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 11.5º North and longitudes 3.11º West and 1.11º 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 ***(will be submitted separately)***

Annex 2 Project cash flow statements ***(will be submitted separately)***

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

ANNEX 3

Technical report on wood anatomy

ITTO project *"Development and implementation of a species identification and timber tracking system in Africa with DNA fingerprints and stable isotopes"*

Report: Wood Anatomy

PD Dr. Gerald Koch and M.Sc. Volker Haag (Thünen Institute of Wood Research)

Introduction

The control of internationally traded timber requires reliable methods for a doubtless identification of the wood species (botanical taxa). The clear identification of the timber is also important for the assessment of product properties "consumer protection" as lower-grade substitute timbers are imported at a distinctly increasing rate. In the context of these major challenges wood anatomy provides the most valuable support for practical wood identification. The methods for the macroscopic and microscopic wood identification are basically established and routinely applied since more than 100 years.

Macroscopic wood identification is based on observations in the three anatomical planes of a wood specimen: transverse (perpendicular to the stem axis), radial (parallel to the stem axis) and tangential (parallel to the stem axis) which can be observed with the unaided eye or with the help of a magnifying lens. The method is suitable for a first reliable determination of the declared taxon. For the macroscopic wood identification, the transverse planes of the specimens are smoothed using a cutter or carpet knife and examined with a hand lens (recommended magnification 10-12x, see Fig. 1).

Fig. 1. Preparation of the transverse plane and macroscopic observation / identification of the timber

For "official" or "judicable" wood identification, microscopic analyses are routinely conducted. Using light microscopic techniques, up to 100 anatomical characters can be used which are internationally standardized according to the IAWA lists of "Microscopic Features for Hardwood and Softwood Identification". The defined microscopic features describe the individual tissue types: vessels, parenchyma and fibres and provide additional information about mineral inclusions as part of a wood "anatomical fingerprint". Overall, the microscopic description of about 6,700 wood timbers (wood genus/species) are currently available and documented in several computerized databases, e.g., InsideWood (2004 onwards) or Commercial timbers (delta-intkey, 2000 onwards).

Material and methods

The Thünen Institute of Wood Research (Wood Anatomical Laboratory) received two collectives of solid wood samples (overall 50 specimens) for microscopic wood identification and verification of the declared botanical nomenclature "blind test on species declaration".

One collective (25 samples with the codes RM_2014 and X2) was provided by WWF, Deutschland (contact person: J. Zahnen). The second collective (25 sample with the codes G2S_S) was submitted by G2S (contact person for the documentation of the results: G. Yene).

For the microscopic wood identification thin sectionings (10 to 20 µm thickness in the three anatomical directions: transversal, radial and tangential) were cut on a sliding microtome from aligned wood blocks (dimension of approx. 5 - 10 mm^3) of the individual 50 samples (Fig. 2).

The wood anatomical structures of the specimens were microscopically investigated using a standard light microscope with polarized light device (magnification of the objectives 4x to 40x) and directly

compared with reference slides (vouchered material of the scientific wood collection RBHw*) and microscopic wood slides prepared within the ITTO project (Fig. 3, Master thesis of. V. Haag).

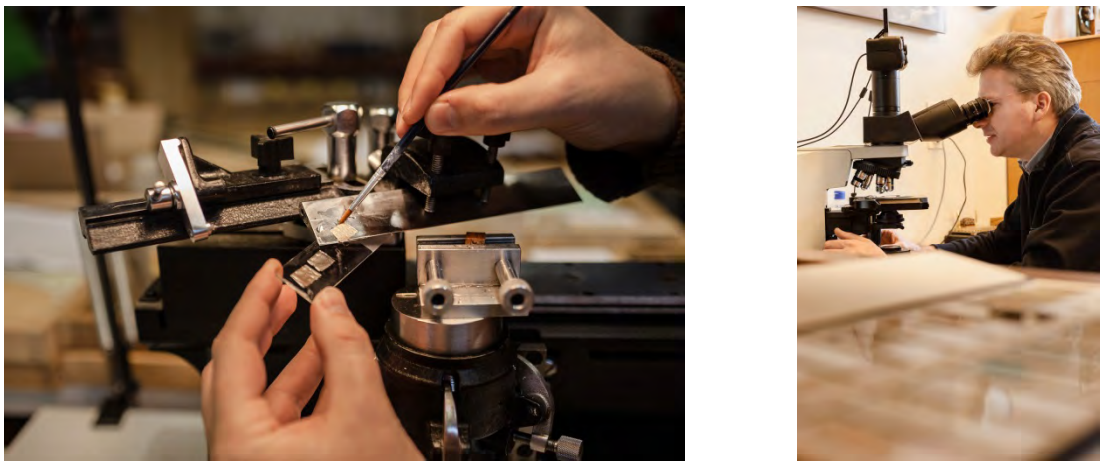

Fig. 2. Preparation (microtome) and microscopic analysis of the wood sections

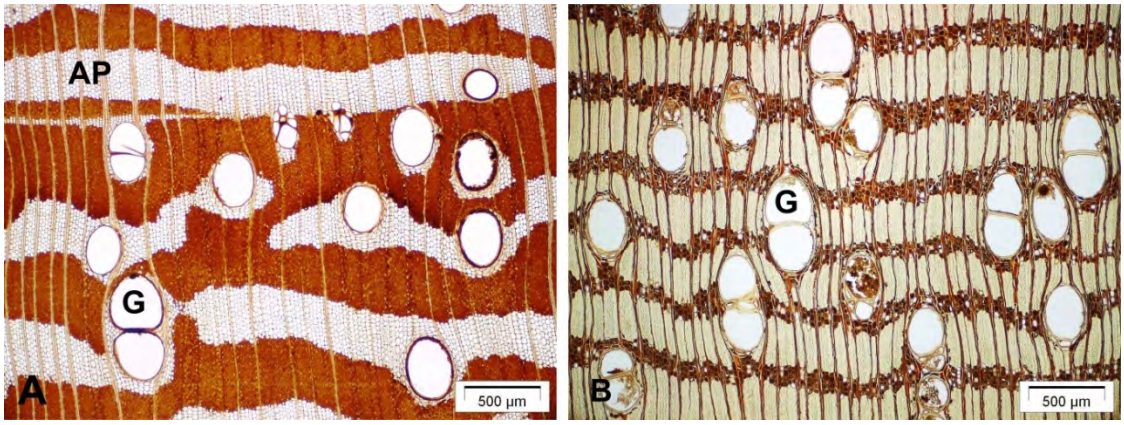

Fig. 3. Microscopic transverse sections of Millettia spp. (left) and Lophira alata (right); references for the microscopic wood identification

The wood anatomical structures of the microscopically identified timber are also compared with the descriptions in the computerised database *Commercial timbers* in the Delta-Intkey-System. This very established database was also applied for the microscopic wood identification of the blind-test samples.

Results

The results of the microscopic wood identification are presented in the following tables Part I (samples provided by the WWF) and Part II (samples provided by the Thünen Institute of Forest Genetics) with the specified information/columns: sample codes, claims, results of microscopic identification and comments.

The evaluation of the provided results reveals the high capability of the wood anatomical analyses: All individual samples (100%) of both collectives were clearly identified on the genus level which defines the relevant trade names according to the EN Standard 13556 *"Nomenclature of timbers used in Europe"* and the requirements of the European Timber Regulation (EUTR). The identification of individual wood species, e.g. *Entandrophragma cylindricum* or *Triplochiton scleroxylon,* etc., was successfully achieved for 56% (part I) and 60% (part II) of the analysed samples. Comments to the "maximal" possible differentiation or identification of the individual samples are provided in detail (see table 1 and 2). The investigated wood blocks and microscopic slides are carefully preserved and documented at the Thünen Institute of Wood Research for additional microscopic analyses or verification.

**RBHw = acronym of the Thünen Wood Collection according to the Index Xylariorum*

Table 1: Results of the microscopic wood identification - Part I: Collectives RM_2014 and X2 provided by WWF, Deutschland

Sample code	Claims on species name	Results of lab	Results of the microscopic wood identification (wood anatomy)	Comments
RM_2014_03	*Milicia excelsa*	false ✖	***Millettia* spp. = Wengé or Panga Panga**	*correct trade name **Wengé / Panga Panga***
RM_2014_04	*Erythrophleum ivorense*	*correct* ✔	***Erythrophleum* spp. = Tali**	*the individual species within the genus **Erythrophleum** can´t be distinguished microscopically*
RM_2014_13	*Khaya ivorensis*	*correct* ✔	***Khaya* spp. = Khaya**	*the individual species within the genus **Khaya** can´t be distinguished microscopically*
RM_2014_37	*Erythrophleum suaveolens*	*correct* ✔	***Erythrophleum* spp. = Tali**	*the individual species within the genus **Erythrophleum** can´t be distinguished microscopically*
RM_2014_39	*Entandrophragma utile*	false ✖	***Aucoumea klaineana* = Okoumé**	*correct trade name **Okoumé***
RM_2014_42	*Entandrophragma angolense*	false ✖	***Nauclea diderrichii* = Bilinga**	*correct trade name **Bilinga***
RM_2014_45	*Afzelia pachyloba*	*correct* ✔	***Afzelia* spp. = Afzelia**	*the individual species within the genus **Afzelia** can´t be distinguished microscopically*
RM_2014_48	*Entandrophragma cylindricum*	false ✖	***Entandrophragma angolense* = Tiama**	*correct declaration **Tiama***
RM_2014_49	*Aningeria robusta*	false ✖	***Baillonella toxisperma = Moabi***	*correct declaration **Moabi***
RM_2014_59	*Aucoumea klaineana*	false ✖	***Afzelia* spp. = Afzelia**	*correct trade name **Afzelia***
RM_2014_60	*Cylicodiscus gabunensis*	false ✖	***Entandrophragma utile*= Sipo**	*correct trade name **Sipo***
X2-57	*Pterocarpus soyauxii*	false ✖	***Pericopsis elata* = Afrormosia**	*correct trade name **Afrormosia** (CITES-species)*
X2-58	*Baillonella toxisperma*	false ✖	***Pouteria* spp. (*Aningeria* spp.) = Aningré**	*correct trade name **Aningré***
X2-59	*Afzelia bipindensis*	*correct* ✔	***Afzelia* spp. = Afzelia**	*the individual species within the genus **Afzelia** can´t be distinguished microscopically*
X2-65	*Guibourtia ehie*	false ✖	***Guibourtia* spp. = Bubinga**	*The wood anatomical characters show best agreement with **Bubinga**; the individual species G. ehie = Ovengkol can be excluded*
X2-66	*Millettia laurentii*	false ✖	***Milicia* cf. *excelsa* = Iroko**	*correct trade name **Iroko***
X2-67	*Khaya grandiflora*	*correct* ✔	***Khaya* spp. = Khaya**	*the individual species within the genus **Khaya** can´t be distinguished microscopically*
X2-68	*Milicia regia*	*correct* ✔	***Milicia* spp. = Iroko**	*the individual species within the genus **Milicia** can´t be distinguished microscopically*
X2-69	*Terminalia superba*	*correct* ✔	***Terminalia superba* = Limba**	*correct declaration*
X2-74	*Pericopsis elata*	false ✖	***Pterocarpus soyauxii* = Padouk**	*correct trade name **Padouk***
X2-75	*Nauclea diderrichii*	false ✖	***Aucoumea klaineana* = Okoumé**	*correct trade name **Okoumé***
X2-76	*Khaya ivorensis*	false ✖	***Entandrophragma cylindricum* = Sapelli**	*correct trade name **Sapelli***
X2-78	*Triplochiton scleroxylon*	*correct* ✔	***Triplochiton scleroxylon* = Abachi**	*correct declaration*
X2-79	*Pericopsis elata*	false ✖	***Cylicodiscus gabunensis* = Okan**	*correct trade name **Okan***
X2-81	*Lophira alata*	*correct* ✔	***Lophira alata* = Bongossi**	*correct declaration*

Table 2: Results of the microscopic wood identification - Part II: Collective G2S_S provided by the Thünen Institute of Forest Genetics

Sample code	Claims on species name	Result of lab	Result of the microscopic wood identification (wood anatomy)	Comments
G2S_S_1.0	*Guibourtia ehie*	false ✗	***Afzelia* spp. = Afzelia**	*correct trade name* ***Afzelia***
G2S_S_1.5	*Baillonella toxisperma*	false ✗	***Afzelia* spp. = Afzelia**	*correct trade name* ***Afzelia***
G2S_S_2.0	*Khaya anthotheca*	correct ✓	***Khaya* spp. = Khaya**	*the individual species within the genus* ***Khaya*** *can´t be distinguished microscopically*
G2S_S_3.5	*Baillonella toxisperma*	correct ✓	***Baillonella toxisperma* = Moabi**	*correct declaration*
G2S_S_5.0	*Entandrophragma cylindricum*	false ✗	***Entandrophragma angolense* = Tiama**	*correct trade name* ***Tiama***
G2S_S_8.0	*Entandrophragma candollei*	false ✗	***Entandrophragma utile* = Sipo**	*correct trade name* ***Sipo***
G2S_S_8.5	*Entandrophragma cylindricum*	false ✗	***Khaya* spp. = Khaya**	*correct trade name* ***Khaya***
G2S_S_10.0	*Milicia excelsa*	false ✗	***Erythrophleum* spp. = Tali**	*correct trade name* ***Tali***
G2S_S_11.5	*Guibourtia* spp.	correct ✓	***Guibourtia* spp. = Bubinga**	*correct declaration*
G2S_S_13.0	*Lophira alata*	correct ✓	***Lophira alata* = Bongossi**	*correct declaration*
G2S_S_13.5	*Lophira alata*	correct ✓	***Lophira alata* = Bongossi**	*correct declaration*
G2S_S_14.0	*Erythrophleum suaveolens*	false ✗	***Milicia* spp. = Iroko**	*correct trade name* ***Iroko***
G2S_S_15.0	*Milicia regia*	correct ✓	***Milicia* spp. = Iroko**	*the individual species within the genus* ***Milicia*** *can´t be distinguished microscopically*
G2S_S_16.5	*Millettia laurentii*	correct ✓	***Millettia* spp. = Wengé (Panga Panga)**	*Millettia laurentii = Wengé and Millettia stuhlmannii* = Panga panga *can´t be distinguished microscopically*
G2S_S_18.5	*Khaya* spp.	false ✗	***Pericopsis elata* = Afrormosia**	*correct trade name* ***Afrormosia*** *(CITES-species)*
G2S_S_20.0	*Terminalia superba*	correct ✓	***Terminalia superba* = Limba**	*correct declaration*
G2S_S_21.5	*Pterocarpus soyauxii*	correct ✓	***Pterocarpus soyauxii* = Padouk**	*correct declaration*
G2S_S_24.0	*Triplochiton scleroxylon*	correct ✓	***Triplochiton scleroxylon* = Abachi**	*correct declaration*
G2S_S_25.5	*Entandrophragma utile*	false ✗	***Mansonia altissima* = Mansonia, Béte**	*correct trade name* ***Mansonia*** *note: Mansonia doesn't belong to the ITTO-species list of the 21 selected taxa*
G2S_S_30.5	*Triplochiton scleroxylon*	correct ✓	***Triplochiton scleroxylon* = Abachi**	*correct declaration*
G2S_S_33.5	*Erythrophleum ivorense*	false ✗	***Lovoa trichilioides* = Dibétou**	*correct trade name* **Dibétou** *note: Dibétou doesn't belong to the ITTO-species list of the 21 selected taxa*
G2S_S_35.5	*Afzelia* spp.	correct ✓	***Afzelia* spp. = Afzelia**	*correct declaration*
G2S_S_38.5	*Nauclea diderrichii*	false ✗	***Guarea* spp. = Bossé**	*correct trade name* **Bossé** *note: Bossé doesn't belong to the ITTO-species list of the 21 selected taxa*
G2S_S_41.5	*Aningeria robusta*	false ✗	***Mansonia altissima* = Mansonia, Béte**	*correct trade name* ***Mansonia*** *note: Mansonia doesn't belong to the ITTO-species list of the 21 selected taxa*
G2S_S_47.5	*Cylicodiscus gabunensis*	correct ✓	***Cylicodiscus gabunensis* = Okan**	*correct declaration*

Conclusions

Regarding the role of wood anatomy in the control of internationally traded timber -*successfully applied within the ITTO blind test*- it can be clearly stated that the microscopic analysis is currently the most feasible and competitive method to identify wood. The microscopic analysis allows access to a large number of references (anatomical description of about 6,700 wood species) including the increasingly traded “lesser known species”. Wood anatomy is routinely applied in the daily control of wood and wood products and false declarations can be proven in a short time. However, the important information about the geographic origin of the timber can’t be determined by wood structure. To obtain this information, an interdisciplinary combination of genetic-, isotope-, and microscopic techniques is a very feasible solution. In general, the methods of macroscopic and microscopic wood identification can be relatively easy transferred to international working groups involved in the control of timber trade (relative low investment costs for the microscopic techniques). However, the reliable identification based on microscopic wood structure requires considerable professional expertise, a rather sophisticated infrastructure, and a well-sorted reference wood collection (Fig. 4).

Fig. 4. Scientific wood collections at the Thünen Institute of Wood Research, Hamburg

ANNEX 4

Technical reports on genetics

Thünen Institute of Forest Genetics	Dr Céline Blanc-Jolivet
	PD Dr Bernd Degen
Université Libre de Bruxelles	Dr Kasso Dainou
	Dr Olivier Hardy2
University od Adelaide	Mr. Duncan Jardine
	Mr. Joey Gerlach
	Dr Elly Dormontt
	Dr Andrew Lowe
Plant Genetic Diagnostics Ltd	BSc. Maike Paulini
	Dr. Aki Michael Höltken

Development of a genetic reference map for Sapelli

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Material and methods

A total of 1192 *Entandrophragma* spp. samples were sampled in Ghana, Ivory Coast, Cameroon, Congo Brazzaville, Democratic Republic of Congo (DRC) and Gabon. Although indication of species identity was provided, we analyzed all samples and not only *Entandrophragma cylindricum* (Sapelli). One individual from Cameroon and one individual from DRC were selected for molecular marker (SNP) discovery through Restriction Site-Associated DNA sequencing (RADSeq). A set of putative SNPs located in the nuclear genome were selected for screening with a MassARRAY technology (Mc Kernan et al. 2002) conducted by the INRA Genome-Transcriptome Facility (GTF). Genotypes from a total of 190 individuals representing the whole distribution range were used to select the final set of SNP loci. All individuals were then genotyped at the selected loci to build the genetic reference data.

To detect genetic structure, we conducted a Bayesian clustering analysis (Pritchard et al. 2000). This method allows the grouping of individuals in genetic entities regardless of geographical origin. We proceeded to grouping of reference individuals based on the results of the cluster analysis and on the country of origin. This data was further used to control the geographical origin of blind samples.

Based on the results of the cluster analysis, we selected 10 individuals for a genome skimming approach using the MiSeq platform. The goal here was to analyze plastid genome (mitochondrial and chloroplastic) to find species-specific markers and to increase the resolution of the reference data based on nuclear SNP discovered with the RADseq method. Putative chloroplastic SNPs were also screened on a MassARRAY platform by the GTF.

Results

RADseq yielded more than 1,000 putative SNP loci. Among those, 131 were organized in four multiplexes for a MassARRAY genotyping. Three groupings were applied to find the loci with the strongest geographical signal: per bloc, per country, per country excluding Western African countries. For each loci and dataset, we estimated the correlation between geographical distance and genetic distance and geographical distance and differentiation index. We selected in priority loci, which had a high correlation at several datasets. Genetic assignment using a Bayesian approach (Rannala & Mountain 1997) by grouping reference samples by country was conducted for all loci and for the reduced set of loci to compare the accuracy of the reduced set of loci compared to results with all loci. A final set of 74 loci (two multiplexes) was defined for the screening of all individuals.

Nine genetic clusters could be identified. Among those, two could be attributed to other species, *Entandrophragma angolese* and *Entandrophragma utile*. A dendrogramm (Figure 1) illustrates the

genetic relationship among the identified clusters and also suggests that cluster 2 might represent another *Entandrophragma* species.

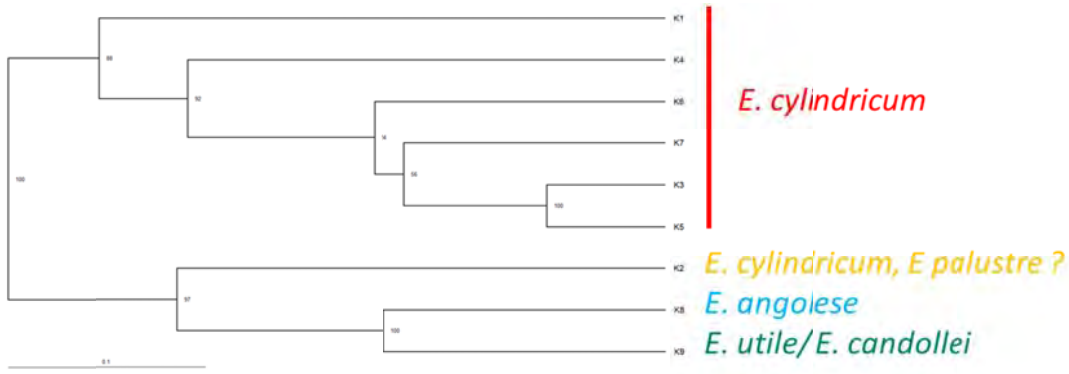

Figure 1: Dendrogramm representing the genetic relationships among the identified genetic clusters

The results highlight species misidentification problems in the field, especially in DRC where a lot of samples declared as *E. cylindricum* were attributed to *E. angolese* by the genetic data. The geographical distribution of the genetic clusters in presented in Figure 2.

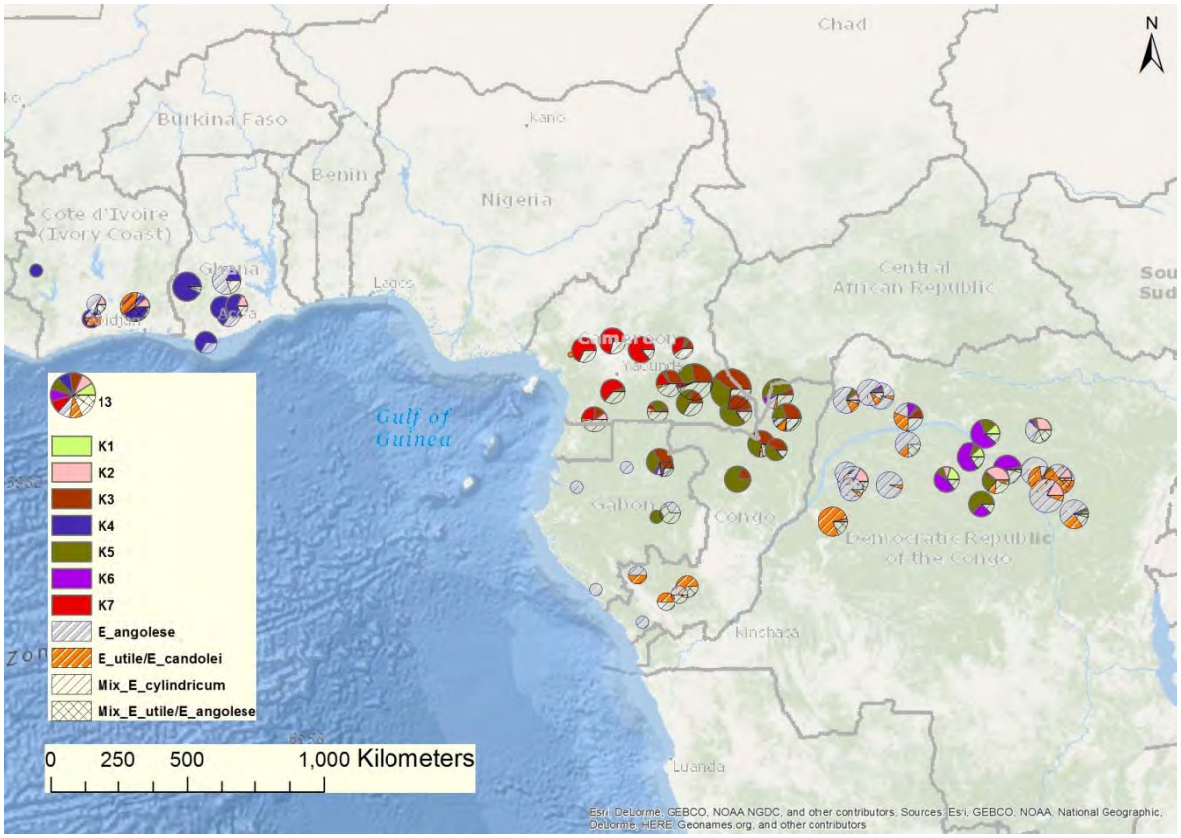

Figure 1: Distribution map of the *Entandrophragma spp.* genetic clusters

The map show that cluster 4 (K4) is restricted to Western Africa, clusters 1 (K1) and 6 (K6) to DRC, cluster 7 (K7) to Cameroon, while clusters 3 (K3) and 5 (K5) are common in all Central African countries. This means if clusters 1, 4, 6 and 7 are observed in a timber sample, then controlling the country of origin is straightforward. However, for clusters 3 and 5, more precise statistical analysis is required. Genome skimming and screening of putative SNPs on 190 individuals allowed the identification of a set of 13 loci from the chloroplast genome for species identification. Unfortunately, no further geographical structure could be identified on *E. cylindricum* samples.

Precision of the reference data

Individual assignment tests were performed using the Bayesian multilocus-approach (Rannala & Mountain 1997) in GDA_NT (Degen, unpublished) and a new approach based genetic distances of individuals (GeoAssign). All individuals from the reference data were self-classified to the country of origin using the leave-one-out approach (self-assignment, Efron 1983). The below table gives the results for the different countries:

Population	**Sample Size**	**Tested ind/**	**Bayesian approach**	**Distance approach**	
			% correct assigned	**% correct assigned**	**% claim accepted**
Cameroon	434	431	61	68	90
Congo_Braz	141	140	51	57	97
DRC	487	420	80	72	92
Gabon	36	36	22	3	84
Ghana	69	66	80	95	89
Ivory Coast	25	23	17	0	64
Total/Mean			**66**	**65**	**90**

The precision measured by the % of correct assigned individuals varies among the countries and statistical approaches from 0% to 95%. Most of the wrong assignment in West Africa is mixing up Ghana and Ivory Coast.

Blind test

For the blind tests we used the reference data and the Bayesian approach based on allele frequencies (Rannala & Mountain 1997). We tested different approaches of classification of the reference data (only classified by country, classified by country and genetic cluster). The results of this approach are given in the blind test reports (Annex 7.1 and Annex 7.2). In addition, we applied the approach based on genetic distances among individuals (Gregorius 1978) and higher thresholds for data completeness and the criteria to reject a claim (Annex 8). The different ways of analyzing the data, treating missing data and the different thresholds for the blind test lead to an overall performance from 50% to 83% correct results for claims on the country of origin.

Discussion

Species identity

The newly developed markers could efficiently assign individuals to species. However, the low sample size for *E. candollei* (six individuals) and potential identification mistakes in the field do not allow the genetic characterization of this species. Indeed, *E. candollei* and *E. utile* individuals were both belonging to the genetic cluster 9 based on nSNPs. However, chloroplast SNPs showed a subdivision of the cluster, but we could not define whether one group corresponds to *E. candollei* and the other to *E. utile*, or whether the second was another species. Further sampling with reliable taxonomic identification is thus needed to clarify the genetic differentiation between *E. candollei* and *E utile*.

The genotypes of individuals from cluster 2 raised a lot of questions. First, cluster 2 was genetically distant to the *E. cylindricum* group (Figure 1), which suggests that those individuals belong to another *Entandrophragma* species. This cluster mostly occurs in central DRC, where the species *E. palustre* is present. Again, sampling of well-taxonomically identified *E. palustre* should help to clarify this question. Second, cluster 2 was found to be very polymorphic at the chloroplast SNPs. It is therefore possible that this group is not genetically homogenous. Low amplification success at the nSNPs, probably due to low DNA quality, and low sampling size probably also artificially led to the grouping of these individuals in one single cluster. This could probably explain the occurrence of cluster 2 in Western Africa.

Genetic structure of Sapelli

Genetic data at the nSNPs clearly identified a strong differentiation among Western Africa and Central Africa. This pattern is also observed in many other species and results from the recolonization from different glacial refugias after the last glaciations. The data show that a contact zone between the two lineages exists in Western Cameroon (hybrid individuals cluster 4 (Western Africa) and 7 (Cameroon). We therefore expect that populations in Nigeria have intermediate genotypes.

The lack of genetic structure in Central Africa has also been reported in other species. Populations found in Cameroon, Congo Brazaville and Gabon probably originated from the same glacial refugia located on the coast. The relative climate homogeneity and the absence of geographical barriers further prevented differentiation in the Congo Bassin region. Populations from center DRC differ from the Congo Basin populations, probably also due to presence of another glacial refugia. Unfortunately, species identification mistakes in the field were very common in the Western DRC, Southern Gabon and Southern Congo Brazaville samples, thereby strongly reducing the number of *E. cylindricum* samples. Therefore we judge the reference data in this area for this species as not sufficient for timber tracking.

Literature

Efron B (1983) Estimating the error rate of a prediction rule - improvement on cross-validation, J. Am. Stat. Assoc. 78 , 316-331.

Gregorius H-R (1978). The concept of genetic diversity and its formal relationship to heterozygosity and genetic distance. Mathematical Bioscience 41: 253-271.

McKernan K, Fujii C, Ziauddin J, Malek J, McEwan P (2002). A high throughput and accurate method for SNP genotyping using Sequenom MassARRAY (TM) system. Am J Hum Genet 71(4): 454-454.

Pritchard JK, Stephens M, Donnelly P (2000). Inference of population structure using multilocus genotype data. Genetics 155(2): 945-959.

Rannala B, Mountain JL (1997). Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences of the United States of America 94(17): 9197-9201.

Development of a genetic reference map for Iroko

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Material and methods

A total of 1920 *Milicia excelsa* and *Milicia regia* samples were sampled in Ghana, Ivory Coast, Cameroon, Congo Brazzaville, Democratic Republic of Congo (DRC), Gabon and Kenya. One individual from Benin and one individual from Kenya, available before sampling activities, were selected for molecular marker (SNP) discovery through Restriction Site-Associated DNA sequencing (RADSeq). A set of putative SNPs located in the nuclear genome were selected for screening with a MassARRAY technology (Mc Kernan et al. 2002) conducted by the INRA Genome-Transcriptome Facility (GTF). Genotypes from a total of 95 individuals representing the whole distribution range were used to select the final set of SNP loci. 1833 individuals were then genotyped at the selected loci to build the genetic reference data.

To detect genetic structure, we conducted a Bayesian clustering analysis (Pritchard et al. 2000). This method allows the grouping of individuals in genetic entities regardless of geographical origin. We proceeded to grouping of reference individuals based on the results of the cluster analysis and on the country of origin. This data was further used to control the geographical origin of blind samples.

Results

RADseq yielded more than 1,000 putative SNP loci. Among those, 138 were organized in four multiplexes for a MassARRAY genotyping. Three groupings were applied to find the loci with the strongest geographical signal: per bloc, per country, per country excluding Western African countries. For each loci and dataset, we estimated the correlation between geographical distance and genetic distance and geographical distance and differentiation index. We selected in priority loci, which had a high correlation at several datasets. Genetic assignment by grouping reference samples by country was conducted for all loci and for the reduced set of loci to compare the accuracy of the reduced set of loci compared to results with all loci (Rannala & Mountain 1997). A final set of 79 loci (two multiplexes) was defined for the screening of all individuals, which included two chloroplastic loci formerly identified (Dainou et al., 2010).

Five genetic clusters could be identified, among those one represented the species *M. regia*, commonly observed in Western Africa. One cluster was only present in Western Africa (K2), while three clusters (K3, K4 and K5) were present in central Africa.K3 was dominant in the Congo Basin, while K4 was restricted to the South-Western area and K5 on the Eastern Coast (Kenya and Eastern DRC) (Figure 1).

Further work was conducted to allow genetic screening of SNPs with PCR-RFLP, which can be easily applied in African laboratories. Among the 79 loci used for the final screening, 23 were selected for the development of a PCR-RFLP approach. Assignment tests showed that the use of these 23 loci reduced self-assignment success of 10% compared to the set of 79 loci.

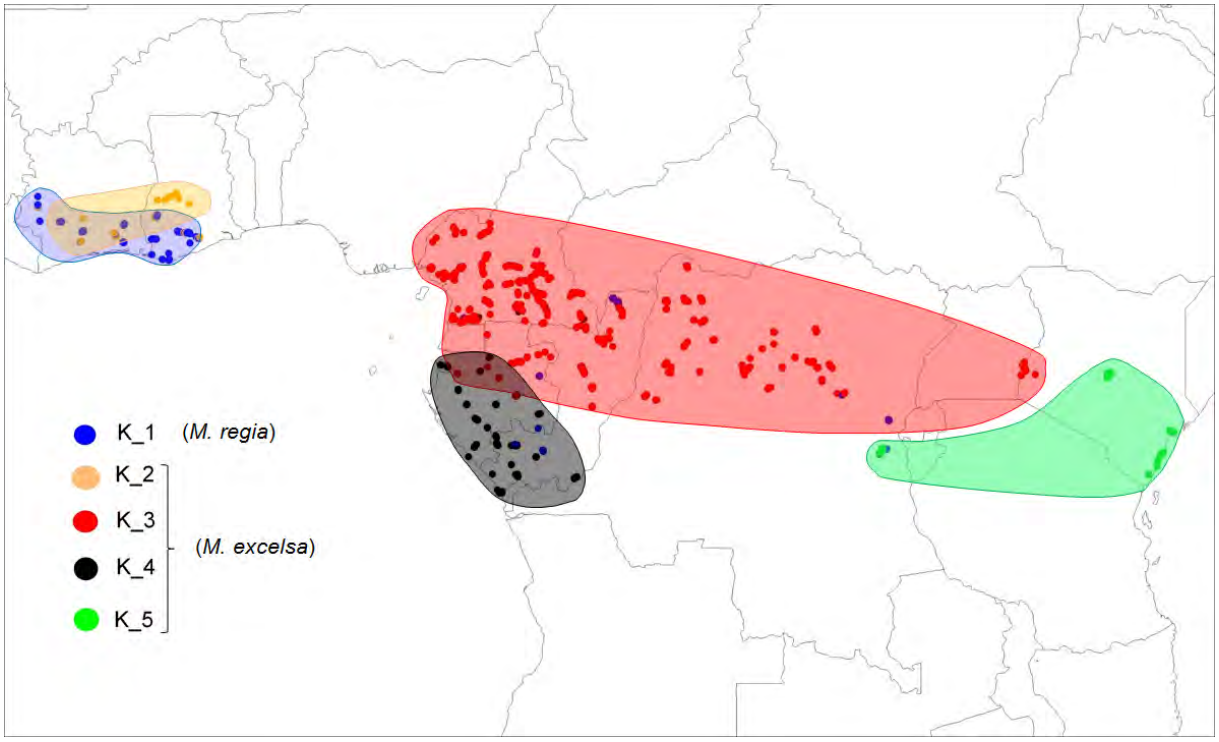

Figure 1: Distribution map of the *Milicia spp.* genetic clusters

Precision of the reference data

Individual assignment tests were performed using the Bayesian multilocus-approach (Rannala & Mountain 1997) in GDA_NT (Degen, unpublished) and a new approach based genetic distances of individuals (GeoAssign). All individuals from the reference data were self-classified to the country of origin using the leave-one-out approach (self-assignment, Efron 1983). The below table gives the results for the different countries:

Population	**Sample Size**	**Tested ind/**	**Bayesian approach**	**Distance approach**	
			% correct assigned	**% correct assigned**	**% claim accepted**
Cameroon	306	305	66	43	83
Congo_Braz	260	259	49	42	87
DRC	412	411	53	45	80
Gabon	252	251	62	71	91
Ghana	46	46	72	42	89
Ivory Coast	101	101	50	94	100
Kenya	103	103	81	77	95
Total/Mean			**59**	**54**	**86**

Blind test

For the blind tests we used the reference data and the Bayesian approach based on allele frequencies (Rannala & Mountain 1997). We tested different approaches of classification of the reference data (only classified by country, classified by country and genetic cluster). The results of this approach are given in the blind test reports (Annex 7.1 and Annex 7.2). In addition, we applied the approach based on genetic distances among individuals (Gregorius 1978) and higher thresholds for data completeness and the criteria to reject a claim (Annex 8). The different ways of analyzing the data and the different thresholds for the blind test lead to an overall performance from 40% to 60% correct results for claims on the country of origin.

Discussion

Species identity

The set of 79 SNP loci could efficiently differentiate the two *Milicia* species. Interestingly, a few individuals from Central Africa were assigned to the *M. regia* cluster and their leaves showed similar morphology. Further analysis could determine that these individuals genetically diverge from *M. regia* samples occurring in Western Africa. This indicates that *M. regia* was formerly widespread in Central Africa.

Genetic structure Iroko

Genetic data at the nSNPs clearly identified a strong differentiation among Western Africa and Central Africa. This pattern is also observed in many other species and results from the recolonization from different glacial refugias after the last glaciations. The presence of distinct genetic group in Kenya indicates the presence of a glacial refugia on the Eastern coast with restricted subsequent dispersal, probably due to geographical barriers (rift valley). The lack of genetic structure in Central Africa has also been reported in other species. Populations found in Cameroon, Northern Congo Brazaville and Northern Gabon (K3) probably originated from the same glacial refugia located on the coast, while the presence of K4 suggests that a second refugia existed more South. The relative climate homogeneity and the absence of geographical barriers further prevented differentiation in the Congo Bassin region.

Reference

Dainou K, Bizoux JP, Doucet JL, Mahy G, Hardy OJ, Heuertz M. (2010). Forest refugia revisited: nSSRs and cpDNA sequences support historical isolation in a wide-spread African tree with high colonization capacity, *Milicia excelsa* (Moraceae). Mol Ecol 19: 4462–4477.

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McKernan K, Fujii C, Ziauddin J, Malek J, McEwan P (2002). A high throughput and accurate method for SNP genotyping using Sequenom MassARRAY (TM) system. Am J Hum Genet 71(4): 454-454.

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Development of Genographic map for Ayous (*Triplochiton* scleroxylon) using SNP markers

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Objective

Develop single nucleotide polymorphic (SNP) markers for ayous, to allow genetic identification and verification of the geographic source of origin of timber.

Material and Methods

Genographic map

SNP marker discovery

Partial genome sequencing for initial single nucleotide polymorphism (SNP) discovery was performed using an in-house reduced representation genome library preparation method modified for double restriction enzyme digest
method (after Vos et al. 1995; van Orsouw et al. 2007) (see Jardine et al. 2015 for more information on the protocol). A total of 48 individuals from 10 populations and 3 countries, representing the geographical distribution of ayous were used in this marker discovery stage. Genomic DNA of these samples was extracted at the Thünen Institute-Forest Genetics (TIFG), with the extractions sent to Adelaide for molecular analysis. The samples were sequenced using an Ion Torrent PGM™ system (Life Technologies), with the subsequent data analysed using the CLC-bio workbench (Qiagen) and Geneious (Biomatters) platforms. A shortlist of 127 potential loci was identified, and a final panel of 117 loci designed into three multiplexes were prepared for genotyping on the Sequenom® MassARRAY® iPLEX™ GOLD platform.

DNA from 90 samples, consisting of the original 48 samples used in the marker discovery, as well as an extra 48 samples were used to screen the potential SNP loci for suitability. Any loci that failed to amplify or found to be uninformative were excluded from further use.

A second set of SNP discovery and development was undertaken by TIFG using three of the reference samples on a Rad-Seq platform (details available from TIFG). A final panel of SNP markers was developed using a combination of the most suitable markers from both the Adelaide and TIFG marker sets. This final panel consisted of 235 markers across six multiplexes and was used to screen all individuals in the subsequent analysis.

Genotyping

A total of 911 individuals, representing 45 populations from five countries (Figure 1), were genotyped using the final SNP panel. Samples were available as either leaf tissue or cambium plugs, and DNA was extracted at the Australian Genome Research Facility (AGRF). Samples and loci that failed to meet a strict 95% sequencing success threshold were removed from further analysis. The remaining individuals and loci (792 and 190 respectively) were analysed for linkage disequalibrium (LD) and Hardy Weinberg (HW) equilibrium (removing outlier loci), heterozygosity and F_{ST}. When a pair of loci was identified as being linked, the locus with the lowest heterozygisity and/or lowest HW ratio was removed. The final dataset consisted of 753 individuals and 105 loci. A STRUCTURE analysis to identify the most appropriate number of genetic clusters. This was done using standard parameters and incorporating a burnin length of 3000000mcmc's and 700000mcmc run length.

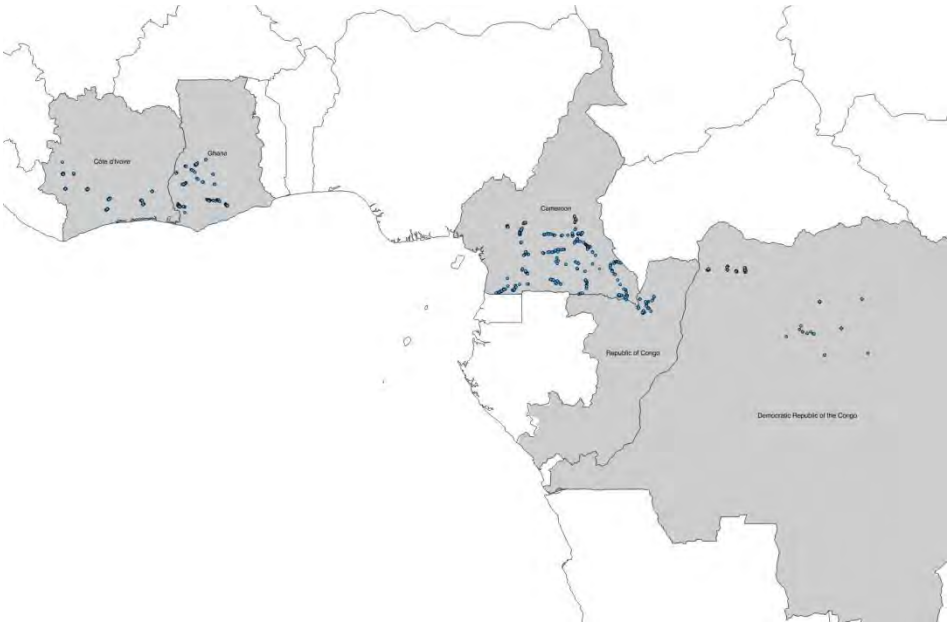

Figure 1. Location of all the samples used in the second round of genotyping

Blind samples

Twenty blind test samples were sent to Adelaide, 10 via WWF and 10 via G2S. DNA was extracted from all samples, using either a modified Analatik Jena innuPREP Plant DNA extraction kit or standard BoTAB for timber protocol. DNA was genotyped using the final SNP marker set on the MassARRAY platform at AGRF. The results from the genotyping of blind samples were screened and filtered to remove samples that had less than a 95% sequencing success rate. The geographic source of samples was estimated using the GeoAssign program, which provides a likely position of test samples as the centroid point of the 10 most genetically similar individuals from the reference data set.

Results and discussion

The results of the STRUCTURE analysis identified that a clustering of K=2 (Figure 2) was the most applicable for the dataset, followed by K=4. The K=4 clustering (Figure 3) was considered to be the most useful for geographic structuring and assignment as the following genetic clusters could be identified; Ghana/Ivory Coast; most of Cameroon; SE Cameroon, northern Republic of Congo and NW DRC; and central DRC.

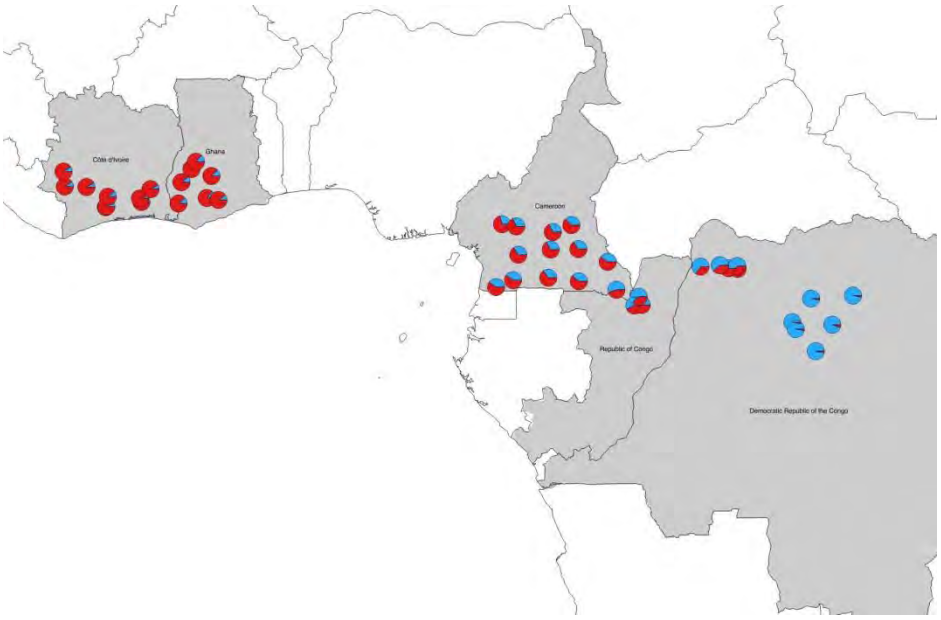

Figure 2. Proportional membership of populations and individuals under K=2 STRUCTURE clustering.

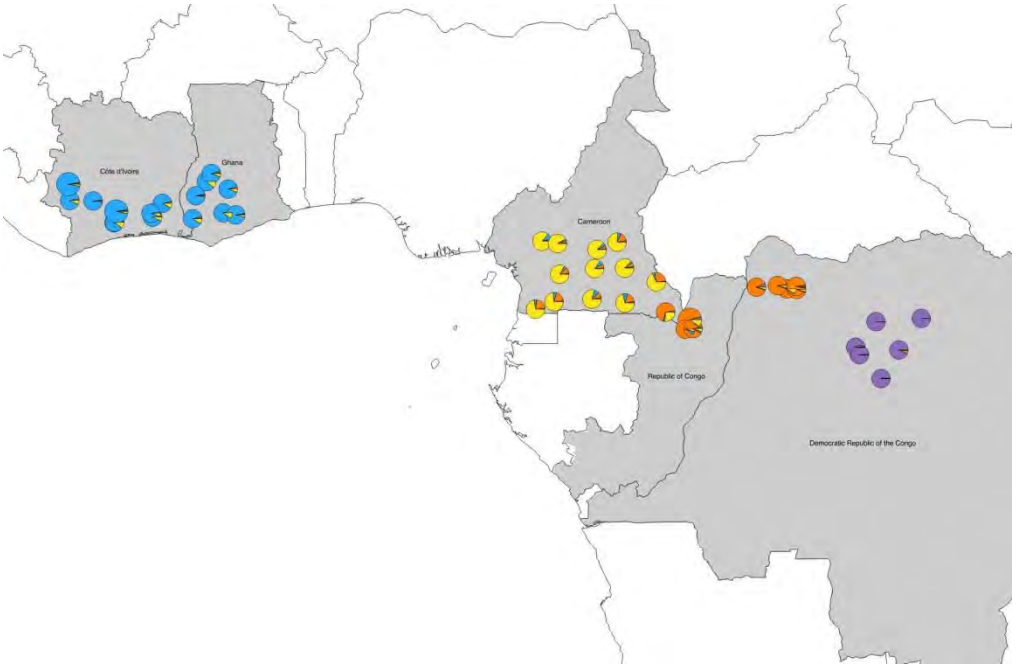

Figure 3. Proportional membership of populations and individuals under K=4 STRUCTURE clustering

For the blind samples (Table 1), 15 out of 20 samples produced high quality DNA that could be reliably genotyped, therefore 75% of tests could be processed. Using the GeoAssign analytical routine to identify the most likely country of origin, the claimed origin was accepted in eight cases and rejected in six cases. Scored against the true origin 11 out of 15 tests (73.3.%) were correctly accepted/rejected.

Sample ID	Claimed origin	GeoAssign origin	Real origin	GeoAssign Reject claim?	Correct?
BT_2014_533	Ghana	Ghana/Ivory Coast	Ghana	No	Yes
BT-2014_543	Cameroon	Cameroon	Gabon	Yes	No
BT_2014_547	DRC	Ghana	Ghana	Yes	Yes
BT_2014_551	CIV	Ivory Coast	Ghana	No	No
BT_2014_563	CIV	Ivory Coast	Ghana	No	No
BT_2014_567	DRC	Ghana	Ghana	Yes	Yes
BT_2014_568	Cameroon	Cameroon	Cameroon	No	Yes
BT_2014_578	Cameroon	Ghana	Ghana	Yes	Yes
BT_2014_594	Ghana	Ghana/Ivory Coast	Ghana	No	Yes
BT_2014_598	Ghana	Ghana	Ghana	No	Yes
G2S_O_T1	Ghana	DNA quality too poor			
G2S_O_T3	Ghana	Ghana	Ghana	No	Yes
G2S_O_T6	Cameroon	DNA quality too poor			
G2S_O_T7	Congo Braz	DNA quality too poor			
G2S_O_T9	DRC	Cameroon	Cameroon	Yes	Yes
G2S_O_T11	Cameroon	Cameroon	Cameroon	No	Yes
G2S_O_T13	Cameroon	Cameroon	Cameroon	No	Yes
G2S_O_T15	Cameroon	DNA quality too poor			
G2S_O_T16	Cameroon	DNA quality too poor			
G2S_O_T18	Cameroon	Ghana	Cameroon	Yes	No
Overall DNA analysis success = 75%			**Correct claim accept/reject = 73.3%**		

Table 1. Blind test results, indicating claimed origin, genetically identified origin and true origin, claim rejection/acceptance and correct determination.

The results provide an insight into where further sampling would be useful and would improve future blind test results, in particular the following locations; Ghana/Ivory Coast, Gabon and southern Congo Braz and DRC and western Cameroon/Nigeria.

ITTO project "Development and implementation of a species identification and timber tracking system in Africa with DNA fingerprints and stable isotopes"

Report: DNA barcoding

Dr. Aki Michael Höltken & BSc. Maike Paulini (Plant Genetic Diagnostics Ltd., Thünen Institute of Forest Genetics)

Background

A genetic barcoding method for the identification of 21 tropical tree species based on timber samples requires molecular markers (a) with low intraspecific but sufficient interspecific variability, (b) a high-copy number of the target DNA fragments because of the low yield of DNA following extraction and (c) short in length due to high degradation of the DNA. The DNA of chloroplasts (cpDNA) combines all these features. It is present in multiple copies per cell, the ring structure of the cp-genome gives higher stability to the DNA molecule and, because cpDNA is maternally inherited, there is no recombination of the cp genome in contrast to nuclear DNA. Various protocols have been published to extract DNA from wood, from recently logged (almost fresh) up to processed timber and timber products from different steps in the chain-of-custody. Most of the approaches are developed to mitigate the effects of contamination of the samples with external DNA and to minimise further demolition of already highly degraded DNA sequences (DE FILIPPIS & MAGEL 1998, DEGUILLOUX et al. 2002, RACHMAYANTI et al. 2006, ASIF & CANNON 2007).

Methodological approach

The selected tree species of this project belong to 9 botanical families and 8 orders. This circumstance poses a huge challenge for the development of a universal DNA barcoding system. Although universal fragments are available for plant species identification, these are mostly not suitable for timber species identification and require major modifications:

1. Official barcoding fragments are too long for amplification from timber samples. Universal primers have to be designed within these DNA sequences to reduce the length of the amplification products.
2. Intergenic sequences are too variable in size as well as in the order of nucleotides. An alignment of the sequences as well as the detection of overlapping DNA sections for the design of universal primers is not possible.
3. Coding sequences seem to display the only option of finding universal barcoding sequences for this large botanical variety of tropical timber species.

Sequencing: After sequencing diverse coding cpDNA regions using reference material provided by project partners (cambium, herbarium dried leaf material) and comparing the sequencing results with (partly) available data from the NCBI data bank, the *rbcL* gene showed the best features for primer design in order to develop barcoding sequences. This fragment is 575 basepairs long and contains, besides some indels of only 1 bp., 95 SNPs (single nucleotide poplymorphisms), a part of them species or genera specific, others family or order specific (see figue 1).

Primer design: When comparing all sequence sites, we found that the alignment revealed no region with sufficient consensus to accommodate a unique single oligonucleotide for use as primer although this fragment is one of the most conservative regions within the cpDNA genome. In all cases, one to three nucleotides were mismatched. When designing primers for this region, we introduced a degenerate site, or "wobble", to compensate for the variability in the target sequence (see figure 1).

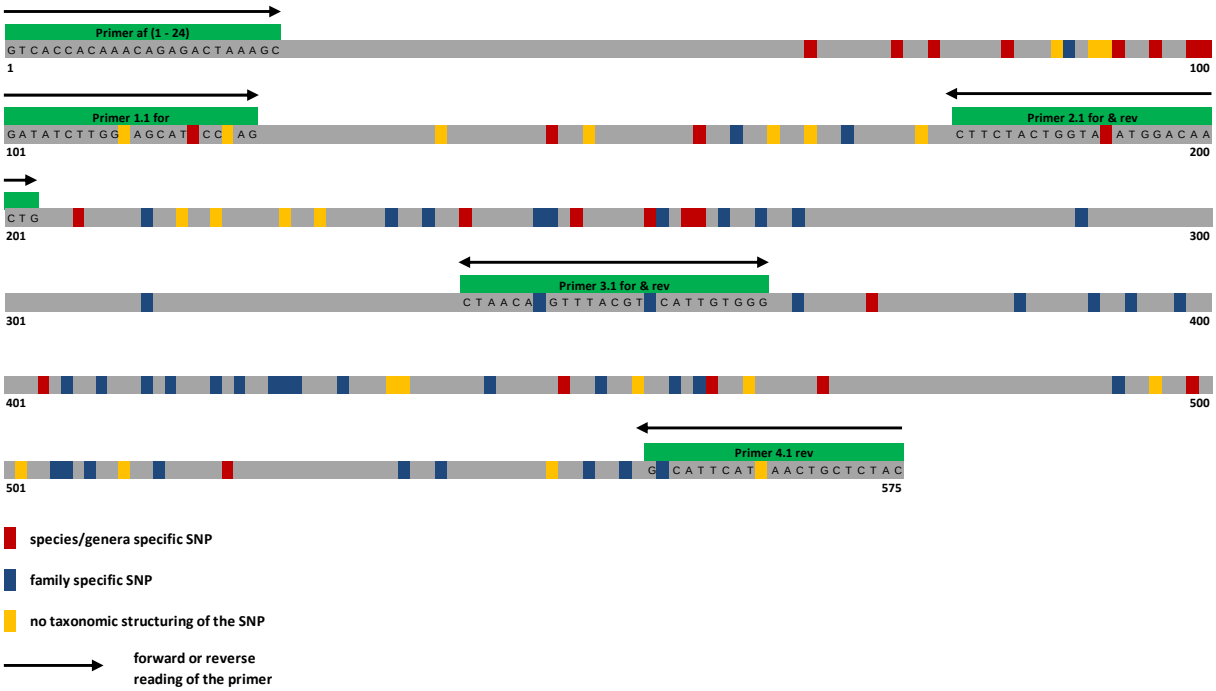

Figure 1: Used cpDNA barcoding sequence (*rbc*L) for differentiating the 22 tropical timber tree species, the forward and reverse primers and the specifity of the detectes SNPs (species/genera or family specific SNPs or SNPs with no taxonomic structuring)

Results and conclusions from the timber sample blind test

95 SNPs (single nucleotide polymorphisms) have been detected within this cpDNA fragment between the different taxonomic species, genera, families and orders (see figure 1). The results of applying this fragment in a blind test on 50 timber samples are shown in table 1.

As positive cases we declined the blind test timber samples for which we could identify the right species or genera. In most of these cases we stopped our identification on the genera level, because most of the listed genera consist of many species world wide for which we have no reference material or which could not been differentiated by the chosen cpDNA marker (see G2S_S_1.0, for example). In many cases there were also no or too bad PCR amplification products for interpretation (no results from the lab) or even wrong interpretation of the outcome of the genetic analysis. Altogether, it turned out to be difficult to apply DNA techniques for the differentiation between taxonomically very distant species, e.g. species belonging to different families or even different taxonomical orders. Due to this high variability of the DNA between taxonomically distant species, the design of well functioning consensus primers requires the introduction of “wobbles”. These might have caused the problems in amplifying the identified barcoding fragments, particularly of the highly degraded DNA. Further, there was no chance to analyse further cpDNA sequences because there was no overlapping information making the outcome of alignment procedures impossible to interpret. For taxonomically distant families and orders it is much more effective to apply morphological methods as used by Gerald Koch.

But a very interesting and useful application of DNA techniques should be considered in cases, in which morphological methods are not sufficient to differentiate between different species. This is the case for very closely related species and has already successfully tested in previous genetic projects (see DNA-based identification of mahogany species [*Swietenia*] Höltken et al. 2011). Here we can circumvent the above mentioned “alignment” problems of the DNA sequences. A claim from the morphological survey/expertise for the genera or the family in the first instance would be and then we can detect the species name with genera or family specific molecular methods. In the case of differentiating closely related tree species we are able to use intergenic sequences *(psb*A-*trn*H, *mat*K-

*trn*H etc.). Further, for species groups that have been studies by next generation sequencing techniques (see RAD-sequencing of *Entandrophragma*) and for which thousands of SNPs have been developed, the MassARRAY Techonology (Agena Bioscience)can be applied for a much more reliable species determination. Further, this new technique requires low DNA quantities after extraction, so that we could differentiate even between species of the *Entandrophragma* genus in this study (see samples G2S_S_5.0, RM_2014_48). Further candidates would be species of the genera *Afzelia*, *Erythrophleum*, *Khaya*, *Milicia* etc. requiring studies on species differentiation.

Table 1: Results of the DNA-barcoding blind test on tropical timber samples

Sample code	Claims on species names	True species names	Lab results		Comment
G2S_S_1.0	*Guibourtia ehie.*	*Afzelia africana*	Afzelia		identified genus right
G2S_S_1.5	*Baillonella toxisperma*	*Afzelia spp*		Pterocarpus	wrong
G2S_S_2.0	*Khaya anthotheca*	*Khaya ivorensis*		Guibourtia	wrong
G2S_S_3.5	*Baillonella toxisperma*	*Baillonella toxisperma*		no reference	wrong
G2S_S_5.0	*Entandrophragma*	*Entandrophragma angolense*	E. angolense		identified species right
G2S_S_8.0	*Entandrophragma candollei*	*Entandrophragma utile*		Guibourtsia	wrong
G2S_S_8.5	*Entandrophragma*	*Entandrophragma utile*	X	X	no results from the lab
G2S_S_10.0	*Milicia excelsa*	*Erythrophleum suaveolens*		Guibourtsia	wrong
G2S_S_11.5	*Guibourtia spp.*	*Guibourtia spp.*	Guibourtsia		identified genus right
G2S_S_13.0	*Lophira alata*	*Lophira alata*		no reference	wrong
G2S_S_13.5	*Lophira alata*	*Lophira alata*	X	X	no results from the lab
G2S_S_14.0	*Erythrophleum suaveolens*	*Milicia excelsa*	X	X	no results from the lab
G2S_S_15.0	*Milicia regia*	*Milicia regia*	Milicia		identified genus right
G2S_S_16.5	*Millettia laurentii*	*Millettia laurentii*	X	X	no results from the lab
G2S_S_18.5	*Khaya spp*	*Pericopsis elata*	X	X	no results from the lab
G2S_S_20.0	*Terminalia superba*	*Terminalia superba*	Terminalia		identified genus right
G2S_S_21.5	*Pterocarpus soyauxii*	*Pterocarpus soyauxii*		Malvaeceae family	wrong
G2S_S_24.0	*Triplochiton scleroxylon*	*Triplochiton scleroxylon*	*Triplochiton*		identified genus right
G2S_S_25.5	*Entandrophragma utile*	*Mansonia altissima*	no reference		exclusion right
G2S_S_30.5	*Triplochiton scleroxylon*	*Sterculia rhinopetala*		*Triplochiton*	wrong
G2S_S_33.5	*Erythrophleum ivorense*	*Lovoa trichiloides*		Pterocarpus	wrong
G2S_S_35.5	*Afzelia spp*	*Afzelia spp*		Erythrophleum ssp.	wrong
G2S_S_38.5	*Nauclea diderrichii*	*Nauclea diderrichii*	X	X	no results from the lab
G2S_S_41.5	*Aningeria robusta*	*Mansonia altissima*		Cyclodiscus	wrong
G2S_S_47.5	*Cylicodiscus gabunensis*	*Cylicodiscus gabunensis*	*Cylicodiscus*		identified species right
RM_2014_03	*Milicia excelsa*	*Millettia laurentii*	X	X	no results from the lab
RM_2014_04	*Erythrophleum ivorense*	*Erythrophleum suaveolens*	Erythrophleum ssp.		identified genus right
RM_2014_13	*Khaya ivorensis*	*Khaya anthotheca*	X	X	no results from the lab
RM_2014_37	*Erythrophleum suoveolens*	*Erythrophleum ivorense*	X	X	no results from the lab
RM_2014_39	*Entandrophragma utile*	*Aucoumea kleineana*	Aucoumea		identified genus right
RM_2014_42	*Entandrophragma angolense*	*Nauclea diderrichii*	Nauclea		identified genus right
RM_2014_45	*Afzelia pachyloba*	*Afzelia bipindensis*	Afzelia		identified genus right
RM_2014_48	*Entandrophragma cylindricum*	*Entandrophragma angolense*	E. angolense		identified species right
RM_2014_49	*Aningeria robusta*	*Baillonella toxisperma*	Sapotaceae family		identified family right
RM_2014_59	*Aucoumea klaineana*	*Afzelia pachyloba*	Afzelia		identified genus right
RM_2014_60	*Cyclodiscus gabunensis*	*Entandrophragma utile*	X	X	no results from the lab
X2-57	*Ptercarpus soyauxii*	*Pericopsis elata*	X	X	no results from the lab
X2-58	*Baillonella toxisperma*	*Aningeria robusta*	X	X	no results from the lab
X2-59	*Afzelia bipindensis*	*Afzelia africana*	X	X	no results from the lab
X2-65	*Guibourtia ehie.*	*Guibourtia tessmanii*		Nauclea	wrong
X2-66	*Millettia laurentii*	*Millicia excelsa*	X	X	no results from the lab
X2-67	*Khaya grandiflora*	*Khaya ivorensis*	X	X	no results from the lab
X2-68	*Milicia regia*	*Milicia excelsa*	X	X	no results from the lab
X2-69	*Terminalia superba*	*Terminalia superba*	Terminalia superba		identified species right
X2-74	*Pericopsis elata*	*Pterocarpus soyauxii*	X	X	no results from the lab
X2-75	*Nauclea diderrichii*	*Aucoumea kleineana*	X	X	no results from the lab
X2-76	*Khaya ivorensis*	*Entandrophragma cylindricum*	X	X	no results from the lab
X2-78	*Triplochyton scleroxylon*	*Triplochyton scleroxylon*	X	X	no results from the lab
X2-79	*Pericopsis elata*	*Cylicodiscus gabunensis*	X	X	no results from the lab
X2-81	*Lophira alata*	*Lophira alata*	X	X	no results from the lab

Nevertheless, on fresh material (leaf, cambium, bud tissues) this chosen *rbc*L fragment turned out to differentiate many tropical timber species very effectively. It can be used as a quick-check procedure improving the true species´ identity of tropical tree sample collections (quality management system).

Literature

HÖLTKEN A.M., SCHRÖDER H., WISCHNEWSKI N., MAGEL E. & FLADUNG M. (2012): Development of DNA-based methods to identify CITES-protected timber species: A case study in the Meliaceae family. *Holzforschung* 66: 97-104.

ANNEX 5

Technical reports on the stable isotopes analysis

Final report ITTO Africa Project - Technical report on the stable isotopes analysis for sapelli

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1 Materials and methods

1.1 Sample receipt and preparation

Reference and blind test samples (n=210 and n=20 respectively) of Sapele (*Entandrophragma cylindricum)* were received at FERA (the Food and Environment Research Agency, UK) and logged into the laboratory information management system, (LIMS) where they were each assigned a unique identifier. Reference samples, as well as 10 of the blind test samples consisted of timber shavings of inner cambium , as technical difficulties meant collectors could not take timber cores from many of the sampling locations. For the reference samples, collection teams took information on timber genus and GPS co-ordinates to aid digital mapping processes (ARC-GIS).

Half of the blind test samples (n=10) consisted of offcuts of sawn timber. For these samples, a hand drill was used to extract powder across the growth rings. Freeze-drying of the timber shavings and powder from the blind test samples was carried out in order to remove any excess moisture and to aid cellulose purification. Following freeze-drying, reference samples (including half the blind test samples) were broken into small pieces enabling transfer in small amounts to an IKA handheld analytical mill where they were pulverised to a fine powder.

1.1.1 Cellulose purification

To reduce the influence of tree morphology (lignin:cellulose ratio) upon the isotope distribution of the raw timber, isotope measurements were standardised by performing the analysis of $\delta^{2}H‰$, $\delta^{13}C‰$ and $\delta^{18}O‰$ isotopic ratios on the isolated alpha cellulose. For this, the method by Brendel *et al.* (2000) was applied in the preparation of the samples.

1.2 Analysis of carbon isotopes in cellulose

Following cellulose purification, 1 mg of cellulose were weighed in duplicate into tin capsules (3.5 x 5 mm i.d.) and sealed before transfer into a 96 position sample tray prior to analysis. Where analysis did not take place on the same day, sample trays were stored in a desiccator.Sealed tin capsules containing the purified cellulose and standards were placed in the autosampler of the elemental analyser (Fisons, Milan, Italy) and purged with helium, before dropping into a vertical quartz tube maintained at a temperature of 1020 °C (Kelly *et al*., 2006). The carrier gas stream was temporarily enriched with oxygen and the sample and tin capsule oxidised in a 'flash' combustion reaction. Quantitative combustion was achieved by passing the gas mixture over two catalyst layers of chromium oxide and silvered copper or cobaltous oxide (Isoprime, Cheadle, UK). The combustion gases were passed first over elemental copper at a temperature of 650 °C to remove residual oxygen and second through a chromatographic column (Porapak PQS, SS, 2 m, 6 x 5 mm) heated at 35 °C. Residual Water was removed from the gas stream before entering the IRMS by a trap containing anhydrous magnesium perchlorate. During the measurement a portion of the effluent from the elemental analyser (ca. 0.5 ml/min) was transferred into the IRMS (Isoprime, Cheadle, UK) using helium carrier gas (ca. 85 mL/min). The signal from ions at $^{m}/_{z}$ 44, $^{m}/_{z}$ 45 and $^{m}/_{z}$ 46 for CO_2 were monitored. The $\delta^{13}C$ values ([‰] vs V-PDB) of the samples were determined by calibration against certified reference materials, IAEA CH3 cellulose and IAEA CH6 sucrose. The IAEA Cellulose and sucrose standards had $\delta^{13}C$ values of -24.72‰ and -10.38‰ respectively, which allowed for a stretch correction calculation to be applied to the sample data.

1.3 Analysis of hydrogen and oxygen isotopes in cellulose

Following cellulose purification, 1 mg of cellulose were weighed in quadruplicate into tin capsules (3.5 x 5 mm i.d.) and sealed before transfer into a 96 position sample tray prior to analysis. Sealed tin capsules containing purified cellulose and standards were placed in the autosampler of a Pyrocube elemental analyser (Elementar, Hanau, Germany), purged with helium, and dropped into a vertical glassy carbon tube with quartz liner maintained at a temperature of 1450°C. The products of thermal decomposition (gaseous H_2 and CO) were passed through a water trap containing sodium hydroxide/phosphorus pentoxide to remove residual moisture. A carbon monoxide trap retained CO while enabling free transfer of H_2 into the IRMS for measurement. Following H_2 analysis the CO trap was heated rapidly, transferring desorbed CO into the IRMS for measurement. During the measurement a portion of the effluent from the elemental analyser (ca. 0.5 ml/min) was transferred into the IRMS (Isoprime, Cheadle, UK) using helium carrier gas (ca. 135 mL/min). The signal from ions at $^{m}/_{z}$ 2, $^{m}/_{z}$ 3 for H_2 and $^{m}/_{z}$ 28, $^{m}/_{z}$ 30 for CO were monitored. The lack of suitable matrix matched certified standards posed a challenge for hydrogen measurements. Ratios of $^{2}H/^{1}H$, the $\delta^{2}H$ values ([‰] vs V-SMOW) were determined by a applying a single point calibration with certified reference material IAEA CH7 Polyethelyne foil ($\delta^{2}H$‰ -100.3‰). $\delta^{18}O$ values were determined by calibration against certified reference materials of benzoic acid, IAEA 601 and 602 ($\delta^{18}O$‰ +23.3‰ and +71.4‰). The benzoic acid standards have very different values, thus allowing for a stretch correction calculation to be applied to the data.

1.4 Blind test samples

Hypothesis testing of the blind test samples was performed using chi-square statistical analysis of the CHO isotopes in the following steps:

1.4.1 For each of the principal components, a local regression model (“loess” as implemented in R version 3.0.2 for Windows) was fitted so that it predicts the isotope ratio from the geographical coordinates (including a latitude:longitude interaction). The smoothing (bandwidth) parameter (referred to as “span” in loess terminology”) was optimized using leave-one-out cross validation.

1.4.2 Chi-square statistics were calculated based on the difference between the actual value of the principal component and the value predicted by the model. For the reference data, the predicted value was based on leave-one-out cross validation while for the blind test data, the prediction was based on the nearest point in a 101*101 grid covering the geographical coordinates of the reference data. This was necessary because “loess” can’t always produce predictions for an irregular set of coordinates (such as the reference data).

1.4.3 The Chi-square statistics for the blind test data were cut off at the empirical 95 percentile of the chi-square statistic for the reference data. This corresponded roughly to the 97% percentile of the theoretical chi-square distribution, indicating that the residuals are slightly more heavy-tailed than the normal distribution.

2 Results of the reference and blind test data

2.1 Reference samples

The stable isotope ratios of cellulose from timber samples originating from different geographical origins were determined by IRMS. Initially, the stable isotope data was processed by CDA to determine if data could provide sufficient discrimination between the respective countries of origin. The multivariate model achieved a correct classification rate of 60%, with ^{18}O and ^{13}C providing the most discrimination. Fig. 1 shows a cross plot of functions derived from the multivariate model using the CHO stable isotopes variables used in the country of origin assignments.

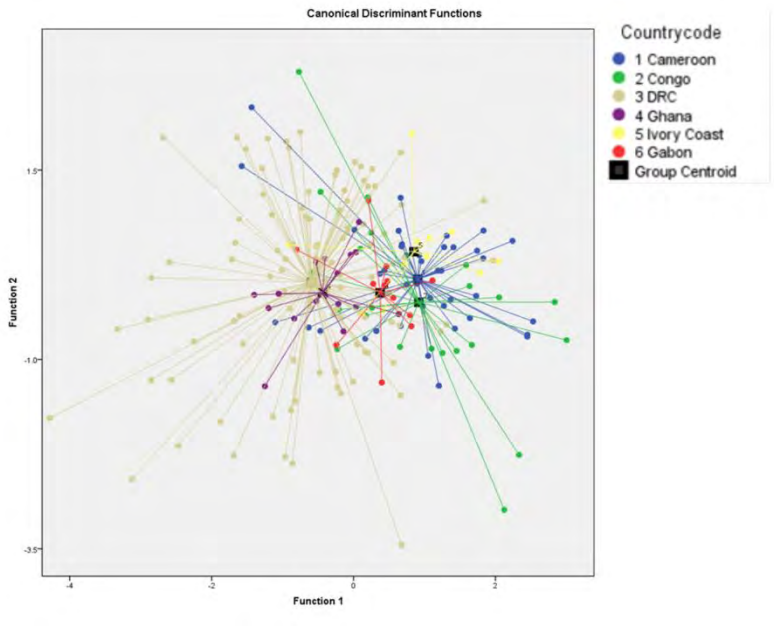

Fig. 1 Functions derived from the statistical analysis of stable isotope variables (^{18}O, ^{13}C and ^{2}H) for the respective country groups.

2.2 Results of the blind test exercise

2.2.1 Claims from blind test

For the country level claims of origin, the chi-squares statistical analysis of the CHO isotope data, acquired from the analysis of reference and blind test samples, was not able to reject the hypothesis that the claims of county origin for the timber were incorrect (Table 1.1). For regional level claims of origin, the chi squares analysis rejected the labelling claim of four of the twenty samples: BT_2014_573, BT_2014_557, G2S_O_E6, G2S_O_E22.

Table 1.1 FERA's country and regional claim for the blind test samples.

ID-number	species	declared country of origin	declared region of origin [GPS and Radius]	Y/N result concerning the declaration of origin: COUNTRY	Y/N result concerning the declaration of origin: REGION
BT_2014_552	Sapelli	DRC	N0.45; E25.8 (200 km Radius)	correct	correct
BT_2014_577	Sapelli	Cameroon	N2.8,E12 (40 km Radius)	correct	correct
BT_2014_510	Sapelli	Ghana	N6.4; **W**1.2 (70 km Radius)	correct	correct
BT_2014_592	Sapelli	Congo Brazzaville	N0.51544; E16.72308 (120 km Radius)	correct	correct
BT_2014_573	Sapelli	DRC	S4.77; E16.9 (120 km Radius)	correct	incorrect
BT_2014_557	Sapelli	DRC	**S**4; E19 (300 km Radius)	correct	incorrect
BT_2014_580	Sapelli	Cameroon	N3.04; E14.5 (90 km Radius)	correct	correct
BT_2014_525	Sapelli	Ghana	N6.3; E0 (75 km Radius)	correct	correct
BT_2014_534	Sapelli	Kongo Brazzaville	**S**3; E15 (100 km Radius)	correct	correct
BT_2014_522	Sapelli	DRC	N1.55115; E21.07416 (300 km Radius)	correct	correct
G2S_O_E4	Sapelli	Ghana		correct	N/A
G2S_O_E6	Sapelli	DRC	South - west (Bandundu province)	correct	incorrect
G2S_O_E8	Sapelli	Cameroon	South (Sangmelima)	correct	correct
G2S_O_E11	Sapelli	Congo	North Region	correct	correct
G2S_O_E13	Sapelli	Congo	North Region	correct	correct
G2S_O_E14	Sapelli	Congo	North Region	correct	correct
G2S_O_E18	Sapelli	DRC	North-West (Equateur province)	correct	correct
G2S_O_E20	Sapelli	DRC	North-West (Equateur province)	correct	correct
G2S_O_E22	Sapelli	DRC	South-West (bandundu province)	correct	incorrect
G2S_O_E24	Sapelli	DRC	North East region (orientale region, Kisangan	correct	correct

2.2.2 Outcome of blind test

Following submission of the blind test results to the WWF and G2S, the real country and regional origin of the samples were revealed enabling assessment of FERA's performance in the blind test exercise (Table 1.2)

Table 1.2 FERA's performance in blind test exercise.

Laboratory	wood species	Blind test partner	Evaluation in blind test (Country claim)			Total samples able to be analysed in blind test (%)	Success rate in blind test (%)	Adjusted success rate in blind test (samples not-analysed excluded) from analysis) (%)
			Right	Wrong	No Answer			
FERA	*Entandrophragma cylindricum* (Sapele)	WWF	7	3	0	100	80	80
		G2S	9	1	0			

3 Conclusions

Canonical discriminant analysis of data acquired from the analysis of $\delta^{2}H$, $\delta^{13}C$ and $\delta^{18}O$ stable isotope ratios in cellulose enabled a 60% country classification rate. The viability of these data to achieve a high degree of country of origin discrimination was promising, and therefore deemed suitable to undergo assessment in the blind test exercise. As shown in Table 1.2, FERA was able to analyse 100% (20/20) of the samples supplied in the blind test, and achieved a 70% success rate for the country claims on the blind test samples supplied by WWF (wood shavings). For the samples received from G2S (sawn timber), a 90% success rate was achieved. The higher performance achieved with the G2S samples may be linked to the presence of a greater number of growth rings. The inclusion of additional isotopes in the statistical model such as ^{15}N, ^{34}S and ^{87}Sr may improve the resolution and predictive power, as may the use of better matrix and delta matched and standards for the calibration of hydrogen data.

Final report ITTO Africa Project - Technical report on the stable isotopes analysis for Iroko

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Background

The stable isotope method is one of the most promising tools in forensic science as it offers the opportunity to verify the authenticity of many materials [Meier-Augenstein 2010]. Today there is a wide array of publications available about the use of stable isotope analysis to determine the provenance of various products such as: wheat, rice, olive oil and animal products such as milk and beef [Kelly 2005]. Stable isotope analysis is an established method to study animal migration [Hobson 1999] and is used in criminal investigations where hair samples are used to predict the provenance of perpetrators [Ehleringer 2008].

The most predominant use of stable isotope analysis is within the food sector where it is regularly used to re-trace and verify the origin of food. The stable isotope method has been incorporated into European regulation: 2729/2000 as the method to authenticate the origin of wine and control mislabelling. Considering that stable isotope analysis is a well-established and robust method widely used in traceability, it is surprising that that it has not yet been applied in the timber industry even though its basic scientific principles dealing with the correlation of cellulose and water were published in the mid-70s [Epstein 1977].

Water plays an essential role in stable isotope traceability. The mean isotopic ratios of hydrogen and oxygen in precipitation are primarily dependent on the annual temperature of specific locations [Dansgaard 1964] and secondarily on other less influential factors such as altitude, latitude and the continental effect [Araguas 2000]. Consequently there is great variability in isotopic patterns in groundwater geographically [Bowen 2002]. Hydrogen and oxygen isotopes in tree cellulose reflect the isotopic patterns observed in groundwater with some modifications, the signature is primarily influenced by the evaporation effects of water [Flanagan 1991a] and secondarily by biochemical fractionation in the anabolism of cellulose [Sternberg 1986, Luo 1992] e.g. with a $\delta^{18}O$ shift of approximately +27‰. The result is that different shifts of $\delta^{18}O$ and δH are observable in tree celluloses. Nevertheless, correlation of the two ratios is still applicable and is implemented in paleoclimatic studies [Burk 1981, Yapp 1982]. Furthermore a progress could be an analysis of lignin methoxy group to achieve a higher correlation with the groundwater [Keppler 2007].

The ratio of carbon isotopes in timber is primarily dictated by the photosynthetic pathway the plant uses [O'Leary 1988], this aspect is not geographically distinct therefore it is not relevant in terms of tracking the origin of timber. On the other hand, there is a strong fractionation in carbon ratios that is dependent on stomata conductance and photosynthetic assimilation [Farquhar 1982]; both are influenced by environmental factors such as humidity, light and temperature. Therefore the carbon ratio in timber reflects the local climate of the area in which it grew and is suitable as an additional parameter to add resolution to provenancing using stable isotopes. This has been utilised where measurements of carbon ratios in tree rings were used as a code in conjunction with climate data to decipher the origin of the wood with a resolution of 114-304km [Kagawa & Leavitt 2009]. However, this application of the stable isotope method is costly and time-consuming.

Another strategy is to analyse the average isotopic ratio of carbon in many tree rings with an adapted sampling and preparation procedure to include additional stable isotopic parameters. Historically, sulphur [Thode 1991] and strontium [Capo 1998] have been used as further parameters to decipher the geographical origin of wood.

The stable isotopes of the bio-elements show the highest fractionation effects in nature with respect to their light mass versions. Natural fractionation systems, such as the global water cycle, produce geographically distinct patterns. With the exception of Strontium, stable isotopes of the heavy elements have no relevance in provenancing. Heavy strontium ^{87}Sr is formed in nature by the radioactive decay of the long-lived rubidium isotope ^{87}Rb. As a result, ^{87}Sr can also reflects the age of the geology. Besides this, geological parameters such as ^{87}Sr can provide further information about product adulteration and geographical provenance [Rummel 2010, Voerkelius 2010].

Ratios of $\delta^{15}N$ in agricultural soils are indicative of the fertilisation method used [Bateman 2007] which result in positive stable isotope ratios, these are notably high in soils fertilised using organic fertilisers such as manure. Positive nitrogen ratios in soil and wood are indicative of forest influenced by fertilisers. On the other hand natural forest tends to have very depleted nitrogen isotope ratios which can be as low as -6‰ [Yoneyama 1990] due to the impact of nitrogen fallout.

The resolution of the origin is increased when combinations of isotope signatures are used, this has been demonstrated in the tracking of larch wood [Horacek 2009] where $\delta^{18}O$ and $\delta^{13}C$ were used to discriminate Siberian from European larch. In a ground-breaking study from 2008 to 2010, six stable isotopes (COHNS and Sr) were used to build a database Tectonia grandis (teak) and Mahogany genus Swietenia (consisting of S. macrophylla, S.mahagoni and S. humilis) species with over 1,000 samples from 18 different countries [Boner 2011]. The developed database was independently tested by WWF using blind test samples (origin and species of samples not declared) to confirm the reliability of the application. 13 out 15 samples were addressed correctly with respect to provenance.

In timber, the stable isotope method is most accurate when used in conjunction additional parameters such as genetics. This was demonstrated in the German GIZ project (GIZ 2010) where samples of Iroko and Sapelli species from Cameroon were mapped using the stable isotopes of the bio elements (COHNS) and genetic parameters, and tested against blind test samples. Alone, the stable isotope method addressed 3 samples incorrectly from 16. In combination with genetic parameters, only 1 sample was addressed incorrectly with both methods.

Material and Methods

Mostly recommended for check of the origin are the stable isotopes of the light bioelements (COHNS): hydrogen, oxygen, carbon, nitrogen, sulphur. Additional the higher isotope of strontium was included in the project as well.

Sample preparation:

Regularly the wood chips are milled into a fine powder using a ball-mill apparatus. After that the powder is extracted in a soxhlet apparatus over 6 hours with methylene chloride and methanol. The powder is dried in a laboratory-type drying cabinet for at least 1 hour.

Finally the samples are stored in air tight sample vials and could be weight in for analysis.

To avoid any equilibration or humidity effect the weight in samples for oxygen and hydrogen analysis are equilibrated overnight in a desiccators with a defined humidity of 10.6 %. Afterwards the samples are vacuum dried for at least 2 hours.

Furthermore the strontium needs an additional preparation. Round about 2 to 4g of wood powder are burned in a combustion furnace at a temperature of 750°C. The ash is transferred in a micro wave heater with round about 10ml nitric acid and 2ml hydrogen peroxide. Digestion temperature: >180°C for >15min. Depending on the samples a purification is necessary to avoid isobaric influence. Therefore purification with Sr resin (Sr-C20-A, Eichrom) is performed.

Equipment:

1. **D/H, $^{18}O/^{16}O$ measurement:**
   The high temperature application uses HT-PyrOH with silicium carbide tube (Hekatech) filled with glassy carbon chips and coal powder. Working temperature for pyrolysis of >1550°C. To gain a higher precision the isotopes are measured in a master / slave configuration with two IRMS (Isoprime, Elementar). Each IRMS is only measuring one isotope: D/H or $^{18}O/^{16}O$
2. **$^{13}C/^{12}C$ measurement**
   EA (Carlor Erba, NA1500) in combination with IRMS (Horizon, NU-Instruments). Working temperature: 1021°C (oxidation), 600°C (reduction)
3. $^{15}N/^{14}N$ measurement
   EA (Carlor Erba, NA1500) in combination with IRMS (Horizon, NU-Instruments). A further addition of a packed column for CO separation is used to get rid of isobaric effect. Working temperature: 1021°C (oxidation), 600°C (reduction)
4. **$^{34}S/^{32}S$ measurement:**
   EA (Hekatech) with IRMS (Optima, VG Instruments). A one tube combustion (oxidation and reduction in one tube) is used to solve any SO3 problem. Furthermore combustion water is directly trapped with magnesium perchlorate at the end of the tube. Working temperature: 1000°C.
5. **$^{87}Sr/^{86}SR$:** ICP-MS (Elan 6100) was used. For correction the isotopic standard: NIST SRM 987 was used.

Results

The German isotopic laboratory Agroisolab was responsible for development of the iroko (Milicia excelsa) database. In total 474 reference samples from seven African countries (Cameroon, Democratic Republik of Congo, Gabon, Ghana, Ivory Coast, Kenya and the Republic of the Congo) were analyzed in the project time. Most of the results will be included in the international GTTN database.

Regularly the water isotopes (D/H and $^{18}O/^{16}O$) represent the main differentiation parameter for origin check. Unfortunately the mapped countries are more or less on the equator line so it could be expected that water isotopes are in tendency very similar. Therefore reference samples from the

costal countries Cameroon, Ghana, Gabon and the Republic of Congo show similar $^{18}O/^{16}O$ and D/H ratios in range of +23.8 to +24.3 ‰ and -48 to -51 ‰ respectively. In contrast the continental country Democratic Republic of Congo shows a tendency to increased $^{18}O/^{16}O$ and D/H ratios of +25,1 ‰ ($^{18}O/^{16}O$) and -45,4 ‰ (D/H). Nevertheless the most increased ratios could be detected in Kenya with an average $^{18}O/^{16}O$ ratio of 26.3 ‰ and an average D/H ratio of -40.4 ‰. These significant increased ratios are in well agreement with the known stable isotopic water situation in these regions [Bowen 2012].

Because of the similarities of the water isotopes the geological stable isotopes ($^{34}S/^{32}S$, $^{15}N/^{14}N$, $^{87}Sr/^{86}Sr$ are getting more relevant for discrimination. Therefore the sulphur ratios are useful to distinguish samples from Ghana to Gabon or Cameroon (figure 2).

Only the combination of all six stable isotopes enables a differentiation of various countries (figure 3). Still yet timber from Gabon and RCB are hardly to discriminate and have broad overlapping with several countries. Therefore further parameters are needed to improve the discrimination. A solution is still the combination of stable isotopes with genetic, rare elements or near infrared data.

Figure 1: Isoscapes of the $^{18}O/^{16}O$ ratios in timber (Milicia excelsa)

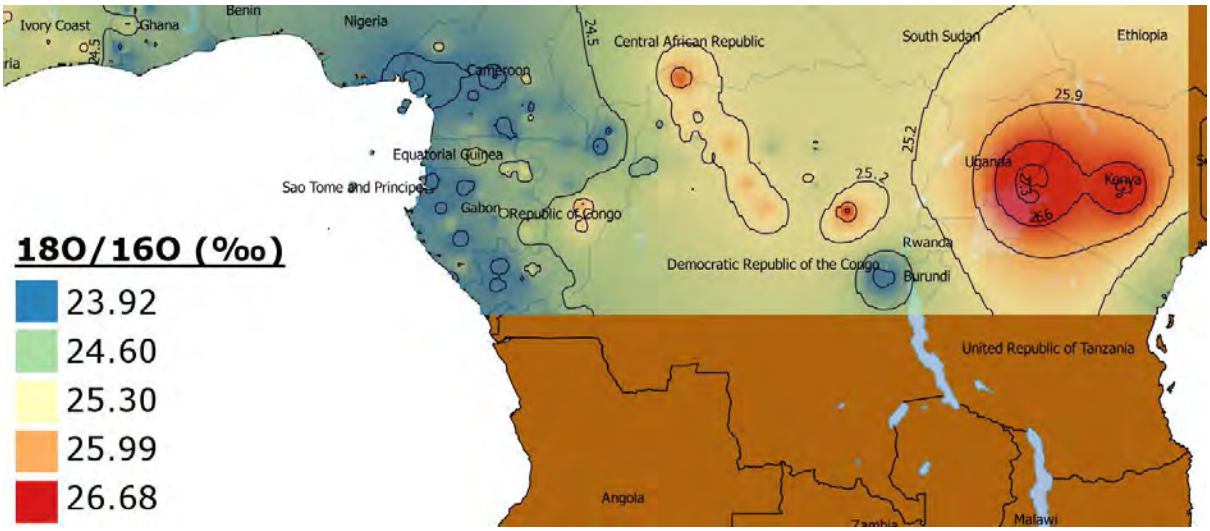


Figure 2: Boxplots of the stable isotopic sulphur ratios of 7 African countries

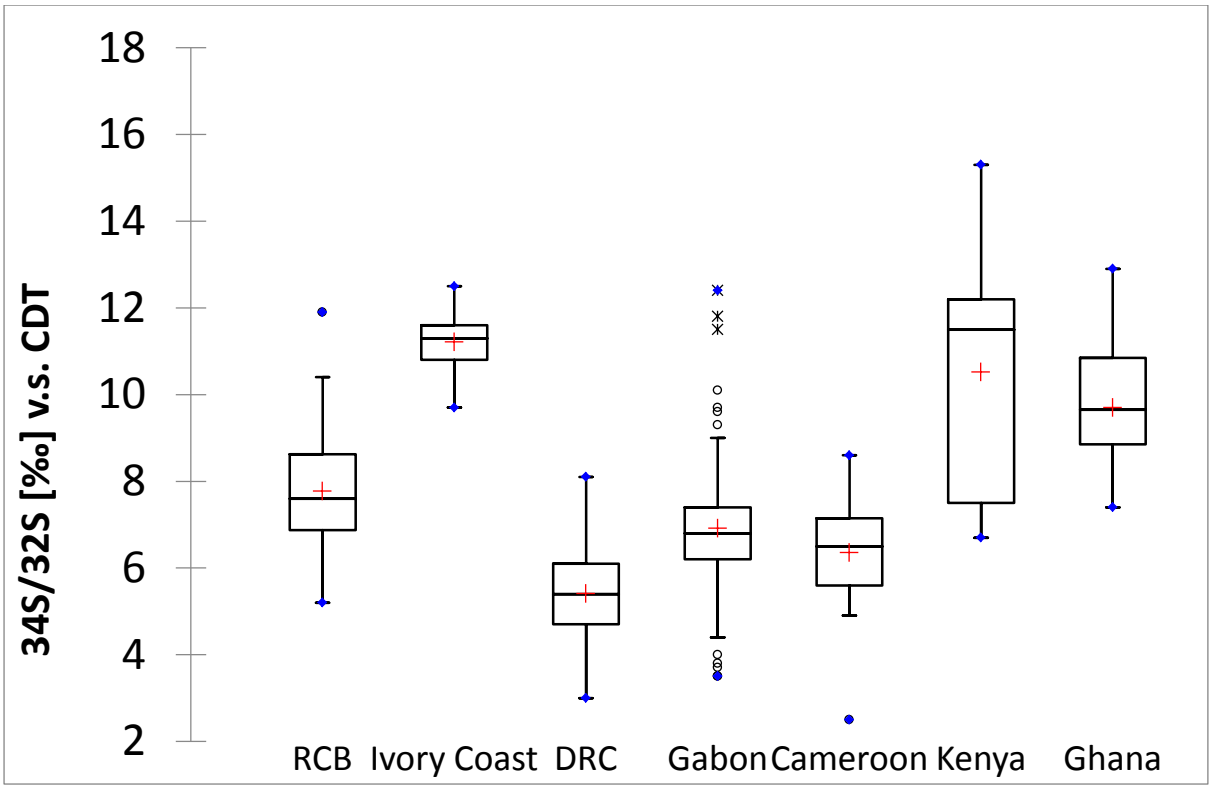
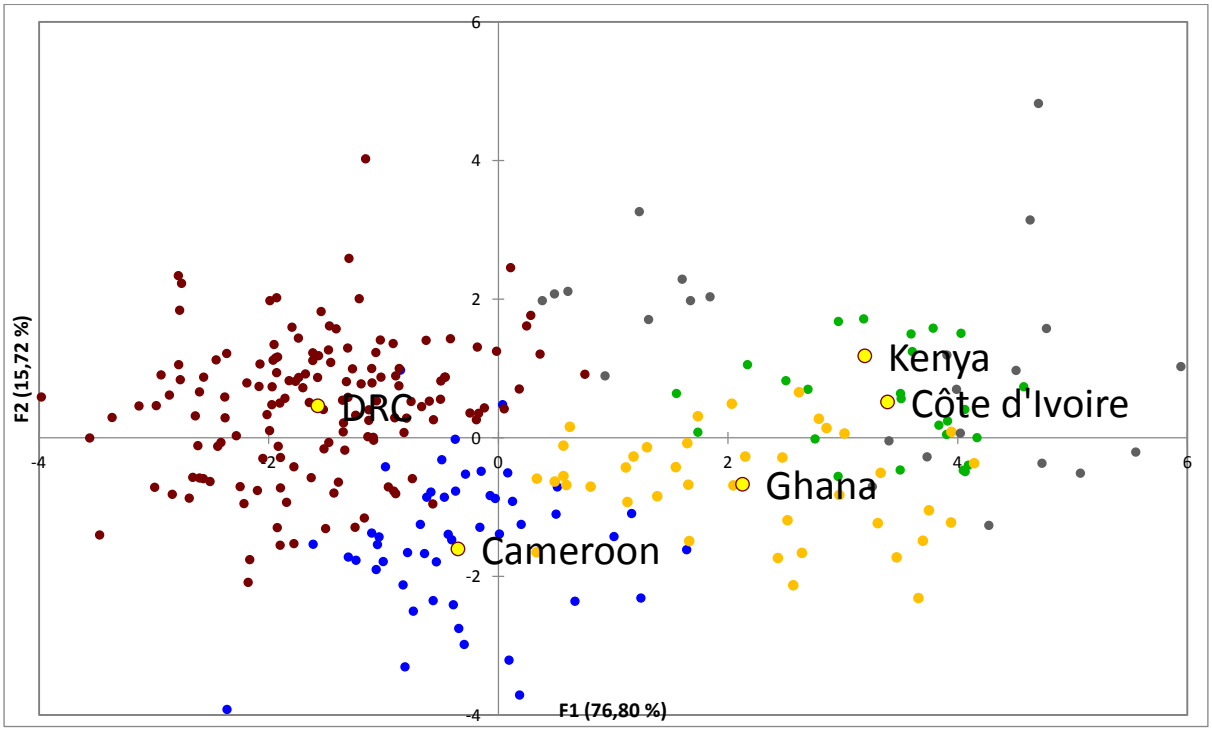


Figure 3: Discriminate analysis (DA) of 5 using stable isotope of biolements (COHNS) and Strontium



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Final report ITTO Africa Project - Technical report on the stable isotopes analysis for ayous

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Materials and Methods

Sample preparation:
Sample preparation started with close inspection and cleaning of the samples. Afterwards they were homogenized in a ball mill (Retsch MM250, Haan, Germany) and weighed into tin (for carbon and nitrogen analyses) and silver (for hydrogen and oxygen analyses) capsules. Before measurement the silver capsules were stored in a desiccator for equilibration. For sulphur isotope measurement a sulphur pre-concentration step had to be performed. The so processed samples were then afterwards also weighed into tin capsules.

Measurements:
For hydrogen and oxygen measurements the silver capsules were introduced into the autosampler of a high-temperature elemental analyser (Flash HT, Thermo Scientific, Bremen, Germany). Pyrolysis at 1450°C converts the samples into gaseous state and a continuous helium flow transports the gas via a ConFLO (Thermo Scientific) to the Delta V isotope ratio mass spectrometer (Thermo Scientific). There the samples are analysed for the isotope composition.

Fig. 1: EA - IRMS (Thermo Scientific)

For carbon, nitrogen and sulphur isotope measurements the tin capsules were introduced into an elemental analyser (EA) via the autosampler. In contrast to the hydrogen and oxygen measurement a thermal combustion (at T=1020°C) takes place in the EA. The further procedure is identical with the procedure for hydrogen and oxygen isotope measurements.

All results are expressed in the conventional δ notation in permil [‰] versus international standards: V-SMOW (Vienna Standard Mean Ocean Water) for hydrogen and oxygen, V-PDB (Vienna Pee Dee Belemnite) for carbon, N_{air} for nitrogen and V-CDT (Vienna Canyon Diabolo Troilite) for sulphur isotope measurements. The results are calibrated against certified standards. For carbon a protein standard (IVA) is used (δC13= -26,98‰) and hydrogen and oxygen results are calibrated against a cellulose standard IAEA CH-3 (δ18O=32,5‰, δ2H=-35,5‰). The maximum standard deviation is about ± 0,2‰ for carbon and nitrogen, ± 3‰ for hydrogen and ± 0,3‰ for oxygen and sulphur isotope ratios.

Results

Carbon isotope ratios: For carbon isotopes the average as well as the range are rather similar for all of the countries. For Cameroon the δC13 values range from -30,3 to -26,5‰, for the Republic of Congo from -31,3 to -28,1‰, for Cote d'Ivoire from -30,5 to -26,5‰, for the Democratic Republic of Congo from -33,3 to -27,9‰ and for Ghana from -29,3 to -26,77‰.

Hydrogen isotope ratios: The δH2 values for Cameroon, the Republic of Congo, Cote d'Ivoire, the Democratic Republic of Congo and Ghana are between -54 and -32‰, -51 and -32‰, -55 and -34‰, -47 and -18‰ and between -56 and -30‰, respectively.

Oxygen isotope ratios: Oxygen isotopes show values between 22,5 and 25,3‰ for Cameroon, 19,4 and 22,7‰ for the Republic of Congo (due to different sample material, see below), 22,9 and 25,1‰ for Cote d'Ivoire, 23,3 and 27,0‰ for the Democratic Republic of Congo and values between 22,7 and 25,1‰ for Ghana.

Additionally also the isotope range for N-, S- and Sr-isotopes have been analysed.

Discussion

Generally, the isotope measurement results are quite good for distinguishing between the investigated countries. In the blind test 13 out of 19 samples were judged correctly.

Significant for the results was the sample material analysed and which varied between countries of origin with respect to the reference samples. Samples from Ghana and Cameroon were wood cores, samples from the Democratic Republic of Congo wood shavings and the samples from the Republic of Congo bark material. These differences in sample material had a significant influence on the results. The oxygen isotope values of the samples of the Republic of Congo were significantly lower than the values of the other investigated countries. As the samples from the countries neighbouring the Republic of Congo do not give similar results, the material is most probably responsible for this difference. The results of the other measured element isotopes of the Republic of Congo are not conspicuous and explainable and no influence due to the sample material obvious.
Wood cores are supposed to provide the most trustworthy results because an average over the annual rings is produced. This is important because isotopic values depend on weather and climate, which can change over the lifetime of a tree. Therefore, small differences between the annual rings are possible. Wood shavings give an offset for the nitrogen isotope

values with respect to the neighbouring countries wood core samples. For the other element isotopes no offset is obvious.

Some of the blind-test samples were located in provinces and regions without available reference data. Some of the declared geographic origins of the blind samples were actually situated hundreds of kilometres away from the next reference samples. This is problematic as comparability is not possible or of only limited use. Different influences of actual geographic origin (for example micro – climate, soil type and thickness, water availability, geology, etc..) can differ from the regions which were investigated.

Outlook

A larger amount of samples will increase the reliability and accuracy of the interpretations. Especially for distinguishing between provinces of the investigated countries of origin the current amount of samples is small as in some of the countries the provinces are just represented by two or three reference samples, and sometimes there is no reference sample at all. The isotope analysis of the elements H, C, O, N, S, Sr is necessary for an optimal interpretation. Also the combination of different methods (isotopes and genetics) certainly will be an important improvement.

ANNEX 6

Report on the ring test results

Ring testing of molecular markers for genetic traceability of *Entandrophragma cylindricum*, *Milicia excelsa* and *Triplochiton scleroxylon*

Céline Blanc-Jolivet 02.09.2015

Aims of the ring test:

Genetic fingerprinting protocols have been recently developed to control declarations of species and region of origin of timber. In well-equipped laboratories, large sets of SNPs markers have screened to build genetic maps of reference for the species *Entandrophragma cylindricum*, *Milicia excelsa* and *Triplochiton scleroxylon*. Application of genetic fingerprinting methods in the producers' countries is a very important achievement to improve detection of false declarations in timber before exportation.

Three African reference laboratories for genetic fingerprinting have been supported by the project to get ready to conduct DNA testing on timber (KEFRI in Nairobi/Kenya; IRET in Libreville/Gabon; FORIG in Kumasi/Ghana). Because cost and time-effective methods are needed to conduct such analysis, sets of very informative molecular markers have been identified. This ring test aims at ensuring the quality of the DNA extraction of fresh and timber material, as well as the successful amplification, with low occurrence of DNA contamination, of the newly developed genetic markers in the African reference laboratories. Another partner Institution (NERC) will also take part to this ring test organized by the Thünen Institute.

Design:

Four molecular markers were identified for each species. Each laboratory will receive both materials to extract (dried leaves and timber) and already extracted DNA from the same individuals. The goal of the test is to amplify all genetic markers and to estimate the fragment size on an agarose or polyacrylamide gel. All selected loci can be used for timber tracking and genotyping can be conducted through sequencing, PCR-RFLP or fragment analysis, depending on the locus.

The Thünen Institute prepared DNA extraction kits, primers and samples for each participating laboratory.

Evaluation of the results:

Each participant will have to report amplification success and fragment size for each sample and locus. **Results have to be communicated to Céline Blanc-Jolivet until 30.06.2015**.

Instructions

Each participant will receive a package containing: DNA extractions kits, four fresh samples from each species, four dried DNA samples from each species, and four primer pairs per species (total: 24 samples and 12 primer pairs). **Other reagents (PCR buffers, agarose, acrylamide, DNA ladder, tubes, tips…) have to be provided by the participants themselves** as formerly explained by email. A budget was attributed to the participants for this purpose (see project description).

The testing might be conducted as follows:

- **Preparation of the samples (timber and leaf)**

Put some of material from the samples SA_1, SA_2, SA_3, SA_4 (Sapelli), IR_1, IR_2, IR_3, IR_4 (Iroko) AY_1, AY_2, AY_3 and AY_4 (Ayous) in tubes and extract the DNA according to the provided protocol with the innuPREP Plant DNA Kit.

- **Preparation of the concentrated DNA stock solution for the provided dried DNA samples**

Dissolve the provided dried DNA samples (1,5 mL Tubes) with 15µL (SA_7; IR_5; AY_7) or 25µL (SA_5, SA_6 and SA_8; IR_ 6, IR_7, IR_8; AY_5, AY_6, AY_8) with sterile water.

- **Dilution of DNA to 10 ng/µL**

Dilute provided dried DNA samples to 10ng/µL for PCR (see table below) as well as samples newly extracted with the InnuPREP Kit (estimate the DNA concentration with the standard protocol applied in your laboratory).

Sample	concentration	comments
SA_5	245 ng/µL	Dilute to 10 ng/ µL
SA_6	966,1 ng/µL	Dilute to 10 ng/ µL
SA_7	-	Dilute 1:10
SA_8	-	Dilute 1:10
IR_5	-	Dilute 1:10
IR_6	433,9 ng/µL	Dilute to 10 ng/ µL
IR_7	-	Dilute 1:10
IR_8	1685,6 ng/µL	Dilute to 10 ng/ µL
AY_5	-	Dilute 1:10
AY_6	270,7 ng/µL	Dilute to 10 ng/ µL
AY_7	-	Dilute 1:10
AY_8	111,9 ng/µL	Dilute to 10 ng/ µL

- **Preparation of the primers**

Use the information on the attached list (Oligonukleotid Synthese Report) to find the appropriate amount of water to add in each tube. The provided primers are marked in color on the list. After adding the water, let the pellet resuspend at room temperature for one or two hours before diluting to 10 µM.

- **Preparation of the PCR**

We provided the optimal PCR protocols used in Thünen, please note that some adjustments have been done for timber samples. Because the reagents might be different in other laboratories, optimization of the protocol might be necessary.

Samples SA_1 to SA_8 (Sapelli) might be tested with primers pairs SA_m20477 F+R, SA_2101 F+R, SA_0387 F+R, and SA_rbcL_1.1a F+ SA_rbcL_2.1a R.
Samples IR_1 to IR_8 (Iroko) might be tested with primers pairs IR_0342 F+R, IR_0536 F+R, IR_3246 F+R and IR_rbcl_2.1a F + IR_ rbcL_3.3a R.
Samples AY_1 to AY_8 (Ayous) might be tested with primers pairs AY_4681 F+R, AY_5909 F+R, AY_1559 F+R, AY_rbcL_3.3a F +AY_rbcL_4.1a R.

- **Visualization of the results**

Amplified DNA should be visualized on an agarose and/or acrylamide gel. Repeat the PCR if necessary. Please fill the following table according to your results and send to Céline Blanc-Jolivet celine.blanc-jolivet@ti.bund.de **until 30.06.2015**.

Results

- **Description of the samples provided**

Blind ID	Real ID	Sample description
SA_1	CIV_12_ENTC_01	Timber
SA_2	Coffee	Leaf- Negative control
SA_3	DRC_3_ENTC_01	Leaf
SA_4	CB_06_ENTC_18	Leaf
SA_5	DRC_3_ENTC_01	DNA from leaf
SA_6	CB_06_ENTC_18	DNA from leaf
SA_7	CIV_12_ENTC_01	DNA from timber
SA_8	H_2O	Negative control
IR_1	Coffee	Leaf- Negative control
IR_2	CB_21_IROE_11	Timber
IR_3	GH_4_IROE_8	Leaf
IR_4	DRC_30_IROE_9	Leaf
IR_5	CB_21_IROE_11	DNA from timber
IR_6	DRC_30_IROE_9	DNA from leaf
IR_7	H_2O	Negative control
IR_8	GH_4_IROE_8	DNA from leaf
AY_1	CIV_03_TRI_11	Timber
AY_2	GH_3_TRI_4	Leaf
AY_3	DRC_02_TRI_05	Leaf
AY_4	Coffee	Leaf- Negative control
AY_5	H_2O	Negative control
AY_6	GH_3_TRI_4	DNA from leaf
AY_7	CIV_03_TRI_11	DNA from timber
AY_8	DRC_02_TRI_05	DNA from leaf

- **Results provided by the participants**

One African laboratory (KEFRI, Nairobi, Kenya) and NERC could successfully amplify DNA and address fragment sizes. Results are reported below. The two other laboratories (IRET, Libreville, Gabon; FORIG, Kumasi, Ghana) did not manage to amplify DNA due to very long delays for chemicals delivery and conservation problems which damaged the products.

Sapelli samples NERC

	SA_m20477 F+R			SA_2101 F+R			SA_0387 F+R			SA_rbcL_1.1a F+ SA_rbcL_2.1a R		
	PCR success (Y/N)	Fragment size	Comments	PCR success (Y/N)	Fragment size	Comments	PCR success (Y/N)	Fragment size	Comments	PCR success (Y/N)	Fragment size	Comments
SA_1	Y	150	165	N	-	80	Y	350	93	Y	100	100
SA_2	N	-	-	N	-	-	N	-	-	Y	100	100
SA_3	N	-	174	N	-	80	N	-	93	Y	100	100
SA_4	Y	150	157	Y	90/(400)	80	Y	100/350	93	Y	100	100
SA_5	Y	160	174	Y	50/90	80	Y*	50	93	Y	100	100
SA_6	Y	150	157	Y	90/(400)	80	Y	100/350	93	Y	100	100
SA_7	Y	160	165	Y	50/90	80	Y	100/350	93	Y	100	100
SA_8	N	-	-	Y*	50	-	Y*	50	-	N	-	-

* the fragment observed at 50 bp was probably a “primer cloud”, so no amplification for this sample

Sapelli samples KEFRI

	SA_m20477 F+R			SA_2101 F+R			SA_0387 F+R			SA_rbcL_1.1a F+ SA_rbcL_2.1a R		
	PCR success (Y/N)	Fragment size	Comments	PCR success (Y/N)	Fragment size	Comments	PCR success (Y/N)	Fragment size	Comments	PCR success (Y/N)	Fragment size	Comments
SA_1	N	-	165	Y	75/100	80	Y	125	93	Y	275	100
SA_2	N	-	-	Y	75/100	-	Y	275	-	Y	125/225	100
SA_3	Y	50/180	174	Y	100	80	Y	125	93	Y	125/225	100
SA_4	Y	50/150	157	Y	100	80	Y	125/375	93	Y	125	100
SA_5	Y	180	174	Y	100	80	N	-	93	Y	125	100
SA_6	Y	150	157	Y	100	80	Y	75/125	93	Y	125	100
SA_7	Y	150	165	Y	50/100	80	Y	125/375	93	N	-	100
SA_8	N	-	-	N	-	-	N	-	-	Y	175	-

Iroko samples NERC

	IR_0342 F+R			IR_0536 F+R			IR_3246 F+R			IR_rbcl_2.1a F + IR_ rbcL_3.3a R		
	PCR success (Y/N)	Fragment size	Comments	PCR success (Y/N)	Fragment size	Comments	PCR success (Y/N)	Fragment size	Comments	PCR success (Y/N)	Fragment size	Comments
IR_1	N	-	-	N	-	-	N	-	-	N	-	190
IR_2	Y	140	140	N	-	104	Y	(180)/500	151	Y	190	190
IR_3	Y	140	140	Y	100	104	Y	180	151	Y	190	190
IR_4	Y	140	140	Y	120	104	Y	180	151	Y	190	190
IR_5	Y	140/300/ (500)	140	Y	180/400	104	Y	(180)/500	151	Y	190	190
IR_6	Y	140	140	Y	120	104	Y	180	151	Y	190	190
IR_7	N	-	-	N	-	-	N	-	-	N	-	-
IR_8	Y	140	140	Y	100	104	Y	180	151	Y	190	190

Iroko samples KEFRI

	IR_0342 F+R			IR_0536 F+R			IR_3246 F+R			IR_rbcl_2.1a F + IR_ rbcL_3.3a R		
	PCR success (Y/N)	Fragment size	Comments	PCR success (Y/N)	Fragment size	Comments	PCR success (Y/N)	Fragment size	Comments	PCR success (Y/N)	Fragment size	Comments
IR_1	N	-	-	Y	125/275	-	Y	150	-	Y	200	190
IR_2	Y	175	140	Y	175/150	104	Y	200/500/700	151	Y	200	190
IR_3	Y	175	140	Y	100/400	104	Y	200	151	Y	200	190
IR_4	Y	175	140	Y	125	104	Y	200	151	N	-	190
IR_5	N	-	140	Y	75/150/430	104	Y	200/500/700	151	Y	200	190
IR_6	Y	175	140	Y	125	104	Y	200	151	Y	200	190
IR_7	N	-	-	Y	75/150	-	N	-	-	N	-	-
IR_8	Y	175	140	Y	75/130	104			151	Y	200	190

Ayous samples NERC

	AY_4681 F+R			AY_5909 F+R			AY_1559 F+R			AY_rbcL_3.3a F +AY_rbcL_4.1a R		
	PCR success (Y/N)	Fragment size	Comments	PCR success (Y/N)	Fragment size	Comments	PCR success (Y/N)	Fragment size	Comments	PCR success (Y/N)	Fragment size	Comments
AY_1	N	-	86	N	-	81	N	-	92	N	-	250
AY_2	N	-	86	N	-	81	N	-	92	N	-	250
AY_3	Y	700	86	N	-	81	N	-	92	N	-	250
AY_4	N	-	-	N	-	-	N	-	-	Y	250	250
AY_5	Y	700	-	N	-	-	N	-	-	N	-	-
AY_6	Y	700	86	Y	90	81	Y	100	92	Y	250	250
AY_7	Y	700	86	Y	90/(200)/(400)	81	Y	100	92	Y	250	250
AY_8	N	-	86	Y	90	81	Y	100	92	Y	250	250

Ayous samples KEFRI

	AY_4681 F+R			AY_5909 F+R			AY_1559 F+R			AY_rbcL_3.3a F +AY_rbcL_4.1a R		
	PCR success (Y/N)	Fragment size	Comments	PCR success (Y/N)	Fragment size	Comments	PCR success (Y/N)	Fragment size	Comments	PCR success (Y/N)	Fragment size	Comments
AY_1	Y	125/175	86	Y	100	81	Y	125	92	Y	240	250
AY_2	Y	125	86	N	-	81	Y	125	92	Y	240	250
AY_3	Y	125	86	Y	100	81	Y	125	92	Y	240	250
AY_4	N	-	-	N	-	-	Y	325/650	-	Y	240	250
AY_5	N	-	-	N	-	-	N	-	-	N	-	-
AY_6	N	-	86	N	-	81	N	-	92	Y	240	250
AY_7	N	-	86	N	-	81	Y	125	92	N	-	250
AY_8	Y	125/175	86	Y	100	81	Y	125	92	Y	240	250

- **Interpretation pf the results**

Each participant could successfully amplify DNA from timber, either extracted by Thünen or extracted by themselves, and for most tested markers. This demonstrates the utility of the InnuPREP Kit for the DNA extraction from timber.

The presence of multiple bands reported by both participants at a few loci reveals the lack of optimization of PCR conditions. All loci were tested by Thünen on the same samples and provided good results with the PCR conditions provided to the participants.

Most discrepancies among the results provided by NERC and the expected fragment sizes result from amplification failure (especially in Ayous extracted by NERC) and PCR optimization problems. Signal at around 50 bp on the agarose gel was misinterpreted as a positive amplification, while it might only be due primer clumps ("primer cloud"). Only one contamination seemed to have occurred (AY_5 at AY_4681 F+R), but the fragment reported is too long, which is not critical for the use of this PCR product for timber tracking.

As for KEFRI, PCR optimization, but maybe also fragment size estimation problems occurred. The participant laboratory mentioned that they did not go through an optimization step, therefore we do not see this as critical. More importantly, seven cases of contamination were observed, with at least two cases corresponding to the expected fragment size in other samples (SA_2 at SA_2101 F+R; IR_1 at IR_3246 F+R), indicating a contamination from the same species. Therefore, more caution should be taken during PCR preparation and during dilution of PCR product aliquots. These results highlight the need of further discussion between Thünen and KEFRI to tackle this problem.

ANNEX 7

Report on the blind test results

WWF
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2015

ITTO-Project PD 620/11 M; Rev. 1
Blind Test Evaluation Report
WWF Germany

ITTO-Project PD 620/11 M; Rev. 1

Blind Test Evaluation Report

WWF Germany

The project "Development and implementation of a species identification and timber tracking system in Africa with DNA fingerprints and stable isotopes" (funded by the International Tropical Timber Organization (ITTO)) focuses on various economically important African timber tree species. Within the project, WWF Germany was contracted to carry out a blind test to generate independent verification and check the efficiency and practical performance of the different methods.

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1 Introduction

The ITTO project "Development and implementation of a species identification and timber tracking system in Africa with DNA fingerprints and stable isotopes" focuses on various economically important African timber tree species. Within the project, WWF Germany was contracted to carry out a blind test to generate independent verification and check the efficiency and practical performance of the different methods. The conditions were fixed in two documents:

- Memorandum of understanding (MoA; 3/2013; Appendix 1)
- Additional Instructions for the blind test of the ITTO-Africa project (2/2015; Appendix 2)

The blind test consisted of two parts:

Part I: species identification (21 African timber species;
Methods: wood anatomy, DNA barcoding)

Part II: verification of the declared origin (3 African timber species;
Methods: DNA fingerprinting, stable isotope method).

According to the MoA specification WWF delivered the agreed number of analysable samples (see expert opinions attached). In agreement with TI (Thünen Institute; Großhansdorf, Germany) the parties agreed to cut down the number of samples (see Additional Instructions for the blind test of the ITTO-Africa project from 2/2015; appendix 2).

For **part I**, the Plant Genetic Diagnostics GmbH (Großhandorf, Germany) conducted the genetic work and the Institute of Wood Research at the Thünen Institute (TI; Bergedorf, Germany) carried out an anatomical analysis of the wood samples.

For **part II**, genetic work was done by the Institute of Forest Genetics at the TI and by University of Adelaide (Australia); and the work on stable isotopes was done by JR/HBLFA Francisco-Josephinum (HBLFVienna, Austria), AgroIsolab GmbH (Jülich, Germany) and by the Food and Environment Research Agency (FERA; York, UK).

In agreement with TI, WWF Germany organized external funding from **World Resources Institute (WRI)** to allow blind test sampling in Africa from standing trees. This request of WWF is based on the conviction that specifically for part II of the blind test results are more expressive and solid if blind test samples derived from authentic material from standing trees taken with GPS-data on site. The WWF blind test comprised of two different material types: 1. wood samples collected from standing trees in the field and 2. sawn wood taken from forest management units (see also chapter “Wood sampling”)

In agreement with TI, WWF Germany engaged **Philipps-University Marburg** (Germany) in a subcontract to provide a collection of wood samples required for both blind test parts. To fulfil the project conditions, Philipps-University Marburg additionally signed a confidentiality agreement.

To verify that the blind test samples provided by Philipps-University Marburg were consistent with the agreed species (part II), WWF arranged a **third party verification** (wood anatomy) - which confirmed the declared species identity (Appendix 4: Sample A_DRC_2014 identical with BT_2014_19 (*Entandrophragma cylindricum*); Sample B_DRC_2014 identical with RM_2014_42 (*Nauclea diderichii*)).

In agreement with TI WWF deposited all questions and results of the blind test at the German Federal Ministry of food and agriculture before the blind test st.

2 Wood sampling

One goal of the test was to create questions close to real cases *"...Both tests should be done using saw timber or equal treated material in order to keep it close to the later practical application of the developed tools..." (MoA)*. On the one hand there was a need to use sawn wood with comparable characteristics to real products. On the other hands there was a need to reach highest quality concerning knowledge about the origin (authentic samples). WWF ranges only samples taken in the field with GPS-data as "A-quality" (see appendix 8).

2.1 Wood samples from standing trees

Between May and November 2014, Philipps-University Marburg collected wood samples for both blind test parts in two different African countries: Democratic Republic of Congo (DRC) and Ghana. The sampling was carried out by two employees from Philipps-University Marburg: Kristina Osen and Sören Kaack supported by several institutions that provided logistic support and official authorizations – see Table 1.
Additionally, WWF collected samples from various cooperating partners supported by internal and external timber experts to full fill all test requirements. All involved partners agreed to maintain strict confidentiality on the identity of the species and the geographic origin of the material.
According to the signed agreement Philipps-University Marburg did not pass additional reference samples, information about the blind test or the involved laboratories to any third party.

Table 1: List of cooperating partners

Partners in DRC:
WWF – DRC
Direction de la gestion forestière du MECNT - Ministère de l'environnement, conservation de la nature, tourisme
ICCN - Institut Congolais pour la Conservation de la Nature
INERA - Institut National pour l'Etude et la Recherche Agronomiques
Logging companies: Sodefor - Société de Développement Forestier and CFT - Compagnie Forestière de Transformation
Field assistant Mr. Bernard Ikati Lisongi (Ir. Eaux et Forêts et Gestion des ressources naturelles de la Université de Kinshasa)
Partners in Ghana:
Forestry Commission, RMSC – Resource Management Support Center
Crops Research Institute Kumasi (for the export licence)

For part I, Philipps-University Marburg collected 59 samples of different genera out of the 21 target species in DRC and Ghana: *Millettia laurentii; Terminalia superba; Pterocarpus soyauxii; Pipdadeniastrum africanum; Nauclea diderrichii; Milicia excelsa; Khaya anthotheca; Guibourtia spp.; Erytrophleum suaveolens; Erythrophleum ivorense; Entandrophragma utile; Entandrophragma cylindricum; Entandrophragma angolense; Baillonella toxisperma; Aucoumea klaineana; Lophira alata; Cylicodiscus gabunensis; Milicia regia; Aningeria robusta; Khaya ivorensis; Afzelia pachyloba; Afzelia bipindensis.*

For part II, Philipps-University Marburg collected 73 wood samples of the three target species *Milicia excelsa*, *Entandrophragma cylindricum* and *Triplochiton scleroxylon*. For *Milicia excelsa*, 23 samples were collected in DRC and 3 samples were collected in Ghana. For *Entandrophragma cylindricum*, 20 samples were collected in DRC and 3 samples in Ghana. For *Triplochiton scleroxylon*, 24 samples were collected in Ghana (see Figure 2).

2.1.1 Sampling procedure

The identity of the target trees was always double-checked by the involved sample takers (trained biologists) and national forest experts on the ground. For the actual sample taking, all cambium material was thoroughly removed from the unambiguously identified target tree, and wood samples were taken with hammer and chisel or with a driller. To prevent any possible mixing of sampling material from different tree individuals, sampling tools like chisel and auger were thoroughly cleaned after each sample taking to entirely remove possible remaining materials from the previously sampled tree. Additionally, GPS data for each target tree was recorded. Pictures were taken from each sample site and tree.

Figure 1: Picture of blind test sampling Philipps-University Marburg; Example blindtest ID "RM_2014_42" (*Nauclea diderrichii*; Bilinga); equal to ID "B_DRC_2014" - Appendix 4

All sample material was sun- and air-dried and stored in paper bags. Those paper bags were packed in plastic bags with silica gel to keep the samples dry and prevent fungi infection. Eache sample was stored in an individual bag and marked with a clear ID, so there was no likelihood of material confusion at any given time. Finally, the dry samples (humidity < 10%) were sent to the corresponding labs for downstream analysis.

The sample amount for part I was collected as follows:
DNA: approx. 75 g of fresh wood material, corresponding to a volume of approx. 2 cm x 5 cm x 10 cm, about the size of a cigarette box. Material was collected either with a chisel or with a driller and comprised both sapwood and heartwood.

Wood anatomy: solid wood cube – minimum the size 1 cm^3. Material was collected with a chisel and comprised both sapwood and heartwood.
The sample amount for part II was collected as follows:

DNA and stable isotope samples (sample taking was identical): approx. 75 g of wet wood material, corresponding to a volume of approx. 2 cm x 5 cm x 10 cm , about the size of a cigarette box. Material was collected either with a chisel or with a driller and comprised both sapwood and heartwood with a mean depth of 8 – 10 cm.

Wood anatomy: solid wood cube– minimum the size 1 cm^3. Material was collected with a chisel and comprised both sapwood and heartwood.

For every target tree, Philipps-University Marburg collected two samples, with one sample from each batch to be kept in Marburg. These additional sample batches were collected for several reasons: a) for safety reasons in case the original batch were to be damaged or lost and b) for further analysis approaches in the future, after successful completion of the blind test.

2.2 Sawn wood samples

Blind test part I:

To complete the set of the 21 target species of part I of the blind test (species verification) WWF purchased sawn wood and veneer samples from three different reliable wood traders. In total, 25 test samples were collected for part I, which included 11 wood samples taken directly from trees, 11 sawn wood samples and 3 veneer samples.

Blind test part II:

The set of 60 blind test samples (3 species x 10 blind test samples) included 6 sawn wood samples (3 x 2). Sawn wood samples were also collected by WWF in order to provide a blind test that reflects the requirements of a realistic investigation set up (as most questionable timber in the trade has been treated and processed similarly). The samples were provided by reliable forest concessionaires and collected on their saw mill sites. Only samples from clearly trackable logs with proven origin were taken. For all samples taken into account, the granted minimum information of origin is the FMU (Forest Management Unit). This was verified by a forestry specialist and in each case by at least one WWF-representative. In cases where the exact GPS coordinates of the standing tree were known, these were given. In cases where only the FMU was known, GPS coordinates of a point near the centre of the FMU were given. As no selected FMU was larger than 30 km in diameter, any tree in the respective FMU is not further away from that centre point than 15 km. All samples were cut from larger timber pieces with hand held saws. Any side of the sample thus received a fresh cut with a thoroughly cleaned saw, minimizing any possibility of contamination (e.g. with extrinsic sawdust). Sawn wood samples were collected via WWF in Cameroon, Congo Brazzaville and Gabon.

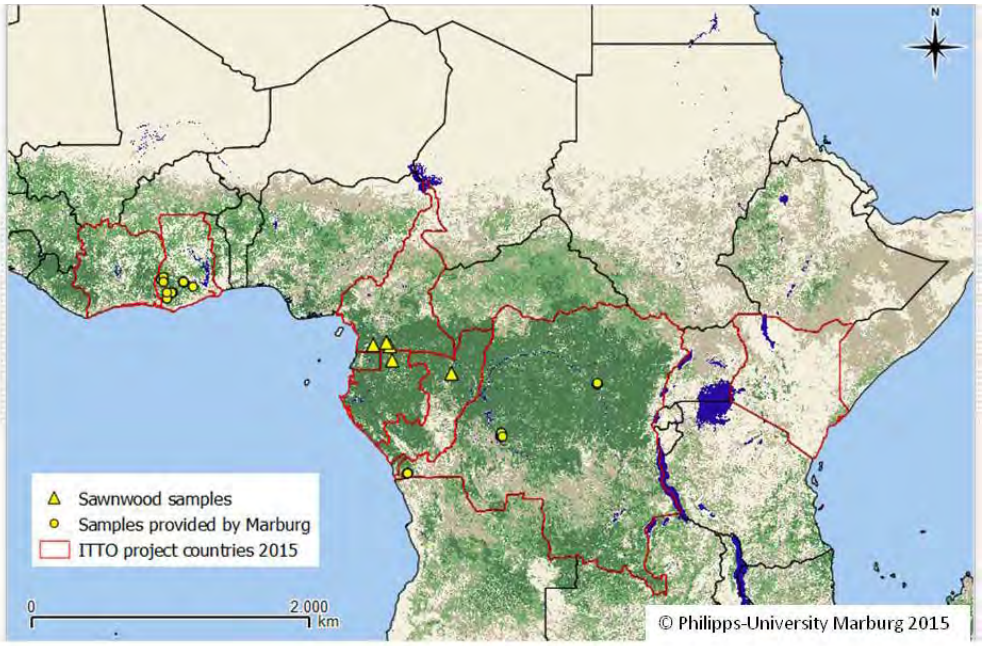

Figure 2: Sample taking locations for *Milicia excelsa, Entandrophragma cylindricum* and *Triplochiton scleroxylon* in ITTO project countries provided by University of Marburg (yellow dots) and WWF (yellow triangles).

3 Blind test design

For both blind test approaches (part I & II) WWF's strategy was to combine basic, middle and more demanding tasks. Another goal was to create questions close to real cases *"...Both tests should be done using saw timber or equal treated material in order to keep it close to the later practical application of the developed tools..." (MoA).*

3.1 Blind test design, part I (species identification)

Within the project scope, the entire species spectrum covered the following 21 target species: *Afzelia spp., Aningeria robusta, Aucoumea klaineana, Baillonella toxisperma, Cylicodiscus gabunensis, Entandrophragma angolense, Entandrophragma cylindricum, Entandrophragma utile, Erythrophleum ivorense, Erythrophleum suaveolens, Guibourtia spp., Khaya spp., Lophira alata, Milicia excelsa, Milicia regia, Millettia laurentii, Nauclea diderrichii, Pericopsis elata, Pterocarpus soyauxii, Terminalia superba* and *Triplochiton scleroxylon.*

The set of samples for the blind test approach included 25 samples from the species spectrum bearing true/false declarations. In agreement with TI, WWF Germany included all 21 target species into the set of declarations to make sure that in case of DNA analysis there was a chance use to use all markers developed during the ITTO-project. According to the MoA agreement the idea was to verify the given species declaration.

Samples were taken partly from the field according to the above mentioned protocol and complemented with samples from reliable wood traders.

Table 2: Blind test, part I – 25 blind test samples; Black columns: information sent to the labs with the question to verify the declared species; Red columns: Real species

ID	Blind test declaration	Real species (Scientific name)	Real species (Common name)
RM_2014_03	*Milicia excelsa*	*Millettia laurentii*	Wenge
RM_2014_04	*Erythrophleum ivorense*	*Erythrophleum suaveolens*	Tali
RM_2014_13	*Khaya ivorensis*	*Khaya anthotheca*	Khaya
RM_2014_37	*Erythrophleum suaveolens*	*Erythrophleum ivorense*	Tali
RM_2014_39	*Entandrophragma utile*	*Aucoumea kleineana*	Ocumé
RM_2014_42	*Entandrophragma angolense*	*Nauclea diderrichii*	Bilinga
RM_2014_45	*Afzelia pachyloba*	*Afzelia bipindensis*	Afzelia
RM_2014_48	*Entandrophragma cylindricum*	*Entandrophragma angolense*	Tiama blanc
RM_2014_49	*Aningeria robusta*	*Baillonella toxisperma*	Moabi
RM_2014_59	*Aucoumea klaineana*	*Afzelia pachyloba*	Afzelia
RM_2014_60	*Cylicodiscus gabunensis*	*Entandrophragma utile*	Sipo
X2-57	*Pterocarpus soyauxii*	*Pericopsis elata*	Afromosia
X2-58	*Baillonella toxisperma*	*Aningeria robusta*	Anigre
X2-59	*Afzelia bipendensis*	*Afzelia africana*	Afzelia
X2-65	*Guibourtia ehie*	*Guibourtia tessmannii*	Bubinga
X2-66	*Millettia laurentii*	*Milicia excelsa*	Iroko / Kambala
X2-67	*Khaya grandiflora*	*Khaya ivorensis*	Khaya
X2-68	*Milicia regia*	*Milicia excelsa*	Iroko / Kambala
X2-69	*Terminalia superba*	*Terminalia superba*	Limba
X2-74	*Pericopsis elata*	*Pteroarpus soyauxii*	Padouk
X2-75	*Nauclea diderrichii*	*Okoumea klaineana*	Okoumé
X2-76	*Khaya ivorensis*	*Entandrophragma cylindricum*	Sapelli
X2-78	*Triplochiton scleroxylon*	*Triplochiton scleroxylon*	Abachi / Ayou
X2-79	*Pericopsis elata*	*Cyclodiscus gabunensis*	Okan
X2-81	*Lophira alata*	*Lophira alata*	Azobé or Bongossi

3.2 Blind test design, part II (verification of the declared origin)

The project focussed on the 3 target species Iroko (*Milicia excelsa*), Sapelli (*Entandrophragma cylindricum*) and Ayou (*Triplochiton scleroxylon*). In order to design the blind test on origin, the scope of available reference material was one decisive parameter, as only reference material taken during the ITTO project was accepted for labs to do the blind test interpretation.

The available reference material was sampled independently from WWF's blind test approach during the first part of the ITTO-project and consists of 5400 leaf, cambium and wood samples from ten countries linked to part I and part II of the blind test [ITTO-tropical forest update 24/1]. From these samples, 828 analysable reference samples were available for the stable isotope method. 3324 analysable reference samples from the comparable number of populations were available for DNA labs.

Figure 3: Distribution of reference sampling locations (5400 samples; ten countries) of the tree tropical African timber species [source: ITTO-tropical forest update 24/1]

In 2015, TI and WWF jointly agreed to only include those countries into the blind test design (list of possible right or wrong declared countries), that count with a minimum set of at least 20 usable reference samples. As a result the list of possible countries of origin includes:

- Ayou (*Triplochiton scleroxylon*)
  - Cameroon
  - Congo Brazzaville
  - Côte d'Ivoire
  - DRC
  - Ghana
- Iroko (*Milicia excelsa*)
  - Cameroon
  - DRC
  - Gabon
  - Ghana
  - Kenya
- Sapelli (*Entandrophragma cylindricum*)
  - Cameroon
  - Congo Brazzaville
  - DRC
  - Ghana

WWF designed the blind test in a way that every participating lab received a collection of samples from one or more of the tree target species with different declared origins. In every sample package WWF included the same number of samples and the same number of basic, middle or more

difficult questions in matters of country and region to stress the distance of declared and real origin as a crucial parameter. (Background information: According to the EU Timber Regulation (EUTR), affected companies have to declare at least the country of origin or, in cases of different risk levels within a country, the region or the Forest Management Unit (FMU)).

In cases where labs only answered the question on the country level, the degree of difficulty is easier than the one predicted in WWF's column. Example:

Table 3: Example to explain the logical framework of the blind test

ID	Declared country	Declared Region	Declared Origin (GPS)	WWF: Declaration is true/false (Country/Region)	WWF: Real Country of Origin	WWF: Real Origin GPS_X	WWF: Real Origin GPS_Y	WWF: Distance [km]; Declared/ real	WWF: predicted level of difficulty
BT_2014_533	Ghana	South-East	N6.3; W0.022 (80 km radius)	r/f	Ghana	N 5.57883	W 2.25545	270	m

Declared origin is Ghana – real country of origin is also Ghana. The predicted middle degree of difficulty ("m") deduce from a wrongly declared region inside Ghana. In case a lab could only answer the question on the country level but not the regional question, the first part of the question was solved (basic level of difficulty) but the trickier challenge (country AND region; predicted middle level of difficulty) was not accomplished.

WWF guarantees that in all cases where the declared country coordinates are wrong, these declarations are at least 150 km away from the correct place of origin.

In agreement with TI, WWF Germany chose only false declarations inside a country in case the country was correctly declared. The region was described precisely via GPS and radius.

3.3 Blind test design Ayou (*Triplochiton scleroxylon*)

Table 4: Blind test, part II; 10 blind test samples (Ayou) Black columns: information sent to the labs with the question to verify the declared origin; Red columns: Real origin, distance and prediction of difficulty (b = basic; m = middle; d = more difficult) concerning combined question country and region.

ID	Declared country	Declared Region	Declared Origin (GPS)	WWF: Declaration is true/false (Country/Region)	WWF: Real Country of Origin	WWF: Real Origin GPS_X	WWF: Real Origin GPS_Y	WWF: Distance [km]; Declared/real	WWF: predicted level of difficulty
BT_2014_533	Ghana	South-East	N6.3; W0.022 (80 km radius)	r/f	Ghana	N 5.57883	W 2.25545	270	m
BT_2014_543	Cameroon	North	N3.15017 E 13.61635 (80 km r.)	f/f	Gabun	N 1.630097	E 12.089700	240	d
BT_2014_547	DRC	South-West	S5; E16 (90 km radius)	f	Ghana	N 6.41525	W 1.20916	2300	m
BT_2014_551	Côte d'Ivoire	South-East	N5.9; W3.75 (25 km radius)	f	Ghana	N 5.35566	W 2.26029	180	d
BT_2014_563	Côte d'Ivoire	South	N6; W5 (100 km radius)	f	Ghana	N 6.49586	W 2.47783	280	d
BT_2014_567	DRC	West	S2; E19 (200 km radius)	f	Ghana	N 6.01586	W 2.04149	2500	b
BT_2014_568	Cameroon	Central	N3.518 E13.44178 (50 km r.)	r/f	Cameroon	N 2.54425	E 11.92747	200	m
BT_2014_578	Cameroon	West	N5; E10 (110 km radius)	f	Ghana	N 6.58086	W 2.38226	1400	b
BT_2014_594	Ghana	Central	N7.75; W0.988 (60 km r.)	r/f	Ghana	N 6.41922	W 2.37705	210	d
BT_2014_598	Ghana	Central - south	N6.4; W1.2 (90 km radius)	r/r	Ghana	N 6.40983	W 1.20743	< 10	b

3.4 Blind test design Iroko (*Milicia excelsa*)

Table 5: Blind test, part II; 10 blind test samples (Iroko)Black columns: information sent to the labs with the question to verify the declared origin; Red columns: Real origin, distance and prediction of difficulty (b = basic; m = middle; d = more difficult) concerning combined question country and region.

ID	Declared country	Declared Region	Declared Origin (GPS)	WWF: Declaration is true/false (Country/Region)	WWF: Real Country of Origin	WWF: Real Origin GPS_X	WWF: Real Origin GPS_Y	WWF: Distance [km]; Declared/real	WWF: predicted level of difficulty
BT_2014_518	Gabun	North-East	N0.84; E13.85 (40 km radius)	f	Congo B.	N 0.8	E 15.95	230	d
BT_2014_526	Ghana	South	N6; W1 (60 km radius)	r/r	Ghana	N 6.41294	W 1.20957	50	b
BT_2014_527	DRC	Central	S2; E19 (180 km radius)	r/r	DRC	S 3.13563	E 19.08678	130	m
BT_2014_549	Kenya	West	0; E35 (100 km radius)	f	DRC	N 0.08401	E 25.19800	900	b
BT_2014_554	Gabun	North	N1.3; E12.225 (60 km radius)	f	Cameroon	N 2.65853	E 10.89303	230	d
BT_2014_566	Kamerun	South	N3.5; E12 (100 km radius)	f	DRC	S 5.36783	E 13.06735	1000	b
BT_2014_569	DRC	North-West	N0.0131; E18.24 (40 km radius)	r/f	DRC	S 3.13551	E 19.08598	380	m
BT_2014_596	Gabun	South	S3.09; E11.1 (50 km radius)	f	DRC	S 5.36419	E 13.06610	400	d
BT_2014_599	Ghana	South-East	N6.3; E0 (70 km radius)	r/f	Ghana	N 6.00239	W 2.04507	230	m
BT_2014_600	DRC	North-West	N2; E21 (200 km radius)	r/f	DRC	S 5.37003	E 13.06686	1200	d

3.5 Blind test design Sapelli (*Entandrophragma cylindricum*)

Table 6: Blind test, part II; 10 blind test samples (Sapelli)Black columns: information sent to the labs with the question to verify the declared origin; Red columns: Real origin, distance and prediction of difficulty (b = basic; m = middle; d = more difficult) concerning combined question country and region.

ID	Declared country	Declared Region	Declared Origin (GPS)	WWF: Declaration is true/false (Country/Region)	WWF: Real Country of Origin	WWF: Real Origin GPS_X	WWF: Real Origin GPS_Y	WWF: Distance [km]; Declared/real	WWF: predicted level of difficulty
BT_2014_510	Ghana	South	N6.4; W1.2 (70 km radius)	r/r	Ghana	N 6.41563	W 1.20895	< 10	b
BT_2014_522	DRC	North-West	N1.55115; E21.07416 (300 km r.)	r/f	DRC	S 3.14157	E 19.08899	570	d
BT_2014_525	Ghana	South-East	N6.3; E0 (75 km radius)	r/f	Ghana	N 6.00600	W 2.04759	230	d
BT_2014_534	Kongo Brazzaville	Central/ South	S3; E15 (100 km radius)	f	DRC	S 3.14682	E 19.09272	450	d
BT_2014_552	DRC	North	N0.45; E25.8 (200 km radius)	r/r	DRC	N 0.08388	E 25.19451	80	b
BT_2014_557	DRC	Central	S4; E19 (300 km radius)	r/f	DRC	N 0.08143	E 25.19737	800	m
BT_2014_573	DRC	South-West	S4.77; E16.9 (120 km radius)	r/f	DRC	S 3.14100	E 19.08616	300	d
BT_2014_577	Kamerun	South	N2.8,E12 (40 km radius)	r/r	Cameroon	N 2.762739	E 11.747475	30	b
BT_2014_580	Kamerun	South-East	N3.04; E14.5 (90 km radius)	f	Congo B.	N 0.8	E 15.95	300	m
BT_2014_592	Kongo Brazzaville	North	N0.51544; E16.72308 (120 km r.)	f	DRC	S 3.14632	E 19.09076	500	m

4 Results

4.1 Results part I (species identification)

4.1.1 Wood anatomy (Thünen Institute)

Table 7: Result blind test part I (species identification) – wood anatomy (Thünen Institute; Bergedorf-Germany; Dr. Gerald Koch). Black columns: table sent back from the lab. Red/coloured columns: additional column with WWF's evaluation of the test (green background colour = declared species was verified correctly / species or genus was identified correctly; white background colour = for this species the method is not able to verify or identify on the species level.)

ID	Claims on species name *Deklarierte Holzart*	Result of lab *Testergebnis*	Result of the microscopic wood identification (wood anatomy) *Ergebnis der mikroskopischen Holzartenbestimmung (Holzanatomie)*	Comments *Kommentare*	WWF blind test: Real species	WWF: evaluation of lab results (verification of the declared species)	WWF: blind test evaluation in detail (identified genus)	WWF: blind test evaluation in detail (identified)
RM_2014_03	*Milicia excelsa*	false	*Millettia* spp. = Wengé or Panga Panga	*correct trade name Wengé / Panga Panga*	*Millettia laurentii*	correct	correct genus identified	no result from lab
RM_2014_04	*Erythrophleum ivorense*	*correct*	*Erythrophleum* spp. = Tali	*the individual species within the genus Erythrophleum can´t be distinguished microscopically*	*Erythrophleum suaveolens*	Verificaiton "correct" linked to the genus level because species verification is not possible with this method here	correct genus identified	no result from lab because species identification is not possible with this method
RM_2014_13	*Khaya ivorensis*	*correct*	*Khaya* spp. = Khaya	*the individual species within the genus Khaya can´t be distinguished microscopically*	*Khaya anthotheca*	Verificaiton "correct" linked to the genus level because species verification is not possible with this method here	correct genus identified	no result from lab because species identification is not possible with this method
RM_2014_37	*Erythrophleum suaveolens*	*correct*	*Erythrophleum* spp. = Tali	*the individual species within the genus Erythrophleum can´t be distinguished microscopically*	*Erythrophleum ivorense*	Verificaiton "correct" linked to the genus level because species verification is not possible with this method here	correct genus identified	no result from lab because species identification is not possible with this method

RM_2014_39	*Entandrophragma utile*	false	*Aucoumea klaineana* = Okoumé	*correct trade name Okoumé*	*Aucoumea klaineana*	correct	correct genus identified	correct species identified
RM_2014_42	*Entandrophragma angolense*	false	*Nauclea diderrichii* = Bilinga	*correct trade name Bilinga*	*Nauclea diderrichii*	correct	correct genus identified	correct species identified
RM_2014_45	*Afzelia pachyloba*	*correct*	*Afzelia* spp. = Afzelia	*the individual species within the genus Afzelia can´t be distinguished microscopically*	*Afzelia bipindensis*	Verificaiton "correct" linked to the genus level because species verification is not possible with this method here	correct genus identified	no result from lab because species identification is not possible with this method
RM_2014_48	*Entandrophragma cylindricum*	false	*Entandrophragma angolense* = Tiama	*correct declaration Tiama*	*Entandrophragma angolense*	correct	correct genus identified	correct species identified
RM_2014_49	*Aningeria robusta*	false	*Baillonella toxisperma = Moabi*	*correct declaration Moabi*	*Baillonella toxisperma*	correct	correct genus identified	correct species identified
RM_2014_59	*Aucoumea klaineana*	false	*Afzelia* spp. = Afzelia	*correct trade name Afzelia*	*Afzelia pachyloba*	correct	correct genus identified	no result from lab because species identification is not possible with this method
RM_2014_60	*Cylicodiscus gabunensis*	false	*Entandrophragma utile*= Sipo	*correct trade name Sipo*	*Entandrophragma utile*	correct	correct genus identified	correct species identified
X2-57	*Pterocarpus soyauxii*	false	*Pericopsis elata* = Afrormosia	*correct trade name Afrormosia (CITES-species)*	*Pericopsis elata*	correct	correct genus identified	correct species identified
X2-58	*Baillonella toxisperma*	false	*Pouteria* spp. (*Aningeria* spp.) = Aningré	*correct trade name Aningré*	*Aningeria robusta*	correct	correct genus identified	no result from lab
X2-59	*Afzelia bipindensis*	*correct*	*Afzelia* spp. = Afzelia	*the individual species within the genus Afzelia can´t be distinguished microscopically*	*Afzelia africana*	Verificaiton "correct" linked to the genus level because species verification is not possible with this method here	correct genus identified	no result from lab because species identification is not possible with this method
X2-65	*Guibourtia ehie*	false	*Guibourtia* spp. = Bubinga	*The wood anatomical characters show best agreement*	*Guibourtia tessmannii*	correct	correct genus identified	no result from lab because species identification is

				with Bubinga; the individual species G. ehie = Ovengkol can be excluded				not possible with this method
X2-66	*Millettia laurentii*	false	*Milicia* cf. *excelsa* = Iroko	*correct trade name Iroko*	*Milicia excelsa*	correct	correct genus identified	correct species identified
X2-67	*Khaya grandiflora*	*correct*	*Khaya* spp. = Khaya	*the individual species within the genus Khaya can´t be distinguished microscopically*	*Khaya ivorensis*	Verificaiton "correct" linked to the genus level because species verification is not possible with this method here	correct genus identified	no result from lab because species identification is not possible with this method
X2-68	*Milicia regia*	*correct*	*Milicia* spp. = Iroko	*the individual species within the genus Milicia can´t be distinguished microscopically*	*Milicia excelsa*	Verificaiton "correct" linked to the genus level because species verification is not possible with this method here	correct genus identified	no result from lab because species identification is not possible with this method
X2-69	*Terminalia superba*	*correct*	*Terminalia superba* = Limba	*correct declaration*	*Terminalia superba*	correct	correct genus identified	correct species identified
X2-74	*Pericopsis elata*	false	*Pterocarpus soyauxii* = Padouk	*correct trade name Padouk*	*Pteroarpus soyauxii*	correct	correct genus identified	correct species identified
X2-75	*Nauclea diderrichii*	false	*Aucoumea klaineana* = Okoumé	*correct trade name Okoumé*	*Aucoumea klaineana*	correct	correct genus identified	correct species identified
X2-76	*Khaya ivorensis*	false	*Entandrophragma cylindricum* = Sapelli	*correct trade name Sapelli*	*Entandrophragma cylindricum*	correct	correct genus identified	correct species identified
X2-78	*Triplochiton scleroxylon*	*correct*	*Triplochiton scleroxylon* = Abachi	*correct declaration*	*Triplochiton scleroxylon*	correct	correct genus identified	correct species identified
X2-79	*Pericopsis elata*	false	*Cylicodiscus gabunensis* = Okan	*correct trade name Okan*	*Cyclodiscus gabunensis*	correct	correct genus identified	correct species identified
X2-81	*Lophira alata*	*correct*	*Lophira alata* = Bongossi	*correct declaration*	*Lophira alata*	correct	correct genus identified	correct species identified

4.1.2 DNA barcoding (Plant Genetic Diagnostics GmbH)

Table 8: Result blind test part I (species identification) – DNA barcoding / fingerprinting (Plant Genetic Diagnostics GmbH; Großhansdorf-Germany; Dr. Aki Michael Höltken). Black columns: table sent back from the lab. Red/coloured columns: additional columns with information about real species and WWF's evaluation of the test (green background colour = declared species was verified in the right way; yellow background colour = formally wrong verification, if the species level is considered but all verifications and identifications on the genus level are correct)

orginal ID	claim	Barcoding- / finger-printing results	final comment	WWF blind test: Real species	WWF: evaluation of lab results (verification of the declared species)	WWF: blind test evaluation in detail (identified genus)	WWF: blind test evaluation in detail (identified)
RM_2014_03	*Milicia excelsa*			*Millettia laurentii*	no result from lab	no result from lab	no result from lab
RM_2014_04	*Erythrophleum ivorense*	YES / -	*E. ivorense* or *E. suaveolens*	*Erythrophleum suaveolens*	Formally wrong verification, if the species level is considered but all verifications and identifications on the genus level are correct	correct genus identified	undetermined remark about identified species
RM_2014_13	*Khaya ivorensis*			*Khaya anthotheca*	no result from lab	no result from lab	no result from lab
RM_2014_37	*Erythrophleum suaveolens*			*Erythrophleum ivorense*	no result from lab	no result from lab	no result from lab
RM_2014_39	*Entandrophragma utile*	NO / NO	*Aucoumea*	*Aucoumea klaineana*	correct	correct genus identified	no result from lab
RM_2014_42	*Entandrophragma angolense*	NO / NO	*Nauclea*	*Nauclea diderrichii*	correct	correct genus identified	no result from lab
RM_2014_45	*Afzelia pachyloba*	YES / -	*Afzelia*	*Afzelia bipindensis*	Formally wrong verification, if the species level is considered but all verifications and identifications on the genus level are correct	correct genus identified	no result from lab
RM_2014_48	*Entandrophragma cylindricum*	- / NO	nuclear and chloropl. SNPs match with *E. angolense*	*Entandrophragma angolense*	correct	correct genus identified	correct species identified
RM_2014_49	*Aningeria robusta*	YES / -	*Sapotaceae* family	*Baillonella toxisperma*	wrong	no result from lab	no result from lab
RM_2014_59	*Aucoumea klaineana*	NO / -	*Afzelia*	*Afzelia pachyloba*	correct	correct genus identified	no result from lab
RM_2014_60	*Cylicodiscus gabunensis*			*Entandrophragma utile*	no result from lab	no result from lab	no result from lab
X2_57	*Pterocarpus soyauxii*			*Pericopsis elata*	no result from lab	no result from lab	no result from lab

					(veneer)		
X2_58	*Baillionella toxisperma*			*Aningeria robusta*	no result from lab (veneer)	no result from lab	no result from lab
X2_59	*Afzelia bipen-densis*			*Afzelia africana*	no result from lab	no result from lab	no result from lab
X2_65	*Guibourtia ehie*	NO / -	Nauclea	*Guibourtia tessmannii*	correct	wrong	no result from lab
X2_66	*Milletia laurentii*			*Milicia excelsa*	no result from lab	no result from lab	no result from lab
X2_67	*Khaya grandiflora*			*Khaya ivorensis*	no result from lab	no result from lab	no result from lab
X2_68	*Milicia regia*			*Milicia excelsa*	no result from lab	no result from lab	no result from lab
X2_69	*Terminalia superba*	YES / -		*Terminalia superba*	correct	correct genus identified	correct species identified
X2_74	*Pericopsis elata*			*Pteroarpus soyauxii*	no result from lab	no result from lab	no result from lab
X2_75	*Nauclea dide-richii*			*Okoumea klaineana*	no result from lab	no result from lab	no result from lab
X2_76	*Khaya ivorensis*			*Entandro-phragma cylind-ricum*	no result from lab	no result from lab	no result from lab
X2_78	*Triplochiton scleroxylon*			*Triplochiton scleroxylon*	no result from lab	no result from lab	no result from lab
X2_79	*Pericopsis elata*			*Cyclodiscus gabunensis*	no result from lab	no result from lab	no result from lab
X2_81	*Lophira alata*			*Lophira alata*	no result from lab	no result from lab	no result from lab

4.2 Results part II (verification of declared origin)

General remark: WWF blind test comprised two levels, firstly declaration on country level and secondly declaration on regional level. It was obligatory to answer the question on country level as this is always the minimum declaration for affected companies e.g. according to EUTR.

4.2.1 DNA fingerprinting; Ayou (University of Adelaide)

Table 9: Result blind test part II (verification of declared origin); Ayou; DNA fingerprinting (University of Adelaide; Australia; Prof. Andrew Lowe). Black columns: table sent back from the lab; Red/coloured additional columns – 1. real country of origin and information if the declaration of country/region was true/false; columns 2. and 3. show WWF's evaluation of the test; Main task: verification of the declared country; Evaluation: green = verification of the declared country/region correct, yellow = verification of the declared country/region undetermined, red = verification of the declared country/region false.

ID	Claimed country of origin	result of lab (country)	result of lab (region)	Notes	WWF: real country; r/f country/region	WWF: blind test evaluation (country)	WWF: blind test evaluation (region)
BT_2014_533	Ghana	uncertain - matched to Ivory Coast/Ghana cluster	uncertain - matched within 500 km	sequinom success = 96%	Ghana r/f		
BT_2014_543	Cameroon	confirmed	confirmed - within 120 km	sequinom success = 94%	Gabun f/f		
BT_2014_547	DRC	rejected	rejected	sequinom success = 93%	Ghana f/f		
BT_2014_551	CIV	confirmed	confirmed matched within 250 km	sequinom success = 97%	Ghana f/f		
BT_2014_563	CIV	confirmed	confirmed matched within 100 km	sequinom success = 97%	Ghana f/f		
BT_2014_567	DRC	rejected	rejected	sequinom success = 97%	Ghana f/f		
BT_2014_568	Cameroon	confirmed	confirmed - matched within 200 km	sequinom success = 97%	Cameroon r/f		
BT_2014_578	Cameroon	rejected	rejected	sequinom success = 96%	Ghana f/f		
BT_2014_594	Ghana	uncertain - matched to Ivory Coast/Ghana cluster	uncertain - matched within 350 km	sequinom success = 93%	Ghana r/f		
BT_2014_598	Ghana	confirmed	uncertain - matched within 150 km	sequinom success = 96%	Ghana r/r		

Table 10: Number of analysable reference samples for Ayou (University of Adelaide):

Country list reference samples	**Number of reference samples**
Cameroon	154
Congo B.	45
Côte d'Ivoire	208
DRC	123
Ghana	122
	652

4.2.2 DNA fingerprinting; Iroko (Thünen Institute of Forest Genetics)

Table 11:Result blind test part II (verification of declared origin); Iroko; DNA fingerprinting (TI of Forest Genetics; Großhansdorf-Germany; Dr. Céline Blanc-Jolivet). Black columns: table sent back from the lab; Red/coloured additional columns – 1. real country of origin and information if the declaration of country/region was true/false; columns 2. and 3. show WWF's evaluation of the test; Main task: verification of the declared country; Evaluation: green = verification of the declared country correct, red = verification of the declared country false.

ID	Declared Country	Claim on region within country	Final conclusion TI	Comments	WWF: real country; r/f country/region	WWF: blind test evaluation (country)	WWF: blind test evaluation (region)
BT_2014_526	Ghana	N6; W1 (60 km Radius)	confirmed		Ghana r/r		no result from lab
BT_2014_600	DRC	N2; E21 (200 km Radius)	rejected		DRC r/f		no result from lab
BT_2014_566	Cameroon	N3.5; E12 (100 km Radius)	confirmed		DRC f/f		no result from lab
BT_2014_596	Gabon	S3.09; E11.1 (50 km Radius)	confirmed		DRC f/f		no result from lab
BT_2014_554	Gabon	N1.3; E12.225 (60 km Radius)	confirmed		Cameroon f/f		no result from lab
BT_2014_549	Kenya	0; E35 (100 km Radius)	rejected	Central Africa	DRC f/f		no result from lab
BT_2014_569	DRC	N0.0131; E18.24 (40 km Radius)	rejected		DRC r/f		no result from lab
BT_2014_527	DRC	S2; E19 (180 km Radius)	confirmed		DRC r/r		no result from lab
BT_2014_518	Gabon	N0.84; E13.85 (40 km Radius)	rejected		Congo-Brazz. f/f		no result from lab
BT_2014_599	Ghana	N6.3; E0 (70 km Radius)	rejected	Ivory Coast	Ghana r/f		no result from lab

Table 12: Number of analysable reference samples for Iroko (TI):

Country list reference samples	Number of reference samples
Cameroon	306
Congo B.	260
Côte d'Ivoire	101
DRC	412
Gabon	252
Ghana	46
Kenya	103
	1480

4.2.3 DNA fingerprinting; Sapelli (Thünen Institute of Forest Genetics)

Table 13: Result blind test part II (verification of declared origin); Sapelli; DNA fingerprinting (Thünen Institute of Forest Genetics; Großhansdorf-Germany; Dr. Céline Blanc-Jolivet). Black columns: table sent back from the lab; Red/coloured additional columns – 1. real country of origin and information if the declaration of country/region was true/false; columns 2. and 3. show WWF's evaluation of the test; Main task: verification of the declared country; Evaluation: green = verification of the declared country/region correct, red = verification of the declared country/region false.

ID	Declared Country	Claim on region within country	Final conclusion Country	Final conclusion region	Comments	WWF: real country; r/f country/region	WWF: blind test evaluation (country)	WWF: blind test evaluation (region)
BT_2014_552	DRC	N0.45;E25.8 (200km Radius)	rejected	rejected		DRC r/r		
BT_2014_577	Cameroon	N2.8,E12 (40km Radius)	confirmed	confirmed		Cameroon r/r		
BT_2014_510	Ghana	N6.4;W1.2 (70km Radius)	confirmed	confirmed		Ghana r/r		
BT_2014_592	Congo. B.	N0.51544;E16.72308 (120km Radius)	confirmed	confirmed		DRC f/f		
BT_2014_573	DRC	S4.77;E16.9 (120km Radius)	confirmed	rejected	Orientale region most likely	DRC r/f		
BT_2014_557	DRC	S4;E19 (300km Radius)	rejected	rejected		DRC r/f		Formally right but possibly it is an artefact of a consecutive fault
BT_2014_580	Cameroon	N3.04,E14.5 (90km Radius)	rejected	rejected	likely region of origin between West Cameroon and Ghana	Congo B. f/f		
BT_2014_525	Ghana	N6.3;E0 (75km Radius)	confirmed	confirmed		Ghana r/f		
BT_2014_534	Congo Brazz.	S3;E15 (100km Radius)	confirmed	rejected	North region	DRC f/f		
BT_2014_522	DRC	N1.55115;E21.07416 (300km Radius)	rejected	rejected		DRC r/f		Formally right but possibly it is an artefact of a consecutive fault

Table 14: Number of analysable reference samples for Sapelli (TI):

Country list reference samples	Number of reference samples
Cameroon	434
Congo B.	141
Côte d'Ivoire	25
DRC	487
Gabon	36
Ghana	69
	1192

4.2.4 Stable Isotopes; Ayou (JR/HBLFA Francisco Josephinum)

Table 15: Result blind test part II (verification of declared origin); Ayou; Stable Isotopes (JR/HBLFA Francisco Josephinum; Wieselburg-Austria; Dr. Micha Horacek). Black columns: table sent back from the lab; Red/coloured additional columns – 1. real country of origin and information if the declaration of country/region was true/false; columns 2. and 3. show WWF's evaluation of the test; Main task: verification of the declared country; Evaluation: green = verification of the declared country correct, red = verification of the declared country false.

ID	Declared Origin	Interpretation	Remarks	WWF: real country of origin	WWF: blind test evaluation (country)	WWF: blind test evaluation (region)
BT_2014_578	Cameroon	NO	Declared origin of blind test sample is outside of regions covered by reference samples. As Sr-isotope value is very high (and thus also not in agreement with the Sr-isotopes of Mount Cameroon) we assume an incorrect declaration. Origin perhaps Cote d´Ivoire?	Ghana f/f		no result from lab
BT_2014_594	Ghana	YES		Ghana r/f		no result from lab
BT_2014_567	DRC	YES	Declared origin of blind test sample is far away from regions covered by reference samples, therefore no information is available for that region. Still, the results are in agreement with reference sample values (except for the Sr-isotope value). As Sr-isotopes are dominated by the bedrock (geology) and we do not have any Sr-isotope information from the declared origin we have no evidence against the declared origin. An alternative origin might be Ghana or Cote d`Ivoire.	Ghana f/f		no result from lab
BT_2014_568	Cameroon	YES		Cameroon r/f		no result from lab
BT_2014_598	Ghana	YES		Ghana r/r		no result from lab
BT_2014_547	DRC	YES	Declared origin of blind test sample is far away from regions covered by reference samples, therefore no information is available for that region. Still, the results are in agreement with reference sample values (except for the Sr-isotope value). As Sr-isotopes are dominated by the bedrock (geology) and we do not have any Sr-isotope information from the declared origin we have no evidence against the declared origin.	Ghana f/f		no result from lab
BT_2014_543	Cameroon	YES		Gabun f/f		no result from lab
BT_2014_563	Ivory Coast	YES		Ghana f/f		no result from lab
BT_2014_533	Ghana	YES		Ghana r/f		no result from lab
BT_2014_551	Ivory Coast	YES		Ghana f/f		no result from lab

General remarks [*HBLFA*]: The reference data set for Ayou(s) only contains the data of 167 instead of intended 210 reference samples (more than 20% less than planned). Of the 167 reference samples around 25% (and perhaps even more as relevant data are missing) have been taken from non-adult (juvenile) trees with diametres of 20cm and less. Furthermore, the reference samples have been collected in 3 ways: A) drill core material, B) tree shavings and C) tree bark. Only the first kind of sample is in accordance with the sampling protocol. Analysis of other sample material (B and C) influences the results obtained. This clearly is visible for example for the tree bark material (e.g. see reference values for Congo d18O-values clearly giving other numbers than the rest of the samples) and differences are also observed between materials A and B. As sampled sites should be characterized by 3-4 trees it becomes obvious that the data set is extremely limited - even for samples within the investigated regions. To our estimate the data set is too small to differentiate between regions within countries.

Table 16: Number of analysable reference samples for Ayou (HBLFA):

Country list reference samples	**Number of reference samples**	**comment**
Côte d'Ivoire	27	
Cameroon	41	
Ghana	34	
DRC	46	
Congo B.	0	19 bark samples - not analysable
	148	

4.2.5 Stable Isotopes; Iroko (Agroisolab GmbH)

Table 17: Result blind test part II (verification of the declared origin); Iroko; Stable Isotopes (Agroisolab GmbH; Jülich-Germany; Dr. Markus Boner). Black columns: table sent back from the lab; Red/coloured additional columns – 1. real country of origin and information if the declaration of country/region was true/false; columns 2. and 3. show WWF's evaluation of the test; Main task: verification of the declared country; Evaluation: green = verification of the declared country/region correct, red = verification of the declared country/region false.

Code	Declared country	Result, country level	Result, regional level	Remarks	WWF: real country; r/f country/region	WWF: blind test evaluation (country)	WWF: blind test evaluation (region)
BT_2014_526	Ghana	Yes, Poor confidence from origin	(Yes)	Samples show signatures which are difficult to predict the origin. Nevertheless an origin from Ghana is still likely. The next best alternative is: Cameroon. Numbers of references in that region is too small to evaluate the region.	Ghana r/r		
BT_2014_600	DRC	Yes, Good confidence from origin	No	Next alternative: Cameroon	DRC r/f		
BT_2014_566	Cameroon	No, Low confidence from Cameroon	/		DRC f/f		no analysis because country-level already „no“
BT_2014_596	Gabon	Yes Poor confidence from origin	(Yes)	The origin from Gabon is still likely. A significant prediction is not possible with that sample. Next alternative: RCB / Cameroon: 30 % Numbers of references (n=4) in that region is too small to evaluate the region.	DRC f/f		
BT_2014_554	Gabon	No, Low confidence from Gabon	/		Cameroon f/f		no analysis because country-level already „no“
BT_2014_549	Kenya	No, Excluded to be from origin Kenya	/		DRC f/f		no analysis because country-level already „no“
BT_2014_569	DRC	Yes, Poor confidence from origin	(Yes)	Overlapping between DRC and Cameroon. Nevertheless origin from DRC is more likely. No direct reference samples available. Therefore the sample is hard to evaluate	DRC r/f		
BT_2014_527	DRC	(No), Poor confidence from origin	/	Overlapping between DRC and Gabon. Therefore only weak probability. Currently an origin from Gabon is more likely.	DRC r/r		no analysis because country-level already „no“

BT_2014_518	Gabon	No, Low confidence from Gabon	/		Congo B. f/f		no analysis because country-level already „no“
BT_2014_599	Ghana	No, Low confidence from Ghana	/	Numbers of references in that region is too small to evaluate the region.	Ghana r/f		no analysis because country-level already „no“

Table 18: Number of analysable reference samples for Iroko (Agroisolab GmbH)

Country list reference samples	**Number of reference samples**	**Comments**
Cameroon	52	
Congo B.	73	10 samples bark or thin branches with bark
Côte d'Ivoire	28	2 samples from bark
DRC	141	8 samples from bark and one thin branch
Gabon	94	
Ghana	40	
Kenya	25	
	453	

4.2.6 Stable Isotopes; Sapelli (FERA Science Ltd.)

Table 19: Result blind test part II (verification of the declared origin); Iroko; Stable Isotopes (FERA Science Ltd.; York-UK) - Result based on reference data collected inside ITTO project and techniques contracted inside the ITTO project (isotopes only). Black columns: table sent back from the lab; ; Red/coloured additional columns – 1. real country of origin and information if the declaration of country/region was true/false; columns 2. and 3. show WWF's evaluation of the test; Main task: verification of the declared country; Evaluation: green = verification of the declared country/region correct, red = verification of the declared country/region false.

ID-number	declared country of origin	Y/N result concerning the declaration of origin: COUNTRY	Y/N result concerning the declaration of origin: REGION	WWF: real country; r/f country/region	WWF: blind test evaluation (country)	WWF: blind test evaluation (region)
BT_2014_552	DRC	Correct	Correct	DRC r/r		
BT_2014_577	Cameroon	Correct	Correct	Cameroon r/r		
BT_2014_510	Ghana	Correct	Correct	Ghana r/r		
BT_2014_592	Congo B.	Correct	Correct	DRC f/f		
BT_2014_573	DRC	Correct	Not correct	DRC r/f		
BT_2014_557	DRC	Correct	Not correct	DRC r/f		
BT_2014_580	Cameroon	Correct	Correct	Congo B. f/f		
BT_2014_525	Ghana	Correct	Correct	Ghana r/f		
BT_2014_534	Congo B.	Correct	Correct	DRC f/f		
BT_2014_522	DRC	Correct	Correct	DRC r/f		

Table 20: Number of analysable reference samples for Sapelli (FERA Science Ltd.)

Country list reference samples	Number of reference samples
Cameroon	39
Congo (B.)	29
DRC	119
Ghana	17
Côte d'Ivoire	11
Gabon	12
	227

5 Summary and overview of the blind test results

Table 21: Summary of the results from blind test part I,species verification

Method	Verification of the declared species (Main question of ITTO blind test)	Identification at family level	Identification at genus level	Identification at species level
Wood anatomy	72 % correct and 28 % correct with limitation (as far as the method allows)	100 % correct	100 % correct	56 % correct
				44 % no result from lab
DNA barcoding	24 % correct and 8 % correct with limitation	32 % correct	28 % correct	8 % correct
	64 % no result from lab	64 % no result from lab	64 % no result from lab	88 % no result from lab
	4 % wrong	4 % wrong	8 % wrong	
				4 % uncertain

Table 22: Summary of the results from blind test part IIdeclaration of origin

Type	Species	Institute	Basic level -country- (Main question of ITTO blind test)	Advanced level -region-
DNA	Ayou	Adelaide	50 % correct	30 % correct
			20 % uncertain	30 % uncertain
			30 % wrong	40 % wrong
DNA	Iroko	TI	40 % correct	No results from lab
			60 % wrong	
DNA	Sapelli	TI	50 % correct	50 % correct
			50 % wrong	20 % consecutive fault
				30 % wrong
Isotope	Ayou	Josephinum	50 % correct	No results from lab
			50 % wrong	
Isotope	Iroko	Agroisolab	70 % correct	20 % correct
			30 % wrong	60 % no analysis
				20 % wrong
Isotope	Sapelli	FERA	70 % correct	50 % correct
		"inside" ITTO	30 % wrong	50 % wrong

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9 Appendix

Appendix 1

THÜNEN

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Ihr Zeichen/Ihre Nachricht vom:	Unser Zeichen/Unsere Nachricht vom:	Datum
		11.03.2013

Memorandum of Agreement im Rahmen des Projekts PD 620/11 M (Rev. 1)/ITTO

Sehr geehrter Herr Zahnen,

anbei übersende ich Ihnen zwei Exemplare der o. g. Vereinbarung. Bitte lassen Sie ein Exemplar durch Herrn Heinrich gegenzeichnen und senden dieses zu meinen Händen zurück.
Vielen Dank.

Mit freundlichen Grüßen
Im Auftrag

Vivien Steinel

MEMORANDUM OF AGREEMENT
between

Johann Heinrich von Thünen-Institute (TI),
Federal Research Institute for Rural Areas, Forestry and Fisheries
Bundesallee 50
D-38116 Braunschweig, Germany

hereinafter referred to as **EXECUTING AGENCY (EA)**

and

World Wild Fund for Nature, Germany (WWF/Germany)
Reinhardtstraße 14
D-10117 Berlin, Germany

hereinafter referred to as **COLLABORATING AGENCY (CA)**

ON THE IMPLEMENTATION OF THE PROJECT **PD 620/11 M (Rev. 1)** of

The International Tropical Timber Organization (ITTO)

"Development and implementation of a species identification and timber tracking system in Africa with DNA fingerprints and stable isotopes"

We, the undersigned authorized representatives of EA and CA; endorse this agreement as the formal document guiding project implementation by both agencies in accordance with the Project Agreement.

SECTION 1. GENERAL RESPONSIBILITIES

EA is the Executing Agency for the project. It will be in charge of general management and administration of all Project activities, and will also be directly responsible for reporting all results to ITTO. It will be responsible for receiving, disbursing, and managing all funds released by ITTO to the project in accordance with ITTO's rules and regulations.

SECTION 2. SPECIAL RESPONSIBILITIES OF THE CA

CA will be a Collaborating Agency for the project implementation. According to the project proposal it shall be responsible for:

a) Activity 1.4: Sampling for the blind testing 50 wood samples belonging to 21 taxa (*Afzelia spp., Aningeria robusta, Aucoumea klaineana, Baillonella toxisperma, Cylicodiscus gabunensis, Entandrophragma angolense, Entandrophragma cylindricum, Entandrophragma utile, Erythrophleum ivorense, Erythrophleum Suaveolens, Guibourtia spp., Khaya spp., Lophira alata, Milicia excelsa, Milicia. Regia, Millettia laurentii, Nauclea diderrichii, Pericopsis elata, Pterocarpus soyauxii, Terminalia superb* and *Triplochiton scleroxylo*) to test the DNA barcodes and wood anatomical tools for species identification.

For this sampling, the country of origin does not matter. It is important to provide wood samples of the 21 taxa but also others that either belongs to the same genus or that are very similar in terms of wood anatomy. The material should be labelled only with numbers and a wrong or false claim on the species name and send to EA.

b) Activity 2.9: Sampling for the blind testing of 60 wood samples to test the power of the genetic and isotopic reference data base to control claims on the country of origin

The 60 wood samples of this test should be composed of 20 wood samples of each of the three species *Milicia excelsa, Entandrophragma cylindricum and Triplochiton scleroxylon.* The samples should partly be from the seven African countries (Cameroon, Central African Republic, Democratic Republic of Congo, Republic of Congo, Gabon, Ghana and Kenya.) and partly from other countries. The samples should include material with correct and false declarations (claims) on the country of origin. All three target species should be covered, but the 20 samples for each species do not need to come from 20 different locations. The material should be labelled only with numbers, the correct species name and a wrong or false claim on the country of origin. The material should be send to EA for further distribution.

Both tests should be done using saw timber or equal treated material in order to keep it close to the later practical application of the developed tools.

CA agrees to deploy the necessary personnel to provide management, administrative, and technical support in project implementation in accordance with the work-plan. CA is aware that very strict confidentiality should be maintained on the identity of the species and the geographic origin of the material. Especially this information on species and origin should not be known or given to the any of the project partner.
The CA needs to organise the material according to the above requirements, distribute it with correct and false claims to the EA, receive the results of the labs, compare it with the correct species or country of origin and make a short report on the outcomes for the EA.

SECTION 3. ACCEPTENCE

CA has to accept the work described in Section 2. and to deliver an evaluation of the blind test. Additional comments to the evaluation or interpretation from others than the CA can be done as long as it is distinguishable to the CA's text. Any change of the CA's blind test evaluation (see sentence 1) has to be confirmed by CA.

SECTION 4. REMUNERATION AND CONTRACT PERIOD

A maximum amount of 20,000 US$ is reserved for the activities of the CA during the contract period 01.04.2013 to 30.09.2014. The agreed remuneration is only due for the proper performance and there are no additional claims for remuneration or fees exceeding the total sum.

The first payment of 10,000 US$ will be made by the EA on 15.04.2013, a second payment of 5,000 US$ will be made when 75% of the total sampling of sections 2a and 2b is completed. A final payment of 5,000 US$ will be done after the submission of a final report detailing the achievement of the sampling objectives and the results of the blind tests. The payment will be done according to invoices from the CA. The payment will be done in Euros: the official UN exchange rate of 1 January 2012 (USD 1 = EURO 0.774) will be applied.

For the preparation of cost statements the CA shall provide the EA with proofs of spent budget. The CA shall keep strict budgetary control over the funds allocated to it and keep such funds, until the time of their actual disbursement, with a bank of commonly recognized high reputation. The CA will keep all original invoices for the cost statements to be prepared for the ITTO by the EA.

If the actual costs of the activities of the CA are less than is provided for in the project budget under this Agreement, the balance remaining unspent on completion of the Project, shall be returned to the EA.

SECTION 5. REPORTING REQUIREMENTS

EA shall be responsible for preparing and submitting to ITTO all progress reports, technical reports, and completion report in accordance with the project document and project agreement. It shall also submit to ITTO needed financial statements on use of projects funds and at the end of the project a final statement of account for ITTO and counterpart funds audited by recognized independent auditors appointed by EA in consultation with ITTO.
CA shall assist in the reporting requirements by providing data, cost statements and information as needed. This Agreement shall take effect upon signing by both agencies.

SECTION 6. USE OF PLANT MATERIAL AND DATA

The EA and CA agree that the material collected for the blind test will be sent to the EA which then sends the material to the agencies responsible for these tests. The collected material and data collected will be used for the purpose of the ITTO project (PD 620/11 M (Rev. 1)) and derived publications. EA and CA will be included in the authors list of all derived publications.

SECTION 7. LIABILITY

The EA is discharged from every liability both to a third party and to CA's losses caused by the fulfilment of this contract.

SECTION 8. WITHDRAWAL

The EA can withdraw from the contract if CA does not meet the deadline for production as agreed in Section 4 neither an adequate respite or if he does not remedy deficiencies in a stipulated period. In case of withdrawal from the contract all the carried out and accepted work will be paid according to the relation of the total and usable work.

SECTION 9. ALTERATIONS OF/AMENDMENTS TO THE CONTRACT

Any changes, modifications and amendments to this contract must be in writing. Should any provision of this contract be or become invalid, this shall not affect the validity of the remaining provisions of the contract.

SECTION 10. JURISDICTION

Venue shall be Braunschweig (Germany)

On behalf of the Executing Agency	On behalf of the Collaborating Agency
Prof. Dr. Folkhard Isermeyer - President of the Thünen-Institute-	Christoph Heinrich - Executive Officer Conservation WWF Germany-
Date: 11. 03. 2013 Place: Braunschweig	Date: 25. 03. 2013 Place: Berlin
Signature:	Signature:

Additional Instructions for the blind test of the ITTO-Africa project

(Agreed between Thünen-Institute and WWF in Berlin the 27/02/2015)

The blind test will be done with 50% of the samples compared to the WWF-MoA contract (3/2013). The other 50% of the samples has been collected by Gersyn Services (G2S). This means from each of the two collections 25 of the 50 samples for the tests of species declarations will be taken and for each of the three species *Entandrophragma cylindicum* (Sapelli), *Milicia excelsa* (Iroko) and *Triplochiton scleroxylon* (Ayous) 10 samples for the tests on declared country of origin will be used.

WWF and G2S will be responsible for two independent blind tests (including test on species and origin). The organisations will provide the Thünen-Institute of Forest Genetics with the list of samples that have been selected for the test. They will provide a table with the sample number and declared tree species for the species test and with the declared country of origin and partly with GPS coordinates combined with a radius that describes a region or with a description of the region in case of G2S. The claims are a mixture of true and false declarations. In case of false declarations of the origin of the WWF samples, false and true origins have to be at least 150 km away from each other.

For a fair test we need to ensure that the two alternative laboratories get samples from the same tree individual, of same quality, same consistence and with the same claimed species or declared origin respectively. G2S delivered the Thünen-Institute for each sample a single piece of saw timber. WWF will (via the University of Marburg) provide most of the samples in kind of saw timber or chiselled from trees in the forest. Each of the samples will be divided in 4 portions of approx. 10g. Each of the involved laboratories will get a set of samples (a full set of the Part II blind test (origin) will go to Thorsten Hinrichs; Ministry BMEL; one full set will go to Bernd Degen TI; three sets á 10 samples (Sapeli, Iroko, Ayou) will go to three different isotope labs and three sets will go to the two genetic lab). There are four blind test samples from the WWF set (sawn wood) without additional reference material at the University of Marburg. WWF will inform Bernd Degen about the samples ID numbers so he can send the samples to the University of Marburg. One complete set will be kept as back up reference at the Thünen-Institute and one complete set of each samples will be sent to Thorsten Hinrichs at the Federal Ministry of Food and Agriculture (BMEL) as reference samples, in case anyone doubts on the identity of the samples. For Part I of the blind test

(species) Bernd Degen ordered the set of 50 samples given by WWF to Gerald Koch. WWF will select from the list 25 out of these 50 samples for the species test. Bernd Degen will forward these 25 samples to Plant Genetic Diagnostics GmbH for the genetic species test. The WWF samples with claims on the origin will be taken from the samples stored at the University of Marburg. The selection of the 10 samples per species should include only those with country claims with at least 20 reference samples (DNA and isotopes). Also from the WWF samples 4 pieces per sample will be created and distributed in the same way as the samples from G2S.

The laboratories can only check claims for which the tools and reference data have been developed in frame of the ITTO project.

This means each of the samples for the species test can be any species but should have a claim on one of the following 21 alternative taxa: *Afzelia spp., Aningeria robusta, Aucoumea klaineana, Baillonella toxisperma, Cylicodiscus gabunensis, Entandrophragma angolense, Entandrophragma cylindricum, Entandrophragma utile, Erythrophleum ivorense, Erythrophleum suaveolens, Guibourtia spp., Khaya spp., Lophira alata, Milicia excelsa, Milicia regia, Millettia laurentii, Nauclea diderrichii, Pericopsis elata, Pterocarpus soyauxii, Terminalia superb and Triplochiton scleroxylon.*

For the samples of the test on declared country of origin the declared origin should be from the list of the following countries:
Milicia excelsa (Iroko): Ghana, DRC, Cameroun, Gabon, Kenya
Entandrophragma cylindricum (Sapelli): Cameroun, Ghana, DRC, Congo Brazzaville
Triplochiton scleroxylon (Ayous): Cameroun., Ghana, DRC, Congo Brazzaville, Ivory coast
The claims on the country/region of origin are either right or wrong. For the WWF samples is guaranteed that the declared country or GPS coordinates that are wrong, are at least 150 km away from the correct place of origin.

Before the analysis of blind test samples starts, WWF and G2S will send the list of claims of the selected samples together with the solution on the true tree species or the true origin to Thorsten Hinrichs at the Federal Ministry of Food and Agriculture (BMEL).

The laboratories analysing the blind test samples should check if the declaration is correct or not. The result of these analyses is for each sample and question (species; origin) a “yes” or “no”. With regard to origin, the labs should verify both the declared country of origin and if given the declared region of origin. They can provide comments on the results. The tests need to be done with the data collected in frame of the ITTO project. In addition the laboratories can provide a second test result for each sample using additional reference samples and /or additional variables (e.g. other isotopes, gene markers, other techniques like NIR). The second test result need to be clearly labelled and identified.

In order to guarantee complete transparency the reference data used for the blind test and the method of data analysis should be provided to any of the involved partners (provider of blind test samples, involved laboratories, and executive agency of the project) including a description of the laboratory methods, statistical approach, used software and formulas. The data should be provided to these parties in a form that experts could recalculate and confirm the analytical approach and conclusions. BMEL stores the samples for a period of one year after the end of the project.

WWF and G2S will – independent from each other - provide the Thünen-Institute of Forest Genetics a short report with the evaluation of the blind test on the comparison of the laboratories result and the true tree species or the true origin. The report should clearly state which results have been generated with reference data of the ITTO project and which result have been elaborated by the laboratories using additional reference data and / or analysed variables.

Appendix 3

RAHMENKOOPERATIONSVEREINBARUNG # 14266

Zwischen dem

WWF Deutschland
Reinhardtstraße 14
10117 Berlin

vertreten durch Sylvia Becker

- im folgenden „**WWF**" genannt -

und der

Philipps-Universität Marburg
Biegenstraße 10
35032 Marburg

vertreten durch die Präsidentin

Ausführende Stelle:
Prof. Dr. Nina Farwig und Prof. Dr. Birgit Ziegenhagen
FB Biologie
Karl-von-Frisch-Straße 8
35032 Marburg

- im folgenden „**UMR**" genannt -

- WWF und UMR werden im Folgenden einzeln oder gemeinsam auch als „Kooperationspartner" bezeichnet -

gültig. Eine weitere Kooperationsvereinbarung oder eine schriftliche Vertragsverlängerung bleibt davon unberührt.

5.2 Jeder Kooperationspartner ist berechtigt die Vereinbarung mit einer Frist von 6 Monaten zu kündigen. Eine Kündigung aus wichtigem Grund unter Beachtung der gesetzlichen Bestimmungen bleibt unberührt.

5.3 Die Kündigung hat schriftlich zu erfolgen.

6. Geheimhaltung

6.1 Die Kooperationspartner verpflichten sich, geheimhaltungspflichtige Informationen und Gegenstände, die geheimhaltungspflichtige Informationen enthalten, wie z.B. Unterlagen, Muster, Materialproben, welche ihnen im Rahmen der Kooperation evtl. vom anderen Kooperationspartner offenbart werden, streng vertraulich zu behandeln, sie Dritten ohne vorherige schriftliche Zustimmung des offenbarenden Kooperationspartners nicht zugänglich zu machen und nur solchen Mitarbeitern des Kooperationspartners zugänglich zu machen, die diese für die Durchführung der im Rahmen dieses Vertrages zu erbringenden Arbeiten benötigen. Darüber hinaus werden sie die ihnen vom jeweils anderen Kooperationspartner offenbarten geheimhaltungspflichtigen Informationen ausschließlich zur Durchführung der Forschungsarbeiten unter dieser Vereinbarung verwenden.

6.2 Die Pflicht zur Geheimhaltung und Nichtverwertung entfällt für Informationen

a) die sich zum Zeitpunkt ihrer Übermittlung nachweislich im Besitz des die Information empfangenden Kooperationspartners befanden;

b) die zum Zeitpunkt der Übermittlung öffentlich bekannt waren;

c) die nach dem Zeitpunkt ihrer Übermittlung ohne Verschulden des die Information empfangenden Kooperationspartners öffentlich bekannt werden;

d) die der die Information empfangende Kooperationspartner nach ihrer Übermittlung durch den offenbarenden Kooperationspartner von einem Dritten erwirbt, sofern dieser Dritte nicht dem offenbarenden Kooperationspartner gegenüber zur Geheimhaltung verpflichtet ist und ein Recht zu einer derartigen Weitergabe hat.

6.3 Alle geheimhaltungspflichtigen Informationen wird der empfangende Kooperationspartner sorgfältig aufbewahren, vor jeder Einsichtnahme Dritter schützen und auf Verlangen jederzeit - spätestens aber bei Beendigung dieser Vereinbarung - dem offenbarenden Kooperationspartner übergeben.

6.4 Die Geheimhaltungspflichten enden fünf Jahre nach Beendigung dieser Vereinbarung gemäß Ziffer 5.

Für den WWF Deutschland

Berlin, den 23.04.14

Sylvia Becker
Kaufmännische Geschäftsleitung

Berlin, den 23.04.14

Annette Heiß
Referentin Kaufm. Projektabwicklung

Für die Philipps-Universität Marburg

Marburg, den 06. Mai 2014

i.V.
Prof. Dr. Katharina Krause
- Präsidentin der Philipps-Universität Marburg -

Marburg, den 08.05.2014

Prof. Dr. Nina Farwig
- Verantwortliche Wissenschaftlerin

Marburg, den 08.05.2014

Prof. Dr. Birgit Ziegenhagen
- Kooperierende Wissenschaftlerin -

Anhang 1: Probenprotokoll: Isotopes, DNA and wood anatomy;
Mandatory methodology and scientific procedures agreed upon by Project Partners
Anhang 2: Budget
Anhang 3: Projektbeschreibung

Appendix 4

Institut für Holztechnologie Dresden · Zellescher Weg 24 · 01217 Dresden · Germany

Herrn Johannes Zahnen
Forstpolitik/Unternehmenskooperation
WWF Deutschland
Reinhardtstraße 18
D-10117 Berlin

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gemeinnützige GmbH
Zellescher Weg 24
01217 Dresden

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Fax: +49 351 4662 211
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www.ihd-dresden.de

Dresden, 10.02.2015

Untersuchungsbericht
Auftrags-Nr.: 1215022

Auftrag:	Mikroskopische Holzartenbestimmungen an zwei Proben aus der Demokratischen Republik Kongo
Auftrag vom:	30.01.2015
Auftraggeber (AG):	Herr Johannes Zahnen, Forstpolitik/Unternehmenskooperation, WWF Deutschland, Reinhardtstraße 18, D-10117 Berlin über Philipps-Universität Marburg, Naturschutzbiologie, Frau Kristina Osen, Karl-von-Frisch-Str. 8, 35043 Marburg
Auftragnehmer (AN):	Institut für Holztechnologie Dresden gemeinnützige GmbH (IHD)
Verantw. Bearbeiter:	Dipl.-Ing. (FH) Björn Weiß

Dr. W. Scheiding
Ressortleiter Biologie/Holzschutz

Der Untersuchungsbericht enthält 8 Seiten einschließlich 13 Fotos. Jede auszugsweise Vervielfältigung bedarf der schriftlichen Genehmigung des IHD. Die Untersuchungsergebnisse beziehen sich ausschließlich auf das untersuchte Material.

1 Auftrag

Das Institut für Holztechnologie gemeinnützige GmbH wurde beauftragt, mikroskopische Holzartenbestimmungen an zwei Proben aus der Demokratischen Republik Kongo durchzuführen.

2 Untersuchungsmaterial

Die beiden Holzproben wurden von Frau Kristina Osen im Mai 2014 in der Demokratischen Republik Kongo genommen und zur Untersuchung gesandt (Foto 1).

Probenbezeichnungen:
A_DRC_2014: *Entandrophragma cylindricum* (Handelsname: Sapelli; Familie Meliaceae),
B_DRC_2014: *Nauclea diderichii* (Handelsname: Bilinga; Familie Rubiaceae).

Probenabmessungen: jeweils etwa 60 mm lang, 30 mm breit und 5 mm dick.

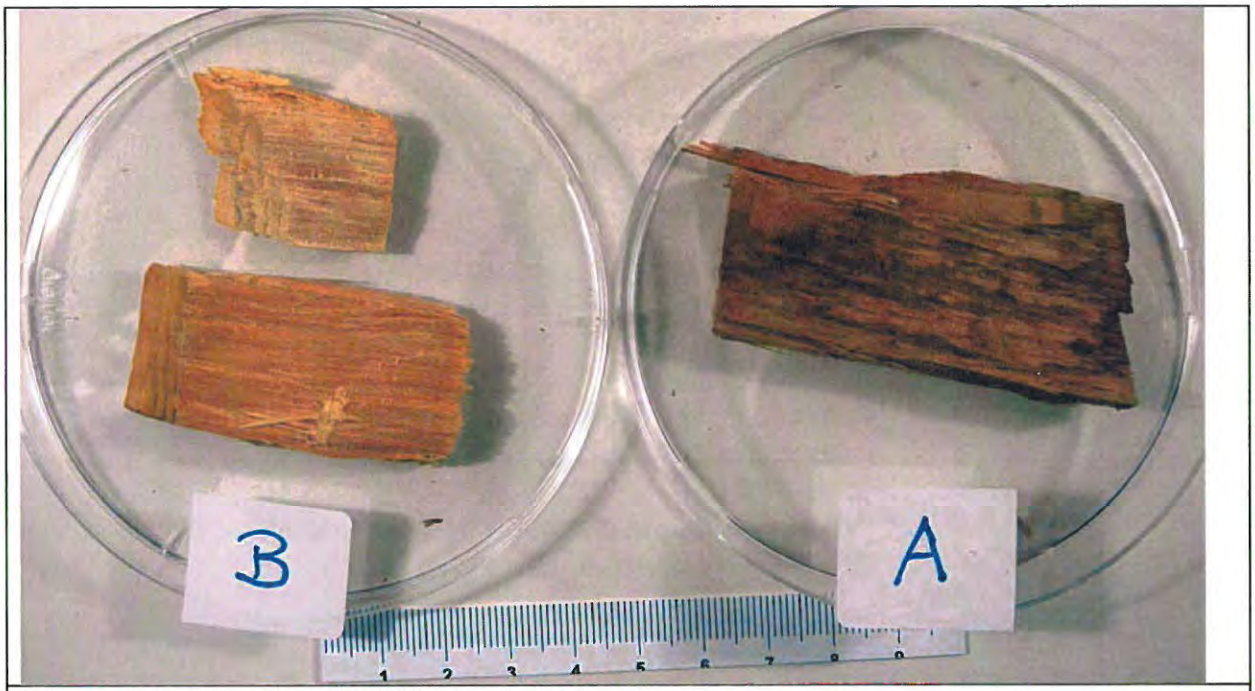

Foto 1: Untersuchungsmaterial Probe A und B

3 Untersuchungsdurchführung

Für die Holzartenbestimmungen wurden vom Untersuchungsmaterial Holzdünnschnitte in den 3 holzanatomischen Hauptschnittrichtungen angefertigt (Querschnitt: Q, Tangentialschnitt: T, Radialschnitt: R). Die Dünnschnitte wurden mit dem Durchlichtmikroskop „ECLIPSE E-800" der Firma Nikon, bei Vergrößerungen von 40- bis 600fach untersucht. Anhand der ermittelten Strukturmerkmale wurden die Holzarten bestimmt.
Über die entsprechende Kopplung mit dem Bildverarbeitungssystem „NIS-Elements D3.2" und der digitalen „Camera DS-Fi1c" wurden Messungen durchgeführt und Fotos erstellt.
Zum Vergleich dienten Holzdünnschnittpräparate, mikrofotografische Abbildungen, Holzstrukturbeschreibungen und Bestimmungsschlüssel.

4 Untersuchungsergebnisse

4.1 Probe A

Folgende Merkmale wurden bei Probe A festgestellt, s. auch Mikrofotos 2-9:

Farbe	– grau bis rötlich (wahrscheinlich Splintholz); Foto 1
Gefäße	– zerstreutporig angeordnet, meist einzeln, auch paarig (Foto 2, 3) – Form rundlich (Foto 4, 5) – wenig zahlreich – Ø etwa 50 ... 115 ... 140 – Gefäßtüpfel sehr klein – vereinzelt rote Kernstoffe vorhanden (Foto 5, 8, 9); waschen intensiv bei der Präparation aus
Längsparenchym	– um die Gefäße (paratracheal-vasizentrisch; par.- vasizentrisch), auch konfluent (Foto 2 - 5) – in weiten Abständen breite tangentiale Bänder, 5 ... 7 Zellen breit; (apotracheal-marginal); Foto 5
Holzstrahlen	– stellenweise stockwerkartig (Foto 8) und stellenweise unregelmäßig angeordnet (Foto 6, 7) – heterogen aufgebaut, mit einer aufrechten Kantenzelle (Foto 7, 9) – Breite 2 ... 6 Zellen, meist 3 bis 4 (Foto 6 - 8) – Höhe: 200 ... 450 ... 660 µm (Foto 7, 8) – vereinzelt Kalziumoxalatkristalle vorhanden
Fasern	– radial angeordnet (Foto 5) – Libriformfasern

(Gefäße ... G, Längsparenchym ... LP, Holzstrahlen ... HS, Fasern ... F)

Bestimmte Holzart: die festgestellten Merkmale der Holzprobe A stimmen mit denen von Sapelli (*Entandrophragma cylindricum*) überein.

Mikrofotos Probe A

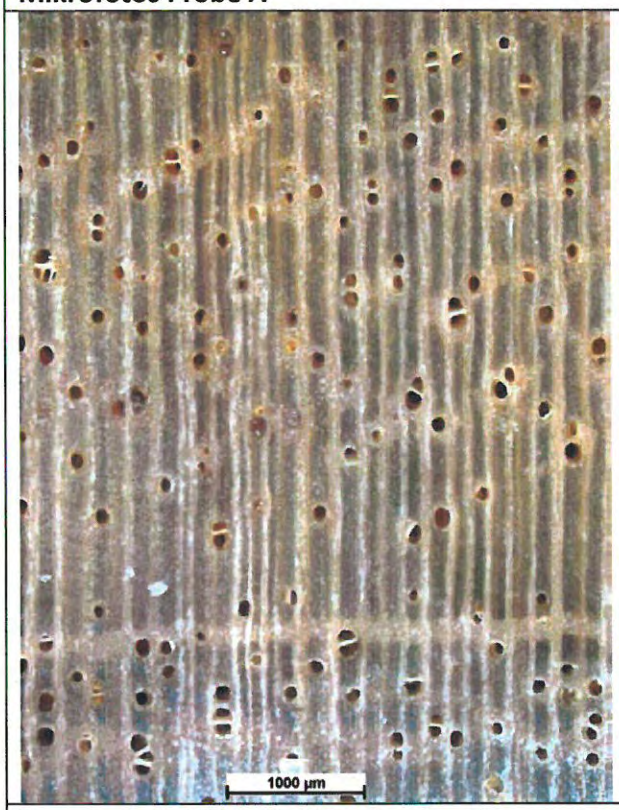

Foto 2: Q, Auflicht; Gefäße einzeln oder paarig; M 20:1

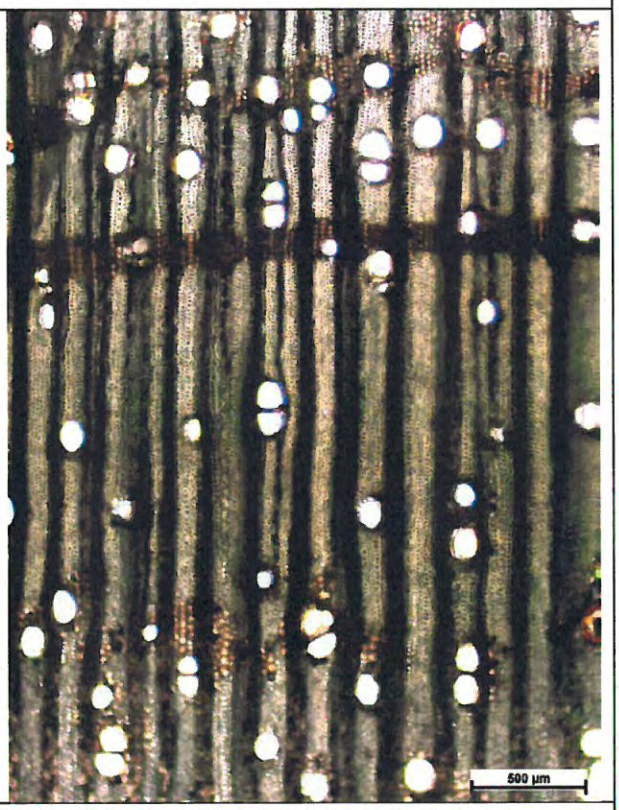

Foto 3: Q, Durchlicht; LP-Band vorhanden (apotracheal-marginal); M 40:1

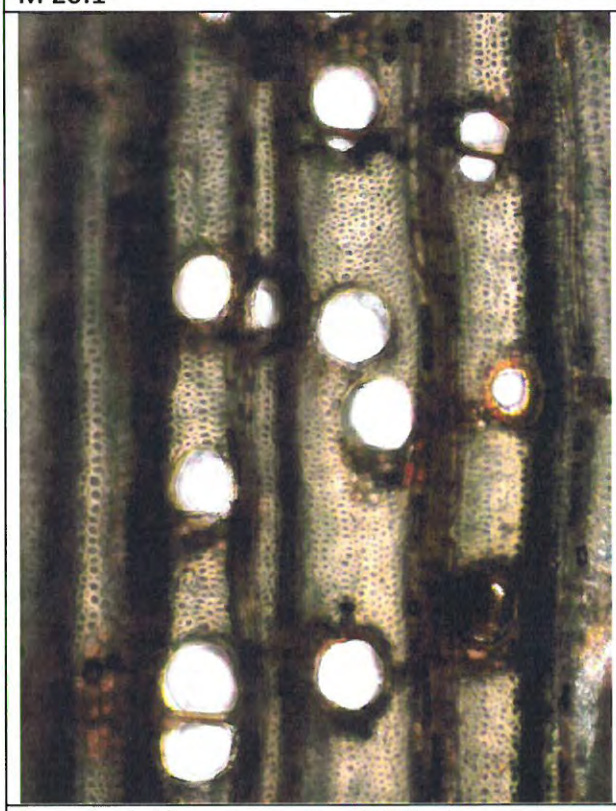

Foto 4: Q; LP auch um die Gefäße (paratracheal-vasizentrisch); M 100:1

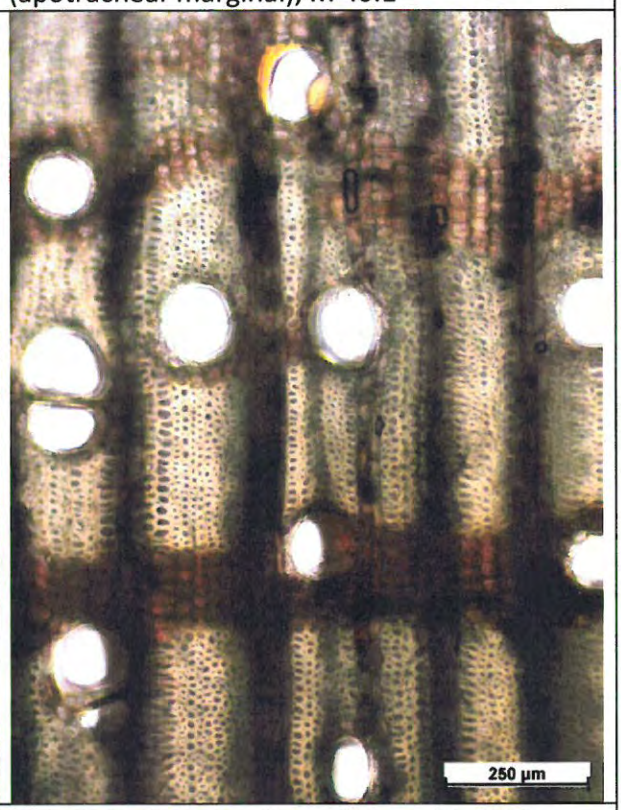

Foto 5: Q; Fasern radial gerichtet; M 100:1

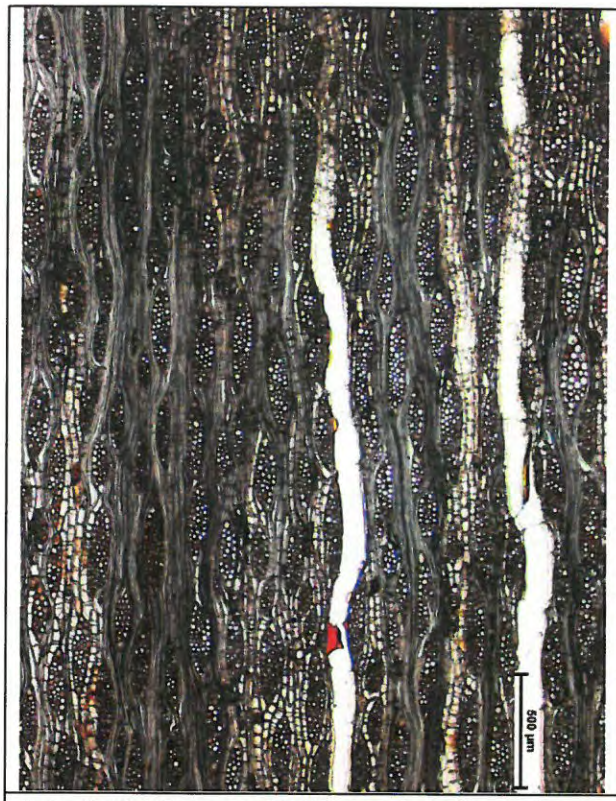

Foto 6: T; HS unregelmäßig und stellenweise mit Stockwerkbau; M 40:1

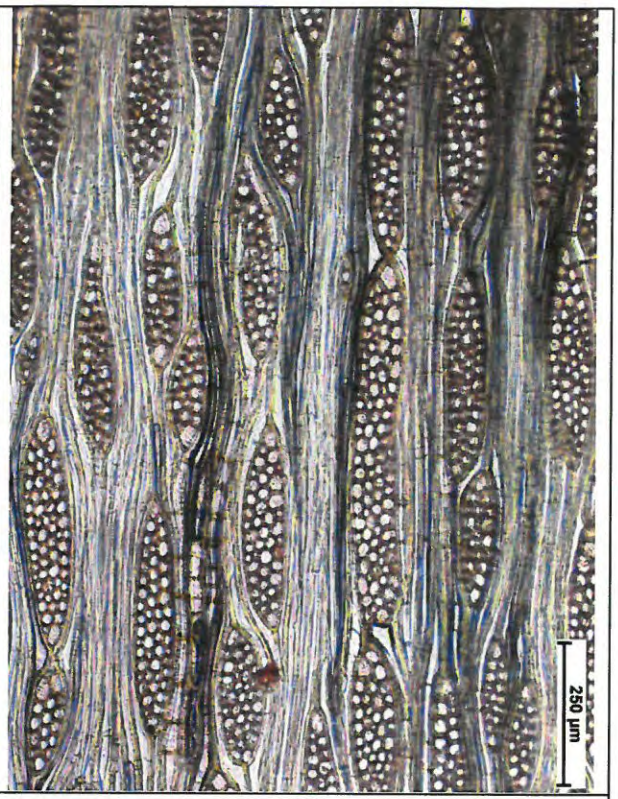

Foto 7: T; HS unregelmäßig angeordnet, M 100:1

Foto 8: T; Bereich mit stockwerkartiger HS-Anordnung; M 100:1

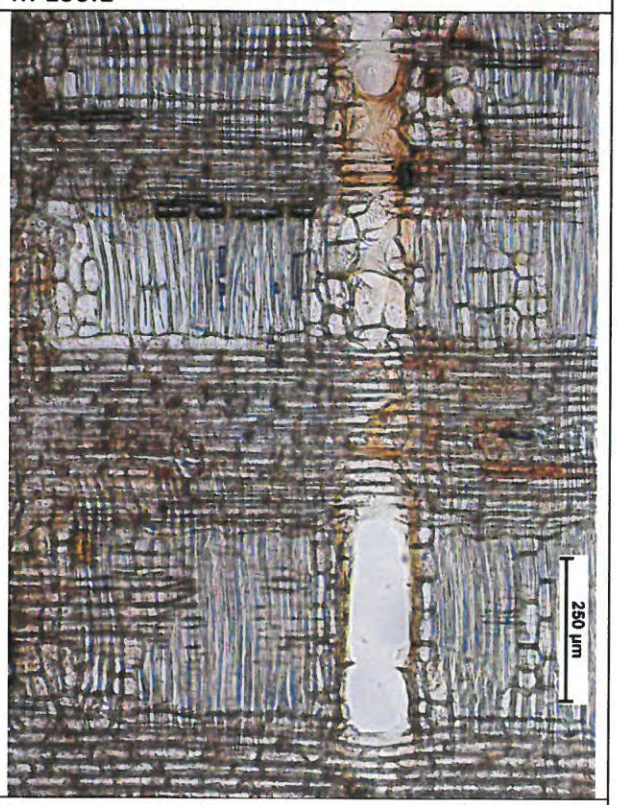

Foto 9: R; HS schwach heterogen aufgebaut; M 100:1

4.2 Probe B

Folgende Merkmale wurden bei Probe B festgestellt, s. auch Mikrofotos 10-13:

Farbe	– gelb
Gefäße	– zerstreutporig angeordnet, meist einzeln, selten auch paarig (Foto 10) – Form leicht oval (Foto 10, 11) – Anzahl gering – Ø etwa 80 ... 160 ... 210 µm
Längsparenchym	– in kurzen tangentialen Linien (apotracheal, tangetial-leiterförmig), Foto 11
Holzstrahlen	– unregelmäßig angeordnet, heterogen aufgebaut, – 1 bis 4 Zellen breit; einzelne HS sehr unregelmäßig zusammengesetzt, aus ein- und mehrschichtigen Teilen bestehend (Foto 12) – Höhe: 150 ... 1300 ... 1700 µm
Fasern	– radial angeordnet (Foto 11) – überwiegend Fasertracheiden

(Gefäße ... G, Längsparenchym ... LP, Holzstrahlen ... HS, Fasern ... F)

Bestimmte Holzart: die festgestellten Merkmale der Holzprobe B stimmen mit denen von Bilinga (*Nauclea diderichii*) überein.

Mikrofotos Probe B

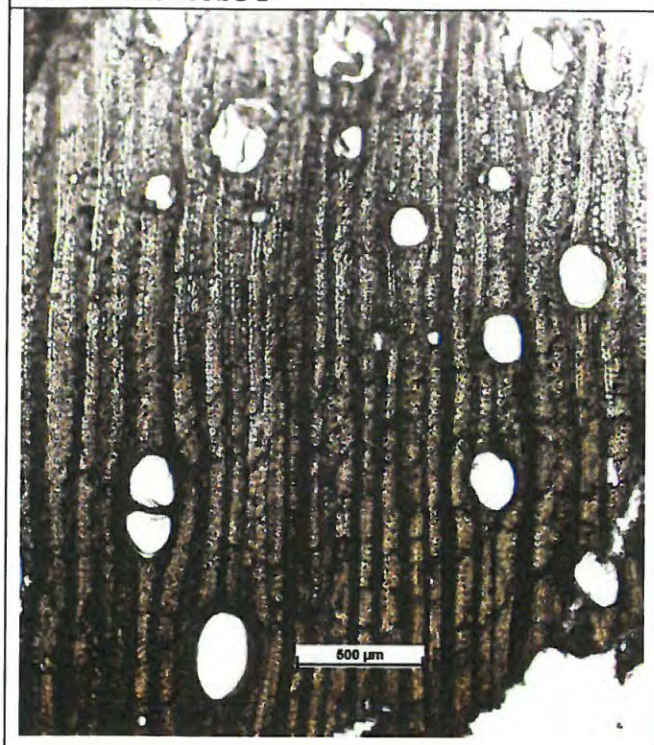

Foto 10: Q, Durchlicht; Gefäße leicht oval; M 40:1

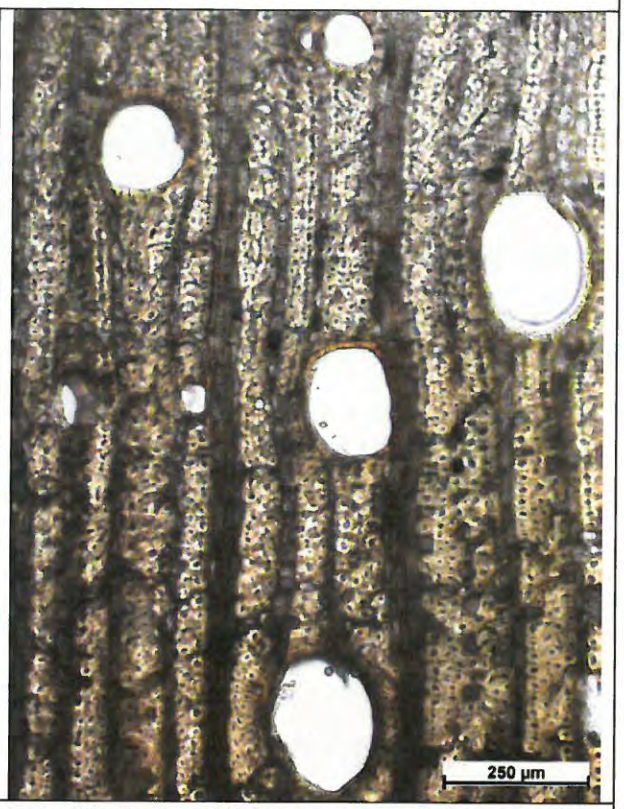

Foto 11: Q, Durchlicht; Fasern radial angeordnet; M 100:1

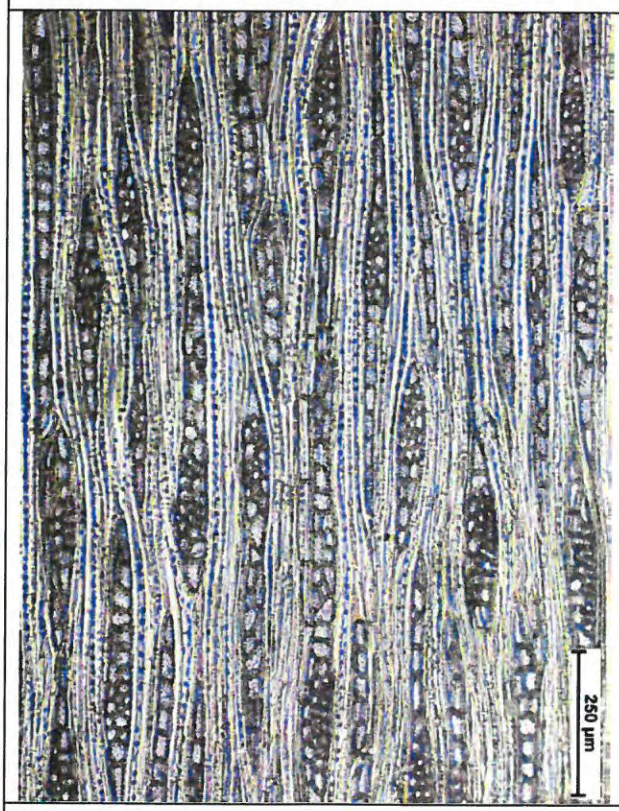

Foto 12: T; HS aus ein- und mehrschichtigen Teilen zusammengesetzt; M 100:1

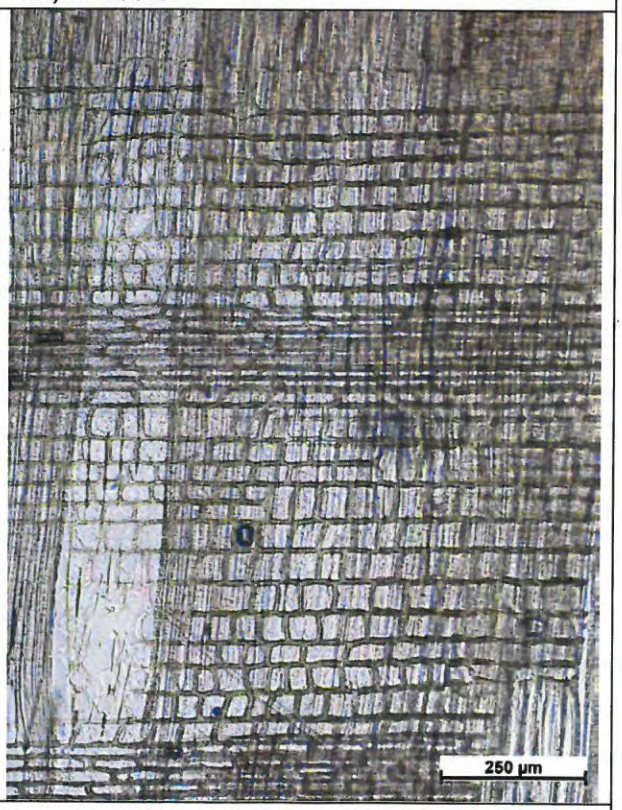

Foto 13: R; HS stark heterogen aufgebaut; M 100:1

5 Zusammenfassung

Bei den makroskopischen und mikroskopischen Untersuchungen der beiden zugesandten Proben wurde festgestellt, dass bei Probe A die Holzstrukturmerkmale mit der Holzart Sapelli (*Entandrophragma cylindricum*) übereinstimmen. Als Vergleich hierzu dienten u.a. zahlreiche Abbildungen aus früheren Untersuchungen von Sapelli aus dem Kongo, an denen ebenfalls zu erkennen ist, dass die Holzstrahlen in vielen Bereichen auch unregelmäßig angeordnet sind.

Die festgestellten Holzstrukturmerkmale der Holzprobe B stimmen mit denen von Bilinga (*Nauclea diderichii*) überein.

Entsprechend kann bestätigt werden, dass es sich bei den beiden Holzproben, die von Frau Kristina Osen im Mai 2014 in der Demokratischen Republik Kongo genommen wurden, um Sapelli *(Entandrophragma cylindricum*) und Bilinga *(Nauclea diderichii*) handelt.

Björn Weiß

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Appendix 5

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Marburg, den 24. November 2014

To Whom it may concern

Wissenschaftliche Anforderungen an einen Bildprobentest zur Validierung verschiedener Analyseverfahren zur Art- und zur Herkunftsidentifizierung

Ziel eines Blindprobentests ist es, unterschiedliche Analyseverfahren auf ihre Praxistauglichkeit zu vergleichen. Dabei muss jedes Analyseverfahren von einem unabhängigen Partner durchgeführt werden, d.h. ein Partner für die Holzanatomie, ein Partner für die Genetik und ein Partner für Stabile Isotopen.

a) Referenzmaterial zu Blindproben
b) Durchführende Partner
c) Probeneigenschaften
d) Probendeklaration

Ad a) Die Blindproben sollten von einem anderen Partner als das Referenzmaterial gesammelt werden, um unabhängige Proben zu gewährleisten. Während Referenzmaterial so beschaffen sein kann, dass eine einfache und sichere Identifizierung der Art und der Herkunft erfolgen kann, muss bei Blindproben darauf geachtet werden, dass für das jeweilige Verfahren spezifische Ansprüche mit maximaler Herausforderung an die Methode bestehen sollten (siehe Ad c).

Ad b) Die durchführenden Partner der drei verschiedenen Analyseverfahren sollten keinen Austausch über die Blindproben haben, um unabhängig voneinander Stärken und Schwächen der verschiedenen Verfahren herauszuarbeiten. Auch hier gilt für das Probenmaterial, dass eine maximale Herausforderung an die jeweilige Methode bestehen muss (siehe Ad c).

Ad c) Um die Stärken und Schwächen der verschiedenen Verfahren zu validieren sind unterschiedliche Probeneigenschaften gefordert. Für die Holzanatomie ist ein massives Stück Holz gefordert, da nur so anatomische Merkmale ersichtlich sin. Weder für die Genetik noch für die Stabilen Isotope dürfen größere Holzstücke verwendet werden, die es ansonsten erlauben würden, zusätzlich zum eigentlichen Verfahren holzanatomische Merkmale zu verwenden. Daher sollten hier vorzugweise Holzspäne, die nach einem standardisierten Protokoll beschafft wurden, genutzt werden. Zudem sollten sie nicht aus Sägemühlen stammen damit keine unerwünschte Kontamination auftreten kann.

Ad d) Um die Stärken und Schwächen der verschiedenen Analyseverfahren zu validieren, ist es notwendig, dass die Proben so deklariert werden, dass keine Rückschlüsse über die Herkunft möglich sind. Identische Proben sollten daher unter unterschiedlicher Deklaration an die durchführenden Partner der jeweiligen Verfahren versendet werden.

N. Farwig

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Appendix 6

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Marburg, den 24. November 2014

To Whom it may concern

Pilzbefall bei Holzproben und mögliche Schwierigkeiten in der molekularen Taxonomie (Taxon-Referenz-Blindproben)

- falls Schimmel einmal unabsichtlich und entgegen aller Gegenmaßnahmen bei der Beschaffung von Holzproben aus den tropischen Regenwäldern auftreten sollte –

Allgemein gilt zu bedenken, dass auch nicht schimmliges pflanzliches Gewebe immer pilzliche DNA durch endophytische Pilze enthält (s.u.). Diesem Sachverhalt müssen per se alle genetischen Art-Nachweismethoden Rechnung tragen.

Wenn Holzproben gelegentlichen geringen Schimmelpilzbefall aufweisen, besteht ein äußerst geringes Risiko bei der molekularen Identifizierung des unbekannten Taxons. Die benutzten molekularen Systeme sind in der Regel als hoch spezifisch für Pflanzen zu werten, so dass „kontaminierende pilzliche" Gewebe nicht erfasst werden. Wenn universelle Systeme (s.u.) verwendet werden sollten, dann besteht zwischen Pflanze und Pilz ein so großer evolutionärer Abstand, dass eine unerwünschte Identifizierung des Pilzes nahezu ausgeschlossen ist. Untersuchungen mit dem Fokus „Tier" sind da anders zu bewerten auf Grund deren größerer evolutionären Nähe zu den Pilzen.

a) durch falsch positive Identifizierung (genetische Kontamination durch den Pilz)
b) oder durch PCR- Inhibierung (biochemische Interaktion in der PCR).

Ad a) Die DNA-Markersysteme, welche derzeit häufig bei Höheren Pflanzen (inkl. Bäumen), eingesetzt werden, um Arten zuverlässig zu identifizieren, stammen aus

i) der Chloroplasten-DNA. In diesem Fall ist ein falsch positives Ergebnis ausgeschlossen, da Pilze keine Chloroplasten besitzen.

ii) aus der Kern-DNA. Wenn hier Varianten aus Internal Transcribed Spacer (ITS) – Regionen der ribosomalen DNA als Marker eingesetzt werden, sind die pflanzenspezifischen PCR-Primer für bestimmte Untereinheiten designed und nicht komplementär genug zur Pilz-DNA. Lediglich bei der Verwendung universeller für Pflanzen und Tiere gleichermaßen nützlichen Sequenz-Barcodes (s.o.) findet man in 0.3 % aller Fälle einmal pilzlich-endophytische Sequenzen, wobei dikotyle Pflanzenarten ein wesentlich geringeres Sequenz-Kontaminationsrisiko als monokotyle tragen (Yao et al. 2010). Aber dieses ist sicher Stand des Wissens aller Laboratorien, die eine sichere genetische pflanzliche Art-Identifizierung anstreben, wobei solche Systeme dann wohl eher gemieden werden. Für die Nutzung von SNP-Markern bzw. einzelnen Punktmutationen in Kerngenen von Pflanzen gilt, dass es oft schon nicht möglich ist, die PCR-Primer von einer Pflanzenart zur verwandten Art zu übertragen, geschweige denn ist nicht davon auszugehen, dass diese Übertragung zwischen Organismenreichen gelänge bzw. unkontrolliert geschähe. Sollten dennoch pilzliche Sequenz-Kontamination in der PCR amplifiziert werden, sind solche Sequenzen in den Genbanken schnell zu erkennen und zu verwerfen, da es sich vermutlich um extrem konservative Sequenzen handelt, die bereits für viele Organismen unter Angabe der Art und Systematik hinterlegt sind.

Ad b) Eine Inhibierung der PCR durch pilzliche Substanzen ist weniger wahrscheinlich als eine PCR-Inhibierung durch die oft als sehr störend bekannten Sekundärstoffe der Tropenhölzer als solchen.
Im Zusammenhang mit Pilzen gibt es interessante Untersuchungen, die beide Systeme „Pilz und Pflanze" aus einem einzigen DNA-Extrakt spezifisch erreichen. So konnten ohne Probleme in mykorrhizierten verholzten Wurzeln sowohl die Wirtspflanze als auch der Pilz jeweils spezifisch molekulargenetisch angesteuert werden und entsprechende spezifische Informationen aus ein und demselben Extrakt gewonnen werden, ohne irgendeine Kreuzreaktion bzw. biochemische Inhibierung der PCR zu erleben (Donges et al., unveröffentlichte Ergebnisse).
Die vorgenannten Erläuterungen treffen für Blindbeprobungen zu, die einem Arterkennungssystem zugeführt werden sollen. Ähnlich verhält es sich für Systeme die INNERHALB von Baumarten gesicherte Identifizierungen von Herkünften bzw. Provenienzen gewährleisten sollen. Es gilt das für Chloroplasten-DNA-Marker gesagte (siehe oben). Sollten individuumbasierte oder frequenzbasierte Verfahren auf der Grundlage von Kern-Mikrosatelliten-Markern angestrebt werden, ist durch die hohe Spezifität der PCR-Primer keine Kreuzkontamination durch den Pilz zu erwarten.

Yao, H., Song, J., Liu, C., Luo, k. et al. (2010). Use of ITS2 Region as the Universal DNA Barcode for Plants and Animals. PLOS One, DOI: 10.1371/journal.pone.0013102.

Sollten die Vertrags-Laboratorien andere als die hier dargestellten genetischen Verfahren verwenden, z.B. andere Markierungssysteme bzw. Verfahren aus dem Methodenkreis der NGS (Next Generation Sequencing) müssten diese vermittelt werden, um auch für diese konkrete Aussagen über Risiken treffen zu können. Auch schließen die mit bestem Wissen oben dargestellten Erläuterungen Wissenslücken nicht aus.

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23.10.2014

Beeinträchtigung der Isotopenverhältnisse durch Schimmelbefall

Sehr geehrter Herr Zahnen,

nach dem derzeitigen Kenntnistand ist nicht davon auszugehen, dass die Isotopenmuster des Holzes durch Schimmelbefall beeinträchtigt werden.
Es ist darauf hinzuweisen, dass die Stabil-Isotopentechnik ein Nachweis eines physikalischen Merkmals ist. Dieses lässt sich durch den Schimmelbefall nicht verändern. Im Gegenteil wird gerade diese Besonderheit ausgenutzt Nahrungsweb-Studien durchzuführen.

Mit freundlichen Grüßen,

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18 Nov 2014

Blindtest

Blindtests dienen dazu, die Aussagekraft einer Methode zu prüfen. Im vorliegenden Projekt Wasserzeichen geht es darum, mit Blindtests zu überprüfen, ob mit der Isotopenanalyse die Aussage zur regionalen Herkunft von Produkten richtig verifiziert werden kann.

Beim Vorgehen ist es unerlässlich, dass die Herkunft der für die Blindtests verwendeten Blindproben zweifelsfrei bekannt ist. Deshalb werden für die Blindtests Proben verwendet, die gemäß dem Verfahren für authentische Referenzproben genommen wurden. Die Probennahme erfolgt bei pflanzlichen Produkten vor der Ernte auf dem Feld. Der Probenahmeort wird zusätzlich durch Bestimmung der GPS-Daten sowie durch Fotos von der beprobten Fläche dokumentiert.

Praxisproben sind für Blindtest nicht geeignet, da bei einer falschen Verifizierung einer Herkunftsaussage durch das Labor nicht ausgeschlossen werden kann, dass die falsche Verifizierung durch die nicht gesicherte Herkunft der Probe bedingt ist.

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"Development and implementation of a species identification and timber tracking system in Africa with DNA fingerprints and stable isotopes"

Wood species and country origin identification blind test

Brief report on the results

Background

Following the sampling realized in different countries in Africa regions in the frame of the ITTO funded project named "Development and implementation of a species identification and timber tracking system in Africa with DNA fingerprints and stable isotopes", two blind test processes have been conducted in order to determine whether the methods elaborated are able to help identify the species or the country origin of a given piece of timber and which can be used to track timber besides the other timber tracking systems existing on the trade.

The first blind test focused on species identification while the second one focused on country identification.

The collecting of samples has been done from West and Central Africa countries coordinated by the consultancy G2S based in Cameroon.

Objectives

- Species identification: the result awaited here was to confirm or not that the wood sample tested belongs to the claim species;
- Country identification: the result awaited was to confirm or not the claim made regarding the country of origin of a wood sample.

Methodology of sampling

Globally, the methodology has been inspired by the terms of reference put at disposal by the project coordinator.

1. Wood sample types

As suggested by the project, wood samples have been removed from sawn timber in order to realized the blind test on the samples which are as close as possible to the form in which the timber from Africa is more and more sold in European countries (note that the approaches used to test have been developed with cambium samples collected during the sampling on trees) because the laboratories should be more confronted to that form of material when realizing the tests of identification if the method is widely adopted for wood tracking in the timber industry.

2. Species identification

During the step one, more than 80 wood samples species have been collected from 21 taxa (list attached in annex 1) in Ghana, Cameroon and Gabon. From that set of samples, 50 have been selected and tagged with code number before being sent to the project coordinator directly from Ghana and Cameroon. Preferably, the samples were collected from sawmills but some have been collected from chain sawmilling operations due to the fact that the concerned species were not found sawed at sawmills (some are generally sold in logs by the logging companies).

From the 50 samples sent to the project coordinator, a list of 25 have finally been issued by consultancy organizations involved (Ghana and Cameroon) and communicated to the project coordinator for testing in laboratories to confirm or not the claim done by the consultancy organizations.

The headings of the final excel file sent were: order number, code numbers, claims on species names, true species names (see the list attached in annex 2).

3. *Country level identification*

Around 75 wood samples have been collected from 3 species (Milicia excelsa, Triplochiton scleroxylon, Entandrophragma cylindricum) in sawmills (majority) and chain sawmilling operations in Ghana, Ivory coast, Cameroon, Congo republic, CAR, DRC and Gabon. From that set of samples, 60 have been selected and tagged for testing purposes.

The samples collected in West Africa have been sent to G2S in Cameroon (in charge of sampling in Central Africa) in order to finalize the samples tagging process and send all the samples together directly from Cameroon to the project coordinator. At the end of selection process, a list of 30 samples has finally be issued by consultancy organizations and communicated to the project coordinator for testing in laboratories to confirm or not the claim done by the consultancy organizations.

As the necessary tools (genetic markers…) were not identify/developed for some target species coming from some countries, it has been agreed that for those species and countries, the true solution (true country origin) couldn't choose/select/concern them but they could be selected/chose among the claims on country origin so that the test can confirm or not the claim.

The headings of the final excel file sent were: order number, code numbers, species names, claims on country, claims on region within the country (for some countries), true country origin (see the list attached in annex 3).

Results

The results are presented in 2 steps:

- Step 1: species identification results by laboratory and method/approach used
- Step 2: country origin identification by laboratory and method/approach used

1. *Species identification results*

Legend for understanding

	Laboratory answer correct when compare to the claim
	Laboratory not correct when compare to the claim
	No answer given by the laboratory regardless the reason or no claim provided by the sampling team

1.1. Wood anatomy approach

The laboratory answer is assessed compare to the claim made for each species (ToR) and at the genus level because the method can't enable to identify the species for some timber species. This help to have a unique approach of evaluation for all the samples (timber species) sent to the laboratory.

Table 1: results of the species identification based on wood anatomy method- Gerald KOCK

	anatomy approach_Gerald KOCK				
Sample code numbers	**Claims on species names**	**True species names**	**Laboratory answer**	**Sampling team evaluation**	**Comments**
G2S_S_1.0	*Guibourtia ehie.*	Afzelia africana	Claim False	*correct*	
G2S_S_1.5	*Baillonella toxisperma*	Afzelia spp	Claim False	*correct*	
G2S_S_2.0	*Khaya anthotheca*	Khaya ivorensis	Claim True	*correct*	the individual species with the genus Khaya can't be distinguished microscopically
G2S_S_3.5	*Baillonella toxisperma*	Baillonella toxisperma	Claim True	*correct*	
G2S_S_5.0	*Entandrophragma cylindricum*	Entandrophragma angolense	Claim False	*correct*	
G2S_S_8.0	*Entandrophragma candollei*	Entandrophragma utile	Claim False	*correct*	
G2S_S_8.5	*Entandrophragma cylindricum*	Entandrophragma utile	Claim False	*correct*	but the species name given is not matching with the name given sampling team (verification going on)
G2S_S_10.0	*Milicia excelsa*	Erythrophleum suaveolens	Claim False	*correct*	
G2S_S_11.5	*Guibourtia spp.*	Guibourtia spp.	Claim True	*correct*	
G2S_S_13.0	*Lophira alata*	Lophira alata	Claim True	*correct*	
G2S_S_13.5	*Lophira alata*	Lophira alata	Claim True	*correct*	
G2S_S_14.0	*Erythrophleum suaveolens*	Milicia excelsa	Claim False	*correct*	
G2S_S_15.0	*Milicia regia*	Milicia regia	Claim True	*correct*	the individual species with the genus Milicia can't be distinguished microscopically
G2S_S_16.5	*Millettia laurentii*	Millettia laurentii	Claim True	*correct*	
G2S_S_18.5	*Khaya spp*	Pericopsis elata	Claim False	*correct*	
G2S_S_20.0	*Terminalia superba*	Terminalia superba	Claim True	*correct*	
G2S_S_21.5	*Pterocarpus soyauxii*	Pterocarpus soyauxii	Claim True	*correct*	
G2S_S_24.0	*Triplochiton scleroxylon*	Triplochiton scleroxylon	Claim True	*correct*	
G2S_S_25.5	*Entandrophragma utile*	Mansonia altissima	Claim False	*correct*	
G2S_S_30.5	*Triplochiton scleroxylon*	*Triplochiton scleroxylon*	Claim True	*correct*	
G2S_S_33.5	*Erythrophleum ivorense*	Lovoa trichiloides	Claim False	*correct*	
G2S_S_35.5	*Afzelia spp*	Afzelia spp	Claim True	*correct*	
G2S_S_38.5	*Nauclea diderrichii*	Nauclea diderrichii	Claim False	*not correct*	the laboratory confirm that the sample received is not from that species (verification with another lab is going on)
G2S_S_41.5	*Aningeria robusta*	Mansonia altissima	Claim False	*correct*	
G2S_S_47.5	*Cylicodiscus gabunensis*	Cylicodiscus gabunensis	Claim True	*correct*	

1.2. Barcoding and fingerprinting approach

Table 2: results of the species identification based on barcoding method- plant genetics diagnosis

			Barcoding approach_plant genetic_Aki		
Sample code numbers	**Claims on species names**	**True species names**	**Laboratory answer_barcoding**	**Sampling team evaluation**	**Comments**
G2S_S_1.0	*Guibourtia ehie.*	Afzelia africana	*claim wrong*	*correct*	
G2S_S_1.5	*Baillonella toxisperma*	Afzelia spp	*claim wrong*	*correct*	
G2S_S_2.0	*Khaya anthotheca*	Khaya ivorensis	*claim wrong*	*correct*	
G2S_S_3.5	*Baillonella toxisperma*	Baillonella toxisperma	*claim wrong*	*incorrect*	sequence not in the reference data set according to the laboratory
G2S_S_5.0	*Entandrophragma cylindricum*	Entandrophragma angolense	***no answer***		according to the lab: no DNA amplification
G2S_S_8.0	*Entandrophragma candollei*	Entandrophragma utile	*claim wrong*	*correct*	
G2S_S_8.5	*Entandrophragma cylindricum*	Entandrophragma utile	***no answer***		according to the lab: low DNA quality or DNA markers do not w ork on this sample
G2S_S_10.0	*Milicia excelsa*	Erythrophleum suaveolens	*claim wrong*	*correct*	
G2S_S_11.5	*Guibourtia spp.*	Guibourtia spp.	*claim true*	*correct*	
G2S_S_13.0	*Lophira alata*	Lophira alata	*claim wrong*	*incorrect*	
G2S_S_13.5	*Lophira alata*	Lophira alata	***no answer***		according to the lab: low DNA quality or DNA markers do not w ork on this sample
G2S_S_14.0	*Erythrophleum suaveolens*	Milicia excelsa	***no answer***		according to the lab: low DNA quality or DNA markers do not w ork on this sample
G2S_S_15.0	*Milicia regia*	Milicia regia	*claim true*	*correct*	
G2S_S_16.5	*Millettia laurentii*	Millettia laurentii	***no answer***		no DNA amplification
G2S_S_18.5	*Khaya spp*	Pericopsis elata	***no answer***		no DNA amplification
G2S_S_20.0	*Terminalia superba*	Terminalia superba	*claim true*	*correct*	
G2S_S_21.5	*Pterocarpus soyauxii*	Pterocarpus soyauxii	*claim wrong*	*incorrect*	
G2S_S_24.0	*Triplochiton scleroxylon*	Triplochiton scleroxylon	*claim true*	*correct*	
G2S_S_25.5	*Entandrophragma utile*	Mansonia altissima	*claim wrong*	*correct*	
G2S_S_30.5	*Triplochiton scleroxylon*	*Triplochiton scleroxylon*	*claim true*	*correct*	
G2S_S_33.5	*Erythrophleum ivorense*	Lovoa trichiloides	*claim wrong*	*correct*	
G2S_S_35.5	*Afzelia spp*	Afzelia spp	*claim wrong*	*incorrect*	
G2S_S_38.5	*Nauclea diderrichii*	Nauclea diderrichii	***no answer***		no DNA amplification
G2S_S_41.5	*Aningeria robusta*	Mansonia altissima	*claim wrong*	*correct*	
G2S_S_47.5	*Cylicodiscus gabunensis*	Cylicodiscus gabunensis	*claim true*	*correct*	

2. *Country origin identification results*

Legend for understanding

	Laboratory answer correct when compare to the claim
	Laboratory not correct when compare to the claim
	No answer given by the laboratory regardless the reason or no claim provided by the sampling team

2.1. Isotope approach

Table 3: results of the country origin identification based on isotopic approach- FERA-Donarski

		Isotope approach_FERA_James DONARSKI								
Sample code numbers	Species	Claims on country	True country origin	laboratory answer_country_isotope (claim true or false)	Sampling team evaluation	Comments	Claim on region within country	laboratory answer_country_isotope (claim true or false)	Sampling team evaluation	Comments
G2S_O_ E4	*Entandrophragma cylindricum*	Ghana	Ghana	***Yes***	*correct*		no claim on region			
G2S_O_E6	*Entandrophragma cylindricum*	DRC	Cameroon	***Yes***	*not correct*		South-West (Bandundu province)	**No**	*incorrect*	as it's incorrect at country level, the answ er given at region level is automatically considered incorrect
G2S_O_ E8	*Entandrophragma cylindricum*	Cameroon	Cameroon	***Yes***	*correct*		South (Sangmelima)	***Yes***	*correct*	
G2S_O_ E11	*Entandrophragma cylindricum*	Congo	Congo	***Yes***	*correct*		North region	***Yes***	*correct*	
G2S_O_ E13	*Entandrophragma cylindricum*	Congo	Congo	***Yes***	*correct*		North region	***Yes***	*correct*	
G2S_O_ E14	*Entandrophragma cylindricum*	Congo	Congo	***Yes***	*correct*		North region	***Yes***	*correct*	
G2S_O_ E18	*Entandrophragma cylindricum*	DRC	DRC	***Yes***	*correct*		North-West (Equateur province)	***Yes***	*correct*	
G2S_O_ E20	*Entandrophragma cylindricum*	DRC	DRC	***Yes***	*correct*		North-West (Equateur province)	***Yes***	*correct*	
G2S_O_ E22	*Entandrophragma cylindricum*	DRC	DRC	***Yes***	*correct*		South-West (Bandundu province)	***No***	*incorrect*	
G2S_O_ E24	*Entandrophragma cylindricum*	DRC	DRC	***Yes***	*correct*		North-East region (Orientale region)	***Yes***	*correct*	

Table 4: results of the country origin identification based on isotopic approach- Agroisolab-BONER

		Isotope approach_Agroisolab_BONER								
Sample code numbers	Species	Claims on country	True country origin	laboratory answer_country _isotope (claim true or false)	Sampling team evaluation	Comments	Claim on region within country	laboratory answer_country _isotope (claim true or false)	Sampling team evaluation	Comments
G2S_O_M1	*Milicia Excelsa*	Ghana	Ghana	***Yes***	*correct*		no claim on region			
G2S_O_M4	*Milicia Excelsa*	Gabon	Congo	***No***	*correct*		North east region	***No***	*correct*	
G2S_O_M5	*Milicia Excelsa*	DRC	Congo	***No***	*correct*		North-West (Equateur province)	***No***	*correct*	
G2S_O_ M6	*Milicia Excelsa*	Cameroon	CAR	***Yes***	*incorrect*		South-west	Possible	*incorrect*	A significant evaluation oft the closer region could only be estimated, because the numbers of reference is too small (n=3)
G2S_O_ M7	*Milicia Excelsa*	Gabon	CAR	***No***	*correct*		North region	***No***	*correct*	
G2S_O_ M10	*Milicia Excelsa*	Gabon	DRC	***Yes***	*incorrect*		North region (Oyem)	No	*incorrect*	as it's incorrect at country level, the answer given at region level is automatically considered incorrect
G2S_O_ M12	*Milicia Excelsa*	DRC	DRC	***Yes***	*correct*		North center (Orientale province)	***Yes***	*correct*	
G2S_O_ M14	*Milicia Excelsa*	Cameroon	Gabon	***Yes***	*incorrect*		Center region (Mbalmayo)	no answer		according to the lab the Numbers of references it too small to evaluate the region (n=3))
G2S_O_ M15	*Milicia Excelsa*	Gabon	Cameroon	***No***	*correct*		Center-West region	***No***	*correct*	
G2S_O_ M20	*Milicia Excelsa*	Cameroon	Cameroon	***Yes***	*correct*		South region (Ebolowa)	no answer		according to the lab the Numbers of references it too small to evaluate the region (n=3))

Table 5: results of the country origin identification based on isotopic approach- HBLFA-HORACEK

		Isotope approach_HBLFA_HORACEK								
Sample code numbers	Species	Claims on country	True country origin	laboratory answer_country _isotope (claim true or false)	Sampling team evaluation	Comments	Claim on region within country	laboratory answer_country_ isotope (claim true or false)	Sampling team evaluation	Comments
G2S_O_T1	*Triplochiton scleroxylon*	Ghana	Ghana	***Yes***	***correct***					no claim on region
G2S_O_T3	*Triplochiton scleroxylon*	Ghana	Ghana	***Yes***	***correct***					no claim on region
G2S_O_T6	*Triplochiton scleroxylon*	Cameroon	Congo	***Yes***	***incorrect***		South region (Ebolowa)	no answer		Answer at regional level not mentioned
G2S_O_T7	*Triplochiton scleroxylon*	Congo	Congo	*no answer*	–	not enough reference samples provided (less than 20).	North region	no answer		not enough reference samples provided (less than 20).
G2S_O_ T9	*Triplochiton scleroxylon*	DRC	Cameroon	***No***	***correct***		North-West (Equateur province)	no answer		Answer at regional level not mentioned
G2S_O_ T11	*Triplochiton scleroxylon*	Cameroon	Cameroon	***Yes***	***correct***		East region (Bertoua)	no answer		Answer at regional level not mentioned
G2S_O_ T13	*Triplochiton scleroxylon*	Cameroon	Cameroon	***Yes***	***correct***		South region	No	incorrect	
G2S_O_ T15	*Triplochiton scleroxylon*	Cameroon	Cameroon	***Yes***	***correct***		South region	No	incorrect	
G2S_O_ T16	*Triplochiton scleroxylon*	Cameroon	Cameroon	***Yes***	***correct***		South region	No	incorrect	
G2S_O_T18	*Triplochiton scleroxylon*	Cameroon	Cameroon	***Yes***	***correct***		East region (Mbang)	no answer		Answer at regional level not mentioned

2.2. Genetic approach

Table 6: results of the country origin identification based on genetic approach- Adelaide-Andrew

				genetic approach_Adelaide_Andrew						
Sample code numbers	Species	Claims on country	True country origin	laboratory answer_country _isotope (claim true or false)	Sampling team evaluation	Comments	Claim on region within country	laboratory answer_country _isotope (claim true or false)	Sampling team evaluation	Comments
G2S_O_T1	*Triplochiton scleroxylon*	Ghana	Ghana	***no answer***		sequinom success < 90%, sample quality not high enough for robust conclusions	no claim			
G2S_O_T3	*Triplochiton scleroxylon*	Ghana	Ghana	***Yes***	*correct*					
G2S_O_T6	*Triplochiton scleroxylon*	Cameroon	Congo	***no answer***		sequinom success < 90%, sample quality not high enough for robust conclusions	South region (Ebolowa)	no answer		sequinom success < 90%, sample quality not high enough for robust conclusions
G2S_O_T7	*Triplochiton scleroxylon*	Congo	Congo	***no answer***		sequinom success < 90%, sample quality not high enough for robust conclusions	North region	no answer		sequinom success < 90%, sample quality not high enough for robust conclusions
G2S_O_ T9	*Triplochiton scleroxylon*	DRC	Cameroon	***No***	*correct*		North-West (Equateur province)	***No***	*correct*	
G2S_O_ T11	*Triplochiton scleroxylon*	Cameroon	Cameroon	***Yes***	*correct*		East region (Bertoua)	***Yes***	*correct*	
G2S_O_ T13	*Triplochiton scleroxylon*	Cameroon	Cameroon	***Yes***	*correct*		South region (Ebolowa)	***Yes***	*correct*	
G2S_O_ T15	*Triplochiton scleroxylon*	Cameroon	Cameroon	***no answer***		sequinom success < 90%, sample quality not high enough for robust conclusions	South region (Ebolowa)	no answer		sequinom success < 90%, sample quality not high enough for robust conclusions
G2S_O_ T16	*Triplochiton scleroxylon*	Cameroon	Cameroon	***no answer***		sequinom success < 90%, sample quality not high enough for robust conclusions	South region (Ebolowa)	no answer		sequinom success < 90%, sample quality not high enough for robust conclusions
G2S_O_T18	*Triplochiton scleroxylon*	Cameroon	Cameroon	***No***	*incorrect*		East region (Mbang)	***No***	*incorrect*	

Table 7: results of the country origin identification based on genetic approach- Thunen-Celine

		genetic approach_Thunen_Celine								
Sample code numbers	Species	Claims on country	True country origin	laboratory answer_country _isotope (claim true or false)	Sampling team evaluation	Comments	Claim on region within country	laboratory answer_country _isotope (claim true or false)	Sampling team evaluation	Comments
G2S_O_ E4	*Entandrophragma cylindricum*	Ghana	Ghana	***No***	*incorrect*		no claim			
G2S_O_E6	*Entandrophragma cylindricum*	DRC	Cameroon	***No***	*correct*		South-West (Bandundu province)	***No***	*correct*	
G2S_O_ E8	*Entandrophragma cylindricum*	Cameroon	Cameroon	***Yes***	*correct*		South (Sangmelima)	***Yes***	*correct*	
G2S_O_ E11	*Entandrophragma cylindricum*	Congo	Congo	***no answer***		not enough loci, bad DNA quality according to the laboratory	North region	***no answer***		not enough loci, bad DNA quality according to the laboratory
G2S_O_ E13	*Entandrophragma cylindricum*	Congo	Congo	***Yes***	*correct*		North region	***Yes***	*correct*	
G2S_O_ E14	*Entandrophragma cylindricum*	Congo	Congo	***Yes***	*correct*		North region	***Yes***	*correct*	
G2S_O_ E18	*Entandrophragma cylindricum*	DRC	DRC	***No***	*incorrect*		North-West (Equateur province)	***No***	*incorrect*	
G2S_O_ E20	*Entandrophragma cylindricum*	DRC	DRC	***No***	*incorrect*		North-West (Equateur province)	***No***	*incorrect*	
G2S_O_ E22	*Entandrophragma cylindricum*	DRC	DRC	***Yes***	*correct*		South-West (Bandundu province)	***Yes***	*correct*	
G2S_O_ E24	*Entandrophragma cylindricum*	DRC	DRC	***Yes***	*correct*		North-East (Orientale region)	***Yes***	*correct*	

Table 8: results of the country origin identification based on genetic approach- Thunen-Celine

		genetic approach_Thunen_Celine								
Sample code numbers	Species	Claims on country	True country origin	laboratory answer_country_ isotope (claim true or false)	Sampling team evaluation	Comments	Claim on region within country	laboratory answer_country _isotope (claim true or false)	Sampling team evaluation	Comments
G2S_O_M1	*Milicia Excelsa*	Ghana	Ghana	***Yes***	*correct*					
G2S_O_M4	*Milicia Excelsa*	Gabon	Congo	***No***	*correct*		North east region	no answer		
G2S_O_M5	*Milicia Excelsa*	DRC	Congo	***Yes***	*incorrect*		North-West (Equateur province)	no answer		
G2S_O_ M6	*Milicia Excelsa*	Cameroon	CAR	***Yes***	*incorrect*		South-west	no answer		
G2S_O_ M7	*Milicia Excelsa*	Gabon	CAR	***Yes***	*incorrect*		North region	no answer		
G2S_O_ M10	*Milicia Excelsa*	Gabon	DRC	***No***	*correct*		North region (Oyem)	no answer		
G2S_O_ M12	*Milicia Excelsa*	DRC	DRC	***Yes***	*correct*		North center (center of Orientale	no answer		
G2S_O_ M14	*Milicia Excelsa*	Cameroon	Gabon	***Yes***	*incorrect*		Center region (Mbalmayo)	no answer		
G2S_O_ M15	*Milicia Excelsa*	Gabon	Cameroon	***No***	*correct*		Center-West region	no answer		
G2S_O_ M20	*Milicia Excelsa*	Cameroon	Cameroon	***Yes***	*correct*		South region (Ebolowa)	no answer		

ANNEX 8

Example for a comparative analysis of the blind test data on geographic claims of the ITTO-Africa Project

Example for a comparative analysis of the blind test data on geographic claims of the ITTO-Africa Project

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(02/11/2015)

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Background

We discussed among all involved partners the results of the blind test during a project meeting in Großhansdorf on the 23.06.2015. The meeting have shown that the different groups used quite variable approaches to analyse the data. The range of methods varied from a pure expert evaluation with no statistics up to advanced statistical approaches. Also the used criteria to reject a claim on origin and to include or exclude individuals of reference and blind tests sets in the analysis were not unique among the groups. Thus the proportion of correct results in the blind test is influenced by two different factors:

a) by the performance of the method and the quality of the reference data
b) by the statistical approach and the criteria to include or exclude incomplete data as well as the thresholds to reject or accept a declaration of origin

I analysed the provided reference data and blind test data of the ITTO project using a unique approach and using the same criteria to judge on claims in order to have a more objective basis to compare the results of the different groups. The raw data were shared with all involved laboratories on the 14/09/2015. The presented approach and the results are thought as a science based contribution to the ongoing discussion of the performance of the methods.

Method

I computed pairwise distance measures between the individual reference data and the test data. For the metric isotope data I made a z-transformation of the different elements and calculated then the city-block-distance among the isotope profile of the different individuals (Deichsel & Trampisch 1985; page 22 ff.). For the SNP-data in genetics I computed the genetic distance of Gregorius among the multilocus genotypes of the different individuals (Gregorius 1978). Then I computed exclusion probabilities to judge on the claim “correct country of origin”. In all cases with at least 10 reference data in the declared country, the

exclusion probability is equal the proportion of cases where the distance among a test sample and a reference sample was higher than the distance of this reference sample and all other reference samples that fulfil the requirements of the claim (e.g. from the same claimed country). The pairs of individuals were included in the analysis only if at least 80% of the data of the two compared individual were measured. Then the reference samples are ordered from the smallest to the largest distance after the distances among a test individual and all reference samples have been computed. A small distance means similar genetic composition or similar stable isotope profile of reference sample and test individual. If the declared country of origin is correct than we would expect a significant higher proportion of reference samples from the declared country of origin among the most similar reference samples. The index RND is computed for all present countries in the reference samples. The Index RND varies between -1 and +1. If the Index RND is equal 0 then the proportion of similar reference samples is exactly as high as randomly expected according to the sample intensity in a country. A positive Index RND indicates an excess and a negative index stands for a deficit of similar reference samples compared to the sample proportion. The departure between observed and expected numbers of similar individuals has been checked with an Exact Fisher's test.

Thresholds and rules to judge on claims

The below presented results were computed based on the following thresholds:

a) test individuals and reference data with less than 80% of the gene markers or stable isotopes completed were not included in the analysis. => "unsolved"
b) for test individuals with an exclusion probability >= 80% the declaration was "rejected"
c) for test individuals with an exclusion probability < 80% but an RND-Index below 0.1 and an alternative country with a significant positive and higher RND-Index => "rejected"
d) all test individuals that could not be rejected by the rules b and c are classified as "accepted_a"
e) all test individuals that could not be rejected by the rules b and c and got a significant positive RND-Index > 0.1 for the declared country are classified as "accepted_confirmed".

Results

The detailed results are given in the attached three tables. For each species there is one table with the direct comparison of isotope and genetic results.

- The isotope data provided results for all test samples data. For the genetics between 17% and 47% of the samples did not have enough SNPs amplified. This is due to the degradation of DNA in timber.
- Based on all test individuals that had sufficient data the success rate of the isotopes varied between 55% and 75%. For the genetic data the proportion of correct results varied between 60% and 83%.
- For the isotopes overall all species 10 out of 60 samples have been wrongly classified as "rejected" (17 %) and for the genetics 4 out of 43 samples (9 %). These are very critical errors in face of a later practical application because it indicates the risk that a correct declaration gets rejected. Further work is needed to see if this error could be minimised with other thresholds during the data analysis. But it also clearly indicates problems with the reference data itself (insufficient number and distribution of reference samples, errors on geographic co-ordinates, too weak spatial structure of the measured variables).
- Over all species there were 28 cases for which both methods got a result and either one (82%) or both methods (18 %) made a wrong decision on the claim. This low rate of overlap in the errors indicates that a combination of methods has the potential to minimise the proportion of errors. The critical point is then the best way to judge which methods gets for a particular case the best and "correct" decision. This requires further work.

References

Deichsel G, Trampisch HJ (1985) Clusteranalyse und Diskriminanzanalyse. Gustav Fischer Verlag: Stuttgart.

Gregorius H-R (1978). The concept of genetic diversity and its formal relationship to heterozygosity and genetic distance. Mathematical Bioscience 41: 253-271.

Results blind test Triplochiton scleroxylon (Ayous)

	Claim	Solution	Isotopes								Genetics							
ID_Sample	Group	Group	Decision	N Cluster	Exclusion Probability	Index Cameroon	Index DRC	Index Ghana	Index Con_Braz	Index CIV	Decision	N Cluster	Exclusion Probability	Index Cameroon	Index Con_Braz	Index DRC	Index Ghana	Index CIV
G2S_O_T1	Ghana	Ghana	accept_a	30	0.69	0.35*	-0.36**	0.25ns	-0.07ns	-0.12ns								
G2S_O_T3	Ghana	Ghana	accept_a	30	0.71	0.30ns	-0.32*	-0.04ns	0.07ns	0.00ns	accept_a	38	0.51	-0.28**	-0.06ns	-0.23**	0.16ns	0.46**
G2S_O_T6	Cameroon	Con_Braz	accept_a	30	0.45	0.30ns	-0.32*	0.25ns	-0.11ns	-0.08ns								
G2S_O_T7	Con_Braz	Con_Braz	reject	30	0.72	0.35*	-0.32*	0.17ns	-0.07ns	-0.08ns								
G2S_O_T9	DRC	Cameroon	reject	30	0.89	0.30ns	-0.36**	0.17ns	-0.07ns	0.00ns	reject	26	0.99	-0.05ns	-0.08ns	-0.24*	0.24ns	0.17ns
G2S_O_T11	Cameroon	Cameroon	accept_a	30	0.71	0.30ns	-0.36**	0.08ns	-0.07ns	0.08ns	accept_a	32	0.43	0.13ns	0.03ns	0.12ns	-0.12ns	-0.18ns
G2S_O_T13	Cameroon	Cameroon	accept_a	30	0.39	0.17ns	-0.36**	0.38*	-0.11ns	-0.04ns	accept_a	19	0.19	0.07ns	0.11ns	0.13ns	-0.20ns	-0.15ns
G2S_O_T15	Cameroon	Cameroon	accept_a	30	0.6	0.26ns	-0.36**	0.17ns	-0.07ns	0.04ns								
G2S_O_T16	Cameroon	Cameroon	accept_a	30	0.63	0.30ns	-0.36**	0.21ns	-0.07ns	-0.04ns								
G2S_O_T18	Cameroon	Cameroon	accept_a	30	0.55	0.30ns	-0.36**	0.17ns	-0.07ns	0.00ns	reject	26	0.89	-0.05ns	-0.08ns	-0.24*	0.24ns	0.17ns
BT_2014_533	Ghana	Ghana	accept_a	30	0.37	0.13ns	-0.32*	0.25ns	-0.11ns	0.08ns	reject	23	0.82	0.06ns	-0.10ns	-0.21ns	0.05ns	0.31ns
BT_2014_543	Cameroon	Gabun	accept_confirmed	30	0.5	0.35*	-0.36**	0.25ns	-0.07ns	-0.12ns	accept_confirmed	28	0.76	0.38*	-0.04ns	-0.09ns	-0.13ns	-0.11ns
BT_2014_547	DRC	Ghana	accept_a	30	0.57	-0.04ns	-0.14ns	0.21ns	-0.11ns	0.12ns	reject	23	0.92	-0.28*	-0.10ns	-0.21ns	0.32ns	0.38ns
BT_2014_551	Ivory_Coast	Ghana	accept_a	30	0.39	-0.13ns	-0.18ns	0.21ns	-0.11ns	0.24ns	accept_a	39	0.76	-0.23*	-0.06ns	-0.19*	0.25*	0.30ns
BT_2014_563	Ivory_Coast	Ghana	reject	30	0.57	0.13ns	-0.27ns	0.33*	-0.11ns	-0.04ns	accept_a	21	0.66	-0.31*	-0.05ns	-0.24ns	0.35ns	0.29ns
BT_2014_567	DRC	Ghana	accept_a	30	0.47	0.04ns	0.05ns	0.00ns	-0.07ns	0.04ns	reject	17	0.85	-0.15ns	-0.06ns	-0.21ns	0.00ns	0.58*
BT_2014_568	Cameroon	Cameroon	accept_confirmed	30	0.47	0.35*	-0.36**	0.08ns	-0.07ns	0.04ns	accept_a	20	0.62	0.07ns	-0.05ns	0.13ns	0.06ns	-0.21ns
BT_2014_578	Cameroon	Ghana	accept_a	30	0.3	0.22ns	-0.32*	0.29ns	-0.11ns	-0.04ns	reject	31	0.88	-0.29**	-0.07ns	-0.24*	0.40**	0.24ns
BT_2014_594	Ghana	Ghana	accept_confirmed	30	0.35	0.00ns	-0.32*	0.38*	-0.11ns	0.08ns	accept_confirmed	24	0.66	-0.28ns	-0.09ns	-0.26*	0.40*	0.19ns
BT_2014_598	Ghana	Ghana	accept_a	30	0.46	0.00ns	-0.14ns	0.25ns	-0.07ns	0.00ns	accept_a	31	0.79	-0.21ns	-0.03ns	-0.24*	0.16ns	0.38*
		Correct	**13**	**65%**							**10**	**67%**						
		Wrong	**7**	**35%**							**5**	**33%**						
		Unsolved	**0**	**0%**							**5**	**25%**						

Results blind test Milicia excelsa (Iroko)																				
	Claim	Solution	Isotopes										Genetics									
ID_Sample	Group	Group	Decision	N Cluster	Exclusion Probability	Index Cameroon	Index Con_Braz	Indec CIV	Index DRC	Index Gabon	Index Ghana	Index Kenya	Decision	N Cluster	Exclusion Probability	Index Cameroon	Index Con_Braz	Index DRC	Index Gabon	Index Ghana	Index CIV	Index Kenya
G2S_O_M1	Ghana	Ghana	accept_confirmed	30	0.68	0.22ns	-0.08ns	-0.04ns	-0.50***	-0.21ns	0.39**	0.04ns	accept_confirmed	39	0.53	-0.26**	-0.22**	-0.39***	-0.22**	0.55***	0.39***	-0.08ns
G2S_O_M4	Gabon	Congo Braz	reject	30	0.83	0.19ns	0.00ns	-0.07ns	-0.15ns	0.08ns	0.00ns	-0.07ns	accept_a	36	0.74	-0.17ns	0.10ns	0.12ns	0.20ns	-0.03ns	-0.06ns	-0.09ns
G2S_O_M5	DRC	Congo Braz	reject	30	0.8	0.19ns	-0.08ns	-0.07ns	0.00ns	0.08ns	-0.04ns	-0.07ns	accept_a	39	0.61	-0.13ns	0.06ns	0.11ns	0.16ns	-0.03ns	-0.08ns	-0.08ns
G2S_O_M6	Cameroon	CAR	accept_confirmed	30	0.8	0.30*	0.08ns	-0.07ns	-0.20ns	-0.21ns	0.04ns	0.00ns	accept_a	68	0.49	-0.06ns	0.04ns	0.08ns	0.14ns	-0.03ns	-0.08*	-0.08*
G2S_O_M7	Gabon	CAR	reject	30	0.9	0.22ns	0.00ns	-0.07ns	-0.05ns	-0.08ns	-0.04ns	0.00ns	accept_confirmed	49	0.67	-0.13ns	0.10ns	-0.14ns	0.37**	-0.04ns	-0.07ns	-0.07ns
G2S_O_M10	Gabon	DRC	reject	30	0.64	0.30*	0.00ns	-0.07ns	-0.15ns	-0.08ns	0.04ns	-0.07ns	reject	50	0.85	0.00ns	0.32**	-0.11ns	-0.05ns	-0.04ns	-0.06ns	-0.07ns
G2S_O_M12	DRC	DRC	accept_a	30	0.67	0.00ns	0.04ns	-0.07ns	0.30ns	-0.17ns	0.04ns	-0.07ns	accept_confirmed	30	0.59	0.08ns	-0.12ns	0.50**	-0.16ns	-0.03ns	-0.07ns	-0.07ns
G2S_O_M14	Cameroon	Gabon	accept_confirmed	30	0.56	0.52***	0.00ns	-0.04ns	-0.20ns	-0.25*	-0.04ns	-0.07ns	accept_a	50	0.52	0.18ns	0.00ns	-0.11ns	0.12ns	-0.04ns	-0.06ns	-0.09ns
G2S_O_M15	Gabon	Cameroon	reject	30	0.7	0.37**	0.00ns	-0.07ns	-0.30ns	-0.08ns	0.07ns	-0.07ns	reject	42	0.76	0.52***	-0.11ns	-0.17ns	-0.03ns	-0.02ns	-0.08ns	-0.08ns
G2S_O_M20	Cameroon	Cameroon	accept_confirmed	30	0.63	0.48***	-0.04ns	-0.07ns	-0.25ns	-0.25*	0.07ns	-0.04ns	accept_confirmed	38	0.51	0.40**	0.00ns	-0.22ns	-0.03ns	-0.03ns	-0.09ns	-0.09ns
BT_2014_518	Gabon	Congo Braz	reject	30	0.9	0.19ns	0.12ns	-0.07ns	0.00ns	-0.17ns	0.00ns	-0.07ns	reject	46	0.73	-0.03ns	-0.03ns	0.39*	-0.11ns	-0.02ns	-0.07ns	-0.07ns
BT_2014_526	Ghana	Ghana	reject	30	0.87	0.33**	0.00ns	-0.07ns	-0.20ns	-0.13ns	0.07ns	-0.07ns	accept_confirmed	47	0.49	-0.27***	-0.21**	-0.38***	-0.21**	0.57***	0.39***	-0.07ns
BT_2014_527	DRC	DRC	accept_a	30	0.59	0.07ns	0.08ns	-0.07ns	0.20ns	-0.08ns	-0.07ns	-0.07ns	accept_a	94	0.56	0.08ns	-0.04ns	0.16ns	0.04ns	-0.03ns	-0.07*	-0.08**
BT_2014_549	Kenya	DRC	reject	30	0.88	0.00ns	-0.12ns	-0.07ns	0.50*	-0.04ns	-0.07ns	-0.07ns	reject	46	0.82	-0.03ns	0.16ns	0.09ns	-0.03ns	-0.02ns	-0.07ns	-0.07ns
BT_2014_554	Gabon	Cameroon	reject	30	0.91	0.41**	0.04ns	-0.04ns	-0.25ns	-0.21ns	0.00ns	-0.04ns	accept_confirmed	36	0.73	0.14ns	-0.10ns	-0.15ns	0.33*	-0.03ns	-0.06ns	-0.09ns
BT_2014_566	Cameroon	DRC	accept_a	30	0.32	0.15ns	-0.04ns	-0.07ns	-0.20ns	0.25ns	-0.04ns	-0.07ns	reject	52	0.68	-0.10ns	0.42***	-0.38***	0.19ns	-0.04ns	-0.08ns	-0.08ns
BT_2014_569	DRC	DRC	accept_a	30	0.61	0.00ns	0.16ns	-0.07ns	0.15ns	-0.13ns	0.00ns	-0.07ns	reject	69	0.74	0.05ns	0.00ns	-0.08ns	0.23*	-0.03ns	-0.08*	-0.08*
BT_2014_596	Gabon	DRC	reject	30	0.36	0.44***	-0.08ns	-0.04ns	-0.50***	-0.13ns	0.21*	-0.07ns	accept_a	37	0.49	-0.24*	0.42**	-0.11ns	0.13ns	-0.03ns	-0.09ns	-0.09ns
BT_2014_599	Ghana	Ghana	reject	30	0.93	0.11ns	0.04ns	-0.07ns	0.20ns	-0.17ns	0.00ns	-0.07ns	accept_confirmed	38	0.65	-0.27**	-0.23**	-0.41***	-0.23**	0.30**	0.66***	-0.09ns
BT_2014_600	DRC	DRC	accept_a	30	0.64	-0.07ns	0.00ns	-0.07ns	0.15ns	0.17ns	-0.04ns	-0.07ns	reject	41	0.58	-0.18ns	0.24ns	-0.33**	0.47***	-0.03ns	-0.08ns	-0.08ns
		Correct	**15**	**75%**									**11**	**55%**								
		Wrong	**5**	**25%**									**9**	**45%**								
		Unsolved	**0**	**0%**									**0**	**0%**								

Results blind test Entandrophragma cylindricum (Sapelli)

	Claim	Solution	Isotopes									Genetics								
ID_Sample	Group	Group	Decision	N Cluster	Exclusion Probability	Index Ghana	Index Gabon	Indec DRC	Index CIV	Index Con_Braz	Index Cameroon	Decision	N Cluster	Exclusion Probability	Index Cameroon	Index Con_Braz	Index DRC	Index Gabon	Index Ghana	Index CIV
G2S_O_E4	Ghana	Ghana	reject	30	0.88	0.00ns	-0.04ns	-0.60*	0.11ns	0.00ns	0.29ns	unsolved		-	-	-	-	-	-	-
G2S_O_E6	DRC	Cameroon	reject	30	0.67	-0.07ns	-0.04ns	-0.67**	0.11ns	-0.04ns	0.46**	unsolved		-	-	-	-	-	-	-
G2S_O_E8	Cameroon	Cameroon	accept_a	30	0.58	0.11ns	-0.04ns	-0.27ns	0.11ns	0.00ns	-0.04ns	accept_a	25	0.71	0.30ns	-0.10ns	0.00ns	-0.04ns	0.00ns	0.00ns
G2S_O_E11	Con_Braz	Con_Braz	accept_a	30	0.58	-0.04ns	-0.04ns	-0.07ns	0.07ns	0.00ns	0.04ns	unsolved		-	-	-	-	-	-	-
G2S_O_E13	Con_Braz	Con_Braz	accept_a	30	0.55	0.00ns	-0.04ns	-0.27ns	0.07ns	0.00ns	0.13ns	unsolved		-	-	-	-	-	-	-
G2S_O_E14	Con_Braz	Con_Braz	accept_a	30	0.52	0.00ns	-0.04ns	-0.27ns	0.07ns	0.00ns	0.13ns	unsolved		-	-	-	-	-	-	-
G2S_O_E18	DRC	DRC	accept_a	30	0.43	0.14ns	-0.04ns	-0.40ns	0.07ns	0.00ns	0.04ns	unsolved		-	-	-	-	-	-	-
G2S_O_E20	DRC	DRC	reject	30	0.44	0.04ns	-0.04ns	-0.60*	0.11ns	0.04ns	0.21ns	accept_a	66	0.63	-0.30ns	0.15ns	0.08ns	0.02ns	-0.08*	-0.02ns
G2S_O_E22	DRC	DRC	accept_a	30	0.57	-0.04ns	-0.04ns	0.00ns	0.04ns	0.00ns	0.04ns	unsolved		-	-	-	-	-	-	-
G2S_O_E24	DRC	DRC	accept_a	30	0.58	-0.04ns	-0.04ns	-0.07ns	0.07ns	0.00ns	0.04ns	accept_confirmed	48	0.48	-0.45ns	0.08ns	0.30**	-0.04ns	-0.09ns	-0.02ns
BT_2014_510	Ghana	Ghana	reject	30	0.96	-0.04ns	0.00ns	-0.87***	0.18ns	0.00ns	0.38*	accept_confirmed	32	0.59	-1.00***	-0.19*	-0.10ns	-0.03ns	0.79***	0.19*
BT_2014_522	DRC	DRC	reject	30	0.55	0.07ns	0.00ns	-0.67**	0.11ns	0.04ns	0.17ns	accept_a	40	0.62	0.13ns	-0.03ns	-0.03ns	0.05ns	-0.03ns	-0.03ns
BT_2014_525	Ghana	Ghana	reject	30	0.85	0.00ns	0.00ns	-0.40ns	0.11ns	0.00ns	0.13ns	accept_confirmed	25	0.68	-1.00***	-0.19ns	-0.14ns	-0.04ns	0.83***	0.12ns
BT_2014_534	Con_Braz	DRC	accept_a	30	0.29	-0.04ns	0.07ns	-0.47ns	0.07ns	0.00ns	0.17ns	accept_a	45	0.6	-0.28ns	-0.05ns	0.15ns	0.05ns	-0.07ns	0.02ns
BT_2014_552	DRC	DRC	reject	30	0.48	-0.04ns	0.11ns	-0.80***	0.07ns	0.00ns	0.33*	accept_a	51	0.49	-0.19ns	0.09ns	0.15ns	-0.02ns	-0.09ns	-0.02ns
BT_2014_557	DRC	DRC	reject	30	0.45	-0.04ns	0.07ns	-0.67**	0.07ns	-0.04ns	0.33*	accept_confirmed	51	0.63	-0.38ns	0.12ns	0.24**	-0.04ns	-0.09ns	-0.02ns
BT_2014_573	DRC	DRC	accept_a	30	0.29	0.11ns	0.04ns	-0.47ns	0.11ns	-0.07ns	0.08ns	accept_confirmed	39	0.56	-0.31ns	0.03ns	0.23*	-0.03ns	-0.06ns	-0.03ns
BT_2014_577	Cameroon	Cameroon	accept_a	30	0.36	-0.04ns	0.07ns	-0.80***	0.07ns	0.11ns	0.25ns	accept_a	39	0.65	0.00ns	0.09ns	0.03ns	-0.03ns	-0.08ns	0.00ns
BT_2014_580	Cameroon	Con_Braz	accept_confirmed	30	0.59	-0.07ns	-0.04ns	-0.53*	0.11ns	-0.04ns	0.38*	unsolved			-	-	-	-	-	-
BT_2014_592	Con_Braz	DRC	accept_a	30	0.23	0.04ns	0.00ns	-0.47ns	0.07ns	0.04ns	0.13ns	accept_a	62	0.6	0.08ns	0.06ns	0.02ns	-0.02ns	-0.09*	-0.02ns
		Correct	**11**	**55%**								**10**	**83%**							
		Wrong	**9**	**45%**								**2**	**17%**							
		Unsolved	**0**	**0%**								**8**	**40%**							

ANNEX 9

Reporting of the control of chain of custody for two species of Khaya

CHAIN OF CUSTODY OF TIMBER RESOURCE IN SAMARTEX IN THE CONTEXT OF TRACKING SYSTEMS FOR LEGAL TIMBER IN GHANA

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Preamble

In recent years, there has been rapid development of international initiatives to deny market access to illegal timber. Governments in producer countries are being encouraged to establish reliable verification and monitoring systems in order to ensure that their timber exports have been legally sourced and produced.

The total estimated land area of Ghana is 23,854,000 ha (FRA 2010) out of which 7,448,000 ha (32.3%) of the vegetation falls within the High Forest Zone (HFZ) while the remaining 16,406,000 ha is savannah woodland. Timber production, which is about 5% of national GDP, is mainly from the HFZ of which about 1,630,000 ha is designated as forest reserves.

Over the last two decades, Ghana has developed and implemented comprehensive forest management systems including monitoring mechanisms such as harvesting, transporting, processing and exporting of timber and timber products as well as procedures for post auditing with the view of reducing leakages along the chain.

This paper highlights the main tracking process as well as challenges including efforts being made by the Forestry Commission (FC) of Ghana to improve and implement a more reliable monitoring and verification system for timber and timber products. This applies to Yoyo forest reserve a concession managed by Sarmartex Plywood and timber company ltd selected for a pilot study under the ITTO project PD 620/11 Rev.1 (M).

Timber production in Ghana may come either from Government designated forest reserves like Yoyo forest reserve where our pilot study was undertaken or from off-reserve areas. Each of these two sources of timber has different management and monitoring mechanism.

Timber Production from Yoyo Forest Reserve

Yoyo Forest reserve is managed for environmental protection or timber production and or both. Yoyo forest reserve covers an area of 231.31km^2 within the wet /moist Evergreen zone of the High Forest Zone in Ghana. It is one of the designated natural forest timber production reserve. Yoyo production Forest Reserve is sub divided into compartments (unit of management) with an average size of about 128 ha. There is a time line (Harvesting Schedule) for logging individual compartments. Logging history and static inventory of the production area is used as a guide in preparation of a Harvesting Schedule (HS) and every HS is prepared using a forty-year (40-yr) felling cycle.

Concessions (group of compartments) or Timber Utilization Contract (TUC) areas in the yoyo forest reserve where the pilot study was undertaken have been allocated to Sarmartex Timber and Plywood Company Ltd through competitive bidding process but regulation of timber cuts within a particular TUC area is done by the Forestry Commission.

Pre Logging Process in Yoyo Forest Reserve

1. **Stock Survey**: This activity is only initiated when a compartment is due for logging within the Yoyo reserve. Two processes are involved: A demarcation of the compartment due for logging, followed by enumeration of all timber species ≥50 cm dbh. Key activities under the enumeration process are the identification of the timber species, diameter measurement of the tree, the coordinates of the tree and assignment of a stock number for the tree (usually written below the point of felling with a scriber). The stock survey is done by the District Stock Survey Team of the Forest Services Division (FSD) of the FC.
2. **Check Survey**: This activity is a quality control measure by the Regional Team of the FSD. It involves a 5% check of the stock survey conducted by the Stock Survey Team.
3. **Stock Map Preparation**: A graphical representation of all the trees captured during stock survey. It shows the position and distribution of the trees within the compartment. This is done manually by the cartography unit, District FSD of Forestry commission.
4. **Yield Calculation**: It involves an application of a conservative formula to determine the number of stems of individual timber species that should be earmarked for felling within the compartment. The formulae are:

   $Z = 0.5Y + 0.2X$ normal formula

   $Z = 0.25Y + 0.2X$ reduced formula for species that have been overexploited in the past

   Where Z = the yield in number of stems, Y = the number of trees above felling limit, X = the number of trees in the 20 cm size class below the felling limit

5. **Yield Map Production**: This map shows only the spatial distribution of all trees in the proposed yield. It also shows the major skidding trails and log dumps for the TUC Holder (Sarmartex) to use during logging in the compartment. This is usually documented manually by the cartography unit, District FSD of Forestry Commission.
6. **Yield Approval**: The stock map, the proposed yield map and yield table are dispatched to the Regional FSD Office for scrutiny and approval.
7. **Yield Endorsement**: Here the Director of Resource Management Support Centre (RMSC) of the FC will vet the approved yield including environmental adherence and make changes if the need arise before a final consent is given for the TUC Holder

(Sarmartex) to start logging operation within the compartment. It is during this phase that softcopies of the final yield tables together with the yield map are released to both the Regional and District FSD offices for monitoring. A copy is also given to the TUC Holder (Sarmartex).

Logging and Transporting of Timber at Yoyo Forest Reserve

Tracking of timber begins from here. Sarmartex conducts his own felling and carting operations using the yield table and the yield map of the Yoyo Forest reserve as a guide. The following verification and monitoring procedures are conducted before the logs are conveyed to the mill:

- All trees felled are crosschecked by FSD Range Supervisor to make sure that the felled tree is part of the yield. Stock number, species name as well as the coordinates of the tree are the main decisive factors
- Tree bole volume parameters (diameter and length) are taken by the Range Supervisor before the felled tree is sectioned into logs by the logging team. These parameters are entered on a Tree Information Form (TIF) where individual trees volume are calculated to fulfill two key objectives:
    - To determine the stumpage value of the felled tree for the TUC Holder (Sarmartex) to pay
    - For tracking the logs that will be obtained from the felled tree to the mill
- Log Measurement and Conveyance Certificates (LMCCs): The District FSD office issues the LMCC to the Sarmartex before the logs could be transported to any destination within the country for milling. This conveyance certificates has other details such as the Reserve and compartment that the logs are coming from, stock numbers of the trees which were felled, the logs obtained from individual trees, their volumes etc
- Authentication of the LMCCs: this activity is done by a representative of the Timber Industry Development Division (TIDD) of the FC at the various road checkpoints and at the mills. Other checks at the mills that are closely monitored by both TIDD and RMSC are the Sawmill Entry Records and Recovery Records

Post Exploitation Checks in Yoyo Forest Reserve

This activity is usually conducted at Yoyo Forest Reserve at the end of a logging operation in a particular compartment by personnel from RMSC. Key activities here are:

1. Conformity to logging standards including compliance to environmental quality
2. All trees harvested are within the approved yield

Issuance of Compartment Closure Certificate in Yoyo Forest Reserve

This activity is initiated after post exploitation checkers are satisfied with the logging operation within the compartments of Yoyo Forest Reserve.

Penalties

When it is detected that some trees have been extracted outside the approved yield at Yoyo Forest Reserve, ten times the value of the tree is surcharged the TUC Holder (Sarmartex). When the offence is serious, the property mark of the Holder is suspended or revoked.

Mill Site Inspection at Yoyo Forest Reserve

Pre Mill: At the Mill a staff of TIDD will conduct log yard inspection at harvesting compartment to mop up leakages and to ensure that all logs are covered by LMCCs.

Contracts are then approved to ensure value for money and for the avoidance of discrepancies in production

Post Mill: Post milling inspection is conducted as a quality control measure at Yoyo, and to ensure that parcel information conforms to contract requirements.

Input-output analysis of logs and processed wood: This activity is done to ensure that logs for processing are from approved sources. It also monitors efficiency and recovery rates at the industry level.

Harbor Inspection: This is where final checks are conducted before the timber products are exported out of Ghana's shores

Reconciliation of harvesting data: The Resource management support centre (RMSC) of the FC will reconcile all harvesting and milling data by collating all trees harvested and milled at Yoyo Forest Reserve within the year as captured by various FC institutions (FSD, TIDD, and RMSC) to determine infractions. The infraction report is then sent to the Chief Executive of FC.

Challenges with the Existing Mode of Timber Tracking

1. Most of the tracking activities are done manually with a lot of paper work causing delays in delivery
2. Duplication of activities and documentation along the chain of custody among various organizations within FC making reconciliation cumbersome and difficult
3. The entire process is subject to human discretion

4. Errors and abuses are detected late along the chain making corrections and punishment ineffective.
5. Misrepresentation of species always difficult to be detected for processed wood. Thus, endangered species could be exploited and mixed up with species that could be traded.

The Way Forward To Enhance Forest Resource Monitoring in Ghana

The Wood Tracking System (WTS) under the Voluntary Partnership Agreement (VPA) aims at ensuring Forest Law Enforcement Governance and Trade (FLEGT). **Helveta**, a consulting agency in collaboration with FC have piloted a system that aimed at transforming the existing manual chain of custody into an electronic one that would improve efficiency and eliminates leakages and problems associated with the existing methods.

The electronic tracking process involves the use of Hand Held Computers to capture stock survey data, and transfer the data into a central station where yield determination, stock mapping and yield mapping would be automated. A new way of monitoring timber harvesting and transporting, processing and exporting were also piloted by HELVETA. However, most of the processes of the HELVETA system are under human discretion which cannot eliminate Errors and abuses associated with the manual tracking.

Currently, Ghana is a signatory to the VPA and is looking for internationally accepted technologies that could support the vigorous manual chain of custody to eliminate errors and abuses associated with the system. The DNA fingerprints technology for identifying species and source of timber when becomes operational could be used along the line of manual chain of custody to eliminate errors and abuses to ensure sustainable forest management and conservation of biodiversity of the forest resource estate in Ghana.

Project Report

M. PAULINI & A. M. HÖLTKEN

DNA-BASED SPECIES IDENTIFICATION AND TIMBER TRACKING OF *KHAYA IVORENSIS AND K. ANTHOTHECA* IN GHANA

in Frame of the ITTO project

"Development and implementation of a species and timber tracking system in Africa with DNA finderprints and stable isotopes (PD620/11 M (Rev. 1))"

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1. Background

The timber company SAMARTEX and the Forestry Research Institute of Ghana (FORIG) in collaboration with the Resource Management Support Center of the Forestry Commission (FC) were seeking support from the company `Plant Genetic Diagnostics´ GmbH and the Thünen-Institute of Forest Genetics to implement a DNA-based wood tracking verification system. This should improve the overall effectiveness of the existing Wood Tracking System (WTS) set up in accordance with the Voluntary Partnership Agreement (VPA) and the due diligence obligations of SAMARTEX in frame of the EU timber regulation.

Illegal logging and trade in illegal timber continues to be a major threat to sustainable forest management in West Africa. The issue has attracted much attention from NGOs, policy makers and the media. Although legal instruments have been established to control logging and trade in illegal timber at both national and regional levels, these instruments are still lacking, providing ample opportunity for fraud and the continuation of illegal activities.

Over the last two decades, Ghana has developed and implemented comprehensive forest management systems, including monitoring mechanisms for harvesting, transportation, processing and exporting of timber and timber products. Many challenges to the effective execution of these systems remain including:

- A reliance on manual tracking activities generating a lot of paper work that causes delays in delivery,
- duplication of activities and documentation along the Chain-of-Custody amongst various organizations within the Forestry Commission making reconciliation cumbersome and difficult.
- The entire process is subject to human discretion and fraud is suspected to be commonplace.
- Errors and abuses are detected late along the chain making corrections and punishment ineffective.

DNA-based methods are now available that resolve these problems and provide timber companies and timber traders a high level of security. DNA provides a scientific, truly independent and infallible platform to validate Chain-of-Custody documentation and eliminate fraud. By comparing the individual genetic profile of a wood sample taken from a tree during the forest inventory with the genetic profile of a wood sample taken after harvesting at another point in the supply chain (e.g. log yard or mill), it is possible to independently validate the paper-based or electronic tracking system ensuring that correct trees have been harvested and processed.

In addition to the proof of correct and legal origin of the timber, the company SAMARTEX has much interest to know the exact botanical species of *Khaya* due to very similar and sometimes overlapping morphological traits making species identification by tree spotting in the field quite difficult (see table 1). This is an economically important information because there are clear differences in wood quality between *K. ivorensis* and *K. anthotheca* and thus needs to be known before felling the wrong trees causing further ecological and economical damage. Moreover, the EU-timber regulations ask for precise declarations of the botanical species.

Table 1: Most important morphological traits of *Khaya anthotheca* and *K. ivorensis*

Trait	*Khaya anthotheca*	*Khaya ivorensis*
Leaflets	2-4 pairs, 8-15 X 4-8 cm	4-7 pairs, 5-14 X 2-6 cm
Laterals	5-9 pairs	5-9 pairs
Ecology	Dry to moist semi-deciduous	Evergreen to moist semi-deciduous
Fruits	6-10 cm diameter, 4-5 valved, <3 (5) mm thick	4-7 cm diameter, 5 valved, <3 (5) mm thick
Bark	Smooth, often pale, with scattered scales	Rough, scaly (smooth when younger

Figure 1: Very similar morphology of *Khaya anthotheca* and *K. ivorensis*

In frame of the ITTO project "Development and implementation of a species identification and timber tracking system in Africa with DNA fingerprints and stable isotopes" we conducted a pilot study with the following three objectives:

1. Development of DNA-fingerprinting methods that can be used to identify the exact *Khaya* species before felling
2. Test of reliability of the DNA-based tree tracking system approach to control a chain-of-custody of *Khaya* timber

2. Material and Methods

2.1 Sampling of plant material

Cambium samples from 400 trees (*Khaya ivorensis* and *K. anthotheca*) were collected in the actual logging zone of the SAMARTEX concession (Fig. 2 and 3). The samples were stored in plastic bags or tubes with silica gel for quick dehydration and DNA conservation. Later, after the felling, wood and veneer samples were colleted at the saw mill and the veneer production facility. Most timber samples include only trees that were part of the before samples individuals in the logging area. After a mission in Ghana, all these timber samples were personally transported to Germany and passed over to PLANT GENETIC DIAGNOSTICS (PGD) Ltd.

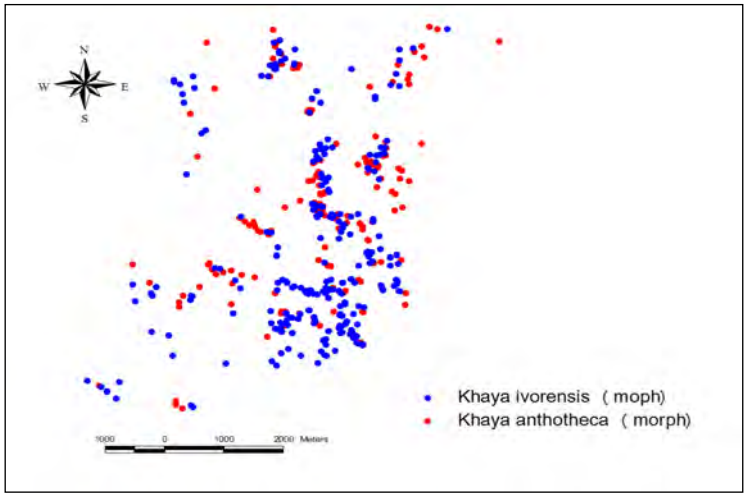

Figure 2: Distribution and morphological species identification of the sampled *Khaya* trees in the concession

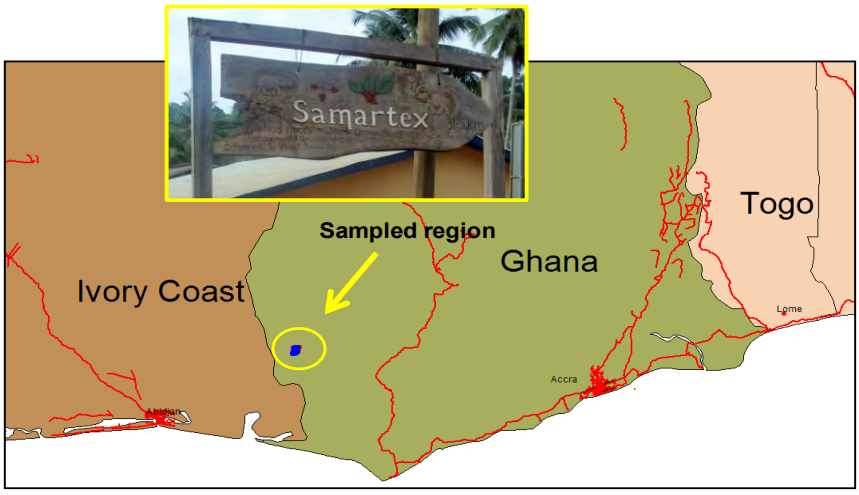

Figure 3: Location of the Samartex logging concession in Ghana

2.2 Lab work

2.2.1 DNA isolation

In the last years various protocols have been developed and published to extract DNA from wood, from recently logged (almost fresh) timber up to processed wood and woody products from different steps in the chain-of-custody (DE FILIPPIS & MAGEL 1998, DEGUILLOUX et al. 2002, RACHMAYANTI et al. 2006, ASIF & CANNON 2007). In this study we used a new protocol which is applied for patent with partners from Germany and Australia and that was already used for up to 1000 years old oak timber samples. This approach is aligned to mitigate the effects of contamination of the samples with external DNA, to minimize further demolition of already degraded DNA sequences and to purify timber DNA from the majority of PCR inhibitors such as cellulose and hemicellulose, lignin (almost phenolic groups), waxes and different pigments and colours.

2.2.2 The selection of DNA markers

In many studies nuclear microsatellites, also known as simple sequence repeats (nSSRs), revealed to be the optimal choice of marker. Due to high variability and codominance this kind of marker is suitable for a combination of both, forensic identification purposes (e.g. individual fingerprints for the development of tracking systems to control chain-of-custody, LOWE et al. 2010) and species identification (HÖLTKEN et al. 2012, 2014). Furthermore, SSRs have been used to study spatial genetic structures and to develop geo-genetic reference maps. The latter method allows an independent determination of the geographic origin of timber without reference sampling during the timber chain-of-custody (see JOLIVET & DEGEN 2012).

Several sets of microsatellites have already been developed for different genera of the Meliaceae family such as *Khaya*, *Swietenia* and *Entandrophragma* (see KARAN et al. 2012, LEMES et al. 2002, LEMES et al. 2003, LI et al. 2010, SEXTON et al. 2010, WHITE & POWELL 1997 etc.). After testing a lot of primer pairs, six loci were assessed to be very suitable to deal with the above mentioned objectives.

Table 2: Primers before and after optimization (sequences, fragment length in base pairs, GC/AT-relation)

	primer information from literature			primer information after optimization		
SSR-Locus	primer-sequences	fragment length	GC/AT	primer-sequences	fragment length	GC/AT
Ks063	F: CAATATAAGGGACAATACTCTCA	206-268 bp	8 / 15	F: CAATATAAGGGACAATACTCTCA	209-228	8 / 15
	R: CAACATAGATCCATCGTGAGT		9 / 12	R: TATACAAACATAGATCCATCGT		8 / 14
Kse2_11	F: TTTCTACCAGTGCGGTCCCT	278-294 bp	11 / 9	F: TTTCTACCAGTGCGGTCCCT	252-270	11 / 9
	R: GTGCAAATTGTGCTGGGGTG		11 / 9	R: GTGCAAATTGTGCTGGGGTG		11 / 9
Kse1_14	F: TGTTCTCCCAGTTATGGCAGTG	159-193 bp	11 / 11	F: GTTCTCCCAGTTATGGCAGTG	134-166	11 / 10
	R: CCGGTGGAAGTGATTTGACCTT		11 / 11	R: CCGGTGGAAGTGATTTGACCT		11 / 10
Ks079	F: TTTCAACTCTTCAATCTTCATCT	87-115 bp	7 / 16	F: CAACTCTTCAATCTTCATCTGG	93-130	9 / 13
	R: GGCACTACCAATATTTTGTTTT		7 / 15	R: GAGACGGGGCACTACCAATA		11 / 9
Kse1_23	F: AATGAGTAATGACAAGAAAGTA	344-394 bp	6 / 16	F: GCTAATGAGTAATGACAAGAAAG	343-383	8 / 15
	R: AATTGGCGGATAGTTGATGT		8 / 12	R: GGCGGATAGTTGATGTATGC		10 / 10
Kse3_29	F: TTAGGCATAACCGAGGAAAC	193-229 bp	9 / 11	F: TAGGCATAACCGAGGAAACAG	168-195	10 / 11
	R: AAGGCTGTCATTGAAGATAGGAG		10 / 13	R: GCCTGTCATTGAAGATAGGAG		10 / 11

2.2.3 Optimisation of the chosen SSR-Markers for the analysis of timber samples

We designed new primers considering high degradation and low quality of DNA in timber. As shown in table 2, we reduced the sizes of several microsatellites fragments, we chose the primer nucleotide sequences in order to achieve high conformity of the annealing temperatures of forward and reverse primers (50-60°C), consisting of the nucleotides Guanin or Cytosin at their 3´- and 5´- ends allowing an optimized annealing of the primers to the single-stranded DNA template. Further, four of the used six microsatellites consist of repeating sequences of three base pairs of DNA providing a much more accurate amplification with lower numbers of "stutter" bands (ssrKS16, Ks063, Kse3-29, Ks079).

In the following we specified the information about the used SSR-fragments. The optimized primer pairs are visualized in grey and the SSR-units (sequence repeats) underlined:

Ks063; GenBank HQ200185.1

TACATATTGTTCTAAAAACAATATAAGGGACAATACTCTCATTATCTATTGATGGTACCTTTAACAATAATATGA
AATTTAAAAAATAATATTGCTATGTTAAAAATAATAAAGCAAATATTCAAAAGAATAACAATATATAGAACAAAA
TAATAATAATAATAATAATAATAATAATAATAAAGGACCTACTTATAATAAAATAAATAAATAAAAACTCAC
GATGGATCTATGTTGGTATATTTAGTTAGTTACAGTGCTACTTGTATTTTTT

Kse1-14; GenBank GU903058.1

TCATGTTCTCATGTTCTCCCAGTTATGGCAGTGGGGTAACCCTTAGGTGCATCATTGAATAACCAGCTTCATGCA
ACTCAGTATTTTTAGGCATAGGCATCCAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGATACCTCAAACA
AGGTCAAATCACTTCCACCGGTAATGTTCCACACAATTCCAGTAGCCCTCTCTATACCAAT

Kse1-23; GenBank GU903059.1

CAAAAGCCTGGAAGTTAATGCAAAAGGTTGAACTTAATTTGACGAAGCTAATGAGTAATGACAAGAAAGTATAAT
TCTATTTTCCTTGAATATGAATCAAAGAGATGGTTAATTTGTCCTATAGTTAGAAGGCAAGTGGCCAGGCTAGCA
AGGCTCAACAATAAATTTATATCGCACAAGCGCCATAACTCCACCATGAGTGAGAAGAAGAAGAAGAAGAAGAAG
AAGAAGAAGCAGAGTAAATTTATTGGTGAGGTTGGTGGAAGCCGCATCTACGTAATCTAGAAAGGCACCTTCCTC
TCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTGCATTTGCCTAGAATAATTA
GATTCCTCATTTAATGCATGGAGAAAAACCATTAAACGCATACATCAACTATCCGCCAATTTTGGTAAGTGCATT

Kse2-11; GenBank: GU903061.1

TAGAGTTCAAAAGGCCATGCACACAAGAAACAACACATACGAGTGCATAAGCCTATTTCTACCAGTGCGGTCCCT
CCTAAAATCATAACAAAAGATTGTGGATAATTGGCCTATAAAATTAACTGCAATTCATGAAGGGTTCAACAAACA
AGTCAAAGAAATTTAGTAAAGATTGGCAAATATTTTCAGACAATAAAGGGTGAGAGCCTGAAAACATTGCAGACA
AATCCATCCTTGTTAACCAATGTCAAAAATGTTACTAGCATGTGTGTGTGTGTGTGTGTGTGTCTCCCCGTAT
CACTCACACCCCAGCACAATTTGCACCTAACACAGCCTGATATATC

Kse3-29; GenBank GU903064.1

GCACTGGATTTAAGAGGCACAAAGACTTATTTAGGCATAACCGAGGAAACAGCCCACAAGCTTTACCTGATAAAT
TGCCAGAATCATCACACTCATCAACTTCCTCTTCACT*TTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC*TATAT
CCTCCCCATAATGAGGTATGACAATAGACTTGTTAACAAGAGGCCTCCTATCTTCAATGACAGCCTTCATAACTT
CTCTTAGTTGCTCAATTGC

Ks079; GenBank: HQ200189.1

TTTTCAACTCTTCAATCTTCATCTGGACACTCTTCTACTTCTTCTTCTTCTTCTTCTTCTTCGCTCAGTTTCTGC
TTTCCAAAACAAAATATTGGTAGTGCCCCGTCTCGTATTTTGCCCCGCAAAAAGCCGACAGATTTTTTGATATGA

2.2.4 Testing amplification success of the chosen microsatellites

Amplification success of the chosen primer pairs was tested using three cambium samples from each of the two *Khaya* species (PCR-conditions in table 3A-D). As expected, the microsatellites with repeats of three base pairs yielded in more accurate amplification results than fragments consisting of two base pair repeats because of a lower amount of slippage events during polymerase activity. Two examples are given in Figure 4 (SSR-Loci Ks079, fig. 5a and b; Kse1-14, fig. 5c and d), showing the results of the fragment length detection on an ABI 3730 genetic analyzer. Altogether, the optimization of the primer pairs resulted in excellent amplification success.

Tables 3A – 3D: The PCR conditions are shown in Tables 2A to 2D.

SSR-Loci: Ks063, Kse2-11, Kse1-14, Kse3-29, Ks079

A

PCR-component	C stock	C final	V_R (µL)
PCR-buffer	10 X	1 X	1,50
$MgCl_2$	25 mM	1,75 mM	1,05
dNTPs	10 mM	0,2 mM	0,30
primer for	10 mM	0,2 mM	0,15
primer rev	10 mM	0,2 mM	0,15
polymerase	5 U/µL	0,4 U/µL	0,12
DNA	10 ng/µL	2 ng/µL	3,00
H_2O			8,73
	total reaction volume		15,00

B **PCR-programme**

First denaturation	94°C	3 min	
Denaturation	94°C	30 sec	
Annealing	57-59°C	45 sec	30 X
Elongation	72°C	1 min	
Final elongation	72°C	10 min	
Conservation	8°C	∞	

SSR-Locus: Kse1-23

C

PCR-component	C stock	C final	V_R (µL)
PCR-buffer	10 X	1 X	1,50
$MgCl_2$	25 mM	2,00 mM	1,20
dNTPs	10 mM	0,2 mM	0,30
primer for	10 mM	0,2 mM	0,15
primer rev	10 mM	0,2 mM	0,15
polymerase	5 U/µL	0,4 U/µL	0,12
DNA	10 ng/µL	2 ng/µL	3,00
H_2O			8,58
	total reaction volume		15,00

D **PCR-programme**

First denaturation	94°C	3 min	
Denaturation	94°C	30 sec	
Annealing	50°C	45 sec	30 X
Elongation	72°C	1 min	
Final elongation	72°C	10 min	
Conservation	8°C	∞	

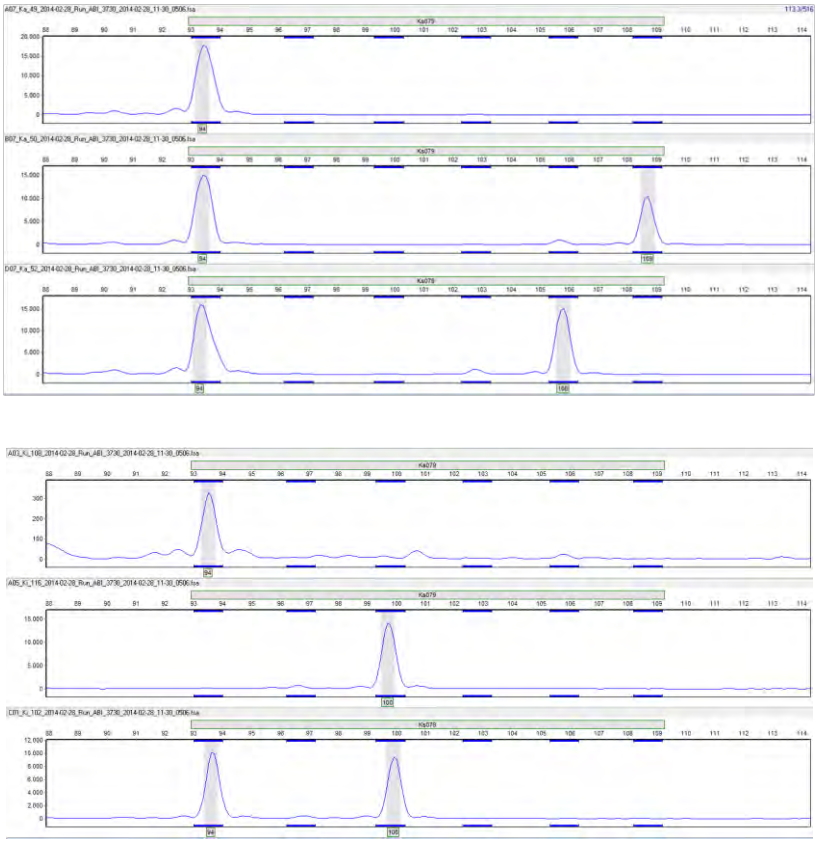

Figure 4a: Fragment lengths of three *Khaya anthotheca* (above) and three *K.ivorensis* (below) samples at the SSR locus Ks079 (3bp-repeats)

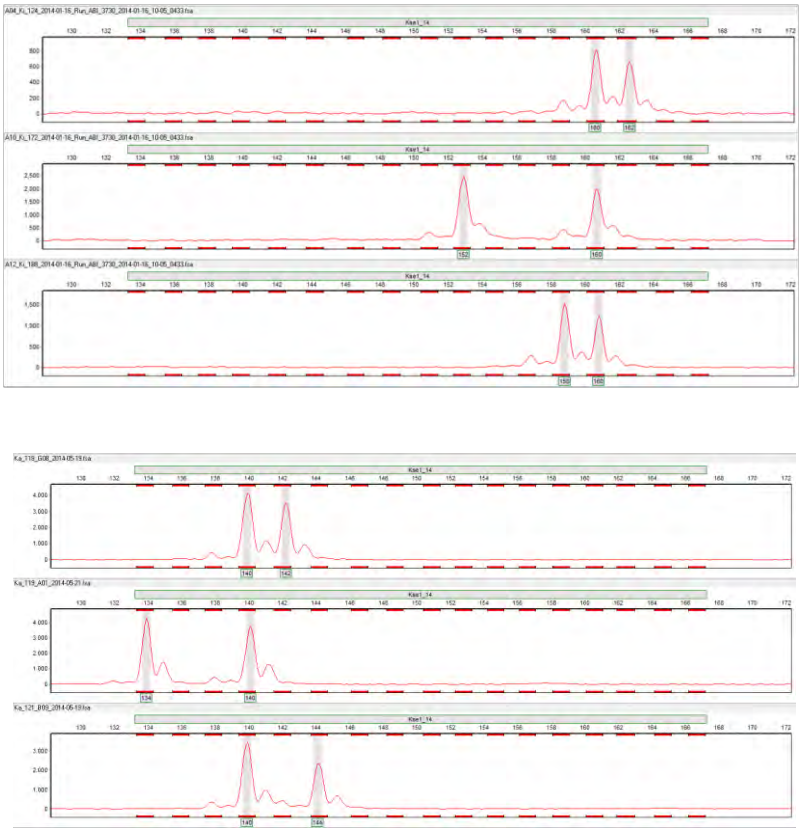

Figure 4b: Fragment lengths of three *Khaya anthotheca* (above) and three *K.ivorensis* (below) samples at the SSR locus Kse1-14 (2bp-repeats)

2.2.5 Population genetic calculations

2.2.5.1 Species identification and hybrid quantification

The assignment of individuals to reproductive groups was carried out using the software STRUCTURE 2.3.2 (PRITCHARD et al. 2009, FALUSH et al. 2003, 2007). This method is suitable to assort individuals to populations with restricted gene flow or to different taxonomic units (species or subspecies), but it also enables to detect hybrid swarms and to quantify the proportion of hybrids. The programme operates on a model-based Bayesian clustering method, which allows conclusions on population structures, reproductive conditions and mating systems, independent of predetermined clustering structures. In the process, the proportions of genetic admixture of each individual are quantified leading to an individual assignment to reproductive units or clusters.

2.2.5.2 Genetic profiles, diversity and differentiation

On the basis of the clustering results, allelic frequencies of the six analysed microsatellite loci were calculated for each of the reproductive group. Differences between clusters are highlighted by assembling the frequency information into allelic profiles (histograms).

Further, the genetic distances of the reproductive groups or clusters were characterized using the genetic differentiation parameter according to GREGORIUS (1974). This parameter measures the proportion of genetic types not shared by both of the populations. It equals half of the sum of the absolute differences of the frequencies of genetic types in populations X and Y. This distance parameter reaches its maximum value 1, if the two populations have no genetic types in common and its minimum value 0 if the two populations have identical genetic structures:

$$d_0 = 0{,}5 \sum_{i=1}^{n_k} |x_i - y_i|$$

x_i and y_i denotes the frequency of the i-th genetic type in population X and Y at the k-th locus

2.2.6 *Timber tracking*

The DNA-isolates of the wood samples, many of them part of the sampled trees in the logging area, were analysed using the six optimized SSR-markers for tracing back the timber chain-of-custody. Microsatellites are commonly accepted, particularly in forensic studies, due to their high variability and high exclusion probability.

3. Results and Discussion

Based on multilocus-SSR data (see appendix 1) we obtained information about the clustering of the genetically studied individuals into reproductive groups incl. hybridization as well as population genetic parameters of the clusters.

3.1 Reproductive groups

3.1.1 Reproductive groups based on genetic data

Compared to other closely related species, we found an exceptionally high genetic differentiation between two groups of individuals originating from a single area within the complex concession structure of the company SAMARTEX (figure 5). For example, in the two oak species *Quercus petraea* and *Q. pubescens*, native to Central and Southern Europe, for which species integrity is also presserved to a high extent, an estimation of species identity based on genetic markers has been shown to be more accurate on the population than on the individual level (HÖLTKEN et al. 2012). In the case of Khaya we are able to precicely differentiate single individuals into reproductively isolated and taxonomic groups. The proportion of potential hybrids is very low (<7%). This low value may also be interpreted as a result of sharing rare alleles by some individuals or as PCR-artefacts by polymerase slippage.

In conclusion, the genetic method developed in this study offers a reliable approach for a clear distinction of taxonomic groups on the individual level in a species mixture of *Khaya ivorensis* and *K. anthotheca*.

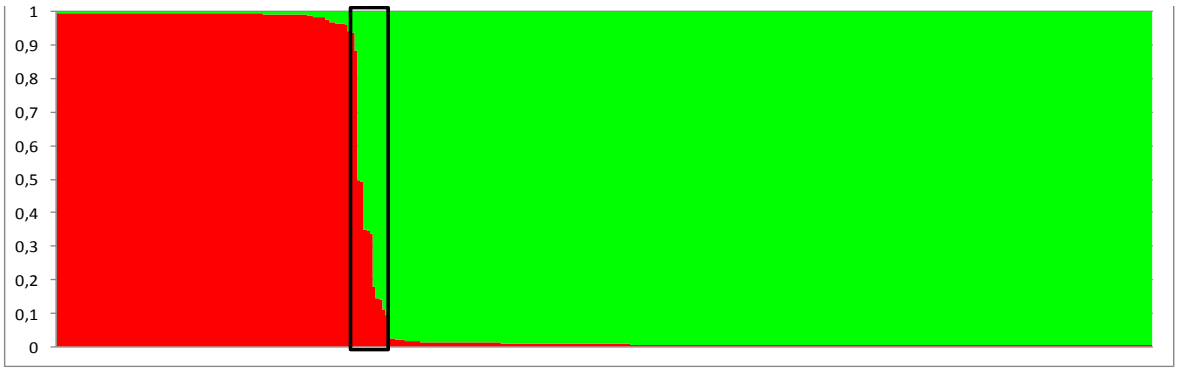

Figure 5: Genetic clustering of the analysed *Khaya* individuals based on STRUCTURE results of six SSR-loci; individuals ordered by genetic admixture

3.1.2 Differences of genetic and morphological characterization

In figure 6 the individuals are ordered by morphological species identification after the analysis of their genetic admixture. Individuals on the left side of the figure were phenologically characterized as *K. anthotheca*, on the right side as *K. ivorensis* (see arrows). The results clearly indicate that the conditions in the field are difficult for species identification by tree spotting, which may be a larger problems in tropical than in deciduous forests (tall trees, worse light conditions, dense shrub layer etc.). High error rates were detected for *K. anthotheca* (XXX%), a more accurate morphological species identifiation could be recorded for *K. ivorensis* (error rate XX%) with the more valuable timber.

In conclusion, this new and ready-to-use genetic approach offers a highly accurate „prescreening“ procedure before harvesting activities in order to evaluate the dominating *Khaya* species within concessions, reducing economical and ecological damage.

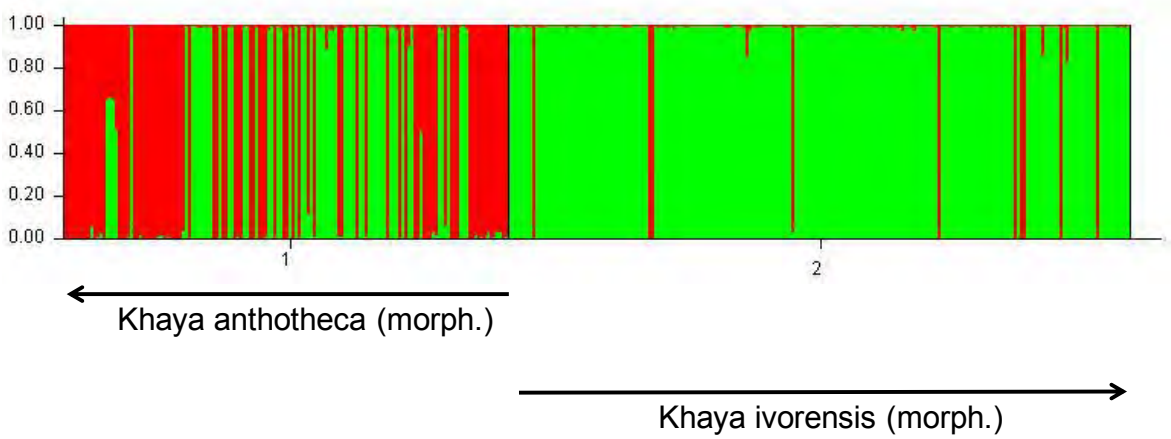

Figure 6: Genetic clustering of the analysed *Khaya* individuals based on STRUCTURE results of six SSR-loci; individuals ordered by morphological species identification; ■=*K. anthoteca* (genetic identification), ■=*K. ivorensis* (genetic identification)

3.2 Genetic structure of the reproductive units

Differences in allelic frequency profiles and a clear genetic differentiation underline the reliability of the developed genetic tool for differentiating the two species *Khaya ivorensis* and *K. anthotheca*.

In figure 7 the allele frequencies of the two reproductive units are shown for each of the six used microsatellite markers. For most of the loci we found two or more dominating alleles, which were found to be more or less fixed to one of the reproductive units (*K. ivorensis* or *K. anthotheca*). For example SSR-locus Ks063: *K. ivorensis* is almost fixed for the alleles 219 and 222, whereas alleles 212 and 215 dominate in *K. anthotheca*. The similar situation holds for the loci Kse2-11, Kse1-14 and Kse3-29. SSR-locus Kse1-23 shows a much higher variability but also low overlapping allelic frequencies. For Kse-079 we detected one dominating allele for both species (allele with 94 basepairs), but differentiating for the alleles 97 to 130.

Altogether, the genetic differentiation d_0 of the gene pool was 0.849. That means, 84.9% of the allelic variants have to be exchanged between the two reproductive groups to obtain identical population genetic structures. Single locus values for this parameters varied between 0.647 and 0.944. These are exceptionally high values for closely related species indicating a very restricted gene flow by hybridization effects (allele swamping).

This selection of microsatellite loci allows a reliable species identification using different statistical approaches. Here we used STRUCRURE 2.3.2 as basic methodology which clusters individuals into groups showing optimals Hardy-Weinberg conditions , but further procedures such as Geneclass based on gene frequencies should also offer very precise results using the developed genetic background data of this study.

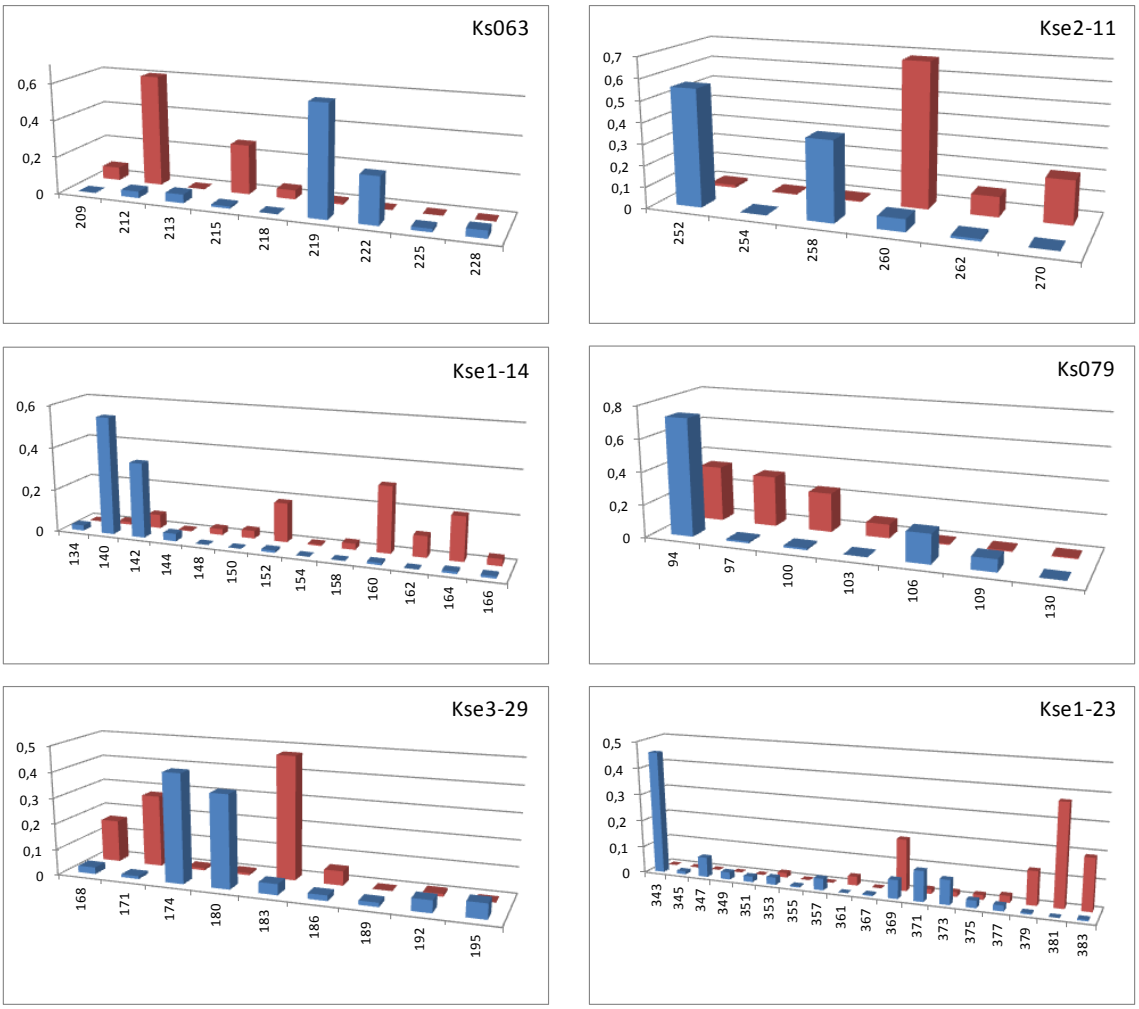

Figure 7: Allelic frequency profiles of the genetically identified reproductive groups in Khaya; ■= K. ivorensis, ■= K. anthotheca

3.3 Timber DNA analysis

More than 200 cambium and timber samples have been analyzed using the above optimized microsatellite primers. Table 4 represents a selection of the results of the timber tracking test. Cambium and corresponding timber samples (from the same putative tree) have been collected in order to evaluate the effectiveness of the chosen DNA marker system to work on woody tissues with low DNA quality and quantity.

Over all, about 50% of the timber samples could be genotyped at least at three of the six microsatellite loci. Due to the high variability of this marker system, this outcome should be enough to detect fraud in the documentation of the chain-of-custody of this high value timber (see serial no. 384, 420 in table 4). Nevertheless, the operators of this new timber tracking system should act with caution, because not all microsatellite fragments turned out to be stable. This is the case for SSR-locus Kse3-29. In several cases some alleles are not amplified at all (e.g. serial no. 213, 357, 421, 426) or the PCR generated shifts according to the length of the repeat motiv (3 bp).

To affirm the obtained results, two repetitions of the timber DNA analyses are recommended. Two further repetitions should be carried in the suspicious cases.

Table 4: A selection of the results concerning the *Khaya* timber tracking test; ■ PCR-misamplification due to low DNA quality; ■ clear wrong declaration of timber

Material	Serial No.	Kse063	Kse063	Kse2_11	Kse2_11	Kse1_14	Kse1_14	Ks079	Ks079	Kse3_29	Kse3_29	Kse1_23	Kse1_23
Kambium	213	215	215	260	270	164	166	100	100	171	183	-1	-1
Timber_1	213a_2	-1	-1	260	270	164	166	100	100	171	171	-1	-1
Timber_2	213b _1	215	215	260	270	164	166	-1	-1	183	183	-1	-1
Kambium	354	219	219	252	258	140	140	94	94	174	180	343	369
Timber_1	354a	219	219	252	258	140	140	-1	-1	-1		-1	-1
Timber_2	354b	219	219	-1	-1	140	140	-1	-1	-1	-1	-1	-1
Kambium	357	218	218	260	270	160	162	100	100	168	183	-1	-1
Timber_1	357a_1	218	218	260	270	160	162	-1	-1	183	183	-1	-1
Timber_2	357a_2	218	218	-1	-1	160	162	100	100	168	183	-1	-1
Timber_3	357b_1	218	218	260	270	160	162	-1	-1	183	183	-1	-1
Timber_4	357b_2	218	218	260	270	160	162	100	100	168	183	-1	-1
Kambium	361	212	212	260	270	152	160	94	94	183	183	383	383
Timber_1	361b_1	-1	-1	260	270	152	160	-1	-1	183	183	-1	-1
Timber_2	361b_2	-1	-1	260	270	152	160	-1	-1	183	183	-1	-1
Kambium	384	212	215	252	258	142	160	-1	-1	174	183	-1	-1
Timber_1	384a	-1	-1	270	270	152	166	-1	-1	183	183	-1	-1
Timber_2	384b	-1	-1	270	270	-1	-1	-1	-1	183	183	-1	-1
Kambium	406	212	218	260	270	150	164	97	97	168	171	-1	-1
Timber_1	406a_1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Timber_2	406a_2	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Kambium	408	219	222	252	258	140	142	106	106	174	174	343	373
Timber_1	408a	-1	-1	252	258	140	142	-1	-1	174	174	343	373
Timber_2	408b	-1	-1	252	258	140	142	106	106	174	174	343	373
Kambium	409	212	215	260	260	150	164	100	103	168	171	-1	-1
Timber_1	409a	212	215	260	260	150	164	100	103	168	171	-1	-1
Timber_2	409b	212	215	260	260	150	164	100	103	168	171	-1	-1
Kambium	410	219	219	252	252	142	144	106	106	180	192	343	343
Timber_1	410a	219	219	252	252	142	144	106	106	180	192	343	343
Timber_2	410b	219	219	252	252	142	144	106	106	180	192	343	343
Kambium	411	212	215	252	258	158	162	-1	-1	183	186	-1	-1
Timber_1	411a	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Timber_2	411b	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Kambium	415	219	222	252	258	140	142	94	94	174	192	349	373
Timber_1	415a	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Timber_2	415b	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Kambium	416	212	212	260	270	152	164	-1	-1	171	183	-1	-1
Timber_1	416a	212	212	260	270	152	164	-1	-1	171	183	-1	-1
Kambium	419	212	212	260	260	148	164	-1	-1	171	183	-1	-1
Timber_1	419a	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Timber_2	419a	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Kambium	420	219	219	252	258	140	140	94	94	174	180	343	375
Timber_1	420a_1	212	212	-1	-1	152	164	-1	-1	-1	-1	-1	-1
Timber_2	420a_2	212	215	260	262	152	164	94	94	171	183	-1	-1
Kambium	421	222	222	252	258	140	142	94	94	168	195	343	369
Timber_1	421a_1	222	222	252	258	140	142	-1	-1	168	168	-1	-1
Timber_2	421b_2	222	222	252	258	-1	-1	94	94	168	195	343	369
Kambium	423	222	222	258	258	140	144	94	94	174	192	343	357
Timber_1	423a	222	222	258	258	140	144	94	94	174	192	343	357
Timber_2	423b	222	222	258	258	140	144	94	94	174	192	343	357
Kambium	426	212	212	260	270	162	164	-1	-1	171	171	-1	-1
Timber_1	426a_1	212	212	260	270	164	164	94	94	171	183	-1	-1
Timber_2	426a_2	212	212	260	270	164	164	-1	-1	171	171	-1	-1
Kambium	429	219	219	252	258	140	142	94	94	174	180	343	343
Timber_1	429a	-1	-1	252	258	140	142	94	94	174	180	343	343
Timber_2	429b	219	219	252	258	140	142	94	94	174	180	343	343
Kambium	430	219	219	258	258	140	142	106	106	174	186	343	373
Timber_1	430a	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Timber_2	430b	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Kambium	432	219	219	258	260	140	140	94	94	180	180	345	357
Timber_1	432a	219	219	-1	-1	140	140	-1	-1	-1	-1	-1	-1
Timber_2	432b	219	219	258	260	140	140	-1	-1	-1	-1	-1	-1
Kambium	435	-1	-1	252	258	140	142	106	106	168	180	343	371
Timber_1	435a_2	-1	-1	252	258	140	142	106	106	168	180	343	371
Timber_2	435b_1	219	219	252	258	140	142	-1	-1	168	183	-1	-1
Kambium	444	212	215	260	260	152	164	-1	-1	-1	-1	-1	-1
Timber_1	444a	212	215	260	260	152	164	-1	-1	168	168	-1	-1
Timber_2	444b	212	215	260	260	152	164	-1	-1	168	168	-1	-1

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ANNEX 10

Technical report on the sampling

Projet PD 620/11 M (Rev. 1)

Développement et application d'un système d'identification des espèces et de traçabilité du bois à l'aide d'empreintes génétiques et d'isotopes stables en Afrique

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Rapport de synthèse sur la phase de récolte des échantillons

Nils Bourland

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1. Objectifs de l'échantillonnage et procédures mises en œuvre

Dans un contexte de lutte contre l'exploitation illégale des bois tropicaux africains, le projet a pour objectif de développer puis valider des outils permettant d'identifier (1) l'espèce exploitée (barcoding et anatomie du bois) et (2) l'origine géographique du bois et des produits dérivés (empreinte génétique et chimie du bois via isotopes stables). Le projet concerne les étendues forestières réparties sur sept pays parmi les principaux exportateurs de bois, à savoir : Cameroun, Côte d'Ivoire[1], Gabon, Ghana, Kenya, République Démocratique du Congo (RDC) et République du Congo (« Congo » dans la suite du document). Sur le terrain, deux procédures d'échantillonnage et de collecte d'échantillons ont été mises en œuvre selon le groupe de taxons concernés et le type d'analyses de laboratoire à effectuer.

La **première procédure** (activité 2.1 du projet : origine géographique) a consisté en la récolte de matériel végétal destiné aux analyses génétiques (morceau de limbe ou fragment de cambium[2]) et de chimie du bois (copeaux[3]) pour les trois principaux taxons du projet (« taxons-cibles prioritaires », ou TCP dans la suite du document) : *Triplochiton scleroxylon* (ayous/obeche/samba), *Milicia* spp. (iroko) et *Entandrophragma cylindricum* (aboudikro/sapelli). Pour ces trois taxons, la taille de l'échantillon à atteindre était de 4.800 individus collectés à fins d'études génétiques (2.000 iroko, 1.600 sapelli et 1.200 ayous) et 720 arbres fournissant les copeaux de bois (300 iroko, 210 pour chacune des deux autres espèces). Le nombre de population[4] ainsi que l'effectif moyen attendu par population et par espèce sont présentés dans le tableau 1.

Tableau 1. Effectifs attendus de l'échantillonnage des trois taxons-cibles prioritaires, ou TCP (développement de méthodes de localisation de l'origine géographique) dans les sept pays-cibles.

Taxons-cibles prioritaires	Nombre de populations	Effectif moyen / pop. en "génétique"	Effectif moyen / pop. en "chimie"	Effectif total en "génétique"	Effectif total en "chimie"
Milicia excelsa	100	20	3	**2.000**	**300**
Entandrophragma cylindricum	80	20	3	**1.600**	**240**
Triplochiton scleroxylon	60	20	3	**1.200**	**180**

La **seconde procédure** (activité 1.1 du projet : identité du taxon) a concerné 21 taxons dits taxons-cibles secondaires (TCS) incluant les 3 TCP (tableau 2). Le but ultime ici étant de développer des méthodes fiables d'identification des taxons, le nombre d'échantillons nécessaire était moindre et fixé à 10 par espèce. Autant que possible, l'échantillonnage de chaque individu devait être double, comprenant un

[1] La République Centrafricaine, initialement concernée par le projet, a été remplacée par la Côte d'Ivoire pour des raisons de sécurité des équipes de terrain (conflit armé en Centrafrique lors de l'échantillonnage).

[2] Le limbe fait référence à un fragment de feuille fraîche couvrant une surface cumulée de 20 à 50 cm², tandis que le fragment de cambium devait également être prélevé sur une surface d'environ 5 cm² à l'état frais.

[3] Le terme « copeaux » fait référence à la sciure récoltée par perçage du tronc à l'aide d'une foreuse ou, lorsque l'utilisation de cet outil s'avérait impossible, aux fragments prélevés à l'aide d'une machette dans le bois frais de l'arbre-cible.

[4] Une population a été définie *a priori* comme étant l'ensemble des tiges d'un taxon donné dans une surface géographique délimitée par un carré de 100 à 150 km de côté.

fragment de feuille ou cambium (barcoding) et un morceau de bois destiné aux études d'anatomie du bois. De plus, l'échantillonnage devait être réparti aussi uniformément que possible sur l'aire de distribution de l'espèce, dans la limite des zones de collecte du présent projet.

Tableau 2. Liste des taxons-cibles secondaires (TCS) devant être échantillonnés à fins de développement de méthodes d'identification du taxon. Le code identifiant chaque espèce lors de l'encodage est également mentionné.

Espèce	**Code**	**Espèce**	**Code**
Afzelia spp.	AFZ	*Lophira alata*	LOP
Aucoumea klaineana	AUC	*Milicia excelsa*	IRO
Baillonella toxisperma	BAI	*Milicia regia*	IRO
Cylicodiscus gabunensis	CYL	*Millettia laurentii*	MIL
Entandrophragma angolense	ENTA	*Nauclea diderrichii*	NAU
Entandrophragma cylindricum	ENTC	*Pericopsis elata*	PER
Entandrophragma utile	ENTU	*Pouteria aningeri / Pouteria altissima*	POU
Erythrophleum ivorense	ERY	*Pterocarpus soyauxii*	PTE
Erythrophleum suaveolens	ERY	*Terminalia superba*	TER
Guibourtia spp.	GUI	*Triplochiton scleroxylon*	TRI
Khaya spp.	KHA		

2. Protocole d'échantillonnage

2.1. Matériel de collecte

Le matériel suivant a été acquis puis transmis aux équipes de terrain :

1. Matériel nécessaire à la collecte, au séchage puis au stockage des morceaux de limbe/cambium : du gel de silice, des enveloppes en papier et sacs hermétiques transparents ;
2. Outillage nécessaire au prélèvement des copeaux/blocs de bois : des kits de "foreuse-visseuse" Makita comprenant chacun, outre l'appareil, trois batteries, deux mèches, un transformateur de courant continu vers du courant alternatif et une mallette de transport ;
3. Matériel de stockage des copeaux et blocs de bois : des sacs en coton ;
4. Matériel de communication : des téléphones-satellite ;
5. Matériel de positionnement géographique : des GPS et piles appropriées ;
6. Petit matériel/consommable : frondes pour la collecte de feuilles, tentes, feutres indélébiles, bottes, imperméables, machettes, trousses de premiers soins, *etc.* ;
7. Un véhicule Toyota Hilux a été acquis dans le cas spécifique du Cameroun pour la réalisation de l'ensemble des travaux.

2.2. Activités et missions planifiées

Pour chaque pays, les activités suivantes ont été chronologiquement mises en œuvre en quatre phases successives (schéma typique) :

1. Phase préparatoire, à Gembloux
   - préparation du plan d'échantillonnage en tenant compte de l'étendue à couvrir, des facilités/difficultés d'accès aux massifs forestiers, et de la présence/absence des TCP et TCS sur base de la littérature disponible ;
   - Identification et sélection d'un partenaire local principal, dans la mesure du possible, pour le suivi régulier des récoltes ;
   - Rédaction des termes de référence propres à chaque zone à couvrir (annexe 2) ;
2. Phase d'initiation, sur le terrain
   - selon le cas, création d'une ou de plusieurs équipes techniques (comprenant chacune une ou plusieurs personnes ;
   - formation des équipes et attribution du matériel ;
   - démarrage de la phase de récolte en présence de l'équipe ;
3. préfinancement de l'activité : 1/3 du budget total dédié à l'échantillonnage local ;

4. Phase de suivi, à Gembloux : suivi à distance de la progression des récoltes : état d'avancement, problèmes rencontrés et solutions possibles, *etc.* ;
5. Phases de reprise des échantillons et de contrôle de la qualité, sur le terrain
   - Réception des échantillons ;
   - Premier contrôle (quantité, qualité du séchage, données disponibles, *etc.*) conduisant à l'acceptation ou au refus de chaque échantillon ;
   - Remise d'une proportion du second tiers du budget à l'équipe au prorata du nombre d'échantillons acceptés / nombre initialement fixé ;
   - Second contrôle qualité via la sélection aléatoire de quelques échantillons et vérification terrain (arbre existant, espèce correctement identifiée) ;
   - Versement du dernier tiers suivant le résultat de l'étape précédente.
6. Phase d'encodage des échantillons, sur le terrain et à Gembloux. Encodage des échantillons et transmission du matériel végétal et du fichier d'encodage à TI-ITTO.

Le tableau 3 dresse la liste des missions effectuées dans le cadre des phases d'initiation, de reprise des échantillons et du contrôle-qualité.

2.3. Constitution des équipes

La constitution des différentes équipes impliquées dans les phases détaillées au paragraphe précédent est détaillée au tableau 4.

2.4. Codification des échantillons

Des codes d'identification ont été attribués à chacun des arbres échantillonnés. Ces codes sont structurés comme suit : « **Code du pays_identifiant de la population_code du taxon_numéro de l'échantillon** ». Le numéro de l'échantillon est attribué par taxon et par population (il recommence par 1 dans chaque bloc ou population). Les codes attribués aux pays sont les suivants[5] :

- Cameroun C
- Congo CO
- Côte d'Ivoire CIV
- Gabon G
- Ghana GH
- Kenya K
- RDC DRC

[5] Un code a également été attribué aux Bénin (« B »), Nigéria (« N ») et République Centrafricaine (« CAR »), 9 échantillons issus de ces pays ayant été transmis à l'équipe de TI-ITTO (Hamburg).

Tableau 3. Principales missions de terrain effectuées par N. Bourland dans le cadre de l'échantillonnage. Plusieurs missions de terrain ponctuelles non détaillées dans le tableau ont été effectuées au Cameroun, pays de lancement des récoltes du projet (essais du matériel/adaptation de la méthodologie).

Mission	Pays	Mois et année	Principales phases concernées
1	Ghana	Mai 2012	Initiation des collectes
2	Cameroun	Octobre 2012	Initiation des collectes
3	RDC	Novembre 2012	Initiation des collectes de l'équipe 7 (*cf*. tableau 4)
4	RDC	Décembre 2012	Initiation des collectes des équipes 6 et 9 (*cf*. tableau 4) Suivi terrain de l'équipe 7
5	Ghana	Janvier 2013	Suivi terrain
6	RDC	Mars 2013	Initiation des collectes de l'équipe 8 (*cf*. tableau 4) Suivi terrain des équipes 6, 7 et 9
7	Congo	Juillet 2013	Initiation des collectes de l'équipe 11 (*cf*. tableau 4)
8	Gabon	Août 2013	Initiation des collectes
9	RDC	Août 2013	Suivi-terrain
10	Kenya	Septembre 2013	Initiation des collectes
11	Côte d'ivoire	Septembre 2013	Initiation des collectes
12	Congo	Septembre 2013	Initiation des collectes de l'équipe 10 (*cf*. tableau 4) Suivi terrain de l'équipe 11
13	RDC	Novembre 2013	Fin des récoltes
14	Congo	Novembre 2013	Suivi terrain des équipes
15	Gabon	Novembre 2013	Suivi terrain
16*	Côte d'ivoire	Novembre 2013	Fin des récoltes

* Mission effectuée par le Dr Taofic Alabi (Gembloux Agro-Bio Tech/ULg).

Les rares individus des pays non concernés par le projet (Bénin et Nigéria) ne respectent pas forcément cette structure de codes individuels. Il en va de même pour la République Centrafricaine qui n'est plus considérée comme pays prioritaire pour l'échantillonnage (*cf*. chapitre 1). Les codes retenus par espèce sont présentés dans le tableau 2. Ainsi par exemple, DRC_08_TRI_19 correspond à la tige n°19 de *T. scleroxylon* échantillonnée dans le bloc (ou population) n°8 en RDC.

Tableau 4. Équipes de chaque pays engagées dans la collecte des échantillons. L'embauche ponctuelle/temporaire de personnel ouvrier/villageois n'est pas répertoriée dans le tableau. Le détail des contacts par pays est présenté en annexe 3.

Équipe	Composition	Pays	Zone attribuée	Partenaire principal (institution)
1	1 botaniste 1 chauffeur	Cameroun	Pays	Université de Yaoundé I
2	1 prospecteur 1 aide 1 chauffeur	Côte d'Ivoire	Pays	Cantonnement forestier
3	1 botaniste	Gabon	Pays	
4	1 botaniste 1 chauffeur*	Ghana	Pays	Forest Research Institute of Ghana
5	1 technicien 1 scientifique	Kenya	Pays	Kenya Forestry Research Institute
6	1 ingénieur forestier	RDC	Mbandaka-Gemena-Lisala-Bumba	Jardin Botanique d'Eala
7	1 ingénieur forestier	RDC	Kisangani	Resources & Synergies Development
8	1 scientifique	RDC	Uvira-Fizi	Resources & Synergies Development
9	1 prospecteur	RDC	Lac Tumba	Centre de Recherche de Mabali
10	1 scientifique	Congo	Nord-Est	Nature Plus asbl
11	1 scientifique	Congo	Sud, Centre et Nord-Ouest	Université Marien Ngouabi

* A certains moments de l'échantillonnage, un scientifique a accompagné l'équipe.

3. Résultats de l'échantillonnage

L'ensemble des échantillons collectés, ainsi que les détails de chaque échantillon (code, pays, bloc, coordonnées GPS, lieu, *etc.*) sont repris dans un fichier Microsoft Excel© accompagnant le présent rapport.

3.1. Échantillons pour les études génétiques (empreinte et barcoding)

Le tableau 5 montre le bilan des collectes effectuées pour les analyses génétiques (origine géographique et "barcoding"). La figure 1 montre la répartition spatiale de cet échantillonnage pour chacune des trois taxons prioritaires.

Tableau 5. Effectifs des échantillons collectés pour les analyses génétiques. La dernière colonne du tableau indique les taux d'échantillonnage en regard des effectifs prévus (rappel : 1.600 pour *E. cylindricum*, 2.000 pour *Milicia* spp., 1.200 pour *T. scleroxylon*, et 10 pour chacun des TCS).

Taxons	Cameroun	Congo	Côte d'Ivoire	DRC	Gabon	Ghana	Kenya	Total	% de l'objectif
Taxons-cibles prioritaires (TCP)									
Entandrophragma cylindricum	218	127	23	527	48	57		***1.000***	*63*
Milicia spp.	327	301	250	685	431	144	113	***2.251***	*>100*
Triplochiton scleroxylon	318	49	224	192		140		***923***	*77*
Taxons-cibles secondaires (TCS)									
Afzelia spp.	3	44	2	41	11	4	4	***109***	*>100*
Aucoumea klaineana		5			5			***10***	*100*
Baillonella toxisperma	6	2		3	4			***15***	*>100*
Cylicodiscus gabunensis	8	2			2	6		***18***	*>100*
Entandrophragma spp.	7	28	3	31	6	16		***91***	*>100*
Erythrophleum spp.	7	25		24	3	1		***60***	*>100*
Guibourtia spp.	3	3		30	7	3		***46***	*>100*
Khaya spp.	3	2	2	5	2	13		***27***	*>100*
Lophira alata	15	78		24	13	3		***133***	*>100*
Millettia laurentii	5	20		9	2			***36***	*>100*
Nauclea diderrichii	6	23	2	25	4	6		***66***	*>100*
Pericopsis elata	5	2		9				***16***	*>100*
Pouteria spp.	3	8	2	3		6		***22***	*>100*
Pterocarpus soyauxii	12	30		62	2			***106***	*>100*
Terminalia superba	11	23	2	12	1	8		***57***	*>100*
Total	***957***	***772***	***510***	***1.682***	***538***	***407***	***117***	***4.983***	

Au total, ce sont environ 4.983 échantillons qui ont été collectés. Les objectifs ont été atteints pour les TCS. En ce qui concerne les TCP, l'objectif a été dépassé pour *Milicia* spp. Pour les deux autres taxons, *E. cylindricum* et *T. scleroxylon*, le taux d'échantillonnage par rapport aux objectifs initialement fixés varie de 63 à 77%. Ce résultat est plus qu'appréciable au vu des nombreuses difficultés qui ont jalonné l'échantillonnage, et qui sont décrits en détail dans le chapitre 4.

Par ailleurs, on notera que la répartition spatiale des collectes est assez homogène, nonobstant les régions d'absence naturelle de certains taxons et les blocs n'ayant pu être échantillonnés. Au total, 108 des 124 populations (blocs) initialement identifiées ont été parcourus, soit 87,1% des prévisions (tableau 6). Certains blocs ont dû être abandonnés pour des raisons budgétaires, tandis que d'autres n'ont pu

être parcourus pour des raisons budgétaires et contraintes de terrain, détaillées en annexe 1 et dans le chapitre 4.

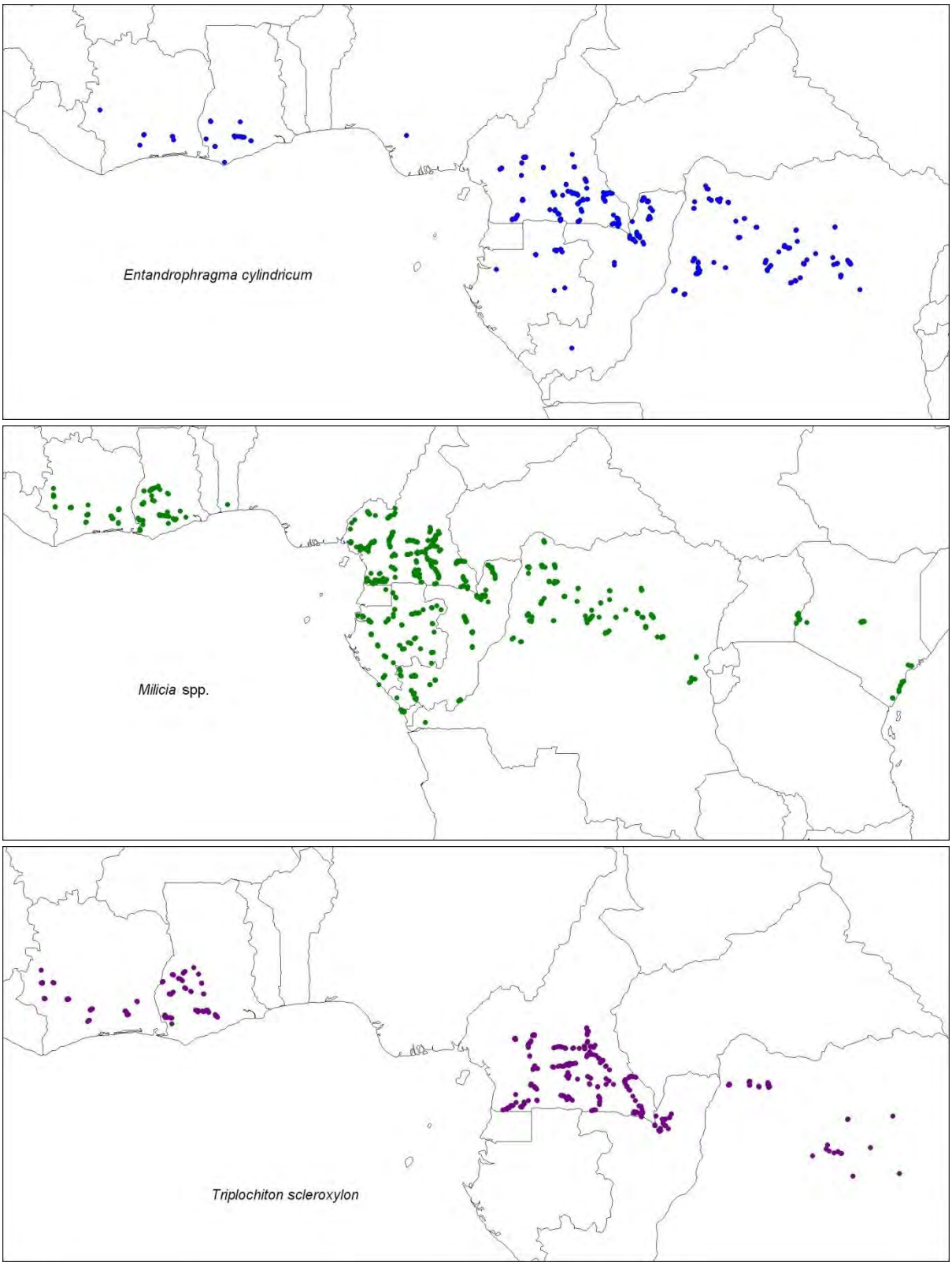


Figure 1. Répartition spatiale des échantillons des TCP collectés dans le cadre du présent projet.

Tableau 6. Taux d'échantillonnage des taxons-cibles prioritaires en termes de populations.

Pays	N° des blocs non échantillonnés	Nombre de blocs échantillonnés	Nombre de blocs prévus	% de l'objectif
Cameroun		17	17	*100*
Congo-Brazzaville	04 – 05 – 10 – 11 – 13 – 14 – 19 – 20	16	24	*67*
Côte d'Ivoire		10	10	*100*
Gabon	09	21	22	*95*
Ghana		8	8	*100*
Kenya	04 – 07	5	*7*	*71*
RD du Congo	18 – 21 – 32 – 33 – 35	31	36	*86*
Total		**108**	**124**	**87**

3.2. Échantillons pour les études chimiques (empreinte isotopique)

Les études chimiques ne concernent que les TCP. Les objectifs initialement définis sont présentés dans le tableau 1. Le tableau 7 dresse le bilan des collectes des échantillons destinés aux analyses chimiques. Les objectifs ont été quasiment atteints dans ce cas-ci, au niveau taxon. De plus, six échantillons d'*Entandrophragma angolense*, non mentionnés dans le tableau 7, ont été collectés.

Tableau 7. Bilan des collectes d'échantillons à fins d'analyses chimiques.

Taxon	Cameroun	Congo Brazzaville	Côte d'Ivoire	DRC	Gabon	Ghana	Kenya	Total	% de l'objectif
Entandrophragma cylindricum	34	29	11	119	12	17		**222**	*92,5*
Milicia spp.	52	77	30	156	94	40	25	**474**	*>100*
Triplochiton scleroxylon	40	20	27	46		34		**167**	*92,8*
Total	***126***	***126***	***68***	***321***	***106***	***91***	***25***	***863***	*>100*

3.3. Échantillons pour les études d'anatomie du bois

Les études d'anatomie de bois concernent tous les taxons cibles. Rappelons qu'à l'image des analyses de "barcoding", l'objectif était de 10 échantillons par taxon. Le tableau 8 dresse le bilan des échantillons collectés par taxon.

Les objectifs ont été atteints pour les TCP et pour bon nombre de TCS. Du fait des difficultés mentionnées dans le chapitre 4, il n'a pas toujours été possible d'obtenir les effectifs totaux prévus pour certains TCS.

Tableau 8. Effectifs des échantillons collectés pour les analyses d'anatomie de bois. La dernière colonne du tableau donne les taux d'échantillonnage en regard des effectifs prévus, soit 10 individus par taxon.

Taxon	Cameroun	Congo Brazzaville	Côte d'Ivoire	Gabon	Ghana	Kenya	Total	% de l'objectif
Taxons-cibles prioritaires								
Entandrophragma cylindricum	1	5	5	2	3		**16**	> 100
Milicia spp.	2	4	11	3	6	4	**30**	> 100
Triplochiton scleroxylon	1	2	10		4		**17**	> 100
Taxons-cibles secondaires								
Afzelia spp.	2	3	2	4	1	2	**14**	> 100
Aucoumea klaineana		2		3			**5**	50
Baillonella toxisperma	1	2		1			**4**	40
Cylicodiscus gabunensis	1	2		2			**5**	50
Entandrophragma spp.	3	8	3	5			**19**	> 100
Erythrophleum spp.	2	4		3	1		**10**	> 100
Guibourtia spp.	1	2		3			**6**	60
Khaya spp.	1	2	2		1		**6**	60
Lophira alata		4		2			**6**	60
Millettia laurentii	1	3		2			**6**	60
Nauclea diderrichii	1	4	2	3			**10**	100
Pericopsis elata	1	1					**2**	20
Pouteria spp.	1		2				**3**	30
Pterocarpus soyauxii	2	4		3			**9**	90
Terminalia superba	1	4	2	1			**8**	80
Total	***22***	***56***	***39***	***37***	***16***	***6***	***176***	*98*

4. Principaux problèmes ayant nui à l'échantillonnage

Plusieurs problèmes ont été rencontrés avant ou pendant la phase de récolte des échantillons. Quinze types principaux sont détaillés ci-après, certains étant étroitement liés à d'autres :

- type 1 – utilisation du matériel
- type 2 – absence d'infrastructures routières et de moyens de transports fiables
- type 3 – coûts prohibitifs des transports et moyens de logement
- type 4 – multiplication des équipes
- type 5 – prix élevé de la main d'œuvre/des services
- type 6 – présence de zones d'insécurité
- type 7 – contraintes de terrain liées au fonctionnement des zones tribales ou à des échéances politiques/administratives
- type 8 – dispersion des tiges ou rareté de certains taxons
- type 9 – mode fonctionnement du service des finances de l'ULg
- type 10 – fiabilité des équipes de terrain
- type 11 – absence de services bancaires fiables/de qualité – transports de fonds
- type 12 – TDR inutilisés/mauvaise localisation des populations
- type 13 – contacts difficiles avec le WWF Allemagne
- type 14 – phase d'encodage non budgétisée et réalisées dans de mauvaises conditions
- type 15 – divers

4.1. Difficultés d'utilisation du matériel

Pays concernés : Cameroun, Congo, Côte d'Ivoire, Gabon, RDC.

Brève description. L'utilisation du matériel tel que décrit au chapitre 2 n'a pas toujours été possible. Les contraintes les plus importantes ont été rencontrées pour l'utilisation de la foreuse. En effet, hormis au Ghana, Kenya et dans certaines parties du Cameroun, les équipes de terrain ont rarement disposé d'une source de courant pour recharger les batteries. Le recours à la foreuse ne pouvait donc qu'être très erratique. De plus, particulièrement au Cameroun, de nombreux villageois ont tout simplement refusé que l'équipe de collecte utilise la foreuse, par crainte d'endommager irrémédiablement le fût des arbres.

Par ailleurs, en RDC où la grande majorité des déplacements se faisait à moto ou à pirogue, la masse importante de la foreuse s'est avérée être un handicap difficile à surmonter. D'autre part, le protocole de travail pour la récolte des échantillons destinés aux analyses isotopiques ne nous est parvenu que le 6 septembre 2013, soit au terme de la majeure partie des campagnes de récolte. Ce protocole mentionne par exemple une profondeur de prélèvement de 20 cm dans la tige, ce qui n'a pas été possible à la machette.

Répercussions sur le projet. La nécessité de travailler à la machette s'est imposée durant une bonne partie de la phase de récolte. Rappelons aussi que nous n'avions pas de retour sur les éventuels problèmes posés par l'échantillonnage à la machette des copeaux de bois.

4.2. Absence d'infrastructures routières et de moyens de transports fiables

Pays concerné(s) : Congo, RDC.

Brève description. Dans le cadre du suivi des activités de terrain, de nombreux trajets aériens (seul moyen de transport envisageable pour relier Mbandaka, Kisangani et Goma en un temps acceptable) ont été effectués avec des compagnies inscrites sur la liste noire de l'UE (Compagnie Africaine d'Aviation/CAA, Gomair). Un appareil Fokker 50 de la CAA qui devait nous permettre d'accéder de Goma à Beni et Bunia s'est écrasé le lundi 4 mars durant son approche sur Goma, soit la veille de notre départ pour Bunia :

http://www.lalibre.be/actu/international/crash-d-un-avion-a-goma-9-personnes-etaient-a-bord-pas-de-belge-51b8f9b4e4b0de6db9c9ca2a

La compagnie CAA n'étant pas fiable (surbooking, vols annulés et horaires modifiés sans préavis ni possibilité de recours/remboursement, pas de service d'assistance, *etc*.), nous avons perdu un trajet « aller » (surbooking non remboursé, retour autorisé) et avons été contraints de voyager dans un avion cargo de la compagnie Gomair pour assurer la continuité de la mission auprès des équipes 6 et 9.

Enfin, en l'absence de stations de carburant en certains lieux, il était nécessaire de voyager – en cas de déplacement motorisé – avec des jerrycans, lesquels ont attisé la convoitise des populations locales, comme des responsables des barrages de contrôles routiers, et donc créé des risques de vol importants.

Répercussions sur le projet. Il y a eu des prises de risques importantes sur certains trajets, et des difficultés/impossibilités d'accès à certaines zones initialement prévues dans l'échantillonnage (cas du parc national de la Salonga en RDC ou des zones marécageuses situées au Nord-Est du Congo). Ces risques concernaient également les équipes de terrain, celles-ci ayant été amenées à travailler dans des conditions extrêmement précaires, et en des endroits isolés parfois très dangereux pour leur sécurité (par exemple dans les provinces Orientale et du Sud-Kivu en RDC).

4.3. Coûts prohibitifs des transports et logements vs. budget disponible

Pays concerné(s) : principalement la RDC.

Brève description. Quelques exemples pour illustrer le fait que le budget disponible n'était pas en adéquation avec les coûts réels sur le terrain :

- location d'un véhicule 4x4 et chauffeur dans les zones de Mbandaka ou Kisangani : 100 à 250 $US/jour, carburant et frais de mission du chauffeur non compris ;
- trajet aéroport (N'Djili) – hôtel : environ 80 $US ;
- nuitée d'hôtel à Goma : 100 à 120 $US ;

- nuitée d'hôtel à Mbandaka : 150 à 180 $US. Alternative : quelques auberges aux conditions plus que douteuses (maximum 3-4 heures/jour d'électricité dans le meilleur des cas, pas d'eau courante, environnement très bruyant, *etc*.) ;
- seule alternative au transport aérien pour effectuer le trajet Kinshasa-Mbandaka : 1000 à 1500 $US par le fleuve en vedette rapide ;
- atteindre la Salonga en un temps raisonnable/acceptable nécessite de 800 à 1000 $US (aller simple);
- *etc*.

Répercussions sur le projet. Le budget extrêmement serré de la phase d'échantillonnage ne permettait pas d'envisager la moindre alternative intéressante ou d'engager des frais supplémentaires importants. Rappelons pourtant que nos premières estimations du budget nécessaire incluaient de tels aléas ou situations imprévues, mais que ces estimations ont été drastiquement réduites par TI-ITTO.

La répercussion de ce budget limité se notait aussi sur les équipes de terrain, souvent tenues d'accepter des conditions de travail déplorables : déplacements à moto sans casque, pas d'assurance accident, harcèlements aux « contrôles » de police/gendarmerie, *etc*.

4.4. Nécessité de multiplier les équipes dans certains pays

Pays concerné(s) : Cameroun, Congo, Gabon, RDC.

Brève description. Principalement en RDC, les vastes étendues à couvrir couplées à des problèmes de type 2 et l'obligation de travailler avec du personnel ayant une parfaite connaissance du terrain, nous ont contraints à travailler avec quatre équipes (tableau 4). Au Congo, des problèmes de types 3 et 5 nous ont contraints à faire appel à des collaborateurs de Nature Plus, certains intervenant sur d'autres activités. Au Cameroun et au Congo, les équipes ont dû être changées pendant la phase d'échantillonnage (voir problème de type 11). Au Gabon, l'équipe 3 a fait appel à des sous-traitants pour réaliser certaines récoltes.

Répercussions sur le projet. D'une manière générale, la multiplication des équipes a entraîné des coûts élevés pour assurer leur suivi (notamment au travers de la multiplication des missions de terrain, mais aussi de la lourdeur des vérifications post-collectes faites au bureau). D'une part les changements opérés au sein des équipes 1 et 11 et, d'autre part, l'utilisation d'intermédiaires par l'équipe 3 n'ont pas été un gage de qualité, notamment dans la phase de gestion des échantillons : dans ces cas particuliers, nous avons procédé à de nombreux rejets d'échantillons, et ce pour diverses raisons (identités taxonomiques erronées des échantillons, échantillons mal séchés, vieilles feuilles ramassées au sol, *etc*.).

4.5. Coûts prohibitifs de la main d'œuvre/des services

Pays concernés : Congo Brazzaville, Ghana, Kenya.

Brève description. Il n'a pas été possible de couvrir l'ensemble des populations de *M. excelsa* au Kenya (annexe 1). Au Ghana, le maillage de l'inventaire a dû être adapté pour tenir compte du budget disponible (nécessité de restreindre le nombre de populations). Toujours au Ghana et toujours par

manque de fonds, nous avons dû "partager" l'équipe de terrain (personnel et véhicule du FORIG) avec le projet IDRC Project #106106-001 ("*Engaging Developing Nations in the International Barcode of Life Project*") afin de réduire les frais. Au Congo, il a été très difficile de trouver des partenaires (refus systématique des budgets proposés car jugés insuffisants ; *cf*. par exemple MM. Jean-Marie Moutsamboté et Félix Koubouana).

Répercussions sur le projet. Afin de compenser les exigences salariales dans ces pays, les conditions de travail des équipes de terrain ont dû être "assouplies", générant les répercussions évoquées pour le type 3 (coûts élevés des transports et moyens de logement).

4.6. Présence de zones d'insécurité

Pays concerné(s) : Côte d'Ivoire, Kenya et RDC.

Brève description. Quelques cas :

- la présence en Côte d'Ivoire d'anciens soutiens (éléments armés) de M. Laurent Gbagbo dans des zones frontalières avec le Libéria rendait ces dernières particulièrement dangereuses ;
- la frontière entre le Kenya et la Somalie était secouée par des enlèvements crapuleux et attentats (en répercussion à l'intervention militaire kenyane), et des règlements de compte à caractères ethniques (conflits récurrents autour du bétail, notamment), *etc*. ;
- en RDC, une large part de l'Ituri, les zones frontalières avec la République Centrafricaine et le Soudan du Sud ainsi que la région couvrant les réserves d'Itombwe et Kahuzy Biega (Ouest du lac Tanganyika) sont des zones de non droit, parcourues par des milices armées qui y commettent régulièrement des atrocités ;
- toujours en RDC, lors de notre séjour à Kisangani, un soulèvement meurtrier a eu lieu, avec des représailles envers les représentants de l'ONU et membres des ONG internationales (impossibilité de sortir du logement, ville paralysée, deux membres de l'ONG Handicap International *sauvées in extremis* d'un lynchage, *etc*.) ;
- toujours en RDC, lors de notre séjour à Goma, le mouvement rebelle M23 a procédé à un bombardement meurtrier de la ville (dernier bombardement avant l'offensive combinée des FARDC et de la Monusco).

Répercussions sur le projet. D'importantes zones n'ont pas pu être échantillonnées. Ces zones sont identifiées en annexe 1, les pertes pour le projet sont quantifiées chaque fois que c'est possible.

4.7. Contraintes liées à des tensions ethniques ou politico-administratives

Pays concernés : Gabon, RDC.

Brève description. Quelques exemples :

- les élections municipales au Gabon en 2013, y compris leurs préparatifs, ont rendu l'accès difficile à certaines localités, voire impossible pendant plusieurs semaines ;

- la phase de recensement des fonctionnaires en RDC a interrompu ou fortement ralenti les activités de terrain pendant plusieurs mois (principalement de mai à août 2013) ;
- toujours en RDC, l'ethnie Ngbaka a refusé l'échantillonnage sur ses terres durant la période des initiations des jeunes, faisant preuve d'agressivité envers l'équipe 6 ;
- dans divers contextes tendus, des pertes de matériel ont été également déplorées : l'équipe 3 s'est faite agressée à cause du GPS, l'équipe 6 a dû abandonner son petit matériel – sacs hermétiques, enveloppes en papier – à la suite de menaces, *etc*.

Répercussions sur le projet. L'échantillonnage a été considérablement ralenti/rendu complexe du fait de ces tensions, sans compter les risques de sécurité encourus par les équipes de terrain.

4.8. Dispersion des tiges ou rareté de certains taxons

Pays concernés : tous.

Brève description. Quelques exemples :

- d'importants coûts d'échantillonnage résultant de longs déplacements (faible efficacité) pour l'échantillonnage d'*E. cylindricum* ;
- faible échantillonnage de *P. aningeri*, voire de *T. scleroxylon* par rapport aux prévisions de départ, résultant d'une surestimation initiale de l'abondance de ces espèces.

Répercussions sur le projet. D'une manière générale, l'échantillonnage des tiges d'*E. cylindricum* a été problématique dans tous les pays à l'exception du Cameroun, eu égard au mode de distribution dispersé et à la faible densité de population de cette espèce. Dans ces conditions, les effectifs obtenus sont plus que satisfaisants, bien qu'inférieures aux estimations initiales.

4.9. Mode de fonctionnement du service des finances de l'ULg

Pays concernés : néant (problème indépendant des pays-cibles).

Brève description. Quelques exemples :

- aucun financement n'est accordé tant que l'avance en cours n'est pas intégralement justifiée ;
- les délais avant le remboursement des notes de frais sont parfois importants ;
- les remboursements sont effectués de manière désordonnée (par exemple plusieurs notes de frais sont transmises, mais certaines ne sont que partiellement remboursées tandis que les autres le sont intégralement) ;
- la très mauvaise communication résultant du nombre élevé d'interlocuteurs du service administratif et financier qui se sont succédés sur ce dossier (4 personnes au total), avec des exigences et réponses plus ou moins claires selon l'interlocuteur, des refus de remboursement non notifiés, *etc*. ;
- l'application de taux de conversion variables sur le franc CFA ;
- la non prise en compte du décalage entre les réalités « occidentales » et celles en vigueur en Afrique (justificatifs ne pouvant être obtenus – corruption –, termes des contrats de travail, *etc*.).

Répercussions sur le projet. Après plusieurs refus d'avance de financement, nous avons rencontré d'importantes difficultés pour pré-financier les collectes/missions de terrain. Les missions n'ont pu être menées à bien qu'avec l'appui de Nature Plus (préfinancement sans taux d'intérêt), avec pour conséquence un suivi plus que pénible de la comptabilité. L'implication de Nature Plus, en termes de gestion administrative et financière, est donc largement sous-estimée, et certainement pas rémunérée à la hauteur des multiples services rendus.

4.10. Fiabilité des équipes de terrain

Pays concernés : Cameroun, Congo, Gabon, RDC.

Brève description. Quelques exemples :

- au Cameroun, des écarts de comportement (conduite inappropriée sur les sites industriels, promesses financières non tenues vis-à-vis du personnel temporaire, *etc*.) de l'équipe 1 ont été notés dans certaines sociétés (Pallisco, Wijma et Alpicam), entraînant des complications inutiles avec les dirigeants desdits sites ainsi que des refus d'accès à certains massifs forestiers (Alpicam) ;
- toujours au Cameroun, le remplacement du botaniste initialement en poste par un doctorant a notamment induit des erreurs d'attribution des codes des échantillons ;
- au Congo, l'équipe 11 a également été remplacée durant le processus, le premier scientifique engagé faisant trop fréquemment des erreurs d'identification (exemple : confusion entre *M. excelsa* et *Ficus* sp.), obligeant à rejeter une partie des collectes effectuées ;
- au Gabon également, comme mentionné ci-avant, le recours à des sous-traitants parfois incompétents a conduit au refus de nombreux échantillons ;
- en RDC, l'équipe 9 (tableau 4) ayant dans un premier temps fait du bon travail (deux blocs), la décision a été prise de lui en attribuer deux autres. Ces deux blocs n'ont jamais été parcourus, l'avance (520 $US) ne nous a jamais été rendue sous le prétexte que cette somme a été dépensée en déplacements, les deux blocs étant finalement jugés trop dangereux (ethnies hostiles).
- toujours en RDC, citons encore de nombreuses négligences lors des collectes : l'équipe 9 a ainsi confondu un jeune *Barteria* sp. avec *Lophira lanceolata*. L'équipe 7 a récolté des feuilles de plantules de *Cola* sp. en lieu et place de *T. scleroxylon*, *etc*. D'une manière générale, il est apparu très difficile de trouver en RDC du personnel compétent (botanique), autonome, fiable (gestion du budget, notamment) et acceptant de travailler au tarif que nous pouvions nous permettre.

Répercussions sur le projet. Pertes financières et perte de deux populations de *M. excelsa* (RDC). Pertes de temps à résoudre les problèmes créés dans les entreprises suite au passage de l'équipe (Cameroun, notamment chez Alpicam qui avait pourtant initialement ouvert sa concession au projet). Abandon de 3 blocs d'échantillonnage (Congo, voir annexe 1) potentiellement riches en TCP.

4.11. Problèmes de transport de fonds

Pays concernés : Cameroun, Congo, Gabon, RDC

Brève description. Hormis dans les grands centres urbains où les transactions bancaires sont envisageables, il n'a pas été possible de retirer sur place d'importantes sommes d'argent. La nécessité de réduire les coûts – budget sous-évalué – et les délais, mais aussi de minimiser les risques (*e.g.* le manque de fonds une fois sur le terrain) nous ont conduit à transporter d'importantes sommes durant tous les déplacements, dépassant parfois 10 000 € (maximum autorisé dans les aéroports européens, sans déclaration aux douanes) en petites coupures pour être autonomes et plus efficaces sur le terrain.

Répercussions sur le projet. Prise de risque importante lors des déplacements internationaux ou locaux, et dans les hôtels (risque de perte ou vol, agressions, *etc*.).

4.12. TDR inutilisés/mauvaise localisation des populations

Pays concernés : tous, sauf le Kenya.

Brève description. A l'exception de l'équipe n°5 (Kenya), aucune n'a lu entièrement les termes de référence qui leur ont été attribués (la version française a été traduite en anglais pour le Ghana). Seule l'équipe n°3 (Gabon) n'a pas hésité à nous interpeller à distance (communications internationales) et à plusieurs reprises lorsqu'elle avait des doutes, bien que cela n'ait pas été suffisant pour éviter toute une série de problèmes observés à la réception des échantillons et/ou à l'encodage.

Répercussions sur le projet. La mission de lancement a chaque fois été fondamentale. Néanmoins, du temps a été par la suite perdu par les équipes lors de prélèvements, séchages et transports d'échantillons n'intéressant pas directement le projet (*Entandrophragma candollei*, *Pouteria altissima*, *etc*.). Des sur- et sous-échantillonnages ont également été constatés pour certaines populations. Enfin, certaines déterminations botaniques n'ont été faites qu'au niveau du genre, sans pour autant qu'un herbier accompagne l'échantillon comme c'était pourtant exigé. Compte tenu de l'organisation du suivi du projet, il était impossible d'éviter ces différentes erreurs, qui témoignent surtout du sérieux des uns et des autres.

4.13. Contacts difficiles avec le WWF-Allemagne

Pays concernés : néant (problème indépendant des pays-cibles).

Brève description. Pour rappel, le WWF-Allemagne était en charge de fournir le protocole d'échantillonnage des copeaux de bois. Dès le lancement du projet, les contacts avec notre interlocuteur (M. Johannes Zahnen) se sont avérés difficiles, puis ont totalement cessé. Il s'en est suivi un retard important dans la transmission du protocole de travail (pour rappel, parvenu en septembre 2013), conduisant à l'échantillonnage des copeaux par des substituts (usage de la machette le plus souvent) qui pourraient s'avérer peu conformes pour l'analyse chimique d'une majorité d'échantillons.

Répercussions sur le projet. En septembre 2013, le WWF Allemagne propose de nous fournir un kit solaire de recharge de la batterie de la foreuse. Nous avons accepté cette proposition même si la phase de récolte des échantillons approchait de la fin. Nous apprenons en décembre 2013 que le kit en question ne pourra être livré qu'en janvier 2014, nous avons donc demandé l'annulation de cette commande. En conclusion, si les conditions de terrain ont nui au prélèvement des copeaux de bois, il nous semble

également évident que la lenteur des réactions des contacts au WWF-Allemagne a également grandement entravé la bonne collecte des échantillons d'analyses isotopiques.

4.14. Encodage non budgétisé et souvent réalisé dans de mauvaises conditions

Pays concernés : néant (problème indépendant des pays-cibles).

Brève description. Le problème principal réside dans le fait que l'encodage des échantillons n'était pas clairement imputé à l'un des partenaires, en l'occurrence ni Gembloux Agro-Bio Tech/ULg, ni Nature Plus, ni TI-ITTO. Dans le souci de mener à bien un échantillonnage digne de ce nom, nous avions toutefois entrepris de réaliser cette tâche à nos frais. Le travail s'est vite avéré extrêmement important, nécessitant une disponibilité qui nous a fait défaut. En définitive, ce fut une bonne partie du laboratoire de foresterie tropicale (FORTROP, ULg) qui fut mobilisée sur cette tâche. Ainsi, les personnes suivantes, de FOTROP/ULg ou d'autres institutions, ont participé à l'encodage, totalisant l'équivalent de 150 homme.jour^{-1} :

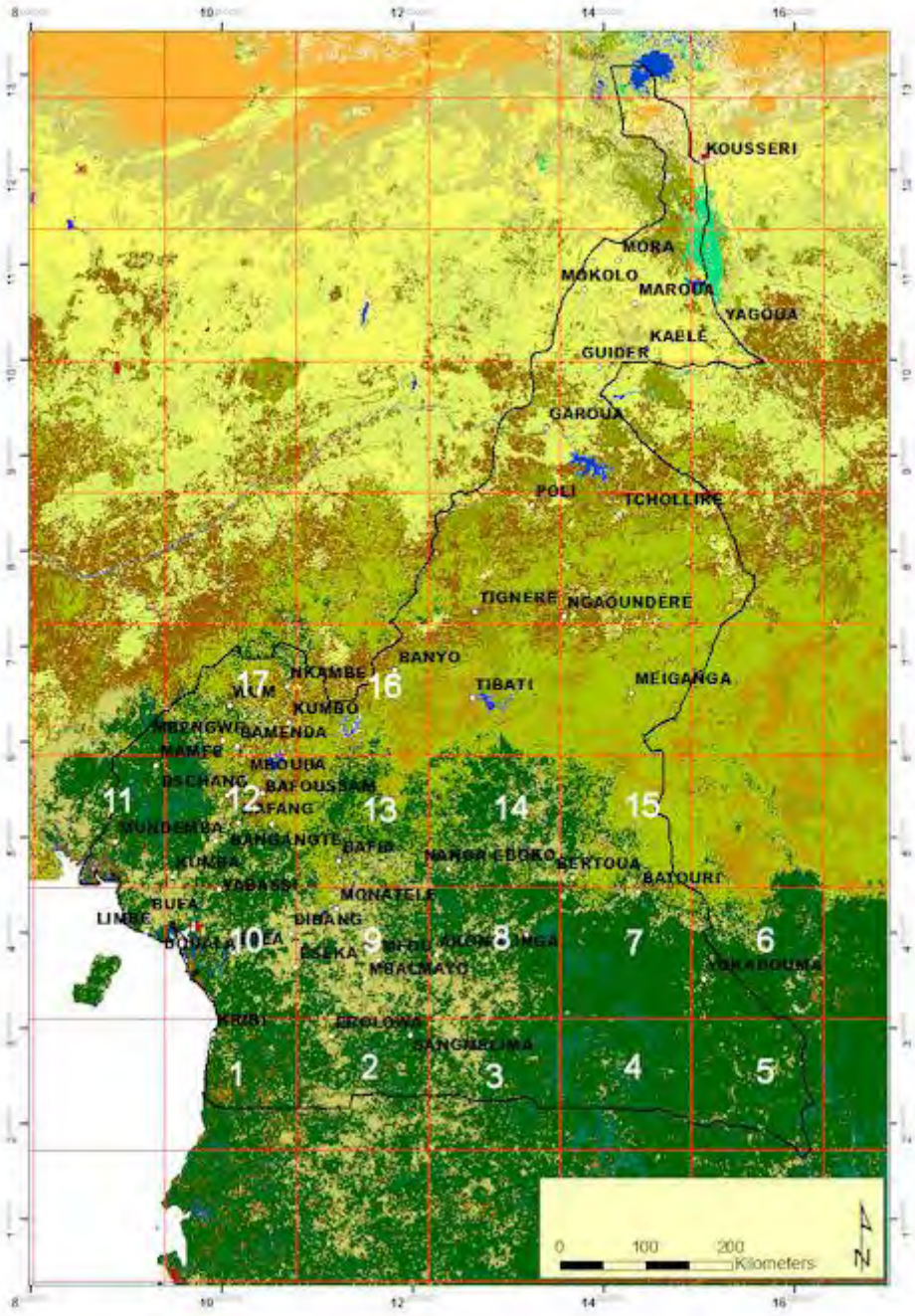
- M. Achille Biwolé (Doctorant, ULg)
- Mlle Anaïs Gorel (Assistante-Doctorante, ULg)
- M. Armel Donkpegan (Doctorant, ULg)
- M. Chauvellin Douh (Doctorant, ULg)
- M. Félicien Tosso (Doctorant, ULg)
- M. Grace Loubota (Etudiant en Master 2, ULg)
- Dr Kasso Daïnou (chercheur Post-Doc, Nature Plus)
- Mme Marie-Ange Golard (Technicienne, ULg)
- Dr Nils Bourland (Coordonnateur du présent contrat, ULg)
- Dr Jean-François Gillet (chercheur Post-Doc, Nature Plus)
- Dr Céline Blanc-Jolivet (chercheuse Post-Doc, TI)
- Dr Henri-Noël Zoéwindé Bouda (Coordonnateur du projet, TI)
- Mme Patricia Hernandez-Schiller (bénévole)

Répercussions sur le projet. L'implication d'un grand nombre d'encodeurs, travaillant parfois simultanément et/ou en dehors des heures de bureau, a permis de finaliser l'encodage des données dans les délais, mais a engendré de nombreuses incohérences (échantillons différents portant les mêmes codes, erreurs d'encodage, non-respect des normes d'encodage prédéfinies, *etc.*). Il a fallu par la suite consacrer un temps important et du personnel pour le "nettoyage" de la base de données. A l'heure où nous écrivons ces lignes, quelques rares incohérences subsistent toujours, et sont corrigées au fur et à mesure qu'elles sont détectées.

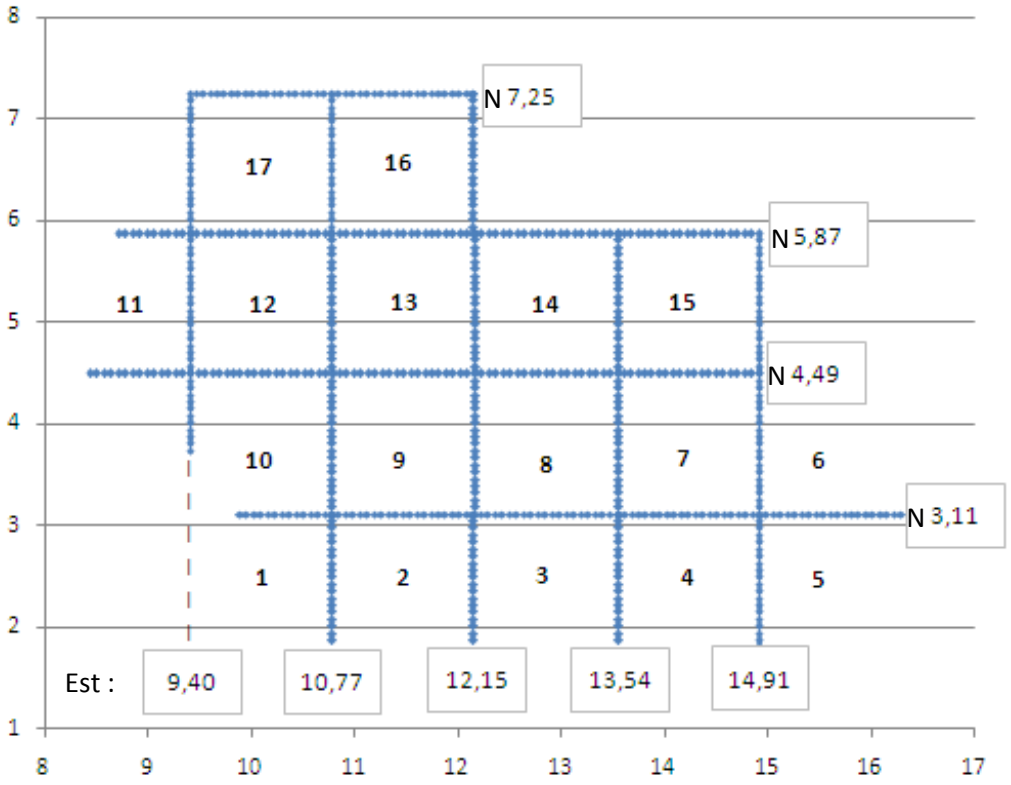
Annexe 1 – Cartes et identifiants des populations échantillonnées

Les dimensions de la maille carrée unitaire (= population) sont précisées entre parenthèses.

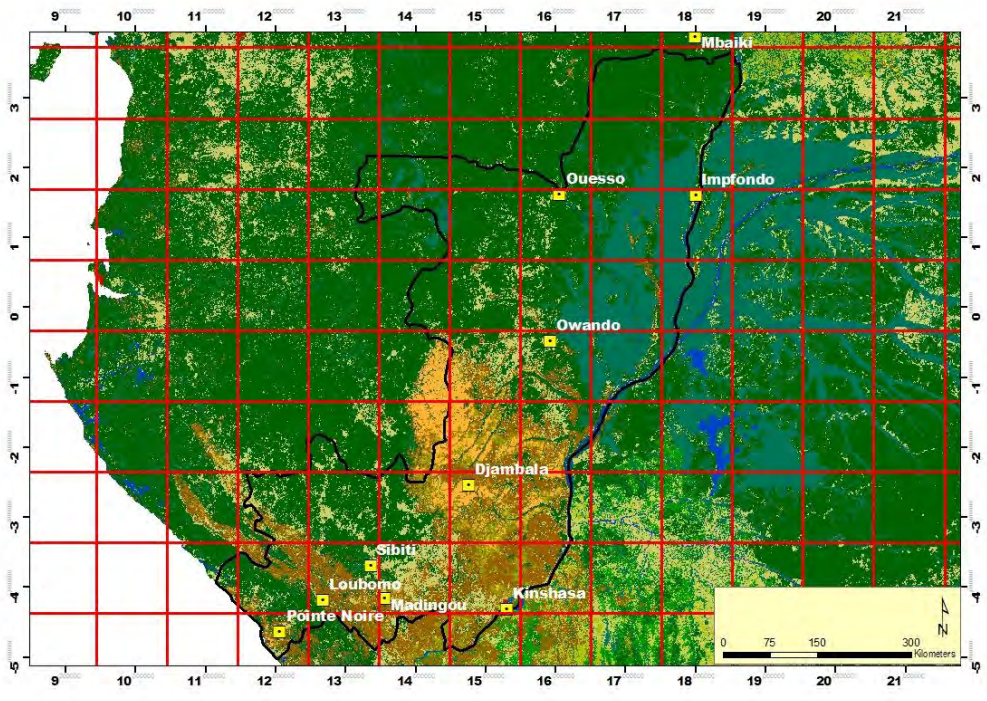
1) Cameroun (150 x 150 km ; équipe 4, tableau 4)



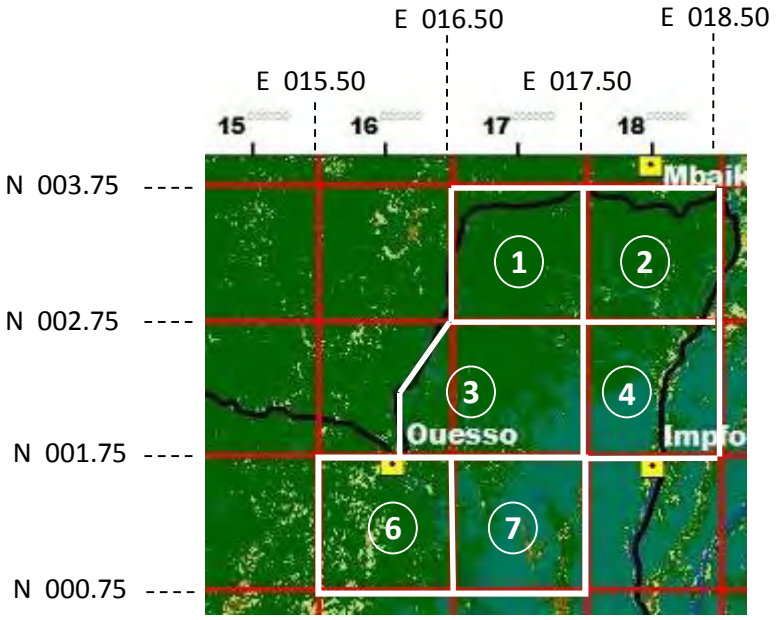
Identifiants des blocs d'échantillonnage et coordonnées correspondantes



2) Congo, vue globale (110 x 110 km)

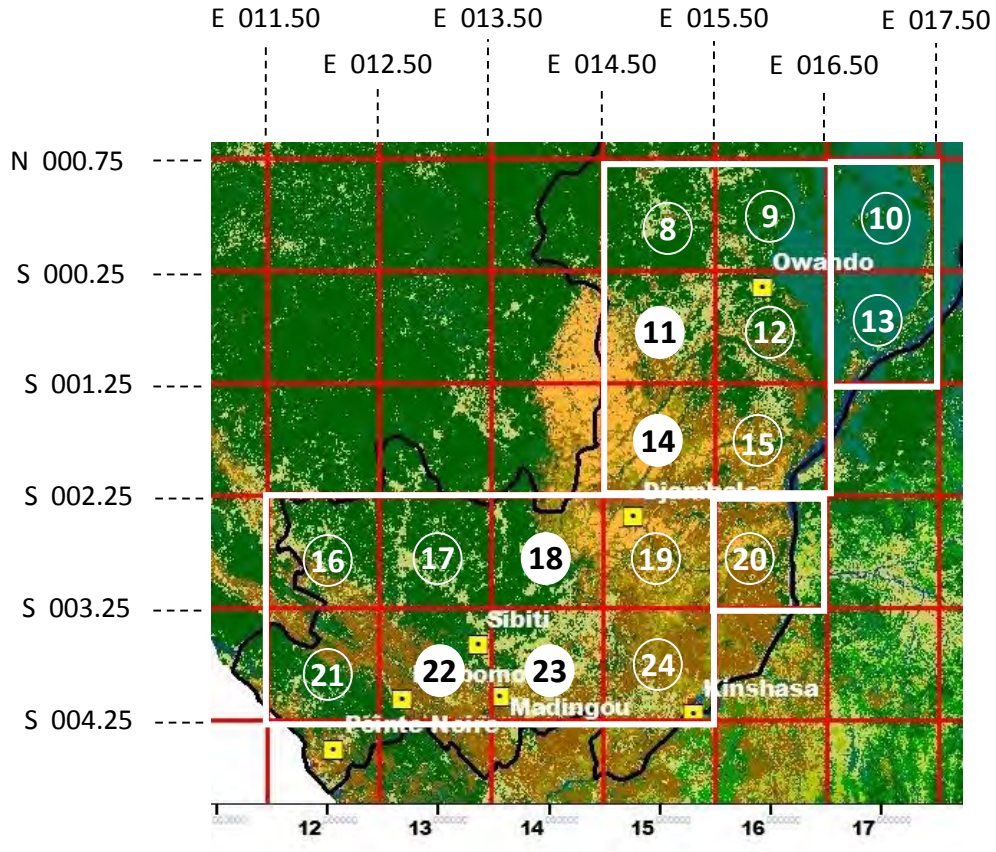


Nord-Congo (tableau 4 : équipe 10, « Nord-Est »)

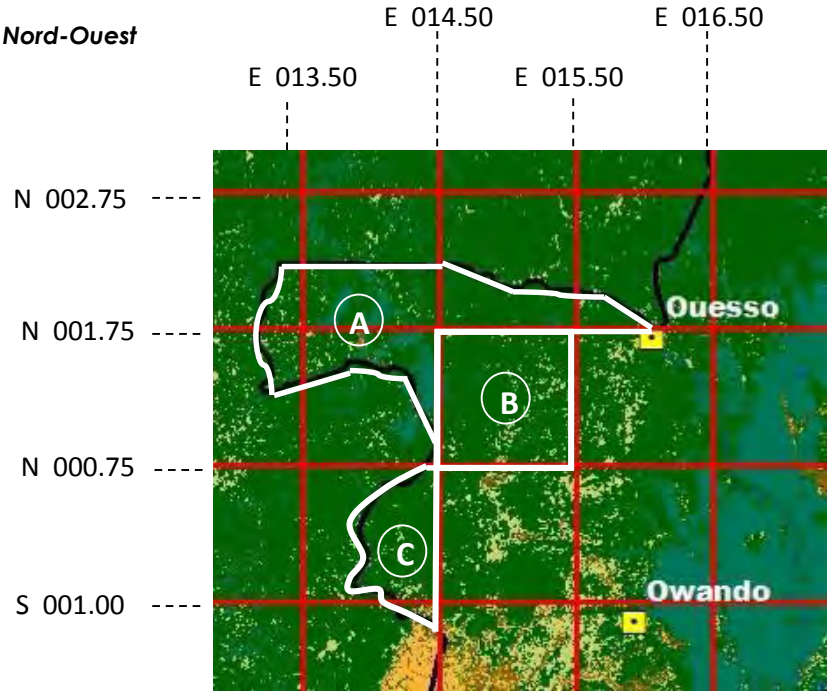


Remarque. Les blocs d'échantillonnage n°2 (en partie seulement) et 4 n'ont pas été parcourus par manque de fonds (ou manque de partenaire pour faire le travail à ce prix). Le bloc n°5 (au sud du n°4) a été abandonné car trop difficilement accessible.

Sud-Congo (tableau 4 : équipe 11, « Sud et Centre »)



Congo Nord-Ouest



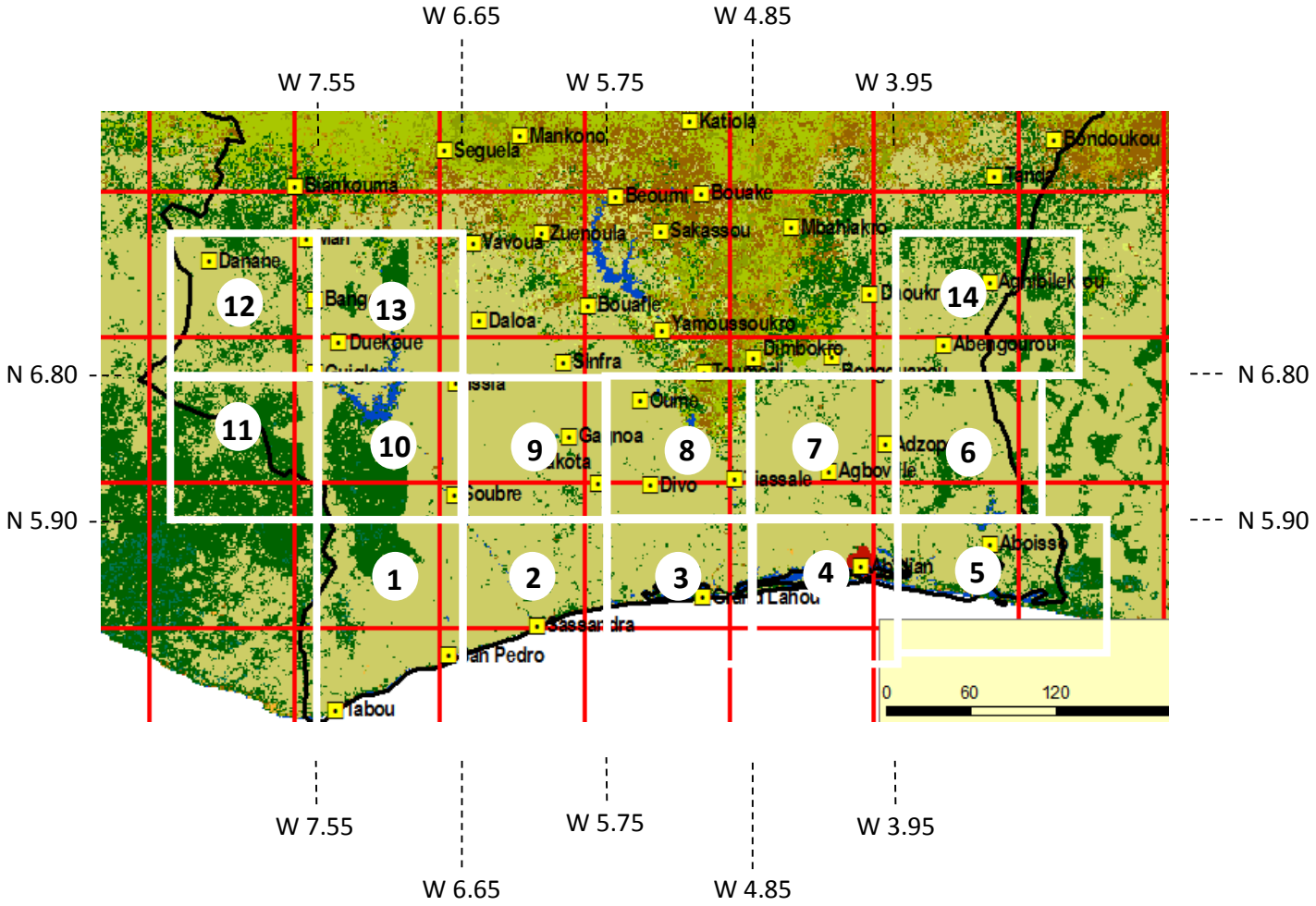
A = Souanké

B = Odzala

C = Deija Woob

Remarque. La zone « Congo Nord-Ouest », bien qu'initialement prévue dans le plan d'échantillonnage, n'a pas été parcourue par manque de financement (*vs.* coûts des services et prestations) et en raison de problèmes d'infrastructures routières au Nord de la République du Congo, soit la perte de trois populations potentiellement riches en taxons-cibles pour le projet (notamment *E. cylindricum* et *T. scleroxylon*).

3) Côte d'Ivoire, vue globale (110 x 110 km ; tableau 4 : équipe 2)

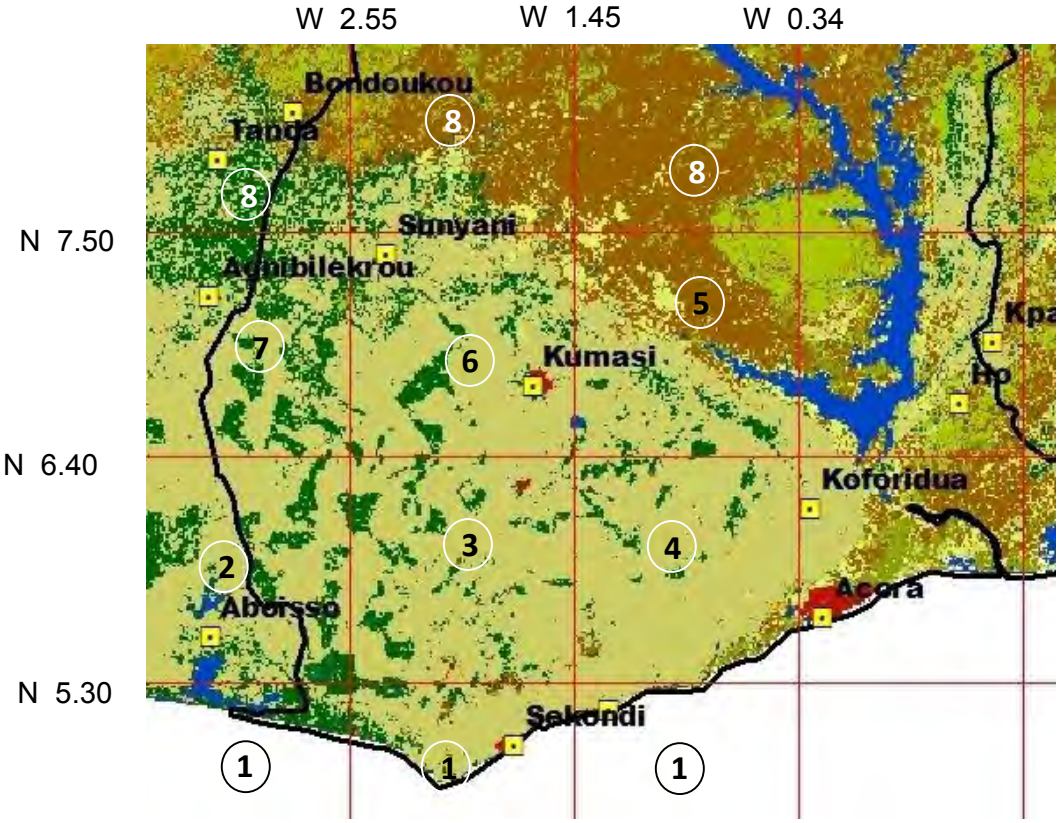


Remarque. Les blocs d'échantillonnage n°13 et 14 n'ont pas été parcourus par l'équipe de terrain par manque de financement, tandis que ceux portant les n°1 et 11 n'ont pas été parcourus en raison des dangers pour le personnel liés aux conséquences de la guerre civile (soit la perte de populations principalement de *M. excelsa* et de *T. scleroxylon*). Dans les TDR, eu égard au budget disponible, l'équipe devait échantillonner un total de 10 populations sur toutes celles proposées.

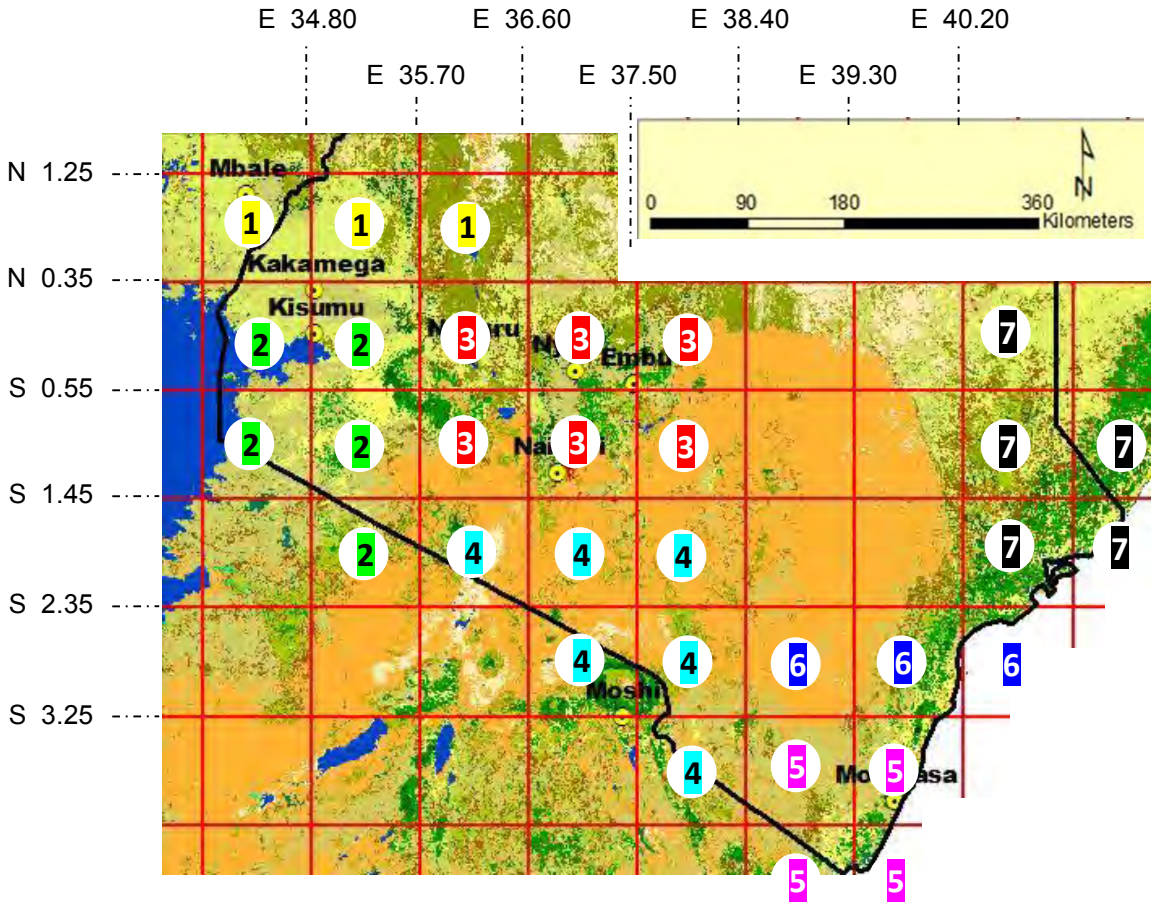
4) Gabon, vue globale (110 x 110 km ; tableau 4 : équipe 3)

Cf. Annexe 2.

5) Ghana, vue globale (120 x 120 km ; tableau 4 : équipe 4)

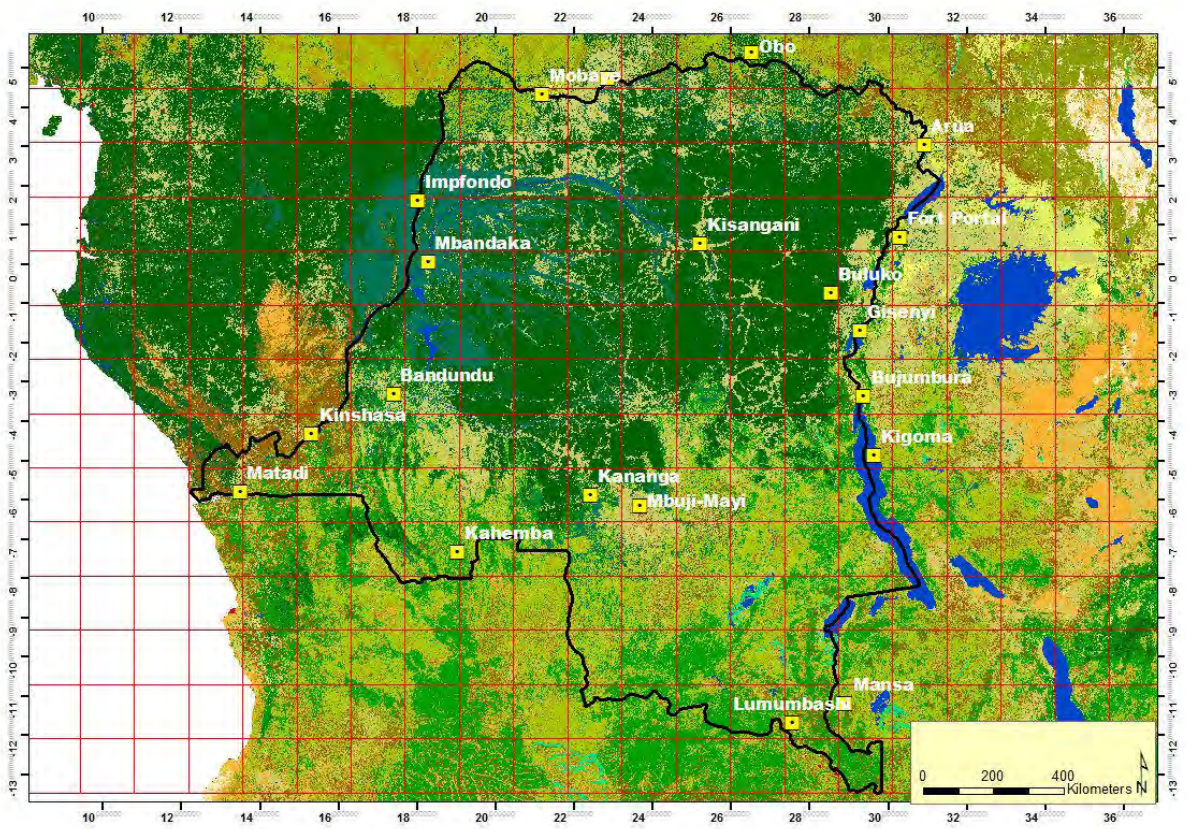


6) Kenya, vue globale (100 x 100 km ; tableau 4 : équipe 5)



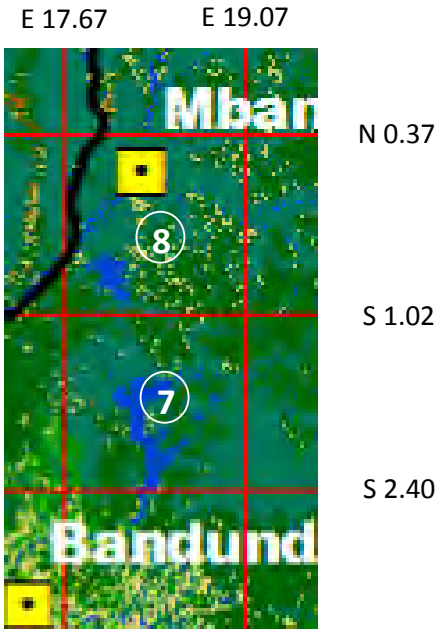
Remarque. Les blocs d'échantillonnage n°4 et 7 n'ont pas été parcourus par l'équipe de terrain par manque de financement et en raison des dangers pour le personnel liés à l'instabilité en Somalie, respectivement (soit la perte d'une population de *M. excelsa* sur six et d'une population d'*A. quanzensis* sur trois).

7) RDC, vue globale (150 x 150 km)



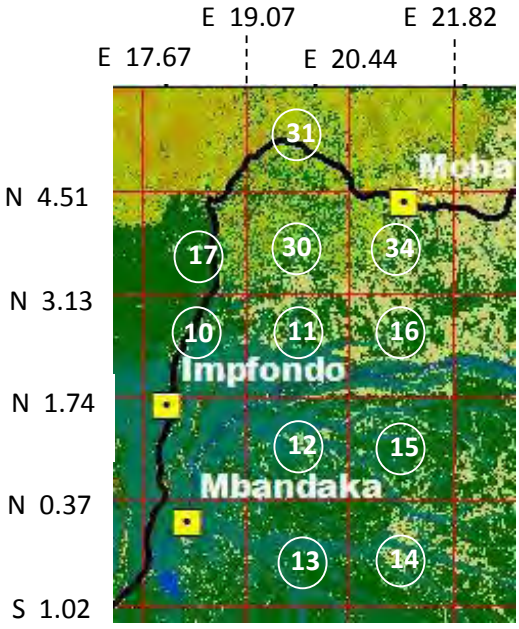
Remarque. Les blocs n°32 et 33 (à l'Est du bloc n°35), un temps envisagés, ont été abandonnés par manque de budget.

RDC zone 1 (tableau 4 : équipe 9, « Mbandaka Sud »)



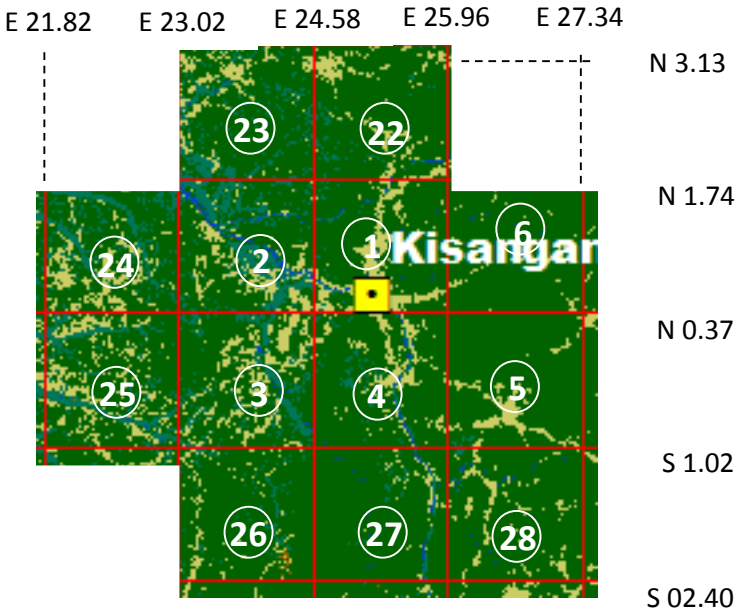
Remarque. Les blocs d'échantillonnage localisés au Sud et à l'Est du bloc 7, non représentés sur la carte, n'ont pas été parcourus en raison du manque d'infrastructures routières/navigables (*vs.* budget trop faible) et en raison de problèmes ethniques/tribaux.

RDC zone 2 (tableau 4 : équipe 6, « Mbandaka Est-Gemena-Lisala-Bumba »)



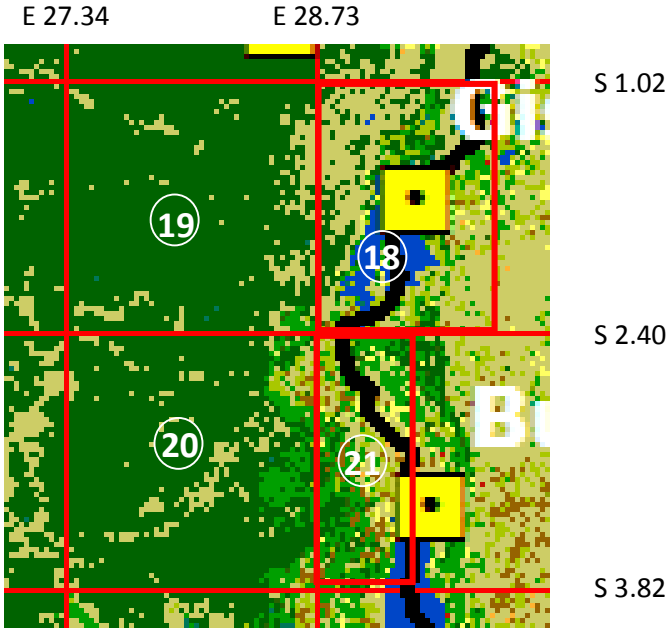
Remarque. Le bloc n°36, non illustré sur la carte, comprend 6 échantillons dont la latitude est comprise entre S 5.56 et S 5.62.

RDC zone 3 (tableau 4 : équipe 7, « Kisangani »)



Remarque. D'importants risques ont été pris par l'équipe de terrain pour mener à bien l'échantillonnage dans le bloc n°28, cette partie de la RDC étant sous le contrôle de mouvements rebelles. Le bloc situé directement à l'est du bloc n°22 a été abandonné, les risques encourus étant trop importants (mêmes raisons que précédemment). Des tiges principalement de *M. excelsa* et de *T. scleroxylon* ont de ce fait été perdues pour le projet.

RDC zone 5 (tableau 4 : équipe 8, « Goma »)



Remarque. D'importants risques ont été pris par l'équipe de terrain pour mener à bien l'échantillonnage dans les blocs n°19 et 20. En effet, cette partie de la RDC était, au moment de la réalisation de l'échantillonnage, sous le contrôle de mouvements rebelles, ce qui a très fortement compliqué l'accès aux deux réserves (Kahuzi Biega et Itombwe) et aux villages environnants. Les blocs n°18 et 21, initialement intégrés au plan d'échantillonnage, ont été abandonnés, les risques encourus étant trop importants (mêmes raisons que précédemment). Des tiges principalement de *M. excelsa* ont de ce fait été perdues pour le projet.

Annexe 2 – Exemples de termes de référence, cas du Gabon

Development and implementation of a species identification and timber tracking system in Africa with DNA fingerprints and stable isotopes

Projet PD 620/11 M (Rev. 1)

Procédure à suivre pour les étapes de récolte, transport, séchage et stockage des échantillons récoltés dans le cadre du projet Zone couvrant le Gabon

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Définitions préalables

Les trois types d'échantillons mentionnés dans le présent document sont :

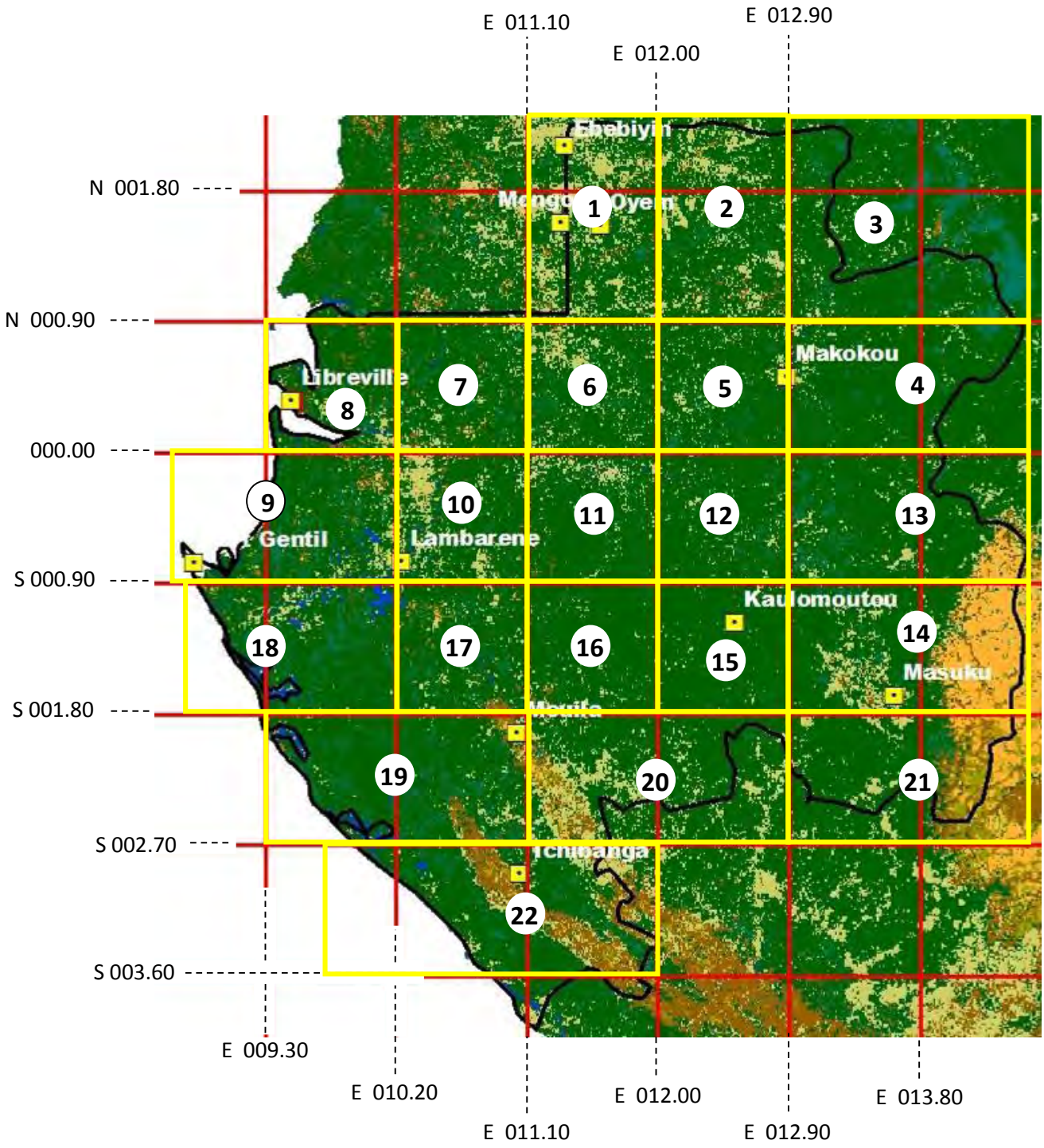
Type 1. Feuille/cambium = un tel échantillon est composé d'un ou de plusieurs morceau(x) de feuille fraîche de l'arbre-cible (si possible de 20 à 50 cm² de surface récoltée) ou de cambium (si possible au moins 5 cm² de surface récoltée), respectivement. Le cas échéant, la surface des feuilles doit être nettoyée (au chiffon) des lichens, poussières, *etc.* La nervure centrale peut être supprimée pour faciliter le séchage. Le récolteur veillera à laisser au moins 50 m entre deux récoltes de feuille d'un même taxon.

Type 2. Copeaux = un tel échantillon est composé de copeaux de bois extraits de la tige de l'arbre-cible sur pied à l'aide d'une machette. A l'endroit du prélèvement (les contreforts et empattements sont privilégiés afin de limiter les dégâts occasionnés à l'arbre) et préalablement à celui-ci, l'écorce est retirée à l'aide de la machette. Les copeaux sont ensuite récoltés et stockés dans les sacs en coton prévus à cet effet.

Type 3. Bois = cet échantillon est constitué d'un morceau de bois. Cet échantillon est prélevé dans le bois d'une tige mature (adulte), après avoir retiré l'écorce. La forme finale et le volume de l'échantillon importent peu (il peu être prélevé à la machette) à la condition qu'il contienne un cube d'au moins 3 cm d'arrête.

Taxons-Cibles Prioritaires (TCP) = il s'agit des trois taxons (*Entandrophragma cylindricum*, *Milicia excelsa* et *Triplochiton scleroxylon*) pour lesquels des prélèvements de feuilles et de copeaux doivent être effectués, au sein de blocs d'échantillonnage géographiquement délimités (voir définition ci-après).

Bloc d'échantillonnage = carré géographique de 110 km de côtés, défini en première approche comme délimitant une population (au sens génétique) de l'un des trois TCP. Un total de 22 blocs a été défini qui couvre l'ensemble des forêts du Gabon (voir la carte insérée ci-après).



Si la latitude est supérieure à **N 000.90** et
la longitude est inférieure à **E 012.00**
alors il s'agit du **bloc n°1** (Oyem-Bitam)

Si la latitude est supérieure à **N 000.90** et
la longitude est comprise entre **E 012.00** et **E 012.90**
alors il s'agit du **bloc n°2** (Minkébé)

Si la latitude est supérieure à **N 000.90** et
la longitude est supérieure à **E 012.90**
alors il s'agit du **bloc n°3** (Mekambo)

Si la latitude est comprise entre **000.00** et **N 000.90** et

la longitude est supérieure à **E 012.90**
alors il s'agit du **bloc n°4** (Makokou Est)

Si la latitude est comprise entre **000.00** et **N 000.90** et
la longitude est comprise entre **E 012.00 et E 012.90**
alors il s'agit du **bloc n°5** (Makokou Ouest)

Si la latitude est comprise entre **000.00** et **N 000.90** et
la longitude est comprise entre **E 011.10 et E 012.00**
alors il s'agit du **bloc n°6** (Mitzic)

Si la latitude est comprise entre **000.00** et **N 000.90** et
la longitude est comprise entre **E 010.20 et E 011.10**
alors il s'agit du **bloc n°7** (Libreville Est)

Si la latitude est comprise entre **000.00** et **N 000.90** et
la longitude est inférieure à **E 010.20**
alors il s'agit du **bloc n°8** (Libreville)

Si la latitude est comprise entre **000.00** et **S 000.90** et
la longitude est inférieure à **E 010.20**
alors il s'agit du **bloc n°9** (Port Gentil)

Si la latitude est comprise entre **000.00** et **S 000.90** et
la longitude est comprise entre **E 010.20 et E 011.10**
alors il s'agit du **bloc n°10** (Ndjolé)

Si la latitude est comprise entre **000.00** et **S 000.90** et
la longitude est comprise entre **E 011.10 et E 012.00**
alors il s'agit du **bloc n°11** (La Lopé)

Si la latitude est comprise entre **000.00** et **S 000.90** et
la longitude est comprise entre **E 012.00 et E 012.90**
alors il s'agit du **bloc n°12** (Lastourville)

Si la latitude est comprise entre **000.00** et **S 000.90** et
la longitude est supérieure à **E 012.90**
alors il s'agit du **bloc n°13** (Okandja)

Si la latitude est comprise entre **S 000.90** et **S 001.80** et
la longitude est supérieure à **E 012.90**
alors il s'agit du **bloc n°14** (Franceville)

Si la latitude est comprise entre **S 000.90** et **S 001.80** et
la longitude est comprise entre **E 012.00 et E 012.90**
alors il s'agit du **bloc n°15** (Koulamoutou)

Si la latitude est comprise entre **S 000.90** et **S 001.80** et
la longitude est comprise entre **E 011.10 et E 012.00**
alors il s'agit du **bloc n°16** (Dibandi)

Si la latitude est comprise entre **S 000.90** et **S 001.80** et

la longitude est comprise entre **E 010.20 et E 011.10**
alors il s'agit du **bloc n°17** (Yombi)

Si la latitude est comprise entre **S 000.90** et **S 001.80** et
la longitude est inférieure à **E 010.20**
alors il s'agit du **bloc n°18** (Omboué)

Si la latitude est comprise entre **S 001.80** et **S 002.70** et
la longitude est inférieure à **E 011.10**
alors il s'agit du **bloc n°19** (Loango-Mouilla)

Si la latitude est comprise entre **S 001.80** et **S 002.70** et
la longitude est comprise entre **E 011.10 et E 012.90**
alors il s'agit du **bloc n°20** (Birougou)

Si la latitude est comprise entre **S 001.80** et **S 002.70** et
la longitude est supérieure à **E 012.90**
alors il s'agit du **bloc n°21** (Birougou Est)

Si la latitude est comprise entre **S 002.70** et **S 004.00**
alors il s'agit du **bloc n°22** (Tchibanga)

Taxons-Cibles Secondaires (TCS) = il s'agit des 19 taxons suivants pour lesquels 2 tige (par taxon) doit faire l'objet d'un prélèvement de bois, feuilles et cambium (les trois échantillons sur la même tige). Ces prélèvements sont indépendants de ceux effectués pour les 3 TCP. Ces TCS sont :

Afzelia spp. (attention, toujours préciser l'espèce !)
Aucoumea klaineana
Baillonella toxisperma
Cylicodiscus gabunensis
Entandrophragma angolense, E. cylindricum, E. utile
Erythrophleum ivorense
Erythrophleum suaveolens
Guibourtia spp. (attention, toujours préciser l'espèce !)
Khaya spp. (attention, toujours préciser l'espèce !)
Lophira alata
Milicia excelsa
Millettia laurentii
Nauclea diderrichii
Pouteria aningeri (= *Aningeria robusta*)
Pterocarpus soyauxii
Terminalia superba
Triplochiton scleroxylon

Phase de récolte des échantillons

1. Lorsque des récoltes, quelles qu'elles soient, sont envisagées dans la concession d'une société forestière, il est impératif de se conformer scrupuleusement à son règlement intérieur (sécurité, bonne conduite, *etc.*) et de fournir au Chef de site (1) en début de mission une présentation orale du travail à accomplir et (2) en fin de mission un compte-rendu du travail accompli.

2. Tout trajet, quel qu'il soit, doit être mis à profit pour réaliser autant de récoltes que possible.

3. Pour des raisons financières évidentes, le récolteur de terrain évitera d'effectuer deux déplacements dans un même bloc d'échantillonnage (surtout si ce bloc est isolé/éloigné), ce qui revient à dire qu'une zone sera quittée si possible uniquement lorsque tous les objectifs y auront été atteints à 100%.

4. Les prélèvements de feuilles sont effectués à l'aide d'une fronde (voir photos 1 et 2), l'objectif étant la récolte d'un ou de plusieurs morceau(x) frais. Les feuilles trouvées au sol ne peuvent convenir (fraiches ou vieilles), elles sont proscrites. Les morceaux de cambium sont prélevés à l'aide d'une lame (couteau ou machette) : 5-6 cm² de matière suffisent (attention au séchage !).

Photos 1 et 2. – Exemples de frondes artisanale (à g.) et industrielle (à d.) utilisées pour la collecte de morceaux de feuilles fraîches dans le houppier de l'arbre-cible.

5. Tant pour les TCP que les TCS, si un doute subsiste lors de la récolte, un échantillon d'herbier est constitué portant les coordonnées GPS de l'échantillon concerné. Ce spécimen d'herbier permettra de confirmer la détermination botanique une fois de retour au bureau.

6. Un spécimen d'herbier, qui pourra ultérieurement servir de référence, sera constitué, pour toute la zone de récolte, pour chacun des taxons (TCS).

7. Si un arbre est échantillonné par exemple pour ses feuilles et ses copeaux, toutes les informations utiles (nom complet de l'espèce, date de récolte, coordonnées GPS, altitude GPS, diamètre estimé de l'arbre en cm) sont inscrites sur l'enveloppe contenant les feuilles. Ces mêmes informations sont inscrites sur (au marqueur indélébile) et dans (au crayon sur un bout de papier) le sachet en coton contenant les copeaux de bois (photo 3).

Le code de récolte est inscrit sur et à l'intérieur du sachet

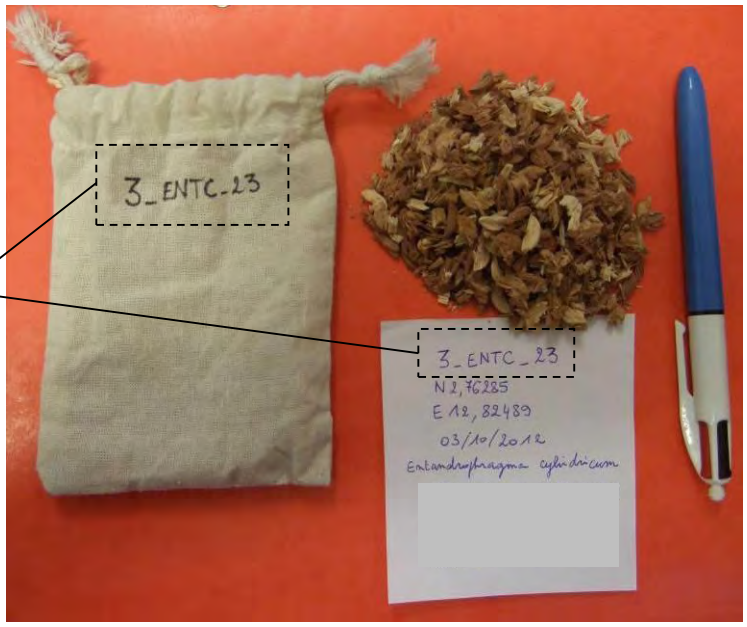


Photo 3. – Aperçu d'un échantillon de copeaux de bois d'*Entandrophragma cylindricum* prélevés au niveau de l'un des empattements de l'arbre-cible. Le sachet en coton contient également une étiquette dûment complétée au crayon gris (« 3_ENTC_23 » dans notre exemple).

8. Afin d'éviter les erreurs de lecture, le récolteur veillera à écrire de manière aussi lisible que possible et à éviter toute rature, imprécision et autre source pouvant conduire à une interprétation erronée.

9. Pour toute récolte (feuilles, copeaux, bois), les coordonnées de l'arbre échantillonné sont mémorisées dans le GPS (si possible au pied de l'arbre) en même temps que le code de récolte.

10. Après chaque récolte, la fiche de suivi (annexée au présent document) des récoltes est dûment complétée. Cette feuille des récoltes permet (1) d'avoir un rapide état d'avancement du travail accompli et (2) d'éviter tout prélèvement en sous-nombre (travail inachevé) ou au contraire excessif (travail inutile). **Dans tous les cas, le principe de précaution sera appliqué : mieux vaut un peu plus que pas assez !**

11. Une croix est effectuée à la machette sur chacun des arbres qui a été échantillonné pour éviter de prendre plusieurs échantillons (par exemple de feuilles) sur un arbre donné

12. La liste des échantillons attendus par le projet est donnée au tableau suivant.

Exemple d'interprétation du tableau : [5 blocs] x [22 tiges par bloc] = [110 échantillons]

Taxon	Nombre d'échantillons de feuilles	Nombre d'échantillons de copeaux	Nombre d'échantillons de bois	Nombre d'échantillons de cambium
Entandrophragma cylindricum	5 x 22 = 110	5 x 5 = 25	2 X 1 = 2	2 X 1 = 2
Milicia excelsa	20 x 22 = 440	20 x 5 = 100	2 X 1 = 2	2 X 1 = 2
Triplochiton scleroxylon	2 x 22 = 44	2 x 5 = 10	2 X 1 = 2	2 X 1 = 2
Afzelia spp.	30 tiges bien réparties		2 X 1 = 2	2 X 1 = 2
Aucoumea klaineana	15 x 1 = 15		2 X 1 = 2	2 X 1 = 2
Baillonella toxisperma	2 X 1 =2		2 X 1 = 2	2 X 1 = 2
Cylicodiscus gabunensis	2 X 1 =2		2 X 1 = 2	2 X 1 = 2
Entandrophragma angolense	2 X 1 =2		2 X 1 = 2	2 X 1 = 2
Entandrophragma utile	2 X 1 =2		2 X 1 = 2	2 X 1 = 2
Erythrophleum ivorense	2 x 1 = 2		2 X 1 = 2	2 X 1 = 2
Erythrophleum suaveolens	2 x 1 = 2		2 X 1 = 2	2 X 1 = 2
Guibourtia spp.	2 X 1 =2		2 X 1 = 2	2 X 1 = 2
Khaya spp.	2 X 1 =2		2 X 1 = 2	2 X 1 = 2
Lophira alata	30 tiges bien réparties		2 X 1 = 2	2 X 1 = 2
Millettia laurentii	2 X 1 =2		2 X 1 = 2	2 X 1 = 2
Nauclea diderrichii	2 X 1 =2		2 X 1 = 2	2 X 1 = 2
Pouteria aningeri (*A. robusta*)	2 x 1 = 2		2 X 1 = 2	2 X 1 = 2
Pterocarpus soyauxii	2 X 1 =2		2 X 1 = 2	2 X 1 = 2
Terminalia superba	2 X 1 =2		2 X 1 = 2	2 X 1 = 2
Nombre total d'échantillons (tous types confondus)	**906**			

Phases de transport et séchage des échantillons

1. Chaque soir, tous les échantillons (feuilles, copeaux, bois, cambium et spécimen d'herbier) en phase de séchage sont contrôlés. Tout est mis en œuvre pour éviter toute contamination par de la moisissure : (a) dans le cas des échantillons de feuilles et cambium le moyen le plus efficace est le remplacement du silica-gel décoloré (et donc imbibé d'eau) ; (b) concernant les copeaux et le bois, les sachets en coton doivent être parfaitement aérés ; (c) enfin, le papier journal des échantillons d'herbier est fréquemment changé.

2. Le cas échant, tout échantillon attaqué, et donc perdu pour le projet, devra être détruit pour éviter la propagation du(des) champignon(s). **Dans tous les cas, le principe suivant sera appliqué : mieux vaut peu de matériel, mais bien conservé, que beaucoup dans un état détérioré !**

3. Lors des transports, les échantillons récoltés, quels qu'ils soient, seront stockés à l'abri de la pluie.

Phase de stockage et transmission des échantillons

1. Chaque échantillon de feuilles/cambium est conditionné dès sa récolte sur le terrain dans une enveloppe en papier sur laquelle sont inscrites au stylo bille l'ensemble des informations utiles (voir photo 4)

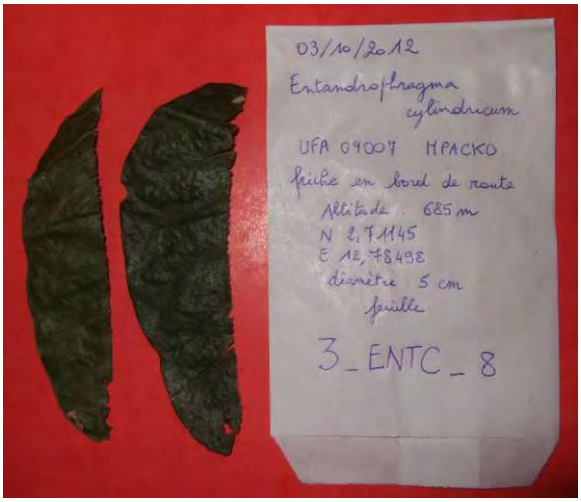


Photo 4. – Exemple d'échantillons de feuilles d'*Entandrophragma cylindricum* conditionnés sur le terrain dans une enveloppe en papier. Toutes les informations requises sont inscrites au stylo bille sur l'enveloppe, y compris la date (dans notre exemple le 3 octobre 2012).

2. Les échantillons de copeaux et de bois sont conditionnés dès leur récolte sur le terrain exclusivement dans un sachet en coton fourni par le projet à cet effet.

3. L'ensemble des échantillons récoltés (feuilles/cambium, copeaux, bois et planches d'herbier) sont stockés dans un lieu sec.

4. Avant la remise des échantillons à Nils Bourland, le collecteur rempli, date et signe la fiche de remise-reprise (en annexe).

Bloc d'échantillonnage

– …… –

Date de début des récolte : _____ / _____ / 20_____

Date de fin des récolte : _____ / _____ / 20_____

Taxon	Feuilles	Copeaux	Cambium	Bois
Milicia excelsa	(22)	(5)	(1)	(1)
Entandrophragma cylindricum	(22)	(5)	(1)	(1)
Triplochiton scleroxylon	(22)	(5)	(1)	(1)
Afzelia spp.	(pas de limite)		(1)	(1)
Aucoumea klaineana	(1)		(1)	(1)
Baillonella toxisperma	(1)		(1)	(1)
Cylicodiscus gabunensis	(1)		(1)	(1)
Entandrophragma angolense	(1)		(1)	(1)
Entandrophragma utile	(1)		(1)	(1)
Erythrophleum suaveolens	(1)		(1)	(1)
Guibourtia spp.	(1)		(1)	(1)
Khaya spp.	(1)		(1)	(1)
Lophira alata	(pas de limite)		(1)	(1)
Milicia regia	(1)		(1)	(1)
Millettia laurentii	(1)		(1)	(1)
Nauclea diderrichii	(1)		(1)	(1)
Pouteria aningeri (*Aningeria robusta*)	(1)		(1)	(1)
Pterocarpus soyauxii	(1)		(1)	(1)
Terminalia superba	(1)		(1)	(1)

Remarque(s) concernant ces récoltes :

Fiche de remise des échantillons

Feuille n° ……… / ………

Coordonnées de l'échantillon	Type d'échantillon (Feuille/cambium = F ; Bois = B ; Copeaux = C ; Herbier = H)

Coordonnées de l'échantillon	Type d'échantillon (Feuille/cambium = F ; Bois = B ; Copeaux = C ; Herbier = H)

Remarque(s) :

………………………., le ……… / ……… / 20……

Annexe 3 – Liste détaillée des principaux partenaires de la phase de récolte du projet

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Côte d'Ivoire	Ministère des Eaux et Forêts Cantonnement d'Agboville www.eauxetforets.gouv.ci	Capitaine Fofana Adama +225 22503564 ; +225 09635501 ; +225 02855514
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ANNEX 10

Minutes of the final conference

ITTO project PD 620/11 Rev.1 (M)

"Development and implementation of a species identification and timber tracking system with DNA fingerprints and stable isotopes in Africa"

1st-2nd July 2015

Douala, Cameroon

The final conference minutes

(Reporters: Dr. Henri-Noël Bouda, Dr. Céline Blanc-Jolivet and Dr. Bernd Degen)

This document summarizes the discussions that took place in Douala (Cameroon) at the final conference of the ITTO project "Development and implementation of a species identification and timber tracking system with DNA fingerprints and stable isotopes in Africa". It is presented in minute's format with discussions reflecting the exact order of the agenda.

Attendees: Seventy participants including representatives from eighteen countries attended the conference (Annex 1).

The meeting started with the opening session. The meeting's facilitator was Mr. David Abouem A. Tchoyi, international consultant and former Minister in charge of Higher Education and Scientific Research in Cameroon.

Objectives of the meeting

The objectives of the meeting were:

a) To present the results of the project
b) To discuss strategies for better use of the projects results
c) To discuss the perspectives of DNA and Stable Isotopes technologies for timber tracking

Wednesday July 1st, 2015

Opening session: 10:30-11:15

The opening session was marked by five speeches:

- A welcome note of the project Coordinator and Director of the Thünen Institute of Forest Genetics (PD. Dr. Bernd Degen),
- A welcome note of the representative of German Federal Ministry of Agriculture and Food (BMEL) (Thorsten Hinrichs),
- A welcome note of the representative of US Forest Service (Shelley Gardner),
- A welcome note of the ITTO representative and project manager from the ITTO headquarters in Japan (Dr. Gerhard Breulmann). During his speech, Dr. Breulmann presented an overview of the various initiatives of ITTO on timber tracking.
- The opening speech of the representative of the Cameroonian Ministry of Forests and Wildlife (Assan Gomse).

Introduction session:

The introduction session started with a short self-introduction of all participants, and then the chairman has presented the agenda of the conference. As an overview, three presentations were made during this introduction session.

1) Background information to the problem of illegal logging and timber regulations (EU, USA, Australia) (Shelly Gardener, USA)

Mrs Gardner presentation was focused on the three main new legal acts concerning illegal logging:

- The Australia Illegal Logging Prohibition Act
- The E.U. Timber Regulation
- And the U.S. Lacey Act

She concluded her speech with some information on the Global Timber Tracking Network, GTTN (**www.globaltimbertrackingnetwork.org**)

2) Practical application of timber tracking methods (genetics, stable isotopes, wood anatomy) (Bernd Degen, Marcus Boner, Volker Haag; Germany). This presentation was divided into 3 parts and presented by 3 experts to cover the important technologies of timber tracking included in the project.
    - Genetics: the control with genetics are on different scales:
        - control of species identity
        - control of geographic origin

    - tree by tree approach to check the chain of custody
- Wood anatomy: this method is to identify the species, but for some cases it is only accurate at genus level
- Stables isotope: Stable isotopes are commonly used to track back the origin of various goods like food. In the context of illegal logging the method is used to verify the declaration of the timber geographic origin.

3) Overview of the ITTO project: objectives, work plan and expected results (Henri Bouda, Germany). This presentation was focussed on: the objectives and outputs, the target countries, the target species and the project partners.

Discussion session:

The discussion following the introduction session was focussed on:

- The robustness of the genetic and isotope methods. While genetics investigates the intrinsic characteristics of the tree to answer the question of the identity and origin, the stable isotope method looks at the differentiation due to the environmental conditions. The two methods are rather complementary.
- The problem related to genetic testing material from plantations (trees from Congo planted in Ivory Coast will have the genetic composition as Congolese trees, even being in Ivory Coast). But generally, such plantations are of less concern in terms of illegal logging. The current project is focussing on natural forests.
- The measurements conducted in the case of stable isotopes analysis.
- What will be the future of the collaboration with the African reference labs after the project is finished and how will the regional collaboration between the African genetic reference labs and the different African timber producer countries work? At the end of the project we made a ring tests including all laboratories. Those laboratories which have successfully participated in the ring tests are good candidates for further support in order to strengthen their skills. Also a regional collaboration among the different African countries and the regional laboratories needs to be stimulated and supported.
- What is the link between the project and the VPA / FLEGT? How could the technologies developed in the project be integrated in the existing tracking systems? How to make these technologies relevant at local level for timber used at the national markets? => The VPAs include the commitment of the timber producer countries to assure the legality of exported timber. For this the techniques developed and data collected for tree species identification and tests on tree origin are very useful. This knowledge can be applied via the three regional genetic reference labs and by direct consultation of the involved western laboratories.

- What are the perspectives for the African countries after this project finishes, especially for countries without a genetic reference lab? => These countries need to find arrangements with the regional genetic reference labs on the application of test techniques (e.g. by sending experts from their countries to the reference labs or by developing service agreements among each other).
- What is the precision of the tracking technologies at borders between countries? => This depends on the spatial pattern of the reference data and the declared position to be verified. For the genetic and stable isotope reference data of the project the precision is on average between 200 to 350 km.

Session 2a: Presentation of the project results:

During this session, the presentations were focused on the genetics and the stable isotopes results

1. Genetics results
    - Genetic structure in *Entandrophragma cylindricum* (Céline Blanc-Jolivet) : the goal is to find molecular markers with strong geographical structure. The distribution of the nine identified genetic groups was presented. It was also possible to distinguish *Entandrophagma* species with the newly developed molecular markers, which raised the problem of species misidentification in the field. The team of Andrew Lowe was also able to identify the occurrence of several species with new generation sequencing.
    - Genetic structure in *Triplochiton scleroxylon* (Andrew Lowe, Australia; and presented by Céline Blanc-Jolivet, Germany) : three genetic groups could be identified, including West Africa (Ghana), Western part of Central Africa (Cameroon) and Eastern part of central Africa (DR Congo).
    - Identification of reference populations for Milicia species (iroko) and adaptation of the protocol for African laboratories (Serge Kasso Dainou, Belgium): the work was held by Nature+, in collaboration with the University of Brussels and was focused on 3 main points:
        - Identify reference populations for wood tracking based on genetic markers (SNP)
        - Assess assignment scores in these reference populations and identify a set of most discriminant genetic markers
        - Develop a protocol applicable in African laboratories

The follow outputs can be drawn from the work done:

- Clear distinction between M. exelsa and M. regia
- Identification of 6 different *Milicia* gene pools
- Increase of the number of markers for a better spatial resolution: **next step**
- 15 reference populations could be improved with more loci
- Assignment scores are generally good for well differentiated gene pools

- Training of 4 African researchers who are now able to contribute to local genetic works

2. Stable isotopes
   - Verifying the declared origin of timber using stable isotopes (Gareth Rees, FERA/UK): the work allowed to establish a reference library (n = 210) using stable isotopes of authentic tropical timber samples from Ghana, Ivory Coast, DRC, Congo, Gabon and Cameroon, to develop novel techniques to classify timber origin, and to develop and optimise the sample extraction methods. Three isotope ratios (D/H, 18O/16O, 13C/12C) have been used and the work was on Sapelli.
   - Stable isotope investigation of *Triplochiton scleroxylon* -Ayous-(Micha Horacek): using the same method and the same istope ratios as FERA/UK, Mr. Horacek's institute (Josephinum Research/Austria). The outcomes are quite similar, allowing discriminating timber from different provenances.

     Project results: Iroko -*Milica Excelsa*- (Markus Boner, Germany): Agroisolab worked with six isotope ratios (D/H, 18O/16O, 13C/12C, 15N/14N, 34S/32S, Sr),

Discussion on the project results

The discussion following the session A of the project results presentation was focussed on:

- The cost-effectiveness of the different methods
- Intellectual property issues concerning the reference data and availability through a database
- Time needed to investigate and have the final result to verify the declaration (time from the collection to the result). It could be done in a couple of weeks for all analysis together (anatomy and confirmation by genetics). The stable isotopes method may take at most a week.
- How to make such technology efficiently useable in the producer countries. It would be useful if for example customs could conduct such analysis. This was identified as a topic to be further discussed during the group work in the afternoon.
- Who is charged for the costs of the analysis? Is it the producer country, the importer country, the company seller or the company buyer? Is it cost-effective? It seems to be clear that the cost will be indirectly charged to the buyer through increase of the price of unit cubic meter.
- What is the progress and effectiveness of the training? After 3 months in the skilled labs, are the trainees able to make the analysis by themselves? => Yes, but the problem will be at equipment level as there are only 3 reference labs in Africa. Also this subject needed to be discussed further during the group work session this afternoon.
- What about the other species (except Iroko, Sapelli and Ayous)? Threatened species are more likely to be abused. The project coordination group explained who the selection of target species

has been done: The selection of target species has been made during an international workshop in Yaoundé in 2011. All countries were involved and the selection has been effective after long discussion and agreement of all participants. Anyway, the door is not closed for other species, as we are running another project on other 7 African species and 7 Latin American species. We are trying to cover most exported species timber in few years.

- What kind of conclusion can be drawn from the tests on origin? Just if the claim is correct or false Or also an indication of the true origin? For the producer countries it is interesting to increase the resolution e.g. more information about the concession of origin

Session 2b: Presentation of the project results

During this session, the presentations were focused on the wood anatomy and the blind tests results, and the technologies transfer matter.

3. Wood anatomy: a microscopic wood identification and verification of the declared botanical nomenclature have been done for 178 solid wood samples in most cases it is possible to get down to the genus level
4. Blind test

The blind tests were organised by 2 different partners: WWF/Germany and G2S/Cameroon. Both operators sent samples to tree species testing and tests on origin to the involved labs. Part of the samples had a correct declaration on species and origin and another part was false. By comparing the laboratories feedback with the true species and true origin conclusions on the performance of the testing technologies were drawn.

5. Technology transfer for genetic timber verification in Africa (Emmanuel Opuni-Frimpong, Ghana). With this presentation, Mr. Opuni-Frimpong displayed the list of trainings held in the genetic reference labs in Africa, and also the complete list of 11 trainees who have spent 3 months in a skilled genetic lab in Europe and/or Australia. He mentioned also the equipment that the reference labs received from the project.
6. Main project outcomes and Conclusions (Bernd Degen, Germany)

In this presentation, Degen summarized the outcomes of the project and drew some conclusions

Main outcomes:

- Pre-project in 2011: discussion on work programme and methods, selection of species
- Samples: over 5000 samples collected for the analysis. The collection has been made by the University of Liège (Belgium), and organized by Dr. Nils Bourland who coordinated many teams in Cameroon, Congo, Ivory Coast, DR Congo, Ghana and Kenya.
- Reference data on tree species identification (21 taxa)
- Pilot study on genetic species identification of Khaya on a forest concession in Ghana:

  - Clear identification of two genetic clusters (species): *Khaya anthotheca* and *Khaya. ivorensis*
  - Very strong genetic differences among the two species (very little evidence for hybridisation)
  - 5% of the *Khaya ivorensis* are classified genetically as *Khaya anthotheca*
  - 40% of the *Khaya anthotheca* are classified genetically as *Khaya ivorensis*
  - Development of new gene marker (SNPs) useful to improve power of tree by tree CoC tracking
- Reference data on geographic origin for 3 species (Sapelli, Iroko and Ayous)
- Blind tests
  - Species identification
    - Wood anatomy => good results => maximal technical resolution reached in many cases
    - DNA-Barcoding => moderate results, much room for improvement
  - Claims on geographic origin (country) analysed with reference data of the project
    - Isotopes: Results for 95 to 100% of the samples. From all samples 65% correct
    - Genetics: Results for 78% of the samples. From all samples 50% correct. From samples with sufficient DNA amplification => 64% correct
- Reference labs in Africa in place: three labs with additional equipment and training for genetics
- Training completed: three training workshops in Africa labs, and 11 trainees in genetic labs in Brussels, Edinburgh, Grosshansdorf and Adelaide

Conclusions

- Sampling: the spatial distribution was not ideal, as some regions are underrepresented. For the future we need:
  - more geographic different sampling points (transects)
  - less individuals per sampling point
  - more information collected per individual (more gene markers, more isotopes)
- DNA-Barcoding. For the future, the following points have to be carefully observed:
  - Preference for approaches using multiple gene region (combination of nuclear and plastid genes)
  - More attention for assignment of "non-target species" => change sampling design
  - More controls to avoid DNA-contamination
- Tools to control claims on geographic origin: Confirmation that both methods: isotopes and genetics are very useful tools to control declarations on origin. In the future, we have to pay attention on the following points:

  - Need to increase the resolution
  - Adding reference samples from low coverage areas
  - Collecting more information per reference sample (more isotopes, more gene markers)
  - Applying both methods
- African genetic reference labs
  - Self-organisation needed to implement control services on national and regional level
  - Participation in future ring and blind tests
  - Support need to be prolonged and efforts should be merged

Discussion on the project results

- What about the use of local knowledge for the species identification? It has not been mentioned during the presentations. How do we draw experience from local knowledge? The answer is that during the sampling, local people have been involved and the local knowledge on species is the 1st mean to identify species that have been sampled.
- Necessity to combine methods: It is clear that combining the methods will improve the results
- There are different types of errors in the blind test: Not finding a mis-declaration is less critical than not confirming a true declaration.
- Automatic analysis should be available in the database to check the origin of a timber sample
- Genetic and isotopic verification could be complementary to existing traceability systems.
- Project results should be more disseminated to the respective decision makers in the countries (forest ministries)

Session 3a: round tables (group discussions)

The participants were divided in two groups to discuss on the following 2 topics:

- Group 1: Application of the new technologies and project results in frame of timber regulations (Chair: Thorsten Hinrichs, Germany)
- - Group 2: Technology transfer – future work of the African regional reference labs (Chair: Joseph Machua, Kenya)

The day ended with these group discussions.

Thursday July 2nd, 2015

The results of the discussions have been presented on the 2nd day of the conference before the closing ceremony and the field trip.

Session 3b: Presentation and discussion of group results and recommendations

Presentation of group 1 results and recommendations

The group raised the list of five questions to discuss, including:

- How would you apply the new technologies?
- What steps to take forward? What improvements do we need to make?
- How do we support the existing regulatory frameworks on certification, tracking and combating illegal logging?
- What are these regulations? What is illegal?
- What is the level of interest among producer and consumer countries in the new technologies?

The discussions came out with the following recommendations:

- The timber producer countries see the need for a training and establishment of an isotope centre in the region. The feasibility and practicability needs further discussion (FERA, Agroisolab and Josephinum research are positive about this possibility)
- Need to make the methods available and affordable, as the new technologies are complementary (and not in competition with) to existing methods being used, e.g. SGS & proper records/documentation
- Need of further training for national/regional experts of the methods requested by several country representatives
- Proposals/suggestions for more reference labs discussed
- Suggestions to expand the list of priority species, at country and regional level. Example given on the global initiative of most target species - 50 each for Africa, South America and Asia. In Africa, the 50 species may well include the list of species mentioned at the beginning of the project
- Improve database by increasing the spatial representation

Presentation of group 2 results and recommendations

The group discussed the following topics:

- Labs equipment
- Trainings
- Collaboration
- Future funding

The following recommendations have been made:

- To maintain collaboration within Africa and with Europe
- To seek funding to reinforce the labs
- To further develop the techniques to get reduce the costs
- To establish isotopes technology labs in Africa

- To take advantage of the training opportunities available from ITTO fellowships
- Thünen Institute (TI) to initiate PHASE II of this project to enhance and promote the use of the developed techniques

Closing session: 10:30-11:15

The speakers thanked the donor of the project, the project partners and co-ordinator and the participants and the people who organized the conference for all the efforts.

Field trip

The field trip consisted of 2 visits:

- The group visited ALPICAM, a timber exporting company with different stages of wood processing and variable wood products (sawing, peeling and slicing).
- At the timber yard of the harbour, the participants saw the last steps of the timber export process before leaving the territory of Cameroon

Annex 1: List of participants to the final conference of the ITTO project – Douala/Cameroon 1st-2nd/07/2015

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