Citation:
Retief, K., West, A.G. and Pfab, M.F. (in press) Can stable isotopes and radiocarbon dating provide a forensic solution for curbing illegal harvesting of threatened cycads? *Journal of Forensic Sciences*. DOI: 10.111/1556-4029.12644

Can stable isotopes and radiocarbon dating provide a forensic solution for curbing illegal harvesting of threatened cycads?

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Accepted article, in press, *Journal of Forensic Sciences*.

Author contributions: AW, MP and KR designed experiment, KR conducted research, AW, MP and KR wrote manuscript.

We acknowledge the South African National Biodiversity Institute and the National Research Foundation for financial assistance.

ABSTRACT

Cycads in South Africa are facing an extinction crisis due to the illegal extraction of plants from the wild. Proving wild origin of suspect *ex situ* cycads to the satisfaction of a court of law is difficult, limiting law enforcement efforts. We investigated the feasibility of using multiple stable isotopes to identify specimens removed from the wild. Relocated and wild specimens from the African genus *Encephalartos* were sampled: *E. lebomboensis* and *E. arenarius.* ^{14}C analysis indicated that a ±30 year chronology could be reliably obtained from the cycads. For *E. arenarius*, pre-relocation tissue was consistent with a wild origin, whereas tissue grown post-relocation was isotopically distinct from the wild for $^{87}Sr/^{86}Sr$ and $\delta^{15}N$. For *E. lebomboensis,* $\delta^{34}S$, $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ were different between relocated and control plants, consistent with the >30 years since relocation. Our findings demonstrate the potential for a forensic isotope approach to identify illegal *ex situ* cycads.

KEYWORDS

forensic science, stable isotope analysis, radiocarbon dating, principal component analysis, illicit trade, *Encephalartos*, conservation, provenance, poaching, strontium

South Africa is the centre of African cycad diversity, containing more than half of the species distributed throughout Africa (1). The African genus *Encephalartos* consists of 66 species (2), of which 29 species are endemic to South Africa (3, 4). Yet, *Encephalartos* species are the most threatened group of plants in South Africa, with 78% of the 37 species classified as threatened (4). Three species are already extinct in the wild and 12 species are classified as Critically Endangered and will shortly face extinction if the current pace of wild plant removal continues (2). Illegal harvesting of wild cycads for private collections and landscaping purposes has resulted in numerous species being pushed towards the brink of extinction and is a significant conservation issue in South Africa (1, 5). Cycads are long-lived plants and take decades to reach a desirable size, promoting the removal of already established plants from the wild (1). Furthermore, cycads become more desirable to collectors as they become rarer, causing inflated values for rare cycads as the cost of finding wild cycads increases (6).

There has been a substantial increase in illegally harvested wild cycads entering the legal cycad market since the early 1990s (3, 5). The strict legislation intended to protect wild cycads is largely ineffective at present, as law enforcement agencies lack capacity to curb illegal removal of wild plants. Additionally, once wild cycads reach collectors, it is extremely difficult to prove that suspect cycads originated from the wild to the satisfaction of a court of law.

The advancement of technology in biodiversity conservation offers potential novel tools for law enforcers to use in regulating legal trade and combating the illegal trade. For example, the use of micro-chipping to mark cycads in the wild has had some success in apprehending and prosecuting poachers and collectors who have obtained these specimens illegally. However, significant drawbacks of this technique are that each cycad needs to be marked prior to the poaching event and many poachers detect the micro-chips using X-ray scanners and then remove them (7). DNA barcoding is useful to confirm species identity and determine which populations they originated from (7-9), thereby identifying genetic origin for investigations (8). Yet, genetic studies have failed to provide conclusive evidence of a wild versus cultivated origin of sampled collections. What is needed is a forensic technique that does not require pre-marking of wild individuals, cannot be removed or altered by the collector and is able to distinguish between wild versus cultivated origins for many years after the poaching event. In this study we explore the potential of using the stable isotopic composition of cycad tissues as such a forensic technique.

The use of stable isotopes in courts is most effective when reinforcing information or providing additional evidence in case investigations (10). This method will potentially improve enforcement and compliance within the cycad trade by targeting the end users, who are often far removed from the poaching events, thereby decreasing the demand for wild mature plants and contributing to the conservation of cycads in the wild. Using stable isotopes as a forensic tracer is potentially invaluable, because poachers are unable to remove the isotopic signature within the plant tissue that grew in the wild, as is currently occurring with micro-chips. Prosecution for possession or removal of wild cycads would also be possible a number of years after the poaching event. Proving legitimacy of cycads in nurseries is challenging for authorities and this forensic technique has significant implications for certifying legal trade both nationally and internationally. The use of stable isotope ratios has the potential to verify the origin of cycads in nurseries, thereby assisting authorities with implementing the provisions of CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora). *Encephalartos* species are included in Appendix I and therefore international trade in wild specimens for commercial purposes is prohibited.

Forensic stable isotope studies have been successful in numerous contexts, including tracing the origin of poached elephant bone and ivory (11), and sourcing drug trafficking routes through tracing the geographic origin of marijuana (12), as well as cocaine and heroin (13). Several isotopes have been used successfully or have potential as geographic indicators. $^{87}Sr/^{86}Sr$ is a useful indicator of the surrounding geology, and has sourced the origin of materials such as bone, ivory or fruit back to their original location, through characteristics of the geology in an area (12, 14, 15). $\delta^{15}N$ within plants is sensitive to changes in source nitrogen (16), and has been used to detect different types of fertilizer Marijuana grew in (17). Thus $\delta^{15}N$ may be useful in detecting differences between plant tissues grown in fertilized gardens versus un-fertilized soils in the wild, possibly linking nitrogen source to growing locality (12). $\delta^{34}S$ is mostly used to detect sources of pollution through aerosols or certain bio-indicator plants (18, 19) and lead stable isotopes are also useful indicators for pollution (20). $\delta^{34}S$ and lead stable isotopes have the potential to differentiate between plants growing in non-polluted versus polluted localities as well as determining pollution source making them potential geological indicators (18-20). $\delta^{13}C$ is strongly related to the availability of soil water, transpiration rates and humidity, because water availability and water use is a key part of CO_2 assimilation (21). $\delta^{13}C$ in combination with $\delta^{18}O$ reflects growing conditions, such as plant water source and water availability, and can be used to identify characteristics of the environment a plant grew in (22, 23). $\delta^{18}O$ has been used extensively to trace the geographic origin of organisms and materials (24, 25), because they vary spatially within rainfall thereby providing signatures specific to the area of origin (23).

Forensic stable isotope studies have been most successful where there is distinct isotopic separation between geographic locations or sources of material and a robust chronology contained within the material of interest (25). In this regard, forensic tracing of South African cycads may be well suited to this technique. Cycads are range restricted with small populations (1), enhancing the probability of distinct isotopic compositions associated with wild populations. Additionally, the majority of poached cycads are relocated far from their natural wild range, increasing the probability of distinct isotopic composition of tissue grown in the relocated location relative to the wild location. Most poached cycads are relocated to the urban areas of the Gauteng Province, whereas the majority of wild cycad populations within South Africa are distributed within the Limpopo, Mpumalanga, KwaZulu-Natal, and Eastern Cape Provinces (26).

The aim of this study was to investigate the use of a forensic isotope approach as a technique to identify cycads that had been removed as mature individuals from a wild location and relocated to a garden. The question asked was: Can stable isotopes and ^{14}C-dating be used to validate the wild origin of cycads with a known relocation history? Using a suite of stable isotope measurements (δ^{13}C, δ^{15}N, δ^{18}O, δ^{34}S, ^{87}Sr/^{86}Sr and ^{206}Pb/^{207}Pb, ^{208}Pb/^{207}Pb) on tissues of a known age (from ^{14}C-dating), we explored the chronological change in isotopic composition of tissues from cycads with a known relocation history and control plants from the wild. We tested three approaches. Firstly, we tested for relative changes in the isotopic composition of external leaf bases in relocated and wild plants. We assumed that for a stable isotope to successfully record a relocation event there would have to be: 1) no statistical difference between pre-relocation tissue in wild and relocated plants, and 2) a significant difference in post-relocation tissue between wild and relocated plants. Secondly, we explored the isotopic “fingerprint” of cycads in a multivariate analysis. We tested whether post-relocation tissue was distinguishable from pre-relocation tissue. Thirdly, we explored the isotopic composition of cellulose from vascular cores in the plants to determine if the post-relocation tissue was isotopically distinct from the wild. Successful validation of these techniques would pave the way for further development of the forensic isotopic approach into a law enforcement tool for combating the illegal trade in endangered cycads.

Methods

Sampling criteria and study species

We identified two case studies for our initial testing of the forensic isotope method. Each case study consisted of a comparison between an individual of known relocation history, currently growing in a location different from the wild habitat, and a control specimen from the wild habitat. The species chosen (*Encephalartos lebomboensis* and *Encephalartos arenarius*) met the following criteria that were deemed to be important for properly testing the technique: (i) an arboreal growth form allowing chronological sampling along the stem, (ii) restricted wild population range, (iii) threatened by poaching, (iv) the existence of at least one individual with a known relocation history that was removed from the wild at least three years ago and grown in an *ex situ* climate that is different from the wild, and (v) accessible plants still remaining in the wild to sample as a control. Practical difficulties in obtaining specimens with a reliable relocation history and sampling wild specimens of threatened and protected species limited our ability to expand the initial sample size beyond 4 individuals. Paucity of sample size is problematic in most cycad studies, especially when removing cycad material (14).

Encephalartos lebomboensis (Endangered A2acd; B1ab(ii,iii,iv,v)+2ab(ii,iii,iv,v)), commonly known as the lebombo cycad, occurs in isolated populations in Kwazulu-Natal, Mpumalanga and Swaziland and grows at high altitudes on rocky slopes or on cliffs and ravines in grasslands or savannas (26, 27). This species is threatened by poaching for collections, which has resulted in considerable population declines over the past 90 years (27). Today, fewer than 5000 individuals remain in the wild (27). One individual of *E. lebomboensis* was sampled at the Kirstenbosch National Botanical Garden, Cape Town. This plant was moved to the garden in 1946 and originated from a wild population in the northern part of KwaZulu-Natal, approximately 1400 km from Cape Town. One control specimen of *E. lebomboensis* was sampled from the wild, in KwaZulu-Natal near the origin (within 80 km) of the relocated specimen at Kirstenbosch. The wild *E. lebomboensis* population in KwaZulu-Natal are found at an elevation of 1100 m above sea level and experience a mean annual precipitation of 1053 mm (28), which peaks in midsummer (29). This is distinct from Kirstenbosch, which experiences winter rainfall and hot, dry summers, with a mean annual precipitation of 1400 mm (30) and is at an elevation of around 150 m above sea level (29). Additionally, the geologies also differ between the two localities; the Cape Town region consists of Cape Peninsula Pluton from the Cape Granite Suite compared to quartzite, which forms part of the Mozaan Group in KwaZulu-Natal (29). These differences in environmental factors between the wild and relocated localities were likely to provide distinct isotopic compositions.

Encephalartos arenarius (Endangered A2acd; B1ab(ii,iii,iv,v)+2ab(ii,iii,iv,v); C1), or the alexandria cycad, occurs in isolated populations distributed on the coastal sand dunes of the Eastern Cape Province (26, 31). This species grows in sandy soils consisting of Quaternary sands (29), and is usually found on sloping hills or dunes under tree canopies, or in open grasslands due to altered habitat for pastures (31). The population has decreased by 50 % over the past 60 years due to illegal collection and habitat loss, and only 850 to 1500 mature individuals remain in the wild (31). One specimen of *E. arenarius* was sampled at the Lowveld National Botanical Garden in Nelspruit, which arrived at the garden in 1992 after it was confiscated from poachers. This cycad originated from a wild population in the Eastern Cape, approximately 1000 km from Nelspruit. Nelspruit has mild to hot sub-tropical conditions, with summer rainfall and mean annual precipitation ranging between 600 mm and 1 100 mm (28). The Lowveld National Botanical Garden is found at an elevation of around 650 m and the geology in the surrounding area consists of granite and gneiss (29). The control specimen for *E. arenarius* was sampled at the same locality that the confiscated cycad at the Lowveld National Botanical Garden originated from. In contrast to Nelspruit, the wild *E. arenarius* population receives between 450 mm and 900 mm of mean annual precipitation throughout the year (28), with peak rainfall during March and November, and grows at an elevation of around 100 m above sea level (29).

Establishing an isotope chronology

In order to establish an isotope chronology that could be used for forensic purposes, we tested two sampling protocols. Firstly, we repeated Vogel and van der Merwe's (14) method of removing leaf bases from the upper, middle and lower sections of the cycad stem (Fig. 1). The upper part of the stem was expected to have the youngest plant tissue with increasing plant tissue age towards the lower leaf bases (14). A minimum of three replicates were collected for the petioles and each section of the stem. Each replicate required three to four leaf bases depending on their size (altogether approximately two grams dry weight), which were removed by shaving the outer part of the leaf base off with a hammer and chisel, about one to two centimetres into the stem. The leaves were cut at their base with secateurs to sample the petioles. Any lichen, moss or algae growing on the leaf bases were scraped off using a sharp razor blade. Samples were dried at 60˚C for 48 hours and ground to a fine powder in a ball mill.

Secondly, we tested a novel sampling method using a hand held increment borer to obtain cores of the vascular tissue from the lower, middle and upper parts of the cycad stem (Fig. 1). We expected the age of the vascular tissue to decrease with increasing height and proximity to the exterior of the stem, with the youngest tissue being at the top of the stem and in the outer parts of the vascular tissue (26). The vascular tissue was sliced out of the cores under a dissecting microscope and then dried at 60˚C for 48 hours. Samples were ground to a fine powder in a ball mill. Between 80 and 200 mg of dry weight sample was reduced to cellulose, following methods described by Leavitt and Danzer (32).

Radiocarbon dating of samples

We determined the chronology of the tissue samples through ^{14}C-dating, allowing us to establish which material grew within the wild or within the new location. All analyses were conducted at the Beta-Analytical Laboratory, Miami, Florida (www.radiocarbon.com). Leaf bases sampled from the upper, middle and lower sections of the relocated *E. lebomboensis* stem and from the upper and middle sections of the relocated *E. arenarius* stem were analysed for ^{14}C. One sample from the inner vascular ring and one sample from the outer vascular ring at the lower section of the relocated *E. lebomboensis* stem were reduced to cellulose. All samples were pre-treated with acid-alkali-acid (AAA) wash, using HCl-NaOH-HCl (33). Before the samples were analysed using accelerator mass spectrometry (AMS), the AAA pre-treated samples were cryogenically purified to carbon dioxide through the combustion of the sample, and then reduced to solid graphite (33).

The ^{14}C was measured as percent modern carbon (pMC), because there was more ^{14}C present in the samples than in the modern reference standard (AD 1950), indicating that the sample originated after the atom bomb testing, which caused an increase in ^{14}C post 1950. The calendar age for the samples were calibrated using percent modern carbon from the "bomb spike" curve (34, 35).

Stable isotope analyses

Leaf base and petiole samples were analyzed for the stable isotopic composition of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), sulfur ($\delta^{34}S$), strontium ($^{87}Sr/^{86}Sr$) and lead ($^{206}Pb/^{207}Pb$, $^{208}Pb/^{207}Pb$).

The core samples that were reduced to cellulose were only analysed for the stable isotopic composition of carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$).

$\delta^{18}O$ and $\delta^{34}S$ were analysed at the Iso-Analytical Laboratory (www.iso-analytical.co.uk) in the United Kingdom using a Europa Scientific 20-20 Isotope-Ratio Mass Spectrometer (IRMS) and a modified Sercon Elemental Analyser. The core samples were weighed (1.0 ± 0.1 mg) into silver capsules (8 mm by 5 mm) for $\delta^{18}O$ analysis and put into a micro-titre plate to dry at 60°C for seven days with standards IAEA-CH-6 (sucrose, $\delta^{18}O_{V\text{-}SMOW}$ = 36.4 ‰) and IAEA-C-3 (cellulose, $\delta^{18}O_{V\text{-}SMOW}$ = 32.2 ‰). The known isotopic signatures for the standards IAEA-CH-6, IAEA-C-3, and IAEA-601 (benzoic acid, $\delta^{18}O_{V\text{-}SMOW}$ = 23.3 ‰) were used as quality control checks during the analysis.

The leaf base and petiole samples were weighed out (6.0 ± 0.1 mg) into tin capsules for $\delta^{34}S$ analysis and then loaded into an automatic sampler with the standards and the catalyst vanadium pentoxide. The SO^+ ion beam was calibrated for the ^{18}O contribution using the following standards: IA-R025 (Iso-Analytical working standard barium sulfate, $\delta\ ^{34}S_{V\text{-}CDT}$ = +8.53 ‰), IA-R026 (Iso-Analytical working standard silver sulfide, $\delta^{34}S_{V\text{-}CDT}$ = +3.96 ‰) and IA-R061 (Iso-Analytical working standard barium sulfate, $\delta^{34}S_{CDT}$ = +20.33 ‰). IA-R061 was also the reference material used for analysing $\delta^{34}S$. The two quality control standards were IA-R061 and IA-R027 (blue whale baleen, $\delta^{34}S_{CDT}$ = +16.3 ‰).

$\delta^{13}C$ and $\delta^{15}N$ was analysed at the Stable Light Isotope Laboratory, Department of Archaeology, University of Cape Town, using a Flash 2000 organic elemental analyser coupled to a Delta V Plus IRMS via a Conflo IV gas control unit (Thermo Scientific, Bremen, Germany). Tin capsules containing 3 ± 0.1 mg of leaf base or petiole samples were analysed for $\delta^{13}C$ and $\delta^{15}N$, and 0.4 ± 0.01 mg of cellulose from the vascular rings was analysed for $\delta^{13}C$.

Isotopic composition of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), sulfur ($\delta^{34}S$) and oxygen ($\delta^{18}O$) is expressed in parts per thousand (‰), relative to the international standard:

$\delta\ ^{X}E = (R_{sample}/\ R_{standard}\ -1)*1000$

Where E, is the element of interest, X is the atomic mass of the heavier isotope, R_{sample} is the ratio of the heavier to the lighter isotope within the sample, $R_{standard}$ is the ratio of the international standard (PDB for $\delta^{13}C$, atmospheric air for $\delta^{15}N$, Canyon Diablo meteorite for $\delta^{34}S$, and Vienna Standard Mean Ocean Water for $\delta^{18}O$).

Strontium ($^{87}Sr/^{86}Sr$) and lead ($^{206}Pb/^{204}Pb$; $^{207}Pb/^{204}Pb$; $^{208}Pb/^{204}Pb$) isotope ratios were analyzed at the Department of Geological Sciences, University of Cape Town. Between 200 mg and 500 mg of ground leaf base and petiole sample was weighed into crucibles for ashing. The furnace temperature was initially set at 300˚C, then increased every hour by 100˚C and left over night once the temperature reached 600˚C.

Between 10 and 50 mg of ashed sample was weighed into 7 ml teflon beakers. The sample preparation and digestion for $^{87}Sr/^{86}Sr$, $^{206}Pb/^{204}Pb$; $^{207}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$ was based on methods described by Pin et al. (36) and Miková and Denková (37). Strontium and lead isotope ratios were analysed using a Nu Instruments Nu Plasma HR instrument. Strontium was analysed as a solution in 200ppb 0.2% HNO_3 following methods outlined by Miková and Denková (37), which was then normalized against a $^{87}Sr/^{86}Sr$ ratio of 0.710255. The results were corrected for rubidium isobaric interference on ^{87}Sr by measuring the signal for ^{85}Rb and the natural $^{85}Rb/^{87}Rb$ ratio (36). A value of 0.1194 and the exponential law was used to correct for the instrumental mass fractionation on the $^{86}Sr/^{88}Sr$ isotope (37).

The lead isotope ratios were analysed as a solution in 50ppb 2% HNO_3 with a Nu Instruments DSN-100 desolvating nebuliser. All of the samples had NIST SRM997Tl standard added to them in the ratio of 10:1 Pb:Tl. A triple lead spike was done by adding an aliquot of $^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$ to the analysis to reduce the standard deviation of the isotope ratios to ~100 ppm (38). The samples were corrected for interference with mercury isotopes (^{204}Hg) by monitoring the presence of ^{202}Hg in the sample (39, 40). The lead isotope ratios were also corrected for instrumental mass fractionation using the exponential law and a value of 2.3889 for the $^{205}Tl/^{203}Tl$ standard that was added to the lead samples and standards (40).

Statistical analysis

Based on ^{14}C-dating and the visible difference seen in growth along the stem of the relocated cycads (see Results, Table 1), we pooled samples within a plant to represent pre-relocation and post-relocation groups for statistical analysis. The same groupings were applied to the wild specimens serving as controls. For a stable isotope ratio to successfully record a relocation event, we assumed that there would have to be: 1) no statistical difference between pre-relocation tissue in wild and relocated plants, and 2) a significant difference in post-relocation tissue between wild and relocated plants.

For *E. arenarius*, relocated in 1992, the middle and lower leaf base samples were grouped together as the pre-relocation group and the petiole and upper leaf base samples were grouped together as the post-relocation group (see Table 1). For *E. lebomboensis*, relocated in 1946, all samples were considered to form part of the post-relocation group (see Table 1). For each species, we tested for differences between the wild and relocated groups by a one-way ANOVA for each stable isotope, followed by a *post-hoc* Tukey test (for *E. arenarius*) if significant differences were found. Analyses were conducted in STATISTICA version 11 (41).

A principal component analysis (PCA) was done in R-gui version 2.15.2 (42) using multiple stable isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, $^{87}Sr/^{86}Sr$ and $^{206}Pb/^{207}Pb$, $^{208}Pb/^{207}Pb$) to test the clustering of leaf base and petiole samples in the wild and relocated specimens. The sulfur concentration in the upper leaf bases and petioles of the E. *arenarius* wild specimen was too low for $\delta^{34}S$ analysis, and $\delta^{34}S$ was therefore excluded from the *E. arenarius* PCA.

The core samples were not well constrained temporally (Table 1), limiting the statistical analyses that could be justifiably performed. Instead we followed two simple approaches. Firstly, we tested for homogeneity of variance between the wild and relocated samples within each species using Levene's Test. We reasoned that relocated specimens would be more likely to show higher variance than specimens from a fixed location. Where variance was homogenous, we tested for differences between the wild and relocated samples within each species using a Student's t-Test. In the case of variance being non-homogenous, we did not perform a Student's t-Test, but rather assessed which samples from the relocated plant fell inside and outside the range expected for a plant remaining in one locality. This range was assumed to be adequately represented by the mean ±2 standard deviations of the wild specimen. Samples outside of this range were likely to be from a different locality than the wild. Analyses were conducted separately for $\delta^{13}C$ and $\delta^{18}O$ in STATISTICA version 11 (41).

Results

Radiocarbon dating

The ^{14}C dates for both relocated specimens suggested that leaf bases would be effective for forensic tracing if the poached cycads were relocated between 6 to 30 years ago. In both plants, there was a sequential increase in age from the top to the base of the stem (Table 1), consistent with findings from Vogel and van der Merwe (14). The ^{14}C results from *E.*

arenarius indicated that the relocation event occurred between the middle and upper portions of the stem (Table 1). This was consistent with the visible change seen in growth, through increased stem diameter and bigger leaf bases, since it was moved to Nelspruit. Unexpectedly, the oldest ^{14}C date for the relocated E. *lebomboensis* specimen was 34 years ago (Table 1), indicating that the entire stem grew subsequent to the plant being relocated in 1946, and did not grow in the wild.

We hypothesized that the inner vascular tissue would be older than the outer vascular tissue, as there were no obvious growth rings. The ^{14}C dates for vascular tissue from the inner and outer portions of the lower core in *E. lebomboensis* supported this hypothesis of the outer vascular tissue being younger than the inner (Table 1).

Isotope chronology in leaf bases

Strontium

For *E. lebomboensis*, the ^{87}Sr/^{86}Sr ratios were significantly different between the relocated and wild specimens (Tables 3 and 4; Fig. 2). As ^{87}Sr/^{86}Sr is a good indicator of underlying geology (12, 14, 15), this result indicates that the entire relocated stem grew in a different locality from the wild specimen. This is consistent with the ^{14}C-dating results (Table 1). For *E. arenarius*, ^{87}Sr/^{86}Sr ratios for the petiole and upper leaf base samples in the relocated specimen were significantly different from the wild specimen (Tables 3 and 4, Fig. 3). However, the middle and lower leaf base samples in the relocated specimen, dated to before the relocation (Table 1), were indistinguishable from the wild plant (Tables 3 and 4, Fig. 3). These results are consistent with a wild origin and subsequent relocation of the relocated *E. arenarius* plant.

Lead

There were no significant differences in $^{206}Pb/^{207}Pb$ between wild and relocated groups for either species (Table 3). There was a significant difference in $^{208}Pb/^{207}Pb$ between wild and relocated groups for *E. lebomboensis*, but no significant differences for *E. arenarius* (Table 3).

Sulfur

The wild and relocated *E. lebomboensis* specimens were siginificantly different in $\delta^{34}S$ (Tables 3 and 4), indicating that $\delta^{34}S$ may be useful in tracing relocations. However, there was considerable variation (>9‰) within the relocated plant. For *E. arenarius*, all groups were significantly different from each other (Tables 3 and 4). However, the pre-relocation groups in wild and relocated plants were siginificantly different (Table 4), refuting the utility of $\delta^{34}S$ as a tracer. Unfortunately, we were unable to compare the post-relocation groups, due to insufficient sulfur concentration in those samples (Table 2). The high degree of variance within plants, as well as the difference seen for the pre-relocation groups indicates that $\delta^{34}S$ may not be a good indicator for locality within cycads.

Carbon

There was no significant difference in $\delta^{13}C$ between the relocated and wild stem for *E. lebomboensis* (Table 3), suggesting that the ratio of internal to external leaf CO_2 concentrations (C_i/C_a) (43) was constant for this species in the two locations. Noticeably, there was little variability in $\delta^{13}C$ within both *E. lebomboensis* specimens and the wild *E. arenarius* specimen (Table 2), indicating little variability in water-use efficiency over the lifetime of stems growing at a consistent location. The ANOVA of *E. arenarius* groups was significant (Table 3). The post-hoc Tukey test showed the pre-relocation groups were significantly different from each other, but the post-relocation groups were not (Table 4). This is the opposite pattern that one would expect if $\delta^{13}C$ were reflecting the relocation event. However, the trend within the relocated *E. arenarius* was distinct from the other stems. It showed a ±3‰ change in $\delta^{13}C$ in the post-relocation tissue relative to pre-relocation tissue (Table 2). In contrast, the control specimens varied by ±1‰ (Table 2). This illustrates that individual plants within a common habitat may have different water-use efficiencies, possibly depending on variations in micro-climate, and emphasizes the need to look at relative shifts within a plant.

Nitrogen

There was no difference in $\delta^{15}N$ between the relocated and wild *E. lebomboensis* specimens, despite the plants growing in different localities (Table 3). For *E. arenarius*, $\delta^{15}N$ was significantly different between post-relocation groups, but not between pre-relocation groups (Tables 3 and 4), consistent with a common origin and subsequent relocation of the relocated plant.

Principal Component Analysis: using multiple isotope tracers

The PCA for the *E. lebomboensis* samples separated the relocated and the wild specimen into two distinct groupings (Fig. 4), indicating that the specimens originated from different environments. The differences between the relocated and wild specimens were driven primarily by $\delta^{13}C$, $\delta^{34}S$ and $^{87}Sr/^{86}Sr$.

The petiole and upper leaf base samples from the relocated *E. arenarius* specimen grouped together in the PCA, whereas the middle and lower leaf base samples grouped together with all samples from the wild specimen (Fig. 5). This suggests that the petioles and upper leaf bases from the relocated plant originated from a different environment (Nelspruit) compared to the middle and lower leaf bases. The middle and lower leaf bases were grouped with the wild specimen, indicating that they originated from the Eastern Cape (Fig. 5). The grouping of the different localities was driven primarily by $\delta^{15}N$, $\delta^{13}C$ and $^{87}Sr/^{86}Sr$ ratios.

Isotopic composition of cellulose in core samples

There was no significant difference in variance between wild and relocated *E. lebomboensis* plants for both $\delta^{13}C$ (F = 0.022, df =59, p = 0.881) and $\delta^{18}O$ (F = 0.530, df =59, p = 0.470). Wild and relocated specimens differed significantly in both $\delta^{13}C$ (t = -8.81, df = 53, p = 0.84 Table 4; Fig. 6) and $\delta^{18}O$ (t = 13.52, df = 59, p = 0.26; Fig. 6). These significant differences in $\delta^{13}C$ and $\delta^{18}O$, as well as the homogenous variance between wild and relocated plants, suggests that these plants grew in separate localities for their entire histories.

There was a significant difference in variance between wild and relocated *E. arenarius* plants for both $\delta^{13}C$ (F = 6.76, df =32, p = 0.014) and $\delta^{18}O$ (F = 12.10, df =32, p = 0.0015). While the variance of the wild *E. arenarius* was comparable to the wild and relocated *E. lebombensis* specimens (Fig.s 6 and 7), the variance of the relocated specimen was approximately 5-fold higher in both $\delta^{13}C$ and $\delta^{18}O$ relative to the wild specimen (Fig. 7). There were several core samples from the relocated *E. arenarius* that fell outside of the mean ±2 standard deviations of the wild specimen (Fig. 7). Due to the lack of temporal control of these core samples, we hestitate to over-interpret these data, other than to assume that the greater variance in $\delta^{13}C$ and $\delta^{18}O$ of the relocated specimen reflects variation in environmental conditions, or stress, associated with a relocation event. However, we note that the relocated upper core sample was most different from the $\delta^{18}O$ in the wild plant (Fig. 7). The upper core is likely to represent the youngest vascular tissue in the plant, and thus probably was formed post-relocation.

Discussion

Our results indicate that stable isotopes have great potential to be used as a forensic tracer to determine the origin of *ex situ* cycads. The *E. lebomboensis* relocated and wild stems were isotopically distinct from each other in $^{87}Sr/^{86}Sr$, $\delta^{34}S$ and $\delta^{18}O$ (Fig. 2, 4 and 6), supporting the ^{14}C results that showed the stems grew in different localities (Table 1). $^{87}Sr/^{86}Sr$ and $\delta^{15}N$ in the most recent tissue from the relocated *E. arenarius* was isotopically distinct from the older tissue, which resembled the isotopic signature of the wild specimen (Fig. 3, 5 and 7). This supports ^{14}C results that the relocated *E. arenarius* specimen originated from the wild and was moved to a new locality in 1992. Taken together, these two case studies indicate that changes in geographic locality can be detected within the isotopic chronology of these cycads, providing a potentially powerful tool to forensically determine the origin of illegally harvested cycads.

The effectiveness of this forensic isotope method relies primarily on two factors. Firstly, an isotopic chronology must exist that reflects the growing conditions of the cycad over the period of forensic interest. Our study suggests that sampling of the leaf bases and petioles is an effective method for establishing an isotope chronology of approximately 30 years. Thus, illegally obtained cycads may be forensically detectable 1 to 30 years after the relocation event, depending on the time required for new leaves to grow within the new locality. It is possible that relocation from the wild may be detectable more than 30 years after removal in slower growing species, as ^{14}C ages of 150 years for *E. transvenosus* (44) and 210 years for *E. eugene-maraisii* (14) have been measured. Our attempts to use vascular tissue from cores to establish a chronology were less successful. The inner vascular tissue was dated to be younger than the lower leaf bases on the outside of the stem. This was contrary to the results from Vogel and van der Merwe (14) who found that ^{14}C-dating of isolated vascular tissue yielded older ^{14}C-ages than the leaf bases. We speculate that the younger ages for the vascular tissue relative to the leaf bases was caused by the interweaving of new vascular tissue with old as the stem grew in width. If this is the case, our vascular tissue core-sampling technique might provide a less reliable means of obtaining a recent chronology than could be obtained from the leaf bases. Additionally, this method is more destructive than the leaf base sampling technique. As such, the leaf base sampling technique currently appears the best method for establishing a recent chronology.

Secondly, for any successful forensic isotope study, there must be a difference in isotopic composition between the wild and relocated areas that can be detected in the chronology. As seen for *E. arenarius*, a compelling case for a wild origin could be made in cycads where tissue predating the relocation event was isotopically indistinct from wild specimens, whereas tissue grown after the relocation event reflected that of the new locality. In this regard, relocations to a similar climate and substrate as the wild population are unlikely to be detectable through this method (25). However, relocations to gardens with different substrates, watering regimes, climate and atmospheric pollution have a higher probability of being detectable (25). In an ideal situation, wild localities would have a unique isotopic "fingerprint" that would serve as a marker of wild origin when analysing suspect cycads. By measuring multiple isotopic tracers, the chance of detecting a unique "fingerprint" amongst different localities can be maximized (13, 15, 45).

In our study, the use of multiple isotopes was effective in separating wild samples from relocated. Particularly promising was $^{87}Sr/^{86}Sr$, which would be the starting point for any future forensic invesitgation and has been successfully used to provenance materials (12). An advantage of using $^{87}Sr/^{86}Sr$ as a forensic tool is that there is little fractionation of $^{87}Sr/^{86}Sr$ within the plant, therefore the isotope ratios are very consistent with little variablilty. The variation of $^{87}Sr/^{86}Sr$ in both *E. lebomboensis* and *E. arenarius* accurately captured the known histories of these plants. There was very little variation in the wild plants, and considerable difference between samples grown in the wild and the relocated locations (Fig. 2 and 3). This suggests that $^{87}Sr/^{86}Sr$ is a very powerful tool for proving wild origin.

Our results indicate that $\delta^{18}O$ has potential as a forensic tracer for cycads. Multiple environmental parameters influence the $\delta^{18}O$ of cellulose, such as water vapour pressure of the atmosphere, air temperature and $\delta^{18}O$ in the water source (46). As these parameters vary spatially across South Africa (47), there is a high probability that the $\delta^{18}O$ in cellulose would be distinct between wild populations and relocated specimens. In our study, the $\delta^{18}O$ in vascular core cellulose clearly distinguished between wild-grown and relocated *E. lebomboensis* specimens (Fig. 6). It is likely that the differences in growing elevation (150 m above sea level for the relocated specimen, 1100 m above sea level for the wild specimen) and precipitation seasons (winter for the relocated specimen, summer for the wild specimen) explain the ~2‰ higher $\delta^{18}O$ values within the relocated *E. lebomboensis* relative to the wild specimen (Fig. 6). We also interpret the 5-fold increase in variance between specimens grown within one location and the relocated *E. arenarius* specimen as an indication that $\delta^{18}O$ is recording aspects of location. Lack of temporal constraint of the *E. arenarius* core samples hinders a more detailed analysis at present. However, improved temporal understanding of $\delta^{18}O$, possibly by sampling $\delta^{18}O$ in the leaf base chronology, would allow a more precise interpretation of the $\delta^{18}O$ in recently relocated cycads.

Our study also demonstrated several other isotopes that appeared promising for recording the provenance of relocated cycads, specifically $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$ and $^{208}Pb/^{206}Pb$ (Table 3). The variation in $\delta^{15}N$ of *E. arenarius* was consistent with the relocation history of the case study (Table 4). $\delta^{15}N$ is potentially a useful tracer as cycads are often fertilized within gardens. Fertilization may create a distinct plant $\delta^{15}N$ composition compared to wild grown plants (17). In a scenario were cycads are moved between similar geologies, $\delta^{15}N$ may be a more informative geographical tracer than $^{87}Sr/^{86}Sr$.

$\delta^{13}C$ of plant material is primarily used for detecting physiological changes within the plant caused by changes in light and water availability (21, 48). While $\delta^{13}C$ will not aid in determining specimen origin, relative changes in $\delta^{13}C$ within the plant may provide an independent measure of relocation to support results from other stable isotope ratios. Our results indicated an increase in $\delta^{13}C$ variance within the relocated *E. arenarius* specimen compared to the other specimens that remained in one locality (Table 2). This increased variance in $\delta^{13}C$ for the relocated specimen was also noted within the vascular core samples (Table 2). Interestingly, there was no difference seen between $\delta^{13}C$ of wild and relocated *E. lebomboensis* leaf bases (Table 3), but there was a significant difference in $\delta^{13}C$ of cellulose from the vascular core samples of wild and relocated plants (Fig. 6). The differences in the $\delta^{13}C$ of the vascular core cellulose are consistent with an interpretation of environmental variability between the wild and relocated specimens. The wild *E. lebomboensis* specimen grew in a more water stressed environment on the edge of a cliff face compared to the relocated specimen that grew in more mesic conditions in a botanical garden. Why was this not recorded in the leaf base $\delta^{13}C$? Possibly the signal was masked by carbon-containing secondary compounds that were included in the leaf base bulk $\delta^{13}C$ measurement, but not in the purifed cellulose. Alternatively, the core samples may reflect a more recent time period than the leaf base samples (Table 1) and might be capturing different scales of environmental variability. This remains an avenue for future research.

Sulfur and lead stable isotopes within plants have successfully been used in detecting polluted localities (18-20). Thus, these isotopes may be valuable for distinguishing between localities when sampling *ex situ* plants relocated to more highly polluted urban areas. In our present study, there were significant differences in $\delta^{34}S$ for wild and relocated specimens of both species. However, the considerable variation in $\delta^{34}S$, together with the inconsistent patterns of variation in specimens of a known relocation history, indicated that $\delta^{34}S$ may not be suitable for fine-scale tracing of provenance in relocated cycads. Lead isotopes were largely unsuccessful at tracing relocations, possibly because our relocated specimens came from botantical gardens with reatively low exposure to atmospheric pollution. Future work could to examine if relocations from pristine wilderness areas to highly polluted urban areas may be detectable using sulfur and lead isotope ratios.

In practice, different sets of isotope tracers may be required for individual forensic applications according to the isotopic variation between the relocated and wild localities. The range-restricted nature of several of the highly desirable cycad species further improves the chances of characterizing a wild “fingerprint”. An advantage of multivariate analyses is that isotopes detecting physiological change within the plant as well as changes specific to locality can be used in combination to understand relocation history. Our results indicate that multivariate analyses allow for the separation of stable isotope ratios into different geographical origins of tissue growth. This makes interpretation of relocation history easier, as well as assisting in identifying unique isotopic “fingerprints” for cycad populations.

Future development

Our two cases studies have shown the potential use of this method for forensic investigation. Future work should focus on replicating this study with other well-documented, relocated specimens, as well as quantifying the isotopic variability within wild populations.

Our study focussed on detecting a difference in isotopic signature within a plant with a known relocation history and comparing it to a known wild control plant. In practice, investigators will be dealing with cycads of unknown history and origin. Future directions of this cycad forensic method would include spatially characterizing the isotopic variability within wild cycad ranges on maps and using this as a reference for unknown cycads in investigations (49). This spatial stable isotope approach has been successfully used for a number of studies (12, 15, 45, 49), and the probability of the unknown cycad originating within the new and wild localities can be tested using process models to predict expected isotope ratios for each locality (50).

In cases where no unique wild "fingerprint" is possible, there is still the possibility of comparing the isotopic composition of the *ex situ* cycad to other cycads from the claimed origin (usually a nursery), and other cycads from the current location and testing whether the isotopic composition of the plant is consistent with any of these locations.

Our findings provide a basis for future research to use stable isotopes and ^{14}C-dating to validate relocation history and origin. We hope that the successful use of this forensic method will provide an effective deterrent for public engaging in the illegal cycad trade.

Acknowledgements

Special thanks to Phakamani Xaba (Kirstenbosch Cycads Living Plants Collection) and Willem Froneman (Lowveld National Botanical Garden) for their advice. Thanks to Dr. Edmund February (Dept. of Biological Sciences, UCT) for use of equipment. Thanks to Karin Behr (SANBI) for organizing permits, and Chris Barichievy (EKZNW), Kenneth Kilani (DEAET), Dylan Davies and Wade Lane (UCT) for field assistance. Thanks to Dr. Petrus Le Roux and Fayrooza Rawoot (Dept. of Geological Sciences, UCT), as well as Ian Newton (Stable Light Isotope Laboratory, UCT) for stable isotope ratio analyses. We acknowledge Ezemvelo KZN Wildlife, Mpumalanga Parks and Tourism Agency, and the Department of Economic Affairs, Environment, and Tourism for issuing permits.

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TABLE 1. The conventional age with error and calibration for the radiocarbon analysis of the relocated *ex situ* specimens, *Encephalartos lebomboensis* (Kirstenbosch National Botanical Garden) and *Encephalartos arenarius* (Lowveld National Botanical Garden).

Specimen	Samples	Conventional age (pMC)	$\delta^{13}C$ (‰)	Calibrated date	Calibration date error
E. lebomboensis	Lower tree core				
(relocated in 1946)	outer vascular ring	112.3 +/- 0.4	-22.5	1993	1992-1994
	inner vascular ring	122.0 +/- 0.6	-22.4	1983	1982-1984
	Leaf bases				
	upper part of stem	106.2 +/- 0.3	-21.3	2005	2003-2006
	middle part of stem	114.7 +/- 0.3	-21.3	1990	1989-1991
	lower part of stem	129.4 +/- 0.6	-25	1979	1978-1979
E. arenarius	Leaf bases				
(relocated in 1992)	upper part of stem	107.4 +/- 0.3	-26.6	2003	2001-2004
	middle part of stem	115.4 +/- 0.3	-25.8	1990	1989-1991

TABLE 2. Mean and standard error for the sulfur, carbon, nitrogen, lead and strontium stable isotope ratios for the relocated and wild *Encephalartos lebomboensis* and *Encephalartos arenarius* specimens.

			$\delta^{34}S$		$\delta^{13}C$		$\delta^{15}N$			$^{206}Pb/^{207}Pb$		$^{208}Pb/^{207}Pb$		$^{87}Sr/^{86}Sr$	
Specimen	sample	n	mean	SE	mean	SE	mean	SE	n	mean	SE	mean	SE	mean	SE
E. lebomboensis	petiole	4	22.58	0.55	-23.01	0.18	-2.32	0.18	2	1.344	0.013	2.283	0.055	0.7110	1.60E-05
	upper leaf base	3	18.20	0.54	-22.21	0.48	-1.07	0.31	2	1.166	0.008	2.395	0.005	0.7109	1.25E-05
	middle leaf base	3	16.40	0.57	-22.74	0.38	-1.48	0.19	2	1.149	0.021	2.395	0.006	0.7107	3.34E-05
	lower leaf base	3	15.54	0.80	-23.86	0.20	-1.28	0.34	2	1.129	0.002	2.395	0.001	0.7110	6.65E-06
E. lebomboensis	petiole	3	11.71	0.64	-22.34	0.18	-1.81	0.08	2	1.175	0.016	2.437	0.009	0.7233	2.88E-05
(wild)	upper leaf base	4	11.16	0.41	-23.58	0.22	-1.18	0.13	3	1.131	0.021	2.392	0.023	0.7231	2.99E-05
	middle leaf base	4	13.88	0.37	-23.29	0.12	-0.70	0.19	3	1.151	0.008	2.415	0.006	0.7228	5.61E-05
	lower leaf base	4	14.59	0.29	-23.52	0.03	-1.57	0.04	3	1.144	0.004	2.410	0.004	0.7233	3.06E-05
E. arenarius	petiole	3	11.81	0.52	-27.48	0.03	-2.21	0.01	2	1.211	0.033	2.393	0.035	0.7259	1.99E-03
	upper leaf base	4	11.58	1.41	-27.13	0.14	-1.52	0.15	3	1.133	0.035	2.388	0.035	0.7282	1.73E-04
	middle leaf base	4	14.25	0.83	-24.64	0.07	-2.29	0.09	3	1.201	0.010	2.414	0.008	0.7110	5.80E-04
	lower leaf base	4	15.16	0.57	-24.19	0.13	-2.23	0.10	3	1.266	0.057	2.395	0.012	0.7101	1.03E-04
E. arenarius	petiole	4			-26.50	0.11	-2.28	0.07	2	1.148	0.022	2.417	0.029	0.7092	4.36E-07
(wild)	upper leaf base	4			-26.71	0.02	-2.54	0.05	3	1.161	0.008	2.417	0.003	0.7092	2.31E-06
	middle leaf base	4	18.72	0.67	-26.08	0.11	-2.40	0.07	3	1.247	0.075	2.328	0.020	0.7093	2.88E-05
	lower leaf base	4	19.77	0.54	-25.48	0.22	-2.46	0.03	3	1.242	0.037	2.384	0.046	0.7092	2.43E-05

TABLE 3. Results from the one-way ANOVA between the wild and relocated *E. lebomboensis* specimens and between the pre-relocation (petiole and upper leaf bases) and post-relocation (middle and lower leaf bases) tissue for the relocated and wild *E. arenarius* specimens.

	$\delta^{34}S$			$\delta^{13}C$			$\delta^{15}N$			$^{87}Sr/^{86}Sr$			$^{206}Pb/^{207}Pb$			$^{208}Pb/^{207}Pb$		
Samples	F	df	p	F	df	p	F	df	p	F	df	p	F	df	p	F	df	p
E. lebomboensis	37.79	1	0.001	1.21	1	0.28	2.13	1	0.16	16886	1	0.001	2.87	1	0.11	5	1	0.039
E. arenarius	45.83	2	0.001	111.2	3	0.001	10.12	3	0.001	401	3	0.001	2.36	3	0.10	2.73	3	0.073

TABLE 4.The mean, standard error (SE) and significance results from the *post-hoc* Tukey test between the relocated and wild *E. lebomboensis* specimens, and between the pre-relocation (petiole and upper leaf bases) and post-relocation (middle and lower leaf bases) groups for the *E. arenarius* specimens.

		$\delta^{34}S$			$\delta^{13}C$			$\delta^{15}N$			$^{87}Sr/^{86}Sr$			$^{206}Pb/^{207}Pb$			$^{208}Pb/^{207}Pb$	
Samples	n	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE
E. lebomboensis (relocated)	13	18.34	±0.80 a	13	-22.96	±0.21 a	13	-1.60	±0.18 a	8	0.7109	±0.0001 a	8	1.197	±0.033 a	8	2.367	±0.021 a
E. lebomboensis (wild)	15	12.94	±0.44 b	15	-23.24	±0.14 a	15	-1.28	±0.12 a	11	0.7231	±0.0001 b	11	1.148	±0.007 a	11	2.411	±0.008 a
E. arenarius																		
petiole & upper LB	7	11.68	±0.78 a	7	-27.28	±0.10 a	7	-1.82	±0.16 a	5	0.7272	±0.0009 a	5	1.164	±0.03 a	5	2.390	±0.023 a
middle & lower LB	8	14.71	±0.50 b	8	-24.42	±0.11 b	8	-2.26	±0.06 a	6	0.7106	±0.0003 b	6	1.234	±0.03 a	6	2.404	±0.008 a
E. arenarius (wild)																		
petiole & upper LB				7	-26.62	±0.06 ac	7	-2.43	±0.16 a	5	0.7092	±0.0001 b	5	1.155	±0.01 a	5	2.417	±0.011 a
middle & lower LB	8	19.30	±0.40 c	8	-25.78	±0.16 c	8	-2.43	±0.04 a	6	0.7092	±0.0001 b	6	1.244	±0.038 a	6	2.356	±0.026 a

Figure captions

FIG. 1. Schematic of the two sampling protocols for establishing an isotope chronology. Petioles were sampled using secateurs, and leaf bases were removed from the upper, middle and lower sections of the cycad stem using a hammer and chisel. Cores were taken from the upper, middle and lower sections of the cycad stem using an increment corer and the vascular rings were cut out of the cores to sample the inner (LCI), middle (LCM) and outer (LCO) sections from the lower tree core, the inner (MCI) and outer (MCO) sections from the middle tree core and one section from the upper core (UC).

FIG. 2. The $^{87}Sr/^{86}Sr$ ratio (mean ±1SE) measured for the petioles and upper, middle, and lower leaf bases sampled from the relocated and wild *Encephalartos lebomboensis* specimens. The two specimens are significantly different throughout the stem reflecting the different growing environments.

FIG. 3. The $^{87}Sr/^{86}Sr$ ratio (mean ±1SE) measured for the petioles and upper, middle and lower leaf bases sampled from the relocated and wild *Encephalartos arenarius* specimens. The two specimens differ only in the material grown within Nelspruit after the relocation in 1992, and the older leaf bases from the relocated plant coincide with the wild control.

FIG. 4. The principal component analysis for the leaf base samples collected for the relocated (Cape Town) and the wild (KwaZulu-Natal) *Encephalartos lebomboensis* specimens. The arrows indicate which isotopes explain the variance within the different samples for the first two components (% variance in brackets).

FIG. 5. The principal component analysis for the leaf base samples collected for the relocated (Nelspruit) and wild (Eastern Cape) *Encephalartos arenarius* specimens. The arrows indicate

which isotopes explain the variance within the different samples for the first two components (% variance in brackets).

FIG. 6. $\delta^{13}C$ and $\delta^{18}O$ (mean ±1SE) from the cellulose extracted vascular tissue for the relocated and wild *Encephalartos lebomboensis* specimens. The two specimens are significantly different indicating different growing localities.

FIG. 7. $\delta^{13}C$ and $\delta^{18}O$ (mean ±1SE) from the cellulose extracted vascular tissue for the relocated and wild *Encephalartos arenarius* specimens. The stippled line indicates the variability within the wild specimen (2xSD) for $\delta^{13}C$ and $\delta^{18}O$.

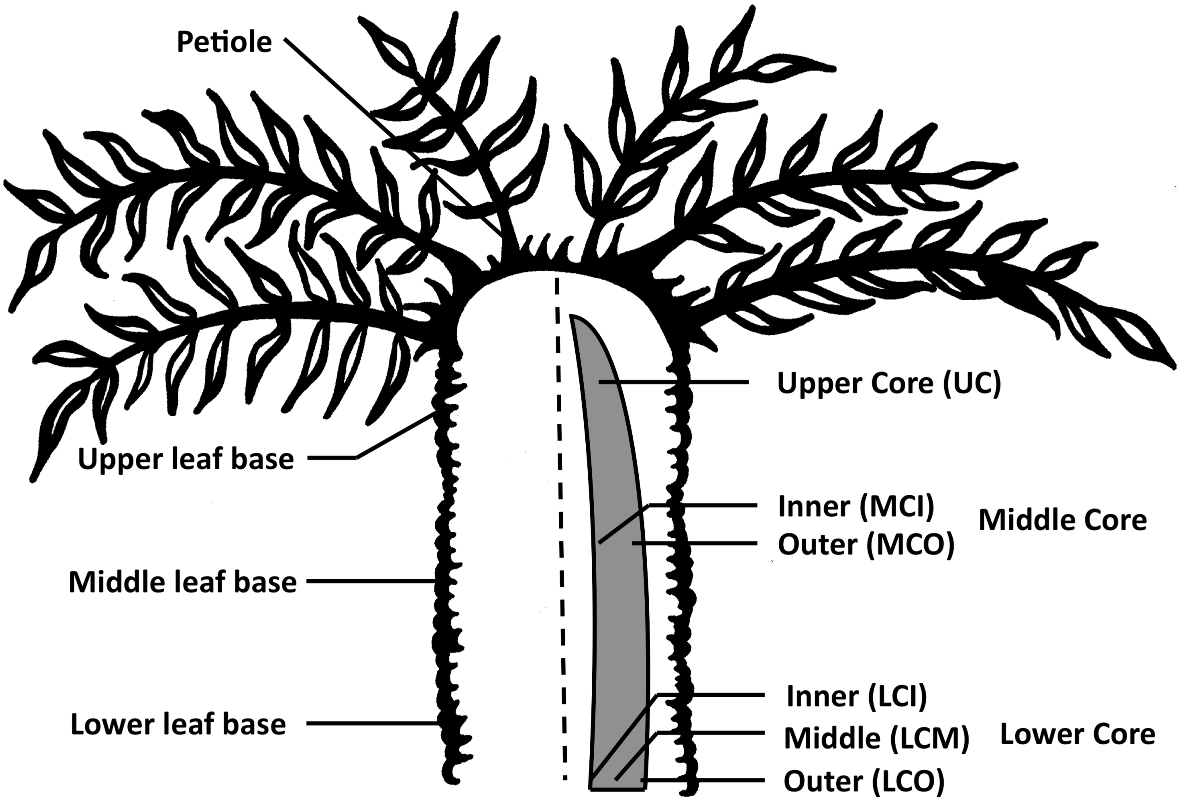
Petiole
Upper Core (UC)
Upper leaf base
Inner (MCI)
Outer (MCO)
Middle Core
Middle leaf base
Inner (LCI)
Lower leaf base
Middle (LCM)
Lower Core
Outer (LCO)

Figure 1

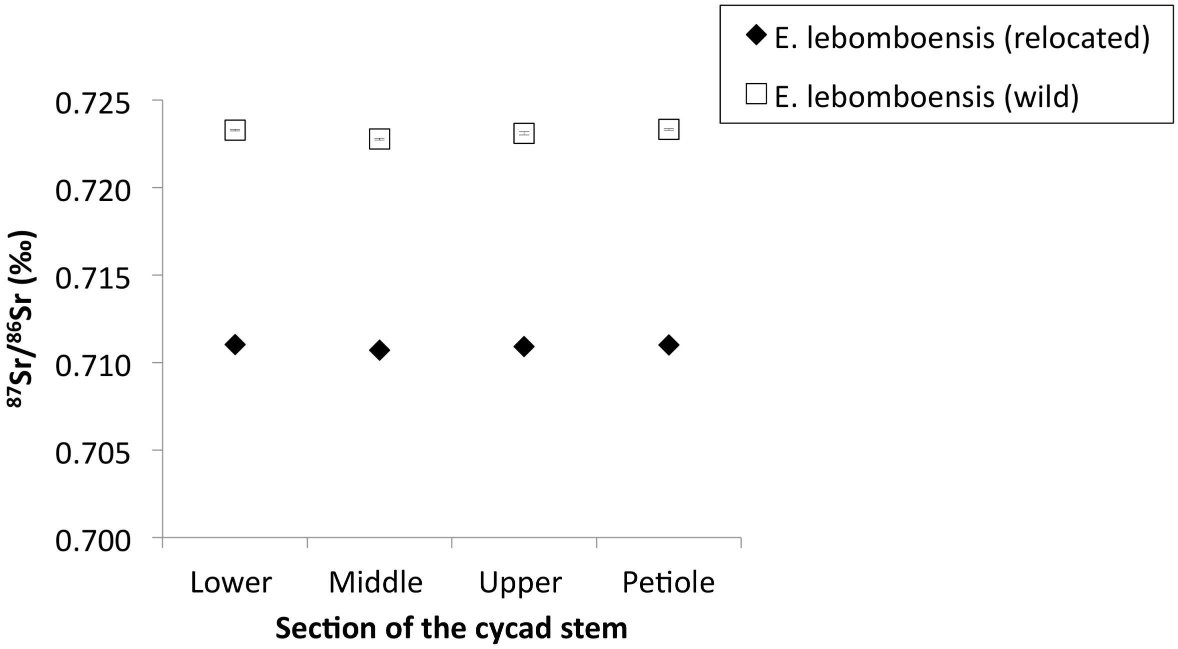
◆ E. lebomboensis (relocated)
☐ E. lebomboensis (wild)
0.725
0.720
0.715
0.710
0.705
0.700
$^{87}Sr/^{86}Sr$ (‰)
Lower
Middle
Upper
Petiole
Section of the cycad stem

Figure 2

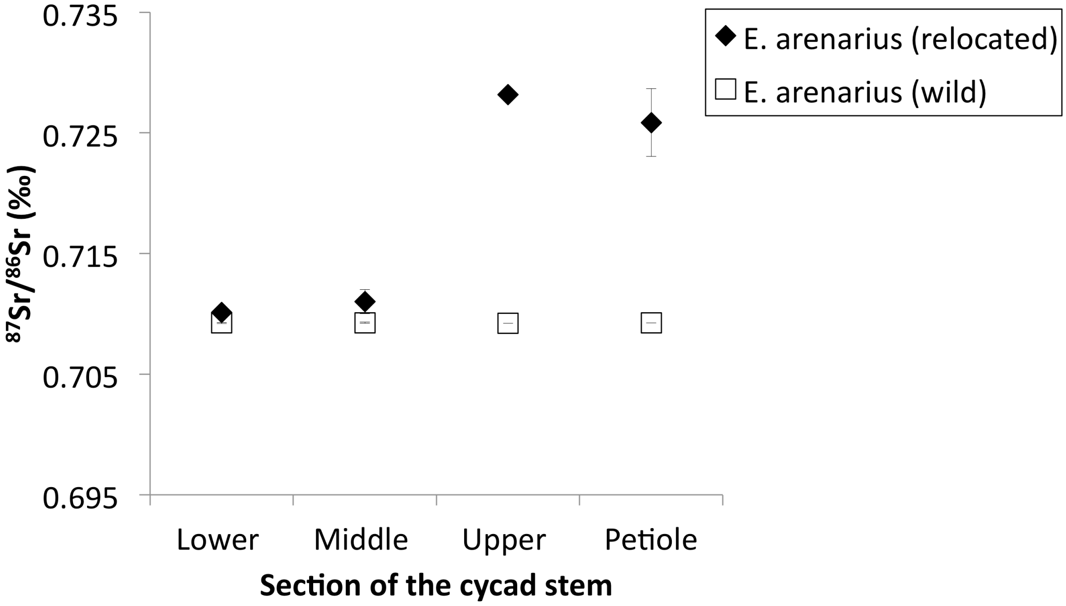
0.735
0.725
0.715
0.705
0.695
$^{87}Sr/^{86}Sr$ (‰)
Lower
Middle
Upper
Petiole
Section of the cycad stem
◆ E. arenarius (relocated)
☐ E. arenarius (wild)

Figure 3

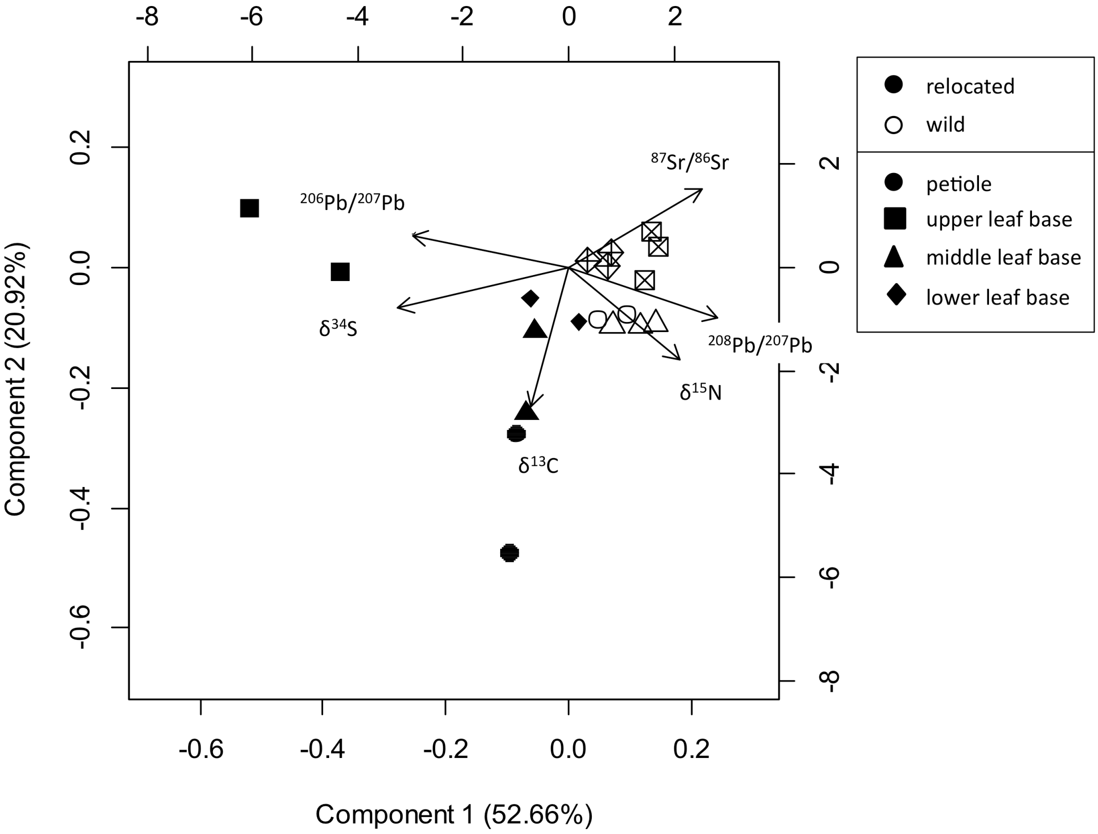
-8
-6
-4
-2
0
2
0.2
0.0
-0.2
-0.4
-0.6
2
0
-2
-4
-6
-8
-0.6
-0.4
-0.2
0.0
0.2
Component 1 (52.66%)
Component 2 (20.92%)
$^{87}Sr/^{86}Sr$
$^{206}Pb/^{207}Pb$
$\delta^{34}S$
$^{208}Pb/^{207}Pb$
$\delta^{15}N$
$\delta^{13}C$
● relocated
○ wild
● petiole
■ upper leaf base
▲ middle leaf base
◆ lower leaf base

Figure 4

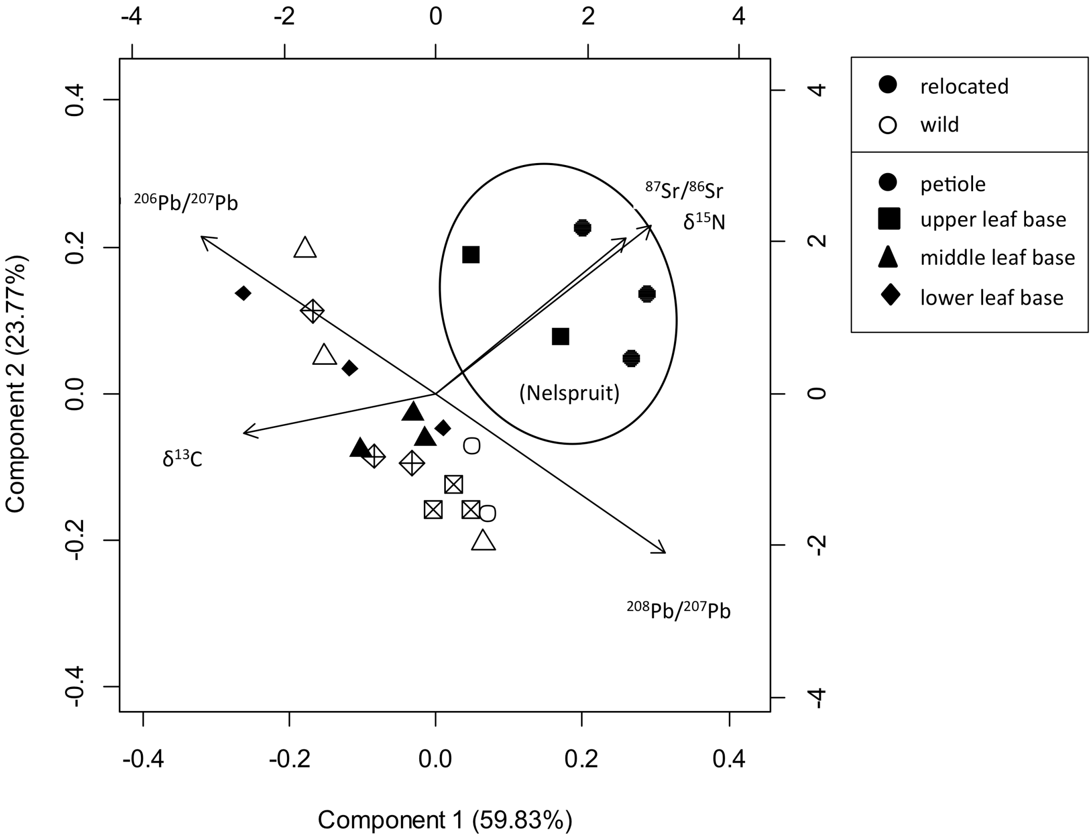
-4
-2
0
2
4
0.4
0.2
0.0
-0.2
-0.4
Component 2 (23.77%)
-0.4
-0.2
0.0
0.2
0.4
Component 1 (59.83%)
²⁰⁶Pb/²⁰⁷Pb
⁸⁷Sr/⁸⁶Sr
δ¹⁵N
δ¹³C
²⁰⁸Pb/²⁰⁷Pb
(Nelspruit)
● relocated
○ wild
● petiole
■ upper leaf base
▲ middle leaf base
◆ lower leaf base

Figure 5

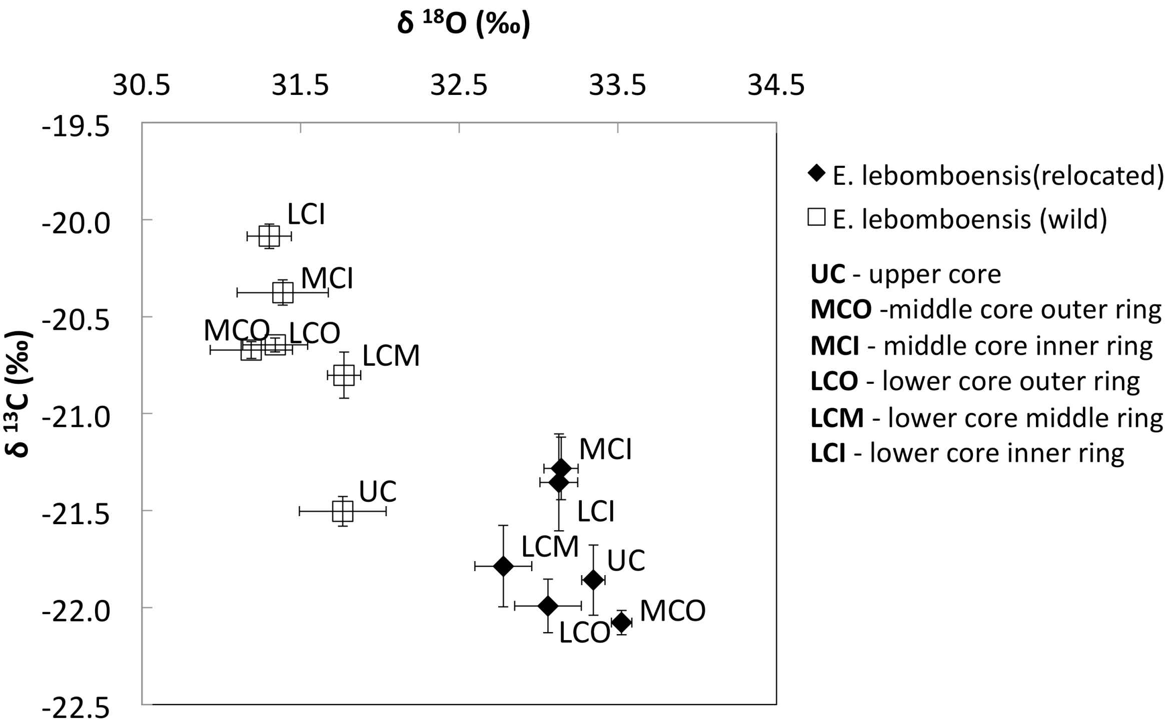
δ ^{18}O (‰)
30.5
31.5
32.5
33.5
34.5
δ ^{13}C (‰)
-19.5
-20.0
-20.5
-21.0
-21.5
-22.0
-22.5
LCI
MCI
MCO
LCO
LCM
UC
MCI
LCI
LCM
UC
MCO
LCO
◆ E. lebomboensis(relocated)
☐ E. lebomboensis (wild)
UC - upper core
MCO -middle core outer ring
MCI - middle core inner ring
LCO - lower core outer ring
LCM - lower core middle ring
LCI - lower core inner ring

Figure 6

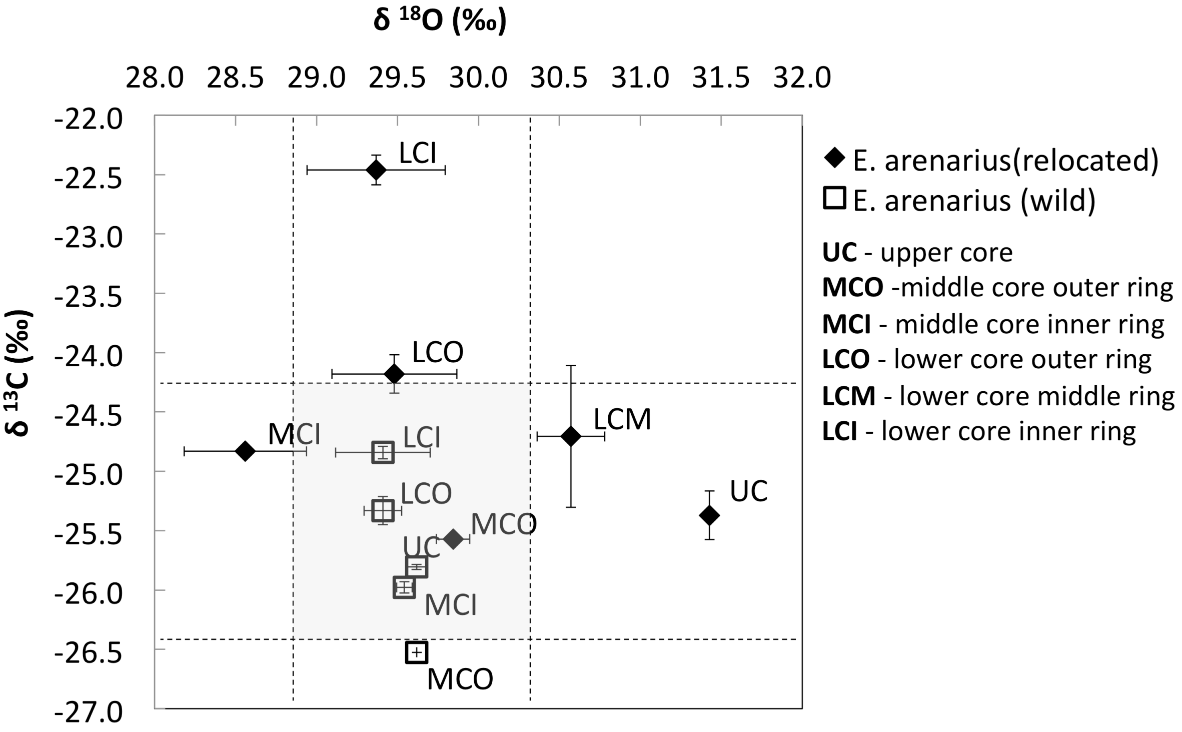
δ ^{18}O (‰)
28.0
28.5
29.0
29.5
30.0
30.5
31.0
31.5
32.0
δ ^{13}C (‰)
-22.0
-22.5
-23.0
-23.5
-24.0
-24.5
-25.0
-25.5
-26.0
-26.5
-27.0
LCI
LCO
MCI
LCI
LCM
LCO
UC
MCO
UC
MCI
MCO
◆ E. arenarius(relocated)
☐ E. arenarius (wild)
UC - upper core
MCO -middle core outer ring
MCI - middle core inner ring
LCO - lower core outer ring
LCM - lower core middle ring
LCI - lower core inner ring

Figure 7