Forest Governance and Policy 2024 Conference (FGP24)
Washington DC, April 29th – May 3rd, 2024

Development of Wood Identification Technologies: Updates from Indonesia

Presented by

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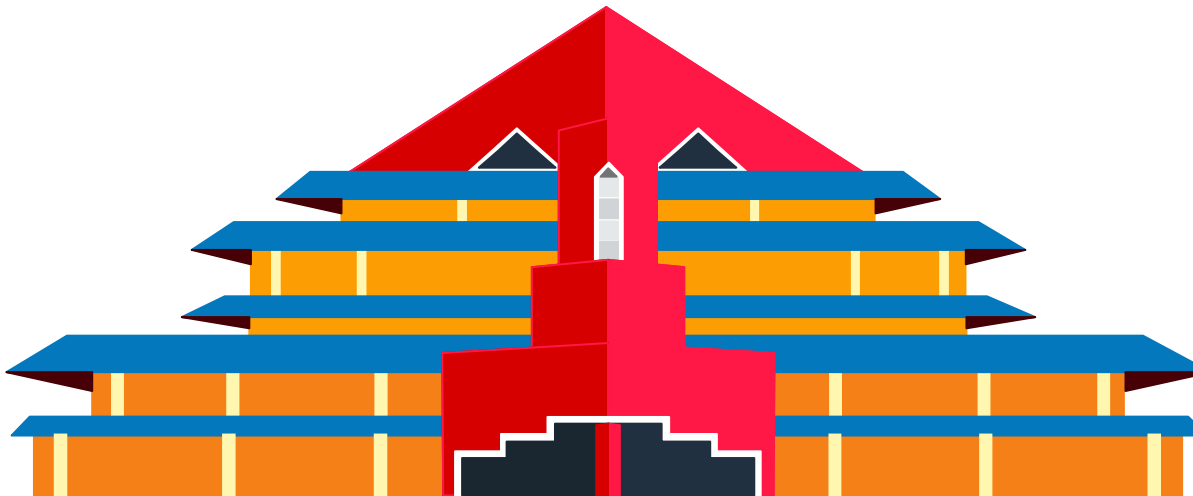

1. Content of Presentation

- Indonesian WoodID Team
- Priority Tree Species
- WoodID Projects in Indonesia
- Practical Examples:
  - Project#1: Shorea Project
  - Project#2: Ebony Project
  - Project#3: WoodID Project
- Key Takeaways

1. Indonesian WoodID Team

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Researcher/Anatomy

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1. Asia's valued trees under threat

- Across Asia, thousands of socio-economically important tree species are threatened. **These species (n=63)** and their genetic resources offer vital sources of income, food and environmental services.

- **Genetic diversity and origin** underlie the species' productivity and allow them to adapt to climate change and other potential threats.

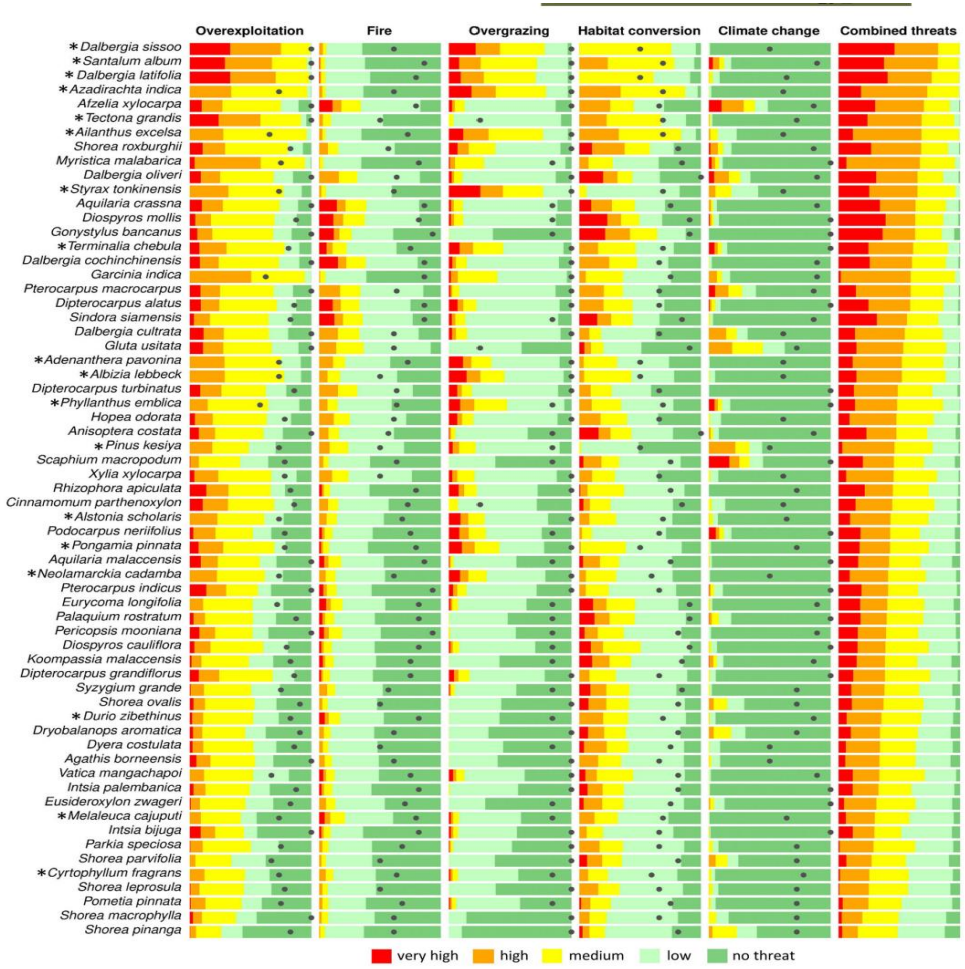
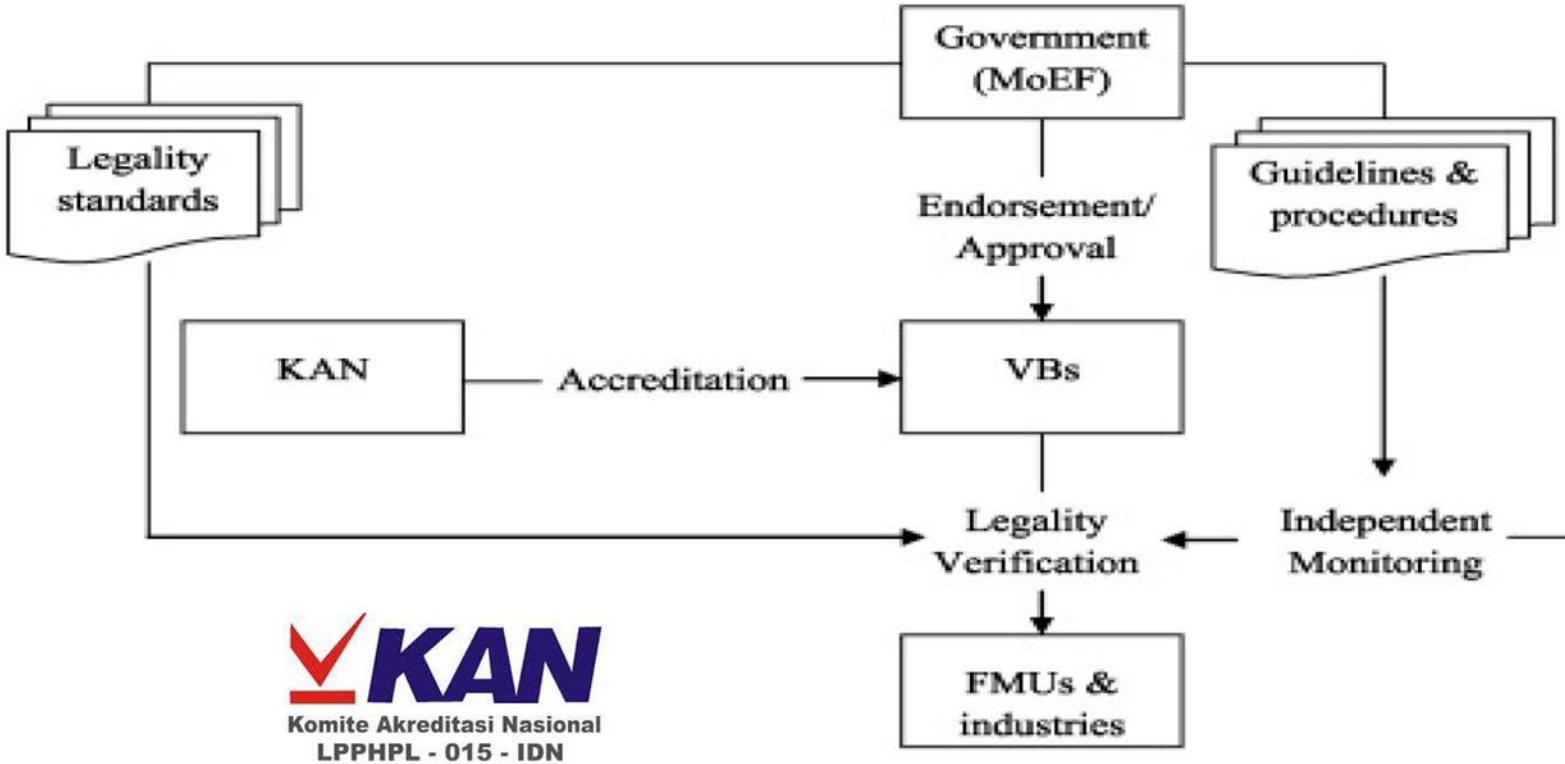


FIGURE 2 Threat sensitivity and vulnerability estimates for 63 tree species relative to five threats and the five threats combined (gray dots, sensitivity values; bars, relative share of distribution range of each species by level of threat [very high, high, medium, low, and no threat]; *, widely cultivated species). Species are in decreasing order of share of distribution range under high or very high vulnerability to combined threats

Gaisberger et al. (2021)

2. WoodID Project in Indonesia

Timber Legality System - SVLK

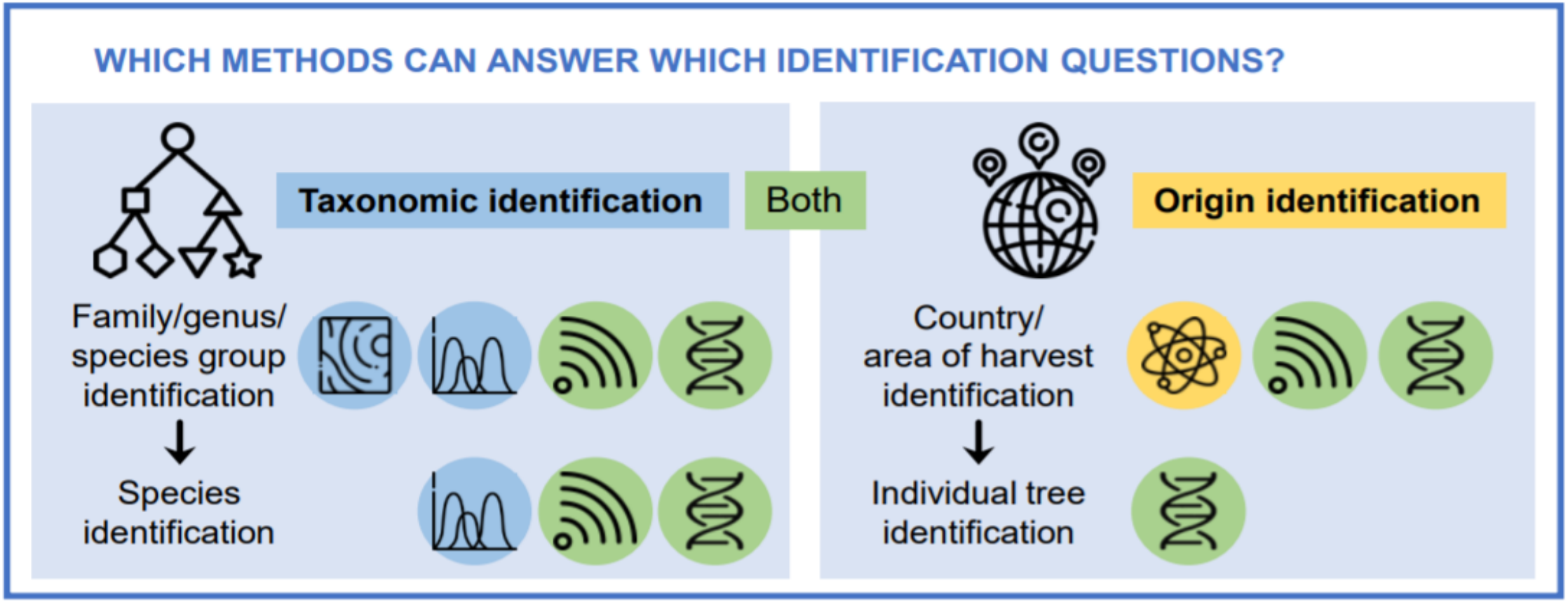


KAN
Komite Akreditasi Nasional
LPPHPL - 015 - IDN
LVLK - 019 - IDN

Source: Maryudi (2016)

2. WoodID Project in Indonesia

Anatomy | DART TOFMS | Stable isotopes | NIR Spectroscopy | Genetics



GTTN (2020)

3. Practical Examples

Genetics

Shorea Project

Genetic Variation of *Shorea* spp (Dipterocarpaceae) in Indonesia

(2002-2007)

Obtaining knowledge of phylogenetic relationships and the amount and spatial distribution of genetic diversity in order to provide information for the development of strategies for the conservation and sustainable utilization of dipterocarps.

NIR Spectroscopy

Genetics

Anatomy

LC-MSMS

Ebony Project

Reference database for Macassar Ebony (*Diospyros celebica*)

(2019-2020)

Setting up a reference data building pipeline for DNA of commercial timber species, *Diospyros celebica* Bakh (Macassar Ebony). Specifically, the project aimed to collect physical timber reference material and extract its associated DNA and other chemicals.

DART TOFMS

Genetics

Anatomy

Stable isotopes

WoodID Project

Indonesian-based wood identification program

(2020-2025)

Setting-up a reference data Building pipeline for physical, molecular, and chemical properties of Indonesian commercial timber species and its application for law enforcement, so that the results of this research can provide wood ID services for the Indonesian timber enforcement community

3.1. Shorea Project

DNA analysis from wood and wood products

Plant Molecular Biology Reporter **24:** 45–55, March 2006

Commentary

Extraction, Amplification and Characterization of Wood DNA from Dipterocarpaceae

YANTI RACHMAYANTI, LUDGER LEINEMANN, OLIVER GAILING and REINER FINKELDEY*
Institute of Forest Genetics and Forest Tree Breeding, Georg-August-Universität Göttingen, Büsgenweg 2, 37077 Göttingen, Germany

Abstract. A successful DNA extraction from wood yielding appropriate DNA quality for PCR amplification allows molecular genetic investigations of wood tissue. Genotypes, the origin of sampled material, and species can be identified based on an investigation of wood if suitable information on genetic variation patterns within and among species is available. Potential applications are in forensics and in the control of the timber and wood trade. We extracted DNA from wood of Dipterocarpaceae, a family that dominates rainforests and comprises many important timber species in Southeast Asia. Several different DNA isolation techniques were compared and optimized for wood samples from natural populations and from wood processing enterprises. The quality of the DNA was tested by spectrophotometry, PCR amplification, and PCR inhibitor tests. An average DNA yield of 2.2 µg was obtained per 50-100 mg of dried wood sample. Chloroplast DNA (cpDNA) regions of different length were amenable to PCR amplification from the extracted DNA. Modification of DNA isolation techniques by the addition of polyvinylpyrrolidone (PVP) addition up to 3.1% into lysis buffer reduced PCR inhibition effectively. In order to evaluate the extraction method, we analyzed leaves and wood from the same tree by PCR amplification, genotyping and sequencing of chloroplast microsatellites.

Key words: chloroplast microsatellites, Dipterocarpaceae, DNA extraction, genotyping, PCR amplification, PCR inhibitor, PVP, sequencing, wood

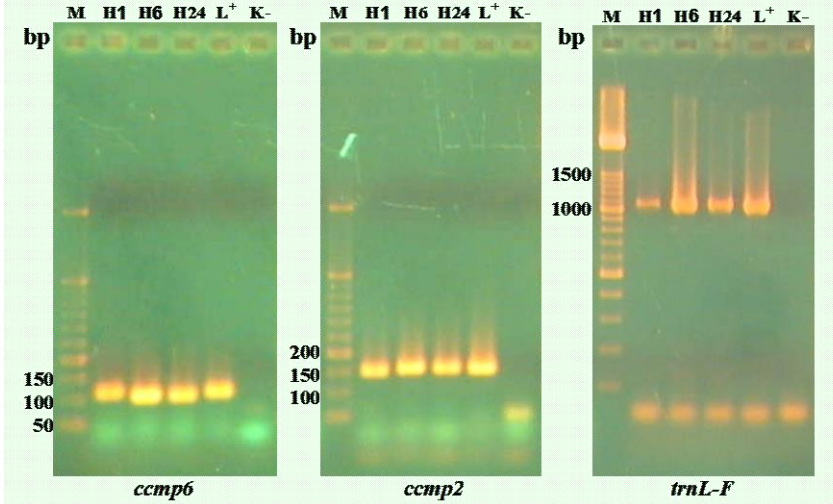
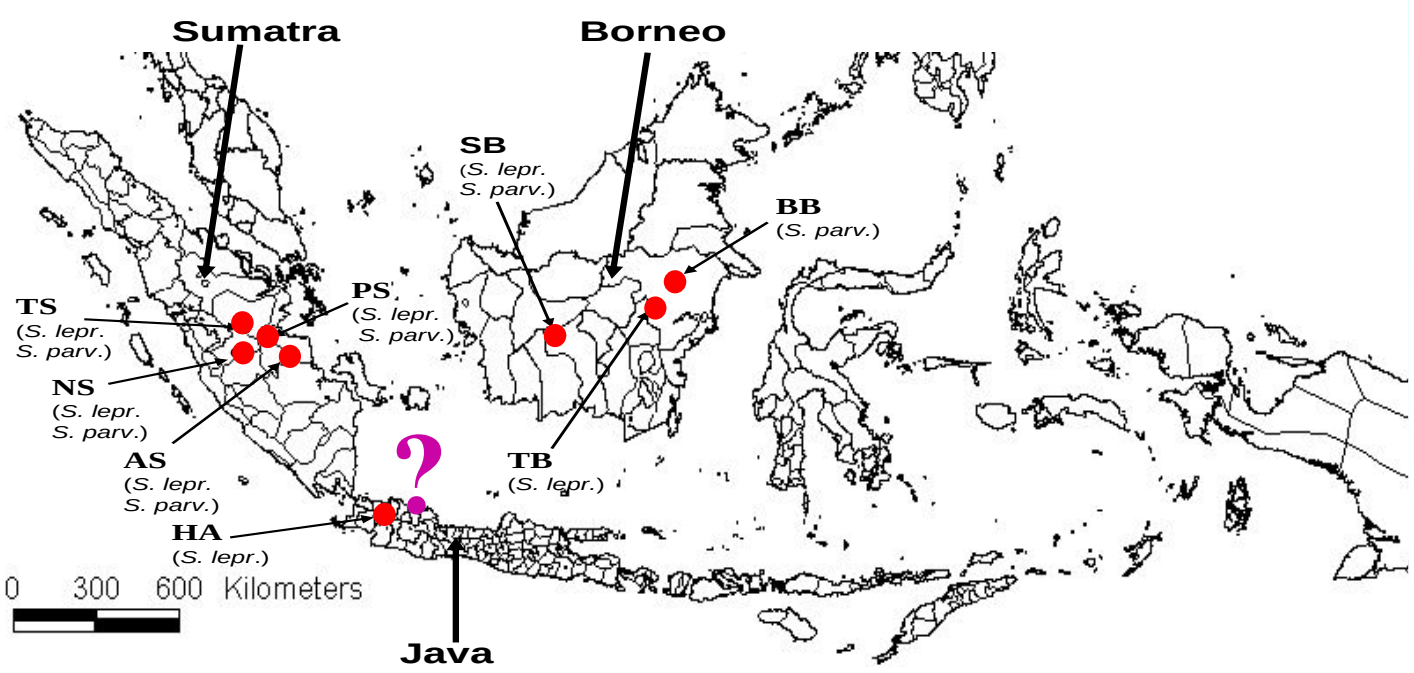
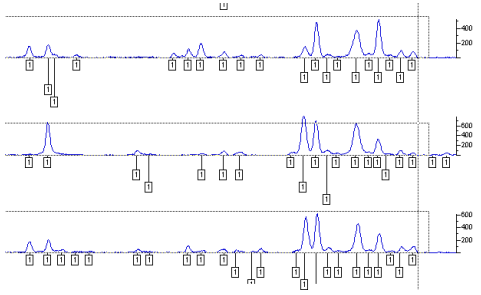


Figure 1. PCR profiles of the DNA samples amplified with primers *trnL-F* (*Taberlet et al., 1991*), *ccmp2* and *ccmp6* (*Weising and Gardner, 1999*).
Length of cpDNA fragment amplified by *ccmp6*, *ccmp2* and *trnL-F* was about 0.1, 0.15 and 1.1 kb, respectively. Samples on gel lanes are: M = Size 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).

3.1. Shorea Project

- Materials: *Shorea leprosula* & *S. parvifolia* (6 populations)
- Methods: AFLP Marker → Database





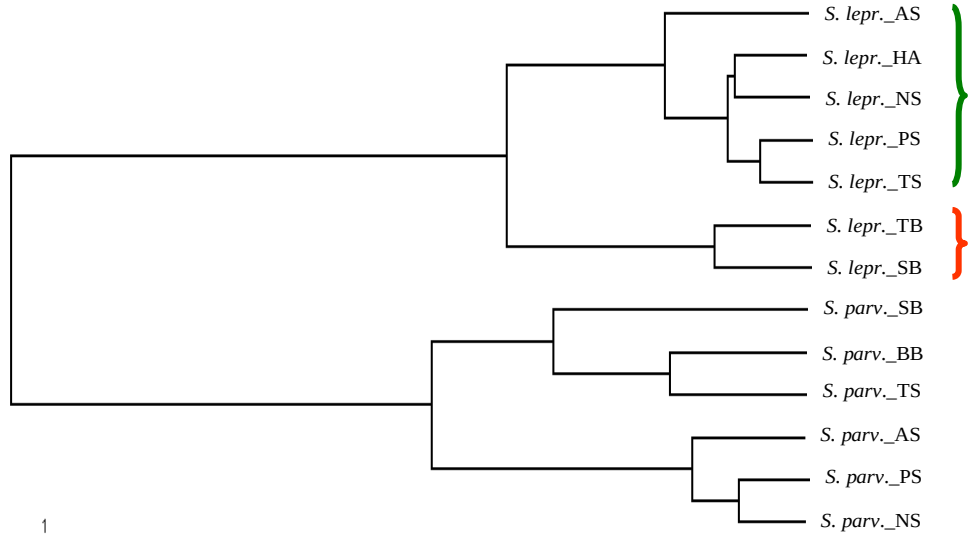
Tree Genetics & Genomes (2006) 2: 225–239
DOI 10.1007/s11295-006-0046-0

ORIGINAL PAPER

Cui-Ping Cao · Reiner Finkeldey ·
Iskandar Zulkarnaen Siregar ·
Ulfah Juniarti Siregar · Oliver Gailing

Genetic diversity within and among populations of *Shorea leprosula* Miq. and *Shorea parvifolia* Dyer (Dipterocarpaceae) in Indonesia detected by AFLPs

Received: 8 November 2005 / Revised: 20 March 2006 / Accepted: 24 May 2006 / Published online: 8 August 2006



Origin:
A= Java
S= Sumatera
B=Borneo

? = Origin?

3.1. Shorea Project

DNA Barcoding of Diptercarps

plants

MDPI

Article

Integrating DNA Barcoding and Traditional Taxonomy for the Identification of Dipterocarps in Remnant Lowland Forests of Sumatra

Carina Carneiro de Melo Moura [1], Fabian Brambach [2], Kevin Jair Hernandez Bado [1], Konstantin V. Krutovsky [1,3,4,5,6], Holger Kreft [2], Sri Sudarmiyati Tjitrosoedirdjo [7], Iskandar Z. Siregar [8] and Oliver Gailing [1,3,*]

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Received: 23 August 2019; Accepted: 17 October 2019; Published: 30 October 2019

check for updates

Abstract: DNA barcoding has been used as a universal tool for phylogenetic inferences and diversity assessments, especially in poorly studied species and regions. The aim of this study was to contrast morphological taxonomy and DNA barcoding, using the three frequently used markers *matK*, *rbcL*, and *trnL-F*, to assess the efficiency of DNA barcoding in the identification of dipterocarps in Sumatra, Indonesia. The chloroplast gene *matK* was the most polymorphic among these three markers with an average interspecific genetic distance of 0.020. The results of the molecular data were mostly in agreement with the morphological identification for the clades of *Anthoshorea*, *Hopea*, *Richetia*, *Parashorea*, and *Anisoptera*, nonetheless these markers were inefficient to resolve the relationships within the *Rubroshorea* group. The maximum likelihood and Bayesian inference phylogenies identified *Shorea* as a paraphyletic genus, *Anthoshorea* appeared as sister to *Hopea*, and *Richetia* was sister to *Parashorea*. A better discriminatory power among dipterocarp species provided by *matK* and observed in our study suggests that this marker has a higher evolutionary rate than the other two markers tested. However, a combination of several different barcoding markers is essential for reliable identification of the species at a lower taxonomic level.

Keywords: *matK*; *rbcL*; *trnL-F*; Dipterocarpoideae; tropical tree diversity; genetic distance; reference DNA library

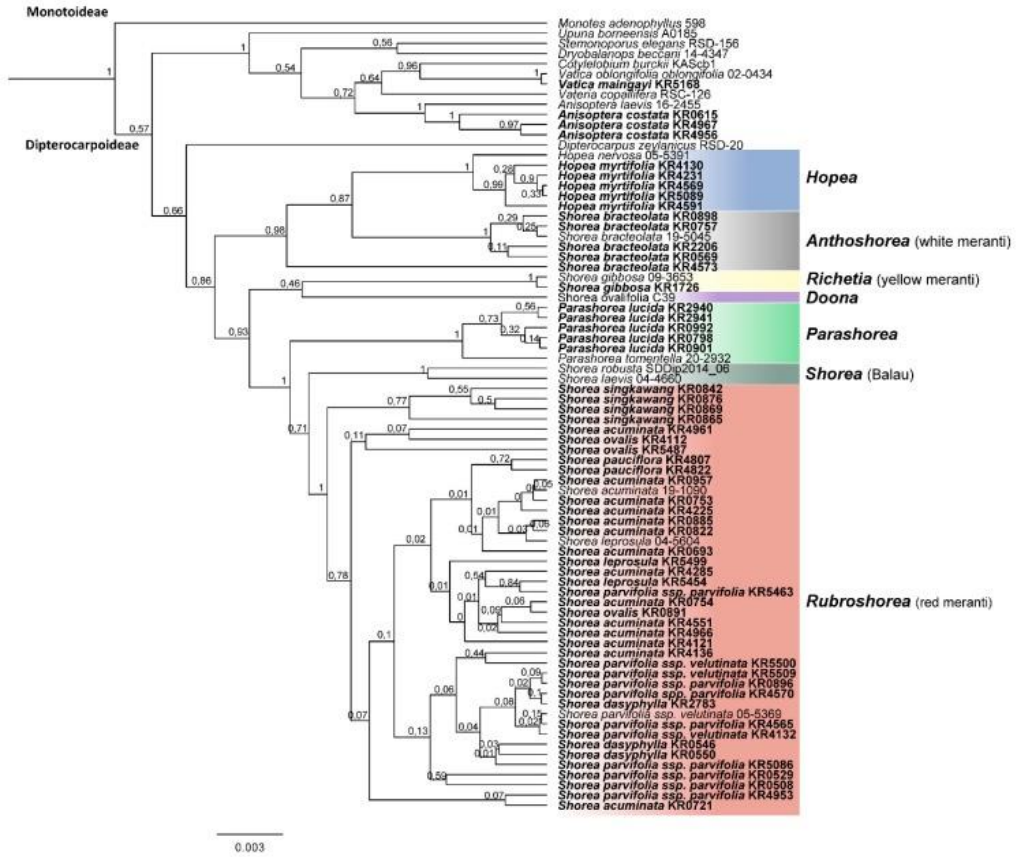
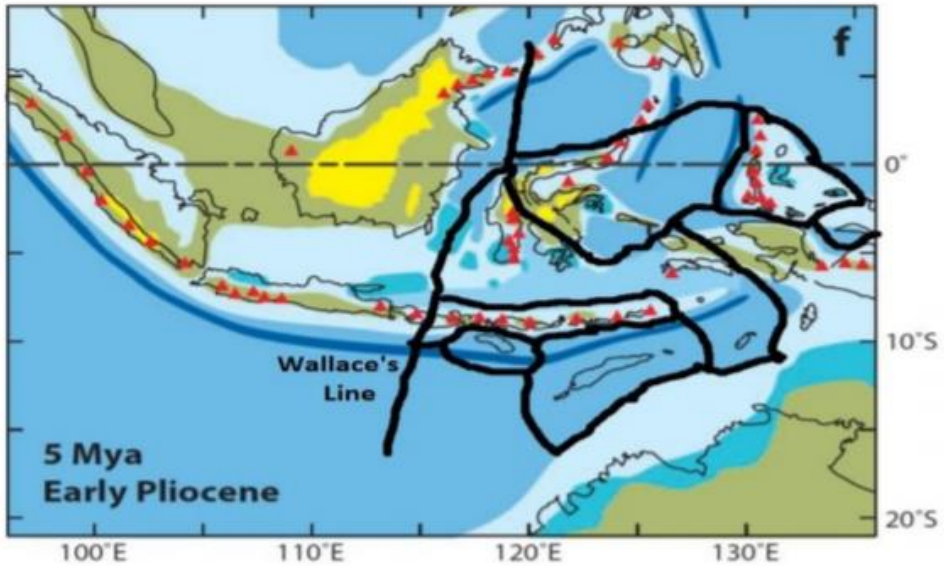


Figure 4 Bayesian inference tree based on the concatenated sequences of the *matK* and *rbcL* markers. The numbers at the tree nodes represent the posterior probability. Tips display species IDs, samples collected for this study are depicted in bold (see Table S1 for details), major clades of Shoreeae are color-highlighted.

3.2. Ebony Project

Sampling from 16 sites (Siregar et al. 2020)



Tectonic history in Wallacea (after Lohman et al 2011, Morley 2000, Hall 2017)

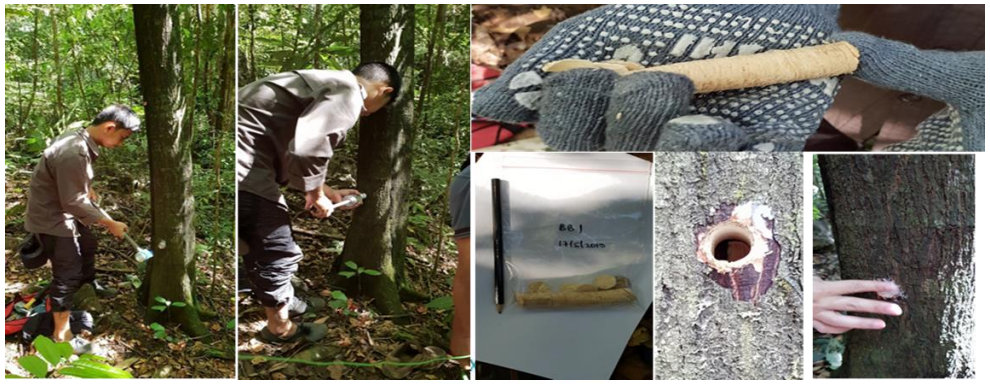
3.2. Ebony Project

Fig. 3. Wood core in a plastic zip lock bag (12 cm x 20 cm).

Fig. 4. Wood core extracted using the pickering punch.

Method: Multi-Analysis

- Genetics
- Anatomy
- NIR Spectroscopy
- Mass Spectrometry (LC MS/MS)



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Method Article

Collecting wood core samples from Macassar ebony (*Diospyros celebica* Bakh.) for multi-purpose analysis using pickering punch

Iskandar Zulkarnaen Siregar[a,b,*], Muhammad Majiidu[a], Fifi Gus Dwiyanti[a,b], Essy Harnelly[c], Ratih Damayanti[d], Lina Karlinasari[e], Mohamad Rafi[a,f], Dewi Anggraini Septaningsih[a], Meaghan Parker-Forney[g]

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[e] Department of Forest Product, Faculty of Forestry and Environment, IPB University, Bogor, Indonesia
[f] Department of Chemistry, Faculty of Mathematics and Natural Science, IPB University, Bogor, Indonesia
[g] World Resources Institute, Washington DC, United State

Wood Core Collection by Peickering Punch

2. Ebony Project

MethodsX 9 (2022) 101728

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Method Article

Collecting wood core samples from Macassar ebony (*Diospyros celebica* Bakh.) for multi-purpose analysis using pickering punch

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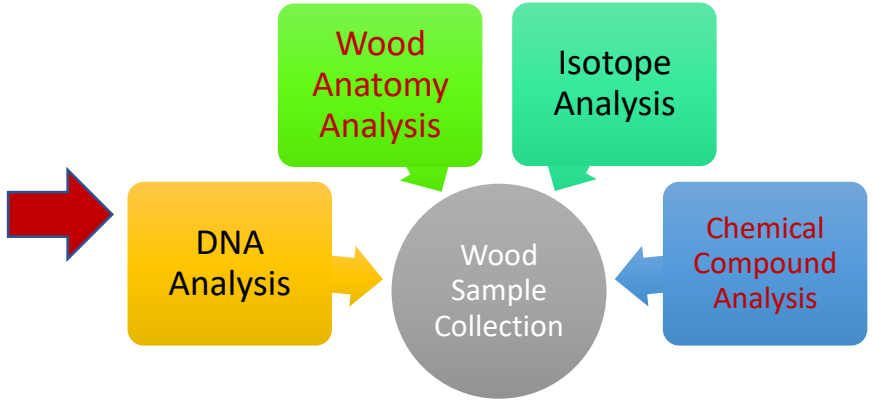
[a] Advanced Research Laboratory, IPB University, Bogor, Indonesia
[b] Department of Silviculture, Faculty of Forestry and Environment, IPB University, Bogor, Indonesia
[c] Department of Biology, Faculty of Mathematics and Natural Science, Syiah Kuala University, Banda Aceh, Indonesia
[d] Research Center for Biomass and Bioproducts, National Research and Innovation Agency (BRIN), Cibinong, Bogor, Indonesia
[e] Department of Forest Product, Faculty of Forestry and Environment, IPB University, Bogor, Indonesia
[f] Department of Chemistry, Faculty of Mathematics and Natural Science, IPB University, Bogor, Indonesia
[g] World Resources Institute, Washington DC, United State

Fig. 3. Wood core in a plastic zip lock bag (12 cm x 20 cm).

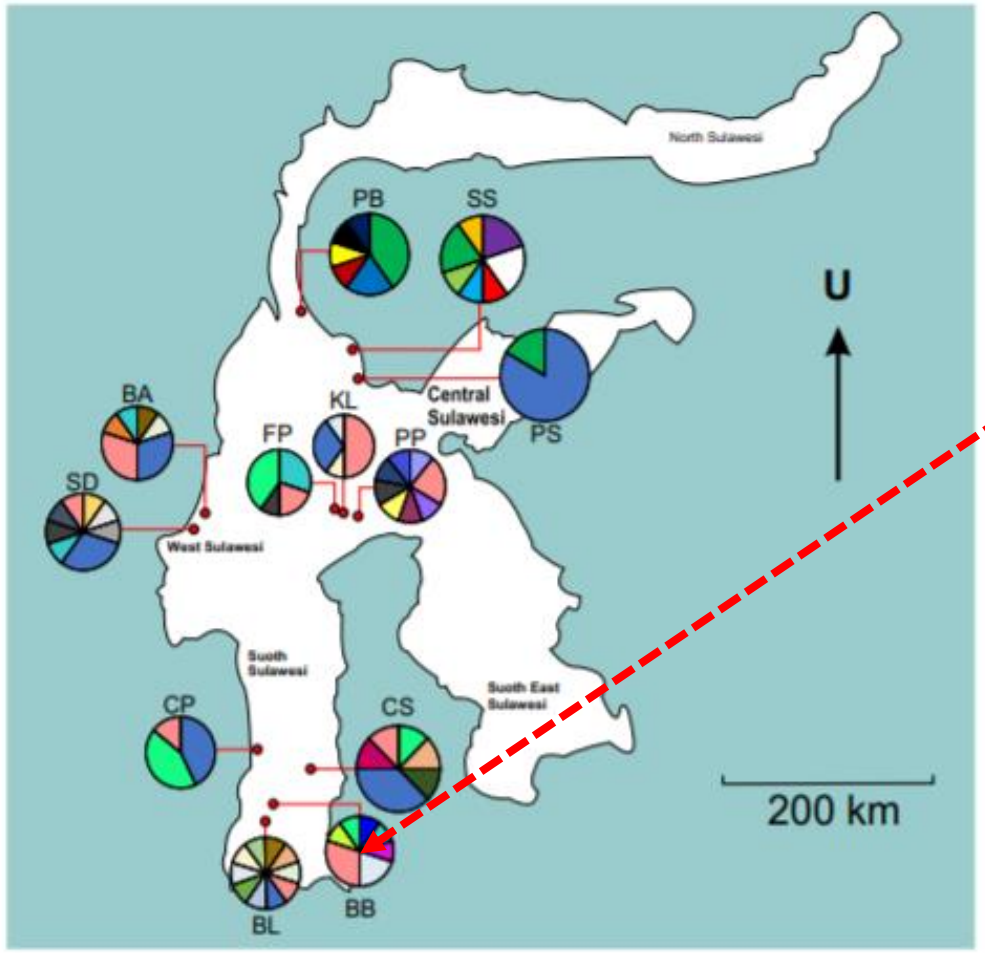
Fig. 4. Wood core extracted using the pickering punch.



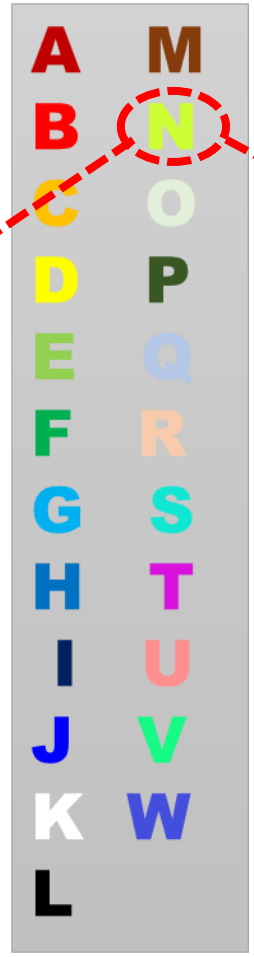
Wood Core Collection by Peickering Punch

3.2. Ebony Project

DNA reference data for Ebony



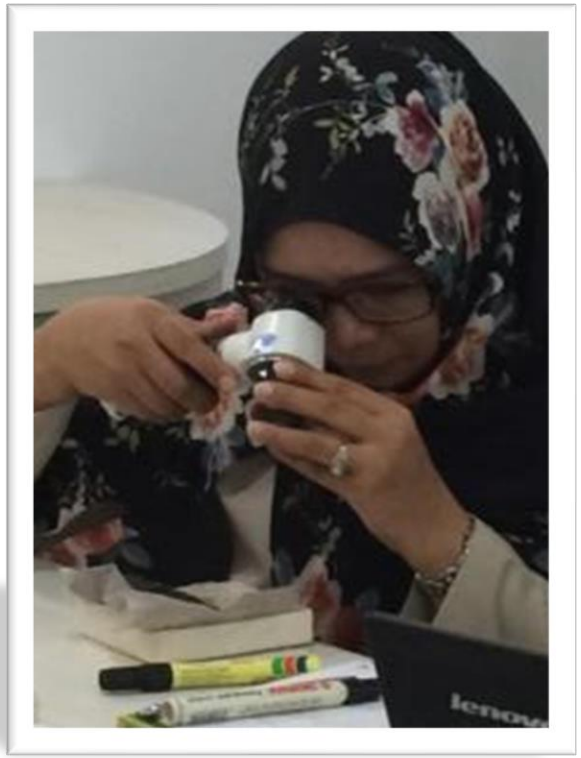
Siregar et al (in prep)

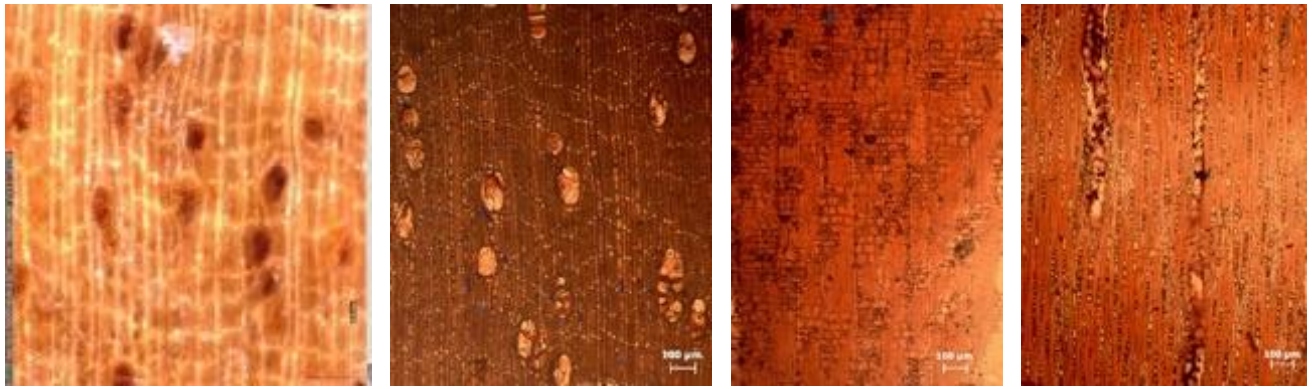


23 haplotypes cpDNA screened based on 5 (five) ccmp loci in Sulawesi (QiAxcel)

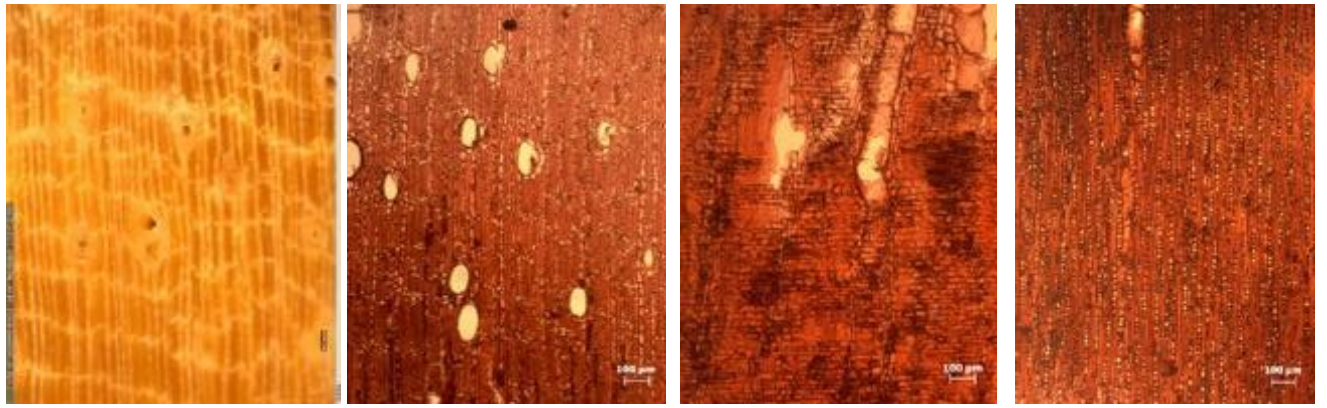
3.2. Ebony Project

Wood anatomy reference data for Ebony





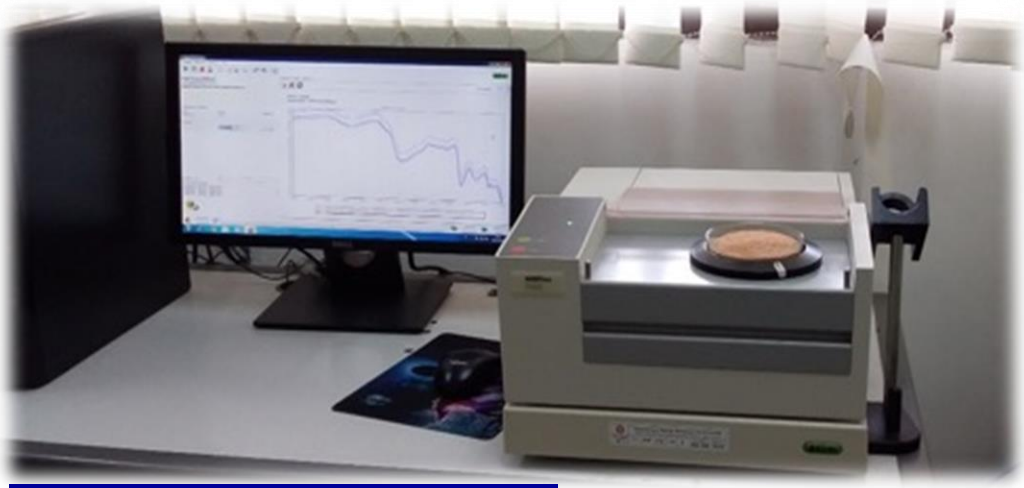
Anatomical structure of *Diospyros celebica* from Batu Ampa (BA)



Anatomical structure of *Diospyros celebica* from Bellabori (BL)

3.2. Ebony Project

NIRs reference data for Ebony



Buchi® NIRFLex N-500

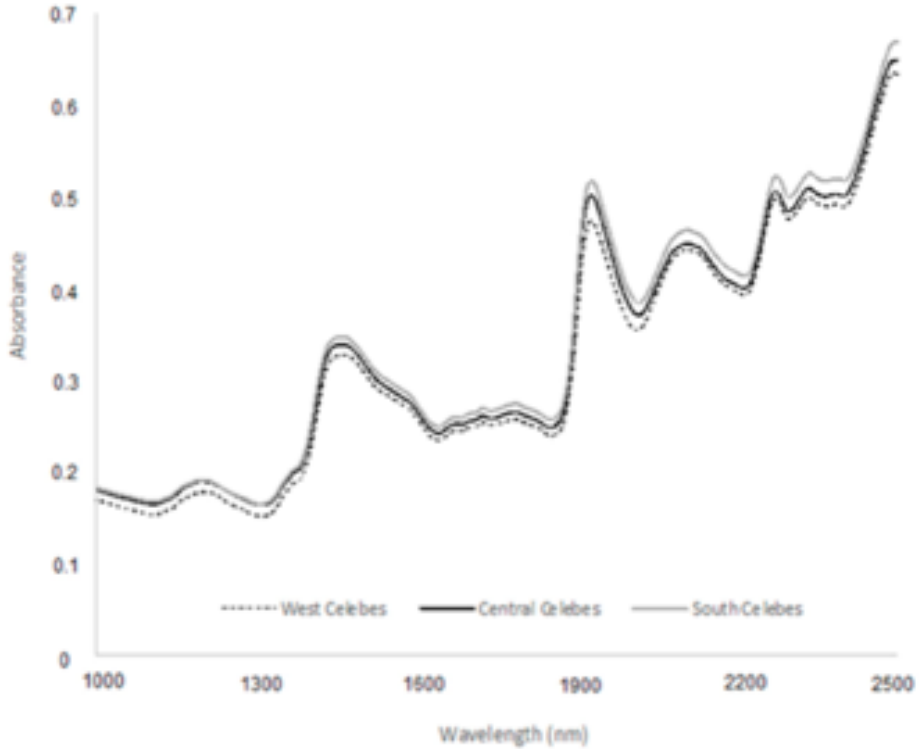


Figure 3. Representative average of original near-infrared (NIR) spectra of ebony wood samples were collected from South Celebes, West Celebes, and Central Celebes.

3.2. Ebony Project

NIRs reference data for Ebony

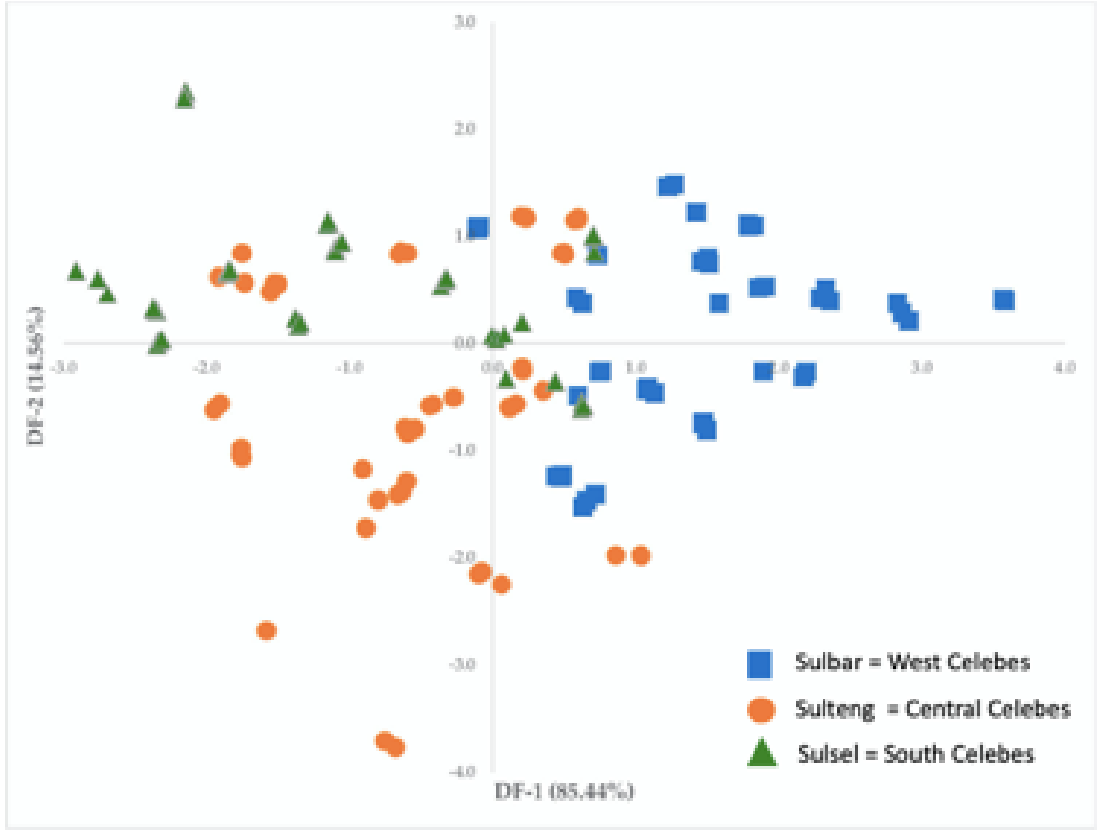


Figure 5. Discrimination ebony wood based on site origin using NIR spectra data processed by principal components analysis–discriminant analysis (PCA–DA).

forests

MDPI

Article

Discrimination and Determination of Extractive Content of Ebony (*Diospyros celebica* Bakh.) from Celebes Island by Near-Infrared Spectroscopy

Lina Karlinasari [1,*], Noviyanti [1], Y. Aris Purwanto [2], Muhammad Majiidu [3], Fifi G. Dwiyanti [4], Mohamad Rafi [5], Ratih Damayanti [6], Essy Harnelly [7] and Iskandar Z. Siregar [4,*]

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3 Molecular Science Research Group, Advanced Research Laboratory, IPB University (Bogor Agricultural University), Kampus IPB Darmaga, Bogor 16680, Indonesia; mmajidu@gmail.com
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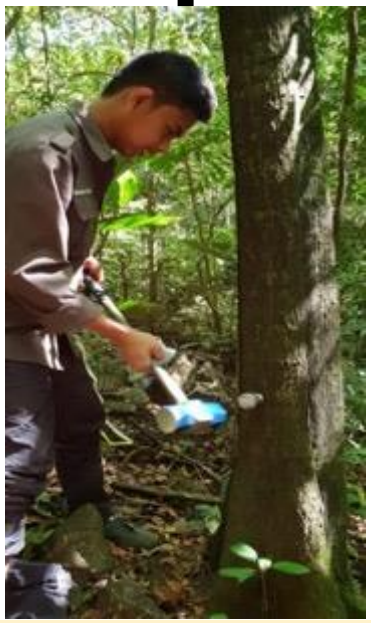
6 Forest Products Research and Development Center, Research, Development and Innovation Agency, Ministry of Environment and Forestry Republic of Indonesia, Jl. Gunung Batu No 5, Bogor 16118, Indonesia; ratih-damayanti@forda-mof.org
7 Department of Biology, Faculty of Mathematics and Natural Science, Syiah Kuala University, Jln. Teuku Nyak Arief Darussalam, Banda Aceh 23111, Aceh, Indonesia; essyharnelly@unsyiah.ac.id
* Correspondence: karlinasari@apps.ipb.ac.id (L.K.); siregar@apps.ipb.ac.id (I.Z.S.)

Abstract: Ebony (*Diospyros celebica* Bakh.) is an endemic plant on Celebes (Sulawesi) island. Extractive

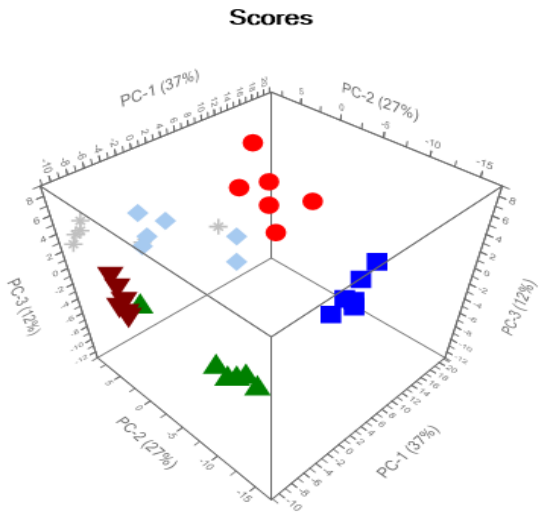
3.2. Ebony Project

Metabolite profiling reference data for Ebony



Sampling *D. celebica* (3 provinces in Celebes)

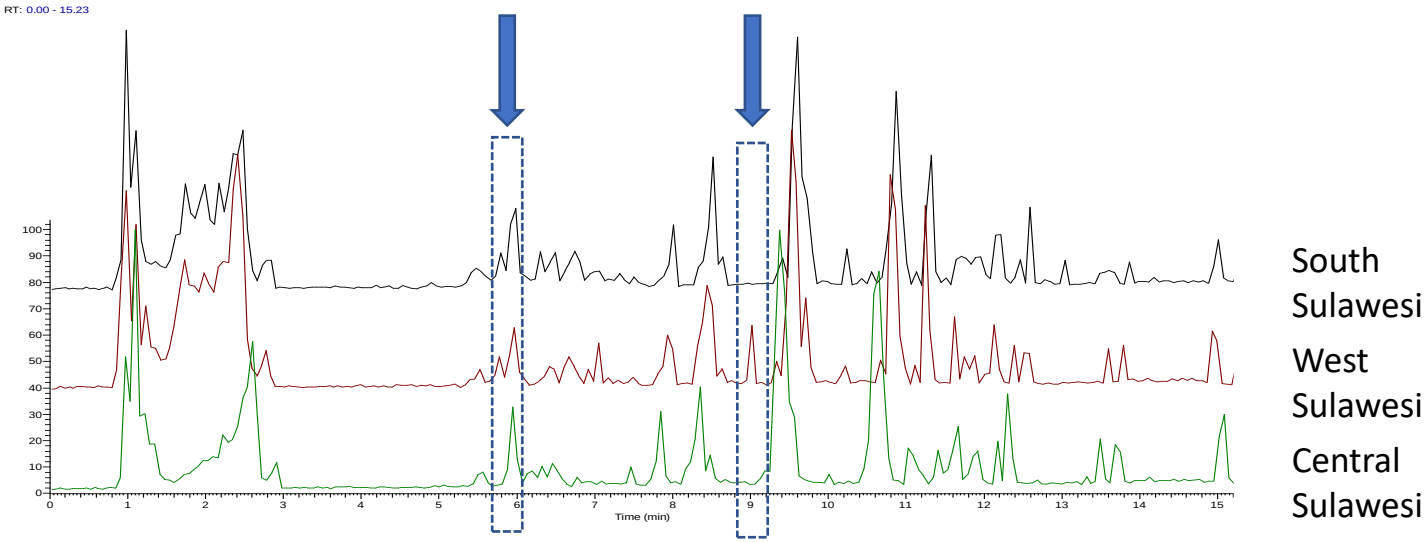
Sample preparation and extraction



multivariate analysis (clustering based on geographical origin)

3.2. Ebony Project

Metabolite profiling reference data for Ebony



UHPLC-Q-orbitrap HRMS Chromatogram of ebony from three provinces in Sulawesi

Diomelquinone A was identified as a marker compound for Sulawesi ebony from West Sulawesi province, while **3-methylplumbagin** and **kaempferol** were markers for South Sulawesi province. These metabolites can be used as diagnostic markers for the growth location of Sulawesi ebony on Sulawesi island, Indonesia.

Wood Science and Technology (2023) 57:211–228
https://doi.org/10.1007/s00226-022-01440-8

ORIGINAL

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Untargeted metabolomics analysis of *Diospyros celebica* Bakh. from three different geographical origins in Sulawesi island using UHPLC-Q-Orbitrap HRMS

Mohamad Rafi[1,2] · Dien Atin Boritnaban[1] · Dewi Anggraini Septaningsih[2] · Fifi Gus Dwiyanti[2,3] · Muhammad Majiidu[2] · Nancy Dewi Yuliana[4] · Lina Karlinasari[5] · Essy Harnelly[6] · Ratih Damayanti[7] · Iskandar Zulkarnaen Siregar[2,3]

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Abstract
Diospyros celebica Bakh is known as Sulawesi ebony and is reported to have quinones as the primary metabolite. The metabolites contained in Sulawesi ebony can be influenced by several factors, one of which is the growth location. This study aims to identify which metabolites are present in Sulawesi ebony wood using UHPLC-Q-Orbitrap HRMS and to determine the origin of Sulawesi ebony wood on Sulawesi Island, Indonesia, in combination with principal component analysis (PCA) and orthogonal partial least square-discriminant analysis (OPLS-DA). Forty-five samples of Sulawesi ebony were sonicated using 80% ethanol and analyzed using UHPLC-Q-Orbitrap HRMS. A total of 35 metabolites were identified based on an in-house database (putative identification). The quinone group is the most present among the identified metabolites in the Sulawesi ebony wood. The resulting base peak chromatograms were preprocessed using correlation optimized warping to align all sample chromatograms before being analyzed with PCA and OPLS-DA. The resulting score plot showed that based on the PCA, the origins could not be distinguished, while OPLS-DA was able to discriminate Sulawesi ebony from the three provinces. Diomelquinone A was identified as a marker compound for Sulawesi ebony from West Sulawesi province, while 3-methylplumbagin and kaempferol were markers for South Sulawesi province. These metabolites can be used as diagnostic markers for the growth location of Sulawesi ebony on Sulawesi island, Indonesia.

3.3. WoodID Project

Indonesian-based Wood Identification Program

WORLD RESOURCES INSTITUTE

(2021-2025)

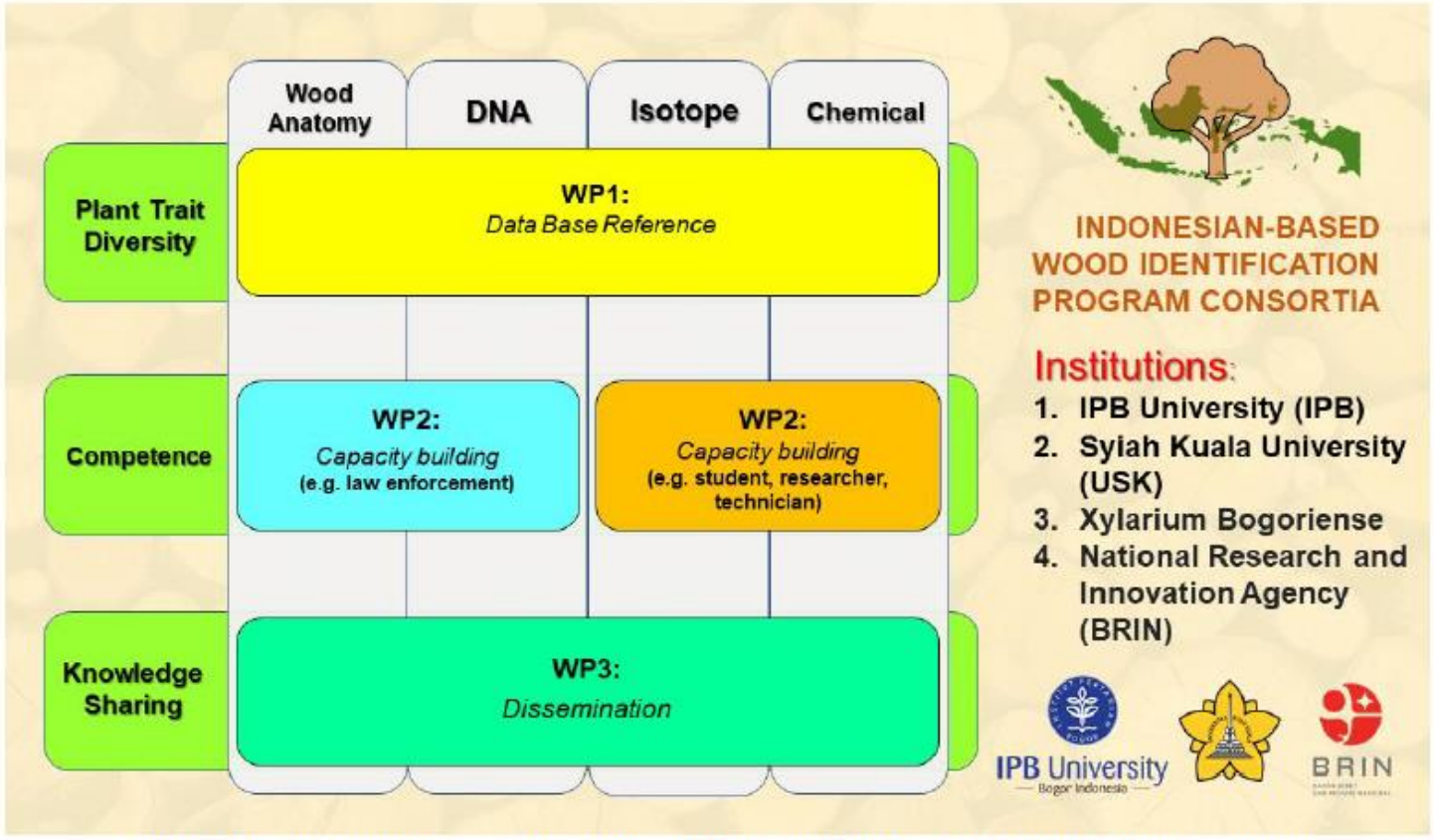

Figure 1. Cross-cutting issue Indonesian-based Wood Identification Program

4. Key Takeaways

- **For the first time, the collection of macassar ebony wood core samples** has been successfully carried out in Sulawesi including herbarium and leaves →Testing the pickering punch in the field was also successful.
- **The wood core can be used for various purposes** →Testing other analyses (NIR/chemical compound and anatomy).
- **This work showed that the scientific advancements resulted from international collaboration** could build the local capacity for building reference database for wood identification.

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Thank You