

MOLECULAR SYSTEMATICS OF THE CASHEW FAMILY  
(ANACARDIACEAE)

A Dissertation

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in

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by  
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Dedicated to my mentors:

Marcia Petersen, my mentor in education

Dr. Frank Watson, my mentor in botany

John D. Mitchell, my mentor in the Anacardiaceae

Mary Alice and Ken Carpenter, my mentors in life

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# Table of Contents

|  |      |
|--|------|
| Acknowledgements .....   | iv   |
| List of Tables .....   | ix   |
| List of Figures .....  | x    |
| Abstract .....   | xiii |
| Chapter 1: Introduction .....  | 1    |
| Fossil Record and Biogeography .....   | 4    |
| Economic Botany .....  | 4    |
| Biochemistry .....   | 9    |
| Taxonomic History .....  | 11   |
| Objectives .....   | 21   |
| Chapter 2: Phylogenetic Position of Anacardiaceae, Burseraceae and Other Allied Families .....                                     | 23   |
| Introduction .....   | 23   |
| Materials and Methods .....  | 28   |
| Results .....  | 32   |
| Discussion .....   | 43   |
| Chapter 3: Molecular Phylogeny of Anacardiaceae: Intrafamilial Classification and Evolutionary Relationships of Noted Genera ..... | 51   |
| Introduction .....   | 51   |
| Materials and Methods .....  | 57   |
| Results .....  | 64   |
| Discussion .....   | 91   |
| Chapter 4: Out of Africa: Taxonomic Split of Madagascan and South African Species of <i>Protorhus</i> Engl. (Anacardiaceae) .....  | 113  |
| Introduction .....   | 113  |
| Materials and Methods .....  | 118  |
| Results .....  | 124  |
| Discussion .....   | 136  |
| Chapter 5: Conclusions .....   | 140  |
| Bibliography .....   | 144  |

|   |     |
|---|-----|
| Appendix A: Taxa Included in Molecular Datasets .....               | 173 |
| Appendix B: Protocol for DNA Extraction of Herbarium Specimens..... | 190 |
| Vita.....   | 193 |

## List of Tables

|   |     |
|---|-----|
| Table 1.1. Family synonymy for Anacardiaceae Lindl., <i>nom. cons.</i> (1830) as delimited in this study.....             | 12  |
| Table 1.2. Proposed infrafamilial classifications of the Anacardiaceae.....   | 13  |
| Table 1.3. Generic affinities of the infrafamilial classification used in this study                                      | 15  |
| Table 1.4. Tribal affinities of the 27 monotypic genera in Anacardiaceae. ....  | 20  |
| Table 1.5. Proposed ordinal affinities of Anacardiaceae based on morphological or molecular data. ....                    | 21  |
| Table 2.1. Sequences of the <i>trnLF</i> primers used in this study.....  | 30  |
| Table 3.1. Generic affinities of the infrafamilial classification used in this study                                      | 53  |
| Table 3.2. Taxon sampling in the <i>matK</i> , <i>trnLF</i> , <i>rps16</i> , and combined datasets.                       | 58  |
| Table 3.3. Sequences of the <i>trnLF</i> primers used in this study.....  | 60  |
| Table 3.4. <i>matK</i> primers used in this study .....   | 61  |
| Table 3.5. Generic placement within the two subfamily system of Anacardiaceae classification outlined in this study. .... | 99  |
| Table 4.1. Selected characteristics of <i>Protorhus</i> .....   | 116 |
| Table 4.2. ITS and ETS primers used in this study. ....   | 120 |
| Table 4.3. Sequences of the <i>trnLF</i> primers used in this study.....  | 121 |

## List of Figures

|   |    |
|---|----|
| Figure 1.1. Ascending and descending epitropous ovules. ....  | 2  |
| Figure 1.2. Schematic of the hypothesized monophyletic Anacardiaceae.....   | 16 |
| Figure 1.3. Schematic of the Burseraceae-Anacardiaceae subtree from the<br>maximum parsimony <i>rbcL</i> phylogeny of selected members of the Sapindales<br>..... | 18 |
| Figure 2.1. Approximate location of <i>trnL</i> F primers used in this study .....  | 30 |
| Figure 2.2. Bootstrap consensus phylogeny of <i>trnL</i> F sequences of<br>Anacardiaceae, Burseraceae, Sapindaceae and Rutaceae.....                              | 34 |
| Figure 2.3. Maximum likelihood phylogram of <i>trnL</i> F sequences of<br>Anacardiaceae, Burseraceae, and Sapindaceae with a Rutaceae outgroup.<br>.....          | 35 |
| Figure 2.4. <i>trnL</i> F subtree of Anacardiaceae from maximum parsimony 50%<br>bootstrap consensus tree.....  | 36 |
| Figure 2.5. <i>trnL</i> F subtree of Burseraceae from maximum parsimony 50%<br>bootstrap consensus tree.....  | 37 |
| Figure 2.6. <i>trnL</i> F subtree of Sapindaceae from maximum parsimony 50%<br>bootstrap consensus tree.....  | 37 |
| Figure 2.7. Coding of morphological character.....  | 39 |
| Figure 2.8. Wind pollination adaptations mapped onto the <i>trnL</i> F maximum<br>parsimony bootstrap consensus tree .....  | 40 |
| Figure 2.9. Presence and absence of resin canals in the phloem mapped onto<br>the <i>trnL</i> F maximum parsimony bootstrap consensus tree.....                   | 41 |
| Figure 2.10. Position of ovules in locules mapped onto the <i>trnL</i> F maximum<br>parsimony bootstrap consensus tree .....                                      | 42 |
| Figure 3.1. Approximate location of <i>trnL</i> F primers used in this study .....  | 60 |
| Figure 3.2. Approximate location of <i>matK</i> primers used in this study .....  | 61 |

|  |    |
|--|----|
| Figure 3.3. Bootstrap consensus phylogeny resulting from phylogenetic analysis of <i>matK</i> sequences of 33 Anacardiaceae ingroup and 5 Burseraceae outgroup taxa. ....  | 66 |
| Figure 3.4. Maximum likelihood tree resulting from phylogenetic analysis of <i>matK</i> sequences of 33 Anacardiaceae ingroup and 5 Burseraceae outgroup taxa .....  | 67 |
| Figure 3.5. Bootstrap consensus phylogeny resulting from phylogenetic analysis of <i>rps16</i> sequences of 55 Anacardiaceae and 5 Burseraceae ingroup and 3 Sapindaceae outgroup taxa .....                           | 68 |
| Figure 3.6. Maximum likelihood tree resulting from phylogenetic analysis of <i>rps16</i> sequences of 55 Anacardiaceae and 5 Burseraceae ingroup and 3 Sapindaceae outgroup taxa .....                                 | 70 |
| Figure 3.7. Bootstrap consensus phylogeny resulting from phylogenetic analysis of <i>trnLF</i> sequences of 81 Anacardiaceae ingroup and 3 Burseraceae outgroup taxa. ....   | 71 |
| Figure 3.8. Strict consensus of 3 maximum likelihood trees resulting from phylogenetic analysis of <i>trnLF</i> sequences of 81 Anacardiaceae ingroup and 3 Burseraceae outgroup taxa .....                            | 72 |
| Figure 3.9. Bootstrap consensus phylogeny resulting from phylogenetic analysis of combined <i>rps16</i> and <i>trnLF</i> sequences of 50 Anacardiaceae and 5 Burseraceae ingroup and 3 Sapindaceae outgroup taxa ..... | 74 |
| Figure 3.10. Bootstrap consensus phylogeny resulting from phylogenetic analysis of combined <i>matK</i> , <i>rps16</i> , and <i>trnLF</i> sequences of 19 Anacardiaceae ingroup and 3 Burseraceae outgroup taxa. ....  | 75 |
| Figure 3.11. Eight morphological, biochemical, and anatomical characters mapped onto the <i>trnLF</i> bootstrap consensus phylogeny.....   | 76 |
| Figure 3.12. Endocarp organization mapped onto the <i>trnLF</i> bootstrap consensus phylogeny.....   | 78 |
| Figure 3.13. Perianth structure mapped onto the <i>trnLF</i> bootstrap consensus phylogeny.....  | 79 |
| Figure 3.14. Dispersal and mechanisms of wind dispersal mapped onto the <i>trnLF</i> bootstrap consensus phylogeny .....   | 80 |

|  |     |
|--|-----|
| Figure 3.15. Genera belonging to the <i>Rhus</i> complex highlighted on the <i>trnL</i> F bootstrap consensus phylogeny.....   | 82  |
| Figure 3.16. Opercula presence and absence mapped onto the <i>trnL</i> F bootstrap consensus phylogeny.....  | 83  |
| Figure 3.17. Leaf complexity mapped onto the <i>trnL</i> F bootstrap consensus phylogeny.....  | 84  |
| Figure 3.18. Taxon distributions mapped onto the <i>trnL</i> F bootstrap consensus phylogeny.....  | 85  |
| Figure 4.1. Map of the ITS and ETS primers used in this study .....  | 121 |
| Figure 4.2. Approximate location of <i>trnL</i> F primers used in this study .....   | 121 |
| Figure 4.3. Strict consensus of 48 most parsimonious trees resulting from phylogenetic analysis of ETS sequences of 35 Anacardiaceae ingroup taxa and 1 Burseraceae outgroup taxon.....                                  | 125 |
| Figure 4.4. Strict consensus of 234 most parsimonious trees resulting from phylogenetic analysis of ITS sequences of 29 Anacardiaceae ingroup taxa and 1 Burseraceae outgroup taxon.....                                 | 126 |
| Figure 4.5. Strict consensus of 448 most parsimonious trees resulting from phylogenetic analysis of <i>trnL</i> F sequences of 34 Anacardiaceae ingroup taxa and 1 Burseraceae outgroup taxon.....                       | 128 |
| Figure 4.6. Strict consensus of 10 most parsimonious trees resulting from phylogenetic analysis of combined ETS and ITS sequences of 26 Anacardiaceae ingroup taxa and 1 Burseraceae outgroup taxon .....                | 129 |
| Figure 4.7. Strict consensus of 8 most parsimonious trees resulting from phylogenetic analysis of combined ETS, ITS, and <i>trnL</i> F sequences of 21 Anacardiaceae ingroup taxa and 1 Burseraceae outgroup taxon ..... | 130 |
| Figure 4.8. ETS, ITS, and <i>trnL</i> F tree from Fig. 4.7 with the number of styles mapped on the phylogeny.....  | 133 |
| Figure 4.9. ETS, ITS, and <i>trnL</i> F tree from Fig. 4.7 with the number of locules mapped on the phylogeny.....   | 134 |
| Figure 4.10. ETS, ITS, and <i>trnL</i> F tree from Fig. 4.7 with the distribution of the species mapped on the phylogeny.....  | 135 |

## Abstract

Anacardiaceae Lindl., the cashew family, is an economically important, primarily pantropically distributed family of 82 genera and over 700 species. This family is well known for its cultivated edible fruits and seeds (mangos, pistachios, and cashews), dermatitis causing taxa (e.g., *Comocladia*, *Metopium*, *Semecarpus*, *Toxicodendron*, etc.), and lacquer plants (*Toxicodendron* and *Gluta* spp.). The taxonomy of Anacardiaceae has not been thoroughly investigated since Engler established the currently used five tribal classification system over 100 years ago. This study evaluated evolutionary relationships of the family using nrDNA and cpDNA sequences. The first part of the study investigated the evolutionary position of Anacardiaceae in relation to closely allied families within the order Sapindales. DNA sequence data for the chloroplast *trnL* intron and 3' exon, and the intergenic spacer between *trnL* and *trnF* (*trnLF*) of Anacardiaceae, Burseraceae, Julianiaceae, Pistaciaceae, Podoaceae, Rutaceae, and Sapindaceae were generated to reconstruct phylogenetic relationships of these families. Julianiaceae, Pistaciaceae, and Podoaceae were all nested within Anacardiaceae. The sister group of Anacardiaceae is Burseraceae.

To understand intergeneric relationships within Anacardiaceae, phylogenies were constructed from sequences of three chloroplast loci (*matK*, *trnLF*, and *rps16*), using maximum parsimony and maximum likelihood as the optimality criteria. Based on these reconstructions and current knowledge of

morphological and anatomical attributes of the Anacardiaceae, the subfamilies of Takhtajan, Anacardioideae (including tribes Anacardieae, Dobineae, Rhoeeae, and Semecarpeae) and Spondioideae (including tribe Spondiadeae), were reinstated. Taxon distributions were mapped onto the phylogeny and the resulting biogeographic patterns were presented as evidence for the complex biogeographical history of the cashew family.

Chloroplast (*trnL*F) and SSU nrDNA (ITS and ETS) loci were sequenced to delimit the generic boundaries and biogeographical history of the Madagascan/African genus *Protorhus*. These findings resulted in the recognition of a new Madagascan endemic genus, *Abrahamia* Randrianasolo ined., segregated from *Protorhus*. From age estimates of the Sapindales, the isolation of Madagascar, and the phylogeny of the African/Madagascan clade of Anacardiaceae, it is unlikely that vicariance played a role in the evolution of Madagascan Anacardiaceae. One possible scenario based on phylogenetic reconstruction is that Anacardiaceae was dispersed over water between Africa and Madagascar a minimum of three times.

## Chapter 1: Introduction

Anacardiaceae Lindl., the cashew family, includes more than 700 species in 82 genera that are primarily distributed pantropically. Some genera, however, extend into the temperate zone. Members of the family are cultivated throughout the world for their edible fruits and seeds, medicinal compounds, valuable timber, and landscape appeal. Some of the products of Anacardiaceae, including mangos (*Mangifera indica* L. and other species), pistachios (*Pistacia vera* L.), cashews (*Anacardium occidentale* L.), and pink peppercorns (*Schinus terebinthifolia* L.), are enjoyed worldwide while other notables such as the pantropical *Spondias* L. fruits, the marula of Africa (*Sclerocarya birrea* (A. Rich.) Hochst.), and the Neotropical fruits of *Tapirira* Aubl., are restricted to localized cultivation and consumption and are not generally transported far distances to larger markets.

The Anacardiaceae includes primarily trees, shrubs, and lianas with resin canals and clear to milky sap. The leaves are estipulate and are usually alternate but may be simple or pinnately compound or rarely bi-pinnate (in *Spondias bipinnata* Airy Shaw and Forman). The flowers are generally not highly conspicuous but are distinctive in having an intrastaminal nectariferous disc and apotropous ovules (an ovule with a raphe that is ventral when ascending and dorsal when descending) that are pendulous and apically, laterally, or basally

attached (see Fig. 1.1). Morphological fruit diversity is exceedingly high with a myriad of types found in the family.

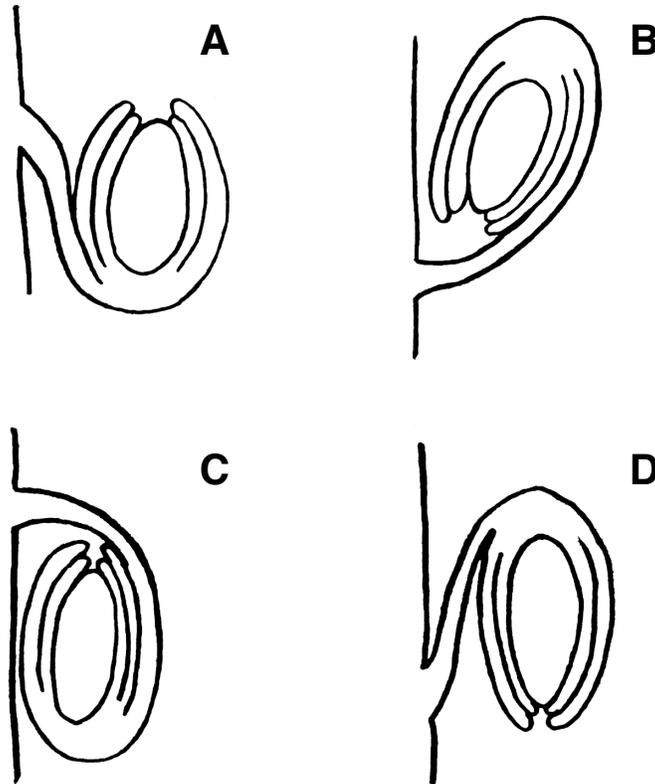


Figure 1.1. Ascending and descending epitropous (as found in Burseraceae: A, B) and apotropous (as found in Anacardiaceae: C, D) ovules. A. Ascending epitropous ovule with a ventral raphe. B. Descending epitropous ovule with a dorsal raphe. C. Ascending apotropous ovule with a dorsal raphe. D. Descending apotropous ovule with a ventral raphe (Redrawn and modified from Geesink et al., 1981).

Although the majority of the family has drupaceous fruits, many of these are variously modified for different mechanisms of dispersal. Several other fruit

types are also represented. Two genera, *Anacardium* L. and *Semecarpus* L. f., have an enlarged edible hypocarp subtending the drupe. One species of *Anacardium*, *A. microsepalum* Loesener, lacks the hypocarp and grows in the flooded forests of the Amazon where it may be fish dispersed (Mitchell and Mori, 1987; J. D. Mitchell, pers. com.). Water dispersal has been reported or purported for three genera, *Mangifera* L., *Poupartiopsis* Randrianasolo ined., and *Spondias*. The variety of mechanisms for wind dispersal seen throughout tribes Anacardieae, Dobineae, and Rhoeeae include subtending enlarged sepals (*Astronium* Jacq., *Hermogenodendron* ined., *Loxostylis* Spreng. Ex Reichb., *Myracrodruon* Allem., *Parishia* Hook. f.), subtending enlarged petals (*Gluta*, *Swintonia*), trichome-covered margins on a globose fruit (*Actinocheita* F. A. Barkley), trichome-covered margins on a flattened fruit (*Blepharocarya* F. Muell., *Ochoterena* F. A. Barkley), elm-like samaras encircled with a marginal wing (*Campylopetalum* Forman, *Cardenasiodendron* F. A. Barkley, *Dobinea* Buch.-Ham. ex D. Don, *Laurophyllus* Thunb., *Pseudosmodingium* Engl., *Smodingium* E. Mey.), samaroid fruits with a single wing (*Faguetia* March., *Loxopterygium* Hook. f., *Schinopsis* Engl.), dry syncarps (multiple fruit, *Amphipterygium* Schiede ex Standl. and *Orthopterygium* Hemsl.), dry achene-like fruit without a wing (*Apteroкарpos* Rizzini), and elongated ciliate pedicles of sterile florets on broken segments of an inflorescence that function much like a tumbleweed (*Cotinus* Mill.). The dry utricle fruits of *Pachycormus* Coville are most likely wind

dispersed but there is no direct report of this in the literature (J. D. Mitchell, pers. com.).

### Fossil Record and Biogeography

Anacardiaceae has a rich fossil record because of its woody growth form and current and historic wide distribution. Anacardiaceae pollen and wood first appears in the Paleocene epoch, 65 to 55 million years ago (Hsu, 1983; Muller, 1984), and is found throughout the world. The origin for the order in which the cashew family occurs, Sapindales, dates back approximately 84 to 65 million years before present (Knobloch and Mai, 1986; Magallón and Sanderson, 2001; Wikström et al., 2001). Anacardiaceae is most likely of Gondwanan origin (Gentry, 1982). This is supported not only by the age of the family that is indicated by the fossil record, but also by its worldwide distribution.

### Economic Botany

As mentioned previously, the major agricultural products enjoyed from the Anacardiaceae are cashews, mangos, pink peppercorns, and pistachios; however, numerous others have high regional value. Edible fruits and/or seeds are cultivated or simply gathered from: *Anacardium giganteum* W. Hancock ex Engl. (cajú- hypocarp), *Antrocaryon* Pierre (tapereba açu, almeixa, antelopes' buttons – fruit and seed), *Bouea* Meissn. (gandaria - fruit), *Buchanania* Spreng. (Cuddapah-almond - fruit and seed), *Choerospondias* B. L. Burt & A. W. Hill

(fruit), *Cyrtocarpa* H. B. & K. (jobillo - fruit), *Fegimanra* Pierre (seed), *Haematostaphis* Hook. f. (blood plum - fruit, seed), *Harpephyllum* Bernh. ex Krauss (kaffir plum - fruit), *Lannea* A. Rich. (Jia, Gumpin - fruit), *Ozoroa* Delile (raison tree - fruit), *Pleiogynium* Engl. (fruit), *Pseudospondias* Engl. (fruit and seed), *Rhus* subgenus *Rhus* (sumac –fruit), *Rhus* subgenus *Lobadium* (skunk brush, aromatic sumac, lemonade berry - fruit), *Schinus* L. (California pepper tree, aroeira- fruit), *Sclerocarya birrea* (marula - fruit), *Searsia* F. A. Barkley (currant bushes, karee, taebos - fruit), *Semecarpus* L. f. (renghas, dhobi nut, marking nut - hypocarp), *Sorindeia* Thou. (osee-efu - fruit), *Spondias* (jobo, hog plum, ciruela, umbu, ubos, caja, tapereba, Spanish plum, jocote, amra, plum tree, etc - fruit), *Tapirira* (paopombo - fruit), and *Trichoscypha* Hook. f. (grape of Gabon – fruit and seed) (J. D. Mitchell, pers. com.).

No Anacardiaceae ranks as a major, internationally important timber tree but many have an important role in smaller timber markets and are valued for their quality wood and rot resistance. One of the most prized rot-resistant anacardiaceous timber comes from species of the South American genus, *Schinopsis*, which has been used extensively in Argentina for railroad ties (J. D. Mitchell, pers. com.). Other timber genera include: *Abrahamia* ined., *Anacardium* L., *Astronium* (gateado, gonçalo alves, aroeira, muiracoatiara), *Camposperma* Thwaites, *Dracontomelon dao* (Blco.) Merr & Rolfe, *Lannea*, *Loxopterygium* (slangenhout, picatón), *Myracrodruon*, *Ozoroa*, *Protorhus* Engl. (red beech), *Schinopsis* (quebracho, braúna), *Sclerocarya* Hochst., *Trichoscypha* (J. D.

Mitchell, pers. com.; Randrianasolo, 1998). The use of the wood varies widely and includes being used in making: matchsticks, cabinetry, bows, charcoal, housing, axe-handles, firewood, bowls, planks, and poles.

Many Anacardiaceae species are also valued for their horticultural appeal. Specimens of *Cotinus*, *Rhus* L., *Schinus*, *Searsia*, *Pistacia chinensis* Bunge, *Pistacia mexicana* Kunth, *Harpephyllum caffrum* Bernh. ex K. Krause, *Lansea coromandelica* (Houtt.) Merr., *Rhodosphaera* Engl., *Smodingium*, and *Toxicodendron* L. are planted for their beautiful inflorescences, infructescences, evergreen foliage, and/or fall foliage. A few agricultural and horticultural species have escaped cultivation and become invasive in their non-native areas.

*Toxicodendron succedaneum* Kuntze, Japanese wax tree, is an Asian species that was originally cultivated in Brazil but escaped after introduction and is now invasive. *Schinus terebinthifolia* (Brazilian pepper tree, pink peppercorns) is another notorious, problematic species in the Everglades of central and southern Florida, the Hawaiian Islands, and various other parts of the subtropics and tropics (Gilman, 1999; Mitchell in Smith et al., 2004). More recently *Pistacia chinensis* has become naturalized and invasive in Texas (J. D. Mitchell, pers. com.).

In addition to its valuable, edible fruits and horticultural importance, some species of Anacardiaceae have long been used for their medicinal properties. *Spondias* and *Rhus* are used extensively by native populations for everything from healing broken bones to treating colds. Other useful genera include

*Anacardium* (bark: astringent, toothache, sore gums, dysentery; leaves: dysentery, diarrhea), *Antrocaryon* (fruit: stomach ache; bark: liver ailments), *Buchanania* Spreng. (fever, skin disease, snake and insect bites, venereal disease, antibiotic), *Haematostaphis* (bark: sleeping sickness), *Heeria* Meissn. (roots: gastro-intestinal illness), *Lannea* (roots: venereal diseases; bark: mouth ulcers, stomach problems; leaves: wounds, laxative; seeds: purgative), *Mangifera* (bark: astringent; sap: anti-syphilitic), *Ozoroa* (roots: intestinal pain, dysentery, migraine, stomach pain, diarrhea, backache, malaria, aphrodisiac; leaves: purgative, vermifuge, lactation promotion, skin diseases), *Pseudospondias* (bark and resin: purgative, diuretic), *Schinus* (bark: purgative, wounds), *Sclerocarya* (bark: wounds; leaves: mouth sores), *Searsia* (leaves and roots: laxative, anti-abortive, gonorrhoea, influenza, wounds), *Sorindeia* (leaves: mouth sores, ulcers, laxative, diuretic), *Spondias* (fruit: diuretic; roots: skin lotion; bark: purgative, leprosy, cough, wounds; leaves: leprosy, parasites, stomach aches), and *Trichoscypha* (resin: miscarriage preventative; bark: wash for smallpox pustules, constipation in infants, hemorrhage associated with pregnancy), (Mitchell *in* Smith et al., 2004).

Approximately 32 of the 82 Anacardiaceae genera contain species known to cause contact dermatitis: *Anacardium*, *Astronium*, *Blepharocarya*, *Bonetiella* Rzed., *Camposperma*, *Cardenasiodendron*, *Comocladia* L., *Cotinus*, *Fegimanra*, *Gluta* L., *Hermogenodendron*, *Holigarna* Buch.-Ham. Ex Roxb., *Lithrea* Hook., *Loxopterygium*, *Loxostylis*, *Mangifera*, *Mauria* Kunth, *Melanochyla*

Hook. f., *Metopium* P.Br., *Myracrodruon*, *Parishia*, *Pentaspadon* Hook.f., *Pseudosmodingium*, *Schinopsis*, *Schinus*, *Semecarpus*, *Smodingium*, *Sorindeia*, *Spondias*, *Swintonia* Griff., *Toxicodendron*, and *Trichoscypha*. Many of these taxa contain variously structured oleoresins that may cause an immune system reaction upon binding with skin proteins (Mitchell, 1990). Humans and other animals allergic to these compounds can have anywhere from a very mild to a deadly reaction depending upon the location of contact, species encountered, and severity of their allergy. The chemistry of the offending compounds has been researched for many taxa (e.g. Backer and Haack, 1938; Hill et al., 1934; Loev, 1952; Tyman and Morris, 1967; Johnson et al., 1972; Gross et al., 1975; Halim et al., 1980; Stahl et al., 1983; Gambaro et al., 1986; Mitchell, 1990; Rivero-Cruz et al., 1997; Drewes et al., 1998), but the cause of the toxicity in others is unknown.

Several of the contact dermatitis causing taxa, infamous for the rash they may cause, may also be used for their tannins and in the lacquerware industry. *Toxicodendron* and *Gluta* resins are used in China, Japan, Thailand, and Vietnam to create decorative, long-lasting wooden art pieces such as trays, jewelry boxes, vases, picture frames, and furniture. Resin collected from the trees is refined and applied to fine wood, increasing the woods' chemical, heat, and humidity resistance. Unfortunately, the oleoresins' activity is not completely suppressed upon drying and lacquerware can continue to cause much discomfort in unsuspecting admirers for years (Kullavanijaya and Ophaswongse, 1997;

Prendergast et al., 2001). One such example was diagnosed by Howard. While he was serving as a US Army consultant, he investigated the painful skin rash experienced by soldiers after sitting on lacquered latrine seats during the Vietnam War (Rodriguez et al., 2003).

## Biochemistry

Toxic compounds and other biochemicals within members of Anacardiaceae have been widely investigated (see review in Aguilar-Ortigoza et al., 2003). Most of these studies have been done at the species or generic level: *Amphipterygium* (Petersen and Fairbrothers, 1983; Wannan and Quinn, 1988; Mata et al., 1991), *Anacardium* (Tyman and Morris, 1967; Pinto 1995; Lima et al., 2002), *Astronium* (Chen and Wiemer, 1984; Alencar et al., 1996), *Blepharocarya* (Wannan et al., 1985), *Buchanania* (Arya et al., 1992), *Gluta* (Du and Oshima, 1985), *Holigarna* (Nair et al., 1952a), *Lannea* (Venkaiah, 1986), *Lithrea* (Gambaro et al., 1986), *Loxostylis* (Drewes et al., 1998), *Metopium* (Rivero-Cruz et al., 1997), *Mangifera* (El-Khalafy and Aly, 1971; El-Khalafy et al., 1971a, 1971b; Cojocarú et al., 1986), *Myracrodruon* (Viana et al., 1997), *Orthopterygium* (Wannan and Quinn, 1988), *Pistacia* L. (Yalpani and Tymann, 1983; Parra et al., 1993), *Rhus* (Chen et al., 1974; Corbett and Billets, 1975; Young, 1976; Aguilar and Zolla, 1982; Bestman et al., 1988; Kurucu et al., 1993; Saxena et al., 1994), *Schinus* (Stahl et al., 1983; Rossini et al., 1996), *Sclerocarya* (Galvez et al., 1991, 1992, 1993), *Semecarpus* (Backer and Haack, 1938; Nair et al., 1952b;

Gedam et al., 1974; Rao et al., 1973; Carpenter et al., 1980; Smit et al., 1995; Oelrichs et al., 1997), *Smodingium* (Eggers, 1974; Findlay et al., 1974; Drewes et al., 1998), *Spondias* (Singh and Saxena, 1976; Tandon and Rastogi, 1976; Corthout et al., 1989; Corthout et al., 1991; Allegrone and Barbeni, 1992; Corthout et al., 1992; Sagrero-Nieves, 1992; Coates et al., 1994; Corthout et al., 1994; Lemos, 1995; Pinto, 1995), *Tapirira* (David et al., 1998), and *Toxicodendron* (Adawadkar and El Sohly, 1983; Du et al., 1984a, 1984b).

Several of these studies investigated the medicinal activity of various extracted compounds such as phenolics (Corthout et al., 1994), esters (Corthout et al., 1992, Galvez, 1992), and tannins (Corthout et al., 1991; Galvez et al., 1994; Viana et al., 1997). Others studied the toxic components such as contact-dermatitis causing compounds (e.g. Hill et al., 1934; Backer and Haack, 1938; Loev, 1952; Johnson et al., 1972; Gross et al., 1975; Halim et al., 1980; Stahl et al., 1983; Gambaro et al., 1986; Mitchell, 1990, Rivero-Cruz et al., 1997; Drewes, 1998) and those responsible for causing nut allergies (Jansen et al., 1992; Fernandez et al., 1995). Drewes et al. (1998) found a great deal of similarity in the structure of the toxic phenolic lipids in *Loxopterygium* and *Smodingium* and thus suggested that the two genera may be more closely related to each other than previously considered. Some of the compounds in Anacardiaceae members have been shown to be defensive in function. These include, among others, antimicrobials (Saxena et al., 1994) and antifungal and/or insect herbivore repelling compounds (Chen and Wiemer, 1984; Cojocararu et al., 1986).

Surveys of the biochemical components of plants can be used not only for medicinal and agricultural use, but for taxonomic purposes as well (McNair, 1929; Reznik and Egger, 1960; Alston and Turner, 1963; etc.). The presence or absence of different chemical compounds is treated much the same way as morphological or anatomical characters in a data matrix. Taxonomic treatments for Anacardiaceae have not been generated with these characters alone, but many have included biochemical traits along with others being considered in the analysis (e.g. Young, 1976; Wannan, 1986; Terrazas, 1994; Aguilar-Ortigoza, 2003;). These and other classifications for the cashew family are discussed in greater detail in the next section.

### Taxonomic History

The family Anacardiaceae was first proposed by Lindley in 1830 but its members have been variously placed in other families including the Blepharocaryaceae Airy Shaw, Cassuviaceae Juss. ex R. Br., Comocladiaceae Martinov, Julianiaceae Hemsl., Lentiscaceae Horaninow, Pistaciaceae Adans., Podoaceae Baill. ex Franch., Rhoaceae Spreng. ex Sadler, Schinaceae Raf., Spondiadaceae Martinov, Sumachiaceae (DC.) Perleb, Terebinthaceae Durande, and Vernicaceae Link (see Table 1.1). Three of these families, Podoaceae, Blepharocaryaceae, and Julianiaceae, are still considered by some taxonomists to be distinct but closely related.

Table 1.1. Family synonymy for Anacardiaceae Lindl., *nom. cons.* (1830) as delimited in this study.

| Family Name                  | Date Proposed | Reason for Synonymy               |
|------------------------------|---------------|-----------------------------------|
| Blepharocaryaceae Airy Shaw  | 1964          | Lumped into Anacardiaceae         |
| Cassuviaceae Juss. ex R. Br. | 1818          | <i>nom. illeg.</i>                |
| Comocladaceae Martinov       | 1820          | Lumped into Anacardiaceae         |
| Julianiaceae Hemsl.          | 1906          | Lumped into Anacardiaceae         |
| Lentiscaceae Horaninow       | 1843          | <i>nom. illeg.</i> = Pistaciaceae |
| Pistaciaceae Adans.          | 1763          | Lumped into Anacardiaceae         |
| Podoaceae Baill. ex Franch.  | 1889          | Lumped into Anacardiaceae         |
| Rhoaceae Spreng. ex Sadler   | 1826          | Lumped into Anacardiaceae         |
| Schinaceae Raf.              | 1837          | Lumped into Anacardiaceae         |
| Spondiadaeae Martinov        | 1820          | Lumped into Anacardiaceae         |
| Sumachiaceae (DC.) Perleb    | 1838          | <i>nom. illeg.</i>                |
| Terebinthaceae Durande       | 1782          | <i>nom. illeg.</i>                |
| Vernicaceae Link             | 1831          | Lumped into Anacardiaceae         |

Podoaceae, including *Dobinea* and *Campylopetalum*, has been separated from the cashew family because the pistillate flowers lack a perianth and are adnate, by their pedicels, to a bract (Takhtajan, 1969, 1980, 1997; Hutchinson, 1973; Willis, 1973; Dahlgren, 1980; Watson and Dallwitz, 1992). Numerous authors recognize a separate Julianiaceae that includes two genera, *Amphipterygium* and *Orthopterygium*, based on the absence of a perianth in the pistillate flowers and those flowers being enclosed in an involucre (Bessey, 1915; Hutchinson, 1926; Wettstein 1935, 1944; Copeland and Doyel, 1940; Gundersen, 1950; Standley and Steyermark, 1949; Barkley, 1957; Melchior, 1964; Stone, 1973; Cronquist, 1988; Watson and Dallwitz, 1992). The monogeneric

Blepharocaryaceae was proposed by Airy Shaw (1965) on the basis of its two species having opposite pinnate leaves and a cupule-like mature pistillate inflorescence. While many authors recognize the affinity of *Blepharocarya* and Anacardiaceae, placement of the genus within the infrafamilial classification of the family has been problematic due to its aberrant morphology (Engler, 1892; Wannan et al., 1987; Wannan and Quinn 1990, 1991).

Table 1.2. Proposed infrafamilial classifications of the Anacardiaceae.

|                             |   |  |              |               |              |
|-----------------------------|---|--|--------------|---------------|--------------|
| Bentham and Hooker 1862     | Anacardieae   | Spondieae  |              |               |              |
| Marchand 1869               | Astronieae, Buchananieae, Mangifereae, Pistacieae, Rhoideae, Semecarpeae, Spondieae, Tapirieae, Thyrsodieae                               |  |              |               |              |
| Engler 1876                 | Astronieae, Buchananieae, Garugeae, Loxopterygieae, Mangifereae, Rhoideae, Semecarpeae, Solenocarpeae, Spondieae, Swintonieae, Tapirireae |  |              |               |              |
| Eichler 1875-78             | 5 groups based on <i>Anacardium</i> , <i>Pistacia</i> , <i>Rhus</i> , <i>Schinus</i> , and <i>Spondias</i>                                |  |              |               |              |
| Engler 1883                 | Mangifereae   | Spondieae  | Semecarpeae  | Rhoideae      |              |
| Engler 1892                 | Mangifereae   | Spondieae  | Semecarpeae  | Rhoideae      | Dobineae     |
| Takhtajan 1987              | Anacardioideae  | Spondioideae<br>Spondieae<br>Rhoideae              | Julanioideae | Pistacioideae | Dobineoideae |
| Takhtajan <sup>1</sup> 1997 | Anacardioideae  | Spondioideae<br>Spondieae<br>Rhoeae                | Julanioideae | Pistacioideae |              |
| Mitchell and Mori 1987      | Anacardieae   | Spondiadeae  | Semecarpeae  | Rhoeae        | Dobineae     |
| Wannan & Quinn 1991         | "Group A" <sup>2</sup><br>"Group A1"<br>"Group A2"  | "Group B" <sup>3</sup><br>"Group B1"<br>"Group B2" |              |               |              |

<sup>1</sup> Recognized a separate Podoaceae

<sup>2</sup> Contains Mitchell and Mori's tribes Semecarpeae and Dobineae, and the members of tribes Rhoeae and Anacardieae not included in Group B

<sup>3</sup> Contains Mitchell and Mori's tribe Spondiadeae and two members each of tribes Rhoeae and Anacardieae

With their 1862 treatment of Anacardiaceae, Bentham and Hooker became the first to formally recognize infrafamilial affinities of the genera within the Anacardiaceae (see Table 1.2). They proposed tribes Anacardieae and Spondieae, distinguished by the presence of a single locule per ovary versus two to five locules, respectively. Marchand (1869) later split the family into nine tribes based on the degree of carpel fusion, ovule insertion on the placenta, the number of locules in the ovary, the number of staminal whorls, and perianth growth after anthesis. Engler's (1876) treatment of the Anacardiaceae for *Flora Brasiliensis* expanded Marchand's tribal system to 11 but in his later treatments reduced that number to four then ultimately five tribes (Engler, 1881, 1883, 1892).

Engler's tribes Dobineae, Mangifereae, Rhoideae, Semecarpeae, and Spondieae, comprise the most widely used classification of Anacardiaceae. Engler circumscribed his tribes using one vegetative and several floral and fruit characters including number of carpels, insertion of the ovule on the placenta, number of staminal whorls, leaf complexity, number of locules in the ovary and fruit, embryo morphology, and stylar insertion on the ovary. Although Engler's three main treatments (1881, 1883, 1892) remain the most detailed and thorough revision of the Anacardiaceae, his tribal descriptions are problematic. Engler used different sets of characters to define each of his tribes, resulting in an overlap in the tribal boundaries. For example, Engler defined his tribe Dobineae by its pistillate flowers lacking a perianth and having a unilocular ovary and defines tribe Anacardieae chiefly by all members having simple leaves, a

character also found in Dobineae. Since this system was established, the addition of numerous genera and species via new discovery and taxonomic revision has further blurred the tribal limits.

Table 1.3. Generic affinities of the infrafamilial classification used in this study (modification of Mitchell and Mori, 1987).

| Tribe       | Affiliated Genera   |
|-------------|---|
| Anacardieae | <i>Anacardium, Androtium, Bouea, Buchanania, Fegimanra, Gluta</i> (including <i>Melanorrhoea</i> ), <i>Mangifera, Swintonia</i>   |
| Spondiadeae | <i>Allospondias, Antrocaryon, Choerospondias, Cyrtocarpa, Dracontomelon, Haematostaphis, Haplospondias, Harpephyllum, Koordersiodendron, Lannea, Operculicarya, Pegia, Pleiogynium, Poupartia, Poupartiopsis</i> ined., <i>Pseudospondias, Sclerocarya, Solenocarpus, Spondias, Tapirira</i>  |
| Semecarpeae | <i>Drimycarpus, Holigarna, Melanochyla, Nothopegia, Semecarpus</i>  |
| Rhoeae      | <i>Abrahamia</i> ined., <i>Actinocheita, Amphipterygium, Apterokarpus, Astronium, Baronia, Blepharocarya, Bonetiella, Camnosperma, Cardenasiodendron, Comocladia, Cotinus, Euroschinus, Faguetia, Haplorhus, Heeria, Hermogenodendron</i> ined., <i>Laurophyllum, Lithrea, Loxopterygium, Loxostylis, Malosma, Mauria, Melanococca, Metopium, Micronychia, Mosquitoxylum, Myracrodruon, Ochoterena, Orthopterygium, Ozoroa, Pachycormus, Parishia, Pentaspadon, Pistacia, Protorhus, Pseudosmodingium, Rhodosphaera, Rhus, Schinopsis, Schinus, Searsia, Smodingium, Sorindeia, Thyrsodium, Toxicodendron, Trichoscypha</i> |
| Dobineae    | <i>Campylopetalum, Dobinea</i>  |

Despite its circumscriptional problems, Engler's classification has been adopted, often with some modification, by many authors such as Melchior (1964), Ding Hou (1978), and Mitchell and Mori (1987). The tribal names and indicated

generic affinities listed in Table 1.3 were revised from those of Mitchell and Mori (1987) who updated Ding Hou's (1978) modification of Engler's classification. These names and tribal affinities reflect recent discoveries and taxonomic revision and are used in this study when referring to present tribal circumscription (Table 1.3).

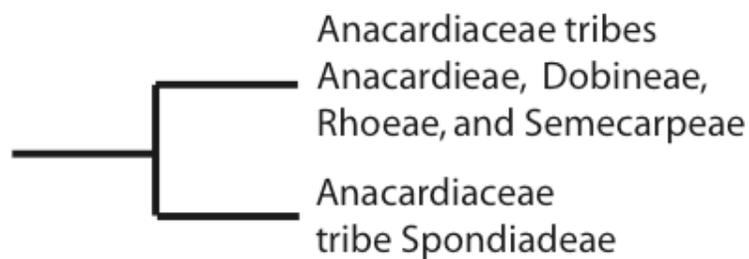


Figure 1.2. Schematic of the hypothesized monophyletic Anacardiaceae as described by Wannan and Quinn (1991).

Wannan and Quinn (1990, 1991) sought a more phylogenetically accurate classification of the Anacardiaceae through floral and pericarp investigation. They preliminarily grouped genera within the family based upon these characters as well as wood anatomy and biflavonoid data. Their investigation identified two tentative groups, A and B, which were each divided into two subgroups, 1 and 2. Wannan and Quinn considered Group A monophyletic while Group B is more or less an artificial assemblage of the remaining genera, not easily placed in a phylogenetic context using morphological and anatomical features. Tribes Anacardieae, Dobineae, Rhoeeae, and Semecarpeae with the exception of *Androtium* Stapf., *Buchanania*, *Camptosperma*, and *Pentaspadon*, were found in

Group A while Group B contains all of Spondiadeae plus the four genera named above (two genera each from Anacardiaceae and Rhoaceae respectively, Fig. 1.2). Wannan and Quinn (1991) designated two genera, *Faguetia* and *Pseudoprotorus* H. Perrier (= Sapindaceae, *Filicium* Thwaites), as unassignable to a group. Several other genera are included in the current study but were not considered by Wannan and Quinn (1991) because these genera have been newly discovered or split from other genera since the time of their publication.

In the first molecular investigation of Anacardiaceae, Terrazas (1994) used sequences of the chloroplast gene *rbcL*, morphology, and wood anatomy data to interpret phylogeny of the family. Her *rbcL* phylogeny found the Anacardiaceae to be paraphyletic, with Burseraceae nested within the cashew family, sister to tribe Spondiadeae (Fig. 1.3). The combined *rbcL*-morphology phylogeny elucidated a monophyletic Anacardiaceae with a decay index greater than five. This phylogeny indicated that the cashew family is comprised of two groups similar to those delimited by Bentham and Hooker (1862) and Wannan and Quinn (1991). Terrazas' Clade A2 contained Spondiadeae plus *Pentaspadon* united by the morphological synapomorphy, multicellular stalked glands on the leaves. Clade A1 contained the remaining genera in the four other tribes and is supported by the morphological and wood anatomical synapomorphies, unicellular stalked leaf glands and the presence of both septate and nonseptate fibers. Her combined data added further support for a revision of the family's infrafamilial classification and showed the traditional five-tribal and

five-subfamilial systems to be artificial. Based on the combined phylogeny, Terrazas proposed that the family be split into two subfamilies, Anacardioideae and Spondioideae.

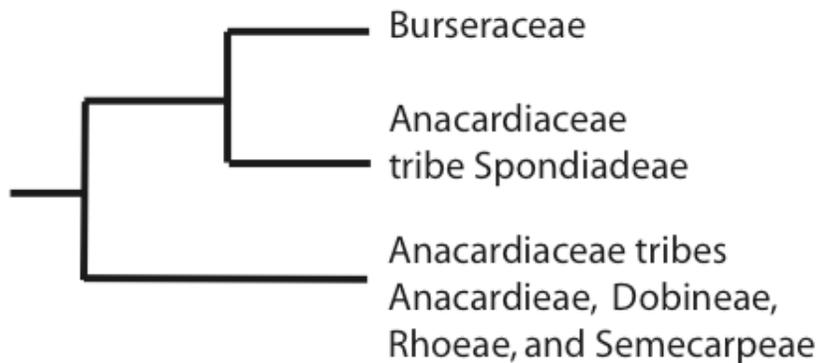


Figure 1.3. Schematic of the Burseraceae-Anacardiaceae subtree from the maximum parsimony *rbcl* phylogeny of selected members of the Sapindales (Terrazas 1994).

Terrazas (1994) also included members of several other closely related families and thus helped to elucidate the position of the cashew family in a larger context. Her combined data indicated that Burseraceae is the sister family to Anacardiaceae, with the supporting synapomorphies of radial canals in the secondary xylem, vertical intercellular secretory canals in the primary and secondary phloem, and the ability to synthesize biflavonyls (Wannan et al., 1985; Wannan, 1986; Wannan and Quinn, 1990, 1991; Terrazas, 1994). This relationship has been previously suggested based on morphological, anatomical, and biochemical data (Gunderson, 1950; Cronquist, 1981; Wannan, 1986;

Takhtajan, 1987; Thorne, 1992) and further supported by DNA sequence data (Gadek et al., 1996; APG, 1998, 2003; Savolainen et al., 2000a, 2000b).

However, the molecular data of both Terrazas (1994) and Gadek et al. (1996) indicated that the relationship of Anacardiaceae and Burseraceae is extremely close. As mentioned above, Terrazas' *rbcL* dataset of 18 Anacardiaceae and three Burseraceae species nested the two families together, only separated based on the inclusion of morphological and anatomical data. Gadek et al.'s *rbcL* dataset of seven Anacardiaceae and three Burseraceae indicated that the two families are monophyletic but their separation is weakly supported by a decay index of only one. Anacardiaceae is distinguished from the Burseraceae anatomically by having a single apotropous ovule per locule and vertical resin ducts in the phloem versus two epitropous ovules per locule and the absence of vertical resin ducts in the Burseraceae (see Fig. 1.1 for an illustration of these ovule positions).

Evaluating the cashew and gumbo limbo families from a morphological perspective is quite revealing of their evolutionary past. While consisting of approximately the same number of species, Anacardiaceae is taxonomically divided into 82 genera while Burseraceae is divided into only 20. Although the historical ecological and evolutionary forces driving this diversity are not yet understood, the disparity in morphological and distributional diversity is quite evident. Anacardiaceae has more fruit diversity than Burseraceae and is present in many more habitats. Burseraceae fruits are dehiscent or indehiscent drupes

while Anacardiaceae fruits are drupes (which may be wind dispersed by various mechanisms or subtended by an enlarged, fleshy hypocarp), syncarps, elm-like samaras, single-winged samaras, dry utricles, or achene-like. It is this diversity in fruit morphology that is primarily responsible for the recognition of so many genera within the cashew family. In many instances fruit characteristics have been interpreted as autapomorphies that have been used to distinguish genera, resulting in an astonishing one third of the recognized genera being monotypic (27 of the 82 genera) (Table 1.4).

Table 1.4. Tribal affinities of the 27 monotypic genera in Anacardiaceae.

| Tribe       | Genera  |
|-------------|---|
| Anacardieae | <i>Androtium</i>  |
| Spondiadeae | <i>Choerospondias, Haematostaphis, Haplospodias, Harpephyllum, Koordersiodendron, Poupartiopsis</i> ined.   |
| Semecarpeae |   |
| Rhoeae      | <i>Actinocheita, Apterokarpos, Bonetiella, Cardenasiodendron, Faguetia, Haplorhus, Heeria, Hermogenodendron</i> ined., <i>Laurophyllum, Loxostylis, Malosma, Melanococca, Mosquitoxylum, Ochoterena, Orthopterygium, Pachycormus, Protorhus, Rhodosphaera, Smodingium</i> |
| Dobineae    | <i>Campylopetalum</i>   |

Historically Anacardiaceae has been placed in the Burserales, Rutales, Sapindales, or Terebinthinae (see Table 1.5). Most modern authors consider it a member of the Sapindales and recent molecular studies at the ordinal level (Gadek et al., 1996) and above (Chase et al., 1993; APG, 1998, 2003; Bremer et al., 1999; Savolainen et al., 2000a, 2000b) have supported this classification.

Table 1.5. Proposed ordinal affinities of Anacardiaceae based on morphological or molecular data.

| Order         | Publications  |   |
|---------------|---|---|
|               | Morphological   | Molecular   |
| Burserales    | Takhtajan 1997  |   |
| Rutales       | Gundersen 1950, Thorne 1992 <sup>1</sup>  |   |
| Sapindales    | Engler 1892, Rendle 1925 <sup>3</sup> , Hutchinson 1926 <sup>3</sup> & 1973, Takhtajan 1954, Dahlgren 1980, Cronquist 1968 <sup>2</sup> , 1981, 1988 <sup>2</sup> , Bhattacharyya & Johri 1998 <sup>3</sup> | Chase et al. 1993 <sup>2</sup> , Gadek et al. 1996 <sup>2</sup> , APG 1998 <sup>2</sup> & 2003 <sup>2</sup> , Bremer et al. 1999 <sup>2</sup> , Savolainen et al. 2000a <sup>2</sup> & 2000b <sup>2</sup> |
| Terebinthinae | Eichler 1875-78 <sup>1,2</sup> , Hallier 1908 <sup>2</sup> (Térébinthines), Wettstein 1935 <sup>1,2,3</sup> , 1944 <sup>1,2,3</sup> , (Terebinthales)   |   |

<sup>1</sup>Sapindales s.s. placed in the same order

<sup>2</sup>Rutales s.s. placed in the same order

<sup>3</sup>Recognized a separate Julianiales or Julianiaceae in Juglandales

## Objectives

The long taxonomic history of the Anacardiaceae illustrates both the confusion of delimiting the family and the problem of organizing the genera into a subfamilial classification. A review of the recent preliminary Anacardiaceae molecular studies of Terrazas (1994) and Gadek et al. (1996) and the morphological and anatomical investigations of Wannan et al. (1986, 1990, 1991) further illuminates the need for a more thorough molecular investigation of Anacardiaceae. The main purpose of this study was to elucidate the phylogeny of the Anacardiaceae using a much more robust sampling of DNA sequence data

from the plastid genes *matK*, *rps16*, the *trnL* intron and 3' exon, and the intergenic spacer between *trnL* and *trnF* (*trnL-trnF*). The resulting cladograms along with previous morphological and anatomical studies were used to (1) reevaluate the subfamilial classification of the family and (2) place its genera in an evolutionary framework. (3) Determining the relationship of the Anacardiaceae and Burseraceae was an essential component of this study. Toward that goal, taxa from other closely related families in the Sapindales were included in this investigation for proper rooting purposes. In addition (4) an in-depth phylogenetic approach was utilized to evaluate *Protorhus* from southern Africa and Madagascar using the plastid *trnLF* region and two nuclear ribosomal regions, ETS and ITS.

## Chapter 2: Phylogenetic Position of Anacardiaceae, Burseraceae and Other Allied Families.

### Introduction

The Sapindales are mostly woody plants with a prominent nectariferous disc and a syncarpous gynoecium usually with one or two ovules per locule (Gadek et al., 1996). The cashew family, Anacardiaceae, and the gumbo limbo family, Burseraceae, are two Sapindalian families that have been closely allied taxonomically and phylogenetically. These two families are united by having radial canals in the secondary xylem, vertical intercellular secretory canals in the primary and secondary phloem, the ability to synthesize biflavonyls, and actinomorphic flowers with an intrastaminal nectariferous disc (Wannan et al., 1985; Wannan, 1986; Wannan and Quinn, 1990, 1991; Terrazas, 1994). Anacardiaceae may be distinguished from Burseraceae by its apotropous ovule, the synthesis of 5-deoxyflavonoids, and only one ovule per locule. In contrast, Burseraceae has an epitropous ovule and two ovules per locule. Sapindaceae is characterized by an extrastaminal nectariferous disc, often zygomorphic flowers, and one, generally apotropous, ovule per locule.

The position of Anacardiaceae and Burseraceae as distinct lineages in an evolutionary context has been placed in question by recent molecular studies. The *rbcL*-based study of Terrazas (1994) found the two families to be paraphyletic. Gadek et al. (1996), in another *rbcL* investigation, sampled broadly within Sapindales and showed the Anacardiaceae and Burseraceae each to be

monophyletic sister taxa supported by a decay index of only one. In trees one step longer, the two families collapsed into a clade supported by a decay index of four. These findings suggested that treating the two families as distinct may be somewhat tenuous.

The positions of three other segregate families, Blepharocaryaceae Airy Shaw, Julianiaceae Hemsl., and Podoaceae Baill. ex Franch., are frequently in question with some authors still treating a fourth, Pistaciaceae Adans., as a separate family. Blepharocaryaceae, a monogeneric Australian family, was segregated by Airy Shaw (1965) from Anacardiaceae by its cupule-like pistillate inflorescence and opposite leaves. Early in its development the pistillate inflorescence is rather open like that of the staminate inflorescence. After initial formation, inflorescence branches and adherent bracts grow around the developing flower, eventually forming the cupule around the fruit (Wannan et al., 1987). Many authors recognize the affinity of this family with the Anacardiaceae, and it appears in the Rhoeeae clade of clade A1 in Terrazas' paraphyletic Anacardiaceae (1994) (*rbcL* phylogeny).

The family Julianiaceae, including the two genera *Amphipterygium* Schiede ex Standl. and *Orthopterygium* Hemsl., has a long, controversial taxonomic history. Its unique fruit and pistillate floral characteristics distinguish the family. Pistillate flowers of these dioecious taxa lack a perianth and are contained within a globose involucre. Stern's (1952) observation that only one of the four to five flowers per inflorescence fully develops and forms a samaroid fruit

is reflective of the uncertainty concerning the fruit morphology of this family. Close inspection of herbarium specimens of *Amphipterygium* and *Orthopterygium* and the illustrations of *Amphipterygium* (= *Juliania*) in Hemsley (1901) indicates that the wind-dispersed fruits of the genus are actually multiples. Cronquist (1981) describes them as dry syncarps: they are wind-dispersed by their elongated and flattened peduncle.

The close affinity of *Amphipterygium* and *Orthopterygium* has been well accepted due to these unusual fruit and floral characteristics. Interpretation of these same features in their classification among flowering plants has been more problematic. Together they comprise family Julianiaceae, which has been placed in the orders Burserales (Takhtajan, 1997), Juglandales (Hutchinson, 1926; Wettstein, et al. 1935, 1944), Julianiales (Melchior, 1964), Rutales (Takhtajan, 1969), and Sapindales (Bessey, 1915; Copeland and Doyel, 1940; Gundersen, 1950; Cronquist, 1968, 1981, 1988; Stone, 1973). Alternatively, they have been treated as sister genera within Anacardiaceae (Hemsley, 1908; Takhtajan, 1954; Thorne, 1973, 1992; Young, 1976; Dahlgren, 1980; Wannan, 1986; Mitchell and Mori, 1987; Wannan and Quinn, 1991;), Burseraceae (Walpers, 1845), or Terebinthaceae (Hallier, 1908).

Podoaceae also contains two genera, *Dobinea* Buch.-Ham. ex D. Don and *Campylopetalum* Forman, and, like Julianiaceae, differs from other members of Anacardiaceae in the morphology of its pistillate flowers. The pistillate flowers lack a perianth and are individually adnate, by their pedicels, to a bract

(Hutchinson, 1969, 1973; Takhtajan, 1969, 1980, 1997; Willis 1973; Dahlgren, 1980; Watson & Dallwitz, 1992; Heng, 1994). Members of Podoaceae have a chromosome number of  $n=7$ , which is lower than any other known Anacardiaceae which are typically  $n \geq 12$ . However, cytological knowledge of the cashew family is limited. Airy Shaw (1965) also found the two genera to have pollen morphology strikingly aberrant for Anacardiaceae but noted that morphological features alone would not be enough to answer the longstanding taxonomic questions of their phylogenetic affinities. *Dobinea* and *Campylopetalum* have had a rather complex taxonomic history, with placement in three different families, Podoaceae (Takhtajan, 1997), tribe Acerineae of the Sapindaceae (Bentham and Hooker, 1862); and tribe Dobineae of the Anacardiaceae (Forman, 1954; Melchior, 1964; Cronquist, 1981; Mitchell and Mori, 1987).

*Pistacia* L. was first proposed as its own family, Pistaciaceae, in 1763 by Adanson based on several morphological characteristics. Members of the genus are dioecious, and have a reduced perianth (consisting of bract-like tepals), plumose styles with associated increased stigmatic surface area, and characteristic pollen morphology with up to eight apertures of poorly defined shape and no colpi (Erdtman, 1971; Mabberley, 1997). Many authors have recognized the affinity of *Pistacia* and tribe Rhoeeae of the Anacardiaceae based on their shared floral traits, while still acknowledging these unique characteristics by placing *Pistacia* in its own tribe or subfamilial group within the cashew family

(Marchand, 1869; Eichler, 1875-78; Takhtajan, 1987, 1997). In *Flora Brasiliensis*, Engler (1876) placed *Pistacia* into tribe Rhoideae (=Rhoeae); a placement that is mirrored by most of the currently used treatments of the family (Engler, 1883; 1892; Mitchell and Mori, 1987).

The current study was undertaken to infer the phylogenetic relationships of Anacardiaceae, Burseraceae, Julianiaceae, Pistaciaceae, and Podoaceae. Sapindaceae was also chosen for robust sampling because it was identified as the sister family to the Anacardiaceae-Burseraceae clade by the most comprehensive molecular study of the Sapindales done to date, (Gadek et al., 1994). While all of these families had previously been considered from a morphological perspective, an inclusive molecular study to elucidate their relationships had not been done. For this reason, analyses of DNA sequence data from three chloroplast loci, the *trnL* intron and 3' exon and the *trnL-trnF* intergenic spacer (referred to hereafter as *trnLF*), are presented here.

Both Gadek et al. (1996) and Terrazas (1994) used the chloroplast gene *rbcL* to investigate the relationships within the Sapindales in a molecular phylogenetic context. Neither of these phylogenies resolved a strongly supported Anacardiaceae or Burseraceae, but instead either found the families to be paraphyletic (Terrazas, 1994) or monophyletic but very weakly supported (Gadek et al., 1996). The *trnLF* is a non-coding chloroplast region that has been shown to be useful at the intrafamilial level for numerous families including, Crassulaceae and related taxa (Ham et al., 1994), Taxodiaceae and

Cupressaceae (Kusumi et al., 2000), and Monimiaceae and related taxa (Renner, 1998). Gielly and Taberlet (1994) found that *trnL*F evolves at more than three times the rate of *rbcL*. This demonstrated level of variability is the reason *trnL*F was chosen for analysis in this study.

## Materials and Methods

**Plant materials and DNA isolation** □ Sampling for this study included 45 ingroup taxa: representing 24 of the 82 genera and all five tribes in Anacardiaceae, nine species in eight genera of Burseraceae, and 12 species in 11 genera of Sapindaceae (see Appendix A for a complete list of taxa and their geographical distributions). One species of Rutaceae was sampled for use as the outgroup. These taxa were selected to represent the morphological diversity across the Anacardiaceae, Burseraceae and Sapindaceae while providing an appropriate designated outgroup representation of the Rutaceae. Fresh and silica-dried materials as well as herbarium specimens were used in this study for DNA extraction. These samples were collected by the author in the field, gathered in herbaria (F, K, LSU, MO, MOR, NY), or contributed by colleagues collecting worldwide. John D. Mitchell and affiliated collectors of The New York Botanical Garden (NY) provided many of the Anacardiaceae silica samples. The laboratory of Dr. Toby Pennington and colleagues, Royal Botanical Garden Edinburgh, also contributed generously to this study with collections from South and Central America. Silica samples of Sapindaceae were provided by Pedro

Acevedo, Department of Botany, Smithsonian Institution, and silica samples of Burseraceae were provided by Douglas Daly, The New York Botanical Garden.

Plant tissue was ground in one of three ways: by hand with a mortar and pestle, in tubes with sterile glass beads and sand placed in a tissue disruptor, or in a FastPrep lysing FP-120 bead mill using lysing matrix "A" tubes containing a ceramic bead and garnet sand (Qbiogene Inc., Carlsbad, CA). Most samples were extracted with the DNeasy Plant Mini Kit (Qiagen Inc., Valencia CA), but modified Doyle and Doyle (1987) and Struwe et al. (1998) methods were also employed. Extractions of herbarium material were done with a modification of the Qiagen protocols and included the addition of 570 mg (30 $\mu$ l) of PCR grade proteinase K (Roche, Indianapolis, IN), 6.5% (30 $\mu$ l)  $\beta$ -mercaptoethanol (BME) and incubation at 42 °C for 12-24 hours on a rocking platform (K. Wurdack designed protocol, pers. com.) (see Appendix B for a complete description of this herbarium extraction protocol).

**DNA amplification and sequencing** □ The chloroplast *trnL*F regions were amplified from extracted total genomic DNA using the polymerase chain reaction (PCR) method. The universal primers c, d, e, and f of Taberlet et al. (1991 and Table 2.1 and Fig. 2.1 below) were used to amplify *trnL*F. Thermal cycling parameters were an initial denaturation of 2 minutes at 97°C; 30 cycles of 94°C for one minute, annealing at 48°C for two minutes, and elongation at 72°C for two minutes; followed by an elongation step of 72°C for 16 minutes.

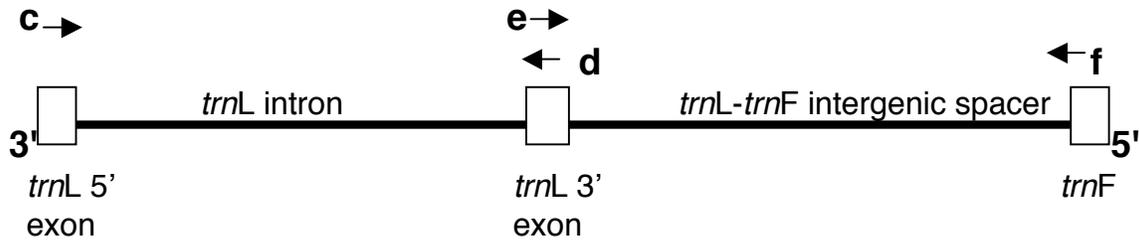


Figure 2.1. Approximate location of *trnLF* primers used in this study (from Taberlet et al. 1991). See Table 2.1 for a list of primers and their reference numbers used here.

Table 2.1. Sequences of the *trnLF* primers used in this study (from Taberlet et al. 1991).

| Name | 5'-3' Sequence       |
|------|----------------------|
| c    | CGAAATCGGTAGACGCTACG |
| d    | GGGGATAGAGGGACTTGAAC |
| e    | GGTTCAAGTCCCTCTATCCC |
| f    | ATTTGAACTGGTGACACGAG |

Successful amplifications were purified using the QIAquick PCR purification kit (Qiagen Inc., Valencia CA). Purified PCR's were quantified by estimation using a Low DNA Mass Ladder (Invitrogen, Carlsbad, CA) and then cycle sequenced using the same primers as were used for amplification (Table 2.1) and ABI Prism<sup>®</sup> BigDye<sup>®</sup> Terminator Cycle Sequencing Ready Reaction Kit version 1 (Applied Biosystems, Foster City, CA). Reactions one quarter the size of the manufacturer's recommendation were run. Cycle sequencing reactions were purified on Sephadex columns and then run on 5% Long Ranger<sup>®</sup> (BioWhittaker Molecular Applications, Rockland, Maine) polyacrylamide gels in an ABI 377XL automated sequencer (Applied Biosystems, Foster City, CA).

**Sequence analysis** □ Sequences were assembled and edited in Sequencher™ 3.1.1 (Gene Codes, Corporation, Ann Arbor, MI). Compiled sequences were initially aligned in Clustal W ver. 1.6 (European Molecular Biology Laboratory 1996, Thompson et al. 1994) and subsequently manually adjusted in MacClade 4.06 (Maddison and Maddison 2003). The dataset was analyzed using PAUP\* 4.0b10 (Swofford 2002) on an iMac G4 with the maximum-parsimony optimality criterion and maximum likelihood. Both analyses were performed on the data with the *trnLF* sequences being treated as a single dataset.

Parsimony analysis was performed using a heuristic search to generate 1000 random taxon addition replicates using equal (Fitch) weights and tree-bisection-reconnection (TBR) branch swapping, holding 10 trees at each step, MulTrees off, and saving only the shortest trees or the shortest from each replicate. The resulting trees were used as starting trees in another round of TBR with MulTrees on. Seventeen gaps (indels) were coded in three different ways to determine their affect on topology and branch support. In the phylogenies presented here, gaps were treated as missing data, poly repeats were included, and branches with a minimum length of zero were collapsed. Support for tree topology was evaluated with 1000 bootstrap replicates using PAUP\* 4.0b10 (Swofford, 2002), 10 random taxon addition replicates using equal weights and TBR branch swapping, holding one tree at each step, MulTrees off. Morphological and anatomical characters (Fig. 2.7) were coded as discrete and mapped onto the *trnLF* bootstrap consensus phylogeny in MacClade 4.06

(Maddison and Maddison 2003) using delayed transformation (DELTRAN) optimization.

For the maximum likelihood analysis, an appropriate nucleotide substitution model was identified using a hierarchical likelihood-ratio test implemented in Modeltest 3.06 PPC (Posada and Crandall 1998) for selection of the best-fit model. The TVM+G+I model, which assumes unequal base frequencies, unequal transition/transversion rates, and among-site rate heterogeneity, provided the best explanation of the data. Heuristic maximum likelihood searches were done using PAUP\* 4.0b10 (Swofford, 2002). Branch lengths were estimated using the TVM+G+I model under the parameters obtained from Modeltest: an estimated transition-transversion ratio, estimated base frequencies, a proportion of invariant sites, among-site rate heterogeneity approximated by a discrete gamma distribution with a shape parameter of 1.8714 and four rate classes.

## Results

The dataset consisted of 1206 characters of which 234 (19.4%) were parsimony informative. The matrix was easily manually aligned and included 17 indels (gaps). Coding gaps as binary characters, missing data, or as a fifth base had no affect on the topology and very little affect on branch support. Length mutations of polynucleotide repeats being included or ignored also had no affect on topology. GenBank accession numbers are shown in Appendix A. Parsimony

analysis resulted in 36 most parsimonious trees on a single island, each of 823 steps in length, a consistency index (CI) of 0.774, a consistency index excluding uninformative characters (RC) of 0.675, a homoplasy index (HI) of 0.226, and a retention index (RI) of 0.872. The 50% bootstrap consensus tree is shown in Figure 2.2. Maximum likelihood analysis generated one tree with a likelihood score of  $-\ln 6330.2518$  (Fig. 2.3). The topology is consistent with the maximum parsimony tree, but is more resolved within the Anacardiaceae and Sapindaceae clades.

Strong support is shown for the sister relationship of Burseraceae and Anacardiaceae (92% bootstrap). Additionally, each of the families has high support for being monophyletic (100% bootstrap for Burseraceae and 91% for Anacardiaceae). Within the Anacardiaceae there are two clades, one containing tribe Spondiadeae (98% bootstrap) and the second containing the other four tribes (Anacardieae, Dobineae, Rhoaeae, and Semecarpeae, 99% bootstrap) (Fig. 2.3). Three families once thought to be segregated from Anacardiaceae by some authors are nested within it in the *trnL*F phylogeny: Julianiaceae (*Amphipterygium adstringens* (Schidl.) Standley and *Orthopterygium huaucui* (A. Gray) Hemsl.), Podoaceae (*Dobinea vulgaris* Buch.-Ham. ex D. Don), and Pistaciaceae (*Pistacia chinensis* Bunge) (Fig. 2.4).

Burseraceae tribal and section affiliations as circumscribed by Daly (in Harley and Daly 1995) are highlighted in Figure 2.5. This figure shows the Burseraceae subtree from the bootstrap consensus phylogeny of *trnL*F

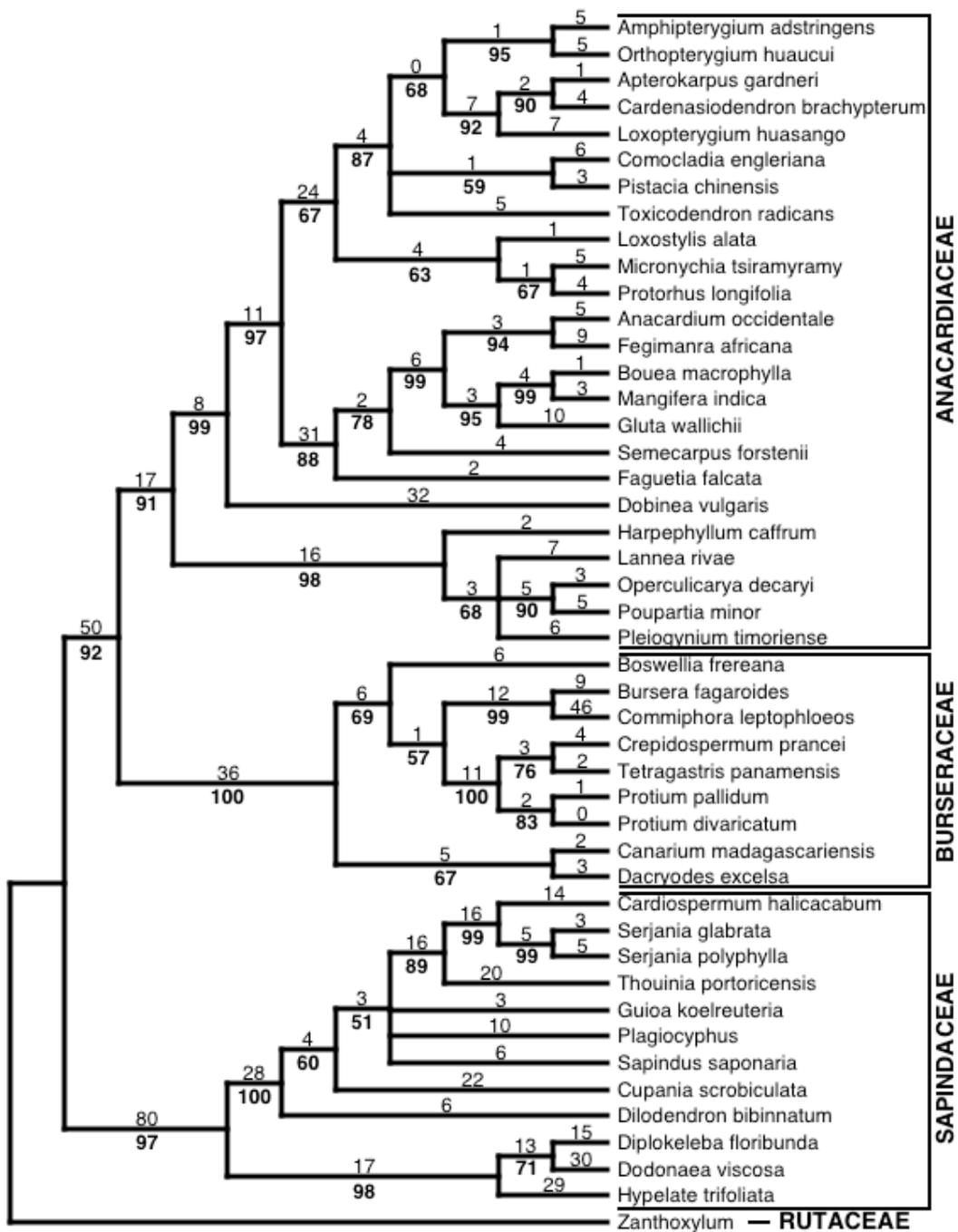


Figure 2.2. Bootstrap consensus phylogeny of *trnLF* sequences of Anacardiaceae, Burseraceae, Sapindaceae and Rutaceae (maximum parsimony). Branch lengths are shown above the branches and bootstrap support is indicated in bold below the branches where greater than 50%. CI=0.774, RC=0.675, RI=0.872, HI=0.226.

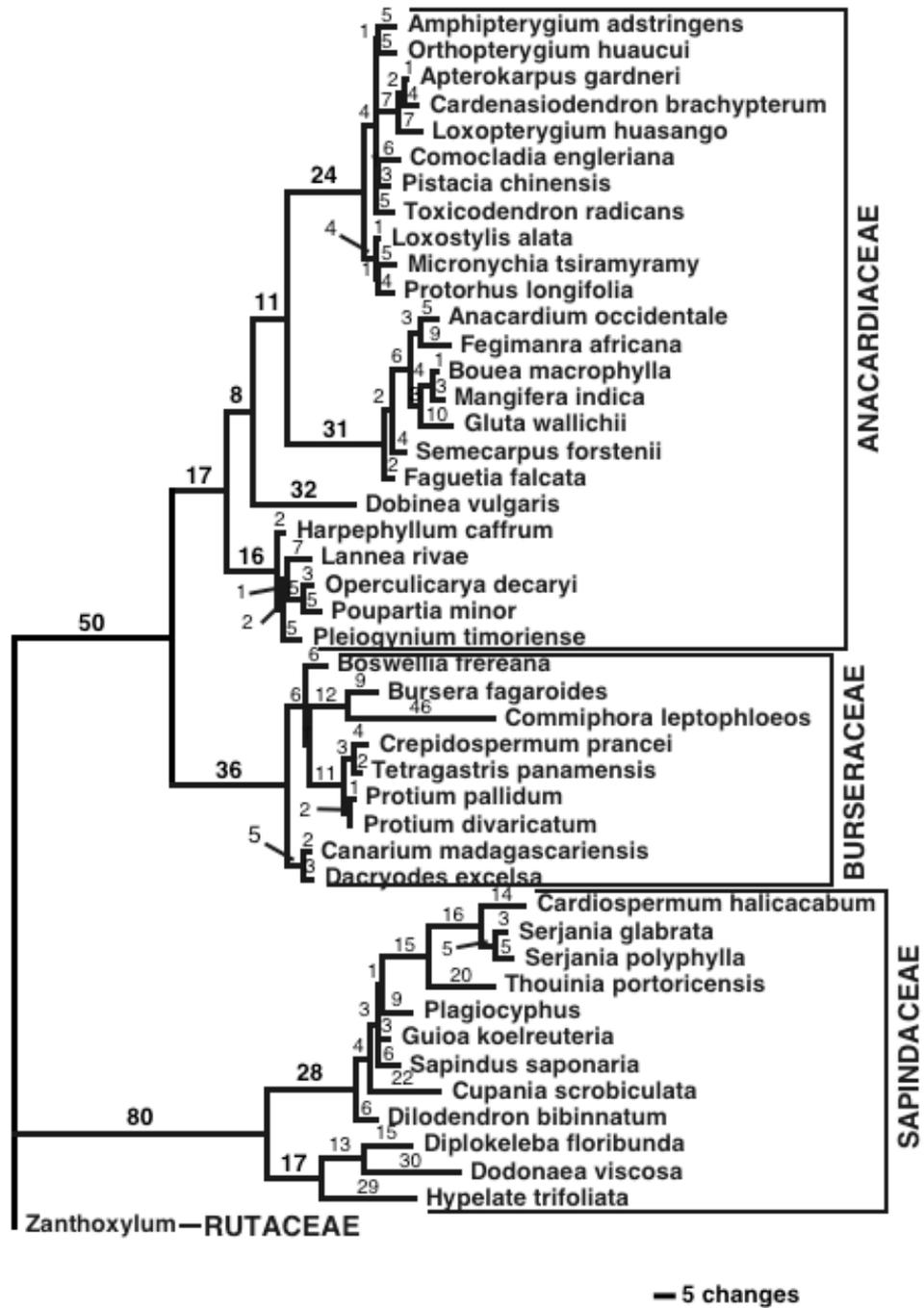


Figure 2.3. Maximum likelihood phylogram of *trnLF* sequences of Anacardiaceae, Burseraceae, and Sapindaceae with a Rutaceae outgroup. Likelihood score 6330.2518; substitution model = TVM+G+I. Branch lengths are shown above branches.

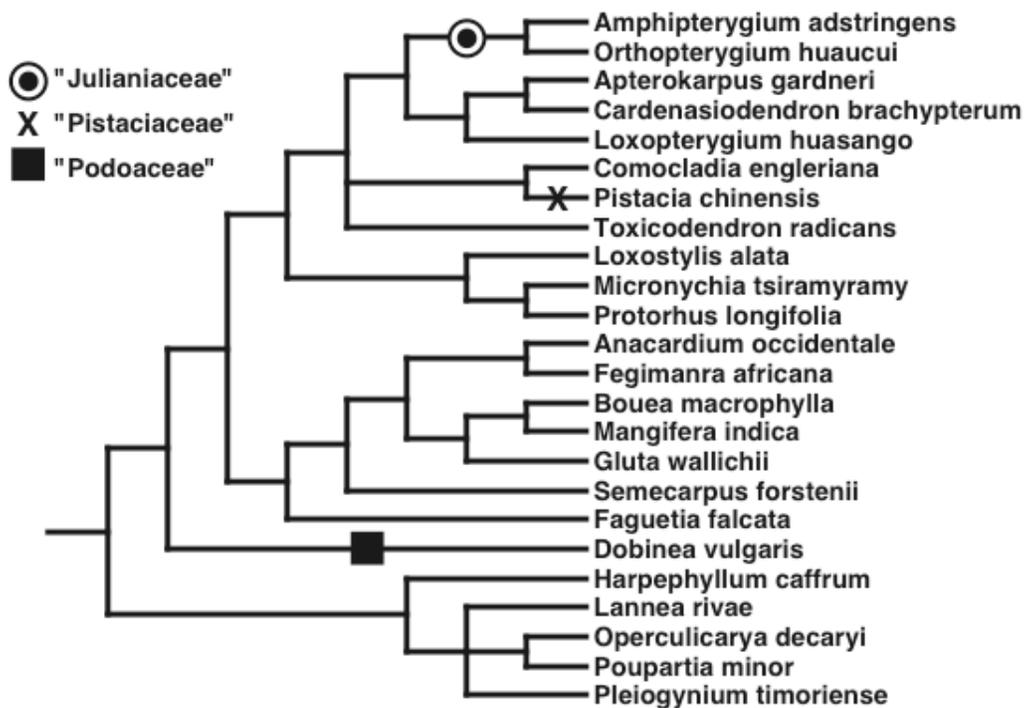


Figure 2.4. *trnLF* subtree of Anacardiaceae from maximum parsimony 50% bootstrap consensus tree shown in Figure 2.3. Families formerly segregated from Anacardiaceae are indicated.

generated in the maximum parsimony search. Two of the four tribes, Proteieae and Canarieae, are monophyletic, and tribe Bursereae is paraphyletic. Proteieae is nested within Bursereae. Tribe Canarieae is sister to the rest of the family.

The subfamilial classification of Sapindaceae is shown in Figure 2.6. As currently circumscribed, the two tribes, Dodonaeoideae and Sapindoideae, are paraphyletic. Dodonaeoideae is nested within Sapindoideae. The monophyly of the Sapindaceae is well supported with 97% bootstrap (Fig. 2.3).

Burseraceae, Rutaceae, Sapindaceae, and tribes Dobineae and Spondiadeae of Anacardiaceae share a 119 base pair indel that has a variable



Figure 2.5. *trnLF* subtree of Burseraceae from maximum parsimony 50% bootstrap consensus tree shown in Figure 2.3. Tribal and sectional affiliations are indicated.

sequence across the four families but is easily aligned. This region is a gap in the *trnLF* sequences for Anacardiaceae tribes Anacardieae, Rhoaeae, and Semecarpeae. Burseraceae, Rutaceae, Sapindaceae and Anacardiaceae tribe Spondiadeae also share other sequence similarities including two smaller indels of six and 11 bases. A four base indel unites Anacardiaceae tribes Anacardieae, Dobineae, Rhoaeae, and Semecarpeae. Burseraceae and Sapindaceae are similarly supported by indels including two five-base and one nine-base indels respectively.



Figure 2.6. *trnLF* subtree of Sapindaceae from maximum parsimony 50% bootstrap consensus tree shown in Figure 2.3. Subfamilial affiliations are indicated.

Morphological characters evaluated in the context of the *trnL*F bootstrap consensus phylogeny are shown in Figure 2.7 and selected characters are mapped in Figures 2.8 to 2.10. The Anacardiaceae-Burseraceae clade is defined by the presence of resin canals in the phloem (character 10 in Fig. 2.7, mapped in Fig. 2.10). This is also the only clade in which the ability to synthesize biflavonoids is found (character 2 in Fig. 2.7), although not all members of the clade produce biflavonoids and many ingroup and outgroup taxa have yet to be investigated.

Burseraceae and Rutaceae have epitropous ovules. Anacardiaceae and Sapindaceae have apotropous ovules (characters 5 and 6 in Fig. 2.7, mapped in Fig. 2.10). It is equivocal as to whether having apotropous ovules is a synapomorphy for the Anacardiaceae as it is equally parsimonious for the common ancestor of the Anacardiaceae-Burseraceae clade to have either character state. Only the inclusion of more distant outgroups beyond Rutaceae would solve this problem. However, the possibility remains that apotropous ovules are a synapomorphy for Anacardiaceae, but this cannot be definitively shown with the current data.

Anacardiaceae is the only clade in which 5-deoxyflavonoids have been found (character 1 in Fig. 2.7). All members of the family that have been surveyed for this biochemical have been found to produce it and it has not been found in any other family in the Sapindales, although most of the taxa for which biochemical surveys have been conducted are not included in this study.

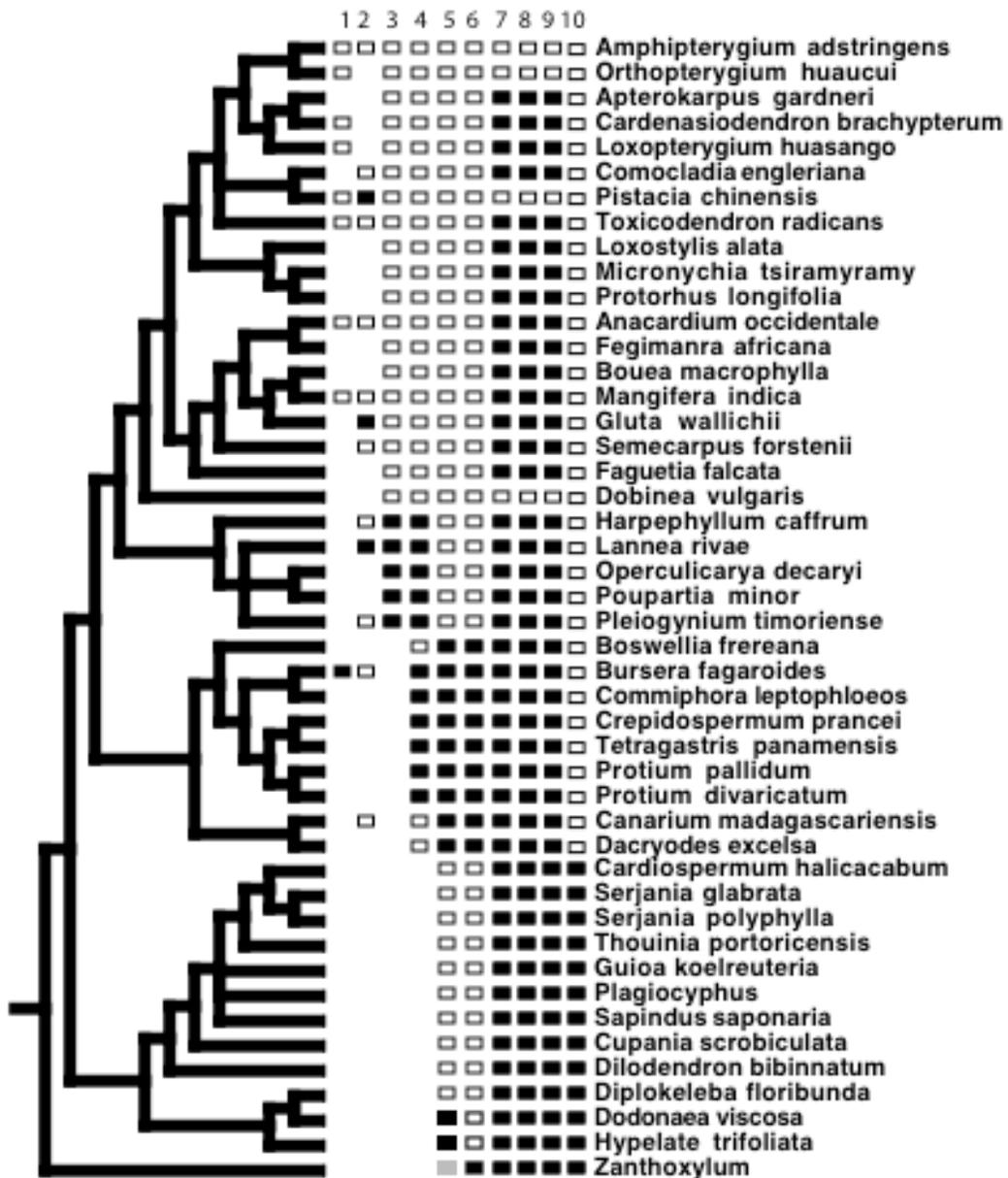


Figure 2.7. Coding of morphological character. Shown next to *trnLF* maximum parsimony bootstrap consensus tree from Figure 2.3. Coding of characters is as follows: (1) 5-deoxyflavonoids, white=present, missing box=unknown; (2) biflavonoids, white=present, black=absent; (3) endocarp, white=regularly arranged layers, black=lacking regularly arranged layers; (4) exocarp, white=thin, black=thick; (5) number of ovules per locule, white=one, black=two, grey=greater than 2; (6) ovule position, white=apotropous, black=epitropous; (7) perianth, white=reduced, black=not reduced; (8) pollen, white=wind adapted, black=not wind adapted; (9) pollination, white=wind, black=animal; (10) resin canals in the phloem, white=present, black=absent (not shown).

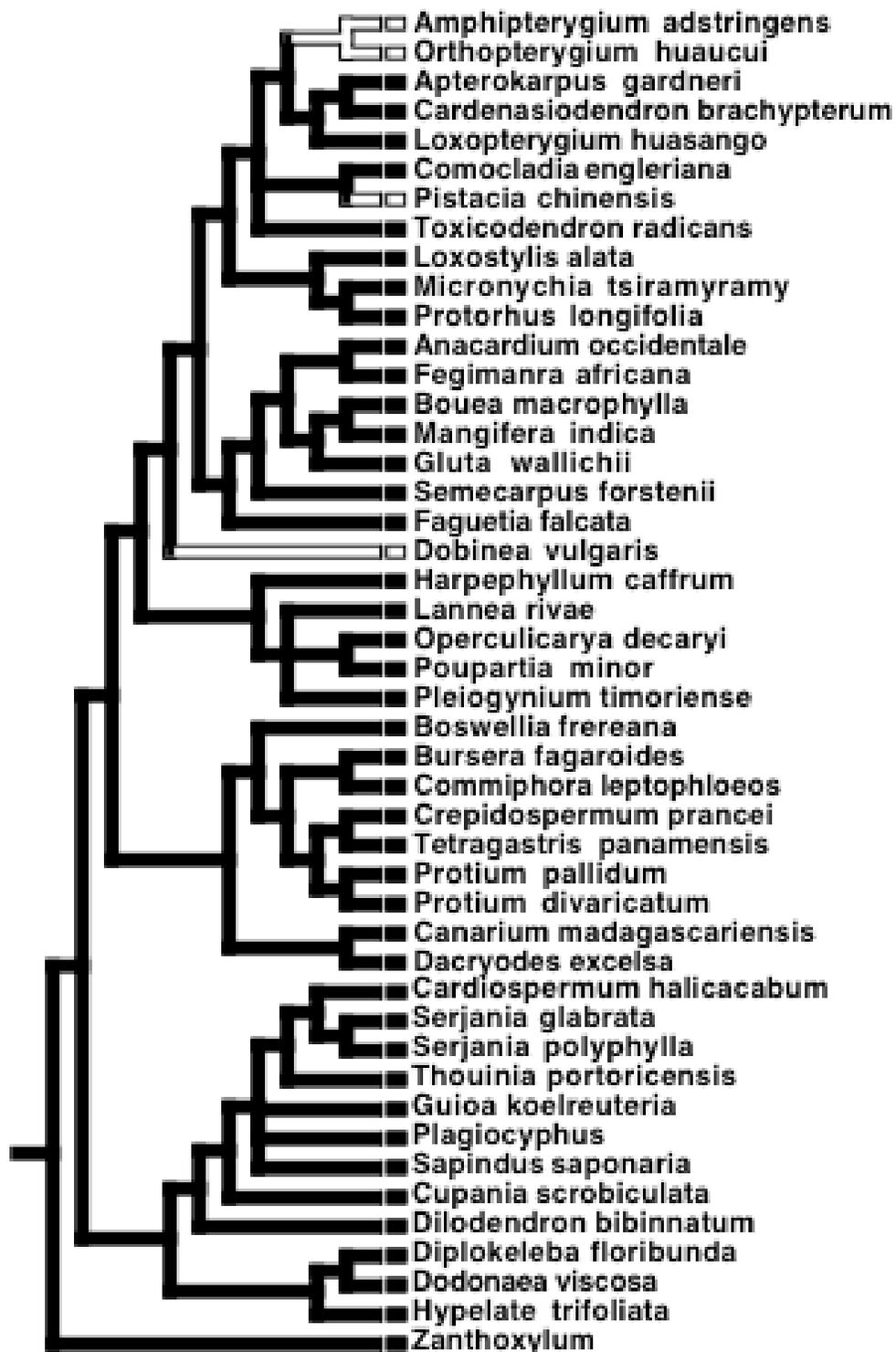


Figure 2.8. Wind pollination adaptations mapped onto the *trnL*F maximum parsimony bootstrap consensus tree shown in Figure 2.3. Reduction in perianth, presence of wind-adapted pollen, and wind pollination are mapped in white.

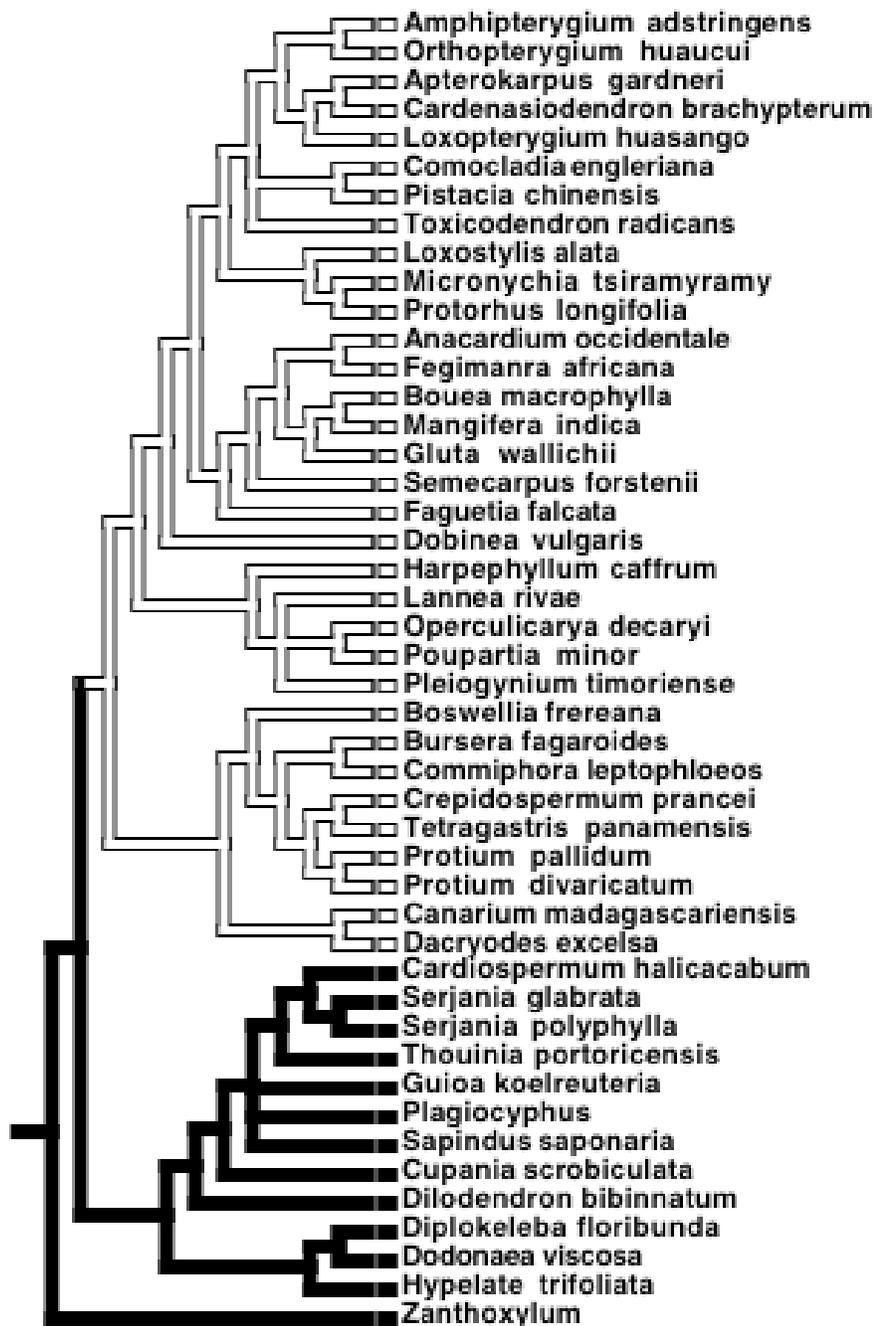


Figure 2.9. Presence and absence of resin canals in the phloem mapped onto the *trnL*F maximum parsimony bootstrap consensus tree shown in Figure 2.3. White=presence of resin canals in phloem, black=absence of resin canals in phloem.

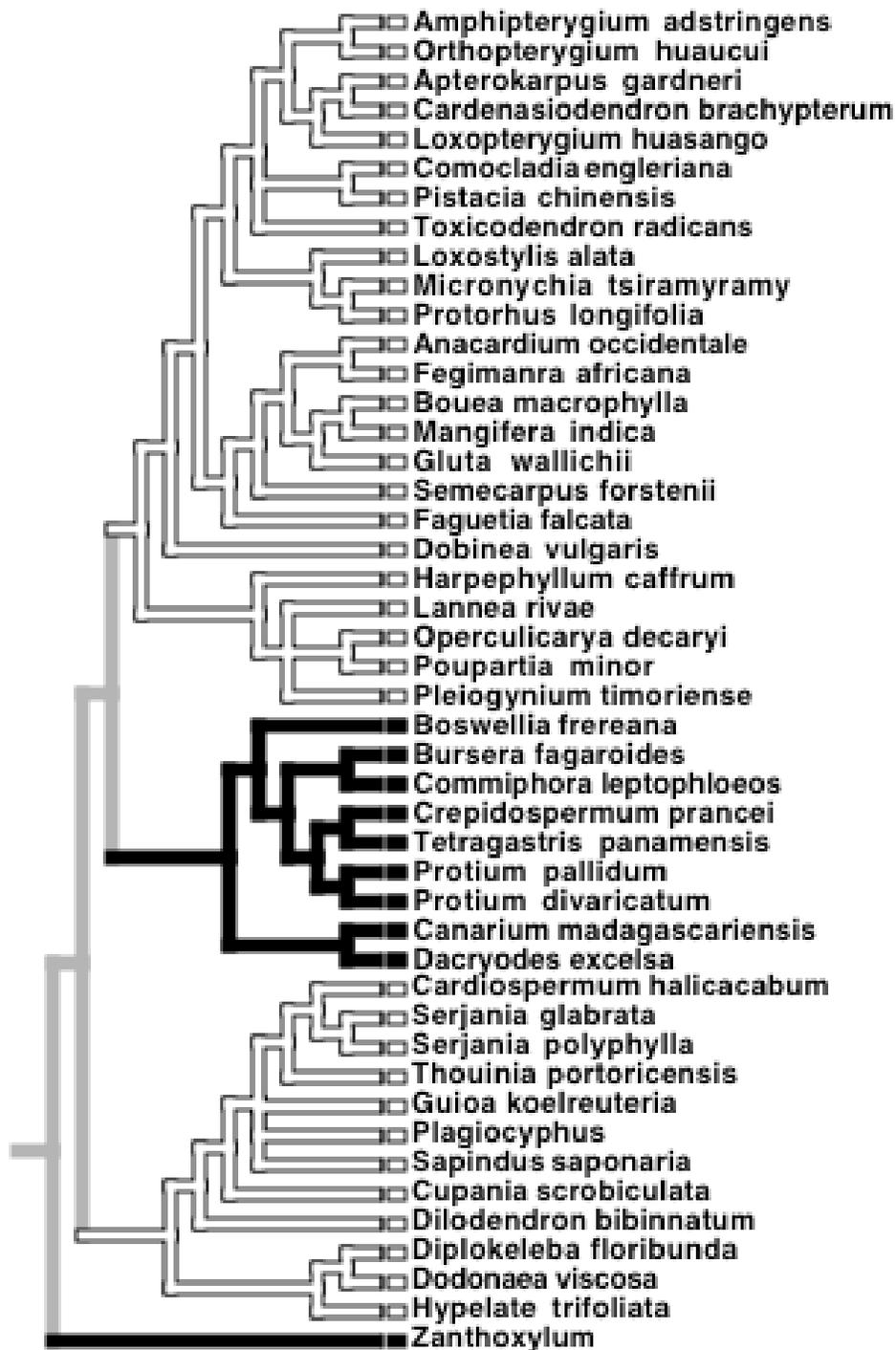


Figure 2.10. Position of ovules in locules mapped onto the *trnL*F maximum parsimony bootstrap consensus tree shown in Figure 2.3. Color-coding is as follows: white=apotropous, black=epitropous, grey=equivocal.

Wind pollination and the associated morphological adaptations of a reduced or absent perianth and pollen with a reduced number of colpi and an increased number of apertures (adaptations increasing the efficiency of wind dispersal) have evolved three times in the family (characters 7, 8, and 9 in Fig. 2.7, mapped in Fig. 2.8).

Thin exocarp tissue is a symplesiomorphic character shared by the upper Anacardiaceae clade and Burseraceae tribe Canarieae (character 4 in Fig. 2.7). Endocarp structure is incompletely known within the Burseraceae and thus the phylogenetically informative status of this character is unclear. An endocarp lacking arranged layers is plesiomorphic because it is found basally in Anacardiaceae but has also been reported for *Canarium* in the Burseraceae (Wannan and Quinn, 1990) (character 3 in Fig. 2.7). An organized endocarp appears to be a synapomorphy of the larger (upper) Anacardiaceae clade (character 3 in Fig. 2.7) but the endocarp structure for most of the Burseraceae taxa has not been investigated.

## Discussion

The finding of a monophyletic Anacardiaceae and Burseraceae in the *trnL*F analyses (Figs. 2.2 and 2.3) adds stronger support to similar results found in recent higher taxonomic level molecular studies that included only a small number of representatives of the cashew and gumbo limbo families (Fernando et al. 1995, Gadek et al. 1996; APG 1998, 2003; Bremer et al. 1999; Savolainen et

al. 2000a, 2000b). This is in contrast with Terrazas' (1994) *rbcl* phylogeny which nests Anacardiaceae and Burseraceae together (with a clade of Spondiadeae and Burseraceae sister to the rest of Anacardiaceae) but agrees with her cladogram based on combined molecular and morphological data that supported the two families as distinct clades.

Anacardiaceae, Burseraceae, and their reported sister family, Sapindaceae (Gadek et al., 1996), are represented in the current study by a greater number of taxa than in any previous investigation. The *trnLF* phylogeny confirms that Anacardiaceae and Burseraceae are sister families with distinct lineages. These two families (including Julianiaceae, Pistaciaceae, and Podoaceae) have the synapomorphy of vertical intercellular secretory (resin) canals in the phloem (Wannan, 1986) (character 10 in Fig. 2.7). They are also the only two families in which the ability to synthesize biflavonoids has been found (Wannan, 1986).

Within Burseraceae, classification has been in fluctuation for some time. The last major revision of the family was done by Engler (1913, 1915, 1931) in which he split the family into three tribes: Boswellieae (Bursereae of Lam, 1932), Canarieae, and Protieae. Daly (in Harley and Daly, 1995) redefined morphological limits of the tribes and established two subtribes for Bursereae (Boswelliinae and Burserinae) in order to reconcile problems of generic placement within the classification. More recently Clarkson et al. (2002) found that the two subtribes are paraphyletic and recommended that if future studies

support their findings, the family should be split into three new tribes reflective of Burseraceae phylogeny: Bursereae (including Canarieae and Boswellinae), Protieae (including Burserinae) and a new, unnamed, tribe including only *Beiselia*.

The phylogeny presented here does not support the Burseraceae clades elucidated in the *rps16* phylogeny of Clarkson et al. (2002) but neither does it support the currently used infrafamilial classification of the Burseraceae. Tribe Canarieae (represented by *Canarium* L. and *Dacryodes* Vahl) is shown as monophyletic and sister to the rest of the family but the monophyletic Protieae is nested within a paraphyletic Bursereae (Fig. 2.5). However, the two groups into which Bursereae is split are equivalent to its two sections proposed by Daly based on morphology (Harley and Daly, 1995). Daly pointed out that the two subtribes are substantially disparate in their morphological features and acknowledged that it may be necessary to elevate them to tribal rank. The *trnLF* phylogeny supports the recognition of the two Bursereae sections as monophyletic (Figs. 2.2, 2.3, and 2.5).

Yet another recent *rps16* phylogeny (Weeks, 2003) contradicts this finding and that of Clarkson et al. This dataset has much more comprehensive sampling within Burseraceae and finds the same monophyletic Protieae sister to Bursereae section Burserinae, but indicates that Bursereae section Boswellinae is sister to Canarieae and that a genus not sampled for the *trnLF* phylogeny, *Beiselia* L. L. Forman, is at the base of the family (Weeks, 2003). It is quite

possible that the absence of *Beiselia* in the *trnLF* dataset has resulted in an inaccurate rooting of the Burseraceae taxa, consequentially finding Canarieae to be sister to the rest of the family instead of sister to Bursereae section Boswellinae.

The subfamilial classification of Sapindaceae is currently in a similar state of uncertainty. Acevedo-Rodríguez and colleagues (in press) commented that the current system of classification of Sapindaceae, including tribes Dodonaeoideae and Sapindoideae is extremely problematic. All recent molecular phylogenies of the family find tribe Dodonaeoideae to be paraphyletic (Gadek et al., 1996; Savolainen et al., 2000b; and an unpublished phylogeny of Johnson and Chase shown in Klaassen, 1999). Dodonaeoideae is paraphyletic and Sapindoideae is polyphyletic in the *trnLF* phylogeny (Fig. 2.6), thus supporting all of the *rbcL* phylogenies in finding the two subfamilies to be artificial. Because this study did not focus on elucidating familial relationships across the order Sapindales (and thus did not include sampling across the order), the status of Sapindaceae as sister to the Burseraceae-Anacardiaceae clade cannot be evaluated in the context of the *trnLF* phylogeny.

Podoaceae, represented by *Dobinea vulgaris*, is nested within Anacardiaceae in the *trnLF* phylogeny (Figs. 2.2-2.4), strengthening the hypothesized location of this group within the cashew family put forth by many previous authors (Engler, 1892; Morot, 1889, Radlkofer, 1890; Forman, 1954; Cronquist, 1981, 1988; Wannan, 1986; Mitchell and Mori, 1987; Takhtajan, 1987,

Wannan and Quinn, 1991). Its position within the family and sister to the clade containing tribes Anacardieae, Rhoeeae, and Semecarpeae, strongly suggests that the Podoaceae is derived within the Anacardiaceae. This relationship is supported by numerous anatomical and morphological similarities including the synapomorphy of an endocarp with regularly arranged layers like those in tribe Rhoeeae (two inner layers with palisade-like sclereids and two outer layers un lignified) (Wannan, 1986; Wannan and Quinn, 1990), carpel morphology similar to that of tribe Anacardieae (unicarpellate but with three vascular bundles in the style to above the locule, possibly indicating two other aborted carpels – likely symplesiomorphic outside of Anacardiaceae but data is incomplete) (Wannan, 1986; Wannan and Quinn, 1991), presence of a single apotropous ovule (also found in Sapindaceae, see Figs. 2.7 and 2.10), wood anatomy (Radlkofer, 1888), and gross morphology (Forman, 1954) (Figs. 2.7 and 2.9).

Similar morphological and anatomical studies have long separated the Julianiaceae from the Anacardiaceae. However, both *Amphipterygium* and *Orthopterygium* are represented in the *trnL*F phylogeny and are found to be monophyletic and nested within the core Rhoeeae clade (Figs. 2.2-2.4). Their placement within the Anacardiaceae is supported by their close resemblance to the family with regard to endocarp anatomy (Fritsch, 1908; Wannan, 1986), glandular leaf hair structure (Fritsch 1908), wood anatomy (Kramer, 1939; Bailey, 1940; Heimsch, 1942; Kryn, 1952; Stern, 1952; Youngs, 1955; Terrazas, 1994), ovule structure (Copeland and Doyel, 1940), serotaxonomy (Petersen and

Fairbrothers, 1983), biflavonoid data (Young, 1976), and pollen structure (Erdtman, 1971) (Fig. 2.7). Of the anatomical evidence that supports these two families being united, Fritsch (1908) wrote, “the Julianiaceae in their anatomical structure show a most marked affinity to the Anacardiaceae, □ so marked, indeed, that it is difficult to hold the two [families] distinct from this point of view.” The current DNA study certainly supports that view and suggests that the Julianiaceae should no longer be recognized and its genera should be transferred to the Anacardiaceae.

The monogeneric Pistaciaceae is distinguished from Anacardiaceae by its reduced flower structure, plumose styles, and unusual pollen morphology. Although these morphological features are aberrant for most of the Anacardiaceae, they are all adaptations for wind pollination, and are shared by the other wind-pollinated genera in the cashew family, *Amphipterygium*, *Orthopterygium*, and *Dobinea* (characters 7-8 in Fig. 2.7). Erdtman (1971) studied the pollen of 20 species of Anacardiaceae and found that the pollen of Julianiaceae and *Pistacia* were very similar. Based on this finding he suggested that the members of Julianiaceae be recognized in Anacardiaceae near *Pistacia*. The placement of *Pistacia* within Anacardiaceae, as indicated in the *trnL* phylogeny (Figs. 2.2-2.4), contradicts many authors' placement of the genus outside of the cashew family (e.g. Marchand, 1869; Eichler, 1875-78; Takhtajan, 1987, 1997). However, Engler's (1876) inclusion of *Pistacia* in tribe Rhoideae (=modern tribe Rhoieae), is supported by the molecular data and is reflected in

most currently used treatments of the family (Engler, 1883, 1892; Mitchell and Mori, 1987). The synapomorphies of a single apotropous ovule per locule place it within the Anacardiaceae (Fig 2.7) and it is further linked to the family by the production of 5-deoxyflavonoids. Morphologically, Pistaciaceae resembles tribe Rhoeeae. The two share several features including three syncarpous carpels, unilocular fruit, and a thin exocarp.

Although the morphological and anatomical characters mentioned above unite Podoaceae, Julianiaceae, and Pistaciaceae with Anacardiaceae, it is difficult to demonstrate the phylogenetic importance of all of them in the context of this phylogeny because many of these traits remain uninvestigated within the Sapindaceae and in the outgroup family, Rutaceae (as well as in other members of the Sapindales). However, when select characters are mapped onto the Anacardiaceae-Burseraceae subtree, it is clear that the often-segregated families and Anacardiaceae have several synapomorphies (Figs. 2.7, 2.9, and 2.10). No material of *Blepharocarya* F. Muell. was obtained for the current study so its position in the context of a monophyletic Anacardiaceae was not evaluated with molecular data. However, two of the same morphological synapomorphies that link the other segregates to Anacardiaceae also link *Blepharocarya* to the cashew family (single apotropous ovule per locule). In addition, the genus has been found to produce 5-deoxyflavonoids, a unique biochemical compound found only in Anacardiaceae within the Sapindales.

The finding of a monophyletic Anacardiaceae including the Julianiaceae, Pistaciaceae, and Podoaceae (Figs. 2.2-2.3) confirms recent ideas about the delimitation of the cashew family including these former segregates (e.g. Mitchell and Mori, 1987; Wannan and Quinn, 1991), and refutes many others who variously segregated these three families from Anacardiaceae (e.g. Hemsley, 1908; Bessey, 1915; Hutchinson, 1926; Wettstein, 1935, 1944; Rendle, 1938; Copeland and Doyel, 1940; Standley and Steyermark, 1949; Gundersen, 1950; Stern, 1952; Barkley, 1957; Melchior, 1964; Stone, 1973; Cronquist, 1988; Watson and Dallwitz, 1992). The morphological synapomorphies along with the *trnL*F phylogeny strengthen the body of evidence in support of recognizing Anacardiaceae inclusive of Julianiaceae, Pistaciaceae and Podoaceae. The data further support the relationship of Burseraceae and Anacardiaceae as distinct sister families.

# Chapter 3: Molecular Phylogeny of Anacardiaceae: Intrafamilial Classification and Evolutionary Relationships of Noted Genera

## Introduction

The Anacardiaceae Lindl. is a widespread and primarily pantropical family of woody plants occurring on every continent except Antarctica. It is notably absent from the floras of northern North America, the southern tip of South America, the Galapagos Islands, northern Eurasia, some Pacific islands, temperate and arid Australia, and New Zealand. Many members of the family are economically important for their edible fruits and seeds. Some of these are widely cultivated (pistachios, cashews, pink pepper corns, and mangos), while others' cultivation is restricted to local farming and/or wild population harvesting (*Rhus* subgen. *Rhus* spp., *Sclerocarya birrea* Hochst., *Semecarpus* L. f. spp., *Spondias* L. spp., *Tapirira* Aubl. spp., etc.). Other Anacardiaceae members are valued for their horticultural appeal, timber, and/or medicinal properties.

Despite the fascinating fruit diversity, wide distribution, and economic importance of the family, Anacardiaceae has not had a thorough systematic assessment since that of Engler (1892) more than one hundred years ago. He recognized five tribes: Dobineeae, Mangifereae, Rhoideae, Semecarpeae, and Spondieae. Engler circumscribed his tribes using vegetative and reproductive characters including the number of carpels, insertion of the ovule on the

placenta, number of staminal whorls, leaf complexity, number of locules in the ovary and fruit, embryo morphology, and insertion of the style on the ovary.

Although Engler's (1881, 1883, 1892) three main treatments together remain the most detailed and thorough revision of the Anacardiaceae, his tribal boundaries are problematic. Because his tribal descriptions were not parallel, placement of genera within them is often dubious and the limits of one tribe are not comparable with the limits of another. For example, Engler defined his tribe Dobineae by its pistillate flowers lacking a perianth and having a single carpel; whereas, tribe Rhoideae was distinguished by a suite of different characters including style insertion and connation, locule number, fruit characters, embryo shape, and several other morphological features. Moreover, tribe Rhoeeae included taxa that lack a perianth. Since this system was established, numerous species and several genera have been added to the family through new discovery and taxonomic reassessment, making the limits of the tribes even more difficult to ascertain.

Recent morphological and anatomical attempts to rectify the subfamilial classification have been preliminary in nature and some consist of rather limited sampling within the Anacardiaceae (Wannan and Quinn, 1990, 1991; Terrazas, 1994). Mitchell and Mori (1987) attempted to salvage the currently used systems and placed all of the genera within a tribal classification system that is designed after those of Engler and Ding Hou (1978). The tribal names and indicated generic affinities listed in Table 3.1 were revised from those of Mitchell and Mori

(1987) to include current ideas of generic placement including several genera, both new to science and new segregates that are yet to be validly published. These names (with the noted exception of some members of the *Rhus* complex) and tribal affiliations will be used in this study when referring to present tribal circumscription.

Table 3.1. Generic affinities of the infrafamilial classification used in this study (modification of Mitchell and Mori, 1987).

| Tribe       | Affiliated Genera  |
|-------------|--|
| Anacardieae | <i>Anacardium, Androtium, Bouea, Buchanania, Fegimanra, Gluta</i> (including <i>Melanorrhoea</i> ), <i>Mangifera, Swintonia</i>  |
| Spondiadeae | <i>Allospodias, Antrocaryon, Choerospodias, Cyrtocarpa, Dracontomelon, Haematostaphis, Haplospodias, Harpephyllum, Koordersiodendron, Lannea, Operculicarya, Pegia, Pleiogynium, Poupartia, Poupartiopsis</i> ined., <i>Pseudospodias, Sclerocarya, Solenocarpus, Spodias, Tapirira</i>  |
| Semecarpeae | <i>Drimycarpus, Holigarna, Melanochyla, Nothopegia, Semecarpus</i>   |
| Rhoeae      | <i>Abrahamia</i> ined., <i>Actinocheita, Amphipterygium, Apterokarpus, Astronium, Baronia*</i> , <i>Blepharocarya, Bonetiella, Camptosperma, Cardenasiodendron, Comocladia, Cotinus, Euroschinus, Faguetia, Haplorhus, Heeria, Hermogenodendron</i> ined., <i>Laurophyllus, Lithrea, Loxopterygium, Loxostylis, Malosma, Mauria, Melanococca, Metopium, Micronychia, Mosquitoxylum, Myracrodruon, Ochoterena, Orthopterygium, Ozoroa, Pachycormus, Parishia, Pentaspadon, Pistacia, Protorhus, Pseudosmodingium, Rhodosphaera, Rhus, Schinopsis, Schinus, Searsia*</i> , <i>Smodingium, Sorindeia, Thyrsodium, Toxicodendron, Trichoscypha</i> |
| Dobineae    | <i>Campylopetalum, Dobinea</i>   |

\*Included in *Rhus* in the phylogenies and text here for purposes of reflecting currently used taxonomy and highlighting the paraphyletic nature of *Rhus s.l.*

Most molecular studies within the family have been focused on genetic diversity and population genetics of crop and timber plants (e.g. *Anacardium* L., Mneney et al., 2001; *Camposperma* Thwaites, Sheely and Meagher, 1996; *Mangifera* L., Yonemori et al., 2002; and *Pistacia* L., Hormaza et al., 1994, 1998; Parfitt and Badenes, 1997; Kafkas and Perl-Treves, 2002) with very little effort being directed toward higher taxonomic level relationships between the genera. Terrazas' (1994) *rbcL* phylogeny and another minor *rbcL* study of the Anacards of Thailand (Chayamarit, 1997) are the only two family-level molecular systematic studies. Both of these included a small sampling of Anacardiaceae (18 species and 16 Thai species, respectively) and thus neither adequately elucidated the phylogeny of the cashew family. Further, the *rbcL* data (Terrazas, 1994) indicated that the Anacardiaceae and Burseraceae are nested together (tribe Spondiadeae is allied with the Burseraceae, sister to the rest of Anacardiaceae) and thus each is paraphyletic. Only when Terrazas' (1994) molecular and morphological data were combined did the resulting cladogram support a monophyletic Anacardiaceae. A clear problem remains of how to properly classify the Anacardiaceae genera so that the larger taxonomic groups in which they occur can be defined morphologically but also reflect the phylogeny of the family.

Although it has been suggested that Anacardiaceae is of Gondwanan origin (Gentry, 1982) and its current and historic wide distribution support this hypothesis, no biogeographical assessment of the family has been conducted

using phylogenetic relationships as evidence of vicariance or dispersal events. Geographical distributions of the taxa were mapped onto the molecular phylogeny in order to look at extant generic relationships in the context of biogeography. Possible historical biogeographical explanations for the current distributions and relationships are evaluated.

The field of systematics has been greatly enhanced in the last 20 years by the development and expansion of molecular data for use in phylogenetic analyses. While these data should not be considered in isolation from morphological and anatomical characteristics of the study organisms, DNA investigations can provide a framework in which morphological data can be considered. Sequence data recently have become much easier to obtain by the development of automated sequencing techniques that facilitate data gathering without using radioactive isotopes. These combined attributes make DNA sequencing attractive for use in phylogenetic studies and was thus chosen for use here.

The current study was undertaken with four main goals: (1) to elucidate a more accurate intrafamilial classification of the family; (2) to reconstruct the relationships of the genera in the cashew family; (3) to identify paraphyletic genera in need of further study and revision; and (4) to test the hypothesis that Anacardiaceae is of Gondwanan origin.

The class II intron *matK* lies within the chloroplast gene *trnK* and codes for maturase (Neuhaus and Link, 1987; Liang and Hilu, 1995). Previous studies

have indicated the utility of *matK* at the infrafamilial level (Johnson and Soltis, 1994; Liang and Hilu, 1996; Kron, 1997; Xiang et al., 1998, etc.) and similar findings from preliminary sequencing tests in the Anacardiaceae also agreed. Parsimony informative variation in *matK* has been reported at 36% (Johnson et al., 1996), 16% in Cornaceae and allies (Xiang et al., 1998), 27% within Ericaceae (Kron, 1997), and 15% in 583 bases of the less variable 3' region in Poaceae (Liang and Hilu, 1996). This level of variability in *matK* made it an appropriate marker for use in this study.

While sequence data from coding genes such as *matK* often provide sufficient variability, non-coding regions have been found to provide more information due presumably to being under less functional evolutionary constraint and thus potentially evolving at a faster rate than coding regions (Clegg et al., 1994; Gielly and Taberlet, 1994; Sang et al., 1997). Previous studies have indicated the utility of *trnL*F (this abbreviation is used for simplicity and refers to the *trnL* intron, *trnL* 3' exon, and the *trnL-trnF* intergenic spacer) at the infrafamilial level (Ham et al., 1994) and similar findings from preliminary sequencing in the Anacardiaceae agree. Gielly and Taberlet (1994) found these loci evolve at more than three times the rate of *rbcL*. The region has been used in numerous family level studies including Acanthaceae (McDade and Moody, 1999; McDade et al., 2000), Amaryllidaceae (Meerow et al., 1999), Gentianaceae (Gielly and Taberlet, 1994, 1996), Rhizophoraceae (Schwarzbach and Ricklefs, 2000), and Zygophyllaceae (Sheahan and Chase, 2000) among others.

The chloroplast *rps16* class II intron was also selected for use in this study. It is located between the two exons of *rps16*, a gene that codes for ribosomal protein small subunit 16, located in the large single-copy region of the chloroplast genome. It has been reported to have evolved two to three times slower than the nuclear ribosomal ITS region and thus has been useful at the intrageneric and infrafamilial levels (Lidén et al., 1997; Oxelman et al., 1997; Asmussen, 1999; Baker et al., 2000; Lee and Downie, 2000; Anderson and Chase, 2001; Clarkson et al., 2002).

The plastid data were first considered alone and then variously combined to assess congruence among the datasets. This enabled comparison of topologies of DNA regions with different functional constraints (i.e. coding vs. non-coding) before combining them to limit spurious results in the separate analyses (Johnson and Soltis, 1998; Wiens, 1998).

## Materials and Methods

**Plant materials and DNA isolation** □ Sampling for this study (Table 3.2), in all but the *rps16* datasets, included representatives of the five Anacardiaceae tribes (see Appendix A for a complete list of taxa). These taxa were selected to represent the morphological diversity within the Anacardiaceae while providing appropriate outgroup representation of the Sapindaceae and/or Burseraceae. Fresh and silica-dried materials as well as herbarium specimens were used in this study for DNA extraction. These samples were collected by the author in the

field, gathered in herbaria (F, K, LSU, MO, MOR, NY), or contributed by colleagues collecting worldwide. Many of the Anacardiaceae silica samples were provided by John D. Mitchell and colleagues at The New York Botanical Garden. The laboratory of Dr. Toby Pennington and colleagues, Royal Botanical Garden Edinburgh also contributed generously to this study with collections from South and Central America. Sapindaceae silica samples were provided by Dr. Pedro Acevedo, Department of Botany, Smithsonian Institute. Burseraceae silica samples were provided by Dr. Douglas Daly, The New York Botanical Garden.

Table 3.2. Taxon sampling in the *matK*, *trnLF*, *rps16*, and combined datasets. Species are listed first, followed by genera in parenthesis.

| Dataset                  | Anacardiaceae | Burseraceae | Sapindaceae |
|--------------------------|---------------|-------------|-------------|
| <i>matK</i>              | 33 (27*)      | 5 (4)       |             |
| <i>rps16</i>             | 55 (45*)      | 5 (5)       | 3 (3)       |
| <i>trnLF</i>             | 81 (57*)      | 3 (3)       |             |
| <i>rps16-trnLF</i>       | 50 (42*)      | 5 (5)       | 3 (3)       |
| <i>matK- rps16-trnLF</i> | 19 (19)       | 3 (3)       |             |

\* Clades of *Rhus s.l.* that have distinct evolutionary origins will be recognized as distinct genera and are counted accordingly.

Plant tissue was ground in one of three ways: by hand with a mortar and pestle, in tubes with sterile glass beads and sand placed in a tissue disruptor, or in a FastPrep lysing FP-120 bead mill using lysing matrix "A" tubes containing a ceramic bead and garnet sand (Qbiogene Inc., Carlsbad, CA). Most samples were extracted with the DNeasy Plant Mini Kit (Qiagen Inc., Valencia CA), but modified Doyle and Doyle (1987) and Struwe et al. (1998) methods were also

employed. Extractions of herbarium material were done with a modification of the Qiagen protocols and included the addition of 570 mg (30 $\mu$ l) of PCR grade proteinase K (Roche, Indianapolis, IN), 6.5% (30 $\mu$ l)  $\beta$ -mercaptoethanol (BME) and incubation at 42 °C for 12-24 hours on a rocking platform (K. Wurdack designed protocol, pers. com., see Appendix B for a complete description of this herbarium extraction protocol).

**DNA amplification and sequencing** □ All of the chloroplast regions utilized were amplified from extracted total genomic DNA using the polymerase chain reaction (PCR) method. The universal primers c, d, e, and f of Taberlet et al. (1991) (Table 3.1) were used to amplify *trnL*F. Those of Oxelman et al. (1997) were used for amplification of *rps16* (*rps16*F: 5'-GTG GTA GAA AGC AAC GTG CGA CTT-3' and *rps19*R2: 5'-TCG GGA TCG AAC ATC AAT TGC AAC-3'). In most cases primers *trnK*-3914F, *psbA*-R, and *trnK*-2R of Johnson and Soltis (1994) were used for amplification of *matK*; however, some taxa proved to be problematic and required the development of new primers for PCR and cycle sequencing (Table 3.3). Thermal cycling parameters for *trnL*F and *rps16* were an initial denaturation of 2 minutes at 97°C; 30 cycles of 94°C for one minute, annealing at 48°C for two minutes, and elongation at 72°C for two minutes; followed by an elongation step of 72°C for 16 minutes. Cycling parameters for *matK* were those of Johnson and Soltis (1994) with an additional denaturation step added at the beginning (initial denaturation of two minutes at 97°C; 30

cycles of 94°C for one minute and thirty seconds, annealing at 48°C for two minutes, and elongation at 72°C for three minutes; followed by an extra elongation step of 72°C for 15 minutes).

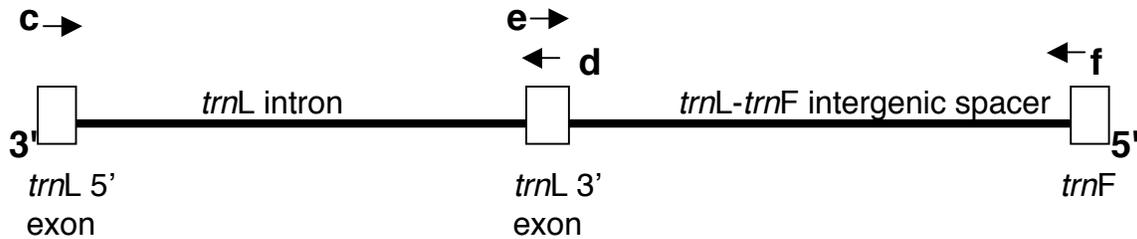


Figure 3.1. Approximate location of *trnLF* primers used in this study (from Taberlet et al., 1991). See Table 3.3 for a list of primers and their reference numbers used here.

Table 3.3. Sequences of the *trnLF* primers used in this study and mapped in Figure 3.1 (from Taberlet et al., 1991).

| Name | 5'-3' Sequence       |
|------|----------------------|
| c    | CGAAATCGGTAGACGCTACG |
| d    | GGGGATAGAGGGACTTGAAC |
| e    | GGTTCAAGTCCCTCTATCCC |
| f    | ATTTGAACTGGTGACACGAG |

Amplified DNA was purified using the QIAquick PCR purification kit (Qiagen Inc., Valencia CA). Purified PCR's were quantified by estimation using a Low DNA Mass Ladder (Invitrogen, Carlsbad, CA) and then cycle sequenced using the same primers as were used for amplification (Tables 3.2, 3.3 and Figs. 3.1, 3.2) and ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit version 1 (Applied Biosystems, Foster City, CA). Reactions one

quarter the size of the manufacturer's recommendation were run. Cycle Sequencing reactions were purified via alcohol precipitation or on Sephadex columns and then run on 5% Long Ranger® (BioWhittaker Molecular Applications, Rockland, Maine) polyacrylamide gels in an ABI 377XL automated sequencer (Applied Biosystems, Foster City, CA).

Table 3.4. *matK* primers used in this study. Primers with reference numbers 1, 8, and 9 are from Johnson and Soltis (1994), all others were designed by the author for use in this study. See Figure 3.2 for a map of primers.

| Reference letter in Figure 3.2 | Name               | 5'-3' Sequence           |
|--------------------------------|--------------------|--------------------------|
| 1                              | <i>trnK</i> -3914F | GGGGTTGCTAACTCAACGG      |
| 2                              | <i>trnK</i> -3F    | AGTYGGGTCKAGTRAATAAA     |
| 3                              | <i>matK</i> -5F    | AAGAGCGATKRKATTGAA       |
| 4                              | <i>matK</i> -4R    | GAKAAGATTGGKTRCGGAG      |
| 5                              | <i>matK</i> -6F    | TCTSCGTAASCAATCTTCTC     |
| 6                              | <i>matK</i> -10R   | CGCTGTGATAATGAGAAAGA     |
| 7                              | <i>matK</i> -7R    | TGAADACRCAGYTGATC        |
| 8                              | <i>trnK</i> -2R    | AACTAGTCGGATGGAGTAG      |
| 9                              | <i>psbA</i> -R     | CGCGTCTCTCTAAAATTGCAGTCA |

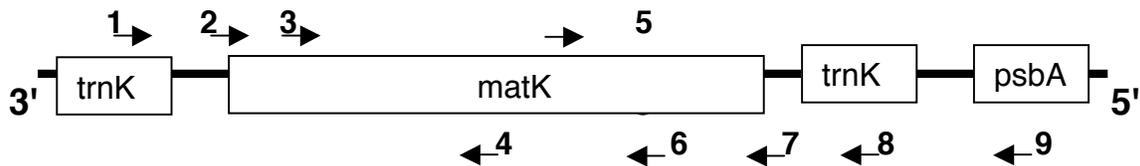


Figure 3.2. Approximate location of *matK* primers used in this study. See Table 3.4 for a list of primers and their reference numbers used here.

**Sequence analysis** □ Sequences were assembled and edited in Sequencher™

3.1.1 (Gene Codes, Corporation, Ann Arbor, MI). Compiled sequences were initially aligned in Clustal W ver. 1.6 (European Molecular Biology Laboratory

1996, Thompson et al., 1994) and subsequently manually adjusted in MacClade 4.0 (Maddison and Maddison 2000). The datasets were analyzed using PAUP\* 4.0b10 (Swofford, 2002) on an iMac G4 with the maximum-parsimony optimality criterion and maximum likelihood. Both analyses were performed on the data with the *matK*, *rps16*, and *trnLF* sequences being treated individually as single datasets. When the data were combined for analysis, only those taxa represented in all datasets were included. All combinations of datasets were evaluated with an incongruence length difference (ILD) test, the partition homogeneity test, in PAUP\* 4.0b10 (Swofford, 2002) (1000 replicate heuristic search, tree-bisection-reconnection (TBR), 10 trees held at each step, MulTrees off). Resulting *P* values were assessed using the standard of Cunningham (1997) ( $p > 0.01$ ). Only those datasets with an ILD test p-value greater than 0.01 were combined.

Parsimony analysis was performed using a heuristic search to generate 1000 replicates of random taxon addition using equal (Fitch) weights and tree-bisection-reconnection (TBR) branch swapping, 10 trees held at each step, MulTrees on, and saving only the shortest trees or the shortest from each replicate. The resulting trees were used as starting trees in another round of TBR with the same parameters as the first and swapping on all trees. Gaps were coded as missing data and branches with a minimum length of zero were collapsed. In order to more effectively locate the optimal tree for these large datasets, the Parsimony Ratchet of Nixon (1999) was implemented in PAUP\*

4.0b10 (Swofford, 2002) using PAUPRat beta version 1 (Sikes and Lewis, 2001) to generate the batch command files. Twenty searches of 200 Ratchet iterations were run for each dataset. A strict consensus of the optimal trees from all 20 searches was then generated using PAUP\* 4.0b10 (Swofford, 2002). Support for tree topology was evaluated with 1000 bootstrap replicates using PAUP\* 4.0b10 (Swofford, 2002) (starting trees generated by random addition, 10 replicates, holding one tree from each step).

Morphological and anatomical characters were coded as discrete and mapped onto the 50% bootstrap consensus *trnL*F tree in MacClade 4.06 (Maddison and Maddison 2003) using delayed transformation (DELTRAN) optimization (Fig. 3.12-3.18). This tree was selected for use in mapping characters because it includes the largest sampling of Anacardiaceae. Distribution and habitat were also mapped onto this tree (Figs. 3.14 and 3.18).

For the maximum likelihood analysis, an appropriate nucleotide substitution model was identified using a hierarchical likelihood-ratio test implemented in Modeltest 3.06 PPC (Posada and Crandall, 1998) for selection of the best-fit model. The TVM+G model, which assumes unequal base frequencies, unequal transition/transversion rates, and among-site rate heterogeneity, provided the best explanation of the data. Heuristic maximum likelihood searches were done using PAUP\* 4.0b10 (Swofford, 2002). Branch length were estimated using the TVM+G model under the parameters obtained from Modeltest: an estimated transition-transversion ratio, estimated base

frequencies, among-site rate heterogeneity approximated by a discrete gamma distribution with four rate classes and a shape parameter of 0.7342 for *matK*, 0.5926 for *trnLF*, and 0.7687 for *rps16*.

## Results

***matK*** □ Parsimony analysis resulted in 40 most parsimonious trees of 1049 steps each, a consistency index (CI) of 0.812, a consistency index excluding uninformative characters (RC) of 0.700, homoplasy index (HI) of 0.188, and a retention index (RI) of 0.862. The 50% bootstrap consensus tree is shown in Figure 3.3. The maximum likelihood tree (log likelihood = -9778.12254) is shown in Figure 3.4. GenBank accession numbers are shown in Appendix A.

***rps16*** □ Parsimony Ratchet analysis resulted 2112 most parsimonious trees of 668 steps each, a CI of 0.768, RC of 0.638, HI of 0.232, and a RI of 0.830. The 50% bootstrap consensus tree is shown in Figure 3.5. The maximum likelihood tree (log likelihood = -5670.68668) is shown in Figure 3.6. GenBank accession numbers are shown in Appendix A.

***trnLF*** □ Parsimony Ratchet analysis resulted in 233 most parsimonious trees of 488 steps each, a CI of 0.779, RC of 0.703, HI of 0.221, and a RI of 0.903. The 50% bootstrap consensus tree is shown in Figure 3.7. The maximum likelihood

tree (log likelihood = -4875.71146) is shown in Figure 3.8. GenBank accession numbers are shown in Appendix A.

**Combined *rps16* and *trnLF*** □ Partition homogeneity tests of the combined matrix showed no significant difference in the phylogenetic signal in the two regions (p-value = 0.64), thus they were combined and analyzed together. Maximum parsimony analysis resulted in 132,589 trees of 1217 steps each, a CI of 0.788, a RC of 0.676, HI of 0.212, and a RI of 0.858. The 50% bootstrap consensus tree is shown in Figure 3.9.

**Combined *matK*, *rps16* and *trnLF*** □ Partition homogeneity tests of the combined matrix showed no significant difference in the phylogenetic signal in the two regions (*matK* vs. *rps16* vs. *trnLF*, p-value = 0.900), thus they were combined and analyzed together. No other combinations of *matK* were run because the partition homogeneity tests showed *matK* vs. *trnLF* (p-value = 0.001) and *matK* vs. *rps16* (p-value = 0.001) to have significantly different phylogenetic signal. Maximum parsimony analysis of the *matK*, *rps16*, and *trnLF*-combined dataset resulted in 16 trees of 1083 steps each, a CI of 0.849, a RC of 0.753, HI of 0.151, and a RI of 0.887. The 50% bootstrap consensus tree is shown in Figure 3.10.

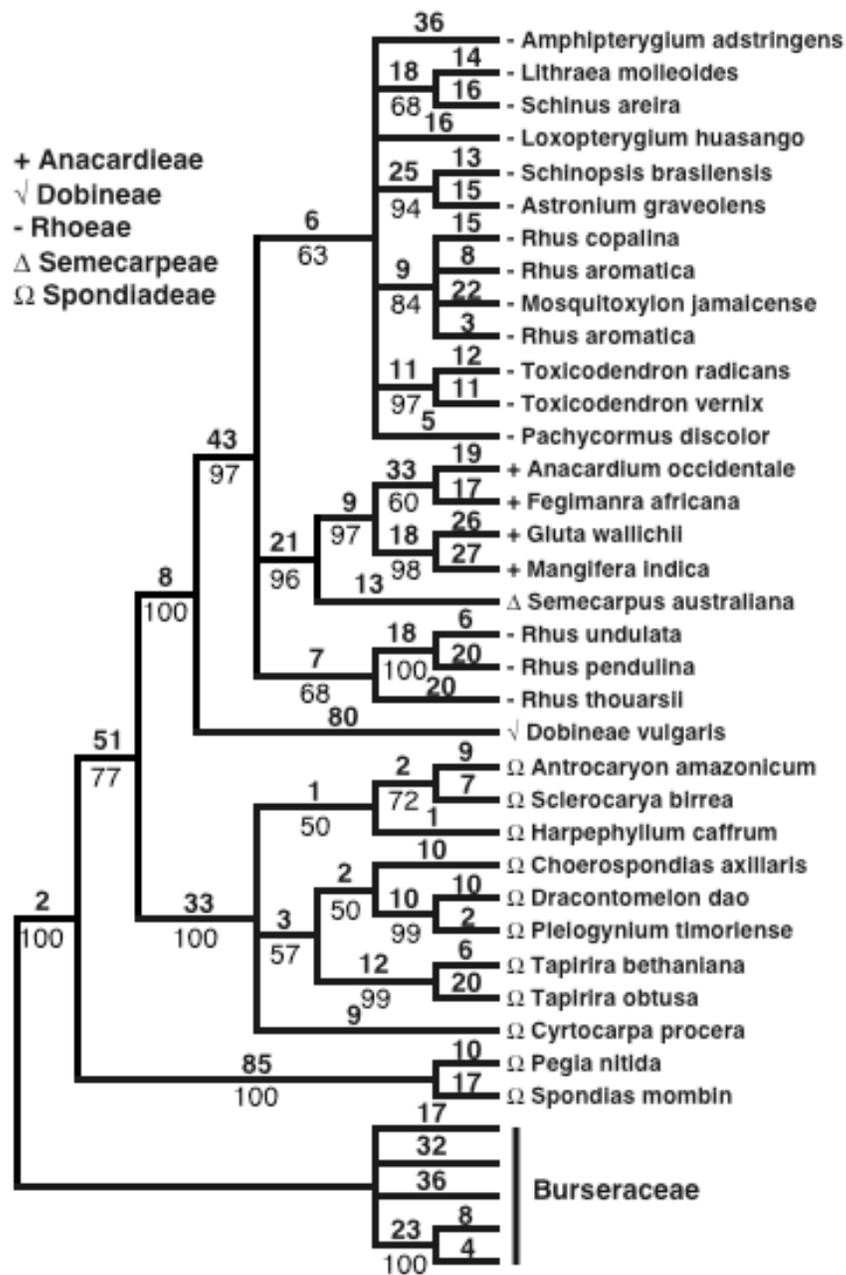


Figure 3.3. Bootstrap consensus phylogeny resulting from phylogenetic analysis of *matK* sequences of 33 Anacardiaceae ingroup and 5 Burseraceae outgroup taxa with bootstrap support ( $\geq 50\%$ ) indicated below branches and branch lengths indicated in bold above branches (1049 steps, CI=0.812, RC=0.700, RI=0.862, HI=0.188). Present Anacardiaceae tribal affiliations are indicated by symbols preceding taxon names.

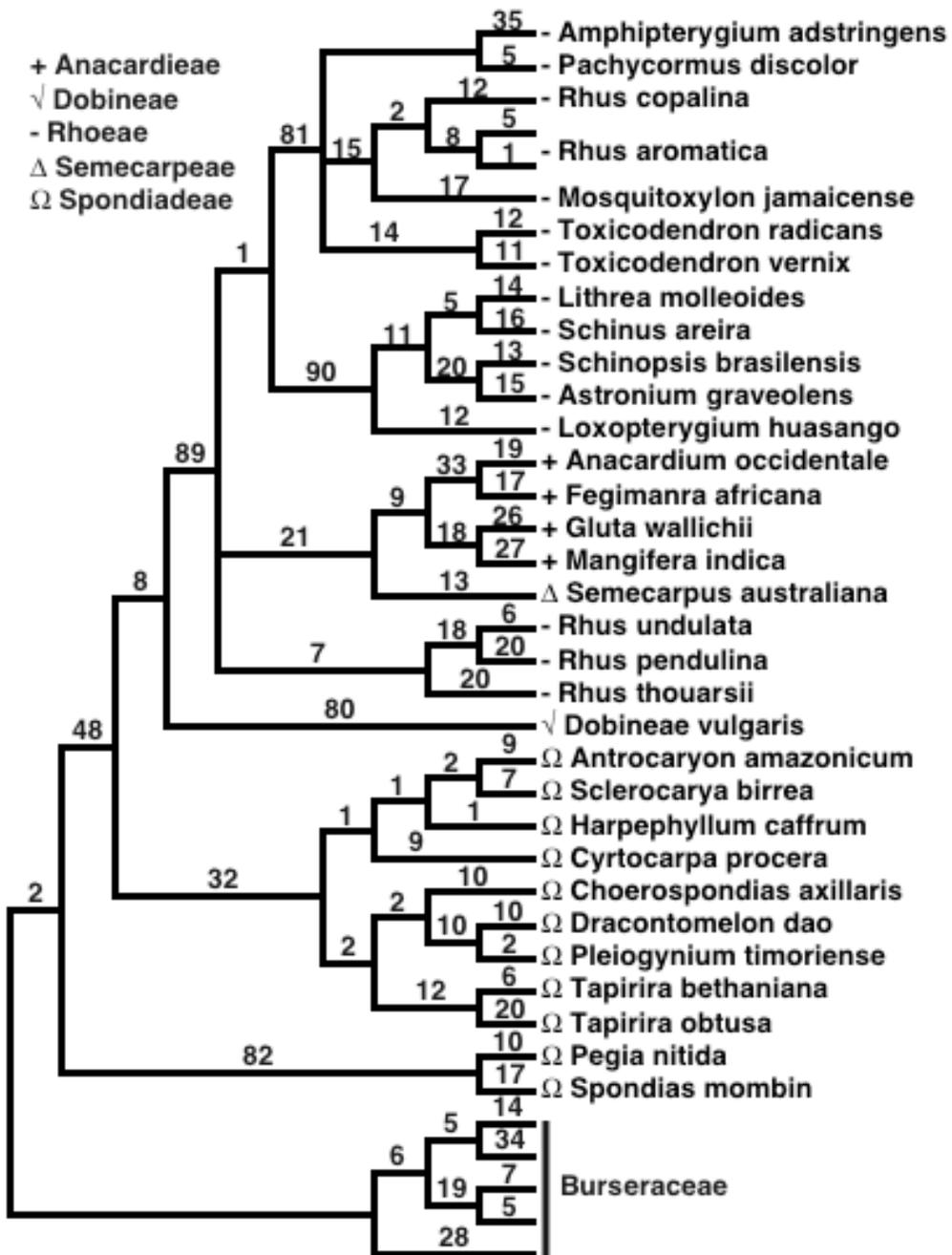
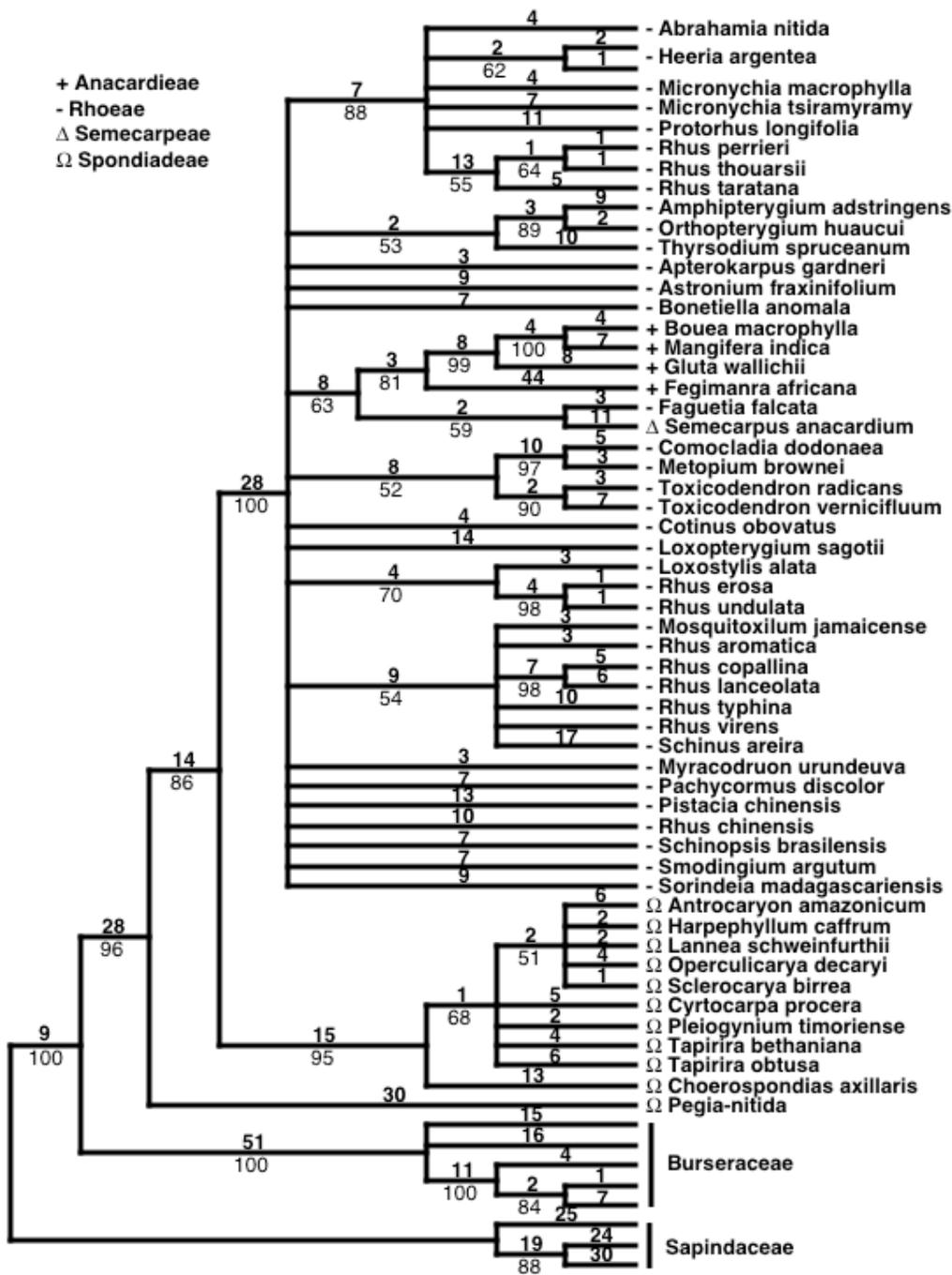


Figure 3.4. Maximum likelihood tree resulting from phylogenetic analysis of *matK* sequences of 33 Anacardiaceae ingroup and 5 Burseraceae outgroup taxa (likelihood score 9778.12254, substitution model = TVM+G). Branch lengths are shown above branches. Present Anacardiaceae tribal affiliations are indicated by symbols preceding taxon names.

Fig 3.5. Bootstrap consensus phylogeny resulting from phylogenetic analysis of *rps16* sequences of 55 Anacardiaceae and 5 Burseraceae ingroup and 3 Sapindaceae outgroup taxa with bootstrap support ( $\geq 50\%$ ) indicated below branches and branch lengths indicated in bold above branches (668 steps, CI=0.768, RC=0.638, RI=0.830, HI=0.232). Present Anacardiaceae tribal affiliations are indicated by symbols preceding taxon names.



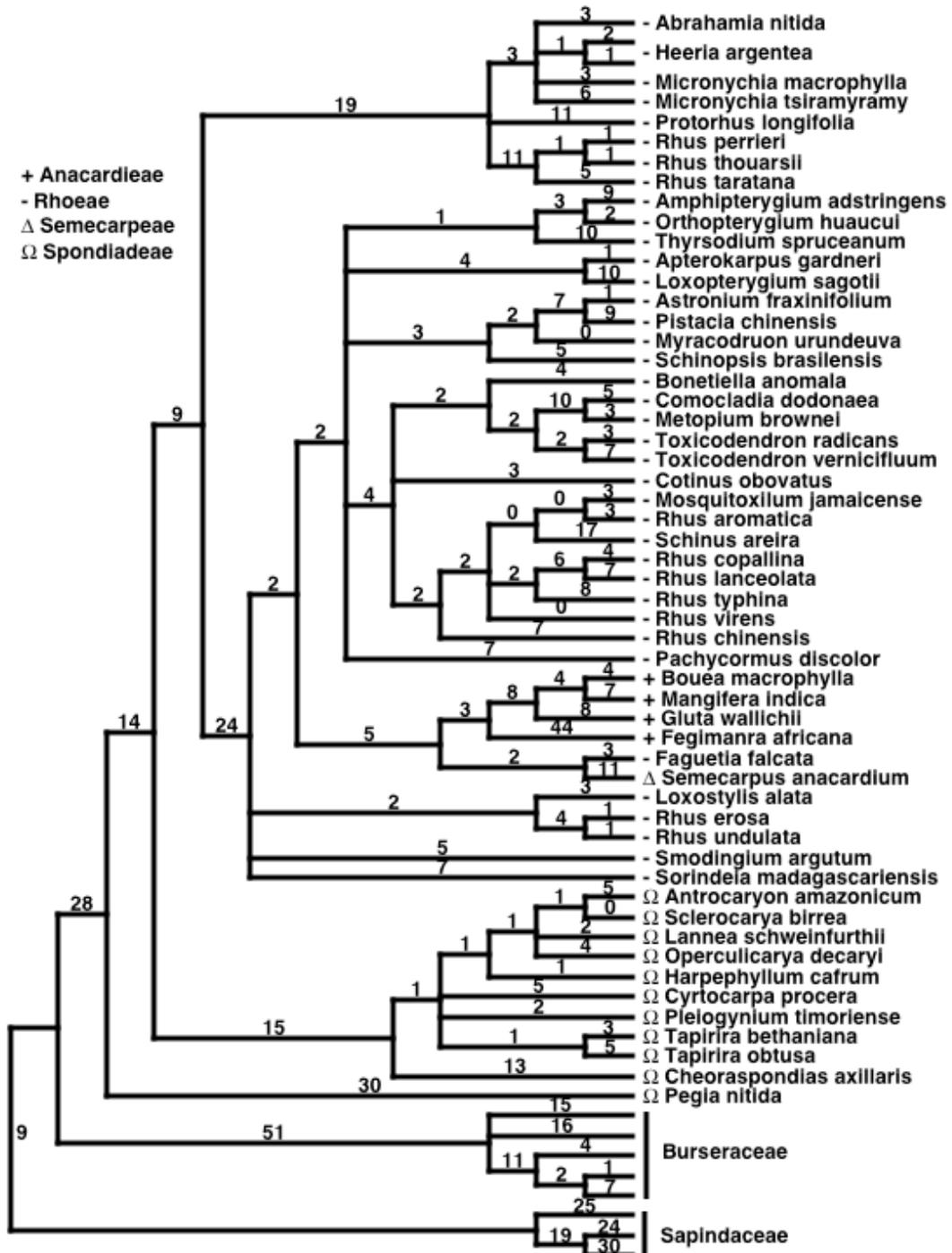


Figure 3.6. Maximum likelihood tree resulting from phylogenetic analysis of *rps16* sequences of 55 Anacardiaceae and 5 Burseraceae ingroup and 3 Sapindaceae outgroup taxa (likelihood score of 5670.68668, substitution model = TVM+G). Branch lengths are shown above branches. Present Anacardiaceae tribal affiliations are indicated by symbols preceding taxon names.

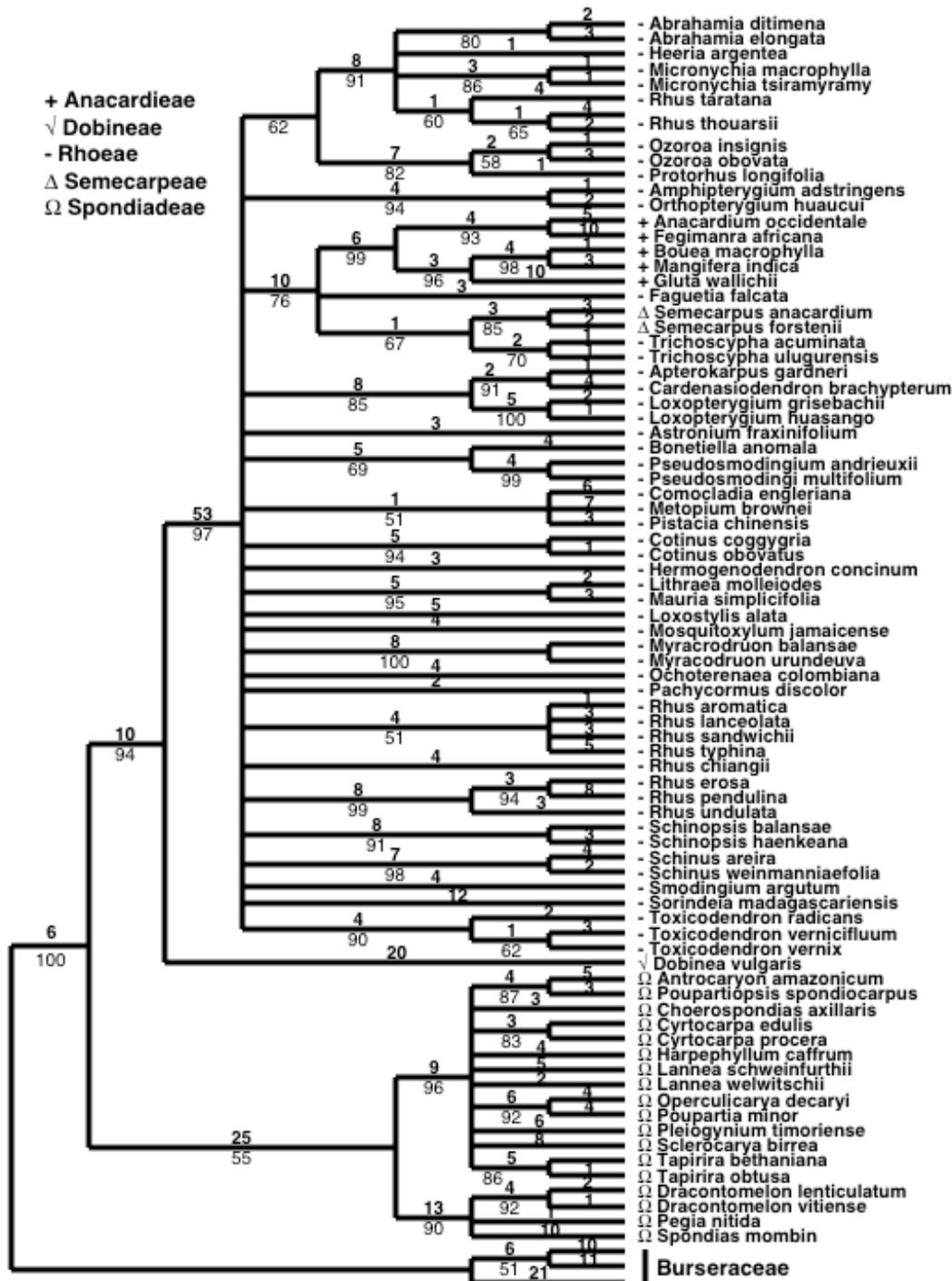
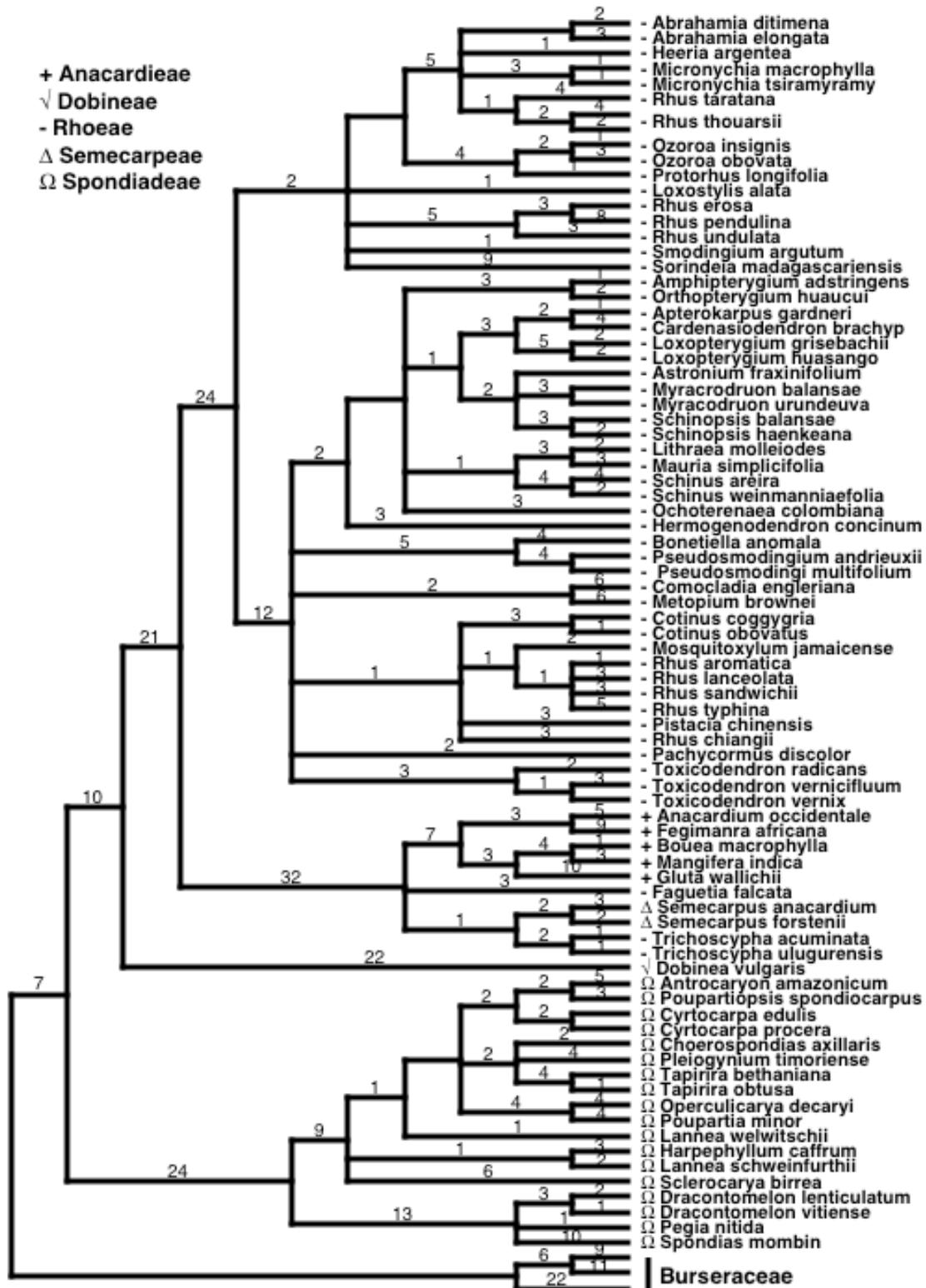


Fig 3.7. Bootstrap consensus phylogeny resulting from phylogenetic analysis of *trnL*F sequences of 81 Anacardiaceae ingroup and 3 Burseraceae outgroup taxa with bootstrap support ( $\geq 50\%$ ) indicated below branches and branch lengths indicated in bold above branches (488 steps, CI=0.779, RC=0.703, RI=0.903, HI=0.221). Present Anacardiaceae tribal affiliations are indicated by symbols preceding taxon names.

Figure 3.8. Strict consensus of 3 maximum likelihood trees resulting from phylogenetic analysis of *trnL*F sequences of 81 Anacardiaceae ingroup and 3 Burseraceae outgroup taxa (likelihood score 4875.71146, substitution model = TVM+G). Branch lengths are indicated above branches. Present Anacardiaceae tribal affiliations are indicated by symbols preceding taxon names.



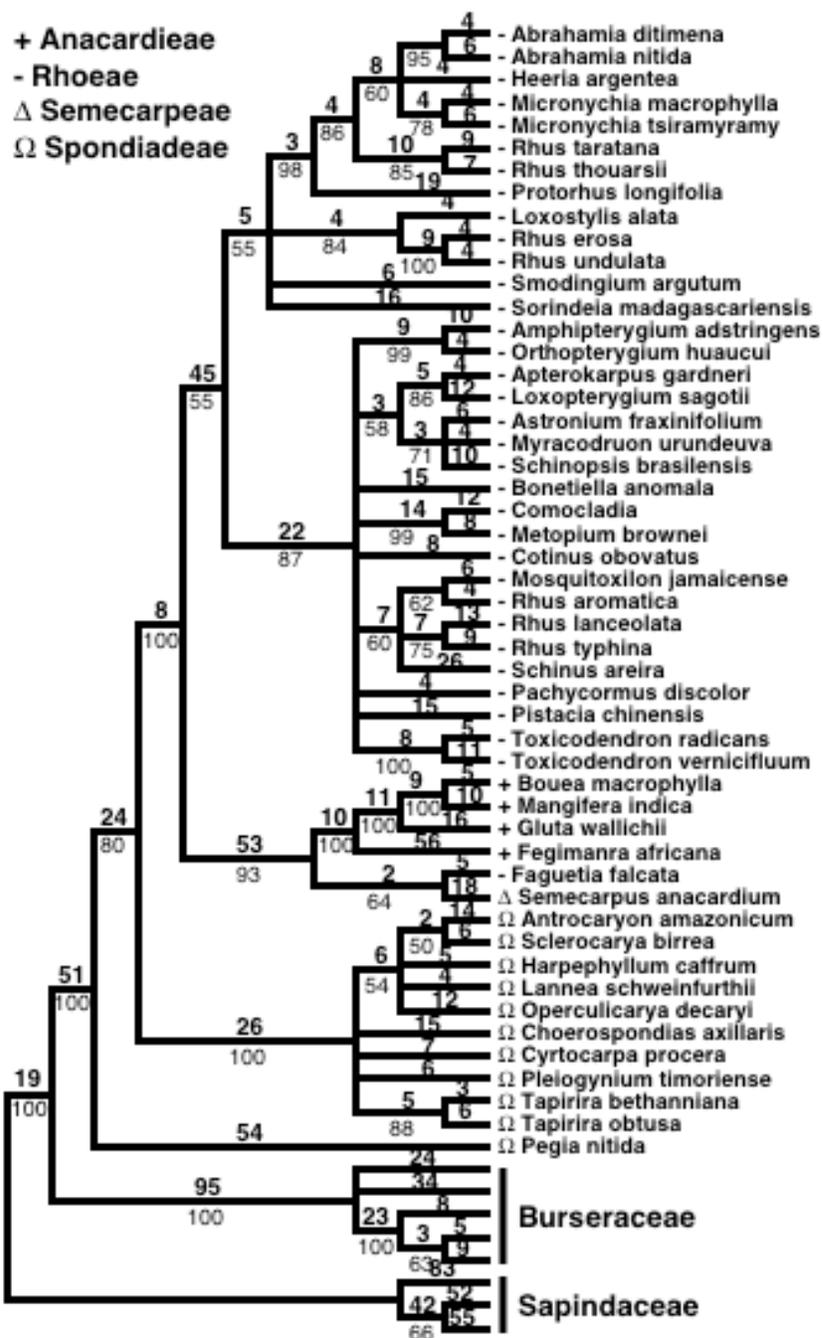


Figure 3.9. Bootstrap consensus phylogeny resulting from phylogenetic analysis of combined *rps16* and *trnL*F sequences of 50 Anacardiaceae and 5 Burseraceae ingroup and 3 Sapindaceae outgroup taxa (1217 steps, CI=0.788, RC=0.676, RI=0.858, HI=0.212). Bootstrap support ( $\geq 50\%$ ) indicated below branches and branch lengths indicated in bold above branches. Present Anacardiaceae tribal affiliations are indicated by symbols preceding taxon names.

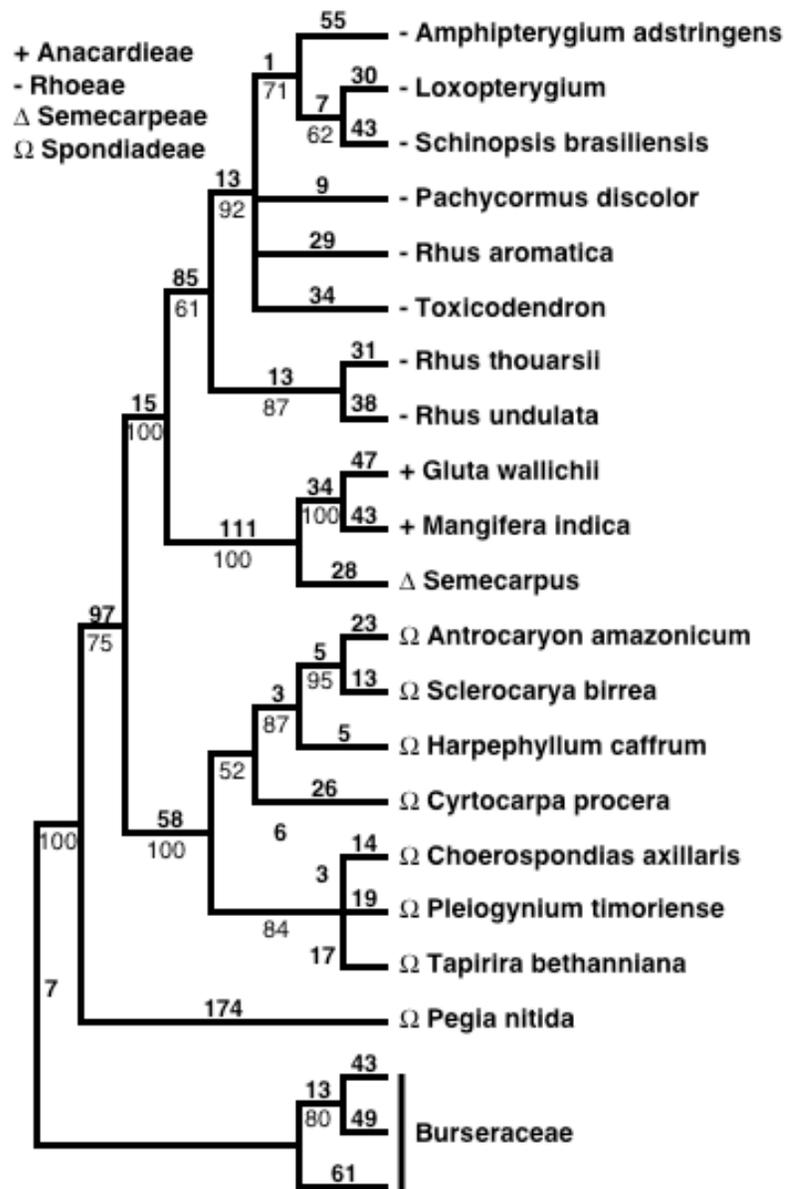
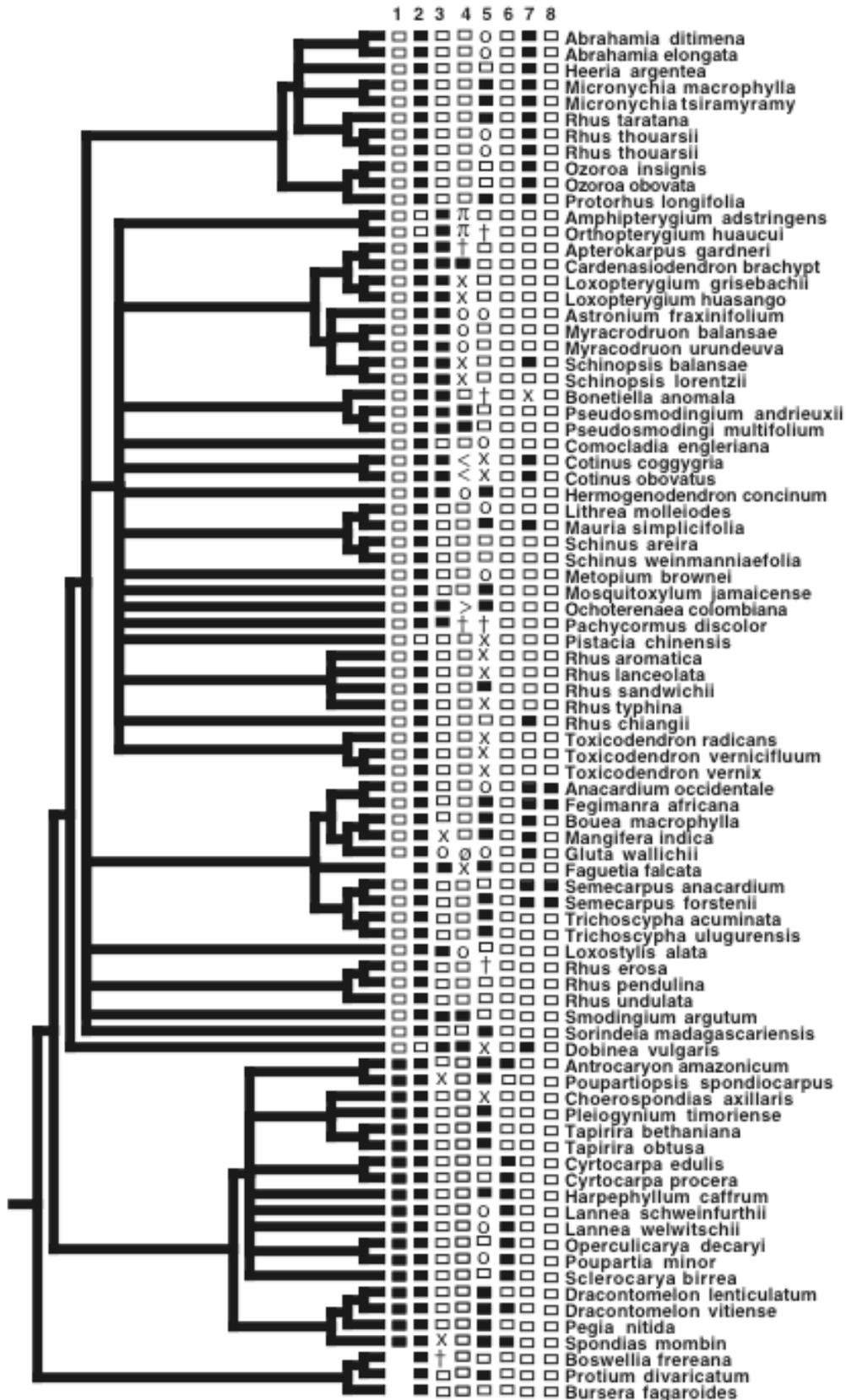


Figure 3.10. Bootstrap consensus phylogeny resulting from phylogenetic analysis of combined *matK*, *rps16*, and *trnLF* sequences of 19 Anacardiaceae ingroup and 3 Burseraceae outgroup taxa (1083 steps, CI=0.849, RC=0.753, RI=0.887, HI=0.151). Bootstrap support ( $\geq 50\%$ ) indicated below branches and branch lengths indicated in bold above branches. Present Anacardiaceae tribal affiliations are indicated by symbols preceding taxon names.

Figure 3.11. Eight morphological, biochemical, and anatomical characters mapped onto the *trnL*F bootstrap consensus phylogeny shown in Fig. 3.7. Coding is as follows: (1) endocarp, white box=*Anacardium*-type, black box=*Spondias*-type, missing box=unknown; (2) perianth, white box=reduced, black box=not reduced; (3) dispersal (all but cross symbol refer to fruit dispersal), white box=animal, black box=wind, x=polymorphic with both animal and water dispersal, cross=seeds wind dispersed, white circle=polymorphic with both animal and wind dispersal; (4) wind dispersed fruit type, white box=none, black box=elm-like samara, x=samaroid with single elongated wing, cross=small dry wingless fruit, white circle=drupe with wings of sepals, pi=dry syncarp, greater than sign=flattened fruit with trichome covered margins, less than sign=inflorescence wind dispersed in tumbleweed fashion, null sign=drupe with wings of petals. (5) habitat, white box=tropical dry forest, black box=tropical moist to wet forest, cross=desert, x=temperate forest, white circle=polymorphic and occurring in both tropical dry and wet forests; (6) opercula, white box=absent, black box=present; (7) leaf complexity, white box= compound, black box=simple or unifoliolate, x=polymorphic with both compound and unifoliolate leaves; (8) hypocarp, white=absent, black=present. See text for discussion of the characters.



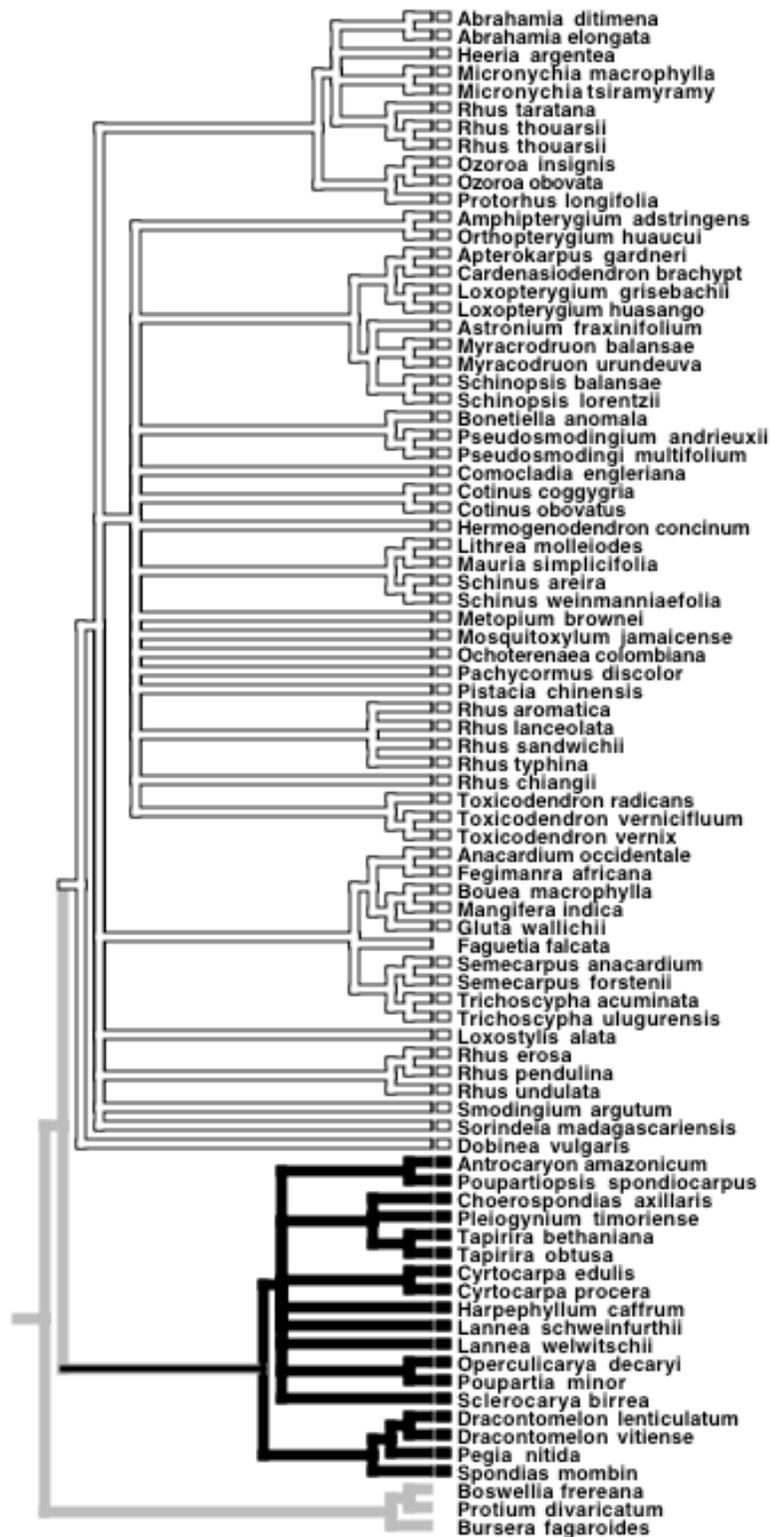


Figure 3.12. Endocarp organization mapped onto the *trnLF* bootstrap consensus phylogeny shown in Fig. 3.7. White=*Anacardium*-type, black=*Spondias*-type, missing box=unknown, grey line=equivocal.

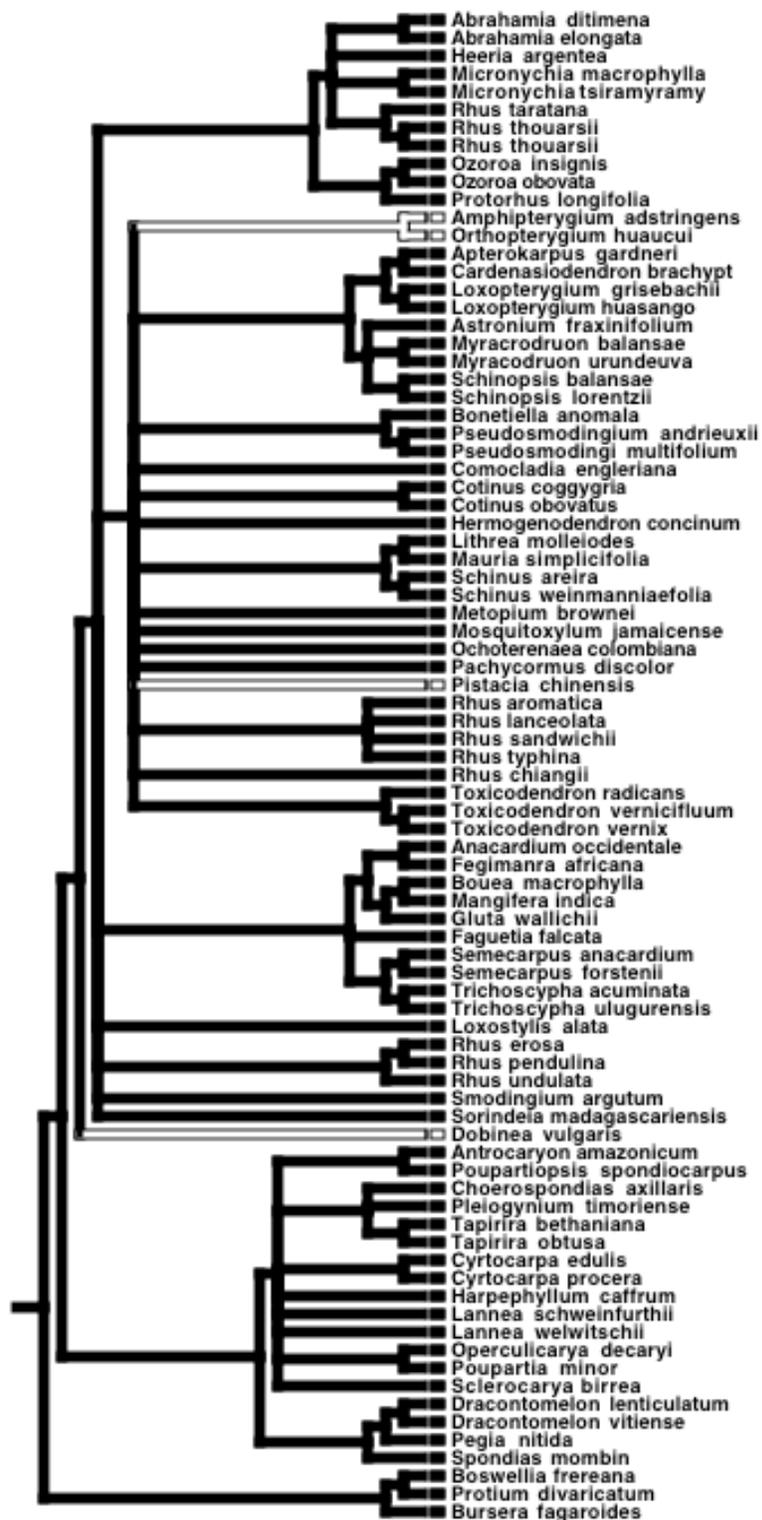
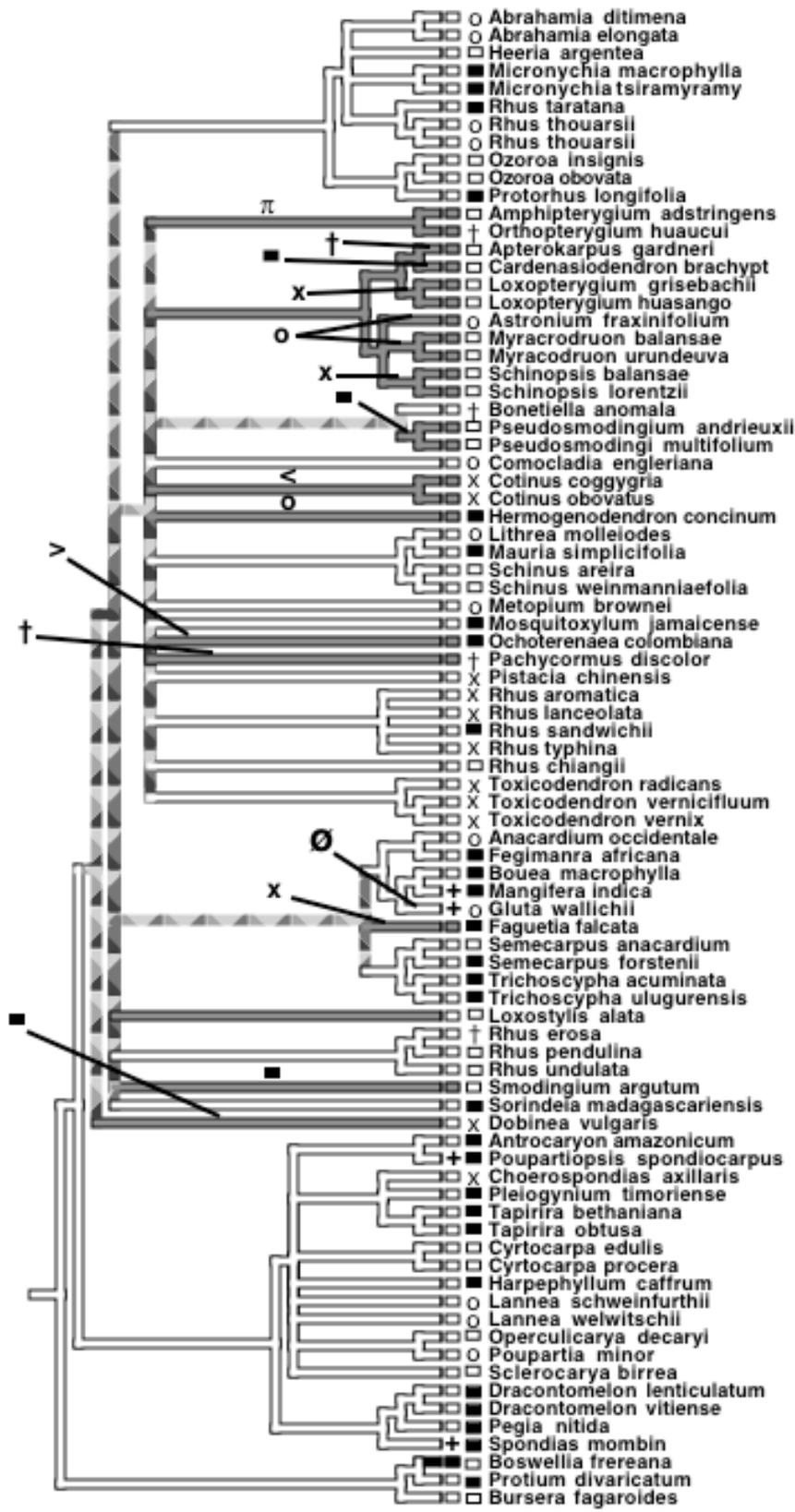


Figure 3.13. Perianth structure mapped onto the *trnL*F bootstrap consensus phylogeny shown in Fig. 3.7. White=reduced, black=not reduced.

Figure 3.14. Dispersal and mechanisms of wind dispersal mapped onto the *trnL*F bootstrap consensus phylogeny shown in Fig. 3.7. Habitat is indicated directly to the left of each taxon name. Characters and habitat are mapped or indicated as follows: dispersal (all but cross symbol refer to fruit dispersal), white box=animal, black box=wind, x=polymorphic with both animal and water dispersal, cross=seeds wind dispersed, white circle=polymorphic with both animal and wind dispersal; wind dispersed fruit type, missing symbol=none, black box=elm-like samara, x=samaroid with single elongated wing, cross=small dry wingless fruit, white circle=drupe with wings of sepals, pi=dry syncarp, greater than sign=flattened fruit with trichome-covered margins, less than sign=inflorescence wind dispersed in tumbleweed fashion, null sign=drupe with wings of petals; habitat, white box=tropical dry forest, black box=tropical moist to wet forest, cross=desert, x=temperate forest, white circle=polymorphic and occurring in both tropical dry and wet forests.



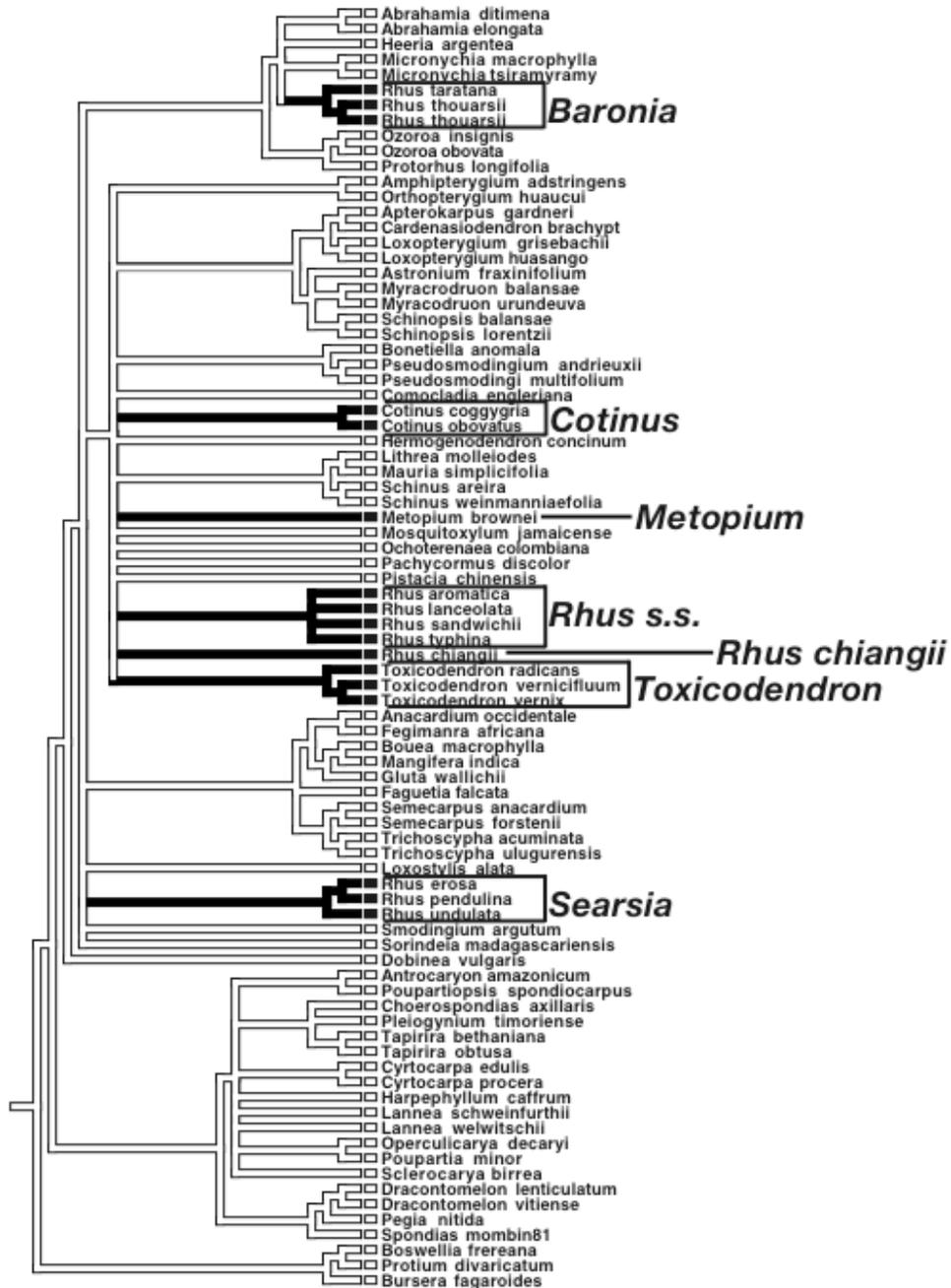


Figure 3.15. Genera belonging to the *Rhus* complex highlighted on the *trnLF* bootstrap consensus phylogeny shown in Fig. 3.7. *Searsia*, *Baronia*, *Rhus chiangii* and *Rhus* s.s. are all referred to as *Rhus* in the text.

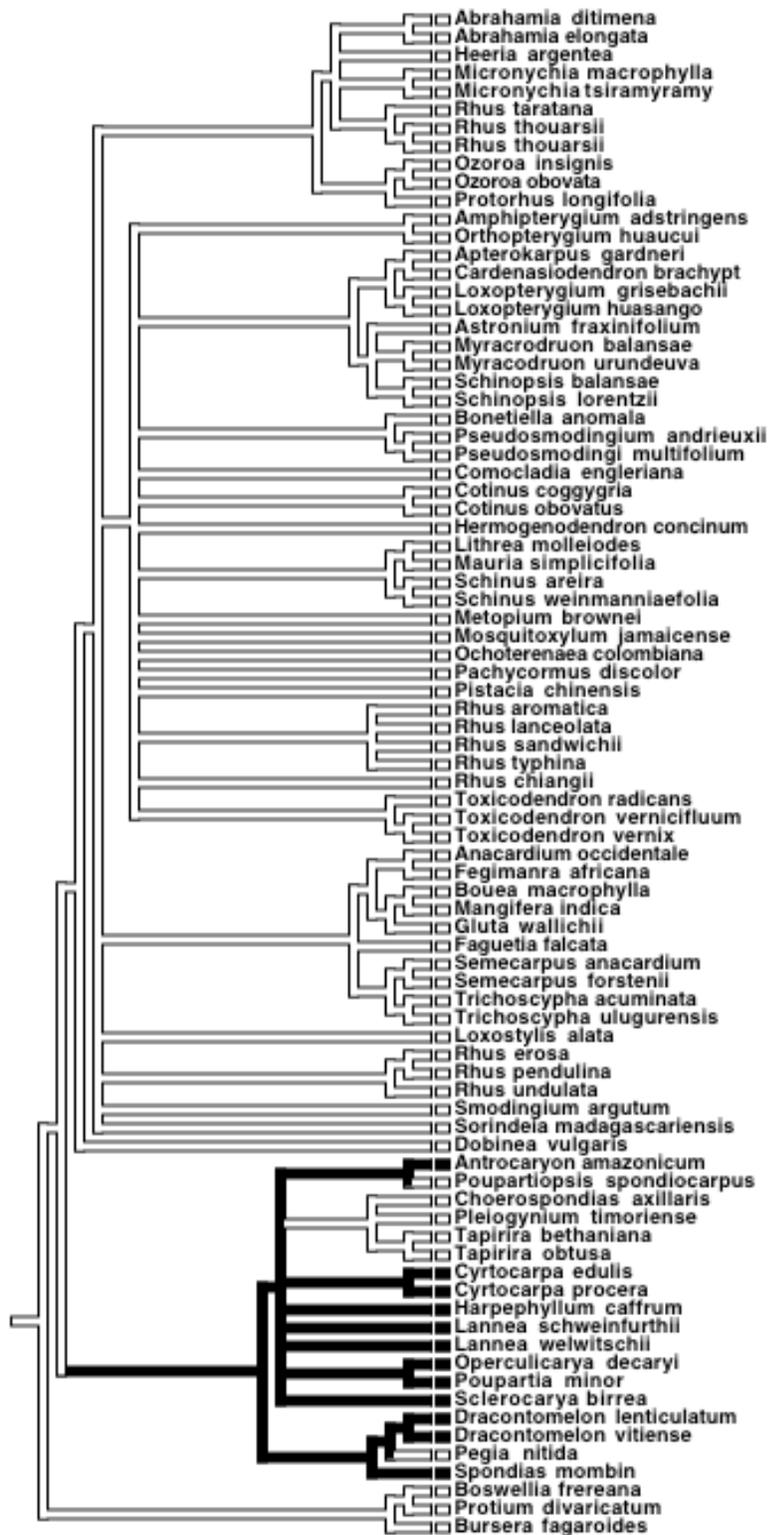


Figure 3.16. Opercula presence and absence mapped onto the *trnL*F bootstrap consensus phylogeny shown in Fig. 3.7. White=absent, black=present;

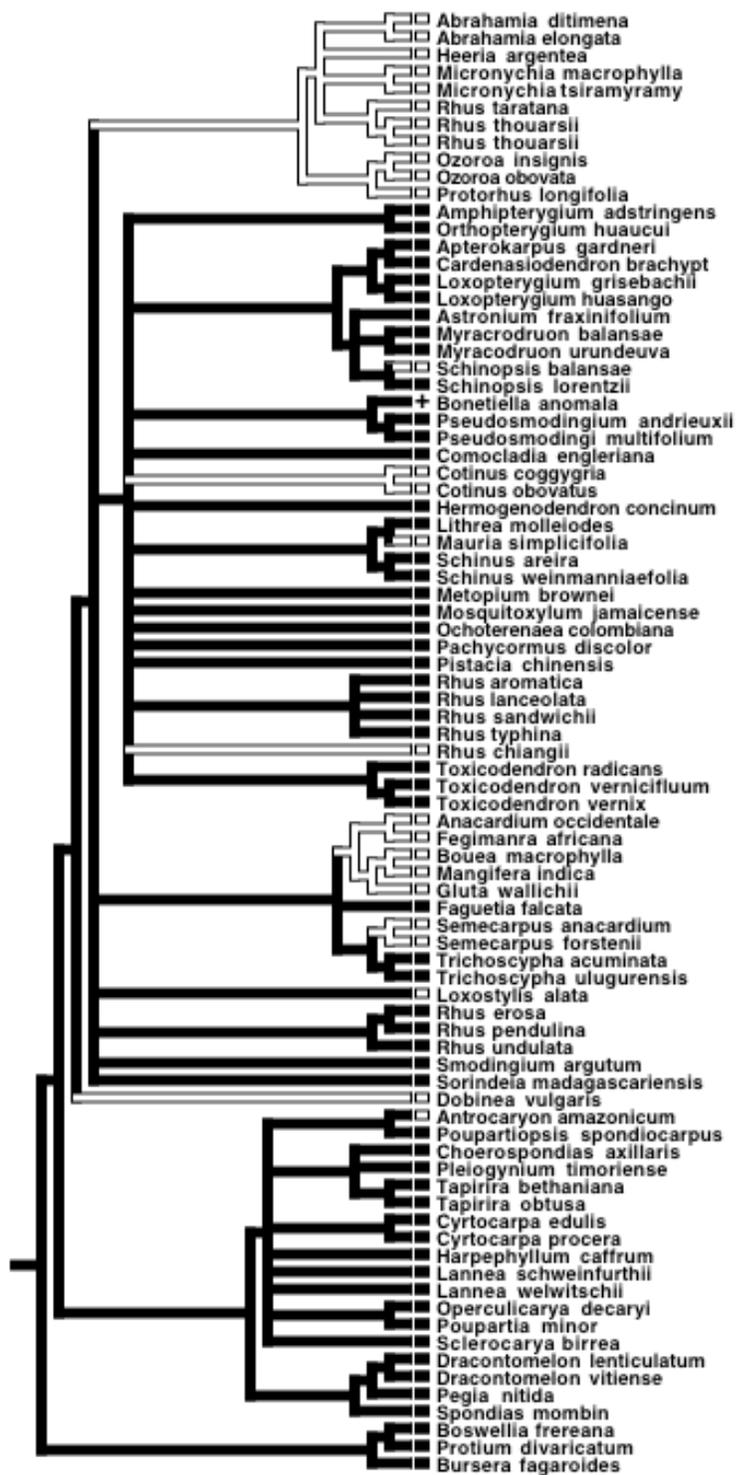
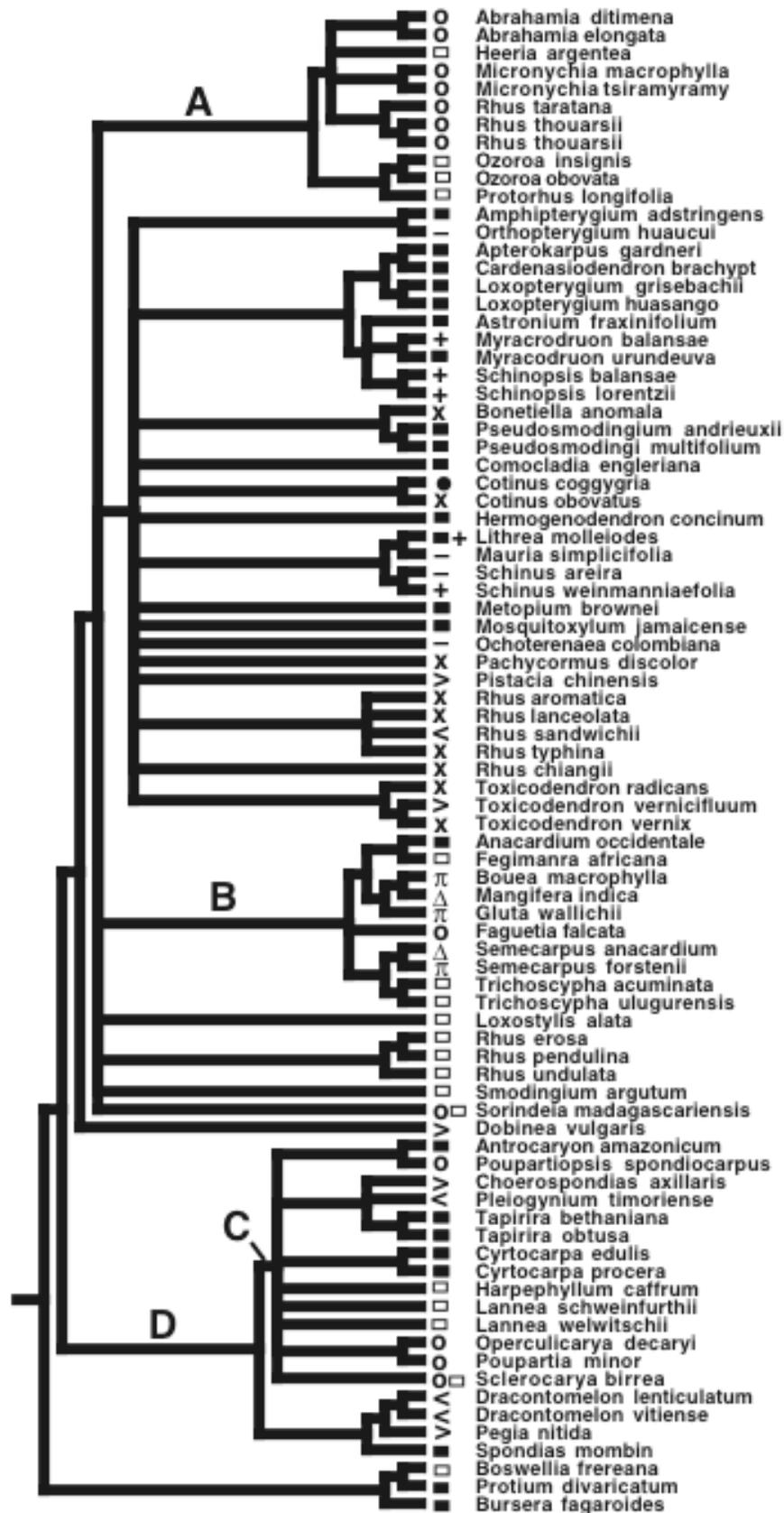


Figure 3.17. Leaf complexity mapped onto the *trnL*F bootstrap consensus phylogeny shown in Fig. 3.7. White = simple or unifoliate, black = compound, plus sign = polymorphic with both compound and unifoliate leaves.

Figure 3.18. Taxon distributions mapped onto the *trnL*F bootstrap consensus phylogeny shown in Fig. 3.7. Coding is as follows: white box=sub-Saharan Africa, black box=Neotropical, x=North American temperate, black circle=temperate Eurasia, pi=Southeast Asia including Malaysia, slash=Andean, greater than sign=east Asia-Himalayan, less than sign=Oceania-Pacific Islands, triangle=Indian subcontinent, white circle=Madagascar, plus sign=South American temperate. See text for a discussion of the labeled clades.



**Relationships** □ Anacardiaceae contains two clades in the *trnLF* phylogenies (Figs. 3.7 and 3.8). In the *trnLF* cladogram, the lower Anacardiaceae clade contains all of the sampled Spondiadeae genera, *Antrocaryon* Pierre, *Choerospondias* B. L. Burt & A. W. Hill, *Cyrtocarpa* H. B. & K., *Dracontomelon* Blume, *Harpephyllum* Bernh. ex Krauss, *Lannea* A. Rich., *Operculicarya* H. Perrier, *Pegia* Coleb., *Pleiogynium* Engl., *Poupartia* Comm. ex Juss., *Poupartiopsis* ined., *Sclerocarya* Hochst., *Spondias*, and *Tapirira* (55% bootstrap, branch length of 25, Fig. 3.7). A monophyletic Spondiadeae is not elucidated in the other phylogenies (Figs. 3.3 – 3.6 and 3.9 – 3.10). The tribe is instead split into two lineages, one containing *Pegia* (Figs. 3.5, 3.6, 3.9 and 3.10) or *Pegia* and *Spondias* (Figs. 3.3 and 3.4) at the base of the family, and the other containing the remaining members of the tribe in a clade sister to the rest of the family (Figs. 3.3 - 3.6 and 3.9 – 3.10).

Anacardieae, Dobineae (only represented in the *matK*, Figs. 3.3 and 3.4, and *trnLF* datasets, Figs. 3.5 and 3.7), Rhoeeae, and Semecarpeae are together monophyletic within the Anacardiaceae (Figs. 3.3 – 3.10). In addition to having overall sequence similarity, this clade was supported by numerous indels in *matK* (eight-base, seven-base, and two six-base indels), *rsp16* (three-base and seven-base indels), and *trnLF* (four-base, six-base, 11-base, and 14-base indels). The same indels are a symplesiomorphy for tribe Spondiadeae and Burseraceae. An endocarp of regularly arranged layers (*Anacardium* type) is probably a synapomorphy for this large clade of four tribes, but this cannot be definitively

shown because the character state is unknown for the included Burseraceae (Fig. 3.11, number 1 and Fig 3.12). However, the endocarp of one Burseraceae genus, *Canarium*, has been investigated and was described as similarly lacking in structure (Wannan and Quinn, 1990) (*Spondias* type) as those of tribe Spondiadeae. Therefore, it is possible that the *Spondias* type of endocarp is symplesiomorphic for Spondiadeae.

*Dobinea* Buch.-Ham. ex D. Don represents tribe Dobineae and is sister to the clade containing members of Rhoeeae, Anacardieae, and Semecarpeae (Figs. 3.3, 3.4, 3.7, and 3.8). This genus has unique sequence autapomorphies that distinguish it, including two five-base indels in *trnLF*. *Dobinea*, tribe Spondiadeae and Burseraceae share several sequence symplesiomorphies including a six-base indel in *matK* and an approximately 117 base indel in *trnLF*. The large *trnLF* indel is somewhat variable (15 base pairs are variable in at least one taxon and the area is interrupted by several smaller gaps including one-base, two-base, six-base, and eight-base indels) but is easily aligned and is absent in all other taxa. *Dobinea*, *Amphipterygium*, *Orthopterygium*, and *Pistacia* all have morphological adaptations for wind pollination but these only appear to be synapomorphies for *Amphipterygium* and *Orthopterygium* (Fig. 3.11, numbers 2 and 3 and Figs. 3.13). Specific mechanisms for wind dispersal are mapped in Figure 3.14, which shows the dry syncarps of *Amphipterygium* and *Orthopterygium* to be the only synapomorphic wind-dispersed fruit type.

Anacardieae is monophyletic in all analyses. It is variously sister to Semecarpeae (*matK*, Figs. 3.3 and 3.4; and combined *matK*, *rps16* and *trnLF*, Fig. 3.10), a clade of Semecarpeae and *Faguetia* March. (*rps16*, Figs. 3.5 and 3.6; *matK*, Figs. 3.3 and 3.4; *rps16*–*trnLF*, Fig. 3.9) or in a clade with Semecarpeae and several members of Rhoeeae (*trnLF*, Figs. 3.7 and 3.8). Rhoeeae is paraphyletic in all analyses with *Trichoscypha* Hook. f. (*trnLF*, Figs. 3.7 and 3.8) and/or *Faguetia* (*rps16*, Figs. 3.5 and 3.6; *trnLF*, Figs. 3.7 and 3.8; and combined *rps16*–*trnLF*, Fig. 3.9) falling outside of the rest of the tribe in a well-supported clade with Anacardieae and Semecarpeae. The combined *matK*, *rps16* and *trnLF* phylogeny (Fig. 3.10) is the only topology that has a monophyletic Rhoeeae, most likely reflective of the extremely small sampling of this tribe (seven of 47 genera).

*Rhus* L. in its broad sense (including several other currently recognized segregate genera: *Actinocheita* F. A. Barkley, *Cotinus* Mill., *Metopium* P.Br., and *Toxicodendron* L.) (Fig 3.15) is polyphyletic. *Toxicodendron* (represented in the datasets variously by *T. radicans* Kuntze, *T. vernicifluum* (Stokes) F.A. Barkley, and/or *T. vernix* (L.) Kuntze) is monophyletic and separate from *Rhus sensu stricto* (including *Rhus* subgenus *Rhus* and *Rhus* subgenus *Lobadium*) (Figs. 3.3-3.9). *T. radicans* and *T. vernicifluum* (92% bootstrap, Fig. 3.5) share a 10-base indel in *rps16*.

*Rhus* subgenus *Rhus* (represented variously by *R. chinensis* Mill., *R. copallina* L., *R. sandwichii* A. Gray, *R. typhina* L., and/or *R. lanceolata* (A. Gray)

Britton) and *Rhus* subgenus *Lobadium* (represented variously by *Rhus aromatica* Aiton and/or *R. virens* Lindh. ex A. Gray) are evolutionary distinct from each other in the *rps16* (Figs. 3.5 and 3.6) and *rps16-trnLF* (Fig. 3.9) and internally unresolved but, together, monophyletic in the *trnLF* phylogeny (Figs. 3.7 and 3.8). The clade including *Mosquitoxylum* Krug & Urb., *Rhus* subgenus *Lobadium*, *Rhus* subgenus *Rhus*, and *Schinus* L. in the *rps16* phylogeny (Fig. 3.5) is supported by 66% bootstrap and an 18-base indel synapomorphy. Within this clade, the clade of *R. copallina* and *R. lanceolata* (64% bootstrap) share a 14-base indel synapomorphy. *Baronia* Baker (*R. taratana* (Baker) H. Perrier and *Rhus thouarsii* (Engl.) H. Perrier) and *Searsia* F. A. Barkley (*Rhus undulata* Jacq., *R. pendulina* Jacq., and *R. erosa* Thunb.) also are distinct from *Rhus s.str.* in all phylogenies (Figs 3.3-3.10). These two *Rhus* segregates are separated from each other in all of the large-sample phylogenies (*rps16*, *trnLF*, and *rps16 - trnLF*) (Figs. 3.5-3.9).

The evolution of wind-dispersed fruits is homoplasious and this trait unites several smaller clades scattered throughout the topology (Fig. 3.11, number 3 and Fig. 3.14). It is primarily associated with dry habitats (Fig. 3.11, number 5 and Fig 3.14). Specific mechanisms of wind dispersal have a similar pattern on the tree (Fig. 3.11, number 4 and Fig. 3.14). One mechanism, dry syncarps, is a synapomorphy for the former Julianiaceae (*Amphipterygium* and *Orthopterygium*).

Opercula are sealing caps in the endocarp that have an important role in the seed germination of some members of tribe Spondiadeae and are a synapomorphy for the tribe (Fig. 3.11, number 6 and Fig. 3.16). They are present in nine of the genera sampled for the *trnF* dataset. It is notable that opercula also appear to have been lost at least three times independently within the clade (Fig. 3.16).

Leaf complexity is a homoplasious character in Anacardiaceae and simple leaves are a synapomorphy for at least four clades (Fig. 3.17). The two clades of *Cotinus* and *Semecarpus*, the African/Madagascan clade at the top of the tree in Figure 3.17, and the Anacardieae clade all have simple leaves.

Current biogeographical distributions of the terminal taxa (reconstruction not shown) highlight several geographical features important for understanding past distributional patterns (Fig. 3.18). Clade A consists of Madagascan and African taxa and clade B contains Gondwanan taxa possibly spreading into Southeast Asia via India. Madagascan taxa appear on the tree in a minimum of three distinct clades (A, B, and C). Clade D contains all representatives of tribe Spondiadeae.

## Discussion

**Tribal affinities** □ Although it is weakly supported, a monophyletic Spondiadeae as is retained in the *trnLF* phylogenies is noteworthy (Figs. 3.7 and 3.8). This tribe is one of the morphologically better defined of Engler's tribes. Members

Spondiadeae generally have thickened endocarps, strongly differentiated exocarps and mesocarps, multilocular fruits, and most have four or more carpels that are generally connate with free styles (*Haematostaphis* Hook. f. has only three carpels and *Solenocarpus* Wight & Arn. only one but neither is represented in this study; Engler, 1883; Wannan, 1986) (Figs. 3.11, number 1 and Fig. 3.12). Wannan and Quinn (1990) describe two endocarp types that occur in the Anacardiaceae, the *Spondias*-type (mass of lignified and irregularly oriented sclerenchyma) and the *Anacardium*-type endocarp (discretely layered and with palisade-like sclereids) (Fig. 3.11, number 1 and Fig. 3.12). The *Spondias*-type is characteristic of the Spondiadeae (also Rhoeeae members *Buchanania* Spreng., *Camptosperma*, and *Pentaspadon* Hook.f.) and is also found in one genus in the Burseraceae, while the *Anacardium*-type characterizes the rest of the family (except *Buchanania*, *Camptosperma*, *Pentaspadon* and possibly others that have not been investigated) (Wannan and Quinn, 1990). Its placement at the base of the Anacardiaceae, close to the Burseraceae, is supported by a very similar endocarp structure shared by Spondiadeae and Burseraceae (Fig 3.11, number 1 and Fig. 3.12). The primary difference between the two is that the endocarp of the Burseraceae is slightly more stratified (Wannan, 1986).

Spondiadeae is the only tribe with ovules pendulous from an apical funicle and in which specialized seed germination structures called opercula occur (Fig 3.11, number 6 and Fig. 3.16). These opercula apparently are a synapomorphy

for the Spondiadeae (Fig. 3.16), although there have been subsequent losses of opercula later on in the evolution of the tribe. Eleven of the 20 genera in the tribe have been found to have opercula (*Antrocaryon*, *Cyrtocarpa*, *Dracontomelon*, *Haematostaphis*, *Harpephyllum*, *Lannea*, *Operculicarya*, *Pleiogynium*, *Pseudospondias* Engl., *Sclerocarya*, and *Spondias*). The soon-to-be described monospecific genus, *Poupartiopsis spondiocarpus* ined., has not yet been thoroughly investigated but opercula are apparently lacking in its thickened endocarp (Schatz, 2001). The germination of many of the other genera has been incompletely studied. Opercula are part of a specialized seed germination structure: they are the sealing caps of pits in the endocarp and vary from being quite woody to rather fleshy. Although most opercula are on the surface of the endocarp, two of the operculate genera, *Spondias* and *Harpephyllum*, have internal opercula. The seed is protected from desiccation and rot by the nearly impenetrable seal of the operculum that remains until the cap is pushed off by the growing embryo upon germination (Hill 1933, 1937). One genus, *Choerospondias*, although not considered to be operculate, does have pits in its endocarp but lacks the sealed caps: fibrous coverings occur over the pits instead. Despite the lack of resolution in the Spondiadeae clade, it is evident that upon further investigation that opercula may be an important morphological feature for defining this lineage (Fig. 3.11, number 6 and Fig. 3.16).

Anacardieae is found to be monophyletic in all analyses (Figs. 3.3-3.10), adding support to evidence provided by traditional taxonomic treatments that this

is a natural group. Members of this tribe are recognizable by having an ovule pendulous from a basal funicle; lateral, gynobasic styles; and simple leaves. Although individually these characters are symplesiomorphic in the family, they are collectively unique here (Engler, 1883; Wannan, 1986; Mitchell and Mori, 1987). Anacardieae is closely allied with Semecarpeae in all analyses (Figs. 3.3-3.10), a relationship supported by the presence of simple leaves (Figs. 3.17 and 3.11, number 7).

The two tribes are allied with each other and to Rhoeeae by the anatomy of their gynoecium and fruit. The Semecarpeae and Anacardieae and some members of Rhoeeae have the synapomorphy of lignification in the outer fruit epidermis, a feature that is absent in the Spondiadeae (Wannan and Quinn, 1990). Although Semecarpeae has three styles and core Anacardieae has only one, Copeland (1961) wrote that, "The vascular system of the pistil [of Anacardieae] is notably similar to those of the tricarpellate pistils of tribe Rhoideae [= Rhoeeae]. It is suggested... that the pistils of these genera are tricarpellate, but so reduced as to have the outward appearance of simple pistils." Thus it seems that Anacardieae and Semecarpeae originated from a tricarpellate ancestor. The loss of an intrastaminal disk can also be seen in this clade: Semecarpeae have an intrastaminal nectariferous disk while core Anacardieae do not (although small gland-like extrastaminal bumps or ridges have been reported in *Mangifera* and *Swintonia* Griff. which some authors presume to be remnants of a disk; Ding Hou, 1978).

It is difficult to draw conclusions about the monophyly of tribe Semecarpeae and impossible to do so for tribe Dobineae based on the current molecular data due to their limited sampling in the datasets. However, the placement of these two tribes is consistent and well supported by the datasets investigated thus far. *Dobinea* is sister to the large Anacardiaceae-Rhoeae-Semecarpeae clade and is not closely allied to any one genus (its purported sister genus, *Campylopetalum* Forman was not sampled for this study). This relatively isolated evolutionary position in the cashew family is consistent with the extremely unusual morphology of this tribe. The pistillate flowers lack a perianth and are adnate, by their pedicels, to a bract (Takhtajan, 1969, 1980, 1997; Hutchinson, 1973; Willis, 1973; Dahlgren, 1980; Watson & Dallwitz, 1992; Heng, 1994). As in other members of the family, the perianth being absent is most likely an adaptation for wind pollination.

Rhoeae's emergence as paraphyletic is consistent with the long-standing difficulty in defining this tribe. Morphological and anatomical characters vary widely across the group and overlap with those of other tribes (see Mitchell and Mori, 1987). Rhoeae is by far the largest tribe with 46 genera (of 82 in the family), which contributes to the problem of finding characteristic morphological and anatomical synapomorphies of the group.

The close affinity of Anacardiaceae and Semecarpeae and the inclusion in this clade of some members of tribe Rhoeae (*Faguetia* and *Trichoscypha*, Figs. 3.5-3.9) leaves the integrity of these three tribes in question and suggests that

the tribes must be re-circumscribed. A similarly radical rearrangement of tribes was previously suggested by Wannan and Quinn (1991) where they split the family into two groups, A and B, each of which was further split into subgroups one and two. In their treatment, Semecarpeae and four Anacardieae genera (*Bouea* Meissn., *Gluta* L., *Mangifera*, and *Swintonia*) are grouped together into 'Subgroup A1 and allied genera'. Subgroup A1 consists of the included Anacardieae members while the Semecarpeae members are considered 'Allied genera.' The subgroup is defined by a unicarpellate gynoecium, reduced number of layer in the endocarp, simple leaves, and a lack of septate fibers in the wood (Wannan and Quinn, 1991). The rest of Anacardieae is scattered into subgroups A2 and B2. The phylogenies presented here indicate that this splitting of Anacardieae is artificial but their grouping of Semecarpeae with Anacardieae is reflective of evolutionary relationships. Interestingly, Wannan and Quinn did not assign *Faguetia* to one of their groups and it is primarily this genus that causes the evolutionary positions of tribes Anacardieae and Semecarpeae to be in question in the phylogenies presented here.

**Proposed classification** □ It is apparent from the relationships elucidated by the chloroplast phylogenies and the current knowledge of Anacardiaceae morphology and anatomy that a new system of classification within the Anacardiaceae is needed. While tribe Spondiadeae is supported as monophyletic (*trnL*F phylogenies, Figs. 3.7 and 3.8, and anatomical and

morphological data, Fig. 3.12), the other tribes are nested within each other. Based on the phylogenies and the taxonomic history, an appropriate system could include three tribes, Spondiadeae, Anacardieae (including Rhoëae and Semecarpeae), and Dobineae or two tribes, Spondiadeae and Anacardieae (including Dobineae, Rhoëae and Semecarpeae). With consideration of former classification systems and current concepts of the morphology of the family, I propose that a two-group system of classification is more appropriate for Anacardiaceae. Because some of the current tribal groupings are still informative within the larger clade (i.e. Dobineae and Anacardieae and possibly Semecarpeae), this new two-group classification within the family should be at the level of subfamily rather than tribe. This ranking will allow for the recognition of tribes within the two subfamilies (and corresponding clades elucidated in the molecular phylogenies). The family was previously split into two subfamilies, Anacardioideae and Spondioideae, by Takhtajan (1987). This classification was also recommended by Terrazas (1994), although she did not reference the subfamilies of Takhtajan nor provide a description or a generic delimitation of her subfamilies. The circumscriptions presented here are an amendment of those outlined by Takhtajan (1987, see also Takhtajan, 1997). The characters listed in the subfamilial descriptions below tentatively define these two subfamilies (placement of the genera within this system is detailed in Table 3.5). In the future, tribes may be recognized to accommodate the morphological and evolutionary uniqueness of groups of taxa within the two subfamilies.

**Anacardioideae** (including Anacardieae, Dobineae, Rhoeeae and Semecarpeae):

Trees, shrubs, rarely vines or perennial herbs. Leaves simple or compound, alternate (usually) or opposite (e.g. *Abrahamia*, *Blepharocarya*, *Bouea*, *Campylopetalum*, and *Ozoroa*) pinnate venation (palmate in *Campylopetalum*). Stamens variable in number; carpels one or three and fused (rarely four to six and partially syncarpous in *Buchanania*); one locule (often by abortion, very rarely two locules in *Camptosperma*); one ovule; apical, basal or lateral ovule insertion; one to three styles, either fused or separate; one to three stigmas; wind and insect pollinated; usually *Anacardium*-type endocarp (discretely layered and with palisade-like sclereids, not found in *Buchanania* Spreng., *Camptosperma*, *Pentaspadon* and possibly others that have not been investigated); exocarp usually thin; animal and wind dispersed fruits. This is the only subfamily in which contact dermatitis-causing taxa occur (i.e. only subfamily that has the ability to produce catechols and other low molecular weight compounds such as resorcinols and other phenols) (Ding Hou, 1978; Mitchell and Mori, 1987; Mitchell, 1990; Wannan and Quinn, 1990).

**Spondioideae** (including Spondiadeae): Trees or shrubs. Leaves compound (rarely simple in *Haplospondias* Kosterm., unifoliolate in some species of *Lannea* or may have both simple and compound leaves in a single individual in *Sclerocarya*). Stamens two times the number of petals; carpels four to five (rarely one in *Solenocarpus* or more than five in *Pleiogynium*); four to five locules

(rarely one or more than five); one ovule per locule; ovules pendulous from an apical funicle; four to five styles; insect pollinated; *Spondias*-type endocarp (mass of lignified and irregularly oriented sclerenchyma); exocarp thick; animal dispersed fruits. This is the only subfamily in which opercula occur (Ding Hou, 1978; Mitchell and Mori, 1987; Wannan and Quinn, 1990).

Table 3.5. Generic placement within the two subfamily system of Anacardiaceae classification outlined in this study.

| Subfamily      | Affiliated Genera  |
|----------------|--|
| Anacardioideae | <i>Abrahamia</i> ined., <i>Actinocheita</i> , <i>Amphipterygium</i> , <i>Anacardium</i> , <i>Androtium</i> , <i>Apterokarpos</i> , <i>Astronium</i> , <i>Baronia</i> , <i>Blepharocarya</i> , <i>Bonetiella</i> , <i>Bouea</i> , <i>Buchanania</i> , <i>Camptosperma</i> , <i>Campylopetalum</i> , <i>Cardenasiodendron</i> , <i>Comocladia</i> , <i>Cotinus</i> , <i>Dobinea</i> , <i>Drimycarpus</i> , <i>Euroschinus</i> , <i>Fegimanra</i> , <i>Faguetia</i> , <i>Gluta</i> (including <i>Melanorrhoea</i> ), <i>Haplorhus</i> , <i>Heeria</i> , <i>Hermogenodendron</i> ined., <i>Holigarna</i> , <i>Laurophyllus</i> , <i>Lithrea</i> , <i>Loxopterygium</i> , <i>Loxostylis</i> , <i>Malosma</i> , <i>Mangifera</i> , <i>Mauria</i> , <i>Melanochyla</i> , <i>Melanococca</i> , <i>Metopium</i> , <i>Micronychia</i> , <i>Mosquitoxylum</i> , <i>Myracrodruon</i> , <i>Nothopegia</i> , <i>Ochoterena</i> , <i>Orthopterygium</i> , <i>Ozoroa</i> , <i>Pachycormus</i> , <i>Parishia</i> , <i>Pentaspadon</i> , <i>Pistacia</i> , <i>Protorhus</i> , <i>Pseudosmodingium</i> , <i>Rhodosphaera</i> , <i>Rhus</i> , <i>Schinopsis</i> , <i>Schinus</i> , <i>Searsia</i> , <i>Semecarpus</i> , <i>Smodingium</i> , <i>Sorindeia</i> , <i>Swintonia</i> , <i>Thyrsodium</i> , <i>Toxicodendron</i> , <i>Trichoscypha</i> |
| Spondioideae   | <i>Allospodias</i> , <i>Antrocaryon</i> , <i>Choerospondias</i> , <i>Cyrtocarpa</i> , <i>Dracontomelon</i> , <i>Haematostaphis</i> , <i>Haplospodias</i> , <i>Harpephyllum</i> , <i>Koordersiodendron</i> , <i>Lannea</i> , <i>Operculicarya</i> , <i>Pegia</i> , <i>Pleiogynium</i> , <i>Poupartia</i> , <i>Poupartiopsis</i> ined., <i>Pseudospondias</i> , <i>Sclerocarya</i> , <i>Solenocarpus</i> , <i>Spondias</i> , <i>Tapirira</i>   |

**Generic affinities** □ Unfortunately, the difficulty in delimiting on the basis of visually identifiable characters is not limited to the traditionally recognized tribes.

Small groupings of genera in the phylogenies are similarly difficult to define morphologically and anatomically. An example of this is seen in one of the clades within the large Rhoeeae clade where a monophyletic group of African and Madagascan genera (i.e. in Figs. 3.7-3.8: *Abrahamia* ined., *Baronia* (*Rhus thouarsii* and *R. taratana*), *Heeria* Meissn., *Micronychia* Oliver, *Ozoroa* Delile, and *Protorhus* Engl.) are united by their old world distributions and having simple leaves (Figs. 3.11, number 7 and Figs. 3.17 and 3.18).

The sister relationship of *Bouea* and *Mangifera* is well supported in the *trnLF* and *rps16* phylogenies (the only datasets in which both occur) (Fig. 3.5-3.9) and has a strong morphological basis as well. They share two fruit characteristics: a thin endocarp consisting of two to three cell layers and a large mesocarp. Wannan (1986) notes that *Mangifera* and *Bouea* have a very specialized pericarp structure. *Bouea* and *Mangifera* are allied with their *trnLF*-indicated sister genus, *Gluta*, by having inferior micropyles, wood with non-septate fibers, and paratracheal and apotracheal parenchyma (Wannan, 1986).

Mitchell and Young (*in* Mitchell and Mori, 1987) consider *Fegimanra* Pierre and *Anacardium* to be sister taxa despite their unusual disjunct distribution. These genera share two unique morphological characteristics (reflexed petals and a fleshy hypocarp) but are divided by their distributions: *Anacardium* is endemic to the Neotropics while *Fegimanra* occurs only in tropical West Africa (Figs. 3.18 and 3.11, number 8). Their sister relationship is well supported in this

study in the *matK* and *trnLF* phylogenies (Figs. 3.3 – 3.4 and 3.7 - 3.8 respectively).

*Amphipterygium* Schiede ex Standl. and *Orthopterygium* Hemsl., formerly recognized as the distinct family Julianiaceae, are shown to be sister genera. This relationship is well supported in the *trnLF* and *rps16* phylogenies as well as by two distinguishing floral features: pistillate flowers lack a perianth and are arranged within a globose involucre. Their fruit structure and development is also unique in the family with several flowers fusing to form a syncarp fruit that is wind dispersed by the expanded and flattened peduncle (Stern, 1952; Fig. 3.11, numbers 3 and 4, and Fig. 3.14). This is the only multiple fruit in the Anacardiaceae.

*Poupartiosis* is a southeastern coastal Madagascan genus soon-to-be described by Randrianasolo (pers. com.). It was originally annotated by Capuron (who never published the name or a description), and was recently rediscovered by Armand Randrianasolo. The lightweight drupes resemble those of *Poupartia* or *Sclerocarya* but are larger in size and appear to be water-dispersed based on their morphology (J. D. Mitchell pers. com.). Although this dispersal mechanism has not yet been observed, if it is found to be true this would be the third report of water dispersal in the family (*Mangifera* and *Spondias* have also been reported to be drift dispersed, see Fig. 3.11, number 3 and Fig. 3.14) (Schatz, 2001). The placement of *Poupartiosis* within the Spondiadeae is consistent with its

morphology (Figs. 3.12 and 3.11, number 1) and other experts' assignment of it to the tribe (J. D. Mitchell pers. com.; Randrianasolo pers. com.; Schatz, 2001).

Santin (1989) revised the Neotropical genera *Astronium* Jacq. and *Myracrodruon* Allem. based on morphological analysis and field observations. She recognized two subgenera within *Astronium*: subgenus *Macrocalyx* with one species, *Astronium concinnum* Schott, and subgenus *Astronium* containing seven other species. Subgenus *Macrocalyx* is distinguished by its unusual asymmetrical pyramidal embryo, bony endocarp, position of the funicle, and very large wings on the fruit. The wings, which are persistent stiffened sepals (Fig. 3.1, number 4 and Fig. 3.14), are approximately twice as long in *A. concinnum* as in the rest of the genus. This morphological evidence eventually led Santin to classify *A. concinnum* in its own genus, *Hermogenodendron* ined. (Mitchell pers. comm.). Unfortunately she never published this new combination, but she did annotate all of the *A. concinnum* specimens at the New York Botanical Garden and the Royal Botanical Gardens at Kew. Although its exact position within tribe Rhoeeae is unresolved in the *trnL*F phylogeny, *Hermogenodendron* is shown to be far removed from *Astronium*, supporting Santin's view that it is a new, unpublished genus.

Wind dispersed fruits occur throughout the family and for the most part appear to have evolved several times independently, either as autapomorphies or as synapomorphies (Fig. 3.11, number 4 and Fig. 3.14). The wind-dispersed syncarps of *Amphipterygium* and *Orthopterygium* were already mentioned and

help to define the clade formerly recognized as Julianiaceae. There is another clade of all wind-dispersed taxa (*Apterokarpos*, *Cardenasiodendron*, *Loxopterygium*, *Astronium*, *Myracrodruon*, and *Schinopsis*) (Fig. 3.11, number 3 and Fig. 3.14) that is not defined by any one morphology. Fruits in this clade include small dry fruits with no wing (*Apterokarpos*), elm-like samaras (*Cardenasiodendron*), samaroids with a single wing (*Loxopterygium* and *Schinopsis*), and drupes with stiffened and expanded sepals (*Astronium* and *Myracrodruon*) (Fig. 3.11, number 4 and Fig. 3.14). Clearly these different mechanisms for wind dispersal are not homologous, but they may have evolved under the influence of a factor common to all of the members of this tribe. All of the species occur in dry tropical forests (Fig. 3.11, number 5 and Fig. 3.14). A majority of the wind-dispersed genera in the *trnL*F phylogeny (13 of 19) occur in dry habitats (deserts or seasonally dry tropical forests). Two occur in the relatively dry temperate forests, and only four are found in tropical moist forests. Thus, it appears that the great diversity of wind-dispersed fruits in Anacardiaceae is more strongly linked to habitat than phylogeny.

*Rhus* (*sensu lato*) is the largest genus in the Anacardiaceae, comprising upwards of 250 species distributed in Africa, Asia, Central America, Europe, Madagascar, North America, and the South Pacific islands. In its broad sense, *Rhus* includes several taxa belonging to subgroups that have variously been recognized as distinct genera making up the *Rhus* complex: *Actinocheita*, *Baronia*, *Cotinus*, *Lobadium* Raf., *Malosma* Nutt. Ex Abrams, *Melanococca*

Blume (= *Duckera* Barkl.), *Metopium*, *Searsia* (= subgenus *Thezera* de Candolle and section Gerontogaeae), *Rhus* (sensu stricto), *Schmaltzia* Desv. emend. Barkley & Reed (elevation of subgenus *Lobadium* to generic level), and *Toxicodendron*. Several of these genera have more popularly been lumped back into *Rhus* in the modern concept of the genus (Fig 3.15). Young (1975) reinstated *Lobadium* as a subgenus of *Rhus*, Perrier de la Bâthie (1946) put Barkley's *Baronia* back into *Rhus*, and several of the other genera are still not universally recognized as segregates from *Rhus* (i.e. *Toxicodendron* and *Searsia*) despite several validly published species transfers. The nomenclature used here reflects Young's concept of *Rhus*, Barkley's (1937) concept of *Baronia* within *Rhus*, and the widely used treatment of *Searsia* within *Rhus*.

This study included representatives of *Rhus* complex members, *Baronia* (*Rhus thouarsii*, *R. perrieri* (Courchet) H. Perrier, and *R. taratana*), *Lobadium* (*Rhus aromatica* and *R. virens*), *Metopium* (*M. brownie* (Jacq.) Urb.), *Rhus* (*R. copallina*, *R. sandwichii*, *R. typhina*, *R. lanceolata*), *Searsia* (*Rhus undulata*, *R. pendulina*, and *R. erosa*), *Toxicodendron* (*T. radicans*, *T. verniciflua*, and *T. vernix*), and *Rhus chiangii* (described as an intermediate between *Rhus* subgenus *Rhus* and subgenus *Lobadium* by Young, 1977). In a recently published molecular phylogeny of SSU rDNA ITS sequences in *Rhus* (*s.l.*), Miller et al. (2001) found the *Rhus* complex to be paraphyletic with *Rhus* (*s.str.*) monophyletic and *Actinocheita*, *Searsia*, and *Toxicodendron* outside of the core group, more closely related to other Anacardiaceae genera. The phylogenies

presented here reflect similarly on the more distant relationships of these segregate genera in finding the *Rhus* complex to be polyphyletic. This result echoes several authors' ideas about the evolutionary relationships of these genera (e.g. Tournefort, 1700; de Candolle, 1825; Engler, 1892; Barkley, 1937, 1942, 1963; Heimsch, 1940; Brizicky, 1963; Gillis, 1971; Young, 1974, 1975, 1978, 1979; Miller et al., 2001).

Perhaps the most surprising aspect of the polyphyletic *Rhus s.l.* is that *Rhus s.str.* was not found to be monophyletic. This result supports Barkley's (1963) elevation of subgenus *Lobadium* to generic status, but it contradicts almost every other major study of core *Rhus*. These include Young's (1975) combining of *Rhus* subgenus *Rhus* and *Rhus* subgenus *Lobadium* based on morphological and biflavonoid data, Barkley's (1937) delimitation of the genus on the basis of it having red fruits covered in glandular trichomes, and the ITS phylogeny of Miller et al. (2001 and Miller, 1998). Due to the large body of evidence for these two subgenera being united and the sample size of only two for subgenus *Lobadium*, further sampling of these two subgenera needs to be done to evaluate thoroughly the monophyly of core *Rhus*.

Miller et al.'s (2001) finding of a paraphyletic subgenus *Rhus* was supported in the *rps16* phylogeny (Figs. 3.5-3.6), which included a more comprehensive sampling of the two subgenera than the other datasets. This dataset included four representatives of subgenus *Rhus* with three of the species forming a clade and one of them, *R. chinensis*, occurring outside of that clade.

Because the Miller et al. phylogeny included more *Rhus s. str.* taxa and was generated from a faster evolving locus, it probably provided a more accurate evolutionary map of the genus. However, the chloroplast phylogenies presented here raise more questions about the monophyly of this genus and indicate that more study is required to settle the nomenclatural problems therein.

*Searsia* was first proposed by Barkley (1942) and encompasses most of the taxa formerly placed in *Rhus* that occur from southern Africa extending north and east into Eurasia. Its placement here outside of the core *Rhus* complex supports the work of previous authors who recognized it as a distinct genus (e.g. Barkley, 1937, 1942, 1963; Gillis, 1971; Young, 1974, 1979; Miller et al., 2001). Barkley (1937) was also the first to segregate *Actinocheita* from *Rhus (s.l.)*. He did so on the basis of it having extremely long, silky, non-glandular, unbranched hairs on the fruit and a very modified disk forming a gynophore. It is clearly distinct from *Rhus (s.str.)* in the *trnL*F phylogeny presented here (Figs. 3.7-3.8).

In 1882, Baker described a new genus, *Baronia*, from Madagascar. Although Baker allied his new genus with *Buchanania* and *Loxostylis* Spreng. ex Reichb. and Engler (1892) recognized its affinities with *Protorhus*, *Baronia* was treated as a member of the Madagascan *Rhus* by Perrier de la Bâthie (1946) in his treatment of the family for the Flora of Madagascar. Since that time, no author has reinstated *Baronia* despite several indicating that it is distinct from all other genera (e.g. Fernandes 1966; Kokwaro and Gillett, 1980; von Teichman, 1996). The three species of *Baronia* were placed in *Protorhus* by Randrianasolo

(1998) in his unpublished thesis, but he has since reconsidered this transfer and now recognizes the genus *Baronia* (Randrianasolo pers. com.). This genus is distinguished from *Rhus* (= *Searsia*) by its three-parted style with capitate stigmas, ovary with three locules (two are lost before maturity), long funicle from which the ovule is pendent, and thick cotyledons. In the phylogenies presented here *Baronia* is evolutionarily removed from *Rhus* (*s.str.*) and *Protorhus*, supporting its reinstatement at the generic level, contradicting the recommendation of Randrianasolo in the most recent evaluation of the genus (Randrianasolo, 1998).

In 1977, Young described *R. chiangii* Young, but the phylogenetic position of this species has since been in question with some authors placing it in *Cotinus* based on its fruit morphology (Rzedowski and Calderón, 1999). In this analysis its position is not resolved within the core Rhoëae clade and thus warrants further investigation. In the *trnL*F phylogeny (Figs. 3.7-3.8), this lack of resolution in the core Rhoëae is also seen in the positions within the tribe of several well-supported monophyletic taxa: *Cotinus* (*C. coggygria* and *C. obovatus*), *Rhus* subgenus *Rhus* (*R. copallina*, *R. sandwichii*, *R. typhina*, *R. lanceolata*), *Searsia* (*Rhus undulata*, *R. pendulina*, and *R. erosa*), and *Toxicodendron* (*T. radicans*, *T. vernicifluum*, and *T. vernix*). All of these *Rhus* complex members occur within the core Rhoëae clade, but their relationships within the clade are unresolved. This study represents an introductory step toward resolving systematic problems with *Rhus* (*s.l.*). Further molecular and morphological studies should be

conducted in order to clarify relationships within this generic complex.

Taxonomic changes based on the results of these studies will formalize unrelated components of the complex as distinct genera.

**Biogeography** □ Gentry (1982) placed Anacardiaceae in his list of Amazonian-centered Gondwanan families but offered no explanation about how this classification was made. Certainly the pantropical distribution of the family supports this theory, which is strengthened by the strong presence of Anacardiaceae in South America, Africa, Madagascar, and the Indian subcontinent (all part of former Gondwanaland). However, there are several genera for which a strictly Gondwanan origin does not fully explain the currently observed disjunctions (i.e. *Spondias*, *Toxicodendron*, *Rhus* s.s., *Pistacia*) and the center of diversity for the family is generally believed to be Malesia (Ding Hou, 1978). For these patterns the North Atlantic land bridge hypothesis (Tiffney, 1985), the Bering Strait land bridge (Scholl and Sainsbury, 1961), and/or other biogeographical explanations must be invoked. An example of this complex biogeographic history is found at the base of the tree (Fig. 3.18, clade D). This clade consists of taxa from South America, Madagascar, sub-Saharan Africa, eastern Asia, and Oceania. While most of these landmasses could have at one time been part of Gondwana, Raven and Axelrod (1974) claim that it is likely that none of Southeast Asia or Indonesia was ever attached to Gondwanaland. The missing Gondwanan links in this clade are Australia and India which could have

transported Anacards north into Asia after their split from Gondwana and subsequent drifting northward (Audley-Charles et al., 1972; Raven and Axelrod, 1974).

Because the sampling of Anacardiaceae is not complete in this phylogeny and most notably because it is particularly depauperate in representation from southern Asia and Australia, it is difficult to thoroughly comment on the biogeographic history of the family as a whole. However, many of the relationships elucidated in the *trnL*F phylogeny present disjunctions of interesting biogeographic implication (Fig. 3.18). Several support a Gondwanan vicariance or early dispersal event. The closest relative to the tropical South American cashew genus, *Anacardium*, is *Fegimanra*, a genus endemic to tropical West Africa and western central Africa (Fig. 3.18, within clade B). When Africa and South America were still part of Gondwana, this area was pushed up against, and later separated from by a relatively narrow water barrier, the west coast of Africa (Scotese, 1997). Thus, the disjunct distribution of these two sister genera can be explained by either a Gondwanan vicariant event or by short or long-distance dispersal over water, depending on the age of the clades.

The role of Madagascar in Gondwanan biogeography has been the focus of a great number of studies (e.g. Rabinowitz et al, 1983; Storey et al., 1995; Murray, 2001; Yoder et al., 2003; Ducousso et al., 2004). Mapping current geographical distributions onto the *trnL*F phylogeny indicates that the Anacardiaceae have been dispersed or experienced a vicariant event between

Madagascar and other landmasses a minimum of three times (Fig. 3.18, clades A, B, and C). At the top of the tree is clade A containing African and Madagascan taxa (*Abrahamia*, *Heeria*, *Micronychia*, *Rhus* (= *Baronia*), *Ozoroa*, and *Protorhus*) and at the base of the tree, in clade C, there are two smaller clades containing Madagascan taxa. Because of the lack of resolution in the Spondioideae clade, the relationship of these two smaller clades is unclear. *Operculicarya* is sister to *Poupartia*, both endemic to the Madagascan-Mascarene region, while *Poupartiopsis* Randrianasolo ined. (also endemic to Madagascar) is sister to the sole South American species of *Antrocaryon*, a mostly sub-Saharan African genus. Unfortunately, none of the African species of *Antrocaryon* were available for inclusion in this study, but this sister relationship between African and South American taxa provides another piece of evidence for Gondwanan vicariance or long-distance over water dispersal. Interestingly, the fruits of the undescribed *Poupartiopsis* are thought to be water-dispersed as the trees grow along the coast in southeastern Madagascar (J. D. Mitchell, pers. com.). Therefore, long distance dispersal via water is not to be discounted completely in this case. The inclusion of African *Antrocaryon* species may help elucidate a more complete biogeographical explanation for this disjunction.

The third distinct clade containing Madagascan taxa (Fig. 3.18, clade B) also contains the clade of *Anacardium* and *Fegimanra* (discussed above). This clade is monophyletic in all analyses (Figs 3.3-3.10) and contains taxa from Southeast Asia, South America, sub-Saharan Africa, the Indian subcontinent,

and Madagascar. Clade B (Fig. 3.18) provides perhaps the best evidence of a Gondwanan origin of Anacardiaceae. The Southeast Asian taxa (*Bouea*, *Gluta*, and *Semecarpus forstenii*) are all sister to taxa or clades of taxa from the Indian subcontinent. One of these genera, *Gluta*, has species that occur on the Indian subcontinent. Because the Indian subcontinent was once a part of Gondwana, Anacardiaceae could have arrived in Southeast Asia via drifting on India from western Gondwana. Furthermore, Clade B has its root in sub-Saharan Africa, the center of western Gondwana (Scotese, 1997). Clearly Gondwana played an important role in the early evolution of Anacardiaceae. However, the North American – Asian (i.e., *Toxicodendron*) and European – North American (i.e. *Cotinus* and *Pistacia*) disjunctions found in the family and the early fossil records present in Laurasian landmasses (Hsu, 1983; Kvacek and Walther, 1998), suggest that the history of the cashew family is more complex than just being of Gondwanan origin.

Future studies will expand the sampling of Anacardiaceae taxa with the hope of generating a complete taxonomic revision for the family. Large, understudied genera (i.e. *Lannea*, *Sorindea* Thou., and *Semecarpus*) and taxa that have traditionally been taxonomically problematic within the family (i.e. *Blepharocarya* F. Muell., *Buchanania*, *Camptosperma*, *Campylopetalum*, *Pentaspadon*, etc) are of special interest. To achieve this, the author has established a field collection and collaboration program to collect Anacardiaceae

in under-collected but extremely taxon-rich areas in Africa (Gabon, Madagascar, Tanzania) and Southeast Asia and the Pacific (New Guinea, Borneo, Thailand).

## Chapter 4: Out of Africa: Taxonomic Split of Madagascan and South African Species of *Protorhus* Engl. (Anacardiaceae)

### Introduction

Madagascar has one of the most unusual biotas on earth with 96% of the flora endemic (Schatz, 2001) and the percentage of endemism in some groups of animals reaching nearly 100% (Yoder et al. 2003). When Gondwana was still intact, Madagascar was sandwiched between western Africa (near present day Somalia and Kenya) and eastern India (Rabinowitz et al., 1983; Schatz, 1996). For this reason, and because of purported subsequent 'rafting' events, the Madagascan flora and fauna have strong links to Africa and Indo-Asia (Schatz, 1996; Yoder, 1996; Murray, 2001; Yoder et al., 2003; Ducouso et al., 2004; Yoder and Yang, 2004). Subsequent to its split from Africa, 165 million years (Myr) ago (Rabinowitz et al., 1983; Coffin and Rabinowitz, 1992; Schatz, 1996), and India 88 Myr ago (Storey et al., 1995), the island continent of Madagascar has been isolated from all other landmasses by large water barriers. Although several different theories on post-Mesozoic land-bridges connecting continental Africa to Madagascar have been proposed (van Stennis, 1962, Eisenberg, 1981; Jolly et al. 1984; McCall, 1997) these have been largely refuted (see the following references for a debunking of the listed theories: McKenzie and Sclater, 1973 for the Cretaceous 'Lemuria' isthmian connection hypothesis; Hag et al., 1987 for the theory that reduced sea levels exposed land masses in the

Mozambique Channel; and Rogers et al., 2000 for the Cenozoic landbridge hypothesis).

The timing of the southward migration of Madagascar (165 Myr to 121 Myr) into its current position suggests that angiosperms were not present when the island separated from Africa. However, several angiosperm lineages had arrived in Madagascar by the time India broke away (88 Myr ago) and started drifting northward, as evidenced by both the timing of the split and pollen deposits from the mid-Cretaceous (Cenomanian) (Storey et al. 1995; Schatz, 1996). These plants may have reached Madagascar from India by land or from Africa by close-proximity water dispersal but subsequent immigrations had to occur by longer distance over-water dispersal (Yoder et al., 2003).

The estimated age of the monophyletic Sapindales, 65 to 84 Myr (Knobloch and Mai, 1986; Magallón and Sanderson, 2001; Wikström et al., 2001), suggests that the order could not have been in Madagascar at the time it split from all other landmasses (88 Myr ago). Therefore, members of the Sapindales, including Anacardiaceae, most likely reached Madagascar via dispersal over water. There were at least three different Anacardiaceae colonizations of Madagascar as indicated by the occurrence of Madagascan taxa in a minimum of three separate clades in the phylogeny of the family (Chapter 2). The study presented here represents a more in-depth study of one of those clades and looks in particular at the taxonomic recognition of a new endemic Madagascan genus, *Abrahamia* Randrianasolo ined., split from the now African

endemic genus *Protorhus* Engl.

The genus *Protorhus* contains trees and shrubs primarily distributed in Madagascar, but with one species endemic to southern Africa. The fruit of the African species, *P. longifolia* (Bernh.) Engl., is eaten by vervet and Samango Monkeys and some birds, and black rhinoceroses eat the bark (Ward, 1980; Coates Palgrave, 1981; von Teichman, 1991). The wood of *P. longifolia* is not water resistant and is prone to rot, but like the Madagascan species of *Protorhus*, it is used locally for beam, plank, and furniture construction (Coates Palgrave, 1981; Randrianasolo, 1998). The sap is used as a depilatory (functioning as a glue on the fingers for easier hair plucking), the aromatic fruits are used as perfume (Coates Palgrave, 1981), and the bark is used for medicinal purposes (Ward, 1980).

These Madagascan and African species of *Protorhus* are separated by more than just the Mozambique Channel and geological history. Their morphology also sets them apart from each other (Table 4.1). Inspection of live plants, specimens, illustrations, and descriptions of *Protorhus* show that the Madagascan species have ellipsoidal and radially symmetrical fruits while those of *P. longifolia* are ovoid and asymmetrical (Engler, 1881, 1892; Perrier de la Bâthie, 1946; Coates Palgrave, 1981; von Teichman, 1991; Randrianasolo, 1998). Another incongruous character in *Protorhus* is the number of locules in the ovary. It was originally described as having trilocular or unilocular-by-abortion ovaries (Engler, 1881; Perrier de la Bâthie, 1946), but von Teichman

(1991) found *P. longifolia* to have consistently unilocular ovaries, suggesting that the earlier records may have been representative only of specimens of the Madagascan species. Cotyledon morphology also varies within the genus along distributional lines. All but one of the Madagascan *Protorhus* have ruminant cotyledons that are inseparable while the African species has separable cotyledons that are not ruminant (Randrianasolo, 1998).

Table 4.1. Selected characteristics of *Protorhus* (after Engler, 1881, 1892; Perrier de la Bâthie, 1946; Coates Palgrave, 1981; von Teichman, 1991; and Randrianasolo, 1998).

| Character    | <i>Protorhus longifolia</i>   | Madagascan <i>Protorhus</i> spp.                                 |
|--------------|---|--|
| Distribution | South Africa  | Madagascar   |
| Fruit        | ovoid; mango-shaped or transversely oblong, asymmetrical            | ellipsoidal, radially symmetrical                                |
| Seed         | lack resiniferous canals, not ruminant; cotyledons easily separable | resiniferous canals making them ruminant; cotyledons inseparable |
| Flower       | 3 short styles, unilocular  | 1 style, trilocular or unilocular by abortion                    |

Engler (1881) described *Protorhus* with eight species but did not designate a type species or specimen. The genus went through a major revision and expansion by Perrier de la Bâthie (1946) before Phillips (1951) designated *P. longifolia* from South Africa, as a lectotype. Perrier de la Bâthie (1946) recognized 15 species in Madagascar and one in Africa. An additional African species, *P. namaquensis*, was described by Sprague in 1913, but subsequently

transferred to *Ozoroa* Delile by von Teichman and van Wyk (1994, see also von Teichman, 1994).

Randrianasolo (1998) recently revised the genus based on traditional monographic and cladistic investigations. In this study he expanded the Madagascan taxa to 19 species and maintained the recognition of only one African taxon. He found these two geographically isolated species groups to be so different that he established a new genus, *Abrahamia*, for the Madagascan species. The characters used to taxonomically separate these two genera are listed in Table 4.1. Although he transferred Barkley's *Baronia* (more commonly recognized in the genus *Rhus*) to *Protorhus* in his 1998 thesis, Randrianasolo has since reconsidered this placement and now recognizes only the type species, *P. longifolia* from Africa, in *Protorhus* (Randrianasolo, pers. com.).

The current study of the molecular systematics of *Protorhus* was undertaken in order to investigate, using molecular phylogenetics, Randrianasolo's recognition of *Abrahamia* as evolutionarily distinct from *Protorhus* and to evaluate the origins of the Madagascan species group in a biogeographic context. Three gene regions were used in this study: the plastid *trnLF* (an abbreviation which refers to the *trnL* intron, *trnL* 3' exon, and the *trnL-trnF* intergenic spacer) and two nuclear ribosomal, the internal transcribed spacer (ITS), and approximately 550 five prime base pairs of the external transcribed spacer (ETS) (Fig. 4.1). The plastid and nuclear ribosomal data were first considered alone and then combined to assess congruence among the datasets.

This enabled comparison of topologies of DNA regions with different functional constraints (i.e. nuclear vs. plastid and concerted evolution in the ribosomal sequences) before combining them to limit misleading results in the separate analyses (Johnson and Soltis, 1998; Wiens, 1998; Soltis et al., 1999).

## Materials and Methods

**Plant materials and DNA isolation** □ Ingroup sampling for this study included 36 taxa in total: 19 Madagascan *Protorhus* taxa (hereafter referred to as *Abrahamia*) representing 13 of the 19 species, the single species of *Protorhus*, and 19 other Anacardiaceae. One Burseraceae was designated as the outgroup (see Appendix A for a complete list of taxa). The non-*Protorhus/Abrahamia* taxa were selected for two reasons: 1) their close relationship to *Protorhus* and/or *Abrahamia*, which was previously elucidated in family-wide phylogenetic studies; or 2) their usefulness in rooting the phylogeny. DNA was extracted from fresh and silica-dried materials as well as herbarium specimens. These samples were directly collected by the author in the field, were gathered in herbaria (LSU, MO, NY), or were contributed by colleagues. Many of the *Abrahamia* samples were provided by Armand Randrianasolo, Missouri Botanical Garden. The Burseraceae sample was provided by Douglas Daly, New York Botanical Garden.

Plant tissue was ground in a FastPrep lysing FP-120 bead mill using lysing matrix "A" tubes containing a ceramic bead and garnet sand (Qbiogene

Inc., Carlsbad, CA). Most samples were extracted with the DNeasy Plant Mini Kit (Qiagen Inc., Valencia CA), but a modified Struwe et al. (1998) method was also employed. Extractions of herbarium material were done with a modification of Qiagen's DNeasy protocols and included the addition of 570 mg (30 $\mu$ l) of PCR grade proteinase K (Roche, Indianapolis, IN), 6.5% (30 $\mu$ l)  $\beta$ -mercaptoethanol (BME) and incubation at 42 °C for 12-24 hours on a rocking platform (K. Wurdack designed protocol, pers. com., see Appendix B for a complete description of this herbarium extraction protocol).

**DNA amplification and sequencing** □ The nuclear ribosomal ITS and ETS and the chloroplast *trnL*F regions were amplified from extracted total genomic DNA using the polymerase chain reaction (PCR) method. The primers of White et al. (1990) (3, 4, and 5 in Table 4.2 and Fig. 4.1) and a forward primer designed by Kenneth Wurdack for angiosperms (Wurdack pers. com.) (6 in Table 4.2 and Fig. 4.1) were used to amplify ITS. ETS was amplified with primer 18S-IGS (Baldwin and Markos 1998) (2 in Table 4.2 and Fig. 4.1) and an internal forward primer designed for Burseraceae by Andrea Weeks (Weeks, 2003) (1 in Table 4.2 and Fig. 4.1). Thermal cycling parameters for ITS were an initial denaturation of 50 seconds at 97°C; 30 cycles of 97°C for 50 seconds, annealing at 53°C for 50 seconds, and elongation at 72°C for one minute and 50 seconds; followed by an elongation step of 72°C for 7 minutes. For ETS, the following parameters were used: initial denaturation of 10 minutes at 95°C; 30 cycles of 94°C for one

Table 4.2. ITS and ETS primers used in this study. ITS5, ITS3 and ITS2 are those of White et al. (1990), 26S-25R was designed for angiosperms by Kenneth Wurdack, primer 18S-IGS is that of Baldwin and Markos (1998), ETS1F was designed for Anacardiaceae-Burseraceae by Andrea Weeks (2003). See Fig. 4.1 for a map of the primers.

| Reference number in Figure 4.1 | Name    | 5'-3' Sequence                          |
|--------------------------------|---------|---|
| 1                              | ETS1F   | TTCGGTATCCTGTGTTGCTTAC                  |
| 2                              | 18S-IGS | GAGACAAGCATATGACTACTGGCA<br>GGATCAACCAG |
| 3                              | ITS5    | CCTTATCATTAGAGGAAGGAG                   |
| 4                              | ITS3    | GCATCGATGAAGAACGCAGC                    |
| 5                              | ITS2    | GCTGCGTTCTTCATCGATGC                    |
| 6                              | 26S-25R | TATGCTTAAAYTCAGCGGGT                    |

minute, annealing at 56°C for two minutes, and elongation at 72°C for two minutes; followed by an elongation step of 72°C for 16 minutes. The universal primers c, d, e, and f of Taberlet et al. (1991) were used to amplify *trnL-F* (Table 4.3 and Fig. 4.2). Thermal cycling parameters were an initial denaturation of 2 minutes at 97°C; 30 cycles of 94°C for one minute, annealing at 48°C for two minutes, and elongation at 72°C for two minutes; followed by a final elongation step of 72°C for 16 minutes.

Successful amplifications were purified using QIAquick PCR purification kit (Qiagen Inc., Valencia CA). Purified PCR products were quantified visually using a Low DNA Mass Ladder (Invitrogen, Carlsbad, CA) and then cycle sequenced using the same primers as were used for amplification (Table 4.3) and ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit version 1 (Applied Biosystems, Foster City, CA). Reactions one quarter the size of the

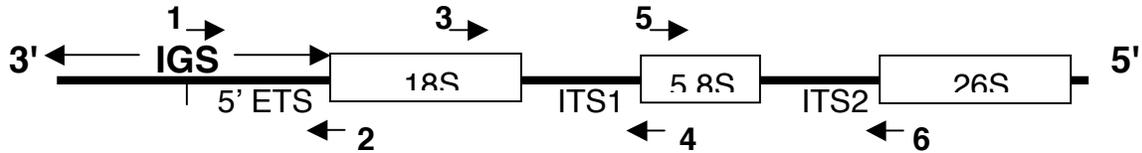


Figure 4.1. Map of the ITS and ETS primers used in this study. See Table 4.2 for primer sequences.

Table 4.3. Sequences of the *trnLF* primers used in this study and mapped in Figure 4.2 (from Taberlet et al., 1991).

| Name | 5'-3' Sequence       |
|------|----------------------|
| c    | CGAAATCGGTAGACGCTACG |
| d    | GGGGATAGAGGGACTTGAAC |
| e    | GGTTCAAGTCCCTCTATCCC |
| f    | ATTTGAACTGGTGACACGAG |

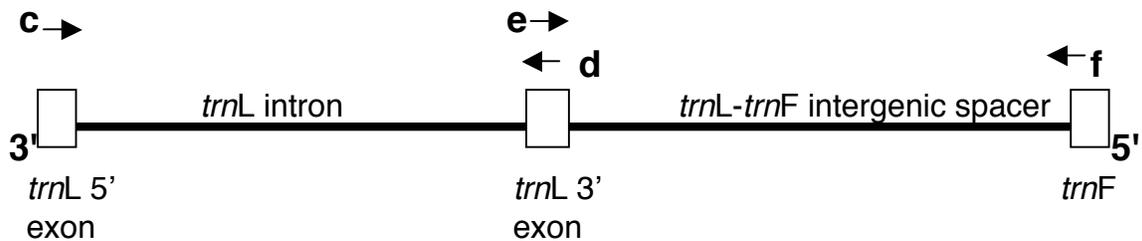


Figure 4.2. Approximate location of *trnLF* primers used in this study (from Taberlet et al., 1991). See Table 4.3 for a list of primers and their reference numbers used here.

manufacturer's recommendation were run. Cycle sequencing reaction products were purified on Sephadex columns and then run on 5% Long Ranger<sup>®</sup> (BioWhittaker Molecular Applications, Rockland, Maine) polyacrylamide gels in an ABI 377XL automated sequencer (Applied Biosystems, Foster City, CA).

**Sequence analysis** □ Sequences were assembled and edited in Sequencher™ 4.1 (Gene Codes, Corporation, Ann Arbor, MI). Compiled sequences were easily aligned and subsequently manually adjusted as taxa were added in MacClade 4.0 (Maddison and Maddison, 2000). The dataset was analyzed using PAUP\* 4.0b10 (Swofford, 2002) on an iMac G4 with the maximum-parsimony optimality criterion and maximum likelihood. Both analyses were performed on the data with the ETS, ITS, and *trnL*F sequences being treated individually as single datasets. When the data were combined for analysis, only those taxa represented in all datasets were included. All combinations of datasets were evaluated with an incongruence length difference (ILD) test, the partition homogeneity test, in PAUP\* 4.0b10 (Swofford, 2002) (1000 replicate heuristic search, tree-bisection-reconnection (TBR), 10 trees held at each step, MulTrees off). Resulting *P* values were assessed using the standard of Cunningham (1997) ( $p > 0.01$ ).

Parsimony analysis was performed using a heuristic search to generate 1000 replicates of random taxon addition using equal (Fitch) weights and tree-bisection-reconnection (TBR) branch swapping, 10 trees held at each step, MulTrees off, and saving only the shortest trees or the shortest from each replicate. The resulting trees were used as starting trees in another round of TBR with MulTrees on. In the phylogenies presented here, gaps were treated as missing data, poly repeats were included, and branches with a minimum length

of zero were collapsed. Support for tree topology was evaluated with 1000 bootstrap replicates using PAUP\* 4.0b10 (Swofford, 2002).

Morphological characters and geographical distributions of the taxa were coded as discrete and mapped onto the combined ETS, ITS, *trn*LF strict consensus phylogeny in MacClade 4.06 (Maddison and Maddison 2003) using delayed transformation (DELTRAN) optimization. This tree was selected for character mapping because it has the greatest resolution of all of the trees while still including sampling of the African and Madagascan genera closely related to the two *Protorhus* species groups (i.e. those in Africa and Madagascar).

For the maximum likelihood analysis, an appropriate nucleotide substitution model was identified using a hierarchical likelihood-ratio test implemented in Modeltest 3.06 PPC (Posada and Crandall 1998) for selection of the best-fit model. Three different best-fit models provided the best explanation of the data, one for each of the gene regions: the TVM+G model was selected for *trn*LF, TVM+G+I for ETS, and TrN+G for ITS (all assume unequal base frequencies, unequal transition/transversion rates, and among-site rate heterogeneity). Heuristic maximum likelihood searches were done using PAUP\* 4.0b10 (Swofford, 2002). Branch lengths were estimated using the best-fit model under the parameters obtained from Modeltest for *trn*LF (an estimated transition-transversion ratio, estimated base frequencies, among-site rate heterogeneity approximated by a discrete gamma distribution with four rate classes and a shape parameter of 0.4954), ETS (an estimated transition-transversion ratio,

estimated base frequencies, a proportion of invariant sites, among-site rate heterogeneity approximated by a discrete gamma distribution with a shape parameter of 2.6791 and four rate classes), and ITS (an estimated transition-transversion ratio, estimated base frequencies, no invariant sites, among-site rate heterogeneity approximated by a discrete gamma distribution with a shape parameter of 0.2534 and four rate classes).

## Results

**ETS** □ A single PCR product as well as single peaks in the sequence chromatograms were obtained for all ETS amplifications and sequences. Therefore, cloning was not undertaken with these taxa. Parsimony analysis resulted in 48 most parsimonious trees of 417 steps each, a consistency index (CI) of 0.607, a consistency index excluding uninformative characters (RC) of 0.404, homoplasy index (HI) of 0.393, and a retention index (RI) of 0.666. Figure 4.3 shows the maximum likelihood tree (log likelihood = -2448.04493) and the strict consensus of the 48 most parsimonious trees generated in this analysis with bootstrap support percentages indicated where greater than 50%. GenBank accession numbers are shown in Appendix A.

**ITS** □ A single PCR product as well as single peaks in the sequence chromatograms were obtained for all ITS amplifications and sequences.

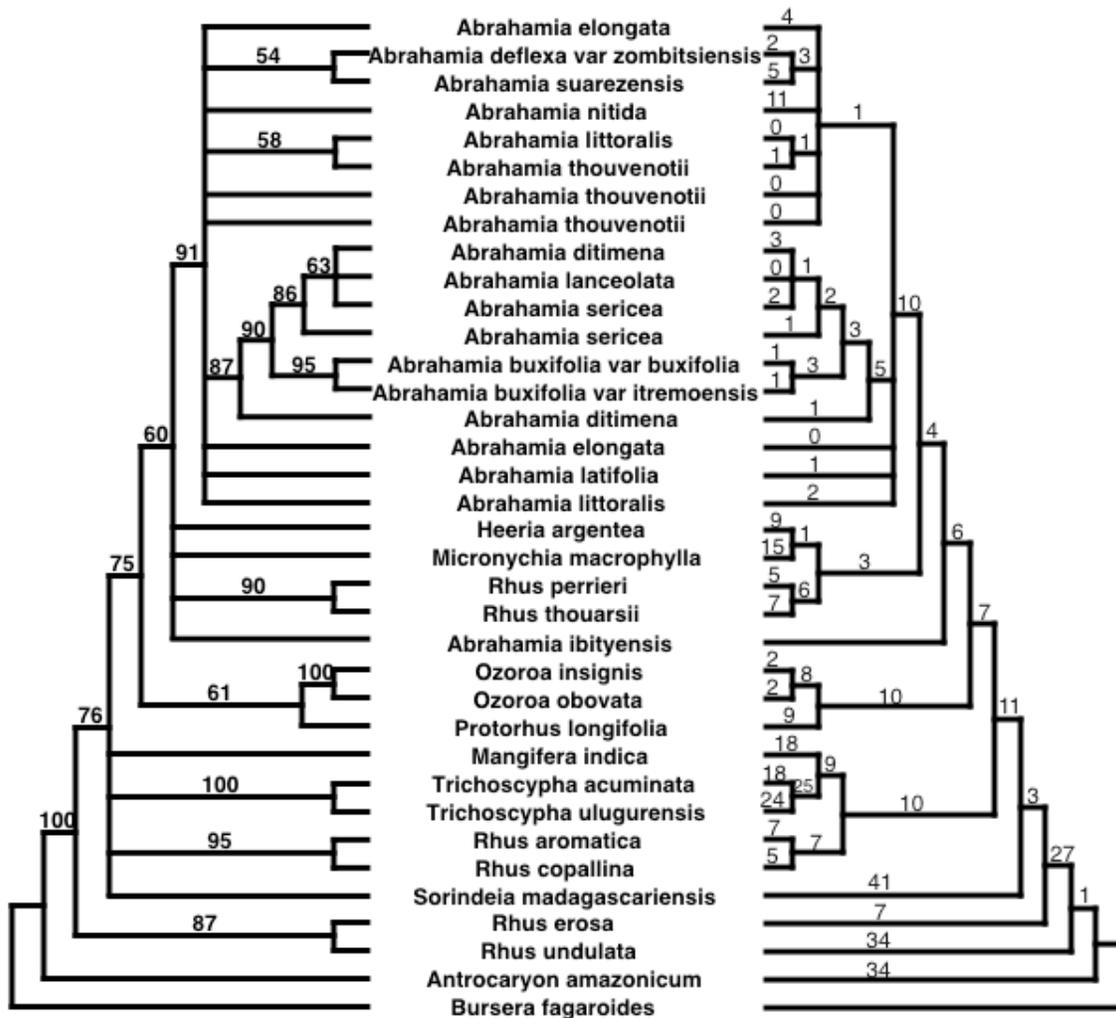


Figure 4.3. Strict consensus of 48 most parsimonious trees resulting from phylogenetic analysis of ETS sequences of 35 Anacardiaceae ingroup taxa and 1 Burseraceae outgroup taxon with bootstrap support ( $\geq 50\%$ ) indicated above branches (left, 417 steps,  $CI=0.607$ ,  $RC=0.404$ ,  $RI=0.666$ ,  $HI=0.393$ ) and the maximum likelihood tree for the same dataset (right, likelihood score 2448.04493, substitution model = TVM+G+I).

Therefore, cloning was not undertaken with these taxa. Parsimony analysis resulted in 234 most parsimonious trees of 784 steps each,  $CI$  of 0.614,  $RC$  of 0.385,  $HI$  of 0.386, and a  $RI$  of 0.627. Figure 4.4 shows the maximum likelihood

tree (log likelihood = -4474.52146) and the strict consensus of the 234 most parsimonious trees generated in this analysis with bootstrap support percentages indicated where greater than 50%. GenBank accession numbers are shown in Appendix A.

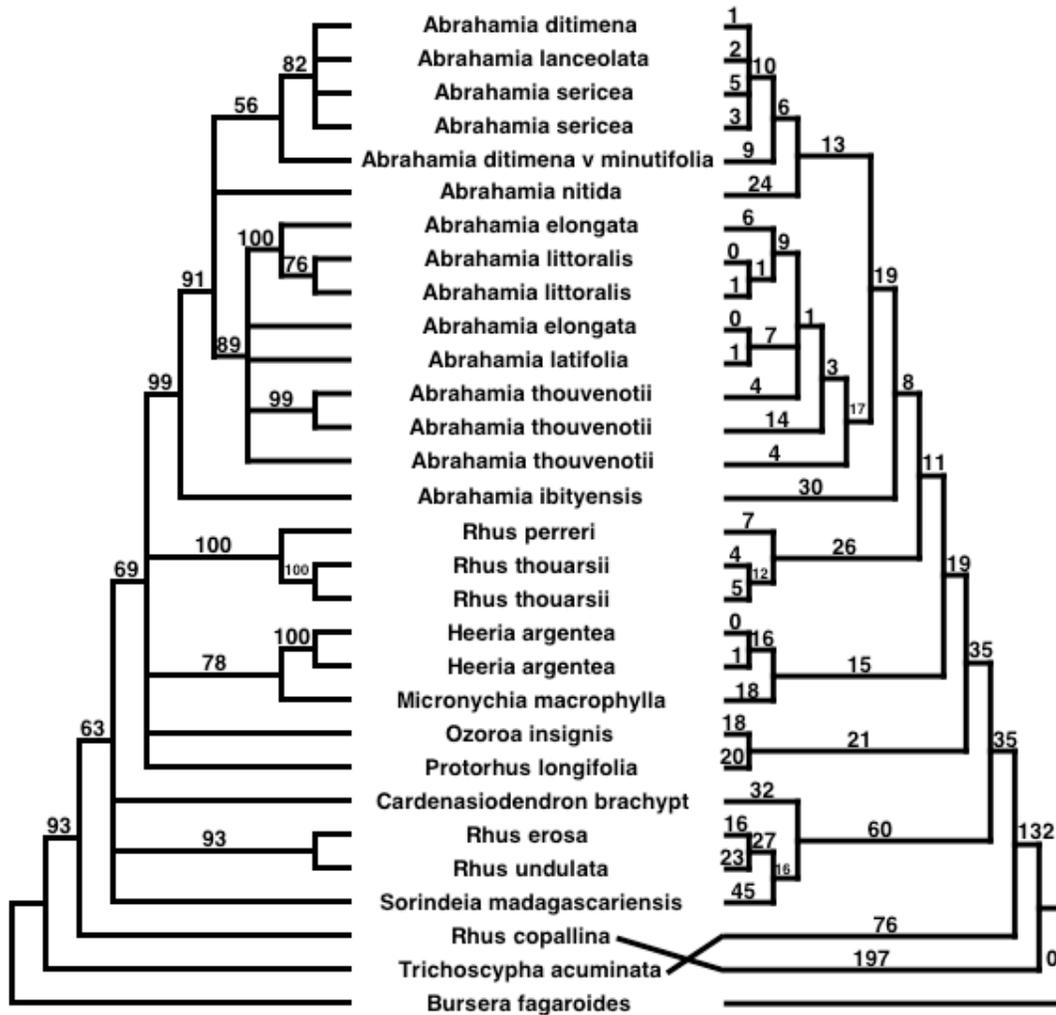


Figure 4.4. Strict consensus of 234 most parsimonious trees resulting from phylogenetic analysis of ITS sequences of 29 Anacardiaceae ingroup taxa and 1 Burseraceae outgroup taxon with bootstrap support ( $\geq 50\%$ ) indicated above branches (left, 784 steps, CI=0.614, RC=0.385, RI=0.627, HI=0.386) and the maximum likelihood tree for the same dataset (right, likelihood score 4474.52146, substitution model = TrN+G).

***trnLF*** □ Parsimony analysis resulted in 448 most parsimonious trees of 206 steps each, a CI of 0.888, a RC of 0.778, HI of 0.112, and a RI of 0.876. Figure 4.5 shows the maximum likelihood tree (log likelihood = -2805.66656) and the strict consensus of the most parsimonious trees generated in this analysis with bootstrap support percentages indicated where greater than 50%. GenBank accession numbers are shown in Appendix A.

**Combined ETS and ITS** □ Partition homogeneity tests of the combined matrix showed no significant difference in the phylogenetic signal of the two regions (p-value = 0.02), thus they were combined and analyzed together. Parsimony analysis resulted in 20 most parsimonious trees of 1042 steps each, a CI of 0.636, a RC of 0.383, HI of 0.364, and a RI of 0.603. Figure 4.6 shows the strict consensus of the most parsimonious trees generated in this analysis with bootstrap support percentages indicated where greater than 50%.

**Combined ETS, ITS, and *trnLF*** □ Partition homogeneity tests of the combined matrix showed no significant difference in the phylogenetic signal of the three regions (p-value = 0.02), thus they were combined and analyzed together. Parsimony analysis resulted in eight most parsimonious trees of 1043 steps each, a CI of 0.707, a RC of 0.432, HI of 0.293, and a RI of 0.612. Figure 4.7 shows the strict consensus of the eight most parsimonious trees generated in

this analysis with bootstrap support percentages indicated where greater than 50%.

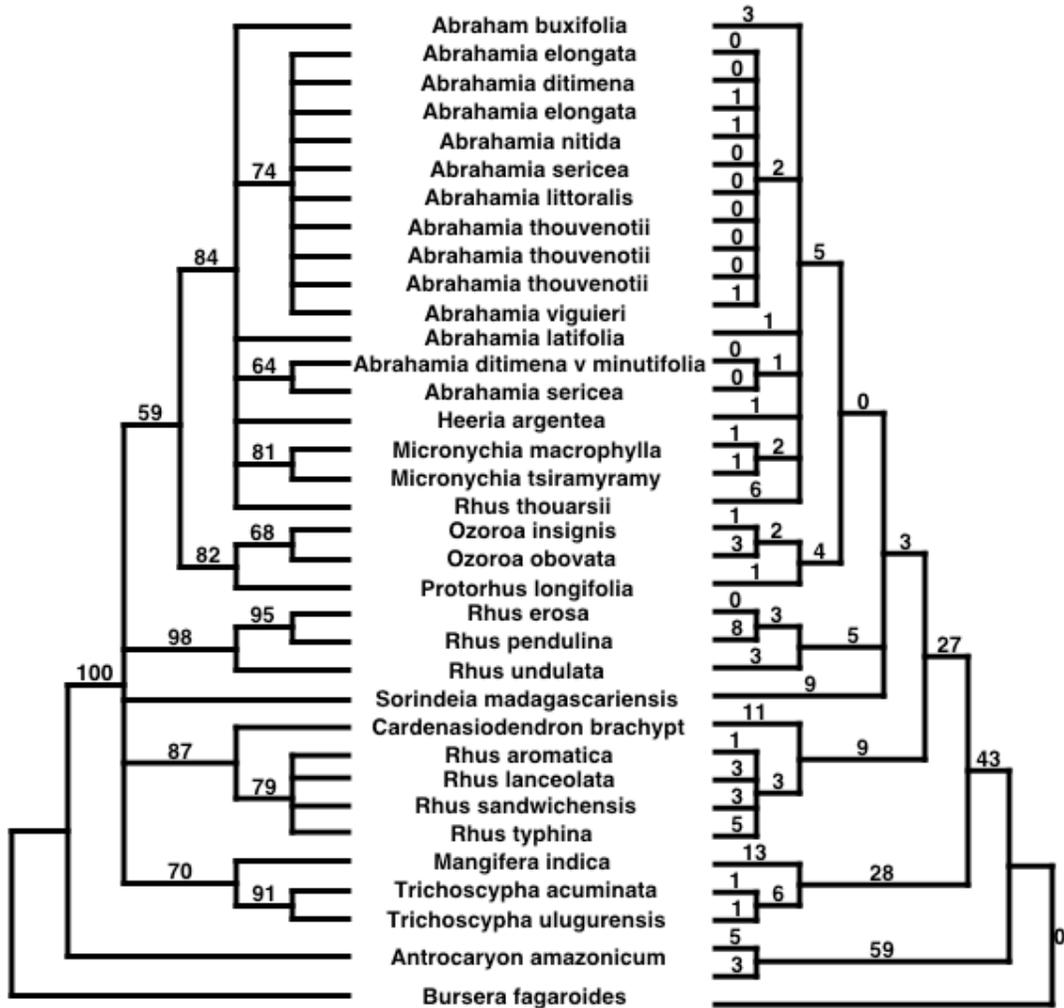


Figure 4.5. Strict consensus of 448 most parsimonious trees resulting from phylogenetic analysis of *trnL*F sequences of 34 Anacardiaceae ingroup taxa and 1 Burseraceae outgroup taxon with bootstrap support ( $\geq 50\%$ ) indicated above branches (left, 206 steps, CI=0.888, RC=0.778, RI=0.876, HI=0.112) and the maximum likelihood tree for the same dataset (right, likelihood score 2805.66656, substitution model = TVM+G).

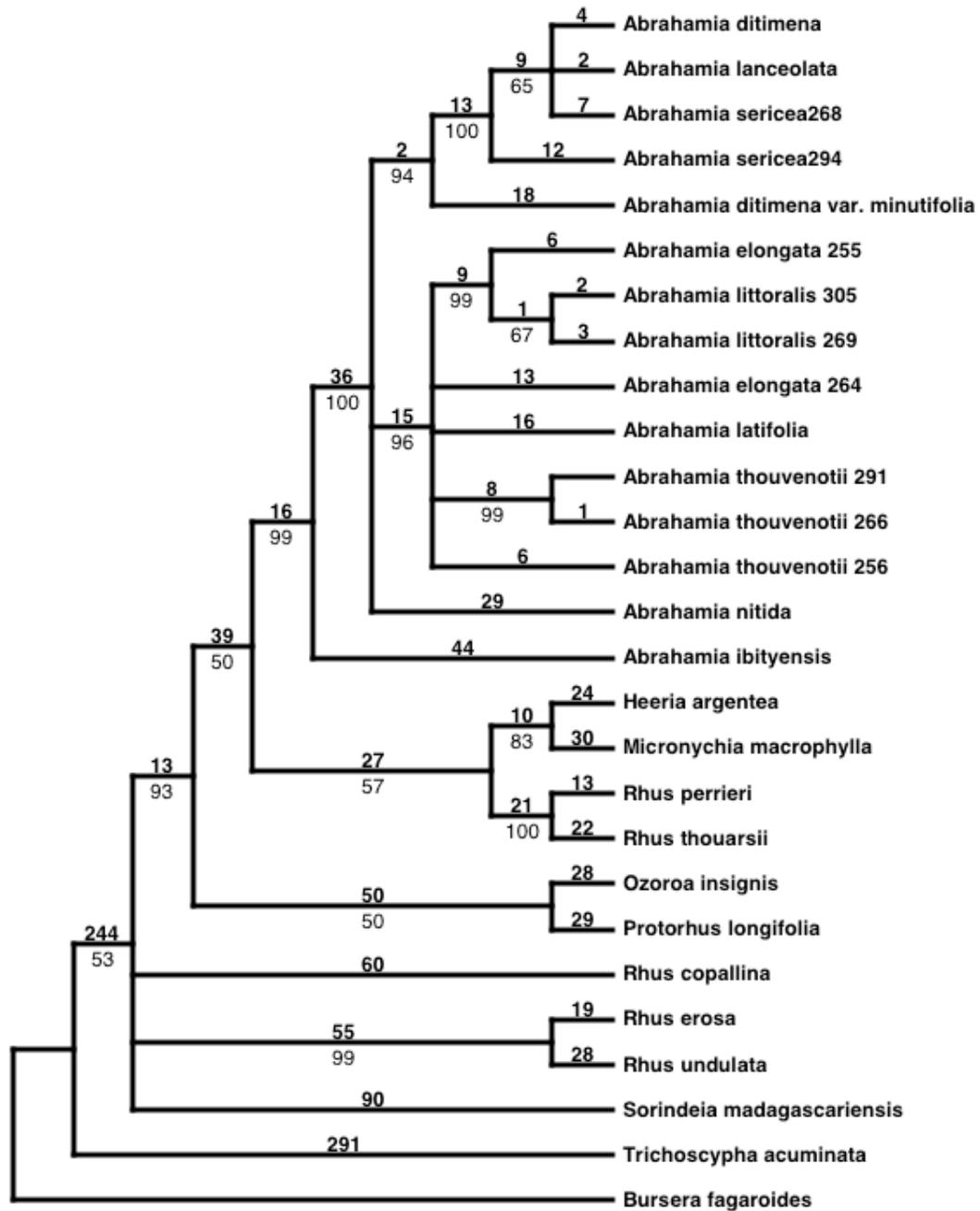


Figure 4.6. Strict consensus of 10 most parsimonious trees resulting from phylogenetic analysis of combined ETS and ITS sequences of 26 Anacardiaceae ingroup taxa and 1 Burseraceae outgroup taxon (1042 steps, CI=0.636, RC=0.383, RI=0.603, HI=0.364). Branch lengths are in bold above branches and bootstrap support ( $\geq 50\%$ ) is indicated below branches.



**Relationships** □ Coding gaps as binary characters or as a fifth base (data not shown) had no effect on the topology and very little effect on branch support compared with analyses treating gaps as missing data. Results are shown with gaps treated as missing data. Length mutations of polynucleotide repeats being included or ignored also had no effect on topology. The ITS, ETS+ITS, and combined ITS, ETS, and *trnL*F phylogenies support a monophyletic *Abrahamia* distinct from *Protorhus* (99%, 99%, and% bootstraps and Figs. 4.4, 4.6, and 4.7, respectively). In the ETS phylogeny (Fig. 4.3), all of the *Abrahamia* species except *A. ibityensis* (H. Perrier) Randrianasolo comb. nov. ined. form a monophyletic group (91% bootstrap), with this one species unresolved in the clade outside of the rest of the genus in the parsimony analysis. When ETS is combined with ITS (Fig. 4.6), *A. ibityensis* is at the base of a monophyletic *Abrahamia* (100% bootstrap).

The only phylogeny in which a single sister taxon is supported for *Abrahamia* is the ETS-ITS-*trnL*F tree (Fig. 4.7) which shows *Rhus thouarsii* (Engl.) H. Perrier (= *Baronia* Baker) as sister to the genus. The ITS and combined ETS-ITS phylogenies have several small clades and individual taxa for which the topology is unresolved in the clade outside of the monophyletic *Abrahamia*. In the combined ETS-ITS tree these include *Heeria* Meissn., *Micronychia* Oliver, *Rhus* L. (= *Baronia*) (Fig. 4.6), and in the ITS tree, these genera and *Ozoroa* Delile and *Protorhus* (Fig. 4.4).

Four *Abrahamia* species, *A. sericea* (Engl.) Randrianasolo comb. nov. ined. (Figs. 4.3, 4.5, 4.6, 4.7), *A. thouvenotii* (Lecomte) Randrianasolo comb. nov. ined. (Figs. 4.3, 4.4), *A. elongata* Capuron ex Randrianasolo ined. (Figs. 4.3, 4.4, 4.6, 4.7), and *A. ditimena* (H. Perrier) Randrianasolo comb. nov. ined. (Figs. 4.3-4.7), are paraphyletic in the phylogenies. *Protorhus* and *Ozoroa* are closely allied in all of the topologies, appearing as sister genera in ETS, *trnLF*, and combined ETS-ITS-*trnLF* in parsimony analysis (Figs. 4.3, 4.5 and 4.7, respectively) and in all of the maximum likelihood analyses (Figs. 4.3 - 4.5).

Support for the sister relationship of *Protorhus* and *Ozoroa* is present in all phylogenies except the combined ETS-ITS (*trnLF* bootstrap 82%, branch length of four in maximum likelihood analysis, Fig. 4.5; combined ETS-ITS-*trnLF* bootstrap 66%, Fig. 4.7; ITS branch length of 21, Fig. 4.4; ETS bootstrap 61%, branch length of 10, Fig. 4.3). *Micronychia* and *Heeria* are sister taxa in a majority of the phylogenies (ITS bootstrap 78%, branch length of 15, Fig. 4.4; ETS branch length of one, Fig. 4.3; ETS-ITS bootstrap 85%, Fig. 4.6; combined ETS-ITS-*trnLF* bootstrap 93%, Fig. 4.7). The two *Micronychia* species in the *trnLF* phylogeny share a five-base indel and are supported by a bootstrap of 81% (Fig. 4.5).

Morphological characters and species distributions are mapped onto the ETS, ITS, and *trnLF* strict consensus of eight most parsimonious trees in Figures 4.8 - 4.10. The distinguishing morphological characters of *Abrahamia*, the number of styles and the number of locules, were selected for their taxonomic

importance. Both of these characters are synapomorphies for the genus (Fig. 4.8 and 4.9).

The species distributions are mapped onto the ETS, ITS, and *trnL*F strict consensus phylogeny in Figure 4.10. There are three endemic Madagascan genera in this phylogeny, *Abrahamia*, *Baronia* (= *Rhus thouarsii*), and *Micronychia*. *Micronychia* is sister to the South African genus *Heeria* in a clade

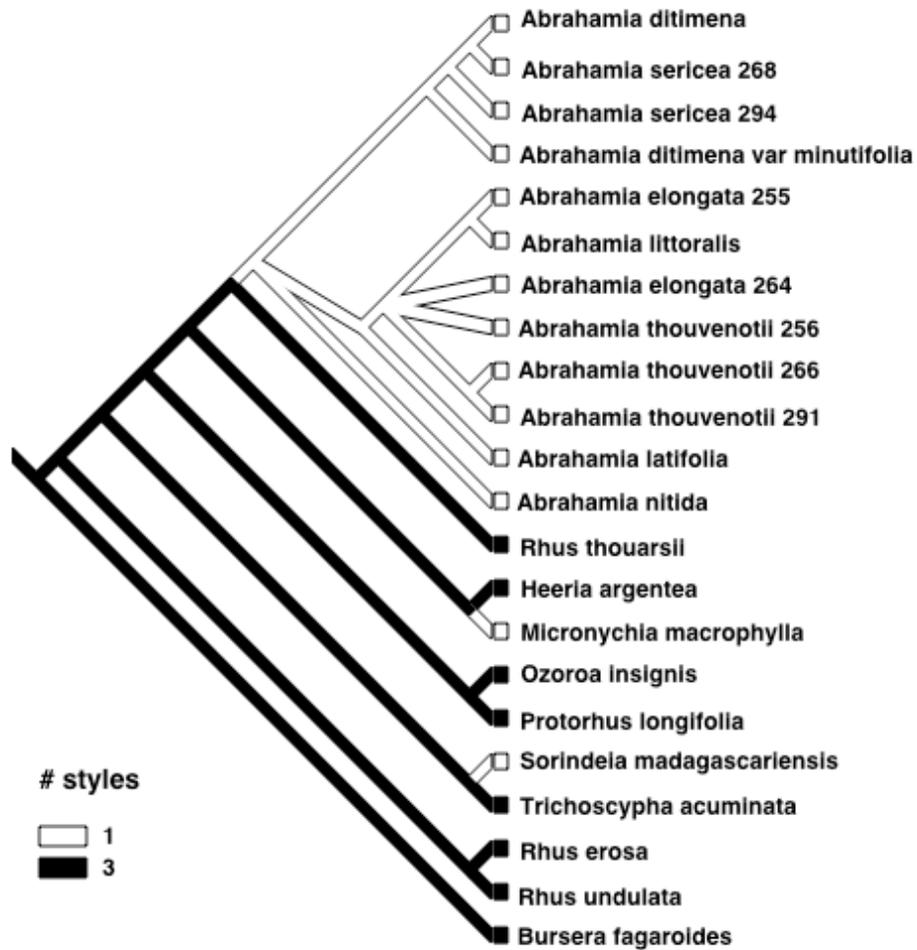


Figure 4.8. ETS, ITS, and *trnL*F tree from Fig. 4.7 with the number of styles mapped on the phylogeny.

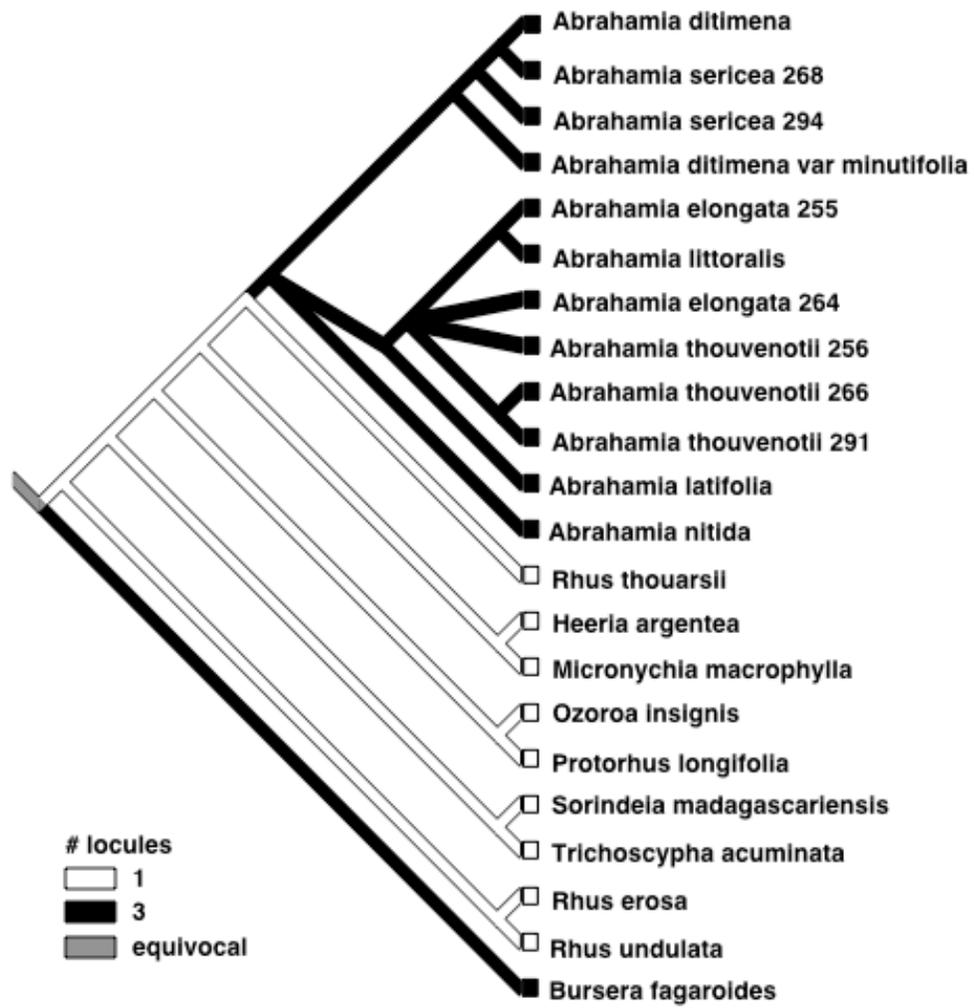


Figure 4.9. ETS, ITS, and *trnL*F tree from Fig. 4.7 with the number of locules mapped on the phylogeny.

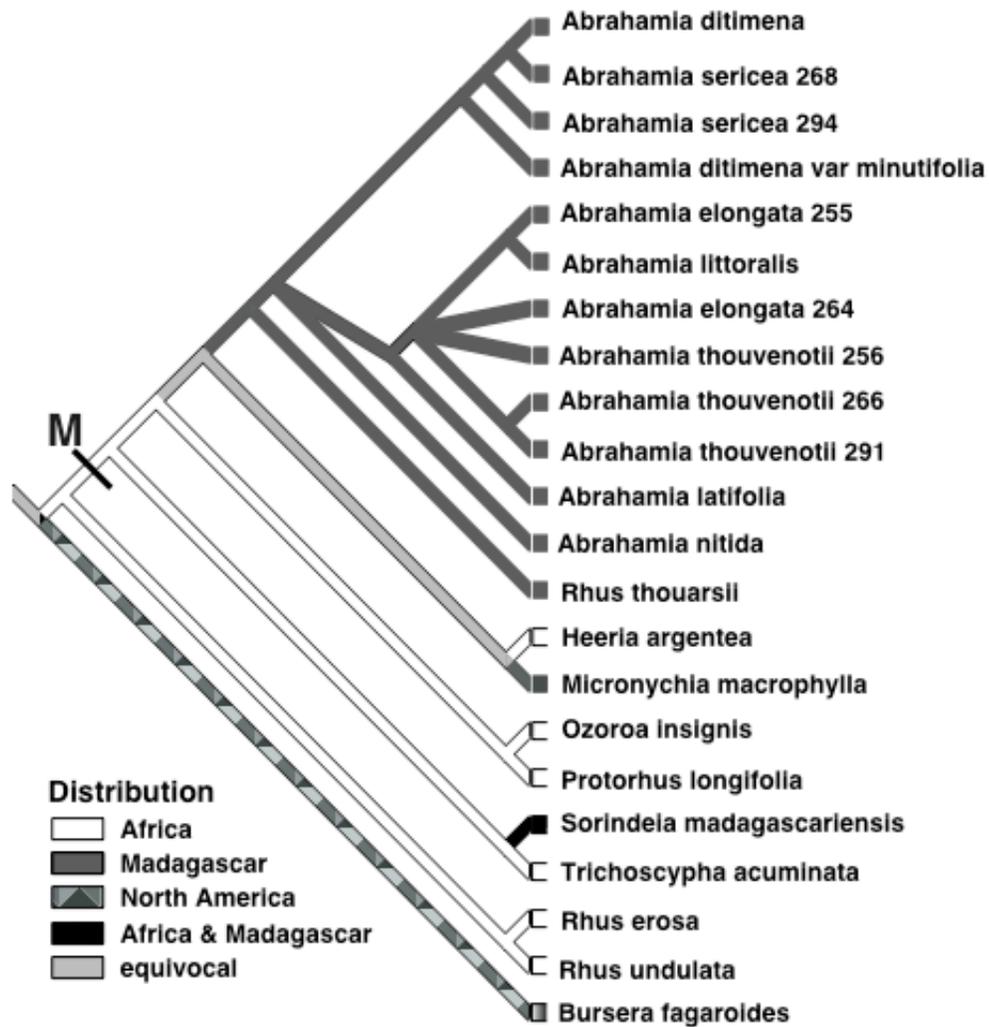


Figure 4.10. ETS, ITS, and *trnL*F tree from Fig. 4.7 with the distribution of the species mapped on the phylogeny. The distribution of *Sorindeia madagascariensis* is coded as polymorphic but is designated with a unique shade for visual purposes.

sister to the other two Madagascan endemic genera (*Abrahamia* and *Baronia*)

(Fig. 4.10). The only other Madagascan taxon in the phylogeny is *Sorindeia*

*madagascariensis*, which occurs in both Africa and Madagascar. The basal-most

Anacardiaceae clade contains the African genus *Searsia* species (designated as

*Rhus erosa* and *R. undulata*) (Fig. 4.10). The root of the clade is a North American representative of the sister family to Anacardiaceae, Burseraceae (*Bursera fagaroides*).

## Discussion

**Taxonomic Relationships** □ The disparate morphological features, number of styles and locules (Figs. 4.8-4.9), in combination with the geographical separation (Fig. 4.10) of the African and Madagascan species of *Protorhus* provide persuasive evidence for the segregation of *Abrahamia*. The morphological characters that best distinguish *Abrahamia* from *Protorhus* are fruit and flower structures (Table 4.1 and Figs. 4.8-4.9). These features have been highlighted in recognizing the two genera as distinct from one another (von Teichman, 1991; von Teichman and van Wyk, 1996; Randrianasolo, 1998). The molecular evidence of DNA sequences from both the plastid and nuclear genomes further supports the recognition of *Protorhus* and *Abrahamia*. This data indicates that these two genera are evolutionarily removed from one another with several lineages occurring between them. This result echoes those of Randrianasolo (1998) while contradicting the two earlier taxonomic studies of this group of taxa that recognized them in only one genus, *Protorhus* (Engler, 1881; Perrier de al Bâthie, 1946). Recent morphological studies have also suggested that *Protorhus* might be comprised of more than one lineage (von Teichman, 1991; von Teichman and van Wyk, 1996).

*Abrahamia ibityensis* appears to be the most basal species in the genus based on the ITS and combined ITS-ETS phylogenies (Figs. 4.4 and 4.6, respectively). The ETS topology indicates that its position is unresolved in the larger clade in which *Abrahamia* occurs (Fig. 4.3). Randrianasolo (1998) notes that this is the only *Abrahamia* species with readily separated cotyledons and peripheral resiniferous canals in the fruit (other *Abrahamia* species have fruit that is resiniferous throughout). Three of the taxa in the sister clades to *Abrahamia* (Figs. 4.3-4.6) also have easily separable cotyledons (*Micronychia macrophylla*, *Rhus thouarsii*, and *R. perrieri* (Courchet) H. Perrier) (Randrianasolo, 1998), thus suggesting that the position of *A. ibityensis* at the base of *Abrahamia* is consistent with morphological trends. In general, evolutionary trends of the morphological characters in this clade are unusual in that while the number of styles decreases from one to three, the number of locules increases from one to three (Figs. 4.8-4.9). The ancestral state of this Anacardiaceae clade was most likely three carpels with or without some degree of fusion. The extant state is to have varying degrees of fusion of the three carpels.

In his thesis, Randrianasolo (1998) differentiates 19 species in *Abrahamia* but recognizes that some specimens are quite aberrant and difficult to assign to a single species. For this reason he suggests that further collection and morphological study may be required to fully delimit the species in *Abrahamia*. Randrianasolo, following his own species delimitation, identified all of the specimens used in this study, but four species, *A. sericea*, *A. thouvenotii*, *A.*

*elongata*, and *A. ditimena*, are polyphyletic in the phylogenies presented here (Figs. 4.3-4.7). Thus, the molecular data also suggests that the species boundaries of this group need to be reevaluated. A more comprehensive phylogeny of all of the *Abrahamia* species is currently being undertaken in order to more accurately delimit the species boundaries of this Madagascan endemic.

**Biogeographic Relationships** □ Angiosperms are estimated to have originated 125 to 135 Myr ago (Soltis et al. 2002), making their appearance in geological time after Madagascar split from the African continent (165 Myr ago). Some 41 to 60 Myr after angiosperms evolved, ancestral members of the order Sapindales appeared (65 to 84 Myr ago). Madagascar was completely free of attachment to other landmasses by 88 Myr ago (Schatz, 1996; Storey et al. 1995), making vicariance a very unlikely explanation of the distribution of Sapindales. Thus, all Sapindalian plants most likely either arrived in or left Madagascar via over-water dispersal, depending on in which landmass the order originated. This is consistent with studies of other organisms that found similar evidence for one or more dispersal events between Africa and Madagascar (i.e. *Begonia*: Plana, 2003; carnivores: Yoder et al., 2003; cichlids: Murray, 2001; primates: Yoder, 1996; *Wolfiella*: Kimball et al., 2003).

Distributional patterns mapped onto the phylogeny (Fig. 4.10) along with the geological age estimates of the Sapindales and the Gondwanan break-up suggest that one clade of Anacardiaceae (clade M in Fig. 4.10) colonized

Madagascar via over-water dispersal from Africa as many as three times.

Another possible explanation of the observed pattern in the clade is that there was a single colonization of Madagascar and then three subsequent re-colonizations of the African continent. Some debate is still ongoing as to the occurrence of a landbridge consisting of small islands in the Mozambique Channel (see McCall, 1997; Rogers et al., 2000; and Yoder et al., 2003). It is possible that dispersal from Africa was a result of island-hopping and not solely over-water dispersal, but regardless of the manner in which they arrived in Madagascar, it is clear from the phylogeny that the endemic genera *Abrahamia*, *Baronia* (= *Rhus thouarsii*), and *Micronychia* evolved subsequent to colonization from the African continent.

## Chapter 5: Conclusions

The Anacardiaceae Lindl. is clearly shown to be monophyletic by all of the molecular data presented in this study. Phylogenies of *trnLF*, *rps16*, and *matK* (Figs. 3.7 – 3.8, 3.5 - 3.6, and 3.3 – 3.4 respectively) all concur that the cashew family is distinct but sister to the gumbo-limbo family, Burseraceae. This finding answers more than a hundred years of confusion regarding the relationship of these two families.

Evidence is also presented that several families that are often excluded from the Anacardiaceae should be recognized within it. The Julianiaceae, Pistaciaceae, and Podoaceae are all shown to be nested within the cashew family and it is therefore recommended that their taxonomy should reflect their evolutionary position and that their species be transferred to Anacardiaceae.

Classification within the Anacardiaceae has been in question since Bentham and Hooker (1862) proposed the first subfamilial system for the cashew family in which they split it into two groups, Anacardieae and Spondieae. Despite most other authors splitting the family into more elaborate systems of subfamilial classification, the molecular results do not refute the original two groups of Bentham and Hooker.

Based on the topologies presented here, a new intrafamilial system of classification is proposed. This system includes two subfamilies: Anacardioideae and Spondioideae. The following characters define these two subfamilies:

**Anacardioideae** (including Anacardieae, Dobineae, Rhoeeae and Semecarpeae):

Trees, shrubs, rarely vines or perennial herbs. Leaves simple or compound, alternate (usually) or opposite (e.g. *Abrahamia*, *Blepharocarya*, *Bouea*, *Campylopetalum*, and *Ozoroa*) pinnate venation (palmate in *Campylopetalum*). Stamens variable in number; carpels one or three and fused (rarely four to six and partially syncarpous in *Buchanania*); one locule (often by abortion, very rarely two locules in *Camptosperma*); one ovule; apical, basal or lateral ovule insertion; one to three styles, either fused or separate; one to three stigmas; wind and insect pollinated; usually *Anacardium*-type endocarp (discretely layered and with palisade-like sclereids, not found in *Buchanania* Spreng., *Camptosperma*, *Pentaspadon* and possibly others that have not been investigated); exocarp usually thin; animal and wind dispersed fruits. This is the only subfamily in which contact dermatitis-causing taxa occur (i.e. only subfamily that has the ability to produce catechols and other low molecular weight compounds such as resorcinols and other phenols) (Ding Hou, 1978; Mitchell and Mori, 1987; Mitchell, 1990; Wannan and Quinn, 1990).

**Spondioideae** (including Spondiadeae): Trees or shrubs. Leaves compound (rarely simple in *Haplospondias* Kosterm., unifoliolate in some species of *Lannea* or may have both simple and compound leaves in a single individual in *Sclerocarya*). Stamens two times the number of petals; carpels four to five (rarely one in *Solenocarpus* or more than five in *Pleiogynium*); four to five locules

(rarely one or more than five); one ovule per locule; ovules pendulous from an apical funicle; four to five styles; insect pollinated; *Spondias*-type endocarp (mass of lignified and irregularly oriented sclerenchyma); exocarp thick; animal dispersed fruits. This is the only subfamily in which opercula occur (Ding Hou, 1978; Mitchell and Mori, 1987; Wannan and Quinn, 1990).

The phylogenetic analysis of the Madagascan genus, *Protorhus* Engl., identifies some problematic species boundaries in Randrianasolo's (1998) treatment of the segregate genus *Abrahamia* ined., but shows this genus to be monophyletic and distinct from *Protorhus*. Biogeographic interpretation of the phylogeny indicated that Anacardiaceae were dispersed between Africa and Madagascar over water a minimum of three times. The result of this study is a recommendation that the new genus be recognized but that species delimitation in the group is re-evaluated, especially for the large-leaved taxa.

Future studies in the Anacardiaceae should focus on the following areas: (1) to collect in areas that are particularly under-represented in most western herbaria but that hold a wealth of Anacardiaceae taxa (i.e. Southeast Asia, Africa, Pacific Islands); (2) to incorporate these collections into the molecular and morphological database in order to more completely examine the biogeographic history of the cashew family and place all genera within the subfamilial classification system (3) to conduct monographic studies of taxonomically

problematic genera and (4) to continue to investigate higher relationships in the Sapindales, particularly in regard to Burseraceae and Anacardiaceae.

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## Appendix A: Taxa Included in Molecular Datasets

Table A.1. List of taxa used in this study. An X indicates that the listed specimen was used in the indicated dataset. The numbers in the dataset columns indicate that the sequence for that species was combined with that of another (referenced by the extraction number) to represent the taxon in a combined dataset where complete sequence representation was not obtained for identical samples.

| Taxon   | Extract # | Voucher Location | Collector # and Locality      | 3 gene | mat +trnL | rps+trnLF | Abrahamia Total | ETS + ITS | ITS      | ETS      | trnLF    | Abrahamia trnLF | trnLF Genera | trnLF Families | rps16 | matK |
|---|-----------|------------------|-------------------------------|--------|-----------|-----------|-----------------|-----------|----------|----------|----------|-----------------|--------------|----------------|-------|------|
| <i>Abrahamia buxifolia</i> var. <i>buxifolia</i> (H. Perrier)<br>Randrianasolo comb. nov. ined.   | 308       | MO               | Randrianasolo 488, Madagascar |        |           |           |                 |           |          | AY594395 | AY594427 | X               |              |                |       |      |
| <i>Abrahamia buxifolia</i> var. <i>itremoensis</i> (H. Perrier)<br>Randrianasolo comb. nov. ined. | 317       | MO               | Baum 47, Madagascar           |        |           |           |                 |           |          | AY594396 |          |                 |              |                |       |      |
| <i>Abrahamia deflexa</i> var. <i>zombitsensis</i> (H. Perrier)<br>Randrianasolo comb. nov. ined.  | 316       | MO               | Phillipson 2853, Madagascar   |        |           |           |                 |           |          | AY594392 |          |                 |              |                |       |      |
| <i>Abrahamia ditimena</i> (H. Perrier)<br>Randrianasolo comb. nov. ined.                          | 254       | MO               | McPherson 17302, Madagascar   |        | X         | X         | X               | AY594361  | AY594393 | AY594429 | X        | X               | X            |                |       |      |
| <i>Abrahamia ditimena</i> var. <i>minutifolia</i> (H. Perrier)<br>Randrianasolo comb. nov. ined.  | 292       | MO               | Randrianasolo 781, Madagascar |        |           |           | X               | X         | AY594373 | AY594405 | AY594436 | X               |              |                |       |      |

Table A.1 continued

|   |     |    |                                |  |  |   |   |   |          |          |          |   |  |  |          |  |
|---|-----|----|--------------------------------|--|--|---|---|---|----------|----------|----------|---|--|--|----------|--|
| Abrahamia elongata Capuron ex Randrianasolo ined.               | 264 | NY | Pell 637, Madagascar           |  |  |   | X | X | AY594363 | AY594391 | AY594428 | X |  |  |          |  |
| Abrahamia elongata  | 255 | MO | Randrianasolo 596, Madagascar  |  |  |   | X | X | AY594362 | AY594394 | AY594430 | X |  |  |          |  |
| Abrahamia ibityensis (H. Perrier) Randrianasoo comb. nov. ined. | 311 | MO | Rogers 82, Madagascar          |  |  |   |   | X | AY594364 | AY594397 |          |   |  |  |          |  |
| Abrahamia lanceolata H. Perrier ex Randrianasoo ined            | 310 | MO | Randrianasolo 501, Madagascar  |  |  |   |   | X | AY594365 | AY594398 |          |   |  |  |          |  |
| Abrahamia latifolia (Engl.) Randrianasolo comb. nov. ined.      | 252 | MO | Randrianasolo 511, Madagascar  |  |  |   | X | X | AY594366 | AY594399 | AY594431 | X |  |  |          |  |
| Abrahamia littoralis Randrianasolo sp. nov. ined.,              | 269 | NY | Pell 609, Madagascar           |  |  |   | X | X | AY594371 | AY594403 | AY594434 | X |  |  |          |  |
| Abrahamia littoralis Randrianasolo sp. nov. ined.               | 305 | MO | Randrianasolo 467a, Madagascar |  |  |   |   |   | AY594367 | AY594400 |          |   |  |  |          |  |
| Abrahamia nitida (Engl.) Randrianasolo comb. nov. ined.         | 253 | MO | Randrianasolo 586, Madagascar  |  |  | X | X | X | AY594368 | AY594401 | AY594432 | X |  |  | AY594582 |  |

Table A.1 continued

|   |     |    |                                     |    |   |  |   |   |          |          |          |   |  |  |  |          |
|---|-----|----|-------------------------------------|----|---|--|---|---|----------|----------|----------|---|--|--|--|----------|
| Abrahamia sericea<br>(Engl.)<br>Randrianasolo<br>comb. nov. ined.           | 268 | NY | Pell 621,<br>Madagascar             |    |   |  | X | X | AY594370 | AY594402 | AY594433 | X |  |  |  |          |
| Abrahamia sericea<br>(Engl.)<br>Randrianasolo<br>comb. nov. ined.           | 294 | MO | Randrianasolo<br>783,<br>Madagascar |    |   |  | X | X | AY594374 | AY594406 | AY594437 | X |  |  |  |          |
| Abrahamia<br>suarezensis<br>Capuron ex<br>Randrianasolo<br>comb. nov. ined. | 303 | MO | PcPherson<br>15,<br>Madagascar      |    |   |  |   |   |          | AY594407 |          |   |  |  |  |          |
| Abrahamia<br>thouvenotii<br>(Lecomte)<br>Randrianasolo<br>comb. nov. ined.  | 256 | MO | McPherson<br>17266,<br>Madagascar   |    |   |  | X | X | AY594375 | AY594408 | AY594438 | X |  |  |  |          |
| Abrahamia<br>thouvenotii  | 266 | NY | Pell 633,<br>Madagascar             |    |   |  | X | X | AY594376 | AY594409 | AY594439 | X |  |  |  |          |
| Abrahamia<br>thouvenotii  | 291 | MO | Randrianasolo<br>779,<br>Madagascar |    |   |  | X | X | AY594372 | AY594404 | AY594435 | X |  |  |  |          |
| Abrahamia viguieri<br>(H. Perrier)<br>Randrianasolo<br>comb. nov. ined.,    | 288 | MO | Randrianasolo<br>776,<br>Madagascar |    |   |  |   |   |          |          | AY594440 | X |  |  |  |          |
| Amphipterygium<br>adstringens<br>(Schldl.) Standley                         | 110 | NY | Panero 4501,<br>Mexico              | 28 | X |  |   |   |          |          |          |   |  |  |  | AY594458 |

Table A.1 continued

|   |     |            |   |     |   |   |  |  |  |          |          |   |   |   |          |          |
|---|-----|------------|---|-----|---|---|--|--|--|----------|----------|---|---|---|----------|----------|
| Amphipterygium adstringens (Schldl.) Standley         | 287 | RBG E      | Pendry 845, Mexico                                  | 110 |   | X |  |  |  |          | AY594496 |   | X | X | AY594583 |          |
| Anacardium occidentale L.□                            | 92  | NY         | Mori 24142, French Guiana                           |     |   | X |  |  |  |          | AY594497 |   | X | X |          | AY594459 |
| Antrocaryon amazonicum (Ducke) B.L. burt & A.W. Hill□ | 93  | NY         | Mitchell 663, NYBG Living Collection from Brazil    | X   | X | X |  |  |  | AY594410 | AY594441 | X | X |   | AY594584 | AY594460 |
| Apterokarpos gardneri (Engl.) Rizzini                 | 191 | NY         | Pirani 2586 Cordeiro, Giulietti & Fernandes, Brazil |     |   | X |  |  |  |          | AY594498 |   | X | X | AY594585 |          |
| Astronium fraxinifolium Schott□                       | 156 | RBG E      | Pendry 505, Bolivia                                 |     |   | X |  |  |  |          | AY594542 |   | X |   | AY594586 |          |
| Astronium graveolens Jacq.□                           | 78  | NY         | Brokaw 81, Belize                                   |     |   |   |  |  |  |          |          |   |   |   |          | AY594492 |
| Bonetiella anomala (I. M. Johnston) Rzedowski         | 175 | F 2093 102 | Johnston, Wendt & Chiang 11488, Mexico              |     |   | X |  |  |  |          | AY594543 |   | X |   | AY594587 |          |
| Boswellia frereana Birdw.□                            | 87  | NY         | Matsthulian 4302, Somalia                           | X   | X | X |  |  |  |          | AY594499 |   | X | X | AY594588 | AY594461 |
| Bouea macrophylla Griff.                              | 57  | NY         | Gentry & Frankie 66957, Peninsular Malaysia         |     |   | X |  |  |  |          | AY594500 |   | X | X | AY594589 |          |

Table A.1 continued

|   |     |          |  |   |   |         |   |   |          |          |          |   |   |   |          |          |
|---|-----|----------|--|---|---|---------|---|---|----------|----------|----------|---|---|---|----------|----------|
| <i>Bursera fagaroides</i><br>Engl.□                                       | 86  | NY       | Mitchell 657,<br>NYBG Living<br>Collection<br>from Mexico    | X | X | X       | X | X | AY594390 | AY594411 | AY594501 | X | X | X | AY594590 | AY594462 |
| <i>Canarium<br/>madagascariense</i><br>Engl.                              | 275 | NY       | Pell 674,<br>Madagascar                                      |   |   |         |   |   |          |          | AY594502 |   |   | X |          |          |
| <i>Cardenasiodendron<br/>brachypterum</i><br>(Loes.) F.A.<br>Barkley□     | 154 | RBG<br>E | Pendry 691,<br>Bolivia                                       |   |   |         |   |   | AY594377 |          | AY594503 | X | X | X |          |          |
| <i>Cardiospermum<br/>halicacabum</i> L.□                                  | 152 | LSU      | Ferguson 228,<br>USA   |   |   |         |   |   |          |          | AY594504 |   |   | X |          |          |
| <i>Choerospondias<br/>axillaris</i> (Roxb.)<br>B.L. Burtt & A.W.<br>Hill□ | 67  | NY       | King, NYBG<br>living<br>collection from<br>Nepal             | X | X | X       |   |   |          |          | AY594544 |   | X |   | AY594591 | AY594463 |
| <i>Commiphora<br/>leptophloeos</i> (Mart.)<br>Gillett                     | 183 | NY       | Figueirêdo<br>(369) Andrade<br>& Oliveira,<br>Brazil         |   |   |         |   |   |          |          | AY594505 |   |   | X |          |          |
| <i>Comocladia<br/>dodoniaea</i> (L.)<br>Urban                             | 297 | NY       | Specht 10,<br>Puerto Rico                                    |   |   | 15<br>9 |   |   |          |          |          |   |   |   | AY594592 |          |
| <i>Comocladia<br/>engleriana</i> Loes□                                    | 159 | RBG<br>E | Pendry 806,<br>Mexico  |   |   | 29<br>7 |   |   |          |          | AY594506 |   | X | X |          |          |
| <i>Cotinus coggygia</i><br>Scop.  | 39  | LSU      | Bamps 8753,<br>France  |   |   |         |   |   |          |          | AY594545 |   | X |   |          |          |
| <i>Cotinus obovatus</i><br>Raf.   | 147 | MOR      | Reichard 386,<br>Morton<br>Arboretum<br>living<br>collection |   |   | X       |   |   |          |          | AY594546 |   | X |   | AY594593 |          |

Table A.1 continued

|  |     |            |  |   |   |     |  |  |  |          |   |   |          |          |
|--|-----|------------|--|---|---|-----|--|--|--|----------|---|---|----------|----------|
| <i>Crepidospermum prancei</i> Daly             | 181 | NY         | Vester 683, Colombia                                 |   |   | X   |  |  |  | AY594507 |   | X | AY594594 |          |
| <i>Cupania scrobiculata</i> Rich.              | 201 | US         | Acevedo 11119, French Guiana                         |   |   | X   |  |  |  | AY594508 |   | X | AY594595 |          |
| <i>Cyrtocarpa edulis</i> (Brandegees) Standley | 174 | F 2120 859 | Elias 10714, Baja, Mexico                            |   |   |     |  |  |  | AY594547 | X |   |          |          |
| <i>Cyrtocarpa procera</i> Kunth                | 103 | NY         | Torres 1240, Mexico                                  | X | X | X   |  |  |  | AY594548 | X |   | AY594596 | AY594464 |
| <i>Dacryodes excelsa</i> Vahl.                 | 105 | NY         | Struwe & Specht 1085, Puerto Rico                    |   |   | X   |  |  |  | AY594509 |   | X |          | AY594465 |
| <i>Dilodendron bibinnatum</i> Radlk.           | 199 | US         | Acevedo 11129, Bolivia                               |   |   |     |  |  |  | AY594510 |   | X |          |          |
| <i>Diplokeleba floribunda</i> N.E. Br.         | 202 | US         | Acevedo 11130, Bolivia                               |   |   |     |  |  |  | AY594511 |   | X |          |          |
| <i>Dobinea vulgaris</i> Buch.-Ham. ex D. Don   | 112 | NY         | Delendick 76.1570, NYBG Living collection from Nepal |   |   | X   |  |  |  | AY594512 | X | X |          | AY594466 |
| <i>Dodonaea viscosa</i> Jacq.                  | 197 | US         | Acevedo 11144, Bolivia                               |   |   | X   |  |  |  | AY594513 |   | X | AY594597 |          |
| <i>Dracontomelon dao</i> (Blco.) Merr & Rolfe  | 99  | NY         | Soejarto, Madulid & Fernando 7822, Philippines       |   |   | 179 |  |  |  |          |   |   |          | AY594467 |

Table A.1 continued

|  |     |                  |   |   |   |         |   |         |          |          |          |          |   |   |   |          |          |
|--|-----|------------------|---|---|---|---------|---|---------|----------|----------|----------|----------|---|---|---|----------|----------|
| Dracontomelon lenticulatum Wilkinson     | 179 | NY               | Motley 2301, Lae Botanical Garden Living Collection, New Guinea |   |   | 99      |   |         |          |          |          | AY594549 |   | X |   |          |          |
| Dracontomelon vitiense Engl.             | 178 | F<br>2127<br>541 | Regaldo & Vodonaivalu 905, Fiji                                 |   |   |         |   |         |          |          |          | AY594550 |   | X |   |          |          |
| Faguetia falcata March.                  | 270 | NY               | Pell 600, Madagascar  |   |   | 24<br>4 |   |         |          |          |          | AY594514 |   | X | X |          |          |
| Faguetia falcata March.                  | 244 | MO               | Randrianasolo 588, Madagascar                                   |   |   | 27<br>0 |   |         |          |          |          |          |   |   |   | AY594598 |          |
| Fegimanra africana Pierre                | 96  | NY               | Reitsma & Reitsma 1257, Gabon                                   |   |   | X       |   |         |          |          |          | AY594515 |   | X | X | AY594599 | AY594489 |
| Gluta wallichii (Hook f.) Ding Hou       | 51  | NY               | Beaman 7065, Borneo   | X | X | X       |   |         |          |          |          | AY594516 |   | X | X | AY594600 | AY594468 |
| Guioa koelreuteria (Blanco) Merr.        | 195 | NY               | Takeuchi 7123, Papua New Guinea                                 |   |   |         |   |         |          |          |          | AY594517 |   |   | X |          |          |
| Harpephyllum caffrum Bernh. Ex K. Krause | 101 | NY               | Lau 1588, Cultivated at Foster Botanical Garden, Hawaii         | X | X | X       |   |         |          |          |          | AY594518 |   | X | X | AY594601 | AY594469 |
| Heeria argentea (Thunb.) Meisn.          | 315 | MO               | Goldblatt s.n., South Africa                                    |   |   |         |   | 18<br>8 | AY594379 |          |          |          |   |   |   | AY594602 |          |
| Heeria argentea (Thunb.) Meisn.          | 188 | NY               | Shantz & Turner 4017, South Africa                              |   |   | X       | X | 31<br>5 | AY594378 | AY594412 | AY594442 |          | X | X |   | AY594603 |          |

Table A.1 continued

|   |            |                        |   |         |   |   |  |  |  |  |          |   |   |          |
|---|------------|------------------------|---|---------|---|---|--|--|--|--|----------|---|---|----------|
| Hermogenodendron<br>concinnum ined.                     | 189        | NY                     | Kubitzki &<br>Poppendieck<br>79-272, Brazil |         |   |   |  |  |  |  | AY594551 | X |   |          |
| Hypelate trifoliata<br>Sw.                              | 198        | US                     | Acevedo<br>11425, Puerto<br>Rico            |         |   | X |  |  |  |  | AY594519 |   | X | AY594604 |
| Lansea rivae<br>(Chiov.) Sacleux                        | 251        | MO                     | Randrianasolo<br>662, Tanzania              |         |   |   |  |  |  |  | AY594520 |   | X |          |
| Lansea<br>schweinfurthii<br>(Engl.) Engl.               | 250        | MO                     | Randrianasolo<br>661, Tanzania              |         |   | X |  |  |  |  | AY594552 | X |   | AY594605 |
| Lansea welwitschii<br>var. welwitschii<br>(Hiern) Engl. | 97         | NY                     | Nemba &<br>Thomas 532,<br>Cameroon          |         |   |   |  |  |  |  | AY594553 | X |   |          |
| Lithrea molleoides<br>(Vell.) Engl.                     | 141        | RBG<br>E<br>AN1<br>5   | Pendry 711,<br>Bolivia                      | 10<br>8 |   |   |  |  |  |  | AY594554 | X |   |          |
| Lithrea molleoides<br>(Vell.) Engl.                     | 108        | NY                     | Mecenas &<br>Leite 81,<br>Brazil            | 14<br>1 |   |   |  |  |  |  |          |   |   | AY594470 |
| Loxopterygium<br>grisebachii Hieron.<br>& Lorentz       | 143-<br>1  | RBG<br>E<br>AN2<br>7-1 | Pendry 678,<br>Bolivia                      |         |   |   |  |  |  |  | AY594555 | X |   |          |
| Loxopterygium<br>huasango Spruce<br>ex Engl.            | 68,<br>139 | RBG<br>E               | Pennington<br>820, Peru                     | 14<br>5 | X |   |  |  |  |  | AY594521 | X |   | AY594471 |
| Loxopterygium<br>sagotii Hook. f.                       | 145        | RBG<br>E<br>AN3<br>5   | Polak 309,<br>Guyana                        | 68      | X |   |  |  |  |  |          |   |   | AY594606 |

Table A.1 continued

|  |     |                           |  |   |   |   |   |   |          |          |          |   |   |   |          |          |
|--|-----|---------------------------|--|---|---|---|---|---|----------|----------|----------|---|---|---|----------|----------|
| <i>Loxostylis alata</i><br>Spreng. f. ex<br>Reichb.      | 238 | NY                        | Mitchell 652,<br>NYBG living<br>collection 488-<br>84A from<br>South Africa                |   |   | X |   |   |          |          | AY594522 |   | X | X | AY594607 |          |
| <i>Mangifera indica</i> L.                               | 285 | NY                        | Mitchell,<br>NYBG living<br>collection   |   |   |   |   |   |          |          | AY594523 |   | X | X |          |          |
| <i>Mangifera indica</i> L.                               | 90  | NY                        | Annable 2769,<br>Fairchild<br>Tropical<br>Gardens<br>Living<br>Collection,<br>Florida, USA | X | X | X |   |   |          | AY594413 |          | X |   |   | AY594608 | AY594472 |
| <i>Mauria simplicifolia</i><br>Kunth                     | 176 | F<br>2145<br>100          | Leiva et al.<br>1552, Peru   |   |   |   |   |   |          |          | AY594556 |   | X |   |          |          |
| <i>Metopium brownei</i><br>(Jacq.) Urb.                  | 69  | NY                        | Brokaw 295,<br>Belize  |   |   | X |   |   |          |          | AY594557 |   | X |   | AY594609 |          |
| <i>Micronychia</i><br><i>macrophylla</i> H.<br>Perrier   | 262 | NY                        | Pell 643,<br>Madagascar  |   |   | X | X | X | AY594380 | AY594414 | AY594443 | X | X |   | AY594610 |          |
| <i>Micronychia</i><br><i>tsiramiramy</i> H.<br>Perrier   | 265 | NY                        | Pell 634,<br>Madagascar  |   |   | X |   |   |          |          | AY594524 | X | X | X | AY594611 |          |
| <i>Mosquitoxylum</i><br><i>jamaicense</i> Krug &<br>Urb. | 79  | NY                        | Brokaw 158,<br>Belize  |   |   |   |   |   |          |          |          |   |   |   |          | AY594490 |
| <i>Mosquitoxylum</i><br><i>jamaicense</i> Krug &<br>Urb. | 185 | MOB<br>OT<br>0490<br>4277 | Rodríguez<br>736, Costa<br>Rica  |   |   | X |   |   |          |          | AY594558 |   | X |   | AY594612 |          |

Table A.1 continued

|   |     |                  |   |         |   |   |   |   |          |          |          |   |   |          |          |          |
|---|-----|------------------|---|---------|---|---|---|---|----------|----------|----------|---|---|----------|----------|----------|
| Myracrodruon urundeuva Allem.                             | 155 | RBG<br>E         | Pendry 724,<br>Bolivia                                      |         |   | X |   |   |          | AY594560 |          | X |   | AY594613 |          |          |
| Myracrodruon balansae (Engl.) Santin                      | 166 | F<br>2046<br>108 | Schinini<br>24043,<br>Paraguay                              |         |   |   |   |   |          | AY594559 |          | X |   |          |          |          |
| Ochoterenaea colombiana F.A. Barkley                      | 165 | F<br>1977<br>808 | Escobar,<br>Folsom, Brand<br>& Sánchez<br>2598,<br>Colombia |         |   |   |   |   |          | AY594561 |          | X |   |          |          |          |
| Operculicarya decaryi H. Perrier                          | 245 | MO               | Randrianasolo<br>627,<br>Madagascar                         |         |   | X |   |   |          | AY594525 |          |   | X | AY594614 |          |          |
| Orthopterygium huaucui (A. Gray) Hemsl.                   | 109 | NY               | Smith 5726,<br>Peru   |         |   | X |   |   |          | AY594526 |          | X | X | AY594615 |          |          |
| Ozoroa insignis subsp. reticulata (Baker f.) J.B. Gillett | 219 | MO               | Randrianasolo<br>680, Tanzania                              |         |   |   | X | X | AY594381 | AY594415 | AY594444 | X |   |          |          |          |
| Ozoroa obovata (Oliv.) A. Fern. & R. Fern.                | 220 | MO               | Randrianasolo<br>707, Tanzania                              |         |   |   |   |   |          | AY594416 | AY594445 | X |   |          |          |          |
| Pachycormus discolor Coville ex Standl.                   | 278 | NY               | Mitchell 653,<br>Baja, Mexico                               | 20<br>7 | X |   |   |   |          |          | AY594562 |   | X |          | AY594493 |          |
| Pachycormus discolor Coville ex Standl.                   | 207 | US               | Acevedo,<br>Living<br>collection                            | 22<br>0 | X |   |   |   |          |          |          |   |   |          | AY594616 |          |
| Pegia nitida Colebr.                                      | 107 | MOB<br>OT        | Zhanhuo 92-<br>254, China                                   | X       | X | X |   |   |          |          | AY594563 |   | X |          | X        | AY594473 |
| Pistacia chinensis Bunge                                  | 153 | LSU              | Pell 546, LSU<br>cultivation,<br>USA                        |         |   | X |   |   |          |          | AY594527 |   | X | X        | AY594617 |          |

Table A.1 continued

|  |     |                  |  |   |   |   |   |   |          |          |          |   |   |   |          |          |
|--|-----|------------------|--|---|---|---|---|---|----------|----------|----------|---|---|---|----------|----------|
| <i>Plagioscyphus</i> sp.<br>Radlk.                           | 276 | NY               | Pell 602,<br>Madagascar  |   |   |   |   |   |          |          | AY594528 |   |   | X |          |          |
| <i>Pleiogynium</i><br><i>timoriense</i> (A. DC.)<br>Leenh.   | 73  | NY               | NYBG living<br>collection,<br>Fruit and<br>Spice Garden<br>Living<br>Collection,<br>Florida, USA | X | X | X |   |   |          |          | AY594529 |   | X | X | AY594618 | AY594474 |
| <i>Poupartia minor</i><br>(Bojer) L. Marchand                | 274 | NY               | Pell 657,<br>Madagascar  |   |   |   |   |   |          |          | AY594530 |   | X | X |          |          |
| <i>Poupartiopsis</i><br><i>spondiocarpus</i><br>ined.        | 295 | MO               | Randrianasolo<br>592,<br>Madagascar  |   |   |   |   |   |          |          | AY594446 | X | X |   |          |          |
| <i>Protium divaricatum</i><br>subsp. <i>fumarium</i><br>Daly | 129 | NY               | Mori 24157,<br>French<br>Guiana  | X | X | X |   |   |          |          | AY594532 |   | X | X | AY594619 | AY594475 |
| <i>Protium pallidum</i><br>Cuatrec.                          | 130 | NY               | Mori 24185,<br>French<br>Guiana  |   | X |   |   |   |          |          | AY594531 |   |   | X |          | AY594476 |
| <i>Protorhus longifolia</i><br>(Bernh.) Engl.                | 261 | MO               | Randrianasolo<br>17-9-97,<br>South Africa  |   |   | X | X | X | AY594382 | AY594417 | AY594533 | X | X | X | AY594620 |          |
| <i>Pseudosmodingium</i><br><i>andrieuxii</i> Engl.           | 228 | F<br>2081<br>115 | Tenorio<br>17041, Mexico   |   |   |   |   |   |          |          | AY594566 |   | X |   |          |          |
| <i>Pseudosmodingium</i><br><i>andrieuxii</i> Engl.           | 163 | F<br>2081<br>115 | Tenorio<br>17041, Mexico   |   |   |   |   |   |          |          | AY594565 |   | X |   |          |          |
| <i>Rhus aromatica</i><br>Aiton                               | 281 | NY               | Mitchell 670,<br>Texas, USA  |   |   |   |   |   |          |          | AY594447 |   |   |   |          | AY594494 |

Table A.1 continued

|   |     |     |  |   |   |   |   |          |          |          |          |   |   |  |          |          |
|---|-----|-----|--|---|---|---|---|----------|----------|----------|----------|---|---|--|----------|----------|
| Rhus aromatica<br>Aiton                   | 46  | LSU | Mayfield 2881,<br>USA  | X | X | X |   |          |          | AY594418 |          | X | X |  | AY594621 | AY594486 |
| Rhus chiangii<br>Young                    | 186 | NY  | Johnston,<br>Wendt,<br>Chiang &<br>Henrickson<br>12221, Mexico                                   |   |   |   |   |          |          |          | AY594567 |   | X |  |          |          |
| Rhus chinensis Mill.                      | 149 | MOR | Altvatter &<br>Hammond<br>1276<br>05<br>7132 V95,<br>Morton<br>Arboretum<br>Living<br>Collection |   |   |   |   |          |          |          |          |   |   |  | AY594622 |          |
| Rhus copallina L.                         | 47  | LSU | In cultivation,<br>Louisiana,<br>USA   |   |   |   |   |          |          |          |          |   |   |  |          | AY594485 |
| Rhus copallina L.                         | 301 | NY  | Mitchell 666,<br>New Jersey,<br>USA  |   |   |   | X | AY594383 | AY594419 |          |          |   |   |  | AY594623 |          |
| Rhus erosa Thunb.                         | 299 | NY  | Stevenson<br>1395170,<br>South Africa  |   |   | X | X | X        | AY594384 | AY594420 | AY594448 | X | X |  | AY594624 |          |
| Rhus lanceolata (A.<br>Gray) Britton      | 283 | NY  | Campbell 39,<br>NYBG living<br>collection from<br>Texas, USA                                     |   |   | X |   |          |          |          | AY594449 | X |   |  | AY594625 |          |
| Rhus<br>pendulina Jacq.                   | 74  | NY  | NYBG living<br>collection from<br>South Africa   |   |   | X |   |          |          |          | AY594450 | X | X |  |          | AY594487 |
| Rhus perrieri<br>(Courchet) H.<br>Perrier | 257 | MO  | Randrianasolo<br>629,<br>Madagascar  |   |   |   | X |          |          | AY594421 |          |   |   |  | AY594626 |          |

Table A.1 continued

|  |     |            |   |   |   |   |    |   |          |          |          |          |   |   |   |          |          |
|--|-----|------------|---|---|---|---|----|---|----------|----------|----------|----------|---|---|---|----------|----------|
| <i>Rhus sandwichii</i> A. Gray           | 279 | NY         | Mitchell, Hawaii, USA   |   |   |   |    |   |          |          |          | AY594451 | X | X |   |          |          |
| <i>Rhus taratana</i> (Baker) H. Perrier  | 267 | NY         | Pell 625, Madagascar  |   |   | X |    |   |          |          |          | AY594568 |   | X |   | AY594627 |          |
| <i>Rhus thouarsii</i> (Engl.) H. Perrier | 263 | NY         | Pell 638, Madagascar  |   |   | X | 28 |   |          |          |          | AY594452 | X | X |   | AY594628 |          |
| <i>Rhus thouarsii</i> (Engl.) H. Perrier | 286 | NY         | Pell 655, Madagascar  |   |   |   | 26 | X | AY594385 | AY594422 | AY594569 |          |   | X |   |          |          |
| <i>Rhus thouarsii</i> (Engl.) H. Perrier | 134 | MOB OT     | Randriansolo 505, Madagascar  | X | X |   |    |   | AY594386 |          |          |          |   |   |   |          | AY594484 |
| <i>Rhus typhina</i> L.                   | 284 | NY         | Mitchell 672, New Jersey, USA   |   |   | X |    |   |          |          |          | AY594453 | X |   |   | AY594629 |          |
| <i>Rhus undulata</i> Jacq.               | 71  | NY         | NYBG living collection, South Africa                                  | X | X | X | X  | X | AY594387 | AY594423 | AY594454 | X        | X |   |   | AY594630 | AY594483 |
| <i>Rhus virens</i> Lindh. ex A. Gray     | 282 | NY         | Mitchell 667, Texas, USA  |   |   |   |    |   |          |          |          |          |   |   |   | AY594631 |          |
| <i>Sapindus saponaria</i> L.             | 194 | NY         | Zanoni, Mejía & Ramirez 15476, Dominican Republic                     |   |   |   |    |   |          |          |          | AY594534 |   |   | X |          |          |
| <i>Schinopsis balansae</i> Engl.         | 150 | MOR 9279 1 | Christenson 1277, USDA subtrop. Hort. Research Unit living collection |   |   |   |    |   |          |          |          | AY594570 |   | X |   |          |          |
| <i>Schinopsis brasiliensis</i> Engl.     | 135 | RBG E      | Bridgewater 1012, RBGE living collection                              | X | X | X |    |   |          |          |          |          |   |   |   | AY594632 | AY594477 |

Table A.1 continued

|  |     |            |   |        |   |   |   |   |          |          |          |   |          |          |
|--|-----|------------|---|--------|---|---|---|---|----------|----------|----------|---|----------|----------|
| <i>Schinopsis lorentzii</i> Engl.                | 160 | RBG E      | Pendry 680, Bolivia                                   |        |   |   |   |   |          |          | AY594571 | X |          |          |
| <i>Schinus areira</i> L.                         | 140 | RBG E AN10 | Pendry 737, Bolivia                                   |        |   | X |   |   |          |          | AY594572 | X | AY594633 | AY594488 |
| <i>Schinus weinmanniaefolia</i>                  |     | NY         | NYBG living collection                                |        |   |   |   |   |          |          | AY594573 | X |          |          |
| <i>Sclerocarya birrea</i> (A. Rich.) Hochst.     | 70  | NY         | NYBG living collection from South Africa              | X      | X | X |   |   |          |          | AY594574 | X | AY594634 | AY594478 |
| <i>Semecarpus anacardium</i> L. f.               | 64  | NY         | Codon & Codon 13, Nepal                               | 162, A |   | X |   |   |          |          | AY594575 | X | AY594635 |          |
| <i>Semecarpus australiensis</i> Engl.            | A   | RBG S      | Edwards, Australia                                    | 162    |   |   |   |   |          |          |          |   |          | AY594479 |
| <i>Semecarpus forstenii</i> Blume                | 162 | F 2126604  | Regalado & Sirikolo 812, Solomon Islands              | 64, A  | A |   |   |   |          |          | AY594535 | X | X        |          |
| <i>Serjania glabrata</i> Kunth                   | 200 | US         | Acevedo 6553, Bolivia                                 |        |   |   |   |   |          |          | AY594536 |   | X        |          |
| <i>Serjania polyphylla</i> (L.) Radlk.           | 209 | US         | Acevedo, cultivated in Puerto Rico                    |        |   |   |   |   |          |          | AY594537 |   | X        |          |
| <i>Smodingium argutum</i> E. Mey. ex Sond.       | 234 | MOR        | Winter 88, Upington Nursery, S. Af, living collection |        |   | X |   |   |          |          | AY594576 | X |          | AY594636 |
| <i>Sorindeia madagascariensis</i> Thouars ex DC. | 248 | MO         | Randrianasolo 653, Tanzania                           |        |   | X | X | X | AY594388 | AY594424 | AY594455 | X | X        | AY594637 |

Table A.1 continued

|  |     |     |   |    |   |   |  |  |  |  |          |   |          |          |          |
|--|-----|-----|---|----|---|---|--|--|--|--|----------|---|----------|----------|----------|
| Spondias mombin L.                               | 81  | NY  | Mitchell, NYBG living collection                      |    | X |   |  |  |  |  | AY594577 | X |          | AY594480 |          |
| Tapirira bethanniana J.D. Mitchell               | 240 | NY  | Mori 24337, French Guiana                             | X  | X | X |  |  |  |  | AY594578 | X | AY594638 | AY594481 |          |
| Tapirira obtusa (Benth) J.D. Mitchell            | 132 | NY  | Mori 24213, French Guiana                             |    |   |   |  |  |  |  |          |   |          | AY594482 |          |
| Tapirira obtusa (Benth) J.D. Mitchell            | 300 | NY  | Mori 24744, French Guiana                             |    |   | X |  |  |  |  | AY594579 | X | AY594639 |          |          |
| Tetragastris panamensis (Engl.) Kuntze           | 193 | NY  | Jiménez 1852, Ramírez & Rojas, Costa Rica             |    |   | X |  |  |  |  | AY594538 |   | X        | AY594640 |          |
| Thouinia portoricensis Radlk.                    | 204 | US  | Acevedo 11435, Puerto Rico                            |    |   |   |  |  |  |  | AY594539 |   | X        |          |          |
| Thyrsodium spruceanum Benth.                     | 296 | NY  | Mori 24215, French Guiana                             |    |   |   |  |  |  |  |          |   |          | AY594641 |          |
| Toxicodendron radicans Kuntze                    | 136 | LSU | Pell 545, USA   | 27 |   | X |  |  |  |  | AY594540 | X | X        | AY594642 | AY594491 |
| Toxicodendron vernicifluum (Stokes) F.A. Barkley | 298 | NY  | Mitchell 660, NYBG Living Collection from South Korea |    |   | X |  |  |  |  | AY594580 | X |          | AY594643 |          |
| Toxicodendron vernix (L.) Kuntze                 | 277 | NY  | Mitchell 673, Pennsylvania, USA                       |    |   | X |  |  |  |  | AY594581 | X |          | AY594495 |          |

Table A.1 continued

|                                  |     |    |                              |  |  |  |   |   |          |          |          |   |   |   |  |
|----------------------------------|-----|----|------------------------------|--|--|--|---|---|----------|----------|----------|---|---|---|--|
| Trichoscypha acuminata Engl.     | 237 | MO | Walters et al. 539, Gabon    |  |  |  | X | X | AY594389 | AY594425 | AY594456 | X | X |   |  |
| Trichoscypha ulugurensis Mildbr. | 221 | MO | Randrianasolo 726, Tanzania  |  |  |  |   |   |          | AY594426 | AY594457 | X | X |   |  |
| Zanthoxylum sp. III.             | 205 | US | Acevedo 11126, French Guiana |  |  |  |   |   |          |          | AY594541 |   |   | X |  |
|                                  |     |    |                              |  |  |  |   |   |          |          |          |   |   |   |  |

## Appendix B: Protocol for DNA Extraction of Herbarium Specimens

This is a protocol for DNA extraction of herbarium specimens using a FastPrep Lysing FP-120 Bead Mill and the Qiagen DNeasy Plant Mini Kit. It is a modification of the manufacturer's (Qiagen Inc., Valencia CA) recommendations and was designed for the Cullman Program for Molecular Systematics Studies at The New York Botanical Garden by Kenneth Wurdack. It is combined here with instructions for using the FastPrep lysing FP-120 bead mill using lysing matrix "A" tubes containing a ceramic bead and garnet sand (Qbiogene Inc., Carlsbad, CA), but a mortar and pestle may be used instead to grind the tissue.

Table B.1 List of reagents and supplies provided in the DNeasy Plant Mini Kit and those that the researcher must provide.

| Included in the Qiagen Kit | NOT included in the Qiagen Kit |
|----------------------------|--------------------------------|
| Buffer AP1                 | FastPrep tubes                 |
| Buffer AP2                 | β-mercaptoethanol              |
| Buffer AP3/E               | proteinase K                   |
| Buffer AW                  | 2.0 ml tubes                   |
| Buffer AE                  | EXTRA Buffer AW                |
| QIAshredder spin columns   | EXTRA 2 ml collection tubes    |
| DNeasy columns             |                                |
| 2 ml collection tubes      |                                |

Before beginning:

- If Buffer AP1 has formed a precipitate, warm to 65 °C to redissolve.
- Preheat Buffer AE to 65 °C in smaller aliquoted tubes.
- Two complete sets of 2.0 ml tubes will be needed during the extraction procedure. They may be labeled before beginning or during periods of incubation / centrifugation.

1. Grind approximately one square centimeter of plant tissue in a FastPrep tube at speed 5 for 10-15 seconds.
2. Make a master mix of extraction buffer containing 400  $\mu$ l AP1, 30  $\mu$ l  $\beta$ -mercaptoethanol, and 30  $\mu$ l proteinase K per each sample.
3. Add 460  $\mu$ l of master mix to each FastPrep tube, place tubes in a plastic bag attached to a rocking incubator at 42 °C for 12-24 hrs.
4. Remove tubes from incubator and add 130  $\mu$ l AP2 to each; incubate on ice for 5 minutes.
5. Centrifuge FastPrep tube for 5 minutes at 13,000 rpm.
6. Apply the supernatant to a purple QIAshredder spin column and centrifuge for 2 minutes at 13,000 rpm.
7. Transfer flow-through to a labeled 2.0 ml tube without disturbing the cell-debris pellet. Record an estimated amount of how much was transfer by pipetting 50  $\mu$ l at a time.
8. Add 1.5 volumes of Buffer AP3/E to the cleared lysate and mix by pipetting.

9. Apply 650  $\mu$ l of the mixture from step 8 to a clear DNeasy mini spin column.  
Centrifuge for 1 minute at 8,000 rpm and discard flow-through.
10. Repeat step 9 with remaining sample. Discard flow-through and collection tube.
11. Place DNeasy column in a new 2 ml collection tube, add 500  $\mu$ l Buffer AW to the DNeasy column and centrifuge for 1 minute at 8,000 rpm. Discard flow-through and collection tube.
12. Repeat AW wash two more times, discarding collection tube each time. On the final wash (3<sup>rd</sup>) centrifuge for 2 minutes to completely dry the membrane.
13. Very carefully transfer the DNeasy column to a labeled 2.0 ml tube and pipette 50  $\mu$ l of preheated Buffer AE directly onto the DNeasy membrane. Incubate for 5 minutes at room temperature then centrifuge for 1 minute at 8,000 rpm to elute.
14. Repeat elution once as described into the same 2.0 ml tube.
15. Transfer the extraction to the labeled final storage screw cap tube.

## Vita

Susan Katherine Pell was born in Muncie, Indiana on November 16, 1972. She spent her early childhood in Indiana, Florida and Iowa before moving to Louisiana where she attended high school. After graduating from University High School in 1991, she enrolled at St. Andrews Presbyterian College in Laurinburg, North Carolina. While at St. Andrews, she completed an honors research project in the Biology Department under the guidance of Dr. Frank Watson. In May of 1995 she graduated with a bachelor of science degree in biology and went to Louisiana State University to work in the laboratory of Dr. Lowell Urbatsch. Dr. Pell began her graduate studies with Dr. Urbatsch in the spring of 1996. In 2002 she left Baton Rouge to take a position as the laboratory manager of the Lewis B. and Dorothy Cullman Program for Molecular Systematics Studies at the New York Botanical Garden where she is still currently employed. She defended her dissertation in February of 2004