IPB University
Bogor Indonesia

Asia-Pacific Forestry Week 2019
Forests for Peace and Well-being
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Wood Identification Tool Development and Application:

Examples from Indonesia

Presented by

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Introduction

Progress – SFM Certification

State Forest Area

Natural Forest

123 MU PHPL certified
49 MU SLK certified
13.4 million ha

Plantation Forest

91 MU PHPL certified
37 MU SLK certified
6.8 million ha

State Owned Forestry Enterprise (Perum Perhutani)

3 MU
PHPL certified
2.4 million ha

Timber Utilization Permit (IPK)

113 units
SLK certified

MU = Management Unit

Private Forest

190 community groups
SLK certified
272.8 thousand ha

Indonesian LEGAL Wood

Industries

Primary Industry

1,291 units
SLK certified

Secondary Industry

1,530 units
SLK certified

Registered Shelter (TPT)

179 units
SLK certified

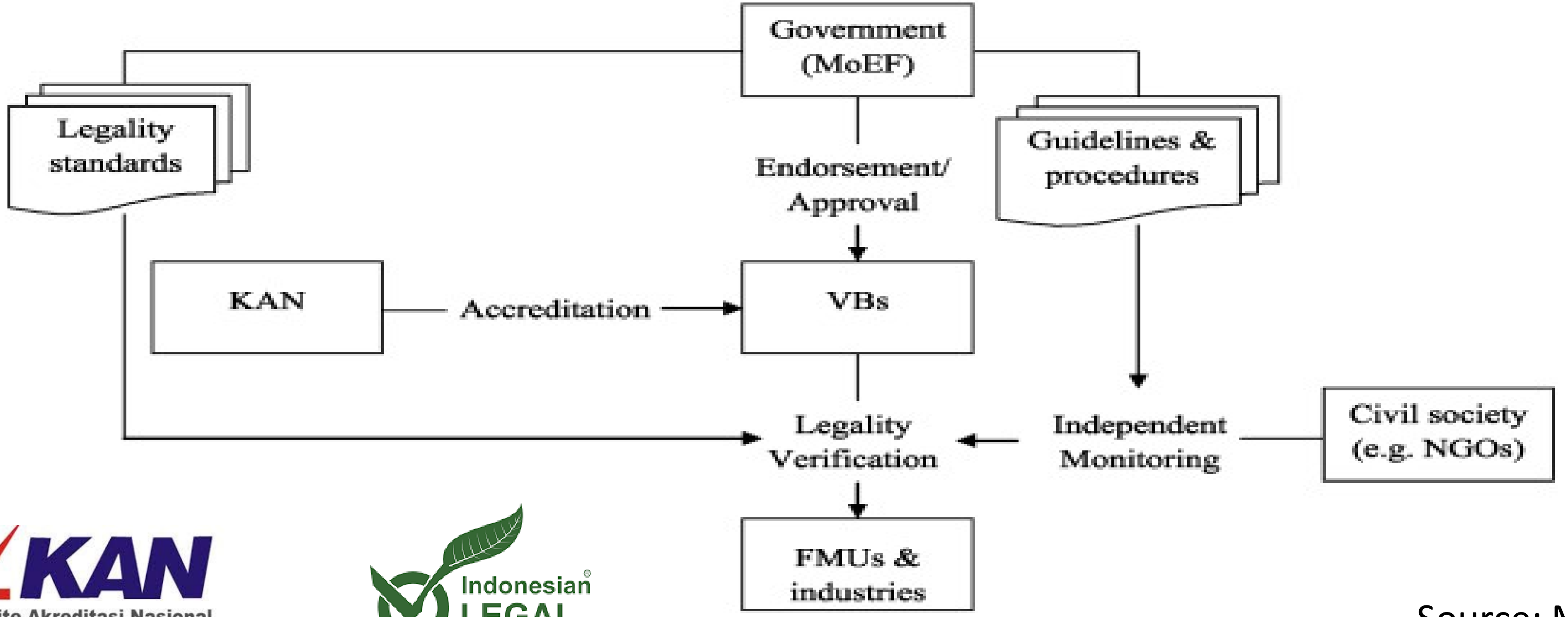
Exporter

101 units
SLK certified

Progress of PHPL (Management of Sustainable Production Forest) and SLK (Certification of Timber Legality) certification, as of December 2017 (DJPHPL-MoEF, 2018)

Introduction

Timber Legality System - SVLK



Komite Akreditasi Nasional
LPPHPL - 015 - IDN
LVLK - 019 - IDN

Indonesian LEGAL Wood

Source: Maryudi (2016)

Introduction

Tree Genetics and Wood DNA Studies in Indonesia

- Red Meranti
- Ironwood
- Ebony

- Population genetics
- DNA Barcodes

Technology for determining origin and species of timber??

Red Meranti & Ironwood Research

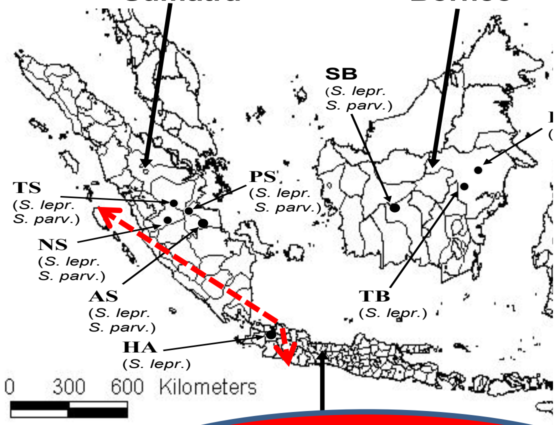
Population genetics, DNA Barcodes & Wood DNA

Results

Population Genetic Studies#1

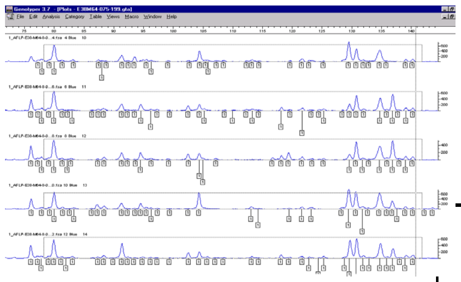
Red Meranti (*Shorea leprosula*)

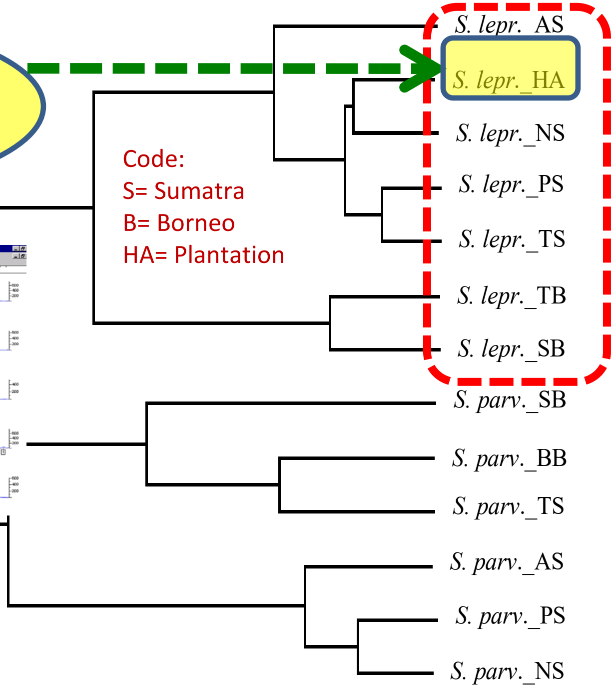
AFLP Markers



Transfer of materials (seeds): Siberut, Sukabumi

FOERDAI-Haurbentes Experimental Forest (He=0.186)





Code:
S= Sumatra
B= Borneo
HA= Plantation

AFLP Fragments

S. lepr= Shorea leprosula
S. parv=Shorea parvofolia

Table 2 Genetic diversity within populations of *S. leprosula* and *S. parvifolia*

Population	Sample size	Polymorphic loci	PPL (%)	n_a	n_e	H_e	H'_e	I
*S. lepr*_HA	34	34	60.71	1.607	1.318	0.186	0.204	0.280
*S. lepr*_TB	23	26	46.43	1.464	1.184	0.113	0.143	0.182
*S. lepr*_AS	9	37	66.07	1.661	1.355	0.208	0.258	0.316
*S. lepr*_PS	11	29	51.79	1.518	1.280	0.163	0.200	0.248
*S. lepr*_TS	22	28	50.00	1.500	1.281	0.160	0.177	0.238
*S. lepr*_NS	16	28	50.00	1.500	1.250	0.151	0.184	0.232
*S. lepr*_SB	18	27	48.21	1.482	1.249	0.145	0.169	0.221
Mean	19	30	53.32	1.533	1.274	0.161	0.191	0.245
Total	133	52	92.86	1.929	1.347	0.211	0.212	0.330
SD				0.260	0.352	0.184		0.251
*S. parv*_BB	16	19	33.93	1.339	1.201	0.115	0.154	0.171
*S. parv*_AS	33	43	76.79	1.768	1.371	0.222	0.238	0.337
*S. parv*_PS	23	33	58.93	1.589	1.228	0.143	0.167	0.228
*S. parv*_TS	14	21	37.50	1.375	1.159	0.097	0.137	0.152
*S. parv*_NS	28	28	50.00	1.500	1.189	0.119	0.143	0.188
*S. parv*_SB	21	30	53.57	1.536	1.211	0.135	0.161	0.214
Mean	22.5	29	51.79	1.518	1.227	0.138	0.167	0.215
Total	135	48	85.71	1.857	1.336	0.205	0.204	0.319
SD				0.353	0.353	0.185		0.256

PPL Percentage of phenotypically polymorphic loci, n_a observed number of alleles per locus, n_e effective number of alleles per locus, H_e Nei's (1973) gene diversity, *I* Shannon's information index (Lewontin 1972), *SD* standard deviation of total values H'_e Nei's gene diversity estimated with the computer program AFLP-SURV 1.0 (Vekemans 2002)

Center of genetic diversity for *S. leprosula* in ex Asia Log Concession (PT. REKI), Sumatra

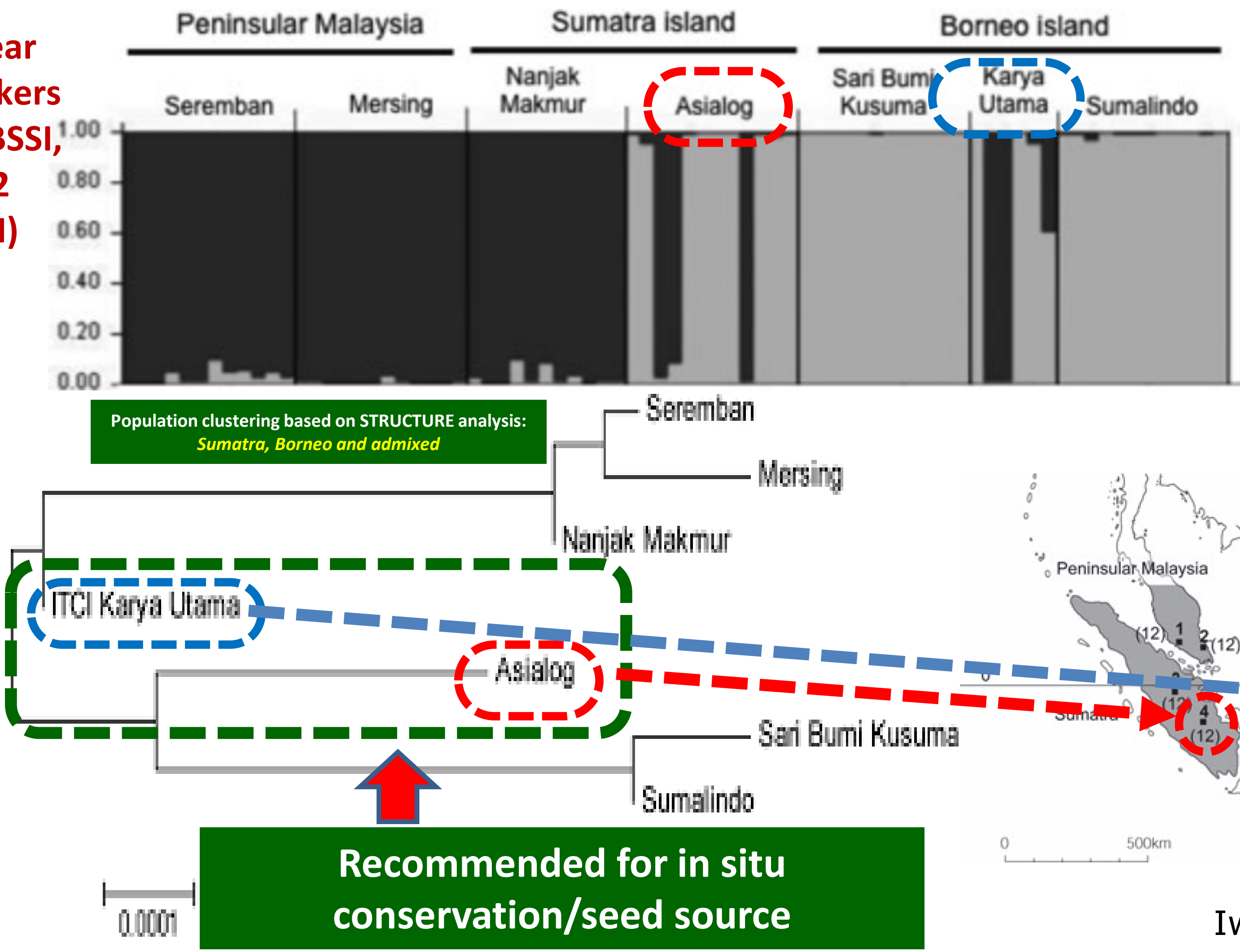
Cao *et al.* (2006)

Results

Population Genetic Studies#2

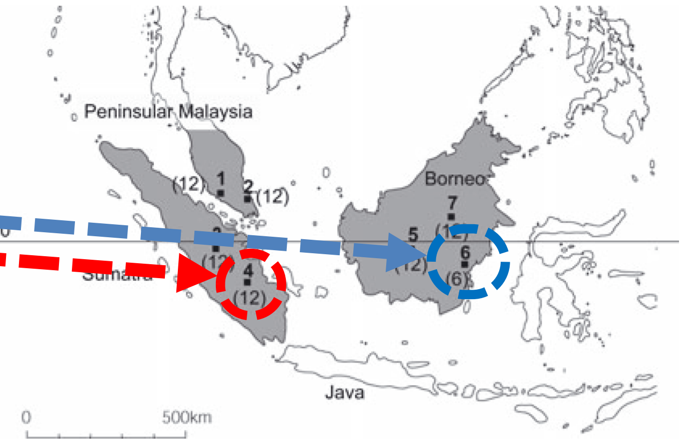
Shorea parvifolia

Five nuclear gene markers (GapC, GBSSI, PgiC, SBE2 and SODH)



Population clustering based on STRUCTURE analysis: ***Sumatra, Borneo and admixed***

Recommended for in situ conservation/seed source

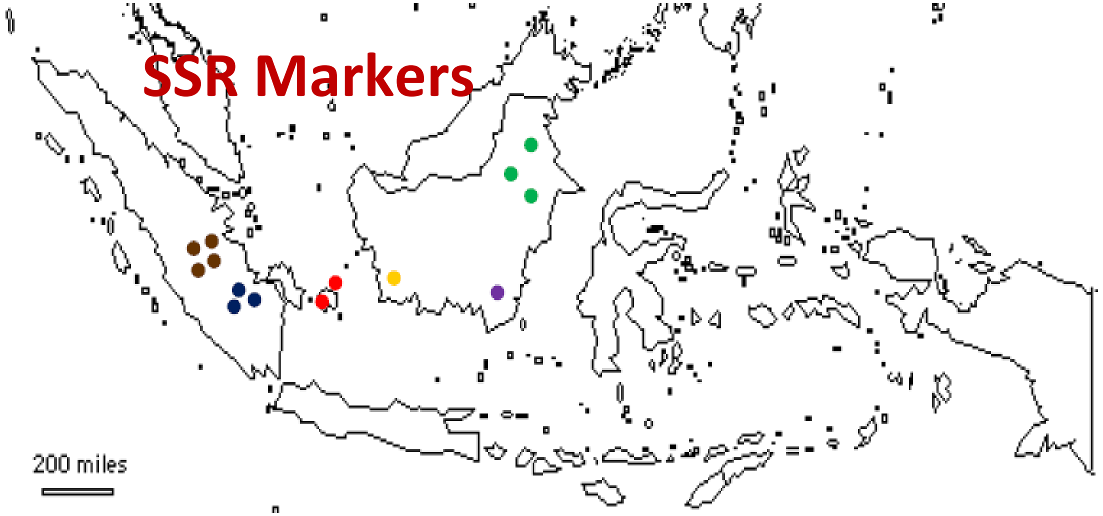


Iwanaga *et al.* (2012)

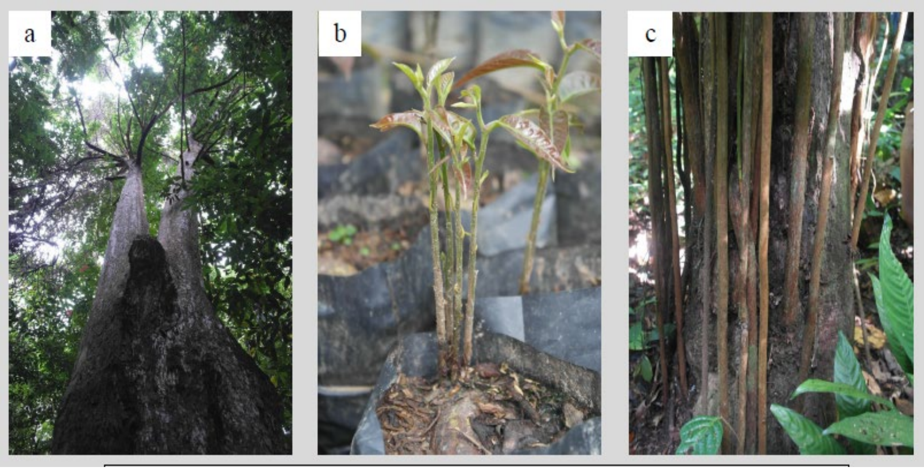
Results

Population Genetic Studies#3

Ironwood (Eusideroxylon zwageri)



Symbol	Provinces	No.population
●	Jambi	4 populations
●	South sumatra	3 populations
●	Belitung	2 populations
●	East Kalimantan	3 populations
●	West Kalimantan	1 population
●	South Kalimantan	1 population



Purba et al. (unpublished)

- Collection of leaves from mature trees and seedlings for DNA extraction throughout the natural distribution area in Indonesia (n= 1048 samples)
- Ten suitable genetic markers (SSR) have been developed
- Population structure of ironwood (*E. zwageri*) were determined by analyzing the level of genetic differentiation within and among populations

Results

DNA Barcodes

The objective is to generate DNA sequences of vascular plant species in Sumatra and combine it with morphological analysis in order to provide a reliable species identification tool for vascular plants in tropical forest

DNA barcode markers (matK and rncL); n= 5100 samples; > 1100 species

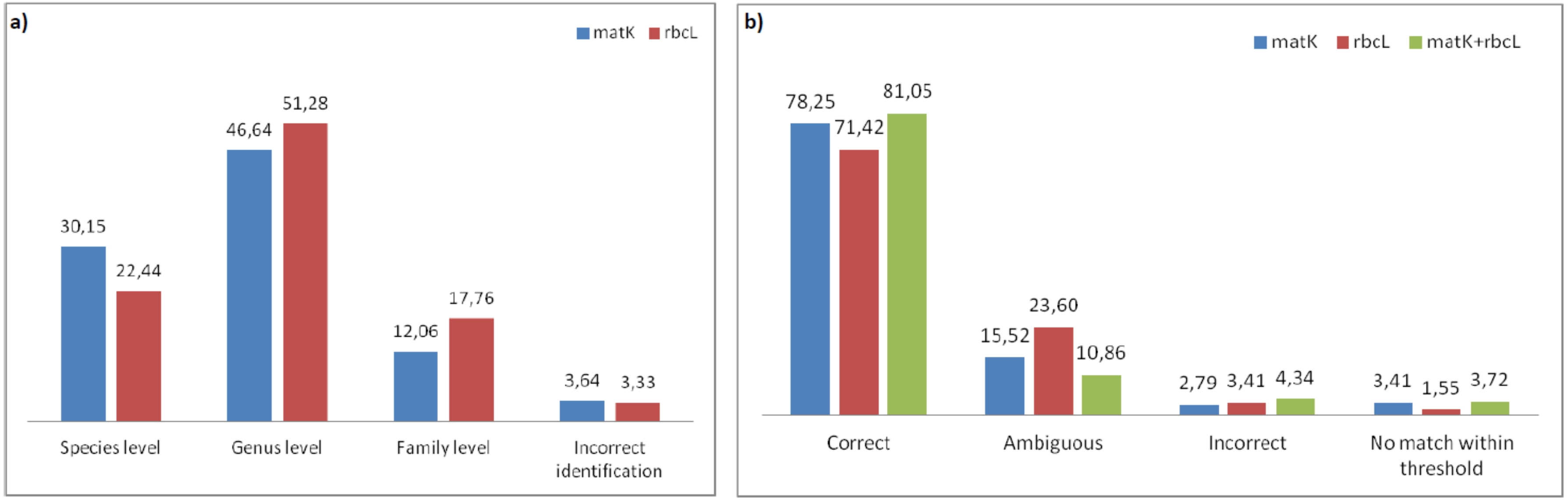


Fig 1. a) Level of match comparison between morphological identification and molecular identification (in percentage) with matK and rbcL as markers, b) Identification success of DNA barcodes (in percentage) using TaxonDNA with matK, rbcl, and combination of both markers

Amandita *et* al. (2019)

Results

DNA Isolation from Wood#1

Shorea spp

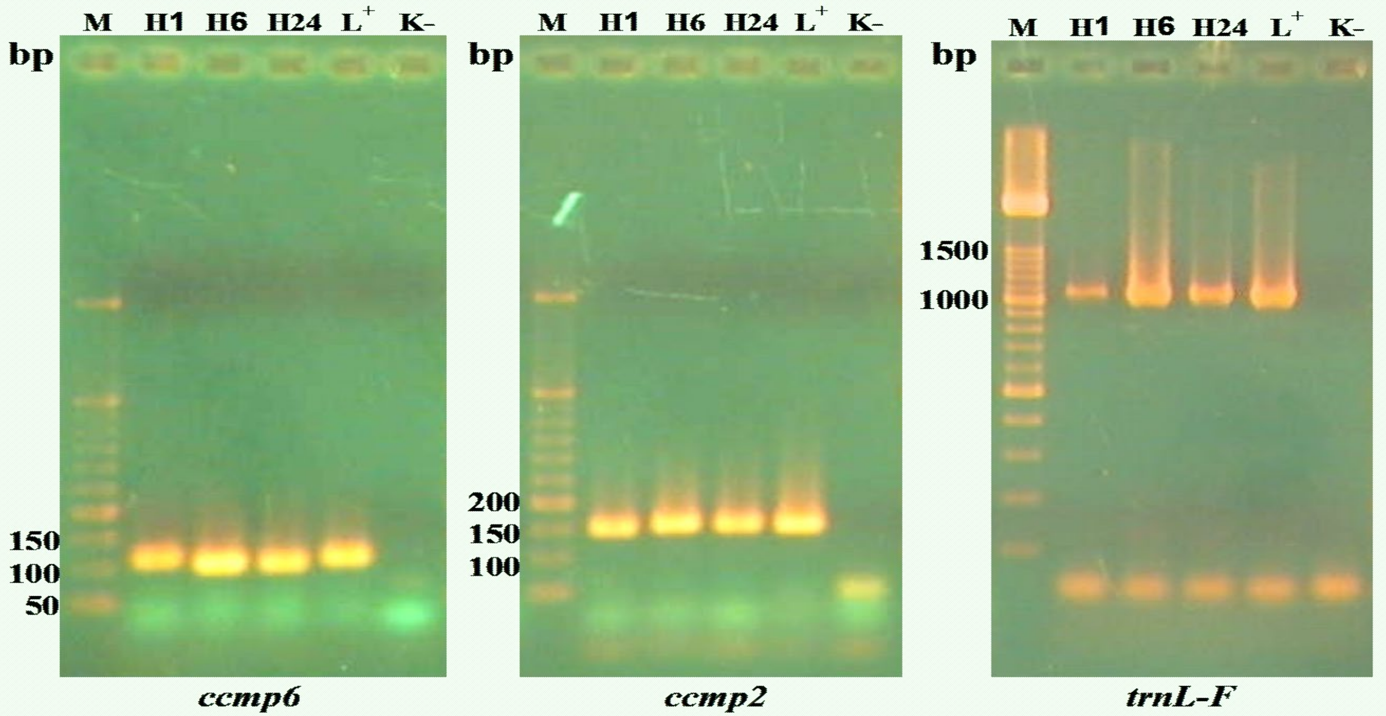


Figure 1. PCR profiles of the DNA samples amplified with primers *trnL-F* (*Taberlet et al., 1991*), *ccmp2* and *ccmp6* (*Weising and Gardner, 1999*).

Length of cpDNA fragment amplified by *ccmp6*, *ccmp2* and *trnL-F* was about 0.1, 0.15 and 1.1 kb, respectively. Samples on gel lanes are: M = Size standard; H1, H6 and H24 = wood DNA of Meranti (botanical name unknown), *Shorea leprosula* and *Shorea ovalis*, respectively; L^+ = Positive control (leaf DNA); K^- = Negative control (water).

Rachmayanti et al. (2006)

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Results

DNA Isolation from Wood#2

Comparison of Two DNA Isolation Methods on 49 Wood Species

Nama Sampel	Sampel K	Sampel S	No K	No S	Nama Sampel	Sampel K	Sampel S	No K	No S
Acacia mangium Willd.	+	+	1	19	*Vitex sp.*	+	-	26	-
Maesopsis eminii Engler	+	+	2	5	*Metrosideros petiolata* (Valeton) Koord.	+	-	27	-
Octomeles sumatrana Miq.	+	-	3	-	*Swietenia sp.*	+	+	28	4
Calophyllum macrocarpum Hook.f	+	-	4	-	*Dracontomelon dao* (Blanco) Merr. & Rolfe	+	-	29	-
Ochroma grandifloa Rowlee	+	-	5	-	*Intsia palembanica* Miq.	+	-	30	-
Pterospermum elongatum	+	-	6	-	*Parashorea tomentella*	+	+	31	18
Hopea laevifolia (Parijs) Endert	+	-	7	-	*Parashorea smythiesii* Wyatt-Smith ex P.S. Ashton	+	-	32	-
Cantleya corniculata (Becc.) R.A. Howard	+	-	8	-	*Pometia pinnata* J.R. Forster & G. Forster	+	+	33	15
Elmerrillia ovalis (Miq.) Dandy	+	-	9	-	*Melia azedarach* L.	+	+	34	9
Hopea sangal Korth	+	-	10	-	*Ganophyllum falcatum* Bl.	+	-	35	-
Durio zibethinus Murray	+	+	11	1	*Azadirachta indica* A. Juss.	+	-	36	-
Agathis sp.	+	+	12	17	*Cinnamomum parthenoxylon* (Jack.) Meisn	+	-	37	-
Diospyros pilosanthera Blanco	+	+	13	14	*Quercus sp.*	+	-	38	-
Gmelina arborea Roxb.	+	+	14	8	*Tristania maingayi* Duthie	+	-	39	-
Vernonia arborea Buch. -Ham.	+	-	15	-	*Alstonia sp.*	+	+	40	11
Tectona grandis L.f.	+	+	16	10	*Altingia excelsa* Noronha.	+	+	41	7
Cassia siamea Lamk.	+	-	17	-	*Grevillea robusta* A. Cunn. Ex R. Br.	+	-	42	-
Koompassia malaccensis ex Benth.	+	-	18	-	*Paraserianthes falcataria* (L.) I. C. Nielsen	+	+	43	2
Sandoricum koetjape (Burm. F.) Merr.	+	-	19	-	*Dalbergia latifolia* Roxb.	+	-	44	-
Dryobalanops aromatica C.F Gaertn	+	+	20	20	*Evodia aromatica* (Sonn.) Pers..	+	-	45	-
Aleurites moluccanus (L.) Willd.	+	-	21	-	*Toona sureni* (Blume.) Merr.	+	-	46	-
Khaya anthotheca (Welw) C. DC.	+	+	22	6	*Pinus merkusii* Junghuhn & de Vriese	+	+	47	13
Dipterocarpus confertus Slooten	+	+	23	16	*Teijsmanniodendron simplicifolium* Merr.	+	-	48	-
Terminalia bialata (Roxb.) Steud.	+	+	24	3	*Hibiscus tilliaceus* L.	+	-	49	-
Eucalyptus deglupta Blume	+	-	25	-	*Shorea parvifolia* Dyer	-	+	-	12

Keterangan: K = Kering, S = Segar

(Ramdhani & Siregar, 2017)

49 wood species

Methods	Wood sample types	DNA markers (barcodes)	Total samples	Amplification success (%)
CTAB	Dry	ITS	49	21 (43%)
		rbcL		10 (20%)
	Fresh	ITS	20	20 (100%)
		rbcL		20 (100%)
QDPMK (Qiagen)	Dry	ITS	49	23 (47%)
		rbcL		13 (27%)
	Fresh	ITS	20	19 (95%)
		rbcL		19 (95%)

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Short Communication:
DNA extraction from stored wood of *Falcataria moluccana* suitable for barcoding analysis

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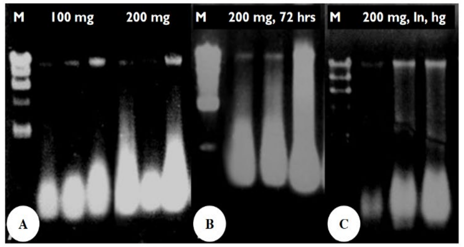


Figure 1. DNA extracted from A) 0.1 (lane 2-4) and 0.2 (lane 5-7) g tissue, frozen 24 hours, B) 0.1 g tissue, frozen 72 hours, and C) 0.2 g tissue with liquid nitrogen and grind using mortar. M: λ *Hind*III

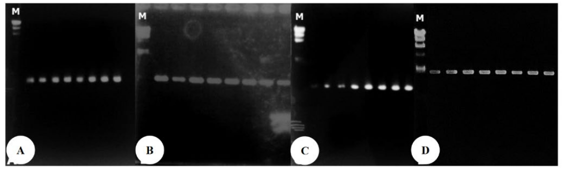


Figure 2. The amplification results using *psb*A-*trn*H intergenic spacer primer with DNA from previous extraction methods as template: A) 100 mg frozen for 24 hours in 30 °C; B) 200 mg frozen for 24 hours in 30 °C; C) 100 mg frozen for 72 hours in 30 °C; D) 200 mg frozen with liquid nitrogen; M: 1 kb DNA ladder.

Falcataria moluccana

Table 1 The nanophotometer result of DNA extracted from all methods used

Starting material	Freezing method	Disrupting method	Maximum DNA yield (ng/µL)	A_{260}/A_{280} ratio
100 mg	24 hrs; -30°C	TissueLyser	257.80	1.87-2.25
200 mg	24 hrs; -30°C	TissueLyser	140.45	1.89-2.86
100 mg	72 hrs; -30°C	TissueLyser	214.35	1.91-4.38
200 mg	Liquid nitrogen	Hand grinding	174.10	1.48-2.84

(Shabrina et al., 2019)

Summary

- Focus on populations of widespread species for **baseline genetic information** only (e.g. genetic structure, diversity) → genetic database development
- Application mostly for identifying **"center of genetic diversity"** → FGR conservation strategy
- Wood samples for DNA isolation is **not standard** (methods, size etc) → only for genetic analysis purpose not for others

Ebony Research in Indonesia

Ebony Research

Progress and Preliminary Results

Iskandar Z. Siregar, Fifi Gus Dwiyanti, Essy Harnelly, Muhammad Majiidu, Lina Karlinasari, Ratih Damayanti, M Rafi, Meaghan Parker-Forney

WORLD RESOURCES INSTITUTE

Introduction

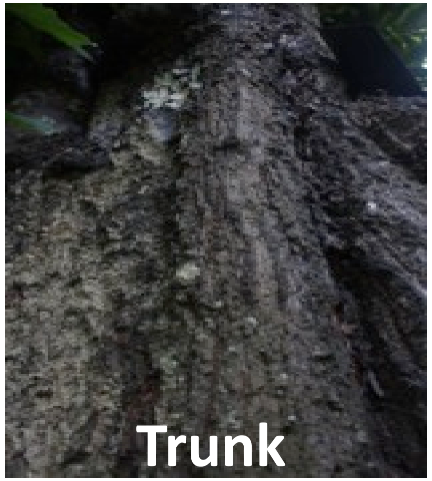
Timber trade of Ebenaceae → 23 commercial species → 6 luxury woods species → 1 endemic species

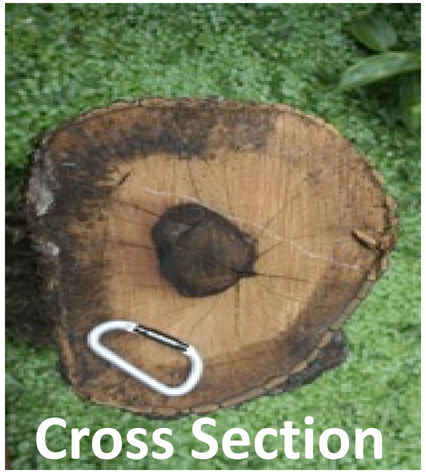
Diospyros celebica
(Ebony)

Djarwanto *et al.* 2017

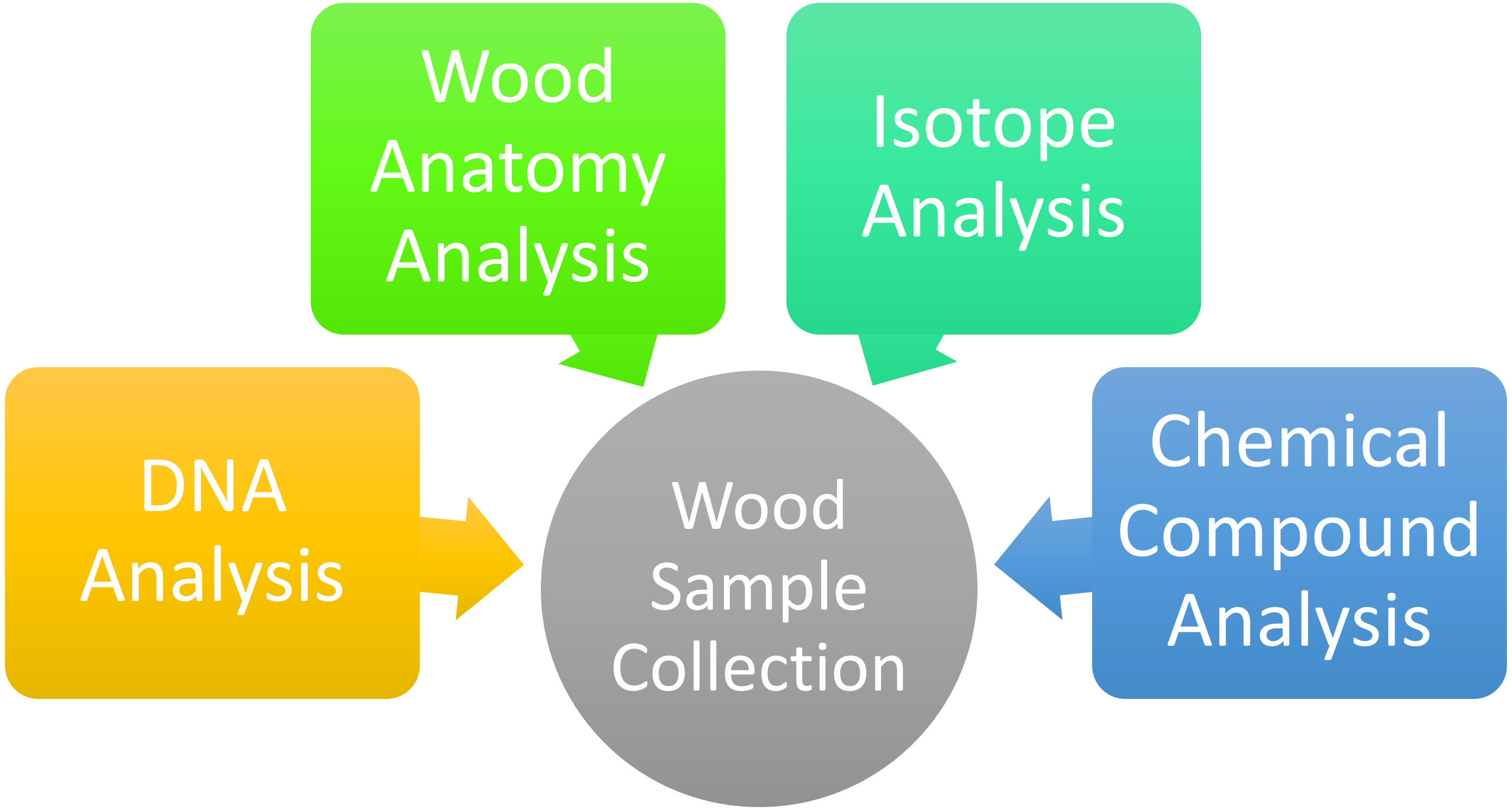
Introduction

- Endemic to Sulawesi Island (South Sulawesi, West Sulawesi, Central Sulawesi and North Sulawesi)
- Slow growing species
- Vulnerable species (IUCN 2019)
- Timber for carving, inlay, furniture and musical instruments
- Problem: **Illegal logging** → Wood identificatin tools?





Methods


Wood Anatomy Analysis
Isotope Analysis
DNA Analysis
Wood Sample Collection
Chemical Compound Analysis

Objectives

- **Main objective**: To setting up a reference data building pipeline for DNA of commercial timber species, *Diospyros celebica* Bakh. (Ebony)

- **Specific objectives**: To collect physical timber reference material and extract its associated DNA for the species *Diospyros celebica* Bakh. (Ebony).

Activity#1:

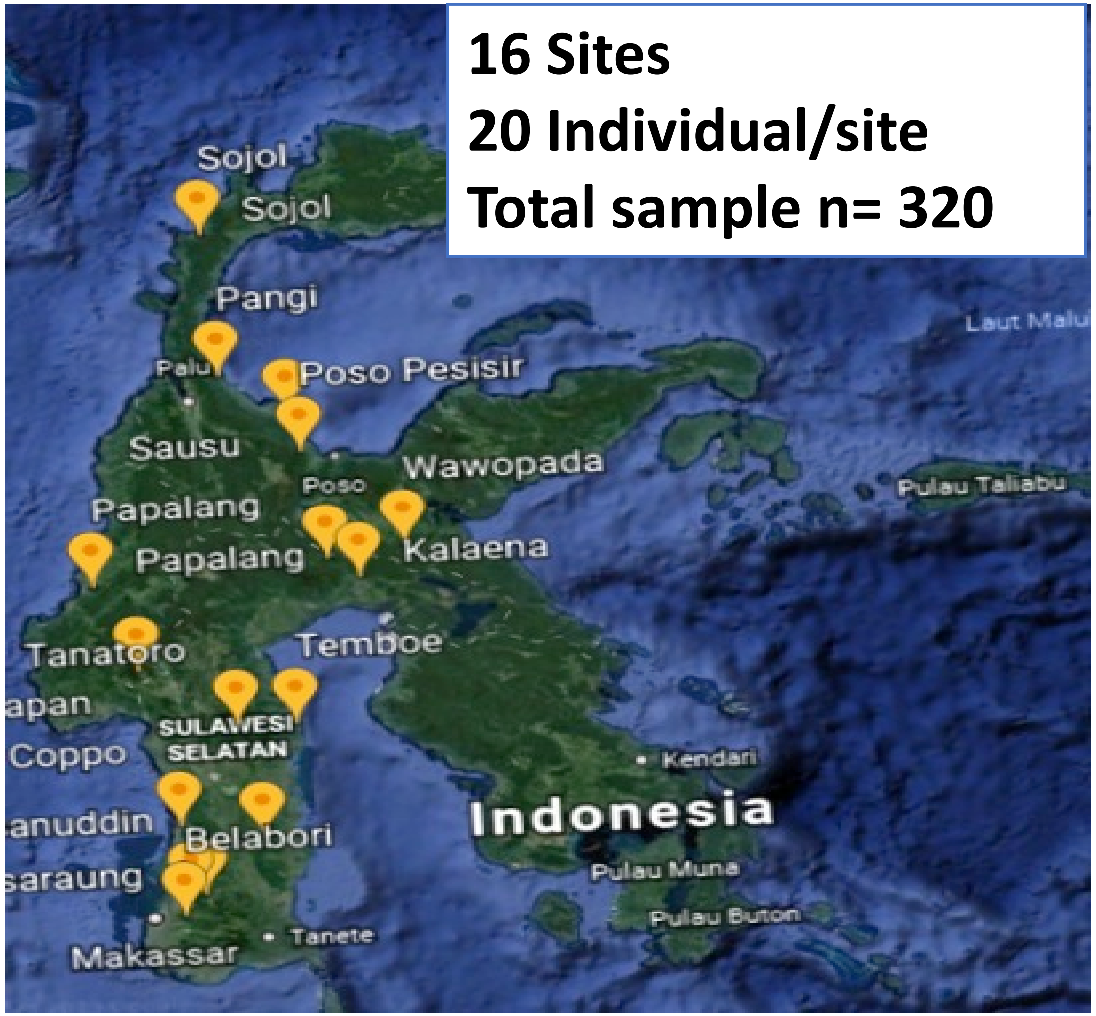
Wood Collection (Concentrated Species: *Diospyros celebica)* in Sulawesi

Objective

To update the current distribution of *Diospyros celebica* in Sulawesi including wood sample collection

D. celebica Wood Sampling Sites

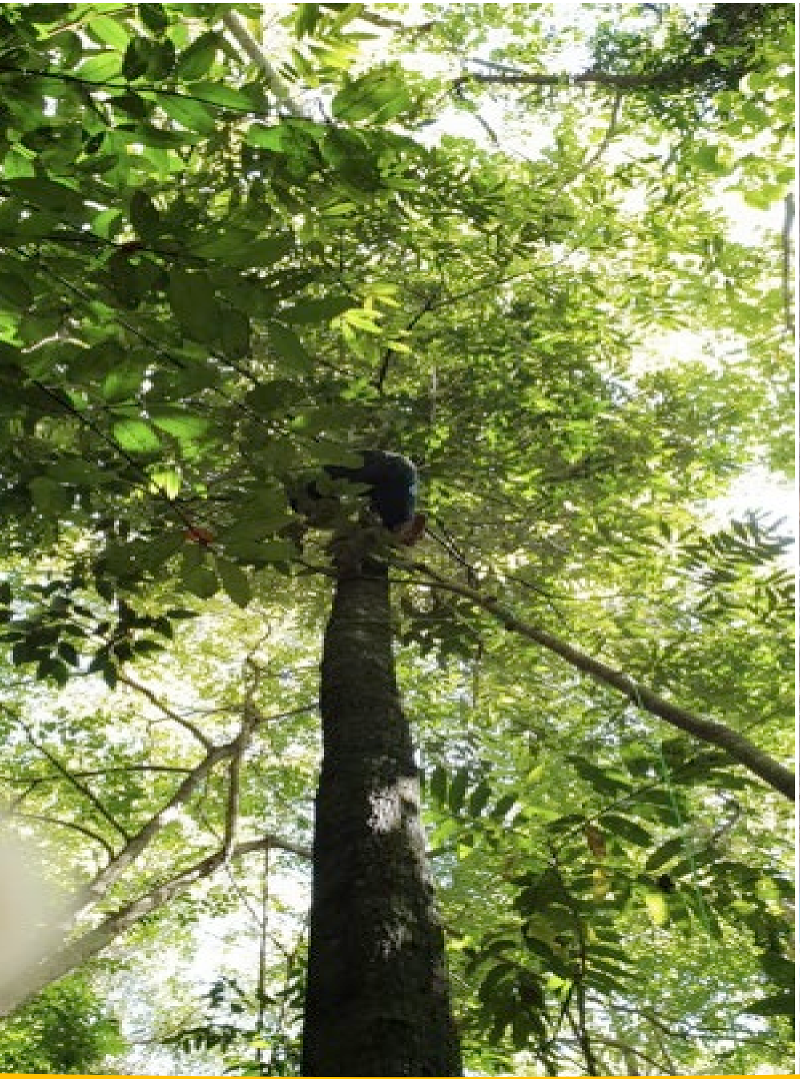
No	Province	Regency	Sub-district	Sampling Sites
1	South Sulawesi	Maros		Batimurung Bulusaraung National Park
				Hasanuddin University Teaching Forest
		Barru	Barru	Coppo Village
		Sidenreng Rappang (Sidrap)	Pituriase	Tana Toro Protection Forest
		Gowa	Parangleo	Bellabori
		Luwu Timur	Mangkutana	Ponda-Ponda Nature Reserve
			Mangkutana	Kalaena Nature Reserve
			Mangkutana	Mango Lembo Village
			Mangkutana	Pegunungan Faruhumpeni Nature Reserve
		Luwu	Larompong	Temboe Education Forest and Tourism
		Bone	Ulaweng	Cani Sirenreng Nature Park
2	West Sulawesi	Mamuju	Papalang	Batu Papang Village
			Papalang	Palado
3	Central Sulawesi	Marowali	Lembo	Wawopada Village
		Parigi Moutong	Sausu	Sausu Village
				Pangi Binangga Nature Reserve
		Poso	Poso Pesisir	Peawa Oti Montane Forest
		Donggala		Gunung Sojol Nature Reserve



16 Sites
20 Individual/site
Total sample n= 320

Field Work: GPS, Tree Measurement

Field Works: Herbarium Collection



Field Works: Leaf Collection

Field Works: Core Wood Collection

Field Works: Core Wood Collection

Activity#2:
DNA Extraction from Ebony (*Diospyros celebica* Bakh.) Dry Wood Samples Collected Using Pickering Punch

Objective

To develop the most efficient method of obtaining DNA from tissues of dry ebony wood

Materials & Methods

1. Core Wood Extraction from Tree Stand Using Pickering Punch (Agroisolab, UK)

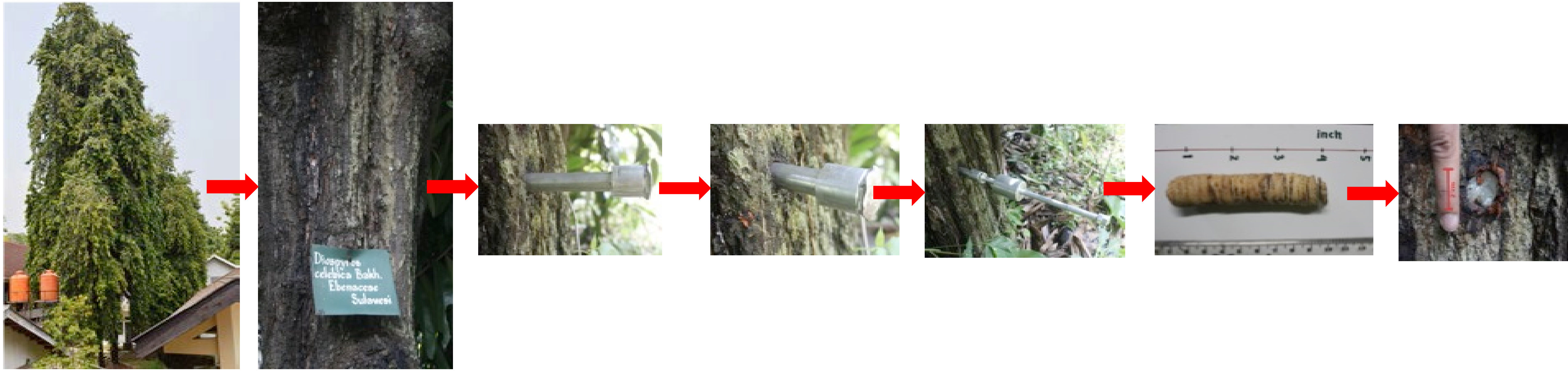


Figure 1. The workflow of ebony wood core extraction using Pickering Punch.

Materials & Methods

2. Genomics DNA Extraction from Dry Wood Using CTAB Method

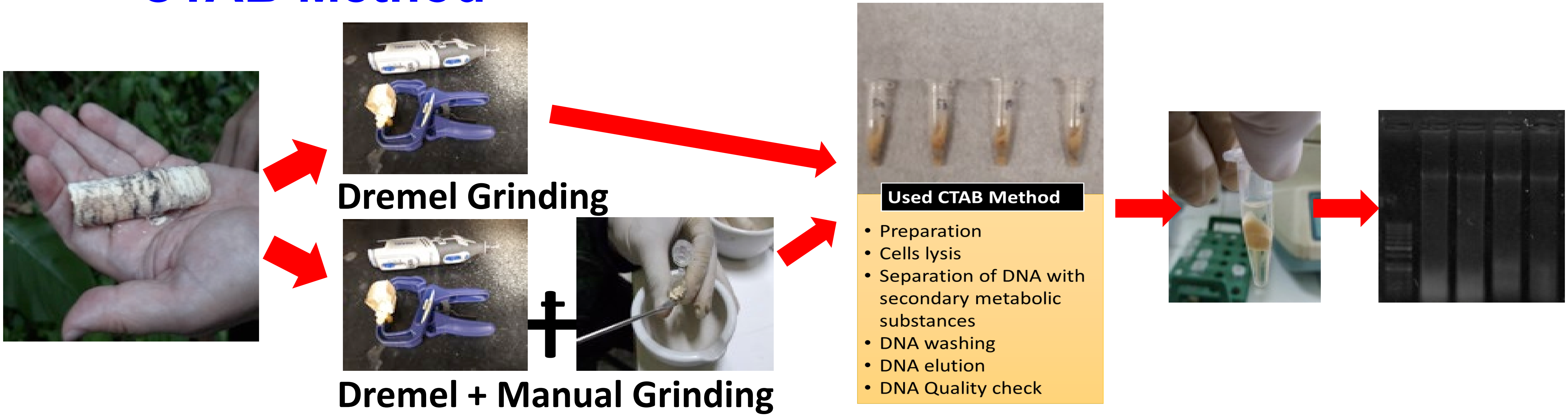


Figure 2. The workflow of ebony dry wood DNA extraction.

Results & Discussion

Table 1 Presents concentrations and quality of the obtained DNA using two different homogenization methods.

Tree No.	Homogenization Method	DNA Conc. (ng/µl)	Purity ($A_{260}/_{280}$)
1	Dremel	215.25	2.532
	Dremel+manual grinding	335.60	2.011
2	Dremel	166.50	2.595
	Dremel+manual grinding	242.75	2.104

Testing the use of Oxford Nanopore's MinION Sequencing Device (in collaboration with Prof Brook Milligan, NMSU)

Closing Remarks

- The **first collection** of samples has been carried out in Sulawesi comprising herbarium, leaves and core wood → Testing the pickering punch.
- Modification of combined the use of **“dremel + manual grinding”** can be recommended for extracting DNA from dry wood → Testing the use of MinION.
- **The core wood** can be used for various purposes → Testing other analyses (isotope, NIR/chemical compound and anatomy).

Thank you