Molecular Phylogenetics and Evolution 48 (2008) 12–22

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

# Phylogeny, biogeography, and ecology of *Ficus* section *Malvanthera* (Moraceae)

Nina Rønsted [a,b,c,*], George D. Weiblen [b], V. Savolainen [a,d], James M. Cook [d,e]

[a] *Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, UK*
[b] *Bell Museum of Natural History, Department of Plant Biology, University of Minnesota, 1445 Gortner Avenue, Saint Paul, MN 55108, USA*
[c] *Department of Medicinal Chemistry, University of Copenhagen, Universitetsparken 2, Copenhagen 2100, Denmark*
[d] *Department of Biological Sciences, Imperial College London, Ascot, UK*
[e] *School of Biological Sciences, University of Reading, Reading RG6 6AS, UK*

ARTICLE INFO

*Article history:*
Received 28 June 2007
Revised 2 April 2008
Accepted 5 April 2008
Available online 14 April 2008

*Keywords:*
Moraceae
*Ficus*
*Malvanthera*
Phylogeny
ITS
ETS
*G3pdh*

ABSTRACT

We conducted the first molecular phylogenetic study of *Ficus* section *Malvanthera* (Moraceae; subgenus *Urostigma*) based on 32 *Malvanthera* accessions and seven outgroups representing other sections of *Ficus* subgenus *Urostigma*. We used DNA sequences from the nuclear ribosomal internal and external transcribed spacers (ITS and ETS), and the glyceraldehyde-3-phosphate dehydrogenase (*G3pdh*) region. Phylogenetic analysis using maximum parsimony, maximum likelihood and Bayesian methods recovered a monophyletic section *Malvanthera* to the exclusion of the rubber fig, *Ficus elastica*. The results of the phylogenetic analyses do not conform to any previously proposed taxonomic subdivision of the section and characters used for previous classification are homoplasious. Geographic distribution, however, is highly conserved and Melanesian *Malvanthera* are monophyletic. A new subdivision of section *Malvanthera* reflecting phylogenetic relationships is presented. Section *Malvanthera* likely diversified during a period of isolation in Australia and subsequently colonized New Guinea. Two Australian series are consistent with a pattern of dispersal out of rainforest habitat into drier habitats accompanied by a reduction in plant height during the transition from hemi-epiphytic trees to lithophytic trees and shrubs. In contradiction with a previous study of *Pleistodontes* phylogeny suggesting multiple changes in pollination behaviour, reconstruction of changes in pollination behaviour on *Malvanthera*, suggests only one or a few gains of active pollination within the section.

## 1. Introduction

Figs (*Ficus*, Moraceae) constitute one of the largest genera of angiosperms, with almost 750 species of terrestrial trees, shrubs, hemi-epiphytes, climbers and creepers occurring in the tropics and subtropics worldwide (Berg and Corner, 2005). All species of figs share the distinctive fig inflorescence (syconium), which is the site of an obligate mutualism with pollinating fig wasps of the family Agaonidae (Cook and Rasplus, 2003). Figs are important genetic resources with high economic and nutritional value. They also play an important role in generating biodiversity in the rainforest ecosystem by setting fruits throughout the year and providing an important source of food for most fruit-eating vertebrates in the tropics (Harrison, 2005). Most insects pollinate passively, but fig wasps are one of a few cases, where active pollination behaviour has evolved (Cook and Rasplus, 2003). While most genera of pollinating wasps are active pollinators, five out of 20 genera contain both passive and active pollinators (Kjellberg et al., 2001).

Recent classification divided the genus into six subgenera based primarily on morphology (Berg, 2003). The monoecious subgenus *Urostigma*, to which section *Malvanthera* belongs, is the largest with about 280 species worldwide, most of them displaying the characteristic hemi-epiphytic habit (banyans and stranglers). *Ficus* section *Malvanthera* Corner (subg. *Urostigma*) includes 23 species of hemi-epiphytes and lithophytes producing aerial, adventitious, or creeping root systems. The section has its primary centre of diversity in Australia and a second centre in New Guinea and the Bismarck Archipelago. A few species extend eastwards into Oceania (e.g. Lord Howe Island, New Caledonia, the Solomon Islands and Vanuatu). Section *Malvanthera* includes species with two very distinct ecologies: hemi-epiphytic stranglers and free-standing trees in the rainforests of Eastern Australia and New Guinea, and lithophytic shrubs and trees occurring in more arid parts of Australia (Dixon, 2003).

The section was established by Corner (1959) who recognized 19 species characterized by features including: a slit-shaped or triradiate ostiole with all bracts descending; syconia with two or three basal bracts; reniform unilocular anthers with crescentic or transverse dehiscence; ovaries attached at the base to the receptacle or imbedded in the receptacle; a red spot at the base or the apex

---

[*] Corresponding author. Address: Department of Medicinal Chemistry, University of Copenhagen, Universitetsparken 2, Copenhagen 2100, Denmark.
*E-mail address:* nr@farma.ku.dk (N. Rønsted).

1055-7903/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2008.04.005

of the ovary; and a stigma that is either simple or bifid. Corner (1959) and again Berg and Corner (2005) noted the similarity between subg. *Urostigma* sections *Malvanthera* and *Galoglychia* in the inflexed apical bracts of the ostiole and internal bracts. Berg and Corner (2005) also noted that similarities in venation suggest a relationship between *Malvanthera* and the *F. benjamina* group of section *Urostigma* subsection *Conosycea*. Molecular phylogenetic studies have shown sections *Conosycea* and *Malvanthera* to be sister taxa (Rønsted et al., 2005).

Corner (1965) subdivided section *Malvanthera* into two series. *Malvanthereae* is distinguished by reniform anthers dehiscing longitudinally and crescentically. The series includes 18 species in four loosely defined subseries, *Eubracteatae*, *Hesperidiiformes*, *Malvanthereae*, and *Platypodeae* based on characters of the basal bracts and whether the ovaries are partly embedded in the receptacle or not. The monotypic series *Cyclanthereae* includes *F. sterrocarpa* from New Guinea, which has depressed-subglobose anthers (Table 1).

Chew (1989) followed Corner (1965) in treating Australian *Ficus*, whereas Australian *Malvanthera* were subsequently revised by Dixon (2001a–d, 2003), who proposed a new subdivison of the section into ser. *Malvanthereae* including species with imbricate basal bracts, and ser. *Hesperidiiformes* with valvate basal bracts (Table 1).

In treating *Ficus* of the Malesian region, Berg and Corner (2005) united the monotypic section *Stilpnophyllum* (*F. elastica*, the rubber fig) and section *Malvanthera* as subsections of an expanded section *Stilpnophyllum* based on similarities between *F. elastica* and the *Malvanthera* species in the venation of the lamina, the length of the stipules and cucullate (hood-shaped) caducous basal bracts. However, *F. elastica* is distinct from the *Malvanthera* species in the shape of the ostiole and in having anthers with separate theca and connate stipules. The Indo-Chinese origin of *F. elastica*, which is widely cultivated, also has little geographical affinity to the Australian and Melanesian *Malvanthera*. A global molecular phylogenetic study (Rønsted et al., 2005) including *F. elastica*, 12 species of *Conosycea* figs and 11 species of *Malvanthera* figs strongly suggested that *F. elastica* is a member of section *Conosycea* with a derivative morphology.

Within subsection *Malvanthera*, Berg and Corner (2005) recognised 18 species in three informal groups, based on characters of the basal bracts and ostiole shape. Berg and Corner (2005) united most of Cornerś species from New Guinea (*F. augusta*, *F. hesperidiiformis*, *F. heteromeka*, *F. mafuluensis*, *F. sterrocarpa* and *F. xylosycia*)

**Table 1**
Historical classification of *Ficus* section *Malvanthera*

| Rønsted et al. (present study, Fig. 1) | Corner (1965) | Dixon (2003) | Berg (2005)[h] |
|---|---|---|---|
| Subsect. *Malvantherae* | | | |
| *F. macrophylla* | Subser. *Malvanthereae* | Ser. *Malvanthereae* | Subsect. *Malvanthera* C |
| *F. pleurocarpa* | Subser. *Hesperidiiformes* | Ser. *Hesperidiiformes* | Subsect. *Malvanthera* C |
| Subsect. *Hesperidiiformes* | | | |
| Ser. *Hesperidiiformes* | | | |
| *F. hesperidiiformis*[a] | Subser. *Hesperidiiformes* | Ser. *Hesperidiiformes* | Subsect. *Malvanthera* A |
| *F. sterrocarpa* | Ser. *Cyclanthereae* | Ser. *Hesperidiiformis* | *F. hesperidiiformes*[a] |
| Ser. *Glandiferae*[b] | | | |
| *F. baola*[c] | *F. obliqua*[c] | *F. obliqua*[c] | Subsect. *Malvanthera* B |
| *F. glandifera* | Subser. *Malvanthereae* | Ser. *Hesperidiiformes* | Subsect. *Malvanthera* B |
| *F. rhizophoriphylla* | Subser. *Malvanthereae* | Ser. *Malvanthereae* | Subsect. *Malvanthera* B |
| Ser. *Xylosyciae* | | | |
| *F. augusta* | Subser. *Hesperidiiformes* | Ser. *Hesperidiiformes* | *F. hesperidiiformes*[a] |
| *F. heteromeka* | Subser. *Hesperidiiformes* | Ser. *Hesperidiiformes* | *F. hesperidiiformes*[a] |
| *F. mafuluensis* | Subser. *Hesperidiiformes* | Ser. *Hesperidiiformes* | *F. hesperidiiformes*[a] |
| *F. xylosycia* | Subser. *Hesperidiiformes* | Ser. *Hesperidiiformes* | *F. hesperidiiformis*[a] |
| Subsect. *Platypodeae* | | | |
| Ser. *Eubracteatae* | | | |
| *F. triradiata* | Subser. *Eubracteatae* | Ser. *Hesperidiiformes* | Subsect. *Malvanthera* A |
| Ser. *Obliquae* | | | |
| *F. cerasicarpa*[d] | *F. leuchotricha*[d] | Ser. *Malvanthereae* | Subsect. *Malvanthera* C |
| *F. lilliputiana*[e] | *F. brachypoda*[e] | Ser. *Malvanthereae* | Subsect. *Malvanthera* C |
| *F. obliqua* | Subser. *Platypodeae* | Ser. *Malvanthereae* | Subsect. *Malvanthera* C |
| *F. platypoda*[d] | Subser. *Platypodeae*[d] | Ser. Malvanthereae | Subsect. *Malvanthera* A |
| *F. subpuberula* | Subser. *Platypodeae* | Ser. *Malvanthereae* | Subsect. *Malvanthera* C |
| Ser. *Crassipeae* | | | |
| *F. crassipes* | Subser. *Hesperidiiformes* | Ser. *Hesperidiiformes* | Subsect. *Malvanthera* A |
| *F. destruens* | Subser. *Platypodeae* | Ser. *Malvanthereae* | Subsect. *Malvanthera* B |
| Ser. *Rubiginosae* | | | |
| *F. atricha*[f] | *F. platypoda*[f] | Ser. *Malvanthereae* | Subsect. *Malvanthera* C |
| *F. brachypoda*[f] | *F. platypoda*[f] | Ser. *Malvanthereae* | Subsect. *Malvanthera* C |
| *F. rubiginosa*[g] | Subser. *Platypodeae* | Ser. *Malvanthereae* | Subsect. *Malvanthera* C |
| *F. watkinsiana* | Subser. *Malvanthereae* | Ser. *Malvanthereae* | Subsect. *Malvanthera* C |

[a] *F. hesperidiiformis* includes *F. augusta*, *F. heteromeka*, *F. mafuluensis*, *F. sterrocarpa*, and *F. xylosycia* in Bergs (2005) classification.
[b] Series *Glandiferae* possibly includes *F. baola* and *F. rhizophoriphylla* based on morphological affinities such as a slit-shaped ostiole (Berg, 2002).
[c] *F. baola* C.C. Berg was raised from *F. obliqua* (Berg 2002).
[d] *F. cerasicarpa* has affinities to *F. platypoda* (Dixon 2001a). *F. cerasicarpa* and *F. platypoda* were included in *F. leuchotricha* in Corner's classification.
[e] *F. lilliputiana* is a new species with affinities to *F. brachypoda* (Dixon 2001b).
[f] *F. atricha* and *F. brachypoda* were included in *F. platypoda* in Corner's classification.
[g] *F. baileyana* was included in *F. rubiginosa* by Dixon (2001d).
[h] *F. elastica* constituted a monotypic section *Stilpnophyllum* in Corner's classification.

under a broadly circumscribed *F. hesperidiiformis* evidently based on the apparent presence of intermediate forms. At the same time, Berg accepted the narrow species concept of Dixon (2003) for the Australian taxa.

The purpose of our study is to (1) infer phylogenetic relationships in section *Malvanthera* using three molecular markers: the internal and external transcribed spacers (ITS and ETS) of nuclear ribosomal DNA, and the glyceraldehyde-3-phosphate dehydrogenase (*G3pdh*) gene, (2) reconstruct the evolution of morphological characters used to subdivide the section in previous treatments, (3) trace the biogeographic history of the section, (4) identify evolutionary changes in habitat use, and (5) retrace the evolution of active pollination.

## 2. Materials and methods

### 2.1. Taxon sampling

Taxon sampling was aimed at testing hypotheses of phylogenetic relationship among recognized species. Phylogeographic analyses of particular complexes are needed to clarify species limits in *Malvanthera* (Haine et al. 2006), as are population genetic analyses to identify the role that hybridization may have played, if any, in the diversification of *Malvanthera* (Machado et al. 2005). We inferred phylogenetic relationships in section *Malvanthera* using three molecular markers: the internal and external transcribed spacers (ITS and ETS) of nuclear ribosomal DNA, and the glyceraldehyde-3-phosphate dehydrogenase (*G3pdh*) gene. We did not include chloroplast DNA markers in the study based on the absence of phylogenetically informative variation in four regions (*rps16* intron, *trnL* intron, *trnL-F* spacer, and *psB–psbF* spacer) within *Ficus* according to Rønsted et al. (2007).

Contrary to Berg and Corner (2005), we follow the circumscription of *Malvanthera* sensu Corner (1965) treating *F. elastica* as an outgroup according to its historical taxonomic and molecular phylogenetic position (Rønsted et al., 2005).

Total genomic DNA was extracted from 29 *Ficus* specimens. In addition 19 ITS sequences, 16 ETS sequences, and 11 *G3pdh* sequences were retrieved from GenBank/EBI (following papers by Weiblen, 2000; Jousselin et al., 2003; Rønsted et al., 2005; Rønsted et al., 2008), resulting in a total of 34 *Malvanthera* and seven outgroups representing the other sections of subgenus *Urostigma*. Specimen determinations, vouchers, localities and GenBank/EBI Accession Nos. (EF545651–EF545669, ETS: EF538767–EF538785, and *G3pdh*: EF538786–EF538801) are listed in Table 2. Species concepts are primarily according to Dixon (2001a–d, 2003), for the Australian taxa and Corner (1959) for the remainder of the section. We included multiple individuals of some species to evaluate species limits and the extent of infraspecific variation. We included samples representing 19 of the 23 species of *Ficus* section *Malvanthera*. Material of *F. cerasicarpa* D.J. Dixon from Northern Australia, *F. rhizophoriphylla* King and *F. mafuluensis* Summerh. from New Guinea, and *F. baola* C.C. Berg from the Solomon Islands, was not available for this study.

### 2.2. DNA extraction, amplification and sequencing

DNA extractions were performed in two ways. Some specimens were extracted using the Qiagen DNeasy plant extraction kit (Valencia, CA, USA) from 15–20 mg of dried leaf-fragments or herbarium material. Other specimens were extracted using 0.2–0.3 g silica dried leaves and a modified version of the 2 × CTAB method of Doyle and Doyle (1987). Before precipitation, an aliquot was purified using the Qiagen PCR purification kit (Qiagen, Inc., Santa Clarita, CA, USA) following the manufacturer's protocols. The remainder of the DNA was purified using a caesium chloride/ethidium bromide gradient (1.55 g $\text{ml}^{-1}$ density) followed by a dialysis and was deposited in the DNA Bank at the Royal Botanic Gardens, Kew (www.rbgkew.org.uk).

The internal and external transcribed spacers, ITS and ETS (Baldwin et al., 1995; Baldwin and Markos, 1998), were amplified using primers 17SE and 26SE (Sun et al., 1994) or ITS4 and ITS5 (White et al., 1990) and Hel1 and 18S ETS (Baldwin and Markos, 1998), respectively. The glyceraldehyde-3-phosphate dehydrogenase (*G3pdh*) region (Strand et al., 1997) was amplified using primers 7F and 9R (Strand et al., 1997).

All three regions were amplified and sequenced following protocols by Rønsted et al. (2008). Both strands were sequenced for each region for the majority of taxa. For some samples, internal primers (GCT ACG TTC TTC ATC GAT GC) and (GCA TCG ATG AAG AAC GTA GC) modified from ITS2 and ITS3, respectively (White et al., 1990) were used for sequencing of the ITS region, and internal primers 286F (TGT ATT CTG GTT GGG TTT C, Rønsted et al., 2008) and 437R (TTC TGA AGC CTG ACA GTG AGG, Rønsted et al., 2008) for sequencing of the *G3pdh* region in addition to the primers used for amplification. Internal primers were used to ensure overlap between sequenced fragments, where chromatograms could not be unambiguously interpreted throughout the whole region.

### 2.3. Phylogeny reconstruction

Sequences were edited and assembled using Sequencer 4.1.2$^{TM}$ software (Gene Codes Corp., Ann Arbor, MI, USA), and all sequences were aligned by eye in PAUP v. 4.0b10 for Macintosh (Swofford, 2002). Phylogenetic analyses were conducted using PAUP v. 4.0b10 (Swofford, 2002). All changes were assessed as unordered and equally weighed (Fitch parsimony; Fitch, 1971).

We analysed the three gene regions separately to identify phylogenetic conflicts among the regions prior to performing a combined analysis. For the separate analyses, we show only the bootstrap tree (Fig. 1A–C) to establish that there were no cases of strong conflict, namely clades that are highly supported by bootstrap analysis in a single-loci analysis (e.g. *G3pdh*) that are incongruent with highly supported clades in another single-locus analysis (e.g. ITS). All data were therefore combined into one analysis, and we consider the combined analysis to provide the best estimate of phylogeny (Fig. 2).

We attempted to avoid potential problems with non-overlapping datasets in the combined analysis by including only samples for which at least ITS was sequenced. Taxon sampling was maximised in two cases by combining different accessions of the same species. We treated as a single taxon two accessions of *F. xylosycia*, which were both from the same site in the Madang Province of Papua New Guinea, and likewise, two accessions of *F. brachypoda*, from the same region of the Australian Northern Territory. In the case of *F. subpuberula*, however, only an ETS sequence was available.

Parsimony analysis of the combined matrix was not exhaustive due to large numbers of equally parsimonious trees at each step in the search. Most parsimonious trees (MP) were obtained using: (i) 1000 replicates of random taxon addition sequence and TBR branch swapping with only 25 trees held at each step to save time by avoiding swapping on suboptimal islands; (ii) the trees collectively found in these 1000 replicates were then used as starting trees for a second search using TBR branch swapping until up to a maximum of 15,000 trees were found. Relative levels of homoplasy in all the datasets were assessed using the consistency index (CI) and the retention index (RI). In all cases RI was at least 0.78, which suggests that multiple islands of equally parsimonious trees are unlikely (Maddison, 1991).

**Table 2**
Voucher information and Genbank accession numbers of *Ficus* material included in this study

| Taxa | Voucher | Origin | ITS | ETS | G3PDH |
|---|---|---|---|---|---|
| *F. atricha* D. J. Dixon | Rønsted 142 (K) | Liv. Col. (BG) 2003, Berg 93.220 | Rønsted 2005 | Rønsted 2005 | EF538786 |
| *F. augusta* Corner | GW597 (MIN) | Madang, PNG 1995 | EF545651 | EF538767 | EF538787 |
| *F. brachypoda* (Miq) Miq. | Collector: John Zammit | Kulgera, NT, Australia, 1997 | EF545652 | EF538768 | – |
| *F. brachypoda* | Dixon/Kerrigan 1101 (DNA) | NT, Australia, 2004 | – | – | EF538788 |
| *F. crassipes* Bailey | Cook 987 | Boonjee, QLD, Australia, 1998 | Rønsted 2005 | Rønsted 2005 | EF538789 |
| *F. destruens* C.T. White | GW943 (MIN) | Cow Bay, QLD 1997 | Weiblen 2000 | EF538769 | EF538790 |
| *F. destruens* | Dixon, PHD 312 | Mount Halifax, Bluewater, QLD | EF545653 | – | – |
| *F. glandifera* Summerh. | | Vanuatu | Rønsted 2005 | Rønsted 2005 | Rønsted 2008 |
| *F. hesperidiiformis* King | B204 (MIN) | Madang, PNG 1998 | EF545654 | EF538770 | – |
| *F. hesperidiiformis* | GW825 (MIN) | Madang, PNG 1997 | Weiblen 2000 | EF538771 | Rønsted 2008 |
| *F. hesperidiiformis* | GW2736 (MIN) | Madang, PNG 2006 | EF545655 | – | – |
| *F. heteromeka* Corner | GW2125 (MIN) | Chimbu, PNG 2004 | EF545656 | EF538772 | EF538791 |
| *F. lilliputiana* D. J. Dixon | Dixon, PHD 424 (DNA) | Kununurra, WA | EF545657 | EF538773 | – |
| *F. macrophylla* Desf. ex. Pers. subsp. *macrophylla* | | | Jousselin 2003 | Jousselin 2003 | – |
| *F. macrophylla* | GW679 (MIN) | Auckland Museum, cult. NZ 1995 | EF545658 | – | – |
| *F. macrophylla* subsp. *columnaris* (C.Moore) P.S.Green | Savolainen/Baker (K) | Lord Howe Island, 2003 | Rønsted 2005 | Rønsted 2005 | EF538792 |
| *F. obliqua* G. Forst. | Cook 2003–6 | Port Douglas, QLD, Australia | EF545659 | EF538774 | EF538793 |
| *F. obliqua* | GW1220 (MIN) | Liv. col. (NOU) 2001 | EF545660 | EF538775 | – |
| *F. platypoda* sensu Dixon | Jacobs/Wilson 5665 (K) | WT, Australia 1988 | Rønsted 2005 | Rønsted 2005 | EF538794 |
| *F. pleurocarpa* F. Muell. | | | Jousselin 2003 | Jousselin 2003 | – |
| *F. pleurocarpa* | Cook 9812/CLV441 | Tully, QLD, Australia 1998 | EF545661 | EF538776 | EF538795 |
| *F. rubiginosa* Desf. ex Vent. | Cook FRYN3 | Yungaburra, QLD 2005 | EF545662 | EF538777 | – |
| *F. rubiginosa* | Rønsted 89 (C) | Liv. col. (C) 2002, E1859-0014 | EF545663 | EF538778 | Rønsted 2008 |
| *F. rubiginosa* | MW Chase 19870 (K) | Liv. col. (K) 2003, K1961-35201 | EF545664 | – | – |
| *F. sterrocarpa* Diels | GW734 (MIN) | Eastern Highlands, PNG 1996 | EF545665 | EF538779 | EF538796 |
| *F. sterrocarpa* | T9414 (MIN) | Eastern Highlands, PNG | EF545666 | EF538780 | EF538797 |
| *F.* cf. *sterrocarpa* | GW1126 (MIN) | East Sepik, PNG 2000 | EF545667 | EF538781 | – |
| *F.* cf. *sterrocarpa* | GW1881 (MIN) | East Sepik, PNG 2003 | EF545668 | EF538782 | EF538798 |
| *F. subpuberula* Corner | Dixon/Kerrigan 1102 (DNA) | NT, Australia 2004 | – | EF538783 | – |
| *F. triradiata* var. *sessilicarpa* Corner | Hind/Herscovitch 6409 (K) | QLD, Australia, 1991 | Rønsted 2005 | Rønsted 2005 | EF538799 |
| *F. watkinsiana* F.M.Bailey | Rønsted 83 (C) | | Rønsted 2005 | Rønsted 2005 | Rønsted 2008 |
| *F. watkinsiana* | Cook (NR256) | Atherton, QLD, Australia 2005 | EF545669 | EF538784 | EF538800 |
| *F. xylosycia* Diels | | Madang, PNG | Weiblen 2000 | – | – |
| *F. xylosycia* | B207 (MIN) | Madang, PNG 1999 | – | EF538785 | EF538801 |
| Outgroup | | | | | |
| *F. americana* Aubl. | | | Rønsted 2005 | Rønsted 2005 | Rønsted 2008 |
| *F. benjamina* L. | | | Jousselin 2003 | Jousselin 2003 | Rønsted 2008 |
| *F. binnendykii* Miq. | | | Jousselin 2003 | Jousselin 2003 | Rønsted 2008 |
| *F. elastica* Roxb. ex. | | | Jousselin 2003 | Jousselin 2003 | Rønsted 2008 |
| *F. ingens* (Miq.) Miq. | | | Rønsted 2005 | Rønsted 2005 | Rønsted 2008 |
| *F. lutea* Vahl | | | Jousselin 2003 | Jousselin 2003 | Rønsted 2008 |
| *F. sundaica* Blume | | | Rønsted 2005 | Rønsted 2005 | Rønsted 2008 |

*Notes:* Abbreviations: Cult, cultivated; Liv. col, Living collections; NT, Northern Territory; NZ, New Zealand; PNG, Papua New Guinea; QLD, Queensland; WT, Western Australia. Herbarium codes: (BG), University of Bergen, Norway; (C), University of Copenhagen, Denmark; (DNA), Department of Natural Resources, Environment, and the Arts, Northern Territory, Australia; (K), Royal Botanic Gardens, Kew, UK; (MIN), University of Minnesota, Saint Paul, USA; (NOU), Institut de la Recherche pour le Developpement, Nouméa, New Caledonia. References: Jousselin 2003, Jousselin et al. (2003); Rønsted 2005, Rønsted et al. (2005); Rønsted 2008, Rønsted et al. (2008).

The combined dataset was also analysed under maximum likelihood (ML). We first selected a best fitting model of molecular evolution using Modeltest (Posada and Crandall, 1998). Model parameters thus obtained were then used in 25 heuristic search replicates under the ML criterion.

Clade support was assessed using non-parametric bootstrap re-sampling and Bayesian analysis of the combined dataset. Bootstrap analyses (Felsenstein, 1985) of the three individual datasets and the combined dataset were carried out using 1000 replicates each consisting of 100 simple addition sequence replicates, with TBR swapping, and a limit of 10 trees retained for each replicate. We defined scores between 50 and 74 bootstrap percentages (BS) as weak support, scores between 75% and 89% BS as moderate support, and scores of greater than 90% BS as strong support. We consider percentages <50 % to be unsupported because such clades are not present in the majority of trees for the re-sampled datasets.

Bayesian analysis of the combined dataset was performed with MrBayes 3.1.2 (Ronquist and Hulsenbeck, 2003). We used an HKY85 model of evolution (lset NST = 2 RATES = equal). The analysis was performed with 2,000,000 generations on four Monte Carlo Markov chains with equal rates and trees sampled every 100 generations (mcmc NGEN = 2,000,000, PRINTFREQ = 1000, SAMPLEFREQ = 100, NCHAINS = 4). The average standard deviation of the split frequencies was 0.0022 after 1,685,000 and also after 2,000,000 generations indicating that there was no great advantage to be gained by sampling additional generations. The first 10,000 trees of low posterior probability were deleted and all remaining trees were imported into PAUP. A majority rule consensus tree was produced showing the posterior probabilities (pp) of all observed bi-partitions. We consider posterior probabilities of at least 0.95 as strong clade support.

To investigate if the Australian lithophytes (*F. atricha*, *F. brachypoda*, *F. lilliputiana*, *F. platypoda*, and *F. subpuberula*) could have originated only once, they were constrained as monophyletic and all other ingroup and outgroup branches were collapsed. This tree was then used as a topological constraint in 1000 replicates of MP analysis in PAUP. We then used the Templeton test (Templeton,

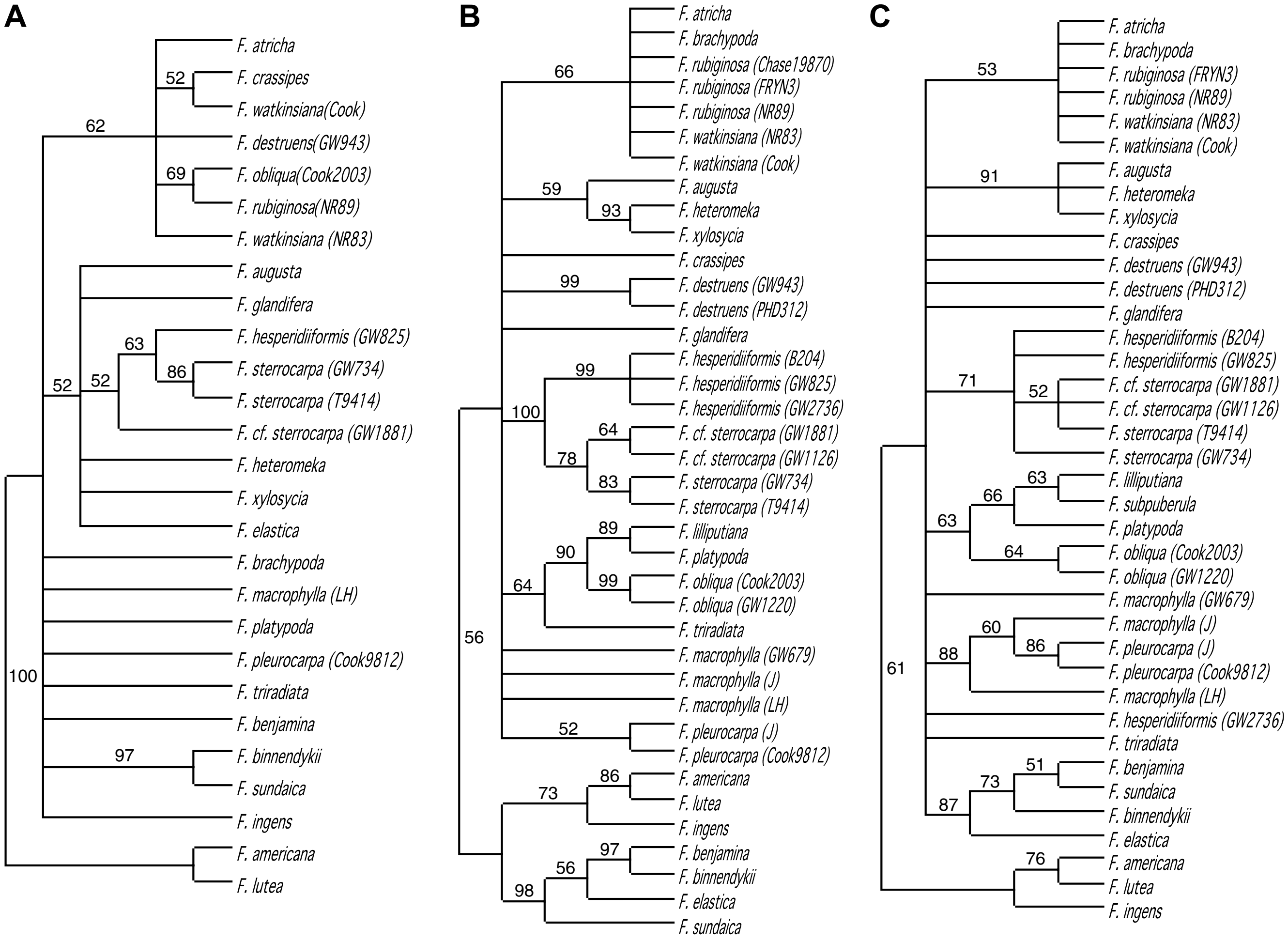


**Fig. 1.** Bootstrap consensus trees from analysis of (A) *G3pdh* nrDNA sequences, (B) ITS nrDNA sequences, and (C) ETS nrDNA sequences. *Ficus americana*, *F. benjamina*, *F. binnendykii*, *F. elastica*, *F. ingens*, *F. lutea*, and *F. sundaica* were designated as an outgroup.

1983) to compare the length of the constrained and the unconstrained trees.

### 2.4. Morphological characters

Five morphological characters used for delimiting subsections, series and informal groups of section *Malvanthera* in previous classifications were scored for all species (Fig. 3). Characters were taken from descriptions in the literature (Corner, 1959; Chew, 1989; Dixon, 2001a–d, 2003; Berg and Corner, 2005) and supplemented by personal observations of herbarium specimens. The characters are described in the legend of Fig. 3. Although seed ovaries embedded in the receptacle or attached at the base to the receptacle has been used to distinguish subseries in the past (Corner, 1965), we found this character difficult to score and we did not include it Fig. 3.

### 2.5. Mapping habitat use

Habitat use was scored for all species (Fig. 3). Character states were taken from descriptions in the literature (Corner, 1959; Chew, 1989; Dixon, 2001a–d, 2003; Berg and Corner, 2005) and supplemented by personal observations of herbarium collections and during fieldwork in Australia and Papua New Guinea. The characters are described in the legend of Fig. 3.

### 2.6. Mapping changes in pollination behaviour

Previous studies have shown a strong association between active pollination, low anther/ovule ratios (floral sex ratio, FRS), and the presence of coxal combs (a line of setae or dense, parallel and long hairs on the fore coxa) on the pollinator, whereas the presence of pollen pockets provide a good, but imperfect index of active pollination (Cook et al. 2004; Jousselin et al. 2003; Kjellberg et al. 2001). Floral sex ratios, the presence or absence of coxal combs, pollen pockets, and pollination behaviour were taken from the literature and supplemented by personal observations of *F. glandifera*, *F. heteromeka*, *F. sterrocarpa* and *F. cf. sterrocarpa* as well as the pollinators of *F. glandifera*, *F. heteromeka*, and *F. cf. sterrocarpa* (see Fig. 4).

Following Cook et al. (2004), we estimated floral sex ratios (FSR) as the proportion of flowers that are male (i.e. anthers/anthers + ovules), by counting and sexing all flowers in one syconium for each of *F. glandifera*, *F. heteromeka*, *F. sterrocarpa* and *F. cf. sterrocarpa*. All syconia were collected from trees growing within their natural ranges.

We mapped pollination behaviour onto the ML tree presented in Fig. 2, collapsing all duplicate samples of the same species, and analysed the patterns of changes using parsimony techniques in MacClade version 4.03 (Maddison and Maddison, 2001). We

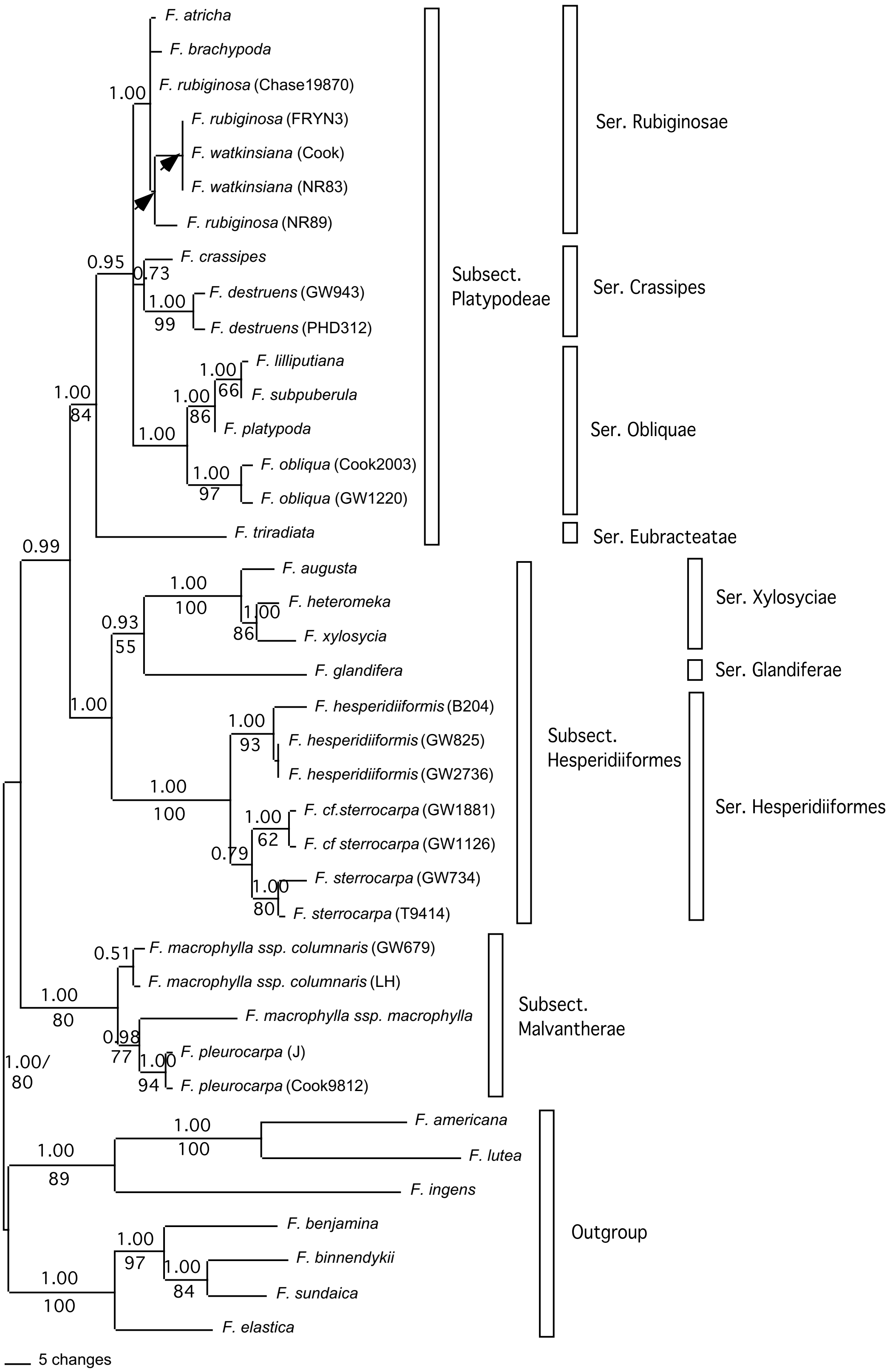


**Fig. 2.** One of 14 maximum likelihood trees with ML branch lengths, and with bootstrap percentages and posterior probabilities below and above branches, respectively. Arrowheads indicate branches that collapse in the strict consensus of 14 ML trees. Our preferred classification of *Malvanthera* suggested in the present study is indicated on the right.

treated behaviour as an unordered, binary (active/passive) character and weighted gains and losses of active behaviour equally in seeking the most parsimonious reconstruction of changes.

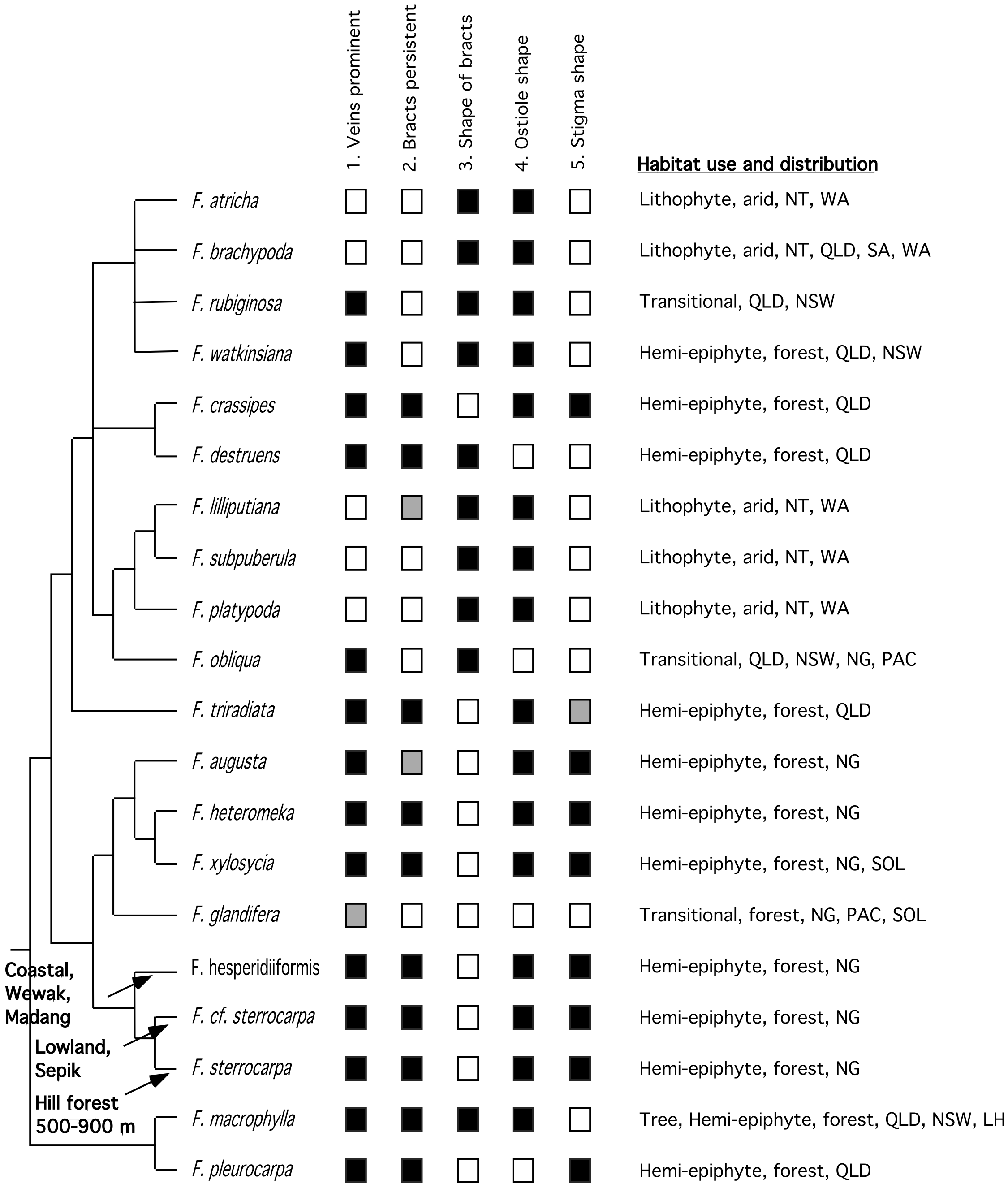


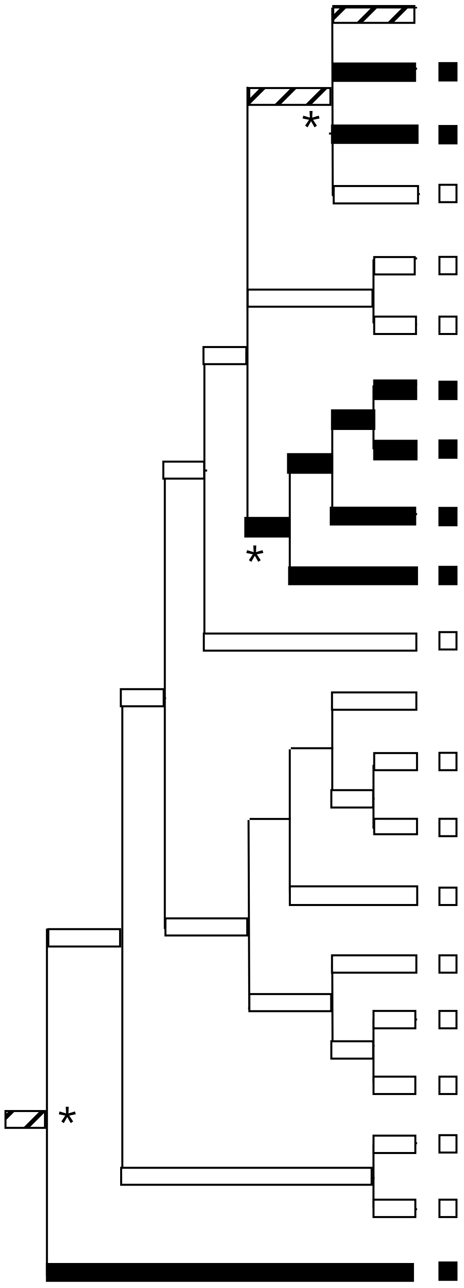
**Fig. 3.** Maximum likelihood (ML) tree from Fig. 2 showing the distribution of macro-morphological characters used in previous classifications and the distribution of biogeography and habitat use. White, black, and grey boxes show character states 0, 1, or polymorphic, respectively. 1. Prominence of basal or primary lateral veins on leaves: basal veins distinct (1) or not (0). 2. Persistence of basal bracts: Basal bracts persistent (1) or cauducous (0). 3. Shape of basal bracts: Bracts imbricate (1) or valvate (0). 4. Ostiole shape: Triradiate (1) or slit-shaped (bilabiate; 0). 5. Shape of stigma: Stigma bifid (1) or simple (0). LH—Lord Howe Island, NG—New Guinea, NSW—New South Wales, NT—Northern Territory, PAC—Pacific islands, QLD—Queensland, SA—South Australia, SOL—Solomon Islands, WA—Western Australia.

## 3. Results

### 3.1. Analyses of the combined dataset

Since there were no strongly supported conflicting clades within the ingroup, we considered it appropriate to directly combine all data. The aligned combined matrix of all three regions contained 32 ingroup taxa and 7 outgroups, and a total of 2024 base pairs of which 186 (9%) were parsimony informative. MP analysis with a limit of 25 trees per replicate produced 4286 trees of 557 steps with CI = 0.75 and RI = 0.78. These trees were used as starting trees in a second search with no limit on the number of trees per replicate, but with a maximum of 15,000 trees. This search produced the maximum number of trees, which were then swapped to completion.

The strict consensus tree of the combined MP analysis is not fully resolved and a considerable number of clades do not obtain more than 50% BS. The ingroup is monophyletic (80% BS). Within the ingroup, we find a soft polytomy with four clades. One clade includes *F. pleurocarpa* and *F. macrophylla* samples



| | 1. FSR | 2. Coxal combs | 3. Pollen pockets | 4. Pollination mode | 5. Pollinator |
|---|---|---|---|---|---|
| *F. atricha* | ????? | ?? | ?? | ?? | *Unknown* |
| ■ *F. brachypoda* | 0.777 | ■ | ■ | ■ | *P. macrocainus* |
| ■ *F. rubiginosa* | 0.125 | ■ | ■ | ■ | *P. imperialis* |
| □ *F. watkinsiana* | 0.399 | □ | ▒ | □ | *P. nigriventris* |
| □ *F. crassipes* | 0.453 | □ | ▒ | □ | *P. addicotti* |
| □ *F. destruens* | 0.246 | □ | ▒ | □ | *P. rigisamos* |
| ■ *F. lilliputiana* | 0.088 | ■ | ■ | ■ | *P. proximus* |
| ■ *F. subpuberula* | ???? | ■ | ■ | ■ | *P. astrabocheilus* |
| ■ *F. platypoda* | 0.153 | ■ | ■ | ■ | *P. cuneatus* |
| ■ *F. obliqua* | 0.109 | ■ | ■ | ■ | *P. greenwoodi* |
| □ *F. triradiata* | 0.315 | □ | ▒ | □ | *P. schizodontus* |
| *F. augusta* | ???? | ?? | ?? | ?? | *Unknown* |
| □ *F. heteromeka* | 0.55 | □ | □ | □ | *P. ex F. heteromeka* |
| □ *F. xylosycia* | 0.332 | □ | □ | □ | *P. riecki* |
| □ *F. glandifera* | 0.51 | □ | □ | □ | *P. blandus* |
| □ F. hesperidiiformis | 0.256 | □ | □ | □ | *P. plebejus* |
| □ *F. cf. sterrocarpa* | 0.61 | □ | □ | □ | *P. ex F. cf. sterrocarpa* |
| □ *F. sterrocarpa* | 0.29 | □ | □ | □ | *P. immaturus* |
| □ *F. macrophylla* | 0.248 | □ | ■ | □ | *P. froggatti* |
| □ *F. pleurocarpa* | 0.533 | □ | ▒ | □ | *P. regalis* |
| ■ *F. ingens* | 0.040 | ■ | ■ | ■ | *Platyscapa soraria* |

**Fig. 4.** Parsimony reconstruction of changes (*) between passive (white) and active (black) pollination behaviour showing the distribution of flower sex ratios (FSR) of *Malvanthera* species and correlated morphology of their pollinators. White and black boxes show character states 0 and 1, respectively. 1. FSRs from Cook et al. (2004), except *Ficus glandifera*, *F. heteromeka*, *F. sterrocarpa* and *F. cf. sterrocarpa*, which were counted for the present study. 2. Coxal combs absent (0) or present (1). 3. Pollen pockets absent (0), present, but small (grey boxes), or present and large (1). 4. Pollination mode passive (0) or active (1). 5. Pollinating *Pleistodontes* species. Wasp data from Cook et al. (2004), Lopez-Vaamonde et al. (2002), and Wiebes (1994), except for *Pleistodontes blandus*, *P. ex. F. heteromeka* and *P. ex. F. cf. sterrocarpa*, which were observed for the present study. Some *Malvanthera* species have more than one pollinator, but no differences in pollination behaviour between pollinators of the same *Malvanthera* species have been described.

(80%), another clade includes *F. hesperidiiformis* and *F. sterrocarpa* (100% BS), a third clade includes *F. augusta*, *F. glandifera*, *F. heteromeka* and *F. xylosycia* (55%), and a last clade includes the remainder of the Australian taxa (84%). The MP consensus tree is not shown, but MP bootstrap support is indicated on the ML tree in Fig. 2.

For the ML analysis, Modeltest suggested TrN + I + G as the best fitting model using both hierarchical likelihood ratio tests (hLRTs) and the Akaike information criterion (AIC). Based on recommendations by Posada and Buckley (2004), we used the model selected by the Akaike criterion for the ML search (Parameters: Lset Base = (0.2393, 0.2548, 0.2819) Nst = 6 Rmat = (1.0000, 3.9211, 1.0000, 1.0000, 4.9209) Rates = gamma Shape = 0.8798 Pinvar = 0.5620). This analysis produced 14 trees and the strict consensus tree provided resolution between and within most of the clades found in the MP analysis. One of the 14 trees is shown in Fig. 2 with Bayesian posterior probabilities and MP bootstrap support indicated above and below the branches, respectively. The ingroup is monophyletic and the first split is between a *F. pleurocarpa* plus *F. macrophylla* clade and the rest of the section. Among the rest of *Malvanthera* we find two major clades. In the first major clade, *F. glandifera* is sister to the *F. augusta*, *F. heteromeka* plus *F. xylosycia* clade, and *F. hesperidiiformis* plus *F. sterrocarpa* is sister to all. In the *F. hesperidiiformis*–*F. sterrocarpa* clade, there were multiple well-supported clades that indicate the presence of at least three species in the complex (see discussion in Section 4.3). The other major clade includes *F. triradiata* as sister to the remainder of the Australian taxa, which form a *trichotomy* of (1) *F. crassipes* and *F. destruens*; (2) *F. lilliputiana F. obliqua*, *F. platypoda*, and *F. subpuberula*; and (3) *F. atricha*, *F. brachypoda*, *F. rubigionosa* and *F. watkinsiana*.

The Bayesian analysis provided a tree that was identical to the ML consensus tree, except that two more clades were resolved, but only with pp = 0.51. These weakly supported clades included *F. crassipes*–*F. destruens* as sister to the *F. atricha*–*F. watkinsiana* clade, and within this clade, *F. brachypoda* as sister to the remainder.

The analysis constraining Australian lithophytes to be monophyletic produced 247 trees, which were only 8 steps longer (565 steps) and were not significantly different from the 15,000 trees from the unconstrained combined analysis ($p = 0.1276$).

## 4. Discussion

### 4.1. Infrasectional relationships of section Malvanthera

The results of our molecular phylogenetic study do not support any of the previous classifications of section *Malvanthera* (Corner, 1965; Dixon, 2003; Berg and Corner, 2005, Table 1). Instead we find correspondence with geography. Two major lineages (subsections *Malvantherae* and *Platypodeae*) are primarily Australian and a third major lineage (subsection *Hesperidiiformes*) has its centre of diversity in New Guinea. Based on these results we propose a new subdivision of section *Malvanthera* that reflects phylogenetic relationships (Table 1, Fig. 2).

### 4.2. Homoplasy of characters previously used for subdivision of section Malvanthera

The connection between phylogenetic subdivisions and biogeography was not apparent in any of the previous subdivisions of section *Malvanthera*, because all of the macro-morphological characters used for subdivision are homoplasious. Most of the characters mapped in Fig. 3 vary even between closely related species. For example, *F. macrophylla* has imbricate bracts, a triradiate ostiole, and a simple stigma, whereas its sister species, *F. pleurocarpa*, has valvate bracts, a bilabiate ostiole, and a bifid stigma. However, the close relationship of *F. macrophylla* and *F. pleurocarpa* is supported by several other lines of evidence. Both species are hemiepiphytes or trees occurring in tropic rainforest in Northeast Queensland and in coastal habitats from Queensland to New South Wales and both species have distinct basal veins, three ostiolar bracts, female florets embedded in the receptacle, and a similar indumentum on the abaxial surface of the leaves.

Dixon (2003) used the shape of the basal bracts to divide ser. *Malvanthereae* with imbricate bracts from ser. *Hesperidiiformes* with valvate bracts. All species from New Guinea have valvate bracts, and Dixon's scheme was the first to unite *F. glandifera* with the other species from New Guinea. However, the Australian *F. pleurocarpa* (subsect. *Malvanthereae*), as well as *F. crassipes* and *F. triradiata* (subsect. *Platypodeae*) also have valvate bracts, and were therefore placed in ser. *Hesperidiiformes* with geographically disjunct species from New Guinea.

Some of the morphological characters used previously to delimit groups are still useful. For example, within subsection *Platypodeae*, the persistence of basal bracts separates series *Eubracteatae* and *Crassipeae* with persistent basal bracts from series *Rubiginosae* and *Obliqua* with cauducous basal bracts. Some characters appear to be associated with aspects of ecology, such as the basal or primary lateral veins of leaves, which are inconspicuous in the Australian lithophytes, but prominent in the rainforest species. In summary, phylogenetic relationships were not reflected in previous subdivisions of *Malvanthera* because the morphological characters used to delimit taxa are quite homoplasious.

### 4.3. Species delimitation within subsection Hesperidiiformes in New Guinea

Berg and Corner (2005) united most of Corner's (1965) New Guinean species under an expanded *F. hesperidiiformis*. Our results suggest that Corneŕs species must be maintained. Based on phylogeny alone, it is clear that an expanded *F. hesperidiiformis* concept is not monophyletic unless it also includes *F. glandifera*, which was retained as a species (Berg and Corner, 2005).

Berg and Corner (2005) recognised no clear separation of *F. hesperidiiformis* and *F. sterrocarpa* and species delimitation in this complex remains uncertain. Our phylogenetic analyses (Figs. 2 and 3) show a clade of coastal samples from the Wewak and Madang provinces, which we recognize as *F. hesperidiiformis*. The *F. sterrocarpa* samples are divided into two clades, a clade with hill forest samples found at 500–900 m, which we recognize as *F. sterrocarpa*, and a second clade with lowland samples from Sepik, which may represent a separate species here designated *F. cf. sterrocarpa*. We think that there are at least three species in this complex, but further studies including more extensive sampling and comparison of morphological characters are needed.

The existence of several species in the *F. hesperidiiformis*–*F. sterrocarpa* complex is further supported by preliminary tests of pairwise distances of ITS among figs and the *cox1* gene among their specific pollinators (Rønsted and Weiblen, unpublished data). Distances between *F. hesperidiiformis* and *F. sterrocarpa* are in the same range as distances between other New Guinean species or between closely related Australian species. Likewise, pairwise distances of *Pleistodontes immaturis*, *P. cf. immaturis*, and *P. plebejus* pollinating *F. sterrocarpa*, *F. cf. sterrocarpa* and *F. hesperidiiformis*, respectively, are comparable to distances between pollinators of closely related Australian *Malvanthera* species (Lopez–Vaamonde et al. 2001).

### 4.4. Biogeography and habitat use within section Malvanthera

We suggest that section *Malvanthera* is of Australian origin (Figs. 2 and 3) given that the sister lineage to the New Guinean *Malvanthera* and most Australian *Malvanthera* is a *F. pleurocarpa*–*F. macrophylla* clade (subsection *Malvanthereae*). *Ficus pleurocarpa* and *F. macrophylla* subsp. *macrophylla* are endemic to the Australian mainland, while *F. macrophylla* subsp. *columnaris* probably represents a later colonization event to Lord Howe Island, where this subspecies is endemic (Dixon, 2001c). Our analyses indicate that subsp. *columnaris* could be the oldest lineage in the clade, but more sampling of both subspecies is needed to clarify this. According to Rønsted et al. (2005), section *Malvanthera* originated at least 41 million years ago and radiated gradually from about 35 million years ago. The section probably colonized New Guinea once (subsection *Hesperidiiformes*) with *F. glandifera* and *F. xylosycia* subsequently colonising other islands in Oceania.

In subsection *Platypodeae*, (Figs. 2 and 3), *F. triradiata* (series *Eubracteatae*), is a rainforest hemi-epiphyte and represents the first diverging lineage, although only well supported by the Bayesian analysis. Series *Crassipeae* (*F. crassipes* and *F. destruens*) are also hemi-epiphytic rainforest species. In series *Obliquae*, the first diverging lineage is *F. obliqua*, which occurs both as a hemi-epiphyte and as a lithophyte throughout its range, whereas the remaining species in this series are all lithophytes. Our results are consistent with a pattern of radiation within series *Obliquae* from the rainforest into drier habitats in Australia with a transition from tall hemi-epiphytic trees to small trees and shrubs. Within series *Rubiginosae*, *F. watkinsiana* is restricted to rainforest, *F. rubiginosa* can both be hemi-epiphytic and lithophytic, whereas *F. atricha* and *F. brachypoda* are both lithophytic shrubs or small trees. Relationships within series *Rubiginosae* are not well resolved in this study, but this clade possibly represents a second radiation from the forest into drier habitats in Australia parallel to series *Obliquae*. A Templeton test showed that trees with all five Australian lithophytes constrained as monophyletic was not significantly different from unconstrained trees, indicating that the transition from forest to arid zones might have happened only once.

However, in addition to the lithophytes, we also find transitional species, such as *F. rubiginosa* and *F. obliqua* in Australia and *F. glandifera* in New Guinea and Oceania. *Ficus glandifera* is part of a New Guinean rainforest clade and is not closely related to the Australian lithophytic and transitional species further supporting the possibility that colonisation of arid environments may have evolved at least twice. In conclusion there seems to be more homoplasy in morphology and ecology within section *Malvanthera* than previous subdivisions of the section indicated. This agrees with a hypothesis of multiple gains of active pollination behaviour in *Pleistodontes* species pollinating section *Malvanthera* (Cook et al., 2004).

### 4.5. Evolution of pollination behaviour

Cook et al. (2004) mapped changes in pollination behaviour onto a phylogeny of *Pleistodontes*. They found that there have been three to six changes in pollination behaviour within *Pleistodontes*, emphasizing that co-evolution does not reach an endpoint and that selection pressures on mutualists are not constant. Changes in pollination behaviour correlated perfectly with changes in anther/ovule ratio in the host figs as also found by Kjellberg et al. (2001) and no evidence was found of phylogenetic restrictions at the species level (Cook et al. 2004). Although all actively pollinated *Malvanthera*, have a simple stigma (Figs. 3 and 4), passively pollinated species either have a bifid or a simple stigma, implying limited correlation between this stigma trait and pollination behaviour.

We used *F. ingens* (subgenus *Urostigma* section *Urostigma*) and its active pollinator *Platyscapa soraria* as an outgroup. As in Cook et al. (2004) the ancestral state for *Malvanthera*/*Pleistodontes* was inferred to be passive pollination, implying one change from active pollination, although this is wholly dependent on outgroup status. Within *Malvanthera*, one change to active pollination is needed for series *Obliquae*, and at least one change to active pollination within the ser. *Rubiginosae*. With the presence of small pollen pockets, *F. watkinsiana* and its pollinator *P. nigriventris*, could represent a secondary loss of active pollination, depending on the true relationship within this series. However, the number of pollination mode changes is not certain and at least one inferred ingroup change depend on weakly supported nodes within subsect. *Platypodeae*. Accordingly, mapping traits associated with pollination behaviour onto our phylogenetic hypothesis for *Malvanthera*, implies only 1–3 changes within the section, in contradiction with 3–6 changes previously inferred by mapping pollination behaviour onto a phylogenetic hypothesis for *Pleistodontes* (Cook et al. 2004).

It is noteworthy, that the pattern of radiation out of the rainforest habitat into drier habitats associated with a transition from tall hemi-epiphytic trees to small trees and shrubs (Fig. 3), also seems to be associated with a gain of active pollination behaviour (Fig. 4). However, both the phylogenies of the figs and the pollinators needs improved resolution and support before we can really understand the patterns and processes of co-evolution in the *Malvanthera*–*Pleistodontes* mutualism.

## Acknowledgments

N.R. thank M. Thomas (K), H.V. Hansen (C), D. Dixon (DNA) and C.C. Berg (BG) for providing plant material, Jean-Yves Rasplus for information on *Pleistodontes blandus*, Dale Dixon, Finn Kjellberg and an anonymous reviewer for comments on an earlier version of this manuscript. This work was partly supported by a *Marie Curie Outgoing International Fellowship* within the 6th European Community Framework Program (N.R./V.S.) and the NERC (UK) (J.M.C.).

## References

Baldwin, B.G., Sanderson, M.J., Porter, J.M., Wojciechowski, M.F., Campbell, C.S., Donoghue, M.J., 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. MO Bot. Gard. 82, 247–277.

Baldwin, B.G., Markos, S., 1998. Phylogenetic utility of the external transcribed spacer (ETS) of 18S–26S rDNA: congruence of ETS and ITS trees of *Calycadenia* (Compositae). Mol. Phyl. Evol. 10, 449–463.

Berg, C.C., 2002. *Ficus baola*, a new species of *Ficus* subgenus *Urostigma* section *Malvanthera* (Moraceae) from the Solomon Islands. Blumea 47, 315–317.

Berg, C.C., 2003. Flora Malesiana precursor for the treatment of Moraceae 1: the main subdivision of *Ficus*: the subgenera. Blumea 48, 167–178.

Berg, C.C., Corner, E.J.H., 2005. Moraceae (Ficus). In: Noteboom, H.P. (Ed.), Flora Malesiana ser. 1, vol. 17. National Herbarium of Nederland, Leiden, The Netherlands, pp. 1–730.

Chew, W.L., 1989. Moraceae. In: George, S. (Ed.), Flora of Australia, vol. 3. Australian Government Printing Service, Canberra, pp. 15–68.

Cook, J.M., Bean, D., Power, S.A., Dixon, D.J., 2004. Evolution of a complex coevolved trait: active pollination in a genus of fig-wasps. Evol. Biol. 17, 238–246.

Cook, J.M., Rasplus, J.-Y., 2003. Mutualists with attitude: coevolving fig wasps and figs. TREE 18, 241–248.

Corner, E.J.H., 1959. Taxonomic notes on *Ficus* Linn., Asia and Australasia. I. Subgen. *Urostigma* (Gasp.) Miq. Gard. Bull. Sing. 17, 368–404.

Corner, E.J.H., 1965. Checklist of *Ficus* in Asia and Australasia with keys to identification. Gard. Bull. Sing. 21, 1–186.

Dixon, D.J., 2001a. A chequered history: the taxonomy of *Ficus platypoda* and *F. leuchotricha* (Moraceae: *Urostigma* sect. *Malvanthera*) unravelled. Austr. Syst. Bot. 14, 535–563.

Dixon, D.J., 2001b. *Ficus lilliputiana* (Moraceae), a new species from the Kimberley region of Western Australia and the Northern Territory. Nuytsia 13, 457–464.

Dixon, D.J., 2001c. Figs, wasps and species concepts: a re-evaluation of the infraspecific taxa of *Ficus macrophylla* (Moraceae: *Urostigma* sect. *Malvanthera*). Austr. Syst. Bot. 14, 125–132.

Dixon, D.J., 2001d. Figuring out the figs: the *Ficus obliqua*—*Ficus rubiginosa* complex (Moraceae: *Urostigma* sect. *Malvanthera*). Austr. Syst. Bot. 14, 133–154.

Dixon, D.J., 2003. A taxonomic revision of the Australian *Ficus* species in the section *Malvanthera* (*Ficus* subg. *Urostigma*: Moraceae). Telopia 10, 125–153.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.

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

Fitch, W.M., 1971. Towards defining the course of evolution: minimum change for a specific tree typology. Syst. Zool. 20, 406–416.

Haine, E.R., Martin, J., Cook, J.M., 2006. Deep mtDNA divergences indicate cryptic species in a fig-pollinating wasp. BMC Evol. Biol. 6, 83.

Harrison, R.D., 2005. Figs and the diversity of tropical rainforests. Bioscience 55, 1053–1064.

Jousselin, E., Rasplus, J.-Y., Kjellberg, F., 2003. Convergence and coevolution in a mutualism: evidence from a molecular phylogeny of *Ficus*. Evolution 57, 1255–1269.

Kjellberg, F., Jousselin, E., Bronstein, J.L., Patel, A., Yokoyama, J., Rasplus, J.-Y., 2001. Pollination mode in fig wasps: the predictive power of correlated traits. Proc. Roy. Soc. Lond. B 268, 113–1121.

Lopez-Vaamonde, C., Rasplus, J.Y., Weiblen, G.D., Cook, J.M., 2001. Molecular phylogenies of figwasps: partial cocladogenesis of pollinators and parasites. Mol. Phyl. Evol. 21, 55–71.

Lopez-Vaamonde, C., Dixon, D.J., Cook, J.M., Rasplus, J.-Y., 2002. Revision of Australian species of *Pleistodontes* (Hymenoptera: Agaonidae) fig-pollinating wasps and their host plant associations. Zool. J. Linn. Soc. 136, 637–683.

Machado, C.A., Robbins, N., Gilbert, T.P., Herre, E.A., 2005. Critical review of host specificity and its coevolutionary implications in the fig/fig-wasp mutualism. Proc. Natl. Acad. Sci. USA 102, 6558–6565.

Maddison, D.R., 1991. The discovery and importance of multiple islands of most-parsimonius trees. Syst. Zool. 40, 315–328.

Maddison, D.R., Maddison, W.P., 2001. MacClade. Sinauer Sunderland, MA, USA.

Posada, D., Buckley, T., 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ration tests. Syst. Biol. 53, 793–808.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Ronquist, F., Hulsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Rønsted, N., Weiblen, G.D., Cook, J.M., Salamin, N., Machado, C.A., Savolainen, V., 2005. 60 million years of co-divergence in the fig-wasp symbiosis. Proc. Roy. Soc. Lond. B 272, 2593–2599.

Rønsted, N., Yektaei-Karin, E., Turk, K., Clarkson, J.M., Chase, M.W., 2007. Species-level phylogenetics of large genera: prospects of studying co-evolution and polyploidy. In: Hodkinson, T., Parnell, J. (Eds.), Reconstructing the Tree of Life: Taxonomy and Systematics of Species Rich Taxa. Systematic Association Series. CRC Press.

Rønsted, N., Weiblen, G.D., Clement, W., Zerega, N., Savolainen, V., 2008. Reconstructing the phylogeny of figs (*Ficus*, Moraceae) to unravel the origin of fig-wasp mutualisms. Symbiosis 45, 45–56.

Strand, A.E., Leebens-Mack, J., Milligan, B.G., 1997. Nuclear DNA-based markers for plant evolutionary biology. Mol. Ecol. 6, 113–118.

Sun, Y., Skinner, D.Z., Liang, G.H., Hulbert, S.H., 1994. Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. Theor. Appl. Genet. 89, 26–32.

Swofford, D.L. 2002. PAUP*: Phylogenetic Methods Using Parsimony (* and other methods), version 4, Sinauer, Sunderland, MA.

Templeton, A.R., 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. Evolution 37, 221–244.

Weiblen, G.D., 2000. Phylogenetic relationships of functionally dioecious *Ficus* (Moraceae) based on ribosomal DNA sequences and morphology. Am. J. Bot. 87, 1342–1357.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols. Academic Press, San Diego, CA, USA, pp. 315–322.

Wiebes, J.T., 1994. The Indo-Australian Agaoninae (pollinators of figs). Proc. K. Ned. Akad. Wet. 92, 1–208.