

Dissertation zur Erlangung des Doktorgrades der
Naturwissenschaften (Dr. rer. nat.)
an der Fakultät für Biologie der Ludwig-Maximilians-
Universität München

STUDYING PLANT EVOLUTION WITH
PHYLOGENETIC, MOLECULAR-CYTOGENETIC, AND
NICHE MODELING APPROACHES

vorgelegt von

Alexander Konstantin Rockinger

München, 11. Oktober 2016

*meiner Familie,
Elfrun, August, und Elena*

PREFACE

Statutory declaration

Erklärung

Diese Dissertation wurde im Sinne von §12 der Promotionsordnung von Prof. Dr. Susanne S. Renner betreut. Ich erkläre hiermit, dass die Dissertation nicht einer anderen Prüfungskommission vorgelegt worden ist und dass ich mich nicht anderweitig einer Doktorprüfung ohne Erfolg unterzogen habe.

Eidesstattliche Erklärung

Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbstständig und ohne unerlaubte Hilfe angefertigt wurde.

Alexander Rockinger, 11. Oktober 2016

(Unterschrift)

1. Gutachter: Prof. Dr. Susanne S. Renner
2. Gutachter: Prof. Dr. Jochen Heinrichs

Tag der Abgabe: 11. Oktober 2016

Tag der Disputation: 19. Dezember 2016

Declaration of contribution

In this thesis, I present the results from my doctoral research, carried out in Munich from December 2013 to August 2016 under the guidance of Prof. Dr. Susanne S. Renner. My thesis resulted in three manuscripts, presented in Chapters 2 to 4, of which two have been published (Chapters 3 and 4) and one is in review (Chapter 2). For the paper in chapter 3, I contributed to the revision of material and did much of the writing while for the other two papers (Chapters 2 and 4) I generated all data and conducted all analyses myself. Writing and discussion involved collaboration with Prof. Renner. I also gave the conference talks and poster presentations listed below.

Alexander Rockinger
(Signature)

Prof. Susanne S. Renner
(Signature)

List of publications

Peer-reviewed journal articles

ROCKINGER, A., FLORES, A.S., RENNER, S.S. In review. Clock-dated phylogeny for 48% of the 700 species of *Crotalaria* (Fabaceae–Papilionoideae) resolves sections worldwide and implies conserved flower and leaf traits throughout its pantropical range. *BMC Evolutionary Biology*.

FLORES, A.S., **ROCKINGER, A.**, SCHÜTZ-RODRIGUES, R., TOZZI, A.M.G.A. 2016. Lectotypifications and taxonomic changes in Brazilian *Crotalaria* L. (Fabaceae). *Phytotaxa* 267, 296–300.

ROCKINGER, A., SOUSA, A., CARVALHO, F.A., RENNER, S.S. 2016. Chromosome number reduction in the sister clade of *Carica papaya* with concomitant genome size doubling. *American Journal of Botany* 103: 1082–1088. [Journal cover]

Oral presentations

ROCKINGER, A. A worldwide phylogeny of the mega-diverse genus *Crotalaria* reveals ancient signal in leaf architecture, but not flowers. *International Symposium and Workshop Legume Morphology: Current knowledge and future directions*. Nov 3, 2015. Botucatu, São Paulo, Brazil

ROCKINGER, A. Progress on the mega-diverse genus *Crotalaria*. *16. Annual meeting of the Gesellschaft für Biologische Systematik (GfBS)*. Mar 19, 2015. Zoologisches Forschungsmuseum Alexander Koenig, Bonn

Poster

ROCKINGER, A. AND S. S. RENNER. What's up with the spirally twisted keel beaks in *Crotalaria* flowers? 23rd Symposium on Biodiversity and Evolutionary Biology of the German Botanical Society, Sept 8–11, 2016, Institute of Systematic Botany and Mycology, University of Munich (LMU)

ROCKINGER, A. AND S. S. RENNER. Nested chromosome number reduction in the sister clade of *Carica papaya* (Caricaceae, Brassicales) with concomitant genome size doubling. *Chromatin Dynamics, 4th Munich Chromatin Symposium*, Mar 10–12, 2016, Biomedical Center Munich, LMU HighTech Campus Martinsried, Munich

ROCKINGER, A. AND S. S. RENNER. Does rarity leave a footprint in herbarium collections?. *International Biogeography Society: 7th Biennial Meeting*. Jan 8–12, 2015. University of Bayreuth

ROCKINGER, A. AND S. S. RENNER. Evolution of the mega-diverse genus *Crotalaria* (Fabaceae) – first results?. *Radiations – Evolutionary Plant Radiations: Where, When, Why & How?* June 12–15, 2014. Universität of Zurich (UZH), Department of Systematic and Evolutionary Botany

CONTENTS

Preface	v
Statutory Declaration	v
Erklärung	v
Eidesstattliche Erklärung	v
Declaration of contribution	vii
List of publications	vii
Oral presentations	viii
Poster	viii
Contents	ix
Summary	1
Chapter 1: General Introduction	3
1.1 Study system 1, the legume genus <i>Crotalaria</i> – phylogenetics of mega-diverse taxa	5
1.2 Study system 2, the Caricaceae – chromosomal change in modern plant systematics	12
1.3 Research questions in the two study systems	14
Chapter 2: Clock-dated phylogeny for 48% of the 700 species of <i>Crotalaria</i> (Fabaceae– Papilionoideae) resolves sections worldwide and implies conserved flower and leaf traits throughout its pantropical range	17
Chapter 3: Lectotypifications and taxonomic changes in Brazilian <i>Crotalaria</i> L. (Fabaceae)	81
Chapter 4: Chromosome number reduction in the sister clade of <i>Carica papaya</i> with concomitant genome size doubling	89
Chapter 5: General Discussion	105
5.1 Phylogenetic relationships and divergence times in <i>Crotalaria</i>	107
5.2 Flower and leaf trait evolution in <i>Crotalaria</i>	109
5.3 Chromosome numbers in <i>Crotalaria</i>	111
5.4 Taxonomic treatment of Neotropical <i>Crotalaria</i>	112
5.5 Chromosome numbers and genome size alterations in Caricaceae	112
5.6 Future research questions on flower functionality in <i>Crotalaria</i>	113
References	117
Acknowledgements	131

SUMMARY

This study uses phylogenetic, molecular-cytogenetic, and climate niche reconstruction approaches to study aspects of the evolution of two groups of flowering plants, the pantropical legume genus *Crotalaria* and the papaya family, Caricaceae. *Crotalaria*, with about 700 species worldwide, is among the largest genera of flowering plants. I constructed the first comprehensive phylogeny of this genus, including 48% of the species from all sections and the entire geographic range, to infer its expansion history and answer the question if flower and leaf traits reflect different climate niches. The Caricaceae comprise only 34 species, two in Africa and 32 in the Neotropics. Using a modern phylogenetic framework, I investigated changes in chromosome numbers and genome sizes, topics that have received much attention in related families, such as Brassicaceae, where polyploidy and genome downsizing are common.

Crotalaria is monophyletic, and molecular clock dating implies that it diverged from its closest relative, a small southern African genus, 23 to 30 Ma ago, with the crown group then beginning to diversify 18 to 29 Ma ago. My trait plotting and reconstruction of ancestral states focus on leaf types (simple and compound) and flower traits, especially keel petals and calyx lobes, which in combination have been used to distinguish eight sections of *Crotalaria*. My data support none of these sections as monophyletic, and all trait states evolved and were lost repeatedly. I used (carefully cleaned) occurrence data from the Global Biodiversity Information Facility to correlate the occurrence of simple or compound leaves with the amount and distribution of rainfall, yearly temperature, elevation, and radiation. When applying phylogenetic correction for the non-independence of inherited traits, my analyses revealed no correlation between climate and leaf type, and flower traits also appear unrelated to climate. All flowers in *Crotalaria* have a piston-type pollination mechanism in which the weight of a bee inserting its proboscis to reach the nectar at the base of the staminal tube exerts force on the keel, triggering the hairy style to emerge, which then brushes pollen through the tip of the keel and deposits it on the bee's abdomen. This corresponds to the abdominal scopa of megachilid bees, a species-rich group of pantropical distribution that includes many species pollinating *Crotalaria*. In a separate paper, I dealt with nomenclature and typification of Brazilian *Crotalaria*, proposing four new synonyms and lectotypes for eleven species.

For *Crotalaria*, I compiled information on its chromosome numbers from the literature; plotting the data on the DNA phylogeny revealed that polyploidy is limited to Neotropical and Asian species. In-depth studies of karyotype and genome size changes require living material, and most cytogenetic studies have therefore focused on temperate or herbaceous species, while tropical trees, even when economically important, have scarcely been studied. I generated chromosome counts for species representing all genera of the Caricaceae, three of which had never had their chromosomes counted. I discovered that a stepwise chromosome number reduction (descending dysploidy) from $18 > 16 > 14$ has occurred in the sister clade of papaya (*C. papaya*). The Mexican *Horovitzia cnidoscoloides* has $2n = 16$ chromosomes, while the two (likewise Mexican) species of *Jarilla* examined both have $2n = 14$ chromosomes. Fluorescent *in situ* hybridization with standard rDNA probes (5S rDNA, 45S rDNA, and *Arabidopsis*-type telomeres) revealed much rDNA variation in the Caricaceae and interstitial telomeric repeats in *Jarilla*. Such interior placements of telomere DNA may be indicators for past chromosomal fusion and rearrangement. Most surprisingly, the species of *Jarilla*, despite having the lowest chromosome numbers in the family, have almost double the genome size of other Caricaceae, suggesting that the repeated chromosomal fusions were accompanied by bursts of transposon amplification. These results have implications for ongoing genome assemblies in the Caricaceae, which are ancestrally dioecious and an important model for sex chromosome evolution.

Chapter 1

GENERAL INTRODUCTION

Plant diversity across the globe is highly unevenly distributed, whether one considers species diversity per surface area or species diversity in extant clades. Despite some mega-diverse taxa having large geographic ranges, spatially limited distribution does not necessarily lead to low rates of diversification. Examples are the genus *Inga*, which occurs throughout the Neotropics and has 300 species (Richardson *et al.* 2001), while *Alnus* has a similarly large range in the Northern hemisphere (with a few species in the Andes), but has only 35 species (Chen and Li 2004). Neither is the age of a clade decisive for its diversity; the crown group of *Inga* is c. 5.9 my old (Richardson *et al.* 2001); that of *Alnus* c. 11 my (Grimm and Renner 2013). Other examples are *Dianthus*, the crown group of which is 1.2–7 my old and has c. 300 species (Valente *et al.* 2010), and *Jarilla*, which is c. 7 my old and has 3 species (Carvalho and Renner 2012, 2013a). Such disparity in species numbers may reflect a clade's success in filling different pollinator niches, edaphic niches, or climate niches, all of which may contribute to reproductive isolation among populations, leading to species formation. Disparity in species numbers may also be due to the vagaries of geographic isolation, especially in island archipelagoes or mountainous regions (e.g., Johnson 2010: Different pollinators in South Africa; Sebastian *et al.* 2012: *Sicyos* [Cucurbitaceae]: Geographic isolation; Holstein and Renner 2011: *Coccinia* [Cucurbitaceae]: Climate and soil). Besides ecological niche differentiation and random geographic isolation, polyploidy and hybridization are well-documented reasons for speciation (Chapman and Burke 2007; Soltis and Soltis 2009; Paun *et al.* 2011). My doctoral research was aimed at exploring plant evolution and diversity at different levels, using a comparative approach and a range of methods, including microscopy, molecular cytogenetics, the inference of climate niches, and the study of flower morphology (as it relates to pollinators), with the overarching goal being to better understand species proliferation in flowering plants. I chose two plant groups of similar age, however highly differing in their species diversity, and spatial distribution, and below I briefly introduce the two groups before specifying my research questions.

1.1 Study system 1, the legume genus *Crotalaria* – phylogenetics of mega-diverse taxa

If we are to understand what may drive diversification (i.e., defined as speciation minus extinction) in flowering plants, we will need to study morphological evolution and niche occupation in both species-rich and species-poor clades. In flowering plants, there are about 50 genera (not necessarily all monophyletic) that have >500 species. Few of the 35

Table 1. The 35 genera of flowering plants with ≥ 700 species with DNA phylogenies. An asterisk marks genera for which only studies with a regional or sectional focus are available.

Rank	Family	Genus	Total species number	Species number sampled (% of total)	Study
1	Fabaceae	<i>Astragalus</i>	c. 3270	48 (c. 1%)	Scherson <i>et al.</i> (2008)
				45 (c. 1%)	Osaloo <i>et al.</i> (2003)
				115 (c. 4%)	Wojciechowski <i>et al.</i> (1999)
2	Orchidaceae	<i>Bulbophyllum</i> *	c. 2032	53 (c. 3%)	Hosseini <i>et al.</i> (2012)
				42 (c. 2%)	Smidt <i>et al.</i> (2011)
3	Cyperaceae	<i>Carex</i>	c. 1990	100 (c. 5%)	Roalson <i>et al.</i> (2001)
4	Rubiaceae	<i>Psychotria</i>	c. 1951	64 (c. 3%)	Nepokroeff <i>et al.</i> (1999)
5	Euphorbiaceae	<i>Euphorbia</i>	c. 1836	352 (c. 19%)	Zimmermann <i>et al.</i> (2010)
6	Begoniaceae	<i>Begonia</i>	c. 1484	268 (c. 18%)	Moonlight <i>et al.</i> (2015)
				30 (c. 2%)	Dewitte <i>et al.</i> (2011)
				30 (c. 2%)	Goodall-Copestake <i>et al.</i> (2010)
				64 (c. 4%)	Forrest <i>et al.</i> (2005)
7	Orchidaceae	<i>Dendrobium</i> *	c. 1371	38 (c. 3%)	Forrest and Hollingsworth (2003)
				32 (c. 2%)	Burke <i>et al.</i> (2008)
8	Fabaceae	<i>Acacia</i>	c. 1353	75 (c. 5%)	Clemens (2003)
				104 (c. 8%)	Murphy <i>et al.</i> (2010)
				11 (c. 1%)	Miller and Bayer (2001)
				59 (c. 4%)	Robinson and Harris (1999)
9	Solanaceae	<i>Solanum</i>	c. 1250	22 (c. 2%)	Bukhari <i>et al.</i> (1999)
				365 (c. 34%)	Särkinen (2013)
				102 (c. 8%)	Weese and Bohs (2007)
				18 (c. 1%)	Bohs and Olmstead (1997)
10	Asteraceae	<i>Senecio</i>	c. 1250	36 (c. 3%)	Olmstead and Palmer (1997)
				186 (c. 15%)	Pelser <i>et al.</i> (2007)
11	Euphorbiaceae	<i>Croton</i>	c. 1223	79 (c. 6%)	Berry <i>et al.</i> (2005)
12	Orchidaceae	<i>Pleurothallis</i>	c. 1120	143 (c. 13%)	Wilson <i>et al.</i> (2013)
13	Myrtaceae	<i>Eugenia</i> *	c. 1113	28 (c. 3%)	Van der Merwe <i>et al.</i> (2005)
14	Piperaceae	<i>Piper</i>	c. 1055	332 (c. 31%)	Jaramillo <i>et al.</i> (2008)
				49 (c. 5%)	Smith <i>et al.</i> (2008)
				51 (c. 5%)	Jaramillo and Manos (2001)
15	Primulaceae	<i>Ardisia</i> *	c. 1046	8 (c. 1%)	Ku and Hu (2014)
16	Myrtaceae	<i>Syzygium</i>	c. 1041	80 (c. 8%)	Craven and Biffin (2010)
				64 (c. 6%)	Biffin <i>et al.</i> (2006)

Rank	Family	Genus	Total species number	Species	Study
				number sampled (% of total)	
17	Ericaceae	<i>Rhododendron</i>	c. 1001	85 (c. 9%)	Goetsch <i>et al.</i> (2005)
18	Melastomataceae	<i>Miconia</i>	c. 1000	216 (c. 22%)	Goldenberg <i>et al.</i> (2008)
19	Piperaceae	<i>Peperonia</i>	c. 1000	51 (c. 5%)	Samain <i>et al.</i> (2008)
				48 (c. 5%)	Smith <i>et al.</i> (2008)
20	Lamiaceae	<i>Salvia</i>	c. 946	65 (c. 7%)	Walker <i>et al.</i> (2004)
21	Ericaceae	<i>Erica</i>	c. 860	379 (c. 45%)	Pirie <i>et al.</i> (2011)
22	Balsaminaceae	<i>Impatiens</i>	c. 851	150 (c. 18%)	Yu <i>et al.</i> (2016)
				86 (c. 10%)	Janssens <i>et al.</i> (2006)
23	Cyperaceae	<i>Cyperus</i>	c. 839	39 (c. 5%)	Larridon <i>et al.</i> (2012)
				18 (c. 2%)	Muasya <i>et al.</i> (2002)
24	Phyllanthaceae	<i>Phyllanthus*</i>	c. 833	8 (c. 1%)	Wurdack <i>et al.</i> (2004)
25	Amaryllidaceae	<i>Allium</i>	c. 815	331 (c. 41%)	Li <i>et al.</i> (2010)
				100 (c. 12%)	Friesen <i>et al.</i> (2006)
26	Orchidaceae	<i>Epidendrum*</i>	c. 800	13 (c. 2%)	Pessoa <i>et al.</i> (2012)
27	Asteraceae	<i>Vernonia</i>	c. 800	—	No data found
28	Orchidaceae	<i>Lepanthes</i>	c. 800	—	No data found
29	Araceae	<i>Anthurium</i>	c. 790	102 (c. 13%)	Carlsen and Croat (2013)
30	Ebenaceae	<i>Diospyrus</i>	c. 767	119 (c. 16%)	Duangjai <i>et al.</i> (2009)
31	Moraceae	<i>Ficus</i>	c. 750	80 (c. 11%)	Ronsted <i>et al.</i> (2008)
32	Caryophyllaceae	<i>Silene*</i>	700+	14 (c. 2%)	Marais <i>et al.</i> (2011)
33	Fabaceae	<i>Indigofera</i>	700+	88 (c. 13%)	Schrire <i>et al.</i> (2003)
34	Oxalidaceae	<i>Oxalis*</i>	700+	50 (c. 7%)	Oberlander <i>et al.</i> (2009)
35	Fabaceae	<i>Crotalaria</i>	c. 700	338 (c. 48%)	Rockinger <i>et al.</i> (in review)

largest genera have been studied phylogenetically (Table 1), with only four studies having a taxon sampling >30% (Goldenberg *et al.* 2008: *Miconia* [Melastomataceae], c. 22% sampled; Jaramillo *et al.* 2008: *Piper* [Piperaceae], c. 31% sampled; Pirie *et al.* 2011: *Erica* [Ericaceae], c. 45% sampled; Li *et al.* 2010: *Allium* [Amaryllidaceae], c. 41% sampled; Särkinen *et al.* 2013: *Solanum* [Solanaceae], c. 34% sampled). Reasons for this include the difficulty of obtaining suitable material for producing a molecular phylogeny with a comprehensive taxon sampling, problems in sorting out names (of which there usually are at least twice as many as ‘accepted’ species), and insufficiently understood geographic ranges (which contributes to unclear species boundaries). These difficulties have led to the situation that most studies of clade diversification

in flowering plants have focused on small groups. Fully conscious of this, I chose one of the 35 largest genera (Table 1) as one of my two study systems, the pantropical legume genus *Crotalaria*, which has some 700 species.

Crotalaria has its center of diversity in Africa and Madagascar, where some 500 of its 700 species occur. However, the genus went through secondary radiations in Australia, India, and South America (Chapter 2). *Crotalaria*s are annual or perennial, herbaceous or woody plants occurring mostly in open, dry habitats on well-drained soil throughout the tropics, subtropics, and southern temperate areas. About 15 species are distributed pantropically due to their use as fiber crops, cattle fodder, and to improve soil nitrogen content (Polhill 1968, 1982). With eight other predominantly southern hemisphere tribes, the *Crotalariaeae* are part of the genistoids (Cardoso *et al.* 2012: Papilionoid-wide molecular analysis).

Common traits among genistoids are a xeromorphic habit, compound leaves with few leaflets or simple leaves, dimorphic stamens, fusion of the filaments, seeds with a hilar lobe from extension of the radicle, and an abundance of alkaloids (Crisp *et al.* 2000; Cardoso *et al.* 2012). All genistoids accumulate alkaloids, usually quinolizidine alkaloids (Van Wyk and Verdoorn 1990; Crisp *et al.* 2000), but the c. 50 (of 700) species of *Crotalaria* so far tested (Mears and Mabry 1971; Flores *et al.* 2009) instead produce hepatotoxic pyrrolizidine alkaloids (PAs). While quinolizidine alkaloids are derived from lysine and have two fused six-membered rings sharing a nitrogen molecule, pyrrolizidine alkaloids occur as complex esters and are derived from ornithine or arginine, they often occur as *N*-oxides. The two types of alkaloids are not known to occur in the same species (Mears and Mabry 1971; Van Wyk 2003). Hepatotoxic pyrrolizidine alkaloids are also present in *Laburnum* (*Genisteae*), *Lebeckia* (*Crotalariaeae*), *Pericopsis* (*Sophoreae*) (Van Wyk 2003; Lewis *et al.* 2005). Recently, Irmer *et al.* (2015) showed that PA biosynthesis in *Crotalaria* depends on nodulation; plants not infected by rhizobial bacteria and therefore not having formed nodules, did not have PAs. These alkaloids can be concentrated in leaves, seeds, nectar or pollen, but the nodules have the highest concentration, and the PAs are transported from there to the above ground parts of the plant. Plant alkaloids are toxic for generalist insect herbivores (Van Dam *et al.* 1995; Hartmann and Ober 2000; Narberhaus *et al.* 2005; Ober and Kaltenecker 2009; Macel 2011), but specialized butterflies, moths, and bees can detoxify PAs, and their larvae feed on pyrrolizidine-containing leaves (the case for moths or butterflies) or nectar or pollen (the case of adult butterflies and bees or bee larvae). An example is *Utetheisa*

ornatrix, an arctiid moth that feeds on *Crotalaria* (as larva) and retains the sequestered PAs through metamorphosis into the adult stage when they are used for the insect's defense and biosynthesis of sexual pheromones (Ferro *et al.* 2006). The work by Irmer *et al.* (2015) and other studies of the chemical defenses in *Crotalaria* have proceeded without any phylogenetically-guided sampling, and indeed the phylogeny resulting from my research shows that the species analyzed for alkaloids so far do not span the diversity of the genus.

Crotalaria flowers are adapted to Megachilidae bees, a worldwide clade of some 4000 species that are grouped in two subfamilies (Fidelinae and Megachilinae), and whose scopa are restricted to the ventral surface of the abdomen. Megachilid bees are found on all continents except Antarctica, and their habitats range from arid zones to tropical rainforests and alpine regions (Gonzalez *et al.* 2012; Danforth *et al.* 2013). Specialized megachilids use the scent of PAs (previous paragraph) to find their pollen and nectar hosts. This has been studied in the Fabaceae genus *Crotalaria*, which is the main host of *Hoplitis parana* and which contains pyrrolizidine alkaloids at least in its vegetative parts and mostly in its seeds (Fletcher *et al.* 2009). This chemical specialization leads directly to the topic of the floral apomorphies of *Crotalaria*. One is a hairy style that functions in its pollination mechanism and fits with the pollen-carrying structures of Megachilidae bees (Figure 1 D; Figures 2 A2–D2). The trichomes on the upper distal section of the style push the pollen out of the keel petal and present it in a position where a bee's lower abdomen will come in contact with it. Another apomorphy of *Crotalaria* is a staminal tube with interlocking upper edges (Polhill 1982, Le Roux and Van Wyk 2012). The closest relative of *Crotalaria* is the southern African genus *Bolusia* (five species), which differs in having a helically coiled keel and a single standard petal appendage (Van Wyk *et al.* 2010; Le Roux and Van Wyk 2012; supported by my data: Chapter 2). Morphological features used to key out species groups (ranked as sections) in *Crotalaria* are the morphology of the keel beak, the lobing of the calyx, and the length of the calyx in comparison to the keel petal, the curvature of the style, shape of the stigma, and position of inflorescence (see Chapter 2 for details). There are few studies on the functioning of the flowers or on plant pollinator interactions, involving two New World (Etcheverry 2000: *C. micans*; Etcheverry 2001: *C. stipularia*; Etcheverry *et al.* 2003: *C. micans*;) and one Asian (Jacobi *et al.* 2005: *C. retusa*) species. *Crotalaria* leaves are usually compound, mostly trifoliolate and more rarely unifoliolate (a compound leaf in which only one leaflet is formed)

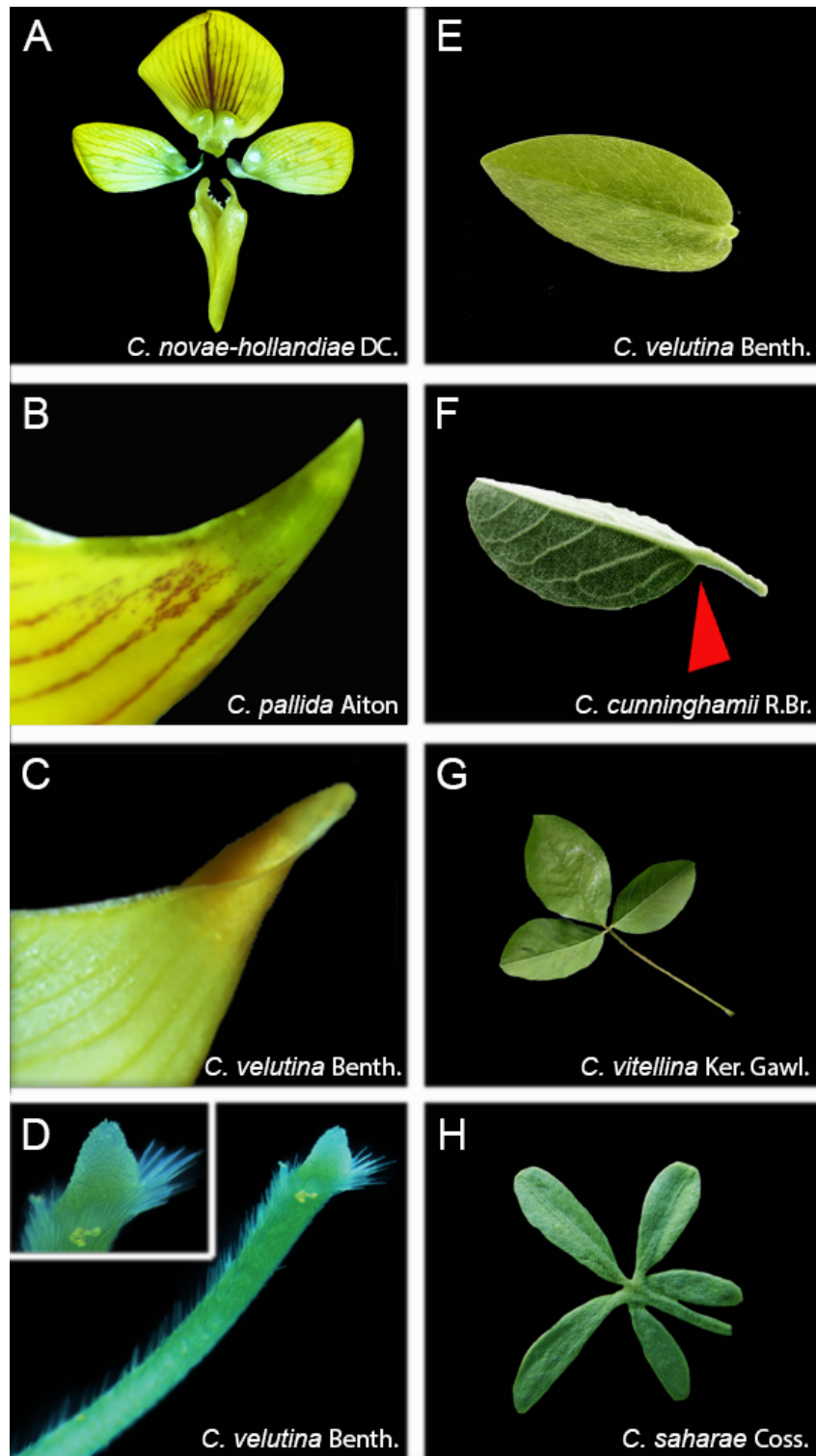


Figure 1. Flowers and leaf morphology. A) Corolla consisting of standard petal, two lateral wing petals, and the lower keel petal (here spirally twisted); B) Straight keel beak; C) Spirally twisted keel beak; D) Styler brush and papillate stigma; E) Simple leaf; F) Unifoliolate leaf, recognizable by a ridge articulation at the base of its single leaflet (red arrow); G) Trifoliolate leaf; H) Multifoliolate leaf.

or multifoliolate (Figures 1 E–H). Simple-leaved species predominantly occur in Asia, and in the Neotropics. No analyses have been carried out on the adaptive value of the leaf types, for example, in drier or more humid habitats.

Building on the work of Bentham (1843), Harvey (1862), Baker (1914), Verdoorn (1928), Wilczek (1953a, b), Hepper (1958), Milne-Redhead (1961), Torre (1962), and Schreiber (1970), Roger M. Polhill (born in 1937 and now retired but still active at the Royal Botanic Gardens Kew), the only living taxonomist to have worked on *Crotalaria* in its entirety (albeit with less focus on the Asian, Australasian, and Neotropical species), established the currently accepted sectional classification of the genus. In its first version (Polhill, 1968), Polhill accepted eleven sections and eight subsections in Africa and Madagascar; species from outside these two regions were briefly mentioned but not classified. Bisby (1970) applied numeric taxonomy to *Crotalaria*. He carried out multivariate analyses on 52 floral-morphological traits measured in herbarium material representing 273 species from Africa and Madagascar. Bisby and Polhill (1973) and Polhill (1982) used the results from these analyses to revise Polhill's (1968) sections, ending up with 8 sections. All post-1982 studies have had a regional focus, such as revisions in Australia (Holland 2002), India (Ansari 2006, 2008; Subramaniam *et al.* 2013, 2015), Brazil (Flores *et al.* 2006, 2016; Flores and Tozzi 2008), and Africa and Madagascar (Le Roux *et al.* 2013, this included 11 species from other regions). The Neotropical species are the least studied, with the exception of Brazil for which Flores (2004) assigned the species occurring in Brazil to four of Polhill's (1982) sections, and evaluated the taxonomic value of chromosome numbers (available for 23 of the 31 Brazilian species) and pyrrolizidine alkaloid profiles (available for 28 of the 31 Brazilian species) (Flores *et al.* 2006, 2009). The only phylogenetic studies including molecular data are by Le Roux *et al.* (2013: 24% of the total species sampled) and Subramaniam *et al.* (2013, 2015: 10% of the total species sampled), and both had a regional focus (Table 1). In these studies, *Crotalaria* is monophyletic, with six of its eight sections para- or polyphyletic. However, the lack of comprehensive taxon sampling has hampered the understanding of phylogenetic relationships within *Crotalaria*, and especially species from Asia outside India, Australasia and the Neotropics have been widely neglected in molecular phylogenies.

Chromosome numbers and karyotype descriptions for species of *Crotalaria* have been published mostly for African and Indian species, but are scarce for the Neotropics (e.g. Boulter *et al.* 1970; Windler 1974; Mangotra and Koul 1991; Palomino and Vázquez 1991; Oliveira and Aguiar-Perecin 1999; Tapia-Pastrana *et al.* 2005; Flores *et al.* 2006). Cytogenetic studies with C-banding and FISH using standard probes have been done in 12 species from four sections. Six of these species have a straight keel beak and belong to section *Chrysocalycinae* (Mondin and Aguiar-Perecin 2011: *C. incana* with $2n = 14$) and *Hedriocarpae* (Morales *et al.* 2011: *C. lanceolata*, *C. mucronata*, *C. ochroleuca*, *C. pallida*, *C. striata*; all with with $2n = 16$); the other six have spirally twisted keel beaks and belong to section *Calycinae* (*C. juncea* with $2n = 16$, *C. paulina* with $2n = 32$, *C. stipularia* with $2n = 32$) and section *Crotalaria* (*C. retusa*, *C. spectabilis*, *C. virgulata*; all with $2n = 16$) (Mondin *et al.* 2007; Mondin and Aguiar-Perecin 2011). It was found that the six species with spirally twisted keel beak had smaller chromosomes than the other six, but the work was done without a phylogenetic framework.

1.2 Study system 2, the Caricaceae – chromosomal change in modern plant systematics

Since Theodor Boveri developed the theory of chromosomal inheritance in 1909 (Baltzer 1964), biologists have been interested in chromosome numbers (Goldblatt and Lowry 2011; Rice *et al.* 2015). Methods for preparing chromosome spreads were established by Cyril D. Darlington in the 1930s, including the squashing and staining techniques still applied today. Since then, chromosome number data have become an important tool in plant systematic research (Stuessy *et al.* 2014). About 60,000 angiosperm species have had their chromosomes counted (Bennett 1998), which amounts to 19% of the estimated 352,000 species of angiosperms (The Plant List 2010, <http://www.theplantlist.org/>). Chromosome numbers in angiosperms range from $n = 2$ to $n = 320$, and can vary among closely related species or even between individuals of a population (Singh and Harvey 1975; Mandáková *et al.* 2013: *Cardamine*). In spite of occasional intra-population variation, chromosome numbers can be useful in the delimitation of species (Guerra 2008). In practice, however, the usefulness of chromosomes in species delimitation is limited by the need to have living material for staining and counting.

The most important role of chromosome number therefore is not their use in taxonomy/systematics, but instead in evolutionary biology because they allow us to infer evolutionary events, such as polyploidy (whole genome multiplication) and dysploidy (increase

or decrease of chromosome numbers involving single chromosomes). Although both phenomena play a role in karyotype evolution, polyploidization has been studied more than dysploidy, at least in plants. Dysploidy has been considered the main reason of changing chromosome numbers in animals (Imai *et al.* 2002), and it also occurs frequently in plants (Escudero *et al.* 2014). Dysploidy mostly happens through fission (gain) and fusion (loss) events: Ascending dysploidy means an increase of the chromosome number through fission, whereas decreasing dysploidy refers to a decrease in number (Guerra 2008; Sousa *et al.* 2014). These processes may be accompanied by loss of material, probably repetitive DNA, although this is still little documented. In the case of fission, a new centromere and two new telomeres have to be generated, whereas in the case of fusion of acrocentric or telocentric chromosomes one of the centromeres has to be inactivated (Schubert *et al.* 1992, 1995). Gradual dysploid reductions in chromosome numbers have been analyzed in most detail in *Arabidopsis* ($n = 8$ to $n = 5$: Yogeewaran *et al.* 2005; Lysak *et al.* 2006) and *Cucumis* ($n = 12$ to $n = 7$: Yang *et al.* 2014).

In my study of the role of chromosomes in speciation, I used traditional chromosome counts and fluorescent *in situ* hybridization (FISH) (Chapter 4). This staining technique utilizes fluorescent probes to detect the position and number of specific loci on a chromosome. It was introduced by Gall and Pardue (1969) and John *et al.* (1969), and initially involved the detection of radioactive DNA or RNA probes by autoradiography. Since then, speedier and safer methods have been developed that utilize haptens and fluorochromes as probes, and differently-colored fluorescent reagents also allow for simultaneous detection of multiple target sequences. The fluorescent signals are captured under a fluorescent microscope equipped with special cameras, and the pictures taken are then overlaid and analyzed with digital image software (Jiang and Gill 1994).

My study system for chromosomal evolution was the Caricaceae family, with 34 species of tropical trees and (straggling) shrubs in six genera and a crown group age of about 27 (22–33) Ma (Carvalho and Renner 2012). Caricaceae is thus about 10 Ma older than *Crotalaria*, which has 20 times more species. The family is most species-rich in the New World and has just two species on any other continent (Africa), while *Crotalaria* is most species-rich in Africa, and occurs on all continents except Antarctica. I chose to study the Caricaceae because the clade is small enough to focus on the role of chromosomal change in its evolution, something not possible in any large group because molecular-cytogenetic work is labor-intensive and requires repeated

access to living material. Such material was available in the Munich greenhouses because of the work of an earlier Ph.D. student, F.A. Carvalho, who had focused on the taxonomy, phylogenetics, and biogeography of Caricaceae (Carvalho and Renner 2012, 2013b).

Based on initial chromosome counts carried out at the start of my research, Caricaceae appear to have undergone a reduction in chromosome numbers during their evolution, and I wanted to understand this dysploidy. Most Caricaceae are dioecious, and *Carica papaya* is sometimes considered a model for studies on the evolution of sex chromosomes (Liu *et al.* 2004; Wu *et al.* 2010; Ming *et al.* 2011; Van Buren *et al.* 2015). X/Y chromosomes that appear to be homologous to the papaya X/Y chromosomes have been discovered in *Vasconcellea parviflora* (Iovene *et al.* 2015). Prior to my research, almost no cytogenetic work had been done on the family probably because Caricaceae are tropical trees (rarely stragglers). Based on comparison of whole-genome sequences of *Arabidopsis thaliana*, *Carica papaya*, *Populus trichocarpa*, and *Vitis vinifera*, Tang *et al.* (2008) proposed that the most recent common ancestor of Caricaceae underwent a polyploidization event, but within Caricaceae, no genome duplication events have been reported. Prior to my studies, fluorescent *in situ* hybridization (FISH) with 18S and 5S rDNA standard probes had been carried out on just three species, *Carica papaya*, *Vasconcellea goudotiana*, and *V. pubescens*, and BAC clones had been used to detect X/Y chromosomes in *Jacaratia spinosa* (Costa *et al.* 2008; Iovene *et al.* 2015).

1.3 Research questions in the two study systems

To advance our understanding of diversification of a mega-diverse group (especially as regards morphological change relative to niche shifts) and to discover clade relationships and ages in *Crotalaria*, I compiled a molecular phylogeny for nuclear and plastid loci, sampling as many species as possible, representing all geographic regions, sections (with their type species), and morphological variation (Chapter 2). For this purpose I combined available sequence data with new data generated by myself, mainly relying on DNA samples from herbarium specimens. Due to their underrepresentation in previous studies, I focused especially on Neotropical and Australasian species. I used molecular-clock dating and species distribution data to infer when and where the crown group of *Crotalaria* originated and how fast subgroups diversified in independent regions with similar or dissimilar conditions. In terms of trait evolution, I asked when and where morphological flower and leaf traits evolved, how conserved they are, and if

they are related to climate niches. I also tested different traits' taxonomic value by plotting them as discrete characters on DNA-based phylogenies and/or by carrying out ancestral state reconstructions. To assess the possible adaptive value of traits, I used both categorical data based on the assessment of species distribution in Köppen-Geiger climate groups and regression analyses with climate and environmental variables for species occurrence data, taking into account phylogenetic structure of the data. This allowed me to address whether flower and leaf traits are correlated with temperature, precipitation, elevation, or sun radiation (Chapter 2).

Apart from considering the evolution of *Crotalaria* on a worldwide scale, I wanted to advance the taxonomic treatment of Neotropical *Crotalaria* (c. 80), the least studied group. This part of my work involved collaboration with Andréia S. Flores from Universidade Federal de Roraima, Brazil. As part of a revision of 31 species of *Crotalaria* known from Brazil, we lectotypified 11 species names and synonymized another four (Chapter 3).

My research questions in the Caricaceae centered on the chromosome number reduction discovered during initial work. Wild plants of nine species representing all genera are cultivated in the greenhouses of the Munich Botanical Garden, which was the precondition for carrying out my research. I counted the diploid chromosome numbers of these nine species, including three species from genera that had never been the focus of any chromosomal work, namely the African *Cylicomorpha parviflora* (one of two species counted) and the Mexican *Horovitzia cnidoscoloides* (*Horovitzia* is monotypic), and *Jarilla caudata* and *J. heterophylla* (two of three species counted). The chromosome number of two of these genera, *Horovitzia* ($2n = 16$) and *Jarilla* ($2n = 14$), deviated from the other genera, and my aim was to find out what might have caused these reductions and whether they occurred in sequence. I used FISH to detect possible traces of past events of chromosomal rearrangements that have lead to lower chromosome numbers. For this purpose I used *Arabidopsis*-type telomeric probes. I also tested chromosome counts of five species in three of the other genera of Caricaceae and applied two other standard probes for 45S and 5S rDNA sites, which are highly conserved among eukaryotes. Lastly, I combined the chromosome count and FISH data with data on genome size to answer the question if chromosome number alterations are related to genome size variation, and I used a dated phylogeny to infer time and direction of changes.

Chapter 2

CLOCK-DATED PHYLOGENY FOR 48% OF THE 700 SPECIES OF *CROTALARIA* (FABACEAE–
PAPILIONOIDEAE) RESOLVES SECTIONS WORLDWIDE AND IMPLIES CONSERVED FLOWER AND LEAF
TRAITS THROUGHOUT ITS PANTROPICAL RANGE.

Rockinger, A., Flores, A.S., and Renner, S.S.

BMC Evolutionary Biology, 2016 [in review]

BMC Evolutionary Biology

Clock-dated phylogeny for 48% of the 700 species of *Crotalaria* (Fabaceae-Papilionoideae) resolves sections worldwide and implies conserved flower and leaf traits throughout its pantropical range

--Manuscript Draft--

Manuscript Number:	
Full Title:	Clock-dated phylogeny for 48% of the 700 species of <i>Crotalaria</i> (Fabaceae-Papilionoideae) resolves sections worldwide and implies conserved flower and leaf traits throughout its pantropical range
Article Type:	Research article
Section/Category:	Phylogenetics and Phylogeography
Funding Information:	
Abstract:	<p>Background: With some 700 species, <i>Crotalaria</i> is among the angiosperm's largest genera. We sampled 48% of these species, representing all sections and geographic regions, for nuclear and plastid DNA markers to infer possible drivers of <i>Crotalaria</i> diversification on a clock-dated phylogeny, focusing on climate niches, flower morphology, leaf types (compound vs. simple), and polyploidy, since chromosome counts are available for about 36% of the sequenced species.</p> <p>Results: <i>Crotalaria</i> is monophyletic and most closely related to African <i>Bolusia</i> (five species) from which it diverged 23 to 30 Ma ago. The analyses reveal that keel petal and sepal morphologies, which are taxonomically important, are conserved in large clades and uncorrelated to climate as statistically assessed with bioclimate data. Compound leaves are the ancestral condition, from which simple leaves evolved a few times, also not clearly correlated with climate. With the current sampling, most of the polyploid species are in one clade and have similar flowers and leaves.</p> <p>Conclusions: Of the 16 genera of <i>Crotalariaeae</i>, most of them centered in Africa, <i>Crotalaria</i> is by far the most species rich and widespread, yet its flower traits and leaf morphology are invariant in large species groups and change in chromosome numbers is limited (with current sampling). <i>Crotalaria</i> thus does not fit common suggestions about 'drivers' of angiosperm diversification, which tend to focus on mountain habitats, climate niches, and diversity of pollination modes.</p>
Corresponding Author:	Susanne Renner Ludwig-Maximilians-Universitat Munchen GERMANY
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Ludwig-Maximilians-Universitat Munchen
Corresponding Author's Secondary Institution:	
First Author:	Alexander Rockinger
First Author Secondary Information:	
Order of Authors:	Alexander Rockinger Andreia Silva Flores, Dr. Susanne Renner
Order of Authors Secondary Information:	

1 **Clock-dated phylogeny for 48% of the 700 species of *Crotalaria* (Fabaceae–**
2 **Papilionoideae) resolves sections worldwide and implies conserved flower and**
3 **leaf traits throughout its pantropical range**

4
5 Alexander Rockinger^{1*}, Andréia Silva Flores², and Susanne S. Renner¹

6 ¹*Systematic Botany and Mycology, University of Munich (LMU) Menzinger Str. 67,*
7 *80638 Munich, Germany*

8 ²*Instituto de Amparo a Ciência, Tecnologia e Inovação de Roraima, Herbário do*
9 *Museu Integrado de Roraima, Av. Brigadeiro Eduardo Gomes s.n., Parque Anauá,*
10 *69305-010 Boa Vista, RR, Brazil*

11 *Correspondence: a.rockinger@gmail.com

12
13 **Background:** With some 700 species, *Crotalaria* is among the angiosperm's largest
14 genera. We sampled 48% of these species, representing all sections and geographic
15 regions, for nuclear and plastid DNA markers to infer possible drivers of *Crotalaria*
16 diversification on a clock-dated phylogeny, focusing on climate niches, flower
17 morphology, leaf types (compound vs. simple), and polyploidy, since chromosome
18 counts are available for 36% of the sequenced species.

19 **Results:** *Crotalaria* is monophyletic and most closely related to African *Bolusia* (five
20 species) from which it diverged 23 to 30 Ma ago. The analyses reveal that keel petal
21 and sepal morphologies, which are taxonomically important, are conserved in large
22 clades and uncorrelated to climate as statistically assessed with bioclimate data.
23 Compound leaves are the ancestral condition, from which simple leaves evolved a
24 few times, also not clearly correlated with climate. With the current sampling, most of
25 the polyploid species are in one clade and have similar flowers and leaves.

26 **Conclusions:** Of the 16 genera of Crotalarieae, most of them centered in Africa,
27 *Crotalaria* is by far the most species rich and widespread, yet its flower traits and leaf
28 morphology are invariant in large species groups and change in chromosome numbers
29 is limited (with current sampling). *Crotalaria* thus does not fit common suggestions
30 about 'drivers' of angiosperm diversification, which tend to focus on mountain
31 habitats, climate niches, and diversity of pollination modes.

32 **Keywords:** Climate niches, GBIF data, molecular clock, trait evolution, flower
33 morphology, leaf architecture

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

34 **Background**

35 Among the World's largest angiosperm genera, *Crotalaria* with some 700 species
36 occupies place 35 ([http://en.wikipedia.org/wiki/List_of_the_largest](http://en.wikipedia.org/wiki/List_of_the_largest_genera_of_flowering_plants)
37 [genera_of_flowering_plants](http://en.wikipedia.org/wiki/List_of_the_largest_genera_of_flowering_plants)). Five of these genera have been studied with a species
38 sampling >20% (Goldenberg *et al.* 2008: *Miconia* [Melastomataceae], 22% of 1057
39 species; Jaramillo *et al.* 2008: *Piper* [Piperaceae], 31% of 1055 species; Pirie *et al.*
40 2011: *Erica* [Ericaceae], 45% of 860 species; Li *et al.* 2010: *Allium* [Amaryllidaceae],
41 41% of 815 species; Le Roux *et al.* 2013: *Crotalaria* [Fabaceae], 24% of 700
42 species). Studies that have used sparser sampling of mega-diverse genera, for
43 example, in *Pedicularis* (Ree 2005), *Ranunculus* (Hörandl *et al.* 2005), and *Begonia*
44 (Moonlight *et al.* 2015), have attributed these groups' high species numbers to
45 differentiation into pollinator niches (*Pedicularis*), habitat differentiation linked to
46 elevation niches (*Begonia*) or a broad range of reproductive strategies (autogamy,
47 apomixis, and vegetative growth in *Ranunculus*), and polyploidy and hybridization,
48 which are known to contribute to speciation and diversification in plants. Studying
49 mega-diverse clades (>500 species) is important for understanding plant evolution,
50 especially the role of supposed 'drivers' of diversity, such as adaptations to different
51 pollinators (May and Sargent, 2009 for a critical review) or biome and climate niche
52 shifts. Here we focus on *Crotalaria*, a pantropical clade of woody or herbaceous
53 species of low stature that mostly occur in open habitats at low to mid-altitudes and
54 that have conspicuous flowers and fruits so that they are relatively easily collected
55 and well represented in collections. The genus does not present particular taxonomic
56 problems due to hybridization or apomixes as does, e.g., *Ranunculus* (Emadzade and
57 Hörandl 2011), and it has benefitted from consistent taxonomic work by Roger Polhill
58 (1968, 1982) and phylogenetic studies focusing on its African and Indian species (Le
59 Roux *et al.* 2013; Subramaniam *et al.* 2013, 2015). Of the 700 species, about 500
60 occur in Africa and Madagascar, 80 in India, 20 in Australia, and 80 in the Americas
61 (Polhill 1982; Lewis *et al.* 2005; Flores and Tozzi 2008). About 15 species are
62 distributed pantropically due to their use as fiber crops, cattle fodder, and erosion
63 control plants (Polhill 1968).

64 *Crotalaria* species have typical papilionoid flowers, composed of standard,
65 wing, and keel petals (Figures 1, inset, 2). These flowers are adapted to bee
66 pollination and especially to Megachilidae, a worldwide clade of some 4000 species
67 (Danforth *et al.* 2013) whose pollen-carrying structures are restricted to the ventral

68 surface of the abdomen. This matches the ventral pollen presentation in *Crotalaria*.
69 The only way for a bee to reach the nectary at the base of the staminal tube is by
70 inserting its proboscis through a central channel at the base of the standard petal.
71 Access to the nectary from the sides is blocked by bulbous or plate-like appendages at
72 the inner base of the standard petal. Pollen transfer occurs while the nectar-drinking
73 bee holds on to the lateral wing petals with its tarsi, depressing the flower's keel with
74 its body weight, which causes the style to emerge from a staminal tube through the
75 keel beak. The upper part of the style bears stiff hairs that brush or scrape pollen
76 grains from the flower's own anthers and deposit them on the bee's abdomen (Lavin
77 and Delgado 1990; Etchevery 2001; Etchevery et al. 2003; Jacobi et al. 2005; Le
78 Roux and Van Wyk 2012). In a revision of *Crotalaria*, Polhill (1982; p. 4) mentioned
79 that the genus could be subdivided by its flower morphology. About half the species
80 have a slightly rostrate keel with a straight beak, bulbous appendages on the standard
81 petal blade and on the claw, and trichomes only on one side of the style (sections
82 *Chrysocalycinae*, *Grandiflorae*, *Hedriocarpae*; our Figure 1). The other half has
83 flowers with a highly rostrate keel with a spirally twisted beak, plate-like appendages
84 that do not extend to the claw, and hairs either along a single spiral line or along both
85 sides of the style (sections *Calycinae*, *Crotalaria*, *Dispermae*). Polhill suggested that
86 a spirally twisted keel beak might allow for a better proportioning of pollen, without
87 indicating whether he was thinking of fewer grains on more bees or grains distributed
88 over a longer duration of anthesis. Sections *Geniculatae* and *Schizostigma* were
89 thought to have an intermediate type of flower (Polhill 1982). The precise function of
90 the beak keel in pollen export during the 36–48 h of anthesis (personal observation of
91 flowers with both keel types) is unknown, but combined with other flower traits, such
92 as the calyx shape (Figure 1, inset), it is an important character for distinguishing
93 sections. Fitting with their bee pollination, *Crotalaria* flowers are yellow, sometimes
94 with red or brownish markings; only a handful of species have white, blue, or
95 greenish flowers. An example of a green-flowered species is *C. cunninghamii*, which
96 is endemic to Australia and is pollinated by honeyeaters (Meliphagidae) (Popic et al.
97 2016).

98 Leaves in *Crotalaria* are usually compound and mostly trifoliolate, more
99 rarely unifoliolate, multifoliolate, or simple. The adaptive value of compound leaves
100 is thought to lie in heat dissipation because there is greater convection than in a
101 simple leaf of equal size, fitting with the prevalence of compound leaves in dry

102 habitats (Vogel 2009). Compound leaves also allow individual leaflets to change their
103 angle of inclination and thereby maximize diffuse light capture at microsites, with the
104 degree of folding also varying diurnally (Shackel and Hall 1979; Vogel 2009). Given
105 the still poor understanding of the adaptive significance of simple vs. compound
106 leaves (Vogel 2009; Warman et al. 2011), we were interested in how their occurrence
107 in *Crotalaria* might correlate with temperature, precipitation, elevation, or sun
108 radiation (Table 1).

109 Chromosome counts have been published for 36% of the sequenced species of
110 *Crotalaria* (e.g. Boulter et al. 1970; Windler 1974; Mangotra and Koul 1991;
111 Palomino and Vázquez 1991; Oliveira and Aguiar-Perecin 1999; Tapia-Pastrana et al.
112 2005; Flores et al. 2006), and 12 species from four sections have been studied with
113 modern molecular-cytogenetic methods, including C-banding and FISH (Mondin et
114 al. 2007; Mondin and Aguiar-Perecin 2011; Morales et al. 2011). This density of
115 chromosomal data allows us to assess the possible role of polyploidy in the evolution
116 of the genus.

117 Here we use three plastid and two nuclear gene regions to reconstruct a
118 phylogeny of 338 (48%) of the 700 species, sampling representatives of all sections of
119 *Crotalaria* and of all 15 other genera of Crotalarieae. Our aim was to resolve the
120 relationships among species groups and then to use phylograms as well as clock-dated
121 versions of the phylogeny to infer the relative evolutionary lability of flower traits,
122 leaf morphology, climate niches, and chromosome numbers in this pantropical genus.

124 **Results**

125 **Closest relatives and age of *Crotalaria*, and leaf and flower evolution in the genus**

126 Along with 15 other genera (with a total of some 517 species of mainly African
127 distribution *Crotalaria* belongs to the Crotalarieae (Lewis et al. 2005; Boatwright et
128 al. 2008; Cardoso et al. 2012), a placement supported here with a sampling of
129 representatives from all Crotalarieae genera. *Crotalaria* is monophyletic and most
130 closely related to the African genus *Bolusia* (5 species), followed by the monospecific
131 likewise African *Euchlora* (Figure 1). Inferred ages for key divergence events under
132 different clock models are summarized in Table 2; their 95% posterior probability
133 intervals overlap, suggesting that the results are robust to choice of priors. The stem
134 age of *Crotalaria* falls between the late Oligocene and the early Miocene, with the
135 divergence from *Bolusia* occurring 23 (18–28) to 30 (21–51) Ma ago (Table 2). The

136 deepest divergences between surviving *Crotalaria* lineages date to between 18 (14–
137 22) and 29 (18–42) Ma ago. The ages of other main clades are shown in Table 2.
138 *Bolusia* and *Euchlora*, as well as most other Crotalarieae have a strictly African
139 distribution, suggesting that *Crotalaria* originated in Africa. From there, Madagascar
140 was reached at least 10 times (Figure 3) while Australia was reached at least five
141 times, both from SE Asia and apparently also from Africa, although denser species
142 sampling would be required to confidently infer closest African/Australian relatives.

143 Within *Crotalaria*, large species groups have almost homogeneous flower
144 morphologies (Figure 2). Thus, bilabiate calyces are almost restricted to Asia,
145 Australasia, and the Neotropics and are rare in Africa (compare Figure 1 to 3,
146 ‘bilabiate calyx clade’), with a few reversals to equally lobed calyces, such as
147 predominate in Africa. Of the 338 sampled species, 177 (52%) have a spirally twisted
148 keel beak and belong to our ‘bilabiate calyx’ and ‘bulbous standard appendages’
149 clades (Figure 1). There is also a species group with truncate calyx tubes that largely
150 corresponds to Polhill’s (1982) section *Hedriocarpae* (most *Crotalaria* have a
151 campanulate calyx). Our ‘core *Chrysocalycinae*’ clade comprises most species of
152 Polhill’s section *Chrysocalycinae* and the *Grandiflorae* (16 species) and *Stipulosae*
153 (14 species). The sister relationship between *C. linearifoliolata* from Somalia and *C.*
154 *persica* from the Horn of Africa to the Arab Peninsula will require renewed
155 assessment of their morphologies; Pohlhill (1982) had placed them in his sections
156 *Schizostigma* and *Hedriocarpae*. Calyces shorter than keel petals are conserved in
157 species groups within the ‘truncate calyx tube’ and the ‘bulbous standard appendages’
158 clades (Figure 2).

159 Leaf architecture in *Crotalaria* is highly conserved, as shown by the ancestral
160 state reconstruction (Figure 3) and the permutation test (estimated $D = -1,26$). All five
161 species of the closest outgroup *Bolusia* have compound (trifoliolate) leaves, while the
162 single species of *Euchlora*, the next closest relative, has simple leaves. Most simple-
163 leaved *Crotalaria* species belong to the ‘bilabiate calyx’ and the ‘core
164 *Chrysocalycinae*’ clades and thus are found outside Africa (compare Figures 3 and 4).
165 Trifoliolate leaves predominate in African and Madagascan species (173 of 205
166 sequenced species of *Crotalaria* that occur in Africa and Madagascar have this leaf
167 type), while 56 of 69 sequenced Asian species have simple leaves.

168
169

170 **Leaf types and climate**

171 Of the 279 species that could be assigned to a climate category, 23 occur in the humid
172 tropics, 125 in the dry tropics, 62 in the arid tropics, and 66 in mild temperate
173 climates (Figure 5A). We have sampled an estimated 60% of the simple leaved and
174 46% of the compound leaved species of *Crotalaria*. The occurrence of simple- and
175 compound-leaved species differs significantly between the humid tropics and the
176 other three climate types (all $p < 0.0002$, df 1) and slightly between arid and mild
177 temperate regions ($p < 0.03$, df 1; Figure 5B, Table 3). Phylogenetically uncorrected
178 linear regression analyses with the *glm* and *logistf* models imply that simple-leaved
179 species have a higher probability of occurring in climates with high precipitation
180 (Figure 5C). Under the phylogenetically corrected *binaryPGLMM* model, these
181 correlations are no longer significant due to the few evolutionary transitions in leaf
182 type and hence low statistical power.

183

184 **Polyploidy in *Crotalaria***

185 We gathered chromosome numbers from the literature for 122 of the 338 sampled
186 *Crotalaria* species and plotted them on the phylogeny (Figure S2). The most common
187 number in *Crotalaria* is $2n = 16$, with 92 species of the 122 species counted having
188 this number. With the current sampling, most polyploids are tetraploid (assuming a
189 base number of 8), with $2n = 32$, and occur in the Neotropics, where they belong to
190 our ‘bilabiate calyx clade.’ Exceptions are *C. tweediana*, a species with $2n = 54$ and
191 endemic to Brazil, and *C. ferruginea* with $2n = 48$ from Southeast Asia and
192 Australasia, and *C. massaiensis* with $2n = 32$ from Kenya.

193

194 **Discussion**

195 **Phylogenetic relationships and major groups in *Crotalaria***

196 *Crotalaria* as traditionally circumscribed (Polhill 1968, 1982) is monophyletic.
197 Morphologically, the five species in the tropical African genus *Bolusia* differ from the
198 700 species of *Crotalaria* by having a helically (not spirally) coiled keel and a single,
199 rather than paired, appendages blocking the nectary access while the single species of
200 *Euchlora*, endemic to the Northern and Western Cape, lacks any appendages blocking
201 nectary access. *Bolusia* has trifoliolate leaves, while *Euchlora hirsuta* has simple
202 leaves. We have sampled between 25 and 79% of the species in Polhill’s (1982) eight
203 sections (four of them with >50% sampled, see Figure 1), and with this sampling all

204 sections are polyphyletic. Le Roux et al.'s (2013) merging of Polhill's sections
205 *Crotalaria* and *Dispermae* (our 'bulbous standard appendages clade') is supported by
206 our results. On the other hand, eight of their eleven sections are rendered polyphyletic
207 by our increased species sampling (only their species-poor sections *Amphitrichae*
208 (four species), *Grandiflorae* (14 species), and *Stipulosae* (13 species) are
209 monophyletic). Additional file 1: Figure S1 shows the sections of Polhill and Le Roux
210 et al. plotted on our tree. A future sectional classification should probably not be
211 undertaken until at least 80% of the species are sampled (currently 48%).

212

213 **Evolution of flower and leaf traits and their distribution in different climate** 214 **types**

215 Flower traits turned out to be uncorrelated to climate, although most of the species
216 occurring in the humid tropics have bilabiate calyces while species occurring in the
217 dry tropics mostly have equally lobed calyces (Figure 1, inset, Figure 2). That flower
218 traits are uncorrelated to climate may reflect that the main pollinators of crotalarias,
219 species in the long-tongued bee family Megachilidae, which includes over 4,000
220 species, are abundant in ecosystems from arid habitats to tropical forests (Danforth et
221 al. 2013). Other important pollinators are the pantropical carpenter bees (*Xylocopa*;
222 Etcheverry et al. 2003; Jacobi et al. 2005). All *Crotalaria* flowers (even the honey-
223 eater pollinated Australian *C. cunninghamii*; Popic et al. 2016) have the same
224 pollination mechanism in which the narrow keel beak serves as a cylinder and the
225 style acts together with the anthers as a piston. No study has compared the effect of a
226 twisted or keel beak on pollen release, and Pohlhill's (1982) suggestion that a twisted
227 keel beak might better proportion pollen release does not match the finding that
228 flowers with and without such keels have the same short life span and are visited by
229 some of the same bee species (*C. micans* and *C. stipularia* flowers function for c. four
230 days, Etcheverry 2001; Etcheverry et al. 2003; Jacobi et al. 2005: *C. retusa* flowers
231 for one day; own observations show that flowers last for 3-4 days in *C. pallida*, and
232 for 4-5 days in *C. cunninghamii*, *C. novae-hollandiae*, and *C. velutina*). It would be
233 worth testing whether species with spirally twisted keel beaks and those with straight
234 beaks produce different amounts of pollen; if the species with twisted beaks produced
235 fewer pollen grains, it should be selectively advantageous for them to release fewer
236 grains per bee visit.

1 237 The range map resulting from occurrences of the 183 of the 700 species of
2 238 *Crotalaria* that are represented in GBIF (Figure 4, inset) illustrates the minimal range
3 239 of the genus but also the highly uneven uploading of data to GBIF, with India
4 240 especially underrepresented. Nevertheless, the GBIF data show that the genus occurs
5 241 not only in the humid tropics (minimum temperature ≥ 18 °C; annual precipitation ≥ 25
6 242 mm (monsoon climate) / ≥ 60 mm (rain forests)), dry tropics (minimum temperature
7 243 ≥ 18 °C; annual precipitation < 60 mm), and arid tropics (minimum temperature < 18
8 244 °C (cold desert and savannah) / ≥ 18 °C (hot desert and savannah), but also in mild
9 245 temperate regions (minimum temperature range 10-22 °C; annual precipitation > 40
10 246 mm). Fitting with a likely origin in dry regions of Africa, the ancestral leaf type in
11 247 *Crotalaria* is the compound leaf, the adaptive advantage of which is thought to lie in
12 248 heat dissipation which is greater in small, thin, mobile leaflets than in simple leaves of
13 249 comparable size (Vogel 2009). In addition, the mobility of leaflets in compound
14 250 leaves may avoid overheating and maximize diffuse light capture (raus: Muraoka et
15 251 al. 1998). The possible adaptive value of simple leaves, by contrast, is poorly
16 252 understood (Warman et al. 2011). We found only four evolutionary transitions from
17 253 compound to simple leaves (Figure 3) and few reversals, with a possible
18 254 overrepresentation of simple leaves in more humid habitats (Figure 5A, B), although
19 255 this was not upheld in the phylogenetically informed analysis. Because of their small
20 256 stature and ease of cultivation, *Crotalaria* species would be suitable for much-needed
21 257 experimental work on the adaptive benefits of leaf architecture (Vogel 2009; Warman
22 258 et al. 2011).

23 259

24 260 **Polyploidy in *Crotalaria***

25 261 Tetraploidy in *Crotalaria* appears to have arisen in the common ancestor of a New
26 262 World clade (crown node marked with an arrow in Additional file 2: Figure S2). Of
27 263 the 46 Neotropical species in our phylogeny, 27 form a clade that is embedded within
28 264 a clade of 73 mostly Asian species (the ‘bilabiate calyx’ clade). Of these 27, 18 have
29 265 had their chromosome numbers counted, and all are polyploid (17 of them have $2n =$
30 266 32; one has $2n = 54$). Windler (1973) suggested that polyploidy might be related to
31 267 self-compatibility, but a high rate of seed set after selfing has been documented in all
32 268 three species of *Crotalaria* studied in this regard: *C. micans*, *C. retusa*, and *C.*
33 269 *stipularia*) (Etcheverry 2001; Etcheverry et al. 2003; Jacobi et al. 2005). However,
34 270 only *C. stipularia* is native to South America and tetraploid, while *C. micans* and *C.*

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

271 *retusa*, both are pantropical due to their use as fibre crops, green manure, and cattle
272 fodder (Polhill 1982), are diploid ($2n = 16$). Two (counted) Asian species close to the
273 Neotropical tetraploid clade, *C. ferruginea* and *C. humifusa*, also are tetraploids as is
274 *C. massaiensis* from Kenya.

275

276 **Conclusion**

277 Our aim was to resolve the main species groups of the mega-diverse pantropical
278 genus *Crotalaria* in order to infer the evolutionary frequency of change in its flower
279 traits, leaf types, and climate niches, and to obtain a first view of the possible role of
280 polyploidy in its evolution. The few transitions from the ancestral compound leaf
281 architecture to simple leaves may be associated with occurrence in climates with
282 higher rainfall, although this was no longer statistically supported in a
283 phylogenetically informed analysis. Flower morphology also is highly conserved
284 throughout the genus. In combination, these findings argue against pollinator niches
285 or climate niches as particularly important in the diversification of *Crotalaria*, while
286 the possible role of chromosomal change (at least in some clades) deserves further
287 study. *Crotalaria* therefore does not fit common suggestions about ‘drivers’ of
288 angiosperm diversification, which tend to focus on mountain habitats, climate niches,
289 and diversity of pollination modes.

290

291 **Methods**

292 **Taxon sampling, plant material, DNA extractions, PCR amplifications,** 293 **sequencing and alignment**

294 Plant material was obtained from specimens deposited in herbaria in Munich (M and
295 MSB), the Museu Integrado de Roraima (MIRR), the Instituto de Botânica (SP), the
296 Universidade de São Paulo (SPF), the Missouri Botanical Garden (MO), the Royal
297 Botanic Gardens in Kew (K), and the Botanical Garden and Botanical Museum in
298 Berlin (B). A few samples were obtained during a field trip in May 2015 to São Paulo
299 and Bahia, Brazil, and two from plants cultivated in the greenhouses of the Munich
300 Botanical Garden. Additional file 3: Table S1 lists all sampled taxa with their voucher
301 information, geographic origin and GenBank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>)
302 accession numbers. Total genomic DNA was extracted from 5–25 mg of leaf tissue,
303 using plant DNA extraction kits (NucleoSpin, Macherey-Nagel, Düren, Germany)
304 according to the manufacturer’s protocol. Polymerase chain reactions (PCR) followed

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

305 standard protocols, using Taq DNA polymerase and 10 primers (Additional file 4:
306 Table S2). PCR products were purified with the ExoSap clean-up kit (Fermentas, St.
307 Leon-Rot, Germany), and sequencing relied on Big Dye Terminator kits (Applied
308 Biosystems, Foster City, CA, USA) and an ABI 3130 automated sequencer. In all, 26
309 chloroplast sequences (*rbcL* gene, *psbA-trnH* intergenic spacer) and 245 nuclear
310 sequences (ribosomal DNA internal transcribed spacers ITS1 and ITS 2, plus the
311 intervening 5.8 S gene, and external transcribed spacer ETS) were newly generated
312 for this study. New sequences were BLAST-searched in GenBank and then aligned
313 with MAFFT v7 (Kato and Standley 2013) using default parameters. To take into
314 account secondary structure, the Q-INS-i multiple alignment strategy was chosen for
315 the ITS and ETS sequences. Minor alignment errors were manually adjusted in
316 Geneious v8.1.8 (Kearse et al. 2012). We first generated separate alignments for the
317 ITS region (381 species, 851 aligned positions), the ETS region (108 species, 606
318 aligned positions), the *matK* gene (135 species, 754 aligned positions), the *rbcL* gene
319 (196 species, 552 aligned positions), and the *psbA-trnH* intergenic spacer (124
320 species, 408 aligned positions). In the absence of statistical conflict (>70% maximum
321 likelihood bootstrap support) among topologies from these matrices, the datasets were
322 concatenated resulting in a matrix of 3171 aligned positions, representing 338 species
323 of *Crotalaria*, 33 species of the other 15 genera within Crotalarieae, and 23 further
324 species belonging to the core genistoid clade (Lavin et al. 2005; Cardoso et al. 2012;
325 our Additional file 3: Table S1). We sampled the eight sections of Polhill (1982) and
326 the 11 of Le Roux et al. (2013) with their type species, except for *Crotalaria clavata*
327 Wight & Arn., the type of section *Hedriocarpae* Wight & Arn., and *Crotalaria*
328 *leptocarpa* Balf.f., the type of section *Schizostigma* Polhill.

330 **Phylogenetic and trait analyses**

331 Phylogenetic trees were estimated using Maximum Likelihood (ML) optimization in
332 RAxML v8.0 (Stamatakis 2014) under the GTR+ Γ substitution model with four rate
333 categories. Statistical support came from bootstrapping under the same model, with
334 1000 replicates.

335 All sequenced species were scored for the following flower trait states: beak
336 of the flower keel (barely twisted = 0, spirally twisted = 1), calyx lobing (equally
337 lobed = 0, bilabiate = 1), length of the calyx compared to length of the keel (shorter
338 than keel = 0, as long as/longer than keel = 1), and diploid chromosome numbers ($2n$

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

339 = 14 = 0; 2n = 16 = 1; 2n = 18 = 2; 2n = 32 = 3; 2n = 42/48 = 4; 2n = 54 = 5). These
340 traits were plotted on a phylogram. Species were also coded for leaf trait states:
341 simple = 0, unifoliolate = 1, trifoliolate = 2, multifoliolate = 3. For leaf type, we
342 carried out ancestral state reconstruction on an ultrametric tree and compared an equal
343 rates model wherein all transition rates among trait states are equal with a
344 symmetrical rate model, which implements equal rates of backward and forward
345 character state transition while the distinct state combinations can have distinct rates,
346 and an all-rates-different model. All trait state codings are shown in Additional file 5:
347 Table S3

348 349 **Molecular clock dating**

350 For molecular clock dating we relied on Bayesian optimization in BEAST v1.8.2
351 (Drummond et al. 2012) and a reduced alignment in which we removed 162
352 *Crotalaria* species with very short (<0.0001) or identical branch lengths, while
353 maintaining the 34 outgroups. We added 23 species of other core genistoids to this
354 dataset to allow for fossil calibration. This resulted in a matrix of 240 species and
355 3,171 aligned positions, of which 183 species are crotalarias (representing all major
356 clades) and the rest are representatives of the core genistoids. To convert genetic
357 branch lengths into absolute times we applied three calibration approaches: (1) A
358 strict clock calibrated with the oldest known legume fossil, a seedpod from western
359 Wyoming that is most similar to the Sophoreae genera *Bowdichia* and *Diploptropis*,
360 dated to at least the Late Paleocene (56 Mya) (Herendeen and Wing 2001; P. S.
361 Herendeen, Chicago Botanical Garden, pers. communication 17 Feb. 2016). We
362 assigned this fossil to the crown node of the *Bowdichia-Diploptropis-Leptolobium*-
363 clade, which we used as the outgroup, with a gamma distribution of shape 1.4, scale
364 2.0, and offset 54, reflecting the minimum age of 56 Mya, and using a normally
365 distributed prior for the clock rate (this allowed 95% of the root node ages to fall
366 between 54.17 and 62.97). (2) Next we used an uncorrelated lognormal (UCLN)
367 relaxed clock model with the same fossil calibration, using a diffuse gamma
368 distribution of shape 0.001, scale 1000.0, offset 0.0 for the ucln.mean rate. (3) Lastly
369 we applied a strict clock with three unlinked partitions for the ITS, ETS, and plastid
370 markers, using a genome substitution rate of 0.00346 subst./site/my for the ITS region
371 following Kay et al. (2006: *Lupinus*) and a plastid genome rate of 0.00056
372 subst./site/my by Palmer (1991) for the combined plastid loci *matK*, *rbcL*, and *psbA*-

1 373 *trnH*. The substitution rate for the ETS partition was estimated, using a diffuse
2 374 gamma distribution as in approach 2. In each BEAST run, we used a pure-birth (Yule)
3 375 tree prior, the GTR+ Γ substitution model, and Monte Carlo Markov chains (MCMC)
4 376 of 100 million generations, with parameters sampled every 10,000th generation.
5 377 Tracer v1.6 (part of the BEAST package; Rambaut et al. 2014) was used to assess
6 378 effective sample sizes (ESS) for all estimated parameters. We used TreeAnnotator
7 379 v1.8.2 (part of the BEAST package) to discard 10% of the saved trees as burn-in and
8 380 to combine trees. Maximum clade credibility trees with mean node heights were
9 381 visualized using FigTree v1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>) and R (R
10 382 Chore Team 2015). We report highest posterior densities intervals, the interval
11 383 containing 95% of the sampled values.
12
13
14
15
16
17
18
19
20
21

384

385 **Geography, traits, climate, and chromosome numbers**

22 386 Species were area-coded according to their natural distribution range, based on
23 387 information from regional floras and taxonomic revisions (Hooker 1879; Polhill 1968;
24 388 Lee 1978; Polhill 1982; Holland 2002; Ansari 2006, 2008; Flores and Tozzi 2008;
25 389 Flores et al. 2016), and the International Legume Database and Information System
26 390 (ILDIS) (Roskov et al. 2005). We then assigned species to five major distribution
27 391 areas: Americas (North, Central, and South America, and the Caribbean); Africa and
28 392 the Middle East; Madagascar including Mauritius, Réunion, and the Seychelles; Asia
29 393 (from the east of Arabian Peninsula to Southeast Asia; and Australasia (comprising
30 394 Australia, Papua New Guinea, and Melanesia).

31 395 To assess the distribution of leaf types in different climate zones, we used a
32 396 categorical approach for which we assigned species to climate zones and also linear
33 397 regression analyses with continuous bioclimate data for georeferenced species records
34 398 coming from the Global Biodiversity Information Facility (GBIF;
35 399 <http://www.gbif.org>). For the categorical approach, each species was assigned to one
36 400 of 14 climate types in the Köppen-Geiger system (Kottek et al. 2006; Peel et al. 2007;
37 401 Wilkerson and Wilkerson 2010; our Table 1), which uses a three-letter code to
38 402 categorize a ‘main climate’ (first letter), annual precipitation distribution (second
39 403 letter), and seasonal temperature (third letter). Species were assigned to the climate
40 404 type found in >70% of their range; 48 species without a determinable main climate
41 405 category were coded as NA and excluded from further analysis, resulting in 279
42 406 species assigned to a climate category. In a second step, we grouped the 14 climate
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

407 types into just four types: humid tropics (Af, Am; Table 3 for definitions), dry tropics
408 (As, Aw), arid (BWk, BWh, BSk, BSh) and mild temperate (Cfa, Cfb, Csa, Csb, Cwa,
409 Cwb). To test if the occurrence of simple and compound leaves differs between the
410 four climate types, we used Pearson's chi-squared test for count data as part of the R
411 package 'stats' (R Chore Team 2015) and posthoc pairwise comparisons with the
412 *chisq.post.hoc* function as implied in the R package 'fifer' (Fife 2014).
413 For the linear regression analyses with continuous data, we queried *Crotalaria* species
414 names in GBIF using the *gbif* function of the R-package 'dismo' (Hijmans et al. 2011)
415 and then filtered the data by removing fossil and literature records, coordinate
416 duplicates within a species, records with a resolution >10 km, and species with fewer
417 than 10 georeferenced records. This resulted in a dataset of 2048 records for 183
418 species, listed in Additional file 5: Table S3 with their trait states. Values for climate
419 parameters were standardized to allow for comparative analyses and were log
420 transformed, if not normally distributed. To identify multicollinearity of predictor
421 variables, we determined variance inflation factors by applying the *vif* function of the
422 R package 'HH' (Heiberger 2016). To determine which climate variable is
423 explanatory for the distribution of leaf types, we applied generalized linear models
424 with the *glm* function of R 'stats' and compared results to logistic regression models
425 applying Firth's correction to the likelihood by using the *logistf* function of the R
426 package 'logistf' (Heinze et al. 2013). We also considered phylogenetic structure in
427 our data by using a permutation test as implemented in the *phylo.d* function of the R
428 package 'caper' (Orme et al. 2013), and the *binaryPGLMM* function of the 'ape'
429 package (Paradis et al. 2004), which performs a linear regression for binary
430 phylogenetic data and simultaneously estimates the strength of phylogenetic signal.
431 For these analyses, it was necessary to simplify the leaf trait coding described under
432 2.2 to simple = 0 or compound = 1, with the latter trait state including trifoliolate,
433 unifoliolate, and multifoliolate. Available chromosome numbers were compiled from
434 the literature and plotted on the phylogenetic tree.

435 436 **Availability of supporting data**

437 All the supporting data are included as additional files under: <http://www.xxxx>
438
439
440

441 **Additional files**

442 **Additional file 1: Figure S1.** The 8 sections of Polhill (1982) and the 11 sections of
443 Le Roux et al. (2013) plotted on a maximum likelihood tree for 372 accessions
444 representing 338 *Crotalaria* species and 33 species of the remaining 15 genera of
445 Crotalariaeae based on 3171 aligned nucleotides of nuclear and plastid loci.

447 **Additional file 2: Figure S2.** Same maximum likelihood tree as in Figure 1 with
448 chromosome numbers for 122 species plotted on the tips and shown as bars to the
449 right. Red arrow marks the crown node of the polyploid Neotropical clade; black
450 arrows mark species in which the stages of anthesis have been studied.

451
452 **Additional file 3: Table S1.** Species used in this study with herbarium vouchers,
453 place of deposition (in a few cases also their barcodes), geographic origin, distribution
454 ranges, and GenBank accession numbers for all sequences. Type species of Polhill's
455 (1982) and Le Roux et al.'s (2013) sections are listed with the respective sectional
456 names and are marked with an asterisk. Newly sequenced species in bold. Native
457 distribution areas are marked by an (N), those where a species has been introduced by
458 an (I), and those where the status is uncertain by an (U).

459
460 **Additional file 4: Table S2.** Primer sequences used in this study (listed 5'- to 3'-end)
461 and applied protocols.

462
463 **Additional file 5: Table S3.** Species list with coding of sections; distribution areas;
464 leaf and flower trait states; species' climate categories; and number of GBIF records.
465 Polhill's (1982) sections (1 = *Grandiflorae*; 2 = *Chrysocalycinae*; 3 = *Hedriocarpace*;
466 4 = *Geniculatae*; 5 = *Schizostigma*; 6 = *Calycinae*; 7 = *Crotalaria*; 8 = *Dispermae*);
467 Le Roux et al. (2013) sections (1 = *Hedriocarpace*; 2 = *Incanae*; 3 = *Schizostigma*; 4 =
468 *Calycinae*; 5 = *Borealigeniculatae*; 6 = *Crotalaria*; 7 = *Stipulosae*; 8 = *Glaucae*; 9 =
469 *Geniculatae*; 10 = *Amphitrichae*; 11 = *Grandiflorae*); distribution areas ("Region";
470 "Region 2"; "Region 3"; "Region 4") (0 = Americas (North, Central, and South
471 America, and the Caribbean); Africa and the Middle East; Madagascar including
472 Mauritius, Réunion, and the Seychelles; Asia (from the east of Arabian Peninsula to
473 Southeast Asia; Australasia (comprising Australia, Papua New Guinea, and
474 Melanesia)); leaf type (0 = simple; 1 = unifoliolate; 2 = trifoliolate; 4 =
475 multifoliolate); leaf type (binary) (0 = simple; 2 = compound); beak of the flower keel
476 ("Keel") (barely twisted = 0; spirally twisted = 1); calyx lobing (equally lobed = 0;
477 bilabiate = 1); length of the calyx compared to length of the keel ("Calyx length")
478 (shorter than keel = 0; as long as/longer than keel = 1); Köppen-Geiger categories (0
479 = Af; 1 = Am; 2 = As; 3 = Aw; 4 = BWk; 5 = BWh; 6 = BSk; 7 = BSh; 8 = Cfa; 9 =
480 Cfb; 10 = Csa; 11 = Csb; 12 = Cwa; 13 = Cwb); Köppen-Geiger major climate group
481 (0 = humid tropics [Af, Am]; 1 = dry tropics [As, Aw]; 2 = arid [BWk, BWh, BSk,
482 BSh]; 3 = mild temperate [Cfa, Cfb, Csa, Csb, Cwa, Cwb]); diploid chromosome
483 numbers ("2n"): 0 = 14; 1 = 16; 2 = 18; 3 = 32; 4 = 42/48; 5 = 54 (reference list for
484 chromosome numbers below table). NA = not available.

485
486 **Competing interests**

487 The authors declare that they have no competing interests.

488

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

489 **Authors' contributions**

490 AR generated the data and carried out all analyses; AR and SSR wrote the paper; ASF
491 contributed material, and helped gathering chromosome and morphological trait data;
492 SSR devised the project and provided financial support.

493
494 **Acknowledgements**

495 We thank M. Silber for assistance in the lab, C. M. Zohner for advice on climate
496 analysis, and S. G. Aninta for help with gathering trait and distribution data. We also
497 thank the curators of the herbaria listed in Additional file 3: Table S1 for granting
498 access to their collections.

499
500 **References**

- 501 Ansari, A.A. 2006. Taxonomic studies on genus *Crotalaria* L. in India - II: infra-
502 generic classification. *J. Econ. Tax. Bot.* 30:570-582.
- 503 Ansari, A.A. 2008. *Crotalaria* L. in India. Bishen Singh Mahendra Pal Singh, Dehra
504 Dun.
- 505 Baldwin, B.G., Markos, S. 1998. Phylogenetic utility of the External Transcribed
506 Spacer (ETS) of 18S–26S rDNA: congruence of ETS and ITS trees of
507 *Calycadenia* (Compositae). *Mol. Phylogenet. Evol.* 10:449-463.
- 508 Boulter, D., Derbyshire, E., Frahm-Leliveld, J.A., Polhill, R.M. 1970. Observations
509 on the cytology and seed proteins of various African species of *Crotalaria* L.
510 (Leguminosae). *New Phytol.* 69:117-131.
- 511 Boatwright, J.S., Le Roux, M.M., Wink, M., Morozova, T., Van Wyk, B.E. 2008.
512 Phylogenetic relationships of tribe Crotalarieae (Fabaceae) inferred from DNA
513 sequences and morphology. *Syst. Bot.* 33:752-761.
- 514 Cardoso, D., de Queiroz, L.P., Pennington, R.T., de Lima, H.C., Fonty, E.,
515 Wojciechowski, M.F., Lavin, M. 2012. Revisiting the phylogeny of
516 papilionoid legumes: new insights from comprehensively sampled early-
517 branching lineages. *Am. J. Bot.* 99:1991-2013.
- 518 Cohen, K.M., Finney, S.C., Gibbard, P.L., Fan, J.X. 2013. The ICS international
519 chronostratigraphic chart. *Episodes* 36:199-204.
- 520 Cubas, P., Pardo, C., Tahiri, H., Castroviejo, S. 2010. Phylogeny and evolutionary
521 diversification of *Adenocarpus* DC.(Leguminosae). *Taxon* 59:720-732.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

522 Danforth, B.N., Cardinal, S., Praz, C.J., Almeida, E.A.B., Michez, D. 2013. The
523 impact of molecular data on our understanding of bee phylogeny and
524 evolution. *Annu. Rev. Entomol.* 58:57-78.

525 Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A. 2012. Bayesian phylogenetics
526 with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.*, 29: 1969-1973.

527 Etcheverry, A.V. 2001. Role of staminal growth in delayed self-pollination of
528 *Crotalaria stipularia* (Fabaceae: Papilionoideae). *Acta Hort.* 561:339-342.

529 Etcheverry, A.V., Westerkamp, C., Protomastro, J.J. 2003. Delayed autonomous self-
530 pollination in the colonizer *Crotalaria micans* (Fabaceae: Papilionoideae):
531 structural and functional aspects. *Plant Syst. Evol.* 239:15-28.

532 Fife, D. 2014. fifer: A collection of miscellaneous functions. R package version 1.0.
533 <https://CRAN.R-project.org/package=fifer>

534 Flores, A.S., Corrêa, A.M., Forni-Martins, E.R., Tozzi, A.M.G.D.A. 2006.
535 Chromosome numbers in Brazilian species of *Crotalaria* (Leguminosae,
536 Papilionoideae) and their taxonomic significance. *Bot. J. Linn. Soc.* 151:271-
537 277.

538 Flores, A.S., Tozzi, A.M.G.D.A. 2008. Phytogeographical patterns of *Crotalaria*
539 species (Leguminosae-Papilionoideae) in Brazil. *Rodriguésia* 59:477-486.

540 Flores, A.S., Rockinger, A., Rodrigues, R.S., Tozzi, A.M.G.D.A. 2016.
541 Lectotypifications and taxonomic changes in Brazilian *Crotalaria* L.
542 (Leguminosae). *Phytotaxa* 267:296-300.

543 Goldenberg, R., Penneys, D.S., Almeda, F., Judd, W.S., Michelangeli, F.A. 2008.
544 Phylogeny of *Miconia* (Melastomataceae): initial insights into broad patterns
545 of diversification in a megadiverse neotropical genus. *Int. J. Plant. Sci.*
546 169:963-979.

547 Heiberger, R.M. 2016. HH Statistical Analysis and Data Display: Heiberger and
548 Holland. R package version 3.1-32. URL [http://CRAN.R-](http://CRAN.R-project.org/package=HH)
549 [project.org/package=HH](http://CRAN.R-project.org/package=HH)

550 Heinze, G., Ploner, M., Dunkler, D., Southworth, H. 2013. logistf: Firth's bias reduced
551 logistic regression. R package version 1.21. [https://CRAN.R-](https://CRAN.R-project.org/package=logistf)
552 [project.org/package=logistf](https://CRAN.R-project.org/package=logistf)

553 Herendeen, P.S., Wing, S. 2001. Papilionoid legume fruits and leaves from the
554 Paleocene of northwestern Wyoming. Botany 2001, Abstracts, Society of
555 America. <http://2001.botanyconference.org/section7/abstracts/26.shtml>

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- 556 Hijmans, R.J., Phillips, S., Leathwick, J., Elith, J. 2011. Package ‘dismo’. Available
557 online at: <http://cran.r-project.org/web/packages/dismo/index.html>.
- 558 Holland, A.E. 2002. A review of *Crotalaria* L. (Fabaceae: Crotalarieae) in Australia.
559 *Austrobaileya* 6: 293-324.
- 560 Hooker, J.D. 1879. The Flora of British India. L. Reeve & Co., Covent Garden,
561 London.
- 562 Hörandl, E., Paun, O., Johansson, J.T., Lehnebach, C., Armstrong, T., Chen, L.,
563 Lockhart, P. 2005. Phylogenetic relationships and evolutionary traits in
564 *Ranunculus* s.l. (Ranunculaceae) inferred from ITS sequence analysis. *Mol.*
565 *Phylogenet. Evol.*, 36, 305-327.
- 566 Jacobi, C.M., Ramalho, M., Silva, M. 2005. Pollination biology of the exotic
567 rattleweed *Crotalaria retusa* L. (Fabaceae) in NE Brazil. *Biotropica* 37: 357-
568 363.
- 569 Katoh, K., Standley, D.M. 2013. MAFFT Multiple Sequence Alignment Software
570 Version 7: Improvements in performance and usability. *Mol. Biol. Evol.* 30:
571 772-780.
- 572 Kay, K.M., Whittall, J.B., Hodges, S.A. 2006. A survey of nuclear ribosomal internal
573 transcribed spacer substitution rates across angiosperms: an approximate
574 molecular clock with life history effects. *BMC Evol. Biol.* 6:36.
- 575 Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S.,
576 Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B.,
577 Meintjes, P., Drummond, A. 2012. Geneious Basic: An integrated and
578 extendable desktop software platform for the organization and analysis of
579 sequence data. *Bioinformatics* 28:1647-1649.
- 580 Kocyan, A., Zhang, L.B., Schaefer, H., Renner, S.S. 2007. A multi-locus chloroplast
581 phylogeny for the Cucurbitaceae and its implications for character evolution
582 and classification. *Mol. Phylogenet. Evol.* 44:553-577.
- 583 Kottek, M., Grieser, J., Beck, C., Rudolf, B., Rubel, F. 2006. World Map of the
584 Köppen-Geiger climate classification updated. *Meteorol. Z.* 15:259-263.
- 585 Lavin, M., Delgado, A. 1990. Pollen brush of Papilionoideae (Leguminosae):
586 morphological variation and systematic utility. *Am. J. Bot.* 77:1294-1312.
- 587 Lavin, M., Herendeen, P.S., Wojciechowski, M.F. 2005. Evolutionary rates analysis
588 of Leguminosae implicates a rapid diversification of lineages during the
589 Tertiary. *Syst. Bot.* 54:575-594.

- 590 Lee, A.T. 1978. Some species of *Crotalaria* in Australia. *Telopea* 1: 319-356.
- 591 Le Roux, M.M., van Wyk, B.-E. 2012. The systematic value of flower structure in
592 *Crotalaria* and related genera of the tribe Crotalarieae (Fabaceae). *Flora* 2012,
593 207:414-426.
- 594 Le Roux, M.M., Boatwright, J.S., Van Wyk, B.-E. 2013. A global infrageneric
595 classification system for the genus *Crotalaria* (Leguminosae) based on
596 molecular and morphological evidence. *Taxon* 62:957-971.
- 597 Lewis, G., Schrire, B.D., Mackinder, B., Lock, M. 2005. Legumes of the world. Royal
598 Botanical Gardens, Kew.
- 599 Mangotra, R., Koul, A.K. 1991. Polyploidy in genus *Crotalaria*. *Cytologia* 56:293-
600 296.
- 601 May, K.M., Sargent, R.D. 2009. The role of animal pollination in plant speciation:
602 Integrating ecology, geography, and genetics. *Annu. Rev. Ecol. Evol. Syst.*
603 40:637-656.
- 604 Mondin, M., Santos-Serejo, J.A., Aguiar-Perecin, M.L.R. 2007. Karyotype
605 characterization of *Crotalaria juncea* (L.) by chromosome banding and
606 physical mapping of 18S-5.8 S-26S and 5S rRNA gene sites. *Genet. Mol. Biol.*
607 30:65-72.
- 608 Mondin, M., Aguiar-Perecin, M.L.R. 2011. Heterochromatin patterns and ribosomal
609 DNA loci distribution in diploid and polyploid *Crotalaria* species
610 (Leguminosae, Papilionoideae), and inferences on karyotype evolution.
611 *Genome* 54:718-726.
- 612 Moonlight, P.W., Richardson, J.E., Tebbitt, M.C., Thomas, D.C., Hollands, R., Peng,
613 C.-I., Hughes, M., Silman, M. 2015. Continental-scale diversification patterns
614 in a megadiverse genus: the biogeography of Neotropical *Begonia*. *J. Biogeo.*
615 42: 1137-1149.
- 616 Morales, A.G., Aguiar-Perecin, M.L.R., Mondin, M. 2012. Karyotype
617 characterization reveals an up and down of 45S and 5S rDNA sites in
618 *Crotalaria* (Leguminosae-Papilionoideae) species of the section *Hedriocarpae*
619 subsection *Macrostachyae*. *Genetic Resources and Crop Evolution* 59:277-
620 288.
- 621 Oliveira, A.C.D., Aguiar-Perecin, M.D. 1999. Karyotype evolution in the genus
622 *Crotalaria* (Leguminosae). *Cytologia* 64:165-174.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

623 Orme, D., Freckleton, R., Thomas, G., Petzoldt, T., Fritz, S., Isaac, N., Pearse, W.
624 2013. Caper: Comparative Analyses of Phylogenetics and Evolution in R. R
625 package version 0.5.2. <https://CRAN.R-project.org/package=caper>.
626 Palmer, J.D. 1991. Plastid chromosome, structure and evolution. In Bogorad, L, Vasil,
627 IK, (eds.), *The Molecular Biology of Plastids*, California: Academic Press, pp.
628 5-53.
629 Palomino, G., Vázquez, R. 1991. Cytogenetic studies in Mexican populations of
630 species of *Crotalaria* L. (Leguminosae-Papilionoideae). *Cytologia* 56:343-
631 351.
632 Paradis E., Claude J., Strimmer K. 2004. APE: analyses of phylogenetics and
633 evolution in R language. *Bioinformatics* 20:289-290.
634 Peel, M.C., Finlayson, B.L., McMahon, T.A. 2007. Updated world map of the
635 Köppen-Geiger climate classification. *Hydrol. Earth Syst. Sci. Discuss.* 4:439-
636 473.
637 Polhill, R.M. 1968. Miscellaneous notes on African species of *Crotalaria* L.: II. *Kew*
638 *Bulletin* 22:169-348.
639 Polhill, R.M. 1982. *Crotalaria* in Africa and Madagascar. CRC Press, Rotterdam..
640 Popic, T.J., Davila, Y.C., Wardle, G.M. 2016. Cheater or mutualist? Novel florivory
641 interaction between nectar-rich *Crotalaria cunninghamii* and small mammals.
642 *Austr. Ecol.* 41:390-398.
643 R Core Team 2015. R: a language and environment for statistical computing. R
644 Foundation for Statistical Computing, Vienna.
645 Rambaut, A, Suchard, M.A., XIE, D., Drummond, A.J. 2014. Tracer v1.6, Available
646 from <http://beast.bio.ed.ac.uk/Tracer>.
647 Ree, R.H. 2005. Phylogeny and the evolution of floral diversity in *Pedicularis*
648 (Orobanchaceae). *Int. J. Plant Sci.* 166:595-613.
649 Roskov, Y.R., Bisby, F.A., Zarucchi, J.L., Schrire, B.D., White, R.J. 2005. ILDIS
650 World Database of Legumes: <http://www.ildis.org>.
651 Sang, T., Crawford, D., Stuessy, T. 1997. Chloroplast DNA phylogeny, reticulate
652 evolution, and biogeography of *Paeonia* (Paeoniaceae). *Am. J. Bot.* 84:1120-
653 1120.
654 Schakel, K.A., Hall, A.E. 1979. Reversible leaflet movements in relation to drought
655 adaptation of cowpeas, *Vigna unguiculata* (L.) Walp. *Funct. Plant Biol.* 6:265-
656 276.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
- 657 Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post
658 analysis of large phylogenies. *Bioinformatics* 30:1312-1313.
- 659 Su, Y.-S., Yajima, M. 2014. R2jags: a package for running JAGS from R. R package
660 version 0.04- 03. URL [http://CRAN.R-project.org/ package=R2jags](http://CRAN.R-project.org/package=R2jags)
- 661 Subramaniam, S., Pandey, A.K., Geeta, R., Mort, M.E. 2013. Molecular systematics
662 of Indian *Crotalaria* (Fabaceae) based on analyses of nuclear ribosomal ITS
663 DNA sequences. *Plant Syst. Evol.* 299:1089-1106.
- 664 Subramaniam, S., Pandey, A.K., Rather, S.A. 2015. A revised circumscription of the
665 species in *Bracteatae* complex (section *Calycinae*) in the genus *Crotalaria* L.:
666 evidence from nuclear and chloroplast markers. *Plant Syst. Evol.* 301:2261-
667 2290.
- 668 Tapia-Pastrana, F., Gallegos-Pacheco, E., De Teodoro-Pardo, C., Mercado-Ruaro, P.
669 2005. New cytogenetic information of two Mexican populations of *Crotalaria*
670 *incana* L. (Leguminosae-Papilionoideae). *Cytologia* 70:207-212.
- 671 Vogel, S. 2009. Leaves in the lowest and highest winds: temperature, force and shape.
672 *New Phytol.* 183:13-26.
- 673 Warman, L., Moles, A.T., Edwards, W. 2011. Not so simple after all: searching for
674 ecological advantages of compound leaves. *Oikos* 120:813-821.
- 675 White, T.J., Bruns, T., Lee, S.J.W.T., Taylor, J.W. 1990. Amplification and direct
676 sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols:*
677 *a guide to methods and applications* 18:315-322.
- 678 Wilkerson, M.S., Wilkerson, M.B. 2010. Koeppen-Geiger-GE.kmz. Greencastle:
679 DePauw University, Greencastle, Indiana, USA.
- 680 Windler, D.R. 1973. Field and garden studies in *Crotalaria sagittalis* L. and related
681 species. *Phytologia* 26:289-354.
- 682 Windler, D.R. 1974. Chromosome numbers for native North American unifoliolate
683 species of *Crotalaria* (Leguminosae). *Brittonia* 26:172-176.

51 **Figure captions**

52
53
54
55 **Figure 1.** Maximum likelihood tree for 372 accessions representing 338 species of
56 *Crotalaria* and 33 species of the remaining 15 genera of Crotalarieae based on 3171
57 aligned nucleotides of nuclear and plastid sequences. Black circles at nodes represent
58 bootstrap values $\geq 70\%$, and branch colors Polhill's (1982) sections (see lower inset
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

691 for their names and species numbers). Upper inset: Morphological characters of
692 Polhill's sections. Photos: A. Rockinger.

693

694 **Figure 2.** Same maximum likelihood tree as in Figure 1 with key flower traits plotted:
695 Keel beak (spirally twisted; barely twisted), calyx (bilabiate; equally lobed, and length
696 of calyx (as long as/longer than keel/shorter than keel). Photos: A. Rockinger.

697

698 **Figure 3.** Maximum likelihood tree with the *Crotalaria* species' distribution areas
699 plotted. Blue: Americas; orange: Africa and Middle East; red: Madagascar; green:
700 Asia including maritime Southeast Asia; purple: Australia, Papua New Guinea, and
701 Melanesia. Inset: worldwide distribution of *Crotalaria* based on 22,225 georeferenced
702 occurrences from GBIF (<http://www.gbif.org>) representing 183 species. Due to
703 uneven collecting effort, India is underrepresented.

704

705 **Figure 4.** Ancestral state reconstruction for simple and compound (unifoliolate,
706 trifoliolate, and multifoliolate) leaves under an all-rates-different model, carried out
707 on a chronogram for 183 *Crotalaria* species, 33 species of other Crotalarieae, and 23
708 species of the remaining genistoids. Pie charts indicate ancestral state probabilities
709 and node bars 95% posterior probability intervals. The geological time scale is in
710 million years and follows Cohen et al. (2013 updated). The fossil seedpod most
711 closely resembles the genera *Bowdichia* and *Diploptropis* (Herendeen and Wing 2001).
712 Photo: P. S. Herendeen.

713

714 **Figure 5.** A) Distribution of *Crotalaria* species in main climate types (Materials and
715 Methods and Table 1); numbers refer to sampled species; B) Distribution of simple-
716 and compound-leaved species in the four climate types; C) Probability of occurrence
717 of simple-leaved species in relation to mean annual precipitation (AP, Bio12).

718

719

720

721

722

723

724

725 **Tables**

726

727 **Table 1.** Correlation coefficient values of the generalized likelihood model (glm) and
 728 the, and variance inflation factors (VIF). Climate and environmental parameters:
 729 Mean annual temperature (MAT, BIO1), temperature annual range (TAR, BIO7),
 730 annual precipitation (AP, BIO12), precipitation seasonality (PS, BIO15), elevation
 731 (EL), average sun radiation (ASR).

732

Parameter	glm	logistf	VIF
MAT	0.92	0.88	1.33
TAR	1.71*	1.61	2.32
AP	3.32***	3.11	1.52
PS	-0.08	-0.09	2.03
ELE	-0.94	-0.9	1.58
ASR	-0.92	-0.88	1.85

733

734

735 **Table 2.** Estimated mean node ages (Ma) for selected divergence events under
 736 different clock models. Ages are in million years, and the values in brackets are the
 737 95% posterior probability intervals.

738

Node of interest	Fossil calibration		Substitution rate calibration
	Strict clock	Relaxed clock	Strict clock
Root	77.5 (63.8–92.4)	87.0 (58.2–122.2)	93.2 (81.0–106.1)
<i>Crotalaria</i> stem node	23.1 (18.4–27.9)	29.5 (21.4–50.6)	29.5 (26.2–33.0)
<i>Crotalaria</i> crown node	17.7 (14.3–21.5)	29.1 (18.3–42.4)	22.9 (20.5–25.4)
Chore			
<i>Chrysocalycinae</i> crown node	14.0 (12.4–19.0)	26.5 (15.8–38.5)	19.3 (16.9–21.7)
2n = 14 clade	11.7 (8.7–14.8)	29.5 (18.3–42.3)	16.5 (13.3–19.6)
Truncate calyx tube clade crown node	12.2 (9.7–15.0)	22.1 (13.0–32.0)	16.4 (14.3–18.5)
<i>Crotalaria linearifoliolata/C. persica</i> clade crown node	16.7 (13.3–20.2)	26.4 (16.5–37.8)	21.6 (19.3–24.1)
Bulbous standard appendages clade crown node	13.6 (10.5–16.7)	22.4 (13.7–32.2)	17.9 (15.3–20.6)
Bilabiate calyx clade crown node	12.4 (9.6–15.1)	20.9 (12.6–30.1)	15.7 (13.4–18.0)

739

740

741

742 **Table 3.** Köppen-Geiger climate categories and their grouping to major climate
 743 groups. Precipitation criteria depend on annual accumulated precipitation (P_{ann}),

744

745

746

747

748

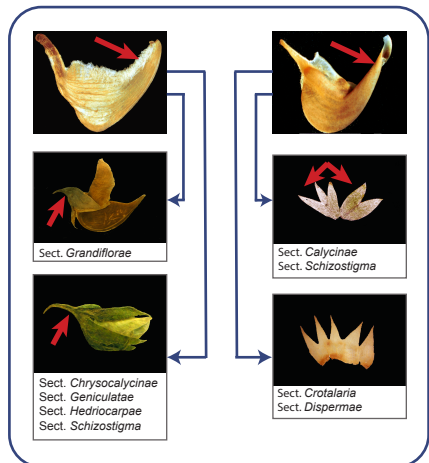
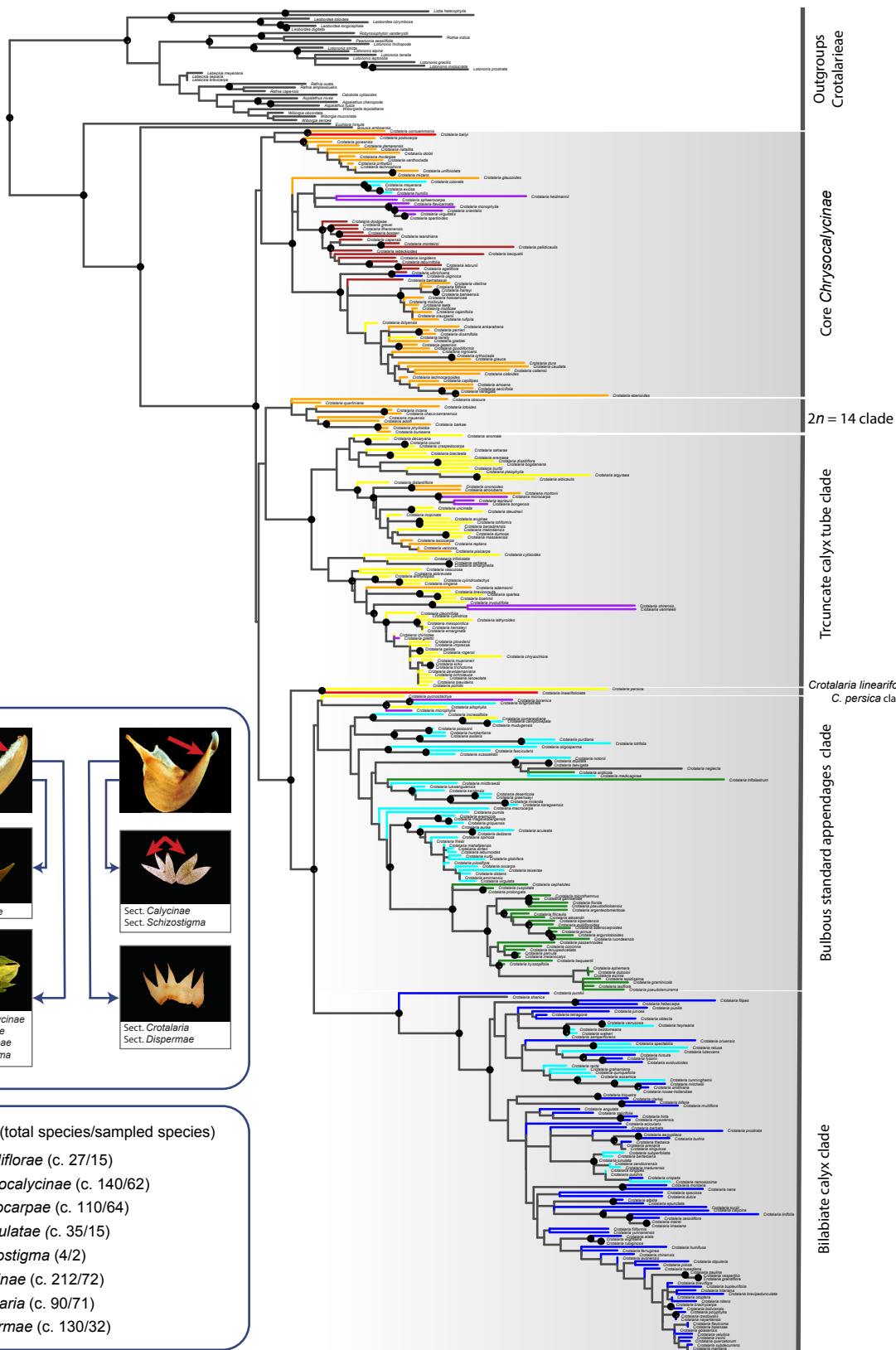
744 monthly precipitation in driest (P_{\min}) and wettest (P_{\max}) month, for the summer and
 745 winter half-years on the hemisphere considered ($P_{\text{smin}}, P_{\text{smax}}, P_{\text{wmin}}, P_{\text{wmax}}$) and dryness
 746 threshold (P_{th} , only for Arid). Dryness threshold (mm) depends on annual temperature
 747 and annual cycle of precipitation. Temperature criteria depend on annual mean near-
 748 surface temperature (T_{ann}), monthly mean temperature of warmest (T_{max}) and coldest
 749 (T_{min}) months, and monthly temperature (T_{mon}). (See Kottek et al. 2006)

Köppen-Geiger climate category	Main climate (first letter)	Annual Precipitation	Seasonal temperature	Major climate group
Af	Equatorial	$P_{\min} \geq 60$ mm	$T_{\min} \geq +18^{\circ}\text{C}$	<i>Humid tropics</i>
Am		$P_{\text{ann}} \geq 25(100 - P_{\min})$		
As		$P_{\min} < 60$ mm in summer		
Aw		$P_{\min} < 60$ mm in winter		
Bsh	Arid	$P_{\text{ann}} > 5 P_{\text{th}}$	$T_{\text{ann}} \geq +18^{\circ}\text{C}$	<i>Arid</i>
Bsk			$T_{\text{ann}} < +18^{\circ}\text{C}$	
Bwh		$P_{\text{ann}} \leq 5 P_{\text{th}}$	$T_{\text{ann}} \geq +18^{\circ}\text{C}$	
Bwk			$T_{\text{ann}} < +18^{\circ}\text{C}$	
Cwa	Mild temperate	$P_{\text{wmin}} < P_{\text{smin}}$ and $P_{\text{smax}} > 10 P_{\text{wmin}}$	$T_{\text{max}} \geq +22^{\circ}\text{C}$	<i>Mild temperate</i>
Cwb			$T_{\text{max}} < +22^{\circ}\text{C}$ and at least 4 $T_{\text{mon}} \geq +10^{\circ}\text{C}$	
Csa		$P_{\text{smin}} < P_{\text{wmin}}, P_{\text{wmax}} > 3 P_{\text{smin}}$ and $P_{\text{smin}} < 40$ mm	$T_{\text{max}} \geq +22^{\circ}\text{C}$	
Csb			$T_{\text{max}} < +22^{\circ}\text{C}$ and at least 4 $T_{\text{mon}} \geq +10^{\circ}\text{C}$	
Cfa		neither Cs nor Cw	$T_{\text{max}} \geq +22^{\circ}\text{C}$	
Cfb			$T_{\text{max}} < +22^{\circ}\text{C}$ and at least 4 $T_{\text{mon}} \geq +10^{\circ}\text{C}$	

751

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Figure 1



- Sections (total species/sampled species)
- Grandiflorae (c. 27/15)
 - Chrysocalycinae (c. 140/62)
 - Hedriocarpae (c. 110/64)
 - Geniculatae (c. 35/15)
 - Schizostigma (4/2)
 - Calycinae (c. 212/72)
 - Crotalaria (c. 90/71)
 - Dispermae (c. 130/32)

Figure 2

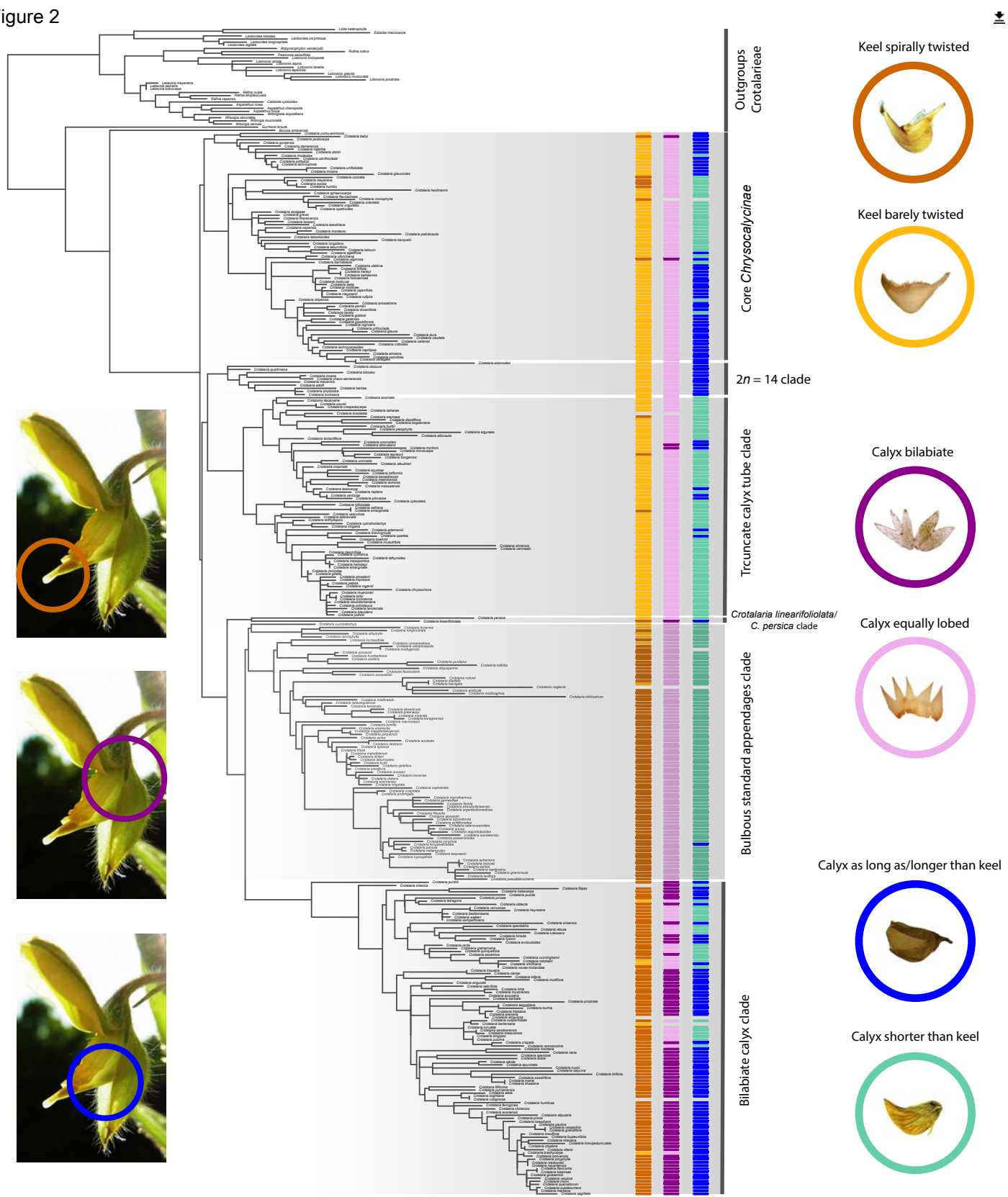
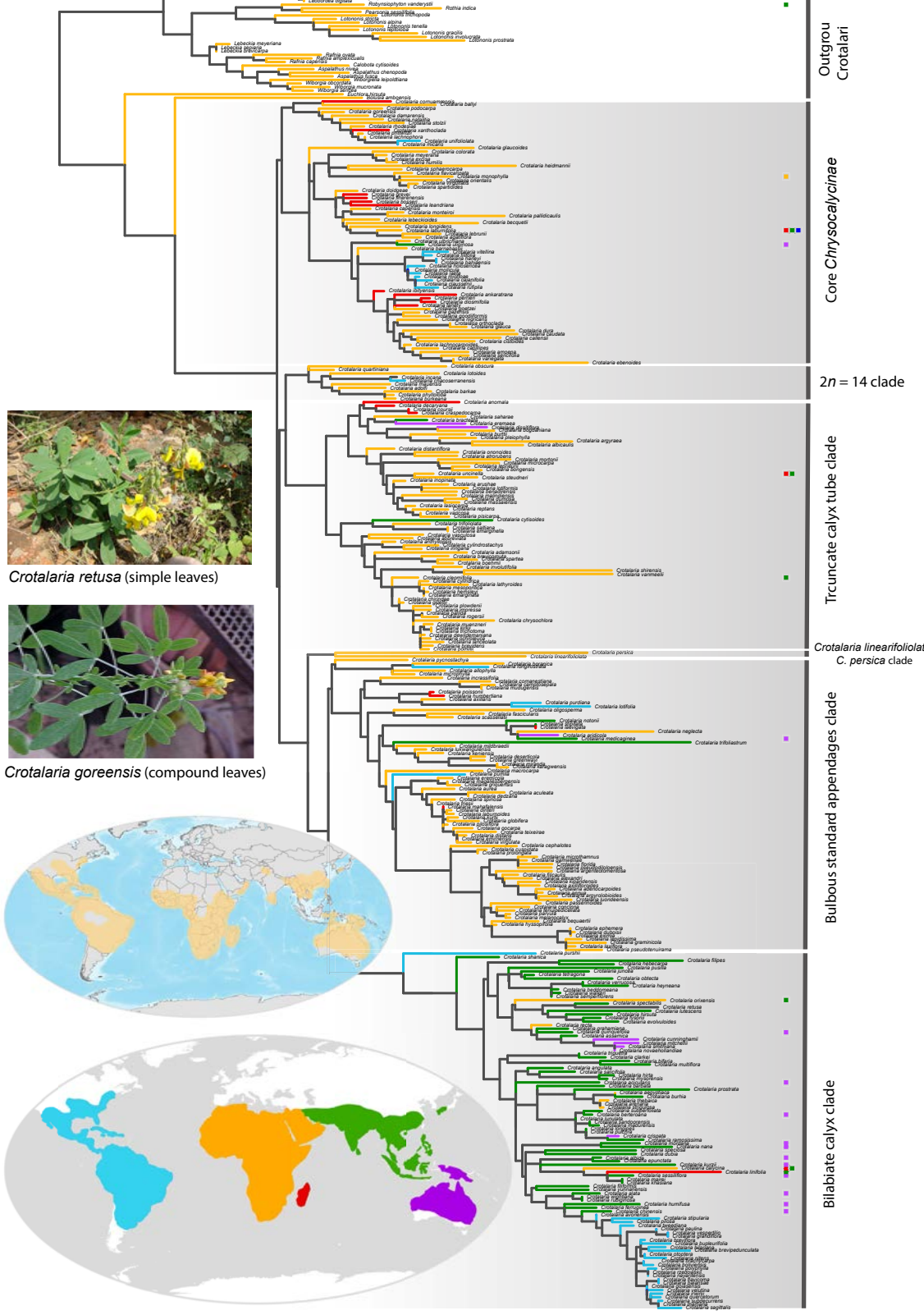


Figure 3



Crotalaria retusa (simple leaves)

Crotalaria gorensis (compound leaves)

Outgroup
Crotalari

Core Chrysochalcinae

2n = 14 clade

Truncate calyx tube clade

Crotalaria linearifoliolata/
C. persica clade

Bulbous standard appendages clade

Bilobate calyx clade

Figure 4

77.5 My



Seedpod, Wyoming, 56 million years
Several calibrations (see Table 2)

Compound leaves

Trifoliolate



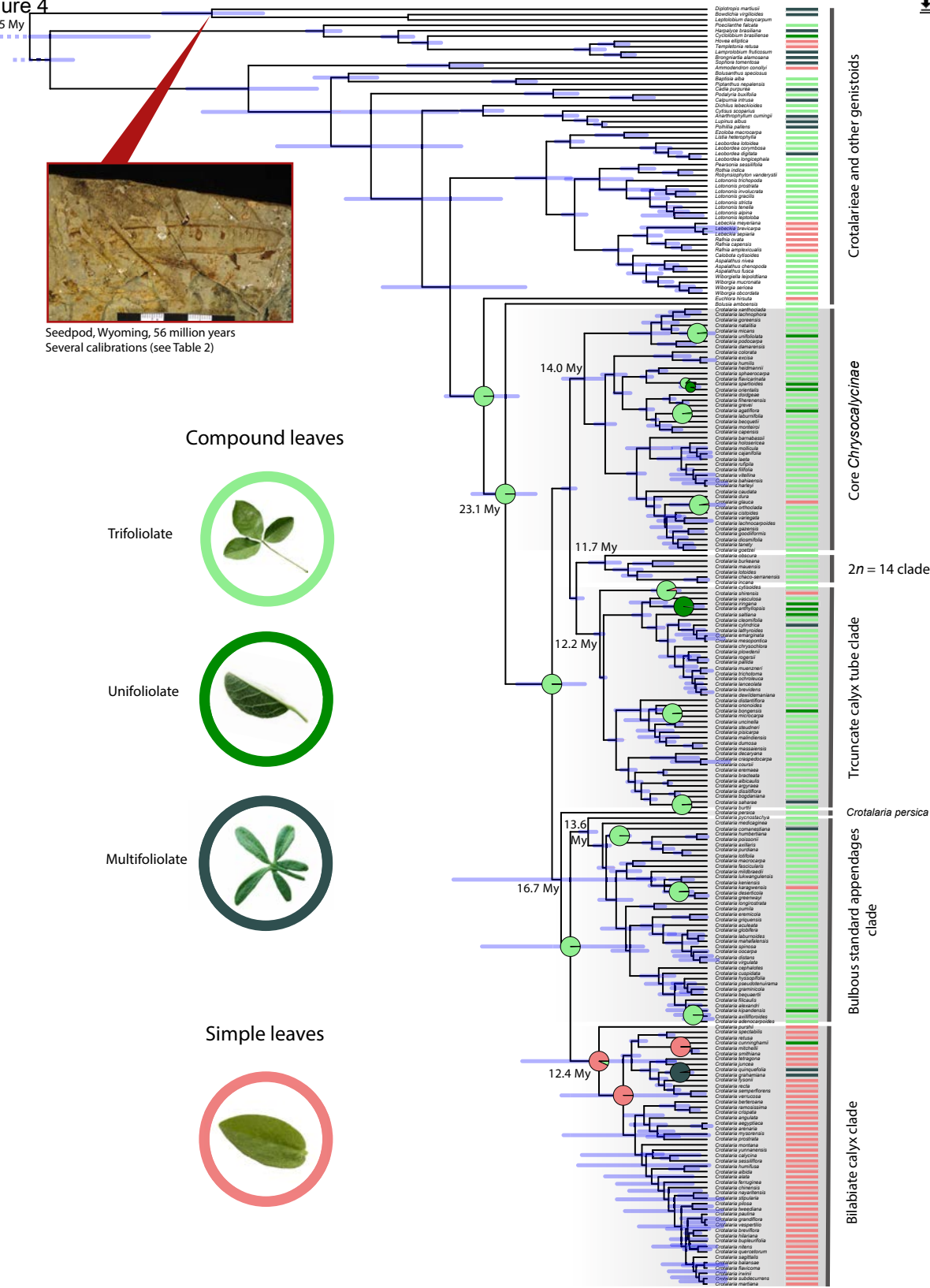
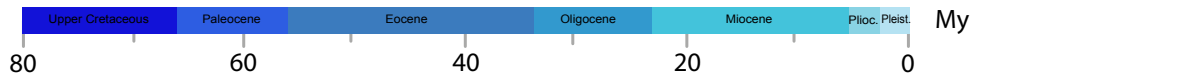
Unifoliolate



Multifoliolate



Simple leaves



Crotalariae and other genistoids

Core Chrysoylcinae

2n = 14 clade

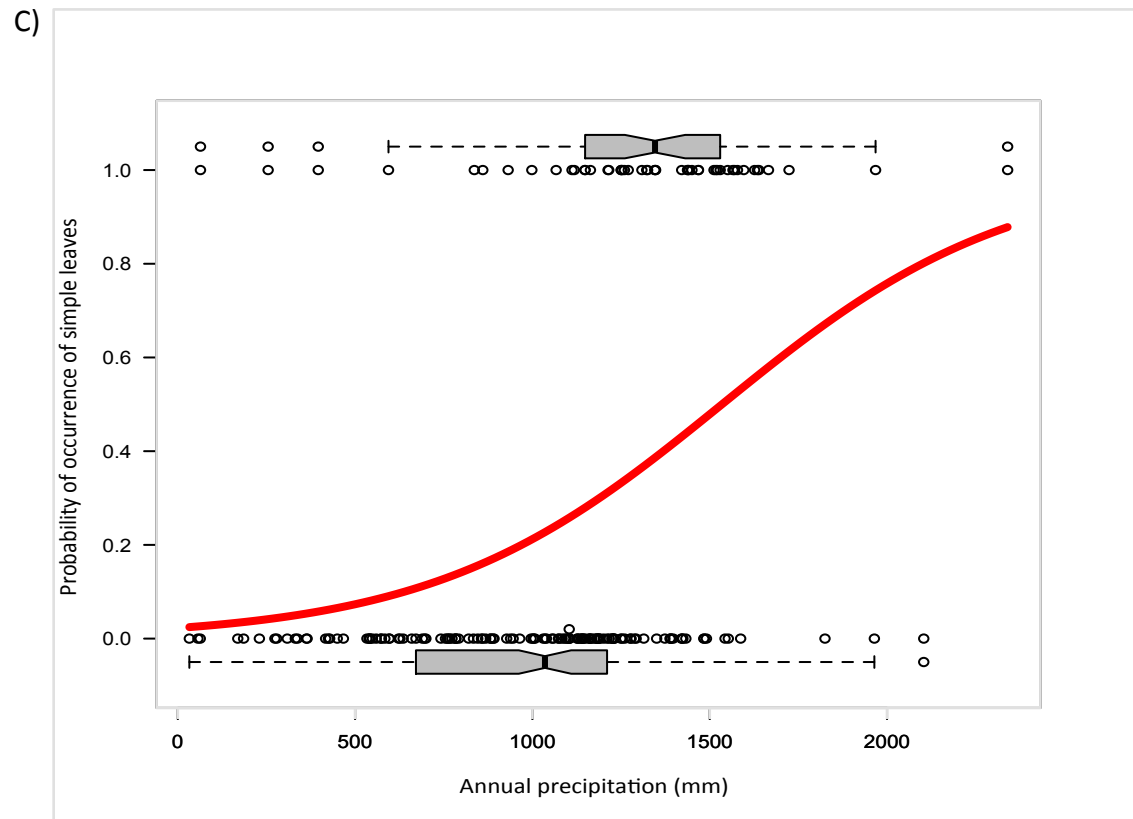
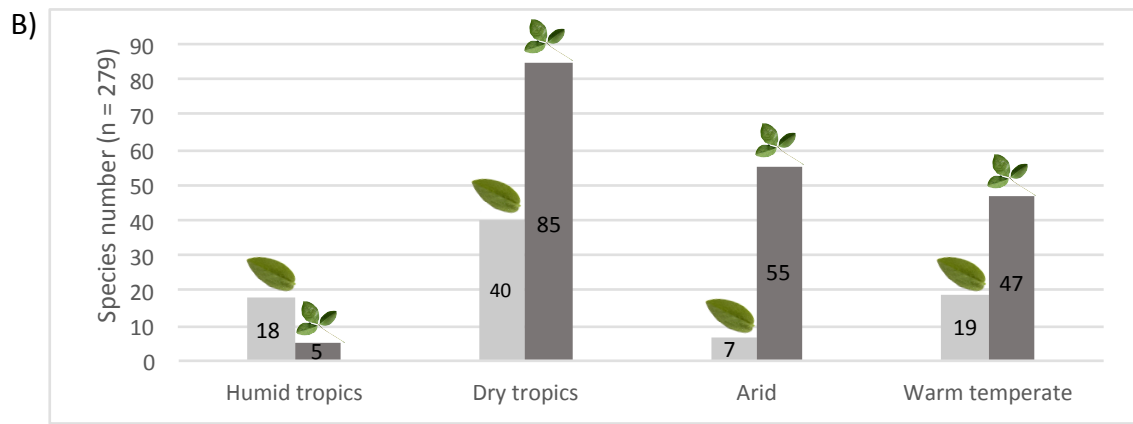
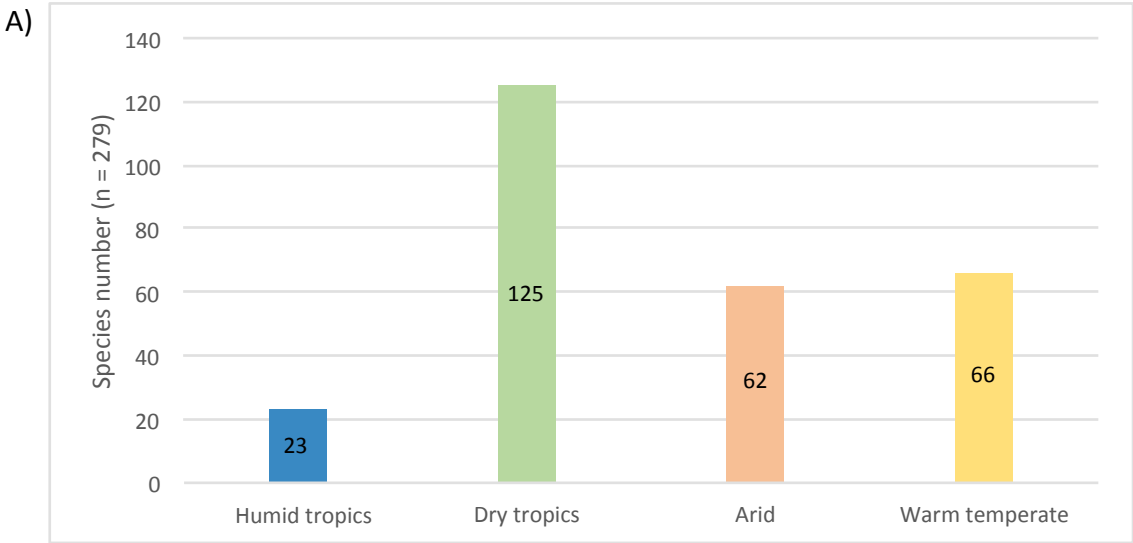
Truncate calyx tube clade

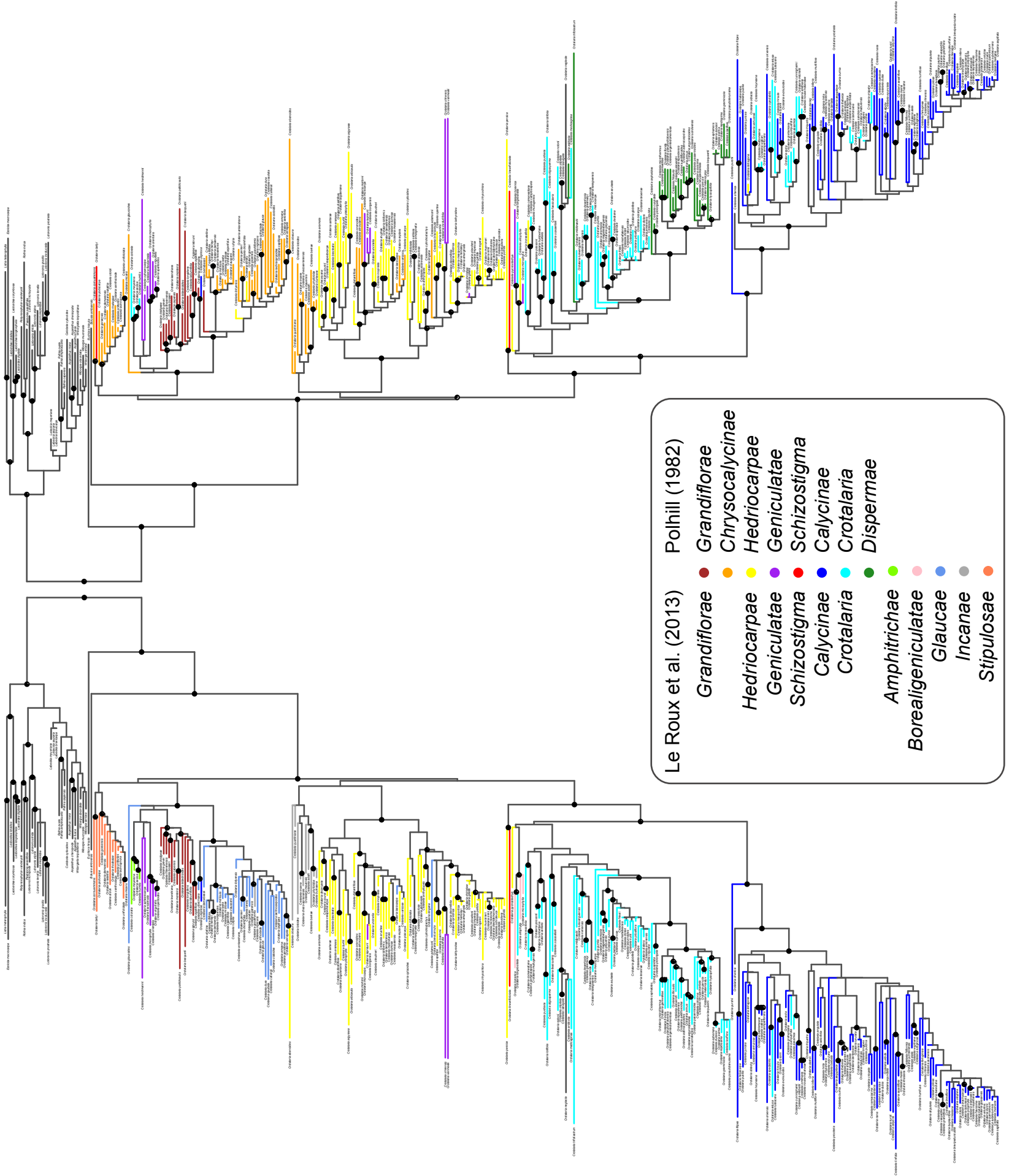
Crotalaria persica

Bulbous standard appendages clade

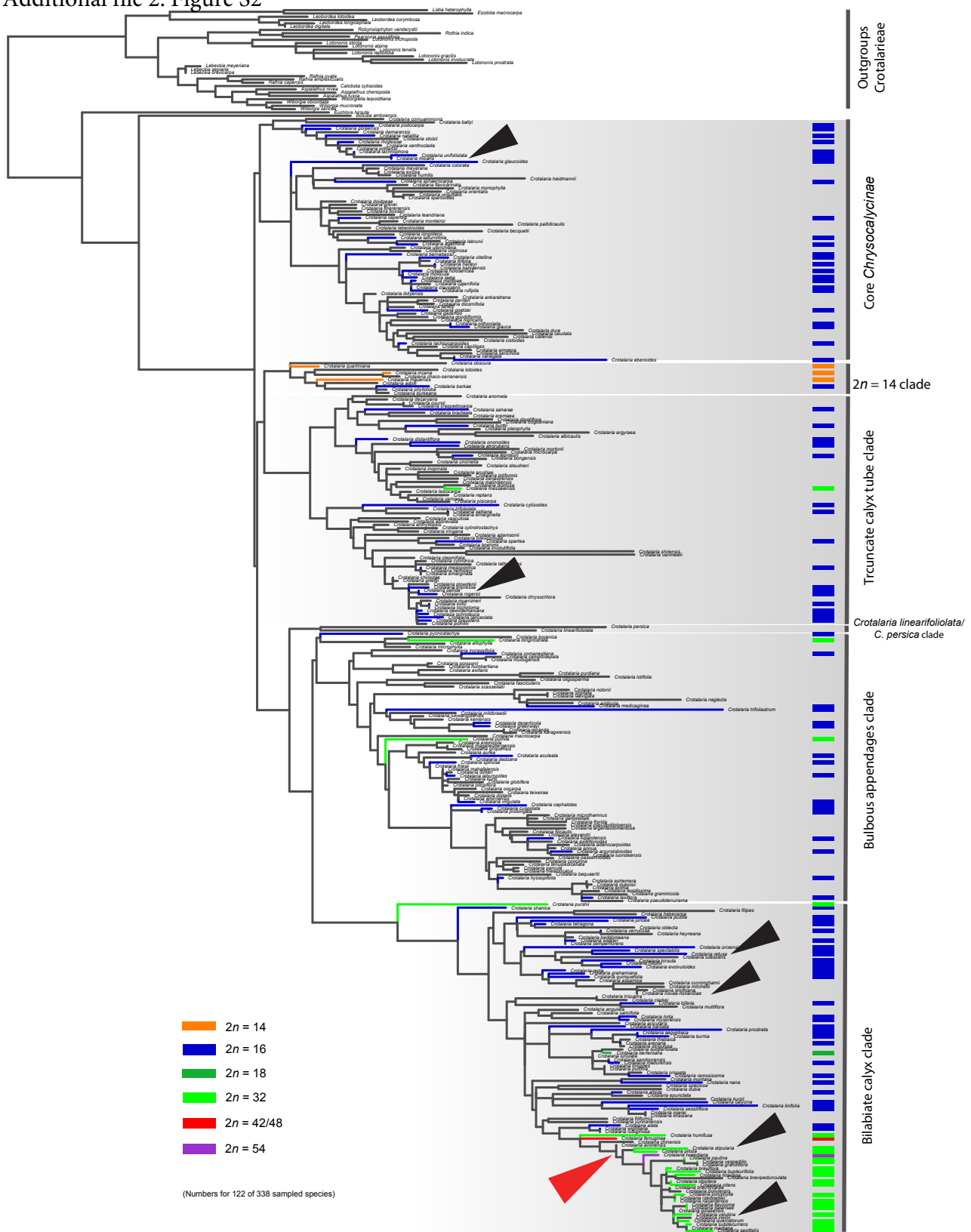
Bilabiate calyx clade

Figure 5





Additional file 2: Figure S2



Additional file 3: Table S1: Species used in this study with herbarium vouchers, place of deposition (in a few cases also their barcodes), geographic origin, distribution ranges, and GenBank accession numbers for all sequences.

Species name	Voucher information and geographical origin	Species distribution range	ETS	ITS	matK	psbA-trnH	rbcl
<i>Anemodendron conollyi</i> Boiss.	N. Beliana and G. Proskuriakova, locality unknown (RNG)	Asia (N): Kazakhstan, Turkmenistan, Uzbekistan	-	EF457705	-	-	-
<i>Anarthrophyllum cumingii</i> (Hook. & Arn.) F. Phil.	A. K. Alnouchie An201, locality unknown (MAF)	South America (N): Chile	-	FJ839486	-	-	-
<i>Aspalathus chenopoda</i> L.*	R. Dahlgren and B. Peterson 350, South Africa (M)	Africa (N): South Africa	KX390757	KX371651	-	-	-
<i>Aspalathus fusca</i> Thunb.	R. Dahlgren and A. Strid 4027, South Africa (M)	Africa (N): South Africa	KX390758	-	-	-	-
<i>Aspalathus nivea</i> Thunb.	R. Dahlgren and B. Peterson 1515, South Africa (M)	Africa (N): South Africa	KX390759	KX371652	-	-	-
<i>Baptisia alba</i> (L.) Vent.*	Jones NCBG-3-00, China (KUN)	North America (N): Canada, United States	-	AJ773348	-	-	-
<i>Bolusia amboensis</i> (Schinz) Harms	ETS, ITS: W. Gless 10091, Namibia (M) matK, psbA-trnH: J. S. Boatwright et al. 248, South Africa (WIND) rbcl: W. Gless 10091, Namibia (K)	Africa (N): Botswana, Namibia, South Africa, Zambia	KX390760	KX371653	-	JQ067549	EU347943
<i>Bowdichia virgiloides</i> Kunth*	R. T. Pennington 477, Brazil (E)	South America (N): Brazil, Colombia, Guyana, Paraguay, Surinam, Venezuela	-	EF457709	-	-	-
<i>Brongniartia alamosana</i> Rydb.	Hu 1120, Mexico (DAV)	North- and Central America (N): Mexico, Sonora	-	AF467022	-	-	-
<i>Codia purpurea</i> (G. Piccoli) Alton	Cameron and Miller 12281b, locality unknown (E)	Africa (N): Ethiopia, Kenya, Somalia; Middle East (N): Oman, Saudi Arabia, Yemen	-	EF457710	-	-	-
<i>Calabotia oytisoides</i> (Berg.) Eckl. and Zeyh.*	P. Goldblatt 7197, South Africa (M)	Africa (N): South Africa	KX390761	KX371654	-	-	-
<i>Calpurnia intrusa</i> (W.T. Alton) E. Mey.*	B.-E. van Wyk 3006, South Africa (JRAU)	Africa (N): Lesotho, South Africa	-	AJ409914	-	-	-
<i>Crotalaria abbreviata</i> Baker f.	ETS, ITS, psbA-trnH: H. Wild 3291, Zimbabwe (M) rbcl: Bigood et al. 3512, Tanzania (UPS)	Africa (N): Tanzania, Congo, Zambia, Zimbabwe	KX390762	KX371655	-	KX390740	JQ041115
<i>Crotalaria acicularis</i> Benth.	M. Ramos 462, Philippines (M)	Asia (N): Bangladesh, Burma, Cambodia, China, Hainan, India, Indonesia, Laos, Myanmar, Nepal, Philippines, Sulawesi, Taiwan, Thailand, Vietnam; Australasia (N): Australia	-	KX371656	-	-	-
<i>Crotalaria aculeata</i> De Wild.	ITS: J. M. Hildebrandt 3485, Madagascar (M) matK, psbA-trnH, rbcl: Mhorro and Backéus 2330, Tanzania (UPS)	Africa (N): Angola, Burundi, Ethiopia, Malawi, Rwanda, Sudan, Tanzania, Uganda, Congo, Zambia; Indian Ocean (I): Madagascar, Reunion	-	KX371657	-	JQ067383	JQ041116
<i>Crotalaria adamsonii</i> Baker f.	F. Malaisse 9506, Congo (MO)	Africa (N): Angola, Congo, Malawi, Mozambique, Tanzania, Congo, Zambia	KX390763	KX371658	-	-	-
<i>Crotalaria adenocarpoides</i> Taub.	P. K. Rwaburindore 5196, Uganda (M)	Africa (N): Burundi, Rwanda, Uganda, Congo	-	KX371659	-	-	-
<i>Crotalaria adolfi</i> Harms	Iversen et al. 87597, Tanzania (UPS)	Africa (N): Tanzania	-	JQ067163	-	JQ067384	JQ041117
<i>Crotalaria aegyptiaca</i> Benth.	A. Shmida s. n. [MSB5784], Israel (MSB)	Africa (N): Egypt, Somalia; Asia (N): Iran; Middle East (N): Israel, Jordan, Oman, Saudi Arabia, Sinai, United Arab Emirates, Yemen	KX390764	KX371660	-	-	-
<i>Crotalaria agatiflora</i> Schweinf.	ITS: P. J. Greenway and Kanuri 13.648, Kenya (M) matK: Fries and Fries 2047, Kenya (UPS)	Africa (N): Burundi, Ethiopia, Kenya, Malawi, Mozambique, Tanzania, Uganda, Congo, Zimbabwe; Asia (I): India; Australasia (I): Australia, New Zealand; South America (I): Colombia	-	KX371661	-	JQ067425	JQ041118

Species name	Voucher information and geographical origin	Species distribution range	ETS	ITS	matK	psbA-trnH	rbcL
<i>Crotalaria alata</i> D.Don	Van Beusekom 3652, Thailand (K)	Africa (I): Tanzania, Uganda, Asia (N): Bangladesh, Bhutan, Cambodia, China, Hainan, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Sri Lanka, Sumatra, Thailand, Vietnam; Australasia (N): Papua New Guinea; Caribbean (U): Indian Ocean (I): Madagascar, Mauritius, Reunion; Pacific Ocean (N): Bismarck-Archipelago	-	JQ067339	-	-	-
<i>Crotalaria albicaulis</i> Franch.	Gilbert et al. 7358, Ethiopia (UPS)	Africa (N): Djibouti, Ethiopia, Somalia	-	JQ067164	-	JQ067385	JQ041119
<i>Crotalaria albida</i> Roth	Ye 2606, China (MO)	Asia (N): Bangladesh, Bhutan, Cambodia, China, Hainan, India, Java, Laos, Lesser Sunda Is, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Sri Lanka, Sulawesi, Sumatra, Taiwan, Thailand, Vietnam; Australasia (N): Papua New Guinea	-	KX371662	-	-	-
<i>Crotalaria alexandri</i> Baker f.	M. G. Gilbert et al. 9201, Ethiopia (K)	Africa (N): Burundi, Ethiopia, Kenya, Malawi, Mozambique, Rwanda, Tanzania, Uganda, Zambia, Zimbabwe	KX390765	KX371663	-	JQ067386	JQ041120
<i>Crotalaria allophylla</i> Thulin * Sect. <i>Borealigeniculatae</i> M.M. Le Roux & B.-E. van Wyk	Thulin et al. 7282, Somalia (UPS)	Africa (N): Somalia	-	JQ067166	-	JQ067387	JQ041121
<i>Crotalaria amoena</i> Baker	Biggood et al. 4565, Tanzania (UPS)	Africa (N): Angola, Tanzania, Congo, Zambia	-	JQ067167	-	-	JQ041122
<i>Crotalaria angulata</i> Mill.	S. S. Subramaniam 1068, India (DUH)	Asia (N): India, Sri Lanka	-	KP698615	-	-	-
<i>Crotalaria ankaratrana</i> R.Vig.	Peltier 5015, Madagascar (P)	Indian Ocean (N): Madagascar	-	JQ067282	-	JQ067498	JQ041124
<i>Crotalaria annua</i> Milne-Redh.	Biggood et al. 4070, Tanzania (UPS)	Africa (N): Angola, Malawi, Tanzania, Congo, Zambia	-	JQ067168	-	-	JQ041125
<i>Crotalaria anomala</i> R.Vig.	Peltier 4944, Madagascar (P)	Indian Ocean (N): Madagascar	-	JQ067281	-	-	JQ041126
<i>Crotalaria anthyllopsis</i> Baker	Biggood and Vollesen 3191, Tanzania (UPS)	Africa (N): Angola, Burundi, Central African Republic, Ethiopia, Kenya, Malawi, Mali, Mozambique, Nigeria, Rwanda, Tanzania, Uganda, Congo, Zambia, Zimbabwe	-	JQ067169	-	-	JQ041127
<i>Crotalaria arenaria</i> Benth.	Eden Foundation 65, Niger (K)	Africa (N): Chad, Mali, Mauritania, Niger, Nigeria, Senegal	-	JQ067333	-	JQ067538	JQ067581
<i>Crotalaria argenteotomentosa</i> R. Wilczek	F. Malaisse 10546, Congo (M)	Africa (N): Congo, Zambia	KX390766	KX371664	-	-	-
<i>Crotalaria argyrea</i> Baker	M. M. Le Roux et al. 59, Namibia (WIND)	Africa (N): Angola, Namibia	-	JQ067302	-	JQ067513	JQ067582
<i>Crotalaria argyrolabioides</i> Baker	Brummit 11355, Malawi (UPS)	Africa (N): Malawi, Mozambique, Tanzania, Congo, Zambia	-	JQ067170	-	JQ067391	JQ041128
<i>Crotalaria andicola</i> Domin	Forster 20890, Australia (MEL)	Australasia (N): Australia	-	JQ067293	-	JQ067506	-
<i>Crotalaria arushae</i> Polhill	M. Richards 23729, Tanzania (M)	Africa (N): Tanzania	KX390767	KX371665	-	-	-
<i>Crotalaria assamica</i> Benth.	GenBank GU396702, unvouchered	Asia (N): China, Hainan, India, Laos, Myanmar, Taiwan, Thailand, Vietnam	-	-	-	GU396702	-
<i>Crotalaria atrorubens</i> Benth.	R. Bartha 7127, Nigeria (M)	Africa (N): Cameroon, Chad, Ghana, Mali, Niger, Nigeria, Senegal, Sudan	-	KX371666	-	-	-
<i>Crotalaria aurea</i> Baker f.	Gliess 13497, Namibia (WIND)	Africa (N): Namibia	-	JQ067157	-	-	JQ041129
<i>Crotalaria avonensis</i> Delaney & Wunderlin	K. R. De Laney 1623, USA (K)	North America (N): United States	KX390768	KX371667	-	-	-
<i>Crotalaria axillaris</i> Alton	Swenson 8, Kenya (UPS)	Africa (N): Angola, Burundi, Central African Republic, Ethiopia, Ghana, Kenya, Malawi, Mozambique, Tanzania, Togo, Uganda, Congo, Zambia	-	JQ067171	-	JQ067392	JQ041130
<i>Crotalaria axillifloroides</i> R. Wilczek	E. A. Robinson 5246, Zimbabwe (M)	Africa (N): Congo, Zambia	KX390769	KX371668	-	-	-

Species name	Voucher information and geographical origin	Species distribution range	ETS	ITS	mark	psbA-trnH	rbcl
<i>Crotalaria bahiensis</i> Windler & Skimmer	Dr. Zehntner 2049, Brazil (M)	South America (N): Brazil	KX390770	KX371669	-	-	-
<i>Crotalaria balanseae</i> Micheli	De Barros 2561, Brazil (SP)	South America (N): Argentina, Brazil, Paraguay	KX390771	KX371670	-	-	-
<i>Crotalaria ballyi</i> Polhill	Gilbert and Thulin 1488, Kenya (UPS)	Africa (N): Kenya	-	JQ067172	-	JQ067393	JQ041131
<i>Crotalaria barbata</i> Wight & Arn.	ITS: Hohenacker s. n. [M-0242309], India (M) psbA-trnH: Wight 610, locality unknown (MEL)	Asia (N): India, Java	-	KX371671	-	JQ067508	-
<i>Crotalaria barcae</i> Schweinf.	H. Ern et al. 1279, Togo (B)	Africa (N): Angola, Botswana, Burkina Faso, Cameroon, Chad, Ethiopia, Ghana, Kenya, Malawi, Mozambique, Namibia, Niger, Nigeria, Somalia, South Africa, Sudan, Tanzania, Togo, Zambia, Zimbabwe	KX390772	KX371672	-	-	-
<i>Crotalaria barnassii</i> Baker f.	B.-E. van Wyk s. n., Botswana (URAU)	Africa (N): Angola, Malawi, Mozambique, Namibia, South Africa, Tanzania, Zimbabwe	-	JQ067322	-	-	JQ067584
<i>Crotalaria bequaertii</i> R. Wilczek	Lovett and Kayombo 4901, Tanzania (UPS)	Africa (N): Congo, Malawi, Rwanda, Tanzania, Uganda, Zambia	-	JQ067173	-	JQ067394	JQ041132
<i>Crotalaria beddomeana</i> Thoth. & A.A. Ansari	Conveny et al. 5844, Australia (MEL)	Asia (N): India, Sri Lanka; Australasia (I): Australia	-	JQ067287	-	JQ067501	JQ041227
<i>Crotalaria benadiensis</i> Chiov.	Gilbert and Thulin 1151, Kenya (UPS)	Africa (N): Somalia	-	JQ067174	-	JQ067395	JQ041133
<i>Crotalaria bequaertii</i> Baker f.	ETS: E. A. Robinson 6494, Zimbabwe (M) ITS, mark, rbcl: Bidgood et al. 4439, Tanzania (UPS)	Africa (N): Angola, Congo, Malawi, Tanzania, Zambia, Zimbabwe	KX390773	JQ067175	-	-	JQ041134
<i>Crotalaria berteriana</i> DC.	S. S. Subramaniam et al. 1073, India (DUH)	Asia (N): India, Indonesia, Sri Lanka, Sumatra; Australasia (N): Papua New Guinea; Caribbean (N): Guadeloupe, Jamaica, Puerto Rico; Indian Ocean (I): Madagascar, Mauritius, Reunion, Seychelles; Pacific Ocean (I): Hawaii	-	KR673341	-	-	-
<i>Crotalaria bifaria</i> L.f.	Kambale SSK-1, India (DUH)	Asia (N): India, Sri Lanka	-	JQ945936	-	-	-
<i>Crotalaria boehmi</i> Taub.	Bidgood et al. 3862, Tanzania (UPS)	Africa (N): Tanzania	-	JQ067176	JQ040997	-	JQ041136
<i>Crotalaria bogdaniana</i> Polhill	Gilbert et al. 8216, Ethiopia (UPS)	Africa (N): Ethiopia, Kenya, Somalia, Tanzania, Uganda	-	JQ067177	JQ040998	-	JQ041137
<i>Crotalaria boliviensis</i> Windler & S.G. Skinner	J. R. I. Ward and M. Mendoza 19077, Bolivia (K)	South America (N): Bolivia	KX390774	KX371673	-	KX390741	-
<i>Crotalaria bongensis</i> Baker f.	ITS: Espirito Santo Explorações Botânicas 3466, Guinea-Bissau (M) mark, psbA-trnH, rbcl: Mhoro 1020, Tanzania (UPS)	Africa (N): Angola, Cameroon, Central African Republic, Kenya, Liberia, Nigeria, Sudan, Tanzania, Uganda, Congo, Zambia	-	KX390756	JQ040999	JQ067399	JQ041138
<i>Crotalaria boranica</i> Baker f.	J. J. F. de Wilde 5935, Ethiopia (M)	Africa (N): Ethiopia, Kenya, Somalia	KX390775	KX371674	JQ041000	KX390745	KX083394
<i>Crotalaria bosseri</i> M. Peitler	Barthelat 1313, Madagascar (P)	Indian Ocean (N): Madagascar	-	JQ067270	-	JQ067489	JQ041140
<i>Crotalaria brachycarpa</i> Benth.	E. Uje 7200, Brazil (K)	South America (N): Brazil	-	KX371675	-	KX390742	-
<i>Crotalaria bracteata</i> D.C.	M. van de Bult 1282, Thailand (M)	Asia (N): Bangladesh, Bhutan, Burma, Cambodia, China, India, Laos, Myanmar, Philippines, Thailand, Vietnam	KX390776	KX371676	-	-	-

Species name	Voucher information and geographical origin	Species distribution range	ETS	ITS	mark	psbA-trnH	rbcl
<i>Crotalaria brevicornuta</i> Polhill	R. M. Polhill and S. Paulo 1926, Tanzania (B)	Africa (N): Tanzania	KX390777	KX371677	-	-	-
<i>Crotalaria brevidens</i> Benth.	ETS: Weston 4936, Costa Rica (MO) ITS, mark, rbcl: Pocs 87065/A, Tanzania (UPS)	Africa (N): Burundi, Cameroon, Central African Republic, Chad, Ethiopia, Kenya, Nigeria, Rwanda, Sudan, Tanzania, Uganda, Congo; Caribbean (I): Dominican Republic, Guadeloupe, Martinique, Puerto Rico, Virgin Is; Central America (I): Costa Rica; North America (I): United States; South America (I): Brazil, Colombia South America (N): Brazil	KX390778	JQ067196	JQ041001	-	JQ041141
<i>Crotalaria breviflora</i> DC.	A. S. Flores et al. 717, Brazil (MIRR)	South America (N): Brazil	KX390779	KX371678	-	-	-
<i>Crotalaria brevipedunculata</i> Windler	Gentry 5311, Mexico (M)	Central America (N): Mexico	-	KX371679	-	-	-
<i>Crotalaria bupleurifolia</i> Cham. & Schltdl.	Gutiérrez MNMG 576, Mexico (MO)	Central America (N): Mexico	KX390780	KX371680	-	-	-
<i>Crotalaria burthia</i> Benth.	V. Mozaifarian 52827, Iran (TARI)	Asia (N): Afghanistan, India, Iran, Pakistan	-	KX371681	-	-	-
<i>Crotalaria burkeana</i> Benth.	Schutte 457, South Africa (JRAU)	Africa (N): Botswana, Mozambique, South Africa, Swaziland, Zimbabwe	-	JQ067127	JQ041002	-	JQ041143
<i>Crotalaria burttii</i> Baker f.	Bidgood et al. 1028, Tanzania (UPS)	Africa (N): Kenya, Tanzania	-	JQ067181	JQ041003	JQ067402	JQ041144
<i>Crotalaria cajaniifolia</i> Kunth.	P. Döbbeler 4786, Costa Rica (M)	Caribbean (U): Caribbean; Central America- Belize (U), Costa Rica (U), Guatemala (U), Mexico (U), Nicaragua (N), Panama (U)	-	KX371682	-	-	-
<i>Crotalaria callensii</i> R. Wiltzek	E. A. Robinson 6022, Congo (M)	Africa (N): Congo	-	KX371683	-	-	-
<i>Crotalaria calycina</i> Schrank* sect. <i>Calycinae</i> Wight & Arn.	ITS: A. Lohrer 2405, Philippines (M) mark, rbcl: Vollesen 4504, Tanzania (UPS)	Africa (N): Angola, Burundi, Central African Republic, Chad, Congo, Ethiopia, Gabon, Ghana, Guinea, Guinea Bissau, Ivory Coast, Mali, Mozambique, Nigeria, Senegal, Sierra Leone, Sudan, Tanzania, Togo, Uganda, Zaire, Zambia; Asia (N): Bangladesh, Bhutan, Cambodia, China, East Timor, Hainan, India, Indonesia, Laos, Lesser Sunda Is, Malaysia, Moluccas, Nepal, Pakistan, Philippines, Ryukyu Is, Sri Lanka, Sulawesi, Sumatra, Taiwan, Thailand, Vietnam; Australasia (N): Australia, Papua New Guinea; Pacific Ocean (N): Bismarck Archipelago	-	KX371684	JQ041004	-	JQ041145
<i>Crotalaria camptosepala</i> Thulin	Thulin et al. 7259, Somalia (UPS)	Africa (N): Somalia	-	JQ067182	JQ041005	-	JQ041146
<i>Crotalaria carpensis</i> Jacq.	ITS: B.-E. van Wyk 1985, South Africa (JRAU) mark: Maurin et al. OM3786, South Africa (PRE) rbcl: B.-E. van Wyk 2933a, South Africa (JRAU)	Africa: Kenya (I), Malawi (N), Mozambique (N), South Africa (N), Swaziland (N), Zimbabwe (N); Asia (I): Nepal; Indian Ocean (I): Mauritius (I), Reunion (I); Pacific Ocean (I): New Zealand	-	EU34788 4	JX905953	-	EU348034
<i>Crotalaria capillipes</i> Polhill	Bidgood et al. 1036, Tanzania (UPS)	Africa (N): Tanzania	-	JQ067183	JQ041006	JQ067404	JQ041147
<i>Crotalaria caudata</i> Baker	Bidgood et al. 4488, Tanzania (UPS)	Africa (N): Angola, Burundi, Cameroon, Mozambique, Nigeria, Tanzania, Uganda, Zaire, Zambia, Zimbabwe	-	JQ067184	-	-	JQ041148
<i>Crotalaria cephalotes</i> A.Rich.	Bidgood et al. 3993, Tanzania (UPS)	Africa (N): Angola, Benin, Burkina Faso, Burundi, Cameroon, Central African Republic, Chad, Ethiopia, Ghana, Guinea, Ivory Coast, Kenya, Malawi, Mali, Mozambique, Nigeria, Rwanda, Senegal, Sudan, Tanzania, Togo, Uganda, Zaire, Zambia, Zimbabwe	-	JQ067185	-	-	JQ041149
<i>Crotalaria chaco-serranensis</i> H.G. Bach & Fortunato	Pérez et al. 4367, Argentina (K)	South America (N): Argentina, Bolivia	KX390781	KX371685	-	KX390753	-
<i>Crotalaria chinensis</i> L.	Sorensen et al. 2254, Thailand (K)	Asia (N): Burma, Cambodia, China, Hainan, India, Indonesia, Laos, Malaysia, Myanmar, Philippines, Sabah, Sarawak, Sumatra, Taiwan, Thailand, Vietnam; Australasia (N): Papua New Guinea	-	JQ067335	-	-	-

Species name	Voucher information and geographical origin	Species distribution range	ETS	ITS	matK	psbA-trnH	rbcl
<i>Crotalaria chirindae</i> Baker f.	Gereau et al. 4502, Tanzania (UPS)	Africa (N): Malawi, Mozambique, Tanzania, Zimbabwe	-	JQ067186	JQ041007	-	JQ041150
<i>Crotalaria chrysocilaria</i> Harms	Bidgood et al. 4756, Tanzania (UPS)	Africa (N): Burundi, Cameroon, Kenya, Rwanda, Sudan, Tanzania, Uganda, Zaire, Zambia	-	JQ067187	JQ041008	-	JQ041151
<i>Crotalaria cistoides</i> Baker	Bidgood et al. 4731, Tanzania (UPS)	Africa (N): Angola, Tanzania, Zambia	-	JQ067188	JQ041009	JQ067408	JQ041152
<i>Crotalaria clarkei</i> Gamble	S. S. Subramaniam 1088, India (DUH)	Asia (N): India	-	JQ945932	-	-	-
<i>Crotalaria clausenii</i> Benth.	A. P. Duarte 5704, Brazil (B)	South America (N): Brazil	KX390782	KX371686	-	-	-
<i>Crotalaria cleomifolia</i> Baker	Nkhoma et al. 284, Zambia (UPS)	Africa (N): Angola, Benin, Burundi, Cameroon, Central African Republic, Ethiopia, Guinea, Kenya, Malawi, Mali, Mozambique, Nigeria, Rwanda, Sierra Leone, Sudan, Tanzania, Togo, Uganda, Zaire, Zambia, Zimbabwe; Asia: Malaysia (I), Malaysia (N), Vietnam (I)	-	JQ067189	JQ041010	-	JQ041153
<i>Crotalaria colorata</i> Schinz * Sect. <i>Amphitrichae</i> M. M. Le Roux & B.-E. van Wyk	W. Giess 5050, Namibia (M)	Africa (N): Namibia, South Africa	KX390783	KX371687	JQ041011	KX390748	JQ041154
<i>Crotalaria comanestiana</i> Volkens & Schweinf.	Mesfin and Vollesen 4311, Ethiopia (UPS)	Africa (N): Djibouti, Ethiopia, Kenya, Somalia, Tanzania	-	JQ067190	JQ041012	JQ067410	JQ041155
<i>Crotalaria concinna</i> Polhill	E. Milne-Redhead and P. Taylor 9513B, Tanzania (B) ETS: Du Puy and Bpravonjariisoa M124, Madagascar (MO)	Africa (N): Tanzania	KX390784	KX371688	-	-	-
<i>Crotalaria cornu-ammonis</i> R. Vig.	ITS, psbA-trnH, rbcl: D. J. Du Puy M59, Madagascar (P) Labat 2124, Madagascar (P)	Indian Ocean (N): Madagascar	KX390785	JQ067267	-	JQ067486	JQ041156
<i>Crotalaria coursii</i> M. Peltier	Du Puy M679, Madagascar (P)	Indian Ocean (N): Madagascar	-	JQ067272	-	JQ067491	JQ041157
<i>Crotalaria craspedocarpa</i> R. Vig.	Du Puy M679, Madagascar (P)	Indian Ocean (N): Madagascar	-	JQ067276	JQ041013	-	JQ041158
<i>Crotalaria crispata</i> Benth.	Chippendale 6848, Australia (MO)	Australasia (N): Australia	-	KX371689	-	-	-
<i>Crotalaria cunninghamii</i> R. Br.	ETS, ITS, rbcl: R. Hill 295, Australia (M) psbA-trnH: A. Rockinger 20161, Australia (M)	Australasia (N): Australia	KX390786	KX371690	-	KX390752	KX364158
<i>Crotalaria cuspidata</i> Taub.	Bidgood et al. 3805, Tanzania (UPS)	Africa (N): Angola, Central African Republic, Nigeria, Tanzania, Zaire, Zambia	-	JQ067191	JQ041014	-	JQ041159
<i>Crotalaria cylindrica</i> A. Rich.	Gilbert and Dagne 8424, Ethiopia (UPS)	Africa (N): Ethiopia, Kenya	-	JQ067201	JQ041015	-	JQ041160
<i>Crotalaria cylindrostachys</i> Baker	ETS, ITS: R. M. Polhill and S. Paulo 1934, Tanzania (B) matK, rbcl: Mhoro 1176, Tanzania (UPS)	Africa (N): Angola, Malawi, Tanzania, Zambia, Zimbabwe	KX390787	KX371691	JQ041016	-	JQ041161
<i>Crotalaria cylisoides</i> DC.	ITS: A. K. Pandey 910, India (DUH, M) psbA-trnH: Pengklai et al. 3001, Thailand (K)	Asia (N): Bangladesh, Bhutan, China, India, Myanmar, Nepal, Thailand	-	JN990123	-	JQ067543	-
<i>Crotalaria damarensis</i> Engl.	Schutte 463b, South Africa (JRAU)	Africa (N): Angola, Botswana, Mozambique, Namibia, South Africa, Zambia, Zimbabwe	-	JQ067141	JQ041018	JQ067361	JQ041163
<i>Crotalaria decaryana</i> R. Vig.	Lefèvre 16, Madagascar (P)	Indian Ocean (N): Madagascar	-	JQ067279	JQ041019	-	JQ041164

Species name	Voucher information and geographical origin	Species distribution range	ETS	ITS	matK	psbA-trnH	rbcL
<i>Crotalaria decziana</i> Polhill	R. B. Kwatha and J. L. Balaka 133, Malawi (MO)	Africa (N): Malawi	KX390788	KX371692	-	-	-
<i>Crotalaria deserticola</i> Baker f.	Bidgood et al. 1197, Tanzania (UPS)	Africa (N): Burundi, Ethiopia, Kenya, Malawi, Mozambique, Rwanda, Tanzania, Uganda, Zaire, Zambia, Zimbabwe	-	JQ067203	JQ041020	JQ067423	JQ041165
<i>Crotalaria dewildemaniana</i> R. Wilczek	Reekmans 9292, Burundi (UPS)	Africa (N): Burundi, Cameroon, Kenya, Rwanda, Tanzania, Uganda, Zaire	-	JQ067197	JQ041021	-	JQ041166
<i>Crotalaria dinteri</i> Schinz	Ujas MIU443, Namibia (WIND)	Africa (N): Botswana, Namibia, South Africa	-	JQ067147	JQ041022	-	JQ041167
<i>Crotalaria diosmifolia</i> Benth.	Du Puy M320, Madagascar (P)	Indian Ocean (N): Madagascar	-	JQ067269	-	JQ067488	JQ041168
<i>Crotalaria dissitiflora</i> Benth.	Bean 12986, Australia (MEL)	Australasia (N): Australia	-	JQ067288	JQ041023	JQ067502	JQ041169
<i>Crotalaria distans</i> Benth.	B.-E. van Wyk 1814, South Africa (JRAU)	Africa (N): Botswana, Lesotho, Malawi, Namibia, South Africa, Zaire, Zambia, Zimbabwe	-	JQ067124	JQ041024	-	JQ041170
<i>Crotalaria distantiflora</i> Baker f.	Gilbert and Dagner 8427, Ethiopia (UPS)	Africa (N): Ethiopia, Kenya, Tanzania	-	JQ067204	JQ041025	JQ067424	JQ041171
<i>Crotalaria doidegae</i> Verd.	B.-E. van Wyk 3042, South Africa (JRAU)	Africa (N): South Africa	-	JQ067131	-	-	JQ692942
<i>Crotalaria dubia</i> Graham	Stocks s. n. [M-0242310], India (M)	Asia (N): Bangladesh, China, India, Myanmar, Thailand	KX390789	KX371693	-	-	-
<i>Crotalaria duboisii</i> R. Wilczek	Bidgood et al. 3984, Tanzania (UPS)	Africa (N): Zaire, Zambia	-	JQ067193	JQ041026	-	JQ041172
<i>Crotalaria dumosa</i> Franch.	Thulin et al. 3751, Ethiopia (UPS)	Africa (N): Ethiopia, Kenya, Somalia	-	JQ067194	JQ041027	-	JQ041173
<i>Crotalaria dura</i> J.M.Wood & M.S.Evans	B.-E. van Wyk 4332, South Africa (JRAU)	Africa (N): Mozambique, South Africa	-	JQ067128	JQ041028	JQ067350	JQ041174
<i>Crotalaria ebenoides</i> (Guill. & Perr.) Walp.* Sect. <i>Chrysocalycinae</i> (Benth.) Bak. f.	Espirito Santo Explorações Botânicas 3509, Guinea-Bissau (M)	Africa (N): Guinea Bissau, Mali, Senegal	-	KX371694	-	-	-
<i>Crotalaria emarginata</i> Benth.	Hedberg et al. 300, Tanzania (UPS)	Africa (N): Kenya, Tanzania	-	JQ067195	JQ041029	-	JQ041176
<i>Crotalaria emarginella</i> Vatke	D. Podlech 35856, Yemen (M)	Africa (N): Djibouti, Ethiopia, Kenya, Somalia, Sudan	KX390790	KX371695	-	JQ067416	JQ041177
<i>Crotalaria emirnensis</i> Benth.	Peltier 2098, Madagascar (P)	Indian Ocean (N): Madagascar	-	JQ067273	-	-	JQ041178
<i>Crotalaria ephemeris</i> Polhill	Bidgood et al. 3893, Tanzania (UPS)	Africa (N): Zaire, Zambia	-	JQ067202	JQ041030	JQ067422	JQ041179
<i>Crotalaria epunctata</i> Dalzell	Manudev 5202, India (DUH)	Asia (N): India	-	JQ945952	-	-	-
<i>Crotalaria eremaea</i> F. Mueller	ETS, ITS, psbA-trnH: Hill 475, Australia (M) rbcL: Vonow 700, South Australia (MO)	Australasia (N): Australia	KX390791	KX371696	-	KX390746	KX357619
<i>Crotalaria eremicola</i> Baker f.	Kolberg and Tholkes HK1637, Namibia (WIND)	Africa (N): Botswana, Mozambique, Namibia, South Africa	-	JQ067154	JQ041031	JQ067375	JQ041180
<i>Crotalaria evoluloides</i> Benth.	S. S. Subramaniam 1039, India (DUH)	Asia (N): India, Sri Lanka, Vietnam	-	JN990124	-	-	-
<i>Crotalaria excisa</i> (Thunb.) Baker f.	B.-E. van Wyk 3108, South Africa (JRAU)	Africa (N): South Africa	-	JQ067137	JQ041032	JQ067357	JQ041181

Species name	Voucher information and geographical origin	Species distribution range	ETS	ITS	matK	psbA-trnH	rbcL
<i>Crotalaria eximia</i> Polhill	Milne-Redhead and Taylor 10496, Tanzania (UPS)	Africa (N): Tanzania	-	JQ067200	-	-	JQ041182
<i>Crotalaria fascicularis</i> Polhill	Roos 838, Ethiopia (UPS)	Africa (N): Ethiopia, Kenya, Uganda	-	JQ067199	JQ041033	-	JQ041183
<i>Crotalaria ferruginea</i> Benth.	S. Isles and A. Vinas 32419, Papua New Guinea (M)	Asia (N): Bangladesh, Bhutan, China, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Philippines, Sri Lanka, Taiwan, Thailand, Vietnam; Australasia (I): Papua New Guinea	-	KX371697	-	-	-
<i>Crotalaria fihenensis</i> R.Vig.	Du Puy M154, Madagascar (P)	Africa (I): Senegal; Indian Ocean (N): Madagascar	-	JQ067275	-	JQ067494	JQ041184
<i>Crotalaria filiculis</i> Baker	Bidgood et al. 3340, Tanzania (UPS)	Africa (N): Angola, Malawi, Mozambique, Tanzania, Zaire, Zambia, Zimbabwe	-	JQ067207	JQ041034	-	JQ041185
<i>Crotalaria filifolia</i> Rose	J. C. Soto Núñez 10449, Mexico (M)	Central America (N): Mexico	-	KX371698	-	-	-
<i>Crotalaria filiformis</i> Benth.	Maxwell 89-1458, Thailand (MO)	Asia (N): Myanmar, Thailand	KX390792	KX371699	-	-	-
<i>Crotalaria filipes</i> Benth.	S. S. Subramaniam and A. K. Pandey 3416, India (DUH)	Asia (N): India	-	JQ945926	-	-	-
<i>Crotalaria flavicarinata</i> Baker f.	Hochobes MH1267, Namibia (WIND)	Africa (N): Botswana, Namibia, Zambia, Zimbabwe	-	JQ067309	JQ067559	-	JQ067586
<i>Crotalaria flavicoma</i> Benth.	A. S. Flores et al. 822, Brazil (MIRR)	South America (N): Brazil	-	KX371700	-	-	-
<i>Crotalaria florida</i> Baker (Welw. ex Bak.)	E. A. Robinson 6096, Zimbabwe (M)	Africa (N): Angola, Tanzania, Zaire, Zambia	-	KX371701	-	-	-
<i>Crotalaria friesii</i> Verd.	Drummond 6843, Zimbabwe (UPS)	Africa (N): Zambia, Zimbabwe	-	JQ067258	-	-	-
<i>Crotalaria fysonii</i> Dunn	S. S. Subramaniam 1035, India (DUH)	Asia (N): India	-	JN990127	-	-	-
<i>Crotalaria gamwelliae</i> Baker f.	Bidgood et al. 4596, Tanzania (UPS)	Africa (N): Tanzania, Zimbabwe	-	JQ067213	JQ041037	-	JQ041188
<i>Crotalaria gazensis</i> Baker f.	Lotter 2062, Mozambique (K)	Africa (N): Mozambique, South Africa, Zimbabwe	-	JQ067315	JQ067563	JQ067523	JQ067587
<i>Crotalaria gillettii</i> Polhill	Mesfin and Kagneu 2423, Ethiopia (UPS)	Africa (N): Ethiopia	-	JQ067216	JQ041038	-	JQ041189
<i>Crotalaria glauca</i> Willd. * Sect. <i>Glaucæ</i> (Benth.) M.M. Le Roux & B.-E. van Wyk	Bidgood et al. 4521, Tanzania (UPS)	Africa (N): Angola, Benin, Burkina Faso, Burundi, Cameroon, Central African Republic, Chad, Congo, Ethiopia, Gabon, Ghana, Guinea, Guinea Bissau, Ivory Coast, Kenya, Malawi, Mali, Mozambique, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, Sudan, Tanzania, Togo, Uganda, Zaire, Zambia, Zimbabwe	-	JQ067230	-	-	-
<i>Crotalaria glaucoides</i> Baker f.	J. Raynal 6603, Senegal (M)	Africa (N): Guinea Bissau, Mali, Senegal	-	KX371702	-	-	-
<i>Crotalaria glabifera</i> E.Mey.	Stirton 11860, South Africa (RAU)	Africa (N): South Africa, Swaziland	-	JQ067125	JQ041039	JQ067347	JQ041190
<i>Crotalaria goetzei</i> Harms	Thulin 7826, Malawi (UPS)	Africa (N): Malawi, Tanzania, Zambia	-	JQ067210	JQ041040	JQ067430	JQ041191
<i>Crotalaria goiasensis</i> Windler & S.G.Skinner	Pereira-Silva et al. 8908, Brazil (CEN)	South America (N): Brazil	-	JX120586	-	-	-
<i>Crotalaria goodiiiformis</i> Vatke	Lotter 2041, Mozambique (K)	Africa (N): Kenya, Mozambique, Tanzania, Zaire	-	JQ067317	JQ067565	-	JQ041192

Species name	Voucher information and geographical origin	Species distribution range	ETS	ITS	matK	psbA-trnH	rbcL
<i>Crotalaria gorensis</i> Gull. & Perr. * Sect. <i>Stipulosae</i> (Baker f.) M.M. Le Roux & B.-E. van Wyk	A. N. Millar 35375, Papua New Guinea (M)	Africa (N): Angola, Benin, Burkina Faso, Burundi, Cameroon, Central African Republic, Chad, Congo, Ethiopia, Gabon, Ghana, Guinea, Guinea Bissau, Ivory Coast, Kenya, Liberia, Malawi, Mali, Mozambique, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, Sudan, Tanzania, The Gambia, Togo, Uganda, Zaire, Zambia, Zimbabwe, Asia: India (I), Malaysia(N); Australia (I); Australia, Papua New Guinea; Indian Ocean (I); Madagascar, Pacific Ocean (I); Bismarck Archipelago; South America (I): Brazil, French Guiana, Guyana Asia (N): India; Indian Ocean (I); Madagascar, Mauritius, Reunion	-	JQ067257	JQ041042	JQ067476	JQ041193
<i>Crotalaria grahamiana</i> Wight & Arn	Bean 6119, Australia (MEL)		-	JQ067294	-	JQ067507	-
<i>Crotalaria graminicola</i> Baker f.	Bidgood et al. 4759, Tanzania (UPS)	Africa (N): Benin, Burundi, Cameroon, Central African Republic, Ghana, Tanzania, Togo, Zaire, Zambia	-	JQ067243	-	-	JQ041194
<i>Crotalaria grandiflora</i> Windler & Skinner (M)	E. Pereira 4539 and Pabst 4920, Brazil (M)	South America (N): Brazil	-	KX371703	-	-	-
<i>Crotalaria greenwayi</i> Baker f.	Abdallah et al. 321, Tanzania (UPS)	Africa (N): Kenya, Tanzania	-	JQ067256	-	JQ067475	JQ041196
<i>Crotalaria grevei</i> Drake	Allorge 2339, Madagascar (P)	Indian Ocean (N): Madagascar	-	JQ067268	-	JQ067487	JQ041197
<i>Crotalaria griquensis</i> Bolus	B.-E. van Wyk 2533, South Africa (JRAU)	Africa (N): South Africa, Swaziland	-	JQ067134	JQ041044	JQ067354	JQ041198
<i>Crotalaria harleyi</i> Windler & Skinner	A. S. Flores et al. 780, Brazil (M)	South America (N): Brazil	KX390793	KX371704	-	KX390749	KX083395
<i>Crotalaria hebecarpa</i> (DC.) Rudd	Subramaniam 1031, India (DUH)	Asia (N): India, Sri Lanka	-	JN990130	-	-	-
<i>Crotalaria heidmannii</i> Schinz	M. M. Le Roux et al. 60, Namibia (WIND)	Africa (N): Angola, Botswana, Namibia, Zimbabwe	-	JQ067320	JQ041045	JQ067527	JQ041199
<i>Crotalaria hemsleyi</i> Milne-Redh.	Thulin and Mhoro 3073, Tanzania (UPS)	Africa (N): Tanzania	-	JQ067225	JQ041046	-	JQ041200
<i>Crotalaria heyneana</i> Wight & Arn.	S. S. Subramaniam 1061, India (DUH)	Asia (N): India	-	JQ945942	-	-	-
<i>Crotalaria hilariana</i> Benth.	A. S. Flores and R. Schütz Rodrigues 445, Brazil (MIRR)	South America (N): Brazil	-	KX371705	-	-	-
<i>Crotalaria hirsuta</i> Willd.	S. S. Subramaniam and A. K. Pandey 5026, India (DUH)	Asia (N): India, Myanmar; Indian Ocean (I): Mauritius	-	KP698652	-	-	-
<i>Crotalaria hirta</i> Willd.	ITS: S. S. Subramaniam and A. K. Pandey 1092 (DUH) unknown (K)	Asia (N): Cambodia, India, Vietnam; Indian Ocean (I): Comoro Is, Madagascar, Mauritius, Mayotte	-	JQ945928	-	JQ067546	-
<i>Crotalaria holosericea</i> Nees & Mart.	psbA-trnH: Peltier and Peltier, locality Coradiv L 6050, Brazil (CEN)	South America (N): Brazil	-	JQ067326	JQ067572	JQ067531	-
<i>Crotalaria humbertiana</i> M. Peltier	Labat 2456bis, Madagascar (P)	Indian Ocean (N): Madagascar	-	JQ067266	JQ041047	JQ067485	JQ041201
<i>Crotalaria humifusa</i> Benth.	S. S. Subramaniam and A. K. Pandey 5029, India (M)	Asia (N): Bhutan, India, Indonesia, Lesser Sunda Is, Malaysia, Myanmar, Nepal, Philippines, Thailand; Australasia (N): Australia, Papua New Guinea	KX390794	-	-	-	-
<i>Crotalaria humilis</i> Eckl. & Zeyh.	B.-E. van Wyk 2882, South Africa (JRAU)	Africa (N): South Africa	-	JQ067138	JQ041048	JQ067358	JQ041202
<i>Crotalaria hyssopifolia</i> Klotzsch	Bidgood et al. 3737, Tanzania (UPS)	Africa (N): Burkina, Burundi, Cameroon, Central African Republic, Chad, Ethiopia, Ghana, Guinea, Guinea-Bissau, Ivory Coast, Kenya, Malawi, Mozambique, Nigeria, Rwanda, Senegal, Sierra Leone, Sudan, Tanzania, Togo, Uganda, Zaire, Zimbabwe	-	JQ067217	JQ041049	-	JQ041203
<i>Crotalaria ibityensis</i> R. Vig. & Humbert	J. M. Hildebrandt 3892, Madagascar (M)	Indian Ocean (N): Madagascar	KX390795	-	-	-	-
<i>Crotalaria impressa</i> Walp.	Rydling et al. 1078, Eritrea (UPS)	Africa (N): Djibouti, Ethiopia, Sudan	-	JQ067229	JQ041050	-	JQ041204

Species name	Voucher information and geographical origin	Species distribution range	ETS	ITS	matK	psbA-trnH	rbcl
<i>Crotalaria incana</i> L. * Sect. <i>incanae</i> (Benth.) Polhill	ETS: M. F. Devecchi MFD176, Brazil (SPF) ITS: Thulin et al. 9114, Somalia (UPS)	Africa (U): Burundi, Cameroon, Ethiopia, Kenya, Malawi, Mozambique, Nigeria, Rwanda, Sierra Leone, Somalia, Tanzania, Uganda, Zaire, Zambia, Zimbabwe; Asia: Bangladesh (I), China (N), India (I), Indonesia (I), Malaysia (N), Moluccas (N), Philippines (I), Singapore (I), Sri Lanka (I), Sumatra (N), Taiwan (N), Thailand (N), Vietnam (N); Australasia (I): Australia, Papua New Guinea; Caribbean (U): Anguilla, Antigua-Barbuda, Bahamas, Barbados, Cayman Is, Cuba, Dominica, Dominican Republic, Grenada, Guadeloupe, Haiti, Jamaica, Netherlands Leeward Is, Puerto Rico, St Kitts-Nevis, St Martin-St Barthelemy, St Vincent, Virgin Is; Central America (U): Belize, Costa Rica, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama; Indian Ocean (U): Madagascar, Mauritius, Reunion; Middle East (I): Saudi Arabia, Yemen; North America (I): United States; Pacific Ocean (I): Hawaii, Marshall Is, Northern Marianas, Society Is, Solomon Is, Vanuatu; South America (U): Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, French Guiana, Galapagos, Guyana, Paraguay, Peru, Venezuela	KX390796	JQ067262	JQ041051	-	JQ041205
<i>Crotalaria incarassifolia</i> Polhill	Thulin et al. 10085, Somalia (UPS)	Africa (N): Ethiopia, Somalia	-	JQ067255	JQ041052	JQ067474	JQ041206
<i>Crotalaria inopinata</i> (Harms) Polhill	Manktelowi et al. 91080, Tanzania (UPS)	Africa (N): Tanzania	-	JQ067253	JQ041053	JQ067472	JQ041207
<i>Crotalaria involutifolia</i> Polhill	Bidgood et al. 4527, Tanzania (UPS)	Africa (N): Cameroon, Tanzania, Zaire, Zambia	-	JQ067249	JQ041054	-	JQ041208
<i>Crotalaria iringana</i> Harms	Bidgood et al. 3343, Iringana (UPS)	Africa (N): Tanzania	-	JQ067218	JQ041055	-	JQ041209
<i>Crotalaria irwinii</i> Windler & S.G. Skinner	H. S. Irwin et al. 21607, Brazil (K)	South America (N): Brazil	KX390797	KX371706	-	KX390743	-
<i>Crotalaria juncea</i> L.	ITS: Chalmers 1878, Australia (M) ITS: S. Subramaniam et al. 1026, India (DUH) matK, psbA-trnH, rbcl: M. M. Le Roux et al. 36, South Africa (JRAU)	Africa (U): Ghana, Kenya, Nigeria, Senegal, South Africa, Tanzania, Togo, Uganda; Asia: Afghanistan (I), Bangladesh (U), Bhutan (N), Burma (U), Cambodia (N), China (N), India (N), Indonesia (I), Iraq (I), Iran Jaya (I), Laos (N), Lesser Sunda Is (N), Malaysia (I), Moluccas (N), Myanmar (N), Nepal (I), Pakistan (I), Philippines (I), Singapore (I), Sri Lanka (I), Sulawesi (N), Surinam (N), Taiwan (N), Thailand (N), Vietnam (N), Uzbekistan (I), Papua New Guinea; Caribbean (I): Antigua-Barbuda, Barbados, Dominican Republic, Haiti, Jamaica, Martinique, Puerto Rico, Virgin Is; Indian Ocean (I): Andaman Is, Madagascar, Mauritius, Reunion, Seychelles; Pacific Ocean (I): Niue; South America (I): Brazil, Colombia, Guyana, Surinam	KX390798	JN990138	JQ041056	JQ067365	JQ041210
<i>Crotalaria karagwensis</i> Taub.	Gilbert et al. 549, Ethiopia (UPS)	Africa (N): Burundi, Cameroon, Ethiopia, Kenya, Rwanda, Tanzania, Uganda, Zaire	-	JQ067228	JQ041058	JQ067448	JQ041212
<i>Crotalaria keniensis</i> Baker f.	Ryman 217, Kenya (UPS)	Africa (N): Ethiopia, Kenya, Tanzania, Uganda	-	JQ067224	JQ041059	JQ067444	JQ041213
<i>Crotalaria khasiana</i> Thoth. & A.A. Ansari	E. E. Maire s. n. [M-0242318], China (M)	Asia (N): Bhutan, India, Myanmar	KX390799	KX371707	-	-	-
<i>Crotalaria kipandensis</i> Baker f.	Bidgood et al. 4060, Tanzania (UPS)	Africa (N): Malawi, Mozambique, Tanzania, Zaire, Zambi, Zimbabwe	-	JQ067223	JQ041060	-	JQ041214
<i>Crotalaria kirkii</i> Baker	Markström and Nilsson 215A, Tanzania (UPS)	Africa (N): Kenya, Mozambique, Tanzania	-	JQ067212	JQ041061	-	JQ041215
<i>Crotalaria kurtzii</i> Schinz	ITS, ETS, psbA-trnH, rbcl: Dr. Leippert 4479, Namibia (M) matK: Mannheimer CM2680, Namibia (WIND)	Africa (N): Namibia	KX390800	KX371708	JQ041062	KX390755	KX083398
<i>Crotalaria kurzii</i> Kurz	M. van de Bult 1216, Thailand (M)	Asia (N): Bangladesh, China, India, Laos, Myanmar, Thailand, Vietnam	KX390801	KX371709	-	-	-

Species name	Voucher information and geographical origin	Species distribution range	ETS	ITS	mark	psbA-trnH	rbcl
<i>Crotalaria laburnifolia</i> L. * Sect. <i>Grandiflorae</i> (Bak.f.) Polhill	B.-E. van Wyk et al. 4630, Ethiopia (JRAU)	Africa (N): Botswana, Burundi, Chad, Djibouti, Ethiopia, Kenya, Malawi, Mozambique, Rwanda, Somalia, South Africa, Sudan, Swaziland, Tanzania, Uganda, Zaire, Zambia, Zimbabwe; Asia (N): Bangladesh, India, Indonesia, Lesser Sunda Is, Malaysia, Sri Lanka, Thailand, Australasia (N): Australia; Indian Ocean: Mauritius (I), Seychelles (N)	-	JX120577	JX120589	JX120592	JX120598
<i>Crotalaria laburnoides</i> Klotzsch	Borhidi et al. 85562, Tanzania (UPS)	Africa (N): Kenya, Malawi, Mozambique, Somalia, South Africa, Tanzania, Uganda, Zaire; Indian Ocean: Aldabra (I), Comoro Is (N)	-	JQ067236	JQ041064	JQ067455	JQ041218
<i>Crotalaria lachnocarpoides</i> Engl.	Kelbessa et al. 10, Malawi (UPS)	Africa (N): Burundi, Ethiopia, Kenya, Malawi, Mozambique, Sudan, Tanzania, Uganda, Zaire, Zambia, Zimbabwe	-	JQ067264	-	-	JQ041219
<i>Crotalaria lachnophora</i> A.Rich.	Lotter 2035, Mozambique (K)	Africa (N): Angola, Burundi, Cameroon, Chad, Ethiopia, Ghana, Ivory Coast, Kenya, Malawi, Mozambique, Nigeria, Rwanda, Senegal, Sudan, Tanzania, Togo, Uganda, Zaire, Zambia, Zimbabwe	-	JQ067316	JQ067564	-	JQ067588
<i>Crotalaria laeta</i> Mart. ex Benth.	ETS, ITS: F. A. M. Santos 18916, Brazil (M) mark, psbA-trnH: Coradiv L 7718, Brazil (CEN)	South America (N): Brazil	KX390802	KX371710	JQ067571	JQ067530	-
<i>Crotalaria laevigata</i> Lam.	ITS: S. Subramaniam 1050, India (DUH) mark, rbcl: Labat 2413, Madagascar (P)	Indian Ocean (N): Madagascar	-	JN990116	JQ041065	-	JQ041220
<i>Crotalaria lanceolata</i> E.Mey.	ETS, ITS, rbcl, psbA-trnH: A. Rockinger 20143, Brazil (M) mark: B.-E. van Wyk 4329, South Africa (JRAU)	Africa (N): Ethiopia, Kenya, Malawi, Mozambique, South Africa, Sudan, Swaziland, Tanzania, Uganda, Zambia, Zimbabwe; Asia (N): China, Taiwan; Caribbean (I): Caribbean-TRP; Indian Ocean (U): Madagascar; North America (I): United States; South America (I): Brazil, Colombia	KX390803	KX371711	JQ067553	KX390750	KX083396
<i>Crotalaria lasiocarpa</i> Polhill	C. F. Paget-Wilkes 1055, Tanzania (MO)	Africa (N): Tanzania, Zambia	KX390804	KX371712	-	-	-
<i>Crotalaria lathyroides</i> Guill. & Perr.	Espirito Santo Explorações Botânicas 3640, Guinea-Bissau (M) Richards 23228, Zambia (UPS)	Africa (N): Benin, Gambia, Guinea-Bissau, Ivory Coast, Liberia, Mali, Senegal, Sierra Leone	KX390805	KX371713	-	-	-
<i>Crotalaria laxiflora</i> Baker	Clement 2024, Madagascar (P)	Africa (N): Tanzania, Zambia	-	JQ067232	JQ041067	JQ067452	JQ041222
<i>Crotalaria leonardiana</i> M.Peltier	ITS, mark: M. M. Le Roux and B.-E. van Wyk 104, South Africa (JRAU)	Indian Ocean (N): Madagascar	-	JQ067284	JQ041068	JQ067500	JQ041223
<i>Crotalaria lebeckioides</i> Bond	rbcl: B.-E. van Wyk 3315e, locality unknown (JRAU)	Africa (N): South Africa	-	JQ067321	JQ067568	-	EU348036
<i>Crotalaria lebrunii</i> Baker f.	Rwaburindore 2744, Uganda (UPS)	Africa (N): Kenya, Uganda, Zaire	-	JQ067250	-	JQ067469	-
<i>Crotalaria lepidissima</i> Baker f.	M. Richards 17152, Angola (B)	Africa (N): Angola, Zaire, Zambia	KX390806	KX371714	-	-	-
<i>Crotalaria lepreurii</i> Guill. & Perr.	H. Ern et al. 1850, Togo (B) Thulin and Warfa 4640, Somalia (UPS)	Africa (N): Angola, Burkina Faso, Cameroon, Central African Republic, Chad, Ghana, Guinea, Guinea-Bissau, Ivory Coast, Mali, Nigeria, Senegal, Sudan, Togo, Uganda, Zaire Africa (N): Somalia	KX390807	KX371715	-	-	-
<i>Crotalaria linearifoliolata</i> Chiov.	Nakaikes n. [M-0242306], Japan (M)	Asia (N): China, India, Myanmar, Ryukyu Is, Sri Lanka, Taiwan; Caribbean (U): Caribbean-TRP; Indian Ocean (N): Nicobar Is	KX390808	-	-	-	-
<i>Crotalaria linifolia</i> L.f.	M. M. Le Roux et al. 101, South Africa (JRAU)	Africa (N): South Africa	-	JQ067307	JQ067557	JQ067516	JQ067589
<i>Crotalaria longidens</i> Verd.	S. S. Subramaniam 1045, India (DUH)	Asia (N): India	-	JN990113	-	-	-
<i>Crotalaria longipes</i> Wight & Arn.	J. Kufer 63, Guatemala (MSB)	Central America (N): Costa Rica, El Salvador, Guatemala, Mexico, Nicaragua, Panama; North America (U): United States	KX390809	KX371716	-	-	-

Species name	Voucher information and geographical origin	Species distribution range	ETS	ITS	matK	psbA-trnH	rbcL
<i>Cratalaria latifolia</i> L.* Sect. <i>Cratalaria</i> M.M. Le Roux & B.-E. van Wyk	I. Urban 3650, Puerto Rico (M)	Caribbean (U); Central America (N); Honduras, Mexico	-	KX371717	-	-	-
<i>Cratalaria latiformis</i> Milne-Redh.	Ryman 94, Kenya (UPS)	Africa (N); Kenya	-	JQ067234	JQ041070	JQ067453	JQ041225
<i>Cratalaria laiooides</i> Benth.	M. M. Le Roux et al. 47, South Africa (JRAU)	Africa (N); Botswana, South Africa, Zimbabwe	-	JQ067299	JQ067551	JQ067510	JQ067590
<i>Cratalaria lukwangulensis</i> Harms	Borhidi et al. 85.142, Tanzania (UPS)	Africa (N); Kenya, Tanzania	-	JQ067211	JQ041071	JQ067431	JQ041226
<i>Cratalaria lunulata</i> Wight & Arn.	R. Wight 615, India (M)	Asia (N); India, Sri Lanka	-	KX371718	-	-	-
<i>Cratalaria luondeensis</i> R. Wilczek	Iversen et al. 87691, Tanzania (UPS)	Africa (N); Mozambique, Tanzania, Zaire	-	JQ067237	-	-	JQ041228
<i>Cratalaria lutescens</i> Dalzell	Fernandes 560, India (K)	Asia (N); India	-	JQ067337	-	JQ067542	-
<i>Cratalaria macrocarpa</i> E. Mey.	Stirton 11885, South Africa (JRAU)	Africa (N); South Africa, Zimbabwe	-	JQ067126	JQ041072	-	JQ041229
<i>Cratalaria madurensis</i> Wight & Arn.	S. S. Subramaniam and A. K. Pandey 1015, India (DUH)	Asia (N); India, Indian Ocean (I); Reunion	-	KP098626	-	-	-
<i>Cratalaria magaliesbergensis</i> A.S. Flores & Sch. Rodr. (<i>Cratalaria brachycarpa</i> (Benth.) Burt Davy ex Verdoorn)	B.-E. van Wyk 1729, South Africa (JRAU)	Africa (N); South Africa	-	JQ067132	-	-	JQ692943
<i>Cratalaria mahajalensis</i> R. Vig.	Miller 6166, Madagascar (P)	Indian Ocean (N); Madagascar	-	JQ067280	-	JQ067496	JQ041230
<i>Cratalaria mairei</i> H. Lev.	He 483, China (M)	Asia (N); China	KX390810	KX371719	-	-	-
<i>Cratalaria malindiensis</i> Polhill	Thulin et al. 7643, Somalia (UPS)	Africa (N); Kenya, Somalia	-	JQ067263	JQ041073	JQ067482	JQ041231
<i>Cratalaria maritima</i> Benth.	A. S. Flores and R. Schütz Rodrigues 983, Brazil (M)	South America (N); Argentina, Brazil, Paraguay	-	KX371720	-	-	-
<i>Cratalaria massaiensis</i> Taub.	Jansell 4571, Kenya (UPS)	Africa (N); Ethiopia, Kenya, Somalia, Uganda	-	JQ067226	JQ041074	JQ067446	JQ041232
<i>Cratalaria mauensis</i> Baker f.	Ryman 132, Kenya (UPS)	Africa (N); Kenya, Tanzania	-	JQ067235	JQ041075	-	JQ041233
<i>Cratalaria medicaginea</i> Lam.* Sect. <i>Dispermae</i> Wight & Arn.	Chantaranothai and Parnell 90/759, Thailand (K)	Asia (N); Afghanistan, Bangladesh, Burma, China, India, Indonesia, Java, Laos, Myanmar, Nepal, Pakistan, Philippines, Sri Lanka, Taiwan, Thailand, Vietnam; Australasia (N); Australia, Papua New Guinea; Indian Ocean (N); Laccadive Is.; Middle East (U); Oman	-	JQ067334	-	JQ067539	-
<i>Cratalaria melanocalyx</i> Polhill	Biggood et al. 3595, Tanzania (UPS)	Africa (N); Tanzania	-	JQ067247	JQ041076	-	JQ041234
<i>Cratalaria mesopotamica</i> Taub.	Rwaburindore 2340, Uganda (UPS)	Africa (N); Burundi, Entral African Republic, Rwanda, Tanzania, Uganda, Zaire; Asia (I); Indonesia	-	JQ067215	JQ041077	-	JQ041235
<i>Cratalaria meyerana</i> Steud.	Williamson 3378, Namibia (JRAU)	Africa (N); Namibia, South Africa	-	JQ067136	-	JQ067356	JQ041236

Species name	Voucher information and geographical origin	Species distribution range	ETS	ITS	matK	psbA-trnH	rbcL
<i>Crotalaria micans</i> Link	A. S. Flores et al. 675, Brazil (MIRR)	Africa (I); Zaire; Asia (I); Bangladesh, China, India, Indonesia, Laos, Malaysia, Nepal, Philippines, Sabah, Sri Lanka, Sumatra, Taiwan, Thailand, Vietnam; Australasia (I): Australia, Papua New Guinea; Caribbean (N): Dominica, Guadeloupe, Martinique, Montserrat, St Kitts-Nevis, St Vincent, Trinidad and Tobago; Central America (N): Mexico, Nicaragua, Panama; Indian Ocean (I): Madagascar, Reunion, Pacific Ocean (I): Bismarck Archipelago, Fiji, Hawaii, Niue, Western Samoa; South America (N): Argentina, Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Paraguay, Peru, Surinam, Uruguay, Venezuela	-	KX371721	-	-	-
<i>Crotalaria microcarpa</i> Benth.	ITS: Bidgood et al. 3341, Tanzania (UPS) ETS: E. A. Robinson 6430, Zambia (M)	Africa (N): Angola, Burkina Faso, Cameroon, Chad, Ethiopia, Ghana, Kenya, Malawi, Mali, Mozambique, Niger, Nigeria, Rwanda, Sudan, Tanzania, Togo, Uganda, Zambia, Zimbabwe	KX390811	JQ067222	JQ041079	JQ067442	JQ041237
<i>Crotalaria microphylla</i> M. Vahl	Thulin 11016, Somalia (UPS)	Africa (N): Chad, Djibouti, Egypt, Ethiopia, Mauritania, Niger, Somalia, Sudan; Middle East (N): Saudi Arabia, Yemen	-	JQ067239	JQ041080	JQ067458	JQ041238
<i>Crotalaria microthamnus</i> R. Wilczek	Bidgood et al. 3726, Tanzania (UPS)	Africa (N): Zaire, Zambia	-	JQ067227	JQ041081	JQ067447	JQ041239
<i>Crotalaria mildbraedii</i> Baker f.	C. Kayombo 596, Tanzania (MO)	Africa (N): Ethiopia, Rwanda, Uganda, Zaire	KX390812	KX371722	-	-	-
<i>Crotalaria mioctone</i> A. S. Flores & A. M. G. Azevedo	Konishita et al. 12_02, Brazil (M)	South America (N): Brazil	KX390813	KX371723	-	-	-
<i>Crotalaria miranda</i> Milne-Redh.	Bidgood et al. 3970, Tanzania (UPS)	Africa (N): Tanzania, Zambia	-	JQ067245	JQ041082	JQ067464	JQ041240
<i>Crotalaria mitchellii</i> Benth.	R. Hill 319, South Australia (M)	Australasia (N): Australia	KX390814	KX371724	-	-	-
<i>Crotalaria molluca</i> Kunth.	N. Arsène s. n. [M-0242304], Mexico (M)	Central America (N): El Salvador, Guatemala, Mexico	KX390815	KX371725	-	-	-
<i>Crotalaria monophylla</i> Germish.	Burrows and Turpin 12245, South Africa (LYD)	Africa (N): South Africa	-	JX120581	JX120590	-	JX120602
<i>Crotalaria montana</i> Roth.	Clarkson 3044, Australia (SP)	Asia (N): Cambodia, China, India, Indonesia, Irian Jaya, Laos, Malaysia, Philippines, Sri Lanka, Taiwan, Thailand, Vietnam; Australasia (N): Australia, Papua New Guinea; Pacific Ocean (N): Bismarck Archipelago	-	KX371726	-	-	-
<i>Crotalaria monteiri</i> Baker f.	Schutte 83, South Africa (JRAU)	Africa (N): Mozambique, South Africa, Swaziland, Zimbabwe	-	JQ067129	JQ041083	JQ067351	JQ041241
<i>Crotalaria mortonii</i> Hepper	H. Em et al. 2044, Togo (B)	Africa (N): Ghana, Ivory Coast, Togo	KX390816	KX371727	-	-	-
<i>Crotalaria mudugensis</i> Thulin	Wieland 4618, Somalia (UPS)	Africa (N): Somalia [African species; full distribution range uncertain]	-	JQ067241	JQ041084	-	JQ041242
<i>Crotalaria muenzneri</i> Baker f.	Bidgood et al. 862, Tanzania (UPS)	Africa (N): Tanzania	-	JQ067220	JQ041085	-	JQ041243
<i>Crotalaria multiflora</i> (Arn.) Benth.	Hepper 4590, Sri Lanka (K)	Asia (N): India, Sri Lanka	-	JQ067336	-	JQ067541	-
<i>Crotalaria mysorensis</i> Roth	Haines 5573, India (K)	Asia (N): Bangladesh, China, India, Indonesia, Irian Jaya, Lesser Sunda Is, Nepal, Pakistan, Sri Lanka	-	JQ067340	-	-	-
<i>Crotalaria nana</i> Burm. f.	S. S. Subramaniam et al. 1063, India (M)	Asia (N): Cambodia, China, India, Indonesia, Myanmar, Nepal, Sri Lanka, Thailand, Vietnam; Australasia (N): Australia; Caribbean (I): Jamaica; Indian Ocean (N): Nicobar Is	-	KX371728	-	-	-
<i>Crotalaria natalitia</i> Meissner	M. M. Le Roux et al. 99, unknown locality (JRAU)	Africa (N): Angola, Burundi, Ethiopia, Kenya, Malawi, Mozambique, Rwanda, South Africa, Sudan, Swaziland, Tanzania, Uganda, Zaire, Zambia, Zimbabwe; Middle East (N): Yemen	-	JQ067304	JQ067554	JQ067362	JQ041244
<i>Crotalaria nayiritensis</i> Windler	Gabriel 4521, Mexico (MO)	Central America (N): Mexico	KX390817	KX371729	-	-	-
<i>Crotalaria neglecta</i> Wight & Arn.	O. Anders 8989, Afghanistan (M)	Asia (N): Afghanistan [full distribution range uncertain]	-	KX371730	-	-	-

Species name	Voucher information and geographical origin	Species distribution range	ETS	ITS	matK	psbA-trnH	rbcl
<i>Crotalaria nigricans</i> Baker	Bidgood et al. 4580, Tanzania (UPS)	Africa (N): Malawi, Mozambique, Tanzania, Zaire, Zambia	-	JQ067251	JQ041086	JQ067470	JQ041245
<i>Crotalaria nitens</i> Kunth	Plant cultivated in the Munich Botanical Garden as accession 01/1488 and vouchered, specimen [M-0242317] at (M)	Central America (N): Belize, Guatemala, Honduras, Mexico; South America (N): Bolivia, Brazil, Colombia, Ecuador, Guyana, Paraguay, Peru	KX390818	KX371731	-	-	-
<i>Crotalaria notanii</i> Wight & Arn.	S. S. Subramaniam and A. K. Pandey 11248, India (M)	Asia (N): India	-	KX371732	-	-	-
<i>Crotalaria novae-hollandiae</i> DC.	ITS: A. Rockinger 201534, Australia (M) matK, rbcL: Forster and Booth 24198, Australia (MEL)	Australasia (N): Australia	-	KX371733	JQ041087	-	JQ041246
<i>Crotalaria obscura</i> DC.	B.-E. van Wyk 2933, South Africa (JRAU)	Africa (N): South Africa	-	JQ067142	JQ041088	JQ067363	JQ041247
<i>Crotalaria obtecta</i> Wight & Arn.	S. S. Subramaniam et al. 1056, India (DUH)	Asia (N): India	-	JN990134	-	-	-
<i>Crotalaria ochroleuca</i> G. Don	Valls JFM 4933, Brazil (CEN)	Africa (N): Angola, Benin, Botswana, Burkina Faso, Burundi, Cameroon, Central African Republic, Chad, Congo, Ethiopia, Gabon, Ghana, Guinea, Guinea Bissau, Ivory Coast, Kenya, Liberia, Malawi, Mali, Mozambique, Niger, Nigeria, Sao Tome and Principe, Senegal, Sierra Leone, Sudan, Swaziland, Tanzania, Togo, Uganda, Zaire, Zambia, Zimbabwe; Asia (I): China; Australasia (I): Australia, Papua New Guinea; Indian Ocean (I): Madagascar; North America (I): United States; South America (I): Brazil	-	JQ067323	JQ067570	-	-
<i>Crotalaria oligosperma</i> Polhill	Thulin et al. 7745, Somalia (UPS)	Africa (N): Ethiopia, Kenya, Somalia	-	JQ067231	JQ041089	JQ067451	JQ041248
<i>Crotalaria ononoides</i> Benth.	Thulin and Mhoro 2874, Tanzania (UPS)	Africa (N): Angola, Benin, Botswana, Burkina Faso, Burundi, Cameroon, Central African Republic, Chad, Congo, Ethiopia, Gabon, Ghana, Guinea, Guinea Bissau, Ivory Coast, Kenya, Liberia, Malawi, Mali, Mozambique, Niger, Nigeria, Sao Tome and Principe, Senegal, Sierra Leone, Sudan, Swaziland, Tanzania, Togo, Uganda, Zaire, Zambia, Zimbabwe; Asia (I): China; Australasia (I): Australia, Papua New Guinea; Indian Ocean (I): Madagascar; North America (I): United States; South America (I): Brazil	-	JQ067330	JQ067577	JQ067536	JQ067593
<i>Crotalaria oocarpa</i> Baker	Gereau and Kayombo 4797, Tanzania (UPS)	Africa (N): Burundi, Ethiopia, Kenya, Malawi, Tanzania	-	JQ067219	-	JQ067439	JQ041249
<i>Crotalaria orientalis</i> Verd.	M. M. Le Roux et al. 91, South Africa (JRAU)	Africa (N): Botswana, Namibia, South Africa, Zimbabwe	-	JQ067313	JQ067561	-	JQ067594
<i>Crotalaria orixensis</i> Willd.	Mesfin and Kagnew 1598, Ethiopia (UPS)	Africa (N): Ethiopia; Asia (N): India, Philippines	-	JQ067260	-	JQ067479	JQ041250
<i>Crotalaria orthoclada</i> Baker	Hedrén et al. 665, Tanzania (UPS)	Africa (N): Angola, Burundi, Cameroon, Kenya, Malawi, Nigeria, Rwanda, Tanzania, Uganda, Zaire, Zambia	-	JQ067259	-	JQ067478	JQ041251
<i>Crotalaria otoptera</i> Benth.	A. S. Flores and R. Schütz Rodrigues 1002, Brazil (MIRR)	South America (N): Brazil	KX390819	KX371734	-	-	-

Species name	Voucher information and geographical origin	Species distribution range	ETS	ITS	matK	psbA-trnH	rbcL
<i>Cratalaria pallida</i> Guill. & Perr.	B.-E. van Wyk 4331, South Africa (JRAU)	Africa (N): Angola, Benin, Burkina Faso, Burundi, Cameroon, Central African Republic, Chad, Congo, Ethiopia, Gabon, Gambia, Ghana, Guinea, Guinea Bissau, Ivory Coast, Kenya, Liberia, Malawi, Mali, Mozambique, Niger, Nigeria, Rwanda, Sao Tome and Principe, Senegal, Sierra Leone, South Africa, Suda, Swaziland, Tanzania, Togo, Uganda, Zaire, Zambia, South Africa, Suda, Swaziland, Tanzania, Togo, Uganda, Zaire, Zambia, Zimbabwe; Asia (N): Bangladesh, Bhutan, Brunei, Cambodia, China, India, Indonesia, Iran, Java, Laos, Lesser Sunda Is, Malaysia, Moluccas, Myanmar, Nepal, Pakistan, Philippines, Sabah, Sarawak, Singapore, Sri Lanka, Sumatra, Taiwan, Thailand, Vietnam; Australasia (I): Australia, New Caledonia, Papua New Guinea; Caribbean (I): Dominica, Jamaica, Martinique, Montserrat, Puerto Rico, St Kitts-Nevis, St Vincent, Trinidad and Tobago; Central America (N): El Salvador, Nicaragua; Indian Ocean: Andaman Is (N), Chagos Archipelago (U), Christmas I (I), Madagascar (U), Mauritius (I), Nicobar Is (N), Seydheilles (I); Pacific Ocean (I): Bismarck Archipelago, Bougainville, Easter Is, Fiji, Guam, Hawaii, Marshall Is, Niue, Northern Marianas, Society Is, Solomon Is, Tonga, Tubai Is, Vanuatu, Western Samoa; South America (I): Argentina, Brazil, Colombia, Ecuador, Guyana, Peru, Venezuela	-	JQ067305	JQ067555	-	JQ067595
<i>Cratalaria pallidicaulis</i> Harms	Nyasisi 498, Zimbabwe (UPS)	Africa (N): Mozambique, Tanzania, Zaire, Zambia, Zimbabwe	-	JQ067254	-	-	JQ0692940
<i>Cratalaria parvula</i> Baker	Bidgood et al. 3775, Tanzania (UPS)	Africa (N): Angola, Burundi, Cameroon, Central African Republic, Malawi, Nigeria, Tanzania, Zaire, Zambia	-	JQ067246	JQ041090	-	JQ041252
<i>Cratalaria passerinoides</i> Taub.	Bidgood et al. 3591, Tanzania (UPS)	Africa (N): Tanzania, Zaire, Zambia	-	JQ067248	JQ041091	JQ067467	JQ041253
<i>Cratalaria pavulina</i> Schrank	A. S. Flores et al. 856, Brazil (MIRR)	Africa (I): Ethiopia, Kenya, Zimbabwe; Caribbean (I): Guadeloupe, Martinique; South America (N): Argentina, Brazil, Colombia, Venezuela	KX390820	KX371735	-	KX390744	-
<i>Cratalaria perrieri</i> R. Vig.	Du Puy M469, Madagascar (P)	Indian Ocean (N): Madagascar	-	JQ067271	JQ041092	JQ067490	JQ041254
<i>Cratalaria persica</i> (Burm.f.) Merr.	V. Mozaffarian 58261, Iran (TAR)	Africa (N): Djibouti, Ethiopia, Socotra, Somalia, Asia (N): Iran, Pakistan; Middle East (N): Oman, South Yemen, United Arab Emirates	KX390821	KX371736	-	KX390747	KX083397
<i>Cratalaria phylloloba</i> Harms	Mhoro 868, Tanzania (UPS)	Africa (N): Tanzania	-	JQ067252	JQ041093	-	JQ041255
<i>Cratalaria pilosa</i> Mill.	Costa N 2242, Brazil (CEN)	Caribbean (U): Caribbean-TNP; Central America (N): Costa Rica, Mexico, Panama; South America (U): Argentina, Brazil, Colombia, Venezuela	-	JQ067329	JQ067575	-	JQ067596
<i>Cratalaria pilsiflora</i> Baker	Brummit and Synge WC104, Malawi (UPS)	Africa (N): Malawi	-	JQ067265	JQ041094	-	JQ041256
<i>Cratalaria piscarpa</i> Baker	Strohhach B55993, Namibia (WIND)	Africa (N): Angola, Botswana, Malawi, Mozambique, Namibia, South Africa, Tanzania, Zambia, Zimbabwe	-	JQ067153	JQ041095	JQ067374	JQ041257
<i>Cratalaria pleiophylla</i> Polhill	Thulin and Warfa 4697, Somalia (UPS)	Africa (N): Ethiopia, Somalia	-	JQ067242	-	JQ067461	JQ041259
<i>Cratalaria plowdenii</i> Baker	Gilbert and Thulin 38, Ethiopia (UPS)	Africa (N): Ethiopia, Middle East (N): Yemen	-	JQ067244	JQ041097	-	JQ041260
<i>Cratalaria podocarpa</i> DC.	M. M. Le Roux et al. 68, Namibia (WIND)	Africa (N): Angola, Botswana, Chad, Ethiopia, Kenya, Malawi, Mali, Mauritania, Mozambique, Namibia, Niger, Senegal, South Africa, Sudan, Tanzania, Uganda, Zambia, Zimbabwe	-	JQ067300	-	JQ067511	JQ067598
<i>Cratalaria poissonii</i> R. Vig.	Labat 3631, Madagascar (P)	Indian Ocean (N): Madagascar	-	JQ067277	JQ041098	JQ067495	JQ041261
<i>Cratalaria polhillii</i> Thulin	Zemede and Birhanu 555, Ethiopia (UPS)	Africa (N): Ethiopia	-	JQ067233	-	-	-
<i>Cratalaria polyphylla</i> L. Riley	R. Mc Naugh 13777, Mexico (K)	Central America (N): Mexico	-	KX371738	-	-	-
<i>Cratalaria prittwiczii</i> Baker f.	Bidgood et al. 3597, Tanzania (UPS)	Africa (N): Angola, Tanzania, Zaire, Zambia	-	JQ067240	JQ041099	-	JQ041262
<i>Cratalaria prolongata</i> Baker	E. A. Robinson 6658, Zambia (M)	Africa (N): Angola, Malawi, Tanzania, Zaire, Zambia	-	KX371739	-	-	-

Species name	Voucher information and geographical origin	Species distribution range	ETS	ITS	matK	psbA-trnH	rbcl
<i>Crotalaria prostrata</i> Willd.	S. S. Subramaniam et al. 902, India (DUH)	Asia (N): Bangladesh, Cambodia, China, India, Indonesia, Myanmar, Nepal, Pakistan, Philippines, Sri Lanka, Sulawesi, Thailand, Vietnam	-	JQ945953	-	-	-
<i>Crotalaria pseudofoliolensis</i> R. Wilczek	F. Moreno 272, Angola (M)	Africa (N): Tanzania, Zaire, Zambia	-	KX371740	-	-	-
<i>Crotalaria pseudotenurama</i> Torre	Bidgood et al. 3590, Tanzania (UPS)	Africa (N): Angola, Burkina Faso, Cameroon, Ethiopia, Ghana, Guinea, Ivory Coast, Kenya, Malawi, Mali, Senegal, Sudan, Tanzania, Zaire, Zambia	-	JQ067209	JQ041100	-	JQ041263
<i>Crotalaria pulchra</i> Andrews	S. S. Subramaniam and A. K. Pandey 5070, India (M)	Asia (N): India; Indian Ocean (I): Mauritius	KX390822	KX371741	-	-	-
<i>Crotalaria pumila</i> Ortega	ETS: ITS: M. Subieta 248, Bolivia (M) mark: Wojciechowski 930, locality unknown (ASU)	Caribbean (N): Caribbean-TRP; Central America (U): Belize, Costa Rica, Guatemala, Mexico, Nicaragua; North America (N): United States; South America (N): Argentina, Bolivia, Ecuador, Galapagos, Peru, Venezuela	KX390823	KX371742	AY386867	-	-
<i>Crotalaria purdiana</i> Senn	C. D. Adams 11504, Jamaica (M)	Caribbean (U): Caribbean-TRP; Central America (N): Mexico; South America (N) Colombia	-	KX371743	-	-	-
<i>Crotalaria purshii</i> DC.	Radford 25035, USA (K)	North America (N): United States	-	JQ067342	-	-	-
<i>Crotalaria pusilla</i> Heyne ex Roth (DC.)	S. S. Subramaniam and A. K. Pandey 3427, India (M)	Asia (N): India	-	KX371744	-	-	-
<i>Crotalaria pycnostachya</i> Benth.	ITS: D. Podlech 36420, Yemen (MSB) mark: Hohbein A9_K1267, Kenya (EA)	Africa (N): Djibouti, Ethiopia, Kenya, Somalia, Sudan, Tanzania, Uganda; Middle East (N): Yemen	-	KX371745	KR735006	-	-
<i>Crotalaria quartiana</i> A. Rich.	I. Frils et al. 3768, Ethiopia (B)	Africa (N): Angola, Burundi, Cameroon, Ethiopia, Kenya, Nigeria, Rwanda, Sudan, Tanzania, Zaire; Middle East (N): Saudi Arabia, Yemen	-	KX371746	-	-	-
<i>Crotalaria quercetorum</i> Brandegee	H. von Türckheim 2016, Guatemala (M)	Central America (N): Guatemala, Mexico, Nicaragua	-	KX371747	-	-	-
<i>Crotalaria quinquefolia</i> L.	S. S. Subramaniam 1060, India (DUH)	Asia (N): Bangladesh, Cambodia, India, Indonesia, Iran, Java, Laos, Malaysia, Myanmar, Nepal, Philippines, Sabah, Singapore, Sri Lanka, Sulawesi, Sumatra, Thailand, Vietnam;	-	JQ945943	-	-	-
<i>Crotalaria ramosissima</i> Roxb.	Powell 1585, Jamaica (MO)	Australia (N): Australia, Papua New Guinea, Caribbean (I): Antigua-Barbuda, Barbados, Cuba, Dominican Republic, Guadeloupe, Haiti, Jamaica, Martinique, Trinidad and Tobago; Indian Ocean (I): Mauritius; Pacific Ocean (I): Bismarck Archipelago, Fiji, Northern Marianas, Samoa, Solomon Is, Vanuatu; South America (I): Guyana, Surinam	-	KX371748	-	-	-
<i>Crotalaria recta</i> A. Rich.	M. M. Le Roux 42, South Africa (JRAU)	Asia: India (N), Vietnam (I); Caribbean (I): Jamaica	-	KX371749	JQ041102	JQ067367	JQ041266
<i>Crotalaria reptans</i> Taub.	H. J. Schlieben 2470, Tanzania (M)	Africa (N): Angola, Burundi, Cameroon, Central African Republic, Ethiopia, Kenya, Malawi, Mozambique, Nigeria, Rwanda, South Africa, Sudan, Swaziland, Tanzania, Uganda, Zaire, Zambia, Zimbabwe	KX390824	-	-	-	-

Species name	Voucher information and geographical origin	Species distribution range	ETS	ITS	matK	psbA-trnH	rbcL
<i>Crotalaria retusa</i> L.	ETS: ITS: A. Rockinger 20142, Brazil (M) psbA-trnH: Costa N2410, Brazil (CEN)	Africa (U): Gambia, Ghana, Guinea, Kenya, Liberia, Mali, Mozambique, Nigeria, Senegal, Sierra Leone, Somalia, Tanzania, Uganda; Asia (N): Bangladesh, Bhutan, Brunei, Cambodia, China, India, Indonesia, Iran, Kalimantan, Laos, Lesser Sunda Is, Malaysia, Moluccas, Myanmar, Nepal, Pakistan, Philippines, Sarawak, Singapore, Sri Lanka, Sumatra, Thailand, Vietnam; Australasia (N): Australia, Papua New Guinea; Caribbean (I): Anguilla, Antigua-Barbuda, Bahamas, Barbados, Cayman Is, Cuba, Dominica, Dominican Republic, Grenada, Guadeloupe, Haiti, Jamaica, Martinique, Montserrat, Netherlands Leeward Is, Puerto Rico, St Kitts-Nevis, St Lucia, St Martin-St Barthelemy, St Vincent; Central America (I): Belize, Costa Rica, Guatemala, Mexico, Nicaragua, Panama; Indian Ocean (I): Comoro Is, Laccadive Is, Madagascar, Maldives, Mauritius, Reunion, Rodrigues, Seychelles; Middle East (N): Oman, Saudi Arabia, Yemen; North America (I): United States; Pacific Ocean: Bismarck Archipelago (N), Fiji (I), Hawaii (I), Marquesas (I), Northern Marianas (I), Samoa (I), Society Is (I); South America (I): Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, Surinam, Venezuela	KX390825	KX371750	-	JQ067529	-
<i>Crotalaria rhodesiae</i> Baker f.	Robinson 5141, Zimbabwe (M)	Africa (N): Malawi, South Africa, Tanzania, Zambia, Zimbabwe	KX390826	-	-	-	-
<i>Crotalaria rogersii</i> Baker f.	Madsen et al. 1502, Senegal (MO)	Africa (N): Malawi, South Africa, Tanzania, Zambia, Zimbabwe	-	JX120582	-	-	-
<i>Crotalaria rubiginosa</i> Willd.	S. S. Subramaniam et al. 1091, India (DUH)	Asia (N): India, Indonesia, Sri Lanka	-	JQ945954	-	-	-
<i>Crotalaria rufipila</i> Benth.	A. S. Flores et al. 424, Brazil (MIR)	South America (N): Brazil	KX390827	KX371751	-	-	-
<i>Crotalaria rzedowskii</i> J.Espinosa	Ventura 2552, Mexico (MO)	Central America (N): Mexico	-	KX371752	-	-	-
<i>Crotalaria sagittalis</i> L.	Beck 21186, Bolivia (M)	Caribbean (N): Cuba, Dominican Republic, Haiti, Jamaica, Puerto Rico; Central America (N): Belize, Costa Rica, El Salvador, Guatemala, Mexico, Nicaragua, Panama; North America (N): United States; South America (N): Bolivia, Brazil, Colombia, Peru, Venezuela	KX390828	KX371753	-	-	-
<i>Crotalaria saharae</i> Coss.	S. Abrahamczyk SA608, Morocco (M)	Africa (N): Algeria, Libya, Mali, Mauritania, Morocco, Niger, Western Sahara	KX390829	-	-	-	-
<i>Crotalaria salicifolia</i> Wight & Arn.	S. S. Subramaniam 1079, India (DUH)	Asia (N): India	-	JQ945949	-	-	-
<i>Crotalaria saltiana</i> Andr.	ITS: Kazmi et al. 65, Somalia (M) matK: Chuang 4723, Taiwan (ASU)	Africa (N): Djibouti, Ethiopia, Kenya, Somalia, Sudan; Middle East (N): Oman, Yemen	KX390830	KX371754	JQ619981	-	-
<i>Crotalaria sandaorensis</i> Gamble	J. Fernandes 344, India (K)	Asia (N): India	KX390831	KX371755	-	-	-
<i>Crotalaria scasselii</i> Chiov.	Kokwaro 1694, Kenya (UPS)	Africa (N): Kenya, Somalia	-	JQ067206	-	JQ067426	-
<i>Crotalaria semperlorens</i> Vent.	S. S. Subramaniam 1037, India (DUH)	Asia (N): India	-	JN990111	-	-	-
<i>Crotalaria sericifolia</i> Harms	Strobach BS5717, Namibia (WIND)	Africa (N): Angola, Namibia	-	JQ067152	JQ041104	JQ067373	JQ041267
<i>Crotalaria sessiliflora</i> L.	Sorensen et al. 6261, Thailand (K)	Asia (N): Bangladesh, Bhutan, Cambodia, China, East Timor, India, Indonesia, Irian Jaya, Japan, Korea, Laos, Lesser Sunda Is, Malaysia, Moluccas, Myanmar, Nepal, Pakistan, Philippines, Ryukyu Is, Sulawesi, Sumatra, Taiwan, Thailand, Vietnam; Australasia (N): Papua New Guinea	-	JQ067338	-	JQ067544	-
<i>Crotalaria shanica</i> Lace	Garrett 1461, Thailand (K)	Asia (N): Myanmar, Thailand	KX390832	KX371756	-	-	-
<i>Crotalaria shirensis</i> (Baker f.) Milne-Redh.	F. J. Breteier 440b, Cameroon (M)	Africa (N): Angola, Burundi, Cameroon, Kenya, Malawi, Mozambique, Tanzania, Zambia, Zimbabwe	KX390833	-	-	-	-

Species name	Voucher information and geographical origin	Species distribution range	ETS	ITS	mark	psbA-trnH	rbcL
<i>Crotalaria smithiana</i> A.T.Lee		Australasia (N): Australia	-	JQ067290	-	JQ067503	-
<i>Crotalaria spartea</i> Baker		Africa (N): Angola, Burundi, Cameroon, Malawi, Nigeria, South Africa, Tanzania, Zaire, Zambia, Zimbabwe	KX390834	KX371757	-	KX390751	-
<i>Crotalaria spartioides</i> DC.	Lutombi and Strohbach DL149, Namibia (WIND)	Africa (N): Botswana, Namibia, South Africa	-	JQ067149	JQ041105	-	JQ041268
<i>Crotalaria speciosa</i> Roth.	R. Wight 679, India (B)	Asia (N): India; Pacific Ocean (I): Mauritius	KX390835	KX371758	-	-	-
<i>Crotalaria spectabilis</i> Roth.	ETS: R. Wight 589, India (M) ITS: S. Subramaniam and A. K. Pandey 1099, India (DUH) mark, rbcL: Abbott 18302, locality unknown (FLAS)	Africa (I): Kenya, Mali, Tanzania; Asia (N): Bangladesh, China, East Timor, India, Malaysia, Myanmar, Nepal, Pakistan, Taiwan, Thailand; Australasia (I): Australia, New Caledonia, Papua New Guinea; Caribbean (I): Bahamas, Cuba, Dominica, Dominican Republic, Guadeloupe, Jamaica, Martinique, Puerto Rico, St Lucia; Central America (I): Mexico, Nicaragua, Panama; Indian Ocean: Andaman Is (N); Madagascar (I); Mauritius (I); Reunion (I); North America (I): United States; Pacific Ocean (I): Gilbert Is, Hawaii, Marquesas, Northern Marianas, Society Is; South America (U): Argentina, Brazil, Colombia, Peru, Venezuela	KX390836	JN990112	KJ772689	-	KJ773415
<i>Crotalaria sphaerocarpa</i> Perr. ex DC.* Sect. <i>Geniculatae</i> Polhill	Schutte 450, South Africa (JRAU)	Africa (N): Angola, Botswana, Central African Republic, Chad, Lesotho, Malawi, Mali, Mozambique, Namibia, Niger, Nigeria, Senegal, South Africa, Sudan, Swaziland, Tanzania, Zambia, Zimbabwe	-	JQ067139	JQ041106	-	JQ041269
<i>Crotalaria spinosa</i> Hochst.	D. Podlech 36399, Yemen (M)	Africa (N): Angola, Burundi, Ethiopia, Kenya, Rwanda, Senegal, Sudan, Tanzania, Uganda, Zambia, Zimbabwe; Middle East (N): Yemen	KX390837	KX371759	-	-	-
<i>Crotalaria steudneri</i> Schweinf.	M. M. Le Roux et al. 80, Namibia (WIND)	Africa (N): Angola, Botswana, Ethiopia, Malawi, Mozambique, Namibia, South Africa, Sudan, Tanzania, Zambia, Zimbabwe	-	JQ067314	JQ067562	JQ067522	JQ067600
<i>Crotalaria stipitata</i> Wight & Arn.	S. S. Subramaniam 1050, India (DUH)	Asia (N): India	-	KP698669	-	-	-
<i>Crotalaria stipularia</i> Desv.	ETS, ITS: ST. G. Beck 6687, Bolivia (M) mark, psbA-trnH: Valls JFM4946, Brazil (CEI)	Caribbean (I): Cuba, Dominica, Dominican Republic, Guadeloupe, Haiti, Jamaica, Martinique, Puerto Rico, St Lucia, St Vincent; South America (N): Argentina, Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Paraguay, Peru, Surinam, Venezuela	KX390838	KX371760	JQ067573	JQ067532	-
<i>Crotalaria stolzii</i> (Baker f.) Polhill	L. C. Leach 10720, Zimbabwe (M)	Africa (N): Kenya, Malawi, Mozambique, Tanzania, Zaire, Zimbabwe	KX390839	-	-	-	-
<i>Crotalaria strigulosa</i> Balf.f.	Mears 87, Yemen (B)	Africa (N): Socotra	KX390840	KX371761	-	-	-
<i>Crotalaria subdecurrens</i> Marth. ex Benth.	A. S. Flores et al. 864, Brazil (MIRR)	South America (N): Brazil	KX390841	KX371762	-	-	-
<i>Crotalaria subperfoliata</i> Wight	S. S. Subramaniam and A. K. Pandey 1035, India (DUH)	Asia (N): India	-	KP698635	-	-	-
<i>Crotalaria tanety</i> Du Puy, Labat & H.E. Ireland	Du Puy M228, Madagascar (P)	Indian Ocean (N): Madagascar	-	JQ067278	JQ041107	-	JQ041271
<i>Crotalaria teixeirae</i> Torre	Hochobes and Lutombi SS389, Namibia (WIND)	Africa (N): Angola, Namibia	-	JQ067156	JQ041108	JQ067378	JQ041272
<i>Crotalaria tenuipedicellata</i> Baker f.	E. A. Robinson 3494, Zambia (M)	Africa (N): Zaire, Zambia	KX390842	KX371763	-	-	-
<i>Crotalaria tetragona</i> Andrews	ETS: C. D. Adams 6420, Jamaica (M) ITS: A. K. Pandey 10017, India (DUH)	Asia (N): Bangladesh, Bhutan, China, India, Laos, Myanmar, Nepal, Thailand, Vietnam; Caribbean (I): Jamaica, St Vincent; Indian Ocean (I): Mauritius	KX390843	JN990110	-	-	-
<i>Crotalaria thebaica</i> (Delile) DC.	J. Léonard 4862, Libya (M)	Africa (N): Chad, Egypt, Sudan; Middle East (N): Saudi Arabia	-	KX371764	-	-	-

Species name	Voucher information and geographical origin	Species distribution range	ETS	ITS	matK	psbA-trnH	rbcl
<i>Crotalaria trichotoma</i> Bojer	ITS: P. O. Schallert 153, USA (M) matK, psbA-trnH, rbcl: file GenBank 'Chen et al. PS0226MT01'	Africa: Kenya (I), Mozambique (N), Sao Tome and Principe (I), Tanzania (N); Asia (I): China, East Timor, India, Indonesia, Irian Jaya, Kalimantan, Malaysia, Philippines, Sri Lanka, Sumatra, Taiwan, Vietnam; Australasia (I): Australia, Papua New Guinea; Caribbean (I): Antigua-Barbuda, Barbados, Dominica, Dominican Republic, Guadeloupe, Jamaica, Martinique, Puerto Rico, St Lucia, Central America (I); El Salvador, Nicaragua; Indian Ocean (I): Madagascar, Mauritius, Reunion, Seychelles; North America (I): United States; Pacific Ocean: Bismarck Archipelago (N), Nauru (I); South America (U): Brazil, Peru	-	KX371765	HM04950 7	GU99670 3	GO436334
<i>Crotalaria trifoliolatum</i> Willd.	Unknown collector [Botanical Survey of India] s. n. [M-0242324], India (M)	Asia (N): Bhutan, India; Pacific Ocean (I): Northern Marianas	KX390844	-	-	-	-
<i>Crotalaria trifoliolata</i> Bak.f.	I. Friis et al. 15074, Ethiopia (C)	Africa (N): Ethiopia	KX390845	KX371766	-	-	-
<i>Crotalaria triquetra</i> Datzell	S. S. Subramaniam et al. 3419, India (DUH)	Asia (N): East Timor, India, Indonesia, Lesser Sunda Is, Sri Lanka	-	JQ945930	-	-	-
<i>Crotalaria tweediana</i> Benth.	JFW 4263, Brazil (CEN)	South America (N): Brazil	-	JQ067328	JQ067574	JQ067574	-
<i>Crotalaria ulbrichiana</i> Harms	Gless and Louitt. 14197, Namibia (IRAU)	Africa (N): Namibia, Zambia, Zimbabwe	-	JX120580	-	-	JX120601
<i>Crotalaria uliginosa</i> C.C.Huang	Wagner 351, India (M)	Asia (N): China, India	KX390846	KX371767	-	-	-
<i>Crotalaria uncinella</i> Lam.	Lewis 574, Madagascar (P)	Africa (N): Mozambique, Tanzania; Asia (N): China, India, Malaysia, Singapore, Taiwan, Thailand, Vietnam; Indian Ocean (N): Madagascar, Mauritius, Reunion	-	JQ067274	-	JQ067493	-
<i>Crotalaria unifoliolata</i> Benth.	L. S. Kinoshita et al. 26, Brazil (M)	South America (N): Brazil	KX390847	KX371768	-	KX390754	-
<i>Crotalaria vanmeelii</i> R.Wilczek	M. R. 24297, Tanzania (M)	Africa (N): Tanzania, Zambia	KX390848	KX371769	-	-	-
<i>Crotalaria varicosa</i> Polhill	R. D. Box 133, Tanzania (K)	Africa (N): Tanzania	KX390849	KX371770	-	-	-
<i>Crotalaria variegata</i> Baker	E. A. Robinson 5616, Zimbabwe (M)	Africa (N): Angola, Malawi, Mozambique, Tanzania, Zaire, Zambia, Zimbabwe	KX390850	KX371771	-	-	-
<i>Crotalaria vasculosa</i> Benth.	Sturton 11796, South Africa (IRAU)	Africa (N): Kenya, Malawi, Mozambique, South Africa, Tanzania, Uganda, Zimbabwe, Asia (I): India; Indian Ocean (I): Mauritius	-	JQ067140	JQ041109	JQ067360	JQ041273
<i>Crotalaria velutina</i> Benth.	ITS, ITS: A. S. Flores et al. 850, Brazil (MIRR) matK, rbcl: Thomas et al. 5911, Brazil (K)	South America (N): Brazil	-	KX077952	JQ067578	-	JQ067601
<i>Crotalaria verrucosa</i> L.	ITS: S. S. Subramaniam 1009, India (DUH) matK, rbcl: Booth and Foggerty 1631, Australia (MEL)	Africa (I): Nigeria, Sierra Leone, Tanzania, Uganda; Asia (N): Bangladesh, Cambodia, China, East Timor, India, Indonesia, Laos, Lesser Sunda Is, Malaysia, Myanmar, Nepal, Philippines, Sabah, Sri Lanka, Sulawesi, Sumatra, Taiwan, Thailand, Vietnam; Australasia (I): Australia, Papua New Guinea; Caribbean: Anguilla (I), Bahamas (N), Barbados (I), Cayman Is (I), Cuba (I), Dominica (I), Dominican Republic (I), Grenada (I), Guadeloupe (I), Haiti (I), Jamaica (I), Martinique (I), Montserrat (I), Netherlands Leeward Is (I), Puerto Rico (I), St Kitts-Nevis (I), St Lucia (I), St Martin-St Barthelemy (I), St Vincent (I); Central America (U): Belize, Costa Rica, Guatemala, Nicaragua, Panama; Indian Ocean (I): Aldabra, Laccadive Is, Madagascar, Mauritius, Reunion, Seychelles; North America (U): United States; Pacific Ocean (I): Bismarck Archipelago, New Zealand, Niue, Society Is, eastern Samoa; South America (I): Brazil, Colombia, French Guiana, Guyana, Surinam	-	JN990109	JQ041110	-	JQ041274
<i>Crotalaria vespertilio</i> Benth.	L. S. Kinoshita et al. 11182, Brazil (M)	South America (N): Brazil	KX390851	KX371772	-	-	-

Species name	Voucher information and geographical origin	Species distribution range	ETS	ITS	matK	psbA-trnH	rbcL
<i>Crotalaria virgulata</i> Klotzsch	Schutte 451, South Africa (JRAU)	Africa (N): Botswana, Malawi, Mozambique, South Africa, Swaziland, Tanzania, Zaire, Zambia, Zimbabwe; Australasia (U): Australia; South America (I): Brazil	-	JQ067133	JQ041111	JQ067353	JQ041275
<i>Crotalaria virgatitas</i> DC.	B.-E. van Wyk 3060, South Africa (JRAU)	Africa (N): Namibia, South Africa	-	JQ067143	JQ041112	-	JQ041276
<i>Crotalaria vitellina</i> Ker. Gawl.	M. F. Devecchi MFD272, Brazil (SPF)	Caribbean (N): Caribbean-TRP; Central America (N): Belize, Costa Rica, El Salvador, Guatemala, Mexico, Panama; South America (N): Brazil, Colombia, Venezuela	KX390852	KX371773	-	-	-
<i>Crotalaria walkeri</i> Arn.	S. S. Subramaniam et al. 1022, India (DUH)	Asia (N): India, Sri Lanka	-	JN990108	-	-	-
<i>Crotalaria wightiana</i> Wight & Arn.	S. S. Subramaniam et al. 1013, India (DUH)	Asia (N): India, Sri Lanka	-	JQ945947	-	-	-
<i>Crotalaria xanthoclada</i> Benth.	Labat 2008, Madagascar (P)	Indian Ocean (N): Madagascar	-	JQ067283	JQ667487	-	JQ692941
<i>Crotalaria yunnanensis</i> Franch.	Forrest 28564, China (MO)	Asia (N): China	KX390853	KX371774	-	-	-
<i>Cyclolobium brasiliense</i> Benth.*	Ratter et al. 7431, locality unknown (E)	South America (N): Bolivia, Brazil, Paraguay	-	AF287637	-	-	-
<i>Cytisus scoparius</i> (L.) Link	Unknown collector [MAF148134], Spain (MAF)	Africa (I): Canary Is, Madeira, Soth Africa; Asia (I) China, India, Japan, Russia in Asia; Australasia (I): Australia, Tasmania; Europe (N): Austria, Belarus, Belgium, Corsica, Czech Republic, Denmark, Estonia, France, Germany, Great Britain, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Moldova, Netherlands, Norway, Poland, Portugal, Romania, Russia in Europe, Sardegna, Slovakia, Spain, Sweden, Switzerland, Ukraine, Yugoslavia; Indoan Ocean (I): Madagascar; North America (I): Canada, United States; Pacific Ocean (I): Antipodean Is, Chatham Is, Hawaii, New Zealand; South America (I): Argentina, Bolivia, Chile	-	AF351119	-	-	-
<i>Dichilus lebeckioides</i> DC. *	Schutte 151, locality unknown (JRAU)	Africa (N): Botswana, Namibia, South Africa, Swaziland, Zimbabwe	-	EU34789	-	-	-
<i>Diplazis martiusii</i> Benth.*	Beck et al. 166, Brazil (US)	South America (N): Brazil, Colombia, Peru, Venezuela	-	AY553711	-	-	-
<i>Euchlora hirsuta</i> (Thunb.) Druce*	J. S. Boatwright 233, South Africa (JRAU)	Africa (N): South Africa	-	JQ067345	-	-	-
<i>Ezoloba macrocarpa</i> (Eckl. & Zeyh.) B.-E. van Wyk & Boatwr.*	Helme 2076, South Africa (NBG)	Africa (N): South Africa	-	FM87593	-	-	-
<i>Harpalycx brasiliana</i> Benth.	R. Schütz Rodrigues 1153, Brazil (RB)	South America (N): Brazil	-	KJ028470	-	-	-
<i>Hovea elliptica</i> (Sm.) DC.	M. D. Crisp 8924, locality unknown (CANB)	Australasia (N): Australia; Pacific Ocean (I): New Zealand	-	AF287640	-	-	-
<i>Lamprolobium fruticosum</i> Benth.*	Clarkson and Neldner 8827, locality unknown (K)	Asia (I): India; Australasia (N): Australia	-	GQ25008	-	-	-
<i>Lebeckia brevicarpa</i> M.M. le Roux & B.-E. van Wyk	M. M. le Roux et al. 4, South Africa (JRAU)	Africa (N): South Africa	-	EU34785	-	-	EU347933
<i>Lebeckia meyeriana</i> Eckl. & Zeyh.	B.-E. van Wyk 3009, South Africa (JRAU)	Africa (N): South Africa	-	EU34785	-	-	EU347905
<i>Lebeckia sepalaria</i> (L.) Thunb.	M. M. le Roux et al. 10, South Africa (JRAU)	Africa (N): South Africa	-	EU34785	-	-	EU347936
<i>Leobordea corymbosa</i> (E. Mey.) B.-E. van Wyk & Boatwr.	J. C. Scheepers 699, South Africa (M)	Africa (N): South Africa, Swaziland	KX390854	KX371775	-	-	-
<i>Leobordea digitata</i> (Harv.) B.-E. van Wyk & Boatwr.	ITS: B.-E. van Wyk 2350, South Africa (JRAU) rbcL: B.-E. van Wyk 2342, South Africa (JRAU)	Africa (N): South Africa	-	EU34777	-	-	EU348057
<i>Leobordea longicephala</i> (B.-E. van Wyk) B.-E. van Wyk & Boatwr.	M. Koekemoer 320, South Africa (M)	Africa (N): South Africa	KX390855	KX371776	-	-	-

Species name	Voucher information and geographical origin	Species distribution range	ETS	ITS	matK	psbA-trnH	rbcl
<i>Leopardia lotioides</i> Del. *	B.-E. van Wyk et al. 4204, locality unknown (JRAU)	Africa (N): Algeria, Angola, Cape Verde, Central African Republic, Chad, Djibouti, Egypt, Ethiopia, Kenya, Libya, Mauritania, Morocco, Namibia, South Africa, Sudan, Tanzania, Uganda, Zimbabwe; Asia (N): India, Iran, Pakistan; Middle East(N): Qatar, Saudi Arabia, Syria, Yemen	-	EU34776 5	-	-	EU348003
<i>Leptolabium dasy carpum</i> Vogel*	L.. P. de Queiroz 13973, Brazil (HUEFS)	South America (N): Brazil	-	JX124514	-	-	-
<i>Listia heterophylla</i> E.Mey. *	ITS: O. H. Volk 5172, Namibia (M) rbcl: B.-E. van Wyk et al. 4207, locality unknown (JRAU)	Africa (N): Botswana, Lesotho, Mozambique, Namibia, South Africa, Swaziland, Zaire, Zambia, Zimbabwe unknown (JRAU)	KX390856	KX371777	-	-	EU348012
<i>Lotanosis alpina</i> (Eckl. & Zeyh.) B.-E. van Wyk	B.-E. van Wyk 1478, locality unknown (JRAU)	Africa (N): South Africa	-	AM26244 6	-	-	-
<i>Lotanosis gracilis</i> Benth.	J. P. H. Acodks 19746, South Africa (M)	Africa (N): South Africa	KX390857	KX371778	-	-	-
<i>Lotanosis involucreta</i> (P. J. Bergius) Benth.	ITS: B.-E. van Wyk 2873, locality unknown (JRAU) rbcl: J. S. Boatwright et al. 116, locality unknown (JRAU)	Africa (N): South Africa	-	EU34780 5	-	-	EU347997
<i>Lotanosis leptoloba</i> Bolus	J. S. Boatwright et al. 185, South Africa (JRAU)	Africa (N): Namibia, South Africa	-	EU34775 7	-	-	EU348077
<i>Lotanosis prostrata</i> (L.) Benth.	ITS: J. S. Boatwright et al. 115, locality unknown (JRAU) rbcl: B.-E. van Wyk 3229, locality unknown (JRAU)	Africa (N): South Africa	-	EU34780 8	-	-	EU348054
<i>Lotanosis stricta</i> (Eckl. & Zeyh.) B.-E. van Wyk	ITS: B.-E. van Wyk 1718, South Africa (JRAU) rbcl: B.-E. van Wyk 1718, locality unknown (JRAU)	Africa (N): South Africa	-	EU34779 3	-	-	EU348091
<i>Lotanosis tenella</i> Eckl. & Zeyh.	Ecklon 1282, South Africa (B)	Africa (N): Lesotho, South Africa	-	KX371779	-	-	-
<i>Lotanosis trichopoda</i> (E. Mey.) Benth.	L. C. C. Liebenberg 7741, South Africa (M)	Africa (N): South Africa	KX390858	KX371780	-	-	-
<i>Lupinus albus</i> L. *	C. Gröger 1401, Greece (M)	Africa (N): South Africa	-	KX371781	-	-	-
<i>Pearsonia sessilifolia</i> (Harv.) Dummer*	M. Weigend 2307, South Africa (M)	Africa (N): South Africa	KX390859	KX371782	-	-	-
<i>Piptanthus nepalensis</i> Sweet*	Wang 0121, China (KUN)	Asia (N): Bhutan, China, India, Myanmar, Nepal; Europe (I): Great Britain	-	AF215922	-	-	-
<i>Podalyria buxifolia</i> Willd.	J. S. Boatwright and Magee 34, South Africa (JRAU)	Africa (N): South Africa	-	AM26149 6	-	-	-
<i>Poaclanthus jalcata</i> (Vell.) Heringer	De Lima 2, Brazil (R)	South America (N): Brazil	-	AF467492	-	-	-

Species name	Voucher information and geographical origin	Species distribution range	ETS	ITS	matK	psbA-trnH	rbcl
<i>Polhillia pallens</i> C.H. Stirt.	ITS: B.-E. van Wyk 2128 (JRAU) rbcl: B.-E. van Wyk 2708, locality unknown (JRAU)	Africa (N): South Africa	-	AM26245 3	-	-	EU347958
<i>Rafnia ampliclexialis</i> Thunb.*	P. Goldblatt 4026, South Africa (M)	Africa (N): South Africa	KX390860	KX371783	-	-	-
<i>Rafnia capensis</i> (L.) Druce	J. S. Boatwright and Magee 26, South Africa (JRAU)	Africa (N): South Africa	-	EU34774 2	-	-	-
<i>Rafnia ovata</i> E.Mey.	Campbell and B.-E. van Wyk 128, South Africa (JRAU)	Africa (N): South Africa	-	AJ744941	-	-	-
<i>Robynsiophyton vanderystii</i> R. Wilczek*	Lisowski 20326, locality unknown (K)	Africa (N): Angola, Zaire, Zambia	-	EU34787 8	-	-	EU347952
<i>Rothia indica</i> (L.) Druce	Rattler s. n. [M-0242323], India (M)	Asia (N): India, Indonesia, Laos, Malaysia, Sri Lanka, Vietnam; Australasia (U): Australia	KX390861	-	-	-	-
<i>Sophora tomentosa</i> L.*	B. Skyes CHR 569752, Cook Islands. Plant in cultivation and not vouchered; Kate Boardman, database manager at Allan Herbarium, 18 July 2016, pers. communication	Africa (U): Ghana, Ivory Coast, Liberia, Mozambique, Nigeria, Sao Tome and Principe, Senegal, Sierra Leone, Tanzania, Togo; Asia (N): Cambodia, China, India, Indonesia, Irian Jaya, Malaysia, Moluccas, Myanmar, Pakistan, Philippines, Sabah, Sarawak, Sri Lanka, Sulawesi, Sumatra, Taiwan, Thailand, Vietnam; Australasia (N): Australia, Papua New Guinea; Caribbean (N): Bahamas, Cayman Is., Dominican Republic, Haiti, Jamaica; Central America (N): Belize, Mexico, Nicaragua, Panama; Indian Ocean (U): Aldabra, Andaman Is., Laccadive Is., Madagascar, Maldives, Mauritius, Nicobar Is., Rodrigues, Seychelles; North America (N): United States; Pacific Ocean (N): Bismarck Archipelago, Fiji, Gilbert Is., Marquesas, Marshall Is., Northern Marianas, Society Is., Solomon Is.; South America (N): Argentina, Brazil, Colombia	-	AY725482	-	-	-
<i>Templetonia retusa</i> (Vent.) R.Br.*	M. D. Crisp 8996, Australia (CANB)	Australasia (N): Australia	-	AF287636	-	-	-
<i>Wilborgia mucronata</i> (L. f.) Druce (M)	S. Soini s. n. [M-0242325], South Africa (M)	Africa (N): South Africa	KX390862	KX371784	-	-	KX083399
<i>Wilborgia obcordata</i> (P.J. Bergius) Thunb.* (JRAU)	J. S. Boatwright et al. 98, South Africa (JRAU)	Africa (N): South Africa	-	EU34774 8	-	-	EU347972
<i>Wilborgia sericea</i> Thunb.	J. S. Boatwright et al. 124, South Africa (JRAU)	Africa (N): South Africa	-	EU34775 5	-	-	EU347968
<i>Wilborgiella leipoldiana</i> (Schltr. ex R. Dahlgren) Boatw. & B.-E. van Wyk*	H. C. Taylor 11787, South Africa (M)	Africa (N): South Africa	KX390863	KX371785	-	-	KX083400

Additional file 4: Table S2: Primer sequences used in this study (listed 5'- to 3'-end) and applied protocols

Protocol	Reactants	Treatments			Amplification cycles
		Pre-melt	Amplification	Final extension	
PCR	Gene or spacer region	Primer sequence (reference)			
	<i>rbcL</i>				
	600f	ATTTATGCCGTTGGAGAGACCG (Kocyan et al. 2007)			
	800r	CAATAACRGCATGCATYGCACGRT (Kocyan et al. 2007)	95°C (30 sec) + 52°C (1 min) + 68°C (1 min)	68°C (10 min)	39
	<i>psbA-trnH</i>				
	<i>psbA</i>	GTTATGCATGAACGTAATGCTC (Sang et al. 1997)	95°C (30 sec) + 52°C (1 min) + 68°C (1 min)	68°C (10 min)	39
	<i>trnH</i>	CGCGCATGTTGATTACAAAATC (Sang et al. 1997)			
	ITS region				
	1	TCCGTAGGTGAACCTGCCG (White et al. 1990)	95°C (30 sec) + 54°C (1 min) + 68°C (1 min)	68°C (10 min)	39
	2	GCTGCGTCTTCATCGATGC (White et al. 1990)			
	3	GCATCGATGAAGAACGCAGC (White et al. 1990)			
	4	TCCTCCGCTTATTGATATGC (White et al. 1990)			
	ETS region				
	281F (Genisteae)	TGCTTCCATTTGCTTGTGCCT (Cubas et al. 2010)	95°C (30 sec) + 54°C (1 min) + 68°C (1 min)	68°C (10 min)	39
18S-IGS	GAGACAAGCATATGACTACTGGCAGGATCAACCAG (Baldwin and Markos 1998)				
ExoSAP cleaning	For 4.0 µl PCR product: 0.03 µl Exonuclease I 0.3 µl Shrimp Alkaline Phosphatase	37°C (15 min) + 80°C (15 min) + 4°C (4 min)	-	-	
Cycle reaction (BigDye Terminator v3.0)	For every 3 µl ExoSAP cleaning product: 1.0 µl Big Dye, 1.5 µl sequencing buffer 5x, and 0.5 µl primer	96°C (1 min)	96°C (10 sec) + 55°C (15 sec) + 60°C (4 min)	35	

Additional file 5: Table S3. Species list with coding of sections; distribution areas; leaf and flower trait states; species' climate categories; number of GBIF records; and chromosome numbers

Species name	Sections Polhill (1982)	Sections Le Roux et al. (2013)	Region 1	Region 2	Region 3	Region 4	Leaf type	Leaf type binary	Keel	Calyx lobing	Calyx length	Köppen-Geiger categories	Köppen-Geiger major climate group	GBIF occurrences	2n
<i>Aspalathus chenopoda</i>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>Aspalathus fusca</i>	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>Aspalathus nivea</i>	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>Bolusia amboensis</i>	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>Calobota cytsoides</i>	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>Crotalaria abbreviata</i>	3	1	1	NA	NA	NA	2	1	0	0	1	3	1	NA	NA
<i>Crotalaria acicularis</i>	6	4	3	4	NA	NA	0	0	1	1	0	1	0	26	NA
<i>Crotalaria oculata</i>	7	6	1	NA	NA	NA	2	1	1	0	1	3	1	58	1
<i>Crotalaria adamsonii</i>	2	8	1	NA	NA	NA	0	0	0	0	0	3	1	NA	NA
<i>Crotalaria adenocarporoides</i>	8	6	1	NA	NA	NA	2	1	1	0	1	3	1	12	NA
<i>Crotalaria adolfi</i>	2	2	1	NA	NA	NA	2	1	0	0	0	13	3	NA	NA
<i>Crotalaria aegyptiaca</i>	6	4	1	NA	NA	NA	0	0	1	1	0	5	2	63	1
<i>Crotalaria agatiflora</i>	1	11	1	NA	NA	NA	1	1	0	0	0	9	3	147	1
<i>Crotalaria alata</i>	6	4	3	4	NA	NA	0	0	1	1	0	7	0	151	1
<i>Crotalaria albicaulis</i>	3	1	1	NA	NA	NA	2	1	0	0	1	7	2	NA	NA
<i>Crotalaria albida</i>	6	4	3	4	NA	NA	0	0	1	1	0	0	0	157	1
<i>Crotalaria alexandri</i>	8	6	1	NA	NA	NA	2	1	1	0	1	3	1	20	NA
<i>Crotalaria allophylla</i>	3	5	1	NA	NA	NA	2	1	0	0	1	NA	1	NA	NA
<i>Crotalaria amoena</i>	6	8	1	NA	NA	NA	2	1	0	0	0	3	1	NA	NA
<i>Crotalaria angulata</i>	6	4	3	NA	NA	NA	0	0	1	1	0	0	0	11	NA
<i>Crotalaria ankaratrana</i>	2	8	2	NA	NA	NA	2	1	0	0	0	13	3	NA	NA
<i>Crotalaria annua</i>	8	6	1	NA	NA	NA	2	1	1	0	1	12	3	NA	NA
<i>Crotalaria anomala</i>	3	1	2	NA	NA	NA	2	1	0	0	1	7	2	NA	NA
<i>Crotalaria anthyllopsis</i>	3	1	1	NA	NA	NA	1	1	0	0	1	3	1	23	NA
<i>Crotalaria arenaria</i>	6	4	1	NA	NA	NA	0	0	1	1	0	5	2	13	1
<i>Crotalaria argyrea</i>	3	6	1	NA	NA	NA	2	1	1	0	1	12	3	NA	NA
<i>Crotalaria argyrolabioides</i>	8	6	1	NA	NA	NA	2	1	0	0	1	5	2	30	NA
<i>Crotalaria aridicola</i>	8	6	4	NA	NA	NA	2	1	1	0	1	7	2	130	NA
<i>Crotalaria arushae</i>	3	1	1	NA	NA	NA	2	1	0	0	1	13	3	NA	NA
<i>Crotalaria assamica</i>	7	6	3	NA	NA	NA	0	0	1	1	1	NA	NA	NA	NA
<i>Crotalaria atrorubens</i>	2	1	1	NA	NA	NA	2	1	0	1	0	NA	NA	NA	1
<i>Crotalaria aurea</i>	7	6	1	NA	NA	NA	2	1	1	0	1	5	2	NA	NA
<i>Crotalaria avonensis</i>	6	4	0	NA	NA	NA	0	0	1	1	0	NA	NA	NA	NA
<i>Crotalaria axillaris</i>	7	6	1	NA	NA	NA	2	1	0	1	0	NA	NA	78	NA
<i>Crotalaria axillifloroides</i>	8	6	1	NA	NA	NA	2	1	1	0	1	12	3	16	NA
<i>Crotalaria bahiensis</i>	2	1	0	NA	NA	NA	2	1	0	0	0	7	2	20	NA
<i>Crotalaria balansae</i>	6	4	0	NA	NA	NA	0	0	1	1	0	8	3	27	3
<i>Crotalaria ballyi</i>	5	7	1	NA	NA	NA	0	0	1	1	0	7	2	NA	NA
<i>Crotalaria barbata</i>	6	4	3	NA	NA	NA	0	0	1	1	0	NA	NA	NA	1
<i>Crotalaria barkae</i>	2	2	1	NA	NA	NA	2	1	0	0	0	NA	NA	NA	1
<i>Crotalaria barnabassii</i>	1	11	1	NA	NA	NA	2	1	0	0	1	7	2	16	1
<i>Crotalaria bequaertii</i>	1	6	1	NA	NA	NA	2	1	0	0	1	13	3	30	NA
<i>Crotalaria beddomeana</i>	7	6	3	NA	NA	NA	0	0	1	0	1	3	1	NA	NA

Species name	Sections Polhill (1982)				Sections Le Roux et al. (2013)				Region 2	Region 3	Region 4	Leaf type	Leaf type binary	Keel	Calyx lobing	Calyx length	Köppen-Geiger categories	Köppen-Geiger major climate group	GBIF occurrences	Zn
	3	8	1	6	1	NA	NA	NA												
<i>Crotalaria benadrensis</i>	3		1		1	NA	NA	NA	2	1	0	0	1	7	2	NA	NA			
<i>Crotalaria bequaertii</i>	8		6	1	NA	NA	NA	NA	2	1	1	0	1	12	3	NA	NA			
<i>Crotalaria berteriana</i>	7		6	3	4	NA	NA	NA	0	0	1	NA	NA	NA	3	25	24	2		
<i>Crotalaria bifaria</i>	6		4	3	NA	NA	NA	NA	0	0	1	1	0	1	0	NA	1	NA		
<i>Crotalaria boehmii</i>	3		1	1	NA	NA	NA	NA	0	0	0	0	1	3	1	NA	NA			
<i>Crotalaria bogdaniana</i>	3		1	1	NA	NA	NA	NA	2	1	0	0	1	3	1	28	NA	NA		
<i>Crotalaria bogdaniana</i>	6		4	0	NA	NA	NA	NA	0	0	1	1	0	NA	NA	NA	NA	NA		
<i>Crotalaria bongensis</i>	4		1	1	NA	NA	NA	NA	1	1	0	0	1	3	1	36	NA	NA		
<i>Crotalaria boranica</i>	4		5	1	NA	NA	NA	NA	2	1	0	0	1	5	2	NA	NA	NA		
<i>Crotalaria bosseri</i>	1		11	2	NA	NA	NA	NA	1	1	0	0	1	8	3	NA	NA	NA		
<i>Crotalaria brachycarpa</i>	2		6	0	NA	NA	NA	NA	2	1	0	0	0	NA	NA	NA	NA	NA		
<i>Crotalaria bracteata</i>	3		1	3	NA	NA	NA	NA	2	1	NA	NA	1	3	1	13	NA	NA		
<i>Crotalaria brevicornuta</i>	3		1	1	NA	NA	NA	NA	1	1	0	0	1	NA	NA	NA	NA	NA		
<i>Crotalaria brevidens</i>	3		1	1	NA	NA	NA	NA	2	1	0	0	1	3	1	84	1	NA		
<i>Crotalaria breviflora</i>	6		4	0	NA	NA	NA	NA	0	0	1	1	0	8	3	97	3	NA		
<i>Crotalaria brevipedunculata</i>	6		4	0	NA	NA	NA	NA	0	0	1	1	0	NA	NA	NA	NA	NA		
<i>Crotalaria bupleurifolia</i>	6		4	3	NA	NA	NA	NA	0	0	1	1	0	5	2	NA	1	NA		
<i>Crotalaria burhia</i>	6		4	0	NA	NA	NA	NA	0	0	1	1	0	NA	NA	NA	NA	NA		
<i>Crotalaria burkeana</i>	2		2	1	NA	NA	NA	NA	2	1	0	0	0	7	2	58	NA	NA		
<i>Crotalaria burttii</i>	3		1	1	NA	NA	NA	NA	2	1	0	0	1	3	1	17	1	NA		
<i>Crotalaria caljanifolia</i>	2		1	0	NA	NA	NA	NA	2	1	0	0	0	NA	NA	238	NA	NA		
<i>Crotalaria callensis</i>	2		8	1	NA	NA	NA	NA	2	1	0	0	0	3	1	NA	NA	NA		
<i>Crotalaria calycina</i>	6		4	1	2	3	NA	NA	0	0	1	1	0	0	0	328	1	NA		
<i>Crotalaria camposepala</i>	7		6	1	NA	NA	NA	NA	2	1	1	0	1	NA	NA	NA	NA	NA		
<i>Crotalaria capensis</i>	1		11	1	NA	NA	NA	NA	2	1	0	0	1	8	3	141	1	NA		
<i>Crotalaria capillipes</i>	2		8	1	NA	NA	NA	NA	2	1	0	0	0	7	2	NA	NA	NA		
<i>Crotalaria caudata</i>	2		8	1	NA	NA	NA	NA	2	1	0	0	0	12	3	28	NA	NA		
<i>Crotalaria cephalotes</i>	8		6	1	NA	NA	NA	NA	2	1	1	1	1	3	1	120	1	NA		
<i>Crotalaria chaco-serranensis</i>	2		2	0	NA	NA	NA	NA	2	1	0	0	0	3	1	NA	NA	NA		
<i>Crotalaria chinensis</i>	6		4	3	4	NA	NA	NA	0	0	1	1	0	0	0	24	NA	NA		
<i>Crotalaria chirindae</i>	3		1	1	NA	NA	NA	NA	2	1	0	0	1	3	1	NA	NA	NA		
<i>Crotalaria chrysochlora</i>	3		1	1	NA	NA	NA	NA	2	1	0	0	1	3	1	39	NA	NA		
<i>Crotalaria cistoides</i>	2		8	1	NA	NA	NA	NA	2	1	0	0	0	7	2	19	NA	NA		
<i>Crotalaria clarkii</i>	6		4	3	NA	NA	NA	NA	0	0	1	1	0	3	1	NA	NA	NA		
<i>Crotalaria clausenii</i>	2		1	0	NA	NA	NA	NA	2	1	0	0	0	NA	NA	NA	NA	1		
<i>Crotalaria cleomifolia</i>	3		1	1	3	NA	NA	NA	3	1	0	0	1	3	1	82	NA	NA		
<i>Crotalaria colorata</i>	7		10	1	NA	NA	NA	NA	2	1	1	0	1	5	2	12	NA	NA		
<i>Crotalaria comanestiana</i>	3		6	1	NA	NA	NA	NA	3	1	0	0	1	7	2	24	1	NA		
<i>Crotalaria concinna</i>	8		6	1	NA	NA	NA	NA	2	1	1	0	0	NA	NA	NA	NA	NA		
<i>Crotalaria cornu-ammonis</i>	2		7	2	NA	NA	NA	NA	2	1	0	0	0	NA	NA	NA	NA	NA		
<i>Crotalaria coursii</i>	3		1	2	NA	NA	NA	NA	2	1	0	0	1	8	3	42	NA	NA		
<i>Crotalaria craspedocarpa</i>	3		1	2	NA	NA	NA	NA	2	1	0	0	1	9	3	12	NA	NA		
<i>Crotalaria crispata</i>	6		4	4	NA	NA	NA	NA	0	0	1	1	0	3	1	193	NA	NA		
<i>Crotalaria cunninghamii</i>	7		6	4	NA	NA	NA	NA	1	1	1	0	1	7	2	683	NA	NA		

Species name	Sections Polhill (1982)				Sections Le Roux et al. (2013)				Region 2	Region 3	Region 4	Leaf type	Leaf type binary	Keel	Calyx lobing	Calyx length	Köppen-Geiger categories	Köppen-Geiger major climate group	GBIF occurrences	Zn	
	8	3	3	2	6	1	1	1													1
<i>Crotalaria cuspidata</i>	8				6	1	NA	NA	NA	NA	2	2	1	1	0	0	1	3	1	11	1
<i>Crotalaria cylindrostachys</i>	3				1	1	NA	NA	NA	NA	2	1	1	0	0	1	7	2	18	NA	NA
<i>Crotalaria cytisioides</i>	3				1	3	NA	NA	NA	NA	2	1	1	0	0	1	NA	NA	25	1	NA
<i>Crotalaria damarensis</i>	2				7	1	NA	NA	NA	NA	2	1	1	0	0	0	7	2	58	NA	NA
<i>Crotalaria decaryana</i>	3				1	2	NA	NA	NA	NA	2	1	1	0	0	1	1	0	39	NA	NA
<i>Crotalaria deadzana</i>	7				6	1	NA	NA	NA	NA	2	1	1	1	0	1	12	3	NA	NA	NA
<i>Crotalaria deserticola</i>	7				6	1	NA	NA	NA	NA	2	1	1	0	0	1	3	1	81	1	1
<i>Crotalaria dewildemaniana</i>	3				1	1	NA	NA	NA	NA	2	1	1	0	0	1	3	1	45	1	1
<i>Crotalaria dinteri</i>	7				6	1	NA	NA	NA	NA	2	1	1	0	0	1	5	2	NA	NA	NA
<i>Crotalaria diasijfolia</i>	2				8	2	NA	NA	NA	NA	2	1	1	0	0	0	13	3	40	NA	NA
<i>Crotalaria dissitiflora</i>	3				1	4	NA	NA	NA	NA	2	1	1	0	0	1	7	2	329	NA	NA
<i>Crotalaria distans</i>	7				6	1	NA	NA	NA	NA	2	1	1	0	0	1	7	2	96	NA	NA
<i>Crotalaria distantiflora</i>	3				1	1	NA	NA	NA	NA	2	1	1	0	0	1	3	1	32	1	1
<i>Crotalaria doidgeae</i>	1				11	1	NA	NA	NA	NA	2	1	1	0	0	1	7	2	18	NA	NA
<i>Crotalaria dubia</i>	6				4	3	NA	NA	NA	NA	0	0	1	1	1	0	12	3	NA	NA	NA
<i>Crotalaria duboisii</i>	8				6	1	NA	NA	NA	NA	2	1	1	0	0	1	12	3	NA	NA	NA
<i>Crotalaria dumosa</i>	3				1	1	NA	NA	NA	NA	2	1	1	0	0	1	5	2	11	NA	NA
<i>Crotalaria dura</i>	2				8	1	NA	NA	NA	NA	2	1	1	0	0	0	3	1	34	NA	NA
<i>Crotalaria ebenoides</i>	2				1	1	NA	NA	NA	NA	1	1	1	0	0	0	NA	NA	NA	1	1
<i>Crotalaria emarginata</i>	3				1	1	NA	NA	NA	NA	2	1	1	0	0	1	2	1	31	NA	NA
<i>Crotalaria emarginella</i>	7				6	1	NA	NA	NA	NA	2	1	1	1	0	1	NA	NA	NA	NA	NA
<i>Crotalaria emirnensis</i>	7				6	2	NA	NA	NA	NA	2	1	1	0	0	1	NA	NA	NA	NA	NA
<i>Crotalaria ephemera</i>	8				6	1	NA	NA	NA	NA	2	1	1	0	0	1	12	3	NA	NA	NA
<i>Crotalaria epunctata</i>	6				4	3	NA	NA	NA	NA	0	0	0	1	1	0	3	1	NA	NA	NA
<i>Crotalaria eremaea</i>	7				6	4	NA	NA	NA	NA	2	1	1	1	0	1	NA	NA	1068	NA	NA
<i>Crotalaria eremicola</i>	7				6	1	NA	NA	NA	NA	2	1	1	0	0	1	5	2	33	NA	NA
<i>Crotalaria evolvaloides</i>	6				4	3	NA	NA	NA	NA	0	0	1	1	1	0	1	0	NA	1	1
<i>Crotalaria excisa</i>	7				10	1	NA	NA	NA	NA	2	1	1	0	0	1	9	3	46	NA	NA
<i>Crotalaria eximia</i>	8				6	1	NA	NA	NA	NA	2	1	1	0	0	1	3	1	NA	NA	NA
<i>Crotalaria fascicularis</i>	7				6	1	NA	NA	NA	NA	2	1	1	0	0	1	3	1	14	NA	NA
<i>Crotalaria ferruginea</i>	6				4	3	NA	NA	NA	NA	0	0	1	1	1	0	0	0	160	4	4
<i>Crotalaria fherenensis</i>	1				11	2	NA	NA	NA	NA	2	1	1	0	0	1	3	1	50	NA	NA
<i>Crotalaria filicaulis</i>	8				6	1	NA	NA	NA	NA	2	1	1	0	0	1	12	3	14	NA	NA
<i>Crotalaria filifolia</i>	2				NA	0	NA	NA	NA	NA	2	1	1	0	0	0	NA	NA	33	NA	NA
<i>Crotalaria filiformis</i>	6				4	3	NA	NA	NA	NA	0	0	0	1	1	0	1	0	NA	NA	NA
<i>Crotalaria filipes</i>	6				4	3	NA	NA	NA	NA	0	0	0	1	1	0	3	1	NA	NA	NA
<i>Crotalaria flavicarinata</i>	4				9	1	NA	NA	NA	NA	2	1	1	0	0	1	7	2	20	NA	NA
<i>Crotalaria flavicomata</i>	6				4	0	NA	NA	NA	NA	2	1	1	1	1	0	3	1	73	3	3
<i>Crotalaria florida</i>	8				6	1	NA	NA	NA	NA	2	1	1	0	0	1	NA	NA	NA	NA	NA
<i>Crotalaria friesii</i>	7				6	1	NA	NA	NA	NA	2	1	1	0	0	1	12	3	NA	NA	NA
<i>Crotalaria fysonii</i>	6				4	3	NA	NA	NA	NA	0	0	0	1	1	0	3	1	10	1	1
<i>Crotalaria gamwelliae</i>	8				6	1	NA	NA	NA	NA	2	1	1	0	0	1	12	3	NA	NA	NA
<i>Crotalaria gazensis</i>	2				8	1	NA	NA	NA	NA	2	1	1	0	0	0	NA	NA	12	NA	NA

Species name	Sections Polhill (1982)				Sections Le Roux et al. (2013)				Region 2	Region 3	Region 4	Leaf type	Leaf type binary	Keel	Calyx lobing	Calyx length	Köppen-Geiger categories	Köppen-Geiger major climate group	GBIF occurrences	Zn
	4	2	1	8	1	2	3	NA												
<i>Crotalaria gillettii</i>	4	1	NA	NA	NA	NA	NA	2	1	0	0	0	1	3	1	1	NA	NA	1	
<i>Crotalaria glauca</i>	2	1	NA	NA	NA	NA	NA	2	0	0	0	0	0	0	0	1	194	1		
<i>Crotalaria glaucoides</i>	2	1	NA	NA	NA	NA	NA	2	1	0	0	0	0	NA	NA	NA	NA	1		
<i>Crotalaria globifera</i>	7	1	NA	NA	NA	NA	NA	2	1	1	1	0	1	9	3	78	NA	NA		
<i>Crotalaria goetzei</i>	2	1	NA	NA	NA	NA	NA	2	1	0	0	0	0	12	3	10	1	NA		
<i>Crotalaria goosii</i>	6	4	0	NA	NA	NA	NA	0	0	1	1	1	0	3	1	NA	NA	NA		
<i>Crotalaria goodii</i>	2	1	NA	NA	NA	NA	NA	2	1	0	0	0	0	3	1	75	NA	NA		
<i>Crotalaria gorenensis</i>	2	1	NA	NA	NA	NA	NA	2	1	0	0	0	0	3	1	459	1	NA		
<i>Crotalaria grahamiana</i>	7	6	1	NA	NA	NA	NA	3	1	1	1	0	1	3	1	40	1	NA		
<i>Crotalaria graminicola</i>	8	1	NA	NA	NA	NA	NA	1	1	1	1	0	1	3	1	50	NA	NA		
<i>Crotalaria grandiflora</i>	6	4	0	NA	NA	NA	NA	0	0	1	1	0	0	3	1	29	NA	NA		
<i>Crotalaria greenwayi</i>	7	1	NA	NA	NA	NA	NA	2	1	1	1	0	1	3	1	36	1	NA		
<i>Crotalaria grevei</i>	1	11	2	NA	NA	NA	NA	2	1	0	0	0	1	3	1	55	NA	NA		
<i>Crotalaria griquensis</i>	7	6	1	NA	NA	NA	NA	2	1	1	1	0	1	7	2	20	NA	NA		
<i>Crotalaria harleyi</i>	2	1	0	NA	NA	NA	NA	2	1	0	0	0	0	3	1	24	1	NA		
<i>Crotalaria hebecarpa</i>	6	4	3	NA	NA	NA	NA	0	0	1	1	1	0	3	1	NA	NA	NA		
<i>Crotalaria heidmannii</i>	4	1	NA	NA	NA	NA	NA	2	1	0	0	0	1	5	2	33	NA	NA		
<i>Crotalaria hemsleyi</i>	3	1	1	NA	NA	NA	NA	2	1	0	0	0	1	3	1	NA	NA	NA		
<i>Crotalaria heyneana</i>	7	6	3	NA	NA	NA	NA	0	0	1	0	0	1	3	1	NA	NA	NA		
<i>Crotalaria hilariana</i>	6	4	0	NA	NA	NA	NA	0	0	1	1	1	0	8	3	30	3	NA		
<i>Crotalaria hirsuta</i>	6	4	3	NA	NA	NA	NA	0	0	1	1	1	0	9	3	NA	1	NA		
<i>Crotalaria hiirta</i>	6	4	3	NA	NA	NA	NA	0	0	1	1	1	0	7	2	NA	1	NA		
<i>Crotalaria holosericea</i>	2	1	0	NA	NA	NA	NA	2	1	0	0	0	0	2	1	103	1	NA		
<i>Crotalaria humbertiana</i>	7	6	2	NA	NA	NA	NA	2	1	1	1	0	1	7	2	19	NA	NA		
<i>Crotalaria humifusa</i>	6	4	3	4	NA	NA	NA	0	0	1	1	1	0	0	0	44	3	NA		
<i>Crotalaria humilis</i>	7	10	1	NA	NA	NA	NA	2	1	1	0	0	1	5	2	27	NA	NA		
<i>Crotalaria hyssopifolia</i>	8	1	1	NA	NA	NA	NA	2	1	1	0	0	1	3	1	91	1	NA		
<i>Crotalaria ibityensis</i>	3	1	2	NA	NA	NA	NA	2	1	0	0	0	1	NA	NA	NA	NA	NA		
<i>Crotalaria impressa</i>	3	1	1	NA	NA	NA	NA	2	1	0	0	0	1	5	2	NA	1	NA		
<i>Crotalaria incana</i>	2	5	1	NA	NA	NA	NA	2	1	1	0	0	0	5	1	1136	0	NA		
<i>Crotalaria incrasifolia</i>	7	6	1	NA	NA	NA	NA	2	1	1	0	0	1	5	2	NA	NA	NA		
<i>Crotalaria inopinata</i>	3	1	1	NA	NA	NA	NA	2	1	0	0	0	1	3	1	NA	NA	NA		
<i>Crotalaria involutifolia</i>	3	1	1	NA	NA	NA	NA	0	0	0	0	0	1	12	3	NA	NA	NA		
<i>Crotalaria iringana</i>	3	1	1	NA	NA	NA	NA	1	1	0	0	0	1	3	1	10	NA	NA		
<i>Crotalaria irwinii</i>	6	4	0	NA	NA	NA	NA	0	0	1	1	1	0	NA	NA	NA	NA	NA		
<i>Crotalaria juncea</i>	6	4	3	NA	NA	NA	NA	0	0	1	1	1	0	1	0	321	1	NA		
<i>Crotalaria karagwensis</i>	7	6	1	NA	NA	NA	NA	0	0	1	1	0	1	3	1	13	NA	NA		
<i>Crotalaria keniensis</i>	7	6	1	NA	NA	NA	NA	2	1	1	1	0	1	3	1	40	NA	NA		
<i>Crotalaria khasiana</i>	6	4	3	NA	NA	NA	NA	0	0	1	1	1	0	NA	NA	NA	NA	NA		
<i>Crotalaria kipandensis</i>	8	6	1	NA	NA	NA	NA	1	1	1	1	0	1	12	3	25	1	NA		
<i>Crotalaria kirikii</i>	3	1	1	NA	NA	NA	NA	2	1	0	0	0	1	3	1	NA	1	NA		
<i>Crotalaria kurtii</i>	7	6	1	NA	NA	NA	NA	2	1	1	1	0	1	7	2	NA	NA	NA		
<i>Crotalaria kurzii</i>	6	4	3	4	NA	NA	NA	0	0	1	1	1	0	12	3	NA	NA	NA		
<i>Crotalaria laburnifolia</i>	1	11	1	2	3	4	4	2	1	1	0	0	1	3	1	348	1	NA		

Species name	Sections Polhill (1982)				Sections Le Roux et al. (2013)				Region 2	Region 3	Region 4	Leaf type	Leaf binary	Keel	Calyx lobing	Calyx length	Köppen-Geiger categories	Köppen-Geiger major climate group	GBIF occurrences	Zn	
	7	2	1	6	1	NA	NA	2													1
<i>Crotalaria laburnoides</i>	7	NA	NA	6	1	NA	NA	2	1	NA	NA	2	1	1	0	1	3	1	1	62	1
<i>Crotalaria lachnocarpoides</i>	2	NA	NA	8	1	NA	NA	2	1	NA	NA	2	1	0	0	0	3	1	1	105	1
<i>Crotalaria lachnophora</i>	2	NA	NA	7	1	NA	NA	2	1	NA	NA	2	1	0	0	0	3	1	1	64	1
<i>Crotalaria laeta</i>	2	NA	NA	8	0	NA	NA	2	1	NA	NA	2	1	0	0	0	3	1	1	18	1
<i>Crotalaria laevigata</i>	3	NA	NA	1	2	NA	NA	2	1	NA	NA	2	1	0	0	1	NA	NA	NA	NA	NA
<i>Crotalaria lanceolata</i>	3	NA	NA	1	1	NA	NA	2	1	NA	NA	2	1	0	0	1	3	1	1	442	1
<i>Crotalaria lasiocarpa</i>	2	NA	NA	8	1	NA	NA	2	1	NA	NA	2	1	0	0	0	3	1	1	NA	NA
<i>Crotalaria lathyroides</i>	3	NA	NA	1	1	NA	NA	2	1	NA	NA	2	1	0	0	1	3	1	1	34	NA
<i>Crotalaria laxiflora</i>	8	NA	NA	6	1	NA	NA	2	1	NA	NA	2	1	0	0	1	3	1	1	NA	NA
<i>Crotalaria leandriana</i>	1	2	NA	11	2	NA	NA	2	1	NA	NA	2	1	0	0	1	9	3	1	NA	NA
<i>Crotalaria lebeckioides</i>	1	1	NA	11	1	NA	NA	2	1	NA	NA	2	1	0	0	1	6	2	1	NA	NA
<i>Crotalaria lebrunii</i>	1	1	NA	11	1	NA	NA	2	1	NA	NA	2	1	0	0	1	3	1	1	NA	NA
<i>Crotalaria lepidissima</i>	8	NA	NA	6	1	NA	NA	2	1	NA	NA	2	1	0	0	1	NA	NA	NA	NA	NA
<i>Crotalaria lepreurii</i>	4	NA	NA	9	1	NA	NA	2	1	NA	NA	2	1	1	0	1	NA	NA	NA	NA	NA
<i>Crotalaria linearifolia</i>	5	NA	NA	3	1	NA	NA	2	1	NA	NA	2	1	1	0	0	5	2	1	NA	NA
<i>Crotalaria linifolia</i>	6	4	2	4	2	3	NA	0	0	NA	NA	0	0	1	0	0	NA	NA	NA	NA	NA
<i>Crotalaria longidens</i>	1	1	NA	11	1	NA	NA	2	1	NA	NA	2	1	0	0	1	7	2	1	NA	NA
<i>Crotalaria longipes</i>	7	3	NA	6	3	NA	NA	0	0	NA	NA	0	0	0	0	1	3	1	1	NA	NA
<i>Crotalaria longirostrata</i>	7	0	NA	6	0	NA	NA	2	1	NA	NA	2	1	1	0	1	NA	NA	NA	NA	NA
<i>Crotalaria latifolia</i>	7	0	NA	6	0	NA	NA	2	1	NA	NA	2	1	0	0	1	NA	NA	NA	173	3
<i>Crotalaria latiformis</i>	3	1	NA	1	1	NA	NA	2	1	NA	NA	2	1	0	0	1	13	3	1	11	NA
<i>Crotalaria latoides</i>	2	1	NA	2	1	NA	NA	2	1	NA	NA	2	1	0	0	0	7	2	1	50	NA
<i>Crotalaria lukwangulensis</i>	7	1	NA	6	1	NA	NA	2	1	NA	NA	2	1	0	0	1	3	1	1	31	NA
<i>Crotalaria lunulata</i>	7	3	NA	6	3	NA	NA	0	0	NA	NA	0	0	0	1	3	1	1	1	NA	NA
<i>Crotalaria luondeensis</i>	8	1	NA	6	1	NA	NA	2	1	NA	NA	2	1	0	0	1	NA	NA	NA	NA	NA
<i>Crotalaria lutescens</i>	7	3	NA	6	3	NA	NA	0	0	NA	NA	0	0	0	1	3	1	1	1	NA	NA
<i>Crotalaria macrocarpa</i>	7	1	NA	6	1	NA	NA	2	1	NA	NA	2	1	0	0	1	8	3	1	17	NA
<i>Crotalaria madurensis</i>	7	3	NA	6	3	NA	NA	0	0	NA	NA	0	0	0	1	3	1	1	1	NA	NA
<i>Crotalaria magaliesbergensis</i>	7	1	NA	6	1	NA	NA	2	1	NA	NA	2	1	1	0	1	NA	NA	NA	NA	NA
<i>Crotalaria mahafalensis</i>	7	2	NA	6	2	NA	NA	2	1	NA	NA	2	1	0	0	1	7	2	1	13	NA
<i>Crotalaria mairei</i>	6	4	NA	4	3	NA	NA	0	0	NA	NA	0	0	1	0	0	NA	NA	NA	NA	NA
<i>Crotalaria mallindensis</i>	3	1	NA	1	1	NA	NA	2	1	NA	NA	2	1	0	0	1	2	1	1	11	NA
<i>Crotalaria martiana</i>	6	0	NA	4	0	NA	NA	0	0	NA	NA	0	0	1	0	0	3	1	1	60	3
<i>Crotalaria massaliensis</i>	3	1	NA	1	1	NA	NA	2	1	NA	NA	2	1	0	0	1	2	1	1	24	3
<i>Crotalaria mauensis</i>	2	2	NA	2	2	NA	NA	2	1	NA	NA	2	1	0	0	0	9	3	1	16	0
<i>Crotalaria medicaginea</i>	7	3	4	6	3	NA	NA	2	1	NA	NA	2	1	0	0	1	13	0	1	1559	1
<i>Crotalaria melinocalyx</i>	8	1	NA	6	1	NA	NA	2	1	NA	NA	2	1	0	0	1	3	3	1	NA	NA
<i>Crotalaria mesopotamica</i>	3	1	NA	1	1	NA	NA	2	1	NA	NA	2	1	0	0	1	3	1	1	47	1
<i>Crotalaria meyeriana</i>	7	10	NA	1	1	NA	NA	2	1	NA	NA	2	1	0	0	1	4	2	1	NA	NA
<i>Crotalaria micans</i>	2	0	NA	7	0	NA	NA	2	1	NA	NA	2	1	0	0	0	8	3	1	662	1
<i>Crotalaria microcarpa</i>	4	1	NA	1	1	NA	NA	2	1	NA	NA	2	1	0	0	1	3	1	1	187	NA
<i>Crotalaria microphylla</i>	4	5	NA	1	1	NA	NA	2	1	NA	NA	2	1	0	0	1	5	2	1	NA	NA
<i>Crotalaria microthamnus</i>	8	6	NA	1	1	NA	NA	2	1	NA	NA	2	1	0	0	1	3	1	1	NA	NA
<i>Crotalaria mildbraedii</i>	7	6	NA	1	1	NA	NA	2	1	NA	NA	2	1	1	0	1	9	3	1	15	NA

Species name	Sections Polhill (1982)				Sections Le Roux et al. (2013)				Region 2	Region 3	Region 4	Leaf type	Leaf type binary	Keel	Calyx lobing	Calyx length	Köppen-Geiger categories	Köppen-Geiger major climate group	GBIF occurrences	Zn
	2	3	4	5	6	7	8	9												
<i>Crotalaria mioctoe</i>	2				0	NA	NA	NA	2	2	1	0	0	0	0	8	3	NA	1	
<i>Crotalaria miranda</i>	7				1	NA	NA	NA	0	0	0	1	0	0	1	3	1	NA	NA	
<i>Crotalaria mitchellii</i>	3				4	NA	NA	NA	0	0	0	0	0	0	1	NA	NA	319	NA	
<i>Crotalaria mollifica</i>	2				0	NA	NA	NA	2	2	1	0	0	0	0	NA	NA	108	NA	
<i>Crotalaria monophylla</i>	4				1	NA	NA	NA	2	2	1	NA	NA	NA	NA	NA	NA	NA	NA	
<i>Crotalaria montana</i>	6				4	NA	NA	NA	0	0	0	1	1	0	0	3	1	NA	1077	
<i>Crotalaria monteroi</i>	1				1	NA	NA	NA	2	2	1	0	0	0	1	3	1	NA	117	
<i>Crotalaria mortonii</i>	2				1	NA	NA	NA	2	2	1	0	1	0	0	NA	NA	NA	NA	
<i>Crotalaria mudugensis</i>	7				1	NA	NA	NA	2	2	1	1	0	0	NA	3	1	NA	NA	
<i>Crotalaria muenzneri</i>	3				1	NA	NA	NA	2	2	1	0	0	1	1	0	0	NA	11	
<i>Crotalaria multiflora</i>	6				4	NA	NA	NA	0	0	0	1	1	0	0	NA	NA	NA	NA	
<i>Crotalaria mysorensis</i>	6				3	NA	NA	NA	0	0	0	1	1	0	0	NA	NA	30	1	
<i>Crotalaria nana</i>	6				4	NA	NA	NA	0	0	0	1	1	0	0	3	1	NA	NA	
<i>Crotalaria natalitia</i>	2				7	NA	NA	NA	2	2	1	0	0	0	0	3	1	NA	178	
<i>Crotalaria nayaritensis</i>	6				4	NA	NA	NA	0	0	0	1	1	0	0	NA	NA	23	3	
<i>Crotalaria neglecta</i>	NA				1	NA	NA	NA	2	2	1	NA	NA	NA	NA	NA	NA	NA	NA	
<i>Crotalaria nigricans</i>	2				1	NA	NA	NA	2	2	1	0	0	0	0	3	1	NA	NA	
<i>Crotalaria nitens</i>	6				4	NA	NA	NA	0	0	0	1	1	0	0	0	0	NA	NA	
<i>Crotalaria notanii</i>	7				3	NA	NA	NA	0	0	0	1	1	0	0	3	1	NA	NA	
<i>Crotalaria novae-hollandiae</i>	7				6	NA	NA	NA	1	1	1	1	0	0	1	7	2	NA	NA	
<i>Crotalaria obscura</i>	2				1	NA	NA	NA	2	2	1	0	0	0	0	9	3	NA	37	
<i>Crotalaria oblecta</i>	6				3	NA	NA	NA	0	0	0	1	1	0	0	3	1	NA	NA	
<i>Crotalaria ochroleuca</i>	3				1	NA	NA	NA	2	2	1	0	0	0	1	3	1	NA	105	
<i>Crotalaria oligosperma</i>	7				6	NA	NA	NA	2	2	1	1	0	0	1	5	2	NA	NA	
<i>Crotalaria omanoides</i>	2				1	NA	NA	NA	2	2	1	0	0	0	0	3	1	NA	116	
<i>Crotalaria occarpa</i>	7				6	NA	NA	NA	2	2	1	1	0	0	1	3	1	NA	26	
<i>Crotalaria orientalis</i>	4				9	NA	NA	NA	1	1	1	0	0	0	1	7	2	NA	19	
<i>Crotalaria orixensis</i>	6				4	NA	NA	NA	0	0	0	1	1	0	0	3	1	NA	15	
<i>Crotalaria orthoclada</i>	2				8	NA	NA	NA	2	2	1	0	0	0	0	3	1	NA	3	
<i>Crotalaria toptera</i>	6				4	NA	NA	NA	0	0	0	1	1	0	0	3	1	NA	1092	
<i>Crotalaria pallida</i>	3				1	NA	NA	NA	2	2	1	0	0	0	1	3	1	NA	NA	
<i>Crotalaria pallidicaulis</i>	1				11	NA	NA	NA	2	2	1	0	0	0	1	12	3	NA	NA	
<i>Crotalaria parvula</i>	8				6	NA	NA	NA	2	2	1	1	0	0	1	12	3	NA	NA	
<i>Crotalaria passerinoides</i>	8				6	NA	NA	NA	0	0	0	1	0	0	1	3	1	NA	NA	
<i>Crotalaria paulina</i>	6				4	NA	NA	NA	0	0	0	1	1	0	0	8	3	NA	69	
<i>Crotalaria perrieri</i>	2				8	NA	NA	NA	2	2	1	0	0	0	0	9	3	NA	NA	
<i>Crotalaria persica</i>	3				1	NA	NA	NA	2	2	1	0	0	0	1	5	2	NA	15	
<i>Crotalaria phylloblobo</i>	2				2	NA	NA	NA	2	2	1	0	0	0	0	3	1	NA	NA	
<i>Crotalaria pilosa</i>	6				4	NA	NA	NA	0	0	0	1	1	0	0	3	1	NA	122	
<i>Crotalaria pilosiflora</i>	7				6	NA	NA	NA	2	2	1	1	0	0	1	12	3	NA	NA	
<i>Crotalaria pisicarpa</i>	2				1	NA	NA	NA	2	2	1	0	0	0	0	7	2	NA	46	
<i>Crotalaria pleiophylla</i>	3				1	NA	NA	NA	3	3	1	0	0	0	1	NA	NA	NA	NA	
<i>Crotalaria plowdenii</i>	3				1	NA	NA	NA	2	2	1	0	0	0	1	13	3	NA	12	
<i>Crotalaria podocarpa</i>	2				7	NA	NA	NA	2	2	1	0	0	0	0	7	2	NA	122	

Species name	Sections Polhill (1982)				Sections Le Roux et al. (2013)				Region 2	Region 3	Region 4	Leaf type	Leaf type binary	Keel	Calyx lobing	Calyx length	Köppen-Geiger categories	Köppen-Geiger major climate group	GBIF occurrences	Zn
	7	3	6	1	2	1	1	2												
<i>Crotalaria poissonii</i>	7	NA	6	NA	2	NA	NA	NA	2	NA	NA	2	1	0	1	7	2	10	NA	
<i>Crotalaria polhillii</i>	3	NA	1	NA	1	NA	NA	NA	2	NA	NA	2	1	0	1	NA	NA	NA	NA	
<i>Crotalaria polyphylla</i>	6	NA	4	NA	0	NA	NA	NA	0	NA	NA	0	0	1	0	NA	NA	NA	3	
<i>Crotalaria prittwitzii</i>	2	NA	7	NA	1	NA	NA	NA	2	NA	NA	2	1	0	0	3	1	NA	NA	
<i>Crotalaria prolongata</i>	8	NA	6	NA	1	NA	NA	NA	2	NA	NA	2	1	1	1	12	3	NA	1	
<i>Crotalaria prostrata</i>	6	NA	4	NA	3	NA	NA	NA	0	NA	NA	0	0	1	0	0	0	0	18	
<i>Crotalaria pseudodilobaensis</i>	8	NA	6	NA	1	NA	NA	NA	2	NA	NA	2	1	0	1	3	1	NA	NA	
<i>Crotalaria pseudatenulirama</i>	8	NA	6	NA	1	NA	NA	NA	2	NA	NA	2	1	1	1	3	1	25	NA	
<i>Crotalaria pulchra</i>	7	NA	6	NA	3	NA	NA	NA	0	NA	NA	0	0	1	1	3	1	NA	NA	
<i>Crotalaria pumila</i>	7	NA	6	NA	0	NA	NA	NA	2	NA	NA	2	1	0	1	NA	NA	465	3	
<i>Crotalaria purdiana</i>	7	NA	6	NA	0	NA	NA	NA	2	NA	NA	2	1	0	1	NA	NA	12	NA	
<i>Crotalaria purshii</i>	6	NA	4	NA	0	NA	NA	NA	0	NA	NA	0	0	1	0	NA	NA	18	3	
<i>Crotalaria pusilla</i>	6	NA	4	NA	3	NA	NA	NA	0	NA	NA	0	0	1	0	3	1	NA	1	
<i>Crotalaria pycnostachya</i>	3	NA	1	NA	1	NA	NA	NA	2	NA	NA	2	1	0	1	13	3	84	1	
<i>Crotalaria quartinaia</i>	2	NA	2	NA	1	NA	NA	NA	2	NA	NA	2	1	0	0	NA	NA	NA	0	
<i>Crotalaria quercetorum</i>	6	NA	4	NA	0	NA	NA	NA	0	NA	NA	0	0	1	0	NA	NA	71	3	
<i>Crotalaria quinquefolia</i>	7	NA	6	NA	3	NA	NA	NA	3	NA	NA	3	1	0	1	0	0	35	1	
<i>Crotalaria ramosissima</i>	7	NA	6	NA	3	NA	NA	NA	0	NA	NA	0	0	1	1	3	1	247	1	
<i>Crotalaria recta</i>	7	NA	4	NA	1	NA	NA	NA	0	NA	NA	0	0	1	0	3	1	142	1	
<i>Crotalaria reptans</i>	2	NA	1	NA	1	NA	NA	NA	2	NA	NA	2	1	0	1	NA	NA	NA	NA	
<i>Crotalaria retusa</i>	7	NA	6	NA	5	NA	NA	NA	0	NA	NA	0	0	1	1	12	3	1108	1	
<i>Crotalaria rhodesiae</i>	2	NA	7	NA	1	NA	NA	NA	2	NA	NA	2	1	0	1	NA	NA	NA	1	
<i>Crotalaria rogersii</i>	3	NA	1	NA	1	NA	NA	NA	2	NA	NA	2	1	0	0	12	3	24	1	
<i>Crotalaria rubiginosa</i>	NA	NA	NA	NA	3	NA	NA	NA	0	NA	NA	0	0	NA	NA	3	1	NA	NA	
<i>Crotalaria rufipila</i>	2	NA	1	NA	0	NA	NA	NA	2	NA	NA	2	1	0	0	3	1	15	1	
<i>Crotalaria rzedowskii</i>	6	NA	4	NA	0	NA	NA	NA	0	NA	NA	0	0	1	0	NA	NA	NA	NA	
<i>Crotalaria sagittalis</i>	6	NA	4	NA	0	NA	NA	NA	0	NA	NA	0	0	1	0	3	1	NA	NA	
<i>Crotalaria saharae</i>	3	NA	1	NA	1	NA	NA	NA	3	NA	NA	3	1	1	1	NA	NA	571	3	
<i>Crotalaria salicifolia</i>	6	NA	4	NA	3	NA	NA	NA	0	NA	NA	0	0	1	0	3	1	NA	NA	
<i>Crotalaria saltiana</i>	3	NA	1	NA	1	NA	NA	NA	2	NA	NA	2	1	0	1	5	2	38	1	
<i>Crotalaria sandozensis</i>	7	NA	6	NA	3	NA	NA	NA	0	NA	NA	0	0	1	1	NA	NA	NA	NA	
<i>Crotalaria scassellatii</i>	7	NA	6	NA	1	NA	NA	NA	2	NA	NA	2	1	0	1	2	1	NA	NA	
<i>Crotalaria semperflorens</i>	7	NA	6	NA	3	NA	NA	NA	0	NA	NA	0	0	1	0	0	0	11	NA	
<i>Crotalaria sericifolia</i>	2	NA	8	NA	1	NA	NA	NA	2	NA	NA	2	1	0	0	7	2	NA	NA	
<i>Crotalaria sessiliflora</i>	6	NA	4	NA	3	NA	NA	NA	0	NA	NA	0	0	1	0	0	0	NA	1	
<i>Crotalaria shanica</i>	NA	NA	NA	NA	3	NA	NA	NA	0	NA	NA	0	0	2	1	NA	NA	145	1	
<i>Crotalaria shrensis</i>	4	NA	9	NA	1	NA	NA	NA	0	NA	NA	0	0	0	1	NA	NA	17	NA	
<i>Crotalaria smithiana</i>	3	NA	1	NA	4	NA	NA	NA	0	NA	NA	0	0	0	0	NA	NA	231	NA	
<i>Crotalaria smithiana</i>	3	NA	1	NA	1	NA	NA	NA	0	NA	NA	0	0	0	0	NA	NA	NA	1	
<i>Crotalaria spartea</i>	4	NA	9	NA	1	NA	NA	NA	1	NA	NA	1	1	0	1	5	2	72	NA	
<i>Crotalaria spartioides</i>	6	NA	4	NA	3	NA	NA	NA	0	NA	NA	0	0	1	0	NA	NA	NA	NA	
<i>Crotalaria speciosa</i>	7	NA	4	NA	3	NA	NA	NA	0	NA	NA	0	0	1	0	12	3	222	1	
<i>Crotalaria spectabilis</i>	4	NA	9	NA	1	NA	NA	NA	2	NA	NA	2	1	0	1	7	2	187	1	
<i>Crotalaria sphaerocarpa</i>	7	NA	6	NA	1	NA	NA	NA	2	NA	NA	2	1	0	1	3	1	120	1	

Chapter 3

LECTOTYPIFICATIONS AND TAXONOMIC CHANGES IN BRAZILIAN *CROTALARIA* L. (LEGUMINOSAE).

Flores, A.S., Rockinger, A., Rodrigues, R.S., and Tozzi, A.M.G.A.

Phytotaxa 267(4), 296–300



Lectotypifications and taxonomic changes in Brazilian *Crotalaria* L. (Leguminosae)

ANDRÉIA SILVA FLORES^{1*}, ALEXANDER ROCKINGER², RODRIGO SCHÜTZ RODRIGUES³ & ANA MARIA GOULART DE AZEVEDO TOZZI⁴

¹Instituto de Amparo à Ciência, Tecnologia e Inovação de Roraima, Herbário do Museu Integrado de Roraima, Av. Brigadeiro Eduardo Gomes s.n., Parque Anauá, 69305-010 Boa Vista, RR, Brazil

²University of Munich (LMU), Systematic Botany and Mycology, Menzinger Str. 67, 80638 Munich, Germany

³Universidade Federal de Roraima. Centro de Estudos da Biodiversidade, Herbário UFRR, Av. Cap. Ene Garcez 2413 69310-000. Boa Vista, RR, Brazil

⁴Universidade Estadual de Campinas, Instituto de Biologia, Departamento de Biologia Vegetal, Rua Monteiro Lobato, 255, 13083-862. Campinas, SP, Brazil

*Corresponding author: andreiasflores@gmail.com

As part of a revision of the species of *Crotalaria* L. (Papilionoideae, Crotalarieae) occurring in Brazil, eleven lectotypifications are proposed for: *Crotalaria acutiflora* Benth., *Crotalaria depauperata* Mart. ex Benth., *Crotalaria holosericea* Nees & Mart., *Crotalaria holosericea* var. *grisea* Benth., *Crotalaria laeta* Mart. ex Benth., *Crotalaria leptophylla* Benth., *Crotalaria rufipila* Benth., *Crotalaria unifoliolata* Benth., *Crotalaria vitellina* Ker-Gawl., *Crotalaria vitellina* var. *glabrata* Benth., and *Crotalaria vitellina* var. *minor* Benth. Four new synonyms are also proposed.

Keywords: Crotalarieae, Fabaceae, Neotropics, Papilionoideae, synonym, taxonomy, typification

Introduction

The pantropical genus *Crotalaria* Linnaeus (1753: 714) comprises approximately 700 species and together with 15 other genera belongs to the mainly African tribe Crotalarieae (Van Wyk 2005; Boatwright *et al.* 2011). Forty-two species are recorded for Brazil, of which 31 are native and 17 endemic (Flores & Tozzi 2008). The morphological key features of *Crotalaria* are a rostrate keel, a hairy style, monadelphous androecium and dimorphic stamens in a 5+5 configuration, paired callosities on the standard petal blade and claw, inflated pods, and simple or compound (unifoliolate, digitately tri- or multifoliolate) leaves (Polhill 1982; Flores & Tozzi 2008; Le Roux *et al.* 2013).

During his taxonomic treatment of *Crotalaria* for the Flora Neotropica, the late Donald Windler (1940–2012) studied the collections of several European herbaria in which he assigned material as lectotypes. However, neither his floristic treatment nor the lectotypifications have been published. As part of a revision of Brazilian *Crotalaria* we propose eleven lectotypifications, adopting the lectotypification assignments made by Windler (as noted on his determination labels) that we found reasonable. However, in some cases we differ from his assignments. We also have synonymized four names in the course of this work.

***Crotalaria grandiflora* Bentham (1839: 429).**

Crotalaria acutiflora var. *grandiflora* (Benth.) Bentham (1859: 23).

Type:—BRAZIL. “Near Cercado”, s.d., *J.B.E. Pohl 1124* (holotype W0052805!).

Crotalaria divaricata Bentham (1839: 429), *syn. nov.*

Type:—BRAZIL. “Rio Reazon”, s.d., *J.B.E. Pohl s.n.* (holotype K000500583!; isotypes FV0077377!, NY00006683!).

Crotalaria acutiflora Bentham (1843: 482).

Lectotype (designated here):—BRAZIL. “Chapada Santa Cruz”, 1836, *J.B.E. Pohl s.n.* (K000500593!).

Lectotype image: <http://specimens.kew.org/herbarium/K000500593>.

In the protologue of *Crotalaria acutiflora*, Bentham (1843) mentioned material collected by Pohl and Vauthier in Brazil as “*C. breviflora*, Benth. in *Herb. Mus. Vind. MS. non DC.*”. While the material of Vauthier could not be found, the Pohl material

in K and W was analyzed. Windler assigned the material W0052804 as lectotype of *C. acutiflora*. We disagree with this choice, however, as this material comprises two specimens mounted on one sheet, stating three collection numbers (“2898, 1125 and 1123”), and three locations (“Chapada S. Cruz, Megaponte & S. Luzia”). This makes the mutual assignment of specimens, collection numbers, and locations impossible. On the other hand, the material in the K herbarium (K000500593) is unambiguous by stating only one location and collection number. In addition, it carries Bentham’s handwriting and, in agreement with the protologue, was labeled “*C. breviflora* Herb. Mus. Vin.”. Thus, we hereby designate this material as lectotype.

Windler & Skinner (1982) considered *Crotalaria acutiflora* and *C. grandiflora* as a single taxon, while Bentham (1859) had treated *C. grandiflora* as a variety of *C. acutiflora*, and considered *C. divaricata* as a distinct species. His distinction was based on vegetative pubescence, being sericeous in *C. acutiflora* and tomentose in *C. divaricata*. Studying the type material led us to the conclusion that the different types of pubescence are not sufficiently distinguishable to segregate two different taxa. We therefore synonymize *C. divaricata* under *C. grandiflora*.

Crotalaria holosericea Nees & Martius in Wied-Neuwied *et al.* (1824: 26).

Lectotype (designated here):—BRAZIL. “Circa Barra da Vareda”, s.d., *M. Wied-Neuwied s.n.* (BR6584061!; isolectotype BR8423450!).
Lectotype image: <http://www.br.fgov.br/RESEARCH/COLLECTIONS/HERBARIUM/detail.php?ID=440743>.

Crotalaria holosericea var. *grisea* Bentham (1859: 30).

Lectotype (designated here):—BRAZIL. “In prov. Piauiensis campis prope Oeiras et alibi”, s.d., *C.F.P. Martius s.n.* (M0187170!).
Lectotype image: <http://plants.jstor.org/stable/10.5555/al.ap.specimen.m0187170>.

In the protologue of *Crotalaria holosericea*, Nees & Martius (1824: 26) cited material from the locations “Circa Barra da Vareda” and “in campis Provinciae Piauiensis”. No material from the latter location could be found, but the locality stated on the identification label of the Wied-Neuwied syntype in the BR herbarium (BR6584061) “Barra da Vareda, in prov. Min. confin. orient.” agrees with the original description. In accordance with Windler’s assignment, we designate it here as lectotype.

Bentham (1859) defined *Crotalaria holosericea* var. *grisea* as an intermediate form between *C. clauseni* and *C. holosericea*. The description was based on the following syntypes: Brazil, “in prov. Piauiensis campis prope Oeiras et alibi: M.” (collected by Martius), and “circa Oeiras frequens et ad rivulum prope Boa Esperanza: Gardner 2104 et 2105”. In agreement with Windler’s assignment, we designate the collection of Martius as lectotype since it displays most completely the defining morphological characters.

Crotalaria laeta Martius ex Bentham (1859: 30).

Crotalaria vitellina var. *laeta* (Mart. ex Benth.) Windler & S.G. Skinner (1982: 186).

Lectotype (designated here):—BRAZIL. “Prov. Piauihy. Brasilia tropica”, 1839, *G. Gardner 2103* (K000187675!; isolectotypes BM000900983!, L0005768!, P02731906!).

Lectotype image: <http://specimens.kew.org/herbarium/K000187675>.

In the protologue of *Crotalaria laeta*, Bentham (1859) mentioned two collections: “Habitat in pascuis amoensis serenis prov. Piauiensis: M” and “Gardner n. 2103”. Windler assigned the material in M (collected by Martius) as lectotype. Although this material actually corresponds to *C. laeta*, it was also included by Bentham (1859) among the syntypes of *C. vitellina* var. *glabrata*. As the material of Gardner in K most closely resembles the original description and is more complete as it has flowers and fruits, we designate it here as lectotype.

Crotalaria maypurensis Kunth (1824: 403).

Type:—VENEZUELA. “Crescit in ripa Orinoci, prope Maypures”, s.d., *A.J.A. Bonpland & F.W.H.A. von Humboldt s.n.* (holotype P00660097!).

Crotalaria depauperata Martius ex Bentham (1859: 30).

Crotalaria maypurensis var. *depauperata* (Mart. ex Benth.) Windler & Skinner (1982: 186).

Lectotype (designated here):—BRAZIL. “in Chapada prope Capellinha ejusdem prov.”, s.d., *A. de St. Hilaire 193* (K000500581!).

Lectotype image: <http://specimens.kew.org/herbarium/K000500581>.

Crotalaria leptophylla Bentham (1839: 430).

Lectotype (designated here):—GUYANA. “Savannahs of the Rupunoony”, s.d., *R. Schomburgk s.n.* (K000500566! [specimen on the right side of sheet]).

Lectotype image: <http://specimens.kew.org/herbarium/K000500566>.

Crotalaria depauperata was described on the basis of four collections: “*Habitat in alpestribus tractus Adamantini Serra de S. Antonio dicti prov. Minarum: M.*”, “*in Chapada prope Capellinha ejusdem prov.: A. de St. Hilaire*”, “*in prov. Goyazensis campis arenosis; Gardner 4113*”, and “*ad Ribeirão da Prata: Pohl*”. All this material has been located and analyzed. Avendaño (2011) assigned the St. Hilaire material in K (K000500581) as lectotype of *C. depauperata*, referring to the additional specimen identification “lectotype” label assigned by Windler in 1980. We agree with this assessment and thus here formalize the choice of this material as lectotype of *C. depauperata*.

In his original description, Bentham (1839) cited the following syntypes for *Crotalaria leptophylla* “*Savannahs of the Rupunoony. Schomburgk.*” and “*On the Rio Preto, in Brasil. Pohl.*” The Pohl material has not been located, but we have studied Schomburgk’s collections in the Kew herbarium. Windler assigned the material K000500565 as lectotype of *C. leptophylla*. We disagree with this assignment since the material K000500566 is the single specimen with the exact locality given in the protologue stated on its identification label. Therefore we here designate the latter material as lectotype.

Crotalaria rufipila Bentham (1859: 28).

Lectotype (designated here):—BRAZIL. “*In saxosis Serra da Piedade*” s.d., *L. Riedel 586* (K000500557! [specimen on the right side of sheet]).

Lectotype image: <http://specimens.kew.org/herbarium/K000500557>.

Bentham (1859) cited the syntypes “*Habitat in prov. Minarum district Adamantium: Gardn. n. 4505.*”, “*in saxosis Serra da Piedade: Riedel*”, “*in campis editis ad Ouro Preto: M.*”, and “*in eadem prov.: A. de St. Hilaire*”. In 1980, Windler labeled the collection of Riedel, deposited in K (K000500557, specimen on the right side of sheet), as his choice for the designation of a lectotype of *C. rufipila*, which we formally designate here.

Crotalaria unifoliolata Bentham (1839: 430).

Lectotype (designated here):—BRAZIL. “*Near Oretiro*”, s.d., *J.B.E. Pohl 1078* (K000500552!; isolectotype W0057044!).

Lectotype image: <http://specimens.kew.org/herbarium/K000500552>.

Bentham (1839) referred to two collections when describing this species: “*Brasil. Schücht*” and “*Near Oretiro. Pohl*”. The Schücht material could not be located. The Pohl material was found in the herbaria K and W. Windler assigned the material in K (K500552) as lectotype of *C. unifoliolata*. In agreement with Windler’s previous assignment, we designate it here as lectotype.

Crotalaria vitellina Ker-Gawler (1820: 447).

Lectotype (designated here):—[Illustration in] Ker Gawler (1820: pl. 447) “The drawing was taken in the hothouse at the nursery of Messrs. Colville, in the King’s Road, Chelsea, where the plant flowers freely and produces seed”, “We are informed that this plant has been only lately introduced from the Brazils”.

Lectotype image: <http://www.biodiversitylibrary.org/page/130909#page/41/mode/1up>.

Crotalaria vitellina var. *glabrata* Bentham (1859: 29), *syn. nov.*

Lectotype (designated here):—BRAZIL. “*Sta Catharina*”, 1820, *A. de St. Hilaire 1719 (catal. C2)*. (P00758473!).

Lectotype image: <http://mediaphoto.mnhn.fr/media/1441328962671FhOX5W197q7K3Hia>.

Crotalaria vitellina var. *minor* Bentham (1859: 29), *syn. nov.*

Lectotype (designated here):—BRAZIL. “*Serra de Araripe*”, 1839, *G. Gardner 2411* (K000187977!).

Lectotype image: <http://specimens.kew.org/herbarium/K000187977>.

Crotalaria vitellina var. *oblongifolia* Bentham (1859: 29), *syn. nov.*

Type:—BRAZIL. “*In Brasilia orientali*”, 1845, *F.C. Raben s.n.* (holotype not located; isotype C10012078!).

According to Ker-Gawler (1820), the description of *Crotalaria vitellina* was based on a cultivated plant originating from Brazil. However, Ker-Gawler did not cite a type collection and no original plant material of *C. vitellina* has been located. We consider the colored illustration (1820: pl. 447) in the protologue to represent part of the original material of *C. vitellina*. It is assumed that the plate is the only surviving original material and we hereby designate it as lectotype.

Bentham (1859) described three varieties of *C. vitellina*, based on the size and pubescence of legume, and leaflet form. He characterized *C. vitellina* var. *glabrata* by having glabrous legumes (vs. sericeous-pubescent in *C. vitellina* var. *vitellina*), *C. vitellina* var. *minor* by its oval or oboval and obtuse leaflets, numerous smaller flowers, and legumes of 1.6–2.2 cm in length (vs. oval-lanceolate leaflets and legumes of 2.5–3.75 cm in *C. vitellina* var. *vitellina*), and *C. vitellina* var. *oblongifolia* by having oblong and obtuse leaflets (vs. oval-lanceolate leaflets in *C. vitellina* var. *vitellina*). Bentham (1859) mentioned numerous smaller flowers in *C. vitellina* var. *minor* and “*flores mediocres v. inter minores*” in *C. vitellina* var. *vitellina*, but no flower size was provided in his description.

Examining a large set of specimens of *C. vitellina* throughout its neotropical distribution range, neither completely glabrous nor mature pods shorter than 2 cm were found. Pods generally are puberulent (with few short and adpressed trichomes) or glabrescent, and 2–3 cm in length. In addition, the leaflet form varies between oblong and elliptic in the same individual (elliptic being predominant). As we have not been able to detect additional diagnostic characters for each variety, we thus recognize a single taxon, *C. vitellina*, and place the three Bentham varieties in synonymy.

In his description of *C. vitellina* var. *glabrata*, Bentham (1859) cited various syntypes: “*ad Rio Jequetinhonha et in prov. Matto Grosso: Pohl*”, “*prope Paratinga prov. S. Pauli*”, “*in pascuis amoensis prov. Piauiensis: M*” and “*ad S. Catharina et prope Rio de Janeiro (forma foliolis majoribus): St. Hilaire*”. We do not agree with Windler’s assignment of a flowering collection of Pohl s.n. “*ad Rio Jequetinhonha*” in the K herbarium (K000500542) as the lectotype of *C. vitellina* var. *glabrata*, because this specimen does not have fruits (glabrous pods are the only defining character of this variety). The syntypes collected by Martius and St. Hilaire have glabrescent fruits, whose short trichomes are mostly found near their base and apex. All Martius syntypes correspond to *C. laeta* by having wings shorter than the keel (in *C. vitellina* wings are as long as or longer than the keel). Among the material of Saint Hilaire deposited in P are several specimens from Rio de Janeiro that cannot be clearly assigned as original material. However, one specimen from the state Santa Catarina was labeled “*C. vitellina* var. *latifolia*” by Bentham. This variety is unpublished and Bentham supposedly referred to this material in the protologue as “*forma foliolis majoribus*” from Saint Hilaire’s collection. Furthermore, this specimen includes mature seed pods. We therefore designate it here as the lectotype of *C. vitellina* var. *glabrata*.

When describing *C. vitellina* var. *minor*, Bentham (1859) cited the following collections: “*in prov. Minarum: Claussen*”, “*In locis saxosis ejusdem prov.: St Hilaire*”, “*in prov. Rio de Janeiro prope Macahe et Praya Grande Gardner n. 1930*” and “*in prov. Cearensi prope Crato: Gardner n. 241*”. In agreement with Windler’s previous assignment, we designate the Gardner material in K (K000187977) as lectotype of this variety.

Bentham (1859) described *C. vitellina* var. *oblongifolia* based on the material “*In Brasilia orientali: Raben (specimen mancum)*”. We understand that the material analyzed by Bentham is an incomplete or a poorly preserved specimen. On the other hand, it was found in C herbarium a complete and well-preserved specimen collected by Raben in Brazil. This specimen has oblong leaflets similar to that described by Bentham for *C. vitellina* var. *oblongifolia*. We assume that it is an isotype of this variety and the holotype remains to be located.

Acknowledgments

We thank the curators of BM, BR, C, F, K, M, NY, P, UEC, and W for loans or digital images of collections. Johannes Walter (Natural History Museum, Vienna), Hans-Joachim Esser (Botanische Staatssammlung München), Gwilym Lewis (Royal Botanical Gardens, Kew), Jefferson Prado (Instituto de Botânica, São Paulo), and Susanne S. Renner (Botanische Staatssammlung München) are gratefully acknowledged for their support in locating type material, nomenclatural advice, or critical comments on the manuscript. ASF was supported by a grant from the Fundação de Amparo à Pesquisa do Estado de São Paulo -FAPESP (proc. 00/11674-2).

References

- Avenidaño, N. (2011) Revisión taxonómica del género *Crotalaria* L. (Fabaceae-Crotalariaeae) en Venezuela. *Acta Botánica Venezuelica* 34: 13–78.
- Bentham, G. (1839) Enumeration of plants collected by Mr. Schomburgk, British Guiana. *Annals of Natural History* 3: 427–438.
<http://dx.doi.org/10.1080/03745483909443258>
- Bentham, G. (1843) Enumeration of the Leguminosae indigenous to Southern Asia and Central and Southern Africa. *The London Journal of Botany* 2: 423–613.

- Bentham, G. (1859) *Crotalaria*. In: Martius, C.F.P. (Ed.) *Flora Brasiliensis* 15(1). F. Fleischer, Lipsiae, pp. 17–32.
- Boatwright, J.S., Wink, M. & van Wyk, B.-E. (2011) The generic concept of *Lotononis* (Crotalariaeae, Fabaceae): Reinstatement of the genera *Euchlora*, *Leobordea* and *Listia* and the new genus *Ezoloba*. *Taxon* 60 (1): 161–177.
- Flores, A.S. & Tozzi, A.M.G.A. (2008) Phytogeographical patterns of *Crotalaria* species (Leguminosae-Papilionoideae) in Brazil. *Rodriguésia* 59: 477–486.
- Ker-Gawler, J.B. (1820) *Crotalaria vitellina*. *The Botanical Register* 6: 447.
- Kunth, K.S. (1824) *Crotalaria*. In: Humboldt, F.W.H., Bonpland, A.J.A. & Kunth, K.S. (Eds.) *Nova Genera et Species Plantarum* 6: 397–406.
- Le Roux, M.M., Boatwright, J.S. & Van Wyk, B.-E. (2013) A global infrageneric classification system for the genus *Crotalaria* (Leguminosae) based on molecular and morphological evidence. *Taxon* 62: 957–971.
<http://dx.doi.org/10.12705/625.1>
- Linnaeus, C. (1753) *Species Plantarum*. Laurentii Salvii, Stockholm, 1200 pp.
- Wied-Neuwied, M., Nees, C.G.D. & Martius, C.F.P. (1824) Beitrag zur Flora Brasiliens. *Nova Acta Physico-Medica Academiae Caesareae Leopoldino-Carolinae Naturae Curiosorum Exhibentia Ephemerides sive Observationes Historias et Experimenta* 12: 1–54.
- Polhill, R.M. (1982) *Crotalaria in Africa and Madagascar*. A.A. Balkema, Rotterdam, 389 pp.
- Van Wyk, B.-E. (2005) Crotalariaeae. In: Lewis, G., Schrire, B., Mackinder, B. & Lock, M. (Eds.) *Legumes of the World*. Royal Botanic Gardens, Kew, pp. 273–281.
- Windler, D.R. & Skinner, S.G. (1982) New taxa and new combinations in the American Crotalaris (Fabaceae). *Phytologia* 50: 185–206.
<http://dx.doi.org/10.5962/bhl.part.16101>

Chapter 4

CHROMOSOME NUMBER REDUCTION IN THE SISTER CLADE OF *CARICA PAPAYA* WITH CONCOMITANT
GENOME SIZE DOUBLING.

Rockinger, A., Sousa, A., Carvalho, F.A., and Renner, S.S.

American Journal of Botany 103(6), 1082–1088 [Journal cover]

Chromosome number reduction in the sister clade of *Carica papaya* with concomitant genome size doubling¹

Alexander Rockinger², Aretuza Sousa, Fernanda A. Carvalho, and Susanne S. Renner

PREMISE OF THE STUDY: Caricaceae include six genera and 34 species, among them papaya, a model species in plant sex chromosome research. The family was held to have a conserved karyotype with $2n = 18$ chromosomes, an assumption based on few counts. We examined the karyotypes and genome size of species from all genera to test for possible cytogenetic variation.

METHODS: We used fluorescent in situ hybridization using standard telomere, 5S, and 45S rDNA probes. New and published data were combined with a phylogeny, molecular clock dating, and C values (available for ~50% of the species) to reconstruct genome evolution.

KEY RESULTS: The African genus *Cylicomorpha*, which is sister to the remaining Caricaceae (all neotropical), has $2n = 18$, as do the species in two other genera. A Mexican clade of five species that includes papaya, however, has $2n = 18$ (papaya), $2n = 16$ (*Horovitzia cnidoscoloides*), and $2n = 14$ (*Jarilla caudata* and *J. heterophylla*; third *Jarilla* not counted), with the phylogeny indicating that the dysploidy events occurred ~16.6 and ~5.5 million years ago and that *Jarilla* underwent genome size doubling (~450 to 830–920 Mbp/haploid genome). Pericentromeric interstitial telomere repeats occur in both *Jarilla* adjacent to 5S rDNA sites, and the variability of 5S rDNA sites across all genera is high.

CONCLUSIONS: On the basis of outgroup comparison, $2n = 18$ is the ancestral number, and repeated chromosomal fusions with simultaneous genome size increase as a result of repetitive elements accumulating near centromeres characterize the papaya clade. These results have implications for ongoing genome assemblies in Caricaceae.

KEY WORDS Brassicales; Caricaceae; descending dysploidy; FISH; genome size doubling; interstitial telomere repeats

On the basis of gene synteny, the ancestor of all Caricaceae, a small family in the order Brassicales, underwent a single whole-genome duplication event (Tang et al., 2008), and chromosome numbers (Rice et al., 2015) and genome sizes (Gschwend et al., 2013) appear to have been stable since. In the related family Brassicaceae, of about the same age but with 10 times as many species, there is evidence of much transposable element amplification and polyploidy, yet a narrow range of small genome sizes over long evolutionary time periods (Lysak et al., 2009). In addition, synteny suggests that Brassicaceae have undergone at least three ancestral polyploidization events (Tang et al., 2008); there are no data for any within-Cariaceae genome duplications yet. The assumption of stability in chromosome number and genome size, however, is based on data from just three (*Carica*, *Jacaratia*, and

Vasconcellea) of the family's six genera. Genome size stability cannot be extrapolated from chromosome number variation or the other way around, as recently documented again for *Carex* (Escudero et al., 2015).

The six genera of Caricaceae comprise 34 species occurring in tropical Africa and Central and South America (Carvalho, 2013 onward). Papaya (*Carica papaya*) is an important tree crop (FAO, 2016) that originated in southern Mexico, and molecular data show that its sister clade consists of the monotypic genus *Horovitzia*, endemic to southern Mexico, and *Jarilla*, three species of herbs endemic to Mexico and Guatemala (Carvalho and Renner, 2012, 2013). Sister to all New World Caricaceae is an African genus (*Cylicomorpha*) with two species. A draft of the papaya genome became available in 2008 (Ming et al., 2008), and since then, considerable effort has gone into understanding the sex chromosomes of *C. papaya* (Liu et al., 2004; Wu et al., 2010; VanBuren et al., 2015). Using 22 BACs distributed along the papaya X and Y chromosome, Iovene et al. (2015) identified homologous chromosome pairs in *Jacaratia spinosa* and *Vasconcellea parviflora*.

¹ Manuscript received 26 March 2016; revision accepted 3 May 2016.

Systematic Botany and Mycology, University of Munich, 80638 Munich, Germany

² Author for correspondence (e-mail: a.rockinger@gmail.com)

doi:10.3732/ajb.1600134

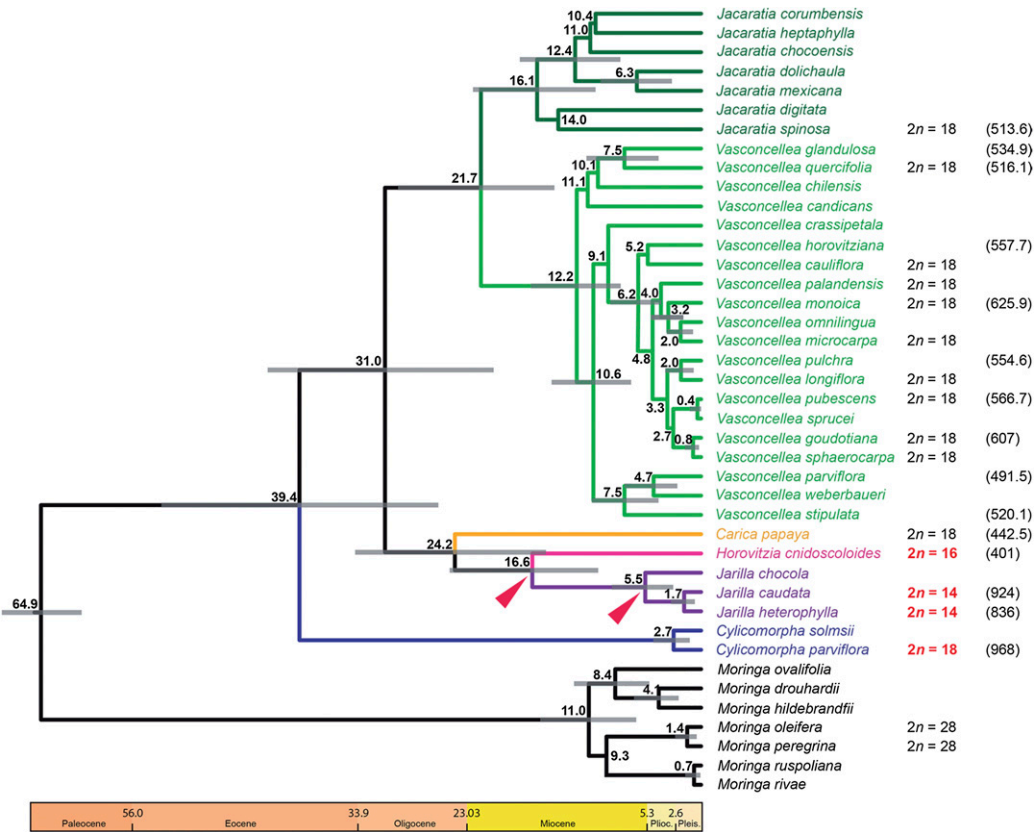


FIGURE 1 Evolutionary relationships within Caricaceae. Branch lengths are proportional to time, and values at nodes indicate divergence times in million years, with bars indicating 95% posterior probability intervals. Chromosome numbers of species with newly counted chromosome numbers are in bold and in red. Chromosome numbers for *Moringa oleifera* are from Silva et al. (2011) and the Chromosome Counts Database (Rice et al., 2015), and for *Moringa peregrina* from Nazari et al. (2012). Values in brackets refer to genome size ranges in millions of base pairs (Mbp) per haploid genome. Arrowheads indicate inferred events of descending dysploidy. The geological time scale is million years and follows Walker et al. (2012).

In spite of the focus on Caricaceae as a model group for the evolution of sex chromosomes, the family has received little attention from molecular cytogeneticists, perhaps because of its assumed conservativeness. Five species have been studied with FISH; Costa et al. (2008) found variation in the number and position of 18S and 5S rDNA sites between *C. papaya*, *V. goudotiana*, and *V. pubescens* (under its synonym *V. cundinamarcensis*), and Iovene et al. (2015), using several BAC clones that were previously mapped to the papaya X/Y chromosomes, found that the presumed sex chromosomes of *J. spinosa* are homomorphic and pair completely. In other species, chromosomes had been counted with traditional means, and all were reported to have a diploid number of $2n = 18$ (Appendix S1, see Supplemental Data with the online version of this article). The remaining three genera have never been studied, yet are disproportionately important because, respectively, they represent the deepest divergence in the Caricaceae (*Cylicomorpha*) and the sister clade to *Carica*. We here used a molecular-clock-dated phylogeny for all species of Caricaceae to infer the direction and timing of changes in chromosome number (with our new data now available for 50% of the species) and FISH to test for the presence of interstitial telomere repeats (ITRs) in species with reduced chromosome numbers, as a possible indication of recent chromosomal fusions. Interstitial telomere repeats have been used as footprints of

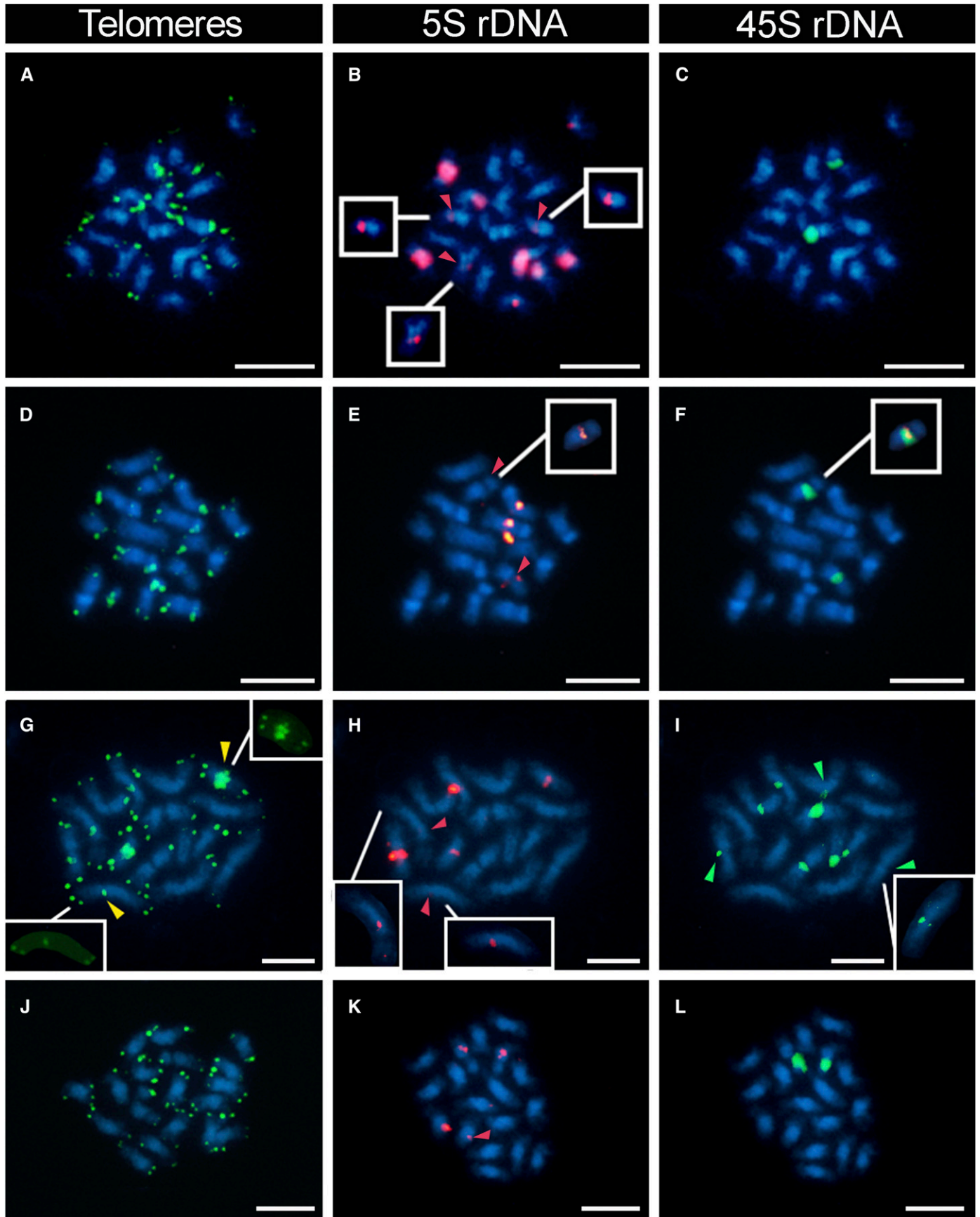
(evolutionarily recent) chromosome fusion in angiosperms and gymnosperms, such as in the legume *Vicia faba*, the Malvaceae *Sideritis montana*, in *Solanum*, in species of the Araceae genus *Typhonium*, and in *Picea* and *Pinus* (Presting et al., 1996; Schmidt et al., 2000; He et al., 2013; Sousa et al., 2014; Sousa and Renner, 2015). We also tested the expectation that genome sizes in Caricaceae would stay within a narrow range (as in Brassicaceae; Lysak et al., 2009), given the absence of polyploidy in the family and the apparent stability of chromosomal homology across some of their genera (Iovene et al., 2015).

MATERIALS AND METHODS

Phylogenetic analysis, molecular clock dating—A molecular phylogeny was generated as done by Carvalho and Renner (2012), using a matrix that includes one accession of each of the 34 species of Caricaceae and seven species of *Moringa* as outgroups. The matrix consisted of five plastid loci (*trnL-trnF*, *rpl20-rps12*, *psbA-trnH* intergenic spacers, *matK* and *rbcl* genes) and one nuclear marker

(the ribosomal DNA internal transcribed spacers ITS1 and ITS2, plus the intervening 5.8S gene) and had 4711 aligned positions. Trees were inferred under maximum likelihood (ML) optimization in RAXML v8.0 (Stamatakis, 2014), using the GTR + Γ substitution model with four rate categories. Bootstrapping under ML used 1000 replicates.

Molecular clock dating relied on the uncorrelated lognormal relaxed clock model implemented in the program BEAST v1.8.0 (Drummond et al., 2012). We again used the GTR + Γ substitution model, a pure-birth (Yule) tree prior, and a Markov chain Monte Carlo (MCMC) chain length of 200 million generations, sampling every 10,000th generation. Log files were examined in the program Tracer v1.6 (Rambaut et al., 2014) to assess convergence and to confirm that effective sampling sizes for all parameters were larger than 200, indicating that MCMC chains were long enough to reach stationarity. The obtained trees were summarized with the program TreeAnnotator v1.8.0, with a 10% burn-in. A maximum credibility tree was then analyzed in the program FigTree v1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>). The divergence of the Caricaceae/Moringaceae has been estimated in three large-scale studies (of flowering plants and of Brassicaceae) that used fossil calibrations (Wikström et al., 2001: 59 million years ago (Ma) (58–61); Beilstein et al., 2010: 69 Ma (105–38); Bell et al., 2010: 67 Ma (45–86)). We



therefore assigned a normally distributed prior with a mean of 65 Ma and a standard deviation of 2 Ma to this node.

Plant material—Wild-collected seeds from Brazil, Mexico, and Kenya of *Carica papaya*, *Cylicomorpha parviflora*, *Horovitzia cnidoscoloides*, *Jacaratia spinosa*, *Jarilla caudata*, *J. heterophylla*, *Vasconcellea cauliflora*, *V. monoica*, and *V. quercifolia* were germinated between 2012 and 2013, and seedlings were cultivated in the greenhouses of the Munich Botanical Garden. Vouchers have been deposited in the Munich herbarium (M) and are listed in Appendix S1.

Preparation of chromosome spreads—Root tips were collected from potted plants between 08:30 and 11:00 hours and pretreated with 70 ppm cycloheximide (Roth, Karlsruhe, Germany) in 2 mM 8-hydroxyquinoline for 2.5 h at room temperature, and for an additional 2.5 h at 4°C to increase the number of metaphase cells (Tlaskal, 1979), then fixed in freshly prepared 3:1 (v/v) ethanol/glacial acetic acid at room temperature overnight, and stored at -20°C until use. Spreads of mitotic metaphase chromosomes were prepared by air-dry dropping as described by Aliyeva-Schnorr et al. (2015), with the following modifications: Fixed root tips were digested with 1% cellulase (w/v; Onozuka RS; Serva, Heidelberg, Germany), 0.4% pectolyase (w/v; Sigma, St. Louis, Missouri, USA), 0.4% cytohelcise (w/v; Sigma) in citric buffer, pH 4.8 for 2 h 50 min at 37°C in a humid chamber, and the freshly prepared fixative consisted of 1:1 (v/v) methanol/glacial acetic acid.

FISH and DNA probes—FISH was performed as described by Sousa et al. (2013) with the inclusion of an ethanol series of 70–90–100%, 2 min each, after the final washes. For localization of the 5S and 45S rDNAs, we used the 18S–5.8S–25S rDNA repeat unit of *Arabidopsis thaliana* in the pBSK+ plasmid, labeled with digoxigenin-11-dUTP (Roche, Basel, Switzerland) by nick translation and a 349-bp fragment of the 5S rRNA gene repeat unit from *Beta vulgaris* cloned into pBSK+ (Schmidt et al., 1994) and labeled with biotin-16-dUTP (Roche) by PCR. Telomere repeats were visualized with the *Arabidopsis*-type telomere probe amplified according to Ijdo et al. (1991) using the oligomer primers (5'-TTTAGGG-3')₅ and (5'-CCCTAAA-3')₅, labeled with digoxigenin-11-dUTP by nick translation. Slides were first analyzed with the probes for telomeres and 5S rDNA. They were then destained and rehybridized with 45S rDNA. At least 20 metaphases and prometaphases were analyzed, except for *Carica papaya* and *Cylicomorpha parviflora* for which at least 10 were analyzed. For *Jarilla caudata* and *Vasconcellea quercifolia*, slides were first analyzed with the 5S and 45S rDNA probes, then destained and rehybridized with the telomere probe. Since little material was available for these last two species (2–7 metaphases analyzed), we show them only in Appendix S2 (see online Supplemental Data). Images were taken with a Leica

DMR microscope equipped with a KAPPA-CCD camera and the KAPPA software. They were optimized for best contrast and brightness using Adobe (San Jose, California, USA) Photoshop CC 2014.

RESULTS

Genome size and chromosome number change in light of the phylogeny—A species of the African genus *Cylicomorpha* (*C. parviflora*) has the largest genome (968 Mbp/haploid genome) in the family, while most New World species have smaller and similar genome sizes (Fig. 1; Appendix S1). The 5-species clade of *Carica*, *Horovitzia*, and *Jarilla* has undergone a dramatic genome size doubling in one of its members, the genus *Jarilla*, while *Carica* and *Horovitzia* have slightly smaller genomes than most other Caricaceae.

Chromosome numbers, descending dysploidy, and distribution of FISH signals, including interstitial telomere repeats—Diploid chromosome numbers of *Carica papaya*, *Cylicomorpha parviflora*, *Jacaratia spinosa*, *Vasconcellea cauliflora*, *V. monoica*, and *V. quercifolia* were confirmed or newly established as $2n = 18$. *Horovitzia cnidoscoloides* has $2n = 16$, and *Jarilla caudata* and *J. heterophylla* have $2n = 14$ (Figs. 2, 3; online Appendices S1–S3). All Caricaceae have submetacentric and metacentric chromosomes, and in species with $2n = 18$ chromosomes, they are of similar size. *Horovitzia cnidoscoloides* ($2n = 16$) has two pairs of chromosomes that are smaller than the remaining six pairs (Fig. 2D–F). The karyotypes of *J. caudata* and *J. heterophylla* ($2n = 14$) each consist of one smaller and six larger chromosome pairs (Fig. 2G–I; Appendix S2A–C). In all species, centromeres, subterminal, and terminal regions are weakly stained or DAPI negative, while pericentromeric and interstitial regions are stained more brightly. Chromosomes of *Carica papaya*, *Horovitzia cnidoscoloides*, *Jacaratia spinosa*, and *Vasconcellea monoica* have strong heterochromatic blocks stained intensely.

The distribution of FISH signals among the nine studied species is summarized in Appendix S3. The number of 5S rDNA sites is variable, ranging from 15 pericentromeric signals in as many chromosomes in *Vasconcellea quercifolia* (Appendix S2E) over six major sites in three pairs and four minor sites in two others in *Carica papaya* (Fig. 2B; Costa et al., 2008 found only six such sites) to four sites in two chromosome pairs in *Horovitzia cnidoscoloides* (Fig. 2E) to just two subterminal sites in one pair in *Cylicomorpha parviflora* (Fig. 3H).

The number of 45S rDNA sites is less variable, with *Carica papaya* (Fig. 2C), *Cylicomorpha parviflora* (Fig. 3I), *Horovitzia cnidoscoloides* (Fig. 2F), *Jacaratia spinosa* (Fig. 2L), and *Vasconcellea cauliflora* (Fig. 3C) all having two sites in one chromosome pair

FIGURE 2 Detection of telomeres and 5S and 45S rDNA signals by FISH in chromosomes of (A–C) *Carica papaya* ($2n = 18$), (D–F) *Horovitzia cnidoscoloides* ($2n = 16$), (G–I) *Jarilla heterophylla* ($2n = 14$), and (J–L) *Jacaratia spinosa* ($2n = 18$). Yellow arrowheads indicate a strong and a weak interstitial telomere repeat (ITR); red arrowheads indicate weak 5S rDNA signals; green arrowheads indicate weak 45S rDNA signals. Insets in G show chromosomes with strong and weak ITRs without being overlapped with DAPI. Insets in B, E, H, I show weak 5S or 45S rDNA signals treated with a differential brightness and contrast. Inset in F shows overlap of adjacent 5S and 45S rDNA signals. The 5S signals were detected with Cy3-conjugated streptavidin (red), and 45S and telomeric signals were detected with FITC-conjugated antidigoxigenin (green). Chromosomes were counterstained with 4',6-diamidino-2-phenylindole (DAPI). Scale bars = 5 μ m.

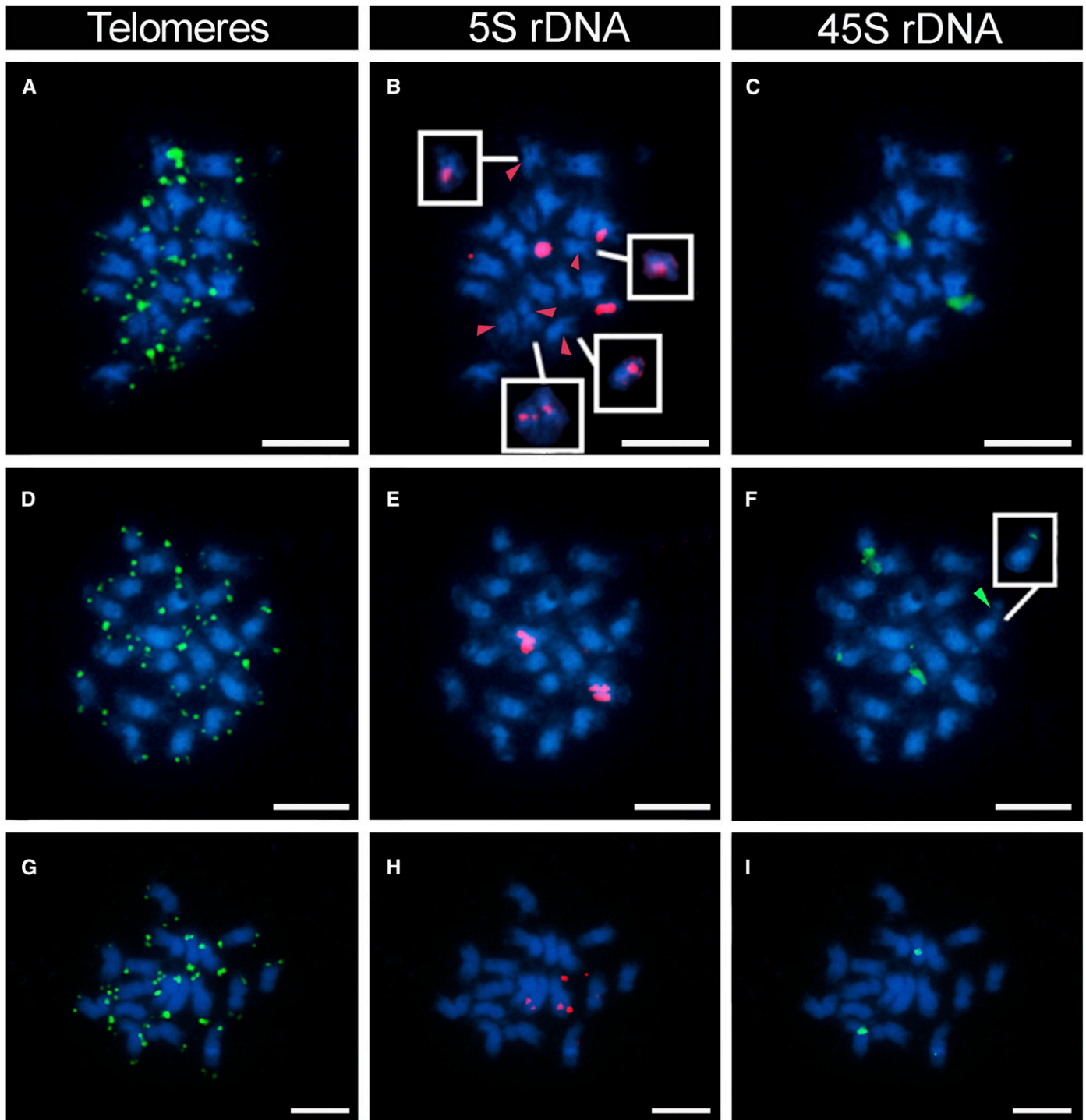


FIGURE 3 Detection of telomeres and 5S and 45S rDNA signals by FISH in chromosomes of (A–C) *Vasconcellea cauliflora* ($2n = 18$), (D–F) *Vasconcellea monoica* ($2n = 18$), and (G–I) *Cylicomorpha parviflora* ($2n = 18$). Red arrowheads indicate weak 5S rDNA signals; green arrowheads indicate weak 45S rDNA signals. Insets in B and F show weak 5S or 45S rDNA signals treated with a differential brightness and contrast. The 5S signals were detected with Cy3-conjugated streptoavidin (red), and 45S and telomeric signals were detected with FITC-conjugated antidigoxigenin (green). Chromosomes were counterstained with 4',6-diamidino-2-phenylindole (DAPI). Scale bars = 5 μm .

(Appendix S2). *Vasconcellea monoica* (Fig. 3F) and *V. quercifolia* (Appendix S2F) have four sites, *Jarilla caudata* (Appendix S2C) has six, and *J. heterophylla* (Fig. 2I) has eight. In *H. cnidoscoloides*, sites are adjacent to the small 5S rDNA sites (Fig. 2F, inset), and sites

syntenic to 5S rDNA sites occur in *Jacaratia spinosa* (Fig. 2K, L), *Jarilla caudata* (Appendix S2B, C), *J. heterophylla* (Fig. 2H, I), *Vasconcellea cauliflora* (Fig. 3B, C), and *V. quercifolia* (Appendix S2E, F).

Telomere signals at terminal loci were present in all species (Fig. 2A, D, G, J; Fig. 3A, D, G; Appendix S2A, D), and the two *Jarilla* species with $2n = 14$ both have pericentromeric ITRs. *Jarilla heterophylla* has six ITRs, which correspond to the number and position of 5S rDNA sites (Fig. 2G, H). *Jarilla caudata* has at least four ITRs, also corresponding to 5S rDNA sites (Appendix S2A, B), and there seem to be six weaker sites; however, due to a lack of sufficient plant material, we can only confirm four ITRs.

DISCUSSION

Descending dysploidy and the temporal decay of interstitial telomere arrays—From the distribution of chromosome numbers on the phylogeny (Fig. 1), it is clear that $2n = 18$ is the ancestral number, with sequential descending dysploidy from 18 to 16 to 14 in the 5-species-large papaya clade. We were unable to obtain material of the third species of *Jarilla*, *J. chocola* (also endemic in Mexico), but the other two species of this genus have $2n = 14$ (Fig. 2G–I; Appendix S2A–C), and the dysploidy from 16 to 14 could thus have occurred in the common ancestor of the genus, which lived about 5.5 Ma, while the preceding dysploidy, from 18 to 16, occurred in the common ancestor of *Jarilla* and *Horovitzia*, which diverged from each other about 16.6 Ma (Fig. 1). Descending dysploidy is relatively common (Lysak, 2014), with well-studied examples in herbaceous Brassicaceae (Yogeeswaran et al., 2005; Lysak et al., 2006; Mandakova and Lysak, 2008; Cheng et al., 2013), *Oxalis* (Vaio et al., 2013), *Cucumis* (Yang et al., 2014), Melianthaceae (Pellicer et al., 2014), and Araceae (Sousa et al., 2014; Sousa and Renner, 2015), but we have not found another instance of descending dysploidy (from 18 to 16 to 14) in a clade of trees and perennial climbers.

The presence of ITRs in the pericentromeric regions of at least two chromosome pairs in *Jarilla caudata* (Appendix S2A) and in three pairs in *J. heterophylla* (Fig. 2G) suggests chromosome rearrangements. Plausible rearrangement scenarios are end-to-end fusions, inversions, and translocations, including parts of chromosomes merging into the pericentromeric region of others (Schubert and Lysak, 2011; Yang et al., 2014; Wang et al., 2015). Large mainly heterochromatic ITRs in pericentromeric regions are known from mammals and plants (Presting et al., 1996; Bolzán and Bianchi, 2006; Ruiz-Herrera et al., 2008; He et al., 2013; Majerová et al., 2014; Yang et al., 2014), and it is thought that they are prone to breakage and rearrangements, fostering their amplification and redistribution together with adjacent satellite repeats, which are abundant in pericentromeric regions (Theuri et al., 2005; He et al., 2013). Repeats are thought to become enriched near former break- and ligation points (Yang et al., 2014; Wang et al., 2015). The high variability of 5S rDNA copies in Caricaceae and their adjacency to the ITRs in both *Jarilla* species also suggest that these sites are linked to the amplification of repetitive elements; most angiosperms have just two 5S sites (Roa and Guerra, 2015). The lack of ITRs in *Horovitzia cnidoscoloides*, which diverged roughly 16.6 Ma, could be explained by the decay of these repeats over time, while they are still detectable in the younger *Jarilla* lineage (roughly 5.5 Myr).

Lowest chromosome numbers correlate with genome size doubling—Contrary to our expectation that genome sizes in Caricaceae would stay within a relatively narrow range (as in Brassicaceae; Lysak et al., 2009), which would have fit the absence of polyploidy in the Caricaceae and the apparent homology of at least

one chromosome pair across two distantly related genera (Iovene et al., 2015), we discovered that the species with the lowest chromosome number have genomes that are twice as large as those of their closest relatives (Fig. 1; Appendix S1). Since there is no evidence of polyploidy, the increase in genome size likely is due to massive bursts in transposon amplification. A possible mechanism is that transposable elements (TEs) “escape” repression and elimination mechanisms by integrating preferentially in or near centromeres (Theuri et al., 2005; Luo et al., 2012). Such TEs, for example, the chromoviruses Tekay, Galadriel, CRM, and Reina, vary greatly in abundance even in very closely related species (Piednoël et al., 2013). The genome size doubling in the common ancestor of *J. caudata* and *J. heterophylla* matches the amplification of telomeric satellite repeats that we observed in their pericentromeric regions, perhaps with an accompanying increase of TEs. Regardless of the causes of the immense DNA increase, it likely contributed to further chromosomal rearrangements and the dysploidy from 18 to 16 to 14.

ACKNOWLEDGEMENTS

We thank Martina Silber for support in the laboratory, Ray Ming for C-values from material of *Cylicomorpha parviflora*, *Horovitzia cnidoscoloides*, and *Jarilla caudata* and *J. heterophylla* that we provided for his genome sequencing work, and two anonymous reviewers for their comments.

LITERATURE CITED

- Aliyeva-Schnorr, L., L. Ma, and A. Houben. 2015. A fast air-dry dropping chromosome preparation method suitable for FISH in plants. *Journal of Visualized Experiments* 106: e53470.
- Beilstein, M. A., N. S. Nagalingum, M. D. Clements, S. R. Manchester, and S. Mathews. 2010. Dated molecular phylogenies indicate a Miocene origin for *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* 107: 18724–18728.
- Bell, C. D., D. E. Soltis, and P. S. Soltis. 2010. The age and diversification of the angiosperms re-revisited. *American Journal of Botany* 97: 1296–1303.
- Bolzán, A. D., and M. S. Bianchi. 2006. Telomeres, interstitial telomeric repeat sequences, and chromosomal aberrations. *Reviews in Mutation Research* 612: 189–214.
- Carvalho, F. A. 2013 onward [continuously updated]. e-Monograph of Caricaceae, version 1, November 2013. <http://herbaria.plants.ox.ac.uk/bol/caricaceae> [accessed 5 February 2016].
- Carvalho, F. A., and S. S. Renner. 2012. A dated phylogeny of the papaya family (Caricaceae) reveals the crop’s closest relatives and the family’s biogeographic history. *Molecular Phylogenetics and Evolution* 65: 46–53.
- Carvalho, F. A., and S. S. Renner. 2013. Correct names for some of the closest relatives of *Carica papaya*: A review of the Mexican/Guatemalan genera *Jarilla* and *Horovitzia*. *PhytoKeys* 29: 63–74.
- Cheng, F., T. Mandakova, J. Wu, Q. Xie, M. Lysak, and X. Wang. 2013. Deciphering the diploid ancestral genome of the mesohexaploid *Brassica rapa*. *Plant Cell* 25: 1541–1554.
- Costa, F. R., T. N. S. Pereira, G. L. Hodnett, M. G. Pereira, and D. N. Stelly. 2008. Fluorescent *in situ* hybridization of 18S and 5S rDNA in papaya (*Carica papaya* L.) and wild relatives. *Caryologia* 61: 411–416.
- Drummond, A. J., M. A. Suchard, D. Xie, and A. Rambaut. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29: 1969–1973.
- Escudero, M., E. Maguilla, J. Loureiro, M. Castro, S. Castro, and M. Luceño. 2015. Genome size stability despite high chromosome number variation in *Carex* gr. *laevigata*. *American Journal of Botany* 102: 233–238.
- FAO. 2016. Food and Agriculture Organization of the United Nations Statistics Division, <http://faostat3.fao.org/search/papaya/E> [accessed 2 February 2016].

- Gschwend, A. R., C. M. Wai, F. Zee, A. K. Arumuganathan, and R. Ming. 2013. Genome size variation among sex types in dioecious and triecious Caricaceae species. *Euphytica* 189: 461–469.
- He, L., J. Liu, G. A. Torres, H. Zhang, J. Jiang, and C. Xie. 2013. Interstitial telomeric repeats are enriched in the centromeres of chromosomes in *Solanum* species. *Chromosome Research* 21: 5–13.
- Ijdo, J., R. Wells, A. Baldini, and S. Reeders. 1991. Improved telomere detection using a telomere repeat probe (TTAGGG)_n generated by PCR. *Nucleic Acids Research* 19: 4780.
- Iovene, M., Q. Yu, R. Ming, and J. Jiang. 2015. Evidence for emergence of sex-determining gene(s) in a centromeric region in *Vasconcellea parviflora*. *Genetics* 199: 413–421.
- Liu, Z., P. H. Moore, H. Ma, C. M. Ackerman, M. Ragiba, Q. Yu, H. M. Pearl, et al. 2004. A primitive Y chromosome in papaya marks incipient sex chromosome evolution. *Nature* 427: 348–352.
- Luo, S., J. Mach, B. Abramson, R. Ramirez, R. Schurr, P. Barone, G. Copenhaver, and O. Folkerts. 2012. The cotton centromere contains a Ty3-gypsy-like LTR retroelement. *PLoS One* 7: e35261.
- Lysak, M. A. 2014. Live and let die: Centromere loss during evolution of plant chromosomes. *New Phytologist* 203: 1082–1089.
- Lysak, M. A., A. Berr, A. Pecinka, R. Schmidt, K. McBreen, and I. Schubert. 2006. Mechanisms of chromosome number reduction in *Arabidopsis thaliana* and related Brassicaceae species. *Proceedings of the National Academy of Sciences, USA* 103: 5224–5229.
- Lysak, M. A., M. A. Koch, J. M. Beaulieu, A. Meister, and I. J. Leitch. 2009. The dynamic ups and downs of genome size evolution in Brassicaceae. *Molecular Biology and Evolution* 26: 85–98.
- Majerová, E., T. Mandáková, G. T. Vu, J. Fajkus, M. A. Lysak, and M. Fojtová. 2014. Chromatin features of plant telomeric sequences at terminal vs. internal positions. *Frontiers in Plant Science* 5(article 593): 1–10.
- Mandakova, T., and M. A. Lysak. 2008. Chromosomal phylogeny and karyotype evolution in $x=7$ crucifer species (Brassicaceae). *Plant Cell* 20: 2559–2570.
- Ming, R., S. Hou, Y. Feng, Q. Yu, A. Dionne-Laporte, et al. 2008. The draft genome of the transgenic tropical fruit tree papaya (*Carica papaya* Linnaeus). *Nature* 452: 991–996.
- Nazari, Z., N. H. Mirzaie, K. G. Bakhshi, and F. Asadicorom. 2012. Karyotypic characteristics of *Moringa peregrina* (Forssk.) Fiori in Iran. *Iranian Journal of Medical and Aromatic Plants* 27: 635–646.
- Pellicer, J., L. J. Kelly, I. J. Leitch, W. B. Zomlefer, and M. F. Fay. 2014. A universe of dwarfs and giants: Genome size and chromosome evolution in the monocot family Melanthiaceae. *New Phytologist* 201: 1484–1497.
- Piednoël, M., G. Carette-Vega, and S. S. Renner. 2013. Characterization of the LTR retrotransposon repertoire of a plant clade of six diploid and one tetraploid species. *Plant Journal* 75: 699–709.
- Presting, G. G., A. Frary, K. Pillen, and S. D. Tanksley. 1996. Telomere-homologous sequences occur near the centromeres of many tomato chromosomes. *Molecular & General Genetics* 251: 526–531.
- Rambaut, A., M. A. Suchard, D. Xie, and A. J. Drummond. 2014. Tracer v1.6. Available from <http://beast.bio.ed.ac.uk/Tracer>.
- Rice, A., L. Glick, S. Abadi, M. Einhorn, N. M. Kopelman, A. Salman-Minkov, J. Mayzel, et al. 2015. The Chromosome Counts Database (CCDB)—A community resource of plant chromosome numbers. *New Phytologist* 206: 19–26.
- Roa, F., and M. Guerra. 2015. Non-random distribution of 5S rDNA sites and its association with 45S rDNA in plant chromosomes. *Cytogenetic and Genome Research* 146: 243–249.
- Ruiz-Herrera, A., S. G. Nergadze, M. Santagostino, and E. Giulotto. 2008. Telomeric repeats far from the ends: Mechanisms of origin and role in evolution. *Cytogenetic and Genome Research* 122: 219–228.
- Schmidt, A., R. L. Doudrick, J. S. Heslop-Harrison, and T. Schmidt. 2000. The contribution of short repeats of low sequence complexity to large conifer genomes. *Theoretical and Applied Genetics* 101: 7–14.
- Schmidt, T., T. Schwarzacher, and J. Heslop-Harrison. 1994. Physical mapping of rRNA genes by fluorescent in-situ hybridization and structural analysis of 5S rRNA genes and intergenic spacer sequences in sugar beet (*Beta vulgaris*). *Theoretical and Applied Genetics* 88: 629–636.
- Schubert, I., and M. A. Lysak. 2011. Interpretation of karyotype evolution should consider chromosome structural constraints. *Trends in Genetics* 27: 207–216.
- Silva, N., A. B. Mendes-Bonato, J. G. C. Sales, and M. S. Pagliarini. 2011. Meiotic behavior and pollen viability in *Moringa oleifera* (Moringaceae) cultivated in southern Brazil. *Genetics and Molecular Research* 10: 1728–1732.
- Sousa, A., N. Cusimano, and S. S. Renner. 2014. Combining FISH and model-based predictions to understand chromosome evolution in *Typhonium* (Araceae). *Annals of Botany* 113: 669–680.
- Sousa, A., F. Fuchs, and S. S. Renner. 2013. Molecular cytogenetics (FISH, GISH) of *Coccinia grandis*: A ca. 3 myr-old species of Cucurbitaceae with the largest Y/autosome divergence in flowering plants. *Cytogenetic and Genome Research* 139: 107–118.
- Sousa, A., and S. S. Renner. 2015. Interstitial telomere-like repeats in the monocot family Araceae. *Botanical Journal of the Linnean Society* 177: 15–26.
- Stamatakis, A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.
- Tang, H., J. E. Bowers, X. Wang, R. Ming, M. Alam, and A. H. Paterson. 2008. Synteny and collinearity in plant genomes. *Science* 320: 486–488.
- Theuri, J., T. Phelps-Durr, S. Mathews, and J. Birchler. 2005. A comparative study of retrotransposons in the centromeric regions of A and B chromosomes of maize. *Cytogenetic and Genome Research* 110: 203–208.
- Trkal, J. 1979. Combined cycloheximide and 8-hydroxyquinoline pretreatment for study of plant chromosomes. *Biotechnic & Histochemistry* 54: 313–319.
- VanBuren, R., F. Zeng, C. Chen, J. Zhang, C. N. Wai, J. Han, R. Aryal, et al. 2015. Origin and domestication of papaya Yh chromosome. *Genome Research* 25: 524–533.
- Vaio, M., A. Gardner, E. Emshwiller, and M. Guerra. 2013. Molecular phylogeny and chromosome evolution among the creeping herbaceous *Oxalis* species of sections *Corniculatae* and *Ripariae* (Oxalidaceae). *Molecular Phylogenetics and Evolution* 68: 199–211.
- Walker, J. D., J. W. Geissman, S. A. Bowring, and L. E. Babcock [compilers]. 2012. Geologic Time Scale, version 4.0. Geological Society of America, . <http://www.geosociety.org/science/timescale>.
- Wang, X., D. Jin, Z. Wang, H. Guo, L. Zhang, L. Wang, J. Li, and A. H. Paterson. 2015. Telomere-centric genome repatterning determines recurring chromosome number reductions during the evolution of eukaryotes. *New Phytologist* 205: 378–389.
- Wikström, N., V. Savolainen, and M. W. Chase. 2001. Evolution of the angiosperms: Calibrating the family tree. *Proceedings of the Royal Society of London, B, Biological Sciences* 268: 2211–2220.
- Wu, X., J. Wang, J. K. Na, Q. Yu, R. C. Moore, F. Zee, S. C. Huber, and R. Ming. 2010. The origin of the non-recombining region of sex chromosomes in *Carica* and *Vasconcellea*. *Plant Journal* 63: 801–810.
- Yang, L., D. Koo, D. Li, T. Zhang, J. Jiang, F. Luan, S. S. Renner, et al. 2014. Next-generation sequencing, FISH mapping, and synteny-based modeling reveal mechanisms of decreasing dysploidy in *Cucumis*. *Plant Journal* 77: 16–30.
- Yogeeswaran, K., A. Frary, T. L. York, A. Amenta, A. H. Lesser, J. B. Nasrallah, S. D. Tanksley, and M. E. Nasrallah. 2005. Comparative genome analyses of *Arabidopsis* spp.: Inferring chromosomal rearrangement events in the evolutionary history of *A. thaliana*. *Genome Research* 15: 505–515.

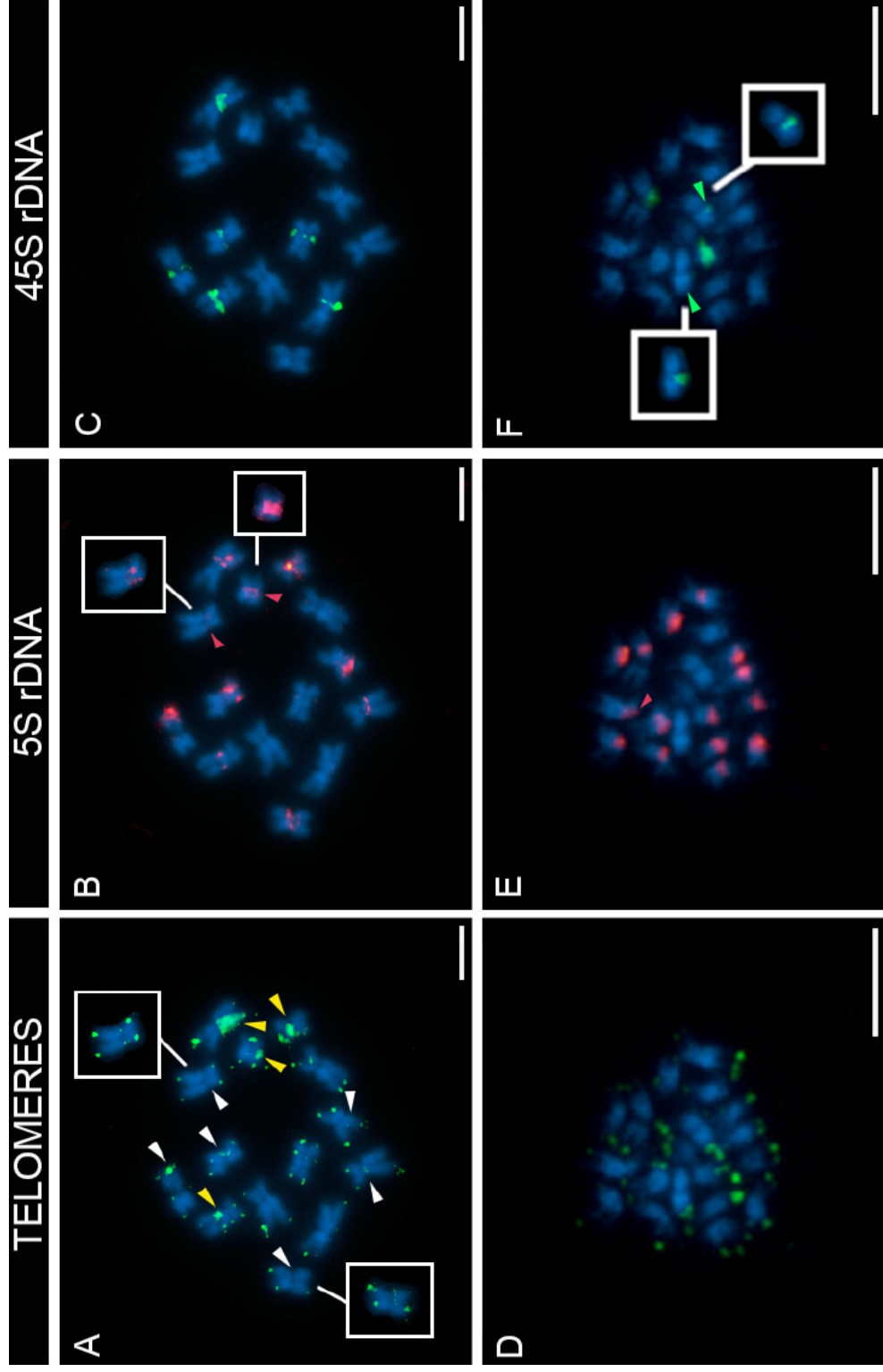


Fig. S1. Detection of telomeres, 5S and 45S rDNA signals by FISH in chromosomes of: A–C, *Jarilla caudata* ($2n = 14$); D–F, *Vasconcellea quercifolia* ($2n = 18$). Yellow arrowheads indicate clear ITR signals; white arrowheads indicate potential weak ITRs; red arrowheads indicate weak 5S rDNA signals in B and a chromosome with a signal while its homologue lacks a signal in E; green arrowheads indicate weak 45S rDNA signals. Insets in A show weak ITRs treated with a differential brightness and contrast. Insets in B and F show weak 5S or 45S rDNA signals treated with a differential brightness and contrast. 5S signals were detected with Cy3-conjugated streptavidin (red), and 45S and telomeric signals were detected with FITC-conjugated anti-digoxigenin (green). Chromosomes were counterstained with 4',6-diamidino-2-phenylindole (DAPI). Scale bars = 5 μ m

Table S1. Chromosome numbers and C-values (genome sizes in millions of base pairs (Mbp) per haploid genome) for the 20 species of Caricaceae so far studied.

Species	Chromosome number; voucher	Genome size; voucher	References for the chromosome number; those for C-values are marked by an *
<i>Carica papaya</i> L.	$2n = 18$; A. Rockinger 2015/7 (M)		This study
	$2n = 18$; none		Simmonds (1954)
	$2n = 18$; none		Datta (1970)
	$2n = 18$; none		Joshi and Ranjekar (1982)
		372 Mbp; none	*Arumuganathan and Earle (1991)
	$2n = 18$; none		Costa et al. (2008)
	$2n = 18$; none		Damasceno et al. (2009)
<i>Cylicomorpha parviflora</i> Urb.	$2n = 18$; none		*Araújo et al. (2010)
	$2n = 18$; none	318 Mbp; none	*Gschwend et al. (2013)
		442.5 Mbp, HCAR 320	
	$2n = 18$; Mark Nicholson s.n. (M)	968 Mbp; none	This study; *R. Ming, pers. comm. Aug. 2013
<i>Horovitzia cnidoscoloides</i> (Lorence & Torres Colín, R.) V.M.Badillo	$2n = 16$; A. Rockinger 2015/5 (M)	401 Mbp; none	This study; *R. Ming, pers. comm. Aug. 2013
	$2n = 18$; A. Rockinger 2015/6 (M)		This study
		$2n = 18$; none	
<i>Jacaratia spinosa</i> (Aubl.) A.DC.	$2n = 18$; HCAR 227		Silva et al. (2012)
		513.6 Mbp; HCAR 227	*Gschwend et al. (2013)
	$2n = 14$; F.A. Carvalho 2240 (M)	924 Mbp; none	This study; *R. Ming, pers. comm. Aug. 2013
<i>Jarilla caudata</i> (Brandege) Standl.	$2n = 14$; F.A. Carvalho 2239 (M)	836 Mbp; none	This study; *R. Ming, pers. comm. Aug. 2013
<i>Vasconcellea cauliflora</i> (Jacq.) A.DC.	$2n = 18$; F.A. Carvalho 2242 (M)		This study
	$2n = 18$; none		Caetano et al. (2008)

Species	Chromosome number; voucher	Genome size; voucher	References for the chromosome number; those for C-values are marked by an *
<i>Vasconcellea glandulosa</i> A.DC.		534.9 Mbp; HCAR 300	*Gschwend et al. (2013)
<i>Vasconcellea goudotiana</i> Triana & Planch.	$2n = 18$; none $2n = 18$; none $2n = 18$; none $2n = 18$; HCAR 167		De Zerpa (1959) Costa et al. (2008) Caetano et al. (2008) Silva et al. (2012) *Gschwend et al. (2013)
<i>Vasconcellea horovitziana</i> (V.M.Badillo) V.M.Badillo		607 Mbp; HCAR 167 557.7 Mbp; HCAR 305	*Gschwend et al. (2013) *Gschwend et al. (2013)
<i>Vasconcellea longiflora</i> (V.M.Badillo) V.M.Badillo	$2n = 18$; none		Caetano et al. (2008)
<i>Vasconcellea microcarpa</i> (Jacq.) A.DC.	$2n = 18$; none		De Zerpa (1959)
<i>Vasconcellea monoica</i> (Desf.) A.DC.	$2n = 18$; F.A. Carvalho 2243 (M) $2n = 18$; none $2n = 18$; none		This study De Zerpa (1959) Damasceno et al. (2009) *Gschwend et al. (2013)
<i>Vasconcellea palandensis</i> (V.M.Badillo, Van den Eynden & Van Damme) V.M.Badillo	$2n = 18$; none	625.9 Mbp; HCAR 171	Caetano et al. (2008)
<i>Vasconcellea parviflora</i> A.DC.		491.5 Mbp; HCAR 180/179	*Gschwend et al. (2013)
<i>Vasconcellea pubescens</i> A.DC.	$2n = 18$; none $2n = 18$; none $2n = 18$; none		Costa et al. (2008) Caetano et al. (2008) Damasceno et al. (2009) *Gschwend et al. (2013)
<i>Vasconcellea pulchra</i> (V.M.Badillo) V.M.Badillo		566.7 Mbp; HCAR 46 554.6 Mbp; HCAR 267	*Gschwend et al. (2013) *Gschwend et al. (2013)

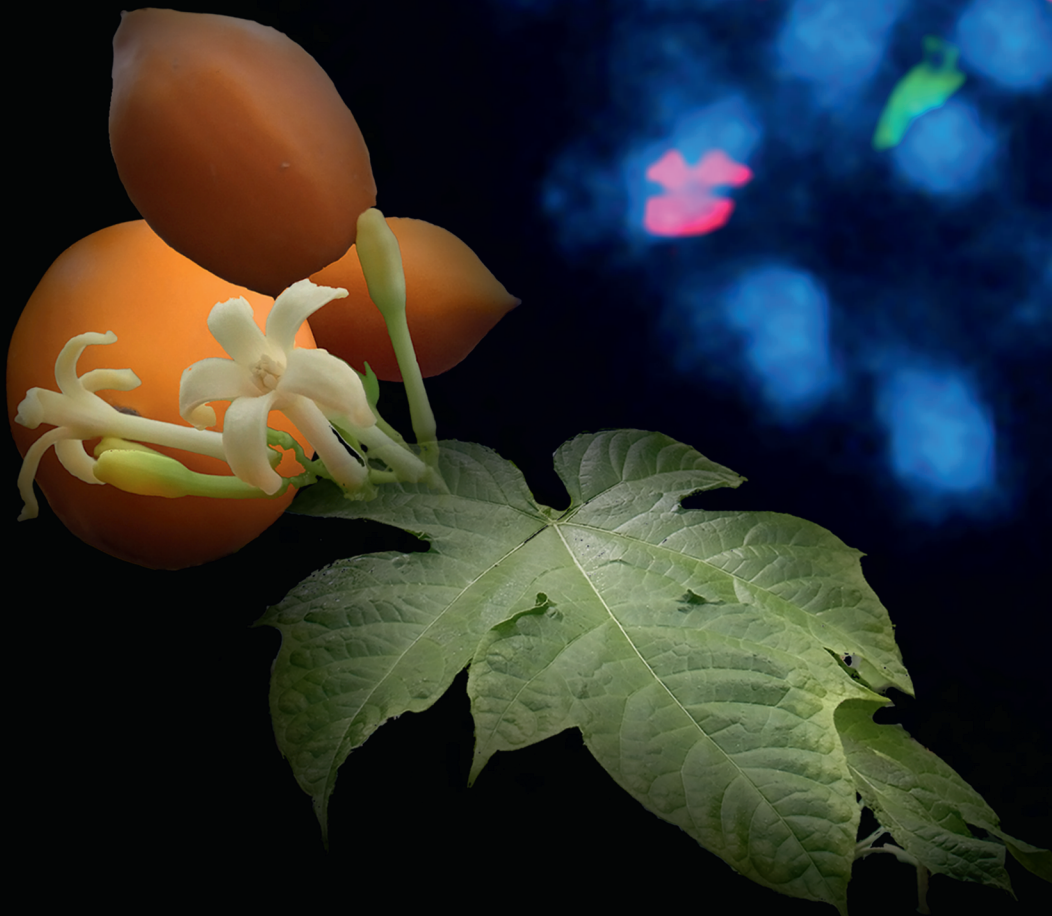
Species	Chromosome number; voucher	Genome size; voucher	References for the chromosome number; those for C-values are marked by an *
<i>Vasconcellea quercifolia</i> A.St.-Hil.	$2n = 18$; F.A. Carvalho 2241 (M)		This study
	$2n = 18$; HCAR 226		Silva et al. (2012)
	$2n = 18$; RS3586	516.1 Mbp; HCAR 226	Bernardello et al. (1990) *Gschwend et al. (2013)
<i>Vasconcellea sphaerocarpa</i> (García-Barr. & Hern.Cam.) V.M.Badillo	$2n = 18$; none		Caetano et al. (2008)
<i>Vasconcellea stipulata</i> (V.M.Badillo) V.M.Badillo		520.1 Mbp; HCAR 177	*Gschwend et al. (2013)

Table S2. FISH results for nine species representing the six genera of Caricaceae. All species have telomere signals at both ends of all their chromosomes, and the table therefore only lists interstitial telomere repeats (ITRs). Asterisks mark syntenic and squares adjacent sites.

Species name	2n	Number / position of ITRs	Number / position of 5S rDNA sites	Number / position of 45S rDNA sites
<i>Carica papaya</i>	18	None (Fig. 2A)	10 / pericentromeric (Fig. 2B)	2 / pericentromeric (Fig. 2C)
<i>Cylicomorpha parviflora</i>	18	None (Fig. 3G)	2 / subterminal (Fig. 3H)	2 / pericentromeric (Fig. 3I)
<i>Horovitzia cnidoscoloides</i>	16	None (Fig. 2D)	4 (of which 2 [■]) / pericentromeric (Fig. 2E)	2 [■] / pericentromeric (Fig. 2F)
<i>Jacaratia spinosa</i>	18	None (Fig. 2J)	4 (of which 2*) / pericentromeric (Fig. 2K)	2* / pericentromeric (Fig. 2L)
<i>Jarilla caudata</i>	14	4–10 [■] / pericentromeric (Fig. S1A)	10 (of which at least 4 [■] and at least 4*) / pericentromeric (Fig. S1B)	6 (of which at least 4*) / pericentromeric (Fig. S1C)
<i>Jarilla heterophylla</i>	14	6 [■] / pericentromeric (Fig. 2G)	6 [■] / pericentromeric (Fig. 2H)	8 (of which 2*) / pericentromeric (Fig. 2I)
<i>Vasconcellea cauliflora</i>	18	None (Fig. 3A)	8 (of which 2*) / pericentromeric (Fig. 3B)	2* / pericentromeric (Fig. 3C)
<i>Vasconcellea monoica</i>	18	None (Fig. 3D)	4 (duplication) / pericentromeric (Fig. 3E)	4 / pericentromeric (Fig. 3F)
<i>Vasconcellea quercifolia</i>	18	None (Fig. S1D)	15 (of which 2*) / pericentromeric (Fig. S1E)	4 (of which 2*) / pericentromeric (Fig. S1F)

American Journal of Botany

June 2016 ♦ Volume 103 ♦ Number 6



Official Publication of the Botanical Society of America, Inc.
www.amjbot.org

Chapter 5

GENERAL DISCUSSION

5.1 Phylogenetic relationships and divergence times in *Crotalaria*

Since I began my work on *Crotalaria* in December 2013, three other studies on the genus that relied on molecular sequence data have come out. Two of them deal with Indian *Crotalaria* (Subramaniam *et al.* 2013, 2015) and both present phylogenies based on nuclear and chloroplast sequences for 10% of the 700 species of *Crotalaria*, including roughly 52% of the total Indian species. The authors focused on the evolution of morphological traits, such as shape of the keel beak and leaves, and combined these data with pod anatomical traits to revise the sectional placement of Indian *Crotalaria*. They synonymized section *Crotalaria* subsection *Bracteatae* under section *Calycinae* (all sections are marked in Chapter 2, Figure 1 and authors of all sections are in Chapter 2, additional file 3: Table S1). A paper by Le Roux *et al.* (2013) is based on a nuclear and chloroplast phylogeny for 24% of the 700 species, and including only two Neotropical, three Asian, and two Australasian species. Even with this limited sampling, the authors raised three subsections of Pohlhill's (1982) section *Chrysocalycinae* to sectional rank (*Glaucæ*, *Incanae*, and *Stipulosæ*), sank subsection *Chrysocalycinae* into section *Hedriocarpæ*, section *Dispermae* into section *Crotalaria*, and introduced two new sections, *Amphitrichæ* and *Borealigeniculatae* (Chapter 2, additional file 1: Figure S1).

For my doctoral research, I built on these studies by adding new sequences for a DNA phylogeny that now samples 338 species (48%), representing all distribution areas and all but one sectional type species. The 140 newly sequenced species are mostly from Asia, Australasia, and the Neotropics; 59 species are from Africa and Madagascar. To rigidly test the monophyly of *Crotalaria*, I generated sequence data for 17 species from the remaining Crotalarieae, which had not been represented in previous molecular studies, so that my outgroup sampling includes all the tribe's 16 genera (Chapter 2, additional file 3: Table S1). For molecular clock analyses, I sequenced an additional species of the other core genistoids. I relied on two nuclear markers as the backbone of my phylogeny, the ITS and ETS region. To improve the resolution, I combined the nuclear sequences with additional chloroplast sequences, namely for the *matK* and *rbcL* genes, and the *psbA-trnH* intergenic spacer. In all, I generated 271 sequences in the course of this study. This resulted in a phylogeny of six well-supported main clades (Chapter 2, Figure 1). I named these clades according to characteristics suitable for distinguishing them readily, and for this I found floral morphological characters and chromosome numbers to be most useful; I focused on characters that are found in most members of a clade and absent in most species belonging to

other clades. As expected in such a species-rich group, no single distinguishing character is shared by all species of any larger clade and lacking in all species of all other clades. As noted by Polhill (1982), a reticulate pattern of interspecific variation of morphological characters pervades the genus, and species can only be grouped by a combination of several characters. Thus, all his sections are defined by character combinations. Le Roux *et al.* (2013) adapted this approach when defining their revised classification of *Crotalaria*. My enhanced species sampling renders eight of the 11 sections of Le Roux *et al.* (2013) polyphyletic (see Chapter 2, additional file 1: Figure S1), and in my view another revision of the sectional classification should await a species sampling of at least 70–80%.

The deepest split within *Crotalaria*, dating to 18–29 Ma, is between the ‘core *Chrysocalycinae*’ clade and the other five clades. This clade harbours most sampled species belonging to Polhill’s section *Chrysocalycinae*, most sampled species of section *Geniculatae*, and all sampled species of section *Grandiflorae*. Most species from Madagascar and most Neotropical species with trifoliolate leaves also belong to this clade. The remainder of clades can be divided into two subclades, one consisting of the ‘ $2n = 14$ ’ clade and the ‘truncate calyx tube’ clade, and the other comprising the ‘*Crotalaria linearifoliolata*/*C. persica*’ clade, the ‘bulbous standard appendages’ clade, and the ‘bilabiate calyx’ clade (Chapter 2, Figure 1). Species of the ‘ $2n = 14$ ’ clade belong to Polhill’s section *Chrysocalycinae* subsection *Incanae* and most of them share the same diploid chromosome number, which is the rarest number occurring in the species of *Crotalaria* that have been studied (see *Chromosome numbers in Crotalaria*). Most species of the ‘truncate calyx tube’ clade share this calyx type and are distributed in Africa, with the exception of two species occurring in Asia, two species endemic to Australia, and a clade of four Madagascan species (Chapter 2, Figures 1 and 3). The ‘*Crotalaria linearifoliolata*/*C. persica*’ clade comprises two species with distinct morphological traits and similar distribution range, reaching from the Horn of Africa to Arabia. The ‘bulbous standard appendages’ clade includes the majority of species from Polhill’s section *Crotalaria* and all species from his section *Dispermae*, both predominantly African with few species in Asia, Australasia, and the Neotropics. The ‘bilabiate calyx’ clade comprises most of both the Asian and Neotropical species, with the latter being embedded within the Asian species. With few exceptions, species in this clade have simple leaves and flowers with a bilabiate calyx and a spirally twisted keel beak (Chapter 2, Figure 1 (inset); Figures 1 C, E in the *General Introduction*).

With the sampling of 48% of the 700 species of *Crotalaria*, and all other 15 genera of Crotalariaeae, *Crotalaria* is strongly supported as monophyletic. Its closest relative is *Bolusia*, with currently five accepted species occurring in tropical Africa south of the equator, and the next closest relative is monotypic *Euchlora* from South Africa. *Bolusia* is morphologically similar to *Crotalaria*, but differs by having a helically coiled keel (Van Wyk *et al.* 2010). Polhill (1968, 1982) suggested a close relationship between *Bolusia* and *Crotalaria*, seeing *Crotalaria cornu-ammonis* from Madagascar, which has an unusually curved keel beak, as ‘prototype’ for the floral morphology of *Bolusia*. Indeed, the phylogeny reveals *C. cornu-ammonis* with its basal position close to *Bolusia amboensis* (Chapter 2, Figure 1). It will be interesting to sample the four other species of *Bolusia* to test the monophyly of that genus.

5.2 Flower and leaf trait evolution in *Crotalaria*

Plotting and ancestral state reconstruction of morphological flower and leaf traits revealed that species-rich groups in *Crotalaria* have almost homogeneous morphological traits, even though each trait has evolved multiple times in the genus (Chapter 2, Figures 2 and 4). Polhill (1968, 1982) was the first to note that about half of the genus has flowers with non-twisted keel beaks, while the rest has flowers with spirally twisted keel beaks. In total, c. 350 species have flowers with spirally twisted keel beaks and 350 species have flowers with straight keel beaks. While most of the species with straight-beaked flowers occur in Africa, most flowers with a twisted beak are distributed throughout Asia, Australasia, and the Neotropics. Bilabiate calyces only occur in species with spirally twisted keel beaks and are almost restricted to Asian and Neotropical species and, with a few exceptions, correlate with simple leaves. Leaf types also are highly conserved, with trifoliolate leaves being most common and predominating in African and Madagascan species, and most simple leaved species belonging to the Asian-Australasian-Neotropical ‘bilabiate calyx’ clade. While multifoliolate leaves occur frequently in Fabaceae and are also found in other genistoids (Chapter 2, Figure 4), only few species of *Crotalaria* have this leaf type. Unifoliolate leaves also occur with low frequency, and neither leaf type seems to be related to geography or ecology. Champagne *et al.* (2007) inferred that the ancestral leaf type in legumes is the compound leaf, and this seems to be true also in *Crotalaria*.

Since *Crotalaria* is mega-diverse, has a pantropical distribution and is well represented in collections, it provides a suitable group for studying parameters putatively related to its high

diversification rate and success in niche adaptation. However, because of the lack of a phylogenetic framework no study prior to mine has addressed these questions. My phylogeny is well-resolved and has a comprehensive and representative taxon sampling in terms of distribution and morphology by sampling between 25–79% of the species in Polhill’s (1982) eight sections (four of them with >50% sampled, Chapter 2, Figure 1) and c. 60% of the simple leaved and c. 46% of the compound leaved species. Based on this sampling, I decided to assess the distribution of *Crotalaria* in climate space and to address the question how leaf and flower morphology is correlated with temperature, precipitation, elevation, and sun radiation. For this purpose, I carried out regression analyses with available species distribution data from GBIF; in total, my dataset contained 22,225 occurrence records for 183 species of *Crotalaria*, reflecting the genus’ entire distribution range, with India slightly undersampled and Australia slightly oversampled. Results showed no correlation with flower morphology, which in hindsight may be explained by all *Crotalaria* flowers being adapted to ventrally-pollen collecting bees (but see *Future research questions on flower functionality in Crotalaria*).

By contrast, regression analyses revealed a correlation between annual precipitation and leaf morphology: With increasing annual precipitation, simple leaved species tend to occur with a higher probability than species with compound leaves (Chapter 2, Figure 5 C). This result was also supported by a categorical approach based on Köppen-Geiger climate categories, in which I assigned the species represented in my phylogeny to four main climate categories: humid tropics, dry tropics, arid zones, and mild temperate regions (Chapter 2, Figures 5 A, B). The results confirmed Polhill’s (1968, 1982) observations, that crotalarias are most frequent in the dry tropics, but newly reveal that simple-leaved species are slightly overrepresented in the humid tropics. With a regression model that took phylogenetic structure of the data into account, these results were no longer significant, as expected from the strong clustering of most simple-leaved species in the ‘bilabiate calyx’ clade, resulting in too few independent origins of simple leaves to infer any correlation with climate parameters (Chapter 2, Figure 4). While morphogenesis and evolutionary patterns of simple- and compound-leaved species have been assessed in angiosperms (e.g. Efroni *et al.* 2010; Geeta *et al.* 2011), their adaptive value under environmental conditions remains poorly understood and studied. Givnish (1978) proposed the ‘cheap throwaway branch hypothesis’, by which he referred to the idea that compound leaves would be beneficial under conditions of competition for light (requiring constant replacement of older

leaves in the dark understory of tropical rain forests) because their long rachis would enable them to function like a branch with multiple simple leaves, yet they would be less costly because no formation and support of woody tissue is required. However, this idea hardly applies to herbaceous and openland species such as crotalarias.

5.3 Chromosome numbers in *Crotalaria*

Chromosome numbers are known for 122 (36%) of the 338 sampled species of *Crotalaria*, and I have plotted these on the phylogenetic tree (Chapter 2, additional file 2: Figure S2) because I wanted to use chromosome numbers as one possible trait to characterize natural groups in the genus (even though this trait is difficult to use in practice because it requires living material for counting). The predominant diploid number in *Crotalaria* is $2n = 16$ (92 species of the 122 counted species have this number). The lowest known number is $2n = 14$ and occurs only in species belonging to my '2n = 14' clade. With the current sampling, with exception of *C. massaiensis* from Kenya, polyploidy is limited to Asian and Neotropical species, and most polyploids occur in the Neotropics and belong to the 'bilabiate calyx' clade and are tetraploids with $2n = 4x = 32$.

Among the Neotropical species, *C. tweediana*, with $2n = 54$ and endemic to Brazil, may be a septaploid with subsequent fusion or loss of two chromosomes. Another species with an atypical chromosome number is the Asian *C. ferruginea*, which may be a hexaploid with $2n = 6x = 48$ (Mangotra and Koul 1979). Both *C. ferruginea* and *C. tweediana* are nested within the Asian and Neotropical tetraploids of the 'bilabiate calyx' clade. Windler (1973, 1974), who hypothesized that the Neotropical tetraploids diverged from Asian *Crotalaria* and reached the Neotropics via long distance dispersal, suggested that polyploidy in the Neotropical species might be related to self-compatibility because genetic incompatibility mechanisms can break down in polyploids (Paun *et al.* 2011). The phylogeny confirms that 27 of the 46 sampled Neotropical species form a clade (crown node marked with an arrow in Chapter 2, additional file 2: Figure S2) that is embedded within a clade of 73 mostly Asian species, and 18 of these 27 Neotropical species are known to be polyploids. However, polyploidy may have arisen already in Asia since the two (counted) Asian species that are closest to the Neotropical clade are also tetraploids.

5.4 Taxonomic treatment of Neotropical *Crotalaria*

Although the species of *Crotalaria* occurring in Brazil are well studied (Flores *et al.* 2006, 2008, 2009, 2016), the remaining Neotropical *Crotalaria* remain the least studied group within the genus. Windler (1973, 1974) recognized 74 species for the Neotropics from southern North America to Paraguay (Chapter 2, Figure 3 (maps)), 59 of them native, while Le Roux *et al.* (2013) give numbers of 64 species for South America and 34 for Central and North America. My contributions to a revision of Brazilian *Crotalaria* are summarized in Chapter 3. The Brazilian savannah (cerrado) probably harbors the greatest species number within the Neotropics. We currently recognize 42 species, of which 31 are native, and 17 endemic, and we propose eleven lectotypifications and four synonymizations. Mexico has been reported as another center of diversity in the Neotropics (Windler 1974; Le Roux *et al.* 2013), however, no recent taxonomic treatments for Central and North America are available. A revision for the entire Neotropical region should be undertaken, especially to clarify the delimitation of a few wide-ranging and polymorphic species, which have only been considered in regional floras, such as *C. rotundifolia* and *C. sagittalis*.

5.5 Chromosome numbers and genome size alterations in Caricaceae

In my research on the chromosomes of Caricaceae I was able to retrieve the chromosome numbers for nine species from all six genera. The numbers of three genera had been unknown before: African *Cylicomorpha* ($2n = 18$), which is sister to all Neotropical Caricaceae, and *Horovitzia* and *Jarilla*, which are sister to *Carica papaya* (Chapter 4, Figure 1). My counts confirm the diploid number $2n = 18$ for the remaining genera *Carica*, *Jacaratia*, and *Vasconcellea*, and have revealed an unexpected chromosome number reduction (decreasing dysploidy) in the closest relatives of papaya, *Horovitzia* ($2n = 16$) and *Jarilla* ($2n = 14$), which happened stepwise between 17 and 6 Ma ago. From the phylogeny, $2n = 18$ clearly is the ancestral chromosome number. While dysploidy is not unusual in plants, and has happened also in the closely related Brassicaceae (e.g. Yogeswaran *et al.* 2005: *Arabidopsis*; Cheng *et al.* 2013: *Brassica rapa*), there have been no reports on other cases of stepwise chromosome number reductions ($18 > 16 > 14$). The most interesting aspect of this reduction is that the species of *Jarilla* have the lowest chromosome numbers, yet the second (*J. caudata*: 924 Mbp) and third (*J. heterophylla*: 836 Mbp) largest genomes in Caricaceae; *Cylicomorpha parviflora* (968 Mbp) has

the largest genome. These genomes are around twice the size of those of other Caricaceae (Chapter 4, Table S1), and this finding is unexpected because there is no evidence of polyploidization events.

The reasons for the chromosome number reduction with simultaneous genome size increase in Caricaceae remain unclear. However, my FISH experiments shed light on the potential mechanisms behind these phenomena: The detection of interstitial telomeric repeats (ITRs) in pericentromeric regions of *Jarilla caudata* and *J. heterophylla* suggests chromosomal rearrangements, such as fusions and fissions, and these events might have involved telomeric satellite repeats becoming enriched in pericentromeric regions (Chapter 4, Figures 2 and S1). These and possibly other linked transposable elements may have caused the massive genome size increase.

5.6 Future research questions on flower functionality in *Crotalaria*

While flower morphology has been important for grouping the many species of *Crotalaria*, flower functionality and plant pollinator interactions have been studied in merely two Neotropical species (Etcheverry 2001: *C. stipularia*, keel beak spirally twisted; Etcheverry 2000 and Etcheverry *et al.* 2003: *C. micans* (today pantropical), keel beak not twisted) and one other pantropical species of presumably Asian origin (Jacobi *et al.* 2005: *C. retusa*, keel beak not twisted). These studies showed that during the 36–48 h of anthesis, all species pass through the same floral developmental stages, with the outer and inner set of anthers dehiscing at different points of time. One day before flower opening (which occurs early in the morning), the outer (antisealous) anthers release their pollen into the keel (Figures 2 B1, B2). Bees that visit the open flower, sit on the closed keel, holding onto the outer side of the wing petals with their tarsi; the outer petal surface has a roughened surface that allows for a better hold of the tarsi (Figure 2a). When inserting its proboscis to reach the nectar at the base of the staminal tube (Figure 2b), the bee's weight exerts force on the keel, which causes the style to emerge and brush out the pollen through the tip of the keel and depositing it on the bee's abdomen. The megachilid bee *Megachile crotalariae* (= *Pseudocentron crotalariae*) pollinates *C. micans*, *C. retusa*, and *C. stipularia* (Etcheverry 2001; Etcheverry *et al.* 2003, Jacobi *et al.* 2005), indicating that a spirally twisted keel does not exclude or attract particular types of bees. In addition to the style, the growing antisealous anthers also push pollen from the antisealous anthers towards the tip of the

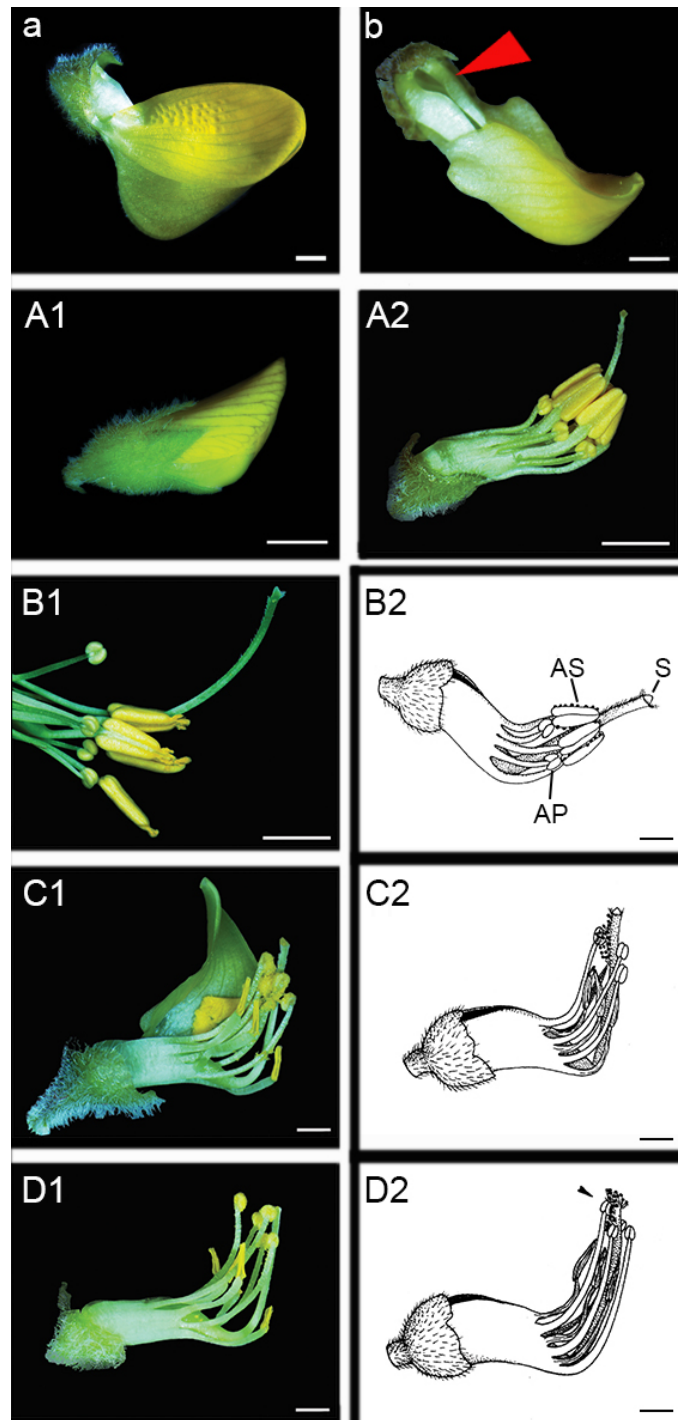


Figure 2. Flower traits and development in *Crotalaria novae-hollandiae*. a) Foot holds for the bees' tarsi (calyx and standard removed); b) Staminal tube with aperture granting access to nectar (red arrow); calyx, standard, and wings removed; A1) Early budstage; A2) Early budstage with corolla and calyx removed; B1 and B2) One day before anthesis, day 1: dehiscence of the antisepalous stamens (AS); C1 and C2) Day 2: Growing of filaments towards the stigma (S) and dehiscence of the antipetalous stamens (AP); D1 and D2) Day 3: Self pollination. Drawings in B2, C2, and D2: *C. stipularia*, modified from Etcheverry (2001). Bars = 1 mm.

keel (Figures 2 C1, C2). Sometime during the 36–48 h-long anthesis, the stigma becomes receptive, allowing for selfing (Figures 2 D1, D2). Experimental and genetic work is needed to assess the contribution of outcrossed versus selfed pollen in *Crotalaria*.

In cultivated plants of three species, I observed the same flower development within the same time frame: *C. novae-hollandiae* (endemic to Australia; keel beak spirally twisted), *C. pallida* (native to Africa, but now pantropical; keel beak not twisted), and *C. velutina* (endemic to Brazil; keel beak spirally twisted). The phylogenetic positions of the five species studied so far are indicated in Figure 3. Polhill (1982) suggested that the twisted beak, which is correlated with higher positioned appendages, restricted to the blade of the standard petal, and in many species also with bilabiate calyces (Figure 3), may require greater effort from the pollinator (i.e., a stronger, heavier bee) to reach the nectar. The twisted keel beak may also result in less pollen being released per visit, allowing for the distribution of pollen onto more individuals, but since all *Crotalaria* flowers have the same short duration of anthesis this cannot be the full explanation. It still needs to be tested whether species with spirally twisted keel beaks and those with non-twisted beaks produce different amounts of pollen and also when exactly their stigmas become receptive. If the species with twisted beaks produced fewer pollen grains, it should be selectively advantageous for them to release fewer grains per bee visit.

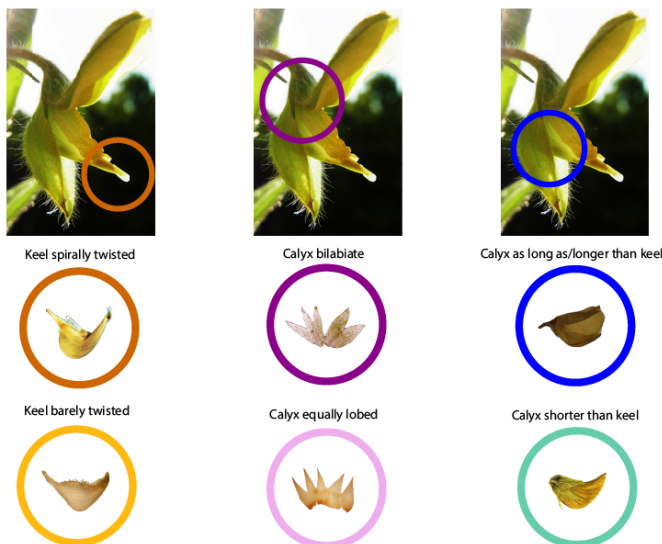
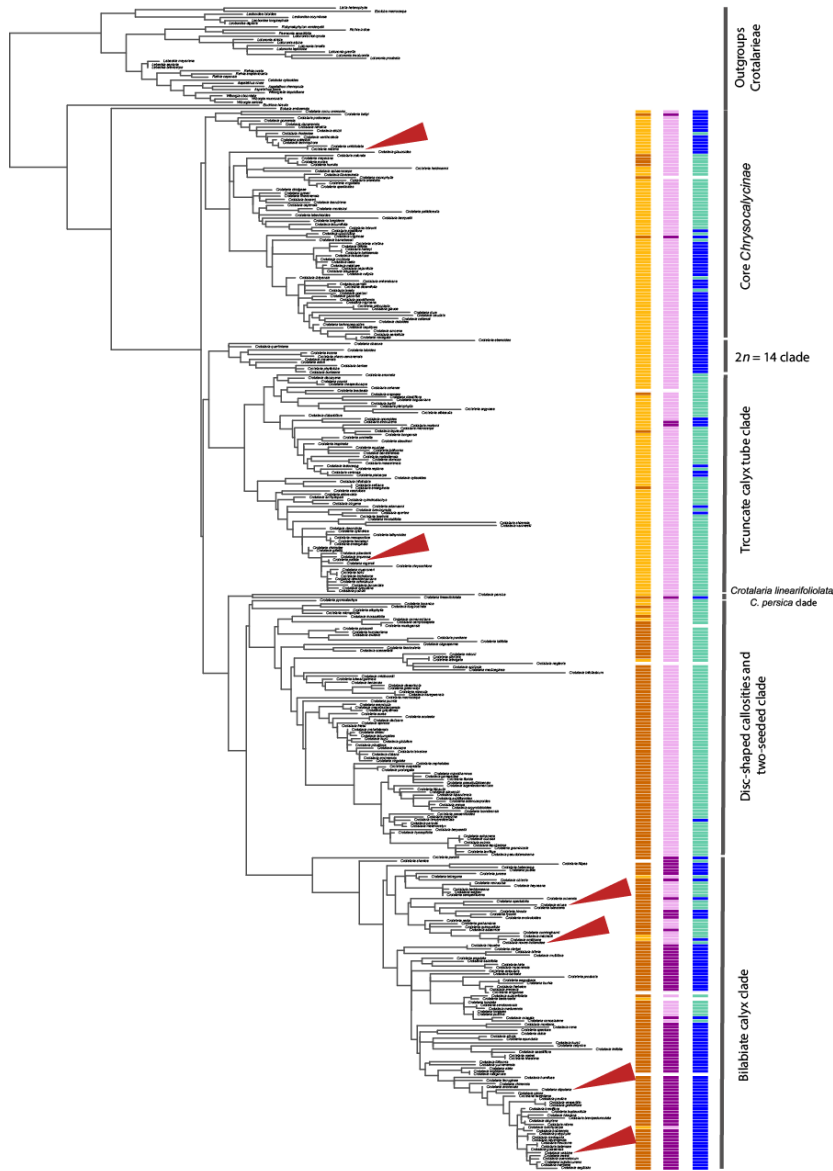


Figure 3. Maximum likelihood tree for 372 accessions representing 338 species of *Crotalaria* and 33 species of the remaining 15 genera of Crotalariaeae based on 3171 aligned nucleotides of nuclear and plastid sequences, with key flower traits plotted: Keel beak (spirally twisted; not twisted), calyx (bilabiate; equally lobed), and length of calyx (as long as/longer than keel; shorter than keel). Arrows: species studied by Etcheverry (2000, 2001), Etcheverry *et al.* (2003), Jacobi *et al.* (2005), and by myself.

REFERENCES

- Ansari, A.A. 2006. Taxonomic studies on genus *Crotalaria* L. in India – II: infra-generic classification. *J. Econ. Tax. Bot.* 30, 570-582.
- Ansari, A.A., 2008. *Crotalaria* L. in India. Bishen Singh Mahendra Pal Singh, Dehra Dun.
- Baker, E.G. 1914. The African species of *Crotalaria*. *J. Linn. Soc. Bot.* 42, 241-425.
- Baltzer, F. 1964. Theodor Boveri. *Science* 144, 809-815.
- Bennett, M.D. 1998. Plant genome values: How much do we know? *Proc. Natl. Acad. Sci. USA* 95, 2011-2016.
- Bentham, G. 1843. Enumeration of the Leguminosae indigenous to Southern Asia and Central and Southern Africa XV. *Crotalaria*. *London J. Bot.* 2, 472–481, 559-593.
- Berry, P.E., Hipp, A.L., Wurdack, K.J., Van Ee, B., and Riina, R. 2005. Molecular phylogenetics of the giant genus *Croton* and tribe Crotonae (Euphorbiaceae sensu stricto) using ITS and *trnL-trnF* DNA sequence data. *Am. J. Bot.* 92, 1520-1534.
- Biffin, E., Craven, L.A., Crisp, M.D., and Gadek, P.A. 2006. Molecular systematics of *Syzygium* and allied genera (Myrtaceae): evidence from the chloroplast genome. *Taxon* 55, 79-94.
- Bisby, F.A., and Polhill, R.M. 1973. The role of taxometrics in angiosperm taxonomy. *New Phytol.* 72, 727-742.
- Bohs, L., and Olmstead, R.G. 1997. Phylogenetic relationships in *Solanum* (Solanaceae) based on *ndhF* sequences. *Syst. Bot.* 22, 5-17.
- Boulter, D., Derbyshire, E., Frahm-Leliveld, J.A., and Polhill, R.M. 1970. Observations on the cytology and seed proteins of various African species of *Crotalaria* L. (Leguminosae) *New Phytol.* 69, 117-131.
- Bukhari, Y.M., Koivu, K., and Tigerstedt, P.M.A. 1999. Phylogenetic analysis of *Acacia* (Mimosaceae) as revealed from chloroplast RFLP data. *Theor. Appl. Genet.* 98, 291-298.
- Burke, J.M., Bayly, M.J., Adams, P.B., and Ladiges, P.Y. 2008. Molecular phylogenetic analysis of *Dendrobium* (Orchidaceae), with emphasis on the Australian section *Dendrocoryne*, and implications for generic classification. *Aust. Syst. Bot.* 21, 1-14.

- Cardoso, D., de Queiroz, L.P., Pennington, R.T., de Lima, H.C., Fonty, E., Wojciechowski, M.F., and Lavin, M. 2012. Revisiting the phylogeny of papilionoid legumes: New insights from comprehensively sampled early-branching lineages. *Am. J. Bot.* 99, 1991-2013.
- Carlsen, M.M., and Croat, T.B. (2013). A molecular phylogeny of the species-rich Neotropical genus *Anthurium* (Araceae) based on combined chloroplast and nuclear DNA. *Syst. Bot.* 38, 576-588.
- Carvalho, F.A., and Renner, S.S. 2012. A dated phylogeny of the papaya family (Caricaceae) reveals the crop's closest relatives and the family's biogeographic history. *Mol. Phylogenet. Evol.* 65, 46-53.
- Carvalho, F.A., and Renner, S.S. 2013a. Correct names for some of the closest relatives of *Carica papaya*: A review of the Mexican/Guatemalan genera *Jarilla* and *Horovitzia*. *PhytoKeys* 29: 63-74.
- Carvalho, F.A., and Renner, S.S. 2013b. The phylogeny of the Caricaceae. Pp. 81-92 in *Genetics and genomics of papaya*, eds. Ming, R. and Moore, P., Springer Verlag.
- Carvalho, F.A., Filer, D., and Renner, S.S. 2015. Taxonomy in the electronic age and an e-monograph of the papaya family (Caricaceae) as an example. *Cladistics* 31: 321-329.
- Champagne, C.E., Goliber, T.E., Wojciechowski, M.F., Mei, R.W., Townsley, B.T., Wang, K., Paz, M.M., Geeta, R., and Sinha, N.R. 2007. Compound leaf development and evolution in the legumes. *Plant Cell*, 19, 3369-3378.
- Chapman, M.A., and Burke., J.M. 2007. Genetic divergence and hybrid speciation. *Evolution* 61, 1773-1780.
- Chávez-Pesqueira, M., Suarez-Montes, P., Castillo, G., and Nunez-Farfan, J. 2014. Habitat fragmentation threatens wild populations of *Carica papaya* (Caricaceae) in a lowland rainforest. *Am. J. Bot.* 101, 1092-1101.
- Chen, Z., and Li, J. 2004. Phylogenetics and biogeography of *Alnus* (Betulaceae) inferred from sequences of nuclear ribosomal DNA ITS region. *Int. J. Plant Sci.* 165, 325-335.
- Cheng , F. , Mandakova, T. , Wu, J. , Xie, Q., Lysak, M., and Wang, X. 2013. Deciphering the diploid ancestral genome of the mesohexaploid *Brassica rapa*. *Plant Cell* 25, 1541-1554.
- Clements, M.A. 2003. Molecular phylogenetic systematics in the Dendrobiinae (Orchidaceae), with emphasis on *Dendrobium* section *Pedilonum*. *Telopea* 10, 247-272.

- Costa, F.R., Pereira, T.N.S., Hodnett, G.L., Pereira, M.G., and Stelly, D.M. 2008. Fluorescent *in situ* hybridization of 18S and 5S rDNA in papaya (*Carica papaya* L.) and wild relatives. *Caryologia* 61, 411-416.
- Craven, L.A., and Biffin, E. 2010. An infrageneric classification of *Syzygium* (Myrtaceae). *Blumea* 55, 94-99.
- Crisp, M.D., Gilmore, S., and Van Wyk, B.E. 2000. Molecular phylogeny of the genistoid tribes of papilionoid legumes. Pp. 249-276 in *Advances in Legume Systematics*, eds. Herendeen, P.S. and Bruneau, A., Royal Botanical Gardens, Kew.
- Cusimano, N., Sousa, A., and Renner, S.S. 2012. Maximum likelihood inference implies a high, not a low, ancestral haploid chromosome number in Araceae, with a critique of the bias introduced by 'x'. *Ann. Bot.* 109, 681-692.
- Danforth, B.N., Cardinal, S., Praz, C.J., Almeida, E.A.B., and Michez, D. 2013. The impact of molecular data on our understanding of bee phylogeny and evolution. *Annu. Rev. Entomol.* 58, 57-78.
- Dewitte, A., Twyford, A.D., Thomas, D.C., Kidner, C.A., and Van Huylbroeck, J. 2011. The origin of diversity in *Begonia*: genome dynamism, population processes and phylogenetic patterns. Pp. 27-52 in *The dynamical processes of biodiversity-case studies of evolution and spatial distribution*, eds. Griollo, O., and Venora, G., Intech.
- Duangjai, S., Samuel, R., Munzinger, J., Forest, F., Wallnöfer, B., Barfuss, M.H.J., Fischer, G., and Chase, M.W. 2009. A multi-locus plastid phylogenetic analysis of the pantropical genus *Diospyros* (Ebenaceae), with an emphasis on the radiation and biogeographic origins of the New Caledonian endemic species. *Mol. Phylogenet. Evol.* 52, 602-620.
- Efroni, I., Eshed, Y., and Lifschitz, E. 2010. Morphogenesis of simple and compound leaves: a critical review. *Plant Cell*, 22, 1019-1032.
- Escudero, M., Martin-Bravo, S., Mayrose, I., Fernandez-Mazuecos, M., Fiz-Palacios, O., Hipp, A.L., Pimentel, M., Jimenez-Mejias, P., Valcarcel, V., Vargas, P., and Luceno, M. 2014. Karyotypic changes through dysploidy persist longer over evolutionary time than polyploid changes. *PLoS One* 9, e85266.
- Etcheverry, A.V. 2000. The role of pollinators and pattern of fruit production in *Crotalaria micans* (Fabaceae: Papilionoideae). In: *VIII International Symposium on Pollination-Pollination: Integrator of Crops and Native Plant Systems* 561, 349-353.

- Etcheverry, A.V. 2001. Role of staminal growth in delayed self-pollination of *Crotalaria stipularia* (Fabaceae: Papilionoideae). *Acta Hort.* 561, 339-342.
- Etcheverry, A.V., Westerkamp, C., and Protomastro, J.J. 2003. Delayed autonomous self-pollination in the colonizer *Crotalaria micans* (Fabaceae: Papilionoideae): structural and functional aspects. *Plant Syst. Evol.* 239, 15-28.
- Ferro, V.G., Guimaraes, P.R., and Trigo, J.R. 2006. Why do larvae of *Utetheisa ornatrix* penetrate and feed in pods of *Crotalaria* species? Larval performance vs. chemical and physical constraints. *Entomol. Exp. Appl.* 121, 23-29.
- Fletcher, M.T., McKenzie, R.A. Blane, B.J., Reichmann, K.G. 2009. Pyrrolizidine alkaloids in *Crotalaria* taxa from northern Australia: risk to grazing livestock. *J. Agr. Food Chem.* 57, 311-319.
- Flores, A.S. 2004. Taxonomia, números cromossômicos e química de espécies de *Crotalaria* L. (Leguminosae-Papilionoideae) no Brasil. PhD thesis, Universidade Estadual de Campinas, Campinas, SP.
- Flores, A.S., Corrêa, A.M., Forni-Martins, E.R., and Tozzi, A.M.G.A. 2006. Chromosome numbers in Brazilian species of *Crotalaria* (Leguminosae, Papilionoideae) and their taxonomic significance. *Bot. J. Linn. Soc.* 151, 271-277.
- Flores, A.S., and Tozzi, A.M.G.A. 2008. Phytogeographical patterns of *Crotalaria* species (Leguminosae-Papilionoideae) in Brazil. *Rodriguésia* 59: 477-486.
- Flores, A.S., Tozzi, A.M.G.A., and Trigo, J.R. 2009. Pyrrolizidine alkaloid profiles in *Crotalaria* species from Brazil: Chemotaxonomic significance. *Biochem. Syst. Ecol.* 37, 459-469.
- Flores, A.S., Rockinger, A., Rodrigues, R.S., and Tozzi, A.M.G.D.A. 2016. Lectotypifications and taxonomic changes in Brazilian *Crotalaria* L. (Leguminosae). *Phytotaxa*, 267, 296-300.
- Forrest, L.L., and Hollingsworth, P.M. 2003. A recircumscription of *Begonia* based on nuclear ribosomal sequences. *Plant Syst. Evol.* 241, 193-211.
- Forrest, L.L., Hughes, M., and Hollingsworth, P.M. 2005. A phylogeny of *Begonia* using nuclear ribosomal sequence data and morphological characters. *Syst. Bot.* 30, 671-682.
- Friesen, N., Fritsch, R.M., and Blattner, F.R. 2006. Phylogeny and new intrageneric classification of *Allium* (Alliaceae) based on nuclear ribosomal DNA ITS sequences. *Aliso* 22, 372-395.

- Gall, J.G., and Pardue, M.L. 1969. Formation and detection of RNA-DNA hybrid molecules in cytological preparations. *Proc. Natl. Acad. Sci. USA* 63, 378-383.
- Geeta, R., Dávalos, L.M., Levy, A., Bohs, L., Lavin, M., Mummenhoff, K., Sinha, N., and Wojciechowski, M.F. 2012. Keeping it simple: flowering plants tend to retain, and revert to, simple leaves. *New Phytol.*, 193, 481-493.
- Givnish, T.J. 1978. On the adaptive significance of compound leaves, with particular reference to tropical trees. Pp. 351-380 in *Tropical Trees as Living Systems.*, eds. Tomlinson, P.B., and Zimmermann, H., Cambridge: Cambridge University Press.
- Goetsch, L., Eckert, A.J., and Hall, B.D. 2005. The molecular systematics of *Rhododendron* (Ericaceae): a phylogeny based upon RPB2 gene sequences. *Syst. Bot.* 30, 616-626.
- Goldenberg, R., Penneys, D. S., Almeda, F., Judd, W.S., and Michelangeli, F.A. 2008. Phylogeny of *Miconia* (Melastomataceae): initial insights into broad patterns of diversification in a megadiverse neotropical genus. *Int. J. Plant. Sci.* 169: 963-979.
- Goldblatt, P., and Lowry, P.P. 2011. The Index to Plant Chromosome Numbers (IPCN): Three decades of publication by the Missouri Botanical Garden come to an end. *Ann. Mo. Bot. Gard.* 98, 226-227.
- Gonzalez, V.H., Griswold, T., Praz, C.J., and Danforth, B.N. 2012. Phylogeny of the bee family Megachilidae (Hymenoptera: Apoidea) based on adult morphology. *Syst. Entomol.* 37, 261-286.
- Goodall-Copestake, W.P., Pérez-Espona, S., Harris, D.J., and Hollingsworth, P.M. 2010. The early evolution of the mega-diverse genus *Begonia* (Begoniaceae) inferred from organelle DNA phylogenies. *Biol. J. Linn. Soc.* 101, 243-250.
- Grimm, G.W., and Renner, S.S. 2013. Harvesting Betulaceae sequences from GenBank to generate a new chronogram for the family. *Bot. J. Linn. Soc.* 172, 465-477.
- Guerra, M. 2008. Chromosome numbers in plant cytotaxonomy: concepts and implications. *Cytogenet. Genome Res.* 120, 339-350.
- Hartmann, T., and Ober, D. 2000. Biosynthesis and metabolism of pyrrolizidine alkaloids in plants and specialized insect herbivores. Pp. 207-244 in *Topics in Current Chemistry: Biosynthesis - Aromatic Polyketides, Isoprenoids, Alkaloids* vol 209, eds. Leeper, F.J., Vederas, J.C., Berlin, Springer.

- Harvey, W.H. 1862. Leguminosae. Pp. 39-47 in *Flora capensis* vol. 2, eds. Harvey, W.H., and Sonder, O.W., Dublin, Hodges, Smith & Co.
- Hepper, F.N. 1958. Papilionaceae, *Crotalaria*. Pp. 544-553 in, *Flora of west tropical Africa*. ed. Keay, R.W.J., London, Crown Agents.
- Holland, A.E. 2002. A review of *Crotalaria* L. (Fabaceae: Crotalariaeae) in Australia. *Austrobaileya* 6: 293-324.
- Holstein, N., and Renner, S.S. 2011. A dated phylogeny and collection records reveal repeated biome shifts in the African genus *Coccinia* (Cucurbitaceae). *BMC Evol. Biol.* 11, 28.
- Hol, W.H.G., and Van Veen, J.A. 2002. Pyrrolizidine alkaloids from *Senecio jacobaea* affect fungal growth. *J. Chem. Ecol.* 28, 1763-1772.
- Hosseini, S., Go, R., Dadkhah, K., and Ainuddin Nuruddin, A. 2012. Studies on maturase K sequences and systematic classification of *Bulbophyllum* in Peninsular Malaysia. *Pak. J. Bot.* 44, 2047-2054.
- Imai, H.T., Satta, Y., Wada, M., and Takahata, N. 2002. Estimation of the highest chromosome number of eukaryotes based on the minimum interaction theory. *J. Theor. Biol.* 217, 61-74.
- Iovene, M., Yu, Q., Ming, R., and Jiang, J. 2015. Evidence for emergence of sex-determining gene(s) in a centromeric region in *Vasconcellea parviflora*. *Genetics* 199, 413-421.
- Irmer, S., Podzun, N., Langel, D., Heidemann, F., Kaltenecker, E., Schemmerling, B., Geilfus, C.-M., Zöhr, C., and Ober, D. 2015. New aspect of plant-rhizobia interaction: Alkaloid biosynthesis in *Crotalaria* depends on nodulation. *Proc. Natl. Acad. Sci. USA* 112, 4164-4169.
- Jacobi, C.M., Ramalho, M., Silva, M. 2005. Pollination biology of the exotic rattleweed *Crotalaria retusa* L. (Fabaceae) in NE Brazil. *Biotropica* 37, 357-363.
- Janssens, S., Geuten, K., Yuan, Y.M., Song, Y., Küpfer, P., and Smets, E. 2006. Phylogenetics of *Impatiens* and *Hydrocera* (Balsaminaceae) using chloroplast *atpB-rbcL* spacer sequences. *Syst. Bot.* 31, 171-180.
- Jaramillo, M.A., and Manos, P.S. 2001. Phylogeny and patterns of floral diversity in the genus *Piper* (Piperaceae). *Am. J. Bot.*, 88, 706-716.

- Jaramillo, M.A., Callejas, R., Davidson, C., Smith, J.F., Stevens, A.C., and Tepe, E.J. 2008. A phylogeny of the tropical genus *Piper* using ITS and the chloroplast intron *psbJ-petA*. *Syst. Bot.* 33, 647-660.
- Jiang, J., and Gill, B.S. 1994. Nonisotopic in situ hybridization and plant genome mapping: the first 10 years. *Genome* 37, 717-725.
- John, H.A., Birnstiel, M.L., and Jones, K.W. 1969. RNA-DNA hybrids at the cytological level. *Nature* 223, 582-587.
- Johnson, S. D. 2010. The pollination niche and its role in the diversification and maintenance of the southern African flora. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 365, 499-516.
- Ku, C., and Hu, J.M. 2014. Phylogenetic and cophylogenetic analyses of the leaf-nodule symbiosis in *Ardisia* subgenus *Crispardisia* (Myrsinaceae): evidence from nuclear and chloroplast markers and bacterial RRN operons. *Int. J. Plant Sci.* 175, 92-109.
- Larridon, I., Bauters, K., Reynders, M., Huygh, W., Muasya, A.M., Simpson, D.A., and Goetghebeur, P. 2013. Towards a new classification of the giant paraphyletic genus *Cyperus* (Cyperaceae): phylogenetic relationships and generic delimitation in C4 *Cyperus*. *Bot. J. Linn. Soc.* 172, 106-126.
- Le Roux, M.M., and Van Wyk, B.-E. 2012. The systematic value of flower structure in *Crotalaria* and related genera of the tribe Crotalarieae (Fabaceae). *Flora* 207, 414-426.
- Le Roux, M.M., Boatwright, J.S., and Van Wyk, B.-E. 2013. A global infrageneric classification system for the genus *Crotalaria* (Leguminosae) based on molecular and morphological evidence. *Taxon* 62, 957-971.
- Lewis, G., Schrire, B.D., Mackinder, B., and Lock, M. 2005. *Legumes of the world*. Royal Botanical Gardens, Kew.
- Li, Q.Q., Zhou, S.D., He, X.J., Yu, Y., Zhang, Y.C., and Wei, X.Q. 2010. Phylogeny and biogeography of *Allium* (Amaryllidaceae: Allieae) based on nuclear ribosomal internal transcribed spacer and chloroplast *rps16* sequences, focusing on the inclusion of species endemic to China. *Ann. Bot.-London* 106, 709-733.
- Liu, Z., Moore, P.H., Ma, H., Ackerman, C.M., Ragiba, M., Yu, Q., Pearl, H.M., and Kim, M.S. 2004. A primitive Y chromosome in papaya marks incipient sex chromosome evolution. *Nature* 427, 348-352.

- Lysak, M.A., Berr, A., Pecinka, A., Schmidt, R., McBreen, K., and Schubert, I. 2006. Mechanisms of chromosome number reduction in *Arabidopsis thaliana* and related Brassicaceae species. *Proc. Natl. Acad. Sci. USA* 103, 5224-5229.
- Macel, M. 2011. Attract and deter: a dual role for pyrrolizidine alkaloids in plant-insect interactions. *Phytochem. Rev.* 10, 75-82.
- Mandáková, T., Kovařík, A., Zozomová-Lihová, J., Shimizu-Inatsugi, R., Shimizu, K.K., Mummenhoff, K., Marhold, K., and Lysak, M.A. 2013. The more the merrier: recent hybridization and polyploidy in *Cardamine*. *Plant Cell*, 25, 3280-3295.
- Mangotra, R., and Koul, A.K. 1979. Chromosome number in *Crotalaria ferruginea* Grah ex Benth. *Sci. Cult.* 45, 252.
- Mangotra, R., and Koul, A.K. 1991. Polyploidy in genus *Crotalaria*. *Cytologia* 56, 293-296.
- Marais, G.A., Forrest, A., Kamau, E., Käfer, J., Daubin, V., and Charlesworth, D. 2011. Multiple nuclear gene phylogenetic analysis of the evolution of dioecy and sex chromosomes in the genus *Silene*. *PLoS One* 6, e21915.
- May, K.M., and R. D. Sargent 2009. The role of animal pollination in plant speciation: Integrating ecology, geography, and genetics. *Annu. Rev. Ecol. Evol. Syst.* 40: 637-656.
- Mears, J.A., and Mabry, T.J. 1971. Alkaloids in the Leguminosae. Pp. 73-178 in *Chemotaxonomy of the Leguminosae*, eds. Harborne, J.B., Boulter, T., and Turner, B.L., London-New York, Academic Press.
- Miller, J.T., and Bayer, R.J. 2001. Molecular phylogenetics of *Acacia* (Fabaceae: Mimosoideae) based on the chloroplast *matK* coding sequence and flanking *trnK* intron spacer regions. *Am. J. Bot.* 88, 697-705.
- Milne-Redhead, E. 1961. Miscellaneous notes on African species of *Crotalaria* L. *Kew Bull.* 15, 157-167.
- Ming, R., Bendahmane, A., and Renner, S.S. 2011. Sex chromosomes in land plants. *Annu. Rev. Plant Biol.* 62, 485-514.
- Mondin, M., Santos-Serejo, J.A., and Aguiar-Perecin, M.L.R. 2007. Karyotype characterization of *Crotalaria juncea* (L.) by chromosome banding and physical mapping of 18S-5.8 S-26S and 5S rRNA gene sites. *Genet. Mol. Biol.*, 30, 65-72.

- Mondin, M., and Aguiar-Perecin, M.L.R. 2011. Heterochromatin patterns and ribosomal DNA loci distribution in diploid and polyploid *Crotalaria* species (Leguminosae, Papilionoideae), and inferences on karyotype evolution. *Genome* 54, 718-726.
- Moonlight, P.W., Richardson, J.E., Tebbitt, M.C., Thomas, D.C., Hollands, R., Peng, C.I., and Hughes, M. 2015. Continental-scale diversification patterns in a megadiverse genus: the biogeography of Neotropical *Begonia*. *J. Biogeogr.* 42, 1137-1149.
- Morales, A.G., Aguiar-Perecin, M.L.R., Mondin, M. 2012. Karyotype characterization reveals an up and down of 45S and 5S rDNA sites in *Crotalaria* (Leguminosae-Papilionoideae) species of the section *Hedriocarpae* subsection *Macrostachyae*. *Genetic Resources and Crop Evolution* 59, 277-288.
- Muasya, A.M., Simpson, D.A., and Chase, M.W. 2002. Phylogenetic relationships in *Cyperus* L. s.l. (Cyperaceae) inferred from plastid DNA sequence data. *Bot. J. Linn. Soc.* 138, 145-153.
- Murphy, D.J., Brown, G.K., Miller, J.T., and Ladiges, P.Y. 2010. Molecular phylogeny of *Acacia* Mill. (Mimosoideae: Leguminosae): evidence for major clades and informal classification. *Taxon* 59, 7-19.
- Narberhaus, I., Zintgraf, V., and Dobler, S. 2005. Pyrrolizidine alkaloids on three trophic levels—evidence for toxic and deterrent effects on phytophages and predators. *Chemoecology* 15, 121-125.
- Nepokroeff, M., Bremer, B., and Sytsma, K.J. 1999. Reorganization of the genus *Psychotria* and tribe Psychotrieae (Rubiaceae) inferred from ITS and *rbcL* sequence data. *Syst. Bot.* 24, 5-27.
- Ober, D., and Kaltenecker, E. 2009. Pyrrolizidine alkaloid biosynthesis, evolution of a pathway in plant secondary metabolism. *Phytochemistry* 70, 1687-95.
- Oberlander, K.C., Emshwiller, E., Bellstedt, D.U., and Dreyer, L.L. 2009. A model of bulb evolution in the eudicot genus *Oxalis* (Oxalidaceae). *Mol. Phylogenet. Evol.* 51, 54-63.
- Oliveira, A.C.D., and Aguiar-Perecin, M.D. 1999. Karyotype evolution in the genus *Crotalaria* (Leguminosae). *Cytologia* 64, 165-174.
- Olmstead, R.G., and Palmer, J.D. 1997. Implications for the phylogeny, classification, and biogeography of *Solanum* from cpDNA restriction site variation. *Syst. Bot.* 22, 19-29.

- Osaloo, S.K., Maassoumi, A.A., and Murakami, N. 2003. Molecular systematics of the genus *Astragalus* L.(Fabaceae): phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacers and chloroplast gene *ndhF* sequences. *Plant Syst. Evol.* 242, 1-32.
- Palomino, G., and Vázquez, R. 1991. Cytogenetic studies in Mexican populations of species of *Crotalaria* L. (Leguminosae-Papilionoideae). *Cytologia* 56, 343-351.
- Paun, O., Forest, F., Fay, M.F., and Chase, M.W. 2011. Parental divergence and hybrid speciation in angiosperms revisited. *Taxon* 60, 1241-1244.
- Pelser, P.B., Nordenstam, B., Kadereit, J.W., and Watson, L.E. 2007. An ITS phylogeny of tribe Senecioneae (Asteraceae) and a new delimitation of *Senecio* L. *Taxon* 56, 1077.
- Pennington, R.T., Lavin, M., Ireland, H., Klitgaard, B., Preston, J., and Hu, J. M. 2001. Phylogenetic relationships of basal papilionoid legumes based upon sequences of the chloroplast *trnL* intron. *Syst. Bot.* 26, 537-556.
- Pessoa, E.M., Alves, M., Alves-Araújo, A., Palma-Silva, C., and Pinheiro, F. 2012. Integrating different tools to disentangle species complexes: a case study in *Epidendrum* (Orchidaceae). *Taxon* 61, 721-734.
- Polhill, R.M. 1968. Miscellaneous notes on African species of *Crotalaria* L.: II. *Kew Bull.* 22, 169-348.
- Polhill, R.M., 1982. *Crotalaria in Africa and Madagascar*. A.A. Balkema, Rotterdam.
- Pirie, M.D., Oliver, E.G.H., and Bellstedt, D.U. 2011. A densely sampled ITS phylogeny of the Cape flagship genus *Erica* L. suggests numerous shifts in floral macro-morphology. *Mol. Phylogenet. Evol.* 61, 593-601.
- Rice, A., Glick, L., Abadi, S., Einhorn, M., Kopelman, N.M., Salman-Minkov, A., Mayzel, J., Chay, O., and Mayrose, I. 2015. The Chromosome Counts Database (CCDB) - a community resource of plant chromosome numbers. *New Phytol.* 206, 19-26.
- Richardson, J.E., Pennington, R.T., Pennington, T.D., and Hollingsworth, P.M. 2001. Rapid diversification of a species-rich genus of Neotropical rain forest trees. *Science* 293, 2242-2245.
- Roalson, E.H., Columbus, J.T., and Friar, E.A. 2001. Phylogenetic relationships in Cariceae (Cyperaceae) based on ITS (nrDNA) and *trnT*-LF (cpDNA) region sequences: assessment of subgeneric and sectional relationships in *Carex* with emphasis on section *Acrocystis*. *Syst. Bot.* 26, 318-341.

- Robinson, J., and Harris, S.A. 2000. A plastid DNA phylogeny of the genus *Acacia* Miller (Acacieae, Leguminosae). *Bot. J. Linn. Soc.* 132, 195-222.
- Ronsted, N., Weiblen, G.D., Clement, W.L., Zerega, N.J.C., and Savolainen, V. 2008. Reconstructing the phylogeny of figs (*Ficus*, Moraceae) to reveal the history of the fig pollination mutualism. *Symbiosis (Rehovot)* 45, 45.
- Samain, M.S., Vanderschaeve, L., Chaerle, P., Goetghebeur, P., Neinhuis, C., and Wanke, S. 2009. Is morphology telling the truth about the evolution of the species rich genus *Peperomia* (Piperaceae)? *Plant Syst. Evol.* 278, 1-21.
- Särkinen, T., Bohs, L., Olmstead, R. G., and Knapp, S. 2013. A phylogenetic framework for evolutionary study of the nightshades (Solanaceae): a dated 1000-tip tree. *BMC Evol. Biol.* 13, 1.
- Scherson, R.A., Vidal, R., and Sanderson, M.J. 2008. Phylogeny, biogeography, and rates of diversification of New World *Astragalus* (Leguminosae) with an emphasis on South American radiations. *Am. J. Bot.*, 95, 1030-1039.
- Schreiber, A. 1970. Fabaceae. Pp. 16-28 in *Prodromus einer Flora von Südwestafrika*, Lfg. 60, ed. Merxmüller, H., Lehre: J. Cramer.
- Schrire, B.D., Lavin, M., Barker, N.P., Cortes-Burns, H., von Senger, I., and Kim, J.-H. 2003. Towards a phylogeny of *Indigofera* (Leguminosae-Papilionoideae): identification of major clades and relative ages. Pp. 269-302 in *Advances in Legume Systematics Part 10, Higher level systematics*, eds. Klitgaard, B.B., and Bruneau, A., Royal Botanic Gardens, Kew.
- Schubert, I., Schriever-Schwemmer, G., Werner, T., and Adler, I.D. 1992. Telomeric signals in Robertsonian fusion and fission chromosomes: implications for the origin of pseudoaneuploidy. *Cytogenet. Genome Res.* 59, 6-9.
- Schubert, I., Rieger, R., and Fuchs, J. 1995. Alteration of basic chromosome number by fusion-fission cycles. *Genome* 38, 1289-1292.
- Sebastian, P., Schaefer, H., Lira, R., Telford, I.R.H., and Renner, S.S. 2012. Radiation following long-distance dispersal: The contributions of time, opportunity, and diaspore morphology in *Sicyos* (Cucurbitaceae). *J. Biogeography* 39, 1427-1438.
- Singh, B.D., and Harvey, B.L. 1975. Selection for diploid cells in suspension cultures of *Happlopappus gracilis*. *Nature* 253, 453.

- Smidt, E.C., Borba, E.L., Gravendeel, B., Fischer, G.A., and Van den Berg, C. 2011. Molecular phylogeny of the Neotropical sections of *Bulbophyllum* (Orchidaceae) using nuclear and plastid spacers. *Taxon*, 60, 1050-1064.
- Smith, J.F., Stevens, A.C., Tepe, E.J., and Davidson, C. 2008. Placing the origin of two species-rich genera in the late cretaceous with later species divergence in the tertiary: a phylogenetic, biogeographic and molecular dating analysis of *Piper* and *Peperomia* (Piperaceae). *Plant Syst. Evol.* 275, 9-30.
- Soltis, P.S., and Soltis, D.E. 2009. The role of hybridization in plant speciation. *Annu. Rev. Plant Biol.* 60, 561-588.
- Sousa, A., Cusimano, N., and Renner, S.S. 2014. Combining FISH and model-based predictions to understand chromosome evolution in *Typhonium* (Araceae). *Ann Bot* 113, 669-680.
- Stuessy, T.F., Crawford, D.J., and Soltis, P.S. 2014. *Plant Systematics – The Origin, Interpretation, and Ordering of Plant Biodiversity*. Koeltz Scientific Books, Königstein (Taunus).
- Subramaniam, S., Pandey, A.K., Geeta, R., and Mort, M.E. 2013. Molecular systematics of Indian *Crotalaria* (Fabaceae) based on analyses of nuclear ribosomal ITS DNA sequences. *Plant Syst. Evol.* 299, 1089-1106.
- Subramaniam, S., Pandey, A.K., and Rather, S.A. 2015. A revised circumscription of the species in *Bracteatae* complex (section *Calycinae*) in the genus *Crotalaria* L.: evidence from nuclear and chloroplast markers. *Plant Syst. Evol.* 301, 2261-2290.
- Tang, H., Bowers, J.E., Wang, X., Ming, R., Alam, M., and Paterson, A.H. 2008. Synteny and collinearity in plant genomes. *Science* 320, 486-488.
- Tapia-Pastrana, F., Gallegos-Pacheco, E., De Teodoro-Pardo, C., and Mercado-Ruaro, P. 2005. New cytogenetic information of two Mexican populations of *Crotalaria incana* L. (Leguminosae-Papilionoideae). *Cytologia* 70, 207-212.
- The Plant List 2010. *Version 1. Published on the Internet*. Available at: <http://www.theplantlist.org/> (accessed 08.08.2016).
- Thoden, T.C., and Boppré, M. 2010. Plants producing pyrrolizidine alkaloids: sustainable tools for nematode management? *Nematology* 12, 1-24.
- Torre, A.R. 1962. *Crotalaria*. Pp. 6–76 in *Conspectus Florae Angolensis* vol. 3, eds. Exell, A.W., and Fernandes, A., Junta de Investigações do Ultramar, Lisboa.

- Valente, L.M., Savolainen, V., and Vargas, P. 2010. Unparalleled rates of species diversification in Europe. *P. Roy. Soc. B-Biol. Sci.* 277: 1489-1496.
- Van Dam, N.M., Vuister, L.W.M., Bergshoeff, C., de Vos, H., and Van der Meijden, E.D. 1995. The “Raison D'être” of pyrrolizidine alkaloids in *Cynoglossum officinale*: Deterrent effects against generalist herbivores. *J. Chem. Ecol.* 21, 507-523.
- Van der Merwe, M.M., Van Wyk, A.E., and Botha, A.M. 2005. Molecular phylogenetic analysis of *Eugenia* L. (Myrtaceae), with emphasis on southern African taxa. *Plant Syst. Evol.* 251, 21-34.
- Van Wyk, B.E. 2003. The value of chemosystematics in clarifying relationships in the genistoid tribes of papilionoid legumes. *Biochem. Syst. Ecol.* 31, 875-884.
- Van Wyk, B.E., and Verdoorn, G.H. 1990. Alkaloids as taxonomic characters in the tribe Crotalarieae. *Biochem. Syst. Ecol.* 18, 503-515.
- Van Wyk, B.-E., Venter, M., and Boatwright, J.S. 2010. A revision of the genus *Bolusia* (Fabaceae, Crotalarieae). *So. Afr. J. Bot.* 76, 86-94.
- Van Buren, R., Zeng, F., Chen, C., Zhang, J., Wai, C.M., Han, J., Aryal, R., Gschwend, A.R., Wang, J., Na, J.K., Huang, L., Zhang, L., Miao, W., Gou, J., Arro, J., Guyot, R., Moore, R.C., Wang, M.L., Zee, F., Charlesworth, D., Moore, P.H., Yu, Q., and Ming, R. 2015. Origin and domestication of papaya Yh chromosome. *Genome Res.* 25, 524-533.
- Verdoorn, I.C. 1928. A revision of the Crotalarias of South and South-East Tropical Africa. *Bothalia* 2, 371-420.
- Walker, J.B., Sytsma, K.J., Treutlein, J., and Wink, M. 2004. *Salvia* (Lamiaceae) is not monophyletic: implications for the systematics, radiation, and ecological specializations of *Salvia* and tribe Mentheae. *Am. J. Bot.*, 91, 1115-1125.
- Weese, T.L., and Bohs, L. 2007. A three-gene phylogeny of the genus *Solanum* (Solanaceae). *Syst. Bot.* 32, 445-463.
- Wilczek, R. 1953a. Papilionaceae Genisteeae Congolanae Novae (*Robynsiophyton*, *Crotalaria*, *Argyrolobium*). *Bull. Jard. Bot. État Bruxelles* 23, 125–211.
- Wilczek, R. 1953b. *Crotalaria*. Pp. 43-273 in *Flore du Congo Belge* vol. 4, eds. Robyns, W., Staner, P., Demaret, F., Germain, R., Gilbert, G., Hauman, L., Homès, M., Jurion, F., Lebrun, J., Van den Abeele, M., and Boutique, R., Institut National pour l'Étude Agronomique du Congo belge, Brussels.

- Wilson, M., Belle, C., Dang, A., Hannan, P., Kellogg, L., Kenyon, C., Low, H., Mochizuki, A., Nguyen, A., Sheade, N., Shan, L., Shum, A., Stayton, T., Volz, C., Vosburgh, H., Wellman, H., and Woolley, M. 2013. A preliminary phylogenetic analysis of *Pleurothallis* sensu lato based upon nuclear and plastid sequences. *Lankesteriana* 13, 139.
- Windler, D.R. 1973. Field and garden studies in *Crotalaria sagittalis* L. and related species. *Phytologia* 26: 289-354.
- Windler, D.R. 1974. Chromosome numbers for native North American unifoliolate species of *Crotalaria* (Leguminosae). *Brittonia* 26, 172-176.
- Wojciechowski, M.F., Sanderson, M.J., and Hu, J.M. 1999. Evidence on the monophyly of *Astragalus* (Fabaceae) and its major subgroups based on nuclear ribosomal DNA ITS and chloroplast DNA *trnL* intron data. *Syst. Bot.* 24, 409-437.
- Wu, X., Wang, J., Na, J.K., Yu, Q., Moore, R.C., Zee, F., Huber, S.C., and Ming, R. 2010. The origin of the non-recombining region of sex chromosomes in *Carica* and *Vasconcellea*. *Plant J.* 63, 801-810.
- Wurdack, K.J., Hoffmann, P., Samuel, R., de Bruijn, A., Van der Bank, M., and Chase, M.W. 2004. Molecular phylogenetic analysis of Phyllanthaceae (Phyllanthoideae pro parte, Euphorbiaceae sensu lato) using plastid *rbcL* DNA sequences. *Am. J. Bot.* 91, 1882-1900.
- Yang, L., Koo, D.H., Li, D., Zhang, T., Jiang, J., Luan, F., Renner, S.S., Henaff, E., Sanseverino, W., Garcia-Mas, J., Casacuberta, J., Senalik, D.A., Simon, P.W., Chen, J., and Weng, Y. 2014. Next-generation sequencing, FISH mapping and synteny-based modeling reveal mechanisms of decreasing dysploidy in *Cucumis*. *Plant J.* 77, 16-30.
- Yogeeswaran, K., Frary, A., York, T.L., Amenta, A., Lesser, A.H., Nasrallah, J.B., Tanksley, S.D., Nasrallah, M.E. 2005. Comparative genome analyses of *Arabidopsis* spp.: Inferring chromosomal rearrangement events in the evolutionary history of *A. thaliana*. *Genome Res.* 15, 505-515.
- Yu, S.X., Janssens, S.B., Zhu, X.Y., Lidén, M., Gao, T.G., and Wang, W. 2016. Phylogeny of *Impatiens* (Balsaminaceae): integrating molecular and morphological evidence into a new classification. *Cladistics* 32, 179-197.
- Zimmermann, N.F.A., Ritz, C.M., and Hellwig, F. H. (2010). Further support for the phylogenetic relationships within *Euphorbia* L. (Euphorbiaceae) from nrITS and *trnL-trnF* IGS sequence data. *Plant Syst. Evol.* 286, 39-58.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank Susanne S. Renner for supervising me in her dedicated manner, for always being available to discuss my ideas, and for pushing me to continuously work hard. Thank you for guiding me, encouraging me, and for advancing my research. Your true passion for science and dedication to deliver high-quality research remain unmatched and are an inspiration to all of us.

I'm indebted to Martina Silber for her guidance and assistance with the lab work. Thanks, Martina, also for our many conversations.

I would like to express my gratitude to Roger M. Polhill for acknowledging my work, for sharing his vast experience and unequalled knowledge on *Crotalaria*. Dear Roger, thank you for your advice and our discussions. I deeply admire your thorough fundamental work on *Crotalaria*!

I also thank my colleague and friend, Andréia S. Flores, for our collaboration and support on a professional and personal level. Aguardo ansiosamente nossos projetos futuros!

I cordially thank my colleagues and friends, near and far, who were part of these almost three years by working and learning together. Thanks for your support: Constantin M. Zohner, Oscar A. Pérez-Escobar, Sidonie Bellot, Aretuza Sousa, Natalie Cusimano, Guillaume Chomiccki, Juanita Gutiérrez Valencia, Aline C. Martins, Eva Facher, Jochen Heinrichs, Julia Bechteler, Dimas Carmo, Rodrigo S. Rodrigues, Rafaela Freitas, Ana Sousa, Amanda Noronha, Fernanda Satori, André Moreira, Marcelo F. Devecchi, Allan C. Pscheidt, Margarida da Rocha Fiuza de Melo, Agnes Scheunert, Andreas Fleischmann, Paulo Gonella, Yasumin S. Lermer, Günter Gerlach, Jutta Babczinsky, Andreas Gröger, Andreas Beck, Carola Irlacher, Rafael Matysiuk, Martina Simbeck, Harald Loose, and Achim Hörmann.

Special thanks to the colleagues and curators who provided material for molecular work: Hans-Joachim Esser (M and MSB), Andréia S. Flores (MIRR), Gwilym Lewis (K), Robert Vogt (B), Maria Candida Henrique Mamede (SP), and Renato de Mello-Silva (SPF).

Agradeço à Antonia e ao Joaquim Rosa, e à Catia Rosa Neves, por sempre terem me recebido com amor e respeito apesar de todo esse tempo à distância. Vocês moram no meu <3

Last but not least, danke ich meinen Eltern, Elfrun und August Rockinger, und meiner Schwester, Elena Rockinger, für ihre bedingungslose Unterstützung und Liebe.