PHYLOGENY OF *Litsea* (LAURACEAE) INFERRED FROM SEQUENCES OF THE CHLOROPLAST GENES *matK* AND *ndhF*

Filogeni *Litsea* (Lauraceae) Berdasarkan Sekuens DNA Gen Kloroplast *matK* dan *ndhF*

Izu Andry Fijridiyanto[1)] and Noriaki Murakami[2)]

[1]Center for Plant Conservation Bogor Botanic Garden, Indonesian Institute of Sciences
Jl. Ir. H. Juanda 13 Bogor 16003
[2]Makino Herbarium, Tokyo Metropolitan University
1-1 Minami-Osawa, Hachioji, Tokyo 192-0397, Japan
Author for correspondence: Izu Andry Fijridiyanto (e-mail: izuandryf@gmail.com)
Manuscript received March 5, 2010; accepted April 15, 2010

Abstrak

Hubungan kekerabatan (filogenetik) *Litsea* dan marga-marga yang berdekatan (*Actinodaphne*, *Lindera* and *Neolitsea*) dari suku Lauraceae telah diamati dengan menggunakan data molekular. Analisis dilakukan pada sekuens nukleotida dari gen-gen kloroplas *matK* dan *ndhF*. Walaupun gen-gen ini dikenal sebagai untaian pengkode yang berevolusi dengan cepat, tetapi untuk taksa tumbuhan yang diamati pada penelitian ini menunjukkan variasi yang rendah. Pohon-pohon filogenetik hasil analisis gabungan dari sekuens nukleotida *matK* dan *ndhF* menunjukkan bahwa marga *Litsea* tidak monofiletik. Di antara empat seksi dari marga *Litsea* (seksi *Tomingodaphne*, seksi *Litsea*, seksi *Conodaphne* dan seksi *Cylicodaphne*), hanya seksi *Litsea* yang monofiletik.

Kata Kunci: filogeni molecular, *Litsea, matK, ndhF*

INTRODUCTION

Litsea is one of the largest genus in 52 recognized genera of the family Lauraceae with about 400 species. The genus is separated from the other genera by having umbellate inflorescences, unisexual and trimerous flowers, 9 stamens, 4-locular anthers, equally or reduced tepals, small to rather large fruit cupules, and leaves arranged alternately or sometimes oppositely. The species of *Litsea* are distributed mostly in Asia, Australia, the Pacific Islands, and America (Rohwer, 1993).

Based on morphological characters *Litsea* is classified into four sections, namely: *Tomingodaphne* (Blume) Hook.f., *Litsea* Benth., *Conodaphne* (Blume) Benth. and *Cylicodaphne* (Nees) Benth. Section *Tomingodaphne* consists of deciduous species. Section *Litsea* is defined by having incomplete or absent perianth segments, a perianth tube not or only slightly enlarged in the fruit, and often more than 12 stamens. Section *Conodaphne* has complete perianth segments, usually nine stamens and a small to slightly enlarged perianth tube in the fruit. Section *Cylicodaphne* is characterized by having penninerved leaves, trimerous flowers, six perianth segments, 12

stamens and an enlarged perianth tube with cup-shaped fruit cupules (Li *et al.*, 1982).

The genus *Litsea* belongs to tribe Laureae whose generic delimitation is still problematic. Moreover, the phylogenetic relationships both within the genus *Litsea* and among this genus and putative related genera are still poorly understood. Close relationships among *Litsea* and its related genera, such as *Actinodaphne*, *Lindera* and *Neolitsea* have been suggested by previous publications, however they have not been well documented.

Nowadays, molecular phylogenetic analyses using DNA sequence data have been proved to be the most powerful way to infer phylogenetic relationships of organisms. DNA fragments could easily be amplified even from a small amount of wild plant samples and its nucleotide sequences are also easily determined using automated DNA sequencer. The molecular phylogenetic trees based on the obtained sequence data often give us clear relationships among the taxonomically problematic taxa. Nucleotide sequence information of chloroplast DNA (cpDNA) has often been useful to resolve phylogenetic relationships of plants. Some rapidly evolved coding regions of cpDNA, such as *matK* and *ndhF* have been used for phylogenetic analyses at low taxonomic levels (Soltis and Soltis, 1998).

This study aims to explore the potential of nucleotide sequences from cpDNA (*matK* and *ndhF*) as sources of information for resolving phylogenetic relationships both within *Litsea* and among *Litsea* and putative related genera.

MATERIALS AND METHODS

Plant Materials

The molecular analyses were performed using 24 species of *Litsea*, one species of sect. *Tomingodaphne*, two species of sect. *Litsea*, nine species of sect. *Conodaphne* and 12 species of sect. *Cylicodaphne*. six species of *Actinodaphne*, four species of *Neolitsea* and five species of *Lindera* were also included in our study. *Machilus rimosa* and *Phoebe exelsa* (tribe Perseae) were used as outgroups, because tribe Perseae has shown to be a sister taxa to the tribe Laureae, to which the genus *Litsea* belongs (Rohwer, 2000; Chanderbali *et al.*, 2001). A complete list of the species examined in this study, along with voucher, GenBank/DDBJ/EMBL accessions and source information is presented in Table 1.

DNA Extraction, PCR Amplification and Nucleotide Sequencing

Leaf samples were collected from the field or cultivated plants and dried in silica gel. Total DNA was extracted following the procedure of Kawahara *et al.* (1995). Polysaccharides and oils were removed using washing buffer solution (0.1 M HEPES pH 8.0, 2% 2-mercaptoethanol, 1% polyvinylpyrrolidone and 0.05 M ascorbic acid). Some DNA samples required further purification using a Qiagen-tip 20 column (Qiagen, Hilden, Germany) following procedures described in Kawahara *et al.* (1995).

Table 1. Plant materials used for this study

Species	Voucher	Source	Accession No. *matK/ndhF*
Actinodaphne glomerata (Blume) Nees	IZ 802	BBG	AB258991/ AB442018
Actinodaphne macrophylla (Blume) Nees var. *angustifolia* Koord. & Valet.	IZ 854	BBG	AB258990/ AB442019
Actinodaphne maingayi Hook. f.	IZ 2068	LNP	AB259062/ AB442021
Actinodaphne malaccensis Hook. f.	IZ 2053	LNP	AB258992/ AB442020
Actinodaphne myriantha Merr.	IZ 2052	LNP	AB259063/ AB442022
Actinodaphne procera Nees	IZ 2057	LNP	AB259064/ AB442023
Lindera erythrocarpa Makino	TI 526	KOC	AB259065/ AB442024
Lindera lucida (Blume) Boerl.	IZ 2010	LNP	AB259066/ AB442026
Lindera obtusiloba Blume	TI 3402	KYO	AB259067/ AB442027
Lindera polyantha (Blume) Boerl.	IZ 876	CBG	AB259068/ AB442028
Lindera umbellata Thunb.	TI 3477	KYO	AB259069/ AB442030
Litsea accedens (Blume) Boerl.	IZ 2066	LNP	AB259070/ AB442031
Litsea caulocarpa Merr.	IZ 2043	LNP	AB259071/ AB442032
Litsea costalis (Nees) Kosterm.	IZ 2041	LNP	AB259072/ AB442033
Litsea cubeba (Lour.) Pers.	IZ 863	CBG	AB259073/ AB442034
Litsea diversifolia Blume	IZ 864	CBG	AB259074/ AB442035
Litsea erectinervia Kosterm.	IZ 2032	LNP	AB259075/ AB442036
Litsea fenestrata Gamble	IZ 2031	LNP	AB259076/ AB442037
Litsea ferruginea (Blume) Blume	IZ 2016	LNP	AB259077/ AB442038
Litsea firma Hook. f.	IZ 835	BBG	AB259078/AB510914
Litsea garciae S.Vidal	IZ 2025	LNP	AB259081/ AB442039
Litsea globularia Ng	IZ 2044	LNP	AB259079/ AB442040
Litsea glutinosa (Lour.) C. B. Rob.	IZ 824	BBG	AB259080/ AB442041
Litsea grandis (Wall.) Hook. f.	IZ 2042	LNP	AB259082/ AB442042
Litsea lancifolia Hook. f. var. *grandifolia* (Stapf) Ng	IZ 2047	LNP	AB259083/ AB442043
Litsea machilifolia Gamble	IZ 2037	LNP	AB259084/ AB442044
Litsea maingayi Hook. f.	IZ 2007	LNP	AB259085/ AB442045
Litsea mappacea (Blume) Boerl.	IZ 871	CBG	AB259086/AB510915
Litsea noronhae Blume	IZ 818	BBG	AB259087/ AB442046
Litsea ochracea (Blume) Boerl.	IZ 2034	LNP	AB259088/ AB511907
Litsea resinosa Blume	IZ 839	BBG	AB259089/ AB442047
Litsea rubicunda Kosterm.	IZ 2026	LNP	AB259090/ AB442048
Litsea sarawacensis Gamble	IZ 2049	LNP	AB259091/AB511908
Litsea tomentosa Blume	IZ 874	CBG	AB259092/ AB442049
Litsea umbellata Merr.	IZ 809	BBG	AB259093/AB510916
Machilus rimosa Blume	IZ 870	CBG	AB259098/ AB442050
Neolitsea aciculata (Blume) Koidz.	IZ 1001	KYO	AB259094/ AB442051
Neolitsea cassia (L.) Kosterm.	IZ 831	BBG	AB259095/ AB442052
Neolitsea javanica (Blume) Backer	IZ 869	CBG	AB259096/ AB442053
Neolitsea sericea (Blume) Koidz.	IZ 852	BBG	AB259097/ AB442054
Phoebe excelsa Nees	IZ 868	CBG	AB259099/ AB442055

The material were collected from Bogor Botanic Garden (BBG) and Cibodas Botanic Garden (CBG), Indonesia; Lambir National Park (LNP), Malaysia; Kochi (KOC) and Kyoto (KYO), Japan. The samples were collected by I.A. Fijridiyanto (IZ) or T. Iwasaki (TI).

Double-stranded DNAs of the chloroplast *matK* region were amplified using the primer pair *trnK* 3914 and *trnK* 2R of Johnson and Soltis (1995). The PCR profile consisted of an initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 2 min and extension at 72°C for 3 min, with a final extension at 72°C for 7 min. Amplification of the chloroplast *ndhF* region was carried out using primer 1 and 2110R described by Olmstead and Sweere (1994). The PCR profile for *ndhF* consisted of initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 2 min and extension at 72°C for 2 min, with a final extension at 72°C for 7 min. After amplification, the PCR products were checked by electrophoresis in 1.0% agarose gels; amplified fragments were then purified using a QIAquick Gel Extraction Kit (Qiagen). For nucleotide sequencing, a BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) was used following the manufacturer's protocols. Sequencing was performed using a 3100 Genetic Analyzer (Applied Biosystems). All primers used for sequencing in this study are listed in Table 2.

Phylogenetic Analyses using Maximum Parsimony and Bayesian Methods

The nucleotide sequences obtained were contiged using Chromas Pro version 1.34 software (Technelysium Pty Ltd., Tewantin, Queensland). After all the overlapping sequences were checked, a contiged sequence for each species was generated. The sequences for all taxa were aligned using BioEdit version 7.0.5.3 (Hall, 1999) and then were adjusted manually.

Phylogenetic analyses based on the maximum parsimony criterion were performed using PAUP* version 4.0b10 (Swofford, 2002) for two kinds of data sets. The first analysis was based either *matK* or *ndhF* data sets. The second was based on combined *matK* and *ndhF* data. Insertions and deletions were treated as missing data. All characters were equally weighted and unordered (Fitch, 1971). Data sets were analyzed by the heuristic search method with tree bisection-reconnection (TBR) branch-swapping and the MULTREES option on, ten replications of sequence addition with the stepwise addition option, and all of the most parsimonius trees (MPTs) were saved. The evaluation of internal support of clades was conducted by bootstrap analysis (Felsenstein, 1985) utilizing 1,000 replicates with TBR branch-swapping and the MULTREES option off. The number of steps, consistency indices (CI) and retention indices (RI) were calculated using one of the MPTs in each analysis using the TREE SCORE command in PAUP*.

Table 2. Primers used for PCR amplification and sequencing of *matK* and *ndhF* regions.

	Primer sequence 5'-3'	Source
matK		
Forward		
909(*trnK*3914F)	GGGGTTGCTAACTCAACGG	Johnson & Soltis (1995)
448	GTGTCAGATATACTAATACC	Rohwer (2000)
805	ACCCTATGGTTGTTCAAAGAC	Rohwer (2000)
1084	CTATTAAGAAATTCGAGACC	Rohwer (2000)
1318	TGTGCTAGAACTTTGTCTCG	Rohwer (2000)
matK-AF	CTATATCCACTTATCTTTCAGGAGT	Ooi *et al.* (1995)
matK-BF	TCAGAGGGATTTGCGTTTATTGTGG	Ooi *et al.* (1995)
Reverse		
2288(*trnK*-2R)	AACTAGTCGGATGGAGTAG	Johnshon & Soltis (1995)
805	GTCTTTGAACAACCATAGGGT	Rohwer (2000)
941	CCGGTTGAGACCACAAGT	Rohwer (2000)
1166	ACGGCTTACTAATGGGATGCC	Rohwer (2000)
1422	TTGGGAAGATCAAAGAAAGA	Rohwer (2000)
1847	ACTAGTCGGATGGAGTAGA	Rohwer (2000)
matK-R	CTGCATATACGCCCAAATCGGTCAA	Ooi *et al.* (1995)
matK-8R	AAAGTTCTAGCACAAGAAAGTCGA	Ooi *et al.* (1995)
ndhF		
Forward		
1	ATGGAACA(GT)ACATAT(CG)AATATGC	Olmstead & Sweere (1994)
536	TTGTAACTAATCGTGTAGGGGA	Olmstead & Sweere (1994)
972	GTCTCAATTGGGTTATATGATG	Olmstead & Sweere (1994)
1318	GGATTAAC(CT)GCATTTTATATGTTTCG	Olmstead & Sweere (1994)
1603	CCT(CT)ATGAATCGGACAATACTATGC	Olmstead & Sweere (1994)
Reverse		
2110R	CCCCCTA(CT)ATATTTGATACCTTCTCC	Olmstead & Sweere (1994)
1603R	GCATAGTATTGTCCGATTCAT(AG)AGG	Olmstead & Sweere (1994)
1318R	CGAAACATATAAAATGC(AG)GTTAATCC	Olmstead & Sweere (1994)
972R	CATCATATAACCCAATTGAGAC	Olmstead & Sweere (1994)
536R	TCCCCTACACGATTAGTTACAA	Olmstead & Sweere (1994)

The congruence between *matK* and *ndhF* data was tested with the incongruence length differences (ILD) test (Mickevich and Faris, 1981; Farris *et al.*, 1994) as implemented in PAUP* (the "partition-homogeneity test").

Bayesian analyses were conducted with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) using a best-fit model of sequence evolution from MrModeltest 2.2 (Nylander, 2004). Using the best model of substitution indicated by the Akaike information criterion (AIC), Bayesian inference analyses were run with four simultaneous Markov chain Monte Carlo (MCMC) chains initially run for 2,000,000 generations, saving the current tree to a file every 100 generations. The sampling of posterior distribution was considered to be adequate if the average standard deviation of split frequencies was <0.01 at the end of each run. The "sump" and "sumt" commands in MrBayes were used to summarize and further investigate for convergence of all parameters of the MCMC run results. Before a majority rule consensus tree was generated, trees produced prior

to log likelihood stabilization and convergence were discarded (burn in = 5000).

RESULTS AND DISCUSSION

Molecular Trees based on Nucleotide Sequences of *matK* and *ndhF*

The aligned nucleotide sequences of *matK* including small parts of the *trnK* intron comprises 1,628 characters. Among these, 1,534 (94.2%) are constant, 46 (2.8%) are parsimony-uninformative and 48 (2.9%) are parsimony-informative characters (Table 3). The distribution of parsimony-informative characters is shown in Fig. 1A. The parsimony-informative characters are mostly distributed along the first 1000 base pairs. The nucleotide sequences of the four species of the genus *Actinodaphne* are identical (*A. glomerata, A. myriantha, A. maingayi* and *A. procera*). Two pairs of identical sequences are also found in the genus *Litsea*, one of which is shared by three (*L. machilifolia, L. globularia* and *L. costalis*) and the other by two different species (*L. erectinervia* and *L. ochracea*). The parsimony analysis resulted in 66 MPTs with a length of 107 steps, CI of 0.897 and RI of 0.929. Topologies of the molecular trees derived from Bayesian and parsimony analyses based on the same dataset of sequences were very similar. The Bayesian trees obtained based on *matK* sequences is shown in Fig. 2. Bootstrap percentages (BP) obtained from parsimony analyses are presented below Bayesian posterior probabilities (PP) on the Bayesian trees.

Table 3. Statistics calculation from parsimony analyses of the separate and combined data matrices of *matK* and *ndhF*.

	matK	*ndhF*	Combined *matK* & *ndhF*
No. of sites	1,628	2,105	3,733
No. of constant sites (%)	1,534 (94.2%)	1,955 (92.9%)	3488 (93.4%)
No. of variable sites (%)	46 (2.8%)	84 (4%)	129 (3.5%)
No. of informative sites (%)	48 (2.9%)	66 (3.1%)	116 (3.1%)
No. of steps (substitution)	107	179	288
No. of MPTs	66	20	44
CI	0.897	0.872	0.878
RI	0.929	0.913	0.923

The aligned nucleotide sequences of *ndhF* comprises 2,105 characters. Among these, 1,955 (92.9%) are constant, 84 (4.0%) are parsimony-uninformative and 66 (3.1%) are parsimony-informative characters (Table 3). Distribution of parsimony-informative characters are shown in Fig. 1B. The parsimony-informative characters are mostly distributed along the last 1000 base pairs. The nucleotide sequences of the two species of the genus *Actinodaphne* are identical (*A. procera* and *A. maingayi*). Two pairs of identical sequences are also found in the genus *Litsea*, each of them was shared by two different species (*L. erectinervia* and *L. ochracea*; *L. fenestrata* and *L. costalis*). The parsimony analysis resulted in 20 MPTs with a length of 179 steps, CI 0.872 and RI 0.913. Topologies of the molecular trees derived from Bayesian and parsimony analyses based on the same dataset of sequences were very similar. The Bayesian trees obtained based on *ndhF* sequences is shown in Fig. 3. BP of parsimony analyses are also presented below PP of Bayesian analysis on the Bayesian tree.

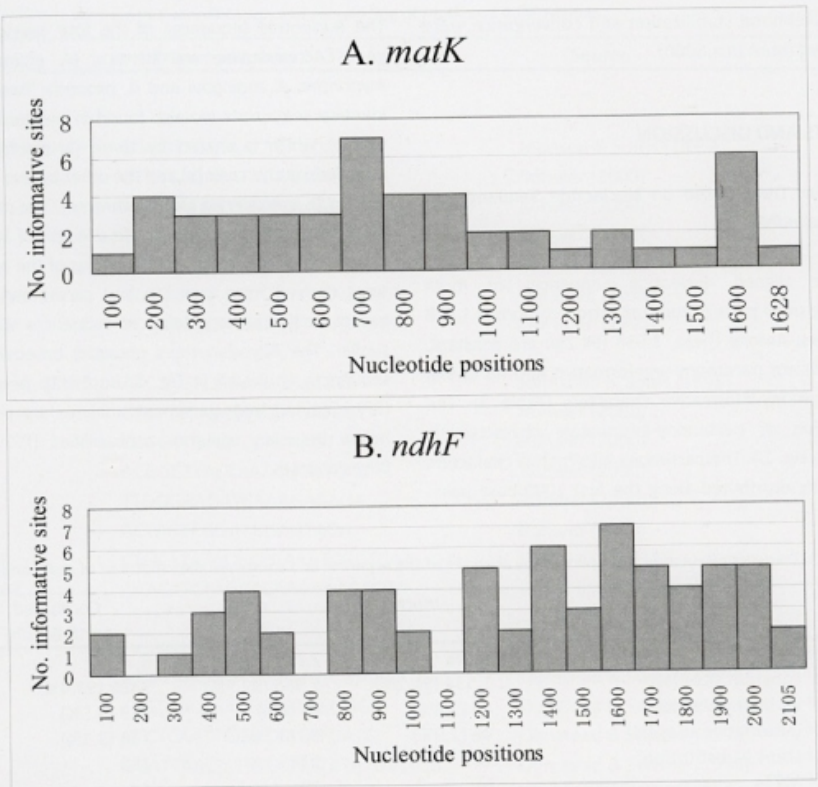

Fig.1. Distribution of parsimony-informative sites in *matK* (A.) and *ndhF* (B.) data sets.

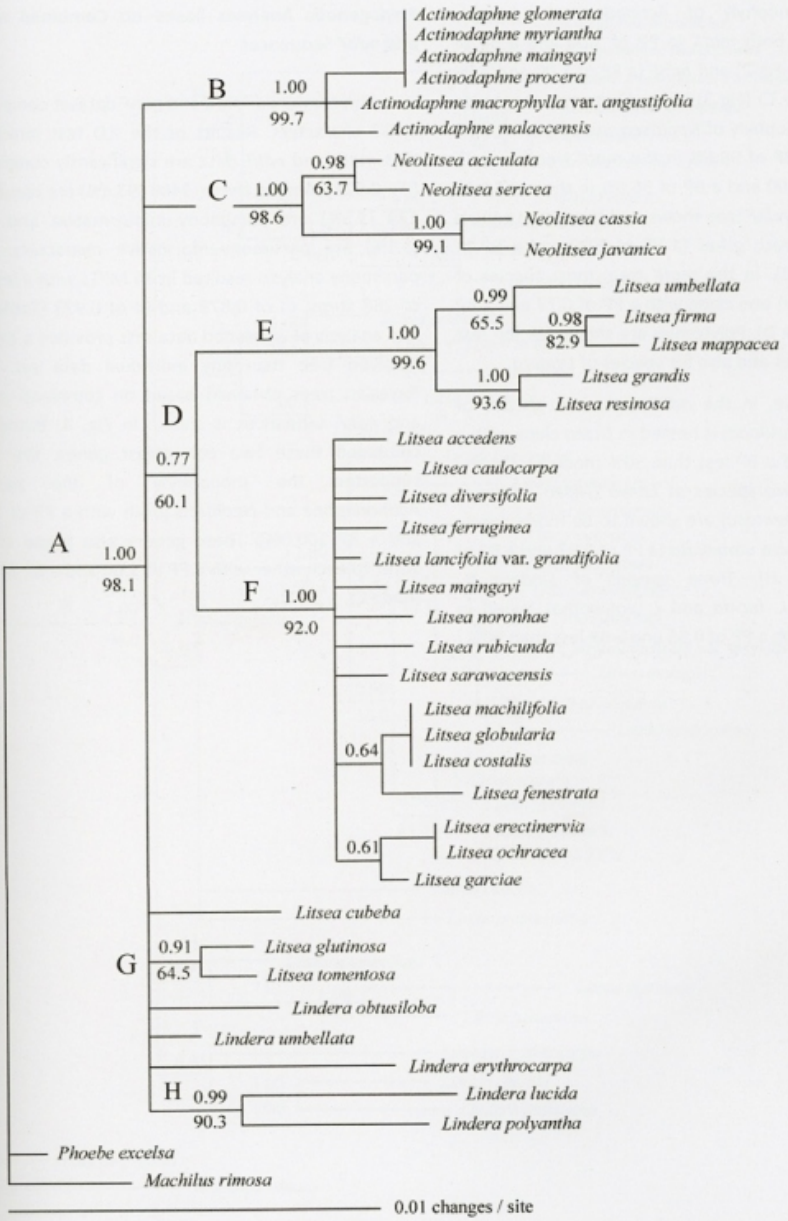

Fig. 2. Bayesian 50% majority-rule consensus tree of *matk* sequences. Numbers above branches are posterior probabilities (>0.5); those below branches are bootstrap frequencies by maximum parsimony analysis (>50%).

The monophyly of *Actinodaphne* is highly supported by both *matK* (a PP of 1.00 and a BP of 99.7; node B) (Fig 2) and *ndhF* (a PP of 1.00 and a BP of 100.0; node C) (Fig 3) trees. These two trees also show the monophyly of *Neolitsea*, supported by a PP of 1.0 and a BP of 98.6% in the *matK* tree (node C) and a PP of 1.00 and a BP of 96.0% in the *ndhF* tree (node D). The *ndhF* tree shows that these two genera are sister to each other (a PP of 1.00 and a BP of 75.8%; node B). In the *matK* tree, most species of *Litsea* cluster in one clade with a PP of 0.77 and a BP of 60.1% (node D). Polytomies are shown for the rest of *Litsea* species and also for species of *Lindera*.

Meanwhile, in the *ndhF* tree, one species of *Lindera* (*L. obtusiloba*) is nested in *Litsea* clade with a PP of 0.97 and a BP less than 50% (node E). On the other hand, two species of *Litsea* (*Litsea glutinosa* and *Litsea tomentosa*) are shown to be more closely related to *Lindera umbellata* (a PP of 0.97 and a BP of 57.9%; node H). Three species of *Lindera* (*L. erythrocarpa*, *L. lucida* and *L. polyantha*) joined in one lineage with a PP of 0.95 and a BP less than 50%.

Phylogenetic Analyses based on Combined *matK* and *ndhF* Sequences

The combined *matK* and *ndhF* dataset comprises 3,733 characters. Results of the ILD test indicated that *matK* and *ndhF* data are significantly congruent (P = 0.928). Among these, 3488 (93.4%) are constant, 129 (3.5%) are parsimony-uninformative and 116 (3.1%) are parsimony-informative characters. The parsimony analysis resulted in 44 MPTs with a length of 288 steps, CI of 0.878 and RI of 0.923 (Table 3). The analysis of combined data sets provided a better resolved tree than any individual data set. The Bayesian trees obtained based on combined *matK* and *ndhF* sequences is shown in Fig. 4. Based on combined these two chloroplast genes, the tree supported the monophyly of the genera *Actinodaphne* and *Neolitsea* (both with a PP of 1.00 and a BP 100.0%). These genera also found to be sister to each other with a PP of 1.00 and a BP 81.6% (node C).

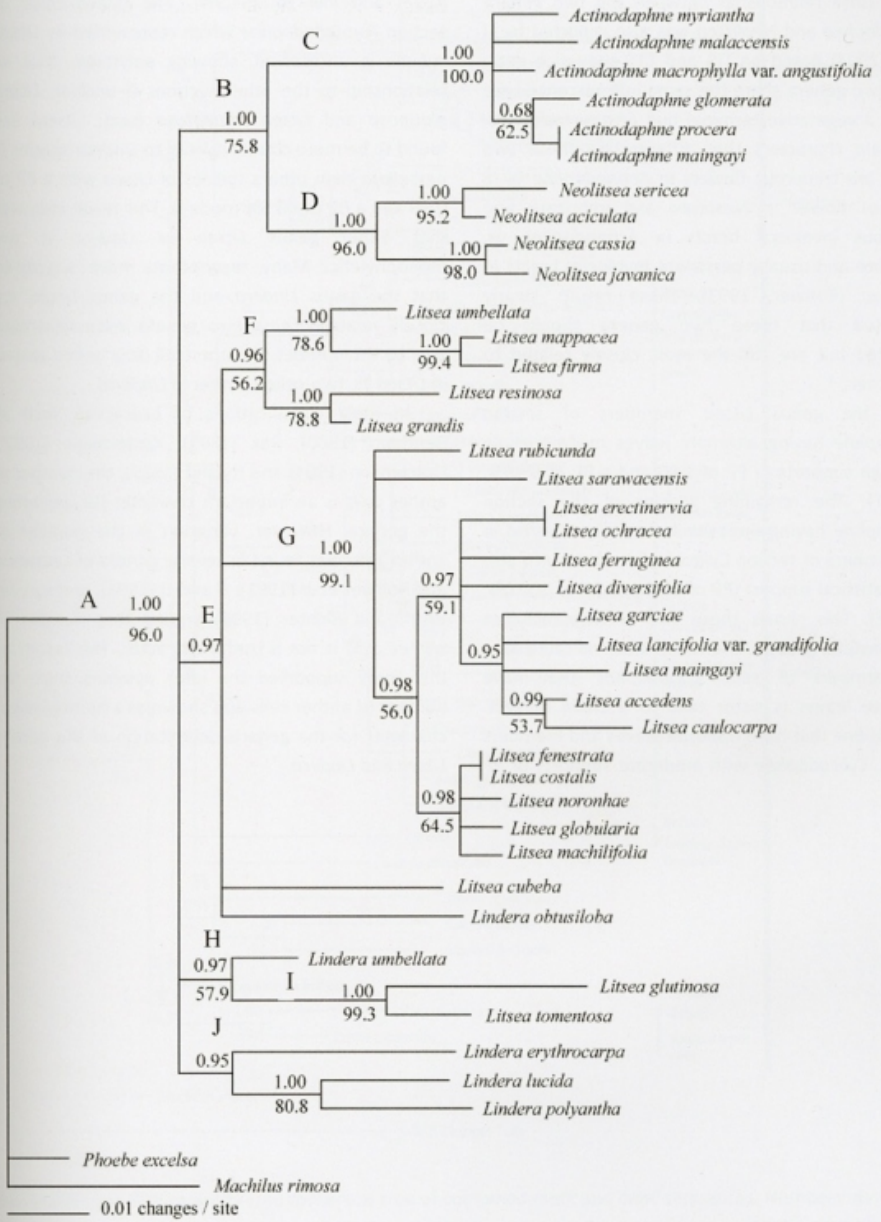

Fig. 3. Bayesian 50% majority-rule consensus tree of *ndhF* sequences. Numbers above branches are posterior probabilities (>0.5); those below branches are bootstrap frequencies by maximum parsimony analysis (>50%).

A close relationship between the two genera *Actinodaphne* and *Neolitsea* was also reported by Li *et al.* (2007) based on ITS and ETS sequence data. These two genera share the same inflorescence type lacking a vegetative terminal bud on the main axis. The main characters that differentiate these two genera are trimerous flowers in *Actinodaphne* vs. a dimerous flower in *Neolitsea* and imbricate and deciduous involucral bracts in *Actinodaphne* vs. decussate and usually persistent involucral bracts in *Neolitsea* (Rohwer, 1993). These results clearly supported that these two genera should be separated but are still the most closely related to each other.

In the genus *Litsea*, members of section *Conodaphne* having alternate leaves made a clade with high supports (a PP of 1.00 and a BP of 99.9%; node F). The remaining species of the section *Conodaphne* having opposite leaves were nested in the members of section *Cylicodaphne* clade with also high statistical support (PP of 1.00 and BP of 100.0%; node E). This shows these two sections are not monophyletic groups. The result also indicated that the members of sect. *Conodaphne* that have alternate leaves is sister taxa to a clade of sect. *Conodaphne* that have opposite leaves and members of sect. *Cylicodaphne* with moderate supports of PP (0.92) and low BP (61.9%). The relationships of section *Tomingodaphne* which represented by *Litsea cubeba* is unresolved, showing polytomy, thus its relationship to the other sections is unclear. *Litsea glutinosa* and *Litsea tomentosa* (sect. *Litsea*) are found to be more closely related to *Lindera* species *L. umbellata* than others species of *Litsea* with a PP of 0.96 and a BP of 66.5% (node I). This result indicates that either genus *Litsea* or *Lindera* is not monophyletic. Many taxonomists have suggested that the genus *Lindera* and the genus *Litsea* are closely related. These two genera were separated only by the number of anther cell (four-celled anther in *Litsea* vs. two-celled anther in *Lindera*).

In most classifications of Lauraceae such as Bentham (1880), Pax (1891), Kostermans (1957), Hutchinson (1964) and Hyland (1989), the number of anther cells is an important character for delimiting the genera. However, variation in the number of anther cells was found in several genera of Lauracea, and Rohwer *et al* (1991), Li and Li (1991), and van der Werff and Richter (1996) argued that number of anther cells is not a useful character. The results of this study supported the later opinions since the number of anther cells was shown as a homoplasious character for the generic delimitation of the genera *Litsea* and *Lindera*.

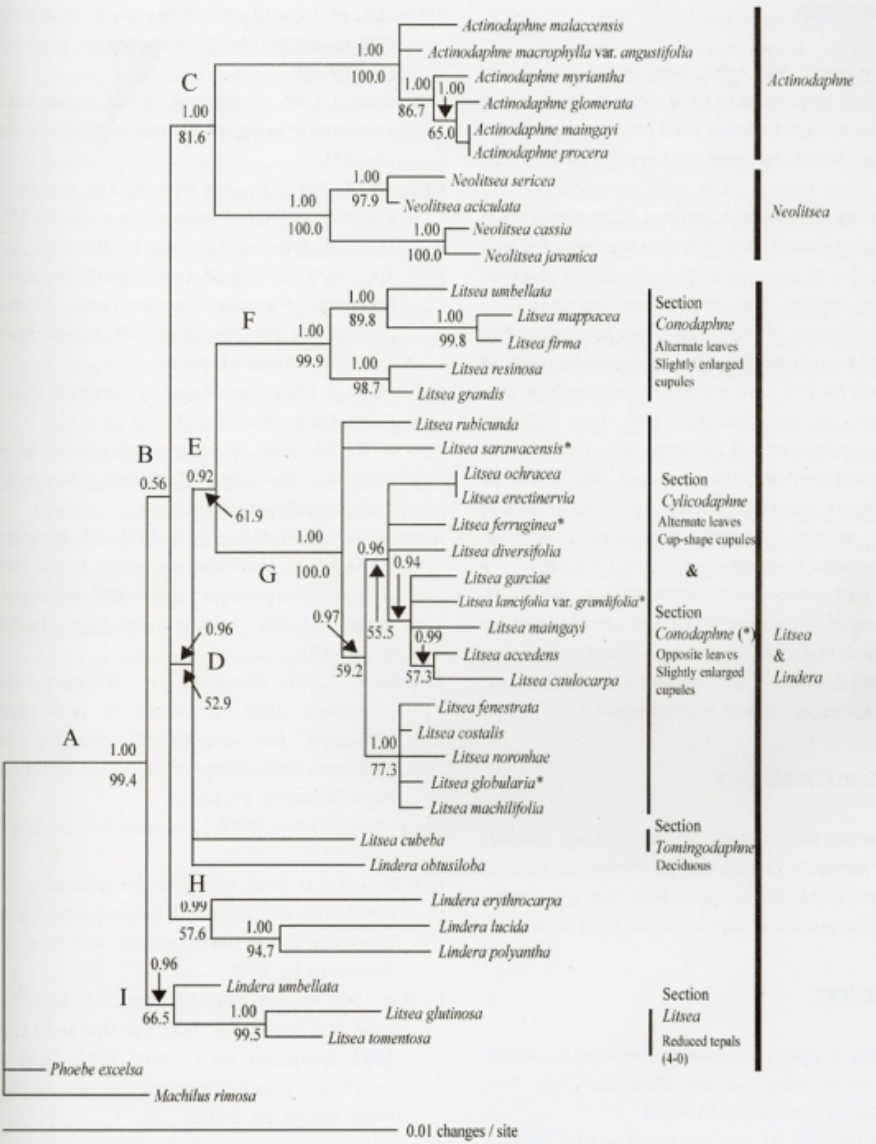

Fig. 4. Bayesian 50% majority-rule consensus tree of combined *matk* and *ndhF* sequences. Numbers above branches are posterior probabilities (>0.5); those below branches are bootstrap frequencies by maximum parsimony analysis (>50%).

CONCLUSIONS

The *matK* and *ndhF*, two coding regions of cpDNA, are considered to have molecular evolutional rates fast enough for lower level phylogenetic studies of higher plants. However, the results showed that these two regions are still conservative for informative phylogenetic analyses of the genus *Litsea* and related genera. This limitation was indicated by a low number of parsimony-informative sites observed in these regions, and also by the fact that even different species of the genus *Litsea* sometimes had identical sequences. Thus, the phylogenetic utility of these loci for this plant group was proven to be low. However, the obtained molecular trees indicated that *Actinodaphne* and *Neolitsea* are monophyletic groups and they are closely related. The results do not support the classifications of Li *et al.* (1982) which divided *Litsea* into four sections. Morphological characters which used for separating sections in *Litsea*, were homoplasious characters. Polyphyly of the genera *Litsea* and *Lindera* was also suggested. Major revision of both *Litsea* and *Lindera* is needed, based on increased sampling and additional molecular and morphological data.

ACKNOWLEDGEMENTS

I would like to thank Ms. Lucy Chong, Sarawak Forest Research Center, for permitting to conduct fieldwork in Lambir National Park and Mr. Takaya Iwasaki for providing plant materials from Japan.

REFERENCES

Bentham, G. 1880. Laurineae. In: Bentham G. and J.D. Hooker (Eds). *Genera Plantarum Vol. 3*. L. Reeve, London. pp. 146–168.

Chanderbali, A.S., H. van der Werff and S.S. Renner. 2001. Phylogeny and historical biogeography of Lauraceae: evidence from the chloroplast and nuclear genomes. *Annals of the Missouri Botanical Garden* 88: 104–134.

Farris, J.S., M. Kallersjo, A.G. Kluge and C. Bult. 1994 Testing significance of incongruence. *Cladistics* 10: 315–319.

Felsenstein, J. 1985. Confidence limit on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

Fitch, W.M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Systematic Zoology* 20: 406–416.

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

Hutchinson, J. 1964. *The Genera of Flowering Plants Vol. 1*. Clarendon Press, Oxford.

Hyland, B.P.M. 1989. A revision of Lauraceae in Australia (excluding *Cassytha*). *Australian Systematic Botany* 2: 135–367.

Johnson, L.A. and D.E. Soltis. 1995. Phylogenetic inference in Saxifragaceae *sensu stricto* and *Gilia* (Polemoniaceae) using *matK* sequences. *Annals of the Missouri Botanical Garden* 82:149-175.

Kawahara, T., N. Murakami, H. Setoguchi and Y.Tsumura. 1995. Procedure of plant DNA extraction for phylogenetic analysis (in Japanese). *Proceedings of the Japan Society of Plant Taxonomy* 11: 13–32.

Kostermans, A.J.G.H. 1957. Lauraceae. *Reinwardtia* 4: 193–256.

Li, H.W. and J. Li. 1991. Notes on the taxonomy and distribution of the genus *Caryodaphnopsis* of Lauraceae and its characteristics. *Acta Botanica Yunnanica* 13: 1–13.

Li, H.W., P.Y. Pai, S.K. Lee, F.N. Wei, Y.T. Wei, Y.C. Yang, P.H. Huang, H.P. Tsui, Z.D. Shia and J.L. Li. 1982. Lauraceae. In: Li, H-W. (Ed.). *Flora of Republicae Popularis Sinicae Vol. 31*. Science Press, Beijing. pp. 1-463.

Li, L., J. Li, J.G Conran and X-W. Li. 2007. Phylogeny of *Neolitsea* (Lauraceae) inferred from Bayesian analysis of nrDNA ITS and ETS sequences. *Plant Systematics and Evolution* 269: 203–221.

Mickevich, M.F. and J.S. Farris. 1981. The implications of congruence in *Menidia*. *Systematic Zoology* 30: 351–370.

Nylander, J.A.A. 2004. MrModeltest2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University. Uppsala, Sweden. Website *http://www.abc.se/~nylander/*

Olmstead, R.G. and J.A. Sweere. 1994. Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. *Systematic Biology* 43: 467–481.

Ooi, K., Y. Endo, J. Yokoyama and N. Murakami. 1995. Useful primer designs to amplify DNA fragments of the plastid gene *matK* from angiosperm plants. *Journal of the Japanese Botany* 70: 328–331.

Pax, F. 1891. Lauraceae. In: Engler A. and K. Prantl (Eds.). *Die naturelichen Pflanzenfamilien Bd. III, 2*. Engelmann, Leipzig. pp. 106–126.

Rohwer, J.G. 1993. Lauraceae. In: Kubitzki K., J.G. Rohwer and V. Bittrich (Eds.). *The Families and Genera of Vascular Plants. Vol. 2*. Springer, Berlin. pp 366–391.

Rohwer, J.G. 2000 Toward a phylogenetic classification of the Lauraceae: evidence from *matK* sequences. *Systematic Botany* 25: 60–71.

Rohwer, J.G., H.G. Richer and H. van der Werff. 1991. Two new genera of neotropical Lauraceae and critical remarks on the generic delimitation. *Annals of the Missouri Botanical Garden* 78: 388–40.

Ronquist, F. and J.P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Soltis, D.E. and P.S. Soltis. 1998. Choosing an approach and an appropriate gene for phylogenetic analysis. In: Soltis D.E., P.S. Soltis and J.J. Doyle (Eds.). *Molecular Systematics of Plants II. DNA Sequencing*. Kluwer Academic, Dordrecht. pp. 1-42.

Swofford, D.L. 2002. *PAUP* Phylogenetic Analysis Using Parsimony*. Sinauer and Associates, Massachusetts.

van der Werff, H. and H.G. Richter. 1996. Toward an improved classification of Lauraceae. *Annals of the Missouri Botanical Garden* 83: 409–418.