Biogeography, Diversification and Extinction risk of Cycads

By

LEDILE THABITHA MANKGA

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Supervisor: Dr K. Yessoufou

Co-supervisor: Dr M. Chitakira

Submitted: February 2020

DECLARATION

Name: Ledile Thabitha Mankga

Student number: 60996471

Degree: Doctor of Philosophy in Environmental Science

Title of a thesis: Biogeography, Diversification and Extinction risk of Cycads

I declare that the thesis hereby submitted to the University of South Africa for the degree DOCTOR of PHILOSOPHY (Environmental Science) is my own and has not been previously submitted for any degree or examination at any higher educational institution.

…………………………… …02 March 2020…….

Signature Date

DISCLAIMER/WARNING

This thesis contains chapters that have been accepted in different scientific Journals for publication. Chapter 2 has been published in AOB Plants and Chapter 3 has been published in South African Journal of Botany. Chapter 4 is published in the speciality section of Phylogenetics, Phylogenomics and Systematics, a section of Frontier Journal in Ecology and Evolution.

ABSTRACT

Cycads have an interesting evolutionary history since they represent the oldest lineage plants that originated ~ 300 million years ago (Ma) in the mid-Permian but re-diversified recently around 12 Ma with C*ycas* and *Encephalartos* being the most rapidly diversified lineages within the cycads group. Several studies have explored the taxonomic relationships, diversification history within the two genera but there's a limited understanding of evolutionary history, biogeography of cycads and what drives cycads to extinction risk. The aim of this study was to provide a better explanation on what predispose cycads to high risk of extinction and also elucidate the biogeography and evolutionary diversification history of the two cycads genera (African genus *Encephalartos* and Asian-Pacific genus *Cycas*). The three main objectives addressed: firstly, the ecological factors predisposing cycads to high risk of extinction was explored. Secondly, reconstruct the most comprehensive phylogeny of the two most diversified cycad genera. Lastly, investigate the evolutionary and historical biogeography of *Encephalartos* and *Cycas*. The risk of extinction using phylogenetic comparative method and fitting cumulative link mixed model on biological, ecological and evolutionary data of cycads on the most threatened lineage in the plant kingdom were investigated. Then, assembled the most complete phylogeny and reconstructed the historical biogeography of *Encephalartos* and *Cycas* using S-DIVA and Binary Bayesian model (BBM) respectively. The nine group of threats to cycads such as habitat loss, overcollection, fire, deforestation, medicinal usages, grazing, invasive alien plants, reproduction failure and the last one linked to climate change impacts flood/drought were identified. But, habitat loss, overcollection, medicinal uses and reproduction failure were clustered on the cycad tree of life suggesting that, closely related species might be exposed to similar threats implying that ecological factors that drives cycads to high risk of extinction were anthropogenically mediated and resulted in vulnerable (VU) category. The phylogenies of the two genera were found to be well supported. *Encephalartos* phylogeny, revealed two major clades following species geographic origins, one southern African clade and one east-central-west African clade. The biogeographic analysis suggests that the genus may have diverged around 9 Ma from southern Africa. Then colonized east-central-west African region through vicariance, suggesting that the eastern rift system in eastern Africa and the west Africa Dahomey Gap acted as a geographical barrier limiting species dispersal. Furthermore, most species accumulated in the last 2.6 Ma and there were no significant shifts in any of the evolutionary events, suggesting that a constant-rate diversification model is best suited for *Encephalartos*. The phylogenetic analysis of the *Cycas* genus pointed to Indochina as the origin of the genus, which may have dispersed firstly across the Pacific Islands during the late Miocene aided by multiple excursions of sea levels and the development of a key innovation (a spongy endocarp) particularly in the seeds of subsection Rumphiae. The colonization of South China, which was initially thought to be the origin of the genus, may have occurred more recently aided by both dispersal and vicariance events. Also, no significant shifts in the evolutionary events that shaped the diversity of *Cycas* was observed. The findings of this study provide the evidence of historical biogeography and the evolutionary events that shaped the current diversity of the genera *Encephalartos* and *Cycas*. Overall, this study is the first to elucidates the pattern of extinction risk in cycads and also to identify that most threats that drives extinction risk of cycads were anthropogenically mediated.

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DEDICATION

I would like to dedicate this thesis to my parents, Mr. Godfrey Nakedi Mankga and the late Mrs. Aria Mathekga Mankga. Ke a leboga Batlokwa!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BF = Bayes factor

BM = Brownian model

BRC = Bayesian relaxed clock

CG = Crown Group

CR = Critically endangered

EN = Endangered

DD = Data deficient

DNA = Deoxyribonucleic acid

ED = Evolutionary distinctiveness

ESS = Effective sample size

Genbank = National Centre for Biotechnology Information

GTR+I+Γ = General time reversal + Gamma + Propotion invariant

ITS = Internal transcribed spacer

IUCN = International union for conversation of nature

KM^2 = Square kilometer

LC = Least concern

Ma = Million years ago

matK = Maturase K

MCMC = Markov chain Monte Carlo

Myr^{-1} = Species per Million years

NT = Near threatened

PCC = Percentage Correct Classified

PHYP = Phytochrome P

rbcL = Ribulose bisphosphate carboxylase gene

SG = Stem Group

TREEANNOTATOR = a program to summarize the information from sample of trees

trnH-psbA = spacer between *trnH* and *psbA* genes

VU = Vulnerable

Computational Packages

BEAST = Bayesian Evolutionay Analysis by sampling trees

BEAUti = an interactive graphical application for designing analysis and generating the control file (BEAST XML file)

BMM = Bayesian Binary Method

DEC = Dispersal-Extinction-Cladogenesis

DIVA = dispersal-vicariance analysis

CLMM = Cumulative link mixed effect model

GEIGER = Analysis of Evolutionary Diversification

LASER = Likelihood Analysis of Speciation/Extinction rates from phylogenies

PASTIS = an R package to facilitate phylogenetic assembly with soft taxonomic inferences

RASP = Reconstruct Ancestral State in Phylogenies

S-DIVA = Statistical Dispersal-Vicariance

TESS = Diversification rate estimation and fast simulation of reconstructed phylogenetic tress under tree-wide-time-heterogeneous birth-death process including mass-extinction events

TRACER = a graphical tool for visualization and diagnostic of MCMC

Chapter 1

General Introduction

1.1 Introduction

Cycads are dioecious and entomophilous plants that developed palm-like habit with stout trunks and large evergreen pinnate leaves (Jones 2002). They share some characteristics with the ferns (e.g. spermatozoa with flagella) and angiosperms (e.g. seeds; Guan 1996; Norstog and Nicholls 1997). The dispersal of cycads seeds is limited to 2-7 km mostly mediated through rodents and small fruit-eating bats (Yang and Meerow 1996). Cycads represent the oldest seed plants lineage that originated ~ 300 million years ago (Ma) in the mid-Permian (Hendricks 1987; Gao and Thomas 1989; Calonje et al. 2017) and reached their greatest diversity in the Jurassic era (Jones 2002; Nagalingum et al. 2011). Geographically, cycads are restricted to tropical and subtropical or warm temperate regions with predominantly summer rainfalls (Jones 2002; Whitelock 2002).

Cycads, because of their unique evolutionary history (Nagalingum et al. 2011) and their conservation status (highly threatened taxonomic group; Yessoufou et al. 2017), attracted much attention recently (e.g. Nagalingum et al. 2011; Yessoufou et al. 2014; Condamine et al. 2015; Xiao and Müller 2015; Yessoufou et al. 2017; Liu et al. 2018). However, the biogeography, the evolution and the extent of the threats facing this taxonomic group, especially at genus level remain less actively explored. This chapter introduces and discusses key topics relevant to the main three chapters of the dissertation.

1.2 Taxonomic changes or debate on Cycads

1.2.1. Families of cycads

Initially, cycads were classified within one family Cycadaceae (de Candolle 1868). Then, Johnson (1959) recognised two additional families that are Stangeriaceae and Zamiaceae. Stevenson (1981) added the family Boweniaceae (genus *Bowenia*). Later, Stevenson (1990) assigned the genus *Bowenia* to the family Stangeriaceae under the subfamily Bowenioideae. Hill et al. (2003) recognized three families i.e. Cycadaceae, Zamiaceae and Stangeriaceae (Table 1.1). However, most recent molecular studies support two families, Cycadaceae and Zamiacaeae (Hill et al. 2003; Chaw et al. 2005; Osborne et al. 2012; Wang and Ran 2014).

The family Cycadaceae represents only one genus, *Cycas* L., with about 116 species (Calonje et al. 2017; Liu et al. 2018) while the family Zamiaceae represent nine genera, comprising of *Ceratozamia* Brongn. (with 27 species), *Bowenia* Hook. Ex Hook.f. (with two species), *Dioon* Lindl. (with 14 species), *Encephalartos* Lehm. (with 65 species), *Lepidozamia* Regel. (with two species), *Microzamia* Miq. (with 41 species), *Zamia* L. (with 71 species), *Stangeria* T.Moore (one species) and *Microcycas* (Miq.) A. (one species) (Osborne et al. 2017) (Table 1.1).

Table 1.1 Biological classification of cycads

Order	**Suborder**	**Family**	**Subfamily**	**Tribe**	**Subtribe**	**Genus**
Cycadales	Cycadineae	Cycadaceae	Cycadoideae			*Cycas*
	Zamineae	Zamiaceae	Stangerioideae			*Stangeria*
			Bowenioideae			*Bowenia*
			Encephalartoideae	Diooeae		*Dioon*
				Encephalarteae	Encephalartinae	*Encerphalartos*
					Macrozamiinae	*Macrozamia*
						Lepidozamia
			Zamioideae	Ceratozamieae		*Ceratozamia*
				Zamieae	Microcycadinae	*Microcycas*
					Zamiinae	*Zamia*
						Chigua

1.2.2. Classification within the family Cycadaceae

Cycas L. is the only genus belonging to the family Cycadaceae; it comprises of six sections, including *Asiorientales*, *Panzhihuaenses*, *Wadeanae*, *Stangarioides*, *Indosinenses*, and *Cycas* (Hill 2004; Liu et al. 2018). The sections were firstly divided into four (Asiorientales, Stangerioides, Indosinensis and *Cycas*) by Hill (1993; 1994) based on the reproductive organs. Then, Hill (2008) and Lindström et al. (2008) divided the section *Cycas* into three subsections (Rumphiae, Endemicae and *Cycas*) and added the two sections *Panzhihuaenses* and *Wadeae* thus resulting into six sections. *Cycas* is the most rapidly diversified clade in the cycads group with 116 accepted species names (Calonje et al. 2017; Yessoufou et al. 2017). There has been a debate on the classifications of this genus based on the number of ovules (Pilger 1926), mophological differences in megasporophyll (Hill 1995; Hill 2004), stem base and pinnate morphology (De Laubenfels and Adema 1998).

Most researchers conducted molecular studies on species level based on limited sampling sizes of *Cycas* to resolve the taxonomic classification (Chaw et al. 2005; Salas-Leiva et al. 2013; Treutlein and Wink 2002; Nagalingum et al 2011; Sangin et al. 2010; Xiao and Möller 2015). The recent study of Liu et al. (2018) revealed six sections of *Cycas*. However, some sections such as *Cycas*, *Indosinenses*, *Wadeae* and *Asiorientales* were resolved but the rest remained uncertain. But Sangin et al. (2010) and Liu et al. (2018) discovered that plastid markers were not able to resolve sections within *Cycas*. While the nuclear datasets was able to resolve six sections of *Cycas* (Xiao and Möller 2015) and the chloroplast dataset produce clades that aligned with geographic regions (Liu et al. 2018). Liu et al. (2018) suggested that this kind of differences in the markers that result in low interspecific variation could be likely due to the recent radiation of the genus.

1.2.3. Cycads within the family Zamiaceae

A higher propotion of the cycads genera belongs to the family Zamiaceae comprising of nine genera (*Zamia* L, *Encephalartos* Lehmn., *Dioon* Lindl., *Ceratozamia* Brongn., *Macrozamia* Miq., *Microcycas* (Miq) A.D.C., *Bowenia* Hook. Ex Hook.f, *Stangeria* T.Moore and *Lepidozamia* Regel. (Chaw et al. 2005; Zgurski et al. 2008). In general, Mexico has been regarded as the center of diversity for Zamiaceae family i.e. *Zamia*, *Dioon* and *Ceratozamia* (Contreras-Medina and Luna-Vegas 2007). *Zamia* species cover the broadest spectrum of habitats in the American regions (Donaldson 2003) and it is the second largest genus within the cycads group. The genus *Zamia* is monophyletic with about 71 species (Osborne et al. 2012) endemic to America with three sections: 1) MegaMexico, including the northern part of Central American Isthmus, 2) Caribbean Island including Florida, and 3) South America including some parts of Costa Rica extending to Panama (Zonneveld and Lindström 2016). Members of this cycads group are distributed from Mexico, southern USA to Central America; Caribbean Islands to south Bolivia (Whitelock 2002). *Zamia* is the only American cycads species that is found in both sides of equator (Whitelock 2002) apart from the genus *Encephalartos* that is endemic to Africa (see Figure 1.1).

The genus *Encephalartos* has 65 species being monophyletic (Nagalingum et al. 2011) with most members of the genus (50%) scattered across southern Africa with one species occurring in West Africa

(*E. barteri*) (Golding and Hurter 2003). Southern Africa has been considered as the center of diversity for most members of *Encephalartos* species (Donaldson 2003; Golding and Hurter 2003). There are five species (*Encelephalartos marunguensis* Devred, *Encelephalartos schmitzii* Malaisse, *Encelephalartos Poggei* Asch, *Encelephalartos schaijesii* Malaisse, Sclavo & Crosiers and *Encelephalartos laurentianus* De Wild) found in Central Africa with no described species in northern Africa. This raises a question of why the genus is unevenly distributed throughout the African continent. Members of the genus *Encephalartos* are characterised with pinnate leaves that contain leaflets that lack articulated midrib that discriminate them from the rest of the cycads group. Also, within the genus *Encephalartos,* species are distinguished by leaf morphology, cone morphology and phenology (i.e. cone reproduction; Voster et al. 2004). Therefore, this morphological taxonomic species placement has resulted in a speculations of molecular phylogenetic relationship within the genus (Voster et al. 2004).

Within the family Zamiaceae, the genus *Dioon* consists of approximately 10 species distributed in Mexico, consisting of one species in Hondarus (Moretti et al. 1993) and with fossil evidence dated back to Eocene in Alaska (Norstog and Nicholls 1997). They consist of grey to blue-green pinnate leaves with non-articulated leaflets lacking midrib. The megasporophylls are broadly flattened, upturned and overlapping. The female cones contain two seeds attached to sporophyll that differentiate it from other genera (Norstog and Nicholls 1997; Figure 1.1). The genus *Ceratozamia* consists of about 27 species found in areas of Mexico, Guatemala and extending to Belize (Whitelock 2002; Osborne et al. 2012). This genus is characterized by prominent sporophylls paired with horns and compound pinnate leaves that are spirally arranged (Haynes 2011; Figure 1.1). The leaflets lack midrib and have parallel veins that are articulated at the base (Hill et al. 2004). The genus *Macrozamia* consists of 41 species (Osborne et al. 2012) endemic to eastern Australia extending to central and south west of Australia (Hill et al. 2004).

Similar to all cycads, the species of *Macrozamia* are dioecious, subterranean, palm-like trunks and have thin, flat leaflets (Chaw et al. 2005). This genus produce a single leaf at a time unlike other genera that produce many leaves that erupts simultaneously (Hill et al. 2004). The smallest genera with less than three species are *Microcycas* (1 species) and *Lepidozamia* (2 species) being endemic to Australia. *Microcycas* has one species (*Microcycas caloma*) which is endemic to Cuba (Hill et al. 2004; Osborne et al. 2012). This species has palm-like shrubs with tall aerial stems that produce many leaves (Osborne et al. 2012). Microsporophylls and megasporophylls are spiral and are closely related to *Zamia* (Hill et al. 2004). The genus *Lepidozamia* has two species (*L. hopei* and *L. peroffskyana*) (Osborne et al. 2012) and they are distributed in the eastern part of Australia. The genus *Lepidozamia* has a unique leaf morphology character orientation of epidermal cells at the axis of pinna being different to other cycads genera (Hill et al. 2004; Condamine et al. 2015). The genus *Bowenia* is distributed in Australia and has only two species *(Bowenia serrulata* and *Bowenia spectabilis*). The species are characterised of fern like shrubs with naked subterranean stem that produce one to many shoots (Hill et al. 2004). Leaves are bipinnate and lack midrib (Hill et al. 2004). Lastly, the genus *Stangeria* has one species (*Stangeria eriopus*) that is found only in South Africa (Hill et al. 2004; Osborne et al. 2012; Salas-Leiva et al. 2013). The species inhabit the coastal grassland and inland forests along the eastern coast part of South Africa. Morphologically, *Stangeria* are fern like with pinnate leaves and leaftlets has midrib with lateral veins (Osborne et al. 2012). It has branched stem, carrot shaped tuberous roots and subterranean leaves (Osborne et al. 2012; Figure 1.1).


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Figure 1.1. Representative of pollen bearing cones of all cycads genera. **A**) *Bowenia serrulata* **B**) *Bowenia spectabilis* **C**) *Ceratozamia decumbens* **D**) *Ceratozamia decumbens* **E**) *Cycas couttsiana* **F**) *Cycas revoluta* **G**) *Dioon* angustifolium **H**) *Dioon angustifolium* **I**) *Encephalartos ferox* seed cone **J**) *Encephalartos ferox* pollen cone **K**) *Lepidozamia hopei* **L**) *Lepidozamia peroffskyana* pollen cone **M**) *Macrozamia lucida* seed cone **N**) *Macrozamia lucida* pollen cone **O**) *Microcycas calocoma* seed cone **P**) *Microcycas calocoma* pollen cones **Q**) *Stangeria eriopus* seed cone **R**) *Stangeria eriopus* pollen cone **S**) *Zamia imperialis* seed cone **T**) *Zamia imperialis* pollen cones. Photos taken from Calonje et al. (2011).

1.3 Biogeography of Cycads

Geographically, cycads are restricted to tropical and subtropical or warm temperate regions with predominantly summer rainfalls (Jones 2002; Whitelock 2002; Figure 1.2), although the distribution is sporadic (Hill et al. 2003). They are usually reffered as the living fossils that originated in the upper Paleozoic more than 300 Ma (Taylor et al. 2012; Pott et al. 2010) and existed with the dinasaurs until to the present age. However, the theory of the "living fossil cycad" has been challenged (Nagalingum et al. 2011; Condamine et al. 2015). The fossil evidence of Cycadaceae family points to Asia (South China) as the origin of the genus *Cycas* (Hill 1995; Xiao and Möller 2015).

From Asia, the genus *Cycas* is further distributed southward to Australia and from eastern Africa, eastward to the Pacific islands (Hill 2004; Figure 1.1). The rapid radiation is the result of vicariant speciation facilitated by the Red River Fault between south China and Indochina as a physical barrier (Xiao and Möller 2015). While the fossil evidence of *Zamia* point to central America as the origin of the genus extending to Carribean region, northern South America and Colombia as the highest species diversity (Zonneveld and Lindström 2016). Also, the genera *Dioon* and *Ceratozamia* are abundant and diverse in Central America (*Microcycas* in Cuba) and Mexico (Contreras-Medina et al. 2007). They are mainly distributed in temperate forests that are associated with mountain chains. The genus

Encephalartos is endemic to southern Africa extending to the eastern Africa, central and some part of western Africa (Golding and Hurter 2003; Fig. 1.2) and has been receiving lots of attention recently. But other genera such as *Macrozamia*, *Lepidozamia* and *Bowenia* hasn't been receiving lots of attention and has been reported in the southern Hemisphere. Their fossils are relatively sparse (Hill 1998) and are distributed near coastal Queensland in north east Australia, North eastern New South Wales (Chamberlain 1912; Johnson 1959; Hill et al. 2019).

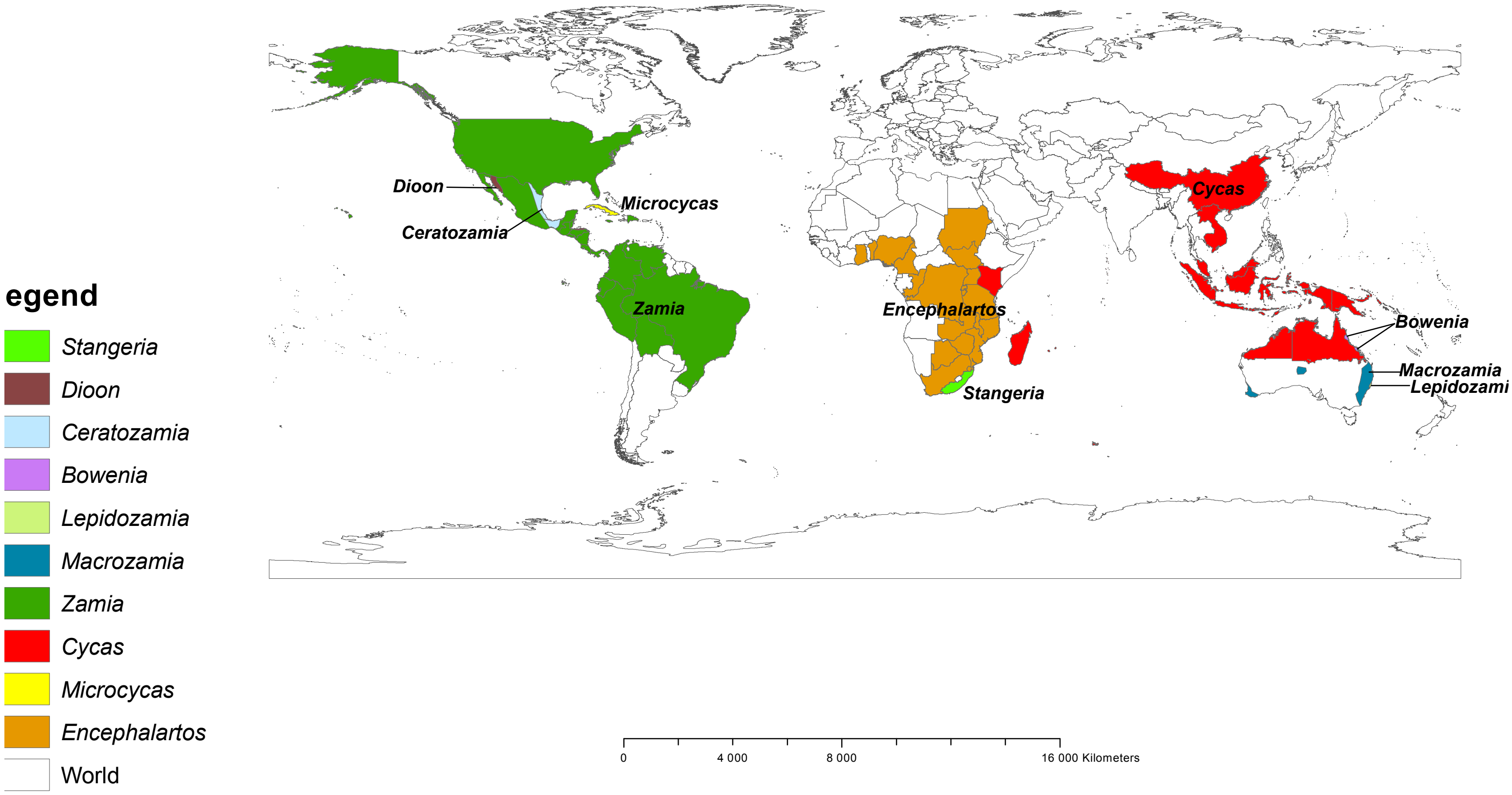

Figure 1.2 The world map indicating the distribution of cycads all over the world

1.4 Evolutionary diversification of Cycads

With all 10 genera that diversified within the cycads group, Nagalingum et al. (2011) indicated that the cycads group is not older than ~12 Ma. This re-diversification, might have been triggered by many things such as several pulses of extinction (van de Schootbrugge et al. 2008; Nagalingum et al. 2011), environmental changes (Crane 1987; van de Schootbrugge et al. 2008) and speciation in the past (Davies and Schaefer 2011). However, the question remains: to what extend does ecological forces (dispersal and vicariance) occurr to shape the current cycads diversity? What ecological forces shaped these events (speciation and extinction)? These are some of the interesting questions that evolutionary biologists asked (Keppel et al. 2009; Wang and Ran 2014; Xiao and Möller 2015).

Molecular biologists have been using various molecular phylogenetic approaches to reconstruct the evolutionary diversification of taxonomic group of interest without references of fossils records (Harvey et al. 1994; Rüber and Zardoya 2005). These phylogenetic alternative methodological approaches can inform evolutionary events regarding the role of past climatic events, evolutionary novelty and adaptive and non-adaptive radiations of various diversification rate in a phylogenetic tree (Harmon et al. 2003; Fordyce 2010). Various methods have been developed to examine diversification rate variations including accumulation of lineage through time (Nee et al. 1994), distribution of tree branch lengths (i.e. parametric rate comparison (PRC)) (Alfaro et al. 2009; Fordyce et al. 2014) and the shape of ordered cladogenic events (Pybus and Harvey 2000; Höhna et al. 2015).

Lineages-through-time plot (LTT plot) is usually represented in a graphical shape. The shape of lineage-through-time plot depends on the evolutionary events that take place during diversification rate. Crisp and Cook (2009) explained these graphical shape theories in the following manner: 1) Linear or Exponential curve shows that species birth and death rate is constant over time. That is, when the death rate decreases, the curve will be linear throughout the diversification period and when the death rate increases the curve will be steep towards the present (Fig. 1.3 A-B) 2) Concave or convex shape indicate that a single rate significant speciation shift increased (convex shape) or decreased (concave shape) within the diversification rate (Fig. 1.3 C-D) 3) If the density dependent shows a steep slope, then it is as an adaptive radiation and an upswing slope that is reffered as anti-sigmoidal curve (Fig. 1.3 E-F).

Therefore, in this study (Chapter 3 and 4) we have applied the LTT plot to address whether the diversification of two genera *Encephalartos* and *Cycas* followed a constant-rate model. That is, if the LTT-plot does not depart significantly from those of the simulated trees under a constant-rate birth-death model.

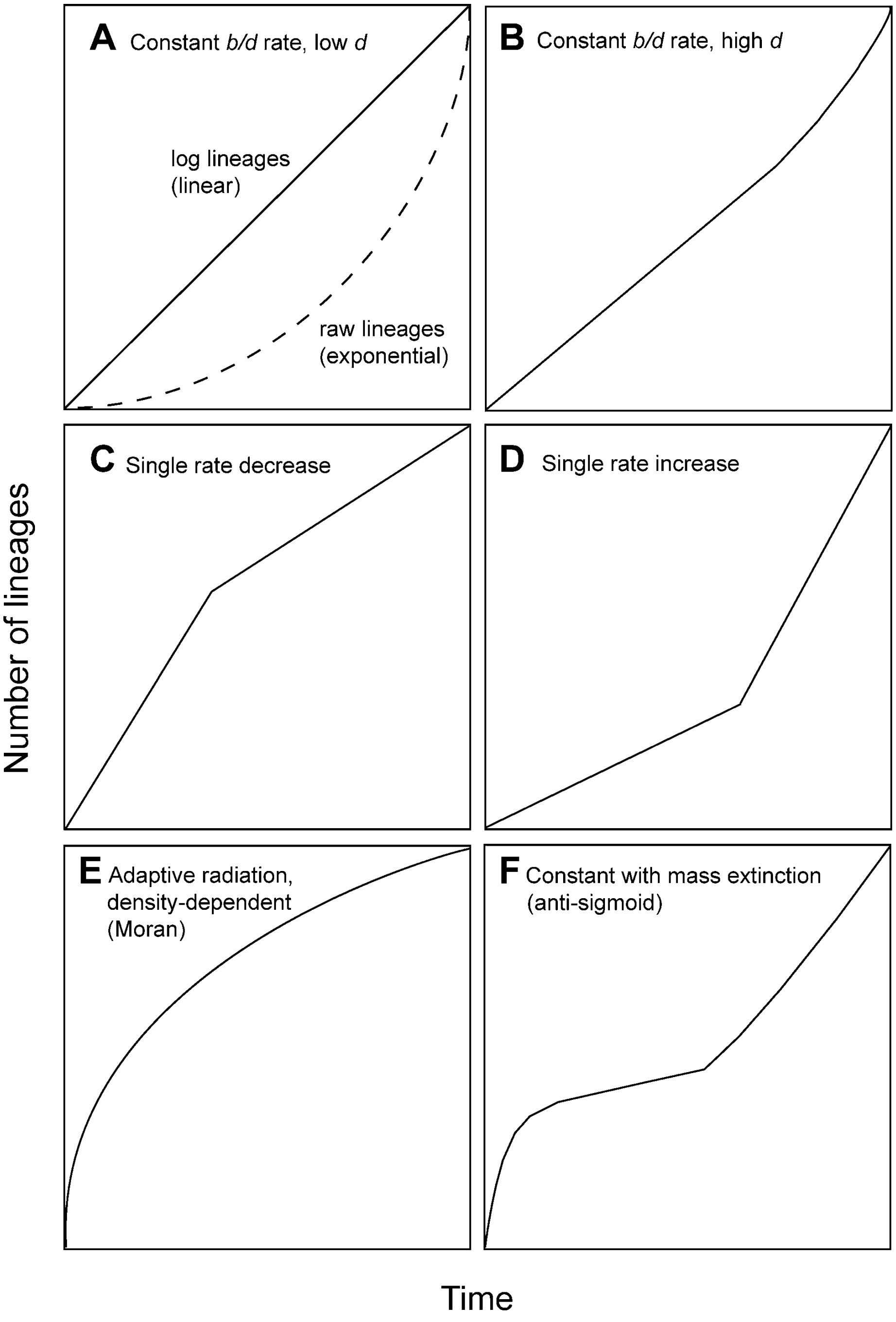

Figure 1. 2. Six possible graphical theoretical expectations of diversification patterns of lineage-through-time plots (adapted from Crisp and Cook 2009). **A**) Exponential curve with constants birth and death rate with low death. **B**) Linear curve with constant birth and death rate with high death **C**) Concave shape with single rate decrease **D**) Convex shape with increased single rate **E**) Adaptive radiation that is density dependent **F**) Anti-sigmoidal shape with constant mass extinction.

1.4.1.Evolutionary diversification of *Encephalartos*

Encephalartos is a most prominent species-rich lineage in southern Africa within the cycads group (Norstog and Nicholls 1997). A significant number of species (~50%) are endemic to South Africa and few species (~40%) occur in tropical regions of Central and East Africa (Golding and Hurter 2003). However, southern Africa experienced severe droughts during the Pliocene/Pleistocene era (Yessoufou et al. 2014) and during these conditions species adapt mophologically to adjust to harsh climate conditions for survival (de Menocal 1995) and promoted species radiation (Treutlein et al. 2005; Yessoufou et al. 2014). The explosive radiation of species can be mediated through different set of ecological forces such as environmental condition (Rull 2012), dispersal and vicariance. Ecological forces might have shaped the current species distribution pattern of this taxonomic group.

Yessoufou et al. (2014)'s study aimed to answer the questions regarding the evolutionary diversification history of the African genus *Encephalartos* excluding the biogeoagraphy of the genus. The researchers, revealed that the overall diversification pattern was punctuated by a mass extinction event (~2.6Ma) that promoted explosive radiation of *Encephalartos* through ecological forces that occurred in southern Africa. Yessoufou et al. (2014), used the Yule prior (pure birth) to generate the phylogenetic tree to investigate the overall diversification of the genus. Usually, a Yule prior is used in a Bayesian relaxed-clock dating a phylogenetic tree of a taxa that underwent extinction events (Condamine et al. 2015). It models the branching process during the reconstruction of a phylogenetic tree assuming a constant speciation rate without an extinction rate while the birth death prior models both the speciation and extinction events. Therefore, in Chapter 3, we re-investigate Yessoufou's findings and the historical biogeography of the genus *Encephalartos* using a complete phylogeny assembled based on the birth death prior.

1.4.2 Evolutionary diversification of *Cycas*

Cycas is the most widely distributed and diverse genus within the cycads group, with 116 species (Calonje et al. 2017; Liu et al. 2018). Its distribution extent to China, southern Japan to southwards Australia extending to eastwards Africa (Whitelock 2002; Chaw et al 2005, Liu et al. 2018). Since the fossil records date back to 200 Ma (Norstog and Nicholls 1997), the morphology of the genus could have changed a little resulting in taxonomic confusion (Yang and Meerow 1996) and the subgeneric classification becoming problematic (Hill 1995, 2004). However, molecular studies have indicated a rapid speciation in the Miocene (Crisp and Cook 2011; Nagalingum et al. 2011; Treutlein and Wink 2002) that resulted in taxonomic confusion within the genus.

Although the phylogenetic relationship within this genus have remained controversial, many scientist have tried to resolve this issue (Treutlein and Wink 2002; Chaw et al. 2005; Sangin et al. 2010; Nagalingum et al 2011; Salas-Leiva et al. 2013; Xiao and Möller 2015). Molecular studies with few sample sizes (Keppel et al. 2008; Xiao and Möller 2015) indicated that *Cycas* evolved from South China to Indochina across the Red River fault and dispersed to Island of South East Asia which was mediated through spongy layer in *Cycas* seeds (Xiao and Möller 2015). Also, this widespread distribution of the genus might have been promoted by the long distance dispersal events across the ocean. The genus *Cycas* has been identified as the most diversified clade (Yessoufou et al. 2017) within the cycads group.

But, the researchers didn't investigate the patterns or forces behind the diversification of the genus. Therefore, in Chapter 4, we investigate the historical biogeography of *Cycas* and elucidate the ecological forces that shaped the current diversity patterns within the cycads group.

1.5 Extinction risk of Cycads

All cycads genera have interesting evolutionary history (Nagalingum et al. 2011) and most of the genera are highly threatened. Almost 70% of cycads species are threatened with a high risk of extinction (Osborne et al. 2012). Therefore, a better understanding of the drivers of cycad's extinction risk is necessary to inform conservation decisions. Nonetheless, there is a disparity in efforts devoted to unravelling the extinction risk patterns of vertebrates versus plants (Davies and Yessoufou 2013; Luiz et al. 2016; see further references in Pellens and Grandcolas 2016). Few studies focus on angiosperms (Yessoufou et al. 2012; Daru et al. 2013; Leao et al. 2014), amphibians (e.g. Sodhi et al. 2008) and veterbrates (Cooper et al. 2008; Ripple et al. 2017). As a result, we are comparatively well informed of the predisposition of vertebrates and, to a lesser extent, angiosperms, to extinction risk as well as how their phylogenetic trees would be affected by species loss (Mooers et al. 2012; Davies and Yessoufou 2013). In contrast, such knowledge is yet to be well demonstrated for gymnosperms, although the latter group is more threatened than angiosperm (IUCN 2010).

Current knowledge indicate that ecological or biological factors (Sodhi et al. 2008; Yessoufou et al. 2012) and evolutionary history (Davies et al. 2011) predispose a particular taxonomic group to risk of extinction. For example, life history trait such as body size predispose vertebrates to extinction risk

(Cardillo 2003), but the role of size in predisposing plants to extinction remains debatable (Bradshaw et al. 2008; Sodhi et al. 2008). In contrast, evidence suggests that extinction risk in plants may be linked to their evolutionary rather than life history (Davies et al. 2011). Also, because phylogenetically conserved traits can be linked to extinction (e.g. phenology; Willis et al. 2008), it becomes necessary to assess the distribution of extinction risk along a phylogenetic tree (Fritz and Purvis 2010; Davies et al. 2011; Yessoufou et al. 2012), although extinction risk is not an evolving trait (Grandcolas et al. 2011).

Such a phylogenetic signal analysis of extinction risk would help predict whether unrelated species [e.g. species with high value of evolutionary distinctiveness (ED); Isaac et al. 2007] or closely related ones are more at risk (Purvis et al. 2000). However, the phylogenetic analysis of extinction risk is traditionally conducted based on IUCN threat categories (Davies et al. 2011). This tradition has recently showed to be potentially misleading especially when the drivers of extinction risk are not taken into consideration (see Schachat et al. 2016). Similarly, conservation decisions could be further misled if the threat status of some species remains unknown (e.g. Data Deficient DD species; Luiz et al. 2016; Veron et al. 2016).

As the susceptibility of species to extinction is linked to their past evolutionary history (Davies et al. 2011), reconstructing the tree of life of a particular taxonomic group will likely inform our understanding of the pattern of extinction risk in the group (Davies et al. 2011; Purvis et al. 2000). It also help understand how the tree of life could be pruned by species loss (Davies and Yessoufou 2013; Purvis et al. 2000). Assembling the cycads tree of life is the first step toward unravelling these questions as well as the temporal dynamic of species accumulation.

Existing attempts to assemble a complete phylogeny failed to include a complete list of cycads diversity (Nagalingum et al. 2011; Forest et al. 2018), and this could potentially limit our understanding of a full picture of cycads diversification history (Nagalingum et al. 2011), the forces driving their biogeographic pattern and how species loss may impact the cycads tree of life. Such understanding would in turn guide actions toward the preservation of the evolutionary diversity accumulated in the tree of life. For example, a strong phylogenetic signal in a threat can guide the prediction of the threat status of a particular species that has not yet been assessed.

1.6 Problem Statement

The ongoing six mass extinction event is characterized by the loss of two-third of biodiversity (Davies and Yessoufou 2013). This loss raises a global concern as humanity relies on the goods and services biodiversity provides, e.g. foods, medicine, pollination, recreation, etc. To understand better what predisposes biodiversity to extinction risk, there has been a huge body of literature devoted to this question, especially with regard to the evolutionary basis of species loss.

However, most of these studies focus on vertebrates (mammals, birds, reptiles). Furthermore, the comparatively few studies on plants focus on angiosperms, with no equivalent efforts devoted to gymnosperms, although gymnosperms are more at risk of extinction. Indeed, cycads for example, a gymnosperm group with ~70% of threatened species, amongs the most threatened group of plants (IUCN 2010; Yessoufou et al. 2017) with an interesting evolutionary and histororical biogeography of the genera at the species level. Cycads genera are sporadically distributed across the globe (Hill et al.

2003) espeacially the genus *Cycas* and *Encephalartos*. What causes or drove the ecological forces (dispersal or vacariance) of the sporadic distribution of each genera remained questionable. For example, the genus *Encephalartos* is endemic to southern Africa and one species (*E. barteri*) is endemic to West Africa (Donaldson 2003) and five species are found in Central Africa with no species in northern Africa. Also, the *Cycas* genus is distributed in the southward Asia to Australia and to the eastward extending to the Pacific island and further to the eastern Africa (Hill 2004). The reconstruction of historical biogeography is commonly used to reveal how species might have been distributed in the past and what could have driven the current geographic pattern of the species. Also, what could have driven the ecological and evolutionary pattern of the species?

As such, there is an urgent need to understand how best this plant group and their evolutionary diversity is distributed and safeguarded the context of the ongoing extinction crisis and historical biogeography in the tropics. To reach this global objective, a recent study (Cooper et al. 2008) demonstrated, again using a vertebrate as a case study, that an integrative approach, that combines biogeography, evolutionary data and extinction risk information is best suited to elucidate how conservation decisions can be designed efficiently to prevent biodiversity loss. Such opportunity of integrated analysis of extinction risk for cycads is missing, precluding us from designing a bigger picture of conservation plan for cycads globally. The present project aims to fill this knowledge gap.

1.7.Rationale and Justification of the study

The rationale for this project is that cycads are the most threatened group of plants, and we still have a poor knowledge of: i) what predisposes them to such a high risk of extinction, ii) how can evolutionary and extinction risk data can be analysed within a historical biogeographic concept?. In general, the future of humanity relies strongly on a continued delivery of ecosystem services (food, medicinal plants, pollination, clean air, erosion control, etc.) by the environment (Millennium Ecosystem Assessment 2005). Unfortunately, and perhaps surprisingly, human activities are driving the loss of the service deliverers, i.e. species and biodiversity, at an unprecedented rate particularly under the tropics (Vamosi and Vamosi 2008) so that scientists are now convinced that we have entered the sixth mass extinction period of human history (Barnosky et al. 2011).

Indeed, species extinction is driven mostly by direct or indirect anthropogenic forces (e.g. unsustainable use of resources, invasion of alien species, climate change, etc). Species loss is the end result of a long process of roughly three stages that define the temporal and spatial dynamic of biodiversity: i) species explosive radiation and accumulation over time (temporal dynamic), ii) species dispersal to occupy ecologically suitable niches (spatial dynamic that defines their historical biogeography) and perform environmental functions (including various ecosystem services), and iii) their extinction.

These three stages are linked such that, for example, the radiation history of species can predispose them to extinction particularly for plants (Davies et al. 2011). As such, a better understanding of the dynamic and functioning process of biodiversity is necessary to guide actions towards an environmental management and conservation that ensures a sustainable delivery of ecosystem services as targeted in the National Environmental Management. Using selected taxa as models (cycads), the present project is designed to fill the knowledge gap on the historical biogeographic and evolutionary process that governed the temporal species accumulation and spatial dynamic of cycads.

1.8. Aim and Objectives

The main aim of the study was to analyse the evolutionary and historical biogeographical predispositions of cycads to high risk of extinction. The specific objectives were as follows:

- **Objective 1**:

  Elucidate the evolutionary and ecological factors predisposing cycads to high risk of extinction

- **Objective 2**:

  To investigate the historical biogeography and evolutionary diversification of the genus *Encephalartos* using a complete phylogeny assembled based on birth-death model.

- **Objective 3**:

  To provide a refined understanding of the evolutionary and ecological processes that shaped the biogeography of the genus *Cycas*. Specifically, we assembled the most comprehensive phylogeny of the genus, which was then used to elucidate its historical biogeography as well as the ecological forces that mediated the observed diversity patterns.

1.9.Layout of the thesis

These study consists of five chapters. The first chapter presents the general introduction to the study. It provides the background to the study and outlines the problem statement and and the study rational. The chapter also presents the aim and objectives of the study as well as the layout of the thesis.

The second chapter focuses on the factors driving the global decline of cycads diversity. This chapter elucidates the evolutionary and ecological factors predisposing cycads to high risk of extinction. This chapter addresses research objective one. Questions addressed in the chapter include: 1) what are the variables that correlate with extinction risk in cycads? 2) Is there any evidence of phylogenetic signal in extinction risk 3) What are the actual causes of extinction risk in cycads 4) In which categories do species with unknown conservation status fall?

The third chapter focuses on the Origin and diversification of the African genus *Encephalartos.* The chapter traces the temporal evolutionary dynamics of diversification of *Encephalartos* and the historical forces that shaped current biogeography of *Encephalartos* in Africa. This chapter investigates the evolutionary processes (speciation, extinction, mass extinction) using five markers (*rbc*La, *mat*K, trH-*psb*A, ITS and PHYP) that shaped the current diversity and biogegraphic distribution of *Encephalartos*. This chapter is published in South African Journal of Botany.

The fourth chapter is entitled, "The genus *Cycas* have diversified from Indochina and occupied its current ranges through vicariance and dispersal events." The objective of this chapter was to provide a good insight of the ecological processes that shaped the current biogeography of the genus in Pacific-

Asia. It provide the most comprehensive phylogeny of 116 *Cycas* species from seven nuclear regions (PHYP, RPB1, HZP, AC3, F3H, SAMS and GTP) and four plastid regions (*trnH*-*psb*A, *psb*M-t*rn*D, *trn*L-*trn*F and *trn*S-*trn*G). This chapter is also published in Frontier Journal Ecology and Evolution.

Chapter 2

Factors driving the global decline of cycads diversity

Ledile T Mankga [1,2] and Kowiyou Yessoufou [3*]

[1] Department of Life and Consumer Sciences, University of South Africa, Florida campus, Florida 1710, South Africa.

[2] Department of Environmental Science, University of South Africa, Science Campus, Florida, South Africa

[3] Department of Geography, Environmental Management and Energy Studies, University of Johannesburg, APK Campus, Auckland Park 2006, Johannesburg, South Africa.

Corresponding author: Dr K Yessoufou

Corresponding author email address: kowiyouyessoufou1@gmail.com

Running head: Correlates of extinction risk in cycads group

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Abstract

Mounting evidence indicates that we are witnessing the sixth mass extinction period. Given the important goods and services biodiversity delivers to humans, there is a need for a continued commitment to investigate what pre-disposes some taxa to greater risk of extinction. Here, we investigate this question using a phylogenetic comparative method and fitting a cumulative link mixed effect model on biological, ecological and evolutionary data of cycads, the most threatened lineage in the plant kingdom. We identified nine groups of threats to cycads, with habitat loss, overcollection, fire and reproduction failure being the most prominent, but only four of these threats (habitat loss, overcollection, medicinal uses and reproduction failure) clustered on the cycad tree of life. This clustering suggests that closely related species may be exposed to similar threats, perhaps because of geographic regionalization of cycads genera. Nonetheless, the diversity of threats and several variables linked to the biology and ecology of cycads correlate with extinction risk (e.g. altitude, height, diameter, geographic range), and different variables seem to be linked to different IUCN status of cycads. Although their predictive power is generally < 50 %, geographic range and maximum diameter stood out as the best predictors particularly for the Vulnerable (VU) category, with a predictive power of 87 % and 69 %, respectively. Using our best model for VU, we predicted all five Data Deficient (DD) species of cycads to be in the VU category. Collectively, our results elucidate the pattern of extinction risk in cycads and, since most threats that we identified as drivers of extinction risk of cycads are anthropogenically mediated, we recommend stronger legislation to regulate human–cycad interactions and the commitment of all governments globally to implement this regulation.

Keywords: Anthropogenic pressure; cycad ecology and biology; data deficient species; evolutionary distinctiveness; species loss; tree of life.

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2.1 Introduction

To gain a better understanding and knowledge of the drivers of extinction risk, is necessary to inform conservation decisions, and predicting future risk could be informed of the historical extinction events. However, there is a disparity in efforts devoted to unravelling these drivers and the extinction risk patterns of vertebrates (e.g. Cooper et al. 2008; Davies and Yessoufou 2013; Luiz et al. 2016; Schachat et al. 2016; Veron et al. 2016, see further references in Pellens and Grandcolas 2016) in comparison to plants, and the only few extinction risk studies that focus on plants prioritize angiosperms (e.g. Sodhi et al. 2008; Yessoufou et al. 2012; Daru et al. 2013; Leao et al. 2014). As a result, we are comparatively well-informed of the pre-disposition of vertebrates and, to a lesser extent, angiosperms, to extinction risk as well as how their phylogenetic trees would be affected by species loss (Davies et al. 2011; Mooers et al. 2012; Davies and Yessoufou 2013). In contrast, such knowledge is yet to be well-demonstrated for gymnosperms, although the latter group is more threatened than angiosperm (e.g. 70 % of cycads are threatened, IUCN 2010; Yessoufou et al. 2017).

Cycads are a group of gymnosperm of particular interest due to their evolutionary history (Nagalingum et al. 2011; Yessoufou et al. 2014; Condamine et al. 2015) and their morphological features shared between ferns and angiosperms (Norstog and Nicholls 1997; Brenner et al. 2003). Their origin dated back to ~300 million years ago (Hendricks 1987), and in the Mesozoic era, cycads exhibited a worldwide distribution (Hermsen et al. 2009). However,

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the age of the extant cycads is much younger (12–2 Ma; Nagalingum et al. 2011), and they are restricted to tropical and subtropical regions of the world. Unfortunately, 70 % of all the 339 cycads taxa (Yessoufou et al. 2017) are threatened with high risk of extinction (IUCN 2010; Osborne et al. 2012; Yessoufou et al. 2017). Current knowledge indicates that ecological or biological factors (Sodhi et al. 2008; Yessoufou et al. 2012) as well as evolutionary history (Davies et al. 2011) pre-dispose a particular taxonomic group to risk of extinction. For example, life history trait such as body size pre-dispose vertebrates to extinction risk (Cardillo 2003), but the role of size in pre-disposing plants to extinction remains debatable (Freville et al. 2007; Bradshaw et al. 2008; Sodhi et al. 2008).

In contrast, evidence suggests that extinction risk in plants may be rather linked to their evolutionary rather than life history (Lozano and Schawartz 2005; Vamosi and Wilson 2008; Davies et al. 2011; Daru et al. 2013). Based on this knowledge, we compiled a list of putative biological and ecological parameters linked to extinction risk in previous studies. This includes altitude, diameter, diversity of threats (i.e. number of threats recorded for each species), generation time, geographic range and height. For example, high-altitude habitats are usually considered a ‘safe heaven’ for ancient but threatened taxa (Fjeldsa and Lovett 1997; Fjeldsa et al. 2012). Also, an early study found a higher richness of threatened species at high altitude (Yessoufou et al. 2012).

In addition, extinction risk in animals has also been linked to body size, generation time and geographic range with the expectations that species with larger size, longer generation time and

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smaller geographic range would be more at risk (Bennett and Owens 1997; Russell et al. 1998; Purvis et al. 2000; Cardillo 2003; Fisher and Owens 2004; Cooper et al. 2008; IUCN 2010). The representatives of body size in the present study are diameter and height (see also Sodhi et al. 2008).

Furthermore, evidence that evolutionarily younger or older taxa tend to be more at risk and is indicative of an evolutionary pre-disposition to extinction (Purvis et al. 2000; Vamosi and Wilson 2008; Davies et al. 2011; Daru et al. 2013). Also, because phylogenetically conserved traits can be linked to extinction (e.g. phenology; Willis et al. 2008), it becomes necessary to assess the distribution of extinction risk along a phylogenetic tree (Fritz and Purvis 2010; Davies et al. 2011; Yessoufou et al. 2012), although extinction risk is not an evolving trait (Grandcolas et al. 2011). Such a phylogenetic signal analysis of extinction risk would help predict whether unrelated species (e.g. species with high value of evolutionary distinctiveness (ED); Isaac et al. 2007) or closely related ones are more at risk (Purvis et al. 2000). However, the phylogenetic analysis of extinction risk is traditionally conducted based on IUCN threat categories (Davies et al. 2011). This tradition has recently showed to be potentially misleading as long as the drivers of extinction risk are not taken into consideration (see Schachat et al. 2016). Similarly, conservation decisions could be further misled if the threat status of some species remains unknown (e.g. Data Deficient, DD, species, Luiz et al. 2016, Veron et al. 2016).

In the present study, our objective is to provide a better explanation of extinction risk in the cycads group. Specifically, we identified and categorized all threats to cycads, tested for phylogenetic signal in the threat categories, and generate the best model of extinction risk that was then used to predict the threat status of DD species.

2.2. Material and Methods

2.2.1 Data Collection

2.2.1.1 Global cycad diversity, IUCN status and categories of threats

In a recent study, our research group compiled a list of 339 cycads taxa following a thorough literature search (e.g. Lindström 2009, Nagalingum et al. 2011, Osborne et al. 2012) and taking into account some synonymous names [see Appendix A]. In the same study, IUCN threat categories for all taxa were also compiled (www.redlist.org, August 2016; Osborne et al. 2012): DD (five taxa), Least Concern (LC: 47 taxa), Near Threatened (NT: 68 taxa), Vulnerable (VU: 78 taxa), Endangered (EN: 70) and Critically endangered (CR: 67 taxa). In the present study, we complemented these data with additional information on different threats to cycads available from various sources including the IUCN database (www.redlist.org, August 2016) [see Appendix A].

2.2.1.2 Cycad tree of life

The phylogenetic tree used in this study is the complete cycad tree comprising 339 taxa

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recently assembled in our research group (see Yessoufou et al. 2017) by combining DNA sequences of the nuclear region PHYP for 199 species (Nagalingum et al. 2011) and taxonomic information following the Thomas et al.'s (2013) approach. This tree is submitted to TreeBase with the submission ID # 20161 and the details of tree reconstruction are available in a recent paper that we published (Yessoufou et al. 2017).

2.2.1.3 Potential predictors of IUCN status

To fit predictive models of IUCN status for all cycads, we compiled a list of putative variables including altitude, diameter, diversity of threats, ED, generation time, geographic range and height. We recorded the minimum and maximum of the altitudinal occurrence of each species from IUCN (2010). Two types of diameters were recorded, the minimum and the maximum diameter. The diversity of threats was defined as the number of threat categories (as defined above) recorded for each species. ED is a metric that approximates the evolutionary ages of each species such that a species with a higher ED value is subtended on a phylogeny by a longer branch (Isaac et al. 2007). ED values for all cycads were compiled from Yessoufou et al. (2017) [see Appendix A]. Data on generation time were retrieved from the IUCN database (www.redlist.org, August 2016). Geographic range data were compiled in two ways; first as surface area of geographic ranges (in km^2) and these data were retrieved from IUCN (2010) and, second, as the number of locations where a species occurs (defined in Osborne et al. 2012). Finally, we documented the minimum and maximum height for each cycads species also from IUCN. Overall, 11 variables were included in our predictive models, and values for all these variables are presented in [see

Appendix A].

2.2.2 Data analysis

All analyses were conducted in R (R Core Team 2015) and detailed below. Prior to analyses, we checked for correlations among all the 11 variables to avoid redundancy. We found that minimum altitude and maximum altitude do correlate, as well as minimum and maximum height [see Figure 2.1]. Therefore, we discarded maximum altitude and maximum height, implying that the analysis on modelling presented below focused only on the remaining nine variables.

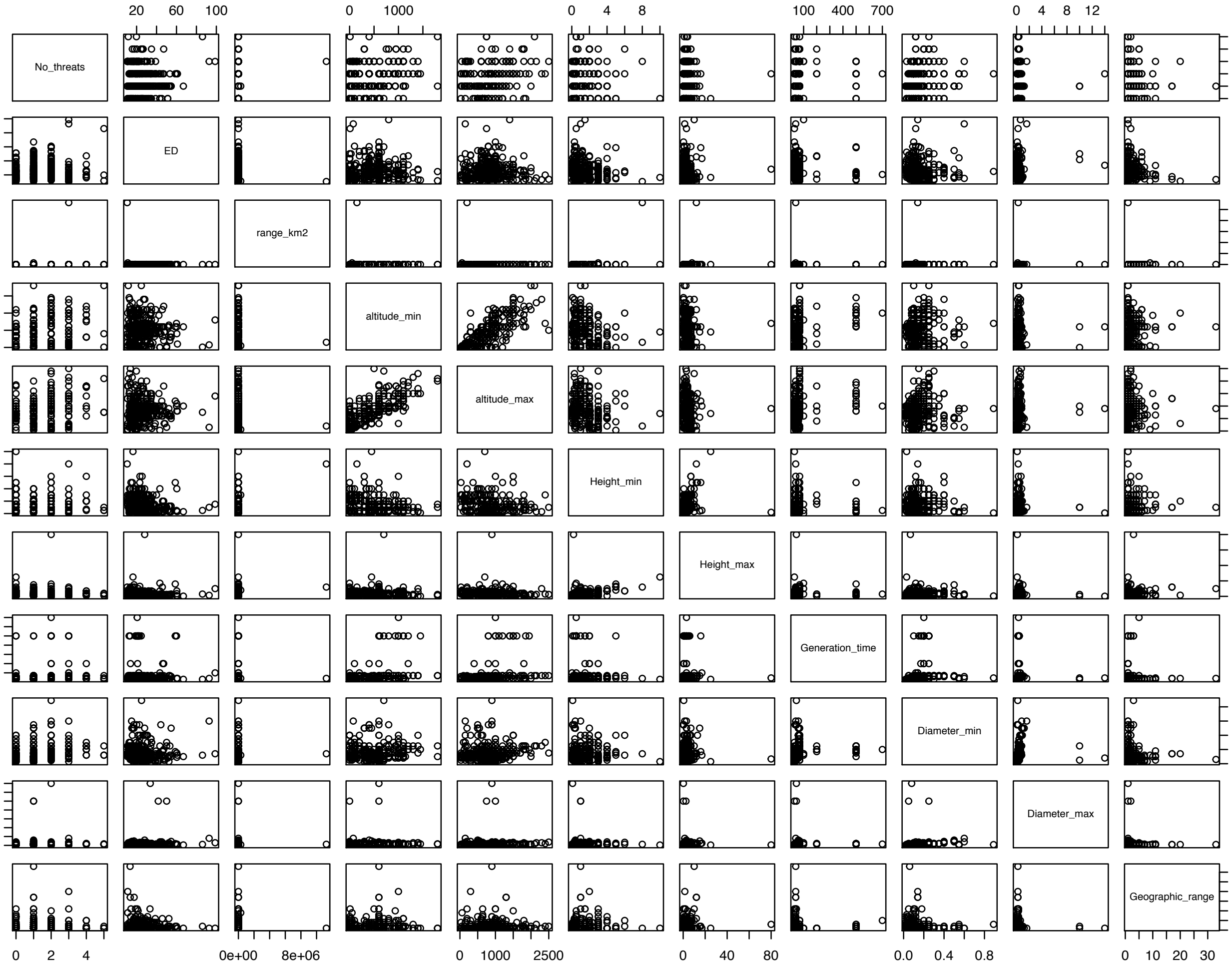

Figure 2.1 Test for autocorrelation among variables

2.2.3. Phylogenetic signal in threat groups to cycads

Each threat identified was coded as follows; 1: when a threat is reported for a species; 0: when a threat is not reported for a species and NA: when information for a species were missing for a given threat [see Appendix A]. Prior to any analysis, missing data were explored using a combination of graphical displays (Prantner 2011), first for threats (see

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Figure 2.1), then for predictors of IUCN status (see Figure 2.3 and 2.4), and lastly, we used the k-nearest neighbour imputation method implemented in the R package VIM (Templ et al. 2016) to impute NA values. Next, the D statistic (Fritz and Purvis 2010) was applied to assess the phylogenetic signal in each threat using the complete cycads phylogeny of Yessoufou et al. (2017). The D statistic provides an estimate of phylogenetic conservatism for binary traits that can be compared with both a random shuffle of trait values at the tips of a phylogeny and a Brownian threshold model (BM; Fritz and Purvis 2010), but we reported here only the significance at random. When $D = 1$ then traits are randomly distributed at the tips of the phylogeny; $D = 0$ corresponds to a BM model; $D < 0$ signifies traits are highly conserved, whereas $D > 1$ signifies traits are over-dispersed on phylogenetic tree. If a D value falls between 0 and 1, then we tested whether this value is statistically different from 1 (random); if so, then we concluded that the observed D value is non-random. If D value is not statistically different from 1, then the observed value is considered as random.

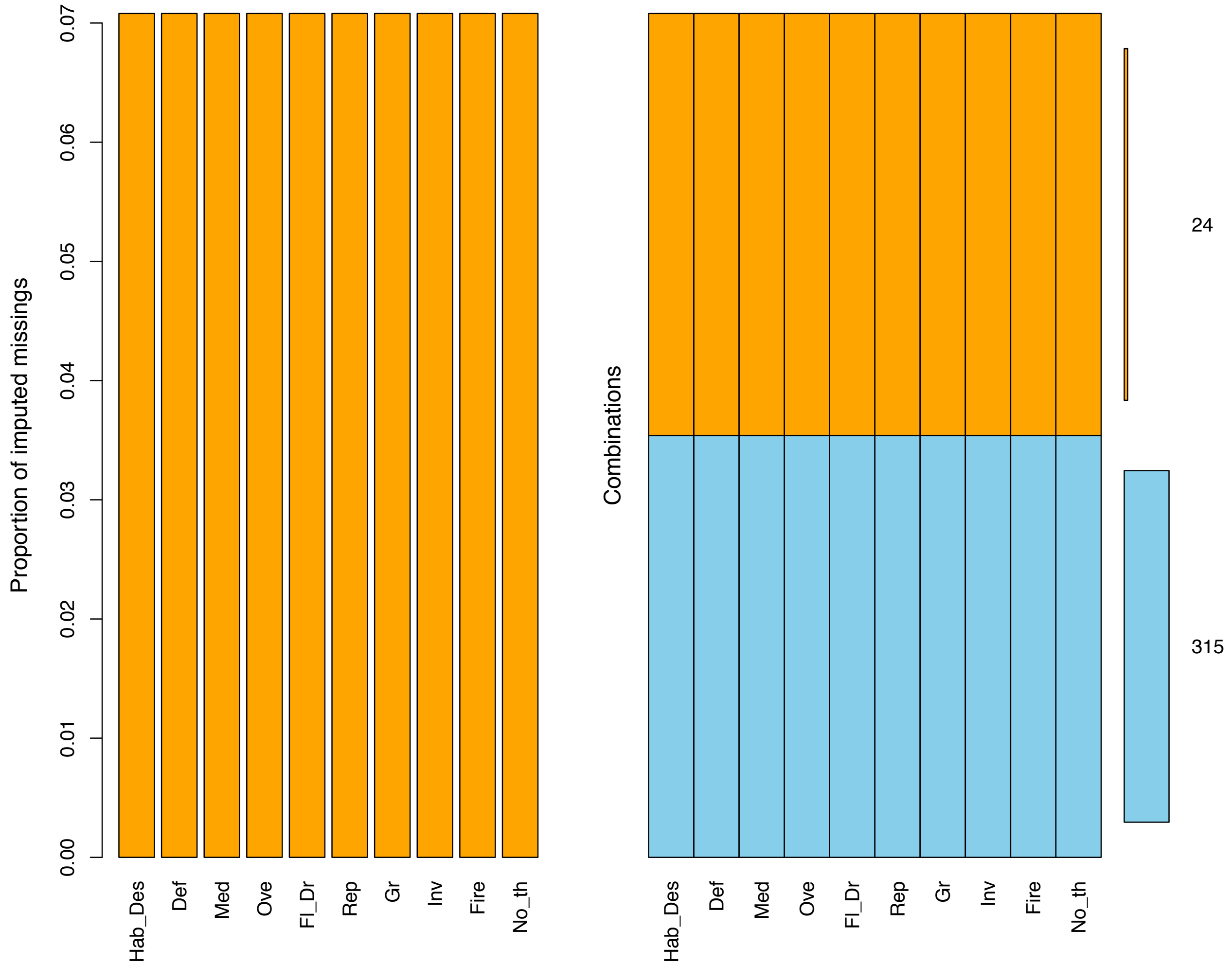

Figure 2.2 Aggregation graphic of the imputed missing data for all threats identified for cycads. Left: Barplots indicating that all threats have the same amount of imputed values; Right: An aggregation plot, showing all existing combinations of imputed (orange) and observed (blue) values. Far Right: small barplot showing the frequencies of different combinations. Threats are coded as follows: Hab_Des = habitat destruction; Def = deforestation; Med = medicinal uses; Ove = overcollection; Fl_Dr = flood/drought; Rep = reproduction failure; Gr = grazing; Inv = invasive species; No_th = number of threats.

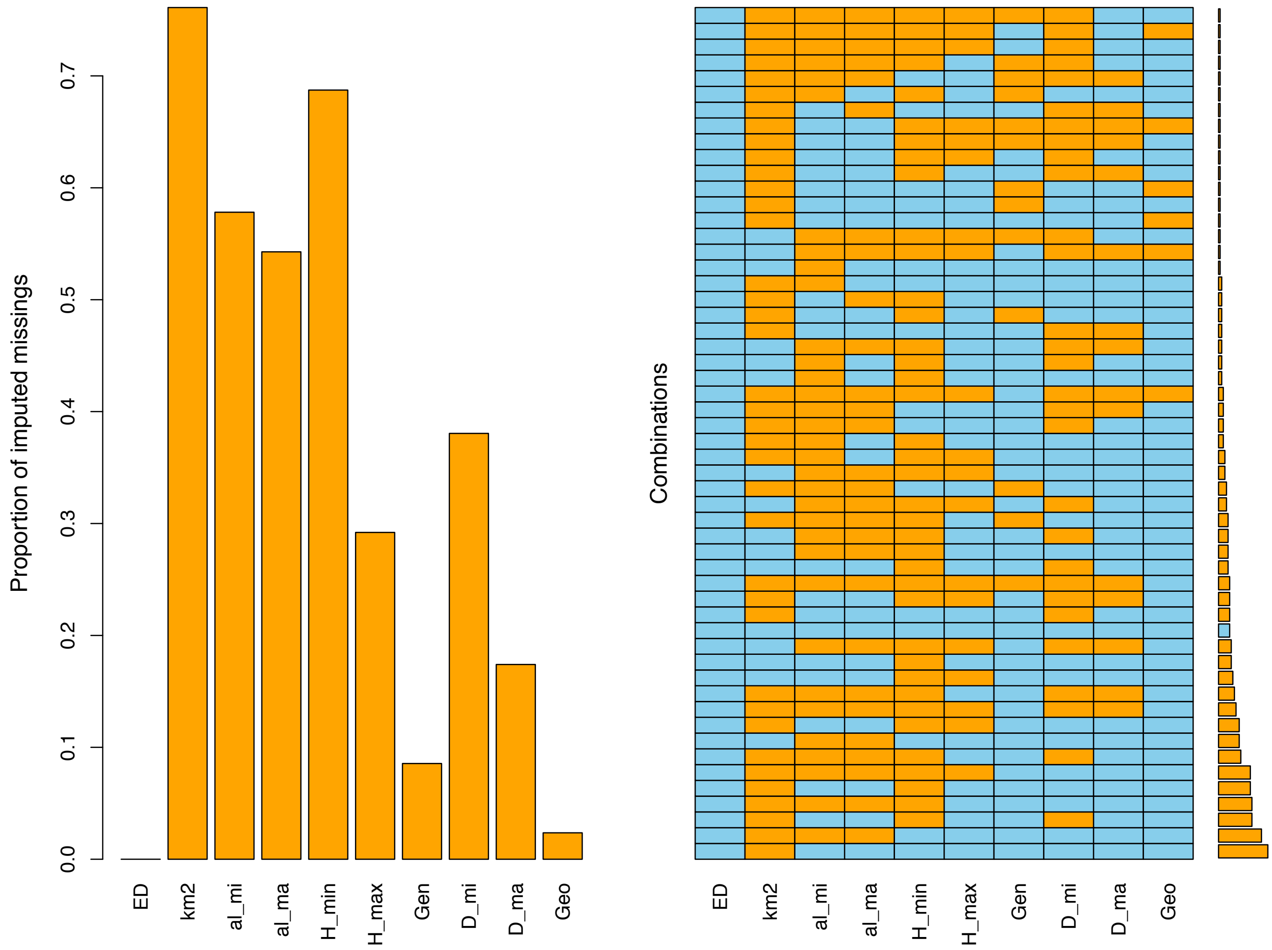

Figure 2.3 Aggregation graphic of tthe imputed data for all predictors of extinction risk included in the study. Left: Barplots indicating geographic range (km^2) and Minimum height have the largest amount of imputed values; Right: An aggregation plot, showing all existing combinations of imputed (orange) and observed (blue) values. Far Right: small barplot showing the frequencies of different combinations. Overall, the aggregation plot shows that a species for which the geographic range (km2) is missing will also likely lack data on altitude and height. The predictors are: ED = evolutionary distinctiveness; km2 = geographic range measured as surface area; al_mi = minimum altitude; al_ma = maximum altitude; H_min = minimum height; H_max = maximum height; Gen = generation time; D_mi = minimum diameter; D_ma = maximum diameter; Geo = geographic range measured as number of locations of species occurrence.

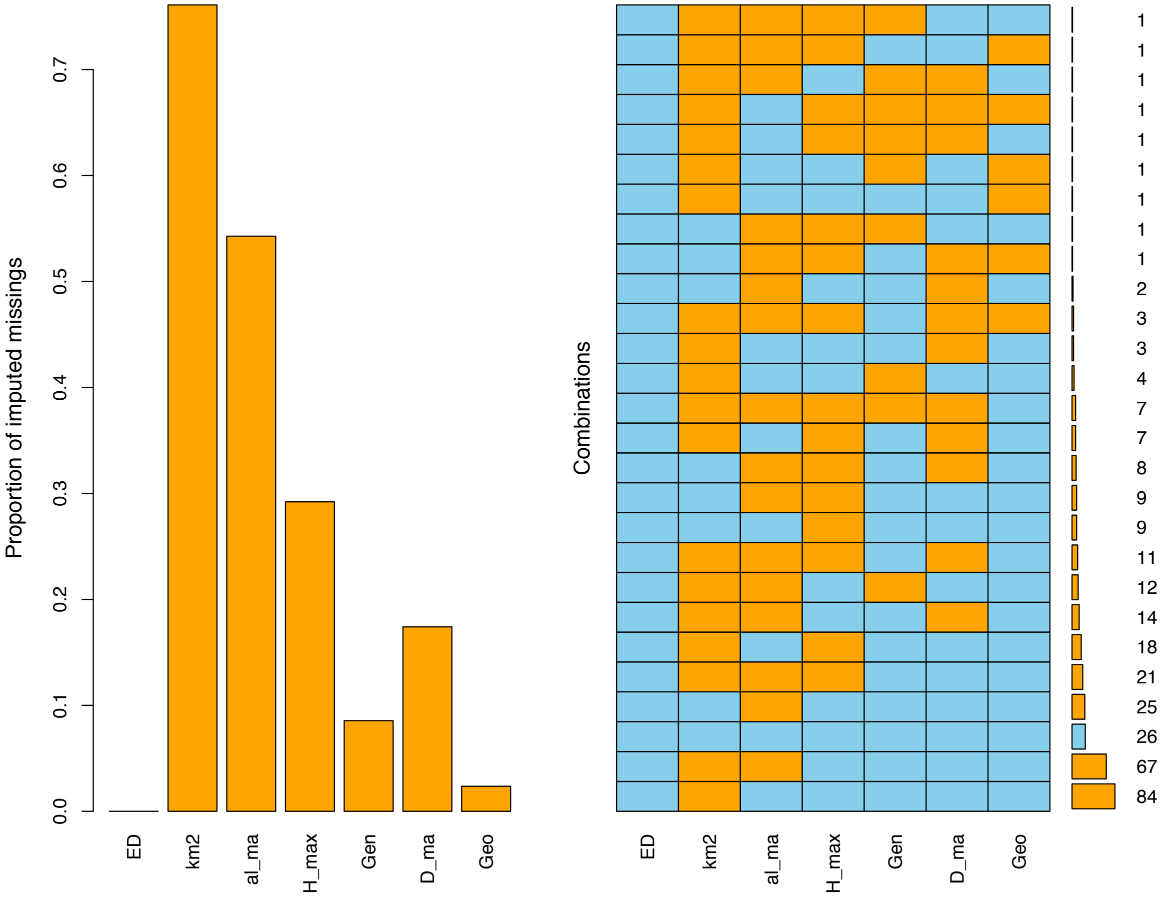

Figure 2.4 Aggregation graphic of the imputed data for some predictors of extinction risk. These predictors are those that have minimum and maximum values as indicated in Fig. 2.3 (e.g. minimum and maximum height); the difference with Fig. 2.3 is that we only show maximum value of predictors unlike in Fig. 2.3 where both maximum and minimum values are shown. Left: Barplots indicating geographic range (km^2) and Minimum height have the largest amount of imputed values; Right: An aggregation plot, showing all existing combinations of imputed (orange) and observed (blue) values. Far Right: small barplot showing the frequencies of different combinations. Overall, the aggregation plot shows that a species for which the geographic range (km2) is missing will also likely lack data on altitude and height. The predictors are: ED = evolutionary distinctiveness; km2 = geographic range measured as surface area; al_mi = minimum altitude; al_ma = maximum altitude; H_min = minimum height; H_max = maximum height; Gen = generation time; D_mi = minimum diameter; D_ma = maximum diameter; Geo = geographic range measured as number of locations of species occurrence.

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2.2.2.4 Predictive models for extinction risk of cycads

We explored the power of each of our nine variables to predict the IUCN threat status of cycads species by fitting the cumulative link mixed effect model (CLMM; Christensen 2013). The IUCN status is a ranked categorical status defined as LC < NT < VU < EN < CR. We preferred the CLMM approach as our modelling method to the machine-learning methods based on a number of advantages the CLMM provides (see Luiz et al. 2016 for details). In summary, CLMM is a better approach as it allows a direct analysis of ranked categorical variables (here IUCN categories: LC < NT < VU < EN < CR) as response variables without necessarily converting them into numerical values (and such conversion is the tradition; e.g. see Mooers et al. 2008, Davies et al. 2011 or Yessoufou et al. 2012). In so doing, CLMM has the advantage of preserving the variance structure of the original ordinal ranks of the categorical response variables, and thus prevents the loss of information generally observed when categorical values are either converted into numerical values or grouped into binomial classifications (e.g. non-threatened vs. threatened categories). CLMM also prevents an unnecessary elevated type I error generally observed when IUCN categories are converted into numerical values where differences between adjacent risk levels are assumed equivalent (e.g. LC = 0 and NT =1 or EN = 3 and CR = 5).

In the models, the response variable is the IUCN status, and the dependent variables used as fixed effects are altitude, diameter, diversity of threats, ED, generation time, geographic range and height. We also included the taxonomic ranks 'Genus' within 'Family' and 'Family' within 'Order' as a random effect in our models to account for potential effects of shared ancestry, using the R function 'clmm' (package 'ordinal', Christensen 2013).

Then, two types of models were generated, univariate and multivariate. For the multivariate model, we conducted model selection using a backward stepwise removal of non-significant fixed-effect terms from the full model, based on log-likelihood ratio tests. The predictive power of our model fit was quantified as the percentage of species whose IUCN status are correctly predicted by the model; this is referred to, in Luiz et al. (2016), as Percentage Correct Classified (PCC). The PCC value was calculated in two ways; first, PCC was calculated for each best model identified considering all species (i.e. overall predictive power of each model) and second, PCC was also calculated for each best model considering species in each IUCN category (i.e. predictive power of each model per IUCN category). Models were fitted using the function 'clm' from the R package 'ordinal' (Christensen 2013). We used the coefficients of the final model to estimate the IUCN threat status of the five DD species (the R function 'predict' implemented in the package 'ordinal').

2.3 Results

Cycads group comprises of 64 % threatened species in the categories VU (23 %), EN (21 %) and CR (20 %), and almost 1 % and 2 % of species are in the categories Extinct in the Wild and DD, respectively (Figure 2.5A). Such a high level of extinction risk is driven mainly by nine categories of threats including predominantly habitat loss (38 %), overcollection (29 %), fire (9 %) and reproduction failure (8 %) and to a lesser extent invasive species (3 %; Figure 2.5B).

In total, five variables that correlate significantly with the extinction risk were identified, of which three correlate negatively [geographic range (measured as number of locations of species occurrence), minimum height and maximum diameter] and the remaining two correlate positively (threat diversity, i.e. number of threats facing each species and minimum altitude; Table 1, Figure 2.5C).

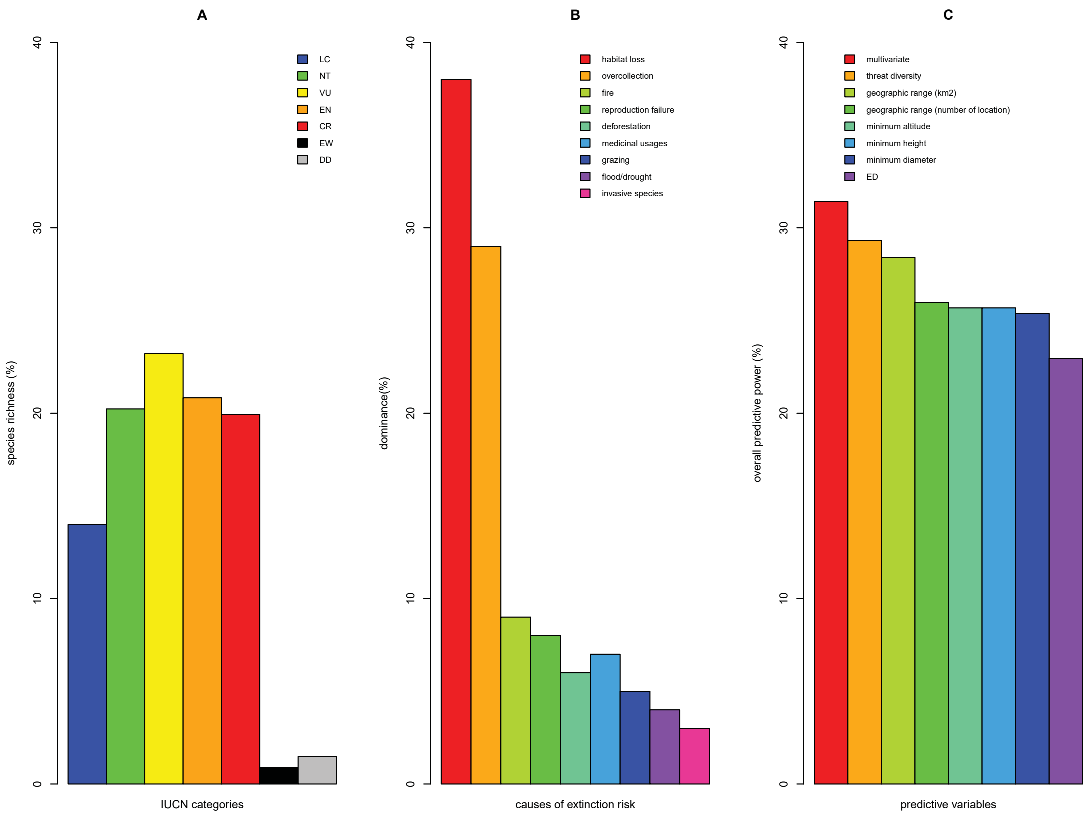


Figure 2.5 General pattern of extinction risk in cycads group. **(A)** Cycad species richness in each IUCN threat categories (LC = least concern; NT = near threatened; VU = vulnerable; EN = endangered; CR = critically endangered; DD = data deficient; EW = extinct in the wild). **(B)** The identified causes of threats to cycad globally, (C) the overall predictive power of all significant correlates of extinction risk of cycads. Multivariate = the best multivariate model, the model include maximum diameter, geographic range (measured as number of locations of species occurrence) and the diversity of threats (Table 2.1).

Table 2.1. Parameters of the cumulative linear mixed effects models with IUCN redlist category as an ordinal categorical response variable. Significant variables are indicated by stars (*) and the number of stars indicates the level of significance. NS= not significant.

Univariate model	Variables	Estimate	Standard error	Z values Test Statistics	Probability values
	Diversity of threats (log +1)	1.531	0.244	6.260	P<0.001***
	ED (log)	0.500	0.256	1.953	P =0.05
	Range (km^2) (log +1)	-0.087	0.055	-1.572	P =0.116
	Minimum altitude (log +1)	0.234	0.061	3.83	P<0.001***
	Minimum height (log)	-0.287	0.111	-2.594	P=0.009**
	Generation time (log)	-0.039	0.312	-0.127	P=0.899NS
	Maximum diameter (log)	-0.404	0.185	-2.177	P=0.029*
	Geographic range (number of locations) (log)	-0.625	0.154	-0.036	P<0.001***
	Diversity of threats (log +1)	1.531	0.244	6.260	P<0.001***
	ED (log)	0.500	0.256	1.953	P= 0.05
	Range (km^2) (log +1)	-0.087	0.055	-1.572	P =0.116
	Minimum altitude (log +1)	0.234	0.061	3.83	P<0.001***
	Minimum height (log)	-0.287	0.111	-2.594	P=0.009**
	Generation time (log)	-0.039	0.312	-0.127	P=0.899^{NS}
	Maximum diameter (log)	-0.404	0.185	-2.177	P=0.029*
	Geographic range (number of locations) (log)	-0.625	0.154	-0.036	P<0.001***
Multivariate model	Maximum diameter (log)	-0.471	0.185	-2.491	P0.012*
	Geographic range (log)	-0.647	0.156	-4.139	P<0.001***
	Diversity of threats (log)	1.554	0.245	6.319	P<0.001***

However, the multivariate model (that includes threat diversity, maximum diameter and geographic range) has the overall highest predictive power of extinction risk (PCC = 33 %) and maximum diameter the lowest (PCC = 24 %; Figure 2.5C). At IUCN category level, although we found that all models (uni- and multivariate) yielded their best prediction for VU category (except for minimum altitude, Figure 2.6), It was also found that different variables are good predictors of different IUCN categories. For example, geographic range (measured as number of locations), maximum diameter and minimum height are excellent predictors of VU (87 %, 69 % and 54 %, respectively); minimum altitude for CR (41 %), diversity of threats for EN (32 %) and VU (33 %; Figure 6).

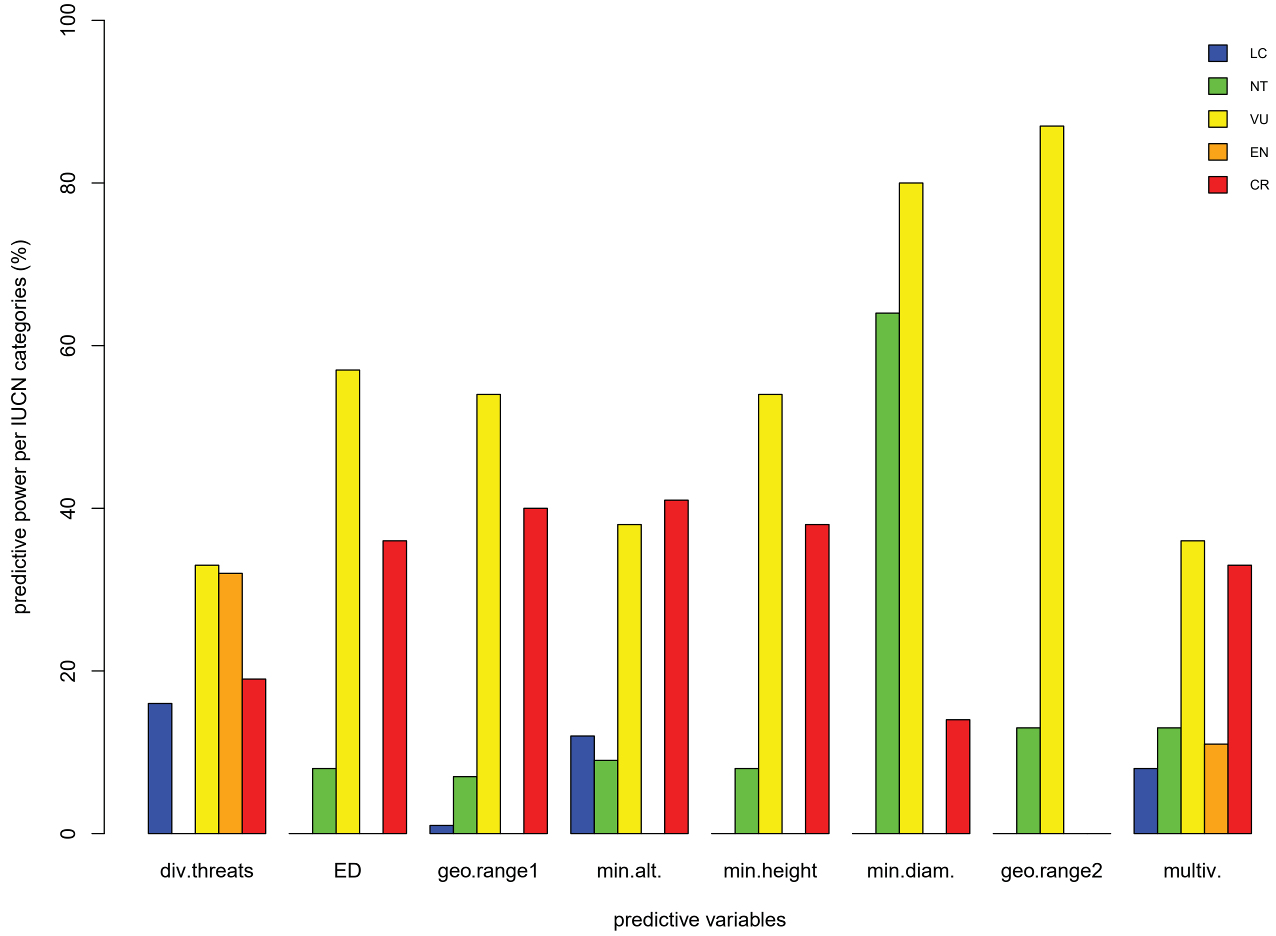
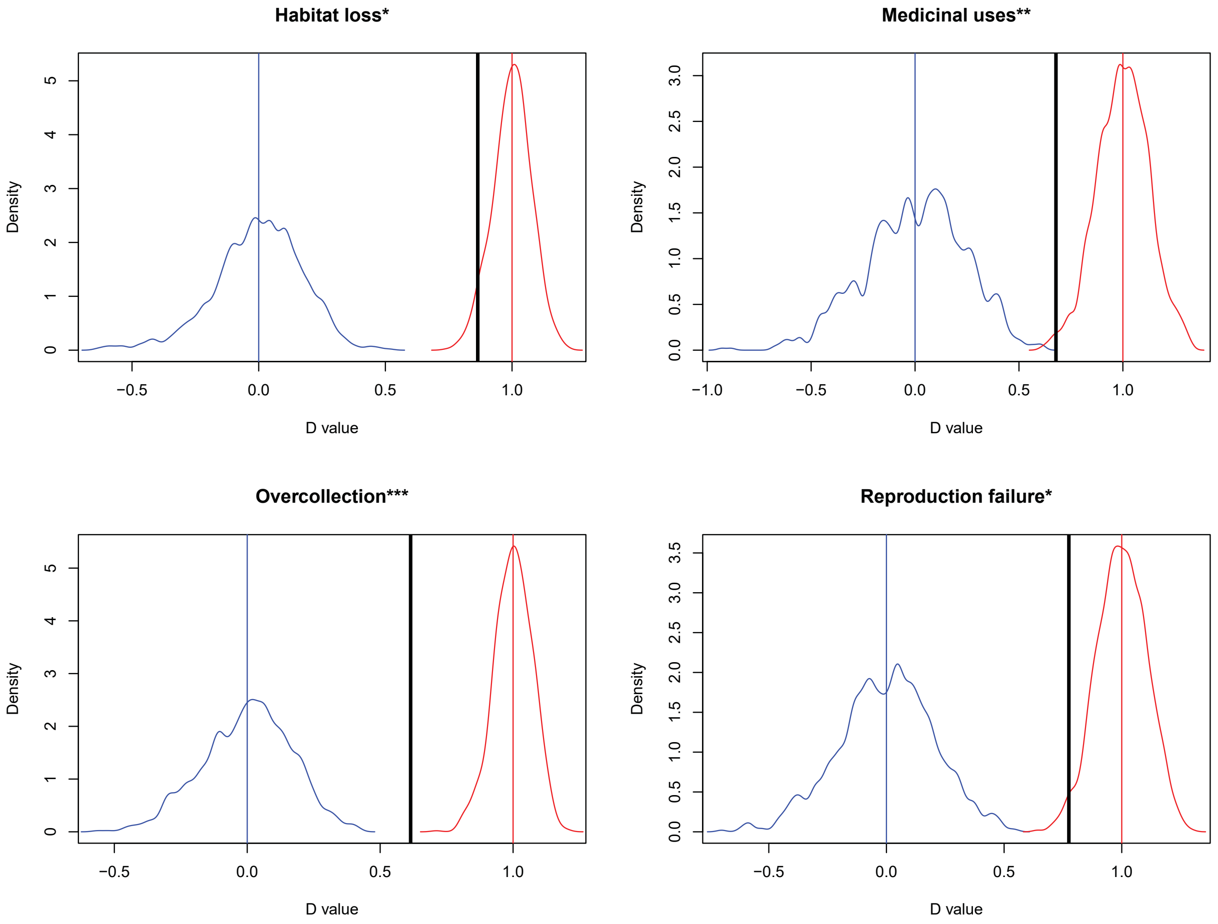


Figure 2.6 Predictive power of all significant correlates of extinction risk in each IUCN categories. LC = least concern, NT = near threatened, VU = vulnerable, EN = endangered, CR = critically endangered.

All these models indicate that the DD species are threatened (VU, EN or CR). In particular, based on the geographic range (number of locations) that showed the highest predictive power for VU (87 %), all DD species (*Cycas aenigma*, *Cycas. indica*, *Cycas sphaerica*, *Ceratozamia brevifrons* and *Zamia lindleyi*) are predicted to be in the VU category. Finally, we further explored the phylogenetic predisposition of cycads to extinction risk. Of the nine categories of threats identified, we found evidence of phylogenetic signal in only four: habitat loss, medicinal uses, overcollection and reproduction failure (Figure 2.7).



Habitat loss*

Medicinal uses**

Overcollection***

Reproduction failure*

Figure 2.7 Results of the tests of phylogenetic signal in the causes of extinction risk using Fritz and Purvis (2010) D Statistics. The graph in blue in the distribution of D values assuming a Brownian Motion (BM) model. The blue vertical lines indicates D = 0 (When the phylogenetic distribution of a parameter is no different from BM). The graph in red is the distribution of D values assuming a random model, and the red vertical line indicates D = 1 (when the phylogenetic distribution of a parameter is no different from random). The bold black line indicates the observed D values. The number of * is indicative of the significance level of the observed D values.

2.4. Discussion

All threats of cycads were summarized into nine categories, of which seven were directly linked to human activities (habitat loss, overcollection, fire, deforestation, medicinal usages, grazing, invasive alien plants), one to the biology of cycads (reproduction failure) and the last one was linked to climate change impacts (flood/drought). This is an indicative of the prominent role human plays in driving the loss of biodiversity (Wake and Vredenburg 2008), particularly under the tropic (cycads are mainly tropical), thus supporting the well-known 'tropical biodiversity crisis' (Vamosi and Vamosi 2008).

Although there is a general trend for closely related species to be threatened (phylogenetic signal) irrespective of the taxonomic group at hand (Purvis et al. 2000; Purvis 2008; Davies et al. 2011; Yessoufou et al. 2012; see Yessoufou and Davies 2016 for further references), a recent study revealed that such evidence does not hold for cycads, i.e. threatened cycads species are not significantly clustered on the cycad tree of life (Yessoufou et al. 2017). The phylogenetic signal analysis is traditionally explored on IUCN threat categories (e.g. Fritz and Purvis 2010; Davies et al. 2011; Yessoufou et al. 2017), but a recent study demonstrated convincingly that the causes of extinction rather than the extinction risk status should be integrated into the phylogenetic comparative analysis of extinction risk (Schachat et al. 2016). As opposed to Yessoufou et al. (2017) who found no evidence for a phylogenetically patterned extinction risk for cycads, our results here indicate that certain causes of extinction of cycads species can be linked to phylogenetic pre-disposition. For example, we found evidence that phylogenetically closely related species are more threatened than expected by habitat loss, overcollection, medicinal uses and reproduction failure. These finding suggests a phylogenetic pre-disposition of cycads to extinction such that closely related species may share similar vulnerabilities in the face of similar threats. Such phylogenetic pre-disposition could be the result of closely related species sharing similar life history traits that evolve along the phylogeny. It could also be because closely related species are in fact exposed to similar threats, given the geographic regionalization of cycads genera (e.g. all species within the genus *Encephalartos* occur in Africa, and all species in the genus *Ceratozamia* occur in the New World, etc.).

We further tested for correlates of extinction risk of cycads fitting a CLMM on nine biological, ecological and evolutionary variables. Two of these variables correlate positively with extinction risk (diversity of threats and minimum altitude). The finding that species facing a high diversity (number) of threat are more at risk of extinction is not a surprise. So, is the positive correlation of extinction with altitude, as higher altitude may be playing the role of refugia for species that are threatened at lower altitude due to human pressure (Sandel et al. 2011; Yessoufou et al. 2012; White and Bennett 2015). Positive correlation of ED, a phylogenetic metric, with extinction risk was previously reported for cycads (e.g. Yessoufou et al. 2017), and this provides support for a phylogenetic pre-disposition of plants to extinction (Vamosi and Wilson 2008, Davies et al. 2011; Condamine et al. 2013; Daru et al. 2013). How does phylogenetic history pre-dispose plant to extinction risk remains to be elucidated. In the Cape Floristic Region, young plant lineages are more at risk (Davies et al. 2011) whilst the opposite trend was recently reported for plant lineages in mangrove ecosystems (Daru et al. 2013). However, the correlation between ED and extinction risk reported in Yessoufou et al. (2017) is not confirmed in the present study for cycads, and this is because Yessoufou et al. (2017) did not account for shared ancestry among species in their analysis.

Furthermore, geographic range (measured as number of geographic locations where species are found; Osborne et al. 2012), minimum height and maximum diameter show a negative correlation with extinction risk. This result for geographic range is not surprising as this variable is one of the keys of IUCN threat categorization system (IUCN 2010). However, a negative correlation of height with extinction is counterintuitive, as we would expect taller species to be more at risk because they are easy to spot by illegal cycads collectors. The finding could perhaps be an indication that shorter cycads might be more VU to a number of threats that we identified above, including flood, invasive plants, and particularly to grazing and fire (see lanky and corky strategy of Dantas and Pausas 2013). The correlation of height with extinction risk could also result in the correlation that we found for diameter, given the well-known allometric relationship between height and diameter (e.g. Mugasha et al. 2013). In particular, species with small diameter may have invested more in vertical growth (i.e. height), and in light of the negative correlation between height and extinction risk, the negative correlation between diameter and extinction risk becomes meaningful. Despite these significant correlations, the overall predictive power of the models generated are only between 24 % and 33 %, indicating that many other variables driving the extinction risk of cycads are not included in the study. This provides room for further investigations of the pre-disposition of cycads to high risk of extinction.

In contrast, we found a strong predictive power while looking at each IUCN threat category level particularly for geographic range (number of locations of species occurrence), which predicts correctly 87 % of VU status. Such strong predictive power per IUCN threat category was used to clarify the status of DD species. All five DD cycad species were predicted to be in the VU category, thus adding five more species to the threatened cycad species richness.

In the present study, we explored what pre-disposes cycads to high risk of extinction. Mainly human induced pressures (e.g. habitat loss, grazing, fire, medicinal usages, etc.), the biology of cycads (e.g. reproduction failure) and climate related variables (e.g. drought, flood) threaten cycads diversity, putting at risk the evolutionary history accumulated in the cycad tree of life over million years. We acknowledge that many other threats particularly linked to the biology and ecology of cycads (e.g. dispersal ability, availability of pollinators, cycad–pollinator interactions, etc.) could also be playing a role in shaping the current extinction risk pattern, but these variables are not evaluated in the present study. We explicitly explored this pattern and reveal a phylogenetic basis for extinction risk of cycads such that phylogenetically closely related species are exposed to similar threats. It is well established that excluding DD species from extinction risk analysis (this is the tradition, Bielby et al. 2006, Yessoufou et al. 2012) is likely to induce bias in decision making process (Whittaker et al. 2005, Luiz et al. 2016) and could therefore mislead our understanding of how extinction risk may prune the tree of life (Veron et al. 2016). More critically, we identified significant (statistically) correlates of extinction risk for cycads, and used predictive models to determine the IUCN threat status of the five DD cycads species. As such, this study allows for a comprehensive picture of the extinction pattern in cycads group and elucidates the evolutionary pre-disposition of plants to extinction risk.

Chapter 3

On the origin and diversification history of the African genus *Encephalartos* Lehm.

Ledile T. Mankga[1,2], Munyaradzi Chitakira[2], and Kowiyou Yessoufou[3]

[1] *Department of Life and Consumer Sciences, University of South Africa, Science Campus, Florida, South Africa*

[2] *Department of Environmental Science, University of South Africa, Science Campus, Florida, South Africa*

[3] *Department of Geography, Environmental Management and Energy Studies, University of Johannesburg, APK campus, Johannesburg, South Africa*

Corresponding author: Mankga LT

Corresponding author email address: mankga.ledile@gmail.com

Running head: Historical biogeography and diversification of the African cycads

Abstract

Of the 10 known genera of cycads, the genus *Encephalartos* is endemic to Africa. Although a number of studies showed interest into the taxonomic relationships within the genus as well as its diversification history, we still have limited understanding of its historical biogeography. In the present study, using five gene markers, we first reconstructed a complete phylogeny of the genus. Then, we reconstructed its historical biogeography based on S-DIVA model. Finally, we fitted the CoMET model to test for significant shifts in evolutionary events driving the current diversity in the genus. Overall, our phylogeny is well supported and reveals two major clades following species geographic origins, one southern African clade and one east-central-west African clade. Our biogeographic analysis suggests that the genus may have diverged around 9 Ma from southern Africa (100% probability) and then colonized the region through dispersal. Although the origin of the east-central-west African clade is uncertain (50% probability), the clade may have diverged from the southern African clade through vicariance. We suggest that the eastern rift system in eastern Africa and the west African *Dahomey Gap* – a dry corridor that breaks pan-African tropical rainforest into different blocks – are geographical barriers limiting dispersal into the rest of the continent whilst, at the same time, promoting the vicariance signature observed. Although most species accumulated in the last 2.6 Ma, there were no significant shifts in any of the evolutionary events, suggesting that a constant-rate diversification model is best suited for the genus and that the rapid species accumulation during the Pliocene–Pleistocene transition may not be as dramatic as initially thought.

Keywords: Birth-death speciation, cycads, diversification, *Encephalartos*, historical biogeography, Yule speciation.

3.1. Introduction

Cycads are ancient seed plants with fossil records dated back to about 270 Ma (Hendricks 1987; Norstog and Nicholls 1997; Davis and Schaefer 2011). They attained their greatest diversity during the dinosaurs' era (Jones 2002) but re-diversified around 12 Ma (Nagalingum et al. 2011). They share similar morphological characters with ferns and angiosperms (Norstog and Nicholis 1997) and are restricted to tropical and subtropical regions (Jones 2002; Whitelock 2002; Donaldson 2003).

Taxonomically, cycads comprise about 300-350 species in 10 genera (Osborne et al. 2012; Calonje et al. 2017; Yessoufou et al. 2017). Of these genera, the genus *Encephalartos*, with its 65 species, is entirely endemic to Africa (Hill and Stevenson 1998). The majority of these species occur in southern Africa: 45 species are endemic to southern Africa, and only one species (*E. barteri* Lehm.) is endemic to West Africa (Donaldson 2003; Golding and Hurter 2003), with no known species in northern Africa. This raises the question of why there is such a disproportionate distribution of the genus on the continent.

To respond to this question, the present study proposes to reconstruct both the historical biogeography and the evolutionary diversification history of the genus. The reconstruction of historical biogeography is one of the approaches commonly used to reveal how species might have been distributed in the evolutionary past and is more likely to point out potential ecological forces (e.g. dispersal, vicariance, etc.) that may have shaped current geographical pattern of a given taxonomic group (McPeek and Brown 2000; Stoks and McPeek 2006; Simões et al. 2016).

In an early study, Yessoufou et al. (2014) attempted to answer the question by investigating the evolutionary diversification history of *Encephalartos* with no focus on the biogeography of the genus. They revealed an overall constant diversification pattern punctuated by a mass extinction event (around ~2.6 Ma) that may have triggered an explosive radiation mediated through ecological opportunities (Lovette and Bermingham 1999). Yessoufou et al. (2014) indicated that this explosive radiation occurred specifically in southern Africa. Although their study provided some insights into how species in the genus may have been accumulated over evolutionary time in Africa, the study employed the Yule priors while reconstructing the phylogenetic tree of the genus. A recent study called for caution with regard to the unquestionable use of Yule priors in Bayesian relaxed-clock dating of phylogenetic tree (see Condamine et al. 2015).

The Yule priors (also known as pure birth) model tree formation assuming constant speciation rate with no extinction while the birth-death priors model tree formation assuming that both speciation and extinction events have occurred. Both prior types have a significant influence on the resulting phylogenetic tree, particularly on the estimation of node ages (Couvreur et al. 2010; Condamine et al. 2015). Condamine et al. (2015) for instance, revealed some striking differences in age estimates and diversification dynamics of cycads when the Yule versus the birth-death priors were employed on the same dataset. They consequently recommended the use of the birth-death priors especially when it is unequivocally known - as is the case for cycads - that the taxonomic group at hand has experienced past extinction events (Niklas 1997; Crepet and Niklas 2009).

In light of Condamine et al.'s recommendation, Yessoufou et al.'s (2014) findings need to be re-investigated, given the influence of model selection (Yule vs. birth death) of tree reconstruction particularly on age estimates and consequently on both evolutionary events and biogeographic analysis. The main aim of the present study was to explain the geographical pattern of the genus *Encephalartos* using a complete phylogeny assembled based on birth-death model and with which the historical biogeography and evolutionary diversification of the genus were investigated.

3.2 Materials and Methods

3.2.1 Reconstruction of a complete phylogeny and estimation of divergence time for the genus *Encephalartos*

To assemble the complete phylogeny used in the present study, we complemented the same DNA matrix (three plastids: *rbc*La, *mat*K, *trnH*-*psb*A, and one nuclear marker: ITS) used in Yessoufou et al. (2014) with one additional nuclear marker (PHYP) retrieved from Nagalingum et al. (2011). The details of DNA dataset information, as well as details of species names and GenBank accession numbers for all DNA sequences are available in Table 3.1. In addition, DNA sequences of the following species were included as outgroups and for calibration purpose (Nagalingum et al. 2011; Yessoufou et al. 2014; Condamine et al. 2015): *Macrozamia plurineria* (L.A.S.Johnson) D.L.Jones, *Macrozamia communis* (L.A.S.Johnson), *Macrozamia pauli-guilielmi* W.Hill & F.Muell., *Lepidozamia peroffskyana* Regel, and *Lepidozamia hopei* (W.Hill) Regel.

Next, based on the combined DNA dataset (all five markers), an XML file was generated using the program BEAUti v.1.8.3 (Heled and Drummond, 2010), and this file was used to reconstruct a dated complete phylogenetic tree employing the Bayesian MCMC approach implemented in the program BEAST v.1.8.3 (Drummond and Rambaut, 2007). The model GTR+I+Γ was selected in the process of dated tree reconstruction based on AIC (Akaike, 1973) evaluated using MODELTEST (Nylander, 2004). In addition, the birth-death prior with uncorrelated relaxed lognormal model for rate variation among branches was selected following Condamine et al.'s (2015) recommendation for cycads. To calibrate the *Encephalartos* tree, we selected uniform priors with minimum and maximum age estimates for nodes calibration, as the normal priors bias the node age estimates (Schenk 2016). The following uniform calibration points were used following Condamine et al. (2015) based on fossil calibration: Cycad CG (235 – 364.7 Ma), Cycad SG (265.1 – 364.7 Ma), *Encephalartos* SG (72.1 – 265.1 Ma), *Lepidozamia* SG (33.9 – 265.1Ma). Monte Carlo Markov Chains (MCMC) were run for 100 million, and trees were sampled every 10,000 generations. The resulting log files including the prior and likelihood values as well as effective sample size (ESS) were analysed using TRACER 1.5 (Drummond and Rambaut, 2007; Rambaut et al. 2013). The ESS values range from 300 to 800 for the age estimates. The first 25 % (i.e. 2500) of the resulting 10,000 trees were discarded and burned using TREEANNOTATOR version 1.6.1 software (Rambaut and Drummond, 2007) to generate the maximum clade credibility (MCC) tree. The node support on the BEAST tree is interpreted as: not supported when PP<0.50, supported when PP=0.60 and strongly supported when PP>0.60.

To further assess the node support on the phylogeny, bootstrap values at each node were also assessed using non-parametric bootstrapping for 1000 pseudo-replicates (Felsenstein 1985). This was also done on a Maximum Parsimony tree assembled in PAUP* v4.0b 10 (Swofford 2002). Bootstraps values were interpreted as: BS > 70% indicate strong support, and BS < 70% indicate weak support (Hillis and Bull 1993; Wilcox et al. 2002).

3.2.2 Historical biogeography

To reconstruct the historical biogeography of the genus, we performed the Statistical Dispersal-Vicariance Analysis (S-DIVA, Yu et al. 2011) as well as the Dispersal-Extinction-Cladogenesis (DEC) model. S-DIVA, instead of the commonly used DIVA was preferred as the former evaluates statistically the alternative ancestral ranges at each node of a phylogenetic tree while accounting for both phylogenetic uncertainty and uncertainty in DIVA optimization. The S-DIVA model considers all possible ancestral ranges at each node in the phylogeny and then calculates the probabilities of each ancestral range at the node (Ali et al. 2011). The DEC model allows for testing specific dispersal hypothesis through time (Ree et al. 2005). To run these two models, the origins of each *Encephalartos* spp. were coded as follows (Osborne et al. 2012): (A) West Africa (Benin, Ghana and Nigeria), (B) Central Africa (Democratic Republic of Congo), (C) East Africa (Tanzania, Kenya, Uganda and Sudan), (D) Southern Africa (Zambia, South Africa, Mozambique, Swaziland, Malawi and Angola).

3.2.3 Diversification analyses

All the diversification analyses were done as implemented in the R package TESS (Höhna et al. 2015).

To assess whether the diversification rates have changed significantly through time, the gamma value (Pybus and Harvey, 2000) was calculated, the LTT (Lineage-Through-Time) plot was reconstructed, and several evolutionary models were tested. The value of gamma was calculated using the R package LASER (Rabosky, 2006). To assess the significance of gamma, the observed gamma was compared to the expected value of gamma under a constant-rate birth-death model. To this end, an MCMC (Markov chain Monte Carlo) simulation was performed to estimate the posterior probability distribution of gamma under this constant-rate model. Specifically, the constant-rate birth-death model was parameterized by drawing rate parameters from the joint posterior densities inferred from the phylogenetic tree. This parameterized model was used to simulate 1000 phylogenies, and these simulated phylogenies were used to calculate the expected values of gamma. Then, the observed value of gamma was compared to the posterior-predictive distribution of these expected values. If the observed value falls near the center of the simulated distribution, then the diversification rates of *Encephalartos* are constant over time. If not, it means that the diversification has significantly changed over time (Höhna et al. 2015).

In addition, the 1000 phylogenies that were simulated were used to reconstruct the posterior-predictive distribution of the corresponding LTT plots (1000 simulated LTT plots). The observed LTT plot for *Encephalartos* was then reconstructed and compared to the simulated LTT plots. If the observed LTT plot falls within the simulated LTT plots, this means that the diversification rate of the genus has been constant over time. Otherwise, the diversification has experienced some evolutionary shifts.

Furthermore, the evolutionary models that explain the diversification patterns depicted by the observed LLT plot were identified. The models tested include a constant-rate birth-death model and three rate-variation models. The rate-variation models include a birth-death model with an exponentially decreasing speciation rate, a birth-death model with piecewise-constant rates (i.e., rates of speciation and extinction change over time but the diversification rate remains constant; Höhna et al. 2015) and a birth-death model of evolution punctuated by a mass-extinction event. Using Bayes Factors (BF; Baele et al. 2013), a pairwise comparison of these models was done to select the best model. For two models M_0 and M_1, BF value was interpreted following Jeffreys (1961): $BF(M_0,M_1) <1$, means M_1 is supported, $1<BF(M_0,M_1)<3.2$ suggests that M_0 is barely worth mentioning, $3.2<BF(M_0,M_1)<10$ indicates a substantial support for M_0, $10<BF(M_0,M_1)<100$ is indicative of a strong support for M_0, and $BF(M_0,M_1) >100$ is interpreted as decisive support for M_0.

Finally, to investigate whether the genus *Encephalartos* experienced some mass extinctions events (if so, when?), the CoMET [Compound Poisson Process (CPP) on Mass Extinction Time)] approach was employed (May et al. 2016). This approach has the advantage of being able not only to fit all possible birth-death models to the data at hand but also to specifically model mass extinction events. The CoMET approach treats the number of speciation-rate shifts, extinction-rate shifts, mass-extinction events as well as the parameters associated with these events as random variables, and then estimates their joint posterior distribution. For this analysis, hyperpriors was set both empirically and *a priori*.

Table 3.1. The species names and Genbank accession numbers of sequences used in the data analyses. All the sequences from *mat*K and *rbc*La, *trn-Hpsb*A, PHYP and ITS were retrieved from GenBank. The "_" indicate DNA sequences that are not available.

Species names	**Accession numbers**				
	matK	**rbcL**	**ITS**	***trnH*-psbA**	**PHYP**
Encephalartos aemulans Vorster	KP979368	KU937269	KX130168	KX152015	JN655975
Encephalartos altensteinii Lehm.	KU937283	KU937293	KX130170	KX152064	JN655976
Encephalartos aplanatus Vorster	KU937199	KU937247	AY335266	KX152090	JN655977
Encephalartos arenarius R.A. Dyer	KU937288	KU937224	KX130176	KX152066	JN655978
Encephalartos barteri Carruth. ex Miq.	JQ046256	JQ025458	AY335310	_	JN655979
Encephalartos brevifoliolatus Vorster	KP979453	JQ025464	KX130181	KX151994	_
Encephalartos bubalinus Melville	KU937212	KU937259	EF612927	_	JN655980
Encephalartos caffer (Thunb.) Lehm.	KU937272	KU937304	KX130182	KX152091	JN655981
Encephalartos cerinus Lavranos & D.L. Goode	KU937194	KU937242	KX130187	KX152092	JN655982
Encephalartos chimanimaniensis R.A.Dyer & Verdoorn	JQ046248	JQ025477	JQ046110	KX151998	_
Encephalartos concinnus R.A. Dyer & I. Verd.	KX130138	JQ025478	KX130188	JQ045968	JN655983
Encephalartos cupidus R.A. Dyer	_	KU937238	KX130189	KX152068	JN655984
Encephalartos cycadifolius (Jacq.) Lehm.	KP979473	JQ025486	KX130193	KX152070	JN655985
Encephalartos dolomiticus Lavranos & D.L. Goode	_	KU937231	KX130197	KX152004	JN655986
Encephalartos dyerianus Lavranos & D.L. Goode	_	KU937234	KX130199	KX152093	JN655987

Encephalartos equatorialis P.J.H.Hurter	KU937213	KU937261	JQ046101	JQ045961	_
Encephalartos eugene-maraisii I. Verd.	KX130142	KX130113	KX130203	KX152072	JN655988
Encephalartos ferox Bertol. f.	KU937202	KU937250	KX130204	JQ046019	JN655989
*Encephalartos friderici-guilielm*i Lehm.	KX130143	KX130114	KX130207	KX152011	JN655990
Encephalartos ghellinckii Lem.	KU937181	KU937229	KX130210	KX152076	JN655991
Encephalartos gratus Prain	KU937201	KU937249	KX130212	KX152077	JN655992
Encephalartos heenanii R.A.Dyer	KU937179	KU937227	JQ046090	KX152078	_
Encephalartos hildebrandtii A. Braun & C.D.Bouché	KU937218	KU937266	_	JQ045949	JN655993
Encephalartos hirsutus P.J.H.Hurter	KU937200	KU937248	KX130218	KX152094	_
Encephalartos horridus (Jacq.) Lehm.	AF410169	KU937244	KX130219	KX152023	JN655994
Encephalartos humilis I. Verd.	KU937197	KU937245	KX130223	KX152025	JN655995
Encephalartos inopinus R.A.Dyer	KU937177	KU937225	KX130227	KX152079	JN655996
Encephalartos ituriensis Bamps & Lisowski	KP979441	JQ025548	_	_	JN655997
Encephalartos kisambo Faden & Beentje	KP979432	KU937263	JQ046152	JQ046013	JN655998
Encephalartos laevifolius Stapf & Burtt Davy	KU937185	KU937233	KX130229	KX152095	JN655999
Encephalartos lanatus Stapf & Burtt Davy	KU937198	KU937246	KX130231	KX152096	JN656000
Encephalartos latifrons Lehm.	KU937282	KU937230	KX130235	KX152098	_
Encephalartos laurentianus De Wild.	KU937220	KU937268	KX130236	KX152081	JN656001

Encephalartos lebomboensis I. Verd.	KU937217	KU937265	KX130239	KX152034	JN656002
Encephalartos lehmannii Lehm.	KU937193	KU937241	KX130242	KX152099	JN656003
Encephalartos longifolius (Jacq.) Lehm.	KU937287	KU937289	KX130244	KX152083	JN656004
Encephalartos macrostrobilus S. Jones & J.Wynants	_	JQ025593	EF612936	EF653159	JN656005
Encephalartos manikensis (Gilliland) Gilliland	KU937208	KU937256	KX130251	KX152101	JN656006
Encephalartos marunguensis Devred	KP979392	JQ025603	JQ046062	JQ045922	_
Encephalartos middelburgensis Vorster	KU937191	KU937239	KX130253	KX152040	JN656007
Encephalartos msinganus Vorster	KU937222	KU937270	JQ046059	JQ045918	JN656008
Encephalartos munchii R.A. Dyer & I. Verd.	KU937210	KU937257	KX130256	KX152102	JN656009
Encephalartos natalensis R.A. Dyer & I. Verd.	KU937187	KU937235	KX130258	KX152103	JN656010
Encephalartos ngoyanus I. Verd.	KU937178	KU937226	KX130260	KX152105	JN656011
Encephalartos nubimontanus P.J.H. Hurter	_	KU937236	KX130263	KX152046	JN656012
Encephalartos paucidentatus Stapf & Burtt Davy	KU937214	KU937262	KX130265	KX152084	JN656013
Encephalartos poggei Asch.	_	JQ025638	JQ046138	JQ045998	_
Encephalartos princeps R.A. Dyer	KU937203	KU937251	KX130268	KX152106	JN656014
Encephalartos pterogonus R.A. Dyer & I. Verd.	JQ046184	KX130125	KX130269	JQ045905	JN656015
Encephalartos relictus P.J.H.Hurter	_	JQ025643	KX130270	KX152049	_
Encephalartos schaijesii Malaisse, Sclavo &Crosiers	_	_	EF653151	_	JN656016

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Encephalartos schmitzii Malaisse	JQ046183	JQ025644	EF653152	JQ045904	JN656017
Encephalartos sclavoi De Luca, D.W. Stev. & A.Moretti	KU937223	KU937271	JQ046130	JQ045996	JN656018
Encephalartos senticosus Vorster	KU937195	KU937243	KX130274	KX152053	JN656019
Encephalartos septentrionalis Schweinf.	_	AF394359	AY335311	_	JN656020
Encephalartos tegulaneus Melville	KP979413	JQ025665	EF653155	JQ046029	JN656021
Encephalartos transvenosus Stapf & Burtt	KU937219	KU937267	KX130278	KX152110	JN656022
Encephalartos trispinosus (Hook.) R.A. Dyer	KU937281	KU937295	KX130279	KX152107	JN656023
Encephalartos turneri Lavranos & D.L. Goode	KU937206	KU937254	KX130283	KX152109	JN656024
Encephalartos umbeluziensis R.A.Dyer	KX130163	KX130131	KX130285	KX152059	_
Encephalartos villosus Lem.	KU937209	KF221187	KX130289	KX152060	JN656025
Encephalartos whitelockii P.J.H. Hurter	KU937204	KU937252	JQ046032	JQ045892	JN656026
Encephalartos woodii Sander	KX130165	KU937237	KX130292	KX152063	JN656027
Lepidozamia hopei Regel	_	KX130133	KX130293	_	JN656028
Lepidozamia peroffskyana Regel	_	KX130134	KX130294	_	JN656029
Macrozamia plurinervia (L.A.S. Johnson) D.L.	_	_	_	_	JN656048
Macrozamia communis L.A.S.Johnson	AF279801	AF531205	EF653158	_	_
Macrozamia pauli-guilielmi W. Hill & F. Muell.	_	_	AF531234	JX215391	JN656046

3.3 Results

3.3.1 Phylogenetic tree of *Encephalartos*

The combined DNA matrix made up of three plastid regions (*rbcL*a, *mat*K and *trnH-psb*A) and two nuclear regions (nrITS and PHYP) includes 5736 characters, 384 potentially parsimony informative sites and 5060 constant characters (Table 3.2). Using this dataset, the phylogenetic tree reconstructed for the genus *Encephalartos* is strongly supported (PP=1.00/BS=94) and suggests that the genus may have diverged around 9 Ma (95% HPD, 8.03 – 11.07; Figures 3.1 & Appendix B). The phylogeny reveals two major clades following species geographic origins, one southern African clade and one east-central-west African clade. The southern African clade, with 45 species out of 65, is the largest clade that radiated at ~6 Ma (95% HPD, 4.37 – 9.10). This clade can be subdivided into three subclades. The first subclade, called *southern African subclade 1* (Figure 3.1), is well supported (PP=0.7/BS=90), and is sister to the rest of the genus with a strongly supported sister relationship (PP=1.00/BS=94). The second subclade, the *southern African subclade 2* (Figure 3.1) is supported (PP=0.60) whereas the *southern African subclade 3* (Figure 3.1) is not supported. The east-central-west African clade is strongly supported (PP=1.00) and is made up of three subclades, that is, one central African subclade embedded within two east African subclades 1 and 2 (PP=0.8 for each of these subclades; Figure 3.1). Finally, the only species from West Africa, i.e. *Encephalartos barteri*, is sister to *E. macrostrobilus* (PP=0.9; Figure 3.1), which is a member of the east African subclades 2 and endemic to Uganda.

Table 3.2 The summary of DNA matrix and Maximum Parsimony statistics of individual gene.

	ITS	**PHYP**	**matK**	***trnH-psbA***	**rbcL**	**Combined dataset**
Number of taxa	68	60	61	65	69	70
Number of characters	1299	1771	853	1257	556	5736
Number of trees	498	623	100	395	17	883
Missing data	>5%	>5%	>5%	>5%	>5%	0
Constant character	930	1255	794	880	539	5060
Parsimony uninformative variable	83	406	48	358	17	292
Parsimony informative site	286	110	11	19	0	384

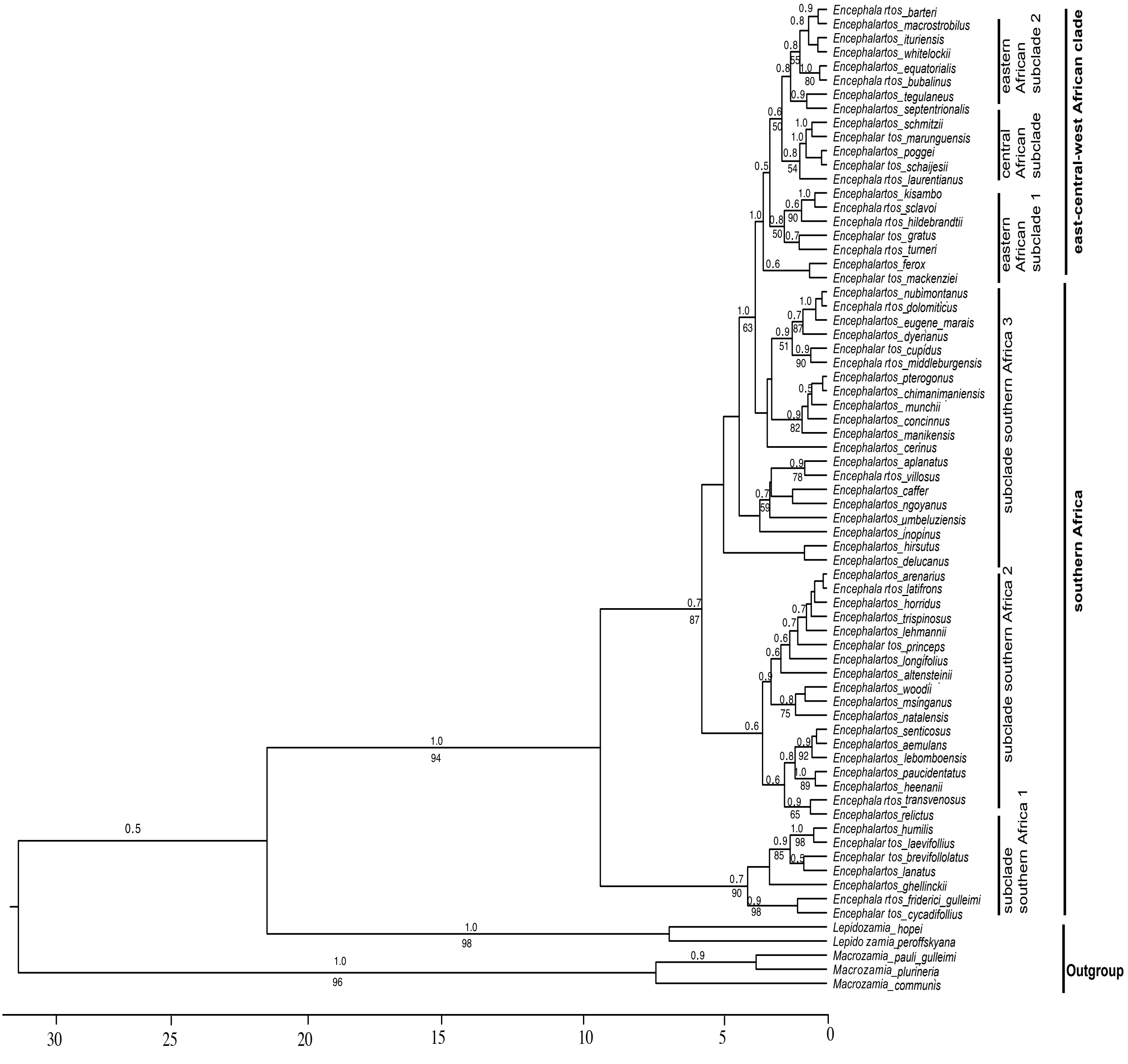

Figure 3.1 A complete phylogetic tree of the genus *Encephalartos* based on the nuclear (ITS and PHYP) and plastid (*rbc*L, *mat*K and *trnH*-*psb*A) markers under a relaxed clock model. Posterior probabilities of supported nodes (PP ≥ 0.9) are showed above branches and Boostrap values below the branches.

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3.3.2 Historical Biogeography: Ancestral Area Reconstruction States

The application of both DEC and S-DIVA models generates similar results (Figures 3.2 and Appendix C, respectively). As showed in Figure 3.1, there are two main clades, one southern African clade and one east-central-west African clade. Firstly, our results point to southern Africa (node I, 100% probability; Figure 3.2), as the origin of the genus *Encephalartos*, which dated back to around 9 Ma (Node I). Specifically, this origin is South Africa (subclades southern Africa 1 and 2) from which the genus may have dispersed to the rest of southern Africa (Figures 3.1 and 3.2) forming the southern African clade. Secondly, although the origin of the east-central-west African clade is uncertain (node II, Figure 2, 50% probability), it may have diverged from the southern African clade through vicariance (node II, Figure 3.2). For example, the east African species *Encephalartos delucanus*, endemic to Tanzania, may have diverged from the South African species *E. hirsutus* aided by vicariance (Figure 3.2). Furthermore, within the east-central-west African, the central African subclade may have diverged from east African subclade (Figures 3.1 & 3.2) also through vicariance (node III, 100% probability, Figure 3.2) and the only west African species *E. barteri* from the east African species *E. macrostrobilus* again through vicariance (Figure 3.2). Explanations of these events (dispersal and vicariance) are summarized in Figure 3.3.

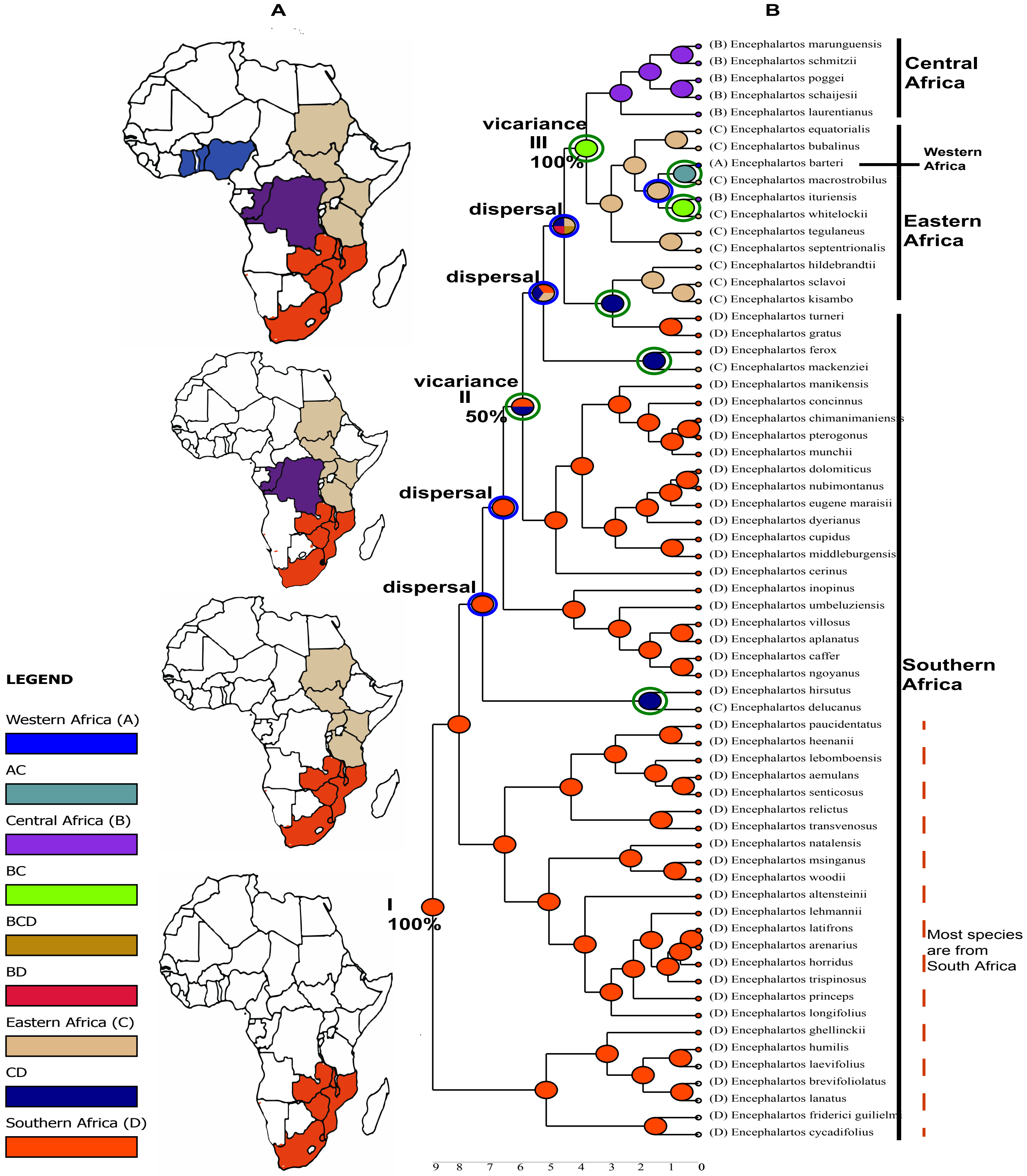

Figure 3.2 The historical biogeographic reconstruction of the genus *Encephalartos* using Statistical-Dispersal Vicariance analysis (S-DIVA) model. A- Delimitation of geographic ranges of species adopted in the present study. B- Phylogeny showing historical biogeography. Pie charts at each node indicate the probabilities of alternative ancestral ranges. The green

rings around the node represent vicariance event and the blue rings represent dispersal event. Key major nodes are indicated as I-III and the probability of the origin at these nodes are also mentioned as %.

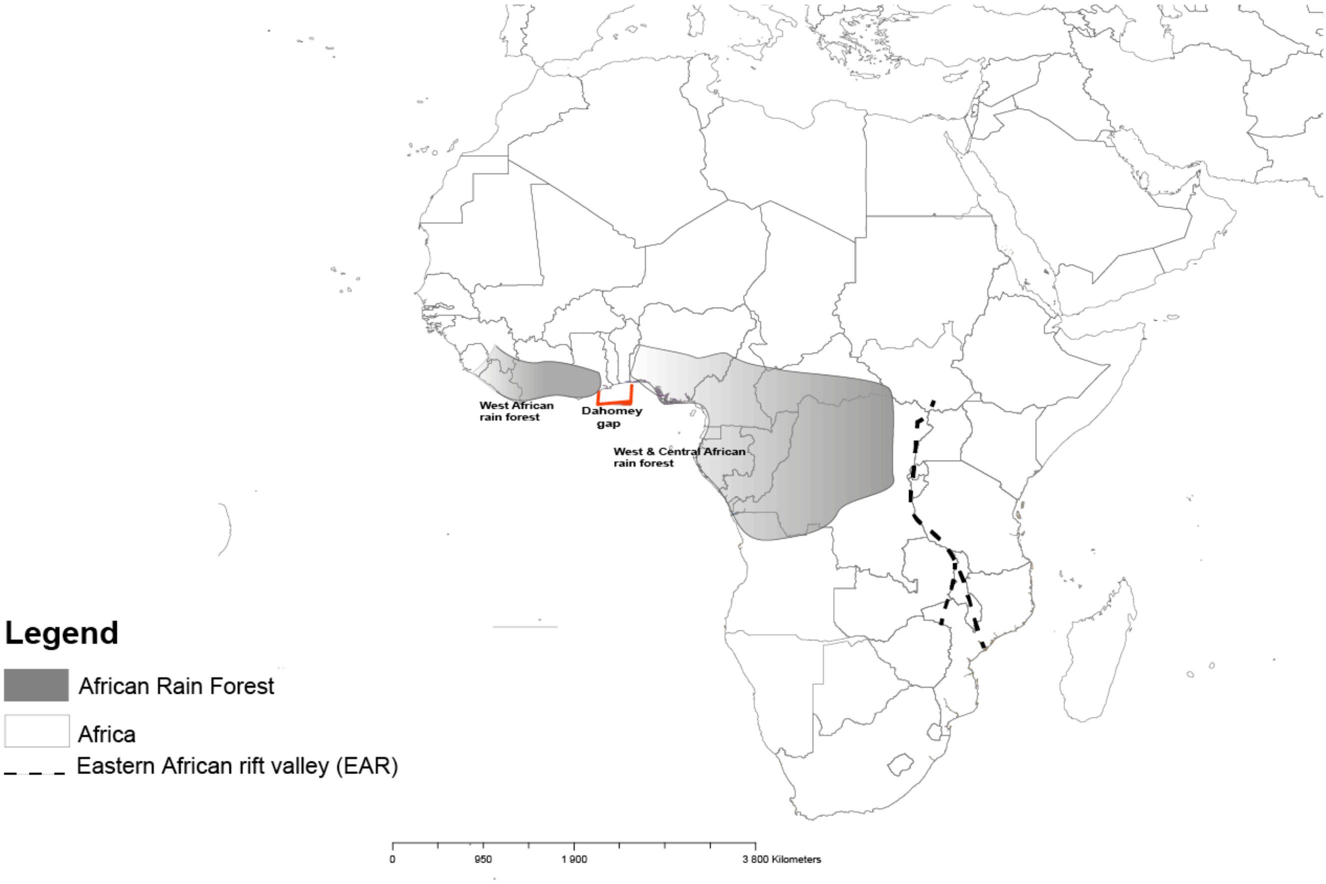

Figure 3.3 The map of Africa showing two potential barriers (Dahomey Gap and Eastern African Rift) for dispersal that may account for vicariance. The Dahomey Gap breaks up African forest into blocks that may have been connected in the past, and the Eastern Rift may have separated East African from the rest of the continent.

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3.3.3 Diversification analysis

The observed gamma is $\Upsilon = -0.788$, and this value falls within the 95% credible interval of its posterior predictive distributions (Figure 3.4a), suggesting that the genus *Encephalartos* may have diversified following a constant-rate model. In addition, the LTT-plot does not depart significantly from those of the simulated trees under a constant-rate birth-death model (Figure 3.4b), further confirming a constant diversification rate for the genus although, we found a rapid diversification in the last 2.6 Ma (Figure 3.4c). Even when testing alternative models using Bayes factors, we also found that the constant birth-death model was strongly supported as the best model (BF = 24.24; Table 3.3). Finally, fitting the CoMET model to test for potential evolutionary events that may have occurred throughout the diversification period of the genus, we found a number of events but none was significant. Specifically, we found that speciation rate remains constant at ~0.45 sp.my^{-1} over time (Figure 3.5A) with some shifts particularly towards the present but none was significant (Figure 3.5B). Also, extinction rate was roughly constant at ~0.1 sp.my^{-1} over time (Figure 3.5C) with no significant shift (Figure 3.5D) whereas evidence of some mass extinction events have been noted (Figure 3.5E) particularly around 9-7 Ma but again with no significant shift (Figure 3.5F).

Table 3.3 The Bayes factor (BF) values calculated for each birth-death model tested for the phylogeny of the genus *Encephalartos*. ConstBD = constant-rate birth-death model, DecrBD = continuously variable-rate birth-death model, EpisodicBD = episodically variable-rate birth-death model, and MassExtinctionBD = explicit mass-extinction birth-death model.

M_0	M_1	BF
ConstBD	MassExtinctionBD	24.244339
EpisodicBD	MassExtinctionBD	20.855570
ConstBD	DecrBD	15.991697
EpisodicBD	DecrBD	12.602928
DecrBD	MassExtinctionBD	8.252642
ConstBD	EpisodicBD	3.388769
ConstBD	ConstBD	0.000000
DecrBD	DecrBD	0.000000
EpisodicBD	EpisodicBD	0.000000
MassExtinctionBD	MassExtinctionBD	0.000000
EpisodicBD	ConstBD	-3.388769

MassExtinctionBD	DecrBD	-8. 252642
DecrBD	EpisodicBD	-12.602928
DecrBD	ConstBD	-15.991697
MassExtinctionBD	EpisodicBD	-20.855570
MassExtinctionBD	ConstBD	-24.244339

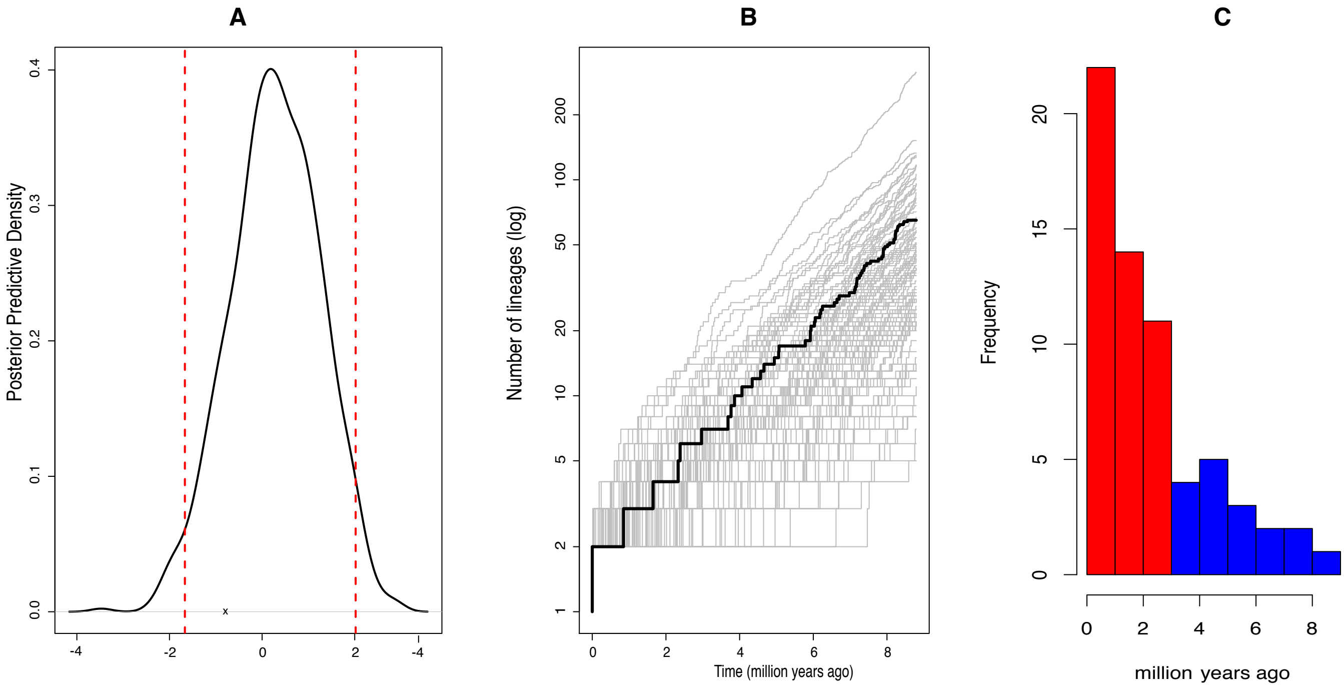

Figure 3.4 Test of diversification of the genus *Encephalartos* showing the absolute fit of the *Encephalartos* tree to the constant rate birth-death model. **A**) Posterior predictive distribution of gamma statistic; red dotted lines indicate 95% credible interval and X shows the position of the observed value of gamma for the genus. **B**) Grey color corresponds to Lineage Through Time (LTT) plot of 1,000 simulated trees and black bold LTT plot corresponds to the

observed LTT plot for the genus. **C**) Histogram indicating the frequency of branching time on the phylogeny of *Encephalartos*; the red color shows the most frequent branching events occurred during the last 2.6 million years and the blue color shows the earlier branching events.

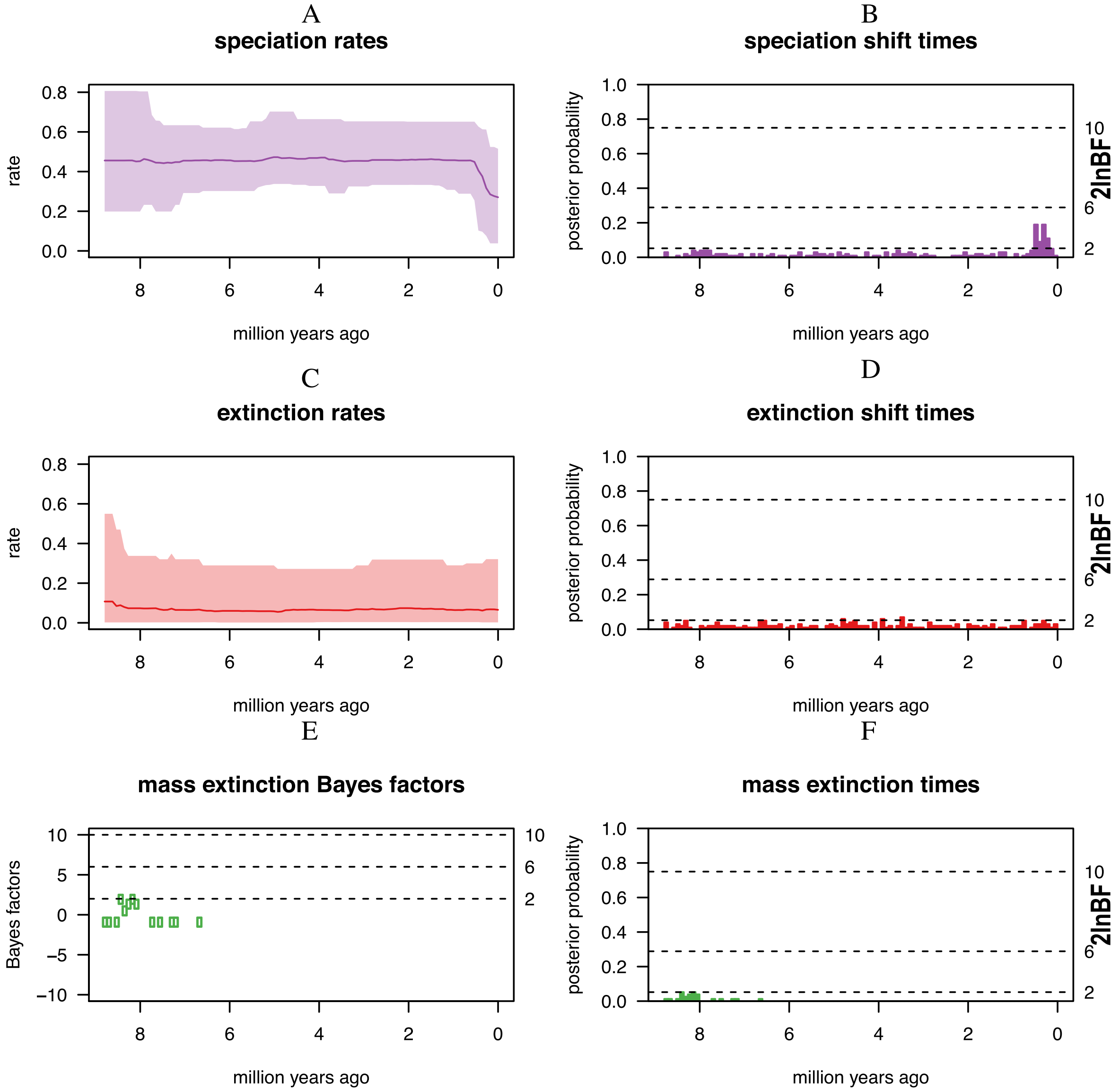

Figure 3.5. Summary of the evolutionary events (A-F) identified using Compound Poisson Process (CPP) on Mass Extinction Time (CoMET) model. The CoMET approach treats the number of speciation-rate shifts, extinction-rate shifts, mass-extinction events as well as the parameters associated with these events as random variables, and then estimates their joint posterior distribution. Hyperpriors are specified empirically, i.e. an automatic empirical hyperprior was set as implemented in the R library TESS (Höhna 2015).

3.4 Discussion

3.4.1 Origin of the genus *Encephalartos*

The phylogeny of the genus *Encephalartos* reconstructed in the present study is better in many respects than the one reconstructed in a recent study for similar purpose (see Yessoufou et al. 2014). First, it is well supported in general (0.7≤PP≤1; Figure 3.1), and shows a better topology that follows geographic origins (Treutlein and Wink 2002; Treutlein et al. 2004) and makes ecological sense (Figure 3.1). For example, the only West African species *E. barteri* is recovered as a sister to the east African species *E. macrostrobilus* with strong support while its relationship was not clear in Yessoufou et al. (2014) who recovered this species embedded within the eastern and central African species. Although both species occur in geographically distinct regions (West Africa for *E. barteri* and East Africa for *E. macrostrobilus*), they share not only similar habitats (rocky areas and savanna) but also occur at similar altitude (400-1400 m for *E. barteri*, Bösenberg 2010; 900-1400 m for *E. macrostrobilus*; Donaldson 2010). Also, the southern African clade recovered in the present study was well supported, and the three subclades found were also supported (except one) as opposed to Yessoufou et al.'s tree reported for the same genus. These differences are certainly the results of the differences in the number of markers used (4 markers including only 1 nuclear region in Yessoufou et al. 2014 versus 5 makers including 2 nuclear regions in the present study). The supported subclade *Southern African 1* (Figure 3.1) are all endemic to the eastern South African mountain, occurs in cool and high elevation grasslands that experience frost and snow (Vorster 2004). The supported subclade *Southern African 2* corresponds to the clade recently identified as the most rapidly diversifying clade within the genus (Yessoufou et al. 2014). It is formed of species that developed underground stems occurring in fire-prone habitats in southern Africa (grassland; Vorster 2004; Yessoufou et al. 2014).

3.4.2 Historical biogeography and diversification

Our analysis revealed that the genus may have originated around 9 Ma from southern Africa and may have first dispersed within southern African region, and then the diversification in the rest of the continent may have been aided by vicariance. In particular, the central African subclade may have diverged from east African subclade through vicariance, and the only West African species, *E. barteri*, may have too diverged from the east African species *E. macrostrobilus* through vicariance. Vicariance events imply that the eastern, central and western African populations of *Encephalartos* were once connected and then were later separated into different populations owing to geographic barriers that prevent continuous gene flow, leading to the radiation of new species.

Indeed, there are several evidences that tropical African forests were once connected as a continuous vegetation type, particularly in the Eocene (Coetzee 1993; Axelrod and Raven 1978; Jacobs et al. 1999). This is evidenced by the floristic similarities reported in several studies across tropical African regions (e.g. White 1979; Wasser and Lovett 1993; Burgess and Clarke 1998; Burgess 2000; Couvreur et al. 2008). In addition, studies indicated that, during the Oligocene-Early Miocene (c. 33-20 Ma) when east African region became arid, this pan-African rainforest first broke up (Axelrod and Raven 1978; Wasser and Lovett 1993; Burgess and Clarke 2000; Morley 2000; Davis et al. 2002). Then, broken forests underwent multiple expansions and contractions from the mid-Tertiary onwards (c. 33-2 Ma) which may have allowed the spread from West-Central Africa to East Africa or vice versa (Coetzee et al. 1993; Wasser and Lovett 1993; Maley 1996; Burgess et al. 1998; Jacobs et al. 1999), thus promoting the diversification by vicariance (Couvreur et al. 2008). Furthermore, a number of studies have also reported geological rifting in East Africa (e.g. East African Rift) as a long-term dispersal barrier separating eastern Africa from the rest of the continent (e.g. Mairal et al. 2017). This barrier (the East African Rift) occurred in the last 9-5 Ma (Macgregor 2015), a period coinciding with the origin of the genus *Encephalartos*. The occurrence of this barrier may have contributed, as reported elsewhere, to allopatric speciation (vicariance) (e.g. Gottelli et al. 2004; Assefa et al. 2007; see more references in Mairal et al. 2017). We suggest, here, that the evidence of vicariance that we found, e.g. between the southern and central-west African clades (recovered in our phylogeny) or between *E. hirsutus* (southern Africa) and *E. delucanus* (eastern Africa), may have been promoted by a combination of multiple expansion and contraction events of African forests and the appearance of geological barriers.

An additional but important historical event, referred to as *Dahomey Gap*, contributed significantly in breaking or disconnecting further the West African forest from the rest of pan-African tropical forest (Salzmann and Hoelzmann 2005; Demenou et al. 2016). The *Dahomey Gap* is a climatically dry corridor (200 km wide; Demenou et al. 2016) that appeared abruptly at the onset of the late Holocene (Salzmann and Hoelzmann 2005). This gap breaks up pan-African tropical forests into upper and lower blocks that disconnect West African forest (upper block) from central-east African forests (lower block) along c. 1000 km-long distance (Couvreur et al. 2008, see Figure 3.3), thus representing a barrier to dispersal between forest blocks (Salzmann and Hoelzmann 2005; Demenou et al. 2016). Recently, Demenou et al. (2016) demonstrated that the *Dahomey Gap* is an effective barrier preventing gene flow between west-central and east African species. This *Dahomey Gap* may have contributed to the sister relationships and the vicariance event that we found between *E. barteri* (West Africa) and *E. macrostrobilus* (East Africa). Did these events, dispersal and vicariance, in the context of climate change, promote significant shifts in the diversification history of the genus?

All our analyses (gamma statistics; LTT plot; Bayes factors) indicated that the accumulation of species in the genus *Encephalartos* follows a constant-rate model. This was previously reported in Yessoufou et al. (2014) who, however, identified a punctual explosive radiation around 2.6 Ma in southern Africa while interpreting the antisigmoidal LTT plot that they found. This radiation corresponds to our *subclade southern Africa 2* in which most species developed underground stems, potentially as key innovation to survive aridity and frequent fire (Maurin et al. 2014) during the Pliocene-Pleistocene transition in southern Africa around 2.6 Ma (Yessoufou et al. 2014). In the present study, although we found that most radiations took place in the last 2.6 Ma (Figure 3.4c), our CoMET analysis did not find these radiations as significant shift, suggesting that the increased radiation in the last 2.6 Ma may not be referred to as explosive radiation or explosive diversification (Givnish 2015). Indeed, the interpretation of antisigmoidal LTT plot as driven by explosive radiation has been questioned (Turgeon et al. 2005; McKenna and Farrell 2006) as one may expect explosive radiation to lead to an increase without plateau. Furthermore, the phylogeny of the genus *Encephalartos* exhibits phylogenetic fuses – long branches from the origin. This is interpreted as evidence for low diversification or mass extinction. Even the antisigmoidal LTT plot is also linked to mass extinction (Crisp and Cook 2009). Our CoMeT analysis, indeed, pointed to extinction events throughout the diversification period of the genus *Encephalartos* (Figure 3.5D) as well as mass extinction events at the origin of the genus (9-6 Ma; Figure 3.5F). This period overlaps with the mass extinction period reported for gymnosperms in general (7–5 Ma; Niklas 1997; Crepet and Niklas 2009). Nonetheless, none of these mass extinction events, based on our analysis, was significant, but they may have promoted species accumulation within the genus through adaptive radiation (Yessoufou et al. 2014; Givnish 2015).

Overall, using a new complete phylogeny for the genus *Encephalartos*, we found that the genus may have originated from southern Africa, then dispersed within the region, and its diversification in the rest of the continent may have been mediated through vicariance. Finally, this diversification follows a constant-rate model and indicates that the massive radiation in the last 2.6 Ma may not be as dramatic as initially reported (Yessoufou et al. 2014) as none of the evolutionary events shows a significant shift.

Chapter 4

The genus *Cycas* may have diversified from Indochina and occupied its current ranges through vicariance and dispersal events

Ledile T. Mankga[1], Munyaradzi Chitakira[2], Thendo Mugwena[3], Kowiyou Yessoufou[3*]

[1]*Department of Life and Consumer Sciences, University of South Africa, Science Campus, Florida, South Africa*

[2]*Department of Environmental Science, University of South Africa, Science Campus, Florida, South Africa*

[3]*Department of Geography, Environmental Management and Energy Studies, University of Johannesburg, APK campus, Johannesburg, South Africa*

***Corresponding author:** Dr K. Yessoufou

Corresponding author's email address: kowiyouy@uj.ac.za

Running head: Historical biogeography and diversification of the genus *Cycas* in the Pacific region

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Abstract

Biogeographically, cycads were once widely distributed but the extant cycads are restricted to tropical and subtropical regions. Their evolutionary history is also fascinating as they originated ~ 300 Ma and re-diversified recently around 12 Ma, with the genus *Cycas* being the most rapidly diversified and widely distributed lineage. Here, we first retrieved DNA sequences from genbank and assembled a complete phylogeny of *Cycas* using molecular markers including a plant DNA barcode *trnH-psbA*. Then, we employed the Bayesian Binary Method to reconstruct the historical biogeography of the extant *Cycas* and finally, using the Bayesian approach for diversification analysis, we explored the evolutionary events that might shape the rapid diversification and wide distribution of *Cycas* across the pacific islands. Our analysis pointed to Indochina as the origin of the genus, which may have dispersed firstly across the Pacific Islands during the late Miocene aided by multiple excursions of sea levels and the development of a key innovation, i.e. a spongy endocarp particularly in the seeds of subsection Rumphiae. The colonization of South China, which was thought to be the origin of the genus, may have occurred more recently aided by both dispersal and vicariance events. However, no significant shifts in the evolutionary events (speciation, extinction, mass extinction) that shaped the diversity of the genus was observed. Our study therefore clarifies the historical biogeography and the evolutionary events that shaped the current diversity of the genus *Cycas*.

Keywords: Cycads, DNA barcode, evolutionary diversification, historical biogeography, late Miocene, sea-level excursions.

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4.1 Introduction

Cycads are dioecious and entomophilous plants that developed palm-like habit with stout trunks and large evergreen pinnate leaves (Jones 2002). They share some characteristics with the ferns (e.g. spermatozoa with flagella) and angiosperms (e.g. naked seeds; Guan 1996; Norstog and Nicholls 1997). The dispersal of cycads seeds is limited to 2-7 km mostly mediated through rodents, small fruit-eating bats and long dispersal via the sea (Yang and Meerow 1996). Cycads represent the oldest lineage plants that originated ~ 300 million years ago (Ma) in the mid-Permian (Hendricks 1987; Gao and Thomas 1989; Calonje et al. 2017) and reached their greatest diversity in the Jurassic era (Jones 2002; Nagalingum et al. 2011). Geographically, cycads are restricted to tropical and subtropical or warm temperate regions with predominantly summer rainfalls (Jones 2002). In total, 10 genera diversified within the cycads group, with the genus *Cycas* being the largest of all (Osborne et al. 2012; Calonje et al. 2017).

Specifically, *Cycas* is the only genus in the family Cycadaceae, a family that is an early-diverging lineage to the cycads phylogenetic tree (Stevenson 1992; Nagalingum et al. 2011). This genus comprises six sections, including Asiorientales, Panzhihuaenses, Wadeanae, Stangarioides, Indosinenses, and *Cycas* (Hill 2004). The genus *Cycas* is the

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most rapidly diversified clade in the cycads group with ~ 112 species (Yessoufou et al. 2017). Fossil evidence points to Asia as the origin of the genus (Hill 1995; see also Xiao and Möller 2015). From Asia, the genus *Cycas* is further distributed southward to Australia, eastern Africa and the Pacific islands (Hill 2004).

In Asia, the genus is distributed across the Red River Fault between South China and the Indochina block, with the Red River potentially constituting a geographical barrier for gene flow (Xiao and Möller 2015). If this barrier was effective, we would expect to detect the signature of vicariance events in the evolutionary history of the genus *Cycas* (Keppel et al. 2008; Xiao and Möller 2015). Then, the widespread distribution of *Cycas* from Asia to Africa, Australia and across the Pacific regions might have been mediated through long distance dispersal. However, the sample analysed in a recent study that tested this hypothesis (Xiao and Möller 2015) was taxonomically limited (only 31 species out of 112), although they included representatives of all six sections of the genus in their analysis. Even in Keppel et al.'s (2008) study, only the subsection Rumphiae of the section *Cycas* was analyzed. As such, their inferences on the evolutionary and ecological processes that shaped the biogeography of *Cycas* may require further investigations. In addition, in their recent analysis of the diversification rate comparison across the cycads tree of life, Yessoufou et al. (2017) revealed a diversification rate heterogeneity across the tree with the genus *Cycas* identified as the most rapidly diversifying clade, and they suggested that this rapid diversification might have mediated their widest geographic distribution. Unfortunately, they did not go further to elucidate the patterns of diversification events within this clade.

In the present study, our aim is to provide a refined understanding of the evolutionary and ecological processes that shaped the biogeography of the genus *Cycas*. Specifically, we assembled the most comprehensive phylogeny of the genus, which was then used to elucidate its historical biogeography as well as the ecological forces that mediated the observed diversity patterns.

4.2 Materials and Methods

4.2.1 A complete list of *Cycas* species to reconstruct a dated *Cycas* phylogeny

The full list of cycads species is still a matter of debate. However, a recent study analyzed a large dataset of informative markers (DNA and taxonomy data) to estimate the total cycads diversity to 116 (100 accepted, 7 subspecies and 9 controversial species; Liu et al. 2018). To assemble a complete phylogeny for the 116 *Cycas* species, we retrieved DNA sequences of seven nuclear regions (PHYP, RPB1, HZP, AC3, F3H, SAMS and GTP) and four plastid regions (*trnH*-*psb*A, *psb*M-*trn*D, *trn*L-*trn*F and *trn*S-*trn*G) of *Cycas* species from GenBank/NCBI (accessed October 2018; Liu et al. 2018). The accession numbers and

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species names are presented in Table 4.1. The dated phylogeny was assembled for 135 species including outgroups (*Bowenia* Hook.ex Hook.f., *Ceratozamia* Brongn, *Dioon* Lindl., *Encephalartos* Lehm., *Lepidozamia* Regel, *Macrozamia* Miq., *Microcycas calocoma* (Miq.) A. DC., *Stangeria eriopus* (Kunze) Baill., *Zamia* L., *Ginkgo biloba* L.) following the Bayesian approach implemented in the BEAST program (Rambaut and Drummond 2007).

The following steps were followed for the BEAST analysis. Firstly, an XML file using BEAUti (Drummond and Rambaut 2007) was generated. Secondly, the best model GTR+I+Γ (based on Akaike information criterion evaluated using MODELTEST; Nylander 2004) was selected as well as the birth-death process prior with uncorrelated relaxed lognormal model for rate variation among branches following Condamine et al. (2015). To calibrate the *Cycas* tree, uniform priors with minimum and maximum age estimates for nodes calibration were selected as the normal priors bias the node age estimates (Schenk 2016). The following uniform calibration points were used following Condamine et al. (2015) based on cycad group: Cycads SG (273.9 – 364.9 Ma), *Dioon* SG (107 – 207.9 Ma), *Encephalartos* SG (97.7 – 192.5 Ma), *Bowenia* SG (88.7 – 174.3 Ma), *Lepidozamia* SG (33.9 – 55 Ma), *Ceratozamia* SG (19.2 – 84.9 Ma), *Cycas* Stem Group (SG) (15.8 – 257.2) Ma, *Zamia* SG (14.6 – 57 Ma). Lastly, MCMC was run for 100 million genetrations with trees sampled every 10 000 generations. At the end of the process of dated tree reconstruction, the ESS values ranged from 200 to 901 for the age estimates; the first 2,000 trees were burnt and the remaining 8,000 trees were combined using TREE ANNOTATOR (Rambaut and Drummond 2007) to generate a maximum clade credibility

(MCC) tree. The node support on this MCC tree is interpreted as follows: not supported (PP<0.50), supported (PP=0.60) and strongly supported (PP>0.60). In addition, the bootstrap node supports on the phylogeny were assessed using PAUP v40b10 (Swofford, 2002) approach. The Maximum Parsimony (MP) tree was performed based on the heuristic search with 1000 random sequences additions keeping 10 trees. The bootstrap values were interpreted as: BS >70% indicates strong support and BS < 70% indicate weak support (Hillis and Bull 1993; Wilcox et al. 2002).

4.2.2 Ancestral area reconstruction states: historical biogeography of *Cycas*

To reconstruct the historical biogeography of the genus *Cycas*, we grouped all species into three categories based on their current geographic distribution (Osborne et al. 2012) and following Xiao and Möller (2015). The category (A) includes species from South China, Taiwan- Ryukyu Archipelago, and Palawan islands (we refer henceforth to A as simply South China). The category (B) includes species from Indochina, and (C) include Islands of Southeast Asia plus the Malay Peninsula, the Indian subcontinent, East Africa and North Australia.

We used both Bayesian Binary Model (BBM) and Dispersal-Extinction-Cladogenesis (DEC) Model analysis implemented in RASP to reconstruct the possible ancestral ranges of the genus *Cycas* on the phylogenetic trees. For BBM analysis, the frequencies of an ancestral range at a node in ancestral reconstructions are averaged over all trees generated by RASP in Bayesian analysis (Yan et al. 2010). To account for uncertainties in phylogeny, we used 20,000 trees from MCMC output generated within BBM model. The MCMC chains were run simultaneously for 5,000,000 generations. The state was sampled every 1000 generations. Fixed JC + G (Jukes-Cantor + Gamma) were used with null root distribution and the maximum number of areas for this analysis was kept as 3.

4.2.3 Diversification analysis

All the diversification analyses were run using R library TESS (Höhna et al. 2015). Firstly, we identified the branching model that fits the diversification of the genus *Cycas* and then compared the number of taxa and the ϒ-statistic (Pybus and Harvey 2000) of the *Cycas* tree to the posterior-predictive distribution of 1000 simulated trees under a constant-rate birth-death model. The constant-rate birth-death model was parameterized by drawing rate parameters from the joint posterior densities inferred from the phylogenetic tree. This parameterized model was used to simulate 1000 phylogenies, which were then used to calculate the expected values of gamma. If the actual number of species fall near the center of the respective posterior-prediction distribution, then the model can be used to simulate the *Cycas* trees, indicating that it provides a good absolute fit and the diversification rates of *Cycas* are constant over time. Conversely, if the summary statistics fell outside the 95% credible interval of the posterior-predictive distributions, then the constant rate birth-death model is not suitable to predict the simulated trees and the diversification has significantly changed over time (Höhna et al. 2015).

In addition, we plotted the posterior-predictive distribution of the lineage accumulation curves (LTT plots for simulated trees) and compared the predictive distribution to the LTT plot for the observed tree. If the observed LTT plot falls within the simulated LTT plots, then the diversification rate of the genus *Cycas* has been constant over time and if not, this means that the diversification has experienced some evolutionary shifts.

Finally, the evolutionary models that explain the diversification patterns depicted by the observed LTT plot were identified. The models tested include a constant-rate birth-death model and three rate-variation models. The rate-variation models include a birth-death model with an exponentially decreasing speciation rate, a birth-death model with piecewise-constant rates (i.e., rates of speciation and extinction change over time but the diversification rate remains constant; Höhna et al. 2015) and a birth-death model of evolution punctuated by a mass-extinction event. Using Bayes Factors (BF; Baele et al. 2013), a pairwise comparison of these models was done to select the best model. For two models M_0 and M_1, BF values were interpreted following Jeffreys (1961). Specifically,

$BF(M_0,M_1) < 1$ means the model M_1 is supported; $1<BF(M_0,M_1)<3.2$ suggests that M_0 is barely worth-mentioning; $3.2<BF(M_0,M_1)<10$ indicates a substantial support for M_0, $10<BF(M_0,M_1)<100$ is indicative of a strong support for M_0, and $BF(M_0,M_1) >100$ is interpreted as decisive support for M_0 (Jeffreys, 1961).

4.2.4 The Compound Poisson Process (CPP) Mass-Extinction Times [CoMET] analysis

To investigate whether the genus *Cycas* has experienced some mass extinctions events (if so, when?), the CoMET [Compound Poisson Process (CPP) on Mass Extinction Time)] approach was employed (May et al. 2016). This approach has the advantage of being able not only to fit all possible birth-death models to the data at hand but also to specifically model mass extinction events. The CoMET approach treats the number of speciation-rate shifts, extinction-rate shifts, mass-extinction events as well as the parameters associated with these events as random variables, and then estimates their joint posterior distribution. For this analysis, hyperpriors were set both empirically and *a priori*.

Table 4.1 The accession numbers and species names of the sequences used in the analyses were downloaded from Genbank/NBCI. The "_" denotes that sequences are not available.

Sampling	**Accession numbers**										
Taxon	*psb*A-*trnH*	*trn*L-*trn*F	*trn*S-*trn*G	*psb*M-*trn*D	*PHY*P	*RPB1*	*HZP*	*AC3*	*F3H*	*SAMS*	*GTP*
Cycas aculeata K.D. Hill & Hiêp	KP117150	KP117204	KX181997	KX182668	KP117123	KP117177	KX182454	KX182098	KX183085	KX182963	KX182336
Cycas aenigma K.D. Hill & A.Lindstr.	KX182220	KX182575	KX181998	KX182669	KX182767	KX182864	KX182455	KX182099	KX183086	KX182964	KX182337
Cycas angulata R.Br.	KX182221	KT991437	KT991499	KT991446	KT991461	KT991479	KX182456	KX182100	KX183087	KX182965	KF309336
Cycas armstrongii Miq.	KX182222	KX182576	KX181999	KX182670	KX182768	KX182865	KX182457	-	KX183088	KX182966	KX182338
Cycas apoa K.D. Hill	KX182223	KX182577	KX182000	KX182671	KX182769	KX182866	KX182458	KX182101	KX183089	KX182967	KX182339
Cycas arnhemica ssp.muninga	KX182224	KX182578	KX182001	KX182672	KX182770	KX182867	KX182459	KX182102	KX183090	KX182968	KX182340
Cycas balansae Warb.	KX182225	KX182579	KX182002	KX182673	KX182771	KX182868	KX182460	KX182103	KX183091	KX182969	KX182341
Cycas brachycantha K.D. Hill, Hiêp &P.K.Loc	KX182226	KX182580	KX182003	KX182674	KX182772	KX182869	KX182461	KX182104	KX183092	KX182970	KX182342
Cycas badensis K.D.Hill	KX182227	KX182581	KX182004	KX182675	KX182773	KX182870	KX182462	KX182105	KX183093	KX182971	KX182343
Cycas bougainvilleana K.D. Hill	KX182228	KT991438	KT991500	KT991447	KT991462	KT991480	KX182463	KX182106	KX183094	KX182972	KX182344
Cycas bifida (Dyer)	KX182229	KX182582	KX182005	KX182676	KX182774	KX182871	KX182464	KX182107	KX183095	KX182973	KF309337

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K.D.Hill											
Cycas beddomei Dyer	KX182230	KX182583	KX182006	KX182677	KX182775	KX182872	KX182465	KX182108	KX183096	KX182974	KX182345
Cycas brunnea K.D.Hill	KX182231	KX182584	KX182007	KX182678	KX182776	KX182873	KX182466	KX182109	KX183097	KX182975	KX182346
Cycas basaltica C.A. Gardner	KX182232	KX182585	KX182008	KX182679	KX182777	KX182874	KX182467	KX182110	KX183098	KX182976	KX182347
Cycas calcicola Maconochie	KX182233	KX182586	KX182009	KX182680	KX182778	KX182875	KX182468	KX182111	KX183099	KX182977	KX182348
Cycas condaoensis K.D. Hill & S.L. Yang	KX182234	GU250507	GU250461	GU250484	KX182779	KX182876	KX182469	KX182112	KX183100	KX182978	KX182349
Cycas conferta Chirgwin	KX182235	KX182587	KX182010	KX182681	KX182780	KX182877	-	-	-	-	KX182350
Cycas chenii	KP117166	KP117220	KX182011	KX182682	KP117139	KP117193	KX182470	KX182113	KX183101	KX182979	KX182351
Cycas changjiangensis N.Liu	KX182236	GU250520	GU250474	GU250497	KX182781	KX182878	KX182471	KX182114	KX183102	KX182980	KX182352
Cycas chevalieri Leandri	KX182237	KX182588	KX182012	KX182683	KX182782	KX182879	KX182472	KX182115	KX183103	KX182981	KX182353
Cycas chamaoensis K.D. Hill	KX182238	GU250505	KX182013	GU250482	KX182783	KX182880	KX182473	KX182116	KX183104	KX182982	KX182354
Cycas curranii (J.Schust.) K.D.Hill	KX182239	KX182589	KX182014	KX182684	KX182784	KX182881	KX182474	KX182117	KX183105	KX182983	KX182355
Cycas collina K.D. Hill, Hiệp & P.K.Loc	KX182240	KX182590	KX182015	KX182685	KX182785	KX182882	KX182475	KX182118	KX183106	KX182984	KX182356
Cycas campestris	KX182241	KX182591	KX182016	KX182686	KX182786	KX182883	KX182476	KX182119	KX183107	KX182985	KX182357

K.D.Hill											
Cycas circinalis L.	KX182242	KX182592	KX182017	KX182687	KX182787	KX182884	KX182477	KX182120	KX183108	KX182986	KX182358
Cycas cairnsiana F. Muell.	KX182243	KX182593	KX182018	KX182688	KX182788	KX182885	KX182478	KX182121	KX183109	KX182987	KX182359
Cycas couttsiana K.D. Hill	KX182244	KX182594	KX182019	KX182689	KX182789	KX182886	KX182479	KX182122	KX183110	KX182988	KX182360
Cycas clivicola K.D. Hill	KX182245	GU250506	GU250460	GU250483	KX182790	KX182887	KX182480	KX182123	KX183111	KX182989	KX182361
Cycas clivicola ssp.lutea	KX182246	KX182595	KX182020	KX182690	KX182791	KX182888	KX182481	KX182124	KX183112	KX182990	KX182362
Cycas debaoensis Y.C. Zhong & C.JChen.	KX182247	KX182596	KX182021	KX182691	KX182792	KX182889	KX182482	KX182125	KX183113	KX182991	KX182363
Cycas diannanensis Z.T. Guan & G.D.Tao	KP117151	KX182597	KT991501	KT991448	KP117124	KP117178	KX182483	KX182126	KX183114	KX182992	KX182364
Cycas dolichophylla K.D. Hill, Hiêp &P.K.Loc	KP117152	KX182598	KX182022	KX182692	KX182793	KX182890	KX182484	KX182127	KX183115	KX182993	KX182365
Cycas desolata P.I.Forst	KX182248	KX182599	KX182023	KX182693	KX182794	KX182891	KX182485	KX182128	KX183116	KX182994	KX182366
Cycas edentata de Laub.	KX182249	GU250513	GU250467	GU250490	KT991466	KT991484	KX182486	KX182129	KX183117	KX182995	KX182367
Cycas elongata (Leandri) D.Yue Wang	KX182250	KX182600	KX182024	KX182694	KX182795	KX182892	KX182487	KX182130	KX183118	KX182996	KX182368
Cycas elephantipes A.Lindstr. & K.D.Hill	KX182251	KX182601	KX182025	KX182695	KX182796	KX182893	KX182488	KX182131	KX183119	KX182997	KX182369

Cycas falcata K.D.Hill	KX182252	KX182602	KX182026	KX182696	KX182797	KX182894	KX182489	KX182132	KX183120	KX182998	KX182370
Cycas furfuracea	KX182253	KX182603	KX182027	KX182697	KX182798	KX182895	KX182490	KX182133	KX183121	KX182999	KX182371
Cycas fugax K.D. Hill, Hiêp & P.K.Loc	KX182254	KT991439	KT991502	KT991449	KT991467	KT991485	KX182491	KX182134	KX183122	KX183000	KX182372
Cycas fairylakea	KX182255	KX182604	KX182028	KX182698	KX182799	KX182896	KX182492	KX182135	KX183123	KX183001	KX182373
Cycas ferruginea F.N. Wei	KX182256	KX182605	KX182029	KX182699	KX182800	KX182897	KX182493	KX182136	KX183124	KX183002	KX182374
Cycas glauca Miq.	KX182257	KX182606	KX182030	KX182700	KX182801	KX182898	KX182494	KX182137	KX183125	KX183003	KX182375
Cycas guizhouensis K.M. Lan & R.F. Zou	KP117153	KP117207	KX182031	KX182701	KX182802	KX182899	KX182495	KX182138	KX183126	KX183004	KX182376
Cycas guizhouensis_thai	KX182258	KX182607	KX182032	KX182702	KX182803	KX182900	-	KX182139	KX183127	KX183005	KX182377
Cycas hoabinhensis P.K.Loc & H.T.Nguyen	KX182259	KX182608	KX182033	KX182703	KX182804	KX182901	KX182496	KX182140	KX183128	KX183006	KX182378
Cycas hongheensis S.Y. Yang & S.L.Yang	KX182260	KT991444	KT991512	KT991459	KT991477	KT991497	KX182497	KX182141	KX183129	KX183007	KX182379
Cycas hongheensis S.Y. Yang & S.L.Yang	KX182261	KT991445	KT991513	KT991460	KT991478	KT991498	KX182498	KX182142	KX183130	KX183008	-
Cycas hainanensis C.J. Chen	KX182262	GU250521	GU250475	GU250498	KX182805	KX182902	KX182499	KX182143	KX183131	KX183009	KX182380
Cycas indica A.Lindstr. & K.D.Hill	KX182263	KX182609	KX182034	KX182704	KX182806	KX182903	KX182500	KX182144	KX183132	KX183010	KX182381
Cycas inermis Lour.	KX182264	KX182610	KX182035	KX182705	KX182807	KX182904	KX182501	KX182145	KX183133	KX183011	KX182382

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Cycas javana (Miq.) de Laub.	KX182265	KX182611	KX182036	KX182706	KX182808	KX182905	KX182502	KX182146	KX183134	KX183012	KX182383
Cycas lacrimans A.Lindstr. & K.D.Hill	KX182266	KX182612	KX182037	KX182707	KX182809	KX182906	KX182503	KX182147	KX183135	KX183013	KX182384
Cycas laotica	KX182267	KX182613	KX182038	KX182708	KX182810	KX182907	KX182504	KX182148	KX183136	KX183014	KX182385
Cycas lindstromii S.L. Yang, K.D. Hill &Hiep	KX182268	GU250509	GU250463	GU250486	KX182811	KX182908	KX182505	KX182149	KX183137	KX183015	KX182386
Cycas longipetiolula D.Y.Wang	KX182269	KX182614	KX182039	KX182709	KX182812	KX182909	KX182506	KX182150	KX183138	KX183016	KX182387
C. lanepoolei C.A.Gardner	KX182270	KX182615	KX182040	KX182710	KX182813	KX182910	KX182507	KX182151	KX183139	KX183017	KX182388
Cycas lingshuiensis G.A.Fu	KX182271	KX182616	KX182041	KX182711	KX182814	KX182911	KX182508	KX182152	KX183140	KX183018	KX182389
Cycas litoralis K.D.Hill	KX182272	KX182617	KX182042	KX182712	KX182815	KX182912	KX182509	KX182153	KX183141	KX183019	KX182390
Cycas macrocarpa Griff.	KX182273	KX182618	KX182043	KX182713	KX182816	KX182913	KX182510	KX182154	KX183142	KX183020	-
Cycas macrocarpa Griff.	KX182274	KX182619	KX182044	KX182714	KX182817	KX182914	KX182511	KX182155	KX183143	KX183021	KX182391
Cycas media R. Br. subsp media K. D. Hill	KX182275	KX182620	KX182045	KX182715	KX182818	KX182915	KX182512	KX182156	KX183144	KX183022	KX182392
Cycas media ssp.banksii	KX182276	KX182621	KX182046	KX182716	KX182819	KX182916	KX182513	KX182157	KX183145	KX183023	KX182393
Cycas media R. Br.	KX182277	KX182622	KX182047	KX182717	KX182820	KX182917	KX182514	KX182158	KX183146	KX183024	KX182394

subsp. ensata K.D.Hill											
Cycas multifrondis D.Y.Wang	KX182278	KX182623	KX182048	KX182718	KX182821	KX182918	KX182515	KX182159	KX183147	KX183025	KX182395
Cycas megacarpa K.D.Hill	KX182279	KX182624	KX182049	KX182719	KX182822	KX182919	KX182516	KX182160	KX183148	KX183026	KX182396
Cyvas micholitzii Dyer	KX182280	KX182625	KX182050	KX182720	KX182823	KX182920	KX182517	KX182161	KX183149	KX183027	KX182397
Cycas maconichie ssp.lanata	KX182281	KX182626	KX182051	KX182721	KX182824	KX182921	KX182518	KX182162	KX183150	KX183028	KX182398
Cycas maconochiei ssp.maconichiei	KX182282	KX182627	KX182052	KX182722	KX182825	KX182922	KX182519	KX182163	KX183151	KX183029	KX182399
Cycas maconichie ssp.viridis	KX182283	KX182628	KX182053	KX182723	KX182826	KX182923	KX182520	KX182164	KX183152	KX183030	KX182400
Cycas multiovula D.Y.Wang	KX182284	KX182629	KX182054	KX182724	KX182827	KX182924	KX182521	KX182165	KX183153	KX183031	KX182401
Cycas multipinnata C.J. Chen & S.Y.Yang	KX182285	KP117209	KX182055	KX182725	KX182828	KX182925	KX182522	KX182166	KX183154	KX183032	KX182402
Cycas miquelii Warburg	KX182286	KX182630	KX182056	KX182726	KX182829	KX182926	-	KX182167	KX183155	-	-
Cycas micronesica K.D. Hill	KX182287	KX182631	KX182057	KX182727	KX182830	KX182927	KX182523	KX182168	KX183156	KX183033	KX182403
Cycas montana A.Lindstr. & K.D.Hill	KX182288	KX182632	KX182058	KX182728	KX182831	KX182928	KX182524	KX182169	KX183157	KX183034	KX182404
Cycas nathorstii J.Schust.	KX182289	KX182633	KX182059	KX182729	KX182832	KX182929	KX182525	KX182170	KX183158	KX183035	KX182405

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Cycas nitida K.D.Hill & A.Lindstr.	KX182290	KX182634	KX182060	KX182730	KX182833	KX182930	KX182526	KX182171	KX183159	KX183036	KX182406
Cycas nongnoochiae K.D. Hill	KX182291	KX182635	KX182061	KX182731	KT991468	KT991486	KX182527	KX182172	KX183160	KX183037	KX182407
Cycas nongnoochiae K.D. Hill	KX182292	KX182636	KX182062	KX182732	KX182834	KX182931	KX182528	KX182173	KX183161	KX183038	-
Cycas ophiolitica K.D. Hill	KX182293	KT991440	KT991504	KT991451	KT991469	KT991487	KX182529	KX182174	KX183162	KX183039	KX182408
Cycas orientis K.D.Hill	KX182294	KX182637	KX182063	KX182733	KX182835	KX182932	KX182530	KX182175	KX183163	KX183040	KX182409
Cycas pachypoda K.D.Hill	KX182295	KT991441	KT991505	KT991452	KT991470	KT991488	KX182531	KX182176	KX183164	KX183041	KX182410
Cycas pectinata A Buch.-Ham.	KP117156	KP117210	KT991506	KT991453	KP117129	KP117183	KX182532	KX182177	KX183165	KX183042	KX182411
Cycas platyphylla K.D. Hill	KX182296	KX182638	KX182064	KX182734	KX182836	KX182933	KX182533	KX182178	KX183166	KX183043	KX182412
Cycas pruinosa Maconochie	KX182297	KX182639	KX182065	KX182735	KX182837	KX182934	KX182534	KX182179	KX183167	KX183044	KX182413
Cycas papuana F.Muell.	KX182298	KX182640	KX182066	KX182736	KX182838	KX182935	KX182535	KX182180	KX183168	KX183045	KX182414
Cycas pranburiensis S.L. Yang & al.	KX182299	KX182641	KX182067	KX182737	KX182839	KX182936	KX182536	KX182181	KX183169	KX183046	KX182415
Cycas petraea A. Lindstr. & K.D. Hill	KX182300	KT991442	KT991507	KT991454	KT991471	KT991489	KX182537	KX182182	KX183170	KX183047	-
Cycas petraea A.	KX182301	KX182642	KX182068	KX182738	KX182840	KX182937	KX182538	KX182183	KX183171	KX183048	KX182416

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Lindstr. & K.D. Hill											
Cycas parvula S.L.Yang ex D. Yue Wang	KP117158	KP117212	KX182069	KX182739	KP117131	KP117185	KX182539	KX182184	KX183172	KX183049	KX182417
Cycas panzhihuaensis L. Zhou & S. Y.Yang	KP117157	KP117211	KT991508	KT991455	KP117130	KP117184	KX182540	KX182185	KX183173	KX183050	KX182418
Cycas riuminiana Porte ex Regel	KX182302	KX182643	KX182070	KX182740	KX182841	KX182938	KX182541	KX182186	KX183174	KX183051	KX182419
Cycas rumphii Miq.	KX182303	KX182644	KX182071	KX182741	KX182842	KX182939	KX182542	KX182187	KX183175	KX183052	KX182420
Cycas revoluta Thunb.	KP117159	GQ273656	AB434465	GQ273612	KP117132	KP117186	KX182543	KX182188	KX183176	KX183053	KX182421
Cycas siamensis Miq.	KP117160	GU250511	GU250465	GU250488	KP117133	KP117187	KX182544	KX182189	KX183177	KX183054	KX182422
Cycas sundaica Miq. Ex A.Lindstr. & K.D.Hill	KX182304	KX182645	KX182072	KX182742	KX182843	KX182940	KX182545	KX182190	KX183178	KX183055	KX182423
Cycas seemannii A. Braun	KX182305	GU250516	GU250470	GU250493	KT991472	KT991490	KX182546	KX182191	KX183179	KX183056	KX182424
Cycas segmentifida D.Yue Wang & C.Y.Deng	KX182306	GU250523	GU250477	GU250500	KX182844	KX182941	KX182547	KX182192	KX183180	KX183057	KX182425
Cycas schumanniana Lauterb.	KX182307	KX182646	KX182073	KX182743	KX182845	KX182942	KX182548	KX182193	KX183181	KX183058	-
Cycas schumanniana Lauterb.	KX182308	KX182647	KX182074	KX182744	KX182846	KX182943	KX182549	KX182194	KX183182	KX183059	KX182426
Cycas simplicipinna (Smitinand) K.D. Hill	KX182309	KX182648	KX182075	KX182745	KX182847	KX182944	KX182550	KX182195	KX183183	KX183060	KX182427

Cycas sancti-lasallei	KX182310	KX182649	KX182076	KX182746	KX182848	KX182945	KX182551	KX182196	KX183184	KX183061	KX182428
Cycas semota K.D. Hill	KX182311	KX182650	KX182077	KX182747	KX182849	KX182946	KX182552	KX182197	KX183185	KX183062	KX182429
Cycas sphaerica Roxb.	KX182312	KX182651	KX182078	KX182748	KX182850	KX182947	KX182553	KX182198	KX183186	KX183063	KX182430
Cycas scratchleyana F.Muell.	KX182313	KX182652	KX182079	KX182749	KX182851	KX182948	KX182554	KX182199	KX183187	KX183064	KX182431
Cycas silvestris K.D.Hill	KP117161	KP117215	KX182080	KX182750	KP117134	KP117188	KX182555	KX182200	KX183188	KX183065	KX182432
Cycas shiwandashanica	KX182314	KX182653	KX182081	KX182751	KX182852	KX182949	KX182556	KX182201	KX183189	KX183066	KX182433
Cycas sexseminifera F.N. Wei	KX182315	GU250524	GU250478	GU250501	KX182853	KX182950	KX182557	KX182202	KX183190	KX183067	-
Cycas sexseminifera F.N. Wei	KX182316	KX182654	KX182082	KX182752	KX182854	KX182951	KX182558	KX182203	KX183191	KX183068	KX182434
Cycas shanyaensis G.A.Fu	KX182317	KX182655	KX182083	KX182753	KX182855	KX182952	KX182559	KX182204	KX183192	KX183069	KX182435
Cycas szechuanensis C.Y. Cheng, W.C.Cheng & L.K.Fu	KP117163	KP117217	KX182084	KX182754	KP117136	KP117190	KX182560	KX182205	KX183193	KX183070	KX182436
Cycas taitungensis C.F.Shen, K.D.Hill, C.H.Tsou & C.J.Chen	KX182318	GU250502	KX182085	GU250479	KT991473	KT991491	KX182561	KX182206	KX183194	KX183071	KX182437
Cycas thouarsii R. Br. ex Gaudich.	KX182319	GU250517	GU250471	GU250494	KX182856	KX182953	KX182562	KX182207	KX183195	KX183072	KX182438
Cycas tuckeri K.D.Hill	KX182320	KT991443	KT991510	KT991457	KT991474	KT991492	KX182563	KX182208	KX183196	KX183073	KX182439

Cycas tropophylla K.D. Hill & P.K. Loc	KP117165	KP117219	KX182086	KX182755	KP117138	KP117192	KX182564	KX182209	KX183197	KX183074	KX182440
Cycas tanqingii D.Yue Wang	KP117164	KX182656	KT991511	KT991458	KP117137	KP117191	KX182565	KX182210	KX183198	KX183075	KX182441
Cycas truncata de Laub.	KX182321	KX182657	KX182087	KX182756	KX182857	KX182954	KX182566	KX182211	KX183199	KX183076	KX182442
Cycas tansachana K.D. Hill & S.L. Yang	KX182322	GU250512	GU250466	GU250489	KT991475	KT991493	KX182567	KX182212	KX183200	KX183077	KX182443
Cycas taiwaniana Carruth.	KX182323	KX182658	KX182088	KX182757	KX182858	KX182955	KX182568	KX182213	KX183201	KX183078	KX182444
Cycas vespertilio A.Lindstr. & K.D.Hill	KX182324	KX182659	KX182089	KX182758	KX182859	KX182956	KX182569	KX182214	KX183202	KX183079	KX182445
Cycas wadei Merr.	KX182325	GU250504	GU250458	GU250481	KT991476	KT991494	KX182570	KX182215	KX183203	KX183080	KX182446
Cycas xipholepis K.D. Hill	KX182326	KX182660	KX182090	KX182759	KX182860	KX182957	KX182571	KX182216	KX183204	KX183081	KX182447
Cycas yorkiana K.D. Hill	KX182327	KX182661	KX182091	KX182760	KX182861	KX182958	KX182572	KX182217	KX183205	KX183082	KX182448
Cycas zambalensis Madulid & Agoo	KX182328	KX182662	KX182092	KX182761	KX182862	KX182959	KX182573	KX182218	KX183206	KX183083	KX182449
Cycas zeylanica (J.Schust.) A. Lindstr. &K. D. Hill	KX182329	GU250518	GU250472	GU250495	KX182863	KX182960	KX182574	KX182219	KX183207	KX183084	KX182450
Bowenia sp Hook.ex Hook.f.	JX402774	AF531185	AY138203	JX402774	JN655955	-	-	-	-	-	KF309332
Ceratozamia sp	KX182330	KX182663	KX182093	KX182762	JN655968	-	-	-	-	-	KX182451

Brongn											
Dioon sp Lindl.	KX182331	GQ273655	AB434427	GQ273609	JN655970	-	-	-	-	-	KF309338
Encephalartos sp. Lehmn	KX182332	KX182664	KX182094	KX182763	JN656000	-	-	-	-	-	KX182452
Lepidozamia sp Regel	KX182333	KX182665	KX182095	KX182764	JN656029	KX182961	-	-	-	-	KF309343
Macrozamia sp Miq.	KX182334	KX182666	KX182096	KX182765	JN656044	-	-	-	-	-	KF309345
Microcycas calocoma (Miq.) A.DC.	-	AF531194	AB434461	GQ273604	JN656055	-	-	-	-	-	KF309346
Stangeria eriopus (Kunze) Baill.	JX416858	AF531184	AB434424	JX416858	JN656056	KX182962	-	-	-	-	KF309347
Zamia sp L.	KX182335	KX182667	KX182097	KX182766	JN656065	-	-	-	-	-	KX182453
Ginkgo biloba L.	JN867578	JN867578	GQ227504	JN867578	KT071989	AY490553	DQ657216	-	AY742228	JF519742	-

4.3 Results

4.3.1 Phylogenetic tree of Cycas

The concatenated DNA matrix consists of seven nuclear regions (PHYP, RPB1, HZP, AC3, F3H, SAMS and GTP) and four plastid regions (*trnH*-*psb*A, *trn*L-*trn*F, *trn*S-*trn*G and *psb*M-*trn*D) is characterized of 10788 characters, 3947 potential parsimony informative, 3257 parsimony uninformative and 3584 constant characters (Table 4.2). The missing data is less than 5% (Table 4.2).

The phylogenetic tree reconstructed is, in general, well supported as the vast majority of nodes have PP>0.70 and BP>80% (Figure 4.1). Further, the ESS values ranged from 200 to 901 for the age estimates, suggesting convergence between posterior distributions and the MCMC estimates. The dated tree indicate two major clades from the cown with a strong support (PP/BP 0.5/99 and 0.9/99) suggesting that the genus *Cycas* may have diverged around 12 Ma (95% HPD, 10.4 – 14.7; Figure 4.1 & Appendix D). Even though the origin of the genus dated back to 12Ma, most *Cycas* diversification was initiated in the Pleistocene and reaches the peak in the Holocene (Figure 4.1). The genus *Cycas* consists of six sections (*Cycas*, *Wadeae*, *Asiorientales*, *Stangerioides*, *Panzhihuaenses* and *Indosinenses*), the section *Cycas* is the largest (67 species out of 116 species), polyphyletic, well supported (PP/BP = 0.8/99 and 1.0/85) and radiated ~2 Ma (95% HPD, 1.09 – 2.6; Figure 4.1). The sections *Stangerioides* and *Indosinenses* are not monophyletic with no support and both most species in these sections radiated ~1 Ma (95% HPD, 0.61-

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1.90; 0.17-2.4 and 1.09-3.03, respectively). However, *Panzhihuaenses* and *Asiorientales* sections with three species are monophyletic with a strong support (PP/BP = 1.0/77; 95% HPD, 0.14 – 0.924; Figure 4.1 & Appendix D). Finally, the section *Wadeae*, consisting of two species that are monophyletic with a strong support (PP/BP = 0.9/96) is the most recently radiated section (95% HPD, 0.01-0.37; Figure 4.1).

Table 4.2 The summary of DNA matrix and MP statistics for the aligned, analyzed and number of informative for each gene regions used in the study

	AC3	***F3H***	***GTP***	***HZP***	***PHYP***	***psb*A-*trnH***	***psb*M-*trn*D**	***RBP1***	***SAMS***	***trn*L-*trn*F**	***trn*S-*trn*G**	**Combined dataset**
Number of taxa	135	135	135	135	135	135	135	135	135	135	135	135
Number of characters	962	748	619	1281	940	730	1342	542	946	1403	1257	10788
Number of trees	68	827	1273	1206	1805	264	2868	702	3981	1761	1342	15321
Missing data	>5%	>5%	>5%	>5%	>5%	>5%	>5%	>5%	>5%	>5%	>5%	0
Constant character	908	140	67	259	30	553	34	66	583	457	487	3584
Parsimony uninformative variable	29	516	67	899	145	78	323	175	323	404	301	3257
Parsimony informative site	25	95	485	123	765	99	985	301	40	542	487	3947

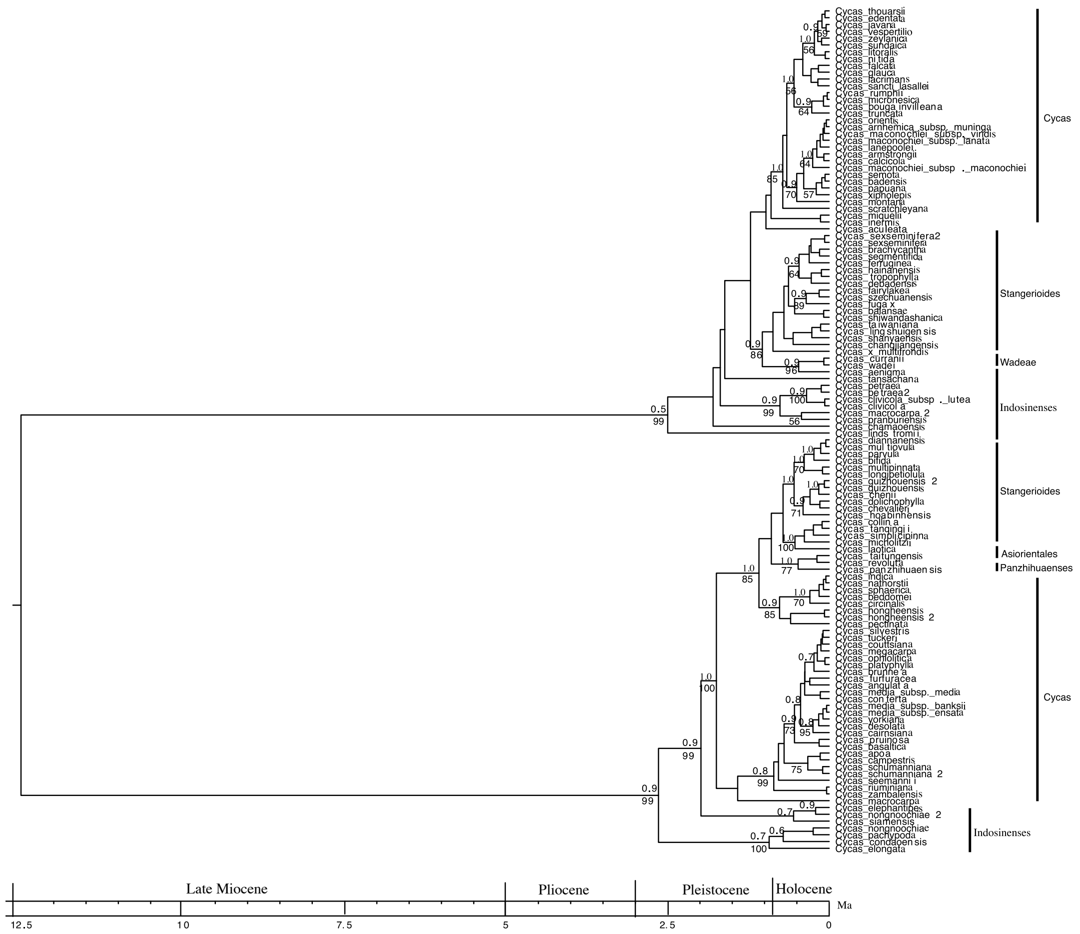

Figure 4.1 A complete Maximum Parsimony (MP) phylogeny tree of the genus *Cycas* from combined seven nuclear genes (PHYP, RPB1, HZP, AC3, F3H, SAMS and GTP) and four chloroplasts (*trnH*-psbA, *trn*L-*trn*F, *trn*S-*trn*G and *psb*M-*trn*D) based on Bayesian Inference. The numbers above the branch represent Bayesian Posterior Probability (PP) and below the branch represent the Maximum Parsimony bootstrap value BP.

4.3.2 Historical biogeography of *Cycas*

The application of both BBM and DEC models generated different results (Figures 4.2 and Appendix E, respectively). The origin of DEC model analysis was unclear with 40% probability while BBM analysis points to Indochina (~99%) as the origin of the genus *Cycas*, which dated back to around 12 Ma (node I, Figure 4.2). Around 2 Ma, the species diverged from Indochina to the Islands of Southeast Asia (node II, probablity 49%), including the Malay Peninsula, the Indian subcontinent, East Africa and North Australia where the diversification was mostly mediated through vicariance (Figures 4.2 & 4.3), although the origin is uncertain because of the unsupported propability that is less than 50%. Around the same time period, i.e. 2 Ma, the *Cycas* species further diversified within Indochina (nodes III, probability 99%), and colonised South China around ~ 1,5 Ma, (node IV, probability 90%) perhaps aided by vicariance (Figures 4.2 & 4.3).

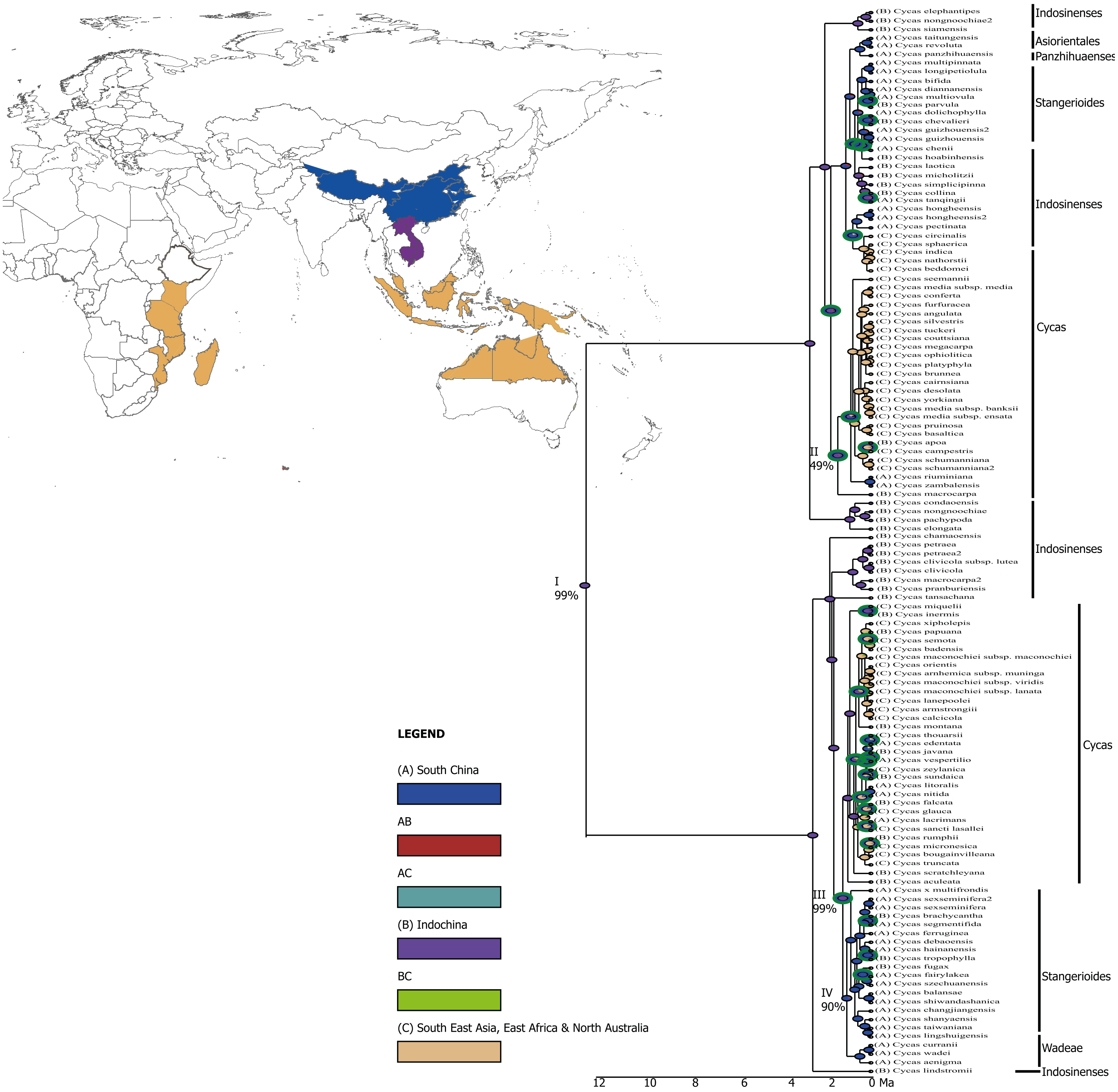

Figure 4.2 A graphical output from RASP showing results of ancestral reconstruction area from Bayesian Binary Method (BBM) analysis. Pie charts at each node show probabilities of alternative ancestral ranges. The green circles around the node represent vicariance events and the blue circles represent dispersal events. Key major nodes representing historical origin of different taxonomic sections are noted A-D and the probability of the origin at these nodes are also mentioned (%).

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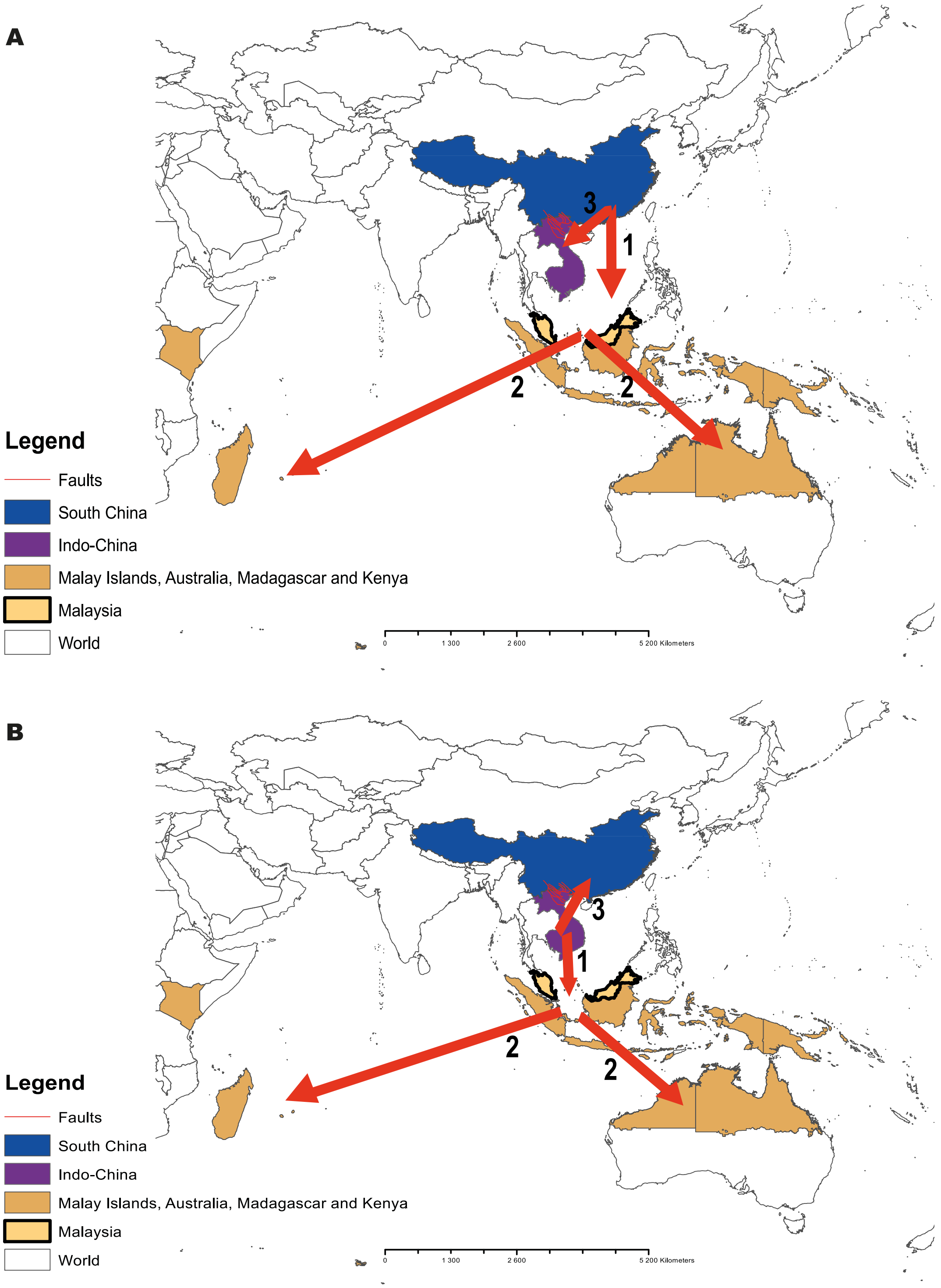

Figure 4.3 A representation of the colonization routes of the genus *Cycas* across the Pacific regions. **A**) Adopted from Xiao and Möller (2015); **B**) our route reconstruction.

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4.3.3 Diversification analysis

Most of the diversification events occurred in the last 2 million years (Figure 4.4 A & B). These diversification events may have followed a constant diversification model as revealed in the following findings. The number of taxa (116) falls within the 95% credible interval of its posterior predictive distributions (Figure 4.5). This means that the constant-rate birth-death model used to reconstruct the predictive distributions provides a good absolute fit to the evolutionary diversification of the genus *Cycas*. In addition, our LTT-plot does not depart significantly from those of the simulated trees under a constant-rate birth-death model (Figure 4.5). These findings indicate a constant diversification over time. Finally, when testing alternative models using Bayes Factors to select the best diversification model, we found that a constant birth-death model is strongly supported (BF =72.40; Table 4.3).

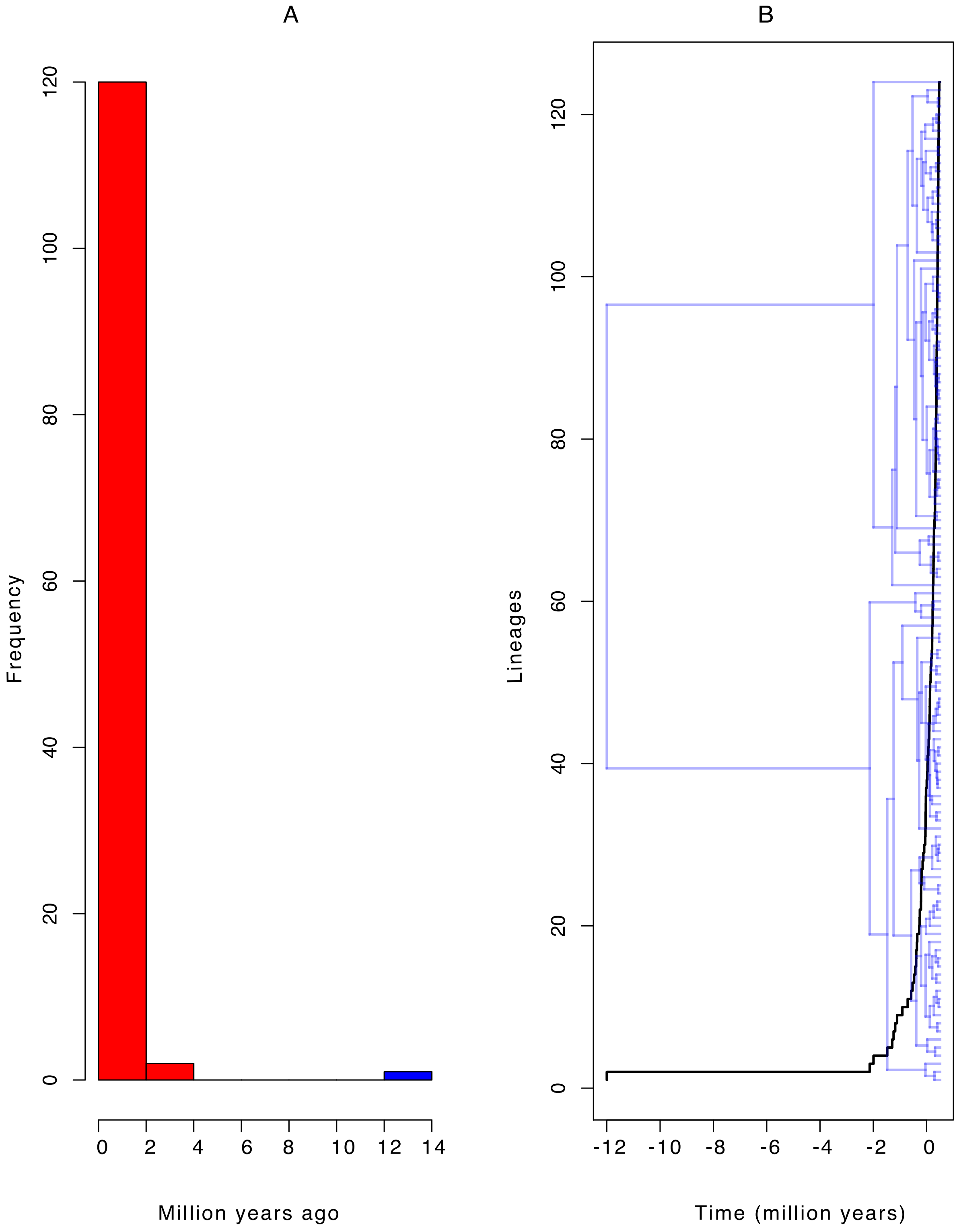

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Figure 4.4 The indication of the diversification pattern of the genus *Cycas*. **A**) Histogram showing the frequency of branching time of the phylogeny of *Cycas*, red colour indicate earlier branching events **B**) Lineage-through-time plot of the the phylogeny of *Cycas*.

Table 4.3 The Bayes factor (BF) values calculated for each birth-death model tested for the phylogeny of the genus *Cycas*. ConstBD = constant-rate birth-death model, DecrBD = continuously variable-rate birth-death model, EpisodicBD = episodically variable-rate birth-death model, and MassExtinctionBD = explicit mass-extinction birth-death model.

M_0	M_1	BF
ConstBD	MassExtinctionBD	72.400192
EpisodicBD	MassExtinctionBD	67.498351
DecrBD	MassExtinctionBD	57.157832
ConstBD	DecrBD	15.242360
EpisodicBD	DecrBD	10.340518
ConstBD	EpisodicBD	4.901841
ConstBD	ConstBD	0.000000
DecrBD	DecrBD	0.000000
EpisodicBD	EpisodicBD	0.000000
MassExtinctionBD	MassExtinctionBD	0.000000
EpisodicBD	ConstBD	-4.901841
DecrBD	EpisodicBD	-10.340518
DecrBD	ConstBD	-15.242360
MassExtinctionBD	DecrBD	-57.157832
MassExtinctionBD	EpisodicBD	-67.498351

MassExtinctionBD	ConstBD	-72.400192

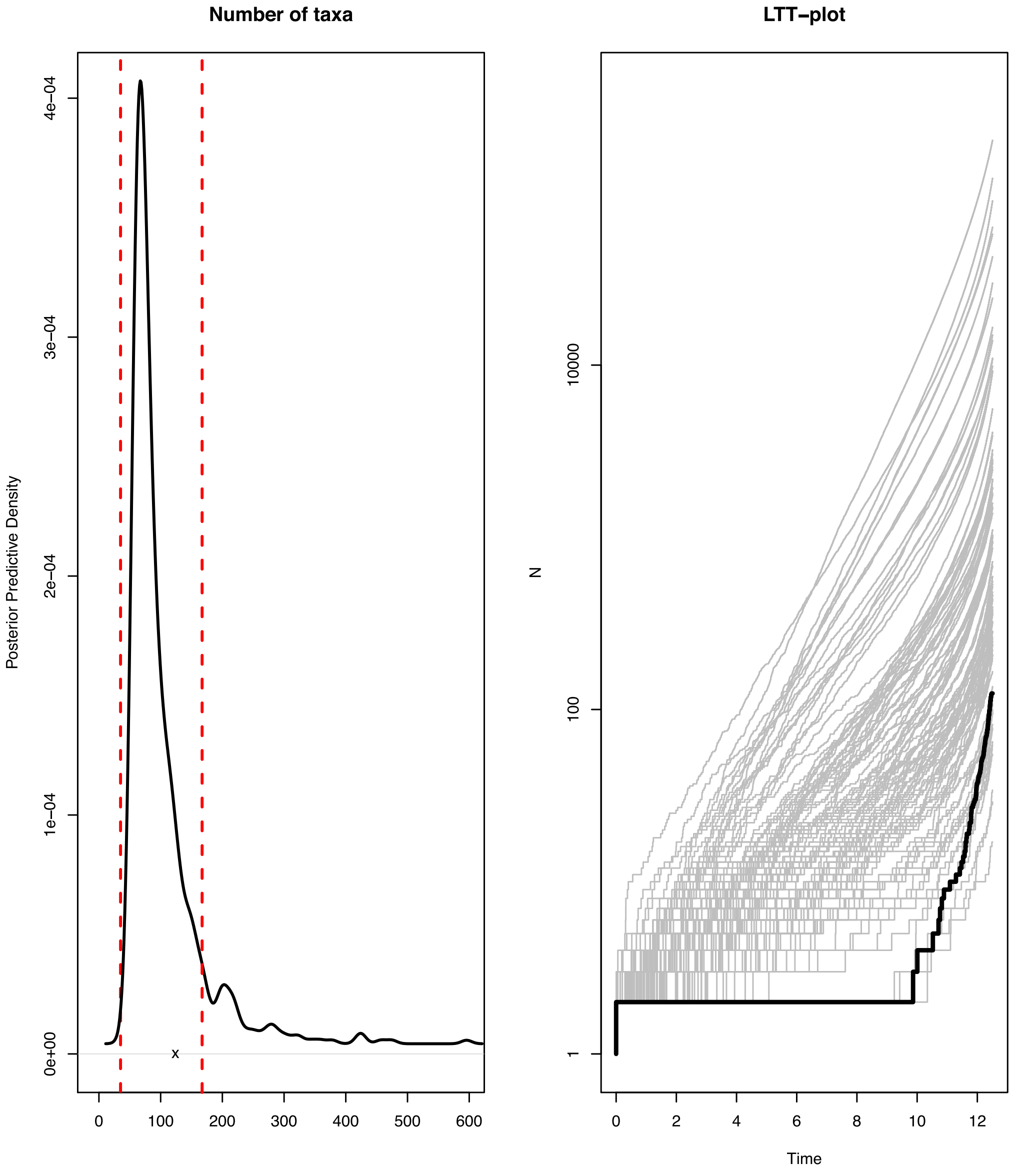

Figure 4.5 Indicate the absolute fit of the *Cycas* tree to the constant rate birth-death model using the posterior predictive simulation. **A**) Shows the number of species, the dashed lines indicate the 95% credible interval and X indicates the location of the species number. **B**) shows Lineage Through Time (LTT) plot of the simulated trees. The dashed lines indicate 95% credible interval and X shows the position of the value of gamma statistic.

4.3.4 The CoMET results

We tested several diversification events that might shape the biogeographical patterns. The diversification hyperpriors were specified *a priori* and empirically. The results of a *priori* hyperpriors are reported below as the results are similar to that of the empirically set priors.

The analysis indicates an initial speciation rate of 3.0 species per million years (Myr^{-1}) around 12 Ma; this rate increased to 3.2 species Myr^{-1} around 10 Ma (Figure 4.6A) and decreased slightly around 8 Ma to 2.9 species Myr^{-1}. The speciation rate remains roughly constant around 7 to 5 Ma (~ 2.9 species Myr^{-1}) and increased sharply to 4.0 species Myr^{-1} around 3 to 1 Ma until to the present (Figure 4.6A). We, however, found a recent speciation shift which indicate the present day (Figure 6B) (2lnBF > 6). Furthermore, the extinction rates remained constant at ~ 0.4 species Myr^{-1} around 12 to 3 Ma and decreased to 0.25 at 3 Ma to present (Figure 4.6C). However, there was no evidence of any significant shift in extinction or mass extinction (2lnBF < 6; Figure 4.6D-F).

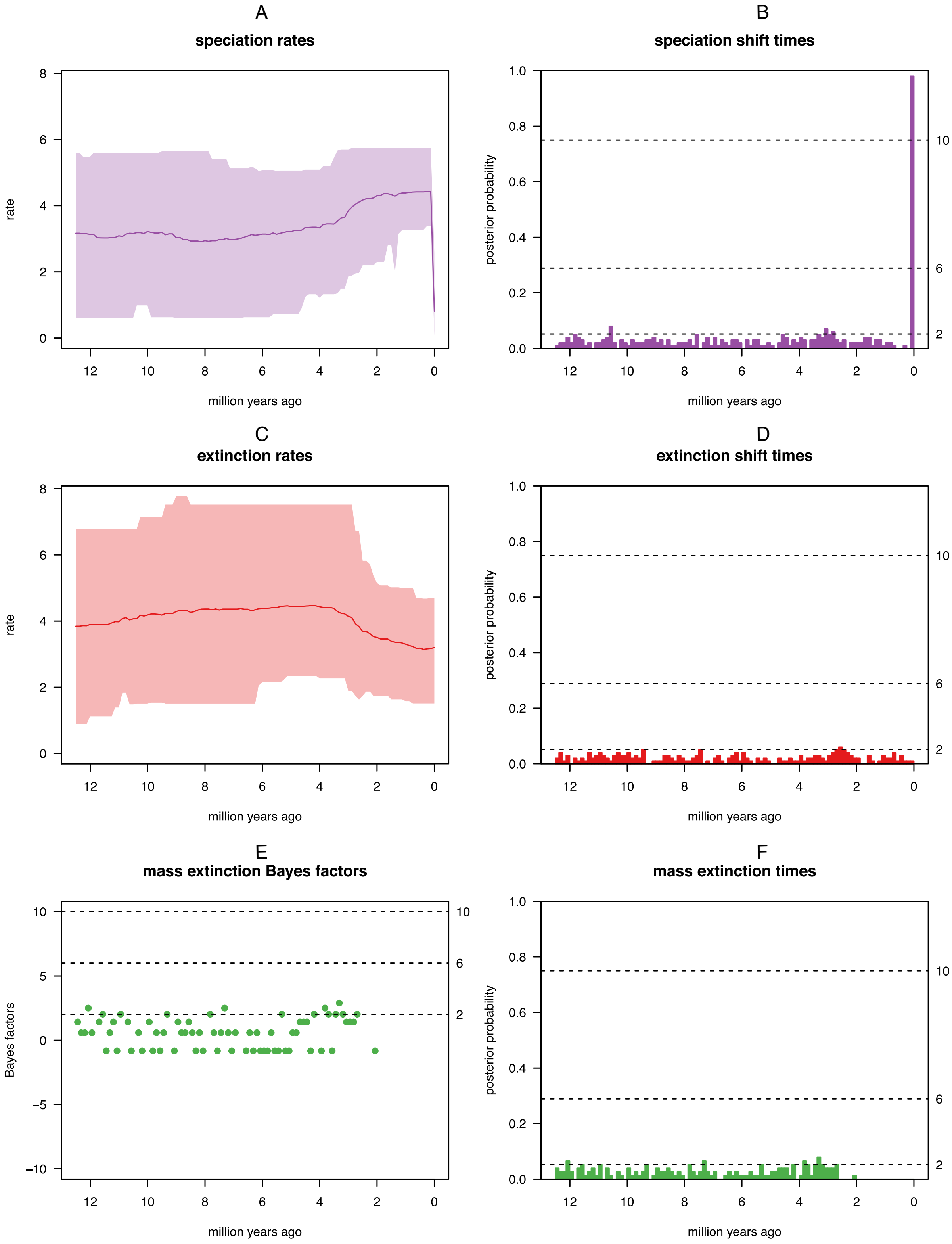

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Figure 4.6 Summary of all evolutionary events (A-F) reported in this study. This results visualized using the CoMET model and the reported results are for the diversification hyperpriors specified as *priori.*

4.4 Discussion

4.4.1 Phylogenetic tree of *Cycas*

Overall, the topology and the node support of the phylogeny of *Cycas* is similar to the most recent treatment of the genus (Liu et al. 2018). Three of the six sections of the genus are polyphyletic (*Cycas*, *Stangerioides*, *Indosinense*) and the remaining sections are monophyletic as previously reported (Xiao and Möller 2015; Liu et al. 2018). Few points are worth highlighting. In our phylogeny, the species *Cycas macrocarpa* and *Cycas pranburiensis* are nested within the section Indosinenses but they were included in the section *Cycas* in previous studies (Liu et al. 2018; Hill and Yang 1999). However, the sections *Cycas* and *Indosinenses* have overlapping distribution pattern in Southeast China and India that might have caused a gene flow within the two sections (Yang and Meerow 1996), making it difficult to distinguish species of these two sections on a phylogeny.

4.4.2 Historical biogeography

The biogeography of the genus *Cycas* has been investigated in recent studies (e.g. Keppel et al. 2008; Xiao and Möller 2015). In their study, Xiao and Möller, (2015) indicated, with high level

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of confidence (~94%), that South China is the origin of the genus. Our analysis, instead, pointed to Indochina as the origin of the genus, which dated back to ~ 12 Ma (evolutionary age of the genus). They also indicated that Indochina was the first to be colonized by the genus through vicariance and dispersal from South China (with a relatively low confidence level, 46%) with a series of late dispersal events across the Malay archipelagos through to Australia and East Africa. In our study, again, the colonization routes are different. Specifically, we found that the colonization route might actually have started, firstly, from Indochina (ancestral area B) to ancestral area (C) (Malay islands southward to Australia and westward to Madagascar, East-Africa) and lastly from Indochina (B) to South China (A).

Indeed, the historical biogeography of the Pacific Island's flora has always been a matter of debate (e.g. see Keppel et al. 2009). This study adds to this debate specifically with regard to the origin and the ecological forces that might drive the distribution of the genus *Cycas* in the region. The differences between our findings and those of Xiao and Möller (2015) could be linked to the differences in the sampling size between both studies. Although, they included representatives of the major sections of the genus into their analysis, only 31 species were analyzed whilst ours includes the complete sample (116 species) of the genus. In addition, our analysis further contradicts theirs in term of the sequences of the colonization events. As opposed to Xiao and Möller (2015), we found that the colonization of South China occurred actually not at an early stage but at the last, after the rest of the genus distribution ranges across the Pacific islands has been colonized. However, our study agrees with Xiao and Möller (2015) concerning the ecological processes (dispersal and vicariance) that might have mediated the colonization. On this aspect, the Red River Fault may have played an important role, which may include the role of a geographic barrier between Indochina and South China (Xiao and Möller 2015; Zheng et al. 2016). This barrier may account for the delay of the colonization of South China in comparison to the early colonization of the Malay archipelagos and the distribution ranges of the genus previously reported (Xiao and Möller 2015).

In this early colonization of the Malay archipelagos, Malesia might have played the role of a source area from which the genus might have dispersed westwards to East Africa and eastwards into the Pacific (centre-periphery hypothesis, Brown 1984; Hampe and Petit 2005; Kawecki 2008; Gaston 2009). The centre–periphery hypothesis provides an explanation to the biogeographical distribution of species from their centre of origin to their peripheral ranges. The hypothesis predicts that populations are more isolated and less abundant towards the periphery of their distribution (Sexton et al. 2009). Although we did not explicitly test this hypothesis in this study, early studies reported an overall decrease in taxonomic diversity of various plant groups from Malesia eastwards in the Pacific region (Corner 1963; van Balgooy 1969; Woodroffe 1987). Even this report holds for *Cycas* as, for example, most *Cycas* species in the subsection

Rumphiae are centred in or around Malesia (Hill 1996b; Keppel et al. 2008).

The debate on the colonization process of the Pacific Islands (Keast and Miller 1996; Ebach and Tangey 2006) evolves around vicariance and long-distance dispersal events (see Keppel et al. 2009). The vicariance biogeography (Nelson and Platnick 1981) was initially believed to be the major force structuring the flora of the Pacific (Whitmore 1973; Ladiges et al. 2003; Heads 2006, 2008; Ladiges and Cantrill 2007). However, the long distance dispersal process has also been central in the early debate (Darwin 1859; Guppy 1906; Ridley 1930; Mayr 1954; Carlquist 1967). Interestingly, mounting evidence, including molecular data, supports the long distance dispersal scenario (Turner et al. 2001; Price and Clague 2002; Winkworth et al. 2002, 2005; Perrie and Brownsey 2007). For the genus *Cycas*, the long distance dispersal is more likely the main event through which the entire geographic ranges of *Cycas* has been colonized (Keppel et al. 2008; Xiao and Möller 2015). There are various scenarios for this dispersal event, including the hitch-hiking, stepping-stones, and long distance dispersal scenarios (Keppel et al. 2009) mediated through a floatation-facilitating layer in the seeds of *Cycas* (Xiao and Möller 2015; Zheng et al. 2016).

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To further elucidate this historical biogeographic process, we explored several evolutionary events that might shape the diversification of *Cycas*, including speciation, extinction, and mass extinctions. Around 12 Ma, we found an initial speciation rate that is very similar to the overall speciation rate reported for gymnosperm in general (Crisp and Cook 2011). However, the overall speciation corresponds to the late Miocene (Tortonian-Messinian), a period characterized in the Pacific regions by frequent sea level excursions (e.g. eight sea level excursions; Aharon et al. 1993). These multiple frequent rises and falls of sea level would likely contribute to a long dispersal of *Cycas* seeds across the Pacific islands through to Australia, Madagascar and East Africa. For example, species in the subsection Rumphiae developed seeds with spongy layer inside the sclerotesta (de Laubenfels and Adema 1998); the "spongy" characteristic of the seeds facilitates the floatation of the seeds, thus promoting a long trans-oceanic dispersal across the pacific islands (de Laubenfels and Adema 1998; Xiao and Möller 2015; Zheng et al. 2017).

Cycads have a fascinating evolutionary history starting around 300 Ma (Hendricks 1987), and the extant cycads re-diversified around 12-2 Ma (Nagalingum et al. 2011). They share morphological characteristics of ferns and angiosperms (Brenner 2003; Norstog and Nicholls 1997), and these characteristics make them a unique taxonomic and evolutionary group. In this group, the genus *Cycas* has recently been identified as the most rapidly diversified and widely

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distributed clade (Yessoufou et al. 2017). Here we build upon this knowledge to reconstruct the historical biogeography and the evolutionary events that might shape the rapid diversification and wide distribution across the pacific islands. Our analysis indicated that Indochina may have been the origin of the genus (but see Xiao and Möller 2015), and that the pacific island may have been first colonized through dispersal way before the genus reaches South China. This dispersal may have been facilitated by multiple excursions of sea level and the development of a key innovation, a spongy endocarp. We also found a number of evolutionary events. Our study therefore clarifies the historical biogeography and the evolutionary events that shaped the current diversity of the genus.

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Chapter 5

Conclusions and Recommendations

This chapter provides a synthesis of the chapters making up this thesis. The chapter presents a summary of the study findings, conclusions and recommendations for practical actions.

Cycads (Cycadopsida: Cycadales) are an interesting ancient seed plants which originated ~300 million years ago and share similar morphological characters with ferns and angiosperms. They were regarded as the major plant communities during Jurassic and Cretaceous era and declined drastically to about 300 species comprising of 10 genera in the mid-late Cretaceous. Undoubtedly, the recent cycads (~300 spp.) originated from synchronous radiation events that dated back to about 12 Ma and inhabit the tropical and subtropical regions with predominant summer rainfalls with two families recognized, Cycadaceae and Zamiaceae and they all appear to be monophyletic.

In total, 10 genera diversified within the cycads group with the two genera *Cycas* and *Encephalartos* being the most diversified genera excluding *Zamia*. These two genera (*Cycas* and *Encephalartos*) extent their distribution to Asia, Northern part of Australia and Africa respectively. Therefore, they represent the most geographically wide-ranging and morphologically diverse species within the cycads group. In this study, we have build upon this knowledge to reconstruct the historical biogeography and the evolutionary events that might have shaped the rapid diversification and widespread distribution of *Encephalartos* and *Cycas* species. It was discovered that the explosive radiations within *Encephalatos* and *Cycas* were mediated or shaped by the global climate change during the Miocene age. This global climate change profoundly transformed the climates of tropical and subtropical biomes by increasing their seasonality and aridity within the environment and also pushed most species to slow morphological transformation as a result of adaptive radiation response driven by changes in climatic regimes of the past.

For example, the African cycads (*Encephalatos*) based on the reconstructed complete phylogeny analysis, the genus originated in southern Africa. Then, diverged around 9 Ma from southern Africa to east-central-west Africa through vicariance and later disperse to northward regions of the continent. Vicariance events simply, imply that the eastern, central and western African populations of *Encephalartos* were once connected and then later separated into different populations owing to geographic barriers that prevent continuous gene flow, leading to the radiation of new species. For example, the tropical African forest was once a continuous vegetation type and then broke up thus promoting species diversification by vicariance. Most species accumulated in the last 2.6 Ma which was mediated by the mass extinctions that opened up ecological niches promoting explosive radiation. Although, there were no significant shifts in any of the evolutionary events, suggesting that a constant-rate diversification model is best suited for the genus and the rapid species accumulation occurred during the Pliocene–Pleistocene.

Similarly, based on the complete phylogentic tree analysis, the genus *Cycas* which was initially thought to be from South China (see Xiao and Moller 2015), was found to be originated in Indochina. The genus may have dispersed firstly across the Pacific Islands during the late Miocene, aided by multiple excursions of sea levels and the development of a key innovation, i.e. a spongy endocarp particularly in the seeds of subsection *Rumphiae*. Then, colonize South China, which was thought to be the origin of the genus, that may have occurred more recently aided by both dispersal and vicariance events. All these colonisations may have further been mediated through a number of evolutionary events but none was significant. Cycads genera have experienced drastic environmental changes and undergone significant extinction which makes them an interesting plant group to reconstruct the evolutionary and historical biogeography.

In the extinction risk study, nine threats of cycads were identified. It was discovered that habitat loss, overcollection, medicinal uses and reproduction failure to be clustered on the cycads tree of life. In such, closely related species were exposed to similar threats but that could be due the geographic regionalization of the genera. Nonetheless, the diversity of threats and several variables linked to the biology and ecology of cycads correlate with extinction risk and different variables seemed to be linked to different IUCN status of cycads. All the five Data Deficient (DD) species of cycads were predicted to be in the VU category

In conclusion, cycads group have witnessed periods of extinction and most drastical environmental changes in the past. As such, this study have predisposed the evolutionary history and extinction risk of the cycads group and most of the threats that were identified as drivers of extinction risk of cycads were anthropogenically mediated. A complete phylogeny for the genus *Encephalartos* revealed that the genus originated in southern Africa and dispersed northward of Africa mediated by both viacariance and dispersal. Similarly, the complete phylogeny of *Cycas* also, indicated Indochina as the origin of *Cycas*. This was mediated by multiple level of sea excursions and a spongy endocarp within the seed of the subsection Rumphiae. Therefore, this study clarifies the historical biogeography and the evolutionary events that shaped the current diversity of the two genera (*Encephalartos* and *Cycas*). But none of the evolutionary events showed a significant shifts.

We recommend legislation to regulate human-cycads interactions and the commitment of all governments globally to implement these regulations. Lastly, the reconstruction of a phylogenetic tree of the two genera *Encephalartos* and *Cycas* can be further resolved with additional markers for further research study. The additional dataset with the whole chloroplast genome data can be carried out for a deeper understanding of the phylogeny and the historical evolution of the two cycads genera.

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Appendices

Appendix A: Raw data for all ecological, biological and evolutionary information

Table S1 Raw data for all ecological, biological and evolutionary information used in this study. GE = Global endangerment; Hab-D = Habitat loss _Destruction; Def = Deforestation; Med = Medicinal uses; Over_P = Overcollection_Poaching; F_D = Flood_Drought; Repr = Reproduction failure; Graz = Grazing; Inv = Invasive plants; No_thr = Number of threats; ED =; range1 = Geographic range KM^2; alt_min = altitude min; alt_max = altitude max; thr_st = threat stature; H_min = height min; H_max = height max; G_time = Geographic time; D_min = Diameter min; D_max = Diameter max; G_range = Geographic range; NT = Not Threatened; T = Threatened.

Species	GE	Hab_D	Def	Med	Over_P	F_D	Repr	Graz	Inv	Fire	No_thr	ED	Range1	alt_min	alt_max	thr_st	H_min	H_max	G_time	D_min	D_max	G_range
Bowenia_serrulata	LC	1	0	0	0	0	0	0	0	0	1	50.14995	NA	30	150	NT	NA	NA	30	0.3	0.25	1
Bowenia_spectabilis	LC	1	0	0	0	0	0	0	0	0	1	50.14995	NA	0	750	NT	NA	NA	30	NA	10	1
Ceratozamia_alvarezii	EN	1	0	0	1	0	0	0	0	0	2	39.5459	16	NA	NA	T	0.1	0.5	45	0.089	0.175	1
Ceratozamia_becerrae	EN	1	0	0	0	0	0	0	0	1	2	30.23118	1000	NA	NA	T	0.1034	0.31	45	0.0548	0.1047	2
Ceratozamia_chimalapensis	CR	1	0	0	0	0	0	0	0	1	2	39.5459	NA	NA	NA	T	0.2	0.1	NA	0.178	0.331	1
Ceratozamia_decumbens	CR	1	0	0	1	0	0	0	0	0	2	33.73552	NA	NA	NA	T	0.09	0.2	NA	0.08	14	1
Ceratozamia_euryphyllidia	CR	1	0	0	0	0	0	0	0	0	1	20.08057	NA	NA	NA	T	0.1034	0.31	45	0.548	0.1047	2
Ceratozamia_fuscoviridis	CR	1	1	1	0	0	0	0	0	0	3	39.03437	NA	NA	NA	T	0.165	0.36	NA	0.14	0.22	1
Ceratozamia_hildae	EN	1	0	0	1	0	0	0	0	0	2	36.39392	NA	NA	NA	T	0.1	0.2	45	0.05	0.25	3

Ceratozamia_hondurensis	CR	1	1	0	1	1	0	1	0	0	5	19.85266	NA	NA	NA	T	NA	NA	45	NA	NA	1
Ceratozamia_huastecorum	CR	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	39.03437	NA	NA	NA	T	NA	NA	45	NA	NA	1
Ceratozamia_kuesteriana	CR	1	0	0	1	0	0	0	0	0	2	25.5329	NA	NA	NA	T	NA	NA	45	NA	NA	1
Ceratozamia_latifolia	EN	1	0	0	1	0	0	0	0	0	2	20.70881	NA	NA	NA	T	0.1	0.2	45	NA	0.1	4
Ceratozamia_matudae	EN	1	0	0	0	0	1	0	0	0	2	24.96245	5000	NA	NA	T	0.1	0.5	45	0.89	0.175	3
Ceratozamia_mexicana	VU	1	1	0	1	0	0	0	0	0	3	20.08057	NA	NA	NA	T	NA	1	45	0.08	0.2	2
Ceratozamia_microstrobila	VU	1	0	0	0	0	0	0	0	0	1	41.76929	1000	NA	NA	T	NA	0.25	45	NA	10	2
Ceratozamia_miqueliana	CR	1	0	0	1	0	0	0	0	0	2	22.91372	NA	NA	NA	T	0.1034	0.31	45	0.548	0.1047	3
Ceratozamia_mirandae	EN	1	0	0	1	0	0	0	0	1	3	34.77023	NA	NA	NA	T	NA	NA	45	NA	NA	1
Ceratozamia_mixeorum	EN	1	1	0	0	0	0	0	0	0	2	42.82403	25	NA	NA	T	0.34	1.25	45	0.14	0.18	1
Ceratozamia_morettii	EN	0	0	0	1	0	0	0	0	0	1	47.26377	10	NA	NA	T	NA	0.3	45	NA	0.08	1
Ceratozamia_norstogii	EN	0	0	0	1	0	0	0	0	0	1	22.39375	1100	NA	NA	T	0.1	0.5	45	0.089	0.175	2
Ceratozamia_robusta	EN	1	0	0	1	0	0	0	0	1	3	20.70881	NA	NA	NA	T	1.5	2	45	NA	0.3	11
Ceratozamia_sabatoi	EN	1	0	0	0	0	0	1	0	0	2	25.5329	NA	NA	NA	T	0.1	0.5	45	0.089	0.175	2

Ceratozamia_santillanii	CR	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	33.73552	NA	NA	NA	T	NA	NA	45	NA	NA	1
Ceratozamia_vovidesii	VU	1	0	0	0	0	0	0	0	1	2	25.92663	NA	1000	1700	T	NA	NA	45	NA	NA	1
Ceratozamia_whitelockiana	EN	1	0	0	0	0	0	0	0	1	2	19.85266	NA	NA	NA	T	0.2	0.3	45	0.12	0.18	1
Ceratozamia_zaragozae	CR	0	0	0	1	0	0	0	0	0	1	30.23118	45	NA	NA	T	NA	NA	45	NA	NA	1
Ceratozamia_zoquorum	CR	1	0	0	1	0	0	0	0	0	2	44.3358	40	NA	NA	T	0.1034	0.31	45	0.548	0.1047	1
Chigua_bernalii	CR	1	0	0	1	1	0	0	0	0	3	92.77871	NA	75	150	T	1	1.4	NA	0.6	1.6	NA
Cycas_aculeata	VU	1	0	0	0	0	0	0	0	0	1	18.227	10	NA	NA	T	NA	NA	30	0.15	0.18	1
Cycas_angulata	LC	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	15.77084	NA	0	30	NT	5	12	NA	0.15	0.25	1
Cycas_annaikalensis	CR	0	0	0	1	0	0	0	0	0	1	20.23621	NA	NA	940	T	NA	5	40	0.19	0.61	1
Cycas_apoa	NT	1	0	0	0	0	0	0	0	0	1	15.77197	NA	NA	NA	NT	NA	2.5	40	NA	NA	3
Cycas_arenicola	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	16.41264	NA	NA	NA	NT	1.5	2.5	40	0.15	0.2	1
Cycas_armstrongii	VU	1	0	0	0	0	0	0	0	1	2	29.42157	NA	NA	NA	T	3	6	40	0.05	0.11	1
Cycas_arnhemica	LC	0	0	0	0	0	0	0	0	1	1	32.74662	NA	NA	NA	NT	1.5	2.5	40	0.12	0.2	1
Cycas_badensis	NT	1	0	0	0	0	0	0	0	0	1	17.56073	NA	NA	NA	NT	NA	8	40	NA	NA	5
Cycas_balansae	NT	1	0	0	1	0	0	0	0	0	2	60.9654	NA	100	800	NT	NA	NA	40	0.12	0.2	1

												1										
Cycas_basaltica	LC	0	0	0	1	0	0	0	0	0	1	14.90585	NA	230	260	NT	2	4	40	0.15	0.23	1
Cycas_beddomei	EN	1	0	1	0	0	1	0	0	1	4	25.56325	388	300	900	T	NA	2	40	0.12	0.23	1
Cycas_bifida	VU	1	0	0	1	0	0	0	0	0	2	18.72094	NA	100	300	T	NA	NA	40	NA	NA	5
Cycas_bougainvilleana	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	24.69741	NA	NA	NA	NT	NA	5	40	NA	NA	3
Cycas_brachycantha	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	51.24649	NA	NA	NA	NT	NA	1	40	0.09	0.12	1
Cycas_brunnea	NT	0	0	0	1	0	0	0	0	0	1	30.05926	NA	NA	NA	NT	2	5	40	0.17	0.23	1
Cycas_cairnsiana	VU	0	0	0	1	0	0	0	0	0	1	21.22965	NA	450	500	T	2	5	40	0.12	0.16	1
Cycas_calcicola	LC	0	0	0	1	0	0	0	1	1	3	17.0605	NA	123	155	NT	2	5	40	0.16	0.22	1
Cycas_campestris	NT	0	0	0	1	0	0	0	0	1	2	19.20191	20000	NA	NA	NT	NA	2.5	40	NA	0.2	2
Cycas_canalis	LC	1	0	0	0	0	1	0	0	1	3	18.227	NA	NA	NA	NT	3	5	40	0.07	0.14	1
Cycas_candida	EN	0	0	0	0	0	0	0	0	1	1	24.9318	55	NA	NA	T	NA	NA	40	NA	NA	1
Cycas_cantafolia	CR	1	0	0	1	0	1	0	0	0	3	17.23081	NA	NA	NA	T	NA	NA	NA	NA	NA	1
Cycas_chamaoensis	CR	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	14.78945	NA	NA	NA	T	NA	10	40	0.14	0.28	1
Cycas_chamberlainii	EN	1	0	0	1	0	0	0	0	0	2	12.51481	NA	615	800	T	NA	NA	40	NA	NA	1
Cycas_changjiangens	EN	1	0	0	1	0	0	0	0	0	2	29.0368	NA	600	800	T	0.3	2.5	40	NA	0.2	1

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Cycas_chevalieri	NT	0	0	0	1	0	0	0	0	0	1	21.68381	NA	NA	NA	NT	NA	1.2	NA	0.08	0.18	4
Cycas_circinalis	EN	1	0	1	1	0	1	0	0	0	4	25.56325	NA	300	1000	T	6	7	40	0.12	0.27	5
Cycas_clivicola	LC	0	0	0	1	0	0	0	0	0	1	11.14557	300000	NA	60	NT	NA	8	40	0.12	0.16	9
Cycas_collina	VU	1	0	0	1	0	0	0	0	0	2	29.82684	20000	400	900	T	NA	NA	40	0.1	0.14	1
Cycas_condaoensis	VU	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	26.07318	20	NA	NA	T	0.2	2.5	40	0.14	0.17	1
Cycas_conferta	NT	1	0	0	1	0	0	0	0	0	2	60.36198	NA	NA	NA	NT	4	7	40	0.09	0.13	1
Cycas_couttsiana	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	31.82362	NA	NA	700	NT	3	7	40	0.14	0.2	1
Cycas_cupida	VU	1	0	0	1	0	0	0	0	0	2	20.61617	60	NA	NA	T	NA	NA	40	NA	NA	1
Cycas_curranii	CR	1	1	0	0	0	0	0	0	0	2	34.0329	NA	NA	NA	T	NA	3	40	NA	NA	1
Cycas_debaoensis	CR	1	1	0	1	0	0	0	0	0	3	10.80662	NA	300	1300	T	NA	NA	40	0.15	0.2	1
Cycas_desolata	VU	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	16.41264	NA	450	550	T	4	7	40	0.15	0.25	1
Cycas_diannanensis	VU	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	11.02612	NA	600	1800	T	NA	0.03	40	0.25	0.35	2
Cycas_dolichophylla	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	18.87451	NA	NA	NA	NT	NA	1.5	40	0.18	0.3	11
Cycas_edentata	NT	1	0	0	0	0	0	0	0	0	1	13.48673	1000	NA	NA	NT	NA	10	40	NA	0.2	33

Cycas_elephantipes	EN	0	0	0	1	0	0	0	0	0	1	29.1319	NA	NA	NA	T	1	3	40	0.15	0.2	1
Cycas_elongata	EN	1	1	0	1	0	0	0	0	0	3	21.47247	NA	50	200	T	2	5	40	0.1	0.2	5
Cycas_falcata	VU	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	11.02612	1350	NA	NA	T	NA	5	40	0.12	0.3	2
Cycas_ferruginea	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	43.33355	7215	NA	NA	NT	NA	1.2	40	0.12	0.18	3
Cycas_fugax	CR	1	0	0	0	0	0	0	0	0	1	37.99666	NA	NA	200	T	NA	NA	40	0.08	0.12	1
Cycas_guizhouensis	VU	1	0	1	1	0	0	0	0	0	3	10.80662	NA	400	1300	T	NA	1	40	0.1	0.15	3
Cycas_hainanensis	EN	1	1	0	0	0	0	0	0	0	2	34.0329	NA	0	1200	T	0.3	3.5	40	NA	0.3	1
Cycas_hoabinhensis	EN	0	0	0	1	0	0	0	0	0	1	24.43046	NA	50	150	T	NA	0.6	40	0.05	0.08	4
Cycas_hongheensis	CR	0	0	0	1	0	1	0	0	0	2	28.09396	NA	400	600	T	1	3	40	0.12	0.15	1
Cycas_inermis	VU	1	0	0	1	0	0	0	0	0	2	37.99666	NA	NA	NA	T	1.5	4	40	0.08	0.14	4
Cycas_javana	EN	1	0	0	0	0	0	0	0	0	1	28.4363	NA	NA	NA	T	2	4	40	0.15	0.2	3
Cycas_lacrimans	EN	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	14.358	NA	NA	NA	T	1	2	NA	NA	NA	1
Cycas_lane-poolei	LC	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	14.37567	NA	300	370	NT	5	8	40	0.12	0.15	1
Cycas_lindstromii	EN	1	0	0	1	0	0	0	0	0	2	20.23621	4280	0	30	T	NA	NA	40	0.05	0.08	4
Cycas_litoralis	NT	1	0	0	0	0	0	0	0	0	1	12.58177	1000	NA	NA	NT	NA	NA	40	NA	NA	NA

Cycas_maconochiei	LC	0	0	0	0	0	0	0	1	1	2	15.93741	NA	0	40	NT	3	7	40	0.09	0.15	1
Cycas_macrocarpa	VU	1	0	0	0	0	0	0	0	0	1	45.82549	NA	NA	NA	T	NA	12	40	NA	NA	6
Cycas_media_ensata	LC	1	0	0	0	0	0	1	0	0	2	15.78573	NA	0	860	NT	3	6	40	0.1	0.18	1
Cycas_media_media	LC	1	0	0	0	0	0	1	0	0	2	17.49625	NA	0	860	NT	3	6	40	0.1	0.18	NA
Cycas_megacarpa	VU	1	0	0	0	0	0	0	0	0	1	18.53765	NA	150	300	T	3	6	40	0.08	0.14	1
Cycas_micholitzii	VU	1	0	0	0	0	0	0	0	0	1	36.43868	NA	130	600	T	NA	NA	40	0.1	0.15	3
Cycas_micronesica	EN	1	0	0	0	0	0	1	1	0	3	10.58724	11250000	NA	NA	T	8	12	40	0.14	0.25	1
Cycas_miquellii	LC	1	0	0	0	0	1	0	0	0	2	21.02826	NA	0	300	NT	NA	NA	NA	NA	NA	NA
Cycas_montana	NT	1	0	0	1	0	0	0	1	0	3	22.60174	NA	NA	NA	NT	NA	1.5	NA	0.3	0.35	1
Cycas_multipinnata	EN	1	0	0	1	0	0	0	0	0	2	18.43632	27040	200	1300	T	NA	NA	40	0.14	0.25	2
Cycas_nathorstii	VU	1	0	1	0	0	0	0	0	0	2	14.358	NA	30	300	T	NA	4.5	40	0.11	0.2	2
Cycas_nitida	NT	1	0	0	0	0	0	0	0	0	1	25.68127	NA	NA	NA	NT	NA	NA	NA	NA	NA	1
Cycas_nongnoochiae	VU	0	0	0	1	0	0	0	0	1	2	19.79711	NA	50	100	T	NA	5	NA	0.1	0.15	1
Cycas_ophiolitica	VU	1	0	0	1	0	0	1	0	0	3	14.90585	NA	150	250	T	2	7	40	0.14	0.2	1
Cycas_orientis	LC	0	0	0	0	0	0	0	0	1	1	28.0939	NA	NA	NA	NT	4	7	40	0.08	0.14	1

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Cycas_pachypoda	CR	0	1	0	1	0	0	0	0	0	2	19.21076	NA	NA	NA	T	0.5	1.5	40	0.12	0.17	2
Cycas_panzhihuaensis	VU	1	0	1	1	0	0	0	0	0	3	30.05926	14500	1100	2000	T	1	3	40	0.15	0.2	2
Cycas_papuana	NT	0	0	0	0	0	0	0	0	1	1	21.47898	NA	NA	NA	NT	NA	2.8	40	NA	NA	2
Cycas_pectinata_A	VU	0	0	1	0	0	0	0	0	0	1	13.66021	NA	600	1300	T	1	12	40	0.14	0.2	17
Cycas_pectinata_B	VU	0	0	1	0	0	0	0	0	0	1	17.56073	NA	600	1300	T	1	12	40	0.14	0.2	17
Cycas_petraea	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	24.43046	60	NA	NA	NT	NA	6	40	0.15	0.2	1
Cycas_platyphylla	EN	0	0	0	1	0	0	0	0	0	1	18.53765	NA	400	750	T	2	4	40	0.1	0.15	1
Cycas_pranburiensis	VU	0	0	0	1	0	0	0	0	0	1	43.33355	NA	5	30	T	1	3	40	0.08	0.1	1
Cycas_pruinosa	LC	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	29.1319	NA	NA	NA	NT	1.5	2.5	40	0.15	0.35	1
Cycas_revoluta	LC	1	0	0	0	0	1	0	0	0	2	23.2936	NA	0	300	NT	0.5	2	40	NA	0.2	1
Cycas_riuminiana	EN	1	0	0	0	0	0	0	0	0	1	14.32454	NA	615	800	T	NA	NA	40	NA	NA	1
Cycas_rumphii	NT	1	0	0	0	0	0	0	0	0	1	14.37567	NA	10	200	NT	3	10	40	0.11	0.2	11
Cycas_saxatilis	VU	1	0	0	0	0	0	0	0	0	1	19.20191	NA	NA	NA	T	0.5	4	40	NA	NA	1
Cycas_schumanniana	NT	1	0	0	0	0	0	0	0	1	2	12.8756	NA	NA	1600	NT	NA	2	40	0.15	0.2	4

Cycas_scratchleyana	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	14.13015	NA	5	900	NT	4	7	40	0.12	0.2	7
Cycas_seemanii	VU	1	0	0	0	0	0	0	0	0	1	21.69839	NA	0	600	T	4	10	40	0.1	0.2	5
Cycas_segmentifida	VU	0	1	0	0	0	0	0	0	0	1	36.43868	NA	600	900	T	NA	0.5	40	0.1	0.23	4
Cycas_semota	NT	0	0	0	1	0	0	0	0	0	1	30.74311	NA	NA	NA	NT	NA	5	40	NA	NA	1
Cycas_sexseminifera	VU	0	0	0	1	0	0	0	0	0	1	14.11838	NA	NA	NA	T	NA	0.6	40	0.06	0.15	4
Cycas_shanyaensis	VU	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	12.51481	10	700	800	T	2.1	3.1	40	0.2	0.25	1
Cycas_siamensis	VU	1	0	0	1	0	0	0	0	1	3	11.14557	NA	NA	300	T	NA	1.5	40	0.14	0.2	20
Cycas_silvestris	VU	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	20.74771	NA	NA	NA	T	0.03	0.04	40	0.1	0.15	1
Cycas_simplicipinna	NT	1	0	0	0	0	0	0	0	0	1	29.82684	NA	600	1300	NT	NA	NA	40	0.08	0.14	5
Cycas_sundaica	LC	1	0	0	1	0	0	0	1	0	3	14.11838	NA	NA	NA	NT	NA	0.05	40	0.2	0.35	1
Cycas_szechuanensis	CR	1	0	0	1	0	0	0	0	0	2	42.39772	NA	NA	NA	T	NA	2	40	0.15	0.25	2
Cycas_taitungensis	EN	0	0	0	1	0	1	0	0	0	2	25.68127	65	400	900	T	0.03	0.06	40	0.25	0.3	1
Cycas_taiwaniana	EN	1	0	0	1	0	0	0	0	0	2	60.36198	NA	400	1100	T	NA	3.5	40	0.15	0.3	1
Cycas_tanqingii	NT	1	0	0	0	0	0	0	0	0	1	20.61617	80	NA	800	NT	NA	2	40	0.25	0.3	2

Cycas_tansachana	CR	1	0	0	1	0	0	0	0	0	2	15.77084	10	NA	400	T	2	5	40	0.1	0.18	1
Cycas_terryana	VU	1	0	0	0	0	0	0	0	0	1	23.2936	NA	NA	NA	T	NA	NA	40	NA	NA	1
Cycas_thouarsii	LC	1	0	0	1	0	0	0	0	0	2	18.7099	NA	0	200	NT	NA	4	40	NA	0.1	6
Cycas_tropophylla	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	51.24649	400	NA	NA	NT	NA	1	40	0.08	0.15	2
Cycas_tuckeri	VU	1	0	0	0	0	0	0	0	1	2	19.79711	15	NA	NA	T	NA	5	40	NA	NA	1
Cycas_vespertilio	NT	0	1	0	0	0	0	0	0	0	1	28.4363	NA	NA	NA	NT	1	3	40	NA	NA	6
Cycas_wadei	CR	1	0	0	1	0	0	0	0	1	3	19.06555	NA	20	50	T	NA	5	40	0.1	0.2	1
Cycas_xipholepis	LC	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	15.93741	NA	NA	NA	NT	NA	6	40	0.1	0.15	1
Cycas_yorkiana	NT	1	0	0	0	0	0	0	0	1	2	15.78573	11530	NA	NA	NT	NA	4	40	NA	NA	1
Cycas_zambalensis	CR	1	0	1	0	0	0	1	0	1	4	20.19838	NA	NA	NA	T	NA	3	40	NA	NA	1
Cycas_zeylanica	VU	1	0	0	0	0	0	0	0	0	1	10.58724	NA	5	50	T	2.3	3.1	40	0.13	0.2	2
Dioon_angustifolium	VU	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	18.87281	NA	NA	NA	T	NA	NA	500	NA	NA	2
Dioon_argenteum	VU	1	0	0	0	0	0	0	0	0	1	20.82433	350	1100	1600	T	NA	3	500	0.18	0.32	1
Dioon_califanoi	EN	1	0	0	1	0	0	0	0	1	3	24.654	126	NA	NA	T	NA	3	500	NA	0.3	2
Dioon_caputoi	EN	1	0	0	1	0	0	0	0	0	2	60.02895	NA	NA	NA	T	NA	1	500	NA	0.25	2
Dioon_edule	NT	1	0	0	1	0	0	0	0	0	2	20.6160	NA	NA	NA	NT	NA	3	700	NA	0.3	5

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Dioon_holmgrenii	EN	1	0	0	1	0	0	0	0	0	2	21.43385	NA	NA	NA	T	NA	6	500	NA	0.4	1
Dioon_mejiae	LC	1	1	0	0	0	0	0	0	0	2	22.89612	NA	NA	NA	NT	NA	1	500	NA	0.25	3
Dioon_merolae	VU	0	0	1	1	0	0	0	1	0	3	22.43078	NA	NA	NA	T	NA	3	500	0.25	0.4	2
Dioon_purpusii	VU	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	21.43385	NA	1000	1500	T	NA	5	500	NA	0.4	1
Dioon_rzedowskii	EN	1	0	0	0	0	0	0	0	0	1	20.82433	25	NA	NA	T	NA	5	500	0.25	0.4	1
Dioon_sonorense	EN	0	0	1	1	1	0	0	0	0	3	22.43078	NA	NA	NA	T	NA	NA	500	NA	NA	2
Dioon_spinulosum	EN	1	0	0	1	0	0	0	0	0	2	58.81312	NA	NA	NA	T	5	16	500	NA	0.4	2
Dioon_stevensonii	CR	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	20.61607	NA	NA	NA	Td	NA	NA	NA	NA	NA	2
Dioon_tomasellii	VU	1	0	0	1	0	0	0	0	0	2	18.87281	NA	600	1850	T	NA	1	500	NA	NA	3
Encephalartos chimanimaniensis	EN	0	0	0	1	0	0	0	0	0	1	26.60507	NA	600	1100	T	NA	1.8	70	NA	0.45	2
Encephalartos fridericiguilielmi	NT	0	0	1	1	0	0	0	0	0	2	21.40559	NA	0	600	T	NA	4	70	0.4	0.6	2
Encephalartos_aemulans	CR	1	0	0	1	0	0	0	0	0	2	19.12329	295	100	600	T	NA	3	70	NA	0.35	1
Encephalartos_altensteinii	VU	1	0	1	1	0	0	0	0	0	3	12.85207	450	100	200	T	4	7	70	0.25	0.35	1
Encephalartos_aplan	VU	0	0	0	1	0	1	0	0	0	2	21.0218	NA	400	1400	T	NA	NA	35	NA	NA	1

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Encephalartos_arenarius	EN	1	0	0	1	0	0	0	0	0	2	23.34009	140	1300	1500	EW	NA	1	70	0.2	0.3	1
Encephalartos_barteri	VU	0	0	0	1	1	0	0	0	1	3	15.62878	NA	1300	2150	NT	0.3	2.6	70	0.25	0.6	3
Encephalartos_brevifoliolatus	EW	0	0	0	1	0	0	0	0	0	1	53.41136	NA	300	700	NT	NA	2.5	70	0.25	0.3	1
Encephalartos_bubalinus	NT	0	0	0	0	0	1	0	0	0	1	22.75749	NA	500	900	T	NA	2	70	NA	0.45	2
Encephalartos_caffer	NT	1	0	0	1	0	0	0	0	0	2	14.30221	NA	NA	1000	T	0.3	0.4	35	0.2	0.25	1
Encephalartos_cerinus	CR	0	0	0	1	0	1	0	0	0	2	17.17823	NA	800	900	T	NA	0.3	35	NA	0.25	1
Encephalartos_concinnus	EN	0	0	1	1	0	0	0	0	0	2	34.9623	58	700	1500	T	NA	3	70	NA	0.45	2
Encephalartos_cupidus	CR	0	0	0	1	1	1	0	0	1	4	47.18981	290	1200	1800	NT	NA	0.75	200	0.2	0.3	1
Encephalartos_cycadifolius	LC	0	0	0	0	0	1	0	0	1	2	13.48624	NA	1200	1950	T	0.5	1.5	500	NA	0.25	1
Encephalartos_delucanus	EN	1	0	0	1	0	0	0	0	1	3	30.92973	NA	1100	1500	T	NA	0.12	70	0.1	0.2	1
Encephalartos_dolomiticus	CR	0	0	0	1	0	1	0	0	0	2	47.18981	0.3	NA	700	T	NA	2	70	NA	0.4	1
Encephalartos_dyerianus	CR	0	0	0	1	0	1	0	0	0	2	12.85207	5	NA	1000	T	NA	4	70	NA	0.6	1
Encephalartos_equatorialis	CR	0	0	0	1	0	1	0	0	1	3	28.26662	NA	1400	1500	T	NA	6	70	0.4	0.6	1
Encephalartos_eugen	EN	0	0	0	1	0	1	0	0	0	2	35.1810	NA	20	100	NT	2.5	4	70	0.3	0.45	1

emaraisii												8										
Encephalartos_ferox	NT	1	0	0	1	0	0	0	0	0	2	19.8147	NA	700	1400	NT	1	2	70	NA	0.3	2
Encephalartos_ghellincki	VU	0	0	0	1	0	0	0	0	1	2	19.8147	NA	700	2400	T	NA	3	70	0.3	0.4	2
Encephalartos_gratus	VU	1	0	0	0	0	0	0	0	0	1	52.39442	NA	650	900	T	NA	2.5	70	NA	0.6	2
Encephalartos_heenanii	CR	1	0	0	1	0	1	0	0	1	4	17.77268	300	750	1750	T	2	4	70	0.25	0.35	2
Encephalartos_hildebrandtii	NT	1	0	1	0	0	1	0	0	0	3	16.6309	NA	0	600	NT	NA	6	70	NA	0.6	5
Encephalartos_hirsutus	CR	0	0	0	1	0	0	0	0	0	1	23.22916	NA	800	1000	T	NA	4	70	0.35	0.4	1
Encephalartos_horridus	EN	1	0	0	1	0	0	0	0	0	2	13.90459	NA	100	400	T	NA	0.3	200	0.2	0.3	1
Encephalartos_humilis	VU	1	0	0	0	0	0	0	0	1	2	24.40141	NA	NA	NA	T	0.35	0.5	NA	0.13	0.18	1
Encephalartos_inopinus	CR	0	0	0	1	0	1	0	0	0	2	46.26647	NA	600	800	T	2	3	200	0.17	0.25	1
Encephalartos_ituriensis	NT	0	0	0	1	0	0	0	0	0	1	27.00078	NA	1100	1200	NT	NA	6	70	NA	0.5	2
Encephalartos_kisambo	EN	1	0	1	1	0	0	0	0	0	3	11.67276	NA	800	1800	T	NA	4	70	NA	0.6	1
Encephalartos_laevifolius	CR	1	1	1	1	0	0	0	0	0	4	24.40141	NA	950	1800	T	3	4	70	0.25	0.35	5
Encephalartos_lanatus	NT	1	1	0	0	0	0	0	0	0	2	34.20526	NA	1200	1500	NT	1	2	70	0.25	0.3	1
Encephalartos_latifrons	CR	1	0	0	1	0	1	0	0	0	3	22.23855	NA	200	600	T	0.025	3	100	NA	NA	1

Encephalartos_laurentianus	NT	0	0	0	1	0	0	0	0	0	1	17.44729	NA	450	550	NT	NA	15	70	NA	1	2
Encephalartos_lebomboensis	EN	0	1	1	1	0	0	0	0	0	3	20.10179	NA	500	1000	T	3	5	70	NA	0.3	3
Encephalartos_lehmannii	NT	0	0	0	1	1	0	1	0	0	3	21.01598	NA	400	1000	NT	1.5	3	200	0.25	0.45	1
Encephalartos_longifolius	NT	1	0	0	0	0	0	0	0	0	1	30.9371	NA	200	700	NT	3	4	70	0.3	0.4	1
Encephalartos_mackenziei	NT	0	0	0	1	0	0	0	0	0	1	24.78282	NA	1800	2000	NT	1.5	2.5	70	NA	NA	1
Encephalartos_macrostrobilus	EN	0	1	0	0	0	0	1	0	0	2	13.7809	50	900	1400	T	NA	2.5	70	0.3	0.4	1
Encephalartos_manikensis	VU	0	0	0	1	0	0	0	0	0	1	16.90228	NA	600	1400	T	NA	1.5	70	NA	0.3	2
Encephalartos_marunguensis	VU	0	0	0	1	0	0	0	0	1	2	27.00078	7500	1400	1700	T	NA	0.4	70	NA	0.15	1
Encephalartos_middleburgensis	CR	1	0	0	1	0	1	0	0	1	4	34.9623	NA	1100	1400	T	NA	7	70	0.3	0.45	1
Encephalartos_msinganus	CR	1	0	0	1	0	1	0	0	0	3	17.77268	10	900	1200	T	NA	3	70	NA	0.35	1
Encephalartos_munchii	CR	0	0	0	1	0	1	0	0	0	2	13.48624	3	1000	1100	T	NA	1	70	NA	0.35	1
Encephalartos_natalensis	NT	0	0	1	1	0	0	0	0	0	2	21.40559	NA	200	1200	NT	3	6.5	70	0.25	0.4	1
Encephalartos_ngoyanus	VU	0	0	0	1	0	0	1	0	0	2	53.41136	NA	200	600	T	NA	0.3	35	NA	0.2	2
Encephalartos_nubimontanus	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	34.26229	NA	NA	1000	EW	NA	2.5	NA	0.35	0.4	1

Encephalartos_paucidentatis	VU	1	0	0	1	0	0	0	0	0	2	23.34009	424	1000	1500	T	6	7	70	0.4	0.7	2
Encephalartos_poggei	LC	0	0	1	0	0	0	0	0	0	1	30.92973	NA	500	1000	NT	NA	2	70	NA	0.3	3
Encephalartos_princeps	VU	1	0	0	1	0	0	0	0	0	2	22.75749	1870	200	800	T	3	5	70	0.3	0.4	1
Encephalartos_pterogononus	CR	0	0	0	1	0	1	0	0	0	2	24.90164	35	700	1000	T	NA	1.5	70	NA	0.4	1
Encephalartos_relictus	EW	0	0	0	1	0	0	0	0	0	1	12.56742	NA	400	600	EW	NA	NA	NA	NA	NA	1
Encephalartos_schaijesii	VU	0	0	0	0	0	1	0	0	1	2	12.56742	NA	1450	1500	T	0.11	0.25	500	0.2	0.33	1
Encephalartos_schmitzii	VU	1	0	0	1	0	0	0	0	1	3	19.82233	NA	1000	1400	T	NA	0.3	500	NA	0.2	2
Encephalartos_sclavoi	CR	1	0	1	1	0	1	0	0	1	5	11.67276	NA	1800	2100	T	NA	1	70	NA	0.35	1
Encephalartos_senticosus	VU	0	0	0	1	0	0	0	0	0	1	20.10179	NA	300	800	T	NA	4	70	NA	0.3	2
Encephalartos_septentrionalis	NT	1	0	0	1	1	0	0	0	0	3	13.7809	NA	500	2500	NT	NA	2.5	70	NA	0.75	2
Encephalartos_tegulaneus	LC	0	0	1	1	0	0	0	0	0	2	13.12263	NA	1400	2300	NT	NA	10	70	NA	0.6	1
Encephalartos_transvenosus	LC	1	0	0	1	0	0	0	0	0	2	23.22916	NA	600	1500	NT	5	13	70	0.4	0.5	1
Encephalartos_trispinosus	VU	0	0	0	1	0	0	0	0	0	1	13.90459	NA	100	600	T	NA	1	NA	0.25	0.3	1
Encephalartos_turneri	LC	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	45.29299	NA	600	1200	NT	NA	3	70	NA	0.8	1

Encephalartos_umbeluziensis	EN	0	0	0	1	0	0	0	0	0	1	28.26662	336	50	120	T	NA	0.3	35	0.2	0.25	2
Encephalartos_villosus	LC	1	0	0	1	0	0	0	0	0	2	19.12329	NA	100	600	NT	NA	0.3	35	NA	0.2	3
Encephalartos_whitelockii	CR	1	0	0	1	0	0	1	0	0	3	34.44208	NA	1000	1300	T	NA	4	70	0.35	0.4	1
Encephalartos_woodii	EW	1	0	0	0	0	0	0	0	0	1	30.9371	NA	NA	NA	EW	3	6	NA	0.4	0.6	1
Lepidozamia_hopei	LC	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	43.35002	NA	0	1000	NT	NA	17	100	NA	0.5	1
Lepidozamia_peroffskyana	LC	1	0	0	0	0	0	0	0	0	1	43.35002	NA	0	1000	NT	4	7	70	NA	0.8	1
Macrozamia_cardiacensis	VU	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	15.18195	14	500	640	T	NA	0.4	60	0.2	0.4	1
Macrozamia_communis	LC	1	0	0	0	0	0	0	0	0	1	15.78572	NA	0	300	NT	NA	1.5	60	0.4	0.9	1
Macrozamia_concinna	LC	0	0	0	0	0	0	0	0	1	1	15.18301	NA	800	1100	NT	NA	NA	60	0.08	0.15	1
Macrozamia_conferta	VU	0	0	0	1	0	0	0	0	0	1	32.28496	423	600	750	T	NA	NA	60	0.15	0.3	1
Macrozamia_cranei	EN	1	0	0	0	0	0	0	0	0	1	54.65748	NA	400	600	T	NA	NA	60	0.1	0.25	1
Macrozamia_crassifolia	VU	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	32.28496	160	340	420	T	NA	NA	60	0.1	0.2	1
Macrozamia_diplomera	LC	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	24.60612	NA	NA	500	NT	NA	NA	60	0.2	0.4	1
Macrozamia_douglasii	LC	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	26.54739	NA	0	150	NT	NA	0.6	60	0.4	0.7	1

Macrozamia_dyeri	LC	0	0	0	0	0	0	0	0	1	1	28.07962	NA	NA	NA	NT	0.4	3	60	0.5	1.2	1
Macrozamia_elegans	EN	1	0	0	1	0	0	0	0	0	2	33.38726	112	120	150	T	NA	NA	60	0.15	0.3	1
Macrozamia_fawcettii	NT	0	0	0	1	0	0	0	0	0	1	32.3706	5500	5	550	NT	NA	NA	60	0.1	0.2	1
Macrozamia_fearnsidei	LC	1	0	0	0	0	0	0	0	0	1	13.06859	NA	300	600	NT	NA	NA	60	0.15	0.35	1
Macrozamia_flexuosa	EN	1	0	0	1	0	0	0	0	0	2	22.74608	NA	NA	NA	T	NA	NA	60	0.08	0.2	1
Macrozamia_fraseri	LC	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	29.77538	NA	NA	NA	NT	NA	3	60	0.4	0.7	1
Macrozamia_glaucophylla	LC	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	24.76862	NA	NA	NA	NT	NA	NA	60	0.2	0.4	1
Macrozamia_heteromera	LC	0	0	0	1	0	0	0	0	0	1	15.2723	NA	NA	200	NT	NA	NA	60	0.5	0.9	1
Macrozamia_humilis	VU	1	0	0	0	0	0	0	0	0	1	45.99116	NA	NA	600	T	NA	NA	60	0.18	0.28	1
Macrozamia_johnsonii	LC	0	1	0	0	0	0	0	0	0	1	54.65748	222	NA	NA	NT	0.3	3	60	0.5	0.9	1
Macrozamia_lomandroides	EN	1	0	0	1	0	0	0	0	0	2	12.53673	NA	NA	NA	T	NA	NA	60	0.1	0.17	1
Macrozamia_longispina	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	23.27557	50	200	700	NT	NA	0.3	60	0.2	0.3	1
Macrozamia_lucida	LC	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	26.54739	NA	30	600	NT	NA	NA	60	0.08	0.2	1
Macrozamia_macdonnelli	LC	0	0	0	1	0	1	0	0	0	2	15.01719	NA	NA	NA	NT	0.4	3	60	0.6	0.8	1

Macrozamia_machinii	VU	0	0	0	1	0	0	0	0	0	1	33.38726	460	320	460	T	NA	NA	60	0.2	0.3	1
Macrozamia_macleayi	LC	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	12.53673	NA	100	500	NT	NA	0.4	60	0.3	0.4	1
Macrozamia_miquelii	LC	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	13.06859	NA	0	500	NT	NA	NA	60	0.2	0.4	1
Macrozamia_montana	LC	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	13.9143	NA	NA	NA	NT	NA	0.6	60	0.25	0.45	1
Macrozamia_moorei	NT	1	0	0	0	0	0	0	0	0	1	16.16684	NA	300	500	NT	2	7	60	0.5	0.8	1
Macrozamia_mountperriensis	LC	1	0	0	0	0	0	0	0	0	1	27.25841	NA	50	400	NT	NA	NA	60	0.25	0.4	1
Macrozamia_occidua	VU	0	0	0	1	0	0	0	0	0	1	15.18301	10	800	1000	T	NA	NA	60	0.1	0.2	1
Macrozamia_parcifolia	VU	0	0	0	1	0	1	0	0	1	3	15.17074	NA	60	220	T	NA	NA	60	0.1	0.2	1
Macrozamia_pauliguilielmi	EN	1	0	0	1	0	0	0	0	0	2	18.55799	NA	5	25	T	NA	NA	60	0.1	0.2	1
Macrozamia_platyrhachis	VU	1	0	0	0	0	1	0	0	0	2	15.78572	NA	NA	NA	T	NA	NA	60	0.25	0.6	1
Macrozamia_plurinervia	EN	1	0	0	1	0	1	0	0	1	4	15.7715	NA	NA	NA	T	NA	NA	60	0.2	0.3	1
Macrozamia_polymorpha	LC	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	18.55799	NA	NA	NA	NT	NA	NA	60	0.1	0.25	1
Macrozamia_reducta	LC	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	13.9143	NA	NA	NA	NT	NA	0.4	60	0.2	0.4	1
Macrozamia_riedlei	LC	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	17.26147	NA	NA	NA	NT	NA	0.3	60	0.25	0.4	1

Macrozamia_secunda	VU	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	24.76862	NA	NA	NA	T	NA	NA	60	0.08	0.15	1
Macrozamia_serpentine	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	15.18195	850	NA	NA	NT	NA	NA	60	NA	NA	1
Macrozamia_spiralis	EN	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	25.61488	NA	NA	NA	T	NA	NA	60	0.08	0.2	1
Macrozamia_stenomera	NT	1	0	0	1	0	0	0	0	0	2	22.74608	NA	NA	NA	NT	NA	NA	60	0.1	0.25	1
Macrozamia_viridis	EN	1	0	0	0	0	0	0	0	1	2	15.17074	1000	NA	NA	T	NA	NA	60	0.1	0.2	1
Microcycas_calocoma	CR	1	0	1	0	0	1	0	0	0	3	98.76153	NA	NA	NA	T	NA	10	100	NA	0.6	1
Stangeria_eriopus	VU	1	1	1	1	0	0	1	0	0	5	86.06692	NA	10	750	T	NA	NA	30	NA	NA	2
Zamia_acuminata	VU	1	0	0	0	0	0	0	0	0	1	32.43504	NA	NA	NA	T	NA	0.4	30	0.07	0.08	3
Zamia_amazonum	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	18.789	NA	NA	NA	NT	NA	2.5	30	0.03	0.08	7
Zamia_amblyphyllidia	VU	1	0	0	1	0	0	0	0	0	2	14.68817	NA	NA	NA	T	NA	NA	30	NA	0.2	NA
Zamia_amplifolia	CR	1	0	0	0	0	0	0	0	0	1	21.18936	NA	NA	NA	T	NA	2.5	30	NA	NA	1
Zamia_angustifolia	VU	1	0	0	0	0	0	0	0	0	1	28.15189	9000	NA	NA	T	NA	NA	30	NA	NA	4
Zamia_boliviana	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	26.80184	NA	NA	NA	NT	NA	NA	30	0.03	0.1	2
Zamia_chigua	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	27.525	NA	NA	NA	NT	NA	2	30	NA	0.15	2

Zamia_cremnophila	EN	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	16.38093	53	NA	NA	T	10	25	30	0.03	0.09	1
Zamia_cunaria	VU	1	0	0	0	0	0	0	0	0	1	39.96309	3140	NA	NA	T	NA	NA	NA	NA	0.1	4
Zamia_decumbens	CR	1	0	0	1	0	0	0	0	0	2	28.15189	NA	NA	NA	T	NA	80	NA	0.067	0.11	3
Zamia_disodon	CR	1	0	0	0	0	0	0	0	0	1	16.34563	NA	NA	NA	T	NA	NA	30	0.05	0.08	1
Zamia_dressleri	EN	1	0	0	0	0	0	0	0	0	1	28.07089	2530	NA	NA	T	NA	NA	30	0.03	0.05	2
Zamia_elegantissima	EN	1	0	0	0	0	0	0	0	0	1	19.60963	100	NA	NA	T	NA	NA	30	NA	NA	2
Zamia_encephalartoides	VU	1	0	0	0	0	0	0	0	0	1	25.66209	266	NA	NA	T	NA	2	30	NA	0.25	1
Zamia_fairchildiana	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	24.51522	NA	NA	NA	NT	0.5	1	30	0.06	0.15	3
Zamia_fischeri	EN	1	0	0	0	0	0	0	0	0	1	25.79944	2770	NA	NA	T	NA	NA	30	0.02	0.08	4
Zamia_furfuracea_A	EN	1	0	0	0	0	1	0	0	0	2	33.70254	630	NA	NA	T	NA	NA	30	NA	0.2	1
Zamia_furfuracea_B	EN	0	0	0	1	0	0	0	0	0	1	21.53129	631	NA	NA	T	NA	NA	30	NA	0.2	1
Zamia_gentryi	CR	1	0	0	0	0	0	0	0	0	1	26.08393	5	NA	NA	T	NA	1.5	30	0.05	0.15	2
Zamia_gomeziana	VU	1	0	0	0	0	1	0	0	0	2	25.3669	NA	NA	NA	T	NA	NA	NA	NA	NA	1
Zamia_hamannii	CR	1	0	1	0	0	0	0	0	0	2	29.18057	NA	NA	NA	T	NA	2.4	NA	0.075	0.2	1
Zamia_herrerae	VU	1	0	0	0	0	0	0	0	0	1	52.4231	NA	NA	NA	T	NA	NA	30	0.03	0.1	6

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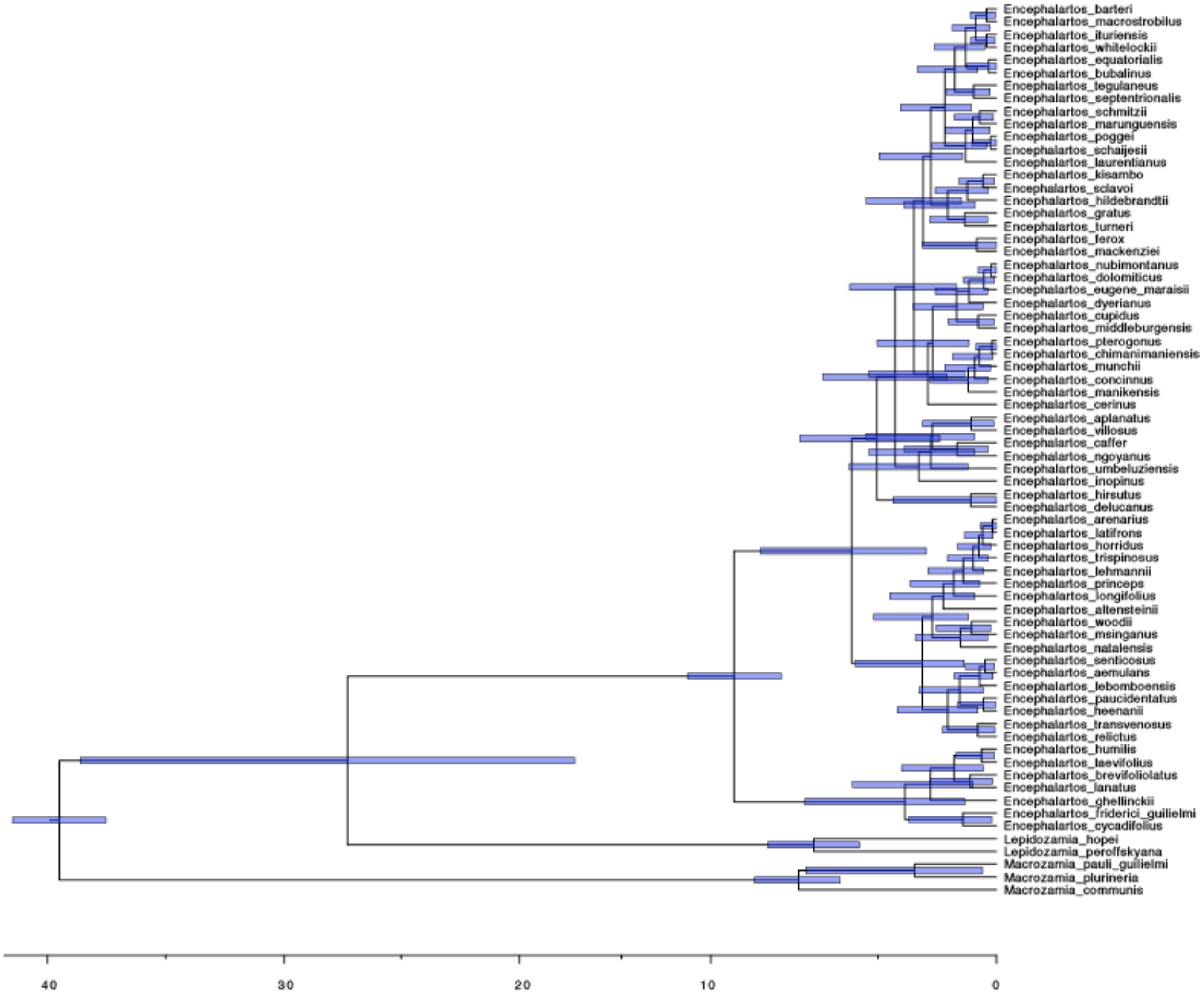
Zamia_hymenophyllidia	CR	1	0	0	0	0	0	0	0	0	1	34.88073	NA	NA	NA	T	NA	NA	30	0.02	0.04	2
Zamia_imperialis	CR	1	0	0	1	0	0	0	0	0	2	34.74487	NA	NA	NA	T	NA	1.1	NA	NA	0.22	3
Zamia_incognita	VU	1	1	0	0	0	0	0	0	0	2	14.74109	NA	NA	NA	T	NA	NA	NA	NA	NA	3
Zamia_inermis	CR	1	0	0	1	0	1	0	0	1	4	26.59268	NA	NA	NA	T	0.7	1.5	30	0.2	0.25	1
Zamia_integrifolia	NT	1	0	0	1	0	0	0	0	0	2	32.54882	NA	NA	NA	NT	NA	1.3	30	NA	0.06	5
Zamia_ipetiensis	EN	1	0	0	0	0	0	0	0	0	1	26.5222	50	NA	NA	T	NA	NA	30	NA	0.1	2
Zamia_katzeriana	EN	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	34.87335	NA	NA	NA	T	NA	NA	30	NA	NA	3
Zamia_kickxii	CR	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	14.68817	NA	NA	NA	T	NA	NA	30	NA	NA	NA
Zamia_lacandona	EN	1	0	0	0	0	0	0	0	1	2	38.76277	3400	NA	NA	T	0.15	0.6	30	0.045	0.08	1
Zamia_lawsoniana	NT	1	0	0	0	0	0	1	0	0	2	33.70254	NA	NA	NA	NT	NA	NA	30	NA	NA	NA
Zamia_lecointei	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	22.97657	NA	NA	NA	NT	NA	NA	30	0.05	0.1	4
Zamia_lindenii	NT	1	1	0	0	0	0	0	0	0	2	21.50499	NA	NA	NA	NT	NA	4	30	0.1	0.3	10
Zamia_loddigesii	NT	1	0	0	0	0	0	1	0	0	2	38.73882	NA	NA	NA	NT	NA	0.3	30	NA	0.12	6
Zamia_lucayana	EN	1	0	0	0	0	0	0	0	0	1	27.68497	13	NA	NA	T	NA	NA	30	NA	NA	2

Zamia_macrochiera	CR	1	0	0	0	0	0	0	0	0	1	45.24072	NA	NA	NA	T	NA	NA	30	0.1	0.2	1
Zamia_manicata	NT	1	0	0	0	0	0	0	0	0	1	29.18057	NA	NA	NA	NT	NA	NA	30	0.02	0.05	3
Zamia_meermanii	EN	1	0	0	1	0	0	0	0	1	3	27.525	NA	NA	NA	T	NA	NA	NA	NA	NA	2
Zamia_melanorrhachis	EN	1	0	0	0	0	0	0	0	0	1	16.38093	NA	NA	NA	T	NA	NA	30	0.05	0.08	3
Zamia_montana	CR	1	1	0	0	0	0	0	0	0	2	23.94414	NA	NA	NA	T	0.5	1.5	30	0.1	0.2	2
Zamia_monticola	CR	0	1	0	0	0	0	0	0	0	1	31.85847	NA	NA	NA	T	NA	0.3	30	0.15	0.2	1
Zamia_muricata	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	21.18936	NA	NA	NA	NT	NA	0.15	30	0.03	0.08	8
Zamia_nesophila	CR	1	0	0	1	0	0	0	0	0	2	31.01836	NA	NA	NA	T	NA	2.8	NA	0.06	0.24	1
Zamia_neurophyllidia	VU	0	0	0	0	1	0	0	0	0	1	48.38625	NA	NA	NA	T	0.6	2	30	0.05	0.12	3
Zamia_obliqua	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	19.60963	NA	NA	NA	NT	0.5	5	30	0.05	0.12	4
Zamia_onan-reyesii	CR	1	0	0	1	0	0	0	0	0	2	31.72165	NA	NA	NA	T	NA	NA	NA	NA	NA	1
Zamia_oreillyi	VU	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	17.15522	NA	NA	NA	T	NA	NA	30	NA	NA	1
Zamia_paucijuga	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	26.08393	NA	NA	NA	NT	NA	NA	30	NA	0.08	6
Zamia_picta	EN	1	0	0	0	0	0	0	0	0	1	25.883	NA	NA	NA	T	NA	NA	30	NA	NA	NA
Zamia_poeppigiana	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	19.07345	NA	NA	NA	NT	NA	3	30	0.1	0.3	7

Zamia_portoricensis	EN	0	0	0	0	0	1	0	0	0	1	16.55332	220	NA	NA	T	NA	NA	30	NA	0.15	1
Zamia_prasina	CR	1	0	0	0	0	0	0	0	0	1	33.45521	NA	NA	NA	T	NA	0.3	30	NA	0.1	11
Zamia_pseudomonticola	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	21.7473	NA	NA	NA	NT	NA	0.3	30	0.05	0.07	2
Zamia_pseudoparasitica	NT	1	1	0	0	0	0	0	0	0	2	34.88073	NA	NA	NA	NT	NA	1	30	NA	0.15	4
Zamia_pumila	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	25.78335	NA	NA	NA	NT	NA	NA	30	0.03	0.25	3
Zamia_purpurea	CR	1	0	0	0	0	0	0	0	0	1	23.94414	NA	NA	NA	T	NA	0.5	30	NA	0.04	2
Zamia_pygmaea	CR	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	24.13965	NA	NA	NA	T	NA	0.02	30	NA	0.04	2
Zamia_pyrophylla	CR	1	0	0	0	0	0	0	0	0	1	25.3669	NA	NA	NA	T	NA	NA	NA	NA	0.1	1
Zamia_restrepoi	CR	1	0	0	1	1	0	0	0	0	3	16.34563	NA	75	150	T	NA	NA	30	NA	NA	1
Zamia_roezlii	NT	0	0	1	0	0	0	0	0	0	1	17.15522	NA	NA	NA	NT	NA	7	30	NA	NA	6
Zamia_sandovalii	NT	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	25.79944	NA	NA	NA	NT	NA	NA	30	NA	NA	1
Zamia_skinneri	EN	1	0	0	0	0	0	0	0	0	1	47.67762	6250	NA	NA	T	1.2	2.4	30	0.075	0.2	1
Zamia_soconuscensis	VU	1	0	0	0	0	0	0	0	0	1	21.53129	NA	NA	NA	T	0.3	0.5	30	0.05	0.25	1
Zamia_spartea	CR	1	0	0	0	0	0	0	0	0	1	41.82245	1235	NA	NA	T	NA	NA	30	NA	0.1	1
Zamia_standleyi	VU	N	N	N	NA	N	N	N	N	N	0	31.0183	NA	NA	NA	T	NA	NA	30	0.05	0.12	7

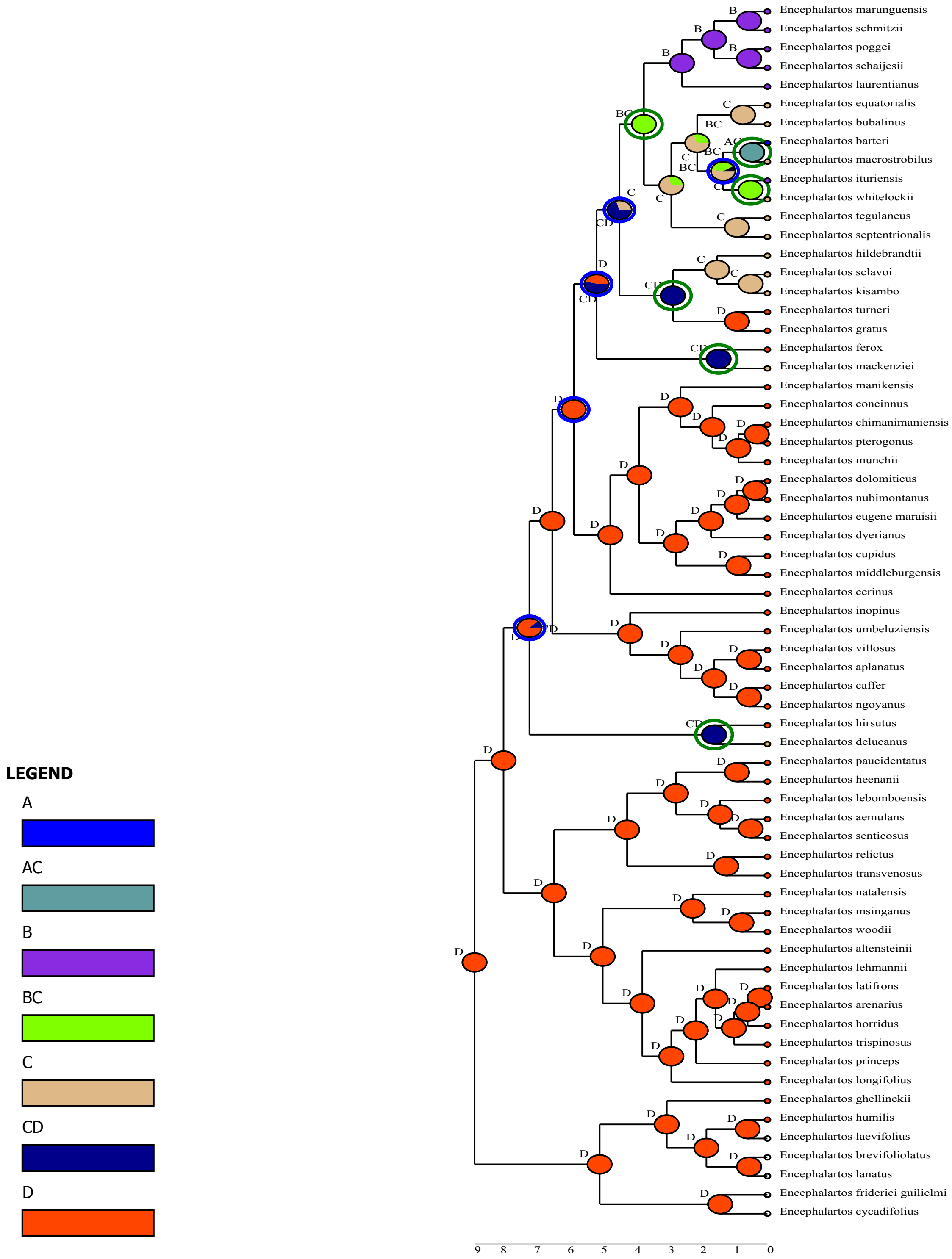
		A	A	A		A	A	A	A	A		6										
Zamia_stricta	VU	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	16.55332	25	NA	NA	T	NA	NA	30	NA	NA	1
Zamia_tolimensis	CR	1	1	0	0	0	0	0	0	0	2	31.59701	NA	NA	NA	T	NA	4	NA	0.1	0.3	1
Zamia_tuerckheimii	NT	1	0	0	0	0	0	0	0	0	1	27.68497	NA	NA	NA	NT	1.5	3	30	NA	NA	1
Zamia_ulei	NT	0	0	0	1	0	0	0	0	0	1	24.13965	NA	NA	NA	NT	NA	1	30	0.04	0.06	7
Zamia_urep	CR	1	0	0	0	0	0	0	0	0	1	38.73882	30	NA	NA	T	0.4	0.5	30	0.025	0.03	1
Zamia_variegata	EN	1	0	0	0	0	0	0	0	0	1	36.92315	NA	NA	NA	T	NA	0.2	30	NA	0.08	4
Zamia_vazquezii	CR	0	1	0	0	0	0	0	0	0	1	66.9213	NA	NA	NA	T	NA	0.3	30	NA	0.1	1
Zamia_wallisii	CR	1	1	0	0	0	1	0	0	0	3	28.07089	NA	NA	NA	T	NA	NA	30	0.03	0.05	1
Zamia_lindleyi	DD	1	0	0	0	0	0	0	0	0	1	19.07345	NA	1143	NA	DD	NA	3	30	0.1	0.3	2
Ceratozamia_brevifrons	DD	1	0	0	0	0	0	1	0	0	2	26.68981	NA	NA	NA	DD	0.09	0.28	45	0.08	0.19	1
Cycas_aenigma	DD	1	0	0	1	0	0	0	0	0	2	12.8756	NA	1100	NA	DD	0.5	4	40	NA	NA	1
Cycas_indicaA.	DD	0	0	1	0	0	0	0	0	0	1	33.75955	NA	934	NA	DD	NA	4	40	0.1	0.23	1
Cycas_sphaerica	DD	0	0	1	0	0	0	0	0	0	1	17.23081	NA	300	1000	DD	NA	5	40	0.09	0.27	1

Appendix B: Maximum credibility tree



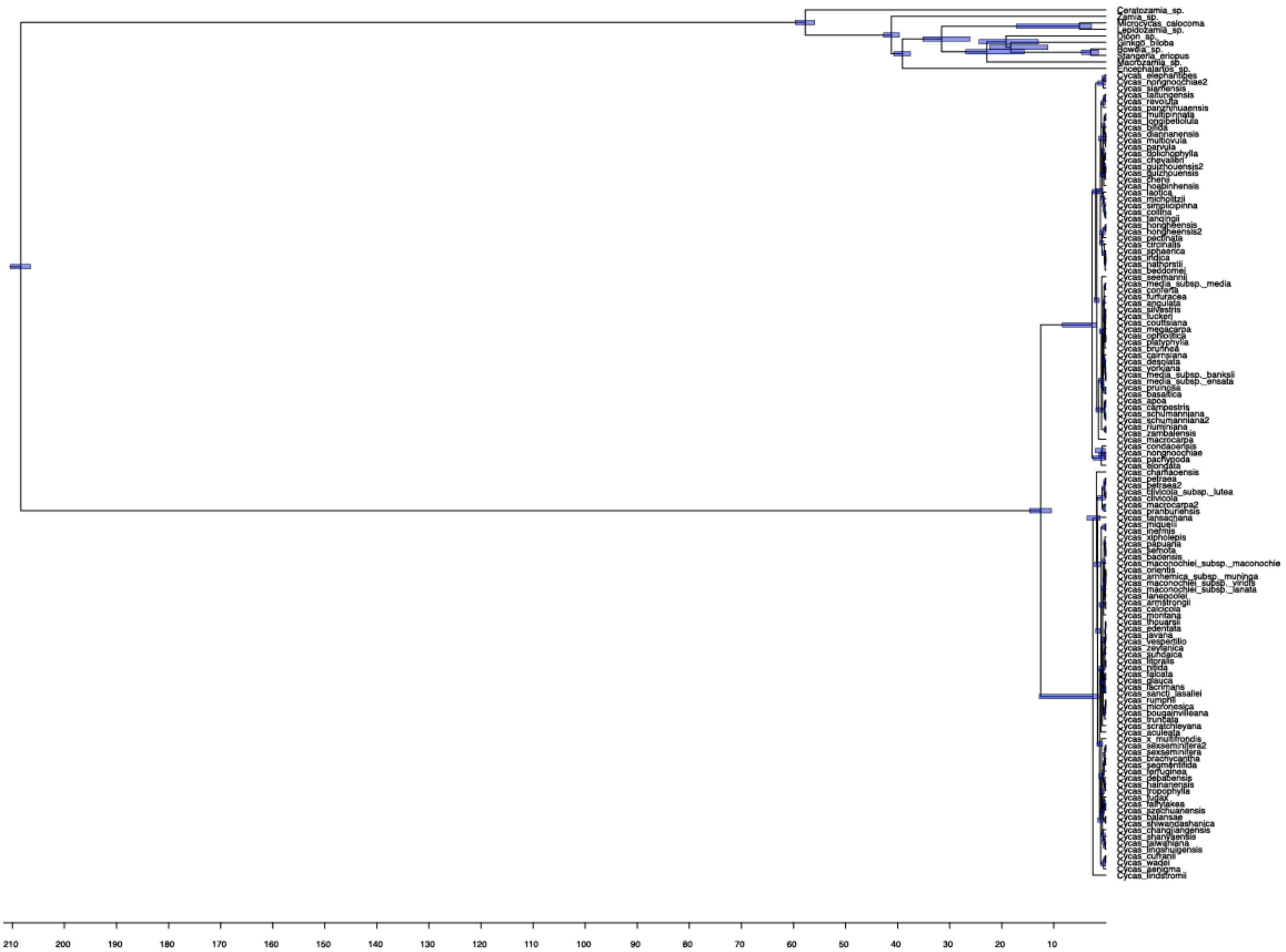
Appendix B: Maximum Clade Credibility (MCC) tree indicating 95% highest posterior density (blue error bars) based on BEAST analysis.

Appendix C: DEC Model analysis for the genus *Encephalartos*



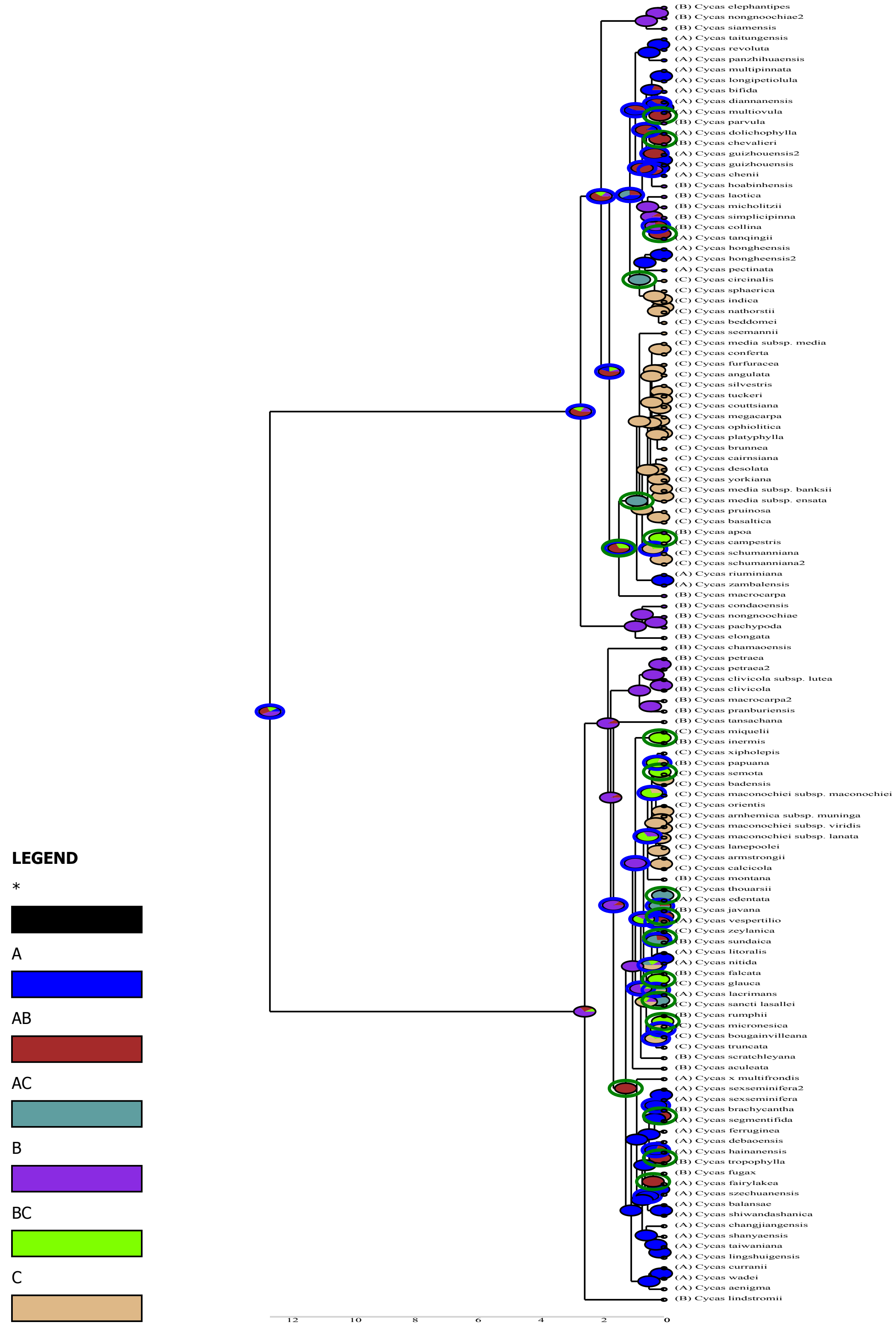
Appendix C: DEC Model Analysis implemented in RASP as a comparison to S-DIVA model

Appendix D: Maximum Clade Credibility (MCC)



Appendix D : Maximum Clade Credibility (MCC) tree indicating 95% highest posterior density (blue error bars) based on BEAST analysis.

Appendix E: DEC Model analysis for the genus *Cycas*



Appendix E: DEC Model analysis implemented in RASP as a comparison to BBM model

Appendix F: Ethical clearance certificate

UNISA university of south africa

UNISA CAES GENERAL ETHICS REVIEW COMMITTEE

Date: 07/07/2017

NHREC Registration # : REC-170616-051
ERC Reference # : 2017/CAES/099
Name : Ms LT Mankga
Student #: 60996471

Dear Ms Mankga

Decision: Referred back
Expiry date 30/07/2017

Researcher(s): Ms LT Mankga
mankglt@unisa.ac.za; (011) 471-3604

Supervisor (s): Dr K Yessoufou
kowiyouy@uj.ac.za

Dr M Chitakira
Chitam1@unisa.ac.za; (011) 471-3220

Working title of research:

Elucidating the historical forces that mediated the biogeography of cycads and shaped their diversification history

Qualification: PhD Environmental Science

Thank you for the application for research ethics clearance by the UNISA CAES General Ethics Review Committee for the above mentioned research.

The **low risk application** was reviewed in compliance with the UNISA Policy on Research Ethics and the UNISA Standard Operating Procedure on Research Ethics Risk Assessment on 06 July 2017. **The application is referred back for revisions and clarifications.**

Open Rubric

The following comments, emanating from the reviews, are tabled for your attention, clarification and/or amendment:

1. The ethics form is poorly completed. The majority of the sections were simply left blank, with no indication whether they are not applicable or whether it was an oversight. Furthermore, in Section D.2 the applicant provides a long list of plants that will be used in the research, yet later on it is stated that this is only a desktop study. If no actual plants are going to be used for this research, the ethics application form should reflect this. The ethics application form should be able to stand on its own, and readers should be able to fully understand the project without reading the research proposal as well. The researcher is requested to submit the corrected application form, upon which ethical clearance will be given.

The revised application and the supporting documents must be submitted to the CAES General Ethics Committee chair Prof EL Kempen and the Research Ethics Administrator Ms M Van Wyk for expedited review by the committee on or before 30 July 2017.

Highlight all changes *made on the application form to streamline the review process. The ethics clearance number,* ***2017/CAES/099****, must be used in all correspondence.*

Failure to submit the clarifications and/or revised document/s by the expiry date will mean that all subsequent submissions related to the project will be regarded as a new application for ethics review.

Data collection activities, as indicated in the application documents, may not commence until final approval has been granted by the CAES General ERC.

It is your responsibility to ensure that the proposed research adheres to the values and principles expressed in the UNISA Research Ethics Policy.

Please note:
If your re-submission does not adhere to the procedure set out above it will not be tabled for ethics review and will be returned to you within 48 hours.

Yours sincerely,

Prof MA Antwi

Acting Chair of CAES General ERC

E-mail: antwima@unisa.ac.za

Tel: (011) 670-9391