Cremastosperma (and other evolutionary digressions)

Molecular phylogenetic, biogeographic, and taxonomic studies in Neotropical Annonaceae

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Moleculair fylogenetische, biogeografische, en taxonomische studies in Neotropische Annonaceae

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Contents

Chapter 1

Introduction

This thesis contains the results of molecular systematic studies of groups in the flowering plant family Annonaceae and a taxonomic revision of the genus *Cremastosperma*, which occurs in the Neotropics. Each Chapter includes its own introduction, but it is my intention to introduce here some of the subjects, approaches, and more important concepts employed. An overview of the contents of the rest of the thesis is also presented.

Work in this thesis falls under what I believe to be two interrelated but very different branches of biological research. These can be summarised as 1) the description of biological diversity (α-taxonomy) and 2) the increase of our understanding of patterns and processes underlying that diversity. The biological diversity under study all belongs to a family of flowering plants known as the Annonaceae. Annonaceae, first described by Jussieu in 1789, grow in the forms of trees, shrubs, and woody vines, and are almost entirely limited to tropical climes, particularly tropical Asia and Africa (the old world tropics), and Latin America (the new world, or Neotropics). Those more familiar with temperate plants might still have encountered Annonaceae in the form of the juice of the cherimoya fruit (*Annona cherimola* Mill.) or in perfumes made with ylang-ylang (*Cananga odorata* (Lam.) Hook. f. & Thoms.).

Annonaceae, taxonomy and systematics

There are around 2,500 species of Annonaceae, 900 of which are found in the Neotropics (Chatrou *et al.*, 2004). These numbers are an estimation on at least two counts: firstly, the biological diversity of much of the world's tropics is very poorly known. New species of plants are described every day (13 new species of *Cremastosperma* have been described in the preparation of this thesis alone). Secondly, the species as a concept is difficult, if not impossible, to define. In this thesis, species are distinguished following the taxonomic species concept of Davis & Heywood (1963), in which they are described as "assemblages of individuals with morphological features in common and separable from other such assemblages by correlated morphological discontinuities in a number of features." This approach represents not so much a concept of what a species actually is, but rather a practical solution for living in a world where species very clearly exist, but where it is not always easy, or even possible, to identify them. Exactly how different two such assemblages have to be in order to constitute separate species remains a subjective decision. This subjectivity cannot, in my opinion, be avoided. This is due, firstly, to the variety of unique circumstances in which speciation events occur. Secondly, should you find grounds on which to accept one particular species concept from the large available selection (the existence of which probably owes a great deal to those various unique circumstances), the demands for data necessary to apply it are often unrealistic. Increases in collections will continue to unearth new variation, which in some cases may challenge views of species delimitations. Species defined following the taxonomic species concept can only be regarded as hypotheses of the underlying biological reality (however that is to be defined). These hypotheses are, however, a vitally important tool for those studying organisms in any branch of biology.

Biological diversity is immense. There are estimated to be more than 78,800 flowering plant species in the Neotropics alone (Smith *et al.*, 2004). Not only do these species need to be described, but this information needs to be organised in such a way that it can be retrieved quickly and easily. This end is served through classifying species into groups, and those groups into larger groups. For example, the species *Cremastosperma cauliflorum*, as described by Robert E. Fries (1931) belongs to the genus *Cremastosperma*. *Cremastosperma*, along with genera such as *Annona* and *Cananga*, is classified within the family Annonaceae, which in turn is grouped with families including Magnoliaceae and Myristicaceae to comprise the order Magnoliales.

A number of studies have presented groupings of genera within Annonaceae following different principles and using different sources of data. *Cremastosperma* has been associated with a number of other mostly Neotropical genera, under the names '*Malmea*-tribe/group' (on the basis of pollen morphology; Walker, 1971) and '*Cremastosperma*-group' (flower morphology; Van Heusden, 1992). These groupings were contradicted to a greater or lesser extent by works based on gross morphology (Fries, 1959) and fruit and seed morphology (Van Setten & Koek-Noorman, 1992), and by phenetic analyses of floral and fruit morphological data (Koek-Noorman *et al.*, 1997). Any assumption that one subset of characters (in itself an effectively arbitrary subdivision of the total variation) is in some way a superior foundation for classification than others requires explicit justification. This is particularly the case where different partitions of characters appear to support different conclusions. However, the same is true for the assumptions made in the interpretation of those characters.

Conflicting results can also be derived from exactly the same data, if the groupings are made following different guiding principles.

Monophyly and phylogeny reconstruction

Whilst the only effective way of delimiting species may rest on the opinion of an expert in a particular group, cladistic methodology (Hennig, 1966) has effectively excluded such subjectivity from higher level classification. The structure provided by evolutionary history can be used to make a system of classification which not only best reflects the information we have for particular species, but also allows us to predict characteristics that have yet to be observed. Groups are only recognised where they include all of the descendents of a common ancestor, and no others. These are identified by the possession of shared derived character states. Such a group is described as monophyletic and otherwise known as a clade. Characteristics can be lost by individual lineages within a clade, and new characteristics can also be gained. Groups defined by the absence of characters which are present in other groups, rather than on the presence of characters inferred to have been acquired in a common ancestor, can have more other characteristics in common with other groups than with each other. The predictive value of such groups is correspondingly lower.

In Annonaceae, the principle of monophyly has been applied on a limited scale and led to some re-classification at the generic level (Chatrou *et al.*, 2000; Mols, 2004). The assumption behind all phylogenetic inference methods is that the input data consist of a set of homologous characters (Sanderson & Shaffer, 2002). However, difficulties in homology assessment and high levels of homoplasy in cladistic analyses using morphological data at higher taxonomic levels in Annonaceae has yielded equivocal results (Doyle & Le Thomas, 1996). This has led to recent studies relying on DNA sequence data alone (Mols *et al.*, 2004; Richardson *et al.*, 2004) or in combination with morphology (Doyle *et al.*, 2000; Mols, 2004). A number of chloroplast DNA (cpDNA) regions have been exploited as sources of phylogenetically informative characters: in particular the widely used *rbcL* gene and *trnL-F* region. Uniparental inheritance and effective lack of recombination means that although phylogenies inferred using cpDNA sequence data may or may not reflect species phylogenies (Nichols, 2001), they can in any case be regarded as gene trees for which the congruence with organismal history becomes greater with increasing time scale (Clegg & Zurawski, 1992) (*i.e.* assuming that reticulation is less frequent between more distantly related plant species). Given the levels of uncertainty and disagreement in the interpretation of morphological variation in Annonaceae, it is not to be expected that the reconstruction of phylogeny with DNA sequences will necessarily identify clades that are supported by all or large parts of the previous evidence. They may not even be straightforward to diagnose. Without knowledge of the organisms themselves, a phylogenetic tree based on DNA sequence data serves little purpose. However, in circumstances such as these it is a particularly powerful tool, providing a greater weight of evidence which can be used to assess the homology and phylogenetic signal of morphological characters with which no single supported result could otherwise be obtained.

Phylogeny reconstruction in this thesis is performed using two different inference methods: maximum parsimony (MP), and Bayesian inference (see Chapter 3). Support for clades is represented mainly by bootstrap percentages (Felsenstein, 1985) under MP, and posterior probabilities (Huelsenbeck *et al.*, 2001) under Bayesian analysis. Bootstrap percentages are regarded as conservative estimations of support (Hillis & Bull, 1993). Relatively higher posterior probabilities have been reported for clades recovered using Bayesian inference (*e.g.* compared with maximum likelihood analysis; Suzuki *et al.*, 2002; and with parsimony analyses; Simmons & Miya, 2004). Although in some cases this has been shown to represent an efficient means of correctly resolving relationships (Simmons & Miya, 2004), it has led some workers to regard these results with scepticism (Suzuki *et al.*, 2002). Support values derived under different methods are impossible to compare directly. In the rare cases where application of different methods appears to give strongly conflicting results (*e.g.* Huelsenbeck, 1997) this may or may not reflect a shortcoming in ones of the techniques (see Siddall & Whiting, 1999). Use of only one such method would of course fail to reveal such a problem. However, in general I consider time to be better invested in improving the data than spent interpreting phylogenies that are either unsupported or not robust to the method applied.

Biogeography

The overall objective of this thesis could be summarised as to describe the diversity of species found in the genus *Cremastosperma*, and investigate how that diversity might have originated. The distribution of the species of *Cremastosperma* is of particular interest: the highest diversity of species of *Cremastosperma* is found in areas surrounding the Andean mountain chain, and they are all but absent from central Amazonia. This 'Andean-centred' distribution is also found in a small number of other genera of Annonaceae. That the constituent species of multiple disparate taxa have been collected in particular areas has often invited the explanation of a common biogeographic history. In the case of Andean-centred taxa the orogeny of the Andean mountain chain has been suggested to be a factor driving their diversification (Gentry, 1982). Most genera of Annonaceae are relatively more diverse in Amazonia. Confirming monophyly in *Cremastosperma* is of primary importance in testing this hypothesis. Amazonian-centred, rather than Andean-centred, distribution patterns could be the result of some possible poly- or paraphyletic groupings of species of *Cremastosperma* and related genera.

A robust phylogeny makes a number of approaches to reconstructing the biogeographic history of organisms possible. Ancestral distribution areas can be inferred (*e.g.* Doyle & Le Thomas, 1997; Doyle *et al.*, 2004) and a cladistic biogeographic approach used to identify congruent area cladograms (*e.g.* Pennington *et al.*, 2004). Recent developments in molecular dating have begun to offer the possibility of being able to demonstrate when diversifications in such groups actually occurred within the time slices of such biogeographic hypotheses. For example, hypotheses corresponding to refuge theory (Haffer, 1969), taking place within the time frame of Pleistocene climatic changes, can be compared to those suggesting speciation to be concentrated earlier in the Tertiary as suggested by other authors (*e.g.* Hooghiemstra & van der Hammen, 1998).

Molecular dating

Molecular dating is subject to a number of sources of error which must be taken into account when assessing the significance of the result (Sanderson & Doyle, 2001). Chloroplast sequences do not adhere to a time calibrated molecular clock (Gaut, 1998). Rate heterogeneity can be addressed using techniques such as nonparametric rate smoothing (NPRS: Sanderson, 1997), penalized likelihood (PL: Sanderson, 2002), or a Bayesian approach (*e.g.* Thorne *et al.*, 1998). These methods assume autocorrelation of rates across a phylogeny (Sanderson *et al.*, 2004). Uncertainty can be associated with the topology of the phylogeny in question (phylogenetic uncertainty), and in the estimation of branch lengths optimised onto it given the character sampling used ('substitutional noise') (Sanderson & Doyle, 2001). Assessing the effects of the latter source of error when using NPRS and PL is possible through bootstrap resampling techniques, and the effects of phylogenetic uncertainty can be avoided by limiting dating to significantly supported nodes.

Absolute, as opposed to relative, ages can only be estimated by calibration using fossil or geological data to set or limit the age of one or more nodes. This process is controversial (see Shields, 2004), often afflicted by considerable uncertainty (Bremer, 2000; Smith & Peterson, 2002; Hedges & Kumar, 2004). Due to the incompleteness of the fossil record, age estimates produced using fossil calibrations will arguably always be underestimations of true ages (Reisz

& Muller, 2004). More accurate results might be achieved if fossils could be used to constrain multiple nodes within the phylogeny (Soltis *et al.*, 2002; Sanderson *et al.*, 2004), the placement of which can be facilitated by assessments of homology made possible by robust phylogenies. The oldest unambiguously identifiable fossil Annonaceae remains have been found in the Maastrichtian of Nigeria (seeds with perichalazal ring and ruminate endosperm; Chesters, 1955) and Colombia (reticulate monosulcate pollen; Sole de Porta, 1971). The ages of these fossils are not suitable to address the question of the age of the family itself, but a number of recent studies have used molecular dating techniques, deriving consistent age estimations for nodes within the Magnoliales (Doyle *et al.*, 2004; Richardson *et al.*, 2004). Richardson *et al.* (2004), largely in agreement with Doyle & Le Thomas (1997), suggested pantropical distributions of clades within Annonaceae to be due to interchange between Africa and South America across the opening Atlantic ocean followed by dispersal across Bering and/or North Atlantic land bridges, rather than the older explanation of vicariance of Gondwana.

Overview

The aims of Chapter 2 are to test monophyly of the genera *Cremastosperma*, Mosannona, Duguetia, and Guatteria by phylogenetic analysis of *rbcL*, *trnL-F* and *matK* cpDNA data. Comparisons of relative timing and preliminary estimates for the absolute ages of their most recent common ancestors (MRCAs) are made, facilitated by the inclusion of all four groups in a single analysis using NPRS (Sanderson, 1997). These estimations are used to investigate whether diversification within these contrasting genera can be shown to have occurred within the same or significantly different time slices. An important consideration in working with *Cremastosperma*, *Mosannona*, *Duguetia*, and *Guatteria* is the possible source of error represented by the large disparity in numbers of taxa available for analysis. In order to assess whether and to what extent the outcome of age estimations might be influenced not only by character (as above) but also by taxon sampling, re-sampling approaches are used and the results compared.

In Chapter 3 Gentry's (1982) explanation for the distribution of Andean-centred taxa is tested. That was that they 1) originated in South America (*i.e.* by vicariance of Gondwana) and 2) were subject to a recent burst of speciation as a result of the orogeny of the Andean mountain chain. Phylogeny reconstruction using eight cpDNA markers (*psbA-trnH*, *ndhF*, *trnT-L*, *trnS-G* and *atpb-rbcL*, as well as *trnL-F*, *rbcL* and *matK*) and different molecular dating techniques (NPRS, PL, Bayesian) are applied in order to:

1) Determine the geographic origin of the Andean-centred genera

Cremastosperma, *Klarobelia*, *Malmea*, and *Mosannona*: identify the sister groups of the four genera (whether these are other Neotropical, or Asian clades), and the age of these sister groups (whether their distribution could have originated by vicariance of Gondwana, dispersal from Africa or dispersal across the Boreotropics).

2) Determine whether the four genera may have congruent evolutionary histories that may all have been affected by the Andean orogeny: testing the monophyly of each of these genera and determining the age of the crown group of each genus.

In Chapter 4 conflicting signal is investigated and the hypothesis of a paralogy in the *trnL-F* regions in Annonaceae is tested, using both PCR-based and phylogenetic analysis techniques. Further conclusions are drawn with respect to the timing of duplication and to the phylogenetic signal contained in both copies. To address the question of functional homology in the two copies, comparisons are drawn between sequences obtained in this study and proposed secondary structures and corresponding functional constraints in the *trnL* gene and Group I intron in land plants (Borsch *et al.*, 2003; Quandt *et al.*, 2004). Positional homology (*i.e.* within or possibly without the chloroplast genome) is less straightforward to determine from sequences alone. A number of potential directions for future research are therefore also discussed.

In Chapter 5 a taxonomic revision of the genus *Cremastosperma* is presented. All 29 recognised species are treated. An identification key, descriptions, illustrations, and a list of exsicatae is provided. Results of phylogenetic analysis using multiple chloroplast markers plus the paralogue of the *trnL-F* region discovered in Chapter 4 are briefly discussed.

Chapter 2

Phylogeny reconstruction and molecular dating in four Neotropical genera of Annonaceae: the effect of taxon sampling in age estimations

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Abstract

Preliminary results are presented comparing the timing of diversification in four predominantly Neotropical genera of Annonaceae; *Cremastosperma*, *Duguetia*, *Guatteria,* and *Mosannona*. With the exception of a few basal lineages, the majority of the ca. 2500 species of the Annonaceae diverge into two major sister clades. One comprises relatively few, larger, genera (including *Duguetia* and *Guatteria*) representing roughly twice as many species in total and with an apparent rate of molecular divergence (revealed by branch lengths in most parsimonious trees) around three times as high as the other (including *Cremastosperma* and *Mosannona*), in which more genera each comprise fewer species. Explanations for the disparity in numbers of species in these four genera are sought by use of phylogeny reconstruction and molecular dating techniques (using nonparametric rate smoothing) aiming to assess monophyly and arrive at preliminary estimates of the relative ages of their most recent common ancestors (MRCAs). The effects, in particular, of taxon and character sampling on date estimates in these genera is assessed and compared. Results in the species rich genus *Guatteria* show higher sampling of crown group taxa resulting in significantly older age estimation for the MRCA.

Keywords: Annonaceae, *Cremastosperma*, *Mosannona*, *Duguetia*, *Guatteria*, phylogeny reconstruction, molecular dating, taxon sampling.

Abbreviations: BS (bootstrap support); LBC (long branch clade); LTT (lineages through time); ML (maximum likelihood); MP (maximum parsimony); MRCA (most recent common ancestor); NPRS (nonparametric rate smoothing); SBC (short branch clade).

Introduction

Annonaceae in the Neotropics

Annonaceae comprises around 2500 species of trees and lianas in ca.130 genera distributed pan-tropically, predominantly in tropical rain forests. Over 900 species are found in the Neotropics (Chatrou *et al.*, 2004), where they represent a significant part of plant diversity, in terms both of numbers of species and of individuals. Studies on α-diversity of Amazonian forests rank Annonaceae among the most abundant families (*e.g.* Valencia *et al.*, 1994; Ter Steege *et al.*, 2000). Relative abundance of Annonaceae is high in areas that have been designated biodiversity hotspots by Myers *et al.* (2000) such as the Chocó/Darién/Western Ecuador region, the tropical Andes, and Brazil's Atlantic forest (Davis *et al.*, 1997).

Neotropical representatives of Annonaceae have been a major focus of systematic research at the Utrecht branch of the National Herbarium of the

Netherlands since the founding of the international Annonaceae project in 1983. This has resulted in taxonomic revisions of a large proportion of the Neotropical genera (*e.g.* Maas & Westra, 1984; 1985; 1992; Murray, 1993; Johnson & Murray, 1995; Chatrou, 1998; Maas & Westra, 2003; Maas *et al.*, 2003), with the remaining unrevised Neotropical genera almost all the subjects of current work. In parallel to alpha taxonomic treatment of Annonaceae, efforts to reconstruct the phylogeny of the family based on morphology (Doyle & Le Thomas, 1996) and DNA sequence data (Bygrave, 2000; Doyle *et al.*, 2000; Mols *et al.*, 2004; Richardson *et al.*, 2004) are providing an increasingly robust framework for the interpretation of the diversity being described. The most recent data gathered to reconstruct the family phylogeny (Chatrou *et al.*, unpublished data) include *rbcL* and *trnL-F* sequences for 220 species, representing over 80 genera, sampled from all continents.

Phylogeny reconstruction in Annonaceae

Annonaceae is classified within the order Magnoliales (APG II, 2003), wherein it is highly supported as sister group to Eupomatiaceae, based on cladistic analysis of morphological and molecular data (Sauquet *et al.*, 2003), with *Anaxagorea* resolved as sister to the rest of the family (Doyle *et al.*, 2000; Sauquet *et al.*, 2003). The further use of morphology for phylogeny reconstruction in Annonaceae has been problematic due to difficulties in homology assessment and high levels of homoplasy (Doyle & Le Thomas, 1996). Results of phylogenetic research based on *rbcL* and *trnL-F* sequence data (Mols *et al.*, 2004; Richardson *et al.*, 2004) support the position of *Anaxagorea* and further divide the rest of Annonaceae between a small clade including *Cananga* and *Cleistopholis*, sister group to a large clade including the majority of species of the family (see Fig. 1).

This large clade is further divided into two sub-clades. Clade A includes roughly 1,500 species, all characterised by an inaperturate pollen condition, almost a thousand of which are divided between 7 species-rich genera: *Annona*, *Artabotrys*, *Duguetia*, *Goniothalamus*, *Guatteria*, *Uvaria*, and *Xylopia*. The remaining 500 species are currently assigned to around 40 smaller genera. Two specialised morphological syndromes within the family are confined exclusively to this clade: fused fruiting carpels, and climbing habit. The second sub-clade (B) is predominantly Asian, but also including all the Neotropical genera with imbricate sepals, one basal ovule, and apocarpous fruits (such as *Malmea*, *Oxandra*, *Ephedranthus*, *Cremastosperma,* and *Mosannona*). It comprises around 700 species (less than half the total number estimated for clade A), divided between around 50 genera, few of which represent more than 40 species. Pollen types in this clade have been characterised as monosulcate or disulculate, however, recent results suggest this pattern to be less straightforward, *i.e.* with cryptic apertures and inaperturate compound pollen forms observed

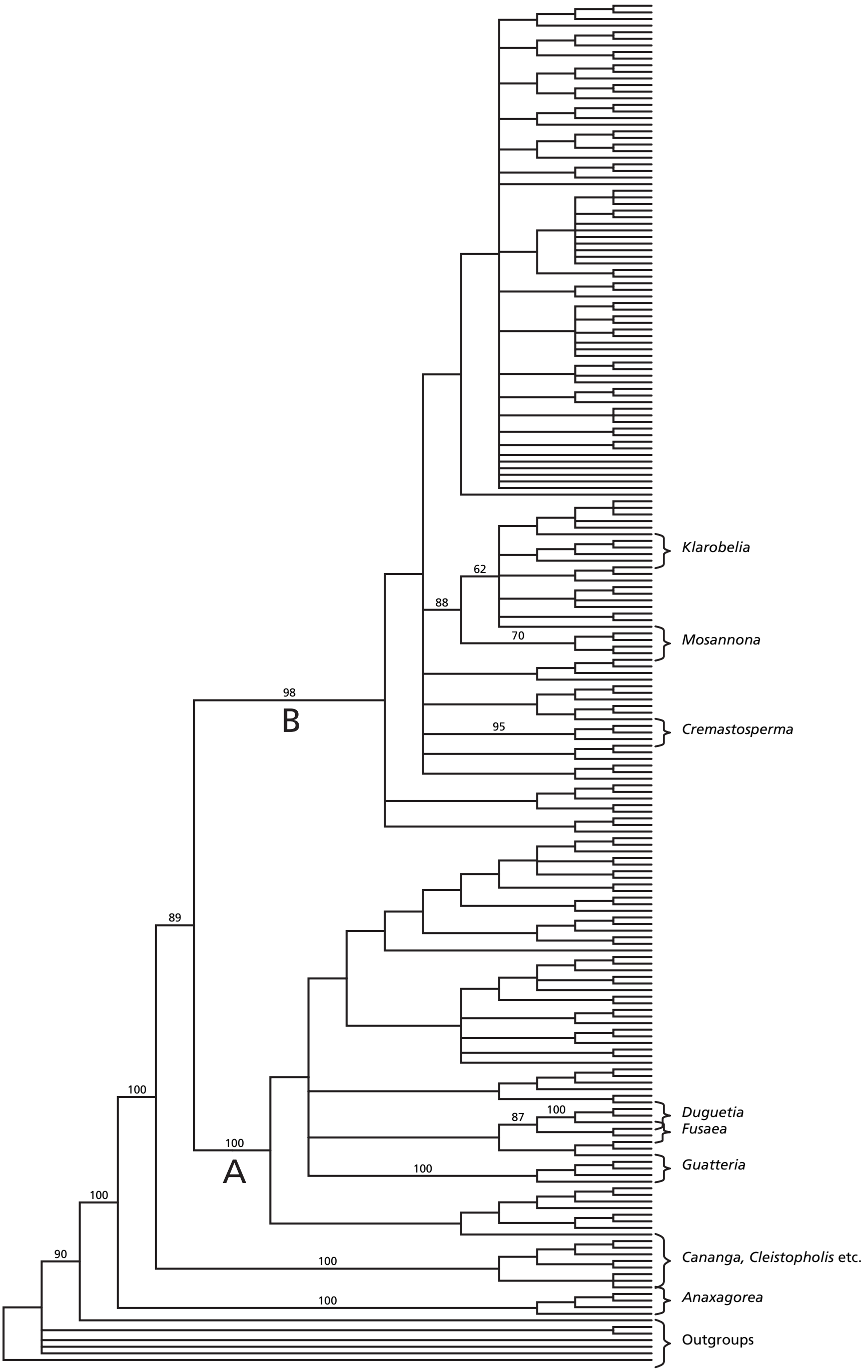

Fig. 1. 50% majority rule bootstrap consensus of Annonaceae (with selected bootstrap frequencies indicated) using Magnoliales and Lauraceae representatives as outgroups, based on phylogenetic analysis of *rbcL* and *trnL-F* DNA sequences (adapted from Richardson *et al.*, 2004). Genera emphasised in this study are indicated, A and B denote long and short branch clades, respectively.

in some groups (Mols *et al.*, submitted). Phylogenetic analysis of both *rbcL* and *trnL-F* plastid DNA sequences reveals a large difference in branch lengths between clades A and B. The mean branch length from the common ancestor to the terminals is twice as long in the former than in the latter (Richardson *et al.*, 2004), hence the informal names 'long branch clade' (A) and 'short branch clade' (B) used here. Though such a phenomenon might be symptomatic of inappropriate rooting, the results of multiple independent analyses (see above) which agree on *Anaxagorea* as sister to the rest of Annonaceae appear to refute this explanation.

The availability of a robust and well-sampled phylogeny facilitates many approaches to the study of plant evolution. The pan-tropical distribution of the Annonaceae enables the study of biogeographic history on a global scale (Richardson *et al.*, 2004). In addition, increasingly dense sampling of taxa allows historical inference at more local scales and recent evolutionary time frames, such as the biogeography of the Central and South American tropics. It further enables one to address other biological questions such as which factors may be important in driving speciation and the evolution of morphological traits. In this chapter we concentrate on four Neotropical genera currently the subject of species-level phylogeny reconstruction projects, *Cremastosperma* and *Mosannona* of the short branch clade, and *Duguetia* and *Guatteria* of the long branch clade (Fig. 2, see Appendix B). We aim both to test monophyly of these genera and to arrive at preliminary estimates for the ages of their most recent common ancestors (MRCAs). This will enable more meaningful comparisons to be made of the contrasting species richness and apparent rates of molecular evolution in these clades, which may in turn contribute to our understanding of the contrasting evolutionary trends that they appear to represent within Annonaceae.

Species level systematics in four Neotropical genera of Annonaceae

Cremastosperma can be distinguished from other Neotropical Annonaceae by the presence of a distinctive groove running down the raised midrib, the parallel organisation of the tertiary veins, and the roof-like structure of the expanded connective above the thecae. The genus currently comprises 17 recognised species, though the completion of taxonomic revision will result in approximately 35 species in total of 'Andean centred' distribution, sensu Gentry (1982) (this thesis, Chapter 5*). The bulk of collections have been made in lowland to premontane forest along the Andes in Peru and Ecuador, with significant diversity extending north through Colombia into Panama and

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***An overestimation of the number of species of *Cremastosperma* was made at the time this paper was accepted for publication. Twenty-nine, plus two insufficiently known, are treated in Chapter 5.**

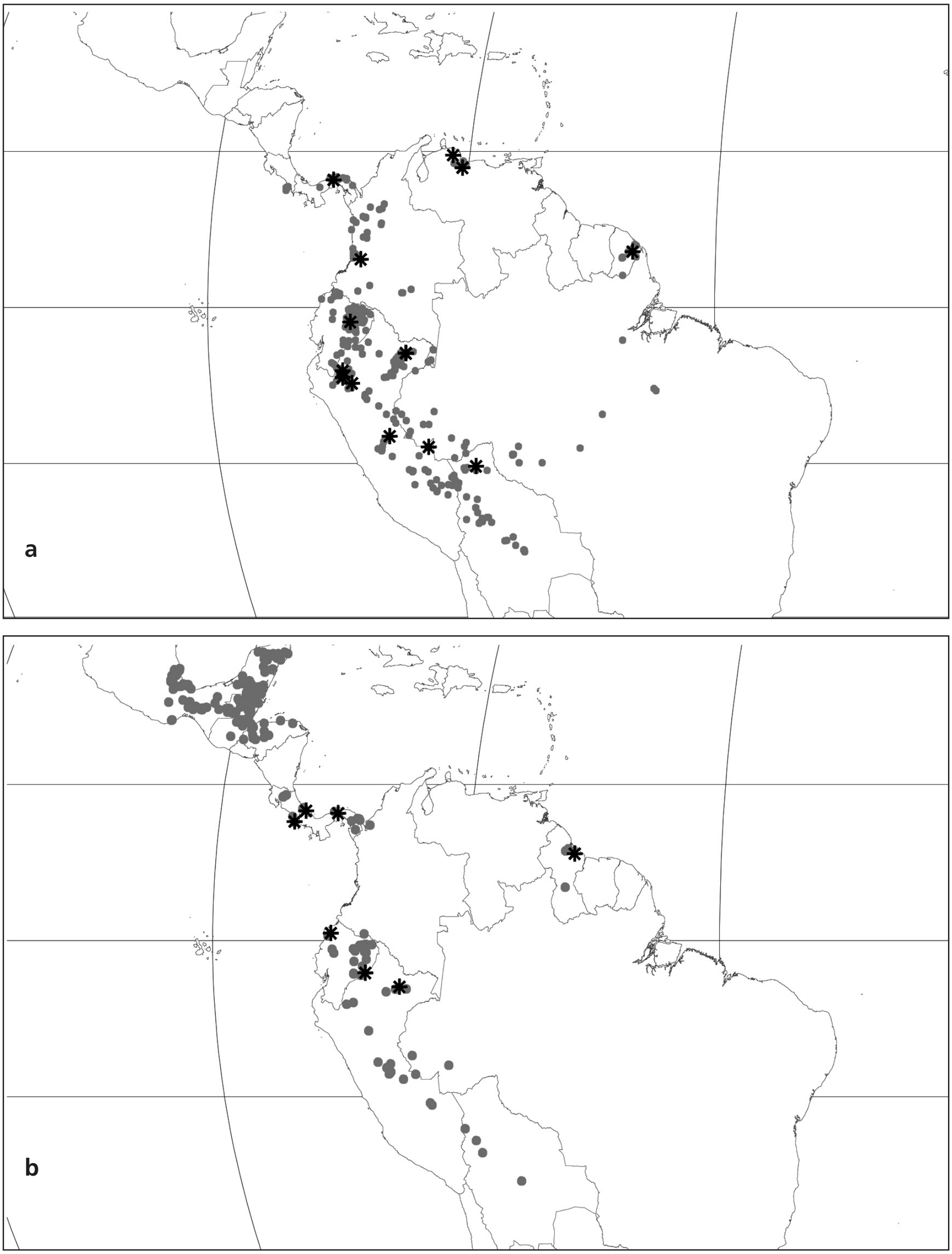

Fig. 3a-d. **Distribution maps: *Cremastosperma* (a), *Mosannona* (b), *Duguetia* (c), and *Guatteria* (d) (collections denoted by grey dots, collections sampled for DNA sequence data denoted by black stars)**

Costa Rica (see Fig. 3a). Only three species* have been collected from further east: *C. macrocarpum* in coastal Venezuela, *C. brevipes* in French Guiana and *C. monospermum* widespread from Peru across northern Bolivia and central

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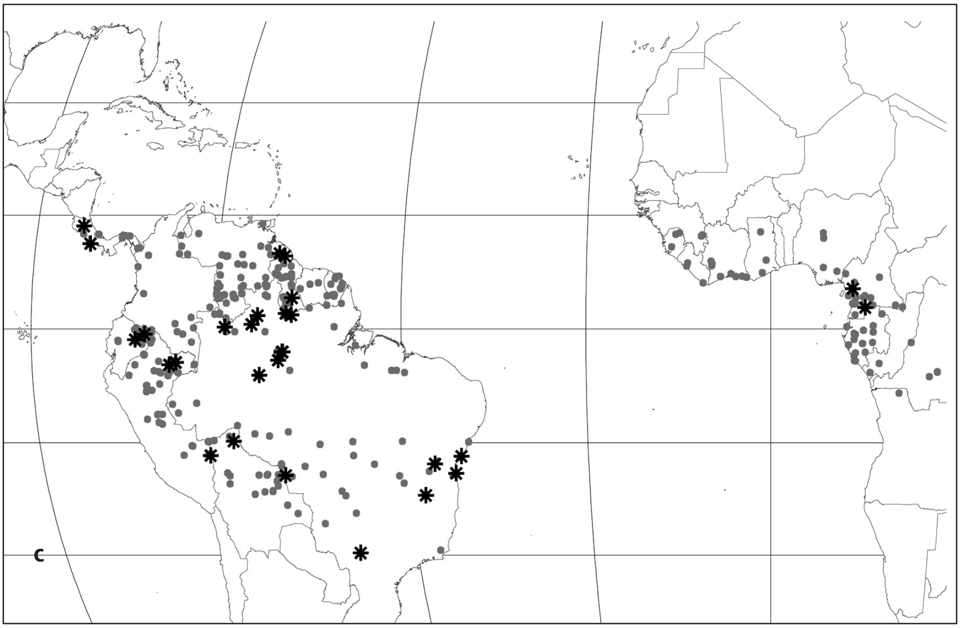
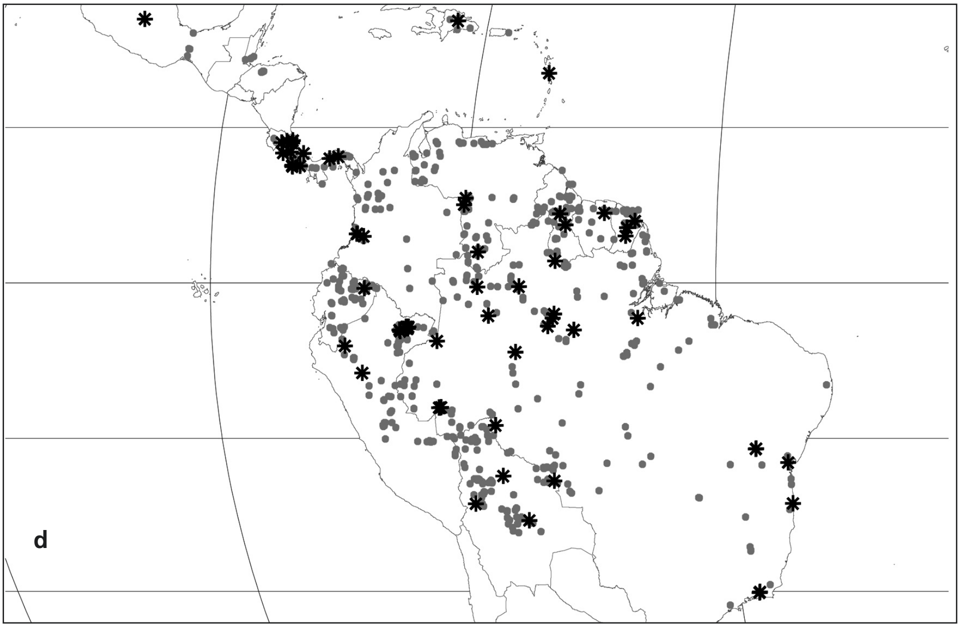
***A fourth, *C. venezuelanum* Pirie, has since been published (Chatrou & Pirie, 2005)**





Brazil. Cladistic analysis of *rbcL* and *trnL-F* sequences does not resolve the sister group of *Cremastosperma* (Richardson *et al.*, 2004), for which a number of clades of Neotropical Annonaceae may be implicated, notably the isolated genera *Malmea* and *Pseudoxandra*.

Mosannona comprises 15 species excluded from the genus *Malmea* (Chatrou, 1998), differing from those of other Annonaceae genera by the combination of a raised midrib and terminal inflorescences (Chatrou *et al.*, 2004). The distribution areas of species of *Mosannona* (see Fig. 3b) are small, with little overlap between areas, the pattern of distribution closely resembling that of the species of *Cremastosperma*. The majority of *Mosannona* species are found in the upper Amazonian forests of Ecuador and Peru, several species occur in Mesoamerica, distributed as far north-west as the Mexican states of Veracruz and Nayarit, and *M. discolor* has been collected in two disjunct areas in Guyana and Suriname. The *Mosannona* clade receives significant support (88% bootstrap, see Fig. 2) on the basis of *rbcL* and *trnL-F* sequence comparison as sister group to a larger clade containing genera including *Oxandra* and *Ephedranthus* and recently described genera formerly representing species of *Malmea*: *Klarobelia* and *Pseudomalmea* (Richardson *et al.*, 2004).

Duguetia occurs in tropical West and Central Africa as well as in the New World, though 92 out of 96 species are Neotropical, distributed evenly across the South American tropics and in Central America north into Costa Rica (see Fig. 3c). *Duguetia* is clearly distinguishable from other genera by the covering of stellate and/or lepidote hairs, and the so-called pseudosyncarpous fruits: the stipeless carpels are held by shallow depressions in the fruiting receptacle, and in addition show various degrees of fusion of the carpels among the different species. A thorough treatment of the genus was assembled in the monograph by Maas *et al.* (2003), and phylogenetic analysis using morphological and anatomical characters revealed a clade comprising the Neotropical genera *Fusaea* and *Duckeanthus* and African *Letestudoxa* and *Pseudartabotrys* as sister group to *Duguetia* (Chatrou *et al.*, 2000). DNA sequence data, however, indicate *Fusaea* alone is sister to *Duguetia* (Richardson *et al.*, 2004).

Comprising more than 250 species, *Guatteria* is the most species rich genus in Annonaceae, with the only exception of the highly polyphyletic old world genus *Polyalthia* (Mols *et al.*, 2004). It is distributed throughout the Neotropics (see Fig. 3d) where it is common in wet forest areas across a relatively wide range of elevation from sea level to around 2,000 m. *Guatteria* resembles Neotropical short branch clade genera such as *Cremastosperma*, in that it also produces single seeded apocarpous fruits from axillary flowers. The *Guatteria* clade is subtended by a long branch leading from a polytomy in the basal portion of the long branch clade (see Fig. 1), leaving the identity of the sister group to *Guatteria* ambiguous. The last revision of the genus dates back to Fries' treatment (1939) and to date *Guatteria* is the only major genus of Neotropical Annonaceae awaiting revision, a task impeded by low morphological and anatomical diversity within the genus.

Preliminary results of phylogenetic analyses with higher levels of taxon sampling in the four genera showed that the combination of *rbcL* and *trnL-F* DNA sequences did not yield sufficient resolution. The large number of species required in order to meaningfully represent *Duguetia* and *Guatteria* in a phylogenetic analysis results in an increased demand for informative characters (Bremer *et al.*, 1999). For *Cremastosperma* and *Mosannona*, although comprising fewer species, the proportion of informative characters to taxa in *rbcL* / *trnL-F* matrices produced was nevertheless low. In general, complete sampling of taxa for plant species level molecular phylogenies remains challenging when working with tropical groups. For instance, in Annonaceae relatively few of the often large numbers of species are in cultivation, and of the rest, many are rarely collected in the wild. Extraction from herbarium material, though often successful, presents challenges for the amplification of target regions (Savolainen *et al.*, 1995). Low quality of many of the available DNA samples prevented large-scale generation of AFLP™ data. Currently no protocol is available for amplifying nuclear encoded marker regions across the Annonaceae. We therefore decided to sample additional characters from the plastid genome, *i.e.* the gene *matK* (encoding a maturase). This region has been shown to be highly effective in reconstructing phylogeny in angiosperms (Hilu *et al.*, 2003) and universal primers are available (Cuénoud *et al.*, 2002).

Molecular dating: a tool for testing hypotheses in Neotropical Annonaceae

Comparison of taxa classified at the generic level becomes meaningful if we have an idea of the time frame within which they diversified. The larger number of species in the genus *Guatteria* might simply indicate diversification over a longer period of time. Molecular dating provides a possibility to test hypotheses for which there is an implicit time frame. For instance, biogeographic hypotheses linking diversifications in groups such as *Cremastosperma* in the Neotropics to relatively recent events such as the Andean orogeny (Gentry, 1982) can be tested, as can those seeking to explain cross-continentally distributed tropical groups such as *Duguetia* according to either long distance dispersal, Tertiary migration or older Gondwanan distributions (*e.g.*, Renner *et al.*, 2001; Davis *et al.*, 2002). Even the relative contributions of the processes of speciation and extinction in producing such patterns can be assessed (Barraclough & Reeves, 2005).

Molecular dating techniques are subject to a number of sources of error which must be taken into account when assessing the significance of the result (Sanderson & Doyle, 2001). Rate heterogeneity can be addressed using techniques such as nonparametric rate smoothing (Sanderson, 1997), which assumes autocorrelation of rates across a phylogeny, and which can incorporate assumption of a gamma distribution of rates across characters. Absolute, as opposed to relative, ages can only be estimated given a (rate-smoothed) ultrametric tree calibrated using fossil or geological data to set or limit the age of one or more nodes. This process is controversial, often afflicted by considerable uncertainty (Bremer, 2000; Smith & Peterson, 2002; Hedges & Kumar, 2004). Further uncertainty can be associated with the topology of the phylogeny in question (phylogenetic uncertainty), and in the estimation of branch lengths optimised onto it given the character sampling used ('substitutional noise') (Sanderson & Doyle, 2001). Assessing the effects of the latter source of error is possible through bootstrap re-sampling techniques, whereas the effects of phylogenetic uncertainty can be avoided by limiting dating to significantly supported nodes.

Error stemming from taxon sampling in the estimation of ages for particular nodes is less straightforward to determine, as the taxon sampling itself may define the node in question. For example, the first branching lineage represented by *Amborella* effectively defines the age of the crown group of Angiosperms. Given that one has identified and included such a lineage in the analysis, methods of molecular dating should provide consistent estimations of the age of the MRCA of the crown group, irrespective of levels of taxon sampling. This is of particular importance since both comprehensively and representatively sampled groups are subject to 'natural taxon sampling bias' in the form of extinction, and techniques to estimate levels of extinction (Nee *et al.*, 1994; Barraclough & Reeves, 2005) are based on the same molecular dating methods. Given the large differences in estimated numbers of extant species between the four genera under study here, (*i.e.* 96 species, and 32 accessions available for *Duguetia*, 250/49 for *Guatteria*, 35/23 for *Cremastosperma* and 15/7 for *Mosannona*) we therefore consider it important to explore whether taxon sampling itself might influence the outcome of the analysis. Thus, we want to test whether dating results from analyses including all available taxa in a study are also observed in analyses using equal sized subsets of those taxa.

The aims of this study were to test monophyly of the genera *Cremastosperma*, *Mosannona*, *Duguetia* and *Guatteria* more thoroughly than Richardson *et al.* (2004) and arrive at preliminary estimates for the ages of their most recent common ancestors (MRCAs). We then used these estimations to investigate whether diversification within these contrasting genera can be shown to have occurred within the same or significantly different time slices. Finally, in order to assess whether and to what extent the outcome of age estimations thus produced might be influenced either by character or taxon sampling, re-sampling approaches were used and results compared.

Materials and methods

Taxon sampling

This study utilises both unpublished and published (Sauquet *et al.*, 2003; Mols *et al.*, 2004; Mols *et al.*, submitted) *rbcL*, *matK* and *trnL-F* sequences from a total of 132 taxa (see Appendix A). Twenty three accessions of *Cremastosperma*, (representing around half of the (ca. 35) described and un-described species in the genus), 7 samples of *Mosannona*, (representing just under half of the species), 32 samples of *Duguetia*, (representing one third of the species) and 49 samples of *Guatteria*, (representing around a fifth of the estimated number of species) were included in this study (geographical localities of specimens sampled are indicated in Appendix A and Fig. 3). In addition, major clades in Annonaceae, including sister or putative sister groups of the four genera as identified by Richardson *et al.* (2004), were represented here by 23 accessions. These represented 11 species each from the long branch and short branch clades (including taxa from across the tropics) plus *Anaxagorea*, sister group to the rest of the Annonaceae. The numbers of accessions were limited in this way in order to restrain computational times to reasonable lengths. Outgroups were selected from other families within the Magnoliales sensu APG (2003), *i.e. Eupomatia* (Eupomatiaceae is sister group of the Annonaceae (Qiu *et al.*, 2000)), *Magnolia* (Magnoliaceae) and *Coelocaryon* (Myristicaceae).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted using a modified cetyl trimethyl ammonium bromide (CTAB) method (Doyle & Doyle, 1987): 50 mg silica dried or herbarium leaf material was homogenised in 1300 µl CTAB and incubated for 20 minutes with 12 µl 2-mercaptoethanol at 65°C, followed by 90 minutes ambient mixing with 1 ml 24:1 chloroform:isoamylalcohol. Following 10 minutes centrifugation at 13,000 rpm, 300 µl supernatant was purified using Wizard DNA purification system (Promega corp.). We omitted isopropanol precipitation, in order to avoid co-precipitation of oxidised material (Savolainen *et al.*, 1995).

PCR amplification conditions were modified depending on the qualities of the DNA sample available. Samples extracted from herbarium material often contain lower quantities of more fragmented DNA, as well as higher levels of PCR inhibiting compounds (Savolainen *et al.*, 1995). In most cases the *rbcL* gene was amplified in two pieces and sequenced using primers 1F/724R (Olmstead *et al.*, 1992) and 636F/1460R (Fay *et al.*, 1997; Fay *et al.*, 1998). Where amplification was unsuccessful (particularly in lower quality herbarium extracted DNA samples) further internal primers, 217F: 5'-GGACTTACCAGCCTTGATCG-3', 922F: 5'-GGTATGCACTTTCGTGTACTAGC-3', 536R: 3'-GGTTATCCGCCAAGAACTACGG-5' and 1104R: 3'-GGCAGAACACCTGGCAAAGAGACC-5' were designed and applied in combination 1F/536R, 217F/724R, 636F/1104R, and 922F/1460R to amplify the gene in four overlapping pieces of ca. 500 bp long. The angiosperm-universal primers described by (Taberlet *et al.*, 1991) were used to amplify and sequence the *trnL* intron (primers C/D) and *trnL-trnF* spacer (primers E/F) separately. Partial *matK* sequences were amplified and sequenced using primers 390F and 1326R (Cuénoud *et al.*, 2002). Where amplification was unsuccessful further internal primers, MintF: 5'-TCCTTTGGAACTGTTCTTGAGC-3' and MintR: 5'-GATCCTGTGCGGTTGAGACC-3' were designed and applied in combination

390F/MintR and MintF/1326R in order to amplify the gene in two overlapping pieces of ca. 500 bp long. A standard PCR protocol was used throughout, with the addition of 0.4 % BSA (which was found to increase amplification in all samples). The PCR profile used for all regions comprised 35 cycles of 30 sec. at 94°C, 1 min. at 55°C, 2 min. at 72°C, with an initial 4 min. at 94°C and final 7 min. at 72°C. PCR products were purified using QIAquick PCR purification kits (Qiagen), sequenced with the PCR primers, and analysed by electrophoresis using an automatic sequencer ABI 3730XL.

DNA sequence, phylogenetic & dating analyses

DNA sequences were edited in SeqMan 4.0 (DNAStar Inc., Madison, WI) and aligned manually, resulting in alignments of 1431 positions (*rbcL*), 1333 positions (*trnL-F*), and 831 positions (*matK*). Gaps in the *trnL-F* alignment were coded as present/absent characters where they could be coded unambiguously, following Simmons & Ochoterena (2000), and areas where the assessment of homology was ambiguous were excluded from the analyses. Sequences were deposited at Genbank (see Appendix A).

Data were analysed using the parsimony algorithm of the software package PAUP* 4.0b10 (Swofford, 2000), under the equal and unordered weights criterion (Fitch parsimony; Fitch, 1971). Support was estimated for the three markers independently and, after checking for significantly supported incongruencies, combined, using a 500 replicate bootstrap analysis with 'full' heuristic searches of 50 random addition sequences, TBR, saving 50 trees each time. A maximum parsimony search was performed on the combined matrix with 500 random taxon additions, saving 100 trees per replicate, using the tree-bisection-reconnection (TBR) branch swapping algorithm. ModelTest 3.06 (Posada & Crandall, 1998) was used to select the substitution model best fitting the (entire) dataset. This substitution model was used to select the most likely of the maximum parsimony topologies as estimated above and calculate branch lengths using the maximum likelihood (ML) criterion as implemented in PAUP*.

In order to test whether our data set exhibited clocklike behaviour a likelihood ratio test was performed on one of the parsimonious tree topologies: that with the highest likelihood, derived from the complete matrix including all taxa. Likelihood of the data with and without constraint of a molecular clock, were calculated and the likelihood ratio statistic compared with χ^2 critical value with 135 degrees of freedom (*i.e.*, number of taxa minus 2). Thereafter, Sanderson's method of nonparametric rate smoothing (NPRS) was applied (Sanderson, 1997) as implemented in the software package r8s (Sanderson, 2002) in order to estimate divergence times.

As stated above, the focus of this chapter is on the relative ages of the four genera. However, to represent these ages within an absolute timescale the molecular clock was calibrated by constraining the MRCA of the clade including all Annonaceae except the genus *Anaxagorea* and the basal clade including *Cananga* (see Fig. 1) to between 65 and 71.3 mya. This age is based on the presence of seeds with lamelliform ruminations in the Maastrichtian of Nigeria (Chesters, 1955). When used as single calibration point in preliminary analyses this age produced a result congruent with the age of the MRCA of the genera *Annona* and *Asimina* (25 mya) as estimated by Wikström *et al.* (2001) (data not shown). The *Annona/Asimina* node was therefore also fixed for further analyses.

Divergence times were estimated for the complete matrix, as well as for character re-sampled (analysis I) and taxon re-sampled matrices (analysis II). In analysis I, confidence limits on branch lengths, reflecting stochasticity in the sampling of character changes ('substitutional noise'), were estimated by 100 replicates of bootstrap re-sampling (as also described in Wikström *et al.*, 2001), including all taxa, with subsequent ML branch length estimation on a constrained tree topology (as above) for each bootstrap replicate. This results in 100 trees comprising a range of estimated lengths for each branch of the topology. Divergence times were then estimated for the 4 nodes representing the MRCAs of each of the four genera in each of these 100 trees and the results summarised giving mean values with standard deviation for specified nodes (the MRCA for each genus) using the 'profile' command in r8s.

In analysis II, 100 smaller matrices of 38 taxa were constructed by excluding randomly all but three species each of *Cremastosperma*, *Mosannona*, *Duguetia* and *Guatteria*, leaving the other Annonaceae and outgroup taxa in (see above). This was done in order to assess whether and to what extent taxon sampling might influence the outcome of age estimations. Each of these 100 taxon re-sampled matrices was then subjected to phylogenetic analysis followed by r8s analysis as above. The oldest of the 100 estimations for the MRCA for each genus (this time represented by three accessions) is then interpreted as the oldest age estimate for the MRCA of that genus. This estimate should be independent of numbers of taxa sampled across the four genera and thus suitable to test whether there is a bias present in the form of uneven taxon sampling in analysis I. Should previous analyses of all available taxa have resulted in a single supported topology, this could have been used to constrain relationships in each of the taxon re-sampled analyses. However, this was not the case. In order to exclude the possibility of phylogenetic error causing erroneously old age estimates, the trees from which the oldest age estimate overall for each genus were obtained was inspected to confirm that the topology did not conflict with the bootstrap consensus including all available taxa (Fig. 4).

Results

Bootstrap analysis of the three markers independently revealed no significantly supported conflict (bootstrap frequencies ≥70%); therefore the data were combined for further analyses. Parsimony analysis of the combined data produced <50,000 most parsimonious trees. Statistics of the three markers separately are given in Table 1, numbers of variable and parsimony informative characters per marker within each of the four genera are given in Table 2. Support estimated using a 500 replicate bootstrap analysis is represented in Fig. 4 (see below). Results were congruent with those produced by Richardson *et al.* (2004; see Fig. 3). Monophyly of clades including all accessions of *Cremastosperma*, *Duguetia*, *Guatteria* and *Mosannona* was supported by bootstrap percentages of 99, 100, 100 and 100 respectively, but sequence variation within the clades was generally low, as reflected by the small numbers of internal nodes supported by <70 % BS (1, 2, 4 and 3 respectively). Numbers of variable characters were higher in the long-branch clade genera *Guatteria* and *Duguetia* than within *Cremastosperma* and *Mosannona*, and those within *Guatteria* were highest of the four (see Table 2). However, the majority of maximum parsimony analyses performed for analysis II resulted in a single most parsimonious estimation of the relationships between the three accessions included for each of the four genera.

Table 1. **Details of maximum parsimony search**

	Length of alignment	variable characters	parsimony informative characters	% variable/ informative characters	CI optimised on tree in Figure 4	RI optimised on tree in Figure 4
rbcL	1387	293	164	21 / 12	0.521	0.885
trnL-F	1179	406	190	34 / 16	0.733	0.927
matK	831	363	198	44 / 24	0.706	0.920

Table 2. **Numbers of variable / parsimony informative characters, according to marker and in total within each of the four genera**

	rbcL	*trnL-F*	*matK*	Total
Mosannona	22/11	17/4	9/1	48/16
Cremastosperma	35/9	22/6	18/6	75/21
Duguetia	74/46	41/8	52/9	167/63
Guatteria	77/43	87/18	49/15	213/76

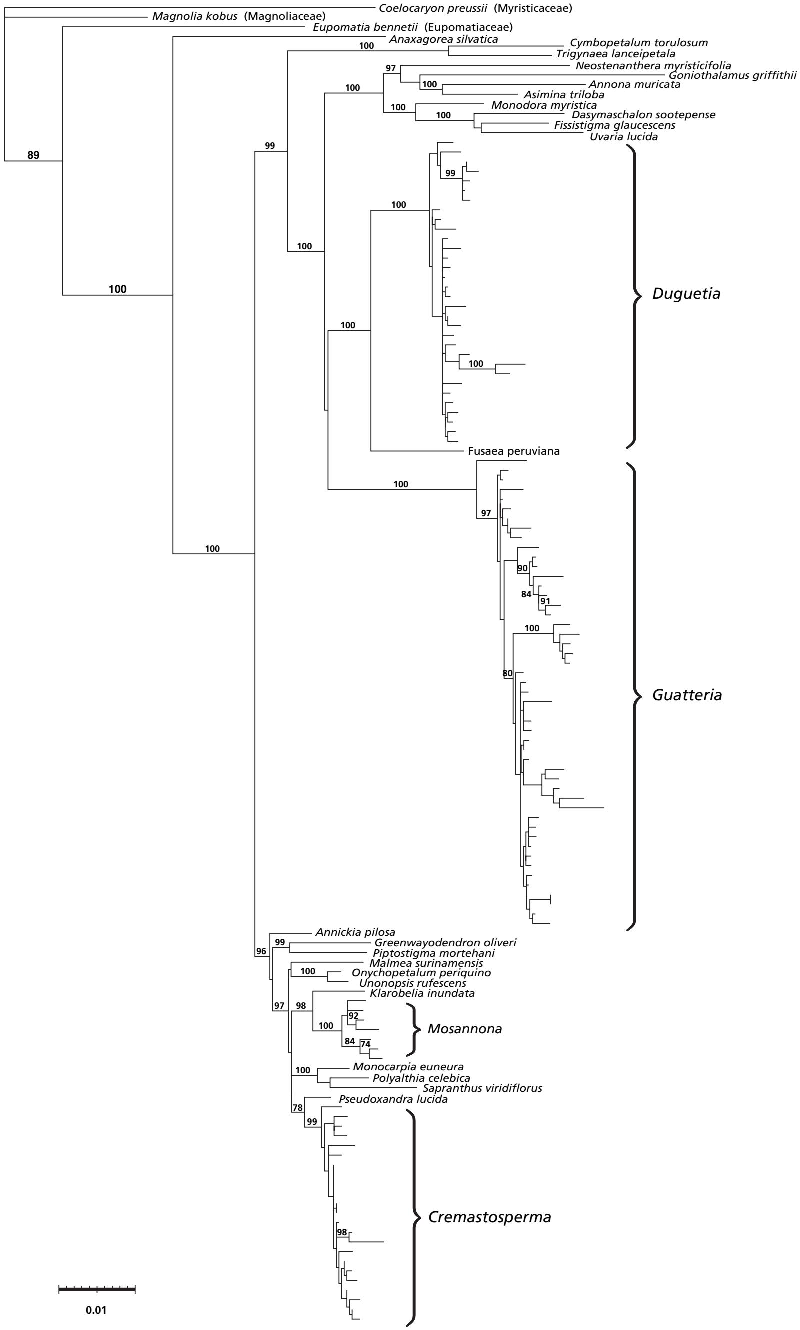


Fig. 4. Phylogram of Annonaceae (this study, analysis I) including all samples of *Cremastosperma*, *Mosannona*, *Duguetia* and *Guatteria*, with Magnoliales outgroups based on *rbcL*, *trnL-F* and *matK*. One of 50,000 most parsimonious trees, the one with highest maximum likelihood (ML) score, is shown, with branch lengths optimised based on the ML model described in the text. Bootstrap values at nodes are based on parsimony analysis. Scale bar indicates 0.01 subst. per site.

The substitution model selected as best fitting the (entire) dataset was the general time reversible model (Tavare, 1986) with a proportion of invariable sites and gamma distributed rate (GTR + I + Γ, $\alpha = 0.995$). One maximum parsimony topology, the one with optimal likelihood of the data, is represented in Fig. 4 with optimised ML branch lengths. Results of a likelihood ratio test rejected a molecular clock ($p < 0.0005$). Results of molecular dating using NPRS are represented in Table 3 in the following way: firstly, the mean age of the MRCAs of the four clades with estimation of error due to substitutional noise; (analysis I). Second, the earliest age estimations derived from the taxon-resampled matrices (analysis II). And finally the difference (where observed) between the minimum age estimated for analysis I, and the value for analysis II, which was not found to exceed the oldest age estimate under analysis I in results for any of the four genera.

Table 3. **Age estimations for the MRCAs of the four genera included in this study. Analysis I (see text) bootstrap re-sampled characters (mean with standard deviation), Analysis II; the oldest age estimates derived from taxon re-sampled matrices, and (the minimum value of) I minus II, where a difference was observed**

	Analysis I (mya)	Analysis II (mya)	I - II
Cremastosperma	35.57 ±7.38	28.82	-
Mosannona	24.01 ± 4.74	22.55	-
Duguetia	29.04 ± 4.52	20.34	4.18
Guatteria	36.65 ± 2.50	19.42	14.73

The latest (youngest) estimated age of the MRCA of *Guatteria*, with confidence limits according to substitutional noise, was significantly older than the earliest estimations for *Mosannona* and *Duguetia*. The mean age estimated for the MRCA of *Cremastosperma* was similar to that of *Guatteria*, however confidence limits for *Cremastosperma*, *Mosannona* and *Duguetia* overlapped, as did those for *Cremastosperma* and *Guatteria* (see Table 3), due to the high standard deviation in the result for *Cremastosperma*.

The earliest age estimations derived from the taxon re-sampled matrices fell within the confidence limits according to substitutional noise in *Cremastosperma* and *Mosannona*. The value for *Duguetia* was slightly younger than the youngest confidence limit according to substitutional noise, and for *Guatteria*, substantially younger. We did not further test for significance of the differences observed as it is not obvious what test /chance distribution should be applied.

Discussion

Phylogeny reconstruction and monophyly of *Cremastosperma*, *Duguetia*, *Guatteria*, and *Mosannona*

Phylogenetic analysis of an increased proportion of species of the genera *Cremastosperma*, *Mosannona*, *Duguetia* and *Guatteria* compared with previous studies has served to support the monophyly of all four genera with a high degree of confidence. Taxa were selected as representatives of major clades in the family, and of the sister groups (or most likely sister groups) of *Cremastosperma*, *Mosannona*, *Duguetia* and *Guatteria*, as revealed by Richardson *et al.* (2004). Such a selection resulted in the reconstruction of topologies congruent with those found in that study, limiting the scope of this chapter to the questions at hand, thus making further discussion of the wider topology here inappropriate.

In our results, resolution of relationships within *Cremastosperma*, *Mosannona*, *Duguetia* and *Guatteria* was low, despite the extra characters sampled as compared with Richardson *et al.* (2004). The number of informative characters contributed to the analyses by *matK* was relatively high (especially given the length of the sequence) and levels of homoplasy comparable to those in *trnL-F*, both rather lower than in *rbcL* (see Table 1), suggesting it to be a useful marker for further application within Annonaceae. However, numbers of characters informative at the intrageneric level, particularly in *Cremastosperma* and *Mosannona*, were low (see Table 2), which is reflected by the lack of resolution in relationships within them in Fig. 4. This suggests that more, and more variable, sequence data is necessary in order to resolve relationships at this level, and that, ideally, AFLP™ markers should be applied as well. Alternatively, low copy nuclear encoded genes may provide such variation, and is in any case necessary in order to attempt to infer species rather than simply chloroplast phylogenies.

Molecular dating

Calibrating a molecular clock by means of fossil data or geological events under- and over-estimates ages respectively, to an unknowable degree, and must be interpreted with caution (Bremer, 2000; Smith & Peterson, 2002; Hedges & Kumar, 2004). In attempting to make a comparison between the ages of the four clades the analysed in this study, the impact of error arising from the calibration of the molecular clock was avoided by including them in a single analysis using the same calibration. This allows direct comparison of relative ages to be made, even if the accuracy of the absolute dates remains un-assessed.

Rate heterogeneity is apparent in Annonaceae, as exemplified by the differences in proportions of variable characters found between the short-branch and long-branch clades. It can be addressed using a number of techniques assuming autocorrelation of rates across the tree, including parametric Bayesian approaches (*e.g.* Thorne *et al.*, 1998) and semi-parametric Penalised Likelihood (Sanderson, 2002). The nonparametric rate smoothing technique (Sanderson, 1997) has been used in a number of studies representing all angiosperms (*e.g.* Sanderson & Doyle, 2001; Wikström *et al.*, 2001; Davies *et al.*, 2004), wherein variation in generation time, a major determining factor in rates of molecular evolution, is far greater than within Annonaceae, where herbaceous habit is not observed. Despite possible drawbacks of the method under particular conditions (Sanderson, 2002), NPRS was used in this study, in order to keep the numbers and durations of the analyses within reasonable bounds, and to enable comparison with results obtained in other studies (*e.g.* Barraclough & Reeves, 2005, and Bakker *et al.*, 2005).

Because combined analysis of *rbcL*, *trnL-F* and *matK* sequences failed to resolve relationships within the four genera it was necessary to select effectively arbitrarily resolved topologies (the most parsimonious trees with the best maximum likelihood scores) with which to make age estimations. Long-branch attraction in one of the examples provided by Sanderson & Doyle (2001) resulted in overestimation of the age of the MRCA of Angiosperms due to spurious placing of *Oryza* as sister group of the Angiosperms. The possibility of such error affecting our age estimations when analysing all taxa cannot entirely be excluded; however, the topology used showed no conflict with provisional results of phylogeny reconstruction in the four genera based on analysis of more characters (data not shown). No further attempt was made to estimate the effect of phylogenetic uncertainty on those date estimations, as the ages of interest, those of the MRCAs of each of the four genera, were represented by nodes which were present in the strict consensus of all most parsimonious trees (not shown) and which received 97-100% bootstrap support.

In the taxon re-sampling technique (Analysis II) detailed here, the ages of the MRCAs of the four genera are represented by the oldest of the age estimates (see Table 3). This is because many of the replications are likely to involve species with relatively recent MRCAs, where the relatively fewer lineages which effectively define the age of the group are missed. Ideally a topology supported by analyses including all taxa would be imposed for each of the taxon re-sampled matrices, and in principle a range of estimations could thus be produced for each node in the topology. At the least, given support for the first-branching ('basal') lineage in a topology, this lineage could be included in all re-sampled matrices in order to arrive at a thorough test of the age of the MRCA. No fully supported topology was available for any of the genera based on these results, and only in *Guatteria* was there support for the first branching lineage within the genus. It was therefore decided to treat each genus equally by re-sampling randomly without imposing any topological constraints. We have no reason to believe that the topologies produced for analysis II would all be congruent with a ‘true’ phylogeny of all the taxa available, however, those points representing the oldest age estimates were inspected and found not to conflict with the supported relationships based on analysis of all taxa (as in Fig. 4).

From the standard deviations according to substitutional noise it appeared that diversifications in *Mosannona* and *Duguetia* occurred within roughly the same period of time, beginning significantly later than that of *Guatteria* (see Table 3). Lack of precision in the result for *Cremastosperma* may be an effect of the low number of variable characters in the DNA sequence data, the difference compared with *Mosannona* explained by the higher number of accessions included. A parametric or semi-parametric approach may be more appropriate for *Cremastosperma*, given the abundance of short branches in a phylogeny of this genus based on this data, as nonparametric rate smoothing tends to over-fit the data in such cases, leading to rapid fluctuations in rate (Sanderson, 2002).

Ages for *Cremastosperma* and *Mosannona* estimated from taxon re-sampling (analysis II) fell within the confidence intervals obtained by re-sampling characters (analysis I). However, the taxon re-sampling estimations for the age of the MRCAs of *Duguetia* and, more markedly, that for *Guatteria*, were younger than the upper confidence limits estimated by character re-sampling. The MRCA of *Guatteria* yielded the most precise result in the character re-sampling analysis judged by the smallest standard deviations, which is possibly a reflection of the higher number of informative characters present in the *Guatteria* data. *Guatteria* was represented by the largest number of accessions in the analysis, *i.e.* 49 compared to 23, 7 and 32 in *Cremastosperma*, *Mosannona* and *Duguetia*, respectively. It would thus be expected that if difference in the number of samples were to bias the result, that this effect would be most noticeable in *Guatteria*.

The observation of this conflict raises a conundrum: should apparently under-representing a species-rich genus such as *Guatteria* by including only three species in the taxon re-sampling lead to an underestimation of the age of the MRCA? Or should including an absolutely higher number of accessions of *Guatteria*, relative to those of the other genera in analysis I, be interpreted as an overestimation in age? If the former conclusion were to be drawn, and molecular dating only be performed on fully sampled clades, do we then need to factor in a more realistic assessment of the levels of sampling, by first estimating levels of extinction? If so, how would we ensure that this estimation itself, based on the same analyses, is not subject to the same uncertainty?

Alternatively, these inconsistencies may be a result of the particular method used. One possible explanation might be multiple substitutions as revealed by increased taxon sampling. ML branch lengths, as used here, should be less affected by this phenomenon than those reconstructed under maximum parsimony.

However, ML may not be entirely immune to this effect (T. Barraclough, pers. com.), and where sequence variation in a clade is relatively higher, as is the case in *Guatteria* (and *Duguetia* to a lesser extent), and homoplasy significant (particularly in *rbcL*; see Table 1), this might magnify the effect. A second possible explanation might be provided by Barraclough & Reeves (2005): bias caused by low sequence variation (characterised by a large number of unresolved inner branches in a phylogeny) leading to an apparent slow down in LTT plots. That this would be apparent in *Guatteria* and *Duguetia*, and not in *Cremastosperma* and *Mosannona* where sequence variation was lower, might be explained in analysis I by the imprecision in the result for *Cremastosperma* and the lower ratio of taxa to variable characters in *Mosannona*.

One of the few supported nodes within *Guatteria* is that between *G. anomala* and the rest of the genus. The earliest age estimations in analysis II for the MRCA of *Guatteria* were derived from re-sampling which included this taxon. Although sampling of *Guatteria* in analysis I was the highest of the four genera, it actually represented the lowest proportion of the total numbers of extant species for the four genera. Targeted sampling throughout the range of geographic and morphological diversity within such a group might be hoped to reveal such anomalies, but even when a larger proportion of the extant species have been sampled the possibility remains that more early branching lineages will be discovered. This would definitively increase the age estimation for the MRCA of the genus. However, given the results shown here, it would seem sensible to investigate whether increased sampling of the younger clade representing the majority of sampled species of *Guatteria* might, less intuitively, and perhaps misleadingly, lead to the same result.

Conclusions

As sister groups, the 'short branch' and 'long branch' clades of Annonaceae are of equal age. However, the latter consists of twice as many species, the majority of these clustered within a small number of genera. How meaningful is this difference? Is it simply a question of taxonomy, or does it reflect a real difference in the evolutionary history of these groups? Could such differences be a result of the morphological innovations, such as syncarpous fruits and liana habit, only found in the long branch clade, and could these have caused differences in rates of speciation and extinction compared with the short branch clade?

Results of phylogeny reconstruction presented here support monophyly in the genera *Cremastosperma*, *Mosannona*, *Duguetia* and *Guatteria*, although more stringent tests would require increased sampling, to a lesser extent in *Duguetia* but especially in *Guatteria* where the proportion of extant species sampled was lowest. Estimation of the ages of the MRCAs of these genera, with confidence limits reflecting error due to finite sampling of stochastically evolving characters, resulted in significantly greater age for *Guatteria* than for *Duguetia* or *Mosannona* (the result for *Cremastosperma* was too imprecise to be able to draw such conclusions).

In addressing questions of taxon sampling, with respect to molecular dating, we encountered an apparent conflict. A more species rich group might either be hypothesised to have originated earlier than one comprising fewer species (such as was first apparent in *Guatteria*), or to have been the result of a recent more rapid radiation. In a large group, achieving complete sampling, or even knowing the proportion of complete sampling that has been achieved, may be a practical impossibility. It would seem intuitively reasonable to assume that, with knowledge of the broad topology of such a group, a subset of taxa can be chosen to represent clades for which ages can then be estimated (for example, in angiosperms: Sanderson & Doyle, 2001; Wikström *et al.*, 2001; Davies *et al.*, 2004). However, results produced here suggest that increased taxon sampling from within a clade (and not simply of lineages 'basal' to it) may result in older age estimations for that clade.

It is necessary to determine whether this result is robust to different methods of age estimation, to use with more resolved phylogenies and different taxonomic groups. The implication, should it indeed prove to be robust, is either that species rich groups may have diversified more recently than a densely sampled analysis might suggest, or alternatively, that a reduced analysis of carefully selected 'placeholder' taxa might underestimate that age. The predominance of large genera in the long-branch clade might reflect evolutionary history, or the figment of a taxonomist's system. Further investigation will be required to discern between these two competing explanations.

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Chapter 3

'Andean-centred' genera in the short branch clade of Annonaceae: testing biogeographic hypotheses using phylogeny reconstruction and molecular dating

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Abstract

A number of Neotropical genera of Annonaceae display 'Andean-centred' distribution patterns, with high species richness on both sides of the Andes mountain range. We test biogeographic hypotheses regarding the origin of Andean centred plant groups by reconstructing phylogeny and estimating the timing of diversifications in four genera: *Cremastosperma*, *Klarobelia*, *Malmea*, and *Mosannona*, using chloroplast DNA sequences of related Annonaceae taxa plus outgroups. Almost all accessions of the four genera formed monophyletic groups nested within a clade including all of the South American-centred 'short branch clade' genera, to the exclusion of two largely Asian and two African clades. We infer a common ancestor of the four genera to have originated in South America, but not by vicariance of an ancestral population on Gondwana. Radiations of these clades could have been influenced by the Andean orogeny, but further tests require greater precision in the molecular dating results.

Keywords: Andes, Annonaceae, biogeography, *Cremastosperma*, *Klarobelia*, *Malmea*, *Mosannona*, Neotropics, phylogeny reconstruction, molecular dating.

Abbreviations: BS (bootstrap support); LBC (long branch clade); MP (maximum parsimony); MRCA (most recent common ancestor); NPRS (nonparametric rate smoothing); PL (penalized likelihood); PP (posterior probability); SAC clade (South American centred clade); SBC (short branch clade).

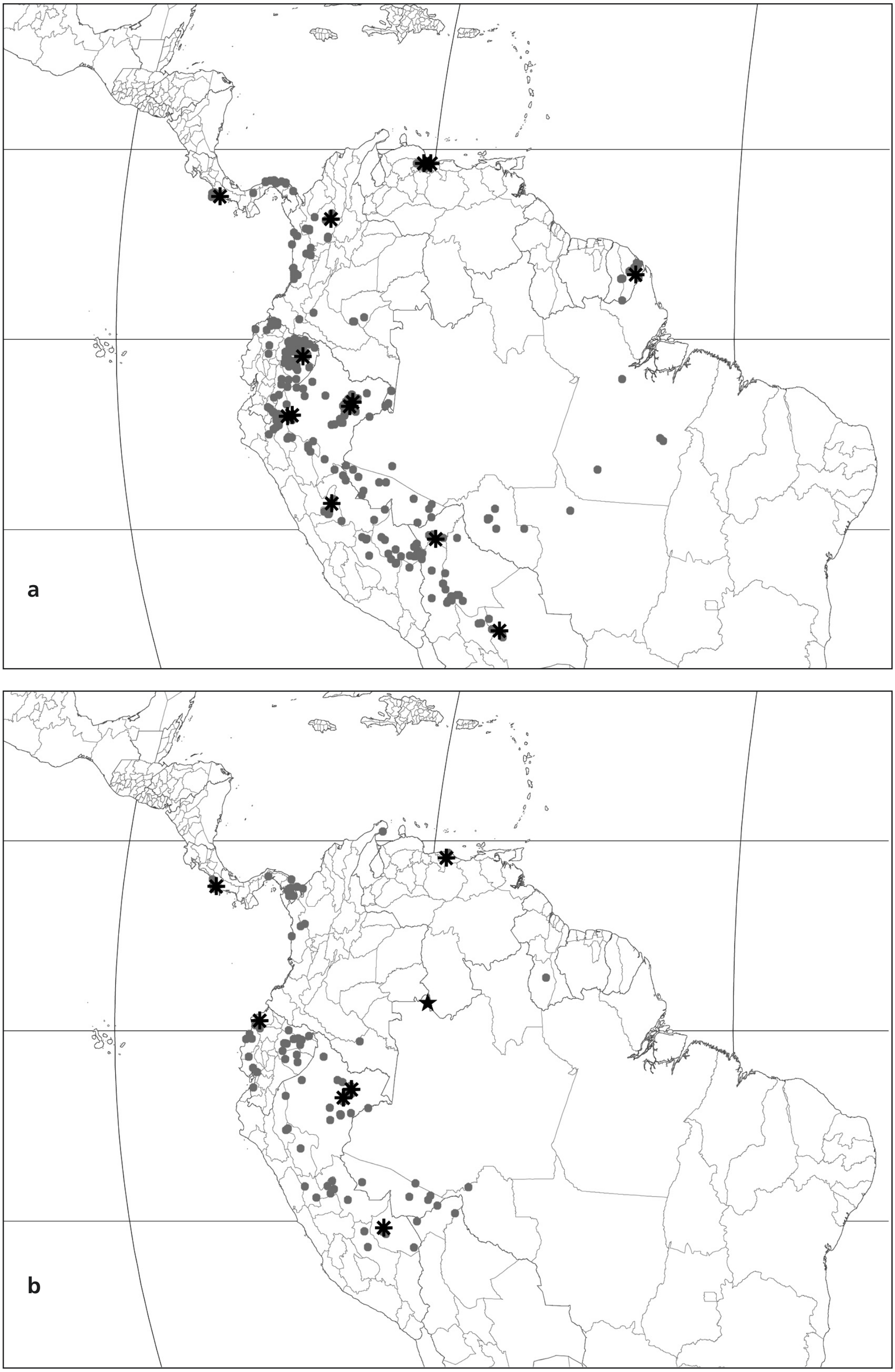
Introduction

Around a third of all flowering plants are found in the Neotropics (tropical America) (Smith *et al.*, 2004). Two areas within the Neotropics; the tropical Andes (including forests on the eastern side of the Andes extending from Colombia through eastern Ecuador and Peru as far south as Bolivia), and the Chocó/Darién/Western Ecuador region (the narrow tropical zone on the Pacific Ocean side of the Andean mountain chain) together house 22,500 endemic plant species representing 7.5% of all species of plants worldwide (Myers *et al.*, 2000). Understanding the origins of these biodiverse areas in the Neotropics will help us to determine why this region is so species rich.

In his 1982 paper, Al Gentry proposed historical biogeographical scenarios that might explain the high floristic diversity of the Neotropics in general, and the areas surrounding the Andes in particular. Gentry considered the majority of South American taxa to be Gondwanan-derived and he distinguished two groups within them on the basis of distribution patterns and growth forms. Amazon-centred taxa, *sensu* Gentry, are largely canopy trees and lianas. Andean-centred taxa, in contrast, are diverse in the lowlands near the base of the mountains and in middle elevation cloud forests, areas corresponding to the tropical Andes and Chocó/Darién/Western Ecuador region, with very poor representation in Amazonia. They are chiefly shrubs, epiphytes and palmettos. Gentry's explanation for the distribution of Andean-centred taxa was that they were subject to speciation as a result of the orogeny of the Andean mountain chain. However, South American geological history of the last few tens of millions of years is complex, and a number of overlapping events may be critical in determining current distribution patterns – in order of decreasing age: the Andean orogeny, closure of Panama isthmus and the numerous episodes of climatic changes occurring throughout the Pleistocene (Burnham & Graham, 1999). Even the relative importance of historical versus present day (ecological) factors in determining current plant species distributions remains a point of contention (*e.g.* see Tuomisto & Ruokolainen, 1997).

The pantropically distributed family Annonaceae comprises around 2500 species of trees and lianas, found predominantly in tropical rain forests. Over 900 species are recognised in the Neotropics (Chatrou *et al.*, 2004), where they represent a significant part of plant diversity, both in terms of number of species and number of individuals (Valencia *et al.*, 1994; Ter Steege *et al.*, 2000). The majority of Annonaceae genera in South America would be considered Amazon-centred according to Gentry, *i.e.* most species are medium to large trees distributed across Amazonia. However, some groups, notably the genera *Cremastosperma*, *Klarobelia*, *Malmea*, and *Mosannona* display markedly Andean centred distributions (lowland to pre-montane forest usually only up to 1,500 m) and are usually small under-story trees.

Cremastosperma can be distinguished from other Neotropical Annonaceae by its raised midrib with a unique longitudinal groove and comprises approximately 35 species. Most species are found along the Andes in Peru and Ecuador, with significant diversity also extending north through Colombia into Panama and Costa Rica (see Fig. 1a). Only four species have been found further east: *C. macrocarpum* and *C. venezuelanum* in coastal Venezuela, *C. brevipes* on the Guiana shield and *C. monospermum* widespread from Peru across northern Bolivia and central Brazil. Of the 19 species originally described under *Malmea*, 12 were subsequently moved to three new genera described by Chatrou (1998) on the basis of leaf, inflorescence and seed characters: *Klarobelia* (now including 12 recognised species), *Mosannona* (14) and *Pseudomalmea* (3). *Malmea* now includes 6 recognised species. The distribution of species of *Klarobelia*, *Malmea*, and *Mosannona* (Fig. 1b, c & d) are markedly similar to those of *Cremastosperma*. In all four genera no single species is found either on both sides of the Andean mountain chain, or distributed across more than one of the further disjunct areas in the Guianas, Venezuela and tropical Andes. However, there are differences: *Mosannona* is distributed further into Central America, with one species found as far north-west as the Mexican


a
b

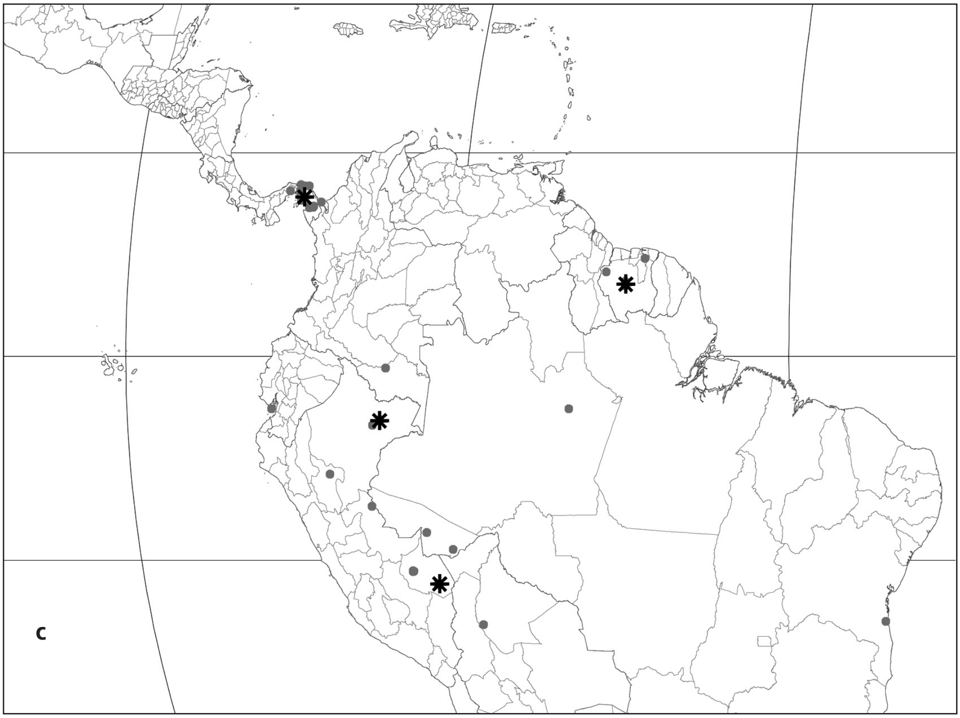


Fig. 1. **Distribution maps: Mollweide projection. Collections denoted by grey dots, location of those sampled for DNA by black asterisks. 1a: *Cremastosperma* 1b: *Klarobelia* (black star: *Pseudephedranthus fragrans*) 1c: *Malmea* 1d: *Mosannona***

states of Veracruz and Nayarit, and, as is also the case for *Malmea*, no species of *Mosannona* have been collected in coastal Venezuela. Further, the type species of *Malmea*, *M. obovata* is only known from one collection from the Atlantic coast of Brazil, and *M. manausensis* is found in the heart of the Amazon basin (Fig. 1c): in neither of these two areas have species of the other three genera been collected.

Phylogeny reconstruction in Annonaceae: are *Cremastosperma*, *Klarobelia*, *Malmea* and *Mosannona* monophyletic?

The informal names long branch clade (LBC) and short branch clade (SBC), have been applied to the two major, pan-tropically distributed clades comprising the majority of species in Annonaceae (see Fig. 2), reflecting the large difference in the rate of molecular change apparent as branch lengths representing inferred changes on phylogenetic trees (Richardson *et al.*, 2004; Pirie *et al.*, this thesis, Chapter 2). Phylogenetic analyses of Annonaceae using plastid *rbcL* and *trnL-F* (Mols *et al.*, 2004; Richardson *et al.*, 2004) and *rbcL*, *trnL-F* and *matK* (Pirie *et al.*, this thesis, Chapter 2) DNA sequence data placed *Cremastosperma*, *Klarobelia*, *Malmea* and *Mosannona*, and a number of other Neotropical genera with imbricate sepals, one basal, lateral, or apical ovule, and apocarpous fruits, in the SBC.

Phylogenetic analyses of Annonaceae presented by Richardson *et al.* (2004) and Pirie *et al.* (this thesis, Chapter 2) indicate that *Cremastosperma*, *Klarobelia*, *Malmea* and *Mosannona* are likely to represent monophyletic groups. However, the former study included limited taxon sampling within the four genera, and revealed only low support for monophyly of *Mosannona*. The latter study included extra sampling of both characters and taxa. The low support for *Mosannona* (Richardson *et al.*, 2004) is probably a result of insufficient character sampling. Increased sampling of species within these genera and their closest relatives, including taxa distributed outside the 'Andean-centred' regions is necessary.

The SBC phylogeny: Pantropical disjunct distribution patterns and their implications for biogeographic hypotheses in the Neotropics

Recent studies of angiosperm groups, including Annonaceae, previously regarded as Gondwanan distributed, have demonstrated using molecular dating techniques that these distributions may have originated later than the estimated timing of separation of its constituent continents (Renner *et al.*, 2001; Davis *et al.*, 2002; Richardson *et al.*, 2004). A combination of the presence of land connections and of suitable climatic conditions made a number of dispersal paths between currently isolated tropical zones available during periods since the break up of Gondwana (Morley, 2003; Pennington & Dick, 2004). The

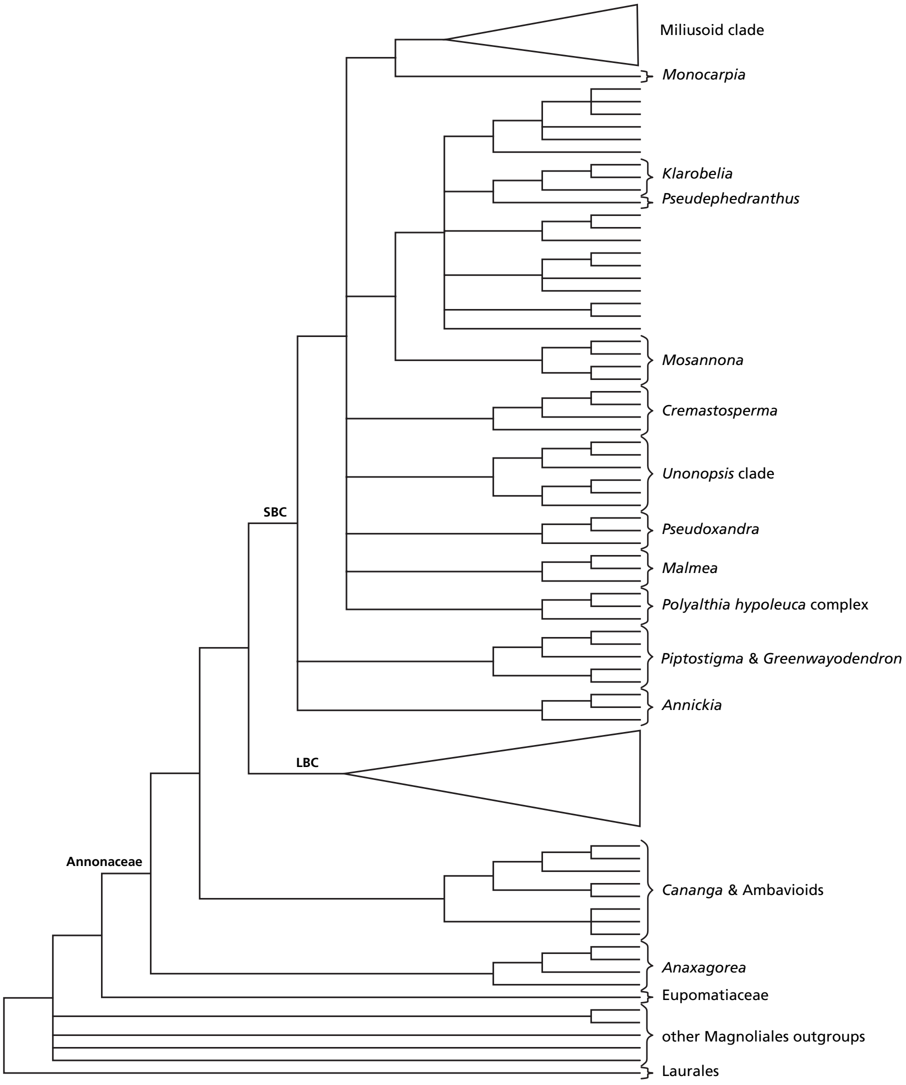


Fig. 2. **Phylogeny of the Annonaceae. Summary of maximum parsimony 50% bootstrap consensus topology adapted from Richardson et al. (2004).**

view that since its split from Africa around 100 mya the geological history of South America is that of an island continent, only recently connected to North America via the Panamanian isthmus in the Pliocene (Burnham & Graham, 1999) may thus underestimate the role of dispersal in the origin of pan-tropical disjunctions (Morley, 2003).

One proposed explanation for the pantropical distribution of clades within Annonaceae is through dispersal across the 'boreotropics'. This dispersal route, unlike the presumption of a common ancestor on Gondwana, would not necessarily involve an ancestral area in South America. The phylogeny of the

SBC and positions of *Cremastosperma*, *Klarobelia*, *Malmea*, and *Mosannona* within it has important implications for possible reconstructions of their biogeographical history. Levels of resolution on the basis of *rbcL* and *trnL-F* do not exclude the possibility that most of the Neotropical SBC genera together could represent a monophyletic group (Richardson *et al.*, 2004). Should in particular the *Cremastosperma* and *Malmea* clades be demonstrated to be sister to other South American centred clades this would indicate a common geographical origin (in the Neotropics) dating back at least as far as the MRCA of those groups. However, should such a Neotropical clade share a most recent last common ancestor with a (largely) Asian clade, then its Neotropical distribution could theoretically be as recent as the age of its crown group. The origin of its constituent species in South America would then need to be reassessed.

The importance of this question for Andean biogeography is that it is currently impossible to reject the possibility that *Cremastosperma* and/or *Malmea* originated in different geographic areas to *Klarobelia* or *Mosannona*: within or without the South American continent, with recent dispersal to South, or Central America followed by radiation. The similarity in their current distribution patterns might not reflect common biogeographic history, and the explanation of Gentry for their diversification, *i.e.* in response to the Andean orogeny, might even fall outside the time frame of their presence in South America.

Aims:

Gentry's explanation for the distribution of Andean-centred taxa was that they 1) originated in South America (*i.e.* Gondwanan) and 2) were subject to a recent burst of speciation as a result of the orogeny of the Andean mountain chain. This represents two hypotheses for the origins of *Cremastosperma*, *Klarobelia*, *Malmea* and *Mosannona* which can be tested using phylogenetic reconstruction and molecular dating techniques. The aims of this study are therefore firstly to determine the sister groups of the four genera and the age of these sister groups. This may help us determine the geographic origin of these Andean centred genera. Secondly, we aim to test the monophyly of each of these genera and also to determine the age of the crown group of each genus. This may help us to determine whether they have congruent evolutionary histories that may all have been affected by the Andean orogeny. Should the similar distribution patterns of these Andean-centred genera in Annonaceae be due to a common biogeographical history, then reconstructing that history might also offer insight into the origins of the high diversity of other taxa in north western South America. Alternatively, the species represented by these Annonaceae genera might have originated in different ways and/or over different periods of time.

Materials and Methods

Taxon sampling

This study largely utilised previously unpublished sequence data, as well as published sequences (Sauquet *et al.*, 2003; Mols *et al.*, 2004; Pirie *et al.*, this thesis, Chapter 2; Chatrou *et al.*, in prep.). Around half the total numbers of described and un-described species for each of the four genera under study were represented by fourteen samples of *Cremastosperma*, six of *Klarobelia*, four of *Malmea* and seven of *Mosannona*. Geographical distribution of specimens sampled is indicated on the distribution maps (see Fig. 1a–d). A total of 77 SBC taxa were sampled, including increased sampling of species representing all related South American genera as represented by Richardson et al. (2004), five Asian and Central American taxa representative of the miliusoid clade, including first-branching lineage *Monocarpia* (Mols *et al.*, 2004), three samples representing the Asian '*Polyalthia hypoleuca* complex' clade (Rogstad, 1989; Mols *et al.*, 2004) and eight representing the African genera *Greenwayodendron*, *Piptostigma*, and *Annickia*, first branching lineages of the SBC (Mols *et al.*, 2004; Richardson *et al.*, 2004). Major clades within the LBC and the first-branching lineages of Annonaceae, the 'Ambavioid' clade and *Anaxagorea* (Doyle & Le Thomas, 1996; Richardson *et al.*, 2004) were represented by sixteen accessions. Outgroups were selected from other families of the order Magnoliales; *Magnolia* and *Liriodendron* (Magnoliaceae) and *Coelocaryon* (Myristicaceae). Genbank accession numbers and voucher details are presented in Appendix A.

Character sampling

For all 96 accessions the chloroplast DNA markers *rbcL*, *trnL-trnF* and *psbA-trnH* were sampled (Matrix 1: see Appendix A), and for 23 of these 96 accessions the additional markers, *matK*, *ndhF*, *trnT-trnL*, *trnS-trnG* and *atpB-rbcL* (Matrix 2: see Appendix A). LBC accessions were excluded in Matrix 2, as high sequence divergence within this clade made homology assessment in the alignment of non-coding markers ambiguous. Having confirmed the best outgroups for the SBC in Matrix 1, both alignment problems and sequencing effort were minimised by the exclusion of more distant outgroups in Matrix 2.

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted using a modified cetyl trimethyl ammonium bromide (CTAB) method (Doyle & Doyle, 1987): 50 mg silica dried or herbarium leaf material was homogenised in 1300 µl CTAB and incubated for 20 minutes with 12 µl 2-mercaptoethanol at 65°C, followed by 90 minutes ambient mixing with 1 ml 24:1 chloroform: isoamylalcohol. After 10 minutes centrifuged at 13,000 rpm, 300 µl supernatant was purified using Wizard DNA purification system (Promega corp.) (*i.e.* without isopropanol precipitation, avoiding the co-precipitation of oxidised material; Savolainen et al., 1995).

PCR amplification conditions were modified depending on the qualities of the DNA sample available. Samples extracted from herbarium material often contain lower quantities of more fragmented DNA, and higher levels of PCR inhibiting compounds (Savolainen *et al.*, 1995). In most cases the *rbcL* gene was amplified in two pieces and sequenced using primers 1F/724R (Olmstead *et al.*, 1992) and 636F/1460R (Fay *et al.*, 1997; Fay *et al.*, 1998). Where amplification was unsuccessful further internal primers, 217F, 922F, 536R and 1104R (Pirie *et al.*, this thesis, Chapter 2), and 376R: 5'-GGGTTCAAAGC TCTACGAGCTCTACG-3' and 444F: 5'-GGTCCGCCCCATGGCATCC-3' were applied in combination 1F/536R, 217F/724R (or 1F/376R, 217F/536R and 444F/724R), 636F/1104R and 922F/1460R to amplify the gene in up to five overlapping pieces of between 300 and 500 bp long. Plant universal primers of Taberlet *et al.* (1991) were used to amplify separately and sequence the *trnL* intron (primers C/D) and *trnL-trnF* spacer (primers E/F). The *psbA-trnH* intergenic spacer was amplified and sequenced using primers psb A and trn H (GUG) (Hamilton, 1999). Partial *matK* sequences were amplified using primers 390F and 1326R (Cuénoud *et al.*, 2002), and MintF and MintR (Pirie *et al.*, this thesis, Chapter 2), in combination 390F/1326R or 390F/MintR and MintF/1326R and sequenced using primers 390F and 1326R. The *ndhF* gene was amplified and sequenced in two overlapping pieces using primers 1, 972 and 2110R (Olmstead & Sweere, 1994) and 1165R (Kim *et al.*, 2001) in combination 1/1165R and 972/2110R. The *trnT-trnL* intergenic spacer was amplified using primers A and B (Taberlet *et al.*, 1991), AintFCrem: 5'-CCGTTCCGGTATTCCAAATCGAGC-3' and ABintR: 5'-CGTTGATGTAT CCGCAATTCAATATG-3' in combination A/B or A/BintR and AintFCrem/B and sequenced using primers A and B. The *trnS-trnG* intergenic spacer was amplified and sequenced using primers trn S (GCU) and trn G (UCC) (Hamilton, 1999) and the *atpB-rbcL* intergenic spacer using primers atprbc3 (complimentary to S20 of Hoot *et al.*, 1995) and atprbc2 (Scharaschkin and Doyle, pers. com.).

A standard PCR protocol was used throughout, with the addition of 1 µl 0.4 % BSA per 25 µl reaction (which was found to increase amplification in all samples), 35 cycles of 30 sec.: 94°C; 1 min.: 55°C; 2 min.: 72°C, with an initial 4 min.: 94°C and final 7 min.: 72°C. PCR products were purified using QIAquick PCR purification kits (Qiagen), sequenced with the PCR primers, and analysed by electrophoresis using an automatic sequencer ABI 3730XL.

Phylogenetic Analysis

DNA sequences were edited in SeqMan 4.0 (DNAStar Inc., Madison, WI) and aligned manually, resulting in alignments of 1496 positions (*rbcL*, including a 34-41 aligned positions long non-coding region on the 3' end), 1402 positions (*trnL-F*), 798 positions (*psbA-trnH*), 843 positions (*matK*), 2043 positions (*ndhF*), 1157 positions (*trnT-trnL*), 953 positions (*trnS-trnG*) and 828 positions (*atpB-rbcL*). Areas of the alignments where the assessment of homology was ambiguous were excluded from the analyses.

Gaps in the alignments were coded as present/absent characters where they could be coded unambiguously, following Simmons and Ochoterena (2000). Two excluded regions, one in *psbA-trnH*, the other in *trnT-trnL*, of 15 and 12 positions respectively, appeared to represent inversions, with around half the accessions possessing almost exact reverse-compliment sequences of the others. Under the assumption that these inversions had occurred with high frequency, the bases in one version were aligned with those of the reverse compliment of the other. These characters displayed little or no homoplasy when optimised onto the bootstrap topologies and were therefore presumed to contain phylogenetic signal and included in further analyses.

Maximum parsimony (MP) analysis: Data were analysed using the parsimony algorithm of the software package PAUP* 4.0b10 (Swofford, 2000), under the equal and unordered weights criterion (Fitch parsimony; Fitch, 1971). The length of the shortest trees were estimated for Matrix 1 using the parsimony ratchet (Nixon, 1999) as implemented using PAUP* and PAUPRat (Sikes & Lewis, 2001). All shortest trees were calculated using the 'branch and bound' method for Matrix 2, and Bremer support (Bremer, 1994) estimated using the program TreeRot (Sorenson, 1999). Support was also estimated using bootstrap analyses of 500 replicates with 'full' heuristic searches of 50 random addition sequences, TBR, saving 50 trees each time. Bootstrap percentages were interpreted following Richardson et al. (2004): 50-74% represents weak support, 75-84% moderate support and 85-100% strong support. Bootstrap analyses were performed on Matrix 2 with and without the *rbcL* sequence data (see Results).

Selecting the best fitting DNA substitution model: ModelTest 3.06 (Posada & Crandall, 1998) was used to select the substitution model best fitting each sequence data partition, and the combined sequence data of Matrix 1, using an arbitrary most parsimonious tree topology as estimated above for Matrix 1 and the most parsimonious topology for Matrix 2.

Bayesian analysis: The combined datasets were also analysed using Bayesian inference, as implemented in MrBayes version 3.0 (Huelsenbeck, 2000). The data was partitioned according to the separate markers used and both rates and substitution models were allowed to vary across the partitions. Prior values for the DNA substitution models were applied to each partition (as determined using ModelTest above). Prior probabilities for all topologies were equal. *Coelocaryon preussii* (Myristicaceae, sister group to rest of Magnoliales; Sauquet *et al.*, 2003) was chosen as the single outgroup taxon permitted by MrBayes in Matrix 1, *Cleistopholis glauca* as outgroup for Matrix 2. MCMC analyses were run for 5,000,000 generations with four simultaneous MCMC chains to calculate posterior probabilities (PP) and one tree per 100 generations was saved. The burn-in values were determined empirically from the likelihood values and 50% majority rule consensus trees calculated together with approximations of the PP for the observed bipartitions.

Molecular dating

Topology: Nodes present in the Bayesian consensus of Matrix 1 and not contradicted by results from Matrix 2 were used to constrain two further MP searches of the sequence data of Matrix 1 ('full' heuristic, 100 random taxon addition sequences, TBR, saving maximum of 50 shortest trees each time), from which single arbitrary most parsimonious topologies were selected. The two searches included (A) all taxa (96 in total), and (B) all taxa minus nine accessions of *Cremastosperma*, three of *Mosannona*, and three of *Klarobelia*, thus leaving each of these clades, and that of *Malmea*, represented by four accessions (including those representing first branching lineages, thus ensuring that the crown nodes remained comparable), in order to explore possible bias in age estimations according to numbers of taxa sampled.

Fossil calibration: The oldest unambiguously identifiable fossil Annonaceae remains have been found in the Maastrichtian of Nigeria (seeds with perichalazal ring and ruminate endosperm; Chesters, 1955) and Colombia (reticulate monosulcate pollen; Sole de Porta, 1971). However, their precise placement on the Annonaceae phylogeny is ambiguous, due to the reticulate nature of the characters available for their identification. The fossil taxon *Archaeanthus* (Dilcher & Crane, 1984) was used following Doyle *et al.* (2004) and Richardson *et al.* (2004) to assign a minimum age of 98 mya to the stem node of Magnoliaceae (due to the distinctive stipules, elongate receptacle and fruits). This interpretation is not entirely uncontroversial: *Archaeanthus* has recently been explicitly excluded from age estimations in angiosperms by Crepet *et al.* (2004). Crepet *et al.* instead used two fossil flowers, *Cronquistiflora* and *Detrusandra* (Crepet & Nixon, 1998), to impose the more conservative minimum age of 90 mya on the Magnoliales. However, results of Doyle *et al.* (2004) and Richardson *et al.* (2004) broadly agreed with ages estimated in angiosperm wide studies (*e.g.* Wikström *et al.*, 2001; Davies *et al.*, 2004), which suggest the calibration of Crepet *et al.* may represent a (greater) underestimation of the true age.

In order to test whether the sequence data of Matrix 1 exhibited clocklike behaviour, a likelihood ratio test was performed on the first of the above (constrained) most parsimonious tree topologies. Likelihood of the data with and without constraint of a molecular clock, were calculated and the likelihood ratio statistic compared with χ^2 critical value with 94 degrees of freedom (*i.e.*, number of taxa minus 2).

Molecular dating using NPRS and PL: This substitution model selected using ModelTest was used to calculate branch lengths for the above topologies based on the original data using the maximum likelihood (ML) criterion as implemented in PAUP*. Confidence limits on branch lengths, reflecting stochasticity in the sampling of character changes ('substitutional noise'), were estimated by 100 replicates of bootstrap re-sampling (as also described in Wikström *et al.*, 2001), with subsequent ML branch length estimation on the constrained tree topology for each bootstrap replicate. This resulted in 100 trees comprising a range of estimated lengths for each branch of the topology.

Thereafter, Sanderson's methods of nonparametric rate smoothing (NPRS) (Sanderson, 1997) and penalized likelihood (PL) (Sanderson, 2002a) were applied as implemented in the software package r8s (Sanderson, 2002b) in order to estimate divergence times. Divergence times were estimated for nodes representing the MRCAs (*i.e.* the crown groups) of each of the four genera, and those representing the MRCAs of a number of further SBC sub-clades (see Table 3), with the Magnoliaceae stem lineage (*i.e.* the root node, once the initial outgroup has been pruned out in the analyses) fixed at 98 million years old. Analyses were performed on the trees with branch lengths derived from the original data, and for each of the 100 trees each with branch lengths derived from bootstrap re-sampled data. The results of the latter analyses were summarised giving mean values with standard deviation for specified nodes using the 'profile' command in r8s.

Molecular dating using a Bayesian technique: Bayesian molecular dating was performed following Rutschmann (2004), Renner (2004), and the PAML (Yang, 1997) and multidivtime (Thorne & Kishino, 2002) manuals. Nucleotide substitutions in the combined sequence data were estimated using PAML's 'baseml' program and the F84 + Γ model (with five rate categories), with the single topology as above. Using the multidivtime package, each baseml output was converted using 'pamlmodelinf' for input in 'estbranches', to estimate branch lengths and calculate the variance-covariance structure of those estimates. These were then used as input for 'multidivtime' to calculate node divergence times with the following settings: 100,000 generations of the Markov chain, sampled every 10 after a burn in of 10,000. Prior number of time units between tip and root: 98 (*i.e.* millions of years), SD of prior: 98, prior rate at root node: 0.0003 (derived from r8s results using PL), nu: 1, SD nu: 1, and the single constraint on node times was the same as the calibration in the r8s analyses as above.

Results

Matrix 1

Both 100 and 1000 iterations of the parsimony ratchet recovered trees of 2137 steps, CI =0.648, RI =0.777. Sequence and alignment lengths, numbers of variable and parsimony informative characters and tree statistics for Matrix 1 are presented in Table 1. The marker *rbcL* provided the lowest total number of parsimony informative characters, despite representing higher numbers of sequenced bases, and exhibited significantly higher levels of homoplasy compared with the other two markers.

Table 1: Details of Matrix 1, maximum parsimony search and best fitting substitution models

Marker	Sequence length	Alignment length	Variable characters	Pars. inf. characters	Pars. inf. indels	[d]CI/RI	Model
[b]*rbcL*	1470-1480	1496	310	187	0	0.519/0.712	GTR+I+Γ
trnL-trnF	912-996	[a]1402	454	270	30	0.729/0.810	K81uf+Γ
psbA-trnH	([c]274) 412-511	798	340	215	10	0.689/0.812	TVM+Γ

[a]Alignment length of Chatrou *et al.*; [b]Including 3' non coding region; [c]*Annona muricata* 0525: large deletion; [d]Optimised onto the combined topology

The best fitting substitution model for all sequence data of matrix 1 estimated using ModelTest was GTR+I+Γ. Best fitting models for each marker individually are presented in Table 1. MP bootstrap analysis and Bayesian inference of Matrix 1 resulted in congruent consensus topologies, that of Bayesian inference being significantly more resolved. Both results are summarised in Fig. 3, with bootstrap support (BS) values below the nodes and posterior probabilities above. Monophyly of *Cremastosperma*, *Malmea*, and *Mosannona* were confirmed by 100% BS. A clade including all accessions of *Klarobelia* plus *Pseudephedranthus* received 90% BS. Further clades supported by >50% BS correspond largely to those revealed by Richardson et al. (2004), except for a clade including all the 'South American centred' SBC taxa (except a single accession, unidentified to genus, falling with 100% BS within the miliusoid/*Monocarpia* clade) with 63% BS. This will be further referred to as the 'SAC clade'. Sampling of taxa within the miliusoid clade was not sufficient

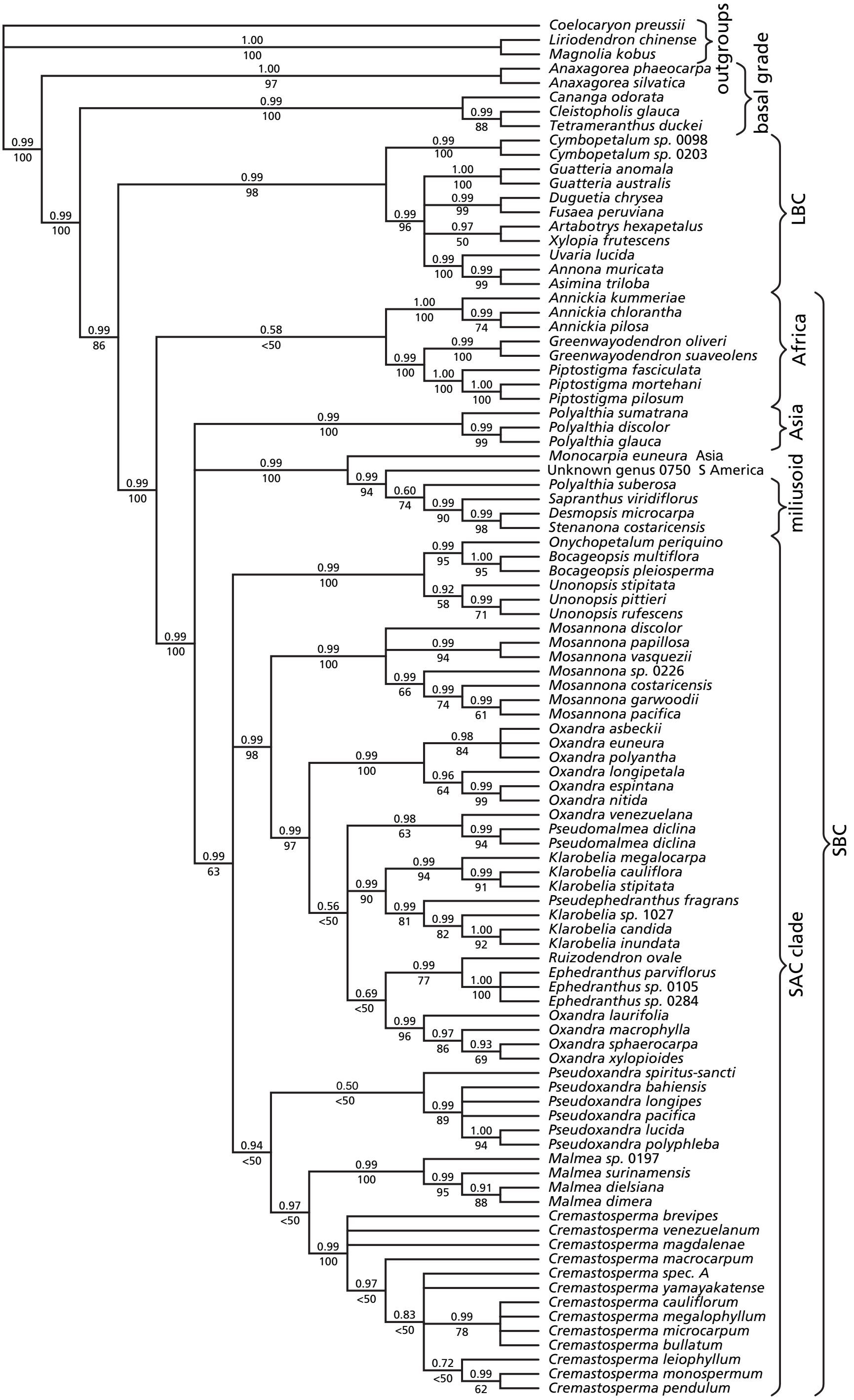


Fig. 3. **Summary of results of phylogeny reconstruction using maximum parsimony and Bayesian analysis: Matrix 1. MP bootstrap support values below the nodes, Bayesian analysis posterior probabilities above.**

to further infer the position of the unidentified accession, and it was omitted from further character sampling. One clade, that of *Malmea* as sister group to *Cremastosperma*, received 97% posterior probability, but less than 50% BS (see results of Matrix 2 below).

Matrix 2

MP branch and bound search of Matrix 2 excluding *rbcL* sequence data resulted in a single tree of 1555 steps, CI =0.871, RI =0.791, presented in Fig. 4 (with posterior probabilities above the nodes and BS values and Bremer support below). Inclusion of *rbcL* resulted in 3 trees of 1754 length, CI =0.851, RI =0.761. Sequence and alignment lengths, numbers of variable and parsimony informative characters and tree statistics for Matrix 2 are presented in Table 2. Levels of homoplasy in *rbcL* were higher than those of all the other markers. The CI values for *rbcL*, *trnL-trnF* and *psbA-trnH* were higher in Matrix 2 than in Matrix 1, which is to be expected given the lower taxon sampling density: less homoplasy is revealed. Values for RI were lower in all three markers in Matrix 2, but that for *rbcL* decreased markedly. Analyses were thus performed on Matrix 2 with and without the *rbcL* sequence data, and results compared.

Table 2: Details of Matrix 2, maximum parsimony search and best fitting substitution models

Marker	Sequence length	Alignment length	Variable characters	Pars. inf. characters	Pars. inf.indels	[f]CI/RI	Model
[c]*rbcL*	1470-1480	[a]1496	127	59	0	0.688/0.554	TrN+I+Γ
matK	843	843	142	69	0	0.881/0.833	K81uf+Γ
ndhF	2043	2043	370	170	0	0.840/0.763	K81uf+Γ
trnT-trnL	641-947	1157	178	68	8	0.877/0.745	TVM+Γ
trnL-trnF	[b]912-996	[a]1402	174	64	7	0.907/0.816	K81uf+Γ
psbA-trnH	([e]274) 412-511	[b]798	120	50	1	0.838/0.782	K81uf+Γ
trnS-trnG	([d]265-) 643-851	953	134	50	0	0.890/0.769	TIM+Γ
atpB-rbcL	745-770	828	135	61	4	0.918/0.874	K81uf+I

[a]Alignment length of Chatrou *et al.*; [b]Alignment/length derived from Matrix 1; [c]Including 3' non coding region; [d]*Malmea dielsiana* 0260: large deletion; [e]*Annona muricata* 0525: large deletion; [e]Optimised onto the combined topology

Best fitting substitution models for each marker as estimated by ModelTest are presented in Table 2. Bootstrap analysis of Matrix 2 resulted in a fully resolved consensus tree, though one node received only weak support (see Fig. 4). The SAC clade received strong support (87% BS). A sister group relationship between *Pseudoxandra* and *Cremastosperma* was also strongly supported (100% BS), contradicting the results of Bayesian (but not of MP) analysis of Matrix 1. The bootstrap consensus when including *rbcL* included two polytomies. The two nodes with Bremer support of only 1 step without *rbcL* (and subject to moderate to weak BS) were no longer recovered when

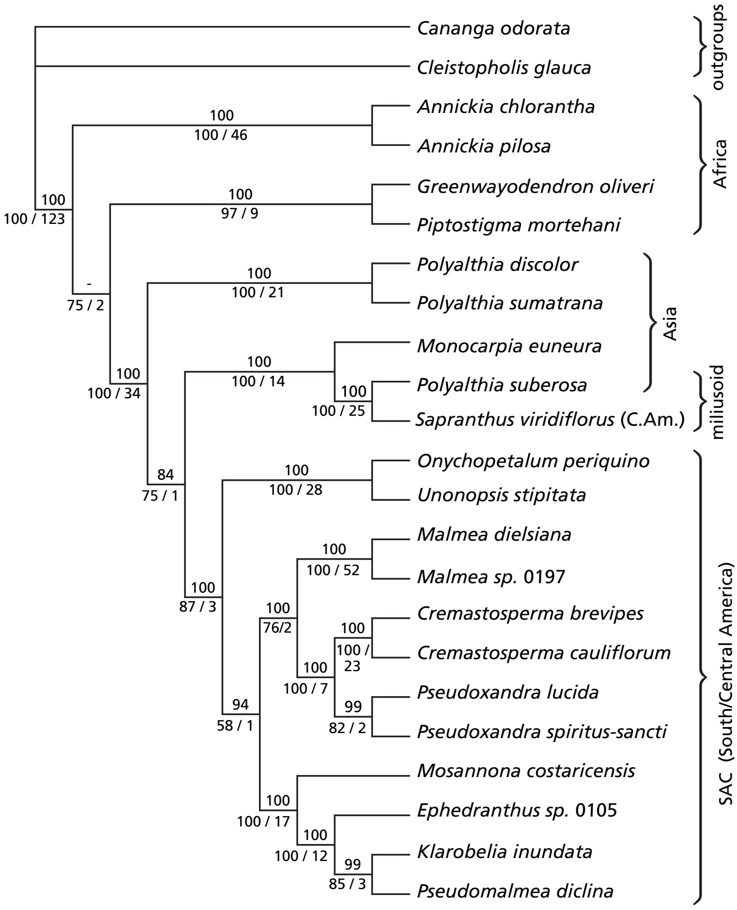


Fig. 4. Summary of results of phylogeny reconstruction using maximum parsimony and Bayesian analysis: Matrix 2 (excluding *rbcL* sequence data). Single most parsimonious topology presented with MP bootstrap and Bremer support values below the nodes, Bayesian analysis posterior probabilities above.

rbcL was included. These were the node of the *Mosannona/Klarobelia* clade as sister to the *Cremastosperma/Malmea/Pseudoxandra* clade (58% BS), and that of the miliusoid/*Monocarpia* clade as sister to the SAC clade (75% BS). BS values across the topology were slightly lower when *rbcL* was included (data not shown). Bayesian analysis recovered a congruent topology, though with one polytomy: relationships between the two African clades and the rest of the ingroup were not resolved.

Molecular dating

The likelihood of the sequence data of Matrix 1 given the constraint topology were significantly different according to clock constrained and unconstrained substitution models ($P<0.01$), indicating rate heterogeneity *i.e.* the rejection of a molecular clock. NPRS, PL and Bayesian (using multidivtime) methods were thus applied to produce full (A) and reduced (B) taxon sampled ultrametric trees. The cross validation test for PL resulted in a smoothing parameter of 31.62. Maximum ages for the MRCAs of the four genera were estimated at *ca.* 20–27 mya using NPRS, 13–16 mya using PL, and 23–28 mya (95% probability) using multidivtime. The maximum age estimated for the Asian/Neotropical crown group was *ca.* 42 mya (NPRS), 37 mya (PL) and 51 mya (multidivtime – 95% probability). Age estimations with standard deviations using NPRS and PL, and mean age estimations with standard deviations and 95% confidence limits derived using multidivtime are presented in Table 3 for key nodes referred to in the discussion and a chronogram for the SBC (based on results using PL) with timing of geological events referred to in the discussion is presented in Fig. 5 (see below).

The bounds for age estimations, according to standard deviations or 95% confidence limits, overlapped for all for nodes compared across the three different methods, and both taxon selections. For results using NPRS and multidivtime all age estimations for nodes including all 96 taxa (A) were older than those produced when only 81 were included (B). The largest apparent difference was observed in the *Cremastosperma* crown node, from which clade the largest number of accessions had been excluded in (B). In contrast, ages produced for the two taxon selections using PL were almost identical, with no consistent difference apparent. More rigorous tests are required to determine whether the results of any of these methods are sensitive to levels of taxon sampling. In this study the widest bounds of the age estimations produced using the different methods are interpreted as the most stringent test for the biogeographic hypotheses.

Table 3: Ages estimations derived using DNA sequence data of Matrix 1 and the nonparametric rate smoothing (NPRS), penalized likelihood (PL) and Bayesian (Multidivtime) methods: (A) including all 97 taxa and (B) including only 4 samples each of *Cremastosperma*, *Mosannona* and *Klarobelia* (82 taxa in total)

	NPRS (A)	NPRS (B)	PL (A)	PL (B)	Multidivtime (A)	Multidivtime (B)
Cremastosperma	**22.33** (S.D. = 4.99)	**13.65** (S.D. = 4.99)	**7.17** (S.D. = 8.53)	**7.54** (S.D. = 5.83)	**16.49** (S.D. = 5.14 95% = 8.01 - 27.73)	**9.07** (S.D. = 3.84 95% = 3.45 - 18.20)
Malmea	**15.90** (S.D. = 4.23)	**13.09** (S.D. = 3.29)	**9.54** (S.D. = 5.35)	**9.88** (S.D. = 5.02)	**13.42** (S.D. = 4.97 95% = 5.49 - 24.76)	**10.47** (S.D. = 4.04 95% = 4.28 - 19.94)
Klarobelia	**15.96** (S.D. = 3.31)	**10.71** (S.D. = 3.01)	**7.69** (S.D. = 5.20)	**7.46** (S.D. = 4.16)	**12.51** (S.D. = 4.59 95% = 5.25 - 23.12)	**9.25** (S.D. = 3.58325 95% = 3.72 - 17.64)
Mosannona	**17.38** (S.D. = 3.28)	**10.22** (S.D. = 2.72)	**8.62** (S.D. = 4.45)	**7.88** (S.D. = 3.77)	**13.58** (S.D. = 4.87 95% = 6.08 - 24.85)	**10.18** (S.D. = 3.82 95% = 4.62 - 19.22)
***Mosannona* west of Andes (1)**	**12.67** (a)	**-**	**6.04** (a)	**-**	**8.76** (S.D. = 3.76 95% = 3.26 - 17.78)	**-**
***Mosannona* east of Andes (2)**	**11.98** (a)	**-**	**5.27** (a)	**-**	**5.97** (S.D. = 3.19 95% = 1.35 - 13.69)	**-**
***Mosannona* / *Klarobelia* clade**	**31.16** (S.D. = 2.95)	**25.57** (S.D. = 2.60)	**19.42** (S.D. = 8.21)	**19.60** (S.D. = 7.91)	**26.40** (S.D. = 6.08 95% = 16.13 - 39.81)	**22.15** (S.D. = 5.40 95% = 12.89 - 33.82)
SAC clade (node 'A')	**37.65** (a)	**33.09** (a)	**24.76** (a)	**25.43** (a)	**31.81** (S.D. = 6.23 95% = 21.23 - 45.44)	**26.66** (S.D. = 5.82 95% = 16.21 - 39.20)
Neotropics / Asia (node 'B')	**39.23** (S.D. = 3.15)	**34.87** (S.D. = 2.77)	**26.29** (S.D. = 9.64)	**27.07** (S.D. = 9.11)	**36.72** (S.D. = 6.60 95% = 25.29 - 50.93)	**31.70** (S.D. = 6.35 95% = 20.25 - 44.94)
***Piptostigma* / *Greenwayodendron* / Neotropics / Asia (node 'C')**	**48.44** (a)	**44.78** (a)	**34.89** (a)	**35.63** (a)	**42.76** (S.D. = 6.83 95% = 30.81 - 56.62)	**38.13** (S.D. = 6.64 95% = 25.83 - 51.833)

[a] No S.D. calculated for nodes subtended by zero-length branches in any of the bootstrap replicate trees

Discussion

'Backbone' phylogeny of the SBC

The resolution obtained in this study suggests that sampling a larger number of characters for a careful selection of placeholder taxa would be an efficient approach for resolving relationships between further clades identified on the basis of wider taxon sampling in Annonaceae. A number of previously unidentified clades were discovered: sister group relationships between *Cremastosperma* and *Pseudoxandra* and between the *Cremastosperma/Pseudoxandra* clade and *Malmea*, and the SAC clade. The latter result in particular has important implications: in the first instance, optimisation of ancestral areas results in the stem nodes of each of the clades forming the SAC clade resolving as Neotropical. Consequently, the minimum age which can be inferred for the presence of their ancestral lineages in the Neotropics is greatly constrained.

However, despite the large amount of DNA sequence data analysed, the numbers of characters supporting the 'backbone' nodes were low. Two nodes in particular are subject to weak or moderate support and no longer recovered when *rbcL* was included in the analyses: the node of the *Mosannona/Klarobelia* clade as sister to the *Cremastosperma/Malmea/Pseudoxandra* clade and that of the miliusoid/*Monocarpia* clade as sister to the South American centred clade. The latter uncertainty effectively renders the optimisation of ancestral areas for the nodes subtending the Asian and SAC clades ambiguous, increasing the maximum possible age for the presence of the SAC clade in the Neotropics (see below and Fig. 5).

Biogeographic history in the SBC of Annonaceae: tracking the origins of Andean-centred genera on the South American continent

The oldest estimations of the age of the SBC crown group produced here (58.76 mya), in agreement with estimations of Richardson et al. (2004) (53.1-62.5 mya s.d. 3.6) were significantly younger than the timing of the Africa-South America break-up (ca. 100 mya; Burnham & Graham, 1999). The age of the MRCA of the SAC clade (Fig. 5, indicated by an arrow and 'A'), estimated at between around 45 and 16 mya here, represents the time after which this lineage can be said with certainty to have been present in the Neotropics. According to the SBC topology presented here, the actual age of the first Neotropical ancestor of the SAC clade could date back as far as the SAC clade stem node (Fig. 5 'B': 51-16 mya). However, should the miliusoid/*Monocarpia* clade prove instead to be sister to the *Polyalthia hypoleuca* clade, this would push this age back as far as that of the *Piptostigma/Greenwayodendron*/Neotropics/Asia node (Fig. 5 'C': 57-26 mya).

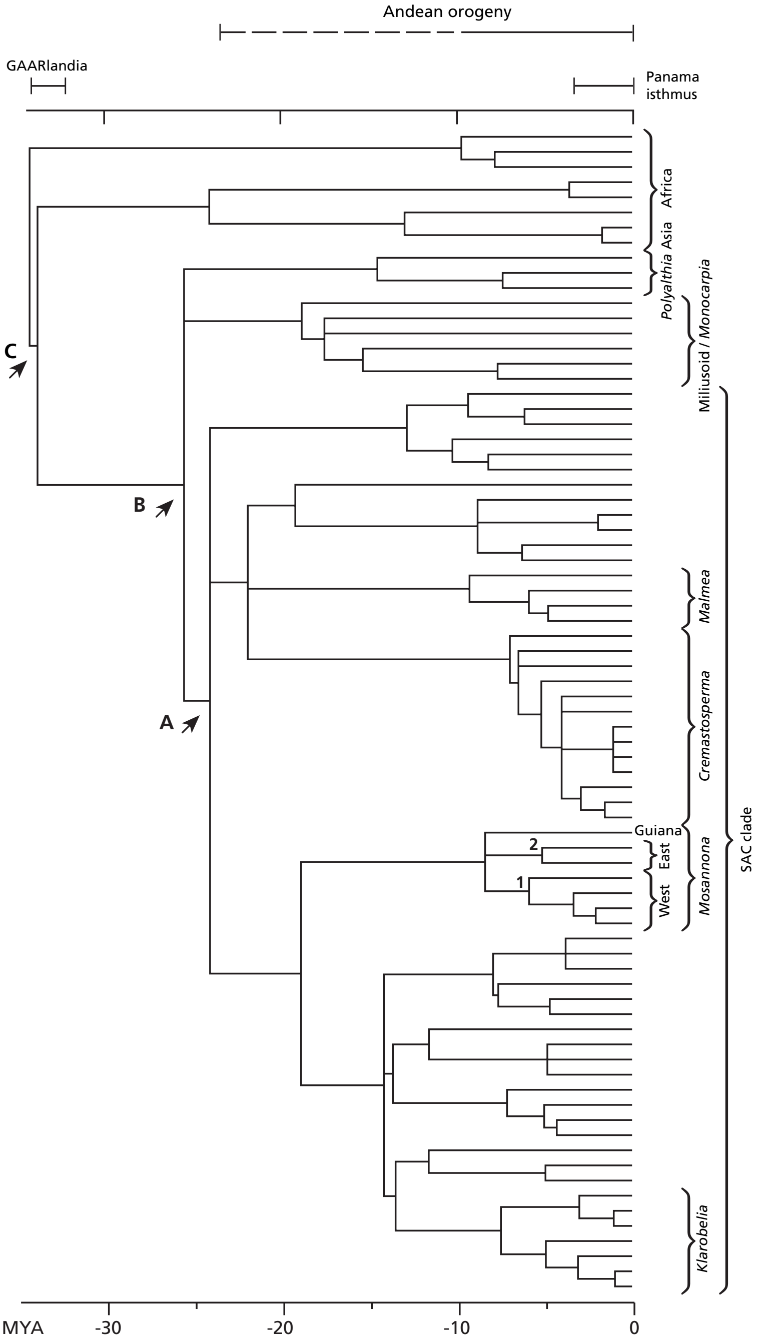


Fig. 5. **Chronogram: branch lengths proportional to time, as estimated using PL. Nodes A, B and C represent minimum ages of a common Neotropical ancestor of the SAC clade according to different topologies as referred to in the text. Nodes 1 and 2 represent the clades in *Mosannona* found west and east of the Andes respectively. Ages are subject to standard deviations (where estimated) of 4.45-9.64 my.**

Neither of these maximum ages are ancient enough to allow explanation of the distribution of the major clades within the SBC through either the splitting of west Gondwana or transatlantic dispersal from Africa to South America (which according to Morley (2003) may have been possible across island chains up to around 76 mya). Furthermore, the corresponding minimum ages are older than the first point at which North and South America were directly connected by the present day Central American land bridge (Fig. 5) (3.5–3.1 mya; Burnham & Graham, 1999).

If these conclusions are interpreted as support for a Boreotropical dispersal of SBC subclades, then South American origin of the SAC clade would have to imply dispersal between North and South America prior to the closure of the Panama isthmus. Both animal and plant fossil evidence suggest that this may have been possible (Morley, 2003). The plate-kinematic model of Pindall *et al.* (1988), is interpreted to suggest two windows of opportunity for dispersal associated with the formation of island chains at the leading edge the east-drifting Caribbean plate (Morley, 2003; Pennington & Dick, 2004). Firstly, the proto-Greater Antilles formed a bridge between Yucatán and Colombia, *ca.* 50 mya, which was subsequently fractured as the plate drifted further east. Secondly, a land mass including the Greater Antilles and Aves Ridge (GAARlandia; Iturralde-Vinent & McPhee, 1999) formed around 35–33 mya which may have provided a dispersal route for around 3 million years, before fragmenting to form the present day Caribbean islands (Morley, 2003). The Panama isthmus itself formed in the Pliocene from the island arc associated with the trailing edge of the Caribbean plate (Pindall *et al.*, 1988).

These two pre-Panama dispersal opportunities appear not to have been utilised by ancestors of the genera *Desmopsis*, *Sapranthus*, *Stenanona*, and *Tridimeris*, which are nested with high support within the otherwise almost exclusively Asian miliusoid clade (Mols *et al.*, 2004). Both *Sapranthus* and *Tridimeris* are endemic to Central America, as are most species of *Desmopsis* and *Stenanona*: the few South American species of these genera are found only on the Pacific coast of Colombia (P. Maas, pers. comm.), apparently limited in their distribution into South America by the barrier presented by the Andean mountain chain (see below). Clades within the SAC clade, such as those of *Cremastosperma*, *Klarobelia*, *Malmea*, or *Mosannona*, are diverse in South America. If they were to have originated in Central America and dispersed south across the Panama isthmus (*i.e.* within the last 3.5 million years), it would be difficult to explain why those miliusoid clades did not. More data is needed to get more accurate dates for the divergences of Central American taxa, and thus better to understand how the distributions of SBC taxa were affected by pre-Panama isthmus land-bridges. However, in the absence of a fully sampled and completely resolved phylogeny of the SAC clade, we consider these results to provide convincing support for a common ancestor of

Cremastosperma, *Klarobelia*, *Malmea*, and *Mosannona* having been present on the South American continent.

Monophyly of *Cremastosperma*, *Klarobelia*, *Malmea*, and *Mosannona*

In general monophyly among genera sampled was confirmed. A possible exception was the genus *Oxandra*, the accessions of which fell within two clades plus one isolated lineage. Monophyly was confirmed in *Cremastosperma*, *Malmea* and *Mosannona*. *Klarobelia* proved broadly monophyletic, with the exception of a single accession. *Pseudephedranthus fragrans*, representing a monotypic genus from the upper Rio Negro on the Brazil/Venezuela/Colombia border, was nested within *Klarobelia*. It was sister to a clade of species not represented by Richardson *et al.* (2004) which together forms the sister group to the rest of *Klarobelia*, thus explaining why its position had not been previously discovered. This result is however curious, given the morphological characters (such as impressed as opposed to raised midrib and open rather than closed bud development) which otherwise appear to represent synapomorphies for *Klarobelia* to the exclusion of *Pseudephedranthus*, and also warrants further investigation. The 'Andean centred' distribution patterns were thus not contradicted, though the unique distribution of *Pseudephedranthus* falls between those of the disjunct localities of other species in the tropical Andes and coastal Venezuela.

Progress towards reconstructing the biogeographic history of *Cremastosperma*, *Klarobelia*, *Malmea*, and *Mosannona*

One biogeographical hypothesis concerning Andean centred groups such as *Cremastosperma*, *Klarobelia*, *Malmea*, and *Mosannona* was that speciation in these groups occurred as a result of the Andean orogeny. The inferred presence of ancestors of the four clades on the South American continent prior to the closure of the Panama isthmus, and confirmation of their distribution patterns through testing monophyly of the accessions of these taxa sampled, failed to reject this hypothesis. A further test was applied using molecular dating techniques to examine the possibility that diversifications in these groups occurred within the time frame of the elevation of the Andes. Burnham & Graham (1999) considered the Andean orogeny to have been an influence in the history of Neotropical vegetation since the Miocene. This would place the effective timeframe for diversifications that might have been associated with the Andean orogeny at anywhere between around 23.3 mya and the present. However, estimations of paleoelevation of the Central and Colombian Andes suggest that much of the Andean uplift occurred in the late Miocene and Pliocene (Gregory-Wodzicki, 2000), when elevations increased by more than 3,500 m (Lundberg & Chernoff, 1992). The central Andes had reached no more than half its modern elevation by 10 mya and the Eastern Cordillera of the Colombian Andes was at no more than 40% of its modern elevation by 4 mya (Gregory-Wodzicki, 2000).

Molecular dating results produced here showed the ages of MRCAs of each of the four genera to fall within roughly the same wide time window (the oldest dates we estimated are those using NPRS that give ages of from 22.33 +/- 4.99 mya to 15.96 +/- 3.31 mya). The Multidivtime and PL methods suggest even younger ages for diversification in these genera that even more clearly fall within the timeframe of Andean orogeny. These dates are consistent with suggestions by Burnham and Graham (1999) that the Andean orogeny has had an effect on the history of Neotropical vegetation since the Miocene. However, imprecision in the results means that we cannot exclude the possibility that speciation within each of the four clades actually occurred during rather different time slices.

That no one species of any of these four genera is to be found on both sides of the Andes provides compelling evidence that the current elevation of the northern Andes forms an effective barrier to dispersal in these groups. Furthermore, preliminary results in *Mosannona* presented here show most of the species sampled to fall into two clades, one from west and one east of the Andes (see Figs 3 and 5). If the north-Andean uplift represents a vicariance event common to this, and other SAC clade genera, then this should be reflected in their species phylogenies, with congruent timing of subsequent divergences. The age of the divergence between *Mosannona* clades on the west and on the east of the Andes (represented by nodes 1 and 2 in Fig. 5, and in Table 3) could be as early as 15 mya, or rather more recent according to our estimates, which is consistent with the limitation in elevation below which species of the SAC clade are found (mostly 1,500 m, very rarely up to around 2,000 m), which would probably place a vicariance explanation for speciation in these groups no earlier than the Pliocene. The absence of miliusoid clade taxa east of the Andes would suggest the timing to have been prior to the closure of the Panama isthmus. However, alternative (or additional) causes of speciation (or indeed extinction) in these groups could have been linked to distribution shifts along the Andean elevational range during climatic changes in the Pleistocene (Hooghiemstra & van der Hammen, 1998).

Conclusions

We consider the fully resolved (though not in all nodes highly supported) topology presented here to represent a credible hypothesis of phylogenetic relationships between the major clades of the SBC – but one which should be further tested with independent data.

The identification of a clade comprising all the SBC genera distributed in South America and mostly only to a limited extent into Central America (the SAC clade), to the exclusion particularly of Asian and Central American endemic clades, suggests a common origin of the SAC clade in South America. Origin of the SAC clade in South America as a result of dispersal across the Boreotropics is supported by the age estimations presented here rather than Gentry's hypothesis of origin by Gondwanan vicariance. Broad monophyly of the four genera leads us to conclude that the distribution patterns as observed are not the arbitrary result of the definition of poly- or paraphyletic groups. The ages estimated for the MRCA of each clade were not significantly different from each other and appear to fall within the time frame of the orogeny of the Northern Andes, though the strength of this test was limited by imprecision in the molecular dating results.

Further testing these biogeographic hypotheses requires the reconstruction of species level phylogenies of *Cremastosperma*, *Klarobelia*, *Mosannona* and *Malmea*. Further work should be concentrated both on finding further age calibration points for the Annonaceae phylogeny and on assessing sources of error in the techniques used to derive ultrametric trees. This approach could shed further light on the dynamic processes of invasion of Central America (Chatrou, 1997), and the origin of high species diversity in tropical America.

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Chapter 4

An ancient paralogue of the cpDNA *trnL* (UAA)-*trnF* (GAA) region in Annonaceae and its application in phylogeny reconstruction

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Abstract

Phylogenetic signal of *trnL-F* in Annonaceae conflicts with that of other chloroplast markers with respect to the position of the Neotropical genus *Unonopsis*. This conflict is shown to be the result of preferential amplification of a paralogous copy of the *trnL-F* region, pseud*trnL-F*, which diverged from *trnL-F* in a direct ancestor of the Annonaceae. The pseud*trnL-F* region evolves at a faster rate than *trnL-F*. Although the exon appears intact, some pseud*trnL-F* intron sequences show signs of disruption of the secondary structure otherwise conserved across land plants. The pseud*trnL-F* region may have phylogenetic utility for some groups of Annonaceae, but until its position in the Annonaceae genome is determined the phylogenetic signal it contains will have to be interpreted with caution.

Keywords: Annonaceae, Magnoliales, *Unonopsis*, conflict, paralogy, lineage sorting, *trnL-F*.

Abbreviations: BS (bootstrap support); LBC (long branch clade); SBC (short branch clade); SAC clade (South American centred clade); MP (maximum parsimony); ML (maximum likelihood); MRCA (most recent common ancestor); PL (penalized likelihood); PP (posterior probability).

Introduction

For the last decade DNA sequences from the chloroplast genome have been widely exploited as an invaluable source of characters for phylogenetic inference in plants. This is largely due to their uniparental mode of inheritance and effective lack of recombination. Phylogenies inferred using chloroplast DNA (cpDNA) sequence data may or may not reflect species phylogenies (Nichols, 2001), but they can in any case be regarded as gene trees for which the congruence with organismal history becomes greater with increasing time scale (Clegg & Zurawski, 1992).

For practical purposes, polymerase chain reaction (PCR) amplification of cpDNA markers is made easier by the mostly large numbers of chloroplasts present in each plant cell. In a solution of total extracted DNA target sequences have higher effective copy number than those of most markers located in the cell nucleus. The practical application and patterns of evolution of different potential cpDNA markers are increasingly well understood. Not only have a number of complete chloroplast genomes been sequenced (*e.g.* Notsu *et al.*, 2002), but regions within the chloroplast have been sequenced for a broad selection of plants (*e.g.* Small *et al.*, 1998; Shaw *et al.*, 2005).

The chloroplast encoded *trnT-F* region

The cpDNA *trnL-F* region in land plants consists of the transfer RNA genes *trnT*$_{\mathrm{UGU}}$, *trnL*$_{\mathrm{UAA}}$ and *trnF*$_{\mathrm{GAA}}$ arranged in tandem, separated by non-coding spacer regions. It is positioned in the large single copy region, approximately 8kb downstream of *rbcL*. The *trnL* gene of cyanobacteria and a number of chloroplast genomes, including that of all land plants, contains a group-I intron positioned between the U and the A of the UAA anticodon loop. This intron is inferred by phylogenetic analysis to have been present in the cyanobacterial ancestor of the plastid lineages of Rhodophyta, Chlorophyta and Glaucocystophyta and subsequently vertically transmitted (Besendahl *et al.*, 2000).

The succession of conserved *trn* genes and the apparent absence of gene rearrangements in the *trnT-F* region facilitated the design of plant universal primers by Taberlet *et al.* (1991), since which in particular the *trnL* intron and *trnL-F* spacer (together, the *trnL-F* region) has become one of the most widely used chloroplast markers for phylogenetic analyses in plants (Quandt *et al.*, 2004). The accumulation of an increasingly large body of sequences of the *trn(T-)L-F* region from a wide range of plants has allowed further study of its structures, functions and evolution (*e.g.* in different orders of flowering plants: Bakker *et al.*, 2000; in basal angiosperms: Borsch *et al.*, 2003; in land plants: Quandt *et al.*, 2004; in bryophytes: Quandt & Stech, 2004).

Use of *trnL-F* in phylogeny reconstruction in Annonaceae

Sequences from the *trnL-F* region (not including the *trnT-L* region or trnL 5' exon) have recently been used in combination with those from further chloroplast markers *rbcL* and *matK* as a source of characters for phylogeny reconstruction in the tropical flowering plant family Annonaceae (Sauquet *et al.*, 2003; Mols *et al.*, 2004; Richardson *et al.*, 2004; this thesis, Chapter 2). The majority of species of Annonaceae fall within two large clades (Richardson *et al.*, 2004). The informally named 'long branch clade' (LBC) represents around 1,500 of the total 2,500 species and is sister group to the 'short branch clade' (SBC), which itself represents a further 700 species (Fig. 1). The remaining species fall within two clades forming a basal grade (this thesis, Chapter 2). As is to be expected from different sequences sampled from the plastid genome (Chase & Cox, 1998), these markers were shown to contain complementary phylogenetic signal, and were thus applied in combined analyses which delivered more resolved phylogenies, subject to higher levels of support, than those derived from each marker individually.

In contrast to this result, subsequent maximum parsimony analysis of sequences of the *trnL-F* region for additional accessions resulted in a position of the Neotropical genus *Unonopsis* conflicting with that derived from other cpDNA markers.

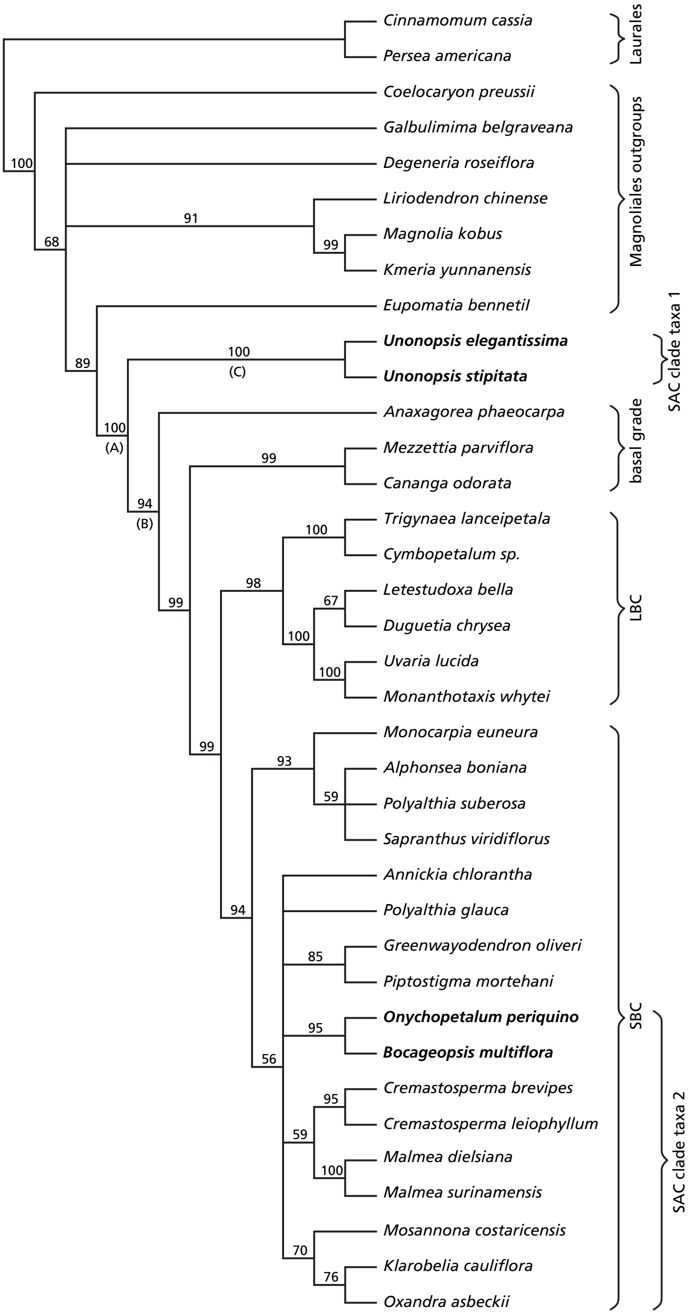


Fig. 1: **Maximum parsimony 50% majority rule bootstrap consensus tree resulting from analysis of the entire *trnL-F* region for a selection of Annonaceae, other Magnoliales, and Laurales outgroups. Bootstrap support is indicated above the nodes. Nodes labelled A, B and C are those defining *Unonopsis* as a monophyletic sister group to Annonaceae.**

Conflicting results in the placement of *Unonopsis* in Annonaceae *trnL-F* phylogeny

Unonopsis has been grouped with two smaller South American genera, *Bocageopsis* and *Onychopetalum* (comprising 4 species each to the 38 of *Unonopsis*), on the basis of morphological similarity (they share small, usually whitish flowers with valvate sepals and petals, and a low number of pitted, flattened seeds in lateral position with a canaliculate raphe and spiniform ruminations;Van Heusden, 1992;Van Setten & Koek-Noorman, 1992). However, monophyly of *Unonopsis* with respect to *Bocageopsis* and *Onychopetalum* is far from certain, and that of each of the three has yet to be stringently tested. In order to attempt this test, and as part of wider phylogenetic research in Annonaceae, we compared sequences from the *trnL-F*, *rbcL*, *matK* and *psbA-trnH* regions for a number of samples of each of the three genera.

Inclusion of the resulting *trnL-F* sequences in phylogenetic analysis with a selection of Annonaceae, other Magnoliales and Laurales *trnL-F* sequences (as used by Mols *et al.*, 2004; Richardson *et al.*, 2004) resulted in a monophyletic *Unonopsis* as sister group to the rest of the Annonaceae (Fig. 1). This result was in direct conflict with the other plastid DNA sequence data. Phylogenetic analysis of multiple chloroplast markers supports monophyly of the *Unonopsis/Bocageopsis/Onychopetalum* clade, placed with high support within the South American centred clade (SAC clade), itself nested within the SBC (this thesis, Chapter 3).

A number of potential problems in phylogeny reconstruction can lead to apparently incongruent results. In particular, weak signal in the data, failings of the inference method under particular conditions, and incorrect assessment of homology - either in sequence alignment, or due to paralogy (Sanderson & Shaffer, 2002). No such immediately obvious errors were apparent. The position of *Unonopsis* in the *trnL-F* phylogeny received high bootstrap support. The alignment of sequences was unambiguous. No unusually large insertion/deletions were observed, nor was the subtending branch representing changes in the two *Unonopsis* sequences unusually long.

The chloroplast genome is rarely subject to recombination. In the absence of recombination, incongruence between the phylogenetic signal of different chloroplast markers could be caused by paralogy, leading to lineage sorting. The node of the most recent common ancestor (MRCA) of putatively orthologous and paralogous sequences in the *trnL-F* (gene) phylogeny would represent the divergence of the two paralogues. This itself could represent the duplication of a region including *trnL-F*. It could alternatively represent a speciation event, followed by hybridisation with an unknown extra-Annonaceae Magnoliales lineage, and heteroplasmy (though this might seem an outside possibility). In either case, the phylogeny presented in Fig. 1 could be observed if in *Unonopsis* one paralogue was amplified by PCR, and in other Annonaceae, the other paralogue.

The inference of lineage sorting leads to two predictions: firstly, *trnL-F* sequences with the same signal as the other chloroplast markers should be (or should have been) present in *Unonopsis*. Secondly, if lineage sorting is the result of a duplication, a second copy of *trnL-F* should be (or should have been) present in all other taxa descending from the node representing the inferred duplication event. According to the preliminary results, thus, in all Annonaceae.

Aims and objectives

In this paper we test the hypothesis that conflicting phylogenetic signal in the *trnL-F* region in Annonaceae is the result of lineage sorting, using PCR-based and phylogenetic analysis techniques. Copy-specific PCR primers were designed based on Annonaceae *trnL-F* sequences obtained previously (Sauquet *et al.*, 2003; Mols *et al.*, 2004; Richardson *et al.*, 2004; this thesis, Chapter 2). These might amplify the different paralogues, if present, both in *Unonopsis* and in other Annonaceae. We then draw further conclusions with respect to the timing of divergence and to the phylogenetic signal contained in both copies.

Support for the hypothesis raises a number of further issues concerning the definition of homology in Magnoliales *trnL-F* regions. To address the question of functional homology, comparisons are drawn between sequences obtained in this study and proposed secondary structures and corresponding functional constraints in the *trnL* gene and Group I intron in land plants (Borsch *et al.*, 2003; Quandt *et al.*, 2004). Positional homology (*i.e.* within or possibly without the chloroplast genome) and the precise origin of paralogues is less straightforward to determine from sequences alone. A number of potential directions for future research, such as the application of additional PCR based or southern blotting techniques, are therefore also discussed.

Materials and Methods

Taxon sampling

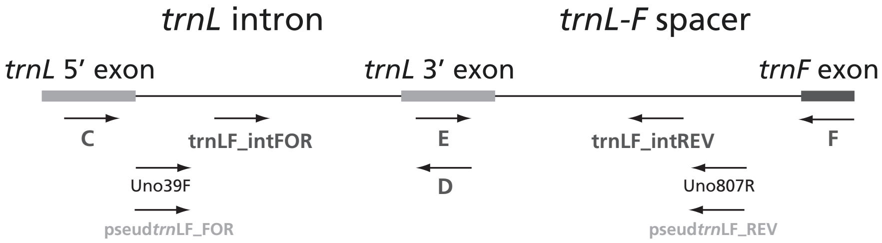
Recent improvements in both phylogenetic resolution and representation of taxa (Sauquet *et al.*, 2003; Mols *et al.*, 2004; Richardson *et al.*, 2004) provide a robust framework for the choice of taxa in phylogeny reconstruction in Magnoliales. This study utilised previously unpublished sequence data, as well as published *trnL-F*, *rbcL*, *matK* and *psbA-trnH* sequences (Kojoma *et* *al.*, 2002; Sauquet *et al.*, 2003; Mols *et al.*, 2004; this thesis, Chapters 2 & 3; Chatrou *et al.*, in prep.; see Appendix A).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted using a modified cetyl trimethyl ammonium bromide (CTAB) method (Doyle & Doyle, 1987): 50 mg silica dried or herbarium leaf material was homogenised in 1300 μl CTAB and incubated for 20 minutes with 12 μl 2-mercaptoethanol at 65°C, followed by 90 minutes ambient mixing with 1 ml 24:1 chloroform: isoamylalcohol. After 10 minutes centrifuged at 13,000 rpm, 300 μl supernatant was purified using Wizard DNA purification system (Promega corp.) (*i.e.* without isopropanol precipitation, avoiding the co-precipitation of oxidised material; Savolainen *et al.*, 1995).

A standard PCR protocol was used throughout, with the addition of 1μl 0.4 % BSA per 25 μl reaction (which was found to increase amplification in all samples), 35 cycles of 30 sec.: 94°C; 1 min.: 55-58°C; 2 min.: 72°C; with an initial 4 min.: 94°C; and final 7 min.: 72°C. PCR products were purified using QIAquick PCR purification kits (Qiagen), sequenced with selected PCR and specially designed sequencing primers (see below), and analysed by electrophoresis using an automatic sequencer ABI 3730XL.

BLAST search (Altschul *et al.*, 1997) was employed using the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST/) to compare the *Unonopsis trnL-Fs* with published sequences. To avoid confusion from this point the *trnL-F* copy homologous with those previously sequenced in Annonaceae will be referred to as '*trnL-F*', and that homologous with those first sequenced only in *Unonopsis* will be referred to as 'pseud*trnL-F*'.



Primer sequences (5′-3′):

Annonaceae partial *trnl-F* / pseud*trnL-F* internal sequencing primers:

trnLF_intFOR: GAGAGAAACATTTCTGGTCGG
trnLF_intREV: GGGCAATCCTGAGCCAAATCC

pseud*trnLF*_specific primers:

pseudtrnLF_FOR: GGAAACCTACTAGTCACTTCC
pseudtrnLF_REV: GGAGATTCCTTGCCCATTATCTG

***Annonaceae trnLF*_specific primers:**

Uno39F: GGAAACCTACTAAGTGAGAACTTCC
Uno807R: GCCCATTCATTATCTGTTC

Fig. 2: Scheme of primers used to amplify and sequence *trnL-F* and pseud*trnL-F*.

For taxa other than *Unonopsis* the *trnL-F* region was amplified and sequenced using plant universal primers of Taberlet *et al.* (1991) in combination C/F or C/D and E/F (the positions and sequences of all primers used to amplify and sequence both copies of the *trnL-F* region are presented in Fig. 2). Further primers were designed to amplify specifically and sequence the different *trnL-F* copies. In *Unonopsis*, *trnL-F* was amplified using primers Uno39F and Uno807R. In other Annonaceae (and in *Unonopsis* samples where sequencing using primers C/D, E/F failed) pseud*trnL-F* was amplified using primers pseudtrnLF_FOR and pseudtrnLF_REV. Uno39F and pseudtrnLF_FOR were designed to anneal to the same region near the beginning of the *trnL* intron where length differences were specific to the different copies, the same being true of Uno807 and pseudtrnLF_REV, located within the *trnL-F* intergenic spacer (Fig. 3). The higher annealing temperature of 58°C was employed to reduce the chances on non-copy-specific annealing in these reactions. Few areas of the alignment displayed (small) copy-specific length differences suitable

```
trnL 3’ exon<=**************..................................trnL intron=>
Persea americana            ????????????GGTATGGAAACCTACTAAGTGATAACTTCCAAATT
Coelocaryon preussii        ????????????GGTATGGAAACCTACTAAGTGGTAACTTCCAAATT
Magnolia kobus              ????????????GGTAGGGAAACCTAcTAAGTGGTACCTTCCAAATT
Eupomatia bennetii          ????????????GGTATGGAAACCTACCAAGTGGTAACTTCCAAATT
Malmea dielsiana            ????????????GGTATGGAAACCTACTAAGTGAGAACTTCCAAATT
Cremastosperma brevipes     GGATTGAGCCTTGGTATGGAAACCTACTAAGTGAGAACTTCCAAATT
Unonopsis elegantissima     GGATTGAGCCTTGGTATGGAAACCTACTA-GTCA---CTTCCAAATT
Unonopsis stipitata         GGATTGAGCCTTGGTATGGAAACCTACTA-GTCA---CTTCCAATCT
pseudtrnLF_FOR                               GGAAACCTACTA-GTCA---CTTCC=>
Uno39F                                       GGAAACCTACTAAGTGAGAACTTCC=>

                            <=.........trnL-F intergenic spacer..........=>
Persea americana            TACAAATGAACATAT-AT----AGGCAAGGAATTTCCATTATTAAAT
Coelocaryon preussii        CACAAATGAACAGATTAT----GGGCAAGGAATCCCCATTATTGAAT
Magnolia kobus              TACAAATGCCCATATTAT----GGGCAAGGAATCTCCATTATTGAAT
Eupomatia bennetii          TACAAATGAACATATTAT----GGGCAAGGAATCTCCATTATTGAAT
Malmea dielsiana            TACAAATGAACAGATAATGAATGGGC---GAATCTCCACTATTGAAT
Cremastosperma brevipes     TACAAATGAACAGATAATGAATGGG----GAATCTCCACTATTGAAT
Unonopsis elegantissima     TACAAATGAACAGATAAT----GGGCAAGGAATCTCCATTATTGAAT
Unonopsis stipitata         TACAAATGAACAGATAAT----GGGCAAGGAATCTCCATTATTGAAT
pseudtrnLF_REV                      <=CAGATAAT----GGGCAAGGAATCTCC
Uno807R                          <=GAACAGATAATGAATGGGC
```

Fig. 3. Copy-specific primer sequences, illustrating length difference in pseud*trnL-F* intron and spacer.

for primer design. Possible primer sequences were far from ideal: firstly, patterns of insertions/deletions inferred in Annonaceae *trnL-F* sequences suggested that annealing sites may not be present in taxa not closely related to *Unonopsis* (length differences were not consistent across all sequences available). Secondly, primers did not conform to the basic principles of primer design (such as >50% GC content, and including two G/Cs on the 3' end). To alleviate the latter problem, PCR products amplified using both sets of copy-specific primers were sequenced using primers trnLF_intFOR and trnLF_intREV, which were designed to anneal within the amplified fragments, following such principles in order to improve efficiency of the sequencing reaction.

Analysis

Two matrices were constructed: 1) a *trnL-F* matrix including *trnL-F* and pseud*trnL-F* (thus with some taxa present twice), 2) a multi-gene matrix also including *rbcL*, *matK* and *psbA-trnH* where *trnL-F* and pseud*trnL-F* were treated as separate partitions (and where taxa were thus present only once). In the latter case *trnL-F* and pseud*trnL-F* sequences (as identified from analysis of the first matrix) were both aligned with non-Annonaceae outgroup *trnL-F* sequences (which were thus present in both partitions), and where Annonaceae pseud*trnL-F* sequences were unavailable (*i.e.* in non-SAC clade taxa) these taxa were excluded.

DNA sequences were edited in SeqMan 4.0 (DNAStar Inc., Madison, WI) and aligned by eye. All areas of the alignment where the assessment of homology was ambiguous were excluded from the analyses. In the analyses where sequences of *trnL-F* (in *Unonopsis*) or pseud*trnL-F* (in other Annonaceae) were incomplete (due to the position of the copy-specific primers), the corresponding stretches of the alignment at both ends were excluded.

Maximum parsimony (MP) analysis: Data were analysed using the parsimony algorithm of the software package PAUP* 4.0b10 (Swofford, 2000), under the equal and unordered weights criterion (Fitch parsimony; Fitch, 1971). The length of the shortest trees were estimated with 'full' heuristic searches of 1000 random addition sequences, TBR, saving 100 trees each time. Support was estimated using bootstrap analyses of 500 replicates with 'full' heuristic searches of 100 random addition sequences, TBR, saving 50 trees each time. Bootstrap percentages were interpreted following Richardson *et al.* (2004): 50-74% represents weak support, 75-84% moderate support and 85-100% strong support. For the multi-gene matrix, support was estimated for the markers independently and in combined analysis.

Selecting the best fitting DNA substitution model: ModelTest 3.06 (Posada & Crandall, 1998) was used to select the substitution model best fitting each sequence data partition for each matrix using a most parsimonious tree topology. For matrix 1 ModelTest was run both with and without non-

Annonaceae sequences in order to check whether omitting the outgroups (and their relatively long branches) could have resulted in different models and parameters.

Bayesian analysis: Bayesian inference was applied as implemented in MrBayes version 3.0 (Huelsenbeck, 2000). The use of Markov chain Monte Carlo analyses (MCMC; Geyer, 1991) in Bayesian inference facilitates heuristic searching of parameter value space for maximum likelihood models of DNA substitution in phylogeny reconstruction (Huelsenbeck *et al.*, 2001). Prior values for the DNA substitution models were applied to each partition (as determined using ModelTest above). Prior probabilities for all topologies were equal. *Persea americana* (Lauraceae) was chosen as the single outgroup taxon permitted by MrBayes for the *trnL-F* matrix (1), *Coelocaryon preussii* (Myristicaceae, sister group to rest of Magnoliales; Sauquet *et al.*, 2003) for the multi-gene matrix (2). In the multi-gene matrix the data was partitioned according to the separate markers and both rates and substitution models allowed to vary across the partitions. MCMC analyses were run for 5,000,000 generations with four simultaneous MCMC chains to calculate posterior probabilities (PP), saving one tree per 100 generations. The burn-in values were determined empirically from the log-likelihood values and 50% majority rule consensus trees were calculated together with approximations of the PP for the observed bipartitions.

r8s analyses: A single MP topology was selected from the three found on heuristic search (as above) of the *trnL-F* matrix (1), having excluded all missing data (leaving 635 characters), and all Annonaceae taxa for which only one copy of *trnL-F* was available (leaving 33 sequences), and constraining the relationships between Magnoliales outgroups to conform to those demonstrated by Sauquet *et al.* (2003). A likelihood ratio test was performed on this topology: likelihood of the data with and without constraint of a molecular clock were calculated, and the likelihood ratio statistic compared with χ^2 critical value with 31 degrees of freedom (*i.e.*, number of sequences minus 2). ML branch-lengths were then calculated using the substitution model calculated as above with a) the original matrix, and b) 100 bootstrap re-sampled matrices. Thereafter, the penalized likelihood (PL) method of Sanderson (2002a) was applied using the program r8s (Sanderson, 2002b) to estimate rates and divergence times (with standard deviations according to variation in branch lengths derived from the bootstrap re-sampled data). To calibrate the rate smoothed tree in absolute time two r8s analyses were performed using the ages of different fossils to impose minimum ages on basal nodes: 1) *Archaeanthus* (Dilcher & Crane, 1984), imposing a minimum age of 98 mya for the Magnoliaceae stem node. 2) *Cronquistiflora* and *Detrusandra* (Crepet & Nixon, 1998), following Crepet *et al.* (2004), to impose the more conservative minimum age of 90 mya on the Magnoliales crown group.

Secondary structure of the *trnL* gene and intron

The secondary structures of the *trnL* gene and intron were calculated for representatives of both *trnL-F* copies. Stem-loop regions were identified by comparison to the structure proposed by Borsch *et al.* (2003) for *Nymphaea odorata*, with further reference to the conserved sequence motifs reported across land plants by Quandt *et al.* (2004). Secondary structures of these regions were then estimated individually using Mfold (Zuker, 2003), except in the case of the more variable P8 region, which was not further considered as the precise structure in angiosperms is not yet fully understood (Quandt, pers. com.), rendering comparison between putatively conserved regions impossible.

Results and discussion

Robustness of the position of *Unonopsis* in the *trnL-F* phylogeny

BLAST search (Altschul *et al.*, 1997) identified chloroplast *trnL-F* regions derived from species of Magnoliaceae – a family of the same order as Annonaceae; the Magnoliales (APG II, 2003; Sauquet *et al.*, 2003) – to be most similar to the *Unonopsis trnL-F* sequences. The assumption of identical divergence rates inherent in BLAST-based methods do not make this a reliable method to infer relatedness of the sequences (Thornton & DeSalle, 2000). However, this would appear to exclude the possibility of the *Unonopsis trnL-F*s being descendents of a more recent common ancestor with chloroplast *trnL-F* sequences from outside the taxonomic scope of our analyses, or with tRNA genes known from other genomic compartments of taxa such as *Arabidopsis*.

Reconstruction of the phylogeny of the entire *trnL-F* region using both Bayesian inference and MP resulted in topologies congruent with that presented in Fig. 1. Alignment length, numbers of included, variable and parsimony informative characters for the (entire) *trnL-F* matrix, CI/RI based on an arbitrary MP topology, and best-fitting model are presented in Table 1. The nodes defining the position of the *Unonopsis* pseud*trnL-F* sequences as monophyletic sister group to the rest of the Annonaceae (A, B and C; Fig. 1) were subject in all cases to strong BS and 100% PP.

Table 1: Details of phylogenetic analysis of the entire *trnL-F* region

Alignment length	of which excluded	Variable characters	Pars. inf. characters	Tree length	CI/RI	Best fitting model
1222	139	359	208	561	0.772/0.881	K81uf+Γ

Explaining the incongruent position of *Unonopsis* sequences in the *trnL-F* phylogeny according to errors in the analyses appears unlikely. In order to test possible sensitivity of the result to alignment ClustalX (Thompson *et al.*, 1997) was applied with default multiple alignment parameters. The entire, unedited, resulting alignment was analysed using MP and Bayesian inference, which recovered nodes A, B, and C with moderate to strong BS and >95% PP (data not shown). Long branch attraction cannot have affected the result, as it can only be manifested through the attraction of long branches (Siddall & Whiting, 1999). In the Annonaceae phylogeny this might be expected to be seen in the erroneous placement of highly divergent sequences as sister groups to one of the more derived LBC taxa, certainly not as sister to the Annonaceae clade. Finally, applying ModelTest with and without non-Annonaceae sequences resulted in the same best fitting substitution model (K81uf+Γ).

A paralogue of *trnL-F* in Annonaceae

The PCR-based approach employed here resulted in the amplification and sequencing of *trnL-F* in *Unonopsis* (forming a monophyletic group with *Bocageopsis* and *Onychopetalum* in the SBC), and of pseud*trnL-F* in accessions of *Bocageopsis*, *Cremastosperma*, *Malmea*, *Onychopetalum* and *Oxandra*: all SBC taxa of the SAC clade forming a monophyletic group with *Unonopsis* pseud*trnL-F* sequences (Fig. 4). Copy-specific amplification was not always successful: in some taxa of the SAC clade (*e.g. Pseudoxandra*), and accessions of further SBC taxa (such as *Annickia* and *Polyalthia*), the pseud*trnL-F* primers instead amplified the original *trnL-F* copy. When applied to accessions of LBC or basal grade taxa, no amplified product was produced. It is possible that the small length differences (indels) used as targets for the copy specific primers are only present in the SAC clade, either representing synapomorphies, or sympleisiomorphies secondarily lost in the other clades sampled. This result offers no direct evidence for the presence or otherwise of pseud*trnL-F* in other clades in Annonaceae.

The location of a duplication event on a gene tree represents its most recent possible age (Thornton & DeSalle, 2000), which in the case of the divergence of *trnL-F* and pseud*trnL-F* is more recent than the MRCA of Annonaceae and Eupomatiaceae, but prior to the generally accepted MRCA of Annonaceae as represented by the stem lineage of *Anaxagorea*. This latter node has been estimated at 57 or 69 mya (Doyle *et al.*, 2004) and 64.9 or 76.8 mya (Richardson *et al.*, 2004), using molecular dating with different fossil calibrations. Similar analyses performed here primarily to estimate the rates of evolution of the two copies of *trnL-F* (see below) were calibrated with two different fossils to estimate the age of the divergence in absolute terms. This resulted in ages of 81.18 mya using the fossil *Archaeanthus*, following Doyle *et al.* (2004) and Richardson *et al.* (2004), and 58.30 mya using *Cronquistiflora*

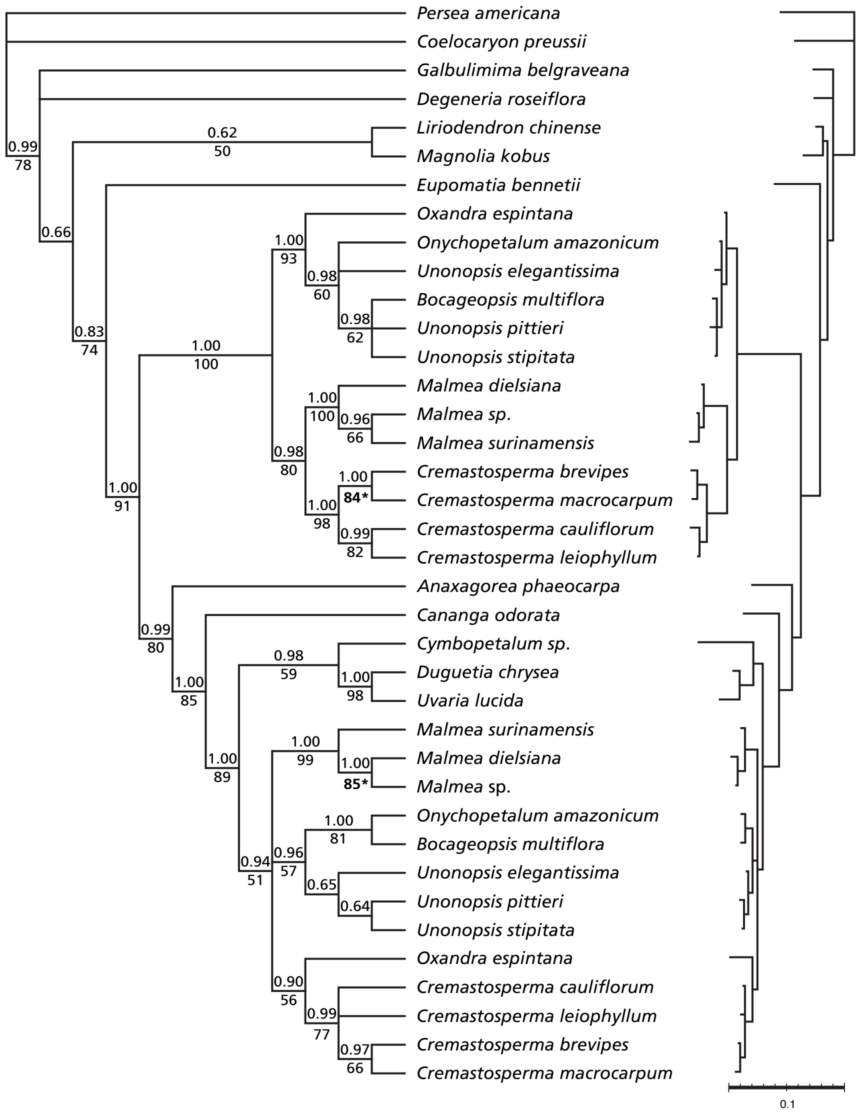


Fig. 4: Bayesian 50% majority rule consensus tree and phylogram resulting from analysis of partial *trnL-F*/pseud*trnL-F*. Posterior probabilities are indicated above the nodes, maximum parsimony bootstrap support (where >50%) below. Bootstrap support values for conflicting nodes referred to in the text are highlighted in bold and with an asterisk (*)

and *Detrusandria* following Crepet *et al.* (2004) respectively, with a standard deviation of 7.38. In the same analyses the age of SAC clade crown group was also estimated, as represented by two different nodes: 1) the MRCA of all the Annonaceae *trnL-F* sequences included and 2) the MRCA of the corresponding pseud*trnL-F* sequences. The resulting ages were not significantly different (Table 2).

Table 2: r8s analyses:

	trnL-F divergence	MRCA SAC clade: *trnL-F*	MRCA SAC clade: pseud*trnL-F*
Age: calibration 1	81.18 mya sd=7.38	34.25 mya sd=7.68	31.61 mya sd=5.94
Age: calibration 2	58.30 mya sd=7.38	21.68 mya sd=7.68	18.08 mya sd=5.94
***Rate** estimated:	0.000612	0.000461	0.000744
mean:	0.000709	0.000490	0.000907
sd:	0.000085	0.000106	0.00014

*changes site^{-1} million years^{-1}

Phylogenetic utility of Annonaceae *trnL-F* copies

Duplicated genes have proved useful in rooting phylogenetic analyses, in particular those in which there are no extant outgroups (an extreme example being that of the tree of life) or where outgroups are too distant (such as in angiosperms; Donoghue & Mathews, 1998; Mathews & Donoghue, 1999). Character polarisation in Annonaceae is uncontroversial: *Anaxagorea* is supported as first branching lineage in cladistic analyses of both morphological and of molecular data (Doyle & Le Thomas, 1996; Doyle *et al.*, 2000; Sauquet *et al.*, 2003). However, were the root of the Annonaceae to be misplaced this could provide an explanation (where no other is yet forthcoming) for the increased rate of evolution (of cpDNA markers) apparent in the LBC. No such conclusion can be drawn from analyses performed here: phylogenetic analysis of *trnL-F* and pseud*trnL-F* sequences resulted in a gene tree in which this rooting is clearly not disrupted (see Figs 1 & 4). Relationships between taxa represented twice were mostly congruent (Fig. 4), with any apparently conflicting nodes subject to PP <95% and/or BS <75%. Analysis of *rbcL*, *matK* and *psbA-trnH* sequences separately also revealed no supported conflict. These partitions were therefore included in a combined analysis which resulted in a topology congruent with those of *trnL-F* and pseud*trnL-F*, with two apparent exceptions (Fig. 5a, compare with Fig. 4): 1) relationships between species of *Malmea*, which were in conflict with those revealed by *trnL-F*, and 2) those between species of *Cremastosperma*, which were in conflict with those revealed by pseud*trnL-F* (BS values of corresponding nodes are bold and highlighted with asterisks in Figs 4 and 5).

Combined analysis resulted in (generally) higher support for a more resolved topology (Fig. 5b), with the exception of lower support for relationships between species of *Malmea*.

Proportions of variable and parsimony informative characters and CI/RI (based on the topology derived from combined analysis) are presented in Table 3. Of the five markers compared, the roughly 600 bp long fragment of pseud*trnL*-

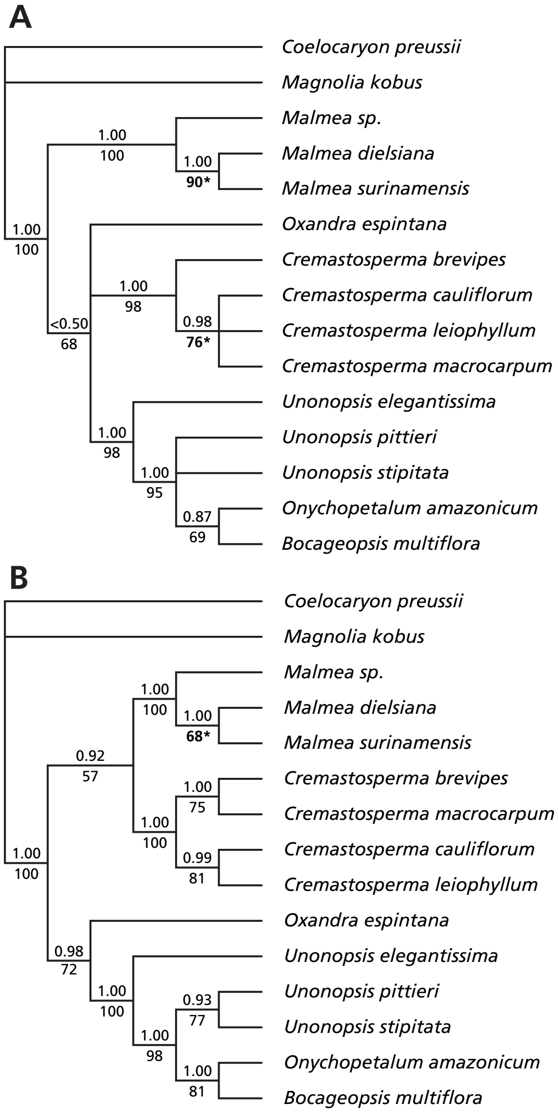


Fig. 5: Bayesian / Maximum parsimony bootstrap consensus trees of a) combined chloroplast DNA markers *rbcL, matK* and *psbA-trnH* and b) *rbcL, matK, psbA-trnH, trnL-F* and pseud*trnL-F*. Bootstrap support values for conflicting nodes referred to in the text are highlighted in bold and with an asterisk (*)

F amplified using the copy specific primers provided the highest proportion of parsimony informative characters: more in total than *rbcL*, which is rather more than twice as long and has to be amplified and sequenced in two pieces. The phylogenetic utility of pseud*trnL-F* would also appear clear comparing the resolution and support values, particularly within *Cremastosperma*, as presented in Fig. 4. The limited conflict apparent in the topologies (see Figs 4 and 5) is not consistently between one partition and the others. It may be a result of either the small numbers of informative characters involved, or the very limited taxon sampling, or both. However, in comparing the two *trnL-F* paralogues with the single corresponding copy found in other plants, two further levels of homology can be considered: functional and positional. These may have implications for the patterns of evolution displayed which might lead us to treat this new potential marker with some caution.

Table 3: Comparison of markers use in combined analyses

Marker	Approx. sequence length	Variable characters: No. / %	Pars. inf. characters: No. / %	CI/RI
(partial) *trnL-F*	600	74 / 12.3	27 / 4,5	0.94/0.88
(partial) pseud*trnL-F*	600	89 / 14.8	50 / 8.3	0.92/0.92
rbcL	1500	122 / 8.1	44 / 2.9	0.83/0.70
matK	850	136 / 16	60 / 7.1	0.95/0.92
psbA-trnH	430	61 / 14.2	10 / 2.3	0.93/0.79
combined	3980	482 / 12.1	191 / 4.8	0.91/0.86

CI/RI calculated using single most parsimonious topology of combined analysis.

Functional homology of Annonaceae *trnL-F* copies

Although examples of paralogues of chloroplast genes have been documented where function in one copy (often to be found in a different genomic compartment) appears to have been lost entirely (see below), genome-wide studies suggest that the vast majority of redundant genes still experience purifying selection (Wagner, 2002). Loss of function in protein coding genes can in many cases be demonstrated, for example where mutations result in disruption of the reading frame or the appearance of 'stop' codons within it. The function of the transfer RNA for which the *trnL* gene itself codes is related to its secondary structure (and also that of the intron found within it). We therefore attempted to assess the functionality of copies of *trnL-F* in Annonaceae by comparison with plant *trnL* introns and 3' exons for which secondary structures have been proposed (by Borsch *et al.*, 2003; Quandt *et al.*, 2004; Quandt & Stech, 2004).

Borsch *et al.* (2003) found across basal angiosperms only 20% of the 95 positions corresponding to proposed stem structures in the *trnL* intron to be variable, attesting to the conservation of its secondary structure. The structure

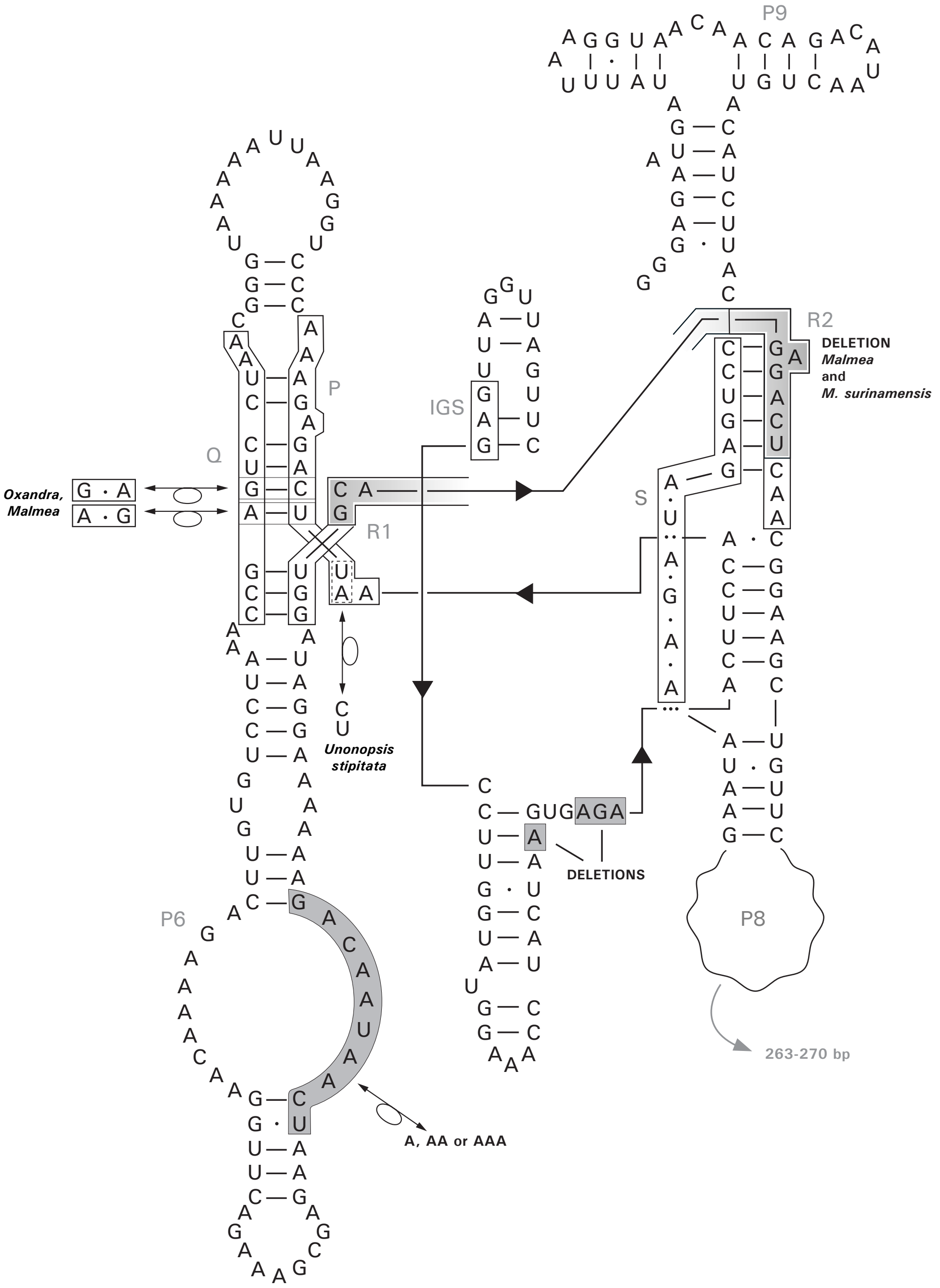


Fig. 6: Secondary structure of the *trnL* intron sequence of *Cremastosperma brevipes.* Conserved sequence motifs are outlined and labelled following Cech *et al.* (1994). Selected differences between this representative *trnL-F* sequence and all or specified pseud*trnL-F* sequences are outlined and labelled, with deletions filled in grey.

presented in Fig. 6 (following Cech *et al.* (1994), based on the secondary structure of group I introns modelled by Michel & Westhof, (1990)), is that of the *trnL* intron sequence of *Cremastosperma brevipes* (see table of samples in Appendix A). The conserved sequence motifs, as described by Quandt *et al.* (2004), and selected differences between this and pseud*trnL-F* sequences are indicated.

The greater number of parsimony informative characters in pseud*trnL-F* sequences in comparison to *trnL-F* sequences is reflected in a higher number of changes occurring in regions inferred to represent stem structures as opposed to loops in *Nymphaea*. Excluding the P6 and P8 regions (for which secondary structures in both copies appeared to differ significantly from that of *Nymphaea*), 11 single base changes are observed in pseud*trnL-F* putative stem regions, in comparison to 5 in the same taxa for *trnL-F*. No compensatory changes were apparent in either *trnL-F* or pseud*trnL-F* sequences analysed. The few base changes apparent in the 3' exon relative to that of *Nymphaea* are limited to within loop structures in both copies. More significantly, a 9 bp long deletion is observed in the pseud*trnL-F* sequences of two of the *Malmea* accessions (representing a synapomorphy for a clade also supported by *rbcL*, *psbA-trnH* and *matK* data but apparently contradicted by *trnL-F*). This deletion includes most of the conserved R1 and R2 sequence motif (as described by Quandt *et al.*, 2004; see Fig. 6), and thus seems likely to represent a significant change in the secondary structure.

Rate of evolution of Annonaceae *trnL-F* copies

The role of selection can be investigated by estimating relative and absolute rates of sequence divergence for different branches in the gene family tree. If mutation rate is constant, differences in divergence rates represent strength of selection (Thornton & DeSalle, 2000). Under the best fitting substitution model (K81uf+Γ), the likelihood of the partial *trnL-F* data given one of the MP topologies was 2022.27. Enforcing a molecular clock resulted in a significantly different likelihood of 2074.00 ($P=<0.01$, chi squared test, 31 degrees of freedom), and the clock hypothesis was thus rejected. We therefore used the penalized likelihood method of Sanderson (2002a) to estimate rates of evolution in different branches of the *trnL-F* gene tree, applying a bootstrapping technique to assess error according to character sampling. The rate at SAC crown node was estimated to be 0.000744 (sd=0.000146) changes per position per million years for pseud*trnL-F*, significantly higher than that estimated for *trnL-F*: 0.000461 (sd=0.000106).

Should we be comparing two chloroplast encoded markers, the difference observed might thus be interpreted to suggest less stringent selection acting on pseud*trnL-F*. However, although one of the two copies of *trnL-F* in Annonaceae is presumably to be found in the expected position in the chloroplast genome, the position of the other (whichever that might be) is unknown. Relative rate differences can be attributable to other evolutionary or population genetic phenomena (Small *et al.*, 1998), some of which, such as background mutational processes, drift, and rates of recombination, differ across genomic compartments. The positional homology of both copies is thus critical to interpreting this higher rate of change. It also determines their mode of inheritance and is thus critical for the interpretation of the phylogenetic signal they contain.

Positional homology in Annonaceae *trnL-F* copies

There are numerous examples of duplicated chloroplast genes in the literature, the position of many of which has been demonstrated. A pseudogene of *rpl*23 is present as in the chloroplasts of many members of Caryophyllales (Clegg & Zurawski, 1992), and partial or complete pseudogenes of *trnF* have been observed as insertions in the *trnL-F* spacer in *Microseris* (Asteraceae, Vijverberg & Bachmann, 1999) and particular lineages of Brassicaceae (Dobes *et al.*, 2004; Koch *et al.*, 2005). Ayliffe *et al.* (1998) identified sequences of plastid homology in the nuclear genome of various angiosperms, as did Millen *et al.* (2001), demonstrating transfers of *inf*A from the chloroplast to the nucleus.

Gene content of the mitochondrial genome is considered particularly dynamic and flexible (Nakazono & Hirai, 1993). Cummings *et al.* (2003) reported hundreds of successful transfers of *rbcL* from the chloroplast to the mitochondrion in flowering plants: of the five examined, all had disrupted reading frames. Nakazono & Hirai (1993) compared mitochondrial and chloroplast gene content in rice: 16 chloroplast sequences between 32 bases and 6.8 kb long (equivalent in total to 19% of rice total chloroplast genome) were discovered in the mitochondrion. The nine intact and three defunct tRNA genes discovered included the 3' *trnL* exon and *trnF*. With the publication of the complete rice mitochondrial genome, Notsu *et al.* (2002) discovered a total of 17 tRNA genes and five pseudo tRNA sequences of chloroplast origin. They additionally identified nuclear sequences of chloroplast origin positioned adjacent to sequences of mitochondrial origin, suggesting transfer to have been via the mitochondrion.

Further approaches to locating copies of *trnL-F* in Annonaceae

A number of possible approaches might identify the positional homology of *trnL-F* copies in Annonaceae. Regions surrounding *trnL-F* and pseud*trnL-F* might contain adjacent sequences of identifiable origin, or those which might suggest a mechanism by which a duplication could have occurred, such as mobile genetic elements (Thornton & DeSalle, 2000). However, either region might represent part of a significantly larger duplicated fragment, and this approach would in any case be likely to suffer either from similarity (amplification of the wrong copy) or parallel divergence (failure of amplification) of primer annealing sequences.

It might be possible to determine the location of the paralogues using southern blotting techniques with mitochondrial or nuclear DNA preparations and PCR products as a probe. However, the quality of DNA samples available for SBC taxa is relatively low (very few are known from cultivation), which might present challenges for isolating mitochondrial, chloroplast or nuclear DNA. Fresh material of other Annonaceae taxa is available from cultivated specimens (mostly LBC taxa such as *Annona* or *Monodora*). However, pseud*trnL-F* sequences, if present (not subject to secondary loss) in LBC taxa, might be highly divergent in comparison to those of the SBC or the other *trnL-F* copy. Similarity less than 70% might result in failure of the gel blot (Fulton *et al.*, 2002).

Conclusions

The discovery of a paralogous copy of *trnL-F* in Annonaceae poses at the same time theoretical and practical problems for phylogeny reconstruction. When using chloroplast markers in Annonaceae, single regions on their own (as defined by the primers available to amplify and sequence them, and generally of less than 2kb length) almost always provide insufficient total numbers of informative characters to arrive at supported topologies. However, the most densely sampled phylogenetic studies of Annonaceae to date (*i.e.* Mols *et al.*, 2004; Richardson *et al.*, 2004) rely on the *trnL-F* region for a large proportion of the informative characters analysed (the rest provided by *rbcL*). Paralogy of *trnL-F* sequences for *Unonopsis* with respect to other available Annonaceae *trnL-F* sequences, might mean a large source of data would be excluded from analyses in which the positions of its species are of interest (*i.e.* monophyly of *Bocageopsis* and *Onychopetalum* or phylogeny of the SBC). More worryingly, although no further 'hard' conflicts have been observed in phylogeny reconstruction in Annonaceae, the known existence of at least one paralogous copy of *trnL-F*, in combination with the limited resolution provided by currently available alternative markers (for most taxa *rbcL* alone), might then call into question results derived from using *trnL-F* alone and perhaps also in combined analyses in which it provides the majority of informative characters.

The presence of these two copies of *trnL-F* in Annonaceae is not be a problem for phylogeny reconstruction: pseud*trnL-F* is identifiable on phylogenetic analysis as forming a monophyletic sister-group to the Annonaceae *trnL-F* clade, and primers developed during this study allow the amplification of *trnL-F* in *Unonopsis*. The phylogenetically informative characters in pseud*trnL-F* sequences may be an unforeseen benefit of this phenomenon. This marker may have limited taxonomic scope (thus far amplification has only been successful in a limited number of closely related taxa), but contains a relatively large amount of information for the length of sequence. Its phylogenetic signal appears to be complementary to that of chloroplast markers, though further sampling of taxa is necessary to test this further. Should both copies of *trnL-F* be found in the chloroplast, or one transferred to the mitochondrion, maternal inheritance should result in congruent gene trees. However, if one copy has been transferred to the nucleus, its different mode of inheritance might result in a conflicting gene phylogeny. This itself could offer valuable insight into the evolutionary history of clades in Annonaceae.

Results presented here serve to confirm the caution with which datasets dominated by characters from one marker should be interpreted. Even widely used cpDNA markers such as the *trnL-F* region can result in a gene tree at odds with those of other markers. In the case of *trnL-F* in Annonaceae, the paralogous copies discovered appear to have a higher rate of change than those orthologous with Annonaceae *trnL-F* sequences previously known. This difference, and possibly also the positions of these changes (in particular the deletion common to two *Malmea* accessions in a conserved region of the intron secondary structure), may indicate relaxed selectional constraint. Alternatively, it could reflect the higher ambient mutation rates characteristic of the nuclear genome. Interpretation of the functional homology of the *trnL-F* and pseud*trnL-F* regions, as well as that of their phylogenetic signal, is therefore dependent on their positional homology, *i.e.* within the Annonaceae genome, and worthy of further study.

Chapter 5

Revision and phylogeny of *Cremastosperma* (Annonaceae)

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Abstract

A taxonomic revision and phylogeny based on DNA sequence data is presented for the Neotropical genus *Cremastosperma* (Annonaceae). Twenty-nine species are recognised, the majority occurring in lowland to premontane wet forest in areas surrounding the Andean mountain chain. Clades are identified corresponding to species distributed either west or east of the Andes, suggesting the Andean orogeny to represent an important vicariance event in the evolution of the group.

Keywords: Annonaceae, *Cremastosperma*, Neotropics, phylogeny reconstruction, taxonomy.

Introduction

The genus *Cremastosperma* R.E.Fr. can most easily be distinguished from other Neotropical Annonaceae with apocarpous, stipitate fruits by its midrib, which is raised on the upper side with a mostly conspicuous longitudinal groove. The individual distributions of its species are restricted to four (disjunct) areas of lowland to premontane tropical forest: 1. the Chocó/Darién/western Ecuador region (the narrow tropical zone to the west of the Andean mountain chain on the Pacific Ocean side of north-western South America) plus central America, 2. the tropical Andes (including forests on the eastern side of the Andes extending from Colombia through eastern Ecuador and Peru as far south as Bolivia), 3. coastal Venezuela and 4. French Guiana.

Taxonomic history of Cremastosperma

Cremastosperma was described by Robert E. Fries (1930) based on a species originally described by Diels (1906) under the genus *Aberemoa: A. pedunculata* Diels, which thus became the type species *Cremastosperma pedunculatum* (Diels) R.E.Fr. Further work by Fries up to 1950 increased the number of species of *Cremastosperma* to 17. In 1931 he described *C. cauliflorum* R.E.Fr., *C. gracilipes* R.E.Fr., *C. leiophyllum* R.E.Fr., and *C. megalophyllum* R.E.Fr., and made four new combinations transferring species from *Cymbopetalum* (*C. monospermum* (Rusby) R.E.Fr.), *Guatteria* (*C. pendulum* (Ruiz & Pav.) R.E.Fr. and *C. poiteaui* (Diels) R.E.Fr.), and *Unonopsis* (*C. polyphlebum* (Diels) R.E.Fr.). In 1934 he described four new species: *C. longicuspe* R.E.Fr., *C. peruvianum* R.E.Fr., *C. guianense* R.E.Fr., and *C. williamsii* R.E.Fr.; in 1937 one: *C. juruense* R.E.Fr. (in which publication he also transferred *C. guianense*, *C. polyphlebum* and *C. williamsii* to the genus *Pseudoxandra*); and in 1939, one: *C. microcarpum* R.E.Fr. plus a variety of *C. monospermum:*

C. monospermum (Rusby) R.E.Fr. var. *brachypodum* R.E.Fr. In the latter paper he also made a new combination: *C. brevipes* (DC.) R.E.Fr., under which he brought both *Guatteria brevipes* DC. and *Cremastosperma poiteaui* (Diels) R.E.Fr. into synonymy. In 1948 and 1950 Fries described his last five new species of *Cremastosperma: C. anomalum* R.E.Fr., *C. killipii* R.E.Fr., and *C. oblongum* R.E.Fr., followed by the first species of the genus from the Pacific coast of Colombia: *C. novogranatense* R.E.Fr. and *C. pacificum* R.E.Fr.

After the death of Fries in 1966, taxonomic work on *Cremastosperma* was resumed subsequent to the establishment of the international Annonaceae project (Maas, 1983). *C. anomalum* was transferred by Maas (in Maas *et al.*, 1986) to *Malmea* and subsequently by Chatrou (1998) to the newly described *Klarobelia*, along with a number of species formerly placed under *Malmea*. Maas (1986) further described one species from Panama (*C. panamense* Maas) and one from Venezuela (*C. macrocarpum* Maas).

The increase in the numbers of collections available for study in the last fifty years has revealed a substantial proportion of previously undescribed species in a number of recently revised Neotropical genera of Annonaceae. The most extreme example may be that of *Pseudoxandra*, revised by Maas & Westra (2003), in which 80% of species treated were new or very recently described. However, *Klarobelia* now comprises 12 species, of which only 3 were described prior to the treatment of Chatrou (1998) (i.e. 75% new). In preparation for a revision of *Cremastosperma* for this thesis, a further 13 species, 45% of the 29 recognised here, have been described.

Pirie & Zapata (2004) described three species from the Marañon basin in northern Peru, and Pirie (in Chatrou & Pirie, 2005) increased the number of species of *Cremastosperma* known from Venezuela to two. However, the greatest underestimation of species diversity appeared to be represented by collections from the Chocó/Darién/western Ecuador region. The general increase in numbers of collections of *Cremastosperma* made since the time of Fries has been restricted in areas of north-western South America. Far fewer were available than for comparable areas on the Amazonian side of the Andean mountain chain. Nevertheless, the degree of morphological differentiation clear from the previously largely undetermined collections that were available led Pirie (2005) to describe eight new species from extra-Amazonian Colombia and Ecuador plus Panama. One additional new species, endemic to Amazonian Ecuador, published in the latter paper brought the total number of recognised species of *Cremastosperma* to 31 (including two species, *C. killipii* and *C. juruense*, which are synonymised in this chapter under *C. longicuspe* and *C. monospermum* respectively).

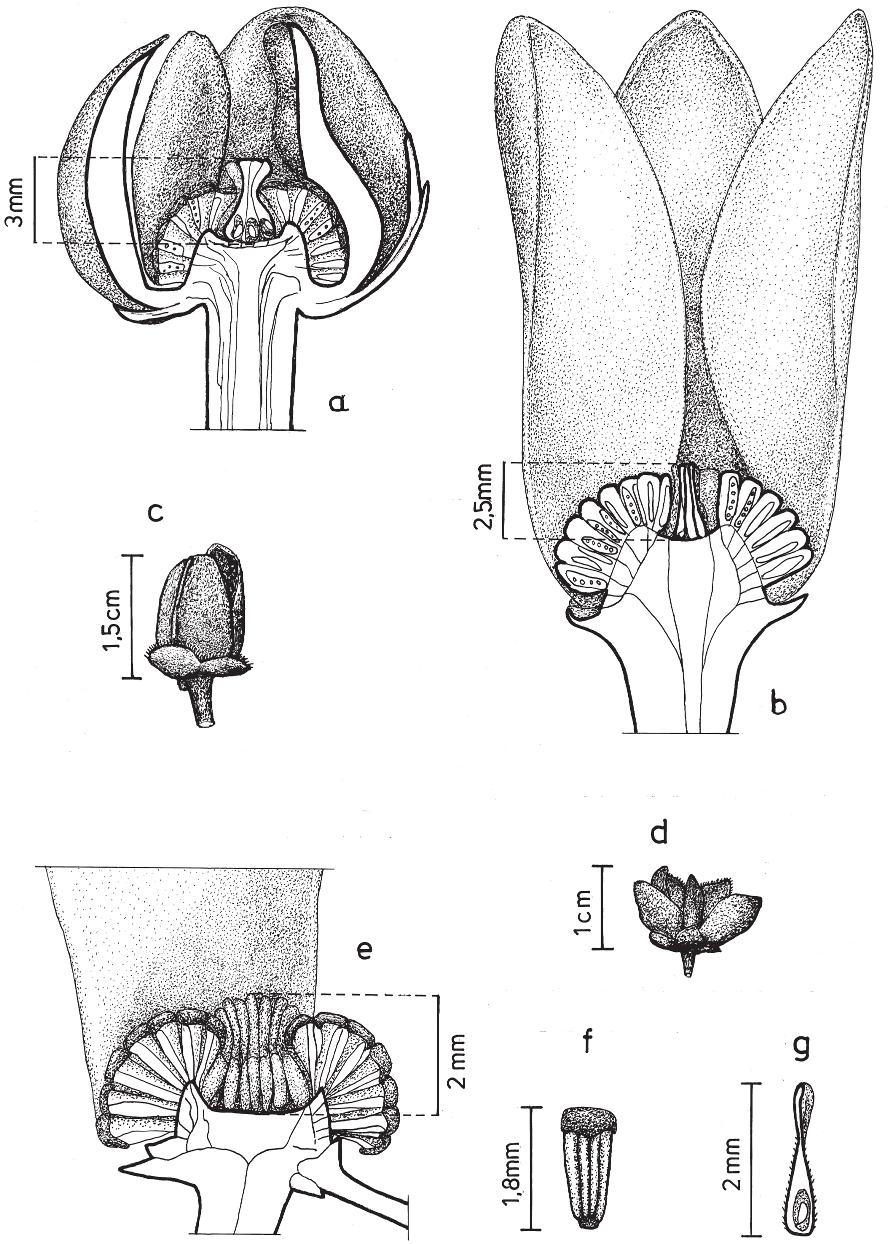


Fig. 1. Flowers of *Cremastosperma*. Fig. 11 from van Heusden (1992). a. *C. microcarpum*: longitudinal section; b. *C. oblongum* R.E.Fr.: longitudinal section; c. *C. monospermum* (Rusby) R.E.Fr.: bud; d. *C. gracilipes* R.E.Fr.: flower; e. & f. *C. cauliflorum* R.E.Fr.: longitudinal section (e) and stamen (f); g. *C. microcarpum*: carpel (a: *Maas et al. 6281*; b: *Maas et al. 4592;* c: *Nelson 763*; d: *Luteyn et al. 4890*; e & f: *Holm-Nielsen et al. 21501*; g: *Prance et al. 3527*)

Position of *Cremastosperma* within Annonaceae

Various morphological characters have been emphasised by different authors in placing *Cremastosperma* in more, or less, formal classifications of Annonaceae. Van Heusden (1992) defined her *Cremastosperma* group (also including the Neotropical genera *Ephedranthus*, *Malmea*, *Oxandra*, *Pseudephedranthus*, *Pseudoxandra*, and *Ruizodendron*) on a combination of floral characters: imbricate, often ciliate sepals and petals; small sepals (rarely over 4 mm long); usually whitish or greenish, sometimes yellowish flowers; and one basal, lateral or apical ovule (Fig. 1 g). The broader *Malmea* group of Walker (1971) also included the Neotropical genera *Unonopsis*, *Bocageopsis*, and *Onychopetalum* and the African genus *Annickia*. It was characterised by solitary, medium to large, sulcate pollen grains. Group 4 of van Setten & Koek-Noorman (1992), defined by transversely grooved or pitted seeds lacking arils, included most of the *Cremastosperma* group genera plus a number of Asian taxa (but neither *Unonopsis*, *Bocageopsis*, and *Onychopetalum*, nor *Annickia*). Amongst these taxa *Cremastosperma* was noted to be exceptional in the combination of a pitted seed wall (Fig. 2 e,f) with spiniform ruminations - a condition shared with *Pseudoxandra* and *Malmea* sensu Chatrou (1998), *i.e.* not including species formerly included in *Malmea* and now representing the genera *Klarobelia*, *Mosannona*, and *Pseudomalmea*.

Molecular phylogenetic studies of Annonaceae using DNA sequence data (Mols *et al.*, 2004; Richardson *et al.*, 2004; this thesis Chapters 2, 3 & 4) have shown strong support for a clade including all the above mentioned taxa plus a large number of Asian species: the 'short branch clade' (the SBC). Results presented in Chapter 3 of this thesis identified a clade including all SBC genera with distributions centred in South America (the SAC clade), corresponding to the *Cremastosperma* group of by van Heusden (1992), plus a clade including *Unonopsis*, *Bocageopsis*, and *Onychopetalum*. Relationships within the SAC clade were not all subject to high support. In particular, the relationship between the latter *Unonopsis* clade and clades representing the *Cremastosperma* group were effectively unresolved. However, sister group relationships were demonstrated between *Cremastosperma* and *Pseudoxandra*, and between this clade and *Malmea*. The combination of seed characters identified by van Setten & Koek-Noorman (1992; see above) for these genera may thus represent synapomorphies for this clade.

Phylogeny and biogeographic history of *Cremastosperma*

That no one species of *Cremastosperma* is found on both sides of the Andean mountain chain suggests that the Andes represents a current barrier to dispersal. The distribution of those species, in areas surrounding the Andes, may further suggest that the Andean orogeny played an important role in

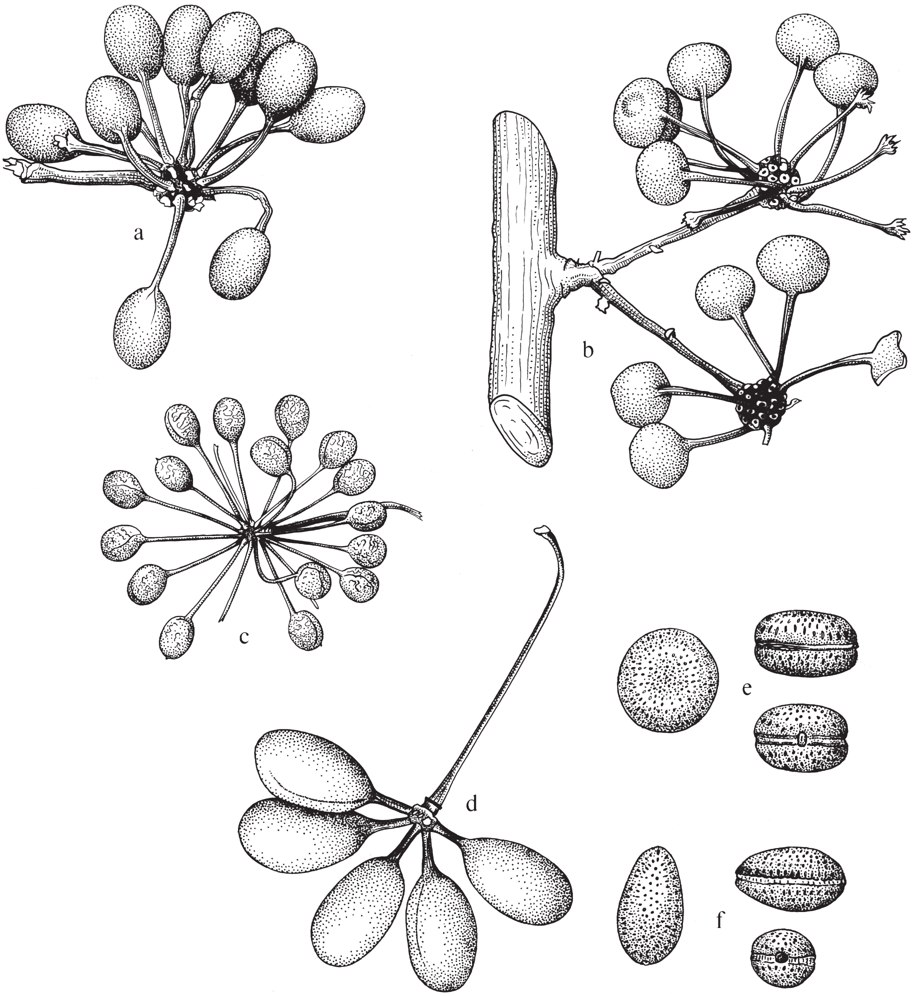


Fig. 2. **Fruits and seeds of *Cremastosperma*. Adapted from plate 8 from van Setten & Koek-Noorman (1992). a. *Cremastosperma megalophyllum* R.E.Fr.; b. & e. *C. cauliflorum* R.E.Fr.; c. *C. microcarpum* R.E.Fr.; d. *C. macrocarpum* Maas; f. *C. monospermum* (Rusby) R.E.Fr. (a: *Brandbyge & Asanza C. 30017*; b, e: *Prance et al. 24094*; c: *Gentry et al. 32153*; d: *Wingfield & van der Werff 6751*; f: *Sperling et al. 6198*)**

the diversification in the genus. Phylogenetic analysis of DNA sequence data suggest that the species of *Cremastosperma* comprise a monophyletic group (this thesis, Chapters 2 & 3), and results of molecular dating (Chapter 3) suggest diversification in this group occurred during the timeframe of the Andean orogeny. A cladistic biogeographic approach could be employed to determine whether Andean-centred distributions found not only in *Cremastosperma*, but also in other clades in Annonaceae (such as *Klarobelia*, *Malmea*, and *Mosannona*, see Chapter 3) may be the result of a common biogeographic history. However, relationships particularly between species of *Cremastosperma* remain as yet largely unresolved.

In this chapter a taxonomic revision of *Cremastosperma* is presented. Twenty-nine species are recognised and two further putative species described unofficially, awaiting further collections. Identification keys are provided. Results of phylogenetic analyses using DNA sequence data are presented.

Phylogeny reconstruction using DNA sequence data

Materials and methods

Taxon sampling: This study largely utilised previously unpublished sequence data, as well as published sequences (Mols *et al.*, 2004; Richardson *et al.*, 2004; this thesis, Chapters 2, 3 & 4; see appendix). Nine SBC outgroup taxa were selected following the results of Chapter 3, including two accessions each of the most closely related genera *Pseudoxandra* and *Malmea*. The 33 accessions of *Cremastosperma* sampled included 19 of the 29 species recognised here, from across the entire geographical distribution, plus *C. spec.* A and *C. spec.* B (some species were thus represented by multiple accessions).

Character sampling: For all 42 accessions the cpDNA markers *rbcL*, *matK*, *trnT-F* and *psbA-trnH* were sampled, following the PCR and sequencing protocols described in Chapters 2 & 3. Amplification and sequencing of a further cpDNA marker, *ndhF*, and of pseud*trnL-F* (see Chapter 4) was only successful in 27 accessions of *Cremastosperma* (representing 18 species), and in pseud*trnL-F* only two outgroups (both species of *Malmea*).

Phylogenetic analysis: DNA sequences were edited in SeqMan 4.0 (DNAStar Inc., Madison, WI) and aligned manually. Areas of the alignments where the assessment of homology was ambiguous were excluded from the analyses. Gaps in the alignments were coded as present/absent characters where they could be coded unambiguously, following Simmons and Ochoterena (2000) simple gap coding principles. Phylogeny was inferred under maximum parsimony (MP) and using Bayesian inference. MP 'full' heuristic searches (1000 iterations, TBR, saving 50 trees per iteration) were performed for the combined data and MP bootstrap support was estimated using PAUP* (Swofford, 2000), for the markers individually and combined, as described in Chapter 3. Combined analyses under Bayesian inference were performed using MrBayes version 3.0 (Huelsenbeck, 2000), also as described in Chapter 3.

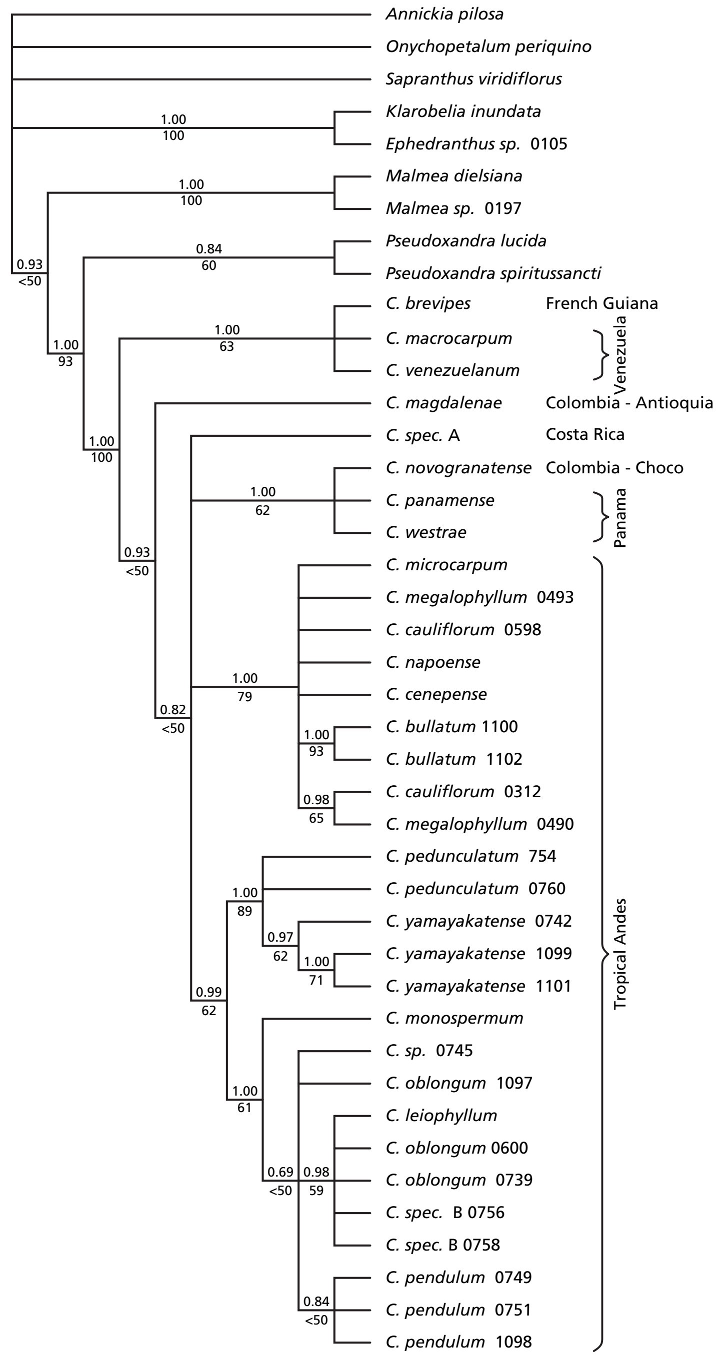


Fig. 3 *Cremastosperma* phylogeny based on *rbcL*, *matK*, *trnT-F*, and *psbA-trnH* (*i.e.* without *ndhF* or pseud*tnL-F* data): posterior probabilities (above the nodes) and bootstrap percentages (below).

Results and Discussion

MP bootstrap analysis of the individual data partitions revealed no supported incongruencies (BS >75%). Data were thus combined in further analyses. MP heuristic search including all taxa but neither *ndhF* nor pseud*trnL-F* resulted in 44501 trees of 539 steps, CI = 0.87, RI = 0.83. The same search strategy with inclusion of *ndhF* and pseud*trnL-F*, and exclusion of ingroup taxa for which both were unavailable, resulted in 148 trees of 832 steps, CI = 0.88, RI = 0.83. Consensus topologies (50% majority rule) derived under MP bootstrap and Bayesian analyses were congruent. Topologies with posterior probabilities (above the nodes) and bootstrap percentages (below) are presented in Fig. 3 (for analyses including all taxa but without *ndhF* or pseud*trnL-F,* data*)* and Fig. 4 (including *ndhF* or pseud*trnL-F* data for fewer taxa).

Table 1: **DNA sequences: variable and parsimony informative characters. Values are made comparable by the inclusion of only *Cremastosperma* and *Malmea* taxa for which *ndhF* and pseud*trnL-F* sequences were available.**

Marker	Approx. sequence length	Variable characters:	Pars. inf. characters	Pars. inf. Indels
[a]*rbcL*	1480	22	13	0
matK	850	42	21	0
ndhF	2050	100	33	0
trnT-trnF	1550-1950	68	34	7
psbA-trnH	400-450	25	10	1
pseud*trnL-F*	600	50	29	1

[a]Including 3’ non coding region

Use of multiple chloroplast markers here (with the possible exception of pseud*trnL-F*, the genomic compartment of which is unknown: see Chapter 4) revealed a number of clades within the genus *Cremastosperma*. Pseud*trnL-F* provided the highest proportion of informative characters for the length of sequence. Given the low numbers of informative characters which cpDNA markers appear to provide at this taxonomic level in Annonaceae (Table 1), this is probably not a realistic strategy for deriving a fully resolved phylogeny of the genus. The development of protocols for more variable markers (such as single copy nuclear genes) is thus of importance to further addressing problems at this level.

The clades revealed correspond largely to geographic areas (see Figs 3&4). The divergence of the Venezuelan and Guianan lineages occurred prior to that leading to clades found either in the tropical Andes, or in the Chocó/Darién/

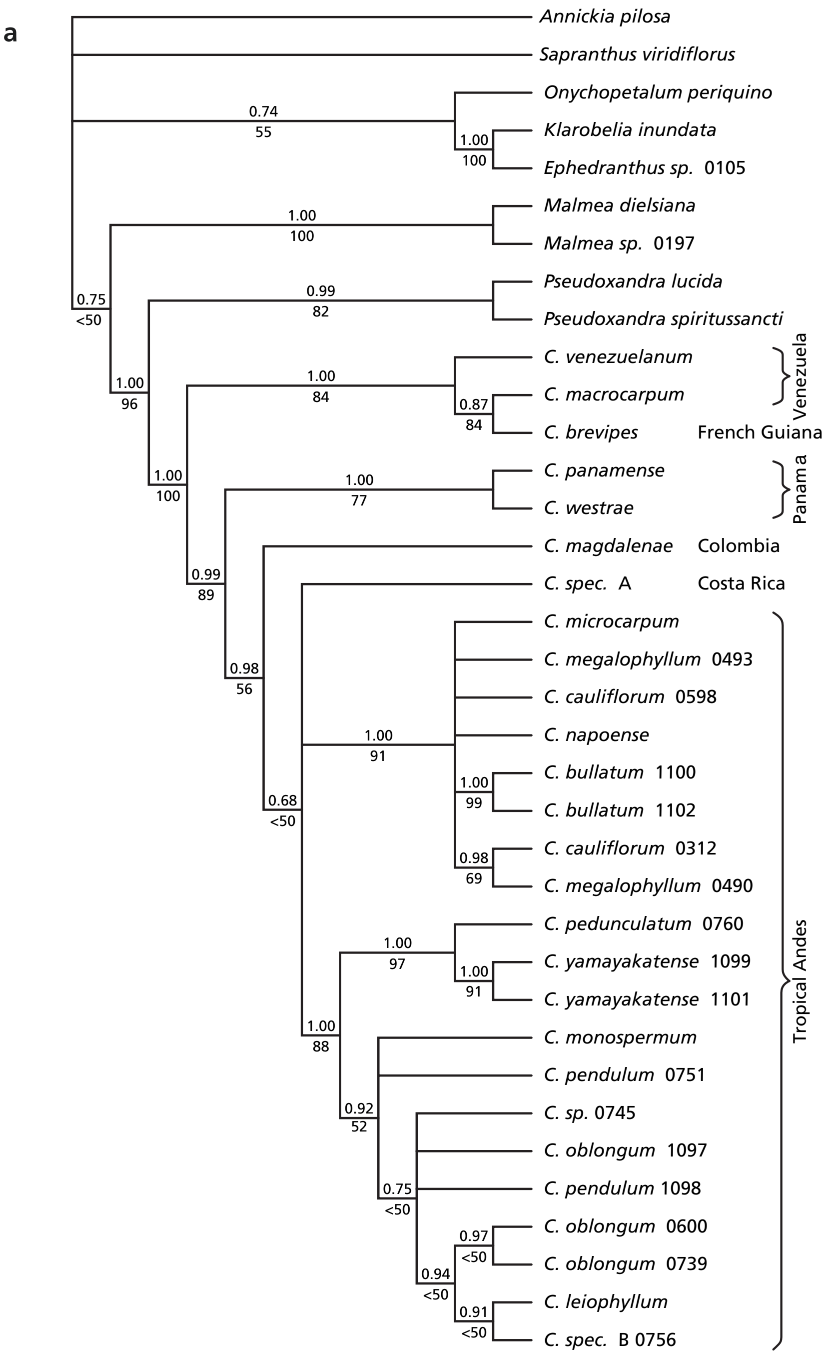

a
Annickia pilosa
Sapranthus viridiflorus
Onychopetalum periquino
Klarobelia inundata
Ephedranthus sp. 0105
Malmea dielsiana
Malmea sp. 0197
Pseudoxandra lucida
Pseudoxandra spiritussancti
C. venezuelanum
C. macrocarpum
C. brevipes
French Guiana
Venezuela
C. panamense
C. westrae
Panama
C. magdalenae
Colombia
C. spec. A
Costa Rica
C. microcarpum
C. megalophyllum 0493
C. cauliflorum 0598
C. napoense
C. bullatum 1100
C. bullatum 1102
C. cauliflorum 0312
C. megalophyllum 0490
C. pedunculatum 0760
C. yamayakatense 1099
C. yamayakatense 1101
C. monospermum
C. pendulum 0751
C. sp. 0745
C. oblongum 1097
C. pendulum 1098
C. oblongum 0600
C. oblongum 0739
C. leiophyllum
C. spec. B 0756
Tropical Andes
0.74
55
1.00
100
1.00
100
0.75
<50
0.99
82
1.00
96
1.00
84
0.87
84
1.00
100
1.00
77
0.99
89
0.98
56
1.00
91
1.00
99
0.68
<50
0.98
69
1.00
97
1.00
91
1.00
88
0.92
52
0.75
<50
0.97
<50
0.94
<50
0.91
<50

Fig. 4: ***Cremastosperma*** **phylogeny based on *rbcL*, *matK*, *trnT-F*, *psbA-trnH*, *ndhF*, and pseud*trnL*-F (taxa for which *ndhF* or pseud*trnL-F* was unavailable are excluded): a. Bayesian 50% consensus topology. Posterior probabilities (above the nodes) and bootstrap percentages (below). b. Phylogram resulting from Bayesian analysis (scale bar indicates 0.01 subst. per site)**

western Ecuador region or Central America (i.e. either the west or the east side of the Andes mountain chain). The tropical Andes species all fall into one of two clades. Relationships between these two clades, a further clade including the Central American species *Cremastosperma panamense* and *C. westrae*, and two isolated lineages corresponding to accessions from Costa Rica (*C. spec.* A) and the Magdalena valley of Colombia (*C. magdalenae*) are unresolved.

These results provide further evidence to suggest the importance of the Andean orogeny as a vicariance event in the history of the evolution of *Cremastosperma*. Further conclusions await a more resolved phylogeny of the genus.

Systematic treatment

Materials and methods

Measurements are based mostly on dried material. Where measurements are derived from material preserved in alcohol (and dimensions therefore often greater, as the structures are not subject to water loss) these are indicated between accolades {}.

Indument: unless otherwise indicated, descriptions of the indument of bracts, sepals, and petals refers to that on the outer side.

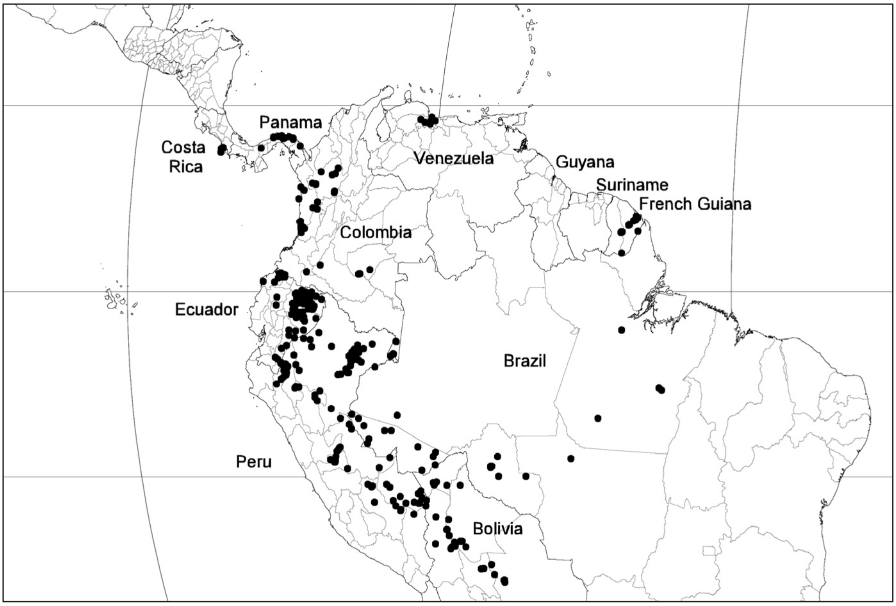
Leaf apex: in a few cases we make a distinction between acuminate and cuspidate (in the sense of abruptly acuminate) leaf apex shapes: this represents an arbitrary, but, the first author believes, nevertheless useful, delimitation within a pattern of continuous variation. An illustration accompanies this character in the key.

Inflorescence: according to Chatrou (1998), the inflorescences of all Annonaceae can be considered as terminal: apparently axillary inflorescences in genera such as *Guatteria*, *Klarobelia*, and *Pseudomalmea* consists of a short shoot, developing primarily from a leaf-axillary position, subtending a terminal pedicel with flower. The distinction between these two structures is in most cases clear, demarcated by an articulation. The short shoot bears a variable number of bracts (referred to here as lower bracts) which in occasional specimens are larger and leaf-like in appearance. In two species of *Cremastosperma* (*C. cauliflorum* R.E.Fr. and *C. napoense* Pirie), a rhipidium is formed by the development of further shoots from the axils of bracts on this short axillary shoot. The short axillary shoot is described here under the convenient term 'peduncle', following Maas & Westra (2003).

Cremastosperma – Map 1

Cremastosperma R.E.Fr. (1930) 46, f. 6a-c. – Type: *Cremastosperma pedunculatum* (Diels) R.E.Fr.

Trees or shrubs (0.5-)1.5-20 m tall; young twigs and petioles glabrous to densely covered with appressed or erect, simple, whitish to golden, up to 1 mm long hairs. *Leaves* distichous, simple, entire, petiolate, exstipulate; lamina elliptic to obovate, or narrowly so, index 1.6-5, chartaceous to coriaceous, glabrous (rarely sparsely covered with appressed or erect, simple, up to 1 mm long hairs) above, glabrous to densely hairy (particularly at the base and on veins) below, base acute, obtuse, or rounded (subcordate to cordate), apex (obtuse, acute) acuminate (or cuspidate), extreme tip rounded, venation brochidodromous, primary vein raised over entire leaf length above with an often conspicuous longitudinal groove particularly in the basal half, secondary veins 5-20(-30) on either side of the primary vein, often with 1-6 intersecondary veins, running parallel to primary vein for a short distance, thereafter angles with primary vein either increasing or decreasing towards the apex (or consistent), sometimes branching, often forming distinct loops, smallest distance between loops and margin 1-7 mm, tertiary veins percurrent (or reticulate). *Inflorescence* of single flowers, or occasionally up to 8 in a rhipidium, pendant, clustered in groups of up to 7, terminal on short axillary shoots (i.e. peduncles) on leafy or leafless twigs, older branches or on the main trunk (then often on brachyblasts). *Indument*: peduncles, pedicels, outer sides of bracts, sepals, and petals glabrous to densely covered with appressed or erect, simple, up to 1 mm long hairs, bracts, sepals, and petals ciliate. 1-several lower bracts, deltate to depressed ovate (rarely narrowly elliptic, leafy), rounded to acute, caducous or persistent; single upper bract attached to pedicel, ovate to deltate, acute to obtuse; closed flower buds (ovoid to triangular) broadly to depressed ovoid. *Flowers* actinomorphic, bisexual, with one whorl of free or slightly connate, imbricate, sepals and two whorls of free, imbricate, petals, green, creamy or yellow in vivo, often black in sicco; sepals and petals thin at margins, occasionally with prominent venation; sepals three, much smaller than petals; petals six, the outer ones ovate, elliptic, or broadly so, the inner ones elliptic to obovate or narrowly so, stamens numerous, spirally arranged, extrorse, inserted on and below a ventral ridge encircling a central depression in the receptacle in which the carpels are inserted, 1-2 mm long, connective appendage transversely rhombic-hexagonal; carpels 20-40, spirally arranged, free, ovary 1-locular, glabrous or hairy, with 1 basal, lateral, or apical ovule, stigma sessile. *Fruit* apocarpous, monocarps 5-40, stipitate, mostly asymmetrical, sometimes strongly so, sometimes with an often excentric apicule, green maturing mostly through red to brown or black in vivo, light brown to black in sicco. *Seeds* 1, basal, lateral or apical, ellipsoid to globose, yellow to



Map 1: **Distribution of *Cremastosperma***

reddish brown, surface deeply to shallowly pitted, lacking an aril, with a raised or sunken raphe encircling seed longitudinally (diagonally), regularly (or more sinuously), ruminations spiniform.

Distribution – Twenty-nine species in the Neotropics: from southern Costa Rica in the north to Bolivia in the south. Most species are distributed in regions surrounding the Andean mountain range, 2 in coastal Venezuela (*Cremastosperma macrocarpum* Maas and *C. venezuelanum* Pirie), 1 in French Guiana (*C. brevipes* (DC.) R.E.Fr.), and 1 widespread across Brazil south of the Amazon river (*C. monospermum* (Rusby) R.E.Fr.).

Habitat and Ecology – Lowland to premontane tropical wet forest, inundated areas, and terra firme. At elevations of 0–2000 m.

Key to species of Cremastosperma

A number of species of *Cremastosperma* cannot always be identified with certainty on the basis of fruiting material alone. Floral characters are of primary importance from lead 12 onwards in the identification key. From this point fruit characters are still included where they are also informative, and in combination with known distributions of the species may still allow successful determination in many cases where flowering material is unavailable. *C. spec.* A (only known from fruits) is not included. It is, however, the only species of the genus known from Costa Rica.

1a. Pedicels >120 mm long in flower, >150 mm long in fruit. Lamina 35–60 cm long – (Pacific coast of Colombia and Ecuador)..**12. C. longipes**
b. Pedicels <120 mm long in flower, ≤150 mm long in fruit. Lamina 8–64 cm long..2

2a. Lamina bullate, densely covered with hairs 1 mm long below and on margin. Pedicels c. 100 to 120 mm long in flower, 110–150 mm long in fruit – (The Peruvian state of Amazonas).......................................**4. C. bullatum**
b. Leaf lamina not, or rarely slightly, bullate, sparsely to densely covered with hairs up to 0.5 mm long or glabrous below. Pedicels <95 mm long in flower, <110 mm long in fruit...3

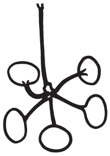
3a. Sepals 7–10 mm long, often persistent, densely covered with hairs c. 0.6 mm long. Stipes 1.5–4 mm long..4b.
Sepals ≤7 mm long, mostly caducous, sparsely to densely covered with hairs <0.6 mm long or glabrous. Stipes >4 mm long..5

4a. Monocarps sparsely to rather densely covered with hairs to 0.2 mm long – (Pacific Coast of Colombia)...................................**19. C. novogranatense**
b. Monocarps glabrous or sparsely covered with hairs to 0.1 mm long – (Panama)..**28. C. westrae**

5a. Leaf apex obtuse to acute, base cordate (rarely rounded), secondary veins 15–30 on each side – (N Venezuela)..........................**27. C. venezuelanum**
b. Leaf apex acuminate to cuspidate, base acute to obtuse (rarely rounded or cordate), secondary veins mostly <20 on each side......................................6

6a. Inflorescence branching, of multiple flowers/fruits...............................7
b. Inflorescence not branching, of single flowers/fruits.............................8

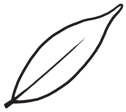
7a. Outer side of sepals and petals densely covered with hairs to 0.4 mm long. Monocarps globose to transversely broadly ellipsoid, rather densely covered with hairs to 0.2 mm long – (Amazonian Brazil, Ecuador, and Peru)..**5. C. cauliflorum**
b. Outer side of sepals and outer petals rather densely to densely covered with hairs to 0.2 mm long, inner petals largely glabrous but with a narrow, dense, sometimes branching band of appressed whitish-golden hairs to 0.2 mm long extending from the base to halfway towards the apex. Monocarps ellipsoid, glabrous – (Amazonian Ecuador)................................**18. C. napoense**



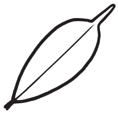
8a. Lamina up to 20 cm long. Inflorescence on main trunk. Pedicels glabrous. Monocarps ellipsoid, strongly asymmetrical – (Pacific coast of Colombia)..**7. C. chococola**
b. Lamina up to 64 cm long. Inflorescence on leafy twigs, thicker branches, or on the main trunk. Pedicels hairy or glabrous. Monocarps globose or ellipsoid, mostly slightly asymmetrical...9

9a. Monocarps hairy (if without fruits, go to lead 12).............................10
b. Monocarps glabrous, or appearing so (sparsely covered with hairs <0.1 mm long)..12

10a. Leaf base cordate to subcordate. Pedicels c. 8 mm long. Monocarps slightly longer than stipes – (The Peruvian state of Amazonas).........**6. C. cenepense**
b. Leaf base acute to obtuse, rarely rounded. Pedicels (5–)12–25 mm long. Monocarps roughly equal to or shorter than stipes....................................11



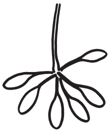
11a. Leaf apex cuspidate, lamina drying green. Flowers covered with hairs c. 0.2 mm long. Monocarps 10–15 mm long – (Amazonian Colombia, Ecuador, and Peru)..**9. C. gracilipes**



b. Leaf apex acuminate, lamina drying brown or greyish green. Flowers covered with hairs c. 0.3 mm long. Monocarps 8–11 mm long – (Amazonian Brazil, Colombia, and Peru).......................................**16. C. microcarpum**

12a. Sepals, petals, and pedicels hairy...13
b. Sepals and petals glabrous, pedicels mostly so.......................................18

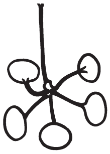
13a. Sepals ≥3 mm long..14
b. Sepals <3 mm long...15

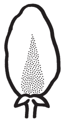


14a. Leaf axillary buds with conspicuous dense indument. Pedicels >25 mm long in flower. Monocarps narrowly ellipsoid, 27-28 mm long – (The Colombian state of Antioquia)...........................**8. C. dolichocarpum**
b. Leaf axillary buds inconspicuous. Pedicels ≤ 25 mm long in flower. Monocarps ellipsoid, up to 15 mm long..11

15a. Pedicels <17 mm long in fruit or flower – (Panama)......**28. C. westrae**
b. Pedicels >17 mm long in fruit or flower..16

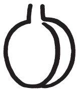
16a. Pedicels >30 mm long in flower – (W Ecuador)..**26. C. stenophyllum**
b. Pedicels <30 mm long in flower...17





17a. Outer side of petals rather densely (evenly) covered with hairs. Monocarps <15 mm long, shorter than stipes, strongly asymmetrical – (The Colombian state of Antioquia)..**1. C. antioquense**
b. Outer side of petals densely covered with hairs at base and in a line leading to the petal apex. Monocarps 22-28 mm long, longer than stipes, slightly asymmetrical – (Pacific coast of Colombia and Ecuador)..**2. C. awaense**

18a. Flower buds depressed ovoid or broadly ovoid-triangular, remaining closed in development..19
b. Flower buds depressed ovoid, opening (loosely) in development.........22

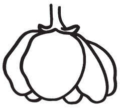


19a. Flower buds depressed ovoid...20

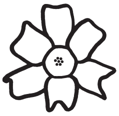
b. Flower buds broadly ovoid-triangular..21

20a. Pedicels >28 mm long in flower and fruit – (Central Peru)...**24. C. pendulum**
b. Pedicels <20 mm long in flower and fruit – (The Peruvian state of Amazonas)..**29. C. yamayakatense**

21a. Lamina ≤ 35 cm long. Pedicels 40-50 mm long in flower. Stipes 8-15 mm long – (Amazonian Bolivia, Brazil, and Peru)...........**17. C. monospermum**
b. Lamina > 35 cm long. Pedicels 18-20 mm long in flower. Stipes 20-40 mm long – (The Peruvian state of Amazonas).......................**25. C. peruvianum**



22a. Petals opening loosely in bud development, mostly covering reproductive organs on drying..23

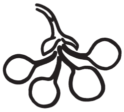


b. Petals opening widely in bud development, reproductive organs mostly exposed on drying...27

23a. Pedicels >30 mm long...24
b. Pedicels <30 mm long...25

24a. Sepals up to 2 mm long. Monocarps 18-24 mm long - (N Venezuela)..... ....**13. C. macrocarpum**
b. Sepals longer than 2 mm. Monocarps 12-17 mm long - (N Peru, Ecuador)...**23. C. pedunculatum**

25a. Sepals ≥ 4 mm long...26
b. Sepals < 4 mm long - (Pacific coast of Colombia)......**21. C. pacificum**



26a. Leaf lamina 20-28 cm long. Pedicels <10 mm long in flower. Monocarps globose - (The Colombian state of Antioquia)... ....**14. C. magdalenae**
b. Leaf lamina 13-57 cm long. Pedicel >10 mm long in flower. Monocarps ellipsoid to broadly so - (Amazonian Colombia, Ecuador, and Peru)............. ....**15. C. megalophyllum**

27a. Pedicels >20 mm long in flower...28
b. Pedicels <20 mm long in flower...29

28a. Pedicels up to 34 mm long in flower. Monocarps drying blackish, asymmetrical, the stipes thickening somewhat where they meet the monocarps - (Bolivia)...**10. C. leiophyllum**
b. Pedicels up to 68 mm long in flower. Monocarps drying light to dark brown or blackish, the stipes not markedly thickening where they meet the monocarps - (S Peru)...**31 C. spec B**

29a. Lamina coriaceous, up to 45 cm long. Sepals ≤2 mm long - (The Brazilian state of Acre and C and S Peru)......................................**20. C. oblongum**
b. Lamina chartaceous, up to 40 cm long. Sepals mostly >2 mm long........ 30

30a. Leaf apex cuspidate - (N Peru)................................**11. C. longicuspe**
b. Leaf apex acuminate..31

31a. Lamina 18-39 cm long, often drying reddish on veins below. Sepals 3-4 mm long. Monocarps ellipsoid - (French Guiana)............**3. C. brevipes**
b. Lamina up to 22 cm long, drying greenish. Sepals 1.5-3 mm long. Monocarps more or less globose - (Panama)....................**22. C. panamense**

Synoptical Key

Numbers cited refer to those used in the key above and descriptions. Those cited under more than one lead are indicated in bold. Where a character state is unknown for a given species the corresponding number is omitted.

1. Leaf axillary buds indument
    - a) Conspicuously hairy (versus inconspicuous and glabrous).
        8-19

2. Leaf length
    - a) <25 cm
        1-2-3-4-5-6-7-8-**9-10-11-13-14-15-16-17-18-20-21-22-23-24-29-30-31**
    - b) 25-35 cm
        1-2-3-4-5-9-10-11-13-14-15-16-17-18-19-20-21-23-24-26-**27-28-30-31**
    - c) >35 cm
        3-5-12-**15-18-19-20-21**-25-**27-28-30**

3. Leaf base
    - a) Cordate, subcordate or rounded (versus obtuse or acute)
        4-6-**19**-27

4. Leaf apex
    - a) Acute or obtuse
        27
    - b) Acuminate
        1-2-3-4-5-6-7-8-10-11-12-13-14-15-16-**17**-18-**19**-20-21-22-23-24-25-26-28-29-30-31
    - c) Cuspidate
        9-11-**17-19**

5. Leaf appearance
    - a) Bullate (versus not bullate)
        4-(**15**)

6. Secondary veins
a) ≥18 (versus <18)
4-15-20-25-**27**

7. Inflorescence position
a) On main trunk
1-4-5-7-**15**-(**20**)-**23-27-30**
b) On thicker branches and leafless twigs
1-2-3-**4-5-8**-10-12-**15-16-17**-18-**19-20**-21-**22-23-25**-26-**27-28-29-30-31**
c) On leafy twigs
1-2-4-6-**8**-9-11-13-14-**15-16-17-19-20-22-23**-24-**25-28-29-31**

8. Inflorescence
a) Branching (versus simple)
5-18

9. Pedicel length (in flower)
a) <10 mm
14-(**16**)-**20**-29
b) 10-20 mm
1-3-**5-9-10**-11-**15-16**-19-**20**-21-22-25-27
c) 21-80 mm
1-2-**5**-8-**9-10**-13-(**15**)-**16**-17-18-**23**-24-26-31
d) >80 mm
4-12-(**23**)

10. Pedicel length (in fruit)
a) <20 mm
3-5-6-**9-10-11-14-15-16-19-20-22-25-27**-28-29-**30**
b) 20-39 mm
1-2-3-5-7-9-10-11-14-15-16-17-18-**19-20**-21-**22-23-25-27-30**
c) 40-100 mm
1-2-5-7-8-13-(**15**)-**17**-(**20**)-**23**-24
d) >100 mm
4-12-(**23**)

11. Pedicel indument
    a) Hairy
        1-2-**3**-4-5-6-8-9-**11**-12-16-18-19-20-**21**-**23**-26-28
    b) Glabrous
        3-7-10-**11**-13-14-15-17-**21**-22-**23**-24-25-27-29-30-31

12. Flower buds
    a) Opening widely
        3-5-10-18-20-22
    b) Opening loosely
        1-2-8-9-11-12-13-14-15-16-19-21-23-26-27-28
    c) Remaining closed
        4-17-24-25-29

13. Closed flower bud shape
    a) Broadly ovoid-triangular (versus depressed ovoid)
        4-17-25

14. Sepals length
    a) ≤2.5 mm
        1-2-**10**-13-(**14**)-**17**-**18**-20-**22**-**23**-24-26-27-28
    b) 2.6-5 mm
        3-5-8-9-**10**-11-12-**14**-**15**-16-**17**-**18**-21-**22**-**23**-25-29-31
    c) 5.1-7.5
        4-**14**-**15**-**19**
    d) >7.5
        19

15. Sepals indument
    a) Hairy
        1-2-4-5-8-9-12-16-18-19-(**20**)-26-28
    b) Glabrous
        3-10-11-13-14-15-17-**20**-21-22-23-24-25-27-29-31

16. Sepals
    a) Mostly persistent
        14
    b) Occasionally and/or partially persistent
        8-15-19-22-28
    c) Caducous
        1-2-3-4-5-9-10-11-12-13-16-17-18-20-21-23-24-25-27-29-30-31

17. Monocarp shape
    - a) Globose to transversely broadly ellipsoid
      5-14-22
    - b) Ellipsoid
      1-2-3-4-6-7-**8**-9-10-11-12-13-15-16-17-18-19-20-21-23-24-25-27-28-29-30-31
    - c) Narrowly ellipsoid
      8

18. Monocarp symmetry
    - a) Strongly asymmetrical (versus slightly so)
      1-7

19. Monocarp length
    - a) <12 mm
      3-5-9-16-17-**22-24-31**
    - b) 12-18 mm
      1-**3**-4-**5**-6-7-**9**-10-11-14-**18-19-20-21-22**-23-**24-25-28**-29-30-**31**
    - c) 18.1-24 mm
      2-12-13-15-**18-19-20-25**-27-28
    - d) >24 mm
      2-8

20. Monocarps
    - a) Hairy (versus (appearing) glabrous)
      4-5-6-9-16-19

21. Stipe length
    - a) <4 mm
      19
    - b) 4-10 mm
      3-5-6-**9-11-13**-14-**15-16-17-19-20-21-22-24-28-31**
    - c) 10.1-20 mm
      1-**2-3**-4-**5**-7-8-**9-10-11-13-15-16-17-18-20-21-22-23-24-25**-27-**28**-29-30-**31**
    - d) 20.1-30 mm
      2-5-10-12-**15-18-22-23-25**
    - e) >30 mm
      (**5**)-**25**

22. Distribution
- a) French Guiana: 3
- b) N Venezuela: 13-27
- c) Central America (Costa Rica, Panama): 22-28
- d) Pacific coast of Colombia and Ecuador: 2-7-12-19-21-26
- e) The Colombian state of Antioquia: 1-8-14
- f) Amazonian Brazil: **5-16-17-20**
- g) Amazonian Colombia: **9-15-16**
- h) Amazonian Ecuador: **5-9-15**-18-**23**
- i) Peru: **4-5-6-9-15-16-17-23-24-25-29-31**
- j) N Peru: **4-5-6-9-15-16-17-23-25-29**
- k) The Peruvian state of Amazonas: **4-6-25-29**
- l) S and Central Peru: **24-25-31**
- m) Bolivia: 10-**17**

1. Cremastosperma antioquense Pirie – Fig. 5; Map 2

Cremastosperma antioquense Pirie (2005) 43, f. 1. – Type: *Soejarto 3586* (holo COL; iso F, GH, HUA, MO), Colombia, Antioquia: Mun. Anorí, Corregimiento Providencia, Buenos Aires, 4 km from Providencia, 500-700 m, 10 Dec. 1972.

Tree c. 5 m tall; young twigs and petioles sparsely covered with appressed brown hairs up to 0.2 mm long or glabrous. *Leaves*: petioles 7-10 by 2-3 mm; lamina narrowly elliptic, 16-27 by 6-9.5 cm (index 2.3-2.8), chartaceous, drying to a mosaic of brown and lighter green on both sides, glabrous on both sides, base obtuse, apex acuminate (acumen 10-15 mm long), primary vein grooved in the basal half, 1-1.5 mm wide at widest point, secondary veins 8-11, intersecondary veins occasional, distance between from 10 mm at the base to 50 mm closer to the apex, angles with primary vein from 50° at the base to 70° closer to the apex, forming distinct loops, smallest distance between loops and margin 3-4 mm, tertiary veins more or less percurrent. *Inflorescence* of single flowers, axillary on leafy twigs or from main trunk, then solitary or clustered in groups of at least two on brachyblasts; peduncles c. 2 by 1.5 mm (in flower), 2-3 by 1.5-2 mm (in fruit); pedicels 20-28 by c. 1 mm diam. at the base, 1.5-2 mm diam. at the apex (in flower), 20-40 by c. 2 mm diam. at the base, c. 3 mm diam. at the apex (in fruit), peduncles and pedicels sparsely covered with appressed whitish-golden hairs to 0.2 mm long; 2 lower bracts, deltate, c. 1 mm long, obtuse, caducous; upper bract attached around halfway along pedicel, deltate, 1 mm long, obtuse, outer side of upper and lower bracts rather densely to densely covered with appressed whitish-golden hairs to 0.2 mm long; closed flower buds not seen; flowers light green, stamens and carpels yellowish or pinkish in vivo, petals dark brown, contrasting to lighter colour of sepals and

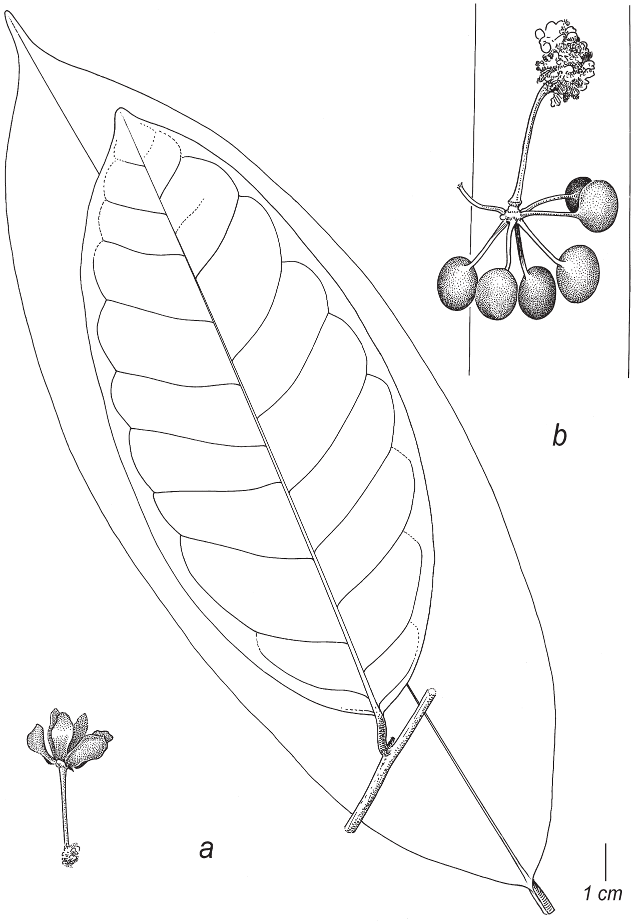


Fig. 5. ***Cremastosperma antioquense*** **Pirie. a. leaf and flower; b. fruit (a:** ***Soejarto 2798*****; b:** ***Soejarto 3586*****)**

pedicels in sicco; sepals fused at base, deltate, appressed, 2–2.5 by 2–2.5 mm, acute, caducous, sparsely to rather densely covered with appressed whitish-golden hairs to 0.2 mm long; outer petals elliptic, c. 12 by 8 mm, inner petals elliptic, 10–12 by 5–6 mm, outer side of outer and inner petals rather densely covered with appressed whitish-golden hairs to 0.2 mm long; receptacle depressed ovoid; androecium 5-7 mm diam., stamens c. 1 mm long, connective appendage roughly rhombic, 0.5–0.7 mm wide, glabrous; gynoecium 1-1.5 mm diam., carpels c. 1.5 mm long, glabrous. *Monocarps,* stipes, and receptacle glabrous, monocarps c. 10, ellipsoid to broadly so, strongly asymmetrical, 13–14 by c. 11 mm, orange to deep red, maturing to black in vivo, dark reddish brown in sicco, with an excentral apicule; stipes orange to deep red in vivo, c. 20 by 1.5 mm; fruiting receptacle depressed ovoid, c. 6 mm diam. *Seeds* ellipsoid, reddish brown with dark pits each surrounded by a raised rim, c. 12 by 9 mm, raphe sunken, regular.

Distribution – Colombia (Antioquia).

Habitat and Ecology – Primary forest. At elevations of 500–700 m. Flowering: February, fruiting: December.

Notes – *Cremastosperma antioquense* is similar to *C. awaense*, particularly in the appearance of the flowers. However, the fruits of the two species are more distinct: in contrast to *C. awaense*, the monocarps of *C. antioquense* are smaller, shorter than the stipes, strongly asymmetrical and entirely glabrous. In addition, none of the collections of *C. awaense* display cauliflory, a condition found in both of the two collections of *C. antioquense*.

2. Cremastosperma awaense Pirie – Fig. 6; Map 3

Cremastosperma awaense Pirie (2005) 45, f. 2. – Type: *Aulestia 842* (holo QCNE; iso U), Ecuador, Carchi: Maldonado, parish of Tobar Donoso, Ethnic Reserve Awá, Sabalera, 900 m, 22 Nov. 1992.

Tree 4–15(–20) m tall, 8–25 cm diam.; young twigs and petioles sparsely to rather densely covered with appressed golden hairs to 0.3 mm long. *Leaves*: petioles 4–11(15) by 1.5–3 mm; lamina narrowly elliptic to slightly obovate, 17–33 by 5.5–13 cm (index 2.2–3.6), chartaceous, brown/grey green above, darker below, veins on underside dark brown, glabrous above, veins sparsely to rather densely covered with appressed golden hairs to 0.3 mm long below, base obtuse to acute, apex accuminate (acumen 10–20 mm long), primary vein 1–3 mm wide at widest point, secondary veins 7–11, intersecondary veins occasional, distance between from 10 mm at the base to up to 60 mm closer to the apex, angles with primary vein from 45–50° at the base to 55–60° closer to the apex, forming loops in the apical half, smallest distance between loops and margin 1–3 mm, tertiary veins with some reticulation. *Inflorescence* of single, solitary flowers, axillary on leafy or leafless twigs; peduncles c. 1.5 by 1 mm (in flower), 1.5–3 by 1–2 mm (in fruit); pedicels 27–28 by c. 1 mm (in flower), 35–60 by 1–2 mm (in fruit), peduncles and pedicels rather densely to densely covered with appressed golden hairs to 0.3 mm long; single lower bract, broadly elliptic, 1–2 by 1–1.5 mm, obtuse, caducous, outer side densely covered with appressed golden hairs to 0.3 mm long; upper bract attached around midway along the pedicel, broadly elliptic, 1–2.5 by 1–2 mm, obtuse, rather densely to densely covered with appressed golden hairs to 0.3 mm long; flower buds depressed ovoid; flowers green or cream in vivo, blackish in sicco; sepals free, deltate, reflexed (appressed in bud), 2–2.5 by 2–2.5 mm, obtuse, caducous, outer side rather densely to densely covered with appressed golden hairs to 0.3 mm long; outer petals elliptic to broadly elliptic, 10–15 by 8–9 mm, inner petals elliptic, 10–15 by 5–6 mm, appearing glabrous but sparsely to rather densely covered with appressed golden hairs to 0.2 mm long on the outer side, denser at the base and in a band leading from the base to the apex of the petals; stamens 1–1.5 mm long, connective appendage c. 1 mm wide; gynoecium c. 2 mm diam., carpels 30–40,

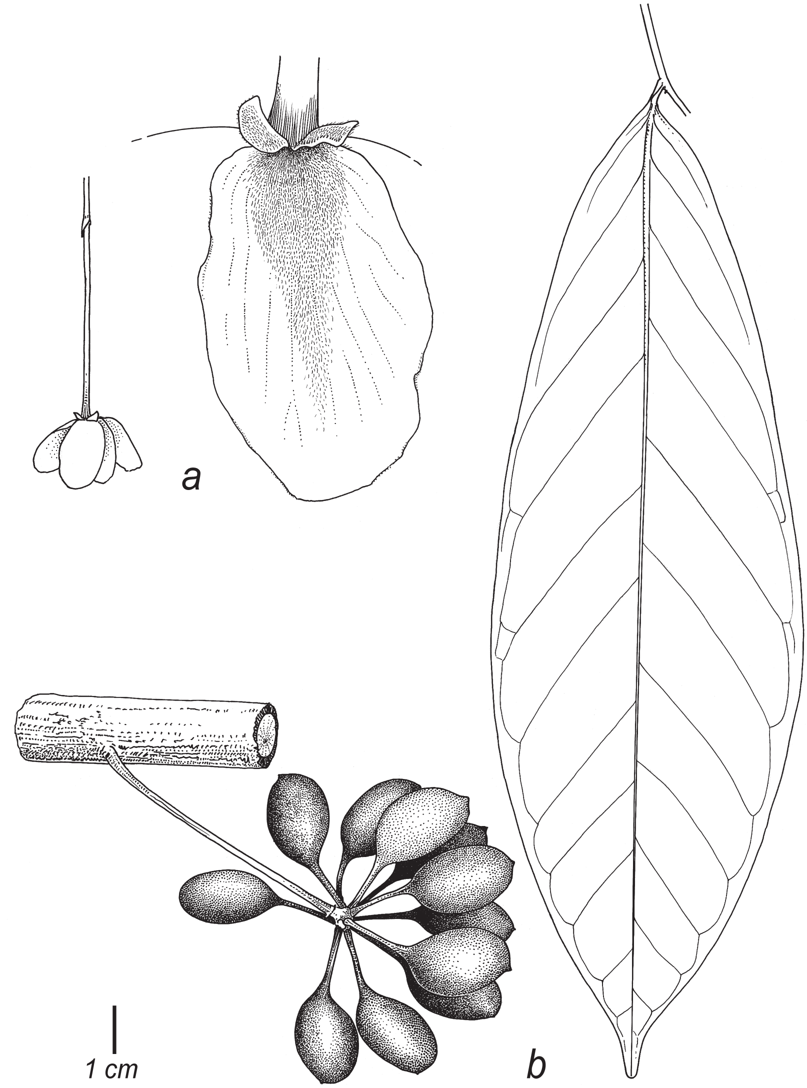


Fig. 6. ***Cremastosperma awaense*** **Pirie. a. flower; b. leaf and fruit (a:** ***Aulestia 842*****; b:** ***Van der Werff 12045*****)**

1-2 mm long, sparsely covered with golden, < 0.1 mm long hairs. *Monocarps* 10-12(-20), ellipsoid, slightly asymmetrical, 22-28 by 12-17 mm, brown in sicco, with an excentral apicule or rarely a nipple-like protuberance; stipes 11-24 by 1-1.5 mm; fruiting receptacle depressed ovoid, 3.5-8 mm diam., monocarps, stipes and receptacle very sparsely to sparsely covered with appressed white hairs, <0.1 mm long. *Seeds* ellipsoid, asymmetrical, yellow-orange with shallow pits, c. 19 by 11 mm, raphe sunken, regular.

Distribution – Pacific coast of Ecuador (Esmeraldas and Carchi) and Colombia (Nariño and Chocó).

Habitat and Ecology – Primary humid to pre-montane tropical forest. At elevations of 0–2,000 m. Flowering: January, September, and November; fruiting: January, February and June to September.

Vernacular names – Colombia: Guasca negra. Ecuador: Cargadera negra, Castaña negro, Huasca negra, Teuug teiug.

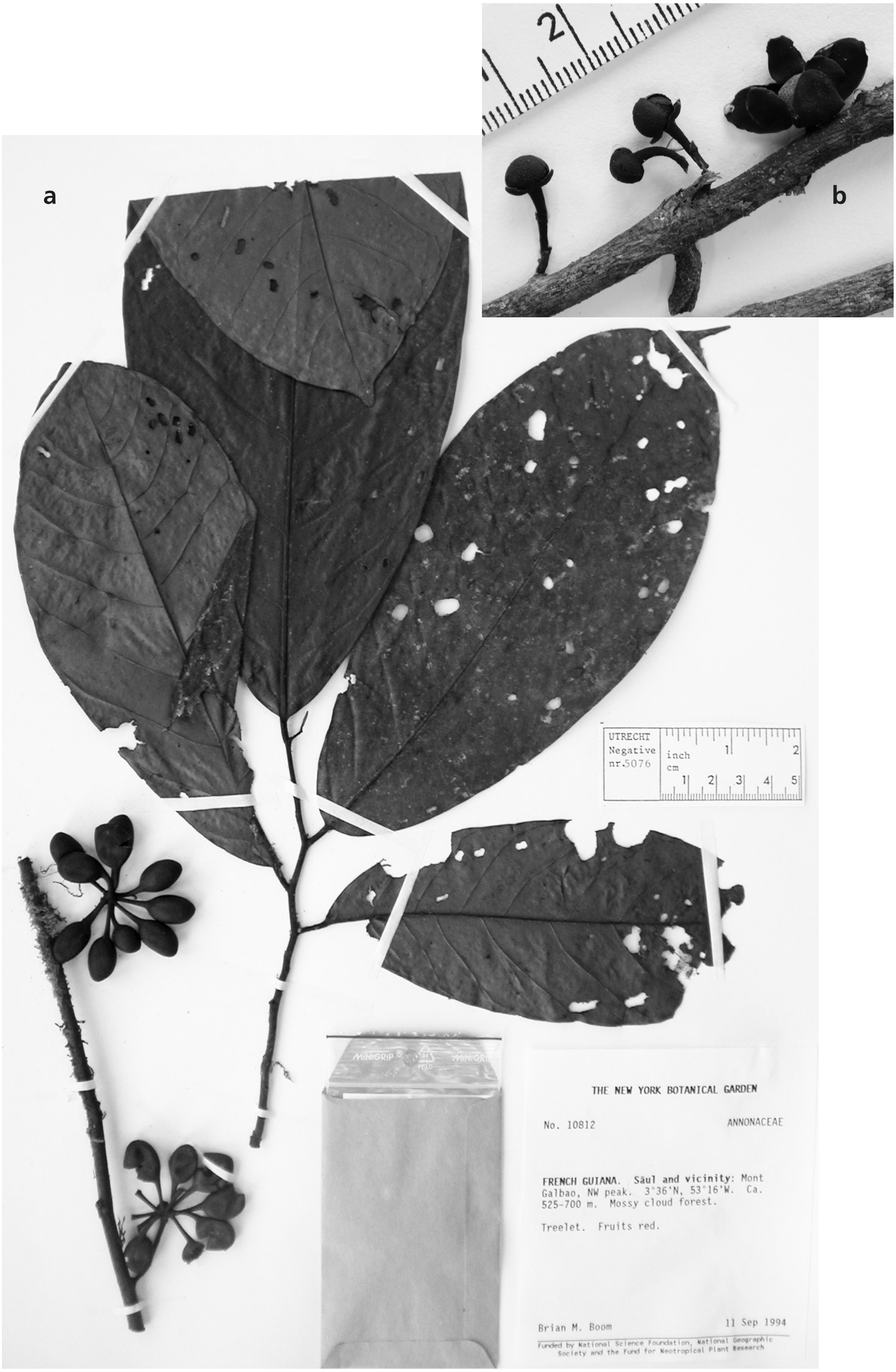
Note – *Cremastosperma awaense* can be distinguished by the unique pattern of indument on the outer sides of the petals: denser at base and in a line leading to the petal apex. The sparse indument of very short (<0.1mm) hairs on the monocarps and stipes are not visible without magnification, and the fruits appear glabrous. This character is also exhibited by some specimens of *C. westrae*. *C. awaense* can easily be distinguished from both *C. westrae* and the geographically closer *C. stenophyllum* Pirie on the basis of the length of the pedicel. That of *C. westrae* is shorter (not exceeding 17 mm) and that of *C. stenophyllum* longer (c. 45 mm in comparison to 27–28 mm in flower).

3. Cremastosperma brevipes (DC.) R.E.Fr. – Fig. 7; Map 4; App. 2, Fig. 2

Cremastosperma brevipes (DC.) R.E.Fr. (1939) 538. – *Guatteria brevipes* DC. in Dunal (1817) 126. – Type: *Martin s.n.* (lecto G, iso BM, K, S), French Guiana, without location.

Cremastosperma poiteaui (Diels) R.E.Fr. (1931) 328. – *Guatteria poiteaui* Diels (1931) 74. – Type: *Poiteau s.n.* (holo G; iso B, F, G), French Guiana, without location, 1819–1821.

Tree 4–20 m tall, 3–20 cm diam.; young twigs and petioles glabrous to sparsely covered with appressed white/yellowish hairs to 0.2 mm long. *Leaves*: petioles 4–9(–12) by 1.5–4 mm; lamina elliptic to obovate (or narrowly so), 18–39 by 7–15 cm (index 2–3.5), chartaceous, brown/green with a reddish tinge on both sides (particularly on the veins on the underside), darker above, glabrous on both sides, base obtuse to rounded (rarely acute), apex acuminate (acumen 5–20 mm long), primary vein 1.5–3 mm wide at widest point, secondary veins 9–15, intersecondary veins 0–2, distance between from 5–10 mm at the base to 35 mm closer to the apex, angles with primary vein from 45–60° at the base to 55–70° closer to the apex, not branching, forming mostly distinct loops, smallest distance between loops and margin 1–3 mm, tertiary veins percurrent. *Inflorescence* of single flowers, solitary or clustered in groups of 2, on leafless twigs; peduncles 1–2 by 1–2 mm (in flower), 2–5 by c. 2 mm (in fruit), sparsely to rather densely covered with appressed white/yellowish c. 0.1 mm long hairs; pedicels 15–20 by 1–2 mm at the base (in flower), 18–23 by c. 2 mm (in fruit), sparsely covered with appressed white/yellowish hairs c. 0.1 mm long or glabrous; 2 lower bracts, deltate, basal to 0.2 mm long, apical 0.3–0.5 mm long, obtuse, mostly



THE NEW YORK BOTANICAL GARDEN

No. 10812 ANNONACEAE

FRENCH GUIANA. Saül and vicinity: Mont Galbao, NW peak. 3°36'N, 53°16'W. Ca. 525-700 m. Mossy cloud forest.

Treelet. Fruits red.

Brian M. Boom 11 Sep 1994

Funded by National Science Foundation, National Geographic Society and the Fund for Neotropical Plant Research

Fig. 7. ***Cremastosperma brevipes*** **(DC.) R.E.Fr. a. fruiting specimen; b. flower buds (a:** ***Boom 10812*****; b:** ***Prévost 3446*****)**

persistent, rather densely covered with appressed white/yellowish hairs c. 0.1 mm long; upper bract mostly attached midway along pedicel, deltate to broadly ovate, 1-2 by c. 1 mm, obtuse or emarginate, sparsely covered with appressed white/yellowish hairs c. 0.1 mm long; closed flower buds depressed ovoid, opening in development; flowers green, sometimes tinged with red around margins of petals or creamy yellow in vivo, reddish or dark brown in sicco, sepals and petals glabrous; sepals connate for 0.5-1 mm, broadly ovate, recurved (rarely appressed), 3-4{-5} by 3.5-5 mm, obtuse, caducous; outer petals elliptic, 12-22 by 7-12 mm, rounded, inner petals narrowly obovate to narrowly elliptic, 10-24 by 4-7 mm, obtuse; androecium c. 6 mm diam.; stamens 1.4-1.6 mm long, connective appendage 0.5-0.7 mm wide; gynoecium c. 1 mm diam.; carpels c. 25, c.2.3 mm long, glabrous. *Monocarps* 7-17, ellipsoid, slightly asymmetrical, 11-17 by 9-11 mm, green maturing to red, reddish brown, dark purple or black in vivo, blackish or reddish brown in sicco, with an excentric apicule; stipes green maturing to red in vivo, 7-14 by 1-1.5(3) mm; fruiting receptacle depressed ovoid, 4-8 mm diam., monocarps, stipes, and receptacle glabrous. *Seeds* broadly ellipsoid to globose, yellowish or orange-brown, lightly pitted, c. 8 by 7-8 mm, raphe raised within a sunken groove, somewhat irregular.

Distribution – French Guiana: region of Saül and Nouragues.

Habitat and Ecology – Primary moist forest. At elevations of 200 – 800 m. Flowering: February, March, May, and October; fruiting: May – October, December, and January.

Vernacular names – French Guiana: Apélému, Maman yawé.

Notes – *Cremastosperma brevipes* is the only species of the genus found in the Guianas. The leaves, when dried, have a characteristic reddish tinge (particularly on the underside). This species is similar to *C. venezuelanum*, but differing in particular by the acuminate as opposed to acute or obtuse leaf apex of *C. venezuelanum*, smaller sepals and monocarps and shorter stipes.

Its use as fish bait has been reported. As of many Annonaceae, the bark of *C. brevipes* has been described as aromatic: on collection *Riera 668* a peppery smell is reported.

4. Cremastosperma bullatum Pirie – Fig. 8; Map 5; App. 2, Fig. 3

Cremastosperma bullatum Pirie in Pirie & Zapata (2004) 8, f. 2, 3-5. – Type: *Pirie et al. 71* (holo U; iso AAU, AMAZ, CUZ, E, F, HAO, HUT, K, MO, MOL, NY, US, USM, WU), Peru, Amazonas: Bagua, District Imaza, community Yamayakat, trail to Putuim, 420 m, 22 Nov. 2003.

Tree 2-10 m tall; young twigs and petioles densely covered with mainly erect golden hairs up to 1 mm long. *Leaves*: petioles 3-7 mm by 2.5-3 mm; lamina elliptic or narrowly so to slightly obovate, 17-28 by 6-11 cm (index 2.4-3.5), chartaceous,

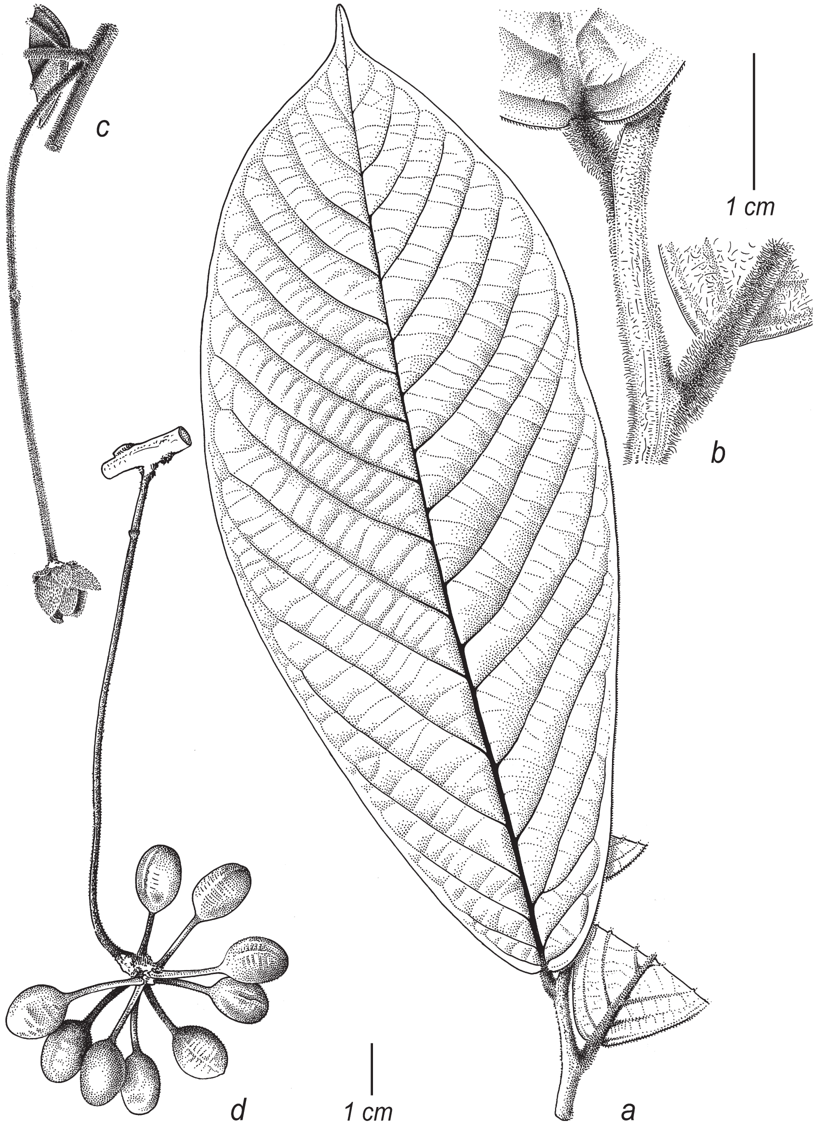


Fig. 8. ***Cremastosperma bullatum*** **Pirie a. leaf; b. leaf base; c. flower; d. fruit (a-c:** ***Vásquez et al. 24891*****; d:** ***Jaramillo, N. et al. 972*****)**

mid brown, occasionally slightly grey above (immature leaves drying black), sparsely covered with mainly erect golden hairs up to 1 mm long or glabrous above, densely so on edge of lamina and on all veins below, base rounded to subcordate, apex acuminate (acumen 5-20 mm long), primary, secondary and tertiary veins sunken in depressions in leaf surface, primary vein 1.5-2 mm wide at widest point, densely covered with mainly erect golden hairs up to 1 mm long above and below, secondary veins 15-20 (intersecondary veins rare), distance between from 6 mm at the base to 16 mm closer to the apex, angles with primary vein consistently around 60-70°, occasionally branching, forming distinct loops, smallest distance between loops and margin 1-1.5 mm; tertiary veins largely percurrent. Inflorescences of single, successively produced, flowers, axillary on leafy branches, on leafless branches and produced from the main trunk (then on brachyblasts); peduncles and pedicels sparsely to rather densely covered with mainly erect golden hairs up to 1 mm long, peduncles 17-20 mm by 1-1.5 mm diam. (in flower), 18-25 mm by c. 1.5 mm (in fruit); pedicels c.100 to 120 mm by c. 1 mm at the base (in flower), 110-150 by c. 1.5 mm (in fruit); bracts densely covered with mainly erect golden hairs up to 1 mm long, single lower bract, elliptic to ovate, 2.5 mm long, 1 mm wide, acute, persistent or partially caducous; upper bract within central third of pedicel length, elliptic to ovate, 2-3 mm long, 1-2 mm wide, acute; closed flower buds depressed ovoid developing to ovoid before opening, green in vivo, brown in sicco; flowers green, maturing to yellow with a basal orange patch on the outside of the outer petals in vivo, golden brown in sicco, outer sides and apical portion of the inner sides of petals and outer sides of sepals densely covered in appressed golden hairs up to 1 mm long, inner sides otherwise glabrous; sepals basally connate, 5-7 by c. 6 mm, deltate, acute, caducous, outer petals broadly ovate, c. 18 by 15 mm, inner petals ovate, concave, c. 25 by 12 mm wide; androecium c. 5 mm diam., stamens 1-1.5 mm long, connective appendage of inconsistent and irregular shape, c. 0.5 mm wide; gynoecium {c. 2.5} mm diam., glabrous. *Monocarps*, stipes, and receptacle sparsely to moderately densely covered with erect golden hairs up to 0.2 mm long, monocarps 8-10, dark brown in sicco, ellipsoid, slightly asymmetrical, c. 15 by 11 mm diam., often with an excentric apicule; stipes 14-16 by 1.5 mm; fruiting receptacle 5-6 mm in diam. *Seeds* ellipsoid, orange-brown, shallowly pitted, c.13 by 10 mm, raphe raised, regular.

Distribution – Peru (Amazonas).

Habitat and Ecology – Primary forest on red clay. At elevations of 300-500 m. Flowering: February and November; fruiting: November and June.

Notes – *Cremastosperma bullatum* can easily be distinguished from all other species of *Cremastosperma* by any one of the number of unique and striking characteristics it displays. The leaf blade has a blistered or bubbled (bullate) appearance, both in the field and when pressed, which is due to the deeply sunken nature of the primary, secondary, and tertiary venation. The indument present on many of its parts is far longer than in any other species in the genus, and, also uniquely in the genus, densely inserted in a halo-like formation around the leaf margin. Other notable characteristics are the unusually long pedicel, the orange colouring of the base of the outer petals of mature flowers, the inner petals considerably longer than the outer petals, and the rounded to subcordate shape of the leaf base.

5. Cremastosperma cauliflorum R.E.Fr. – Fig. 2 b, e, 9; Map 5; App. 2, Fig. 4

Cremastosperma cauliflorum R.E.Fr. (1931) 330. – Type: *Klug 902* (holo B; iso F, NY, US), Peru, Loreto: Mishuyacu, near Iquitos, 100 m, Feb.–Mar. 1930.

Tree 2–20 m tall, 4–25 cm diam.; young twigs and petioles glabrous to rather densely covered with appressed or erect golden hairs to 0.5 mm long. *Leaves*: petioles 4–12(–16) by 2–4(–6) mm; lamina elliptic to obovate or narrowly so, (14–) 20–61 by 5–14(–22) cm (index 2.3–3.7), chartaceous, olive/brown green above, darker below, glabrous above except for base of primary vein sparsely covered with appressed or erect hairs to 0.3 mm long, base, primary and secondary veins sparsely to rather densely covered with appressed or erect golden hairs to 0.5 mm long below, base acute to obtuse, apex acuminate (acumen 5–45 mm long), primary vein verrucose (particularly at the base), deeply grooved for most of length, 1.5–3.5(–5) mm wide at widest point, secondary veins (6–)10–17, occasionally 1–2 intersecondary veins, distance between from 4 mm at the base to up to 40 mm closer to the apex, angles with primary vein from 45–70° at the base to 45–60° closer to the apex, not branching, forming mostly distinct loops, smallest distance between loops and margin 1–5 mm, tertiary veins percurrent. *Inflorescence* of 1–5 flowers, branching, solitary or clustered in groups of up to 7, on thick leafless twigs or on main trunk (then often on brachyblasts); peduncles 3–12(–15) by 1–1.5(–3) mm (in flower), 3–15 by 1–3 mm (in fruit); pedicels 10–45 by 1–3 mm at the base (in flower), 15–45 by 1–3 mm (in fruit), peduncles and pedicels rather densely to densely covered with mainly erect golden hairs c. 0.3 mm long, often with hairs more densely covering the articulation point between shoot and pedicel; single lower bract (from the axil of which short shoots subtending new flowers develop), deltate, 1.5–2 mm long, acute, caducous, densely covered with mostly appressed golden hairs to 0.3 mm long; upper bract attached around midway along pedicel, broadly to very broadly ovate or deltate, 2–4 mm long, obtuse or acute, outer side densely covered with appressed or erect golden hairs to 0.3 mm long; closed flower buds depressed ovoid, opening in development; flowers (pale) green, creamy white, greenish yellow or yellow in vivo, brownish yellow or brown with orange, dark brown or black base in sicco, outer side of sepals and petals densely covered with erect or appressed golden hairs to 0.4 mm long, inner side of sepals and petals sparsely to rather densely covered with erect hairs to 0.4 mm long or glabrous, base glabrous; sepals free, broadly to very broadly ovate-deltate, mostly recurved, 3–5 by 4–6 mm, obtuse, caducous; outer petals elliptic to broadly elliptic, 10–25(–32) by 9–17 mm, inner petals elliptic, 11–21(–32) by 6–11 mm; androecium 7–10 mm diam., stamens 1.7–2.1 mm long, connective appendage roughly rhombic to diamond-shaped, 0.7–1 mm wide; gynoecium 2–3 mm diam., carpels c. 40, 2–2.3 mm long, sparsely to rather densely covered with mostly appressed golden hairs to 0.2 mm long. *Monocarps* 9–41, globose to transversely broadly ellipsoid, slightly asymmetrical, 8–13 by 10–14 mm, green maturing to orange, red, brown and black

Fig. 9. ***Cremastosperma cauliflorum*** **R.E.Fr. a. fruiting specimen; b. inflorescence (a:** ***Prance et al. 24094*****; b:** ***Vásquez & Jaramillo 11423*****)**

in vivo, blackish brown or brown in sicco, sometimes with an apicule at or near the apex, monocarps, stipes and receptacle rather densely covered with erect golden hairs to 0.2 mm long; stipes 7-23(-32) by 1-2 mm; fruiting receptacle depressed ovoid, 4-11 mm diam. *Seeds* broadly ellipsoid to globose, orange, pitted, 9-10 by 9-10 mm, raphe sunken, regular.

Distribution – Amazonian Colombia, Ecuador, Peru, and Brazil (Acre, Amazonas).

Habitat and Ecology – Moist primary forest, mostly non-inundated areas, on clayey or lateritic soil or white sand. At elevations of 100 – 500 m. Flowering: June, August, October-February; fruiting: May-February.

Vernacular names – Colombia: Espintana blanca. Ecuador: Mantach, Moncapatahue, Piton, Uñetahue, Uñitague. Peru: Bara caspi, Espintana.

Notes – *Cremastosperma cauliflorum* is one of the only two species of the genus displaying a branched inflorescence. It can be discerned from the other, *C. napoense*, by the presence of indument on the monocarps and stipes and by the greater length and density of hairs on the inflorescences.

The wood is aromatic, flowers reported as vanilla scented.

6. Cremastosperma cenepense Pirie & Zapata – Fig. 10; Map 5

Cremastosperma cenepense Pirie & Zapata (2004) 13, f. 2, 9. – Type: *Rojas et al. 269* (holo U; iso AMAZ, HUT, MO, USM); Peru, Amazonas: prov. Condorcanqui, Río Cenepa region, community Mamayaque, 400 m, 11 Aug. 1997.

Tree c. 10 m tall; young twigs and petioles sparsely (axillary buds densely) covered with appressed golden hairs c. 0.1 mm long. *Leaves*: petioles 4-7 by 1-2 mm; lamina narrowly elliptic, 12-22 by 4-8 cm (index 2.7-3), chartaceous, grey-yellow green above, light brown or yellowish green below, glabrous on both sides, base cordate to subcordate, apex acuminate (acumen 8-10 mm long), primary vein 1-1.5 mm wide at widest point, secondary veins 7-12, intersecondary veins occasional, distance between from 2-5 mm at the base to 15-25(35) mm closer to the apex, angles with primary vein from 90-80° at the base to 60-50° closer to the apex, forming distinct loops, smallest distance between loops and margin 2-5 mm, tertiary veins more or less percurrent. *Inflorescence* of single, solitary flowers, axillary on leafy twigs; peduncles c. 2 mm by 2 mm (in fruit); pedicels c. 8 mm by 2 mm at the base (in fruit), peduncles and pedicels sparsely covered with appressed golden hairs c. 0.1 mm long; 2 lower bracts, caducous; upper bract attached midway along pedicel, caducous; flowers not observed. *Monocarps* 8-10, blackish brown in sicco, ellipsoid, slightly asymmetric, 14-15 by 9-11 mm, with an excentric apicule, monocarps, stipes, and receptacle rather densely covered with appressed golden hairs c. 0.1 mm long; stipes 7-8 mm by c. 1.5 mm; fruiting receptacle 4-7 mm diam. *Seeds* ellipsoid, golden brown shallowly wrinkled (immature), c. 12 mm by 7 mm, raphe sunken, regular.

Distribution – Peru (Amazonas), in the area of the Cenepa River (a tributary of the Marañon River).

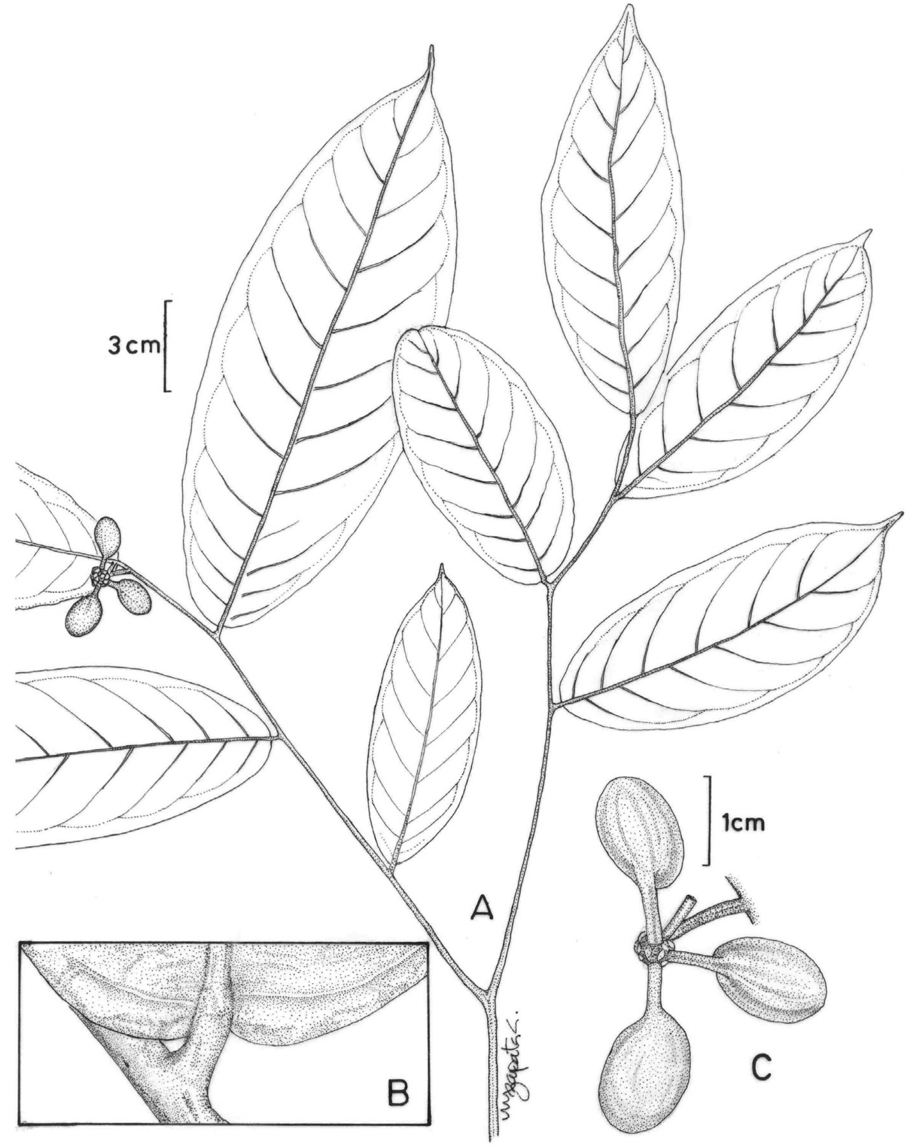


Fig. 10. ***Cremastosperma cenepense*** **Pirie & Zapata. A. fruiting twig; B. leaf base; C. fruit (A, C: *Rojas 269*; B: *Kayap 1078*)**

Habitat and Ecology – Primary forest. At elevations of 250–400 m. Fruiting: July and August.

Notes – *Cremastosperma cenepense* is similar to *C. yamayakatense* and *C. gracilipes*. It differs in the shape of the leaf base (cordate or subcordate as opposed to acute in *C. yamayakatense* and *C. gracilipes*), the indument on the fruits (rather dense as opposed to almost always absent in *C. yamayakatense*), and lengths of the pedicel (shorter than that of *C. gracilipes*) and stipes (shorter than those of *C. yamayakatense*).

7. Cremastosperma chococola Pirie – Fig. 11; Map 2

Cremastosperma chococola Pirie (2005) 47, f. 3. – Type: *Gentry & Forero 7286* (holo MO; iso COL), Colombia, Chocó: Alto de Buey, 500-1200 m, 8 Jan. 1973.

Tree c. 5 m tall; young twigs and petioles glabrous. *Leaves*: petioles 5-8 by 1.5-2 mm; lamina narrowly elliptic, 11-20 by 4-5.5 cm (index 3.7-4), chartaceous, dark/olive brown, shiny above, lighter pinkish brown, matt below, glabrous above and below, base acute to cuneate, apex acuminate (acumen 7-10 mm long), primary

Fig. 11. ***Cremastosperma chococola*** **Pirie. a. leaf and fruit (*****Gentry & Forero 7286*****)**

vein c. 1 mm wide at widest point, verrucose below, secondary veins 8-10, no intersecondary veins, distance between from 5 mm at the base to 29 mm closer to the apex, angles with primary vein from c. 60° at the base to 60-70° closer to the apex, forming distinct loops, smallest distance between loops and margin 2.5-3.5 mm, tertiary veins reticulate. *Inflorescence* of single flowers, solitary or clustered in groups of at least two, on brachyblasts on the main trunk; peduncles, 2-3 by 1-1.5 mm (in fruit); pedicels 38-42 by 1 mm diam. at the base, 1 mm diam. at the apex (in fruit), peduncles and pedicels glabrous; lower bract(s) not observed; upper bract attached within basal half of pedicel, ovate, c. 1 by 0.7 mm, obtuse, glabrous; flowers not observed. *Monocarps* 10-13, ellipsoid, strongly asymmetrical (stipes inserted within basal half of longest axis), 13-14 by 10-11 mm, with an excentral, to 0.2 mm long, apicule, green maturing through red to dark blue in vivo, dark brown in sicco, glabrous; stipes 15-18 by c. 1 mm increasing to 1.5 diam. when mature, glabrous; fruiting receptacle depressed ovoid, 4-5 mm diam., glabrous. *Seeds* ellipsoid, orange-brown, pitted, 9-11 by 6-8 mm, raphe sunken, regular.

Distribution - Pacific coast of Colombia (Chocó).

Habitat and Ecology - Tropical wet forest. At elevations of 0-1200 m. Fruiting: January and June

Notes - The strongly asymmetric monocarps of *Cremastosperma chococola* resemble those of *C. antioquense*, and collections of both species display cauliflory (though not exclusively so in *C. antioquense*) with inflorescences inserted on similar brachyblasts. However, *C. chococola* can easily be distinguished by its small, narrowly elliptic leaves with typical pinkish-brown colour on the underside, and by the absence of hairs on the pedicels.

8. Cremastosperma dolichocarpum Pirie - Fig. 12; Map 3

Cremastosperma dolichocarpum Pirie (2005) 49, f. 4. - Type: *Sánchez et al. 415* (holo U; iso COL), Colombia, Antioquia: Frontino, Nutibara, upper watershed of Río Cuevas, 15 Jul. 1986.

Tree 6-18 m tall, 15-22 cm diam.; young twigs and petioles sparsely covered with white-yellow appressed hairs 0.3-0.5 mm long. *Leaves*: petioles 3-6 by 1.5-2 mm, often with warts extending up primary vein; axillary buds densely covered with white-yellow appressed hairs 0.3-0.5 mm long; lamina elliptic to narrowly so, 14-24.5 by 6-10 cm (index 1.6-3.1), chartaceous or subcoriaceous, mid-dark brown above, lighter below, glabrous above, sparsely covered with white-yellow appressed hairs 0.3-0.5 mm long (particularly on veins) below, base obtuse-acute (narrowly cuneate), apex acuminate (acumen 4-15 mm long), primary vein not conspicuously grooved, 1-2 mm wide at widest point; secondary veins (5-)7-9(-11), intersecondary veins occasional, distance between from 5 mm at the base to 30 mm closer to the apex, angles with primary vein 40-50° at the base to 50-70° closer

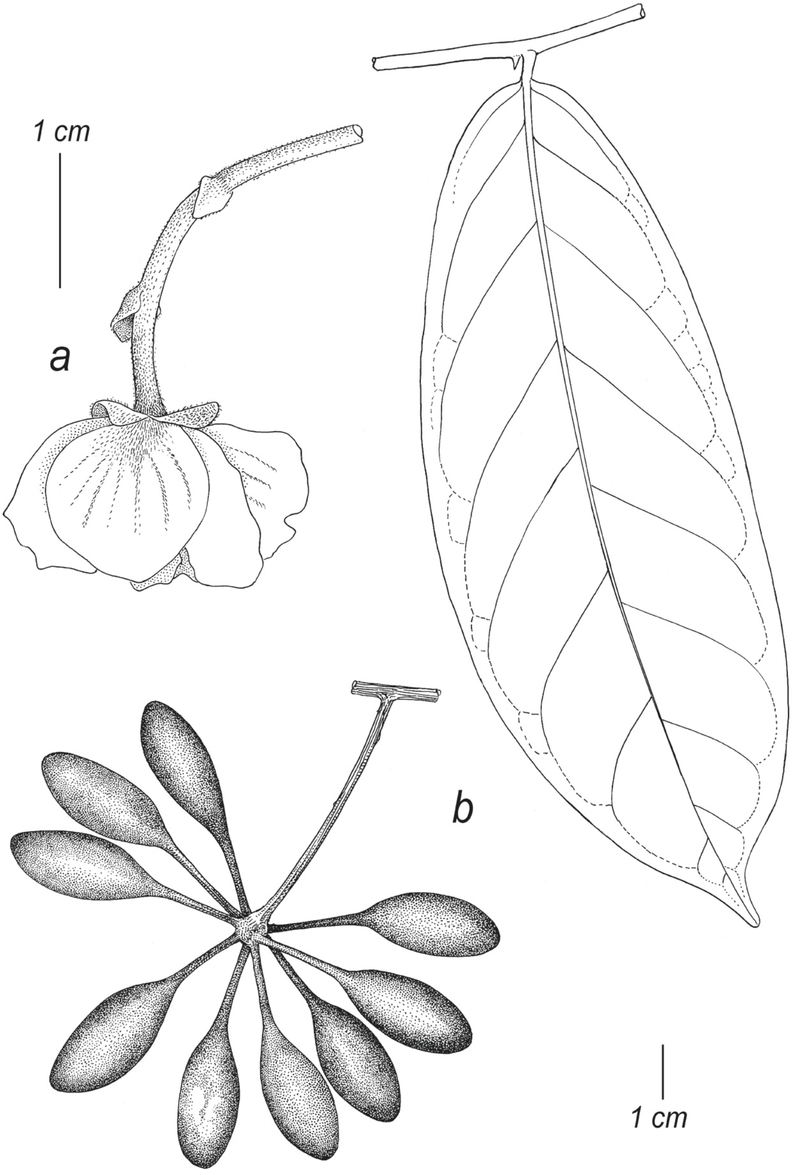


Fig. 12. ***Cremastosperma dolichocarpum*** **Pirie. a. flower; b. leaf and fruit (a:** ***Callejas 3110*****; b:** ***Sánchez 415*****)**

to the apex, not branching, forming distinct loops in the apical half–two thirds, smallest distance between loops and margin 1.5–4 mm; tertiary veins percurrent with significant reticulation. *Inflorescences* of single flowers solitary or clustered in groups of 2 (or more), produced from leafy twigs or leafless branches; peduncles of two internodes, the second 1.2–4 mm long, c. 1 mm diam. (in flower), approx. 2 mm long, 2 mm diam. (in fruit); pedicels 28–47 mm long, c. 1 mm diam. at the base, 1–1.5 mm diam. at the apex (in flower), 40–55 mm long, 1.5–2 mm diam.

at the base, 2-2.5 mm diam. at the apex (in fruit); peduncles and pedicels rather densely covered with white-yellow appressed hairs 0.3-0.5 mm long; two lower bracts (one on each internode), the apical one persisting later into flowering, 1-1.5 by 0.7-1 mm, broadly ovate, obtuse, caducous, rather densely covered with white-yellow appressed hairs 0.3-0.5 mm long; upper bract broadly to narrowly ovate, 1-3.5 by 1-2 mm, obtuse, persistent, densely covered with white-yellow appressed hairs 0.3-0.5 mm long; flower buds depressed ovoid; flowers green maturing to yellow with formaline-like scent in vivo, brown outside and black inside in sicco; sepals free, ovate, appressed, 3-4 mm long, obtuse, occasionally persistent on less mature fruit, densely covered with white-yellow appressed hairs 0.3-0.5 mm long; outer petals ovate to broadly so, 10-15 by 9-11 mm, inner petals ovate, 10-16 by 7-8 mm densely covered with white-yellow appressed hairs 0.3-0.5 mm long; receptacle ovoid to depressed ovoid; androecium 3-5 mm diam., stamens 1-1.2 mm long, connective appendage c. 1 mm wide, glabrous; gynoecium 1.5-2 mm diam., carpels 0.5-0.6 mm long, glabrous. *Monocarps* 10-20 (fully ripe fruit not observed), black in sicco, ellipsoid or narrowly so, 27-28 mm long, 11-12 mm diam., with an excentric apicule (obvious only in immature fruit); stipes 17-19 mm by 1.5-2 mm; monocarps and stipes sparsely covered with golden appressed hairs < 0.1mm long or glabrous; fruiting receptacle broadly ovoid, 4-5 mm diam., often sparsely covered with white-yellow appressed hairs 0.3-0.5 mm long. *Seeds* ellipsoid to narrowly so, c. 17 mm long and 7 mm diam., dark brown and wrinkled, raphe impressed, encircling seed diagonally.

Distribution – Colombia (Antioquia): northern and western foothills of the Cordillera Occidental.

Habitat and Ecology – At elevations of 1200-1500 m. Flowering: May and December; fruiting: May, July, and December.

Notes – *Cremastosperma dolichocarpum* can be distinguished from other species of *Cremastosperma* by the unique long-ellipsoid monocarps after which the species is named and identified even when sterile by the conspicuous axillary buds with dense indument (otherwise only observed in *C. novogranatense*, where they are less conspicuous).

9. Cremastosperma gracilipes R.E.Fr. – Fig. 1 d, 13; Map 4

Cremastosperma gracilipes R.E.Fr. (1931) 325. t. 26. – Type: *Tessmann 4748* (holo B; iso S), Peru, Loreto: upper Río Marañon, Puerto Melendez, below Pongo de Manseriche, 155 m, 13 Dec. 1924.

Tree or shrub 0.5-10 m tall; young twigs and petioles glabrous to sparsely covered with appressed brown hairs to 0.4 mm long. *Leaves*: petioles 2-8 by 1-2.5 mm; lamina elliptic to obovate or narrowly so, 11-28 by 3-10 cm (index 2-4(-4.7)), chartaceous, (pale) greyish or brownish green on both sides, often more greyish

Fig. 13. ***Cremastosperma gracilipes*** **R.E.Fr. a. fruiting specimen; b. flower (a:** ***Hurtado 3019*****; b:** ***Palacios 1651*****)**

above, glabrous on both sides, base acute to obtuse or rounded, apex cuspidate (cusp 10-35 mm long), primary vein lightly grooved for basal third, 1-1.5 mm wide at widest point, more or less verrucose below, secondary veins 8-17, often 1-3 intersecondary veins, distance between from c. 5 mm at the base to up to 25(-30) mm closer to the apex, angles with primary vein rather variable, from

45-80° at the base to 60-80° closer to the apex, forming distinct loops, smallest distance between loops and margin 2-6.5 mm, tertiary veins percurrent. *Inflorescence* of single, solitary flowers, on leafy twigs; peduncles 1-4 by c. 1 mm (in flower), 2-5 by 1.5-2 mm (in fruit); pedicels (12-)15-25 by 1 mm diam. at the base (in flower), 14-30 by 1-1.5 mm (in fruit), peduncles and pedicels rather densely covered with more or less erect brown hairs 0.2 mm long; 2 lower bracts, deltate, c. 1 mm long, caducous, rather densely covered with more or less erect brown hairs 0.2 mm long; upper bract attached around midway along the pedicel, ovate or broadly so, 1-3 by c. 1 mm, obtuse or acute, outer side sparsely to rather densely covered with appressed or erect whitish hairs to 0.2 mm long; flower buds depressed ovoid; flowers green to greenish yellow, pale yellow or cream in vivo, dark brown with a lighter brown calyx in sicco, outer sides of sepals and petals sparsely to rather densely covered with erect or appressed whitish hairs to 0.2 mm long, inner sides glabrous to sparsely covered with appressed whitish hairs to 0.2 mm long (or inner petals papillate); sepals free, broadly ovate to deltate, recurved, 3-4 by 2.5-4 mm, obtuse, caducous; outer petals (broadly) elliptic to ovate, 9-15 by 7-12 mm, inner petals elliptic, obovate, or narrowly so, 8-16 by 4-7 mm; androecium c. 5 mm diam., stamens 1.2-1.5 mm long, connective appendage roughly rhombic to diamond shaped, 0.7-0.8 mm wide; gynoecium c. 2 mm diam., carpels c. 25, c. 2.2 mm long, sparsely covered with erect golden hairs 0.1 mm long. *Monocarps* 3-23, ellipsoid, slightly asymmetrical, 10-15 by 7-9 mm, with an excentric apicule, green maturing to pink or yellow through to red, purple and black in vivo, reddish or dark brown in sicco, monocarps, stipes and receptacle sparsely to rather densely covered with erect whitish hairs 0.1 mm long; stipes green maturing to pink or yellow to red in vivo, 7-17 by 1-1.5 mm, increasing to 3 mm diam. when mature; fruiting receptacle 3-8 mm diam. *Seeds* ellipsoid, orange-brown, shallowly pitted, 5-8 by 3.5-6 mm, raphe sunken, regular.

Distribution – Amazonian Colombia, Ecuador, and Peru.

Habitat and Ecology – Often primary, but also secondary inundated and non-inundated forest. At elevations of 100-500 m. Flowering: January and April – August; fruiting: throughout the year.

Uses – infusions of the leaves are reported as being used to treat stomach pains.

Vernacular names – Ecuador: Ansuelo caspi muyo, Ayacara, Daycabome.

Notes – *Cremastosperma gracilipes* most closely resembles *C. microcarpum*. The hairs on the flowers are shorter and less dense, which results in their drying a darker brown. The leaves are further distinctive in the shape of the apex (markedly cuspidate with an often long drip-tip) and in the green colour they consistently retain on drying. The leaves of *C. longicuspe* are similar, but in contrast to *C. gracilipes* both flowers and fruit are entirely glabrous.

10. Cremastosperma leiophyllum R.E.Fr. – Fig. 14; Map 6; App. 2, Fig. 1a, b; 5

Cremastosperma leiophyllum R.E.Fr. (1931) 328. – *Guatteria leiophylla* Diels (1931) 77, non (Donn. Smith) Saff. – Type: *Buchtien 705* (holo B; iso US), Bolivia, La Paz: Mapiri, San Carlos, 850 m, 2 Dec. 1926.

Annona nitida Ruiz & Pav. in López (1959) 429, t. 488, non Martius (1841), nom. nud.

Guatteria rusbyi J.F.Macbr. (1929) 171. – *Guatteria lucida* Rusby (1927) 245, non C. Presl. – Type: *O.E. White 913* (holo NY), Bolivia, Beni: Covendo 630 m, 26 Aug. 1921.

Shrub or *tree* 3–20 m tall, 3–18 cm diam.; young twigs and petioles glabrous. *Leaves*: petioles 4–12 by 1–3(–4) mm; lamina (narrowly) obovate to narrowly elliptic, 12–28 by 4–9(–12) cm (index 2–3.9), chartaceous, often green or greenish brown, greyish above with darker or reddish veins, glabrous on both sides, base acute to obtuse, apex acuminate (acumen 5–15 mm long), primary vein 1.5–3 mm wide at widest point, verrucose, secondary veins 7–13, intersecondary veins 1–6, distance between from 14–24 mm at the base to 10–25 mm closer to the apex, angles with primary vein from 60–80° at the base to 40–50° closer to the apex, not branching forming distinct loops, smallest distance between loops and margin 2–4(–6) mm, tertiary veins percurrent. *Inflorescence* of single flowers, solitary or clustered in groups of up to 4, on older, leafless twigs; peduncles 1–2 by 1–2 mm (in flower), 2–4 by 1.5–3 mm (in fruit), sparsely covered with appressed golden <0.1 mm long hairs or glabrous; pedicels 18–34 by 1–1.5 mm at the base (in flower), 18–34(–43) by 1–3 mm (in fruit), glabrous; 1–3 lower bracts, depressed ovate, c. 0.5 by 1 mm, obtuse, caducous, glabrous; upper bract attached around midway along pedicel, ovate to broadly so, c. 1.5 by 1 mm, obtuse, glabrous; closed flower buds depressed ovoid, opening early in development; flowers green maturing to yellow or creamy yellow in vivo, dark yellow, reddish brown, or dark brown in sicco; sepals free, very broadly ovate-triangular, recurved, 2–3 by 2–3 mm, obtuse, caducous, sepals and petals glabrous; outer petals elliptic, 12–15 by 8–11 mm, rounded, inner petals elliptic, 13–15 by 6–8 mm; androecium c. 7 mm diam., pinkish in vivo, stamens 1.4–1.8 mm long, connective appendage c. 0.8 mm wide; gynoecium c. 2 mm diam., carpels 2–2.2 mm long, glabrous. *Monocarps*, stipes, and receptacle glabrous, monocarps 6–30, ellipsoid, asymmetrical, 14–17 by 8–9 mm, green maturing to yellow, orange-red and red in vivo, black (reddish brown when immature) in sicco, with an excentric apicule; stipes 16–26 by 1–1.5 mm; fruiting receptacle 4–9 mm diam. *Seeds* ellipsoid, light brown, pitted c. 12 by c. 7 mm, raphe sunken, regular.

Distribution – Bolivia (Beni, Cochabamba, La Paz, Santa Cruz).

Habitat and Ecology – Mostly in primary wet or moist forest, also in mildly disturbed areas, often on slopes or terraces, on sandstone soils. At elevations of 200–1000 m. Flowering: February, May, July through September, November, and December; fruiting: February through August, November, and December.

Fig. 14. *Cremastosperma leiophyllum* R.E.Fr. a. fruiting specimen; b. flower (a & b: *Seidel & Schulte 2265*)

Notes – *Cremastosperma leiophyllum* is the most southerly distributed species of the genus, and of the two found in Bolivia, the only endemic. Bud development in *C. leiophyllum* is open (as opposed to that of *C. monospermum*). It most closely resembles *C. spec.* B (which is not found in Bolivia), from which it can best be distinguished by the characteristic shape (asymmetrical, the stipes thickening somewhat where they meet the monocarps) and colour (blackish) of the mature fruits when dried.

11. Cremastosperma longicuspe R.E.Fr. – Fig. 15; Map 5

Cremastosperma longicuspe R.E.Fr. (1934) 203. – Type: *Poeppig s.n.* (lecto LE; iso S), Peru, Loreto: Maynas, anno 1831.

Cremastosperma killipii R.E.Fr. (1948) 3, P. I a-b. – Type: *Killip & Smith 29020* (holo US), Peru, Loreto: Yurimaguas, lower Río Huallaga, 135 m, Aug.-Sep. 1929.

Tree or *shrub* 1.5-20 m tall; young twigs and petioles sparsely covered with appressed whitish or golden hairs to 0.2 mm long. *Leaves*: petioles 4-14 by 1-3 mm; lamina elliptic, obovate, or narrowly so, 10-27 by 3-11 cm (index 1.5-5), chartaceous, green or greyish green above, green or brownish green below, glabrous above, very sparsely covered with appressed whitish hairs to 0.2 mm long particularly on veins below, base acute (rarely obtuse), apex cuspidate (cusp 20-35 mm long), primary vein 1-2 mm wide at widest point, secondary veins 7-15, intersecondary veins 0-1(-4), distance between from 7-15 mm at the base to 9-18 mm closer to the apex, angles with primary vein from 60-80° at the base to 40-50° closer to the apex, rarely branching, forming distinct loops, smallest distance between loops and margin 2-4 mm, tertiary veins more or less percurrent. *Inflorescence* of single flowers solitary (or clustered in groups of 2), on leafy twigs; peduncles 2-5 by c. 1 mm (in flower), 4-10 by 1-2 mm (in fruit), sparsely to rather densely covered with appressed golden hairs to 0.2 mm long; pedicels 10-14 by 1-1.5 mm at the base (in flower), 11-20 by 1-2 mm (in fruit), sparsely covered with appressed golden hairs to 0.2 mm long or glabrous; 2 lower bracts of unequal dimensions, basal lower bract deltate, c. 0.5 by 0.5 mm, acute, caducous, apical lower bract narrowly elliptic, c. 1.5 by 0.5 mm, rounded, caducous, lower bracts sparsely covered with appressed golden hairs to 0.1 mm long or glabrous; upper bract attached near base or midway along pedicel, ovate, c. 2 by c. 1 mm, acute, sparsely covered with appressed golden hairs to 0.1 mm long or glabrous; closed flower buds not seen; flowers yellowish in vivo, brown in sicco; sepals free, deltate, appressed or recurved, 3-4 by 3-4 mm, acute, caducous, sepals and petals glabrous; outer petals broadly elliptic, 10-12 by 9-12 mm, inner petals broadly elliptic, c. 11 by 10 mm; androecium not seen; gynoecium not seen. *Monocarps*, stipes, and receptacle glabrous, monocarps 6-13(-36), ellipsoid, slightly asymmetrical, 12-13 by 8-10 mm, white, red, deep red, or deep purple in vivo, reddish brown to dark brown or black in sicco, with an excentric apicule

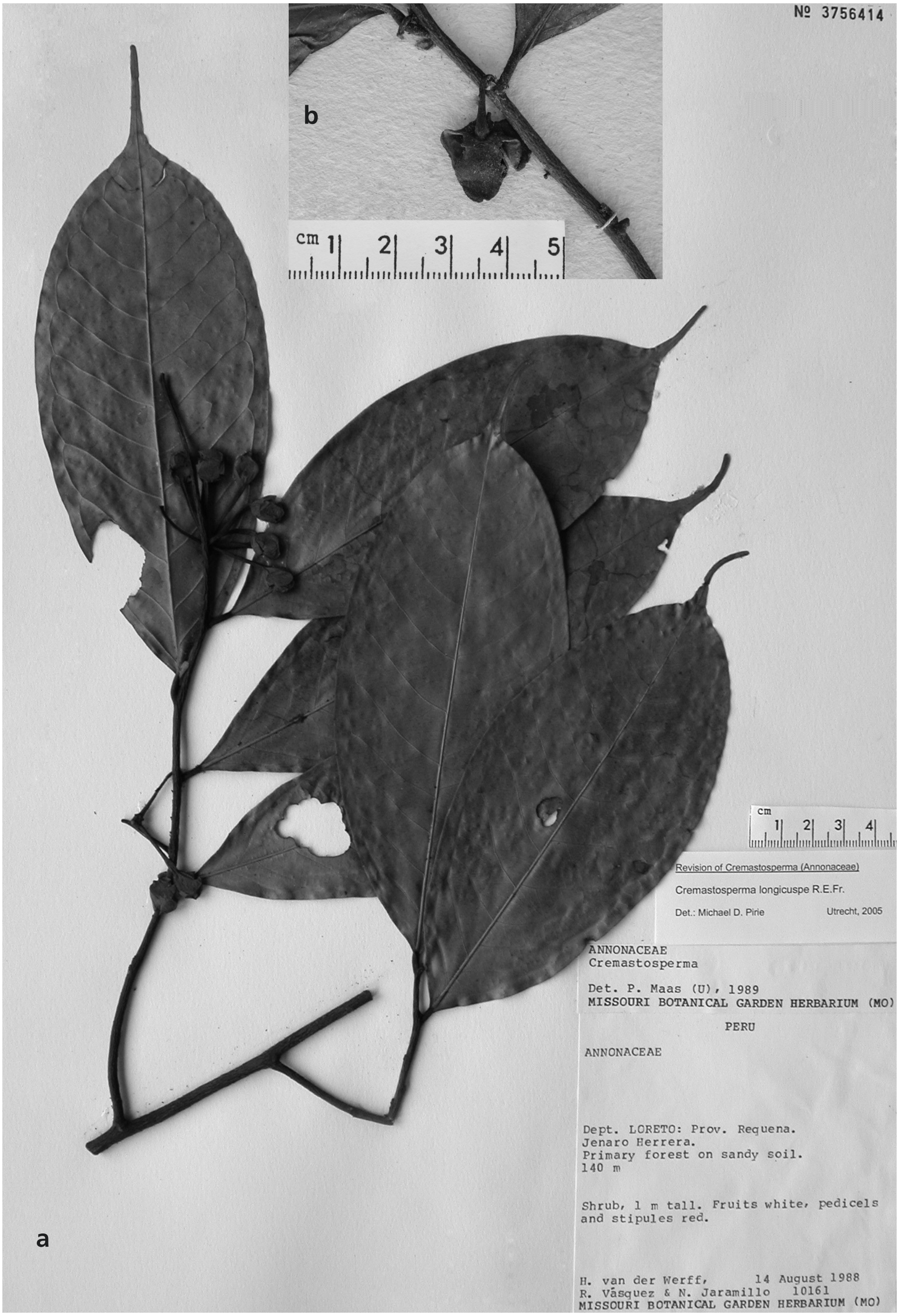


Fig. 15. *Cremastosperma longicuspe* R.E.Fr. a. fruiting specimen; b. flower (a: *van der Werff 10161*; b: *Poeppig s.n.*)

when unripe; stipes 10–19 by c. 2 mm; fruiting receptacle 4–5(–9) mm diam. *Seeds* ellipsoid, orange or reddish brown, 8–9 by 5–6 mm, raphe sunken, regular.

Distribution – Peru (San Martín and Loreto), most collections found in the basin of the Río Huallaga.

Habitat and Ecology – Primary tropical wet forest on sandy or white sand soil. At elevations of 140–200 m. Flowering: August–October; fruiting: February–June, August, September, and November.

Notes – *Cremastosperma longicuspe* most closely resembles *C. gracilipes*, particularly in the shape of the leaf. However, in contrast to *C. gracilipes*, the fruits and flowers are glabrous. In describing *C. killipii*, Fries (1948) noted its similarity to *C. longicuspe*. The leaves of the type specimen of *C. killipii* are unusually broad, but leaf and other characters otherwise fall within the variation found in *C. longicuspe*, including the notable cuspidate apex.

12. Cremastosperma longipes Pirie – Fig. 16; Map 2

Cremastosperma longipes Pirie (2005) 51, f. 5 – Type: *Forero et al. 6576* (holo COL; iso MO), Colombia, Chocó: San José del Palmar, mouth of Río Torito (tributary of Río Hábita), west slope, 3 Mar. 1980.

Tree 4.5–15 m tall; young twigs and petioles black, verrucose, sparsely to rather densely covered with white-golden appressed hairs c. 0.4 mm long. *Leaves*: petioles 10–15 mm long, 2.5–7 mm diam.; lamina elliptic to narrowly so, 35–60 by 10–25 cm (leaf index 2.3–3), chartacous to subcoriacous, olive/dark brown above, lighter below, glabrous above, sparsely covered with white-golden appressed hairs c. 0.4 mm long on veins below (densely so developing leaves), base acute, apex acuminate (acumen 10–15 mm long), primary vein deeply grooved in basal half, 2–6 mm wide at widest point; secondary veins 10–16, intersecondary veins rare, distance between from 10 mm at the base to 80 mm closer to the apex, angles with primary vein 45° at the base to 60–70° closer to the apex, not branching, forming distinct loops in the apical half to third of the leaf, smallest distance between loops and margin 3–4 mm; tertiary veins mainly percurrent. *Inflorescences* of single, pendulous flowers, produced from leafless branches; peduncles 5–8 mm long, c. 1 mm diam. (in flower), c. 4 mm long, 2 mm diam. (in fruit); pedicels 90 (less mature) – 210 mm long, c. 1 mm diam. at the base, 1.5 mm diam. at the apex (in flower), c. 240 mm long, 2 mm diam. at the base, 3 mm diam. at the apex (in fruit); peduncles and pedicels sparsely to rather densely covered with white-golden appressed hairs c. 0.4 mm long; single lower bract, broadly elliptic, 1–2 by c. 1 mm, acute, caducous, densely covered with white-golden appressed hairs c. 0.4 mm long; upper bract attached on lower half of pedicel, elliptic, 1.5–3 by c. 1 mm, acute, densely covered with white-golden appressed hairs c. 0.4 mm long; closed flower buds not seen; flowers green (immature) in vivo, medium brown in sicco; sepals free, triangular to broadly trullate,

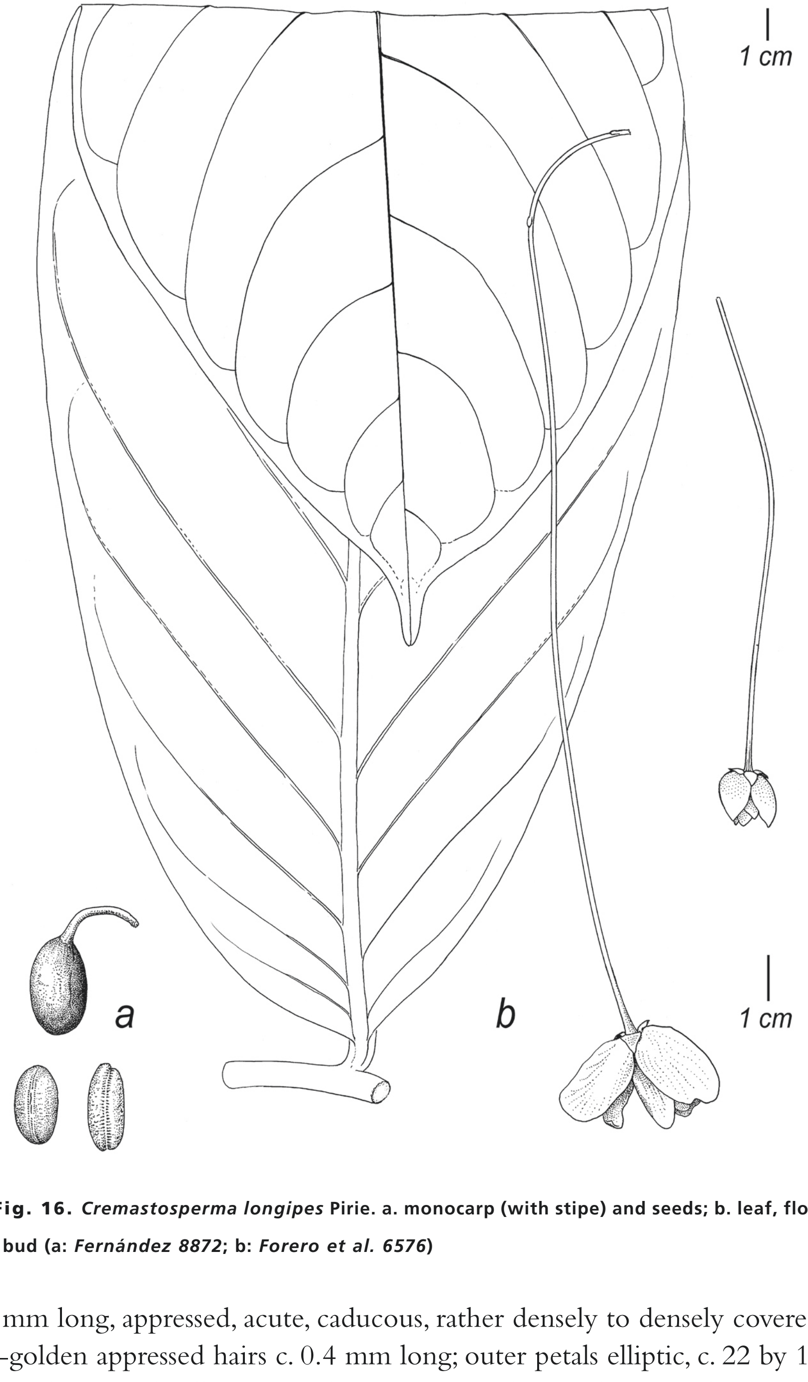


Fig. 16. ***Cremastosperma longipes*** **Pirie. a. monocarp (with stipe) and seeds; b. leaf, flower, and flower bud (a:** ***Fernández 8872*****; b:** ***Forero et al. 6576*****)**

3–4.5 mm long, appressed, acute, caducous, rather densely to densely covered with white-golden appressed hairs c. 0.4 mm long; outer petals elliptic, c. 22 by 12 mm, inner petals narrowly elliptic, c. 22 by 6 mm, sparsely to rather densely covered with white-golden appressed hairs c. 0.4 mm long; stamens, c. 1.2 mm long, connective appendage rhombic, 1 mm wide, glabrous. *Monocarps* c. 20, black in sicco, ellipsoid, slightly asymmetrical, c. 20 mm long, 12 mm diam., without an apicule, glabrous; stipes c. 25 mm long, 2 mm diam., glabrous; fruiting receptacle ovoid, 8 mm in diam, sparsely covered with white-golden appressed hairs c. 0.4 mm long. *Seeds* ellipsoid, 18-20 mm long, 8-9 mm diam., orange/brown, with many shallow pits, raphe slightly raised, encircling seed longitudinally.

Distribution – Pacific coast of Ecuador (Esmeraldas) and Colombia (Chocó and Riseralda).

Habitat and Ecology – Humid lowland to premontane forest. At elevations of 280-1400 m. Flowering: January and March; fruiting: September.

Notes – *Cremastosperma longipes* can easily be distinguished from other species of the genus by the exceptional length of the pedicel, after which the species is named. The flowers and fruits of most species of *Cremastosperma* are borne on pedicels less than 50 mm long, with rare exceptions such as *C. pedunculatum* and *C. bullatum* never exceeding 150 mm in length, significantly shorter than those of *C. longipes*. In addition, leaves of *C. longipes* are unusually large, equalling the maximum dimensions observed in *C. megalophyllum*, a more densely collected species from Amazonian Colombia, Ecuador, and Peru.

13. Cremastosperma macrocarpum Maas – Fig. 2 d, 17; Map 9

Cremastosperma macrocarpum Maas in Maas *et al.* (1986) 253, f. 2, 3b. – Type: *van der Werff & Vera, Flora Falcón 937* (holo U), Venezuela, Falcón: Sierra de San Luis, above Santa María, 1300 m, 26 Jul. 1979.

Tree 5-10 m tall; young twigs and petioles glabrous. *Leaves*: petioles 3-10 by 1-3 mm; lamina narrowly elliptic to elliptic, 10-30 by 4-9(12) cm (index 2.3-3.7), chartaceous, green, brownish green or brown on both sides, darker above, glabrous on both sides, base obtuse to rounded, apex acuminate (acumen 5-10 mm long), primary vein grooved in basal half, 1-2 mm wide at widest point, verrucose, secondary veins 6-12, intersecondary veins 1-2, distance between from 7-15 mm at the base to 7-23 mm closer to the apex, angles with primary vein from 60-80° at the base to 45-60° closer to the apex, not branching, forming mostly distinct loops, smallest distance between loops and margin 1-5 mm, tertiary veins mostly percurrent. *Inflorescence* of single, solitary flowers, on leafy twigs; peduncles 1 by 0.5-1 mm (in flower), 1-2 by 1-2 mm (in fruit), sparsely covered in appressed golden hairs to 0.1 mm long or glabrous; pedicels 35-45 by 0.5-1 mm at the base up to 2.5 mm diam. at the apex (in flower), 40-65 by 1-1.5 mm at the base up to 5 mm diam. at the apex (in fruit), glabrous; single lower bract, depressed ovate, 0.5 by 1 mm, rounded, caducous, rather densely covered with appressed golden hairs to 0.1 mm long; upper bract at or near base of pedicel, ovate, c. 1.5 by 0.8 mm, obtuse, glabrous; closed flower buds not seen; flowers pale greenish yellow with green base or cream-coloured in vivo, black in sicco, sepals and petals glabrous; sepals free, broadly to depressed ovate, recurved, 1.5-2 by 1.5-2 mm, obtuse, caducous;

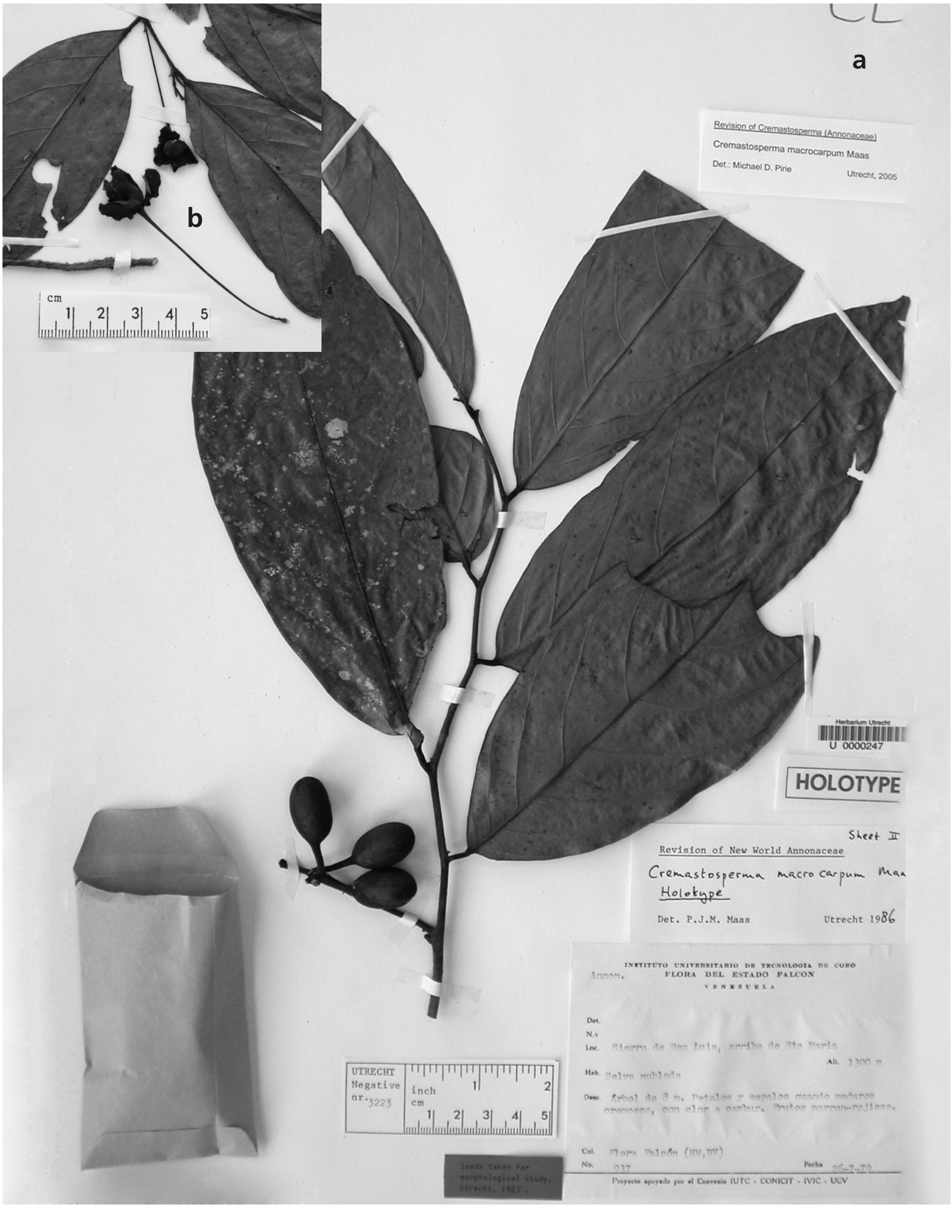


Fig. 17. ***Cremastosperma macrocarpum*** **Maas. a. fruiting specimen; b. flower (a & b:** ***van der Werff & Vera, Flora Falcón 937*****)**

outer petals ovate, 11-14 by 8-9 mm, obtuse, inner petals obovate, 15-16 by c. 7 mm, obtuse; androecium c. 6.5 mm diam., gynoecium not seen. *Monocarps*, stipes, and receptacle glabrous, monocarps 7-18, ellipsoid, slightly asymmetrical, 18-24 by 12-14 mm, very small strongly excentric apicule, green maturing to yellowish brown, brown or purple-black in vivo, reddish brown or dark brown in sicco; stipes 7-14 by c. 2 mm; fruiting receptacle 5-8 mm diam. *Seeds* ellipsoid, orange-brown, shallowly pitted, c. 20 by 8 mm, raphe sunken, regular.

Distribution – Venezuela (Falcón and Yaracuy).

Habitat and Ecology – Primary or secondary evergreen cloud forest. At elevations of 700–1500 m. Flowering: July; fruiting: March, May – July, October, and December.

Notes – One of only two species of *Cremastosperma* found in Venezuela, *C. macrocarpum* can most easily be distinguished from *C. venezuelanum* by its smaller leaves (10–30 cm as opposed to 30–53 cm long) and longer pedicels (40–65 mm as opposed to 16–22 mm in fruit).

14. Cremastosperma magdalenae Pirie – Fig. 18; Map 3

Cremastosperma magdalenae Pirie (2005) 53, f. 6. – Type: *Escobar & Folsom 3309* (holo NY, 2 sheets; iso HUA, U), Colombia, Antioquia: San Luis, Medellín-Bogotá highway, 8.1 km E of bridge over Río Caldera, 980–1020 m, 13 Mar. 1983.

Tree 3–7 m tall; young twigs and petioles slightly canaliculate and/or verrucose, glabrous. *Leaves*: petioles 6–14 mm long, 2–3 mm diam.; lamina narrowly elliptic, 20–28 by 7–9 cm (index 2.5–3.1), chartaceous to subcoriaceous, olive to more lime green or brown above, darker below, glabrous on both sides, base obtuse to acute, apex acuminate (acumen 5–10 mm long), primary vein deeply grooved in basal 1/2 – 3/4, occasionally verrucose below, c. 2 mm wide at widest point, glabrous, secondary veins 9–14, often 2 or 3 intersecondary veins, distance between from 4–5 mm at the base to 20–35 mm closer to the apex, angles with primary vein from 40–50° at the base to 70–80° closer to the apex, occasionally branching, occasionally forming more or less indistinct loops in the apical half, smallest distance between loops and margin 2–4 mm, tertiary veins rather reticulate. *Inflorescences* of single flowers, solitary or clustered in groups of two, axillary on leafy twigs; peduncles 2–3 mm long, c. 2 mm diam. (in fruit); pedicels c. 7 mm long, 1 mm diam. at the base, 1.5 mm diam. at the apex (in flower), 16–20 mm long, 1.5–2 mm diam. at the base, c. 3 mm diam. at the apex (in fruit), peduncles and pedicels glabrous, two lower bracts, the apical one depressed triangular, c. 1.5 by 2 mm, acute, persistent, glabrous, upper bract attached around midway along pedicel, broadly ovate to deltate, 0.6–2.5 by 0.8–2.5 mm wide, actute to obtuse, glabrous; closed flower buds not seen, flowers in vivo immature light green, black in sicco, sepals and petals glabrous; sepals fused for basal 1 mm, broadly to very broadly ovate, appressed, (2–)5–7 by (2–) c. 5 mm, acute, mostly persistent, outer petals elliptic, c. 12 by 7 mm, inner petals narrowly elliptic, c. 12 by 5 mm; androecium 2.5–2.7 mm diam., stamens c. 0.7 mm long, connective appendage rhombic, 0.3–0.4 mm wide. *Monocarps* 20–30, globose, symmetrical, 12–13 mm long, 12–13 mm diam., green maturing to red in vivo, black in sicco, glabrous, with a slightly excentral, 0.25 mm long apicule; stipes (immature) 9–10 mm long, 1–1.5 mm diam., glabrous; fruiting receptacle depressed

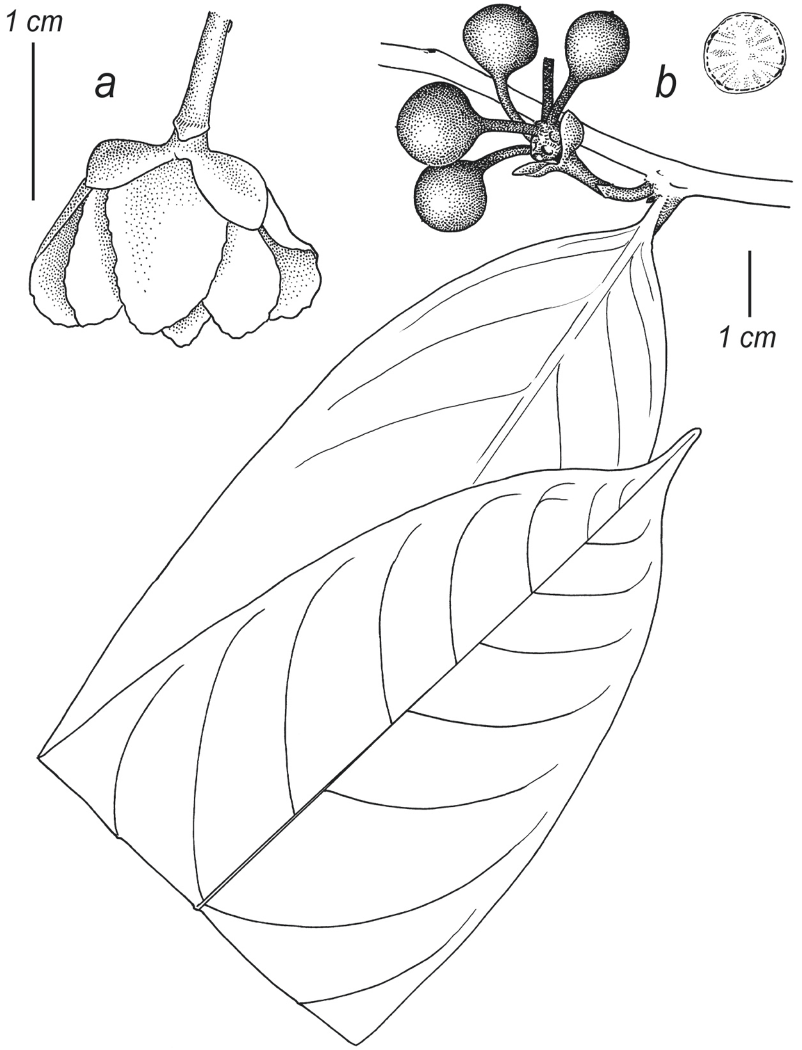


Fig. 18. ***Cremastosperma magdalenae*** **Pirie. a. flower; b. fruiting twig and cross section of seed (a:** ***Hernández 251*****; b:** ***Escobar & Folsom 3309*****)**

ovoid, 7–9 mm diam. (only immature fruits seen). *Seeds* globose, shallowly pitted with a papery outer layer, c. 13 by 11 mm, orange-brown, raphe neither raised nor sunken, regular.

Distribution – Colombia (Antioquia), west side of the Magdalena valley.

Habitat and Ecology – Disturbed primary or secondary forest. At elevations of 670–1200 m. Flowering and fruiting: March.

Note – *Cremastosperma magdalenae* Pirie can be distinguished from other species of the genus by the combination of globose monocarps and large sepals which mostly persist into fruiting (one slightly differing collection, *Cárdenas 2899*, displays immature fuits with smaller sepals only persistent on one of the two duplicates studied). Also noteworthy are the relatively short pedicels and the absence of indument on all parts. The absence of hairs on fruits and flowers reveals the blackish colour typical of specimens of *Cremastosperma* upon drying. Both *C. panamense* Maas and *C. pacificum* R.E.Fr. (a species found on the Pacific coast of Colombia) also lack indument, but, amongst other differences, the sepals of both species are much smaller and do not persist into fruiting.

15. Cremastosperma megalophyllum R.E.Fr. – Fig. 2 a, 19; Map 7; App. 2, Fig. 6

Cremastosperma megalophyllum R.E.Fr. (1931) 329. – Type: *Ducke RB19620* (holo S; iso B, RB), Peru, Loreto: Boca de Pebas, 23 Oct. 1927.

Tree or *shrub* 3-15 m tall, 2-15(-35) cm diam.; young twigs and petioles glabrous. *Leaves*: petioles 5-22 by 1.5-9 mm; lamina obovate to elliptic, or narrowly so, 13-57 by 3-26 cm (index 2-5.1), chartaceous to coriaceous, shiny on both sides, secondary veins often impressed above (giving slightly bullate appearance), (dark) greyish green or brown above, more brown or green below, glabrous on both sides, base obtuse to rounded, rarely acute or decurrent, apex acuminate (acumen (5-)10-30 mm long), primary vein conspicuously grooved in basal half, 1-5 mm wide at widest point, secondary veins (5-)8-21, intersecondary veins often 1-2(-3), distance between from 5-25 mm at the base to 9-35(-43) mm closer to the apex, angles with primary vein from (30-)45-70(-80)° at the base to (30-)40-75° closer to the apex, rarely branching, forming distinct loops, smallest distance between loops and margin 1-5 mm, tertiary veins percurrent. *Inflorescence* of single flowers, solitary or clustered in groups of up to 3, on leafy or leafless twigs or on the main trunk; short axillary shoot, 4-7(-8) by 0.7-1 mm (in flower), 4-10 by 1.5-4 mm (in fruit), glabrous or sparsely covered with whitish appressed hairs to 0.1 mm long; pedicels 10-20(-32) by c. 1 mm at the base, up to 2(-2.5) mm diam. at the apex (in flower), 15-30(-40) by 1.5-3 mm at the base, up to 4 mm diam. at the apex (in fruit), glabrous; 3 lower bracts, deltate, c. 1 by 1 mm, acute, caducous, sparsely covered with whitish appressed hairs to 0.1 mm long or glabrous; upper bract attached in apical half of pedicel, broadly ovate, 1-3 by 1-2 mm, obtuse, glabrous; closed flower buds depressed ovoid, opening loosely in development; flowers green maturing to yellow in vivo, black in sicco, sepals and petals glabrous; sepals free or connate for 1 mm, broadly ovate to triangular, appressed, open and conspicuous whilst petals still closed in young buds, 4-6 by 4-6 mm, obtuse to acute, sometimes briefly or partly persistent; outer petals broadly elliptic, 11-18 by 9-15 mm, obtuse, inner petals obovate, 10-16 by 5-7 mm, obtuse; androecium diam. unknown, stamens 1.3-1.8 mm long, connective appendage 0.7-1 mm wide; gynoecium diam. unknown,

Revision of Cremastosperma (Annonaceae)
Cremastosperma megalophyllum R.E.Fr.
Det.: Michael D. Pirie Utrecht, 2005

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ECUADOR

ANNONACEAE
Cremastosperma

Napo: Orellana Cantón

Parque Nacional Yasuní. Carretera de Maxus en construcción, Km 12-16. Bosque húmedo Tropical. Bosque primario.
00°31'S 76°32'W 240 m

Arbusto de 6 m de altura. Frutos verdes en cabezuela.
6 marzo 1993

Jorge Zuleta 175

Fig. 19. ***Cremastosperma megalophyllum*** **R.E.Fr. a. fruiting specimen; b. flower buds (a: *Zuleta 175*; b: *Palacios 3270*)**

carpels 1.5-2 mm long. *Monocarps*, stipes, and receptacle glabrous, monocarps 6-32, ellipsoid to broadly ellipsoid, (slightly) asymmetrical, 12-20 by 9-14 mm, green maturing to yellow, orange, purple and black in vivo, reddish or dark brown or black in sicco, often with an excentric apicule; stipes 10-30 by 1-2 mm; fruiting receptacle 3-12 mm diam. *Seeds* broadly ellipsoid, reddish-orange, pitted, c. 12 by 9-10 mm, raphe raised (more so when seeds immature), regular.

Distribution – Amazonian Peru, Ecuador, and Colombia.

Habitat and Ecology – Primary and secondary premontane or lowland rainforest, sometimes inundated, on red (oxisols/lateritic) or sometimes volcanic soils. At elevations of 100-1200 m. Flowering: April-June, September-January; fruiting: throughout the year.

Vernacular names – Ecuador: Caramoyu; Oñetahue (the plant itself), Oñetahuemo (the fruit) (Huaorani); Anchu panga cara caspi (wide-leaved bark wood) , Ayacara, Cucha casa caspi, Ichilla cara caspi (small bark tree), Lynshtimoia, Tara caspi or T'zinytala (Quechua); Mandachi (Shuar). Peru: Bara.

Notes – Despite its name, the size of leaves of *Cremastosperma megalophyllum* varies from large to relatively small with respect to those of other species of the genus. This variation is also apparent in the size of the fruits, which are similar to those of *C. napoense*, but which in contrast are never borne on a branching inflorescence. *C. megalophyllum* is best distinguished by the shape of the glabrous, black-drying flowers: the large sepals open earlier, and to a greater extent, than the petals (in contrast to those of *C. napoense*, bud development of which is open from an early stage and the lighter colour (particularly of the pedicel) of which indicates the presence of indument).

16. Cremastosperma microcarpum R.E.Fr. – Fig. 1 a, g, 2 c, 20; Map 7; App. 2, Fig. 7

Cremastosperma microcarpum R.E.Fr. (1939) 559. – Type: *Krukoff 6151* (holo S; iso A, F, G, GB, K, MICH, MO, NY, U, US), Brazil, Amazonas: Mun. Humaitá, Rio Madeira, near Tres Casas, 18 Sep. 1934.

Tree or *shrub* 2-20 m tall, 2.5-18.5 cm diam.; young twigs and petioles glabrous to rather densely covered with appressed and/or erect white or golden hairs 0.3-0.4 mm long. *Leaves*: petioles 2-12 by 1.5-3 mm; lamina narrowly elliptic (narrowly obovate), 13-31 by 4-12 cm (index 1.8-3.8(-4.8)), chartaceous, green, greyish or brownish green or brown on both sides, shiny above, veination below often yellowish, glabrous above, glabrous to rather densely covered with appressed or erect white hairs to 0.2 mm long at the base and on primary and secondary veins below, base acute to obtuse, rarely rounded or narrowly cuneate, apex acuminate (acumen 10-40 mm long), primary vein 1-2 mm wide at widest point, more or less verrucose on both sides, lightly grooved for around half of length, secondary veins 7-15, often 1-4 intersecondary veins, distance between from c. 5 mm at the base to c. 20 mm closer to the apex, angles with primary vein mostly from 45-60° at the base to 60-80° closer to the apex, not branching, forming distinct loops, smallest

FLORA OF PERU
f Systematic Botany, Utrecht (U)

iorum R.E. Fries

Det. E.C.H. van Heusden 1985
Loreto.
Prov. Maynas: Quistacocha, near Iquitos.
Alt. - 100 m

Swampy place.

Tree, 7 m tall, 12 cm diam.; cortex bitter, yellow within; leaves bullate, dark green and shiny above, greyish-green with yellowish-brown midrib below; pedicels reddish-green, calyx green at the outer side, green with pink base at the inner side; corolla buds greyish at outer side, outer petals brownish at outer side, inner side and inner petals creamy-yellow, stamens pinkish; stipes reddish, monocarps brownish-red.

Separate: flowers and fruits in spirit
Woodnr Uw 30309

Maas, P.J.M. nr 6281
13 Nov 1984

Revision of Cremastosperma (Annonaceae)
Cremastosperma microcarpum R.E.Fr.
Det.: Michael D. Pirie Utrecht, 2005

Fig. 20. ***Cremastosperma microcarpum*** **R.E.Fr. a. leaves; b. flowers and flower buds; c. fruit (a & b:** ***Maas et al. 6281*****; c:** ***Vásquez & Jaramillo 9350*****)**

distance between loops and margin 2–7 mm, tertiary veins percurrent. *Inflorescence* of single flowers, solitary or clustered in groups of up to 3, on leafy or leafless twigs; peduncles (3–)5–15 by c. 1 mm (in flower), 4–15 by 1–2 mm (in fruit); pedicels (5–) 12–24 by c. 1 mm at the base (in flower), 10–25 by 1–2 mm (in fruit), peduncles and pedicels sparsely to rather densely covered with appressed or erect whitish hairs to 0.3 mm long; 1 to several lower bract(s), the basal-most small and scale-like, those more apical mostly (long) elliptic, occasionally leaf-like, 2–6(–60) by c.1 mm, acute, caducous, rather densely covered with appressed white hairs to 0.3 mm long; upper bract attached mostly on the basal half of the pedicel, ovate to deltate, 1.5–2.5 by 1–1.5 mm, obtuse or acute, sparsely to rather densely covered with appressed or erect golden hairs to 0.3 mm long; closed flower buds very broadly to depressed ovoid, opening loosely in development; flowers green, maturing to brown, (pale) yellow, cream or white outside, cream or yellow inside, calyx green or dark brown outside, green with a pink base inside in vivo, pale (orange-) brown or brown with dark or reddish brown base in sicco, sepals and petals rather densely to densely covered with appressed or erect golden hairs (whitish close to the edges) to 0.3 mm long; sepals free or connate for 0.5 mm, broadly ovate to deltate, not reflexed, 3–4{–6} by 2.5–4{–6} mm, obtuse, caducous; outer petals ovate to very broadly ovate, rounded, 11–18{–19} by 10–17 mm, inner petals elliptic to (narrowly) obovate, obtuse, 10–16{–22} by 5–8{–10} mm; androecium c. 7 mm diam., stamens 1.3–1.5 mm long, connective appendage 0.6–0.8 mm wide, glabrous; gynoecium c. 1 mm diam., carpels 2–2.5{–2.9} long, sparsely covered with erect whitish hairs to 0.1 mm long. *Monocarps* (8–)17–33, ellipsoid to broadly ellipsoid, asymmetrical, 8–11 by 6–8 mm, often with an oblique longitudinal groove corresponding to the seed raphe, green maturing to pink or orange through purple or brownish red, brown and black in vivo, dark or reddish brown in sicco, with an excentric apicule, monocarps, stipes and receptacle sparsely to rather densely covered with erect whitish hairs c. 0.1 mm long; stipes 8–16 by c. 1 mm; fruiting receptacle 4–8 mm diam. *Seeds* broadly ellipsoid, orange brown, pitted, 6–8 by 5–6 mm, raphe sunken, somewhat irregular.

Distribution – Amazonian Peru, Brazil, and Colombia.

Habitat and Ecology – Forest inundated by white (várzea) or black (tahuampa) water, on yellowish, lateritic soil. At elevations of 80–200 m. Flowering: March, July, and September; fruiting: throughout the year.

Notes – *Cremastosperma microcarpum* resembles most closely *C. gracilipes*, from which it differs in the denser, longer hairs on the flowers and acuminate as opposed to cuspidate leaf apex. The hairy flower resemble somewhat those of *C. cauliflorum*, but which cannot be confused as *C. microcarpum* never exhibits a branching inflorescence. In addition, the monocarps of *C. cauliflorum* are larger than those of *C. microcarpum* and characteristically globose to transversely broadly ellipsoid as opposed to ellipsoid.

17. Cremastosperma monospermum (Rusby) R.E.Fr. – Fig. 1 c, 21; Map 4; App. 2, Fig. 8

Cremastosperma monospermum (Rusby) R.E.Fr. (1931) 193. – *Cymbopetalum monospermum* Rusby (1910) 505 ("Symbopetalum"). – Type: *R.S. Williams* 670 (holo NY; iso K), Bolivia, La Paz: San Buenaventura, 470 m, 12 Nov. 1901.

Cremastosperma juruense R.E.Fr. (1937) 282. – Type: *Krukoff 4697* (holo NY; iso S), Brazil, Amazonas: Basin of Rio Juruá, near mouth of Rio Embira (tributary of Rio Tarauacá), 6 Jun. 1933.

Cremastosperma monospermum (Rusby) R.E.Fr. var. *brachypodum* R.E.Fr. (1939) 559. – Type: *Dahlgren & Sella 162* (holo S; iso B, F), Brazil, Pará: Boa Vista on the Tapajós River, 5 May 1929.

Tree or *shrub*, 1–12 m tall, 4–10 cm diam.; young twigs and petioles glabrous. *Leaves*: petioles 5–10 by 1–3 mm; lamina obovate, elliptic or narrowly so, (8–)10–35 by 4–12 cm (index 2–3.5), chartaceous, green to brown, darker above, veins often reddish below, glabrous on both sides or rarely sparsely covered with appressed whitish to 0.4 mm long hairs on primary vein below, base acute to obtuse, rarely narrowly cuneate, mostly decurrent, apex acuminate to cuspidate (acumen/cusp 5–30 mm long), primary vein 1–2 mm wide at widest point, secondary veins 6–10, intersecondary veins 0–3, distance between from 10–18 mm at the base to 12–24 mm closer to the apex, angles with primary vein from 70–80° at the base to 40–50° closer to the apex, rarely branching, forming mostly distinct loops, smallest distance between loops and margin 2–6 mm, tertiary veins mostly reticulate. *Inflorescence* of single flowers solitary (or clustered in groups of 2), on leafy or leafless twigs; peduncles 2–10 by 0.5–1 mm (in flower), 2–10 by 1.5–2 mm (in fruit), sparsely covered with appressed to erect golden to 0.1 mm long hairs or glabrous; pedicels 40–50(–70) by 0.5–1 mm at the base (in flower), (12–)22–73 by 1–1.5 mm (in fruit), green or reddish in vivo, glabrous; 2 lower bracts, deltate, c. 0.8 by 0.8 mm (occasionally large and leafy), obtuse, caducous, sparsely covered with appressed golden 0.1 mm long hairs or glabrous; upper bract mostly attached around half way along pedicel, broadly ovate or deltate, 1–2.5 by c. 1 mm, acute, obtuse, rounded or truncate, outer side sparsely covered with appressed golden 0.1 mm long hairs or glabrous; flower buds broadly ovoid-triangular, remaining closed (or nearly so) throughout development; flowers green, maturing to creamy yellow, yellow or orange in vivo, dark or reddish brown or black in sicco; sepals and petals glabrous, sepals free or connate for c. 1 mm, broadly ovate or deltate, appressed, patent or recurved, 2–4 by 2–3 mm, acute or obtuse, mostly caducous; outer petals ovate, 9–14 by 6–8 mm, inner petals elliptic to ovate, or narrowly so, 10–13 by 4–5 mm; androecium not seen; gynoecium not seen. *Monocarps*, stipes and receptacle glabrous, monocarps 10–29, ellipsoid to broadly so, slightly asymmetrical, 9–11 by 7–8 mm, green maturing to pink, maroon, red or (blue-) black in vivo, brown, dark or reddish brown or black in sicco, with an excentric apicule; stipes (6–)8–15 by c.

Fig. 21. ***Cremastosperma monospermum*** **(Rusby) R.E.Fr. a. fruiting specimen; b flower buds (a:** ***Nuñez & Timaná 12152*****; b:** ***Cid Ferreira et al. 6301*****)**

1 mm; fruiting receptacle 3-8 mm diam. *Seeds* broadly ovoid, reddish brown, pitted, pits appear black with raised rim, 8-10 by 6-7 mm, raphe sunken, regular.

Distribution - Bolivia (Beni, La Paz, Pando), Brazil (Acre, Pará, Rondônia), and widespread across Peru.

Habitat and Ecology – Primary and secondary lowland forest, occasionally on poorly drained soils or brown latosols. At elevations of 200-500 m. Flowering: April, July – December; fruiting: throughout the year.

Vernacular names: Bolivia: Yohisi. Peru: Ayacbara.

Notes – *Cremastosperma monospermum* is the most widespread species of the genus – the only found both along the eastern foothills of the Andes as far south as Bolivia and across Brazil south of the Amazon. It is best distinguished by the shape of the flower bud: roughly triangular with an obtuse apex, apparently remaining closed throughout development, with the petals not opening fully even at maturity. In most other species of the genus the flower bud opens during development. *C. pendulum* and *C. yamayakatense* also exhibit glabrous, closed flower buds, but the shape in both is depressed ovoid. The latter also has a short, sturdy pedicel, very different to *C. monospermum*, the flower of which is borne on a slender, and often long (though rather variable), pedicel.

The authors do not consider it useful to recognise sub-specific taxa within *C. monospermum*. The variation in stipe length and thickness represented by the type of var. *brachypodum*, described by Fries, falls within that of the species as a whole and is therefore synonymised here.

18. Cremastosperma napoense Pirie – Fig. 22; Map 3

Cremastosperma napoense Pirie (2005) 54, f. 7. – Type: *Alvarado 267* (holo U; iso AAU, MO, QCNE), Ecuador, Napo: Cantón Archidonia, foothills south of Volcano Sumaco, km 50 on Hollín – Loreto road, community Huahua Sumaco, 1100 m, 3 May 1989.

Tree 5-20 m tall, 10-15 cm diam.; young twigs and petioles rather densely covered with appressed whitish-golden hairs to 0.2 mm long. *Leaves*: petioles 8-12(-18) by 3-4 mm; lamina narrowly elliptic, 17-42 by 7-13 cm (index 1.8-3.9), chartaceous, olive green or brown on both sides, venation darker below, glabrous above, rather densely covered with appressed whitish-golden hairs to 0.2 mm long on veins below, base acute, apex acute to acuminate (acumen 5-15 mm long), primary vein grooved over entire leaf length, verrucose at the base, 3-4 mm wide at widest point, secondary veins (8-)10-15, occasionally 1 or 2 intersecondary veins, distance between from 4-9 mm at the base, 20-50(-60) mm in the centre to 10-30 mm closer to the apex, angles with primary vein from 40-50° at the base to 70-80° closer to the apex, occasionally branching, forming more or less distinct loops in the apical half, tertiary veins percurrent. *Inflorescence* of 1-8 flowers, branching, solitary or clustered in groups of 2, on leafless twigs and branches; peduncles 7-22 by 1-1.5 mm (in flower), 10-22 by 2.5-3 mm (in fruit); pedicels 25-38 by c. 1 mm diam. at the base, 1.5-2 mm diam. at the apex (in flower), 25-38 by 2-3 mm diam. at the base, 2-4 mm diam. at the apex (in fruit), peduncles and pedicels

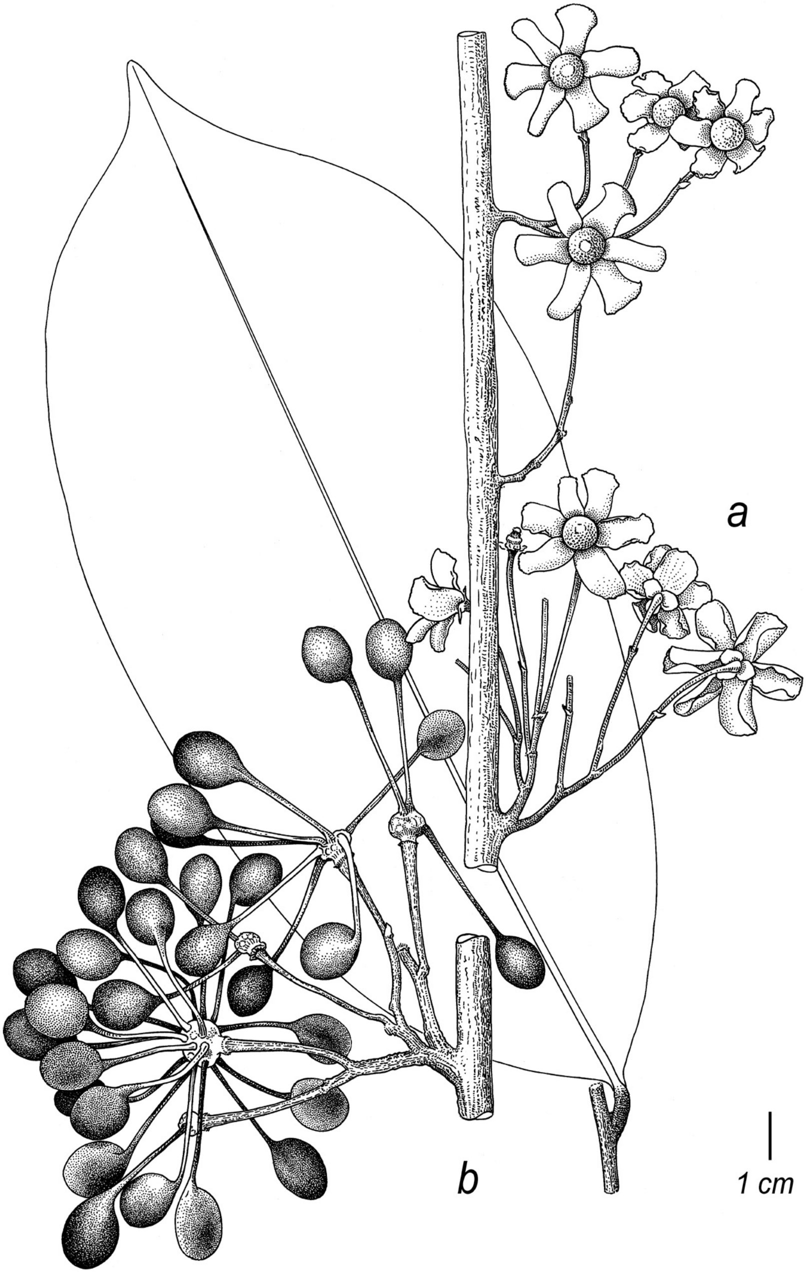


Fig. 22. ***Cremastosperma napoense*** **Pirie. a. inflorescences; b. infrutescence and leaf (a:** ***Cerón 2986*****; b:** ***Alvarado 267*****)**

rather densely to densely covered with appressed whitish to golden hairs to 0.2 mm long; single lower bract, caducous; upper bract attached around halfway along the pedicel, broadly to depressed elliptic or broadly to depressed ovate, 1–2 by 1.5–1.8 mm, obtuse, outer side densely covered with appressed whitish-golden hairs to

0.2 mm long; flower buds depressed ovoid, opening early in development; flowers green maturing to greenish yellow or cream in vivo, brown in sicco; sepals fused for first 0.5 mm, deltate, appressed (basal 1 mm of sepals and petals reflexed), 2.5–3 by c. 3 mm, acute or obtuse, caducous, inner side glabrous, outer side rather densely to densely covered with appressed whitish-golden hairs to 0.2 mm long; outer petals elliptic, 8–15 by 5–9 mm, inner petals elliptic, 12–14 by 5–7 mm, inner side of inner and outer petals glabrous, most of outer side of outer petals rather densely to densely covered with appressed whitish-golden hairs to 0.2 mm long (towards the margins and apex glabrous), outer side of inner petals largely glabrous but with a narrow, dense, sometimes branching band of appressed, whitish-golden, to 0.2 mm long hairs extending from the base to halfway towards the apex; receptacle depressed ovoid; androecium c. 6 mm diam., stamens 1–1.5 mm long, connective appendage roughly hexagonal, 0.5–0.8 mm wide, glabrous; gynoecium c. 2 mm diam., carpels up to c. 40, c. 1.5 mm long, glabrous or sparsely covered with appressed, whitish-golden, to 0.2 mm long hairs. *Monocarps* 16–37, ellipsoid, asymmetrical, 12–20 by 10–13 mm, green maturing to dark purple or black in vivo, black in sicco, glabrous; stipes 20–30 by 2–3 mm, glabrous; fruiting receptacle depressed ovoid, 7–12 mm diam., glabrous. *Seeds* ellipsoid, brown, lightly furrowed (not pitted), c. 13 by 9 mm, raphe neither sunken nor raised, encircling seed longitudinally.

Distribution – Ecuador (Napo, one collection in Pastaza).

Habitat and Ecology – Primary pluvial premontane forest, often on volcanic soils but also reported growing on limestone. At elevations of 600–1300 m. Flowering: September, November, December, and February; fruiting: August to December, March to May.

Vernacular name – Ecuador: Ayacara.

Notes – The characteristic pattern of indument on the inner petals of *Cremastosperma napoense* appears to be unique for the genus. The species can be further distinguished by the combination of a branching inflorescence and glabrous fruits. The only other species in the genus with such an inflorescence is *C. cauliflorum*, which differs both in the presence of brown indument on the (characteristic globose to transversely broadly elliptic) monocarps and in the dense covering of much longer hairs on the flowers.

19. Cremastosperma novogranatense R.E.Fr. – Fig. 23; Map 2

Cremastosperma novogranatense R.E.Fr. (1950) 329. – Type: *Cuatrecasas 17573* (holo S; iso F, US), Colombia, El Valle: Costa del Pacifico, Río Cajambre, Silva, 5–80 m, 5–15 May 1944.

Tree 8–20 m tall, 5–13 cm diam.; young twigs and petioles densely covered with appressed or erect golden to whitish hairs to 0.4 mm long. *Leaves*: petioles (6–) 10–20 by 3–5 mm; axillary buds densely covered with appressed to erect golden

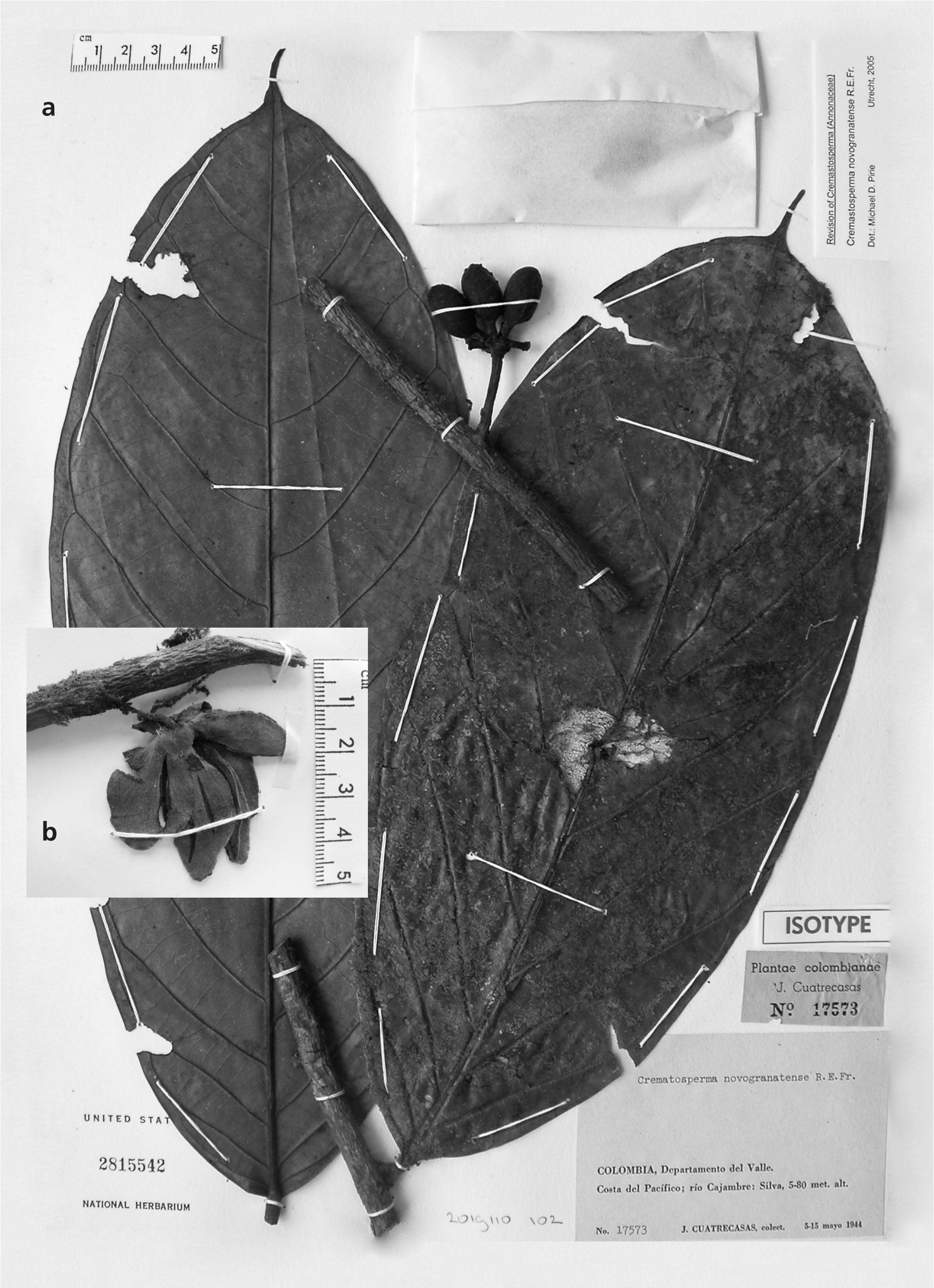


Fig. 23. ***Cremastosperma novogranatense*** **R.E.Fr. a. fruiting specimen; b. flower (a:** ***Cuatrecasas 17573*****; b:** ***Cuatrecasas & Willard 26031*****)**

to whitish hairs to 0.4 mm long; lamina elliptic to obovate or narrowly so, (24–) 32–50 by 11–21 cm (index 2.2–2.8), chartaceous, pale brownish green to greyish green above, pale brownish green below, glabrous above, veins sparsely to rather densely covered with appressed golden to whitish hairs to 0.5 mm long below, base obtuse to rounded, apex acuminate to cuspidate (acumen 15–40 mm long), primary vein 2–4 mm wide at widest point, secondary veins 10–13, intersecondary veins 1–3, distance between from 4 mm at the base to up to 40 mm closer to the apex, angles with primary vein from 50–70° at the base to 45–55° closer to the apex, not branching forming mostly distinct loops, smallest distance between loops and margin 2–7 mm, tertiary veins percurrent. *Inflorescence* of single flowers, solitary (or clustered in groups of 2), on leafy or leafless twigs; peduncles 2–3 by c. 2 mm (in flower), 2–4 by 2.5–3 mm (in fruit); pedicels 10–20 by c. 2 mm (in flower), 13–27 by 2.5–3 mm (in fruit), peduncles and pedicels sparsely to densely covered with appressed golden hairs to 0.4 mm long; single lower bract, ovate, c. 2 by 1.5 mm, acute, often persistent, outer sides of bracts, of sepals and of petals densely covered with appressed golden hairs to 0.6 mm long; upper bract in the lower half of the pedicel, broadly to depressed ovate, 2–3.5 by 1.5–2 mm, acute; flower buds globose; flowers pale green, sepals light brownish green in vivo, outer side of sepals and petals dark yellow or greyish green, inner side reddish brown or dark brown in sicco; sepals free, ovate to broadly ovate, appressed or patent, 7–10 by 7–8 mm, obtuse, often persistent, with prominent venation; outer petals elliptic, 17–29 by 10–16 mm, obtuse, inner petals narrowly elliptic, 28–33 by 9–12 mm, obtuse; androecium not seen; stamens 1.6–1.9 mm long, connective appendage 0.8–1 mm by 0.5–0.8 mm wide; gynoecium not seen. *Monocarps*, stipes, and receptacle sparsely to rather densely covered with appressed brown hairs to 0.2 mm long, monocarps 3–14, ellipsoid to broadly ellipsoid, asymmetrical, 16–22 by 10–13 mm, yellow, orange, red or pale brown in vivo, blackish brown to black in sicco, with an excentric apicule; stipes 1.5–4 by 2 mm; fruiting receptacle 4–10 mm diam. *Seeds* ellipsoid, yellowish-brown, very shallowly pitted, c. 16 by c. 10 mm, raphe sunken, irregular.

Distribution – Pacific coast of Colombia (El Valle).

Habitat and ecology – Rain forest. At elevations of 0–130 m. Flowering: September and October; fruiting: May, September.

Notes – *Cremastosperma novogranatense* can be distinguished by its almost sessile monocarps and by the large and densely hairy flowers with unusually large, often persistent, sepals. It is most similar to *C. westrae* Pirie, the sepals of which are much smaller, and indument in general of shorter, less dense hairs.

20. Cremastosperma oblongum R.E.Fr. – Fig. 24; Map 6; App. 2, Fig. 9

Cremastosperma oblongum R.E.Fr. (1948) 4. – Type: *Killip & Smith* 23622 (holo US; iso NY, S), Peru, Junín: Río Pinedo, N of La Merced, 30 May 1929.

Tree or *shrub* 2–12 m tall, 6–15 cm diam.; young twigs and petioles sparsely covered with appressed golden hairs to 0.4 mm long. *Leaves*: petioles 5–16 by 2–

Fig. 24. ***Cremastosperma oblongum*** **R.E.Fr. a. fruiting specimen; b. flower (a:** ***Foster & d'Achille 10224*****; b:** ***Maas et al. 4592*****)**

4 mm; lamina elliptic, obovate or narrowly so, 13-45 by 5-12 cm (index 2.5-4), coriaceous, shiny grey-green or brown above, dull brown below, glabrous above, sparsely covered with appressed golden hairs to 0.4 mm long on veins below, base acute (rarely cordate), apex acuminate (acumen (5-)10-20 mm long), primary

vein grooved in basal half, 2–4 mm wide at widest point, secondary veins 8–19, intersecondary veins often 1(–2), distance between from 8–24 mm at the base to 12–27 mm closer to the apex, angles with primary vein from 40–70° at the base to 20–60° closer to the apex, sometimes branching, forming distinct loops, smallest distance between loops and margin 2–5 mm, tertiary veins mostly percurrent. *Inflorescence* of single flowers clustered in groups of up to three, on leafy or leafless twigs (occasionally on main stem, then on brachyblasts); peduncles c. 2 by 1 mm (in flower), 2–5 by 1.5–3 mm (in fruit); pedicels 7–18 by c. 1 mm at the base (in flower), 12–35(–55) by 1.5–3 mm (in fruit), peduncles and pedicels sparsely covered with appressed whitish hairs to 0.1 mm long; 2 or 3 lower bracts, deltate, 0.5–1 by 0.5–1 mm, obtuse, caducous or persistent, sparsely covered with appressed whitish hairs to 0.1 mm long; upper bract attached in basal half of pedicel, shallowly triangular, c. 1 by c. 2 mm, obtuse, persistent, sparsely covered with appressed whitish hairs to 0.1 mm long; no closed flower buds seen; flowers green maturing to cream, yellow or orange/yellow in vivo, black in sicco; sepals free, deltate, often recurved, c. 2 by 2 mm, acute, caducous, sparsely covered with appressed whitish hairs < 0.1 mm long or glabrous; petals glabrous, outer petals obovate to narrowly so, c. 16 by 6–8 mm, , inner petals elliptic to narrowly so, 15–16 by 4–6 mm; androecium c. 8 mm diam., stamens 1–1.5 mm long, connective appendage 0.5–0.8 mm wide; gynoecium c. 1.5 mm diam., stigmas sparsely covered with erect golden hairs < 0.1 mm long. *Monocarps*, stipes, and receptacle glabrous, monocarps 6–20, ellipsoid, asymmetrical, 16–20 by 10–14 mm, green maturing through orange or red to black in vivo, brown or black in sicco, with an excentric apicule; stipes green maturing to red in vivo, 9–17 by 2–3 mm; fruiting receptacle 7–12 mm diam. *Seeds* ellipsoid, reddish brown, pitted, c. 13 by 8 mm, raphe raised, regular.

Distribution – Central and southern Peru (Cuzco, Huánuco, Junín, Loreto, Madre de Dios, Pasco, San Martín, and Ucayali), and adjacent Brazil (Acre).

Habitat and Ecology – Primary, often upland, rainforest, on white sands, brown latosols and limestone soils. At elevations of 100–1300 m. Flowering: September, December; fruiting: March–July, November–January.

Vernacular names – Peru: Bara caspi, Carahuasca, Carahuasca amarilla, Hicoja, Palo blanco, Tortuga blanca.

Notes – *Cremastosperma oblongum* is best discerned from the most similar other species on the basis of floral characters: the sepals are small and recurved (unlike *C. megalophyllum*) and borne on short pedicels, whilst bud development is open (not the case in *C. yamayakatense*). The leaves are also distinctive: rather leathery with a greyish colour on the upper side, with secondary veins forming conspicuous loops and often narrowly elliptic. Fruiting specimens display more variation – particularly in the length of the pedicel. In particular, cauliflorous specimens from the Peruvian department of Pasco have longer pedicels, as does the type specimen itself.

21. Cremastosperma pacificum R.E.Fr. – Fig. 25; Map 3

Cremastosperma pacificum R.E.Fr. (1950) 330. - Type: *Cuatrecasas 17463* (holo S; iso F, US), Colombia, El Valle: Costa del Pacífico, Río Cajambre, Silva, 5-80 m, 5-15 May 1944.

Tree 3–15 m tall, 2.5–25 cm diam.; young twigs and petioles sparsely to rather densely covered with appressed golden hairs to 0.4 mm long or glabrous. *Leaves*: petioles 8–16 by 2–4 mm; lamina elliptic to obovate, or narrowly so, 19–41 by 9–16 cm (index 2.1–3.1), chartaceus to slightly coriaceous, brown, brownish green, or greyish green and shiny above, brown, pale brown or greenish brown below, glabrous above, glabrous or sparsely to densely covered with appressed golden hairs to 0.4 mm long particularly on veins below, base acute, apex acuminate (acumen 10–20 mm long), primary vein shallowly grooved at base, 2–3 mm wide at widest point, secondary veins 7–12, intersecondary veins occasionally 1–2, distance between from 8 mm at the base to up to 55 mm closer to the apex, angles with primary vein from 30–50° at the base to 50–70° closer to the apex, not branching, often forming distinct loops for the apical third, smallest distance between loops and margin 1.5–2 mm, tertiary veins percurrent. *Inflorescence* of single solitary flowers, on leafless twigs; peduncles c. 1 by 1 mm (in flower), 2–3 by c. 2 mm (in fruit), rather densely to densely covered with appressed golden or whitish hairs to 0.2 mm long; pedicels 12–20 by c. 1 mm (in flower), 22–35 by 1.5 mm (in fruit), longitudinally furrowed, glabrous or sparsely to rather densely covered with appressed gold or whitish hairs to 0.2 mm long; single lower bract, deltate, 1–1.5 by c. 1 mm, obtuse or acute, occasionally persistent, densely covered with appressed gold or whitish hairs 0.2 mm long; upper bract in the lower half of the pedicel, deltate, 1–1.5 by 1–1.5 mm, rounded, outer side sparsely to rather densely covered with appressed gold or whitish hairs to 0.2 mm long or glabrous; flowers pale greenish yellow, or pale green in vivo, black or dark brown in sicco, sepals and petals glabrous; sepals free, very broadly ovate, 3 by 3–3.5 mm, obtuse, caducous; outer petals elliptic to broadly elliptic, 16 by 11–12 mm, inner petals obovate, 15 by 7 mm, obtuse; androecium not seen; stamens 1.4 mm long, connective appendage 0.6 mm wide; gynoecium not seen. *Monocarps* (2–)7–21, ellipsoid, slightly asymmetrical, 15–18 by 10–12 mm, green (immature) in vivo, black or dark brown in sicco, with an excentric apicule, monocarps, stipes and receptacle glabrous; stipes 10–18 by 1 mm; fruiting receptacle depressed ovoid, 3–9 mm diam. *Seeds* ellipsoid, yellow, furrowed and lightly pitted, c. 10 by 8 mm, raphe raised within sunken groove, regular.

Distribution – Pacific coast of Colombia (El Valle and Chocó).

Habitat and Ecology – Tropical wet and pluvial forest, reported as growing on yellow clay with alluvial substrate. At elevations of 5–100 m. Flowering: December and August; fruiting: April and May.

Notes – *Cremastosperma pacificum* appears similar to a number of other species characterised by the absence of (visible) indument on flowers and fruits. Most

Fig. 25. ***Cremastosperma pacificum*** **R.E.Fr. a. fruiting specimen; b. flower (a:** ***Cuatrecasas 17463*****; b:** ***Sánchez et al. 323*****)**

similar are *C. magdalenae* and *C. megalophyllum*, the sepals of both of which are much larger (4-7 mm long, as opposed to up to 3 mm in *C. pacificum*). From the limited floral material available, bud development in *C. pacificum* would not appear to be open, with bud shape similar to that of *C. yamayakatense*. In contrast to both *C. yamayakatense* and *C. panamense* (with open bud development) the monocarps of *C. pacificum* are relatively large (> 15 mm long, as opposed to up to 14 mm) and around the same length as, rather than longer than, the stipes.

22. Cremastosperma panamense Maas – Fig. 26; Map 2

Cremastosperma panamense Maas in Maas *et al.* (1986) 254, f. 5 & 6 – Type: *Johnston 1812* (holo MO; iso A, MICH), Panama, Canal Zone: NW part of Canal Zone, area W of Limon Bay, Gatun Locks and Gatun Lake, Maru Towers, 7 Apr. 1956.

Tree or *shrub* 1.5-7(-20) m tall, 3-10 cm diam.; young twigs and petioles glabrous. *Leaves*: petioles 2-10 by 1-2.5 mm, caniculate above, verrucose or furrowed; lamina narrowly elliptic, 8-22 by 2-7 cm (index 2.7-4.7), chartaceous, green, brownish green, or greyish green (or brown) above, (pale) green, (pale) brownish green (or brown) below, shiny on both sides, glabrous on both sides, base acute to obtuse, decurrent, rarely narrowly cuneate, apex acuminate (acumen 5-25 mm long), primary vein occasionally shallowly grooved at the base, 1-1.5 mm wide at widest point, verrucose below, secondary veins 5-10, intersecondary veins 1-4, distance between from 5-20 mm at the base to 15-25 mm closer to the apex, angles with primary vein from 35-55(-70)° at the base to 60-75° closer to the apex, not branching, forming mostly distinct loops, smallest distance between loops and margin 2-5 mm, tertiary veins largely reticulate with little distinction between tertiary and quaternary veins. *Inflorescence* of single, solitary flowers, on leafy or leafless twigs; peduncles 1-3 by 0.5-1 mm (in flower), 1-3 by 1.5-2 mm (in fruit), sparsely covered with erect whitish to golden hairs to 0.1 mm long; pedicels 12-20 by 0.5-0.8 mm at the base (in flower), 13-22 by 1-2 mm (in fruit), glabrous; (1-)2-3(-several) lower bract(s), deltate, c. 0.5 by 0.5 mm, obtuse, mostly caducous, sparsely covered with erect whitish to golden hairs to 0.1 mm long; upper bract halfway along the pedicel, ovate to depressed ovate, 1-1.5 by 0.7-1mm, acute, obtuse or emarginate, glabrous; closed flower buds very broadly ovoid, opening in development; flowers green when immature, maturing to white, cream, or (pale) yellow in vivo, yellow brown, dark brown, or blackish brown in sicco, sepals and petals glabrous; sepals free, broadly to very broadly ovate, appressed or patent, 1.5-3 by 1.5-3 mm, obtuse, caducous or persistent; outer petals narrowly ovate to narrowly elliptic, 7-18 by 4-6 mm, obtuse, inner petals narrowly ovate to narrowly elliptic, 10-25 by 3-5 mm, obtuse; androecium diam. unknown, stamens 1.2-1.5 mm long, connective appendage 0.7-0.9 mm wide; gynoecium diam. unknown, carpels c. 35, 2 mm long, glabrous. *Monocarps*, stipes, and receptacle glabrous, monocarps (2-)8-

Fig. 26. *Cremastosperma panamense* Maas. a. fruiting specimen; b. flowers (a: *Johnston 1812*; b: *Pérez 832*)

30, more or less globose, slightly asymmetrical, 8-13 by 7-11 mm, green maturing to yellow, orange, red, or black in vivo, pale brown, reddish brown, or brown in sicco, with an excentral apicule; stipes 7-21 by 1-1.5(-3) mm; fruiting receptacle 3-8 mm diam. *Seeds* broadly ellipsoid, globose or transversally ellipsoid, light brown, pitted, 7-12 by 6-10 mm, raphe sunken, regular.

Distribution - Panama (Coclé, Colón, Panamá, and San Blas).

Habitat and Ecology - Evergreen tropical wet forest, cloud forest or low swampy places or in disturbed areas. At elevations of 0-800 m. Flowering: April, May, July, and August; fruiting: throughout the year.

Vernacular names - Panama: Palo santo, Sate wawa, Waras gid.

Notes - *Cremastosperma panamense* appears similar to *C. magdalenae*, but can be distinguished by the smaller size of the sepals, which additionally persist less frequently into fruiting. *C. pacificum* and *C. chococola* are both geographically close (Pacific coast of Colombia) and share the characters of glabrous pedicels, (flowers) and fruits. However, the pedicels of *C. chococola* are considerably longer, and the shape of the larger monocarps of *C. pacificum* (ellipsoid as opposed to roughly globose), as well as the larger leaves, allow easy distinction in both cases. The colour of the relatively small leaves (drying consistently green), the relatively large distance between the loops of their secondary veins and the margin, and the reticulate and indistinct nature of tertiary and quaternary venation of *C. panamense* are also distinctive.

23. Cremastosperma pedunculatum (Diels) R.E.Fr. - Fig. 27; Map 7

Cremastosperma pedunculatum (Diels) R.E.Fr. (1930) 48. - *Aberemoa pedunculata* Diels (1906) 409. - Type: *Weberbauer 4558* (holo B; iso F, G, MOL), Peru, San Martín: Moyobamba, 1100-1200 m, 1906.

Tree 4-15 m tall, 8-15 cm diam.; young twigs and petioles glabrous. *Leaves*: petioles 4-15 by 1-3 mm; lamina elliptic to obovate, or narrowly so, 12-27 by 4-10 cm (index 2-3.6), chartaceous, (dark) greyish brown above, blackish brown with darker veins below, glabrous above, glabrous to sparsely covered with appressed golden hairs to 0.6 mm long, particularly on veins below, base acute to rounded, decurrent, apex acuminate (acumen 5-20 mm long), primary vein 1.5-2.5 mm wide at widest point, secondary veins 7-13, intersecondary veins often 1-3, distance between from 5 mm at the base to 15-20 mm closer to the apex, angles with primary vein inconsistent, 40-60° at the base and closer to the apex, not branching, forming distinct loops, smallest distance between loops and margin 1-4 mm, tertiary veins more or less percurrent. *Inflorescence* of single flowers clustered in groups of up to 2, on leafy or leafless twigs or main trunk; peduncles 2-10 by c. 1 mm (in flower), 5-15 by c.1.5 mm (in fruit), sparsely to rather densely covered with appressed golden or whitish hairs 0.1- 0.4 mm long; pedicels (30-)35-75(-95) by c.

Fig. 27. ***Cremastosperma pedunculatum*** **(Diels) R.E.Fr. a. flowering twig; b receptacle, illustrating insertion of carpels; c. carpel with single apical/lateral ovule (a-c:** ***Weberbauer 4558*****, reproduced from Fries (1930))**

1 mm at the base, to 3 mm diam. at the apex (in flower), (30-)55-85(-110) by 1-1.5 mm at the base, to 3 mm diam. at the apex (in fruit), sparsely to rather densely (at the base) covered with appressed golden or whitish hairs 0.1- 0.4 mm long or glabrous; 1-several lower bracts, elliptic, c. 1.5 by 1 mm, acute, caducous, sparsely to

rather densely covered with appressed golden or whitish hairs 0.1- 0.4 mm long; upper bract attached in variable position on pedicel, ovate to very broadly ovate, 1-2.5 by 1-2 mm, acute, obtuse, or rounded, sparsely to rather densely covered with appressed golden or whitish hairs 0.1- 0.4 mm long or glabrous; closed flower buds very broadly ovoid to globose, opening loosely in development; flowers green, maturing to green-violet, yellow or pale cream-yellow, inner petals with purple base in vivo, dark brown or reddish brown in sicco, sepals and petals glabrous; sepals free or connate for 1 mm, broadly to very broadly ovate or broadly ovate-triangular, appressed, patent or recurved, 2.5-4 by 2.5-4 mm, obtuse, mostly persistent; outer petals elliptic to broadly elliptic, 11-17 by 7-13 mm, inner petals elliptic, obovate or narrowly so, 11-19 by 4-8 mm, obtuse or rounded, petals with prominent venation; androecium c. 7 mm diam., stamens 1.3-1.8 mm long, connective appendage 0.5-0.8 mm wide; gynoecium c. 1.5 mm diam., glabrous, carpels 2-2.2 mm long. *Monocarps* 3-27, ellipsoid to broadly ellipsoid, asymmetrical, 12-17 by 10-12 mm, green (immature) in vivo, black, dark brown, or reddish brown in sicco, with an excentric apicule, monocarps, stipes and receptacle glabrous; stipes 11-21 by 1.5-2 mm; fruiting receptacle 4-10 mm diam. *Seeds* ellipsoid to broadly ellipsoid, reddish brown, pitted, c. 10 by c. 7 mm, raphe sunken, regular.

Distribution – Peru (San Martín and Cajamarca), Ecuador (Zamora-Chinchipe). Two collections of less certain affinity have been made further north in Ecuador (Pastaza, Morona-Santiago) and one in Colombia (Caquetá).

Habitat and Ecology – Premontane and montane primary and secondary forest, sometimes inundated, mainly on soils with calcareous bedrock. At elevations of 850-1800 m (except the single specimen collected in Pastaza (Ecuador) at 360 m). Flowering: July, October-December; fruiting February, July, October, and December.

Notes – The length of the pedicels of *C. pedunculatum* is only matched or exceeded by those of *C. bullatum* (distinguished by the bullate appearance of the leaves and dense, long, indument on most parts) or *C. longipes* (from the western side of the Andes, and with much longer pedicels and larger leaves).

The holotype of *Guatteria socialis*, *C. Schunke 395*, was determined by Diels as *C. pedunculatum*. Fries (1931) deliberately omitted placing *G. socialis* in synonymy under *C. pedunculatum*, citing differences in the reported growth form. The type is reported by the collector to be a liana. This has not been recorded for any other collections of *C. pedunculatum* (although it is reported for the type specimen of *C. oblongum*). In addition, the collection was made in the central Peruvian department of Junin, much further south than the known distribution of *C. pedunculatum* (in northern Peru and Ecudor). A photo of the holotype (not the specimen itself) was available to the authors. The collection appears to be of a *Cremastosperma* (the leaves with a raised midrib), but includes only immature buds. Although Maas *et al.* (1994) listed *G. socialis* as a taxonomic synonym of

C. pedunculatum, we consider the available evidence insufficient to assign this specimen to a particular species in *Cremastosperma*.

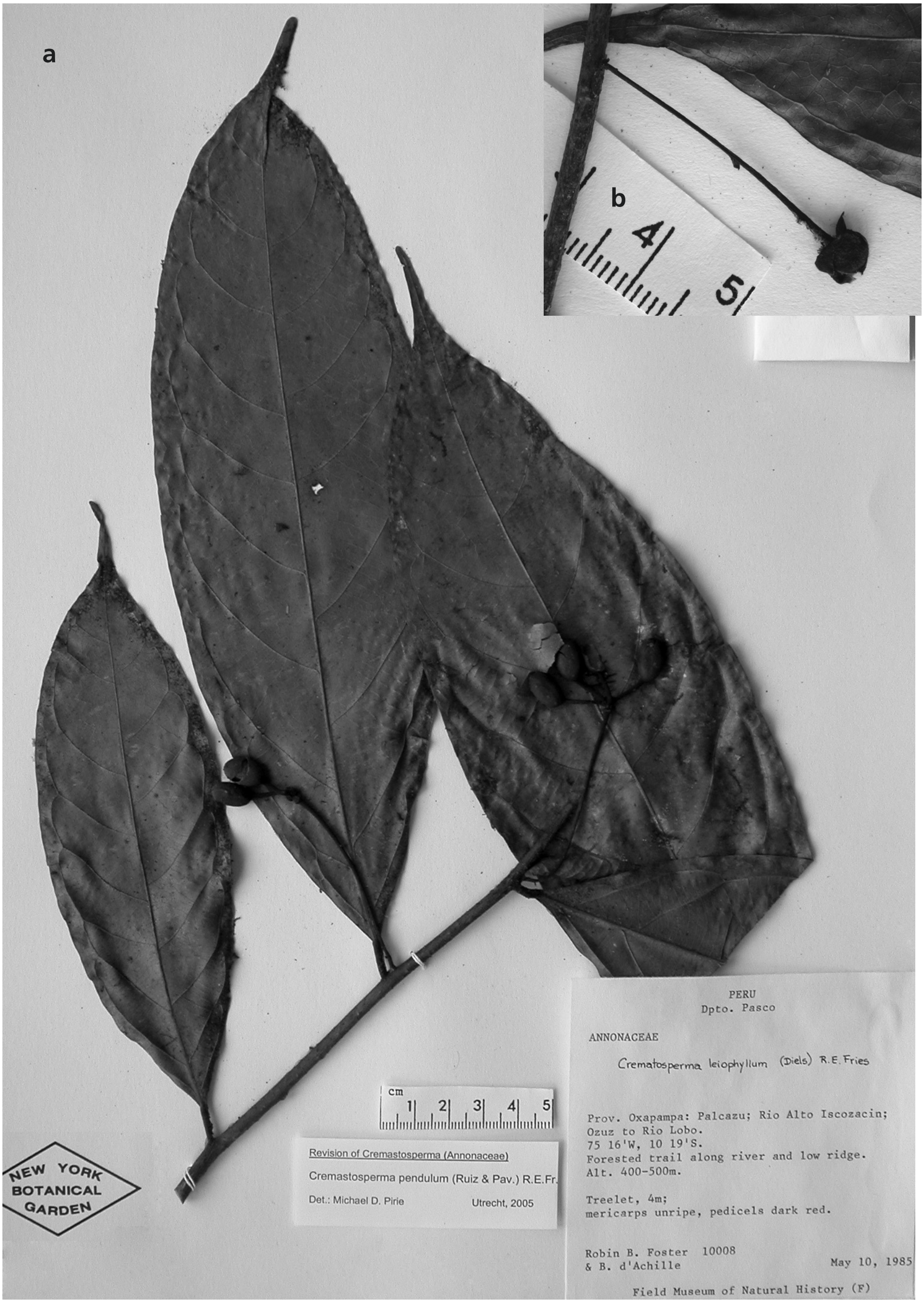
24. Cremastosperma pendulum (Ruiz & Pav.) R.E.Fr. – Fig. 28; Map 8; App. 2, Fig. 10

Cremastosperma pendulum (Ruiz & Pav.) R.E.Fr. (1931) 325. – *Guatteria pendula* Ruiz & Pav. (1798) 146. – Typus: *Pavón s.n.* (holo G), Peru, without location.

Tree or *shrub* 4–10 m tall; young twigs and petioles glabrous. *Leaves*: petioles (3–)6–13 by 1–3 mm; lamina narrowly elliptic, 7–30 by 3.5–10 cm (index 2.9–4.2), chartaceous, drying green (darker above), glabrous above, sparsely covered with appressed whitish hairs to 0.2 mm long particularly on veins or glabrous below, base acute, apex acuminate (acumen 15–25 mm long), primary vein 1–2 mm wide at widest point, deeply grooved in basal half, secondary veins 7–10, intersecondary veins often 1(–2), distance between from 9–16 mm at the base to 11–25 mm closer to the apex, angles with primary vein from 50–60° at the base to 45–50° closer to the apex, sometimes branching, forming distinct loops, smallest distance between loops and margin 2–4 mm, tertiary veins more or less percurrent. *Inflorescence* of solitary flowers on leafy twigs; peduncles 0.5–1.5 by c. 0.5 mm (in flower), 3–5 by 0.8–1 mm (in fruit), sparsely covered with appressed whitish hairs < 0.1 mm long or glabrous; pedicels 28–70 by 0.3–0.5 mm at the base, to 1 mm diam. at the apex (in flower), 50–70 by 0.5–1 mm at the base, to 1.5 mm diam. at the apex (in fruit), green or purple in vivo, glabrous; 1 or 2 lower bracts, deltate, c. 0.5 by 0.5 mm, acute, caducous, rather densely covered with appressed whitish hairs < 0.1 mm long; upper bract attached halfway along pedicel, elliptic or deltate, 0.5–1 by 0.5–1 mm, acute or obtuse, sparsely covered with appressed whitish hairs < 0.1 mm long or glabrous; flower buds depressed ovoid, petals remaining closed (or nearly so) throughout development; flowers green in vivo, black in sicco, sepals and petals glabrous; sepals fused for basal 0.5 mm, elliptic, appressed (particularly in bud) or recurved, c. 2 by 1.5 mm, obtuse, caducous; outer petals ovate, 5–6 by 3–4 mm, inner petals ovate, c. 3.5 by 2 mm; androecium c. 4 mm diam., connective appendage 0.5–0.8 mm wide; gynoecium c. 1 mm diam., glabrous. *Monocarps* 6–11, ellipsoid, slightly asymmetrical, 10–13 by 7–9 mm, monocarps and stipes green maturing to dark reddish brown in vivo, medium to dark brown in sicco, with an excentric apicule, monocarps, stipes, and receptacle glabrous; stipes 9–15 by 1–2 mm; fruiting receptacle depressed ovoid, 3–6 mm diam. *Seeds* ellipsoid, reddish brown, pitted, c. 13 by 7 mm, raphe sunken, regular.

Distribution – Peru (Huánuco, Pasco, Ucayali).

Habitat and Ecology – Primary and secondary tropical lowland and upland forest on brown and red latosols. At elevations of 180–500 m. Flowering: January, August, September, and November; fruiting: May, July, August.



PERU
Dpto. Pasco

ANNONACEAE

Crematosperma leiophyllum (Diels) R.E. Fries

Prov. Oxapampa: Palcazu; Rio Alto Iscozacin;
Ozuz to Rio Lobo.
75 16'W, 10 19'S.
Forested trail along river and low ridge.
Alt. 400-500m.

Treelet, 4m;
mericarps unripe, pedicels dark red.

Robin B. Foster 10008
& B. d'Achille May 10, 1985

Field Museum of Natural History (F)

Revision of Cremastosperma (Annonaceae)

Cremastosperma pendulum (Ruiz & Pav.) R.E.Fr.

Det.: Michael D. Pirie Utrecht, 2005

Fig. 28. ***Cremastosperma pendulum*** **(Ruiz & Pav.) R.E.Fr. a. fruiting specimen; b. flower bud (a:** ***Foster & d'Achille 10008*****; b:** ***Foster 9842*****)**

Notes – *Cremastosperma pendulum* can be distinguished by its long thin pedicel in combination with the shape of the small flower buds which remain closed throughout development: it differs from *C. yamayakatense* in the greater length of pedicel and from *C. monospermum* in the depressed ovoid rather than triangular flower bud.

25. Cremastosperma peruvianum R.E.Fr. – Fig. 29; Map 7

Cremastosperma peruvianum R.E.Fr. (1934) 204. – Type: *Tessmann 4176* (holo B; iso S), Peru, Loreto: upper Río Marañon, Pongo de Manseriche, 160 m, 1 Oct. 1924.

Tree 2–7 m tall; young twigs and petioles glabrous. *Leaves*: petioles 3–15 by 4–8 mm, verrucose or transversally furrowed; lamina narrowly elliptic to narrowly obovate, 38–64 by 11–17(–24) cm (index 2.7–4.4), chartaceous or coriaceous, pale olive or brownish green on both sides, glabrous, base obtuse, rounded, or cordate, often asymetrical, apex acuminate (acumen 15–35 mm long), primary vein deeply grooved in basal half, 3–5 mm wide at widest point, glabrous, secondary veins 20–26, intersecondary veins 1–2, distance between from 2–10 mm at the base, 20–40 mm in the centre, 10–20 mm closer to the apex, angles with primary vein from 55–65° at the base to 70–80° closer to the apex, not branching, forming mostly distinct loops, smallest distance between loops and margin 2–4 mm, tertiary veins percurrent. *Inflorescence* of single solitary flowers, on leafy or leafless twigs; short axillary shoot, 1–1.5 by 1–1.5 mm (in flower), c. 3 by c. 2.5 mm (in fruit), sparsely covered with appressed or erect golden hairs < 0.1 mm long or glabrous; pedicels 18–20 by c. 1 mm at the base (in flower), 20–25 by c. 2 mm (in fruit), red in vivo, glabrous; 2 lower bracts, elliptic, c. 1 by 0.5 mm, obtuse, caducous, sparsely covered with appressed golden hairs < 0.1 mm long; upper bract attached near the base of the pedicel, (broadly) ovate, 1.5–3 by c. 1.5 mm, rounded, glabrous; flower buds broadly ovoid-triangular, remaining loosely closed in development; flowers green, maturing to bright yellow (green at the base) outside, brown inside in vivo, orange or yellowish brown (sepals and bracts lighter) in sicco, sepals and petals glabrous; sepals free, broadly ovate, appressed, c. 4 by 3.5 mm, obtuse, caducous; outer petals elliptic to narrowly obovate, 15–29 by 8–13 mm, obtuse, inner petals narrowly elliptic to narrowly obovate, (13–)21–25 by 4–8 mm; androecium diam unknown, stamens 1.5–1.9 mm long, connective appendage 0.6–0.8 mm wide; gynoecium diam. unknown, carpels c. 2 mm long. *Monocarps*, stipes, and receptacle glabrous, monocarps 3–20, ellipsoid, asymmetrical, 16–19 by 12–13 mm, green maturing to yellow, purple or black in vivo, reddish brown, dark brown or black in sicco, without apparent apicule; stipes 20–40 by 2 mm; fruiting receptacle 4–9 mm diam. *Seeds* broadly ellipsoid, reddish brown, pitted, 11 by 10 mm, raphe sunken, regular.

Distribution – Peru (Amazonas and Loreto).

Habitat and Ecology – Primary, non-inundated forest, on white sand or red clay. At elevations of 170–400 m. Flowering: September and October; fruiting: April, June – August, and October.

Vernacular names – Peru: Achuana, Chiwanim.

Fig. 29. *Cremastosperma peruvianum* R.E.Fr. a. fruiting specimen; b. flower bud (a: *Knapp et al. 7645*; b: *Díaz et al. 8225*)

Notes – *Cremastosperma peruvianum* can be distinguished from other species of the genus by its long, relatively narrow leaves, and long stipes. The flower resembles that of *C. monospermum* in shape, but is larger, with relatively larger sepals, and borne on a shorter, thicker pedicel.

26. Cremastosperma stenophyllum Pirie – Fig. 30; Map 2

Cremastosperma stenophyllum Pirie (2005) 56, f. 8. – Type: *Knapp & Mallet 6159* (holo QCNE), Ecuador, Pichincha: 'Tinalandia', km 112 on the road to Santo Domingo de los Colorados from Quito, 500-1000 m, 15 Jan. 1984.

Tree c. 10 m tall, c. 20 cm diam.; young twigs and petioles rather densely covered with appressed golden hairs to 0.2 mm long. *Leaves*: petioles 5-9 by 2-2.5 mm; lamina narrowly elliptic, 25-30 by 6.5-8 cm (index 3.8-4.3), chartaceous, minutely verrucose, greyish green above, green below, very sparsely covered with appressed yellowish-white hairs to 0.2 mm long below and on veins above, base acute, apex acuminate (acumen 20-25 mm long), primary vein 1-1.5 mm wide at widest point, verrucose, secondary veins 8-10, intersecondary veins occasional, distance between from 5 mm at the base to 50 mm closer to the apex, angles with primary vein from 45-55° at the base to 70-80° closer to the apex, not branching, not forming loops, tertiary veins with some reticulation. *Inflorescence* of single flowers (1 flower observed) on brachyblasts on thicker twigs or branches; peduncle c. 1.5 by c. 1 mm (in flower); pedicels c. 45 by c. 1 mm (in flower), peduncles and pedicels and outer side of bracts (densely), sepals (densely), and petals (sparsely to rather densely) covered with appressed yellowish-white hairs to 0.2 mm long; 2 lower bracts, deltate, c. 1 mm long, obtuse; upper bract attached on basal half of pedicel, ovate, c. 1.5 by 0.8 mm, acute; flowers green, maturing to yellow in vivo, light brown with dark brown patches at the base of the petals in sicco; sepals deltate, 2 mm long, obtuse; outer petals elliptic, c. 18 by 8 mm, inner petals elliptic, c. 18 mm long (diam. unknown). *Fruit* not seen.

Distribution – Ecuador, (Pichincha and Bolívar). At elevations of 500-1200 m.

Habitat and Ecology – Secondary vegetation with primary elements. Flowering: January.

Notes – Only two collections of *Cremastosperma stenophyllum* one of which sterile, have been observed by the author. However, these are consistently distinct from all other species of the genus. *C. stenophyllum* can be distinguished even when sterile by the conspicuously green-drying, narrowly elliptic leaves. The flower somewhat resembles those of *C. awaense* but both the pedicel and leaf acumen are longer and *C. stenophyllum* also lacks the distinctive pattern of indument on the petals of *C. awaense*: the hairs are instead evenly distributed on the outer surfaces.

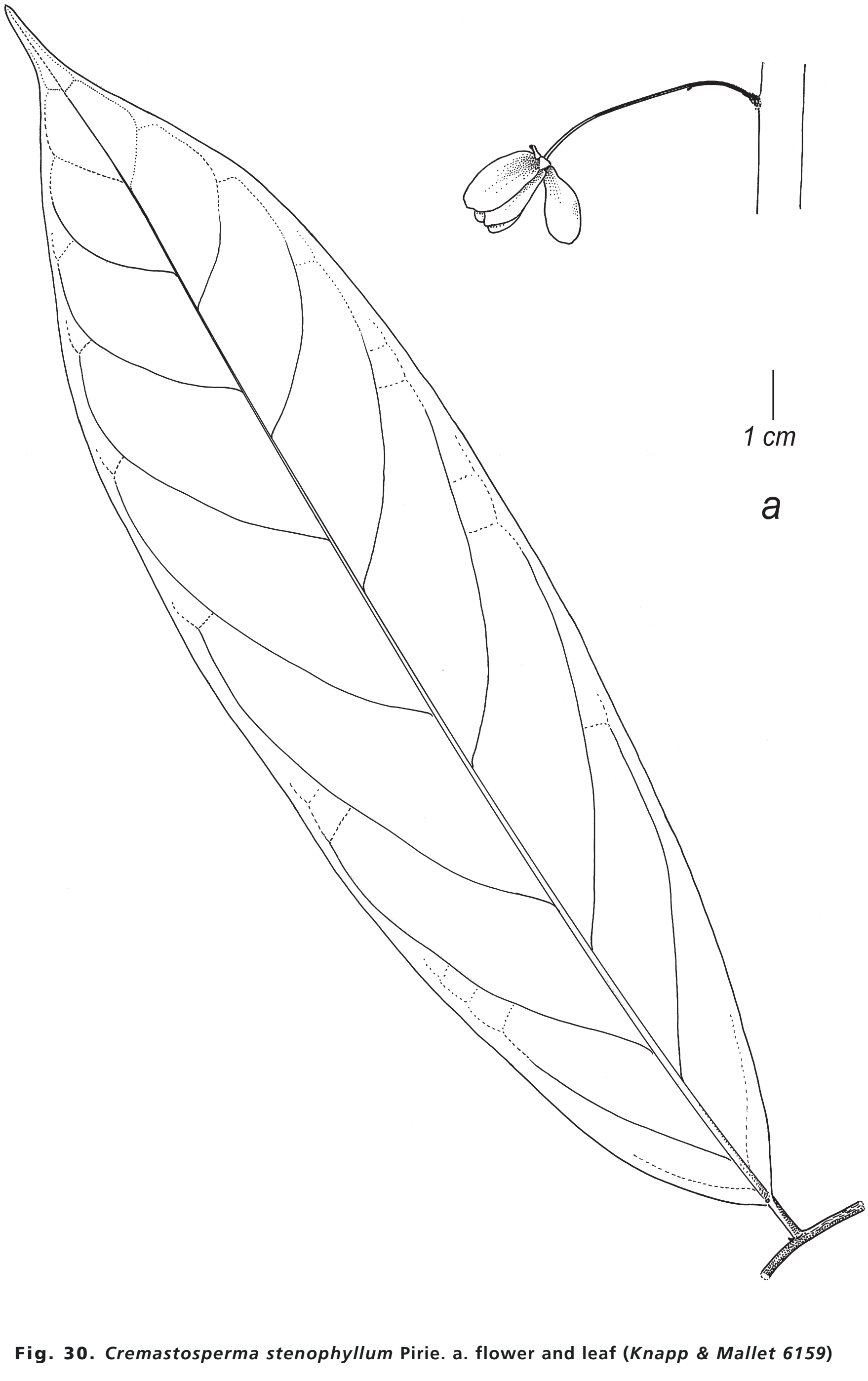


Fig. 30. *Cremastosperma stenophyllum* Pirie. a. flower and leaf (*Knapp & Mallet 6159*)

27. Cremastosperma venezuelanum Pirie – Fig. 31; Map 9

Cremastosperma venezuelanum Pirie in Chatrou & Pirie (2005) 34, f. 1. – Type: *Diaz & Niño 231* (holo U; iso NY), Venezuela, Carababo: Autonomo Mora, Watershed of Río Morón, 700–1100 m, 3 May 1991.

Tree 7–10 m tall; young twigs and petioles glabrous. *Leaves*: petioles 10–20 mm long, 3–4 mm diam.; lamina narrowly elliptic, 30–53 by 9–15 cm (index 3 – 3.6), chartacous to subcoriacous, shiny, dark brown above, olive green/greyish/light to dark brown below, glabrous on both sides, base cordate (rarely rounded), apex obtuse to acute, primary vein 2.5–5 mm wide at the widest point, secondary veins 15–30, intersecondary veins occasional, distance between from 12 mm at the base to 40 mm closer to the apex, angles with primary vein from 90° at the base to 60–50° closer to the apex, rarely branching, forming more or less indistinct loops, smallest distance between loops and margin 2–3 mm; tertiary veins showing some reticulation. *Inflorescence* of single flowers clustered in groups of up to 6, produced from leafless branches or from the main trunk; peduncles c. 2 by 1 mm (in flower), 3–5 by 1.5–3 mm (in fruit); pedicels c. 11 by 1 mm at the base (in flower), 16–22 by 1.5–2 mm (in fruit), glabrous; single lower bract, very broadly triangular, 0.5–1 mm long, obtuse, persistent, glabrous; upper bract attachment variable within central 80% of length, c. 1 by 1.5 mm, depressed triangular, obtuse, glabrous; closed flower buds not seen; flowers dark brown to black in sicco, sepals and petals glabrous; sepals free, depressed triangular, reflexed, c. 1 by 1.5 mm, acute to obtuse, persistent on less mature fruits; outer petals elliptic, c. 18 by 10 mm, inner petals narrowly elliptic, c. 21 by 6 mm; androecium c. 4 mm diam., stamens c. 1 mm long, connective rhombic, c. 0.4 mm wide; gynoecium c. 1.8 mm diam., carpels 0.6–0.7 mm long, glabrous. *Monocarps* 20–35, ellipsoid, asymmetrical, 17–20 by 12–13 mm, black in sicco, with a strongly excentric apicule, monocarps, stipes, and receptacle glabrous; stipes 15–22 by 1.5–2 mm; fruiting receptacle 5–12 mm diam. *Seeds* ellipsoid, orange-brown, shallowly pitted, 15–17 by 13–16 mm, raphe raised, regular.

Distribution – Venezuela (Aragua and Carabobo).

Habitat & Ecology – Understorey of primary, moist, evergreen forest. At elevations of 350–1100 m. Fruiting: April and May; flowering: August.

Note – *Cremastosperma venezuelanum* is best distinguished from other species of *Cremastosperma* by its distinctive acute to obtuse leaf apex (as opposed to acuminate or cuspidate in other species). The combination of cordate (rarely rounded) leaf base, the large angles of the secondary with the primary veins near the base of the leaves and the lack of indument on any parts is also unique. Only one other species of *Cremastosperma* has been collected in Venezuela: *C macrocarpum* Maas, which has longer pedicels and larger monocarps with shorter, thicker stipes.

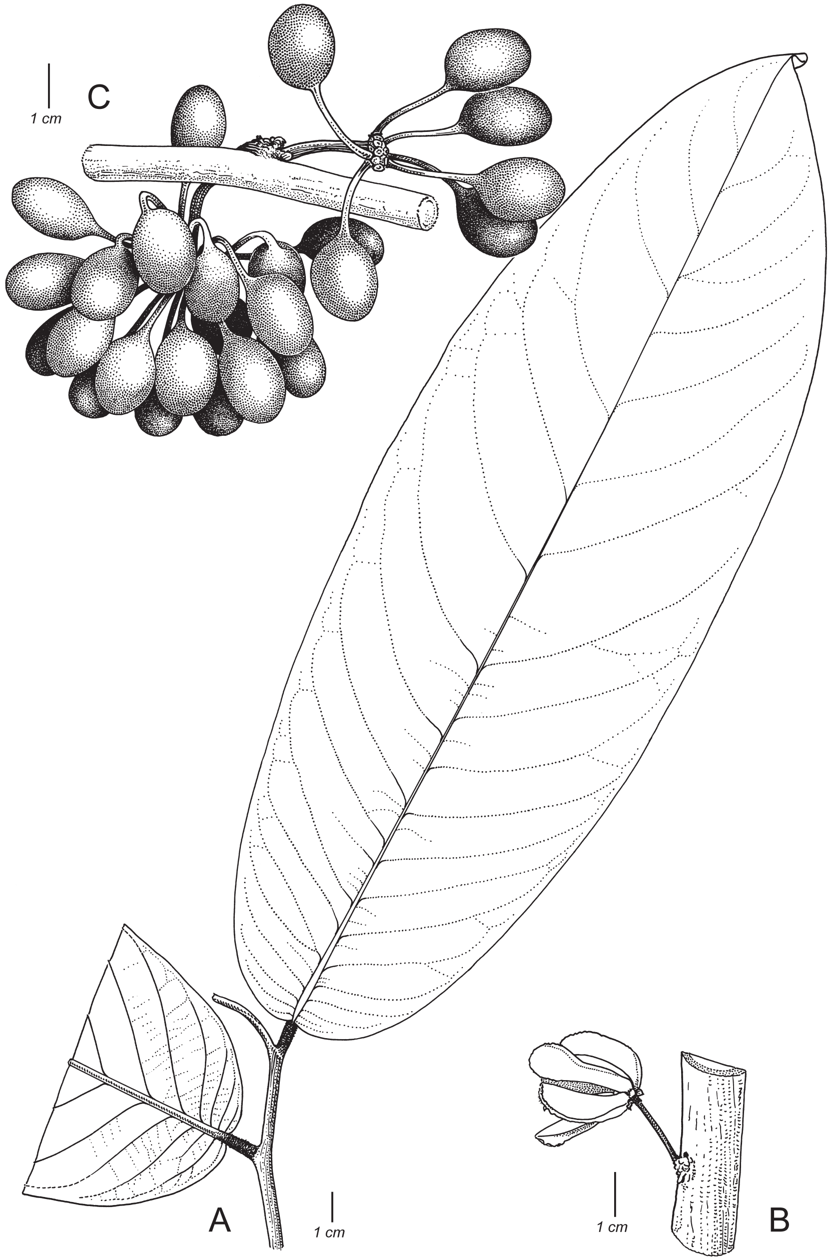


Fig. 31. *Cremastosperma venezuelanum* Pirie. A. leaves; B. flower; C. immature fruit (A, C: *Edwards 397*; B: *Steyermark 94314*)

28. Cremastosperma westrae Pirie – Fig. 32; Map 3

Cremastosperma westrae Pirie (2005) 58, f. 9. – Type: *Liesner 682* (holo MO), Panama, Panamá: road from El Llano to Carti-Tupile, 12 miles above Pan-American Highway, 200-500 m, 13 Mar. 1973.

Tree or *shrub* 4–8 m tall; young twigs and petioles sparsely to densely covered with appressed brown hairs c. 0.1 mm long. *Leaves*: petioles 6-20 by 3-6 mm; lamina narrowly elliptic to slightly obovate or narrowly so, (15-)30-50 by (8-)12-20 cm (index 1.6-3), chartaceus to coriaceus, dark to olive green or brown above, lighter below, glabrous above, sparsely covered with appressed whitish hairs 0.1 mm long on veins below, base acute to rounded, apex acuminate (acumen 10-20 mm long), primary vein 2-5 mm wide at widest point, secondary veins 8-12, no intersecondary veins, distance between from 5-10 mm at the base to 40-60 mm closer to the apex, angles with primary vein from 50-80° at the base to 45-60° closer to the apex, not branching, forming more or less distinct loops in apical half, smallest distance between loops and margin 2-5 mm, tertiary veins mostly percurrent. *Inflorescence* of single, solitary flowers, on leafy or leafless twigs; peduncles, 2–3 by 2–3 mm (in fruit); pedicels 6–17 by 2–3 mm (in fruit), peduncles and pedicels rather densely to densely covered with erect whitish hairs 0.1 mm long; single lower bract, caducous; upper bract attached in the basal half of the pedicel, depressed ovate, c. 1.5 by 2 mm, rounded, outer side densely covered with appressed brown hairs 0.2 mm long; flower buds depressed ovoid, flowers yellow in vivo, black with yellow indument in sicco; sepals free or fused for basal 0.5 mm, deltate, appressed, 2.5-3 by 2.5-3 mm, rounded, often persistent, densely covered (outside, sparsely inside) with appressed brown hairs 0.2 mm long; outer petals elliptic to slightly ovate, c. 9 by 5 mm, rather densely to densely covered (outside, sparsely inside) with appressed brown hairs c. 0.2 mm long, inner petals elliptic, c.8 by 4 mm, rather densely to densely (towards the apex outside, sparsely inside and at base) covered with appressed brown hairs 0.2 mm long; androecium c. 6 mm diam., stamens c. 1.3 mm long, connective appendage c. 0.7 mm wide, glabrous; gynoecium c. 1 mm diam., carpels sparsely covered with erect brown hairs <0.1 mm long. *Monocarps* 6–10, ellipsoid, slightly asymmetrical, 18–22 by 10–12 mm, green, maturing to yellow, orange, red or black in vivo, reddish to blackish brown in sicco, with a small excentric apicule, monocarps and stipes sparsely to rather densely covered with erect whitish hairs <0.1 mm long or glabrous; stipes 4–14 by 1-2 mm; fruiting receptacle depressed ovoid, 3-6 mm diam., densely covered with erect whitish hairs <0.1 mm long. *Seeds* ellipsoid, reddish brown, surface wrinkled and slightly pitted, c. 16 by 10 mm, raphe slightly sunken, encircling seed longitudinally.

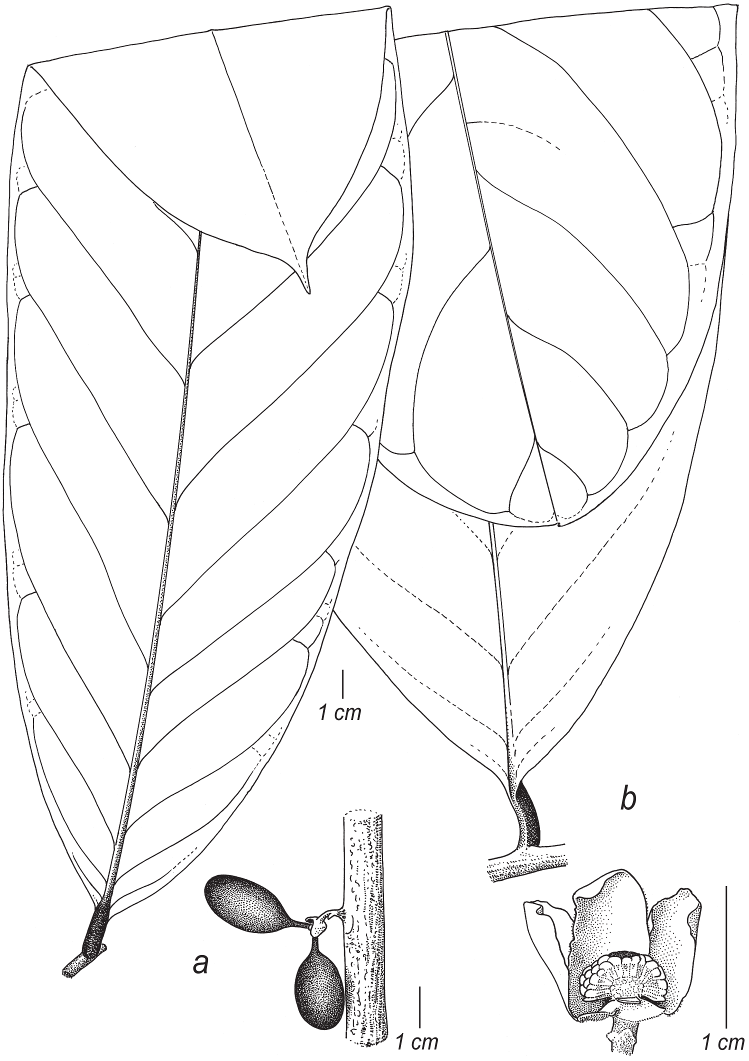


Fig. 32. ***Cremastosperma westrae*** **Pirie. a. fruit and leaf (acuminate apex); b. flower and leaf (apex broken off) (a:** ***de Nevers 4475*****; b:** ***Liesner 682*****)**

Distribution – Panama (Darién, San Blas, and Panamá).

Habitat and Ecology – Primary seasonal evergreen forest on red clay. At elevations of 50–600 m. Flowering: March; fruiting: January, March, July, November, and December.

Notes – *Cremastosperma westrae* is most similar to *C. novogranatense*: it differs in having longer stipes, smaller sepals, and less dense, shorter, hairs on the petals. The shape of the fruits of *C. pacificum* bear a resemblance to those of *C. westrae*. A clear distinction can be made due to the presence of indument on flowers and fruits in *C. westrae*: those of *C. pacificum* are glabrous.

C. westrae is reported to contain cardiac glycosides (W. Wint, on *Sudgen 613*).

Cremastosperma westrae is named after the Dutch botanist Lubbert Y. Th. Westra, whose lifelong dedication to plant systematics includes, in the last two decades, a great contribution to the taxonomy of Neotropical Annonaceae. His encyclopaedic knowledge remains an invaluable and much appreciated resource to colleagues at the Utrecht branch of the National Herbarium of the Netherlands and further afield.

29. Cremastosperma yamayakatense Pirie – Fig. 33; Map 8; App. 2, Fig. 11

Cremastosperma yamayakatense Pirie in Pirie & Zapata (2004) 10, f. 2, 6–8. – Type: *Pirie et al. 57* (holo U; iso CUZ, HAO, HUT, K, MO, NY, USM), Peru, Amazonas: Bagua, District Imaza, community Yamayakat, trail to Putuim, 340 m, 22 Nov. 2003.

Tree 1.5–8 (–20) m tall; young twigs and petioles shallowly grooved, glabrous. *Leaves*: petioles 5–10 mm long, 1–5 mm diam.; lamina narrowly elliptic, 11–24 (–38) by 3.5–8 (–13) cm (index 2.4–3.4), chartaceous, olive-grey green above, light brown below, glabrous on both sides, base acute, apex shortly acuminate to acuminate (acumen 10–25 mm long), primary vein grooved in basal quarter to third, 1–4 mm wide at widest point, secondary veins 8–10 (–14), intersecondary veins occasional, distance between from 5–10 mm at the base to 10–30 mm closer to the apex, angles with primary vein from 70–80°, the angle thereafter decreasing and subsequently increasing again towards the leaf margin, not branching, forming distinct loops, smallest distance between loops and margin 2–6 mm, tertiary veins largely percurrent with some reticulation. *Inflorescences* of single, successively produced, flowers, axillary on leafy branches and on older (leafless) branches (then on brachyblasts); peduncles c. 1 by 1 mm (in flower), 1–3 by 2–2.5 mm (in fruit), sparsely covered with golden hairs c. 0.1 mm long; pedicels 5–7 by c. 1.5 mm at the base (in flower), 8–15(–20) by 2–2.5 mm at the base to 4 mm diam. at the apex (in fruit), glabrous; single lower bract, deltate, 1–2 by 1–2 mm, acute, mostly caducous in fruit, rather densely covered with golden hairs 0.1 mm long; upper bract inserted within basal half of pedicel, deltate, 1–2 by 1–2 mm, acute, glabrous; flower buds depressed ovoid, remaining closed in development; flowers green maturing to yellow in vivo, black in sicco, sepals and petals glabrous; sepals basally connate, deltate, appressed, c. 3 by 3 mm, rounded, caducous, rarely persistent; outer petals ovate, 10–15 by 8–12 mm, inner petals elliptic, c. 12 by 6 mm; androecium 6–7 mm diam., stamens c. 1 mm long, connective appendage c. 0.5 mm wide; gynoecium c. 3 mm diam., carpels length and indument unknown. *Monocarps* 10–22, green maturing through red to black in vivo, black in sicco, ellipsoid, slightly asymmetrical, 12–14 by 7–8 mm, with an excentric apicule, glabrous or sparsely

Revision of Cremastosperma (Annonaceae)
Cremastosperma yamayakatense Pirie
Det.: Michael D. Pirie Utrecht, 2005

PERU

ANNONACEAE

Amazonas: Bagua Province

Distrito Imaza: Región del
Marañon. Comunidad de Yamayakat,
Río Marañon. Bosque primario.
04°55'S 78°19'W 550 m

Arbusto de 2.5 m. de alto, frutos
rojos, flores verdes.
06 Marzo 1995

N. Jaramillo y R. Apanu 584
Proyecto auspiciado por la John D. and
Catherine T. MacArthur Foundation
MISSOURI BOTANICAL GARDEN HERBARIUM (MO)

Fig. 33. ***Cremastosperma yamayakatense*** **Pirie. a. fruiting specimen with old flower (and loose petals); b. flower (a:** ***Jaramillo & Apanu 584*****; b:** ***Barbour 4432*****)**

covered with golden hairs <0.1 mm long; stipes green maturing to red in vivo, 11-12 by c. 1.5 mm increasing to 3 mm when ripe, glabrous or sparsely covered with golden hairs <0.1 mm long; fruiting receptacle 5-10 mm in diam, glabrous. *Seeds* ellipsoid, reddish-brown with small black pits surrounded by a slightly raised rim, 9-13 by 6-7 mm, raphe sunken, regular.

Distribution - Peru (Amazonas), watershed of the upper Río Marañon.

Habitat and Ecology - Primary and secondary forest. At elevations of 200-1000 m. Flowering: November, January-March; fruiting: throughout the year except December and April.

Notes - *Cremastosperma* yamayakatense resembles two other species of *Cremastosperma*; *C. gracilipes*, which has been collected in the departments of Napo and Pastaza in Ecuador, Loreto in Peru and in adjacent Colombia, and *C. cenepense*, from the Cenepa region of Amazonas, Peru, with which its distribution therefore overlaps. The most important differences between *C. yamayakatense* and *C. gracilipes* are in the flowers. *C. gracilipes* is characterised by flower buds which open during development and which bear indument on all parts. In contrast, the flower buds of *C. yamayakatense* bear virtually no indument and appear to remain closed throughout development, the petals only opening slightly when the flowers are mature. Additionally, the flowers of *C. gracilipes* are borne on longer more slender pedicels than those of *C. yamayakatense*. *C. yamayakatense* differs from *C. cenepense* in the shape of the leaf base (acute in *C. yamayakatense,* cordate to subcordate in C. cenepense) and the length of the stipes (longer than the monocarps in *C. yamayakatense*, shorter than the monocarps in *C. cenepense*). The lack of flowering material of *C. cenepense* makes further distinction currently impossible.

Flowering and fruiting specimens of *C. yamayakatense* of around 1.5 m tall were observed in the province of Bagua, though specimens collected both in this area and particularly those collected further north into the province of Condorcanqui, in the area of the Río Cenepa, have been recorded as reaching heights of 6-8 m and in one case 20 m tall. Differences between collections from these two regions have been observed: The leaves of Condorcanqui specimens are generally larger and the fruits have a slight indument whereas those of the Bagua collections are glabrous. In the absence of floral material from the Cenepa region it is assumed that these specimens do represent the same species due to the short pedicel, leaf base shape (which excludes the possibility of their representing specimens of *C. cenepense*) and leaf venation.

Dubious species

Guatteria socialis J.F.Macbr. (1929) 171. – Type: *C. Schunke 395* (holo F), Peru, Junín: Chanchamayo Valley, 1500 m, Oct. 1924–1927.

The type specimen of *G. socialis* represents a species of *Cremastosperma*, but to which species it might belong is not clear due to the immature state of the flower. Only a photo has been seen by the authors.

Insufficiently known species

30. Cremastosperma spec. A – Fig. 34; Map 2

Tree 3–7 m tall, 5–7 cm diam.; young twigs and petioles very sparsely covered with appressed whitish hairs to 0.2 mm long or glabrous. *Leaves*: petioles 7–15(–20) by 2–4 mm; lamina narrowly obovate or elliptic, 21–44 by 5–12 cm (index 2.6–3.4), chartaceous, green or brown above and below, veins darker below, glabrous above, very sparsely covered with appressed whitish hairs to 0.2 mm long, especially on veins, or glabrous below, base acute or obtuse, apex acuminate (acumen 11–20 mm long), primary vein shallowly grooved near base, 1.5–2 mm wide at widest point, secondary veins 7–10, intersecondary veins 0–1, distance between from 10–13 mm at the base to 25–30 mm closer to the apex, angles with primary vein from 70–80° at the base to 45–60° closer to the apex, sometimes branching, forming distinct loops in apical half of leaf, smallest distance between loops and margin 2–3 mm, tertiary veins mostly percurrent. *Inflorescence* of single flowers solitary on leafless twigs or clustered in groups on brachyblasts on the main stem; peduncles 4 by 4 mm (in fruit); pedicels 18–23 by 3 mm diam. at the base, 3–4 mm diam. at the apex (in fruit), peduncles and pedicels glabrous; lower bract(s), upper bract and flowers not seen. *Monocarps* 20–32, ellipsoid (broadly so in immature specimens), slightly asymmetrical, 16–17 by 11–12 mm, green maturing to yellowish, orange and purple in vivo, black in sicco, with a small excentric apicule, monocarps, stipes and receptacle glabrous; stipes 17–18 by 2 mm; fruiting receptacle 14 mm diam. *Seeds* ellipsoid, reddish brown, shallowly pitted, c. 12 by 10 mm, raphe sunken, regular.

Distribution – Costa Rica: Osa peninsula.

Habitat and Ecology – Tropical wet forest. At elevations of 40–300 m. Fruiting: July, September.

Notes – On the basis of the fruits *Cremastosperma spec.* A appears distinct, and its distribution in Costa Rica is the furthest north into Central America of any species of the genus. However, it is the authors opinion that formal description should await the availability of flowering material.

The fruits are reported to occasionally be inhabited with fiercely biting dolichotenne ants.

Revision of Cremastosperma (Annonaceae)

Cremastosperma spec. A

Det.: Michael D. Pirie Utrecht, 2005

MUSEO NACIONAL
COSTA RICA
ANNONACEAE dup.= 2
Cremastosperma

Det.

PUNTARENAS: Reserva Forestal Golfo Dulce
Rincón de Osa. Quebrada Banegas.
8°40'25"N 83°31'20"W 40-100 m
Arbolito de 7 m. Frutos naranja y morado
pegados al tallo. Pedúnculo naranja.

Reinaldo Aguilar 467 25 September 1991

HERBARIO NACIONAL DE COSTA RICA (CR)
INSTITUTO NACIONAL DE BIODIVERSIDAD//MISSOURI BOTANICAL GARDEN (MO)

Fig. 34. *Cremastosperma spec. A*. fruiting specimen (*Aguilar 467)*

Specimens examined:

Costa rica: **Puntarenas:** *Aguilar 467* (INB, MO), Reserva Forestal Golfo Dulce Rincón de Osa, Quebrada Banegas; *Aguilar 4371* (INB), Canton de Osa; *Chatrou et al. 103* (U), Cantón Osa, Bahia Chal, road to La Palma; *Gentry et al. 78657* (F, INB, MO), Fila before Rancho Quemado, near Rincón, Osa Peninsula. Transect 1; *Herrera 3972* (INB), Parque Nacional Corcovado, Cerro Brujo, cabeceras de Quebrada Vaquedano; *Kernan 1224* (U), Parque Nacional Corcovado, Sirena, Los Patos Forest; *Schatz et al. 1002* (MO, U, WIS); S of the new road from the Interamerican Highway at Chacarita to Rincón de Osa, between the road and the Golfo Dulce; *Zamora 2312* (INB, U), Osa.

31. Cremastosperma spec. B – Fig. 35; Map 6

Tree or *shrub* 3-8 m tall, 3-10 cm diam.; young twigs and petioles sparsely covered with appressed whitish hairs to 0.3 mm long. *Leaves*: petioles 7-10 by 1.5-3 mm; lamina elliptic to obovate, or narrowly so, 10-28(-34) by 7-10(-12) cm (index 1.9-3.5), chartaceous to coriaceous, green (or greenish brown), darker above, lighter or more brown with darker or reddish veins below, glabrous above, sparsely covered with appressed whitish hairs to 0.3 mm long particularly on veins below, base acute to obtuse (rarely rounded), apex acuminate (acumen 6-20 mm long), primary vein 1.5-3 mm wide at widest point, secondary veins 7-10, intersecondary veins 0-1, distance between from 11-20 mm at the base to 12-32 mm closer to the apex, angles with primary vein from 70-80° at the base to 40-50°closer to the apex, rarely branching, mostly forming distinct loops, smallest distance between loops and margin 2-5 mm, tertiary veins mostly percurrent. *Inflorescence* of single (very rarely branching) flowers, solitary, axillary on leafy or leafless twigs or thicker branches; peduncles (1-)3-5 by 1-2 mm (in flower), 3-9 by c. 2 mm (in fruit), rather densely covered with appressed to erect whitish hairs to 0.2 mm long; pedicels 20-45(-68) by c. 1 mm at the base (in flower), 22-80 by 1-2.5 mm (in fruit), pink, purple or reddish in vivo, glabrous; 2 lower bracts of unequal dimensions, basal lower bract depressed ovate, c. 0.5 by 1 mm, obtuse, sometimes persistent, densely covered with appressed to erect whitish hairs to 0.2 mm long, apical lower bract elliptic, c. 1.5 by 1 mm, obtuse, sometimes persistent, sparsely to rather densely covered with appressed to erect whitish hairs to 0.2 mm long; upper bract attached around midway along pedicel, (broadly) ovate, 1.5-2 by c. 1-1.5 mm, obtuse or rounded, persistent, sparsely covered with appressed to erect whitish hairs to 0.2 mm long; flower buds broadly ovoid, opening in development; flowers green maturing to yellow or white and yellowish at base in vivo, dark to yellowish light brown sometimes tinged with red, darker at the base in sicco, sepals and petals glabrous; sepals free, deltate or triangular, appressed to recurved, c. 3 by 2-3 mm, acute or obtuse, caducous or sometimes briefly persistent; outer petals elliptic, 14-17 by 6-8 mm, inner petals elliptic to narrowly so, 16-17 by 5-7 mm; androecium c. 6 mm diam., connective appendage to 0.8 mm wide; gynoecium c. 1.5 mm diam., carpels

Revision of Cremastosperma (Annonaceae)

Cremastosperma spec. B

Det.: Michael D. Pirie Utrecht, 2005

PERU: Madre de Dios

Cremastosperma monospermum (Rusby) R. E. Fries

Tree, 6 m.; flowers (all save one picked up fallen on ground) largely cauliflorous; petals greenish with purple sheen, especially below, in 2 whorls of 3; calyx of 3 sepals that are darker green and form the unopened flower bud; flowers on purple pedicel; stamens orange and in a dense ring about green centralmost carpels; fruit green on purple stalks.

At 250 m. along the Big Tree Trail.

Explorer's Inn, near the confluence of Rio Tambopata and Rio La Torre, 39 km. SW. of Puerto Maldonado, 12° 50' S. and 69° 20' W.

S. F. Smith, D. A. Bell, J. U. McClammer, Jr., F. O. Smith, and M. E. Timana # 1578 Jan. 24, 1989

Fig. 35. ***Cremastosperma spec. B*****. a. fruiting and flowering specimen; b. flower (a: *Smith, S. et al. 1578*; b: *Smith, S. et al. 794*)**

glabrous. *Monocarps* 10–25(–32), ellipsoid (broadly so when immature), slightly asymmetrical, 8–12 by 6–8 mm, green maturing to greenish purple and brown in vivo, light to dark brown or blackish in sicco, with an excentric apicule, monocarps, stipes, and receptacle glabrous; stipes 8–13 by c. 2 mm; fruiting receptacle 4–10 mm diam. *Seeds* ellipsoid, light brown, shallowly pitted, c. 11 by 6 mm, raphe sunken, regular.

Distribution – Peru (Madre de Dios and Cuzco).

Habitat and Ecology – Primary and secondary moist and wet forest, occasionally on floodplains. At elevations of 210–670 m. Flowering: August – December; fruiting: January – May, August.

Notes – A number of the specimens grouped under *Cremastosperma spec.* B have in the past been identified as *C. leiophyllum* or *C. monospermum*, two well known species found relatively nearby in Bolivia and widespread across Bolivia, Brazil, and Peru respectively. *C. spec.* B is distinguished here from *C. leiophyllum* (with which distributions do not overlap) on the basis of the shape and colour of the monocarps: those of *C. leiophyllum* dry conspicuously black with a characteristic asymmetrical shape not observed in fruits of *C. spec. B* Flower buds of *C. monospermum* remain closed, with a distinctive triangular shape, throughout development, as opposed to the open flowers of *C. spec.* B In the area of Madre de Dios where the distributions of *C. monospermum* and *C. spec.* B overlap, variation in the otherwise relatively consistent leaf shape of *C. monospermum* appears to be greater, and non-flowering specimens of the two are often not discernable. Morphological variation in specimens here grouped under *C. spec.* B is relatively wide – particularly in the size, shape and texture of leaves and length of pedicel. Including part, or the total variation, of *C. spec. B* within that of either *C. monospermum* or *C. leiophyllum* would, in the opinion of the first author, neither be justified nor helpful for their recognition. However, the specimens currently defining *C. spec.* B are grouped more on the basis of the absence of characters present in those species than on characters that unambiguously diagnose it in its own right. Limited availability of collections, particularly from the department of Cuzco, could also mean that this grouping represents more than one species. For this reason, this it is described here under an unofficial name, awaiting further material on which to base a more satisfactory solution.

Selection of other specimens (30) examined:

Peru: **Cuzco:** *Núñez 12951* (U, USM), Quispicanchi; *Núñez et al. 10146* (MO, U), La Convención, below Echarate, upper Río Urubamba, Manguyari. **Madre de Dios:** *Foster 11495* (U, USM), Manu, Parque Nacional Manu. Río Manu: Tayakome; *Foster et al. 3105* (F), Manu, Shintuya, 1 km up small stream from Río Alto Madre de Dios; *Smith, S.F. et al.1578* (NY, U, USM), Tambopata, Explorer's Inn, near the confluence of Río Tambopata and Río La Torre, 39 km SW of Puerto Maldonado; along the Big Tree Trail.

Excluded species

Cremastosperma anomalum R.E.Fr. (1948) 4, pl. 1c–d. – Type: *Killip & Garcia 33600* (holo S; iso COL, UC, US), Colombia, Chocó: Bahia Solano, near Ciudad Mutis, along Quebrada Jella, 0–75 m, 21–23 Feb. 1939.

≡ **Klarobelia anomala** (R.E.Fr.) Chatrou (1998) 123, f. 2.

Cremastosperma guianense R.E.Fr. (1934) 205. – Type: *Davis 102* (holo K), Guyana, Apoteri: Rupununi River, 21 July 1931.

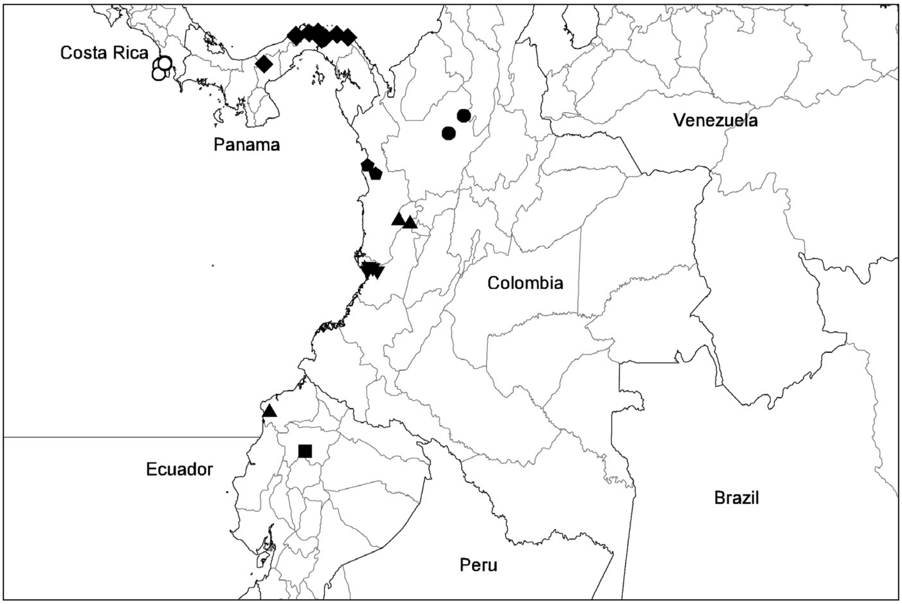
≡ **Pseudoxandra lucida** R.E.Fr. (1937) 230, f. 3a–e.

Cremastosperma polyphlebum (Diels) R.E.Fr. (1931) 331. – Type: *Ule 5628* (holo B; iso F, G, K, MG, S), Brazil, Acre: Rio Juruá-Mirim, Aug. 1901.

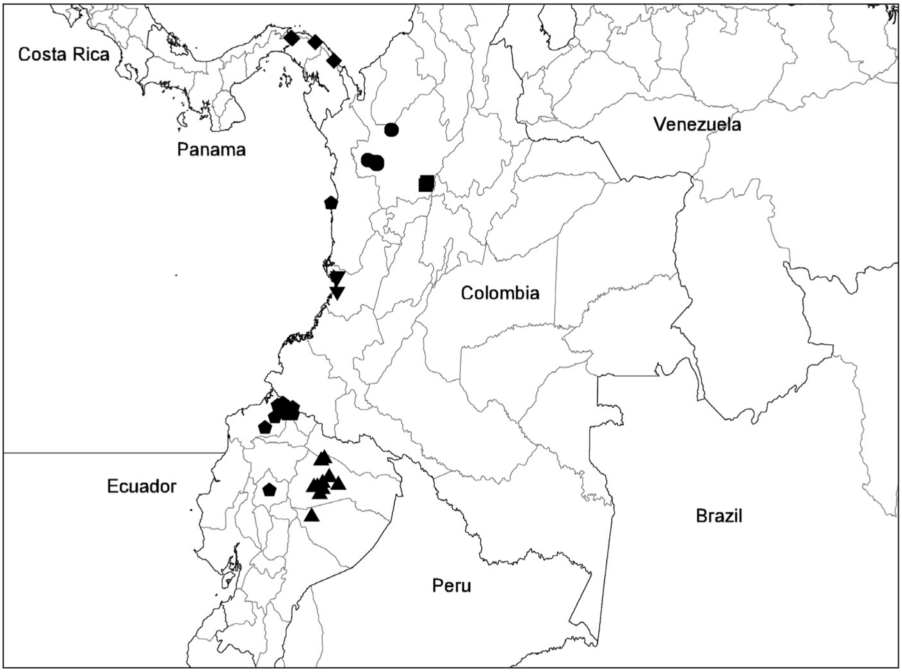
≡ **Pseudoxandra polyphleba** (Diels) R.E.Fr. (1937) 230.

Cremastosperma williamsii R.E.Fr. (1934) 206. – Type: *Ll. Williams 3960* (holo F; iso S), Peru, Loreto: Yurimaguas, Recreo, 23 Oct. 1929.

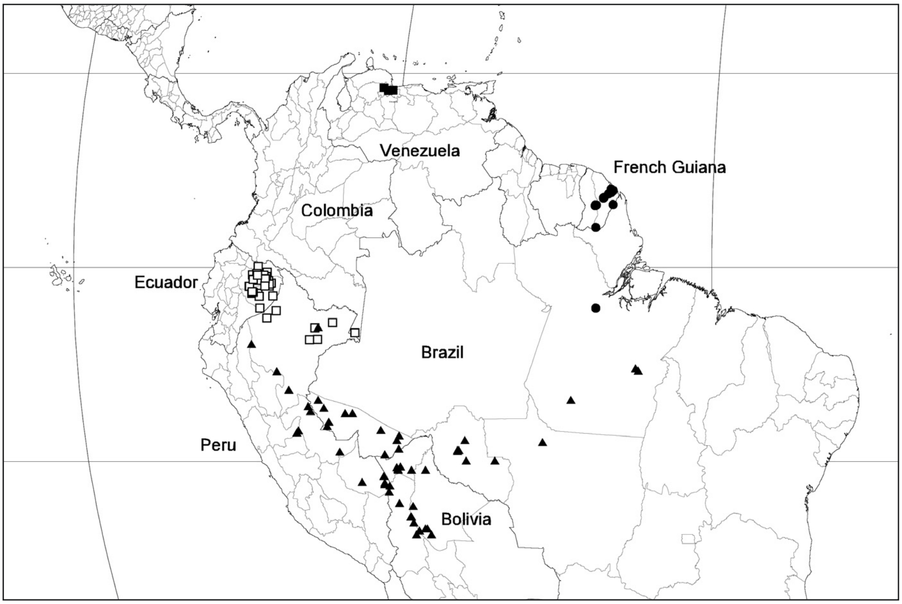
≡ **Pseudoxandra williamsii** (R.E.Fr.) R.E.Fr. (1937) 227, f. 2b,c.



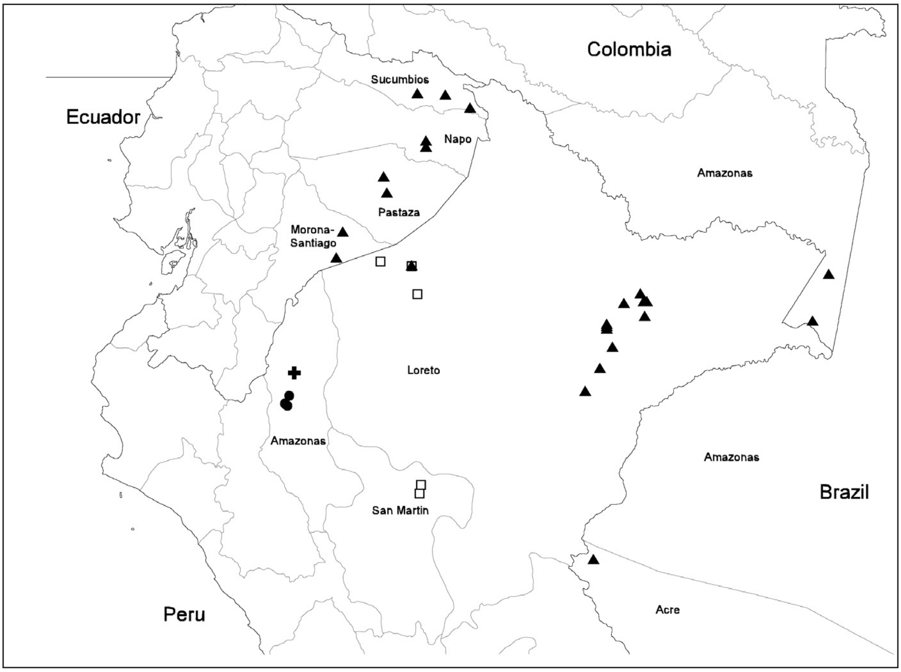
Map 2. Distribution of *Cremastosperma antioquense* Pirie (●), *C. chococola* Pirie (⬟), *C. longipes* Pirie (▲), *C. novogranatense* R.E.Fr. (inverted ▼), *C. panamense* Maas (◆), and *C. stenophyllum* Pirie (■)



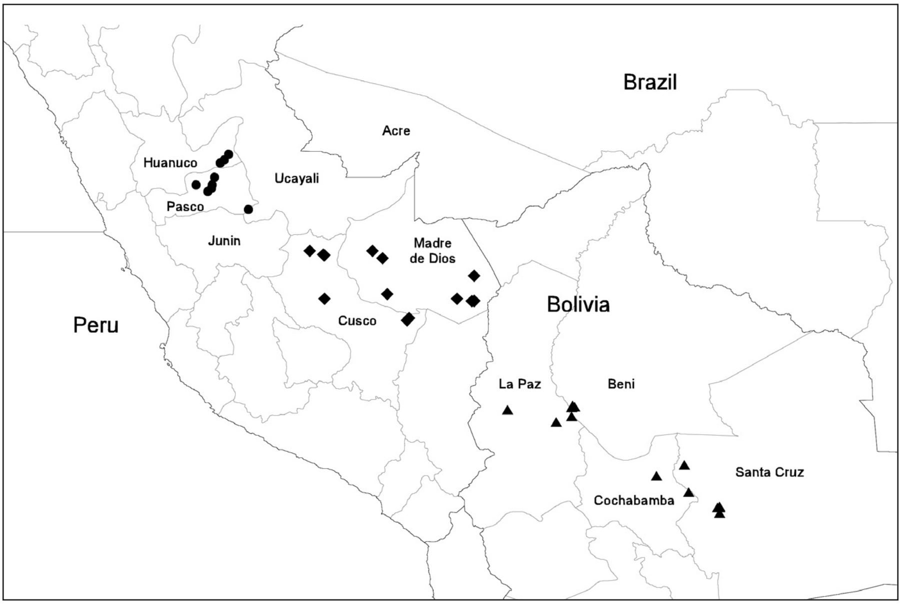
Map 3. Distribution of *Cremastosperma awaense* Pirie (⬟), *C. dolichocarpum* Pirie (●), *C. magdalenae* Pirie (■), *C. napoense* Pirie (▲), *C. pacificum* R.E.Fr. (△), and *C. westrae* Pirie (◆)



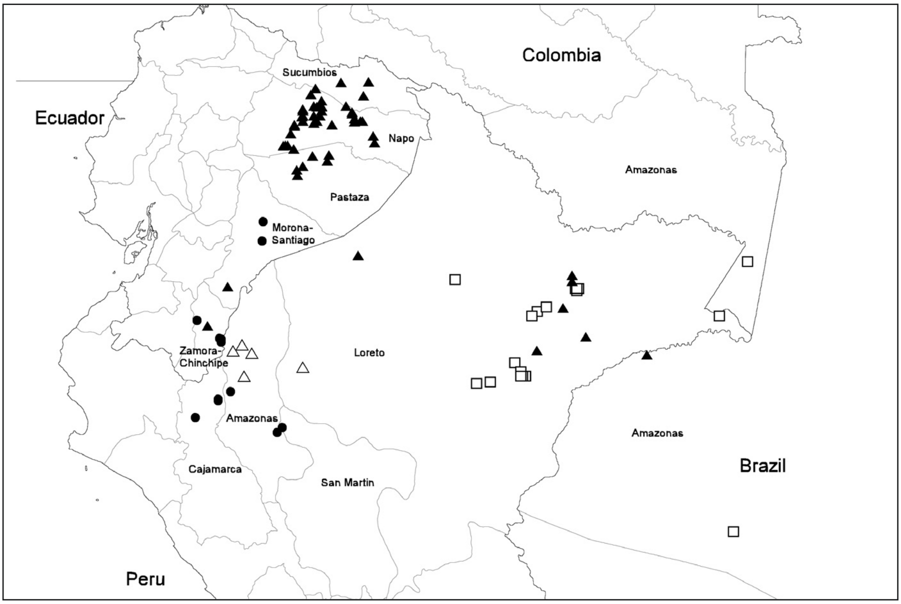
Map 4. Distribution of *Cremastosperma brevipes* (DC.) R.E.Fr. (●); *C. gracilipes* R.E.Fr. (□); *C. macrocarpum* Maas (■); *C. monospermum* (Rusby) R.E.Fr. (▲)



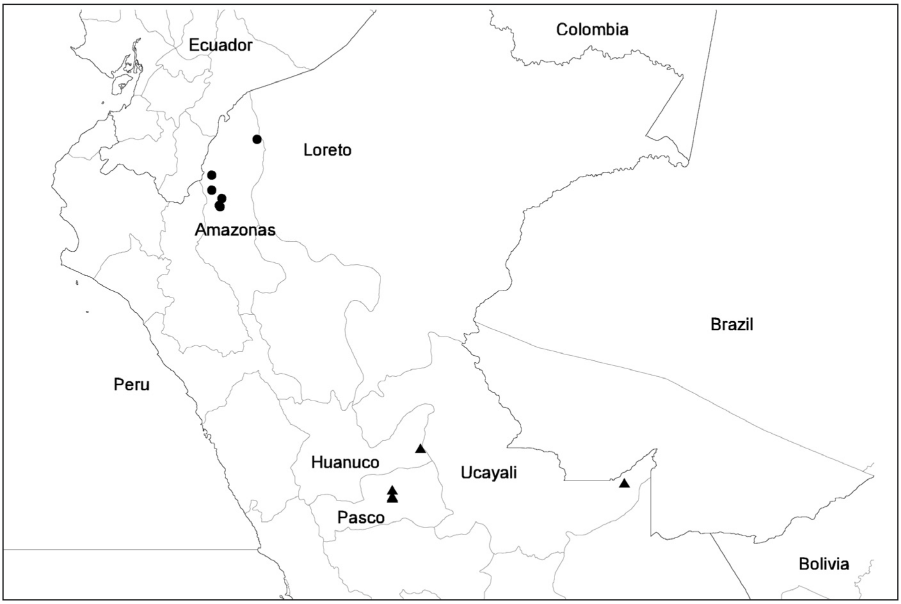
Map 5. Distribution of *Cremastosperma bullatum* Pirie (●); *C. cauliflorum* R.E.Fr. (▲; *C. cenepense* Pirie & Zapata (✚); *C. longicuspe* R.E.Fr. (☐)



Map 6. Distribution of *Cremastosperma leiophyllum* R.E.Fr. (▲); *C.oblongum* R.E.Fr. (●); *C. spec.* B (◆)



Map 7. Distribution of *Cremastosperma megalophyllum* R.E.Fr. (▲); *C. microcarpum* R.E.Fr. (□); *C. pedunculatum* (Diels) R.E.Fr. (●); *C. peruvianum* R.E.Fr. (△)



Map 8. Distribution of *Cremastosperma pendulum* (Ruiz & Pav.) R.E.Fr. (■); *C. yamayakatense* Pirie (●)

Map 9. Distribution of *C. macrocarpum* Maas (☐); *C. venezuelanum* Pirie (▲)

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Identification list

Collections are identified by the first collector and collector number only. The abbreviations behind the collector numbers refer to the following taxa:

Cremastosperma
ant = *antioquense*
awa = *awaense*
bre = *brevipes*
bul = *bullatum*
cau = *cauliflorum*
cen = *cenepense*
cho = *chococola*
dol = *dolichocarpum*
gra = *gracilipes*
lei = *leiophyllum*
lon = *longicuspe*
lonp = *longipes*
mac = *macrocarpum*
mag = *magdalenae*
meg = *megalophyllum*
mic = *microcarpum*
mon = *monospermum*
nap = *napoense*
nov = *novogranatense*
obl = *oblongum*
pac = *pacificum*
pan = *panamense*
ped = *pedunculatum*
pen = *pendulum*
per = *peruvianum*
ste = *stenophyllum*
ven = *venezuelanum*
wes = *westrae*
yam = *yamayakatense*
spA = *spec.* A
spB = *spec.* B

Acevedo-Rodrigues 7512: meg; 7523: meg; 7543: gra; 8635: spB; 9132 spB — Acosta Solís 6429: ste — Aguilar 467: spA; 691: mon; 764: mon; 905: spB?; 4371: spA — Alcorn 11: lon? — Alvarado 228: nap; 267: nap; 298: meg; 427: nap — Alvarez 2405: gra — Ancuash 262: per; 1324: yam; 1517: per — Arrazola 134: lei — Aulestia, M. 637: awa; 842: awa; 1184: awa; 1513: gra; 1726: gra; 2127: gra; 3238: cau; 3395: cau: 3520: gra — Ayala 15: cau; 2432: meg; 3485: mic.

Balslev 2418: gra; 4634: gra; 62310: gra — Barbour 4432: yam; 4851: spB; 4856: spB; 5111: spB — Barclay 4755: gra — Barrier 5166: bre — Beck 1675: lei; 8234: mon; 10092: mon; 12221: mon; 16540: mon; 19323: mon — Beltran 3245: spB; 3580: obl; 5488: meg; 5782: mic — Berlin 218: per; 1588: yam; 1950: per; 2079: per —- Betancur 3309 (changed on label to 33011): dol?; 6043: awa — Boom 5039: mon; 10812: bre — Bourdy GB1743: mon; GB1828: mon — Brandbyge 30017: meg; 30472: gra; 31854: cau; 32225: cau; 32566: meg; 32589: gra; 32681: gra; 32685: gra; 33337: gra; 33434: gra; 33503: cau — Buchtien 705: lei; 706: lei — Bulnes 502: obl.

Callejas 3110: dol — Camp E1311: ped — Campbell 9026: mon — Campos 4160: ped; 4213: ped; 4268: ped — Cárdenas 2899: mag — Cazalet 7528: cau — Cerón 404: meg; 612: meg; 702: gra; 1268: meg; 1353: gra; 2321: gra; 2361: gra; 2378: meg; 2428: gra; 2483: meg; 2500: meg; 2675: gra; 2986: nap; 3009: gra; 3031: meg; 3036: meg; 3037: gra; 3125: meg; 3939: gra; 3949: gra; 4159: meg; 5358: gra;

5436: gra; 5983: gra; 6663: nap; 9324: meg —- Chatrou 103: spA; 208: mic; 224: cau; 227: mic; 233: cau; 238: cau; 259: meg; 265: meg; 267: gra; 268: meg — Chávez 70: yam — Cheta 6/173: cau — Cid Ferreira 2724: mon; 2868: mon; 4507: mon; 4556: mon; 4590: mon; 4673: mon; 5262: cau; 6301: mon; 9959: meg; 10019: cau; 10120A: mon; 10286: mon — CID-JH 6-235: cau — Clark 3158: meg — Coello 60: gra — Cogollo 6039: dol; 6040: dol; 6044: dol; 6052: dol; 6056: dol; 6345: dol; 7869: dol — Cornejo, F. 2276: mon; 2546 spB? — Cornejo, S.X. 4493: gra — Cornejo, X. 6451: awa; 6702: ped — Cremers 15331: bre — Croat 18494: cau — Cuatrecasas 10575: meg; 10726: cau; 11125: cau; 11331: meg; 17463: pac; 17573: nov; 21288: nov; 26031: nov.

Dahlgren 162: mon — Daly 5764: mic; 6186: meg; 6498: mon; 6573: mon; 6630: mon; 6686: mon; 6909: mon; 7347: mon; 7450: mon; 8043: mon; 11719: mon — Davidse 23634: pan — Davidson 9869: mic — Del Carpio 1833: mic; 1923: mic; 1942: mic; 2213: mic; 8009: mon — Devia 2875: nov?; 3762: pac; 4065: pac; 5335: nov — De Nevers 3601: pan; 3906: pan; 4129: pan; 4475: wes; 4571: pan; 4898: pan; 5189: pan; 5221: pan; 5260: pan; 6171: pan; 6513: pan — DeWalt 662: mon — Díaz, S. 825: mon; 1229: cau; 1411: gra; 6851: yam; 8225: per; 8375: yam; 8899: mon; 9563: mon — Díaz, W. 231: ven; 274: mac — Dik 323: meg; 1732: gra — Ducke RB19620: meg — Dwyer 3314: pan; 10248: pan.

Edwards 397: ven — Escobar 3309: mag — Espina 3188: cho — Espinoza 129: meg; 399: cau; 578: gra.

Faber-Langendoen 476: nov; 1208: pac — Fernández 8872: lonp — Feuillet 10239: bre; 10337: bre — Figuieredo 853: mon; 906: mon — Flora Falcón 937 (= Van der Werff 937): mac — Flores 165: obl: 1241: obl — Folsom 6155: pan — Forero 6576: lonp — Forget 305: bre — Foster 2477: spB?; 2512: spB; 3105: spB; 3128: spB; 3157: spB; 3418: spB?; 3502: spB?; 5004: spB?; 5083: spB; 5292: spB?; 6026: spB?; 6589: spB; 8235: spB?; 9482: pen; 10008: pen; 10224: obl; 11495: spB; 12549: mon; 13422: mon — Freire 407: meg; 468: nap — Freitas 8: mic — Fróes 23962: cau.

Galdames 1162A: pan; 1585: pan — García 390: cho — Gentry 5070: pan; 7286: cho; 8828: pan; 8910: mon; 21928: gra; 25111: mic; 29126: cau; 32009: spB; 32153: mic; 35298: pac; 35562: pac; 37169: cau; 38085: mic; 38127: mic; 41879: obl; 42985: mic; 43174: mic; 43776: mic; 45355: ped; 45371: ped; 45481: ped; 45510: ped; 52233: mon; 56210: mic; 57078: nov; 58434: mon; 60032: gra; 60865: cau; 65601: cau; 68663: mon; 70042: awa; 72190: mic; 72235: cau; 72995: lonp; 76343: cau; 76357: mic; 78657: spA; 79039: dol; 80543: ped; 80904: ped — GHS 703: obl — Gómez-Pompa 3067: pan — Gonzales 53: mon — Graham 635: pen; 1713: mon — Grández 222: mic; 491: mic; 1591: cau; 2032: mic; 2488: gra - de Granville 390: bre; B5186: bre; 5670: bre; 5672: bre; 7555: bre; 7672: bre; 7799: bre; 8714: bre; 8876: bre — Grenand 1509: bre — Grijalva 305: meg — Gudiño 310: meg; 1123: meg; 1787: gra.

Harling 17581: gra — Hedin 57: spB?; 74: spB?; 94: mic; 96: mic — Helme 742: mon — Hequet 432: bre — Hernández 251: mag - Herrera, G. 3972: spA; Herrera, H. 1122: wes; 1231: pan — Hinojosa 1313: lei — Hodges 111: yam; 112: yam — Holm-Nielsen 903: meg; 21501: cau; 22082: meg — Hurtado 160: nap; 186: nap; 322: nap; 896: nap; 941: nap; 1385: meg; 2096: nap; 2430: meg; 2445: meg; 2448: meg; 3005: meg; 3019: gra; 3156: meg.

Irvine 374: meg; 734: meg — Irwin 47562: bre.

Jacquemin 2349: bre — Jaramillo, J. 1420: ped; 3533: meg; 3577: gra; 8352: meg; 8364: meg; 12840: nap; 13489: ped; 30773: gra — Jaramillo, N. 403: yam; 584: yam; 686: cau; 918: yam; 942: bul; 972: bul; 1110: yam; 1248: yam; 1409: yam — Jardim 597: mon; 759: mon; 2408: mon — Johnston 1555: pan; 1812: pan — Juncosa 736: mag.

Kanehira 215: ped? — Kayap 33: yam; 631: per; 1078: cen — Kernan 1224: spA — Killeen 3080: lei; 3085: lei; 3110: lei; 3238: lei — Killip 23622: obl; 27902: mon; 28537: lon; 28961: mon; 29020: lon; 37752: ven — Klug 902: cau; 3069: meg; 3726: ped — Knapp 6159: ste; 6354: mon?; 6450: spB; 6588: ped; 7178: lon?; 7183: lon?; 7215: lon; 7645: per; 7864: lon?; 8203: lon — Krukoff 4697: mon; 4748: mic; 4975: mic; 6151: mic; 10556: lei — Kuhlmann 1426: lon — Kvist 892: mic; 1127: mic; 1265: mic; 1355: mic.

Lawesson 39560: gra; 39598: meg — Lescure 549: bre; 2200: meg — Lewis, M. 37979: lei — Lewis, W.H. 11166: lon; 11831: lon; 12186: lon — Liesner 682: wes; 1362: pan; 9763: mac — Lima 582: mon — Lleras P16879: cau — Lowrie 463: mon — Luteyn 4890: gra; 8630: gra; 9057: gra.

Maas 4592: obl; 6271: cau; 6281: mic; 8064: bre; 8222: mic; 8289: mic; 8300: mic; 8577: meg; 8595: meg; 9029: cau; 9148: obl; 9251: cau; 9328: bre; 9559: pan; P13079: mon - Martin s.n. bre — Mathias 5510: mic; 6007: mon — McDaniel 2570: pen; 17020: mic; 20264: mic; 20677: mic; 20761: cau — McDonagh 181: pan; 291: wes — McPherson 9957: pan; 11046: pan; 14039: pan — Meneces 395: lei — Miller 794: meg; 947: pan — Mitchell 26-87: mon — Monsalve 1816: nov — Monteagudo 4933: obl — Morawetz 13-9888: pen; 110-25985: obl — Moretti 12: bre — Mori 2906: pan; 2918: pan; 5527: pan; 6404: pan; 8758: bre; 15079a: bre; 15583: bre; 22721: bre; 22744: bre — Murillo 565: cau — Murray 1520: pan.

Naessany 106: lei — Nee 34402: mon; 34992: mon; 35033: mon; 38126: lei; 38135: lei; 40971: lei; 41022: lei — Neill 6359: meg; 7195: gra; 7210: meg; 7568: gra; 7649: nap; 7883: meg; 8089: nap; 8466: meg; 9088: nap; 9118: gra; 9180: nap; 9334: lei; 9589: ped; 9967: gra; 10005: meg; 11048: nap; 11065: ped?; 11095: gra; 13201: ped? — Nelson 680: mon; 763: mon — Núñez 10146: spB; 10589: mon; 10785: mon; 11068: mon; 11202: mon; 12152: mon; 12446: spB; 12951: spB; 16303: mon; 16898: obl; 19079: spB; 19232: spB; 19240: spB; 19495: spB; 21737: spB.

Oldeman T19: bre; B634: bre; B636: bre; B2165: bre — Oliver 3681: wes — Öllgaard 35223: gra; 38866: gra; 99056: gra — Ownbey 2715: nap.

Palacios 783: gra; 1620: meg; 1651: gra; 2212: nap; 2311: meg; 2416: gra; 2492: gra; 2494: meg; 2871: meg; 2937: meg; 2992: gra; 3270: meg; 3276: meg; 4150: nap; 4215: meg; 4261: meg; 4323: meg; 4759: gra; 4902: meg; 5123: meg; 5485: nap; 7043: gra; 7602: cau; 8043: cau; 8646: ped; 8925: meg; 10278: meg; 10462: gra; 10663: meg; 11016: meg — Pariona 983: obl — Pennington 12266: nap — Pérez 832: pan — Phillips 545: mon — Pinkley 558: meg — Pipoly 12331: mic; 12982: cau; 14155: meg; 16951: dol; 17006: dol; 17073: dol — Pirie 2: lei; 3: lei; 4: mon; 5: mon; 7: obl; 33: pen; 35: pen; 54: pen; 57: yam; 58: yam; 59: yam; 60: yam; 66: bul; 68: bul; 71: bul; 80: yam; 94: bul — Pitman 195: gra — Plowman 2551: mic; 7248: gra — Poeppig 1750: pen; 2091: lon — Prance 3527: mic; 3579: mic; 5354: mon; 7885: mon; 13079 (= Maas P13079): mon; 13464: mic; 23783: cau; 24094: cau — Prévost 2284: bre; 2310: bre; 3446: bre.

Quelal 191: awa — Quipuscoa 2324: lon; 2361: lon.

Raffauf 102: cau — Ramirez 4881: ped?; 9517: mon — Revelo 264: meg — Revilla 2265: cau; 3327: mic — Riéra 668: bre; 685: bre; 1564: bre — Rimachi 1027: meg; 2397: cau; 7588: cau; 7597: obl; 10637: lon — Rodriguez 724: yam; 1112: per; 1152: bul; 1758: ped — Rojas 0255: cen; 0269: cen; 666: mon; 1014: ped — Romero-Castañeda 3369: awa — Romoleroux 1834: gra; 1883: gra — Rosa 2426: mon — Rubio 320: gra; 812: cau; 2181: awa; 2397: nap — Rudas 2260: mic; 4514: cau — Rueda 952: mon — Ruiz, H. 19-28: lei; 19117: pen — Ruíz M., J. 1748: lon? — Ruiz, M. 8712: mic — Ruíz Z., T. 3499: mac.

Sabatier 486: bre; 1486: bre — Saldías 530: lei — Sánchez 323: pac; 415: dol — Sastre 503: meg; 5670: bre; 5771: bre — Scharf 76: bre; 77: bre; 78: bre — Schatz 1002: spA; 1077: pan - Schunke, C. 200: cau — Schunke V., J. 2554: mon; 3320: mon?; 3828: mon?; 4068: mon?; 5645: obl; 5829: obl; 6412: mic; 7251: ped; 7591: mon?; 8484: mon?; 10907: obl — Scolnik 581: lei — Seidel 2003: lei; 2265: lei; 2584: mon; 2674: mon; 2878: lei; 2893: lei; 3543: lei; 4531: mon; 4660: mon; 7234: lei; 7280: mon; 7290: lei - Silva, M.G. 5398: mon - Silva, M.N. 322: mon; 354: mon — Silveira 767: mon; 1464: mon — Smith, D.N. 2031: obl; 2385: pen; 4611: ped; 4633: ped; 6613: obl; 6850: obl; 13253: lei; 13834: mon; 14006: lei; 14058: lei; 14148: lei; 14216: mon — Smith, S.F. 137: spB?; 504: spB; 654: spB; 794: spB; 1577: spB; 1578: spB — Soejarto 1275: meg; 2798: ant; 3586: ant — Solomon 3523: mic; 6606: lei — Sperling 5784: mon; 6198: mon — Sprague 351: meg — Stahl 2931: ped — Stein 2577: meg; 3036: gra — Steyermark 94314: ven; 123804: mac — Sugden 613: wes — Sytsma 2884 pan.

Teixeira 480: mon; 520: mon — Tessmann 4176: per; 4748: gra — Timaná 1010: spB; 1537: mon; 1563: mon; 1593: mon; 1617: mon; 1632: mon; 1673: mon; 1799: mon; 1822: mon; 1824: mon; 3219: mon — Tipaz 1428: awa; 1718: awa; 1912: awa — Tirado 591: awa; 1083: awa — Tostain 33: bre — Tredwell 22: mic — Tunqui 573: yam — Tyson 3314: pan.

Valencia 67352: meg; 68807: meg — Van der Werff 937: mac; 10161: lon; 12045: awa; 13001: ped?; 13022: ped; 13838: cau —Vargas C., C. 1113: lei; 1193: lei;

1194: lei —Vargas, H. 671: meg; 1025: meg —Vásquez 1938: cau; 1969: meg; 2994: meg; 4423: gra; 4559: lon; 6932: mon; 7237: meg; 8010: mic; 8100: mic; 8425: cau; 9350: mic; 10088: mic; 10637: cau; 11192: meg; 11423: cau; 11438: cau; 12295: mic; 12358: cau; 12655: cau; 12675: gra; 12829: mon; 12862: mic; 13007: gra; 13552: mic; 13690: mic; 16885: meg; 17376: gra; 18236: cau; 18990: per; 19055: yam; 19322: spB; 24428: meg?; 24891: bul; 25605: spB —Villa 1499: meg —Villiers 2639: bre.

Wallnöfer 112-7488: mon; 13477: bre; 13499: bre; 25-19588: mon; 117-28988: obl — Warush Juwa RBAE119: cau — Weberbauer 4558: ped — White 913: lei — Whitmore 717: meg; 737: gra; 854: meg; 871: gra — Williams, Ll. 670: mon; 2197: gra; 4092: lon; 4919: mon; 5085: mon; 5287: mon; 5296: mon; 7423: obl — Wingfield 6751: mac — Woytkowski 7128: lon; 7592: meg.

Young, H.J. 219: spB —Young, K. 193: mon.

Zak 4202: meg; 5272: cau — Zamora 444: meg; 518: meg; 581: gra; 657: meg; 671: meg; 2312: spA — Zuleta 175: meg.

Index to scientific names

Accepted taxa are in roman type, and synonyms in italics. Numbers refer to the species number as used in this revision, excluded names are indicated by excl, nomina dubia by nom.dub.

Summary

This thesis presents results of molecular systematic and biogeographic studies of groups in the flowering plant family Annonaceae, and a taxonomic revision of the genus *Cremastosperma*, which occurs in the Neotropics.

In Chapter 2 preliminary results are presented comparing the timing of diversifications in four predominantly Neotropical genera of Annonaceae; *Cremastosperma*, *Duguetia*, *Guatteria*, and *Mosannona*. Explanations were sought for the disparity in numbers of species in these four genera. Phylogeny reconstruction was used to assess their monophyly and molecular dating techniques (applying the nonparametric rate-smoothing method) used to arrive at preliminary estimates of the relative ages of their most recent common ancestors (MRCAs). The effects of taxon and character sampling on date estimates in these genera were assessed and compared. Higher sampling of crown group taxa of the species-rich genus *Guatteria* resulted in significantly older age estimation for its MRCA. This represents a potentially serious bias in a widely used molecular dating method, which in this case made further comparison of species richness in the four genera meaningless. Molecular dating techniques should be assessed for sensitivity to levels of taxon sampling under differing conditions.

In Chapter 3 a South American-centred clade was identified (the SAC clade). The SAC clade comprises all the short-branch clade (SBC) genera distributed in South America and mostly only to a limited extent into Central America, but not those endemic to Asia and Central America. In the absence of a fully resolved phylogeny of the SAC clade, this was interpreted to suggest a common origin in South America. The age estimations (produced using three different rate smoothing methods) supported the hypothesis that the SAC clade originated in South America by dispersal across the Boreotropics. Gentry's hypothesis of a more ancient origin by Gondwanan vicariance was rejected, as was the possibility of later dispersal from Africa. Monophyly was confirmed in *Cremastosperma*, *Malmea*, and *Mosannona*. The monotypic

genus *Pseudephedranthus* was found to be nested within *Klarobelia*, the species of which otherwise formed a monophyletic group. The conclusion was drawn that the Andean-centred distribution patterns as observed are not the arbitrary result of the definition of poly- or paraphyletic groups. The ages estimated for the MRCAs of each clade were not significantly different from each other. Although the strength of this test was limited by imprecision in the molecular dating results, these ages appeared to fall within the time frame of the orogeny of the Northern Andes. To test these hypotheses further, species level phylogenies of *Cremastosperma*, *Klarobelia*, *Mosannona* and *Malmea* are needed. Additional data should also be sought to test the age calibration of the Annonaceae and wider Magnoliales phylogeny. This approach could shed further light on the dynamic processes of the recent invasion of Central America, and the origin of high species diversity in tropical America.

In Chapter 4 an ancient paralogue of the widely used chloroplast marker *trnL-F* was discovered. The divergence of the paralogous copies was inferred to have taken place in a common ancestor of the Annonaceae. Primers were developed to preferentially amplify and sequence the different paralogues, and the resulting sequences were compared in order to assess their functional homology and phylogenetic utility. Pseud*trnL-F* (the paralogue not normally sequenced) evolves at a faster rate than *trnL-F* and appears to have complementary phylogenetic signal. It may represent a useful phylogenetic marker in itself. Although the exon appears to be intact, some pseud*trnL-F,* intron sequences show signs of disruption of the secondary structure which is otherwise conserved across land plants. They may thus be non-functional. The higher evolutionary rate of pseud*trnL-F* could reflect reduced selective pressure due to loss of function. However, it might also be the result of transfer to the nucleus where the rate of change is generally higher. This could have implications for the interpretation of its phylogenetic signal. Future research should attempt to determine both the whereabouts and origin of *trnL-F* and pseud*trnL-F* in the Annonaceae genome.

In Chapter 5 a taxonomic revision and phylogeny of the genus *Cremastosperma* was presented. Twenty-nine species are recognised. Thirteen have been described as new during this project. *C. bullatum* Pirie, *C. cenepense* Pirie & Zapata, and *C. yamayakatense* Pirie from the basin were described following a field expedition in 2003 to Peru. A new species from northern Venezuela (*C. venezuelanum* Pirie) was described; one from Amazonian Ecuador (*C. napoense* Pirie), seven from Colombia and western Ecuador (*C. antioquense* Pirie, *C. awaense* Pirie, *C. chococola* Pirie, *C. dolichocarpum* Pirie, *C. longipes* Pirie, *C. magdalenae* Pirie, and *C. stenophyllum* Pirie) and one from Panama (*C. westrae* Pirie). A further two putative species were described informally awaiting availability of further collections. Two species plus one variety were brought into synonymy: *C. killipii* R.E.Fr. under *C. longicuspe* R.E.Fr. and both *C. juruense* R.E.Fr. and *C. monospermum* (Rusby) R.E.Fr. var. *brachypodum* R.E.Fr. under *C. monospermum*.

A resolved phylogeny can be used to infer the ancestral distributions of a clade, and possible factors behind its radiation. Multiple chloroplast DNA markers and pseud*trnL-F* were applied to reconstruct the phylogeny of 19 of the 29 species of *Cremastosperma*. A number of clades were revealed. These included species limited to particular geographic areas. The divergence of the Venezuelan and Guianan lineages occurred prior to those of the clades found either in the tropical Andes, or in the Chocó/Darién/western Ecuador region or Central America (i.e. either west or east of the Andes mountain chain). The tropical Andes species all fell into one of two clades. Relationships between these two clades, a further clade including the Central American species *Cremastosperma panamense* Maas and *C. westrae*, and two isolated lineages corresponding to accessions from Costa Rica (C. spec. A) and the Magdalena valley of Colombia (*C. magdalenae*), remain unresolved. These results provide further evidence to suggest the importance of the Andean orogeny as a vicariance event in the history of the evolution of *Cremastosperma*. Further conclusions await a more resolved phylogeny of the genus.

Nederlandse Samenvatting

Dit proefschrift bevat de resultaten van een onderzoek naar enkele Neotropische plantengroepen binnen de Annonaceae, een familie van bloemplanten waarvan de soorten in regenwouden over de hele wereld voorkomen. Het gaat zowel om moleculair systematisch en biogeografisch onderzoek, als om een taxonomische revisie van het genus *Cremastosperma*.

In hoofdstuk 2 worden de eerste resultaten weergegeven van een vergelijkend onderzoek naar de diversificatie van vier voornamelijk Neotropische genera, *Cremastosperma*, *Duguetia*, *Guatteria* en *Mosanonna*. Met gebruik van methoden zoals fylogenie-reconstructie en moleculaire dateringstechnieken (in dit geval nonparametric rate-smoothing: NPRS) werden verklaringen gezocht voor de verschillen in het aantal soorten binnen deze vier genera. Dit werd gedaan door de monofylie van deze genera vast te stellen en een eerste schatting te maken van de relatieve ouderdom van hun eerste gemeenschappelijke voorouders. Ook werd het effect bepaald van het aantal taxa en kenmerken op de schatting van de ouderdom van deze genera. In het geval van het soortenrijke genus *Guatteria* had het opnemen van meer soorten uit de kroongroep in de analyse een significant hogere ouderdomsschatting voor de meest recente gemeenschappelijke voorouder tot gevolg. Deze mogelijk ernstige tekortkoming van de veelgebruikte moleculaire dateringsmethode maakt verdere gedetailleerdere vergelijking van soortenrijkdom tussen de genera zinloos. De gevoeligheid van moleculaire dateringsmethoden voor het aantal taxa dat bemonsterd wordt moet verder bestudeerd worden.

In hoofdstuk 3 wordt een clade, de zogenaamde SAC-clade (South American centred clade), geïdentificeerd die zich voornamelijk in Zuidamerikaanse gebieden bevindt. Deze bevat alle genera van de zogenaamde short-branch clade (SBC) die in Zuid-Amerika en voor een beperkt deel in Centraal-Amerika voorkomen, maar niet de genera die endemisch zijn voor Azië en Centraal-Amerika. Ook al is de fylogenie van het SAC-clade niet helemaal opgelost, de resultaten suggereren toch een gemeenschappelijk oorsprong van de SAC-clade in Zuid-Amerika. De schattingen van de ouderdom, die gemaakt zijn met gebruik van drie verschillende 'rate smoothing' methoden, ondersteunen de hypothese dat de SAC-clade in Zuid-Amerika is ontstaan na verspreiding via de Bering Straat. Zowel Gentry's hypothese dat dit patroon ontstaan is door opsplitsing (vicariantie) van Gondwana, als de mogelijkheid van een meer recente verspreiding vanuit Afrika, worden hierdoor verworpen. Monofylie werd bevestigd voor de genera *Cremastosperma*, *Malmea* en *Mosannona*. Het monotypische genus *Pseudephedranthus* is genest binnen *Klarobelia*, een genus dat afgezien daarvan een monofyletische groep vormde. Dit leidt tot de conclusie dat de geobserveerde verspreidingspatronen rondom de Andes niet het arbitraire resultaat zijn van de definitie van poly- of parafyletische groepen. Er zijn geen significante verschillen geconstateerd tussen de schattingen van de ouderdom van de meest recente gemeenschappelijke voorouders van elke clade. Alhoewel de kracht van deze test gelimiteerd is door de onnauwkeurige resultaten van de moleculaire datering, lijken deze schattingen binnen de tijdperiode waarin het noordelijk deel van de Andes is ontstaan te vallen. Om deze hypothesen verder te onderzoeken zijn fylogenieëen op soortniveau van *Cremastosperma*, *Klarobelia*, *Mosannona* en *Malmea* nodig. Aanvullende data moet ook worden gegenereerd om de ouderdomsschatting van Annonaceae en andere Magnoliales te testen. Deze benadering zou het dynamische proces van de recente invasie in Centraal-Amerika en de oorsprong van de hoge biodiversiteit in tropisch Amerika kunnen onthullen.

In hoofdstuk 4 wordt de ontdekking van een paraloge kopie van de veelgebruikte chloroplast marker *trnL-F* beschreven. Deze twee kopieën zijn gedivergeerd in een van de gemeenschappelijke voorouders van de Annonaceae. Primers werden ontwikkeld om de verschillende paraloge kopieën te amplificeren en te sequencen. De gevonden sequenties werden daarna met elkaar vergeleken om de functionele homologie en het fylogenetisch gebruik van de kopieën vast te stellen. Pseud*trnL-F*, de paraloge kopie die normaal niet gesequenced wordt, evolueerde sneller dan *trnL-F*. Het bevat complementaire fylogenetische signalen en is daardoor zelf ook bruikbaar als fylogenetische marker. Alhoewel het exon intact lijkt, vertonen sommige sequenties van het pseud*trnL-F* intron tekenen van verstoring van de secundaire structuur, die in landplanten normaliter geconserveerd is gebleven. Het is dus mogelijk dat deze kopie haar functie verloren heeft. Het is eveneens mogelijk dat de hogere evolutiesnelheid van pseud*trnL-F* de selectiedruk weergeeft die ontstaan is door het verlies aan functionaliteit. Deze hogere snelheid zou echter ook het gevolg kunnen zijn van verplaatsing van de paraloge kopie naar de celkern, waar de mutatiesnelheid in het algemeen hoger is. Dit kan ook implicaties hebben voor de interpretatie van het fylogenetische signaal van deze sequenties. Aanvullend onderzoek zal de plaats van *trnL-F* en pseud*trnL-F* in het Annonaceae-genoom moeten aantonen.

In hoofdstuk 5 worden een taxonomische revisie en een fylogenie van het genus *Cremastosperma* gepresenteerd. De taxonomische studie heeft geleid tot de erkenning van 29 soorten, waarvan er 13 nieuw zijn voor de wetenschap. De drie soorten *C. bullatum* Pirie, *C. cenepense* Pirie & Zapata, and *C. yamayakatense* Pirie komen allen voor in de vallei van de Río Marañon in Peru, en deze werden beschreven na een verzameltocht naar Peru in 2003. Daarnaast werden één nieuwe soort uit Noord Venezuela (*C. venezuelanum* Pirie), één uit Amazonisch Ecuador (*C. napoense* Pirie), zeven uit Colombia en west Ecuador (*C. antioquense* Pirie, *C. awaense* Pirie, *C. chococola* Pirie, *C. dolichocarpum* Pirie, *C. longipes* Pirie, *C. magdalenae* Pirie, and *C. stenophyllum* Pirie), en één uit Panama (*C. westrae* Pirie) beschreven. Ook zijn twee mogelijk nieuwe soorten informeel beschreven in afwachting van aanvullende collecties. Twee soorten en een variëteit zijn gereduceerd tot synoniemen en ondergebracht onder de volgende soorten: *C. killipii* R.E.Fr. onder *C. longicuspe* R.E.Fr., en *C. juruense* R.E.Fr. en *C. monospermum* (Rusby) R.E.Fr. var. *brachypodum* R.E.Fr. onder *C. monospermum*.

Een opgeloste fylogenie kan gebruikt worden om de distributie van de voorouders in een clade en de mogelijke factoren achter de radiatie te reconstrueren. Hiertoe werd de fylogenie van *Cremastosperma* gereconstrueerd, aan de hand van meerdere chloroplast DNA merkers en pseud*trnL-F*. Een aantal clades binnen *Cremastosperma* wordt goed ondersteund door bootstrap percentages, en correspondeert in het algemeen met duidelijk omgrensde gebieden in Zuid Amerika. De Venezuelaanse en Guiaanse afstammingslijnen splitsten eerder af dan afstamminglijnen die in de laaglandbossen op de uitlopers van de Andes, en in Centraal Amerika en het gebied Chocó/Darién/westelijk Ecuador voorkomen – met andere woorden, ten oosten en ten westen van de Andes. De verwantschappen tussen twee clades die samen de soorten ten oosten van de Andes bevatten, een derde clade met de Centraalamerikaanse soorten *C. panamense* en *C. westrae*, alsmede de soorten *C.* spec. A (uit Costa Rica) en *C. magdalenae* (uit de vallei van de Río Magdalena in Colombia) blijven onopgelost. De resultaten suggereren dat de scheiding van populaties door het ontstaan van de Andes een belangrijke rol heeft gespeeld in de evolutionaire historie van *Cremastosperma*. Een beter opgeloste fylogenie is nodig om verdergaande conclusies te trekken.

Resumen

Esta tesis presenta los resultados de la sistemática molecular y la biogeografía de grupos pertenecientes a la familia de plantas con flor, Annonaceae, y la revisión taxonómica del género *Cremastosperma*, los cuales se encuentran en el Neotrópico.

En el capítulo 2 se presentan los resultados preliminares de la comparación de los tiempos de diversificación de cuatro géneros predominante Neotropicales de la familia Annonaceae: *Cremastosperma*, *Duguetia*, *Guatteria*, y *Mosannona*. Se buscaron explicaciones para la disparidad en el número de especies de estos cuatro géneros. La reconstrucción de la filogenia fue utilizada para determinar su monofilia y se emplearon técnicas de datación molecular (aplicando nonparametric rate-smoothing: NPRS, por su nombre y siglas en ingles) para estimar las edades preliminares relativas de sus ancestros comunes más recientes (MRCAs por sus siglas en ingles). En estos géneros se evaluaron y compararon los efectos del muestreo de taxones y caracteres en las estimaciones de edad. Un muestreo dentro del "crown group" (por su nombre en ingles) del rico genero en especies *Guatteria*, dio una estimación de la edad significativamente mas vieja para su MRCA. Esto representa una tendencia potencialmente seria de un método extensamente usado de datación molecular, el cual en este caso dio comparaciones adicionales sin sentido de la riqueza de especies en los cuatro géneros. Las técnicas de datación molecular deben ser evaluadas para la sensibilidad de los niveles de muestreo de los taxones bajo diferentes condiciones.

En el capítulo 3 se identifico un clado llamado "South American-centred" (el clado SAC). El clado SAC contiene todos los géneros del clado de la rama corta (the short-branch clade, SBC, en ingles) distribuidos en Suramérica y solamente a una extensión limitada en Centro América, pero no aquellos endémicos en Asia y Centro América. En ausencia de una filogenia completamente resuelta del clado SAC, esto se interpreta sugiriendo un origen común en Suramérica. Las estimaciones de edad (producidas usando tres métodos de "rate smoothing", como su nombre en ingles) apoyaron la hipótesis que el clade SAC se originó en Suramérica por la dispersión a través del estrecho Bering. Se rechazo la hipótesis de Gentry de un origen más antiguo por la vicarianza de Gondwana, al igual que la posibilidad de una dispersión posterior desde África. Se confirmo la monofilia de *Cremastosperma*, *Malmea*, y *Mosannona*. El género monotípico *Pseudephedranthus* se encontró dentro de Klarobelia, especies las cuales de otra manera formaban un grupo monofilético. Se concluyo que los patrones de distribución centro Andina como se observan no son el resultado arbitrario de la definición de grupos poli- o parafileticos. Las edades estimadas de cada clado por los MRCAs no fueron significativamente diferentes el uno del otro. Aunque el poder de esta prueba se vio limitada por la imprecisión en los resultados de datación molecular, estas edades parecen caer dentro del periodo de tiempo de la orogenia de los Andes del norte. Para probar estas hipótesis, son necesarias filogenias a nivel de especie de *Cremastosperma*, *Klarobelia*, *Mosannona* y *Malmea*. Se debe también buscar datos adicionales para probar la calibración de edad de la familia Annonaceae y de la filogenia más amplia de Magnoliales. Este enfoque podría adicionalmente dar alguna luz en los procesos dinámicos de la reciente invasión de Centro América, y el origen de la alta diversidad de especies en la América tropical.

En el capítulo 4 se descubrió un paralogo antiguo del *trnL-F*, marcador del cloroplasto extensamente usado. Se deduce que la divergencia de la copias de los paralogos ocurrió en un antepasado común de Annonaceae. Se desarrollaron primers para amplificar y secuenciar los diferentes paralogos, y las secuencias resultantes fueron comparadas para determinar su homología funcional y utilidad filogenética. Pseud*trnL-F* (el paralogo no normalmente secuenciado) evoluciona a una rata más rápida que *trnL-F* y parece tener una señal filogenética complementaria. Puede representar en sí mismo un marcador filogenéticamente útil. Aunque el exón parece estar intacto, algunas secuencias del intrón del pseud*trnL-F* muestran signos de interrupción de la estructura secundaria, la cuál se conserva de otra manera a través de las plantas terrestres. Pueden por lo tanto no ser funcionales. La alta rata evolutiva del pseud*trnL-F* podría reflejar la presión selectiva reducida debido a la pérdida de función. Sin embargo, este también puede ser el resultado de la transferencia al núcleo donde la rata de cambio es generalmente más alta. Esto podría tener implicaciones para la interpretación de su señal filogenética. Futura investigaciones deben intentar determinar tanto el lugar como el origen de *trnL-F* y del pseud*trnL-F* en el genoma de la familia Annonaceae.

En el capítulo 5 se presenta la revisión taxonómica y la filogenia del género *Cremastosperma*. Se reconocen veinte nueve especies. Trece se han descrito como nuevas durante este proyecto. *C. bullatum* Pirie, *C. cenepense* Pirie & Zapata, and *C. yamayakatense* Pirie fueron descritas después de una expedición a campo al Perú en el 2003. Se describió una nueva especie del norte de Venezuela (*C. venezuelanum* Pirie); una de la Amazonia Ecuatoriana

(*C. napoense* Pirie); siete de Colombia y del occidente del Ecuador (*C. antioquense* Pirie, *C. awaense* Pirie, *C. chococola* Pirie, *C. dolichocarpum* Pirie, *C. longipes* Pirie, *C. magdalenae* Pirie y *C. stenophyllum* Pirie) y una de Panamá (*C. westrae* Pirie). Dos supuestas especies más fueron descritas informalmente esperando la disponibilidad de más colecciones. Dos especies más una variedad fueron sinonimia: *C. killipii* R.E.Fr. bajo *C. longicuspe* R.E.Fr. y tanto *C. juruense* R.E.Fr. como *monospermum* (Rusby) R.E.Fr. var. *brachypodum* R.E.Fr. bajo *C. monospermum*.

Una filogenia resuelta puede ser usada para inferir la distribución ancestral de un clado, y los posibles factores detrás de su radiación. Los múltiples marcadores de ADN del cloroplasto y el pseud*trnL-F* fueron aplicados para la reconstrucción de la filogenia de 19 de las 29 especies de *Cremastosperma*. Se revelaron un número de clados. Éstos incluyeron especies limitadas a unas áreas geográficas particulares. La divergencia de los linajes venezolanos y de la Guyana ocurrieron antes de los aquellos ocurridos en los clados encontrados en los Andes tropicales, o en la región de Chocó/Darién/Ecuador occidental o América Central (ej. a cualquiera de los dos lados de la Cordillera de los Andes al occidente o al oriente). Todas las especies de los Andes tropicales caen dentro de uno de dos clados. Permanecen sin resolver las relaciones entre estos dos clados, un clado de las especies de Centro América *C. panamense* Maas y *C. westrae*, y dos linajes aislados correspondientes a las especies de Costa Rica (*C. spec.* A) y a la del valle del Magdalena en Colombia (*C. magdalenae*). Estos resultados proporcionan evidencia adicional para sugerir la importancia de la orogenia andina como un evento de vicariance en la historia de la evolución de *Cremastosperma*. Otras conclusiones aguardan una filogenia resuelta del género.

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Curriculum Vitae

Michael D. Pirie was born in London on the 18th of August 1977. In 1995 he completed his secondary school education at King Edward's School, Witley, with A levels in biology, chemistry, and music. In October of that year he began further study in biology at University College, London. During the three year B.Sc. programme he followed courses including 'Genetics and Evolution', 'Plant Reactions to Environmental Stress', 'Population Genetics', and 'Conservation Biology'. In 1998 he graduated with upper second class honours, having submitted a thesis based on a biological survey (results of which were subsequently presented in Pirie & Goldsmith, 1999). In November and January of 1998/99 and February and March of 1999 he volunteered as a field worker at the Australian Trust for Conservation Volunteers in New South Wales and Victoria, and at the South Slough Estuarine Research Reserve, in Oregon, USA, respectively. In October 1999 he began the course 'The Biodiversity and Taxonomy of Plants' at the University of Edinburgh and Royal Botanic Garden Edinburgh, gaining the degree of Master of Science in September 2000, with a thesis entitled 'Biogeography and Systematics of *Centrolobium* (Fabaceae)'. In November 2000 he moved to the Netherlands to begin research for a Ph.D. as 'Assistent in Opleiding' at the Utrecht University branch of the National Herbarium of the Netherlands (NHN). He followed courses both at the NHN (Systematics of Neotropical Plant Families – Utrecht; Advanced Topics in Phylogeny Reconstruction – Leiden) and at the University of Copenhagen, Denmark (Phylogenetic Systematics and Historical Biogeography). His contract was extended for 3 months in 2004 in order to help the preparation and teaching of a new course 'Advanced Evolutionary Biology' for the Utrecht MSc programmes 'Plant Biology' and 'Biogeology'. As part of his Ph.D. research he made collecting trips to Bolivia (October–December 2001, with Chatrou) and Peru (October–December 2003), resulting in the publication of new species (Chatrou & Pirie, 2003; Pirie & Zapata C., 2004) and a contribution to the Catalogue of the Vascular Plants of Bolivia (Chatrou *et al.*, in press). Further publications arising from his Ph.D. research include Pirie *et al.* (2005 –

Chapter 2; submitted – Chapters 3 & 4). He collaborated actively with workers of the Annonaceae project across the NHN (Mols *et al.*, 2004; Richardson *et al.*, 2004; Mols *et al.*, submitted) and presented results at the Nordic Botany meeting (Utrecht 2001), Young Systematists meeting (London 2002), Annonaceae workshop (Malaga, 2002) and Systematics Association biennial meeting (Dublin 2003). He has been awarded a bursary to attend and present results at the next SA meeting in Cardiff (August 2005).

In July 2005 he will begin a three year postdoc position researching systematics and biogeography of the grass subfamily Danthonioideae at the Institute for Systematic Botany, Zürich, Switzerland.

Appendix A

Details of accessions sampled for DNA sequence data. Table 1: Accession details and *rbcL*, *trnL-trnF*, *matK*, and *psbA-trnH* genbank accession numbers. Table 2: *ndhF*, *trnT-trnL*, *trnS-trnG*, *atpB-rbcL* and pseud *trnL-F* genbank accession numbers.

Unless otherwise indicated, sequences were published as original results of chapters of this thesis. DNA ID refers to UDNA database identification number, unless prefixed with JB (Mols, Leiden) or MWC (Chase, Jodrell lab, Royal Botanic Gardens Kew). Voucher specimens for DNA samples extracted at the National Herbarium of the Netherlands (NHN) are held at herbaria as indicated by the following acronyms: NHN – Leiden University (L); New York Botanical Garden (NY); Missouri Botanical Garden (MO); NHN – Utrecht University (U); United States National Herbarium (US); and NHN – Wageningen University (WAG).

Appendix A: Table 1

Taxon	Origin	Voucher	DNA ID	*rbcL*	*trnL-trnF*	*matK*	*psbA-trnH*
Coelocaryon preussii Warb.	Gabon	*Wieringa, J.J. et al. 3640* (WAG)	20	AY743437	AY743456	AY743475	
Kmeria yunnanensis	China	unknown			AY158183[a]		
Liriodendron chinense Sargent	[g]China	*Chatrou, L.W. et al. 279* (U)	521	AY841593[b]	AY841670[b]		AY841424
Magnolia kobus DC.	[g]Japan	*Chatrou, L.W. et al. 278* (U)	520	AY743438	AY743457	AY743476	AY841425
Cinnamomum cassia Blume	China	*Izu experimental station 21*			AB054241 AB054233[c]		
Persea americana Mill. cv. *accnum*	[g]Neotropics	*Chatrou, L.W. 479* (U)	484	AY841592[b]	AY841669[b]		
Galbulimima belgraveana (F. Muell.) Sprague	Australasia	*Qiu, Y-L. 90034* (NCU)			AY220415[d]		
Eupomatia bennettii F.Muell.	Australia	unknown			Prov. 50/51[d]		
Degeneria roseiflora J.M. Milller	Fiji	*J.M. Miller 1189* (SUVA)			AY220414 AY220361[d]		
Alphonsea boniana Finet & Gagnep.	Vietnam	*Kessler, P.J.A. 3116* (L)	JB57	AY318965[e]	AY319077[e]	AY518809[f]	
Anaxagorea silvatica R.E.Fr.	Brazil - Espirito Santo	*Maas, P.J.M. et al. 8836* (U)	113	AY743439	AY743458	AY743477	AY841427
Anaxagorea phaeocarpa Mart.	Ecuador - Napo	*Maas, P.J.M. et al. 8592* (U)	498	AY238952[b]	AY231284 AY238944[d]	AY238960	AY841426

Taxon	**Origin**	**Voucher**	**DNA ID**	***rbcL***	***trnL-trnF***	***matK***	***psbA-trnH***
Annickia chlorantha (Oliv.) Setten & Maas	Gabon	*Sosef, M.S.M. 1877* (WAG)	976	AY841594[b]	AY841671[b]	AY841393	AY841442
Annickia kummeriae (Engl. & Diels) Setten & Maas	Tanzania	*Johnson, D.M. 1942* (OWU)	MWC7004	AY238959[b]	AY231285 AY238945[d]	AY238961[d]	AY841443
Annickia pilosa (Exell) Setten & Maas	Gabon	*Sosef, M.S.M. 1803* (WAG)	977	AY743450	AY743469	AY743488	AY841444
Annona muricata L.	[g]Neotropics	*Chatrou, L.W. 468* (U)	525	AY743440	AY743459	AY743478	AY841428
Artabotrys hexapetalus (L.f.) Bhandari	[g]India	*Chatrou, L.W. 470* (U)	529	AY238953[b]	AY231286 AY238946[d]	AY238962[d]	AY841429
Asimina triloba (L.) Dunal	[g]North America	*Chatrou, L.W. et al. 276* (U)	517	AY743441	AY743460	AY743479	AY841430
Bocageopsis multiflora (Mart.) R.E.Fr.	Guyana	*Jansen-Jacobs, M.J. et al. 5789* (U)	190	AY841600[b]	AY841678[b]	DQ018262	AY841445
Bocageopsis pleiosperma Maas	Brazil - Amazonas	*Miralha, J.M.S. et al. 300* (U)	332	AY841601[b]	AY841679[b]		AY841446
Cananga odorata (Lam.) Hook.f & Thomson	Costa Rica - Limón	*Chatrou, L.W. et al. 93* (U)	239	AY841602[b]	AY841680[b]	AY841394	AY841431
Cleistopholis glauca Engl. & Diels	Gabon	*Wieringa, J.J. et al. 3278* (WAG)	198	AY841603[b]	AY841681[b]	AY841395	AY841432
Cremastosperma brevipes (DC.) R.E.Fr.	French Guiana	*Scharf, U. 76* (U)	601	AY743527	AY743573	AY743550	AY841447
Cremastosperma bullatum Pirie	Peru - Amazonas	*Pirie, M.D. et al. 71* (U)	1100	AY743537	AY743583	AY743560	AY841459

Taxon	**Origin**	**Voucher**	**DNA ID**	***rbcL***	***trnL-trnF***	***matK***	***psbA-trnH***
Cremastosperma bullatum Pirie	Peru - Amazonas	*Pirie, M.D. et al. 94* (U)	1102	DQ018235	DQ018188	DQ018276	DQ018256
Cremastosperma cauliflorum R.E.Fr.	Brazil - Acre	*Maas, P.J.M. et al. 9029* (U)	598	AY743525	AY743571	AY743548	DQ018240
Cremastosperma cauliflorum R.E.Fr.	Peru - Loreto	*Chatrou, L.W. et al. 224* (U)	312	AY743519	AY743565	AY743542	AY841448
Cremastosperma cenepense Pirie & Zapata	Peru - Amazonas	*Rojas, R. 255* (U)	1269	DQ018236		DQ018277	DQ018257
Cremastosperma gracilipes R.E.Fr.	Ecuador - Napo	*Chatrou, L.W. et al. 267* (U)	491	AY743521	AY743567	AY743544	
Cremastosperma leiophyllum R.E.Fr.	Bolivia - Santa Cruz	*Pirie, M.D. et al. 2* (U)	594	AY743523	AY743569	AY743546	AY841449
Cremastosperma macrocarpum Maas	Venezuela - Falcón	*Wingfield, R. 6751* (U)	741	AY743528	AY743574	AY743551	AY841450
Cremastosperma magdalenae Pirie	Colombia - Antioquia	*Escobar, L.A. de 3309* (U)	1270	AY841521	AY841535	DQ018279	AY841460
Cremastosperma megalophyllum R.E.Fr.	Ecuador - Napo	*Maas, P.J.M. et al. 8595* (U)	87	AY743517	AY743563	AY743540	
Cremastosperma megalophyllum R.E.Fr.	Ecuador - Napo	*Chatrou, L.W. et al. 268* (U)	490	AY743520	AY743566	AY743543	DQ018239
Cremastosperma megalophyllum R.E.Fr.	Ecuador - Napo	*Chatrou, L.W. et al. 259* (U)	493	AY743522	AY743568	AY743545	AY841451
Cremastosperma microcarpum R.E.Fr.	Peru - Loreto	*Maas, P.J.M. et al. 8289* (U)	82	AY743516	AY743562	AY743539	

Taxon	**Origin**	**Voucher**	**DNA ID**	***rbcL***	***trnL-trnF***	***matK***	***psbA-trnH***
Cremastosperma microcarpum R.E.Fr.	Peru - Loreto	*Chatrou, L.W. et al. 208* (U)	303	AY319058[e]	AY319172[e]	AY518874[f]	AY841452
Cremastosperma monospermum (Rusby) R.E.Fr.	Bolivia - Pando	*Pirie, M.D. et al. 4* (U)	596	AY743524	AY743570	AY743547	AY841453
Cremastosperma monospermum (Rusby) R.E.Fr.	Peru - Loreto	*Killip, E.P. 28961* (NY)	753	AY743533	AY743579	AY743556	
Cremastosperma napoense Pirie	Ecuador - Napo	*Neill, D. 7649* (U)	737	DQ018224	DQ018177	DQ018265	DQ018242
Cremastosperma novogranatense R.E.Fr.	Colombia - El Valle	*Devia A., W. 5335* (MO)	744	AY743529	AY743575	AY743552	
Cremastosperma oblongum R.E.Fr.	Peru - Pasco	*Pirie, M.D. et al. 7* (U)	1097	DQ018232	DQ018185	DQ018273	DQ018253
Cremastosperma oblongum R.E.Fr.	Brazil - Acre	*Maas, P.J.M. et al.* 9148 (U)	600	AY743526	AY743572	AY743549	DQ018241
Cremastosperma oblongum R.E.Fr.	Peru - Loreto	*Morawetz, W. 25985* (U)	739	DQ018225	DQ018178	DQ018266	DQ018243
Cremastosperma panamense Maas	Panama	*Miller, J.S. 947* (U)	747	AY743530	AY743576	AY743553	DQ018246
Cremastosperma pedunculatum (Diels) R.E.Fr.	Peru - San Martín	*Gentry, A.H. 45510* (U)	760	AY743538	AY743584	AY743561	DQ018251
Cremastosperma pedunculatum (Diels) R.E.Fr.	Ecuador - Zamora-Chinchipe	*Gentry, A.H. 80904* (U)	754	DQ018228	DQ018181	DQ018269	DQ018248
Cremastosperma pendulum (Ruiz & Pav.) R.E.Fr.	Peru - Pasco	*Pirie, M.D. et al. 33* (U)	1098	DQ018233	DQ018186	DQ018274	DQ018254

Taxon	**Origin**	**Voucher**	**DNA ID**	***rbcL***	***trnL-trnF***	***matK***	***psbA-trnH***
Cremastosperma pendulum (Ruiz & Pav.) R.E.Fr.	Peru - Huánuco	*Morawetz, W. 9888* (U)	749	AY743531	AY743577	AY743554	DQ018247
Cremastosperma pendulum (Ruiz & Pav.) R.E.Fr.	Peru - Ucayali	*Graham, J.G. 635* (U)	751	AY743532	AY743578	AY743555	AY841454
Cremastosperma peruvianum R.E.Fr.	Peru - Amazonas	*Rodríguez R., E. 1112* (U)	755	AY743534	AY743580	AY743557	
Cremastosperma sp.	Peru - San Martín	*Woytkowski, F. 7128* (MO)	745	DQ018227	DQ018180	DQ018268	DQ018245
Cremastosperma spec. A	Costa Rica - Osa	*Chatrou, L.W. et al. 103* (U)	245	AY841522	AY841536		AY841455
Cremastosperma spec. B	Peru - Madre de Dios	*Foster, R.B. 3418* (US)	758	DQ018230	DQ018183	DQ018271	DQ018250
Cremastosperma spec. B	Peru - Madre de Dios	*Smith, S.F. 1577* (US)	756	DQ018229	DQ018182	DQ018270	DQ018249
Cremastosperma venezuelanum Pirie	Venezuela - Carababo	*Steyermark, J.A. 94314* (NY)	757	AY743536	AY743582	AY743559	AY841457
Cremastosperma westrae Pirie	Panama	*Oliver, R.L. 3681* (MO)	761	DQ018231	DQ018184	DQ018272	DQ018252
Cremastosperma yamayakatense Pirie	Peru - Amazonas	*Pirie, M.D. et al. 57* (U)	1099	AY743535	AY743581	AY743558	AY841458
Cremastosperma yamayakatense Pirie	Peru - Amazonas	*Vásquez, R. 19055* (MO)	742	DQ018226	DQ018187	DQ018267	DQ018244
Cremastosperma yamayakatense Pirie	Peru - Amazonas	*Pirie, M.D. et al. 80* (U)	1101	DQ018234	DQ018179	DQ018275	DQ018255
Cymbopetalum sp.	Costa Rica	*Chatrou, L.W. et al. 44* (U)	203	AY841523	AY841537	DQ018258	AY841434

Taxon	**Origin**	**Voucher**	**DNA ID**	***rbcL***	***trnL-trnF***	***matK***	***psbA-trnH***
Cymbopetalum sp.	Bolivia - Santa Cruz	*Maas, P.J.M. et al. 8811* (U)	98	AY841609[b]	AY841687[b]		AY841433
Cymbopetalum torulosum G.E.Schatz	Costa Rica - La Selva	*Chatrou, L.W. et al. 54* (U)	210	AY743442	AY743461	AY743480	
Dasymaschalon sootepense Craib	Thailand	*Kessler, P.J.A. 3201* (L)	998	AY743443	AY743462	AY743481	
Desmopsis microcarpa R.E.Fr.	Costa Rica - Limón	*Chatrou, L.W. et al. 85* (U)	234	AY319059[e]	AY319173[e]	AY518804[f]	AY841461
Duguetia bahiensis Maas	Brazil - Bahia	*Amorim, A.M. et al. 800* (U)	475	AY738152	AY740564	AY740532	
Duguetia cadaverica Huber	Guyana	*Jansen-Jacobs, M.J. et al. 5868* (U)	339	AY738153	AY740565	AY740533	
Duguetia calycina Benoist	Guyana	*Jansen-Jacobs, M.J. et al. 5661* (U)	348	AY738154	AY740566	AY740534	
Duguetia cauliflora R.E.Fr.	Guyana	*Jansen-Jacobs, M.J. et al. 5687* (U)	357	AY738155	AY740567	AY740535	
Duguetia chrysea Maas	Brazil - Amazonas	*Maas, P.J.M. et al. 8052* (U)	361	AY841613[b]	AY841691[b]	AY740536	AY841435
Duguetia chrysea Maas	Brazil - Amazonas	*Maas, P.J.M. et al. 8053* (U)	362	AY738156	AY740568	AY740536	
Duguetia confinis (Engl. & Diels) Chatrou	Gabon	*Wieringa, J.J. & F.I. van Nek 3290* (WAG)	365	AY738157	AY740569	AY740537	
Duguetia confusa Maas	Costa Rica	*Chatrou, L.W. et al. 42* (U)	201	AY738158	AY740570	AY740538	
Duguetia echinophora R.E.Fr.	Brazil - Amazonas	*Maas, P.J.M. et al. 8046* (U)	372	AY738159	AY740571	AY740539	

Taxon	Origin	Voucher	DNA ID	*rbcL*	*trnL-trnF*	*matK*	*psbA-trnH*
Duguetia guianensis R.E.Fr.	Guyana	*University of Guyana, course Neotropical Botany 33* (U)	153	AY738160	AY740572	AY740540	
Duguetia hadrantha (Diels) R.E.Fr.	Peru - Loreto	*Chatrou, L.W. et al. 181* (U)	288	AY738161	AY740573	AY740541	
Duguetia lucida Urb.	Bolivia - Beni	*Chatrou, L.W. et al. 367* (U)	732	AY738162	AY740574	AY740542	
Duguetia macrocalyx R.E.Fr.	Guyana	*Jansen-Jacobs, M.J. et al. 3011* (U)	401	AY738163	AY740575	AY740543	
Duguetia macrophylla R.E.Fr.	Peru - Loreto	*Maas, P.J.M. et al.*	403	AY738164	AY740576	AY740544	
Duguetia marcgraviana Mart	Bolivia - Santa Cruz	*Chatrou, L.W. et al. 327* (U)	729	AY738165	AY740577	AY740545	
Duguetia megalocarpa Maas	Brazil - Amazonas	*Maas, P.J.M. et al. 8045* (U)	406	AY738166	AY740578	AY740546	
Duguetia moricandiana Mart.	Brazil - Bahia	*Carvalho, A.M.V. de 3322* (U)	408	AY738167	AY740579	AY740547	
Duguetia neglecta Sandwith	Guyana	*Jansen-Jacobs, M.J. et al. 5655* (U)	410	AY738168	AY740580	AY740548	
Duguetia odorata (Diels) J.F.Macbr.	Peru - Loreto	*Chatrou, L.W. et al. 207* (U)	302	AY738169	AY740581	AY740549	
Duguetia panamensis Standl.	Costa Rica - Limón	*Chatrou, L.W. et al. 97* (U)	242	AY738170	AY740582	AY740550	
Duguetia peruviana (R.E.Fr.) J.F.Macbr.	Ecuador - Guayas	*Maas, P.J.M. et al. 8571* (U)	476	AY738171	AY740583	AY740551	

Taxon	**Origin**	**Voucher**	**DNA ID**	***rbcL***	***trnL-trnF***	***matK***	***psbA-trnH***
Duguetia pycnastera Sandwith	Brazil - Amazonas	*Miralha, J.M.S. et al. 241* (U)	416	AY738172	AY740584	AY740552	
Duguetia quitarensis Benth.	Peru - Madre de Dios	*Chatrou, L.W. et al. 123* (U)	261	AY738173	AY740585	AY740553	
Duguetia riedeliana R.E.Fr.	Brazil - Rio de Janeiro	*Maas, P.J.M. et al. 8819* (U)	424	AY738174	AY740586	AY740554	
Duguetia salicifolia R.E.Fr.	Brazil - São Paulo	*Cordeiro, I. et al. 915* (U)	426	AY738175	AY740587	AY740555	
Duguetia sessilis (Velloso) Maas	Brazil - Rio de Janeiro	*Maas, P.J.M. et al. 8838* (U)	427	AY738176	AY740588	AY740556	
Duguetia sooretamae Maas	Brazil - Espirito Santo	*Maas, P.J.M. et al. 8827* (U)	428	AY738177	AY740589	AY740557	
Duguetia staudtii (Engl. & Diels) Chatrou	Cameroon	*Andel, T.R. van et al. 3290* (U)	854	AY738178	AY740590	AY740558	
Duguetia stelechantha (Diels) R.E.Fr.	Brazil - Amazonas	*Maas, P.J.M. et al. 8058* (U)	451	AY738179	AY740591	AY740559	
Duguetia surinamensis R.E.Fr.	Brazil - Amazonas	*Maas, P.J.M. et al. 8057* (U)	455	AY738180	AY740592	AY740560	
Duguetia ulei (Diels) R.E.Fr.	Brazil - Amazonas	*Miralha, J.M.S., 243* (U)	335	AY738181	AY740593	AY740561	
Duguetia uniflora (DC.) Mart.	Brazil - Amazonas	*Coêlho, D. INPA 3711* (U)	457	AY738182	AY740594	AY740562	
Duguetia yeshidan Sandwith	Guyana	*Jansen-Jacobs, M.J. et al. 6129* (U)	461	AY738183	AY740595	AY740563	

Taxon	Origin	Voucher	DNA ID	*rbcL*	*trnL-trnF*	*matK*	*psbA-trnH*
Ephedranthus parviflorus S.Moore	Brazil - Mato Grosso	*Prance, G.T. et al. 19246* (U)	31	AY841615[b]	AY841693[b]		AY841462
Ephedranthus sp.	Brazil - Espirito Santo	*Maas, P.J.M. et al. 8826* (U)	105	AY841616[b]	AY841694[b]	AY841396	AY841463
Ephedranthus sp.	Peru - Loreto	*Chatrou, L.W. et al. 173* (U)	284	AY319061[e]	AY319175[e]		AY841464
Fissistigma glaucescens Merrill	Hong Kong	*Law, C.L & Kendrick, R.* (L)	1020	AY743444	AY743463	AY743482	
Fusaea peruviana R.E.Fr.	Peru - Loreto	*Chatrou, L.W. et al. 179* (U)	287	AY743445	AY743464	AY743483	AY841436
Goniothalamus griffithii Hook.f. & Thoms.	Thailand	*Kessler, P.J.A. 3188* (L)	1019	AY743446	AY743465	AY743484	
Greenwayodendron oliveri (Engl.) Verdc.	Ghana	*Jongkind, C.C.H. et al. 1795* (WAG)	958	AY743451	AY743470	AY743489	AY841465
Greenwayodendron suaveolens Engl. & Diels var. suaveolens	Gabon	*McPherson, G. 15802* (WAG)	959	AY841524	AY841538		AY841466
Guatteria aeruginosa Standl.	Costa Rica	*Chatrou, L.W. et al. 66* (U)	221	AY740958	AY741007	AY740909	
Guatteria alata Maas & van Setten	Panama	*Mori, S.A. 2894* (U)	776	AY740959	AY741008	AY740910	
Guatteria allenii R.E.Fr.	Panama	*Mori, S.A. 2952* (U)	777	AY740960	AY741009	AY740911	
Guatteria alutacea Diels	Bolivia - Santa Cruz	*Chatrou, L.W. et al. 339* (U)	578	AY740961	AY741010	AY740912	

Taxon	**Origin**	**Voucher**	**DNA ID**	***rbcL***	***trnL-trnF***	***matK***	***psbA-trnH***
Guatteria anomala R.E.Fr.	Mexico	*Ishiki, M. et al. 2233* (U)	150	AY740962	AY741011	AY740913	AY841437
Guatteria atra Sandwith aff.	Guyana	*Gopaul, D. & Maas, P.J.M. 2791* (U)	715	AY740963	AY741012	AY740914	
Guatteria australis A.St.-Hil. aff.	Brazil - Rio de Janeiro	*Lobão, A.Q. & Fiaschi, P. 499* (U)	512	AY740964	AY741013	AY740915	AY841438
Guatteria blainii (Griseb.) Urb.	Dominican Republic	*Maas, P.J.M. 6443* (U)	785	AY740965	AY741014	AY740916	
Guatteria brevicuspis R.E.Fr.	Brazil - Amazonas	*Prance, G.T.16328* (U)	801	AY740966	AY741015	AY740917	
Guatteria caribea Urb.	Dominican Republic	*Tuxill, J. 89* (U)	786	AY740967	AY741016	AY740918	
Guatteria chiriquiensis R.E.Fr.	Costa Rica	*Chatrou, L.W. et al. 43* (U)	202	AY740968	AY741017	AY740919	
Guatteria diospyroides Baill.	Costa Rica - Limón	*Chatrou, L.W. et al. 84 (U)*	233	AY740969	AY741018	AY740920	
Guatteria discolor R.E.Fr. aff.	Brazil - Acre	*Maas, P.J.M. et al. 9030 (U)*	552	AY740970	AY741019	AY740921	
Guatteria dumetorum R.E.Fr.	Panama	*FLORPAN 2497* (U)	779	AY740971	AY741020	AY740922	
Guatteria elata R.E.Fr.	Peru - Loreto	*Chatrou, L.W. et al. 252* (U)	328	AY740972	AY741021	AY740923	
Guatteria elegantissima R.E.Fr.	Colombia - El Valle	*Gentry, A.H. 56948* (U)	833	AY740973	AY741022	AY740924	
Guatteria foliosa Benth.	Bolivia - Santa Cruz	*Chatrou, L.W. et al. 325* (U)	575	AY740974	AY741023	AY740925	
Guatteria glabrescens R.E.Fr.	Brazil - Rio de Janeiro	*Maas, P.J.M. et al. 8816* (U)	99	AY740975	AY741024	AY740926	
Guatteria guianensis (Aubl.) R.E.Fr.	Brazil - Amazonas	*Webber, A.C. et al. 1884* (U)	560	AY740976	AY741025	AY740927	

Taxon	**Origin**	**Voucher**	**DNA ID**	***rbcL***	***trnL-trnF***	***matK***	***psbA-trnH***
Guatteria heterotricha R.E.Fr.	Colombia - El Valle	*Monsalve B., M. 1262* (U)	829	AY740977	AY741026	AY740928	
Guatteria hyposericea Diels aff.	Bolivia - Beni	*Chatrou, L.W. et al. 375* (U)	580	AY740978	AY741027	AY740929	
Guatteria inuncta R.E.Fr.	Costa Rica	*Liesner, R.L. 14631* (U)	780	AY740979	AY741028	AY740930	
Guatteria inundata Mart.	Peru - Loreto	*Chatrou, L.W. et al. 191* (U)	291	AY740980	AY741029	AY740931	
Guatteria jefensis Barringer	Panama	*Valdespino, I.A. 685* (U)	781	AY740981	AY741030	AY740932	
Guatteria latifolia (Mart.) R.E.Fr.	Brazil - Rio de Janeiro	*Lobão, A.Q. 544* (U)	510	AY740982	AY741031	AY740933	
Guatteria liesneri D.M. Johnson & N.A. Murray	Brazil - Amazonas	*Cid F., C.A. 8403* (U)	792	AY740983	AY741032	AY740934	
Guatteria macropus Mart.	Brazil - Bahia	*Pirani, J.R. 2725* (U)	790	AY740984	AY741033	AY740935	
Guatteria maypurensis Kunth	Guyana	*Jansen-Jacobs, M.J. et al. 5416* (U)	182	AY740985	AY741034	AY740936	
Guatteria megalophylla Diels	Peru - Loreto	*Chatrou, L.W. et al. 216* (U)	309	AY740986	AY741035	AY740937	
Guatteria multivenia Diels	Ecuador - Napo	*Maas, P.J.M. 8611* (U)	822	AY740987	AY741036	AY740938	
Guatteria oligocarpa Mart.	Brazil - Bahia	*Maas, P.J.M. 7006* (U)	811	AY740988	AY741037	AY740939	
Guatteria olivacea R.E.Fr.	Peru - Loreto	*Chatrou, L.W. et al. 209* (U)	304	AY740989	AY741038	AY740940	
Guatteria oliviformis Donn.Smith	Costa Rica - Monteverde	*Chatrou, L.W. et al. 80* (U)	230	AY740990	AY741039	AY740941	
Guatteria ouregou (Aubl.) Dunal	French Guiana	*Scharf, U. 85* (U)	679	AY740991	AY741040	AY740942	

Taxon	**Origin**	**Voucher**	**DNA ID**	***rbcL***	***trnL-trnF***	***matK***	***psbA-trnH***
Guatteria parvifolia R.E.Fr.	Brazil - São Paulo	*Gottsberger, G.K. 573007* (U)	809	AY740992	AY741041	AY740943	
Guatteria pittieri R.E.Fr.	Colombia - El Valle	*Werff, H.H. van der 9767* (U)	819	AY740993	AY741042	AY740944	
Guatteria pudica N.Zamora & Maas	Costa Rica - Osa	*Chatrou, L.W. et al. 107* (U)	249	AY740994	AY741043	AY740945	
Guatteria punctata (Aubl.) R.A. Howard	French Guiana	*Molino, J.F. 1593* (U)	482	AY740995	AY741044	AY740946	
Guatteria puncticulata R.E.Fr.	Peru - Loreto	*Chatrou, L.W. et al. 172* (U)	283	AY740996	AY741045	AY740947	
Guatteria recurvisepala R.E.Fr.	Costa Rica - La Selva	*Chatrou, L.W. et al. 61* (U)	217	AY740997	AY741046	AY740948	
Guatteria rotundata Maas & van Setten	Panama - San Blas	*Mori, S.A. 5531* (U)	783	AY740998	AY741047	AY740949	
Guatteria rupestris Mello-Silva & Pirani	Brazil - Minas Gerais	*CFCR 4116* (U)	803	AY740999	AY741048	AY740950	
Guatteria schomburgkiana Mart.	Guyana	*Scharf, U. 60* (U)	696	AY741001	AY741050	AY740952	
Guatteria scytophylla Diels	Brazil - Amazonas	*Maas, P.J.M. et al. s.n.* (U)	559	AY741002	AY741051	AY740953	
Guatteria sellowiana Schltdl. aff.	Brazil - Rio de Janeiro	*Lobão, A.Q. 557* (U)	511	AY741003	AY741052	AY740954	
Guatteria sessilicarpa Maas & van Setten	Panama - Panama	*McPherson, G. 2599* (U)	784	AY741004	AY741053	AY740955	

Taxon	**Origin**	**Voucher**	**DNA ID**	***rbcL***	***trnL-trnF***	***matK***	***psbA-trnH***
Guatteria sp.	Brazil - Rio de Janeiro	*Lobão, A.Q. et al. 565* (U)	513	AY741000	AY741049	AY740951	
Guatteria tonduzii Diels	Costa Rica - Osa	*Chatrou, L.W. et al. 121* (U)	259	AY741005	AY741054	AY740956	
Guatteria villosissima A.St.-Hil.	Brazil - Bahia	*Lobão, A.Q. 630* (U)	564	AY741006	AY741055	AY740957	
Klarobelia candida Chatrou	Peru - Madre de Dios	*Gentry, A.H. et al. 43466* (U)	1029	AY841525	AY841539		AY841467
Klarobelia cauliflora Chatrou	Peru - Loreto	*Chatrou, L.W. et al. 161* (U)	277	AY841627[b]	AY841705[b]		AY841468
Klarobelia inundata Chatrou	Peru - Loreto	*Chatrou, L.W. et al. 205* (U)	301	AY743452	AY743471	AY743490	AY841469
Klarobelia megalocarpa Chatrou	Ecuador - Esmeraldas	*Maas, P.J.M. et al. 8521* (U)	486	AY319062[e]	AY319176[e]	AY518866[f]	AY841470
Klarobelia sp.	Venezuela - Distr. Federal	*Meier, W. & S. Llamozas 3685* (U)	1027	AY841526	AY841540		AY841471
Klarobelia stipitata Chatrou	Costa Rica - Osa	*Chatrou, L.W. et al. 113* (U)	253	AY841628[b]	AY841706[b]		AY841472
Letestudoxa bella Pellegr.	Gabon	*Wieringa, J.J. & T.Nzabi 2797* (WAG)	145	AY841629[b]	AY841707[b]		
Malmea dielsiana R.E.Fr.	Peru - Madre de Dios	*Chatrou, L.W. et al. 122* (U)	260	AY238955[b]	AY231288 AY238948[d]	AY238964[d]	AY841473
Malmea dimera Chatrou	Panama - Panama	*Croat, T.B. 34626* (U)	39	AY841631[b]	AY841709[b]		AY841474
Malmea sp.	Peru - Loreto	*Chatrou, L.W. et al. 8* (U)	197	AY841527	AY841541	AY841397	AY841475

Taxon	**Origin**	**Voucher**	**DNA ID**	***rbcL***	***trnL-trnF***	***matK***	***psbA-trnH***
Malmea surinamensis Chatrou	Suriname	*Jansen-Jacobs, M.J. et al. 6207* (U)	850	AY743453	AY743472	AY743491	AY841476
Mezzettia parviflora Becc.	Indonesia	*Okada 3388* (L)	JB32	AY318983[e]	AY319095[e]		
Monanthotaxis whytei (Stapf) Verdc.	[g]Africa	*Chatrou, L.W. 475* (U)	533	AY841635[b]	AY841713[b]		
Monocarpia euneura Miq.	Indonesia	*Slik, F. 2931* (L)	JB106	AY318998[e]	AY319111[e]	AY518865[f]	AY841477
Monodora myristica (Gaertn.) Dunal	[g]Ivory Coast	*Chatrou, L.W. 477* (U)	535	AY743447	AY743466	AY743485	
Mosannona costaricensis R.E.Fr.	Costa Rica - Limón	*Chatrou, L.W. et al. 90* (U)	237	AY743510	AY743496	AY743503	AY841479
Mosannona discolor (R.E.Fr.) Chatrou	Guyana	*Jansen-Jacobs, M.J. et al. 6000* (U)	186	AY743511	AY743497	AY743504	AY841480
Mosannona garwoodii Chatrou & Welzenis	Panama - Barro Colorado Island	*Garwood, N.C. 3129* (U)	50	AY743512	AY743498	AY743505	AY841481
Mosannona pacifica Chatrou	Ecuador - Esmeraldas	*Maas, P.J.M. et al. 8531* (U)	487	AY743513	AY743499	AY743506	AY841482
Mosannona papillosa Chatrou	Ecuador - Napo	*Pitman, N.* (U)	974	AY743514	AY743500	AY743507	AY841483
Mosannona sp.	Costa Rica - Monteverde	*Chatrou, L.W. et al. 71* (U)	226	AY743509	AY743495	AY743502	AY841478
Mosannona vasquezii Chatrou	Peru - Loreto - Yanamono	*Chatrou, L.W. et al. 226* (U)	313	AY319064[e]	AY319178[e]	AY518869[f]	AY841484
Neostenanthera myristicifolia (Oliv.) Exell	Gabon	*Wieringa, J.J. et al. 3566* (WAG)	54	AY743448	AY743467	AY743486	

Taxon	**Origin**	**Voucher**	**DNA ID**	***rbcL***	***trnL-trnF***	***matK***	***psbA-trnH***
Onychopetalum amazonicum R.E.Fr.	Brazil - Para	*Sperling, C.R. et al. 5925* (U)	972	DQ018222	DQ018175	DQ018261	DQ018237
Onychopetalum periquino (Rusby) D.M.Johnson & N.A.Murray	Bolivia - Beni	*Chatrou, L.W. et al. 425* (U)	17	AY319065[e]	AY319179[e]	AY518876[f]	AY841485
Oxandra asbeckii (Pulle) R.E.Fr.	Guyana	*University of Guyana, course Neotropical Botany UG-NB-55* (U)	629	AY841639[b]	AY841717[b]		AY841486
Oxandra espintana (Spruce ex Benth.) Baill.	Peru - Madre de Dios	*Chatrou, L.W. et al. 133* (U)	268	AY319066[e]	AY319180[e]	DQ018260	AY841487
Oxandra euneura Diels	Peru - Loreto	*Chatrou, L.W. et al. 249* (U)	326	AY841640[b]	AY841718[b]		AY841488
Oxandra laurifolia (Sw.) A.Rich	Dominican Republic	*Maas, P.J.M. et al. 8375* (U)	27	AY841528	AY841542		AY841489
Oxandra longipetala R.E.Fr.	Costa Rica - Osa	*Chatrou, L.W. et al. 114* (U)	254	AY841641[b]	AY841719[b]		AY841490
Oxandra macrophylla R.E.Fr.	Peru - Loreto	*Chatrou, L.W. et al. 204* (U)	300	AY841642[b]	AY841720[b]		AY841491
Oxandra nitida R.E.Fr.	Brazil - Rio de Janeiro	*Maas, P.J.M. et al. 8821* (U)	103	AY841529	AY841543		AY841492
Oxandra polyantha R.E.Fr.	Peru - Loreto	*Chatrou, L.W. et al. 215* (U)	308	AY841643[b]	AY841721[b]		AY841493
Oxandra sphaerocarpa R.E.Fr.	Peru - Loreto	*Maas, P.J.M. et al. 8226* (U)	80	AY841644[b]	AY841722[b]		AY841494

Taxon	**Origin**	**Voucher**	**DNA ID**	***rbcL***	***trnL-trnF***	***matK***	***psbA-trnH***
Oxandra venezuelana R.E.Fr.	Costa Rica - Osa	*Chatrou, L.W. et al. 120* (U)	258	AY841645[b]	AY841723[b]		AY841495
Oxandra xylopioides Diels	Peru - Loreto	*Chatrou, L.W. et al. 165* (U)	280	AY841646[b]	AY841724[b]		AY841496
Piptostigma fasciculata (De Wild.) Boutique	Ghana	*Jongkind, C.C.H. et al. 1862* (WAG)	954	AY841647[b]	AY841725[b]		AY841497
Piptostigma mortehani De Wild.	Gabon	*Wieringa, J.J. et al. 2779* (WAG)	505	AY743454	AY743473	AY743492	AY841498
Piptostigma pilosum Oliv.	Cameroon	*Wieringa, J.J. 2030* (WAG)	956	AY841648[b]	AY841726[b]		AY841499
Polyalthia celebica Miq.	Indonesia	*Mols, J.B. 9* (L)	JB	AY319016[e]	AY319130[e]	AY518827[f]	
Polyalthia discolor Diels	Papua New Guinea	*Takeuchi & Ama 16394* (L)	JB114	AY319021[e]	AY319135[e]	AY518872[f]	AY841500
Polyalthia glauca (Hassk.) Boerl.	Indonesia	*Mols, J.B. 20* (L)	JB17	AY319023[e]	AY319137[e]		AY841501
Polyalthia suberosa (Roxb.) Thwait.	[g]India	*Chatrou, L.W. 480* (U)	536	AY238956[b]	AY231289 AY238949[d]	AY238965[d]	AY841502
Polyalthia sumatrana (Miq.) King	Malaysia	*SAN 143918* (L)	JB22	AY319039[e]	AY319153[e]	AY518873[f]	AY841503
Pseudephedranthus fragrans (R.E.Fr.) Aristeg.	Venezuela - Amazonas	*Maas, P.J.M. et al. 6878* (U)	33	AY841651[b]	AY841729[b]		AY841504
Pseudomalmea diclina (R.E.Fr.) Chatrou	Peru - Loreto - Yanamono	*Chatrou, L.W. et al. 211* (U)	305	AY319068[e]	AY319128[e]	AY841398	AY841506
Pseudomalmea diclina (R.E.Fr.) Chatrou	Peru - Madre de Dios	*Chatrou, L.W. et al. 136* (U)	270	AY841530	AY841544		AY841505

Taxon	**Origin**	**Voucher**	**DNA ID**	***rbcL***	***trnL-trnF***	***matK***	***psbA-trnH***
Pseudoxandra bahiensis Maas	Brazil - Bahia	*Mori, S.A. 12051* (U)	1008	AY841653[b]	AY841731[b]		AY841508
Pseudoxandra longipes Maas	Colombia - El Valle	*Monsalve B., M. 1168* (U)	1007	AY841532	AY841546		AY841509
Pseudoxandra lucida R.E.Fr.	Peru - Loreto	*Chatrou, L.W. et al. 213* (U)	307	AY319076[e]	AY319190[e]	AY319190[f]	AY841510
Pseudoxandra pacifica Maas aff.	Colombia - El Valle	*Rooden, J. van et al. 565* (U)	1005	AY841531	AY841545		AY841511
Pseudoxandra polyphleba (Diels) R.E. Fr.	Peru - Loreto	*Maas, P.J.M. et al. 8227* (U)	81	AY841654[b]	AY841732[b]		AY841512
Pseudoxandra spiritus-sancti Maas	Brazil - Espirito Santo	*Maas, P.J.M. et al. 8833* (U)	110	AY841533	AY841547	AY841399	AY841513
Ruizodendron ovale (Ruiz & Pav.) R.E.Fr.	Ecuador - Napo	*Maas, P.J.M. et al. 8600* (U)	88	AY841657[b]	AY841735[b]		AY841514
Sapranthus viridiflorus G.E.Schatz	Costa Rica - La Selva	*Chatrou, L.W. et al. 55* (U)	211	AY319051[e]	AY319165[e]	AY743493	AY841515
Stenanona costaricensis R.E.Fr.	Costa Rica - Braulio Carillo	*Chatrou, L.W. et al. 67* (U)	222	AY319069[e]	AY319183[e]		AY841516
Tetrameranthus duckei R.E.Fr.	Brazil - Amazonas	*Stevenson, D.W. et al. 1002* (U)	28	AY841658[b]	AY841736[b]		AY841439
Trigynaea lanceipetala D.M.Johnson & N.A.Murray	Peru - Loreto	*Chatrou, L.W. et al. 234* (U)	318	AY743449	AY743468	AY743487	
Unknown genus *sp.*	Peru - Huánuco	*Tello 3416* (NY)	750	AY841520	AY841534		AY841456
Unonopsis elegantissima R.E.Fr.	Peru - Loreto	*Chatrou, L.W. et al. 250* (U)	327	DQ018223	DQ018176	DQ018263	DQ018238

Taxon	Origin	Voucher	DNA ID	*rbcL*	*trnL-trnF*	*matK*	*psbA-trnH*
Unonopsis pittieri Saff.	Costa Rica - Braulio Carillo	*Chatrou, L.W. et al. 68* (U)	223	AY841661[b]	AY841739[b]	DQ018264	AY841517
Unonopsis rufescens (Baill.) R.E.Fr.	French Guiana	*Orava, C.E. 9* (U)	714	AY743455	AY743474	AY743494	AY841518
Unonopsis stipitata Diels	Peru - Loreto	*Chatrou, L.W. et al. 253* (U)	329	AY841662[b]	AY841740[b]	AY841400	AY841519
Uvaria lucida Benth. subsp. virens (N.E.Br.) Verdc.	[g]West Africa	*Botanische Tuinen 84GR00334* (U)	538	AY238957	AY231290 AY238950[d]	AY238966[d]	AY841440
Xylopia frutescens Aubl.	Costa Rica - Osa	*Chatrou, L.W. et al. 106* (U)	248	AY841667[b]	AY841745[b]		AY841441

[a]Wang, Y.-L. *et al.* unpublished; [b]Chatrou *et al.* unpublished; [c]Kojoma *et al.* (2002); [d]Sauquet *et al.* (2003); [e]Mols *et al.* (2004); [f]Mols *et al.* (submitted); [g]Cultivated in Utrecht University Botanic Garden

Appendix A: Table 2

Taxon	DNA ID	*ndhF*	*trnT-trnL*	*trnS-trnG*	*atpB-rbcL*	pseud*trnL-F*
Annickia chlorantha (Oliv.) Setten & Maas	976	AY841401	AY841571	AY841550	AY841370	
Annickia pilosa (Exell) Setten & Maas	977	AY841402	AY841572	AY841551	AY841371	
Bocageopsis multiflora (Mart.) R.E.Fr.	190					DQ018199
Cananga odorata (Lam.) Hook.f & Thomson	239	AY841403		AY841548	AY841372	
Cleistopholis glauca Engl. & Diels	198	AY841404		AY841549	AY841373	
Cremastosperma brevipes (DC.) R.E.Fr.	601	AY841405	AY841573	AY841552	AY841374	DQ018191
Cremastosperma bullatum Pirie	1102	DQ018142	DQ018172			DQ018221
Cremastosperma bullatum Pirie	1100	DQ018140	DQ018170			DQ018220
Cremastosperma cauliflorum R.E.Fr.	598	DQ018125	DQ018150			DQ018206
Cremastosperma cauliflorum R.E.Fr.	312	AY841406	AY841574	AY841553	AY841375	DQ018192
Cremastosperma cenepense Pirie & Zapata	1269		DQ018173			

Taxon	DNA ID	*ndhF*	*trnT-trnL*	*trnS-trnG*	*atpB-rbcL*	pseud*trnL-F*
Cremastosperma leiophyllum R.E.Fr.	594	DQ018123	DQ018148			DQ018193
Cremastosperma macrocarpum Maas	741	DQ018129	DQ018154			DQ018194
Cremastosperma magdalenae Pirie	1270	DQ018143	DQ018174			
Cremastosperma megalophyllum R.E.Fr.	493	DQ018122	DQ018147			
Cremastosperma megalophyllum R.E.Fr.	490	DQ018121	DQ018146			
Cremastosperma microcarpum R.E.Fr.	303	DQ018120	DQ018145			DQ018204
Cremastosperma monospermum (Rusby) R.E.Fr.	596	DQ018124	DQ018149			DQ018205
Cremastosperma napoense Pirie	737	DQ018127	DQ018152			DQ018208
Cremastosperma novogranatense R.E.Fr.	744		DQ018156			
Cremastosperma oblongum R.E.Fr.	600	DQ018126	DQ018151			DQ018207
Cremastosperma oblongum R.E.Fr.	739	DQ018128	DQ018153			DQ018209
Cremastosperma oblongum R.E.Fr.	1097	DQ018137	DQ018167			DQ018217
Cremastosperma panamense Maas	747	DQ018131	DQ018158			DQ018211
Cremastosperma pedunculatum (Diels) R.E.Fr.	760	DQ018135	DQ018165			DQ018215

Taxon	**DNA ID**	***ndhF***	***trnT-trnL***	***trnS-trnG***	***atpB-rbcL***	**pseud*trnL-F***
Cremastosperma pedunculatum (Diels) R.E.Fr.	754		DQ018161			
Cremastosperma pendulum (Ruiz & Pav.) R.E.Fr.	749		DQ018159			
Cremastosperma pendulum (Ruiz & Pav.) R.E.Fr.	751	DQ018132	DQ018160			DQ018212
Cremastosperma pendulum (Ruiz & Pav.) R.E.Fr.	1098	DQ018138	DQ018168			DQ018218
Cremastosperma sp.	745	DQ018130	DQ018157			DQ018210
Cremastosperma spec. A	245	DQ018119	DQ018144			DQ018203
Cremastosperma spec. B	758		DQ018164			
Cremastosperma spec. B	756	DQ018133	DQ018162			DQ018213
Cremastosperma venezuelanum Pirie	757	DQ018134	DQ018163			DQ018214
Cremastosperma westrae Pirie	761	DQ018136	DQ018166			DQ018216
Cremastosperma yamayakatense Pirie	1101	DQ018141	DQ018171			
Cremastosperma yamayakatense Pirie	1099	DQ018139	DQ018169			DQ018219
Cremastosperma yamayakatense Pirie	742		DQ018155			
Ephedranthus sp.	105	AY841407	AY841575	AY841554	AY841376	
Greenwayodendron oliveri (Engl.) Verdc.	958	AY841408	AY841576	AY841555	AY841377	

Taxon	DNA ID	*ndhF*	*trnT-trnL*	*trnS-trnG*	*atpB-rbcL*	pseud*trnL-F*
Klarobelia inundata Chatrou	301	AY841409	AY841577	AY841556	AY841378	
Malmea dielsiana R.E.Fr.	260	AY841410	AY841578	AY841557	AY841379	DQ018195
Malmea sp.	197	AY841411	AY841579	AY841558	AY841380	DQ018196
Malmea surinamensis Chatrou	850					DQ018197
Monocarpia euneura Miq.	JB106	AY841412	AY841580	AY841559	AY841381	
Mosannona costaricensis R.E.Fr.	237	AY841413	AY841581	AY841560	AY841382	
Onychopetalum amazonicum R.E.Fr.	972					DQ018198
Onychopetalum periquino (Rusby) D.M.Johnson & N.A.Murray	17	AY841414	AY841582	AY841561	AY841383	
Oxandra espintana (Spruce ex Benth.) Baill.	268					DQ018189
Piptostigma mortehani De Wild.	505	AY841415	AY841583	AY841562	AY841384	
Polyalthia discolor Diels	JB114	AY841416	AY841584	AY841563	AY841385	
Polyalthia suberosa (Roxb.) Thwait.	536	AY841417	AY841585	AY841564	AY841386	
Polyalthia sumatrana (Miq.) King	JB22	AY841418	AY841586	AY841565	AY841387	
Pseudomalmea diclina (R.E.Fr.) Chatrou	305	AY841419	AY841587	AY841566	AY841388	
Pseudoxandra lucida R.E.Fr.	307	AY841420	AY841588	AY841567	AY841389	
Pseudoxandra spiritus-sancti Maas	110	AY841421	AY841589	AY841568	AY841390	
Sapranthus viridiflorus G.E.Schatz	211	AY841422	AY841590	AY841569	AY841391	
Unonopsis elegantissima R.E.Fr.	327					DQ018200
Unonopsis pittieri Saff.	223					DQ018201
Unonopsis stipitata Diels	329	AY841423	AY841591	AY841570	AY841392	DQ018202

Appendix B

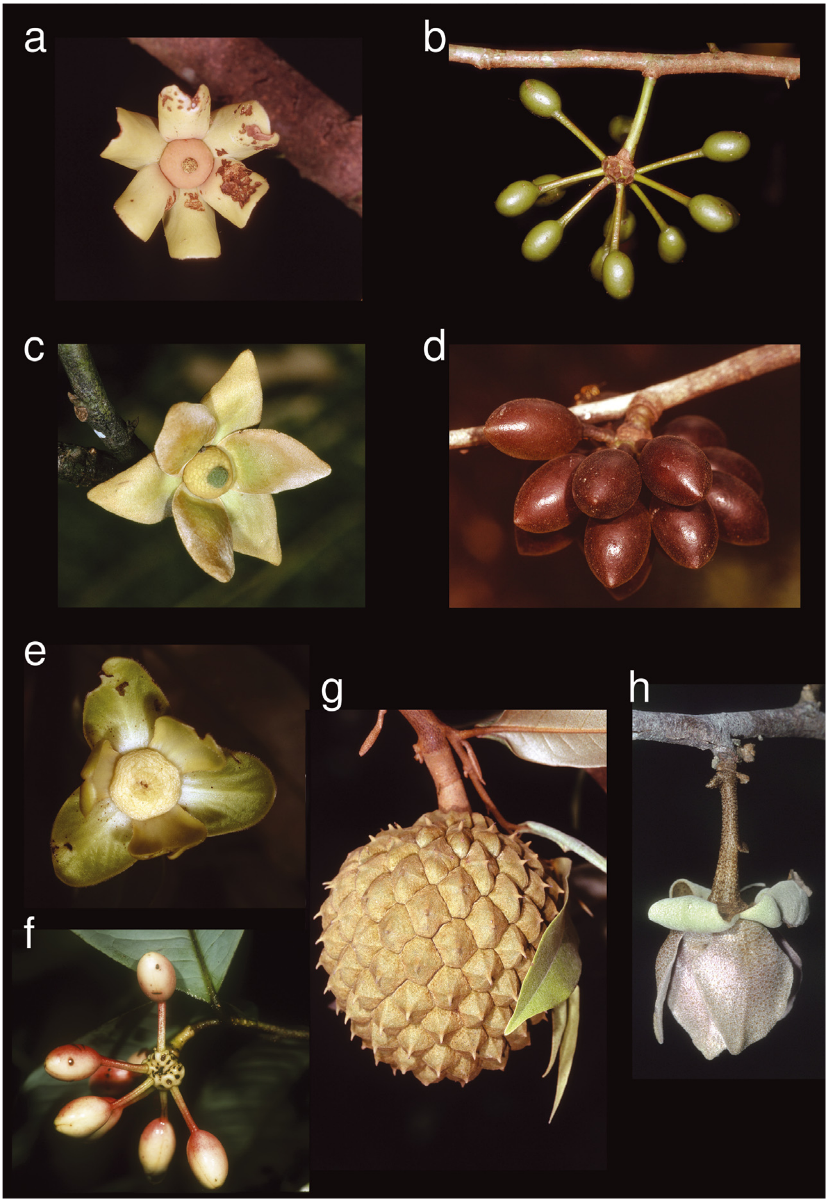

Fig. 1 (Chapter 2, Fig. 2.) (a) *Cremastosperma leiophyllum R.E.Fr. (Pirie et al. 2*, Bolivia), flower before anthesis; (b) *Cremastosperma leiophyllum* R.E.Fr. (*Pirie et al. 2*, Bolivia), immature fruit; (c) *Guatteria megalophylla* Diels (*Chatrou et al. 387*, Bolivia), flower before anthesis; (d) *Guatteria megalophylla* Diels (*Chatrou et al. 387*, Bolivia), almost mature fruit; (e) *Mosannona vasquezii* Chatrou (*Chatrou et al. 226*, Peru), flower before anthesis; (f) *Mosannona* sp. (*Chatrou et al. 72*, Costa Rica), immature fruit; (g) *Duguetia marcgraviana* Mart. (*Chatrou et al. 327*, Bolivia), immature fruit; (h) *Duguetia marcgraviana* Mart. (*Chatrou et al. 329*, Bolivia), flower at anthesis.

Photographs by L.W. Chatrou.

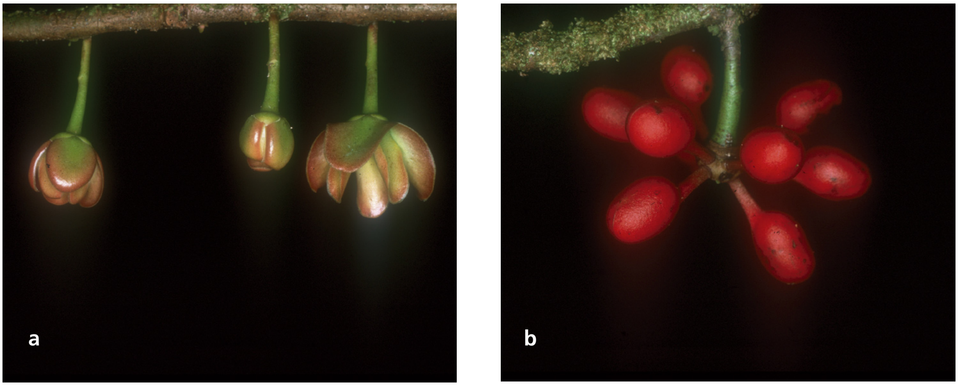

Fig.2. *Cremastosperma brevipes* (DC.) R.E.Fr. a. flower buds; b. fruit (French Guiana; photos: S. Mori)

Fig. 3. *Cremastosperma bullatum* Pirie a. leaf base, illustrating bullate ('bubbled') appearance and long golden hairs; b. flowers (a: *Pirie et al. 71*; b: *Pirie et al. 94*, Peru - Amazonas; photos: MDP)

Fig. 4. *Cremastosperma cauliflorum* R.E.Fr. a. branching inflorescence; b. illustrating closure of inner petals on maturing of flower (*Maas et al. 9021*, Brazil - Acre; photos P.J.M. Maas)

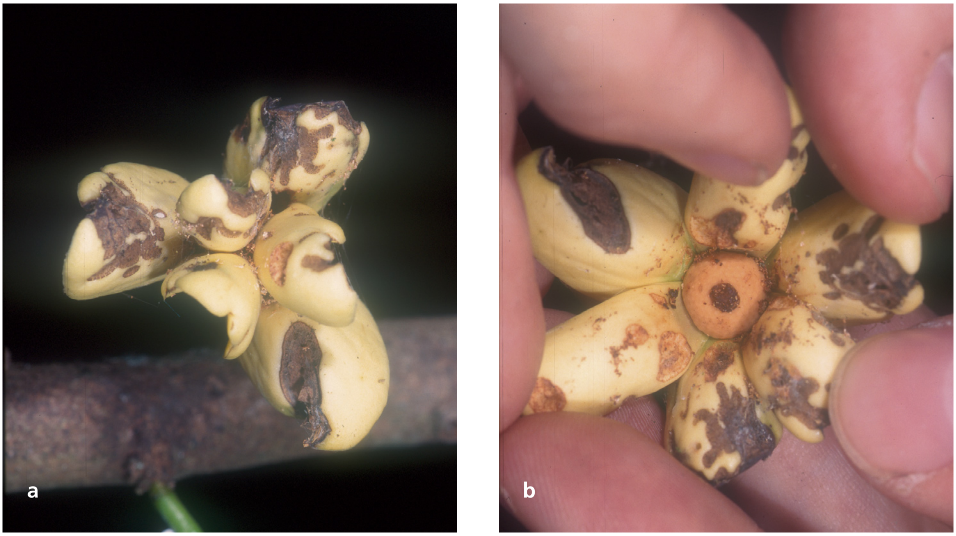

Fig. 5. *Cremastosperma leiophyllum* R.E.Fr. flower at anthesis: a. illustrating closure of inner petals on maturing of flower; b. colour change in stigmas (compare with Appendix 2.1a) (*Pirie et al. 2*, Bolivia - Santa Cruz; photos L.W. Chatrou)

Fig. 6. *Cremastosperma megalophyllum* R.E.Fr. a. habit; b. mature fruit (a: *Maas et al. 8577*; b: *Maas et al. 8595*, both Ecuador - Napo, photos P.J.M. Maas)

Fig. 7. *Cremastosperma microcarpum* R.E.Fr. a. & b. flower; c. fruit (a & b: *Maas et al. 8222*; c: *Maas et al. 6281*, both Peru - Loreto, photos P.J.M. Maas)

Fig. 8. *Cremastosperma monospermum* (Rusby) R.E.Fr. a. habit; b. leaf (illustrating often cuspidate leaf apex); c. flower bud (*Pirie et al. 5*, Bolivia - Pando; photos L.W. Chatrou)

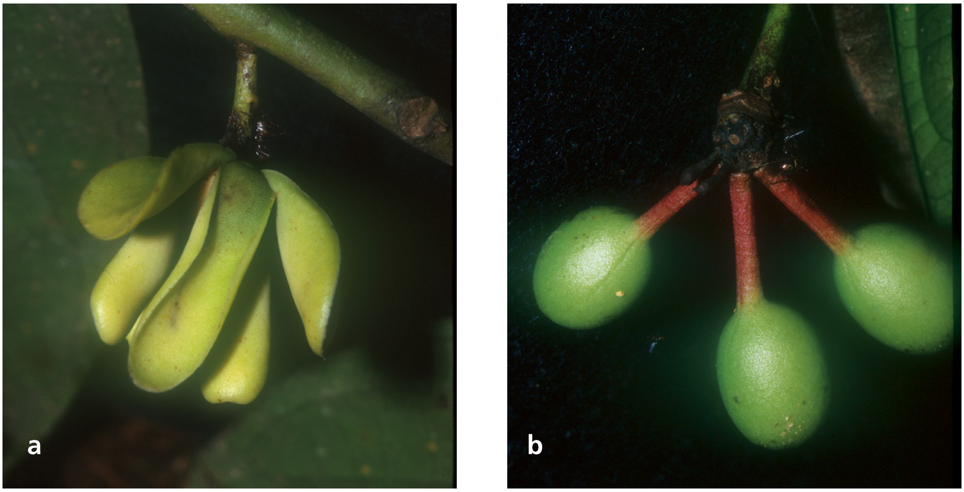

Fig. 9. *Cremastosperma oblongum* R.E.Fr. a. flower; b. fruit (*Maas et al. 9148*, Brazil - Acre; photos P.J.M. Maas)

Fig. 10. ***Cremastosperma pendulum*** **(Ruiz & Pav.) R.E.Fr. habit and flower bud (*Pirie et al. 33*, Peru - Pasco; photo MDP)**

Fig. 11. *Cremastosperma yamayakatense* Pirie a. flower bud; b. habit and fruit; c. mature fruit (a: *Pirie et al. 57*; b: *Pirie et al. 58*; c: *Pirie et al. 60*; all Peru - Amazonas, photos MDP)

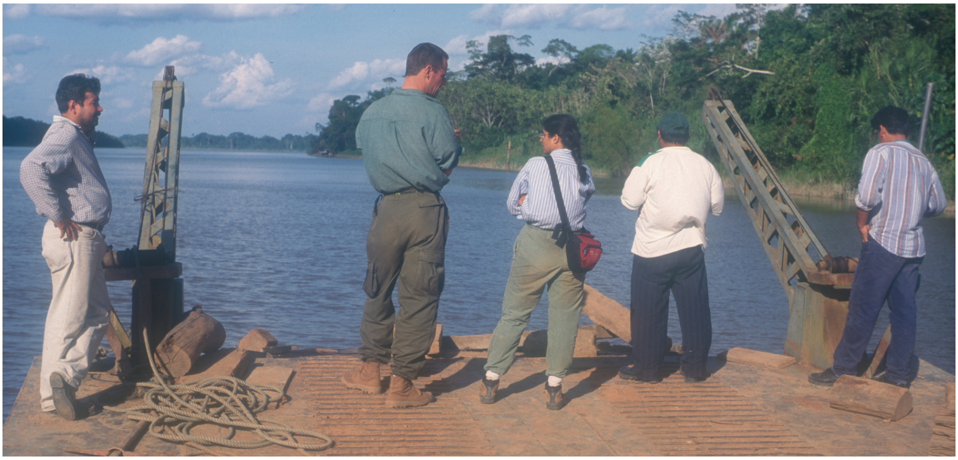

Fig. 12. a. Bolivia - Pando, Nov. 2001. From left: A. Saucedo; L.W. Chatrou, T. Scharaschkin (then at the University of California, Davis); N. Divico (Don Nico); C. Salas;

Fig. 12 b. Peru - San Martín: Estación 'Biodiversidad', Dec. 2003. From left: T. Diaz F.(Universidad Nacional San-Martín, Tarapoto); M. Zapata C. (Universidad Privada Antenor Orrego, Trujillo); R. van Velzen and M. Botermans (NHN - Wageningen); M. Gonzales I.; MDP.

Fig. 12 c. Peru - Amazonas: Comunidad Yamayakat, Nov. 2003. R. Apanu N. (left); J.S. Kasen; displaying local craft.