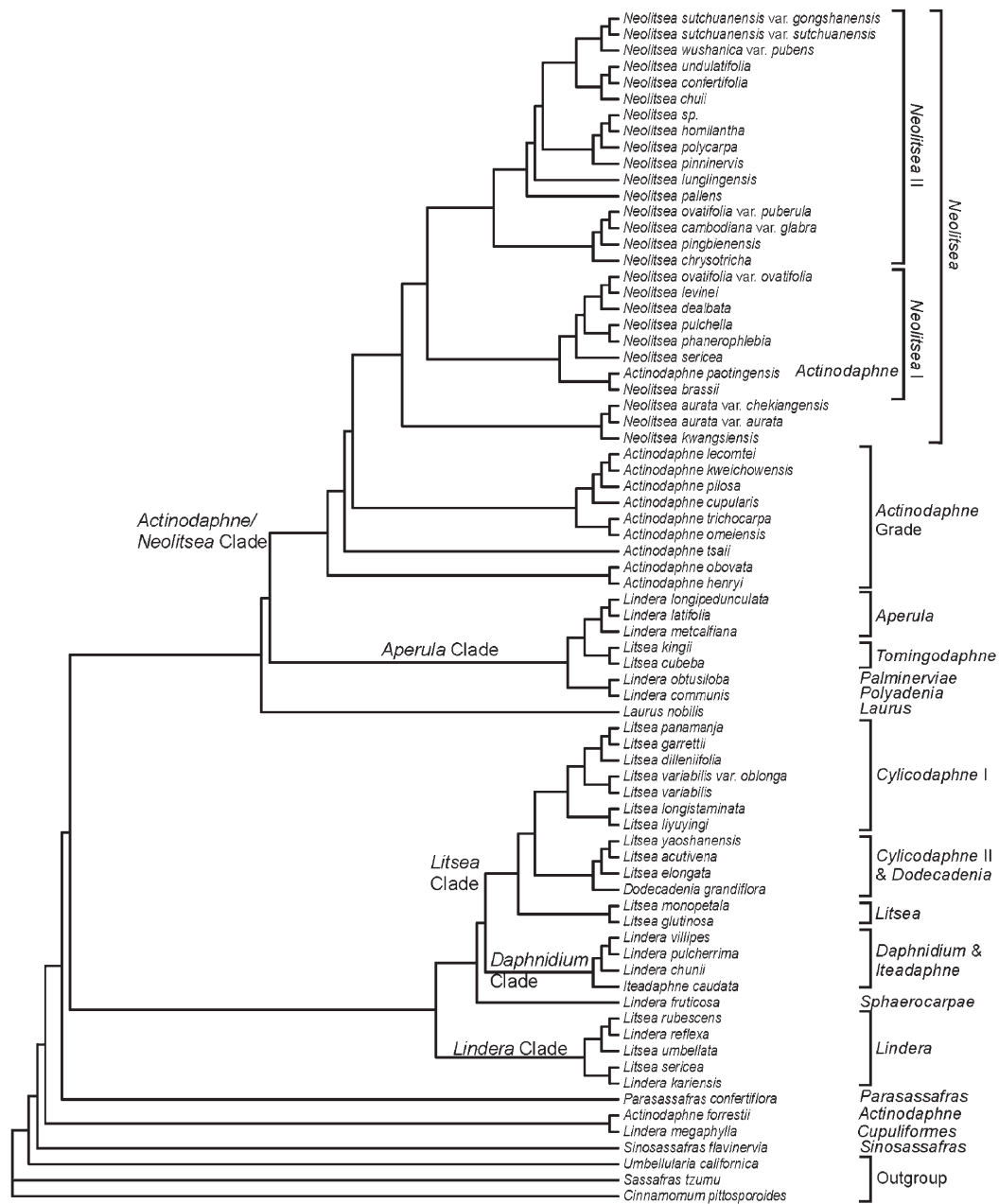


Figure 4. Maximum likelihood tree for Laureae of the unweighted combined ITS and ETS sequence data.

that the former may not be as closely related to *Parasassafras* as previously suggested (Rohwer, 1993), although these two genera were placed successively as basal to the *Lindera* clade in the SW analysis (albeit without bootstrap support) and formed a clade in the combined molecular/morphology analysis, with three morphological synapomorphies. *Sinosassafras flavinervia* has a single or two(to three) pseudo-umbels clustered in the leaf axils and always

bears several pseudo-umbels on axillary short shoots, whereas *L. communis* only has a single or rarely two(to three) pseudo-umbels clustered in leaf axils. Nevertheless, in both taxa, the terminal buds on the axillary short shoots are poorly developed or reduced.

The *Aperula* II clade contained two subgroups (none of which actually belong to *Lindera* sect. *Aperula* s. str.): (1) *Litsea cubeba* and *L. kingii*; and (2) *Lindera megaphylla* and *Actinodaphne forrestii*, and three of

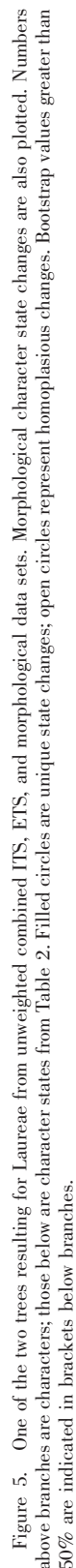


Figure 5. One of the two trees resulting for Laureae from unweighted combined ITS, ETS, and morphological data sets. Morphological character state changes are also plotted. Numbers above branches are characters; those below are character states from Table 2. Filled circles are unique state changes; open circles represent homoplasious changes. Bootstrap values greater than 50% are indicated in brackets below branches.

these (*Litsea cubeba*, *Lindera megaphylla*, and *A. forrestii*) also formed a clade in the study by Li et al. (2004). *Litsea cubeba* and *L. kingii* (sect. *Tomingodaphne*) are united by being deciduous, with naked terminal buds, and differ mainly in the absence of leaf pubescence in *L. kingii* (Long, 1984). Li et al. (2004) also noted that there was a close relationship between *Lindera megaphylla* (sect. *Cupuliformes*) and *A. forrestii*, and that there were micromorphological grounds for the splitting of *Actinodaphne* s.l. This is further supported by a preliminary molecular study of *Actinodaphne*, which found the genus to be polyphyletic within *Laureae* (Li et al., 2006). Although Li (1985) and Tsui (1987) had previously regarded *Lindera* sect. *Cupuliformes* as being possibly related to *Litsea* sect. *Cylicodaphne* because of their similar fruit cupules (despite belonging to different genera), this was not supported by our study, in which section *Cylicodaphne* itself was polyphyletic. Furthermore, *Lindera megaphylla* has large leaves aggregated near the top of branchlets (an unusual character in *Lindera*), making it morphologically more similar to some *Actinodaphne* species. *Lindera megaphylla* also bears a pair of pseudo-umbels on each side of an axillary short shoot with a vegetative terminal bud, whereas *A. forrestii* has several sessile pseudo-umbels clustered on an axillary short shoot that also produces a vegetative terminal bud. This suggests that *L. megaphylla* and *A. forrestii* may share a common inflorescence ontogeny and could help to explain why *A. forrestii* was separated from other *Actinodaphne* species in the analyses of Li et al. (2004, 2006) and the current study.

The largely basal and isolated position of *Lindera obtusiloba* (sect. *Palminerviae*) is unusual. This species is deciduous, and its pseudo-umbels are borne in an axillary, mixed bud (leaves and inflorescences together) covered by scales. This kind of mixed bud also occurs in *Sassafras*. According to this character and the later appearance of *L. obtusiloba* in the fossil record, Tsui (1987) suggested that *Lindera* evolved from *Sassafras* during the Miocene, possibly in response to the onset of cooler, more seasonal climates, although this assumes the correct assignment of fossils to extant genera, and generic definition based on vegetative anatomical and morphological features, which is an area of ongoing research.

Neolitsea consistently formed a terminal monophyletic lineage within the *Neolitsea*–*Actinodaphne* clade, agreeing with the *matK* analysis of Li et al. (2004). The present study (with many more taxa) also shows that *Neolitsea* as a clade is defined by dimerous flowers (apparently a reduction from a trimerous ancestral condition), clustered/verticillate leaf ar-

rangement, triplinerved venation, and decussate, persistent involucral bracts, and that splitting the genus just on leaf venation differences, as suggested as a possibility by Li et al. (2004), is not warranted.

Although two main subclades (*Neolitsea* I and II) were found in all the analyses, the precise composition of these varied, with some taxa moving between them or to a basal grade position depending on the analysis used, possibly due to what is still a relatively small sample size for such a large genus. Similarly, the absence of previously defined morphology-based sections within *Neolitsea* makes “representative” sampling more difficult. As they currently stand, neither *Neolitsea* I nor II has definitive synapomorphies, and they do not differ consistently in leaf venation or inflorescence features. Leaf arrangement seems to be important for two of the larger subclades, with the species in Ia being verticillate, and those of IIb being alternate. Similarly, IIa above *N. penninervis* shows ovoid-ellipsoid fruit, although this feature also occurs in some *Neolitsea* species from subclades I and IIb. Accordingly, the composition of the clades within *Neolitsea* and the nature of any supporting morphological characteristics are the focus of ongoing research.

Similarly, the inclusion of *Actinodaphne paotingensis* inside *Neolitsea* by the ME and ML analyses warrants study. This placement may be related to the instability of the number of floral parts, as there can be six to eight perianth lobes and nine to 15 fertile stamens in this species.

The combined molecular and morphological phylogeny indicates that *Neolitsea* is terminal above *Actinodaphne*, despite previous morphological studies suggesting that *Neolitsea* is closest to *Litsea* (Kostermans, 1957; Hyland, 1989; Rohwer, 1993). Van der Werff (2001) noticed that the inflorescence of *Actinodaphne* is unlike that of other *Laureae*, and several species (e.g., *A. pilosa* (Lour.) Merr., *A. henryi* Gamble, and *A. obovata* (Nees) Blume) have a paniculate or racemose inflorescence enclosed by imbricate, early deciduous involucral bracts. In our study, the *Neolitsea*–*Actinodaphne* clade was defined by the possession of verticillate leaves (later becoming terminally clustered in *Neolitsea*) and thyrsoid inflorescences lacking a terminal bud. This latter inflorescence feature is very different from the brachyblast-type short shoot seen in *Laurus* or the *Litsea*, *Lindera*, and *Aperula* clades, which display instead a pseudo-umbellate inflorescence with a vegetative terminal bud and decussate, tardily deciduous involucral bracts that enclose each pseudo-umbel. Rohwer (1993: fig. 87C–E) suggested that reduction from paniculate or racemose inflorescences led to the sessile pseudo-umbellate inflorescences

seen in *Neolitsea*, and this agrees with its position as a derived terminal above *Actinodaphne*. Furthermore, the clustered pseudo-umbels seen in *Actinodaphne* and *Neolitsea* differ from those of *Litsea* or *Lindera*, as the former are thyrsoïd, lack terminal buds, and are clustered in leaf axils, whereas the latter are arranged along leafless short shoots and bear a vegetative terminal bud.

Within *Neolitsea*, the separation of *N. ovatifolia* var. *ovatifolia* from variety *puberula* Yen C. Yang & P. H. Huang was unexpected. It may just reflect the widespread distribution and/or regional differentiation within this species, but certainly warrants further study to clarify species and varietal limits in this taxon.

Li (1985) and Tsui (1987) suggested an evolutionary series for the inflorescences in *Litsea* and *Lindera*, and our results concur with their hypotheses. The flowers occur in pseudo-umbels enclosed by decussate involucre bracts and are arranged along a leafless axillary short shoot with a terminal bud that can grow into a normal vegetative branch after flowering. A raceme bearing pseudo-umbels arising from normal growth of the peduncles and growth of the internodes in the short shoot was a synapomorphy for the *Laurus-Litsea-Lindera-Aperula* I clade, with reduction in several distal branches to create fasciculate clusters. In other basal groups, the peduncles and internodes of the short shoot are frequently reduced, so that the flowers are arranged in spikelike pseudo-umbels (the sessile inflorescence condition appears to be plesiomorphic in the Laureae). In *Iteadaphne* and *Dodecadenia*, although they follow this basic racemose pattern, the number of flowers per involucre or pseudo-umbel is reduced to one. In both cases, they seem to represent reduced members of otherwise pseudo-umbellate clades, suggesting that the pattern is convergent.

Previous morphology-based studies recognized considerable variability in both *Litsea* and *Lindera* and variously subdivided them into sections (Bentham, 1880; Hooker, 1890; Li et al., 1984; Tsui, 1987). Hooker (1890) and Li et al. (1984) recognized four sections within *Litsea* (*Litsea*, *Conodaphne*, *Cylicodaphne*, and *Tomingodaphne*) based on habit, leaves, floral characters, inflorescences, and fruit cupules. *Lindera* was similarly divided into eight sections (*Lindera*, *Sphaerocarpaceae*, *Palminerviae*, *Aperula*, *Cupuliformes*, *Daphnidium*, *Polyadenia*, and *Uniumbellatae*) by Tsui (1987). Although traditional generic delimitations based on two-celled versus four-celled anthers were not supported by our study, several monophyletic subclades are evident that do correspond, in part, to some of these previously recognized sections, and help to shed light on their phylogenetic relationships, as well as character

evolution, within the clades. For example, the *Litsea* clade was synapomorphic for four-locular anthers, albeit with *Iteadaphne* embedded within it as a reversal.

Within the *Litsea* clade, *L. glutinosa* (sect. *Litsea* and type species for the genus) and *L. monopetala* (sect. *Conodaphne*) were consistently resolved as sister taxa, with moderate support in the ME and Bayesian analyses. Although clustered on both molecular as well as combined data, the pair is characterized by a lack or incompleteness of the perianth lobes (absent in *L. glutinosa* and early deciduous in *L. monopetala*) (How, 1956).

The *Cylicodaphne* I and II subclades, corresponding to members of *Litsea* sect. *Cylicodaphne* s.l., were well supported as separate in most of the analyses, and, even in the Bayesian analysis where they formed a single clade, *Cylicodaphne* I was supported and terminal above an unsupported *Cylicodaphne* II grade. Although *L. variabilis* was placed in section *Conodaphne* by Li et al. (1984), it was treated as a member of section *Cylicodaphne* by Hooker (1890) and our results support its return to that section in *Cylicodaphne* I. Within *Cylicodaphne* I, although some species have eight perianth lobes, they all have pseudo-umbellate racemose inflorescences arranged along leafless short shoots, a feature that they share with the *Litsea* subclade (at least in part) and *Iteadaphne*, although there were no morphological synapomorphies to define *Cylicodaphne* I. In *Cylicodaphne* II, the species have one to several clustered pseudo-umbels on an axillary short shoot and ovoid-elliptical fruits, supporting the inclusion of *Dodecadenia*.

Lindera fruticosa (sect. *Sphaerocarpaceae*) was related to *Iteadaphne* and section *Daphnidium* in the SW analysis, despite its possession of an umbel with an elongate peduncle and its deciduous habit. Nevertheless, it was placed as a less-derived member of the *Lindera* clade in the ME and ML analyses, with which it shares a deciduous habit and possession of a single or few pseudo-umbels clustered on the short shoot. Its basal position may be related to the intermediate situation of leaf venation, as it can possess both triplinerved and pinninerved leaves. However, as none of our analyses showed bootstrap support for the position of this taxon, its associations must be regarded with caution for the present, pending further studies.

The *Lindera* clade consists of species from section *Lindera* and members of *Litsea* sections *Conodaphne* and *Tomingodaphne*. In deciduous habit, *Lindera kariensis* W. W. Sm. (sect. *Lindera*) is very like *Litsea sericea* (Wall. ex Nees) Hook. f. (sect. *Tomingodaphne*), except for the difference in number of anther

cells. Nevertheless, the placement of the evergreen *Litsea umbellata* (Lour.) Merr. (sect. *Conodaphne*) within the group is unusual, as all other members of this clade are deciduous. *Litsea umbellata* also appears unusual within the clade, as its cupule bears persistent tepals. Nevertheless, it is possible that deciduous species that bear cupules without persistent tepals may have evolved from evergreen ancestors with persistent cupule tepals, and in section *Conodaphne* s. str., there are species with and without persistent cupule tepals. Similarly, section *Tomingodaphne* contains two vegetative terminal shoot bud forms in the axillary short shoots: naked versus scaly (Hooker, 1890).

Rohwer (1993) and van der Werff and Richter (1996) recognized a large portion of what we now regard as the Laureae, including *Actinodaphne*, *Dodecadenia*, *Iteadaphne*, *Lindera*, *Litsea*, *Neolitsea*, *Parasassafras*, and *Sinosassafras*. Although *Sassafras* and *Umbellularia* were also included, they should be removed based on the results of Chanderbali et al. (2001), Li et al. (2004), and the present study. This is further supported by the possession of racemose inflorescences in *Sassafras* and the presence of bisexual flowers with extrorse anther cells in the third whorl in *Umbellularia*.

In our study, we sampled species representing all genera and most sections in the core Laureae, and it is clear that a major disparity exists between our molecular phylogenetic results and more traditional morphology-based taxonomic concepts of generic and infrageneric classification in the tribe. For example, both current and earlier analyses (Li & Christophel, 2000; Li et al., 2004) confirm that using two- versus four-celled anthers to separate *Litsea* s.l. from *Lindera* s.l. results in polyphyletic or paraphyletic genera (Rohwer et al., 1991; Rohwer, 1993; van der Werff & Richter, 1996; Li & Christophel, 2000; Li et al., 2004). This character should, therefore, be used with caution in Laureae classifications, although it does seem to be useful for defining some of the higher-level clades in the group (e.g., the *Litsea* clade s. str.).

The significance of other traditional characters often used at the generic level, such as dimerous or trimerous flowers, also needs re-evaluation. For example, *Laurus* and *Neolitsea* have been related previously based on their dimerous flowers, but not in our analyses. Similarly, the nature and arrangement of basal inflorescence involucre bracts (i.e., early deciduous vs. tardily deciduous; imbricate vs. decussate) are traditionally important characters for delimiting genera. However, whereas *Actinodaphne*, *Parasassafras*, and *Sinosassafras* can be distinguished from other Laureae by the possession of early deciduous,

imbricate bracts, these character states are convergently homoplasious in our analyses. This suggests that although potentially diagnostic for relating the latter two genera, the character is of limited phylogenetic value, especially given the position of *Actinodaphne* as a basal grade below *Neolitsea*.

Nevertheless, the clades identified in this study do provide opportunities to examine evolution of specific morphological characters in Laureae, a task already initiated by Li and Christophel (2000), and of particular interest will be studies of inflorescence development. The most common inflorescence form in Laureae is pseudo-umbels clustered in the leaf axils, but our analyses show that there are apparently at least two different ways to produce this structure. One, suggested by previous studies (Li, 1985; Tsui, 1987) and seen in *Laurus* and the *Litsea*, *Lindera*, and *Aperula* I and II clades, results from shortening of the internodes in the short shoot with a vegetative terminal bud. This vegetative terminal bud may be normal and scaly or naked, well developed and large, poorly developed, or even reduced. Sometimes the terminal vegetative shoot along with one or two lateral fertile short shoots with pseudo-umbels merge to form a single axillary mixed bud, and the peduncle of pseudo-umbels and the internodes of the short shoot may be developed or reduced. Given the positions of these taxa toward the base of the trees, this feature appears to be plesiomorphic in Laureae.

The second, derived condition, seen in the *Neolitsea*–*Actinodaphne* clade, results instead from shortening of a thyrsoid inflorescence axis and lacks a vegetative terminal bud. However, given the lack of deep-branch support for the major lineages in this study, definitive conclusions about the phylogeny of inflorescence ontogeny must await further studies.

In conclusion, our study indicates the need for caution in the use of morphological similarity for assessing affinities between taxa in the Laureae. Traditional characteristics of habit, leaf venation, inflorescence, and floral structure appear in many cases to have been the result of convergent and/or parallel evolution and, therefore, may not be indicative of evolutionary affinity or useful for taxon delimitation at higher levels. Added to this is the possibility that some features such as pseudo-umbels may not be homologous. Nevertheless, the study identifies areas in which future research may help to clarify or correct problems of homology and ontogenetic convergence. It provides a hypothesis for possible phylogenetic relationships in Laureae, albeit based on a single, rapidly evolving genome, and gives direction for future studies using multiple independent and possibly more conservative markers to assess the phylogenetic hypotheses that our results indicate.

This will provide the foundation for a revised phylogeny-based classification of the Laureae in which reliable synapomorphies are backed by data from a range of sources.

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