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FOREST GENETIC RESOURCE MANAGEMENT THROUGH DNA TECHNOLOGY

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ABSTRACT

Illegal harvesting of forest resources is a major threat to the sustainability of forest ecosystems. At the Forest Research Institute Malaysia (FRIM), comprehensive DNA barcoding and profiling databases of important forest plant species have been developed since 2009 for species identification, timber tracking and source of origin verification. At present, a total of 366 populations throughout Malaysia and about 15,000 individual samples were used to establish the species, population and individual identification databases. The species identification database enables the taxonomic classification of a suspected timber, while the population identification database can reveal the source of origin up to population level. If DNA profile of a suspected timber matches that of its original stump, the individual identification database can estimate the probability of a random match using a subpopulation-cum inbreeding model to rule out the possibility of matching by chance. Four standard operating procedures (SOPs) for DNA forensics in wood tracking have been developed to facilitate the use of these databases. Together with the SOPs, these DNA databases significantly enhance the capacity of forest officials to curb the problem of illegal logging, while supporting the industries through plant species authentication. Furthermore, these databases have also been used to develop various conservation and management guidelines to strike a balance between conservation and utilisation of forest genetic resources.

Keywords: DNA barcoding, DNA profiling, Short tandem repeat (STR), DNA forensics, conservation genetics

1 INTRODUCTION

Malaysia is rich in forest genetic resources. There are over 15,000 species of vascular plants in Malaysia (UNEP-WCMC, 2004), and among them, more than 3,000 species can reach timber size (Wyatt-Smith 1952 [revised 1999]; Wong 1982 [revised 2002]). Of this total, there are 408 commercially valuable species and about 600 species classified as lesser-known timber (Menon 1967 [revised 2004]; Wong 1982 [revised 2002]; MTC 2019). However, from 2017 to 2021, 201 cases of illegal logging have been recorded in Peninsular Malaysia (Mohamed Zin et al. 2022).

Illegal logging undermines the development of the timber industry and the government's efforts in the sustainable management and utilization of the country's forest genetic resources. To combat this issue effectively, accurate identification of timber species and tracking their geographic origins throughout the supply chain are essential. In the past, spectrometry and isotopic methods have been employed to differentiate wood samples from various geographic locations, allowing for the determination of their origins with varying degrees of certainty. However, these methods are influenced by local environmental factors and can show inconsistencies among individuals from the same population.

Currently, Malaysian foresters rely on wood anatomy evidence to connect suspected timber thefts to their source trees. Unfortunately, this approach is inadequate, as it only allows for identification at the timber trade name or group level rather than at the species or individual level. This limitation underscores the need for more reliable and precise methods to ensure effective enforcement against illegal logging.

New methods for linking a timber log to its population and stump of origin would represent a crucial forensic tool for tracing stolen logs, aiding in the control of illegal logging, and supporting chain-of-custody practices for certifying timber from sustainably managed forests. The unique properties of DNA within timber can serve as an effective tracking and monitoring tool to verify the legality of suspected timber in the context of illegal logging and certification processes.

DNA profiling has been extensively used in human forensics for legal proceedings, such as proving guilt or innocence, resolving paternity issues, identifying remains of missing persons or disaster victims, and establishing citizenship through blood relationships in immigration cases. Similarly, in forestry, DNA can be applied in three key ways for wood tracking and forensic investigations:

1. DNA Barcoding: Markers with high species resolution can identify the taxonomic origin of a wood product, determining its genus or species.
2. Chloroplast and Short Tandem Repeat (STR) Markers: These markers can provide sufficient geographical resolution to identify the geographic origin of a wood product.
3. Highly Polymorphic STR Markers: A set of these markers can be used to create DNA profiling databases for individual identification, allowing an illegally harvested log to be matched to its original stump.

By leveraging these advanced DNA techniques, we can enhance our efforts to combat illegal logging and ensure sustainable forest management, while supporting the industries through plant species authentication.

2 DEVELOPMENT OF PLANT DNA BARCODING AND DNA PROFILING DATABASES

The timber industry is one of the main contributors to the country's revenue. In 2023, the value of timber product exports reached RM21.84 billion (DOSM & MTIB). However, Malaysia's exports of forest-based timber products to international markets are becoming increasingly challenging due to the need to comply with various international regulations, such as the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), the Canadian Wild Animal and Plant Protection and Regulation of International and Interprovincial Trade Act, the United States Lacey Act, the United Kingdom Timber Regulation (UKTR), the European Union (EU) Forest Law Enforcement, Governance and Trade (FLEGT) initiative, the EU Timber Regulation (EUTR), and the Australian Illegal Logging Prohibition Act.

Due to the large number of species and the difficulty of identifying timber at the species level, closely related species groups are often designated with the same timber trade name for management and trade purposes. For example, all species of *Dipterocarpus* are referred to as Keruing. Similarly, *Calophyllum* species are called Bintangor, and Syzygium/Eugenia are referred to as Kelat. In other cases, entire genera like *Madhuca*, *Palaquium*, and *Payena* are collectively known as Nyatoh, while whole families, such as Burseraceae, are referred to as Kedondong (Menon 1967, revised 2004). Only a few timber trade names correspond to a single species, such as Kempas (*Koompassia malaccensis*) and Chengal (*Neobalanocarpus heimii*).

Accurate, rapid, and cost-effective identification and authentication of timber species are essential for enforcing forestry regulations and ensuring sustainable forest management in the country. Sustainable forest management is crucial for ensuring that forest genetic resources can be utilized by both current and future generations. To achieve this goal, knowledge of species diversity and pre-harvest surveys of logging concessions, as well as the enforcement of forestry regulations at timber inspection stations, must be conducted at the species level, not just by timber trade names. In cases of illegal logging investigations, identifying the species of stolen wood becomes a vital element for forensic evidence.

Conventionally, the identification and authentication of timber species rely on morphological characteristics such as leaves, stems, flowers, fruits, and wood anatomy. While this method is cost-effective, morphological identification has certain limitations, including (i) insufficient variation, (ii) subjectivity in analysis, (iii) environmental influences, (iv) some characteristics are only expressed at certain growth stages, and (v) for wood anatomy, most identifications can only reach the group level (trade names) and not the species level. Additionally, once a tree has been processed into timber, many of the identifying morphological features are lost. To address this issue, the unique DNA characteristics present in wood can serve as an alternative method. DNA barcoding techniques rely on using short DNA sequences within the plant genome as tags (DNA barcodes) for species identification (Hebert 2003). The underlying

principle is that each plant species has a unique variation of short DNA sequences that distinguishes it from others.

Given the importance of a reliable system for identifying and authenticating timber species for enforcing forestry regulations and enhancing forest management capabilities, DNA techniques have been comprehensively developed at the Forest Research Institute Malaysia (FRIM) since 2009. Currently, DNA barcode database for the identification and authentication of plant species has been generated for 699 timber species (154 timber trade names), 314 herbal species (Tnah et al. 2019), 70 wild fruits species (Tnah et al. 2024), and 27 CITES-listed species, namely Karas (*Aquilaria* spp. Lee et al. 2022) and Ramin (*Gonystylus* spp. Ng et al. 2016). Whereas, DNA profiling database for timber detection and origin verification has been established for 12 important tropical tree species, namely, *Neobalanocarpus heimii* (Chengal: Tnah et al. 2009, 2010a, 2010b, 2012, 2022, 2024), *Gonystylus bancanus* (Ramin Melawis: Ng et al. 2009, 2016), *Rubroshorea platyclados* (Meranti Bukit: Ng et al. 2009, 2013, 2017), *Intsia palembanica* (Merbau: Ng et al. 2020), *Aquilaria malaccensis* (Karas: Lee at al. 2022), *Rubroshorea leprosula* (Ng et al. 2022), *Koompassia malaccensis* (Kempas), *Rhizophora apiculata* (Bakau Minyak), *Rhizophora mucronata* (Bakau Kurap), *Rubroshorea curtisii* (Meranti Seraya) *Dryobalanops aromatica* (Kapur) and *Dryobalanops oblongifolia* (Keladan). A total of 398 populations throughout Malaysia and 12,656 individual samples were used to establish the DNA databases of 12 species (Figure 1). These databases enable the harvested logs to be matched against the original tree stumps with a high degree of accuracy.

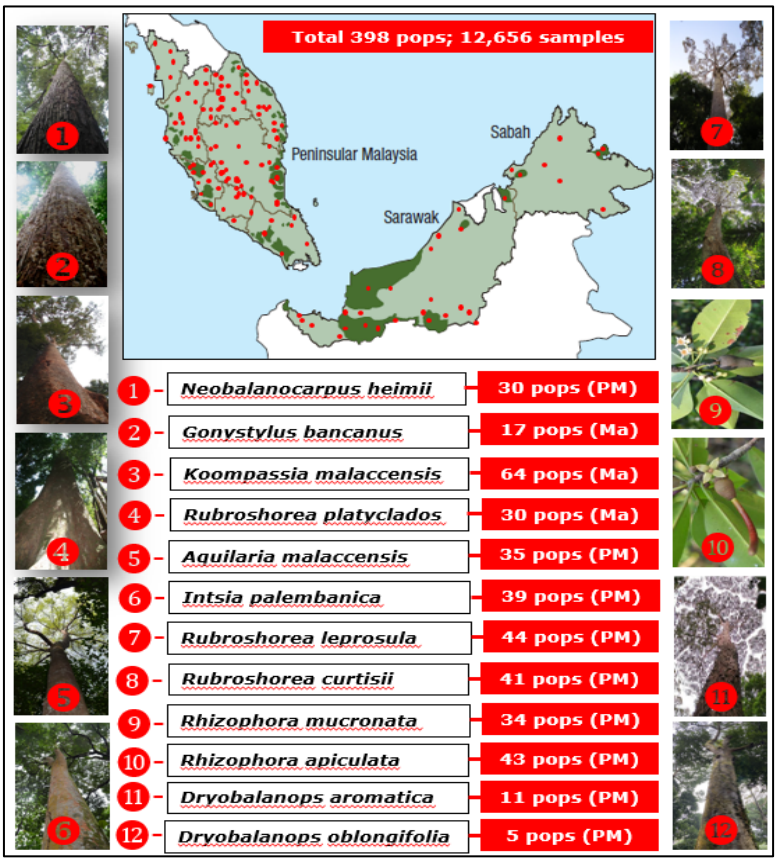

Figure 1 A total of 398 populations throughout Malaysia and 12,656 individual samples were used to establish the DNA profiling databases.

3 DEVELOPMENT OF STANDARD OPERATING PROCEDURES

For forensic applications, the developed DNA database includes four standard operating procedures (SOPs) for the identification and detection of plants (Lee et al. 2020):

- SOP 1: Specimen collection in the field
- SOP 2: Isolation and purification of DNA from cambium, and wood core
- SOP 3: DNA sequencing for species and population identification
- SOP 4: Short tandem repeat (STR) genotyping for population and individual identification

Additionally, “SOP 1: Specimen collection in the field” has been implemented by forest enforcement officers throughout Peninsular Malaysia, as outlined in the Director-General of Forestry Circular No. 1/2020 – "Procedure for Collecting Wood Samples at Forest Offense Sites for DNA-Based Wood Identification Analysis."

4 DEVELOPMENT OF DNA DATABASE MANAGEMENT SYSTEM OF FOREST RESOURCES

To effectively manage the extensive data generated by DNA barcoding and profiling of forest resources, FRIM has created a centralized system known as the DNA Database Management System of Forest Resources (Figure 2). This platform is designed for the storage, management, and application of data, enabling relevant stakeholders to strike a balance between conservation and the sustainable utilization of forest resources.

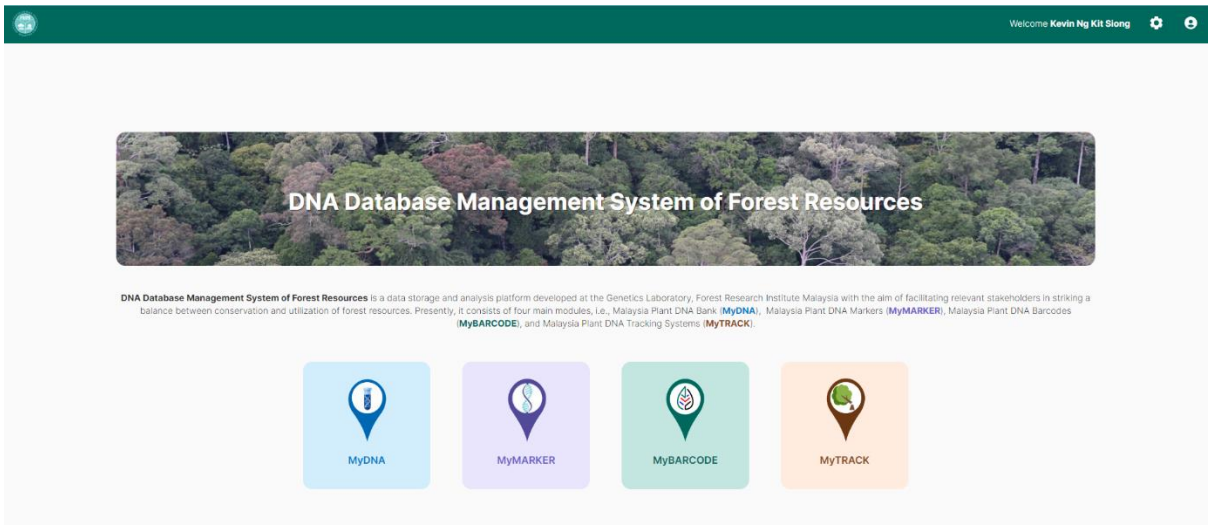

Figure 2 DNA Database Management System of Forest Resources.

The system consists of four main modules:

a) Malaysia Plant DNA Bank (MyDNA) holds the information of FRIM DNA Bank established in 2009 to serve as a repository of DNA samples of forest genetic resources for use by present and future generations in plant research. To date, the DNA Bank

comprises 1336 plant species from 643 genera and 146 families, primarily commercial timber species, herbal and medicinal plants, edible fruit species, and critically endangered plant species, totalling approximately 31,614 samples. The DNA samples are stored in a -80°C freezer, with about 30% tagged with herbarium specimens.

b) Malaysia Plant DNA Markers (MyMARKER) comprises STR primer sequences of important timber species, herbal medicinal plants and critically endangered plant species. These species-specific primers were developed in-house over the years using conventional as well as next-generation sequencing (NGS) approaches. In addition, MyMARKER provides primer information of universal chloroplast and ITS markers. This primer resource is useful for various application such as genetic diversity study, DNA barcoding, phylogeography, timber tracking and others.

c) Malaysia Plant DNA Barcodes (MyBARCODE) comprises reference DNA barcoding databases developed for plant species identification since 2009. High-quality DNA sequences of selected barcoding regions were generated for important tropical timber species, herbal medicinal plants, wild fruit trees and CITES-listed plant species. These databases have been used to assist forest enforcement officers as well as stakeholders of wood, herbal, food and insurance industries in species authentication and identification (Malaysia Book of Records 2017).

d) Malaysia Plant DNA Tracking Systems (MyTRACK) comprises of DNA profiling databases developed for important timber species since 2009 for timber tracking. The DNA-based timber tracking system is developed based on genetic data of samples collected throughout Malaysia. It is the first in the world for tropical tree species (Malaysia Book of Records 2012). These databases have been used to provide forensic evidences for timber tracking and origin verification since 2015 (Malaysia Book of Records 2019).

5 TRAINING OF INVESTIGATING AND ENFORCEMENT OFFICERS ON THE USE OF DNA METHODS FOR INVESTIGATING TIMBER THEFT CASES

Since 2015-2023, more than a dozen DNA Forensic Workshops have been organized in collaboration with the Department of Forestry of Peninsular Malaysia (JPSM) to train and familiarize investigative and enforcement officers from both JPSM and the State Forestry Departments (JPN) on the use of DNA methods for investigating timber theft cases along with the above mentioned four SOPs (Figure 3).

Figure 3 Forestry Science and DNA Forensics Course for Timber Detection held from 11-15 June 2023, organized by JPSM at Sg. Petani, Kedah. Participants were trained in DNA forensic techniques both theoretically and practically.

6 DNA FORENSIC EVIDENCE FOR CASES OF ILLEGAL LOGGING

These databases are currently being used to support officials from the Forestry Department of Peninsular Malaysia (JPSM), the State Forestry Departments (JPN), and the Department of Wildlife and National Parks Peninsular Malaysia (PERHILITAN) in providing DNA forensic evidence for prosecuting illegal loggers under Section 15 of the National Forestry Act 1984 (amended 2022) and the Wildlife Conservation Act 2010 (amended 2022). Since 2015, FRIM has collaborated with these agencies to provide crucial DNA forensic evidence in cases of illegal logging (Table 1).

Table 1 FRIM helps provide DNA forensic evidence for cases of illegal logging.

No.	Year	Department	Report No.	Date of Report
1	2015	JPN Selangor	MGF0115	30 Sep 2015
2	2016	PERHILITAN	MGF0116	25 Aug 2016
3	2017	JPN Selangor	MGF0117	04 Jan 2017
4	2017	PERHILITAN	MGF0817	07 Jun 2017
5	2018	JPSM	MGF0118	10 Apr 2018

6	2019	JPSM	MGF0319	25 Sep 2019
7	2019	JPSM	MGF0519	04 Nov 2019
8	2020	JPSM	MGF0120	15 Jan 2020
9	2020	JPSM	MGF0220	28 Jan 2020
10	2020	JPN Johor	MGF0320	21 Mei 2020
11	2020	JPN Kelantan/JPSM	MGF0520	02 Jul 2020
12	2020	JPN Kedah/JPSM	MGF0620	03 Jul 2020
13	2020	JPN Kelantan/JPSM	MGF0420	24 Jul 2020
14	2020	JPN Kelantan/JPSM	MGF0820	01 Okt 2020
15	2021	JPN Pahang/JPSM	MGF0121	06 Feb 2021
16	2021	JPN Pahang/JPSM	MGF0521	12 Dis 2021
17	2023	JPSM	MGF0123	26 Apr 2023

JPN – Jabatan Perhutanan Negeri
JPSM – Jabatan Perhutanan Semenanjung Malaysia

7. CONCLUSION

The establishment of DNA barcoding and profiling databases for key plant species will greatly enhance the ability of Forest Department officials to combat illegal logging, ultimately promoting the conservation and sustainable use of Malaysia's forest resources. For the timber industry, these databases can be invaluable for identifying and authenticating timber species. Additionally, they hold the potential to certify timber and timber products in compliance with international regulations and consumer standards. This initiative also underscores Malaysia's commitment to forest conservation in response to global anti-tropical wood campaigns.

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