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Research Article

Molecular phylogeny of *Ficus* section *Ficus* in China based on four DNA regions

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Abstract We carried out molecular phylogenetic analyses to examine the phylogenetic relationship of *Ficus* section *Ficus* (Moraceae) based on 22 species, 73 samples of the section, and 37 species, 41 samples representing other sections of *Ficus*. Four DNA sequences from the nuclear ribosomal internal transcribed spacer and the plastid *trnH-psbA*, *psbK-psbI*, and *atpF-atpH* intergenetic regions were selected. Phylogenetic analyses using maximum parsimony, maximum likelihood, and Bayesian methods revealed that section *Ficus* was not monophyletic unless subsection *Ficus* was removed and that subsection *Frutescentiae* was monophyletic when excluding species such as *F. tikoua* Bureau, *F. pedunculosa* Miq., and *F. neriifolia* Sm. *Ficus tikoua* should be transferred from subgenus *Ficus* to subgenus *Sycomorus*. *Ficus tuphapensis* Drake should be transferred into subsection *Frutescentiae* from section *Eriosycea*. Our results also supported the placement of *F. henryi* Warb. ex Diels and *F. subincisa* Buch.-Ham. ex Sm. in subgenus *Sycidium.*

Key words *atpF-atpH*, ITS, phylogeny, *psbK-psbI*, section *Ficus*, *trnH-psbA*.

Ficus L. (Moraceae) is one of the largest and most diverse genera of flowering plants, with over 700 species of terrestrial trees, shrubs, hemi-epiphytes, climbers, and creepers occurring in most tropical and subtropical forests throughout the world (Berg, 1989; Berg & Corner, 2005), and figs are thought to be key ecological species of tropical ecosystems (Janzen, 1979; Leighton & Leighton, 1983; Lambert & Marshall, 1991; Korine et al., 2000). The species of this genus share such traits as a distinctive inflorescence (syconium) and pollination syndrome with fig wasps (Corner, 1940; Janzen, 1979; Wiebes, 1979; Berg, 1989, 1990; Berg & Wiebes, 1992). The interactions between figs and their pollinators perhaps represent the most specialized mutualism, considered a model system for the study of coevolution (Jousselin et al., 2003).

Corner (1965) published the first modern and comprehensive *Ficus* classification comprising four subgenera (*Ficus* L., *Urostigma* (Gasp.) Miq., *Sycomorus* (Gasp.) Miq., and *Pharmacosycea* (Miq.) Miq.) based on characters of leaf anatomy (e.g. distribution of cystoliths), staminate flowers, pistillate flowers, and fruitlets. Later, Berg (1989) regrouped the main subdivisions of Corner's classification on the basis of not only morphological features, but also traits related to reproduction and pollination systems. He recognized two main groups in *Ficus*: the *Pharmacosycea–Urostigma* group (comprising subgenera *Pharmacosycea* and *Urostigma*); and the *Ficus–Sycidium–Sycomorus* group (comprising subgenera *Ficus* and *Sycomorus*), which provided great insights for his later revision (Berg, 2003a). Berg (2003a) divided the genus *Ficus* into six subgenera based on the previous molecular phylogenetic analyses (Herre et al., 1996; Weiblen, 2000), pollination systems (Berg & Wiebes, 1992; Wiebes, 1994, 1995), and morphological characters. Compared with the classification of Corner (1965), it included two more subgenera, *Sycidium* (Miq.) Mildbr. & Burret and *Synoecia* (Miq.) Miq., and one expanded subgenus *Sycomorus*, species of which were all transferred from subg. *Ficus*. By that, subg. *Ficus* became the smallest group of dioecious species (Berg, 2003b) including ca. 57 species divided into two sections. Section *Ficus* L. comprises ca. 30 species, most of which occur in the Sino-Himalayan region, some extending to NE Africa, the Mediterranean, Korea, and Japan (Berg & Corner, 2005), and are elements of *Flora of China* (Zhou & Gilbert, 2003). This section includes shrubs or small trees with a usually whitish indumentum, 2–4 stamens, and a lamina with cystoliths, and is subdivided, according to the shape of lamina and the position of staminate flowers, into two subsections

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Ficus L. (lamina cordate to ovate and palmately -lobed to -fid; staminate flowers mostly near the ostiole) and *Frutescentiae* Sata (lamina with entire margins; staminate flowers mostly scattered). Some species (e.g. *F. deltoidea* Jack and *F. oleifolia* King) are epiphytic or epilithic. Other species (e.g. *F. ischnopoda* Miq.) are primarily rheophytic or live in steambeds. This section is morphologically variable: several species, such as *F. carica* L., *F. pyriformis* Hook. & Arn., *F. ischnopoda*, and *F. variolosa* Lindl. ex Benth., can be easily distinguished from their close relatives; but to others in subsect. *Frutescentiae*, the major morphological characters used to distinguish such species (the shape, size, and texture of lamina or stipules; length of petiole; length of peduncule; position of staminate flower; color, diameter of the syconia; attenuation extent of the inflorenscence base) are so changeable that they may overlap among species, making it difficult to delimit. At present, there are no comprehensive phylogenetic studies on section *Ficus*, and only nine species in total were involved in previous studies (e.g. Herre et al., 1996; Jousselin et al., 2003; Rønsted et al., 2005; Baraket et al., 2009; Renoult et al., 2009; Azuma et al., 2010; Roy et al., 2010; Zerega et al., 2010; Xu et al., 2011). As China is the diversity centre of sect. *Ficus* (ca. 75% natively occurring in China), phylogenetic research fully utilizing Chinese species of this section is helpful to understand the phylogenetic relationship of the inter- and intrasections of subg. *Ficus* distributed in other regions, to deepen the understanding of the history of fig–fig wasp coevolution, and to provide support for taxonomic studies.

The internal transcribed spacer (ITS) region of nrDNA possesses moderate interspecific varition and has been proved to be the primary source of characters for phylogenetic analyses at lower taxonomic levels (Baldwin et al., 1995). However, according to previous studies in *Ficus*, more sequences are needed to analyze for a reliable *Ficus* phylogenetic tree. Our recent *Ficus* barcoding work verified that there are proper levels of informative sites in the plastid *trnH-psbA*, *psbK-psbI*, and *atpF-atpH* intergenic regions (Li et al., 2012). The intent of this study is to explore the value of the three plastid markers in low level phylogenetic studies and to reconstruct the phylogenetic relationships of section *Ficus* by combining them with ITS sequences.

1 Material and methods

1.1 Taxon sampling

We based our phylogenetic analyses on a total of 59 species and 114 samples, including 22 species and 73 samples belonging to sect. *Ficus*. For each species of the core section, at least two samples from different populations were examined except *F. neriifolia* Sm., *F. ovatifolia* S. S. Chang, *F. palmata* Forssk., and *F. johannis* Boiss., which had only one sample each; other related sections of *Ficus* were represented by at least one species. Material was obtained directly from the field or from cultivated plants (botanical collections) (see Table S1). Fresh leaves were dried by silica gel for DNA extraction. Voucher specimens were deposited in the herbarium of East China Normal University (HSNU).

Previous molecular studies based on plastid regions (Herre et al., 1996; Sytsma et al., 2002; Datwyler & Weiblen, 2004; Zerega et al., 2005) have shown that the tribe Castilleae is the closest relative of *Ficus*. Section *Pharmacosycea* (Miq.) Benth. & Hook. f. was previously used as the outgroup because of its retained primitive characters (Herre et al., 1996), but we found it was sister to subsect. *Ficus* and/or some other sections (see Figs. 1, S1, S2) in our constructing trials. So we selected *Antiaris toxicaria* Lesch. as the outgroup in our analyses.

1.2 DNA extraction, amplification, and sequencing

Total DNA was extracted in two ways. Some was extracted from 10 mg dried leaves according to the protocol of Doyle & Doyle (1987); some was extracted using the Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China) from 30 mg silica gel-dried leaves.

Primers and amplification protocols for all four regions are listed in Table 1. Polymerase chain reaction (PCR) was carried out through a TaKaRa TP600 thermocycler (Otsu, Shiga, Japan) and PCR products were purified using the TIANgel Midi Purification Kit (Tiangen Biotech).

Both strands were sequenced for each region and all taxa at Life Technologies (Shanghai, China) and sequencing primers are the same as the amplification primers.

1.3 Phylogenetic analyses

The sequences were edited and assembled using the software Seqman, a subprogram of DNASTAR (Burland, 2000) and afterwards aligned using the CLUSTALW option in Mega 5 (Tamura et al., 2011).

The low levels of variation detected made it pointless to construct phylogenetic relationships using the three plastid regions alone, so we analyzed the ITS matrix, the combined plastid matrix, and the combined nuclear–plastid matrix.

We used three methods, maximum parsimony (MP), Bayesian inference, and maximum likelihood (ML), to construct phylogenetic relationships.

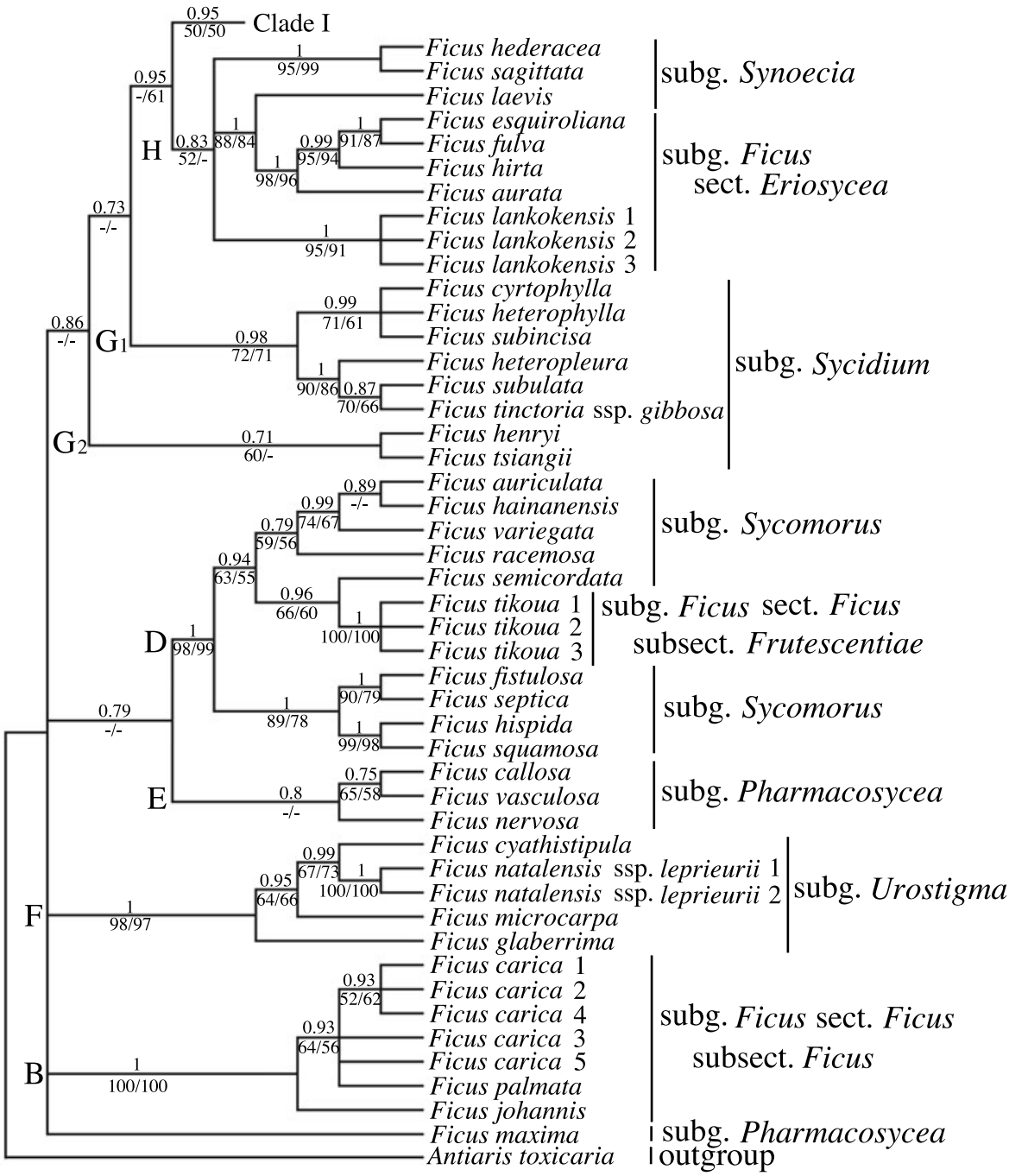

Fig. 1. Phylogenetic tree from the Bayesian analysis of the nuclear ribosomal internal transcribed spacer of *Ficus* section *Ficus* and relatives. Bayesian posterior probabilities are shown above branches, and the bootstrap values (%) of maximum likelihood (ML) and maximum parsimony (MP) analyses below branches (MLBS/MPBS). The text at the right margin indicates the taxonomic position of clades according to Berg (2003a). –, Branches not supported by MLBS or MPBS; A–I, clades A–I.

Parsimony analyses were carried out using PAUP version 4.0b10 (Swofford, 2003) for Macintosh. All characters were assessed as unordered and equally weighted. Gaps were coded as missing data. Most parsimonious trees (MP) were obtained using 1000 replicates of random taxon addition sequence, carried out using the heuristic search option and tree bisection–reconnection (TBR) branch swapping saving multiple trees (MulTrees).

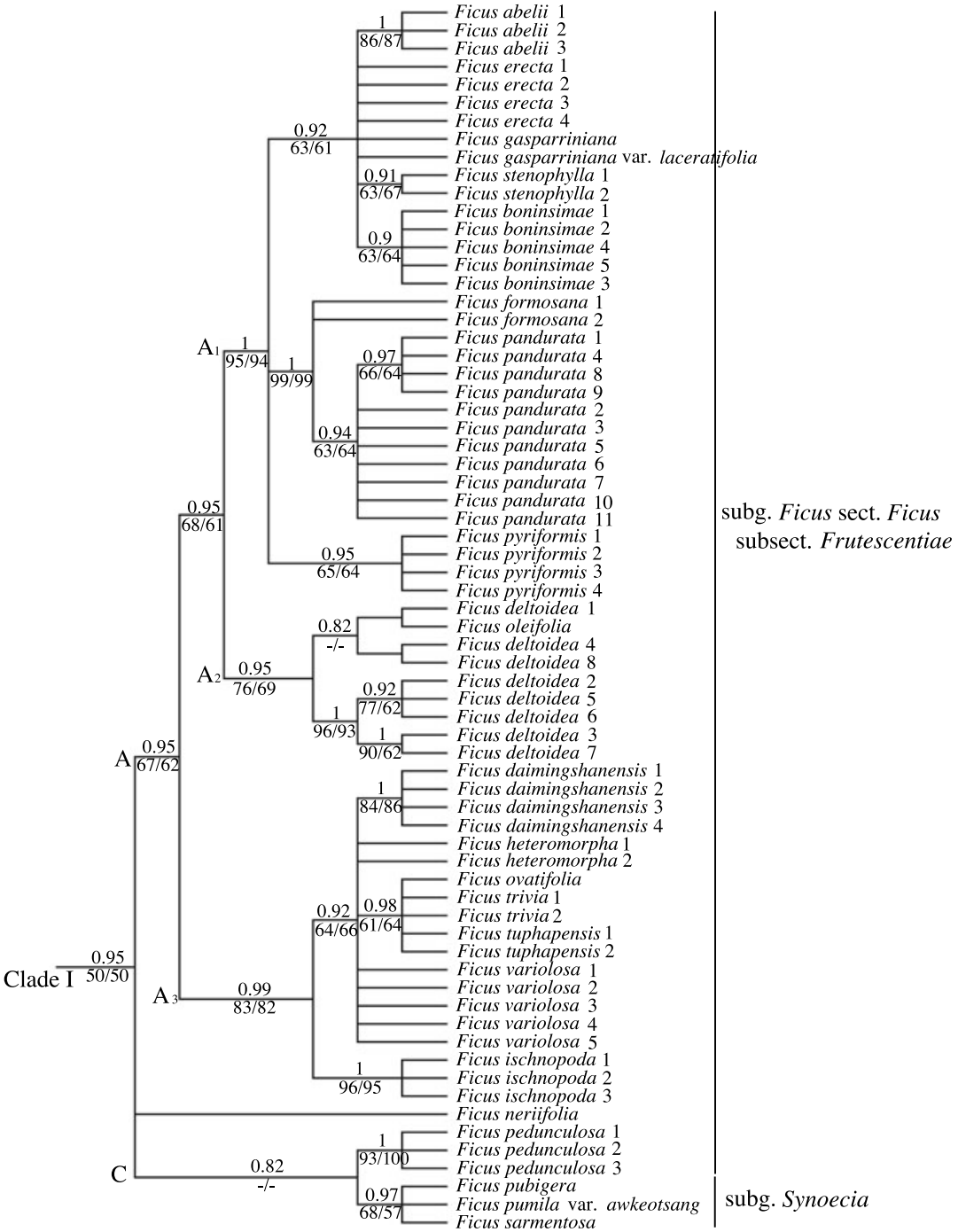

Fig. 1. Continued.

Homoplasy levels were assessed using the consistency index and the retention index. Bootstrap analyses (Felsenstein, 1985) were carried out using 1000 replicates, each consisting of 100 random addition sequence replicates with TBR swapping, and a limit of 1000 trees.

For Bayesian and ML analyses, individual and combined datasets were tested for the appropriate model of nucleotide evolution with MrModelTest 2.3 (Nylander, 2004). The optimal model according to the Akaike information criterion was then implemented for the analyses (Posada & Buckley, 2004).

Table 1 Sequence amplification and sequencing of the internal transcribed spacer (ITS) and plastid *trnH-psbA*, *psbK-psbI*, and *atpF-atpH* intergenic regions of *Ficus*

Sequence	Primers	Amplication protocol
ITS	ITS4 TCCTCCGCTTATTGATATGC ITS3 GCATCGATGAAGAACGTAGC (White et al., 1990)	94 °C 5 min; 94 °C 1 min, 50 °C 45 s, 72 °C 1 min, 30 cycles; 72 °C 5 min
trnH-psbA	psbA3′f GTTATGCATGAACGTAATGCTC trnH f CGCGCATGGTGGATTCACAATCC (Kress & Erickson, 2007)	95 °C 4 min; 94 °C 30 s, 55 °C 1 min, 72 °C 1 min, 35 cycles; 72 °C 10 min
psbK-psbI	psbK f TTAGCCTTTGTTTGGCAAG psbI r AGAGTTTGAGAGTAAGCAT (Lahaye et al., 2008)	94 °C 5 min; 94 °C 30 s, 50 °C 30 s, 72 °C 40 s, 35 cycles; 72 °C 5 min
atpF-atpH	atpF f ACTCGCACACACTCCCTTTCC atpH r GCTTTTATGGAAGCTTTAACAAT (Lahaye et al., 2008)	94 °C 5 min; 94 °C 30 s, 50 °C 30 s, 72 °C 40 s, 35 cycles; 72 °C 5 min

For the Bayesian inference using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003), we carried out 3 000 000 generations of four Monte Carlo Markov chains with equal rates until the average deviation of split frequencies fell well below 0.01. Trees were sampled every 100 generations. The first 25% trees before stationarity were discarded as burn-in, and the remaining trees were used to construct majority-rule consensus trees with posterior probabilities (PP) values shown. We defined the PP value between 0.8 and 0.89 as moderate support and over 0.9 as high support.

The ML analyses were carried out using Garli version 0.951-GUI (Zwickl, 2006). Default parameters were used for the Garli searches. A total of 1000 bootstrap replicates were also carried out using Garli. The files obtained from Garli were then used to construct majority-rule consensus tree using PAUP 4.0b10.

We defined the bootstrap support percentages between 50 and 70 as weak support, percentages between 71 and 89 as moderate support, and percentages more than 90 as high support.

2 Results

In the course of our study, 92 ITS, 93 *trnH-psbA*, 83 *psbK-psbI*, and 86 *atpF-atpH* sequences were obtained and these sequence data have been submitted to the GenBank databases. An additional 24 sequences were retrieved from GenBank (see Table S1).

Detailed characteristics of each matrix are shown in Table 2. For the separate and combined datasets, all phylogenetic methods yielded similar phylogenetic patterns with the Bayesian trees, so we indicated the MP and ML bootstrap percentages on the Bayesian trees (Figs. 1–3).

2.1 Analysis of ITS matrix

The aligned ITS matrix consisted of 114 ingroup taxa and one outgroup taxon. Of the 669 sites included in the analysis, 255 (38.12%) were variable, and 151 (22.57%) were potentially parsimony-informative. The two subsections of section *Ficus* can be found in different parts of Fig. 1, which infers that there clearly is no sister relationship.

Clade I (PP = 0.95; BS = 50%) is an important clade, split into clade A, clade C, and *F. neriifolia*. Clade A (PP = 0.95; maximum likelihood bootstrap support (MLBS) = 67%; maximum parsimony bootstrap support (MPBS) = 62%) comprises the core group of subsect. *Frutescentiae*: clade A_1 (PP = 1; MLBS = 95%; MPBS = 94%) contains morphologically confusing species with chartaceous lamina; clade A_2 (PP = 0.95; MLBS = 76%; MPBS = 69%) contains species

Table 2 Characteristics of individual and combined datasets of the internal transcribed spacer (ITS) and plastid *trnH-psbA*, *psbK-psbI*, and *atpF-atpH* intergenic regions of *Ficus*

Dataset	No. of taxa	Aligned length (bp)	Variable sites	Parsim-info sites	MP tree length	CI	RI	Garli ML score
ITS	114	669	255 (38.12%)	151 (22.57%)	656	0.6341	0.8329	−5009.2565
trnH-psbA	93	499	74 (14.83%)	22 (4.41%)	–	–	–	–
atpF-atpH	85	664	42 (6.33%)	20 (3.01%)	–	–	–	–
psbK-psbI	82	519	53 (10.21%)	23 (4.43%)	–	–	–	–
Combined plastid sequences	80	1682	169 (10.05%)	65 (3.86%)	382	0.7335	0.7855	−4609.4965
Combined plastid and nuclear sequences	80	2346	371 (15.81%)	187 (7.97%)	1134	0.6631	0.7639	−8768.2325

Maximum likelihood (ML) analyses were carried out using Garli version 0.951-GUI (Zwickl, 2006). –, No available data; CI, consistency index; MP, most parsimonious; Parsim-info, parsimony-informative; RI, retention index.

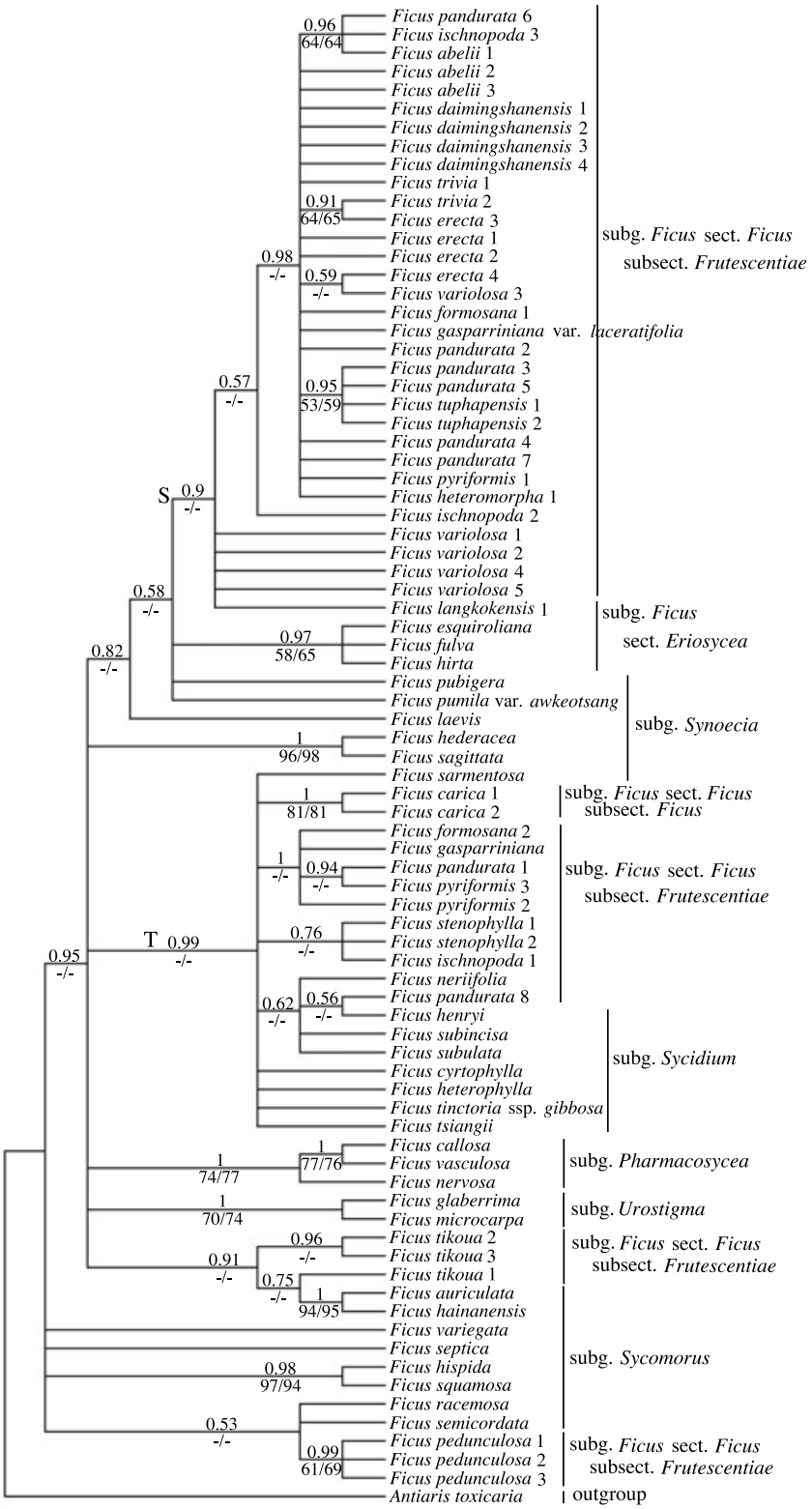

Fig. 2. Phylogenetic tree from the Bayesian analysis of the combined plastid dataset of *Ficus* section *Ficus* and relatives. Bayesian posterior probabilities are shown above branches, and the bootstrap values (%) of maximum likelihood (ML) and maximum parsimony (MP) analyses below branches (MLBS/MPBS). The text at the right margin indicates the taxonomic position of clades according to Berg (2003a). –, Branches not supported by MLBS or MPBS; S, clade S; T, clade T.

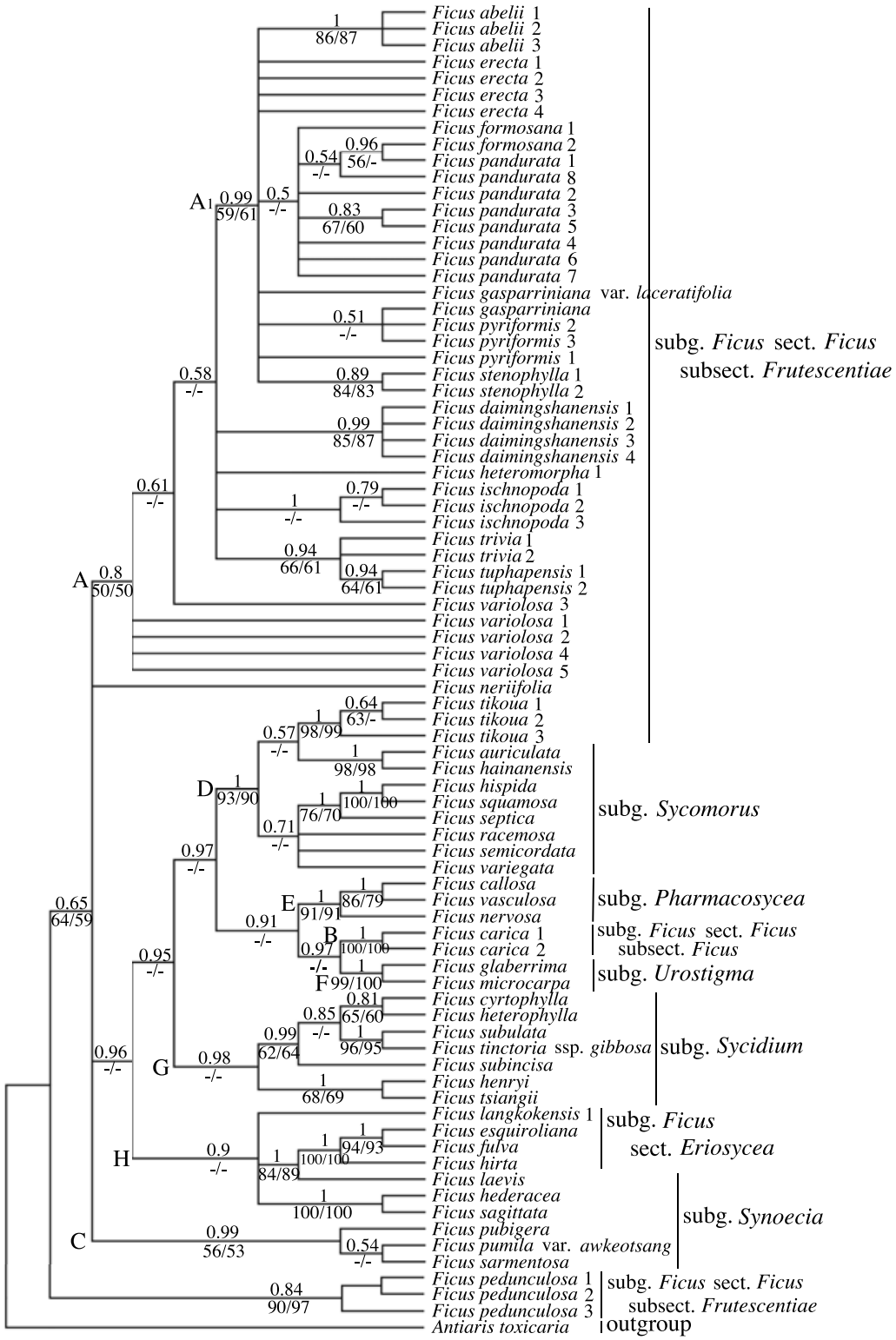

Fig. 3. Phylogenetic tree from the Bayesian analysis of the combined internal transcribed spacer and plastid datasets of *Ficus* section *Ficus* and relatives. Bayesian posterior probabilities are shown above branches, and the bootstrap values (%) of maximum likelihood (ML) and maximum parsimony (MP) analyses below branches (MLBS/MPBS). The text at the right margin indicates the taxonomic position of clades according to Berg (2003a). –, Branches not supported by MLBS or MPBS; A–H, clades A–H.

centered in western Malesia and often found on nutrient-poor substrates (like sandy soils) (Berg & Corner, 2005); and clade A_3, supported by all three methods (PP = 0.99; MLBS = 83%; MPBS = 82%), contains species with thickly chartaceous or thinly coriaceous lamina. Clades A_1 and A_2 form a clade (PP = 0.95; MLBS = 68%; MPBS = 61%) sister to clade A_3. Clade C, with moderate support from the Bayesian analysis (PP = 0.82), comprises four climbers or creepers (*Ficus pedunculosa* Miq. of subsect. *Frutescentiae* and three species of subg. *Synoecia*).

Clade H, supported to be sister to clade I (PP = 0.95; MPBS = 61%), contains all the representative species of sect. *Eriosycea* and additional species belonging to subg. *Synoecia* (*F. laevis* Blume, *F. hederacea* Roxb., and *F. sagittata* Vahl). Clade G_1 plus G_2 contain eight species belonging to subg. *Sycidium*. *Ficus callosa* Willd., *F. vasculosa* Wall. ex Miq., and *F. nervosa* B. Heyne ex Roth form clade E (belonging to subg. *Pharmacosycea*), which is sister to clade D, a highly supported clade belonging to subg. *Sycomorus* including *F. tikoua* Bureau (PP = 1; MLBS = 98%; MPBS = 99%). Clade F (PP = 1; MLBS = 98%; MPBS = 97%) consists of all the representative species of subg. *Urostigma*. Clade B (PP = 1; MLBS = 100%; MPBS = 100%) includes all three species of subsect. *Ficus*.

2.2 Analysis of combined plastid matrix

The aligned plastid matrix consisted of 80 ingroup taxa and 1 outgroup taxon. Of the 1682 sites included in the analysis, 169 (10.05%) were variable and 65 (3.86%) were potentially parsimony-informative. The Bayesian tree is shown in Fig. 2.

The tree topology resulted from combined plastid regions was inconsistent with the ITS tree. Unlike on the ITS phylogeny, the resolution of relationships within this section was poor. Almost all species of section *Ficus* can be found in two clades: the majority of the samples are included in clade S; those remaining are in clade T also comprising the whole samples of subg. *Sycidium* and *F. sarmentosa* Buch.-Ham. ex Sm. *Ficus pedunculosa* and *F. tikoua* are out of the two clades. To other subgenera, only species belonging to subg. *Pharmacosycea* and *Urostigma* are clustered together.

2.3 Analysis of combined ITS and plastid matrix

The aligned combined matrix consisted of 80 ingroup taxa and one outgroup taxon. Of the 2346 sites included in the analysis, 371 (15.81%) were variable and 187 (7.97%) were potentially parsimony-informative.

The Bayesian tree is shown in Fig. 3. The ingroup is first split into several major clades.

The first clade (PP = 0.8; BS = 50%), namely clade A, is the core group of subsect. *Frutescentiae*, which is a polytomy including several smaller clades. Among clade A, clade A_1 contains morphologically confusing species, and species like *F. formosana* Maxim., *F. pandurata* Hance, *F. pyriformis*, *F. gasparriniana* Miq., and *F. erecta* Thunb. are not monophyletic, differing from the results in the ITS matrix analyses. However, there are also such subclades clustered by samples of all the individuals of *F. ischnopoda* (PP = 1), *F. abelii* Miq. (PP = 1; MLBS = 86%; MPBS = 87%), and *F. daimingshanensis* S. S. Chang (PP = 0.99; MLBS = 85%; MPBS = 87%).

Unlike the ITS analysis, clade C (PP = 0.99) only includes three species of subg. *Synoecia* (*F. pumila* var. *awkeotsang* (Makino) Corner, *F. pubigera* (Wall. ex Miq.) Kurz, and *F. sarmentosa*) and *F. pedunculosa* becomes the outermost clade among the ingroup. Clade H (PP = 0.9; BS < 50%) is not well resolved, comprising four species of sect. *Eriosycea* (*F. esquiroliana* H. Lév., *F. fulva* Reinw. ex Blume, *F. hirta* Vahl, and *F. langkokensis* Drake) and three species of subg. *Synoecia* (*F. laevis*, *F. hederacea*, and *F. sagittata*). Clade G (PP = 0.98) contains all representative species of subg. *Sycidium*. *Ficus carica* (clade B) is shown as the sister group of subg. *Urostigma* (clade F). Clade E (subg. *Pharmacosycea*) gains strong support (PP = 1; MLBS = 91%; MPBS = 91%). In clade D, *F. tikoua* (sect. *Ficus* sensu Corner, 1965) is sister to other species belonging to subg. *Sycomorus*, which is highly supported by the three phylogenetic methods (PP = 1; MLBS = 93%; MPBS = 90%).

In agreement with previous results (Rønsted et al., 2005, 2008; Xu et al., 2011), subg. *Ficus* is shown to mainly include three distinct lineages in our study, but the placements of some other species (e.g. *F. tikoua*, *F. pedunculosa*, and *F. neriifolia*) are obviously out of these lineages.

3 Discussion

Although the total number of parsimony-informative characters increased from 151 in the separate ITS matrix to 187 in the combined matrix with the whole regions, the percentage of parsimony-informative characters did decrease (Table 2). The three plastid regions raised the resolution of some clades involving the intersubgeneric relationship in the combined analysis, but they could not resolve the intrasectional relationship of sect. *Ficus*. However, the infrageneric phylogenetic pattern shown in our analysis highlighted that the recognition of six subgenera may need deep discussion, for instance, subg. *Synoecia* would be split and

the placement of subsect. *Ficus* needed re-examination. So more variable regions as suggested by Rønsted et al. (2006) can be used to explore the intrasectional phylogenetic relationship.

As can be seen from Fig. 1, subsect. *Frutescentiae* was split into three highly supported clades: clade A_1 with complicated inter- and intraspecific variation in morphological characters, all shrubs; clade A_2 confined to the western Malesian region; and clade A_3 with a thick papery or thin coriaceous lamina. Berg & Corner (2005) subdivided subsect. *Frutescentiae* into the *F. deltoidea* group (clade A_2 in Fig. 1) and the *F. pedunculosa* group (clades A_1 and A_3 in Fig. 1) based on distribution region, the terminalia mode of branching, and the growing environment, but, distinctly, clades A_1 and A_2 form a sister group to clade A_3 in our study, making the *F. pedunculosa* group paraphyletic with regards to the *F. deltoidea* group.

Morphologically, partially due to the lack of prominent differentiating characters and considerable infraspecific variation, which is not abrupt but gradual, it is difficult to delimit species of subsect. *Frutescentiae* (Berg, 2011), which is also reflected in our DNA-based phylogenetic analyses (see Figs. 2, 3). In the present study, *F. erecta*, *F. pandurata*, *F. formosana*, *F. stenophylla* Hemsl., *F. gasparriniana*, *F. pyriformis*, and *F. abelii* form the highly supported clade A_1 (Fig. 1/Fig. 3: PP = 1/0.99; MLBS = 95%/59%; MPBS = 94%/61%), which means they are phylogenetically closely related entities. Samples of the same species share similar ITS sequences, but their plastid sequences vary a lot. For example, for the *trnH-psbA* spacer, *F. formosana* 1 and 2, *F. pyriformis* 1, 2, and 3, *F. pandurata* 1, 2, and 3 have obvious insertions and deletions (indels). As samples of the same species were collected from different populations, such indels might be the result of divergent evolution of the same species in different parts of the distribution range. Interestingly, for the *trnH-psbA* spacer, particular samples of different species (e.g. *F. pyriformis* 2 and *F. pandurata* 3, *F. formosana* 2 and *F. gasparriniana*), show clear similarities to each other, which could be explained, compared with ample variation in the nuclear genome, by separate origins of what we now call one species with convergent or parallel evolution in the specific characters. Instead of the prior cluster of infraspecific samples, similarities in interspecific sequence could cause crossed clusters of interspecific samples. To these closely related species, interspecific hybridization may be responsible in part for the taxonomic difficulty and gene differentiation. It is known that host shift is a frequent event during the coevolution of figs and their pollinating wasps, which allows interspecific hybridization and hence generates the introgression of the chloroplast genome (Renoult et al., 2009).

The clade formed by subsect. *Ficus* (*F. carica* and *F. palmata* in our study) was not sister to subsect. *Frutescentiae* but closely related to the monoecious subg. *Urostigma*. Thus, molecular evidence did not support the placement of subsect. *Ficus* as classified by Berg (2003b).

Corner (1965) put *F. henryi* Warb. ex Diels and *F. subincisa* Buch.-Ham. ex Sm. in sect. *Ficus*. Berg (2003c) transferred them to subg. *Sycidium* based on characters of the staminate flowers, distichous leaves, and the not fully amplexicaul stipules. It can be seen from Fig. 3 that *F. henryi* and *F. subincisa* show affinity to *F. tsiangii* Merr. ex Corner, *F. cyrtophylla* (Wall. ex Miq.) Miq. and other species belonging to subg. *Sycidium* (clade G, PP = 0.98), which indicates a close relationship with subg. *Sycidium* and confirms Berg's taxonomic deposition.

In our analyses, the three samples of *F. tikoua* cluster together and compose a clade (clade D) with species belonging to subg. *Sycomorus*, which receive strong support (PP = 1; MLBS/ MPBS > 90%). The typical feature of subg. *Sycomorus* is cauliflory, and the figs of *F. tikoua* occur on older creeping stems, which can be considered homologous to the cauliflory of subg. *Sycomorus*. In the classification system of Berg (2003a), the attachment position of figs has been recognized as an important taxonomic feature, so we suggest that *F. tikoua* should be transferred to subg. *Sycomorus*.

In addition, *F. langkokensis*, *F. pedunculosa*, and *F. neriifolia* (belonging to subg. *Ficus* sensu Berg, 2003b) do not cluster together with other members of subg. *Ficus*, but form a single clade or group with other subgenera. In Figs. 1 and 3, *F. langkokensis* shows close relationship with sect. *Eriosycea*, which is in accordance with the treatment of Berg & Corner (2005) to place *F. langkokensis* in sect. *Eriosycea* based on the diagnostic occurrence of cystoliths in the laminas. The close relationship of *F. pedunculosa* to subg. *Synoecia*, suggested partially by their creeping life form, gains moderate support in Fig. 1, but poor support in Fig. 3. With regards to *F. neriifolia*, no clear evidence was obtained either in ITS or combined analyses.

Ficus tuphapensis Drake has been included in subsect. *Eriosycea* (Corner, 1965; Berg & Corner, 2005). Our research indicates that *F. tuphapensis* clearly belongs to subsect. *Frutescentiae* and is closely related to *F. trivia* Corner. The diagnostic characters such as laminas with cystoliths, figs occurring in axils, and staminate flowers with 2–3 stamens also support this hypothesis.

In summary, our phylogenetic framework is not sufficient for reconsidering the classification of sect. *Ficus*. But it is possible that clade A and clade B, supported by both ITS and the combined datasets, may be recognized as new taxonomic units; *F. tuphapensis* may be transferred into subsect. *Frutescentiae*; *F. tikoua* should be transferred out of subg. *Ficus*. With the taxonomic problems elucidated in this study, the future research of section *Ficus* should be focused on a robust phylogenetic reconstruction by sampling more problematic individuals and those species not included in our analysis, and finding more informative DNA markers. In addition, careful examination of morphological characters and coevolutionary patterns of the section would also be essential to achieve a better understanding of the evolution of the section.

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Supplementary material

The following supplementary material is available for this article at http://onlinelibrary.wiley.com/doi/10.1111/j.1759-6831.2012.00221.x/suppinfo:

Table S1. Details of material included in this study

Fig. S1. Phylogenetic tree from the likelihood analyses of internal transcribed spacer dataset of *Ficus* section *Ficus* and relatives in this study. Bootstrap values (%) over 50% are shown above branches.

Fig. S2. Phylogenetic tree from the parsimony analyses of internal transcribed spacer dataset of *Ficus* section *Ficus* and relatives in this study. Bootstrap values (%) are shown above branches.

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