Evolution and classification of figs (*Ficus*, Moraceae) and their close relatives (Castilleae) united by involucral bracts

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Figs and fig wasps are a classic example of an obligate pollination mutualism. Decades of work untangling the ecology and evolution of these organisms has simultaneously contributed to development of the fields of mutualism, coevolution and plant–insect interactions at large. With > 800 species, figs (*Ficus*, Moraceae) are among some of the larger genera of angiosperms. Phylogenetic studies of Moraceae have supported the clade Castilleae as the sister lineage of *Ficus*. Compared to *Ficus*, Castilleae have many fewer species (60 species in 11 genera), suggesting changes in rates of diversification along these two branches. Relatively little is known about Castilleae compared to *Ficus*, and we argue that defining the clade comprising *Ficus* and Castilleae, hereafter Involucrata, focuses attention on opportunities for comparative studies of pollination mutualisms and diversification rates. In this study, we define Involucrata and propose a revised classification scheme that accounts for the phylogenetic reconstruction based on the most comprehensive sampling of this group to date. Moving forward, this classification will better guide and support evolutionary, ecological and comparative pollination biology studies of this group.


INTRODUCTION

With at least 800 named species, *Ficus* L. accounts for more than half of the species diversity of the mulberry family, Moraceae (c. 1100 species; Clement & Weiblen, 2009). Phylogenetic analyses of Moraceae have strongly supported Castilleae as sister to *Ficus* based on plastid (Datwyler & Weiblen, 2004), nuclear (Zerega et al., 2005) and morphological data (Clement & Weiblen, 2009). *Ficus* has been central to advancing study of pollination mutualisms, coevolution and cospeciation (Bronstein, 1988; Herre, 1989; Herre & West, 1997; Lopez-Vaamonde et al., 2001; Weiblen, 2001; Weiblen, Yu & West, 2001; Weiblen & Bush, 2002; Cook & Rasplus, 2003; Jousselin, Rasplus & Kjellberg, 2003; Weiblen, 2004; Machado et al., 2005; Rønsted et al., 2005; Marussich & Machado, 2007; Silvieus, Clement & Weiblen, 2007; Jackson et al., 2008; Jousselin et al., 2008; Herre, Jandér & Machado, 2008; Cruaud et al., 2012a; Cruaud et al., 2012b; McLeish & van Noort, 2012; Conchou et al., 2014; Bain et al., 2016; Rodriguez et al., 2017). *Ficus* spp. occur in tropical and...
subtropical regions worldwide and include trees, hemi-
epiphytes, epiphytes, shrubs, climbers, rheophytes and
lithophytes. In Castillea, Castilleae are a group of 11
genera and 60 species of trees and shrubs with four
species distributed in the Paleotropics and 56 species
in the Neotropics. *Ficus* and Castilleae diverged from
one another at least 65 Mya (Zerega et al., 2005),
and the striking difference in contemporary species
richness suggests differing rates of diversification.

Together, *Ficus* and Castilleae differ from other
Moraceae in having involucral bracts that subtend
the inflorescences on a disc or urn-shaped receptacle.
In Castillea, the involucral bracts do not completely
enclose the inflorescence like they do in *Ficus*. The
positioning of these bracts has profound implications
for their reproductive ecology. In *Ficus*, the involucral
bracts form a tight pore, or ostiole, at the apex of the
receptacle. Mated pollinating wasps force themselves
through this opening into the cavity of the fig
(syconium) where they pollinate flowers, lay eggs and
usually die. Pollinator offspring emerge from galls
inside the fig to mate and collect pollen from staminate
flowers before exiting in search of other receptive
figs. In contrast to the ‘cradle to grave’ relationship
between figs and their pollinating wasps, Castilleae
inflorescences are only partially enclosed by involucral
bracts thereby allowing pollinators to come and go.
From the limited study of pollination in Castillea, wind
(Osmaston, 1965; Croat, 1978) and insect (Sakai,
Kato & Nagamasu, 2000; Zerega, Mound & Weiblen,
2004) pollination syndromes are present. As in *Ficus*,
insect-pollinated Castilleae are also involved in brood-
site pollination mutualisms in which pollinators mate
and lay eggs in the inflorescences. Pollination by thrips
has been documented for two species of Castilleae,
*Antiaropsis decipiens* (K.Schum. (endemic to New
Guinea; Zerega et al., 2004) and *Castilla elastica*
Sess. (widespread in the Neotropics; Sakai et al.,
2000).

Comparative study of *Ficus* and Castillea can
offer insights into the evolution of morphological and
molecular diversity, pollination ecology, diversification
rates and historical dispersal patterns. However, aside
from family-level phytogenetic studies (Datwyler &
Weiblen, 2004; Zerega et al., 2005; Clement & Weiblen,
2009), *Ficus* and Castilleae have seldom been the subject
of comparative work (Clement, 2008; Moe, Clement &
Weiblen, 2012). Comparing Castillea and fig pollination
syndromes, Moe et al. (2012) hypothesized that the
nature of the pollinator reward and the number of floral
visits by a pollinator may account for the difference in
diversification in these two lineages. For instance,
fig wasp offspring develop in galled or fertilized fig
ovules. When wasp offspring fare better in pollinated
flowers, pollination can increase wasp fitness and the
fig can furthermore reduce pollen production to the
benefit of pollinator production. Thrips pollinating
Castillea do not depend on successful pollination as
thrips eat pollen and mate on male inflorescences.
Selective pressure on host choice also differs among fig
and Castilleaa pollination syndromes. In many species,
foundress fig wasps lose their wings and antennae on
entering a fig so that they cannot reach another tree,
probably resulting in intense selection to discern host
quality before host selection. Castillea pollinators can
visit multiple inflorescences per generation with little
consequence for visiting a non-rewarding inflorescence.
Differing selective pressures resulting from the nature
of these pollination interactions may have impacted the
evolutionary trajectory of both lineages (Moe et al., 2012).
Further testing of this hypothesis requires additional
study of pollination biology of Castillea and an improved
phylogenetic framework for *Ficus* and Castillea.

Our current understanding of *Ficus* classification is
largely based on a massive Malesian revision of *Ficus*
initiated by Corner and completed by Berg after Corner’s
death (Berg, 2003a, b, c, d, e, 2004a, b; Berg & Corner,
2005) building on earlier work (summarized in Corner,
1965). Berg's classification based on morphological and
anatomical characters emphasized emphasis on vegetative
characters compared to Corner’s treatments that
focused on floral and fruit characters (Corner, 1965).
Ultimately, Berg & Corner (2005) subdivided *Ficus*
into six subgenera: (1) *Pharmacosycea* (Miq.) Miq.
(monoecious); (2) *Urostigma* (Gasp.) Miq. (monoecious)
(3) *Ficus* (gyno-dioecious); (4) *Sydidiun* (Miq.) Mildbr.
& Burret (gyno-dioecious); (5) *Synoecia* (Miq.) Miq.
(gyno-dioecious) and (6) *Sycomorus* (Gasp.) Miq. (gyno-
dioecious and monoecious). Subgenera *Pharmacosycea*,
*Sydidiun*, *Sycomorus* and *Urostigma* are distributed
from the Pacific to West Africa, with subgenera
*Pharmacosycea* and *Urostigma* additionally including
a Neotropical section. Subgenera *Ficus* and *Synoecia*
are almost exclusively restricted to the Malesian region
and mainland Asia (Berg, 2003a).

The most recent comprehensive molecular
phylogenetic analysis of 200 *Ficus* spp. supported the
monophyly of subgenera *Sydidiun*, *Sycomorus* and
*Synoecia*, but subgenera *Ficus*, *Pharmacosycea* and
*Urostigma* were paraphyletic (Cruaud et al., 2012b)
concurring with prior work on phylogenetic trees for
*Ficus* (Weiblen, 2000; Jousselin et al., 2003; Rønsted
et al., 2005; Rønsted et al., 2008a; Xu et al., 2011).
Although many sections and subsections in these
subgenera were not monophyletic, several supported
classes do broadly correspond to published sections
[Adenosperma Corner, Americanae Miq., Eriosycea
Miq., Galoglychia Gasp., Oreosycea (Miq.) Miq.,
Pharmacosycea (Miq.) Benth.& Hook.f, Syccocarpus
Miq., Sycomorus (Gasp.) Miq.] and subsections
[Conosycea (Miq.) C.C.Berg, Ficus, Frutescentiae Sata,
Given that phylogenetic evidence only partly supports previous taxonomic treatments based on morphology, there is much potential for confusion.

Relationships along the backbone of the phylogenetic tree for *Ficus* remain unsupported, and conflicts between ribosomal DNA and low-copy nuclear gene trees are not resolved (Cruaud et al., 2012b; Harrison et al., 2012). Further, a recent phylogenetic reconstruction from whole plastids representing 59 *Ficus* spp. (Bruun-Lund et al., 2016) provided strong support for relationships deep in the phylogenetic tree for *Ficus*. However, a number of conflicts were identified and await increased resolution and clade support from phylogenetic trees reconstructed from nuclear gene regions for further investigation.

Similar to *Ficus*, the current classification of Castilleae is primarily based on morphology. Castilleae are trees, generally diagnosed by unisexual inflorescences with discoid to cup-shaped receptacles, bracts subtending the inflorescence (involucre), large seeds, sepalate wood fibres and the lack of cystoliths. Molecular phylogenetic analysis of plastid (*ndhF*; Datwyler & Weiblen, 2004) and nuclear (*26S*; Zerega et al., 2005) sequence data in addition to morphology (Clement & Weiblen, 2009) supported the unity of Castilleae, including *Antiaropsis* K.Schum, *Poulsenia* Eggers and *Sparattosyce* Bureau (formerly part of tribe Artocarpeae, breadfruit and relatives) plus all eight genera of Neotropical Castilleae (Datwyler & Weiblen, 2004). Morphological analysis of the tribe further supported two subtribes, Antiaropsineae, comprising *Antiaropsis* and *Sparattosyce*, and Castillineae, including the remaining nine genera (Clement & Weiblen, 2009). As Castilleae have only been treated in the context of Moraceae, revision of classification of Castilleae awaits molecular phylogenetic study.

To facilitate further comparative work among *Ficus* and Castilleae, we present an improved phylogenetic framework for both clades. First, we propose the name Involucrata for the well-supported clade including Castilleae and *Ficus*. This name reflects a key morphological feature shared between the two lineages, involucral bracts. Next, we present a molecular phylogenetic tree of 307 *Ficus* spp. and 43 species of Castilleae, the most robust species sampling of the group to date. Finally, using the current classification of *Ficus* and Castilleae based on morphology (Berg, 1977; Berg & Corner, 2005; Berg, Corner & Jarrett, 2006), we use the phylogenetic tree reconstructed here as a framework to suggest revisions to the classification of Involucrata that now reflect evolutionary relationships. The clade Involucrata includes the reciprocally monophyletic tribes Castilleae and Ficeae.

**MATERIAL AND METHODS**

**TAXON SAMPLING**

To assess the current classification and describe the evolutionary relationships of *Ficus* and Castilleae, we assembled the most comprehensive data matrix to date, sampling representatives of all 11 genera of Castilleae and > 40% of 800 named *Ficus* spp. Data were assembled in two matrices. The first data matrix focused on phylogenetic reconstruction of Involucrata and included 133 taxa. Taxon sampling included 94 *Ficus* spp. (two or three species per major clade; Cruaud et al., 2012b), 39 species of Castilleae representing all 11 genera, and *Artocarpus* J.R.Forst. & G.Forst. (Artocarpeae, Moraceae) as an outgroup. This data set included three gene regions: the internal transcribed spacer region of nuclear ribosomal DNA (ITS), glyceraldehyde 3-phosphate dehydrogenase (*G3pdh*) and granule bound starch synthase (*GBSSI*; Supporting Information, Supplementary Table S1). The second matrix focused on *Ficus* and included 307 *Ficus* spp. adding > 100 species to the most recent comprehensive phylogenetic sample (Cruaud et al., 2012b). Our sampling included the type species of traditionally recognised sections of *Ficus* wherever possible. We designated *Antiaropsis decipiens*, *Castilla elastica*, *Poulsenia armata* (Miq.) Standl. and *Sparattosyce dioica* Bureau as outgroups to root the phylogenetic tree. This data set included six gene regions: ITS; external transcribed spacer region (ETS) and four low-copy nuclear gene regions (*G3pdh*, *GBSSI*, glutamine synthase (*ncpGS*) and, for the first time for *Ficus*, Mg-protoporphyrin monomethyl ester cyclase (*At103*) (Supporting Information, Supplementary Table S1).

Leaf material for sequencing newly added species was obtained from herbaria (A, AAU, F, HON, HUH, K, LAE, MIN, MO, PUH, UNAM), living collections (BG, BR, C, HITBC, K, NBG, REU) and recent field collections (Supporting Information, Supplementary Table S1). New data (> 400 = 34% of analysed sequences) were combined with data from prior phylogenetic work on Moraceae (Weiblen, 2000; Jousselin et al., 2003; Machado et al., 2005; Rensted et al., 2005, 2008a; Silvieus et al., 2007; Jackson et al., 2008; Renoult et al., 2009; Azuma et al., 2010; McLelish et al., 2011; Xu et al., 2011; Cruaud et al., 2012b; Harrison et al., 2012; Kasumi et al., 2012; Chantarasuwat et al., 2015). GenBank accessions for all taxa are available in Supporting Information, Supplementary Table S1.

**DNA EXTRACTION, AMPLIFICATION AND SEQUENCING**

Total genomic DNA was extracted from 15–30 mg of dried leaf-fragments or herbarium material following Rensted et al. (2008a). Amplification of ITS,
ETS, G3pdh, ncpGS and GBSSI for all Ficus spp. was performed following Cruaud et al. (2012b) and references therein. Amplification of At103 followed protocols by Li et al. (2008). Amplification primers are listed in Supporting Information, Supplementary Table S2.

ITS, G3pdh and GBSSI for genera of Castilleae were amplified in a 25 μL reaction using 1x TaKaRa Ex Taq buffer (2mM MgCl₂; Otsu, Shiga, Japan), 0.2 mM each dNTP, 10 μM bovine serum albumin (BSA), 12–25 μM forward and reverse primers (Supporting Information, Supplementary Table S2), 1.25 U TaKaRa Ex Taq DNA polymerase and c. 20 ng of genomic DNA. In instances where ITS amplification was not successful, a nested PCR approach was used by first amplifying a larger region encompassing ITS with 25 μM of external primers 17SE and 26SE (Sun et al., 1994), followed by a second PCR using 1 μL of the previous PCR product, and 25 μM of ITS4 and ITS5. Thermal cycler conditions for all ITS amplifications were: 94 °C for 2 min, 25 cycles of 94 °C for 1 min, 50 °C for 1 min, 70 °C for 2 min, followed by 72 °C for 7 min. Thermal cycler conditions for G3pdh were: 95 °C for 3 min 30 s, 35 cycles of 95 °C for 1 min, 49 °C for 1 min, 70 °C for 2 min, followed by 72 °C for 7 min. Thermal cycler conditions for GBSSI followed a ‘stepdown’ protocol modified from Evans et al. (2000) as follows: 94 °C for 3 min, 2 cycles of 94 °C for 1 min, 58 °C for 1 min, 72 °C for 2 min, 2 cycles of 94 °C for 1 min, 54 °C for 2 min, 72 °C for 2 min, 2 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 2 min, and 24 cycles of 94 °C for 1 min, 48 °C for 2 min, 72 °C for 2 min, followed by 72 °C for 20 min. PCR products were column purified using a Qiagen PCR cleanup kit (Qiagen, Valencia, CA, USA) and quantified using a Turner Quantech Fluorometer (Barnstead-Thermolyne, Dubuque, IA, USA) using Hoechst 33258 dye prior to sequencing.

All ITS, ETS, G3pdh, ncpGS and At103 PCR products were directly sequenced. GBSSI and ITS amplicons showing signs of divergent alleles in direct sequencing were cloned prior to sequencing using either a TOPO-TA (Invitrogen, Carlsbad, CA, USA) or Stratagene PCR cloning kit (Agilent Technologies, Santa Clara, CA, USA) following manufacturer protocols. Transformed bacteria were grown overnight on LB + ampicillin agar plates at 37 °C. Eight to ten colonies per PCR product were screened using PCR for insert size. Three positive clones per accession were grown in LB + ampicillin broth overnight at 37 °C and plasmids were isolated using Qiagen Plasmid Isolation kit (Qiagen, Valencia, CA, USA). In other cases, the gene region of interest was cleaned directly from the clone screen PCR using a Qiagen PCR cleanup kit.

Previously published ETS trees for Ficus have been in conflict with other nuclear genes, as the ETS tree failed to recover a monophyletic Ficus subgenus Sycomorus because section Syccocarpus formed a separate clade sister to subgenus Urostigma (excluding subsection Urostigma) (e.g. Rønsted et al., 2008a). Multiple copies of ETS in Ficus have been suspected (Cruaud, pers. comm.; NR pers. obs.) and potential problems with ETS paralogy have been reported (Calonje et al., 2009). We explored the problem in Ficus by resampling species from clades in conflict and not in conflict among the ETS and other trees. Our sampling included: section Syccocarpus (F. condensa King, F. fistulosas Reinw. ex Blume, F. hispida Blanco and F. scortechinii King), section Adenosperma (F. ochroclora Ridl., F. pseudopalmia Blanco and F. itoana Diels), and section Sycomorus (F. sur Forsk., F. sycomorus L. and F. vallis-choudae Delile) covering subgenus Sycomorus, subsection Conosycea (F. drupacea Thunb.) and subsection Urostigma (F. lacor Buch.-Ham). In an effort to capture a greater proportion of ETS paralogues potentially present, we relaxed PCR conditions by lowering the annealing temperature from 49 to 45 °C, increasing the number of cycles from 25 to 40, and extending the duration of the preemt from 2 min 30 s to 4 min. We also designed and used a Ficus specific primer (ETS-Fic1, Supporting Information, Supplementary Table S2), and cloned all PCR products. We column purified and sequenced six to nine clones per accession (except for F. hispida in which only three amplicons were recovered).

Sequencing for all cleaned PCR products was performed using Big Dye v.3.1 sequencing reagents and protocols (Applied Biosystems, Foster City, CA, USA). Sequencing reactions were performed in 10 μL reactions with 20 ng PCR product or 200 ng of isolated plasmids. Sequencing primers for each gene region are listed in Supporting Information, Supplementary Table S2. Products were visualized and data were collected on an ABI 377 automated DNA sequencer (Applied Biosystems). Sequences were assembled using Sequencer v.4.6 (Gene Codes Corp., Ann Arbor, MI, USA) or Geneious v.R6-7 (www.biomatters.com). Individual gene regions within each data set were first aligned using MAFFT (Katoh & Standly, 2013) and manually inspected.

**Phylogenetic Analyses**

Trees for each gene region were reconstructed using maximum likelihood and Bayesian inference for Involucrata and Ficus. Prior to analysis, the best fitting model of sequence evolution was determined using jModeltest v.2.1.4. (Darriba et al., 2012) following the AIC criterion (Posada & Buckley, 2004). In the Involucrata dataset, TIM3+G, TVM+I+G and TIM2+I+G was selected for G3pdh, ITS and GBSSI,
respectively. For *Ficus*, a GTR+G model of sequence evolution was selected for ITS, ETS and *G3pdh*, and TIM2+G, TPM2uf+G and TPM3uf+I+G were selected for *ncpGS*, GBSSI and *At103*, respectively. Maximum likelihood analyses were performed in Garli v.2.01.167 (Zwickl, 2006) and repeated five times, each time using a random starting tree and allowing model parameters to be estimated. Support was assessed using 500 bootstrap replicates in Garli (Zwickl, 2006). As these models are nested within the general time reversible model, all matrices were analysed with a GTR+G model for Bayesian analyses. Bayesian analyses were run with MrBayes v.3.2.1 (Huelsenbeck & Ronquist, 2001) for 30milion generations. Stationarity was assessed using the Trace option in Geneious v.R7 (Biomatters, Ltd) and with Tracer v.1.5 (Rambaut, 2007), and the first 25% of trees sampled in the posterior distribution were removed as burnin.

Before concatenation in a combined analysis, trees were visually inspected and compared for supported (using bootstrap and posterior probabilities) topological congruence. Using PartitionFinder (Lanfear et al., 2012), we determined the best partitioning strategy and models of sequence evolution for the combined datasets. The combined analyses of the *Ficus* and *Involucrata* datasets were conducted using the same analysis protocols as described for individual gene regions.

**RESULTS**

**CONGRUENCE OF PHYLOGENETIC TREES FOR INVOLUCRATA**

The ML and Bayesian analyses recovered similar topologies but with different levels of clade support. Bayesian analyses often had higher support for relationships as compared to ML bootstrap analyses (Fig. 1, TreeBase accession S24008). Here, we recovered congruent relationships among the trees with one exception. Subsection *Urostigma* was recovered as monophyletic in the ITS tree (bootstrap (BS) = 97, posterior probability (PP) = 1) but not the *G3pdh* tree (subsection *Urostigma* was not fully sampled in GBSSI tree; Fig. 1, TreeBase accession S24008). As the dedicated analysis of *Ficus* offered an expanded sampling of this clade, a detailed description of relationships recovered in trees resulting from that analysis will be described in the *Ficus* phylogenetic tree section below.

With respect to the Castilleae clade in the *Involucrata* analyses, *Castilla, Helicostylis* Trécul and *Maquira* Aubl. were recovered as monophyletic (Fig. 1). *Antiaris* Lesch. and *Poulsonia* are monotypic, and *Antiaropsis* and *Sparattosyce* were each represented by one of the two species. *Naucleopsis* Miq. was recovered as monophyletic in *G3pdh* and ITS trees (Fig. 1, TreeBase accession S24008). However, two clades of *Naucleopsis* spp. were consistently recovered in all trees with one clade containing *N. glabra* Spruce, *N. kruckovii* (Standl.) C.C. Berg, *N. ulei* (Warb.) Ducke and *N. imitans* (Ducke) C.C.Berg and a second clade containing *N. caloneura* Ducke, *N. guianensis* (Mildbr.) C.C. Berg and *N. ternstroemiiflora* (Mildbr.) C.C. Berg. *Perebea* Aubl. and *Pseudolmedia* Trécul were not consistently recovered among the trees. The paraphyly of *Perebea* was due to the exclusion of *Perebea mollis* (Poepp. & Endl.) Huber and *P. rubra* (Trécul) C.C. Berg, which formed a clade independent of other *Perebea* spp. (Fig. 1). The core *Perebea* clade often did not include *P. guianensis* Aubl., but there was little support for excluding it. *Pseudolmedia* was recovered as monophyletic in the GBSSI tree and two well-supported *Pseudolmedia* clades were recovered by ITS. These relationships differ as ITS suggested *P. laevis* (Ruiz & Pav.) J.F.Machr. and *P. macrophylla* Trécul are sister taxa (BS = 100, PP = 1), whereas GBSSI placed *P. laevis* as sister to all *Pseudolmedia* including *P. macrophylla* (BS = 90, PP = 1; Fig. 1, TreeBase accession S24008). *G3pdh* did not recover a clade containing *Pseudolmedia* as *P. laevigata* Trécul and *P. rigida* (Klotzsch & H.Karst.) Cuatrec. (which are well-supported sister taxa in all three gene trees) were more closely related to *Perebea mollis* and *P. rubra* (BS = 73, PP = 1; Fig. 1, TreeBase accession S24008).

Few well-supported relationships among genera of Castilleae were recovered in the gene tree analyses. Neotropical taxa were supported as a clade only by ITS (BS = 86, PP = 1; Fig. 1, TreeBase accession S24008), and none of the trees recovered the relationship of the Palaeotropical to Neotropical genera due to lack of resolution. ITS strongly supported a clade containing *Pseudolmedia, Perebea, Helicostylis* and *Maquira* (BS = 91, PP = 1; Fig. 1, TreeBase accession S24008) and GBSSI was unresolved for these nodes. The *G3pdh* tree conflicted with this clade; this tree recovered a clade of *Pseudolmedia, Perebea* and *Helicostylis* (BS = 88, PP = 1; Fig. 1, TreeBase accession S24008) to the exclusion of *Maquira*. Instead, *Maquira* was recovered as sister to *Naucleopsis* with moderate to strong support (BS = 71, PP = 0.98). Further, in the clade containing *Pseudolmedia, Perebea* and *Helicostylis*, the placement of *Pseudolmedia rigida* and *P. laevigata* (as described above) conflicted with both the ITS and *G3pdh* trees.

**COMBINED ANALYSIS OF INVOLUCRATA**

Although there were supported conflicts when comparing the trees, many of these supported conflicts
Figure 1. Phylogenetic trees from individual (upper left panel) and combined (main tree) maximum likelihood analyses of Involucrata using ITS, G3pdh and GBSSI. Thickened branches represent posterior probabilities greater than 0.95, and maximum likelihood bootstrap values are indicated above the branches (main tree only). Genera in Involucrata are
were only supported by the results of the Bayesian analysis and had low to moderate support in the ML bootstrap analysis. As such, we chose to combine our trees in a total evidence analysis, recognizing that more data will be needed in the future to resolve deeper relationships of the group.

Combining the ITS, G3pdh and GBSSI data improved the resolution and clade support of the Involucrata phylogenetic tree (Fig. 1). All genera of Castilleae, except *Perebea*, were strongly supported with high bootstrap support and high posterior probabilities (Fig. 1). *Perebea* was recovered as paraphyletic as *P. mollis* and *P. rubra* formed a well-supported clade outside of *Perebea* (BS = 82, PP = 1; Fig. 1) similar to results of the tree analyses. *Antiaris decipiens* and *Sparattosyce dioica* were sister taxa (BS = 99, PP = 1; Fig. 1) and formed a clade sister to all other Castilleae (BS = 86, PP = 0.99; Fig. 1). *Antiaris toxicaria* was recovered as sister to *Mesogyne insignis* (BS = 96, PP = 1; Fig. 1), and this clade was recovered as sister to the well-supported clade of Neotropical Castilleae (BS = 94, PP = 1; Fig. 1). In Neotropical Castilleae, *Poulsenia* was recovered as sister to all other Castilleae (BS = 91, PP = 1; Fig. 1). Here, *Maquira* was well supported as sister to *Helicostylis*, *Perebea* and *Pseudolmedia* (BS = 78, PP = 1; Fig. 1), similar to the placement in the ITS and GBSSI trees. *Pseudolmedia laeavigata* and *P. rigida* were recovered in a larger clade of *Pseudolmedia* as opposed to *Perebea rubra* and *P. mollis* as observed in the G3pdh tree.

**TREE CONGRUENCE FOR FICUS**

The final data set included 307 *Ficus* spp. Numbers of species sampled for each gene region were as follows: *At103* – 140, ETS – 244, ITS – 311, G3pdh – 209, GBSSI – 60 and ncpGS – 79. No strongly supported conflicts between individual datasets were recovered. Individual analysis of the *At103* region provided limited resolution and support but did not conflict with previous findings (phylogenetic reconstruction not shown).

Amplification success of the ETS region was improved considerably using the new *Ficus* specific primer ETS-Fic1 (Supporting Information, Supplementary Table S2) resulting in the addition of 39 new sequences of the ETS region (Supporting Information, Supplementary Table S1). The targeted sampling of ETS using relaxed PCR conditions recovered two copies of the ETS region for several accessions from section *Symocarpus* (*F. condensa*, *F. fistulosa*, *F. hispida* and *F. scortechinii*) and section *Adenosperma* (*F. adenosperma*). We found that the He11 primer used in previous studies preferentially amplified a paralogous copy of ETS for some taxa, which resulted in the polyphyly of subgenus *Symocarpus* recovered in previous studies. Using the new *Ficus* specific primer ETS-Fic1, we successfully amplified the presumably correct copy resulting in new sequences placing section *Symocarpus* and all members of section *Adenosperma* with the remainder of subgenus *Symocarpus* as supported by ITS and other genes and morphology. Using the ETS-Fic1 primer (Supporting Information, Supplementary Table S2), the new ETS data recovered a monophyletic subgenus *Symocarpus*. All ETS sequences of section *Symocarpus* and *F. adenosperma* produced prior to this study that represent a paralogous copy were excluded from the data matrix prior to the final analysis.

**COMBINED ANALYSIS FOR FICUS**

The emerging picture of the phylogenetic tree of *Ficus* (Figs 2, 3A–F) was largely consistent with sections or subsections proposed by morphology and provided a coherent global framework, although infrageneric relationships remain uncertain and many relationships were not well supported. The extensive sampling in the present study allowed for interpretation of relationships of several taxa that have been difficult to place using morphology.

Three of the six subgenera (Berg & Corner, 2005), namely *Sycidium* (80% BS/PP = 0.99), *Symocarpus* (97% BS/PP = 1.00) and *Symoecia* (100% BS/PP = 1.00), were monophyletic, whereas subgenera *Ficus*, *Pharmacosycea* and *Urostigma* were polyphyletic. The American section *Pharmacosycea* (100% BS/PP = 1.00) was sister to the remainder of *Ficus* (68% BS/PP = 0.90), although this was not strongly supported. Relationships in the remainder of *Ficus* were not well resolved, but a number of clades were well supported. Section *Oreosycea* (Miq.) Miq. is divided between two clades consisting of subseries *Albipilae* Corner (100% BS/PP = 1.00) and the remainder of section *Oreosycea* (77% BS/PP = 1.00). Subgenus *Urostigma* is also split into a clade with subsection *Urostigma* (100% BS/PP = 1.00) represented by different colours consistent between the trees based on individual loci and the combined phylogenetic tree. In *Ficeae*, clades corresponding to named sections have been collapsed where possible (full tree not shown). For the three trees, G3pdh, ITS and GBSSI, clades have been collapsed based on genus or clades with a genus to compare relationships among these groups in each tree (all trees are available in TreeBase accession S24008).

PP = 1.00) and a larger clade (100% BS/PP = 1.00) including the remainder of the former subgenus Urostigma. Sections Urostigma (Gasp.) Endl. and Stilpnophyllum Endl. are polyphyletic. Subgenus Ficus is split into three clades corresponding to the Ficus carica L. group (100% BS/PP = 1.00), which is unplaced, and sections Frutescentiae Sata (92% BS/PP = 1.00) and Eriosycea Miq. (100% BS/PP = 1.00), which form a clade (98% BS/PP = 1.00) together with subgenus Synoezia (Miq.) Miq. (100% BS/PP = 1.00).

Figure 2. Cladogram based on relationships reconstructed from the maximum likelihood analysis of the six-locus Ficus dataset (detailed tree: Fig. 3A–F) providing an overview of the current phylogenetic understanding of relationships in Ficus. Approximate number of species in each clade indicated to the left of each clade name, and the subgeneric classification based on Berg & Corner (2005) indicated on the righthand side of the coloured boxes. ML bootstrap support indicated as follows: thickened branch = 95–100%, thin branch = 70–94%, and dashed branches = < 69%; posterior probability > 0.95 indicated with an asterisk.
DISCUSSION

PHYLOGENETIC TREE FOR INVOLUCRATA

Here we introduce the name Involucrata to represent the clade containing Ficus and Castilleae. With striking variation in numbers of species, genetic diversity and morphology, we discuss differences in historical biogeography, molecular evolution and pollination ecology between Ficus and Castilleae to propose future research on evolutionary mechanisms driving the diversification of these two lineages.

The centre of diversity for Castilleae is in the Neotropics, whereas the centre of diversity for Ficus is in the Palaeotropics, specifically Borneo and New Guinea (Berg 2005b; Berg et al., 2006). Our study of the phylogenetic tree of Castilleae strongly supports the monophyly of Neotropical Castilleae, suggesting a single colonization event to the New World tropics. In contrast, Ficus probably colonized the Neotropics twice, as phylogenetic studies of Ficus have recovered two well-supported clades of Neotropical Ficus that diversified at different points in evolutionary history (Jousselin et al., 2003; Rønsted et al., 2005; Rønsted et al., 2008a; Cruaud et al., 2012b). Molecular phylogenetic analysis of Ficus tentatively identified the Neotropical section Pharmacosycea as sister to all other lineages of the genus (Herre et al., 1996; Rønsted et al., 2005; Rønsted et al., 2008a; Cruaud et al., 2012b; Bruun-Lund et al., 2016; Zhang et al., 2018), although the crown group of section Pharmacosycea diversified only 16 Mya and long after the origin of Ficus at least 75.0–48.5 Mya (Rønsted et al., 2005; Zhang et al., 2018). Estimates of the crown age of Castilleae (50.0–31.2 Mya) predate the diversification of Neotropical Ficus (Rønsted et al., 2005; Zerega et al., 2005; Xu et al., 2011; Cruaud et al., 2012b; Zhang et al., 2018). Differences in the number of colonization events and in the timing of diversification, seen in light of differences in historical climate and biogeographical events (e.g. the Andean uplift; Machado et al., 2018), should inform our comparison of diversification rates between the two lineages.

Highly specific pollination mutualisms, like the fig–fig wasp interaction, have been hypothesized to increase rates of speciation (Stebbins, 1981), although studies in yuccas and yucca moths have shown the opposite (Smith et al., 2008). Pollination syndromes of the sister group (Castilleae) are worthy of consideration in terms of how they might influence speciation and extinction (Sakai et al., 2000; Zerega et al., 2004; Moe et al., 2012). It remains unknown if thrips and Castilleae depend on each other for survival, as thrips may be able to breed elsewhere, and Castilleae could receive pollen from other insects. Research dedicated to assessing the probability of extinction in the two lineages given their pollination syndromes ought to examine the degree to which speciation and extinction rates are associated with diversification (Moe et al., 2012).

If we consider the morphological evolution of figs and Castilleae as it relates to pollination biology, some of the traits associated with the fig–fig wasp pollination mutualism evolved in the common ancestor of Ficus and Castilleae (Clement & Weiblen, 2009). For instance, the appearance of an involure, which is correlated with a shift from wind to insect pollination, occurred prior to the split between Ficus and Castilleae (Datwyler & Weiblen, 2004; Clement & Weiblen, 2009). Although the involure is not exclusive to Ficus, tracking subsequent modifications of this trait is important in understanding the evolution of fig pollination where pollinators, hatched in the functional male figs, are part of the male investment of the plant (Anstett, Hossaert-McKey & Kjellberg, 1997). Comparisons of molecular evolutionary rates, morphologies and pollination syndrome are needed to identify factors affecting rates of diversification.

PHYLOGENETICS AND TAXONOMY OF CASTILLEAE

Strong support was recovered for the monophyly of the Neotropical taxa (Fig. 1) also recovered in prior phylogenetic studies of the family (Zerega et al., 2005). In this group, monotypic Poulsenia was recovered as sister to all other Neotropical Castilleae. Poulsenia has several unique characters that separate it from the remainder of Castilleae including prickles and the loss of septate wood fibres (Berg, 2001).

Perebea was consistently recovered as paraphyletic in the individual and combined analyses (Fig. 1, TreeBase accession S24008). Perebea section Noyera (Trécul) Engl., including P. rubra and P. mollis, did not group with the rest of the genus. Noyera Trécul (Trécul, 1847) was first designated as a genus with the description of Noyera rubra Trécul. The genus was later reduced to a section of Perebea (Engler, 1889) and also included P. mollis. Ducke (1922) reinstated Noyera including N. mollis (Poeppe. & Endl.) Ducke, N. rubra and later a third species, N. glabrifolia Ducke (Ducke, 1992). In 1972, Noyera was again reduced to a section of Perebea (Berg, 1972), and P. rubra was reduced to a subspecies of P. mollis. Later, P. mollis subsp. rubra (Trécul) C.C.Berg was reinstated as P. rubra, and P. glabrifolia was reduced to P. rubra subsp. glabrifolia (Ducke) C.C.Berg (Berg, 2001). Section Noyera differs from the rest of Perebea in having pluricellular globose capitulate hairs on the lower leaf surface, filiform stigmas and inner involucral bracts that are long and incurred prior to anthesis (Berg, 1972, 2001). Based on molecular evidence and these diagnostic features, we recommend reinstating the
Figure 3A–F. Maximum likelihood tree of the combined analysis of six gene regions for 307 *Ficus* spp. ML bootstrap support indicated as follows: thickened branches = 95–100%, thin branches = 70–94% and dashed branches = < 69%.

Figure 3 Continued. Posterior probability > 0.95 indicated with an asterisk. Species included in phylogenetic analysis of *Ficus* for the first time marked in bold. Proposed names for monophyletic groups of figs are indicated to the right of each clade throughout the figure. A. Synoecia, Frutescentiae and Eriosycea. B. Asperae, Phaeopilosae, Palaeomorphe and Sinoscidium. C. Sycomorus, Adenosperma and Sycomorus spp. D. Oreosycea, Urostigma, Albiplae, Caricae, and Pharmacosycea. E. Galoglychia and Americanae. F. Conosycea and Malvanthera.
Figure 3 Continued.
Figure 3 Continued.
Figure 3 Continued.
Figure 3 Continued.
genus Noyera with N. mollis and N. rubra as the sole members.

An alternative taxonomic proposal would be to expand the circumscription of Perebea to encompass Pseudolmedia. However, Pseudolmedia, has recognizably distinct morphology that supports maintaining it as a genus for practical reasons. All Pseudolmedia spp. are dioecious with uniflorous pistillate inflorescences (Berg, 1972, 1977, 2001). Further, ITS and GBSSI phylogenetic trees support the monophyly of Pseudolmedia, but the G3pdh tree recovered a paraphyletic Pseudolmedia. Although more data are needed to investigate this conflict among trees, the relationships recovered by the ITS and GBSSI trees, not G3pdh, are corroborated by morphology.

Our analysis supported the monophyly of Helicostylis and confirmed the position of the morphologically distinct H. tovarensis (Klotzsch & H.Karst) C.C.Berg as sister to all other Helicostylis (Fig. 1). Helicostylis tovarensis differs from the rest of the genus on account of free rather than basally connate tepals in pistillate flowers, which are uniflorous rather than multiflorous, and one or two staminate inflorescences per leaf axil (Berg, 1972).

Although a combined analysis strongly supported the monophyly of all genera of Castilleae except Perebea (and apart from the three monotypic genera, Poulsenia, Antiaris and Mesogynae Engl.), tree analysis of the Involutcrata data set shed light on a number of conflicts. As the analysis was based on just two low-copy nuclear genes and the internal transcribed spacer region of ribosomal DNA, there is much room for conflict among diverging trees. Specifically, the placement of Maquira and the monophyly of Pseudolmedia were called to question by G3pdh (Fig. 1). We speculate that the G3pdh tree is discordant with the Castilleae species tree based on nuclear ITS, GBSSI, 26S (Zerega et al., 2005; Zerega, Nur Supardi & Motley, 2010), plastid ndhF region (Datwyler & Weiblen, 2004) and morphology. Although the source of the conflict is unknown at this time, some possibilities include having sampled a divergent allele or paralogue for Maquira. Regardless, use of this gene region in the future will require further investigation of the G3pdh gene history in Involutcrata. Other conflicts were observed but supported only by Bayesian posterior probabilities that have been shown to consistently over estimate branch support (Huelsenbeck et al., 2002; Erixon et al., 2003).

PHYLOGENETICS AND TAXONOMY OF FICUS

Compared to the most recent comprehensive phylogenetic studies (Xu et al., 2011; Cruaud et al., 2012b), the present study increased taxon sampling by 42 species that were not included in any of the previous studies, introduced data from a gene region, AT103 (new to phylogenetic studies of Ficus), and reduced the amount of missing data in the matrix adding c. 140 new sequences for Ficus. The topology obtained from the At103 region was consistent with prior phylogenetic studies of Ficus (e.g. Cruaud et al., 2012b). Of the Ficus spp. included for the first time here (highlighted in bold, Fig. 3A–F), most are placed in the same clades as their closest relatives predicted from their current classification sensu Berg & Corner (2005). The inclusion and verification of the placement of these taxa in a comprehensive phylogenetic framework provides stronger evidence for the current circumscription of clades and infrageneric relationships of Ficus.

Some taxa that have been difficult to classify based on their morphology were also included in this phylogenetic analysis of Ficus for the first time. For example, inclusion of additional taxa from subgenus Sycidium including F. tsiangii Corner as a second representative of the Sinosycidium group (section Sinosycidium Corner) helped to confidently identify four major subclades of subgenus Sycidium (groups Palaeomorphe, Phaeopilosae, Sinosycidium and Sycidium; Fig. 3D). On the other hand, additional sampling of the Oreosycea and Synoecia clades highlighted the need for further revision of these groups as emerging subclades do not reflect the current morphological classification (Fig. 3A, B). Taxonomic implications of this most comprehensive phylogenetic framework are discussed next.

CURRENT CLADES TO GUIDE THE CLASSIFICATION OF FICUS

The comparison of morphology-based classification to phylogenetic reconstruction of evolutionary relationships among Ficus identified taxonomic revisions that are needed to guide future evolutionary studies of the clade. Whether the use of rank-based or rank-free taxonomy is applied to future revisions of Ficus, applying names to monophyletic groups should be central to either approach. In our species sampling of Ficus, we attempted to include the type species of former sections to help circumscribe clades. However, this was not always possible; in such cases, we relied on identifying clades based on classically accepted concepts of sections. Ultimately, we propose the recognition of a number of clades in Ficus that in some cases reinforce the classification of Berg & Corner (2005) and in other cases depart from it to provide clarity and precision when communicating about Ficus diversity.

The set of clade names proposed here more accurately recognizes the evolutionary history of Ficus. Wherever
possible, we applied names historically associated with
groups of *Ficus*, and in some cases (e.g. Mixtiflores)
new names were proposed for new assemblages of
species. Each clade name is presented in conjunction
with the closest Linnaean name and rank when
possible for comparison to prior publications on
*Ficus* classification. Figure 2 should be referenced for
interpreting the relationships and hierarchy of the
clades presented in the following discussion. Although
we do not formally revise fig taxonomy here as further
resolution and support for many clades are wanting, we
encourage future revisionary work to consider a rank-
free taxonomy given the number of clades researchers
would want to regularly discuss due to the size and
complex evolutionary history of the group (e.g. shifts in
breeding system, pollinator behaviour, habit etc.).

**Synoeia**

This clade (Fig. 3A; 100% BS/PP = 1.00) corresponds
to *Ficus* subgenus *Synoeia* (Miq.) Miq., one of the
three subgenera that are monophyletic. This clade
includes c. 72 species of dioecious root climbers in
Asia and Australasia (Berg, 2003d; Berg & Corner
sections *Rhizoclodus* Endl. (primarily in New Guinea)
and *Kissosycea* Miq. (primarily in Borneo), which are
not clear-cut based on morphology; these sections are
not resolved by the present molecular study. Notably,
there is a clade consisting of *F. sarmentosa* Buch.-Ham.
ex Sm. and *F. diversiformis* Miq. *Ficus sarmentosa*
is traditionally considered a member of section
*Rhizoclodus*, but is a variable species with affinities to
the *Punctata* group of section *Kissosycea* (Berg & Corner,
2005). *Ficus diversiformis* is traditionally considered a
member of the Malesian section *Kissosycea*, but it is
one of only two species confined to mainland Asian
(Berg & Corner, 2005). The other species, *F. hederacea*
Roxb., was not sequenced for this study. *Ficus pumila*
L. is also a root climber traditionally included in
section *Rhizoclodus*, but previous studies (e.g. Ransted,
2008a) have shown that *F. pumila* is more closely
related to traditional *Ficus* spp. of section *Frutescentiae*
(subgenus *Ficus*), showing that the root-climbing habit
has evolved at least twice. A few other root climbers
such as the essentially Sino–Himalayan *F. laevis* Desf.
and *F. pubigera* (Wall. ex Miq.) Miq. also show affinities
to members of subgenus *Ficus* (Berg & Corner, 2005).
*Ficus laevis* was not sequenced for this study, but
*F. pubigera* is imbedded in section *Rhizoclodus*.

**Frutescentiae**

This clade (Fig. 3A; 92% BS/PP = 0.87) corresponds
to section *Ficus* subsection *Frutescentiae* Sata and
consists of 25–30 species including *F. pumila* and
*F. iidaiana* Wilson, mostly from the Sino–Himalayan
region and eight species from western Malesia. The
*Frutescentiae* clade is closely related to the *Eriosycea* and
*Synoeia* clades.

**Eriosycea**

This clade (Fig. 3A; 100% BS/PP = 1.00) corresponds to
section *Eriosycea* Miq. with c. 34 species ranging from
Sino–Himalaya to New Guinea. The *Eriosycea* and
*Frutescentiae* clades are closely related to the *Synoeia*
clade and together this group forms a well-supported
clad (Fig. 3A; 98% BS/PP = 0.98), which has also
been resolved in previous studies. However, subgenus
*Ficus* to which *Frutescentiae* and *Eriosycea* have been
placed, is polyphyletic on account of the position of
section *Ficus* (see the discussion on the Caricaceae clade).

**Sycidium**

This clade (Figs. 2, 3B; 80% BS/PP = 0.81) corresponds to
subgenus *Sycidium* (Miq.) Berg & Corner, which is
another of the three monophyletic subsections of *Ficus*.
*Sycidium* includes c. 110 dioecious species primarily in
Asia and Australasia with approximately ten species in
Africa and Madagascar (Berg, 2003e; Berg & Corner,
2005). The *Sycidium* clade also largely corresponds to
section *Sycidium sensu Corner 1965*, but excluding
series *Pungentes* Corner [F. minnahassae (Teifjsm. &
de Vriese) Miq. and *F. pungens* Reinw. ex Blume], which
Berg transferred to subgenus *Sycosyceus*, and including
section *Sinosyceae* and series *Sinosyceae* (Berg,
2003e). Berg (2003e) subdivided subgenus *Sycidium*
to two sections based primarily on differences in
growth habit and the flowers; section *Palaeomorphe*
was based on adventitious roots and hermaphroditic
flowers with ovules galled by pollinators, and section
*Sycidium* without aerial adventitious roots. In the
present study, four major clades are recognized,
which may be ranked as sections if stronger support is
obtained in the future (*Palaeomorphe Phaeopilosae*,
*Sinosyceae* and *Asperae* clades). Three Asian
mainland species constituting section *Sinosyceae*
are sister to the remaining subclades.

**Asperae**

This clade (Fig. 3B; 55% BS/PP = 0.56) corresponds to
section *Asperae* (Miq.) Berg & Corner, excluding
*Phaeopilosae* (King) Corner and *Sinosyceae* Corner.
We recommend referring to this clade as Asperae
rather than *Sycidium* to reduce confusion because this
clade is nested in the larger clade *Sycidium* (Fig. 2).
The name Asperae refers to *F. aspera*, the type species
of subgenus *Sycidium* being included in the former
section *Sycidium*. The delimitation of this clade and
its subdivisions may need revision once data including more species becomes available.

**Phaeopiloseae**

This constitutes a well-supported clade (Fig. 3B; 92% BS/PP = 0.91) of species endemic to New Guinea and tropical Australia largely corresponding to the *Conocephalifolia* group *sensu* Berg including *F. wassa* Roxb. and *F. copiosa* Steud. but excluding *Ficus gul* Lauterb. & K.Schum. As a result, the *Phaeopiloseae* clade is confined to Eastern New Guinea and North Queensland. *Ficus complexa* Corner, the type species for Corner’s series *Phaeopiloseae*, as well as a number of other species included in Corner’s series *Phaeopiloseae* or in Bergs *Conocephalifolia* group were not included in this study so that the circumscription and name of the *Phaeopiloseae* clade is uncertain at present.

**Palaeomorphe**

This clade (Fig. 3B; 60% BS/PP = 0.65) corresponds to section *Palaeomorphe* (King) Berg & Corner and includes c. 30 species of climbers or hemi-epiphytes with aerial adventitious roots. The name refers to the frequent presence of hermaphroditic flowers instead of male ones, with an ovule capable of becoming a gall.

**Sinosiscidium**

This clade (Fig. 3B; 100% BS/PP = 1.00) corresponds to the monotypic Chinese section *Sinosiscidium* Corner (*F. tsiangii*) and subsection *Ficus* series *Sinosisycea* Corner comprising *F. henryi* Diels and *F. subincisa* Sm. from mainland Asia. *Ficus subincisa* was not included in this study. The species of section *Sinosiscidium* are atypical in *Sycomidium* in that they present elongate stigmas in female figs and two anthers per male flower in male figs, two traits probably linked to being passively pollinated. Passive pollination has not been reported for any other species of subgenus *Sycomidium*.

**Sycomorus**

This clade (Fig. 3C; 97% BS/PP = 1.00) corresponds to subgenus *Sycomorus* (Gasp.) Miq., which is the final subgenus of *Ficus* supported as monophyletic in phylogenetic reconstructions. Sycomorus includes members of sections *Sycomorus s.l.* (18 species including former section *Neomorphe*), *Sycocarpus* (86 species) and *Adenosperma* (20 species). In addition, this group includes a number of smaller sections (*sensu Berg & Corner, 2005*) with difficult affinities, namely *Dammaropsis* (Warb.) C.C.Berg (five species), *Hemicardia* C.C.Berg (three species), *Papuasyce* (Corner) C.C.Berg (three species) and *Bosscheria* (Teijsm. & de Vriese) C.C.Berg (two species). Corner (1965) only included the monoecious section *Sycomorus* in subgenus *Sycomorus*. However, based on early molecular studies (Weiblen 2000; Jousselin et al., 2003), morphological evidence (Corner, 1967; Berg, 1989; Weiblen, 2000) and a shared genus of pollinating wasps (*Ceratosolen*), Berg & Corner (2005) transferred a number of dioecious sections from Corner’s (1965) subgenus *Ficus* into an enlarged subgenus *Sycomorus*, which we here refer to as the Sycomorus clade.

Two preceding molecular studies including more taxa (Rønsted et al., 2005, 2008a) did not find support for such an expanded subgenus *Sycomorus*, but this was attributed to lack of resolution and informative characters using limited DNA sequence information. Undiscovered paralogous copies of ETS were problematic in Rønsted et al. (2005, 2008a). Here we have identified and removed erroneous copies of ETS and included homologous ETS sequences for this group; as a result, *Sycomorus* was recovered as monophyletic.

Relationships in the *Sycomorus* clade were not well supported in this study and are likely to change with future analyses, but we would expect to recover clades largely corresponding to sections *Sycomorus s.l.*, *Sycocarpus* and *Adenosperma* once the many difficult taxa in the subgenus *Sycomorus* clade are placed. Sections *Sycocarpus* and *Adenosperma* are both resolved with low support. Section *Sycomorus s.l.* is not resolved (Fig. 3C), and we therefore refrain from informally naming these clades at this time.

**Section Papuasyce** of Berg & Corner (2005) includes three species, *F. itoana* Diels and *F. microdicyta* Diels endemic to New Guinea and New Britain and *F. pritchardii* Seem. endemic to Fiji (Berg & Corner, 2005). Section *Papuasyce* was listed as subsection *Papuasyce* in section *Sycocarpus* by Corner (1965). Section *Papuasyce* and section *Adenosperma* lack the nodal glands typical of section *Sycocarpus* Berg & Corner (2005). The dioecious *F. itoana* and the monoecious *F. microdicyta* are sisters in the present study, whereas *F. pritchardii* was not included.

**Section Dammaropsis** includes five species, *F. dammaropsis* Diels, *F. pseudopalma* Blanco, *F. vulgaris* Merr., *F. solomonensis* Rech. and *F. theophrastoides* Seem. ranging from the Philippines to the Solomon Islands. Corner (1965) placed *F. dammaropsis* as subsection *Dammaropsis* and *F. solomonensis* and *F. theophrastoides* in subsection *Auriculisperma*, as series *Theophrastoides* in section *Sycocarpus*. *Ficus pseudopalma* and *F. vulgaris* was included as series *Pseudopalmae* and *Rivulares* respectively in subsection *Ficus* by Corner (1965). In the present analysis, all of these species except
F. solomonsensis are included and their relationship is unresolved among members of section Adenosperma of Berg & Corner (2005b), with which they share spirally and terminally arranged and more or less conspicuously tufted leaves (Berg, 2004a; Berg & Corner, 2005).

Section Hemicardia of Berg & Corner (2005) was originally described as series Prostratae in section Sycomium (subgenus Sycomium; Corner, 1965). Sycomium Hemicardia is supported by free tepals, and one or two anthers per male flower, is primarily Sino–Himalayan and includes F. koutumensis Corner, F. prostrata (Wall. ex. Miq.) Miq. and F. semicordata Buch.-Ham. ex Sm., the latter extending to Malesia.

Berg (2004a) noted the closer morphological affinities of section Hemicardia to section Sycomus than to any of the other sections of the subgenus. In the present analysis, F. koutumensis is not included, but F. prostrata and F. semicordata form a clade (Fig. 3C; 98% BS/PP = 1.00) with uncertain affinity.

Section Bosscheria of Berg & Corner (2005) includes F. minnannahassae and F. pungens ranging from the Philippines to New Guinea. Section Bosscheria of Berg & Corner (2005) forms a clade, which is embedded in the Sycomorus group in the present analysis. They are atypical in the subgenus because of their small figs and flowers.

Sycomorus

This clade (Fig. 3C; 68% BS/PP = 0.71) corresponds to section Sycomorus Miq and includes 86 species.

Adenosperma

This clade (Fig. 3C; 68% BS/PP = 0.51) largely corresponds to section Adenosperma Corner and comprises 20 species.

Oreosycea

This clade (Fig. 3D; 77% BS/PP = 0.62) corresponds to the Palaeotropical section Oreosycea (Miq.). Miq. tentatively including most of subsections Glandulosae C.C. Berg and Pedunculatae Sata sensu Berg & Corner (2005), but excluding subseries Albipilae (Berg, 2003a; Berg & Corner, 2005). Corner (1959) placed section Oreosycea in subgenus Pharmacosycea (Miq.) Miq., but molecular phylogenetic evidence has suggested section Oreosycea is more closely related to subgenus Sycomorus; however, this is not well-supported (54% BS/PP < 0.50 in this study) or consistent. Berg & Corner (Berg, 2003b; Berg & Corner, 2005) divided section Oreosycea into subsections Glandulosae C.C. Berg (including series Austrocaledoniceae Corner) and series Nervosae Corner and Pedunculatae (including subseries Vascularae Corner and subseries Albipilae Corner).

Urostigma

This clade (Fig. 3D; 100% BS/PP = 0.99) corresponds to section Urostigma sensu Corner 1960. Due to the placement of section Urostigma in this phylogenetic analysis and prior studies of Ficus (Jousselin et al., 2003; Rønsted et al., 2005, 2008a), subgenus Urostigma is polyphyletic. The Urostigma clade should be recognized independently from the remaining sections of the former subgenus Urostigma (refer to the Mixtiflores discussion). Additionally, Berg & Corner (2005) expanded section Urostigma uniting Corner's sections Urostigma, Leucozyne and Conosycea, which is not supported by this study. The Sino–Himalayan F. orthoneura H.Lév. & Vanoit appears to be sister to the rest of (sub)section Urostigma (100% BS/PP = 1.00). Ficus orthoneura, F. hookeriana Corner (also Sino–Himalayan, but not included in this study) and F. cornelisiana Chantaras & Y.Q. Peng (Chantarasuwan et al., 2014) present a mixture of characters of section Urostigma and section Conosycea and were placed in their own series in section Urostigma by Corner (1965). In a recent study of (sub)section Urostigma (Chantarasuwan et al., 2015), F. madagascariensis C.C. Berg (not included here) was found to be sister to the remainder of the (sub)section and the next diverging clade consisted of F. orthoneura and F. hookeriana.

Albipilae

This clade (Fig. 3A; 100% BS/PP = 1.00) corresponds to subseries Albipilae Corner and comprised two African species, F. varifolia Warb. and F. dicranostyla Mildbr., and F. albipila (Miq.) King that occurs from Thailand to Australia. Morphological study of subseries Albipilae also assigns F. capillipes Gagnep. from mainland Asia and the Madagascan F. assimilis Baker and F. ampana C.C. Berg to this group; these have not yet been included in phylogenetic studies. The Albipilae clade can be distinguished from the Oreosycea clade primarily by the presence of hairs on the inner surface of the fig receptacle. The exact circumscription of the Albipilae clade awaits comprehensive species sampling.

Caricaceae

This clade (Fig. 3D; 100% BS/PP = 1.00) includes only the domesticated Mediterranean F. carica and F. palmata Roxb. extending from north-eastern Africa to Pakistan. Together with F. iidaiana Wilson from...
Bonin Island (Japan), these three species formerly constituted Ficus section Ficus subsection Ficus Berg & Corner, but F. iidaiana is a member of Frutescentiae in the present study. The traditional subgenus Ficus is polyphyletic consisting of three strongly supported major clades, Caricae, Eriosycea and Frutescentiae, corresponding to clear-cut subdivisions by Berg & Corner (2005; Berg, 2003c). The relationship of the Caricae clade is uncertain. Ficus carica is the type of genus Ficus.

Mixtiflores
This clade (Fig. 3D; 100% BS/PP = 1.00) corresponds to subgenus Urostigma (Gasp.) Miq. excluding section Urostigma (Gasp.) Miq and includes c. 265 monoecious species in two subclades, one (100% BS/PP = 1.00) consisting of section Conosycea Corner (98% BS/PP = 0.99) and (sub)section Malvanthera Corner (100% BS/PP = 0.99), and the other (100% BS/PP = 1.00) including section Galoglychia (Gasp.) Endl. (66% BS/PP = 0.68) and section Americanae Miq. (100% BS/PP = 1.00). In all the species, the staminate flowers are scattered among the pistillate flowers in the fig cavity.

Galoglychia
This clade (Fig. 3E; 66% BS/PP = 0.68) corresponds to the African section Galoglychia (Gasp.) Endl. Early studies (Rønsted et al., 2005, 2007, 2008a) suggested that Galoglychia is paraphyletic to Americanae, but monophyly of Galoglychia has been confirmed by later studies (Renoult et al., 2009; Cruaud et al., 2012b). Detailed phylogenetic studies of section Galoglychia were published by Rønsted et al. (2008b) and Renoult et al. (2009). Based on nuclear sequences, Rønsted, Salvo & Savolainen (2007) found that Galoglychia consists of two major clades in Africa, possibly corresponding to two main centres of diversity. One clade comprises members of subsections Platypyllae (Mildbraed & Burret) C.C.Berg and Chlamydodorae (Mildbraed & Burret) C.C.Berg, are more concentrated in East Africa, and extend to Madagascar and neighbouring archipelagos (Comoros, Mascarenes, Aldabra Islands and Seychelles) and is sister to Americanae in the study by Rønsted et al. (2007). The other main clade (includes members of subsections Caulocarpae (Mildbraed & Burret) C.C.Berg, Cyathistipulae (Mildbraed & Burret) C.C.Berg, Crassicostae (Mildbraed & Burret) C.C.Berg and Galoglychia, which are concentrated in West and Central Africa (Berg, 1986). Renoult et al. (2009) found discordance of highly variable plastid data with the nuclear data, possibly caused by introgressive hybridization. In the present study, the six subclades are evident, but their relationships are not well supported.

Americanae
This clade (Fig. 3E; 100% BS/PP = 1.00) corresponds to Neotropical section Americanae Miq. including c. 110 species of hemi-epiphytes with low sequence variation possibly representing a rapid radiation. A detailed study of the Americanae clade has been published by Machado et al. (2018).

Conosycea
This clade (Fig. 3F; 99% BS/PP = 0.99) corresponds to section Conosycea (Miq.) Corner (Corners, 1965) plus Corner’s acceptance of section Stilpnophyllum Endl. (Ficus elastica Roxb.) and section Leucogyne (F. amplissima Sm. and F. rumphii Bl.), which Berg & Corner (2005) considered members of section Urostigma s.s. (= subsection Urostigma).

A number of clades are resolved in section Conosycea, some of which correspond to traditional series and subspecies, but the subdivisions proposed by Corner (1965) and Berg and Corner (2005) are not reflected.

Malvanthera
This clade (Fig. 3F; 98% BS/PP = 0.99) corresponds to section Malvanthera Corner, which was reduced to subsection rank by Berg & Corner (2005). The Malvanthera clade includes 23 Australasian species with centres of diversity in New Guinea and Australia. The section was included in section Stilpnophyllum Endl. by Berg & Corner (2005) together with F. elastica, but phylogenetic evidence shows that F. elastica is a member of the Conosycea clade and section Stilpnophyllum sensu Berg & Corner (2005) is therefore polyphyletic. A detailed phylogenetic tree of the Malvanthera clade was published by Rønsted et al. (2008b) and relationships in that study are mirrored in the present study including the same sampling for the section. Rønsted et al. (2008b) also highlighted problems with the species concept of Berg & Corner (2005) for Malvanthera. In particular Berg & Corner (2005) united the majority of the New Guinea species under F. hesperidiiformis King, which is not supported by phylogenetic evidence (Rønsted et al., 2008b), and at the same time Berg & Corner (2005) kept a narrow species concept for the Australian species.

Pharmacosycea
This clade (Fig. 3D; 100% BS/PP = 1.00) corresponds to section Pharmacosycea (Miq.) Benth. & Hook.,
includes c. 25 species restricted to the Neotropics and was recovered as sister to all other *Ficus* spp. Polyphyly of subgenus *Pharmacosycea* has been firmly established in molecular phylogenetic trees (e.g. Weiblen, 2000; Rønsted et al., 2005, 2008a; Cruaud et al., 2012b). Morphologically, the *Pharmacosycea* clade is similar to the Old World section *Oreosycea* s.s., the remaining section of subgenus *Pharmacosycea* (*sensu* Berg & Corner, 2005). However, former subgenus *Pharmacosycea* is polyphyletic and all three sections of this subgenus (*Oreosycea*, *Albipilae*, *Pharmacosycea*; Fig. 2) should be recognized as independently evolving lineages. Relationships in section *Pharmacosycea* were recently evaluated by Pederneiras, Romanieu-neto & Mansano (2015), although species names were not fully clarified.

**TAXONOMIC IMPLICATIONS**

A formal revision of *Ficus* awaits additional taxon sampling, but it is our hope that this comprehensive view of the phylogenetics of *Ficus* and recognition of well-supported clades will allow researchers to more easily discuss and describe the evolution and diversity of figs by making use of these informal clade names. In particular, we would advocate that further revision of Moraceae would formally recognize Involucrata either as a clade in a rank-free taxonomy or at the appropriate rank in a rank-based classification system, as many key evolutionary events happened along this branch. For *Ficus*, we strongly recommend abandoning the names associated with non-monophyletic subgenera of figs and instead use the proposed clade names until further taxonomic revision. In Castilleae, we reinstate the genus *Noyera* based on the molecular phylogenetic evidence presented in this paper.  

Type species: *Noyera rubra* Trécul.  

**CONCLUSIONS**

Despite the extensive study of *Ficus* due to its striking diversity and brood-site pollination mutualism, the deep evolutionary history of the group cannot be understood without attention to and comparison with its closest relatives, Castilleae. We introduce the clade Involucrata to recognize that *Ficus* and Castilleae comprise a group united by a trait that is central to their inflorescence morphology and pollination syndromes, the involucral bracts. Here, with the first intensive sampling of Castilleae and the most comprehensive phylogenetic reconstruction of *Ficus* to date, we delineate and name clades that are well supported to guide sampling in future studies of Involucrata and highlight those aspects of phylogenetic tree that warrant further investigation.

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**REFERENCES**


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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

**Supplementary Table S1.** Voucher information for Involucrata.

**Supplementary Table S2.** Amplification and sequencing primers used with ITS, G3pdh, and GBSSI.