Supplementary material

**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\*”.

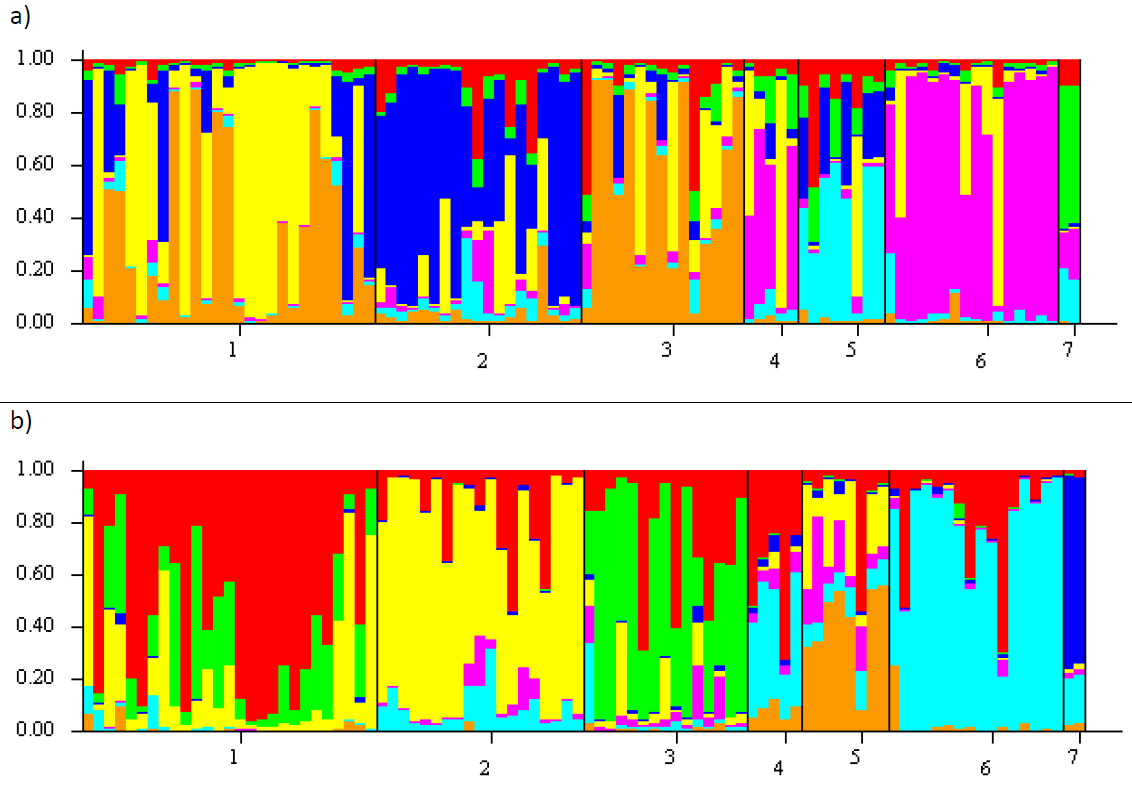
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Species** | **Region** | **Main aims** | **Key findings** | **Reference** |
| *Ceratozamia fuscoviridis* D.Moore (Zamiaceae) | Mexico | Effect of anthropogenic disturbance of species diversity using microsatellites and ISSR | Clustering of taxa corresponded to level of disturbance instead of geographic distance  Negative relationship between level of genetic diversity and degree of disturbance | (García-Montes et al., 2020) |
| *Encephalartos woodii* hort.(Zamaiceae) | South Africa | 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 | (Prakash et al., 2008) |
| *Cycas debaoensis* Y.C.Zhong & C.J.Chen (Cycadaceae) | South China | Determine genetic differentiation | Phenetic analysis reveals two clusters of grouped populations showing relative similarity of all populations to one another.  Genetic diversity among populations was high | (Jianguang et al., 2005) |
| *Cycas diannanensis* Z.T.Guan & G.D.Tao (= *Cycas parvula*) and *Cycas balansae* Warb. (Cycadaceae) | South China | Examine level and distribution of genetic variation in two species | Populations of *C. diannanensis* with lower spatial separation had low expected heterozygosity and very low genetic differentiation compared to the more widely distributed *C. balansae* | (Xiao et al., 2005) |
| *Encephalartos* Lehm. species (Zamiaceae) | African continent | Phylogenetic relationships between 52 *Encephalartos* species using ISSRs,  ITS and rbcLa sequences, and morphological and geographical characters | 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 | (Treutlein et al., 2005) \* |
| *Cycas guizhouensis* K.M.Lan & R.F.Zou(Cycadaceae) | South China | Genetic diversity | Low genetic diversity within populations and high differentiation among populations  Evidence of potential drift and inbreeding | (Long‐Qian et al., 2004) |
| *Cycas fairylakea* D.Yue Wang(Cycadaceae) | South China | Genetic diversity | Species clustered into two groups with UPGMA and Principal components analysis in agreement | (Wang et al., 2006) |
| Dioon spinulosum Dyer ex Eichler  (Zamiaceae) | Egypt | 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 | (Marwa et al., 2020) |
| *Cycas taitungensis* C.F.Shen, K.D.Hill, C.H.Tsou & C.J.Chen(Cycadaceae) | Taiwan | 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 | (Liao et al., 2018) |
| *Cycas beddomei* Dyer(Cycadaceae) | India | Genetic diversity | 15 out of 20 ISSR primers produced unambiguous bands  Phenetic analysis clusters species in two groups corresponding to different sampling locations | (Rout, G.R., Jadhao, K.R., Swain, D. and Panda, 2014) |
| *Cycas balansae* Warb complex (Cycadaceae) | South China | Taxonomy of *Cycas balansae* complex | ISSRs revealed low genetic diversity  Phenetic analysis with UPGMA reveals five distinct clusters corresponding to species | (Xiao et al., 2006) |
| *Dioon caputoi* De Luca, Sabato & Vázq.Torres  *Dioon planifolium* Salas-Mor., Chemnick & T.J.Greg  (Zamiaceae) | Mexico | 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 | (Cabrera-Toledo et al., 2019) |
| *Zamia inermis* Vovides, J.D.Rees & Vázq.Torres  (Zamiaceae) | Mexico | 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 | (Iglesias-Andreu and Favian-Vega, 2021) |
| *Zamia furfuracea* L.f  (Zamiaceae) | Mexico | 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 | (Octavio-Aguilar et al., 2017) |
| *Cycas* *circinalis* L. | India | Sex determination in commercially important plants using RAPD and ISSR | Identified a male specific RAPD marker in *C. circinalis*  No male or female sex-specific ISSR markers were found | (Gangopadhyay et al., 2007) |
| *Ceratozamia* *mexicana* Brongn. (Zamiaceae) | Mexico | 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 | (Sánchez-Coello et al., 2018) |
| *Ceratozamia mexicana* Brongn.  (Zamiaceae) | Mexico | 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 | (Guadalupe Sánchez-Coello et al., 2012) |

**Table S2***.* 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.

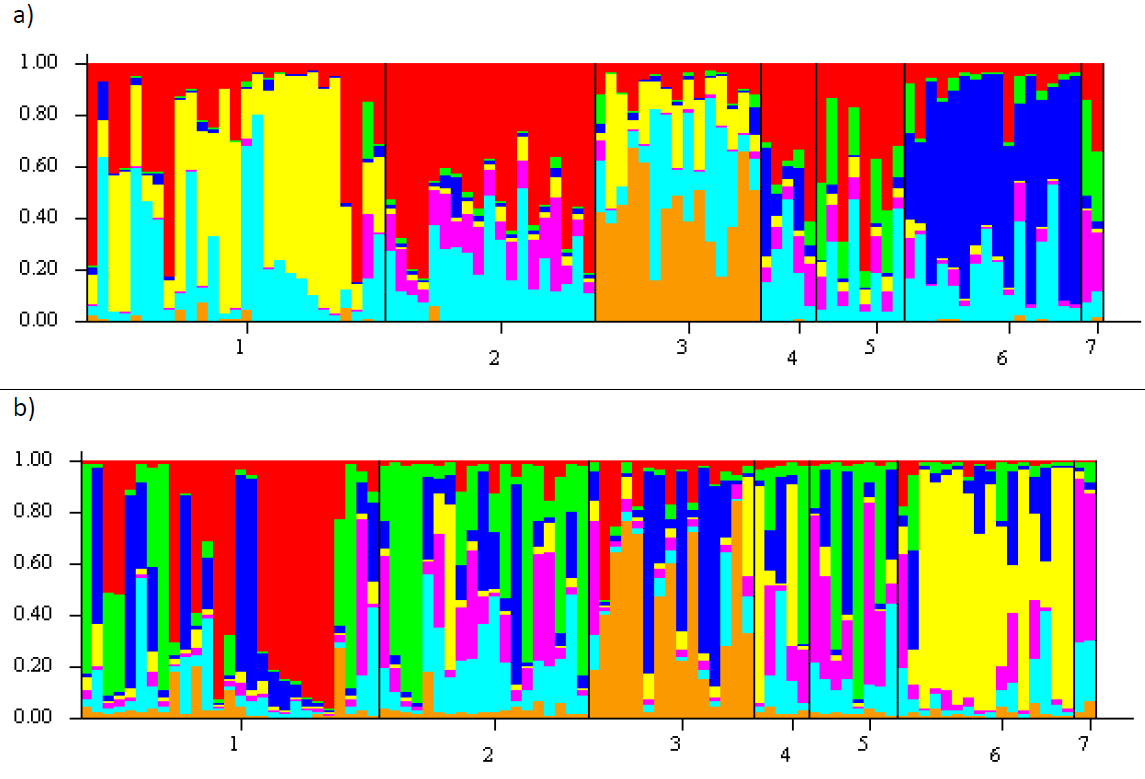
|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Distance coefficient** | **Tree type** | **RFU cut-off value** | | | | | |
|  |  | **50rfu** | | **100rfu** | | **200rfu** | |
|  |  | r | t | r | t | r | t |
| DICE – DICE (1945) | UPGMA | 0.8094 | 11.7986 | 0.69475 | 15.5792 | 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 S3**. List of all plants sampled in this study.

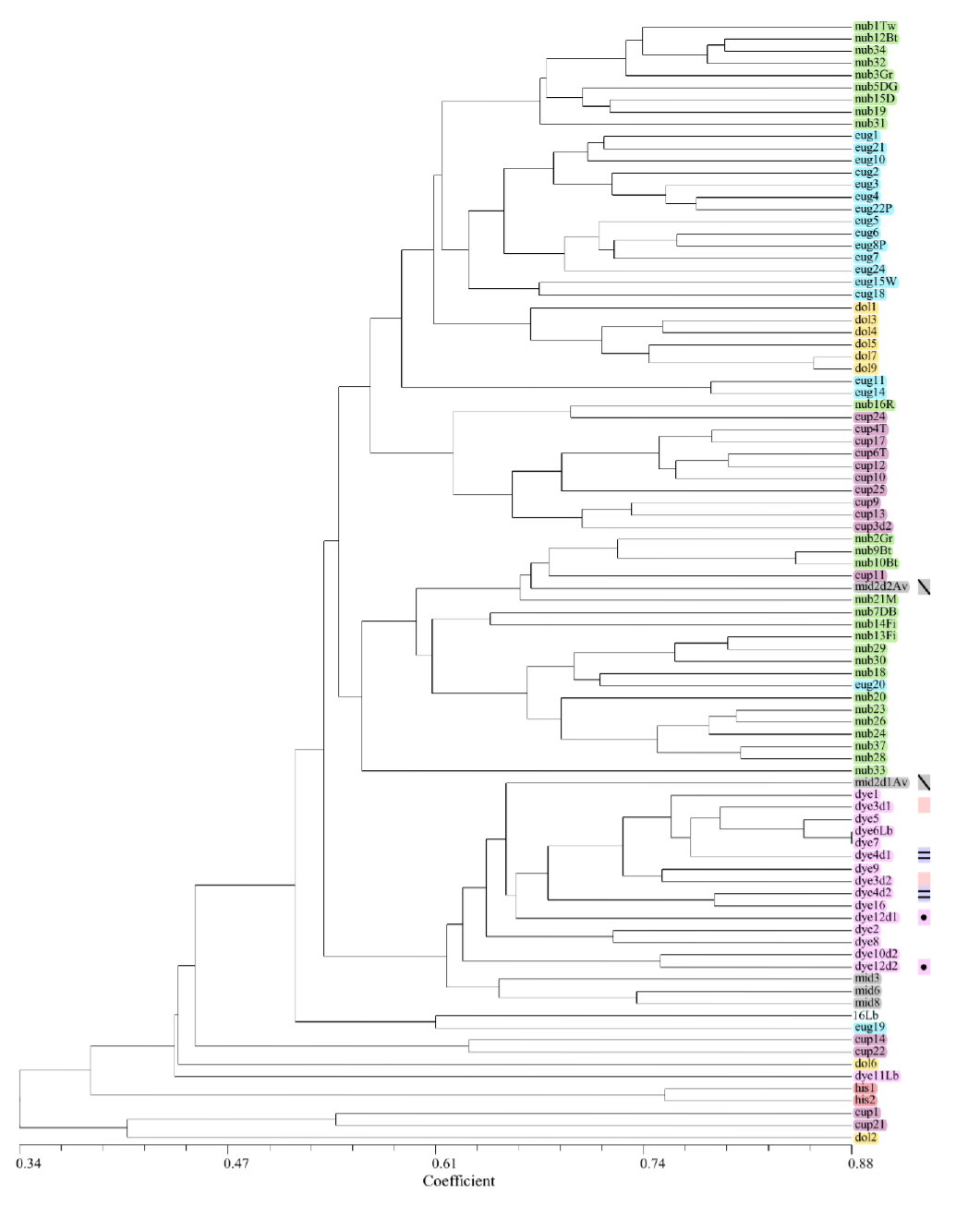
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Final ID | Extraction ID | Batch | Species | Locality/phenotype | Source |
| cup1 | 142ECUP002R | 2 | *E. cupidus* |  | Ridgemere |
| cup10 | 155ECUP019R | 2 | *E. cupidus* | Ohrigstad River | Ridgemere |
| cup11 | 156ECUP030R | 2 | *E. cupidus* | 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 |



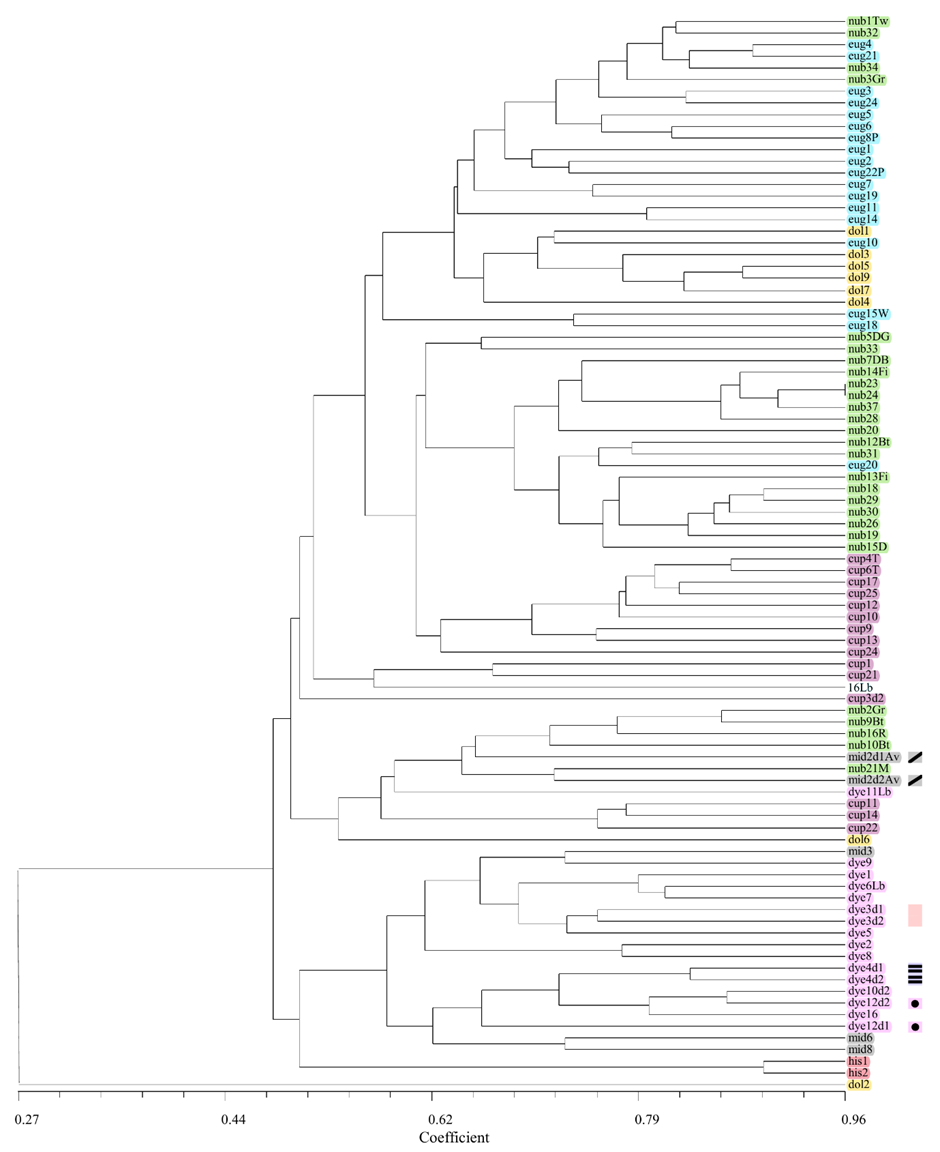
**Figure S1.** STRUCTURE barplots showing the proportion of membership of samples assigned to K = 7 clusters within the *Encephalartos eugene-maraisii* complex. Results are based on ISSR fragments scored at a 100 relative fluorescence unit (rfu) cut-off value. The dataset was tested using the Standard STRUCTURE model (a) and the LOCPRIOR model (b) that accounts for known locality data prior to the run. Colours represent each of the predefined clusters to which each sample is assigned. Species are numbered 1 – 7 on the x-axis, from left to right: *E. nubimontanus*, *E. eugene-maraisii*, *E. cupidus*, *E. middelburgensis*, *E. dolomiticus*, *E. dyerianus*, *E. hirsutus*.



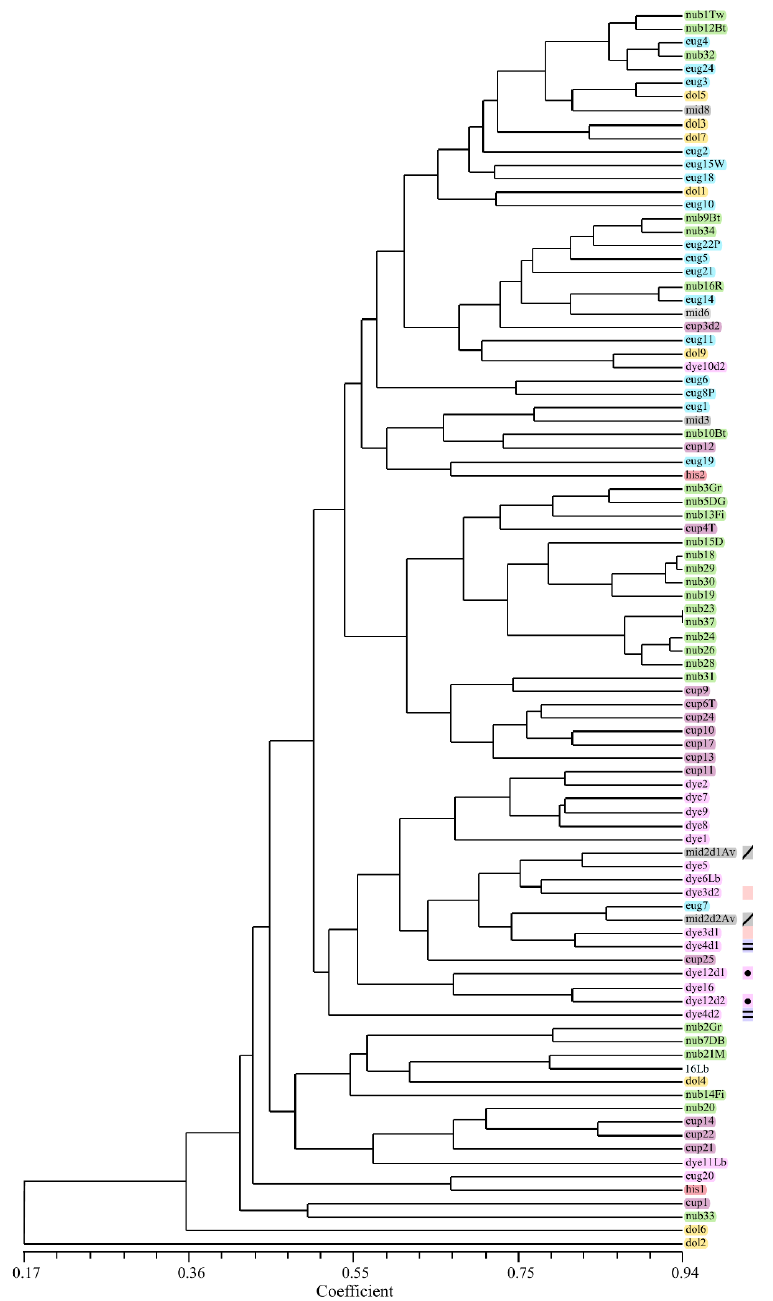
**Figure S2.** STRUCTURE barplots showing the proportion of membership of samples assigned to K = 7 clusters within the *Encephalartos eugene-maraisii* complex. Results are based on ISSR fragments scored at a 200 relative fluorescence unit (rfu) cut-off value. The dataset was tested using the Standard STRUCTURE model (a) and the LOCPRIOR model (b) that accounts for known locality data prior to the run. Colours represent each of the predefined clusters to which each sample is assigned. Species are numbered 1 – 7 on the x-axis, from left to right: *E. nubimontanus*, *E. eugene-maraisii*, *E. cupidus*, *E. middelburgensis*, *E. dolomiticus*, *E. dyerianus*, *E. hirsutus*.



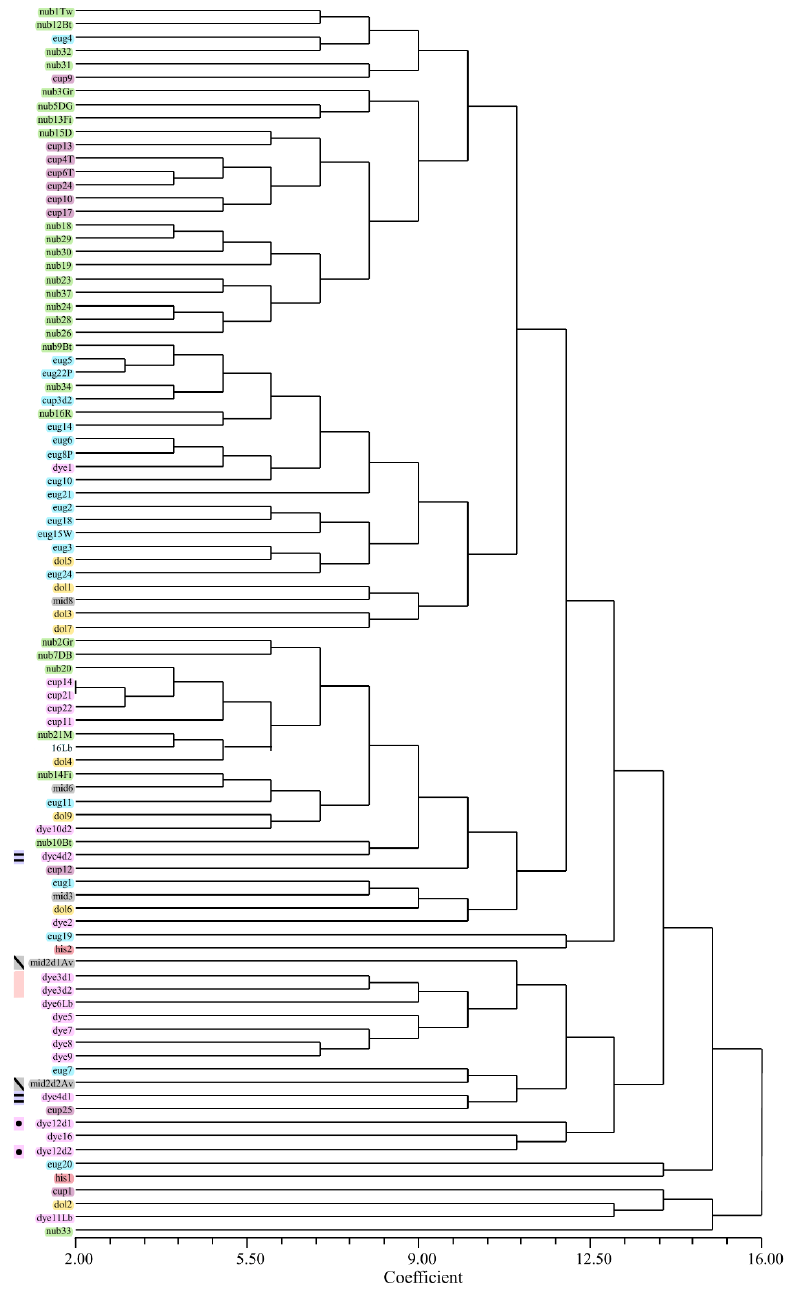
**Figure S3.** UPGMA dendrogram of the *Encephalartos eugene-maraisii* complex based on ISSR markers at a relative fluorescence unit (rfu) cut-off of 50rfu. Band presence and absence was used to compute genetic distances using the DICE coefficient. Colour shading indicates each species group, and specimen names are represented by the first three letters of their species epithet, corresponding toTable S5. Sample duplicates, representing material obtained from the same plant, but extracted in a different DNA extraction batch, are indicated by the coloured rectangles.



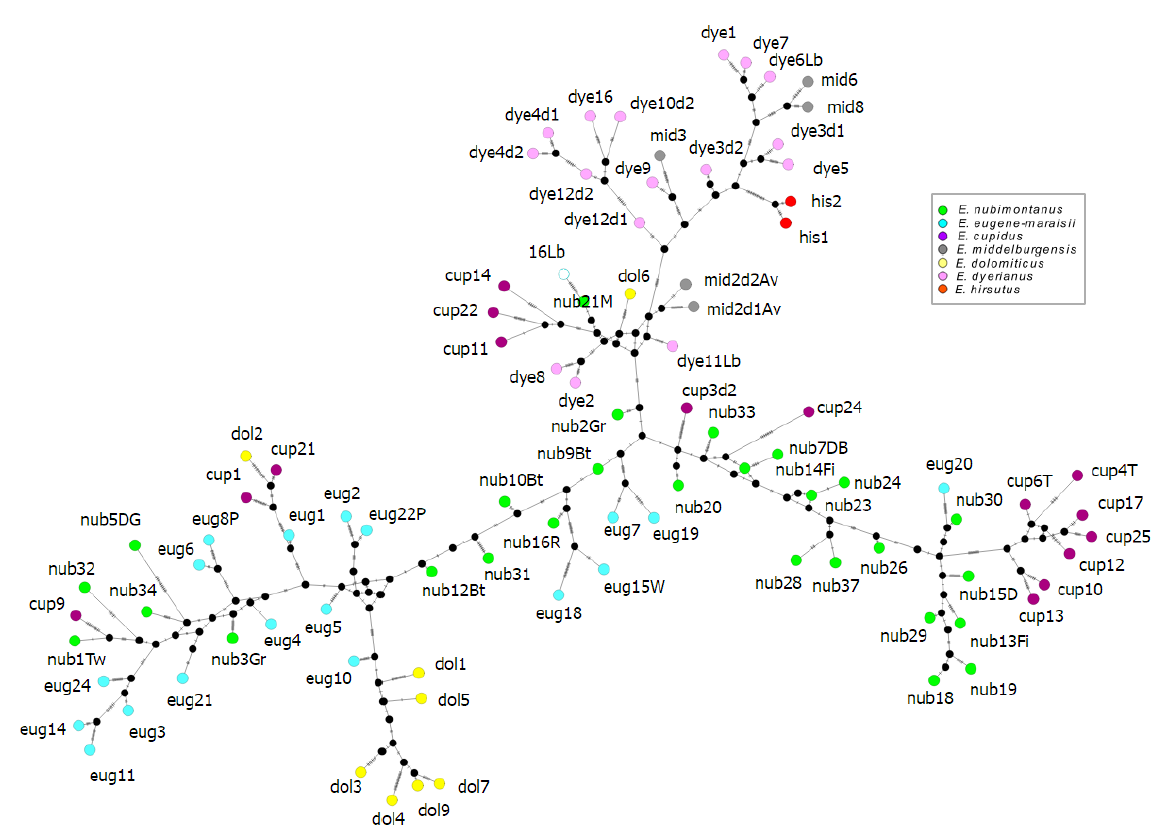
**Figure S4**. UPGMA dendrogram of the *Encephalartos eugene-maraisii* complex based on ISSR markers with a minimum band intensity of 100 relative fluorescence units (rfu). Band presence and absence was used to compute genetic distances using the DICE coefficient. Colour shading indicates each species group, and specimen names are represented by the first three letters of their species epithet, corresponding toTable S5. Sample duplicates, representing material obtained from the same plant, but extracted in a different DNA extraction batch, are indicated by the coloured rectangles.



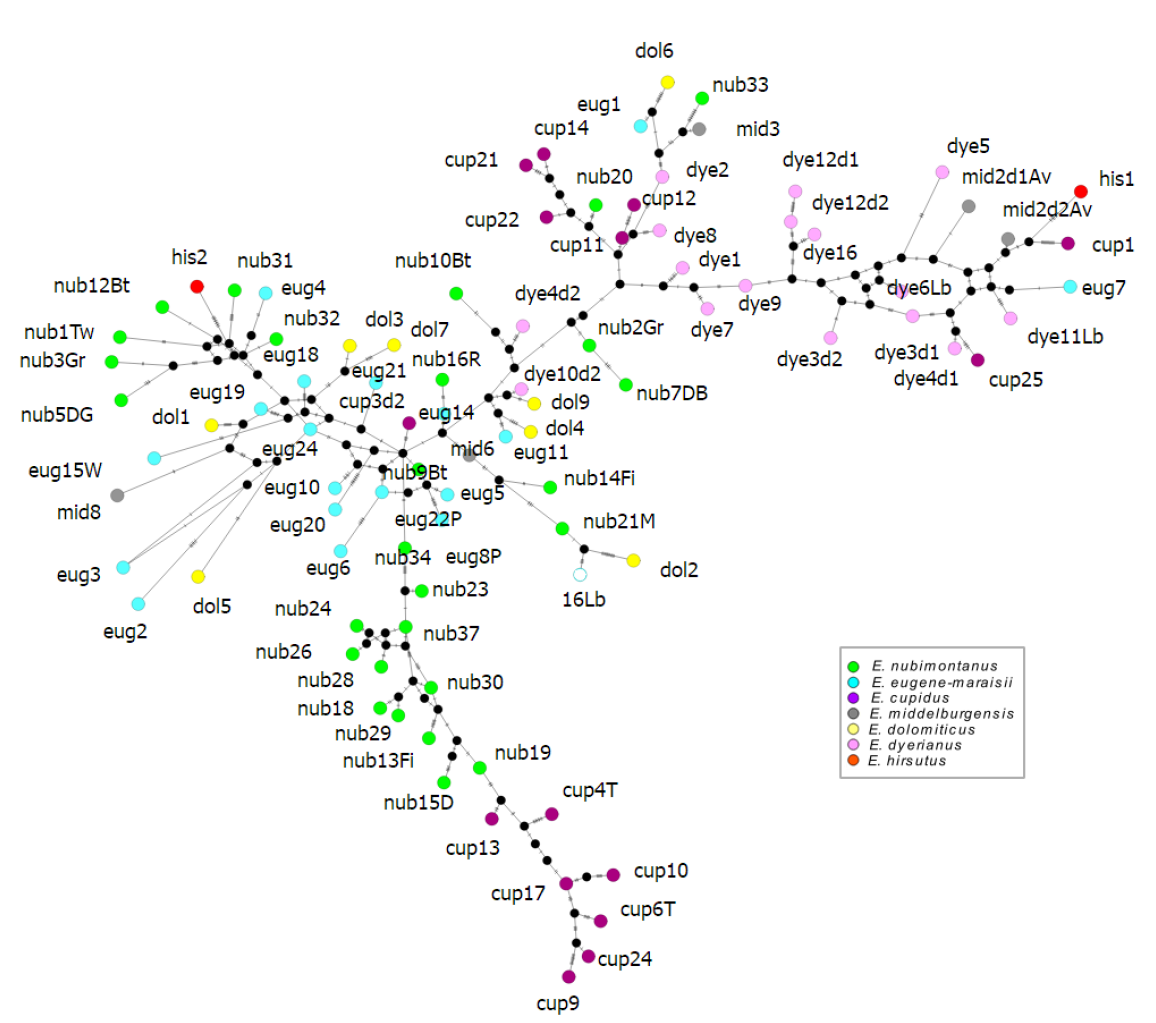
**Figure S5.** UPGMA dendrogram of the *Encephalartos* *eugene-maraisii* complex based on ISSR markers at a relative fluorescence unit (rfu) cut-off of 200rfu. Band presence and absence was used to compute genetic distances using the DICE coefficient. Colour shading indicates each species group, and specimen names are represented by the first three letters of their species epithet, corresponding toTable S5. Sample duplicates, representing material obtained from the same plant, but extracted in a different DNA extraction batch, are indicated by the coloured rectangles.



**Figure S6**. Neighbor Joining dendrogram of the *Encephalartos eugene-maraisii* complex based on ISSR markers at a relative fluorescence unit (rfu) cut-off of 200rfu. Band presence and absence was used to compute genetic distances using the DICE coefficient. Colour shading indicates each species group, and specimen names are represented by the first three letters of their species epithet, corresponding toTable S5. Sample duplicates, representing material obtained from the same plant, but extracted in a different DNA extraction batch, are indicated by the coloured rectangles.



**Figure S7**. 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 S8**. 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.