Supplementary Materials

Table S1. Summary of studies employing the ISSR method applied to cycads based on searches performed in Web of Science Core collection and Google Scholar using the search terms "Cycad* AND "ISSR" OR "intersimple sequence repeat*".

Species	Region	Success of ISSRs	Main aims	Key findings	Reference
Ceratozamia fuscoviridis (Zamiaceae)	Mexico	Lower information content compared to microsatellites. Supplements microsatellite data	Effect of anthropogenic disturbance of species diversity using microsatellites and ISSR	Clustering of taxa corresponded to level of disturbance instead of geographic distance Negative relationship between level of genetic diversity and degree of disturbance	[153]
Encephalartos woodii (Zamaiceae)	South Africa	Successful	Comparing specimens using 15 RAPD and 5 ISSR primers	*E. woodii* collections of Ngoye and Krantzkloof types found to not be genetically identical, suggesting different parents	[154]
Cycas debaoensis (Cycadaceae)	South China	Successful	Genetic diversity	Phenetic analysis reveals two clusters of grouped populations showing relative similarity of all populations to one another. Genetic diversity among populations was high	[155]
Cycas diannanensis (= *Cycas parvula*) and C. *balansae* (Cycadaceae)	South China	Successful	Examine level and distribution of genetic variation in two species	Proximate populations of *C. diannanensis* had low expected heterozygosity and very low genetic differentiation compared to the more widely distributed *C. balansae*	[156]
Encephalartos species (Zamiaceae)	African continent	Unsuccessful	Phylogenetic relationships between 52 *Encephalartos* species using ISSRs, ITS and rbcLa sequences,	ISSRs were unable to provide sufficient resolution to resolve phylogenetic relationships of taxa Morphological, geographical and nuclear DNA characters provided more insight and resolution of phylogenetic relationships	[86] *

			morphological and geographical characters		
Cycas guizhouensis (Cycadaceae)	South China	Successful	Genetic diversity	Low genetic diversity within populations and high differentiation among populations Evidence of potential drift and inbreeding	[26]
Cycas fairylakea (Cycadaceae)	South China		Genetic diversity	Species clustered into two groups with UPGMA and Principal components analysis in agreement	[32]
Dioon spinulosum (Zamiaceae)	Egypt	Not very effective compared to SCoT markers	Species identification using ISSR and Start Codon Translation (SCoT) markers	SCoT markers more informative with more polymorphic bands than ISSR markers More ScoT markers were suitable for identifying D. spinulosum than ISSR markers	[42]
Cycas taitungensis (Cycadaceae)	Taiwan	Successful, but less so than microsatellites	Genetic diversity using microsatellites and ISSRs Identifying genetic units for conservation	Bayesian MCMC analysis revealed presence of inbreeding in populations and decline in genetic variability. Management units for conservation were identified Distinct genetic units were found in in situ and ex situ populations Genetic variability estimates were much lower with ISSR markers than microsatellites	[157]
Cycas beddomei (Cycadaceae)	India	Successful	Genetic diversity	15 out of 20 ISSR primers produced unambiguous bands Phenetic analysis clusters species in two groups corresponding to different sampling locations	[158]
Cycas balansae complex (Cycadaceae)	South China	Successful	Taxonomy of *Cycas balansae* complex	ISSRs revealed low genetic diversity Phenetic analysis with UPGMA reveals five distinct clusters corresponding to species	[90]
Dioon caputoi *Dioon planifolium* (Zamiaceae)	Mexico	Successful	Fine scale genetic variation at different spatial separation	Genetic variation correlated to spatial separation, but in a species-specific manner Data supplements the study of population demographics of species	[159]

Zamia inermis (Zamiaceae)	Mexico	Successful	Sex identification using ISSRs	Located a marker associated with the expression of female traits Tendency found for separate clustering of male and females with Principal coordinates analysis	[36]
Zamia furfuracea (Zamiaceae)	Mexico	Successful	Population demographics and fine scale genetic structure of species	Identified three gene neighbourhoods Genetic composition of age groups and between populations was determined and substantial loss of genetic variability in juveniles discovered Provided useful insights supplementing demographic data and aiding conservation Demystifies population and spatial dynamics and the influence of external factors like dispersal and pollination	[33]
Cycas circinalis L.	India	Unsuccessful	Sex determination in commercially important plants using RADP and ISSR	Identified a male specific RADP marker in *C. circinalis* No sex male or female specific ISSR markers were found	[35]
Ceratozamia mexicana Brongn. (Zamiaceae)	Mexico	Successful	Sex determination and SCAR marker development from ISSRs	ISSRs used as precursors to developing SCAR markers associated with male plants Needly gene associated with sexual expression	[160]
Ceratozamia mexicana Brongn. (Zamiaceae)	Mexico	Successful	Optimised DNA isolation and ISSR-PCR protocol for species	Modified CTAB method used for fresh, young cycad material Included a pre-soak in 5 M NaCL solution	[142]

Table S2. Species within the *Encephalartos eugene-maraisii* complex with their threat status and year of its description. Known synonyms by which taxa were described are also shown. Data is derived from the Online world list of cycads, https://www.cycadlist.org/ (accessed on 12 August 2024).

Species	Region	Year described	Threat status	Synonyms	Known variants
Encephalartos eugene-maraisii	Limpopo	1945	EN	*Encephalartos eugene-maraisii subsp. eugene-maraisii*	Palala Kranzberg Kranzkop Waterberg
Encephalartos cupidus	Mpumalanga	1971	CR		True *cupidus* "Giant *cupidus*" – potentially E. *nubimontanus* Robusta or an alternative form
Encephalartos dolomiticus	Limpopo	1988	CR	*Encephalartos verrucosus*	True *dolomiticus*
Encephalartos dyerianus	Limpopo	1988	CR	*Encephalartos graniticola*	True *dyerianus* "*levubuensis*" (potentially undescribed species)
Encephalartos middelburgensis	Mpumalanga	1989	CR	*Encephalartos eugene-maraisii* subsp. *middelburgensis*	True *middelburgensis* Avontuur (only male specimens encountered)
Encephalartos nubimontanus	Limpopo	1995	EW	*Encephalartos venetus*	Prevalent Fine dwarf Fishtail Pinna Strydom Tunnel Munchi Trichardtsdal Green Wide Pinna Robustus

				Giant Rough *cupidus*
				Giant Fine *cupidus*
Encephalartos hirsutus	Limpopo	1996	CR	

Table S3 Summary of selected samples for study based on gel outcome. Sample amplification was labelled "good" when amplified bands were clearly visible on the gels (Figure S2), and "bad" when they were absent

Sample and primer C Mao	Gel score	Sample and primer E 864	Gel score	Sample and Primer F 856	Gel score	Sample code
1C	good	1E		1F	good	eug9W
3C	good	3E	good	3F	good	eug21
7C	good	7E	good	7F	good	eug22P
8C	good	8E	good	8F	bad	eug23P
9C	good	9E	good	9F	bad	eug24
10C	good	10E	good	10F	good	eug1
11C	good	11E	good	11F	good	eug2
12C	good	12E	good	12F	good	eug3
13C	good	13E	good	13F	good	eug4
16C	good	16E	good	16F	bad	eug5
17C	good	17E	good	17F	good	eug6
18C	good	18E	good	18F	good	eug7
19C	good	19E	good	19F	good	eug8P
20C	good	20E	good	20F	good	eug10
22C	good	22E	good	22F	bad	eug11
23C	good	23E	bad	23F	good	eug12
24C	good	24E	bad	24F	bad	eug13
25C	good	25E	good	25F	bad	eug14
26C	bad	26E	good	26F	good	eug15W
27C	good	27E	good	27F	bad	16Lb
28C	good	28E	bad	28F	bad	eug17
29C	good	29E	good	29F	bad	eug18
32C	good	32E	bad	32F	bad	eug19
33C	good	33E	good	33F	bad	eug20
34C	good	34E	good	34F	good	his1
35C	good	35E	good	35F	good	his2
39C	good	39E	bad	39F	bad	nub32
41C	good	41E	bad	41F	good	nub33
43C	good	43E	bad	43F	good	nub34
48C	bad	48E	good	48F	bad	nub35
50C	good	50E	good	50F	bad	cup3d2
52C	bad	52E	good	52F	bad	cup24
53C	no	53E	good	53F	good	cup25
62C	trace	62E	good	62F	good	mid6
63C	of	63E	good	63F	bad	mid2d2Av
64C	sample	64E	good	64F	good	mid8
74C	good	74E	good	74F	good	dol3
75C	good	75E	good	75F	good	dol4
77C	good	77E	good	77F	good	dol5
78C	bad	78E	good	78F	bad	dol6
80C	good	80E	good	80F	good	dol7

81C	bad	81E	good	81F	bad	
83C	good	83E	good	83F	good	dol9
85C	bad	85E	good	85F	good	dye11Lb
87C	good	87E	good	87F	good	dye3d2
89C	good	89E	good	89F	good	dye4d2
91C	good	91E	good	91F	bad	dye10d2
93C	good	93E	good	93F	good	dye16
94C	good	94E	good	94F	good	dye12d2
			Cohort 2			
107C	good	107E	good	107F	good	nub1Tw
108C	bad	108E	good	108F	good	nub2Gr
109C	good	109E	good	109F	good	nub3Gr
110C	bad	110E	good	110F	bad	nub4Gr
111C	good	111E	good	111F	good	nub5DG
112C	bad	112E	good	112F	good	nub6D
113C	bad	113E	good	113F	good	nub7DB
114C	bad	114E	good	114F	good	nub8D
115C		115E	good	115F	good	nub9Bt
116C	good	116E	good	116F	good	nub10Bt
117C		117E	good	117F		nub11Sp
118C	good	118E	good	118F	good	nub12Bt
119C	good	119E	good	119F	good	nub13Fi
120C		120E	good	120F	good	nub14Fi
121C	good	121E	good	121F	good	nub15D
122C		122E	good	122F	good	nub16R
123C		123E	good	123F	good	nub17D
124C	good	124E	good	124F	good	nub18
125C	good	125E	good	125F	good	nub19
126C		126E	good	126F	good	nub20
128C		128E	good	128F	good	nub21M
129C		129E	good	129F		nub22Ft
131C		131E	good	131F	good	nub23
133C		133E	good	133F	good	nub24
134C		134E	good	134F	good	nub25
135C	good	135E	good	135F	good	nub26
136C		136E	good	136F	good	nub37
137C		137E	good	137F	good	nub28
138C	good	138E	good	138F	good	nub29
139C	good	139E	good	139F	good	nub30
141C	good	141E	good	141F	good	nub31
142C	good	142E		142F	good	cup1
143C		143E		143F	good	cup2
145C		145E		145F	good	cup3d1
147C	good	147E	good	147F	good	cup4T
148C		148E		148F	good	cup5T
150C	good	150E	good	150F	good	cup6T
151C		151E		151F	good	cup7T

152C		152E		152F	good	cup8
154C	good	154E	good	154F	good	cup9
155C	good	155E	good	155F	good	cup10
156C		156E	good	156F	good	cup11
157C	good	157E	good	157F	good	cup12
158C	good	158E	good	158F	good	cup13
159C		159E		159F	good	cup14
161C		161E		161F	good	cup15
164C		164E		164F	good	cup16
165C	good	165E	good	165F	good	cup17
166C	good	166E		166F	good	cup18
169C		169E	good	169F		cup19
170C	good	170E		170F	good	cup20
171C	good	171E		171F	good	cup21
174C	good	174E	good	174F	good	cup22
175C	good	175E	good	175F		cup23
176C		176E		176F	good	mid1
177C	good	177E	good	177F	good	mid2d1Av
178C	good	178E	good	178F	good	mid3
181C	good	181E	good	181F	good	mid4
185C		185E	good	185F	good	mid5
186C	good	186E	good	186F	good	dol1
187C	good	187E		187F	good	dol2
188C	good	188E	good	188F	good	dye1
189C		189E	good	189F	good	dye2
190C	good	190E	good	190F	good	dye3d1
191C	good	191E	good	191F	good	dye4d1
192C	good	192E		192F	good	dye5
193C	good	193E	good	193F	good	dye6Lb
194C	good	194E	good	194F	good	dye7
196C	good	196E	good	196F	good	dye8
198C	good	198E	good	198F	good	dye9
199C		199E		199F	good	dye10d1
200C		200E		200F	good	dye11
201C		201E	good	201F		dye12d1

Table S4. Cophenetic correlation values (r) and t -values showing the extent to which UPGMA trees derived from distance matrices of respective similarity coefficients, fit the datasets. Data are arranged by minimum band intensity (relative fluorescence units, rfu) and from highest r-value. Trees produced from standardised Euclidean and Manhattan distances are also shown.

Distance coefficient	Tree type	RFU cut-off value					
		50rfu		100rfu		200rfu	
		r	t	r	t	r	t
DICE – DICE (1945)	UPGMA	0.8094	11.7986	0.69475	15.5792	0.71523	12.6501
J – Jaccard (1908)	UPGMA	0.81149	13.1634	0.72079	18.478	0.73528	14.9337
K1 – Kulcznski (1927) coef. 1	UPGMA	0.75491	13.6306	0.84352	36.5891	0.83691	30.7461
K2 – Kulcznski (1927) coef. 2	UPGMA	0.73371	14.4069	0.70329	15.6134	0.71218	12.6602
O – Ochiai (1957)	UPGMA	0.80731	11.7822	0.6988	15.6277	0.71096	12.6282
PHI – phi	UPGMA	0.79508	12.1712	0.70696	16.1855	0.69869	13.1679
RR – Russel and Rao (1940)	UPGMA	0.88238	10.5089	0.81662	11.2702	0.81367	11.4547
Euclidean distance	UPGMA	0.79887	13.3991	0.736	17.7936	0.71845	15.1323
Manhattan distance	UPGMA	0.69813	15.6775	0.67458	21.5935	0.65628	15.2665
SM – Simple Matching	UPGMA	0.70461	12.9729	0.66026	18.2035	0.63741	13.1314
UN1 – “unnamed” coef. 1	UPGMA	0.66961	12.4717	0.65027	18.1519	0.62853	12.7104
UN2 – “unnamed” coef. 2	UPGMA	0.81546	14.8146	0.75244	21.4056	0.75533	17.7002
UN3 – “unnamed” coef. 3	UPGMA	0.78531	20.9135	0.86157	37.3712	0.81065	28.7192
UN5 – “unnamed” coef. 5	UPGMA	0.80153	12.1907	0.69732	16.0344	0.71417	13.4151
Y – Yule (1911)	UPGMA	0.7859	9.9835	0.55659	9.8533	0.64114	9.5787
DICE – DICE (1945)	NJ	-0.47017	-8.0962	-0.29193	-6.1135	-0.52836	-9.6449
J – Jaccard (1908)	NJ	-0.55374	-9.3346	-0.36788	-9.3598	-0.51923	-9.7077
K1 – Kulcznski (1927) coef. 1	NJ	-0.69221	-14.8578	-0.4351	-13.7926	-0.58122	-16.0262
K2 – Kulcznski (1927) coef. 2	NJ	-0.49912	-7.9988	-0.3862	-8.4618	-0.38373	-6.4997
O – Ochiai (1957)	NJ	-0.51031	-9.0625	-0.3764	-7.6594	-0.37952	-6.1808
PHI – Phi	NJ	-0.51479	-9.0697	-0.2089	-3.65	-0.41201	-7.0555
RR – Russel and Rao (1940)	NJ	-0.26776	-4.7001	-0.11976	-3.1332	-0.16614	-4.7405
Euclidean distance	NJ	0.53859	10.3391	0.24643	4.6292	0.5636	11.1529
Manhattan distance	NJ	0.44849	8.4883	0.44553	10.573	0.42152	12.4922
SM – Simple Matching	NJ	-0.50293	-10.5151	-0.43859	-14.6196	-0.31934	-10.311
UN1 – “unnamed” coef. 1	NJ	-0.36636	-6.1756	-0.43197	-14.2131	-0.42653	-11.011
UN2 – “unnamed” coef. 2	NJ	-0.62535	-11.3851	-0.54452	-15.9192	-0.47702	-9.1826
UN3 – “unnamed” coef. 3	NJ	-0.5929	-23.1388	-0.56111	-29.1071	-0.58183	-20.7647

UN5 – "unnamed" coef. 5	NJ	-0.46182	-7.8936	-0.37989	-7.8434	-0.4368	-7.5074
Y – Yule (1911)	NJ	-0.50894	-7.6932	-0.3069	-5.0216	-0.34696	-5.4206

Table S5. List of all plants sampled in this study.

Final ID	Extraction ID	Batch	Species	Locality/phenotype	Source
cup1	142ECUP002R	2	*E. cupidus*		Ridgemere
cup10	155ECUP019R	2	*E. cupidus*	Ohrigstad River	Ridgemere
cup11	156ECUP030R	2	*E. cupidus*	Bourkes Luck	Ridgemere
cup12	157ECUP031R	2	*E. cupidus*	Bourkes Luck	Ridgemere
cup13	158ECUP032R	2	*E. cupidus*	Swadini	Ridgemere
cup14	159ECUP035R	2	*E. cupidus*	Swadini	Ridgemere
cup15	161ECUP057R	2	*E. cupidus*	Strydom Tunnel	Ridgemere
cup16	164ECUP060R	2	*E. cupidus*	Above Tunnel	Ridgemere
cup17	165ECUP061R	2	*E. cupidus*	Above Tunnel	Ridgemere
cup18	166CUP1L	2	*E. cupidus*	Steenveld Farm: Blyde River canyon Nature Reserve	Lowveld BG
cup19	169CUP38L	2	*E. cupidus*		Lowveld BG
cup2	143ECUP004R	2	*E. cupidus*		Ridgemere
cup20	170CUP9L	2	*E. cupidus*	Bambi Area Helvetia section	Lowveld BG
cup21	171CUP47L	2	*E. cupidus*		Lowveld BG
cup22	174CUP58L	2	*E. cupidus*		Lowveld BG
cup23	175CUPn/n#2L	2	*E. cupidus*		Lowveld BG
cup24	52ECUP015R	1	*E. cupidus*	Ohrigstad River	Ridgemere
cup25	53ECUP016R	1	*E. cupidus*	Ohrigstad River	Ridgemere
cup3d1	145ECUP006R	2	*E. cupidus*	Brett Tunnel	Ridgemere
cup3d2	50ECUP006R	1	*E. cupidus*	Brett Tunnel	Ridgemere
cup4T	147ECUP008R	2	*E. cupidus*	Type Locality	Ridgemere
cup5T	148ECUP009R	2	*E. cupidus*	Type Locality	Ridgemere
cup6T	150ECUP011R	2	*E. cupidus*	Type Locality	Ridgemere
cup7T	151ECUP013R	2	*E. cupidus*	Type Locality	Ridgemere
cup8	152ECUP014R	2	*E. cupidus*	Ohrigstad River	Ridgemere
cup9	1554ECUP018R	2	*E. cupidus*	Ohrigstad River	Ridgemere
dol1	186EDOL001R	2	*E. dolomiticius*		Ridgemere
dol2	187EDOL007R	2	*E. dolomiticius*		Ridgemere

dol3	74EDOL002R	1	*E. dolomiticius*		Ridgemere
dol4	75EDOL003R	1	*E. dolomiticius*		Ridgemere
dol5	77EDOL005R	1	*E. dolomiticius*		Ridgemere
dol6	78EDOL006R	1	*E. dolomiticius*	Downs	Ridgemere
dol7	80DOL2L	1	*E. dolomiticius*		Lowveld BG
dol9	83DOLnn2L	1	*E. dolomiticius*		Lowveld BG
dye1	188EDYE003R	2	*E. dyerianus*		Ridgemere
dye10d1	199DYE30L	2	*E. dyerianus*		Lowveld BG
dye10d2	91DYE30L	1	*E. dyerianus*		Lowveld BG
dye11	200DYE82L	2	*E. dyerianus*		Lowveld BG
dye11Lb	85dyelebuvu	1	*E. dyerianus*		
dye12d1	201DYE90L	2	*E. dyerianus*		Lowveld BG
dye12d2	94DYE90L	1	*E. dyerianus*		Lowveld BG
dye16	93DYE97L	1	*E. dyerianus*		Lowveld BG
dye2	189EDYE004R	2	*E. dyerianus*		Ridgemere
dye3d1	190EDYE006R	2	*E. dyerianus*		Ridgemere
dye3d2	87EDYE006R	1	*E. dyerianus*		Ridgemere
dye4d1	191EDYE010R	2	*E. dyerianus*		Ridgemere
dye4d2	89EDYE010R	1	*E. dyerianus*		Ridgemere
dye5	192EDYE009R	2	*E. dyerianus*		Ridgemere
dye6Lb	193EDYE013R	2	*E. dyerianus*	levubuensis	Ridgemere
dye7	194DYE8L	2	*E. dyerianus*		Lowveld BG
dye8	196DYE87L	2	*E. dyerianus*		Lowveld BG
dye9	198DYE15L	2	*E. dyerianus*		Lowveld BG
eug1	10EEUG013R	1	*E. eugene-maraisii*	Geelhoutkloof type	Ridgemere
eug10	20EEUG025R	1	*E. eugene-maraisii*	Umkomaas Waterberg	Ridgemere
eug11	22eug2UP	1	*E. eugene-maraisii*	Palala (male)	UP
eug12	23eug3UP	1	*E. eugene-maraisii*	Marakele	UP
eug13	24eug4UP	1	*E. eugene-maraisii*	Marakele	UP
eug14	25eug5UP	1	*E. eugene-maraisii*	likely Kransberg	UP
eug15W	26EUG8L	1	*E. eugene-maraisii*	Waterberg	UP
16Lb	27euglebuvuA	1	unknown	levubuensis?	
eug17	28EUG13L	1	*E. eugene-maraisii*		Lowveld BG
eug18	29EUG14L	1	*E. eugene-maraisii*		Lowveld BG

eug19	32EUG28L	1	*E. eugene-maraisii*		Lowveld BG
eug2	11EEUG014R	1	*E. eugene-maraisii*	Thabazimbi	Ridgemere
eug20	33EUG32L	1	*E. eugene-maraisii*		Lowveld BG
eug21	3EEUG004R	1	*E. eugene-maraisii*	Sterkspruit Potgietersrus	Ridgemere
eug22P	Palala	1	*E. eugene-maraisii*		Ridgemere
eug23P	8EEUG010R	1	*E. eugene-maraisii*	Palala	Ridgemere
eug24	9EEUG012R	1	*E. eugene-maraisii*	Bokpoort	Ridgemere
eug3	12EEUG016R	1	*E. eugene-maraisii*	Palala Plato	Ridgemere
eug4	13EEUG018R	1	*E. eugene-maraisii*	Palala	Ridgemere
eug5	16EEUG021R	1	*E. eugene-maraisii*	Kransberg	Ridgemere
eug6	17EEUG022R	1	*E. eugene-maraisii*	Kransberg	Ridgemere
eug7	18EEUG023R	1	*E. eugene-maraisii*	Kransberg	Ridgemere
eug8P	19EEUG024R	1	*E. eugene-maraisii*	Palala	Ridgemere
eug9W	1EEUG002R	1	*E. eugene-maraisii*	Waterberg	Ridgemere
his1	34EHIS009R	1	*E. hirsutus*		Ridgemere
his2	35his2A	1	*E. hirsutus*		
mid1	176EMID006R	2	*E. middelburgensis*		Ridgemere
mid2d1Av	177EMID003R	2	*E. middelburgensis*	Avontuur	Ridgemere
mid2d2Av	63EMID003R	1	*E. middelburgensis*	Avontuur	Ridgemere
mid3	178MID2L	2	*E. middelburgensis*	Nelspruit	Lowveld BG
mid4	181MID59L	2	*E. middelburgensis*		Lowveld BG
mid5	185MID87L	2	*E. middelburgensis*		Lowveld BG
mid6	62EMID002R	1	*E. middelburgensis*		Ridgemere
mid8	64EMID004R	1	*E. middelburgensis*		Ridgemere
nub10Bt	116ENUB020R	2	*E. nubimontanus*	Strydom Tunnel/Big tooth	Ridgemere
nub11Sp	117ENUB023R	2	*E. nubimontanus*	Strydom Tunnel/Small pinna	Ridgemere
nub12Bt	118ENUB024R	2	*E. nubimontanus*	Strydom Tunnel/Big tooth	Ridgemere
nub13Fi	119ENUB025R	2	*E. nubimontanus*	Fine	Ridgemere
nub14Fi	120ENUB026R	2	*E. nubimontanus*	Strydom Tunnel gorge/Fine	Ridgemere
nub15D	121ENUB027R	2	*E. nubimontanus*	Dolomitica	Ridgemere
nub16R	122ENUB032R	2	*E. nubimontanus*	Mafefe/Robusta	Ridgemere
nub17D	123ENUB036R	2	*E. nubimontanus*	W of Trichards Dal/Dolomitica	Ridgemere
nub18	124ENUB040R	2	*E. nubimontanus*	NE of Penge	Ridgemere
nub19	125ENUB041R	2	*E. nubimontanus*	NE of Penge	Ridgemere

nub1Tw	107ENUB008R	2	*E. nubimontanus*	Kromellenboog/Twisted rachis	Ridgemere
nub20	126ENUB042R	2	*E. nubimontanus*	NW of Penge	Ridgemere
nub21M	128ENUB044R	2	*E. nubimontanus*	Near Strydom Tunnel/Miniature	Ridgemere
nub22Ft	129ENUB057R	2	*E. nubimontanus*	Fishtail	Ridgemere
nub23	131NUB1L	2	*E. nubimontanus*	Thabagolo-Confiscated	Lowveld BG
nub24	133NUB4L	2	*E. nubimontanus*		Lowveld BG
nub25	134NUB6L	2	*E. nubimontanus*		Lowveld BG
nub26	135NUB14L	2	*E. nubimontanus*		Lowveld BG
nub28	137NUB18L	2	*E. nubimontanus*		Lowveld BG
nub29	138NUB20L	2	*E. nubimontanus*		Lowveld BG
nub2Gr	108ENUB010R	2	*E. nubimontanus*	E of Kromellenboog Mine/Green	Ridgemere
nub30	139NUB22L	2	*E. nubimontanus*		Lowveld BG
nub31	141NUB28L	2	*E. nubimontanus*		Lowveld BG
nub32	39ENUB022R	1	*E. nubimontanus*	MH Big tooth near Strydom Tunnel	Ridgemere
nub33	41ENUB030R	1	*E. nubimontanus*	MH Dolomitica Giant near Downs	Ridgemere
nub34	43ENUB033R	1	*E. nubimontanus*	MH Mafefe Robusta Fishtail near Strydom Tunnel	Ridgemere
nub35	48NUB3L	1	*E. nubimontanus*		Lowveld BG
nub37	136NUB17L	2	*E. nubimontanus*		Lowveld BG
nub3Gr	109ENUB011R	2	*E. nubimontanus*	E of Kromellenboog Mine/Green	Ridgemere
nub4Gr	110ENUB012R	2	*E. nubimontanus*	E of Kromellenboog Mine/Green	Ridgemere
nub5DG	111ENUB013R	2	*E. nubimontanus*	Downs/Dolomitica Giant	Ridgemere
nub6D	112ENUB014R	2	*E. nubimontanus*	Downs/Dolomitica	Ridgemere
nub7DB	113ENUB015R	2	*E. nubimontanus*	Downs/Blue Dolomitica	Ridgemere
nub8D	114ENUB016R	2	*E. nubimontanus*	Downs/Dolomitica	Ridgemere
nub9Bt	115ENUB019R	2	*E. nubimontanus*	Strydom Tunnel/Big tooth	Ridgemere
Excluded	CUP9L	1	*E. cupidus*	Mpumulanga -Bambi Area, Helvetia section	Lowveld BG
Excluded	CUP34L	2	*E. cupidus*		Lowveld BG
Excluded	CUP35L	2	*E. cupidus*		Lowveld BG
Excluded	CUP53L	2	*E. cupidus*		Lowveld BG
Excluded	CUP54L	2	*E. cupidus*		Lowveld BG
Excluded	CUPn/n#1L	1	*E. cupidus*	Broader leaflets	Lowveld BG
Excluded	ECUP005R	1	*E. cupidus*	Tunnel	Ridgemere
Excluded	ECUP005R	2	*E. cupidus*	Tunnel	Ridgemere
Excluded	ECUP007R	2	*E. cupidus*	Type Locality	Ridgemere

Excluded	ECUP010R	2	*E. cupidus*	Type Locality	Ridgemere
Excluded	ECUP013R	1	*E. cupidus*	Type Locality	Ridgemere
Excluded	ECUP028R	1	*E. cupidus*	Bourkes Luck	Ridgemere
Excluded	ECUP029R	1	*E. cupidus*	Bourkes Luck	Ridgemere
Excluded	ECUP033R	1	*E. cupidus*	Swadini	Ridgemere
Excluded	ECUP034R	1	*E. cupidus*	Swadini	Ridgemere
Excluded	ECUP036R	2	*E. cupidus*	Swadini	Ridgemere
Excluded	ECUP053R	1	*E. cupidus*	Giant Cupidus	Ridgemere
Excluded	ECUP058R	2	*E. cupidus*	Strydom Tunnel	Ridgemere
Excluded	ECUP059R	2	*E. cupidus*	Above Tunnel	Ridgemere
Excluded	DOL3L	1	*E. dolomiticius*		Lowveld BG
Excluded	DOLn/n#1L	1	*E. dolomiticius*		Lowveld BG
Excluded	DOLn/n#3L	1	*E. dolomiticius*		Lowveld BG
Excluded	E.dolA	1	*E. dolomiticius*		abel
Excluded	EDOL004R	1	*E. dolomiticius*		Ridgemere
Excluded	DYE101L	2	*E. dyerianus*		Lowveld BG
Excluded	DYE15L	1	*E. dyerianus*		Lowveld BG
Excluded	DYE45L	1	*E. dyerianus*		Lowveld BG
Excluded	DYE72L	2	*E. dyerianus*		Lowveld BG
Excluded	DYE82L	1	*E. dyerianus*		Lowveld BG
Excluded	DYE97L	2	*E. dyerianus*		Lowveld BG
Excluded	DYEn/n#1L	1	*E. dyerianus*	Finer leaf, Long leaflet	Lowveld BG
Excluded	EDYE009R	1	*E. dyerianus*		Ridgemere
Excluded	EDYE013R	1	*E. dyerianus*	levubuensis	Ridgemere
Excluded	EEUG003R	1	*E. eugene-maraisii*	Waterberg	Ridgemere
Excluded	EEUG005R	1	*E. eugene-maraisii*	Kransberg	Ridgemere
Excluded	EEUG007R	1	*E. eugene-maraisii*	Kransberg	Ridgemere
Excluded	EEUG009R	1	*E. eugene-maraisii*	Palala	Ridgemere
Excluded	EEUG019R	1	*E. eugene-maraisii*	Kransberg	Ridgemere
Excluded	EEUG020R	1	*E. eugene-maraisii*	Kransberg	Ridgemere
Excluded	eug kransH	1	*E. eugene-maraisii*	Kranskop	Hannes
Excluded	EUG15L	1	*E. eugene-maraisii*		Lowveld BG
Excluded	EUG18L	1	*E. eugene-maraisii*		Lowveld BG
Excluded	eug1UP	1	*E. eugene-maraisii*		UP

Excluded	mid avontA	1	*E. middelburgensis*		Abel
Excluded	MID12L	1	*E. middelburgensis*	Loskop Dam Nature Reserve	Lowveld BG
Excluded	MID13L	1	*E. middelburgensis*	Mpumulanga	Lowveld BG
Excluded	MID29L	1	*E. middelburgensis*	Lammerkop	Lowveld BG
Excluded	MID32L	2	*E. middelburgensis*		Lowveld BG
Excluded	MID43L	2	*E. middelburgensis*		Lowveld BG
Excluded	MID5L	1	*E. middelburgensis*	TPA_Garden Genebank	Lowveld BG
Excluded	MID61L	2	*E. middelburgensis*		Lowveld BG
Excluded	MID69L	2	*E. middelburgensis*		Lowveld BG
Excluded	MID75L	2	*E. middelburgensis*		Lowveld BG
Excluded	EMID001R	1	*E. middelburgensis*	RH	Ridgemere
Excluded	EMID005R	1	*E. middelburgensis*	Theunis Bester/ ex Ferguson	Ridgemere
Excluded	EMID006R	1	*E. middelburgensis*		Ridgemere
Excluded	EMID014R	1	*E. middelburgensis*	RH Avontuur	Ridgemere
Excluded	EMID019R	1	*E. middelburgensis*	MH Doringkop	Ridgemere
Excluded	NUB25L	2	*E. nubimontanus*		Lowveld BG
Excluded	ENUB009R	1	*E. nubimontanus*	N of Kromellenboog Mine/Twister robust cupidus	Ridgemere
Excluded	ENUB017R	1	*E. nubimontanus*	Tunnel/Munchii	Ridgemere
Excluded	ENUB018R	1	*E. nubimontanus*	Tunnel/Munchii	Ridgemere
Excluded	ENUB021R	1	*E. nubimontanus*	Strydom Tunnel/cupidus form	Ridgemere
Excluded	ENUB029R	1	*E. nubimontanus*	S of Downs/Dolomitica	Ridgemere
Excluded	ENUB037R	1	*E. nubimontanus*	W of Trichards Dal/Dolomitica	Ridgemere
Excluded	ENUB038R	1	*E. nubimontanus*	E of Penge	Ridgemere
Excluded	ENUB039R	1	*E. nubimontanus*	NE of Penge	Ridgemere
Excluded	ENUB059R	2	*E. nubimontanus*	Robusta	Ridgemere
Excluded	ENUB062	1	*E. nubimontanus*	Penge	Ridgemere

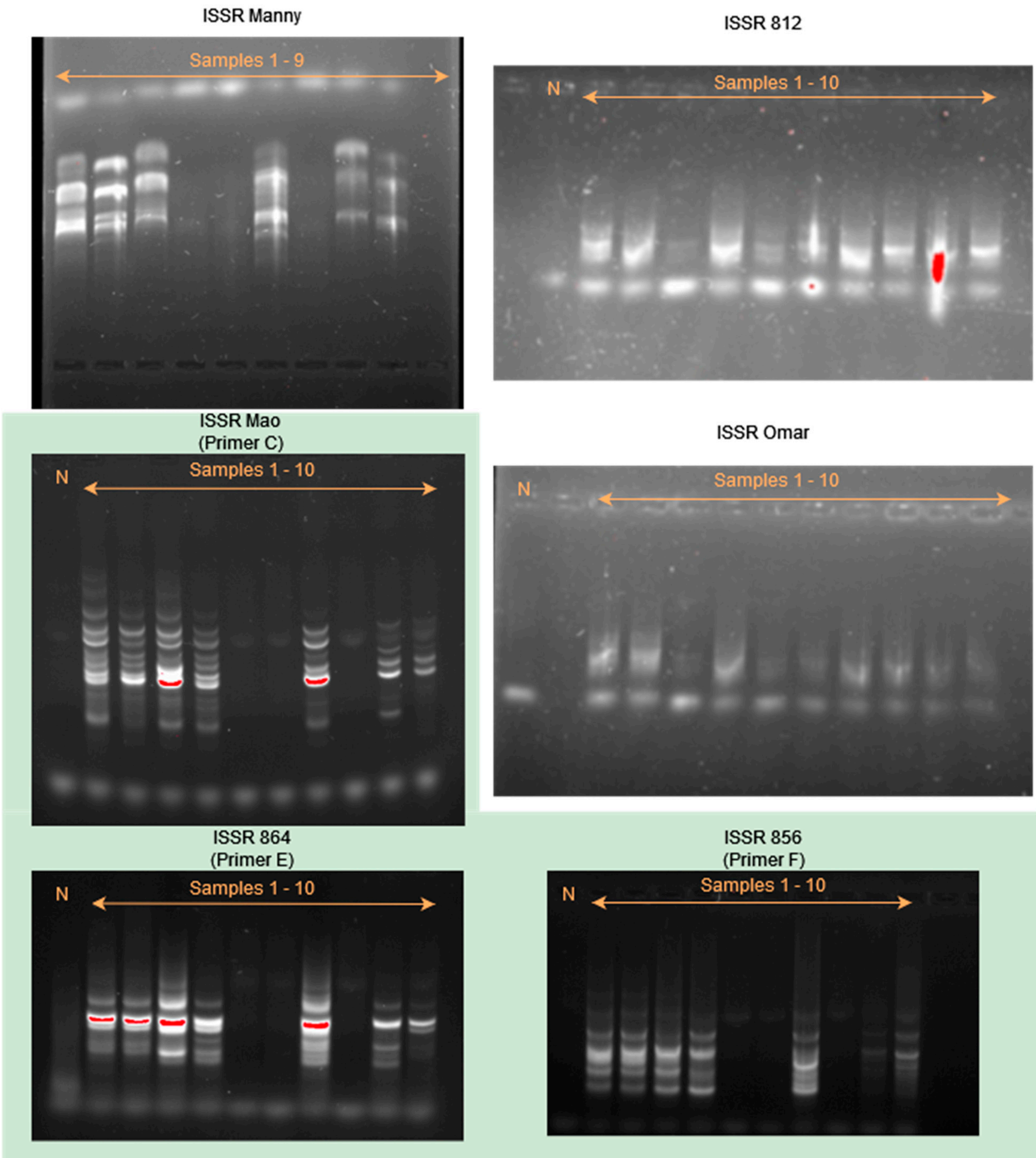


Figure S1. Gels comparing different primers tested for this study. A subset of 10 cycad samples were used to determine which primers were best suited for the study. Primers highlighted in green were selected for this study.

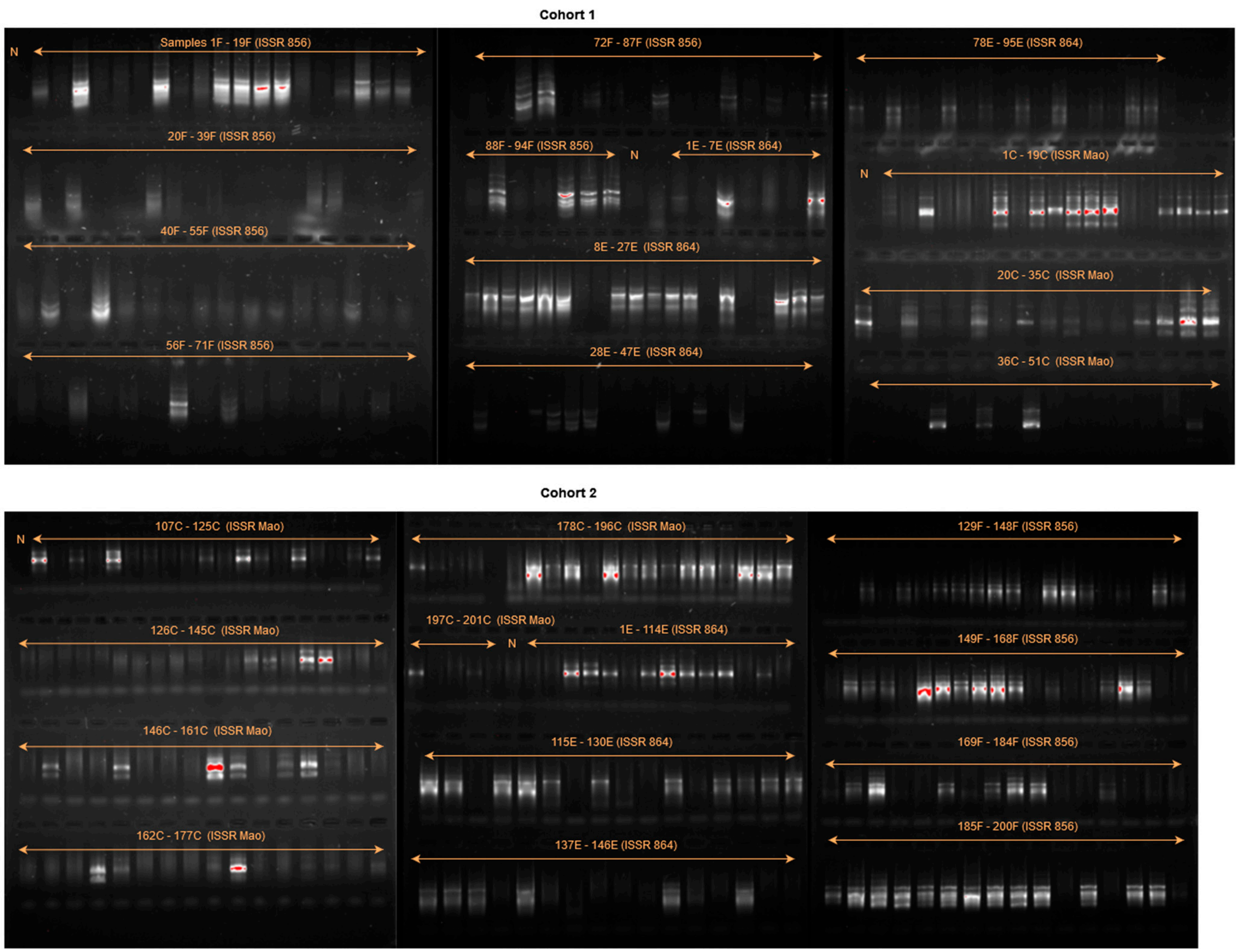
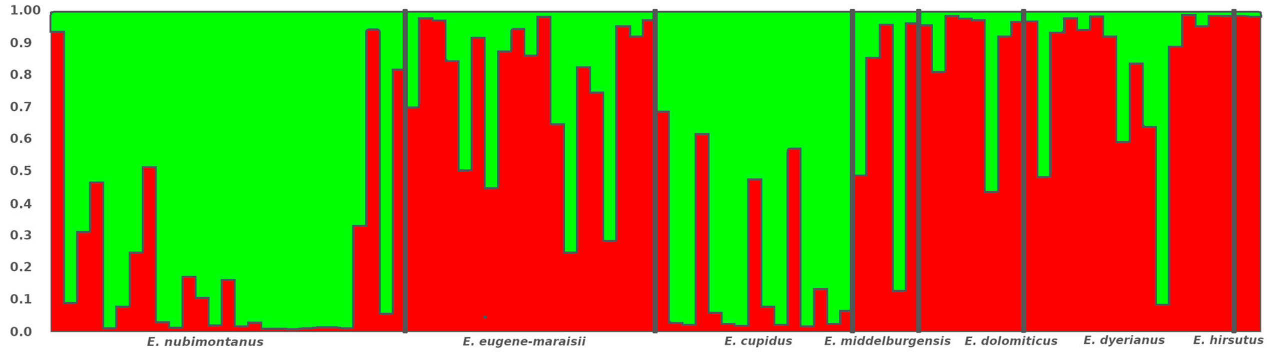
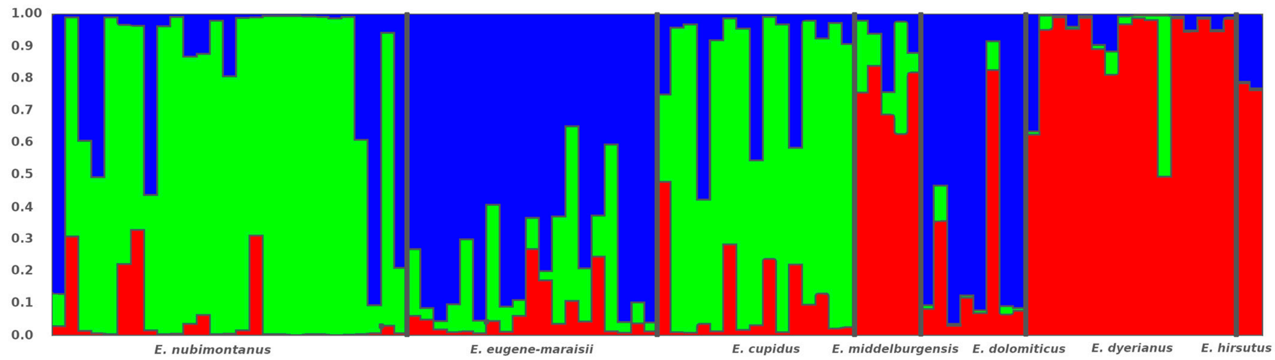


Figure S2. Gels showing amplification results of samples using the selected ISSR primers**.** Each sample (numbered 1 - 201) was amplified with the selected primers indicated by the letter C (ISSR Mao), E (ISSR 864) or F (ISSR 856). Samples were selected for our study based on gel outcomes for all three primers. Samples which did not amplify for all three primers were excluded from the study (see Table S3). N denoted the position of the negative control included with each PCR run. Cohorts 1 and 2 represent the two separate batches in which sample DNA was extracted.

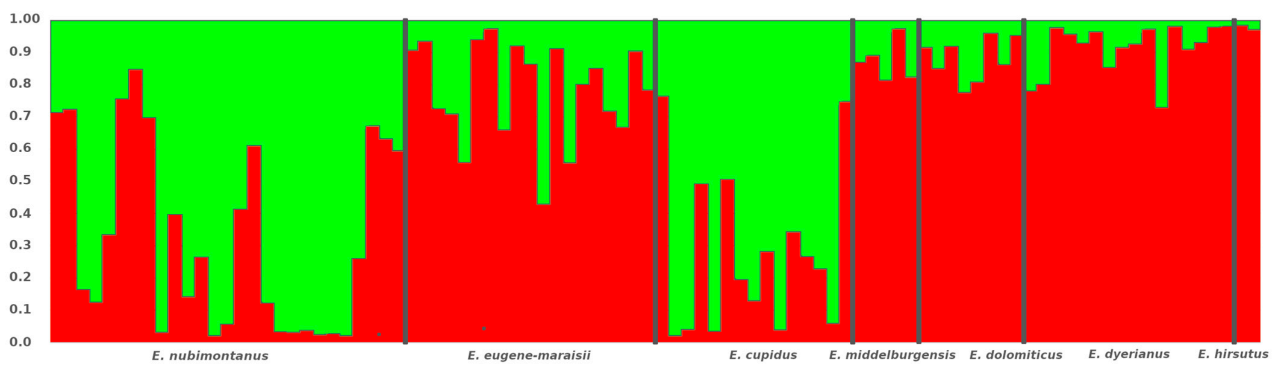


(a)

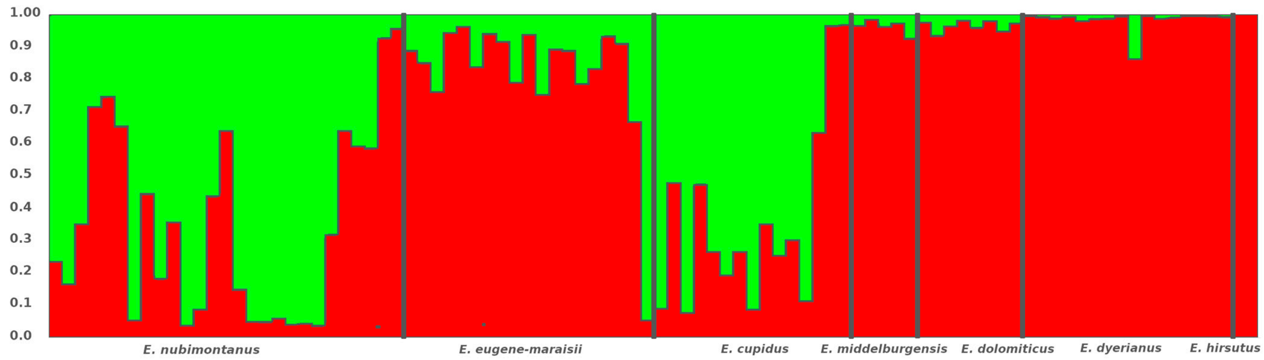


(b)

Figure S3. STRUCTURE barplots showing the proportion of membership of samples assigned to K = 7 clusters within the *Encephalartos eugene-maraisii* complex. Results are based on ISSR fragments scored at a 100 relative fluorescence unit (rfu) cut-off value. The dataset was tested using the Standard STRUCTURE model (a) and the LOCPRIOR model (b) that accounts for known locality data prior to the run.

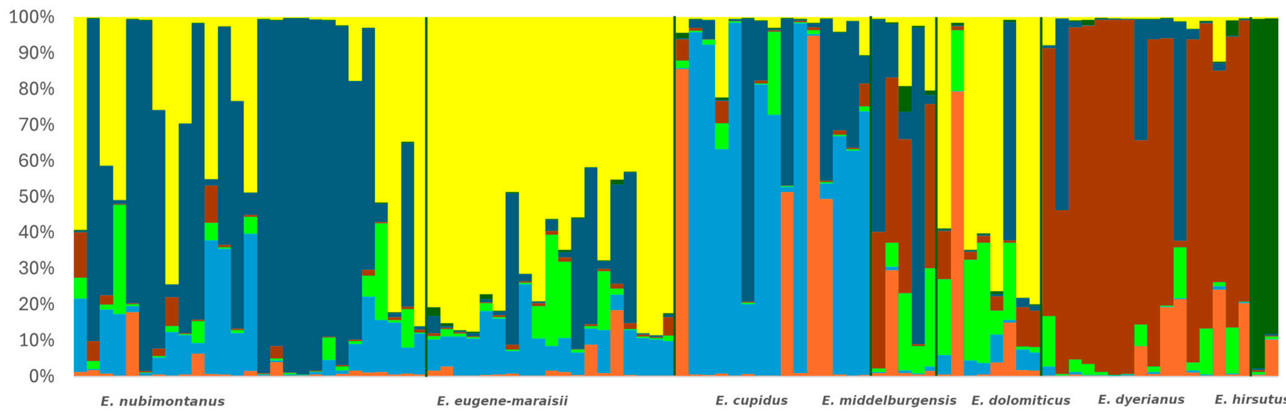


(a)

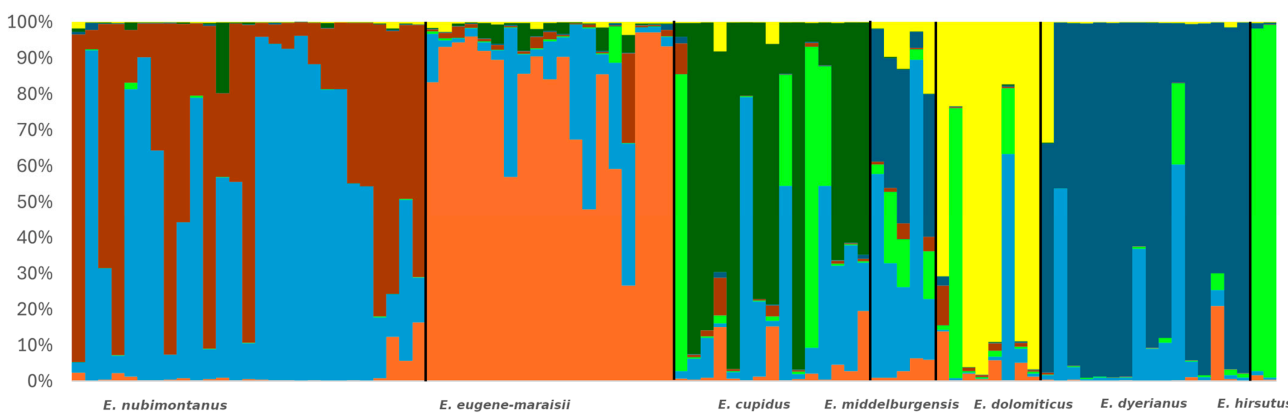


(b)

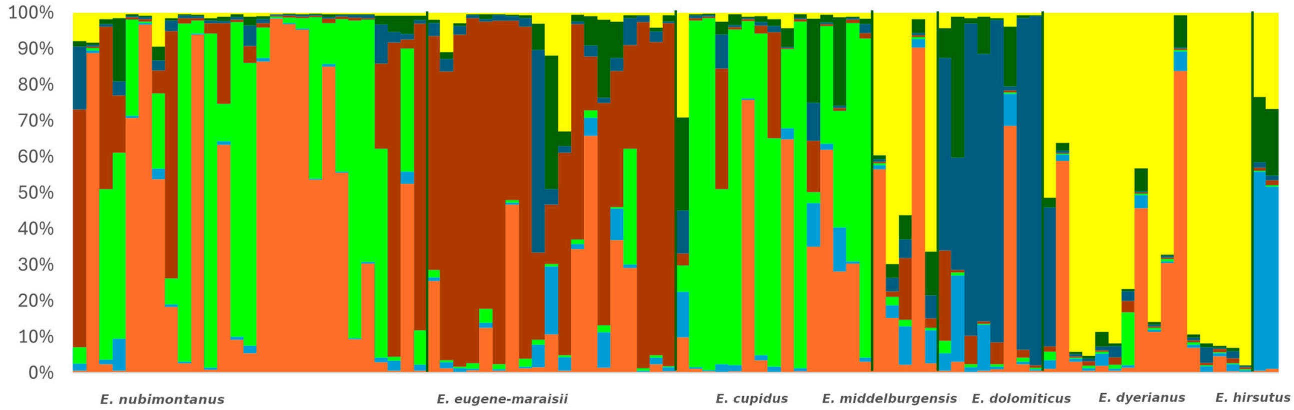
Figure S4. STRUCTURE barplots showing the proportion of membership of samples assigned to K = 7 clusters within the *Encephalartos eugene-maraisii* complex. Results are based on ISSR fragments scored at a 200 relative fluorescence unit (rfu) cut-off value. The dataset was tested using the Standard STRUCTURE model (a) and the LOCPRIOR model (b) that accounts for known locality data prior to the run.



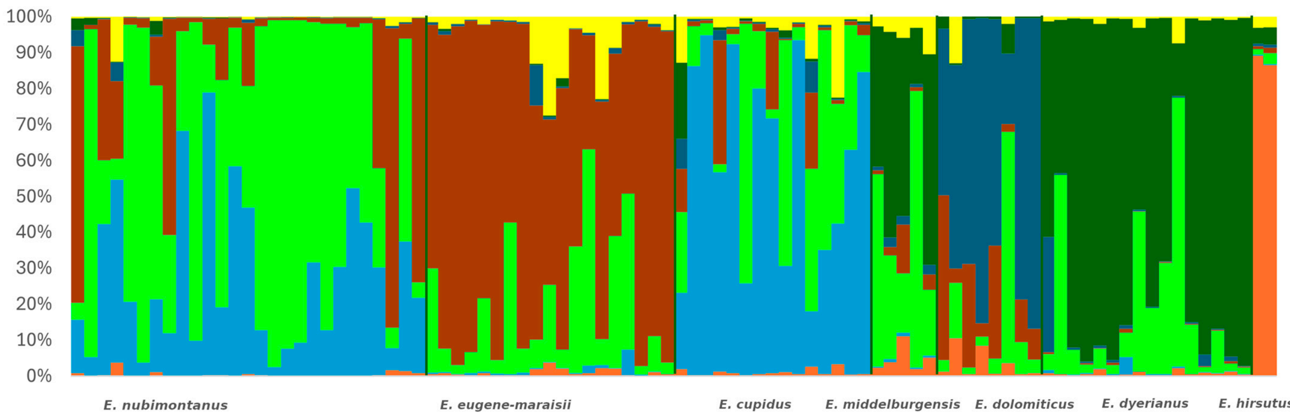
(a)



(b)


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
E. nubimontanus
E. eugene-maraisii
E. cupidus
E. middelburgensis
E. dolomiticus
E. dyerianus
E. hirsutus

(c)


100%
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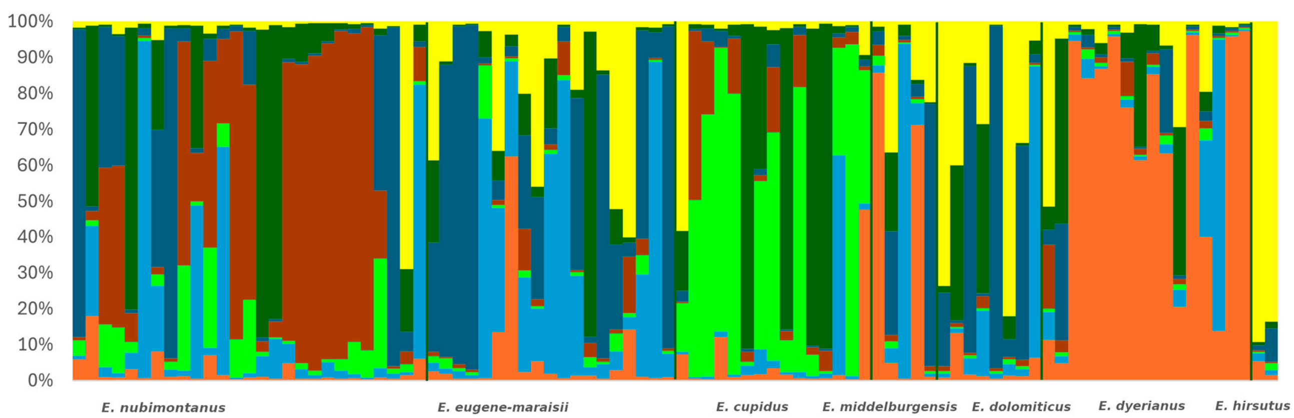
10%
0%
E. nubimontanus
E. eugene-maraisii
E. cupidus
E. middelburgensis
E. dolomiticus
E. dyerianus
E. hirsutus

(d)


100%
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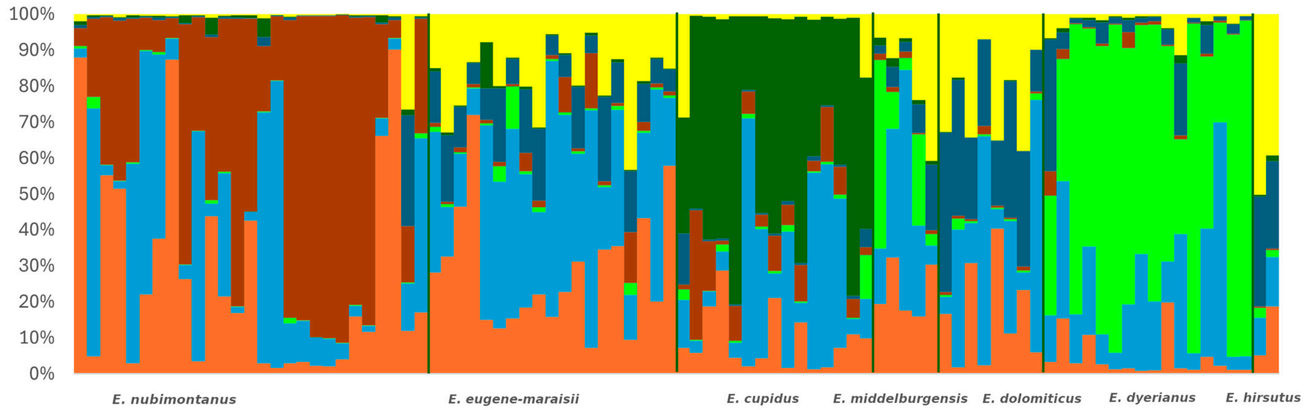
E. nubimontanus
E. eugene-maraisii
E. cupidus
E. middelburgensis
E. dolomiticus
E. dyerianus
E. hirsutus

(e)



(f)

Figure S5. STRUCTURE barplots showing the proportion of membership of samples assigned to K = 7 clusters within the *Encephalartos eugene-maraisii* complex. Results are based on ISSR fragments scored at a 50 (a,b), 100 (c,d) and 200 (e,f) relative fluorescence unit (rfu) cut-off value. The dataset was tested using the Standard STRUCTURE model (a,c,e,) and the LOCPRIOR model (b,d,f) that accounts for known locality data prior to the run.

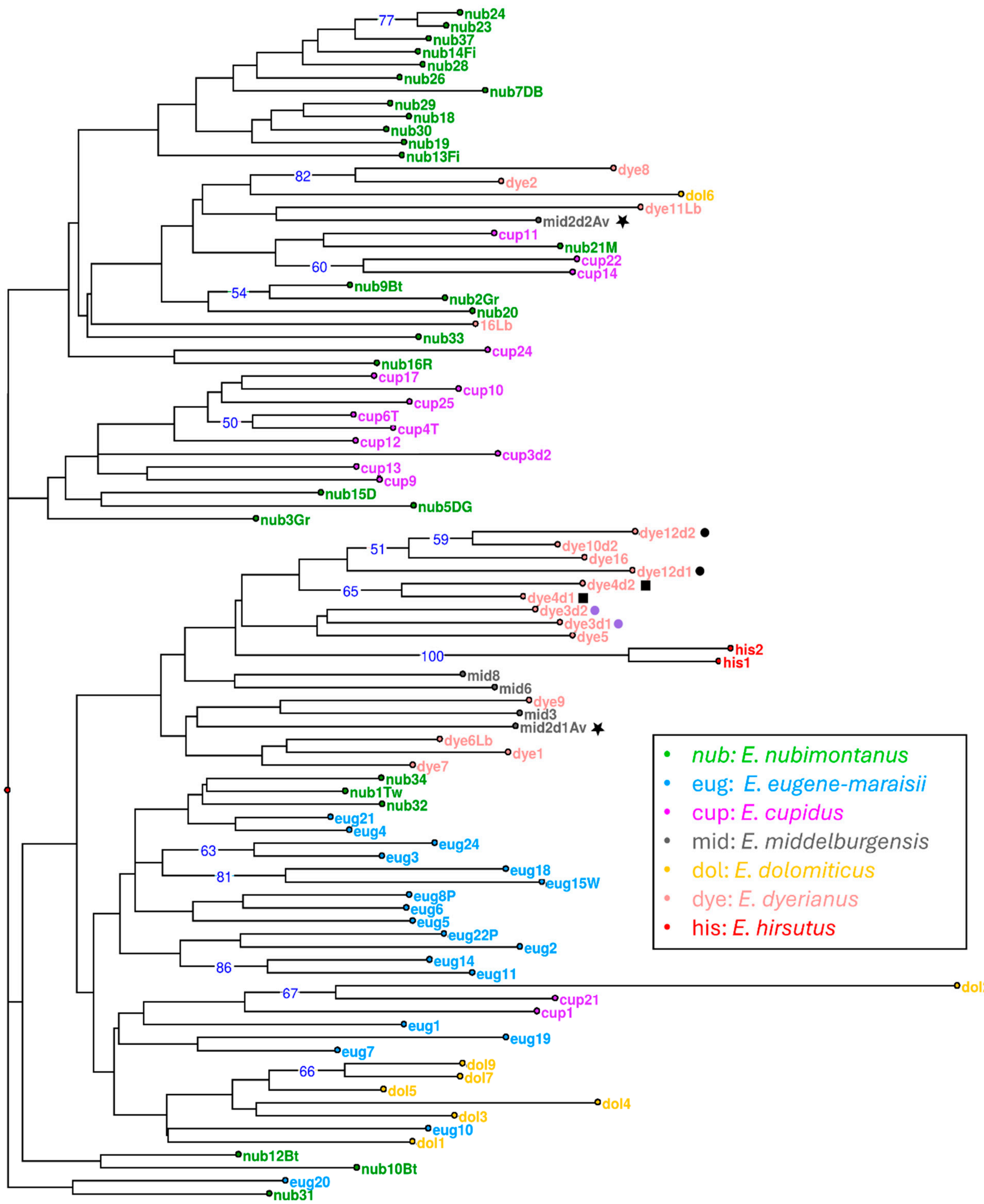


Figure S6. Neighbor joining dendrogram of the *Encephalartos eugene-maraisii* complex based on ISSR markers at a relative fluorescence unit (rfu) cut-off of 100rfu. Bootstrap support greater than 50% is indicated for applicable nodes. Band presence and absence was used to compute genetic distances using the DICE coefficient. The colour of each sample corresponds to its species and sample names are represented by the first three letters of their species epithet. Sample duplicates, representing material obtained from the same plant, but extracted in a different DNA extraction batch, are indicated by the symbols.

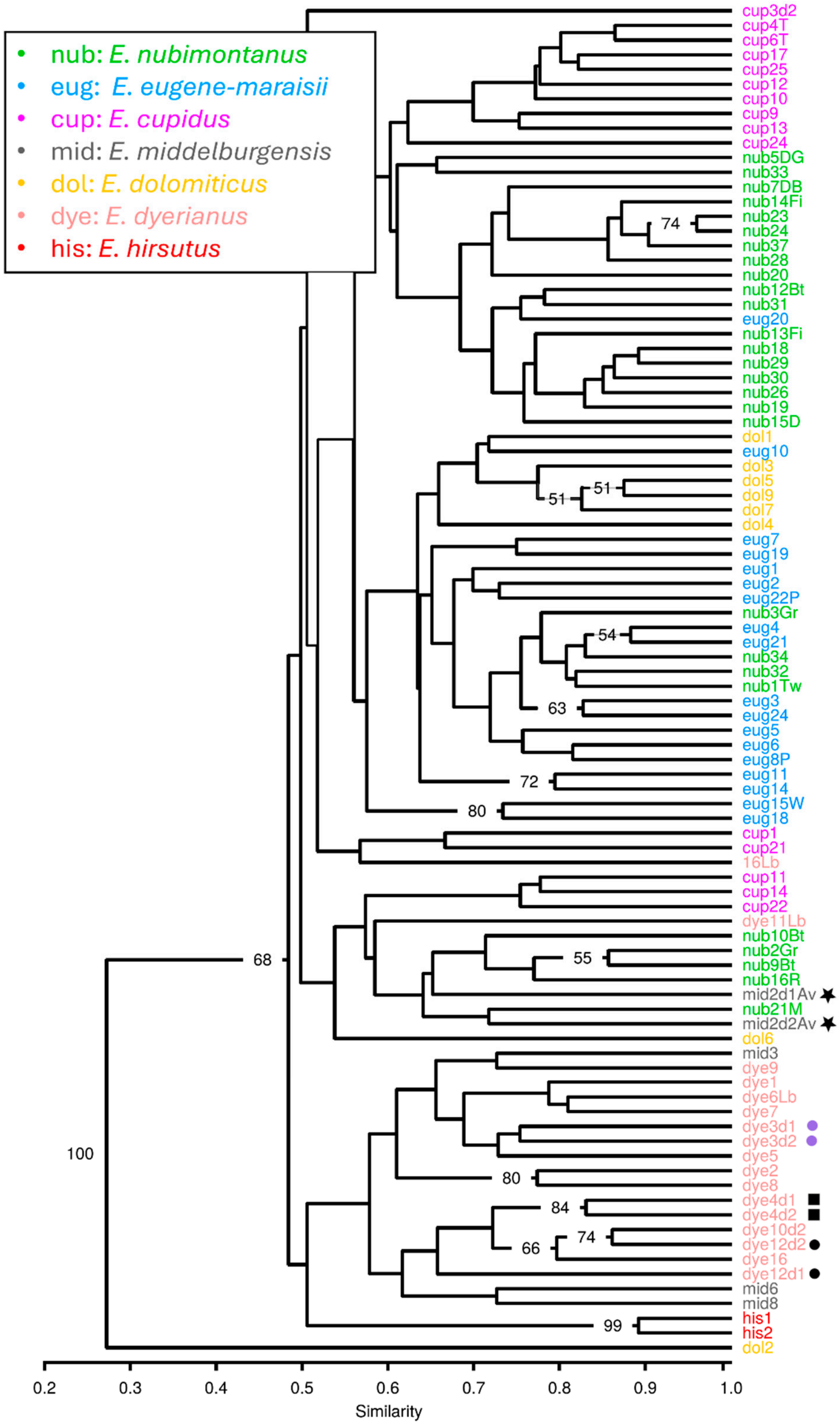


Figure S7. UPGMA dendrogram of the *Encephalartos eugene-maraisii* complex based on ISSR markers with a minimum band intensity of 100 relative fluorescence units (rfu). Bootstrap support greater than 50% is indicated for applicable nodes. Band presence and absence was used to compute genetic distances using the DICE coefficient. Specimen names are represented by the first three letters of their species epithet. Sample duplicates, representing material obtained from the same plant, but extracted in a different DNA extraction batch, are indicated by the symbols.

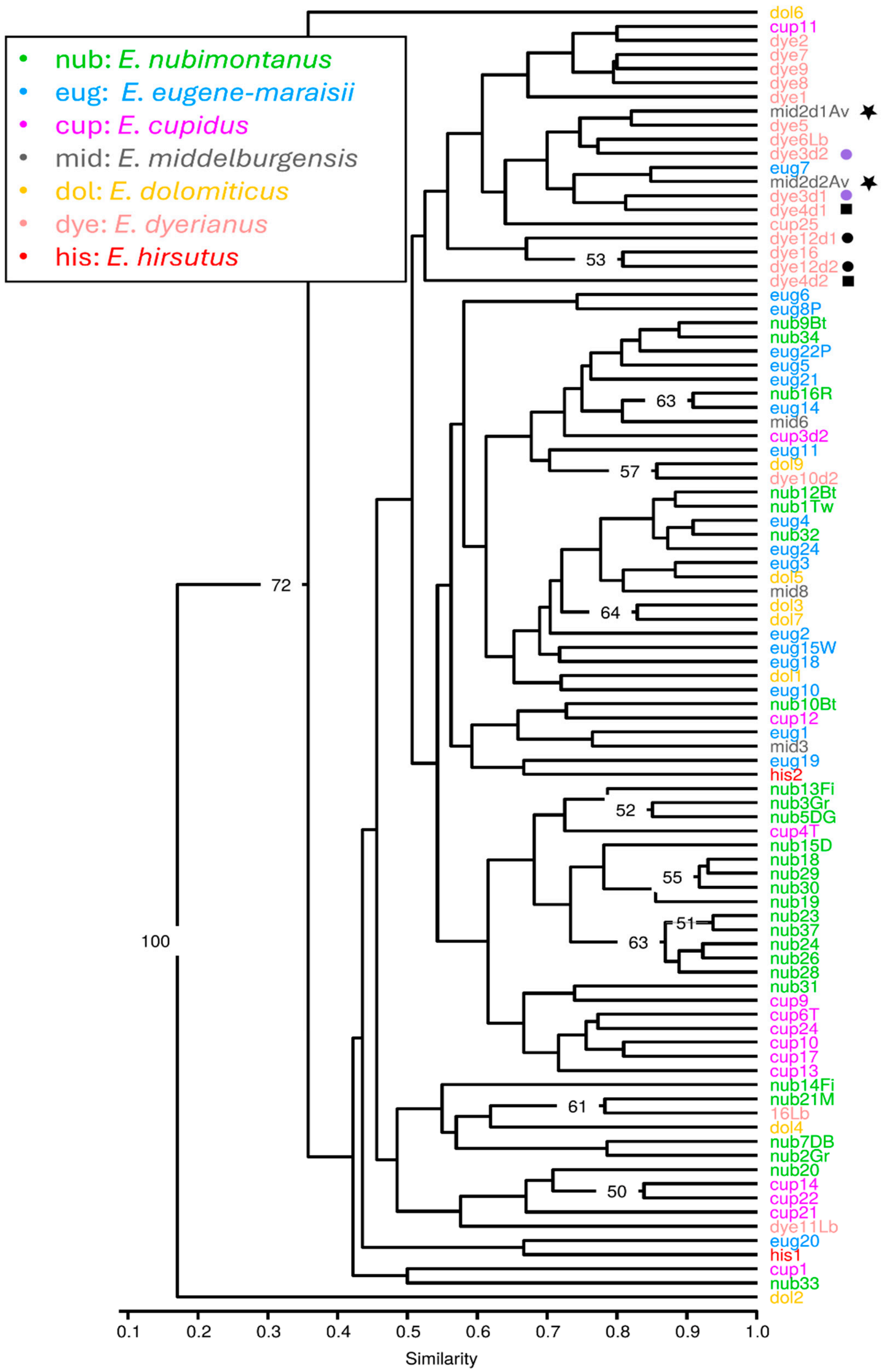


Figure S8. UPGMA dendrogram of the *Encephalartos eugene-maraisii* complex based on ISSR markers at a relative fluorescence unit (rfu) cut-off of 200rfu. Bootstrap support greater than 50% is indicated for applicable nodes. Band presence and absence was used to compute genetic distances using the DICE coefficient. Specimen names are represented by the first three letters of their species epithet. Sample duplicates, representing material obtained from the same plant, but extracted in a different DNA extraction batch, are indicated by the symbols.

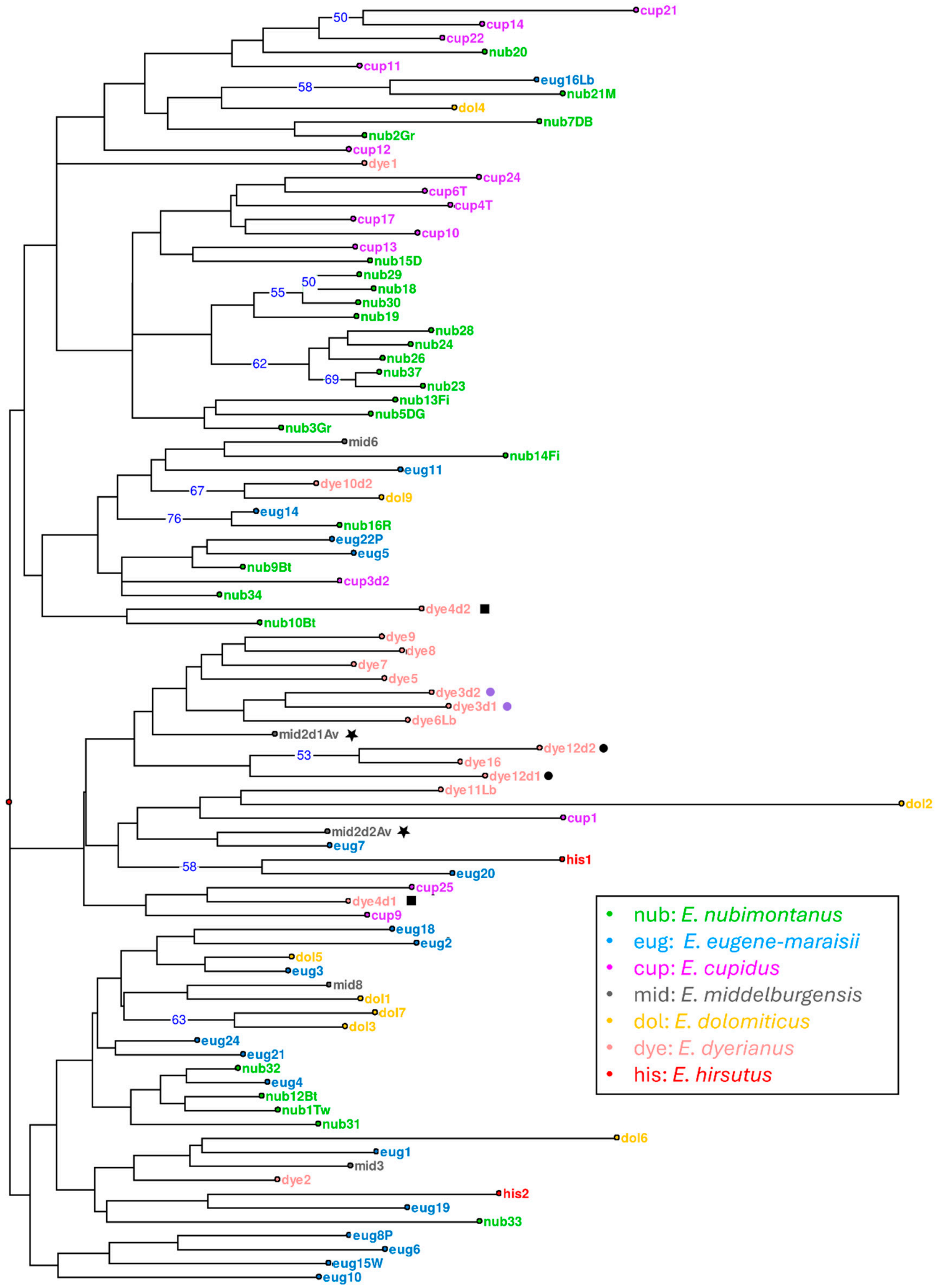


Figure S9. Neighbor Joining dendrogram of the *Encephalartos eugene-maraisii* complex based on ISSR markers at a relative fluorescence unit (rfu) cut-off of 200rfu. Bootstrap support greater than 50% is indicated for applicable nodes. Band presence and absence was used to compute genetic distances using the DICE coefficient. Specimen names are represented by the first three letters of their species epithet, corresponding to Table S5. Sample duplicates, representing material obtained from the same plant, but extracted in a different DNA extraction batch, are indicated by the symbols.

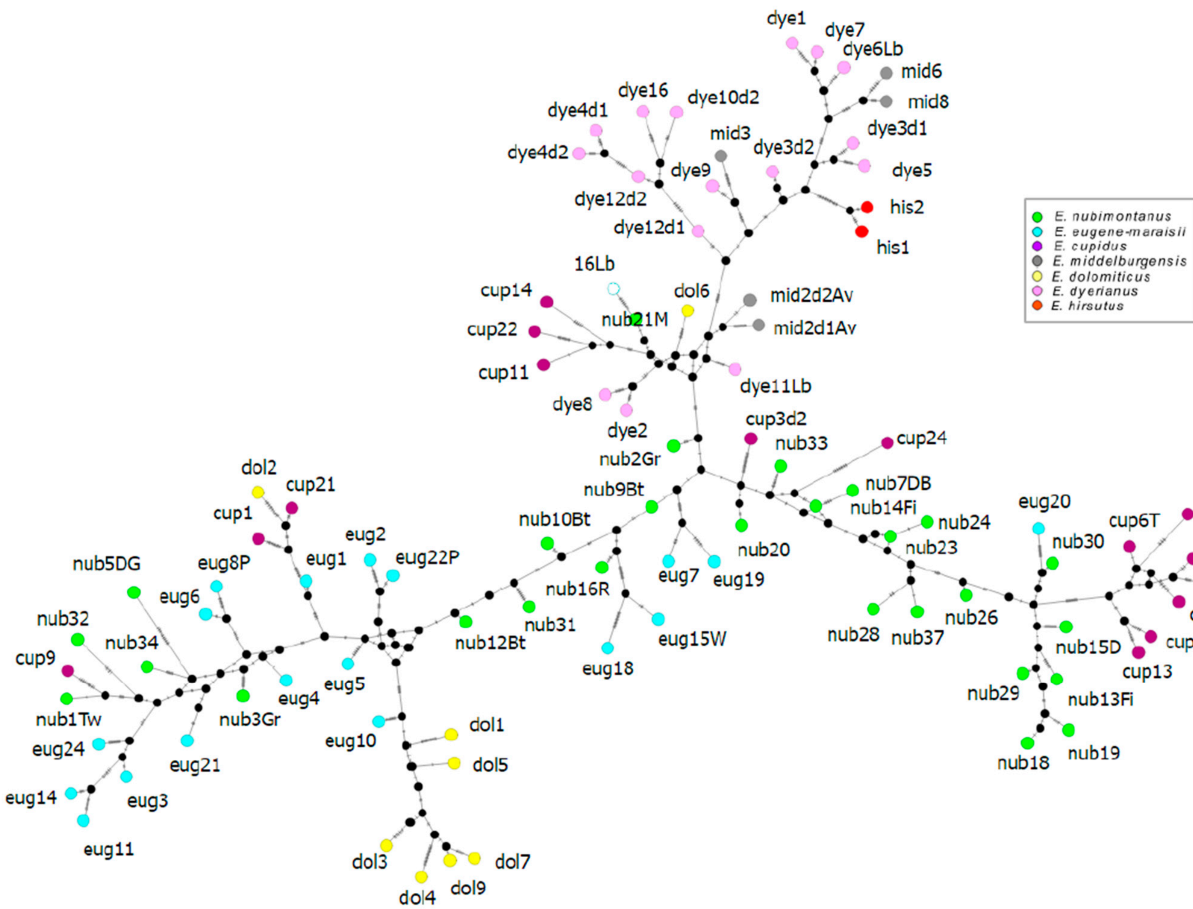


Figure S10. Median joining networks of the *Encephalartos eugene-maraisii complex* based on ISSR markers with a minimum band intensity of 100 relative fluorescent units (rfu). Colours denote the species of each sample in this study.

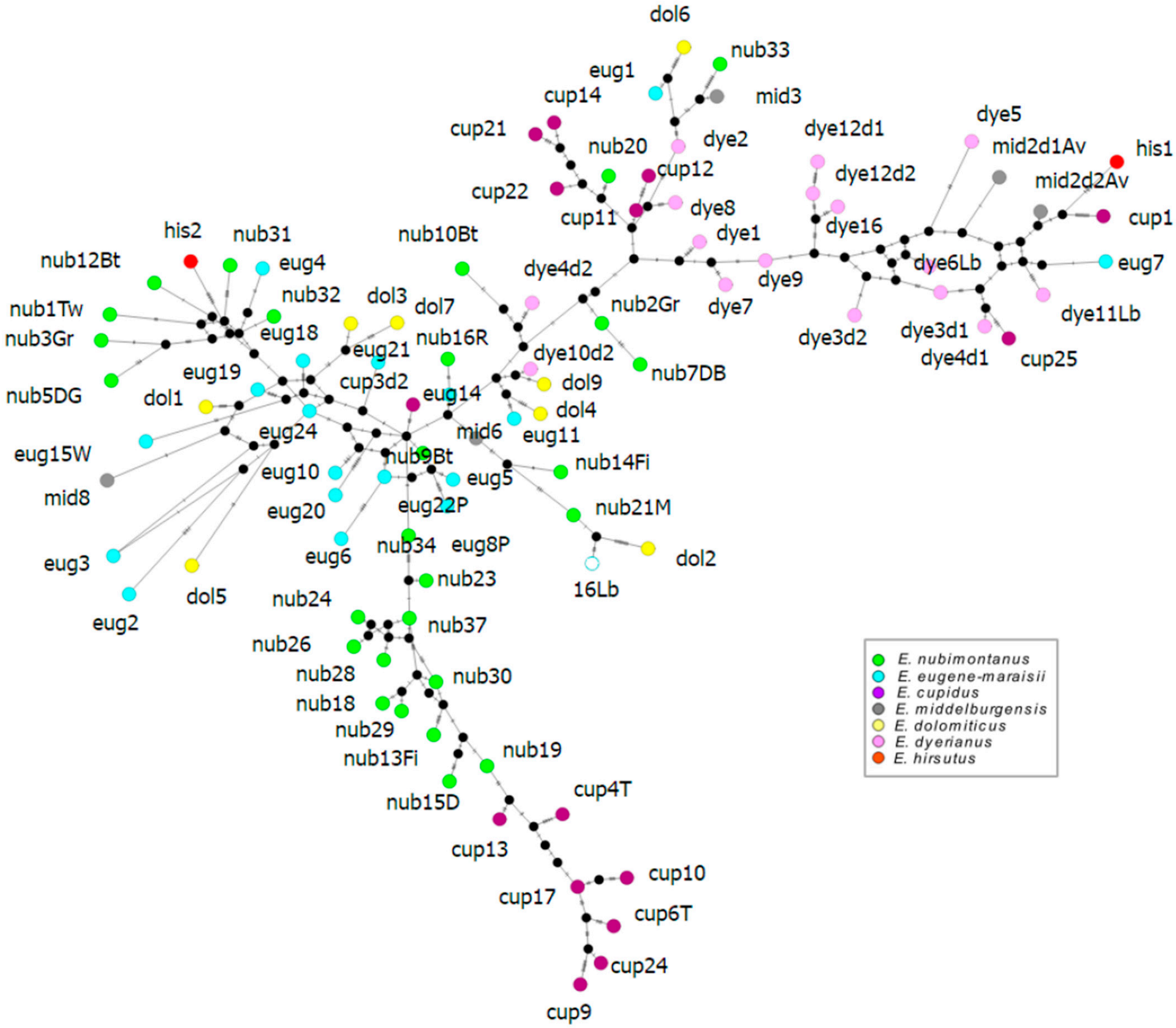


Figure S11. Median joining network of the *Encephalartos eugene-maraisii complex* based on ISSR markers with a minimum band intensity of 200 relative fluorescent units (rfu). Colours denote the species of each sample in this study.