



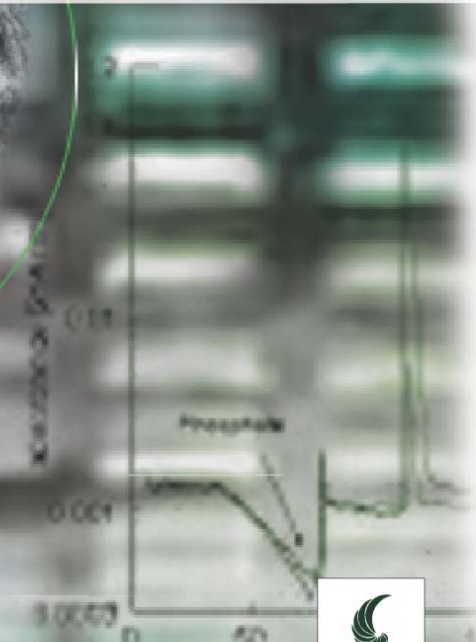
Darwin Initiative

CCMA

Certification to support conservation
of endangered Mexican desert cacti

Annual Report

Dr Julie A. Hawkins
Dr Rolando T. Barcenás Luna



Darwin Project Information.

Project Ref. Number	Ref 14-059
Project Title	Certification to support conservation of endangered Mexican desert cacti
Country(ies)	Mexico
UK Contractor	University of Reading
Partner Organisation(s)	University of Querétaro
Darwin Grant Value	£240 106
Start/End dates	September 2005 to September 2008
Reporting period and annual report number (1,2,3..)	1 April 2006 to 31 March 2007 Annual Report number 2.
Project website	www.uaq.mx/ccma
Author(s), date	Dr Julie A Hawkins; Dr Rolando T Bárcenas Luna 30 th March 2007

1. Project Background.

Cactus collection by amateur enthusiasts and commercial organisations, and illegal trade in cactus plants and seeds present a significant challenge to Mexico in terms of meeting its obligations as a signatory of the Convention on Biological Diversity (CBD). Wild collected cacti from any locality cannot be traded or exported for commercial purposes, but current Mexican legislation allows collection, under permit, of “mother plants”. These plants can be propagated and the resulting plants marketed or exchanged. Limiting the negative impact of collection on wild populations has been difficult in the past because it is not possible to ascertain whether a plant has been wild-collected or propagated. This project aims to support the conservation, sustainable harvest and use of Mexican desert cacti by providing molecular tools which can be used to identify plants to the species level, to determine their parentage and to locate the populations that they were collected from originally. DNA fingerprinting and barcoding tools are applied here for the first time to cacti in trade.

The central purpose of the project is to support the conservation, sustainable harvest and use of Mexican desert cacti and to ensure stake-holders get a fair share of benefits arising out of exploitation by the horticultural trade.

2. Project Partnerships

In June 2006 our first Reading PDRA, Denise Hardesty, left for Australia to take up a permanent full-time position. We appointed Dr Sara Hughes to replace her. Denise had built excellent relationships with the Mexican partners, and so we were careful to ensure that the transition was smooth. The Mexican PI, Rolando Bárcenas, visited the UK during the hand over period. This meant that both PIs and both the incoming and outgoing PDRAs were able to share information together to limit the impact of the staff change.

In the last annual report we noted that CONACYT had funded an application made by staff at MEXU to develop research into the phylogenetics of cacti. Despite our best efforts we haven't been able to dovetail the field and laboratory activities of the CONACYT and the Darwin teams, though

Professor Hernández at MEXU remains an active and involved member of the Darwin team. Although we haven't been working together in the field or in the laboratory, we have agreed that our outputs should be complementary, with the Darwin team focussed on identification and conservation applications of the sequence data, and the CONACYT team focussed on evolutionary interpretation.

In August 2006 Julie Hawkins organised a meeting at the Linnean Society which was attended by Dr Alejandro Casas, an expert on the domestication and reproductive biology of columnar cacti and other plants of the Tehuacán Valley in Central Mexico. She was able to discuss the Darwin project with him, and he is interested to get involved in the Tehuacán valley field activities scheduled for the next reporting period.

3. Progress.

3.1 Progress in carrying out project activities

Reviewer's comments on the last annual report suggested that we might make the technical aspects of the project clearer. In this annual report we have separated the two main technical thrusts of the project, the microsatellite and the sequencing work, into separate sections. A third section describes field activities. This reflects the "division of labour" between the Mexican technician and the UK PDRA, as well as the complementary outputs of the project relating to identification and certification.

a. Microsatellites:

Our goals were refined following ongoing stakeholder dialogue. We have selected two species which are important in trade. Using microsatellites we will identify unique microsatellite fingerprints for selected mother individuals for each species. We will then facilitate marketing of these two genotypes as "green" products, "green" in the sense that profits return to local communities in Mexico to support conservation. We have selected *Echinocactus grusonii* and *Ariocarpus bravoanus* as our first plants to market. Microsatellite screening of these species is proceeding as follows:

We have developed 53 microsatellite primers for *E. grusonii* and 51 for *A. bravoanus*. This year, we have concentrated our efforts on further developing and characterising the *E. grusonii* microsatellites. We have completed the field collection and DNA extraction of *E. grusonii* for the microsatellite work. We have collected a total of 222 individuals from two disjunct populations. These are the only two known natural populations of *E. grusonii*. One of these populations (in Zacatecas) is new to science. The interest in this new population in the popular cactology press (Unger and Huber, 2005; Fitz Maurice et al, 2006) suggests there will be collecting pressure on the natural population, and a market for certified plants. The second population (in Querétaro) is already in decline and there are fewer plants left in the wild. The site was flooded during the construction of a dam, and we have sampled (non-destructively) a representative sample of the remaining plants and all of the plants which were relocated to El Charco Botanic Gardens, Guanajuato when the dam was flooded.

All 53 *E. grusonii* primer pairs were screened for potential polymorphism using a bulking technique described by Cryer et al. (2005). Twenty were identified as having potential and taken forward for screening on individuals. All 222 individuals have been screened against all 20 primers. The results have been

promising, with just a small number of primers being monomorphic, with the majority polymorphic between individuals or between populations. We are currently finalising results for an article to be submitted to Molecular Ecology Notes. We are aiming to submit by the middle of May. The finalisation of the microsatellite development work includes investigating the potential of the *E. grusonii* primers to amplify microsatellites in other species in anticipation of the development of certification schemes for other cacti species.

We are planning our survey so that the markers we develop could find forensic application, as well as being applied to the certification scheme. It is quite possible that plants from Zacatecas will be brought to market following the illegal collection of seed from the site. Having demonstrated that the Zacatecas population is genetically distinct from the plants from Querétaro, we can determine which populations a plant in trade was sourced from. We have sourced samples of *E. grusonii* from plants in trade from six outlets in Asia. We will access plants in trade in Europe and America when the risk of frost is over, and plants can be delivered to us safely. By screening traded plants we can test whether *E. grusonii* currently in circulation is from the well-known Querétaro population. If so, will strengthen the case against any party selling plants from Zacatecas, should any authority seek to make a prosecution. We are seeking advice on this matter, and have negotiated to obtain plants seized by customs in the USA for screening.

Microsatellite development and screening has been carried out in Reading by the UK PDRA. She was assisted by the Mexican technician, Victor Rodriguez, who was in Reading for training from January to June 2006.

References Cryer NC, Butler DR, Wilkinson MJ (2005) High throughput, high-resolution selection of polymorphic microsatellite loci for multiplex analysis. *Plant Methods*, **1**, 3. Fitz Maurice WA, Fitz Maurice B, Etter J, Kristen M (2006) *Echinocactus grusonii*, a new location for the Golden Barrel. *Cactus World* 24 (4) 169-173. Unger G, Huber R (2005) Neuer Standort von *Echinocactus grusonii* Hildmann. *Kaktusblüte* (Wiesbadener Kakteenschau) 22 20-22;

Summary: key milestones relating to ssrs up to end of sixth quarter

- ssr markers developed ✓
- ssr screening completed for 50% of primer pairs across all accessions ✓

b. DNA sequencing:

Our goal is to make DNA sequence data for two regions available for Mexican desert cacti, so that sequence-based DNA barcode methods can be used to identify plants to species.

Most of the DNA sequencing is being carried out in Mexico. The Mexican technician trained at the University of Reading from January to June 2006 is managing all of the sequencing activities at the University of Querétaro. Having experimented with sequencing services available in Mexico, we are now getting best results by sending cyclosequencing products to Korea (Macrogen). All other aspects of DNA sequencing are proceeding well in

Mexico. The second of three Mexican trainees, Alberto Prado, arrived in the UK on March 24th to begin a three month training period.

As reported in the last Annual Report, we experienced some challenges in getting laboratory work underway in Mexico. These were due to 1. Delay in signing MoUs which meant that funds could not be transferred to Mexico on time. 2. Delays in purchasing equipment because of delays in transferring funds and 3. Difficulties in identifying a first trainee to travel to the UK. As a consequence the first trainee was the technician, and so six months of Mexican technician time were effectively lost.

We have sought to remedy this situation in order to get the Mexican side of the laboratory activities up-to-speed as follows. Firstly, the Mexican PI, Rolando Bárcenas, has been employing a technical assistant for Victor Rodriguez, the Mexican technician. We have reallocated funds for Victor's salary in Mexico which were not used while he was in England to technical support. In this way, that over the course of the whole project, we have three full years of technician time in Mexico as well as three Mexican trainees visiting the UK (with one of those trainees being the technician). Secondly, Rolando Bárcenas has recently purchased a second PCR machine so that the expanded team does not find access to the PCR machine a limiting factor. Thirdly, Alberto Prado, the second trainee just arrived in Reading, will direct most of his time to being trained in and carrying out sequencing.

Another challenge has been the selection of an appropriate region for sequencing. Initially we planned to sequence a small part of the matK region of the chloroplast genome, the region which has been selected by international barcoding initiatives, plus a second barcoding region. However, we found that the distribution of SNPs was fairly constant across the whole of the 2500bp of the matK region, but that other barcoding regions piloted were low in SNPs. We therefore decided to select two amplicons from the matK region as our two regions, though strictly speaking only one of these is a CBOL-flagged barcode region. We experimented with sequencing the whole matK region, so that a single long range sequence could be submitted to Genbank. However, we found some parts of the sequence are difficult to sequence through in our material.

Summary: key milestones relating to sequencing up to end of sixth quarter

- 50% of species (325 species, 400 individuals, including multiple accessions collected, determined, extracted and sequenced.
- *Delivered to date* – total number of species collected and determined is 268; total number of sequences is 420, representing 210 species. Collection ✗ sequencing ✓

c. Fieldwork:

Collection of species is a bottleneck. In our last annual report we noted that fieldwork had been delayed because of the delays in transferring funds and because the technician had been in the UK. We have now completed fieldwork for the *E. grusonii* microsatellite work and have half of the known *Ariocarpus* populations in hand for the second tranche of microsatellite work. Most of the outstanding fieldwork is necessary to provision material for the sequencing, and we have struggled to make up the fieldwork. Rolando

Bárcenas has many responsibilities in his position as lecturer at the University of Querétaro which make it difficult for him to make up the fieldwork time for the trips which had been scheduled in year 1. We have agreed that there are two strategies that can help us get up to date with fieldwork – 1. Employ capable field assistants so that the group can split into two teams of two (it isn't safe to work alone in the Mexican desert) so that six week's field work can be completed in three weeks. 2. Encourage our project partners from MEXU to carry out one of our longer trips without Rolando Bárcenas. Although the quality of DNA is far lower, and quantity of material available is low, we are considering approaching Darwin for permission to make up missing taxa with material collected from the MEXU herbarium. Provisionally we have permission from MEXU, and we have carried out pilot studies which show that the quality of even quite old herbarium material is sufficient for DNA extraction and amplification. We were surprised that a succulent plant which is subject to long and slow drying could be used to source DNA, but are pleased that this offers an opportunity to enhance our sampling.

Summary: key milestones relating to fieldwork to end of sixth quarter

- Six week collecting trip to S. Baja carried out. ✓ This trip was delayed from Year 1 due to delays in transferring funds. It was rescheduled to March 2007 as a three week trip with two teams of three.
- Four two-week trips to Chihuahuan desert carried out. Completed, though in shorter duration visits ✓
- Six week trip to N. Baja and NE Sonora carried out. Not yet completed ✗ strategies to secure N. Baja plants and NE Sonora plants developed.

3.2 Progress towards project outputs

The overall project outputs, as outlined in the Logical Framework, are as follows:

- A report on methodology/policy for certification (the stakeholders report).
- A collection of tissue and DNA for development and testing
- Low cost, robust DNA technologies developed and transferred through establishment of laboratories in Mexico
- University-level training of Mexican scientists in the UK provisions skills to support the technology transfer
- Dissemination of research findings through peer-reviewed scientific publications
- Promotion of awareness of the Certification Scheme amongst growers and consumers through an information leaflet

Methodology/policy for certification: the stakeholder's report is now disseminated. This will guide further development of the methodology/policy for certification. The second stakeholder's meeting will refine many of the initial ideas developed in the stakeholder's report. Market research will also guide the way in which certification is implemented.

Collection of tissues/DNA: the tissues and DNA used for sequencing are stored in the new laboratory at UAQ. Duplicate samples are held in Reading.

Low cost, robust DNA technologies developed and transferred through establishment of laboratories in Mexico: the laboratory at UAQ (called the Darwin Laboratory in recognition of the funding from the Darwin Initiative) is now fully operational. To date the technologies established are PCR and sequencing. Low-cost methods for screening microsatellites are under development in Reading for transfer later in the project.

University-level training of Mexican scientists in the UK provisions skills to support the technology transfer: Victor Rodriguez received six months training in the UK and Alberto Prado is currently in the middle of his three-month training period.

Dissemination of research findings through peer-reviewed scientific publications . Our first publication describing the microsatellite primers developed for *E. grusonii* is ready to be submitted this month. We are planning additional publications.

Promotion of awareness of the Certification Scheme amongst growers and consumers through an information leaflet: we anticipate that the leaflet will be developed to accompany the certified plants when they are marketed.

The detailed project outputs for the third, fourth, fifth and sixth quarters of the project are mostly ongoing activities from year 1. All outputs for this period are listed as detailed in the grant application, and commented on.

- 2006/03 Mexican Exchange student takes Intensive Course in Molecular Systematics. ✓ Victor Rodriguez took this course which ran from 28th March to 7th April.
- 2005/09 onwards. Mexican Technician for >3 years. Victor Rodriguez returned to Mexico in June 2006 and has been working as the Mexican Technician since that time. The student chosen for the first exchange and the technician appointed are the same person. Thus for the first six months of the project there was no technician in place in Mexico. Rolando has appointed an assistant technician to assist Victor so that the full complement of three years technician time is available.
- 2006/03 in this month a second exchange student arrived in the UK for training. As stated above the Mexican Technician and the first exchange student were the same person. This second exchange student is otherwise not employed by the project. Although this second exchange student for this period is not listed in the project outputs in the application (an error), they were budgeted for and their training is noted in the implementation timetable as a milestone. One more student will be trained in 2008; in total three Mexicans receive training in the UK, one of whom was subsequently employed as the project's technician. ✓ Alberto Prado the second exchange student, arrived in the UK.
- 2006/03 onwards. Four UoR staff spend 26-30 weeks in total in Mexico. ✓ 4 weeks spent by PDRA in 2006/02 to 03 2007/03. Reporting at a minimum of one international conference. ✓ In March 2007 Rolando Bárcenas was invited to make a presentation about the Darwin Project's work to an international conference organised by the Fulbright Academy of Science and Technology (FAST) and held in Panama City. His travel and accommodation was sponsored by the US Department of Justice

3.3 Standard Output measures

Table 1. Project Outputs (According to Standard Output Measures).

Code No.	Description	Year 1 Total	Year 2 Total	Year 3 Total	Year 4 Total	TOTAL
3	Intensive Course	1				1
5	Employ technician	✓	ongoing			✓
6A/6B	Exchange student	1 technician)	1			2
8	UK staff in Mexico	ongoing	ongoing			✓
9	Stakeholder's report	1				1
14A	Stakeholders' workshop	1				1
14B	International Conference		1			1
15A/C	Press coverage	1	1			2

Table 2: Publications

Type *	Detail (title, author, year)	Publishers (name, city)	Available from (e.g. contact address, website)	Cost £
Spanish language version of the website	Rodriguez and Hawkins	n/a	http://www.uaq.mx/ccma	n/a
Plantsman article	Certification scheme to clamp down on illegal Mexican cacti (Anita Gress, June 2006)	Royal Horticultural Society, Wisley.	http://www.uaq.mx/naturales/biologia/cma/ENGLISH/03-Publications-In-News/Publications.html	n/a
Biodiversitas article	Comercio de cactáceas mexicanas y perspectivas para su conservación (Rolando Bárcenas, September 2006)	CONABIO	http://www.uaq.mx/naturales/biologia/cma/ENGLISH/03-Publications-In-News/Publications.html	n/a
Book chapter	Comercio espinoso: un recurso científico contra el tráfico ilegal, en Mexico valor de origen, Barcenas, R.T. 2006	Santander, Mexico City	Special order from publishers	n/a

3.4 Progress towards the project purpose and outcomes

The project outcome will be the DNA-based CBD and CITES compliant certification scheme for nursery-propagated, traded cacti and the establishment in Mexico of DNA fingerprinting for certification and identification. We are progressing towards these outcomes on two fronts 1. Technically, through data collection and training. 2. Politically, through ongoing stakeholder dialogue and by taking the first steps to negotiate Prior Informed Consent (PIC) to market certified plants.

3.5 Progress towards impact on biodiversity, sustainable use or equitable sharing of biodiversity benefits

We anticipate that the impact on biodiversity, sustainable use and equitable sharing will be felt in the following ways. When certified plants are marketed this will reduce the market share of unsustainably harvested wild plants. The successful negotiation and implementation of Prior Informed Consent (PIC) in accordance with the CBD will ensure that the profits arising from the marketing of certified plants will be distributed equitably. Thus the impact of marketing certified plants will be felt once the plants are marketed. Progress towards marketing of certified plants suggests this will be possible towards the end of the project or shortly after it is completed. Bringing the plants to market depends of the successful negotiation of PIC, and the timescale for this is difficult to predict.

4. Monitoring, evaluation and lessons

To date our monitoring has been through informal channels, sharing spreadsheets electronically as attachments and through skype. We had originally planned to use a log-in password protected website to upload checklists and data, but we plan to continue as we are because we find these means effective. We plan to use designer time which has not been used for the development of password access to develop logos and marketing materials. Opportunities for face-to-face discussions have continued to be invaluable, particularly the visit by Rolando Bárcenas in June 2006 at the handover between the outgoing and incoming PDRA.

The biggest challenge we have faced to date relates to the timing and implementation of fieldwork. At the project's outset Rolando Bárcenas had only just been formally appointed to a position as lecturer at the University of Querétaro so we didn't yet know what his workload would be. We anticipated that his employers would give him a light administrative and teaching load so that he could lead all of the fieldwork personally. This hasn't transpired, and it is difficult for Rolando to lead the number of fieldtrips scheduled. We have learnt the lesson that it is desirable to explicitly state who would be leading and taking part in each fieldtrip so that sponsoring institutions are obliged to adjust workloads to permit those staff to attend. The delays in fieldwork are limiting the availability of fresh material for DNA sequencing, and so we will be approaching Darwin to discuss the possibility of deviating from the implied work plan and supplementing fresh material with existing herbarium samples.

5. Actions taken in response to previous reviews.

In the previous review the reviewer commented on the potential to extend the project by exploiting transferability of microsatellites between species. In the last year we have investigated the transferability of three of the SSR primers, screening a set of 47 closely related species. Transferability was higher than we expected, with 32 species being amplified by at least one primer. We will complete screens with all primers and report on primer transferability in the manuscript about to be submitted to Molecular Ecology Notes. Transferability

opens the possibility of launching more than two certified genotypes, as noted in section 7, impact and sustainability.

The reviewer noted that the stakeholders' report could not be accessed from the website. We have overcome some technical issues to ensure that the stakeholder report is now available.

The reviewer also noted that it wasn't clear how many species/genera the certification was intended to cover. In response to this point we hope that we have made the second annual report much more explicit so that the review process can be more thorough.

Finally, the reviewer noted that the sustainability of the project relies on the lab work being self-funding through a "green tax" on certified cacti sold. This, the reviewer noted further, is difficult to ascertain without costings. We have been working hard on this aspect of the project, speaking to additional stakeholders and experts. This is reported in detail in section 7, impact and sustainability.

In June 2006 our first Reading PDRA, Denise Hardesty, left for Australia to take up a permanent full-time position. We appointed Dr Sara Hughes to replace her. Denise had built excellent relationships with the Mexican partners, and so we were careful to ensure that the transition was smooth. The Mexican PI, Rolando Bárcenas, visited the UK during the hand over period. This meant that both PIs and both PDRAs were able to share information together to limit the impact of the staff change.

In the last annual report we noted that CONACYT had funded an application made by staff at MEXU to develop research into the phylogenetics of cacti. Despite our best efforts we haven't been able to dovetail the field and laboratory activities of the CONACYT and the Darwin teams, though Professor Hernández at MEXU remains an active and involved member of the Darwin team. Although we haven't been working together in the field or in the laboratory, we have agreed that our outputs should be complementary, with the Darwin team focussed on identification and conservation applications of the sequence data, and the CONACYT team focussed on evolutionary interpretation.

In August 2006 Julie Hawkins organised a meeting at the Linnean Society which was attended by Dr Alejandro Casas, an expert on the domestication and reproductive biology of columnar cacti and other plants of the Tehuacán Valley in Central Mexico. She was able to discuss the Darwin project with him, and he is interested to get involved in the Tehuacán valley field activities scheduled for the next reporting period.

6. Other comments on progress not covered elsewhere.

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7. Sustainability.

As our reviewer noted, the sustainability of the project relies on the lab work being self-funding through a “green tax” on certified cacti sold. We have been working hard to create the conditions in which this objective can be met.

Actions taken (which are over and above the Darwin objectives) have been to:

1. Seek private investment to carry out market research and to identify outlets for marketing in the UK. Market research by ANGLE plc (a venture management and consulting company specialising in the commercialisation of technology) began in January 2007.
2. Start the process of negotiating Prior Informed Consent (PIC) agreements with the appropriate Mexican bodies so that plants can be brought to market legally and in full compliance with CBD.
3. Start micro propagation of the certified *E. grusonii* certified genotype so that it is ready to be brought to market in the final year of the Darwin project, with a media event as the first ever DNA certified plants are sold.

These activities will mean that we have lots to report to the stakeholders group at our next meeting in year 3 of the project. In full negotiation with the stakeholders we hope that during the course of the project we are able to initiate trading and establish fair and equitable dispersal of any profits arising. This largely depends on the timescale for PIC agreements. Because the microsatellite markers are transferable it is possible that we will be able to consider the development of a portfolio of certified cacti that could reach the market.

8. Dissemination.

The following dissemination took place:

1. Website launched in Spanish - target audience Mexican cactus growers, Mexican authorities, international cactologists and collectors.
2. Article published in *The Plantsman* - target audience UK botanists and horticulturalists.
3. Article published in the *Biodiversitas* newsletter – target audience Mexican conservationists.
4. Book chapter, *Comercio de cactáceas Mexicanas y perspectivas para su conservación* (Rolando Bárcenas, September 2006).
5. Conference presentation made at Fullbright Academy meeting in Panama City.

9. .

Annex 1. Report of progress and achievements against Logical Framework for Financial Year: 2006/2007.

Project summary	Measurable Indicators	Progress and Achievements April 2005-Mar 2006	Actions required/planned for next period
<p>Goal: To draw on expertise relevant to biodiversity from within the United Kingdom to work with local partners in countries rich in biodiversity but poor in resources to achieve</p> <ul style="list-style-type: none"> The conservation of biological diversity, The sustainable use of its components, and The fair and equitable sharing of the benefits arising out of the utilisation of genetic resources 			
<p>Purpose <i>(insert original project purpose statement)</i></p> <p>to support the conservation, sustainable harvest and use of Mexican desert cacti and to ensure stake-holders get a fair share of benefits arising out of exploitation by the horticultural trade</p>	<p><i>(insert original purpose level indicators)</i></p> <ul style="list-style-type: none"> dialogue between stakeholders initiated and ongoing dialogue informed by technological developments and research findings for targeted cacti development and implementation of a DNA-based CBD and CITES compliant certification scheme supported by DNA-based identification tools 	<p><i>(report impacts and achievements resulting from the project against purpose indicators – if any)</i></p> <ul style="list-style-type: none"> ongoing dialogue continues following first stakeholder reports ongoing market research with a view to supporting CBD-compliant commercialisation databases of SSR and sequence data which will underpin species identification, and identification of geographical origin and parentage are growing 	<p><i>(report any lessons learned resulting from the project & highlight key actions planning for next period)</i></p> <ul style="list-style-type: none"> key actions include the second stakeholder meeting and negotiation of prior informed consent because of difficulties in carrying out fieldwork we will approach Darwin seeking permission of supplement field-collected with herbarium collected material for DNA extraction and development of molecular databases

Annex 1 continued. Report of progress and achievements against Logical Framework for Financial Year: 2005/2006.

Outputs			
<i>(insert original outputs – one per line)</i>	<i>(insert original output level indicators)*1</i>	<i>(report completed activities and outcomes that contribute toward outputs and indicators)</i>	<i>(lessons learned resulting from the project & highlight key actions planning for next period)</i>
report on methodology/policy for certification	stakeholders' report prepared	The stakeholders report is now available in hard copy and electronically in Spanish and in English language version son our website.	We have spoken to others who have experience of negotiating Prior Informed Consent. Negotiation of PIC is a priority for future work in commercialisation.
collection of tissue and DNA for development and testing	field and lab work make DNA available	We have collected 268 specimens for DNA sequencing work and more than 220 for the microsatellite work.	We are still finding organisation of fieldwork challenging, and are exploring new strategies as described in section 5b.
low cost, robust DNA technologies developed and transferred	new knowledge on sequence variation and SSRs in Mexican desert cacti appropriate fingerprinting tools methodologies developed training manual prepared	We have developed SSR primers that are variable and informative of geographical origin and individual identity for the target species, <i>Echinocactus grusonii</i> and preliminary results suggest they are widely transferable. The sequence data we have collected is able to distinguish species.	The training manual will be prepared and delivered in the next reporting period. We originally set out to develop SSR primers for two species, <i>Echinocactus grusonii</i> and <i>Ariocarpus bravoanus</i> . We now find that the <i>E. grusonii</i> primers may be sufficient for our <i>Ariocarpus</i> needs. Had we known that the primers were so widely transferable we would have chosen two much more distantly related species for the primer development work.
university-level training	courses and training exchanges equip 3 Mexican scientists to take project forward	The second student is arrived in UK for three months training in March 2007.	We have decided to keep all materials for DNA extraction as duplicates in Mexico and the UK because it is inconvenient to have to courier material over when it is apparent that additional material is required for the students laboratory work.
peer-reviewed scientific publications information leaflet	scientific publications prepared	The first of the Molecular Ecology Primer Notes is in draft form ready for submission next month.	We have been monitoring recent publications relevant to our project and will continue to do so.

*1 in the original Logical Framework there was not one to one correspondence between outputs and indicators; several indicators are indicators for multiple outputs. I have edited the presentation of the indicators to provide one to one correspondence although this has required some rewording.

Annex 2.

Project summary	Measurable Indicators	Means of verification	Important Assumptions
<p>Goal:</p> <p>To draw on expertise relevant to biodiversity from within the United Kingdom to work with local partners in countries rich in biodiversity but poor in resources to achieve</p> <ul style="list-style-type: none"> • the conservation of biological diversity, • the sustainable use of its components, and • the fair and equitable sharing of benefits arising out of the utilisation of genetic resources 			
<p>Purpose</p> <p>to support the conservation, sustainable harvest and use of Mexican desert cacti and to ensure stake-holders get a fair share of benefits arising out of exploitation by the horticultural trade</p>	<ul style="list-style-type: none"> • dialogue between stakeholders initiated and ongoing • dialogue informed by technological developments and research findings for targeted cacti • development and implementation of a DNA-based CBD and CITES compliant certification scheme supported by DNA-based identification tools 	<ul style="list-style-type: none"> • stakeholder meeting held, and stakeholders attend; follow-up identifies way forward in light of technological and scientific developments • field and molecular research carried out • low-cost, robust, reliable and reproducible fingerprinting methods for identification of species and genotypes developed and implemented in Mexico 	<ul style="list-style-type: none"> • all stakeholders are able to attend meetings • safety of fieldwork in Sonoran border regions is such that fieldwork possible there • UK and Mexican staff available. UAQ continues to maintain laboratories, and access to herbaria in MEXU and UAQ possible
<p>Outputs</p> <ul style="list-style-type: none"> • report on methodology/policy for certification • collection of tissue and DNAs for development and testing • low cost, robust DNA technologies developed and transferred • university-level training • peer-reviewed scientific publications • information leaflet 	<ul style="list-style-type: none"> • stakeholders' report, manual and scientific publications prepared • field and lab work make DNA available • new knowledge on sequence variation and SSRs in Mexican desert cacti • appropriate fingerprinting tools methodologies developed • courses and training exchanges equip 3 Mexican scientists to take project forward in Mexico 	<ul style="list-style-type: none"> • all publications available in hardcopy and electronically • database of material collected and extracted • sequence data exploited as SSR and SNP markers implemented in Mexico • 2 Mexican scientists awarded 10 European Credits each for intensive course; training exchanges happen 	<ul style="list-style-type: none"> • suitable technician and exchange scholars can be identified and employed • fieldwork successful • permissions already granted to sample from herbarium specimens extended • DNA extraction methods already developed in Reading for Opuntioidei cacti are applicable across other groups
<p>Activities</p> <ol style="list-style-type: none"> 1. Stakeholders' meetings. 2. Field and laboratory work. 3. Courses and training. 4. Reports, publications and publicity. 	<p>Activity Milestones (Summary of Project Implementation Timetable)</p> <ol style="list-style-type: none"> 1. 2005/09 and 2007/12. 2. Ongoing; completed 2008/06. 3. In UK: 2006/03-09, 2008/10-12 and 2006/10-12, in Mexico 2007/08. 4. Stakeholders' report 2006/06; manual 2008/09; leaflet 2008/09; electronic web-based identification tool 2008/09; press releases 2005/11 and 2008/11; peer reviewed scientific papers submitted by 2008/09. 		