INTERNATIONAL TROPICAL TIMBER ORGANIZATION (ITTO)
THEMATIC PROGRAMME ON
TRADE AND MARKET TRANSPARENCY (TMT)

TITLE	ESTABLISHMENT OF A FULLY DOCUMENTED REFERENCE SAMPLE COLLECTION AND IDENTIFICATION SYSTEM FOR ALL CITES-LISTED *DALBERGIA* SPECIES AND A FEASIBILITY STUDY FOR *DIOSPYROS* AND LOOK-ALIKE SPECIES
SERIAL NUMBER	TMT-SPD 022/15 (I)
COMMITTEE	Thematic Programme on Trade and Market Transparency (TMT)
SUBMITTED BY	Institute of Integrative Biology (IBZ), ETH Zurich
LANGUAGE	English

Summary

With increasing international demand for precious tropical timber and the difficulties to prevent illegal logging, pressure on source countries is rising to ensure legal and sustainable timber trade. In order to help source countries with trade control, the parties of the Convention of International Trade in Endangered Species (CITES) agreed on listing all *Dalbergia* and *Diospyros spp*. populations from Madagascar on Appendix II. The listing of these species is helping CITES authorities and other involved institutions to better control the trade of wood products derived from these precious timbers on the international market and is an important step towards a sustainable use of these species. Nonetheless, to enforce CITES regulations, reliable and fast identification techniques for logs and wood products are needed.

The main objectives of the here proposed project are to 1) establish a fully documented reference sample collection for all *Dalbergia* species currently listed in CITES Appendix I-III and 2) to finalize identification systems that are being developed based on DNA analysis and wood anatomy for all *Dalbergia* species currently listed in CITES. Experience gained from *Dalbergia* will then be used to 3) assess the feasibility of developing an identification system for the species-rich ebony genus *Diospyros* and 4) to assess the feasibility of including look-alike species of both taxa. Reaching these goals will help Madagascar with the implementation of the Action plan for *Dalbergia* and *Diospyros* agreed at the CoP16.

EXECUTING AGENCY	Plant Ecological Genetics (PEG), Institute of Integrative Biology (IBZ), ETH Zurich, Switzerland
COLLABORATING AGENCIES	University of Antananarivo, Department of Plant Biology and Ecology (DBEV) - Madagascar Consejo Nacional de Áreas Protegidas (CONAP) – Guatemala
SUPPORTING AGENCIES	Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Switzerland Missouri Botanical Garden (MBG), U.S.A.
DURATION	13 months
APPROXIMATE STARTING DATE	May 2015

BUDGET AND PROPOSED SOURCES OF FINANCE	SOURCE	CONTRIBUTION IN US$
	ITTO	200,000
	ETH	20,000
	TOTAL	**220,000**

PART I: CONTEXT

Origin/Background

Madagascar is one of the world's leading biodiversity hotspots (Myers et al., 2000) and the Malagasy forests harbor a vast diversity of precious woods (Randriamalala and Liu, 2010). When sold on the international market, timber from Madagascar is highly sought after and achieves high commercial values (Barrett et al., 2010). The ever increasing demand for precious timber on the international market has led to a massive increase of illegal exploitation of rosewood, palisander and ebony species in Madagascar in recent years (Ballet et al., 2011). Controlling the international trade with illegally logged timber from Madagascar is currently not feasible, because species identification and provenance assignment are not possible from logs (where morphological traits used in species identification such as flowers, bark and fruits have been removed) and reliable tools for species and provenance identification remain to be improved and validated. These limitations facilitate the illegal export of precious timber from Madagascar under names of legally traded timber species. Consequently, measures are needed to stop illegal logging and to protect the species from extinction.

As a first step towards trade regulation of *Dalbergia* and *Diospyros*, some of the most widely exploited species have been listed in CITES Appendix I (*D. nigra*), Appendix II (*D. cochinchinensis, D. granadillo, D. retusa and D. stevensonii*) or Appendix III (*D. darienensis, D. tucurensis*). The *Dalbergia* populations from Madagascar have been included in Appendix II at the most recent CoP16. Guatemala has requested the listing of two more species of that genus in Appendix III and further countries will most likely propose to list endangered *Dalbergia* species. It is now the task of all countries with natural occurrences of listed *Dalbergia* and *Diospyros* species to establish a chain of custody to ensure legal and sustainable international trade. To develop this chain of custody, key partners including the CITES Secretariat, the CITES Plants Committee, the CITES and the ITTO member countries, the main importing countries, as well as national and international research and conservation organizations should be included.

To facilitate adequate implementation of regulations for CITES listed *Dalbergia* and *Diospyros spp*., identification tools need to be developed and implemented. The here proposed project builds on the knowledge of two ongoing research projects: a) Sonja Hassold's PhD project that develops molecular identification tools for *Dalbergia spp.* from Madagascar, and b) Dr. Harisoa Ravaomanalina's development of a wood anatomy atlas for *Dalbergia* and *Diospyros spp*. from Madagascar. In the course of these projects it was found that a reference collection with well documented and identified specimens is needed before identification systems can be fully developed and validated. The establishment of such a reference collection for all CITES-listed *Dalbergia* species is therefore a central element of the here-proposed project. Samples from this reference collection will be sent to collaborating laboratories around the world that are using complementary approaches (e.g. isotope and chemical analyses) with the goal to establish a joint dataset to evaluate the most efficient and powerful identification methods for *Dalbergia* species. Experience gained from *Dalbergia* will then be used to assess the feasibility of developing an identification system for Malagasy ebony (*Diospyros*), because the genus *Diospyros* in Madagascar is highly diverse (an ongoing taxonomic revision of Malagasy *Diospyros* by P.P. Lowry II and G. Schatz revealed the existence of 113 species that are

new to science) and therefore identification methods suitable for *Dalbergia* may not be suitable for *Diospyros*. Further, we will assess the possibility of including look-alike species of *Dalbergia* and *Diospyros* into a future identification system for both genera.

PART II: THE PROJECT

1. Project Objectives

The main goal of this project is to strengthen capacities of the CITES authorities to implement the convention for all species of the genera *Dalbergia* and *Diospyros* that are listed in CITES Appendix I-III and their look-alike species.

Therefore the main objectives intended to complete during this project are:

1) To establish a fully documented reference sample collection for all *Dalbergia* species currently listed in CITES Appendix I-III that is suitable for establishing and validating different identification systems, including DNA and isotope analyses, wood anatomy, and mass spectrometry
2) To finalize identification systems that are being developed based on DNA analysis (Sonja Hassold) and wood anatomy (Dr. Harisoa Ravaomanalina) for all *Dalbergia* species currently listed in CITES Appendix I to III
3) To assess - based on the available experience from *Dalbergia* - the feasibility of developing an identification system, with the same methods used for *Dalbergia*, for the species-rich ebony genus *Diospyros*. *Diospyros* from Madagascar (currently listed in CITES Appendix II) will be used as a case study
4) To evaluate whether it will be possible to efficiently include look-alike species of *Dalbergia* and *Diospyros* into a future identification system for both genera

Justification

The precious woods of Madagascar are highly threatened as a consequence of massive illegal exploitation, forest clearing, and slash-and-burn agriculture (Patel, 2007; Styger et al., 2007). High demand for these timbers on the international market drives illegal logging in Madagascar (Barrett et al., 2010). It is to date impossible to identify logs to species level and to identify the provenance of timber that appears on the international market, which makes it impossible for customs authorities to distinguish legally from illegally harvested logs. Suitable identification tools are therefore needed to provide traceability and evaluate legality and thus to support the conservation of the species (Degen and Fladung, 2007; Novaes et al., 2009). A variety of strategies have been developed in recent years that help with species identification (Horacek et al., 2009; Lancaster and Espinoza, 2012; Lowe and Cross, 2011; Pastore et al., 2011).

In compliance with this project is the CITES listing in 2013 accompanied with an action plan, which includes providing identification material and tests for use in CITES enforcement when implementing the Convention for species of *Dalbergia* and *Diospyros* from Madagascar. The main goal in this project is to strengthen capacities in terms of developing identification methods, laboratory protocols and

guidelines for use by customs officers to apply timber identification techniques for all CITES listed species of these two genera.

Moreover, Guatemala, Madagascar and Asian range states are asked to facilitate access to voucher samples to support the development of identification techniques, tests and guidelines. Within this project we propose to establish a fully documented reference sample collection that is suitable for establishing and validating different identification systems, including DNA and isotope analyses, wood anatomy, and mass spectrometry for *Dalbergia*, and explore the feasibility of developing identification systems for *Diospyros* and look-alikes of both genera.

1.1 Problems to be addressed

In order to allow reliable assignment of logs to species and provenance, a high quality reference database is key. Such a database should ideally be based on reference samples for all species of *Dalbergia* and *Diospyros*. These reference samples encompass herbarium vouchers that include all traits (typically including flowers and fruits) required by taxonomists to identify the species and further include multiple samples from different tissue types that can be used to characterize the species. The biggest challenge for timber trade control nowadays is the lack of reliable species identification techniques for logs derived from CITES-listed species, as well as the possible confusion with look-alike species.

Without the development and validation of new identification techniques and joint efforts it will not be possible to distinguish legal from illegal logs. Currently, several research groups worldwide are using different techniques for species identification. These include i) wood anatomy (macroscopic and microscopic level), ii) molecular methods (DNA barcoding, genotyping), iii) stable isotopes, iv) mass spectrometry and v) automated imaging. A one-day workshop held in Hamburg (Germany, 13 June 2014) with members of various research groups revealed that a combination of different methods may be required for reliable species identification and provenance assignment. The establishment of a fully documented reference sample collection suitable for establishing and validating different identification systems will help us to establish a joint database with the ultimate goal to allow reliable species identification and provenance assignment.

1.2 Intended situation after project completion

We aim to achieve the following goals:

- Increased identification capacities of CITES listed *Dalbergia* species
- Availability and applicability of at least two timber identification methods (wood anatomy and DNA analysis) that can used alone or in combination for the identification of CITES-listed *Dalbergia* species
- Improved research co-operations among laboratories worldwide using complementary identification techniques
- The feasibility of developing a species-identification method for *Diospyros* species and look-alikes of *Dalbergia* and *Diospyros* spp. has been assessed

Benefits for other Parties of CITES dealing with tropical timbers:

- Improved capacity to manage and regulate trade in listed *Dalbergia* species
- Increased capacity to implement CITES legislation because of developed guidelines and protocols
- Availability of a fully documented reference sample collection for all CITES listed *Dalbergia* species
- Start to build up a fully documented reference sample collection for CITES listed *Diospyros* and look-alike species of *Dalbergia* and *Diospyros* spp.

1.3 Target beneficiaries

All countries trading with tropical timber and timber products will benefit from the implementation of this project.

The main beneficiaries in Guatemala and in Madagascar, at the national level, will be: a) scientific authorities and customs officers because of available identification techniques to control trade in *Dalbergia* species; b) the Guatemalan and Malagasy governments because they will have a better understanding of how many species are of economic interest and how trade control can be improved to ensure sustainability and conservation of these species; c) stakeholders, the forest departments, universities and exporters who can make use of the developed fully documented reference database to control shipments, to teach students how to apply the techniques, to rise awareness and to conserve the species.

The beneficiaries at the international level will be: a) customs officers and other control bodies because of available identification techniques to control trade in *Dalbergia* species (logs and eventually also processed wood and wood products), b) wood traders to ensure legality of sold timber, c) organizations working on forest related matters, d) CITES management authorities issuing export and import permits, e) CITES scientific authorities improving decision making about forest management systems, traceability and ensuring sustainability in trade.

1.4 Risks

From the two previous projects of Sonja Hassold and Dr. Harisoa Ravaomanalina we know that obtaining permits for sampling and collecting fertile voucher material for the reference database of the species in Madagascar can be difficult and time consuming, but is key for a successful development of identification tools. The advantage of this proposal is the available experience and knowledge from the two previous studies concerning sampling strategy and sampling sites. Further sampling will be less difficult because of well-chosen sites and available knowledge of species occurrence. In addition, we will visit well-known sites repetitively in order to collect fertile material. With this strategy we aim to overcome the problem of lacking well-identified reference samples. With our newly developed sampling protocol we will be able to collect samples that are also of use to other research groups with other sample needs. Moreover, the sample size per species is of great importance, in order to assess the level of variation in the chosen traits (morphological, chemical or molecular) within species.

Limits: In order to collect fertile material the sampling should take place in the rainy season, but in this season the risk of cyclones and floods is high, which may negatively influence the sampling success. Good planning beforehand and some flexibility in time are necessary to use the best collecting times. The scarcity of some hardwood species may become a limiting factor during sample collection, as it is important to have many samples from the same species to test the reliability of the identification techniques and to capture genetic variation.

Furthermore, illegal logging of precious hardwood nowadays occurs mostly inside protected areas in Madagascar. Consequently, access to sampling sites may not always be possible due to safety concerns, but good planning and strong connections with park authorities will help identifying safe sampling sites.

2. Outputs

The expected outputs of the project are:

Objective 1: **To establish a fully documented reference sample collection for all *Dalbergia* species currently listed in CITES Appendix I-III that is suitable for establishing and validating different identification systems, including DNA and isotope analyses, wood anatomy, and mass spectrometry**

Output 1.1: Availability of a fully documented reference collection for all CITES listed *Dalbergia* spp.

Output 1.2: Availability of a fully documented reference collection for a subset of CITES listed *Diospyros* and look-alike species from Madagascar, Guatemala and from as many countries with natural occurrences of these species as possible.

Objective 2: **To finalize identification systems that are being developed based on DNA analysis (Sonja Hassold) and wood anatomy (Dr. Harisoa Ravaomanalina) for all *Dalbergia* species currently listed in CITES Appendix I to III**

Output 2.1: Availability of a molecular identification method to identify CITES listed *Dalbergia* species

Output 2.2: Validated wood anatomy atlas that incorporates variation among individuals of the same species

Output 2.3 Generating scientific expertise for the identification of CITES-listed *Dalbergia* species

Objective 3: **To assess - based on the available experience from *Dalbergia* – the feasibility of developing an identification system, with the same methods used for *Dalbergia*, for the species-rich ebony genus. *Diospyros* from Madagascar (currently listed in CITES Appendix II) will be used as a case study**

Output 3.1: Available case study for developing an identification method for CITES listed *Diospyros* species

<u>Objective 4:</u> **To evaluate whether it will be possible to efficiently include look-alike species of *Dalbergia* and *Diospyros* into a future identification system for both genera**

Output 4.1: Available feasibility study for developing an identification method for look-alikes of *Dalbergia* and *Diospyros* spp.

3. Activities

Output 1.1: Availability of a fully documented reference collection for all CITES listed *Dalbergia* spp.

Activity 1.1.0 Prepare sampling strategy and apply for collection permits for Madagascar and Guatemala

Activity 1.1.1 Coordination of sampling strategy in Madagascar and Guatemala

Activity 1.1.2 Field campaign for sample collection in Madagascar and Guatemala

Activity 1.1.3 Identification of the collected samples by taxonomic specialists

Activity 1.1.4 Ask for exportation permits for the collected samples

Activity 1.1.5 Shipment of samples to collaborating laboratories

Output 1.2: Availability of a fully documented reference collection for a subset of CITES listed *Diospyros* and look-alike species from Madagascar, Guatemala and from as many countries with natural occurrences of these species as possible

Activity 1.2.0 Prepare sampling strategy and apply for collection permits for Madagascar and Guatemala

Activity 1.2.1 Coordination of sampling strategy in Madagascar and Guatemala

Activity 1.2.2 Field collection of *Dalbergia, Diospyros* and look-alike species in Madagascar and Guatemala

Activity 1.2.3 Collaboration with other countries with natural occurrences of the species to expand the reference collection

Activity 1.2.4 Identification of the collected samples by taxonomic specialists

Activity 1.2.5 Ask for exportation permits for the collected samples

Activity 1.2.6 Shipment of samples to collaborating laboratories for analysis

Output 2.1: Availability of a molecular identification method to identify CITES listed *Dalbergia* species

Activity 2.1.0: Genotype all CITES listed *Dalbergia spp.*

Activity 2.1.1: Develop guidelines to conduct molecular analysis (towards an applicable method)

Output 2.2: Validated wood anatomy atlas that incorporates variation among individuals of the same species

Activity 2.2.0 Complete the lab work for the wood anatomy atlas including more individuals of the same species

Activity 2.2.1 Complete the new catalogue for wood anatomical properties for use by customs officers

Output 2.3: Generating Scientific expertise for the identification of CITES-listed *Dalbergia* species

Activity 2.3.0: Verify the reliability of the reference database for identification of target species

Activity 2.3.1: Develop guidelines to implement identification techniques

Output 3.1: Available case study for developing an identification method for CITES-listed *Diospyros* species.

Activity 3.1.0: Perform experiments to check if the same protocols used for *Dalbergia* can be used for CITES-listed *Diospyros* species.

Activity 3.1.1: Start to genotype well described and documented *Diospyros* species

Activity 3.1.2: Modify protocols and guidelines if necessary to conduct molecular analysis

Output 4.1: Available feasibility study for developing an identification method for look-alikes of *Dalbergia* and *Diospyros* spp.

Activity 4.1.0: Perform experiments to check if the same protocols used for *Dalbergia* can be used for look-alikes of *Dalbergia* and *Diospyros* spp.

Activity 4.1.1: Start to genotype well described and documented look-alike species of *Dalbergia* and *Diospyros*.

Activity 4.1.2: Modify protocols and guidelines if necessary to conduct molecular analysis

5. Work Plan

The Work Plan is presented in **Annex 1.**

6. Budget

6.1 Total Project Budget by Activity

		TOTAL
10.	**Project Personnel**	
	11. National Experts	
	12. National Consultants	32’600
	13. Other labor	
	14. Fellowships and Training	
	15. International Experts	
	16. International Consultants	77’000
	19. **Component Total**	109’600
20.	**Sub-contracts**	
	21. Sub-contract (with A)	
	22. Sub-contract (with B)	
	29. **Component Total**	
30.	**Duty Travel**	
	31. Daily Subsistence Allowance	3’000
	32. International Travel	5’000
	33. Transport Costs	2’000
	39. **Component Total**	10’000
40.	**Capital Items**	
	41. Premises	18’000
	42. Land	
	43. Vehicles	
	44. Capital Equipment	3’000
	49. **Component Total**	21’000
50.	**Consumable Items**	
	51. Raw materials (Lab materials)	60’000
	52. Spares	
	53. Fuel and Utilities	2’800
	54. Office Supplies	5’600
	59. **Component Total**	68’400
60.	**Miscellaneous**	
	61. Sundry	
	62. Auditing	2’000
	63. Monitoring	3,000
	64. Contingencies	6’000
	69. **Component Total**	11’000
70.	**Executing Agency Management Costs (6%)**	
	79. **Component Total**	
100.	**GRAND TOTAL**	220’000

6.2 Project Budget by Source

Budget Components	**Source**			
	ITTO	*Government*	*Other Source (ETH Zurich)*	**Total**
10. Project personnel	109'600			109'600
20. Sub-contracts				
30. Duty travel	10'000			10'000
40. Capital items	3'000		18'000	21'000
50. Consumable items	66'400		2'000	68'400
60. Miscellaneous	11'000			11'000
70. Executing Agency Management Costs				
Total	200'000		20'000	220'000

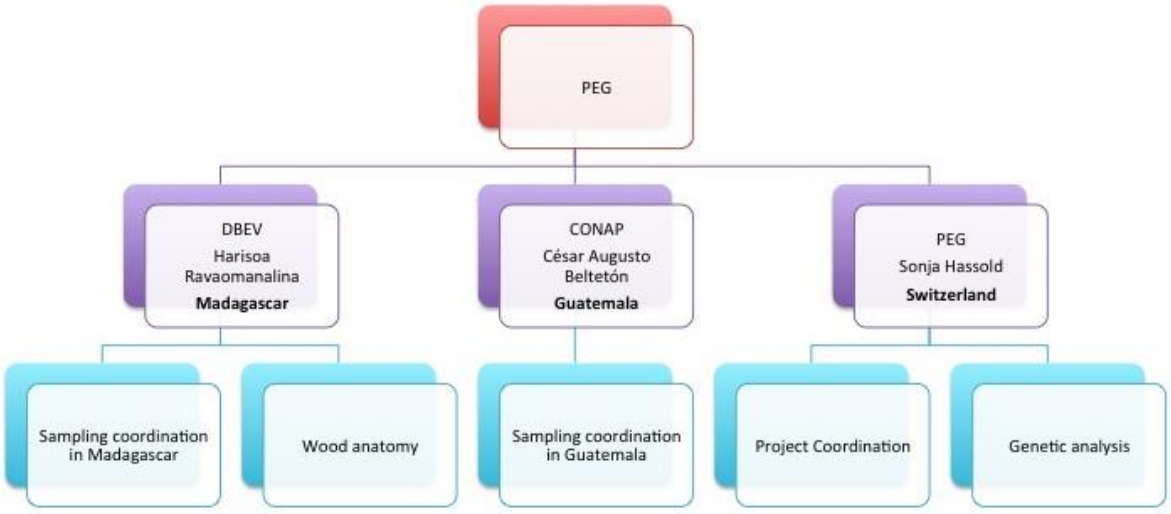
PART III: OPERATIONAL ARRANGEMENTS

1. Management Structure

(a) Blocks organized by different institutions:
- Sampling will be organized by Dr. Harisoa Ravaomanalina and carried out by MBG in Madagascar
- In Guatemala sampling will be organized by CONAP
- DNA analysis is carried out by Sonja Hassold at ETH Zurich, Switzerland
- Analysis of wood anatomy is carried out by Dr. Harisoa Ravaomanalina at DBEV in Antananarivo, Madagascar

(b) The overall organization stays with the PEG institution at ETH Zurich in Switzerland

(c) The organization chart:



2. Monitoring, Reporting and Evaluation

(a) *Project Progress Reports* – provide short three-monthly progress reports based on achievement of Project Outputs in the Work Plan.
(b) *Project Completion Report* – will be available by the end of 2015

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OUTPUTS/ACTIVITIES	RESPONSIBLE PARTY	SCHEDULE (in months) 2015-2016													
		8	9	10	11	12	1	2	3	4	5	6	7	8	9
Output 1.1: Availability of a fully documented reference collection for all CITES listed *Dalbergia* spp.															
Activity 1.1.0 Prepare sampling strategy and apply for collection permit for Madagascar and Guatemala	DBEV, CONAP, PEG														
Activity 1.1.1 Coordination of sampling strategy in Madagascar and Guatemala	DBEV, CONAP														
Activity 1.1.2 Field campaign for sample collection in Madagascar and Guatemala	DBEV, CONAP														
Activity 1.1.3 Identification of the collected samples by taxonomic specialists	DBEV, CONAP														
Activity 1.1.4 Ask for exportation permits for the collected samples	DBEV, CONAP														
Activity 1.1.5 Shipment of samples to collaborating laboratories	PEG, DBEV, CONAP														
Output 1.2: Availability of a fully documented reference collection for a subset of CITES listed *Diospyros* and look-alike species from Madagascar, Guatemala and from as many countries with natural occurrences of these species as possible															
Activity 1.2.0 Prepare sampling strategy and apply for collection permits for Madagascar and Guatemala	DBEV, CONAP, PEG														
Activity 1.2.1 Coordination of sampling strategy in Madagascar and Guatemala	DBEV, CONAP, PEG														
Activity 1.2.2 Field campaign for sample collection of *Dalbergia, Diospyros* and look-alike species in Madagascar and Guatemala	DBEV, CONAP, PEG														
Activity 1.2.3 Collaboration with other countries with natural occurrence of the species to expand the reference collection	PEG														
Activity 1.2.4 Identification of the collected samples by taxonomic specialists	DBEV, CONAP, PEG														
Activity 1.2.5 Ask for exportation permits for the collected samples	DBEV, CONAP														
Activity 1.2.6 Shipment of samples to collaborating laboratories for analysis	PEG, DBEV, CONAP														
Output 2.1 Availability of a molecular identification method to identify CITES-listed *Dalbergia* species															
Activity 2.1.0 Genotype all CITES-listed *Dalbergia spp.*	PEG														
Activity 2.1.1 Develop Guidelines to conduct molecular analysis (towards an applicable method)	PEG														
Output 2.2 Validated wood anatomy atlas that incorporates															

variation among individuals of the same species																
Activity 2.2.0 Complete the lab work for the wood anatomy atlas including more individuals of the same species	DBEV															
Activity 2.2.1 Complete the new catalogue for wood anatomical properties for use by customs officers	DBEV															
Output 2.3 Generating Scientific expertise for the identification of CITES-listed *Dalbergia* species																
Activity 2.3.0 Verify the reliability of the reference database target species	PEG, DBEV															
Activity 2.3.1 Develop guidelines to implement identification techniques	PEG, DBEV															
Output 3.1 Available case study for developing an identification method for CITES listed *Diospyros* species	DBEV, PEG															
Activity 3.1.0 Perform experiments to check if the same protocols used for *Dalbergia* can be used for CITES listed *Diospyros* species	DBEV, PEG															
Activity 3.1.1 Start to genotype well described and documented *Diospyros* species	PEG															
Activity 3.1.2 Modify protocols and guidelines if necessary to conduct molecular analysis	PEG															
Output 4.1 Available feasibility study for developing an identification method for look-alikes of *Dalbergia* and *Diospyros* spp.																
Activity 4.1.0 Perform experiments to check if the same protocols used for *Dalbergia* can be used for look-alikes of *Dalbergia* and *Diospyros* spp.	DBEV, PEG															
Activity 4.1.1 Start to genotype well described and documented look-alike species of *Dalbergia* and *Diospyros*	PEG															
Activity 4.1.2 Modify protocols and guidelines if necessary to conduct molecular analysis	DBEV, PEG															
Final report	PEG, DBEV, CONAP															