

**PROJECT PROGRESS REPORT TO THE
INTERNATIONAL TROPICAL TIMBER ORGANISATION (ITTO)
Submitted by Double Helix Tracking Technologies Pte Ltd**

**Pilot Implementation of a DNA traceability system for *Pericopsis elata* in
forest concessions and sawmills in Cameroon and Congo.**

PERIOD OF REPORT	01 March 2014 to 30 November 2014
PROJECT NUMBER	PP-A/39-162A
EXECUTING/IMPLEMENTING AGENCY	Ministry of Forest Economy and Sustainable Development, Congo Ministry of Forestry and Wildlife (MINFOF), Cameroon
COLLABORATING AGENCIES	Double Helix Tracking Technologies, Singapore Association of Timber and Forest Industries (ATFI), Cameroon
DURATION	12 months
START DATE	December 2013
PROJECT BUDGET	US\$ 303 500
Project coordinator	Double Helix Tracking Technologies Pte Ltd 3 Science Park Drive #02-12/25 The Franklin Singapore 118223
DoubleHelix staff	Darren Thomas Germain YENE YENE

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1. DESCRIPTION OF WORK IMPLEMENTED DURING THE PERIOD

This project seeks to demonstrate that, using DNA techniques, *Pericopsis elata* logs and sawn timber can be traced back to specific stumps from controlled concessions. The proposed DNA traceability system will secure controlled supply chains, detecting log substitution and associated document fraud and allowing for timely corrective actions to be implemented.

The main outputs are: (1) development of genetic markers for *P. elata* suitable for DNA fingerprinting, (2) Capacity building and training of local teams in DNA sample collection and storage, (3) Implementation of DNA traceability in three controlled supply chains from pre-harvest to point of export.

Principle activities and key results since project launch in March 2014 are as follows:

- Successful trial DNA extraction from *P. elata* sawn timber. Initial tests against *P. elata* sawn timber samples have shown that sufficient DNA can be extracted from the timber on a routine basis. See **Appendix A** for DNA extraction report.
- Organisation and conduct of two stakeholder workshops. One in Kribi, Cameroon and the other in Brazzaville, Republic of Congo. The workshop objectives were to explain the goals of the project, work through questions raised by the participants and conduct training. Germain YENE and Darren THOMAS from DoubleHelix, together with Dr. Henri BOUDA from the Thünen Institut attended to conduct training on DNA sample collection, handling and storage. In Cameroon we were able to combine the workshop and training for both *P. elata* and *Prunus africana* projects. Agendas, attendance lists and photographs from the workshops are presented in **Appendix B**.
- Subsequent to the workshops and training, field missions were organised to collect DNA samples of *P. elata* that will form the genetic reference data set. The goal of collecting 10 individuals from each population was exceeded. The teams were able to collect 30 individual samples from each population. This means that we have the sampling stock to develop both a tree to stump traceability system but also a system to discriminate between different populations (harvesting regions) in the future although this is outside the immediate scope of this project. DNA sample records are presented in **Appendix C**, together with photographs from the fieldwork.
- Meetings have been conducted with industry in Cameroon to learn about the *P. elata* supply chain and manufacturing processes. Supply chain processes have been mapped and we are in the process of designing the sampling plan to collect samples from multiple stages of transportation and production as part of the system implementation phase.

2. EXECUTION OF THE WORKPLAN

2.1 Workplan review

Table 1 below indicates the project implementation in comparison with the detailed workplan provided to ITTO on 23 March 2014. The approved workplan schedule is marked in grey, with the actual implementation indicated by the hatched red cells.

Table 1: Activity schedule in comparison with workplan

OUTPUTS/ACTIVITIES	RESPONSIBLE PARTY	Month												STATUS
		Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	
Output 1.1														
1.1.1 Trial DNA extraction	DoubleHelix													Completed
1.1.2 Population sampling	CNIAF, ANAFOR, DoubleHelix													In progress; start delayed and field work slow.
1.1.3 DNA marker development	DoubleHelix													In progress
Output 1.2														
1.2.1 Workshops and training	CNIAF, ANAFOR, DoubleHelix													First workshop complete (Cameroon and Congo)
Output 1.3														
1.3.1 Verification system development	DoubleHelix													In progress
1.3.2 Field implementation	CNIAF, ANAFOR, DoubleHelix													In progress
1.3.3 Ongoing DNA verification	DoubleHelix													Not started
1.3.4 Statistical analysis	DoubleHelix													Not started

2.2 Progress in implementation of the activities

Table 2: Progress of activities conducted during report period

Activity	Percentage executed	Originally planned completion date	Estimated completion date
1.1.1 Trial DNA extraction	100	April 2014	Complete
1.1.2 Population sampling	90	June 2014	November 2014
1.1.3 Genetic marker development	40	December 2014	February 2015
1.2.1 Opening workshop	100	May 2014	Complete
1.3.1 System development	75	July 2014	December 2014
1.3.2 Field implementation	25	September 2014	January 2015

2.3 Inputs applied

To date, DoubleHelix has received USD 80,000 in funds that have been applied towards:

- Project management - field coordination for workshop and sampling; system design; project administration.
- Purchase of sampling consumables (silica gel and hole punches);
- Laboratory costs (manpower and consumables) for trial DNA extraction and marker development;
- DoubleHelix expenses (flights and accommodation relating to workshop attendance for DoubleHelix and Thünen Institut staff);

2.4 Outputs achievement

Output 1.1 – Development of genetic markers

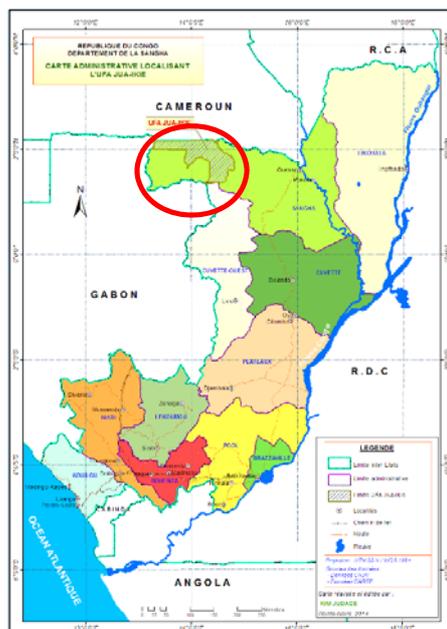
A preliminary test to verify that DNA can be extracted from *P. elata* sawn timber was successfully completed. The results of this DNA extraction test is presented in **Appendix A**.

Following the workshop and training, teams were sent out to collect wood samples from multiple populations in Cameroon and Rep. of Congo. A map of DNA sample collection areas is presented in **Figure 1** below. As of the date of this report, samples are still waiting to be sent from Cameroon pending export approval from the Governments of Cameroon and Congo, and subsequent import approval from Australian customs authorities.

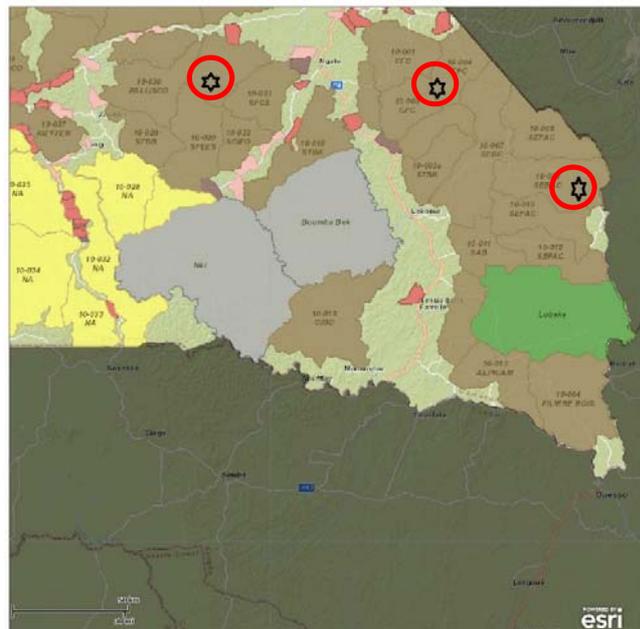
Genetic markers suitable for DNA fingerprinting for *P. elata* are now in progress of being developed. Following field sampling, an initial set of 5 reference DNA samples has been delivered to the laboratory in Australia, enabling the start of genome sequencing to generate candidate genetic markers. Full screening of markers must wait until delivery of all remaining reference samples.

Figure 1 – Locations of *P. elata* populations in Cameroon and Rep. of Congo where DNA samples are being collected.

Republic of Congo sampling location



Cameroon sampling locations



Output 1.2 – Capacity building and training of local teams

The first set of workshops was carried out in Kribi, Cameroon and Brazzaville, Rep. of Congo. These were well attended by members of the Executing Agency, industry and education. Agendas, attendance lists and photographs from the workshops are presented in **Appendix B**.

Output 1.3 – Implementation of DNA verification system

Meetings have been held with ANAFOR and industry participants in Cameroon to discuss the design of a routine sampling strategy from trees and sawn timber.

3. CRITICAL ANALYSIS OF PROJECT PROGRESS

Project delays are attributable to difficulties working in the local environment.

We experienced difficulties in relatively straight-forward tasks, such as sourcing and delivery of sampling consumables such as silica gel and hole punches which delayed the start of sampling activities by three weeks. Unexpectedly, it was impossible to find willing local suppliers of silica gel in Cameroon. In the end, a supplier in South Africa was found, leading to higher than expected costs of delivery to Cameroon and Congo.

Further delays were experienced during the collection of reference DNA samples from target tree populations due to a combination of availability of the local staff trained during the workshops and wet weather (wood samples should not be collected during wet weather since moisture in samples can lead to mould and sample contamination). We continue to experience delays in the delivery of samples to Australia due to a bureaucratic hold up in the issuance of export documentation.

Analysis of reference samples is a limiting step in the project progress, and subsequent activities of genetic marker development and system development have been delayed as a result. Additional time should be put aside for future projects to anticipate for such delays. On the other hand, since harvesting and processing of *P. elata* only takes place from January onwards, it is likely that even with marker development on schedule we would have had to delay start of system implementation to coincide with the start of harvest operations.

During the opening workshops, we were able to research and understand the supply chain mechanism of *P. elata* in Cameroon and Rep. of Congo. It is expected that the envisaged system of tree-by-tree matching will work with *P. elata* sources originating from the Rep. of Congo, since existing document systems allow for individual matching of logs between harvest and at various control points along the transport route.

In Cameroon however, logs are processed into sawn timber. Sawn timber product can only be assigned to a set of logs cut on a specific day. We can foresee that applying a one-on-one matching system in this instance would be cost-prohibitive, since a single sample of sawn timber would need to be tested against multiple logs. In this case, we recommend that a population analysis approach would be more appropriate and will recommend this type of approach in the final project report.

4. CONCLUSIONS

Achievement of project objectives has been delayed but is still expected to be completed. Project duration is likely to extend by another 3 months to June 2015.

Better understanding of the *P. elata* supply chains in Cameroon and Republic of Congo means we expect to be able to successfully implement the system using the envisaged tree-by-tree methodology, but recommend using a different population methodology for processed supply chains that allow assignment to populations (geographic areas) rather than to individual trees/stumps. This would better fit the objectives of the ITTO-CITES project whilst requiring less disruption on the part of industry.

Whilst it is still necessary to send wood samples abroad for testing, it is recommended that we implement a process together with ITTO and the Executive Agencies to fast-track requests for export authorization of wood samples and avoid future delays in the sending of samples to Australia.

As per the Special Service Agreement (E) E14/06, we request the disbursement of the second set of funds of USD 50,000.

Responsible for the Report



Name: Darren Thomas

Position: Executive Director

Date: 24 December 2014

Appendix A – Trial DNA extraction report

Job number: 1087_ITO	Trial DNA Extraction Report for <i>Pericopsis elata</i>
Approved by: Dr. Eleanor Dormontt	Date: Thursday, 1 May 2014

Trial DNA Extraction Report for *Pericopsis elata*

Report written by Ms. Bianca Dunker

All laboratory and analysis work was undertaken by Ms. Bianca Dunker and Mr. Duncan Jardine, under the supervision of Dr. Eleanor Dormontt at the University of Adelaide.

Background

The University of Adelaide has been contracted to undertake a feasibility test on samples of *Pericopsis elata*, to assess whether DNA can be successfully extracted from timber. Five wood samples from different individuals were received from the Thünen Institute, Germany in April 2014. For the purposes of this analysis, the University of Adelaide accepts the species claim and has not undertaken any independent verification of the source of the samples provided.

Methodology

DNA extraction and quantification

Samples were prepared by shaving small sections of the wood with a lathe and undertaking a custom developed DNA extraction methodology (patent pending) designed for timber samples. Three replicates of each sample were included, giving a total of 15 separate DNA extraction procedures. The quantity of DNA present in each extract was measured using a Qubit® 2.0 Fluorometer (Life Technologies).

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Job number: 1087_ITO	Trial DNA Extraction Report for <i>Pericopsis elata</i>
Approved by: Dr. Eleanor Dormontt	Date: Thursday, 1 May 2014

PCR amplification

Extracted DNA from each of the five wood samples was used as a template for polymerase chain reaction (PCR) amplification of three chloroplast microsatellite loci which amplify in the majority of plants. This process was undertaken with DNA diluted to 10% (v/v) to facilitate dilution of inhibitory compounds in the solution.

Results

DNA extraction and quantification

Quantifiable DNA was successfully extracted from all three DNA extractions from each of the five wood samples (Table 1).

Table 1: DNA concentrations of the extracted samples of *Pericopsis elata*

Samples	ng/ml
1	3.47
2	2.13
3	6.63
4	3.24
5	3.47
6	2.30
7	2.24
8	9.80
9	4.42
10	3.71
11	1.80
12	3.13
13	2.11
14	2.48
15	2.71

Job number: 1087_ITO	Trial DNA Extraction Report for <i>Pericopsis elata</i>
Approved by: Dr. Eleanor Dormontt	Date: Thursday, 1 May 2014

PCR amplification

Strong PCR product bands were obtained using the second and the third chloroplast microsatellite primer pairs (Figure 1). No amplification was present for any samples using the first primer pair. Samples from all individuals produced strong product bands. This result implies that usable DNA was successfully extracted from all samples.

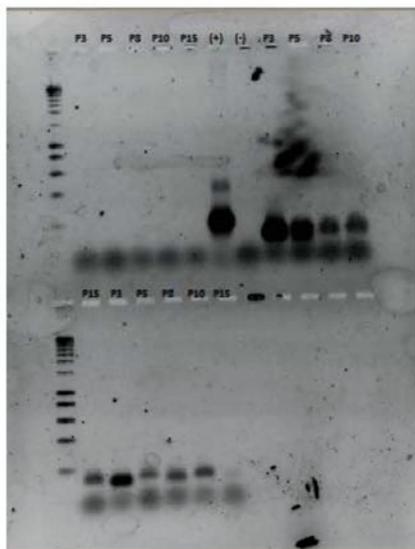


Figure 1: Agarose gel result of PCR amplification. Each column represents a single extracted sample. Sample names are abbreviated. Positive (+) and negative (-) controls were included to validate PCR procedures. A size standard ladder was used to allow PCR product size estimation from the gel.

Conclusions and Recommendations

Based on the DNA extraction trials undertaken at the University of Adelaide on samples of five individuals of *Pericopsis elata* it is our expert opinion that DNA verification of *Pericopsis elata* supply chains is feasible.

Appendix B – Workshop agenda, attendance and photographs

Agenda

DoubleHELIXXX

#02-12/25 The Franklin – Science Park I
Singapore 118223 T +65 6227 9706
www.doublehelixtracking.com
GST/Reg No. 200812136R

DNA Chain-of-Custody workshop agenda

DRAFT

Day 1

- 08:30 **Participants registration**
- 09:00 **Opening remarks - Government, ITTO representatives**
- 09:30 **Photo/Picture with all the participants - Facilitator**
- 10:00 **Introduction and update on the ITTO-CITES programme - Jean Lagarde-Betti (ITTO)**
- 10:30 **Introduction to the project for *pericopsis elata* and *prunus africana***
Darren Thomas, Germain Yene Yene (DoubleHelix)
- 11:00 **Coffee break**
- 11:30 **Overview of using DNA markers to control origin and Chain-of-Custody**
Henri Bouda (Thünen Institut), Darren Thomas
- 12:00 **Practical results of a project using DNA for wood tracking (realized in Cameroon)**
Henri Bouda (Thünen Institut),
- 12:30 **Discussion - Facilitator**
- 13:00 **Lunch**
- 14:00 **Introduction and desk-based training on DNA sampling - Henri Bouda (Thünen Institut), video**
 - a. Tree anatomy
 - b. Tools
 - c. Sampling procedure
 - d. Sample recording and storage
 - e. Logistics
- 15:00 **Coffee break**
- 15:30 **Formulation of sampling plan and schedule for *p.elata* and *prunus africana* + discussion**
Jean Lagarde Betti, all.
- 16:30 **END OF DAY 1**

Day 2 – Field visit

- 08:30 **Participants grouping at the hotel**
- 09:00 **Departure to the forest reserve of Bidou**
- 09:30 **Arrival at the forest reserve site**
- 10:00 **Training in the forest reserve**
 - a. Sampling practice for all participants - led by Henri Bouda, Germain Yene Yene
 - b. Adjustment of sampling procedures for *p.elata* and *prunus africana* - Darren Thomas
- 12:00 Return travel
- 12:30 Debriefing (if necessary)
- 13:00 Lunch
- 14:00 END OF DAY 2

Participant list Congo Brazzaville

REPUBLIQUE DU CONGO

 MINISTERE DE L'ECONOMIE FORESTIERE
 ET DU DEVELOPPEMENT DURABLE

 PROGRAMME CONJOINT OIBT/CITES
 « Gestion durable de l'Afromosia dans le Bassin du Congo »

Activité : n° PP-A/39-162 A "Mise en œuvre pilote d'une traçabilité génétique du bois d'Afromosia dans les concessions forestières et scierie au Congo"



Cniatf_2004@yahoo.fr

LISTE DES PARTICIPANTS DE L'ATELIER "Traçabilité Génétique de L'Afromosia"

N°	Noms et Prénoms	Structure d'Origine	Contacts
1	DEMBE LOUVINGUILA TENDA Hélène	Université Marien NGOUABI	06 926 65 51
2	DONGOU GOPO GAMANTALEY Annick	Direction des Forêts	06 667 66 85
3	LEBOKA Nicolas	CNIAF	
4	YOKA Joseph	Professeur Univ. Marien NG	05 521 31 93
5	MAKAYA CHANDER Lhy - Moeny	Direction des Forêts	05 521 67 78
6	MAKELE-LADHY Bushnel Charmand	Université Marien NGOUABI	06 979 43 54
7	NZOBADILA Espoir Keigerlain	Université Marien NGOUABI	06 979 47 82
8	NDOBOLO MANKERI Nadina	CNIAF	06 651 89 85
9	KOMBO Jean Claude	CNIAF	06 682 57 58
10	NGONGOUYOU Yvon	CNIAF	05 561 19 82
11	MBANTSO née OFOUNGUINI Judith Léna	CNIAF	06 620 08 45

12	Mme Marie Odette ITANGO	Attachée de Presse au MEFDD	05 500 76 33
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16	YENE Germain	DoubleHélix	+237 995 086 83
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18	BANZOUZI Jean Claude	Coordonnateur National du Projet	06 624 73 44
19	BIKOUNDA Stéphanie	Responsable des Sites	066 60 64 15
20	IKOKA Joséphine	Secrétaire du Projet	06 601 46 48
21	IKOA OKANDZA Basile	Société Civile	06 959 06 00
22	BOSSEMBE Christian	Cabinet du Ministre	
23	LIKIBI Félix	Cabinet du Ministre	
24	EKEKE Réve	Cabinet du Ministre	
25	NGOUALA Patrice	CNIAF	06 642 48 04
26	MPATI Basile	CNIAF	05 558 13 76
27	OSSEBI Alain Bienvenu	Assistant, chargé des Opérations	05 526 02 11

1. Atelier de lancement et de formation



Cérémonie officielle



Expert de DoubleHelix montrant un sachet de Silica gel, et séance d'extraction de carotte de bois, lors de l'atelier



Extraction d'échantillons en forêt



Suite extraction d'échantillons en forêt

Appendix C – Sample of DNA collection records and photographs

Pays: REPUBLIQUE DU Congo							Forêt/Région: VFA Jua-1Kie	
Espèce: <i>Pericopsis elata</i> / <i>Afrosmia</i>							Population N°: 1	
Date de collecte: du 15/18 au 15/18 2014							Echantillonneur: NOUNGOU YOUN	
N°	N° échantillons	Nom du site	Nom/N° du bloc	Type échantillon	Spécimen	Matériel vivant/mort	Latitude	Longitude
1	PE-A01-1-NY	VFA Jua-1Kie	06	Cambium	Arbre debout	vivant	02° 03' 52,8"	014° 21' 51,9"
2	PE-A02-1-NY	VFA Jua-1Kie	06	Cambium	Arbre debout	vivant	02° 03' 53,7"	014° 21' 57,7"
3	PE-A03-1-NY	VFA Jua-1Kie	06	Cambium	Arbre debout	vivant	02° 03' 48,2"	014° 22' 02,8"
4	PE-A04-1-NY	VFA Jua-1Kie	06	Cambium	Arbre debout	vivant	02° 03' 45,8"	014° 22' 03,1"
5	PE-A05-1-NY	VFA Jua-1Kie	06	Cambium	Arbre debout	vivant	02° 03' 53,9"	014° 21' 53,3"
6	PE-A06-1-NY	VFA Jua-1Kie	06	Cambium	Arbre debout	vivant	02° 04' 05,0"	014° 21' 53,3"
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8								
9								
10								
11								
12								
13								

NB Voir explications sur le remplissage de ce formulaire en annexe A du protocole d'échantillonnage

Pays: REPUBLIQUE DU Congo							Forêt/Région: VFA Jua-1Kie	
Espèce: <i>Pericopsis elata</i> / <i>Afrosmia</i>							Population N°: 1	
Date de collecte: du 15/18 au 15/18 2014							Echantillonneur: BANZOUZI Ram	
N°	N° échantillons	Nom du site	Nom/N° du bloc	Type échantillon	Spécimen	Matériel vivant/mort	Latitude	Longitude
1	PE-A20-1-BR	VFA Jua-1Kie	-	Cambium	Arbre vivant	vivant	01° 47' 32,8"	014° 38' 02,0"
2	PE-A22-1-BR	VFA Jua-1Kie	-	Cambium	Arbre debout	vivant	01° 47' 35,8"	014° 37' 59,5"
3	PE-A24-1-BR	VFA Jua-1Kie	-	Cambium	Arbre debout	vivant	01° 48' 01,1"	014° 38' 03,1"
4	PE-A26-1-BR	VFA Jua-1Kie	-	Cambium	Arbre debout	vivant	01° 48' 09,5"	014° 37' 58,7"
5	PE-A28-1-BR	VFA Jua-1Kie	-	Cambium	Arbre debout	vivant	01° 48' 16,0"	014° 38' 00,1"
6	PE-A30-1-BR	VFA Jua-1Kie	-	Cambium	Arbre debout	vivant	01° 48' 14,2"	014° 37' 58,1"
7	PE-A32-1-BR	VFA Jua-1Kie	-	Cambium	Arbre vivant	vivant	01° 48' 16,0"	014° 37' 59,9"
8	PE-A36-1-BR	VFA Jua-1Kie	-	Cambium	Arbre vivant	vivant	01° 48' 23,8"	014° 38' 03,3"
9	PE-A34-1-BR	VFA Jua-1Kie	-	Cambium	Arbre vivant	vivant	01° 48' 20,1"	014° 37' 59,9"
10								
11								
12								
13								

NB Voir explications sur le remplissage de ce formulaire en annexe A du protocole d'échantillonnage

Pays: République du Congo						Forêt/Région: UFA-TALA-TALA		
Espèce: <i>Pericopsis elata</i> / <i>Athromesia</i>						Population N°: 2		
Date de collecte: du 20 au 20 2014						Echantillonneur: BANZOUZI Romain		
N°	N° échantillons	Nom du site	Nom/N° du bloc	Type échantillon	Spécimen	Matériel vivant/mort	Latitude	Longitude
1	PE-A01-2-BR	UFA TALA-TALA	02	Cambium	Arbre défilé	vivant	01°36'49,5"	014°52'48,3"
2	PE-A03-2-BR	UFA TALA-TALA	02	Cambium	Arbre défilé	vivant	01°37'49,5"	014°50'34,7"
3	PE-A04-2-BR	UFA TALA-TALA	02	Cambium	Arbre défilé	vivant	01°38'09,3"	014°50'51,7"
4	PE-A05-2-BR	UFA TALA-TALA	02	Cambium	Arbre défilé	vivant	01°38'16,3"	014°50'46,1"
5	PE-A06-2-BR	UFA TALA-TALA	02	Cambium	Arbre défilé	vivant	01°38'21,4"	014°50'47,8"
6								
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NB Voir explications sur le remplissage de ce formulaire en annexe A du protocole d'échantillonnage

Pays: République du Congo						Forêt/Région: UFA TALA-TALA		
Espèce: <i>Pericopsis elata</i> / <i>Athromesia</i>						Population N°: E		
Date de collecte: du 20 au 20 2014						Echantillonneur: NGONCOU YVES		
N°	N° échantillons	Nom du site	Nom/N° du bloc	Type échantillon	Spécimen	Matériel vivant/mort	Latitude	Longitude
1	PE-A02-2-NY	UFA TALA-TALA	01	Cambium	Arbre défilé	vivant	01°37'49,5"	014°50'34,7"
2	PE-A07-2-NY	UFA TALA-TALA	01	Cambium	Arbre défilé	vivant	01°37'55,9"	014°50'58,6"
3	PE-A08-2-NY	UFA TALA-TALA	01	Cambium	Arbre défilé	vivant	01°34'11,6"	015°10'11,1"
4	PE-A09-2-NY	UFA TALA-TALA	01	Cambium	Arbre défilé	vivant	01°44'35,7"	015°14'18,6"
5	PE-A10-2-NY	UFA TALA-TALA	01	Cambium	Arbre défilé	vivant	01°53'43,9"	015°16'56,4"
6	PE-A11-2-NY	UFA TALA-TALA	01	Cambium	Arbre défilé	vivant	01°53'41,9"	015°16'54,9"
7								
8								
9								
10								
11								
12								
13								

NB Voir explications sur le remplissage de ce formulaire en annexe A du protocole d'échantillonnage

Photos of collection in Cameroon

