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Phylogenomic Study of *Monechma* Reveals Two Divergent Plant Lineages of Ecological Importance in the African Savanna and Succulent Biomes

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Abstract: *Monechma* Hochst. s.l. (Acanthaceae) is a diverse and ecologically important plant group in sub-Saharan Africa, well represented in the fire-prone savanna biome and with a striking radiation into the non-fire-prone succulent biome in the Namib Desert. We used RADseq to reconstruct evolutionary relationships within *Monechma* s.l. and found it to be non-monophyletic and composed of two distinct clades: Group I comprises eight species resolved within the *Harnieria* clade, whilst Group II comprises 35 species related to the Diclipterinae clade. Our analyses suggest the common ancestors of both clades of *Monechma* occupied savannas, but both of these radiations (~13 mya crown ages) pre-date the currently accepted origin of the savanna biome in Africa, 5–10 mya. Diversification in the succulent biome of the Namib Desert is dated as beginning only ~1.9 mya. Inflorescence and seed morphology are found to distinguish Groups I and II and related taxa in the Justicioid lineage. *Monechma* Group II is morphologically diverse, with variation in some traits related to ecological diversification including plant habit. The present work enables future research on these important lineages and provides evidence towards understanding the biogeographical history of continental Africa.

Keywords: Africa; biome; RADseq; *Monechma*; *Justicia*; phylogeny; plant diversity

1. Introduction

The Acanthaceae Juss. (Lamiales) are amongst the most diverse and ecologically important vascular plant families in sub-Saharan Africa. They are, for example, the sixth most species-rich family in the Flora of Ethiopia and Eritrea region, the Flora of Tropical East Africa region (Kenya, Tanzania, Uganda), Mozambique and Namibia; the seventh richest in Cameroon and South Sudan; and the ninth richest in Guinea [1–4]. Lineages of Acanthaceae have diversified in a wide range of habitats ranging from hyper-arid desert to tropical rainforest, and are species-poor only in low-nutrient environments such as on the deep Kalahari Sands of southern Africa and the fynbos of the Cape

Floristic Region. In many parts of the continent, Acanthaceae form a dominant constituent of the ground flora such that they provide important ecosystem services and are of economic importance as fodder for livestock and native herbivores [5–7]. Many species of Acanthaceae in sub-Saharan Africa are highly range-restricted and of high conservation concern [6,8–10]. However, despite their obvious importance, our understanding of the diversity and evolutionary history of Acanthaceae is incomplete and many major taxonomic challenges persist [11–16].

One of the most diverse and frequently encountered groups of Acanthaceae in sub-Saharan Africa is the pantropical genus *Justicia* L., taken in a broad sense (i.e., *Justicia* s.l.) [17,18]. Although displaying a large range of morphological diversity, plants of *Justicia* s.l. are readily recognised by the combination of a bilabiate corolla with a rugula (i.e., a stylar furrow on the internal corolla surface), an androecium of two fertile stamens, no staminodes, complex anthers, often with markedly offset thecae and/or with appendages, and 2–4 (–6) colporate pollen with pseudocolpi or with rows of insulae adjacent to the apertures [13,14]. However, recent molecular phylogenetic studies on *Justicia* and allied genera—together comprising the Justicioid lineage—using evidence from six molecular markers [13,14] have demonstrated that *Justicia* s.l. is grossly paraphyletic, with several major, morphologically distinct lineages embedded within it. In order to maintain a broadly circumscribed *Justicia* including morphologically similar taxa such as *Anisotes* Nees, *Anisostachya* Nees, *Monechma* Hochst and *Rungia* Nees, the entire Justicioid lineage would potentially have to be treated as a single genus [13]. This is highly undesirable as it would require subsuming several species-rich genera that are easily separated morphologically, including *Dicliptera* Juss. and *Hypoestes* R. Br. The only plausible alternative, therefore, is to subdivide *Justicia* s.l. into a number of segregate genera [15]. However, only 12–15% of all members of the Justicioid lineage have been phylogenetically sampled to date and many sampling deficiencies need to be addressed before fully informed taxonomic decisions can be made [13].

One such group highlighted as ripe for further taxonomic work is the genus *Monechma* Hochst. s.l. (Figure 1) [13]. *Monechma*, or *Justicia* sect. *Monechma* (Hochst.) T. Anderson, is a group of over 40 species confined to continental Africa and Arabia, with the exception of one species (i.e., the type species, *M. bracteatum* Hochst.) that extends to India (Figure 2). Species of *Monechma* combine the characters of *Justicia* listed above with 2- (rarely 4-) seeded capsules bearing compressed seeds with smooth surfaces [19–21]. However, Kiel et al. [13], upon sampling six species (seven accessions) of *Monechma*, found that this group is not monophyletic and instead separated into two distinct and widely separated clades. *Monechma* Group I, which includes the type species, falls within the Core *Harnieria* clade together with members of *Justicia* sect. *Harnieria* (Solms-Laub.) Benth. (Figure S1). *Monechma* Group II, for which only two species were sampled [13], falls within the Diclipterinae clade, sister to core Diclipterinae: *Kenyancanthus ndorensis* (Schweinf.) I. Darbysh and C.A. Kiel + (*Hypoestes* + *Dicliptera*) (Figure S1).

Attempts to reconcile this unexpected result with morphological evidence [13] suggested that, based on the limited sampling, the two clades could potentially be separated by differences in inflorescence form. *Monechma* Group I was considered to be a predominantly tropical African clade in which the flowers are arranged in 1–few-flowered cymes aggregated into axillary and/or terminal spikes or fascicles, with the bracts markedly differentiated from the leaves. Group II, considered to be a predominantly southern African clade, includes species that have single- or rarely 2-flowered (sub) sessile axillary inflorescences, which can together sometimes form weakly defined terminal spikes, but with the bracts largely undifferentiated from the leaves. In a subsequent study of *Justicia* sect. *Monechma* in Angola [22], this subdivision was expanded upon and the differences in inflorescence form were used to place the majority of Angolan species within Group I. This included both annual, ruderal species, *M. bracteatum* and *M. monechmoides* (S. Moore) Hutch., as well as perennial species of usually fire-prone habitats, such as *M. scabridum* (S. Moore) C.B. Clarke and allies. That study treated these species within *Justicia* in view of the uncertainty over application of the name *Monechma* but noted that species of Group I may ultimately revert to being referred to under *Monechma* following more comprehensive molecular studies [22].

Figure 1. Morphological diversity in *Monechma* s.l. All species pictured have been sampled in the current study. (**A**) *M. monechmoides* (E.A. Tripp, Namibia, collected as *Tripp and Dexter 787*); (**B**) *M. bracteatum* (Mozambique, B. Wursten); (**C**) *M. debile* (C.A. Kiel, Kenya, *Kiel 173*); (**D**) *Justicia* sp. B of Flora Zambesiaca (B. Wursten, Mozambique, *Wursten 1792*); (**E**) *M. ciliatum* (K. Schumann, Burkina Faso); (**F**) *M. ndellense* (A. Thiombiano, Burkina Faso); (**G**) *M. depauperatum* (W. McCleland, Mali); (**H**) *M. rigidum* (D.J. Goyder, Angola, *Goyder 8210*); (**I**) *M. virgultorum* (D.J. Goyder, Angola, *Goyder 8471*); (**J**) *M. serotinum* (E.A. Tripp, Namibia, *Tripp et al. 4068*); (**K**) *M. grandiflorum* (E.A. Tripp, Namibia *Tripp et al. 2034*); (**L**) *M. calcaratum* (E.A. Tripp, Namibia, *Tripp et al. 2043*); (**M**) *M. distichotrichum* (E.A. Tripp, Namibia, *Tripp et al. 2072*); (**N**) *M. leucoderme* (E.A. Tripp, Namibia, *Tripp et al. 2083*); (**O**) *M. mollissimum* (E.A. Tripp, Namibia, *Tripp et al. 2071*); (**P**) *M. desertorum* (L. Nanyeni, Namibia); (**Q–S**) *M. divaricatum*; (**Q**) (E.A. Tripp, Namibia, *Tripp and Dexter 885*); (**R**) (E.A. Tripp, Namibia, *Tripp and Dexter 779*); (**S**) (I. Darbyshire, Namibia); (**T**) *M. genistifolium* (I. Darbyshire, Namibia); (**U** & **V**) *M. cleomoides*; (**U**) (E.A. Tripp, Namibia, *Tripp and Dexter 829*); (**V**) (E.A. Tripp, Namibia, *Tripp et al. 1960*); (**W**) *M. tonsum* (E.A. Tripp, Namibia, *Tripp and Dexter 813*); (**X**) *M. salsola* (I. Darbyshire, Namibia, *Klaassen et al. 2537*). E, F & G reproduced from S. Dressler, M. Schmidt and G. Zizka, African Plants—A Photo Guide: www.africanplants.senckenberg.de [23] with kind permission from the authors and photographers.

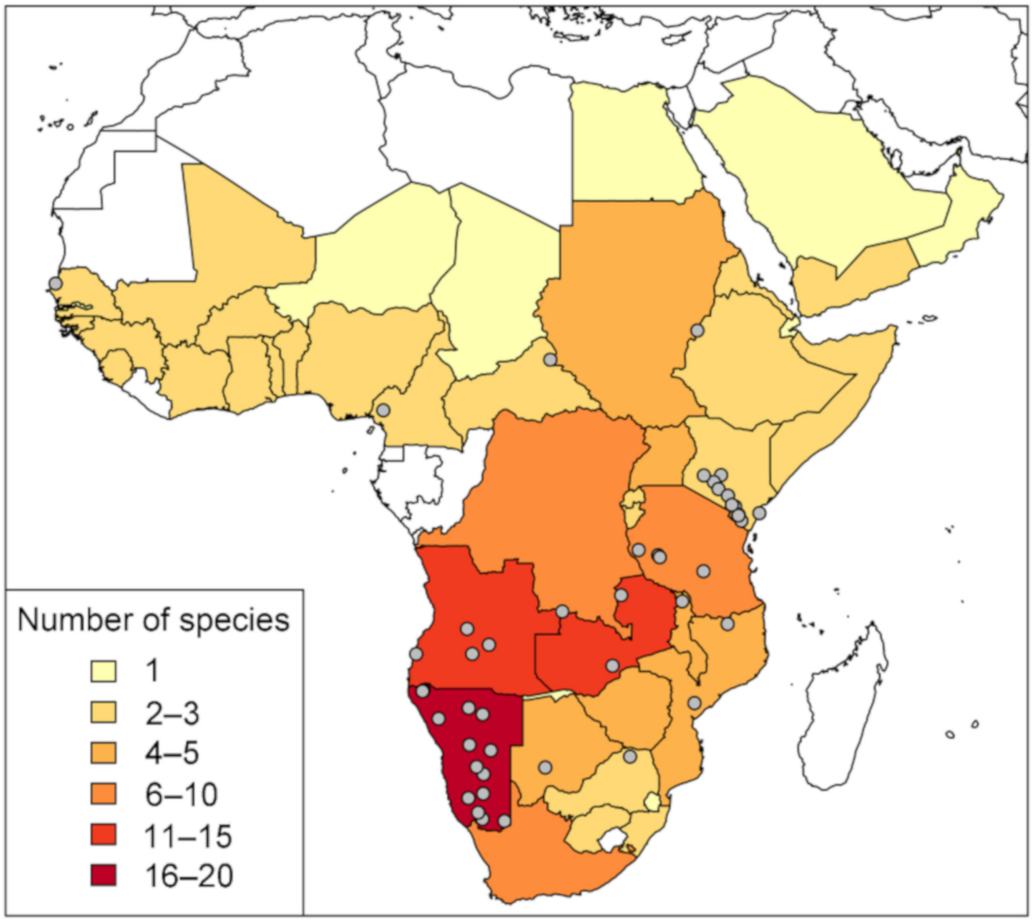

Figure 2. Distribution of *Monechma* s.l. in Africa and Arabia; species richness per TDWG Level 3 geographic region. Note: this is the global distribution of *Monechma* s.l. except that one species (*M. bracteatum*) extends to India. Grey dots represent the samples used in the current study (see Table 1).

Table 1. Taxon and source of plant material from which DNA was extracted for sequencing. Taxa are listed in alphabetical order by genus and species.

Taxon	Source Specimen	Country	Latitude	Longitude
Dicliptera maculata Nees subsp. *usambarica* (Lindau) I. Darbysh.	*Kiel et al. 157* (RSA)	Kenya	−0.1791	35.6317
Dicliptera paniculata (Forssk.) I. Darbysh.	*Kiel et al. 166* (RSA)	Kenya	−2.6910	38.1639
Hypoestes forskaolii (Vahl) R. Br.	*Kiel et al. 144* (RSA)	Kenya	−1.8087	37.5864
Hypoestes triflora (Forssk.) Roem. & Schult.	*Kiel et al. 151* (RSA)	Kenya	−0.7033	36.4346
Justicia anagalloides (Nees) T. Anderson	*Kiel et al. 174* (RSA)	Kenya	−3.4144	38.4262
Justicia attenuifolia Vollesen	*Golding et al. 8* (K)	Mozambique	−12.1739	37.5494
Justicia cordata (Nees) T. Anderson	*Kiel et al. 159* (RSA)	Kenya	−2.5514	37.8933
Justicia cubangensis I. Darbysh. & Goyder	*Goyder et al. 8068* (K)	Angola	−14.5897	16.9072
Justicia eminii Lindau	*Bidgood et al. 930* (K)	Tanzania	−7.9167	35.6000
Justicia fanshawei Vollesen	*Smith et al. 2010* (K)	Zambia	−9.8529	28.9441
Justicia flava (Forssk.) Vahl	*Kiel et al. 146* (RSA)	Kenya	−1.8082	37.5765
Justicia heterocarpa T. Anderson	*Kiel et al. 158* (RSA)	Kenya	−1.2745	36.8146
Justicia kirkiana T. Anderson	*Kiel et al. 177* (RSA)	Kenya	−3.8407	38.6681
Justicia odora (Forssk.) Lam.	*Tripp et al. 4073* (COLO)	Namibia	−17.6041	12.8872
Justicia phyllostachys C.B. Clarke	*Bidgood et al. 6871* (K)	Tanzania	−6.7833	32.0667
Justicia platysepala (S. Moore) P.G. Mey.	*Tripp and Dexter 4119* (COLO)	Namibia	−22.3833	18.4073
Justicia platysepala (S. Moore) P.G. Mey.	*Tripp et al. 6907* (COLO)	Angola	−12.8929	13.4947
Justicia platysepala (S. Moore) P.G. Mey.	*Tripp et al. 6919* (COLO)	Angola	−14.9700	12.9040
Justicia pseudorungia Lindau	*Kiel et al. 185* (RSA)	Kenya	−3.2222	40.1218
Justicia sp. B. of Flora Zambesiaca	*Bester 11112* (K)	Mozambique	−18.5622	34.8731

Table 1. *Cont.*

Taxon	Source Specimen	Country	Latitude	Longitude
Justicia striata (Klotzsch) Bullock	*Kiel et al. 145* (RSA)	Kenya	−1.8082	37.5765
Justicia tetrasperma Hedrén	*Kahurananga et al. 2582* (K)	Tanzania	−6.1994	30.3536
Justicia tricostata Vollesen	*Bidgood et al. 5606* (K)	Tanzania	−8.4500	31.4833
Justicia tricostata Vollesen	*Gillis 11441* (RSA)	Zambia	−15.5470	28.2472
Justicia unyorensis S. Moore	*Kiel et al. 163* (RSA)	Kenya	−2.5514	37.8933
Justicia vagabunda Benoist	*Tripp et al. 1544* (RSA)	China	21.9449	101.2735
Kenyacanthus ndorensis (Schweinf.) I. Darbysh. & C.A. Kiel	*Luke et al. 17084* (K)	Kenya	−0.1499	37.0238
Monechma australe P.G. Mey.	*Tripp et al. 2028* (RSA)	Namibia	−23.7117	17.2600
Monechma bracteatum Hochst.	*Kiel et al. 161* (RSA)	Kenya	−2.5514	37.8933
Monechma bracteatum Hochst.	*Friis et al. 13545* (K)	Ethiopia	11.5285	35.1075
Monechma calcaratum Hochst.	*Tripp and Dexter 2043* (RSA)	Namibia	−25.8755	17.7929
Monechma ciliatum Hochst. ex Nees	*Merklinger 2013-9-55* (K)	Senegal	15.3181	−16.7758
Monechma cleomoides C.B. Clarke	*Klaassen et al. 2530* (K)	Namibia	−21.2978	15.2803
Monechma cleomoides C.B. Clarke	*Tripp et al. 1995* (RSA)	Namibia	−17.8023	12.3261
Monechma cleomoides C.B. Clarke	*Tripp et al. 1960* (RSA)	Namibia	−19.8212	14.1870
Monechma cleomoides C.B. Clarke	*Tripp et al. 1999* (RSA)	Namibia	−17.5193	12.2674
Monechma debile Nees	*Friis et al. 10459* (K)	Ethiopia	13.8167	39.5500
Monechma debile Nees	*Kiel et al. 173* (RSA)	Kenya	−3.3496	38.4483
Monechma depauperatum C.B. Clarke	*Etuge 4446r* (K)	Cameroon	5.0833	9.7167
Monechma desertorum C.B. Clarke	*Oliver et al. 6379* (K)	Namibia	−27.4028	17.3833
Monechma distichotrichum P.G. Mey.	*Tripp et al. 2067* (RSA)	Namibia	−28.0878	19.5131
Monechma distichotrichum P.G. Mey.	*Tripp et al. 2072* (RSA)	Namibia	−27.9074	17.6788
Monechma divaricatum C.B. Clarke	*Tripp and Dexter 808* (RSA)	Namibia	−18.7071	17.2921
Monechma divaricatum C.B. Clarke	*Tripp and Dexter 783* (RSA)	Namibia	−19.5546	17.7329
Monechma divaricatum C.B. Clarke	*McDade et al. 1275* (RSA)	South Africa	−22.8833	29.6667
Monechma divaricatum C.B. Clarke	*Tripp et al. 1970* (RSA)	Namibia	−19.6156	13.2550
Monechma divaricatum C.B. Clarke	*Tripp et al. 2023* (RSA)	Namibia	−23.3475	17.0788
Monechma divaricatum C.B. Clarke	*Tripp et al. 1961* (RSA)	Namibia	−19.8429	14.1279
Monechma divaricatum C.B. Clarke	*Tripp et al. 2029* (RSA)	Namibia	−23.7117	17.2600
Monechma divaricatum C.B. Clarke	*Tripp et al. 2039* (RSA)	Namibia	−26.4395	18.1855
Monechma divaricatum C.B. Clarke	*Tripp and Dexter 4800* (COLO)	Namibia	−20.3351	17.5604
Monechma genistifolium C.B. Clarke	*Tripp and Dexter 775* (RSA)	Namibia	−21.9340	16.6867
Monechma genistifolium C.B. Clarke	*Wanntorp & Wanntorp 339* (K)	Namibia	−21.5125	16.0314
Monechma grandiflorum Schinz	*Tripp and Dexter 2034* (RSA)	Namibia	−24.3024	17.8223
Monechma incanum C.B. Clarke	*Mott 1124* (K)	Botswana	−23.7656	22.8097
Monechma incanum C.B. Clarke	*Puff 780416-2/2* (RSA)	South Africa	−27.9471	22.6925
Monechma leucoderme C.B. Clarke	*Tripp and Dexter 2044* (RSA)	Namibia	−25.8755	17.7929
Monechma leucoderme C.B. Clarke	*Tripp et al. 2083* (RSA)	Namibia	−26.2326	16.5967
Monechma mollissimum (Nees) P.G. Mey.	*Balkwill et al. 11787* (RSA)	South Africa	−28.9489	18.2433
Monechma mollissimum (Nees) P.G. Mey.	*Tripp et al. 2071* (RSA)	Namibia	−27.9231	17.7338
Monechma monechmoides (S. Moore) Hutch.	*Aiyambo et al. 323* (K)	Namibia	−19.4713	17.7469
Monechma monechmoides (S. Moore) Hutch.	*Tripp and Dexter 785* (RSA)	Namibia	−19.4713	17.7469
Monechma monechmoides (S. Moore) Hutch.	*Bingham 11019* (K)	Zambia	−15.1667	27.1667
Monechma ndellense (Lindau) J. Miège & Heine	*Harris & Fay 2150* (K)	C.A.R.	9.1667	23.2167
Monechma rigidum S. Moore	*Goyder 8210* (K)	Angola	−12.5683	16.4931
Monechma salsola C.B. Clarke	*Klaassen et al. 2537* (K)	Namibia	−19.2528	14.0044
Monechma salsola C.B. Clarke	*Klaassen et al. 2544* (K)	Namibia	−19.1944	13.0861
Monechma salsola C.B. Clarke	*Tripp and Dexter 6934* (COLO)	Angola	−14.5999	12.3703
Monechma scabridum S. Moore	*Congdon 584* (K)	Zambia	−11.1664	24.1850
Monechma serotinum P.G. Mey.	*Tripp et al. 4066* (COLO)	Namibia	−17.5117	12.9696
Monechma spartioides (T. Anderson) C.B. Clarke	*Tripp et al. 2064* (RSA)	Namibia	−28.0878	19.5131
Monechma sp.	*Tripp and Dexter 834* (RSA)	Namibia	−17.6070	12.9523
Monechma subsessile C.B. Clarke	*Bidgood et al. 6793* (K)	Tanzania	−6.6167	31.9333
Monechma tonsum P.G. Mey.	*Nyatoro et al. 29* (K)	Namibia	−18.1367	13.8953
Monechma tonsum P.G. Mey.	*Tripp and Dexter 813* (RSA)	Namibia	−18.9546	16.6243
Monechma varians C.B. Clarke	*Synge WC437* (K)	Malawi	−10.3500	33.8833
Monechma virgultorum S. Moore	*Goyder 8471* (K)	Angola	−13.8519	18.2589

1.1. Ecological Importance of Members of Monechma s.l.

Members of *Monechma* s.l. are widespread in sub-Saharan Africa (Figure 2). While a significant number of species of the group occur in fire-prone vegetation corresponding to the savanna biome, the group becomes particularly abundant and diverse (18+ species) in the deserts and shrublands of southwest Africa, centered in southern Angola, Namibia and the Northern Cape region of South

Africa [24], which represents one of the main extensions of the succulent biome in Africa [25] (Figure 3). The tropical succulent biome is less well-known than the tropical savanna biome; both experience seasonality in water availability, but the succulent biome differs from savanna in rarely experiencing fire [26]. Within southwest Africa, *Monechma* frequently forms a major component of the dominant ground flora, often in combination with one or more of three other distantly related lineages in the Acanthaceae family that have diversified independently in this region: *Barleria* L. (Barlerieae) [7], *Blepharis* Juss. (Acantheae) [5] and *Petalidium* Nees (Ruellieae) [6], with both *Barleria* and *Petalidium* represented by over 25 spp. in Namibia alone [27]. The parallel radiation of species in these four genera within the succulent biome in southwest Africa is remarkable, and together result in the Acanthaceae being amongst the most important plant families in the region. In view of the exceptional ecological importance of these genera, it is essential that we have a strong understanding of the species diversity and evolutionary history of these groups. Taxonomic studies of the Namibian radiation of *Monechma* are ongoing as part of the *Flora of Namibia* programme [28]; however, phylogenetic investigation of the evolutionary history of the group has been lacking to date.

Figure 3. The habitat and abundance of *Monechma* in Namibia. (**A**) *M. genistifolium* (bright green) together with *Petalidium englerianum* (Schinz) C.B. Clarke (silver-green) near Outjo (I. Darbyshire); (**B**) *M. tonsum* together with *Petalidium variabile* C.B. Clarke s.l. near Opuwo, collected as *Nyatoro et al. 29* (I. Darbyshire); (**C**) *M. spartioides* c. 30 km W of Ariamsvlei (E.A. Tripp, collected as *Tripp et al. 2064*); (**D**) *M. salsola* near Umbaadjie, collected as *Klaassen et al. 2537* (I. Darbyshire).

Elsewhere in tropical Africa, members of *Monechma* s.l. can be an important constituent of the fire-prone savanna biome of both the Sudanian and Zambesian phytogeographic regions [29], for example *M. depauperatum* (T. Anderson) C.B. Clarke in the Sudanian region and *M. scabridum* in the Zambesian region. Other species such as *M. bracteatum* and *M. monechmoides* favour open habitats with high light availability and so can be common in disturbed, ruderal environments.

1.2. Aims of the Present Study

The present study intends to reconstruct evolutionary relationships within *Monechma* s.l. in the context of the wider classification of the Justicioid lineage and towards understanding the diversification of this ecologically important lineage. A RADseq phylogenetic approach is used in light of the considerable success that this method has provided in resolving phylogenetic relationships within other major lineages of Acanthaceae, including *Petalidium* [6], *Louteridium* S. Watson [30], Ruellieae [31], *Barleria* [32] and New World *Justicia* [33]. The sampling of species of *Monechma* s.l. is here expanded to include ca. 75% of the accepted taxonomic diversity and, in many cases, to include multiple accessions per species with the goal of assessing reciprocal monophyly of such lineages. Specifically, we aim to (a) test prior delimitation of the two clades of *Monechma*; (b) identify and/or confirm morphological traits that diagnose the recognised clades; (c) present a first assessment of the biogeographical history of the genus; (d) place all known species of *Monechma* s.l. into a taxonomic context through a combination of molecular and morphological evidence; and (e) provide a phlyogenetic framework to assist with ongoing and future monographic and floristic work on *Monechma* s.l. and allies in the Justicioid lineage.

2. Materials and Methods

2.1. Sampling

In total, 80 accessions were sampled. Of these, 59 accessions represent 32 of the total 42 species (76%) currently accepted in *Monechma* or in *Justicia* sect. *Monechma*, plus three taxa that are unidentified to species or represent currently undescribed species. The sampling was designed to capture the full range of morphological variation within *Monechma* s.l. as well as to include two or more accessions of morphologically variable species wherever possible. To help delimit broader-scale relationships, we also included 29 accessions spanning major clades of the Justicioid lineage [13]. *Justicia pseudorungia* Lindau of the *Rungia* clade [13] was used as an outgroup for rooting our phylogenetic hypothesis. Leaf tissue for molecular analyses was sampled from either field-collected plant material dried in silica gel or herbarium specimens. Table 1 includes taxon names, source locality and voucher number for all accessions used in this study excluding the removed samples (see Section 2.3); these are mapped on Figure 2.

2.2. DNA Isolation and Sequencing Methods

ddRADseq data (double digest restriction-associated DNA) were used to reconstruct phylogenetic relationships among *Monechma*. At the University of Colorado (Boulder, CO, USA) and Rancho Santa Ana Botanic Garden (RSABG) (Claremont, CA, USA), DNA was extracted from dried leaf tissue using a CTAB protocol [34]. ddRAD libraries were constructed at RSABG using a modified version of that used in [6], which was originally adapted from [35]. A full description of this protocol is published in [6], with details briefly outlined here. All genomic DNA was normalized to ~30 ng/µL before digestion and library construction. Extracted DNA underwent double restriction enzyme digestion using *EcoRI* and *MseI* for 3 h at 37 °C followed by 65 °C for 45 min. Illumina sequencing oligos together with in-line, variable-length barcodes were annealed to the *EcoRI* cut site and ligated onto digested fragments. Illumina oligos were similarly annealed to the *MseI* cutsite. Barcoded ligation products were pooled and cleaned using a Qiagen gel extraction kit. We excised fragments from the gel between 200−700 bp to reduce the effects of dimer and to provide more precise amplification of the targeted region. The gel-purified ligations were amplified using the following PCR reaction: 8.6 µL of water, 4 µL of Phusion HF buffer, 0.5 µL of each Illumina primer (10 µM), 0.6 µL DMSO, 0.6 µL DNTPs, 0.2 µL Phusion. Fifteen cycles of PCR were conducted to amplify the cleaned, ligated products. The reaction was repeated once to ameliorate stochastic differences in PCR amplification. Agarose gels were used to assess amplification and size of the PCR products and amplicon concentrations were evaluated using a Qubit fluorometer 2.0. The custom-tagged products of the PCR reactions were pooled and sent to the University of Colorado's Biofrontiers Next-Gen Sequencing Facility for quality control and further

size selection. BluePippin was used to select a fragment range between 200 and 500 bp to reduce the sequenced genome. Libraries from the 80 samples were pooled to yield a final combined library that was submitted for 1 × 75 sequencing on an Illumina NextSeq v2 High Output Sequencer at Biofrontiers.

2.3. Phylogenetic Reconstruction

We assessed sequencing quality of raw data using FastQC [36]. Data were filtered, trimmed, and demultiplexed using iPYRAD 0.9.31 [37,38]. Of the 80 taxa sampled, four accessions—*Monechma* sp. (specimen: *Tripp et al. 1985*), *Rhinacanthus angulicaulis* I. Darbysh. (*Kiel et al. 170*), *Justicia flava* (Forssk.) Vahl (*Kiel et al. 146*) and *Justicia striolata* Mildbr. (*Congdon et al. 794*)—were removed because of too few loci (i.e., values < 40). Information on the number of ddRAD reads per sample and loci in the assembly for each accession sampled in our study are provided in Table S1. As a result, our final sampling contained 76 accessions, which included 58 accessions of *Monechma* representing 34 taxa (32 accepted species). Of these taxa, 13 were represented by two or more accessions to account for species with broad geographical distributions and/or variation in morphology (Table 1). The de novo assembly parameters for our final dataset are as follows: the minimum required sequence length (to retain a read) = 35 bp; minimum coverage for retaining a cluster = 6; maximum low quality bases = 5; clustering threshold (level of sequence similarity in which two sequences are identified as homologous) = 0.90; minimum number of samples that must have data at a given locus to be retained = 20; maximum number of alleles per site in consensus sequence = 2. We also conducted 3 additional de novo assemblies exploring the number of minimum samples required to retain a locus (i.e., 4, 10, 30). The final RADseq phylogenomic dataset is available in Sequence Read Archive (SRA) under the BioProject number PRJNA635173.

2.4. Phylogenetic Analyses

We implemented two approaches for estimating phylogenetic relationships among *Monechma* s.l.: (1) a Maximum Likelihood (ML) analysis using the concatenated RAD sequence data from all loci derived from the iPYRAD [37,38] assembly and (2) a coalescent-based approach using quartet-based phylogenetic inference under a multispecies coalescent theory framework that used the concatenated RAD sequence data described above, but randomly sampled one SNP per locus. We conducted our ML analyses using IQ-TREE 1.6.10 [39]. The best model of nucleotide substitution and across-site heterogeneity in evolutionary rates was inferred using ModelTest-NG 0.1.5 [40]. The best-fit model was selected based on the corrected Akaike's information criterion. Node and branch supports were obtained from 1000 nonparametric bootstrap replicates under the best inferred model (GTR + G). We constructed quartet-based coalescent phylogenetic inferences using the program Tetrad [41] in iPYRAD [37,38] and assessed node support with 1000 bootstraps. The SVDquartets algorithm [42], implemented in Tetrad [41], uses multi-locus unlinked SNP data to infer the topology among all possible subsets of four samples under a coalescent model. The resulting set of quartet trees are combined and constructed into a species tree. Because the underlying model assumes that the examined SNPs are unlinked, Tetrad subsamples a single SNP from every locus separately for every quartet set in the analysis from the .snps.hdf5 file produced from the iPYRAD output and repeats this subsampling method independently in each bootstrap replicate. This method maximizes the number of unlinked SNP information in the analysis. For both ML and Tetrad analyses, we considered branches to be supported when bootstrap values were >90%, while bootstrap values < 70% were considered unsupported.

2.5. Hypothesis Testing

Six alternative phylogenetic hypotheses were examined using the Shimodaira Approximately Unbiased (AU) tests [43]. Constraint trees were constructed in Mesquite v.2.72 [44]. For each constraint, all aspects of relationships were constructed as a single polytomy, with the exception of the hypothesis under consideration. The constraint trees were loaded into IQ-TREE [39] and run with the settings and model as described above. The best trees from the unconstrained and constrained analyses were

combined into a single file and loaded into IQ-TREE and likelihood scores were compared using the AU test with RELL-optimization and 10,000 bootstrap replicates.

2.6. Divergence Time Estimation

To provide temporal context to the evolutionary history of *Monechma* and close relatives, we estimated divergence times using the most likely tree from our concatenated ML analysis. We pruned this tree to contain a single representative for each ingroup taxon, resulting in a total of 49 species. The singleton tree was rate-smoothed and ultrametricized using penalized likelihood under a relaxed model, where rates are uncorrelated across branches [45] as implemented with the *chronos* function in ape v 5.1 [46] of R v 3.6.0 ("Planting of a Tree") [47]. A best-fit smoothing parameter (lambda) of 1.0 was selected following the cross-validation approach and chi-square test as implemented in treePL [48], testing eight values between 0–1000 distributed on a log-scale. A single fossil calibration for a minimum age date of 11.5 my was used to constrain the most recent common ancestor of the Justicioid lineage. This fossil was previously assessed as both reliably identified and dated [49]. Fossil #32 [49] from the Middle Miocene is a dicolporate pollen grain with distinctive round insulae that laterally flank the apertures [50]; the latter of these traits is known only among Justicioids [13,14,51]. We also used a 35 my maximum date for our calibration, which is the estimated age for Justicieae as a whole [49].

2.7. Biome Evolution and Climatic Niche

We reconstructed an ancestral biome state of lineages to elucidate the history of biome occupancy and biome switching in *Monechma* s.l. For all taxa in our ultrametric tree, species presence/absence in four biomes was determined based on ecoregions [52], as follows: (1) tropical and subtropical grasslands, savannas and shrublands (hereafter savanna biome); (2) deserts and xeric shrublands (hereafter succulent biome); (3) tropical and subtropical broadleaf forests (hereafter forest biome), and (4) montane grasslands and shrublands (hereafter montane biomes). Ancestral state reconstructions were implemented with the *rayDISC* function in the corHMM 1.13 package [53]. This function assumes a constant rate of evolution across all branches and permits polymorphic character states that account for the probability of either state when calculating the likelihood at ancestral nodes. We compared two distinct Markov models of discrete character evolution: the equal rates (ER) or Mk model, which assumes a single rate of transition among all possible states, and the all rates different (ARD) or the AsymmMk model [54,55], which allows different rates for each possible transition. We also examined a symmetrical model (SYM), which specifies equal rate transitions in either direction between pairs of states but permits different rates between different pairs. Model fit was tested by comparing AICc values, from which we selected the model that best fits the data while minimizing the number of parameters [56].

Given asymmetrical patterns of standing diversity in *Monechma* s.l., specifically far greater species richness and abundance in southwestern portions of the range of this lineage, we sought to delimit climatic niche preferences among species throughout the range. We first downloaded 19 WorldClim Bioclimatic variables available in the WorldClim database [57] at 30 arc-seconds resolution [58]. We then extracted bioclimatic data for taxa in our ultrametric tree using latitude and longitude of collections in the R package raster [59]. We visualized changes in two climatic variables: BIO7 = temperature annual range (BIO5 − BIO6: minimum temperature of the warmest month − minimum temperature of the coldest) and BIO12 = annual precipitation (mm), using the *contmap* function in the package phytools [60]. The mapping is accomplished by estimating ancestral states at internal nodes using ML with the *fastAnc* function and then interpolating the states along each edge using Equation (2) of [61]; see [62].

2.8. Morphological Studies

A survey of morphological traits that have been found to be taxonomically informative in past studies of both *Monechma* s.l. and the wider Justicioid lineage was conducted for all relevant taxa

in order to interpret results of the RADseq analyses. We focused on the following morphological traits: plant habit, inflorescence form, details of the androecium including arrangement of anther thecae and details of the staminal appendages, pollen morphology, and seed number, size, shape and indumentum. Most observations were made on herbarium specimens held at K, RSA and COLO (herbarium abbreviations follow [63]) but with additional observations made via access to digital images of type specimens on JSTOR Global Plants [64] and other online repositories of herbarium specimen images. For pollen morphology, unacetolyzed pollen from selected taxa was mounted on aluminum stubs using double-sided sticky tape and coated with gold using a PELCO SC-7 system (Ted Pella, Redding, CA, USA). The coated samples were observed at 10 kV on a Hitachi SU3500 (Hitachi, Tokyo, Japan) scanning electron microscope (SEM) at Rancho Santa Ana Botanic Garden. Chromosome number was also considered through reference to relevant cytological studies.

The geographic distribution of each accepted taxon was delimited using the Level 3 codes of the TDWG geographic scheme for recording plant distributions [65].

3. Results

3.1. Phylogenetic Results

The phylogenies inferred using ML for each of the four concatenated data sets were congruent despite variation in the proportion of missing data (Figure 4, Figures S2–S4). The datasets containing more missing data (i.e., larger alignment files with lower min tax values) yielded similar or identical topologies to the datasets containing fewer missing data (i.e., smaller alignment files with higher min tax values; Figures S2 and S3). However, topologies of the latter, in particular the dataset with minimum samples per locus = 30, had lower bootstrap supports for relationships along the backbone of the phylogeny (Figure S4). We here present the results of the concatenated dataset with the minimum samples per locus set at 20 (Figure 4), which contained 5718 loci and 468,892 SNPs. We chose this assembly because it contains the least amount of missing data without losing resolution (see results from Tetrad analysis, below) while also maximizing the amount of genome data utilized. The coalescent analysis (Figure S5) using the final genotype matrix from the de novo assembly (468,892 SNPs and 20,000,000 quartet sets) resulted in a similar species-level topology to that inferred from the concatenated ML analysis of data. However, the resulting topology inferred from the Tetrad analysis exhibits low resolution along the backbone and thus ambiguous relationships among major clades. Overall, there were no strongly supported topological conflicts between the ML vs. Tetrad analyses (Figure 4 and Figure S5).

Overall, the phylogenetic results from all analyses concur with the findings of the earlier studies [13] (Figure S1) that *Monechma* s.l. is polyphyletic and that species previously placed in this genus (or in *Justicia* sect. *Monechma*) are resolved in one of two clades, with the exception of *M. varians* (see below). Our data reject strict monophyly of *Monechma* s.l. ($p < 0.001$; Table 2).

Monechma Group I (ML: 100% BS; Figure 4) was resolved in the *Harnieria* clade of the Justicioid lineage and is here composed of six species (*M. bracteatum*, *M. debile*, *M. monechmoides*, *Justicia eminii*, *J. tetrasperma* and *J. sp. B* of Flora Zambesiaca; [18]). This clade is sister to *Justicia odora* of *Justicia* sect. *Harnieria*, and these together are sister to the other four sampled members of sect. *Harnieria*. Results from an AU test do not reject the monophyly of *Justicia* sect. *Harnieria* (i.e., *J. unyorensis*, *J. heterocarpa*, *J. striata*, *J. phyllostachys* and *J. odora*; $p = 0.393$). Species of *Monechma* Group I for which two or more accessions were sampled (i.e., *M. debile*, *M. bracteatum*, *M. monechmoides*) were each resolved as reciprocally monophyletic (Figure 4).

Monechma Group II (ML: 98% BS; Figure 4) was resolved as a sister to all sampled members of the Diclipterinae clade (i.e., two species each of *Dicliptera* and *Hypoestes*; *Justicia vagabunda* and *Kenyacanthus ndorensis*). *Monechma* Group II is here composed of 26 species and includes two major clades. The first clade (ML: 100% BS; Figure 4) consists of six tropical African species (*M. ciliatum*, *M. depauperatum*, *M. ndellense*, *M. scabridum*, *M. subsessile* and *Justicia attenuifolia*). The second major

clade (ML: 100% BS; Figure 4) contains the remaining 17 sampled species of tropical and southern African *Monechma*, primarily those of the succulent biome radiation, in addition to *Justicia fanshawei*, *J. cubangensis*, and *J. tricostata*, all of which were described in *Justicia* sect. *Monechma*. Most species of *Monechma* Group II for which two or more accessions were sampled (i.e., *M. distichotrichum*, *M. divaricatum*, *M. genistifolium*, *M. incanum*, *M. leucoderme*, *M. mollissimum* and *M. salsola*) were each resolved as reciprocally monophyletic (Figure 4). Sampled accessions of *Monechma cleomoides* and *M. tonsum* were not resolved as reciprocally monophyletic and instead were resolved as part of a clade containing *M. genistifolium*, *M. australe*, and *M. salsola*. Results of an AU test also reject the monophyly of *M. cleomoides* and *M. tonsum* ($p < 0.001$; Table 2).

Monechma varians was here resolved as sister to *Justicia kirkiana* of the *Tyloglossa* clade (ML: 96% BS; Figure 4). Together, these taxa are sister to Core Diclipterinae + *Monechma* Group II and the *Harnieria* clade, but with weak support (ML: 80% BS; Figure 4).

Justicia platysepala, which was originally placed in *Monechma* (*M. platysepalum* S. Moore) but more recently has been included in *Justicia* [26,66], was here resolved in a clade consisting of *J. anagalloides* and *J. cordata* (ML: 94% BS; Figure 4). This clade is sister to all other sampled in-group taxa in our dataset.

Figure 4. The most likely phylogenetic hypothesis for relationships among *Monechma* s.l. generated from ddRADseq loci. *Monechma* s.l. is not monophyletic and is resolved in two major clades: *Monechma* Group I (grey box) and *Monechma* Group II (blue box). The type species, *Monechma bracteatum*, is denoted in Group I. Collection numbers are listed after species names where multiple accessions were sampled. Asterisks [*] indicate 100% ML bootstrap and dashes [-] indicate <70% ML bootstrap.

Table 2. Results of alternative phylogenetic hypothesis testing using the Shimodaira Approximately Unbiased (AU) test among *Monechma*. H0 = results from present study; H1 = alternative hypotheses based on earlier classification or morphological patterns.

Hypothesis	logL	D (logL)	Reject?	*p*-Value
H0. *Monechma* s.l. is not monophyletic	−1201,860.278			
H1. *Monechma* s.l. (excluding *M. varians*) is monophyletic	−1209,690.731	7830.5	Yes	<0.0001
H0. *Justicia* sect. *Harnieria* is not monophyletic	−1201,860.278			
H1. *Justicia* sect. *Harnieria* is monophyletic, i.e., *Monechma* Group I is not embedded within this section	−1201,862.068	1.7897	No	0.393
H0. *M. ciliatum* is a member of *Monechma* Group II	−1201,860.278			
H1. *M. ciliatum* is not a member of *Monechma* Group II	−1208,510.036	6649.8	Yes	<0.0001
H0. *M. cleomoides* + *M. tonsum* is not monophyletic	−1201,860.278			
H1. *M. cleomoides* including *M. tonsum* is monophyletic	−1203,255.026	1394.7	Yes	<0.0001
H0. *M. cleomoides* is not monophyletic	−1201,860.278			
H1. *M. cleomoides* is monophyletic	−1204,005.563	2145.3	Yes	<0.0001
H0. *M. tonsum* is not monophyletic	−1201,860.278			
H1. *M. tonsum* is monophyletic	−1203,255.026	1394.7	Yes	<0.0001

3.2. Divergence Times

Our divergence time analyses using penalized likelihood estimated that *Monechma* Group I plus *Justicia odora* of the *Harnieria* clade originated around 22 mya (stem group) and began to diversify around 18 mya (crown), with *Monechma* Group I specifically diversifying at approximately 12.3 mya (crown; Figure 5). Our analyses estimate that *Monechma* Group II originated around 22.5 mya (stem) and began diversifying around 13.4 mya. Within Group II, however, the succulent biome radiation is estimated to have begun diversifying as recently as 1.9 mya (Figure 5).

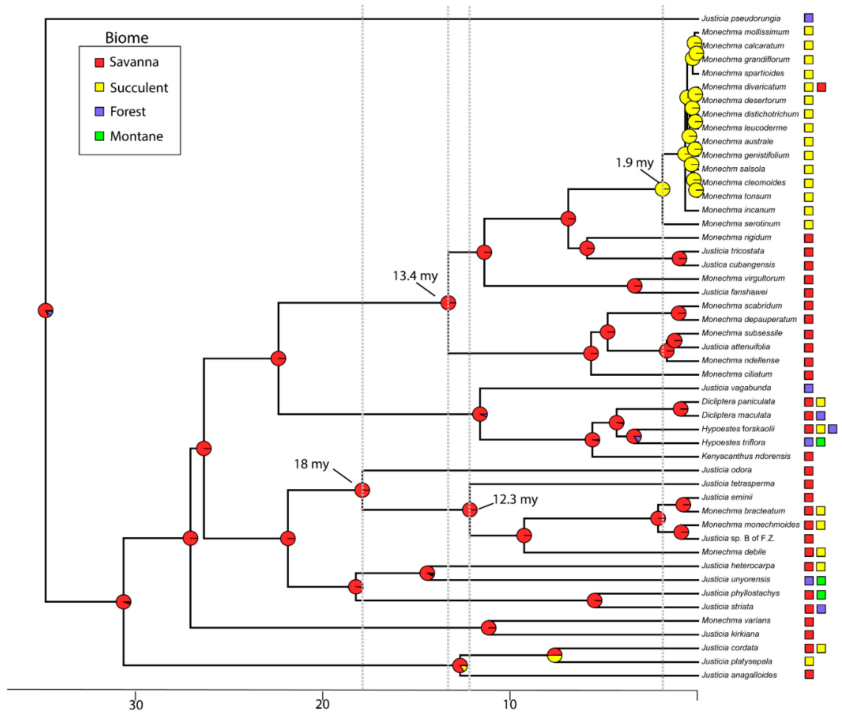

Figure 5. Divergence time estimation from penalized likelihood and biome evolution for *Monechma* Groups I and II. Ancestral state reconstruction: circles at nodes are color coded to reflect ancestral character states with sizes of differently colored wedges indicating likelihood of presence of each state at that node.

3.3. Biome Evolution and Climatic Niche

In our analyses examining transitions among biomes, the common ancestor of the *Harnieria* clade + *Monechma* Group I was most likely distributed in savannas (ER model based on AICc values; Figure 5; Table S2). The ancestor of *Monechma* Group II similarly most likely occupied savannas, with subsequent shifts into the succulent biome including deserts and xeric shrublands (Figure 5). Throughout *Monechma* s.l. and allies, our results suggest there have been rare shifts to tropical forests and montane environments from savanna ancestors (Figure 5).

Ancestral state reconstruction of climatic variables suggests marked shifts in temperature and precipitation regimes during evolution of the succulent biome radiation of Group II. Species in this group (i.e., the "*cleomoides*" clade) have diversified into drier habitats with greater ranges of temperature extremes in comparison to the others in Group II (Figure 6) as well as the remainder of sampled species in our analyses, including those of *Monechma* Group I.

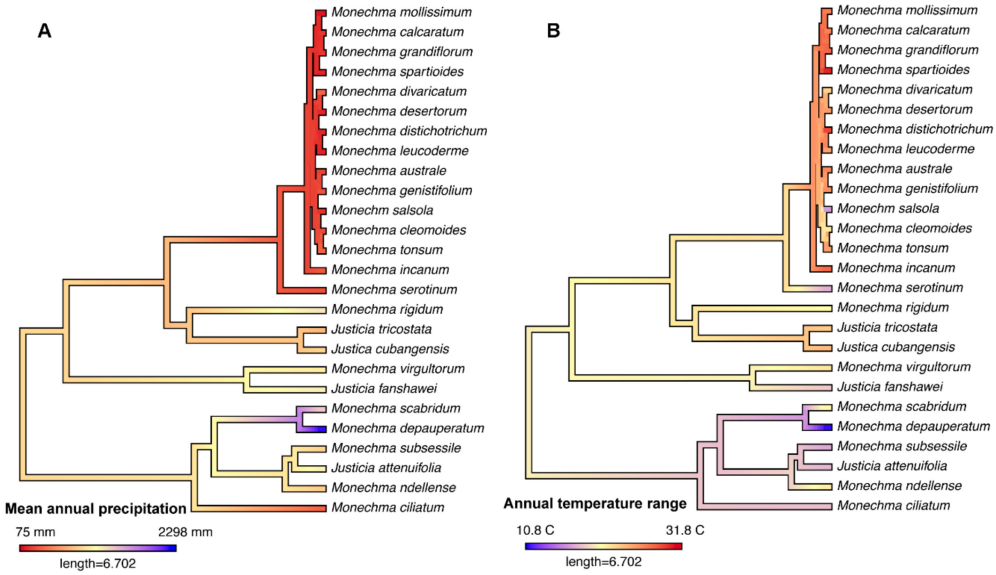

Figure 6. Visualization of WorldClim variables along the nodes and branches of *Monechma* Group II. (**A**) mean annual precipitation (BIO12); (**B**) temperature annual range (BIO7). Species with red color represent species from (**A**) drier climates with a (**B**) greater range of temperature extremes. Raw values were plotted using the *contMap* function in package phytools.

3.4. Taxonomically Informative Morphological Traits

Our analyses indicated inflorescence form and seed morphology are the most informative morphological characters for separation of *Monechma* Groups I and II. Characters that were found not to be diagnostic for these two clades were morphology of the corolla and androecium and pollen type. Plant habit is not diagnostic but was found to be closely aligned to the phytogeography and ecology of the species within these two clades. Further discussion of results of our morphological survey are presented in the discussion below.

4. Discussion

4.1. Ecology and Biogeography of Monechma Groups I and II

Plants of *Monechma* Group I are slender, annual or perennial herbs with brittle leafy stems. In *J. tetrasperma* and sometimes in *J. eminii*, mature plants can be somewhat shrubby with a woody rootstock but they still retain brittle, slender stems. Species of this clade typically favour open habitats with moderate to high light availability, often in areas that regularly burn and can be considered part

of the savanna biome. Several of the species, such as *M. bracteatum*, *M. debile* and *M. monechmoides*, favour disturbed, ruderal habitats although they do not become troublesome weeds. Both the range in growth habit and the favoured habitat types observed in *Monechma* Group I is closely similar to that in *Justicia* sect. *Harnieria* to which *Monechma* Group I is closely allied (see Section 4.2.2 below).

Plants of *Monechma* Group II vary considerably in growth form (see Section 4.2.3), which is again linked closely to ecology. Most species in this clade are perennial herbs or shrublets but *M. ciliatum*, *M. desertorum* and some forms of *M. divaricatum* are annual herbs, though the latter two can be much-branched. Species of the fire-prone savanna biome in the Sudanian and Zambesian phytogeographic regions [29] are typically perennial herbs that produce fertile shoots from a woody base and rootstock that are burnt back during the dry season (similar to geoxylic suffrutices). The exception is *M. ciliatum*, which does not perennate. Species from drier, non-fire prone habitats, particularly in the deserts and xeric shrublands comprising a major extension of the succulent biome in southwest Africa, are most often shrublets with intricate branching (Figure 3).

The majority of species in *Monechma* s.l. occur in the savanna biome and this is reconstructed as the ancestral biome state of the lineage (Figure 5). Our analyses suggest the origin of the lineage at ~31 mya, but the savanna biome is thought to have originated 5–10 mya with the spread and increased dominance of fire-prone C4 grass lineages [67,68]. Indeed, in Africa, phylogenetic evidence suggests that the origin of most lineages of 'underground trees' (geoxylic suffrutices) that place their woody biomass underground to protect it from fire dates to within the last 2 myrs, after the origin and spread of the savanna biome [69]. Either previous studies have grossly inaccurately dated the timing of the origin of the fire-prone savanna biome or *Monechma* s.l. originated in some other biome, with most lineages subsequently shifting to the savanna biome once that biome as we now know it originated; c.f. [70] (Figure 5b). Under the latter scenario, the previous biome(s) occupied by *Monechma* s.l. may have no modern-day analogue, while species in the lineage may have possessed traits that predisposed them to successfully colonise the savanna biome; c.f. [71].

While figuring out the exact timing of colonisation of the savanna biome by *Monechma* s.l. may require further paleobotanical and geological evidence, it seems likely based on our analyses that the ancestors of most extant species were found in savanna except for the conspicuous radiation of >15 species in *Monechma* Group II within the succulent biome (Figure 5). The latter clade, predominantly composed of species in Namibia and neighbouring countries, may have originated as early as ~7 mya (stem age), but seems to have begun substantial diversification within the last one million years (crown age for clade comprising 14 of the 15 species phylogenetically sampled in the clade) (Figure 5). The recency of this radiation is reminiscent of radiation of a distantly related genus of Acanthaceae, *Petalidium*, which similarly has undergone very recent radiation in the succulent biome in southwest Africa, with 39 species originating in the last 0.5 myrs [6]. Indeed, it has been argued that arid environments can facilitate rapid diversification of plant lineages [72], including a suggestion that such has been the case for *Monechma* in this region [24]. These results are surprising, however, as the Namib Desert, which forms the core of the distribution of the succulent biome in southwest Africa, is thought to be among the oldest deserts on Earth, dating to at least 55–80 mya [73]. Clearly, further research, phylogenetic and otherwise, is needed to understand the biogeographical history of this understudied, yet biologically unique region.

4.2. Taxonomic Implications

4.2.1. Morphological and Cytological Traits for the Separation of *Monechma* Groups I and II

Our results confirm that *Monechma* s.l. is a non-monophyletic assemblage of species from two widely separated clades within the Justicioid lineage. In line with earlier studies [13], *Monechma* Group I is nested within the Core *Harnieria* clade whilst *Monechma* Group II is allied to the Diclipterinae clade (Figure 4). However, the constituent species of the two clades of *Monechma*, as revealed by our detailed sampling, do not concur with that interpreted from the limited sampling in earlier studies [13,22].

Specifically, the majority of newly sampled species from tropical Africa with primarily terminal inflorescence spikes bearing the bracts ± highly differentiated from the leaves were not resolved among the *Monechma* Group I clade (Figure 4), as was inferred by those earlier studies [13,22]. Instead, they are resolved in a series of clades within *Monechma* Group II, which is otherwise made up of predominantly southern African species with ± undifferentiated bracts. Thus, the enumeration of *Monechma* Group I in Angola [22] includes taxa from two clades. In light of the present results, the morphological grounds for the separation of the *Monechma* Group I and Group II requires reassessment.

Although quite variable across *Monechma* s.l., growth habit is not diagnostic for separation of these two clades, instead being more closely linked to ecology, which varies particularly within Group II (see Section 4.1) (Figures 5 and 6). Similarly, we do not find traits in the corolla and androecium to be informative. In fact, these traits are remarkably uniform across *Monechma* Group I and II and also across allied taxa in the wider Justicioid lineage, for example in the *Harnieria* and *Tyloglossa* clades. All have the combination of a short and relatively broad corolla tube with tube length usually ≤ the lips (rarely tube > lips e.g., in *M. grandiflorum*) and with prominent transverse ridges ("herring-bone" patterning) on the lower lip (Figure 1); and anthers with offset and often oblique thecae with a prominent pale appendage on the lower theca. In both *Monechma* Groups I and II, the appendage is often bifurcate or even trifurcate at the apex, but this is inconsistent and anthers on the same plant can have entire and divided appendages.

Pollen morphology is often informative in the classification of Acanthaceae [13,14,74], hence pollen morphology has previously been reviewed across the Justicioid lineage [13]. That study found that members of the *Harnieria* clade, including *Monechma* Group I, have bicolporate pollen with each aperture flanked by lines of insulae; see [13] (Figure 11C, D, G and H). The same pollen type was found in the only sampled member of *Monechma* Group II, *M. divaricatum*. We examined one additional species from *Monechma* Group I and two additional species from Group II and further confirm these results (Figure 7). Therefore, pollen type does not distinguish between the two Groups. However, it is noteworthy that this pollen type in Group II is different from other sampled members of Diclipterinae in which the pollen is usually tricolporate-hexapseudocolpate, although in *Rhinacanthus virens* (Nees) Milne-Redh. the pollen is tricolporate with insulae; see [13] (Figure 11).

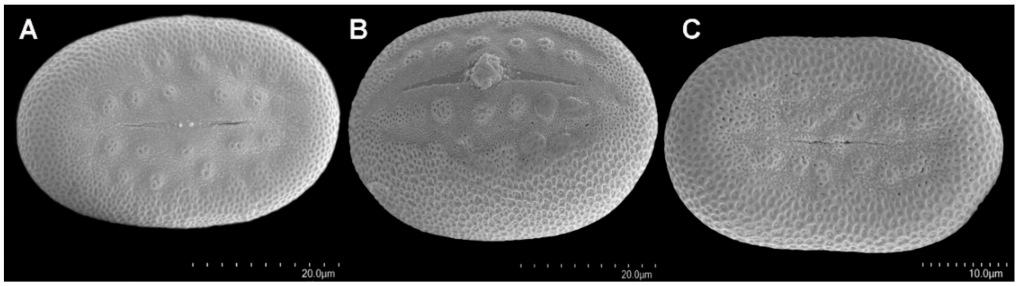

Figure 7. Pollen morphology of *Monechma* Groups I and II. (**A**) and (**B**) *Monechma* Group II: (**A**) *M. leucoderme* (source specimen: *Tripp et al. 2083*, RSA); (**B**) *M. incanum* (*Puff et al. 780416*, RSA). (**C**) *Monechma* Group I: *M. bracteatum* (*Kiel et al. 142*, RSA).

Inflorescence morphology varies considerably within the Justicioid lineage and has been used as an important character in past classification schemes, e.g., [51]. There is considerable variation in inflorescence form in *Monechma* s.l. [19], but based on results presented herein, separation of the two clades using this character is not as straightforward as that proposed in earlier studies [13,22]. In *Monechma* Group I, the flowers are held in axillary or mixed axillary and terminal spikes in which the bracts are highly differentiated from the leaves and are ± broadly elliptic, ovate or obovate (Figure 1A–D). Only in rare cases in Group I is the terminal spike longer than the axillary spikes (or more rarely axillary spikes are absent, e.g., in some specimens of *M. debile*). Each inflorescence unit within the spike can have a single flower, but often contains two or more flowers. The inflorescence

arrangement of *J. tetrasperma* has previously been analyzed in detail [19]. That study found that the dichasial units in the proximal portion of the spike have two pairs of bracts, the upper pair of which are highly uneven in size (i.e., three conspicuous bracts and one inconspicuous bract), while units in the distal portion of the spike have only one ± equal pair of bracts. This arrangement is not observed in *Monechma* Group II where the inflorescences are simpler: most species have single-flowered (rarely 2-flowered) inflorescence units per bract, with either one or two inflorescence units per node. In most species from the succulent biome of southern Africa (e.g., *M. cleomoides*, *M. divaricatum* etc.), the bracts are ± undifferentiated from the leaves and so the flowers are axillary (Figure 1K–S,U–X), although, in some species (e.g., *M. genistifolium*, Figure 1T), they form weakly defined terminal spikes. Most species from savanna biome in tropical Africa have bracts that are clearly differentiated from the leaves and have flowers that are held in well-defined terminal spikes, occasionally with additional, usually shorter spikes in the distal-most leaf axils (Figure 1E–I). The only known exception to this is *J. fanshawei*, which has short axillary and terminal spikes (primarily the former). Species of Group II with well-defined spikes typically have bracts that are proportionately narrow and linear or lanceolate. *Justicia kasamae* Vollesen from Zambia (not sampled in our RADseq analysis but included in Group II on the basis of morphology) is an exception, having imbricate bracts that are broad and elliptic to obovate [18].

Capsules of both clades of *Monechma* typically have only two seeds developed due to early abortion of the upper two ovules [19], although all four ovules mature in *J. tetrasperma* of *Monechma* Group I. Seeds are uniform in *Monechma* Group I, being small, 2–3 mm in diameter [17,18], lenticular with a sharp rim and mottled surface, ± symmetrical in cross section and lacking a ridge on one surface, and are glabrous (Figure 8A–C). Seeds of *Monechma* Group II are much more varied in terms of size, shape, surface characteristics and indumentum (Figure 8D–J); this is discussed in more detail in Section 4.2.3. In summary, the seeds of many species in Group II are larger than in Group I and/or they are less strongly compressed with a more rounded rim; they are often asymmetric in a cross section and often have a ± conspicuous longitudinal ridge on one side. Seed colour varies from black to mottled grey or sometimes (e.g., in *M. divaricatum*; Figure 8I) intricately patterned and coloured. Seeds can be glabrous or can have trichomes (Figure 8D,E). Critically, those species of Group II with small, lenticular, glabrous seeds (e.g., *M. desertorum*, Figure 8H) are ± markedly asymmetric in a cross section, with one surface convex and the other often flat or even slightly concave, and have a conspicuous ridge on one side, quite unlike those of Group I.

Chromosome number has also been found to vary considerably across Acanthaceae [75–78]. However, as far as is known, very few chromosome counts are available for *Monechma* s.l. Within *Monechma* Group I, two independent counts of $2n = 28$ have been reported for *Justicia debilis* Lam. (=*M. debile*) [79]. Counts of $2n = 26$ and $2n = 28$ are common in *J.* sect. *Harnieria* to which *Monechma* Group I is closely allied (and also other Old World *Justicia*), but the count within that clade is variable and some species have $2n = 40$ (–50) [76]. Within *Monechma* Group II, a count of $n = 11$ was recorded for *M. ciliatum* [75]. This differs notably from the count of $n = 15$, which is otherwise characteristic of the Diclipterinae clade [13]. Further studies are required to confirm the consistency of the chromosome counts within, and differences between, the two *Monechma* clades.

In summary, the morphological and cytological differences between the two widely separated clades of *Monechma* are subtle and diagnosis is somewhat hindered by significant morphological variation within each clade, particularly among Group II. However, differences in inflorescence form and seed characteristics, potentially together with differences in chromosome number, are informative in separating these two clades. The constituent species for these two clades are listed in Table 3, with those species not sampled in the RADseq phylogeny being placed based on their morphology.

Figure 8. Seeds of *Monechma* Groups I and II. (**A**–**C**) *Monechma* Group I: (**A**) *M. debile* (source specimen: *Gatheri et al. 79/5*, Kenya); (**B**) *M. bracteatum* (Kisena 1401, Tanzania); (**C**) *Justicia eminii* (*Bidgood et al. 3486*, Tanzania). (**D**–**J**) *Monechma* Group II: (**D**) *M. scabridum* (*Fanshawe F46*, Zambia); (**E**) *M. ciliatum* (*Dere F.H.7047*, Ghana); (**F**) *M. virgultorum* (*Frisby & Maiato 4183*, Angola); (**G**) *J. tricostata* (*Bidgood et al. 3450*, Tanzania); (**H**) *M. desertorum* (*Kolberg & Tholkes HK2038*, Namibia); (**I**) *M. divaricatum* (*Tripp & Dexter 2023*, Namibia); (**J**) *M. mollissimum* (*Salter 1436*, South Africa). All specimens at K.

Table 3. Currently accepted species in *Monechma* s.l., their placement in *Monechma* Groups I and II and their distribution. ˆ denotes that the species has been sampled in the current RADseq phylogeny. Species that were not sampled have been placed based on morphology; placement of these taxa in the clades of *Monechma* Group II should be considered provisional. Combinations in *Monechma* are used wherever available, in preference to combinations in *Justicia*. Geographic range follows TDWG Level 3 codes [65].

Clade	Constituent Species	Distribution
Monechma Group I	ˆ*Monechma bracteatum* Hochst.	Africa: ANG, BOT, ERI, ETH, KEN, MLW, MOZ, NAM, NAT, SOM, SUD, TAN, TVL, UGA, ZAI, ZAM, ZIM; Asia: IND, OMA, YEM
	ˆ*Monechma debile* (Forssk.) Nees	Africa: DJI, ERI, ETH, KEN, SOM, SUD, TAN; Asia: SAU, YEM
	ˆ*Monechma monechmoides* (S. Moore) Hutch.	Africa: ANG, BOT, MLW, MOZ, NAM, TVL, ZAM, ZIM
	Justicia carnosa Hedrén	Africa: SOM
	ˆ*Justicia eminii* Lindau	Africa: BUR, MLW, RWA, TAN, UGA, ZAI, ZAM
	ˆ*Justicia tetrasperma* Hedrén	Africa: TAN, ZAI, ZAM
	ˆ*Justicia* sp. B of Flora Zambesiaca	Africa: MOZ
	Justicia sp. C of Flora Zambesiaca	Africa: ZAM
Monechma Group II		
"*ciliatum/scabridum*" clade	ˆ*Monechma ciliata* (Jacq.) Milne-Redh.	Africa: BEN, BKN, BUR, CAF, CHA, CMN, GAM, GHA, GNB, GUI, ETH, IVO, MLI, MLW, NGA, NGR, RWA, SEN, SIE, SUD, SOSUD, TAN, TOG, UGA, ZAI, ZAM
	ˆ*Monechma depauperatum* (T. Anderson) C.B. Clarke	Africa: BEN, CAF, CMN, GHA, GNB, GUI, IVO, MLI, NGA, SEN, SIE, SOSUD, TOG, ZAI
	ˆ*Monechma ndellense* (Lindau) J. Miège & Heine	Africa: BKN, CAF, GHA, GUI, MLI, SEN, SUD, TOG

Table 3. *Cont.*

Clade	Constituent Species	Distribution
	^*Monechma scabridum* (S. Moore) C.B. Clarke	Africa: ANG, ZAI, ZAM
	^*Monechma subsessile* (Oliv.) C.B. Clarke	Africa: ANG, BUR, KEN, RWA, TAN, UGA, ZAI, ZAM, ZIM
	^*Justicia attenuifolia* Vollesen	Africa: MOZ, TAN
"*virgultorum*" clade	^*Monechma virgultorum* S. Moore	Africa: ANG
	^*Justicia fanshawei* Vollesen	Africa: ZAM
"*tricostatum*" clade	*Monechma glaucifolium* S. Moore	Africa: ANG
	Monechma lolioides (S. Moore) C.B. Clarke	Africa: ANG
	^*Monechma rigidum* S. Moore	Africa: ANG
	^*Justicia cubangensis* I. Darbysh. & Goyder	Africa: ANG
	Justicia eriniae I. Darbysh.	Africa: ANG
	Justicia laeta S. Moore	Africa: ANG
	^*Justicia tricostata* Vollesen	Africa: TAN, ZAM
"*serotinum*" clade	^*Monechma serotinum* P.G. Mey.	Africa: NAM
"*cleomoides*" clade	^*Monechma australe* P.G. Mey.	Africa: CPP, NAM
	^*Monechma calcaratum* Schinz	Africa: NAM
	Monechma callothamnum Munday	Africa: NAM
	^*Monechma cleomoides* (S. Moore) C.B. Clarke	Africa: ANG, NAM
	Monechma crassiusculum P.G. Mey.	Africa: NAM
	^*Monechma desertorum* (Engl.) C.B. Clarke	Africa: NAM
	^*Monechma divaricatum* (Nees) C.B. Clarke	Africa: ANG, BOT, CPP, CPV, MOZ, NAM, NAT, OFS, SWZ, TVL, ZAM, ZIM
	^*Monechma distichotrichum* (Lindau) P.G. Mey.	Africa: CPP, NAM
	^*Monechma genistifolium* (Engl.) C.B. Clarke	Africa: NAM
	^*Monechma grandiflorum* Schinz	Africa: NAM
	^*Monechma incanum* (Nees) C.B. Clarke	Africa: BOT, CPP, NAM, OFS
	^*Monechma leucoderme* (Schinz) C.B. Clarke	Africa: NAM
	^*Monechma mollissimum* (Nees) P.G. Mey.	Africa: CPP, NAM
	Monechma robustum Bond	Africa: CPP
	^*Monechma salsola* (S. Moore) C.B. Clarke	Africa: ANG, NAM
	Monechma saxatile Munday	Africa: CPP
	^*Monechma spartioides* (T. Anderson) C.B. Clarke	Africa: CPP, NAM
	^*Monechma tonsum* P.G. Mey.	Africa: NAM
Monechma Group II incertae sedis	*Justicia kasamae* Vollesen	Africa: ZAM

4.2.2. Relationship of *Monechma* Group I to *Justicia* sect. *Harnieria*

Our results confirm a close relationship between *Monechma* Group I and *Justicia* sect. *Harnieria* (henceforth sect. *Harnieria*). Sect. *Harnieria* is found to be paraphyletic, with *J. odora* being sister to *Monechma* Group I, although monophyly of sect. *Harnieria* cannot be rejected ($p = 0.393$; Table 2). This result concurs closely with the findings of earlier studies [13], where a larger sample of species of sect. *Harnieria* was included than in the current study, and where *J. capensis* Thunb. and *J. odora* together were found to be sister to *Monechma* Group I. A number of morphological similarities have been noted between sect. *Harnieria* and *Monechma* s.l. [19], including general corolla shape, presence of conspicuous transverse ridges ("herring-bone" patterning) on a large portion of the lower corolla lip, and biaperturate pollen with insulae, as well as a similar inflorescence form between some members of *Monechma* and sect. *Harnieria*. These similarities, together with the fact that *J. tetrasperma* has an intermediate fruit type, have been used in support of reducing *Monechma* s.l. to a section of *Justicia* [19].

The principle difference between *Monechma* Group I and sect. *Harnieria* is in the fruits. *Monechma* Group I usually have 2-seeded capsules (4-seeded in *J. tetrasperma*) and seeds with a smooth testa. Those of sect. *Harnieria* have 4-seeded capsules with tuberculate seeds, although some species are heterocarpic with highly modified single-seeded indehiscent fruits in addition to the typical dehiscent capsules [13,76].

Variation in sculpturing of the seed testa has been observed within other lineages of Acanthaceae. For example, apparently closely allied members of the genus *Isoglossa* Oerst. in East Africa can have either a rugose testa (e.g., *I. floribunda* C.B. Clarke, *I. grandiflora* C.B. Clarke) or smooth testa (e.g., *I. mbalensis* Brummitt, *I. ufipensis* Brummitt) [80]. Furthermore, within the Justicioid lineage, seeds with a smooth testa are not unique to the two clades of *Monechma*: smooth seeds are observed in several other taxa in *Justicia* s.l. apparently unrelated to the two clades of *Monechma*. These include

the group of species *J. grisea* C.B. Clarke, *J. rendlei* C.B. Clarke and *J. salvioides* Milne-Redh. from East Africa, and *J. crebrinodis* Benoist and allies from Madagascar. The *J. crebrinodis* group also have 2-seeded capsules, but are otherwise very different morphologically to the clades of *Monechma* and molecular phylogenetic evidence confirms that they are not closely related [81]. This evidence suggests that variation in seed number and sculpturing may hold only limited taxonomic value at the generic rank within the Justicioid lineage and that it might, therefore, be advisable to treat *Monechma* Group I and sect. *Harnieria* as a single taxonomic unit. Nevertheless, further studies, including more thorough molecular sampling of sect. *Harnieria*, are required to fully decipher relationships within that group and in relation to *Monechma* Group I.

4.2.3. Morphological Variation within *Monechma* Group II

As noted in Section 4.2.1, *Monechma* Group II as recircumscribed here includes a range of morphological variation. Based on the results of the RADseq phylogeny, two major clades are noted (see Results), the latter of which contains several minor clades that can be delimited on morphological grounds and may form the basis for a future classification; these are summarised below. The constituent species for each of these clades are listed in Table 3, with those species not sampled in the RADseq phylogeny being placed based on their morphology.

(i) The "*ciliatum/scabridum*" clade, which contains species of fire-prone savanna biome in tropical Africa, largely associated with the Sudanian and Zambesian phytogeographic regions [29]. These species all share terminal spiciform inflorescences (sometimes with additional spikes in the uppermost leaf axils) and bracts that are ± highly modified from the leaves in both size and shape. The widespread West and Central African species *Monechma ciliatum* is unique in this clade in being an annual herb and in having unusual bristly trichomes on the seeds, restricted to tufts at the apex and base of the seeds, the two tufts being oriented in opposite directions (Figure 8E). All other species in this clade, such as *M. depauperatum*, *M scabridum* and *M. subsessile*, are suffruticose herbs (see Section 4.1). Their seeds are at first finely white-puberulous but later glabrescent. They are rounded or oblate in face view and are compressed but with rounded margins and have one face concave when young, the other face convex and with a ± conspicuous central ridge (Figure 8D). Our data reject the exclusion of *M. ciliatum* from *Monechma* Group II ($p < 0.001$; Table 2).

(ii) The "*virgultorum*" and (iii) the "*tricostatum*" clades, which comprise suffruticose herbs of southern tropical Africa, mainly associated with the Zambesian phytogeographic region [29]. As in the "*ciliatum/scabridum*" clade, they have predominantly terminal spiciform inflorescences with highly modified bracts, the exception being *Justicia fanshawei*, which has short axillary and terminal spikes. The seeds in these clades are less compressed than in the "*ciliatum/scabridum*" clade and are glabrous (Figure 8F,G). Several members of these two clades have secund inflorescence spikes in which only one of each pair of bracts is fertile, but this is not universal, for example both *M. rigidum* and *J. tricostata* can have opposite flowers along the spike. The "*tricostatum*" clade differs from the "*virgultorum*" clade in having prominently 3-veined calyx lobes, bracts and bracteoles, the veins often being a markedly different colour from the intercostal surfaces. In *M. virgultorum* and *M. fanshawei*, the calyces are at most only weakly 3-veined with only the midvein ever prominent.

(iv) The "*serotinum*" clade.*Monechma serotinum*, a rare species endemic to the Kaokoveld of Namibia, occupies a position in the phylogeny between the tropical African, fire-prone savanna clades outlined above and the group of species that are concentrated in the non-fire prone deserts and bushlands of southern Africa, i.e., the "*cleomoides*" clade discussed below. *Monechma serotinum* is also somewhat intermediate in morphological terms. It has a well-defined lax terminal spike with reduced bracts in comparison to the leaves as in most members of the savanna clades, but it has the dwarf shrubby habit of many species of the "*cleomoides*" clades (see below), in keeping with its non-fire prone habitat. The seeds of this species are glabrous, compressed, and asymmetric in cross section, with one face convex.

(v) The "*cleomoides*" clade. The remainder of the taxa in *Monechma* Group II are included in a single, large clade which comprises southern African taxa of dry, non-fire prone habitats including deserts and bushlands of the succulent biome. Most of the species are dwarf shrublets, often intricately branched and sometimes with gnarled lignified mature branches, although *M. desertorum* and some forms of *M. divaricatum* are annual herbs [20]. These species are united by having single-flowered axillary inflorescences with the bracts undifferentiated or not markedly differentiated from the leaves, although in some species such as *M. genistifolium* the flowers can together form ill-defined leafy terminal spikes. Many species have a complex indumentum comprising multiple trichome types (often both eglandular and glandular), and in some taxa the trichomes can be branched; for example, *M. incanum* has biramous trichomes on the vegetative parts and *M. calcaratum* has stellate trichomes on the stems [20]. The calyx lobes in this clade are either prominently single-veined or the venation is obscure. *Monechma divaricatum* is notable for having only four calyx lobes with no evidence of a vestigial fifth lobe; all other species in this clade (and elsewhere in *Monechma* Group II) have five-lobed calyces. The seeds in this clade are always glabrous, usually small and compressed, with either a rounded or sharp rim and ± asymmetric in cross section, with one face being more convex than the other and often having a prominent central ridge (Figure 8H–J).

The "*cleomoides*" clade is notable for containing several taxonomically challenging taxa, particularly regarding three highly variable aggregate species: *M. cleomoides*, *M. divaricatum* and *M. spartioides*. We sampled multiple accessions of the former two species. Whilst *M. divaricatum* is monophyletic, albeit with significant phylogenetic diversity, *M. cleomoides* is resolved as polyphyletic. Three accessions of that species are resolved in a clade that also contains two accessions of *M. tonsum*. These two taxa are separated primarily by differences in indumentum: *M. tonsum* has a short velvety indumentum whilst that of *M. cleomoides* usually includes ± dense mixed short and long shining trichomes (Figure 1U–W). Our results suggest that this difference in indumentum may be of limited taxonomic significance. A fourth accession of *M. cleomoides* (*Klaassen et al.* 2530) is resolved as sister to a clade containing *M. genistiifolium* and *M. australe*, which is difficult to reconcile with the morphological evidence, in view of the fact that these two species are morphological dissimilar to *M. cleomoides.* An AU test, however, rejects the monophyly of *M. cleomoides* and *M. tonsum* in addition to a monophyletic *M. cleomoides* + *M. tonsum* relationship ($p < 0.001$; Table 2).

4.2.4. The Status of *Monechma varians* and *Justicia platysepala*

Monechma varians is a rare species, confined to the Nyika Plateau of Malawi. It was recently transferred to *Justicia* sect. *Monechma* [18], although with a note that the capsule and seeds of this species had not been seen. The RADseq data place *M. varians* outside either of the two "*Monechma*" clades, it instead being resolved as sister to *J. kirkiana* in the *Tyloglossa* clade. A specimen of *M. varians* at K, *Synge WC437*, was annotated by M. Hedrén in 2000, stating "a *Justicia* close to *J. linearispica* C.B. Cl[arke]. Capsule probably 4-seeded, inflorescences as in *linearispica*". We concur with this suggestion as these two species are morphologically similar, and earlier molecular phylogenetic studies have placed *Justicia linearispica* within the *Tyloglossa* clade [13].

Justicia platysepala was originally described in *Monechma* on the basis of it having two- (or one-) seeded capsules. However, the seeds of this species—together with the related species *J. guerkeana* Schinz, also previously described in *Monechma* as *M. clarkei* Schinz—are tuberculate, and thus quite unlike those of *Monechma* s.l. Our results confirm that *J. platysepala* does not belong within either of the two clades of *Monechma*.

5. Conclusions

The findings of this study confirm that the genus *Monechma* (or *Justicia* sect. *Monechma*), as previously circumscribed, represents two widely separated clades. Our findings provide insights into the evolutionary histories of these two clades. Particularly striking is the relatively recent radiation (diversifying ca. 1.9 mya) in *Monechma* Group II into the ancient deserts and xeric shrublands of the

succulent biome in southwest Africa. While colonisation of the succulent biome may have involved relaxed selection on traits required to survive regular fires that are present in the savanna biome, it required adaptation to higher water deficits (evidenced by lower precipitation throughout the year) and greater extremes of low and high temperatures (higher annual temperature range). Clearly, this clade in *Monechma* Group II was able to adapt, as the radiation now accounts for more than half of the current species diversity in *Monechma* Group II despite the much longer evolutionary history of the clade within the savanna biome of tropical Africa. Our results support the need for future research to further understand the biogeographical history of these centers of biodiversity in Africa.

Given that *Justicia* s.l. is highly paraphyletic and encompasses all the taxa within the Justicioid lineage, and given the desire to avoid losing valuable taxonomic information that would be incurred through an all-encompassing *Justicia*, the only plausible option is to recognise distinct clades within *Justicia* s.l. as discrete genera and to seek morphological characters in support of these segregations. Our results show that *Monechma* Groups I and II are distinguishable from one another, albeit subtly so, by differences in the inflorescence structure and seed morphology. These two clades should therefore be elevated to generic status, and a forthcoming study will address the nomenclatural implications of recognising them as separate genera. Detailed studies employing NGS techniques, comparable to that presented in the current work, are required across the Justicioid lineage in order to delimit other genera in this complex but ecologically important plant group.

Supplementary Materials: The following are available online at http://www.mdpi.com/1424-2818/12/6/237/s1, Figure S1: Summary phylogeny of the majority-rule consensus tree from Bayesian analysis illustrating the 10 major clades of the Justicioid lineage from [13]. Within the *Harnieria* clade, species of *Monechma* Group I are sister to *Justicia odora*. Embedded within the Diclipterinae clade, species of *Monechma* Group II are sister to *Kenyacanthus ndorensis* + (*Hypoestes* + *Dicliptera*). Thickened branches are supported by ≥0.98 Bayesian posterior probability and ≥70% maximum likelihood bootstrap. Size of clades corresponds to the number of taxa sampled in each clade, Figure S2: The most likely phylogenetic hypothesis generated from ddRAD-seq loci from the iPYRAD de novo assembly with the minimum sample to retain a locus set to four. Asterisks [*] indicate 100% ML bootstrap and dashes [-] indicate <70% ML bootstrap, Figure S3: The most likely phylogenetic hypothesis generated from ddRAD-seq loci from the iPYRAD de novo assembly with the minimum sample to retain a locus set to 10. Asterisks [*] indicate 100% ML bootstrap and dashes [-] indicate <70% ML bootstrap, Figure S4: The most likely phylogenetic hypothesis generated from ddRAD-seq loci from the iPYRAD de novo assembly with the minimum sample to retain a locus set to 30. Asterisks [*] indicate 100% ML bootstrap and dashes [-] indicate <70% ML bootstrap, Figure S5: Phylogenetic relationships among the samples included in our study based on quartet multispecies coalescent analyses of loci resulting from the iPYRAD assembly. Numbers at nodes represent percent support across 1000 replicate quartet analyses. Asterisks [*] indicate 100% support, Table S1: Taxon, source of plant material, number of ddRAD reads per sample and number of loci per sample in our final assembly. Taxa are listed in alphabetical order by genus and species. Table S2: Results of tests for the best-fit model of evolution for biome in the ancestral state reconstruction analyses. The model in bold was selected.

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References

1. Darbyshire, I.; Kordofani, M.; Farag, I.; Candiga, R.; Pickering, H. *The Plants of Sudan and South Sudan: An Annotated Checklist*; Royal Botanic Gardens, Kew: Richmond, UK, 2015.
2. Darbyshire, I.; Timberlake, J.; Osborne, J.; Rokni, S.; Matimele, H.; Langa, C.; Datizua, C.; de Sousa, C.; Alves, T.; Massingue, A.; et al. The endemic plants of Mozambique: Diversity and conservation status. *PhytoKeys* **2019**, *136*, 45–96. [CrossRef] [PubMed]
3. Onana, J.M. The Vascular Plants of Cameroon. A taxonomic checklist with IUCN assessments. In *Flore du Cameroon*; National Herbarium of Cameroon: Yaoundé, Cameroon, 2011; Volume 39.
4. Gosline, G.; Cheek, M.; Bidault, E.; van der Burgt, X.M.; Challen, G.; Couch, C.; Couvreur, T.; Darbyshire, I.; Dawson, S.; Goyder, D.; et al. Known plant diversity of the Republic of Guinea increased by 30% in a new taxonomically curated checklist. Unpublished work.
5. Vollesen, K. *Blepharis (Acanthaceae): A Taxonomic Revision*; Royal Botanic Gardens, Kew: Richmond, UK, 2000.
6. Tripp, E.A.; Tsai, Y.H.E.; Zhuang, Y.; Dexter, K. RADseq dataset with 90% missing data fully resolves recent radiation of *Petalidium* (Acanthaceae) in the ultra-arid deserts of Namibia. *Ecol. Evol.* **2017**, *7*, 7920–7936. [CrossRef] [PubMed]
7. Darbyshire, I.; Tripp, E.A.; Chase, F.M. A taxonomic revision of Acanthaceae tribe Barlerieae in Angola and Namibia. Part 1. *Kew Bull.* **2019**, *74*, 1–85. [CrossRef]
8. Darbyshire, I. Taxonomic notes and novelties in the genus *Isoglossa* (Acanthaceae) from east Africa. *Kew Bull.* **2009**, *64*, 401–427. [CrossRef]
9. Tripp, E.A.; Dexter, K.G. Taxonomic novelties in Namibian *Ruellia* (Acanthaceae). *Syst. Bot.* **2012**, *37*, 1023–1030. [CrossRef]
10. Darbyshire, I.; Luke, Q. *Barleria mirabilis* (Acanthaceae): A remarkable new tree species from west Tanzania. *Kew Bull.* **2016**, *71*, 13. [CrossRef]
11. McDade, L.A.; Daniel, T.F.; Kiel, C.A. Towards a comprehensive understanding of phylogenetic relationships among lineages of Acanthaceae *s.l.* (Lamiales). *Am. J. Bot.* **2008**, *95*, 1136–1152. [CrossRef] [PubMed]
12. McDade, L.A.; Daniel, T.F.; Kiel, C.A. The *Tetramerium* Lineage (Acanthaceae, Justicieae) revisited: Phylogenetic relationships reveal polyphyly of many New World genera accompanied by rampant evolution of floral morphology. *Syst. Bot.* **2018**, *43*, 97–116. [CrossRef]
13. Kiel, C.A.; Daniel, T.F.; Darbyshire, I.; McDade, L.A. Unraveling relationships in the morphologically diverse and taxonomically challenging "justicioid" lineage (Acanthaceae: Justicieae). *Taxon* **2017**, *66*, 645–674. [CrossRef]
14. Kiel, C.A.; Daniel, T.F.; McDade, L.A. Phylogenetics of New World 'justicioids' (Justicieae: Acanthaceae): Major lineages, morphological patterns, and widespread incongruence with classification. *Syst. Bot.* **2018**, *43*, 459–484. [CrossRef]
15. Darbyshire, I.; Kiel, C.A.; Daniel, T.F.; McDade, L.A.; Luke, W.R.Q. Two new genera of Acanthaceae from tropical Africa. *Kew Bull.* **2019**, *74*, 1–25. [CrossRef]
16. Tripp, E.A.; Darbyshire, I.; Daniel, T.F.; Kiel, C.A.; McDade, L.A. Revised classification of Acanthaceae including treatment of previously unplaced genera and worldwide dichotomous keys. Unpublished work.
17. Vollesen, K. *Justicia* . In *Flora of Tropical East Africa*; Beentje, H., Ed.; Royal Botanic Gardens, Kew: Richmond, UK, 2010; pp. 495–601.
18. Vollesen, K. *Justicia* . In *Flora Zambesiaca*; Timberlake, J.R., Martins, E.S., Eds.; Royal Botanic Gardens, Kew: Richmond, UK, 2015; Volume 8, pp. 162–224.

19. Hedrén, M. *Justicia tetrasperma* sp. nov.: A linking species between *Justicia* and *Monechma* (Acanthaceae). *Nordic J. Bot.* **1990**, *10*, 149–153. [CrossRef]
20. Munday, J. *Monechma* . In *Flora of Southern Africa*; Leistner, O.A., Ed.; National Botanical Institute: Pretoria, South Africa, 1995; Volume 30, pp. 47–61.
21. Hedberg, I.I.; Ensermu Kelbessa, E.S.; Sebsebe Demissew, P.E. (Eds.) Ensermu Kelbessa Acanthaceae. In *Flora of Ethiopia & Eritrea*; The National Herbarium, Addis Ababa University: Addis Ababa, Ethiopia; The Department of Systematic Botany, Uppsala University: Uppsala, Sweden, 2006; Volume 5, pp. 345–495.
22. Darbyshire, I.; Goyder, D.J. Notes on *Justicia* sect. *Monechma* (Acanthaceae) in Angola, including two new species. *Blumea* **2019**, *64*, 97–107. [CrossRef]
23. Dressler, S.; Schmidt, M.; Zizka, G. *African Plants—A Photo Guide*; Forschungsinstitut Senckenberg: Frankfurt/Main, Germany, 2014. Available online: www.africanplants.senckenberg.de (accessed on 26 February 2020).
24. Munday, J. The distribution of *Monechma* (Acanthaceae) species in southern Africa. *Bothalia* **1983**, *14*, 575–578. [CrossRef]
25. Ringelberg, J.J.; Zimmermann, N.E.; Weeks, A.; Lavin, M.; Hughes, C.E. Biomes as evolutionary arenas: Convergence and conservatism in the trans-continental succulent biome. *Glob. Ecol. Biogeogr.* **2020**. [CrossRef]
26. Schrire, B.D.; Lavin, M.A.T.T.; Lewis, G.P. Global distribution patterns of the Leguminosae: Insights from recent phylogenies. *Biol. Skr.* **2005**, *55*, 375–422.
27. Klaassen, E.; Kwembeya, E. (Eds.) *A Checklist of Namibian Indigenous and Naturalised Plants*; Occasional Contributions No. 5; National Botanical Research Institute: Windhoek, Namibia, 2013.
28. Chase, F.M. Flora of Namibia: *Monechma*. Unpublished work.
29. White, F. *Vegetation of Africa: A Descriptive Memoir to Accompany the UNESCO/AETFAT/UNSO Vegetation Map of Africa*; UNESCO: Paris, France, 1983.
30. Daniel, T.F.; Tripp, E.A. *Louteridium* (Acanthaceae: Acanthoideae: Ruellieae: Trichantherinae): Taxonomy, phylogeny, reproductive biology, and conservation. *Proc. Calif. Acad. Sci. Ser. 4* **2018**, *65*, 41–106.
31. Tripp, E.A.; Darbyshire, I. *Mcdadea*: A new genus of Acanthaceae endemic to the Namib Desert of Southwestern Angola. *Syst. Bot.* **2020**, *45*, 200–211. [CrossRef]
32. Comito, R.P. A RADseq Phylogeny of *Barleria* (Acanthaceae) Resolves Fine-Scale Relationships. Master's Thesis, California State University, Long Beach, CA, USA, 2019.
33. Kiel, C.A.; Tripp, E.A.; Fisher, A.E.; McDade, L.A. Is pollen involved too? Floral traits and pollinators in New World *Justicia* (Acanthaceae). In Proceedings of the Conference Presentation, Botany 2019 Conference, Tucson, AZ, USA, 27–31 July 2019. Available online: https://2019.botanyconference.org/engine/search/index.php?func=detail&aid=770 (accessed on 30 April 2020).
34. Doyle, J.J.; Doyle, J.L. A rapid DNA isolation procedure for small amounts of fresh leaf tissue. *Phytochem. Bull.* **1987**, *19*, 11–15.
35. Parchman, T.L.; Gompert, Z.; Mudge, J.; Schilkey, F.D.; Benkman, C.W.; Buerkle, C.A. Genome-wide association genetics of an adaptive trait in lodgepole pine. *Mol. Ecol.* **2012**, *21*, 2991–3005. [CrossRef] [PubMed]
36. Andrews, S. *FastQC: A Quality Control Tool for High Throughput Sequence Data*; Babraham Institute: Cambridge, UK, 2017. Available online: https://www.bioinformatics.babraham.ac.uk/projects/fastqc/ (accessed on 26 February 2020).
37. Eaton, D.A.R. PyRAD: Assembly of *de novo* RADseq loci for phylogenetic analysis. *Bioinformatics* **2014**, *30*, 1844–1849. [CrossRef] [PubMed]
38. Eaton, D.A.R.; Overcast, I. ipyrad: Interactive assembly and analysis of RADseq datasets. *Bioinformatics* **2020**. [CrossRef] [PubMed]
39. Nguyen, L.-T.; Schmidt, H.A.; von Haeseler, A.; Minh, B.Q. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Mol. Biol. Evol.* **2015**, *32*, 268–274. [CrossRef] [PubMed]
40. Darriba, D.; Posada, D.; Kozlov, A.M.; Stamatakis, A.; Benoit, M.; Flouri, T. ModelTest-NG: A new and scalable tool for the selection of DNA and protein evolutionary models. *Mol. Biol. Evol.* **2020**, *37*, 291–294. [CrossRef] [PubMed]

41. Eaton, D.A.R.; Spriggs, E.L.; Park, B.; Donoghue, M.J. Misconceptions on missing data in RAD-seq phylogenetics with a deep-scale example from flowering plants. *Syst. Biol.* **2017**, *66*, 399–412. [CrossRef] [PubMed]
42. Chifman, J.; Kubatko, L. Quartet inference from SNP data under the coalescent model. *Bioinformatics* **2014**, *30*, 3317–3324. [CrossRef] [PubMed]
43. Shimodaira, H. An approximately unbiased test of phylogenetic tree selection. *Syst. Biol.* **2002**, *51*, 492–508. [CrossRef] [PubMed]
44. Maddison, W.P.; Maddison, D.R. *Mesquite: A Modular System for Evolutionary Analysis*, Version 2.72; 2009. Available online: http://mesquiteproject.org (accessed on 26 February 2020).
45. Paradis, E. Molecular dating of phylogenies by likelihood methods: A comparison of models and a new information criterion. *Mol. Phylogenet. Evol.* **2013**, *67*, 436–444. [CrossRef] [PubMed]
46. Paradis, E.; Claude, J.; Strimmer, K. APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics* **2004**, *20*, 289–290. [CrossRef] [PubMed]
47. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2019. Available online: http://www.R-project.org/ (accessed on 15 April 2020).
48. Smith, S.A.; O'Meara, B.C. treePL: Divergence time estimation using penalized likelihood for large phylogenies. *Bioinformatics* **2012**, *28*, 2689–2690. [CrossRef] [PubMed]
49. Tripp, E.A.; McDade, L.A. A rich fossil record yields calibrated phylogeny for Acanthaceae (Lamiales) and evidence for marked biases in timing and directionality of intercontinental disjunctions. *Syst. Biol.* **2014**, *63*, 660–684. [CrossRef] [PubMed]
50. Mautino, L.R. Nuevas especies de palinomorfos de las formaciones San José y Chiquimil (Mioceno Medio y Superior), noroeste de Argentina. *Rev. Bras. Paleontol.* **2011**, *14*, 279–290. [CrossRef]
51. Graham, V.A.W. Delimitation and infra-generic classification of *Justicia* (Acanthaceae). *Kew Bull.* **1988**, *43*, 551–624. [CrossRef]
52. Burgess, N.; Hales, J.A.; Underwood, E.; Dinerstein, E.; Olson, D.; Itoua, I.; Schipper, J.; Ricketts, T.; Newman, K. *Terrestrial Ecoregions of Africa and Madagascar: A Conservation Assessment*; Island Press: Washington, DC, USA, 2004.
53. Beaulieu, J.M.; O'Meara, B.C.; Donoghue, M.J. Identifying hidden rate changes in the evolution of a binary morphological character: The evolution of plant habit in campanulid angiosperms. *Syst. Biol.* **2013**, *62*, 725–737. [CrossRef] [PubMed]
54. Pagel, M. Inferring evolutionary processes from phylogenies. *Zool. Scr.* **1997**, *26*, 331–348. [CrossRef]
55. Lewis, P.O. A likelihood approach to estimating phylogeny from discrete morphological character data. *Syst. Biol.* **2001**, *50*, 913–915. [CrossRef] [PubMed]
56. Posada, D.; Buckley, T.R. Model selection and model averaging in phylogenetics: Advantages of Akaike Information Criterion and Bayesian approaches over likelihood ratio tests. *Syst. Biol.* **2004**, *53*, 793–808. [CrossRef] [PubMed]
57. WorldClim. Maps, Graphs, Tables, and Data of the Global Climate. Available online: http://worldclim.org (accessed on 15 April 2020).
58. Fick, S.E.; Hijmans, R.J. WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. *Int. J. Climatol.* **2017**, *37*, 4302–4315. [CrossRef]
59. Hijmans, R.J. *Raster: Geographic Data Analysis and Modeling R Package*; 2014. Available online: https://rdrr.io/cran/raster/ (accessed on 15 April 2020).
60. Revell, L.J. phytools: An R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* **2012**, *3*, 217–223. [CrossRef]
61. Felsenstein, J. Phylogenies and the comparative method. *Am. Nat.* **1985**, *125*, 1–15. [CrossRef]
62. Revell, L.J. Two new graphical methods for mapping trait evolution on phylogenies. *Methods Ecol. Evol.* **2013**, *4*, 754–759. [CrossRef]
63. Thiers, B. Index Herbariorum: A Global Directory of Public Herbaria and Associated Staff. New York Botanical Garden's Virtual Herbarium. Continuously Updated. Available online: http://sweetgum.nybg.org/science/ih/ (accessed on 26 February 2020).
64. JSTOR Global Plants. Available online: https://plants.jstor.org/ (accessed on 26 February 2020).

65. Brummitt, R.K. *World Geographical Scheme for Recording Plant Distributions*, 2nd ed.; International Working Group on Taxonomic Databases for Plant Sciences (TDWG), Hunt Institute for Botanical Documentation, Carnegie Mellon University: Pittsburgh, PA, USA, 2001. Available online: http://www.tdwg.org/standards/109 (accessed on 26 February 2020).
66. Immelman, K.L. *Justicia* . In *Flora of Southern Africa*; Leistner, O.A., Ed.; National Botanical Institute: Pretoria, South Africa, 1995; Volume 30, Part 3; pp. 18–46.
67. Beerling, D.J.; Osborne, C.P. The origin of the savanna biome. *Glob. Chang. Biol.* **2006**, *12*, 2023–2031. [CrossRef]
68. Edwards, E.J.; Osborne, C.P.; Strömberg, C.A.; Smith, S.A.; C4 Grasses Consortium. The origins of C4 grasslands: Integrating evolutionary and ecosystem science. *Science* **2010**, *328*, 587–591. [CrossRef] [PubMed]
69. Maurin, O.; Davies, T.J.; Burrows, J.E.; Daru, B.H.; Yessoufou, K.; Muasya, A.M.; Van der Bank, M.; Bond, W.J. Savanna fire and the origins of the 'underground forests' of Africa. *New Phytol.* **2014**, *204*, 201–214. [CrossRef] [PubMed]
70. Eiserhardt, W.L.; Couvreur, T.L.; Baker, W.J. Plant phylogeny as a window on the evolution of hyperdiversity in the tropical rainforest biome. *New Phytol.* **2017**, *214*, 1408–1422. [CrossRef] [PubMed]
71. Ackerly, D.D. Adaptation, niche conservatism, and convergence: Comparative studies of leaf evolution in the California chaparral. *Am. Nat.* **2004**, *163*, 654–671. [CrossRef] [PubMed]
72. Stebbins, G.L. Aridity as a stimulus to plant evolution. *Am. Nat.* **1952**, *86*, 33–44. [CrossRef]
73. Goudie, A. Namib Sand Sea: Large dunes in an ancient desert. In *Geomorphological Landscapes of the World*; Migon, P., Ed.; Springer: Dordrecht, Germany, 2009; pp. 163–169.
74. Scotland, R.W.; Vollesen, K. Classification of Acanthaceae. *Kew Bull.* **2000**, *55*, 513–589. [CrossRef]
75. Miège, J.; Josserand, N. Nombres chromosomiques d'èspeces africaines et malgaches. *Candollea* **1972**, *27*, 283–292.
76. Hedrén, M. *Justicia* sect. *Harnieria* (Acanthaceae) in tropical Africa. *Acta Univ. Upsal. Symb. Bot. Upsal.* **1989**, *29*, 1–141.
77. Daniel, T.F.; Chuang, T.I. Chromosome numbers of New World Acanthaceae. *Syst. Bot.* **1993**, *18*, 283–289. [CrossRef]
78. Daniel, T.F. Chromosome numbers of miscellaneous Malagasy Acanthaceae. *Brittonia* **2006**, *58*, 291–300. [CrossRef]
79. Grant, W.F. A cytogenetic study in the Acanthaceae. *Brittonia* **1955**, *8*, 121–149. [CrossRef]
80. Darbyshire, I.; Vollesen, K. Ensermu Kelbessa *Acanthaceae (Part 2)*. In *Flora of Tropical East Africa*; Beentje, H.J., Ed.; Royal Botanic Gardens, Kew: Richmond, UK, 2010.
81. McDade, L.A.; Daniel, T.F.; Darbyshire, I.; Kiel, C.A. Justicieae II: Resolved placement of many genera and recognition of a new lineage sister to Isoglossinae. Unpublished work.