ITTO project PD 620/11 Rev.1 (M):
Development and implementation of a species identification and timber tracking system with DNA fingerprints and isotopes in Africa

COMPLETION REPORT

01.02.2012 to 31.07.2015

Thünen Institute of Forest Genetics

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Executive Summary

Illegal logging and associated trade are the cause of many economic and ecological problems both in timber producer and timber consumer countries. Although many legal instruments such as the EU timber trade regulation and US Lacey Act for example, have been established to combat illegal logging and trade of illegally sourced timber, suitable robust tools to identify tree species and geographic origin are still lacking. DNA fingerprints and measurement of stable isotope ratios use characters inherent to the timber which are impossible to falsify. The combination of both methods guarantees a high spatial resolution and a strong statistical power for a cost-effective control of origin of wood and wood products.

This project is the direct outcome of the ITTO pre-project TFL-PPD 023/10 Rev.1 that has been led by the Thünen Institute of Forest Genetics (TI) in collaboration with The Forest Trust (TFT) from October 2010 to May 2011. During the pre-project the Thünen Institute of Forest Genetics, together with 14 collaborative agencies prepared the full proposal and submitted it to the ITTO.

The practical objective of the project was to improve the transparency and effective management of wood supply chains and to increase domestic and international trade of legally produced tropical timber. More specifically, the main goal was the development and implementation of species identification and timber tracking system using DNA fingerprinting and isotopic analyses for three commercial timber tree species from seven African countries.

The project has been initially accepted for a duration of 36 months (from February 2012 to January 2015), with a total budget of US$ 1,695,342.00. Before the end of the 1st year of the project, an additional fund allowed the extension of the project, taking into account a pilot study on the timber tracking chain of custody. The additional budget of US$ 130,000.00 from the US Forest Service and from the Australian government increased the project budget to US$ 1,825,342.00. By the end of the project 2nd year, the project steering committee authorized an extension until July 31st 2015 because of delays in sampling activities. To support the costs of coordination activities during the six months prolongation, additional work on isotopes, and organisation of an international conference on the project results, the main funder of the project (G) agreed to increase its contribution, leading the project total budget to US$ 2,046,092.54.

The main outcomes of the project can be summarized as follow:

1. Pre-project stage:

   Two workshops were organised in order to prepare the final proposal. Scientific issues and methods were discussed in Hamburg (Germany) on 1-3 March 2011, while a workshop in Yaoundé (Cameroon) with African partners focused on the selection of study species and the needs for training.

2. Sampling

   Reference sampling for species identification (21 taxa) consisted on 177 timber samples for anatomical analysis and 280 leaf or cambium samples for the development of DNA barcodes. For verification of geographic origin, reference samples of the three study species (Sapelli, *Entandrophragma cylindricum*; Iroko, *Milicia excelsa* + *M. regia*; and Ayous *Triplochiton scleroxylon*) with known geographic coordinates were taken from all participating African countries except for the Central African Republic, due to an unstable political situation. A total of 869 timber samples were collected for the development of the isotopic reference data, and 4221 leaf or cambium samples for the genetic reference data.

3. Reference data on tree species identification (21 taxa)

   All species could be identified at the genus level with wood anatomy, with 75% identified at the species level. DNA barcoding was based on the sequencing of the chloroplast fragment rbcl and provided 80% exclusion at the genus level and 20% at the species level.

4. Pilot study on genetic species identification of *Khaya* species on a forest concession in Ghana

   In a forest concession of the company SAMATEX analysis of nuclear DNA revealed two strongly differentiated genetic clusters corresponding to the species *Khaya ivorensis* and *Khaya anthotheca* with low evidence of hybridisation. Five percent of the *Khaya ivorensis* trees were classified genetically as *Khaya anthotheca* and 40% of the *Khaya anthotheca* trees were classified genetically as *Khaya ivorensis* therefore suggesting misidentification of *Khaya anthotheca* trees in the forest. Development of new gene marker (SNPs) should be useful to allow tracking of individual trees along the Chain of Custody.

5. Reference data on geographic origin

   A strong spatial structure was observed in all three species and both DNA and isotopic methods. At the country level, self-assignment success ranged between 30 and 100% and large differences were observed among countries and regions. The average spatial precision of self-assignment was 200 to 500 km.

6. Blind tests

   Blind testing of timber samples revealed high species identification success with wood anatomy. The blind tests on the country of origin generated correct results for 55 % to 83 % of the analysed wood samples. Accuracy could be improved with a better geographical coverage of reference samples in some regions, a higher number of molecular markers and isotopes analysed. Generally the resolution of the reference data was too small to check claims for small scale regions within countries.

7. Reference laboratories in Africa:

   Three African genetic laboratories (FORIG in Kumasi, Ghana; IRED Libreville, Gabon; and KEFRI in Nairobi, Kenya) were provided with additional equipment and training.

8. Training

   Three training workshops in Africa were held on a) basic molecular genetic techniques, b) on genetic data analysis, and c) wood anatomy. Between 16 and 30 participants attended the workshops. Intensive training for a period of three months for 11 trainees was provided by genetic laboratories based in Brussels, Edinburgh (UK), Grosshansdorf (Germany) and Adelaide (Australia)

During the final conference in July 2015, project outcomes were demonstrated. Further, challenges encountered in the project implementation were discussed. This lead to the following recommendations:

1. Sampling

The spatial distribution of sampling was not ideal, with regions that were either underrepresented or had a high density of sampling points. A more regular sampling scheme (transect) with more sampling points and less individuals per location is recommended. Also, more information per individual should be sampled (more molecular markers and isotopes). Critical topics that require more attention for the future are the correct measurements of the geographical co-ordinates, the taxonomical identification of the sampled individuals and the correct treatment of sampled material.

2. DNA-Barcoding

   As shown for the species in the genus *Entandrophragma* the species identification can be significantly improved by the use of several nuclear and chloroplast DNA markers. In general, a combination of several gene regions provides more reliable DNA barcoding compared to the monogenic approach used. Attention should be drawn to the possible inclusion of non-target species in the reference sampling to avoid mistakes in identification. Also, DNA contamination of tested timber DNA samples should be avoided and processes sufficiently controlled.

3. Tools to control claims on geographical origin

   We now have confirmation that both isotopic and genetic methods are very useful tools for the control of declarations on origin. To increase spatial resolution, more reference samples should be acquired, both methods should be jointly applied and as many information sources as possible should be utilised (i.e. additional isotopes and molecular markers).

4. African genetic reference laboratories

   A ring test showed that the genetic reference laboratories in Kenya and Ghana are able to amplify DNA from the study species. In Gabon, there is still a need to improve the lab organisation. The main problems identified were delays in delivery of chemicals and problems with the power supply. The next steps should be to facilitate self-organisation to allow the setting up of control services at national and regional levels. Further participation in blind and ring tests may provide opportunities to improve the organisation, to identify the problems and to strengthened collaboration with other African and Western laboratories.

General recommendations:

The recommendations made during the final conference of the project are focused on two main outcomes: (1) the application of the new technologies and project results in frame of timber regulations and (2) the technology transfer (future work of the African regional reference labs).

Concerning the use of the new technologies, the following recommendations were made to improve efficiency in frame of timber regulations:

1. A need for training and establishment of an isotope laboratory in Africa was suggested by African participants. However, the feasibility of this requires further discussion. This topic has already been discussed during the pre-project and was identified as not possible within the time frame of the ITTO project since in contrast to the genetics, no isotope laboratories were available in the seven African project countries.
2. The methods available in Africa, should be robust, cost-effective and complementary to methods currently in use (paper documentation, SGS).
3. Further training should be available for national and regional experts directly in the three African reference laboratories.
4. More reference laboratories should be established in Africa.
5. The list of priority species should be extended to country and regional levels. A global initiative of most important target species was suggested which could include 50 species in each continent (Africa, South America and Asia).
6. Spatial resolution of the reference database should be increased by adding more reference samples.

Suggestions for improvement of technology transfer included:

1. Collaboration between African and Western laboratories should be actively maintained, and further funding should be provided for the laboratories and for training opportunities (for example with ITTO fellowships).
2. The methods should be further developed and validated to improve robustness and cost-effectiveness.
3. The Thünen Institute (TI) was asked to initiate a PHASE II of this project to enhance and promote the use of the developed techniques.

1. Project Identification

1.1. Context

The seven African countries (Cameroon, Central African Republic - CAR, Democratic Republic of Congo - DRC, Congo Republic, Gabon, Ghana and Kenya) cover a total land area of 4,758,610 km² and have a population of more than 158.7 million inhabitants (2009). In general, 62.1% of the population live in rural areas and are largely dependent on subsistence agriculture and natural resources to survive. The less populated countries (Gabon and Congo) have a higher percentage of their population living in urban areas.

There is a widespread poverty in the sub-region. DRC, CAR and Kenya - which when combined represent more than 69.4 % of the total subpopulation - are classified among the lowest income countries in the world (GNI/inhabitant < 995 USD according to the World Bank ranking 2011). Additionally, Cameroon and Congo are classified as lower middle income countries. Only Gabon, representing less than 1% of the population is classified as an upper income country. There is also a high degree of disparity in the distribution of the national wealth (Gini index) and this is usually in disfavour of the rural populations (World Bank, 2011).

Data on industrial logging contribution to the country Gross Domestic Product (GDP) is available for three countries, and range from 4 to 13%. Assessing actual levels of illegal activity is very difficult and can be imprecise because data is not available for all countries.

The forests of Cameroon, CAR, DRC, Congo, and Gabon, and relict forests of Kenya form part of the Congo Basin, which constitutes the second largest area of dense tropical rainforest in the world. It stretches from the coast of the Gulf of Guinea in the west to the mountains of the Albertine Rift in the east, covering approximately seven degrees of latitude on either side of the equator, and is mostly within the Guinea-Congo forest structure. In the west of Cameroon and the east of the Democratic Republic of Congo, they also include the Afromontane forests. Ghana is located on the West Coast of Africa, about 450 km north of the equator between latitudes 4 and 11.5° North and longitudes 3.11° West and 1.11° East. The southern part of Ghana is within the West African rainforest block, at the western end of the Dahomey Gap. Though Kenya is less forested than the other countries, its strategic position in eastern Africa with a direct access to the Indian Ocean makes it an important timber transit country.

1.2. Origin and problem

This project is the direct outcome of the ITTO pre-project TFL-PPD 023/10 Rev.1 led by the Thünen Institute of Forest Genetics (TI) in collaboration with The Forest Trust (TFT) from October 2010 to May 2011. The objectives of the pre-project were to:

- Provide an overview on the situation in the participating African countries, in particular on forest exploitation, trade in different tree species, illegal logging, timber exports to Europe and timber tracking activities and projects
- Present results on existing genetic and isotopic spatial patterns for tree species in the region
- Discuss the planning of the work during the main project
- Select high priority tree species for timber tracking and species identification in the participating African countries
- Define the contributions of the different genetic and isotope laboratories
- Clarify the methods used in the project (gene markers, isotopes, sampling design) and estimate the required budget
- Develop a first draft of the project grant chart
- Present the project to potential stakeholders and discuss their expectations and potential contributions
- Develop a strategy for the acquisition of funding for the project

For this purpose, two workshops were held with potential partners and stakeholders: one in Hamburg (Germany) from 1st to 3rd of March 2011, and one in Yaoundé (Cameroon) from 23rd to 24th of March 2011. 32 representatives from ten countries (Europe, Singapore, Australia and USA) and 50 representatives from 10 countries (seven African project countries, Germany, USA and Singapore) participated to the workshop in Hamburg and Yaoundé respectively.

The key problem identified within the main project was the inefficient tree species identification and control of timber origin in Africa, leading to poor enforcement of existing laws and regulations in timber trade designed to reduce the impact of illegal logging. Falsified traceability documents suggest the presence of illegal timber in the market where no efficient control has been applied. Furthermore, the costs associated with illegal logging are relatively low, which does not support production of legal timber. The absence of tamper-proof methods in traceability systems suggests technological problems and lack of data. For more than 100 years, wood anatomical approaches have been known to be very useful for the identification of tree species, but determination keys are missing for many important African timber species. Further, identification of species within a genus is not possible in some cases. An alternative is the genetic barcoding, which uses genetic differences among species. Unfortunately, little genetic information was available for most African species and genetic barcodes still needed to be developed. Genetic and isotopic fingerprints are two complementary and very reliable methods used to control the origin of timber. Although a previous study demonstrated the use of these techniques at the forest concession level in Cameroon for two species (Iroko: *Milicia excels* and Sapelli: *Entandrophragma cylindricum*), reference data over the species' distribution range was still lacking before the project. Another problem hindering the application of genetic, isotopic and wood anatomy methods directly in the timber producer countries was the lack of availability of equipment and training. Furthermore, no private initiatives had been forthcoming from various stakeholders (private sector, African governments, NGOs, development agencies) to integrate these methods in certification schemes to ensure legality along the chain of custody, due most likely to insufficient information on the possibilities offered by the genetic, isotopic and wood anatomy tools.

2. Project Objectives and implementation strategy

Objectives

The main objective of this project was to improve the transparency and effective management of supply chains, and to increase domestic and international trade of legally produced tropical timber

The project had three specific objectives:

- to develop and implement a timber tracking system using DNA and stable isotope profiling for 3 important timber species in Africa: Iroko (*Milicia excelsa* + *M. regia*), Sapelli (*Entandrophragma cylindricum*) and Ayou (*Triplochiton scleroxylon*),
- to improve the tools available for the identification of tree species with the emphasis on CITES protected species and species that could be confounded with them,
- to transfer knowledge and capacity building in producer countries.

Strategy

The main outcome of the project should be an enforcement of laws and regulations on international timber trade and CITES protection. Thus, the important stakeholders for the implementation of these outcomes are governmental and forestry authorities in timber producer and timber consumer countries. In several cases, these institutions also represent the ITTO focal point of the country (e.g. the Forestry Commission in Ghana). During the pre-project phase, representatives of these groups participated in the two workshops in Hamburg and Yaoundé. Also, the project co-coordinator discussed details of the project implementation with the forestry commission of Ghana in Kumasi in April 2011. The intention was to keep the stakeholders in the project involved as much as possible through common meetings, workshops, and by providing a permanent update on the project progress with electronic newsletters.

Beside the public authorities, responsible forestry companies and timber traders as well as NGOs (WWF, EIA etc.), have shown an interest in applying the new enforcement tools to prove the authenticity of timber. We intended to integrate them as much as possible directly into the project. Thus, forest companies were important partners in the sampling phase of plant material for the large scale reference data to check country of origin. Additionally, two pilot studies of the DNA-based individual tree tracking along the chain of custody were originally planned in collaboration with timber companies. It was anticipated that timber traders would agree to contribute material for blind testing. In addition, meetings and workshops were planned as well as the provision of newsletters to keep these groups involved throughout the duration of the project.

An important element of the capacity building in Africa was the creation of three genetic reference laboratories, the aim of which was to enable the timber producer countries to administer at least a part of the controls themselves. For each of the three reference laboratories, one responsible laboratory in Europe was assigned to focus on the support and training of the partner institutions. It was agreed that the Thünen-Institute of Forest Genetics will be responsible for the reference laboratory in Kumasi (Ghana), the University of Brussels (Belgium) for the laboratory in Libreville (Gabon), and NERC Centre for Ecology and Hydrology (UK) for the laboratory in Nairobi (Kenya).

Assumptions and risks

The sampling of the three timber species plant material for the development of the reference databases for assignment of geographical origin was labour-intensive and complicated since it required close coordination between teams in different countries, which in turn needed access to remote locations, and to training of the teams. A two-step sampling approach was agreed involving first genetic and isotopic screening of 2/3 of all samples followed by a first data analysis and the remaining 1/3 sampling according to the first results and identified high priority sampling regions. The sampling was coordinated by the team of Prof. Dr. Jean Louis Doucet from the Gembloux Agro-Bio Tech (Belgium). This team has extensive experience of sampling in West and Central Africa.

The application of DNA markers to assign species and geographic origin to processed timber assumes that the extracted DNA is of sufficient quality. In order to minimise the risk of poor quality DNA, particular emphasis was placed on further development the DNA extraction protocols, including the use of short DNA fragments for genetic fingerprinting, which are less sensitive to template degradation as is usually observed for timber. Assignment of the geographic origin of timber assumes that the underlying spatial genetic pattern of DNA-markers and stable isotopes in the natural distribution area of the tree species is of sufficient strength. To this end, the development of a high number of DNA-markers for each of the three species was planned using a next-generation DNA sequencing approach. Furthermore, the attention was to combine results from genetic and isotope analyses to ascertain the country of origin. The plan was to have five different laboratories working on genetic methods, three laboratories working on the stable isotopes, and three genetic reference laboratories established in Africa. A ring-test was also planned in order to address whether the different laboratories could yield comparable results. Since it was anticipated that the forest authorities and logging companies in the different African countries might retain strong reservations over the developed reinforcement tools, they were directly involved in the project as much as possible. It was anticipated that successful results from the blind tests would convince these stakeholders of the suitability of the enforcement tools.

The capacity and training component of the project was of great interest to the African countries. For the genetic method, there are three laboratories identified that will be further developed as reference centres, but for the isotopes, no laboratory facility was available in the seven countries. This might cause particular preconceptions against the application of stable isotopes because the work needs to be done entirely outside of Africa.

The total budget of the project originally included two satellite projects covering a complementary part of the ITTO work program. For this part, proposals have been submitted by the University of Adelaide and the Thünen-Institute at the Australian Research Council (requested additional budget of 736,000 USD) and by the Ghana Forestry Commission to the ACP-FLEGT call (requested additional budget of 134,000 USD). From these two proposals the one submitted at the Australian Research Council got supported. But the financial support was significantly reduced compared to the requested amount in the proposal. Thus we changed the expected outputs according to that.

3. Project performance

Planned activities	**Realized activities**
Specific objectives	
Development and implementation of a timber tracking system with DNA and stable isotopes for 3 important timber species in Africa: Iroko (*Milicia excelsa* + *M. regia*), Sapelli (*Entandrophragma cylindricum*) and Ayous (*Triplochiton scleroxylon*)	A timber tracking system with DNA and stable isotopes is set for the three species. Reference maps are made for each species.
Improvement of the tools to identity tree species with focus on CITES protected species and species that could be confounded with them	Based on the wood anatomy and the DNA analyses, the species identification is now much more precise. DNA technology helps to distinguish *K. anthotheca* from *K. ivoirensis*
Transfer of know-how and capacity building in producer countries	Three genetic reference labs are equipped, three training workshops were organised and 11 trainees visited western laboratories
Output 1: 20 African tree species have been identified by wood anatomy and DNA barcode	
1.1. Sampling of wood probes and cambium or leaves from 200 individuals trees	Fully completed
1.2. Wood anatomical study of 20 tree species	Fully completed
1.3. DNA barcoding of 20 tree species	Fully completed
1.4. Blind testing of 50 samples from unknown origin belonging to 20 species based on wood anatomy and barcoding analysis	Fully completed

Planned activities	Realized activities
Output 2: Genetic and stable isotopes reference data to control the country of origin for three important timber species	
2.1. Sampling of cambium or leaves from 4800 individuals trees and wood samples from 720 trees belonging to 3 species (240 locations, 20 samples for the genetics and 3 samples for the isotopes)	Fully completed
2.2. Optimisation of DNA extraction protocols for wood	Fully completed
2.3. Gene marker (chloroplast and nuclear microsatellites, SNPs) development for Iroko	Fully completed, concentration on SNPs
2.4. Gene marker (chloroplast and nuclear microsatellites, SNPs) development for Sapelli	Fully completed, concentration on SNPs
2.5. Gene marker (chloroplast and nuclear microsatellites, SNPs) development for Ayou	Fully completed, concentration on SNPs
2.6. DNA fingerprinting of 2000 Iroko trees	Completed for a reduced number of individuals (1833 individuals)
2.7. DNA fingerprinting of 1400 Sapelli trees	Nearly completed as planned (1192 individuals)
2.8. DNA fingerprinting of 1400 Ayous trees	Completed for a reduced number of individuals (652 individuals)
2.9. Blind testing of 60 samples from unknown origin belonging to 3 species based on DNA fingerprinting	Fully completed
2.10. Blind testing of 50 samples from species based on DNA fingerprinting	Fully completed
2.11. Stable isotopes fingerprinting 300 Iroko trees	Exceeded according to an enlargement of the partners contract (420 individuals). Three additional isotopes (Sr, S and N) and more individuals have been used to increase the spatial resolution
2.12. Stable isotopes fingerprinting of 210 Sapelli trees	Almost completed as planned (209 individuals)
2.13. Stable isotopes fingerprinting of 210 Ayou trees	Almost completed as planned (167 individuals)
2.14. Blind testing of 60 samples from unknown origin belonging to 3 species based on stable isotopes fingerprinting	Fully completed

Planned activities	Realized activities
Output 3: African timber producer countries equipped & their personal trained for timb species identification & control of origin	
3.1. Endowment of small DNA fingerprints laboratory equipment to African participants countries	Fully completed
3.2. Training in skilled labs	Each of 11 trainees had a three months research and training in western laboratories
3.3. Training in African labs	Three training workshops were organised in the three respective reference laboratories
3.4. Supporting the development of the reference labs	The three reference labs have received additional equipment
3.5. Ring tests to setup same level of lab standards	Only the laboratory from Libreville/Gabon did not complete the ring test, for technical reason (electricity crash)
Output 4: Demonstration of control of chain of custody have been done with 1 tree species and the stakeholders have been involved	
4.1. Sampling of cambium of 400 Khaya trees and wood of 200 Khaya	Fully completed
4.2. Development of markers for Khaya	Fully completed
4.3. DNA fingerprinting of 400 Khaya trees	Fully completed
4.4. One week training of FORIG team on genotyping	Fully completed
4.5. Reporting	Fully completed
Output 5: Project co-ordination	
5.1. Executive agency coordination	Fully completed
5.2. Kick-off meeting	Fully completed
5.3. Stakeholders and partners meetings	Four meetings held in addition to the three workshops held in African laboratories and the kick-off meeting
5.4. Steering committee meetings	Three steering committee meetings
Schedule	
Starting date: 01.01.2012	Starting date: 01.02.2012
Duration: 36 months	Duration: 42 months. 6 months extension due to the sampling and other activities delay
Total amount of expenditures	
USD 2,046,092.97	USD 2,046,092.97

4. Project outcome, target beneficiaries involvement

Achievement of the project specific objectives

The specific objective of the project was *the development and implementation of species identification and timber tracking system with DNA fingerprints and stable isotopes for three commercial timber tree species in seven African countries*.

As indicators for the success of the achievement of this specific objective we listed in the project proposal the following three topics:

1. By 2014, a species identification based on wood anatomy and DNA barcode is available for 20 African timber species
2. By 2015, a DNA and stable isotopes fingerprints timber tracking system is ready for use for three African timber species
3. By 2015, African partners are doing independently timber tracking with DNA fingerprints in Africa

As visible in this report and its technical annexes the indicators 1 and 2 can be positively verified and the achievement of these elements can be confirmed. The work of the project has been published (Degen & Bouda 2015) and scientific publications on the methods and reference data are in preparation. The reference data are ready to place them in the online data base of the Global Timber Tracking Network (GTTN). The involved collaborative agencies and the executive agency are receiving wood samples of the African species from the private sector and public authorities to check claims on tree species and geographic origin. The third indicator on the application of the timber tracking with DNA fingerprints in Africa can be positively confirmed for the established regional genetic laboratories in Kumasi (Ghana) and Nairobi (Kenya).

Situation at project completion as compared to pre-project situation

At the end of the project the identification of tree species has also been improved or the 20 taxa involved in the study. Based on the wood anatomy and the DNA analysis, the species identification is now much more precise. Now with gene markers we can much better distinguish among the different high value timber species within the important geni Entandrophragma and Khaya (output 1).

Now a timber tracking system with DNA and stable isotopes is set for the three species: Iroko (*Milicia excelsa* and *M. regia*), Sapelli (*Entandrophragma cylindricum*) and Ayou (*Triplochiton scleroxylon*). Reference maps are made for each species. Blind tests organised by independent operators have been executed to check the performance of the methods and the quality of the reference data. A pilot study on the genetic tracking along a chain of custody for Khaya has been made in collaboration with SAMARTEX (a timber company based in Ghana) and the forest research institute of Ghana (FORIG). This study and the blind tests have demonstrated the efficiency of the new technology to check claims on species and origin. We are aware of the limitations and remaining gaps of the methods and reference data. But these limitations do not hinder the general application of the methods. Moreover, they should be carefully considered for judgments on claims on species and geographic origin (output 2).

The transfer of knowledge and the capacity building in producer countries in the area of genetics and wood anatomy could be archived as planned. Among the 7 African countries involved in the project implementation, only Kenya had a moderately equipped genetic laboratory before the project started. The equipment in the other two laboratories was rudimentary. At the completion of the project, these two laboratories are sufficiently equipped and the KEFRI's laboratory was reinforced. The tree laboratories are now ready to perform basic work on timber DNA. The trainings held at these laboratories and the intensive trainings in western laboratories for eleven trainees from all seven African countries have created long term human capacity (output 3).

In terms of physical environment, sectorial policies and programs, the impact of the project still need more time to be visible. The awareness of the African authorities on the need of efficient controls for legal harvests and timber trade increased a lot during the project phase. Further the establishment of additional laboratories for DNA testing was announced for Cameroon and Congo. The collaboration with the Thünen Institute and other western laboratories continues after the end of the project. In cooperation with the timber sector new initiatives and pilot studies on DNA based timber tracking have been started (e.g. for *Prunus africana* and *Pericopsis elata* in Cameroon and DRC) and timber associations are looking to implement tracking methods of the project (Association Technique Internationale des Bois Tropicaux - ATIBT).

Participation of the target beneficiaries and use of the project results

The participation of project beneficiaries to its implementation can be noticed at different levels of the project life.

- Sampling: the collaborative agencies, as well as timber companies and administrative staff in charge of forest in the target countries have been involved in the sampling, either directly for sample collection on the field, or by assisting samplers and by helping to obtain the official permissions for the sampling..
- Trainings and meetings: stakeholders have been involved in the trainings at African laboratories and the African ITTO members as beneficiaries have been fully involved in the selection of candidates for the intensive training in western labs. Sessions in project meetings were dedicated to stakeholders.
- African reference laboratories are ready to operate after support with equipment and training.

Part of the project results is already being used by the beneficiaries. The samples collected for the project have been duplicated and the host institutes are using the duplicated samples for their own applications. The equipped labs are also working know. E. g. in Gabon, one of the trainees is actually using the regional laboratory to complete the molecular genetics work of his master thesis. The improved knowledge in wood anatomy is used by the African colleagues for species identification. In the day to day work, the laboratories of the collaborative agencies and the executive agency are using the methods and reference data developed in the project for requests from the timber sector and public authorities on species identification and controls on timber origin.

Project sustainability after project completion

The laboratories of the western project partners are regularly applying the methods and reference data for tests on claims on African timber.

The collaboration is continuing between the Thünen Institute and the project beneficiaries:

- A similar project on the reference maps of 16 tree species (including 8 tree species from Africa and 8 from Latin America) is being implemented, in collaboration with FORIG in Ghana. With this project, the number of target countries is extended with 3 more countries in Africa (Ivory Coast, Liberia and Nigeria) and 4 countries in Latin America (Brazil, Bolivia, Peru and French Guiana).
- The project beneficiaries in Congo and Cameroon are planning to open a basic molecular lab and they are negotiating with Thünen Institute to host the trainees who will manage the labs. A trainee from Congo has already spent 2 months at the Thünen lab and will come back in April to complete his training.

The three regional genetic reference laboratories in Africa continue to be operational and further support is given, particularly for FORIG in frame of other funding. The same is aimed for the other laboratories. As discussed during the final conference also the establishment of stable isotopes laboratories is requested for the target region in Africa. Most of the partners involved in the project will continue their co-operation as member of the Global Timber Tracking Network (GTTN).

5. Assessment and analysis

Based on the achievement of the five main expected outputs, the following assessment and analysis can be made:

Output 1: 21 African tree species have been identified by wood anatomy and DNA barcode

This output has been achieved (24 species have been identified by wood anatomy and 21 species have been identified by DNA barcodes). The blind tests have shown interesting results for both anatomy and barcoding, but also demonstrating a necessity to improve sampling to guarantee sufficient reference material and to avoid DNA-contamination during genetic analysis of timber. The technical reports on wood anatomy and on DNA barcoding are given in annexes 3 and 4.4.

Output 2: Genetic and stable isotopes reference data to control the country of origin for three important timber species

The development of markers and the genetic screening of the reference samples (i.e. creation of the genetic reference databases) have been completed for the three species (Iroko, Ayous and Sapelli). The stable isotopes fingerprinting, using three isotopes (hydrogen, carbon and oxygen) has also been completed for the three species. For Iroko, an additional three isotopes (strontium, sulphur and nitrogen) have been analysed in order to increase discrimination at the regional level. The results of the blind test are still under discussion. The main topics comprise common and comparable statistical approaches, comparable thresholds for the decision to reject or accept a claim and common thresholds for data completeness. The blind tests results communicated by the blind test operators WWF and G2S do not reflect these requests for a unified approach. The technical reports on the development of reference maps are shown in annex 4.1 - 4.3 and in annex 5.1 - 5.3, respectively for genetic and stable isotope analyses. A technical report on the blind test is given in annexes 7.1 and 7.2 and a proposal for a common data analysis on the blind test samples is given in annex 8.

Output 3: African timber producer countries equipped and their personal trained for timber species identification and control of origin

The three workshops planned in African laboratories have been completed, as well as the stakeholders meetings. Four meetings have been organised, in addition to the three training workshops and the kick-off meeting.

The African reference laboratories (FORIG, IRET and KEFRI) received additional equipment, chemicals and support with increased capability. The training in the skilled laboratories was completed for 11 trainees at the Thünen Institute in Germany, the NERC in the UK, the University of Brussels in Belgium and the University of Adelaide in Australia.

The standardisation and ring test was completed for two reference laboratories (KEFRI and FORIG) but was unsuccessful for IRET due to power supply problems during the test. The laboratory of NERC in the UK also participated to the ring test. A technical report of the ring test is given in annex 6.

In order to share the results of the project with the participants and interested stakeholders, a final conference has been held in Douala, Cameroon (1st - 2nd July 2015). The minutes of the final conference are presented in annex 10.

Output 4: Demonstration of control of chain of custody have been done with one tree species and the stakeholders have been involved

The activities connected to this output are completed. A report on the cost/benefit analysis of the control of chain of custody of two species of Kaya is given in annex 9.

Output 5: Project co-ordination

All planned meeting and coordination activities were completed. During the final conference of the project in Douala/Cameroon in July 2015, the coordination and collaborative agencies presented the results of the project and advocated for their implementation by decision makers at national and regional level.

6. Lessons learned

The lessons learned from the project implementation are drawn from the four key activities of the project

1. Sampling
    - For all species, ideal spatial distribution of sample points was not achieved. Some regions are underrepresented, while others have a high density of populations.
    - It would be preferable to collect data from an increased number of geographic sampling points (transects) and to collect less individual samples per sampling point. The genetic variation of SNP gene markers with two different alleles is low. Thus a lower number of individuals (n=10) per sample point could be taken while maintaining sufficient estimation of allele frequencies.
    - For more precise results, it is necessary to collect more information per individual (more gene markers, more isotopes)
    - In future projects, further training and implementation of quality controls for the collection of reference samples are required.

2. DNA-Barcoding
    - As has been demonstrated by use of a large set SNPs for the genus *Entandrophragma sp.*, that the use of a combination of several gene regions provides a much more reliable DNA barcoding.
    - It is important to pay closer attention to identification of “non-target species” and include them in the sampling (as predefined outliers).
    - Cross-contamination of DNA during genetic analysis of timber must be completely avoided.

3. Tools to control claims on geographic origin

    From the blind test results, we can conclude that both isotopic and genetic methods are very useful tools in the enforcement of declarations on geographical origin. In order to increase spatial resolution in the reference data, we recommend to:

    - Add reference samples from the low coverage areas

- Collect more information per reference sample (more isotopes, more gene markers)
- Apply both methods in a combined manner
- Analyse the reference data and provide an objective recommendation for which methods would provide a more reliable geographic claim (e.g. from the results of self-assignment tests provided for countries and regions)
- Work on common statistical approaches for data analysis
- Work on and identify further available data and information to support interpretation of isotope results.

4. African (genetic) reference laboratories

   A ring test showed that the genetic reference laboratories in Kenya and Ghana are able to amplify DNA from the study species. In Gabon, there is still a need to improve infrastructure (power supply) and the organisation. We further recommend:

   - A better and more intensive dialog and coordination among the African countries to establishment genetic timber control services at national and regional levels
   - The participation in future ring and blind tests
   - To support further collaboration among western and African laboratories
   - To involve African isotope specialists in future projects in order to increase and include existing knowledge, and contact the isotope facility in Kenya to learn about its status and obstacles needed to overcome for better use of this lab.
   - To include training for African isotope scientists.

7. Conclusions and Recommendations

In addition to the achievement of the expected outputs, the implementation of the project has provided a valuable opportunity to build strong professional relationships between western and African laboratories, and between isotope, genetic and wood anatomy experts. During the final conference of the project, further recommendations have been made to support a better implementation of the findings. These recommendations are focused on two main outcomes: the application of the new technologies and project results in frame of timber regulations and technology transfer (future work of the African regional reference labs).

Concerning the use of the new technologies, the following recommendations were given to improve efficiency in frame of timber regulations:

1. An identified need for training and establishment of an isotope laboratory in Africa (Note: This topic was discussed during the workshops in the pre-project. Apparently there was - in contrast to the genetics - no functional stable isotope laboratory in the 7 African countries. Thus because of this lack of basic infrastructure we could not include technology transfer for this technique in frame of the ITTO project). Efforts should be undertaken to reopen the facility in Kenya, if feasible.
2. The methods should be available in Africa, be robust, cost-effective and complementary to existing methods (paper documentation, SGS).
3. Further training should be offered for national and regional experts
4. Additional reference laboratories should be established in Africa
5. The list of priority species should be extended and other timber producer countries should be included.
6. The spatial resolution of the reference database should be increased.

Suggestions for improvement of technology transfer included:

1. The Collaboration between African and Western laboratories should be actively maintained, and funding should be provided for the laboratories and for training opportunities, for example with ITTO fellowships.
2. The methods should be further developed to increase robustness and cost-effectiveness. In particular, the isotope technology should be made available in Africa.
3. The Thünen Institute (TI) was motivated to apply for a second of this project to enhance and promote the use of the developed techniques

Annexes

Annex 1 Project financial statement

Annex 2 Project cash flow statements

Annex 3: Technical report on wood anatomy

Annex 4: Technical report on genetics

4.1: Technical report on the development of a genetic reference map for Sapelli

4.2: Technical report on the development of a genetic reference map for Iroko

4.3: Technical report on the development of a genetic reference map for Ayous

4.4. Technical report on the development of barcoding of 2 species of Kaya

Annex 5: technical reports on the stable isotopes analysis

5.1: Technical report on the stable isotopes reference map for Sapelli

5.2: Technical report on the stable isotopes reference map for Iroko

5.3: Technical report on the stable isotopes reference map for Ayous

Annex 6: Report on the ring test results

Annex 7: Report on the blind test results

7.1: Technical Report results blind test WWF

7.2: Technical Report results blind test G2S

Annex 8: Report on one approach of a common data analysis of the blind test data

Annex 9: Reporting of the control of chain of custody for two species of Khaya

Annex 10: Technical report sampling

Annex 11: Minutes final conference