

TAXONOMY AND PHYLOGENOMICS OF CHRYSOBALANACEAE

Critical Appraisal

TAXONOMY AND PHYLOGENOMICS OF CHRYSOBALANACEAE

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Word count

Chapters 1–6 contain 13,326 words.

ABSTRACT

The Chrysobalanaceae is a pantropical woody plant family of 546 species and 27 accepted genera. Its centre of diversity is the Amazon where it is among the most numerically important in both number of species and individual trees in forest tree inventories. The family has been extensively studied at the alpha-taxonomic level, culminating in a comprehensive account of all taxa; however, molecular phylogenetics revealed the non-monophyly of several genera, casting doubts as to the relationships suggested by the last classification, published in 2003. The first molecular phylogenetic analyses used single loci (Sanger sequences) and clades were weakly supported, hindering interpretation of results. Phylogenomics, using complete genomes, provided better results with more robust support for clades and uncovered novel relationships among genera. Here I outline the taxonomic history of the family and trace the journey from major taxonomic circumscriptions based on morphology and discuss key findings and contributions from my molecular phylogenetic research starting from 2006, and how results both confirm and contradict the traditional taxonomy. Our analyses retrieve six clades, among them the earliest diverging clade composed of *Bafodeya* from West Africa and *Kostermanthus* from Australasia, and a species-rich clade, the Neotropical clade, of ca. 400 species in 11 neotropical genera that includes the three largest, *Couepia*, *Hirtella* and *Licania*, previously considered distantly related. This clade also has a single West African monospecific genus, *Afrolicania*. Results confirm that genera previously described under *Parinari* are distinct and more distantly related than previously thought. The most recent molecular phylogenetic studies completed in 2020, suggest the family diversified in the Eocene-Oligocene transition, ca. 38.9 Mya and support a palaeotropical origin, most probably in Africa, and shows that rates of diversification were highest in the Neotropics. The relationships uncovered in my recent molecular studies have provided a monophyletic classification of

genera that is essential for producing accurate conservation assessments and supports hypotheses on the role of species diversification within a historic biogeographical context.

KEY WORDS *Couepia*, *Hirtella*, *Licania*, *Parinari*, molecular phylogenetics, Neotropics, Pantropics

PREFACE

This thesis is based on the publications of my taxonomic and molecular phylogenetic research on the pantropical plant family Chrysobalanaceae. The study sets out to bring the morphological work together with novel molecular techniques to present an evolutionary taxonomy of the family. I began work as a botanist in the Amazon rainforest in the city of Manaus, Amazonas, Brazil studying several different plant families. One of the families that stood out significantly was the Chrysobalanaceae, which was ecologically important and numerically abundant in lowland Amazon rainforests. Amazonia appeared to be its centre of diversity. During this time of intensive fieldwork, I became thoroughly familiar with all aspects of the biology of genera and species of Chrysobalanaceae, such as habitat preferences, pollination biology, and seed dispersal, as a basis for providing knowledge on the taxonomy of the family. My first research position was with a forest ecology project led by the World Wildlife Fund, the Biological Dynamics of Forest Fragments Project, later managed by the Smithsonian Institute, where I worked for almost four years starting in 1989. After that, I worked at the National Institute of Amazon Research (INPA) for five years where I was part of a team working on the Flora da Reserva Ducke Project, funded by the Department for International Development (DFID). The project was based in a forest reserve outside the city of Manaus and its aim was to publish a field guide and flora of the reserve. I worked with Chrysobalanaceae, among several other plant families. The results were taxonomic accounts of the families and an illustrated field guide entitled Flora da Reserva Ducke (Ribeiro et al. 1999). Afterwards I moved to the U.K. in 1998 and worked at the Royal Botanic Gardens, Kew focusing on the Chrysobalanaceae as a whole, refining my knowledge on the taxonomy of genera and species that I was familiar with and those that I was not yet familiar with, mostly from the Palaeotropics. At Kew I worked under the supervision of Prof. Sir Ghilleen Prance, the world specialist on Chrysobalanaceae. This work at Kew led to my first major publication

in 2003. I co-authored the World Flora of Chrysobalanaceae, a two-volume comprehensive taxonomic account of the family which included 531 species in 18 genera. After that I embarked on studying and revising the taxonomy of the large genera *Couepia*, *Licania* and *Hirtella*. This led to molecular research in the Jodrell Laboratory at Kew Gardens in collaboration with Prof. Mark Chase starting in 2007. I gradually incorporated all the other genera for a comprehensive study at the molecular level because my research showed that several genera were poly- and paraphyletic and that their classification needed to be re-addressed.

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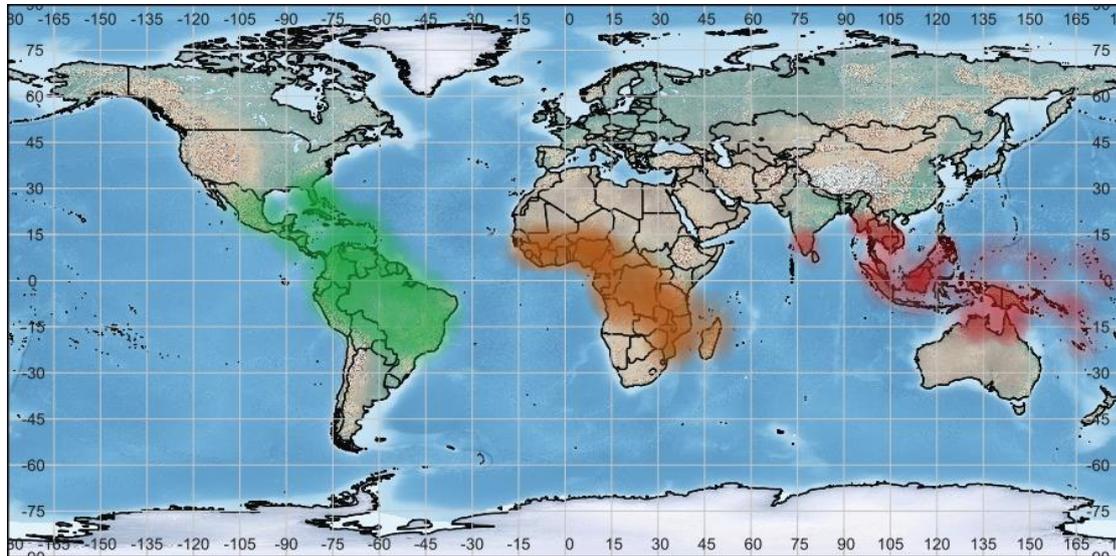
I would like to thank Dr. Andrew Lack for the encouragement on embarking on this thesis and the always positive guidance throughout; Prof. Ghilleen T. Prance for the mentorship and the insightful discussions on Chrysobalanaceae; Dr. Martin Cheek for the constant support and help with technicalities during my thesis; and Dr. Xander van der Burgt for the guidance and support. I also thank Prof. Susan Brooks and Jill Organ who provided much help with matters to do with the degree programme, which made all the difference.

CHAPTER 1: INTRODUCTION

1.1. Introduction to the family

Chrysobalanaceae is a pantropical woody plant family currently comprised of ca. 546 species in 27 genera (Prance & Sothers 2003a, Sothers *et al.* 2014, Sothers *et al.* 2020), found predominantly in lowland rainforests, and in a range of other habitats, such as savannas, and in dry, flooded, high altitude, gallery, and coastal forests. The Neotropics harbours the greatest generic and species diversity, including the three largest genera, *Licania* Aubl., *Hirtella* L. and *Couepia* Aubl., and with ca. 431 species. Of the 16 neotropical genera, 11 are found in the Amazon region, highlighting the importance of the family in this biome. Its centre of diversity is the Amazon Basin where it is usually among the families with the highest number of individuals in forest inventories (Cardoso *et al.* 2017, ter Steege *et al.* 2016). In the Palaeotropics the family is represented in Africa and Madagascar by 70 species, and in Australasia by 45 species (Prance & Sothers 2003a, b, Bardon *et al.* 2013); although represented by a much smaller number of species, they are important components of woodland savannas and primary forests (Figure 1). Several species and genera are island endemics, occurring solely in Madagascar, New Guinea, and New Caledonia. Genera are mostly endemic to each tropical region, with only two, *Parinari* Aubl. and *Maranthes* Blume, native to all three tropical regions; and *Chrysobalanus* naturalised in Australasia. Several species are of economic value and provide important food and timber for local or small to medium scale commercial purposes, and some species have medicinal properties.

Figure 1. Distribution of Chrysobalanaceae and the currently accepted genera by geographic region, with number of species for genera and total number of species.



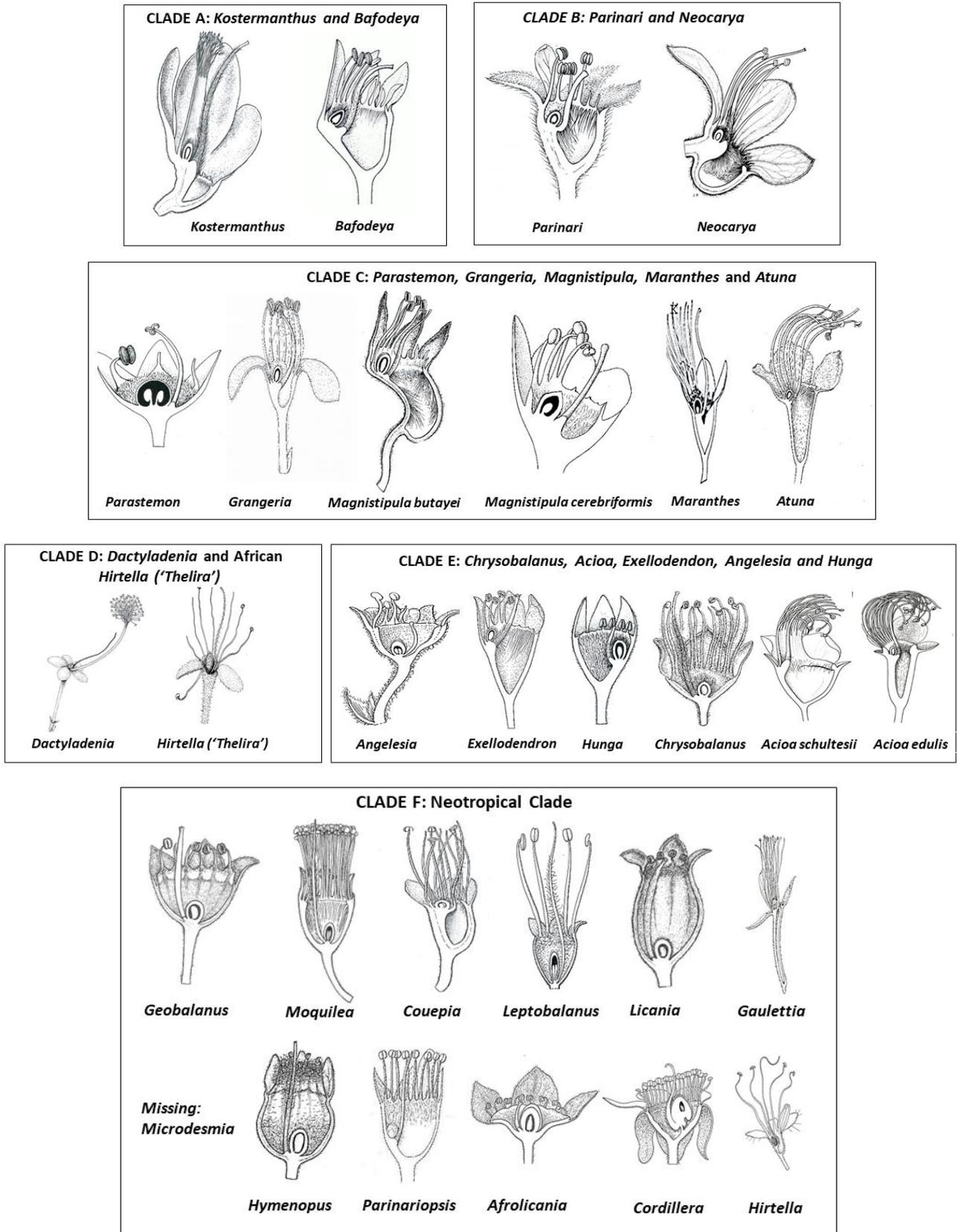
NEOTROPICS	spp.	AFRICA	spp.	AUSTRALASIA	spp.
<i>Acioa</i>	6	<i>Dactyladenia</i>	30	<i>Kostermanthus</i>	3
<i>Couepia</i>	65	<i>Bafodeya</i>	1	<i>Atuna</i>	8
<i>Exellodendron</i>	5	<i>Neocarya</i>	1	<i>Hunga</i>	11
<i>Hirtella</i>	107	<i>Hirtella</i>	2	<i>Parastemon</i>	3
<i>Licania</i>	100	<i>Afrolicania</i>	1	<i>Angelesia</i>	3
<i>Maranthes</i>	1	<i>Maranthes</i>	10	<i>Maranthes</i>	1
<i>Parinari</i>	19	<i>Parinari</i>	10	<i>Parinari</i>	15
<i>Chrysobalanus</i>	3	<i>Chrysobalanus</i>	1	<i>Chrysobalanus</i>	1
<i>Cordillera</i>	1				
<i>Gaulettia</i>	9	<i>Magnistipula</i>	12		
<i>Geobalanus</i>	3	<i>Grangeria</i>	2		
<i>Hymenopus</i>	28				
<i>Leptobalanus</i>	31				
<i>Microdesmia</i>	2				
<i>Moquilea</i>	49				
<i>Parinariopsis</i>	1				
TOTAL GENERA	16	TOTAL GENERA	10	TOTAL GENERA:	8
TOTAL SPECIES:	431	TOTAL SPECIES:	70	TOTAL SPECIES:	45

The family is distinguished by the gynobasic style and the presence of silica in the wood, two synapomorphies not shared by its sister clade, comprised of the families Dichapetalaceae, Euphroniaceae, Trigoniaceae and Balanopaceae (Angiosperm Phylogeny Group IV; Prance & Sothers 2003a). Other distinct morphological features of Chrysobalanaceae include the position of the ovary on the receptacle, which can be either at the base, on the wall or at the mouth of the receptacle; the number of locules in the ovary, of either 1 or 2; the flowers with or without petals; and some genera with distinct mechanisms of seedling escape. In addition, other characters that have shaped the taxonomy of the family include stamen number, which varies from 3 to over 300; the stamen arrangement around the receptacle, unilaterally or in a semi or complete circle; the fusion of filaments, which are mostly free, or less often fused at the base, or fused for half the length (Figure 2).

Species now in Chrysobalanaceae were first cited by Linnaeus (1738) and linked to Rosaceae by Jussieu (1789). Prance (1963) and Prance & White (1988) revised the taxonomy of the family, and proposed several significant taxonomic changes, such as the breakup of the genus *Parinari*, which had previously included most taxa with a bilocular ovary (and even with a unilocular ovary), and which was treated as heterogeneous and artificial. On the other hand, *Licania* was circumscribed as a genus with the central character of a unilocular ovary, positioned essentially at the base of the receptacle, which was shared by all the species.

Prance divided the family into two tribes (Prance 1963), and then four (Prance & White 1988). The World Flora volumes on Chrysobalanaceae (Prance & Sothers 2003a, b), the last major revision of the family, treated 18 genera, both neotropical and palaeotropical, with a few further changes in the family's taxonomy; the tribal classification was abandoned and no other subfamilial classification was proposed. Currently the family is comprised of 27 accepted genera in six well-supported clades.

Figure 2. Floral line drawings of genera showing selected features: receptacle shape, free or fused stamens, included or exerted stamens, ovary position on the receptacle (apical or basal).



The advent of molecular phylogenetic techniques brought about insights for further taxonomic changes at generic level and the extent to which the Chrysobalanaceae was classified artificially came to light. Preliminary molecular studies did not support the previous taxonomic tribal circumscription and highlighted that the tribal classification was inadequate. The first molecular study for Chrysobalanaceae was that of Dissanayake (1999). Several genera were shown to be poly- and paraphyletic. The relevant taxonomic rearrangements were carried out to maintain the monophyly of genera (Sothers & Prance 2014, Sothers et al. 2014, 2016). Further work on the taxonomy of a few genera remains to be done.

In summary, the Chrysobalanaceae is a family of ecological significance and economic value for local communities (Prance & White 1988, Prance & Sothers 2003, Sothers et al. 2020). It holds an important place in ecologically important biomes, such as Amazon lowland primary rainforests and African woodland savannas and other dry forests. Despite extensive knowledge of its taxonomy, several questions remain regarding the generic relationships, character evolution, and diversification history.

1.2. Taxonomy: Morphology versus molecular inference

Taxonomy, the classification, and identification of species is fundamental for the compilation of checklists for countries, regions, conservation areas, and for conservation assessments of species. A robust taxonomic framework also supports research into medicinal plants, ecological studies, such as pollination and other biological interactions and biotic studies, as well as establishing the geographic distribution of species. The foundations of modern plant taxonomy were laid down based on morphological observation and analysis using the naked eye and a stereoscopic microscope. Over time new and novel techniques were added but it was only in the last 30 years that plant taxonomy has been impacted by molecular

techniques and DNA analyses, allowing for an evolutionary approach that has revealed relationships among species never previously hypothesised.

The taxonomy of the Chrysobalanaceae has been challenging and is notable for parallelisms, mentioned in previous works (Prance & White 1988), making it a difficult family to work with based on morphological characters alone. Genera and species were considered closely related based on characters such as the position of the ovary on the receptacle, number of locules in the ovary, and number of stamens. Although the taxonomy of the family appeared to work well based on these morphological features, the first molecular study into the family raised doubts as to the monophyly of the tribes as circumscribed at the time (Dissanayake 1999). The pioneering molecular work of Dissanayake showed that the largest genus, the pantropical *Licania*, was closely related to *Hirtella* and *Couepia*, rather than close to other genera in tribe Chrysobalaneae, such as *Chrysobalanus* L. and *Parastemon* A.DC., that also has the ovary at the base of the receptacle. Results also confirmed the monophyly of *Parinari* and of genera that had previously been placed in synonymy of *Parinari*, namely *Atuna* Raf., *Bafodeya* Prance ex F.White, *Kostermanthus* Prance, *Neocarya* (DC.) Prance, *Maranthes*, *Hunga* Pancher ex Prance, *Exellodendron* Prance by Prance (1963). In addition, most of these genera were not closely related to *Parinari* in her phylogeny.

The early molecular work (Dissanayake 1999, Yakandawala 2010) raised more questions than were answered and this suggested that further molecular research in the Chrysobalanaceae was necessary. The aims for further research were to expand the sampling of taxa to increase the number of accessions and target the unsampled genera and problematic species, or those with distinct morphological features from the genera in which were placed, and those with disjunct geographic distributions. Characters that needed to be further investigated included the number of ovules and the placement of the ovary on the receptacle, since not all genera with a bilocular ovary occurred together in a clade, e.g., those previously

placed in *Parinari*. Genera with the ovary at the base as well as at the mouth of the receptacle appeared closely together, e.g., *Licania*, *Couepia*, *Hirtella*, rather than distantly related.

The results of molecular analyses highlighted that in previous morphologically based taxonomic studies too much emphasis was placed on certain characters that did not yield a monophyletic system. For example, the position of the ovary on the receptacle, which was previously used to classify tribe Chrysobalanaceae and genera such as *Licania* but was not supported as a synapomorphy. Therefore, the taxonomy of the family was considered well delimited until molecular studies indicated that more research was needed to resolve infra-familial relationships. Molecular studies have therefore been of great value in redefining genera and their relationships, resulting in a better taxonomic delimitation.

1.3. The research programme

The Chrysobalanaceae is an important family in terms of its role and significance in the ecology of major biomes. The taxonomic history of the family has been somewhat disputed and although the last comprehensive taxonomic work appeared well circumscribed, molecular phylogenetic studies pointed to several disparities and unsupported relationships among genera. Here, these are addressed from the last taxonomic comprehensive account to a complete phylogenomic reconstruction of the family.

The research programme outlines the taxonomic history of the Chrysobalanaceae, discusses how distinct morphological features shaped its initial classification and outlines key findings and contributions from molecular research and how the results both confirm and contradict the traditional taxonomic circumscription. The last taxonomic account of the family (Prance & Sothers 2003a, b) proposed a classification that was already disputed by preliminary

molecular studies (Dissanayake 1999) and questions persisted regarding taxa that displayed discordant features and disjunct geographic distributions.

The overall aims of my study here are to bring the taxonomy of the Chrysobalanaceae in line with molecular phylogenetic evidence based on a fully resolved genomic framework and to propose a classification expressing the evolutionary history of all genera and species of Chrysobalanaceae. A fully resolved molecular phylogeny is essential to support further research and other studies in the family, such as the high neotropical diversity, evolutionary patterns, conservation genetics and biogeography of the family, and to integrate morphological, ecological, and phylogenetic studies in our understanding of the family.

CHAPTER 2: TAXONOMIC HISTORY OF CHRYSOBALANACEAE

2.1. Historic background of family

The formal taxonomy of the family began with Linnaeus (1753), who cited two American taxa in his *Species Plantarum*: *Chrysobalanus icaco* L., and *Hirtella americana* L., the latter first published in *Hortus Cliffortianus* (Linnaeus 1738). Aublet (1775) described a further six South American genera: *Licania*, *Tachibota* Aubl. (a synonym of *Hirtella*), *Parinari*, *Couepia*, *Moquilea* Aubl. and *Acioa* Aubl. Jussieu (1789) placed all genera of Aublet, along with *Chrysobalanus*, and *Grangeria* Commers. ex Juss. (Jussieu 1789), under Rosaceae in two of its seven tribes, due to their shared character of the gynobasic style. Brown (1818) was the first to suggest family rank to Chrysobalanaceae, characterised by the gynobasic style, erect embryo and ovule, and a tendency towards zygomorphy; he included the following nine genera in his concept of the family: *Acioa*, *Chrysobalanus*, *Couepia*, *Grangeria*, *Hirtella*, *Licania*, *Moquilea*, *Parinari* and *Thelira* Thouars. Subsequently, taxonomists either treated the group as a tribe or subfamily of Rosaceae (Martius & Zuccarini 1832, Candolle 1825, Meisner 1837a,b, Hooker 1865, Baillon 1869, Focke 1891; Hutchinson 1964) or as a distinct family (Lindley 1836; Bentham 1840; Endlicher 1840, 1842; Mueller 1857; Fritsch 1889; Prance 1963).

The delimitation of genera has changed over time (Table 1). Infra-familial divisions were first proposed by Baillon (1869) who treated the group in Rosaceae and subdivided the genera based on the symmetry of the flower as follows: group A: *Chrysobalanus*, *Licania* (including *Moquilea*), *Lecostemon* Benth. (Rhabdodendraceae) and *Stylobasium* Desf. (Stylobasiaceae); and group B: *Grangeria*, *Hirtella*, *Couepia*, *Parinari*, *Acioa*, and *Parastemon*. Fritsch (1889) treated the genera as a family separated into three subfamilies,

Chrysobalaneae, Lecostemoneae, and Stylobasieae. Focke (1891) treated the genera as a tribe of Rosaceae with two subtribes, based on the symmetry of the flowers: Chrysobalaninae comprised of *Chrysobalanus*, *Grangeria*, *Moquilea*, *Licania*, *Lecostemon* (now in Rhabdodendraceae) and *Stylobasium* (now in Stylobasiaceae); and Hirtellinae with *Hirtella*, *Couepia*, *Parinari*, *Acioa*, *Angelesia* Korthals, and *Parastemon*.

Prance (1963) carried out the first major revision of the taxonomy of the group, since Fritsch's treatment (1889). He excluded from the family the doubtful genera *Lecostemon* and *Stylobasium*, subdivided all the genera into two groups based on floral symmetry, following Focke (1891), and clarified the generic limits of problematic genera such as *Parinari* and *Licania*. He redefined *Parinari* leading to the description of several new genera and the reinstatement of old genera, such that *Parinari* was disbanded into eight smaller genera: *Parinari*, *Bafodeya*, *Neocarya*, *Maranthes*, *Atuna*, *Exellodendron*, *Hunga*, and *Kostermanthus*; most had been assigned to *Parinari* based solely on the character of the bilocular ovary, and one of the new segregate genera (*Kostermanthus*) even had a unilocular ovary. This splitting of genus *Parinari* based on morphology is supported by molecular analyses (Bardon et al. 2016, Sothers et al. 2016). On the other hand, the broadly circumscribed *Licania* was maintained by him as a large pantropical genus of species with mainly unilocular ovary and a sub-actinomorphic floral symmetry with the ovary at or near the base of the receptacle. Later, the genus was subdivided into five subgenera (Prance 1972), a concept that persisted until the advent of molecular studies, which showed that the genus was polyphyletic and *Licania* was split into several genera (Sothers & Prance 2014, Sothers et al. 2016).

Prance & White (1988) proposed a new classification of four tribes based on such characters as stamen exertion, arrangement and number of stamens, position of the ovary on the receptacle, and number of locules in the ovary. The 17 genera known at the time were assigned to the following tribes: Chrysobalaneae, Parinarieae, Couepieae and Hirtelleae. In the

most recent and comprehensive taxonomic account of the family (Prance & Sothers 2003a, b), this tribal classification was discarded and none other presented. The first molecular studies at the infra-familial level cast doubts on this classification of tribes and more studies were needed to understand the relationships among genera.

Table 1. Historic tribal delimitation of accepted genera of Chrysobalanaceae over the past two centuries. Asterisk* indicates genera in synonymy.

Baillon 1869	Focke 1891	Prance 1972	Prance & White 1988	Chave et al. 2020
Group A Chrysobalanus LICANIA (*Moquilea) PARINARI	Subtribe <i>Chrysobalaninae</i> Chrysobalanus Grangeria LICANIA Moquilea	Tribe <i>Chrysobalaneae</i> Chrysobalanus Parastemon Grangeria LICANIA (*Angelesia, Moquilea, Geobalanus) Afrolicania	Tribe <i>Chrysobalaneae</i> Chrysobalanus Parastemon Grangeria LICANIA (*Angelesia) Afrolicania	Clade A Bafodeya Kostermanthus Clade B PARINARI Neocarya Clade C Maranthes Atuna
Group B Acioa HIRTELLA Parastemon Grangeria COUEPIA	Subtribe <i>Hirtellinae</i> Acioa HIRTELLA Parastemon Angelesia PARINARIUM COUEPIA	Tribe <i>Hirtelleae</i> Acioa (*Dactyladenia) HIRTELLA Magnistipula Hunga PARINARI (*Atuna, Bafodeya, Exellodendron, Kostermanthus, Maranthes, Neocarya) COUEPIA	Tribe <i>Hirtelleae</i> Dactyladenia HIRTELLA Magnistipula Kostermanthus Atuna Tribe <i>Parinarieae</i> PARINARI Neocarya Exellodendron Hunga Bafodeya Tribe <i>Couepieae</i> Acioa COUEPIA Maranthes	Clade D Magnistipula Parastemon Grangeria Clade D Dactyladenia HIRTELLA (*Thelira) Clade E Acioa Hunga Exellodendron Angelesia Chrysobalanus Clade F Geobalanus Moquilea COUEPIA Leptobalanus LICANIA Gaulettia Microdesmia Hymenopus1 Afrolicania Cordillera Parinariopsis Hymenopus2 HIRTELLA
Figure legend: PARINARI: green LICANIA: blue COUEPIA: red HIRTELLA: orange				
8 genera	10 genera	10 genera	17 genera	27 genera

2.2. Traditional taxonomy and circumscription of key genera: *Parinari*, *Licania*, *Couepia* and *Hirtella*

The taxonomic history of genera has followed a different path and over the centuries authors have disagreed on generic limits and the taxonomy of the three largest genera, *Couepia*, *Hirtella* and *Licania*. *Parinari* has also been problematic and as a result these four genera have undergone rearrangements and circumscriptions. Prance, who studied the family extensively, assigned each of these genera to separate tribes (Prance & White 1988) and therefore did not consider them as closely related. *Licania* was included in tribe Chrysobalaneae with its unilocular ovary positioned at the base of the receptacle, *Parinari* in tribe Parinarieae with bilocular ovary at the mouth of the receptacle, *Couepia* in tribe Couepieae, and *Hirtella* in tribe Hirtelleae, both with a unilocular ovary at the mouth of the receptacle. Of the 27 currently accepted genera (Sothers et al. 2020), 19 have been included in or have been directly associated with one of the four genera cited above, and therefore my discussion will focus on them.

Parinari has been accepted as a genus since it was described by Aublet in 1775, and Jussieu (1789) mentioned the bilocular ovary as a defining character, but Bentham's (1849) greater emphasis on the bilocular ovary led to the misconception of *Parinari* to the extreme point where any species with a bilocular ovary was described in *Parinari*, thereby creating an artificial concept of the genus. When Prance (1963) revised the family, he found that *Parinari* should be divided into eight genera, five of which he described as new: *Exellodendron* (Prance 1972), *Hunga* (Prance 1979), *Bafodeya* and *Neocarya* (White 1976), and *Kostermanthus* (Prance 1979). Two more older genera with bilocular ovaries that had been synonymised into *Parinari* were reinstated, *Atuna* by Kostermans (1969), and *Maranthes* by Prance (1966). Most African species of *Maranthes* were originally described under *Parinari* (Prance & White 1988). All these genera were segregates of *Parinari* and shared the character of a bilocular ovary,

except for *Kostermanthus*, but apart from that they were distinct in several other features, such as the leaves, the position of the ovary on the receptacle, number of stamens, fruits, and even by germination mechanisms. Despite the greatly reduced circumscription of the genus, it remains one of only two pantropical genera in the family, with ca. 39 species.

Licania has also always been accepted as a genus and was described along with *Moquilea*, *Couepia* and *Acioa* (Aublet 1775), but the latter three genera were variously considered as accepted genera or as synonyms in the past; *Moquilea* either as a synonym of *Licania* or as an accepted genus; *Couepia* as a synonym of *Moquilea* or as accepted; and *Acioa* as a synonym of *Couepia* or *Moquilea* or as accepted. All four genera are endemic to the Neotropics and are currently accepted genera.

Bentham (1840) accepted *Licania* and *Moquilea* as distinct genera. He delimited *Licania* as species with the following characters: 4-5 petals or apetalous, stamens less than 15 and ovary at the base of the receptacle. He divided the genus into five sections: *Batheogyne*, *Leptobalanus*, *Microdesmia*, *Eulicania* and *Hymenopus*. *Moquilea* was delimited as having petals, more than 30 stamens and the ovary at the base of the receptacle. Hooker (1867) accepted both genera but reordered Bentham's sections so that *Licania* included sections *Eulicania* and *Hymenopus*, and *Moquilea* sections *Eumoquilea*, *Leptobalanus* and *Microdesmia*. As a result, both genera included not only species with ovary at the base of the receptacle but also species with and without petals, unlike Bentham's delimitation. Fritsch (1888) accepted *Licania* and *Moquilea* but later merged *Moquilea* with *Licania* (Fritsch 1889) and subsequently this concept of *Moquilea* as a synonym was followed thereafter in most classifications (Hallier 1903; Prance 1963, 1972, 1989, Prance & White 1988; Prance & Sothers 2003a).

Prance & White (1988) subdivided *Licania* into five subgenera: subgen. *Licania*, subgen. *Moquilea*, subgen. *Parinariopsis*, subgen. *Angelesia* and subgen. *Afrolicania*; the latter

two comprised of species from Australasia and Africa, respectively, and the other subgenera of exclusively neotropical taxa. *Licania* subgen. *Licania* and subgen. *Moquilea* were both further subdivided into sections; subgen. *Parinariopsis* contained only one species. The genera *Angelesia* (Korthals 1854) and *Afrolicania* Mildbr. (Mildbraed 1921) were also placed in synonymy of *Licania* based on the unilocular ovary positioned at the base of the receptacle. However, *Angelesia* and *Afrolicania* were both later reinstated to generic level, *Afrolicania* based on morphological features (Prance & Sothers 2003a) and *Angelesia* based on molecular studies (Sothers & Prance 2014). In the last revision of the family (Prance & Sothers 2003a) *Licania* remained the largest genus, with *Moquilea* as a synonym. Molecular analyses showed that *Licania* was highly polyphyletic, confirming that *Afrolicania* and *Angelesia* were not closely related to *Licania*, and further that the other subgenera and sections were also polyphyletic (Sothers et al. 2016).

Moquilea was accepted as a distinct genus from *Licania* until Grisebach (1857) merged it under *Licania*. Hooker (1865) reinstated *Moquilea*, but Baillon (1868) merged it under *Licania* again; different authors treated these two genera differently but mostly *Moquilea* was treated as a synonym of *Licania*. Prance (1972) treated several species under a new subgenus of *Licania*, which he named subgen. *Moquilea*. However, molecular analyses showed that *Licania* was polyphyletic and to maintain monophyly, *Moquilea* was re-instated (Sothers et al. 2016). *Moquilea* also has a basal ovary but has exerted stamens.

Couepia was described by Aublet (1775) but was placed in synonymy of *Moquilea* (Martius 1827). Meisner (1837a,b) and Endlicher (1840, 1842) treated *Couepia* and *Acioa* as synonyms of *Moquilea* and Bentham (1840) restored *Couepia* to generic rank. The genus has the ovary positioned at the mouth of the receptacle and mostly an elongated receptacle, these two characters differentiating it from *Licania* and *Moquilea*; all three genera have a unilocular ovary. Prance (1972) divided *Couepia* into superspecies groups, but these were not supported

in molecular phylogenetic analyses (Sothers et al. 2014). One superspecies group of Prance (1972), the parillo group, was excluded from *Couepia* and described as the new genus *Gaulettia* Sothers & Prance by Sothers et al. (2014); and one anomalous species was excluded and described as a new genus, *Cordillera* Sothers & Prance (Sothers et al. 2016), following molecular sequence data which had showed the genus *Couepia* to be polyphyletic. Currently *Couepia* is circumscribed as a neotropical genus of ca. 65 species (Sothers et al. 2020).

Hirtella was first cited by Linnaeus (1738) and then formally described by him in 1753 based on collections from Central America. This genus is distinguished by the unilocular ovary positioned at the mouth of the receptacle; it has always been an accepted. *Thelira* was described from African collections (Du Petit-Thouars 1806) and was first treated as a distinct genus from *Hirtella* until Baillon (1868) placed *Thelira* in synonymy of *Hirtella* and this concept was followed thereafter. Morphologically, the species are very similar, sharing the main features of the ovary at the mouth of the receptacle, the few and unilaterally placed stamens, often the presence of bracteolar glands, and the unilocular ovary. Molecular analyses, however, indicate these genera are in fact distinct but *Thelira* at present remains in synonymy under *Hirtella*.

The few genera that have not generally been associated with either *Parinari*, *Licania*, *Couepia* or *Hirtella* have in some cases had species described under other genera but historically have mostly been accepted by authors. *Dactyladenia* Welw. (Welwitsch 1859), a genus disregarded for much of its existence, was previously treated as a synonym of *Acioa* (Exell 1928) until it was finally re-established as a distinct genus (Prance & White 1979). Both these two genera share with *Kostermanthus* a distinct feature in the family, that of the fused stamens into a strap or ligule for at least half their length. *Chrysobalanus*, *Grangeria*, and *Parastemon* all have a unilocular ovary positioned at the base of the receptacle (except laterally positioned in *Grangeria*) and morphologically are like *Licania* and were in fact placed in tribe

Chrysobalaneae because of these shared features. Molecular analyses confirm that these genera are not closely related to *Licania* at all.

2.3. Morphological characters used in the traditional taxonomy

Chrysobalanaceae displays distinct features that are also quite variable. The floral and fruit characters traditionally used for classifications within the family include, 1) the symmetry of the receptacle, sub-actinomorphic (due to the gynobasic style) with the ovary placed centrally at the base of the receptacle to strongly zygomorphic, with the ovary at the mouth of the receptacle; 2) receptacle shape, shallow to cylindrical and elongate; 3) presence or absence of petals; 4) stamen number, varying from 2 to 300; 5) stamen arrangement around the disk, unilateral, in a complete, or an almost complete circle; 6) free or fused filaments; 7) the position of the ovary on the receptacle at the mouth, on the wall, or at the base; 8) the ovary either uni- or bi-locular; and 9) specific germination mechanisms for seedling escape. Of these characters, the number of locules in the ovary and the position of the ovary on the receptacle have been central to the taxonomy of the family and have shaped the delimitation of genera since the description of the seven genera by Aublet (1775); see Figure 2 in Chapter 1 for the floral morphological features of genera.

Traditionally the character used the most in the taxonomic delimitation of the family is ovary locularity; all genera divide into those with 1- and those with 2-locular ovaries. All the genera with a bilocular ovary have the ovary positioned at the mouth of the receptacle, but genera with a unilocular ovary have the ovary at the base or at the mouth of the receptacle. Ovary locularity and position on the receptacle appear to be good diagnostic characters at generic level but did not support the tribal classification as proposed by Prance & White (1988).

Three of the four tribes included genera with a unilocular ovary, tribe Chrysobalaneae, tribe Couepieae and tribe Hirtelleae.

One of the central and uniting characters of *Licania* is the unilocular ovary. This has determined the classification of *Licania* as closely related to other genera, but unlike *Parinari*, other genera with a unilocular ovary have the ovary positioned at the mouth of the receptacle, such as *Couepia* and *Hirtella*.

Other distinct characters in the family include the hair-filled stomatal cavities on the lower leaf surface that only occur in some species of *Parinari*, *Gaulettia*, *Leptobalanus* (Benth.) Sothers & Prance, *Licania* and *Microdesmia* (Benth.) Sothers & Prance. This character appears to be an adaptation for growing in drier habitats. *Gaulettia* (Sothers et al. 2014) was proposed as a new genus and segregated from *Couepia* based on molecular studies and the central character uniting the nine species is the deeply reticulate leaf venation and stomatal cavities, associated with a tendency to occupy drier habitats. Different types of glands are associated with several genera in the family, and these include laminar, petiolar, bracteolar, and glands on the calyx. These extra-floral glands are most probably associated with plant-animal (insect) interactions. Taxonomically, they are important diagnostic features of some genera, such as the pair of petiolar glands found in species of *Licania* and *Maranthes*, and the bracteolar glands found only in *Hirtella* and *Dactyladenia*.

Despite extensive work on the morphology of the family, doubts about some genera and species remained, such as the monophyly of *Hirtella*, with a disjunct distribution, occurring in the Neotropics and in eastern Africa and Madagascar, the segregate genera of *Parinari* as accepted genera, the monophyly of *Magnistipula*, the disparate species within *Couepia*, such as the *parillo* group and the two red-flowered and bat-pollinated species of *Couepia*. Questions regarding genera and their central features, for example the relationships of genera with bilocular ovaries, and the monophyly of *Licania*, which incorporated several subgenera all with

unilocular ovaries at the base of the receptacle needed to be investigated. And so, the emphasis of my work has been to search for molecular evidence to produce a classification that is monophyletic and that reflects the evolutionary relationships among these 27 genera. I will show that molecular analyses have changed our view and allowed us to understand evolutionary relationships among genera more clearly.

CHAPTER 3: MOLECULAR PHYLOGENETIC RESEARCH IN CHRYSOBALANACEAE

3.1. Early research on molecular phylogenetics and family relationships

Molecular phylogenetic advances provided tools to investigate some of the more problematic taxonomic questions, such as the monophyly of genera and their relationships, and have increasingly helped to elucidate the processes contributing to the evolution and biodiversity of plant groups. These discoveries have been significantly revealing in the Chrysobalanaceae and led to changes in our understanding of relationships within the family.

In the groundbreaking molecular phylogenetic paper, Chase et al. (1993) confirmed that the Chrysobalanaceae was not closely related to Rosaceae, despite sharing similar floral features. Other studies placed the family in the order Malpighiales in a highly supported clade composed of the families Euphroniaceae, Dichapetalaceae, Trigoniaceae and Balanopaceae (Dissanayake 1999, Litt & Chase 1998, Wurdack & Davis 2009, Yakandawala et al. 2010).

Dissanayake (1999) was the first to present a molecular phylogeny of the family. Her analyses included only the nuclear ITS and plastid *rbcL* DNA markers, together with morphology. The study confirmed that the family was not closely related to Rosaceae and showed that the relationships among genera were not concordant with the tribal classification proposed by the most current classification of the family at the time (Prance & White 1988). In addition, the non-monophyly of *Licania* was also suggested and its relationship with other genera were not in line with previous ideas. An interesting result was the putative basal genera, which was an unexpected indication that genera such as *Badodeya*, *Kostermanthus* and *Parinari* had diversified earlier than other genera. Also, the study supported the break-up of *Parinari* into smaller segregate genera (Prance 1963, Prance & White 1988). Her study

provided much needed insights into the evolutionary relationships among genera but only had a small number of species and did not include questionable taxa. Clades were poorly resolved, and relationships among genera could not be confidently inferred. The molecular markers used did not provide a clear and robust cladogram and, in many instances, relationships appeared as a polytomy (i.e., unresolved).

Prance & Sothers (2003a) incorporated results from Dissanayake's work (1999) and abandoned the previous classification of four tribes since the relationships among genera were not supported by her molecular study. The early studies with molecular phylogenetics were revealing but showed that more work was needed at the generic and species level especially improved sampling to target multiple accessions of genera and dubious taxa. In addition, analyses needed to be more robust, and as such more individual markers were needed to add to the already sequenced regions. Essentially, these studies brought about more questions than answers to our understanding of the evolution of the family and so I felt challenged to improve on our understanding of the evolutionary relationships of genera.

A key point in the molecular research on the Chrysobalanaceae was the choice of species to be sequenced. A detailed and thorough taxonomic knowledge of the taxa was crucial for selecting odd taxa for sampling and here my previous field experience and taxonomic work proved valuable. I was able to select species and genera that should be further explored regarding their position in the family, as well as genera that had not been adequately investigated in previous studies. Some taxa that were important sampling choices included *Couepia longipendula* Pilg. and *C. dolichopoda* Prance; species of *Couepia* in the parillo superspecies group, such as *C. parillo* A.DC.; the Afromalagasy species of *Hirtella*; *Couepia platycalyx* Prance; *Couepia recurva* Prance; and *Licania parinariopsis* (Huber) Prance. Other species in genera that had not yet been sequenced were also selected, such as *Kostermanthus heteropetalus* Prance, species from the three subgenera of *Magnistipula*, and multiple species

of the larger genera, mainly *Licania*, *Hirtella*, *Parinari* and *Couepia*. These species were selected because they were unique within the genera they were placed in, either because of their morphology or their habitat and geographic distribution. Some of these taxa were not included in the earlier molecular studies (Dissanayake 1999; Yakandawala et al. 2010).

3.2. Molecular research of genera using individual markers (2006–2014)

In my molecular work I experimented with several markers to find those that worked best for a more robust phylogeny of Chrysobalanaceae. Not all markers show good resolution among taxa analysed for all plant groups. I settled for three plastid markers, *rbcL*, *matK* and *ndhF*, and two nuclear, ITS and *Xdh* for my molecular analyses. A biological phenomenon presented by the family that I became aware of during my molecular research was that the Chrysobalanaceae generally was not robustly supported in the molecular phylogenetic analyses as compared to other families, even when several markers were used. This is a biological factor that has also been observed in other plant families and the only solution was to either use many markers, which was time consuming for the level of results obtained, or sequence complete genomes, which was later incorporated into our studies (section 3.3).

My in-depth study of the genus *Couepia* was the first of a single genus in the family to sequence multiple accessions of the same species and genera (Sothers et al. 2014); over 50 % of species were sampled. At the time of the study, *Couepia* was the third largest genus in the family, comprised of 71 species. Its main morphological features are the unilocular ovary located at the mouth of the receptacle. The study found that *Couepia* was polyphyletic as circumscribed at the time; species of *Couepia* were found in four separate lineages and this result spurred drastic taxonomic re-arrangements. A new genus, *Gaulettia* (Sothers et al. 2014), was described from nine species which formed a morphological group, the parillo superspecies

group (Prance 1972). This new genus is distinct from *Couepia* by the deeply reticulate leaf venation which often also display distinct hair-filled stomatal cavities, a character not present in any other species of *Couepia*. Two species which stood out in *Couepia* were included in a clade with *Acioa*, a genus not closely related to *Couepia*. These two species, formerly in *Couepia*, were the only red flowered bat-pollinated species, in contrast to the white moth or bee pollinated flowers of all other species of *Couepia*. Therefore, these two species were transferred to *Acioa*, *Acioa longipendula* (Pilg.) Sothers & Prance and *A. dolichopoda* (Prance) Sothers & Prance. Two other species of *Couepia* required new combinations into other genera; one was an Andean high-altitude species which appeared in the clade of neotropical *Hirtella*, and a new combination, *H. recurva* (Prance) Sothers & Prance, was made. And finally, the only species in *Couepia* that had the ovary on the wall of the receptacle rather than at the mouth was removed from *Couepia* and tentatively included in *Licania* as *L. platycalyx* (Prance) Sothers & Prance (Sothers et al. 2014). This species was later described by me as a new monospecific genus which was named *Cordillera* (Sothers et al. 2016).

The study by Bardon et al. (2013) was the first to propose a historical biogeographic scenario for the family based on a molecular phylogeny. We used six markers, including ITS, *rbcL*, *matK*, *psbA-trnH*, *ndhA*, *atp1-atpH* and *psbD-trnT*. The study confirmed the polyphyly of *Licania* from an independent dataset that corroborated findings by Sothers et al. (2014). The increased number of accessions of *Licania* included in the Bardon et al. (2013) study greatly improved the phylogeny of the family, paving the way for the necessary taxonomic readjustments in *Licania* (Sothers et al. 2016).

The break-up of *Licania*, which was previously circumscribed as pantropical, began with the resurrection of the West African genus *Afrolicania* following preliminary molecular and morphological analyses (Dissanayake 1999, Prance & Sothers 2003a). With increased sampling of genera and species in molecular studies led by me, *Angelesia*, restricted to

Australasia, was also re-instated to generic level (Sothers & Prance 2014); previously it was a subgenus of *Licania*. All molecular studies also pointed to the polyphyly of the neotropical species of *Licania* (Sothers et al. 2014, Bardon et al. 2013) and the re-circumscription of the neotropical *Licania* into eight segregate genera (Sothers et al. 2016) was necessary to maintain its monophyly. The eight genera are *Geobalanus* Small, *Moquilea*, *Licania*, *Leptobalanus*, *Microdesmia*, *Parinariopsis* (Huber) Sothers & Prance, *Cordillera* and *Hymenopus* (Benth.) Sothers & Prance. These genera had very different relationships than expected; *Geobalanus* is sister to the entire Neotropical clade; *Moquilea* is sister to *Couepia*; *Licania* and *Leptobalanus* sister to each other; and *Hymenopus* remains paraphyletic, with one sub-clade sister to *Hirtella* and the other sub-clade of *Hymenopus* sister to *Parinariopsis* and *Afrolicania*; although support for the latter three genera is low (Chave et al. 2020).

These results also provided evidence that *Licania* and *Chrysobalanus* are in separate clades and not closely related, despite being morphologically similar and previously in a tribe together with *Grangeria* and *Parastemon*. The molecular analyses also confirmed that *Licania* was not pantropical but like *Couepia*, endemic to the Neotropics; previous classifications treated *Licania*, *Couepia* and *Hirtella* as not closely related (Prance & Sothers 2003a, b; Prance & White 1988) and placed them in separate tribes. Results also supported the break-up of *Parinari* into eight segregate genera as proposed by Prance (Prance 1963, Prance & White 1988), and confirmed that these new genera were not all closely related to *Parinari*.

Despite the great advances made in the re-assessment of genera and the new taxonomic re-arrangements, it was clear that a phylogenomic framework was needed rather than using individual molecular markers for providing more information on generic relationships within the Chrysobalanaceae and for producing a fully resolved comprehensive phylogeny of the family, which was still lacking. Some of the results only indicated relationships but with low support despite using more markers, and therefore conclusions were based on poor evidence.

3.3. Molecular research of genera using complete genomes (2016–2020)

Next generation sequencing of whole plastid genomes has provided a fully resolved tree for Chrysobalanaceae that includes all genera, in addition to presenting generally highly supported clades (Chave et al. 2020). This set of results represents the best analyses of the evolutionary relationships among genera available to date. This feat was possible with the collaborative effort between a team working on the historic biogeography of the family and the work I had been developing on the revised taxonomy of the family. Independent datasets were important to validate much of both studies and moving forward to fill in gaps in sampling, which hinged on detailed and comprehensive taxonomic knowledge of the family.

Bardon et al. (2016) were the first to publish a complete plastome of Chrysobalanaceae and built on the previous biogeographical study using plastid genomes for every genus in the family. The earlier molecular analyses based on Sanger sequences did not provide robust support for relationships among some of the clades, and support was low particularly among species. Bardon et al.'s (2016) work was based on more sampling than previous studies and included all genera bar one, *Bafodeya*. It was a basis for comparative studies between the previous phylogenies and the current phylogenomic framework (Chave et al. 2020). It also included many more species for many genera, which improved resolution of the phylogeny, allowing for better analyses. Crucially, it included *Bafodeya*, a genus that had not been properly sequenced before. Some of the key outputs included the discovery of the genera *Bafodeya* and *Kostermanthus* forming a clade sister to the rest the family, followed by the *Parinari* clade, which went against previous ideas, such as that of genera with ovaries at the base of the receptacle as being more primitive. This study confirmed the evolutionary position of these genera, which had been postulated before but without support.

My study also confirmed the polyphyly of *Licania*, supporting the recircumscription of the neotropical taxa into eight separate genera (Sothers et al. 2016). This result was interesting in that it clearly highlighted how the previous perception of relationships was very different from an evolutionary perspective (Table 1 in Sothers et al. 2016). The segregate genera of neotropical *Licania* were included in the same clade but with different sister relationships altogether. In many cases these were novel relationships that will allow further studies into why some genera are genetically more closely related to morphologically distinct genera. For example, *Couepia* and *Moquilea* appear as sister genera even though the placement of the ovary on the receptacle differs markedly; on the other hand, both genera share the exerted stamens, a feature that distinguishes *Moquilea* from *Licania*. *Licania* and *Leptobalanus*, the only two Neotropical genera that lack petals are sister, but the apetalous *Afrolicania*, the only non-neotropical genus in the clade, is sister to a clade of *Hymenopus* and not to *Licania* and *Leptobalanus*.

The phylogenomic studies in Chrysobalanaceae have been many-fold. They have increased the support of clades in the analyses, and therefore produced more robust trees that may be used more reliably for inferring hypotheses, such as historical biogeography and to investigate morphological synapomorphies; the results from previous molecular studies have been confirmed with more confidence and provided new insights into relationships among genera and doubtful relationships have been re-examined. Significantly the morphology of genera could be analysed in a different light and an arrangement of genera based on a phylogenomic taxonomy could be proposed for the family.

In Chave et al. (2020) we produced the most up to date phylogeny of the Chrysobalanaceae based on a total of 163 plastomes and 156 species, compared to the previous study which included plastomes from only 51 species (Bardon et al. 2016). Our latest study confirmed the monophyly of more genera and supported the view that much of the neotropical

extant flora has arisen in situ in the last 15 Mya (Chave et al. 2020). Our latest robust molecular phylogenetic framework allows us to investigate more confidently the relationships among genera and to understand evolutionary adaptations and the historic biogeography of the family.

CHAPTER 4: A REVISED TAXONOMY OF CHRYSOBALANACEAE

4.1. A new circumscription of *Licania*

The results obtained from molecular analyses were pivotal for resolving taxonomic issues within the family and to understand relationships among genera from an evolutionary perspective. Combining both molecular and morphological knowledge was the way forward for a classification that worked.

The molecular research I carried out found that the pantropical *Licania* as circumscribed by Prance & White (1988) was highly polyphyletic, despite the central feature shared by all taxa previously included in it, the unilocular ovary at or near the base of the receptacle (Figure 3). For a taxonomy that is more user-friendly we described the features that distinguish the segregate genera of *Licania* as the following: the presence or absence of petals, the number of stamens, the length of stamens and their insertion around the receptacle, among others (Table 2 & Fig. 3 in Sothers et al. 2016).

Two palaeotropical genera were excluded from *Licania* sensu Prance & White (1988). *Afrolicania* was the first to be excluded based on its morphology (Prance & Sothers 2003a) and molecular analyses (Dissanayake 1999). It differs from all other *Licania* by the androdioecious flowers. It is the only African genus included in the large Neotropical clade and is sister to one of the sub-clades of *Hymenopus*, another genus segregated from *Licania* (Sothers et al. 2016). *Angelesia*, an Australasian genus with three species displays few distinct morphological characters from *Licania* but genetically it was found to be closely related to *Acioa*, *Hunga* and *Exellodendron*, and diversified much earlier than *Licania* (Sothers & Prance 2014, Bardon et al. 2016). The main character that separated *Angelesia* from *Licania* and its other segregates was the unequal sepals (Figs. 19 and 20 in Prance & Sothers 2003a).

The neotropical segregate genera of *Licania* all fall within the same large clade, the Neotropical clade (Bardon et al. 2016, Sothers et al. 2016, Chave et al. 2020). The main synapomorphy for all genera in this clade is the unilocular ovary. The first genus to diversify in the clade is *Geobalanus*, sister to all other genera, with the ovary at the base of the receptacle; the genus has the northern-most distribution, occurring from southern USA through to Mexico and Central America. *Moquilea* is sister to *Couepia*; these two genera share the free, exerted stamens with a tendency to be more numerous, the glabrous or hairy leaves lacking stomatal cavities, and the unilocular ovary; but differ by the position of the ovary at the base in *Moquilea* and at the mouth of the receptacle in *Couepia*.

Licania and *Leptobalanus* are sister, and they share the apetalous flowers, the fewer stamens, and the ovary at the base of the receptacle; they differ by the exerted stamens in *Leptobalanus* and included in *Licania*. Apetaly has appeared three times in Chrysobalanaceae, in *Licania*, *Leptobalanus* and in *Afrolicania*; all three genera are in the Neotropical clade; *Afrolicania* is the only African species in this clade.

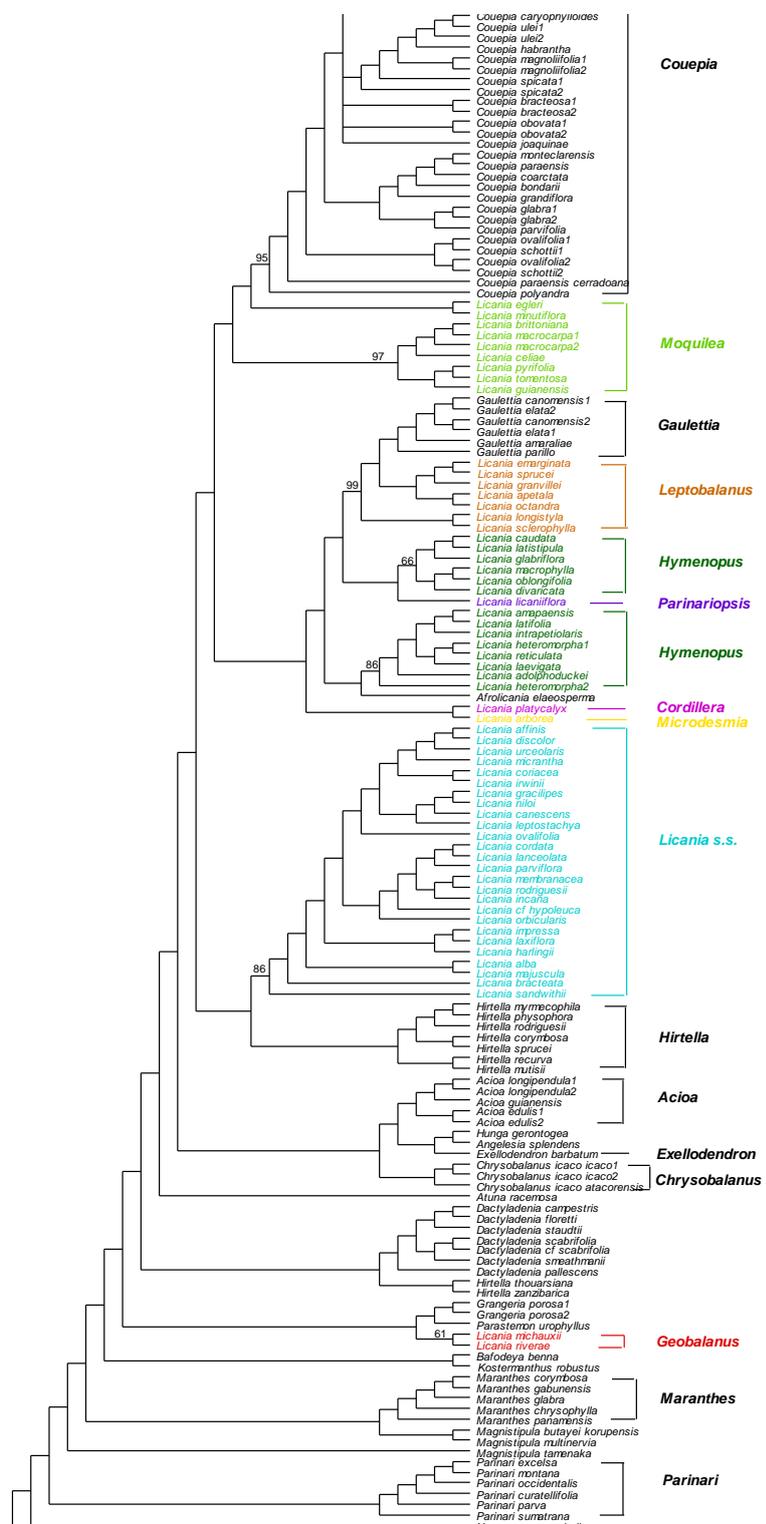
Hymenopus is paraphyletic and appears in two clades, one sister to *Afrolicania* and the other to *Hirtella*. Although *Hymenopus* was described as a segregate genus of *Licania* (Sothers et al. 2016), in molecular analyses the species are divided into two separate clades, but the two clades have low support and there are no apparent features to split the genus into two separate ones. Therefore, we chose to keep them together in one genus until further studies and more sampling of species is available. *Hymenopus* shares with *Hirtella* the presence of petals but differs by having included stamens and the ovary at the base of the receptacle. It shares with *Afrolicania* the ovary at the base of the receptacle and differs markedly by several floral features. The relationships of *Hymenopus* to its sister genera are interesting as they are distinct morphologically; further investigation is needed to clarify these relationships and sort out the taxonomy of *Hymenopus*.

Parinariopsis is distinct from all other segregates of *Licania* in the Neotropics by having the bracteoles enclosing groups of flowers and the ovary inserted on the wall of the receptacle; it shares with some segregate genera of *Licania* the presence of petals, the number of stamens that equal the receptacle in length or are slightly exerted. It should be noted here that the position of the ovary on the wall of the receptacle is also present in only a few species of *Hirtella* and of *Parinari*.

Cordillera and *Microdesmia* are sister, but molecular phylogenetic analyses received poor support (Bardon et al. 2016, Sothers et al. 2016). Later Chave et al. (2020) found *Microdesmia* to be sister to one subclade of *Hymenopus*, and *Cordillera* sister to *Parinariopsis*, both relationships based on phylogenomic analyses, which helped clarify their position. These two genera have distinct distributions and are species-poor genera; *Cordillera* is a monospecific genus widely distributed in the Andean region and *Microdesmia* has two species with almost allopatric distributions, one in Central America and western Amazonia and the other in eastern Brazil. Both have leaves with distinctly deeply reticulate venation and stomatal cavities.

Although the analyses to date have progressed our understanding of the relationships among genera, some genera should be further investigated. Additional sampling will provide further insights into the paraphyly of *Hymenopus*. Because of the difficulties of sequencing taxa and of gaining high support for some clades further studies should be based on phylogenomic analyses. Careful sampling should also continue to target taxa that are morphologically interesting and those that are geographically distinct.

Figure 3. Phylogenetic tree highlighting the segregate genera of neotropical *Licania* (based on Sothers et al. 2016).



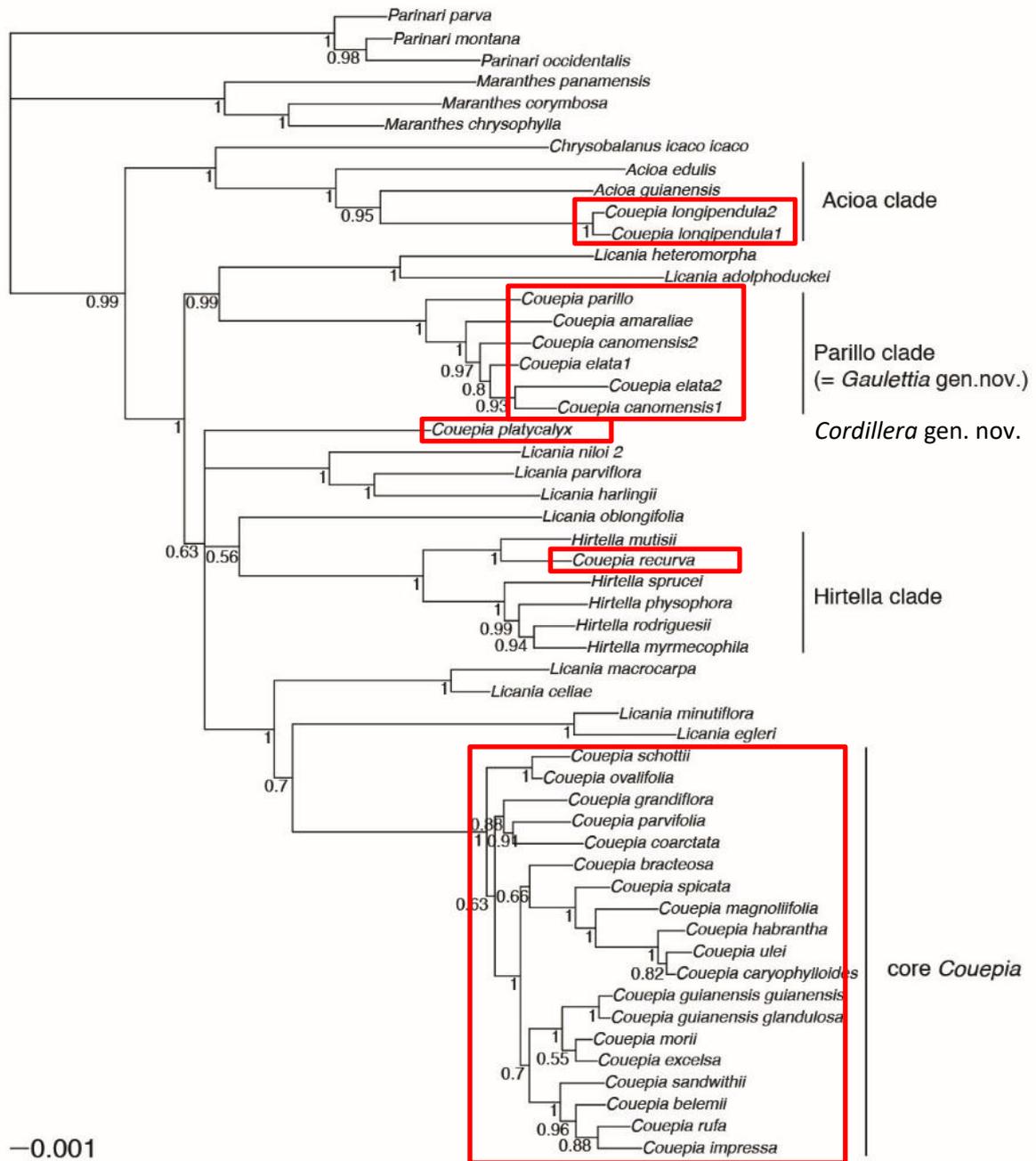
4.2. A new circumscription of *Couepia*

Couepia was one of the seven neotropical genera described by Aublet (1775). The last classification placed this genus in tribe Couepieae (Prance & White 1988), together with *Acioa*, *Maranthes* and *Atuna*, the latter two with a bilocular ovary, unlike *Couepia* that has a unilocular ovary. All four genera share the exserted stamens, and the ovary at the mouth of the receptacle. But Dissanayake (1999) showed that the tribal classification accepted until then was not consistent with the molecular analyses. *Couepia* was distantly related to *Acioa*, *Maranthes* and *Atuna*. More recent studies have found that *Maranthes* and *Atuna* are in the same clade, and *Acioa* is related to other genera altogether.

I carried out a study focusing on revising *Couepia*, sampling more species than previous studies and included a total of 35 species, ca. 50% of the total number of species. Molecular analyses showed that *Couepia* was polyphyletic (Fig. 4), with species occurring in four different lineages (Sothers et al. 2014). This was unexpected as morphologically *Couepia* had always been one of the more stable genera in the classification of the family. Its distinct features are the ovary at the mouth of the receptacle, mostly far-exserted stamens and a tendency for free and more numerous stamens, from 20 to over 300, the unilocular ovary and fruits with smooth or verrucose epicarps. Most species comprised a large clade of core *Couepia*, sister to *Moquilea*.

One clade made up of species of *Couepia* that was not included in the core *Couepia* clade (Fig. 4) was one composed of nine species which all had a unique feature that separated them from core *Couepia*; the species had a deeply reticulate venation on the underside of the leaf, often with hair-filled stomatal cavities. These nine species were transferred by me to a new genus, *Gaulettia*, described to accommodate them (Sothers et al. 2014). This genus is sister to one subclade of *Hymenopus* and *Parinariopsis* (Bardon et al. 2016).

Fig. 4. Phylogenetic tree highlighting the polyphyly of *Couepia* sensu lato (based on Sothers et al. 2014).



My phylogenetic study also tested the position of two species distinct from all others in *Couepia* by the red, bat pollinated flowers, and long pendent flagelliflorous inflorescences. These two species were previously classified as *Couepia*, but molecular analyses confirmed their placement in *Acioa* in a clade not close to *Couepia* (Fig. 4), their distinct morphology contrasting with that of other species of *Couepia* which are white-flowered and mostly moth and bee pollinated. New combinations were made to accommodate them in *Acioa*, as *A. longipendula* and *A. dolichopoda* (Sothers et al. 2014).

One species of *Couepia* sampled in the same study was included in the clade with other species of *Hirtella* (Fig. 4), and therefore this species was transferred to genus *Hirtella* with the new combination, *H. recurva* (Sothers et al. 2014). This species had been dubiously placed in *Couepia* when described by Prance (1972) but was different from most species of core *Couepia* by the elongate and narrow receptacle tube, the reduced number of stamens (17), and the fruit was unknown when described, but has later been found to match the morphology of *Hirtella* fruit. Morphologically it differs somewhat from *Hirtella* but was found to be highly supported in the *Hirtella* clade (Sothers et al. 2014).

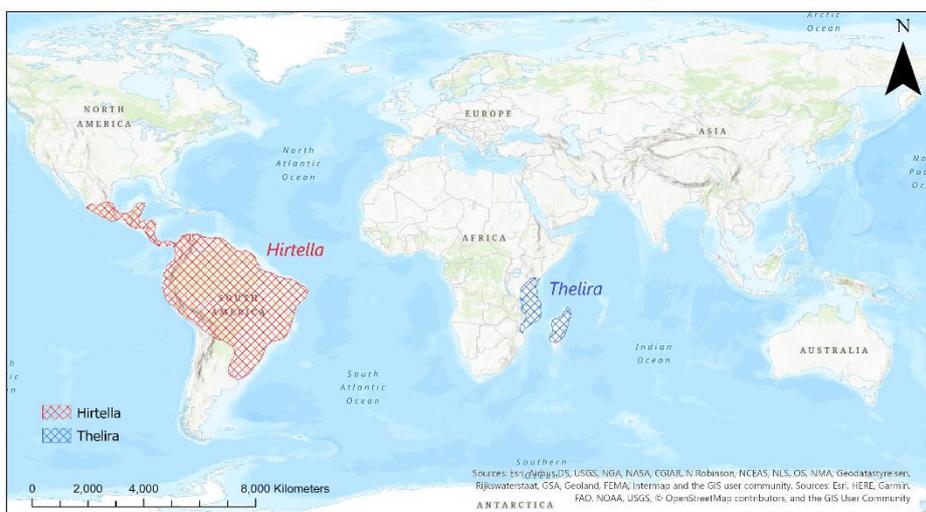
Couepia platycalyx was considered unique in the genus by having the ovary positioned on the wall of the receptacle, unlike all other *Couepia* which had the ovary at the mouth of the receptacle. The molecular analyses found that this species did not form part of the core *Couepia* clade, but its position was not clear (Sothers et al. 2014). Further analyses with more sampling (Bardon et al. 2016, Sothers et al. 2016) found it to be distinct from *Couepia* and *Licania* and a new genus, *Cordillera* Sothers & Prance (Fig. 4), was described to accommodate it (Sothers et al. 2016).

As a result of this work, *Couepia* is a highly supported monophyletic genus sister to *Moquilea*. It has 62 species and is endemic to the Neotropics, with a distribution spanning Mexico through Central America, and throughout South America down to Paraguay.

4.3. A new circumscription of *Hirtella*

Hirtella, the largest genus of Chrysobalanaceae following the break-up of *Licania*, was found to be paraphyletic in molecular phylogenetic analyses (Sothers et al. 2016, Bardon et al. 2016, Chave et al. 2020). The Afromalagasy species of *Hirtella* were found to be in a clade distant from the neotropical species, sister to *Dactyladenia*, an African genus with a very distinct morphology from *Hirtella* but sharing a few characters, such as the unilocular ovary at the mouth of the receptacle and the glands on the bracts and bracteoles. The most striking feature of *Hirtella* is the geographic disjunction represented by the two clades of *Hirtella* and clearly the Afromalagasy taxa represents a separate genus (Fig. 5). The genus *Thelira* (Du Petit-Thouars 1806) was placed in synonymy of *Hirtella* by Baillon (1868) as morphologically both were very similar and difficult to tell apart. This classification was accepted by all later authors, but molecular studies have found the neotropical and Afromalagasy *Hirtella* to be genetically distinct, so *Thelira*, originally described for the Afromalagasy *Hirtella*, should be re-instated.

Figure 5. Distribution of *Hirtella*, highlighting the disjunct distribution of the neotropical and the Afromalagasy taxa.



The 108 neotropical species of *Hirtella* are monophyletic. In the current classification they are subdivided into two sections, sect. *Hirtella* and sect. *Myrmecophila*. Most species are placed in sect. *Hirtella*, but a group of seven species that display leaf bases modified into ant pouches (leaf domatia) make up sect. *Myrmecophila*. In our most current molecular analyses (Chave et al. 2020) these seven species do not form a clade and are all sister to different species of *Hirtella*, a result that should be further investigated, since morphologically all seven species share similar leaf and floral features.

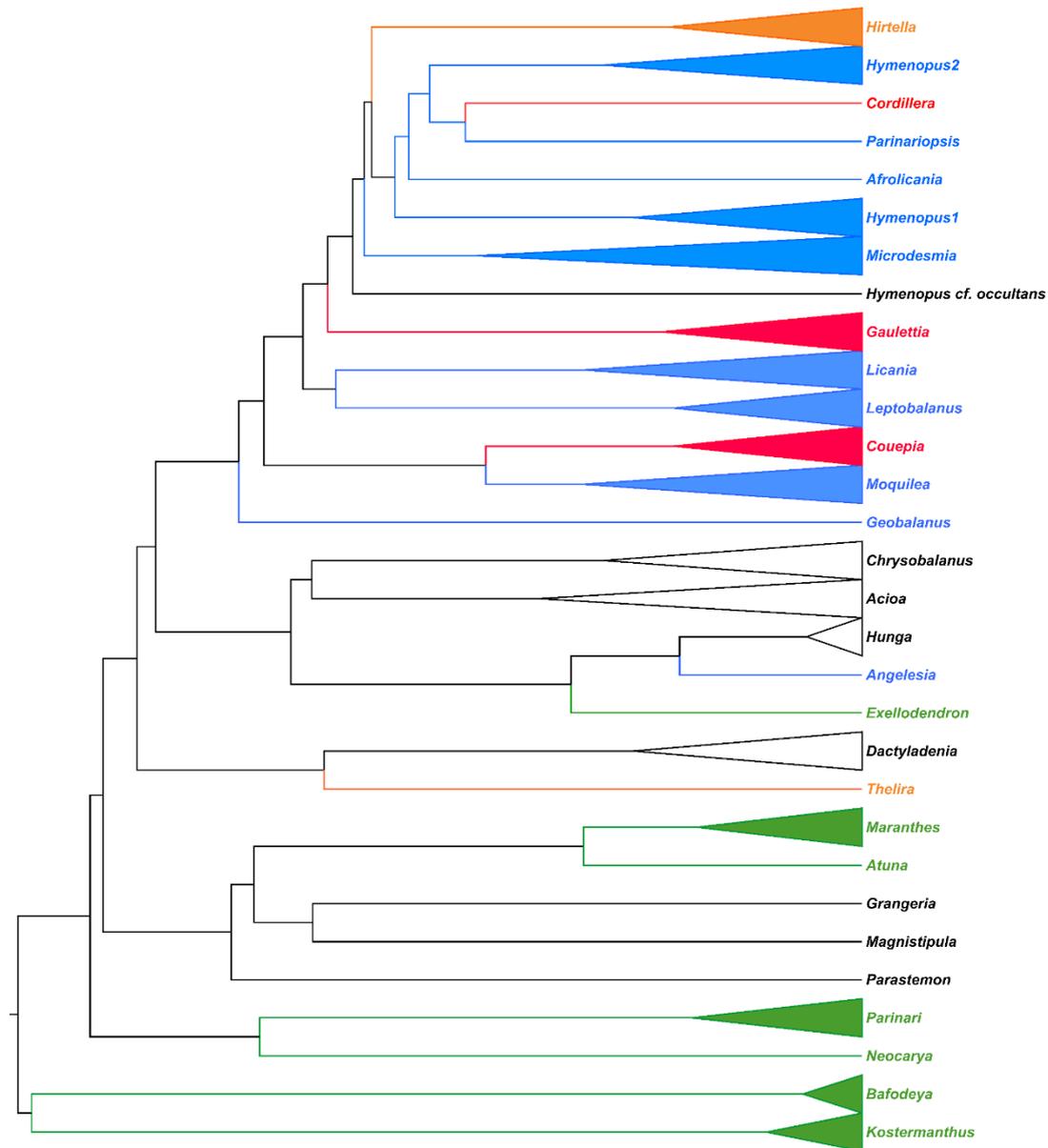
4.4. A new circumscription of *Parinari*

Molecular analyses have corroborated the classification proposed by Prance (1963) that split *Parinari* into eight smaller genera: *Atuna*, *Bafodeya*, *Exellodendron*, *Hunga*, *Maranthes*, *Neocarya*, *Kostermanthus*, and *Parinari*. All the segregate genera are monophyletic and supported as accepted genera, and four, *Hunga*, *Atuna*, *Maranthes* and *Exellodendron*, are not at all closely related to *Parinari* (Fig. 6). Although the bilocular ovary was a character shared by most of the genera previously placed in *Parinari*, these genera were found to be placed in four of the six major clades of Chrysobalanaceae (Bardon et al. 2016).

Bafodeya and *Kostermanthus* are the earliest diverging lineages (Fig. 6) and are quite distinct morphologically, such as by the number of locules in the ovary; the next clade is made up of *Parinari* and *Neocarya* as sister genera, and they share not only the bilocular ovary and other floral features but also the deeply reticulate leaf venation and the same type of seed germination mechanism by basal obturators, a unique feature in the family. *Maranthes* and *Atuna* follow together in a separate clade with *Magnistipula*, *Parastemon* and *Grangeria*; they share a similar receptacle shape and the exserted stamens. *Exellodendron* and *Hunga* are in the same clade, and do not share any morphological features apart from the bilocular ovary; they

are closely related to *Acioa* and *Angelesia*, which differ from the former two genera by the unilocular ovary.

Figure 6. Phylogenetic tree of all genera and clades of Chrysobalanaceae (from Chave et al. 2020). Colours follow the taxonomic historical delimitation of genera in Table 1 (Chapter 2). Legend: *Licania* = blue; *Parinari* = green; *Couepia* = red; *Hirtella* = orange.



For the Palaeotropics, *Magnistipula* is the only other genus, apart from *Hirtella*, that remains paraphyletic. Its taxonomy is yet to be resolved to provide a monophyletic arrangement to the genus. More sampling is needed to fully resolve the classification of the three subgenera of *Magnistipula*.

Phylogenomics has provided an important method to further understand relationships among genera. These insights have helped in making the necessary changes in the taxonomy of the family and have also provided insights into the historic biogeography of the family, which is the subject of the following chapter.

CHAPTER 5: HISTORIC BIOGEOGRAPHY OF CHRYSOBALANACEAE AND CHALLENGES

5.1. Understanding diversification of the family and relationships among genera

Historical biogeography is the study of how species distributions have changed over time. Botanists have long sought to explain patterns of species richness and distributions. The longest standing debate regarding historic biogeography relates to how plants diversified from a ‘centre of origin’. Did speciation take place by dispersal of plants into other regions or did speciation take place by vicariance, i.e., through in situ physical separation; and how have the different genera and species evolved over time and what were the selective pressures, i.e., climate, geomorphology, animal interactions and abiotic factors. These are questions that we seek to answer by studying phylogenetic relationships.

Hypotheses regarding the diversification of plant families have varied widely. Two opposing hypotheses have been proposed for the origins of Chrysobalanaceae. The first postulated a neotropical origin for the family, based on the extraordinary high number of species for that region as opposed to other tropical areas (Raven & Axelrod 1974, Gentry 1982); the three most species-rich genera, *Couepia*, *Hirtella* and *Licania*, are neotropical. The second, by Prance & White (1988), suggested the family originated in Australasia due to the morphological variability of genera found there, particularly that of *Parinari*. This genus is uniform throughout its pantropical range, but in Australasia four species are distinct from the rest, which suggested to those authors that the genus had diversified over a long period of time. Over time the theory that high species numbers equate to species origins was contested and with the advent of molecular phylogenetics a clearer picture across plant groups emerged.

The first study to address the historic biogeography of the Chrysobalanaceae based on a small sampling of 74 species was the work by Bardon et al. (2013). Our study used Sanger sequences (individual loci) from 7 markers for analyses and postulated that the family originated in the Palaeotropics ca. 80 Mya; and that it spread through long distance dispersal into the Neotropics at least four times, with subsequent in situ diversification each time, beginning 40–60 Mya, and with at least one back-dispersal to the Palaeotropics. Our analyses also found that family members had experienced more extinction, speciation, and net diversification in the Neotropics, therefore higher and more rapid speciation rates in the Neotropics were the cause of higher species diversity. This study was the first to show rapid speciation in the lowlands of eastern and central Amazonia, as opposed to other studies which focused on high diversity in the Andean region of South America.

The fast development of new techniques for the use of DNA in plant phylogenetic research led to improved sequencing and sampling by Bardon et al. (2016). In this study we reconstructed the phylogeny of the family based on Sanger sequences from previous studies (Bardon et al. 2013, Sothers et al. 2014) and for the first time complete plastomes of 51 species of Chrysobalanaceae. The study found that the family diversified starting in the late Eocene to early Oligocene transition, 33.4 Mya. Ancestral area reconstruction confirmed a palaeotropic origin for the family with several transoceanic dispersal events. The main Neotropical clade likely resulted from a single migration event from Africa around 28 Mya, with subsequent rapid diversification, triggered by habitat specialisation during the complex geological and palaeoclimatic history of the Neotropics.

The fossil record for Chrysobalanaceae is poor and several of the fossils have been unreliably assigned to the family. All fossils dated before the Oligocene-Miocene (ca. 23 Mya) have been rejected (Bardon et al. 2016), following new findings that came to light (Jud et al. 2016) and that prompted reanalyses. The only definite fossil that can be reliably placed in

Chrysobalanaceae is the fossil flowers of *Licania dominicensis* (Poinar, Chambers & Brown) Chambers & Poinar from amber deposits in the Dominican Republic, dated from the early Miocene, 20–15 Mya (Poinar et al. 2008, Chambers & Poinar 2010).

Chrysobalanaceae diversified from its sister group, Balanopaceae, Dichapetalaceae, Euphroniaceae and Trigoniaceae, long after the breakup of Gondwana, ca. 40 Mya; thus, long distance dispersals must have played an important role in the establishment of the extant diversity of Chrysobalanaceae in tropical rainforests. We now know that different plant families have had a greater capacity for dispersal over long distances than previously thought (Renner 2004). Research has shown that dispersals occurred from Africa to the Neotropics in families such as Lauraceae (Chanderballi et al. 2001), Meliaceae (Muellner et al. 2006), and Zingiberaceae (*Renealmia*; Särkinen et al. 2007); and from South America to Africa, as seen in Rapateaceae and Bromeliaceae (Givnish et al. 2004).

The fruits of Chrysobalanaceae have been associated with a diversity of dispersal modes and several genera have been cited for their ability to disperse through long distances, by water, or animal vectors such as primates, and birds (Prance & Mori 1983, Prance & White 1988, Renner 2004). Chrysobalanaceae are successful at colonising and adapting to different habitats, particularly in lowland tropical forests. The fruits of *Couepia* are primarily dispersed by mammals such as bats, rodents, and primates. The fruit of *Parinari* can be dispersed in several ways, including birds, bats, primates, fish, elephants, squirrels, and by water, fresh and possibly sea water (Prance & White 1988).

In Australasia three species are widely distributed: *Atuna excelsa* (Jack) Kosterm., *Angelesia splendens* Korth. and *Maranthes corymbosa* Blume. *Atuna excelsa* and *Chrysobalanus icaco* are dispersed by both water and animals (Prance & White 1988). The fruit of *Atuna excelsa* withstands salt-water and is also known to be dispersed by ocean currents; although the fruit are large, they have a hard pericarp that aid in flotation (Prance &

Mori 1983). *Angelesia splendens* is dispersed by the fruit pigeon, *Ducula aenea*, and this may explain its widespread distribution in Australasia. Fruits of *Maranthes corymbosa* are dispersed by birds, some of which are strong, long-distance flyers (Prance & White 1988). These documented dispersals are evidence that the Chrysobalanaceae is adapted for long distance dispersals and should be considered when reconstructing the historical biogeography of the family.

5.2. The early diverging lineages and the Palaeotropics

All the molecular phylogenetic analyses to date showed that the earlier diverging lineages and clades comprised mostly palaeotropical genera (Bardon et al. 2013, 2016, Sothers et al. 2014, 2016). In Bardon et al. (2016; Fig. 1) *Kostermanthus* was sister to all other Chrysobalanaceae, and we postulated that *Bafodeya* was also among the early-diverging lineages. Chave et al. (2020) confirmed the position of *Bafodeya* in a clade with *Kostermanthus* (Fig. 7). However, this relationship is difficult to explain because *Bafodeya* and *Kostermanthus* are very different morphologically and geographically. The latter is Australasian with three species and a unilocular ovary, and *Bafodeya* is West African with one species and a bilocular ovary. One suggestion for this may be that taxa that linked these two genera in the past have become extinct. An interesting point to mention is that both have similar habitat preferences that are shared with *Euphronia* in the Euphroniaceae, one of the sister families to Chrysobalanaceae (Chave et al. 2020). *Euphronia* has a restricted distribution in sandy soils within lowland Amazon forests, and *Kostermanthus* also occurs in sandy soils.

Parinari and *Maranthes* are also earlier diverging, both with a pantropical distribution and with distinct morphological features such as seed germination adaptations, and species represented in dry as well as in wet tropical regions. These two pantropical genera appear to

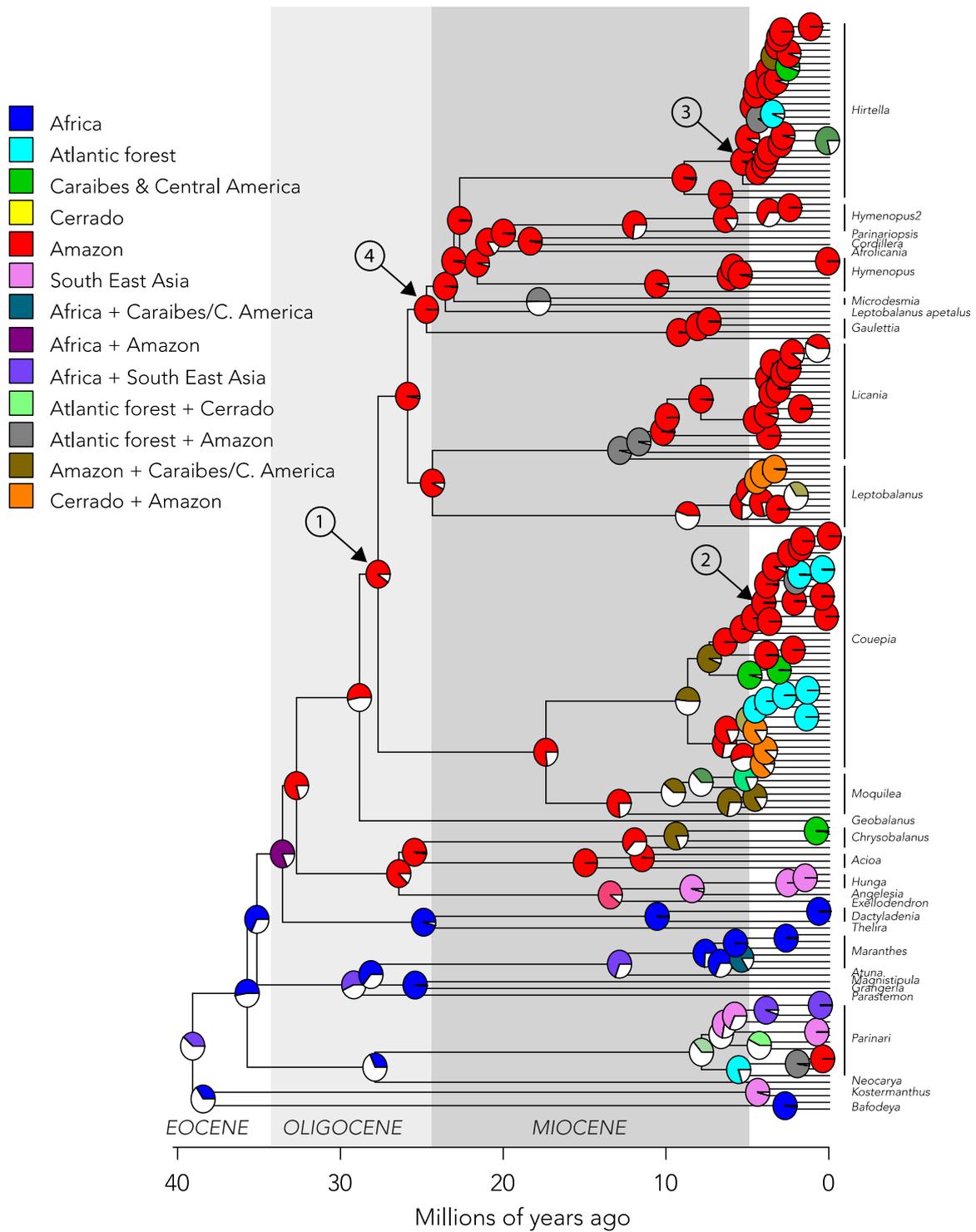
have had separate diversification histories although our analyses suggest an African origin for both genera. *Parinari* first spread into Australasia and later diversified by a secondary dispersal event into the Neotropics from Africa. *Maranthes* is represented in West Africa by 12 species and has a single species in Central America and one widely distributed in Australasia. This genus may have diversified taking a different route, such as via a northern land bridge, or transoceanic long-distance dispersal but the exact pathways remain unclear.

Other putative dispersal routes that could support the diversification of *Maranthes* and other earlier diverging genera include the Boreotropical hypothesis via northern land bridges in the Eocene, 35-54 Mya (Tiffney 1985, Morley 2000). During this period forest covered much of the northern temperate regions and may have provided adequate conditions for the movement of plants. This dispersal route has been postulated for several plant families, such as Burseraceae, Meliaceae, Annonaceae, Lauraceae, Moraceae, Sapotaceae and Melastomataceae, which all arrived in South America via the Boreotropics from Africa (Pennington & Dick 2010). The Beringian route (Wolfe 1972, 1975), via North America and Asia is another route postulated for the arrival of plant groups in the Neotropics via the Palaeotropics, such as Malpighiaceae (Davis et al. 2004). *Maranthes* may have arrived in Central America via this route. But our dating (Bardon et al. 2016, Chave et al. 2020) suggests that the family was only present in the Neotropics after the Eocene megathermal maximum. Long distance dispersal via oceans (island hopping, bird dispersal, ocean currents) should also be considered for genera and clades with disparate or wide distributions within Chrysobalanaceae. *Maranthes* may have spread this way due to the distribution of its extant species. A transpacific long distance dispersal route is a putative scenario for the occurrence of *Maranthes panamensis* (Prance) Prance in Central America; it is very close morphologically to the single Australasian species of *Maranthes*, *Maranthes corymbosa* Blume.

Other earlier diverging genera have distinct diversification histories and although we are slowly dating these and understanding their relationships, their diversification history is still somewhat unclear. *Chrysobalanus* has a transatlantic distribution and has naturalised throughout Australasia. One species occurs on both sides of the Atlantic and our analysis suggests a long-distance dispersal from South America to Africa. The Afromalagasy *Hirtella* ('*Thekira*') are distinct from the neotropical species and although morphologically similar, the Afromalagasy species diverged earlier than the neotropical species. *Dactyladenia*, a West African genus, is sister to the Afromalagasy *Hirtella*. *Magnistipula* is in a clade which includes *Maranthes* and likely had an African origin, but the dispersal scenario is difficult to reconstruct. It remains a genus in which we have not yet established monophyletic relationships and is represented by 12 species in West Africa and one in Madagascar. Further work will be needed to determine the best way to split this genus in two. The genera in this clade have disjunct distributions, such as *Parastemon* and *Grangeria* in Australasia and Madagascar respectively, and *Atuna* in Australasia, and one Pantropical genus (*Maranthes*).

The early diverging genera have morphological features that are specific germination adaptations (*Maranthes*, *Parinari*, *Parastemon*, *Neocarya*), although *Bafodeya* and *Kostermanthus* do not have any specific germination mechanisms. More is known about the neotropical taxa because previously that has been the focus of my studies. With the improved dataset I can continue to piece together the reticulate diversification history of the palaeotropical genera of Chrysobalanaceae.

Figure 7. Ancestral reconstruction of Chrysobalanaceae (reproduced from Chave et al. 2021).



5.3. The break-up of *Licania* and its implications in the Neotropics

Diversification of the Amazon rainforests began in the Tertiary (65 Mya), and the geologic and climatic history of South America has undergone many upheavals over the past 65 million years (Jaramillo et al. 2010). These major geological events have had effects on the movement and distribution of plants throughout Central and South America, including the Chrysobalanaceae. The uplift of the Andes is now considered a major event in shaping present-day landscapes and ecosystems of the Amazon region (Hoorn et al. 2010), and predates the closing of the Isthmus of Panama, that led to the draining of the Lake Pebas system in western Amazonia (in the Neogene) and changed the course of the Amazon River, from flowing west-northwest to its present eastward flow that became fully established by ca. 7 Mya. The closing of the Isthmus of Panama, which connected Central and South America by a land bridge, occurred from ca. 3.5–2.8 Mya (O’Dea et al. 2016, Bacon et al. 2015). It is thought to have triggered the Great American Biotic Interchange (GABI), an event that allowed plants and animals to move more easily between the two land masses previously separated by a large body of water (Stehli & Webb 1985, Cody et al. 2010). This event may have facilitated the movement of certain species less adapted to dispersal over long distances. Some species of *Parinari* have large and heavy fruits with edible mesocarps, so are more adapted for mammal dispersal, unlike some smaller fruited species that are well-adapted to dispersal by water.

Other hypotheses formulated to explain plant species richness in the Amazon region have been proposed in the past. Haffer (1969) proposed his refuge theory during the Pleistocene ice ages (ca. 2.5 Mya) after studying bird distributions in the Amazon basin, setting the stage for identifying areas of plant endemisms within Amazonia. Prance (1973, 1977, 1982a, 1982b) used data on the distribution of Chrysobalanaceae and other families, to propose a similar theory for plants. This correlated closely with Haffer’s (1969) areas and with distribution data

from other biological groups (Brown 1972, Vanzolini 1970) and by palynological evidence (van der Hammen 1974). This theory has been criticised (Colinvaux et al. 2000, 2001; Bush 1994) and, clearly, climatic fluctuations of the Pliocene and Pleistocene may have played a role but have yet to be further investigated based on speciation events (Bardon et al. 2016).

Prance (1972, 1973, 1989) further suggested that disjunct species in genera of Chrysobalanaceae present in the Amazon and Atlantic coastal forests was suggestive of a previously continuous wet forest that extended from the Amazon to the east and south, reaching the coastal Atlantic forests. During warming periods (interglacial periods), forests expanded and may have reached extensive ranges, alternating with the retraction of forests, which were taken over by savannas during glacial periods of climatic cooling in the late Miocene (Jaramillo et al. 2010). This is thought to have given rise to the dry diagonal, an extensive area spanning central Brazil northeastwards towards the coastal regions, dominated by the *cerrado* and *caatinga* biomes. Many *cerrado* lineages are highly adapted to fires; *Parinari obtusifolia*, *Licania dealbata* and *Couepia grandiflora* are examples of dry biome adaptations in the Chrysobalanaceae.

The Neotropical clade, which has over 400 species and nine genera, most likely diversified around 28 Mya, from a single African migration event, followed by rapid diversification (Chave et al. 2020). *Geobalanus*, the earliest diverging genus of the Neotropical clade, comprises three species and has a more northern geographic distribution, from southern USA southwest to Central America. Of the three largest genera in the family, *Licania* (102 species) and *Couepia* (64 species) diversified from ca. 23.6 Mya and 19.5 Mya, respectively, and the neotropical species of *Hirtella* (108 species) diversified from around 17.2 Mya.

The break-up of genera, such as *Parinari* and *Licania*, into smaller genera makes sense from a biogeographical viewpoint. The distribution of the eight segregate genera of *Licania* are broadly allopatric, with some overlap, but each genus displays a general distribution pattern

different from its sister genus. For example, *Moquilea* occurs throughout eastern South America and in Brazil, but it is much more diverse than *Couepia* in western northern South America. Morphologically the segregate genera of *Licania* all share the ovary at the base of the receptacle and although it was considered a feature that indicated a close relationship it has proved not to be a synapomorphy for these genera. The sister relationships of these genera are often with genera that are quite distinct morphologically, such as *Moquilea* and *Couepia*.

Hymenopus still remains paraphyletic in our molecular analyses. One subclade, which contains the type species, is sister to *Afrolicania*, and the other subclade is sister to *Hirtella*. There appears to be no morphological distinction among species of *Hymenopus* in the two subclades, but genetically they are distinct. These relationships need further investigation and as with other genera discussed previously, habitat specialisation may play a role. The three genera, *Hirtella*, *Hymenopus* and *Afrolicania*, are linked to sandy soil habitats, *Hymenopus* within lowland tropical forests, *Afrolicania* in West Africa, and *Hirtella* in a variety of sandy soil habitats throughout Central America and tropical South America. *Hymenopus* is an Amazon-centred genus, but some species occur in eastern Brazil along coastal regions of the southeast. The position of *Afrolicania* in the Neotropical clade suggests a single dispersal event from the Neotropics to Africa around 24 Mya.

Our new and more robust phylogeny has advanced our understanding of the evolution of the family and has contributed to piecing together the historic biogeographical scenario mostly of the Neotropics for now. The break-up of *Licania* and *Parinari* into smaller genera has highlighted morphological and habitat differences that previously were undetected, and that have helped clarify relationships. We can now build an improved scenario of the historic biogeography of the family and focus on gaps in our knowledge and use further novel techniques in molecular phylogenetics. One of these is the Angiosperm353 probe kit, a toolkit for the targeted sequencing of nuclear genes from flowering plants (Baker et al. 2021). A

phylogenetic hypothesis based on nuclear genomes can provide a parallel analysis to compare with our current plastome phylogenetic reconstruction which represents only maternally inherited genes. And finally, more studies need to be carried out on the historic biogeography of the Chrysobalanaceae in the Palaeotropics.

CHAPTER 6: THE ORIGINAL CONTRIBUTION TO KNOWLEDGE REPRESENTED BY THE PUBLISHED WORK

6.1. The papers presented for this thesis

The eleven published works presented here for this thesis constitute a significant body of my own research. Five papers are first-authored and six are co-authored. Five of the papers are taxonomy-centred, with significant contributions to the taxonomy of the family, including the description of new genera to science and new combinations. Three of the papers are based on the historical biogeography of the family with contributions on the diversification of the family in the Neotropics and the first study to use plastomes for phylogenomic analyses in the family.

Prance, G.T. & Sothers, C.A. 2003a. Chrysobalanaceae 1: *Chrysobalanus* to *Parinari*, Species Plantarum: Flora of the World Part 9: 1–319. ISBN 0 642 56832 4.

Prance, G.T. & Sothers, C.A. 2003b. Chrysobalanaceae 2: *Acioa* to *Magnistipula*, Species Plantarum: Flora of the World Part 10: 1–268. ISBN 0 642 56833 2.

Pollard, B.J.; Sothers, C.A. & Prance, G.T. 2004. A new subspecies of *Magnistipula* Engl. (Chrysobalanaceae) from the Bali Ngemba Forest Reserve. In: The Plants of Bali Ngemba Forest Reserve, Cameroon – A Conservation Checklist. Eds: Y. Harvey, B.J. Pollard, I. Darbyshire, J.-M. Onana & M. Cheek. 1–154 pp. RBG, Kew.

Sothers, C. 2010. *Couepia* in Forzza, R.C. et al. (eds.) Catálogo de Plantas e Fungos do Brasil. Instituto de Pesquisas Jardim Botânico do Rio de Janeiro. Vol. 1, 871 pp. ISBN 978-85-8874-242-0. <http://books.scielo.org>

Bardon, L., Chamagne, J., Dexter, K.G., Sothers, C.A., Prance, G.T. & Chave, J. 2013. Origin

- and evolution of Chrysobalanaceae: insights into the evolution of plants in the Neotropics. *Botanical Journal of the Linnean Society* 171: 19–37. <https://doi.org/10.1111/j.1095-8339.2012.01289.x>
- Sothers, C., Prance, G.T., Buerki, S., de Kok, R. & Chase, M.W. 2014. Taxonomic novelties in Chrysobalanaceae: towards a monophyletic *Couepia*. *Phytotaxa*: 172(2): 176–200. <http://dx.doi.org/10.11646/phytotaxa.172.3.2>
- Sothers, C. & Prance, G.T. 2014. Resurrection of *Angelesia*, a southeast Asian genus of Chrysobalanaceae. *Blumea* 59: 103–105. <http://dx.doi.org/10.3767/000651914X684880>
- Bardon, L., Sothers, C., Prance, G.T., Malé, P.-J.G., Xi, Z., Davis, C.C., Muriene, J., Garcia-Villacorta, R., Coissac, E., Lavergne, S. & Chave, J. 2016. Unraveling the biogeographical history of Chrysobalanaceae from plastid genomes. *American Journal of Botany* 103(6): 1–14. <https://doi.org/10.3732/ajb.1500463>
- Sothers, C., Prance, G.T. & Chase, M.W. 2016. Towards a monophyletic *Licania*: a new generic classification of the polyphyletic Neotropical genus *Licania* (Chrysobalanaceae). *Kew Bulletin* 71: 58. <https://www.jstor.org/stable/44991497>.
- Sothers, C.A. & Prance, G.T. 2018. Flora das cangas de Carajás, Pará, Brasil: Chrysobalanaceae. *Rodriguesia* 69(3): 1085–1091. <http://rodriguesia.jbrj.gov.br>.
- Chave, J., Sothers, C., Iribar, A., Suescun, U., Chase, M.W. & Prance, G.T. 2020. Rapid diversification rates in Amazonian Chrysobalanaceae inferred from plastid genome phylogenetics. *Botanical Journal of the Linnean Society* 194: 271–289. <https://doi.org/10.1093/botlinnean/boaa052>

6.2. Novel discoveries: new genera, new sister relationships and a new vision of the family

The research papers presented here have helped establish relationships among genera that had previously been challenged. The molecular phylogenetic studies provided novel insights for the description of new genera and new combinations in several genera of Chrysobalanaceae. My ongoing research has kept up with improved tools in phylogenomics and used them for studies in the family. This has provided a classification that reflects more accurately the evolutionary relationships within the family.

In addition, my studies have made a significant contribution to knowledge of the historical biogeography and diversification of the Chrysobalanaceae. My studies have shown that the family likely had a palaeotropical origin in the Miocene, ca. 38 million years ago, rather than an earlier diversification in the Neotropics as postulated almost 50 years ago. The distribution of species and genera, and the unique morphological features of the Chrysobalanaceae render it as a group that can be used to test hypotheses on character evolution, historic biogeography of tropical South American landscapes, dispersal, and subsequent radiation in the Neotropics and Palaeotropics, and understanding past geomorphological events and historic climate change.

The importance of molecular phylogenetic studies that help clarify the taxonomy and evolutionary relationships of Chrysobalanaceae supports studies in other areas of botanical research. The data are important tools for biodiversity studies and conservation assessments, and as a result several species have been included in IUCN and local Red Data lists (Calderón et al. 2002). Checklists of protected areas and regions, and species conservation assessments are vital tools for protecting the environment (Sothers & Prance 2018). Fieldwork is an important element in studying plants and my research in the field has helped me to have a comprehensive vision of the Chrysobalanaceae.

My research represented by the papers presented for this thesis has provided a much-improved phylogeny of the family that has created monophyletic rather than the previously polyphyletic genera that existed when I began this study. Specifically, my research has resulted in:

1. The description of two genera new to science (*Gaulettia* and *Cordillera*), and eight subgenera and sections of *Licania* that were elevated to generic status.
2. New combinations for over 100 species that had been classified in other genera.
3. The first in depth analyses of genera of Chrysobalanaceae, such as *Couepia*, *Licania* and *Hirtella*, using a molecular approach.
4. The recircumscription of genera in the family, from previously 18 to currently 27 genera.
5. The first study to publish the complete plastomes of all genera of Chrysobalanaceae and the first study using phylogenomics to study the historical biogeography of the family.
6. The discovery of morphological characters that are synapomorphies, characters that are represented by all species in a particular group, for clades and lineages, and those that are homoplasious, characters that are not specific to one clade or lineage.
7. Uncovering evidence of environmental adaptations of species and genera, e.g., leaves with stomatal cavities.
8. The understanding of the historic biogeography and implications for the diversification of the family.

6.3. Future research

The work presented here provides an evolutionary system for the Chrysobalanaceae where almost all genera are monophyletic. Two genera remain to be reclassified to achieve monophyly (*Hirtella* and *Magnistipula*), currently the theme of two papers being written by me as first author. The 27 morphologically well-defined genera fall into six distinct clades that are considerably different from the relationships previously proposed. The splitting of both *Parinari* and *Licania* over time into several smaller genera has enabled a satisfactory evolutionary arrangement of the family. Taxonomically, three genera, *Magnistipula*, *Hymenopus* and the Afromalagasy *Hirtella*, remain to be further investigated to determine their exact position within this new system and make the necessary taxonomic changes and rearrangements that achieves the monophyly of these genera.

In addition to the pending taxonomic issues mentioned above, other questions to be addressed in future studies include: Are there key innovations correlated with diversification in the Neotropics and Palaeotropics? Is there any significance of the reduction in ovary number of locules? What is the functional significance of the position of the ovary on the receptacle? What are the morphological synapomorphies of each clade?

Future studies should focus on floral and fruit morphological features and pollination and dispersal to investigate hypotheses for the ovary positioned at the mouth of the receptacle as an adaptation to allow for more nectariferous tissue and nectar production (e.g., *Maranthes*, *Acioa*), or to create more space for nectar storage (*Couepia*, *Hirtella*, *Gaulettia*), and consequently pollinator specialisation (i.e., butterflies, moths, bats).

Further studies should also explore the diversity of fruit morphology and the four types of germination mechanisms that have been identified. Specific germination mechanisms appear in the following genera: lateral plates in *Maranthes*, *Parastemon* and *Grangeria*, and basal

obturators, or plugs, in *Parinari* and *Neocarya*. These features may be to protect seeds, and the bilocular ovary adapted to adverse climatic conditions such as dry habitats. Features such as lines of weakness, and unilocular ovary, may be better adapted to rainforests, e.g., rapid germination, better adaptation for dispersal. Habitat niche specialisation should also be investigated. Characters that appear to support niche specialisation for dry habitats in the family that have been identified include stomatal cavities, such as *Gaulettia* and *Microdesmia*, and seed germination mechanisms, such as *Parinari*, *Neocarya* and *Maranthes*.

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APPENDIX I. STATEMENTS OF CONTRIBUTION OF THE PAPERS PRESENTED FOR THIS THESIS

Paper 1. Statement of contribution

Paper to be considered as part of the PhD by published work:

Prance, G.T. & Sothers, C.A. 2003a. Chrysobalanaceae 1: *Chrysobalanus* to *Parinari*, Species Plantarum: Flora of the World Part 9: 1–319.

Background: This book, the first of two volumes, is a taxonomic treatment of the 531 species in 18 genera of Chrysobalanaceae worldwide. This volume treats ten genera and provides an introduction, keys to genera, species and subspecies, full taxonomic descriptions for the family, genera and species, citation of specimens, habitat, uses, geographic distribution, and maps for each species. In addition, selected illustrations of species for all genera are included. The publication represents the most comprehensive account of the family to date.

Contribution of the candidate: I, Cynthia Sothers, contributed with the incorporation of data on each species and subspecies and revising all aspects of descriptions, geographic distribution, and incorporation of new collections of species and subspecies for citation. I was responsible for the final revision and editing of the manuscript, in close contact with the series editors. Specifically:

1. I revised each species and subspecies description, updating previous descriptions and information based on new data, such as description of flowers, fruits, habitat, and new occurrences of distribution.
2. I was responsible for the database for the elaboration of the maps for each species and subspecies.
3. I compiled and selected all the illustrations to be published, as well as selecting additional species to be illustrated and providing specimens for use by the botanical artists.
4. I visited the following herbaria to analyse specimens and incorporate any additional information to the publication: BM (London), BR (Brussels), FHO (Oxford), G (Geneva), P (Paris), RB (Rio de Janeiro), WAG (Wageningen).

Statement of contribution: As co-author of the paper cited above, I endorse the statement of contribution made by Cynthia Sothers.

Name, institute, email

Signature

Date

Prof. G.T. Prance

9 October 2020



Paper 2. Statement of contribution

Paper to be considered as part of the PhD by published work:

Prance, G.T. & Sothers, C.A. 2003b. Chrysobalanaceae 2: *Acioa* to *Magnistipula*, Species Plantarum: Flora of the World Part 10: 1–268.

Background: This book, the second of two volumes, treats eight of the 18 genera of Chrysobalanaceae worldwide. It includes an introduction, keys to genera, species and subspecies, full taxonomic descriptions for the family, genera and species, citation of specimens, geographic distribution, notes, and maps for each species. In addition, selected illustrations of species in all genera are included. The publication represents the most comprehensive account of the family to date.

Contribution of the candidate: I, Cynthia Sothers, contributed with the incorporation of data on each species and subspecies and revising all aspects of descriptions, geographic distribution, and incorporation of new specimen collections for citation in the manuscript. I was responsible for the final revision and editing of the manuscript, in close contact with the series editors. Specifically:

1. I revised each species and subspecies description, updating previous descriptions or information based on new data, such as description of flowers, fruits, habitat, and new occurrences of distribution.
2. I was responsible for the database for the elaboration of the maps for each species and subspecies.
3. I compiled and selected all the illustrations to be published, as well as selecting additional species and subspecies to be illustrated and providing specimens for the botanical artists.
4. I visited the following herbaria to analyse specimens and incorporate any additional information to the publication: BM (London), BR (Brussels), FHO (Oxford), G (Geneva), P (Paris), RB (Rio de Janeiro), WAG (Wageningen).

Statement of contribution: As co-author of the book cited above, I endorse the statement of contribution made by Cynthia Sothers.

Name, institute, email

Signature

Date

Prof. G.T. Prance



9 October 2020

Paper 3. Statement of contribution

Paper to be considered as part of the PhD by published work:

Pollard, B.J.; **Sothers, C.A.** & Prance, G.T. 2004. A new subspecies of *Magnistipula* Engl. (Chrysobalanaceae) from the Bali Ngemba Forest Reserve. In: The Plants of Bali Ngemba Forest Reserve, Cameroon – A Conservation Checklist. Eds: Y.Harvey, B.J.Pollard, I.Darbyshire, J.-M.Onana & M.Cheek. 1–154 pp. RBG, Kew.

Background: This paper describes a new subspecies of *Magnistipula*, an African genus of Chrysobalanaceae, in a conservation area in Cameroon.

Contribution of the candidate: I, Cynthia Sothers, was directly involved in the description of this new subspecies. Specifically:

1. I confirmed the taxon was new to science.
2. I checked the description and measurements.
3. I partly wrote and edited the manuscript.

Statement of contribution: As co-author of the paper cited above, I endorse the statement of contribution made by Cynthia Sothers.

Name, institute, email

Signature

Date

Prof. G.T. Prance

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9 October 2020

Paper 5. Statement of contribution

Paper to be considered as part of the PhD by published work:

Bardon, L., Chamagne, J., Dexter, K.G., **Sothers, C.A.**, Prance, G.T. & Chave, J. 2013. Origin and evolution of Chrysobalanaceae: insights into the evolution of plants in the Neotropics. *Botanical Journal of the Linnean Society* 171: 19–37.

Background:

This paper represents one of the first molecular phylogenetic analysis of the family, including the historical biogeography of 17 of the 18 genera of Chrysobalanaceae. This paper led the way for taxonomic re-arrangements towards the monophyly of several genera and helped understand the relationships among genera.

Contribution of the candidate: I, Cynthia Sothers, was responsible for data analyses and revising the manuscript. Specifically:

1. I provided specialist identification for the majority of taxa.
2. I provided half of the silica dried DNA samples for the molecular analyses.
3. I revised and critically reviewed the data analyses and the manuscript as a whole.

Statement of contribution: As co-author of the paper cited above, I endorse the statement of contribution made by Cynthia Sothers.

Name, institute, email

Signature

Date

Prof. G.T. Prance

A handwritten signature in blue ink, appearing to read 'G.T. Prance', with a long horizontal stroke extending to the right.

9 October 2020

Paper 6. Statement of contribution

Paper to be considered as part of the PhD by published work:

Sothers, C., Prance, G.T., Buerki, S., de Kok, R. & Chase, M.W. 2014. Taxonomic novelties in Chrysobalanaceae: towards a monophyletic *Couepia*. *Phytotaxa*: 172(2): 176–200.

Background: This paper concerns the genus *Couepia* which appeared as polyphyletic in molecular phylogenetic analyses, with species of *Couepia* appearing in four separate clades. As such, several taxonomic changes were made, including the description of a new genus to science, and four new combinations, in three separate genera.

Contribution of the candidate: I, Cynthia Sothers, took the leading role in conceiving and writing this paper. Specifically:

1. I performed the majority of the DNA extractions, PCR sequencing, and lab and sequence analyses at the Jodrell Lab, Kew Gardens.
2. I wrote the paper, the generic descriptions, the key to species, prepared the tables, and compiled the data for the new combinations.
3. I selected specimens for the illustration.
4. I prepared the maps for all species.

Statement of contribution: As co-authors of the paper cited above, we endorse the statement of contribution made by Cynthia Sothers.

Name, institute, email

Signature

Date

Prof. G.T. Prance

A handwritten signature in black ink, appearing to read 'G.T. Prance', written over a light green rectangular background.

9 October 2020

Paper 7. Statement of contribution

Paper to be considered as part of the PhD by published work:

Sothers, C. & Prance, G.T. 2014. Resurrection of *Angelesia*, a Southeast Asian genus of Chrysobalanaceae. *Blumea* 59: 103–105.

Background: This paper presents new data based on molecular phylogenetic analyses of Chrysobalanaceae and resulted in genus *Angelesia* being recovered as a distinct segregate genus from *Licania* sensu lato.

Contribution of the candidate: I, Cynthia Sothers, took the leading role in conceiving and writing this paper. Specifically:

1. I checked the nomenclature of the species to make appropriate taxonomic changes.
2. I added citation of additional specimens based on the Kew herbarium collection.
3. I carried out molecular lab work and phylogenetic analyses that resulted in this genus being upgraded from subgenus to generic level.
4. I wrote the manuscript.

Statement of contribution: As co-author of the paper cited above, I endorse the statement of contribution made by Cynthia Sothers.

Name, institute, email

Signature

Date

Prof. G.T. Prance

9 October 2020

A handwritten signature in blue ink, appearing to read 'G.T. Prance', with a long horizontal flourish extending to the right.

Paper 8. Statement of contribution

Paper to be considered as part of the PhD by published work:

Bardon, L., **Sothers, C.**, Prance, G.T., Malé, P.-J.G., Xi, Z., Davis, C.C., Murienne, J., Garcia-Villacorta, R, Coissac, E., Lavergne, S. & Chave, J. 2016. Unraveling the biogeographical history of Chrysobalanaceae from plastid genomes. *American Journal of Botany* 103(6): 1–14.

Background: This paper presents a time-calibrated phylogenomic analysis of the family and includes further sampling from that of previous molecular analyses. Genera and species from across the distribution, focussing on Neotropical taxa, are robustly supported, highlighting the importance of genomes in phylogenetic analyses. Diversification rates are also presented.

Contribution of the candidate: I, Cynthia Sothers, had an active role in the sampling of species and genera, as well as major input into the manuscript itself. Specifically:

1. I contributed with acquiring half of the accessions for the study.
2. I contributed to data analyses.
3. I critically revised the manuscript and made important comments on the taxonomy and hypotheses of the evolutionary history of the family.

Statement of contribution: As co-authors of the paper cited above, we endorse the statement of contribution made by Cynthia Sothers.

Name, institute, email

Signature

Date

Prof. G.T. Prance

A handwritten signature in black ink, appearing to read 'G.T. Prance', written over a light blue horizontal line.

9 October 2020

Paper 9. Statement of contribution

Paper to be considered as part of the PhD by published work:

Sothers, C., Prance, G.T. & Chase, M.W. 2016. Towards a monophyletic *Licania*: a new generic classification of the polyphyletic Neotropical genus *Licania* (Chrysobalanaceae). *Kew Bulletin* 71: 58.

Background: This paper resolves taxonomic issues resulting from molecular analyses that recovered the large genus *Licania* sensu lato as polyphyletic. The genus was split into 8 segregate genera as a result of this publication.

Contribution of the candidate: I, Cynthia Sothers, took the lead role in writing and analyses of this paper. Specifically:

1. I wrote the manuscript, including the keys to genera and species.
2. I selected the taxa for sampling, including fieldwork for missing taxa.
3. I carried out all the lab work and phylogenetic analyses in the Jodrell Lab at Kew Gardens.
4. I prepared all the figures and tables presented in the paper.

Statement of contribution: As co-authors of the paper cited above, we endorse the statement of contribution made by Cynthia Sothers.

Name, institute, email

Signature

Date

Prof. G.T. Prance

A handwritten signature in black ink, appearing to read 'G.T. Prance', written over a light green rectangular background.

9 October 2020

Paper 10. Statement of contribution

Paper to be considered as part of the PhD by published work:

Sothers, C.A. & Prance, G.T. 2018. Flora das cangas de Carajás, Pará, Brasil: Chrysobalanaceae. *Rodriguésia* 69(3): 1085–1091.

Background: This publication forms part of the flora of a critical conservation area with distinct ecological characteristics, in the Amazon region.

Contribution of the candidate: I, Cynthia Sothers, took the lead role in writing and verifying specimen collections. Specifically:

1. I wrote the manuscript, including the species descriptions and keys.
2. I checked determinations, made dissections and took floral and fruit measurements of specimens.
3. I requested a specimen loan from a Brazilian herbarium (BHCB), and also analysed collections from the Kew herbarium (K).
4. I checked the illustration for the paper.

Statement of contribution: As co-author of the paper cited above, I endorse the statement of contribution made by Cynthia Sothers.

Name, institute, email

Signature

Date

Prof. G.T. Prance

9 October 2020

A handwritten signature in blue ink, appearing to read 'G.T. Prance', with a long horizontal flourish extending to the right.

Paper 11. Statement of contribution

Paper to be considered as part of the PhD by published work:

Chave, J., Sothers, C., Iribar, M., Suescun, U., Chase, M.W. & Prance, G.T. 2020. Rapid diversification rates in Amazonian Chrysobalanaceae inferred from plastid genome phylogenetics. *Botanical Journal of the Linnean Society* 194: 271–289.

Background: This paper adds important phylogenomic data following on from the publication Bardon et al. 2016. Here we present complete genomes for all taxa in the phylogenetic analyses which make important contributions for knowledge of the Amazonian clades of Chrysobalanaceae. This study is the first biogeographical analysis incorporating complete genome from all genera of Chrysobalanaceae and represents the most up to date molecular phylogeny for the family.

Contribution of the candidate: I, Cynthia Sothers, had an active role in the sampling of species and genera, as well as major input into the manuscript itself. Specifically:

1. I worked closely on writing the manuscript and discussions on the ideas presented in the paper.
2. I contributed with acquiring half of the accessions for the study.
3. I was responsible for checking the taxonomy of all the species and genera cited in the paper and on the discussion of the problematic taxa.
4. I critically revised the manuscript and made important comments on the taxonomy and hypotheses of the evolutionary history of the family.

Statement of contribution: As co-authors of the paper cited above, we endorse the statement of contribution made by Cynthia Sothers.

Name, institute, email

Signature

Date

Prof. G.T. Prance



13 October 2021