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PHYLOGENETIC RELATIONSHIPS OF FUNCTIONALLY DIOECIOUS *FICUS* (MORACEAE) BASED ON RIBOSOMAL DNA SEQUENCES AND MORPHOLOGY[1]

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Figs (*Ficus*, Moraceae) are either monoecious or gynodioecious depending on the arrangement of unisexual florets within the specialized inflorescence or syconium. The gynodioecious species are functionally dioecious due to the impact of pollinating fig wasps (Hymenoptera: Agaonidae) on the maturation of fig seeds. The evolutionary relationships of functionally dioecious figs (*Ficus* subg. *Ficus*) were examined through phylogenetic analyses based on the internal transcribed spacer (ITS) region of nuclear ribosomal DNA and morphology. Forty-six species representing each monoecious subgenus and each section of functionally dioecious subg. *Ficus* were included in parsimony analyses based on 180 molecular characters and 61 morphological characters that were potentially informative. Separate and combined analyses of molecular and morphological data sets suggested that functionally dioecious figs are not monophyletic and that monoecious subg. *Sycomorus* is derived within a dioecious clade. The combined analysis indicated one or two origins of functional dioecy in the genus and at least two reversals to monoecy within a functionally dioecious lineage. The exclusion of breeding system and related characters from the analysis also indicated two shifts from monoecy to functional dioecy and two reversals. The associations of pollinating fig wasps were congruent with host fig phylogeny and further supported a revised classification of *Ficus.*

Key words: breeding system evolution; coevolution; dioecy; *Ficus*; Moraceae; phylogeny; pollination.

The genus *Ficus* (Moraceae) includes some 750 species of woody plants occurring in most tropical and subtropical forests throughout the world (Berg, 1989). These species of trees, shrubs, climbers, and hemiepiphytic stranglers are recognized by a specialized inflorescence and pollination syndrome (Janzen, 1979; Berg, 1990b). Resembling a fleshy fruit, the fig is an enlarged receptacle enclosing many unisexual flowers that are accessible only by a tightly bract-filled opening or ostiole. The closed inflorescence, or syconium, protects the flowers against most parasites except for diminutive insects capable of entering through the opening (Berg, 1990a). The interior of the inflorescence is the location of an obligate mutualism with pollinating seed predators, fig wasps in the family Agaonidae of parasitic Hymenoptera (Chalcidoidea). Interactions between figs and fig wasps are among the best known examples of reproductive interdependence between plants and their pollinators (Bronstein, 1992). In addition, fig wasps are specialized to the extent that unique pollinator species are associated with most fig species (Ramirez, 1970; Wiebes, 1979; but see Rasplus, 1994, 1996; Kerdelhue, Hochberg, and Rasplus, 1997).

The intertwined life cycles of figs and pollinators, together with their extreme specificity, are the basis for speculation on the nature and extent of coevolution involved (Ramirez, 1974; Wiebes, 1979). Functionally dioecious figs are of special interest due to evolutionary conflicts with pollinators regarding the exploitation of seed resources (Grafen and Godfray, 1991). Until the present, our knowledge of fig breeding system evolution has been shaped by taxonomy (Corner, 1985), anatomy (Beck and Lord, 1988; Verkerke, 1989), ecology (Galil, 1973; Kjellberg et al., 1987; Corlett, 1993; Weiblen, Spencer, and Flick, 1995; Patel and McKey, 1998), and pollinator behavior (Hossaert-McKey, Gibernau, and Frey, 1994; Ware and Compton, 1994). However, phylogenetic studies are few, and the relationships of the functionally dioecious figs have not been examined in detail (Yokoyama, 1995; Herre et al., 1996).

Morphologically, figs are monoecious or gynodioecious according to the representation of the unisexual florets within the syconium (Figs. 1–3). The gynodioecious species are functionally dioecious, with the separation of sexual function resulting from the interaction of pollinator wasps with florets in two types of figs on separate plants (Weiblen, Spencer, and Flick, 1995). Inside the protogynous syconia, female fig wasps deliver pollen to the pistillate florets while laying their eggs in a fraction of fig ovaries. The fate of the ovaries in these species is influenced by the interaction of pollinator ovipositors with dimorphic pistillate florets in the two types of figs (Ganeshaiah et al., 1995). Long-styled florets in seed figs (Fig. 1) have ovaries that are fertilized but are inaccessible to pollinator ovipositors (Galil, 1973). On the other hand, short-styled florets in gall figs (Fig. 2) enable pollinators to deposit their eggs in proximity to fig ovules (Verkerke, 1989). Wasp larvae occupy the ovaries and feed on endosperm. Later in development, staminate florets in gall figs release pollen during the eclosion and mating of the adult wasps. The wingless males then chew an exit from the fig and the winged, pollen-bearing females escape in search of receptive figs in which to complete their life cycle.

Functional dioecy in *Ficus* is a unique product of genetic

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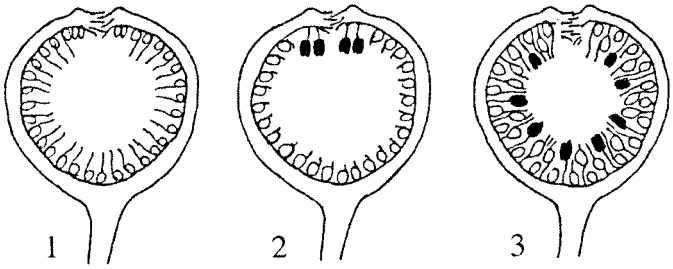


Figs. 1–3. The distribution of unisexual florets in figs (syconia) of monoecious and gynodioecious *Ficus*. There are two types of figs on separate plants in gynodioecious *Ficus*, seed figs containing long-styled pistillate florets (Fig. 1) and gall figs containing short-styled pistillate florets and staminate florets (Fig. 2). Monoecious species have a single type of fig containing pistillate florets with styles of varying length and staminate florets (Fig. 3). Anthers are blackened to indicate the position of staminate florets near the ostiole in Fig. 2 and dispersed among the pistillate florets in Fig. 3.

factors controlling floral development and the impact of pollinator larvae on seed maturation (Valdeyron and Lloyd, 1979). The genetics of sex determination in functionally dioecious *Ficus* is known from crossing studies in the edible fig, *F. carica* L. (Storey, 1975). A pair of linked loci affecting style lengths and the abortion of staminate florets is responsible for gynodioecious morphology. A stable ratio of progeny results from crosses between heterozygous gall figs (*GgAa*) and homozygous seed figs (*ggaa*); *G* is dominant for short-styled pistillate florets, while *g* is recessive for long-styled florets, and *A* is dominant for the development of staminate florets, while *a* is recessive for the abortion of staminate florets. The evolutionary sequence leading to the origin or loss of this unique sex-determining mechanism is unknown (Valdeyron and Lloyd, 1979), although selective pressures such as environmental seasonality and the impact of non-pollinating wasps have been proposed (Kerdelhue and Rasplus, 1996).

A phylogenetic analysis of the functionally dioecious figs and their relatives was undertaken to examine breeding system evolution, pollinator relations, and *Ficus* classification. In the last century, *Ficus* was split into several genera (Gasparrini, 1844; Miquel, 1862) that became the basis for a subgeneric classification after the genus was reunited (Miquel, 1867a, b). Miquel classified the functionally dioecious species in four subgenera based on floral characters. Almost a century later, Corner (1965) united functionally dioecious figs under subg. *Ficus* in his reclassification of the genus (Table 1). *Ficus* can be found in all three tropical regions, but the majority of species occur in Asia, Malesia, and Australia. Functionally dioecious subg. *Ficus* is restricted to the Paleotropics, and Malesia is the center of diversity in terms of species richness and endemism. For example, functionally dioecious figs comprise an estimated 343 species out of the 503 species in the region (68%; Berg, 1989). In addition, five of the eight functionally dioecious sections are centered in Indo-Australia (Table 1). Species within this region were the focus of the study.

The primary source of characters for phylogeny reconstruction was the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. ITS sequences have proven useful for resolving phylogenetic relationships at lower taxonomic levels in plants due to high interspecific variability (Baldwin et al., 1995). As a supplement to ITS sequences, morphological characters for *Ficus* were analyzed separately and in combination. Whether or not to combine morphological and molecular data sets in a single analysis has been a subject of considerable debate in the systematic literature (Bull et al., 1993; de Queiroz, Donoghue, and Kim, 1995). Assuming that different data sets share the same phylogenetic history, systematists have argued that inferences based on all the available data are more likely to be correct than inferences based on a subset of the data (Kluge, 1989; Barrett, Donoghue, and Sober, 1991). However, conflict between data sets can result from systematic error, rate heterogeneity, or from data sets not sharing the same history.

Separate analyses have the advantage of highlighting points of conflict, but if incongruence is due to random errors in phylogeny estimation, then a combined analysis may provide the best estimate of phylogeny (de Queiroz, Donoghue, and Kim, 1995). A conditional approach favors combined analyses in the event of weak incongruence while favoring separate analyses in the event of strong incongruence. Considerations on separate vs. combined analysis of ITS and morphological data sets for *Ficus* were explored using statistical tests. Herre et al. (1996) suggested that *Ficus* morphology may yield in-

TABLE 1. The classification and distribution of *Ficus* L. according to Corner (1965) and summarized by Berg (1989). The arrangement of sections within subgenera is alphabetical.

Subgenus	Section	No. spp.	Distribution
Ficus	*Adenosperma* Corner	23	Indo-Australia
	Ficus	60	Indo-Australia, Asia and Africa
	Kalosyce (Miq.) Corner	20	Indo-Australia and Asia
	Neomorphe King	6	Indo-Australia and Asia
	Rhizocladus Endl.	55	Indo-Australia and Asia
	Sinosycidium Corner	1	Asia
	Sycocarpus Miq.	75	Indo-Australia and Asia
	Sycidium Miq.	105	Indo-Australia, Asia and Africa
Pharmacosycea Miq.	*Oreosycea* (Miq.) Corner	50	Indo-Australia and Africa
	Pharmacosycea	20	America
Sycomorus (Gasp.) Miq.	*Sycomorus*	13	Africa and Indo-Australia
Urostigma (Gasp.) Miq.	*Americana*	120	America
	Conosycea (Miq.) Corner	65	Indo-Australia, Asia and Africa
	Galoglychia (Gasp.) Endl.	75	Africa
	Leucogyne Corner	2	Asia
	Malvanthera Corner	20	Indo-Australia
	Stilpnophyllum Endl.	1	Asia
	Urostigma	20	Indo-Australia, Asia and Africa

TABLE 2. *Ficus* species selected for phylogenetic analysis. Arrangement within subgenera and sections is alphabetical. Pollinating fig wasps (Agaoninae) associated with each *Ficus* species are also indicated. Pollinator subgenera are abbreviated as (*C*)*eratosolen*, (*R*)*othropus*, (*S*)*trepitus*, and (*V*)*alisia*. Voucher numbers and localities of specimens deposited at A are listed. All collections were made by the author except for *F. auriculata* (Ho), *F. botryoides* (Kerdelhue), *F. punctata* (Laman), *F. racemosa* (Spencer), and *F. sur* (Kerdelhue). GenBank accession numbers are GBAN-AF165374–GBAN-AF165419.

Subgenus	Section	Species	Pollinator	Voucher, locality
Ficus	*Adenosperma*	*adenosperma* Miq.	*Ceratosolen* (*C.*) *adenospermae* Wiebes	GW553, New Guinea
		ochrochlora Ridley	*Ceratosolen* (*C.*) sp.	GW735, New Guinea
	Ficus	*grossularioides* Burm. f.	*Blastophaga* (*V.*) *malayana* Wiebes	GW858, Borneo
		padana Burm. f.	*Blastophaga* (*V.*) *intermedia* Grandi	GW1066, Java
	Kalosyce	*punctata* Thunb.	*Wiebesia punctatae* Wiebes	TL1022, Borneo
		ruginerva Corner	*Wiebesia* sp.	GW854, Borneo
	Neomorphe	*auriculata* Lour.	*Ceratosolen* (*C.*) *emarginatus* Mayr	H726, Ho, China
		nodosa Teysm. et Binn.	*Ceratosolen* (*C.*) *nexilis* Wiebes	GW603, New Guinea
		robusta Corner	*Ceratosolen* (*C.*) sp.	GW952, New Guinea
		semivestita Corner	*Ceratosolen* (*C.*) *grandii* Wiebes	GW700, New Guinea
		variegata Bl.	*Ceratosolen* (*C.*) *appendiculatus* Mayr	GW682, Austraila
	Rhizocladus	*baeuerlenii* King	*Wiebesia* sp.	B121, New Guinea
		odoardi King	*Wiebesia* sp.	GW708, New Guinea
	Sycidium	*conocephalifolia* Ridley	*Kradibia jacobsi* (Wiebes)	D7, New Guinea
		copiosa Steud.	*Kradibia copiosae* (Wiebes)	G057, New Guinea
		phaeosyce Laut. et K. Schum.	*Kradibia* sp.	B142, New Guinea
		pungens Bl.	*Ceratosolen* (*C.*) *nanus* Wiebes	GW539, New Guinea
		tinctoria Forst. f.	*Liporrhopalum* c.f. *gibbosae* Hill	G067, New Guinea
		trachypison K. Schum.	*Kradibia* sp.	GW950, New Guinea
		wassa Roxb.	*Kradibia wassae* (Wiebes)	G051, New Guinea
		virgata Reinw. ex Bl.	*Liporrhopalum virgatae* Hill	GW704, New Guinea
	Sycocarpus	*bernaysii* King	*Ceratosolen* (*R.*) *hooglandi* Wiebes	GW541, New Guinea
		botryocarpa Miq.	*Ceratosolen* (*R.*) *corneri* Wiebes	D3, New Guinea
		dammaropsis Diels	*Ceratosolen* (*S.*) *abnormis* (Wiebes)	B34, New Guinea
		hispidioides S. Moore	*Ceratosolen* (*R.*) *dentifer* Wiebes	G053, New Guinea
		itoana Diels	*Ceratosolen* (*S.*) *armipes* Wiebes	GW622, New Guinea
		microdictya Diels	*Ceratosolen* (*S.*) sp.	GW954, New Guinea
		septica Burm. f.	*Ceratosolen* (*C.*) *bisulcatus* (Mayr)	GW836, New Guinea
		theophrastoides Seem.	*Ceratosolen* (*S.*) *vissali* Wiebes	GW826, Solomon Is.
Pharmacosycea	*Oreosycea*	*albipila* (Miq.) King	*Dolichoris* sp.	GW1070, Java
		edelfeltii King	*Dolichoris inornata* Wiebes	GW821, New Guinea
		hombroniana Corner	*Dolichoris* sp.	GW953, New Guinea
	Pharmacosycea	*insipida* Willd.	*Tetrapus costaricanus* (Grandi)	V08, Venezuela
		maxima Mill.	*Tetrapus americanus* Mayr	B02, Brazil
Sycomorus	*Sycomorus*	*botryoides* Baker	*Ceratosolen* (*C.*) *blommersi* Wiebes	GW841, Madagascar
		racemosa L.	*Ceratosolen* (*C.*) *fusciceps* (Mayr)	GW940, Australia
		sur Forssk.	*Ceratosolen* (*C.*) *capensis* Grandi	GW840, Tanzania
Urostigma	*Americana*	*pertusa* L.	*Pegoscapus silvestrii* Grandi	V09, Venezuela
	Conosycea	*microcarpa* L.	*Eupristina* (*P.*) *verticillata* Waterson	GW535, New Guinea
		pellucido-punctata Griff.	*Waterstoniella brevigena* Wiebes	GW868, Borneo
	Malvanthera	*destruens* C.T. White	*Pleistodontes rigisamos* Wiebes	GW943, Australia
		hesperidiiformis King	*Pleistodontes plebejus* Wiebes	G825, New Guinea
		xylosycia Diels	*Pleistodontes rieki* Wiebes	G066, New Guinea
	Urostigma	*prasinicarpa* Elm.	*Platyscapa ficheri* Wiebes	GW827, Solomon Is.
		superba Miq.	*Platyscapa corneri* Wiebes	GW851, Borneo
		virens Ait.	*Platyscapa coronata* (Grandi)	GW555, New Guinea

correct estimates of phylogeny because of convergent evolution in reproductive traits; however previous studies did not specifically test this proposition. With regard to the question of breeding system evolution, the issue of including characters of interest in phylogeny reconstruction was examined using sensitivity analysis (de Queiroz, 1996; Donoghue and Ackerly, 1996).

MATERIALS AND METHODS

The evolutionary relationships of functionally dioecious figs were examined through phylogenetic analyses of 46 species (Table 2). Sampling included representatives of all *Ficus* subgenera and all major sections of the genus apart from *Galoglychia*, from which DNA could not be obtained. The choice of ITS and morphology as sources of characters did not permit the inclusion of other Moraceae as outgroups in the phylogenetic analysis, due to difficulties associated with sequence alignments and the assessment of homology (see Discussion). Evidence from the chloroplast gene *rbcL* (Herre et al., 1996) and morphology (Berg, 1989) suggests that the neotropical *Ficus* sect. *Pharmacosycea* is a sister group to the rest of *Ficus.* Two representatives of sect. *Pharmacosycea* were designated as outgroups. Sampling of the monoecious subgenera included 15 species representing the sections *Oreosycea*, *Urostigma, Conosycea, Malvanthera, Americana,* and *Sycomorus.* In addition, 29 species comprising 8% of subg. *Ficus* and at least two representatives of each functionally dioecious section were sampled, excluding monotypic sect. *Sinosycidium.*

Sources of DNA included leaves preserved in silica gel in the field, herbarium specimens <10 yr old, and fresh leaves harvested from cultivated plants. Genomic DNA was extracted from 10 to 20 mg of dried leaves (30–50 mg when fresh). The protocol of Doyle and Doyle (1987) was modified

to avoid problems associated with DNA isolation from leaves containing latex. Leaves were ground in liquid N_2 and incubated at 60°C in a 400-μL solution of 2X CTAB buffer with 4% polyvinyl pyrrolidone (molecular weight 40 000) and 0.8 μL of ß-mercapto-ethanol. After 1 h, samples were centrifuged for 5 min, and the aqueous supernatant was twice extracted with 400 μL of phenol: chloroform:isoamyl alcohol (25:24:1). The supernatant was extracted a third time with chloroform:isoamyl alcohol (24:1). DNA extracts were cleaned with a GENECLEAN II® kit (BIO 101, Carlsbad, California, USA), serially diluted, and amplified with a PCR reagent system (Gibco BRL, Rockville, Maryland, USA).

Primers ITS4 and ITS5 (White et al., 1990) were used for amplification of the region including the two internal transcribed spacers and the 5.8S subunit of nuclear ribosomal DNA. The thermal conditions for amplification included: (A) denaturation at 96°C (2 min); (B) two cycles of denaturation at 94°C (30 s), annealing at 40°C (30 s), and extension at 72°C (60 s); (C) 35 cycles as in (B) but with annealing at 55°C (30 s); and (D) final extension at 72°C (4 min). Polymerase chain reaction (PCR) products were quantified on 0.4% agarose gels using a Low DNA Mass™ ladder (Gibco BRL, Rockville, Maryland, USA) and single bands were purified with a QIAquick™ PCR purification kit (QIAGEN, Valencia, California, USA). PCR products were cycle sequenced in both directions using primers ITS2, ITS3, ITS4, and ITS5 (White et al., 1990). ITS2 and ITS3 sequencing primers were redesigned for *Ficus* (5′-GCATCGATGAAGAACGTAGC-3′ and 5′-GGAAGGAGAAGTCGTAACAAGG-3′, respectively). Sequences were collected using Long Ranger™ polyacrylamide gels (FMC Bioproducts, Rockland, Maine, USA) and a 377 PRISM™ sequencer with DNA Sequencing Analysis software version 2.1.1 (PE Biosystems, Foster City, California, USA). Chromatograms were edited with Sequencer™ software (Gene Codes, Ann Arbor, Michigan, USA) and aligned manually. The aligned sequences are deposited in TreeBASE (http://www.herbaria.harvard.edu/treebase/index.html). Thirty-three ambiguous positions corresponding to 4.3% of the aligned sequences were excluded from analysis. Thirty-four gaps remained in the aligned sequence following the exclusion of these ambiguous sites. Nineteen autapomorphic indels were treated as missing data. The presence or absence of 15 remaining indels was coded in a supplemental set of characters, and all indel positions were excluded from analyses of the aligned sequences.

Molecular cloning examined heterogeneity among ITS paralogues in functionally dioecious figs. ITS heterogeneity within species was explored because the inclusion of divergent paralogues and pseudogenes in phylogenetic analysis has the potential to yield inaccurate estimates of species phylogeny (Buckler, Ippolito, and Holtsford, 1997). PCR products from four species were cloned and sequenced for comparison with the results of direct sequencing. In addition, ten ITS clones each from *F. nodosa* and *F. variegata* were sequenced to look for the presence of heterologous ITS copies within species. ITS PCR products were ligated and transformed using the pGEM®-T Easy Vector System (Promega Corporation, Madison, Wisconsin, USA). Transformed cells were screened with ampicillin, and recombinant plasmid DNA was isolated using the Wizard® Plus Miniprep DNA purification system (Promega Corporation, Madison, Wisconsin, USA).

Sixty-four discrete morphological characters were selected from the taxonomic literature (Corner, 1933, 1955, 1958, 1960a, b, 1961, 1965, 1967, 1969, 1970a, b, 1976, 1978) and by examination of living plants and more than 800 herbarium collections. Sixty-one characters with two to five states were potentially informative in phylogenetic analysis (Appendix).

Phylogenetic analyses were performed with PAUP* version 4.0b2 for Power Macintosh computers (Swofford, 1998). Under the optimality criterion of parsimony, heuristic searches were conducted according to PAUP* default settings, except that 1000 random addition sequence replicates were used with MAXTREES set to increase without limit. All characters were unordered and weighted equally. Uninformative characters were excluded from all analyses. Bootstrap resampling (Felsenstein, 1985) and decay analyses (Bremer, 1988; Donoghue et al., 1992) were used to estimate clade robustness. Bootstrapping involved heuristic searches with 10 000 replicates and a random addition sequence with $N = 1$. In the case of ITS and combined analyses, the option to save multiple equally parsimonious trees per replicate was disabled to reduce the search times on Power Macintosh 7300 and Macintosh G3 computers. Decay analyses were performed using the program Autodecay version 2.9.5 (T. Eriksson) with ten random addition sequence replicates per heuristic search.

Congruence between morphological and molecular data sets was explored with the incongruence length difference test (ILD; Farris et al., 1994) and Templeton tests (Templeton, 1993; Larson, 1994) as implemented in PAUP*. Each data set was analyzed to find the most parsimonious trees compatible with constraint trees from the rival data set. For example, morphological data were analyzed to find the shortest trees compatible with the shortest trees from a separate analysis of the ITS data. Constraint trees from the rival data set included the strict consensus tree, bootstrap consensus trees (50, 70, and 90%), and a most parsimonious tree selected at random. Most parsimonious trees from the constrained and unconstrained searches were selected at random and compared using a nonparametric sum of signed ranks test. In addition to statistical measures of conflict, comparisons of bootstrap values between conflicting nodes in the separate analyses were used to identify points of weak and strong incongruence between morphological and ITS trees.

RESULTS

Nuclear ribosomal DNA—Amplification of ITS from *Ficus* yielded single bands, and cloning results from four species agreed with the results of direct sequencing at >99% of nucleotide positions. Results from cloning and direct sequencing differed from each other at three to seven positions, and no two clones from the same plant differed by more than five and eight positions of the aligned sequence (~1%) in *F. nodosa* and *F. variegata*, respectively. The location of nucleotide differences among ten clones from each species was scattered such that clones could not be grouped below the level of species. This kind of heterogeneity was suggestive of errors by DNA polymerase during cycle sequencing reactions possibly induced by high GC content in the ITS region. Overall, the results of cloning and direct sequencing suggest that heterogeneity in the ITS region did not pose a major problem for *Ficus* phylogeny reconstruction.

Manually aligned ITS sequences for 46 species were 761 bp in length including 33 positions with ambiguous alignment. Parsimony analyses of ITS alone were based on 643 bp excluding the ambiguous positions and indel positions coded as supplemental characters. One hundred and sixty-five nucleotide positions (25.6%) were potentially informative. In addition, 15 out of 35 indels were potentially informative. Analysis of the 180 characters combined found a single island of 208 most parsimonious trees of 453 steps (consistency index or CI = 0.55). The strict consensus was congruent with the bootstrap consensus at 29 of 31 nodes with >50% support (Fig. 4). Two clades with bootstrap values <60% did not appear in the strict consensus but are shown in Fig. 4. A clade with *F. pungens* as sister to subsect. *Sycocarpus* and a clade with *F. septica* as sister to the rest of subsect. *Sycocarpus* were compatible with 158 and 50 out of 216 most parsimonious trees, respectively.

In agreement with results from *rbcL* (Herre et al., 1996), the neotropical and paleotropical sections of subg. *Pharmacosycea* did not form a clade (Fig. 4), and there was marginal support from ITS for the paraphyly of sect. *Oreosycea*. Monoecious subg. *Urostigma* was not monophyletic due to the position of sect. *Urostigma* as sister to a functionally dioecious clade, but support for this relationship was weak. Subgenus *Ficus* was polyphyletic and divided into two highly supported clades. One functionally dioecious clade included the well-supported and monophyletic sects. *Ficus, Kalosyce, Rhizocladus,* and *Sycidium*, excluding *F. pungens.* The other clade included functionally dioecious sects. *Adenosperma, Neomor-*

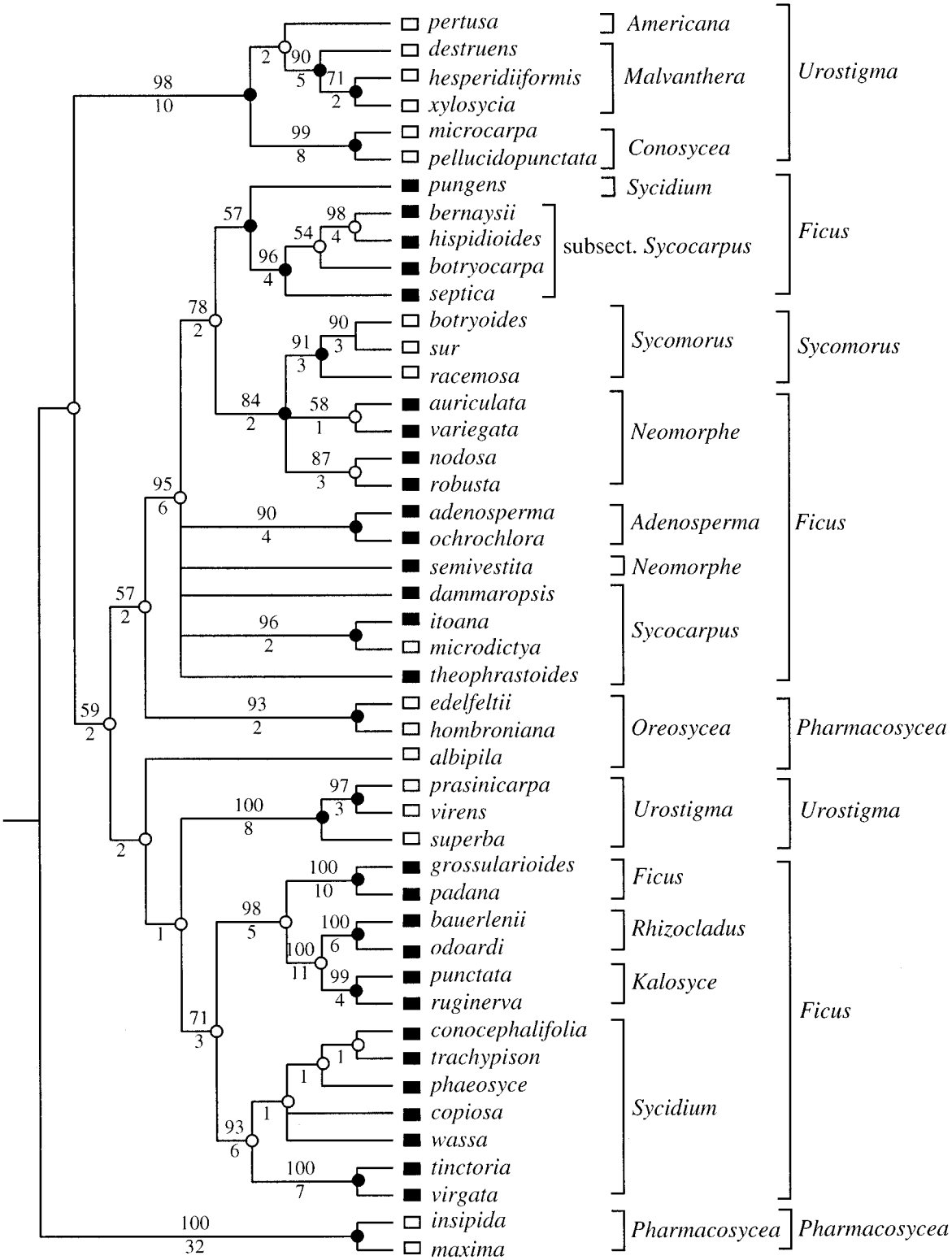


Fig. 4. The strict consensus of 218 ITS trees modified to show two additional clades recovered in the 50% bootstrap consensus (see Results). Bootstrap percentages and decay values are listed above and below the branches, respectively. Closed circles indicate those nodes that are congruent with the morphological strict consensus (Fig. 5). Open circles indicate conflicting nodes. *Ficus* sections and subgenera are shown in brackets. Open and closed bars mark monoecious and functionally dioecious species, respectively.

phe, Sycocarpus, F. pungens, and monoecious subg. *Sycomorus.* Relationships within this clade were not well resolved, although monophyly of sect. *Adenosperma,* subsect. *Sycocarpus,* and subg. *Sycomorus* were each highly supported. In addition, the derivation of monoecious subg. *Sycomorus* within functionally dioecious subg. *Ficus* received strong bootstrap support.

Morphology—The morphological analysis yielded six most parsimonious trees of 339 steps (CI = 0.47). The strict consensus was congruent with the bootstrap consensus at 20 out of 21 nodes with >50% support. (Fig. 5). A clade representing neotropical sect. *Pharmacosycea* with 57%, shown in Fig. 5, was not present in the strict consensus due to the position of *F. albipila* as sister to *F. insipida* in the most parsimonious trees. Morphological analysis indicated that subg. *Ficus* was not monophyletic and that monoecious subg. *Sycomorus* was derived within a paraphyletic sect. *Neomorphe.* The functionally dioecious figs including subg. *Sycomorus* were sister to monoecious subg. *Urostigma.* These three subgenera were derived within a paraphyletic subg. *Pharmacosycea.* However, morphological support for subgeneric relationships was relatively weak, as indicated by low bootstrap values at deep nodes compared to shallow nodes. Monoecious subg. *Urostigma* had a bootstrap value of 64%, but support for the paraphyly of sect. *Oreosycea* was lacking. The monophyly of monoecious sects. *Conosycea, Malvanthera,* and *Urostigma* was upheld in the morphological analysis, in contrast to dioecious sects. *Sycidium, Sycocarpus,* and *Neomorphe,* which were polyphyletic or paraphyletic. However, highly supported clades of functionally dioecious figs included sects. *Adenosperma, Ficus, Kalosyce,* and *Rhizocladus.*

Conflict and congruence—Comparison of morphological and ITS consensus trees (Figs. 4–5), showed that 18 of 45 nodes were in absolute agreement. Nineteen nodes in the ITS tree were not recovered in the morphological analysis. Similarly, 21 nodes in the morphological consensus were not found in the ITS consensus. However, most conflicting nodes were weakly supported (<50% bootstrap) in one analysis or the other, and most nodes with >50% support were compatible with the rival consensus tree. For example, 18 of 21 nodes in the morphological consensus having >50% support were in agreement with the ITS consensus. Although ten nodes with >50% support in the ITS consensus were not recovered in the morphological analysis, none were contradicted by bootstrap values >60% in the morphological consensus. However, the ILD test indicated significant conflict between the morphological and ITS data sets. The interpretation of this result is ambiguous because the ILD test does not distinguish among alternative hypotheses for conflict. In particular, it is unclear whether conflict results from different phylogenetic histories, rate heterogeneity, or systematic error in either data set (de Queiroz, Donoghue, and Kim, 1995).

Results of Templeton tests of incongruence are summarized in Table 3. ITS sequence data strongly rejected the shortest morphological trees. Similarly, the morphological data rejected the shortest ITS trees. However, taking into account clade robustness in the separate analyses had a strong impact on the test results. For example, ITS sequences marginally rejected the morphology-based 50% bootstrap consensus and morphological data significantly rejected the ITS 50% bootstrap consensus, but neither ITS nor morphological data sets rejected their rival 70 or 90% bootstrap consensus trees. Therefore, statistically significant conflict between ITS and morphology was limited to weakly supported nodes in the separate analyses, and it appeared that weak incongruence due to systematic error or rate heterogeneity could account for the significant ILD (Bull et al., 1993).

Combined analyses—The combined analysis recovered eight most parsimonious trees of 747 steps (CI = 0.50) on two tree islands. The strict consensus was congruent with the bootstrap consensus at all but three of 30 nodes with >50% bootstrap values (Fig. 6). A weakly supported clade (54%) including sect. *Neomorphe,* subsect. *Sycocarpus,* and subg. *Sycomorus* was recovered in three of the eight trees (Fig. 6). Seven trees from one island showed the sister relationship of *F. nodosa* and *F. robusta* that was recovered in the bootstrap consensus. In addition, a clade including subg. *Ficus* plus subg. *Sycomorus* was recovered in the bootstrap consensus (63%) but not in the strict consensus due to a difference between tree islands. Furthermore, sect. *Oreosycea* was not monophyletic in any of the most parsimonious trees, but the precise relationships of species in sect. *Oreosycea* differed between tree islands. The island of seven trees supported a clade mostly consisting of functionally dioecious figs, with *F. albipila* as its sister group (Fig. 7). A single most parsimonious tree on the other island placed *F. albipila* as sister to a functionally dioecious clade including sects. *Ficus, Kalosyce, Rhizocladus,* and *Sycidium* (Fig. 8). In this tree, *F. edelfeltii* plus *F. hombroniana* were sister to a clade including subg. *Urostigma* and the other functionally dioecious clade (sects. *Adenosperma, Neomorphe, Sycocarpus,* with monoecious subg. *Sycomorus*).

The combined analysis supported the monophyly of subg. *Sycomorus* and *Urostigma,* but subg. *Ficus* and *Pharmacosycea* were either polyphyletic or paraphyletic, depending on the tree island. Separate and combined analyses agreed on the derivation of monoecious subg. *Sycomorus* within a clade of functionally dioecious figs. Also in agreement with results from *rbcL* (Herre et al., 1996), neotropical sect. *Pharmacosycea* and paleotropical sect. *Oreosycea* did not form a clade. Within monoecious subg. *Urostigma,* there was strong support for the monophyly of the Indo-Australian sects. *Conosycea, Malvanthera,* and *Urostigma.* Two strongly supported clades containing functionally dioecious figs were also recovered in the combined analysis, but it was not entirely clear whether these were sister groups (Fig. 6). The first of these clades was functionally dioecious in its entirety and included sects. *Ficus, Kalosyce, Rhizocladus,* and *Sycidium* (excluding *F. pungens*). Bootstrap and decay values strongly supported the monophyly of each of these sections and the exclusion of *F. pungens* from *Sycidium.* Relationships within *Sycidium* were resolved but mostly not supported by high bootstrap values. Sections *Kalosyce* and *Rhizocladus* were strongly supported sister groups, and this clade was sister to sect. *Ficus.*

A second major clade of functionally dioecious figs, including *F. pungens,* sects. *Adenosperma, Neomorphe,* and *Sycocarpus,* with monoecious subg. *Sycomorus,* had high bootstrap support (89%). However, basal relationships within this clade were not well resolved in the combined analysis. Although sect. *Sycocarpus* was clearly not monophyletic, relationships within the section were mostly unresolved. Section *Neomorphe* was not monophyletic due to the highly supported relationship of *F. semivestita* to sect. *Adenosperma.* Members of

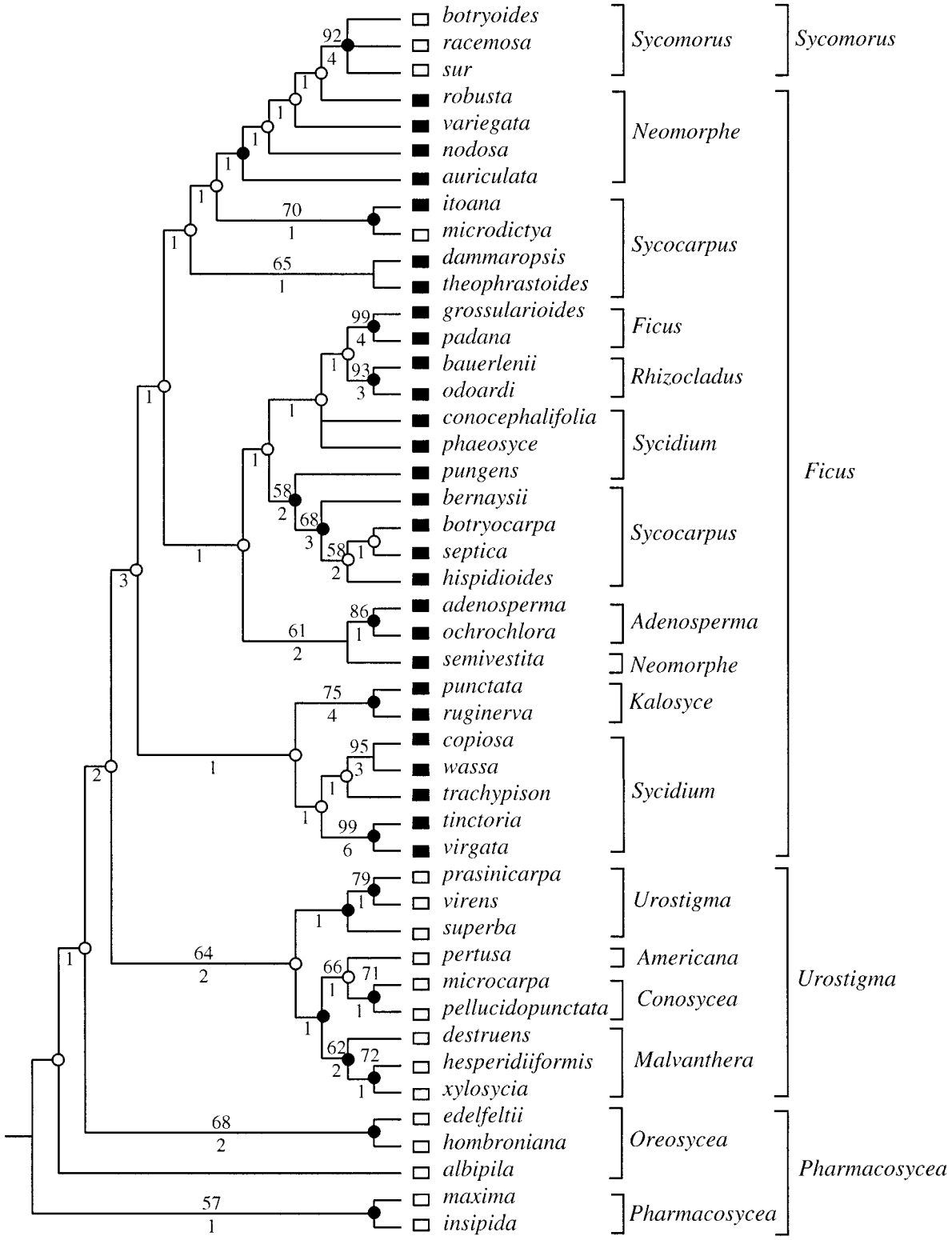


Fig. 5. The strict consensus of six trees resulting from the morphological analysis, modified to show an additional clade recovered in the 50% bootstrap consensus (see Results). Bootstrap percentages and decay values are listed above and below the branches, respectively. Closed circles indicate those nodes that are congruent with the ITS strict consensus (Fig. 4). Open circles indicate conflicting nodes. *Ficus* sections and subgenera are shown in brackets. Open and closed bars mark monoecious and functionally dioecious species, respectively.

TABLE 3. Templeton test results for incongruence between morphological and ITS data sets. For each data set, the tree length (L) resulting from rival constraint searches was compared to the length of shortest trees resulting from unconstrained searches. The largest sum of the signed and ranked differences for *N* characters was used to compute the nonparametric test statistic (*Z*).

Comparison	L	Rank sum	*N*	*Z*	*P*
ITS data and tree vs.					
morphology MP tree	567	115.5	96	−8.6348	<0.0001
morphology strict consensus	555	123.0	94	−8.6687	<0.0001
morphology 50% bootstrap	464	77.5	23	−2.0298	0.0424
morphology 70% bootstrap	453	1.5	2	0.0000	1.0000
morphology 90% bootstrap	453	0	2	—	—
Morphological data and tree vs.					
ITS MP tree	385	77.0	38	−4.3727	<0.0001
ITS strict consensus	372	125.0	37	−3.5736	0.0004
ITS 50% bootstrap	362	107.0	31	−2.8950	0.0038
ITS 70% bootstrap	353	106.5	26	−1.8557	0.0635
ITS 90% bootstrap	349	120.0	26	−1.5410	0.1233

sect. *Neomorphe* (excluding *F. semivestita*) belong to a well-supported clade including monoecious subg. *Sycomorus.* The sister relationship between functionally dioecious *F. itoana* and monoecious *F. microdictya* also received strong support in the combined analysis.

DISCUSSION

ITS and functionally dioecious fig phylogeny—ITS provided insights on the relationships of dioecious figs due to moderate levels of interspecific sequence variation and low levels of intraspecific heterogeneity. Polymorphisms reported for ITS in some plant species (cf. Wendel, Schnabel, and Seelanan, 1995; Campbell et al., 1997) were not detected in *Ficus.* Divergent ITS paralogues appear to be common in lineages having a history of hybridization and polyploidy (Buckler, Ippolito, and Holtsford, 1997). However, natural hybridization and polyploidy are rare in *Ficus* (Storey, 1975). Cytology has been examined in over 100 species and most are diploid ($2n = 26$), with exceptions such as a sterile triploid ($3n = 39$) cultivar of *F. elastica* Roxb. (e.g., Condit, 1964).

Although ITS was phylogenetically informative, the ability to resolve relationships within *Ficus* was limited. Manual alignment of ITS sequences within *Ficus* was straightforward, but sequences were highly diverged compared to other Moraceae and alignment across other genera in the family was complicated by the presence of overlapping indels. This required that tree rooting be based on additional molecular, morphological, and paleontological data (Brues, 1910; Berg, 1989; Herre et al., 1996). Low levels of sequence variability among closely related species also limited the utility of ITS for resolving phylogenetic relationships within most sections. For example, nearly identical ITS sequences were obtained from closely related taxa, such as *F. odoardi* and *F. bauerlenii* (sect. *Rhizocladus*) or *F. bernaysii* and *F. hispidioides* (subsect. *Sycocarpus*). Additional gene regions are needed to corroborate the results based on ITS and morphology.

Morphology and functionally dioecious fig phylogeny—The use of morphological characters in phylogeny reconstruction is worthy of consideration when examining the relationships of the functionally dioecious figs. It has been suggested that convergence in functional traits might bias our conclusions about phylogenetic relationships (Wiebes, 1994b; Herre et al., 1996). Examples of correlated traits include: (1) the construction of the ostiole in figs and pollinator head shape (van Noort and Compton, 1996); (2) the distribution of staminate florets in figs and pollinator behaviors or structures associated with pollen collection (Ramirez, 1978); and (3) fig breeding system and pollinator ovipositor lengths (Ramirez, 1980). However, it can be argued that most characters, whether morphological or molecular, exhibit homoplasy, and this is not sufficient for their exclusion from phylogenetic analysis (Donoghue and Sanderson, 1992). In this study, ITS sequences and morphology showed similar levels of homoplasy in *Ficus* (CI = 0.54 and CI = 0.46, respectively). Morphological features (Appendix) also provide useful touchstones for recognizing clades within *Ficus,* and lists of apomorphies are described in Weiblen (1999).

Tests of incongruence—Significant incongruence was detected between the morphological and ITS data sets by both ILD and Templeton tests (Table 3). However, it is important to ask whether statistically significant conflict, as measured by these tests, should be interpreted as evidence of different phylogenetic histories or rate heterogeneity (Bull et al., 1993). Incongruence may also be due to systematic error, and Templeton tests are potentially informative in this regard because they can take into account levels of support for rival clades in separate analyses. Templeton test results were highly sensitive to the choice of rival constraint trees. If weakly supported clades were included in rival constraint trees, the data significantly rejected the null hypothesis that random errors in phylogeny estimation account for length differences in rival trees. However, the null hypothesis was not rejected if only strongly supported clades were included. This was true for both ITS and morphology when rival constraint trees were limited to clades with >70% and >90% bootstrap support. The arbitrary level of bootstrap support considered ''strong'' seemed reasonable based on empirical studies of phylogenetic accuracy (Hillis and Bull, 1993).

The overall results provided little evidence of strong incongruence between data sets, although several instances of local incongruence deserve further consideration. For example, ITS and morphological analyses differed with regard to the monophyly of subg. *Urostigma.* Mophological and combined analyses placed subg. *Urostigma* in a clade with 65 and 64% bootstrap support, respectively (Figs. 5–6). On the other hand, ITS alone placed sect. *Urostigma* as the sister to a functionally dioecious clade with 59% support (Fig. 4). Decay analysis for ITS indicated that five additional steps were required to con-

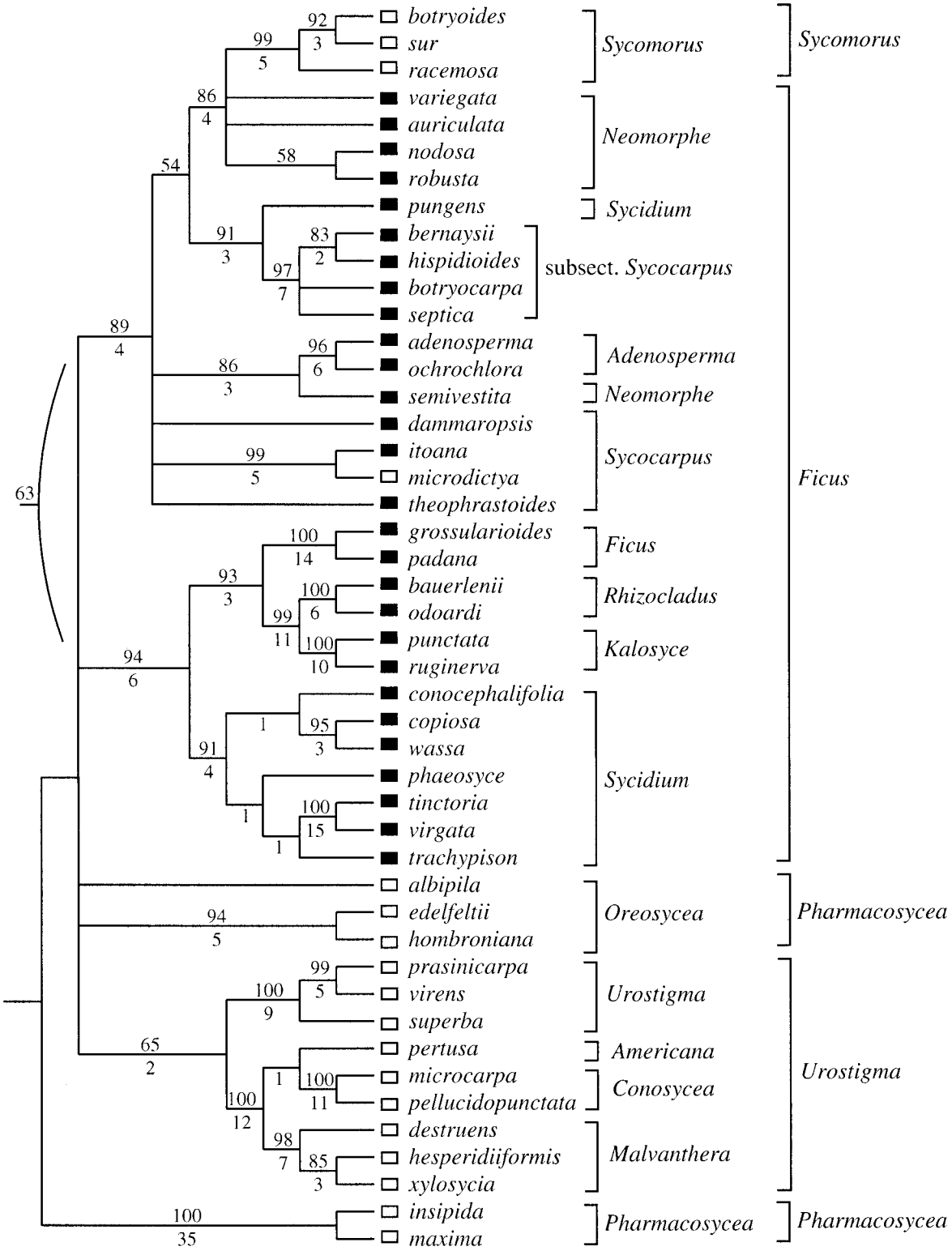
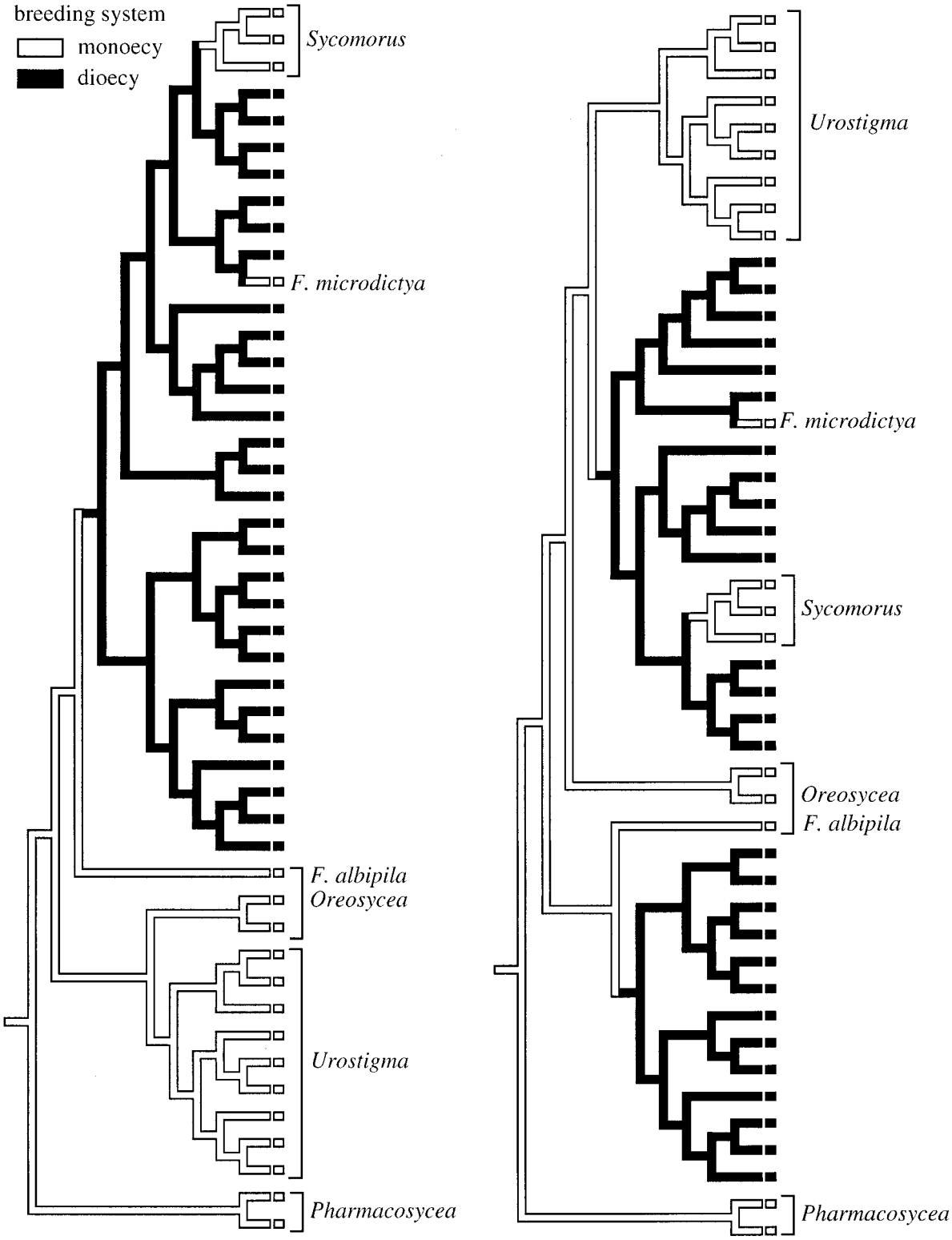


Fig. 6. The strict consensus of eight most parsimonious trees recovered in the combined ITS and morphological analysis with two additional clades recovered in the 50% bootstrap consensus (see Results). A third clade with 63% support (indicated parenthetically) was present in seven of the eight most parsimonious trees. Bootstrap percentages and decay values are listed above and below the branches, respectively. *Ficus* sections and subgenera are shown in brackets. Open and closed bars mark monoecious and functionally dioecious species, respectively.



Figs. 7–8. Alternative reconstructions of fig breeding system under parsimony. (7) One of seven trees from the first island of most parsimonious trees showing a single origin of functional dioecy from monoecy and two reversals to monoecy within the functionally dioecious clade. (8) The single tree from the second island showing two independent origins of functional dioecy and two reversals to monoecy within one of the functionally dioecious lineages. Only monoecious taxa are labeled for simplicity.

tradict the separation of sect. *Urostigma* from the rest of subg. *Urostigma.* However, reciprocal Templeton tests of local incongruence were not significant ($P = 0.20$ and $P = 0.37$ for ITS and morphology, respectively). Another local conflict involved a clade including *F. botryocarpa, F. hispidioides,* and *F. septica* with 58% bootstrap support in the morphological analysis vs. a rival clade including *F. bernaysii, F. botryocarpa,* and *F. hispidioides* with 54% bootstrap support in the ITS analysis. Both clades were present in the combined most parsimonious trees, and support from separate analyses was relatively weak (<60%). In future exploration of local conflicts, it might be possible to minimize the effects of systematic error through modification of character weighting under parsimony or alteration of rate parameters under maximum likelihood (de Queiroz, Donoghue, and Kim, 1995; Huelsenbeck, Bull, and Cunningham, 1996).

Comparisons of bootstrap values as measures of relative clade support indicated that the results of combined analyses were better supported than either separate analysis. Compared to ITS, bootstrap values for 15 nodes increased in the combined analysis, while support for six nodes decreased. Compared to the separate morphological analysis, bootstrap support for 21 nodes increased in the combined analysis, while none decreased. In the absence of evidence for strong incongruence, the combined data provided the best supported estimate of functionally dioecious fig phylogeny. Similar conclusions have been reached in studies of other plant groups (Manos, 1997; Kelley, 1998; but see Mason-Gamer and Kellogg, 1996). Classification, breeding system evolution, and associations with pollinators will be discussed in terms of the combined analysis (Figs. 6–8). However, inferences from the combined phylogeny should be regarded as preliminary until corroborated by analyses of additional genes and taxa.

Classification of functionally dioecious figs—Phylogenetic analysis of ITS and morphological characters sheds light on the traditional classification of functionally dioecious figs (Corner, 1965) and the revised classification of *Ficus* based on pollinator taxonomy (Ramirez, 1977; Berg, 1989). Although some groups appear to be monophyletic, the functionally dioecious figs are not (Fig. 6). In general, Corner's subgeneric classification (Table 1) is not supported by the result of ITS, morphology, or combined analyses (Figs. 4–6). *Pharmacosycea* is not monophyletic, and, in spite of morphological similarity, neotropical sect. *Pharmacosycea* and paleotropical sect. *Oreosycea* do not form a clade. Based on the combined analysis, *Urostigma* may be monophyletic, but separate analyses disagree in this regard. There is no tree supporting the monophyly of sect. *Oreosycea* although ITS and combined analyses support the existence of two major clades of functionally dioecious figs derived within paraphyletic sect. *Oreosycea.* Whether the functionally dioecious clades are sister groups, however, is unclear from the combined analyses.

Separate and combined analyses indicate that monoecious *Sycomorus* is monophyletic and nested in a clade of functionally dioecious *Ficus.* The close relationship of monoecious *Sycomorus* and functionally dioecious *Ficus* was first noted by Miquel (1867) and again by King (1887), but Corner (1960b) kept *Sycomorus* separate on the sole basis of breeding system. Ramirez (1977) proposed a revised classification based on pollinator taxonomy that included sects. *Adenosperma, Neomorphe, Sycocarpus, Sycomorus,* and all *Ceratosolen*-pollinated *Sycidium* in subg. *Sycomorus.* A *Ceratosolen*-pollinated clade was indeed recovered in the phylogenetic analysis, although basal relationships within it were not well resolved. Phylogenetic analyses support the view of Berg (1989) that sect. *Adenosperma* and sect. *Sycocarpus* are closely related. Also in agreement with Berg (1989), a clade including sect. *Neomorphe,* subsect. *Sycocarpus,* and subg. *Sycomorus* is well supported in the ITS and combined analyses.

One entirely functionally dioecious clade has no parallel in Corner's classification, but instead corresponds to subg. *Ficus* sensu Ramirez (1977), including sects. *Ficus, Kalosyce, Rhizocladus* and *Sycidum,* excluding all *Ceratosolen*-pollinated species. Within this clade there are two distinct groups that were recognized by Berg (1989), one including sect. *Sycidium* and the other sects. *Ficus, Kalosyce* plus *Rhizocladus.* The combined phylogenetic analysis also strongly supports the monophyly of each of these sections, excluding all *Ceratosolen*-pollinated *Sycidium,* which Ramirez (1977) transferred into his revised subg. *Sycomorus.* In general, the combined phylogenetic analysis supports the alternative classification of Ramirez (1977).

Breeding system evolution—Phylogenetic analysis indicates one or two independent origins of functional dioecy from monoecy in *Ficus,* depending on which island of most parsimonious trees is examined (Figs. 7–8). The first island included seven equally parsimonious trees with monoecious *F. albipila* as sister to a clade containing functionally dioecious subg. *Ficus* plus monoecious subg. *Sycomorus* (Fig. 7). The second tree island showed monoecious subg. *Urostigma* as sister to one clade of functionally dioecious figs and *F. albipila* as sister to another functionally dioecious clade (Fig. 8). Both separate and combined analyses unequivocally suggested two reversals from functional dioecy to monoecy within one of the functionally dioecious lineages.

It has been argued that the characters of interest should be excluded from phylogenetic analysis in order to avoid circularity and bias in studies of character evolution. Indeed, morphological characters are sometimes excluded from phylogenetic analyses on the grounds that convergence in function can lead to inaccuracy and such arguments are often the basis for preferential use of independent molecular data (Herre et al., 1996; Van Noort and Compton, 1996). However, morphological and molecular data may show similar levels of homoplasy (Donoghue and Sanderson, 1992), and molecular data may also show convergence (Naylor and Brown, 1998). Furthermore, it is possible that excluding the characters of interest can yield biased or inaccurate results (Luckow and Bruneau, 1997), while sensitivity analyses can examine the effect of excluding characters on inferences of character evolution (de Queiroz, 1996; Donoghue and Ackerly, 1996).

Exclusion of breeding system from the combined analysis resulted in one most parsimonious tree similar to the second island from the analysis based on all characters (Fig. 8). The tree indicates that monoecy is ancestral, that functional dioecy has evolved twice, and that two reversals to monoecy have occurred in one of the functionally dioecious lineages. Exclusion of additional characters possibly linked to breeding system, such as the presence of staminodes in seed figs, setose long-styled florets, and funnelform stigmas in short-styled florets, resulted in the same topology. Exclusion of all morphological characters on the grounds that they are not independent of breeding system also results in two gains of functional dioecy and two losses. Therefore, the inclusion or exclusion of

morphological characters did not have an impact on the inferences of breeding system evolution, although whether functional dioecy evolved once or twice was unclear from the combined analysis. It was not possible to obtain recent collections of monoecious *F. pritchardii* Seem., which may represent a third reversal to monoecy within a functionally dioecious lineage. Overall, morphology and pollinator associations suggest its placement with *F. pungens* and subsect. *Sycocarpus* (Wiebes, 1963).

Corner (1965) delimited subgenera on the basis of breeding system, but this character appears to be more homoplasious than morphology in general. The consistency index of breeding system ranged from 0.25 to 0.33, depending on the tree island from the combined analysis, compared to 0.46 for morphology overall. The weight that Corner placed on breeding system led to splitting monoecious *Sycomorus* from functionally dioecious *Neomorphe* in spite of shared features including cauliflory and buttresses. Similarly, monoecious *F. microdictya* was at one time classified with sect. *Oreosycea* (Corner, 1965). However, Corner (1962, 1970b) recognized the close relationship of monoecious *F. microdictya* to functionally dioecious *F. itoana,* and phylogenetic analysis shows the monoecy of *F. microdictya* to be a reversal within a functionally dioecious lineage. Why, then, did Corner divide the figs primarily according to breeding system? Although shifts in breeding system are widespread in flowering plants, taxonomists have often recognized genera and subgenera on the basis of breeding systems (Renner and Ricklefs, 1995). Corner (1985) viewed the evolution of functional dioecy as irreversible. Contrary to expectations based on taxonomic evidence, shifts from dioecy to monoecy in angiosperms may be more common than the reverse (Weiblen, Oyama, and Donoghue, 2000).

Ficus is unique in that functional dioecy results from genetic factors controlling floral development combined with the impact of pollinator larvae on seed maturation (Storey, 1975). Evidence from selection models suggests that pathways to dioecy in flowering plants may involve a gynodioecious intermediate step through the evolution of male sterility (Charlesworth and Charlesworth, 1978), and agents of selection favoring functional dioecy in *Ficus* have been suggested (Kerdelhue and Rasplus, 1996). However, there is not yet a compelling explanation for the loss of functional dioecy. It would seem that a mutation for increased ovipositor length enabling successful egg-laying in seed figs would favor a reversal to monoecy. However, the absence of pollen in seed figs prevents any offspring of a long-ovipositor mutant from founding an F2 generation. This may be precisely why the genes for style length and male sterility are linked in *F. carica* (Storey, 1975). Could reversal to monoecy in functionally dioecious lineages involve the disruption of these linked loci? The possibility of pollinator behavior and morphology driving the evolution of fig breeding systems in different directions is an interesting area for future investigation.

Congruence with pollinator classification—Agreement between fig and pollinator classifications has provided a basis for speculation on the extent of coevolution between *Ficus* and the Agaonidae (Ramirez, 1974; Wiebes, 1979; Corner, 1985). The close correspondence of fig and pollinator taxonomy could be interpreted as direct evidence for cospeciation, but congruence could also be a taxonomic artifact. Artificial agreement could arise if information from one group contributed to the classification of the other. Corner classified most of *Ficus* without knowledge of fig wasp taxonomy (but see pp. 395–396 in Corner, 1962). On the other hand, Wiebes (1994a) admitted the influence of the botanical classification in his concepts of pollinator species and genera. Phylogenetic relationships provide valuable information for identifying instances of conflict and congruence in the taxonomy of the associated groups. Phylogenetic information can then be used to evaluate whether particular cases reflect evolutionary events or taxonomic artifacts.

Agreement between fig and pollinator classification is generally supported by phylogenetic analyses based on ITS sequences and fig morphology (Fig. 9). Remarkably, there was no homoplasy in the associations of pollinator genera (CI = 1.00). Seven out of 12 pollinating genera were each associated with well-supported clades of host figs (>50% bootstrap; Fig. 6). Five of these clades represent taxonomic groups including: (1) *Blastophaga*-pollinated sect. *Ficus,* (2) *Tetrapus*-pollinated sect. *Pharmacosycea,* (3) *Lipporhopalum*-pollinated subsect. *Paleomorphe,* (4) *Pleistodontes*-pollinated sect. *Malvanthera,* and (5) *Platyscapa*-pollinated sect. *Urostigma.* In addition, a clade including sects. *Kalosyce* and *Rhizocladus* is pollinated by *Wiebesia.* One of the major clades of functionally dioecious figs is pollinated by *Ceratosolen,* corresponding subg. *Sycomorus* sensu Ramirez (1977), as designated on the basis of pollinator associations. However, some genera were associated with paraphyletic groupings of *Ficus.* Paraphyletic sect. *Oreosycea,* for example, is pollinated by *Dolichoris.* Also, *Kradibia*-pollinated sect. *Sycidium* is paraphyletic with respect to the *Lipporhopalum*-pollinated species.

Specific cases of conflict between taxonomy and phylogeny also invite discussion. The *Ceratosolen*-pollinated clade, in particular, does not agree with the classification of Corner (1965). In this instance the conflict between fig and pollinator taxonomy is reconciled upon consideration of phylogenetic relationships. Corner (1960a) related *F. pungens* to *Kradibia*-pollinated subsect. *Sycidium,* while Wiebes (1963) placed the pollinator of *F. pungens* under *Ceratosolen.* Corner (1960a) placed *F. pungens* in sect. *Sycidium* on the sole basis of having free tepals. However, in light of evidence from fig morphology, ITS sequences, and pollinator relationships, it is clear that *F. pungens* is a member of the *Ceratosolen*-pollinated clade.

Another interesting case concerns the placement of *F. semivestita.* Corner (1960b) described the species under sect. *Neomorphe* based on leaves, gall figs, and growth form. In spite of similarity in buttresses, girth, and height, *F. semivestita* is the only member of sect. *Neomorphe* with axillary figs. However, Corner's original description was incomplete because seeds were unknown at the time. Recent seed collections provided additional characters supporting the placement of *F. semivestita* in sect. *Adenosperma,* including the presence of a gynobasic style and auriculiform achenes. Wiebes (1963) suggested a close relationship between *Ceratosolen grandii,* the pollinator of *F. semivestita,* and *C. appendiculatus,* the pollinator of *F. variegata* (sect. *Neomorphe).* This affinity, however, was based on two homoplasious morphological characters, and phylogenetic relationships inferred from mitochondrial genes indicate that *C. grandii* is more closely related to pollinators of sect. *Adenosperma* than to *C. appendiculatus* (G. Weiblen, unpublished data). The results once again suggest that the classification of functionally dioecious figs can be improved based on phylogenetic analyses.

The overall congruence between fig and pollinator classifi-

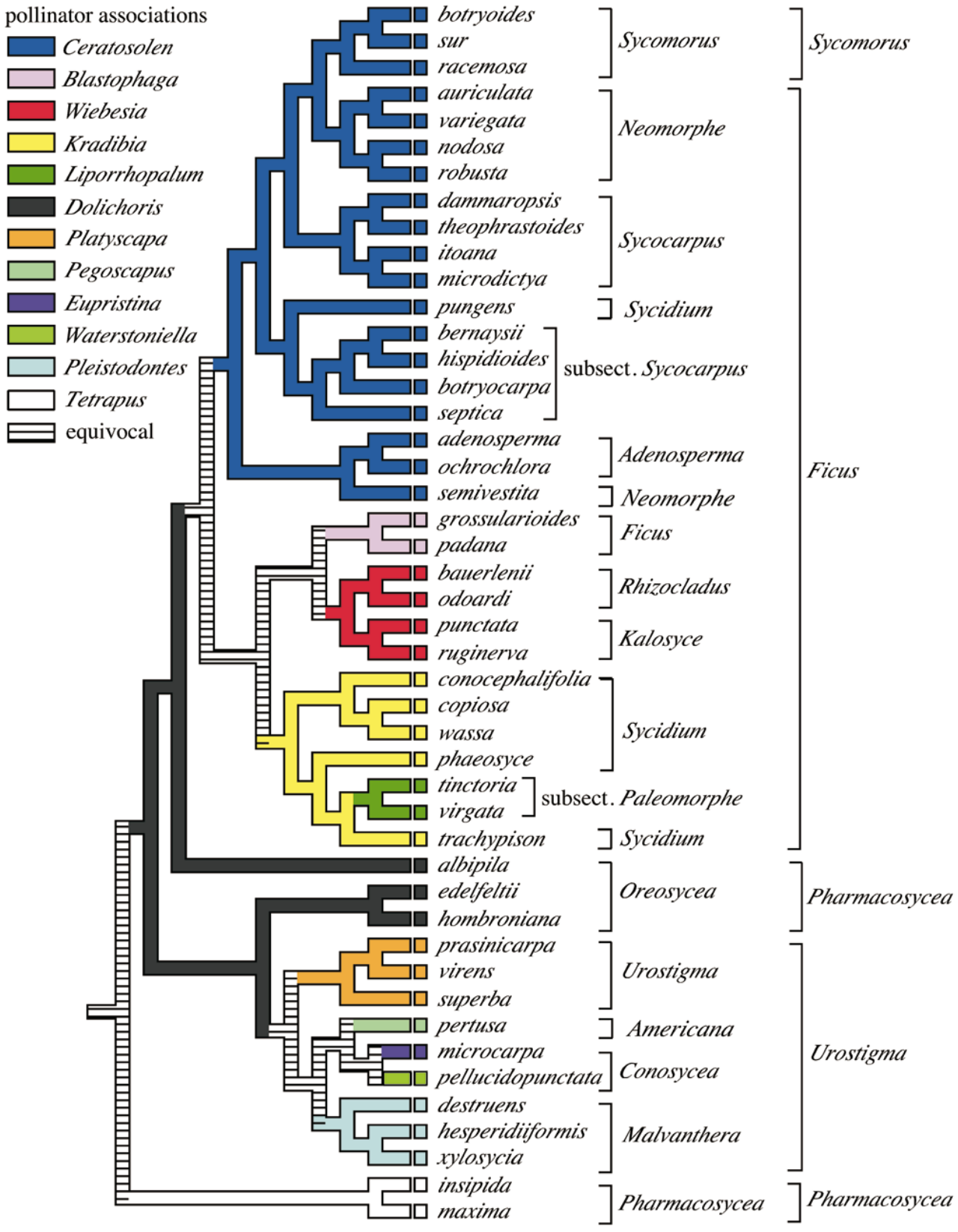


Fig. 9. The associations of pollinating fig wasp genera mapped on one of the most parsimonious trees from the combined analysis of fig morphology and ITS sequences. *Ficus* sections and subgenera are shown in brackets.

cations is striking (Fig. 9), although fig phylogeny alone cannot distinguish between taxonomic artifacts and coevolutionary processes as explanations for congruent patterns. Reciprocal phylogenetic studies are needed to address this point, given that recent classification of pollinators was not independent of fig taxonomy (Wiebes, 1994a). However, it can be concluded from phylogenetic analyses of nuclear ribosomal DNA sequences and morphology that the traditional classification of functionally dioecious figs is not supported. Subgenus *Ficus* is not monophyletic, and multiple reversals to monoecy have occurred within a functionally dioecious clade. Studying the evolution of fig breeding systems in relation to pollinators using a phylogenetic approach may further improve our understanding of fig/pollinator coevolution.

LITERATURE CITED

BALDWIN, B. G., M. J. SANDERSON, J. M. PORTER, M. F. WOJCIECHOWSKI, C. S. CAMPBELL, AND M. J. DONOGHUE. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 247–277.

BARRETT, M., M. J. DONOGHUE, AND E. SOBER. 1991. Against consensus. *Systematic Zoology* 40: 486–493.

BECK, N. G., AND E. M. LORD. 1988. Breeding system in *Ficus carica*, the common fig. I. Floral diversity. *American Journal of Botany* 75: 1904–1912.

BERG, C. C. 1989. Classification and distribution of *Ficus*. *Experientia* 45: 605–611.

———. 1990a. Differentiation of flowers and inflorescences of Urticales in relation to their protection against breeding insects and pollination. *Sommerfeltia* 11: 13–34.

———. 1990b. Reproduction and evolution of *Ficus* (Moraceae): Traits connected with the adequate rearing of pollinators. *Memoirs of the New York Botanical Garden* 55: 169–185.

BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42: 795–803.

BRONSTEIN, J. 1992. Seed predators as mutualists: ecology and evolution of the fig/pollinator interaction. *In* E. A. Bernays [ed.], Insect-plant interactions, 1–47. CRC Press, Boca Raton, Florida, USA.

BRUES, C. T. 1910. The parasitic Hymenoptera of the Tertiary of Florissant, Colorado. *Bulletin of the Museum of Comparative Zoology at Harvard College* 54: 1–125.

BUCKLER, E. S., A. IPPOLITO, AND T. P. HOLTSFORD. 1997. The evolution of ribosomal DNA: divergent paralogues and phylogenetic implications. *Genetics* 145: 821–832.

BULL, J. J., J. P. HUELSENBECK, C. W. CUNNINGHAM, D. L. SWOFFORD, AND P. J. WADDELL. 1993. Partitioning and combining data in phylogenetic analysis. *Systematic Biology* 42: 384–397.

CAMPBELL, C. S., M. F. WOJCIECHOWSKI, B. G. BALDWIN, L. A. ALICE, AND M. J. DONOGHUE. 1997. Persistent nuclear ribosomal DNA sequence polymorphism in the *Amelanchier* agamic complex (Rosaceae). *Molecular Biology and Evolution* 14: 81–90.

CHARLESWORTH, B., AND D. CHARLESWORTH. 1978. A model for the evolution of dioecy and gynodioecy. *American Naturalist* 112: 975–997.

CONDIT, I. 1964. Cytological studies in the genus Ficus. III. Chromosome numbers in sixty-two species. *Madroño* 17: 153–155.

CORLETT, R. T. 1993. Sexual dimorphism in the reproductive phenology of *Ficus grossularioides* Burm. *f.* in Singapore. *Malayan Nature Journal* 47: 149–155.

CORNER, E. J. H. 1933. A revision of the Malayan species of *Ficus*: *Coveliia* and *Neomorphe*. *Malayan Branch Royal Asiatic Society* 11: 1–65.

———. 1955. Revision of *Ficus*. *Flora Malesiana Bulletin* 11: 428–430.

———. 1958. An introduction to the distribution of *Ficus*. *Reinwardtia* 4: 325–355.

———. 1960a. Taxonomic notes on *Ficus* Linn., Asia and Australasia. I–IV. *Gardens' Bulletin Singapore* 17: 368–485.

———. 1960b. Taxonomic notes on *Ficus* Linn., Asia and Australasia. V–VI. *Gardens' Bulletin Singapore* 18: 1–69.

———. 1961. Taxonomic notes on *Ficus* Linn., Asia and Australasia: Addendum. *Gardens' Bulletin Singapore* 18: 83–99.

———. 1962. Taxonomic notes on *Ficus* Linn., Asia and Australasia: Addendum II. *Gardens' Bulletin Singapore* 19: 385–401.

———. 1965. Check-list of *Ficus* in Asia and Australasia with keys to identification. *Gardens' Bulletin Singapore* 21: 1–186.

———. 1967. *Ficus* in the Solomon Islands and its bearing on the Post-Jurassic history of Melanesia. *Proceedings of the Royal Society of London, Series B* 253: 23–159.

———. 1969. *Ficus* sect. *Adenosperma*. *Philosophical Transactions of the Royal Society of London, Series B* 256: 319–355.

———. 1970a. *Ficus* subgen. *Ficus*. Two rare and primitive pachycaul species. *Philosophical Transactions of the Royal Society of London, Series B* 259: 353–381.

———. 1970b. *Ficus* subgen. *Pharmacosycea* with reference to the species of New Caledonia. *Philosophical Transactions of the Royal Society of London, Series B* 259: 383–433.

———. 1976. The climbing species of *Ficus*: derivation and evolution. *Philosophical Transactions of the Royal Society of London, Series B* 273: 359–386.

———. 1978. *Ficus dammaropsis* Bl. and the pedunculate species of *Ficus* subgen. *Sycocarpus*. *Philosophical Transactions of the Royal Society of London, Series B* 281: 373–406.

———. 1985. *Ficus* (Moraceae) and Hymenoptera (Chalcidoidea): figs and their pollinators. *Biological Journal of the Linnean Society* 25: 187–195.

DE QUEIROZ, K. 1996. Including the characters of interest during tree reconstruction and the problems of circularity and bias in studies of character evolution. *American Naturalist* 148: 700–708.

———, M. J. DONOGHUE, AND J. KIM. 1995. Separate versus combined analysis of phylogenetic evidence. *Annual Review of Ecology and Systematics* 26: 657–681.

DONOGHUE, M. J., AND D. A. ACKERLY. 1996. Phylogenetic uncertainties and sensitivity analyses in comparative biology. *Philosophical Transactions of the Royal Society of London, Series B* 351: 1241–1249.

———, R. G. OLMSTEAD, J. F. SMITH, AND J. D. PALMER. 1992. Phylogenetic relationships of Dipsacales based on rbcL sequences. *Annals of the Missouri Botanical Garden* 79: 333–345.

———, AND M. J. SANDERSON. 1992. The suitability of molecular and morphological evidence in reconstructing plant phylogeny. *In* P. S. Soltis, D. E. Soltis, and J. J. Doyle [eds.], Molecular systematics of plants, 340–368. Chapman and Hall, New York, New York, USA.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small amounts of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

FARRIS, J. S., M. KALLERSJO, A. G. KLUGE, AND C. BULT. 1994. Testing significance of incongruence. *Cladistics* 10: 315–319.

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

GALIL, J. 1973. Pollination in dioecious figs: pollination of *Ficus fistulosa* by *Ceratosolen hewitti*. *Gardens' Bulletin Singapore* 26: 303–311.

GANESHAIAH, K. N., P. KATHURIA, R. U. SHAANKER, AND R. VASUDEVA. 1995. Evolution of style-length variability in figs and optimization of ovipositor length in their pollinator wasps: a coevolutionary model. *Journal of Genetics* 74: 25–39.

GASPARRINI, G. 1844. Nova genera quae super nonnullis Fici speciebus struebat, Naples.

GRAFEN, A., AND H. C. J. GODFRAY. 1991. Vicarious selection explains some paradoxes in dioecious fig-pollinator systems. *Proceedings of the Royal Society of London, Series B* 245: 73–75.

HERRE, E. A., C. A. MACHADO, E. BERMINGHAM, J. D. NASON, D. M. WINDSOR, S. S. MCCAFFERTY, W. V. HOUTEN, AND K. BACHMANN. 1996. Molecular phylogenies of figs and their pollinator wasps. *Journal of Biogeography* 23: 521–530.

HILLIS, D. M., AND J. J. BULL. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42: 182–192.

HOSSAERT-MCKEY, M., M. GIBERNAU, AND J. E. FREY. 1994. Chemosensory attraction of fig wasps to substances produced by receptive figs. *Entomologia Experimentalis et Applicata* 70: 185–191.

JANZEN, D. H. 1979. How to be a fig. *Annual Review of Ecology and Systematics* 10: 13–51.

KELLEY, L. M. 1998. Phylogenetic relationships in *Asarum* (Aristolochiaceae) based on morphology and ITS sequences. *American Journal of Botany* 85: 1454–1467.

KERDELHUE, C., M. E. HOCHBERG, AND J. Y. RASPLUS. 1997. Active polli-

nation of *Ficus sur* by two sympatric fig wasp species in West Africa. *Biotropica* 29: 69–75.

———, AND J. Y. RASPLUS. 1996. The evolution of dioecy among *Ficus* (Moraceae): an alternative hypothesis involving non-pollinating fig wasp pressure on the fig-pollinator mutualism. *Oikos* 77: 163–166.

KING, G. 1887. On some new species of *Ficus* from New Guinea. *Journal of the Asiatic Society of Bengal* 55: 394–411.

KJELLBERG, F., P. H. GOUYON, M. IBRAHIM, M. RAYMOND, AND G. VALDEYRON. 1987. The stability of the symbiosis between dioecious figs and their pollinators: a study of *Ficus carica* L. and *Blastophaga psenes* L. *Evolution* 41: 693–704.

KLUGE, A. J. 1989. A concern for evidence and a phylogenetic hypothesis of relationships among Epicrates (Boidae, Serpentes). *Systematics Zoology* 38: 7–25.

LARSON, A. 1994. The comparison of morphological and molecular data in phylogenetic systematics. *In* B. Schierwater, B. Streit, G. P. Wagner, and R. DeSalle [eds.], Molecular ecology and evolution: approaches and applications, 371–390. Birkhauser, Basel, Switzerland.

LUCKOW, M., AND A. BRUNEAU. 1997. Circularity and independence in phylogenetic tests of ecological hypotheses. *Cladistics* 13: 145–151.

MANOS, P. S. 1997. Systematics of *Nothofagus* (Nothofagaceae) based on rDNA spacer sequences (ITS): taxonomic congruence with morphology and plastid sequences. *American Journal of Botany* 84: 1137–1155.

MASON-GAMER, R., AND E. A. KELLOGG. 1996. Testing for phylogenetic conflict among molecular data sets in the Triticeae (Gramineae). *Systematic Biology* 45: 524–545.

MIQUEL, F. A. W. 1862. Over de geographische verspreiding der Ficeae, met een nader onderzoek omtrent de soorten, welke in Amerika, noordelijk van de landengte van Panama, voorkomen. *Mededelingen Koninklijke Akademie van Wetenschappen* 13: 1–34.

———. 1867a. Annotationes de Ficus speciebus. *Annales Museum Botanicum Lugduno-Batavum* 3: 260–300.

———. 1867b. Artocarpeae. *Annales Museum Botanicum Lugduno-Batavum* 3: 210–235.

NAYLOR, G. J. P., AND W. M. BROWN. 1998. *Amphioxus* mitochondrial DNA, chordate phylogeny, and the limits of inference based on comparison of sequences. *Systematic Biology* 47: 61–76.

PATEL, A., AND D. MCKEY. 1998. Sexual specialization in two tropical dioecious figs. *Oecologia* 115: 391–400.

RAMIREZ, W. B. 1970. Host specificity of fig wasps (Agaonidae). *Evolution* 24: 680–691.

———. 1974. Coevolution of *Ficus* and Agaonidae. *Annals of the Missouri Botanical Gardens* 61: 770–780.

———. 1977. A new classification of *Ficus. Annals of the Missouri Botanical Garden* 64: 296–310.

———. 1978. Evolution of mechanisms to carry pollen in Agaonidae (Hymenoptera Chalcidoidea). *Tijdschrift voor Entomologie* 121: 279–293.

———. 1980. Evolution of the monoecious and dioecious habit in *Ficus* (Moraceae). *Brenesia* 18: 207–215.

RASPLUS, J. Y. 1994. The one-to-one specificity of the *Ficus*-Agaoninae mutualism: how casual? *In* L. J. G. van der Masen, X. M. v. d. Burgt, and J. M. v. M. d. Rooy [eds.], The biodiversity of African plants, 639–649. Kluwer Academic Publisher, Dordrecht, The Netherlands.

RENNER, S. S., AND R. E. RICKLEFS. 1995. Dioecy and its correlates in the flowering plants. *American Journal of Botany* 82: 596–606.

STOREY, W. B. 1975. Figs. *In* J. Janick and J. N. Moore [eds.], Advances in fruit breeding, 568–589. Purdue University Press, West Lafayette, Indiana, USA.

SWOFFORD, D. L. 1998. Phylogenetic analysis using parsimony (*and other methods). Sinauer, Sunderland, Massachusetts, USA.

TEMPLETON, A. R. 1993. The ''Eve'' hypothesis: a genetic critique and reanalysis. *American Anthropologist* 95: 51–72.

VALDEYRON, G., AND D. G. LLOYD. 1979. Sex differences and flowering phenology in the common fig, *Ficus carica* L. *Evolution* 33: 673–685.

VAN NOORT, S., AND S. G. COMPTON. 1996. Convergent evolution of agaonine and sycoecine (Agaonidae, Chalcidoidea) head shape in response to the constraints of host fig morphology. *Journal of Biogeography* 23: 415–424.

VERKERKE, W. 1989. Structure and function of the fig. *Experientia* 45: 612–622.

WARE, A. B., AND S. G. COMPTON. 1994. Responses of fig wasps to host plant volatile cues. *Journal of Chemical Ecology* 20: 785–802.

WEIBLEN, G. D. 1999. Phylogeny and ecology of dioecious fig pollination. Ph.D. dissertation, Harvard University, Cambridge, Massachusetts, USA.

———, R. K. OYAMA, AND M. J. DONOGHUE. 2000. Phylogenetic analysis of breeding system evolution in monocotyledons. *American Naturalist* 155: 46–58.

———, H. SPENCER, AND B. FLICK. 1995. Seed set and wasp predation in dioecious *Ficus variegata* from an Australian wet tropical forest. *Biotropica* 27: 391–394.

WENDEL, J. F., A. SCHNABEL, AND T. SEELANAN. 1995. Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton. *Proceedings of the National Academy of Sciences, USA* 92: 280–284.

WHITE, T. J., T. BRUNS, S. LEE, AND J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White [eds.], PCR pProtocols, 315–322. Academic Press, San Diego, California, USA.

WIEBES, J. T. 1963. Taxonomy and host preferences of Indo-Australian fig wasps of the genus *Ceratosolen* (Agaonidae). *Tijdschrift voor Entomologie* 106: 1–112.

———. 1979. Co-evolution of figs and their insect pollinators. *Annual Review of Ecology and Systematics* 10: 1–12.

———. 1994a. Agaonidae (Hymenoptera Chalcidoidea) and *Ficus* (Moraceae): fig wasps and their figs, xiii (*Ceratosolen* & additions). *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen Series C* 97: 123–136.

———. 1994b. The Indo-Australian Agaoninae (pollinators of figs), North-Holland, Amsterdam, The Netherlands.

YOKOYAMA, J. 1995. Insect-plant coevolution and speciation. *In* R. Arai, M. Kato, and Y. Doi [eds.], Biodiversity and evolution, 115–130. National Science Museum Foundation, Tokyo, Japan.

Appendix. *Ficus* reproductive characters and vegetative characters coded for phylogenetic analysis. The rationale for delimitation of character states is outlined in Weiblen (1999). The matrix of morphological characters for 46 species used in the phylogenetic analysis is available at http://www.herbaria.edu/treebase/index.html and at http://www.botany.org.bsa/ajbsupp/v87.

1. Breeding system [0] monoecious or [1] gynodioecious. 2. Syconia [0] solitary or paired in the axils of expanded leaves (axillary) or [1] additionally or entirely produced on leafless branchlets (cauliflorous). 3. Syconia on leafless branchlets with [0] short internodes or [1] with elongated internodes. Only applicable to cauliflorous taxa. 4. Syconia with [0] two or [1] three basal bracts. 5. Basal bracts [0] caducous or [1] persistent in ripe syconia. 6. Basal bracts [0] glabrous or glabrescent, [1] pubescent (with persistent hairs but not rough like sandpaper) or [2] scabrid (rough like sandpaper due to raised cystoliths). 7. Syconia [0] sessile or [1] pedunculate. 8. Syconium basal bracts [0] at the bottom, [1] between the bottom and the top or [2] at the top of the peduncle. Only applicable to pedunculate taxa. 9. Peduncles [0] glabrous, [1] pubescent, [2] scabrid, or [3] muricate (with epidermal hooks 0.5–2 mm in length). Only applicable to pedunculate taxa. 10. Syconia [0] without lateral bracts or transverse ridges, [1] with lateral bracts or [2] with transverse ridges on the receptacle. 11. Syconium outer epidermis [0] glabrous, [1] pubescent, [2] scabrid, [3] muricate or [4] pustulate (covered with lenticels). 12. Syconia [0] without or [1] with sclereids in the receptacle. 13. External ostiolar bracts [0] two or three or [1] more than three, or [2] sunken and not visible on fig exterior. 14. Internal ostiolar bracts [0] overlapping (in cross-section) or [1] inflexed and not overlapping. 15. Syconium lumen [0] dry or [1] fluid-filled during the interfloral phase. 16. Syconium inner epidermis [0] without or [1] with glandular hairs. 17. Pistillate florets [0] all without pedicels (sessile) or [1] varying within syconia from sessile to pedicellate. 18. Pedicels of pistillate florets [0] glabrous or [1] setose. Only applicable to pedicellate taxa. 19. Pistillate perianth with tepals [0] free, [1] fused at the base, [2] fused completely along their length, or [3] without tepals in seed figs. 20. Pistillate perianth with tepals [0] glabrous or [1] pubescent on the abaxial surface. Not applicable to seed figs lacking tepals. 21. Pistillate perianth margins [0] entire, [1] ciliate (hairy) or [2] dentate (toothed). Not applicable to seed figs lacking tepals. 22. Pistillate perianth [0] white (without pigment) or [1] red. Not applicable to seed figs lacking tepals. 23. Style [0] subterminal to lateral or [1] gynobasic. 24. Style [0] glabrous in all florets or [1] setose in long-styled florets. Only applicable to taxa with dimorphic pistillate florets. 25. Style [0] not divided or [1] divided at the apex. 26. Stigma [0] clavate or [1] funnel-

shaped. Only applicable to functionally dioecious taxa. 27. Ovary [0] white (without pigment), [1] with a red spot near the base of the style or [2] red throughout. 28. Ovaries embedded in the receptacle [0] none or [1] those of seed-producing florets. 29. Achene [0] not flattened or auriculiform or [1] auriculiform to flattened; more than twice as long as wide. Applicable only to viable achenes at maturity. 30. Achene [0] smooth or [1] tuberculate. 31. Achene [0] with a single ridge arising from the hilum or [1] with a forked, double ridge arising from the hilum. 32. Staminate florets [0] dispersed, scattered among the pistillate florets or [1] ostiolar (clustered around the ostiole). 33. Staminate florets [0] without pistillodes, [1] with pistillodes or [2] with functional gall ovaries. 34. Staminate florets [0] without or [1] with staminodia. Not applicable to monoecious species. 35. Stamens per floret [0] one, [1] two or [2] varying from one to three. 36. Staminate florets [0] sessile or [1] pedicellate. 37. Staminate perianth with tepals [0] free (not fused), [1] fused at the base or [2] fused completely along their length and splitting at anthesis. 38. Staminate perianth with tepals [0] glabrous or [1] pubescent on the abaxial surface. 39. Filaments [0] without epidermal hairs at the base or [1] with epidermal hairs at the base. 40. Anthers [0] not mucronate or [1] mucronate. 41. Anthers [0] with two thecae or [1] with one theca. 42. Growth habit [0] hemiepiphytic or strangling, [1] climbing, or [2] free-standing. 43. Buttresses in mature trees [0] less than 0.5 m or [1] more than 1 m in height. Not applicable to epiphytes, hemiepiphytes, climbers, shrubs or immature trees. 44. Growth [0] continuous or [1] discontinuous (deciduous). 45. Branches [0] orthotropic or [1] plagiotropic by apposition. 46. Twigs [0] solid or without spongy pith or [1] hollow or with spongy pith. 47. [omitted]. 48. Twigs [0] glabrous or glabrescent, [1] pubescent, with persistent hairs, but not rough like sandpaper or [3] scabrid due to raised cystoliths. 49. Twigs [0] without waxy glands below the node, [1] with a waxy gland below the node or [2] with two glands below the node. 50. Stipules [0] tightly clasping the bud at the apex or [1] bent away from the bud at the apex. 51. Stipules [0] glabrous or [1] pubescent on the abaxial surface. 52. Stipules [0] caducous or [1] persistent. 53. Latex [0] white or [1] yellow. 54. Ptyxis [0] rolled in bud or [1] folded in bud. 55. Phyllotaxis [0] spiral or [1] distichous. 56. Petioles [0] glabrous, [1] pubescent, [2] scabrid, or [3] muricate. 57. Leaves (laminae) [0] cuneate to rounded or [1] cordate at the base. 58. Leaves [0] symmetric or [1] asymmetric. 59. Leaf glands [0] none, [1] one, at the base of the midrib, [2] one, in the axil of one of the basal veins, [3] two, in the axils of both basal veins, [4] in the axils of basal and secondary veins, or [5] only in the axils of lateral veins. 60. Tertiary veins [0] parallel to secondary veins, [1] reticulate (not parallel or perpendicular to secondary veins), or [2] scalariform (perpendicular to secondary veins). 61. Leaf margin [0] entire or [1] serrate to dentate. 62. Leaf epidermis [0] glabrous, [1] pubescent, [2] scabrid, or [3] muricate. 63. Cystoliths [0] none, [1] abaxial, [2] on both sides of the leaf, or [3] adaxial. 64. Stomata [0] not aggregated or [1] aggregated in sunken and foveate areoles.