CORNELL UNIVERSITY FOUNDED A.D. 1865

The systematics of Dombeya Cav. and its near relatives (Dombeyaceae)

by Cynthia Karen Skema

This thesis/dissertation document has been electronically approved by the following individuals:

Luckow,Melissa A (Chairperson)

Doyle,Jeffrey J (Minor Member)

Nixon,Kevin C (Minor Member)

THE SYSTEMATICS OF *DOMBEYA* CAV. AND ITS NEAR RELATIVES (DOMBEYACEAE)

A Dissertation
Presented to the Faculty of the Graduate School
of Cornell University
In Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy

by
Cynthia Karen Skema
August 2010

THE SYSTEMATICS OF *DOMBEYA* CAV. AND ITS NEAR RELATIVES (DOMBEYACEAE)

Cynthia Karen Skema, Ph. D.

Cornell University 2010

The species-rich and morphologically diverse genus *Dombeya* Cav. (Dombeyaceae) is a spectacular example of the remarkable diversity of the biota of Madagascar where evolution has followed a unique trajectory. Like other large genera, *Dombeya* remains a taxonomic hurdle in understanding the Malagasy flora. Over 5000 base pairs of sequence data from five noncoding plastid markers and ITS of *Dombeya* and relatives, including 87 accessions from 68 species and 10 genera, are used to investigate patterns of molecular evolution in *Dombeya* and hypothesize a phylogeny using parsimony and Bayesian methods. These results are synthesized with morphological observations and used as a means for evaluating the generic delimitation and infrageneric taxonomy of *Dombeya*. Plastid introgression is invoked to explain incongruence between closely related taxa in plastid versus ITS phylogenies. The topology of the combined analysis of all molecular regions generally corresponds with morphology and includes four major groups. The majority of the Malagasy species and all African species of *Dombeya* sampled form a monophyletic clade and are defined as *Dombeya sensu stricto*. A second clade includes winged-seed dombeyoids: the highly autapomorphic *Eriolaena*, *Helmiopsis*, *Helmiopsiella* and *D. linearfolia*. This molecular data and morphology support the transfer of *D. linearifolia* to *Helmiopsis*. A third, morphologically coherent clade of *D.* subsect. *Macranthae* plus *D. moratii* are consistently excluded from *Dombeya s.*

str. in both plastid and nuclear phylogenies; these taxa are segregated from *Dombeya* as the new genus *Andringitra*. The fourth clade contains only Mascarene endemics: *Trochetia*, *Ruizia*, and a portion of the *Dombeya* from these islands. Differing relationships between these four groups in plastid versus ITS phylogenies suggest incomplete lineage sorting, possibly indicating the rapid divergence of these lineages. Molecular data provide little support for the infrageneric taxonomy of *Dombeya*. Revisionary work needed in *Dombeya* is begun with a study of section *Astrapaea*, a group distinguished by pendulous inflorescences and long staminal tubes. Broader species circumscriptions than those previously adopted are proposed because traditional taxonomic characters in the section overlap between species and sometimes vary within individuals or populations. One new species, *D. gautieri*, and one new subspecies, *D. cannabina* subsp. *antsifotrensis*, are described.

BIOGRAPHICAL SKETCH

Cynthia Skema greatly enjoyed attending Bryn Mawr College, in Bryn Mawr, Pennsylvania, from which she attained a B.A. in Biology in 1998. She went on to receive an M.Sc. in 2003 from the University of Edinburgh, Scotland in a joint program with the Royal Botanic Garden Edinburgh. «Comme beaucoup d'intellectuels elle ne pouvait pas dire simplement les choses simples.» (Modifié de Proust, *À l'ombre des jeunes filles en fleurs*, 1919).

To my grandfather, Ralph T. Castronova, who taught me the value of hard work

ACKNOWLEDGMENTS

I would like to thank my major advisor, Dr. Melissa Luckow, for her encouragement, understanding and enthusiasm over the years as I wound my way through the maze of my graduate research. She was instrumental in the development of my dissertation project because she understood both what it meant to work in Madagascar and why it was important to do so. I owe a debt of gratitude to her and my entire committee, Dr. Laurence Dorr, Dr. Jeffrey Doyle and Dr. Kevin Nixon, for sharing with me their expertise and wisdom from long experience. I thank them for their patience and keen readiness to answer any question at any time.

I would like to thank all of the people at Cornell University who made these past six years enjoyable and productive for me, particularly the students, faculty and staff of the L. H. Bailey Hortorium and the Department of Plant Biology. I am particularly grateful for the enthusiastic, knowledgeable and interesting fellow students with whom I was lucky enough to share my graduate experience: Shannon Straub, Mariana Yazbek, Janelle Burke, Jim Cohen, Jeremy Coate, Caroline Kellogg, Tee Havananda, Jackie Salazar, Marcela Martínez Millan, Liz Hermsen, Mary Futey, Simon Gunner and Scott Simono. Inevitably, one of them always knew how to do whatever it was I was struggling to figure out in my own research and I thank them for always being willing to share their knowledge. Thanks to Dan Ilut for crunching sequence data to help me find nuclear gene regions. Iván Maureira-Butler and Bernard Pfeil were post-docs who both helped me get on my feet when I was just beginning my graduate career. I thank the staff (past and present) at BH, Bob Dirig, Peter Fraissinet, Anna Stalter and Sherry Vance, for tolerating my invasion of the fourth floor and for all of their kind advice and assistance with loans and specimens. Jim Reveal kindly shared his nomenclatural expertise and spent time poring over the old taxonomic literature of *Dombeya* with me and for that I am indebted. Other Hortorium faculty and

researchers who deserve thanks for their support and assistance are David Bates, Alejandra Gandolfo, Sang-Hun Oh, Sue Sherman-Broyles, Jane Doyle, Jerrold Davis and Bill Crepet.

Countless generous people helped make the dissertation field work I completed in Madagascar and Thailand possible. First and foremost, I would like to thank the Missouri Botanical Garden, especially all the staff in Madagascar, for facilitation of my field work and help in acquiring collecting permits. I would particularly like to thank the following people who lent me additional assistance, shared their botanical knowledge, found critical specimens for me, offered friendship to a strange vazaha, went out in the field with me and even enabled me to make my very first trip to Madagascar: Lalao Andriamahefarivo, Sylvie Andriambololonera, Roger Lala Andriamiarisoa, Tefy Andriamihajarivo, Chris Birkinshaw, Christian Camara, Adolphe Lehavana, Jeannie Raharimampiona, Hans Rajaonera, Charles Rakotovao, Richard Randrianaivo, Fidisoa Ratovoson, and most especially Cynthia Hong-Wa.

The staff at Parc Botanique et Zoologique de Tsimbazaza in Antananarivo were essential to the completion of every hafitra mission I undertook. Marie Elisette Rahelivololona, Jaqueline Razanatsoa and Hèléne Mavo Razanatsoa provided wonderful assistance and camaraderie in the field. I would particularly like to thank Mavo for her companionship on the majority of the *Dombeya* collecting trips—her kindness and laughter were infectious and welcome companions to a road weary botanist. I thank Dan Turk for giving me free rein to collect in his nursery and for sharing with me his vast knowledge of Malagasy and Malagasy plants (down to individual trees in very precise locations). His enthusiasm for hafitra may well exceed even my own and I am grateful for the kind hospitality that he and his family always graced me with in Tana. Christian Camara, Solo Rapanarivo and Frank Almeda wrote helpful letters of support for my work. Amir Antoy and Joanna Luks were key players in my various attempts to improve my language skills in both Malagasy and French.

The following local guides were critical to finding many of the field localities and plants important to this work: Arthur Fetiharison, Pela August, Jean Pierre Lahitsara, Jean Baptiste Mota, Joseph Rahabialady, Zavasone Razafindranisa, Florent DJi, Florent Andrianantenaina, Edmond Ramisy, Rivo Rajoharison, Pascal Andriamamonjisoa, Emmanuel Jaosily and Romulus Rakotomandimby. I thank them for their kind, often quizzical, assistance and apologize to anyone I inadvertently missed off the list. I would particularly like to thank Velorazana and Raymond from Manongarivo for teaching me how to make a bâton magique and Bezoma and the boys of Andreba for singing the whole way up the hill *and* back down the other side.

For the Thailand trip, I thank Rachun Pooma, Sommanussa Sangrit, and Prachaya Srisanga for facilitating my collecting work and Marisa Guptarak and Gernot Huber for being indomitable field assistants and fantastic hosts.

I thank the curators, staff and students at BKF, CMU, MO, P, QBG and US for their help and hospitality during my visits to study specimens housed at those facilities. I would like to thank the following herbaria for loan of materials: BM, BR, G, HUH, K, P, and US. Brian Fisher, Peter Fraissinet and James Hayden kindly identified insects that I found on my *Dombeya* specimens.

I thank Philip Smith for his engaging tales of magnoliid evolution that got me hooked on botany in the first place and then brought me back to beloved Edinburgh for a great year at the Royal Botanic Garden Edinburgh. I thank the staff of that institution for all that they taught me about plants and the botanist’s life, particularly Toby Pennington for his cheery encouragement and support that instrumentally helped me on my way to a Ph.D.

I am happy to acknowledge the following agencies for funding for field work, lab research, conferences or other financial support: American Society of Plant

Taxonomists, Botanical Society of America, Cornell University Department of Plant Biology, Cornell University Graduate School, Peter H. Davis Expedition Funds, Knudson Fellowship, McClintock Award of Cornell University, Harold E. Moore, Jr. Funds and the Oleg Polunin Fund. I was honored to be the recipient of a National Science Foundation Doctoral Dissertation Improvement Grant (DEB-0709969).

I can not thank my family enough for their encouragement, enthusiasm and long tolerance of their very own zubrilla. My parents never flagged in their support of me, and their firm conviction that I could succeed buoyed my sinking spirits often. Their own hard work, accomplishments, generosity, compassion and endless curiosity about the world around them have always filled me with awe—I only hope I can succeed in following in their footsteps. My sister, brother and sister-in-law have long inspired me to embrace all adventures and read anything and everything, if only so that I could appear to be half as interesting, funny and smart as they really are. I thank my nephews for reminding me that playing and laughing are essential daily exercises. Most of all I would like to thank my kick-ass wife for her cherished companionship, for keeping me alive in my sprint to the finish, and for teaching me to not only see the little things, but to savor them. I look forward to all our future adventures together.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

DOMBEYA GAUTIERI (DOMBEYACEAE), A REMARKABLE NEW SPECIES FROM MADAGASCAR

ABSTRACT

Dombeya gautieri Dorr & Skema *sp. nov.* is described from Madagascar and illustrated. It is remarkable for its pinnatipartite leaves, reduced number of fertile stamens, petaloid staminodes, deeply divided style, and minute, 2-carpellate ovary.

INTRODUCTION

Dombeyaceae (alternatively Malvaceae *s.l.*: Dombeyoideae or Sterculiaceae *p.p.*) as presently circumscribed include 20 genera and ca. 350 species (Bayer, 2003). An important centre of diversification for the family is Madagascar where seven genera and ca. 200 species occur. *Dombeya* Cav. is the most species rich genus in the family and the vast majority of its ca. 215 species are endemic to Madagascar. Arènes (1959) recognised 187 species in Madagascar and in the last fifty years there have been relatively few changes to his enumeration. Barnett and Dorr (1986) described one new species and one new variety and they (Barnett, 1988b; Dorr, 2001) also transferred three of the *Dombeya* species Arènes (1959) recognised to related genera. Recently, Applequist (2009) described two new species of *Dombeya* subgen. *Xeropetalum* (Delile) K. Schum. from Madagascar. Seyani (1991), who revised the African species of *Dombeya*, considered six endemic Malagasy species recognised by Arènes (1959) to be synonymous with *D. acutangula* Cav. It is therefore noteworthy that floristic inventories by Swiss and Malagasy botanists led by Laurent Gautier (G) in the Daraina region of northeastern Madagascar have yielded the following remarkable new species of *Dombeya*.

Dombeya gautieri Dorr & Skema, ***sp. nov.*** Species foliis pinnatipartitis, staminibus 5 fertilus, staminodiis 5 petaloidis, stylo bipartito et ovario biloculare a congeneribus diversa. Typus: Madagascar, Antsiranana. *L. Gautier, S. Wohlhauser, L. Nusbaumer & P. Ranirison* LG 4578 (holotypus US; isotypi G, MO, P, TEF).

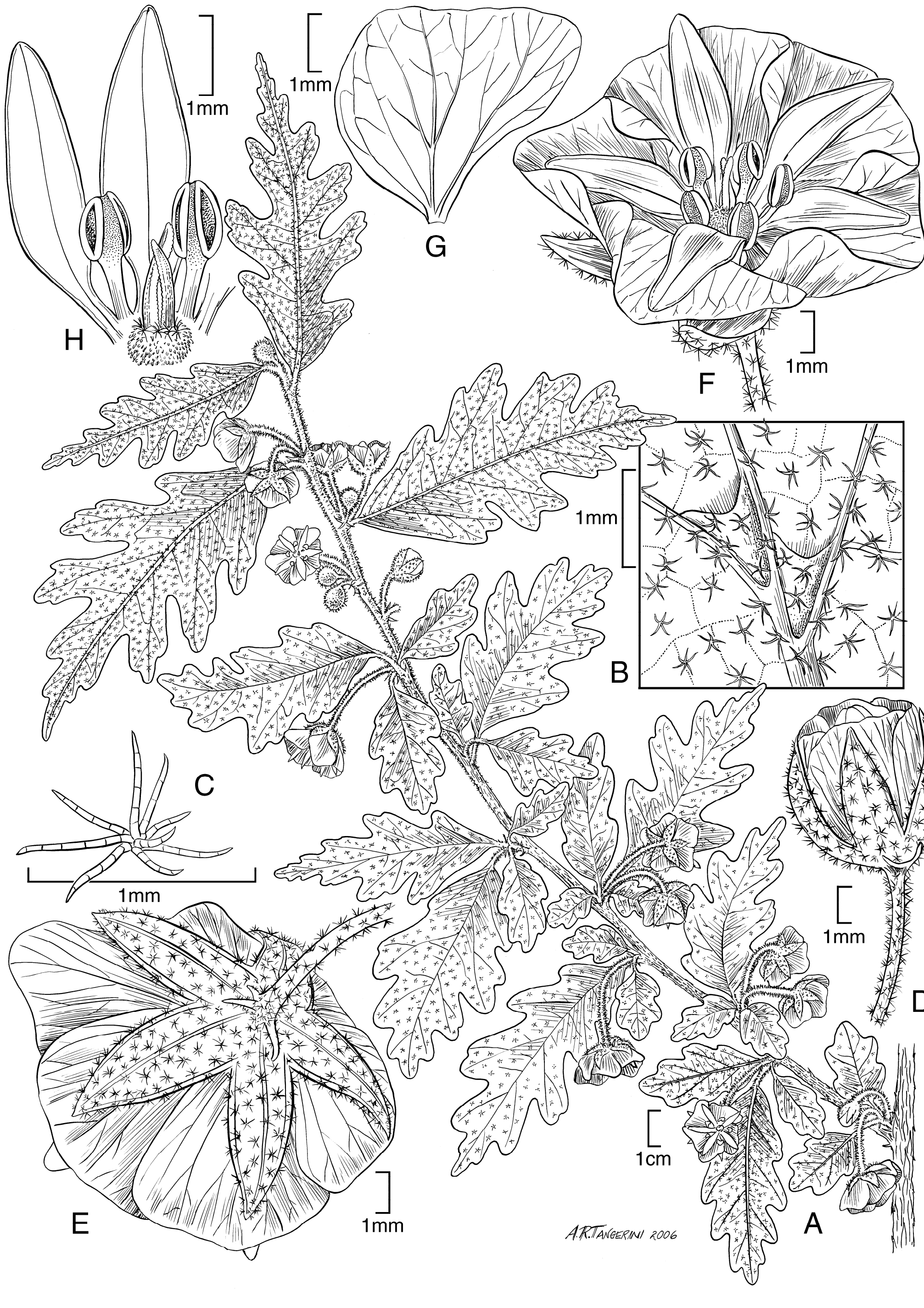
Shrub 2.5 m tall, d.b.h. 2.5 cm in diam. *Stems* dark reddish-brown, either highly condensed with very short (<2 mm) internodes (brachyblasts) or expanded with longer (>10 mm) internodes, new growth densely stellate-pubescent with long-armed hairs, older growth sparsely stellate-pubescent to glabrescent, the bases of stellate hairs initially persisting as dark papillate-like structures. *Leaves* alternate (sometimes appearing whorled on brachyblasts), petioles 2 – 3 mm long, densely stellate-pubescent; leaf blades ovate to narrowly ovate, 3.3 – 5.7 × 2.0 – 2.9 cm, pinnatipartite and appearing "querciform," margin sinuate, base obtuse to rounded (lowermost lobes unequal, weakly to strongly asymmetric), apex acute to obtuse or rounded, sparingly pubescent above with simple, setose or 2-armed hairs and more densely pubescent on veins with multi-armed stellate hairs, sparingly stellate-pubescent below with multi-armed stellate hairs especially on 1° and 2° veins; pocket domatia below in the form of a fine web-like membrane in the axils of 1° and 2° veins, 1 – 2 mm in length. Stipules 2 – 3 mm long on expanded shoots, <1 mm long on brachyblasts, briefly triangular at base, subulate above, densely to moderately stellate-pubescent becoming glabrescent. *Inflorescences* axillary simple cymes, 2 – 3-flowered (when 2-flowered youngest, centre bud missing); peduncles less than 1 mm long, pedicels 6 – 10 mm long, densely stellate-pubescent. Epicalyx of 3 bracts directly subtending flower, bracts acicular, 0.5

– 1 mm long, stellate-pubescent. *Flower* buds spherical, ca. 1 × 1 mm, sepals valvate, sepal apices connate. Calyx 5-parted, fused for less than 1 mm, then free for 2.5 – 3 mm in length, externally heterotrichous with a bed of smaller shorter-armed hairs beneath longer-armed stellate hairs, internally glabrous except for a zone of papillate (probably nectariferous) tissue where the sepals are adpressed to the corolla. Corolla obovate, strongly asymmetric, 2.5 – 3 × 3.5 – 4 mm, glabrous, white. Androecium fused into a very short tube, ca. 0.5 mm tall; stamens 5, free filaments less than 0.5 mm long, anthers basifixed, 0.5-1 mm long, connective not prolonged; staminodes 5, petaloid, spathulate narrowing to a rounded-acute apex, 3 – 3.5 mm long, white. Pollen spherical, spiny (visible at 10× magnification). Gynoecium minute, ca. 0.25 × 0.5 mm, eusyncarpous, 2-carpellate, with a prominent apical paracarpous zone; septa each including a large mucilage duct; ovules 2 per carpel, with basal-axile placentation, erect, anatropous, apotropous (abaxially curved); style ca. 0.6 – 0.8 mm long (in bud), deeply divided (bipartite); stigmatic surface not well-demarcated, presumably the interior apical portion of each style branch. *Fruit* and *seed* unknown. Fig. 1.1.

Distribution. — Endemic to Madagascar, where it evidently is restricted to the Solaniampilana-Maroadabo forest northwest of Daraina.

Specimens Examined. — MADAGASCAR. Prov. Antsiranana: sous-préfecture de Vohemar, commune rurale de Daraina, Daraina, forêt de Solaniampilana-Maroadabo (13°05.69’S, 49°34.89’E), 100 m, 11 March 2004, *L. Gautier, S. Wohlhauser, L. Nusbaumer & P. Ranirison* LG 4578 (holotype US; isotypes G, MO, P, TEF).

Figure 1.1. *Dombeya gautieri*: habit (**A**); detail of leaf undersurface showing multi-armed stellate hairs and pocket domatia (**B**); multi-armed stellate hair (**C**); flower bud early anthesis (**D**); flower from below showing minute epicalyx and ribbed sepals (**E**); flower from above showing petals, petaloid staminodes, stamens, and divided style (**F**); petal (**G**); detail of flower (calyx and corolla removed) showing ovary, style, anthers, and petaloid staminodes (**H**). All from *Gautier et al.* LG 4578. Drawn by Alice Tangerini.


H
1mm
1mm
G
F
1mm
B
1mm
C
1mm
D
1mm
E
1mm
1cm
A
A.R.TANGERINI 2006

Habitat. — The type was collected in one of a series of forested massifs situated between the Loky and Manambato rivers in northeast Madagascar. Gautier & al. (2006) describe the whole region as being underlain by Precambrian crystalline rock with a few volcanic intrusions and the Solaniampilana-Maroadabo forest as being a 1726 ha remnant of dry forest ("forêt dense sèche") at 100 m altitude with a tree canopy 10-20 m in height.

Conservation Status. — Data deficient (IUCN 2001). This species is only known from one collection.

Etymology. — The species is named in honour of the collector of the type and only known material, the Swiss botanist Laurent Gautier (b. 1960).

Notes. — Although Pentapetaceae has priority over Dombeyaceae, the proposal by Doweld and Reveal (2007) to conserve the latter name was recently approved by the Committee for Vascular Plants (Brummitt 2009). We assume that this action will be ratified by the General Committee and the next International Botanical Congress and therefore adopt the more widely used family name.

DISCUSSION

A number of characters associated with *Dombeya gautieri* are remarkable for being either unique or uncommon amongst the Dombeyaceae. Amongst the 20 genera included in the family, only *Dombeya* as presently construed can accommodate this new species. The pinnatipartite leaves of *D. gautieri* are striking and otherwise unknown amongst the Dombeyaceae (Figs. 1.1A, 1.2). Indeed, the foliage is so remarkable that we are surprised that *D. gautieri* has escaped notice until now and suspect it must be quite localised in its distribution. Deeply lobed adult leaves occur in only a few other species of Dombeyaceae and invariably the lobing is palmate. In Madagascar, leaves of *D. palmatisecta* Hochr. are 5 – 9-palmatipartite, those of *D.*

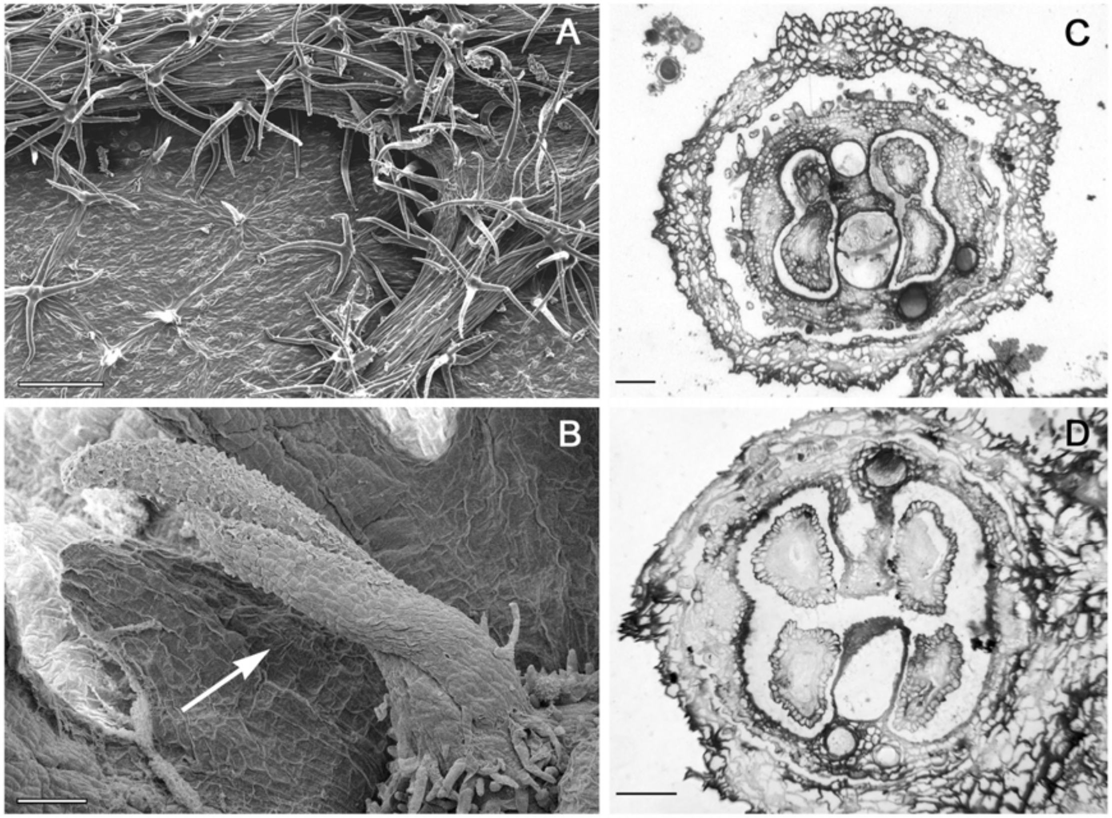
Figure 1.2. *Dombeya gautieri*. Branchlet showing flowers with prominent petaloid staminodes alternating with fertile anthers (*Gautier et al.* LG 4578). Photo by Laurent Gautier, © Conservatoire et Jardin botaniques de la Ville de Genève, Switzerland.

roseiflora Arènes are 3 – 5-palmatilobed, and some leaves of the heterophyllous *D. urenoides* Hochr. (= *D. acutangula sensu* Seyani) are 5-palmatipartite. In the Mascarene Islands, *Ruizia cordata* Cav., *D. populnea* (Cav.) Baker, and *D. acutangula* Cav. (Friedmann, 1987) are heterophyllous and the profound lobing in each species is associated with the juvenile leaves only. This clearly is not the case with *D. gautieri* and we see no close relationship amongst these genera and species.

The abaxial leaf surfaces of *Dombeya gautieri* have distinctive pocket domatia that consist of web-like flaps of tissue (Figs. 1.1B, 1.3A). Similar but less well-developed and less conspicuous domatia occur in several African species of *Dombeya*, notably *D. autumnalis* I. Verd. and *D. cymosa* Harv. (Seyani, 1991). Domatia also occur in most, but not all species of *Nesogordonia* Baill. (Barnett, 1988a), where they consist of tufts of hairs without the web-like flaps of tissue. There is no survey of domatia (either presence or type) within the Dombeyaceae, but we suspect that even if such data existed they likely would not be informative about generic relationships.

The androecium of *Dombeya gautieri* is remarkable in that the fertile stamens are reduced to five in number, each one alternating with a petaloid staminode. Within the genus *Dombeya*, only *D. lokohensis* Arènes and *D. marojejyensis* Arènes have a similar androecial pattern; five fertile stamens alternating with five staminodes. In both of these species, however, the staminodes are ligulate (not petaloid), the leaves are undivided (not pinnatipartite) and the vestiture is lepidote (not stellate). Five fertile stamens alternating with five petaloid staminodes is characteristic of the related genus *Melhania* Forssk., and the small, acicular epicalyx of *D. gautieri* also is reminiscent of the epicalyx found in *Melhania* sect. *Broteroa* (K. Schum.) Arènes. A close relationship with *Melhania*, however, is ruled out since the ovary in the latter genus is invariably 5-carpellate. In addition, the petals of *Melhania* are without exception yellow (not white).

Figure 1.3. *Dombeya gautieri*: detail of pocket domatium [scale bar = 200 Φm] (**A**); detail of style branches (in bud), with minute simple hairs at apex of ovary [scale bar = 100 Φm] (**B**); cross section of ovary (apex) showing two locules with 2 seeds in each locule and one large mucilage duct in each septum [scale bar = 100 Φm] (**C**); idem. (base) [scale bar = 100 Φm] (**D**). All from *Gautier et al.* LG 4578.


A
C
B
D

The petaloid staminodes of *Dombeya gautieri* (Figs. 1.1F, 1.1H, 1.2) are similar to those of *Helmiopsis* H. Perrier, a Malagasy endemic, and the colour of the petals (white) is also a shared character (Fig. 1.2). However, *Helmiopsis* has 10 – 30 (versus 5) fertile stamens and a 3 – 5-carpellate (versus 2-carpellate) ovary. It would be difficult to accommodate *D. gautieri* within *Helmiopsis* without radically recircumscribing the latter genus.

The gynoecium of *Dombeya gautieri* is minute and 2-carpellate (Fig. 1.3 C, D). Among the Dombeyaceae, only species of *Dombeya* subgen. *Xeropetalum* and *Paradombeya sinensis* Dunn also have 2-carpellate gynoecia. (All species of *Dombeya* subgen. *Dombeya* and the other two species of *Paradombeya* Stapf have 5-carpellate gynoecia). Both species of the African genus *Harmsia* K. Schum. appear to be 2-carpellate, but their gynoecia have a false septum and are in fact unilocular (Jenny & al., 1999). Irrespective of carpel number *Paradombeya* differs from *D. gautieri* in usually having 15 (versus 5) fertile stamens, yellow (versus white) petals, and a very briefly divided (versus bipartite) style. Bayer (2003), presumably based on personal observation, described *Paradombeya* as having (1 –) 3 (– 5) stamens per bundle. We have not been able to verify that species or specimens of *Paradombeya* diverge from the general plan of 15 stamens in five bundles.

The style of *Dombeya gautieri* is distinctive; it is 2-parted with each branch free to the base (Fig. 1.3B). Few species of *Dombeya* or other genera of Dombeyaceae have style branches that are completely divided. Arènes (1958) described a series *Brevicolumnae* that includes several species (e.g., *D. halapo* Arènes or *D. punctata* subsp. *ficulnea* (Baill.) Arènes) with style branches divided to the base, but all the species in the series are 5-carpellate. The only species of *Dombeya* that is 2-carpellate and approaches having a completely divided style is *D. apikyensis* Arènes (Arènes 1958), which also has undivided leaves and a completely glabrous gynoecium.

Friedmann (1987) noted that the style branches of *Ruizia cordata* Cav. are essentially free ("pratiquement libres") and that those of *Astiria rosea* Lindl. are briefly united at the base ("faiblement soudés à la base").

One intron and four intergenic spacers of chloroplast DNA of *Dombeya gautieri* have been sequenced and the results included in an ongoing study of the phylogeny of Dombeyaceae (Chapter 2). These molecular data, beyond placing *D. gautieri* within a broadly construed *Dombeya*, are uninformative about the best placement of this species but provide evidence of the need for a reevaluation of generic boundaries in this group. At present, we believe this remarkable new species is best placed in *Dombeya*.

ACKNOWLEDGEMENTS

We are indebted to Laurent Gautier (G) for sending us this collection of *Dombeya gautieri* and permitting us to reproduce his photograph of same (Fig. 1.2); Rose Gulledge (US) for sectioning its ovary, taking the SEMs, and preparing the plates; Jay Horn (US) for helping us interpret its ovary; Alice Tangerini (US) for illustrating the species; and Stanley Yankowski (US) for advising and guiding us on plant anatomical techniques. The research of C. Skema was supported by NSF Doctoral Dissertation Improvement Grant DEB-0709969.

LITERATURE CITED

Applequist, W. L. 2009. Two new species of *Dombeya* (Malvaceae) from Madagascar. *Novon* 19(3): 289-294.

Arènes, J. 1958. Les *Dombeya* de Madagascar et des Comores. *Candollea* 16: 247-449.

Arènes, J. 1959. Sterculiacées. In: H. Humbert (ed.), *Flore de Madagascar et des Comores, 131^{e} Famille*. Firmin-Didot et C^{ie}, Paris.

Barnett, L. C. 1988a. Systematics of *Nesogordonia* Baillon (Sterculiaceae). Unpubl. Ph.D. Dissertation, University of Texas, Austin, Texas.

Barnett, L. C. 1988b. New combinations in the genus *Helmiopsiella* (Sterculiaceae). *Bull. Mus. Natl. Hist. Nat., sér. 4, sect. B, Adansonia* 10(1): 69-76.

Barnett, L. C. & Dorr, L. J. 1986. A new species and variety of *Dombeya* (Sterculiaceae) from Madagascar. *Bull. Mus. Natl. Hist. Nat., sér. 4, sect. B, Adansonia* 8(4): 365-371.

Bayer, C. 2003. Malvaceae. In: K. Kubitzki & C. Bayer (eds.), *The families and genera of vascular plants. V. Flowering plants-dicotyledons: Malvales, Capparales and non-betalain Caryophyllales*. Springer-Verlag, Berlin.

Brummitt, R. K. 2009. Report of the Nomenclature Committee for Vascular Plants: 60. *Taxon* 58(1): 280-292.

Dorr, L. J. 2001. A new combination in the endemic Malagasy genus *Helmiopsis* (Malvaceae-Dombeyoideae). *Adansonia, sér. 3*, 23(1): 151-153.

Doweld, A. & Reveal, J. L. 2007. (1764) Proposal to conserve the name Dombeyaceae. *Taxon* 56(1): 265-266.

Friedmann, F. 1987. Sterculiacées. In: J. Bosser, Th. Cadet, J. Guého & W. Marais (eds.), *Flore des Mascareignes: La Réunion, Maurice, Rodrigues 53.* The Sugar Industry Research Institute, Mauritius.

Gautier, L., Ranirison, P., Nusbaumer, L. & Wolhauser, S. 2006. Aperçu des massifs forestiers de la région Loky-Manambato. In: S. M. Goodman & L. Wilmé (eds.), *Inventaires de la faune et de la flore du nord de Madagascar dans la région Loky-Manambato, Analamerana et Andavakoera*. Centre d'Information et de Documentation Scientifique et Technique, Antananarivo.

IUCN 2001. *IUCN Red List categories and criteria, version 3.1*. IUCN Species Survival Commission. Gland, Switzerland and Cambridge, UK.

Jenny, M., Bayer, C. & Dorr, L. J. 1999. *Aethiocarpa* reduced to *Harmsia* (Malvaceae: Dombeyoideae). *Taxon* 48(1): 3-6.

Seyani, J. H. 1991. The genus *Dombeya* in continental Africa. *Opera Bot. Belg*. 2: 1-186.

CHAPTER 2

MOLECULAR PHYLOGENY OF *DOMBEYA* OF MADAGASCAR: INCONGRUENCE, INTROGRESSION, INTRAGENOMIC POLYMORPHISM & LOW MOLECULAR VARIATION IN A SPECIES-RICH GENUS

ABSTRACT

Over 5000 base pairs of data from five noncoding chloroplast markers and ITS were sequenced for *Dombeya* and its near relatives. Despite its morphological diversity, the species-rich genus *Dombeya* showed remarkably low molecular variation. Phylogenies inferred from parsimony and Bayesian analyses of the combined chloroplast data were incongruent with those for ITS. The most striking example of incongruence was found in *Dombeya* section *Astrapaea*, where three chloroplast clades grouped by geographic locality in contrast to two ITS clades that were concordant with morphology. These patterns were interpreted as evidence for chloroplast introgression, a process that is seemingly limited to near relatives in the wild. Incomplete lineage sorting was evident in the different relationships between the four major groups of taxa in the study in the chloroplast versus ITS phylogenies, possibly indicating the divergence of these lineages in a short time frame. A new approach for constructing and analyzing haplotypes from polymorphic direct sequences was developed to assess phylogenetic signal in individuals with intragenomic polymorphisms in ITS.

INTRODUCTION

Although *Dombeya* (Dombeyaceae or Dombeyoideae, Malvaceae *s.l.*) is distributed in continental Africa, Madagascar, the Comoro and Mascarene islands, the evolution of the genus is largely a Malagasy story. With roughly 180 of its 210 species endemic to Madagascar, *Dombeya* alone comprises around 2% of the island's flora (estimating 10,000-12,000 species total; Gautier & Goodman, 2003). These species of *Dombeya* are shrubs or trees with a wide distribution across the island (Arènes, 1959). They occur as frequent, but not dominant, members of the plant communities across most of the complex mosaic of habitats in Madagascar. *Dombeya* is one of a handful of species-rich and largely endemic plant genera in Madagascar that are in need of study and represent fascinating examples of evolutionary radiations within this unique flora. Yet, little is known about molecular processes at work beneath the morphological diversity shown in Madagascar's megadiverse plant genera.

Dombeya encompasses a wide range of morphological diversity, particularly in the species from Madagascar. The broad morphological spectrum arises from variation in indument, inflorescence structure, flower size, epicalices, androecial length and fusion, and number of stamens and carpels. Particular suites of characters have allowed for taxonomic definition of morphologically distinct species (e.g., Arènes, 1959), but fine gradations in other characters can be seen across flocks of sympatric or parapatric species. Such patterns of morphology may indicate some level of gene flow in these morphological arrays of species, but no molecular investigations have yet assessed this possibility. One such example occurs in *Dombeya* section *Astrapaea*, a group characterized by strikingly long androecial tubes (up to 4 cm), equal to or surpassing the length of the tightly convolute petals, as well as dense, pendulous inflorescences. Members of section *Astrapaea* are distinguished by combinations of leaf and stipule characters (Chapter 4), but exceptions to these “rules” have been observed. Species occur in sympatry through most of the distribution of the section and overlap in flowering times. In its entirety, the section presents a continuum of quantitative floral characteristics and overlapping qualitative characteristics. Two vegetatively distinct individuals will often show parallels in their floral features, e.g., share a distinct petal shape. To test if gene flow resulting from sympatry is at the root of these intergrading morphological patterns, accessions of *D. hilsenbergii* and *D. baronii* were sampled from three geographic regions on the island. These two species are the widest ranging and most broadly sympatric of species within the section, and are morphologically distinct by indument, leaf and stipule characteristics. They represent either extreme on the continuum of floral characters within sect. *Astrapaea*.

Interfertility among species of *Dombeya*, as shown by limited experimentation in artificial crosses for ornamentals, provides evidence that gene flow could occur. Inclusion of one experimental hybrid, *D.* × *cayeuxii*, in the analysis allows study of the molecular patterns and inheritance of a hybrid with known parentage, and presents a model of recent hybridization against which to compare potential wild hybrids. *Dombeya* × *cayeuxii* resulted from a cross between the two most morphologically divergent parents of any of the cultivated hybrids. It is widely cultivated and commonly seen in hothouses around the world or planted out in (sub)tropical botanical gardens. Created by Henri Cayeux in 1895 from a cross between *D. burgessiae* (≡ *D. mastersii*) and *D. wallichii* (André, 1897), it manifests near perfect morphological intermediacy between its parents. Both parental species are in the pentacarpellate *Dombeya* subg. *Dombeya*, but belong in different sections within the subgenus (Arènes, 1959).

This research aimed to use molecular phylogenies of *Dombeya* to evaluate patterns of molecular evolution, as well as levels of molecular variation, which underlie the morphological diversity and proliferation of species in the genus. Sequence data from both nuclear and chloroplast genomes allow investigation into potential instances of hybridization or introgression by comparison of the biparentally inherited nuclear loci with the maternally inherited plastids (Doyle, 1992). Comparison of gene trees also enables evaluation of the potential for lineage sorting or orthology/paralogy conflation in any of the datasets (Doyle, 1992; Wendel & Doyle, 1998), as a means for developing a more robust hypothesis of species relationships and molecular evolution.

ITS was employed as a marker to further investigate the potential for evolutionary histories involving hybridization or introgression in species of *Dombeya*. ITS is one of the most widely used molecular markers in hypothesizing phylogenetic relationships among plants, despite its potential to be phylogenetically misleading (Alvarez & Wendel, 2003; Nieto Feliner & al., 2007). Concerted evolution has the potential to erase the evolutionary history in these ribosomal spacers; such possibilities can only be evaluated by comparison to other independent datasets. Yet, where concerted evolution is incomplete, the converse situation can be true: an opportunity exists to see multiple evolutionary histories in one taxon, i.e., the byproducts of hybridization or introgression (Wendel & Doyle, 1998; Alvarez & Wendel 2003). Multiple studies utilizing ITS have found evidence for individuals being of hybrid origin (e.g., Soltis & Soltis, 1991; Kim & Jansen, 1994; Sang & al., 1995b; Shi & al., 2006).

The objectives of the present study were to: i) assess molecular variation among the morphologically diverse *Dombeya* of Madagascar and ii) evaluate congruence between nuclear (ITS) and plastid (*trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE* and *ndhA* intron) phylogenies of these taxa and their near relatives. A morphological evaluation and the taxonomic implications of these phylogenies are presented in Chapter 3.

MATERIALS & METHODS

Taxon sampling. — Sequences were obtained for 87 accessions of 68 species of 10 genera in the Dombeyaceae with a focus on sampling species of *Dombeya* from Madagascar. Data from previous molecular phylogenies (Bayer & al., 1999; Alverson & al., 1999; Nyffeler & al., 2005; Won, 2009; Le Péchon & al, in press), observations of morphology (Le Péchon & al., 2009; pers. obs.), and a larger molecular phylogenetic study of Dombeyaceae with broader taxonomic sampling (Skema, in prep.) indicated that the genera *Nesogordonia*, *Corchoropsis* and *Pentapetes* could serve as outgroups for the study and that the genera *Eriolaena*, *Helmiopsiella*, *Helmiopsis*, *Ruizia*, *Trochetia* and *Trochetiopsis* should also be sampled given their close relationships with various species of *Dombeya*. All but one of the 18 subsections of *Dombeya* (Arènes, 1959) were sampled and species within the subsections and geographical representatives within species were included where possible.

Fine-scale sampling efforts were concentrated in *Dombeya* section *Astrapaea*, the group deemed most likely to be monophyletic given the putative synapomorphy of a strikingly long staminal tube and otherwise coherent morphology. Individuals representing the two broadly sympatric species, *D. baronii* and *D. hilsenbergii*, were sampled from across their distributions in sympatric pairs from three sites: Montagne d'Ambre, Zahamena and Ranomafana. The only taxa of *D.* sect. *Astrapaea*, *D. hafotsy* and *D. wallichii*, which occur in the far south were also sampled.

Twenty-seven of the accessions in the study came from cultivated sources: thirteen of these are known to be of wild origin (i.e., grown from wild-collected seed or cuttings) and fourteen are of unknown origin (i.e., potentially grown from seed or cuttings of individuals in cultivation). Table 2.1 lists accessions with gene regions sequenced for each and voucher data.

Table 2.1. Accessions included in study with voucher specimen data, gene regions sequenced and collection locations (or provenance, if cultivated). (Herbarium is listed for the unicate or a single duplicate of each voucher.) * Individual known to be or most likely grown from seed or cuttings collected from wild populations.
** Individual potentially grown from seed or cuttings derived from cultivated individuals.

Taxon	**Voucher (Herbarium)** (gene regions sequenced; -- indicates sequence not acquired for that region)	**Collection location; or, if cultivated, provenance (and site of cultivation in parentheses)**
Corchoropsis crenata	*Won et al. 1943* (DGU) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Mt. Bulmo, Korea
Dombeya acerifolia	*C. Skema et al. 221* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Mantadia National Park (Andasibe), Toamasina, Madagascar
***Dombeya acutangula* 1 (var. *rosea*)**	*R. Bone 37* (MAU) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated**, exact provenance unknown, Mauritius (private garden of Claudia Baider, Mauritius)
***Dombeya acutangula* 2**	*Chase18955* (K) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Cascade Mourouk, Rodrigues, Mascarenes
Dombeya amaniensis	*Kayombo & Nkawamba 2212* (MO) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Morogoro, Ulanga District, Tanzania
Dombeya angustipetala	*C. Skema et al. 139* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Ranomafana National Park, Fianarantsoa, Madagascar
***Dombeya australis* 1**	*C. Skema et al. 249* (BH) (--, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Mandritsara (S of Vangaindrano), Fianarantsoa, Madagascar
***Dombeya australis* 2**	*C. Skema et al. 374* (BH) (--, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Mandena forest (N of Fort Dauphin), Toliara, Madagascar
Dombeya autumnalis	*Goldblatt & Manning 10473* (MO) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Pilgrim’s Rest, Mpumalanga, South Africa
***Dombeya baronii* 1**	*C. Skema et al. 404* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Zahamena Natural Reserve, Toamasina, Madagascar
***Dombeya baronii* 2**	*C. Skema et al. 306* (BH) (ITS, -- , -- , *psbMtrnD*, *petLpsbE*, --)	Montagne d'Ambre National Park, Antsiranana, Madagascar

Table 2.1. (Continued)

Taxon	**Voucher (Herbarium)** (gene regions sequenced; -- indicates sequence not acquired for that region)	**Collection location; or, if cultivated, provenance (and site of cultivation in parentheses)**
Dombeya baronii 3	*C. Skema et al. 147* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Ranomafana National Park, Fianarantsoa, Madagascar
Dombeya befotakensis	*C. Skema et al. 141* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Ranomafana National Park, Fianarantsoa, Madagascar
Dombeya borraginea 1	*C. Skema et al. 315* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, --)	Sahafary, Antsiranana, Madagascar
Dombeya borraginea 2	*C. Skema et al. 330* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Betsimiranjana, Antsiranana, Madagascar
Dombeya borraginopsis	*C. Skema et al. 378* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	W of Fort Dauphin, Toliara, Madagascar
Dombeya burgessiae 1	*Chase 14849* (K) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Chyulu Hills, Kenya
Dombeya burgessiae 2	*C. Skema et al. 475* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated**, provenance unknown (Palermo Botanical Garden, Sicily)
Dombeya cacuminum	*C. Skema et al. 207* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated**, provenance unknown (school CEG Avaradoha, Antananarivo, Madagascar)
Dombeya cannabina (subsp. _antsifotrensis_)	*C. Skema et al. 194* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Andringitra National Park, Fianarantsoa, Madagascar
Dombeya × _cayeuxii_ 1	*C. Skema 84* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated**, provenance unknown (Climatron, Missouri Botanical Garden, Missouri, USA)
Dombeya × _cayeuxii_ 2	*C. Skema et al. 206* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated**, provenance unknown (school Andrefa An Ambohijanahary, Antananarivo, Madagascar)
Dombeya coria	*C. Skema et al. 212* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Analamazaotra Special Reserve (Andasibe), Toamasina, Madagascar

Table 2.1. (Continued)

Taxon	**Voucher (Herbarium)** (gene regions sequenced; -- indicates sequence not acquired for that region)	**Collection location; or, if cultivated, provenance (and site of cultivation in parentheses)**
Dombeya dolicophylla	*C. Skema et al. 237* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	18 km S of National Route 2 towards Lakato, Toamasina, Madagascar
Dombeya elegans	*T. Le Péchon 18* (P) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	La Réunion
Dombeya erythroclada	*C. Skema et al. 142* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Ranomafana National Park, Fianarantsoa, Madagascar
Dombeya farafanganica	*W. Applequist 255* (MO) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Vohipao (S of Vangaindrano), Fianarantsoa, Madagascar
Dombeya ferruginea	*R. Bone 33* (MAU) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated*, Trois Mamelles Mountain, Mauritius (private garden of Claudia Baider, Mauritius)
Dombeya gautieri	*L. Gautier 4578* (US) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Daraina (NW of Vohemar), Antsiranana, Madagascar
Dombeya greveana	*C. Skema* 103 (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated*, provenance unknown (nursery of D. Turk, Antananarivo, Madagascar)
***Dombeya hafotsy* 1**	*W. Applequist 231* (MO) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Midongy du Sud National Park, Fianarantsoa, Madagascar
***Dombeya hafotsy* 2**	*W. Applequist 232* (MO) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Midongy du Sud National Park, Fianarantsoa, Madagascar
***Dombeya hilsenbergii* 1**	*C. Skema et al. 151* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Ranomafana National Park, Fianarantsoa, Madagascar
***Dombeya hilsenbergii* 2**	*C. Skema et al. 310* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Montagne d'Ambre National Park, Antsiranana, Madagascar
***Dombeya hilsenbergii* 3**	*C. Skema et al. 421* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Zahamena Natural Reserve, Toamasina, Madagascar
***Dombeya hilsenbergii* 4**	*C. Skema et al. 214* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Analamazaotra Special Reserve (Andasibe), Toamasina, Madagascar

Table 2.1. (Continued)

Taxon	**Voucher (Herbarium)** (gene regions sequenced; -- indicates sequence not acquired for that region)	**Collection location; or, if cultivated, provenance (and site of cultivation in parentheses)**
Dombeya laurifolia	*C. Skema 96* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated*, provenance unknown (nursery of D. Turk, Antananarivo, Madagascar)
Dombeya lecomteopsis	*C. Skema et al. 52* (BH) (ITS, --, --, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Ambatofinandrahana, Fianarantsoa, Madagascar
Dombeya leiomacrantha	*C. Skema et al. 199* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Andringitra National Park, Fianarantsoa, Madagascar
Dombeya leucomacrantha	*C. Skema et al. 201* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Andringitra National Park, Fianarantsoa, Madagascar
Dombeya linearifolia* subsp. *linearifolia	*Service Forestier 29211* (MO) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Ankarana National Park, Antsiranana, Madagascar
Dombeya linearifolia* subsp. *sely	*F. Ratovoson et al. 1228* (MO) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Montagne de Français, Antsiranana, Madagascar
Dombeya lucida	*C. Skema et al. 210* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Mandraka, Antananarivo, Madagascar
Dombeya macrantha	*C. Skema 90* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated*, provenance unknown (nursery of D. Turk, Antananarivo, Madagascar)
Dombeya magnifolia	*C. Skema et al. 135* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, --)	Ranomafana National Park, Fianarantsoa, Madagascar
Dombeya mandenensis	*C. Skema et al. 373* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Mandena forest (N of Fort Dauphin), Toliara, Madagascar
Dombeya cf. marivorahonensis	*C. Skema et al. 319* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Ankarana National Park, Antsiranana, Madagascar
Dombeya mauritiana	*R. Bone 34* (MAU) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated*, Magenta, Mauritius (private garden of Claudia Baider, Mauritius)

Table 2.1. (Continued)

Taxon	**Voucher (Herbarium)** (gene regions sequenced; -- indicates sequence not acquired for that region)	**Collection location; or, if cultivated, provenance (and site of cultivation in parentheses)**
Dombeya modesta	*C. Skema et al. 389* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Zahamena Natural Reserve, Toamasina, Madagascar
Dombeya mollis	*C. Skema 109* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated*, provenance unknown (nursery of D. Turk, Antananarivo, Madagascar)
Dombeya montana	*C. Skema et al. 185* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Andringitra National Park, Fianarantsoa, Madagascar
Dombeya moratii	*Service Forestier 23509* (MO) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Ifandena (between Ihosy and Ankaramena), Fianarantsoa, Madagascar
Dombeya muscosa	*C. Skema et al. 198* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Andringitra National Park, Fianarantsoa, Madagascar
Dombeya palmatisecta	*C. Skema et al. 351* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Manongarivo Special Reserve, Antsiranana, Madagascar
Dombeya populnea	*R. Bone 25* (MAU) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Yemen, Mauritius
Dombeya reclinata	*T. Le Péchon 6* (P) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	La Réunion
Dombeya rottleroides	*C. Skema et al. 337* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Ramena river valley, Antsiranana, Madagascar
***Dombeya rubifolia* 1**	*J. Burke & M. Yazbek 69* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated**, provenance unknown (Fairchild Botanical Garden, Florida, USA)
***Dombeya rubifolia* 2**	*J. Burke & M. Yazbek 70* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated**, provenance unknown (Fairchild Botanical Garden, Florida, USA)
Dombeya sahatavyensis	*C. Skema et al. 409* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Zahamena Natural Reserve, Toamasina, Madagascar

Table 2.1. (Continued)

Taxon	**Voucher (Herbarium)** (gene regions sequenced; -- indicates sequence not acquired for that region)	**Collection location; or, if cultivated, provenance (and site of cultivation in parentheses)**
***Dombeya* × *seminole* (= *D. burgessiae* × *D. burgessiae* 'Rosemound')**	*J. Burke & M. Yazbek 72* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated**, provenance unknown (Fairchild Botanical Garden, Florida, USA)
Dombeya sp.	*Chase 273* (K) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Ibadan, Nigeria
Dombeya stipulacea	*C. Skema et al. 153* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	W of Ranomafana National Park, Fianarantsoa, Madagascar
***Dombeya superba* 1**	*W. Applequist 261* (MO) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Ankarana forestry station (Manombo, SW of Midongy du Sud), Fianarantsoa, Madagascar
***Dombeya superba* 2**	*Service Forestier 23593* (MO) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Farafangana, Fianarantsoa, Madagascar
Dombeya tiliacea	*V. Leyman S4079* (BR) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated**, Pretoria, South Africa (Belgium National Botanical Garden, Belgium)
Dombeya urschiana	*C. Skema 100* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated*, provenance unknown (nursery of D. Turk, Antananarivo, Madagascar)
Dombeya venosa	*C. Skema 94* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated*, provenance unknown (nursery of D. Turk, Antananarivo, Madagascar)
Dombeya viburniflora	*C. Skema et al. 183* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Andringitra National Park, Fianarantsoa, Madagascar
Dombeya viburnifloropsis	*C. Skema et al. 180* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	12 km south of Fianarantsoa, Fianarantsoa, Madagascar
***Dombeya wallichii* 1**	*C. Skema et al. 372* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Mandena forest (N of Fort Dauphin), Toliara, Madagascar

Table 2.1. (Continued)

Taxon	**Voucher (Herbarium)** (gene regions sequenced; -- indicates sequence not acquired for that region)	**Collection location; or, if cultivated, provenance (and site of cultivation in parentheses)**
Dombeya wallichii **2**	*V. Leyman S4083* (BR) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated**, provenance unknown (Belgium National Botanical Garden, Belgium)
Dombeya wallichii **3**	*V. Leyman S4084* (BR) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated**, provenance unknown (Belgium National Botanical Garden, Belgium)
Dombeya wittei	*V. Leyman S4078* (BR) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated**, Democratic Republic of Congo (Belgium National Botanical Garden, Belgium)
Eriolaena candollei	*C. Skema et al. 439* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	native tree in Queen Sirikit Botanic Garden, Chiang Mai Province, Thailand
Helmiopsiella ctenostegia	*Chase 33737* (K) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated*, Toliara, Madagascar (Kew Botanic Gardens, London, UK)
Helmiopsiella madagascariensis	*Chase 33738* (K) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated*, Bejangoa crossroads, Toliara, Madagascar (Kew Botanic Gardens, London, UK)
Helmiopsis bernieri	*C. Skema et al. 288* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Baie des Dunes, Ramena, Antsiranana, Madagascar
Helmiopsis pseudopopulus	*C. Skema et al. 328* (BH) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Camp Orangea, Ramena, Antsiranana, Madagascar
Nesogordonia sp. nov.	*J. Rabenantoandro 1711* (US) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	wild collection, provenance unknown, Madagascar
Pentapetes phoenicea	*C. Skema s.n.* (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated**, provenance unknown (Cornell University, New York, USA)
Ruizia cordata **1**	*H. S. Cubey 128* (E) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated**, provenance unknown (Royal Botanic Garden Edinburgh, Scotland)
Ruizia cordata **2**	*V. Leyman s.n.* (BR) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated**, Réunion (Belgium National Botanical Garden, Belgium)

Table 2.1. (Continued)

Taxon	**Voucher (Herbarium)** (gene regions sequenced; -- indicates sequence not acquired for that region)	**Collection location; or, if cultivated, provenance (and site of cultivation in parentheses)**
Trochetia blackburniana	*R. Bone s.n.* (MAU) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	Petrin Reserve, Mauritius
Trochetia parviflora	*R. Bone 36* (MAU) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated*, Corps de Garde Mountain, Mauritius (private garden of Claudia Baider, Mauritius)
Trochetia uniflora	*R. Bone 35* (MAU) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated*, Trois Mamelles Mountain, Mauritius (private garden of Claudia Baider, Mauritius)
Trochetiopsis erythroxylon	*Chase 18170* (K) (ITS, *trnCycf6*, *ycf6psbM*, *psbMtrnD*, *petLpsbE*, *ndhA*x)	cultivated*, High Peak, St. Helena (Kew Botanic Gardens, London, UK)

Marker sampling. — Using primers from Shaw & al. (2005, 2007), fifteen noncoding chloroplast regions were screened for a panel of ten taxa including outgroups to assess ease of amplification and sequencing and to evaluate the molecular variability of each region. Of these, the following five proved useful: intergenic spacers *trnCycf6*, *ycf6psbM*, *psbMtrnD* and *petLpsbE*, and the intron of *ndhA* (hereafter called *ndhA*x). The nuclear ribosomal region of the internal transcribed spacer 1, the gene 5.8S, and internal transcribed spacer 2 (hereafter called, in its entirety, ITS) was also sequenced. ITS has been used by previous workers to hypothesize relationships for the dombeyoids of the Mascarenes (Le Péchon & al., in press) and so was sequenced to compare both with previous studies and the chloroplast data generated here. ITS was further characterized and analyzed (see section below) to evaluate the phylogenetic signal found in intragenomic polymorphisms within ITS.

DNA isolation, amplification & sequencing. — Total genomic DNA was extracted from both herbarium specimens and leaves collected in the field and dried on silica. Extractions were done using one of two methods. The first method was a variation of a CTAB extraction developed by Permingeat & al. (1998), which was scaled down for use with 20 mg of leaf tissue and modified by the addition of 2% polyvinylpyrrolidone and 0.2% *β*-mercaptoethanol to the extraction buffer. The second method utilized the Qiagen DNeasy plant mini extraction protocol and reagents (Qiagen, Inc., Valencia, California, U.S.A.), with the modification of 12-48 hours incubation at 42ºC in Qiagen buffer AP1 plus 18 mAu of proteinase K every 12 hours (Wurdack, 2004). The modified CTAB protocol was primarily used for samples extracted from leaves dried on silica, though it worked successfully for some herbarium specimens. The majority of herbarium specimen extractions were completed with the modified DNeasy protocol.

Markers were amplified by polymerase chain reactions (PCR) using the TaKaRa Ex Taq Hot Start version reagents (Takara Bio Inc., Shiga, Japan) in the following amounts: 2.5 µL 10X ExTaq buffer, 250 µM each dNTP, 0.75 µM forward primer, 0.75 µM reverse primer, 0.7 U ExTaq, and 2 µL of genomic DNA for a 25 µL reaction. PCRs of dilution series of genomic DNA aliquots in water showed that a 1:20 dilution yielded the most product across accessions and markers. Thus a 1:20 dilution of whole DNA in water was used in most PCRs. A few accessions extracted from herbarium specimens that had a very low yield of DNA were amplified with undiluted whole genomic DNA.

For the chloroplast regions, PCR thermal cycles were 94ºC for 5 minutes; 40 cycles of 94ºC for 30 seconds, 55ºC for 30 seconds, and 72ºC for 1 minute; 72ºC for 5 minutes. The three regions *ndhA*x, *psbMtrnD* and *petLpsbE* were each amplified and sequenced separately. The primers used for amplifying and sequencing the fragment *ndhA*x, which was 1.3 kilobases (k.b.) in length, were ndhAx1 (5'GCY CAA TCW ATT AGT TAT GAA ATA CC 3') and ndhAx2 (5' GGT TGA CGC CAM ARA TTC CA 3') (Shaw & al., 2007) and three primers developed for this study to aid in sequencing around two long homopolymers, ndhAx.intF (5' GAT ATA ATC CGT ATC ATG 3'), ndhAx.intR1 (5' ATT TCG TTC CTG ATA GTC 3'), and ndhAx.intR2 (5' CTC ATA CGG CTC CTC G 3'). The region *psbMtrnD* (0.6 k.b.) was amplified and sequenced with the primers psbMF (5' AGC AAT AAA TGC RAG AAT ATT TAC TTC CAT 3') and trnD^{GUC}R (5' GGG ATT GTA GTY CAA TTG GT 3') (Shaw & al., 2005). The region *psbEpetL* (1.0 k.b.) was amplified and sequenced with the primers petL (5' AGT AGA AAA CCG AAA TAA CTA GTT A 3') and psbE (5' TAT CGA ATA CTG GTA ATA ATA TCA GC 3') (Shaw & al., 2007). To sequence spacers *trnCycf6* and *ycf6psbM*, the continuous region from *trnC* through to *psbM* was amplified as one fragment (1.5-1.7 k.b. in length) and sequenced for its entire length, thus sequence of the short gene *ycf6*, which sits between those two spacers, was included in the datasets for some taxa. The primers used for amplifying *trnCpsbM* were trnC^{GCA}F (5' CCA GTT CRA ATC YGG GTG 3') and psbMR (5' ATG GAA GTA AAT ATT CTY GCA TTT ATT GCT 3') (Shaw & al., 2005) and sequencing primers included the internal primers ycf6F (5' ATG GAT ATA GTA AGT CTY GCT TGG GC 3'), ycf6R (5' GCC CAA GCR AGA CTT ACT ATA TCC AT3') (Shaw & al., 2005), and two primers developed for this study to aid in sequencing around a problematic homopolymer, psbM.intR (5' ATC GGG ATC CCT TTT AC 3') and ycf6.intF (5' TAT AWG GAC AAT GAG G 3').

For ITS, PCR thermal cycles were 94ºC for 3 minutes; 35 cycles of 94ºC for 30 seconds, 55ºC for 1 minute, and 72ºC for 1 minute; 72ºC for 10 minutes. Amplification of ITS resulted in a fragment of 0.8 k.b. and was done with the primers ITS.leu (5' GTC CAC TGA ACC TTA TCA TTT AG 3') and ITS4 (5' TCC TTC CGC TTA TTG ATA TGC 3') (Baum, 1998; ITS4 modified by 1 base pair from White & al., 1990). Both of these primers were used for sequencing ITS, but for some taxa, three additional internal primers designed for this study were used to aid in sequencing: ITS.intF (5' GAC TCT CGG CAA CGG 3'), ITS.intR (5' ACA CCC AGG CAG GCG TGC 3') and ITS4.alt (5' CTG ACC TGG GGT CGC 3').

All PCR products were visualized in 1% agarose gels stained with ethidium bromide and their fragment lengths checked against a size standard. Each PCR reaction was cleaned with an enzyme solution of 0.12 volume of the PCR reaction, consisting of 0.02 volume antarctic phosphatase (at 20000 U/mL; New England Biolabs, Ipswich, Massachusetts, U.S.A.) and 0.02 volume exonuclease I (at 5000 U/mL; New England Biolabs, Ipswich, Massachusetts, U.S.A.) in 0.08 volume 10X ExTaq PCR buffer, and incubated at 37ºC for 45 minutes and then 90ºC for 10 minutes. Sequencing reactions were completed using ABI Prism BigDye Terminator v.3.1 cycle sequencing reagents and run on an Applied Biosystems Automated 3730 DNA Analyzer (Applied Biosystems, Foster City, California, U.S.A.).

As quality control measures, sequences from each region, which were either randomly chosen or those found to sit on a particularly long branch, were compared to databased sequences, using the "blastn" algorithm in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi), as a means of corroborating their identities via sequence similarity to a relative. As alignment to relatives in GenBank can reveal little about potential intrastudy contamination issues, five percent of the accessions, randomly chosen, were extracted anew, amplified, and sequenced for all regions. These sequences were checked against the original sequences for those accessions and not a single base pair differed.

Matrix construction & sequence divergence. — Sequences from each region were aligned by eye. Insertion-deletion events (indels) were coded using the "gapcode" program v.2.1 (Ree, 2008) which implements a simple indel coding method (Barriel, 1994; Simmons & Ochoterena, 2000). The sequencing system employed for this study was observed to reliably distinguish the number of base pairs in a homopolymer string up to 5 base pairs, but often had sequencing difficulties above that number. For example, the system could differentiate between 4 or 5 base pairs in a poly-A run but not between 5 or 6. Therefore, the indel coding output from the "gapcode" program was modified by deleting any characters that coded indels resulting from strings of homopolymers of greater than 5 base pairs. Parsimony informative characters were quantified and matrices for analysis were made in Winclada v.1.7 (Nixon, 2002). All individual gene regions were analyzed both with and without indel coding and evaluated. The ITS dataset underwent further evaluation than the individual chloroplast regions, as described below. One concatenated matrix was made of all the chloroplast regions combined.

Sequence divergence estimates were calculated for each pair of taxa using PAUP* v.4.0 (Swofford, 2001) across the following datasets (with model used in parentheses): *psbEpetL* alone (TVM + G), *ndhA*x alone (K81uf + I), the entire chloroplast dataset concatenated (TVM + G) and ITS alone (GTR + I + G). Models used for estimating divergence were chosen for each dataset under the Akaike Information Criterion (Akaike, 1974) as implemented in ModelTest v.3.7 (Posada & Crandall, 1998) utilizing likelihood values and a neighbor joining tree generated in PAUP* (Swofford, 2001).

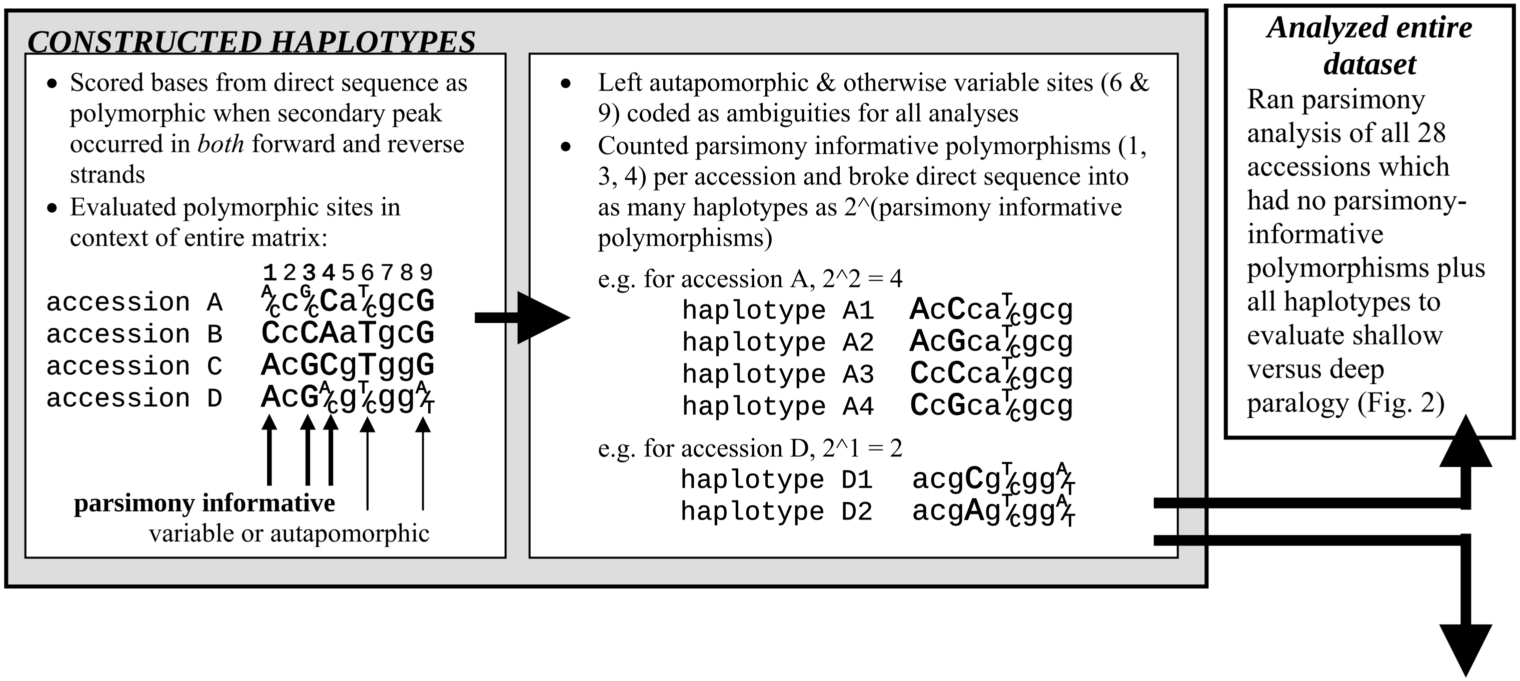
Further characterization & analysis of the ITS dataset. — Careful inspection determined that the presence of pseudogenes of ITS was unlikely in this dataset. Despite a relatively low G+C content (52% for the entirety of ITS, 53% for 5.8S alone), the 5.8S region was 164 b.p. long, which is within the known functional range, for all accessions and had no indels. Structural features of 5.8S and ITS1 (as summarized in Nieto Feliner, 2007) that were identifiable by eye were found in the matrix for all sequences. The 5.8S region contained two variable sites, both of which were parsimony informative characters (PICs). ITS1 had 131 variable sites (42% of ITS1 characters) and 70 PICs (23% of ITS1 characters) and ITS2 had 114 variable sites (48% of ITS2 characters) and 61 PICs (25% of ITS2 characters). The appreciable difference in variable sites between ITS1 and ITS2 versus 5.8S most likely indicates selective constraint on the 5.8S region. Branch lengths in topologies resulting from individual analyses of 5.8S versus ITS1/2 were too low to test for putative pseudogenes among the dataset by means of a tree-based approach (Bailey & al., 2003). All of the above indicate that amplicons of ITS sequenced in this study were most likely functional.

Incomplete concerted evolution across the tandem arrays of rDNA loci in the nuclear genome of these dombeyoids was evident in the abundance of polymorphic sites in the ITS region. Intra-individual sequence polymorphisms of two base calls at one site existed in multiple accessions. No accession had intra-individual indel polymorphisms and therefore direct sequences were always readable. Base pairs in electropherograms from direct sequencing of ITS were scored as polymorphic when there was a secondary peak, no matter what height (though both peaks were often equal in height), beneath a primary peak in *both* the forward and reverse strands. There was no evidence of primer bias in the PCR reactions associated with the polymorphisms. Multiple amplifications of each polymorphic accession returned identical sequences and these polymorphic direct sequences were deemed representative of all the polymorphisms that exist in ITS for these accessions given these PCR conditions.

ITS polymorphisms evident in species of *Dombeya* may be an indication of reticulations in their evolutionary history. A tree-based approach for evaluating phylogenetic signal of the polymorphic bases was devised using haplotypes derived from direct sequences. All parsimony analyses for this approach were conducted in TNT v.1.0 (Goloboff & al., 2008) using 1000 random addition replicates followed by 1000 ratchet iterations, drift and tree fusion and a swap to completion holding 5000 trees (commands: "rs0; hold 20001; rat: it1000upf5dow5; drift: iter 50; mu=rep1000ho20; ratchet; drift; tfuse; ho5000; bbreak=tbr; nel;"). This approach, as summarized in Fig. 2.1, entailed a series of steps, as follows:

1. Polymorphic sites were evaluated in the context of the complete ITS matrix to determine if they had the potential to be informative to parsimony analysis or if they were autapomorphic or otherwise variable (see Fig. 2.1 for examples). Otherwise variable characters are those for which the same polymorphic states occurred at more than one accession, but did not overlap with more than one state seen in the accessions without polymorphisms. Neither these characters nor autapomorphies can provide

Figure 2.1. Flowchart explaining procedure for constructing and analyzing ITS haplotypes derived from direct sequence data.



CONSTRUCTED HAPLOTYPES

- Scored bases from direct sequence as polymorphic when secondary peak occurred in *both* forward and reverse strands
- Evaluated polymorphic sites in context of entire matrix:

```
             1 2 3 4 5 6 7 8 9
accession A  A/C c G/C C a T/C g c G
accession B  C c C A a T g c G
accession C  A c G C g T g g G
accession D  A c G A/C g T/C g g A/T
```

parsimony informative

variable or autapomorphic

- Left autapomorphic & otherwise variable sites (6 & 9) coded as ambiguities for all analyses
- Counted parsimony informative polymorphisms (1, 3, 4) per accession and broke direct sequence into as many haplotypes as 2^(parsimony informative polymorphisms)

e.g. for accession A, 2^2 = 4

```
haplotype A1  AcCcaT/Cgcg
haplotype A2  AcGcaT/Cgcg
haplotype A3  CcCcaT/Cgcg
haplotype A4  CcGcaT/Cgcg
```

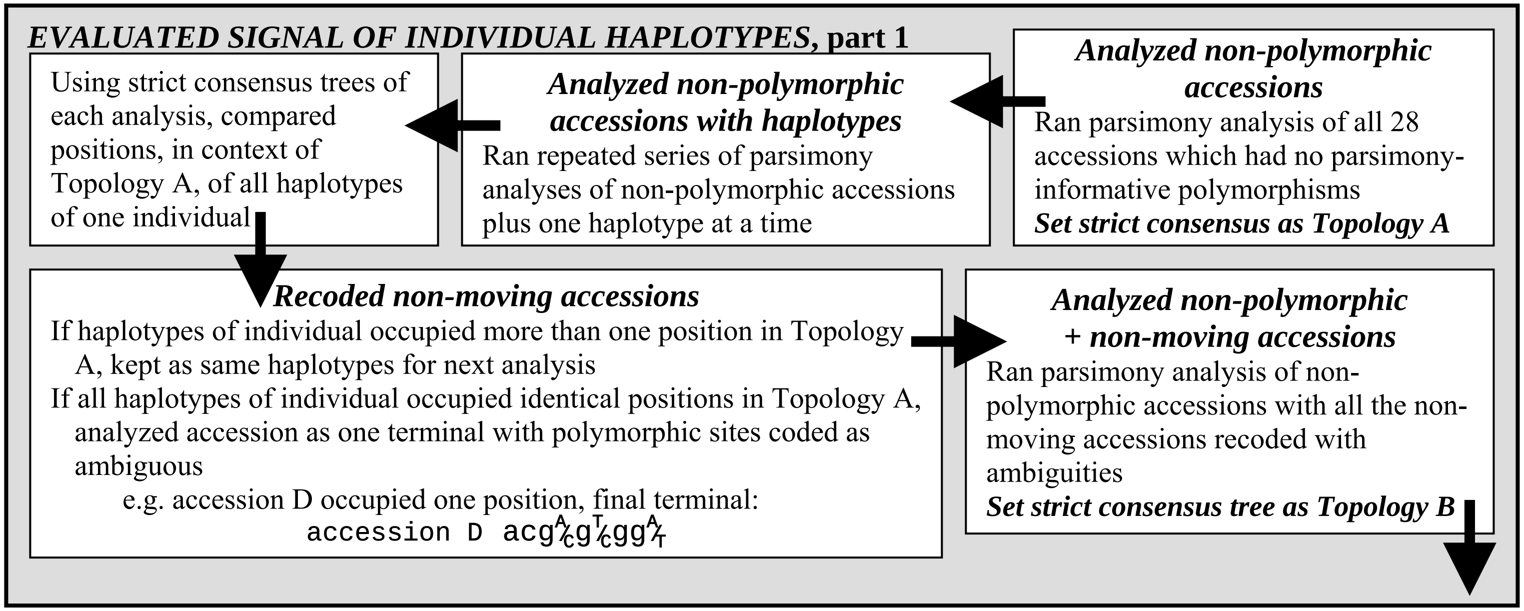
e.g. for accession D, 2^1 = 2

```
haplotype D1  acgCgT/CggA/T
haplotype D2  acgAgT/CggA/T
```

Analyzed entire dataset

Ran parsimony analysis of all 28 accessions which had no parsimony-informative polymorphisms plus all haplotypes to evaluate shallow versus deep paralogy (Fig. 2)

Figure 2.1 (Continued)



***EVALUATED SIGNAL OF INDIVIDUAL HAPLOTYPES*, part 1**

Analyzed non-polymorphic accessions

Ran parsimony analysis of all 28 accessions which had no parsimony-informative polymorphisms

Set strict consensus as Topology A

Analyzed non-polymorphic accessions with haplotypes

Ran repeated series of parsimony analyses of non-polymorphic accessions plus one haplotype at a time

Using strict consensus trees of each analysis, compared positions, in context of Topology A, of all haplotypes of one individual

Recoded non-moving accessions

If haplotypes of individual occupied more than one position in Topology A, kept as same haplotypes for next analysis

If all haplotypes of individual occupied identical positions in Topology A, analyzed accession as one terminal with polymorphic sites coded as ambiguous

e.g. accession D occupied one position, final terminal:

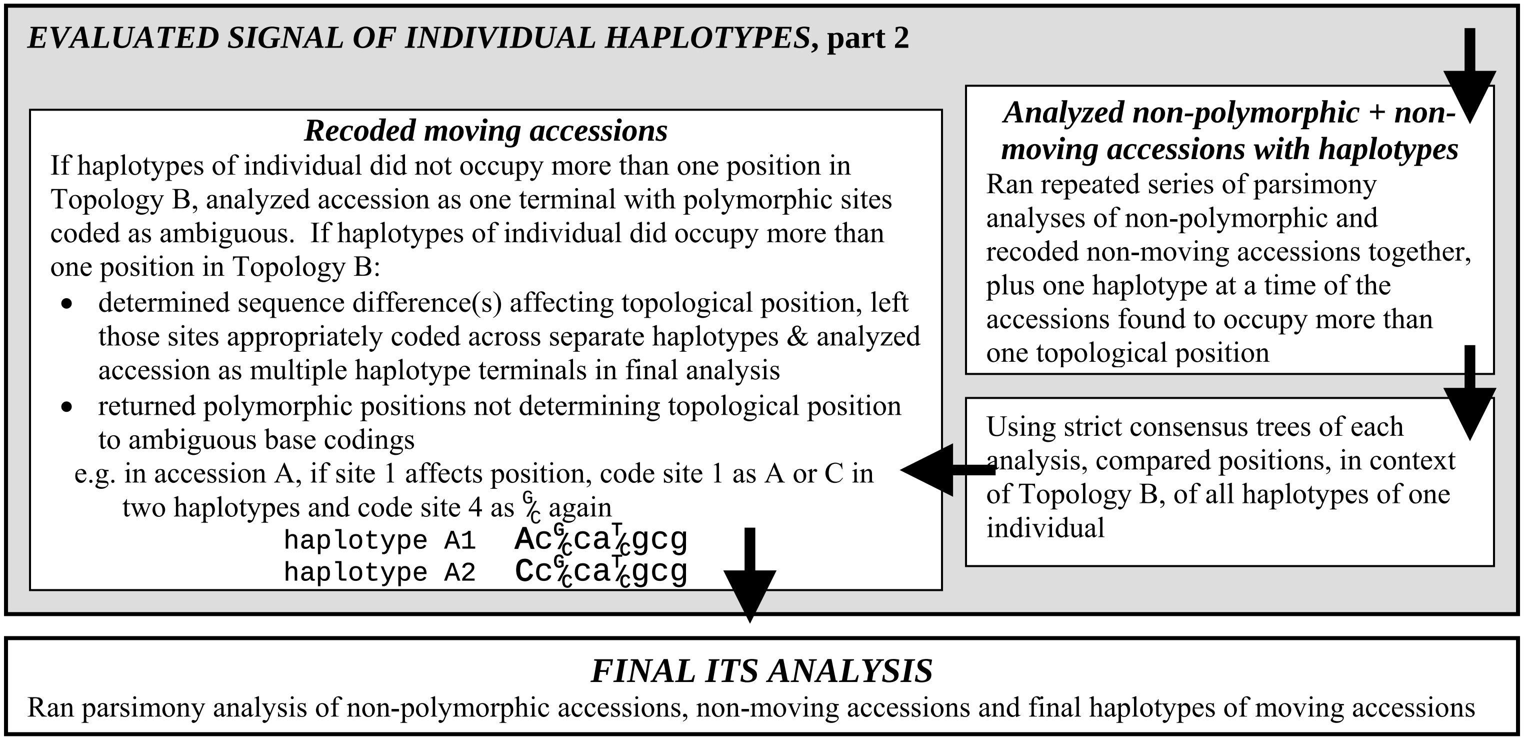
`accession D` acg$^{A}/_{C}$g$^{T}/_{C}$gg$^{A}/_{T}$

Analyzed non-polymorphic + non-moving accessions

Ran parsimony analysis of non-polymorphic accessions with all the non-moving accessions recoded with ambiguities

Set strict consensus tree as Topology B

Figure 2.1 (Continued)



***EVALUATED SIGNAL OF INDIVIDUAL HAPLOTYPES,* part 2**

Recoded moving accessions

If haplotypes of individual did not occupy more than one position in Topology B, analyzed accession as one terminal with polymorphic sites coded as ambiguous. If haplotypes of individual did occupy more than one position in Topology B:

- determined sequence difference(s) affecting topological position, left those sites appropriately coded across separate haplotypes & analyzed accession as multiple haplotype terminals in final analysis
- returned polymorphic positions not determining topological position to ambiguous base codings

e.g. in accession A, if site 1 affects position, code site 1 as A or C in two haplotypes and code site 4 as $^{G}/_{C}$ again

haplotype A1 Ac$^{G}/_{C}$ca$^{T}/_{C}$gcg
haplotype A2 Cc$^{G}/_{C}$ca$^{T}/_{C}$gcg

Analyzed non-polymorphic + non-moving accessions with haplotypes

Ran repeated series of parsimony analyses of non-polymorphic and recoded non-moving accessions together, plus one haplotype at a time of the accessions found to occupy more than one topological position

Using strict consensus trees of each analysis, compared positions, in context of Topology B, of all haplotypes of one individual

FINAL ITS ANALYSIS

Ran parsimony analysis of non-polymorphic accessions, non-moving accessions and final haplotypes of moving accessions grouping information and so were coded as ambiguities in all analyses. An "autapomorphy" is defined here in the context of an individual or a species; these same sites could be synapomorphies among particular ribotypes within one individual (Doyle & Davis, 1998).

2. Considering parsimony informative polymorphisms only, every possible haplotype of each polymorphic taxon was scored as a separate terminal.

3. A parsimony analysis was conducted with all the ITS sequences that had no parsimony informative polymorphisms in the ITS region, hereafter called the "non-polymorphic" subset of taxa, and all of the haplotypes constructed from polymorphic accessions. This analysis provided a means of evaluating if polymorphisms occurred within a species or individual or if they occurred across species ("shallow" versus "deep" paralogy, Bailey & al., 2003).

A subseries of parsimony analyses (steps 4-9) were conducted to investigate each haplotype individually for phylogenetic signal, for the following reasons. Parsimony analysis of an additive dataset, such as completely unhomogenized ITS ribotypes, would show any ribotypes resulting from hybridization sitting in two places in the topology, near one parental ribotype or the other parental ribotype (McDade 1990, 1992). Where concerted evolution is in action, the resulting "recombinant" ribotypes of hybrids could also be placed in a position between the two parents, often incurring deresolution of one or both clades including a parent (McDade, 1990, 1992). An added complication for ITS sequences is the potential for mutations occurring within a hybrid individual with divergent ribotypes to spread via concerted evolution and create intra-individual synapomorphies that could support a clade of otherwise divergent ribotypes. If an ITS phylogeny showed little molecular variability, i.e., topological resolution was derived from few supporting characters, such ribotype synapomorphies could swamp out the few characters providing phylogenetic signal and cause further deresolution of the topology.

4. A parsimony analysis of the non-polymorphic subset of taxa was conducted and the resulting strict consensus tree was set as Topology A.

5. Each putative haplotype of each polymorphic accession was then added, one by one (using "taxcode" commands in TNT), to a repeated series of parsimony analyses, each one consisting of the non-polymorphic subset of taxa and one haplotype. Strict consensus trees of each analysis were used to compare topological positions, in the context of Topology A, of all the haplotypes of one individual.

6. The "moving" accessions, i.e., any polymorphic accession for which haplotypes occupied more than one position in Topology A, were kept as the same haplotypes for the next analysis. The "non-moving" accessions, i.e., any polymorphic accession for which all haplotypes occupied the same position in Topology A, had their polymorphic sites recoded as ambiguities and were analyzed as one terminal in the final analysis.

7. A parsimony analysis was conducted of ITS sequences from the non-polymorphic subset of taxa and the recoded non-moving accessions. The strict consensus topology of this analysis was set as Topology B.

8. Each putative haplotype of each moving accession was then added, one by one, to a second repeated series of parsimony analyses, each one consisting of the non-polymorphic subset of taxa, all non-moving accessions and one haplotype. Strict consensus trees of each analysis were used to compare positions, in the context of Topology B, of all the haplotypes of one individual.

9. If the haplotypes of an individual occupied more than one position in Topology B, the polymorphic site that was affecting the topological position was determined by the sequence differences between differently placed haplotypes. That site was left

scored as individual states across haplotypes and the accession was analyzed as multiple haplotype terminals in the final analysis. The remainder of the polymorphic sites (if any), i.e., those that did not affect topological position, were scored as ambiguous.

10. The final analysis of the ITS dataset included i) non-polymorphic accessions, ii) non-moving accessions and iii) moving accessions broken into haplotypes representing the entirety of their polymorphisms relevant to their topological position. The methods for analysis of this final ITS matrix are discussed below in the "Phylogenetic analyses" section.

In sampling ribotype diversity, constructing haplotypes from direct sequence of polymorphic ITS regions is at least as effective as cloning, if not more so. This is only true given clean and readable direct sequences (i.e., sequence lacking indels). Unlike cloning, haplotype construction samples every variant possible from a direct sequence and does not accidentally sample PCR artifacts; however, both methods fail as a means for detecting cryptic variation. Although cloning haplotypes from polymorphic ITS pools has been shown to capture sufficient haplotype diversity to discern past hybridization events (e.g., Campbell & al., 1997), there is little evidence that full sampling of all the ribotypes present in an individual is achieved by the routine sampling of clones. For example, only four of 29 clones were repeat ribotypes in Rosselló & al. (2006) and even more thorough attempts at sampling ITS resulted in only nine of 90 total clones as repeat ribotypes (Razafimandimbison & al., 2004). Cloning has also been shown to be ineffective at sampling ribotype variation not evident in direct sequences (Rauscher & al., 2002). Cloning can also introduce error by sampling PCR artifacts— artifacts which are swamped out in direct sequence reads and have no impact on the haplotype construction method.

It is possible that analysis of a complete set of haplotypes constructed from direct sequences may actually provide more information than cloning. For example, a dataset of haplotypes can be generated from the direct polymorphic sequence from a known hybrid in which all polymorphisms are accounted for (i.e., haplotypes are completely additive for all polymorphisms apparent in direct sequence), yet none of the haplotypes group with one of the two progenitors in a phylogenetic analysis. This scenario could easily represent the results derived from the routine cloning of ribotypes from a hybrid. Furthermore, if concerted evolution was currently in progress but still incomplete in that known hybrid's nrDNA, haplotype construction could even provide more information than an actual dataset of every ribotype extant in that individual (generated, for instance, by next generation sequencing). In such a case, the haplotype construction method could reconstruct a parental haplotype, that no longer exists (at least in a direct sequence) in the hybrid individual but once did, that provides the phylogenetic signal necessary to group with a progenitor. If the haplotype construction method provided these results in a putative hybrid of unknown progenitors, other markers could then be employed to investigate the relationships to the hypothesized parent.

At the least, constructing and analyzing each potential haplotype provides a conservative approach to appraising phylogenetic signal in polymorphic ITS sites. One by one phylogenetic analysis of the constructed haplotypes ameliorates the impact of ribotype recombinants on topological resolution and can even help pinpoint which sequences are most likely recombinant (if sufficient signal is retained in the direct sequence to distinguish parental ribotypes).

Lastly, limited cloning was done to compare haplotypes created for these taxa based on their polymorphic direct sequences with those found via cloning. The ITS regions of four taxa were cloned, two individuals of *D.* × *cayeuxii*, a known hybrid, which showed polymorphisms at 11 and 13 sites, and two taxa (*D. superba* 1 and *D. burgessiae* 2). Three PCR reactions (methods described above) of ITS were pooled and then cloned using the Invitrogen TOPO TA Cloning Kit for Sequencing (Invitrogen, Carlsbad, California, U.S.A.). The ITS regions from a total of seventeen clones were then amplified, sequenced, and analyzed (both singly and all together) with the rest of the ITS matrix.

Phylogenetic analyses. — Parsimony analyses were done on individual gene regions with and without indel coding. Both parsimony and Bayesian analyses were done on the combined chloroplast dataset. Parsimony analyses were conducted in TNT v.1.0 (Goloboff & al., 2008) and trees visualized in Winclada v.1.7 (Nixon, 2002). Two methods for parsimony analysis, each analyzing only parsimony informative characters, were compared and found to give identical results. The first type of parsimony analysis was a modified version of the TNT analysis spawned from Winclada and began with 2000 random addition sequences each holding 20 trees that underwent TBR swapping, followed by 5000 iterations of the ratchet (percent probability of upweighting and downweighting each set to 5, otherwise default settings), then 50 cycles of drift (default settings), and 5 rounds of tree fusion (default settings), from which all the final most parsimonious trees were swapped to completion (or until 1,000,000 trees were saved) using TBR. The second type of parsimony analysis followed that of Little (2006) and began with 5000 random addition sequence replicates, each replicate undergoing TBR swapping holding 20 trees and 20 iterations of the ratchet (settings as above except 10% of parsimony informative characters set for perturbation), from which all the final most parsimonious trees were swapped to completion (or until 1,000,000 trees were saved) using TBR. Support values were measured by conducting 10,000 replicates of a bootstrap analysis in TNT, with each replicate consisting of 20 random addition sequence replicates using TBR swapping and holding 20 trees and 200 iterations of the ratchet. The strict consensus trees of each bootstrap replicate were used to calculate percent frequency support values on the strict consensus tree for the corresponding dataset in Winclada. All strict consensus trees discussed in this study have ambiguously supported clades collapsed ("nel" in TNT).

Models used in the Bayesian analysis of the combined chloroplast dataset were chosen under the Akaike Information Criterion (Akaike, 1974) as implemented in MrModelTest v.2 (Nylander, 2004) utilizing likelihood values and a neighbor joining tree generated in PAUP* v.4.0 (Swofford, 2001). The Bayesian analysis was completed in MrBayes (Ronquist & Huelsenbeck, 2003) running two simultaneous runs of 10 chains each for 20,000,000 generations sampling every 1000 generations. Data in the analysis were partitioned by spacer or gene region. Partitions and models used for each were as follows: *ndhA*x, GTR + I; *trnCycf6*, GTR; *ycf6* gene, JC; *ycf6psbM*, HKY; *psbMtrnD*, HKY; *psbEpetL*, GTR + G. Stationarity was assumed to have been reached when the average standard deviation of split frequencies and potential scale reduction factor, both calculated by MrBayes, measured less than 0.01 and 1, respectively, and effective sample size, as calculated in Tracer v.1.5 (Rambaut & Drummond, 2009), measured well above 200 for every parameter. Burn-in for the analysis was conservatively set at 10% of the generations (2,000 sampled trees), although stationarity was seen to be reached prior to this point.

RESULTS

Matrix information & sequence divergence. — Of the individual chloroplast gene regions, *psbEpetL* was the most variable, followed by *ndhA*x , *ycf6psbM*, *trnCycf6* and lastly *psbMtrnD* (Table 2.2). The gene *ycf6* held no parsimony informative characters. *Nesogordonia* alone possessed a 139 base pair insertion in the

Table 2.2. Summary of matrix characteristics for the gene regions sequenced. The section labeled "no outgroups" excludes the taxa *Nesogordonia*, *Corchoropsis*, *Pentapetes* and *Trochetiopsis*; the section labeled "major *Dombeya* clade" is as indicated in chloroplast analysis. Sequence divergence statistics were calculated only for ITS, the most variable chloroplast regions (*ndhA*x and *psbEpetL*), and the combined chloroplast dataset.

	ndhAx	***trnCycf6***	***ycf6psbM***	***psbMtrnD***	***psbEpetL***	**cp**	**ITS**	**cp + ITS**
Number of taxa	87	87	87	87	87	87	85	87
Aligned length (bp)**	1330	764	924*	605	805	4428*	715	5143*
Inferred number of indels	23	22	33*	10	21	76*	49	125*
Length of indels (bp)	1-14	1-24	1-35	1-10	1-17	1-35	1-10	1-35
all taxa								
% PICs without indels	2.9	1.6	2.6	2.1	2.9	2.5	18.6	4.7
% PICs with indels	3.6	2.7	3.0	2.4	3.8	3.0	19.4	5.3
% sequence divergence	0-5.3	--	--	--	0-3.7	0-4	0-38	--
no outgroups								
% PICs without indels	1.5	0.7	0.8	1.2	1.6	1.2	13.0	2.8
% PICs with indels	1.8	1.5	1.1	1.5	2.3	1.6	13.1	3.1
% sequence divergence	0-0.9	--	--	--	0-1.3	0-0.8	0-12	--
major *Dombeya* clade								
% PICs without indels	0.8	0.5	0.4	0.3	1.0	0.7	5.5	1.3
% PICs with indels	1.0	1.1	0.6	0.3	1.5	0.9	5.4	1.4
% sequence divergence	0-0.6	--	--	--	0-1.1	0-0.5	0-3.5	--

*excluding a 139 bp insertion seen in *Nesogordonia*; **excluding characters from indel coding *ycf6psbM* region. The aligned matrix of combined chloroplast regions for all taxa had 4773 characters, including indel coding; 3.2% of the characters were parsimony informative and 0.7% of PICs were indel characters. Almost 50% of the relatively few PICs that the chloroplast did possess were a result of molecular divergence between the outgroups and the rest of the taxa in the analysis (1.6% PICs for all chloroplast regions combined *without* the genera *Nesogordonia*, *Corchoropsis*, *Pentapetes* and *Trochetiopsis*). Of the 764 characters of the aligned ITS matrix for all taxa, 19.4% of the characters were parsimony informative and 2.0% of PICs were indel characters. With outgroups excluded, ITS retained 13.1% PICs.

Pairwise sequence divergence was roughly an order of magnitude higher for ITS than for the chloroplast as a whole. (The highest sequence divergence of the most variable, individual chloroplast region— *psbEpetL* or *ndhA*x— is reported here after that given for the five chloroplast regions calculated together.) Maximum sequence divergence between an outgroup accession and an ingroup accession was 38% for ITS and 4% for the chloroplast (5.3% for *ndhA*x). Between two ingroup members (any accessions excluding *Nesogordonia*, *Corchoropsis*, *Pentapetes* and *Trochetiopsis*), the highest sequence divergence was 12% for ITS and 0.8% for the chloroplast (1.3% for *psbEpetL*). Within the major *Dombeya* clade divergence in ITS was 3.5% at its highest and 0.47% for the chloroplast (1.1% in *psbEpetL*). (See Table 2.2 for estimates of sequence divergence and Figure 3A for clade designations.)

Further analyses of the ITS dataset. — Of 143 polymorphic sites found in the ITS dataset, five sites, all autapomorphic, were in 5.8S, while the remaining 138 sites were in the intergenic spacers (46 in ITS1 and 31 in ITS2) and 77 of them (~53%) were parsimony informative. Two-state polymorphisms were present in 248 cells in the ITS matrix, roughly 0.4% of the total matrix cells.

Seventy-two accessions in the study (~83% of accessions sampled) showed polymorphic sites at least once in their sequence. Of these, 59 accessions contained parsimony informative polymorphisms (~68% of accessions). Polymorphic sites per accession varied from one to 13, but only eleven accessions (*D. befotakensis*, two individuals of *D. burgessiae*, *D. coria*, *D. laurifolia*, *D. montana*, *D. palmatisecta*, *D. superba* 2, *D. tiliacea* and two individuals of *D.* × *cayeuxii*) had more than three polymorphic sites. Only the two individuals of the known hybrid *D.* × *cayeuxii* had more than eight parsimony informative polymorphic sites (one had 11 sites, the other 13). Excluding *D.* × *cayeuxii*, accessions with parsimony informative polymorphisms required the construction of a total of 880 haplotypes. Only one individual of *D.* × *cayeuxii*, which by itself necessitated the construction of 2056 haplotypes, was included in the haplotype analyses.

Parsimony analysis of all the haplotypes plus the "non-polymorphic" accessions resulted in over 5000 most parsimonious trees with a length (L) of 3463, a consistency index (CI) of 12 and a retention index (RI) of 55. The resulting strict consensus is shown in Fig. 2.2. The majority of haplotypes showed polymorphisms confined within individuals or species, forming intra-individual or intraspecific clades, e.g., a clade of four *Helmiopsis pseudopopulus* haplotypes from one individual or a clade of four *Ruizia cordata* haplotypes from two individuals. One instance of a polymorphism shared across species was shown by a clade formed of one haplotype each from *D. muscosa* and *D. leiomacrantha* supported as separate from the other haplotypes of each of these two species (marked in Fig. 2.2). Also seen in this analysis were four interspecific polytomies (numbers 1-4, Fig. 2.2), e.g., haplotypes of *D. stipulacea*, *D. montana*, *D. coria*, *D. befotakensis*, *D. acerifolia* and *D. rottleroides* form one large polytomy (#2 in Fig. 2.2) with non-polymorphic sister species.

Figure 2.2. Strict consensus tree of parsimony analysis of the non-polymorphic ITS subset of taxa with all haplotypes of polymorphic sequences derived from direct sequence. Number after a taxon name differentiates individuals of the same species. Numbers in parentheses after a taxon name denote the number of haplotypes constructed for that accession. Brackets signify a set of haplotypes that grouped as a clade. Examples of deep and shallow paralogy are marked, as well as four (1-4) polytomies where haplotypes of an accession are undifferentiated from interspecific accessions. Circles mark "moving" accessions that occupied two positions in the last round of one-by-one haplotype analyses, excluding the two individuals of *D.* X *cayeuxii* which are represented here by three clones each (A, B and C).

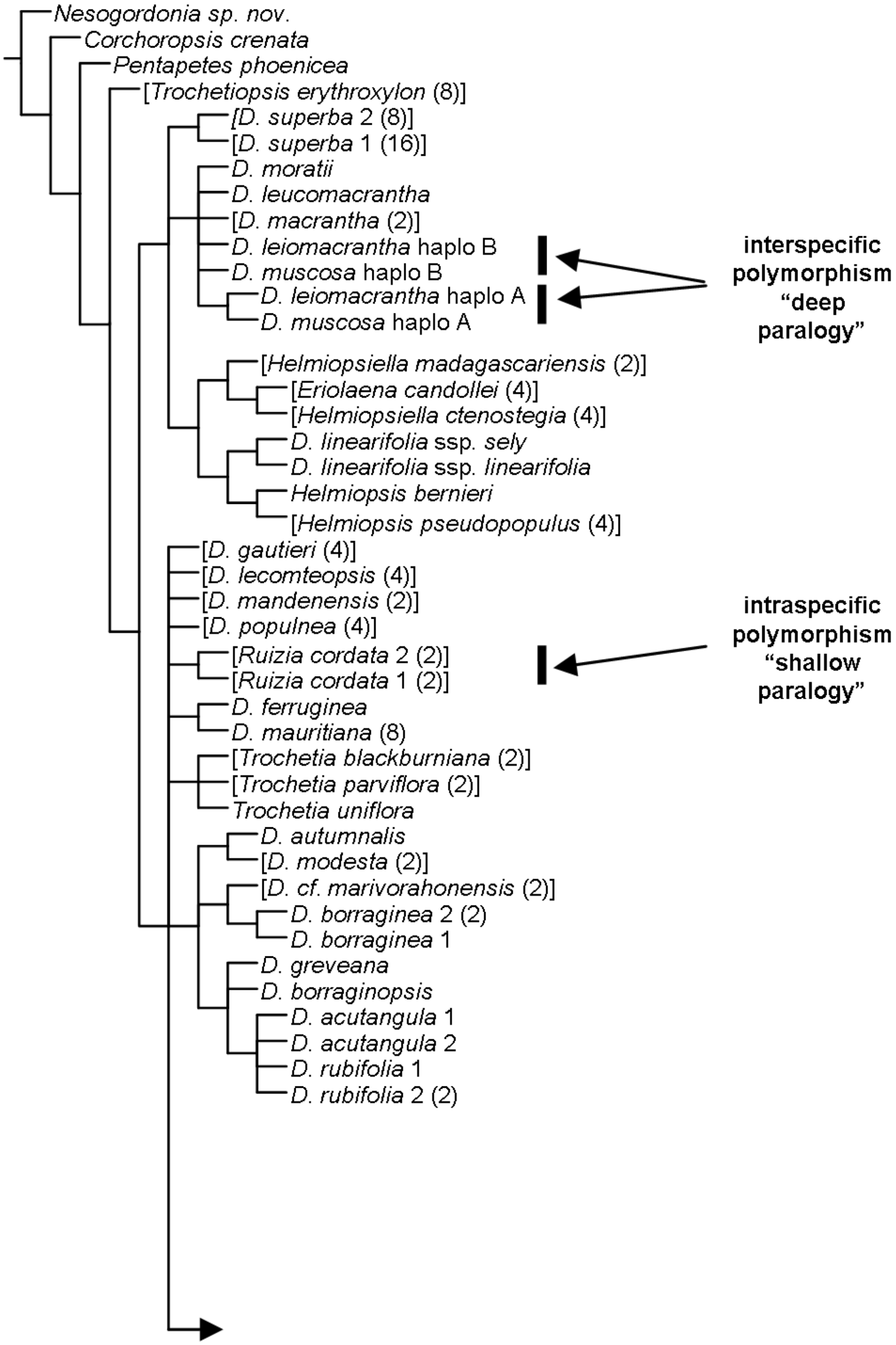
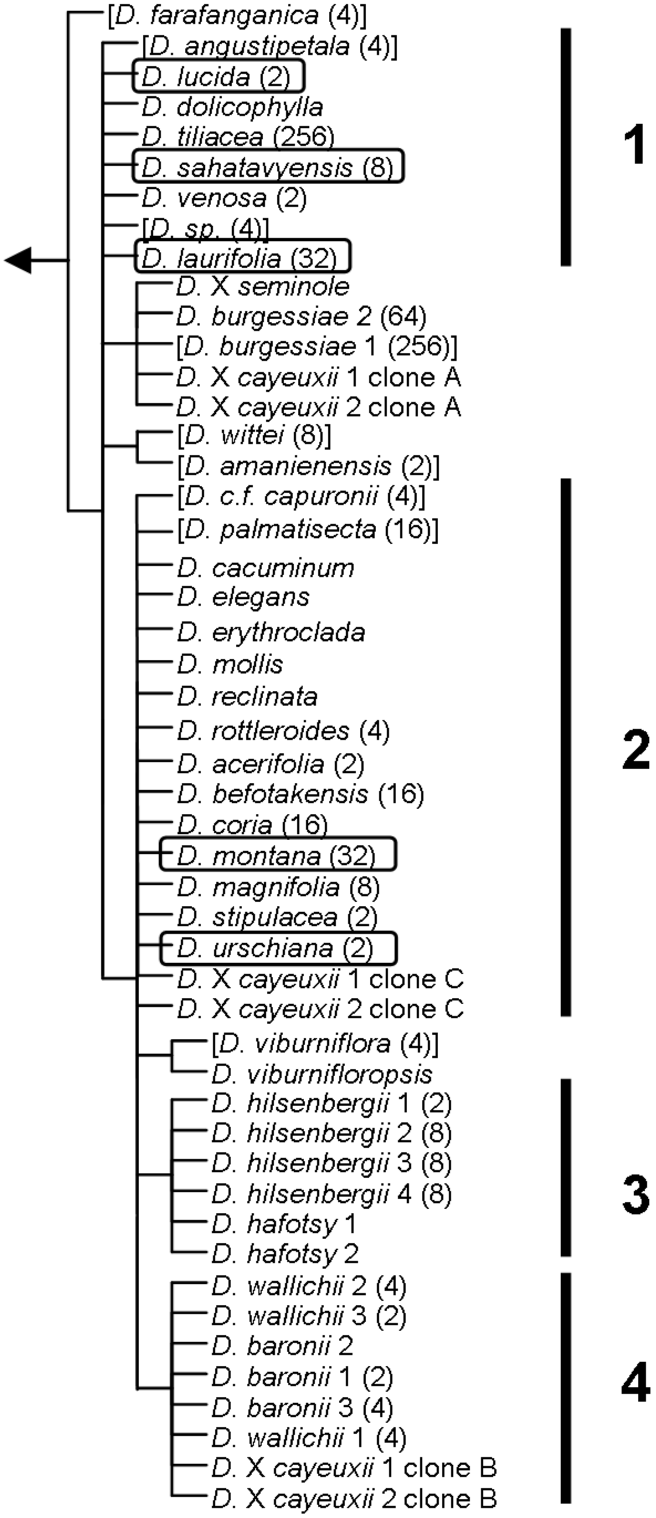

Nesogordonia sp. nov.
Corchoropsis crenata
Pentapetes phoenicea
[Trochetiopsis erythroxylon (8)]
[D. superba 2 (8)]
[D. superba 1 (16)]
D. moratii
D. leucomacrantha
[D. macrantha (2)]
D. leiomacrantha haplo B
D. muscosa haplo B
D. leiomacrantha haplo A
D. muscosa haplo A
[Helmiopsiella madagascariensis (2)]
[Eriolaena candollei (4)]
[Helmiopsiella ctenostegia (4)]
D. linearifolia ssp. sely
D. linearifolia ssp. linearifolia
Helmiopsis bernieri
[Helmiopsis pseudopopulus (4)]
[D. gautieri (4)]
[D. lecomteopsis (4)]
[D. mandenensis (2)]
[D. populnea (4)]
[Ruizia cordata 2 (2)]
[Ruizia cordata 1 (2)]
D. ferruginea
D. mauritiana (8)
[Trochetia blackburniana (2)]
[Trochetia parviflora (2)]
Trochetia uniflora
D. autumnalis
[D. modesta (2)]
[D. cf. marivorahonensis (2)]
D. borraginea 2 (2)
D. borraginea 1
D. greveana
D. borraginopsis
D. acutangula 1
D. acutangula 2
D. rubifolia 1
D. rubifolia 2 (2)
interspecific polymorphism “deep paralogy”
intraspecific polymorphism “shallow paralogy”

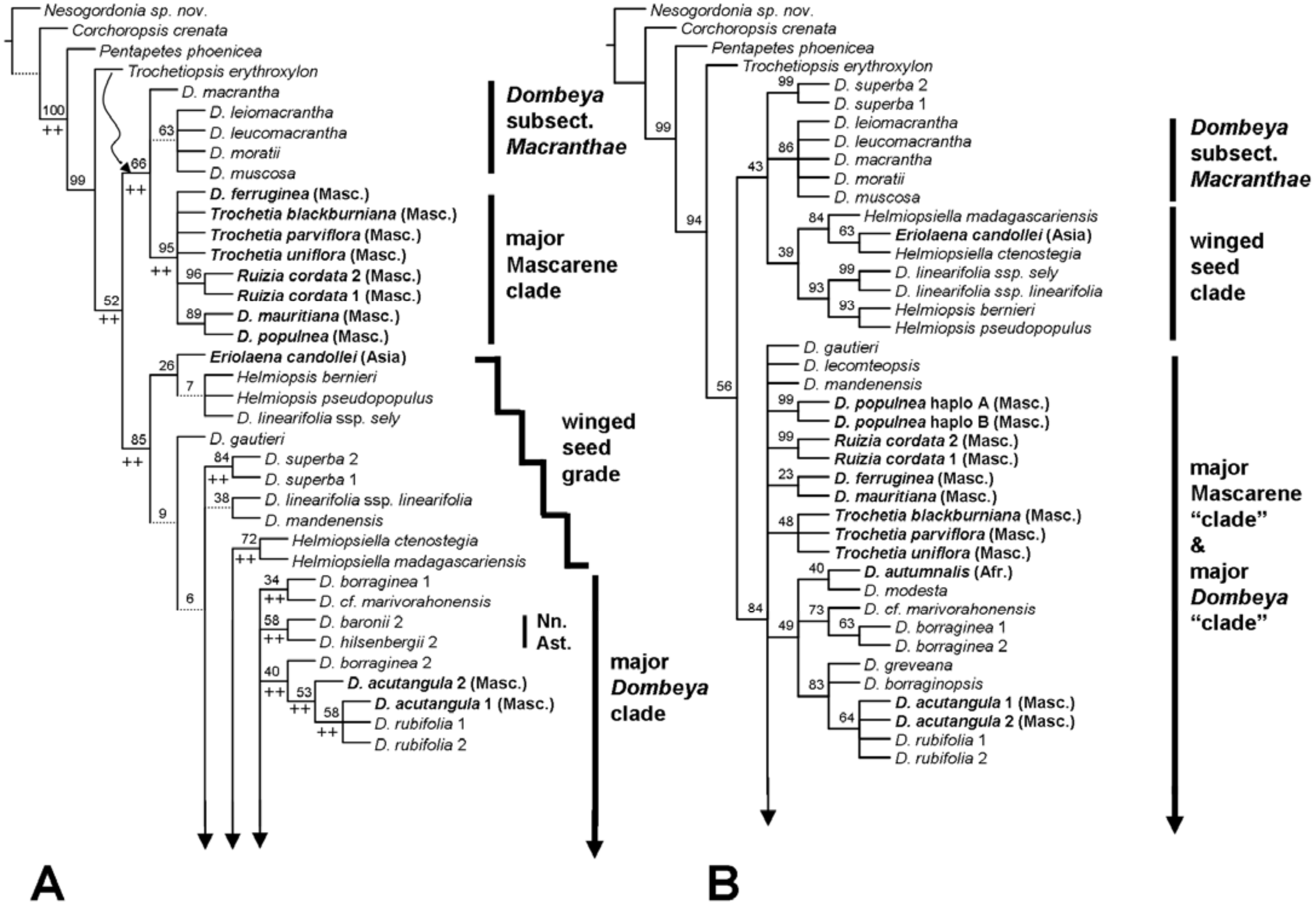
Figure 2.2 (Continued)



The non-polymorphic subset of taxa included the outgroups *Nesogordonia*, *Corchoropsis* and *Pentapetes* and one accession of each of the major clades (clades with >2 members) found in all permutations of the analyses. Parsimony analysis of the non-polymorphic subset of taxa resulted in three most parsimonious trees (L = 380, CI = 85, RI = 78; topology not shown). In the haplotype analyses, no accession occupied more than two positions. Eight "moving" accessions (*D. laurifolia*, *D. lucida*, *D. montana*, *D. populnea*, *D. sahatavyensis*, *D. urschiana*, and the two individuals of *D.* × *cayeuxii*) occupied two positions in the strict consensus trees produced from the repeated parsimony analyses adding one putative haplotype terminal at a time to the reduced non-polymorphic ITS subset of taxa (see flowchart, Fig. 2.1). Of these, the two positions of *D. lucida* haplotypes and the two positions of *D. populnea* haplotypes were very similar, respectively, and differed from one another only by collapsing/resolving one node in their two respective areas of the topology. Once the "non-moving" accessions, which had parsimony informative polymorphic characters that did *not* affect their position in the topology, were added back into the ITS analysis, all of the haplotypes of the same "moving" accessions were still found to hold two positions, except for *D. lucida* that sat in only one position. Clones of *D.* × *cayeuxii* were used as terminals in the final ITS analysis. The remaining five "moving" accessions, excluding *D. lucida* and two individuals of *D.* × *cayeuxii*, were analyzed in the final analysis as ten haplotypes, two per accession.

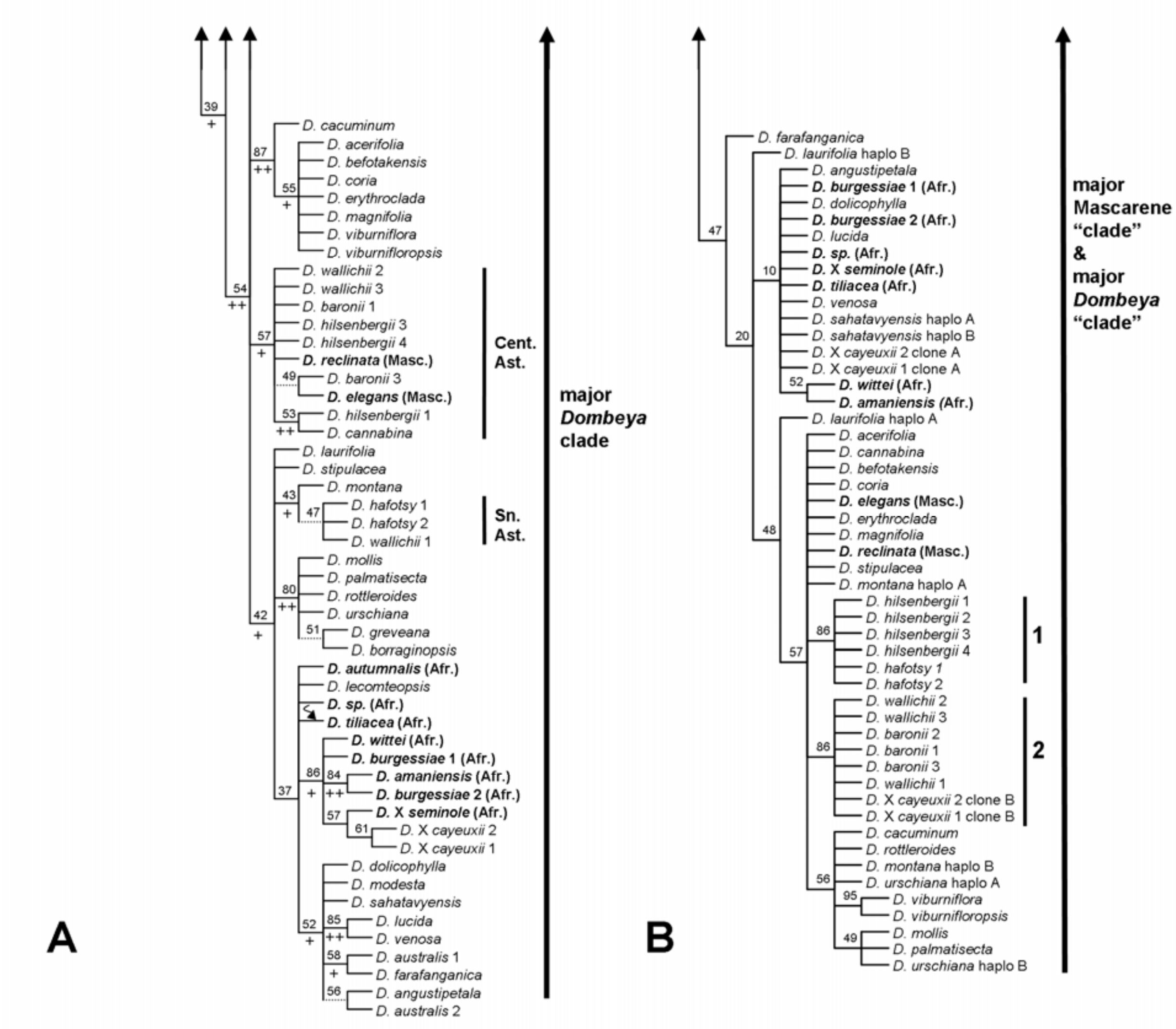
Analysis of this final ITS dataset of 92 terminals resulted in over 1 million most parsimonious trees (L = 571, CI = 69, RI = 79). The topology of the strict consensus tree (see Fig. 2.3B) reflected both the topologies resulting from analysis of the non-polymorphic subset of taxa alone ("Topology A" of Fig. 2.1) and the non-polymorphic subset of taxa plus "non-moving" accessions ("Topology B" of Fig. 2.1); the general underlying relationships among these taxa did not change by the addition of

Figure 2.3. Strict consensus tree resulting from parsimony analysis of the combined chloroplast dataset with the results of Bayesian analysis mapped on (**A**) and strict consensus tree resulting from parsimony analysis of the ITS dataset (**B**). In tree (A), clade "Nn. Ast." corresponds to *Dombeya* sect. *Astrapaea* from northern Madagascar, clade "Cent. Ast." to *D.* sect. *Astrapaea* from central-eastern Madagascar and clade "Sn. Ast." to *D.* sect. *Astrapaea* from southern Madagascar. In tree (B), clades 1 & 2 correspond to two clades of *Dombeya* sect. *Astrapaea* that are each concordant with morphology and specific delimitations. Values above branches indicate bootstrap percentages from 10,000 replicates. Hashed lines show nodes that collapse in Bayesian analysis. Symbols below branches indicate Bayesian posterior probabilities, where 0.9<+<1.00 and ++ = 1.00. Arrows indicate nodes that move in Bayesian analysis. Excluding outgroups (*Nesogordonia*, *Corchoropsis*, *Pentapetes*, and *Trochetiopsis*), names in bold indicate an accession originating from outside of Madagascar with the region of provenance given in parentheses (Afr. = Africa; Masc. = Mascarenes). Numbers after a name differentiate individuals sampled from one species. Putative haplotypes of five accessions are labeled "haplo A" or "haplo B" and four clones of two individuals of *D.* X *cayeuxii* are labeled as "clone A" or "clone B."



Nesogordonia sp. nov.
Corchoropsis crenata
Pentapetes phoenicea
Trochetiopsis erythroxylon
D. macrantha
D. leiomacrantha
D. leucomacrantha
D. moratii
D. muscosa
D. ferruginea (Masc.)
Trochetia blackburniana (Masc.)
Trochetia parviflora (Masc.)
Trochetia uniflora (Masc.)
Ruizia cordata 2 (Masc.)
Ruizia cordata 1 (Masc.)
D. mauritiana (Masc.)
D. populnea (Masc.)
Eriolaena candollei (Asia)
Helmiopsis bernieri
Helmiopsis pseudopopulus
D. linearifolia ssp. sely
D. gautieri
D. superba 2
D. superba 1
D. linearifolia ssp. linearifolia
D. mandenensis
Helmiopsiella ctenostegia
Helmiopsiella madagascariensis
D. borraginea 1
D. cf. marivorahonensis
D. baronii 2
D. hilsenbergii 2
D. borraginea 2
D. acutangula 2 (Masc.)
D. acutangula 1 (Masc.)
D. rubifolia 1
D. rubifolia 2
100
++
99
52
++
66
++
63
95
++
96
89
26
7
85
++
9
84
++
38
6
72
++
34
++
58
++
40
++
53
++
58
++
Dombeya subsect. Macranthae
major Mascarene clade
winged seed grade
Nn. Ast.
major Dombeya clade
A
Nesogordonia sp. nov.
Corchoropsis crenata
Pentapetes phoenicea
Trochetiopsis erythroxylon
D. superba 2
D. superba 1
D. leiomacrantha
D. leucomacrantha
D. macrantha
D. moratii
D. muscosa
Helmiopsiella madagascariensis
Eriolaena candollei (Asia)
Helmiopsiella ctenostegia
D. linearifolia ssp. sely
D. linearifolia ssp. linearifolia
Helmiopsis bernieri
Helmiopsis pseudopopulus
D. gautieri
D. lecomteopsis
D. mandenensis
D. populnea haplo A (Masc.)
D. populnea haplo B (Masc.)
Ruizia cordata 2 (Masc.)
Ruizia cordata 1 (Masc.)
D. ferruginea (Masc.)
D. mauritiana (Masc.)
Trochetia blackburniana (Masc.)
Trochetia parviflora (Masc.)
Trochetia uniflora (Masc.)
D. autumnalis (Afr.)
D. modesta
D. cf. marivorahonensis
D. borraginea 1
D. borraginea 2
D. greveana
D. borraginopsis
D. acutangula 1 (Masc.)
D. acutangula 2 (Masc.)
D. rubifolia 1
D. rubifolia 2
99
99
86
43
94
84
63
39
99
93
93
56
99
99
23
48
40
73
63
84
49
83
64
Dombeya subsect. Macranthae
winged seed clade
major Mascarene “clade” & major Dombeya “clade”
B

Figure 2.3 (Continued)



haplotypes. The two putative haplotypes of each of the "moving" accessions held the same positions in the final strict consensus that they occupied when analyzed individually with the non-polymorphic subset of taxa (see Fig. 2.3B for positions).

The direct sequences of the two hybrid individuals of *D.* × *cayeuxii* when compared to their parental species (*D. wallichii* and *D. burgessiae*), showed 14 variable sites. Nine of them were additive, wherein both individuals of *D.* × *cayeuxii* had polymorphisms (e.g., K) representing the bases of either parent (parent 1 had T, parent 2 had G). Other sites where one parent had a polymorphism (e.g., Y) that included the single base pair of the other parent (e.g., T), variously showed: i) two hybrids fix to a single base (both T), ii) two hybrids retain the polymorphism (both Y), or iii) one hybrid retained the polymorphism (Y) and one hybrid fixed to the single base (T). The remaining two sites showed one hybrid with a novel base in a polymorphism (Y), unlike either parent (both C), and lastly, both hybrids exhibiting a novel base (T) in the form of a polymorphism (W) including the A either parent possessed. At no position did the hybrids possess a non-polymorphic, completely novel base not seen in either parent. In analyses of each haplotype added singly to the non-polymorphic subset of taxa, the haplotypes of *D.* × *cayeuxii* were seen to sit in multiple positions, all of which were bounded by the positions of the two parents. The hybrid haplotypes were sometimes sister to one parent or the other, but could also occupy positions between either, sometimes collapsing one or both of the clades in which the parents belonged with or without collapse of the other clades positioned between them.

Cloned sequences from the four accessions (four clones of *D. superba* 2, two clones of *D. burgessiae* 2 and 11 clones for two individuals of *D.* × *cayeuxii*) were additive to their direct sequences, meaning all polymorphisms were accounted for across the clones (except for *D. burgessiae* 2, with only two clones sequenced, for which two polymorphisms were unresolved). None of the clone sequences for any one accession were identical nor did any show sequence differences not seen in the original direct sequences. Results from parsimony analyses of the clone sequences added singly to the non-polymorphic subset of taxa were identical to the results of the same analyses with putative haplotypes (those constructed from polymorphic direct sequences): *Dombeya superba* clones sat in one position, *D. burgessiae* 2 clones sat in one position, and *D.* × *cayeuxii* clones sat in multiple positions, as described above. Two clones from each individual of *D.* × *cayeuxii* that grouped with either parent were chosen to include in the final analysis of ITS.

Phylogenetic Analyses. — Parsimony analyses of the combined chloroplast regions with indel coding yielded nine most parsimonious trees (L = 498, CI = 91, RI = 91; strict consensus tree, Fig. 2.3A). The nine most parsimonious trees differed in only two places. In both cases, the changes to the tree topology resulted from a ‘wildcard’ taxon moving around in a confined area of the tree. *Dombeya lecomteopsis* moved into and out of a clade with D. *sp.* Kew and *D. tiliacea* due to one missing PIC and *D. moratii* moved among its sister taxa *D. leiomacrantha*, *D. leucomacrantha* and *D. muscosa*, also due to one missing PIC. A parsimony analysis of the combined chloroplast dataset omitting the two characters that were lacking for *D. lecomteopsis* and *D. moratii* resulted in one most parsimonious tree which was identical to the strict consensus of the nine most parsimonious trees resulting from analysis of the entire chloroplast dataset. The concatenated chloroplast sequence data produced one consistent set of relationships with little conflict or homoplasy.

Few clades in the chloroplast topology garnered moderate (>75%) bootstrap support (BS). *Nesogordonia*, *Corchoropsis* and *Pentapetes* were well supported as outgroups, but *Trochetiopsis*, although consistently placed as an outgroup by parsimony analysis, had few characters supporting its position as separate from the ingroups (BS = 52%). The genera *Eriolaena*, *Helmiopsis*, *Helmiopsiella*, *Ruizia*, and *Trochetia* were all nested within *Dombeya sensu lato*. The clade with the majority of taxa from the Mascarenes was the most supported clade in the chloroplast dataset (BS = 95%) and included the genera *Trochetia* (3 spp.) and *Ruizia* as well as three Mascarene species of *Dombeya* (with *D. mauritiana* and *D. populnea* as well-supported sister taxa, BS = 96%). (Other Mascarene species occur elsewhere in the tree and are marked as such in Fig. 2.3). A clade that excluded the outgroups, *Dombeya* subsect. *Macranthae*, and the major Mascarene clade was fairly well supported (BS = 85%). Most species of *Dombeya* formed a poorly supported clade (BS = 54%) and is hereafter called the "major *Dombeya* clade." The genera *Eriolaena*, *Helmiopsis* and *Helmiopsiella* were placed as sister taxa to the major *Dombeya* clade in a series of very poorly supported nodes with *D. linearifolia*, *D. gautieri* and *D. mandenensis* interdigitated among these three other genera. The major *Dombeya* clade had three moderately well-supported clades within it (as denoted by the first name in the clade from the top): 1) clade marked by *D. cacuminum* (BS = 87%), 2) clade marked by *D. mollis* (BS = 80%), and 3) clade marked by *D. wittei* and including only 5-carpellate African species (BS = 86%). Other high bootstrap values supported a sister relationship between *D. amaniensis* and *D. burgessiae* 1 (BS = 84%), a sister relationship between *D. lucida* and *D. venosa* (BS = 85%), or simply showed support for relationships between intraspecific individuals (e.g., *D. superba* accessions or accessions of the monospecific genus *Ruizia*). The phylogeny of the combined chloroplast data hypothesized by Bayesian analysis differs little from that of parsimony, except that it placed *Trochetiopsis* sister to the clade of *D.* subsect. *Macranthae* plus the major Mascarene clade, underlining the close relationship this "outgroup" may have to particular members of *Dombeya s. l.* Bayesian analysis also collapsed both poorly-supported branches separating *D. gautieri* from the taxa surrounding it to form a polytomy (see Fig. 2.3A).

In contrast to the single signal of the chloroplast data, parsimony analysis of the ITS dataset alone produced over 1 million trees (L = 571, CI = 69, RI = 79; strict consensus tree, Fig. 2.3B). Conflicting characters, independent of the polymorphisms, were apparent in the ITS dataset and relationships between most clades in ITS changed across the many most parsimonious trees resulting in the multiple polytomies seen in the strict consensus. As in the combined chloroplast analysis, analysis of the ITS matrix alone showed *Nesogordonia*, *Corchoropsis* and *Pentapetes* well-supported as outgroups, and *Trochetiopsis* consistently placed as an outgroup but again with low support (BS = 56%). Unlike the chloroplast dataset, ITS supported a sister relationship between both subspecies of *D. linearifolia* and *Helmiopsis* (BS = 93%) and placed *Eriolaena* within *Helmiopsiella* (BS = 84%), and united these two clades as sister clades with low support (BS = 39%). The ITS dataset also supported *Dombeya* subsect. *Macranthae* as a clade (BS = 86%) and included the members of the major Mascarene clade separately (i.e., not grouped as a clade) within the major *Dombeya* clade (BS = 84%). Analysis of ITS alone also differed from analysis of the combined chloroplast regions by its moderately supported clades within the major *Dombeya* clade, which were as follows: 1) clade marked by *D. greveana*, including multiple taxa with scorpioid cymes (BS = 83%), 2) clade marked by *D. hilsenbergii* 1 (BS = 86%; Fig. 2.3B, clade 1), and 3) clade marked by *D. wallichii* 2 (BS = 86%; Fig. 2.3B, clade 2). Relationships between intraspecific individuals supported in the combined chloroplast analysis were also supported by ITS along with a well-supported sister relationship between *D. viburniflora* and *D. viburnifloropsis* (BS = 95%), as well as between the two intragenomic putative haplotypes of *D. populnea*.

DISCUSSION

Molecular variation in *Dombeya*. —Meaningful comparisons of molecular diversity are difficult to make across genera given differences in distribution and species numbers. The tendency to report in the literature total sequence variation for all taxa in a study, which often includes outgroups that are phylogenetically distant, also complicates comparisons. Nonetheless, the levels of molecular variation in *Dombeya* (5.5% PICs in ITS) are on par with other paleotropical plant genera with diversity around the Indian Ocean basin. *Gaertnera*, more widespread but less species-rich than *Dombeya* (Rubiaceae; 68 spp.; Africa, Madagascar, Mascarenes, Sri Lanka and southeast Asia), had 4.9% PICs in ITS (Malcomber, 2002). Low levels of sequence divergence were commented upon for the Malagasy species of *Coffea*, another native to Africa, Madagascar and the Mascarenes (Maurin & al., 2007). *Coffea* and its near relative *Psilanthus* had 12.9% PICs for ITS, similar to the level of molecular diversity seen in *Dombeya s.l.* (13.0%), but an exact number of PICs for *Coffea* alone was not reported (Maurin & al., 2007). Sequence divergence for ITS within the major *Dombeya* clade (3.5% maximum divergence) is less than or falls within the range seen in other island endemics that include far fewer species. *Dendroseris* with eleven species had an average sequence divergence of 2.67% in ITS (Sang & al., 1994) and *Robinsonia* with seven species had an average of 6.26% (Sang & al., 1995a). Both of these genera of Asteraceae are endemic to the Juan Fernandez Islands. Four species from three genera of the Hawaiian silversword alliance sequenced for ITS had a maximum sequence divergence of 3.2% (Baldwin, 1992).

Molecular variation appears remarkably low for the major *Dombeya* clade given its morphological diversity. Yet, research into the genetics of speciation and domestication (e.g., Bradshaw & al., 1998; Doebley, 2004; Bouck & al., 2007) has shown that few loci, or multiple loci in single linkage groups, can underlie striking morphological changes. Such genetically localized drivers may be at work in creating the previously described morphological variability in *Dombeya*; a similar scenario has been proposed to account for the diversity of inflorescence structures seen in *Gaertnera* (Malcomber, 2002). In general, a lack of molecular divergence may suggest that many of the clades within *Dombeya* are recent in origin. Chloroplast introgression and incomplete concerted evolution in ITS (both discussed below) preclude the use of molecular dating methods to estimate a time frame for the divergence of such clades.

Introgression. — The chloroplast dataset provided evidence for chloroplast introgression, most obviously in the case of *Dombeya* sect. *Astrapaea*. Three poorly supported plastid clades of *D.* sect. *Astrapaea* separated conspecifics and instead grouped accessions based on geography (Fig. 2.3A). The northern clade included the pair of accessions from Montagne d'Ambre (*D. baronii* 1 and *D. hilsenbergii* 2). The southern clade included two specimens from Midongy du Sud (*D. hafotsy* 1 and 2) as well as an accession of *D. wallichii* from near Fort Dauphin. The last clade included eight accessions of *D.* sect. *Astrapaea* from eastern and central Madagascar, including a pair of species from Ranomafana (*D. baronii* 3 and *D. hilsenbergii* 1) and a pair from Zahamena (*D. baronii* 1 and *D. hilsenbergii* 3). To interpret this topology as an indication of the actual sister relationships for these accessions would require extraordinary convergent evolution of striking floral characteristics across these clades followed by convergent evolution of leaf and stipule characteristics for the *D. baronii* accessions from either clade and likewise for the *D. hilsenbergii* accessions. Even if all three clades (presently in a polytomy) were supported as sister to one another, allowing the floral characteristics to evolve only once, convergent evolution of leaf and stipule characteristics would still have had to occur more than once.

Similar to the plastid data, ITS formed three lineages from the accessions of *D.* sect. *Astrapaea*, and not a single, monophyletic clade. In contrast to the plastid phylogeny, ITS supported two *D.* sect. *Astrapaea* clades that corresponded to morphology rather than geography. (The third lineage had one member, *D. cannabina*, and sat in a position basal to either group.) These two major sect. *Astrapaea* clades grouped all conspecific accessions and furthermore united *D. hilsenbergii* and *D. hafotsy*, two species which share a unique combination of floral characteristics for the section (Chapter 4). The best explanation of these data is that introgression, specifically cytoplasmic introgression and not nuclear, has occurred between sympatric species of *D*. sect. *Astrapaea* within these three general geographical areas.

Introgression may also account for phylogenetic placement of the following accessions, not in *D.* sect. *Astrapaea*: i) *D. australis* 1 from Mandritsara grouping with *D. farafanganica* from nearby Vohipao (though with poor support) rather than with conspecific *D. australis* 2 that was collected to the south near Fort Dauphin; and ii) placement of the African species of *D. lecomteopsis* and *D. autumnalis* near other continental African species of *Dombeya* in the chloroplast tree despite their more phylogenetically distant positions in the ITS topology. There is little geographic pattern in other clades present in the chloroplast topology and therefore no further indications of local cytoplasmic introgression.

Although introgression has evidently occurred between some species of *Dombeya* in Madagascar, it must have limits as the chloroplast phylogeny does not correspond strictly to geographic localities, as seen in other introgressed taxa (e.g., *Echinacea*, Flagel & al., 2008). Evidence for introgression in field-collected accessions occurs only between fairly closely related species within *Dombeya*. A number of accessions sampled from the same collecting localities as the species pairs from *D.* sect.

Astrapaea did not group with them, e.g., *D. angustipetala* (sect. *Xeropetalum*) or *D. befotakensis* (sect. *Dombeya*) from Ranomafana or *D. sahatavyensis* (sect. *Xeropetalum*) from Zahamena, nor did other accessions sampled from the same collecting localities, e.g., *D. viburniflora* (sect. *Dombeya*), *D. cannabina* (sect. *Astrapaea*) and *D. leiomacrantha* (sect. *Trochetiantha*), all from Andringitra. This scenario of introgression between closely related species but not between phylogenetically distant species is also seen in *Quercus*, wherein the closely related species *Q. robur*, *Q. pubescens*, *Q. frainetto* and *Q. petraea* often share cytotypes by geographic region but sympatric *Q. cerrinus* do not (Curtu & al. 2007) and in *Fraxinus*, wherein species of one section, *F. angustifolia* and *F. excelsior*, routinely share chloroplast haplotypes but have no haplotype in common with sympatric *F. ornus*, a species of another section (Heuertz & al., 2006). Artificial crosses between cultivated *Dombeya* suggest that reproductive isolation in this group is not derived from genetic incompatibilities. Some level of reproductive isolation must exist in the wild, perhaps via pollinator specialization or phenological isolation.

Interpreting ITS polymorphisms. — Rapid concerted evolution and intragenomic sequence homogeneity have long been seen as the norm for ITS sequences in plants (Baldwin & al., 1995), but a growing body of literature provides evidence for intragenomic ITS polymorphisms in numerous taxa, both in cases involving putative pseudogenes (e.g., Razafimandimbison & al., 2004; Harpke & Peterson, 2006) and those concerning apparently functional paralogs only (e.g., Rosselló & al., 2006; Rosselló & al., 2007). Although ITS polymorphisms are more evident than before, the interpretation of such sequences is not necessarily straightforward. Widespread polymorphisms in ITS across the dombeyoids sampled here indicate that the mutation rate of the nrDNA gene family outpaces molecular drive (Dover 1982, 1989), but what is critical to the use of these gene sequences in phylogenetics is the tempo of mutation and concerted evolution compared to cladogenesis (or speciation) (Sanderson & Doyle, 1992; Alvarez & Wendel, 2003; Nieto Feliner & Rosselló, 2007).

Determination of whether polymorphisms represent intra-individual or intraspecific ITS paralogs irrelevant to phylogenetic inference, or whether they cross species boundaries and could therefore confound phylogenetic analysis, can only be achieved via phylogenetic analysis (Bailey & al., 2003). The polymorphisms seen in *Dombeya* and its near relatives showed both patterns of paralogy (Fig. 2.2). "Shallow" paralogy (that of individual or intraspecific paralogs, Bailey & al., 2003) was evident for the majority of individuals and/or species exhibiting ITS polymorphisms in this study and will be discussed no further. One instance of "deep" paralogy (that of interspecific paralogs, Bailey & al., 2003) may exist between two species within *D.* subsect. *Macranthae*. Only one point mutation supports this interspecific clade, making it difficult to interpret whether it is a chance parallel mutation occurring independently in each species or an actual instance of ribotype diversity shared across species boundaries. Either way, a close relationship between these two species is obvious from morphology and no inferences of relationships among the species of the *D.* subsect. *Macranthae* clade affect the arguments presented here. Of greater concern and difficult to interpret were the four interspecific polytomies (labeled 1-4 in Fig. 2.2) in which ITS haplotypes from one individual were as closely related to sister species as they were to other ITS haplotypes from the same individual and genome. These polytomies reflect both conflicting characters, in some cases intragenomic polymorphisms, as well as a simple lack of information, i.e., insufficient molecular variation in ITS, to differentiate some of these taxa.

Multiple processes could have created the conflicting suites of characters seen in ITS. Incomplete lineage sorting is one possibility and is all the more likely given the widespread extant intraindividual ribotype diversity observed. A second possibility is chance parallel mutations occurring in genetically isolated species forming pseudosynapomorphies (Doyle & Davis, 1998). These are a common enough occurrence with little remedy in any dataset, but of particular note here given how many clades within the major *Dombeya* clade are supported by single characters. Hybridization is another possibility, and one that is further complicated by the likelihood of mutations and concerted evolution within the hybrid lineage creating synapomorphies across what were once phylogenetically divergent ribotypes inherited from the progenitors of the hybrid line. The one-by-one analyses of haplotypes (including clones) with the "non-polymorphic" ITS sequences should have mitigated complications caused by phylogenetic grouping of intra-individual ribotype synapomorphies. These analyses should also have shown evidence of past reticulation events, if they existed, by the placement of different haplotypes from one individual in multiple, particular positions in the ITS topology.

The meaning and usefulness of the method proposed here for analyzing haplotypes depends on both the extent of sampling of the non-polymorphic subset of taxa and the reliability of the topology retrieved with just these taxa. If the non-polymorphic subset of taxa only included individuals from one or two clades from the full ITS topology, these analyses would be of little use. Fortuitously, the non-polymorphic subset of taxa for these dombeyoids included representatives of each clade found in each permutation of the ITS analyses. The second point concerns the potential for lineage sorting. If, for example, all taxa were derived from an ancestral population with a suite of ITS ribotypes, and the non-polymorphic taxa simply represented those species that had fixed to one of these ancestral ribotypes (or a derivation of one) sooner than the polymorphic taxa, the analysis of the non-polymorphic taxa will not reflect evolutionary history. Given general congruence between ITS and the chloroplast within the major *Dombeya* clade, this latter scenario seems unlikely.

With these caveats, the one-by-one haplotype analyses helped determine potential underlying causes of the polymorphisms and pinpointed particular taxa that were creating the polytomies found in the analysis of the non-polymorphic subset of taxa with all the constructed haplotypes. Indeed, the five "moving" accessions that continued to occupy two disparate topological positions in the last round of one-by-one haplotype analyses were all accessions that sat within the polytomies in the analysis of all haplotypes of ITS (Fig. 2.2, taxon names circled).

Of these "moving" accessions, the easiest to interpret was the textbook example of a reticulate history shown by the known hybrid, *D.* × *cayeuxii*. Some clones and haplotypes constructed from polymorphic direct sequences of *D.* × *cayeuxii* were placed close to one (*D. burgessiae*) or the other progenitor (*D. wallichii*) in the ITS topology, as would be expected from an additive dataset (McDade, 1990, 1992). Other haplotypes or clones sat basal to either progenitor as would be expected for a non-additive dataset capable of showing intermediacy, e.g., a recombinant DNA sequence (Wendel, 1995) or a morphological character (McDade, 1992). The presence of both additive and non-additive patterns are not surprising because concerted evolution is still in progress. These same two hybrid individuals of *D.* × *cayeuxii* grouped with the maternal progenitor of the cross (*D. burgessiae*; André, 1897) in the chloroplast phylogeny. The ITS polymorphisms seen in *D.* × *cayeuxii* exceed the number of polymorphisms in the other sequences and show a nearly additive pattern (as detailed in Results) to the ITS sequences of either progenitor. The time frame since the creation of *D.* × *cayeuxii*, about one century, is similar to that of the natural hybrid *Tragopogon mirus* that also shows additivity of nrDNA data, in the form of restriction sites, from either progenitor (Soltis & Soltis, 1991). The example of *D.* × *cayeuxii* differs markedly from plant groups in which hybrids, artificial or natural, have shown complete concerted evolution (e.g., Chase & al., 2003), sometimes remarkably swiftly, such as the hybrid of two species of *Armeria* that had already homogenized to one parental ribotype in only two generations (Fuertes Aguilar & al., 1999).

Interpretation of polymorphisms in the final "moving" accessions other than *D.* × *cayeuxii* is more complex. *Dombeya populnea* will not be discussed because it was not in a polytomy in the analysis of all ITS sequences including haplotypes and both of its haplotypes were sister to one another in the final ITS phylogeny. In contrast, *Dombeya laurifolia*, *D. montana*, *D. sahatavyensis* and *D. urschiana* created two of the polytomies (Fig. 2.2, numbered 1 and 2) seen in analysis of all ITS sequences including haplotypes; each had haplotypes that occupied two positions in the final ITS phylogeny. Comparison of the position of these individuals in the chloroplast topology against their positions in the ITS topology reveals little about the cause of their polymorphisms in ITS.

None of these individuals show morphological intermediacy to the two clades to which they are closest in the ITS tree, except for one interesting character in *D. laurifolia.* In the ITS topology, one or the other haplotype of *D. laurifolia* sat next to two clades of Malagasy species of *Dombeya* that differ by their carpel number: 2-3 versus 4-5 carpels. The species *D. laurifolia* usually has three carpels, but sometimes has four or five (Hochreutiner, 1926; Arènes, 1959; pers. obs.). This characteristic is not unique to *D. laurifolia* and has been reported for other species of *Dombeya* (e.g., *D. longicuspis*, Hochreutiner, 1926; *D. spectabilis* and even *D. sahatavyensis*, Arènes, 1959; *D. mandenensis*, pers. obs.), but it is notable as an "intermediate" morphological characteristic given its positions in the ITS phylogeny. Yet, it still seems doubtful that *D. laurifolia* is of hybrid origin, given its uninformative position in the chloroplast tree and the fact that its ITS haplotypes differ by one base pair. The evaluation of *D. montana*, *D. sahatavyensis* and *D. urschiana* for morphological intermediacy is more difficult because ITS posits relationships of their haplotypes to very morphologically similar clades (or to one clade, as is the case for *D. sahatavyensis*).

It seems most likely that the two positions of *D. laurifolia*, *D. montana*, *D. sahatavyensis* and *D. urschiana* in the ITS phylogeny result either from pseudosynapomorphies, particularly considering each haplotype within an individual differs from the other by only one base pair, or from retention in these individuals of the ribotype diversity of a common ancestor which was lost to concerted evolution in other lineages.

Regardless of the reason for the multiple phylogenetic relationships seen in ITS for some of the polymorphic accessions, from a phylogenetic perspective the pattern of overall relationships within these ITS sequences is evidence of a disconcerting lack of concordance between the tempos of cladogenesis and concerted evolution within these ribotypes. A similarly complex relationship of polymorphic ITS haplotypes was seen in a molecular phylogeny including the few species of *Aponogeton* (Aponogetonaceae) endemic to Madagascar and hypotheses of their hybrid or polyploid origin were suggested but not further investigated (Les & al., 2005). The frequent intragenomic variability coupled with low intergenomic variability of ITS in these dombeyoids echoes that found in a study of ITS and ETS sequences of a malvoid relative, *Sidalcea* (Malvaceae; Andreasen & Baldwin, 2003), wherein the polymorphisms were attributed to hybridization and gene flow within young clades, further corroborated by overlapping distributions and morphology as well as low molecular variability. All of these conditions are reminiscent of the situation seen in *Dombeya*, and the causalities may equally be similar. Shared polymorphisms across clades may indicate the recent divergence of these species from one another. Further evaluation of the phylogenetic utility of these ITS sequences made in light of other independent datasets, such as from other nuclear genes, is desirable.

Incongruence between chloroplast & ITS. — Incongruence between chloroplast and ITS datasets is shown in the relationships between the four major groups of taxa found in these phylogenies (Fig. 2.3): i) major Mascarene group, ii) major *Dombeya* clade, iii) taxa with winged seeds, and iv) *D*. subsect *Macranthae*. Differing placements of *D. superba*, *D. gautieri* and *D. mandenensis* contribute to the incongruence, which may also include the genus *Trochetiopsis*. Although placed consistently as an outgroup by parsimony analysis, *Trochetiopsis* was poorly supported as such and Bayesian analysis placed it sister to a clade of *Dombeya* subsect. *Macranthae* plus the major Mascarene clade in an analysis of the chloroplast data (Fig. 2.3A). The chloroplast tree also showed a sister relationship between *Dombeya* subsect. *Macranthae* and the major Mascarene clade, and placed all the taxa with winged seeds among the early diverging lineages of *Dombeya s. str.* ITS (Fig. 2.3B) formed a clade of the winged seed taxa, placed them sister to *D.* subsect. *Macranthae* and left the major Mascarene clade in a polytomy with the early diverging lineages of *Dombeya s. str.* Few of these relationships in either topology were well supported.

Neither conflation of orthology and paralogy nor ancient bouts of chloroplast introgression between ancestors to these four clades of dombeyoids can be ruled out as potential explanations for the incongruence, particularly given the numerous paralogs evident in the ITS dataset and the plastid introgression observed among closely related extant accessions. Nonetheless, lineage sorting seems the most likely explanation for the incongruent patterns observed. The few characters supporting the alternative relationships among these four groups (and possibly *Trochetiopsis*) may indicate that the time frame in which these clades diverged from one another was short. Alleles are less likely to come to fixation during short intervals of evolutionary time (Pamilo & Nei, 1988), i.e., at short internodes on a phylogenetic tree. Lack of fixation of alleles between events of cladogenesis increase the chances of incomplete lineage sorting and potentially conflicting phylogenies across markers (Maddison & Knowles, 2006; e.g., Poe & Chubb, 2004). More molecular data could help determine the relationships among these major clades, but such data could just as easily suggest further alternative scenarios of relationships. Preliminary data from putatively single copy nuclear regions indicate alternative scenarios of relationships than those presented by markers sampled here for these same taxa (Skema, unpubl. data).

CONCLUSIONS

Low molecular variation within both the chloroplast and ITS in *Dombeya* is comparable to other taxa of similar distribution and other island endemics, yet all these taxa possess far fewer species than *Dombeya*. The lack of molecular divergence may suggest that clades within *Dombeya* are relatively young. Plastid introgression within three broad geographic regions in Madagascar (north, central-east, and south) is evident in some species of *Dombeya*. Despite the interfertility of phylogenetically distant species of *Dombeya*, inferred plastid introgression in the wild seems limited to closely related species. Shared polymorphisms in ITS may likewise indicate gene flow across species, or possibly young clades in which fixation of ribotypes is still in progress. *Dombeya* × *cayeuxii*, a known hybrid from cultivation, exhibited ITS ribotypes of both parents and the plastid haplotype of its maternal parent. Incongruence between plastid and ITS phylogenies between four major groups of dombeyoids may indicate that these clades diverged from one another within a short time frame. These results underscore a need for evaluation of both chloroplast and nuclear markers before inferences of species phylogenies, speciation processes and diversification rates are made from sequence data. *Dombeya* could be an ideal system in which to study the speciation processes that have generated Madagascar's diverse flora because of it high levels of microendemism and the wide distribution of its species across the complex mosaic of habitats on Madagascar and all the phytogeographical domains (Humbert, 1965). Yet, such questions can not be approached until after the development of a robust understanding of the molecular evolution and systematics of this genus in a phylogenetic framework.

LITERATURE CITED

Akaike, H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19: 716-723.

Alvarez, I. & Wendel, J. F. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* 29: 417-434.

Alverson, W. S., Whitlock, B. A., Nyffeler, R., Bayer, C. & Baum, D. A. 1999. Phylogeny of the core Malvales: Evidence from *ndhF* sequence data. *American Journal of Botany* 86: 1474-1486.

André, E. 1897. *Dombeya cayeuxii*. *Revue Horticole* 69: 544-545.

Andreasen, K. & Baldwin, B. B. 2003. Nuclear ribosomal DNA sequence polymorphism and hybridization in checker mallows (*Sidalcea*, Malvaceae). *Molecular Phylogenetics and Evolution* 29: 563-581.

Arènes, J. 1959. Flore de Madagascar et des Comores, Sterculiacées. Firmin-Didot, Paris.

Bailey, C. D., Carr, T. G., Harris, S. A. & Hughes, C. E. 2003. Characterization of angiosperm nrDNA polymorphism, paralogy and pseudogenes. *Molecular Phylogenetics and Evolution* 29: 435-455.

Baldwin, B. G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: An example from the Compositae. *Molecular Phylogenetics and Evolution* 1(1): 3-16.

Baldwin, B. G., Sanderson, M. J., Porter, J. M., Wojciechowski, M. F., Campbell, C. S. & Donoghue, M. J. 1995. The ITS regions of nuclear ribosomal DNA: A valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 247-277.

Barriel, V. 1994. Phylogénies moléculaires et insertions-délétions de nucléotides. *C. R. Acad. Sci. Paris, Sciences de la vie* 317: 693-701.

Baum, D. A., Small, R. L. & Wendel, J. F. 1998. Biogeography and floral evolution of baobabs (*Adansonia*, Bombacaceae) as inferred from multiple data sets. *Systematic Biology* 47: 181-207.

Bayer, C., Fay, M. F., de Bruijn, A. Y., Savolainen, V., Morton, C. M., Kubitzki, K., Alverson, W. S. & Chase, M. W. 1999. Support for an expanded family concept of Malvaceae within a recircumscribed order Malvales: A combined analysis of plastid *atpB* and *rbcL* DNA sequences. *Botanical Journal of the Linnean Society* 129: 267-303.

Bouck, A., Wessler, S. R. & Arnold, M. L. 2007. QTL analysis of floral traits in Louisiana iris hybrids. *Evolution* 61: 2308-2319.

Bradshaw Jr., H. D., Otto, K. G., Frewen, B. E., McKay, J. K. & Schemske, D. W. 1998. Quantitative trait loci affecting differences in floral morphology between two species of monkeyflower (*Mimulus*). *Genetics* 149: 367-382.

Campbell, C. S., Wojciechowski, M. F., Baldwin, B. B., Alice, L. A. & Donoghue, M. J. 1997. Persistent nuclear ribosomal DNA sequence polymorphism in the *Amelanchier* agamic complex (Rosaceae). *Molecular Biology and Evolution* 14: 81-90.

Chase, M. W., Knapp, S., Cox, A. V., Clarkson, J. J., Butsko, Y., Joseph, J., Savolainen, V. & Parokonny, A. S. 2003. Molecular systematics, GISH and the origin of hybrid taxa in *Nicotiana* (Solanaceae). *Annals of Botany* 92: 107-127.

Curtu, A. L., Gailing, O., Leinemann, L. & Finkeldey, R. 2007. Genetic variation and differentiation within a natural community of five oak species (*Quercus* spp.). *Plant Biology* 9: 116-126.

Doebley, J. 2004. The genetics of maize evolution. *Annual Review of Genetics* 38: 37-59.

Dover, G. 1982. Molecular drive: A cohesive mode of species evolution. *Nature* 299: 111-117.

Dover, G. 1989. Linkage disequilibrium and molecular drive in the rDNA gene family. *Genetics* 122: 249-252.

Doyle, J. J. 1992. Gene trees and species trees: Molecular systematics as one-character taxonomy. *Systematic Botany* 17: 144-163.

Doyle J. J. & Davis, J. I. 1998. Homology in molecular phylogenetics: A parsimony perspective. Pp. 101-131 in Soltis, D. E., Soltis, P. S. & Doyle, J. J. (eds.), *Molecular Plant Systematics II: DNA Sequencing.* Kluwer Academic, Norwell, MA, USA.

Flagel, L. E., Rapp, R. A., Grover, C. E., Widrlechner, M. P., Hawkins, J., Grafenberg, J. L., Álvarez, I., Young Chung, G. & Wendel, J. F. 2008. Phylogenetic, morphological and chemotaxonomic incongruence in the North American endemic genus *Echinacea*. *American Journal of Botany* 95: 756-765.

Fuertes Aguilar, J., Rosselló, J. A. & Nieto Feliner, G. 1999. Nuclear ribosomal DNA (nrDNA) concerted evolution in natural and artificial hybrids of *Armeria* (Plumbaginaceae). *Molecular Ecology* 8: 1341-1346.

Gautier L. & Goodman, S. M. 2003. Introduction to the flora of madagascar. Pp. 229-250 in Goodman, S. M. and Benstead, J. P. (eds.), *The Natural History of Madagascar.* The University of Chicago Press, Chicago.

Goloboff, P. A., Farris, J. S. & Nixon, K. C. 2008. TNT, a free program for phylogenetic analysis. *Cladistics* 24: 774-786.

Harpke, D. & Peterson, A. 2006. Non-concerted ITS evolution in *Mammillaria* (Cactaceae). *Molecular Phylogenetics and Evolution* 41: 579-593.

Heuertz, M., Carnevale, S., Fineschi, S., Sebastiani, F., Hausman, J. F., Paule, L. & Vendramin, G. G. 2006. Chloroplast DNA phylogeography of European ashes, *Fraxinus* spp. (Oleaceae): Roles of hybridization and life history traits. *Molecular Ecology* 15: 2131-2140.

Hochreutiner, B. P. G. 1926. Monographie des *Dombeya* de Madagascar. *Candollea* III: 5-120.

Humbert H. 1965. Description des types de végétation. Pp. 46-78 in Humbert, H. and Cours Darne, G. (eds.), *Notice de la carte. Madagascar.* Travaux de la Section Scientifique et Technique de l'Institut Français de Pondichérry.

Kim, K. & Jansen, R. K. 1994. Comparisons of phylogenetic hypotheses among different data sets in dwarf dandelions (*Krigia*, Asteraceae): additional information from internal transcribed spacer sequences of nuclear ribosomal DNA. *Plant Systematics and Evolution* 190: 157-185.

Le Péchon, T. 2009. Systématique des Dombeyoideae (Malvaceae, ex-Sterculiaceae) des Mascareignes: Approches morphologique et moleculaire. Ph.D. thesis, l'Université Pierre et Marie Curie, Paris, France.

Le Péchon, T., Cao, N., Dubuisson, J. & Gigord, L. D. B. 2009. Systematics of Dombeyoideae (Malvaceae) in the Mascarene archipelago (Indian Ocean) inferred from morphology. *Taxon* 58: 519-531.

Les, D. H., Moody, M. L. & Jacobs, S. W. L. 2005. Phylogeny and systematics of *Aponogeton* (Aponogetonaceae): The Australian species. *Systematic Botany* 30: 503-519.

Little, D. P. 2006. Evolution and circumscription of the true cypresses (Cupressaceae: *Cupressus*). *Systematic Botany* 31: 461-480.

Maddison, W. P. & Knowles, L. L. Inferring phylogeny despite incomplete lineage sorting. *Systematic Biology* 55: 21-30.

Malcomber, S. T. 2002. Phylogeny of *Gaertnera* Lam. (Rubiaceae) based on multiple DNA markers: Evidence of a rapid radiation in a widespread, morphologically diverse genus. *Evolution* 56: 42-57.

Maurin, O., Davis, A. P., Chester, M., Mvungi, E. F., Jaufeerally-Fakim, Y. & Fay, M. F. 2007. Towards a phylogeny for *Coffea* (Rubiaceae): Identifying well-supported lineages based on nuclear and plastid DNA sequences. *Annals of Botany* 100: 1565-1583.

McDade, L. 1990. Hybrids and phylogenetic systematics I: Patterns of character expression in hybrids and their implications for cladistic analysis. *Evolution* 44: 1685-1700.

McDade, L. 1992. Hybrids and phylogenetic systematics II: The impact of hybrids on cladistic analysis. *Evolution* 46: 1329-1346.

Nieto Feliner, G. & Rosselló, J. A. 2007. Better the devil you know? Guidelines for insightful utilization of nrDNA ITS in species-level evolutionary studies in plants. *Molecular Phylogenetics and Evolution* 44: 911-919.

Nixon, K. C. 2002. WinClada version 1.86. Published by the author, Ithaca, NY, USA.

Nyffeler, R., Bayer, C., Alverson, W. S., Yen, A., Whitlock, B. A., Chase, M. W. & Baum, D. A. 2005. Phylogenetic analysis of the Malvadendrina clade (Malvaceae s.l.) based on plastid DNA sequences. *Organisms, Diversity and Evolution* 5: 109-123.

Nylander, J. A. A. 2004. MrModeltest version 2. Program distributed by the author, Evolutionary Biology Centre, Uppsala University.

Pamilo, P. & Nei, M. 1988. Relationships between gene trees and species trees. *Molecular Biology and Evolution* 5: 568-583.

Permingeat, H. R., Romagnoli, M. V., Sesma, J. I. & Vallejos, R. H. 1998. A simple method for isolating DNA of high yield and quality from cotton (*Gossypium hirsutum* L.) leaves. *Plant Molecular Biology Reporter* 16: 89.

Poe, S. & Chubb, A. L. 2004. Birds in a bush: Five genes indicate explosive evolution of avian orders. *Evolution* 58: 404-415.

Posada, D. & Crandall, K. A. 1998. Modeltest: Testing the model of DNA substitution. *Bioinformatics* 14: 817-818.

Rambaut, A. & Drummond, A. J. 2009. Tracer version 1.5: MCMC trace analysis package. Published by the author, Edinburgh, Scotland.

Rauscher, J. T., Doyle, J. J. & Brown, A. H. D. 2002. Internal transcribed spacer repeat-specific primers and the analysis of hybridization in the *Glycine* *tomentella* (Leguminosae) polyploid complex. *Molecular Ecology* 11: 2691-2702.

Razafimandimbison, S. G., Kellogg, E. A. & Bremer, B. 2004. Recent origin and phylogenetic utility of divergent ITS putative pseudogenes: A case study from Naucleeae (Rubiaceae). *Systematic Biology* 53: 177-192.

Ree, R. 2008. Gapcode.py, a python script for coding indels in sequence alignments as binary characters for phylogeny reconstruction. Program distributed by author, http://www.reelab.net/home/software.

Ronquist, F. & Huelsenbeck, J. P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.

Rosselló, J. A., Cosín, R., Boscaiu, M., Vicente, O., Martínez, I. & Soriano, P. 2006. Intragenomic diversity and phylogenetic systematics of wild rosemaries (*Rosmarinus officinalis* L. s.l., Lamiaceae) assessed by nuclear ribosomal DNA sequences (ITS). *Plant Systematics and Evolution* 262: 1-12.

Rosselló, J. A., Lázaro, A., Cosín, R. & Molins, A. 2007. A phylogeographic split in *Buxus balearica* (Buxaceae) as evidence by nuclear ribosomal markers: When ITS paralogs are welcome. *Journal of Molecular Evolution* 64: 143-157.

Sanderson, M. J. & Doyle, J. J. 1992. Reconstruction of organismal and gene phylogenies from data on multigene families: Concerted evolution, homoplasy and confidence. *Systematic Biology* 41: 4-17.

Sang, T., Crawford, D. J., Kim, S. & Stuessy, T. F. 1994. Radiation of the endemic genus *Dendroseris* (Asteraceae) on the Juan Fernandez Islands: Evidence from sequences of the ITS regions of nuclear ribosomal DNA. *American Journal of Botany* 81: 1494-1501.

Sang, T., Crawford, D. J., Stuessy, T. F. & Silva O., M. 1995a. ITS Sequences and the phylogeny of the genus *Robinsonia* (Asteraceae). *Systematic Botany* 20: 55-64.

Sang, T., Crawford, D. J. & Stuessy, T. F. 1995b. Documentation of reticulate evolution in peonies (*Paeonia*) using internal transcribed spacer sequences of nuclear ribosomal DNA: Implications for biogeography and concerted evolution. *Proceedings of the National Academy of Sciences, U.S.A.* 92: 6813-6817.

Shaw, J., Lickey, E. B., Beck, J. T., Farmer, S. B., Liu, W. S., Miller, J., Siripun, K. C., Winder, C. T., Schilling, E. E. & Small, R. L. 2005. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* 92: 142-166.

Shaw, J., Lickey, E. B., Schilling, E. E. & Small, R. L. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. *American Journal of Botany* 94: 275-288.

Shi, S., Qiu, Y., Li, E., Wu, L. & Fu, C. 2006. Phylogenetic relationships and possible hybrid origin of *Lycoris* species (Amaryllidaceae) revealed by ITS sequences. *Biochemical Genetics* 44: 198-208.

Simmons, M. P. & Ochoterena, H. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369-381.

Soltis, P. S. & Soltis, D. E. 1991. Multiple origins of the allotetraploid *Tragopogon mirus* (Compositae): rDNA evidence. *Systematic Botany* 16: 407-413.

Swofford, D. L. 2001. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0. Sinauer, Sunderland, MA, USA.

Wendel, J. F. 1995. An unusual ribosomal DNA sequence from *Gossypium gossypioides* reveals ancient, cryptic, intergenomic introgression. *Molecular Phylogenetics and Evolution* 4: 298-313.

Wendel J. F. & Doyle, J. J. 1998. Phylogenetic incongruence: Window into genome history and molecular evolution. Pp. 265-296 in Soltis, D. E., Soltis, P. S. & Doyle, J. J. (eds.), *Molecular Systematics of Plants II: DNA Sequencing.* Kluwer Academic, Norwell, MA, USA.

White T. J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322 in Innis, M., Gelfand, D., Sninsky, J. & White, T. (eds.), *PCR Protocols: A Guide to Methods and Applications.* Academic Press, San Diego.

Won, H. 2009. Phylogenetic position of *Corchoropsis* Siebold & Zucc. (Malvaceae *s.l.*) inferred from plastid DNA sequences. *Journal of Plant Biology* 52: 411-416.

Wurdack, K. J., Hoffmann, P., Samuel, R., de Bruijn, A., van der Bank, M. & Chase, M. W. 2004. Molecular phylogenetic analysis of Phyllanthacaeae (Phyllanthoideae *pro parte*, Euphorbiaceae *sensu lato*) using plastid *rbcL* DNA sequences. *American Journal of Botany* 91: 1882-1900.

CHAPTER 3

SEPARATING *DOMBEYA* (DOMBEYACEAE) OF MADAGASCAR FROM THE DOMBEYOIDS: MORPHOLOGICAL EVALUATION OF A MOLECULAR PHYLOGENY AND A NEW SEGREGATE GENUS, *ANDRINGITRA*

INTRODUCTION

Dombeya Cav., *nom. cons.* (Dombeyaceae or Dombeyoideae, Malvaceae *s.l.* or Sterculiaceae *pro parte*) is a paleotropical genus of approximately 210 species. Nineteen species are in continental Africa (one extends onto the Arabian peninsula), fifteen in the Mascarenes, and approximately 180 on the island of Madagascar; all but one, *D. acutangula sensu* Seyani, are endemic to each geographical area (Arènes, 1959; Friedmann, 1987; Seyani, 1991). *Dombeya* represents a spectacular example of the remarkable diversity of the megaflora of Madagascar where evolution has largely followed its own amazing trajectory. With so many species endemic to Madagascar, *Dombeya* is one of the big plant genera of the island, comprising about 2% of its flora.

The species of *Dombeya* from Madagascar have not been treated as a whole since the completion of the volume on Sterculiaceae for the *Flore de Madagascar et des Comores* (Arènes, 1959) and the genus is in need of revision. The flora treatment preceded a major influx of plant collections from the island (Gautier & Goodman, 2003) and many recent specimens remain unstudied. Specialists have found the current infrageneric taxonomy inadequate for the Malagasy species (e.g., Barnett & Dorr, 1986; Applequist, 2009a) and other workers have found it equally problematic for the African (Seyani, 1991) and Mascarene species (Friedmann, 1987; Le Péchon &

al., 2009). *Dombeya* and other large genera remain as taxonomic hurdles in understanding the flora of Madagascar. This paper is a taxonomic evaluation of molecular phylogenetic work centered around the *Dombeya* of Madagascar, sampling throughout the diversity of the genus and its near relatives.

Cavanilles (1786, 1787) described *Dombeya*, and the two principal enumerations of species within the genus were completed much later by Hochreutiner (1926) and Arènes (1958). Friedmann (1987) revised the species of *Dombeya* from the Mascarenes and their near relatives, the endemic genera *Astiria* (presumably extinct), *Ruizia* and *Trochetia*. Shortly thereafter Seyani (1991) delimited nineteen species from the over 120 names previously used for the *Dombeya* of Africa. More recent taxonomic work included the description of a few new species in *Dombeya* (Barnett & Dorr, 1986; Applequist, 2009a; Skema & Dorr, in press; Le Péchon, in prep.), but for some time *Dombeya* taxonomy centered mainly around removing particular species from the genus upon discovering that they had winged seeds and should be placed in *Helmiopsis* or *Helmiopsiella* (Barnett, 1988a; Dorr, 2001). The need for fruits of these species for identification to genus attests to the similarity of their floral morphology. Indeed, a number of genera in Dombeyaceae conform to one general floral plan: epicalyx present, calyx and corolla pentamerous, androecium fused in a tube from which arise stamens alternating with five staminodes, and a syncarpous gynoecium that develops as a capsular fruit.

The morphological characters that distinguish dombeyoid genera relevant to this study are as follows (summarized in Table 3.1). As currently circumscribed, *Dombeya* can be differentiated from other dombeyoids by a scarious perianth that persists beneath fruits and the presence of five staminodes in the androecium (pers. obs.; Arènes, 1959; Bayer & Kubitzki, 2003). *Ruizia* and *Astiria* have a similarly persistent perianth, but both lack staminodes. *Ruizia* has ten carpels and free styles (Cavanilles,

Table 3.1. Comparison of key morphological features of *Dombeya* and related genera.[1]

Genus	number of epicalyx bracts	internal face of calyx	corolla	number of staminodes, carpels	fruit	seeds
Dombeya	3	glabrous, usu. 1 glandular patch[2]	persistent*	5, 2-5	globose capsule	wingless
Astiria	3	glabrous, 1 glandular patch	persistent	0*, 5	globose capsule	wingless
Ruizia	3	glabrous, 1 glandular patch	persistent	0*, 10*	indehiscent 10-parted "capsule"	wingless
Trochetia	1* (spathiform)	glabrous, 1 glandular patch	caducous	5, 5	globose capsule	wingless, rarely rudimentarily winged
Trochetiopsis	3	± pubescent*	persistent	5, 5	globose capsule	wingless
Eriolaena	3	pubescent, 2* glandular patches	caducous	0*, 4-10	ovoid-conical capsule*	apically winged*
Helmiopsis	3	glabrous, usu. 1 glandular patch	caducous	5, (3 or) 5	ovoid-conical capsule*	apically winged*
Helmiopsiella	3	glabrous	caducous	5, 5-10	ovoid-conical capsule*	apically winged*

[1] Characteristics scored from observation of herbarium specimens or from the literature (Friedmann, 1987; Barnett, 1988a; Applequist, 2009b). [2] Glandular tissue on the internal face of the calyx occurs in one or two patches of papillae at the base of each sepal, and has been observed to be nectariferous in most species. * Marks a character that helps distinguish the genus.

1786; Lindley, 1844) unlike *Astiria* and many *Dombeya* which have five carpels and a single, apically divided style. *Trochetia* bears an early caducous and spathiform epicalyx of one piece (Cordemoy, 1895; Friedmann, 1987), by which it is clearly separated from *Dombeya* (and most Dombeyaceae) which have three bracts for an epicalyx. *Trochetiopsis* was segregated from *Trochetia* because it has a 3-parted epicalyx (rather than 1-parted), a persistent perianth (rather than caducous) and 5-10 stamens (rather than 15) (Marais, 1981). Pubescence on the internal face of the sepals of *Trochetiopsis* (Marais, 1981) differentiates it from the internally glabrous sepals of *Dombeya*. *Helmiopsis* and *Helmiopsiella* stand apart from these other dombeyoids by their ovoid woody fruits and winged seeds. *Helmiopsiella* differs from *Helmiopsis* by having staminodes opposite the sepals, a lack of nectariferous tissue on the calyx or corolla and pubescence within the ovary (Arènes, 1956a). *Eriolaena* is described in detail below.

Molecular studies (Bayer & al., 1999; Alverson & al., 1999; Nyffeler & al., 2005) of the "core" Malvales (Bombacaceae, Malvaceae, Sterculiaceae and Tiliaceae, as traditionally recognized) have significantly advanced our understanding of the taxonomy of Dombeyaceae. These studies provided the current concept of the family, the core of which was the tribe Dombeyeae, a morphologically coherent group recognized in traditional taxonomy (e.g., Schumann, 1890; Edlin, 1935) and generally thought to include *Astiria*, *Cheirolaena*, *Corchoropsis*, *Dombeya*, *Harmsia*, *Melhania*, *Paradombeya*, *Paramelhania*, *Pentapetes*, *Ruizia*, *Trochetia* and *Trochetiopsis*. To these taxa were added genera previously placed in other tribes of the traditional Sterculiaceae (*Eriolaena* from Eriolaeneae; *Pterospermum* from Helictereae; *Helmiopsis* and *Helmiopsiella* from Helmiopsideae) or Tiliaceae (*Burretiodendron*, *Schoutenia*), as well as the variously placed *Nesogordonia* (e.g., Tiliaceae, Engler, 1907; Mansonieae in Buettneriaceae, Edlin, 1935). The use of chimeric terminals and (necessarily) incomplete taxon sampling left unanswered questions about numerous relationships in Dombeyaceae, but these analyses created an important first hypothesis of evolutionary relationships in the family. These phylogenies supported earlier arguments that removed *Nesogordonia* from the Helmiopsideae *sensu* Arènes (1959), due primarily to different derivations of the wings on their seeds, but nevertheless associated *Nesogordonia* both to *Pterospermum* and to Dombeyeae based on morphology and anatomy (Barnett, 1988b). Won (2009) recently confirmed the inclusion of *Corchoropsis* in the Dombeyaceae with molecular data, a genus whose inclusion was previously suggested from morphology (Takeda, 1912; Tang, 1992).

Current questions about phylogenetic relationships within Dombeyaceae largely surround the placement of the *Dombeya* of Madagascar. Recent morphological and molecular studies of the dombeyoids of the Mascarenes have shown that *Dombeya* is not monophyletic and includes within it the genera *Astiria*, *Helmiopsis*, *Trochetia*, and *Ruizia* (Le Péchon, 2009; Le Péchon & al., 2009). Yet, neither study adequately addressed the taxonomic elephant in the room, the *Dombeya* of Madagascar. *Dombeya* is easily the largest genus in Dombeyaceae, with *Melhania* second largest (60 species; 17% of the family), and the *Dombeya* from Madagascar alone make up the bulk (60%) of the species in the family. Given the variation seen in these species of *Dombeya* from Madagascar in indument, inflorescence structure, flower size, androecial length and fusion and number of stamens and carpels, the relationships of these Malagasy species to other dombeyoids are still in question and have the potential to change the currently held knowledge of evolutionary relationships and character evolution for the family.

Other remaining taxonomic puzzles within Dombeyaceae concern the placement of a few anomalous taxa, such as *Eriolaena* and *Dombeya* subsect. *Rigidae*. *Eriolaena* (tribe Eriolaeneae: e.g., de Candolle, 1822; Edlin, 1935) has many unique characters such as two nectariferous patches on the inner sepals, lack of staminodes, many anthers diverging from various points along the length of the androecial tube, a (sometimes) tetramerous perianth, and basally pubescent and clawed petals reflexing by an S-shaped bend back towards the sepals. The androecial characteristics and the (sometimes) highly divided epicalyx bracts inspired de Candolle (1822) to describe *Eriolaena* as an evolutionary link between Sterculiaceae and Malvaceae *s. str*. Despite the unique characteristics of *Eriolaena*, evidence from androecial morphology (van Heel, 1966), embryology (Tang, 2009), inflorescence structure (Bayer, 1999), pollen morphology (Erdtman, 1952) and wood anatomy (Chattaway, 1932; Barnett, 1988b) suggests it has a general affinity to the tribe Dombeyeae. Perrier de la Bâthie (1944) argued for its close relationship to *Pterospermum* and *Helmiopsis*; Barnett (1987a, 1988b) associated it with *Helmiopsiella* and *Helmiopsis* (the only two members of the tribe Helmiopsideae) primarily based on characteristics of the winged seeds. Barnett (1987a) further proposed including both Helmiopsideae and Eriolaeneae within Dombeyeae, due to similarities of the cotyledons, flowers, seeds and wood anatomy. Although shown to be sister to *Helmiopsiella* and one chimeric taxon from *Dombeya* in a molecular phylogeny (Won, 2009), the exact phylogenetic relationship of *Eriolaena* to other dombeyoids remains unclear.

Dombeya subsect. *Rigidae* was erected by Arènes (in *D.* sect. *Capricornua*; 1958) to accommodate *D. linearifolia* and *D. rigida*, the only two of the 187 species of *Dombeya* he treated that have glands on the lower half of their petals. In 2001, Dorr discovered that *D. rigida* had a short, marginal wing on its seed and transferred it to *Helmiopsis*. Applequist (2009b), in a recent revision of *Helmiopsis,* rejected Dorr's taxonomy on the grounds that the narrow marginal seed wing of *D. rigida* and the long apical seed wing of *Helmiopsis* are not necessarily homologous, and left these taxa in

Dombeya. The phylogenetic relationships and best taxonomic placement of these two unusual species is still uncertain.

The most recent infrageneric treatment for the Malagasy species of *Dombeya* was that of Arènes (1958, 1959), and the key features of his delimitations to section are summarized in Table 3.2. The first infrageneric division, into subgenera, is based on carpel number: 2-3 carpellate species in *D.* subg. *Xeropetalum*; (4-)5 carpellate species in *D.* subg. *Dombeya* (based on Schumann, 1900). Each subgenus contains a section of species with scorpioid cymes (sections *Paracapricornua* and *Capricornua*). Within *D.* subg. *Xeropetalum* Arènes further divided on inflorescence type with umbels in *D.* sect. *Decastemon* and cymes in *D.* sect. *Xeropetalum*. Within *D.* subg. *Dombeya*, all

Table 3.2. Morphological characteristics employed by Arènes (1958, 1959) in the latest treatment[1] of the infrageneric taxonomy of *Dombeya*.

Subgenera		**Sections**	
Xeropetalum	flowers 2-3-carpellate (sometimes 4 or even 5 carpels)	*Paracapricornua*	scorpioid cymes
		Decastemon	typical umbels
		Xeropetalum	umbellate, corymbose or paniculate cymes
Dombeya	flowers 5-carpellate (sometimes 3 or 4 carpels)	*Capricornua*	scorpioid cymes
		Trochetiantha	solitary flowers
		Astrapaea	long staminal tube
		Paracheirolaena [2]	epicalyx bracts pinnately lobed
		Dombeya	lacks above diagnostic characters

[1] This treatment is only presented here to the rank of section. [2] The sole species of sect. *Paracheirolaena*, *D. ctenostegia*, has since been transferred to *Helmiopsiella* (Barnett, 1988a).

solitary-flowered species were put in *D.* sect. *Trochetiantha* (based on Baillon, 1885). The two sections *D.* sect. *Astrapaea* and *D*. sect. *Paracheirolaena* were diagnosed by strikingly long staminal tubes and pinnately lobed epicalyx bracts, respectively. *D*. sect. *Dombeya*, with the greatest number of species in the subgenus, included various inflorescence types (umbellate cymes, typical umbels) but was only united by its lack of the diagnostic characteristics of the other sections in the subgenus. Infrageneric characters based on inflorescence structure and others utilizing the shape of the androecial tube (not summarized here) were highly homoplasious on a phylogeny of dombeyoids of the Mascarenes, except for a clade of taxa with scorpioid cymes (Le Péchon, 2009).

With particular emphasis on sampling *Dombeya* from Madagascar, this study aimed to i) evaluate the generic delimitation and infrageneric taxonomy of *Dombeya*, ii) resolve the relationships of *Eriolaena* and *Dombeya* subsect. *Rigidae* to other Dombeyaceae, and iii) synthesize the results of the molecular phylogeny with morphology and begin the taxonomic revision of the *Dombeya* of Madagascar where required.

MATERIALS & METHODS

Sampling & Laboratory Methods. — Sequences were obtained for 87 accessions of 68 species of 10 genera in the Dombeyaceae with a focus on species of *Dombeya* from Madagascar. *Nesogordonia*, *Corchoropsis* and *Pentapetes* were chosen as outgroups because they are morphologically distinct from *Dombeya* and because previous molecular phylogenies place them outside *Dombeya* (Bayer & al., 1999; Alverson & al., 1999; Nyffeler & al., 2005; Won, 2009). The choice of ingroup taxa was based on a larger study of the dombeyoids with broader taxonomic sampling (Skema, in prep.). Samples of the following genera, for which multiple species were obtained when possible, were included: *Eriolaena*, *Helmiopsiella*, *Helmiopsis*, *Ruizia*, *Trochetia* and *Trochetiopsis*. Within *Dombeya,* 73 specimens were sampled representing 55 of the roughly 210 species in the genus; infrageneric representation included all of the eight sections and 17 of the 18 subsections as recognized by Arènes (1959). The only subsection not sampled was subsect. *Humbertianae*, which has one member, *D. humbertiana*. Table 3.3 lists accessions included in the study, voucher data and, where appropriate, their taxonomic classification following the last treatment of the *Dombeya* of Madagascar (Arènes, 1959).

Five chloroplast noncoding regions (intergenic spacers *trnCycf6*, *ycf6psbM*, *psbMtrnD* and *petLpsbE*, and the intron of *ndhA*; Shaw & al., 2005 and 2007) and the nuclear ribosomal region of the internal transcribed spacer 1, the gene 5.8S, and internal transcribed spacer 2 (hereafter called, in its entirety, ITS; Baum & al., 1998 and White & al., 1990) were sequenced. Details of taxon and marker sampling as well as methods for DNA extractions, amplification of markers, primers used and sequencing procedures can be found in Chapter 2.

Matrices & Phylogenetic Analysis. — Sequences were aligned by eye and insertion-deletion events (indels) were coded using the "gapcode" program v.2.1 (Ree, 2008) which implements a simple indel coding method (Barriel, 1994; Simmons & Ochoterena, 2000; further details in Chapter 2). All individual gene regions were analyzed both with and without indel coding. The ITS dataset showed many intra-individual polymorphisms and underwent further evaluation and analyses, as detailed in Chapter 2. The data from the five plastid markers were concatenated into a combined matrix and analyzed. Comparison of analyses of the plastid data versus ITS alone provided evidence for plastid introgression between closely related species. Both intraindividual polymorphism in ITS and plastid introgression among species are biological phenomena that must be evaluated (see Chapter 2), but neither

Table 3.3. Accessions included in study with voucher specimen data, collection location (or provenance, if cultivated), and infrageneric placement down to subsection in treatment of *Dombeya* (Arènes, 1958, 1959). (Herbarium is listed for the unicate or a single duplicate of each voucher.) * Individual known to be or most likely grown from seed or cuttings collected from wild populations. ** Individual potentially grown from seed or cuttings derived from cultivated individuals.

subgenus	**section**	**subsection**	**Taxon**	**Voucher (Herbarium)**	**Collection location; or, if cultivated, provenance (and site of cultivation in parentheses)**
--	--	--	***Corchoropsis crenata***	*Won et al. 1943* (DGU)	Mt. Bulmo, Korea
Dombeya	Dombeya	Utriculiferae	***Dombeya acerifolia***	*C. Skema et al. 221* (BH)	Mantadia National Park (Andasibe), Toamasina, Madagascar
Dombeya	--	--	**_Dombeya acutangula_ 1 (var. _rosea_)**	*R. Bone 37* (MAU)	cultivated**, exact provenance unknown, Mauritius (private garden of Claudia Baider, Mauritius)
Dombeya	--	--	**_Dombeya acutangula_ 2**	*Chase 18955* (K)	Cascade Mourouk, Rodrigues, Mascarenes
Dombeya	--	--	***Dombeya amaniensis***	*Kayombo & Nkawamba 2212* (MO)	Morogoro, Ulanga District, Tanzania
Xeropetalum	Xeropetalum	Glabrae	***Dombeya angustipetala***	*C. Skema et al. 139* (BH)	Ranomafana National Park, Fianarantsoa, Madagascar
Xeropetalum	Decastemon	Decantherae	**_Dombeya australis_ 1**	*C. Skema et al. 249* (BH)	Mandritsara (S of Vangaindrano), Fianarantsoa, Madagascar
Xeropetalum	Decastemon	Decantherae	**_Dombeya australis_ 2**	*C. Skema et al. 374* (BH)	Mandena forest (N of Fort Dauphin), Toliara, Madagascar
Xeropetalum	--	--	***Dombeya autumnalis***	*Goldblatt & Manning10473* (MO)	Pilgrim's Rest, Mpumalanga, South Africa

Table 3.3 (Continued)

subgenus	**section**	**subsection**	**Taxon**	**Voucher (Herbarium)**	**Collection location; or, if cultivated, provenance (and site of cultivation in parentheses)**
Dombeya	Astrapaea	Somanga	***Dombeya baronii* 1**	*C. Skema et al. 404* (BH)	Zahamena Natural Reserve, Toamasina, Madagascar
Dombeya	Astrapaea	Somanga	***Dombeya baronii* 2**	*C. Skema et al. 306* (BH)	Montagne d'Ambre National Park, Antsiranana, Madagascar
Dombeya	Astrapaea	Somanga	***Dombeya baronii* 3**	*C. Skema et al. 147* (BH)	Ranomafana National Park, Fianarantsoa, Madagascar
Dombeya	Dombeya	Utriculiferae	***Dombeya befotakensis***	*C. Skema et al. 141* (BH)	Ranomafana National Park, Fianarantsoa, Madagascar
Xeropetalum	Paracapricornua	--	***Dombeya borraginea* 1**	*C. Skema et al. 315* (BH)	Sahafary, Antsiranana, Madagascar
Xeropetalum	Paracapricornua	--	***Dombeya borraginea* 2**	*C. Skema et al. 330* (BH)	Betsimiranjana, Antsiranana, Madagascar
Dombeya	Capricornua	Greveanae	***Dombeya borraginopsis***	*C. Skema et al. 378* (BH)	W of Fort Dauphin, Toliara, Madagascar
Dombeya	--	--	***Dombeya burgessiae* 1**	*Chase 14849* (K)	Chyulu Hills, Kenya
Dombeya	--	--	***Dombeya burgessiae 2***	*C. Skema et al. 475* (BH)	cultivated**, provenance unknown (Palermo Botanical Garden, Sicily)
Dombeya	Dombeya	Cymoso-Umbellatae	***Dombeya cacuminum***	*C. Skema et al. 207* (BH)	cultivated**, provenance unknown (school CEG Avaradoha, Antananarivo, Madagascar)

Table 3.3 (Continued)

subgenus	**section**	**subsection**	**Taxon**	**Voucher (Herbarium)**	**Collection location; or, if cultivated, provenance (and site of cultivation in parentheses)**
Dombeya	Astrapaea	Cannabinae	***Dombeya cannabina* (subsp. *antsifotrensis*)**	*C. Skema et al. 194* (BH)	Andringitra National Park, Fianarantsoa, Madagascar
--	--	--	***Dombeya* × *cayeuxii* 1**	*C. Skema 84* (BH)	cultivated**, provenance unknown (Climatron, Missouri Botanical Garden, Missouri, USA)
--	--	--	***Dombeya* × *cayeuxii* 2**	*C. Skema et al. 206* (BH)	cultivated**, provenance unknown (school Andrefa An Ambohijanahary, Antananarivo, Madagascar)
Dombeya	Dombeya	Utriculiferae	***Dombeya coria***	*C. Skema et al. 212* (BH)	Analamazaotra Special Reserve (Andasibe), Toamasina, Madagascar
Xeropetalum	Xeropetalum	Glabrae	***Dombeya dolicophylla***	*C. Skema et al. 237* (BH)	18 km S of National Route 2 towards Lakato, Toamasina, Madagascar
Dombeya	Dombeya	Stipulaceae	***Dombeya elegans***	*T. Le Péchon 18* (P)	La Réunion
Dombeya	Dombeya	Coroniferae	***Dombeya erythroclada***	*C. Skema et al. 142* (BH)	Ranomafana National Park, Fianarantsoa, Madagascar
Xeropetalum	Decastemon	Decantherae	***Dombeya farafanganica***	*W. Applequist 255* (MO)	Vohipao (S of Vangaindrano), Fianarantsoa, Madagascar

Table 3.3 (Continued)

subgenus	**section**	**subsection**	**Taxon**	**Voucher (Herbarium)**	**Collection location; or, if cultivated, provenance (and site of cultivation in parentheses)**
Dombeya	Dombeya	Cymoso-Umbellatae	***Dombeya ferruginea***	*R. Bone 33* (MAU)	cultivated*, Trois Mamelles Mountain, Mauritius (private garden of Claudia Baider, Mauritius)
--	--	--	***Dombeya gautieri***	*L. Gautier 4578* (US)	Daraina (NW of Vohemar), Antsiranana, Madagascar
Dombeya	Capricornua	Greveanae	***Dombeya greveana***	*C. Skema 103* (BH)	cultivated*, provenance unknown (nursery of D. Turk, Antananarivo, Madagascar)
Dombeya	Astrapaea	Cannabinae	***Dombeya hafotsy* 1**	*W. Applequist 231* (MO)	Midongy du Sud National Park, Fianarantsoa, Madagascar
Dombeya	Astrapaea	Somanga	***Dombeya hafotsy* 2**	*W. Applequist 232* (MO)	Midongy du Sud National Park, Fianarantsoa, Madagascar
Dombeya	Astrapaea	Cannabinae	***Dombeya hilsenbergii* 1**	*C. Skema et al. 151* (BH)	Ranomafana National Park, Fianarantsoa, Madagascar
Dombeya	Astrapaea	Cannabinae	***Dombeya hilsenbergii* 2**	*C. Skema et al. 310* (BH)	Montagne d’Ambre National Park, Antsiranana, Madagascar
Dombeya	Astrapaea	Cannabinae	***Dombeya hilsenbergii* 3**	*C. Skema et al. 421* (BH)	Zahamena Natural Reserve, Toamasina, Madagascar
Dombeya	Astrapaea	Cannabinae	***Dombeya hilsenbergii* 4**	*C. Skema et al. 214* (BH)	Analamazaotra Special Reserve (Andasibe), Toamasina, Madagascar

Table 3.3 (Continued)

subgenus	**section**	**subsection**	**Taxon**	**Voucher (Herbarium)**	**Collection location; or, if cultivated, provenance (and site of cultivation in parentheses)**
Xeropetalum	Xeropetalum	Floribundae	***Dombeya laurifolia***	*C. Skema 96* (BH)	cultivated*, provenance unknown (nursery of D. Turk, Antananarivo, Madagascar)
Xeropetalum	Decastemon	Longipedicellatae	***Dombeya lecomteopsis***	*C. Skema et al. 52* (BH)	Ambatofinandrahana, Fianarantsoa, Madagascar
Dombeya	Trochetiantha	Macranthae	***Dombeya leiomacrantha***	*C. Skema et al. 199* (BH)	Andringitra National Park, Fianarantsoa, Madagascar
Dombeya	Trochetiantha	Macranthae	***Dombeya leucomacrantha***	*C. Skema et al. 201* (BH)	Andringitra National Park, Fianarantsoa, Madagascar
Dombeya	Capricornua	Rigidae	***Dombeya linearifolia* subsp. *linearifolia***	*Service Forestier 29211* (MO)	Ankarana National Park, Antsiranana, Madagascar
Dombeya	Capricornua	Rigidae	***Dombeya linearifolia* subsp. *sely***	*F. Ratovoson & al. 1228* (MO)	Montagne de Français, Antsiranana, Madagascar
Xeropetalum	Xeropetalum	Longifoliae	***Dombeya lucida***	*C. Skema et al. 210* (BH)	Mandraka, Antananarivo, Madagascar
Dombeya	Trochetiantha	Macranthae	***Dombeya macrantha***	*C. Skema 90* (BH)	cultivated*, provenance unknown (nursery of D. Turk, Antananarivo, Madagascar)
Dombeya	Dombeya	Utriculiferae	***Dombeya magnifolia***	*C. Skema et al. 135* (BH)	Ranomafana National Park, Fianarantsoa, Madagascar

Table 3.3 (Continued)

subgenus	**section**	**subsection**	**Taxon**	**Voucher (Herbarium)**	**Collection location; or, if cultivated, provenance (and site of cultivation in parentheses)**
Xeropetalum	Decastemon	Decantherae	***Dombeya mandenensis***	*C. Skema et al. 373* (BH)	Mandena forest (N of Fort Dauphin), Toliara, Madagascar
Dombeya	Capricornua	Greveanae	***Dombeya cf. marivorahonensis***	*C. Skema et al. 319* (BH)	Ankarana National Park, Antsiranana, Madagascar
Dombeya	Assonia	--	***Dombeya mauritiana***	*R. Bone 34* (MAU)	cultivated*, Magenta, Mauritius (private garden of Claudia Baider, Mauritius)
Xeropetalum	Xeropetalum	Villosae	***Dombeya modesta***	*C. Skema et al. 389* (BH)	Zahamena Natural Reserve, Toamasina, Madagascar
Dombeya	Dombeya	Utriculiferae	***Dombeya mollis***	*C. Skema 109* (BH)	cultivated*, provenance unknown (nursery of D. Turk, Antananarivo, Madagascar)
Dombeya	Dombeya	Utriculiferae	***Dombeya montana***	*C. Skema et al. 185* (BH)	Andringitra National Park, Fianarantsoa, Madagascar
--	--	--	***Dombeya moratii***	*Service Forestier 23509* (MO)	Ifandena (between Ihosy and Ankaramena), Fianarantsoa, Madagascar
Dombeya	Trochetiantha	Macranthae	***Dombeya muscosa***	*C. Skema et al. 198* (BH)	Andringitra National Park, Fianarantsoa, Madagascar
Dombeya	Dombeya	Coroniferae	***Dombeya palmatisecta***	*C. Skema et al. 351* (BH)	Manongarivo Special Reserve, Antsiranana, Madagascar
Dombeya	Assonia	--	***Dombeya populnea***	*R. Bone 25* (MAU)	Yemen, Mauritius

Table 3.3 (Continued)

subgenus	**section**	**subsection**	**Taxon**	**Voucher (Herbarium)**	**Collection location; or, if cultivated, provenance (and site of cultivation in parentheses)**
Dombeya	Dombeya	Utriculiferae	***Dombeya reclinata***	*T. Le Péchon 6* (P)	La Réunion
Dombeya	Dombeya	Cymoso-Umbellatae	***Dombeya rottleroides***	*C. Skema et al. 337* (BH)	Ramena river valley, Antsiranana, Madagascar
Dombeya	Capricornua	Greveanae	***Dombeya rubifolia* 1**	*J. Burke & M. Yazbek 69* (BH)	cultivated**, provenance unknown (Fairchild Botanical Garden, Florida, USA)
Dombeya	Capricornua	Greveanae	***Dombeya rubifolia* 2**	*J. Burke & M. Yazbek 70* (BH)	cultivated**, provenance unknown (Fairchild Botanical Garden, Florida, USA)
Xeropetalum	Xeropetalum	Floribundae	***Dombeya sahatavyensis***	*C. Skema et al. 409* (BH)	Zahamena Natural Reserve, Toamasina, Madagascar
Dombeya	--	--	***Dombeya* × *seminole* (= *D. burgessiae* × *D. burgessiae* 'Rosemound')**	*J. Burke & M. Yazbek 72* (BH)	cultivated**, provenance unknown (Fairchild Botanical Garden, Florida, USA)
--	--	--	***Dombeya sp.***	*Chase 273* (K)	Ibadan, Nigeria
Dombeya	Dombeya	Stipulaceae	***Dombeya stipulacea***	*C. Skema et al. 153* (BH)	W of Ranomafana National Park, Fianarantsoa, Madagascar
Dombeya	Trochetiantha	Superbae	***Dombeya superba* 1**	*W. Applequist 261* (MO)	Ankarana forestry station (Manombo, SW of Midongy du Sud), Fianarantsoa, Madagascar

Table 3.3 (Continued)

subgenus	**section**	**subsection**	**Taxon**	**Voucher (Herbarium)**	**Collection location; or, if cultivated, provenance (and site of cultivation in parentheses)**
Dombeya	Trochetiantha	Superbae	***Dombeya superba* 2**	*Service Forestier 23593* (MO)	Farafangana, Fianarantsoa, Madagascar
Dombeya	--	--	***Dombeya tiliacea***	*V. Leyman S4079* (BR)	cultivated**, Pretoria, South Africa (Belgium National Botanical Garden, Belgium)
Dombeya	Dombeya	Cymoso-Umbellatae	***Dombeya urschiana***	*C. Skema 100* (BH)	cultivated*, provenance unknown (nursery of D. Turk, Antananarivo, Madagascar)
Dombeya	Dombeya	Cymoso-Umbellatae	***Dombeya viburnifloropsis***	*C. Skema et al. 180* (BH)	12 km south of Fianarantsoa, Fianarantsoa, Madagascar
Xeropetalum	Xeropetalum	Villosae	***Dombeya venosa***	*C. Skema 94* (BH)	cultivated*, provenance unknown (nursery of D. Turk, Antananarivo, Madagascar)
Dombeya	Dombeya	Utriculiferae	***Dombeya viburniflora***	*C. Skema et al. 183* (BH)	Andringitra National Park, Fianarantsoa, Madagascar
Dombeya	Astrapaea	Involucratae	***Dombeya wallichii* 1**	*C. Skema et al. 372* (BH)	Mandena forest (N of Fort Dauphin), Toliara, Madagascar
Dombeya	Astrapaea	Involucratae	***Dombeya wallichii* 2**	*V. Leyman S4083* (BR)	cultivated**, provenance unknown (Belgium National Botanical Garden, Belgium)
Dombeya	Astrapaea	Involucratae	***Dombeya wallichii* 3**	*V. Leyman S4084* (BR)	cultivated**, provenance unknown (Belgium National Botanical Garden, Belgium)

Table 3.3 (Continued)

subgenus	**section**	**subsection**	**Taxon**	**Voucher (Herbarium)**	**Collection location; or, if cultivated, provenance (and site of cultivation in parentheses)**
Dombeya	--	--	***Dombeya wittei***	*V. Leyman S4078* (BR)	cultivated**, Democratic Republic of Congo (Belgium National Botanical Garden, Belgium)
--	--	--	***Eriolaena candollei***	*C. Skema et al. 439* (BH)	native tree in Queen Sirikit Botanic Garden, Chiang Mai Province, Thailand
Dombeya	Paracheirolaena	--	***Helmiopsiella ctenostegia***	*Chase 33737* (K)	cultivated*, Toliara, Madagascar (Kew Botanic Gardens, London, UK)
--	--	--	***Helmiopsiella madagascariensis***	*Chase 33738* (K)	cultivated*, Bejangoa crossroads, Toliara, Madagascar (Kew Botanic Gardens, London, UK)
--	--	--	***Helmiopsis bernieri***	*C. Skema et al. 288* (BH)	Baie des Dunes, Ramena, Antsiranana, Madagascar
--	--	--	***Helmiopsis pseudopopulus***	*C. Skema et al. 328* (BH)	Camp Orangea, Ramena, Antsiranana, Madagascar
--	--	--	***Nesogordonia sp. nov.***	*J. Rabenantoandro 1711* (US)	wild collection, provenance unknown, Madagascar
--	--	--	***Pentapetes phoenicea***	*C. Skema s.n.*	cultivated**, provenance unknown (Cornell University, New York, USA)

Table 3.3 (Continued)

subgenus	**section**	**subsection**	**Taxon**	**Voucher (Herbarium)**	**Collection location; or, if cultivated, provenance (and site of cultivation in parentheses)**
--	--	--	***Ruizia cordata* 1**	*H. S. Cubey 128* (E)	cultivated**, provenance unknown (Royal Botanic Garden Edinburgh, Scotland)
--	--	--	***Ruizia cordata* 2**	*V. Leyman s.n.* (BR)	cultivated**, Réunion (Belgium National Botanical Garden, Belgium)
--	--	--	***Trochetia blackburniana***	*R. Bone s.n. (MAU)*	Petrin Reserve, Mauritius
--	--	--	***Trochetia parviflora***	*R. Bone 36* (MAU)	cultivated*, Corps de Garde Mountain, Mauritius (private garden of Claudia Baider, Mauritius)
--	--	--	***Trochetia uniflora***	*R. Bone 35* (MAU)	cultivated*, Trois Mamelles Mountain, Mauritius (private garden of Claudia Baider, Mauritius)
--	--	--	***Trochetiopsis erythroxylon***	*Chase 18170* (K)	cultivated*, High Peak, St. Helena (Kew Botanic Gardens, London, UK)

automatically precludes a simultaneous or “total evidence” analysis of all the sequence data.

Two matrices of the “global” dataset (all chloroplast regions combined with ITS) were analyzed to investigate any possible effects of ITS polymorphisms on phylogenetic conclusions. One global matrix included ITS with intraindividual polymorphisms coded as polymorphisms (i.e., either possible state) whereas the second global matrix included ITS with putative haplotypes constructed from the polymorphic ITS data as separate terminals. The second matrix included two haplotypes from each of five accessions (see Chapter 2 for a list) that were found to occupy disparate positions in a series of phylogenetic analyses of polymorphism in ITS alone. The ITS haplotypes of each of these five accessions were concatenated with identical chloroplast data sequenced for the relevant accession. Further details of the ITS analyses, the haplotypes and their construction can be found in Chapter 2.

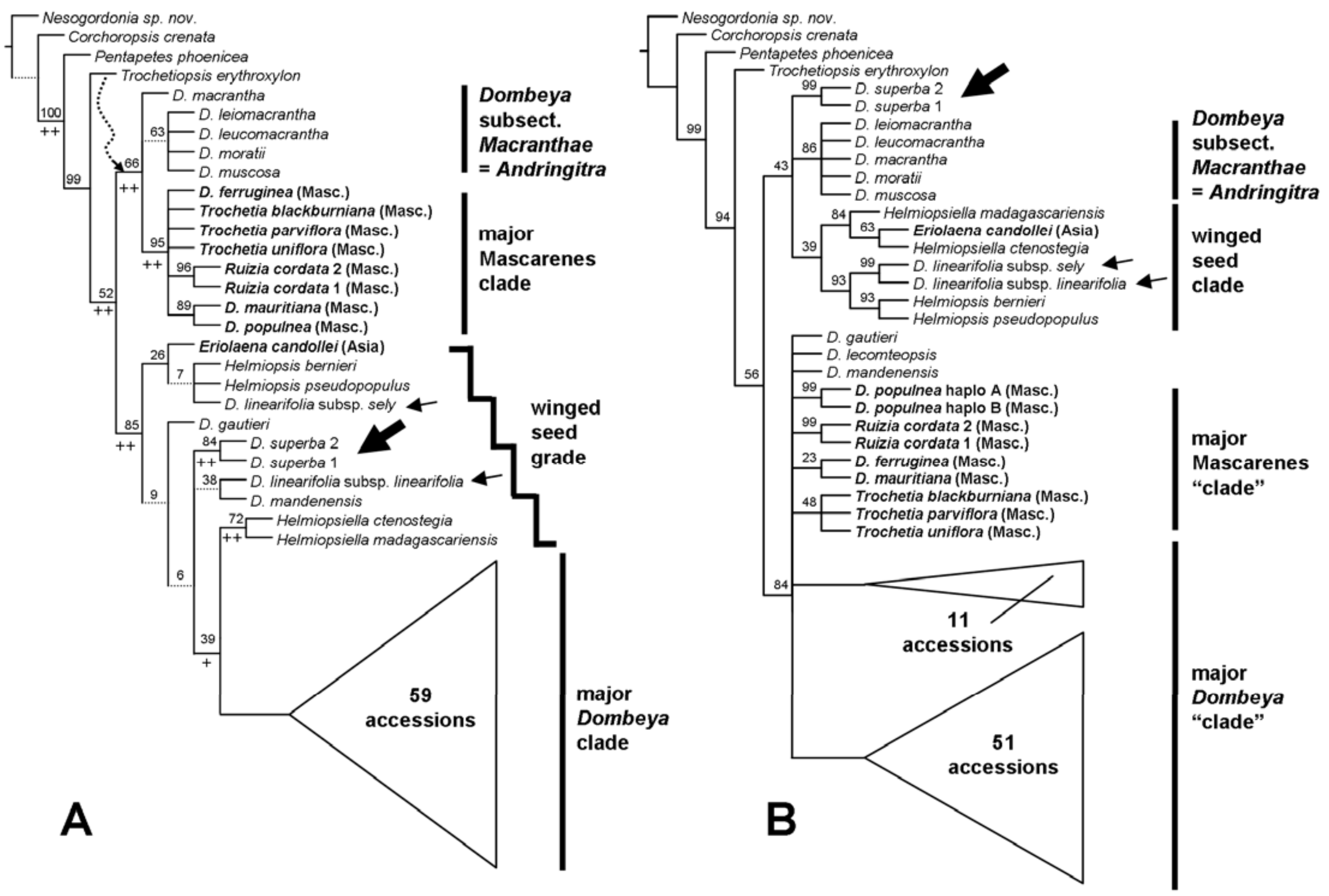
Not all gene regions were sequenced for all taxa: four accessions (*D. magnifolia, D. borraginea* and two accessions of *D. australis*) lack one gene region each, one accession (*D. lecomteopsis*) lacks two regions and one accession lacks three regions (*D. baronii* 2). (Details of missing regions are in Chapter 2, Table 2.1.) Each of the taxa with missing regions was removed, one by one, and then all six simultaneously, from a series of parsimony analyses of the global dataset that were completed in TNT (Goloboff & al., 2008; “rs0; hold 20001; rat: it1000upf5dow5; drift: iter 50; mu=rep1000ho20; ratchet; drift; tfuse; ho5000; bbreak=tbr; nel;”). Topologies of the resulting strict consensus trees were compared to evaluate the effect of these missing data on the phylogeny.

Exact details of the analytical methods are elaborated upon in Chapter 2, but a summary of analyses specific to the global dataset is as follows. Phylogenies were hypothesized for the global dataset using both parsimony methods and Bayesian inference. Parsimony analysis was conducted in TNT v.1.0 (Goloboff & al., 2008) and trees visualized in Winclada v.1.7 (Nixon, 2002). Parsimony analysis used random addition sequences followed by TBR swapping, then ratchet, drift and tree fusion iterations. Support values were measured by conducting 10,000 replicates of a bootstrap analysis in TNT and strict consensus trees of each bootstrap replicate were used to calculate percent frequency support values on the strict consensus tree for the corresponding dataset in Winclada. All strict consensus trees discussed in this study have ambiguously supported clades collapsed. Models used in the Bayesian analysis were chosen under the Akaike Information Criterion (Akaike, 1974) as implemented in MrModelTest v.2 (Nylander, 2004) utilizing likelihood values and a neighbor joining tree generated in PAUP* v.4.0 (Swofford, 2001). Bayesian phylogenetic analysis was completed in MrBayes (Ronquist & Huelsenbeck, 2003) sampling every 1000 generations for 20,000,000 generations. Data in the analysis were partitioned by spacer or gene region and models used for each were as follows: *ndhA*x, GTR + I; *trnCycf6*, GTR; *ycf6* gene, JC; *ycf6psbM*, HKY; *psbMtrnD*, HKY; *psbEpetL*, GTR + G; ITS1, GTR+ G; 5.8S, JC; ITS2, GTR + G. Burn-in for the analysis was conservatively set at 10% of the generations.

RESULTS

Matrices & Phylogenetic Analyses. — Only clades of particular relevance to the morphological evaluation of these dombeyoids and groups that have incongruent placement between the chloroplast and nuclear topologies are presented in the strict consensus summary trees of the combined chloroplast analysis and ITS analysis (Fig. 3.1). Full phylogenies resulting from both the chloroplast and ITS analyses and details of parsimony informative characters (PICs) per gene region can be found in Chapter 2.

Figure 3.1. Summary of strict consensus trees for four major groups of dombeyoids. Parsimony analysis of combined chloroplast dataset on which Bayesian topology is mapped (**A**). Parsimony analysis of ITS dataset (**B**). Both figures are condensed from Chapter 2 and accessions omitted consist only of species of *Dombeya*. Solid arrows mark taxa (*D. superba*, large arrow; two subspecies of *D. linearifolia*, small arrows) whose incongruent positions between the chloroplast and ITS phylogenies are discussed in the text. The dashed arrow indicates a node that moved in the Bayesian analysis. Dashed lines show nodes that collapse in Bayesian analysis. Values above branches indicate bootstrap percentages from 10,000 replicates. Symbols below branches indicate Bayesian posterior probabilities, where 0.9<+<1.00 and ++ = 1.00. Excluding outgroups (*Nesogordonia*, *Corchoropsis*, *Pentapetes*, and *Trochetiopsis*), names in bold indicate an accession originating from outside of Madagascar with the region of provenance given in parentheses (Afr. = Africa; Masc. = Mascarenes). Numbers after a name differentiate individuals sampled from one species.


Nesogordonia sp. nov.
Corchoropsis crenata
Pentapetes phoenicea
Trochetiopsis erythroxylon
D. macrantha
D. leiomacrantha
D. leucomacrantha
D. moratii
D. muscosa
D. ferruginea (Masc.)
Trochetia blackburniana (Masc.)
Trochetia parviflora (Masc.)
Trochetia uniflora (Masc.)
Ruizia cordata 2 (Masc.)
Ruizia cordata 1 (Masc.)
D. mauritiana (Masc.)
D. populnea (Masc.)
Eriolaena candollei (Asia)
Helmiopsis bernieri
Helmiopsis pseudopopulus
D. linearifolia subsp. sely
D. gautieri
D. superba 2
D. superba 1
D. linearifolia subsp. linearifolia
D. mandenensis
Helmiopsiella ctenostegia
Helmiopsiella madagascariensis
59 accessions
100
++
99
66
++
63
95
++
96
89
52
++
26
7
85
++
84
++
38
72
++
9
6
39
+
Dombeya subsect. Macranthae = Andringitra
major Mascarenes clade
winged seed grade
major Dombeya clade
A
Nesogordonia sp. nov.
Corchoropsis crenata
Pentapetes phoenicea
Trochetiopsis erythroxylon
D. superba 2
D. superba 1
D. leiomacrantha
D. leucomacrantha
D. macrantha
D. moratii
D. muscosa
Helmiopsiella madagascariensis
Eriolaena candollei (Asia)
Helmiopsiella ctenostegia
D. linearifolia subsp. sely
D. linearifolia subsp. linearifolia
Helmiopsis bernieri
Helmiopsis pseudopopulus
D. gautieri
D. lecomteopsis
D. mandenensis
D. populnea haplo A (Masc.)
D. populnea haplo B (Masc.)
Ruizia cordata 2 (Masc.)
Ruizia cordata 1 (Masc.)
D. ferruginea (Masc.)
D. mauritiana (Masc.)
Trochetia blackburniana (Masc.)
Trochetia parviflora (Masc.)
Trochetia uniflora (Masc.)
11 accessions
51 accessions
99
99
94
86
43
84
63
39
99
93
93
56
99
99
23
48
84
Dombeya subsect. Macranthae = Andringitra
winged seed clade
major Mascarenes "clade"
major Dombeya "clade"
B

Parsimony analysis of the combined chloroplast regions with indel coding yielded nine most parsimonious trees with a length (L) of 498, a consistency index (CI) of 91, and a retention index (RI) of 91 (Fig. 3.1A). The concatenated chloroplast sequence data produced one consistent set of relationships with little conflict or homoplasy, and the nine most parsimonious trees differed only by a few sister relationships between species due to missing data (see Chapter 2 for details). The phylogeny of the combined chloroplast data hypothesized by Bayesian analysis differed little from that of parsimony, except that *Trochetiopsis* was placed sister to the clade of *D.* subsect. *Macranthae* plus the major Mascarene clade, underlining the close relationship this "outgroup" may have to particular members of *Dombeya s. l.* Bayesian analysis also collapsed the two poorly-supported branches separating *D. gautieri* from the taxa surrounding it to form a polytomy (Fig. 3.1A). In contrast to the consistent signal of the chloroplast data, parsimony analysis of the ITS dataset alone produced over 1 million trees (L = 571, CI = 69, RI = 79; Fig. 3.1B). Conflicting characters, independent of the polymorphisms, were apparent in the ITS dataset. Relationships between most clades in ITS changed across the many most parsimonious trees and resulted in the multiple polytomies seen in the strict consensus.

Analysis of the global dataset (chloroplast plus ITS) with the inclusion of the ten haplotypes constructed from the ITS dataset resulted in a strict consensus tree (data not shown) identical in topology to the strict consensus of the global analysis without the haplotypes (i.e., polymorphisms were coded as polymorphisms), except that it placed the two haplotypes from each accession as sister to one another. Haplotypes from the known hybrid *D.* × *cayeuxii* that have chloroplast regions of their maternal parent (*D. burgessiae*) but ITS regions of their paternal parent (*D. wallichii*) decreased resolution in the global analysis tree. Therefore, in the final matrix for global analysis, only chloroplast sequence from *D.* × *cayeuxii* combined with maternal ITS sequence from *D.* × *cayeuxii* clones was included for each of the two individuals sampled for this taxon, and paternal ITS sequences were omitted from the global analysis.

The aligned matrix of the global dataset, complete with indel coding for all regions, had 5537 characters of which 5.5% were parsimony informative with 0.9% indel coding PICs. Roughly half of the PICs in the combined dataset came from the five chloroplast regions and the other half from ITS. Analysis of the global dataset resulted in 31,860 most parsimonious trees (L = 1109, CI= 77, RI = 80; Fig. 3.2, strict consensus). Whereas the analysis of the chloroplast data alone showed one fairly consistent set of relationships, the addition of ITS introduced into the analyses characters conflicting with the chloroplast characters, resulting in many more most parsimonious trees and a lower CI.

Removal of each, and then all, of those terminals that lacked sequences for one or more markers did not increase resolution of the topologies for the global analysis, except for one accession, *D. baronii* 2. The removal of *D. baronii* 2 changed the strict consensus resulting from the global analysis not because of the sequence data actually missing for this accession but because the removal of its shared synapomorphy with *D. hilsenbergii* 2 in *psbEpetL* changed the balance of conflicting characters between ITS and the chloroplast datasets. Therefore, *missing* data for these accessions were not seen to impact the resulting topologies and the accessions were kept in the analyses.

In the topology of the strict consensus tree of the global analysis (Fig. 3.2), both subspecies of *D. linearifolia* formed a clade (BS = 97%) that was sister to a clade of both species of *Helmiopsis* (BS = 92%) with high support (BS = 94%). *Eriolaena* was sister to both species of *Helmiopsiella* (BS = 72%). These two clades (*Eriolaena* + *Helmiopsiella*; *Helmiopsis* + *D. linearifolia*) formed a clade (BS = 67%) with *D. superba* as their sister (BS = 66%), both with low support. This entire clade was sister

Figure 3.2. Strict consensus tree of parsimony analysis of the global dataset (plastid plus ITS). The topology resulting from Bayesian analysis is mapped onto the parsimony tree until the node followed by two arrows, after which the parsimony topology continues on next page (**A**, outlined by solid box) and the Bayesian topology continues on the following page (**B**, outlined by dashed box). A black circle marks the node defining *Dombeya sensu stricto*. Support values and labeling of accessions and nodes moving/collapsing between types of analyses are the same as in Fig. 3.1.

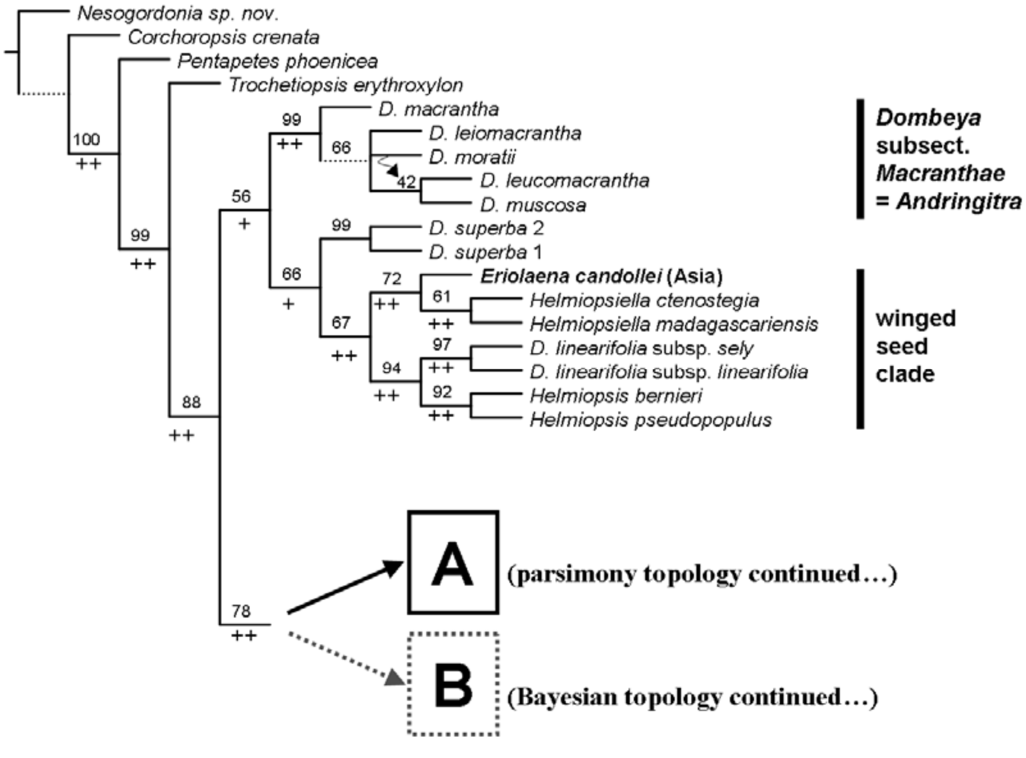

Nesogordonia sp. nov.
Corchoropsis crenata
Pentapetes phoenicea
Trochetiopsis erythroxylon
D. macrantha
D. leiomacrantha
D. moratii
D. leucomacrantha
D. muscosa
D. superba 2
D. superba 1
Eriolaena candollei (Asia)
Helmiopsiella ctenostegia
Helmiopsiella madagascariensis
D. linearifolia subsp. sely
D. linearifolia subsp. linearifolia
Helmiopsis bernieri
Helmiopsis pseudopopulus
100
++
99
++
88
++
56
+
99
++
66
42
99
66
+
72
++
61
++
67
++
97
++
94
++
92
++
78
++
Dombeya subsect. Macranthae = Andringitra
winged seed clade
A
(parsimony topology continued…)
B
(Bayesian topology continued…)

Figure 3.2. (Continued)

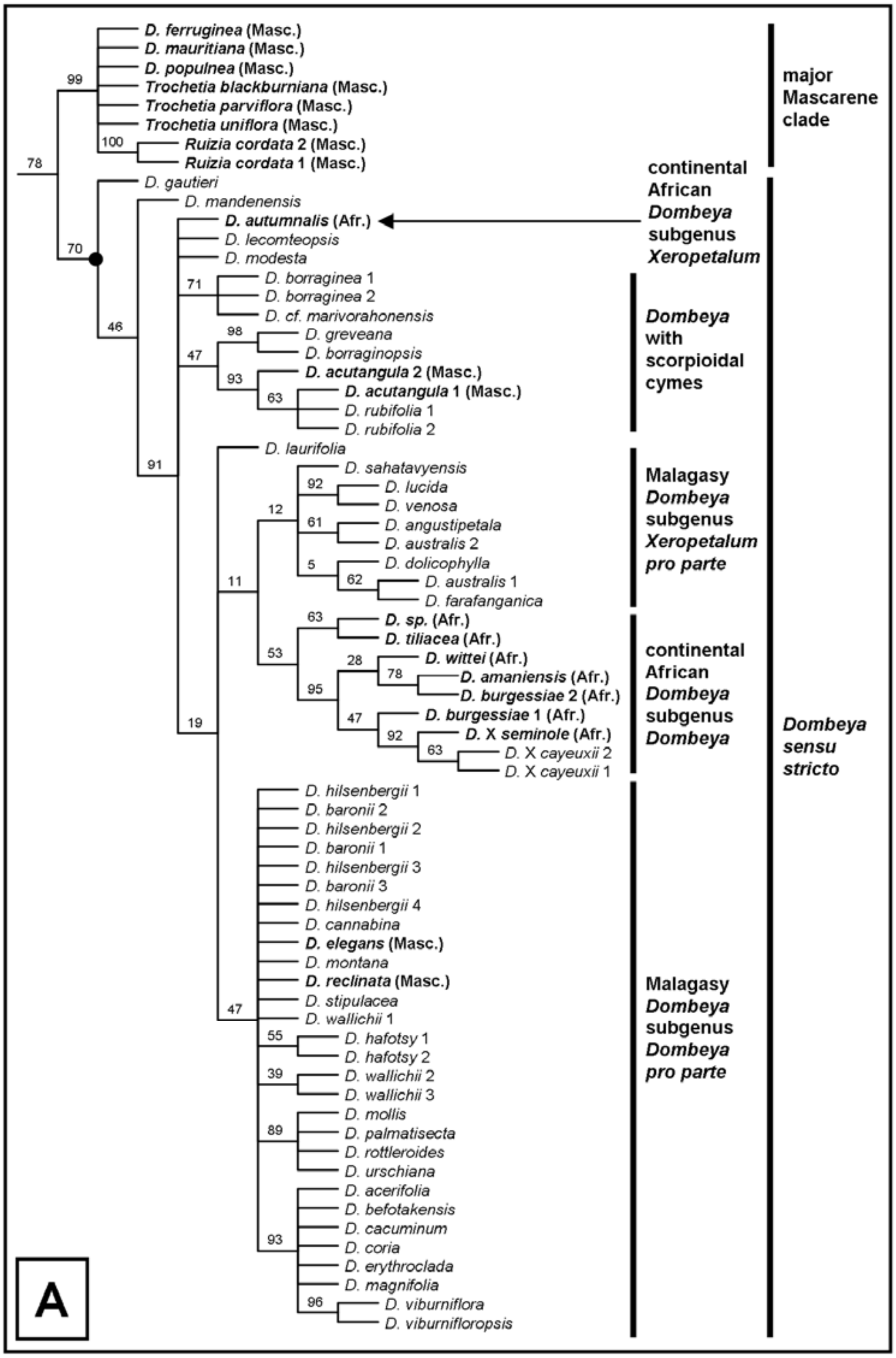
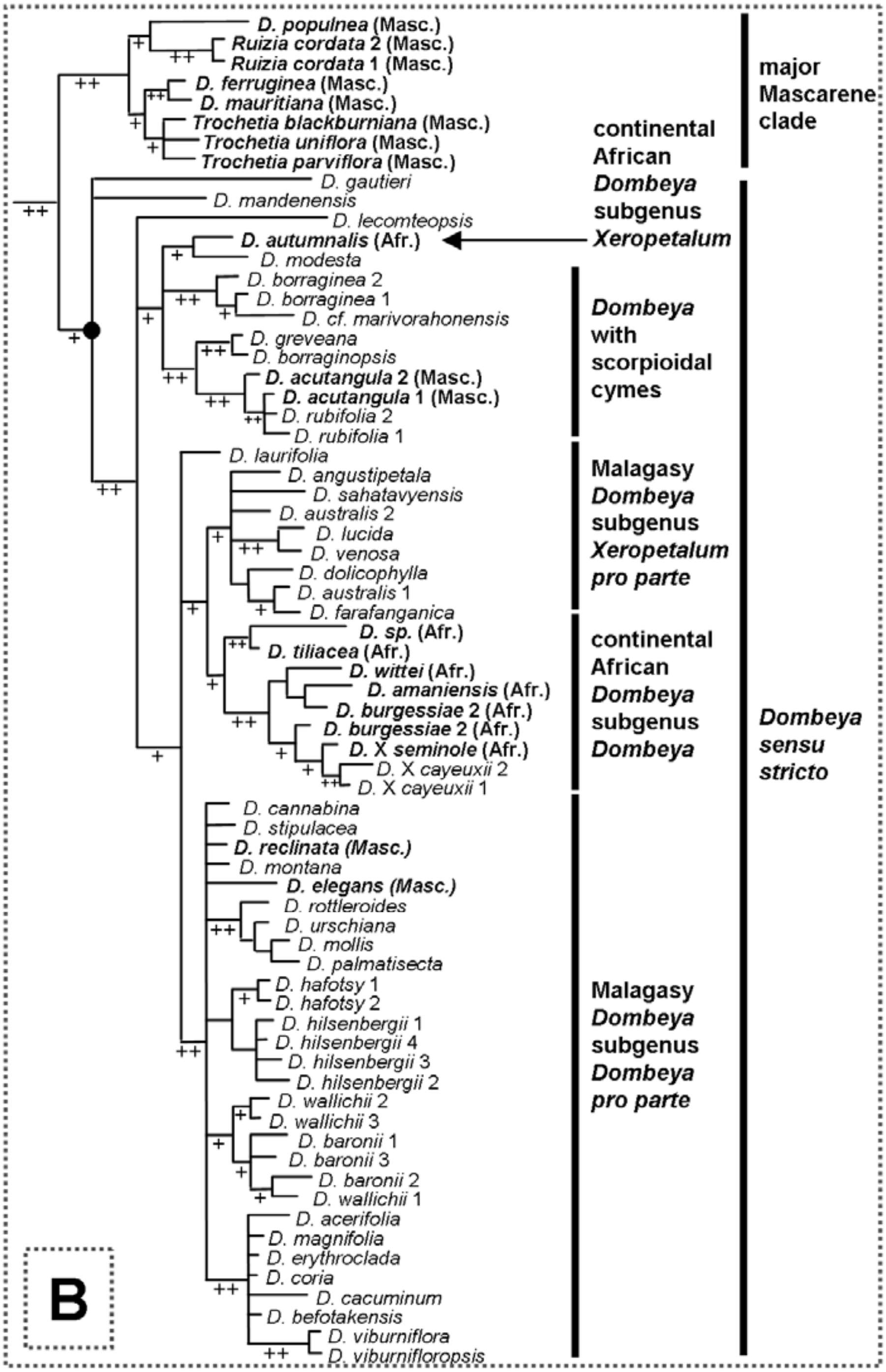


Figure 3.2. (Continued)



to a well-supported clade of *Dombeya* subsect. *Macranthae* that included *D. macrantha* (BS = 99%), but again only with low support (BS = 56%). The "major Mascarene clade," the clade with the majority of taxa from the Mascarenes sampled for this study, was well supported (BS = 99%) in the global analysis. (Other Mascarene species occur elsewhere in the tree and are marked as such in Fig. 3.2). The "major *Dombeya* clade," the clade with the majority of species of *Dombeya* sampled for the study but excluding *D. gautieri* and *D. mandenensis*, was also well supported (BS = 91%). The major Mascarene clade was sister to the major *Dombeya* clade (BS = 78%), with *D. gautieri* and *D. mandenensis* sitting between them as successive sisters to the major *Dombeya* clade with moderate or low support (BS = 70% and 46%, respectively). The combined analysis of the global dataset using Bayesian inference retained more resolution (Fig. 3.2) than the parsimony analysis.

DISCUSSION

Lack of monophyly of *Dombeya*. — *Dombeya* is paraphyletic, with *Eriolaena*, *Helmiopsiella*, *Helmiopsis*, *Ruizia* and *Trochetia* nested within it (Fig. 3.2). These results are concordant with previous work done on the dombeyoids of the Mascarenes (Le Péchon, 2009; Le Péchon & al., 2009), but add *Helmiopsiella* and *Eriolaena* to the list of taxa that have diverged from within *Dombeya sensu lato*. The general generic relationships in the chloroplast topology, (((*Dombeya p.p.* + *Helmiopsiella*) + *Eriolaena*) + *Ruizia*), are the same as those of the latest molecular study analyzing more slowly evolving chloroplast regions (*rbcL*, *atpB*, *ndhF*) (Won, 2009), but better sampling of *Dombeya* in this study allows an understanding of the placement of disparate lineages of *Dombeya* among the other genera. The inclusion of the genera *Helmiopsiella*, *Helmiopsis* and *Eriolaena* provides the first molecular data to demonstrate that both the tribes Helmiopsideae and Eriolaeneae are not merely nested within the tribe Dombeyeae but within *Dombeya sensu lato*. This and various anatomical and morphological similarities, as outlined by Barnett (1987a), provide ample reason to follow Barnett in considering both Eriolaeneae and Helmiopsideae as synonyms of Dombeyeae, but the exact membership of Dombeyeae is left for future work (Skema, in prep.).

The paraphyly of *Dombeya* is not surprising given the broad morphological variation within the genus and the few characters differentiating it from other dombeyoid genera. More unexpected is the monophyly of the majority (all but ten species) of *Dombeya* sampled for this study (Fig. 3.2), particularly given the still broad morphological variation included within this clade. *Dombeya sensu stricto* is delimited here (Fig. 3.2, black circle) based on the results of the combined analysis, as it represents the most robust phylogenetic hypothesis. Although their positions are poorly supported, *D. gautieri* and *D. mandenensis* are conservatively considered as the earliest diverging lineages of *Dombeya s. str.*, whereas the Malagasy species of *D. superba*, *D. linearifolia*, *D. moratii* and those in *D.* subsect. *Macranthae* are excluded from *Dombeya s. str.*

Roughly one-third of the species of *Dombeya* from the Mascarenes are excluded from *Dombeya s. str.*, as shown in this study and previous molecular work (Le Péchon, 2009). Each of these studies utilized ITS, but sampled different chloroplast regions (except for *trnDpsbM*). *D. ferruginea*, *D. mauritiana*, and *D. populnea* form a clade with *Trochetia* and *Ruizia*, all of which are endemic to the Mascarenes (Fig. 3.2). Another Mascarene endemic, *D. rodriguesiana*, was shown by Le Péchon (2009) to be included in a clade with *D. superba*, *D. macrantha* and one species of *Helmiopsis*, all of which fall outside of *Dombeya s. str.* in the present analysis. This relationship between morphologically disparate species of *Dombeya* (e.g., *D. rodriguesiana* and *D. macrantha*) may lend added significance to the minor point that

D. rodriguesiana has epicalyx bracts persistent beneath fruits, given that all *D.* subsect. *Macranthae* show the same characteristic. The present study nests *Dombeya reclinata* and *D. elegans* among Malagasy species of *D.* subg. *Dombeya* (Fig. 3.2), a topological position generally congruent with the results of Le Péchon (2009) in which they were sister to *D.* sect. *Dombeya*. Le Péchon (2009) sampled seven other species of Mascarene *Dombeya* that were included in this clade as well. The taxonomy of these Mascarene species is a work in progress (Le Péchon, in prep.).

Evolutionary patterns & geographic distributions. — The relationships between the geographical areas in which these dombeyoids occur are complex, as shown in the combined analysis (Fig. 3.2), assuming that even a single character supporting a clade is meaningful for this group of dombeyoids which have such little molecular divergence. In this topology, the Mascarene taxa nest within the Malagasy *Dombeya* in at least three clades, but better sampling has shown a fourth separate clade of Mascarene *Dombeya* in the placement of *D. rodriguesiana* near *D. superba* (Le Péchon, 2009). The continental African species of *Dombeya* sit in two positions within the Malagasy *Dombeya* (*D. autumnalis* and continental African *D.* subg. *Dombeya* clade in Fig. 3.2). All the species with scorpioid cymes, potentially representing a single species (*D. acutangula sensu* Seyani), most likely span across all three regions, Madagascar, the Mascarenes and continental Africa, although only taxa from Madagascar and the Mascarenes are sampled here. *Trochetiopsis*, a genus endemic to St. Helena island in the Atlantic Ocean, sits between an Asian (*Pentapetes*) and Malagasy taxa (majority of ingroup). The Asian genus *Eriolaena* is sister to endemic Malagasy taxa. These dombeyoids provide significant evidence of the strong connections between the floras of Africa, Madagascar, other Indian Ocean islands and India/South Asia (Schatz, 1996; as reviewed in Yoder & Nowak, 2006), and possibly for the extreme dispersability of these plants. Similar patterns of complex and repeated interchange between the African and Indian Ocean island floras are seen in tree ferns (Janssen, 2008).

Consideration of certain morphological characters. — The persistence of floral parts – epicalyx bracts, sepals, petals, and even the androecium – in these dombeyoids can differ temporally but this distinction is seldom noted. Although floral maturation through time may hamper recognition of perfectly discrete categories, it is useful to create rough categories of persistence. For epicalyx bracts (also called bracteoles), "early caducous" bracts are those rarely seen under the youngest floral buds or more usually seen only as scars beneath the buds. "Caducous" bracts can be found beneath buds but are usually not seen beneath flowers. Bracts "persistent into flowering" can be seen beneath flowers but not fruits, whereas bracts "persistent into fruiting" remain in position throughout the entire maturation of the gynoecium into fruit. This last distinction is often unrecognized in the literature, with bracts persistent only through flowering and those persistent through fruiting both simply described as "persistent" (e.g., Arènes 1959).

A character commonly used in differentiating among some of these dombeyoids is the position of the staminodes relative to the petals or sepals. *Dombeya* and *Helmiopsiella* are characterized as having staminodes opposite the sepals whereas *Helmiopsis* has staminodes opposite the petals (Arènes, 1959). These characterizations generally stand but are problematic for certain species. Observations of specimens for this study have shown that different species can occupy many positions across the gradation from one extreme (opposite petals) to the other (opposite sepals). It is possible to see species with staminodes that are not exactly opposite the petals nor the sepals, but somewhere in between. A similar observation was made by Applequist (2009b), who noted some variation even within individuals. van Heel (1966: 298, Fig. 231 therein) described the complexity of the positions of these parts of the androecium of various Dombeyeae, illustrating, for example, that in *D. wallichii* the staminodes sit halfway between the point of insertion of the sepals and the petals. He further argued that the position of the androecial parts, and consequently that of the gynoecium, may be dependent on the contortion of the petals. For these reasons, reliance on this character is avoided in evaluating the morphology of taxa in this study.

Another character of the androecium that varies between species, and may potentially be of taxonomic use, is the prolongation of connective tissue between the apex of the thecae. Although van Heel (1966) characterizes all Dombeyeae in his study as having prolonged connective tissue on their anthers, observations for this study demonstrate that connective extensions occur only sporadically among the Dombeyeae and its relatives. This character may have taxonomic utility in differentiating species, but probably not higher ranks, as it seems to vary among closely related taxa. For example, prolonged connectives were seen in *Helmiopsiella madagascariensis* but not *Helmiopsiella ctenostegia*, and in *D. linearifolia* subsp. *linearifolia* but not *D. linearifolia* subsp. *sely*. Prolonged connectives were also seen in *Trochetiopsis erythroxylon*, *Helmiopsis bernieri*, *Nesogordonia perpulchra* and *Helmiopsiella poissonii*. Reports of this character in yet other species exist, such as in *Trochetia uniflora* (de Candolle, 1822). Seyani (1991) describes variation even within species of *Dombeya* from continental Africa for this character (e.g., *D. torrida*).

Two different types of pubescence may occur internally within the gynoecia of the taxa in this study. The first is intralocular pubescence, found within the carpels along the internal face of the outer ovary wall. Intralocular pubescence can straddle the dehiscence suture line of the loculicidal capsules or be fused to the septum distally where it meets the outer ovary wall, and sometimes extends on to the internal face of the base of the ovary. The second type of pubescence is found inside the central axis of the ovary, where all the septae meet, and for ease of discussion this will be called "centraseptal" pubescence. Both forms of pubescence co-occur in *Pentapetes phoenicea* and *Helmiopsiella ctenostegia*, intralocular pubescence alone can be seen in *Helmiopsiella madagascariensis* and centraseptal pubescence alone occurs in *Trochetiopsis erythroxylon*, *Helmiopsiella leandrii*, and sporadically within *Dombeya s. l.* The presence of such types of pubescence has been used taxonomically as one character differentiating *Helmiopsiella* (pubescent) from *Helmiopsis* (glabrous) (e.g., Arènes, 1956a; Barnett, 1988a; Dorr, 2001). It is of note that Seyani (1991, p.36) found pubescence in the "inside of the locules" to be too variable within species of African *Dombeya* to be of use taxonomically, though it is not clear whether he meant intralocular or centraseptal pubescence.

Placement of the highly apomorphic *Eriolaena*. — *Eriolaena*, usually assigned to its own tribe Eriolaeneae, nested in these phylogenies within tribe Helmiopsideae. Specifically, ITS placed *Eriolaena* within a clade of *Helmiopsiella* and sister to a clade of *Helmiopsis* and *D. linearifolia* (Fig. 3.1B), whereas chloroplast data included it in a clade with *Helmiopsis* and *D. linearifolia* subsp. *sely* (Fig. 3.1A). Perrier de la Bâthie (1944) was the first to recognize a similarity between *Eriolaena* and *Helmiopsis*, presumably due to their winged seeds as he also lists *Pterospermum* as similar. His paper predated the description of *Helmiopsiella*. Barnett (1988b) noted substantive similarities between *Eriolaena*, *Helmiopsis* and *Helmiopsiella* in the capsules (woody and ovoid-conical in shape) and seeds (with long apical wings). She also clearly differentiated between the apically winged seeds of *Helmiopsis*, *Helmiopsiella* and *Eriolaena* where the funiculus runs through the body of the wing and the apically winged seeds of *Pterospermum* where the funiculus runs along the margin of the wing. [Contrary to Bayer & Kubitzki (2003, in their key to Dombeyoideae), neither *Helmiopsis* nor *Helmiopsiella* have basal placentation, but, in fact, have axile placentation like *Eriolaena* and most dombeyoids.] Barnett (1987a) asserted that these fundamental similarities of the fruits of *Helmiopsis*, *Helmiopsiella* and *Eriolaena* are strong indications that they are homologous structures. Parsimony analysis of morphological characters (Le Péchon & al., 2009) also placed *Eriolaena* and *Helmiopsis* in a clade together, along with *Trochetia*, supported by the synapomorphies of capsule shape and winged seeds with vasculature as already described. Caducous petals as well as these fruit and seed similarities provide morphological support for these three genera as a clade. They are also the only genera of the dombeyoids included in this analysis that contain species with yellow petals.

***Dombeya* subsection *Rigidae*.** — Arènes (1958) erected *D.* subsect. *Rigidae* to accommodate *D. rigida* and *D. linearifolia* and described the glands on the lower half of their petals as diagnostic for the subsection. These two species have long been considered sister to one another based on morphology (Hochreutiner, 1907), but their taxonomic placement within *Dombeyaceae* has been recently debated. Dorr (2001) moved *D. rigida* to *Helmiopsis* due to the discovery of a short, marginal wing on its seed and that it has staminodes opposite the petals, nectariferous tissue on the petals and glabrous locules. In revising *Helmiopsis*, Applequist (2009b) argued that the narrow marginal wing on the seed of *D. rigida* may not be homologous with the long apical wing on the seed of *Helmiopsis*, and left these two species in *Dombeya* following Arènes (1958). Applequist (2009b, Table 1 therein) also presented other characteristics differing between *Helmiopsis* and *D.* subsect. *Rigidae*, as follows: lepidote vs. stellate pubescence; dichotomously branching cymes or geminate or solitary flowers vs. scorpioid cymes; glandular tissue on the sepals vs. none; epicalyx bracts rapidly caducous or not enveloping buds vs. epicalyx bracts sometimes enveloping buds.

In the ITS analysis both subspecies of *D. linearifolia* form a clade that is sister to a clade of *Helmiopsis* with high support (99% and 93% respectively; Fig. 3.1B, marked by small arrows). Placement of these same subspecies differs in the chloroplast analysis (Fig. 3.1A, marked by small arrows): *D. linearifolia* subsp. *sely* groups with *Helmiopsis* and *Eriolaena*; *D. linearifolia* subsp. *linearifolia* is sister to *D. mandenensis*. In contrast to the ITS tree, the supporting nodes have poor bootstrap support. An accession of *D. rigida* from which DNA was extracted had insufficient chloroplast sequence to include in this study, but its ITS sequence grouped it in a trichotomy with both subspecies of *D. linearifolia* with 98% bootstrap support (data not shown). Given the difference in bootstrap support between the chloroplast versus ITS analyses, molecular data more strongly support a close relationship of *D. linearifolia* to *Helmiopsis* as posited by Dorr (2001).

Careful consideration of morphological characters (summarized in Table 3.4) can also be brought to bear on the competing molecular hypotheses. A character hypothesized to link *D.* subsect. *Rigidae* to *Dombeya s. str*. are scorpioid cymes (Applequist, 2009b), an inflorescence type not seen in *Helmiopsis* or *Helmiopsiella*. Scorpioid cymes are seen in both *Dombeya* sect. *Paracapricornua* and *Dombeya* sect. *Capricornua*, in which *D*. subsect. *Rigidae* was included (Arènes, 1958). These three taxa also have similar epicalyx bracts: two small, elliptic to obovate to oblanceolate bracts subtended by one larger, ovate bract that may loosely enclose the other two and the floral bud. Despite the morphological similarities, molecular data do not support a relationship between *D. linearifolia* and any of the species with scorpioid cymes in *Dombeya s. str*. However, *Helmiopsiella poissonii* does possess epicalyx bracts as seen in *D.* subsect. *Rigidae*, despite not having scorpioid cymes. In this species, two smaller, elliptical to ovate bracts are subtended by a larger, widely ovate bract that is fully adpressed to each of the smaller bracts, and all three enclose the young floral bud

Table 3.4. Comparison of key morphological characteristics* of *Dombeya* subsect. *Rigidae* (*D. linearifolia* and *D. rigida*; outlined in black) and putative sister taxa.

genus	indument	inflorescence	persistence of epicalyx	glandular tissue on calyx	glandular tissue on corolla	petals persistent?	fruit	seeds
Helmiopsiella	stellate (lepidote and stellate on *H. poissonii*)	solitary or paniculate or 3-flowered cymes	(early) caducous	absent (present in *H. poissonii*)	absent	no	ovoid-conical capsule	apically winged
Helmiopsis	lepidote (lepidote and stellate on *H. calciola*)	solitary or geminate or dichotomous cymes	early caducous	present	present in sect. *Glandulipetalae*, absent in sect. *Helmiopsis*	no	ovoid-conical capsule	apically winged (wingless in *H. sphaerocarpa*)
D. linearifolia subsp. *sely*	stellate	scorpioid cymes	caducous	present	present	no	globose capsule	apically winged**
D. linearifolia subsp. *linearifolia*	stellate	scorpioid cymes	caducous	absent	present	no	depressed-globose capsule	marginally winged
D. rigida	stellate	scorpioid cymes	caducous	absent	present	no	globose capsule	marginally winged
Dombeya sensu stricto	stellate or lepidote	geminate or (bi)umbellate or scorpioid or paniculate or corymbose cymes	early caducous to persistent beneath fruits	present (rarely absent)	absent	yes	globose to depressed-globose capsule	wingless

* Characteristics scored from observation of herbarium specimens or from the literature (Arènes, 1959; Barnett, 1988a; Dorr, 2001; Applequist, 2009b). ** Observations of these apical wings were made from ovules on a flowering specimen (*F. Ratovoson* 1228), not mature seeds.

within. Although not included in the molecular analysis, *Helmiopsiella poissonii* would likely be a member of the winged seed clade given its fruit (woody ovoid-conical capsule) and seed (apically winged) morphology. The shape of the fruit is another similar characteristic between *D*. subsect. *Rigidae* and *Dombeya s. str.* The (depressed-)globose capsules of *D*. subsect. *Rigidae* are like those of most *Dombeya s. str.* and clearly differ from the ovoid-conical capsules of *Helmiopsiella* and most *Helmiopsis*, yet *Helmiopsis sphaerocarpa* has a globose fruit (Barnett, 1987b). The globose shape of the capsule of *D. linearifolia* subsp. *linearifolia* is particularly similar to that of *D. mandenensis* with which it is grouped in the chloroplast phylogeny (Fig. 3.2A).

The characters of indument, persistence of the epicalyx and presence/absence of glandular tissue on the calyx offer little support of a closer relationship of *D*. subsect. *Rigidae* to *Dombeya s. str*. than to *Helmiopsis*/*Helmiopsiella* (Table 3.4) because the characters vary within all three groups. [See Dorr (2001) for further discussion of use of indument as a generic character.] Position of the staminodes and presence/absence of pubescence within the gynoecium are likewise problematic taxonomic characters and are not used to evaluate the morphological affinities of *D.* subsect. *Rigidae* (see discussion under "Consideration of certain morphological characters" above).

In contrast, the presence/absence of glandular tissue on the corolla, petal persistence, and style and seed characteristics all suggest *D.* subsect. *Rigidae* is more closely related to *Helmiopsis*/*Helmiopsiella* (Table 3.4). Among the species surveyed here, glandular tissue on the corolla is only present in *Helmiopsis* sect. *Glandulipetalae* and *D.* subsect. *Rigidae*. Caducous petals characterize *Helmiopsiella*, *Helmiopsis*, and *D.* subsect. *Rigidae* whereas only persistent petals are found in *Dombeya s. str*. The upright, wedge-like lobes on the styles of both subspecies of *D. linearifolia* in this study are more like the styles of *Helmiopsis* than those of *Dombeya* *s. str*. Styles in *Dombeya s. str*. show varying degrees of fusion and stigmatic lobes vary in length from very long to minute, rounded structures, but on average its style and lobes are thinner than the stout, fleshy ones in both *Helmiopsis* and *Helmiopsiella*. The assertion that seeds of *D. linearifolia* are wingless (Applequist, 2009b) conflicts with observations done for the present study: all specimens of *D. linearifolia* subsp. *linearifolia* and *D. rigida* examined had short marginal wings on their seeds. Indeed, the specimen of *D. linearifolia* subsp. *sely* had ovules with an apically oriented wing almost equal in length to its ovule proper. Seeds of *Dombeya s. str.* are wingless, generally tetragonal and slightly wider at the distal end.

In summary, molecular data, caducous and glandular petals, stout style lobes and variously winged seeds all argue for the exclusion of *D.* subsect. *Rigidae* from *Dombeya s. str*. Given its phylogenetic placement and the fact that Dorr (2001) has already transferred *Dombeya rigida* to a more broadly construed *Helmiopsis*, *D. linearifolia* is here likewise transferred (see “Further new combinations” within “Taxonomic Treatment” below). It is worth emphasizing that generic circumscription between *Helmiopsis* and *Helmiopsiella* is still unclear. *Dombeya* subsect. *Rigidae* and species such as *Helmiopsis sphaerocarpa* (not included in this study) and *Helmiopsiella poissonii* (see morphological discussion below) straddle the boundary between *Helmiopsis* and *Helmiopsiella* and additional sampling is needed to sort out the relationships of species in this group.

***Species anomalae*: More data necessary.** — New morphological observations of the species of *Helmiopsiella poissonii* and *Helmiopsiella leandrii* are summarized here given their potential relevance to the placement of *D.* subsect. *Rigidae* and the delimitations between *Helmiopsis* and *Helmiopsiella*, despite the omission of these species from the molecular study. Study of the few specimens available (including the only one in fruit) of *Helmiopsiella poissonii* indicate that this species has morphological affinities to *Helmiopsiella*, *Helmiopsis* and *D.* subsect. *Rigidae*. The woody conical fruit and long apically-winged seeds of *Helmiopsiella poisonii* are similar to both *Helmiopsis* and *Helmiopsiella*. The characteristics of *Helmiopsiella poisonii* that are most common in *Helmiopsis* include (sparse) fimbriate scales on leaves, axillary inflorescences, a patch of papillate glands on the internal base of the sepals and internally glabrous mature fruits. On the other hand, *Helmiopsiella poissonii* has the stellate indument on the gynoecium that has been argued to be diagnostic of the genus *Helmiopsiella*. The last character is also seen in *D.* subsect. *Rigidae*, which also shares a similar morphology of the epicalyx bracts with *H. poissonii*. Sampling of *Helmiopsiella poissonii* in future studies could prove critical in unraveling phylogenetic relationships between *Helmiopsis*, *Helmiopsiella* and *D*. subsect. *Rigidae*.

In contrast, morphology suggests that *Helmiopsiella leandrii* would most likely group in a phylogenetic analysis with the species of *Helmiopsiella* sampled in the molecular work presented here. Its androecium particularly resembles that of *Helmiopsiella madagascariensis*, but with a multiplication of stamens, and as Barnett (1988a) argued, the androecium does not appear to be triseriate as described by Arènes (1956b). This species is only unusual for *Helmiopsiella* in that its petals have been reported to sometimes persist beneath fruits (Barnett, 1988a).

The last anomalous species, *Dombeya superba*, is placed differently in the ITS and chloroplast topologies (Fig. 3.1, marked by large arrows). It sits in a trichotomy with *D*. subsect. *Macranthae* and the winged seed clade in the ITS tree (Fig. 3.1B), but next to a clade of *D. linearifolia* and *D. mandenensis*, with *D. gautieri* as sister to both, in the chloroplast tree (Fig. 3.1A). In the global analysis, *D. superba* is placed as sister to the winged seed clade (Fig. 3.2). Sister relationships of *D. superba* to any other taxon are poorly supported in all of these analyses; only the sister relationship between the two accessions of *D. superba* sampled are highly supported. Morphological affinities of *D. superba* to both *D.* subsect. *Macranthae* and *Eriolaena* best corroborate the placement of *D. superba* in the global analysis; no morphological characters link *D. superba* to *D. linearifolia*, *D. mandenensis*, *D. gautieri*, *Helmiopsis* or *Helmiopsiella*. *D. superba* resembles *Eriolaena* in the uncommon organization of its androecia, wherein stamens clearly diverge from the abaxial face of a long fused androecial tube at various heights below its apex. They differ in that *Eriolaena* has only fertile stamens arising from the apex of the androecial tube, lacks staminodes and has many stamens, whereas *D. superba* has five, prominent, petaloid staminodes at the apex of its androecial tube and only 15 stamens. The gynoecium of *D. superba* is similar to that of *D.* subsect. *Macranthae* except it is longer and bears no centraseptal pubescence, at least in the immature fruit available for study. Both taxa bear ≥20 ovules, a number high for these dombeyoids, in two rows within each of five locules. Further collections (particularly with mature fruit) and sampling of taxa and characters are needed to clarify sister relationships and provide a clearer morphological basis for placement. At this time, it seems prudent to leave *D. superba incertae sedis*.

Infrageneric relationships within *Dombeya s. str.* — *Dombeya gautieri*, *D. mandenensis*, and *D. lecomteopsis* stand out within *Dombeya s. str*. by their exclusion from the two major (but poorly supported) clades of *Dombeya* in the ITS phylogeny (Fig. 3.1B). Also, *Dombeya gautieri* and *D. mandenensis* receive the least support for their inclusion into *Dombeya s. str.* in the global analysis (Fig. 3.2). All three species possess morphological autapomorphies; *D. mandenensis* and *D. lecompteopsis* may well represent place holders for unique clades of *Dombeya* given further sampling.

The morphology of *D. gautieri* mirrors its intermediate position within both the chloroplast and ITS phylogenies: very close to *Dombeya* but not so far from other genera. When described, the placement of this species into a genus was problematic given its pastiche of characters reminiscent of multiple different genera as well as its autapomorphies (e.g., white flowers with five single stamens between 5 petaloid staminodes, 2-carpellate gynoecium, acicular persistent epicalyx bracts, domatia on leaves; querciform leaves and nearly bipartite style) (Skema & Dorr, in press).

Dombeya mandenensis, although morphologically a seemingly straightforward member of *Dombeya* subg. *Xeropetalum* (e.g., usually tricarpellate ovary, lepidote indument), is excluded from that (poorly supported) clade in the molecular phylogenies (Figs. 3.1, 3.2). Field observations showed that *D. mandenensis* produces exudate on the youngest shoots (including the inflorescences) and ants (*Crematogaster* sp.) cover the plants, particularly near the shoot apices and peduncles beneath the flowers. When the exudate dries, it forms a concretion of white flakes and stellate hairs; it is unclear what organ is secreting the substance. An aphid-like insect was also seen on the stems. Associations between aphids and ants have long been known to exist (Way, 1963) and associations between other insects (Diaspididae, Hemiptera) and ant species in Madagascar have been documented (Ben-Dov, 2010). Arènes (1959) described *D. mandenensis* as "mucilaginous" and the majority of his *D.* subseries *Lavasoenses* are described in the same way. Further sampling may well show that these species form a clade within *Dombeya*, marked by an association with ants, and may be an example of a biotic interaction driving tropical diversification (e.g., Léotard & al., 2008).

Dombeya lecomteopsis is the only species sampled from roughly fifteen species of *D.* sect. *Decastemon* subsect. *Longipedicellatae*. The group has half-inferior ovaries, an unusual characteristic in a family of superior ovaries. The relationship of this species (or potentially a clade of species with half-inferior ovaries) to other *Dombeya* remains unclear given different positions in the chloroplast and ITS trees. Chloroplast data place *D. lecomteopsis* in a poorly supported clade with African species of

Dombeya and *D.* subg. *Xeropetalum* species from Madagascar (Chapter 2, Fig. 2.3A); ITS puts it with *D. gautieri* and *D. mandenensis* as described above (Fig. 3.1B).

As previously mentioned, the most recent infrageneric treatment for *Dombeya* mostly relied on carpel number and inflorescence type (Arènes 1959; Table 3.2). Subgenus *Xeropetalum* was recognized as having 2-3 carpels and subgenus *Dombeya* as having 4-5 carpels per flower. Discounting the three splinter lineages (*D. gautieri*, *D. mandenensis* and *D. lecomteopsis*, discussed above) and the species with scorpioid cymes (discussed below), most of which are 2-3-carpellate, carpel number loosely corresponds to evolutionary lineages on the molecular tree (Fig. 3.2). There are two lineages of 5-carpellate *Dombeya*, one in Madagascar and one in Africa. Likewise there are two lineages of 2-3-carpellate *Dombeya*, again one in Madagascar and one in Africa. Carpel number may prove useful for distinguishing infrageneric groups in *Dombeya*, but it will not be as simple as one subgeneric division.

Arènes used inflorescence characters (or, more correctly, characters of the polytelic synflorescences; Bayer, 1999) to recognize sections within his subgenera. In general, inflorescence characters are not corroborated by the molecular data, with one notable exception: species with scorpioid cymes may form one distinct lineage within *Dombeya s. str.* Seyani (1991) viewed all *Dombeya* with scorpioid cymes as one species, *D. acutangula*. He synonymized most of the species recognized by Arènes (1959, the names for which were used here) and characterized *D. acutangula* as a 2-5 carpellate species diagnosable by scorpioid cymes. Seyani's taxonomic lumping may prove to be correct and further revision and study of the continuity of morphological characters across the Malagasy species are certainly in order, as some specimens morphologically fall between or outside of the described species (e.g., *D. cf. marivorahonesis* of this study). These results are concordant with the molecular phylogeny of Le Péchon (2009), in which the 5-carpellate *D. acutangula* taxa from the Mascarenes formed a well-supported clade.

The placement of species with scorpioid cymes relative to other taxa differs depending on the type of data and the method of analysis. Parsimony analyses of ITS (Chapter 2, Fig. 2.3B) and Bayesian analysis of the global dataset (Fig. 3.2) group them as two lineages within one clade with *D. modesta* and *D. autumnalis* (neither of which have scorpioid cymes). Parsimony analyses of chloroplast data alone (Chapter 2, Fig. 2.3A) and the global dataset (Fig. 3.2) group them as two separate lineages in a polytomy with various other clades.

Within *D.* subg. *Xeropetalum*, where taxon sampling was unfortunately limited, few relationships received even moderate bootstrap support and no distinction is seen between *D.* sect. *Decastemon* (umbellate inflorescences) and *D.* sect. *Xeropetalum* (cymes). Likewise, neither ITS nor chloroplast data support subsections within *D.* sect. *Dombeya* as recognized by Arènes (1958, 1959). Arènes' separation of cymose-umbellate species in subsection Cymoso-Umbellatae (*D. rottleroides*, *D. urschiana*, *D. cacuminum*, *D. viburnifloropsis* sampled here) from the typical-umbellate species in the three other subsections of *D.* sect. *Dombeya* is unwarranted. Furthermore, intermediates between "cymose" umbels and "typical" umbels can be found throughout *D.* sect. *Dombeya* calling into question the recognition of two distinct characters. Seyani (1991) employed a much more complex series of classes for inflorescence types in the *Dombeya* of continental Africa and believed these characters to be of taxonomic use, at least in differentiating between closely related species.

No support exists in the molecular data for subsections *Coroniferae* or *Utriculiferae* within *D.* sect. *Dombeya* (Arènes, 1958). *Dombeya* subsect. *Coroniferae* was defined by short and straight androecial tubes, whereas *D.* subsect. *Utriculiferae* has pouch-like androecial tubes that narrow at their apex (Arènes, 1958). Species of these subsections are interdigitated in the phylogenies (e.g., *D*. subsect. *Coroniferae*: *D. erythroclada*, *D. palmatisecta*; *D.* subsect. *Utriculiferae*: *D. mollis*, *D. acerifolia*, *D. befotakensis*). Le Péchon & al. (2009; Le Péchon, 2009) also found no support for the infrageneric divisions within *D.* subg. *Dombeya*.

The general lack of support for many of the phylogenetic relationships within *Dombeya s. str.*, the conflict shown between the chloroplast and nuclear datasets and the fact that collecting is already underway to allow for denser sampling in future phylogenetic studies of this large genus, all provide sufficient reason to wait before hypothesizing new delimitations for infrageneric groups within *Dombeya s. str.*

Evaluating morphological corroboration for the incongruent topologies of chloroplast versus ITS. — The chloroplast and ITS datasets showed incongruent relationships between the four major groups of taxa found in these phylogenies. The chloroplast tree (Fig. 3.1A) posited a sister relationship between *Dombeya* subsect. *Macranthae* and the major Mascarene clade, and placed all the taxa with winged seeds among the early diverging lineages of *Dombeya s. str.* The ITS tree (Fig. 3.1B) placed a clade of the winged seed taxa sister to *D.* subsect. *Macranthae*; the taxa of the major Mascarene group formed a polytomy with the early diverging lineages of *Dombeya s. str.* None of these relationships were well supported. Differing placements of *D. superba*, *D. gautieri* and *D. mandenensis* also contributed to the incongruence. Even in the global analysis of ITS and chloroplast (Fig. 3.2), wherein a winged seed clade sat sister to *D*. subsect. *Macranthae* and a major Mascarene clade sister to a monophyletic *Dombeya s. str.*, bootstrap values for the relationships presented approached only moderate support (BS = 56%, 78%, respectively). An evaluation of morphological characters may help determine which topology is most corroborated: ITS, the chloroplast or the combined analysis.

Morphology supports the ITS tree (Fig. 3.1B) over that of the chloroplast (Fig. 3.1A) because it places taxa with winged seeds and taxa with glandular petals, all described previously, in their respective clades and minimizes reversals between caducous and persistent petals. Neither *D. superba* nor *D. mandenensis* has winged seeds, and *D. mandenensis* has clearly persistent petals (like the remainder of *Dombeya s. str.*). The persistence of the petals of *D. gautieri* is unknown as it has only been collected in flower, but its young ovules show no signs of nascent wings (Skema & Dorr, in press). These morphological characters support the monophyly of both the winged seed taxa and the glandular petal taxa but they do not tell us whether or not these taxa should be placed next to *Dombeya s. str.*

Characters for which *Eriolaena* seems intermediate to *D.* subsect. *Macranthae* or *D. superba* on the one hand and *Helmiopsiella* and *Helmiopsis* on the other also provide more morphological corroboration for the ITS topology than the chloroplast topology. *Eriolaena* shows persistence of the epicalyx into the flowering stage and may be intermediate between *D*. subsect. *Macranthae* with epicalyx bracts that persist below fruits and the majority of species of *Helmiopsis* and *Helmiopsiella* with their early caducous epicalyx bracts. *Dombeya superba* has a long androecial tube (35 mm), more similar to the length of that in *Eriolaena* (10-20 mm) than the characteristically short androecial tubes of *Helmiopsis* and *Helmiopsiella* (≤ 2 mm), but androecial fusion and length may be less reliable characteristics. Long androecial tubes are also seen in *Dombeya s. str.* (e.g., up to 40 mm in *D.* sect. *Astrapaea*) in close phylogenetic proximity to species with much shorter androecial tubes. In the uncommon arrangement of the stamens coming off its androecial tube, *Eriolaena* is most like *D. superba* (Barnett, 1988b), as described previously. Often differentiated from other dombeyoids by its many stamens, the number of stamens in *Eriolaena* is actually similar to that of *D.* subsect. *Macranthae* and *Helmiopsiella leandrii*, although both of the latter have all of their stamens diverging at or very near the apex of the tube. The occurrence of 8 or more ovules per locule in *Eriolaena* could be interpreted as another intermediate state between the many ovules of *D. superba* (~20) and some *D.* subsect. *Macranthae* and the 2(-4) ovules per locule seen in *Helmiopsiella* and *Helmiopsis*.

The presence of pinnatilobed or fimbriate epicalyx bracts and centraseptal pubescence in both *Eriolaena* and *Helmiopsiella*, or in at least some species of each, was noted by Barnett (1988b), but critical to this discussion is that *Dombeya* subsect. *Macranthae* also possesses both of these characters. Among the dombeyoids studied here, pinnatilobed, fimbriate and highly divided epicalices are unique to *Eriolaena*, *Helmiopsiella* and *D.* subsect. *Macranthae* and are not seen in *Dombeya s. str*. Some species of *Eriolaena* possess entire, ovate epicalyx bracts (e.g., *Eriolaena spectabilis*) reminiscent of those of *D. superba*. Centraseptal pubescence is also seen occasionally among members of *Dombeya s. str.* (e.g., *D. palmatisecta*). Despite its many morphological autapomorphies, *Eriolaena* seems to demonstrate affinities to *D. superba*, *D.* subsect. *Macranthae* and *Helmiopsis*/*Helmiopsiella*.

All these taxa, and *Eriolaena* in particular, tip the balance of morphological characters in favor of a phylogenetic hypothesis more similar to the placement of the winged seed clade in the ITS phylogeny than to that of the winged seed grade in the chloroplast tree, but all of these features are mapped most parsimoniously onto the topology resulting from the global analysis of the chloroplast and ITS data together (Fig. 3.2).

***Dombeya* subsection *Macranthae*.** — Although members of *Dombeya* subsect. *Macranthae* are not the only species of *Dombeya* to be excluded from *Dombeya s. str.* in these analyses, they are the only taxa distinct from *Dombeya s. str.* in *both* the chloroplast and ITS phylogenies (Fig. 3.1). Four of the five species of this subsection were sampled for this study with only *D. seyrigii* missing. ITS supports a clade of the four species sampled from *D.* subsect. *Macranthae* plus *D. moratii* with 86% bootstrap support. In the chloroplast data, only *D. macrantha* is omitted from the group and placed sister to it in a trichotomy. The global analysis grouped all five species with high support (BS=99%).

Arènes (1958) recognized *D.* subsect. *Macranthae* as having flowers with articulated pedicels that bear two bracts midway along their length. He placed the subsection within *D.* sect. *Trochetiantha* because of their solitary flowers. These species form a morphologically cohesive group, with relatively large, pink or red flowers, persistent perianth parts, fascicular stamens, linear staminodes and large capsular fruits with many seeds and sometimes tomentose centraseptal pubescence. They are unique among the Dombeyaceae in that the epicalyx bracts are pinnately or palmately divided to dissected and persist, along with the perianth, into the fruiting stage.

Dombeya moratii, although it has 3-flowered umbels rather than solitary flowers (Barnett & Dorr, 1986), should be included within this group because it possesses the above combination of morphological features and is a member of the clade both in the chloroplast and ITS trees. Solitary flowers are not so uncommon or unlikely to evolve that they must connote a synapomorphy for *D.* subsect. *Macranthae* to the exclusion of *D. moratii*. To the contrary, a nearly identical analog exists in the morphologically coherent genus *Trochetia*, where large, solitary flowered species with articulated pedicels bearing two bracts (*T. granulata, T. uniflora, T. boutoniana, T. blackburniana*) form a natural group with species sporting 3-flowered umbels (*T. parviflora* and *T. triflora*; Friedmann, 1987).

Dombeya as traditionally circumscribed is clearly paraphyletic (this study; Le Péchon, 2009; Le Péchon & al., 2009) and as such its delimitations must be revised.

Recognition of *D.* subsect. *Macranthae*, expanded to include *D. moratii*, at the generic level is a logical place to begin this work given the morphological coherence of these species and the consistent distinctiveness of this lineage in the molecular analyses.

TAXONOMIC TREATMENT

Andringitra Skema, **gen. nov.** ≡ *Dombeya* Cav. subsect. *Macranthae* Arènes in Candollea 16: 281. 1958 – Type: ***Andringitra macrantha*** (Baker) Skema, ***comb. nov.*** ≡ *Dombeya macrantha* Baker in J. Linn. Soc., Bot. 21: 325. 1884. – Type: Betsiléo, in Felsspalten, Apr 1881, *J. M. Hildebrandt 3895* (lectotype, designated by Arènes in Candollea 16: 283. 1958: K!, image seen; isolectotypes: P 00044722!, P 00044721!, images seen).

Genus epicalycum bracteis palmatis vel pinnatis divisis ad dissectis et ad maturitatem fructus persistentibus a confamilis differt.

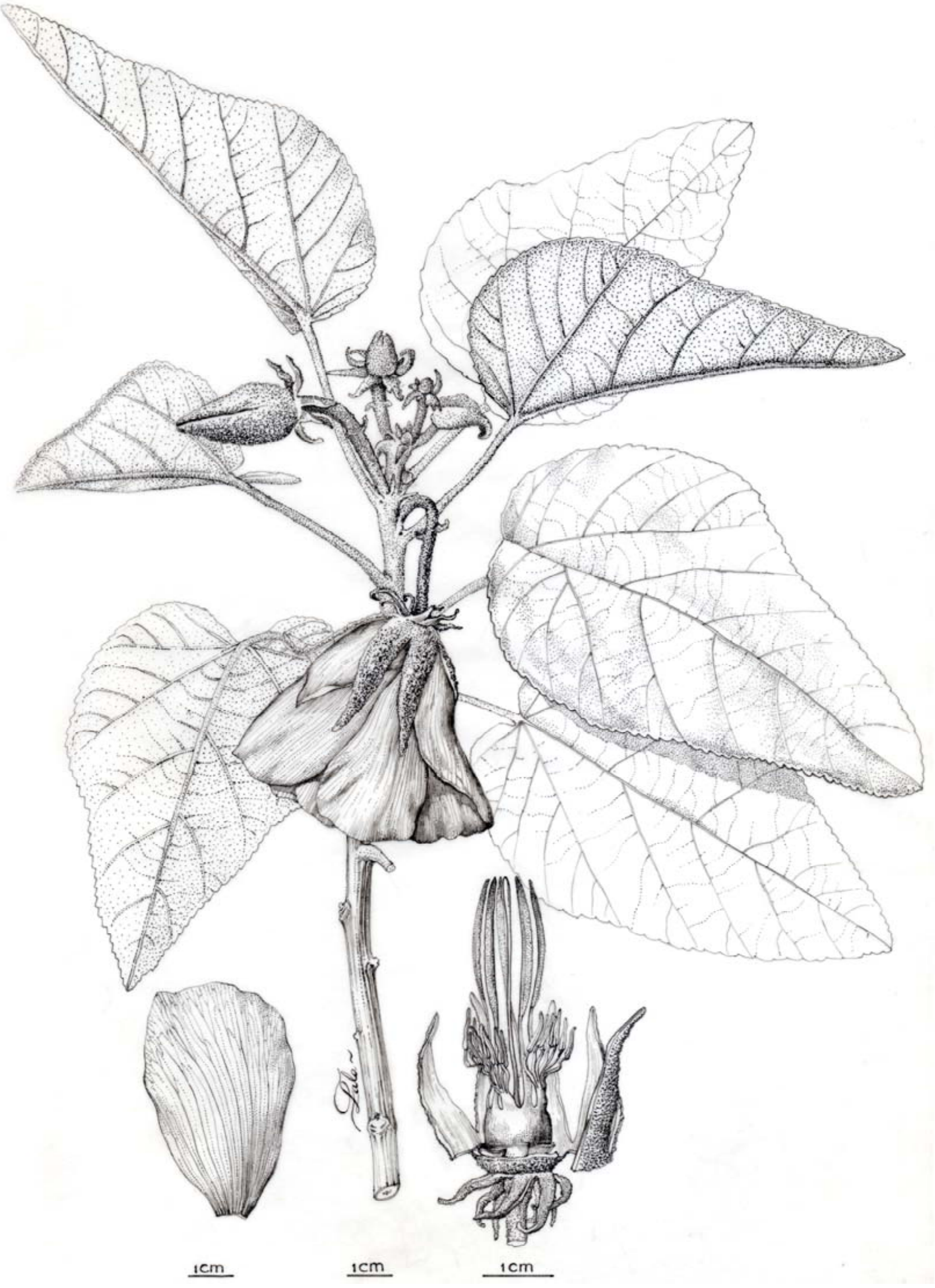
Shrubs or small trees, 1-4(-6) m. Leaves (narrowly) ovate to lanceolate to narrowly oblong, unlobed or occasionally palmately 3-lobed, the lateral lobes much shorter than median (*A. moratii*); apex acute to acuminate; base very shallowly to deeply cordate; margin entire to crenulate to serrulate. Stipules caducous or persistent, acicular to lanceolate to pinnatilobed to palmately divided or dissected. Inflorescences axillary, usually solitary-flowered or sometimes in 3-flowered umbels (*A. moratii*), and bearing two caducous or persistent bracts where the floral axis is articulated. Flowers hermaphroditic, large (2.5 – 6 cm). Epicalyx of three bracts directly subtending flower and persistent beneath fruits; bracts palmately or pinnately divided to dissected, sometimes fimbriate, laciniate or even 6- or 9-parted, usually free or sometimes shortly fused to one another basally. Sepals 5, valvate, shortly connate, abaxially glabrous to densely pubescent, adaxially glabrous with single ovate patch of nectariferous papillae basally. Petals 5, free, convolute, asymmetric, obovate, pink or red, sometimes with white striping or orangish tinge. Androecium monadelphous and coroniform; staminal tube 3-8(-10) mm long; stamens 15-45(-55), arising from apex of staminal tube in fascicles of 3-9(-11) alternating with staminodes; anthers linear to slightly sagittate, longitudinally dehiscent; staminodes 5, linear to lanceolate to narrowly spathulate, densely warty on abaxial surface of distal portion, surpassing stamens and often almost equal to style in length. Ovary superior, 5-locular, ovoid to globose, densely pubescent; placentation axile, ovules 8-20 per carpel, sometimes in two rows; style 1, sometimes striated or shallowly pentacostulate, 5-parted, lobed or toothed distally. Fruit ovoid to globose loculicidal capsule with brown pubescence, sometimes tomentosely pubescent in central axis. Seeds tetragonal, brown, dull. Cotyledons bipartite. Fig. 3.3.

The following new combinations are proposed:

Andringitra leiomacrantha (Hochr.) Skema, **comb. nov.** ≡ *Dombeya leiomacrantha* Hochr. in Candollea 3: 104. 1926. – Type: massif d'Andringitra sur granite, vers 1600 m. alt., Sep 1911, *H. Perrier de la Bâthie 5597* (lectotype, designated by Arènes in Candollea 16: 281. 1958: P!, image seen; isolectotype: G!, image seen).

Andringitra leiomacrantha subsp. ***angustata*** (Arènes) Skema**, comb. nov.** ≡ *Dombeya leiomacrantha* subsp. *angustata* (Hochr.) Arènes in Candollea 16: 282. 1958. ≡ *Dombeya leiomacrantha* var. *angustata* Hochr. in Candollea 3: 106. 1926. – Type: massif de l'Andringitra (Iratsy): vallées de la Riambava et de l'Antsifotra et montagnes environnantes, 27 Novembre – 8 Décembre 1924, *H. Humbert 3806*

Figure 3.3. *Andringitra macrantha* (Baker) Skema: branch with solitary flowers (**A**), petal (**B**), detail of flower with petals removed, persistent epicalyx subtends flower, sepals shown partially cut away, androecium with fascicular stamens alternating with linear staminodes equal in height to style apex (**C**). *C. Skema 12*. Illustration by Roger Lala Andriamiarisoa.


1cm
1cm
1cm

(lectotype, designated by Arènes, l.c.: P!, image seen; isolectotype: G!, image seen)

Andringitra leucomacrantha (Hochr.) Skema, **comb. nov.** ≡ *Dombeya leucomacrantha* Hochr. in Candollea 3: 106. 1926. – Type: brousse éricoïde, massif d'Andringitra, vers 2400 m., Feb. 1922, *H. Perrier de la Bâthie 14398* (lectotype, designated by Arènes in Candollea 16: 284. 1958: P!, image seen; isolectotype: G!, image seen).

Andringitra moratii (L. Barnett & Dorr) Skema, **comb. nov.** ≡ *Dombeya moratii* L. Barnett & Dorr in Bull. Mus. Natl. Hist. Nat., sect. B, 8: 365. 1986. – Type: 1er étage de la Brioche ou "Fandrana" Alt. ~1200 m. P.K. 545 à 64 km d'Ihosy à Ambalavao, May 1971, *G. Cremers 1541* (holotype: P!, image seen).

Andringitra muscosa (Hochr.) Skema, **comb. nov.** ≡ *Dombeya muscosa* Hochr. in Candollea 3: 109. 1926. – Type: partie ouest du massif d'Andringitra, granite, 1600 m. d'alt., (date illegible), *H. Perrier de la Bâthie 5599* (lectotype, designated by Arènes in Candollea 16: 283. 1958: P!, image seen; isolectotype: G!, image seen).

Andringitra seyrigii (Arènes) Skema, **comb. nov.** ≡ *Dombeya seyrigii* Arènes in Candollea 16: 282. 1958. – Type: lisières supérieures de la forêt d'Analakoky au S.E. [southeast] d'Ampandrandava vers 1400 m d'alt., 1945, *A. Seyrig 844* (lectotype, designated here: P 00044968!, image seen; isolectotype: P 00044969!, image seen).

The generic name is taken from the Malagasy name for the massif in Fianarantsoa Province, which is the home of the majority of species of the genus. This generic name serves as homage to the wonderful, unique, mountainous habitat in which many of these spectacular, large-flowered plants can be found rising above ericoid brush in the open, mid- to high-altitude bushlands around Peak Boby. *Andringitra* includes six species, all endemic to Madagascar. *Andringitra macrantha* is the widest ranging and northernmost (e.g., Ambositra, Ibity, Itremo) in the distribution, though it is also recorded from Andringitra massif. *Andringitra leiomacrantha* and *A. muscosa* are known only from Andringitra and *A. moratii* occurs nearby to the north and west in the areas of Ambalavao and Ankaramena. *Andringitra leucomacrantha* can be found at Andringitra and also the nearby Peak Ivohibe and further south to Beampingaratra (north of Andohahela). *Andringitra seyrigii* also occurs in the south, in mid-altitude areas around Betroka and Isoanala in Toliara province.

Andringitra differs from other genera in Dombeyaceae by having conspicuous, palmately or pinnately divided to dissected epicalyx bracts that are persistent into the fruiting stage along with the perianth. The genus is in need of revision. The last treatment was by Arènes (1959, as *Dombeya* subsect. *Macranthae*) and recent collections stretch some of the boundaries of the morphological descriptions from that work. For example, new specimens of *A. muscosa*, originally described from a single collection, have the highly crispate and laciniate epicalyx bracts characteristic of the species but differ from the type specimen in the indument and length of these bracts (i.e., they are shorter than the calyx lobes) and indument of the leaves. Additional collections must be made before a full reappraisal of the genus can be completed because multiple species remain undercollected; for instance, the fruits of *A. moratii* are yet unknown.

Secondary pollen presentation, on the warty abaxial face of the staminodes and adaxial petal apices, was observed for many species of *Andringitra* in the field and seems to be the rule. Indument density varies widely across, and even within, species, from densely tomentose on nearly all organs (some *A. macrantha*) to completely glabrous (*A. leiomacrantha* subsp. *leiomacrantha*). Pubescence on the leaves and calyx, is often comprised of stalked, stellate hairs, presumably the "*écailleux-étoilé*" of Arènes (1959: 195, etc.). These hairs are quite striking visually and can have a stalk of varying length (~0.05 – 0.15 mm) with few (5-8) to many (10-20) arms radiating in various directions from the apex of the stalk. Some hairs even have tiers of radiating arms at the apex and the base.

Further new combinations.

Helmiopsis linearifolia (Hochr.) Skema, **comb. nov.** ≡ *Dombeya linearifolia* Hochr. in Annuaire Conserv. Jard. Bot. Genève 11-12: 1. 1908 [30 Dec 1907]. – Type: nord du Madagascar, 1835, *A. C. J. Bernier 340 pro min. parte* (lectotype, designated by Arènes in Candollea 16: 297. 1958: G 00015437!, image seen).

Helmiopsis linearifolia subsp. **sely** (Arènes) Skema, **comb. nov.** ≡ *Dombeya linearifolia* subsp. *sely* Arènes in Candollea 16: 297. 1958. – Type: Montagne des Français, Diégo-Suarez, 1954, *Service Forestier 9734* (lectotype, designated here: P 00044702!, image seen; isolectotypes: K, P 00044703!, TEF!, images seen).

LITERATURE CITED

Akaike, H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19: 716-723.

Alverson, W. S., Whitlock, B. A., Nyffeler, R., Bayer, C. & Baum, D. A. 1999. Phylogeny of the core Malvales: Evidence from *ndhF* sequence data. *American Journal of Botany* 86: 1474-1486.

Applequist, W. L. 2009a. Two new species of *Dombeya* (Malvaceae) from Madagascar. *Novon* 19: 289-294.

Applequist, W. L. 2009b. A revision of the Malagasy endemic *Helmiopsis* (Malvaceae *s.l.*). *Annals of the Missouri Botanical Garden* 96: 521-540.

Arènes, J. 1956a. Contributions a l'étude des Sterculiacées de Madagascar XVIII: Un genre malgache de Sterculiacées nouveau pour la science. *Bulletin du Muséum, ser. 2* 28: 150-152.

Arènes, J. 1956b. *Dendroleandria g. n. madagascariensis*. *Mémoires de l'Institut Scientifique de Madagascar, séries B* 7: 65-67.

Arènes, J. 1958. Les *Dombeya* de Madagascar et des Comores. *Candollea* 16: 247-449.

Arènes, J. 1959. *Flore de Madagascar et des Comores, Sterculiacées.* Firmin-Didot, Paris.

Baillon, H. E. 1885. Liste des plantes de Madagascar. *Bulletin Mensuel de la Société Linnéenne de Paris* I: 491-496.

Barnett, L. C. 1987a. Tribal realignment of certain paletropical Sterculiaceae. *American Journal of Botany Supplement* 74: 724.

Barnett, L. C. 1987b. An unusual new species of *Helmiopsis* H. Perrier (Sterculiaceae) from Madagascar. *Annals of the Missouri Botanical Garden* 74: 450-452.

Barnett, L. 1988a. New combinations in the genus *Helmiopsiella* (Sterculiaceae). *Bulletin du Museum National d'Histoire Naturelle, sect. B, Adansonia* 10: 69-76.

Barnett, L. 1988b. Systematics of *Nesogordonia* Baillon (Sterculiaceae). Ph.D. thesis, University of Texas, Austin. U.M.I., Ann Arbor, MI.

Barnett, L. C. & Dorr, L. J. 1986. A new species and variety of *Dombeya* (Sterculiaceae) from Madagascar. *Bulletin du Museum National d'Histoire Naturelle, sect. B, Adansonia* 8: 365-371.

Barriel, V. 1994. Phylogénies moléculaires et insertions-délétions de nucléotides. *C. R. Acad. Sci. Paris, Sciences de la vie* 317: 693-701.

Baum, D. A., Small, R. L. & Wendel, J. F. 1998. Biogeography and floral evolution of baobabs (*Adansonia*, Bombacaceae) as inferred from multiple data sets. *Systematic Biology* 47: 181-207.

Bayer, C. 1999. The bicolor unit— homology and transformation of an inflorescence structure unique to core Malvales. *Plant Systematics and Evolution* 214: 187-198.

Bayer C. & Kubitzki, K. 2003. Malvaceae. Pp. 225-311 in Kubitzki, K. and Bayer, C. (eds.), *The Families and Genera of Vascular Plants,* vol. V. Springer-Verlag, Berlin.

Bayer, C., Fay, M. F., de Bruijn, A. Y., Savolainen, V., Morton, C. M., Kubitzki, K., Alverson, W. S. & Chase, M. W. 1999. Support for an expanded family concept of Malvaceae within a recircumscribed order Malvales: A combined analysis of plastid *atpB* and *rbcL* DNA sequences. *Botanical Journal of the Linnean Society* 129: 267-303.

Ben-Dov, Y. 2010. On new taxa and some described armoured scale insects (Hemiptera: Diaspididae) living in the galleries of the ant *Melissotarsus insularis* Santschi (Hymenoptera: Formicidae) in Madagascar. *Zootaxa* 2368: 49-58.

Cavanilles, A. J. 1786. *Secunda Dissertatio Botanica*. Madrid.

Cavanilles, A. J. 1787. *Tertia Dissertatio Botanica*. Madrid.

Chattaway, M. 1932. The wood of the Sterculiaceae I. Specialisation of the vertical wood parenchyma within the subfamily Sterculieae. *New Phytologist* 31: 119-132.

Cordemoy, J. 1895. *Flore de l'Ile de la Réunion.* Paul Klincksieck, Paris.

de Candolle, A. P. 1822. Sur quelques genres nouveaux de la famille des Buttneriacées. *Mémoires du Muséum d'Histoire Naturelle* 9: 97-114 + plates.

Dorr, L. J. 2001. A new combination in the endemic Malagasy genus *Helmiopsis* (Malvaceae-Dombeyoideae). *Adansonia*, *series 3* 23: 151--153.

Edlin, H. L. 1935. A critical revision of certain taxonomic groups of the Malvales. *New Phytologist* 34: 1-20.

Engler, A. 1907. Tiliaceae africanae III: Cistanthera. *Botanische Jahrbucher fur Systematik, Pflanzengeschichte und Pflanzengeographie* 39: 577-579.

Erdtman, G. 1952. *Pollen morphology and plant taxonomy.* Almqvist & Wiksells, Uppsala.

Friedmann, F. 1987. Sterculiacées. In: J. Bosser, Th. Cadet, J. Guého & W. Marais (eds.), *Flore des Mascareignes: La Réunion, Maurice, Rodrigues 53.* The Sugar Industry Research Institute, Mauritius.

Gautier L. & Goodman, S. M. 2003. Introduction to the flora of madagascar. Pp. 229-250 in Goodman, S. M. and Benstead, J. P. (eds.), *The Natural History of Madagascar.* The University of Chicago Press, Chicago.

Goloboff, P. A., Farris, J. S. & Nixon, K. C. 2008. TNT, a free program for phylogenetic analysis. *Cladistics* 24: 774-786.

van Heel, W. A. 1966. Morphology of the androecium in Malvales. *Blumea* 8: 177-394.

Hochreutiner, B. P. G. 1926. Monographie des *Dombeya* de Madagascar. *Candollea* III: 5-120.

Hochreutiner, B. P. G. 1907. Malvaceae et Sterculiaceae novae. *Annuaire du Conservatoire et du Jardin Botaniques de Genève* 11: 1-9.

Janssen, T., Bystriakova, N., Rakotondrainibe, F., Coomes, D., Labat, J. & Schneider, H. 2008. Neoendemism in Madagascan scaly tree ferns results from recent, coincident diversification bursts. *Evolution* 62: 1876-1889.

Le Péchon, T. 2009. Systématique des Dombeyoideae (Malvaceae, ex-Sterculiaceae) des Mascareignes: Approches morphologique et moleculaire. Ph.D. thesis, l'Université Pierre et Marie Curie, Paris, France.

Le Péchon, T., Cao, N., Dubuisson, J. & Gigord, L. D. B. 2009. Systematics of Dombeyoideae (Malvaceae) in the Mascarene archipelago (Indian Ocean) inferred from morphology. *Taxon* 58: 519-531.

Léotard, G., Saltmarsh, A., Kjellberg, F. & Mckey, D. 2008. Mutualism, hybrid inviability and speciation in a tropical ant-plant. *Journal of Evolutionary Biology* 21: 1133-1143.

Lindley, J. 1844. *Astiria rosea*. *Edwards's Botanical Register* 30: 27, t.49.

Marais, W. 1981. *Trochetiopsis* (Sterculiaceae), a new genus from St. Helena. *Kew Bulletin* 36: 645-646.

Nixon, K. C. 2002. *WinClada version 1.86.* Published by the author, Ithaca, NY, USA.

Nyffeler, R., Bayer, C., Alverson, W. S., Yen, A., Whitlock, B. A., Chase, M. W. & Baum, D. A. 2005. Phylogenetic analysis of the Malvadendrina clade (Malvaceae s.l.) based on plastid DNA sequences. *Organisms, Diversity and Evolution* 5: 109-123.

Nylander, J. A. A. 2004. *MrModeltest version 2.* Program distributed by the author, Evolutionary Biology Centre, Uppsala University.

Perrier de la Bâthie, H. 1944. Les genres *Nesogordonia* Baillon et *Helmiopsis g. n.* de Madagascar. *Bulletin de la Société Botanique de France* 91: 226-232.

Ree, R. 2008. *gapcode.py, a python script for coding indels in sequence alignments as binary characters for phylogeny reconstruction.* Program distributed by author, http://www.reelab.net/home/software.

Ronquist, F. & Huelsenbeck, J. P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.

Schatz G. E. 1996. Malagasy/Indo-australo-malesian phytogeographic connections. Pp. 73-84 in Lourenço, W. R. (ed.), *Biogéographie de Madagascar.* ORSTOM, Paris.

Schumann, K. 1890. Sterculiaceae. Pp. 69-99 in Engler, A. and Prantl, K. (eds.), *Die Naturlichen Pflanzenfamilien.* Engelmann, Leipzig.

Schumann, K. 1900. Sterculiaceae africanae. Pp. 1-140 in Engler, A. (ed.), *Monographien afrikanischer Pflanzen-familien und-Gattungen.* Engelmann, Leipzig.

Seyani, J. H. 1991. *Opera Botanica Belgica, vol. 2: The Genus* Dombeya *(Sterculiaceae) in Africa.* National Botanical Garden of Belgium, Meise.

Shaw, J., Lickey, E. B., Beck, J. T., Farmer, S. B., Liu, W. S., Miller, J., Siripun, K. C., Winder, C. T., Schilling, E. E. & Small, R. L. 2005. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* 92: 142-166.

Shaw, J., Lickey, E. B., Schilling, E. E. & Small, R. L. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. *American Journal of Botany* 94: 275-288.

Simmons, M. P. & Ochoterena, H. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369-381.

Skema, C. & Dorr, L. J. In press. *Dombeya gautieri* (Dombeyaceae), a remarkable new species from Madagascar. *Kew Bulletin* 65(3).

Swofford, D. L. 2001. *PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0.* Sinauer, Sunderland, MA, USA.

Takeda, H. 1912. The genus *Corchoropsis*. *Bulletin of Miscellaneous Information, Royal Botanic Gardens, Kew*365.

Tang, Y. 1992. The systematic position of *Corchoropsis* Sieb. et Zucc. *Cathaya* 4: 131-150.

Tang, Y., Gao, H. & Xie, J. 2009. An embryological study of *Eriolaena candolleii* Wallich (Malvaceae) and its systematics implications. *Flora* 204: 569-580.

White T. J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322 in Innis, M., Gelfand, D., Sninsky, J. & White, T. (eds.), *PCR Protocols: A Guide to Methods and Applications.* Academic Press, San Diego.

Way, M. J. 1963. Mutualism between ants and honeydew-producing Homoptera. *Annual Review of Entomology* 8: 307-344.

Won, H. 2009. Phylogenetic position of *Corchoropsis* Siebold & Zucc. (Malvaceae *s.l.*) inferred from plastid DNA sequences. *Journal of Plant Biology* 52: 411-416.

Yoder, A. D. & Nowak, M. D. 2006. Has vicariance or dispersal been the predominant biogeographic force in Madagascar? Only time will tell. *Annual Review of Ecology and Evolutionary Systematics* 37: 405-431.

CHAPTER 4

REVISION OF *DOMBEYA* SECTION *ASTRAPAEA* (DOMBEYACEAE)

ABSTRACT

Dombeya section *Astrapaea* includes five species endemic to Madagascar and the Comoros. Molecular and morphological data support the inclusion of these species within *Dombeya*. The section is distinguished within the genus by pendulous inflorescences and long staminal tubes. Morphological study demonstrated that characters previously used in the taxonomy of *D.* sect. *Astrapaea* overlap between species and sometimes even vary significantly within individuals or populations, leading to a broader circumscription of some species than that of previous workers. This treatment summarizes the taxonomic history, morphology, nomenclature and distribution of *D.* sect. *Astrapaea*. One new subspecies, *D. cannabina* subsp. *antsifotrensis*, is described and a dichotomous key to the species is provided.

INTRODUCTION

Dombeya Cav. *nom. cons.* (Dombeyaceae or Dombeyoideae, Malvaceae *s.l.* or Sterculiaceae *pro parte*) is a paleotropical genus with roughly 200 species. An astounding 175 species are found on Madagascar and the Comoro Islands, 15 in the Mascarenes (Friedmann, 1987), and 19 in continental Africa (from where one, *D. torrida*, extends onto the Arabian peninsula) (Seyani, 1991). All species, except for *D. acutangula sensu* Seyani, are endemic to each geographical area. *Dombeya* alone comprises around 2% of the flora of Madagascar and as such is a fine example of the remarkable biotic diversity found on the island. The most recent and complete taxonomic study of the Malagasy species of *Dombeya* was that of Arènes (1959) for the *Flore de Madagascar*; subsequent revisionary studies have treated only the African (Seyani, 1991) and Mascarene species (Friedmann, 1987). The present revision of section *Astrapaea* begins the needed taxonomic reappraisal of species delimitations within the *Dombeya* of Madagascar and integrates new data gathered from generic-level molecular and taxonomic studies (Chapters 2 and 3) into the sectional treatment.

Dombeya section *Astrapaea* contains five woody species of trees or shrubs endemic to Madagascar and the Comoros. The section occurs predominantly along waterways in the remaining rainforests on the island, resulting in a narrow distribution running parallel to the eastern coast and up into the far north. However, these species are neither limited to rainforests nor a riverine habitat, and can be found in secondary, montane and sublittoral forests. They are also often left standing in agricultural fields and on roadsides due to the usefulness of the inner bark as rough cordage. Section *Astrapaea* is easily distinguished from the remainder of *Dombeya* by a long androecial tube equal to or greatly surpassing the petals in length. This section was chosen to begin the revision of the large and complex genus *Dombeya* because the distinctive morphology of these species indicated they may form a monophyletic group.

TAXONOMIC HISTORY

It is a testament to how striking is the morphology of *Dombeya* sect. *Astrapaea*, particularly the staminal tube, that the first two species named from this group were described in new genera, *Astrapaea wallichii* (Lindley, 1821) and *Hilsenbergia cannabina* (Bojer, 1841). The only other early taxonomic record for the section was by Hooker (1837) who, working in part from some of the same collections as Bojer (1841), described this plant as *Dombeya cannabina*. Hooker discussed how the species possessed characters of both *Dombeya* and *Astrapaea* and suggested the potential synonymy of these genera. Whether species of sect. *Astrapaea* belonged in *Dombeya* or not would remain an open question for the next 30-60 years.

Lindley's description (1821) of *Astrapaea wallichii* was based on a curious specimen sent to him from Nathaniel Wallich, curator of the Botanic Garden of Calcutta, via A. B. Lambert, a gentleman, botanist, and avid collector of plant specimens (Miller, 1970). The many massive, red-flowered umbels hanging below the broad leaves of the large shrub grown from seed at Cambridge impressed Lindley (1821: ad t.14) so greatly that he wrote of it, "…we think, if *Rafflesia* be excepted, it must be one of the most superb plants in the world." He justified assigning the species to a new genus *Astrapaea*, rather than to *Dombeya* (or *Pentapetes*), citing the involucre of bracts subtending the umbellate inflorescence, the "rolled together" petals, the long staminal tube and the lack of an epicalyx as distinguishing characteristics. In fact, all species of sect. *Astrapaea* have three epicalyx bracts, tightly convolute petals and a long staminal tube. The umbellate inflorescence with an involucre of bracts beneath is unique to *D. wallichii*. Bojer (1841) subsequently recognized the new genus *Hilsenbergia* based on his own collections from Madagascar. He noted the dense corymbose cymes under the generic description, and, indeed, it is a feature that separates *Hilsenbergia cannabina* from both *Astrapaea wallichii* and *Dombeya*.

In the latter half of the 19th century, two more species of sect. *Astrapaea* with corymbose cymes were described in *Dombeya*, *D. hilsenbergii* (Baillon, 1885b) and *D. baronii* (Baker, 1887), but the majority of the taxonomic work involving sect. *Astrapaea* from this time returned to Hooker's earlier question of generic delimitation. Lines were drawn and redrawn for generic circumscriptions as each author weighed the various characters overlapping in *Astrapaea*, *Dombeya* and *Hilsenbergia*. For example, Planchon (1850) argued that *Astrapaea* and *Dombeya* were good genera, although he would include *Hilsenbergia* as a section within *Dombeya*. In contrast, Endlicher (1865) synonymized *Hilsenbergia* with *Astrapaea* but maintained them as separate from *Dombeya*. Neither Bentham and Hooker (1862) nor Baillon (1875, 1885a) mention *Hilsenbergia*, but both readily synonymized *Astrapaea* with *Dombeya*. Finally, *Astrapaea* was formally placed as a section in *Dombeya* near the close of the century (Gómez, 1890).

The 20th century saw Hochreutiner (1926) describe two new species within sect. *Astrapaea*. Later, in revising *Dombeya* for the *Flore de Madagascar*, Arènes (1958) added eight new species to the section and proposed the first infrasectional taxonomy for the group. His three subsections, with their salient features, are as follows: i) subsect. *Involucratae* — umbellate inflorescences subtended by a large involucre of bracts, staminal tube equal to corolla in length; ii) subsect. *Cannabinae* — corymbose cymes, staminal tube long-surpassing corolla, leaves ovate and cordiform; and iii) subsect. *Somanga* — corymbose cymes, staminal tube long-surpassing corolla, leaves suborbicular or (sub)palmately lobed. The species belonging to each of these subsections as well as a comparison of Arènes' work (precursor in 1958; treatment in 1959) and the work presented here can be seen in Table 4.1. General differences in characters recognized in the present work and that of Arènes are discussed where relevant in the Morphology section below.

MATERIALS & METHODS

This revision is based on roughly 200 herbarium specimens. *Dombeya* section *Astrapaea*, like many taxa in Madagascar, is under collected. The number of specimens of *D.* sect. *Astrapaea* collected for this study augmented previously collected herbarium specimens by 50% and provided useful insights into morphological variation within the group. Nonetheless, further collections are needed, particularly of *D. hafotsy*, wild *D. wallichii* and individuals from the Comoros. I have followed the phylogenetic species concept (Nixon & Wheeler, 1990) in delimiting species, attempting to recognize the smallest units of individuals supported by unique character combinations.

Table 4.1. Lineages proposed by molecular data (Chapter 2) and species proposed in this work for *Dombeya* sect. *Astrapaea* and a comparison to the last treatment of the group (Arènes 1958, 1959). Shading of Arènes' species signifies his subsection designations: species in unshaded (white) cells were placed in *D.* subsect. *Cannabinae*; species in grey cells in *D.* subsect. *Somanga*; *D. wallichii* in the black cell in *D.* subsect. *Involucratae*.

lineages proposed by molecular data	**species proposed in current work**	**species of Arènes**
A	***hilsenbergii***	***hilsenbergii***
		macropoda
	hafotsy	***hafotsy***
		tsiapetrokensis
		somanga
B	***cannabina***	***cannabina***
		capuronii
		condensata
C	***wallichii***	***wallichii***
	baronii	***mandrakensis***
		ankazobeensis
		alleizettei
		perrieri
		baronii

Measurements were generally made from herbarium materials rehydrated in boiling water with some detergent added. Length measurements of leaf lamina were made from the apex of the leaf to the basal-most portion of the lamina, i.e., the longest observable length of the blade. Midvein length measurements were made from the apex of the leaf to the insertion point of the lamina on the petiole. Colors described are from personal observations in the field, photographs, or herbarium label data;

where colors pertain only to dried plant materials, this is noted. Where color descriptions are omitted (e.g., *D. hafotsy*), no information from living plants was available. Localities for the distribution maps were primarily obtained from GPS coordinates when available for a specimen, but for specimens that lacked these data and were unambiguously from a known locality, georeference points were used for their coordinates. Georeference points were derived from GPS data from my field collections or from the online Gazetteer to Malagasy Botanical Collecting Localities (Schatz & Lescot, 2003). Specimens mapped with georeference points are denoted with the latitude and longitude in brackets in the specimens cited for each species. Vernacular names are in Malagasy except where noted in parentheses and explanation of these names is provided in brackets when known. For the widely cultivated *D. wallichii*, only specimens from Madagascar are mapped. Barcode numbers are noted for herbarium sheets only where they are required to differentiate between multiple duplicates of a collection housed in one herbarium or for specimens for which no or very few label data are known (e.g., collector or collector number unknown).

PHYLOGENY & SECTIONAL DELIMITATION

Not surprisingly given the morphological similarities, the inclusion of *Astrapaea* in *Dombeya* is supported by recent molecular phylogenic work (Chapter 2). These data were derived from sequences of ITS and five noncoding plastid regions obtained from thirteen individuals, sampling each of the five species. As delimited in Chapter 3, *Dombeya s.str.* includes most traditional members of the genus *Dombeya*, excluding only *D.* subsect. *Macranthae*, *D. superba*, *D. linearifolia* and some species of the Mascarenes. Recognition of *D.* sect. *Astrapaea* as a genus would require considerable division of *Dombeya* as currently recognized and the creation of multiple morphologically similar genera. Such a taxonomy is unwarranted and *Astrapaea* is considered to fit well as a section within *Dombeya*.

The monophyly of sect. *Astrapaea* is neither supported nor refuted by the phylogenetic work (Chapters 2 & 3). Parsimony analysis (Chapter 3, Fig. 3.2) of combined plastid and nuclear data placed all accessions of sect. *Astrapaea* as members of a large comb with various species from *Dombeya* sect. *Dombeya* from Madagascar and the Mascarenes. No molecular phylogeny grouped all accessions of sect. *Astrapaea*, nor did any phylogeny show much resolution between any of the small clades occurring within *Dombeya s.str.* Further sampling of molecular markers could help address the question of monophyly of *Astrapaea*. Given the remarkable suite of morphological characters common to the section, it seems likely these species will prove to be monophyletic and they are considered as such here.

Within the genus, sect. *Astrapaea* appears to be most closely related to the Malagasy members of sect. *Dombeya*. Relationships within *Dombeya* are being reevaluated and the reappraisal of generic delimitations are a work in progress (Chapter 3) as we further develop our understanding of evolution within Dombeyaceae. As a result of this flux, neither a generic description of *Dombeya* nor a key to the sections within the genus is provided here. This treatment focuses solely on *Dombeya* section *Astrapaea*.

SPECIES RELATIONSHIPS & DELIMITATIONS

A comparison of plastid and nuclear phylogenies of species of sect. *Astrapaea* suggested a complex evolutionary history for the group (Chapter 2, particularly Fig. 2.3). The three clades of sect. *Astrapaea* in the plastid phylogeny corresponded to the geographic location of specimens rather than species boundaries and morphological characteristics. In contrast, the nuclear phylogeny, showed three major lineages of sect. *Astrapaea*: i) lineage A, *D. hafotsy* + *D. hilsenbergii*; ii) lineage B, *D. cannabina* and iii) lineage C, *D. wallichii* + *D. baronii* (see Table 4.1 above). These ITS clades corresponded more closely to morphology and united individuals from within single species. From these data, plastid introgression has been hypothesized. Multiple, morphologically coherent species, that are often sympatric for some portion of their distribution, share regional plastid haplotypes. Observations of individuals morphologically intermediate between the three broadly distributed, and often sympatric, species of sect. *Astrapaea* (*D. baronii*, *D. cannabina* and *D. hilsenbergii*) in one particular locality (Vohiparara, Fianarantsoa province) support the hypothesis for gene flow across species in the section. Discussion of these putative hybrids can be found under the species description of *D. baronii*.

Morphology within sect. *Astrapaea* generally corroborates relationships derived from the ITS phylogeny. Lineage A, *D. hilsenbergii* and *D. hafotsy*, are very morphologically similar and share a unique combination of characters: a continuous patch of nectariferous tissue within the fused calyx cups, corymbose cymes and bi-ovulate locules. Clear synapomorphies for lineage C, *D. wallichii* and *D. baronii*, are more difficult to determine given the remarkable autapomorphies of *D. wallichii*, but both species tend to occupy the larger and longer end of the spectrum of most quantitative characters measured. The relationship of *D. cannabina* to other members of Astrapaea is unclear and additional molecular work is needed, especially since the only accession sequenced was the rarer subspecies *D. cannabina* subsp. *antsifotrensis*.

The major difficulty in delimiting species within sect. *Astrapaea* derives from the variability that exists within single populations, individuals and even inflorescences. Flower size, in all of its components (calyx length, corolla length, etc.), forms a continuous spectrum across the section, as do stipule and leaf size, and petiole and peduncle lengths. Natural breaks in these quantitative, continuous characters are scant in sect. *Astrapaea*. Although species may generally occupy one end or the other of the continuum of these quantitative characters, they clearly overlap in the values of their ranges for most traits. Trichomes also vary in details of number and length of rays, but when considered on a gross scale (e.g., presence or absence of pubescence on various organs) can provide characters used in circumscription. Other characters include (dis)continuity of nectar patches on the sepals, inflorescence type and ovule number.

MORPHOLOGY

Habit. — Species in *Dombeya* sect. *Astrapaea* are usually trees, frequently found with their trunks growing horizontally out over or along streams, but they also sometimes take the form of large, often sprawling, shrubs. They readily produce sucker growth when the primary trunk is cut and, as a result, individuals in cultivated fields or in disturbed or secondary forests are often formed of a collection of tightly-packed stems.

Bark & wood. — Wood of *Dombeya* has been included in comparative studies at the familial (Chattaway, 1932) and generic levels (*Nesogordonia*; Barnett, 1988). Seyani (1991) surveyed the wood of African species of *Dombeya* for useful taxonomic characters and found few. None of these studies included species from *D*. sect. *Astrapaea*. The wood of *Astrapaea* is too soft to be used for timber, but the inner bark of *Dombeya* is commonly used in Madagascar as rough cordage due to its fibrous nature. This utilization of the bark is reflected in the common name “hafotra” (or “hafitra”) that the Malagasy apply to plants that provide fiber or bast, especially species of *Dombeya* and sect. *Astrapaea* (Richardson, 1885).

Indumentum. — Pubescence in *Dombeya* section *Astrapaea* is primarily of a tufted form that is commonly described as stellate in the taxonomic literature. Both tufted and stellate trichomes are composed of multiple rays, cells resulting from the division of one epidermal cell in a plane perpendicular to the organ surface. In tufted trichomes, these rays grow in various directions from the organ surface, whereas in stellate trichomes the rays grow (roughly) parallel to the organ surface (Jain & Singh, 1973; Rao, 1987). Tufted, but not stellate, trichomes occur in sect. *Astrapaea*. The term “stellate” has been traditionally used to describe these trichomes and so I will follow convention even though tufted may be the technically correct term. Simple hairs also occur in sect. *Astrapaea*. Under a dissecting microscope, stellate hairs appear to be both septate and aseptate, whereas simple hairs are always septate. In a comparative study of trichomes in various members of Sterculiaceae, the simple septate and stellate septate hair types were observed to be unique to *Dombeya* × *cayeuxii*, a hybrid for which *D. wallichii* is one parent (Rao, 1987).

Glandular trichomes also occur in sect. *Astrapaea* in the form of short-stalked glands with a rounded head, often orangish to reddish in color; these are referred to as glandular pubescence in the species descriptions. They are best seen under a dissecting microscope, but are visible in the field with a hand-lens or by an often lustrous or viscous appearance of the organs bearing them. Such an appearance is presumably derived from exudate of the glands. Glandular pubescence occurs sporadically in most species in the section but in *D. cannabina* is denser and occurs on more organs (stipules, leaves, petiole, and peduncle).

Stipules. — Section *Astrapaea* bears obvious stipules, but as they are often caducous they are usually only seen at the shoot apices. Stipules are generally ovate, the truncate base broadly attached to the shoot and leaving a noticeable scar. Stipules of *D. baronii* (Fig. 4.1A) are densely pubescent and usually broadly ovate with an abruptly acuminate apex. Those of *D. wallichii* are the largest of the section, up to 1 cm larger than other species, and are pubescent and ovate, often with an acuminate apex. Stipules of *Dombeya cannabina*, *D. hafotsy* and *D. hilsenbergii* (Fig. 4.1B) are glabrescent and narrowly ovate with a narrowly acute apex. This last morphotype is most extreme in *D. hilsenbergii*, where the shoot apex can sport a collection of highly crispate, long, almost triangular, stipules.

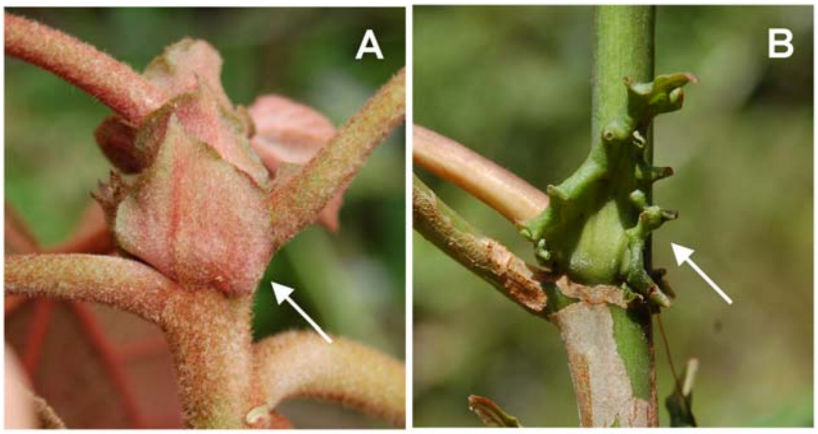


Figure 4.1. Stipules of *Dombeya baronii* (**A**) and *D. hilsenbergii* (**B**).

Leaves. — Leaves in the section are either entire or palmately lobed. Leaf shape is some degree of ovate or orbicular. As already discussed, a previous treatment (Arènes, 1958) distinguished subsections based on two leaf morphotypes: ovate and cordiform versus suborbicular or subpalmately-lobed. Although this general distinction in leaf type is often observed (e.g., the always cordiform *D. hilsenbergii* versus a particularly aceriform *D. baronii*), exceptions are common and one shape can grade into the other. For example, some “cordiform” leaves are clearly orbicular. Also, a plant with three-lobed leaves can be found growing in the same population as a plant with unlobed leaves to which it is morphologically identical in all other aspects (e.g., *Applequist et al. 231* and *232* of *D. hafotsy*). Therefore, I chose not to use these two leaf shapes as characters in making delimitations.

Putative pocket domatia usually occur on the abaxial leaf surface of *D. cannabina* (and rarely in *D. baronii*). The domatia are found in the axils where the primary veins meet, at the apex of the petiole, or where the primary veins meet the secondary veins (most often only on the median primary). They are formed from a solid flap of tissue derived from the distal face of the vein, stretching across the axil in an arc, like the webbing on a webbed-toe. These domatia correspond to the pocket form (O'Dowd and Willson, 1989) of acarodomatia (Lundströem, 1887). Similar pocket domatia have been observed in other species of *Dombeya* (Skema & Dorr, in press) and domatia formed from tufts of hairs in other dombeyoids (*Nesogordonia*; Barnett, 1988). Leaf domatia may attract inhabitants, such as mites, that reduce herbivore pressures. Experimental studies with *Gossypium* have shown a higher abundance of arthropod predators of herbivores to inhabit leaves to which experimental "domatia" (tufts of cotton fibers) have been added (Agrawal & al., 2000).

The leaf margin in species of sect. *Astrapaea* is usually minutely crenulate with each unit of rounded edge terminating apically in a ± rounded tooth that is directed apically. The distance between these units often decreases towards the leaf apex. The depth of these margin characters is a very small fraction of the laminar width in all species. The extent to which the margin is rounded and the length and shape of the tooth vary as much between individuals as between species. Thus these margin characters have not been used in delimiting taxa and belabored descriptions of the variations within each species are of little use. Such margin patterns will simply be referred to as minutely serrulate.

Inflorescence. — The pendulous presentation of inflorescences in all species of sect. *Astrapaea* is unique in *Dombeya* (Figs. 4.2 & 4.3). The corymbose cymes of *D. baronii*, *D. cannabina*, *D. hafotsy* and *D. hilsenbergii* are also unique in *Dombeya* and form dense heads making a brush-like platform of the anthers apically (Fig. 4.2). The umbellate inflorescence is one of the multiple autapomorphies that differentiate *D. wallichii* from the remainder of sect. *Astrapaea* (Fig. 4.3). It is similar to the corymbose cymes in density and also pendulous.

Figure 4.2. Corymbose cymes of *Dombeya cannabina* (**A**), with young off-white inflorescence next to older inflorescence in which an orange-brown scarious perianth surrounds developing fruits, *D. baronii* (**B**) and *D. hilsenbergii* (**C**), with flowers at various stages of development.

Bracts and epicalyx bracts. — The four species of sect. *Astrapaea* with corymbose cymes have a series of distally decrescent inflorescence bracts subtending each branching point within the inflorescence (Fig. 4.2B). *Dombeya wallichii*, with an umbellate inflorescence, has similarly distally decrescent bracts aggregated in a dense involucre at the base of the umbel (Fig. 4.3A). The bracts are generally caducous in both types of inflorescence and older inflorescences that have lost their bracts can appear superficially different from conspecific but younger inflorescences still retaining the bracts.

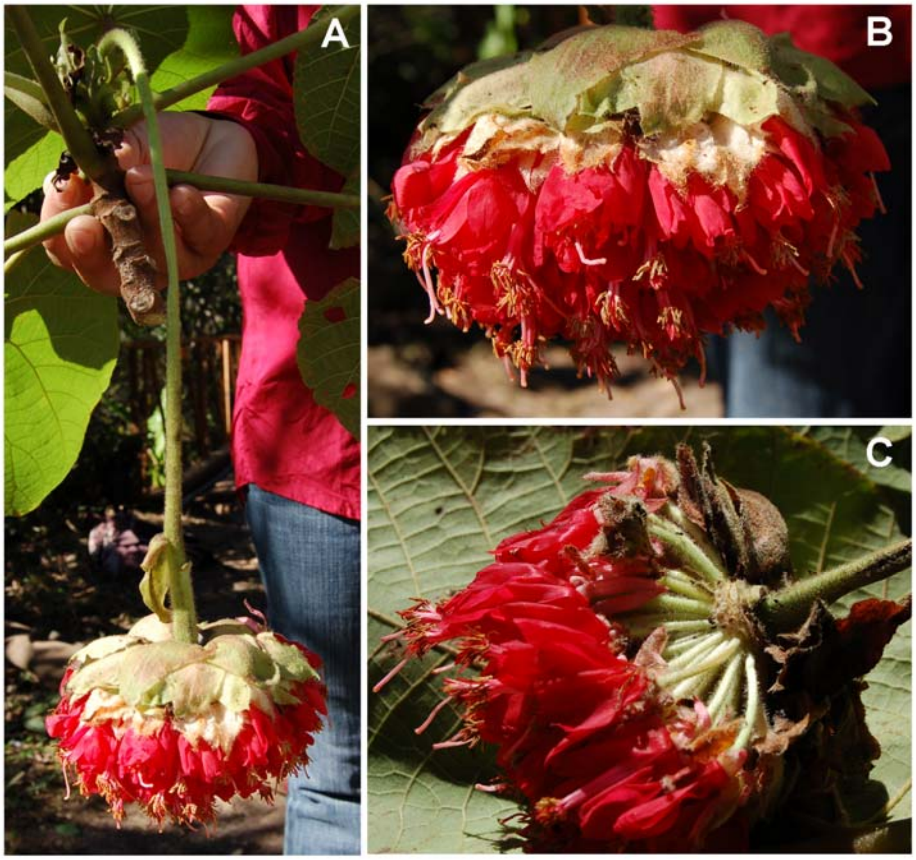


Figure 4.3. Umbellate cyme of *Dombeya wallichii* on long, pendulous peduncle (**A**). An involucre of bracts subtending umbel (**A**), staminal tube roughly equal to corolla length (**B**) and an umbel (**C**, half the flowers removed to reveal pedicels) are all unique characteristics of *D. wallichii*.

Although size of inflorescence bracts forms a continuum within the section, bracts of *D. baronii* and *D. wallichii* (lineage C, see Table 4.1; also Figs. 4.2B & 4.3) are usually larger and can be used to diagnose the species.

All *Dombeya* sect. *Astrapaea*, like all Dombeyaceae, have three epicalyx bracts. In *Astrapaea* they usually subtend the flower by one to a few millimeters. Shape of epicalyx bracts vary as much within an individual as they do between species in the section, sometimes taking on fantastically irregular shapes.

Flowers. — Flowers of sect. *Astrapaea* differ little from the prototypical *Dombeya* flower in most aspects. All floral parts are persistent, meaning the calyx, corolla and androecium are retained after anthesis. They achieve a dry and papery consistency and hang about the fruit as it matures (Fig. 4.2A). Section *Astrapaea* is exceptional in that their petals do not lie open as in other species of *Dombeya*, but closely surround the staminal tube and are approximately parallel to it.

The size of the calyx, in both the length of the fused base and the free lobes above, and the size of the petals are quantitative traits that also form a continuum across the species within the section. Lineage C (*D. baronii* and *D. wallichii*) generally has larger flowers, whereas lineage A (*D. hilsenbergii* and *D. hafotsy*) can sport remarkably small flowers. *Dombeya baronii* is the only species in which the calyx lobes sometimes exceed the petals in length. The degree of sepal fusion in sect. *Astrapaea* varies within all species. The ranges of the length of sepal fusion seen in each species, except *D. wallichii*, overlap with one another. *Dombeya wallichii* is unique in that its sepals are free (or sometimes minutely fused) at their base.

Glandular papillate trichomes occur on the adaxial surface of the calyx in all species of sect. *Astrapaea* and also most species of *Dombeya s.str.* and some related genera (see Chapter 3 for further discussion). These papillate patches are nectariferous. I have observed one droplet of nectar resting upon the adaxial surface at each point where two petals meet in the open flowers of *Dombeya* sect. *Dombeya*. The nectar is wicked to this position from the papillate patch lying directly beneath it. Vogel (2000) defines this as “secondary nectary presentation” because the reward is only indirectly available. Although less visible within the tightly convolute petals, sect. *Astrapaea* also produces nectar in the same manner. Nectar often rains out of a pendulous inflorescence that is in full bloom when it is cut down for collection. In most species of *Dombeya* I have observed, the nectariferous papillae form discrete, usually ovate, patches, one at the base of each calyx lobe. This arrangement always occurs in *D. baronii* and *D. wallichii*. In contrast, both *D. hilsenbergii* and *D. hafotsy* have contiguous papillae patches, i.e., the entire inner surface of the fused portion of the calyx is papillate. *Dombeya cannabina* seems to grade between the two extremes in this feature, frequently having discrete papillate patches but sometimes showing nearly abutting patches or even basally contiguous and apically lobed patches.

The secondary pollen presentation on apical regions of the adaxial face of the calyx found in other species of *Dombeya* (e.g., Prenner, 2002; pers. obs.) is lacking in sect. *Astrapaea*.

Androecium. — The androecium of sect. *Astrapaea* is a much elongated version of that seen in species of *Dombeya* outside the section. The filaments are fused into a staminal tube that surrounds the gynoecium. This staminal tube extends apically to equal or barely surpass the length of the corolla (*D. wallichii*) or extends far beyond the corolla, to 4 cm (all other species; compare Fig. 4.2 and Fig. 4.3.) Fascicles of stamens, with varying degrees of fusion among their members, stand above the apex of the tube interdigitated with five staminodes. All species in the group have a single fascicle of fertile stamens borne between each pair of staminodes except *D. wallichii* that sometimes has two fascicles. Each fascicle can have 2-5 anthers diverging at various points along its length, although single free anthers can appear in any of the species. The total number of stamens per flower varies from (10–)15(–30). *Dombeya hilsenbergii*, *D. hafotsy* and *D. cannabina* usually have fewer total anthers per flower (10–15) than either *D. baronii* (15–25) or *D. wallichii* (20–30), although the values overlap.

The staminodes in sect. *Astrapaea* diverge from the apex of the staminal tube but sit internally to the fertile stamens. They are less conspicuous than the staminodes in most species of *Dombeya* given their narrow shape and the numerous anthers behind which they hide. If they have a function in the flower, it is not an obvious one. Other species of *Dombeya* present pollen secondarily on the staminodes, but such a presentation of pollen in sect. *Astrapaea* would be of little use because the staminodes have no height advantage over the stamens and are situated behind them. In bud, the position of the staminodes may physically shield the style apex from its own pollen, but only in flowers where the style is not exserted beyond the height of the staminodes before pollen release.

The degree of fusion among the parts at the apex of the staminal tube was a taxonomic character utilized by Arènes (1958, 1959). In his keys, he classified anthers as (sub)sessile, short-stipitate ("*brièvement pédicellées-stipitées*") or long-stipitate ("*longuement pédicellées-stipitées*") (Arènes, 1959: 208, 219). Arènes provided no definitions of the various parts of the androecium beyond this classification. In this study, the anther stipe is considered to be the fused portion of the fascicle above the staminal tube but below the divergence of the first anther. Measurements of anther stipe length, staminode length and free filaments of the stamens diverging off the tubes or fascicles show that no quantitative gaps exist in these lengths from sessile to long-stipitate anthers. Furthermore, the degree of fusion can vary within a population, an individual and even an individual flower. The gradation of these categories makes them unsuitable for specific delimitations. Nevertheless, the five species of the section sit at different, although overlapping, points on the continuum of these traits. Once again, the anthers and staminodes of *D. baronii* and *D. wallichii* reside at the longer end of the spectrum, often having visibly long-stipitate fascicles and free filaments in the androecium.

Additionally, there seems to be a temporal aspect to the degree of androecial fusion that further complicates the quantification of these characters and their use in identification. Androecial fusion varies according to the maturity of a flower, with all parts seemingly becoming less fused as the androecium matures. Further discussion on this point is made under the description for *D. cannabina*.

Pollen. — Pollen is 3-porate, spheroidal and spiniferous across most genera in Dombeyaceae (von Mohl, 1835; Rao, 1950; Erdtman, 1952). The first study of pollen in Sterculiaceae (first according to Seyani, 1991) included both *D. wallichii* and *D. cannabina* but reported little beyond the description just given (van Mohl, 1835). Interestingly, Rao (1950) attributed the unusual size variation seen in pollen of *D. spectabilis* as a potential indicator of apomixis or hybridization in the species. Detailed palynology studies of the African species of *Dombeya* showed an unusual variability within *D. buettneri* to have 3- or 4-porate pollen grains (Seyani, 1991).

Gynoecium. — The compound ovary of sect. *Astrapaea* contains five carpels, each of which can contain 2–6 ovules. Lineage A, the corymbose *D. hilsenbergii* and *D. hafotsy*, have two ovules per locule, a trait they share with the umbellate *D. wallichii*. The remaining two species, *D. cannabina* and *D. baronii*, have 3–6 ovules per carpel. All of the ovules usually develop into seeds, but fewer seeds are sometimes produced in carpels with more ovules (e.g., four seeds develop from six ovules) and seed production can vary between carpels in one ovary.

The style is erect, surrounded closely by the androecial tube, and has five apical, often unequal, stigmatic lobes of variable length. Nearly all species have some pubescence at the base of the style. The length to which the pubescence extends distally is highly variable, as much between individuals as between species, and is not a useful taxonomic character. Similarly, the length of the exsertion of the style beyond the apex of the staminal tube has not been found to be of taxonomic value due to differences observed between individuals in a population (Fig. 4.4) and inflorescences within an individual. Details of these observations are in the discussion for *D. cannabina*.

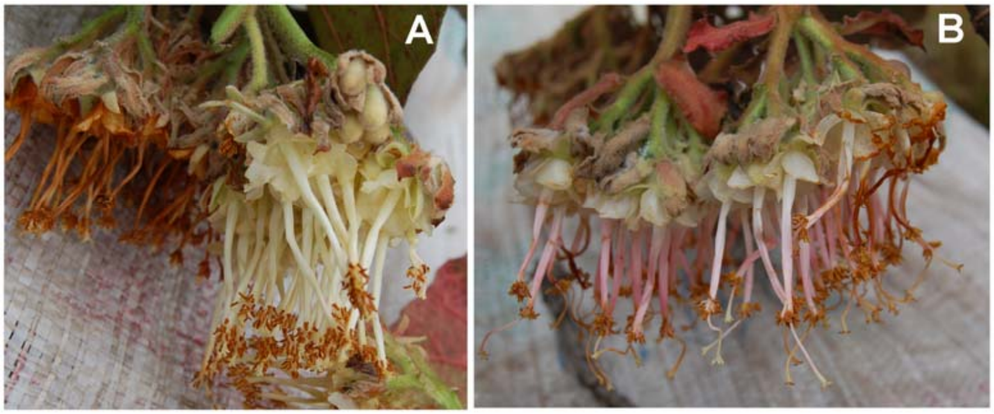


Figure 4.4. Inflorescences from two individuals of *Dombeya cannabina* growing adjacent on a hillside that differ by the length of style exsertion from the staminal tube: barely exserted in young and old inflorescences (**A**; *Skema et al. 177*) versus greatly exserted (**B**; *Skema et al. 178*).

Fruit. — In *D*. sect. *Astrapaea*, fruits are 5-parted, loculicidal capsules with a tough, but not woody, fruit wall. Some variation in the shape of the capsule exists within the section, but the single form that holds constant throughout a species is the 5-angular capsule found in *D. wallichii*. In this fruit, the carpels form five narrow lobes projecting outward from the central axis of the capsule. A few specimens of *D. baronii* have ovaries with carpel lobes that project laterally, possibly suggesting that it has a shallowly 5-angular fruit. This is speculative because I have not seen specimens of *D. baronii* with fully matured fruits. Within both species of lineage A, *D. hilsenbergii* and *D. hafotsy*, there is a tendency for the carpels to grow apically during fruit maturation without equivalent growth of the central axis of the fruit. Such growth results in a globular capsule at the center of which is a deep depression in which the style disappears. The point of attachment of the style sits at a mere fraction of the height of the entire fruit. These fruits are referred to as “recessed globose.” Some individuals of *D. cannabina* also show such apical growth of the fruit, but to a much lesser extent.

Seeds. — Seeds within *D.* sect. *Astrapaea* are of a tetragonal form, expanding slightly at the distal end, as in most species of *Dombeya*.

Chromosome numbers. — Chromosome numbers for *Dombeya* include $2n = 46$, 54 and 56 (Seyani, 1991). No chromosome counts have been reported for species of *Dombeya* section *Astrapaea*.

NATURAL HISTORY & THE ISOLATION OF SPECIES

Very little is known about the natural history of *Dombeya* section *Astrapaea*. The brush-like head of anthers, a sweet but yeasty smell and considerable nectar production in the flowers of the corymbose species of *D.* sect. *Astrapaea* suggest the possibility of a moth or bat pollinator. Of particular interest is if there is a difference in pollinators of the corymbose versus umbellate cymes in the group; color and inflorescence structure make it seem likely. No formal studies of floral visitors in any of the species of sect. *Astrapaea* have been made, but I have observed bees visiting the flowers of *D. baronii*, *D. cannabina* and *D. wallichii*.

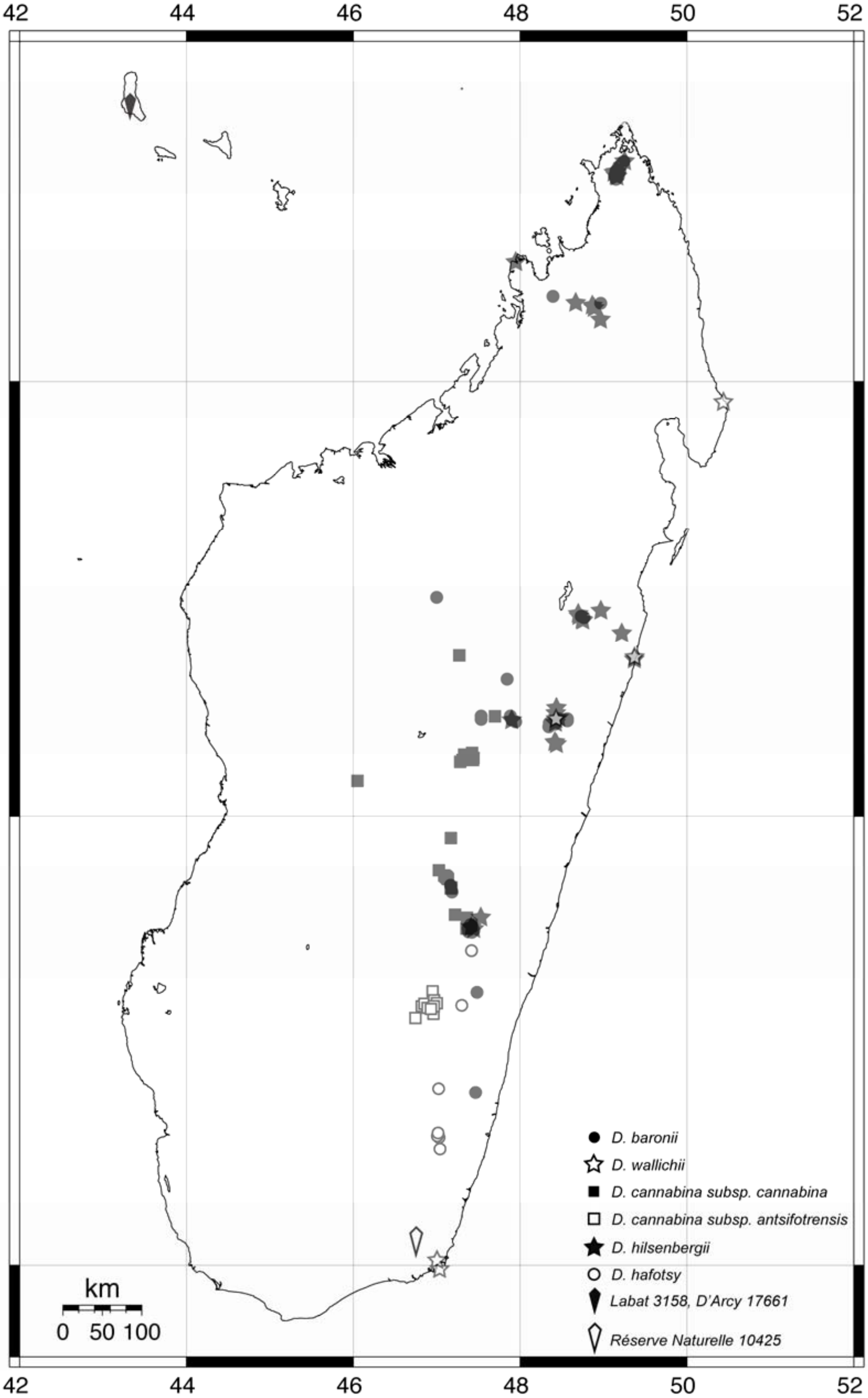
Fruit dispersal is likewise little understood. The capsular fruits reside within a scarious staminal tube sitting within scarious petals and sepals. Larger-sized fruits often break through the staminal tube during development. Field observations suggest that the entire inflorescence must degrade and fall before the carpels and seeds are freed from their scarious prison. Such a process would seem to hinder rather than encourage seed dispersal. There is no visible reward associated with any part of the fruit or seed, nor are there any morphological signs for adaptation to water dispersal despite the typical streamside habitat of these species.

Dombeya sect. *Astrapaea* is presumably outcrossing, but this assumption has not been tested. Cryptically dioecious species of *Dombeya* reside in the Mascarenes (Friedmann, 1987; Humeau & al., 1999) and one monoecious species has been reported from Madagascar (*D. tremuliformis*; Arènes, 1959), but no studies have been done to determine if cryptic dioecy occurs among the Malagasy species. Style exsertion can vary within a species in sect. *Astrapaea* (e.g., see discussion for *D. cannabina*; Fig. 4.4). It is unclear if this is part of a protogyny/protandry system of floral development or if these hermaphroditic and seemingly cosexual flowers could be functionally unisexual. Individuals were observed with fully matured flowers (i.e., beginning fruit maturation) that had the styles still nestled among the anthers. It is unlikely that these flowers are cross-pollinated. Such an arrangement may provide an opportunity for selfing, or these flowers may be functionally male, but neither hypothesis has been tested. Sexual dimorphism has been recorded in the cryptically dioecious species from the Mascarenes (Humeau & al., 1999; Humeau & Thompson, 2001) and no such morphological patterns were observed in the floral parts of sect. *Astrapaea*.

Dombeya, particularly sect. *Astrapaea*, seem to carry a remarkably heavy pest load. Leaves of *Dombeya* in the wild are often reduced to lace by bevies of tiny Coleoptera. Inflorescences of *D. wallichii* serve as a sort of crèche for Noctuidae; these moth larvae can be found nestled throughout the bracts, pedicels and flowers (pers. obs.). Noctuid larvae attack foliage of species of *Dombeya* in other sections (e.g., *D. burgessiae*, Soderholm, 1973). Seed predation is apparent from herbarium specimens. Positive associations between sect. *Astrapaea* and insects may be equally extensive judging from morphological clues (e.g., putative pocket domatia and generally glandular indument of *D. cannabina*). Pests from the Anobiidae and Dermestidae and non-pests from the Nitidulidae, Staphylinidae and Cantharidae were all identified on a single collection of *D. cannabina* (*Skema et al. 111*).

Overlap in the distributions of *D. cannabina*, *D. baronii* and *D. hilsenbergii* (particularly for the latter two; Fig. 4.5) indicates that they are not maintained by geographical isolation, nor is there evidence of genetic isolation mechanisms. Morphological data suggest that hybridization between these three species exists in the region around Vohiparara (Ranomafana; see further discussion under "Hybridization at Vohiparara" at end of treatment) and molecular data demonstrate gene flow among all species of the section at multiple localities across their entire distribution. Thus, temporal or ecological species isolation mechanisms are likely to be acting in sect. *Astrapaea*. Field observations and label data from herbarium specimens suggest that the most concentrated time of flowering is earlier in *D. baronii* (July; potentially the same for *D. wallichii* but the data are very few) than in *D. hafotsy* (August), *D. cannabina* (September) or *D. hilsenbergii* (September), but broad overlap in flowering times exists across all species in the section. Further studies into the preferred substrates, microclimates, pest pressures and symbiotic relationships could uncover potential selective factors that enable species isolation. Pollinator specificity may also be implicated. Whatever the mechanism(s) for isolation, it is clear that it is not a perfect system. Comparative studies between regions where hybridization occurs and where it does not could prove useful for further understanding the biological boundaries of these species.

Figure 4.5. Distribution map of all species of *Dombeya* section *Astrapaea*. Symbols denoting species have a degree of transparency to help show sympatry.


42
44
46
48
50
52
-15
-20
-25
km
0 50 100
● D. baronii
☆ D. wallichii
■ D. cannabina subsp. cannabina
□ D. cannabina subsp. antsifotrensis
★ D. hilsenbergii
○ D. hafotsy
Labat 3158, D'Arcy 17661
Réserve Naturelle 10425

TAXONOMY

NOTE: The stellate hairs of sect. *Astrapaea* vary in density, and length and number of rays. Although a general description of these hairs is required to portray the species well, an excessively detailed description of these variable hairs would encumber communication. Therefore, stellate hairs are summarized using the following terms. (*Length categories are also used to describe simple hairs.)

Ray Length*:

minute (length < 0.1 mm)
short (0.1 ≤ length < 0.5 mm)
medium (0.5 ≤ length < 1 mm)
long (length ≥ 1 mm)

Number of rays:

pauciradiate (less than 4 arms)
multiradiate (4–10 arms)
megaradiate (11 or more arms)

Dombeya Cav. *nom. cons.* sect. **Astrapaea** (Lindl.) Baill. ex M. Gómez, Anales Soc. Esp. Hist. Nat. 19(2): 216. 1890. Validated by an indirect reference, via H. Baillon (Bull. Mens. Soc. Linn. Paris 1: 495. 1885), to *Astrapaea* Lindl., Coll. Bot. ad t.14. 1821. *Astrapaea* Lindl., l.c. —TYPE: *Astrapaea wallichii* Lindl. [=*Dombeya wallichii* (Lindl.) Baill.].

Dombeya sect. *Hilsenbergia* (Bojer) Baker, J. Linn. Soc., Bot. 22: 450. 1887. Validated by a reference to *Hilsenbergia* Bojer, Rapp. Annuel Trav. Soc. Hist. Nat. Ile Maurice 11: 44. 1841. (For use of the rank of section see under *D. insignis* Baker, l.c.). *Hilsenbergia* Bojer, l.c. —TYPE: *Hilsenbergia cannabina* Bojer [=*Dombeya cannabina* Hils. & Bojer ex Hook.].

Shrubs or trees, 3–20 m tall. Bark grey to brown. Stipules (or stipular scars) conspicuous; margin entire. Petioles (3–)5–21(–26) cm long. Leaf lamina 8–29(–31) cm long, (6–)9–25(–31) cm wide; unlobed or palmately 3-, (5-, 7- or 9-)lobed; apex usually acute to acuminate; base usually cordate; venation palmate, basal veins (5–)7–9(–11). Inflorescences axillary, pendulous, umbellate (*D. wallichii*) or corymbose cymes, flowers numerous (>25); peduncles (6–)12–35(–40) cm long. Inflorescence bracts decrescent distally, aggregated beneath umbel (*D. wallichii*) or subtending each branching point in corymb. Epicalyx bracts 3, irregularly shaped, spathulate, linear, oblanceolate, ovate or oblong. Calyx persistent, valvate in bud; sepals 5, free (*D. wallichii*) or basally fused. Petals persistent, tightly convolute, 5, free, glabrous, red (*D. wallichii*) or white to cream to pale salmon. Androecium persistent, monadelphous, fertile stamens (10–)15(–30); staminodes 5, white to cream, often pink or red (particularly in young flowers); staminal tube (15–)20–37(–43) mm long, equal to (*D. wallichii*) or greater than corolla in length, closely surrounding gynoecium, white to cream, sometimes red- or pink-tinged (particularly in young flowers); filaments branching off apex of staminal tube. Anthers linear, subbasifixed, longitudinally dehiscent, dithecal, yellow when immature, brown after dehiscence; pollen spiny. Ovary superior, mucilagenous; placentation axile or basal-axile; carpels 5; ovules 2–6, collateral; style (21–)30–40(–53) mm long, white to cream, often pink- or red-tinged at apex (particularly in young flowers), when pubescent the trichomes pauci- or multiradiate minute- to medium-stellate; stigma (4–)5-lobed. Fruit loculicidal capsule, 2–10 mm long, 3–10 mm in diameter, glabrate. Seeds tetragonal, dull, brown.

Although placement of *Astrapaea* at the rank of section is often attributed to Baillon (1885a; e.g., Hochreutiner, 1926 or Arènes, 1958), his intentions as to the rank of *Astrapaea* in this work are ambiguous. He did not include it within the presented list of sections for the genus *Dombeya* and wrote (1885a: 482) only "Donc, sans compter les *Astrapaea*, *Assonia*, etc., depuis longtemps réintégrés dans le genre *Dombeya*, nous inscrivons dans celui-ci comme sections: *Dombeyella… Melhaniella… Dombeyantha… Trochetiella… Trochetiantha… Trochetina*." Five years later, in an article that included a description of a cultivated specimen of *D. wallichii* (=*Astrapaea penduliflora*) grown in Cuba, Gómez (1890) explicitly placed *Astrapaea* as a section within *Dombeya* via an indirect reference to Lindley's description (1821) of *Astrapaea wallichii* by citing Baillon's earlier, ambiguous statement (1885a).

Key to the species of *Dombeya* section *Astrapaea*

1a. Staminal tube equal to or just surpassing corolla in length, inflorescences umbels subtended by dense involucre of bracts. **5. *D. wallichii***

1b. Staminal tube long surpassing corolla in length, inflorescences corymbs with bracts subtending each floral branch. **2**

  2a. Adaxial surface of leaves glabrous, sometimes glandular or lustrous. **3**

    3a. Abaxial surface of leaves glabrous and without pocket domatia, ovules 2 per carpel. **4. *D. hilsenbergii***

    3b. Abaxial surface of leaves pubescent with short-stellate hairs and usually with pocket domatia, ovules more than 2 per carpel. **2. *D. cannabina***

  2b. Adaxial surface of leaves ± pubescent. **4**

    4a. Flowers small (≤ 14 mm long), single continuous nectariferous patch within calyx cup, ovules 2 per carpel. **3. *D. hafotsy***

    4b. Flowers large (≥ 16 mm long), five discontinuous nectariferous patches within calyx cup, ovules more than 2 per carpel. **1. *D. baronii***

1. Dombeya baronii Baker, J. Linn. Soc., Bot. 22: 450. 1887. — TYPE: MADAGASCAR. *R. Baron 3432* (holotype: K –scanned image!; isotypes: G!, P!).

Dombeya alleizettei Arènes, Candollea 16: 295. 1958. — TYPE: MADAGASCAR. [Antananarivo]: “La Mandraka,” 4 “8$^{\text{bre}}$” 1905, *C. Alleizette 424* (holotype: P!).

Dombeya ankazobeensis subsp. *ankazobeensis* Arènes, Candollea 16: 293. 1958. — TYPE: MADAGASCAR. *R. Decary 14377* (lectotype, designated here: P 00037333!; isolectotype: P 00037334 –scanned image!).

Dombeya ankazobeensis subsp. *befotakensis* Arènes, Candollea 16: 294. 1958. — TYPE: MADAGASCAR. [Fianarantsoa]:“Befotaka (Pr. de Farafangana),” 16 Aug 1926, *R. Decary 4807* (lectotype, designated here: P 00037335!; isolectotypes: P 00037336 –scanned image!, P 00500311).

Dombeya mandrakensis Arènes, Candollea 16: 290. 1958. — TYPE: MADAGASCAR. [Antananarivo]: “La Mandraka,” 19 Jun 1942, *Herbier du Jardin Botanique Tananarive* [*P. Boiteau*] *5050* (lectotype, designated by Arènes, l.c.: P!; isolectotype: TAN).

Dombeya perrieri Arènes, Candollea 16: 292. 1958. — TYPE: MADAGASCAR. [Antsiranana]: “Centre-Nord, dans le forêt d’Ambre entre 1000 et 1400 m,” Sep 1926, *H. Perrier 17771* (lectotype, designated here: P 00044911!; isolectotype: P 00044912 –scanned image!).

Shrubs or trees to 10 m; stems green, usually appearing rufous or pinkish due to pubescence, glabrescent or often moderately to densely pubescent with multiradiate short- to medium-stellate hairs. Stipules caducous or rarely persistent, (6–)11–18(–25) mm long, 6–13(–20) mm wide, ovate to widely ovate or rarely oblong, sometimes slightly undulate, apex abruptly acuminate or rarely acute to narrowly acute, color as per shoots, both surfaces densely pubescent, often more densely on inverted “V” and more moderately towards margin, with pauci- or multiradiate short- or medium-stellate hairs, or rarely simple long hairs, often two distinct sizes of hairs co-occurring. Petioles (5–)12–23(–26) cm long, color as per stems, pubescence as per shoots, rarely sparsely glandular. Leaf lamina (9.5–)17–23(–31) cm long, (9.5–)16–25(–31) cm wide, very widely ovate to orbicular to oblate, palmately 3- or 5- or 7- or 9-lobed, often with three larger and two smaller lobes, or sometimes unlobed, usually bullate; (lobe) apex acute to narrowly acute or rarely acuminate; base deeply cordate, sometimes shallowly cordate; margin minutely serrulate; adaxially green sometimes with pink tinge, moderately to densely or rarely sparsely pubescent with pauci- or multiradiate short-stellate hairs, rarely glandular; abaxially green sometimes with pink tinge, densely or rarely moderately pubescent with multiradiate medium- or rarely short-stellate hairs; veins whitish-green or appearing pink or red or rufous due to pubescence, basal veins (5–)7–9, midvein (8–)12–22(–29) cm long. Inflorescences corymbose cymes; peduncle (7.5–)12.5–31.5(–40) cm long, color as per shoots, densely pubescent at least distally with multiradiate short-stellate hairs, often with 2 bracts borne 1–4.5 cm below the corymb; peduncle bracts caducous or sometimes persistent, 16–30 mm long, (7–)11–18(–20) mm wide, ovate to widely ovate to orbicular, sometimes undulate, apex acute to narrowly acute to acuminate, green usually appearing pink or sometimes rufous due to pubescence (drying gray to brown), moderately to densely pubescent with multiradiate short- to medium-stellate hairs. Inflorescence bracts caducous, (14–)18–29(–36) mm long, (3–)5–12(–16) mm wide, color as per peduncle bracts, both surfaces densely pubescent with multiradiate short- to medium-stellate hairs. Inflorescence axes green sometimes appearing pinkish or rufous due to pubescence, moderately to densely pubescent with multiradiate short-stellate hairs. Pedicels 2–29 mm long, color and pubescence as per inflorescence axes. Epicalyx bracts caducous or persistent, 10–35 mm long, 2–7 mm wide, greenish- or pinkish-white, moderately to densely pubescent with multiradiate short- to long-stellate hairs. Calyx 14–22(–25) mm long, color as per epicalyx bracts, fused basally 2–4(–5) mm; abaxially densely pubescent with multiradiate short- to long-stellate hairs sometimes with greatly varying lengths of rays on single trichomes; adaxially glabrous with discrete widely ovate to oblong patches of nectariferous papillate tissue at base of each lobe; lobes (9–)12–18(–21) mm long, (1.7–)2.1–3.1(–4) mm wide, often unequal in width (to 1 mm) within one flower, lanceolate to narrowly oblong with acute apex. Petals (14–)17–20(–24) mm long, (7–)9–12(–15) mm wide, elliptic to obovate or oblong, white to cream to pale salmon (drying orange to orangish-brown). Staminal tube (21–)24–37(–43) mm long; staminodes (3–)4.2–6.6(–8.9) mm long, oblanceolate with acute apex; fertile stamens in fascicles of 3–5, one fascicle between each pair of staminodes, fascicles with stipes (0.7–)1.2–2.3(–3.4) mm long; filaments free above staminal tube or stipe by 0.3–4.9 mm; anthers 2–5 mm long. Ovary 1.2–2.3(–2.8) mm long, (1.9–)2.7–3.2(–3.7) mm in diameter, globose to depressed globose, densely pubescent and more so apically with multiradiate minute-stellate hairs beneath multiradiate medium- to long-stellate hairs with long erect trichome arms forming dense stand at apex; (3–)4–6 ovules per locule; style (25–)35–40(–53) mm long, glabrous to densely pubescent to proximal ½ of the length or rarely for entire length within androecial tube; stigma lobes (0.4–)0.6–0.8(–1.2) mm long. Fruit 3–5 mm long, 4.5–8 mm in diameter, globose, sometimes shallowly 5-costulate or slightly recessed globose.

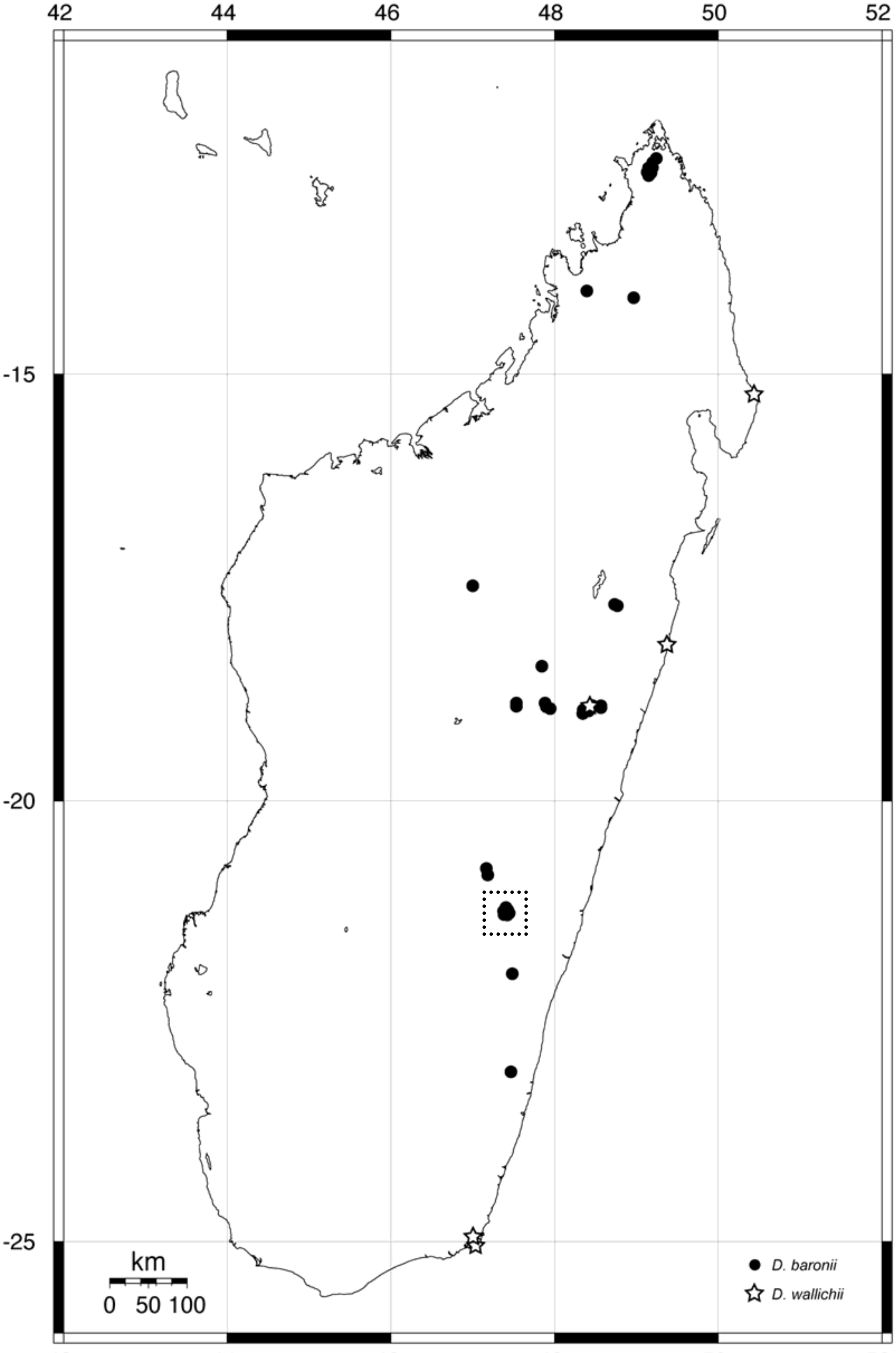
Phenology. Flowering May to September, concentrated in June and July.

Distribution (Fig. 4.6). Northern, eastern, central and south-central Madagascar; montane forest, moss-lichen forest, rainforest, secondary vegetation/savoka; often streamside; to 2000 m.

Vernacular names. Hafidrano [*Dombeya* near the water], hafomena [red *Dombeya*], hafotra [*Dombeya*], hafotra bonetaka, hafotra manampoza [*Dombeya* to use as packing around crabs], makaranga [a name more often used for *Macaranga*, Euphorbiaceae], badju (Comoran).

ADDITIONAL SPECIMENS EXAMINED. **Madagascar.** ANTANANARIVO: forest near Anjozorobe, beside river, 18º26'30"S, 47º50'30"E, *A. Rakotozafy et al. 2700* (MO, P, TAN, US); Antananarivo, Missouri Botanical Garden house, 18º55'09"S, 47º32'08"E, *G. Schatz 3749* (MO); city of Antananarivo, in front yard of Missouri Botanical Garden house, 18º55'8.3"S, 47º32'8.6"E, *C. Skema et al. 88* (BH, MO, P, TAN, US); Centre Mandraka, [18º54"18'S, 47º54'4"E], *R. Descoings 2758* (MO, TAN).—ANTSIRANANA: Montagne d'Ambre, 12º35'10"S, 49º09'10"E, *B. DuPuy et al. MB205* (P, TAN); Tsaratanana massif, trail from Mangindrano up south ridge of the Maromokotro, 14º05'S, 48º58'E, *A. Gentry 11576* (K, MO); SW of Antsiranana, near Joffreville, Parc National Montagne d'Ambre, near sommet d'Ambre, 12º35'S, 49º09'E, *S. Malcomber et al. 2366* (MO, P, TAN, US); Centre Nord forêt d'Ambre, [12º28'S, 49º12'E], *H. Perrier de la Bâthie 17771* (P); Manongarivo, [48º23'30"S, 14ºE], *H. Perrier de la Bâthie 5383* (P); Centre (Nord) Massif de la Montagne d'Ambre aux environs du Grand Lac, [12º25'S, 49º16'E], *Service Forestier 29195* (P); National Park Montagne d'Ambre, commune Joffreville, fokotany Morafeno, 1.5 km from road to Gite d'Etape on trail to Grand Lac on overgrown roadside, 12º31'49.4"S, 49º10'21.6"E, *C. Skema et al. 302* (BH, TAN); National Park Montagne d'Ambre, commune Joffreville, fokotany Morafeno, 1.5 km from road to Gite d'Etape on trail to

Figure 4.6. Distribution map of *Dombeya baronii* and *D. wallichii*. The dashed box refers to a detailed map of putative hybrids (*D. baronii* × *D. hilsenbergii*, *D. baronii* × *D. cannabina*) from Vohiparara shown in Fig. 4.9.


42
44
46
48
50
52
-15
-20
-25
km
0 50 100
● D. baronii
☆ D. wallichii

Grand Lac on overgrown roadside, 12º31’49.0”S, 49º10’21.2”E, *C. Skema et al. 303* (BH, P, TAN, US); National Park Montagne d'Ambre, commune Joffreville, fokotany Morafeno, 1.5 km from road to Gite d'Etape on trail to Grand Lac on overgrown roadside, 12º31’49.2”S, 49º10’21.4”E, *C. Skema et al. 304* (BH, TAN); National Park Montagne d'Ambre, commune Joffreville, fokotany Morafeno, 1.7 km from road to Gite d'Etape on trail to Grand Lac on side of path, 12º31’49.6”S, 49º10’21.4”E, *C. Skema et al. 305* (BH, MO, P, TAN, US); National Park Montagne d'Ambre, commune Joffreville, fokotany Morafeno, 1.9 km from road to Gite d'Etape on trail to Grand Lac on side of path, 12º31’49.9”S, 49º10’21.8”E, *C. Skema et al. 306* (BH, P, TAN); National Park Montagne d'Ambre, commune Joffreville, fokotany Morafeno, on path 3 km from Lac Maudit, 12º34’19.4”S, 49º9’23.8”E, *C. Skema et al. 309* (BH, P, TAN).—FIANARANTSOA: Sarobaratra forêt de l'ouest du pays Sihanaka, [21º58’60”S, 47º28’60”E], *Herbier Jardin Botanique Tananarive 2929* (P); Parc National de Ranomafana, parcelle #3, Talatakely, 21º15’S, 47º27’E, *A. Kotozafy 119* (MO, TAN); Parc National de Ranomafana, parcelle I, à l'est de Vohiparara, près de la region touristique, à 100 m de la route, 21º16’S, 47º24’E, *R. Rakoto & D. Turk 225* (MO, P, US); Ranomafana National Park, parcelle III, Talatakely trail system, piste AA about 300 m from Debut Circuit Varijasy, 21º16’2.5”S, 47º25’26.8”E, *C. Skema et al. 147* (BH, TAN); Ranomafana National Park, parcelle III, Talatakely trail system, 170 m north of bridge over Fompohona stream on piste AA, 21º16’2.5”S, 47º25’26.8”E, *C. Skema et al. 149* (BH, MO, P, TAN, US); Ranomafana National Park, parcelle III, Talatakely trail system, about 50 m on left side heading south from piste AA bridge over Fompohona stream, 21º16’2.5”S, 47º25’26.8”E, *C. Skema et al. 150* (BH, MO, P, TAN, US); on Route National 25, 2 km east of Vohiparara on south side of road, just next to Namorano river in bend in road, 21º14’25.3”S, 47º23’36.7”E, *C. Skema et al. 154* (BH, MO, P, TAN, US); Ranomafana National Park, about 100 m along Andranofady stream in Analatatatra forest to north of Route Nationale 25 about 2 km east of Vohiparara village, 21º13'26.1"S, 47º24'22.1"E, *C. Skema et al. 164* (BH, TAN); Ranomafana National Park, about 300 m along Andranofady stream in Analatatatra forest to north of Route Nationale 25 about 2 km east of Vohiparara village, 21º13'12.8"S, 47º24'25.8"E, *C. Skema et al. 166* (BH, TAN); Ambatofitorahana village, on Route Nationale 7, PK 301 on east side of road from village towards Ambositra, 20º48'56.1"S, 47º10'54.7"E, *C. Skema et al. 172* (BH, MO, P, TAN, US); turned west off Route Nationale 7 one half of a km south of PK 295 at Andoharena village, drove 2 km, took path N to the open forest of Ankarena, 20º46'54.4"S, 47º9'50.2"E, *C. Skema et al. 175* (BH, MO, P, TAN, US); Ranomafana National Park, parcelle 3, S of National Road 25 at 7 km W of Ranomafana, Talatakely trail system, 21º15'30"S, 47º25'E, *D. Turk et al. 530* (MO).—MAHAJANGA: Tampoketsa d'Ankazobe, [17º30'S, 47º0'E], *R. Decary 14377* (P).—TOAMASINA: Analamazoatra, [18º55'60"S, 48º25'E], *C. Alleizette s.n.* (P); Fanovana, [18º55'S 48º34'E], *R. Decary 18066* (K, P); Fanovana, [18º55S, 48º34'E], *R. Decary 18137* (P); Analamazoatra, [18º55'60"S, 48º25'E], *H. Perrier de la Bâthie 5483*(G, P); fivondronana Ambatondrazaka, firaisana Manakambahiny Est, Sahamalaza, Androrangabe, forêt d'Ampitsahambe, dans la Reserve Naturelle Integrale de Zahamena, 17º43'S, 48º44'E, *F. Ratovoson 708* (MO); Analamazoatra Special Reserve, at bridge (Pont Pisciculture) over Analamazoatra River, about 20 m west of fish farm, 18º56'15.8"S, 48º25'2.3"E, *C. Skema et al. 213* (BH, P, TAN); fokotany Antsapanana-Andasibe, on National Route 2, 0.5 km west of turnoff to Andasibe, just on south side of bridge over River Analamazoatra on RN2, 18º57'54"S, 48º21'0.9"E, *C. Skema et al. 233* (BH, P, TAN); fokotany Antsapanana-Andasibe, 7 km south of National Route 2 on road to Lakato, next to River Sahatandra, near village of Ankanihenitsara, 19º0'18.9"S, 48º20'38.2"E, *C. Skema et al. 235* (BH, MO, P, TAN, US); Ampitsahambe forest, commune Manakambahiny Est, fokotany Sahamalaza, 50 m from Onibe River near path to Androrangabe, 17º43’51.7”S, 48º45’56.8”E, *C. Skema et al. 404* (BH, MO, P, TAN, US); Ankafana, *W. Deans Cowan s.n.* (P).—PROVINCE UNKNOWN: Analamabitso, Bemarivo, *H. Perrier de la Bâthie 5613* (G, P); forêt d'Analamazoabi, *H. Perrier de la Bâthie 14890* (P); bord de riviere Andavaka (RN no.4), *Service Forestier 7458* (K, MO, P).

Dombeya baronii can be distinguished from the other species of *D*. sect. *Astrapaea* by its dense pubescence on most organs. It also often has the largest flowers and inflorescence bracts of *Astrapaea* species with corymbose cymes. Aceriform leaves (suborbicular lamina with ≥ five palmate lobes) are also diagnostic of this species but do not occur in all individuals.

Characters based on fusion of the androecium above the staminal tube or the length of the exserted style are of little value in differentiating species of sect. *Astrapaea*, as discussed elsewhere (see Morphology section in Introduction or the discussion under *D. cannabina*). The lack of utility of these characters resulted in the placement of four species (including two subspecies) from Arènes’ work (1958, 1959) into synonymy with *D. baronii*. Yet, considerable variation exists within *D. baronii*, perhaps the most of any species in sect. *Astrapaea*. Given that no clear breaks were observed in that variation, despite the breadth of character ranges, I found it best to recognize one variable species at this time.

Two specimens from the Comoros (*Labat 3158* and *D’Arcy 17661*, both from Mt. Karthala, Grand Comore) are difficult to place, sharing characters with both *D. baronii* and *D. cannabina*. The Comoran specimens have the dense pubescence on almost all parts (e.g., petioles, peduncles, abaxial leaf surface, shoots) that is common to *D. baronii*. They resemble *D. cannabina* in having only sparsely pubescent adaxial leaf surfaces, and shorter, more radiate pubescence everywhere (including the abaxial leaf surface), but they lack the pocket domatia seen in most (but not all) members of that species. More importantly, they have the eglandular, densely pubescent stipules seen in all *D. baronii*; glandular, glabrous stipules are seen in all *D. cannabina*. More collections are needed to clarify the placement of the Comoran populations.

Morphology suggests that particular specimens from one region are hybrids of *D. baronii* × *D. hilsenbergii* and *D. baronii* × *D. cannabina*. Characteristics of these specimens are discussed at the end of the treatment (see section "Hybridization at Vohiparara"). The putative hybrids are mapped in detail in Fig. 4.9 and their general locality highlighted in Fig. 4.6.

2. Dombeya cannabina Hils. & Bojer ex Hook., Bot. Mag. 64: ad t. 3619. 1837. — TYPE: MADAGASCAR. [Antananarivo]: "Hab: in provini Emirina, et, Be-tani mena" *W. Bojer s.n.* (lectotype, designated here: K –scanned image!; isolectotypes: BM!, G –scanned image!, P!, W)

Hilsenbergia cannabina Bojer, Rapp. Annuel Trav. Soc. Hist. Nat. Ile Maurice 11: 45. 1841. *Dombeya cannabina* Bojer ex Hochr., Candollea 3: 111. 1926. *nom. illeg. non* Hook. (1837). — TYPE: MADAGASCAR. [Antananarivo]:"in sylvis montibus Angavou, prov: Emirina," *W. Bojer s.n.* (lectotype, designated by Hochr., l.c.: G –scanned image!; isolectotypes: BM!, K!, P!, W)

Dombeya capuronii Arènes, Candollea 16: 289. 1958. — TYPE: MADAGASCAR. [Antananarivo]: "Manjakatompo, massif de l'Ankaratra, bords des ruisseaux, lieux humides," 2 Oct 1948, *Service Forestier* [*R. Capuron s.n.*] *5* (lectotype, designated here: P 00039952 –scanned image!; isolectotype: P 00039953!).

Dombeya condensata Hochr., Candollea 3: 114. 1926. — TYPE: MADAGASCAR. [Antananarivo]: "Imerina: Tananarivo, Bei einem Dorfe." Sep 1880, *J. M.*

Hildebrandt 3531 (lectotype, designated by Arènes in Candollea 16, 291. 1958: G!; isolectotypes: BM!, P!, W).

Shrubs or trees to 20 m; stems green with or without pink- or red-tinge or appearing tan due to pubescence (subsp. *antsifotrensis*), glabrous or glabrescent or rarely moderately pubescent with simple long hairs (subsp. *antsifotrensis*). Stipules caducous or rarely persistent, (6–)9–17(–29) mm long, (2–)3–7(–10) mm wide, ovate to lanceolate or triangular, undulate, apex narrowly acute, margin involute, green or pink or red (drying brown or black), glabrous, moderately to densely glandular, rarely sparsely glandular, often lustrous. Petioles (3–)5–10(–17) cm long, color as per stems, glabrous or sometimes glabrate, sometimes pubescent in a strip along one side or forming wedge extending down from apex, rarely sparsely pubescent over entirety, with multiradiate short-stellate hairs or rarely simple long hairs (subsp. *antsifotrensis*), sparsely to moderately glandular or rarely not, often lustrous. Leaf lamina (8–)11–18(–29) cm long, (6–)8–15(–25) cm wide, ovate, sometimes widely ovate or elliptical to orbicular, unlobed or rarely palmately, shortly 3-lobed, rarely shallowly bullate; (lobe) apex narrowly acute, sometimes acuminate, to 2 cm; base cordate, sometimes deeply cordate; margin minutely serrulate, rarely entire; adaxially green, glabrous or sometimes evenly but very sparsely pubescent on all veins with minute- to short-stellate hairs, sparsely to moderately glandular, sometimes lustrous; abaxially light green sometimes with pink- to red-tinge, moderately to densely pubescent, trichomes on lamina multiradiate and short-stellate, trichomes on veins often megaradiate and shorter and less dense than those on lamina, often sparsely to moderately glandular on veins, rarely also with simple long trichomes on both lamina and veins (subsp. *antsifotrensis*); veins off-white to (yellowish-)green or pink to red or appearing brown due to pubescence, basal veins 7–9, midvein (7–)9–15(–25) cm long; pocket domatia present, rarely absent. Inflorescences corymbose cymes; peduncle (6–)9–16(–24) cm long, green sometimes with pink- to red-tinge, glabrous or glabrate, rarely sparsely pubescent or moderately in strip along one side or only above bracts, with multiradiate short- to medium-stellate hairs or rarely simple long hairs (subsp. *antsifotrensis*), often sparsely to moderately glandular, sometimes lustrous, often with (1–)2(–3) bracts borne (0–)1–3(–7) cm below the corymb at unequal distances; peduncle bracts persistent or sometimes caducous, 10–14(–24) mm long, 4–8(–12) mm wide, ovate, sometimes undulate, apex acuminate, green sometimes with pink- to red-tinge or pink or red, glabrous, sometimes glandular. Inflorescence bracts caducous or sometimes persistent, (8–)11–14(–24) mm long, (2–)3–7(–12) mm wide, color as per peduncle bracts, both surfaces glabrous to densely pubescent, sometimes pubescence concentrated in inverse "V" or increases in density on consecutive bracts distally, with multiradiate minute- to short-stellate hairs, glandular or not. Inflorescence axes green sometimes with pink- to red-tinge or appearing off-white to rufous due to pubescence, glabrous to glabrescent or moderately pubescent, sometimes pubescence only on interior face of axes, with multiradiate minute to medium-stellate hairs, glandular or not. Pedicels 1–15(–29) mm long, color as per inflorescence axes, moderately to densely pubescent with multiradiate short- to medium-stellate hairs. Epicalyx bracts caducous or persistent, (6–)8–13(–18) mm long, (0.5–)2–4(–8) mm wide, whitish-green sometimes with pink-tinge (drying grey or brown), pubescence per inflorescence bracts. Calyx (8.5–)11–14(–15.5) mm long, whitish or light green sometimes with pink-tinge, fused basally (1.5–)3–4(–4.5) mm; abaxially densely pubescent with multiradiate short-stellate hairs; adaxially glabrous, rarely sparsely pubescent with pauciradiate short-stellate or simple short hairs, nectariferous papillate tissue at base varying in form from separate ovate patches at base of each lobe to conjoined patches within fused cup; lobes (5.5–)8–10(–11) mm long, (2–)2.4–2.9(–3.8) mm wide, ovate to lanceolate. Petals (9–)10–15(–16) mm long, (4–)5–8(–11) mm wide, elliptic to obovate to oblanceolate, white to cream to pale salmon (drying orange to orangish-brown). Staminal tube (22–)25–37(–42) mm long; staminodes (1.5–)2–3(–4.3) mm long, oblanceolate with acute apex; fertile stamens in fascicles of (2–)3(–4), one fascicle between each pair of staminodes, fascicles sometimes sessile or with stipes (0.3–)0.5–0.8(–1.6) mm long; one stamen often (sub)sessile in each triad, filaments of other two free above stipe by 0.4–0.8(–1.9) mm; anthers (1.6–)2–3(–3.2) mm long. Ovary (1.1–)1.3–1.9(–2) mm long, (1.8–)2–2.7(–3) mm in diameter, globose to depressed globose, densely pubescent and more so apically with multiradiate minute-stellate hairs beneath multiradiate medium- to long-stellate hairs with long erect trichome arms forming dense stand at apex; (3–)4(–6) ovules per locule; style (23–)33–43(–52) mm long, glabrous to densely pubescent to varying heights of the length often decreasing in density distally; stigma lobes (0.5–)0.8–1.2(–1.6) mm long. Fruit 2.2–3.3 mm long, 3.3–4.4 mm in diameter, globose or slightly recessed globose.

Vernacular names. Hafotra (fotsy) [(white) *Dombeya*], alampona [or “halampona,” a name more often used for *Hibiscus*, Malvaceae], hafidrano [*Dombeya* near the water; a name more often used for *D. baronii*], hafotra beravy [big-leaved *Dombeya*].

Key to the subspecies of *Dombeya cannabina*:

1a. Simple long hairs absent from abaxial surface of leaf, petiole and peduncle.

D. cannabina subsp. _cannabina_

1b. Simple long hairs present on abaxial surface of leaf (along with stellate hairs), often also on petiole and peduncle, at least apically.

D. cannabina subsp. _antsifotrensis_

Dombeya cannabina subsp. cannabina

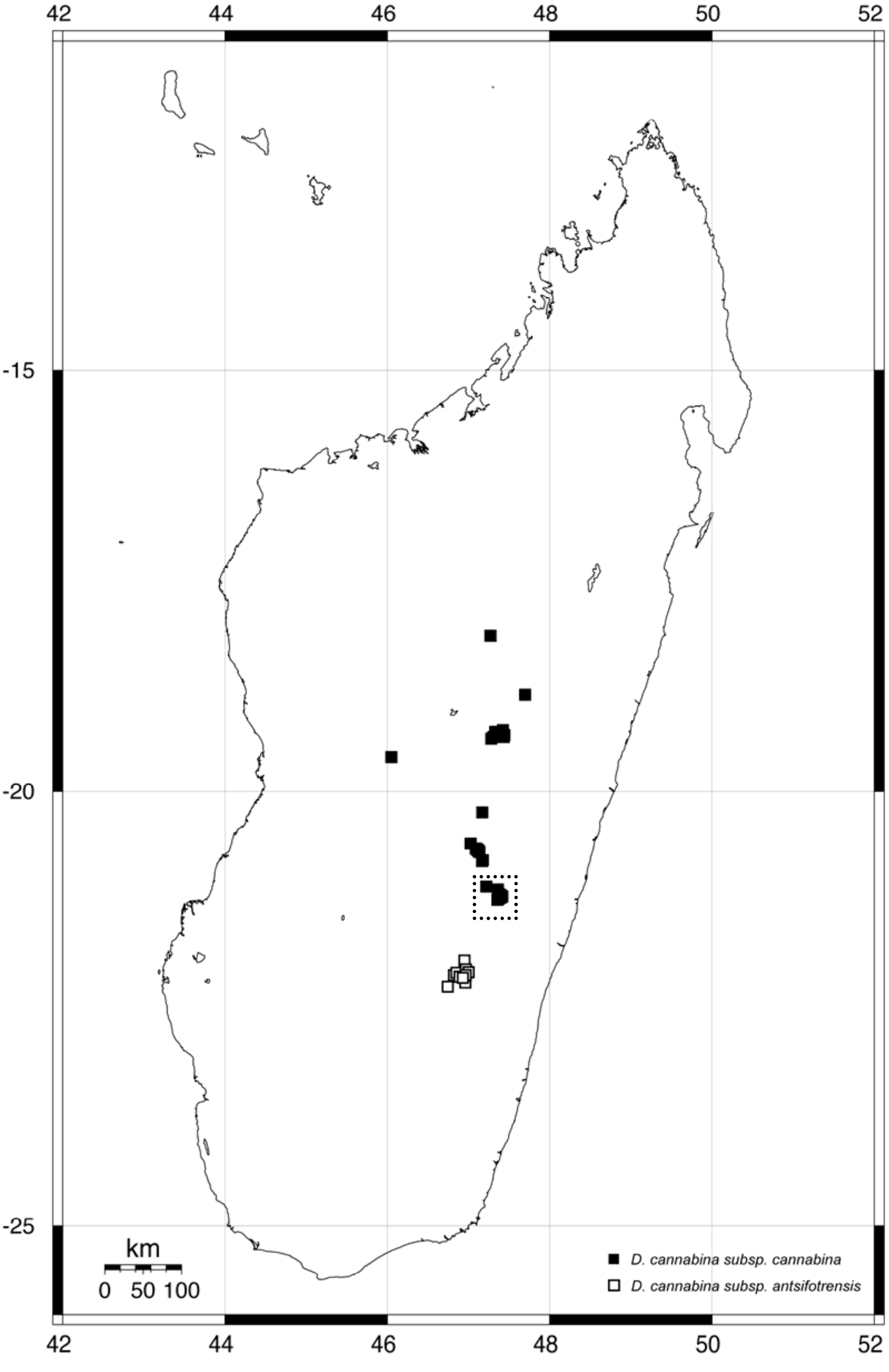
Shoots glabrous or glabrescent. Petioles glabrous or sometimes glabrate, sometimes pubescent in a strip along one side or forming wedge extending down from apex, rarely sparsely pubescent, with multiradiate short-stellate hairs. Leaf lamina abaxially moderately to densely pubescent, trichomes on lamina multiradiate and short-stellate, trichomes on veins often megaradiate and shorter and less dense than those on lamina, often sparsely to moderately glandular on veins. Peduncle glabrous or glabrate, rarely sparsely pubescent or moderately so in strip along one side or only above bracts, with multiradiate short- to medium-stellate hairs.

Phenology. Flowering July to October, concentrated in September.

Distribution (Fig. 4.7). Central Madagascar; humid forest, secondary vegetation/savoka; often streamside; to 2250 m.

ADDITIONAL SPECIMENS EXAMINED. **Madagascar.** ANTANANARIVO: au dessus de Manjakatompo (Ankaratra), [19º19’20”S, 47º25’20”E], *Bosser 16026* (MO, P, TAN); Ankaratra, Ouest Ambatolampy, [19º20’S, 47º20’E], *G. Cremers 2785* (MO, P, TAN); env. Tananarive, Ambohibe, *R. Decary s.n.* (MO); Massif de L'Ankaratra, Flanc Oriental du Tsiafajavona, Manjakatompo, [19º19’S, 47º25’E], *R. Decary et al. 4536* (G, K, P, TAN); Centre, forêt d'Ambohitantely km 130 route de Majunga, [18º10’S, 47º16’E], *R. Descoings 2847* (MO, TAN); environs de Tananarive, *H. Perrier de la Bathie 14801* (G, P); Centre, vestiges de forêt dans un vallon, au lieu dit Ambatondradama au Nord d'Ambohimanga (Tananarive), [19º36’S, 46º3’E], *Service Forestier 18026* (MO, P); Centre, Manjakatompo, versant oriental du Massif de l’Ankaratra, *Service Forestier 18788* (K, MO, P); Manjakatompo, next to stream across road from fish farm., 19º21’27”S, 47º18’48.3”E, *C. Skema et al. 110* (BH, MO,

Figure 4.7. Distribution map of *Dombeya cannabina* subsp. *cannabina* and *D. cannabina* subsp. *antsifotrensis*. The dashed box refers to a detailed map of putative hybrids (*D. cannabina* × *D. hilsenbergii*, *D. cannabina* × *D. baronii*) from Vohiparara shown in Fig. 4.9.


42
44
46
48
50
52
-15
-20
-25
km
0
50
100
■ D. cannabina subsp. cannabina
□ D. cannabina subsp. antsifotrensis

P, TAN, US); Manjakatompo, Analafandrina forest., 19º21’23”S, 47º19’3.1”E, *C. Skema et al. 111* (BH, P, TAN); fokotany Andohamandry (Sleeping Head), 3 km towards Manakavaly off Route Nationale 2, about 30 km east of Antananarivo, on left side heading away from RN2, 18º51’23.6”S, 47º41’48.9”E, *C. Skema et al. 208* (BH, MO, P, TAN, US); environs de Tananarive Ambohibe, sur l'emplacement d'un ancien village, *R. Decary 6690* (G, P).—FIANARANTSOA: Fianarantsoa à Ranomafana 48 km de Fianarantsoa, *L. Allorge & Y. Veyret 534* (P); Ambatofitorahana P.K. 300 route du Sud, [20º49’S, 47º10’E], *J. Bosser 9759* (P, TAN); 10 km W of Ivato on Route #35, [20º40’S, 47º09’E], *T. Croat 29602* (MO, TAN); on Route #7, 39 km N of Ambositra, [20º15’S, 47º10’E], *T. Croat 29439* (MO, TAN); Parc National de Ranomafana, parcelle #3, Talatakely, [21º19’12”S, 47º24’36”E], *A. Kotozafy 119A* (MO, P, US); between Ambositra and Ambatofinandrahana, on Route Nationale 35, 8 km W of Ivato, on river bank S of road, 20º39’20”S, 47º10’10”E, *P. Phillipson et al. 4065* (MO, TAN); vicinity of Ranomafana National Park, between 0.5 and 2 km west of Vohiparara along National Road 45 next to river, 21º15’S, 47º23’E, *J. Randrianasolo & D. Turk 86* (MO); south of the town Ambohimahasoa right along Route Nationale 7 on west side, 55 km north of Fianarantsoa, 21º6’55.8”S, 47º13’21”E, *C. Skema et al. 130* (BH, MO, P, TAN, US); Ranomafana National Park, on Route Nationale 45 on right side heading towards Vohiparara from Route Nationale 7 just past a bridge on Namorano river, 21º15’21.9”S, 47º21’36”E, *C. Skema et al. 134* (BH, P, TAN); on Route Nationale 45, 2-2.5 km west of Vohiparara village, next to bridge over small tributary into Namorano river, 21º14’52”S, 47º22’36.6”E, *C. Skema et al. 156* (BH, MO, P, TAN, US); on Route Nationale 45, 5 km west of Vohiparara village on same side of road as Namorano River, 21º15’2.1”S, 47º21’49.5”E, *C. Skema et al. 157* (BH, MO, P, TAN, US); on Route Nationale 45, 5 km west of Vohiparara village on same side of road as Namorano River, 21º15’2.1”S, 47º21’49.5”E, *C. Skema et al. 158* (BH, TAN); on Route Nationale 45, 6 km west of Vohiparara village next to Namorano River, 21º15’19.1”S, 47º21’36.8”E, *C. Skema et al. 160* (BH, TAN); Ranomafana National Park, on path along northern branch of Namorano River heading northwest about 5 km from Vohiparara on Route Nationale 25, 21º12’50.9”S, 47º21’51.4”E, *C. Skema et al. 170* (BH, P, TAN); Ranomafana National Park, northern branch of Namorano River heading northwest less than 1 km from Vohiparara on Route Nationale 25 on far side of water from road, 21º14’13.3”S, 47º22’36.1”E, *C. Skema et al. 171* (BH, MO, P, TAN, US); on Route Nationale 7, one quarter of a km south of PK 295 north of Ambatofitorahana village, 20º47’2.1”S, 47º10’37.6”E, *C. Skema et al. 176* (BH, MO, P, TAN, US); commune Ivato, on Route Nationale 35 about 5 km west of Ivato, on north side of road behind house, 20º39’52.6”S, 47º9’7.2”E, *C. Skema et al. 177* (BH, P, TAN, US); commune Ivato, on Route Nationale 35 about 5 km west of Ivato, on north side of road behind house, 20º39’52.6”S, 47º9’7.2”E, *C. Skema et al. 178* (BH, MO, P, TAN, US); commune Ivato, on Route Nationale 35 about 5 km west of Ivato at a bend in the road on the south side, 20º39’52.6”S, 47º9’7.2”E, *C. Skema et al. 179* (BH, MO, P, TAN, US); on Route Nationale 35, about 40 km east of Ambatofinandrahana near metal bridge over stream, 20º37’17”S, 47º1’46.3”E, *C. Skema et al. 182* (BH, MO, P, TAN, US).—PROVINCE UNKNOWN: [locality unknown], *W. Deans Cowan s.n.* (BM barcode 000929027); [locality unknown], *W. Deans Cowan s.n.* (BM barcode 000929026); bords de l'Ingalona, *H. Perrier de la Bâthie 5397* (G, P); Central Madagascar, *R. Baron 715* (K); Central Madagascar, *R. Baron 3267* (BM, P); [locality unknown], *R. Baron 3487* (P); Central Madagascar, *R. Baron 3518* (K, P); Central Madagascar, *R. Baron 4520* (BM); Interior, *W. Bojer s.n.* (P barcode 00500375); prov: Emirina and Betanimena, *W. Bojer s.n.* (P barcode 00500374); station piscicole, P.K. 22 route du Sud, *J. Bosser 14498* (MO, TAN); [locality unknown], *R. Viguier & H. Humbert 1572* (G, P); [locality unknown], [collector unknown], [from Hooker herbarium] (K); [locality unknown], [collector unknown], (P barcode 500289); [locality unknown], [collector unknown], (P barcode 500376).

Dombeya cannabina subsp. antsifotrensis Skema *subsp. nov.* — TYPE:

> MADAGASCAR. Fianarantsoa: "Andringitra National Park, Andohanantsifotra forest, southeast of Camp Andriampotsy, on steep slope next to source of Antsifotra stream," 30 Sep 2006, *C. Skema et al. 205,* (holotype: BH!; isotypes: P!, TAN!)

A *D. cannabina* subsp. *cannabina* pilis adjectis erectis simplicibus ad pedunculis petiolis et folliis abaxialis differt.

Indument of shoots, petiole, abaxial leaf and peduncle as described for *D. cannabina* subsp. *cannabina* but with the addition of simple long hairs on the shoot apex, petiole and peduncle, at least apically, and on the undersurface of the leaf.

Phenology. Flowering July to September.

Distribution (Fig. 4.7). South-central Madagascar; eriocoid brush, secondary vegetation/savoka; often streamside and among rocks; to 2100 m.

ADDITIONAL SPECIMENS EXAMINED. **Madagascar.** FIANARANTSOA: localité Ambodoiandahy, Center Sendrisoa, District Ambalavao, [22°0'S, 46°57'E], *Réserve Naturelle 2258* (P); localité RN V [Andringitra], Canton Sendrisoa, District Ambalavao, [22°15'S, 46°45'E], *Réserve Naturelle 3099* (K, P); commune Sendrisoa, fokotany Namoly, on periphery of Andringitra National Park, along trail to Circuit

Imaitso about 6.5 km from Gite d'Etape, right next to trail on edge of the small Amporomahery creek, 22°8'48.8"S, 46°56'32.8"E, *C. Skema et al. 184* (BH, MO, P, TAN, US); commune Sendrisoa, fokotany Namoly Est, in Andringitra National Park, on east side of Antsifotra River about 1 km south of Maharoaky village, 22°9'39"S, 46°55'50"E, *C. Skema et al. 188* (BH, TAN); commune Sendrisoa, fokotany Namoly Est, in Andringitra National Park, on west side of Antsifotra River about 1.5 km south of Maharoaky village, 22°9'48.1"S, 46°55'48.4"E, *C. Skema et al. 190* (BH, P, TAN); commune Sendrisoa, fokotany Namoly Est, in Andringitra National Park, on west side of Antsifotra River about 1.5 km south of Maharoaky village., 22°9'48.1"S, 46°55'48.4"E, *C. Skema et al. 191* (BH, P, TAN, US); commune Sendrisoa, fokotany Namoly Est, in Andringitra National Park, on west side of Antsifotra River about 1.5 km south of Maharoaky village., 22°9'48.1"S, 46°55'48.4"E, *C. Skema et al. 192* (BH, TAN); Andringitra National Park, on path to Pic Boby at very beginning where trail placard with map is placed, maybe 20 m towards peak on right, on trail side of bank of River Zomandao, 22°7'55"S, 46°53'30.5"E, *C. Skema et al. 194* (BH, P, TAN); Andringitra National Park, near very first camp site at beginning of trail to Pic Boby, 22°8'7.3"S, 46°53'26.1"E, *C. Skema et al. 196* (BH, P, TAN).

Dombeya cannabina can be recognized by its densely pubescent abaxial leaf surface, glabrous (rarely glabrescent) adaxial leaf surface and generally glandular indument (always on stipules; often on leaves, petioles, peduncles and inflorescence bracts). The concept of *D. cannabina* as circumscribed here is broader than in past work and I synonymize *D. condensata* and *D. capuronii* based on the coherence of the characters just outlined. The new subspecies, *D. cannabina* subsp. *antsifotrensis*, is described to accommodate a collection of specimens from Andringitra that fit *D. cannabina* in all respects but have the addition of long, simple hairs on the abaxial surface of their leaves, and also frequently on the petiole and peduncle (at least apically). These specimens were collected primarily along the Antsifotra stream, for which the subspecies is named.

I have found the taxonomic characters traditionally used in recognizing *D. cannabina s.str.* and its segregates overlapping, inconstant through floral maturation or within populations, or, where considering ovule number, incorrect. When Hochreutiner (1926) described *D. condensata* he stated that it had four ovules, whereas *D. cannabina* had two. The specimens of *D. cannabina* that I have examined, including the type and all of those cited by Hochreutiner (1926) and Arènes (1958, 1959), typically had four ovules per locule but sometimes 3, 5 or 6. Only two specimens— *Perrier de la Bâthie 5397* and a sheet from Hooker's herbarium at Kew (collector unknown)— had two ovules per locule. (The latter specimen has other unusual characteristics and is discussed further below.) The continual presentation of *D. cannabina s.str.* as bi-ovulate in contrast to the 4-ovulate *D. condensata* can only be seen as an error given the rarity of the bi-ovulate condition in these specimens and is therefore not a useful character in differentiating among them.

Hochreutiner (1926) also cited denser inflorescences, thicker and more densely pubescent leaves, and a style that is exserted beyond the staminal tube and pubescent until the point of exsertion as characters to differentiate *D. condensata* from *D. cannabina*. The only material Hochreutiner had of *D. cannabina* was the type (*Bojer 1833*) that seems to have only young leaves and what is an uncharacteristically small inflorescence, perhaps accounting for some of the attributes he ascribed as marked differences between the two species. Lastly, a style pubescent for its entire length within the staminal tube is rare within the entirety of sect. *Astrapaea*, although it does occur.

Dombeya cannabina and *D. condensata* differ in their descriptions in the later treatment of Arènes (1958, 1959) by the following characters, respectively: i) stipules entire versus pinnatilobed, ii) anthers (sub)sessile versus short-stipitate, and iii) style not or just barely exserted versus long-exserted. Although the type of *D. condensata*, from which the stipule description and illustration seem to have come, does have the rare condition of seemingly persistent stipules (an uncommon condition across all species of sect. *Astrapaea*), the stipules are not pinnatilobed but, in fact, highly undulate.

There is no quantifiable difference between the androecia of specimens identified by Arènes to species with subsessile anthers versus those of specimens identified to species with short-stipitate anthers. The lengths of the free and fused portions of filaments of all of these androecia form a continuum. Observations of flowers at different stages of maturation on the same inflorescence showed that these androecial parts are more congested previous to and during anther dehiscence, and then less fused afterwards. It is unclear if this is because the free parts of the androecium continue to elongate after pollen release or if the fusion between these parts eventually separates (it is very easy to "unzip" the stamens from one another), but all androecia have longer free apical parts after pollen release. Notably, all the specimens determined as *D. cannabina* by Arènes, and thus characterized as having "subsessile" anthers, were in the stage of pollen release when collected.

The length to which the style is exserted beyond the staminal tube is the final character that was used in past treatments to distinguish *D. condensata* (style long-exserted), *D. capuronii* (style shortly exserted) and *D. cannabina s.str.* (style not or barely exserted). More complete collections clearly show that the length to which the style is exserted can vary within an individual according to the stage of flowering (*P. Phillipson et al. 4065, Skema et al. 130*). On these specimens, flowers that were releasing pollen when collected have a style tucked within the apex of the androecial tube or among the anthers whereas flowers already spent of their pollen have exserted styles. Style exsertion was also observed to vary within an individual even when all flowers were at the same stage of flowering (*Skema et al. 110, 208*), and also within a population (*Skema et al. 177, 178*, two adjacent plants on a hillside; see Fig. 4.4). The uniformity of all of these specimens in their stipules, leaves and other inflorescence characters leads me to treat them as one species with variable style exsertion.

I have found no account by Arènes in the literature of what characters motivated him to describe *D. capuronii* (Arènes, 1958). He described the single specimen for the species as having a glabrous adaxial leaf surface ("*indument foliaire nul à la face supérieure très glabre*"), but, in fact, it has the very sparse pubescence on the veins characteristic of *D. condensata sensu* Arènes (Arènes, 1959: 214). He characterized the anthers of *D. capuronii* as short-stipitate but they have no measurable difference to the subsessile anthers of *D. cannabina sensu* Arènes. Perhaps the difference was that the specimen had a single style exserted shortly beyond the staminal tube on one flower in the entire inflorescence, leading him to describe *D. capuronii* with style shortly exserted. Whatever the reasoning, the specimens from Ankaratra fit well within the expanded concept of *D. cannabina* presented here and thus *D. capuronii* is placed in synonymy.

Two specimens (a sheet from Hooker's herbarium at Kew, collector and locality unknown; *Descoings 2847* from Ambohitantely, westernmost point on distribution map) tentatively assigned to *D. cannabina* are somewhat unusual for the species. Both specimens have the glandular stipules and pocket domatia characteristic of *D. cannabina* but have unusually small flowers for the species and lack the normal dense pubescence found on the abaxial leaf surface. Furthermore, the specimen from Kew has trilobed leaves and two ovules per locule. The specimen collected by Descoings has sparse pubescence on the stipules, a character not seen in other *D. cannabina*. Further collections from the western limits of the distribution of sect. *Astrapaea* in Madagascar may help clarify the interpretation of these uncharactertistic features.

Morphology suggests that specimens from Vohiparara are hybrids of *D. cannabina* × *D. hilsenbergii* and *D. cannabina* × *D. baronii*. These specimens are discussed at the end of the treatment (see section "Hybridization at Vohiparara") and mapped in Figs. 4.7 and 4.9.

3. Dombeya hafotsy Arènes, Candollea 16: 289. 1958. — TYPE: MADAGASCAR. Fianarantsoa: "Midongy du Sud, Province de Farafangana, bord de rivière en forêt," 21 Aug 1926, *R. Decary 4959* (holotype: P!; isotypes: BM!, TAN, US!).

Dombeya somanga Arènes, Candollea 16: 295. 1958. — TYPE: MADAGASCAR. Fianarantsoa: "Andrambovato, Tolongoina, Fort Carnot," 25 Jul 1952, *Service Forestier 5596* (lectotype, designated here: P 00044972 –scanned image!; isolectotype: MO!, P 00044973!).

Dombeya tsiapetrokensis Arènes, Candollea 16: 295. 1958. — TYPE: MADAGASCAR. Fianarantsoa: "Tsiapetrokensis, Ankarimbelo, Fort Carnot," 17 Jul 1952, *Service Forestier 6529* (lectotype, designated here: P 00046551!; isolectotypes: P 00046552 –scanned image!, P 00500281).

Trees to 8 m; stems green or brown, glabrescent or often pubescent with multi- or megaradiate minute-stellate hairs. Stipules caducous, 8–17(–25) mm long, 4–8 mm wide, ovate, undulate, apex narrowly acute to acuminate, to 7 mm, margin often involute, green or appearing rufous due to indument, both surfaces moderately to densely pubescent with multiradiate minute-stellate hairs. Petioles (6–)9–13(–19) cm long, green or appearing rufous due to indument, moderately to densely pubescent with multi- or megaradiate minute-stellate hairs. Leaf lamina 12–20(–27) cm long, 9–18(–22) cm wide, ovate to orbicular, unlobed or palmately 3-lobed; (lobe) apex narrowly acute to acuminate, to 1 cm; base cordate, sometimes deeply cordate; margin minutely serrulate; adaxially glabrescent or sparsely to moderately pubescent with multi- to megaradiate minute-stellate, or rarely short-stellate, hairs, often denser and megaradiate on veins; abaxially moderately to densely pubescent with multi- to megaradiate minute-stellate, or rarely short-stellate, hairs, often denser and megaradiate on veins; basal veins 7–9, midvein 10–17(–23) cm long. Inflorescences corymbose cymes; peduncle 15–41 cm long, glabrate or moderately pubescent with multi- or megaradiate minute-stellate hairs, often with (1–)2 bracts borne 2–8 cm below the corymb; peduncle bracts caducous or persistent, 13–20 mm long, 5–7 mm wide, ovate, sometimes undulate, apex acuminate, pubescence as per stipules. Inflorescence bracts caducous, (13–)18–24 mm long, (3–)5–8(–9) mm wide, pubescence as per petiole. Inflorescence axes pubescent as per peduncle. Pedicels 2–15(–23) mm long, pubescence as per peduncle. Epicalyx bracts caducous or persistent, (3–)6–13 mm long, (1–)2–4 mm wide, densely pubescent with multiradiate short-stellate hairs. Calyx 7–11 mm long, fused basally 2–4 mm; abaxially densely pubescent with multiradiate short-stellate hairs; adaxially glabrous or glabrescent with continuous patch of nectariferous papillate tissue within fused cup; lobes 4–7.5 mm long, 1.8–3 mm wide, ovate to lanceolate. Petals 9–12(–14) mm long, 4–6(–8) mm wide, obovate or oblong, white to cream. Staminal tube 15–30(–35) mm long; staminodes (1.4–)2(–3.4) mm long, linear or oblanceolate with acute apex; fertile stamens in fascicles of (2–)3(–4), one fascicle between each pair of staminodes, fascicles sessile or with stipes 0.5–2 mm long; filaments free above stipe by 0.5–1.2 mm; anthers 1.7–2.2 mm long. Ovary 0.5–1.6 mm long, 1.4–2.6 mm in diameter, globose to depressed globose, densely pubescent and more so apically with multiradiate minute-stellate hairs beneath multiradiate medium- to long-stellate hairs with long erect trichome arms forming dense stand at apex; 2 ovules per locule; style 21–42 mm long, glabrous or sparsely to moderately pubescent to no more than ½ of the length often decreasing in density distally; stigma lobes 0.3–1 mm long. Fruit 3–4 mm long, 5–6 mm in diameter, recessed globose, 5-lobed apically.

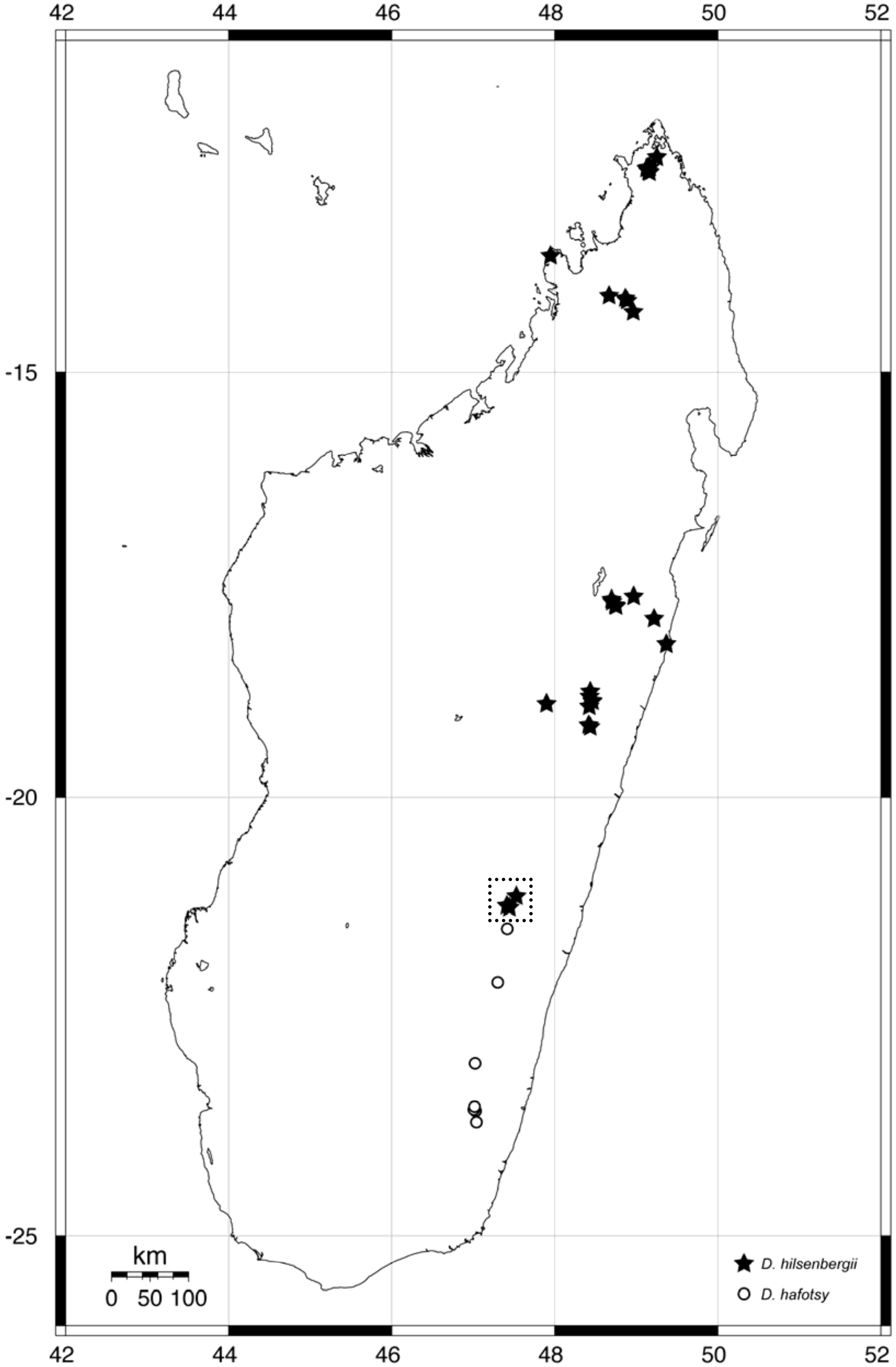
Phenology. Flowering July to September, concentrated in August. Fruiting November.

Distribution (Fig. 4.8). Southern and south-central Madagascar; primary forest; streamside; to 700 m.

Vernacular names. Hafotsy [white *Dombeya*], hafotra ambaniakondro [*Dombeya* under the banana tree], somanga(na), morodrano.

ADDITIONAL SPECIMENS EXAMINED. **Madagascar.** FIANARANTSOA: Midongy du Sud, Volovelo, near roadsides along road to Ranomena, both sides of road, near Volovelo River, fokontany Amboniasy, 23º24’26”S, 47º01’28”E, *W. Applequist et al. 231* (MO); Midongy du Sud, Volovelo, near roadsides along road to Ranomena, both sides of road, near Volovelo River, fokontany Amboniasy, 23º34’26”S, 47º01’28”E, *W. Applequist et al. 232* (MO); Atsimo-Antinamana Region, Vahgaindrano, Fakutan County, Midongy du Sud (Midongy Atsimo), road to Befotaka forest, 23º34’26”S, 47º01’13”E, *R. Bussman et al. 15026* (MO); Atsimo-Antinamana Region, Vahgaindrano, Midongy du Sud (Midongy Atsimo), Amhagavelo, Fkt. Telorano, *R. Bussman et al. 15067* (MO).—PROVINCE UNKNOWN: *R. Baron 3258* (K).

Figure 4.8. Distribution map of *Dombeya hafotsy* and *D. hilsenbergii*. The dashed box refers to a detailed map of putative hybrids (*D. hilsenbergii* × *D. cannabina*, *D. hilsenbergii* × *D. baronii*) from Vohiparara shown in Fig. 4.9.


42
44
46
48
50
52
-15
-20
-25
km
0 50 100
★ D. hilsenbergii
○ D. hafotsy

Dombeya hafotsy is most closely related to *D. hilsenbergii* and shares with it bi-ovulate locules and a continuous patch of nectariferous papillae within the fused calyx cup. It can be distinguished from the generally glabrous *D. hilsenbergii* by its pubescence of minute-stellate hairs on most parts.

Arènes (1958) placed *D. hafotsy* in subsect. *Cannabinae* due to the unlobed leaves and *D. tsiapetrokensis* and *D. somanga* in subsect. *Somanga* due to the usually 3-lobed leaves. Characteristics of leaf lobing have been found to vary within individuals and populations (see further discussion under "Leaves" in Morphology section above) and are not useful taxonomic characters. Here these three species are treated as one given their similarities in stipule shape, leaf and stipule indument, and smaller corymbs.

A specimen collected by Rakotoson (*Reserve Naturelle 10425*) from Andohahela is an unusual collection that is most similar to *D. hafotsy* and *D. hilsenbergii* due to its bi-ovulate locules and continuous patch of nectariferous papillae within the fused calyx cup. It differs from *D. hafotsy* in its glabrescence on most parts and from both *D. hafotsy* and *D. hilsenbergii* in that where it does have pubescence, on the petiole and peduncle, the trichomes are long and simple. These simple hairs are similar to the indument that *D. cannabina* subsp. *antsifotrensis* has in addition to the typical *D. cannabina* subsp. *cannabina* pubescence. The lack of other *D. cannabina* characteristics (pocket domatia, glandular indument, densely pubescent abaxial leaf surfaces) and the presence of bi-ovulate locules make this specimen a poor fit for *D. cannabina* subsp. *antsifotrensis*, despite the similar trichomes. This specimen underlines the need for further collections of sect. *Astrapaea* from southern and south-central Madagascar.

4. Dombeya hilsenbergii Baill., Bull. Mens. Soc. Linn. Paris 1: 495. 1885 (as "*Hilsenbergii*"). — TYPE: MADAGASCAR. "du Sud," *L. Humblot s.n.* (lectotype, designated here: P 00042047!; isolectotypes: G!, P 00042046 –scanned image!).

Dombeya macropoda Hochr., Candollea 3: 112. 1926. — TYPE: MADAGASCAR. “sur les bords de l’Ivondrona, dans les hauts du fleuve,” 1833, *J. Goudot s.n.* (holotype: G –scanned image!; isotype: P!).

Shrubs or trees to 20 m; stems green or brown, glabrous, shoot apices sometimes viscous or lustrous. Stipules caducous or rarely persistent, 10–30 mm long, 3–12 mm wide, ovate to lanceolate or triangular, crispate or sometimes undulate, apex narrowly acute, margin involute, green to light brown (drying dark brown or black), sometimes with reddish-pink tinge, both surfaces moderately to densely pubescent with whitish minute-stellate hairs, appearing glabrescent (and gray-colored on dried specimen), sometimes sparsely glandular. Petioles 4–19 cm long, green, sometimes with reddish-pink tinge, glabrous. Leaf lamina 9–31 cm long, 7–26 cm wide, elliptical to widely ovate, oblong or (sub)orbicular; unlobed or very rarely palmately 3-lobed; apex acuminate, to 5.5 cm long, rarely narrowly acute; base deeply cordate, sometimes shallowly cordate, rarely rounded; margin minutely serrulate, sometimes shallowly crenulate or rarely entire; adaxially green to silvery-blue, glabrous or pubescent with minute-stellate hairs on and just adjacent to veins, sometimes sparsely to moderately glandular, sometimes lustrous; abaxially lighter green, sometimes with reddish-pink tinge, glabrous or pubescent on and just adjacent to veins with minute-stellate hairs, trichomes particularly dense at leaf base, sometimes moderately to densely glandular; young leaves sometimes reddish-pink and often more glandular than mature leaves; veins green, sometimes red on abaxial surface, basal veins 5–11, midvein 7–30 cm long. Inflorescences corymbose cymes; peduncle 8–38 cm long, green, glabrous or rarely glabrescent with minute-stellate hairs, sometimes sparsely glandular, often with

2 bracts borne 1–4(–11) cm below the corymb; peduncle bracts caducous or persistent, 7–22 mm long, 2–8 mm wide, narrowly ovate to ovate, undulate or crispate, apex narrowly acute to acuminate, green or brown, glabrous to glabrescent to ± densely pubescent with multiradiate small-stellate hairs. Inflorescence bracts caducous or persistent, 10–20 mm long, 4–8 mm wide, color and pubescence as per peduncle bracts. Inflorescence axes green, indument as per the inflorescence bracts, sometimes viscous. Pedicels 1–26 mm long, green, glabrescent or pubescent with ± dense multiradiate small-stellate hairs, sometimes viscous. Epicalyx bracts caducous or persistent, 4.5–13.5 mm long, 1.5–6 mm wide, (grayish-)green or brown, densely pubescent with multiradiate short-stellate hairs. Calyx (6.2–)7.9–10.5(–10.8) mm long, whitish-green, fused basally (1.7–)2.2–3.6(–4.5) mm, abaxially densely pubescent with multiradiate short-stellate hairs; adaxially glabrous with continuous patch of nectariferous papillate tissue within fused cup; lobes (4.2–)5–6.8(–7.6) mm long, (1.6–)1.8–2.6(–3.2) mm wide, ovate to lanceolate. Petals 8.5–13 mm long, 3.5–8 mm wide, elliptic to obovate to oblanceolate, white to cream to pale salmon (drying orange to orangish-brown). Staminal tube (18–)22–29(–33) mm long; staminodes 1.5–2.6(–4) mm long, oblanceolate with acute apex; fertile stamens in fascicles of (2–)3, one fascicle between each pair of staminodes, fascicles sessile or with stipes 0.5–1(–3.1) mm long; one stamen often (sub)sessile in each triad, filaments of other two free above stipe by 0.2–1(–2.7) mm; anthers (1.2–)1.5–2.3(–3.3) mm long. Ovary 0.7–2 mm long, 1.3–2.6 mm in diameter, globose to depressed globose, densely pubescent and more so apically with multiradiate minute-stellate hairs beneath multiradiate medium- to long-stellate hairs with long erect trichome arms forming dense stand at apex; 2 ovules per locule; style 21–43 mm long, sparsely pubescent to proximal ½ of the length; stigma lobes 0.5–1.2 mm long. Fruit 2–3.5 mm long, 3–6 mm in diameter, recessed globose or rarely globose.

Phenology. Flowering July to October, concentrated in September.

Distribution (Fig. 4.8). Eastern and northern Madagascar; humid forest, cloud forest, secondary vegetation/savoka; often streamside; to 1475 m.

Vernacular names. Hafotra ambaniakondro [*Dombeya* under the banana tree], (hafotra) somanga(na), hafopotsy [white *Dombeya*], mampoza [may refer to using leaves as packing around crabs, similar to "hafotra manampoza"], mokarana [possible variant of "makaranga," a name more often used for *Macaranga*, Euphorbiaceae], tsomangamena.

ADDITIONAL SPECIMENS EXAMINED. **Madagascar.** ANTANANARIVO: Mandraka, on north side of Route Nationale 2, 18º54'18.1"S, 47º54'3.8"E, *C. Skema et al. 211* (BH, MO, P, TAN).—ANTSIRANANA: À sud d'Antsiranana, près de Joffre-Ville, Parc National Montagne d'Ambre au campement de Chris, 12º35'S, 49º09'E, *O. Andrianantoanina & B. Rochsceohclher 302* (BH, MO, P, US); à sud d'Antsiranana, près de Joffre-Ville, Parc National Montagne d'Ambre au Grand Lac sommet d'Ambre, 12º35'S, 49º09'E, *O. Andrianantoanina & B. Rochsceohclher 347* (MO, P, US); des roussettes au grand Lac de la montage d'Ambre, [12º25'S, 49º15'E], *A. Homolle 80* (P); montagnes entre le haut Sambirano et le haut Maivarano entre Mangindrano et Ampanompia, ravin vers Ampanompia, [14º7'S, 48º52'E], *H. Humbert 18166* (P); Réserve Naturelle IV [Tsaratanana], Canton Marovato, District Ambanja, [14º05'S, 48º53'E], *Réserve Naturelle 2899* (P); Beangona, Ambanja, [14º05'S, 48º40'E], *Réserve Naturelle 7369* (K, P, TAN); western domain, Parc National Montage d'Ambre, 7 km SW of Ambohitra (Joffreville), 12º33'S, 49º08'E, *G. Schatz et al. 1500* (MO, P, TAN, US); Parc National d'Ambre, 7 km SW of Ambohitra (Joffreville), 12º33'S, 49º08'E, *G. Schatz 2397* (MO, P, TAN); National

Park Montagne d'Ambre, commune Joffreville, fokotany Morafeno, on trail on way down from Grand Lac to Gite d'Etape, 12º32’29.8”S, 49º10’4.5”E, *C. Skema et al. 310* (BH, TAN).—FIANARANTSOA: base nord du Pic d'Ivohibe, *R. Decary 5265* (K, P); Ranomafana National Park, Parcelle I, near village of Miaranomy, Anosimasina, 21º09’S, 47º32’E, *S. Malcomber et al. 1593* (MO, P, TAN, US); Andrambovato, Tolongoina, Fort Carnot, [21º31’S, 47º23’E], *Réserve Naturelle 50* (P); bord voie Ampanaherana, [21º29’15”S, 47º19’20”E], *Service Forestier 1606* (P); Ampamaherana, [21º29’15”S, 47º19’20”E], *Service Forestier 2040* (MO, P, TAN); Ankazomanitra, village le plus proche Teoby, canton Anosibe, [21º55’S, 46º58’E], *Service Forestier 26780* (P); Ranomafana National Park, parcelle III, 15 m down Cascade trail off Talatakely trail, *C. Skema et al. 138* (BH, TAN); Ranomafana National Park, parcelle III, Talatakely trail system, about 100-120 m south from piste AA bridge over Fompohona stream, 21º15’S, 47º25’E, *C. Skema et al. 151* (BH, MO, P, TAN); along National Road 25 at 3-7 km W of Ranomafana, north side of road between Ambatolahy and Ambodiamontana, [21º15’30”S, 47º25’E], *J. Solo & J. Randrianasolo 14* (BH, MO, P, US).—MAHAJANGA: Fivondronana Behalanana, Commune Mangindrano, River Antsahotelo, 14º13’26’S, 48º57’49”E, *P. Antilahimena 764* (BH, MO); forêt d'Analamisakana près du village d'Analabe, tampoketsa près de Tsaratanana (herbier de la station agricole de l'Alaotra), *G. Cours 1619* (MO, P, TAN); Ampotaka, Befandriana Nord, [15º14’S, 48º39’E], *Herbier Jardin Botanique Tananarive 5327* (P).—TOAMASINA: Parc National de Zahamena, Andranofantsona, Manakambahiny I, Ambodimangavalo, 17º39’07”S, 48º58’14”E, *N. Andrianjafy 92* (MO); Fanovana *R. Decary* 18136 (P); Lakato district de Moramanga, [19º10’S, 48º25’E], *R. Decary 18238* (G, P); Lakato district de Moramanga, [19º11’S, 48º26’E], *R. Decary 18354* (P); Toamasina, Andasibe (Perinet) Forest NE of graphite mine, 18º53’S, 48º28’E, *P. Phillipson 2103* (MO, P, TAN, US); Réserve Naturelle 3

[Zahamena], Manaka Est, [17º44'S, 48º45'E], *Réserve Naturelle 3150* (K, P); Réserve Naturelle I [Betampona], Canton Amboaviana, District Tamatave, [17º55'S, 49º13'E], *Réserve Naturelle 4259* (P); Réserve Naturelle 3 [Zahamena], Manaka Est, [17º43'S, 48º44'E], *Réserve Naturelle 4464* (P); Analamazaotra Special Reserve, at bridge (Pont Pisciculture) over Analamazaotra River, about 20 m west of, and on the same side of water as, fish farm, 18º56'15.8"S, 48º25'2.3"E, *C. Skema et al. 214* (BH, P, TAN); Mantadia National Park, PK 18 heading north on road past Vakona Lodge and Mantadia entrance gate, on bank of River Iofa, on west side of bridge just south of graphite mine, 18º46'4.2"S, 48º25'56.5"E, *C. Skema et al. 229* (BH, MO, P, TAN, US); Mantadia National Park, PK 10-11 on road heading north past Vakona Lodge and Mantadia entrance gate, on slope above road on east side, 18º49'14.5"S, 48º26'7"E, *C. Skema et al. 232* (BH, P, TAN); commune Manakambahiny Est, fokotany Sahamalaza, just outside village of Androrangabe on path to village of Nonokambo, 17º44'12.3"S, 48º45'30.3"E, *C. Skema et al. 421* (BH, MO, P, TAN, US).—TOLIARA: Ankazomasina Réserve Naturelle, canton Manakambahimy Est, Ambatondrazaka, [17º42'S, 48º43'E], *Réserve Naturelle s.n.* (P barcode 00500285); col. de Tanatana, district de Fort Dauphin, *R. Decary 10391* (P).—PROVINCE UNKNOWN: Hafy [central Madagascar], *Alleizette 788* (P); Central Madagascar, *R. Baron 1414* (BM); *R. Baron 1556* (P); chiefly from northwest Madagascar, *R. Baron 5496* (K, P); between Tamatave and Antananarivo, *Meller s.n.* (P barcode 00500354, K); between Tamatave and Antananarivo, *Meller s.n.* (K); [locality unknown], *Ursch s.n.* (P).

Dombeya hilsenbergii can be distinguished from the remainder of section *Astrapaea* by its glabrous (or glabrescent) leaves and peduncles. Hochreutiner (1926) and then Arènes (1958, 1959) differentiated *D. macropoda* from *D. hilsenbergii* by viscous vs. not viscous shoots, narrow vs. broad inflorescence bracts, and long-stipitate vs. subsessile anther fascicles. Observation of specimens with both subsessile anther fascicles and viscous shoots as well as individual specimens that had young inflorescences with subsessile anthers and older inflorescences with seemingly long-stipitate fascicles argue that *D. macropoda* should be considered a synonym of *D. hilsenbergii*. Difficulties in the application of Arènes' use of androecial fusion characters for taxonomic purposes, particularly their potential to change temporally within individuals, are of relevance to this new delimitation and are discussed further in the Morphology section and in the discussion of *D. cannabina* (both above).

Morphology suggests that hybrids of *D. cannabina* × *D. hilsenbergii* and *D. baronii* × *D. hilsenbergii* occur in the Ranomafana region (shown in dashed box in Fig. 4.8). These putative hybrids are discussed in the section "Hybridization at Vohiparara" at the end of the treatment and mapped in detail in Fig. 4.9.

5. *Dombeya wallichii* (Lindl.) Baill., Bull. Mens. Soc. Linn. Paris 1: 495. 1885.

Astrapaea wallichii Lindl., Coll. Bot. ad t.14. 1821 (as "*Wallichii*"). — TYPE: [INDIA. West Bengal: Calcutta], Hort. Bot. Calcutta [Calcutta Botanic Garden], [between 1812-1821], *Wallich s.n.* [subsequently numbered 1161 by Wallich in 1828] (lectotype, designated by Arènes in Candollea 16: 287. 1958: K – scanned image!; isotypes: BM!, G –scanned image!).

Astrapaea penduliflora DC., Mém. Soc. Phys. Genèv. 4: 90. 1828 (as "*Astrapœa*"). *Dombeya penduliflora* (DC.) M. Gómez, Anales Soc. Esp. Hist. Nat. 19(2): 216. 1890.— LECTOTYPE, designated here: t. 691 in Ker Gawl, Bot. Reg. 9: t. 691. 1823.

Shrubs, 3–10 m (at least in cultivation); stems rufous, moderately or usually densely pubescent with multiradiate short- to medium-stellate hairs. Stipules caducous or persistent, (22–)27–37(–41) mm long, 12–23 mm wide, narrowly ovate to ovate, slightly undulate to undulate, apex narrowly acute to acuminate, light green sometimes with pink-tinge, sparsely to moderately pubescent, denser along midvein in inverted "V," with pauci- or multiradiate short- to medium-stellate hairs and sometimes with simple short to long hairs and sometimes ciliate. Petioles 8.5–21 cm long, light green, glabrate or sparsely to densely pubescent, at least at apex, with pauci- or multiradiate medium-stellate hairs. Leaf lamina (12–)25–29 cm long, (10–)19–25 cm wide, orbicular to widely ovate, unlobed or sometimes palmately 3-lobed; apex acuminate or rarely acute; base deeply cordate, rarely shallowly cordate; margin minutely serrulate; adaxially green, sparsely to densely pubescent, usually denser on veins, with pauci- or multiradiate short- to medium-stellate hairs and sometimes with simple medium hairs; abaxially light green, pubescence as per adaxial surface; veins yellowish-green to brown, basal veins 7–9, midvein (11.5–)19–20(–24.5) cm long. Inflorescences umbellate cymes; peduncle (16–)19–31(–38) cm long, light green with tan or rufous indument, glabrous or glabrate or densely pubescent, denser at apex, with pauci- or multiradiate medium- to long-stellate hairs and sometimes with simple long hairs, sometimes with 2 bracts borne 1.5–2 cm below the corymb; peduncle bracts caducous, 30–45 mm long, 15–25 mm wide, ovate with narrowly acute to acuminate apex, green to whitish-green sometimes with pink-tinge, both surfaces sparsely to moderately pubescent with pauci- or multiradiate short- to long-stellate hairs and sometimes with simple medium to long hairs. Inflorescence bracts forming a conspicuous involucre directly subtending the umbel, caducous or persistent, 31–49 mm long, (17–)20–25 mm wide, color and pubescence as per peduncle bracts. Pedicels 15–25 mm long, whitish-green, moderately to densely pubescent with paucior multiradiate long-stellate hairs and sometimes with simple long hairs. Epicalyx bracts, caducous or persistent, 15–21 mm long, 1–4 mm wide, off-white, glabrescent to sparsely pubescent with pauciradiate long-stellate and simple long hairs. Calyx (15–)18–20(–23) mm long, (2.4–)3.1–3.8(–4.4) mm wide, lanceolate to narrowly oblong with acute apex, off-white sometimes with pink-tinge; abaxially glabrescent or sparsely to moderately pubescent with pauci- or multiradiate long-stellate hairs and sometimes with simple long hairs; adaxially glabrous with discrete very widely ovate to depressed ovate patches of nectariferous papillate tissue at base of each sepal. Petals (24–)26–34(–36) mm long, 6–14 mm wide, elliptic to obovate or oblong, (clear) red. Staminal tube 20–32 mm long; staminodes 5–10 mm long, oblanceolate with acuminate apex; fertile stamens free or in fascicles of 2–4, 1–2 fascicles between each pair of staminodes, fascicles with stipes 0.6–4 mm long; filaments free above staminal tube or stipe by 0.6–9 mm; anthers 2.9–4.6 mm long. Ovary (1.9–)2.6–5 mm long, 2.4–4 mm in diameter, ovoid with 5 angular lobes, stellate in cross section, densely pubescent with evenly distributed multiradiate minute- to medium-stellate hairs; 2 ovules per locule; style 30–40 mm long, glabrous to moderately pubescent basally or to proximal ½ of the length; stigma lobes 0.5–0.7 mm long. Fruit 5–10 mm long, 7–10 mm in diameter, 5-angular with each carpel forming a narrow laterally-projecting lobe.

Phenology. Flowering July.

Distribution (Fig. 4.6). East coast of Madagascar; rainforest (?) and sublittoral forest; often streamside; to 28 m.

Vernacular names. Ma(n)kilody, tsingafiafy.

ADDITIONAL SPECIMENS EXAMINED. **Madagascar.** ANTSIRANANA: Ambohitralalana, Antalaha, [15º14’24”S, 50º26’24”E], *Reserve Naturelle 4435* (P, TAN).—TOAMASINA: forêt de l'Analamazoatra, [18º54’S, 48º25’48”E], *C. Alleizette 652* (P); fluvi Ivoundron [river Ivondro], Takasouha, [18º12’S, 49º22’E], *W. Bojer s.n.* (P); in prov. Bé-tani-mena, ins. Madagascar, *C. Hilsenberg s.n.* (BM).—TOLIARA: sides of sluggish river Nempoy, Fort Dauphin, [25º3’S, 47º2’E], *G. Scott Elliot 3003* (BM, K, P); Fort Dauphin, *G. Scott Elliot s.n.* (BM); Mandena Forest, Rio Tinto conservation site of QMM, Parcelle M15, commune Ampasy Nahampoana, fokotany Ampasy, 24º57’3.4”S, 46º59’59.3”E, *C. Skema et al. 372* (BH, MO, P, TAN, US).—PROVINCE UNKNOWN: Central Madagascar, *R. Baron 1615* (BM, P); [no locality], *J. Breon s.n.* (P); [no locality], *L. Chapelier s.n.* (P); [no locality], *J. Forbes, s.n.* (BM); [no locality], *Richard s.n.* (P).

CULTIVATED SPECIMENS EXAMINED. **Belgium.** Jardin Botanique National de Belgique, *V. Leyman S4083* (BR); Jardin Botanique National de Belgique, *V. Leyman S4084* (BR). **Brazil.** RIO DE JANEIRO: botanical garden, Rio de Janeiro, *H. Curran 348* (US).—STATE UNKNOWN: Brasilien, in einem garten, Rŭdio (US). **Comoros.** ANJOUAN: Anjoun (?), *Richard s.n.* (P barcode 00500275). **El Salvador.** AHUACHAPAN: Ahuachapan, Laguna Verde, *K. Sidwell et al. 795* (BM). **India.** WEST BENGAL: Hort. Botan. Calcutt., 1819, *N. Wallich s.n.* (BM); Ex. ins. Mauritio introd. in hort. bot. Calc., 1815, [collector unknown] (BM); Jardin de Calcutta, [collector unknown] [from Herb. L. Pierre] (P barcode 00500238, 00500239). **Java.** Cult. Hort. Bogor., Java, 1903, [collector unknown] (US). **Mauritius.** de Madagascar naturalisé à l’Île de France, [date unknown] *Richard s.n.* (P); Mauritius, 1895, *Telfair s.n.* (P); Mauritius, 5 Oct 1827, [collector unknown] (US). **Morocco.** RABAT-SALÉ-ZEMMOUR-ZAER: Rabat, Maroc, *J. Lewalie 8587* (BM). **Peru.** LIMA: Lima, Peru, 1912, *H. Forbes s.n.* (BM). **Réunion.** Ile Bourbon, 1836, *A. Delessert s.n.* (P); Île Bourbon (cult.), 1841, *Hombron s.n.* (P); jardin bot. Ile Bourbon, 1841, *M. Richard s.n.* (P); hort. bot. Bourbonne, *Richard s.n.* (P); Jard. Bot. de Bourbon, *Richard s.n.* (P); Jard. Bot. de Bourbon, [collector unknown] (P barcode 00500272). **United Kingdom.** Palm House, Kew, 3 Feb 1888, [collector unknown] (K); Temp. House, Kew, 18 Jan 1924, [collector unknown] (K). **U.S.A.** MISSOURI: greenhouse, Tower Grove, 28 Jan 1861, [collector unknown] [from herbarium of Joseph Tarrigan Monell] (MO); Mo. Bot. Gard., Jan 1892, [collector unknown] [from Herbarium of Thos. A. Williams] (US); greenhouse, Missouri Botanical Garden, 18 Jan 1958, *J. Norton s.n.* (MO). **Country Unknown.** Montgomery, E. Co. of Africa, 1856, *Martin s.n.* (G); [no locality], [collector unknown] (BM barcode 000929036); [no locality], [collector unknown] (P barcode 00500380); [no locality], [collector unknown] [from herbarium Al. de Bunge] (P barcode 00500235); [no locality], [collector unknown], [from herbarium Bosch] (P barcode 00500274); [no locality], [collector unknown] [from herbarium Munro] (K).

Dombeya wallichii can be distinguished easily from the rest of sect. *Astrapaea* by its umbellate inflorescence subtended by a conspicuous involucre of large bracts (Fig. 4.3). *Dombeya wallichii* is most likely to be confused with *D.* × *cayeuxii* André and the name *D. wallichii* is often misapplied to this cultivated hybrid. The morphological resemblance is not surprising given that *D.* × *cayeuxii* is a cross between *D. wallichii* (paternal) and *D. mastersii* (≡ *D. burgessiae*; maternal), done by Cayeux in 1895 in Lisbon (André 1897). *Dombeya* × *cayeuxii* differs from *D. wallichii* in that the former's stipules and bracts (of the peduncle, involucre, epicalyx) are narrower, the flowers are more open and the petals are light pink instead of red. Also, the proportions of the androecia differ between these taxa in two ways. Firstly, the staminal tube of *D. wallichii* is almost equal to or just surpassing the corolla in length, whereas that of *D.* × *cayeuxii* is roughly half the length of the corolla. Secondly, the length of the staminodes in *D. wallichii* are ¼ to ½ the length of the androecial tube, whereas the length of these parts are roughly equal to one another in *D.* × *cayeuxii*. A detailed description of *D.* × *cayeuxii* is provided by Sealy (1965). Contrary to Sealy (1965), I have found that *D.* × *cayeuxii* can sometimes have unlobed leaves (i.e., they are not always palmately 3-lobed) and caducous stipules (i.e., they are not always persistent).

Arènes (1958) listed BM as the herbarium housing the lectotype of *Dombeya wallichii*, but no specimen of Wallich 1161 is known to exist at BM. This error is corrected here by designation of a sheet of Wallich's collection at K, that is labeled 1161.1. (The sheet has its designation from Wallich's list handwritten in the upper right-hand corner and is noted as such to differentiate it from a second sheet also labeled Wallich 1161.1 at K). This sheet was clearly the element used by Lindley (1821) as it is a near perfect match to the specimen illustrated in the plate accompanying his description of *Astrapaea wallichii*. The lectotype for *Astrapaea penduliflora* DC. is designated here as the plate accompanying Ker's description of "Rosy Astrapaea" (1823). Ker considered this living specimen from Kew to be an *Astrapaea wallichii*, but de Candolle believed the plant to be a new species and described it as such. De Candolle clearly stated he based this assessment on the plate accompanying Ker's work and a cultivated, living specimen. There is no indication that a dried specimen was ever made of the cultivated plant, hence the designation of the plate as the lectotype.

The native distribution of *D. wallichii* is poorly understood. The majority of specimens of this species have little or no locality data associated with them and most were collected before 1900. I know of only two collections of *D. wallichii* that were made in the last 100 years, one from the northern east coast of Madagascar (Antalaha, *Reserve Naturelle 4435*) and one from the far southern east coast (Mandena, *Skema et al.* 372). Whether the third collection that may postdate 1900 (*C. Alleizette 652* from Analamazoatra, Toamasina province) is from a cultivated or wild individual is unknown. It seems likely that this specimen was cultivated given that it is the only one of five collections of known locality that did not come from sublittoral forest. In addition, neither I, nor apparently any other collectors, have (re)found *D. wallichii* in the well-collected forest of Analamazoatra. For such an unusual, large-flowered and colorful plant to be so little collected is mysterious. Whether *D. wallichii* has been extirpated from a once broader distribution or has simply been undercollected over the last century remains unclear. A passage written by Reverend Wm. Ellis (1859: 264), on a trip south from Tamatave along inland waterways of the east coast of Madagascar, intimates this species might have once been more common in the wild:

> "*But the most magnificent objects were the fine trees of Astrapaea Wallichii, or viscosa. ... I had seen a good-sized plant growing freely at Mauritius, but here it was in its native home, luxuriating on the banks of the stream, its trunk a foot in diameter, its broad-leaved branches stretching over the water, and its large pink, globular, composite flowers, three or four inches in diameter, suspended at the end of a fine down-covered stalk nine inches or a foot in length. These, hanging by hundreds along the course of the stream, surpassed anything of the kind I had seen or could possibly have imagined. I frequently met with the astrapaea afterward, but always growing near the water, and its branches frequently stretching over the lake or river.*"

The exact provenance of the cultivated *D. wallichii* is also unknown. Historical data suggest that *D. wallichii* arrived in cultivation in Europe via Mauritius and India. The type specimen (*Wallich 1161*) most certainly came from cultivated material. Wallich (1828) noted "H.B.C. & Madagascar" for this collection in a list summarizing details of his specimens; H.B.C. being his shorthand for plants he had cultivated at the Botanic Garden of Calcutta (de Candolle & Radcliffe-Smith, 1981). His notation of

Madagascar must refer only to the country of origin of the species, rather than the collection locality, because Wallich never visited Madagascar. Yet, he did visit Mauritius in 1812 (de Candolle & Radcliffe-Smith, 1981) and it seems plausible he collected seed from *D. wallichii* there (as assumed by previous authors, e.g., Ker, 1823) that he grew at Calcutta Botanic Garden upon his return to India, although I have found no record of this. Although it is currently unknown when and how *D. wallichii* arrived in Mauritius, there was a considerable amount of transport of living plants for cultivation between many of the islands of the Indian Ocean in the 19th century (Bojer, 1837; Bréon 1820 as in Dorr, 1997).

Molecular data (Chapter 2) suggest that at least one of the origins of cultivated *D. wallichii* is central Madagascar. A plastid phylogeny (see Introduction for details) shows three clades of sect. *Astrapaea* that correspond to geography rather than morphology. Three specimens of *D. wallichii* are included in this phylogeny, two from cultivation (*V. Leyman S4083*, *S4084*) and one from the wild in southern Madagascar (*Skema et al. 372*). The two cultivated accessions sit in the *sect. Astrapaea* plastid clade from central Madagascar while the wild collection is found in the southern clade. These data suggest that the wild ancestor of cultivated *D. wallichii* may have grown in Antananarivo, northern Fianarantsoa or Toamasina provinces, although multiple introductions of this species into cultivation are still a possibility.

HYBRIDIZATION AT VOHIPARARA

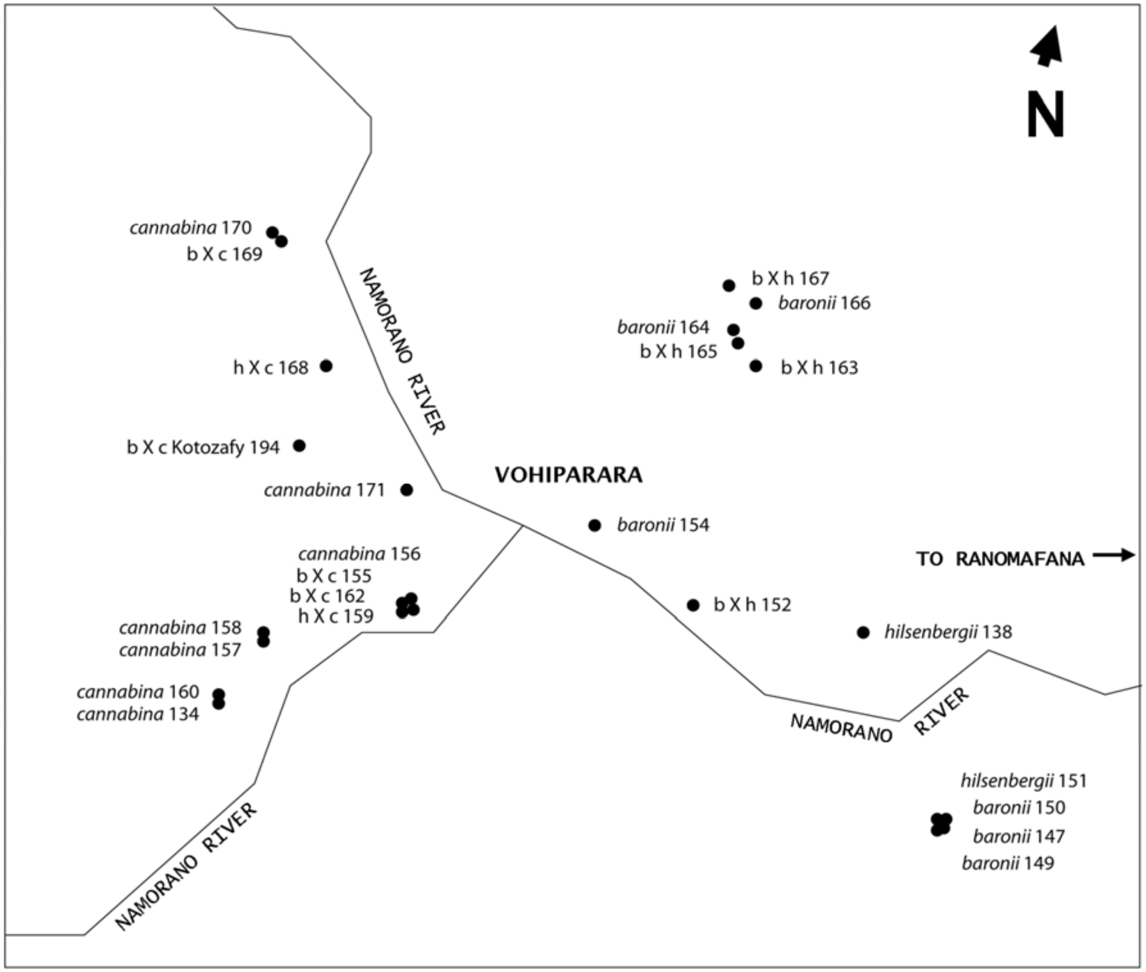
I consider a number of specimens (as listed below) that I collected from along the Namorano river around the village of Vohiparara (Ranomafana region of Fianarantsoa province) to be putative hybrids between *D. baronii*, *D. cannabina* and *D. hilsenbergii*. These specimens demonstrate various levels of morphological intermediacy between the putative progenitor species, all three of which occur sympatrically at that locality (see Fig. 4.9 for map). The forests along the Namorano river and its smaller tributaries are dense with sect. *Astrapaea*, and it was apparent even in the field that each tree looked slightly different from its neighbors. Further morphological examination of specimens found some that simultaneously exhibited characters of both putative progenitors. One such example (*Skema et al. 155*) had a glabrous and densely glandular adaxial stipule surface (like *D. cannabina*) combined with a densely pubescent and eglandular abaxial stipule surface (like *D. baronii*), and also showed a similar split of progenitor features on either surface of the leaves. Other specimens presented a single character of a putative parent in a morphological background of the other putative parent, such as a specimen collected by Skema et al. (167). This specimen was pubescent on all parts, had a palmately-lobed leaf and long, free filaments above the staminal tube (all characters of *D. baronii*) but with 2 ovules (like *D. hilsenbergii*). Molecular data (Chapter 2) suggest that gene flow exists between species in sect. *Astrapaea*, thus the existence of hybrids is not beyond reason. Future work could investigate if morphometric analyses or molecular data further corroborate the hybrid status of these specimens. Beyond that work, the question would remain whether hybrids occur in other regions of sympatry that have not yet been sampled or if Vohiparara, in its substrate or microclimates or pollinator fauna, has unique characteristics that erode (or overlap) potential selective factors that maintain these three species as distinct elsewhere.

D. baronii* × *D. hilsenbergii

Madagascar. FIANARANTSOA: Ranomafana National Park, on Route National 25, 3 km east of Vohiparara on south (Namorano river) side of road, 21º14’50”S, 47º24’6.4”E, *C. Skema et al. 152* (BH, MO, P, TAN, US); Ranomafana National Park, 40 m along Andranofady stream in Analatatatra forest to north of Route Nationale 25

Figure 4.9. Map of collection locations of putative hybrids between three sympatric species that occur along the Namorano river system near Vohiparara (Ranomafana, Fianarantsoa province). Map is a detail of area shown in dashed box in each of Figs. 4.6, 4.7, and 4.8. Circles represent collection localities. Numbers correspond to collection numbers of Skema et al., except for one specimen (no. 194) collected by Kotozafy et al. that is labeled as such. Specimens are labeled as:

putative hybrids:		**“pure” species:**	
b × c	*D. baronii* × *D. cannabina*	baronii	*D. baronii*
b × h	*D. baronii* × *D. hilsenbergii*	cannabina	*D. cannabina*
h × c	*D. hilsenbergii* × *D. cannabina*	hilsenbergii	*D. hilsenbergii*


N
cannabina 170
b X c 169
NAMORANO RIVER
b X h 167
baronii 166
baronii 164
b X h 165
b X h 163
h X c 168
b X c Kotozafy 194
VOHIPARARA
cannabina 171
baronii 154
cannabina 156
b X c 155
b X c 162
h X c 159
TO RANOMAFANA
b X h 152
cannabina 158
cannabina 157
hilsenbergii 138
cannabina 160
cannabina 134
NAMORANO RIVER
hilsenbergii 151
baronii 150
baronii 147
baronii 149
NAMORANO RIVER

about 2 km east of Vohiparara village, 21º13’31.9”S, 47º24’25.4”E, *C. Skema et al. 163* (BH, P, TAN, US); Ranomafana National Park, about 100 m along Andranofady stream in Analatatatra forest to north of Route Nationale 25 about 2 km east of Vohiparara village, 21º13’26.1”S, 47º24’22.1”E, *C. Skema et al. 165* (BH, TAN); Ranomafana National Park, about 500 m along Andranofady stream in Analatatatra forest to north of Route Nationale 25 about 2 km east of Vohiparara village, 21º13’7.1”S, 47º24’17.1”E, *C. Skema et al. 167* (BH, MO, P, TAN, US).

D. baronii* × *D. cannabina

Madagascar. FIANARANTSOA: Parc National de Ranomafana, parcelle #2, à l'Ouest de la ville de Vohiparara, 21º14’S, 47º22’E, *A. Kotozafy et al. 194* (BH, MO, P, TAN, US); Ranomafana National Park, on Route Nationale 45, 2-2.5 km west of Vohiparara village, 21º14’52”S, 47º22’36.6”E, *C. Skema et al. 155* (BH, MO, P, TAN, US); Ranomafana National Park, along Route Nationale 45, 2 km west of Vohiparara village on south side of Namorano River, 21º14’53.2”S, 47º22’32.9”E, *C. Skema et al. 162* (BH, P, TAN); Ranomafana National Park, on path along northern branch of Namorano River heading northwest about 5-6 km from Vohiparara on Route Nationale 25, 21º12”52.6’S, 47º21’56.2”E, *C. Skema et al. 169* (BH, MO, P, TAN, US).

D. cannabina* × *D. hilsenbergii

Madagascar. FIANARANTSOA: Ranomafana National Park, on Route Nationale 45, about 5 km west of Vohiparara village, next to very small bridge on bank of Namorano River, 21º14’52”S, 47º22’36.6”E, *C. Skema et al. 159* (BH, TAN); Ranomafana National Park, on Route Nationale 25 along northern branch of Namorano River heading northwest from Vohiparara, at first bridge about 2-2.5 km from Vohiparara on far side of river from national road, 21º13’34.9”S, 47º22’8.8”E, *C. Skema et al. 168* (BH, P, TAN, US).

DOUBTFUL AND EXCLUDED NAMES

Astrapaea acutangula Cav. ex Barb. Rodr., Hort. Flum. 42. 1893 [1895], *nom. nud.*

Astrapaea bornea Usteri, Guia Bot. Praça Rep. e Jard. Luz 41. 1919.—TYPE: unknown.—A name of unknown application.

Astrapaea mollis Hort. ex Miq. & Groenewegen, Cat. Hort. Amstelod. 271. 1857, *nom. nud.*

Astrapaea tiliifolia Sweet, Hort. Brit. 1: 58. 1826, as *tiliaefolia, nom. nud.*

Astrapaea viscosa Sweet, Hort. Brit. 1: 58. 1826, *nom. nud.*

Dombeya hilsenbergii var. *macrandra* Hochr., *nom. nud., in sched.*

Hilsenbergia cannabina Bojer, Hortus Maurit. 42. 1837, *nom. nud.*

Pentapetes speciosa Wallich in Lindl., Coll. Bot. ad t.14. 1821, *nom. nud., pro. syn.*

LITERATURE CITED

Agrawal, A. A., Karban, R. & Colfer, R. G. 2000. How leaf domatia and induced plant resistance affect herbivores, natural enemies and plant performance. *Oikos* 89: 70-80.

André, E. 1897. *Dombeya cayeuxii*. *Revue Horticole* 69: 544-545.

Arènes, J. 1958. Les *Dombeya* de Madagascar et des Comores. *Candollea* 16: 247-449.

Arènes J. 1959. Sterculiacées. Pp. 1-537 in Humbert, H. (ed.), *Flore de Madagascar et des Comores.* Firmin-Didot et C^{ie}, Paris.

Baillon, H. E. 1875. *The Natural History of Plants.* L. Reeve & Co., London.

Baillon, H. E. 1885a. Liste des plantes de Madagascar. *Bulletin Mensuel de la Société Linnéenne de Paris* I: 491-496.

Baillon, H. E. 1885b. Constitution du genre Dombeya. *Bulletin Mensuel de la Société Linnéenne de Paris* I: 491-496.

Baker, J. G. 1887. Further Contributions to the Flora of Madagascar. *Journal of the Linnean Society* XXII: 441-537.

Barnett, L. 1988. Systematics of *Nesogordonia* Baillon (Sterculiaceae). Ph.D. thesis, University of Texas, Austin. U.M.I., Ann Arbor, MI.

Bentham, G. & Hooker, J. D. 1862. *Genera Plantarum.* Lovell Reeve and Williams and Norgate, London.

Bojer, W. 1837. *Hortus Mauritianus ou énumeration des plantes, exotiques et indigènes, qui croissent à l'île Maurice, disposées d'après la méthode naturelle.* Aimé Mamarot et Compagnie, Mauritius.

Bojer, W. 1841. Espèces nouvelles de plantes à Madagascar et aux îles Comoroes. *Rapport Annuel sur les Travaux de la Société d'Histoire Naturelle de l'Ile Maurice* 11: 44-45.

Bréon, J. N. 1820. *Catalogue des plantes cultivées aux jardins botanique et de naturalisation de l'Île Bourbon.* Imprimerie du Gouvernement, St. Denis.

Chattaway, M. 1932. The wood of the Sterculiaceae I. Specialisation of the vertical wood parenchyma within the subfamily Sterculieae. *New Phytologist* 31: 119-132.

de Candolle, R. & Radcliffe-Smith, A. 1981. Nathaniel Wallich, MD, PhD, FRS, FLS, FRGS (1786 – 1854) and the Herbarium of the Honourable East India Company, and their relation to the de Candolles of Geneva and the *Great Prodromus*. *Botanical Journal of the Linnean Society* 83: 325-348.

Dorr, L. J. 1997. *Plant Collectors in Madagascar and the Comoro Islands.* Continental Printing, Belgium.

Ellis, W. 1859. *Three visits to Madagascar during the years 1853-1854-1856.* J. W. Bradley, Philadelphia.

Endlicher, S. 1865. *Genera plantarum secundum ordines naturales disposita.* Fridericum Beck, Universitatis Bibliopolam, Vienna.

Erdtman, G. 1952. *Pollen morphology and plant taxonomy.* Almqvist & Wiksells, Uppsala.

Friedmann, F. 1987. Sterculiacées. Pp. 1-50 in Bosser, J., Cadet, T., Guého, J. & Marais, W. (eds.), *Flore des Mascareignes: La Réunion, Maurice, Rodrigues* 53. The Sugar Industry Research Institute, Mauritius.

Gómez de la Maza, D. M. 1890. Catálogo de las Periantiadas Cubanas. *Anales de la Sociedad Española de Historia Natural* 19: 215-216.

Hochreutiner, B. P. G. 1926. Monographie des *Dombeya* de Madagascar. *Candollea* III: 5-120.

Hooker, J. D. 1837. *Dombeya cannabina*. *Botanical Magazine* 64: t. 3619.

Humeau, L., Pailler, T. & Thompson, J. D. 1999. Variation in the breeding system of two sympatric *Dombeya* species on La Réunion Island. *Plant Systematics and Evolution* 218: 77-87.

Humeau, L. & Thompson, J. D. 2001. The allometry of flower size dimorphism in dioecious *Dombeya* species on La Réunion. *Ecology Letters* 4: 221-228.

Jain, D. K. & Singh, V. 1973. Structure and ontogeny of trichomes in *Dombeya natalensis* Sond. *Journal of the Indian Botanical Society* 52: 235-241.

Ker, J. B. 1823. *Astrapaea wallichii*. *The Botanical Register* 9: t. 691.

Lindley J. 1821. *Astrapaea wallichii*, t. 14. in Anonymous *Collectanea botanica.* R. and A. Taylor, London.

Lundströem, A. N. 1887. Pflanzenbiologische Studien. II. Die Anpassung der Pflanzen an Thiere. *Nova acta Regiae Societatis scientiarum upsaliensis,* Series 3: XIII.

Miller, H. S. 1970. The herbarium of Aylmer Bourke Lambert. *Taxon* 19: 489-553.

von Mohl, H. 1835. *Sur la structure et les formes des grains de pollen*. *Annales des Sciences Naturelles* 2: 334.

Nixon, K. C. & Wheeler, Q. D. 1990. An amplification of the phylogenetic species concept. *Cladistics* 6: 211-223.

O'Dowd, D. J. & Willson, M. F. 1989. Leaf domatia and mites on Australasian plants: Ecological and evolutionary implications. *Biological Journal of the Linnean Society* 37: 191-236.

Planchon, J. E. 1850. Dombeya Ameliae. *Flore des serres et des jardins de l'Europe* 6: 225-227.

Prenner, G. 2002. Secondary pollen presentation on petals of *Dombeya cacuminum* Hochr. (Sterculiaceae). *Stapfia* 80: 323-326.

Rao, C. V. 1950. Pollen grains of Sterculiaceae. *Journal of the Indian Botanical Society* 28: 130-137.

Rao, S. R. S. 1987. Structure, distribution and classification of plant trichomes in relation to taxonomy: Sterculiaceae. *Feddes Repertorium* 98: 127-135.

Richardson, J. 1885. *A new Malagasy-English dictionary.* The London Missionary Society, Antananarivo.

Schatz, G. E. & Lescot, M. 2003. Gazetteer to Malagasy Botanical Collecting Localities. http://www.mobot.org/mobot/research/madagascar/gazetteer/: May 2010.

Sealy, J. R. 1965. *Dombeya × cayeuxii. Curtis' Botanical Magazine* 174: t. 473.

Seyani, J. H. 1991. *Opera Botanica Belgica, vol. 2: The Genus Dombeya (Sterculiaceae) in Africa.* National Botanical Garden of Belgium, Meise.

Skema, C. & Dorr, L. J. In press. *Dombeya gautieri* (Dombeyaceae), a remarkable new species from Madagascar. *Kew Bulletin* 65(3).

Soderholm, P. K. 1973. *Dombeya* 'Seminole' and *D*. 'Pinwheel,' new cultivars for landscaping in the subtropics. *Florida State Horticultural Society* 86: 452-453.

Vogel, S. 2000. The floral nectaries of Malvaceae *sensu lato* - a conspectus. *Kurtziana* 28: 155-171.

Wallich, N. 1828. A numerical list of dried specimens of plants in the East India Company's Museum, collected under the superintendence of Dr. Wallich of the Company's Botanic Garden at Calcutta. *IDC microfiche 1049, card 3*.