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Phylogeny and biogeography of *Sassafras* (Lauraceae) disjunct between eastern Asia and eastern North America

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Abstract. *Sassafras* (Lauraceae) consists of three species disjunct between eastern Asia (*S. tzumu* and *S. randaiense*) and eastern North America (*S. albidum*). Phylogenetic analysis based on sequences of nuclear ribosomal ITS and three chloroplast non-coding regions (*rpl*16, *trn*L-F, and *psb*A-*trn*H) showed that *Sassafras* is monophyletic and that the eastern North American *S. albidum* is sister to the clade of its two eastern Asian counterparts. Their intercontinental divergence was estimated to be 13.80 ± 2.29–16.69 ± 2.52 million years ago (mya) using the penalized likelihood method with the ITS and three chloroplast markers. Biogeographic analyses combined with fossil evidence suggest that *Sassafras* has a relict distribution in the Northern Hemisphere without a Gondwanan link. The divergence time of the two eastern Asian species (the continental Chinese *Sassafras tzumu* and *S. randaiense* endemic to Taiwan) is estimated to be 0.61 ± 0.75–2.23 ± 0.76 mya. *Sassafras randaiense* from Taiwan was most likely derived from an ancestor from continental China.

Key words: Disjunction, eastern Asia, eastern North America, Lauraceae, *Sassafras*, Taiwan.

Introduction

Lauraceae are one of the basal angiosperm families with fossils dating back to the mid-Cretaceous (Drinnan et al. 1990, Eklund and Kvaček 1998, Qiu et al. 1999). About 50 genera are currently recognized with 2500–3000 species (Wood 1958, Rohwer 1993, van der Werff and Richter 1996). They are mainly distributed throughout the tropical and subtropical regions. Biogeographic studies suggested that the basal lineages of Lauraceae were sorted into two main geographic groups of Gondwanan or Laurasian origins by the late Cretaceous (Chanderbali et al. 2001). Relatively few modern species occur in the temperate zones, although Lauraceae may have been abundant in the northern landmass of Laurasia during the warmer periods in the early Tertiary (Eklund 2000). Further analyses of disjunct Laurasian taxa are necessary to provide insights into their diversification in the Northern Hemisphere.

The disjunct distribution of plants between eastern Asia and eastern North America is a well-known and classical biogeographic pattern in the Northern Hemisphere. Studies on this pattern have so far mostly focused on taxa from the temperate regions (see reviews by Wen 1999, 2001), such as *Liriodendron* L. (Parks and Wendel 1990), *Aralia* L. sect. *Dimorphanthus* (Miq.) Miq. (Wen 2000), *Liquidambar* L. (Hoey and Parks 1991, Shi et al. 1998, Ickert-Bond and Wen 2006), *Phryma* L. (Lee et al. 1996, Xiang et al. 2000, Nie et al. 2006a), and *Symplocarpus* R. A. Salisbury ex Nuttall (Wen et al. 1996, Nie et al. 2006b). *Sassafras* is one of the few representatives of Lauraceae with a disjunct distribution primarily in the north temperate zone extending into the subtropical regions in eastern Asia and eastern North America (Fig. 1). The genus provides a good opportunity to investigate biogeographic diversification of Lauraceae in the temperate regions of the Northern Hemisphere.

Sassafras is a well circumscribed genus including only three species (Li et al. 1984). *Sassafras tzumu* (Hemsley) Hemsley and *S. randaiense* (Hayata) Rehder are from eastern Asia with the former common in central and southern China at altitudes from 150–1900 m and the latter scattered in evergreen broadleaved forests of the Central Ranges in Taiwan at altitudes of 900–2400 m (Li 1963, Li et al. 1984). The North American species [*S. albidum* (Nutt.) Nees] occurs throughout eastern North America and is considered to be the northernmost representative of Lauraceae (Little 1998). The three species differ primarily in their floral structure. *Sassafras randaiense* is different from *S. tzumu* in the former's 2-locular (vs. 4-locular) anthers. The North American *S. albidum* is highly distinct from the eastern Asian congeners. *Sassafras albidum* lacks a pistillode and a fourth staminal whorl consisting of three staminodes in the male flowers. Its female flowers have six staminodes (vs. nine relatively well-developed but perhaps

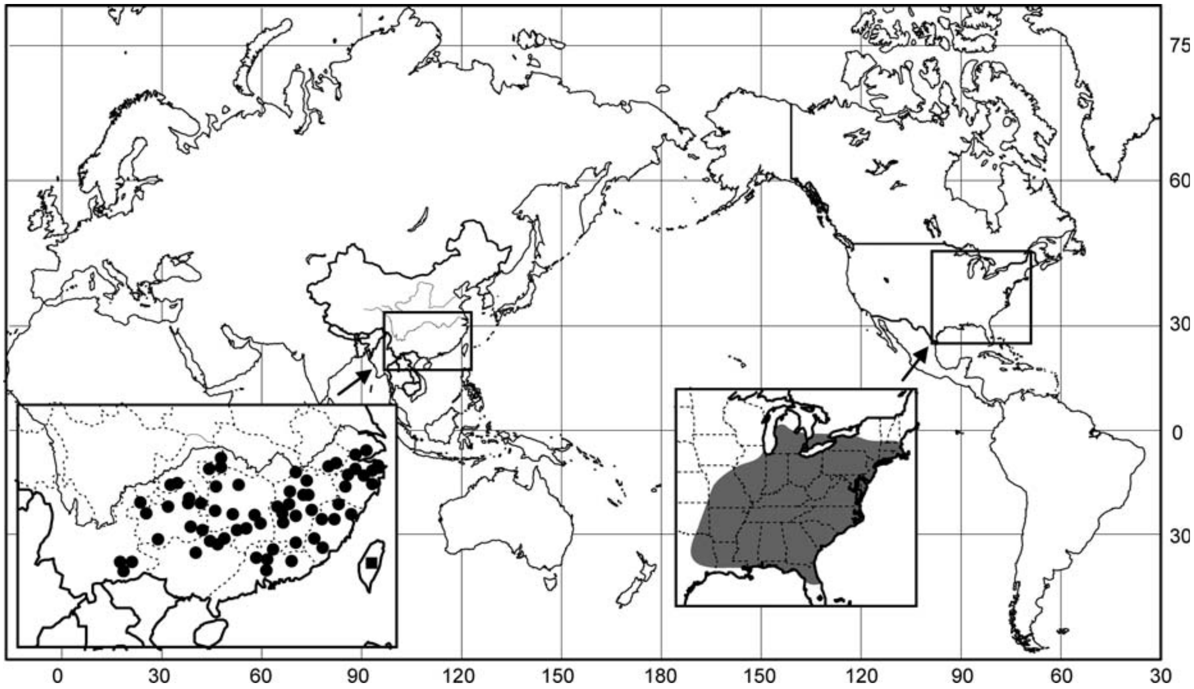

Fig. 1. Distribution of *Sassafras* showing disjunction between eastern Asia and eastern North America. The shaded area, the black circles, and the solid square represent *S. albidum*, *S. tzumu* and *S. randaiense*, respectively

non-functional stamens and three staminodes in the female flowers in the Asian species). The North American species is dioecious, but the reproductive biology of the Asian species has not been well documented and needs to be carefully examined.

Sassafras has usually been included in the tribe Laureae based on its dioecious breeding system, botryoid inflorescences with involucral bracts, and introrse anther cells of the third whorl (Li et al 1984, Rohwer 1993). In addition to these morphological characters, van der Werff and Richter (1996) also regarded *Sassafras* to be closely related to the *Litsea* complex on the basis of their unique accentuated growth ring in secondary xylem and phloem, especially their absence of marginal parenchyma and their presence of phloem fibers. The monophyly of Laureae including *Sassafras* is, however, not supported in the analysis using nuclear ITS and chloroplast *trn*L-F, *rpl*16, *psb*A-*trn*H, and *trn*K sequences (Chanderbali et al. 2001, Rohwer and Rudolph 2005).

The taxonomic and nomenclatural history of *Sassafras* is complex (Blake 1918, Fernald 1936). The genus was established by Presl (1825) based on the North American *S. albidum*, which was originally described by Linnaeus (1753) as *Laurus sassafras* L. the name *Sassafras officinale* Nees and Eberm. was previously applied to the North American species (e.g. in Rehder 1920, Keng 1953). Because the first valid specific epithet for the species was from the name of *Laurus albida* Nuttall published in 1818, *Sassafras albidum* (Nuttall) Nees has been accepted as the correct name for the North American species (Fernald 1936). Rehder (1920) recognized *Sassafras* s. str. as a monotypic genus including only *S. albidum*. Hemsley (1891) initially described *S. tzumu* (Hemsley) Hemsley from central China as *Lindera tzumu* Hemsley and *Litsea laxiflora* Hemsley based on two different, but incomplete specimens (Hemsley 1891). In 1907, Hemsley used *Sassafras tzumu* for the central Chinese species and placed *Litsea laxiflora* as its synonym. *Sassafras randaiense* (Hayata) Rehder from Taiwan was transferred from *Lindera* by Rehder in 1920. The differences among the three *Sassafras* species also led some botanists to assign them to separate genera. *Sassafras tzumu* was considered as belonging to *Pseudosassafras*, to distinguish it from the North American species (Lecomte 1912). Because of the 4- (vs. 2-) sporangiate separation anthers in *S. randaiense*, this species from Taiwan was proposed to be the sole member of the genus *Yushunia* (Kamikoti 1933).

Previous phylogenetic analyses of Lauraceae (e.g. Chanderbali et al. 2001) did not sample *Sassafras randaiense* from Taiwan. The primary difference between *S. randaiense* and *S. tzumu* is the locular number in their anthers, a character that used to be considered important for the Lauraceae taxonomy (Rehder 1920) and has been discussed repeatedly by many authors (e.g. Rohwer et al. 1991, van der Werff 2001). It is thus necessary to test the monophyly of *Sassafras* with the unusual species from Taiwan sampled. The divergence time between the eastern Asian *S. tzumu* and the eastern North American *S. albidum* was estimated to be about 12 mya based on a molecular clock of ITS sequences calibrated with a geologic event of the West Gondwana breakup (90 mya, Chanderbali et al. 2001). Objectives of this paper are to (1) test the monophyly of *Sassafras* with all three species sampled, (2) construct the phylogenetic relationships of the genus, and (3) estimate the divergence times of *Sassafras* using the penalized likelihood method with calibrations from fossil and geological evidence.

Materials and methods

Phylogenetic analysis. Total DNA was obtained from ca. 15 mg silica-gel-dried leaf material using Dneasy (QIAGEN) extraction kits. The PCR amplification of chloroplast sequences was conducted following that of Chanderbali et al. (2001) for *rpl*16, *psb*A-*trn*H, and *trn*L-F regions. Because of the difficulties in amplifying the ITS regions of *Sassafras* with the primers (LAUR 1 and ITS B) as used in Chanderbali et al. (2001) and Li et al. (2004), we designed a new reverse primer (LAUR 2: 5′- CGCCTGACCTGGGGT -3′).

Dimethylsulfoxide (DMSO, 10%) was added to the PCR reactions of ITS to prevent the formation of secondary structures. Sequences were then aligned with ClustalX version 1.83 (PC version) (Thompson et al. 1997), followed by manual adjustment in BioEdit (Hall 1999).

Sassafras belongs to the Perseeae–Laureae clade (core Lauraceae), one of the largest clades in Lauraceae. It is sister to the *Chlorocardium* – *Mezilaurus* clade based on sequences of ITS, *rpl*16, *trn*L-F, *psb*A-*trn*H, *mat*K, and *trn*K (Rohwer 2000, Chanderbali et al. 2001, Rohwer and Rudolph 2005). Nine species representing eight genera from these two clades (see Appendix and Fig. 2) were used as outgroup. Phylogenetic analyses were conducted using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (Rannala and Yang 1996). The heuristic searches for MP analysis used 100 random taxon addition replicates, tree bisection - reconnection (TBR) branch swapping, and collapse of zero-length branches, and character state changes were equally weighted in the analysis. Gaps were treated as missing data. Bootstrap support (BS) for each clade was estimated based on 1000 replicates with heuristic search options using parsimony. The appropriate model of DNA substitution for the maximum likelihood analysis was determined using Modeltest version 3.6 (Posada and Buckley 2004). Once the best-fit model was determined, maximum likelihood searches were performed for the data set (Felsenstein 1981).

Bayesian inference was performed using MrBayes version 3.0 (Huelsenbeck and Ronquist 2001). The MCMC algorithm was run for 2,000,000 generations with 4 incrementally heated chains, starting from random trees and sampling one out of every 100 generations. The first 2000 to 5000 trees were discarded as burn-in, depending on when chains appeared to have become stationary, and the remaining trees were used to construct the Bayesian consensus tree. Internodes with posterior probabilities (PP) ≥ 95% were considered statistically significant.

The incongruence length difference (ILD) test of ITS vs. combined chloroplast sequences (*psb*A-*trn*H, *rpl*16, and *trn*L-F) was conducted in PAUP* (Farris et al. 1994) to assess potential conflicts from different DNA fragments.

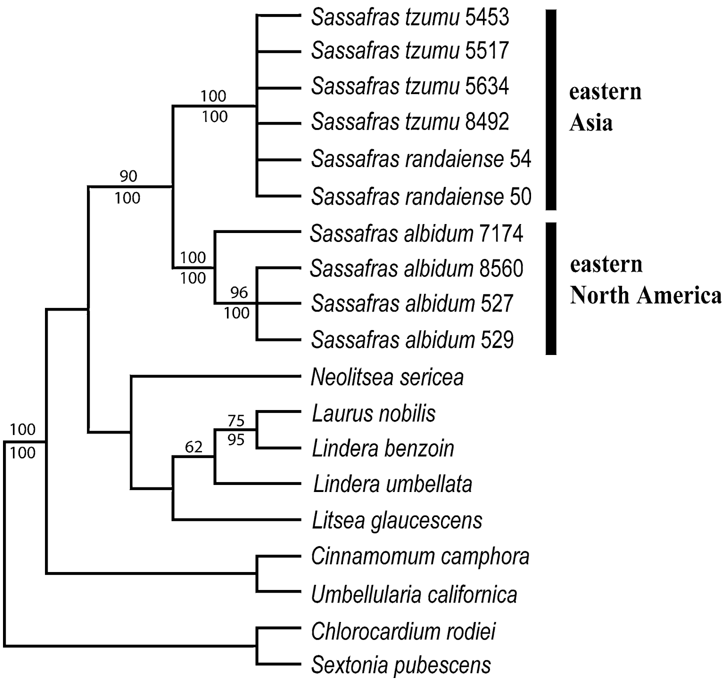

Fig. 2. Strict consensus tree of *Sassafras* (Lauraceae) based on the combined ITS, *trn*L-F, *rpl*16, and *psb*A-*trn*H data (tree length = 456 steps, CI = 0.80, and RI = 0.70). The bootstrap values in 1000 replicates are shown above the lines and the Bayesian Markov chain Monte Carlo (MCMC) posterior probabilities higher than 95% are indicated under the lines

Biogeographic analysis. Using ITS data, Chanderbali et al. (2001) estimated the divergence times of *Sassafras* with its close relatives in the Perseeae – Laureae group (clade C in Fig. 3). We herein employed multiple markers (ITS, *psb*A-*trn*H, *rpl*16, and *trn*L-F) for this purpose, and sampled 48 taxa in the whole Lauraceae and close taxa with sequences of 45 species obtained from GenBank (see Appendix). A likelihood ratio test (Felsenstein 1988) was done to test whether the data evolved in a clock-like fashion. This test resulted in $P < 0.05$, suggesting that rate constancy

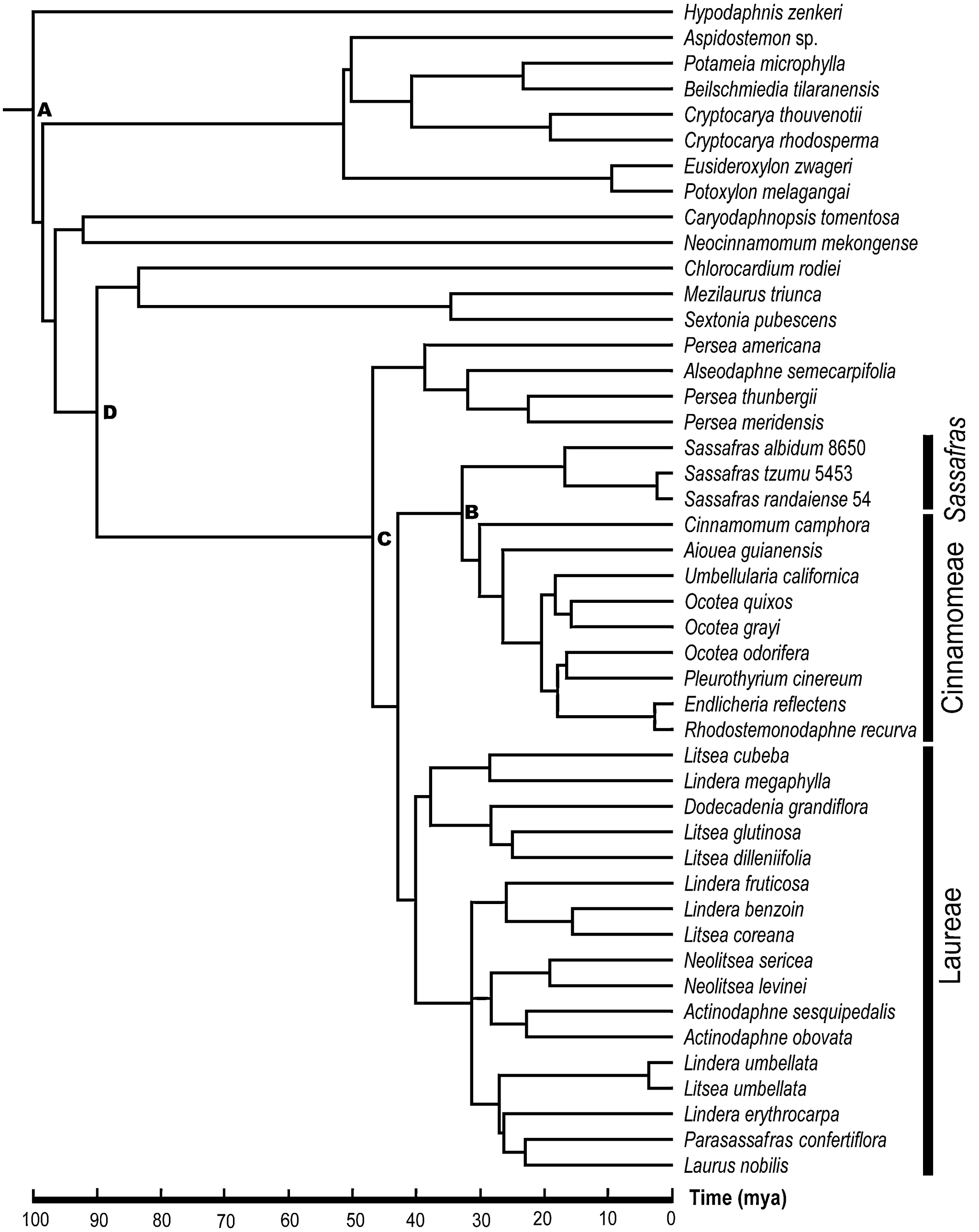

Fig. 3. Chronogram of *Sassafras* and taxa from Lauraceae based on the maximum likelihood tree of combined ITS, *trn*L-F, *rpl*16, and *psb*A-*trn*H matrix. Divergence times are shown as estimated using the penalized likelihood method with the following constraints enforced: node A as 100 mya and node D as 90 mya

in this data set was not supported. The penalized likelihood (PL) method (Sanderson 2002) was then used to estimate divergence times.

The PL method is a semiparametric approach using rate smoothing to allow for robust estimation of node ages in the presence of rate variation between lineages (Sanderson 2002). Ages of nodes in the tree were estimated using penalized likelihood rate smoothing value under a truncated newton algorithm with the program r8s version 1.71 (Sanderson 2003). A cross-validation analysis was performed to obtain the most likely smoothing parameter. Confidence intervals around the age estimates were calculated using nonparametric bootstrapping as used in Baldwin and Sanderson (1998) and Nie et al. (2005).

Fossil records of Lauraceae included flowers, fruits, inflorescences, leaves, and wood ranging from the early Cretaceous to the late Tertiary (Herendeen 1991, Eklund 2000, Frumin et al. 2004). However, there are few reliable fossils which can be unambiguously assigned to the extant group of Lauraceae (Eklund and Kvaček 1998, Eklund 2000). The extinct genus *Mauldinia* is the earliest unquestionable lauraceous fossil. It is characterized by its unique and distinct compound inflorescences with flattened bilobed lateral units bearing sessile 3-merous flowers (Drinnan et al. 1990, Frumin et al. 2004). It was first described from the early Cenomanian of the Cretaceous (ca. 100 mya) in North America (Drinnan et al. 1990). We thus fixed the age of the Lauraceae root as 100 mya (node A in Fig. 3), as required by the PL method. Paleogeographic evidence of West Gondwana breakup showed that direct land connections between Africa and South America were lost by the Upper Mid-Cretaceous, ca. 90 mya (Sclater et al. 1977, Parrish 1993). Therefore, the clade D in Fig. 3 including South American *Chlorocardium – Mezilaurus* clade and Old World core Lauraceae (clade C) are calibrated with this biogeological scenario of 90 mya, as used in Chanderbali et al. (2001).

Sassafrasoxylon Brezinová & Süss. was a fossil wood from the late Cretaceous (the Santonian-Maastrichtian) of the northern Antarctic Peninsular region (Poole et al. 2000). It resembles the extant *Sassafras* taxa in being distinctly ring-porous. It also has vessel elements with simple perforation plates and occasional scalariform plates with relatively few bars in the narrowest latewood vessels, and commonly multiseriate rays (Poole et al. 2000).

Suspicions also have been provided by the authors since morphological comparisons are based on isolated pieces of wood fossils (Poole et al. 2000). Therefore, our estimates calibrated with geological event can be used to test the relationship of this *Sassafras*-like wood fossil to *Sassafras*.

Table 1. Voucher information and GenBank accession numbers for *Sassafras* used in this study

Taxon	Voucher	Locality	ITS	*rpl*16	*psb*A-*trn*H	*trn*L-F
Sassafras tzumu (Hemsley) Hemsley	*J. Wen 5453* (F)	China: Hubei	EF491208	EF491228	EF491217	EF491239
	J. Wen 5517 (F)	China: Jiangxi	EF491209	EF491229	EF491218	EF491240
	J. Wen 5634 (F)	China: Yunnan	EF491210	EF491230	EF491219	EF491241
	J. Wen 8492 (F)	China: Zhejiang	–	EF491231	EF491220	EF491242
S. randaiense (Hayata) Rehder	*J. Chen 50* (F)	China: Taiwan	EF491211	EF491232	EF491221	EF491243
	J. Chen 54 (F)	China: Taiwan	EF491212	EF491233	EF491222	EF491244
S. albidum (Nutt.) Nees	*J. Wen* 7174 (F)	USA: Illinois	EF491213	EF491234	EF491223	EF491245
	J. Wen 8560 (US)	USA: Virginia	EF491214	EF491235	EF491224	–
	Nie & Meng 527 (US)	USA: Virginia	–	EF491236	EF491225	EF491246
	Nie & Meng 529 (US)	USA: Virginia	EF491215	EF491237	EF491226	EF491247
Lindera benzoin (L.) Blume	*J. Wen 8653* (US)	USA: Virginia	EF491216	EF491238	EF491227	EF491248

Results

The ITS and the chloroplast (*rpl*16, *trn*L-F, and *pbs*A-*trn*H) data were shown to be congruent ($P > 0.05$ in the ILD test). The combined ITS and cpDNA matrix comprised 2976 aligned positions, only 129 of which were parsimony-informative. When gaps were treated as missing data, two MPTs were generated with a length of 456 steps, a consistency index (CI) of 0.80 (CI excluding uninformative characters = 0.63), and a retention index (RI) of 0.70. The strict consensus tree with BS and Bayesian posterior probability (PP) for each clade is shown in Fig. 2. The phylogenetic analysis supported the monophyly of *Sassafras* (BS = 90%, PP = 100%). The two Asian species (*Sassafras tzumu* and *S. randaiense*) formed a clade with robust support (BS = 100%, PP = 100%), and then they were sister to the eastern North American *S. albidum* (Fig. 2).

The pairwise distances of ITS among populations of *Sassafras tzumu* is between 0 and 0.339%. No sequence variation was detected in populations within *S. randaiense* and within *S. albidum*. The lack of sequence variation in the widespread *S. albidum* may be due to our limited infraspecific sampling at present. The highest divergence (1.908–2.454%) was between populations of the Asian *S. tzumu* and the North American *S. albidum*. The sequence divergence between the Taiwan and the North American species was 1.735–1.957%. The two Asian species (*S. tzumu* and *S. randaiense*) had a relatively low sequence divergence (0.160–0.643%). The divergence of the chloroplast *trn*L-F and *psb*A-*trn*H among the three species was similar to that of the ITS. The *rpl*16 sequences showed no variation among populations of *S. tzumu* and *S. randaiense*, whereas the populations of *S. albidum* had divergence of 0.11–0.442%. The combined nuclear ITS and the chloroplast data diverged between 0.529–1.435% between the intercontinental species of *S. tzumu* and *S. albidum*, and 0.055–0.175% between the two Old World species of *S. tzumu* and *S. randaiense*.

We estimated the ranges of divergence times of *Sassafras* populations using the population pair with the highest pairwise distance (*Sassafras tzumu Wen 5453* and *S. albidum Wen 8560*) as well as the pair with the lowest pairwise distance (*S. tzumu Wen 8492* and *S. albidum Wen 7174*). The PL analysis estimated the divergence times as 13.80 ± 2.29 -16.69 ± 2.52 mya for *Sassafras tzumu* – *S. albidum*, and 0.61 ± 0.75–2.23 ± 0.76 mya for *S. tzumu* – *S. randaiense*, when node D (Fig. 3) was constrained to be 90 mya. The stem age of *Sassafras* (clade B in Fig. 3) was estimated as 33.02 ± 2.00 mya. The radiation time of the core Lauraceae (clade C in Fig. 3) was also obtained to be 43.54 ± 1.96 mya in our analyses.

Discussion

Phylogenetic relationships. The monophyly of *Sassafras* is well supported in our analysis (BS = 90%, PP = 100%, Fig. 2). In comparison with other close relatives of Laureae, the synapomorphies of *Sassafras* include their racemose inflorescences, slender pedicellate flowers and enlarged, involucre-like bud-scales. *Sassafras* is also unusual in Lauraceae in having the combination of the following wood characters: distinctly ring-porous wood, simple perforation plates but occasional scalariform in latewood vessels and commonly 4- or 5- seriate rays (van der Werff and Richter 1996, Poole et al. 2000).

Samples of *Sassafras tzumu* and *S. randaiense* from eastern Asia form a clade (BS = 100%, PP = 100%) sister to the North American *S. albidum* (BS = 90%, PP = 100%, Fig. 2). The two Asian species are considered to be less specialized than the North American species. The Asian species have nine stamens, three staminodes, and a pistillode in the staminate flowers, and 12 staminodes in the pistillate flowers similar in appearance to the stamens and staminodes of the staminate flowers (Wood 1958). *Sassafras randaiense* is close to *S. tzumu*, differing primarily in its 2-locular anthers whereas anthers of the latter have 4 locules. This character has led to the artificial assignment of many morphologically similar taxa to different genera, such as *Litsea* Lam. (4-locular) and *Lindera* Thunb. (2-locular), and *Parasassafras* D. G. Long (4-locular) and *Sinosassafras* H. W. Li (2-locular) (Long 1984, Li 1985). Nuclear ITS and chloroplast *matK* data do not support the monophyly of *Litsea* nor *Lindera*. Instead species from these two genera are grouped into different clades, and several other genera (e.g. *Neolitsea* (Benth.) Merr., *Actinodaphne* Nees, and *Iteadaphne* Blume) are nested within them (see Fig. 2 in Li et al. 2004). The sister relationship between *Parasassafras* and *Sinosassafras* was supported by the *mat*K data, but not by the combined ITS and *mat*K data in Li et al. (2004). The close relationship between *Sassafras tzumu* and *S. randaiense* is well supported by the combined chloroplast and the nuclear DNA sequences in our study.

Below we also provide a taxonomic key to the species of *Sassafras* to facilitate the identification of the three species:

1. Staminode and pistillode absent in male flowers; staminodes 6 in female flowers; anthers all 4-locular.................. ***S. albidum***
1. Staminode and pistillode present in male flowers; staminodes 6 + 3 + 3 in female flowers; anthers 4- or 2-locular
   2. Leaves ovate or obovate, entire or 2–3-lobed; anthers of various whorls all 4-locular .... ***S. tzumu***
   2. Leaves rhombic-ovate, those on infertile branchlets entire or 2–3-lobed but those on fertile branchlets entire; anthers 2-locular............................... ***S. randaiense***

Biogeography. Based on sequences of ITS and three chloroplast markers (*rpl*16, *trn*L-F, and *pbs*A-*trn*H), the divergence time of *Sassafras* between eastern Asia and eastern North America was estimated to be 13.80 ± 2.29 – 16.69 ± 2.52 mya. Our estimate employed two constraint points, one oldest fossil record of Lauraceae and the other of the geological event of West Gondwana breakup. The earliest inflorescence and flower fossil of Lauraceae is the extinct genus *Mauldinia* from the late Cretaceous. This fossil was used to fix the age of the root of Lauraceae (node A in Fig. 3). Chanderbali et al. (2001) reported the divergence time between *S. albidum* and *S. tzumu* to be about 13 ± 4 mya based on the HKY-Γ distance of ITS sequences using a molecular clock with similarly calibrate point of the West Gondwana breakup (90 mya). Our estimate using multiple molecular markers and the PL method is thus similar to theirs.

As hypothesized by Wolfe (1972, 1975), the "boreotropical flora" reached regions of the high paleolatitudes in the Northern Hemisphere during the early Tertiary. Lauraceae may be abundant in Laurasia in the warm periods during the Paleocene and the Eocene along with the development of "boreotropical flora" because of its abundant fossil records (Eklund 2000). Subsequent climatic changes from the Miocene to the late Tertiary largely eliminated the boreotropical floristic elements from the high latitudes in the Northern Hemisphere, leading to disjunction of ancestral lineages of modern tropical plants between North America and Eurasia. *Sassafras* may be one of the plant groups which survived in a few "refugia" of the Northern Hemisphere, such as eastern Asia and eastern North America (Wen 1999, Eklund 2000, Milne and Abbott 2002).

Morphological and molecular evidence undoubtedly support the inclusion of *Sassafras* in the core Lauraceae group, but its phylogenetic position is still uncertain presumedly due to the early rapid diversification or radiation in this clade (Li and Christophel 2000, Rohwer 2000, Chanderbali et al. 2001, Rohwer and Rudolph 2005). In Chanderbali et al. (2001), its sister relationship to the Laureae was enforced with topological constraint of the monophyly of the Laureae. Without the topological constraint, *Sassafras* was sister to the Cinamomeae including the *Ocotea* complex and the *Cinnamomum* group (see Fig. 3 in Chanderbali et al. 2001), despite that they are often considered to be morphologically similar to Laureae. Similar results have been found in Rohwer and

Rudolph (2005) based on complete *trn*K sequence data. The radiation time of the core Lauraceae was estimated as 43.54 ± 1.96 mya in our analysis, similar to what Chanderbali et al. (2001) estimated the age of the radiation within this clade as 44 ± 7 mya. Our results also strongly suggested that this genus originated in one of the more recent radiation within the family in the early Eocene (Chanderbali et al. 2001, Rohwer and Rudolph 2005).

The stem age of *Sassafras* (clade B in Fig. 3) was estimated to be 33.02 ± 2.00 mya. This result is much younger than the age based on the wood fossil *Sassfrasoxylon gottwaldii* (83 mya), which was recently found from the northern Antarctic Peninsular region in the late Cretaceous (Poole et al. 2000). Anatomically, this fossil shows nearly no difference from wood of the extant *Sassafras* in being distinct ring-porous. A clear link between *Sassfrasoxylon gottwaldii* and extant *Sassafras* was hypothesized by the authors. They also proposed that the ancestor of *Sassafras* first appeared in the Gondwanaland and later radiated into the Northern Hemisphere. However, cautions have been raised against this interpretation because it cannot be assumed that these *Sassafras*-like fossils belong to the extant genus as they were compared from isolated pieces of wood and may represent a stem taxon or another lauraceous genus that developed growth rings in a seasonal and polar environment. In our study, *Sassfrasoxylon gottwaldii* is interpreted as a case of striking convergence to *Sassafras* in a seasonal climate rather than evidence for a Gondwanan origin of the genus, as suggested in Rohwer and Rudolph (2005).

The divergence of the two eastern Asian species (the mainland Chinese *Sassafras tzumu* and *S. randaiense* from the Taiwan Island) is relatively recent, and estimated to be 0.61 ± 0.75 – 2.23 ± 0.76 mya. Taiwan lies at the boundary between the Eurasian and Pacific plates and is a constituent of the south of the Ryukyu arc along the western edge of the Pacific Ocean. It has a relatively recent geologic history and had its origin in the middle/late Miocene (ca. 9 mya). Taiwan did not attain its modern form until about 5–6 mya (Teng 1990, Lu and Hsu 1992, Sibuet and Hsu 1997, 2004, Chiang and Schaal 2006). Since their emergence from the Pacific Ocean, these continental islands gradually acquired their floras and faunas primarily from the Eurasian mainland (Wang 1987). Most island plants thus have close phylogenetic links to their mainland relatives. Temperate forests extended across the continental shelf in eastern Asia are suggested to have provided potential links among Taiwan, mainland China, Korea and Japan during the glacial periods (Qian and Ricklefs 2000). The divergence of the two Asian species is consistent with the relatively recent formation of the Taiwan island. The 2-locular anthers in *Sassafras randaiense* were considered to be more derived than the 4-locular anthers (in *S. tzumu*) in *Sassafras* (Keng 1953). The disjunction of *Sassafras tzumu* and *S. randaiense* may be explained by dispersal from mainland China to Taiwan. The drupe with a weakly developed endocarp of *Sassafras* favors bird dispersal, which is relatively common for Lauraceae (Moore and Willson 1982, Rohwer 1993). Phylogeographic studies on the Taiwan relict *Cycas taitungensis* (Huang et al. 2001) indicate a pattern of colonization from the Asian continent eastward to Taiwan. The other alternative entry of *Sassafras* into Taiwan may be from the Japanese Archipelago via long distance dispersal, as proposed in *Chamaecyparis* Spach, *Michelia compressa* (Maxim.) Sarg., and *Trochodendron aralioides* Siebold & Zucc. (Wang et al. 2003). These taxa occur in both Taiwan and Japan but are not found in continental Asia. *Sassafras* is, however, not present in Japan and Korea, and the migration of *Sassafras* from Japan to Taiwan is quite unlikely.

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Appendix. Taxa from Lauraceae and two outgroups obtained from GenBank with accession numbers used in this study

Taxa	ITS	*trn*L-F	*rpl*16	*psb*A-*trn*H
Actinodaphne obovata (Nees) Blume	AY265398	–	–	–
Actinodaphne sesquipedalis Hook.f.	AF272247	AF268695	AF268239	AF268787
Aiouea guianensis Aubl.	AF272251	AF268696	–	AF268780
Alseodaphne semecarpifolia Nees	AF272252	AF268797	–	AF268799
Aspidostemon sp. van der Werff 12737	–	AF268701	AF268251	AF268819
Beilschmiedia tilaranensis Sa. Nishida	–	AF129014	AF127265	AF129045
Caryodaphnopsis tomentosa van der Werff	–	AF268705	AF268248	AF268807
Chlorocardium rodiei (R.H. Schomb.) Rohwer, H.G. Richt. & van der Werff	AF272258	AF268706	AF268245	AF268802
Cinnamomum camphora (L.) J.Presl	AF272260	AF129020	AF129019	AF129048
Cryptocarya rhodosperma Hyland	–	AF268711		AF268817
Cryptocarya thouvenotii (Danguy) Kosterm.	–	AF232035	AF232745	AF261997
Dodecadenia grandiflora Nees	AY265397	–	–	–
Endlicheria reflectens	AF272274	AF268717	–	AF268758
Eusideroxylon zwageri Teijsmann & Binnendijk	–	AF268718	AF268252	AF268820
Hypodaphnis zenkeri (Engl.) Stapf	–	AF232746	AF232746	AF261998
Illigera luzonensis (C. Presl) Merr. (outgroup)	–	AF052199	AF127264	AF129057
Laurus nobilis L.	AY265392	AF268722	AY832239	AF268785
Lindera benzoin (L.) Blume	AF272283	AF268725	–	AF268788
Lindera erythrocarpa Makino	AF272284	AF268726	AB237262	–
Lindera fruticosa Hemsl.	AY265410	–	–	–
Lindera megaphylla Hemsl.	AY265406	–	–	–
Lindera umbellata Thunb.	AF272285	AF268727	AB237267	AF268789
Litsea coreana H. Lév.	AF272286	AF268728	–	AF268791
Litsea cubeba (Lour.) Pers.	AY265402	–	–	–
Litsea dilleniifolia P.Y. Pai & P.H. Huang	AY265405	–	–	–
Litsea glutinosa (Lour.) C.B.Rob.	AY265403	–	–	–
Litsea umbellata (Lour.) Merr.	AY265404	–	–	–
Mezilaurus triunca van der Werff	AF272287	AF268729	AF268246	AF268804
Monimia ovalifolia Thou. (outgroup)	–	AF054896	AF127269	AF129065
Neocinnamomum mekongense (Hand.-Mazz.) Kosterm.	–	AF268731	AF268249	AF268806
Neolitsea levinei Merr.	AY265401	–	–	–
Neolitsea sericea (Blume) Koidz.	AF272296	AF268732	AF268243	AF268792
Ocotea grayi van der Werff	AF272301	AF268736	AF232747	–
Ocotea odorifera (Vellozo) Rohwer	AF272309	AF268738	–	AF268762
Ocotea quixos (Lam.) Kosterm.	AF272313	AF232037	–	AF261999
Parasassafras confertiflora (Meisn.) D.G. Long	AY265395	–	AF268238	AF268790
Persea americana Mill.	AF272322	AF268744	–	–
Persea meridensis L.E. Kopp	AF272325	AF268745	–	AF268797
Persea thunbergii (Siebold & Zucc.) Kosterm.	AF272327	AF268746	–	AF268798
Potameia microphylla Kosterm.	–	AF268750	AF268250	AF268816
Potoxylon melagangai (Symington) Kosterm.	–	AF268748	–	AF268821
Rhodostemonodaphne recurva van der Werff	AF272333	–	–	–
Sextonia pubescens van der Werff	AF268808	AF232038	AF232748	AF262000
Sinosassafras flavinervia (C.K. Allen) H.W. Li	AY265394	–	–	–
Umbellularia californica (Hook. & Arn.) Nutt.	AF272337	AF268755	–	AF268777

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