



A stand with various plant products at the Faraday traditional medicine market, Johannesburg. Photo by Zandisile Shongwe.

# CYCADS TRACKED THROUGH DNA BARCODES

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**SETTING THE SCENE:** The cycad fossil record dates back to the early Permian (280 million years ago) and possibly the Carboniferous (320 million years ago) making them the world's oldest extant seed plants.

Cycads flourished during the Mesozoic – the age of the dinosaurs – but, unlike the dinosaurs, have survived three mass extinction events in Earth's history.

The devastating loss of cycads, a tragedy largely unnoticed, is very similar to that of the rhino poaching crisis. Cycads are classified as the world's most threatened plant group. Cycad artist Douglas Goode stated in 1989: "cycads could be referred to as the rhino horn of the plant kingdom". South Africa is currently at risk of losing 50% of its cycads within

the next two-10 years, a predicament recently described by SANBI's Michele Pfab as "the South African cycad extinction crisis".

South African representatives of the order Cycadales, include *Stangeria eriopus* and 37 *Encephalartos* species of which three are Extinct in the Wild, twelve Critically Endangered, four Endangered, seven Near Threatened, nine Vulnerable, and only three species of Least Concern (see March 2016 issue of *Veld & Flora*).

*Encephalartos brevifoliolatus* and *E. nubimontanus*, two of the three South African species classified as Extinct in the Wild, became extinct between 2003 and 2010. Numerous populations of cycads have been virtually obliterated by 'collectors'. For example, populations of *Encephalartos cerinus*, first described in 1989, have recently been plundered so heavily, that in less than 30 years, this species is on the verge of becoming Extinct in the Wild with only an estimated 20 to 70 plants remaining!

## LEGISLATION AND CYCADS

South African legislation is among some of the world's strictest laws controlling cycad trafficking. All *Encephalartos* species are listed in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and as Threatened or Protected Species in terms of the National Environmental Management: Biodiversity Act (NEM:BA; Act 10 of 2004).

Conservation officials are often left helpless, however, when monitoring illegal trafficking of species and products derived from cycad species. Helpless, for reasons including:

- Traders deliberately misidentifying threatened cycads as more common species that are less likely to attract the attention of law enforcement agencies;
- Species-level identification is nearly impossible when plants are stripped of their leaves for transport purposes; and,
- It is impossible to identify species from fragments, such as bark material, sold on the *umuthi* (traditional medicine) markets.

## WHAT IS DNA BARCODING?

DNA barcoding involves using a short, agreed-upon region of a genome (genetic

material) as a unique identifier for each species. These short gene sequences – known as DNA barcodes – can be used to identify a species, even if only a small fragment of plant material is available. In 2009, the Consortium for the Barcode of Life (CBOL) Plant Working Group proposed the standardised use of the gene sequences *rbclA* and *matK*, found in chloroplasts, as the core DNA plant barcodes.

## HOW CAN DNA BE USED?

Accurate identification is essential to enforce the highest penalties for illegal cycad trafficking. The emergence of DNA barcoding is providing an invaluable tool in tackling this challenge. Various botanical studies have already demonstrated the impact of DNA barcoding, which has been used to discover and identify unknown species, resolve taxonomic problems, detect substitution in herbal products in North America, aid with traditional medicine market surveys – allowing identification of commercialised medicinal plants in northern Morocco – and scrutinise the illegal trade of endangered plant species, such as wild-harvested orchids in Nepal.

## THE SOUTH AFRICAN CONNECTION

In response to an increase in cycad poaching, South Africa's officials are turning to DNA barcoding to safeguard our cycads. A cycad DNA barcoding

project was launched in 2010 at the African Centre for DNA Barcoding (ACDB), University of Johannesburg, with the aim of creating a DNA barcode reference library for African *Encephalartos* species in an attempt to control illegal cycad collection. This project is in collaboration with the International Barcode of Life and the Barcode of Wildlife projects (see Box 1).

The library, representing 64 of the 65 African *Encephalartos* species, is now complete. For each species we have barcoded at least five duplicate samples from different geographical regions. Thus, we are now able to compare a small fragment of cycad material – a fragment of stem, seed, seedlings or mature specimen without leaves that cannot be identified morphologically – with the cycad DNA library in order to provide accurate identification.

### BOX 1: LARGE-SCALE BARCODING INITIATIVES

**International Barcode of Life Project** ([www.ibol.org](http://www.ibol.org)): In 2011, the Barcode of Life Project was launched in Canada. The largest biodiversity genomics initiative ever undertaken, it is an international effort to build a digital identification system for the planet's estimated 10-100 million species.

**Barcode of Wildlife Project** ([www.barcodeofwildlife.org](http://www.barcodeofwildlife.org)) – The Barcode of Wildlife Project, funded by Google and managed by the Consortium for the Barcode of Life, aims to demonstrate the value of DNA-barcoding to investigate and prosecute wildlife crime in five developing countries. Goals of the project include: 1) to build a public database of reference barcode records for traded species and their close relatives and look-alikes, against which the barcode sequences of confiscated material can be compared; 2) to train researchers, technicians, border inspectors, prosecuting attorneys in the participating countries in DNA barcoding and its applications; and, 3) to test DNA barcodes as a real-world tool for enforcement through implementation of operational, cost-effective barcoding programmes.



Confiscated material following illegal harvesting of mature *Encephalartos* specimens. Photo by Eastern Cape Department of Economic Development, Environmental Affairs and Tourism.

The process takes less than 24 hours from the reception of the material to identification. We have assisted in several police cases related to cycads, as well as tracked the illegal trade of cycads at traditional medicine markets (see Box 2).

Unfortunately, population-level markers (e.g. amplified fragment length polymorphisms - AFLPs) are not yet available for *Encephalartos* species, making it impossible to infer from

which wild population these plants originate. The ACDB laboratory is now moving towards building a genetic profile of cycad species using AFLPs. This is a long-term project since we would need to create a comprehensive AFLP genotype reference library of all wild populations. The project is feasible and would significantly improve cycad law enforcement in South Africa. However, cost and accessibility to the various cycad populations around the country are presently limiting factors.



Dinosaur sculpture at Kirstenbosch. Photo by André Wessels.

## BOX 2: EXPOSING THE ILLEGAL TRADE IN ENCEPHALARTOS SPECIES AT THE FARADAY AND WARWICK TRADITIONAL MEDICINE MARKETS USING DNA BARCODING

**Background:** Illegal plant harvesting for the medicinal trade has increased in South Africa and resulted in the decline of cycad populations, including complete loss of sub-populations. *Encephalartos* is traded at traditional medicine markets in the form of bark strips and stem sections, thus determining the actual species traded presents a major challenge due to lack of characteristic plant identification markers.

**Methodology:** We used DNA barcoding to identify cycads sold at Faraday, and Warwick traditional medicine markets. Market samples were sequenced for the core DNA barcodes (*rbcLa* and *matK*), as well as the two additional regions *trnH-psbA* and *nrITS*. The barcoding database for cycads at the University of Johannesburg was utilised to assign query samples to known species.

**Results:** Our study indicated that there is currently a lucrative trade in cycad species at traditional medicine markets. Market samples identified were: *E. ferox* (Near Threatened), *E. lebomboensis* (Endangered), *E. natalensis* (Near Threatened), *E. senticosus* (Vulnerable), and *E. villosus* (Least Concern).

**Significance:** The study validates the use of DNA barcoding in identifying *Encephalartos* species rapidly, with incredible accuracy, particularly when morphological evidence is lacking. The study highlights the undercover trade in *Encephalartos* species at traditional medicine markets, which are not adequately researched and recognised in official government documents or databases. Now solutions are being provided to address conservation of our most endangered plant species.

**Source:** Williamson, J. et al. Exposing the illegal trade in *Encephalartos* species at the Faraday and Warwick traditional medicine markets in South Africa using DNA barcoding (Submitted to *Genome*).

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