

# Phylogeny and taxonomy of *Eurya* (Ternstroemiaceae) from Taiwan, as inferred from ITS sequence data

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**ABSTRACT.** *Eurya* is the largest genus in the Ternstroemiaceae. The 13 *Eurya* species in Taiwan constitute an important element in various habitats from low to high elevated forests in the island. In an attempt to understand the interspecies relationships and geographical history of these 13 species, we sequenced 123 samples representing 32 *Eurya* species from Taiwan, the Ryukyus, continental China, and Southeast Asia at the nuclear ITS region. Sixteen species of five other genera of Ternstroemiaceae were also incorporated. Molecular phylogenetic trees show that the *Eurya* species studied form a monophyletic group, organized into five clades with geographical correlations. All the 17 *Eurya* species from Taiwan and the Ryukyus studied, except *E. loquaiana*, together constitute two coherent, but distantly related clades: one clade stands at a basal and isolated position, with four out of its five species endemic to these two regions; they are probably Tertiary elements of northern China or their direct descendants. The other clade is much more advanced and has strong affinity to the *Eurya* in southeast China, which suggests that the members were probably originated from the Quaternary flora of southeast China. Ecological partitioning is thought to be the major mechanism for the speciation of four Taiwan-endemic species from *E. chinensis*.

**Keywords:** *Eurya*; ITS; Ternstroemiaceae; Phylogeny; Taiwan; The Ryukyus.

## INTRODUCTION

Taiwan is situated at the junction of the Ryukyu and the Luzon Arcs, and is separated from Fujian Province, China by the 150 km wide Taiwan Strait (Figure 1). When the overall flora of this region became explored in the early 20<sup>th</sup> century, it became clear that the flora of Taiwan mainly originated from continental China (Wilson, 1920; Masamune, 1934; Kanehira, 1936; Li, 1957; Hosokawa, 1958) and the elements from the Philippines were limited and restricted to the very south (Merrill, 1926; Li and Keng, 1950; Li, 1953); while the flora of the Ryukyus (up to the Amami group) was considered as an extension of the flora of Taiwan, separated from the flora of Japan (Kyushu Island and to the north) (Masamune, 1934; Sonohara et al., 1952; Hosokawa, 1958). These earlier viewpoints on the floristic affinities have been supported by later floristic analyses (Liu and Teruya, 1980; Hsieh et al., 1994; Shen, 1997; Hsieh, 2002). A well accepted explanation based on geological/geographical information is that the Taiwan Strait is mostly only 60-80 m deep and served as a bridge connecting continental China and Taiwan several times during the Pleistocene glacial episodes, whereas the Bashi Strait between Taiwan and Luzon Island is deeper than 2,000 m and these islands have never been connected (Shen, 1996). Our comparison on the native genera of

the Ryukyus listed in the “Flora of the Ryukyus, South of Amami Island” (Hatusima and Amano, 1994) also reveals that 96% (650 out of 672) of its native genera are also native to Taiwan.



Figure 1. Map of SE Asia and E Asia.

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These phytogeographical studies of Taiwan were large-scaled analyses of the entire floras or ligneous floras of these regions. In recent years, adopting molecular tools in phytogeographical studies on the plants of Taiwan has become prevalent; however, the majority were focused on the inter-population relationships within a single species, e.g., *Cycas taitungensis* (Huang et al., 2001), *Cyclobalanopsis glauca* (Huang et al., 2002); *Kandelia candel* (Chiang et al., 2001), *Michelia formosana* (Lin, 2001; Lu et al., 2002), *Myrica japonica* (Cheng et al., 2000), *Trochodendron aralioides* (Wu et al., 2001), and *Tritanotrichum oldhamii* (Wang et al., 2004). Congeneric species have rarely been investigated, e.g., *Gentiana* (Chen, 2001). The goal of our study is to approach the phylogeography of a genus from Taiwan by analyzing it together with congeneric species occurring in the Ryukyus and Fujian Province, China. The genus *Eurya* was chosen for three reasons. First, we have been working on a revision of *Eurya* in Taiwan and have acquired an essential species concept of the genus in this region. Second, the total 19 *Eurya* species occurring in the Ryukyus and Taiwan exhibit complex distribution patterns. Five are endemic to the Ryukyus (*E. osimensis*, *E. ryukyuensis*, *E. sakishimensis*, *E. yaeyamensis*, and *E. zigzag*), eight to Taiwan (*E. crenatifolia*, *E. glaberrima*, *E. hayatai*, *E. leptophylla*, *E. nanjenshanensis*, *E. renegechinensis*, *E. septata*, and *E. strigillosa*). Three occur in both Taiwan and SE China, but not in Japan/the Ryukyus (*E. chinensis*, *E. loquaiana*, *E. nitida*). One is present in Taiwan and probably in the Philippines as well (*E. gnaphalocarpa*) (Ling, 1998), one is present in the Ryukyus (and Japan) and China, but not in Taiwan (*E. japonica*), and one occurs in all three regions (*E. emarginata*). Thirdly, the ratio of endemic *Eurya* species is high in Taiwan (8/13=62%) and the Ryukyus (5/7=71%); thus, *Eurya* appears to be a good subject for phytogeographical research.

*Eurya*, comprising c. 130 species (Ling, 1998), was the largest of the nine genera in the subfamily Ternstroemiaceae of Theaceae; but in the recent angiosperm phylogeny system, Ternstroemiaceae is removed from Theaceae and combined with Pentaphragmaceae (Pentaphragmaceae) to form Ternstroemiaceae (Stevens, 2006). The genus *Eurya* distributes from SE Asia, E Asia, to the Pacifics, with continental China as the center of diversity (Ling, 1998). The species of *Eurya* are often common or even dominant in various habitats in Taiwan, such as riverbanks, coastal forests, windy mountain slopes, or cold and humid forests. In this study, for the taxonomic treatment of *Eurya*, we followed the Manual of Taiwan Vascular Plants (Liu et al., 1998) for the species in Taiwan, with the addition of one new species, *E. septata* (Wu et al., 2003). For *Eurya* in the Ryukyus, besides the six species recognized in the Flora of Okinawa and the Southern Ryukyu Islands (Walker, 1976) and the Check List Vascular Floras of Ryukyu Islands (Shimabaku, 1997), we also accepted *E. ryukyuensis* as an independent species. For *Eurya* in continental China, the Flora of China (Ling, 1998) was followed.

## MATERIALS AND METHODS

**Sampling:** All the 13 *Eurya* species distributed in Taiwan, five out of the seven occurring in the Ryukyus, and 12 out of the 18 occurring in Fujian, China were collected in the field. (The 12 Fujian species were collected from Fujian, Guangdong, and/or Hong Kong). *Eurya* species from other areas and five other genera in Ternstroemiaceae, including *Adinandra*, *Cleyera*, and *Euryodendron* of tribe Freziereae and *Anneslea* and *Ternstroemia* of tribe Ternstroemiaceae, were also incorporated (Table 1, Appendix 1). Leaf materials were immediately put in silica gel after detachment in the field; while voucher specimens were deposited in the herbaria listed in Table 1. Sequencing for chloroplast DNA regions *psbA-trnH* and *trnL-trnF* was carried out in 24 and 4 species, respectively (Appendix 2); because their respective sequence variations were too low for interspecies comparison, no more sequencing was continued (details provided in the Results). Sequencing for the nuclear ITS (internal transcribed spacer) DNA region was carried out in 123 samples of 32 *Eurya* species and 23 samples of 14 species from the other five genera (Table 1). The rather extensive sampling was intended to have a preliminary survey on the ITS intraspecific variation of *Eurya* species and to ascertain the suitability of inferring ITS sequences in the present phylogenetic study. The design of the sampling was that for each *Eurya* species occurring in Taiwan and the Ryukyus at least three samples should be sequenced. When two or more con-specific samples exhibited identical sequences, no more sequencing for that species was required and that sequence type would be chosen to represent the species and registered in the GenBank; otherwise, more samples should be sequenced until the requirement was fulfilled. As for the species from other regions and of other genera, the amount of samples was not very many, thus most of the samples were sequenced. The number of con-specific samples in this study ranged from one to 27. For those *Eurya* species with wide distribution ranges, multiple con-specific samples were usually chosen from different countries/islands.

### **DNA extraction, amplification, and sequencing.**

Total DNA was extracted from silica gel-dried or herbarium material following the protocols described in Struwe et al. (1998).

**ITS.** Amplification of the internal transcribed spacer (ITS) region which is composed of ITS1, the 5.8 gene, and ITS2 was done by using primers ITS1eu1 (Urbatsch et al., 2000) and ITS4 (White et al., 1990). The PCR program was set as 94°C for 5 min followed by 35 cycles of 94°C for 1 min, 56°C for 1 min, 72°C 1 min, and a single cycle of 72°C for 7 min. Sequencing was done by using ITS1eu1 and ITS4 primers.

***trnL-trnF.*** Amplification of the *trnL-trnF* which is composed of *trnL* intron, *trnL3'* gene and *trnL-trnF* intergenic spacer was done by using primers TabC and

**Table 1.** List of ITS sequencing samples. The total 146 samples include 123 of 32 *Eurya* species and 23 of 14 species of other five genera of Ternstroemiaceae. The registered accession number and sequence variation among the con-specific samples are provided. Vouchers are deposited at HAST (Herbarium of Academia Sinica, Taiwan, ROC). Three sequences obtained from GenBank are listed as footnotes.

Species name	Distribution range	No. of samples	Con-specific samples with identical ITS sequence. The sequence and one voucher (the one with *) are registered in the Genbank	GenBank accession number	Samples with 1, 2, or 3 sites different from the registered sequence after alignment
<i>Eurya acuminata</i> DC. var. <i>acuminata</i>	S China, SE Asia	1	<i>Tsou</i> 1538*	AY626852	
<i>Eurya acuminata</i> var. <i>wallichiana</i> Dyer	Thailand	1	<i>Maxwell</i> 2004-233*	AY626883	
<i>Eurya acuminatissima</i> Merr. & Chun	C & S China	2	<i>Tsou et al.</i> 1813*, 1814	AY626853	
<i>Eurya amplexifolia</i> Dunn	S China (incl. Fujian)	1	<i>Tsou</i> 1578*	AY626854	
<i>Eurya chinensis</i> R. Br. var. <i>chinensis</i> ( <i>E. chinensis</i> -T)	Taiwan, SE China (Fujian)	10	<i>Tsou et al.</i> 1250, 1327, 1330*, 1378, 1379, 1423, 1425	AY626855	1 site: <i>Tsou et al.</i> 1269 2 sites: <i>Tsou et al.</i> 1258, 1720
<i>Eurya chinensis</i> R. Br. var. <i>chinensis</i> ( <i>E. chinensis</i> -C)	Taiwan, SE China (Fujian)	7	<i>Tsou et al.</i> 1583* 1792, 1794, 1800, 1836, 1837, 1891	AY626856	
<i>Eurya chinensis</i> var. <i>glabra</i> Hu et L. K. Ling	Taiwan, SE China (Fujian)	3	<i>Tsou et al.</i> 1802*, 1892	AY937210	1 site : <i>Chen, C.C.</i> 7403
<i>Eurya crenatifolia</i> (Yamamoto) Kobuski	Taiwan	9	<i>Tsou et al.</i> 1253*, 1333	AY626857	1 site: <i>Tsou et al.</i> 1604, 1614 2 sites: <i>Tsou et al.</i> 1319, 1485 3 sites: <i>Tsou et al.</i> 1381, 1487, 1512
<i>Eurya disticha</i> Chun	S China	2	<i>Tsou et al.</i> 1973*, 1975	AY626858	
<i>Eurya distichophylla</i> Hemsf	SE China (Fujian), Vietnam	3	<i>Tsou et al.</i> 1577*, 2016	AY626859	1 site: <i>Tsou et al.</i> 2113
<i>Eurya emarginata</i> Makino	Taiwan, SE China (Fujian), Ryukyus	4	<i>Tsou et al.</i> 1399, 1400*, 1495	AY626860	1 site: <i>Tsou et al.</i> 1252
<i>Eurya glaberrima</i> Hayata	Taiwan	7	<i>Tsou et al.</i> 1376, 1414*, 1652	AY626861	1 site: <i>Tsou et al.</i> 1601, 1603 2 sites: <i>Tsou et al.</i> 1370 3 sites: <i>Tsou et al.</i> 1374
<i>Eurya gnaphalocarpa</i> Hayata	Taiwan, (Philippines?)	5	<i>Tsou et al.</i> 1260, 1331*, 1864	AY626862	1 site: <i>Tsou et al.</i> 1764 2 sites: <i>Tsou et al.</i> 1314
<i>Eurya groffii</i> Merr.	SW & SE China (Fujian)	2	<i>Tsou et al.</i> 1890*, 1894	AY626863	
<i>Eurya hayatai</i> Yamamoto	Taiwan	5	<i>Tsou et al.</i> 1626*, 1627; <i>Su, Mong Huai</i> 227, 282	AY626864	2 sites: <i>Su, Mong Huai</i> 109

Table 1. (Continued)

Species name	Distribution range	No. of samples	Con-species samples with identical ITS sequence. The sequence and one voucher (the one with *) are registered in the Genbank	GenBank accession number	Samples with 1, 2, or 3 sites different from the registered sequence after alignment
<i>Eurya hebeclados</i> Ling	SE China (Fujian)	2	<i>Tsou et al. 1944*</i>	AY626865	1 site: Zhang, Wen Ju, 2000-12-18-c
<i>Eurya impressinervis</i> Kobuski	SE & SW China	1	<i>Ye, Hua Gu 2003-5-30-a*</i>	AY626866	
<i>Eurya japonica</i> Thunb.	E China, Japan, Ryukyus, Korea	4	<i>Tetsuo Denda 2000-12-6-a*</i> , Zhang, Wen Ju, 2001-9-19-a, 2001-9-19-b	AY626867	1 site: Tetsuo Denda 2000-12-6-b
<i>Eurya laotica</i> Gagnep.	SE Asia	1	<i>Tsou et al. 1620*</i>	AY626868	
<i>Eurya leptophylla</i> Hayata	Taiwan	7	<i>Tsou et al. 1355*</i> , 1356	AY626869	1 site: Tsou et al. 1634, 1647, Lin, Chia Hua 523 2 sites: Tsou 1503 3 sites: Tsou 1456
<i>Eurya loquatiana</i> Dunn	Taiwan, SE China (Fujian)	3	<i>Tsou et al. 1316*</i> , 1352	AY626870	1 site: Tsou et al. 1341
<i>Eurya macartneyi</i> Champ.	SE & S China (Fujian)	3	<i>Tsou et al. 1579*</i> , 1839	AY626871	1 site: Tsou et al. 1831
<i>Eurya muricata</i> Dunn	SE China (Fujian)	2	Zhang, Wen Ju, 2000-12-18-a*	AY626872	2 sites: Zhang, Wen Ju, 2000-12-18-b
<i>Eurya nanjenshanensis</i> (Hsieh, Ling, & Yang) Yang & Lu	Taiwan	3	<i>Tsou et al. 1419*</i> , 1422	AY626873	1 site: Tsou 1421
<i>Eurya nitida</i> Korthals	Taiwan, SE China (Fujian), SE Asia	7	<i>Tsou et al. 1279, 1328, 1582, 1798, 1826*</i> , 1868	AY626874	1 site: Tsou et al. 1806
<i>Eurya quinquelocularis</i> Kobuski	SW China, Vietnam	1	<i>Ye, Hua Gu 2002-6-29-b*</i> , 2002-6-29-e	AY626875	
<i>Eurya reugechiensis</i> Yamamoto	Taiwan	4	<i>Tsou et al. 1342*</i> , Su, Mong Huai 241, 242	AY626876	1 site: Tsou et al. 1367
<i>Eurya rubiginosa</i> Chang	S China	1	<i>Tsou et al. 1580*</i>	AY626877	
<i>Eurya ryukyuenensis</i> Masamune	Ryukyus	4	<i>Tsou et al. 1436*</i> , Wang, Chun Neng 1520, Wang, Jenn Che 2003-10-5	AY626878	1 site: Wang, Jenn-Che 2003-10-6
<i>Eurya sakishimensis</i> Hatusima	Ryukyus	2	Wang, Chun Neng, s.n., Tsou et al. 1301*	AY626879	1 site: Tsou et al. 1442
<i>Eurya septata</i> Wu, Hsu, & Tsou	Taiwan	6	<i>Tsou et al. 1476, 1477*</i> , 1499	AY626880	1 site: Tsou et al. 1470, 1500 2 sites: Tsou et al. 1471
<i>Eurya strigillosa</i> Hayata	Taiwan, Ryukyus	3	<i>Tsou et al. 1335*</i> , 1650	AY626881	2 sites: Tsou et al. 1623

Table 1. (Continued)

Species name	Distribution range	No. of samples	Con-species samples with identical ITS sequence. The sequence and one voucher (the one with *) are registered in the Genbank	GenBank accession number	Samples with 1, 2, or 3 sites different from the registered sequence after alignment
<i>Eurya subintegra</i> Kobuski	S China (Fujian), Vietnam	2	<i>Ye, Hua Gu 2002-6-29-c*</i> , <i>Tsou et al. 1987</i>	AY626882	
<i>Eurya weissiae</i> Chun	SE & S China (Fujian)	2	<i>Ye, Hua Gu 2002-6-26-b*</i> ; <i>Ye, Hua Gu s.n.</i>	AY626884	
<i>Eurya yaeyamensis</i> Masamune	Ryukyus	3	<i>Tsou et al. 1282, 1540*</i>	AY626885	1 site: <i>Tsou et al. 1439</i>
<i>Adinandra dumosa</i> Jack	SE Asia	1	<i>Tsou 1539*</i>	AY626844	
<i>Adinandra elegans</i> How & Ko ex Chang	S China (Fujian)	1	<i>Tsou 1584*</i>	AY626845	
<i>Adinandra formosana</i> Hayata	Taiwan	3	<i>Tsou 827 (AF089713)</i> , <i>Tsou et al. 1493, 1546*</i>	AY626846	
<i>Adinandra lasiostyla</i> Hayata	Taiwan	2	<i>Tsou et al. 1334, 1346*</i>	AY626847	
<i>Adinanda milleitii</i> (Hook. & Arn.) Benth. & Hook. Ex Hance	SE & SW China (Fujian)	2	<i>Tsou et al. 1799*</i> , 1889	AY626848	
<i>Adinandra yaeyamensis</i> Ohwi	Ryukyus	1	<i>Tsou et al. 1303*</i>	AY626849	
<i>Anneslea fragrans</i> Wall. var. <i>lanceolata</i> Hayata	Taiwan	1	<i>Tsou et al. 1099*</i>	AY626887	
<i>Cleyera japonica</i> Thunb.	Taiwan, SE & SW China (Fujian), Japan, Ryukyus	3	<i>Tsou et al. 1296*</i> , 1446, 1631	AY626850	
<i>Cleyera morii</i> Masamune	Taiwan	2	<i>Tsou et al. 858*</i> , 1272	AY626851	
<i>Euryodendron excelsum</i> Chang	S China	1	<i>Tsou et al. 1590*</i>	AY626886	
<i>Ternstroemia gymnanthera</i> (Wight et Arn.) Sprague	Worldwide distributed (J. R. T. & F.)	2	<i>Tsou et al. 1098*</i>	AY626888	1 site: <i>Tsou et al. 1304</i>
<i>Ternstroemia kwangtungensis</i> Merr.	SE China (Fujian), Vietnam	1	<i>Tsou et al. 1585*</i>	AY626889	
<i>Ternstroemia luteoflora</i> Ling	SE & SW China (Fujian)	2	<i>Tsou et al. 1587*</i>	AY626890	2 sites: <i>Tsou et al. 1842</i>
<i>Ternstroemia microphylla</i> Merr.	SE China (Fujian)	1	<i>Tsou et al. 1586*</i>	AY626891	

Notes: Three ITS sequences obtained from the GenBank are AY096023 (*Adinandra latifolia*), AY096025 (*Cleyera pachyphylla*), and AF396453 (*Ternstroemia impressa*).

TabF (Taberlet et al., 1991). The PCR program was set as 94°C for 5 min followed by 30 cycles of 94°C for 1 min, 49°C for 1 min, 72°C for 1 min 30 sec, and a single cycle of 72°C for 7 min. Sequencing was done by using primer TabC and TabF, when necessary, TabD and TabE primers were used in addition (Taberlet et al., 1991).

***trnH-psbA*.** Amplification of the *trnH-psbA* intergenetic spacer was done by using primers *trnH* (Tate and Simpson, 2003) and *psbA* (Sang et al., 1997). The PCR program was set as 96°C for 5 min followed by 35 cycles of 96°C for 50 sec, 53°C for 1 min, 72°C for 30 sec, and a single cycle of 72°C for 5 min. Sequencing was done by using *trnH* and *psbA* primers.

PCR products were cleaned with the QIAquick PCR purification kit (Qiagen, Valencia, California, USA). Sequencing was done on an ABI 377 automated DNA sequencer.

**Sequence alignment.** Sequences were assembled from both directions and ambiguous sites were checked against the electropherograms. All the sequence data were aligned with the GCG program (Wisconsin Package Version 10.3) and then adjusted manually by using Se-AL (Rambaut, 1996).

### Phylogenetic relationship analysis

Cladistic analysis was performed with Maximum Parsimony by using PAUP\* 4.0b10 (Swofford, 2002) and Bayesian analysis by using MrBayes version 3.0b4 (Huelsenbeck and Ronquist, 2001). In the parsimony analysis all characters were unordered and weighted equally (Fitch, 1971). The data matrix was analyzed by employing an heuristic tree search with 1000 replicates with stepwise to create the initial trees, and a sequence addition and tree-bisection-reconnection (TBR) branch swapping were set. The MaxTrees was set to 10000. Relative support was estimated with the bootstrap option in PAUP\* employing a heuristic search with 1000 replicates. In the Bayesian analysis, MrModel test version 3.6 (Posada and Crandall, 1998) was adopted to estimate the parameters, the Markov chain Monte Carlo algorithm was set as four simultaneous chains, and a tree was saved every 5000 generations. Two million generations were performed in each analysis. Trees from the burn-in period were discarded, and a 50% majority rule consensus tree was constructed from the remaining trees.

## RESULTS

### Chloroplast DNA sequences

The *psbA-trnH* sequences of the 20 *Eurya* species examined were uniformly 416 bp long and 16 of them showed exactly the same sequence (Table 2); as for the other four species, *E. acuminata* and *E. groffii* had two single substitution mutations each, and *E. glaberrima* and *E. distichophylla* one substitution mutation (Table 2). The total six variations were singletons. Sequencing of the *trnL-trnF* region was carried out in four distantly

distributed *Eurya* species (Table 2). Three of them showed identical *trnL-trnF* sequences. As for the fourth species, it had a deletion of 27 bp. Both *psbA-trnH* and *trnL-trnF* regions seemed unsuitable for the phylogenetic analysis of *Eurya*, this part of sequencing work was thus intermitted.

### Nuclear ITS sequences

The length of ITS in the six genera of Ternstroemiaceae ranged from 632 to 648 bases before alignment and was 673 after alignment. Within *Eurya* the length was fairly uniform, with 637 bases long in 27 species, 638 bases in three species, and 632 in one; this segment included the ITS1 247-248 bases long, 5.8S 164 bases long, and ITS2 221-227.

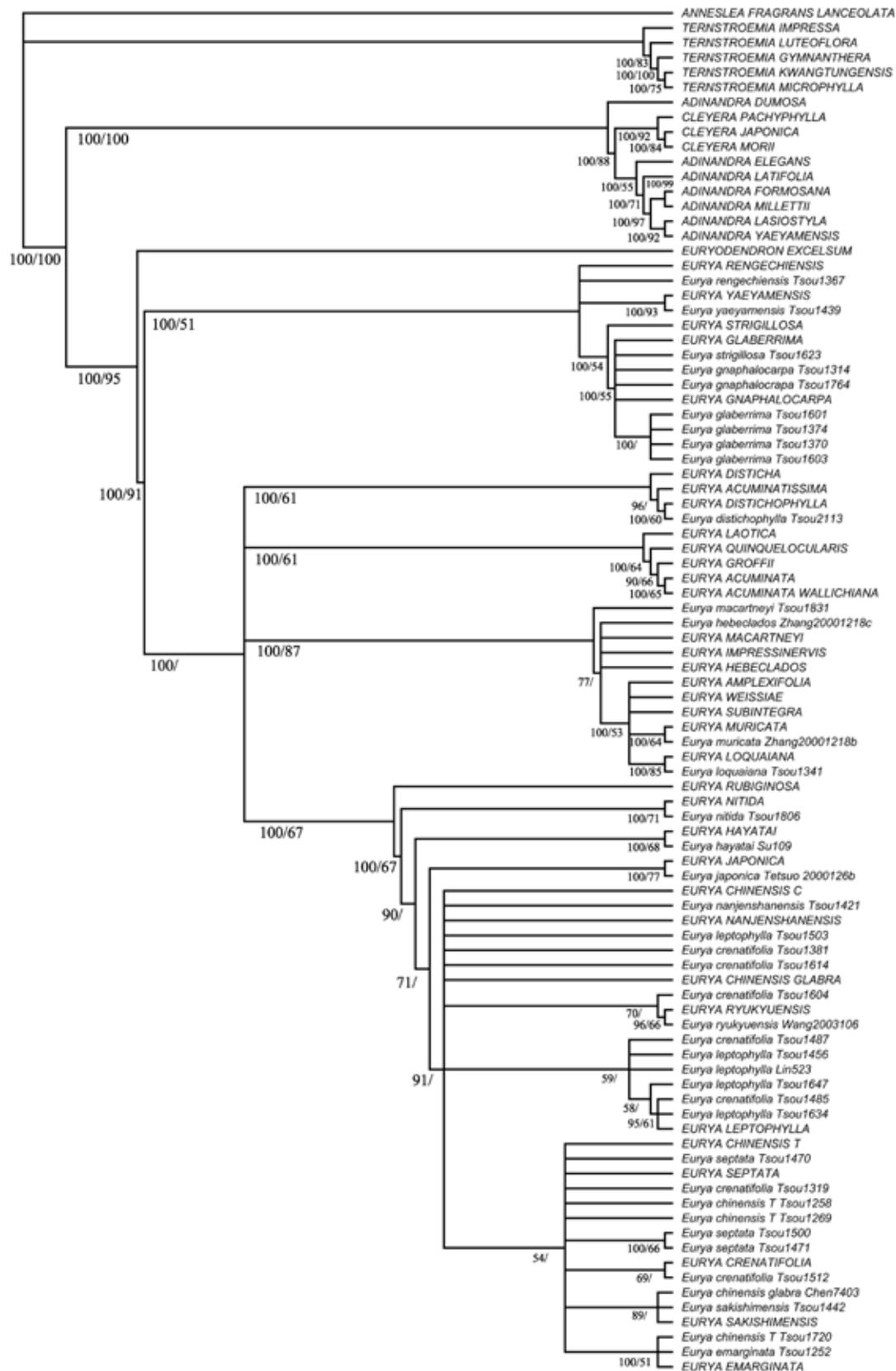
### ITS sequence variation

The number of con-specific samples of *Eurya* species varied from one to 27. For the species with two or more samples, limited intraspecific variation did exist in most of them; nevertheless, the representative sequence of each species, which was generated from at least two con-specific samples, usually emerged within a small sample size, and the other con-specific sequence types differed from the representative at only one to three sites (Table 1). Most of these intraspecific variations were independent, single substitution mutations within the intron and a small percentage were insertions or deletions. The exceptions were found in *E. crenatifolia* and *E. leptophylla* where their ITS sequences were much variable. It is worthy to mention that *Eurya chinensis* was much extensively surveyed (20 samples) because its morphological variations were great and many individuals with different features were sampled (Table 1); but it turned out that ITS sequence was highly conserved among the con-specific samples of *E. chinensis* that seven out of the 10 samples from Taiwan exhibited the same type (*E. chinensis*-T) and all nine samples from Hong Kong and Guangdong, China showed the same type (*E. chinensis*-C). These two dominant sequence types, *E. chinensis*-T and *E. chinensis*-C differ at only one site that the former has a C whereas the latter has a T at base number 200 in the aligned matrix (Table 2). It is important to note that a C at base 200 characterizes all the samples of Taiwanese *E. chinensis*, *E. emarginata*, and *E. septata* and the Ryukyu's *E. sakishimensis*; whereas a T is found in the remaining species. Further discussions on this point will be given in Discussion. In the combined data matrix including the representative sequences of the 32 *Eurya* species, the total variation site is 62 bases in the aligned matrix (Table 2).

### ITS distance within *Eurya*

The distance between any two of the 32 *Eurya* species studied ranges from 0 to 16 bases, under the condition that the representative sequences are compared (Tables 1, 2). Identical ITS sequences were found in two pairs of species, *E. chinensis*-T and *E. septata*, and *E. chinensis*-C and *E. nanjenshanensis*. These two pairs actually differ





**Figure 2.** The gene tree based on 92 ITS sequence types produced from 49 species of six genera. This is the MajRule tree generated from the parsimony analysis, with total characters=673, informative characters=148, tree length=347, CI=0.6945, RI=0.9132, and 10000 trees retained. Numbers showing on the branches are percentage of the presence of the clade among the 10000 trees (numerator) and bootstrap values larger than 50 (%) (denominator). The bootstrap values were based on 800 replicates.

is supported. The species trees (Figures 3, 4) were then produced based on the 52 representative sequences which representing 49 species (Table 1) by using the most parsimony and Bayesian analyses. In general, the MajRule gene tree and the two species trees are highly congruent in their topologies (Figures 2, 3, 4). Their common and important points revealed in these trees are:

1. Among the ingroups, *Adinandra* and *Cleyera* together form a clade with 100% bootstrap support, this clade is sister to *Euryodendron* and *Eurya*. The three species of *Cleyera* are embedded within the seven *Adinandra* species, further consideration on the unification/separation of these two genera is necessary. The two *Adinandra* species endemic to Taiwan, *A. formosana* and *A. lasiostyla*, differing at 19 sites, are not the closest to each other. The lowland *A. formosana* forms a clade with the SE China distributed *A. millettii*, with only one site in difference; the highland *A. lasiostyla* forms a clade with the Ryukyu endemic *A. yaeyamensis*, with six sites different, but sharing seven substitution mutations of their own.
2. *Euryodendron*, a monotypic genus, is sister to and the closest to the *Eurya* clade. The status of *Euryodendron* as a monotypic genus is strongly supported in this study.
3. *Eurya*, with 32 species studied, appears as a monophyletic genus. The 32 species unanimously form five clades and the allocation of these species in five clades is exactly the same in all these MajRule trees (Figures 2, 3, 4). The relationships of these five clades are better revealed in MP trees than in Bayesian trees since the five clades are arranged in three levels in the former, but are parallel in the latter. These five clades possess strong geographical constraints and are thus defined:
  - (1) Ryukyu-Taiwan (RT) endemic group — this clade is sister to the other four clades in MP MajRule trees (Figures 3, 4). Five species are included, with *Eurya yaeyamensis* endemic to the Ryukyus, and *E. reneichiensis*, *E. strigillosa*, and *E. glaberrima* endemic to Taiwan. *Eurya gnaphalocarpa* is reported as also occurring in the Philippines and Taiwan (Ling, 1998). Among these five species, the former two are sister to the latter three.
  - (2) SE Asia-SW China group — four species are included, *E. acuminata*, *E. laotica*, and *E. groffii* with a distribution range in SE Asia and *E. quinquelocularis* in SW China. It is interesting that *E. acuminata wallichiana* shows intermediate sequencing between *E. groffii* and *E. acuminata* (Table 2).
  - (3) S China group — *E. acuminatissima*, *E. disticha* and *E. distichophylla* form a small clade, they mainly distributed in the southern provinces of China.
  - (4) SE China group — eight species, viz., *E.*

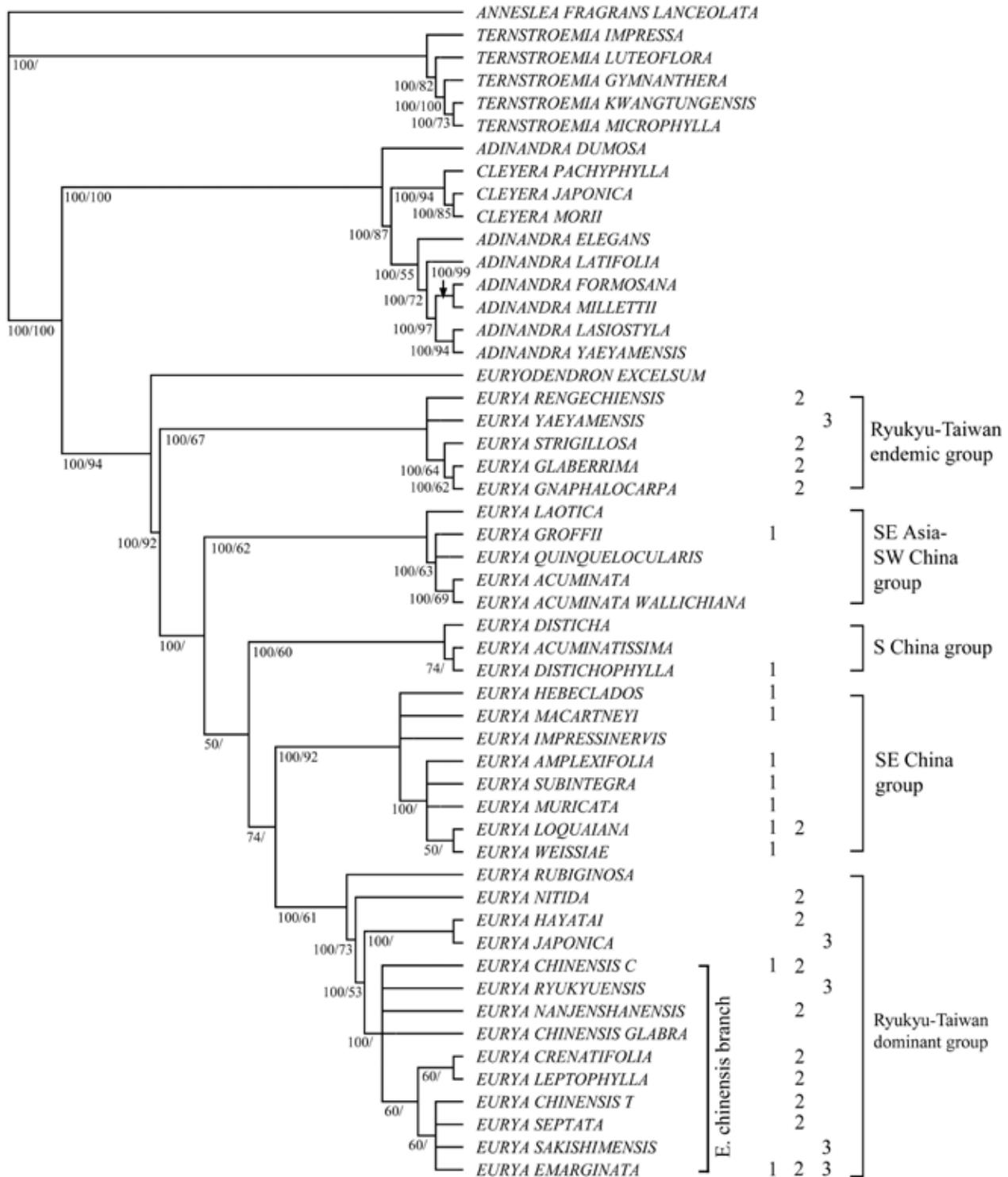
*amplexifolia*, *E. herbeclados*, *E. impressinervis*, *E. loquaiana*, *E. marcartneyi*, *E. muricata*, *E. subintegrifolia*, and *E. weissiae* form a well supported clade. All these species are confined to SE China except that *E. loquaiana* also extends to Taiwan.

- (5) Ryukyu-Taiwan (RT) dominant group — twelve species are included in this clade. The most basal one, *E. rubiginosa*, is only present in Guangdong, China. At the next level is *E. nitida*, occurring in continental China and Taiwan. Among the remaining 10 species, a clade consisting of *E. hayatai* and *E. japonica* is sister to the other eight species. *Eurya japonica* occurs in continental China and Japan and *E. hayatai* is endemic to Taiwan. The remaining eight species are endemic to either Taiwan or the Ryukyus except for the widely distributed *E. chinensis*. Since *E. chinensis* stands as the center of these eight species, as the sequences are concerned, these eight are collectively termed as an *E. chinensis* branch. This *E. chinensis* branch is successfully established in Taiwan and the Ryukyus. The sequence variations among them are so low that the resolution of this branch is very poor. It is worthy to note that in this ladder-like arrangement of this big clade, species distribution range shifts stepwise from SE China (*E. rubiginosa*) to China + Taiwan (*E. nitida*), to E China-Taiwan-Japan (*E. chinensis*, *E. hayatai*, *E. japonica*), and then to Taiwan-Ryukyu endemism (*E. crenatifolia*, *E. leptophylla*, *E. nanjenshanensis*, *E. ryukyensis*, *E. sakishimensis*, and *E. septata*).

In summary, the 32 *Eurya* species studied form five well supported clades (Figures 2, 3, 4). Seventeen out of the 18 *Eurya* species occurring in Taiwan and the Ryukyus are confined to two clades, the R-T endemic and the R-T dominant clades; these two clades are situated respectively at the most basal and the most derived positions in the MP MajRule trees. The remaining Taiwanese *E. loquaiana* is grouped with seven SE China distributed species. The 12 *Eurya* species occurring in Fujian, on the other hand, are spread in four out of the five clades.

## DISCUSSION

The nuclear ITS gene (18S-5.8S-26S) has hundreds to thousands copies arrayed as tandem repeats on the chromosomes. Its phylogenetic inference has been extensively employed by plant taxonomists (Alvarez and Wendel, 2003), but more questions on the impact of its sequence polymorphism on the phylogenetic reliability have been raised (Alvarez and Wendel, 2003; Andreasen and Baldwin, 2003; Bailey et al., 2003). In order to ascertain the suitability of ITS being used in the present study, a lot of efforts were made to collect and sequence multiple samples for each *Eurya* species in Taiwan and neighboring regions. We sequenced 3-27



**Figure 3.** Species tree one based on 52 ITS sequence types representing the total 49 species. This is the Majrle tree generated from the parsimony analysis, with total characters=673, informative characters=141, tree length=309, CI=0.6958, RI=0.9035, and 121 trees retained. Bootstrap supports higher than 50% are shown beneath the branches. Numbers showing on the branches are percentage of the presence of the clade among the 121 trees (numerator) and bootstrap values larger than 50 (%) (denominator). The bootstrap values were based on 1000 replicates. The five clades of the *Eurya* species are defined, and the Arabic numerals 1, 2, and 3 are designated to the distribution range covering Fujian, Taiwan, and the Ryukyus, respectively.



**Figure 4.** Species tree two with the same dataset as species tree two. This is the MajRule tree generated from the Bayesian analysis. The posterior probabilities higher than 50% are shown beneath the branches. Asterisks (\*) indicate the presence of anther septation, two species are unknown in this regard and marked with a “?”, the remaining species have no anther septa.

samples for every *Eurya* species in Taiwan and most species in the Ryukyus; in general, for most of these species a specific and dominant sequence type was found after a small sampling. Identical ITS sequences are commonly obtained from con-specific samples collected from different countries/islands (e.g., *E. chinensis*, *E. emarginata*, *E. japonica*, *E. macartneyi*, *E. nitida*, *E. ryukyuensis*, and *Cleyera japonica*) or from localities hundreds kilometers away (e.g., *E. glaberrima*, *E. loquaiana*, *E. septata*, *E. strigillosa*, and *Adinandra formosana*) (Table 1, Appendix 1). As for con-specific samples collected from the same population, their ITS sequences are even more homogeneous. The tendency of retaining a specific ITS sequence type within a species resulting from the concerted evolution has been reported in many plant groups (Wendel et al., 1995; Sang et al., 1995); we believe that concerted evolution is affecting *Eurya* as well. In addition, in our integrated analysis, all the 92 sequence types generated in this study were incorporated to produce the gene tree (Figure 2), the intraspecific sequence variations of most species represent shallow paralogy which indicates that they would not adversely affect species tree reconstruction (Figures 2, 3, 4). In conclusion, we believe that ITS sequence is reliable for this phylogenetic study of *Eurya*. In the *E. chinensis* lineage the interspecific sequence variations are usually low or even none; whereas the intraspecific variations of *E. chinensis*, *E. crenatifolia*, and *E. leptophylla* are greater than the interspecific variations; thus the sequence types of these three species may mix with a few other species in several small clades. The much greater intraspecific sequence variation of two Taiwan-endemic species, *E. crenatifolia* and *E. leptophylla*, might be due to their slower concerted evolution relative to mutation rates and incomplete lineage sorting, etc. (Andreasen and Baldwin, 2003). It is noteworthy to point out that the dominant sequence type of the species with multiple samples is also the consensus sequence for each species. They were chosen to be registered in the GenBank and used for interspecies interpretations (Table 1).

### Taxonomic implications

The genus *Eurya* has a rather large number of species, c. 130; and criteria used for discriminating species are poor and often microscopical. The present sequencing work shows that the ITS sequences of the 32 *Eurya* species differ at 62 sites (Table 2) and the greatest distance between two species is 16 mutations, which is potentially useful for taxonomic treatment of *Eurya* at theoretical and practical levels. During the course of this study, a few taxonomic problems were resolved by using the ITS sequencing data:

1. Taxonomic treatments of *E. chinensis* in Hong Kong. *Eurya chinensis* and *E. nitida* are the most common *Eurya* species in Hong Kong and sympatric in numerous sites. Usually they can be easily distinguished by the thick pubescence and the smaller leaves in *E. chinensis* and the lack of pubescence and slightly larger leaves in *E. nitida*; however, in many windward mountainous sites, abundant typical (hairy) *E. chinensis* individuals are mixed with a small percentage of glabrous individuals and the latter are not readily distinguishable from *E. chinensis* or *E. nitida*. Sometimes, there are hairy individuals, but with much smaller (c. 1/2 of that of *E. chinensis*) and thicker leaves, which are similar to the Taiwan-endemic *E. crenatifolia* in gross morphology. Local taxonomists use the name, *E. chinensis* var. *glabra* to the glabrous individuals and *E. chinensis* var. *chinensis* to the small-leaved ones (Lai et al., 2004). Owing to the lack of any further studies, we sequenced eight individuals of *E. chinensis* from Hong Kong including the three phenotypes (typical, glabrous, and small-leaved). Interestingly, their ITS sequences came out the same as the *E. chinensis*-C type collected from Guangdong, China (Table 1). The results strongly support the taxonomic treatment of these phenotypes under *E. chinensis*. In addition, this preliminary study suggests that *E. chinensis* possesses great morphological plasticity and may well sustain severe environments where other *Eurya* species can not. The populations surviving in the harsh conditions may express greater morphological diversity.
2. Do *E. acuminata* and *E. japonica* occur in Taiwan? In the most recent treatments of *Eurya* in Taiwan, i.e., Flora of Taiwan (Hsieh et al., 1996), Manual of Taiwan Vascular Plants (Liu et al., 1998), and Flora of China (Ling, 1998), the recognition of *E. acuminata* and *E. japonica* is controversial. The distribution of *Eurya acuminata* in Taiwan is reported in the first and the third treatments; and that of *E. japonica* in the third. Nonetheless, our samples of *E. acuminata* var. *acuminata* collected from Singapore and *E. acuminata* var. *wallichiana* from N Thailand are well separated from Taiwanese *Eurya* in both morphological and ITS sequence aspects (Table 2). Plants in Taiwan previously determined as *E. acuminata* were mostly of *E. loquaiana*, probably due to their similar acuminate leaf apices. For clarifying the issue of *Eurya japonica*, samples were collected from Zhe-Jiang Province, China and Okinawa Island, Japan and they showed the same morphological characteristics and identical ITS sequences (Table 1, Appendix 1); but in the sequence aspect, *E. japonica* differs from those *Eurya* in Taiwan at five sites (*E. hayatai* and *E. nitida*) or more. Among the Taiwanese *Eurya*, the endemic *E. hayatai* is often misidentified as *E. japonica* because they are similar morphologically; with the major distinction on the leaf lower surface where the venation is clear in *E. japonica*, but nearly invisible in *E. hayatai*. These two species are so far closely related as suggested by the species trees (Figures 3, 4). In this study, ITS sequencing work helps to delete the presence of *E. acuminata* and *E. japonica* in the current flora of Taiwan.
3. Taxonomic status of *Eurya ryukyuensis*, *E. nanjenshanensis*, and *Cleyera morii*. *Eurya*

*ryukyuensis*, a Ryukyu-endemic taxon, was first published as an independent species by Masamune in 1935, but was transferred to a variety of *E. emarginata* by Hatushima in 1956 which was followed in all the important floras of that area, such as Flora of Okinawa and the Southern Ryukyu Islands (Walker, 1976) and Check List of the Vascular Flora of the Ryukyu Islands (Shimabuku, 1997). ITS sequencing data shows that *E. ryukyuensis* is different from all the other *Eurya* species studied. The ITS distance from *E. ryukyuensis* to *E. emarginata* is four sites and the shortest distance is found in *E. chinensis* (sequence type of *E. chinensis*-C), with two different bases (Table 2). The treatment of *E. emarginata* var. *ryukyuensis* is thus not supported. *Eurya nanjenshanensis* was published as *E. nitida* var. *nanjenshanensis* in Flora of Taiwan (Hsieh et al., 1996), mainly due to the glabrous feature; but was then recognized as a distinct species in the Manual of Taiwan Vascular Plants (Liu et al., 1998). The ITS sequence of *E. nanjenshanensis* differs from that of *E. nitida* at four sites, but is the same as the *E. chinensis*-C sequence type. Again, the treatment of *E. nitida* var. *nanjenshanensis* is not supported. *Cleyera morii*, a Taiwan endemic, was first published as a variety of *Eurya*, *Eurya ochracea* Szyzy. var. *morii* Yama. by Yamamoto in 1927. It was then transferred to *Cleyera japonica* Thunb. var. *morii* (Yama.) Masa. by Masamune (1935) and then changed to *C. morii* (Yama.) Masa. in 1939. Nonetheless, *C. japonica* var. *morii* was preferred by Kobuski (1938) and adopted in later important treatments, such as Flora of Taiwan, the First Edition (Li, 1976) and the Second Edition (Hsieh et al., 1996), the Manual of Taiwan Vascular Plants (Liu et al., 1998), and Flora of China (Ling, 1998). Our three samples of *C. japonica* collected from the Ryukyus and Taiwan have identical ITS sequence and the two samples of *C. morii* collected from two populations in Taiwan have identical sequences as well. These two species differ at five sites, which strongly supports an independent species status of *C. morii*, in addition to their morphological distinctions (Hsieh et al., 1996). In summary, the present sequencing work supports the independent species status of *E. ryukyuensis* and *E. nanjenshanensis*. Also, *Cleyera morii*, differing from *C. japonica* at five sites, also deserves the status of a species.

4. Taxonomic value of anther septation in *Eurya*. Large scaled taxonomic studies on *Eurya* have been poor; the most recent is by Ling (1998) in the Flora of China. Since his earlier revision (1966) on *Eurya*, Ling has strongly stressed the importance of anther septa and even classified the genus as two subgenera based on this single criterion. This work, though the sampling covers only one fourth of the total number of species, shows that anther septa could have evolved from nonseptate ancestors in different lineages independently (Figure 4). We assume that the evolution of anther septa in *Eurya* is possibly associated with pollination advantages, i.e.,

retaining pollen grains in the anthers in the up-side-down floral orientation. Taxonomic applications of this character in *Eurya* classification are better restricted to species discrimination.

### Phylogeography of *Eurya* of Taiwan

Our study included all the 13 species of *Eurya* occurring in Taiwan, five of the seven (71%) occurring in the Ryukyus, and 12 of the 18 (67%) in Fujian Province, China, and several others from SW China and SE Asia. Increasing the number of species and the regions of sampling will certainly give better resolution of the interspecies relationships. Nonetheless, the relationships of the species occurring in Taiwan and the Ryukyus that they constitute two coherent but distantly related groups plus one isolated species (*E. loquaiana*) is strongly indicated. First, the intimacy among the five species in the RT endemic clade and that among the eight in the RT dominant clade are obvious by the high ITS sequence similarity and the possession of several synapomorphic sites within each clade, which is also reflected in the high bootstrap value. Second, the distant relationship between these two clades as shown in Table 2 and Figures 3 and 4 can not be refuted because members of these two groups constantly have 10 or more different sites at the ITS region. Thirdly, *E. loquaiana* is closely associated with a few members of SE China clade with a strong support of 92% bootstrap value (Figure 4). Its difference from other *Eurya* species in Taiwan ranges from 10 to 15 sites (Table 2) and its isolated position within the *Eurya* in Taiwan is definite. Phylogenetically, *Eurya* species of Taiwan and the Ryukyus form two coherent, but distantly related groups, plus one isolated species, is distinct and unlikely to be altered even when more samples are analyzed.

On the basis of the species relationships of *Eurya*, which we here revealed, a few phylogeographical points can be deduced:

1. Among the seven *Eurya* species occurring in the Ryukyus, five are here studied. They are either embedded in the RT endemic clade (e.g., *E. yaeyamensis*) or in the RT dominant clade (e.g., *E. emarginata*, *E. japonica*, *E. ryukyuensis*, and *E. sakishimensis*). The remaining two *Eurya* species yet studied are rare and endemic *E. osimensis* and *E. zigzag*. Morphologically, *E. osimensis* is very similar to the Taiwan-endemic *E. strigillosa* in general features and especially in possessing yellowish, long (c. 0.6-0.8 mm) hairs on terminal buds and branches; it has even been treated as a variety of the latter by Masamune (1955). *Eurya zigzag* is very similar to another Ryukyu-endemic *E. yaeyamensis* which is embedded in RT endemic clade grouped with four species from Taiwan. Generally speaking, the majority of *Eurya* in the Ryukyus show close affiliations with the *Eurya* in Taiwan. But there are other facts to be considered. First, the endemic rate of the *Eurya* in the Ryukyus is very high (5/7); second, *E. japonica* distributes in E.

China, Japan, and Korea, but not in Taiwan. Thirdly, the only *Eurya* species shared by the Ryukyus and Taiwan, i.e., *E. emarginata*, is probably of avian seed dispersal because it is distributed in the coastal regions of many islands in SE China, Taiwan and the Ryukyus; thus its coexistence in these two regions does not imply any pre-existing land bridges between the two regions. And fourthly, no *Eurya* species is currently distributed to the Ryukyus and Taiwan only. In conclusion, our preliminary analysis suggests that the rather close linkage between the *Eurya* of the Ryukyus and Taiwan was based on ancient interactions; the prolonged isolation of the Ryukyus probably since the middle of Pleistocene (Kimura, 2000) results in the very high endemism of *Eurya*. No recent interactions through land bridges between the Ryukyus and Taiwan can be identified in the case of *Eurya*.

2. The phylogeography of the *Eurya* in Taiwan is a little more complicate. The four species in RT-endemic clade show comparatively close associations with the Ryukyu members, which is probably caused by ancient interactions. Some species (e.g., *E. hayatai*, *E. loquaiana*, *E. nitida*) show affiliations with extant SE China or E China members. And, the remaining six species, including four endemic species, the SE China-Taiwan distributed *E. chinensis*, and *E. emarginata*, form an intimate lineage, with *E. chinensis* as the center (the *E. chinensis* lineage) (Figures 3, 4). These nine species, as a whole, are connected with the current continental progenitors. In this connection, the origin of the four endemic species in the *E. chinensis* lineage can be discussed. *Eurya crenatifolia*, *E. leptophylla*, *E. nanjenshanensis*, and *E. septata* possess identical ITS sequences to that of *E. chinensis*, either to the representative of SE China (*E. chinensis*-C) or Taiwan (*E. chinensis*-T). As we suggested in the Results, *E. chinensis* has great ability in living in diverse habitats and developing great morphological diversities. It is possible that during the several Pleistocene glacial periods when Taiwan was connected with Fujian (Zhao, 1982; Shi et al., 1986; Hsieh and Shen, 1994; Voris, 2000), *E. chinensis* as a member of SE China flora could have migrated to Taiwan several times. Speciation originated from *E. chinensis* might have taken place in different ecological habitats and at different geological times, and then gave rise to these four Taiwan-endemic taxa. *Eurya crenatifolia*, characterized by tiny and thick leaves, is adapted to windy and humid environment, mainly in northern Taiwan; *E. leptophylla*, characterized by small and thin leaves, is restricted to higher elevations; *E. nanjenshanensis*, with glabrous and slightly obovate leaves, is found in the tropical southern lowlands; and *E. septata*, differing from *E. chinensis* in having septate anthers and slightly larger leaves, can well survive in disturbed habitats (Wu et al., 2003). As for the other three Taiwan-endemic species, *E. glaberrima*, *E. hayatai*, and *E. strigillosa*, since their closest

relatives are not yet evident, no interpretations on their endemism can be provided.

In summary, this study shows that the current composition of the *Eurya* in Taiwan is derived from various sources, which may well reflect the complicate phytogeographical history of Taiwan. It has long been noticed that the highlands of Taiwan not only possess much higher rate of endemic species (Hsieh, 2002), but also retain a great number of Tertiary relics from N China (Li, 1957; Liu, 1988; Shen, 1996, 1997), whereas the lowlands of Taiwan have strong affinity with the lowlands of SE China (Hsieh et al., 1994). The rationale is that during the Tertiary the climate of N China was warm and humid. By the end of Tertiary and the beginning of Quaternary when global temperature decreased drastically and the cycles of glacial/interglacial episodes proceeded, the Tertiary flora of N China migrated southwards (Liu, 1988). A considerable percentage of this flora completely vanished in N China and SE China during the middle Pleistocene, but a great number of species were retained in the highlands in Taiwan and Japan to the east, and Yunnan, Guaxie, Sichuan Provinces of China to the west (Yang and Hsu, 1980; Liu, 1988, Shen, 1996). Such floristic similarity between SW China and the highlands of Taiwan was noticed as early as 1920 by Wilson. In our study, the RT endemic group of *Eurya* stands at the basal most position and is distant from the remaining *Eurya* in Taiwan, and it is not close to *Eurya* species from other regions, either. This group is likely to be an isolated and ancient group based on the information available. The four Taiwanese species in the RT endemic clade are restricted to the middle and upper elevated forests in the Central Mountain Range where many well known Tertiary elements are accommodated, for examples *Amentotaxus*, *Chamaecyparis*, *Fagus*, *Hedyderia*, *Juglans*, *Kalopanax*, *Keteleria*, *Taiwania*, *Taxus*, etc. (Shen, 1996). The other group of *Eurya*, the RT dominant clade, shows high affinities with *Eurya* species currently present in SE China (Fujian Province), and most of them are distributed in lowlands. Therefore, we propose that the five species of RT endemic clade are most likely Tertiary elements from N China or their direct descendants which evolved in Taiwan. The remaining nine *Eurya* species in Taiwan are mostly members or derivatives of the Quaternary flora of the SE China. Along with the uplifting of Taiwan during the late Tertiary and Quaternary, earlier immigrants tend to be found in higher elevated areas in Taiwan. Ecological partitioning is suggested as an important mechanism for the speciation of several Taiwan-endemic species from different populations of *E. chinensis* at probably different geological times.

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**Appendix 1.** Information of collection site of the 146 vouchers listed in Table 1.

Species name	Collection site
<i>Eurya acuminata</i> DC. var. <i>acuminata</i>	Singapore: Tsou 1538*.
<i>Eurya acuminata</i> var. <i>wallichiana</i> Dyer	N. Thailand: Maxwell 2004-233*; Maxwell & Euder 1.
<i>Eurya acuminatissima</i> Merr. & Chun	Hong Kong: Tsou et al. 1813*, 1814.
<i>Eurya amplexifolia</i> Dunn	China - Guangdong: Tsou 1578*.
<i>Eurya chinensis</i> R. Br. var. <i>chinensis</i>	<i>E. chinensis</i> -T Taiwan - Taipei: Tsou et al. 1269, 1327, 1330*, 1378, 1379; Nantou: Tsou et al. 1258; Pingdong: Tsou et al. 1250, 1423; Yilan, Turtle Isl.: Tsou et al. 1720. Ryukyu - Iriomote Isl.: Tsou et al. 1425.
	<i>E. chinensis</i> -C China - Guangdong: Tsou et al. 1583*; Hong Kong: Tsou et al. 1792, 1794, 1800, 1836, 1837, 1891.
<i>Eurya chinensis</i> var. <i>glabra</i> Hu et L. K. Ling	Taiwan - Yilan, Turtle Isl., Chen, C.C. 7403; Hong Kong: Tsou et al. 1802*, 1892.
<i>Eurya crenatifolia</i> (Yamamoto) Kobuski	Taiwan - Taipei: Tsou et al. 1253*, 1319, 1381, 1512; Hsinchu: Tsou et al. 1485, 1487; Taichung: Tsou et al. 1333; Yilan: Tsou et al. 1604, 1614.
<i>Eurya disticha</i> Chun	China - Guangdong: Tsou et al. 1973*, 1975.
<i>Eurya distichophylla</i> Hemsl	China - Guangdong: Tsou et al. 1577*, 2016; Fujian: Tsou et al. 2113.
<i>Eurya emarginata</i> Makino	Taiwan - Kinmen Isl.: Tsou et al. 1399, 1400*; Taipei: Tsou et al. 1252, 1495.
<i>Eurya glaberrima</i> Hayata	Taiwan - Taoyuan: Tsou et al. 1370, 1374, 1376; Taichung: Tsou et al. 1414*; Kaohsiung: Tsou et al. 1652; Yilan: Tsou et al. 1601, 1603.
<i>Eurya gnaphalocarpa</i> Hayata	Taiwan - Taichung: Tsou et al. 1331*; Nantou: Tsou et al. 1260, 1864; Yilan: Tsou et al. 1314, 1764.
<i>Eurya groffii</i> Merr.	Hong Kong: Tsou et al. 1890*, 1894.
<i>Eurya hayatai</i> Yamamota	Taiwan - Hsinchu: Su, Mong Huai 227, 282; Taichung: Tsou et al. 1626*, 1627; Kaohsiung: Su, Mong Huai 109.
<i>Eurya hebeclados</i> Ling	China - Guangdong: Tsou et al. 1944*; Zhejiang: Zhang, Wen Ju, 2000-12-18-c.
<i>Eurya impressinervis</i> Kobuski	China - Guangdong: Ye, Hua Gu 2003-5-30-a*.
<i>Eurya japonica</i> Thunb.	Japan - Nago: Tetsuo Denda 2000-12-6-a*, 2000-12-6-b; China - Shanghai: Zhang, Wen Ju 2001-9-19-a, 2001-9-19-b.
<i>Eurya laotica</i> Gagnep.	Vietnam - Sapa: Tsou et al. 1620*.
<i>Eurya leptophylla</i> Hayata	Taiwan - Hsinchu: Tsou et al. 1355*, 1356; Nantou: Lin, Chia Hua 523; Tsou et al. 1456, 1634; Kaohsiung: Tsou 1647; Taidong: Tsou 1503.
<i>Eurya loquaiana</i> Dunn	Taiwan - Hsinchu: Tsou et al. 1352; Nantou: Tsou et al. 1341; Yilan: Tsou et al. 1316*.
<i>Eurya macartneyi</i> Champ.	China - Guangdong: Tsou et al. 1579*; Hong Kong: Tsou et al. 1831, 1839.

## Appendix 1. (Continued)

Species name	Collection site
<i>Eurya muricata</i> Dunn	China - Zhejiang: Zhang, Wen Ju 2000-12-18-a*, 2000-12-18-b.
<i>Eurya nanjenshanensis</i> (Hsieh, Ling, & Yang) Yang & Lu	Taiwan - Pingdong: Tsou et al., 1419*, 1421, 1422.
<i>Eurya nitida</i> Korthals	Taiwan - Taipei: Tsou et al. 1279, 1328. China - Guangdong: Tsou et al. 1582. Hong Kong: Tsou et al. 1798, 1806, 1826*, 1868.
<i>Eurya quinquelocularis</i> Kobuski	China - Guangdong: Ye, Hua Gu 2002-6-29-b*, 2002-6-29-e.
<i>Eurya regechiensis</i> Yamamota	Taiwan - Nantou: Tsou et al. 1342*, 1367; Su, Mong Huai 241, 242.
<i>Eurya rubiginosa</i> Chang	China - Guangdong: Tsou et al. 1580*.
<i>Eurya ryukyuensis</i> Masamune	Ryukyu - Iriomote Isl.: Tsou et al. 1436*; Wang, Chun Neng 1520; Wang, Jenn Che 2003-10-6; Okinawa: Wang, Jenn Che 2003-10-5.
<i>Eurya sakishimensis</i> Hatusima	Ryukyu - Iriomote Isl.: Tsou et al. 1301*, 1442; Wang, Chun Neng, s.n.
<i>Eurya septata</i> Wu, Hsu, & Tsou	Taiwan - Taipei: Tsou et al. 1470, 1471; Nantou: Tsou et al. 1476, 1477*; Taidong: Tsou et al. 1499.
<i>Eurya strigillosa</i> Hayata	Taiwan - Nantou: Tsou et al. 1335*; Kaohsiung: Tsou et al. 1650, 1623.
<i>Eurya subintegra</i> Kobuski	China - Guangdong: Ye, Hua Gu 2002-6-29-c*; Tsou et al. 1987.
<i>Eurya weissiae</i> Chun	China - Guangdong: Ye, Hua Gu 2002-6-26-b*, Ye, Hua Gu s.n.
<i>Eurya yaeyamensis</i> Masamune	Ryukyu - Iriomote Isl.: Tsou et al. 1282, 1439, 1540*.
<i>Adinandra dumosa</i> Jack	Singapore: Tsou 1539*.
<i>Adinandra elegans</i> How & Ko ex Chang	China - Guangdong: Tsou 1584*.
<i>Adinandra formosana</i> Hayata	Taiwan - Hsinchu: Tsou et al. 1493; Taipei: Tsou 827 (AF089713), Tsou 1546*.
<i>Adinandra lasiostyla</i> Hayata	Taiwan - Nantou: Tsou et al. 1334, 1346*.
<i>Adinanda millettii</i> (Hook. & Arn.) Benth. & Hook. Ex Hance	Hong Kong: Tsou et al. 1799*, 1889.
<i>Adinandra yaeyamensis</i> Ohwi	Ryukyu - Iriomote Isl.: Tsou et al. 1303*.
<i>Anneslea fragrans</i> Wall. var. <i>lanceolata</i> Hayata	Taiwan - Pingdong: Tsou et al. 1099*.
<i>Cleyera japonica</i> Thunb.	Ryukyu - Iriomote Isl.: Tsou et al. 1296*, 1446. Taiwan - Kaohsiung: Tsou et al. 1631.
<i>Cleyera morii</i> Masamune	Taiwan - Taipei: Tsou et al. 858*, 1272.
<i>Euryodendron exclesum</i> Chang	China - Guangdong: Chung-Shan Univ., Tsou et al. 1590*.
<i>Ternstroemia gymnanthera</i> (Wight et Arn.) Sprague	Taiwan - Taipei: Tsou et al. 1098*, 1304.
<i>Ternstroemia kwangtungensis</i> Merr.	China - Guangdong: Tsou et al. 1585*.
<i>Ternstroemia luteoflora</i> Ling	China - Guangdong: Tsou et al. 1587*; Hong Kong: Tsou et al. 1842.
<i>Ternstroemia microphylla</i> Merr.	China - Guangdong: Tsou et al. 1586*.

**Appendix 2.** List of *psbA-trnH* and *trnL-trnF* samples and their accession numbers. Vouchers are deposited at HAST (Herbarium, Academia Sinica, Taiwan)

Species	Accession number	Voucher information
<b><i>psbA-trnH</i></b>		
<i>Eurya acuminata</i> DC. var. <i>acuminata</i>	AY943244	<i>Tsou 1538</i> , Singapore
<i>Eurya acuminata</i> var. <i>wallichiana</i> Dyer	AY943245	<i>Maxwell 2003-6-13</i> , N Thailand
<i>Eurya acuminatissima</i> Merr. & Chun	AY943246	<i>Tsou et al. 1813</i> , Hong Kong
<i>Eurya amplexifolia</i> Dunn	AY943247	<i>Tsou 1578</i> , Guangdong, China
<i>Eurya disticha</i> Chun	AY943248	<i>Ye, Hua Gu 2002-6-26</i> , Guangdong, China
<i>Eurya distichophylla</i> Hemsl	AY943249	<i>Tsou et al. 1577</i> , Guangdong, China
<i>Eurya emarginata</i> Makino	AY943250	<i>Tsou et al. 1400</i> , Kinmen Isl., Taiwan
<i>Eurya glaberrima</i> Hayata	AY943251	<i>Tsou et al. 1414</i> , Taichung, Taiwan
<i>Eurya gnaphalocarpa</i> Hayata	AY943252	<i>Tsou et al. 1331</i> , Taichung, Taiwan
<i>Eurya groffii</i> Merr	AY943253	<i>Tsou et al. 1890</i> , Hong Kong
<i>Eurya hayatai</i> Yamamoto	AY943254	<i>Tsou et al. 1626</i> , Taichung, Taiwan
<i>Eurya hebeclados</i> Ling	AY943261	<i>Zhang, Wen Ju, 2000-12-18-c</i> , Zhejiang, China
<i>Eurya impressinervis</i> Kobuski	AY943255	<i>Ye, Hua Gu 2003-5-30-a</i> , Guangdong, China
<i>Eurya laotica</i> Gagnep	AY943256	<i>Tsou et al. 1620</i> , Sapa, Vietnam
<i>Eurya macartneyi</i> Champ	AY943257	<i>Tsou et al. 1579</i> , Guangdong, China
<i>Eurya nitida</i> Korthals	AY943258	<i>Tsou et al. 1826</i> , Hong Kong
<i>Eurya quinquelocularis</i> Kobuski	AY943259	<i>Ye, Hua Gu 2002-6-29-b</i> , Guangdong, China
<i>Eurya rubiginosa</i> Chang	AY943260	<i>Tsou et al. 1580</i> , Guangdong, China
<i>Eurya strigillosa</i> Hayata	AY943263	<i>Tsou et al. 1335</i> , Nantou, Taiwan
<i>Eurya subintegra</i> Kobuski	AY943264	<i>Ye, Hua Gu 2002-6-29-c</i> , Guangdong, China
<i>Eurya weissiae</i> Chun	AY943262	<i>Ye, Hua Gu s.n.</i> , Guangdong, China
<i>Euryodendron exclesum</i> Chang	AY943269	<i>Tsou et al. 1590</i> , Guangdong, China
<i>Ternstroemia gymnanthera</i> (Wight et Arn.) Sprague	AY943270	<i>Tsou et al. 1098</i> , Taipei, Taiwan
<i>Ternstroemia kwangtungensis</i> Merr.	AY943271	<i>Tsou et al. 1585</i> , Guangdong, China
<i>Ternstroemia luteoflora</i> Ling	AY943272	<i>Tsou et al. 1587</i> , Guangdong, China
<b><i>TrnL-trnF</i></b>		
<i>Eurya emarginata</i> Makino	AY943273	<i>Tsou et al. 1716</i> , Turtle Is., Taiwan
<i>Eurya glaberrima</i> Hayata	AY943274	<i>Tsou et al. 1603</i> , Yilan, Taiwan
<i>Eurya strigillosa</i> Hayata	AY943275	<i>Tsou et al. 1650</i> , Pingtung, Taiwan
<i>Eurya acuminata</i> var. <i>wallichiana</i> Dyer	AY943276	<i>Maxwell 2004-233</i> , N Thailand

# 台灣產厚皮香科柃木屬植物的親緣及分類—利用 ITS 片斷的 DNA 序列來推論

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柃木屬係厚皮香科中最大的一屬。台灣的柃木屬含十三個種，是低海拔至高海拔許多森林中在數量上相當重要的一群。為了要了解台灣柃木屬的種間關係及其生物地理史，我們利用比較 ITS DNA 序列的方法研究了採自台灣、琉球、中國大陸及東南亞三十二種柃木，一百二十三個樣品。厚皮香科中另外五個屬十六個種亦同時比較。所得的分子親緣樹顯示本次研究的 32 個柃木種構成一單源的一支，其下則分為五個具有地理關係的小群。採自台灣及琉球的十七個種之中十六種，即除了細枝柃木之外，分屬於這五群中的二群。一群僅含五個種，而四個即為台灣/琉球特有種，居於較孤立而古老的位置。我們推論這一群可能係來自中國北方第三紀植群的一些份子或它們直接的後代。另一群含十一種則居於源起較晚的位置，並顯示與現今華南地區柃木有較親近的關係。我們推論這一支可能係第四紀時由華南而來，而其中四個台灣的特有種則可能係米粹柃木分布在不同生態環境，經由生態區格的種化機制而產生。

**關鍵詞：**柃木屬；ITS；厚皮香科；親緣關係；台灣；琉球。