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Darwin Initiative

Final Report

1. Darwin Project Information

Project Reference No.	*162/12/008*
Project title	*DNA banking, phylogeny and conservation of the South African flora*
Country	*South Africa*
UK Contractor	*Royal Botanic Gardens, Kew (RBG Kew)*
Partner Organisation (s)	*South African National Biodiversity Institute (SANBI; formally the National Botanical Institute) University of Cape Town (UCT) University of Stellenbosch (US) University of Johannesburg (UJ, formally Rand Afrikaans University)*
Darwin Grant Value	*£116,187*
Start/End date	*01/06/2003 – 31/03/2006*
Project website	*http://www.sanbi.org/research/dnabank.htm*
Author(s), date	*Dr Vincent Savolainen & Mr Martyn Powell (RBG Kew, UK), Dr Gail Reeves, Ms Ingrid Nanni & Ms Ferozah Conrad (SANBI, South Africa), 11 August 2006*

2. Project Background/Rationale

South Africa possesses a unique flora with three global biodiversity hotspots within its borders. The wealth of botanical knowledge and expertise in South Africa is reflected in its extensive herbarium collections, housed by both the SANBI and academic institutions. This project was motivated by the need for South Africa to take control of its genetic resources, and to utilize and allow access to them according to CBD protocols and current national legislation. DNA banking has represented one such avenue to address these shortcomings, and thus this project has enabled the financial input and skills and knowledge transfer required to establish a truly modern biodiversity facility for

high-profile research and conservation in South Africa.

3. Project Summary

- **Purpose and Outputs:**

At the outset of the funding cycle in 2003 the project aims were to: (i) archive in a DNA bank the genetic material of at least one species from nearly all ca. 2000 South African flowering plant genera, of which 70% sp. are endemic; (ii) provide the necessary facility to extract DNA and preserve it appropriately, and to allow researchers to have access to plant DNA extracts to be used in applied and fundamental science; (iii) through collaboration with Kew's CBD Unit, to implement the necessary legal agreements for material transfer and benefit sharing of these genetic resources; (iv) train South African researchers and students in high-profile biotechnologies; (v) produce a 'phylogenetic tree of life' of South African plant genera and use this tree to identify areas of endemicity and high priority for conservation.

- **Modifications:**

The outputs have been modified in the following way and approved by the Darwin Secretariat throughout the previous reporting exercise. Our original collecting target (of one representative of every genus in the flora) proved to be overly ambitious, and hampered by severe droughts in the Northern and Western Cape (both areas of high endemicity). We therefore amended our collecting target to 800 genera, although we have now substantially exceeded this target (1,237 genera collected to date). As a result of this revised target we have also restricted our plans to build a phylogenetic tree using *rbcL* sequences to that of the Cape flora (which is made up of 947 genera), focusing on building a comprehensive tree for the Cape, we have also increased our sequencing effort from 500 *rbcL* sequences (initial target) to over 750.

- **CBD articles:**

The project contributes mostly to six of the articles under the CBD (see Appendix 1) and especially article 16: Access to and Transfer of Technology, and article 12: Research and Training. The acquisition of the high-tech equipment required to set-up a DNA banking facility was a huge boost for the SANBI DNA laboratory and this facilitated the training of a South African DNA Bank manager and a large number of South African researchers and students in DNA extraction and sequencing techniques. In addition, the research by a post-doctoral fellow, who was integrated in this project, to produce the phylogenetic tree of the Cape flora would not have been possible without the DNA bank. This research has fostered collaboration between taxonomists and conservationists, and the researchers involved have been engaged in area-wide analyses to use phylogenetic diversity as an additional tool for identifying areas of future conservation efforts. This is groundbreaking research that has benefited tremendously from the DNA banking facilities at SANBI.

- **Success in meeting objectives:**

(i) Genetic material from at least one species of 1,237 South African genera has been archived in the South African DNA bank. The total number of DNA extracts now stored in the DNA bank stands at 4,414.
(ii) The facilities to extract and for the long term storage of DNA are now available at SANBI. SANBI has also created a SANBI-financed DNA Bank manager post to ensure that the collections are properly curated, and available to researchers for applied and fundamental science (Ms Kholiwe Balele who was trained by the project as the DNA bank manager resigned to take up a managerial position at the South African Institute for Aquatic Biodiversity (SAIAB); SANBI has appointed Ms Lache Rossouw to replace her.)
(iii) Kew and SANBI have implemented the necessary legal agreements for material transfer and benefit sharing of these genetic resources. At the start of the project, a

workshop was organised and attended by K. Davis, C. Williams and V. Savolainen from Kew, and G. Reeves and M. Wolfson from SANBI, to set up the basis for these agreements.
(iv) Thirty-nine students from various academic institutes in South Africa have been trained in the molecular laboratory at SANBI in biotechnologies and molecular systematics.
(v) A 'phylogenetic tree of life' of the Cape plant genera has been produced and is being used by the researchers to identify areas of endemicity and high priority for conservation (the draft paper discussing these results is to be submitted to the journal *Nature* by the end of August, manuscript enclosed with this report). The project hoped to produce a 'tree of life' for all South African plant genera but this proved to be too optimistic. The Cape plant genera collected by this project represent 32% of all the genera collected in the South African DNA Bank, so the Cape 'tree of life' represents a large proportion of the collected DNAs. In addition to the stated objectives of the project, the frequent contact of project participants with conservation managers, as well as the popular and scientific articles written by project participants, contributed much towards raising awareness of the possibilities of using DNA banking as a conservation tool amongst conservation managers and civil society groups interested in conservation. The project also stimulated research collaboration between systematists and conservation scientists at the very successful workshop in Cape St Francis in March 2005.
(vi) Two significant books have been written: a DNA banking manual has been produced, which includes protocols for molecular techniques (co-published by the Royal Botanic Gardens, Kew and the World Conservation Union, IUCN), along with a photographic guide to the South African flora (published by Briza Publications). In addition, a special issue of the *Philosophical Transactions of the Royal Society, London*, on DNA barcoding was edited by project leader V. Savolainen and one paper reported on some of the results from this project. A letter to the journal *Science* also publicised this project.

4. Scientific, Training, and Technical Assessment

- **Research:**

Several collecting trips took place during the project in various parts of the country, although with a particular focus on the Cape region:

- March 2004: Trip to Gifberg Mountain (Felix Forest and John Manning).
- September 2004: Day trip to Fernkloof Nature Reserve with Darwin workshop participants. We were shown around the reserve by volunteer guides who helped us collect several new genera for the bank.
- September 2004: As part of the Darwin workshop, several participants joined a collecting trip in the Cedarberg Mountains.
- November 2004: Felix Forest and Terry Trinder-Smith (Bolus Herbarium, University of Cape Town) collected in the Eastern Cape. They received assistance and helpful advice from several people during this trip, including Richard Cowling (Nelson Mandela Metropolitan University) and Tony Dold (Rhodes University). About 80 new genera for the bank were collected during these ten days.
- November 2004: Gail Reeves visited Mpupalanga and received the help of Mervyn Lotter.
- March 2005: Day trip to Fernkloof Nature Reserve with students from the molecular techniques training course.
- August 2005: Collecting trip to the Northern Cape (Ingrid Nänni, John Manning and Felix Forest).

- Several day trips made by Félix Forest and colleagues around Cape Town at various times of the year during the project.
- Many collections were made by Peter Goldblatt, Nick Helme and David Styles. The project paid for each new genus collected by these botanists and they provided us with many rare species and species that grow in inaccessible areas
- Several interesting and valuable genera were collected by Caryl Logie and the Fourcade Botanical Club.

Based on material collected during these fieldtrips, plus DNAs previously available at Kew's DNA bank and duplicated to South Africa, we have built a phylogenetic tree of the Cape flora. Out of the 943 genera currently recognized within the Cape, we included 735 in our phylogenetic tree of the Cape genera of which nearly 400 were collected for the project. The main research output is a conservation analysis of the distribution of phylogenetic diversity in the Cape, which was compared with traditional species richness indices. This paper will be submitted to *Nature* by the end of August 2006. We enclose below the first paragraph of this paper and the full manuscript is attached to this report.

Summary of paper (see also Appendix V): **"Preserving the evolutionary potential of floras in biodiversity hotspots"**. Given limited resources, one of the biggest challenges for conservation biology is to provide conservation planners with ways to prioritise efforts. Much attention has been placed on so-called global hotspots of species richness and endemism (Lamoureux et al. 2006). However, conservation of evolutionary processes is now acknowledged as a priority in the face of rapid global change. Phylogenetic diversity (PD) measures the evolutionary pathways that connect a given taxon assemblage, so that high PD identifies collections of taxa with increased evolutionary potential (Faith 1992). Recent studies concluded that taxon richness was a good proxy for PD (Polasky et al 2001; Rodrigues and Gaston 2002; Rodriguez et al 2005). Here, in an undisputed biodiversity hotspot, we show that taxon richness is decoupled from PD, and fails to capture evolutionary potential when selecting priority areas for conservation. We have compiled a biome-wide phylogenetic analysis of the flora of the Cape of South Africa and identified lineages of medicinal and economic utility. We found that although taxon richness points to the floristic assemblages of the western part of the Cape as the most diverse, for a given number of taxa, PD and evolutionary potential are significantly higher in the eastern part of the region. Furthermore, given the realities of conservation planning, where it is marginal gains that matter, the localities that offer gains in PD are not well predicted by gains based on taxon counts. Our analyses also demonstrate that utility, like diversification (Davies et al 2004), is a labile phylogenetic character, so that PD provides the best strategy for retaining both. As our knowledge is about to encompass the entire tree of life, we will be able to identify those key regions that maximise future options, both for the continuing evolution of life on earth and for its benefit to society.

Several master projects at SANBI, UJ and UCT were also completed under this Darwin initiative (see 15. Appendix II outputs); as an example of the research being done we provide summaries for three MSc projects.

"Conservation genetics of the critically endangered South African cycad *Encephalartos latifrons*." MSc. project: Jessica da Silva. Percy Fitzpatrick Institute, University of Cape Town, and Kirstenbosch Research Centre, SANBI. Using the AFLP fingerprinting technique this project revealed that the collection of *E. latifrons* in the *ex situ* collection at Kirstenbosch Botanic Garden represents a significant portion of the remaining genetic diversity of the species. As a result of this project, approximately three quarters of the plants remaining in the wild (ca. 60 accessions) are now represented in the DNA bank at SANBI. Jessica received a distinction for her thesis and was awarded

third prize for her poster at Cambridge University's Conservation Biology Conference for young scientists in 2005. The paper is currently in preparation to be submitted to Conservation Biology (co-authors: G Reeves (SANBI) and T Hedderson (UCT) and J.S. Donaldson (SANBI)).

"Species-level phylogenetic reconstruction of the African cycad genus *Encephalartos"*. MSc. Project: Amelia Mabunda, Kirstenbosch Research Centre, SANBI and University of the Western Cape. Using a combination of DNA sequences and AFLP markers this project has attempted to reconstruct the species-level phylogenetic history of the genus *Encephalartos*. As a result of this project ca. 70% of the described species of *Encephalartos* are now represented in the DNA bank at SANBI. Amelia aims to submit her MSc thesis by November 2006. She is now also working as part of the SANBI team on the consortium for the barcode of life project, which is coordinated by Kew and aims at developing a universal DNA barcode for land plants.

"Determination of genetic variability in South African species of *Carpobrotus"*. MSc. project: Angeline Khunou, Kirstenbosch Research Centre, SANBI and University of the Western Cape. Using AFLP markers this project focused on two species of *Carpobrotus* indigenous to South Africa that are commonly used for their medicinal properties, *C. edulis* and *C. acinaciformis*. This project demonstrated that hybrids between the two species are common in overlapping parts of their geographical range. This can lead to great difficulties in morphological identification. Approximately 100 accessions are now housed in the SANBI DNA bank representing 'pure' *C. edulis* and *C. acinaciformis* and their hybrids. Angeline passed her MSc in 2005 and the paper is currently being completed for submission to Molecular Ecology (co-authors G Reeves, J Bishop, F Weitz and C Klak).

- **Training and capacity building activities:**

The project has provided capacity building for SANBI staff and others. For example, at the start of the project, Kholiwe Balele (SANBI's DNA bank manager) visited the Jodrell Laboratory and received in-house training in DNA extraction techniques from Kew's DNA bank managers, Edith Kapinos and Laszlo Csiba. As a follow up, Kew's DNA bank managers both visited Kirstenbosch in 2005 to continue training Kholiwe. In turn Kholiwe instructed numerous students and visitors in DNA extraction during her three years as DNA bank manager of the SANBI facility. She also trained one of the lab's intern students, who subsequently was appointed in her place when Kholiwe left SANBI to take up a position with the South African Institute for Aquatic Biodiversity (SAIAB).

The training of South African students has also been a key component of the project, in total 39 students were trained over the four one-week training courses that were organised (see Appendix VI for list of attendees). The first course was held in July-August 2003 (11 students), with subsequent courses in March 2004 (15 students), October 2004 (five students) and March 2005 (8 students). The students from the universities of Cape Town, Stellenbosch and the Western Cape were part of a one-year Masters degree in 'Systematics and Biodiversity Science' and the one-week course at Kirstenbosch was included as part of a module on: 'The uses of molecular data in biodiversity science'.

The training was organised as one-week intensive courses in 'Biotechnology and Molecular Techniques' held at SANBI's molecular laboratory in Kirstenbosch and run by some of the staff working there (Gail Reeves, Ferozah Conrad, Angeline Khunou and Kholiwe Balele as well as post-doc fellow Félix Forest). The course was divided between laboratory and computer practical components. The course involved hands-on experience of molecular biology techniques such as DNA extraction, PCR and cycle

sequencing. In specific relation to the aims of the DNA banking project, the students extracted DNA material to be deposited into the bank and then produced *rbcL* sequences. The computer practical was designed to expose the students to the theory and practice of building phylogenetic trees. In pairs they were required to download published DNA sequences from Genbank (representing coding and non-coding data sets for a given plant group), align these sequences by eye in PAUP, and then to conduct the necessary tree searches in PAUP. Both laboratory and computer practicals were preceded each day by short lectures explaining the theoretical background to the subsequent session. The need for phylogenetic data in the search for accurate and predictive classification systems was stressed during these lectures, in addition to the relevance to conservation planning and needs assessment. At the end of the week the university supervisors were invited to attend 10-15 minute presentations by the students on the outcomes of their computer practical. The extent to which the students had grasped phylogenetic theory and its wider implications to systematics and conservation was thereby assessed. All students were awarded an overall percentage based upon the performance in the lab during the week in addition to their final presentation. These marks contributed towards their MSc degree or BSc. Hons.

As another activity for capacity building, several MSc students from UJ (S. Boatwright, C. Motsi, C. Robinson, P. Naude, N. du Toit, A. van Nickerk, J. Moeaha & M. Rautenbach) visited Kew for some practical experience in our molecular systematics section, usually spending at least one month in the Jodrell Laboratory. They were supervised by V. Savolainen and M. Powell; several publications will be derived from this work: e.g. one paper by Boatrwight, Savolainen, van Wyk, Schutte-Vlok & van der Bank entitled "The enigmatic genus *Cadia*: An example of reversal to actinomorphy in legumes and the phylogeny of Podalyrieae" has just been submitted to *Mol. Phyl. Evol*.

Project Impacts

The project has contributed towards the protection of genetic resources within South Africa; in particular the modification of SANBI's material transfer agreement to allow DNA samples to be passed to outside institutions through the DNA bank has been a significant step forward. In terms of the Biodiversity Strategy and Action Plan it ensures that spatial conservation assessments can use phylogenetic information in future assessments.

The establishment of the DNA bank at SANBI is recognised as an important factor in helping South Africa meet its obligations under the CBD, as Action Plan 50.4 for South Africa targets the number of genomes stored in DNA banks as an indicator of the sustainable use of biological resources and the equitable sharing of the benefits (linking to sub-target 3.1 of the CBD's 2010 Biodiversity Target).

The table below provides examples of what trainees under this project are now doing:

Trained under the Darwin Project	Name	Current situation post-training
Trained DNA Bank manager	Kholiwe Balele	Manager of the fish collections at the SAIAB in Grahamstown, South Africa

Current DNA bank manager	Lache Rossouw	Newly appointed DNA bank manager at the Kirstenbosch Research Centre
PhD Student	Ferozah Conrad	Manager of the molecular laboratory at the Kirstenbosch Research Centre
PhD Student	Christopher Cupido	Scientist at the Compton Herbarium, Kirstenbosch Research Centre
MSc graduate	Angeline Khunou	Scientist in the molecular laboratory at the Kistenbosch Research Centre
MSc graduate	Jessica Da Silva	Currently looking for a PhD studentship
MSc student	Amelia Mabunda	Writing up MSc thesis and working as a technician for the plant DNA barcoding project
Attendee of one-week training course	Ntsikelelo Lester	Holder of a three-month internship at SANBI, and now an MSc student at Stellenbosch University working on plant systematics

The project has strengthened the collaborative links between the UK and the South African partners, and this is highlighted by the ongoing research interests and projects that these two institutions share. The success of the project has had a considerable impact on the collaboration between RBG Kew and SANBI, as it has undoubtedly had a major influence on the successful Darwin post-project application, which will enable the two institutions to continue working closely together on the results of this project. In addition, SANBI have been invited to join an international project on "Establishing a Universal DNA barcode for Land Plants", funded by the Sloane and Moore Foundations, which is coordinated by RBG Kew. Similarly, Kew has helped UJ set up a project on "DNA barcoding of the flora of the Kruger Park for Biodiversity and Conservation", implemented by UJ and funded by the South African NRF. Finally, as another example of the fruitful collaborations that have been derived in part from the Darwin project, all South African partners will be organising a symposium on understanding the causes of hyper-diversity in the Cape using molecular phylogenetic data in late 2007-early 2008. The journal *Mol. Phyl. Evol.* has just accepted to publish the papers resulting from this symposium, with several contributions from SANBI, UJ, US, UCT, etc, and PI V Savolainen (Kew) and T. Verboom (UCT) will be acting as associate editors.

5. Project Outputs

- **Project outputs:**

The project has been highly successful in meeting most of its objectives (see Appendix II), and the major outputs are summarised below.

A DNA banking facility has been established to enable the extraction and preservation of the South African angiosperm flora. There are currently 4,414 DNA extracts archived in the DNA bank, representing at least one species of 1,237 South African genera, and these are accessible to researchers wishing to use them in applied and fundamental science. The original target of collecting at least one species from nearly all ca. 2,000 genera proved to be too ambitious given the problems encountered with extended drought in the country and field collections (see section 9).

The success of the DNA bank has led to SANBI turning the DNA bank manager post into

a permanent position, which will ensure the longevity of the collections and enable the bank to expand and remain available for research scientists.

The required legal agreements have been put into place to allow material transfer to and from the DNA bank, and to ensure appropriate benefit sharing of these genetic resources.

Biotechnological and Molecular Systematic training of South African researchers and students has also been an unqualified success, with 39 students from multiple academic institutions having received training in the molecular laboratory at SANBI.

A phylogenetic 'tree of life' has been produced for the Cape plant genera, and is being used to identify areas of endemicity and high priority for conservation (paper to be submitted to *Nature*, see section 4). Originally it was intended to produce such a phylogenetic tree for all South African plant genera, but this proved to be too optimistic and as such the focus area was restricted to the Cape flora.

Several significant publications have been produced as a result of this project, these include: a manual on DNA banking which includes biotechnology protocols and all of the necessary information required to set-up a DNA banking facility; a field guide to the South African flora; a special issue of the *Transactions of the Royal Society* on DNA barcoding and three papers (one letter and one book chapter published, one paper remaining to be submitted). In addition, several publications based on material from the DNA bank have been or will be published in high profile scientific journals (we have listed two of those in Appendix III).

- **Dissemination of project outputs:**

The dissemination of the project outputs has been to as broad an audience as possible, including both scientific and non-scientific factions of the community.

Information for researchers interested in obtaining DNA samples is available on the SANBI website. This will continue to be developed as SANBI is undertaking a complete review of the current website to give it a new look, and make it more attractive and user friendly. The website developers have been in contact with the DNA Bank staff to discuss how the database should be accessed.

The DNA banking manual produced by the project has been distributed to a wide international audience, and an additional 30 copies have been sent to SANBI for distribution within Africa. It is also available for purchase from the IUCN and Kew websites.

Over the course of the project seven international conferences (e.g. Southern Connection Conference in Cape Town, Evolution meeting in Alaska) were attended where the results of the Darwin project were disseminated. In addition five workshops were also organised/attended by people working on the project, and these also involved the presentation of findings from the project.

Regular updates on the DNA Bank project are available in all three issues of the new "Newsletter of SANBI's Plant Conservation Projects", which is a full colour pamphlet distributed to researchers, conservation managers, policy makers in government departments and civil society interest groups. Items about the project have also been published in Kew Scientist, freely available on the web and distributed to many botanical institutes.

6. Project Expenditure

The project has under spent by a total of £12,004. The majority of this occurred during the first year of the project, as expenditure on capital items (the ultracentrifuge) and salaries was £10,467 less than the budget allowed for. The remaining £1,537 was unspent during the final year of the project, when again salary costs where lower than what had been budgeted for.

7. Project Operation and Partnerships

The South African National Biodiversity Institute was the host-country lead partner. The other partners were the University of Cape Town, the University of Stellenbosch and the University of Johannesburg, who were all partners from the initiation of the project. Subsequently Professor Richard Cowling from the Nelson Mandela Metropolitan University participated in the Cape St Francis workshop and will collaborate in conservation publications emanating from the project.

To ensure that the project maximised resources, a number of additional experts from a variety of institutions where involved at various stages.

The table below lists the people from other organisations who have been involved in project workshops:

Prof Andrew Balmford	Cambridge University, UK
Dr Jonathan Davies	University of Virginia, USA
Dr Daniel Faith	Australian Museum, Sydney, Australia
Dr Angélique Corthals	American Museum of Natural History, USA
Dr Richard Grenyer	University of Virginia, USA

SANBI is responsible for the South African National Spatial Biodiversity Assessments so information from the DNA Bank project can feed straight into the next assessment. Project partners will continue to work together during the 2-year Post-Project funded by the Darwin Initiative.

8. Monitoring and Evaluation, Lesson learning

- **Monitoring and Evaluation:**

The overall purpose of the project was to establish a long-term facility for the extraction and storage of plant DNA extracts in South Africa. Aligned with this we identified four areas in which the impacts of this endeavour could be maximised, and thus progress of the project over three years was monitored according to these four main activities as outlined in the logical framework. They were: (1) workshops; (2) fieldwork and laboratory research; (3) courses, lectures and practicals; and (4) manual, publications and publicity.

The Logical Framework Approach to project management was utilised for the duration of the project, with monitoring carried out in accordance with the four areas described above. Originally the project website was intended to be the primary source of monitoring the project's progress, but given the problems that were encountered (see section 6), regular phone and email contact between the project partners allowed for regular monitoring, evaluation and updating. In addition to this, the five workshops that were organised, and spread over the three years, allowed the project partners to meet and provided an excellent forum for monitoring the progress of the project against the Logframe.

Financial monitoring was principally carried out between the Finance Department of Kew (Mr Des Bennett & Mr George Sarkis) and Ms Ingrid Nänni of SANBI.

Additionally, an external evaluation of the DNA Bank project was carried out as part of SANBI's five-yearly external review on the laboratory in general.

- **Lesson learning**

The first lesson we have learned concerns the field collecting. We had difficulty in mobilising botanists to collect plants for the project and we were also too optimistic regarding the number of genera that could be collected. There were two main obstacles to collecting the ca. 2,000 plant genera in South Africa. Firstly the extended drought in the country meant that many plants were either difficult to find, or at least difficult to identify conclusively because of the lack of flowering material available. Secondly, we underestimated the effort involved in collecting and therefore underestimated the cost. We had assumed that researchers would easily be persuaded to collect material for the DNA Bank, but this was not the case as most researchers collect dedicated species only (often within a very few genera) and were reluctant to undertake extra collecting and identifications which would impact on their own research time. As a result the project participants had to undertake, or organise, all of the plant collecting and identifications. This meant that all of the collecting expenses were met by the project funds and therefore limited the extent of fieldwork. However, the post-doctoral fellow appointed by UCT and SANBI to work on the project undertook much of the collecting and a few renowned South African plant collectors also agreed to collect on behalf of the project for very modest financial contributions towards their field expenses. Without these collections the South African DNA Bank would be much poorer. For similar projects, in

the future, we would increase the budget for field collecting.

The second main lesson we have learned concerns the management of such a large multi-institutional and two-country project. There are inherent difficulties in managing a project that involves a diverse and complex group of international institutions, and on the whole we believe that these difficulties have been overcome throughout the duration of the project. However, at times, it was difficult to deal with both the challenging scientific aspects of the project and the day-to-day administration. There have been a few hiccups and we feel that the best solution is to decouple to a certain extent the responsibilities for the overall running of the project (by PI and coPI) and the day-to-day administration by dedicated staff. We also note that compared to the UK research councils, the Darwin Initiative is heavier in terms of reporting exercise whilst it usually provides smaller grants. We perfectly understand the logic behind this but then the PI and coPI are sometimes overloaded with the detailed administration of the project and monitoring of the many figures required by the Darwin Initiative. In the post-project and another Darwin project led by V. Savolainen, we have now budgeted for some staff time for project management, so that the UK and host-country leaders can dedicate more of their time discussing and dealing with the bigger issues of the projects, i.e. science and conservation.

Finally it has taken more time than expected to duplicate the samples in the SANBI DNA bank and about 2,000 DNAs are still to be duplicated and sent to Kew.

9. Actions taken in response to annual report reviews

The issues raised in annual report reviews throughout the duration of the project have always been carefully considered, discussed amongst project collaborators and responded to. The actions taken in response are summarised below:

- Following concerns about the logistics and viability of carrying out a phylogenetic study on the entire flora of South Africa, the focus was restricted to the flora of the Cape Region.

- Reviewers were unsure about how we would tackle the process of taking our raw DNA bank and phylogenetic data and transforming them into valuable information for conservation. The strategy was finalised following a workshop with conservation planners and evolutionary biologists/geneticists. It was decided to use the phylogenetic data to produce phylogenetic diversity indices for the Cape Region, and to determine whether areas of higher phylogenetic diversity have received priority for conservation (see draft paper enclosed).

- The training course on molecular biology techniques, both practical and analytical, has been integrated into the programme for honours year students at UCT/SANBI in the host country.

- Although a number of papers were included as project outputs in the 2nd Annual report, given that they had been produced using material derived from the South African DNA bank, there was uncertainty over whether these publications stemmed directly from the Darwin project. As a result, they were separated from other publications and have been reported as ‘added-value’.

10. Darwin Identity

The Darwin logo was included in all popular articles and on distributed information relating to the project (e.g. procedures for placing specimens in silica gel). The project was called the "Darwin Initiative DNA Bank Project" and all correspondence from SANBI (e.g. permit applications to the Nature Conservation organisations) was conducted under this name.

The DNA banking manual and the field guide to the wild flowers of South Africa both display the Darwin logo, as do all issues of the SANBI conservation projects newsletter.

Several presentations have been given at high-profile international conferences by people involved in the project, ensuring that the scientific community has been made aware of the Darwin DNA bank project in South Africa. Examples of such presentations include those by Vincent Savolainen, Gail Reeves and Félix Forest at Evolution 2004, USA, Cycad 2005, Mexico, and Radiations of the Cape flora 2004, Switzerland. In the host country, presentations were made by G. Reeves to the Parliamentary portfolio committee on Environmental Affairs and Tourism; the Nelspruit Plant Specialist group; the University of Johannesburg post-graduate presentations; and tutorials at the University of Cape Town. Posters, bearing the Darwin logo, and talks were presented at the southern Connections 2004 conference by Ferozah Conrad, M de Villiers, A. Khunou and V. Savolainen, by F. Conrad and F. Forest at the Barcode of Life 2005 and Jessica da Silva at the student conservation conference.

This project was also clearly endorsed in the South African 2nd National CBD Report, especially with regard to Article 7 and Article 9, e.g. *"Together with the Kew Royal Botanic Gardens, the NBI [SANBI] has established a DNA Bank in South Africa. It will represent a unique archive of plant genetic diversity in South Africa, holding over 2 200 genomes from all genera. It will also serve as a resource to facilitate the discovery of novel genes and for the identification of areas of high priority for conservation. "*

11. Leverage

- **Salary for one post-doc to work within the project:**

SANBI and the University of Cape Town jointly funded the Smuts post-doctoral fellowship that was awarded to Dr Félix Forest to work on the Darwin Initiative DNA Bank Project (salary component was plus the "in-kind" support from SANBI in terms of work space, internet connectivity, computer use etc). Dr Forest undertook a large portion of the plant collecting in the Cape and, with the help of one intern at Kew, produced the phylogenetic 'tree of life' using the Cape genera. Dr Forest also instigated the region wide analysis of the data by organising the Phylogenetic Diversity workshop in Cape St Francis in March 2005. There was also considerable funding supplied by the Darwin Initiative for the post-doc, and most of his travel, conference and seminar and laboratory costs were borne by the project.

- **DNA barcoding of land plants:**

As a direct result of the success of the DNA bank project, and the large quantity of data the bank holds, SANBI have been invited to join an international consortium designated to establishing a standard DNA barcode for land plants. This project has received significant funding from the Sloane and Moore Foundations and is managed by RBG Kew. Following the recent Southern African Regional DNA Barcoding meeting, hosted by SANBI, the Consortium for the Barcode of Life have publicised the DNA banking manual (produced by this project) in their report of the meeting.

- **Fingerprinting:**

Gauteng Nature Conservation committed R80 000 to assess the feasibility of generating genetic fingerprints for cycads threatened by trade. This kind of project can only be

undertaken if the appropriate facility is available for long term storage of DNA extracts, which the SANBI's DNA bank now is.

- **Post-project:**

The success of the initial project has resulted in the award of an additional, post-project, grant from the Darwin Initiative of £98,297 ("Integrating Evolutionary History and Phylogenetic Measures of Biodiversity into Conservation Planning").

12. Sustainability and Legacy

The DNA Bank will endure – SANBI has committed to funding the salary of the DNA Bank manager and the running costs of the DNA Bank. SANBI has committed to funding the DNA Bank manager post in a permanent position. This post is valued at R130,000 (R94,000 salary plus benefits) per annum.

The project partners have extensive collaboration and this will persist into the future. Kew and SANBI collaborate on a number of projects within all sections of the two organisations.

To further ensure the continued collaboration between the partner institutions, all South African material in the Kew DNA bank is labelled, and any requests that Kew receives for such material are forwarded on to the South African DNA bank. This has happened on approximately fifteen occasions so far. Any plant material of South African origin that is extracted at Kew is duplicated and sent to the South African DNA bank according to the terms of our MoU.

An additional, post-project, grant from the Darwin Initiative has been secured (see above) in order to continue working with the data generated from the project. From this post-project, SANBI and Kew plan to publish at least four papers for the scientific audience, co-authored by both parties, on: (i) a region wide conservation analysis of PD using the "Acocks data"; (ii) a hotspots-wide study using plant genera (extension to the Cape analysis of initial project); (iii) a phylogeny-based conservation assessment in the Gouritz region (i.e. a mega corridor that links a number of conservation areas and farms/private lands into a single conservation area; it is situated in a biodiversity hotspot but is also heavily impacted by agricultural land use); (iv) an assessment of species threat from plant phylogenetic data.

13. Value for money

The project represents excellent value for money for a number of reasons. The establishment of a DNA banking facility at SANBI has attracted significant additional funding, and has led to the inclusion of Kirstenbosch Research Centre in an international DNA barcoding project, funded by the Sloane and Moore foundations, to establish a universal barcode for all land plants.

As a result of the data collected by the project, a post-project application has successfully been submitted to the Darwin Initiative, thereby allowing further research into integrating evolutionary history and phylogenetic measures of biodiversity into conservation planning to take place.

The scientific publicity created by the project has been considerable, most notably as a result of a letter in the prestigious scientific journal *Science*. This letter referenced the DNA bank project at SANBI and the Darwin Initiative. Equivalent publicity would be extremely costly.

As a result of the success of the project SANBI have made the DNA bank manager post a permanent position, thus ensuring the future of the bank, which is of great benefit to the institution, researchers and conservation planners.

With Kew, SANBI's has now one of largest DNA banks for plants, which represents an enormous boost for in-country research on biodiversity and conservation.

Appendix I: Project Contribution to Articles under the Convention on Biological Diversity (CBD)

Please complete the table below to show the extent of project contribution to the different measures for biodiversity conservation defined in the CBD Articles. This will enable us to tie Darwin projects more directly into CBD areas and to see if the underlying objective of the Darwin Initiative has been met. We have focused on CBD Articles that are most relevant to biodiversity conservation initiatives by small projects in developing countries. However, certain Articles have been omitted where they apply across the board. Where there is overlap between measures described by two different Articles, allocate the % to the most appropriate one.

Project Contribution to Articles under the Convention on Biological Diversity		
Article No./Title	**Project %**	**Article Description**
6. General Measures for Conservation & Sustainable Use	10%	Develop national strategies that integrate conservation and sustainable use.
7. Identification and Monitoring	Potentially through DNA barcoding	Identify and monitor components of biological diversity, particularly those requiring urgent conservation; identify processes and activities that have adverse effects; maintain and organise relevant data.
8. In-situ Conservation		Establish systems of protected areas with guidelines for selection and management; regulate biological resources, promote protection of habitats; manage areas adjacent to protected areas; restore degraded ecosystems and recovery of threatened species; control risks associated with organisms modified by biotechnology; control spread of alien species; ensure compatibility between sustainable use of resources and their conservation; protect traditional lifestyles and knowledge on biological resources.
9. Ex-situ Conservation		Adopt ex-situ measures to conserve and research components of biological diversity, preferably in country of origin; facilitate recovery of threatened species; regulate and manage collection of biological resources.
10. Sustainable Use of Components of Biological Diversity	An improved phylogeny will make it easier to identify near relatives of economically and medicinally important species.	Integrate conservation and sustainable use in national decisions; protect sustainable customary uses; support local populations to implement remedial actions; encourage co-operation between governments and the private sector.

11. Incentive Measures		Establish economically and socially sound incentives to conserve and promote sustainable use of biological diversity.
12. Research and Training	30%	Establish programmes for scientific and technical education in identification, conservation and sustainable use of biodiversity components; promote research contributing to the conservation and sustainable use of biological diversity, particularly in developing countries (in accordance with SBSTTA recommendations).
13. Public Education and Awareness	10%	Promote understanding of the importance of measures to conserve biological diversity and propagate these measures through the media; cooperate with other states and organisations in developing awareness programmes.
14. Impact Assessment and Minimizing Adverse Impacts		Introduce EIAs of appropriate projects and allow public participation; take into account environmental consequences of policies; exchange information on impacts beyond State boundaries and work to reduce hazards; promote emergency responses to hazards; examine mechanisms for re-dress of international damage.
15. Access to Genetic Resources	10%	Whilst governments control access to their genetic resources they should also facilitate access of environmentally sound uses on mutually agreed terms; scientific research based on a country's genetic resources should ensure sharing in a fair and equitable way of results and benefits.
16. Access to and Transfer of Technology	30%	Countries shall ensure access to technologies relevant to conservation and sustainable use of biodiversity under fair and most favourable terms to the source countries (subject to patents and intellectual property rights) and ensure the private sector facilitates such assess and joint development of technologies.
17. Exchange of Information	10%	Countries shall facilitate information exchange and repatriation including technical scientific and socio-economic research, information on training and surveying programmes and local knowledge
19. Bio-safety Protocol		Countries shall take legislative, administrative or policy measures to provide for the effective participation in biotechnological research activities and to ensure all practicable measures to promote and advance priority access on a fair and equitable basis, especially where they provide the genetic resources for such research.
Total %	**100%**	

14. Appendix II Outputs

Please quantify and briefly describe all project outputs using the coding and format of the Darwin Initiative Standard Output Measures.

Code	Total to date (reduce box)	Detail (←expand box)
Training Outputs		
1a	Number of people to submit PhD thesis	Ferozah Conrad and Christopher Cupido registered at UCT – to complete 2006 **(Target: 2 PhDs submitted – TARGET MET)**
1b	Number of PhD qualifications obtained	
2	Number of Masters qualifications obtained	Jessica da Silva (UCT/SANBI) Graham Rowe (UCT/SANBI) Angeline Khunou (UWC/SANBI) Colette Robinson (UJ) Cynthia Moleboherg Motsi (UJ) Paul Naude (UJ) Nicole du Toit (UJ) Anemari van Nickerk (UJ) Stephen Boatwright (UJ) Jerminah Moeaha (UJ) Marline Rautenbach (UJ) **(Target: 4 Masters obtained – TARGET EXCEEDED)**
3	Number of other qualifications obtained	
4a	Number of undergraduate students receiving training	20 honours students attended a one-week course in biotechnology and molecular techniques **(Target: 36 students – TARGET NOT MET)**
4b	Number of training weeks provided to undergraduate students	20 (one week per student) **(Target: 36 training weeks – TARGET NOT MET)**
4c	Number of postgraduate students receiving training (not 1-3 above)	19 MSc students attended a one-week course in biotechnology and molecular techniques **(Target: 4 students – TARGET EXCEEDED)**
4d	Number of training weeks for postgraduate students	247 (one week per student plus MSC project with > 12 weeks each) **(Target: 48 weeks – TARGET EXCEEDED)**
5	Number of people receiving other forms of **long-term** (>1yr) training not leading to formal qualification(i.e not categories 1-4 above)	1, Kholiwe Balele, trained as DNA bank manager **(Target: 1 DNA bank manager – TARGET MET)**
6a	Number of people receiving other forms of **short-term** education/training (i.e not categories 1-5 above)	

Code	Total to date (reduce box)	Detail (←expand box)
6b	Number of training weeks not leading to formal qualification	Members of staff attended five workshops: 1. Initiation of project help at RBG Kew; 2. Workshop to introduce project to wider academic community in SA held at Kirstenbosch; 3. Second workshop at Kirstenbosch; 4. Phylogenetic diversity workshop held in Port Elizabeth; 5. Final workshop held at Kirstenbosch. **(Target: 9 members of staff attending 4 weeks of workshops – TARGET MET)**
7	Number of types of training materials produced for use by host country(s)	1 DNA banking manual published (January 2006); Kew DNA extraction protocols used at SANBI **(Target: 1 manual on DNA banking – TARGET MET)**
Research Outputs		
8	Number of weeks spent by UK project staff on project work in host country(s)	Savolainen & Powell 2 weeks in September 2003; Savolainen 1 week in January 2004; Savolainen & Davis 2 weeks in September 2004; Savolainen 2 weeks in March 2005; Csiba 2 weeks in July 2005; Kapinos 2 weeks in August 2005; Savolainen & Powell 1 week in February 2006. **(Target: 3 people for 6-9 weeks – TARGET EXCEEDED)**
9	Number of species/habitat management plans (or action plans) produced for Governments, public authorities or other implementing agencies in the host country (s)	0
10	Number of formal documents produced to assist work related to species identification, classification and recording.	'A photographic guide to the wildflowers of South Africa'. Published December 2003. **(Target: 1 field guide – TARGET MET)**
11a	Number of papers published or accepted for publication in peer reviewed journals	2 papers published (Savolainen and Reeves, 2004, *Science*; Savolainen and Forest, 2006, in *Plant Species-level Systematics*); 1 to be submitted (Forest *et al*., *Nature*) **(Target: 1 paper published; 2 submitted – TARGET NEARLY MET – ONE PAPER STILL NEEDS TO BE SUBMITTED TO *NATURE*, SEE ENCL)**

Code	Total to date (reduce box)	Detail (←expand box)
11b	Number of papers published or accepted for publication elsewhere	
12a	Number of computer-based databases established (containing species/generic information) and handed over to host country	1 access database handed over to SANBI and subsequently imported into a newly developed in-house database **(Target: 1 database and website – TARGET MET)**
12b	Number of computer-based databases enhanced (containing species/genetic information) and handed over to host country	
13a	Number of species reference collections established and handed over to host country(s)	1 DNA bank **(Target: 1 DNA bank – TARGET MET)**
13b	Number of species reference collections enhanced and handed over to host country(s)	Herbarium vouchers for the DNA bank **(Target: 1 set of herbarium vouchers – TARGET MET)**

Dissemination Outputs		
14a	Number of conferences/seminars/workshops organised to present/disseminate findings from Darwin project work	5 workshops organised. July 2003, September 2003, September 2004, March 2005, February 2006. **(ADDITIONAL OUTPUT; although we decided to organise workshops at the start of the project, these outputs were not in table 23 of the initial application)**
14b	Number of conferences/seminars/ workshops **attended** at which findings from Darwin project work will be presented/ disseminated.	Southern Connections 2004 Cape Town (Savolainen, Reeves, Conrad and Khunou); Evolution 2004 Colorado (Savolainen & Reeves); Recent Radiations in the Cape Flora Zurich (Savolainen & Reeves); Barcode of Life London (Savolainen, Forest & Conrad); International Cycad conference Mexico (Reeves); Conservation Biology Cambridge (Da Silva); Evolution 2005 Alaska (Savolainen & Forest). **(Target: 2 conferences attended – TARGET EXCEEDED)**
15a	Number of national press releases or publicity articles in host country(s)	
15b	Number of local press releases or publicity articles in host country(s)	3 items in SANBI newsletter **(Target: 2 press releases – TARGET EXCEEDED)**

15c	Number of national press releases or publicity articles in UK	3 item in Kew Scientist October 2003, Oct 2005 & April 2006 **(Target: 2 items – TARGET EXCEEEDED)**
15d	Number of local press releases or publicity articles in UK	
16a	Number of issues of newsletters produced in the host country(s)	3 Newsletters of SANBI's conservation projects **(Target: 0 ADDITIONAL OUTPUT)**
16b	Estimated circulation of each newsletter in the host country(s)	500 per issue
16c	Estimated circulation of each newsletter in the UK	
17a	Number of dissemination networks established	
17b	Number of dissemination networks enhanced or extended	'Barcoding of Life' and "SABiGG South African Big Genera Group' networks enhanced. **(Target: SABiGG network to be enhanced – TARGET EXCEEDED)**
18a	Number of national TV programmes/features in host country(s)	
18b	Number of national TV programme/features in the UK	
18c	Number of local TV programme/features in host country	
18d	Number of local TV programme features in the UK	
19a	Number of national radio interviews/features in host country(s)	1 radio interview by G Reeves on Johannesburg radio station **(Target: 0 ADDITIONAL OUTPUT)**
19b	Number of national radio interviews/features in the UK	
19c	Number of local radio interviews/features in host country (s)	
19d	Number of local radio interviews/features in the UK	
Physical Outputs		
20	Estimated value (£s) of physical assets handed over to host country(s)	£34 807, ultracentrifuge and computer **(Target ultracentrifuge and computer – TARGET MET)**
21	Number of permanent educational/training/research facilities or organisation established	DNA bank facility established **(Target: 1 plant DNA bank – TARGET MET)**
22	Number of permanent field plots established	
23	Value of additional resources raised for project	Minus 80 degree fridge (£8000); Smuts' Memorial Botanical fellowship (UCT/SANBI) to F Forest (200000 ZAR); Leverhulme funded academic interchange network grant (UK) on South African plants (£107020)

15. Appendix III: Publications

Provide full details of all publications and material that can be publicly accessed, e.g. title, name of publisher, contact details, cost. Details will be recorded on the Darwin Monitoring Website Publications Database that is currently being compiled.

Mark (*) all publications and other material that you have included with this report

Type * (e.g. journals, manual, CDs)	**Detail** (title, author, year)	**Publishers** (name, city)	**Available from** (e.g. contact address, website)	**Cost £** incurred to the Darwin project (NOT price)
Book	*A photographic guide to the wildflowers of South Africa*. J Manning (2003)	Briza Publications, Pretoria, South Africa	http://www.briza.co.za/	2200
Journal letter	*A plea for DNA banking*, V Savolainen and G Reeves (2004)	AAAS	Science 304: 1445	0
Journal	*DNA barcoding of life*. V Savolainen, RS Cowan, AP Vogler, GK Roderick and R Lane, eds (2005)	The Royal Society, London, UK	http://www.pubs.royalsoc.ac.uk/	188
Book chapter	*Species-level phylogenetics from continental biodiversity hotspots.* V. Savolainen and F. Forest, Pp 17-30 (2005)	A.R.G. Gantner Verlag, Ruggell, Liechtenstein	In Regnum Vegetabile ISBN 3-906166-39-2	0
Manual	*DNA and Tissue Banking for Biodiversity and Conservation: Theory, Practice and Uses*. V Savolainen, MP Powell, K Davis, G Reeves and A Corthals, eds (2006)	Royal Botanic Gardens, Kew, UK; IUCN	http://www.iucn.org/bookstore/ ISBN 1-84246-119-2	2128

* Journal	*Preserving the evolutionary potential of floras in biodiversity hotspots*. F. Forest *et al.*	To be submitted to *Nature*.		0
Examples of 'Added-value' papers resulting from DNA bank data, but not direct project outputs				
Journal	*Environmental causes for plant biodiversity gradients*. TJ Davies, TG Barraclough, V. Savolainen and MW Chase (2004)	Royal Society, London	Phil. Trans. Roy. Soc. B. 359: 1645-1656	0
Journal	*Energy, area and diversification in the species-rich flowering plant family Iridaceae*. TJ Davies, V Savolainen, MW Chase, P Goldblatt and TG Barraclough (2005)	ASN	American Naturalist 166: 418-425	Cost covered by Imperial College London

16. Appendix IV: Darwin Contacts

To assist us with future evaluation work and feedback on your report, please provide contact details below.

Project Title	DNA banking, phylogeny and conservation of the South African flora
Ref. No.	162/12/008
UK Leader Details	Kew PLANTS PEOPLE POSSIBILITIES
Name	Dr Vincent Savolainen
Role within Darwin Project	Principal Investigator
Address	Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3DS, UK
Phone	
Fax	
Email	
Other UK Contact (if relevant)	
Name	
Role within Darwin Project	
Address	
Phone	
Fax	
Email	
Partner 1	SOUTH AFRICAN national biodiversity institute SANBI
Name	Ms Ingrid Nänni
Organisation	South African National Biodiversity Institute
Role within Darwin Project	Managed the Darwin DNA Bank budget at SANBI, but has been co-ordinating the project administration since the resignation of Dr Gail Reeves from SANBI.
Address	Private Bag X7, Claremont 7735
Fax	
Email	

Appendix VI: Students receiving 'Biotechnology and Molecular Techniques' training.

2003

Department of Applied Herbal Sciences
University of the Western Cape
Honours students

Kholiwe Balele
Ernest Maboza
Andre Braaf
Rehana Surve
Amelia Mabunda
Samantha Thamburan
Nthabiseng Mashaphu
Olivia de Wit
Cindy Lee Knowles
Mo-eed Salie
Moosa Joseph

2004

Department of Applied Herbal Sciences
University of the Western Cape
MSc students

Lezane Benjamin
Siphokazi Makaka
Ntsikelelo Lester
Farzana Parker
Pumla Mabeka

Systematics and Biodiversity Science Masters students
Universities of the Western Cape, Stellenbosch and Cape Town

Pulcherie Bissieugou
Charline Obone
Graham Rowe
Rugengamaanzo Nkubana
David Gwynne-Evans
Amelia Mabunda
Lee-Anne Mannie

Rand Afrikaans University
Botany Honours students

James Stephen Boatwright
Marie-Jeanne Cilliers
A.F. Heumi Youmi
Phindile Simelane
Marline Rautenbach

Conservation and Biodiversity Honours students

Roxanne Green
Jonathan Barker

Masters student

Jeminah Moeaha

March 2005

Systematics and Biodiversity Science Masters students
Universities of the Western Cape, Stellenbosch and Cape Town

Margaret Herron
Julia Watson
Nicola Wheat
Ntsikelelo Lester
One name missing

SA Herbal Science and Medicine Institute
University of the Western Cape Honours students

Tumiso Gasebo
Vathiswa Bengu

School of Pharmacy
University of the Western Cape Masters student

James Mukinda