Above left: Cycads in front of the main building at the University of Johannesburg
Above: Cycad collection at Kirstenbosch Botanical Gardens, Cape Town

DNA BARCODING OF ENDEMIC CYCADS

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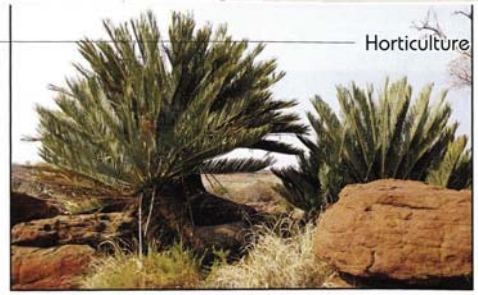
Cycads evolved approximately 300 million years ago and represent the oldest seed plants on earth. Today they are highly endemic and among the most endangered groups of plants worldwide, with approximately 50% of the species included in the IUCN (International Union for Conservation of Nature) Red List and 82% of those listed as threatened or worse.

This is also true for the African endemic species of the genus *Encephalartos* with all of the 65 species classed as CITES (Convention on International Trade in Endangered Species) Appendix I, meaning that trade in wild material is banned and trade in cultivated material is strictly controlled. For some taxa it is already too late; *Encephalartos woodii* (Wood's cycad) is extinct in nature and there is no known female specimen on earth, which makes it possibly the most sought-after cycad in the world. John Medley Wood discovered one clump of male plants in 1895 on the fringes of the Ngoye forest in KwaZulu-Natal and despite numerous excursions in the Ngoye-Mtunzini area, no other specimens of *Encephalartos woodii* have ever been found.

Although new species are being discovered, the exploitation by humans has resulted in many being listed as critically threatened or endangered. In South Africa, hosting more than half of the continent's cycad diversity, some cycad colonies have been virtually wiped out by collectors seeking samples. When a botanist a few years ago discovered a new species, *Encephalartos cerinus*, thieves plundered so many of the plants that they nearly wiped out the species within weeks of its discovery. A search on the Internet or even the Cycad Society of South Africa's web page would deliver more articles on theft than any other matter. For example in November 2009, half a million rands worth of cycads were stolen from the Durban Botanical Gardens. Given the current situation, many species may become extinct before they are discovered.

Left: Stangeria eriopus - the Natal grass cycad is endemic to southern Africa

Right: Male cones of *Encephalartos transvenosus* (Modjadji Cycad)



The rare blue cycad, *Encephalartos middelburgensis*, in its natural habitat near Middelburg, Mpumalanga

Historically, *Encephalartos* species have been used as a source of starch by indigenous people (hence the Afrikaans name "broodboom") but this practice seems to have disappeared in South Africa. More commonly, a section of the outer bark is harvested and sold at 'muti' markets as part of the substantial local trade in medicinal plants. However, by far the largest trade in *Encephalartos* is for horticultural purposes. Collectors are prepared to pay up to 6 000 pounds for a large specimen of a rare species, encouraging a flourishing but illegal trade in these plants and causing many species to become endangered due to the pressure from wild collecting.

Although this paints a bleak picture for species of *Encephalartos*, South Africa has some of the world's strictest laws controlling cycad trafficking, though conservation officials are often overburdened in their efforts especially when it comes to identification. This is due to the fact that when plants are illegally harvested, they are most often transported only as trunks where identification is nearly impossible, and plants sold on the 'muti' markets are often only fragments impossible to identify using conventional means. Accurate tools for identification are therefore essential. In South Africa a permit, issued by Nature Conservation Law, must accompany large specimens of any *Encephalartos* species. However the large amounts of plants, coupled with the difficulty in distinguishing closely related species, often results in misidentification while waiting lists grow ever longer. Misidentification also has a large impact on the artificial pollination of these endangered plants, which is necessary in cultivation. As cycads are dioecious (male and female cones occurring on separate individuals), it is important to use the correct species or even form for exact pollination. The development of a genetic-based tool for identification would thus provide an unparalleled advantage and opportunity for scientists and laymen alike. DNA barcoding is one such technique that is relatively simple to apply and yet can distinguish even between closely related species or forms without expert assistance. An international team of 52 scientists (Consortium for the Barcode of Life Plant Working Group under the leadership of Dr Pete Hollingsworth), including three researchers from the University of Johannesburg, has concluded a four-year effort to find a standard "plant DNA barcode". The findings were published in the Proceedings of the National Academy of Science in which a combination of two gene regions, taken from the plastid genes (rbcL and matK), were selected as barcodes for land plants.

DNA barcoding works by comparing the sequence of DNA bases from a short part of the genome that is standardised between plant groups. It is intended as a reliable, cost-effective tool for documenting biodiversity research, controlling disease vectors, pests and invasive species, protecting endangered species and other regulatory areas in which species identification is critical. DNA barcodes are currently available for 65 000 species derived from more than 700 000 specimens on the Barcode of Life Data Base (BOLD). The aim of iBOL (International Barcode of Life initiative) is to deliver barcodes for 500 000 species by January 2014. In the foreseeable future, the intention is to see this idea of reading plants' genetic barcodes translated into a portable device ("barcoder") that can be taken into any environment to quickly and easily analyse any plant samples, comparing it to a vast data base of information and allowing almost instantaneous identification. Although this stage is still far off, there is no major technological barrier for the development of the barcoder. iBOL anticipates that the development of the device will occur in two stages - firstly a table-top instrument and secondly hand-held barcoders.

To this end a DNA barcoding project on cycads has been launched at the University of Johannesburg, with the goal of creating a library of reference barcoding sequences for all *Encephalartos* species along with *Stangeria eriopus* (the only other African endemic cycad), which will enable any relevant party to identify specimens. This project forms part of a global initiative to DNA barcode all the trees of the world (TreeBOL) within the next five years. This DNA barcode library will allow fully automated identifications for most specimens because of the digital format of the library, which will greatly improve the ability to monitor, understand and manage biodiversity with substantial scientific, forensic and economic benefits. Law enforcement will be able to use the barcode of an unidentified specimen and then compare it with the reference barcode to find the matching species. From a scientific viewpoint, the analysis of the genetic variation found might give greater clarity in terms of relationships between species, classification problems and even new species or forms and will be the first step towards a complete taxonomic revision of the genus at large.

The beauty of these plants seems to be their undoing, with the strong horticultural appeal translated into high threat levels and endangered status. This project will not only help law enforcement but will assist legislators, conservationists and scientists in the fight to solve this problem with a new technique on an ancient group of plants. **lsa**

Photos by Olivier Maurin and Philip Rousseau