

# Savanna fire and the origins of the 'underground forests' of Africa

Olivier Maurin<sup>1\*</sup>, T. Jonathan Davies<sup>1,2\*</sup>, John E. Burrows<sup>3,4</sup>, Barnabas H. Daru<sup>1</sup>, Kowiyou Yessoufou<sup>1,5</sup>, A. Muthama Muasya<sup>6</sup>, Michelle van der Bank<sup>1</sup> and William J. Bond<sup>6,7</sup>

<sup>1</sup>African Centre for DNA Barcoding, Department of Botany & Plant Biotechnology, University of Johannesburg, PO Box 524, Auckland Park, 2006 Johannesburg, Gauteng, South Africa;

<sup>2</sup>Department of Biology, McGill University, 1205 Ave Docteur Penfield, Montreal, QC H3A 0G4, Canada; <sup>3</sup>Buffelskloof Herbarium, PO Box 710, Lydenburg 1120, South Africa;

<sup>4</sup>Department of Plant Sciences, University of Pretoria, Private Bag X20, Hatfield 0028, Pretoria, South Africa; <sup>5</sup>Department of Environmental Sciences, University of South Africa, Florida Campus, Florida 1710, Gauteng, South Africa; <sup>6</sup>Department of Biological Sciences, University of Cape Town, Rondebosch 7701, Western Cape, South Africa; <sup>7</sup>South African Environmental Observation Network, Private Bag X7, 7735, Rhodes Drive, Newlands, Western Cape, South Africa

Author for correspondence:

T. Jonathan Davies

Tel: +1 514 398 8885

Email: j.davies@mcgill.ca

Received: 19 March 2014

Accepted: 11 June 2014

New Phytologist (2014) 204: 201–214

doi: 10.1111/nph.12936

**Key words:** fire adaptation, geoxyllic suffrutex, phylogeny, Pliocene, savanna, trees of southern Africa, underground forests.

## Summary

- The origin of fire-adapted lineages is a long-standing question in ecology. Although phylogeny can provide a significant contribution to the ongoing debate, its use has been precluded by the lack of comprehensive DNA data. Here, we focus on the 'underground trees' (=geoxyles) of southern Africa, one of the most distinctive growth forms characteristic of fire-prone savannas.
- We placed geoxyles within the most comprehensive dated phylogeny for the regional flora comprising over 1400 woody species. Using this phylogeny, we tested whether African geoxyles evolved concomitantly with those of the South American cerrado and used their phylogenetic position to date the appearance of humid savannas.
- We found multiple independent origins of the geoxyle life-form mostly from the Pliocene, a period consistent with the origin of cerrado, with the majority of divergences occurring within the last 2 million yr. When contrasted with their tree relatives, geoxyles occur in regions characterized by higher rainfall and greater fire frequency.
- Our results indicate that the geoxyllic growth form may have evolved in response to the interactive effects of frequent fires and high precipitation. As such, geoxyles may be regarded as markers of fire-maintained savannas occurring in climates suitable for forests.

## Introduction

Savannas, ecosystems with continuous grass cover and variable woody cover, are one of the world's major biomes, reaching their greatest extent in the seasonally dry tropics. Over large parts of their climate range, savannas occur in mosaics with forests, leading to the idea that they are products of anthropogenic fire and deforestation. However, there is accumulating evidence that, worldwide, savannas are of ancient origin. Dated molecular phylogenies point to the origins of the C<sub>4</sub> grasses that dominate savannas in the Oligocene (earliest at 32 million yr ago (Ma)) with considerable diversification already occurring by the Miocene (Christin *et al.*, 2008, 2014; Edwards *et al.*, 2010; Bouchenak-Khelladi *et al.*, 2014). The savanna biome first became a prominent component of tropical vegetation from the late Miocene (*c.* 8 Ma) according to isotopic evidence from palaeosols and fossil teeth (Cerling *et al.*, 1997). Thus, savannas were a major component of tropical vegetation millions of years before

humans began to fell and burn forests. Studies have indicated that, once savannas began to spread, they expanded rapidly, reaching their maximum extent during Pleistocene glacial periods. Today, they cover *c.* 20% of the world's vegetated land surface (Collinson, 1988; Bond, 2008).

The causes of this rapid spread and the long delay between the origins of C<sub>4</sub> grass lineages and their expansion into the savanna biome have been topics of intense research interest over the past 20 yr (Cerling *et al.*, 1997; Sage, 2004; Keeley & Rundel, 2005; Beerling & Osborne, 2006; Osborne, 2008; Edwards *et al.*, 2010; Scheiter *et al.*, 2012). Ehleringer *et al.* (1997) were the first to suggest a general hypothesis for the appearance of C<sub>4</sub> grasses, arguing from photosynthetic considerations that C<sub>4</sub> grasses would first have outcompeted their C<sub>3</sub> precursors in low latitudes with warm growing seasons and when atmospheric CO<sub>2</sub> dropped below 500 ppm. They suggested that this threshold was passed in the late Miocene, explaining the rapid global expansion of savannas from that time. However, subsequent studies, using a variety of proxies, have shown that CO<sub>2</sub> dropped below the 500 ppm threshold in the Oligocene, much earlier than the rise of the

\*These authors contributed equally to this work.

savanna biome, but consistent with the origin of  $C_4$  grasses and other lineages with  $CO_2$ -concentrating mechanisms (Pagani *et al.*, 2002; Arakaki *et al.*, 2011; Beerling & Royer, 2011).

Although photosynthetic advantage may explain how  $C_3$  grasses were outcompeted by  $C_4$  grasses, it does not explain how grasses outcompeted trees, and therefore why grasslands replaced ancestral forests.  $C_4$  grasses are intolerant of shading and are rare or absent in closed forest understories (Ehleringer, 1978; Sage, 2001). For the savanna biome to have expanded, forests would have had to retreat. Increasing aridity is one potential pathway to forest retreat. Phytolith studies in central North America have shown that forests were replaced by  $C_3$  grasslands, which were, in turn, replaced by  $C_4$  grasses during the late Miocene (Strömberg, 2005). The mechanism for forest retreat has been attributed to increasing aridity from the Oligocene. Forest retreat as a result of growing aridity has also been invoked to explain the spread of grasses in Pakistan and Europe (Strömberg, 2011).

However, a climate-based hypothesis for the distribution of savannas does not explain why many contemporary  $C_4$  savannas occur as alternative states to closed forests in tropical landscapes (Hirota *et al.*, 2011; Lehmann *et al.*, 2011; Staver *et al.*, 2011a). The presence of forests indicates climatic conditions that can support closed vegetation. Fire is increasingly being recognized as a key factor maintaining contemporary grasslands in a climate that can support forests (Bond *et al.*, 2005; Lehmann *et al.*, 2011; Staver *et al.*, 2011b; Hoffmann *et al.*, 2012).  $C_4$  grassy biomes currently account for *c.* 80% of the world's burnt area per year (Chuvieco *et al.*, 2008; van der Werf *et al.*, 2010), and many humid savannas burn several times in a decade and some burn twice within a year (Chuvieco *et al.*, 2008; Archibald *et al.*, 2013). The combination of high grass productivity given sufficient moisture, low decomposition rates of  $C_4$  grasses and a dry season suitable for burning every year provides the essential ingredients for the frequent fires characteristic of  $C_4$  savannas (Bond *et al.*, 2003; Bond, 2008). Savanna fires can penetrate forest margins and, depending on the rates of postburn forest recovery, facilitate savanna advance into forest habitat (Kellman, 1984; Hoffmann *et al.*, 2009, 2012; Murphy & Bowman, 2012). Because tree recovery from injury, such as fire damage, is particularly slow at low  $CO_2$ , there may additionally have been synergies between fire-maintained savannas vs closed forests and atmospheric composition (Bond & Midgley, 2000, 2012; Beerling & Osborne, 2006; Kgope *et al.*, 2010; Scheiter *et al.*, 2012).

The demonstration of an empirical link between fire and the advance of savanna into forest has been a challenge. Charcoal records from marine cores show an exponential increase in fire activity coincident with the expansion of the savanna biome (Herring, 1985; Morley & Richards, 1993; Keeley & Rundel, 2005; Hoetzel *et al.*, 2013). Unfortunately, there is no equivalent terrestrial fossil record. The landscapes in which fires are currently most prominent are deeply weathered and not conducive to fossil preservation. Thus, the fossil evidence is currently weighted towards the aridity route for forest retreat and savanna expansion (Strömberg, 2011). Dated molecular phylogenies provide an alternative tool to explore the origins of fire-adapted

lineages and biomes (e.g. Bytebier *et al.*, 2011; He *et al.*, 2011; Midgley & Bond, 2011).

Simon *et al.* (2009) and Simon & Pennington (2012) used phylogenetic methods to infer the history of fire activity in Brazilian savannas (cerrado), and estimated the origin of woody plants restricted to the cerrado as  $< 10$  Ma, with most savanna lineages dated as  $< 5$  Ma, consistent with isotopic evidence for the timing of the spread of  $C_4$  grassy biomes. Thus, far from being an ancient vegetation type (e.g. Cole, 1986), cerrado is more likely to be a recently evolved biome. Savanna lineages differ from their forest relatives in a suite of fire-adapted traits: thick bark, reduced height and large underground storage organs (Coutinho, 1982, 1990; Hoffmann *et al.*, 2003, 2004, 2009, 2012). One of the most distinctive growth forms characteristic of savannas is the geoxylic suffrutex (White, 1979; 'geoxyle' of Simon & Pennington, 2012). These are functionally herbaceous plants with 'woody xylopodia underground but only limited and often short lived aerial shoots' (Simon & Pennington, 2012; see also Coutinho, 1982, 1990; Appezzato-da-Glória *et al.*, 2008). Xylopodia are underground structures consisting of 'a lignified complex of root and shoot tissue with a high capacity to resprout and produce new shoot buds' (Appezzato-da-Glória *et al.*, 2008), and may provide an alternative adaptive escape route from fire. White (1979) described this growth form for African savannas and likened these plants to underground trees with branches buried and only the shoot tips and leaves emerging – he referred to them eloquently as Africa's 'underground forests'. We use the term 'geoxyles' for underground trees hereafter.

Here, we locate the origins of the geoxyle life-form on the first comprehensive phylogeny of African woody plants to explore the origins of African savannas. First, we test whether woody species with this putatively fire-adapted growth form emerged at similar times to those of South American cerrado. Second, we explore the origins of the geoxyle life-form and consider whether these species provide a marker for the appearance of humid savannas through the fire mechanism of forest retreat. Last, we contrast the diversity of lineages contributing to the woody flora of African savannas with the flora of the South American cerrado.

## Materials and Methods

Our study area includes the Zambezan region between  $8^{\circ}5'S$  and  $34^{\circ}5'S$  latitude and  $11^{\circ}7'W$  and  $40^{\circ}9'E$  longitude. It encompasses 12 countries, including Angola, Botswana, Democratic Republic of the Congo, Lesotho, Malawi, Mozambique, Namibia, South Africa, Swaziland, Tanzania, Zambia and Zimbabwe, with the Atlantic and Indian Oceans delimiting the region in the west and east, respectively.

## Taxon sampling

A total of 1400 (of the *c.* 2200) woody plant species, comprising 117 families and 562 genera (Coates Palgrave, 2002; Schmidt *et al.*, 2007; Boon, 2010; Van Wyk *et al.*, 2011; Germishuizen & Meyer, 2013), were sampled over a period of 6 yr in southern Africa (Supporting Information Notes S1), including 53 of the *c.*



200 geoxyle taxa recorded for the region (Notes S2). We follow White's (1976) definition of geoxyles as plants that have a perennial below-ground woody root/stem, flowering and fruiting on seasonal and short-lived (resprouted) stems that do not exceed 1 m tall, and occur in areas receiving annual rainfall above 750 mm. As construed by White (1976), the term 'geoxylic suffrutex' is confined to those woody underground trees that belong to genera whose species are mainly otherwise trees and shrubs. He excluded from his definition those genera that have similar subterranean growth forms but which lack large tree relatives, for example *Acalypha*, *Eriosema*, *Gnidia*, *Hypericum*, *Indigofera*, *Phyllanthus*, *Syncolostemon*, *Tephrosia* and *Vernonia*. White's definition includes the most striking examples of closely related tall trees and functionally herbaceous 'underground trees' (Figs 1–3).

### Phylogeny reconstruction

DNA extractions from leaf material, polymerase chain reactions and sequencing for the two plant DNA barcoding regions (*rbcLa* and *matK*) (CBOL Plant Working Group, 2009) were conducted using standard protocols (Hajibabaei *et al.*, 2005; Ivanova *et al.*,

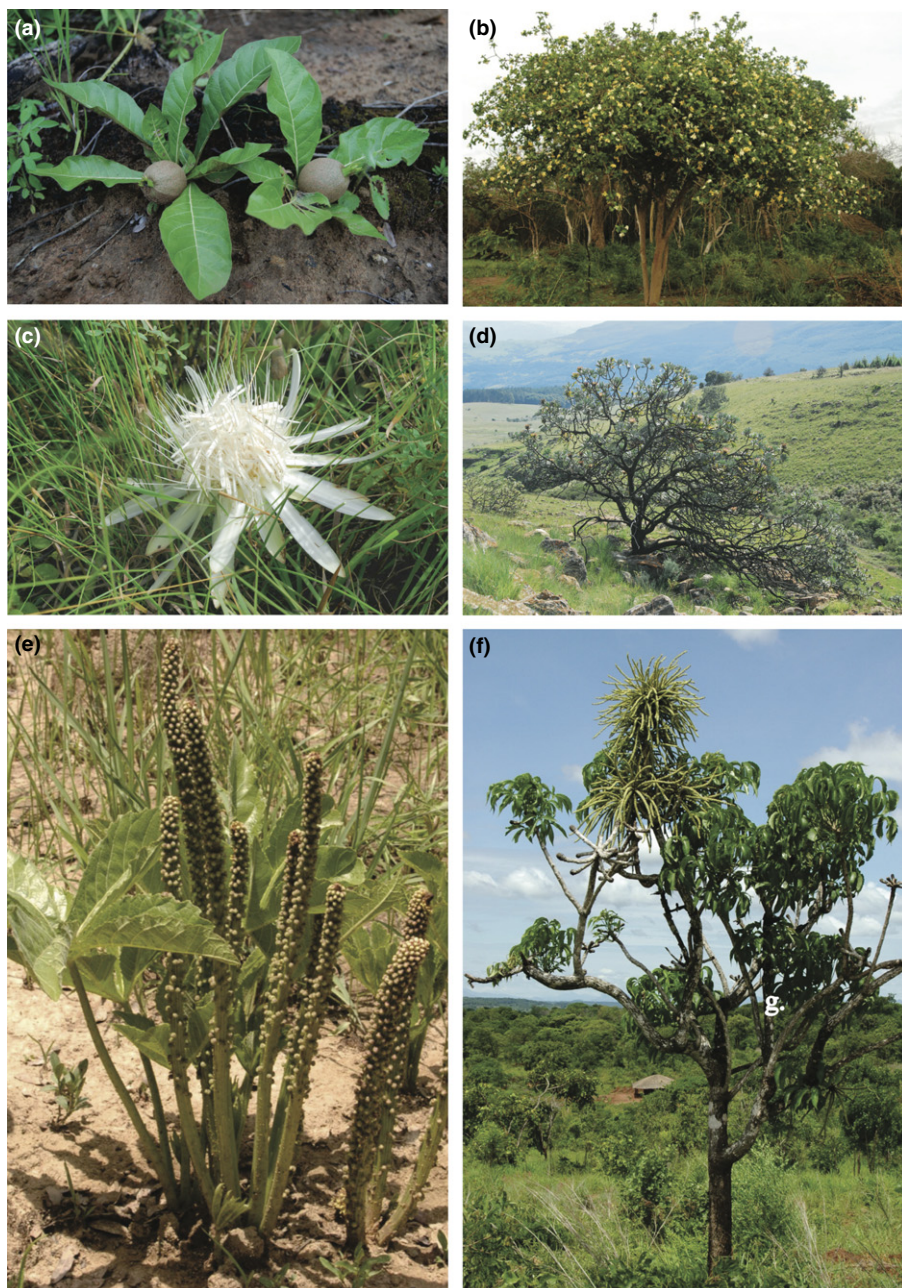
2008). Complementary strands were assembled and edited using Sequencher v.4.8 (Gene Codes, Ann Arbor, MI, USA). The *matK* alignment was performed using Transalign (Bininda-Emonds, 2005). The combined dataset comprised 552 and 942 base pairs for *rbcLa* and *matK* regions, respectively. Voucher specimen information and GenBank accession numbers are listed in Notes S2 and on the BOLD DataSystem ([www.boldsystems.org](http://www.boldsystems.org)). The phylogeny was reconstructed using 1400 taxa representing 117 families and 562 genera of Gymnosperms and Angiosperms. A total of 53 geoxyle taxa, representing 22 Angiosperm Phylogeny Group (APG) families, were included in the matrix.

A maximum likelihood (ML) analysis was performed on the combined dataset using RAxML-HP2 v.7.2.6 (Stamatakis *et al.*, 2008) on the CIPRES cluster (Miller *et al.*, 2009), and enforcing topological constraints assuming the APG III backbone from Phylomatic v.3 (Webb & Donoghue, 2005). The phylogeny was rooted using representatives of Acrogymnospermae (*Callitris*, *Cupressus*, *Cycas*, *Encephalartos*, *Juniperus*, *Pinus*, *Podocarpus*, *Stangeria*, *Widdringtonia* and *Zamia*) (Cantino *et al.*, 2007; Soltis *et al.*, 2011). Branch lengths were then



**Fig. 1** Examples of geoxyles. (a) *Lannea edulis* (Anacardiaceae); (b) *Combretum platypetalum* ssp. *oatesii* (Combretaceae); (c) *Morella pilulifera* (Myricaceae); (d) *Parinari capensis* ssp. *capensis* (Chrysobalanaceae); (e) *Elephantorrhiza elephantina* (Fabaceae); (f) *Dichapetalum cymosum* (Dichapetalaceae); (g) *Ziziphus zeyheriana* (Rhamnaceae). Photographs courtesy of John Burrows (a) and Olivier Maurin (b–g).



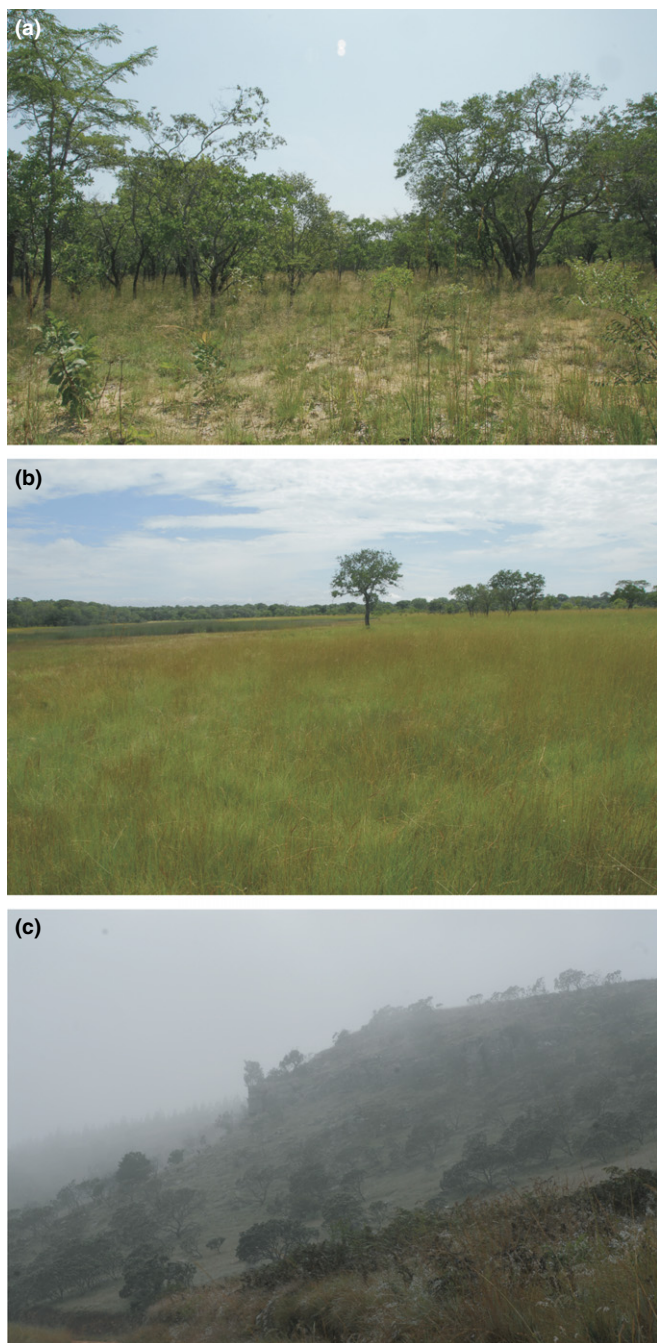


**Fig. 2** Geoxyles and their tree relatives. (a) *Gardenia subacaulis* (Rubiaceae); (b) *Gardenia ternifolia*; (c) *Protea paludosa* ssp. *secundifolia* (Proteaceae); (d) *Protea roupelliae* ssp. *roupelliae*; (e) *Cussonia corbisieri* (Araliaceae); (f) *Cussonia arborea*. Photographs courtesy of John Burrows (a, b, e, f) and Olivier Maurin (c, d).

calibrated in millions of years using a Bayesian Markov chain Monte Carlo (MCMC) approach implemented in BEAST v.1.4.8 (Drummond & Rambaut, 2007), keeping the tree topology fixed. First, the RAxML starting tree was adjusted so that branch lengths satisfied all fossil prior constraints, using PATHd8 v.1.0 (Britton *et al.*, 2007). Second, we assumed an uncorrelated log-normal (UCLN) model for rate variation among branches and the GTR+I+ $\Gamma$  model of sequence evolution for each partition based on the Akaike information criterion evaluated using Modeltest v.2.3 (Nylander, 2004). Third, we used 28 fossil calibration points from Bell *et al.* (2010) (Table S1) as minimum age constraints on the stem node of each group, except for the root of the Eudicots, which was set at 124 million yr, with a log-normal

distribution following Bell *et al.* (2010). We performed four independent runs of MCMC, each for 100 million generations, sampling every 1000 generations. We assessed the MCMC log files for convergence using the effective sample size (ESS) statistics in Tracer v.1.5 (Drummond & Rambaut, 2007). The BEAST analysis reported ESS values > 100, indicating that the posterior estimates were not unduly influenced by autocorrelation. We combined the resulting tree files from the four runs in LogCombiner v.1.7.5 (Drummond & Rambaut, 2007), downsampling 1 in 20 000 trees, and discarding the first 25% trees as burn-in. The maximum clade consensus tree, with means and 95% highest posterior density (HPD) intervals, was generated with TreeAnnotator v.1.7.5 (Drummond & Rambaut, 2007).





**Fig. 3** Three habitats rich in geoxyles. (a) Open woodland in north-west Zambia; (b) seasonally waterlogged grassland in north-west Zambia; (c) highveld habitat in Lydenburg, illustrating the misty conditions characteristic of the South African Highveld. Photographs courtesy of Olivier Maurin.

### Statistical analysis

To explore the divergences between African forest and savanna trees, we categorized each species as occurring predominantly in one of savanna, forest or the fynbos of the Cape Floristic Region (CFR). Savannas were characterized by the presence of a  $C_4$  grassy layer which forests lack (Ratnam *et al.*, 2011), and were defined as tree–grass mixtures where  $C_4$  grasses form a

near-continuous herbaceous layer (Ratnam *et al.*, 2011). Forests, by contrast, were defined as closed woody vegetation casting too much shade to support a continuous  $C_4$  grassy layer. We did not distinguish between the different forest types, such as rainforests, dry deciduous forests or Afro-temperate evergreen forests. The biomes of the CFR are open shrublands, including fynbos, a fire-prone heathland and succulent shrublands (Mucina & Rutherford, 2006). We then used the phylogeny to identify sister species pairs falling exclusively within one biome type, and extracted their times of divergence. The phylogeny for southern African trees represents a regional sample of arborescent species, that is a particular small sample of angiosperms, thus making ancestral state reconstruction problematic because of the very large number of missing species (i.e. non-tree species and trees found outside of southern Africa). Importantly, our estimates of divergence times are conservative because nodes subtending sister pairs are less likely to be split by missing taxa than are nodes deeper in the tree. For example, it will be less common for sister species found within the African savanna biome to have a closer relative outside southern Africa – which would bias us towards the overestimation of divergence times. We assume that the sampling is equal among biomes (forest, 44.3%; savanna, 48.4%; fynbos, 7.3%), a reasonable assumption given the sampling protocol. We note that the shrubland vegetation of the fynbos is naturally tree species poor.

We compare the distribution of evolutionary ages between African savanna and forest sister taxa using a Wilcoxon rank sum test. For the reasons described above, we do not attempt to reconstruct ancestral ecologies directly, but we suggest that if sister taxa occur within the same biome, it is probable that they diverged within this biome. The oldest sister divergence within a biome may thus provide an approximate minimum age for the biome. Next, we examine evolutionary splits between geoxyles and their tree sisters. Here, we include only unambiguous independent shifts to a geoxyle life-form, where a geoxyle species is nested within a more inclusive tree clade (Table 1), thereby allowing us to infer directionality. The geoxyle life-form has apparently arisen multiple times in a few clades, possibly accompanied by reversals to a tree life-form (e.g. *Fadogia*, *Clerodendrum*, *Elephantorrhiza* and *Salacia*) (Fig. 1). However, taxon sampling within these lineages was insufficient to accurately reconstruct the location of evolutionary transitions, and we therefore restricted our analysis to the unambiguous shifts in life-form identified between sister groups.

For each tree–geoxyle pair, we contrasted differences in ecological traits (spines, toxicity, fruit type, root type and seed size) and environment across their geographical distribution (mean annual precipitation, mean annual temperature, mean elevation and fire frequency) (Table 1). Trait data were recorded from the literature (Coates Palgrave, 2002; Schmidt *et al.*, 2007; Plants of Southern Africa, 2012). Fire frequency, precipitation, temperature and elevation variables were obtained by extracting the mean, minimum and maximum values within each species distribution. Precipitation, temperature and elevation variables were obtained from the worldclim database (Hijmans *et al.*, 2005) and fire return frequency from Archibald *et al.* (2010). Statistical significance was

**Table 1** Ecological and environmental data characterizing southern African geoxyles and their tree sister groups

Scientific name (voucher)	Sisters	Max height (m)	Spines	Poisonous	Fruit type coding (0 = dry; 1 = fleshy)	Fruit volume (cm <sup>3</sup> ) <sup>a</sup>	Seed size (cm) <sup>b</sup>	Fire return interval	MAP (mm)	MAT (°C)	Mean elevation (m)
<i>Carissa praetermisa</i> (OM2650)	2	3	1	0	1	NA	0.5	3.3	968	24.1	60
<i>Carissa tetramera</i> (RBN210)	2	3	1	0	1	0.52	NA	2.5	759	21.8	NA
<i>Caesaria</i> sp. (BB12551)	1	1	0	0	0	4.19	NA	1.7	1081	24.8	220
<i>Casearia</i> sp. nov. (Abbott9191)	1	20	0	0	0	4.189	NA	2.2	924	17.8	630
<i>Combretum engleri</i> (OM1025)	3	4	0	0	0	14.14	NA	2.6	499	21.8	1120
<i>Combretum platypetalum</i> (OM2092)	3	3	0	0	0	28.51	NA	4.0	986	21.3	1070
<i>Dichapetalum cymosum</i> (OM2117)	4	0.5	0	0	0	13.09	NA	2.6	496	20.9	1070
<i>Dichapetalum barbosa</i> (OM2374)	4	NA	0	0	0	0.79	NA	NA	NA	NA	90
<i>Dissotis canescens</i> (BB12691)	5	1.8	0	0	0	0.00013	0.75	3.6	1015	22.0	780
<i>Dissotis princeps</i> (OM3806)	5	3	0	0	0	0.00045	NA	2.8	932	20.0	790
<i>Erythrina abyssinica</i> (OM2095)	6	10	1	0	0	NA	1.2	2.9	779	20.3	920
<i>Erythrina acanthocarpa</i> (OM3916B)	6	2	1	0	0	0.36	NA	1.7	647	15.7	1030
<i>Erythrina caffra</i> (BS0057)	6	20	1	1	0	NA	0.8	1.8	737	18.3	410
<i>Erythrina humeana</i> (OM741)	7	4	1	0	0	NA	0.8	2.5	807	19.1	680
<i>Erythrina zeyheri</i> (OM1589)	7	0.5	1	0	0	0.042	1.7	2.5	750	16.6	1450
<i>Eugenia albanensis</i> (BB7021)	8	0.4	0	0	1	1.77	NA	2.2	845	20.1	390
<i>Eugenia capensis</i> A (BB12289)	9	4	0	0	1	1.77	NA	2.4	778	23.3	70
<i>Eugenia capensis</i> (Abbott9225)	9	4	0	0	1	4.19	NA	2.6	860	21.2	NA
<i>Eugenia verdoorniae</i> (Abbott9122)	8	3	0	0	1	4.19	NA	2.3	1027	17.7	470
<i>Ficus capreifolia</i> (OM2566)	10	7	0	0	1	6.28	NA	3.0	743	22.7	420
<i>Ficus pygmaea</i> (MWC20237)	10	3	0	0	1	1.15	NA	4.7	1111	21.4	990
<i>Gardenia cornuta</i> (OM2241)	11	5	0	0	1	9.42	NA	2.3	830	19.9	470
<i>Gardenia resiniiflua</i> (OM1272)	11	7	0	0	1	0.0014	0.35	2.6	619	21.5	800
<i>Gardenia subcaulis</i> (BB12202)	11	0.3	0	0	1	85.08	0.5	3.7	1063	21.4	1070
<i>Jasminum fluminense</i> (OM273)	12	9	0	0	1	NA	NA	2.1	602	20.7	830
<i>Jasminum quinatum</i> (T416)	12	0.4	0	0	1	NA	NA	2.5	772	16.3	1440
<i>Lannea discolor</i> (RL1235)	13	15	0	0	1	0.26	NA	2.6	648	20.4	950
<i>Lannea edulis</i> (OM1991)	13	0.3	0	0	1	0.40	NA	4.1	1008	21.3	1020
<i>Leptactina benguelensis</i> (BB11158)	14	0.4	0	0	1	1.51	0.3	4.3	1124	21.3	1090
<i>Leptactina delagoensis</i> (OM1598)	14	4	0	0	1	0.0080	NA	2.7	736	23.3	220
<i>Leucospermum gerrardii</i> (MWC26648)	15	0.4	0	0	0	NA	NA	3.1	974	17.2	900
<i>Leucospermum saxosum</i> (BB12687)	15	2	0	0	0	NA	NA	2.4	995	18.2	1090
<i>Lopholaena coriifolia</i> (OM & MvdB41)	16	2	0	0	0	NA	NA	2.4	709	18.3	1270
<i>Lopholaena disticha</i> (OM3909)	16	1	0	0	0	NA	NA	2.6	837	17.8	1080
<i>Maerua andradae</i> (LT1802)	17	0.3	0	NA	1	NA	NA	3.6	1053	25.3	290
<i>Maerua juncea</i> (OM1592)	17	5	0	NA	1	14.73	NA	2.0	611	21.5	570
<i>Maerua rosmarinoides</i> (OM1476)	17	5	0	NA	1	NA	NA	2.5	841	18.7	770
<i>Milletia makoudensis</i> (LT1723)	18	1.2	0	0	0	8.25	NA	1.9	1059	24.9	250
<i>Millettia usaramensis</i> (OM2433)	18	10	0	0	0	22.16	0.6	3.3	889	22.7	520
<i>Morella brevifolia</i> (OM3812)	19	0.4	0	0	1	0.014	NA	2.8	833	16.4	1050
<i>Morella serrate</i> (Abbott9173)	19	10	0	0	1	0.014	NA	2.6	716	18.0	960
<i>Ochna arborea</i> (CS03)	20	12	0	0	1	0.26	NA	NA	NA	NA	810
<i>Ochna confusa</i> (OM3828)	20	2	0	0	1	0.34	NA	3.0	1015	20.5	1090
<i>Ozoroa</i> sp. (BB8074)	21	0.6	0	0	1	0.11	NA	2.7	961	20.3	590
<i>Ozoroa albicans</i> (BB8988)	21	1	0	0	1	NA	NA	1.5	694	21.1	580
<i>Ozoroa laetans</i> (BF12423)	21	1.5	0	0	1	0.28	NA	1.8	706	20.5	670
<i>Ozoroa paniculosa</i> (OM1948)	21	6	0	0	1	0.37	NA	2.0	523	19.9	1050
<i>Parinari capensis</i> ssp. <i>incohata</i> (OM3613)	22	2.5	0	0	1	0.79	NA	2.2	796	22.6	100
<i>Parinari excels</i> (BB10672)	22	35	0	0	1	13.09	NA	NA	NA	NA	NA
<i>Paropsia braunii</i> (BB10672)	34	10	0	0	0	0.79	NA	2.6	742	23.9	180
<i>Paropsia brazenana</i> (Fishwick sn)	34	1	0	0	0	3.39	NA	4.6	1046	21.5	1000
<i>Protea gaguedi</i> (Turpin471)	24	10	0	0	0	NA	NA	NA	NA	NA	1140
<i>Protea parvula</i> (OM3817)	24	0.16	0	0	0	NA	NA	2.5	878	16.6	1340
<i>Ritchiea capparoides</i> (LT1805)	25	4	0	NA	1	NA	NA	NA	NA	NA	NA
<i>Ritchiea pygmaea</i> (LT1801)	25	0.4	0	NA	1	NA	NA	3.0	1074	25.1	210
<i>Searsia dentata</i> (OM2251)	28	5	0	0	1	0.034	0.25	NA	NA	NA	1250
<i>Searsia leptodictya</i> (RL1655)	27	9	0	0	1	0.065	0.25	2.3	609	19.0	1110
<i>Searsia pendulina</i> (OM1984)	26	10	0	0	1	0.014	0.63	NA	NA	NA	970

Table 1 (Continued)

Scientific name (voucher)	Sisters	Max height (m)	Spines	Poisonous	Fruit type coding (0 = dry; 1 = fleshy)	Fruit volume (cm <sup>3</sup> ) <sup>a</sup>	Seed size (cm) <sup>b</sup>	Fire return interval	MAP (mm)	MAT (°C)	Mean elevation (m)
<i>Searsia pondoensis</i> (BT10242)	26	1	0	0	1	0.065	0.25	2.6	890	17.6	980
<i>Searsia pygmaea</i> (BL7355)	27	0.2	0	0	1	0.059	0.25	3.0	1114	17.6	1050
<i>Searsia tumulicola</i> ssp. <i>meeseana</i> (OM3818)	28	0.7	0	0	1	0.11	0.21	2.3	875	17.4	1280
<i>Searsia wilmsii</i> (OM3910)	26	0.5	0	0	1	0.065	0.25	2.4	826	18.9	940
<i>Tetracera boiviniana</i> (BB9126)	29	4.5	0	0	0	0.94	0.05	NA	NA	NA	NA
<i>Tetracera masuiana</i> (BB11174)	29	1	0	0	0	1.33	0.45	3.9	1169	22.3	990
<i>Ziziphus abyssinica</i> (OM2582)	30	13	1	0	1	14.14	0.7	3.4	774	23.0	650
<i>Ziziphus zeyheriana</i> (OM3913)	30	1.2	1	0	1	0.27	0.5	2.3	637	19.3	1020

MAP, mean annual precipitation; MAT, mean annual temperature; NA, not available.

<sup>a</sup>Assuming spheroid fruits.

<sup>b</sup>Seed size is measured as the longest axis of the seed.

evaluated using McNemar's test for binary traits, and a Wilcoxon signed rank test for continuous variables (Wilcoxon, 1945). When one of the sisters contained more than one species, contrasts were taken using the average of the species values. Our sister pair approach parallels Felsenstein's (1985) method of independent contrasts and, although it does not make use of all the information contained within the phylogenetic tree, sister comparisons provide a statistically robust method for comparative analyses (Barraclough *et al.*, 1998).

### Cerrado vs African savanna

To compare the phylogenetic affinities between the woody vegetation of the Brazilian cerrado and that of the African savanna, we extracted the list of woody plants and shrubs native to African savanna from the barcode phylogeny already described (586 species) and matching life-forms for cerrado listed by Ratter *et al.* (2012) (<http://cerrado.rbge.org.uk/>; 848 species). We then reconstructed a supertree of African savanna plus cerrado species using the Phylomatic online tool (Webb & Donoghue, 2005), and transformed branch lengths into millions of years using the BLADJ algorithm as implemented in Phylocom version 4.2 (Webb *et al.*, 2008), calibrated with node dates from Wikström *et al.* (2001). Taxonomic mismatches (i.e. species or genera that could not be matched to higher taxa currently recognized by the APG (APG III, 2009)) reduced the final species set to 548 and 809 species for African savanna and cerrado, respectively.

Phylogenetic structure for the regional floras was first assessed by evaluating the proportion of shared branch lengths between floras using the PhyloSor index of phylogenetic beta-diversity following Bryant *et al.* (2008), as implemented in the Picante R-library (Kembel *et al.*, 2010). Significance was determined by randomizing the placement of taxa across the phylogeny (999 replicates). Second, we compared the standardized effect size of the mean pairwise phylogenetic distance between species (the net relatedness index (NRI) of Webb *et al.*, 2002) within each flora with a null model in which the same number of species are drawn at random from the phylogeny.

### Results

Evolutionary splits between African savanna tree sister species are significantly younger than between forest tree species (1.36 vs 3.15 Ma, median age for savanna splits and forest splits, respectively;  $W = 4555$ ,  $P < 0.001$ , Fig. 4), suggesting that speciation events in African savanna have been more recent than in forest, and/or that some forest tree species might either have closest relatives outside southern Africa or non-tree relatives.

The distribution of the geoxyle life-form in Africa is phylogenetically dispersed (Fig. 5), indicating multiple independent evolutionary origins across disparate branches of the angiosperm tree of life, matching observations in cerrado Fabaceae, within the species-rich genus *Mimosa*, and, more generally, evidenced by the

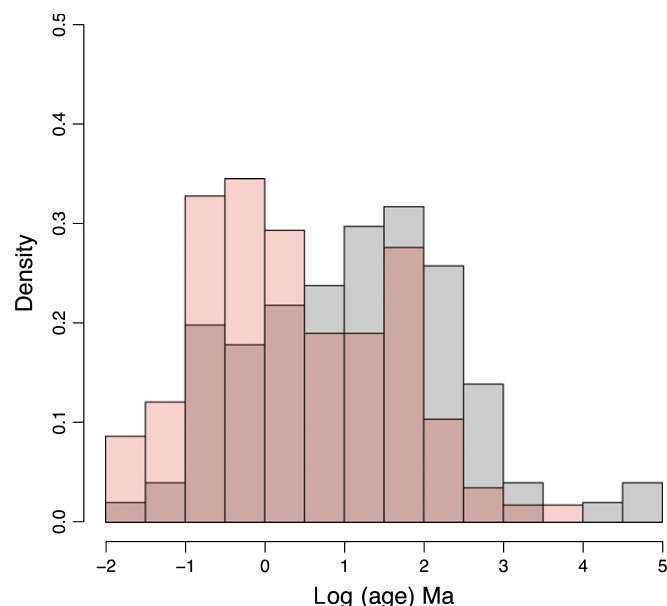
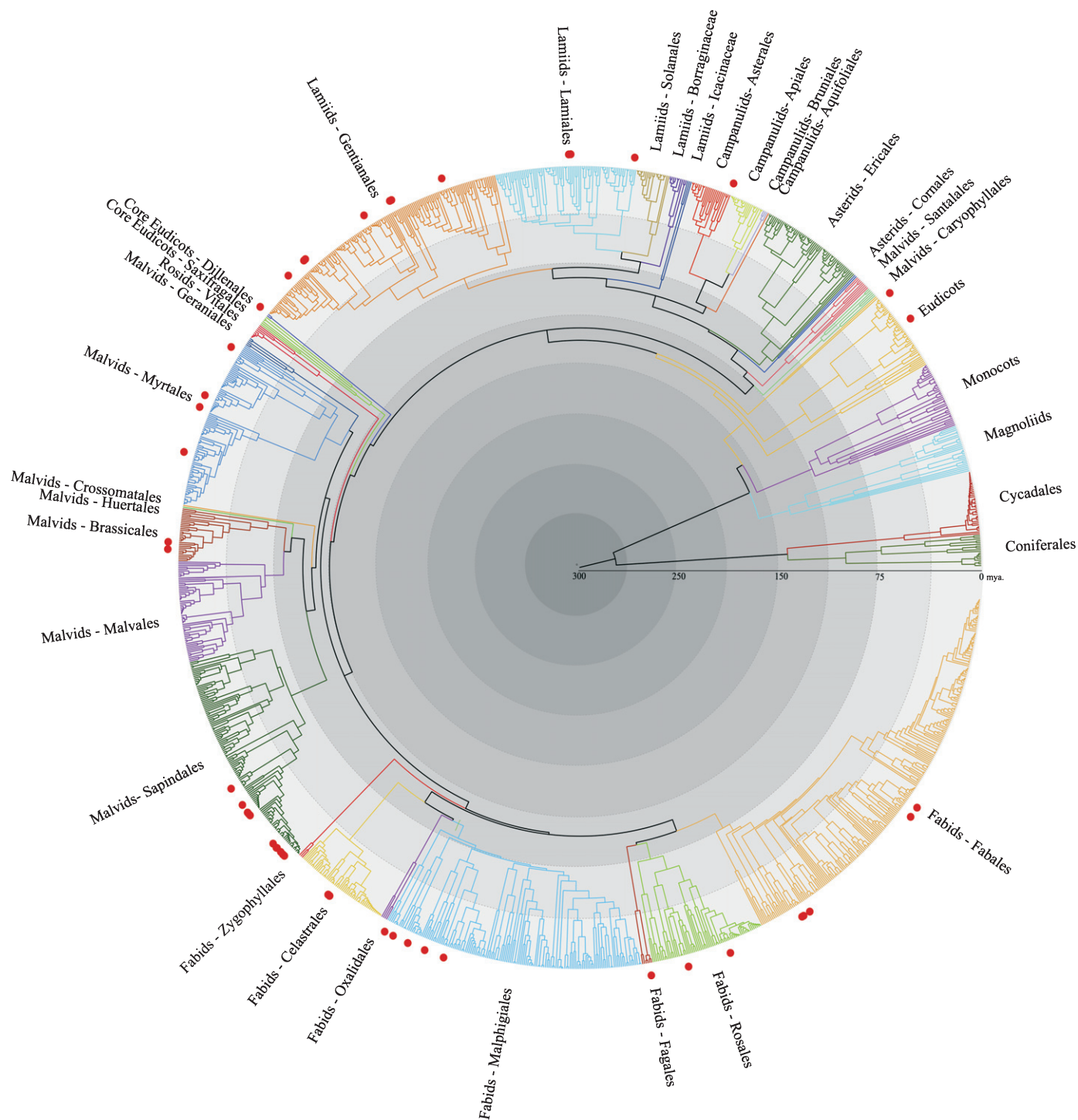


Fig. 4 Distribution of evolutionary splits, in millions of years ago (Ma), between savanna (pink) and forest (grey) sister taxa from the dated phylogenetic tree of southern African trees. Areas of overlap are indicated in brown.





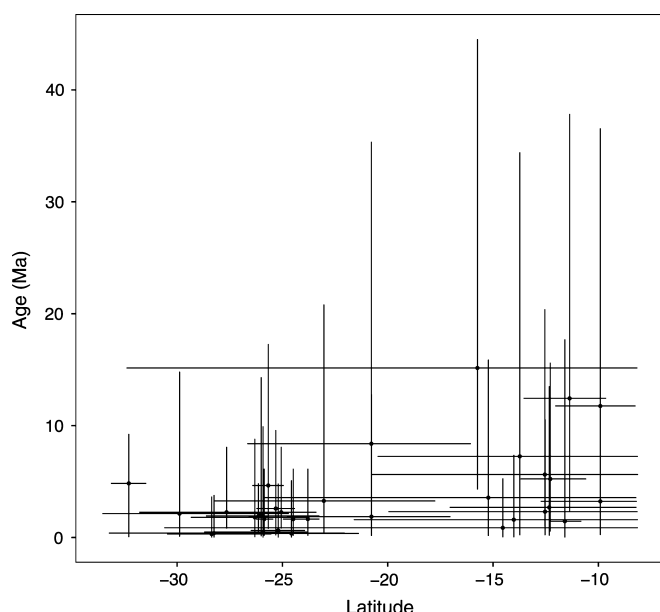
**Fig. 5** Phylogeny of southern African woody flora reconstructed on the basis of DNA barcodes using a maximum likelihood approach after transforming branch lengths to millions of years ago by enforcing a relaxed molecular clock and multiple calibrations. Phylogenetic position of geoxyle life-form indicated in red.

occurrence of cerrado geoxyles across numerous genera and families (Simon *et al.*, 2009). Frequently, a single geoxyle species is nested within a species-rich tree clade, suggesting recent origins of the life-form. Geoxyles have evolved independently in all major lineages (orders) of eudicots, occurring in 30 plant families collected in our study, and arising multiple times in Fabaceae

and Rubiaceae. In a few lineages (e.g. *Fadogia*), we observed multiple origins of the geoxyle habit, suggesting a complex evolutionary history, possibly including reversal to a tree habit, but current taxon sampling does not allow us to accurately resolve independent origins at such fine scales, and we therefore restrict our analysis to single congeneric transitions.



We identified 30 independent transitions to a geoxyle life-form occurring across 26 genera (Table 1). Geoxyles have a median divergence date of 2.28 Ma, with a maximum age of 15.15 Ma for *Dissotis canescens*, which is unusual in that it tends to favour marshy environments. The vast majority of divergences have occurred within the last 2 Ma (Fig. 6). We find no significant ecological differences in geoxyles when compared with their tree sisters (Table 2); they are indistinguishable in fruit type, seed size, root type and herbivore defences. The principal ecological feature that distinguishes geoxyles from their close relatives is their large difference in height ( $V=378$ ,  $P<0.01$ ). However, geoxyles differ significantly in their



**Fig. 6** Scatter plot of divergence times (million years ago (Ma)) between geoxyle life-forms and their tree sister groups against the latitudinal midpoint of the geoxyle geographical distributions ( $n = 36$ ). Horizontal error bars represent latitudinal range extents of geographical distributions; vertical error bars indicate maximum and minimum divergence time estimates between sister groups from the Bayesian posterior distribution of dated trees.

**Table 2** Sister group contrasts between geoxyle life-forms and their tree relatives for various ecological and climatic variables

Variable	Test	Test statistic	<i>P</i> value
Height	Wilcoxon	$V = 378$	$< 0.01$
Spines	McNemar	NA	ns
Poisonous	McNemar	NA	ns
Fruit type	McNemar	NA	ns
Seed size	Wilcoxon	$V = 78.5$	0.78
Mean fire frequency	Wilcoxon	$V = 57$	0.02
Mean annual precipitation	Wilcoxon	$V = 31$	$< 0.01$
Mean annual temperature	Wilcoxon	$V = 133$	0.85
Mean elevation	Wilcoxon	$V = 133$	0.85

McNemar, McNemar's  $\chi^2$  test; NA, not available; ns, not significant; Wilcoxon, Wilcoxon signed rank test.

geographical distribution, being found in regions characterized by higher average rainfall ( $V = 31$ ,  $P < 0.01$ ) and greater fire frequency ( $V = 57$ ,  $P = 0.02$ ) than their tree relatives (Table 2). Furthermore, we observe a striking latitudinal gradient in divergence times (Fig. 6). The mean age of evolutionary splits is significantly younger moving towards more southerly latitudes ( $F = 9.10$ ,  $P = 0.005$ ,  $r^2 = 0.23$  for the phylogenetic regression between latitude and log age implemented in the caper R library (Orme *et al.*, 2012) and allowing lambda to take its ML value). However, the correlation is driven by an absence of older splits at high southern latitudes, whereas, at more tropical latitudes, we find both young and old divergences.

In the supertree of the woody taxa of the cerrado and African savanna (Fig. S1), we find that, unsurprisingly, there is less phylogenetic overlap between the two floras than expected by chance (PhyloSor index = 0.15 in comparison with the null expectation of 0.266, min = 0.246, max = 0.284). However, although cerrado taxa show phylogenetic under-dispersion (NRI = 1.747,  $P = 0.038$ ), perhaps reflecting some *in situ* radiations within a few lineages, the reverse is not true; African savanna taxa are not significantly clustered on the phylogeny (NRI = 0.339,  $P > 0.05$ ).

## Discussion

We have shown that the geoxyle habit evolved independently multiple times in Africa; however, it is always associated with the savanna biome. If we can identify the driving forces in the evolution of this life-form, we might, therefore, also provide insights into the origin and spread of savanna.

Previous work has suggested that shifts from forest to savanna were probably frequent in the South American cerrado (Pennington *et al.*, 2006; Simon *et al.*, 2009). Here, we show that African savanna trees represent an even more phylogenetically dispersed set of taxa, suggesting that shifts from the forest to savanna biome in Africa were at least as frequent. A paucity of endemic genera in both cerrado (Simon *et al.*, 2009) and African savanna further supports parallels in the evolutionary origins of these biomes and a history of frequent shifts from forest to savanna with only few radiations within the savanna biome (see also Oliveira-Filho *et al.*, 2013). In addition, we find that evolutionary divergences between African savanna trees are younger than those between forest trees. If we assume that evolutionary splits between extant species both occupying the same habitat type indicate a shared ancestor within that habitat, our results support a relatively young age for the African savanna biome – that is an absence of older splits between African savanna trees reflects limits to the upper age of the biome. Geoxyles have a median divergence time of 2.28 Ma, but with origins of many taxa dated to within the last 2 million yr, perhaps indicative of a more recent expansion of savanna. Further, we find a latitudinal gradient in age of geoxyle origins in Africa, with an absence of older ages in the south. If the evolution of the geoxyle life-form can be used as a marker for the presence of fire-maintained savanna ecosystems, our results would suggest a gradual expansion of African savanna from the equator to higher latitudes over the past few million years.

## The evolution of 'underground trees'

Four major factors have, at various times, been put forward as evolutionary drivers of the geoxyle habit in Africa. Burt Davy (1922) hypothesized that winter frost on the South African Highveld was the main factor responsible for woody plant species escaping underground; in the South African context, cold temperatures may well have been a contributing factor to the evolution of underground trees. It is also possible that some woody species evolved the geoxyle habit in response to mammal herbivory, particularly on the grassy plains of the South African Highveld (Steenkamp *et al.*, 2001). Africa, with its historically vast herds of antelope and other grazing ungulates, would have had much of its open areas subjected to incidents of severe herbivory. However, the region of greatest geoxyle diversity, the northern areas of the Zambezi Domain (White, 1983), receives little or no frost and the Zambezi Region is dominated by miombo woodlands, a habitat type noted for its paucity of herbivores as a result of the poor nutritional quality of its forage (Frost, 1996), and a long, harsh dry season of almost 7 months (Rodgers *et al.*, 1996). Further, we find no general relationship between the geoxyle life-form and temperature or elevation, and the prevalence of herbivore defences (e.g. presence of spines or poisons) does not differ between geoxyles and their tree relatives. It would seem, therefore, that neither frost nor herbivory has been the major selective pressure in the evolution of the geoxyle habit.

Frank White, in his landmark paper on the underground forests of Africa (White, 1976), attributed the rise of the geoxyle habit mainly to edaphic factors. He pointed out that the open grassy edaphic seasonal wetlands, which are a characteristic feature of the south-central African landscape, support a number of geoxyles, particularly along the margins of these open wetlands or 'dambos'. These seasonally waterlogged grassy depressions often occur on sandy, oligotrophic soils, which are anaerobic when waterlogged. However, although a handful of geoxyles (*Ficus pygmaea*, *Syzygium guineense* ssp. *huillense*, *Erythrina baumii*, *Protea baumii*) are confined to dambos, the majority are not. For example, the region of high geoxyle diversity in north-eastern South Africa (this paper) have no endemic wetland geoxyle species, whereas, in the geoxyle-rich coastal grasslands of the Maputaland region, the geoxyles grow away from the numerous wetland depressions (Matthews *et al.*, 1999). Notably, these grassy dambos are subjected to frequent burning, and it is possible that the open, sunny environment of the dambos would also provide advantages to a dwarf woody plant that would otherwise be shaded in the taller adjacent woodlands. This link with fire brings us to our final putative driver of the geoxyle habit.

Geoxyles in Africa are restricted to savanna habitats or upland grasslands (geoxyles are absent from closed forests). They occur almost exclusively in higher rainfall savannas with frequent fires (White, 1979). Indeed, the seasonal or regular burning of the woodlands and grasslands of Africa is

the most often quoted factor driving the evolution of the geoxylic suffrutex habit in Africa (White, 1976 (in part); Vollesen, 1981; Lock, 1998, 2006; Matthews *et al.*, 1999), as well as in the South American cerrado (Simon *et al.*, 2009; Simon & Pennington, 2012). Although the majority of savanna trees have developed features to withstand fire (thick bark, fire-resistant shoots), geoxyles may have escaped fire by developing their woody component below ground, thus minimizing their resource input into annual vegetative growth to the benefit of flower and fruit production. Most conclusive is the fact that it is clear that, wherever geoxylic suffrutices occur in Africa, fire is a regular feature in the landscape. Our results show that the occurrence of the geoxyle life-form correlates significantly with fire and precipitation, suggesting that both may have contributed to driving trees underground.

How would precipitation and fire select for trees developing underground? Areas of high precipitation should favour forest development, and the proportion of African landscapes covered by forest increases along a precipitation gradient (Lehmann *et al.*, 2011; Staver *et al.*, 2011a). However, greater precipitation also correlates with high grass productivity, thus favouring frequent fire occurrence that maintains the savanna state (Bond, 2008; Lehmann *et al.*, 2011; Staver *et al.*, 2011a,b). Fire is a major selective pressure. Population studies have shown that a key demographic bottleneck for savanna trees is the transition from juvenile plants growing below the flame zone to adult plants taller than flame height and resistant to fire damage. Saplings may persist for decades in the flame zone (the 'firetrap') without growing into mature trees (Trollope, 1984; Scholes & Archer, 1997; Williams *et al.*, 1999; Higgins *et al.*, 2000, 2007; Werner *et al.*, 2006; Prior *et al.*, 2009; Werner & Franklin, 2010; Bond *et al.*, 2012; Werner & Prior, 2013).

We suggest that the geoxylic growth form is advantageous in areas experiencing the interactive effects of frequent fires and high precipitation. The underground trees of Africa can be regarded as markers of fire-maintained savannas occurring in climates suitable for forests (extensive African savannas also occur in arid climates where fires are rare; Lehmann *et al.*, 2011). Poor growing conditions that reduce growth rates of juvenile trees would also result in reduced probabilities of reaching fire-proof sizes and transitioning to tall mature trees. Geoxyles are common where site conditions reduce growth rates, such as on seasonally waterlogged and/or low-nutrient soils, or, in South Africa, at high-altitude sites with cold winters (White, 1979). Thus, geoxyles appear to be an example of heterochrony (a change in the relative timing and/or rate of developmental processes; Li & Johnston, 2000) with ancestral tree growth forms adopting a dwarf stature from which, unlike their tree ancestors, they are able to flower and fruit.

## Fire and the savanna biome

Our prime aim in this study was to use the origin of geoxyles to help date the emergence and spread of fire-dependent



African savannas. However, the limited literature on geoxyles, especially in Africa, raises other questions, including the general nature of fire adaptations in savannas. Fire-adaptive traits have been extensively studied in crown fire regimes, especially in Mediterranean-type shrublands (Keeley *et al.*, 2012). Fire-adaptive traits in the surface fire regimes of savannas are not nearly as well studied or understood. As noted by White (1979), geoxyles appear to be a convergent life-form in South American and African savannas. The link with fire in cerrado has been elaborated by Simon *et al.* (2009) and Simon & Pennington (2012). For African savannas, we have argued above that the growth form has evolved in response to the selective pressures imposed by frequent grass-fuelled fires and soil or climatic conditions that slow woody growth rates. Curiously, geoxyles are absent in northern Australian savannas despite similar fire regimes and soils to those of Africa and Brazil (White, 1979). They have also not been reported, perhaps because they have not been recognized, in the higher rainfall savannas of South and South-East Asia (White, 1979) or the pine savannas of North America (Noss, 2012). Within Africa, White noted a striking difference in the richness and abundance of geoxyles in the savannas of the Zambesian region (this study) contrasting with savannas of the Sudanian region (north of the equator) where they are rare and poor in species. The patchy occurrence of geoxyles in savannas from different geographical regions is perhaps to be expected given the youth of the biome. As noted above, both African savannas and the cerrado have few if any endemic genera, consistent with a young age for the biome in both regions (Simon *et al.*, 2009; this study). The coincidence of a largely Pliocene age for flammable savanna origins in Africa and cerrado is striking. The causes of the abrupt and apparently near-simultaneous assembly and spread of these fire-dependent savannas are unknown, but are an area of active research (Keeley & Rundel, 2005; Scheiter *et al.*, 2012).

## Conclusions

We explored the origin of mesic savannas in Africa using geoxyllic suffrutices, White's underground forests of Africa, as markers for fire-maintained ecosystems. Our results suggest that these savannas first appeared in the tropics with more recent speciation at lower latitudes in southern Africa. Dates of origin of the geoxyles are mostly from the Pliocene (<5.3 Ma), consistent with the appearance of woody fire-maintained cerrado plants in Brazil (Simon *et al.*, 2009; Simon & Pennington, 2012). As the taphonomy of fossil savanna sites biases against the detection of fire-maintained savannas, we suggest that phylogenetic approaches are particularly useful in tracing the origin of more humid savannas. As more complete phylogenies become available for clades from the African fire-maintained savannas, and as more species are sampled for fire-adaptive traits, more refined dates of origin should become available. Nevertheless, this study provides the first evidence for dates of emergence of higher rainfall savannas in Africa, and supports the role of fire in their origins. Furthermore,

our study suggests independent origins of this growth form from those of Brazilian cerrado. The fire frequencies characteristic of seasonally humid savannas are perhaps the highest in Earth's history. The diverse responses of plants to this extreme selective regime warrant further study.

## Acknowledgements

We thank the Government of Canada through Genome Canada and the Ontario Genomics Institute (2008-OGI-ICI-03), the International Development Research Centre (IDRC), Canada and the University of Johannesburg for financial support, and various local and international authorities who granted plant collection permits. We thank Mike Bingham and Sandie Burrows for valuable discussions during the preparation of the manuscript. We thank two anonymous reviewers for comments on an earlier draft of the manuscript.

## References

- Angiosperm Phylogeny Group. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* 161: 105–121.
- Appenzato-da-Glória B, Cury G, Kasue Misaki Soares M, Rocha R, Hissae Hayashi A. 2008. Underground systems of Asteraceae species from the Brazilian Cerrado 1. *The Journal of the Torrey Botanical Society* 135: 103–113.
- Arakaki M, Christin P-A, Nyffeler R, Lendel A, Eggli U, Ogburn RM, Spriggs E, Moore MJ, Edwards EJ. 2011. Contemporaneous and recent radiations of the world's major succulent plant lineages. *Proceedings of the National Academy of Sciences, USA* 108: 8379–8384.
- Archibald S, Lehmann CE, Gómez-Dans JL, Bradstock RA. 2013. Defining pyromes and global syndromes of fire regimes. *Proceedings of the National Academy of Sciences, USA* 110: 6442–6447.
- Archibald S, Scholes RJ, Roy DP, Roberts G, Boschetti L. 2010. Southern African fire regimes as revealed by remote sensing. *International Journal of Wildland Fire* 19: 861–878.
- Barraclough TG, Nee S, Harvey PH. 1998. Sister-group analysis in identifying correlates of diversification – comment. *Evolutionary Ecology* 12: 751–754.
- Beerling DJ, Osborne CP. 2006. The origin of the savanna biome. *Global Change Biology* 12: 2023–2031.
- Beerling DJ, Royer DL. 2011. Earth's atmospheric CO<sub>2</sub> history by proxy. *Nature Geoscience* 4: 418–420.
- Bell CD, Soltis DE, Soltis PS. 2010. The age and diversification of the angiosperms re-visited. *American Journal of Botany* 97: 1296–1303.
- Bininda-Emonds O. 2005. TransAlign: using amino acids to facilitate the multiple alignment of protein-coding DNA sequences. *BMC Bioinformatics* 6: 156.
- Bond WJ. 2008. What limits trees in C<sub>4</sub> grasslands and savannas? *Annual Review of Ecology, Evolution and Systematics* 39: 641–659.
- Bond WJ, Cook G, Williams RJ. 2012. Which trees dominate in savannas? The escape hypothesis and eucalypts in northern Australia. *Austral Ecology* 37: 678–685.
- Bond WJ, Midgley GF. 2000. A proposed CO<sub>2</sub>-controlled mechanism of woody plant invasion in grasslands and savannas. *Global Change Biology* 6: 865–870.
- Bond WJ, Midgley GF. 2012. CO<sub>2</sub> and the uneasy interactions of trees and savanna grasses. *Philosophical Transactions of the Royal Society of London Series B* 367: 601–612.
- Bond WJ, Midgley GF, Woodward FI. 2003. What controls South African vegetation – climate or fire? *South African Journal of Botany* 69: 79–91.
- Bond WJ, Woodward FI, Midgley GF. 2005. The global distribution of ecosystems in a world without fire. *New Phytologist* 165: 525–538.
- Boon D. 2010. *Pooley's trees of Eastern South Africa – a complete guide*. Durban, South Africa: Flora and Fauna Publication Trust.

- Bouchenak-Khelladi Y, Muasya AM, Linder HP. 2014. A revised evolutionary history of Poales: origins and diversification. *Botanical Journal of the Linnean Society* 175: 4–16.
- Britton T, Anderson CL, Jacquet D, Lundqvist S, Bremer K. 2007. Estimating divergence times in large phylogenetic trees. *Systematic Biology* 56: 741–752.
- Bryant JB, Lamanna C, Morlon H, Kerkhoff AJ, Enquist BJ, Green JL. 2008. Microbes on mountainsides: contrasting elevational patterns of bacterial and plant diversity. *Proceedings of the National Academy of Sciences, USA* 105: 11505–11511.
- Burt J. 1922. The suffrutescent habit as an adaptation to environment. *Journal of Ecology* 10: 211–219.
- Bytebier B, Antonelli A, Bellstedt DU, Linder HP. 2011. Estimating the age of fire in the Cape flora of South Africa from an orchid phylogeny. *Proceedings of the Royal Society Series B* 278: 188–195.
- Cantino PD, Doyle JA, Graham SW, Judd WS, Olmstead RG, Soltis DE, Soltis PS, Donoghue MJ. 2007. Towards a phylogenetic nomenclature of Tracheophyta. *Taxon* 56: 822–846.
- CBOL Plant Working Group. 2009. A DNA barcode for land plants. *Proceedings of the National Academy of Sciences, USA* 106: 12794–12797.
- Cerling TE, Harris JM, McFadden BJ, Leakey MG, Quade J, Eisenmann V, Ehleringer JR. 1997. Global vegetation change through the Miocene/Pliocene boundary. *Nature* 389: 153–158.
- Christin PA, Besnard G, Samaritani E, Duvall MR, Hodkinson TR, Savolainen V, Salamin N. 2008. Oligocene CO<sub>2</sub> decline promoted C<sub>4</sub> photosynthesis in grasses. *Current Biology* 18: 37–43.
- Christin PA, Spriggs E, Osborne CP, Strömberg CAE, Salamin N, Edwards EJ. 2014. Molecular dating, evolutionary rates, and the age of the grasses. *Systematic Biology* 63: 153–165.
- Chuvieco E, Giglio L, Justice C. 2008. Global characterization of fire activity: toward defining fire regimes from Earth observation data. *Global Change Biology* 14: 1488–1502.
- Coates Palgrave M. 2002. *Keith Coates Palgrave trees of southern Africa*, 3<sup>rd</sup> edn. Cape Town, South Africa: Struik.
- Cole M. 1986. *Savannas: biogeography and geobotany*. London, UK: Academic Press.
- Collinson AS. 1988. *Introduction to world vegetation*, 2<sup>nd</sup> edn. London, UK: Unwin Hyman Ltd.
- Coutinho LM. 1982. Ecological effects of fire in Brazilian cerrado. In: Huntley BJ, Walker BH, eds. *Ecology of tropical savannas*. Berlin, Germany: Springer, 273–291.
- Coutinho LM. 1990. Fire in the ecology of the Brazilian cerrado. In: Goldammer JG, ed. *Fire in the tropical biota – ecosystem process and global challenges*. Berlin, Germany: Springer, 82–105.
- Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7: 214.
- Edwards EJ, Osborne CP, Stromberg CAE, Smith SA, the C<sub>4</sub> Grasses Consortium. 2010. The origins of C<sub>4</sub> grasslands: integrating evolutionary and ecosystem science. *Science* 328: 587–591.
- Ehleringer JR. 1978. Implications of quantum yield differences on the distributions of C<sub>3</sub> and C<sub>4</sub> grasses. *Oecologia* 31: 255–267.
- Ehleringer JR, Cerling TE, Helliker BR. 1997. C<sub>4</sub> photosynthesis, atmospheric CO<sub>2</sub>, and climate. *Oecologia* 112: 285–299.
- Felsenstein J. 1985. Phylogenies and the comparative method. *The American Naturalist* 125: 1–15.
- Frost P. 1996. The ecology of miombo woodlands. In: Campbell B, ed. *The miombo in transition: woodlands and welfare in Africa*. Bogor, Indonesia: Centre for International Forestry Research, 11–55.
- Germishuizen G, Meyer NL. 2013. Plants of southern Africa: an annotated checklist. *Strelitzia*, vol. 14. Pretoria, South Africa: National Botanical Institute.
- Hajibabaei M, De Waard JR, Ivanova NV, Ratnasingham S, Dooh RT, Kirk SL, Mackie PM, Hebert PDN. 2005. Critical factors for assembling a high volume of DNA barcodes. *Philosophical Transactions of the Royal Society London Series B* 360: 1959–1967.
- He T, Lamont BB, Downes KS. 2011. *Banksia* born to burn. *New Phytologist* 191: 184–196.
- Herring JR. 1985. Charcoal fluxes into sediments of the North Pacific Ocean: the Cenozoic record of burning. In: Sundquist ET, Broecker WS, eds. *The carbon cycle and atmospheric CO<sub>2</sub>: natural variations Archaeal to present*. Washington DC, USA: American Geophysical Union, 32, 419–422.
- Higgins SI, Bond WJ, February EC, Bronn A, Euston-Brown DIW, Enslin B, Govender N, Rademan L, O'Regan S, Potgieter ALF *et al.* 2007. Effects of four decades of fire manipulation on woody vegetation structure in savanna. *Ecology* 88: 1119–1125.
- Higgins SI, Bond WJ, Trollope WSW. 2000. Fire, resprouting and variability: a recipe for tree–grass coexistence in savanna. *Journal of Ecology* 88: 213–229.
- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965–1978.
- Hirota M, Holmgren M, Van Nes EH, Scheffer M. 2011. Global resilience of tropical forest and savanna to critical transitions. *Science* 334: 232–235.
- Hoetzel S, Dupont L, Schefuß E, Rommerskirchen F, Wefer G. 2013. The role of fire in Miocene to Pliocene C<sub>4</sub> grassland and ecosystem evolution. *Nature Geoscience* 6: 1027–1030.
- Hoffmann WA, Adasme R, Haridasan M, Carvalho M, Geiger EL, Pereira MAB, Gotsch SG, Franco AC. 2009. Tree topkill, not mortality, governs the dynamics of alternate stable states at savanna forest boundaries under frequent fire in central Brazil. *Ecology* 90: 1326–1337.
- Hoffmann WA, Geiger EL, Gotsch SG, Rossatto DR, Silva LCR, Lau OL, Haridasan M, Franco AC. 2012. Ecological thresholds at the savanna–forest boundary: how plant traits, resources and fire govern the distribution of tropical biomes. *Ecology Letters* 15: 759–768.
- Hoffmann WA, Orthen B, Franco AC. 2004. Constraints to seedling success of savanna and forest trees across the savanna–forest boundary. *Oecologia* 140: 252–260.
- Hoffmann WA, Orthen B, Nascimento PKV. 2003. Comparative fire ecology of tropical savanna and forest trees. *Functional Ecology* 17: 720–726.
- Ivanova NV, Fazekas AJ, Hebert PDN. 2008. Semi-automated membrane based protocol for DNA isolation from plants. *Plant Molecular Biology Reporter* 26: 186–198.
- Keeley JE, Bond WJ, Bradstock RA, Pausas JG, Rundel PW. 2012. *Fire in Mediterranean ecosystems: ecology, evolution and management*. Cambridge, UK: Cambridge University Press.
- Keeley JE, Rundel PW. 2005. Fire and the Miocene expansion of C<sub>4</sub> grasslands. *Ecological Letters* 8: 683–690.
- Kellman M. 1984. Synergistic relationships between fire and low soil fertility in neotropical savannas: a hypothesis. *Biotropica* 16: 158–160.
- Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26: 1463–1464.
- Kgope BS, Bond WJ, Midgley GF. 2010. Growth responses of African savanna trees implicate atmospheric CO<sub>2</sub> as a driver of past and current changes in savanna tree cover. *Austral Ecology* 35: 451–463.
- Lehmann CER, Archibald SA, Hoffmann WA, Bond WJ. 2011. Deciphering the distribution of the savanna biome. *New Phytologist* 191: 197–209.
- Li P, Johnston MO. 2000. Heterochrony in plant evolutionary studies through the twentieth century. *The Botanical Review* 66: 57–88.
- Lock JM. 1998. Aspects of fire in tropical African vegetation. In: Huxley CR, Lock JM, Cutler DF, eds. *Chorology, taxonomy and ecology of the floras of Africa and Madagascar*. Kew, UK: Royal Botanic Gardens, 181–189.
- Lock JM. 2006. The seasonally dry vegetation of Africa: parallels and comparisons with the neotropics. In: Pennington RT, Lewis GP, Ratter JA, eds. *Neotropical savannas and seasonally dry forests: plant diversity, biogeography and conservation*. London, UK: CRC Press, 450–465.
- Matthews WS, van Wyk AE, van Rooyen N. 1999. Vegetation of the Sileza Nature Reserve and neighbouring areas, South Africa, and its importance in conserving woody grasslands of the Maputland Centre of Endemism. *Bothalia* 29: 151–167.
- Midgley J, Bond WJ. 2011. Pushing back in time: the role of fire in plant evolution. *New Phytologist* 191: 5–7.
- Miller MA, Holder MT, Vos R, Midford PE, Liebowitz T, Chan L, Hoover P, Warnow T. 2009. *The CIPRES portals*. [WWW document] URL [http://www.phylo.org/sub\\_sections/portal](http://www.phylo.org/sub_sections/portal) [accessed November 2012].



- Morley RJ, Richards K. 1993. Gramineae cuticle: a key indicator of Late Cenozoic climatic change in the Niger Delta. *Review of Palaeobotany and Palynology* 77: 119–127.
- Mucina L, Rutherford MC. 2006. *The vegetation of South Africa, Lesotho and Swaziland*. Pretoria, South Africa: South African National Biodiversity Institute.
- Murphy BP, Bowman DMJS. 2012. What controls the distribution of tropical forest and savanna? *Ecology Letters* 15: 748–758.
- Noss RF. 2012. *Forgotten grasslands of the South: natural history and conservation*. Washington, DC, USA: Island Press.
- Nylander JAA. 2004. *Modeltest v2*. Program distributed by the author. Uppsala, Sweden: Evolutionary Biology Centre, Uppsala University.
- Oliveira-Filho AT, Cardoso D, Schrire BD, Lewis GP, Pennington RT, Brummer TJ, Rotella J, Lavin M. 2013. Stability structures tropical woody plant diversity more than seasonality: insights into the ecology of high legume-succulent-plant biodiversity. *South African Journal of Botany* 89: 42–57.
- Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W. 2012. *caper: comparative analyses of phylogenetics and evolution in R*. R package version 0.5. [WWW document] URL <http://CRAN.R-project.org/package=caper> [accessed November 2012].
- Osborne CP. 2008. Atmosphere, ecology and evolution: what drove the Miocene expansion of  $C_4$  grasslands? *Journal of Ecology* 96: 35–45.
- Pagani M, Freeman KH, Ohkouchi N, Caldeira K. 2002. Comparison of water column  $[CO_{2aq}]$  with sedimentary alkenone-based estimates: a test of the alkenone- $CO_2$  proxy. *Paleoceanography* 17: 21–1–21–12.
- Pennington RT, Richardson JE, Lavin M. 2006. Insights into the historical construction of species-rich biomes from dated plant phylogenies, neutral ecological theory and phylogenetic community structure. *New Phytologist* 172: 605–616.
- Plants of Southern Africa. 2012. *Native plants of southern Africa*. South African National Biodiversity Institute. [WWW document] URL [www.plantzafrica.com](http://www.plantzafrica.com) [accessed November 2012].
- Prior LD, Murphy BP, Russell-Smith J. 2009. Environmental and demographic correlates of tree recruitment and mortality in north Australian savannas. *Forest Ecology and Management* 257: 66–74.
- Ratnam J, Bond WJ, Fensham RJ, Hoffmann WA, Archibald S, Lehmann CE, Sankaran M. 2011. When is a 'forest' a savanna, and why does it matter? *Global Ecology and Biogeography* 20: 653–660.
- Ratter JA, Bridgewater S, Ribeiro JF, Fonseca-Filho J, Rodrigues da Silva M, Milliken W, Pullan M, Pott A, Oliveira-Filho A, Durigan G *et al.* 2012. *Analysis of the floristic composition of the Brazilian cerrado vegetation IV*. Presentation of a Revised Data-Base of 367 Areas. [WWW document] URL <http://www.cerrado.rbge.org.uk> [accessed 20 November 2012].
- Rodgers A, Salehe J, Howard G. 1996. The biodiversity of miombo woodlands. In: Campbell B, ed. *The miombo in transition: woodlands and welfare in Africa*. Bogor, Indonesia: Centre for International Forestry Research, 12.
- Sage RF. 2001. Environmental and evolutionary preconditions for the origin and diversification of the  $C_4$  photosynthetic syndrome. *Plant Biology* 3: 202–213.
- Sage RF. 2004. The evolution of  $C_4$  photosynthesis. *New Phytologist* 161: 341–370.
- Scheiter S, Higgins SI, Osborne CP, Bradshaw C, Lunt D, Ripley BS, Taylor LL, Beerling DJ. 2012. Fire and fire-adapted vegetation promoted  $C_4$  expansion in the late Miocene. *New Phytologist* 195: 653–666.
- Schmidt E, Lotter M, McClelland W. 2007. *Trees and shrubs of Mpumalanga and Kruger National Park*. Johannesburg, South Africa: Jacana Education.
- Scholes RJ, Archer SR. 1997. Tree–grass interactions in savannas. *Annual Review of Ecology and Systematics* 28: 517–544.
- Simon MF, Grether R, de Queiroz LP, Skema C, Pennington RT, Hughes CE. 2009. Recent assembly of the Cerrado, a Neotropical plant diversity hotspot, by *in situ* evolution of adaptations to fire. *Proceedings of the National Academy of Sciences, USA* 106: 20359–20364.
- Simon MF, Pennington T. 2012. Evidence for adaptation to fire regimes in the tropical savannas of the Brazilian Cerrado. *International Journal of Plant Sciences* 173: 711–723.
- Soltis DE, Smith SA, Cellinese N, Wurdack KJ, Tank DC, Brockington SF, Refulio-Rodriguez NF, Walker JB, Moore MJ, Carlswald BS *et al.* 2011. Angiosperm phylogeny: 17 genes, 640 taxa. *American Journal of Botany* 98: 704–730.
- Stamatakis A, Hoover P, Rougemont J. 2008. A rapid Bootstrap Algorithm for the RAxML Web-Servers. *Systematic Biology* 75: 758–771.
- Staver AC, Archibald S, Levin SA. 2011a. The global extent and determinants of savanna and forest as alternative biome states. *Science* 334: 230–232.
- Staver AC, Archibald S, Levin SA. 2011b. Tree cover in sub-Saharan Africa: rainfall and fire constrain forest and savanna as alternative stable states. *Ecology* 92: 1063–1072.
- Steenkamp Y, Kellerman MJS, van Wyk AE. 2001. Fire, frost, waterlogged soil or something else: what selected for the geoxylic suffrutex form in Africa? *PlantLife* 25: 4–6.
- Strömberg CAE. 2005. Decoupled taxonomic radiation and ecological expansion of open-habitat grasses in the Cenozoic of North America. *Proceedings of the National Academy of Sciences, USA* 102: 11980–11984.
- Strömberg CAE. 2011. Evolution of grass and grassland ecosystems. *Annual Review of Earth and Planetary Sciences* 39: 517–544.
- Trollope WSW. 1984. Fire in savanna. In: Booysen PV, Tainton NM, eds. *Ecological effects of fire in South African ecosystems*. Berlin, Germany: Springer-Verlag, 149–176.
- Van Wyk B, van der Berg E, Coates Palgrave M, Jordaan M. 2011. *Dictionary of names for southern African trees, 1<sup>st</sup> edn*. Pretoria, South Africa: Briza.
- Vollesen K. 1981. *Catunaregam pygmaea* (Rubiaceae) – a new geoxylic suffrutex from the woodlands of SE Tanzania. *Nordic Journal of Botany* 1: 735–740.
- Webb CO, Ackerly DD, Kembel SW. 2008. Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics* 18: 2098–2100.
- Webb CO, Ackerly DD, McPeck MA, Donoghue MJ. 2002. Phylogenies and community ecology. *Annual Review of Ecology and Systematics* 33: 475–505.
- Webb CO, Donoghue MJ. 2005. Phylomatic: tree assembly for applied phylogenetics. *Molecular Ecology Notes* 5: 181–183.
- van der Werf GR, Randerson JT, Giglio L, Collatz GJ, Mu M, Kasibhatla PS, Morton DC, DeFries RS, Jin Y, van Leeuwen TT. 2010. Global fire emissions and the contribution of deforestation, savanna, forest, agricultural, and peat fires (1997–2009). *Atmospheric Chemistry and Physics* 10: 11707–11735.
- Werner PA, Cowie ID, Cusack JS. 2006. Juvenile tree growth and demography in response to feral water buffalo in savannas of northern Australia: an experimental field study in Kakadu National Park. *Australian Journal of Botany* 54: 283–296.
- Werner PA, Franklin DC. 2010. Resprouting and mortality of juvenile eucalypts in an Australian savanna: impacts of fire season and annual sorghum. *Australian Journal of Botany* 58: 619–628.
- Werner PA, Prior LD. 2013. Demography and growth of subadult savanna trees: interactions of life history, size, fire season, and grassy understory. *Ecological Monographs* 83: 67–93.
- White F. 1976. The underground forests of Africa: a preliminary review. *The Gardens' Bulletin, Singapore* 24: 57–71.
- White F. 1979. The Guineo-Congolian Region and its relationship to other phytochoria. *Bulletin Jardin Botanique National de Belgique* 49: 11–55.
- White F. 1983. *The vegetation of Africa. A descriptive memoir to accompany the Unesco/AETFAT/UNSO vegetation map of Africa*. Paris, France: Unesco.
- Wikström N, Savolainen V, Chase MW. 2001. Evolution of the angiosperms: calibrating the family tree. *Proceedings of the Royal Society Series B* 268: 2211–2220.
- Wilcoxon F. 1945. Individual comparisons by ranking methods. *Biometrics Bulletin* 1: 80–83.
- Williams RJ, Cook GD, Gill AM, Moore PHR. 1999. Fire regime, fire intensity and tree survival in a tropical savanna in Northern Australia. *Australian Journal of Ecology* 24: 50–59.

## Supporting Information

Additional supporting information may be found in the online version of this article.

**Fig. S1** Phylogenetic distribution of Cerrado and Savanna taxa on the Phylomatic supertree with branch lengths scaled to millions of years.

**Table S1** Calibration points and age constraints used in divergence time estimations

**Notes S1** Provisional list of African geoxylic suffrutices occurring south of the Equator.

**Notes S2** List of taxa included in phylogeny with voucher information and GenBank accession numbers.

Please note: Wiley Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.



## About New Phytologist

- *New Phytologist* is an electronic (online-only) journal owned by the New Phytologist Trust, a **not-for-profit organization** dedicated to the promotion of plant science, facilitating projects from symposia to free access for our Tansley reviews.
- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as ready' via *Early View* – our average time to decision is <25 days. There are **no page or colour charges** and a PDF version will be provided for each article.
- The journal is available online at Wiley Online Library. Visit **www.newphytologist.com** to search the articles and register for table of contents email alerts.
- If you have any questions, do get in touch with Central Office (np-centraloffice@lancaster.ac.uk) or, if it is more convenient, our USA Office (np-usaoffice@lancaster.ac.uk)
- For submission instructions, subscription and all the latest information visit **www.newphytologist.com**