INTEGRATION OF DNA BARCODING AND WOOD ANATOMY FOR THE IDENTIFICATION OF SELECTED TIMBERS

by

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THESIS

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DECLARATION

I hereby declare that the thesis entitled "Integration of DNA barcoding and wood anatomy for the identification of selected timbers" is a bonafide record of research done by me during the course of research and that this thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar titles, of any other University or Society.

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CERTIFICATE

Certified that the thesis, entitled "Integration of DNA barcoding and wood anatomy for the identification of selected timbers" is a record of research work done independently by **Azhar Ali A (2018-17-008)** under my guidance and supervision and that it is not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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CERTIFICATE

We, the undersigned members of the advisory committee of Mr. Azhar Ali A, a candidate for the degree of Master of Science in Forestry with major in Forest Products and Utilization, agree that the thesis entitled "Integration of DNA barcoding and wood anatomy for the identification of selected timbers" may be submitted by Azhar Ali A, in partial fulfillment of the requirement for the degree.

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INTRODUCTION

INTRODUCTION

Timber has played a vital role in the daily lives of people from time immemorial. However, putting the right timber species to the right use remains a challenge especially when the availability is considered. The shortage of timber supply from domestic sources widens the demand-supply gap for timber which may lead to adulteration practices in the trade of wood. The accurate identification of species is the only way to counteract these concerns in wood marketing.

Traditional methods of wood identification are based on physical characters and anatomical features of the wood species concerned. However, this approach has some major limitations, such as phenotypic plasticity, where distinct individuals within the same species may show variation due to environmental conditions or local adaptations. Alternatively, two distinct but related species may sometimes show similar anatomical characters which further complicates the process of identification. In the case of timber, the traditional way requires more expertise and understanding of wood biology or anatomy. For the precise taxonomic analysis in such situations, integration of DNA barcoding with the above said physical and anatomical features can serve as foolproof tools.

The DNA barcode is a small fragment of DNA from standardized genome regions that distinguish related species and is similar to barcodes used in supermarkets to identify different products. The concept behind this is that one species can be differentiated from the other by the uniqueness of a short sequence stretch from a reference gene belonging to that species (Hebert and Gregory, 2005). The DNA sample can access an unlimited pool of genetic data. Vijayan and Tsou (2010) described DNA barcoding as the process of identification of species based on diversity in nucleotides of short DNA fragments. The identification process of animals is well established by this method, with the identification of Cytochrome Oxidase subunit 1 (COI) as a standard barcode (Hebert *et al*., 2003). For most of the fungi and plants, the Cytochrome Oxidase subunit 1 (COI) region is not appropriate because the evolution of mitochondrial genes in these groups is much

slower. Hollingsworth *et al*. (2009) suggested two short regions of plastid DNA which are rbcL and matK as the best alternatives.

For the determination of identity as well as the provenance of a timber sample, the use of inbuilt DNA properties serves as an important aid (Asia Forest Partnership, 2005). DNA barcoding has been found successful to distinguish the geographical origin of one timber from another. This is very helpful in tackling issues related to illegal logging and tracing and in forest certification (Deguilloux *et al*., 2003). It is also used in individual identification where one illegally felled log can be matched with its stump (Tnah *et al*., 2010).

The DNA extraction from fresh tissue is regularly done in tropical forest studies and yet, dry wood extraction of DNA has not been adequately realized. Even though DNA extraction from wood is difficult as the inner portion of the wood is fully composed of dead tissues, certain DNA fragments are still attached to the cell wall (Cano-Gauci and Sarkar, 1996). At the same time, extraction from cambium and sapwood yields a significant volume of DNA (Rachmayanti *et al*., 2009). The various challenges in extracting DNA from wood are the presence of PCR inhibitors in the wood samples, decay by microorganisms, and exposure of wood to the natural degenerative process (Lee and Cooper, 1995).

The scope of DNA barcoding is unlimited in the field of forestry which constitutes the discrimination of forest seedlings, large-scale biodiversity surveys, and in counteracting adulteration of forest products (Chase *et al*., 2007). The genetic identification techniques require confirmed databases for reference. DNA barcoding is considered a promising method for species identification in the context of increased illegal logging and trade of threatened species as it is applicable for both wood and wood products. The limitation to effective use of DNA barcoding in wood markets is the lack of a robust and accurate DNA reference database of barcodes from wood. In this context, the creation of a DNA barcode database of common timbers in trade will be highly useful as a reference. This study aims at the

creation of a database integrating DNA barcoding with physical and anatomical characters of some important timber species currently used in Kerala.

The present study was carried out with the following specific objectives:

1. To confirm the authenticity of the wood samples based on their physical and anatomical characters.
2. To create a DNA Barcode database for *seven* selected timber species viz., Indian Sal (*Shorea robusta* Gaertn), Mangium (*Acacia mangium* Willd), Indian sandalwood (*Santalum album* Linn), Western red cedar (*Thuja plicata* Donn), Red sanders (*Pterocarpus santalinus* L.F), Mulluvenga (*Bridelia retusa* Spreng), Malabar neem (*Melia dubia* Cav) which are popularly traded in Kerala.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Timber has played a vital role in the daily lives of people from time immemorial. However, putting the right timber species to the right use remains a challenge especially when we consider its availability. The shortage of timber supply from domestic sources widens the demand-supply gap for timber which may lead to adulteration practices in wood markets. We are in need of an unbiased system of wood identification to counteract unwarranted instances owing to the adulteration of traded commercial timbers. Although various keys for wood identification based on physical and anatomical properties are available, they can't always serve as an authentic source as the conventional identification techniques may fail as the age of the sample is very high and additionally need expertise and understanding of wood biology. Also, these properties show variations based on the conversion and end uses of timber. In this regard, this study aims at the creation of a database integrating DNA barcoding with the physical and anatomical characteristics of some important timber species traded in Kerala. The literature relating to this work *i.e.*, "Integration of DNA barcoding and wood anatomy for the identification of selected timbers" is reviewed and debated under the following headings:

1. Identification studies in Indian timbers
2. Identification studies of imported timbers
3. Literature on vessels
4. Literature on rays
5. Barcode based timber identification studies
6. The literature on DNA extraction, PCR, and Sequencing

2.1. IDENTIFICATION STUDIES IN INDIAN TIMBERS

The identification of timber based on the micro and macro properties of wood has a very long history. Wallich (1832) who was the Superintendent of Calcutta Botanical Garden prepared a list of Indian timbers, which is known to be

the oldest timber identification manual and consists of 457 species of Indian timbers and their description. He was appointed to several botanical missions by the Governor-General of India, especially in 1820-21, to Nepal and Burmese territory in 1826-27. He collected wood of native species on his such expeditions, which were sent to England, and deposited at the India House. The wood characters of each wood were labeled as their characteristics. Appended to the catalog are two valuable tables of experiments on the strength of some of the most important kinds of Indian timber, made by Mr. Kyd, at Calcutta, and by Major H. Campbell, in the gun-carriage manufactory at Cassipur, near Calcutta.

The oldest published record of the Timber identification manual was compiled by Troup (1909) who was a forest economist in FRI, Dehra Dun. He collected information of 553 species, which were India's major commercial timbers then. The publication aimed at helping foresters in identifying timbers based on their general characteristics and making decisions about the suitability of the timber for its specific purposes.

Lushington (1919) analyzed and published the gross anatomical characters of the timbers of the Madras presidency. His publication comprises descriptions of the timber species growing in Madras presidency along with the comparison of its characters with those imported during that time. A detailed account of India's commercial timbers is provided by Gamble (1922) that included a summary of common field identification features including colour, weight, hardness, grain characters, etc., backed by transverse wood section photomicrographs.

The common commercial timbers of India and their uses by Trotter (1929), a forest economist of Forest Research Institute, Dehra Dun comprises the description of 86 species of common Indian woods and also includes information about their seasoning, strength, sources of supply price, etc.

Pearson and Brown (1932) worked on the commercially important timbers of India. They published a paper entitled "Commercial timbers of India" which includes the wood anatomy of 320 trees species under 53 different families. The

book presented notes about the distribution, physical properties, mechanical properties, supply, and uses of each timber along with minute anatomy. The description is accompanied by transverse section photomicrographs of timbers and categorization as per their end uses. Chowdhury (1945) came up with a brief description of 26 Burmese timbers with gross structural features including Andaman padauk and teak. The attributes involve growth rings characters, characters of rays, pore arrangement, and parenchyma. Trotter (1959) provided information about 150 commonly trading timbers of India. His publication contains details like species name, vernacular and trade names, the weight of the timber, seasoning quality, their strength, durability, uses, supply sources, and price for every timber together with a brief description of the characteristics of the timber. He categorized the timbers according to their purposes to allow users to choose the best-suited timber species for their needs.

The Wood Anatomy branch of FRI, Dehra Dun, published series of a comprehensive compilation named, "Indian Woods" which deals with the Indian Timber (Chowdhury and Ghosh, 1958; Rao, 1963; Rao and Purkayastha, 1972). This publication mainly focused on the structure, identification, and use of timbers in the Indian subcontinent and Burma. The wood structure of certain shrubs was also briefly described, in addition to trees.

The gross and minute anatomical features of twenty-five commercial timbers of the State of Madras were recorded by Harikrishnan (1960). He mainly focused on the general properties and gross features of commonly grown timber species such as *Dalbergia latifolia, Mangifera indica, Adina cordifolia, Lagerstroemia microcarpa, Toona ciliata, Mesua ferrea, Artocarpus heterophyllus, Grewia tiliifolia, Pterocarpus marsupium, Palaquium ellipticum, Tectona grandis, Terminalia crenulata,* etc. He also mentioned the usage of each timber species. During this time, Ghosh (1960) put forward the importance of dichotomous keys related to the wood identification and he explained the ways to use a key.

Sharma (1964) carried out the morphological and anatomical investigations on *Artocarpus sp.* Forst. The method he adopted was dissections under a binocular microscope, hand sections, and microtome sections of variable thickness of the fruit of different ages. Rao and Juneja (1971) provided the key for identification to discern 50 common Indian commercial timbers.

The anatomical characteristics of important commercial timbers of Andamans were studied by Purkayastha *et al.* (1976). He published a very detailed overview of 36 timbers, which included the general properties, minute anatomy, gross structure, along with notes concerning their availability and uses. This manual aims to ease the identification of all the Andamans' important timbers in the field as well as in the laboratory.

A manual comprising of anatomical characters of 162 commercial common timbers of Kerala was published by Nazma *et al*. (1981). It aided in the identification of timbers based on their general characters and gross anatomy. The local name, trade name, distribution, tree characteristics, working properties, distribution, strength, and timber usage in addition to the identification characters were also given in this manual.

Bhat (1994) published a book on the wood properties of certain lesser-known trees of Kerala which include physical features like color, texture, grain, basic density, heartwood content, and shrinkage of 21 tree species in addition to their anatomical characters. His study found that a good proportion of the lesser-known native hardwoods have the timber value that can be exploited to make optimum use of the resource.

The Handbook of Lesser-Known Timbers by Bhat *et al*. (2007) is a ready reference source for users of timber to get to know the less-known domestic market timbers, especially in Kerala. The handbook includes the properties and uses of 77 timbers, of which 52 are imported species.

Saxena and Gupta (2011) studied the wood anatomy of the Indian subcontinent's family Salvadoraceae, with particular regard to the ultra-structure of the vessel wall. Their research explains the wood microstructure of *Azima tetracantha, Salvadora oleoides,* and *S. persica,* the members of the Salvadoraceae family in the Indian Subcontinent. For the separation of the species, an identification key was established based on anatomical features of the wood. Research using SEM showed the presence of vesturing in *Salvadora*'s inter-vessel pits.

A comprehensive study was conducted on the anatomical and physical properties of poplar wood by Pande (2012). He differentiated the wood of *Populus. ciliata, P. alba, P. euphratica,* and *P. deltoides* based on their anatomical and physical properties. He also reported the color differentiation of wood as; grey or brownish-grey in *P. ciliata*, sapwood white and heartwood red-colored and black near the pith in *P. euphratica*, whereas white often with a red or yellowish tinge in *P. alba* and white to off-white in *P. deltoides.*

Singh *et al*. (2013) studied the variations in wood anatomical properties of certain *Terminalia* species of Assam. They mainly focused on four species of *Terminalia* namely *T. arjuna, T. bellirica, T. chebula,* and *T. myriocarpa* to study variations in their wood elements. It was observed that the selected *Terminalia* species had diffuse-porous wood with indistinct growth rings in *T. arjuna* and *T. chebula* and distinct growth rings in *T. bellirica* and *T. myriocarpa*. Minimum and maximum vessel frequency were observed as 2 per mm^2 (*T. myriocarpa*) and 14 per mm^2 (*T. chebula*), while minimum and maximum rays per mm were observed as 8 per mm and 16 per mm in all selected species of *Terminalia* except *T. myriocarpa*. Tyloses were observed only in *T. myriocarpa*. Analysis of variance showed significant differences among the wood element dimension of all selected species. Fiber length showed a positive and significant correlation with vessel length and wood density and negative and significant correlation with fiber diameter but a non-significant and negative correlation with fiber wall thickness.

The physical, mechanical, and anatomical properties of mahogany which is exotic for South India have been studied by Anoop *et al.* (2014). They mainly targeted on the evaluation of different wood properties of *S. macrophylla* and the connection between these properties. They also tried to give reasons for the variation in the timber quality of specified species when it is grown outside its natural range. Integrating growth observations with information on parameters of wood quality would help to take judicious management decisions to raise and manage these species in the State.

Gupta (2016) studied the variations in the wood anatomy of hardwood species collected from the coal mines of Jharkhand. She succeeded in studying the detailed anatomical characters of 19 hardwood species collected from the biggest coal mine of India. This study brings out various facets of wood anatomy concerning mining stress by testing the hypotheses statistically.

Sahoo *et al.* (2017) studied the variations in vessel morphological parameters viz., vessel frequency, vessel diameter, vessel area, and vessel length of *Artocarpus hirsutus* wood from trees grown in different climatic zones of Thrissur district, Kerala. Significant variation was found only among agro-climatic zones for vessel length and not between girth classes within zones. In this study vessel area, diameter and frequency did not show any significant variation between three zones as well as between girth classes. Eco-anatomical properties like vulnerability and vessel mesomorphy were also calculated and vulnerability showed a significant difference between the three zones. Vessel mesomorphy values of anjily wood indicated that the species is mesic in nature. As the information on the wood properties of *Artocarpus hirsutus* is very scarce, this study can provide important details regarding the wood properties of this species.

2.2. IDENTIFICATION STUDIES OF IMPORTED TIMBERS

Large-scale urbanization and industrialization have created a massive gap in our country's demand and timber supply. The ban on all forms of felling in the natural forest has also made a significant contribution to the shortfall of timber. To

counteract the inadequate supply, the timber industry has resorted to large-scale imports of timbers from outside the country. A significant number of timbers are imported into our market which is mainly used for construction purposes. These timbers include Meranti (*Shorea sp*.), Pyinkado (*Xylia dolabriformis Benth.*), Teak (*Tectona grandis L.f*), Greenheart (*Ocotea rodiaei* (Schomb) Mez.), Kempas (*Koompassia malaccensis Maing*.), Kusia (*Nauclea diderrichii Kuntze*), Malaysian Padauk (*Pterocarpus sp.*) and Brazilian Rosewood (*Dalbergia nigra Fr. All*.). However, hardly any work has been done on identifying the above timbers, which were imported into the country. There is also much scattered and fragmentary information available about these timbers from the studies done in their native states.

Bond (1914) prepared a wood identification manual for hardwood and softwood species that are native to Tennessee. He prepared reliable information on how to identify wood using a hand-magnifying lens. It also provided a wood identification key for some common Tennessee species, a list of key species characteristics and a list of companies selling wood identification sample sets.

Photomicrographs are much reliable in the identification of certain timber species. Such 504 photomicrographs of softwoods and hardwoods of the world are provided in a work by Howard (1941a). He proposed that the minor anatomical photos would negate any confusion about a wood specimen's identity and sometimes even serve as a witness before the courts. Contemporary to the above study, Miles (1978) published a book with photomicrographs of important world timbers.

Greenheart (*Ocotea rodiaei*), a species used for lintels on Barbuda are studied and a brief note on identification was published in 1945. A series of manuals were published by Menon (1955; 1959; 1971) which describes the distinction between some of the confusing groups of timbers viz., light red, white and yellow Meranti using anatomical characters.

Microscopic features like growth ring, porosity, distribution of pores, parenchyma arrangements, odour, color, presence or absence of gum ducts, along with tree characters and distribution of some commonly imported timber species are put into his study by Titmuss (1971). Farmer (1972) published descriptions of 117 commercially important hardwoods which include hemlocks, merantis, kusia, kempas, fir, etc. He aims to provide information that will help hardwood users select and process the timber best suited for their purposes, taking into account the individual characteristics of timbers such as colour, grain, weight, strength, working properties, wood bending properties, natural durability, and preservation.

An elaborative work was done by Rendle (1970) where he compiled a three-volume book in the timbers of the world. He illustrated 200 species of better-known world timbers in colour with precise descriptions. The work helps industrialists and architects to select timbers based on their characters and uses. The third volume mainly considered the timbers from Asia, Australia, and New Zealand. Titmuss (1971) has provided details of the world's 252 commercial timbers with respect to their distribution and macroscopic features that help to identify timbers in the field, and that in some cases the microscopic features together with illustrations. This manual will help to identify some of the Indian market's commercially used timbers. Edlin (1977), who gave a description of 40 timbers commonly used in the world along with a collection of the actual wood samples, emphasized the importance of general and anatomical features for identification.

Kywe and Soe (1983) compiled an identification key of 25 commercial Burmese timbers based on the morphological and anatomical characteristics, which aid in understanding and identification of Burmese commercial timbers. The characteristics of the wood along with a brief description of the plant were given in the publication. Although Pearson and Brown (1932), in their "Commercial Timbers of India" have included some of the Burmese timbers along with those of the Indian ones, they have dealt only with the selected and limited species of Burmese timbers.

In 1984, a Forest Products Technologist named Martin Chudnoff conducted studies on the tropical trees of the world. He compiled the descriptions of 370 species or genera of tropical trees and their timbers grouped based on their regional origin: Africa, Tropical America, Southeast Asia, and Oceania. His work was mainly based on physical and mechanical properties, processing characteristics, and uses. Data was being gathered, evaluated, and synthesized from world literature. Extensive technical data tables are coded to allow for an easy comparison of species properties and to assist in the selection of woods best suited for end-use (Chudnoff, 1984).

A study was conducted on Kempas, (*Koompassia malaccensis*), a lesser-known species grown in Indonesia by Chong (1992). Newman *et al.* (1996). He studied the members of Dipterocarpaceae which occur in Malaysia's rainforests. Dichotomous keys were prepared based on forest characters, silvicultural information, wood, wood anatomy and uses. He also included the line drawings of leaves and fruits.

IAWA feature list in association with DELTA, option INTKEY (interactive wood identification) was set up by Richter and Trockenbrodt (1993; 1996). Currently, a dataset with 200 common timbers in trade is available, and new entries are made as time allows. The English and German versions of such keys are available. This key can also be used to access explaining notes and illustrations.

Five genera of Magnoliaceae consisting of 59 native species and 2 introduced species of China were taken into the study by Liangt *et al.* (1993). They described the wood anatomy such as its diffuse porosity, scalariform to opposite vessel wall pitting, scalariform perforations with few bars, etc. Their study proves that wood anatomical characters somewhat overlap between *Magnolia* and *Manglietia* and these genera are distinguishable using their wood anatomical properties. They also found the wood anatomy to be similar in the *Michelia* and *Magnolia* and *Kmeria* is the only genus in which crystals were observed.

Monteoliva and Olivera (1994) developed a system of identification of Argentinian woods. They used suitable features for describing the local woods along with the IAWA feature lists. They have found greater differences between earlier literature descriptions and their findings, which proved that the existing wood database is useful but more additions are required for the refining of the database.

An interactive software-based computer key was prepared by Brunner *et al.* (1994) which is composed of macroscopic properties of 115 most important tree species of Guinea. The end-users were forest products industry personnel. The key was published along with a synoptic key, which consists of information on the distribution, appearance, wood structure and properties of those species. The computer-based interactive identification keys were prepared for different species including tropical woods (Tochigi *et al.*, 1984), Japanese woods (Kuroda,1987; Izumoto and Hayashi, 1990), and Chinese woods (Yang and Cheng, 1990; Zhang *et al.*, 1986). Wood anatomy and identification were taught at the University of Stellenbosch using a software-based interactive programme (Wheeler and Baas, 1998).

Wood anatomical characters of 100 Guyanese timbers were studied by Miller and Detienne (2001). Complete anatomical descriptions and a dichotomous key were prepared for these 100 species which enabled the users for accurate timber identification. Their work was stick on to the IAWA List of Microscopic Features for hardwood Identification.

E. A. Wheeler and his coworkers published a paper dealing with the global variations in dicot wood anatomy. The publication was based on the database of inside wood and recommended inside wood resources for further studies (Wheeler *et al.*, 2007). It included comprehensive information on the ecological and geographical range of each species and on the diameter of vessels, density and cell length that would help promote the use of wood anatomical characteristics as indicators for macroclimate and ecology. Their work paved the way for

identification and tracing the ecophysiological history of woody dicots. They also showed the variation in anatomical patterns within families and orders.

Herbarium specimens of 10 common timber species of seven families in Nigeria were identified by Jayeola *et al.* (2009) in the study on the use of wood properties in the identification of selected timber species in Nigeria. They searched for the stable taxonomic micro attributes of each wood sample. The diagnostic features include characters of the treachery elements, in particular, the vessel; fiber and ray structure; intercellular canal and phloem parenchyma. All the 10 species showed variations in its grain from coarse or rough to smooth with a lustrous or glossy surface. *Afzelia toxicana var africana, and Cordia millenii*, of the 10 species studied, three possess hard texture, five fairly hard while two are soft. *Terminalia ivorense* and *Triplochyton scleroxylon* are coarse-grained while *Antiaris toxicana, Khaya ivoremse, Mansonia altissima, Milicia excelsa* and *Tectona grandis* are smooth-grained and lustrous.

Esteban and de Palacios (2009) conducted a comparison study of species of the family Abietoidae (Pinaceae) in which they included 51 species of the family. They assessed the systematic significance of the wood structure in that group. The characters which they studied include; the presence of traumatic and normal resin canal, the axial parenchyma characters, and the ray structure. Their comparative study of the wood anatomy of the family brings out the monophyly of the genera *Abies*, *Cedrus, Keteleeria, Nothotsuga, Pseudolarix*, and *Tsuga* which possess axial parenchyma having nodular transverse end walls in the regions at the boundaries of growth rings. The presence of crystals in the ray parenchyma along with pitted horizontal and nodular end walls of ray parenchyma cells are also added to the distinguishing characters. They subdivide a subfamily into two groups based on the characters of the axial resin canal whereas *Abies, Cedrus, Pseudolarix* and *Tsuga* possess axial resin canals, and *Keteleeria* and *Nothotsuga*, have both normal axial resin canal and a specific arrangement of traumatic axial resin canal. The monophyly of the group formed by *Abies, Keteleeria, Nothotsuga, Pseudolarix* and

Tsuga was proved using the molecular phylogenetic studies, with an exception of the inclusions of *Cedrus*.

Gasson *et al.* (2010) used quantitative wood anatomy, naive Bayes classification, and principal component analysis for the wood identification of *Dalbergia nigra.* The Brazilian rosewood (*Dalbergia nigra*) was in the market for more than 300 years. They distinguished *D. nigra* from similar species using qualitative and quantitative wood anatomy in combination with statistical analysis. They approached the study in three ways: (1) a comparison of qualitative and quantitative wood anatomy; (2) using an unsupervised classification technique, PCA; and (3) with a supervised classification technique, naive Bayes classification. Their study was focused on six Latin American species of *Dalbergia*, which are commercially important and having similarities in overall appearance and/or anatomy with that of *D. nigra.* The study showed that *D. cearensis* showed high degrees of variability among other species in having high vessel frequency (10 vessels per mm^2). *D. miscolobium* showed high ray frequency (100 rays per 10 mm^2) and the maximum number of axially fused rays than other species subjected to observations. Unidentified specimens can be determined as 'not nigra' with no false negatives using classification from Naïve Bayes. This proves that while wood anatomy alone is unlikely to provide the level of identification certainty required by legislation such as CITES; it is being used as a comparatively inexpensive and straightforward means of reducing the number of specimens that would require a more comprehensive study.

Gasson *et al.* (2011) made anatomical studies on the CITES-listed tree species. The wood anatomy of all angiosperm and conifer tree taxa currently listed under CITES is illustrated with light micrographs of low to high-power magnification. Their diagnostic wood characteristics are given in numerical codes obtained from IAWA Hardwood (Wheeler *et al.,* 1989) and Softwood (Richter *et al*., 2004). Lists of microscopic identification features for wood are summarised in two appendices. These explanations and illustrations may be used to identify the genus when carefully compared to look-alike non-CITES-listed timbers illustrated

and described in the web-database of Inside Wood or present in reference wood collections.

The wood anatomical variables and their relation to individual tree morphology and site conditions of tropical trees were studied by Fichtler and Worbes (2012). They studied 35 angiosperm families consisting of 83 genera and 111 species. The samples they collected were stem discs of 139 individual species. The samples were procured from the Tropical stem-disc collection of the Crop Science, Agronomy in the Tropics, University of Gottingen, Germany. These disc samples were subjected to early tree-ring studies. They observed the relationship between anatomical wood variables, the connexions between anatomical wood variables with individual tree morphology and tree age, the relationships between anatomical wood traits and tree growth to climate variables, variations in anatomical wood variables within and between families and sites, the relationship of wood variables to the phylogenetic background, etc. They came up with a strong correlation of 'vessel diameter' with other anatomical variables as well as with climate parameters and tree morphology. Their results emphasize that the tree size and crown exposure to light had a strong impact on the vessel size, thereby explaining the hydraulic stem architecture.

The wood density and anatomical variations of some mangrove species were taken into the study by Santini *et al.* (2012). They mainly concentrated on the mangrove species of New Zealand and Australia. They studied the microanatomical features of the species at the cellular level and came up with a conclusion that the high wood density was associated with large xylem vessels and thick fiber walls. Their work also showed that the wood density tends to increase with a decrease in phloem cells per growth layer of wood. The results of this work also proved the positive correlations of xylem vessels and wood density.

A comparative study of the wood anatomy of 15 woody species in North-eastern Mexico was conducted by Maiti *et al.* (2016). They developed a new method of species identification and quality determination of species using the

variations in wood anatomical characters as well as the hydrological architecture of the species. Their results showed great variabilities in morphology, length, wall lignification of fiber cells in the woods among species. They also showed that the lignification contributes to the high strength qualities of the timber and could be used for furniture, and the wood with low lignification and high parenchymatous cells could be put into use for soft poles and fences. They explained the suitability of woods with a broad lumen and thin walls for use in paper manufacturing and pulping.

Hidayat *et al.* (2017) have done a comparative study on the anatomical characters of Moluccas a local tree species of Indonesia. Moluccan ironwood (*Intsia bijuga*), linggua (*Pterocarpus indicus*), red meranti *(Shorea parvifolia*), and gofasa (*Vitex cofassus*) were the species he considered for the study. The microanatomical observations were taken from the cross, radial, and tangential sections, and the dimensions of vessels, rays and fibers were measured for the quality evaluations. The results showed the presence of crystals in Moluccann Ironwood, Linggua and Gosfasa and resin canals in Red Meranti. Further evaluation showed that higher density Moluccan ironwood had thicker fibre walls, a higher amount of ray number, and wider rays than other species. Red meranti had ray height and fibre length values greater than the other three species.

2.3. LITERATURE ON VESSELS

A study conducted by Carlquist (1975) proved the relationships between the vessel morphology and ecology. Short vessel elements with simple perforation plates are characteristics of xeric conditions while scalariform ones remain restricted to plant taxa with a mesic or alpine ecology. Carlquist (1975) and Baas *et al*. (1983) explained that the more number of narrow vessels are the characteristics of xeromorphic wood in separate studies. Zimmerman (1983) came up with a correlation between vessel radius and matter conductivity i.e., conductivity is directly proportional to the vessel radius's fourth power. Carlquist (1975) put forth the idea that narrow vessels only permit water flow at slow rates.

A phenomenon called vessel grouping favors safety, common in the arid desert and the Mediterranean (Carlquist, 1989). The vessel size may be limited in arid and semi-arid environments in order to reduce inter vessel pitting and embolism by air seeding, a phenomenon described as gas being drawn through pit membrane pores (Wheeler *et al*., 2005; Sperry and Hacke, 2004). Pit membrane porosity between adjacent vessels could be designed to resolve the conflict between functional requirements to minimize vascular resistance, which favors thin porous membranes and limit the spread of embolism requiring robust membranes and smaller pores. Indeed, a decrease in the total area of the pit per vessel causes a decrease in the average size of the membrane pores and thus an increase in cavitation safety (Wheeler *et al*., 2005).

Positive correlations of the grouping of vessels and cavitation resistance had been stated by Lens *et al.* (2011). He described that the surrounding active vessels continue to maintain the 3D conductive pathway even if a particular vessel in a group embolizes.

The increased mechanical strength of Xeric species is characterized by the presence of vessels and imperforate tracheary elements with very thick walls and narrow lumen (Sperry, 2003). Alves and Angyalossy (2002) explained that vessels with a larger diameter are more efficient in conduction but are not so safe and in more prone to embolism, but vessels with lower diameters are safe although it reduces the conductivity of matters. The vessel diameter should not only be linked to sap transport, as it is also related to the higher or lower mechanical resistance of woods. Hence large-diameter vessels can lead to weaker woods compared to smaller-diameter vessels in woods. However, it is possible to keep high conductivity and mechanical resistance by combining large vessel diameter with fibers whose walls provide the resistance required of the tissue as a whole (Tyree *et al.*, 1994).

Hacke *et al*. (2009) proposed a central hypothesis of vascular transport, which describes that the safety of xylem from cavitation resistance is attained by

sacrificing the efficiency of the hydraulic transport system. In many situations, plants seem to have efficient xylem vessels that suggest selection against an inefficient set of traits. Xylem traits may reflect specific environmental or ecological/ selected relationships (Hacke *et al.,* 2009).

Trees growing in stressed conditions have vessels with a smaller diameter as the tree shift to safety by sacrificing its conduction efficiency (Tyree *et al*., 1994). Their stress avoidance strategy results in the competitiveness and survival of tree species along with its capacity to adjust and optimize its hydraulic architecture by vessel size and frequency differences to changing environments year by year (Fonti *et al.,* 2010). With these sites, the high variation in vessel diameter can be attributed to different plant strategies and a strong impact of individual tree morphology (Fitchler and Worbes, 2012).

Along with the vessel diameters, the pit features also result in the embolism of the vessel cells (Dickison, 2000). The small pits (Baas *et al.*, 1983) with tiny apertures (Tyree *et al*., 1994; Sperry and Hacke, 2004) offer a strategy for reducing the occurrence of cavitation irrespective of vessel size. The high vulnerability index is more efficient in the transport of water, but more vulnerable to embolism. Studies stress the importance of variability in diameter and frequency and the grouping of vessels in order to adapt water transport under various environmental conditions (Longui *et al*. 2012). Xylem development is suspended growing under pollution stress (Rajput *et al*., 2008).

2.4. LITERATURE ON RAYS

Many scientists including Alves and Alfonso (2002), used rays for wood ecological studies. Carlquist (1988) mentioned that there is a need for studying the relationship between ray frequency and environmental conditions, which was usually avoided by wood scientists and ecologists. Hardwood xylem contains about 17 % of rays on an average and may reach up to 30 % (Haygreen and Bowyer, 1982). Ray height and ray width were greater than in normal wood in wound altered wood of *Aspidosperma quebracho-blanco* (Bravo, 2010).

Chimelo and Mattos-filho (1988) studied the Brazilian woods and came up with a conclusion that wider rays are seen in Brazilian Cerrado and Caatinga vegetation (xeric conditions) species in comparison with the mesic forest species. A similar study showed that Brazilian species showed higher rays in species of more xeric habits and environmental stresses (Halbwachs and Kisser, 1967; Eckstein *et al.*, 1974; Grill *et al*., 1979; Kartusch and Halbwachs, 1985; Luchi *et al*., 2005).

Investing in parenchyma at stressed sites ensures a better and faster supply of reserves promoting quick flushing and earlywood production (Dunisch and Puls, 2003) which means that the investment in parenchyma is not arbitrary but simply reflects carbohydrate storage mechanisms and mobilization as a function of specific phenological behavior initiated by the limiting and dominating external growth factor. In addition, a greater number of parenchyma cells may be vital in the refilling of cavitated vessel mechanisms (Brodersen *et al*., 2010). Trees from less stressed sites appear to be able to invest more carbon in fiber cell structures that offer protection from mechanical and chemical pressures while stressed sites necessitate as much investment in parenchyma structures to ensure better growth (Poorter *et al*., 2009).

The ray height curve of the highly stressed site grew slightly in the second half of the 1970s, similar to the findings of Halbwachs and Kisser (1967). Different patterns of the trends discussed lead to a conclusion that a pulse-type disturbance on the strongly stressed site has modified stem growth and wood structure (Wimmer, 2002).

Novruzova (1972) claims a higher percentage of ray tissue in xerophytic species when compared with those in mesic sites and Chalk (1955) mentioned that wood samples with narrow rays (eg. Uniseriate rays) have a smaller proportion of the wood devoted to ray tissue whereas samples with wider rays have a higher proportion of wood present as ray tissue.

2.5 BARCODE BASED TIMBER IDENTIFICATION STUDIES

Using the high throughput DNA sequencing techniques, Hebert *et al.* (2003; 2004) carried out different experiments and suggested the application of universally amplified, highly variable, and short DNA barcode regions for species identification. The accuracies can be increased up to individual levels as well (Moritz, 2004) and can be applied in biodiversity surveys. DNA barcodes should have high interspecific variability which leads to accurate species identification and should possess highly conserved sites so as to be sequenced simply with standard protocols.

Some DNA barcode regions are already being commonly used in species identification. Coding regions of the small subunits (SSU) of rRNA genes are being used in the identification of Prokaryotes (Barns *et al*., 1996). For the animal kingdom, the mitochondrial cytochrome oxidase C gene (CO1) is being used as the universal barcoding region (Hebert *et al*., 2003), the same region shows enough variability and can also be used in distinguishing bird species (Hebert *et al*., 2004), fish (Ward *et al*., 2005), spiders (Greenstone *et al*., 2005) and certain butterflies (Hajibabaei *et al*., 2006). Studies were being conducted to assess the usefulness of CO1 for barcoding fungi (Seifert *et al*., 2007), diatoms (Evans *et al*., 2007), and red algae (Robba *et al*., 2006). Even if the mitochondrial marker cytochrome c oxidase I (CO1) plays a successful role in animals (Floyd *et al.*, 2009), studies conducted by Meier *et al.* (2006) in dipterans showed low success rates as well.

The significant developments in the field of sequencing technologies improved the success rates of DNA barcode studies (Hebert *et al*., 2003). The prime plot of DNA Barcoding is for the robust identification of various lifeforms on Earth, using pre-defined segments of molecular sequence data. DNA barcoding has been employed in the error-free identification of fauna. For analyzing the DNA barcode data with utmost accuracy, certain approaches and tools have been developed (Bertolazzi *et al*., 2009; Chu *et al*., 2009; Kuksa and Pavlovic, 2009 Austerlitz *et al*., 2009; Sarkar *et al*., 2008). Since its beginning in the early 2000s, the DNA barcode studies matures and resulted in a new and improved method in species

identification science. Even though the taxonomic species identification studies were not replaced or replicated with the advancing DNA barcode studies (Hebert and Gregory, 2005; Ebach and Holdrege, 2005), the future of developing a catalog of plant species was secured with the advent of a short molecular sequence helpful in identification (Schindel and Miller, 2005).

Hollingsworth *et al*. (2011) defined three important principles of DNA barcoding. The stated principles for DNA barcoding include standardization, minimalism, and scalability. These principles explain the importance of the selection of the barcode region which involves choosing one or a few standard loci which can be sequenced without much effort and reliably with good success rates. These standards should be able to work in very large and diverse sample sets, resulting in easily comparable data to distinguish species from one another. Standard animal barcoding loci (CO1) meet the above criteria well (Hebert *et al*., 2003).

In animal cell DNA barcoding studies, the loci CO1, which is a haploid, uniparentally-inherited, single locus that exhibits high levels of discriminatory power, is used to distinguishing species (Fazekas *et al*., 2009). CO1 is a coding region that codes for proteins. They were present in a high-copy number per cell. The main advantage of these gene loci is that it is not prone to drastic length variations, strong secondary structure, micro-inversions, or frequent mononucleotide repeats. High-quality sequences can be recovered, using these characters along with well-developed primer sets, even from poorly preserved samples. But in plant DNA barcoding studies, the CO1 loci can't be considered as a universal locus for barcoding because of the relatively low rates of nucleotide substitution (Fazekas *et al*., 2008).

There are some limitations for the CO1 being used in plant species identification, which is, higher plant mitochondrial sequences are highly invariable (Chase, 2005), and a rapid rate of structural evolution (changes in chromosome fragment positions, incorporation of foreign DNA) is common in higher plant

mitochondria (Palmer, 1992), making it difficult to align and evolve the sequence information. (Kress *et al*., 2005). There is a need for finding and standardizing alternative DNA barcode regions other than the mitochondrial genome (Kress *et al*., 2005; Chase *et al*., 2007). Researchers focus on short representative DNA sequences from nuclear or non-coding and coding plastid regions (Newmaster *et al*., 2008; Ford *et al*., 2009).

Several pieces of literature describe that more challenging is the search for suitable genomic regions in plants. The ITS (Internal Transcribed Spacer) of the ribosomal DNA along with several regions in the plastid genome such as *rbcL, rpoC1, rpoB, ycf5, psbA-trnH, trnL, atpF-atpH, psbK-psbI*, etc., can be considered as better candidates for plant DNA barcoding (Meier *et al*., 2006; Savolainen, 2005; Chase, 2005; Kress and Wolf, 2005; Shaw *et al*., 2007; Taberlet *et al*., 2007; Lahaye *et al*., 2008; Ford *et al*., 2009). The members of the Consortium for the Barcode of Life (CBoL) have proposed the usage of only two markers namely *rbcL* and *matK* to barcode the land plants (Hollingsworth *et al*., 2009a), they also pointed out the drawback of low species identification rates of 70 %, and the limitation of the resolution to be distributed evenly across the land plant species. Four barcode regions *viz. rbcL, matK, trnH* and ITS2, of the genus *Salacia* (Celastraceae), were amplified using recommended specific primers with an efficiency of 100 % (Dev *et al*., 2015).

The studies of Chase (2007) showed that the applications of plant DNA barcoding can be usefully extended in distinguishing among the seedlings of forest species or applied when carrying out detailed biodiversity surveys in situations where taxonomic recognition is difficult. Even though the DNA barcoding in tropical plants possesses more difficulties than temperate species, studies on some species are published including the works on the *Compsoneura* genus of Myristicaceae, genus *Inga* in the Fabaceae, and the orchid family (Newmaster *et al.,* 2008). Studies conducted by the extraction of DNA is expected to be very difficult in tropical plant species, due to the presence of secondary metabolites (Coley and Barone, 1996). The higher concentration of secondary metabolites not

only hinders the DNA extraction protocols but also results in a reduction of the total performance of DNA barcoding (Friar, 2005). Moreover, the rate of lineage diversification in tropics is often high, leading to the frequent occurrence of explosive radiation (Linder, 2008; Richardson *et al.,* 2001). Researches in genus *Inga* confirmed that the efficiency of DNA barcode-based identification will be less when species tend to have lots of close relatives and reduced levels of interspecific divergence (Newmaster *et al.,* 2008), this limitation should be expected in other groups also (Couvreur *et al*., 2008). Starr *et al*. (2009) confirmed that DNA barcoding concepts will be more difficult for tree species than for non-woody species.

Gonzalez *et al*. (2009) conducted studies for developing protocols for identifying Amazonian timbers using DNA barcode, for each marker they obtained sequences for up to 430 of the total sampled individuals. More than 90 % of the samples showed high-quality sequences for *rpoc1, rbcL*, *rpoB,* and *tnrL* markers. Markers such as *psbA-trnH* and ycf5 showed lower sequencing success. The success of *matK* sequencing was only 70 %. The lowest sequencing success was obtained with ITS, which amplified in only 40 % of their samples.

DNA extracted from wood can put into different applications including forensic forestry and combating illegal trade if good quality DNA can be obtained from dried wood. The DNA from the dried wood of *Neobalanocarpus heimii* was used for providing general guidelines in DNA based authenticity testing. Cambium and sapwood showed high efficacy of DNA extraction than heartwood tissues (Tnah *et al*., 2012). The identity and provenance of felled trees can be determined accurately with the inbuilt unique properties of DNA within the timber if quality DNA can be retrieved from dried wood (Asia Forest Partnership, 2005). The origin or the source of timber can be differentiated from one another by utilizing the geographical structural variations in the chloroplast DNA (cpDNA) marker (Deguilloux *et al*., 2003a; Tnah *et al*., 2009), thereby combating illegal logging and aid in forest certification. The individual species identification can be done by using a highly polymorphic nuclear short tandem repeat (nSTR) marker in DNA extracted

from dried wood, where the illegal log could match its actual stump (Tnah *et al*., 2010), however, the fundamental challenge to use this DNA track-back system relies on the possibility to extract DNA from dried wood.

The standard gene regions *viz; rbcL, matK, and tnrH-psbA* in the chloroplast genome were analyzed to distinguish the wood adulterants East Indian sandalwood, and successfully utilized the single nucleotide polymorphisms (SNPs) identified with *rbcL* and *trnH-psbA* sequence of *Erythroxylum monogynum Roxb.* as well as with matK sequence of *Osyris wightiana Wall.* for the detecting adulterants of East Indian sandalwood. *Osyris wightiana* shows more similarities with *the S. album and* grouped together in the dendrogram (Dev *et al*., 2014).

However, DNA extracted from wood is usually of a low quality which adversely affects the success of DNA barcode studies, and The large fragments associated with the standard barcoding region are not possible to sequence often, which emphasizes the need for developing the shortest informative regions to reach successful identification through DNA barcoding. Successful extractions of DNA from the wood of older age up to 80 years are possible but the length of the DNA barcoding will be reduced correspondingly with time (Jiao *et al*., 2014). Jiao *et al*, (2014) extracted large quantities of DNA from the sapwood of *Aquilaria sinensis*, but the DNA extracted from the heartwood region is feeble and showed less amplification. The results showed that the average quantities of DNA from fresh sapwood and heartwood were 8.01 and 4.39 ng mg^{-1}. He explained the possibility of live parenchyma cells in the sapwood region, which increases the amount of DNA. Also, the DNA may get degraded during the process of heartwood formation.

There are routine studies happening in the extraction of DNA from fresh samples, but the challenges of extracting DNA from dried wood is not yet been coped up, only a few studies guide us in such perspective; *Robinia* species (de Fillippis and Magel, 1998), Oak (Dumolin *et al*., 1999; Deguilloux *et al*., 2002), *Gonystylus bancanus* (Asif and Cannon, 2005) and dipterocarps (Rachmayanti *et*

al., 2006; 2009). Studies showed that the extraction of DNA from the dried wood will not be straight forward (Deguilloux *et al*., 2002; Asif and Cannon, 2005). Chemicals used in preservation, natural degenerative process, microorganism decay and the presence of secondary metabolites hinder the process of extraction of high-quality DNA and limit amplification (Cano-Gauci, 1996; Deguilloux *et al*., 2002).

The use of DNA barcoding in specimen identification is accepted but criticized for seeking to circumvent the need for basic taxonomy when used for species discovery (Will *et al*., 2005). The success rate of DNA barcode-based identification is low when we understudied clades containing closely related species (Meyer and Paulay, 2005). DNA barcoding can be employed in forensic identification purposes as the capabilities increase and the cost of sequencing comes down (Iyengar, 2014; Linacre and Tobe, 2011). The future utility of DNA barcode methods for forensic timber identification can be foreseen by the availability of extensive online sequence databases (Ratnasingham and Hebert, 2007). The main barrier to using DNA barcoding for identification extensively is due to the lack of a reliable DNA reference library (Hartvig *et al*., 2015; Hassold *et al*., 2016; Zheng *et al*., 2016). There were studies attempting the use of molecular markers in identifying different timber species, but the prerequisite of a large sampling of wood tissue and effective DNA barcode to serve as a reference database has often limited its practical application for accurate identification (Bhagawat *et al*., 2005; Hartvig *et al.,* 2015; Hassold *et al*., 2016; Yu *et al*., 2017).

Many researchers proposed that multiple markers will be needed in order to overcome these limitations and to obtain adequate species discriminations. Different suggestions were coming up from different researchers following silico and laboratory-based assessments, these suggested barcode regions involved various combinations of seven plastid markers. Chase *et al*. (2007), propose the combination; *rpoC1+rpoB+matK or rpoC1+maiK+trnH-psbA*. Kress and Erickson (2007) proposed the combination of *rbcL+trnH-psbA and atpF-H+psbKI+matK* (Lahaye *et al.,* 2008). The lack of comparative data including all

candidate markers and a broad taxonomic sample were the biggest challenges in reaching an agreement on a standard barcode locus for plants (Lahaye *et al.*, 2008).

A direct comparison of seven candidate loci was done by two research groups. The results showed not much difference in the performance of different combinations of the seven selected markers and also they were not able to find a marker that is perfect in every aspect (Fazekas *et al*., 2008; Hollingsworth *et al*., 2009b). The research was done by Seberg and Petersen also pointed out the same conclusions (Seberg and Petersen, 2009). Discrimination of six *Pterocarpus* species using a single barcode was not sufficient to give an accurate resolution among the studied *Pterocarpus* species. When combining two to four barcodes, the highest rate of discrimination (100 %) was obtained by matK + ndhF-rpl32 + ITS2 and matK + rbcL + ITS2. Also, combinations of the barcodes that included ITS2 yielded higher rates of success than other combinations of chloroplast DNA barcode (Jiao *et al.,* 2018).

DNA barcoding is superior to traditional taxonomy in many ways (Hebert *et al.,* 2003; Hajibabaei *et al.,* 2007), and is relatively fast, accurate, and easy to use in wood identification studies because it is not affected by the developmental stage and morphology (Chase *et al*., 2007; Lu *et al*., 2015), does not require years of practical expertise and the necessary lab equipment are essentially ubiquitous in molecular biology labs across the world. Quoting the note of the plant Working Group of Consortium of the Barcode of Life, an ideal DNA barcode should have a highly universal primer pair, show high success rates of PCR amplification and sequencing and provide maximal species discrimination at the species level (Hebert *et al*., 2003; Kress *et al.,* 2005; Hollingsworth *et al.,* 2009b). Species-level identification of animals and medicinal plants can be effectively done through DNA barcoding (Zemlak *et al*., 2009; Zang *et al*., 2016), also can be aided in biodiversity assessment (Lahaye *et al*., 2008; Yan *et al.,* 2015) and used in monitoring the illegal trade of wildlife species (Baker, 2008, Baker *et al*., 2010; Gathier *et al*., 2013; Chang *et al*., 2016).

2.6 LITERATURE ON DNA EXTRACTION, PCR, AND SEQUENCING

The success of every DNA barcoding study mainly depends on the extractability of DNA from the specified plant part. DNA can be successfully extracted from both cytoplasmic as well as mitochondrial cells using different protocols. Apart from the animal cell, plant cells have a cell wall which makes the process of DNA extraction tougher. The presence of extractives and secondary metabolites will also hinder the availability of uncontaminated DNA.

Careful sample preparation is necessary for the successful DNA extraction, following tissue lysis, and isolating nucleic acids. The enzymatic digestion is used for the lysis of the tissue, usually, with Proteinase K, which helps in the degradation of proteins and rapidly deactivates nucleases otherwise the nucleases will degrade the DNA during the isolation and purification processes. The nucleic acids are distinguished from other cellular components by using isolation buffers and specially designed filter columns. The quality, quantity and purity of the extracted DNA depend on the condition of the biological source used in the extraction process. The biological sample collected from the field must be well preserved for the success of the barcode study. The exposure of the sample to killing agents like acetate, ethyl alcohol, or cyanide results in the degradation of DNA in museum specimens (Rohland *et al*., 2004; Chakraborty *et al*., 2006; Gilbert *et al*., 2007). Extraction of utilizable DNA from preserved museum samples are a bit challenging due to the presence of fixatives like formaldehyde or other aldehyde mixtures (France, 1996; Chase and Albert, 1998; Chatigny, 2000; Schander and Kenneth, 2003; Santos *et al*., 2008).

Gonzalez *et al.* (2009) developed a new and modified CTAB protocol from leaves and cambium tissue for the extraction of cytoplasmic DNA with high success rates. A Biosprint 15 workstation (Qiagen, CA) was used for the total DNA extraction, following the manufacturer`s protocols. Silica-gel membrane-based DNA extraction methods provide benefits such as ease of processing, comparatively low cost, and a high sample throughput (Tzschucke *et al*., 2002).

Genomic DNA was extracted with at most efficiency from the silica gel dried samples of the genus *Salacia* (Celastraceae), using the DNeasy Plant Mini Kit (Qiagen, California, USA) following the manufacturer's protocol with slight modifications (Dev *et al*., 2015). Muellner *et al*. (2011) extracted total genomic DNA from silica dried leaf as well as from herbarium specimen of the Meliaceae family following the cetyltrimethylammonium bromide (CTAB) procedure of Doyle and Doyle (1987).

A comparative study of three DNA extraction protocols was conducted in dry wood of *Neobalanocarpus heimii* (Dipterocarpaceae) for forensic DNA profiling and timber tracking using DNeasy Plant Mini Kit (Qiagen), modified CTAB protocol (Murray and Thompson, 1980) and modified CTAB with PTB (N-phenacylthiazolium bromide) protocol. DNA extraction from dried wood using the DNeasy Plant Mini Kit (Qiagen) was following the manufacturer's instruction. The CTAB was following the protocol proposed by Murray and Thompson (1980), and for CTAB with PTB protocol, an addition of 5.0 ml of 0.1 M PTB (Prime Organics) in the extraction mixture is made. The result showed that the overall efficacy of DNA extraction was higher for the cambium and sapwood than for heartwood tissues. Of the three-extraction method used DNeasy Plant Mini Kit yielded the maximum quantity of DNA (Tnah *et al*., 2011).

Specialized protocols were recommended for taxa containing high quantities of polysaccharides, mucopolysaccharides, polyphenols, resins, or other secondary metabolites. These substances tend to bind with the nucleic acid during the extraction procedure and interfere with reactions (Winnepenninckx *et al*., 1993; van Moorsel *et al*., 2000; Pirtilla *et al*., 2001; Nishiguchi *et al*., 2002; Thomson, 2002; Skujiene and Soroka, 2003). Many researchers worked to improve the availability of utilizable DNA in good quality and quantity including; nematodes (Bhadury *et al*., 2006), tardigrades (Schill, 2007; Sands *et al*., 2008), copepods (Schizas *et al*., 1997), collembolans (Porco *et al*., 2010), ticks (Hill and Gutierrez, 2003; Halos *et al*., 2004; Mtambo *et al*., 2006), fungi (Zhang *et al*., 1996; Fredricks *et al*., 2005; Muller *et al*., 1998), plants (Csaikl *et al*., 1998; Doyle and Doyle, 1987;

Sheperd *et al*., 2003; Ribeiro and Lovato, 2007) and dried sandalwood (Dev *et al*., 2014).

Asif and Cannon (2005) yielded only low-quantities of DNA with less quality from processed wood of *Gonystylus bancanus.* Both the CTAB and the QIAGEN kit yielded DNA could not be amplified even though a detectable amount of DNA was present. The presence of Maillard products in the DNA or extractives such as terpenes, polyphenolics and polysaccharides resulted in the low DNA yield and failed PCR (Shepherd *et al*., 1997). The Maillard reaction of proteins and carbohydrates results in the DNA oxidation (Evershed *et al*., 1997). The condensation products of reducing sugars with primary amines (proteins) are Maillard products (Poinar *et al*., 1998), which could reduce the quality and yield f extracted DNA significantly.

DNA extraction from dried wood tissue is been more problematic, particularly from the heartwood region. The presence of high ranges of inhibitors like carbohydrates and phenols, and iron (for buried materials) will result in the yield of good quality DNA. All the inhibitors adversely affect the polymerase chain reaction (PCR), and other downstream processes involved in the successful DNA sequencing program. For improving the DNA harvest quality and to get good results in PCR, a number of components can be added to the DNA extraction step, including Proteinase K, Chelex®100 and PVP360 (Lowe, 2007). The Stoffel fragments and DSMO could help in increasing the rates of PCR for highly contaminated samples (Speirs *et al.,* 2009).

Studies have been conducted and succeeded in extracting good quality DNA from even processed and dried wood. Deguilloux *et al.* (2002; 2003b; 2004) successfully extracted DNA from dried wood of Oak timber of different ages and preserved under different conditions. Using contamination-exclusion techniques, Gugerli *et al*. (2005) developed protocols for extracting DNA from ancient sources of timber and successfully extracted and amplified DNA up to 500 bp from buried timbers that were up to 600 years old (Dumolin *et al*., 1999), 350 bp from buried

timber of age 3600 years (Tani *et al.,* 2003), 200 bp from marine timber of age 3600 years (Speirs *et al.,* 2009). Successful DNA extraction has been made from the xylarium specimens of different *Dalbergia* species. The specimens consist of heartwood, sapwood, and twigs. The samples were disrupted using an EFM freezer mill 6770 (SPEX SamplePrep, Metuchen, NJ, USA). DNA extraction was conducted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The average DNA quantity following the total genomic DNA isolation from sapwood, heartwood and twigs was 18.9, 10.6, and 28.8 ng mg^{-1} respectively (Yu *et al*., 2017).

During a study for identifying the Amazonian tree species with DNA barcodes, Gonzalez *et al*. (2009) standardized an amplification protocol for different gene regions for tree species. They performed amplification of coding plastid regions such as *rbcLa* (first part of the *rbcL* gene), *rpoC1, rpoB, matK, ycf5*; the non-coding regions *trnL* and *psbA-trnH*, and the nuclear region ITS. The standardized PRC mix included 0.2 µl of GoTaqh® 51 U/µl (Promega), 10 µl of 56 buffer, 1 µl of 20 µM for each primer, 1 µl of dNTP 10 mM, 1 µl of DNA template, and H2O for a final volume of 50 µl. MinElute PCR Purification Kit (Qiagen, CA) following the manufacturer's protocols were used for the purification of PCR products.

Both the cpDNA and mtDNA regions of the PTB extracted samples of *Gonystylus bancanus* were successfully amplified, one intergenic spacer region on the chloroplast genome (*atpbE* and *rbcL*) produced 800 bp long amplicon. But the extraction and amplification of DNA from a wooden desk older than 50 years was totally a failure, maybe due to the age of the wood used. The successful extraction of useful DNA from various wood samples from a range of plant taxa for PCR amplification and DNA sequencing is a necessary first step in developing DNA fingerprinting techniques for the monitoring and management of the tropical timber trade. The ability to amplify both long and short regions of DNA will allow the inclusion of standard DNA sequencing loci, as well as traditional microsatellite loci, in the genetic database (Asif and Cannon, 2005).

Good quality PCR bands were obtained successfully for the Mahogani family (Meliaceae) with the six coding plastid regions *rpoC1q, rpoB, accD, psbBT-N,* as well as non-coding plastid *trnhSG* and nuclear ITS, except with the *trnK* and *matK-2.1a* and *matK-3.2* primers with which PCR products only be obtained for a few samples. In the case of *YCF5,* all primer combinations were worked; the combinations of *YCF5-4* and *YCF5-1* show only weak bands. The primer combination *rpoC1-1* and *rpoC1-4* were used for the *rpoc1* gene, also *rpoC1-2* and *rpoC1-3* were used for the samples which showed less amplification with the former combination. Amplicons containing more than 600 bps are obtained with *rpoC1-1* and *rpoC1-4*, whereas the amplicon size for the primer combination *rpoC1-2* and *rpoC1-3* was about 400-450 bps. The primer combination *rpob-2* and *rpoB-4* were used for the amplification of the *rpoB* gene, the reaction resulted in amplicons of size 300bps (Muellner *et al*., 2011). The recovery success rate of DNA extracted from the Xylarium wood samples of six commercial *Pterocarpus* species was the highest for *ndhF-rpl32* (90 %), followed by *matK* (82 %) and *rbcL* (70 %), while *ITS2* exhibited the lowest rate (67 %). In total, 123 sequences generated in this work were deposited to GenBank (Jiao *et al*., 2018).

The amplicons were sequenced using BigDyeH Terminator cycle sequencing chemistry (v3.2; ABI; Warrington, Cheshire, UK). The sequencing reaction was carried out in a 10 ml reaction in ABI sequencer. The sequencing was carried out in both directions. SequencherTM 4.8 (gene Codes Corp., Ann Arbor, Michigan, USA) was used for visually inspecting and assembling the DNA fragments (Gonzalez *et al*., 2009). Their research results also showed that 10 % of the marker *psbA-trnH* was having difficulty in sequence from the 3' end. Muellner *et al*. (2011) used *ABI 3730* capillary sequencer (Applied Biosysytems, Inc., Warrington, Cheshire, UK) and a CEQ 8800 Genetic Analysis System (Beckman. Coulter, Krefeld, Germany), for sequencing the amplified DNA of the Mahogany family (Meliaceae).

The amplification reactions performed in different species of *Dalbergia* using the eight plant DNA barcodes *viz*. the chloroplast DNA *matK* and *trnL*, the

chloroplast intergenic spacer (*trnV-trnM1, trnV-trnM2, trnC-petN, trnHpsbA*, and *trnS-trnG*) and the nuclear DNA region *ITS2*; showed high success rate (>90%) for *trnV-trnM1*, *trnH-psbA* and *ITS2*, whereas *trnC-petN* showed lower PCR amplification efficiency of 44 % of the total sample. The bidirectional PCR showed 100 % efficiency for all the eight loci (Yu *et al.*, 2017), and developed 302 new sequences in *Dalbergia* which are added on to the existing database.

MATERIALS AND METHODOLOGY

MATERIALS AND METHODS

3.1 SAMPLE COLLECTION

Wood samples of seven tree species were collected from sawmills and timber dealers across Kerala. The imported timber samples were collected from Steepex India Trading LLP, Koyilandi, Calicut. (Table 1). The leaf samples of accessible species were collected in order to confirm the DNA sequences extracted from wood.

Table 1. Details of timber species collected for the present study

Sl no:	**Scientific Name**	**Trade name**	**Common name**	**Family**
1.	*Shorea robusta* Gaertn	Red Balau	Sal Tree	Dipterocarpaceae
2.	*Acacia mangium* Willd	Brown sal wood	Black wattle	Fabaceae
3.	*Santalum album* Linn	Sandal	East Indian sandalwood	Santalaceae
4.	*Pterocarpus santalinus* L.F	Red Sanders	Red Sanders	Fabaceae
5.	*Bridelia retusa* Spreng	Seikche Wood	Spinous Kino Tree	Phyllanthaceae
6.	*Melia dubia* Cav	Malai Vembu	Malabar Neem	Meliaceae
7.	*Thuja plicata* Donn	Cedar Wood	Western Cedar	Cupressaceae

The features which were used in the identification studies include:

(a) General features:

These include features that can be directly observed without the aid of a microscope. The major general features which were studied included:

1. Color
2. Weight
3. Odor

(b) Anatomical features:

These include features that can be observed with the aid of a microscope. The major features which were studied included:

1. Vessel tangential diameter
2. Vessel frequency
3. Ray width
4. Ray frequency
5. Fiber length

(c) Physical properties

The major features which were studied include:

1. Specific Gravity

3.2. TECHNICAL PROCEDURES

3.2.1. Sectioning

A small block from the samples was cut using an electric woodcutter and was soaked in hot water using a Rotek water bath at 70^{o}C for 24 hours. Then small blocks of 1x1x1cm were chiseled out from the soaked sample for the sectioning. The blocks were then subjected to microtomy. Transverse sections (TS), tangential longitudinal section (TLS), and radial longitudinal section (RLS) of 15 - 20 µm thickness were prepared using a Leica Sledge Microtome (Leica SM 2000R).

3.2.2. Staining

The sections were stained using safranin; the best sections were selected and passed through an alcohol series of 70%, 90%, and 95% for 5 minutes each. These sections were then passed through acetone for 3 minutes and air-dried using a tissue paper and were kept in 100% xylene for 3 hours. Sections were then mounted in a clean slide using DPX. The slides were kept dry for one day.

3.2.3. Image analysis

The permanent slides were analyzed with the help of a Catcam 500E microscope camera which was mounted on a Motic BA210 trinocular microscope. Photomicrography of the transverse, radial, and tangential sections and the measurements of the vessels and rays were taken using an image analyzing software (Catymage, Catalyst Biotechnology Pvt. Ltd)

The anatomical features covered included the arrangement, distribution, frequency, and size of the various cell elements *viz*; vessels, fibers, and ray parenchyma in the wood. These characters included both microscopic and macroscopic characters. For explaining macroscopic features, terminologies used by Rao and Juneja (1971) were made use of and for describing microscopic features, the IAWA list of microscopic features for hardwood identification (Wheeler *et al.*, 1989) and the timbers of Kerala (http://trees.kau.edu/), an open site of KAU was used. In addition, the expertise of highly expert wood anatomists was also used for the final identification of the wood.

3.3. DATA COLLECTION

3.3.1. Measurement of vessel and ray dimensions:

From the permanent slides prepared, observations like vessel diameter, vessel frequency from the transverse sections (TS), and ray width, ray frequency from the longitudinal sections (TLS) were calculated using the image analysis software. The diameter of the vessels and, the width and height of the rays were

calculated using the line tool. The vessel frequency was calculated by counting the number of vessels randomly selected area per field and using the equation;

$$\text{Vessel frequency} = \text{number of vessels} \times 10^6/\text{area in } \mu m^2.$$

The ray frequency can also be calculated by using the line tool. The number of rays covering a unit straight line gives the frequency of ray per µm.

3.3.2. Fiber Morphology:

3.3.2.1 Maceration

The collected wood samples of each species were macerated for measuring fiber characteristics mainly, fiber length. The maceration of the sample was done by Jeffrey's method, using Jeffry's solution. Jeffrey's solution was prepared by mixing 10 g Potassium dichromate and 14 ml Nitric acid. Chips of stem shavings were taken from the sample materials. These chips were boiled in the maceration fluid for 15-20 minutes in test tubes so that individual fibers separated. Then these test tubes were kept for 5-10 minutes so that the fibers settled at the bottom. The solution was discarded and the resultant material was thoroughly washed in distilled water until traces of acid were removed. The samples were stained using safranin and mounted on a temporary slide using glycerine as the mountant. Temporary slides were observed under the microscope. Measurement of fibre dimension was carried out using an image analyzer (CatCam 500E series).

3.3.2.2 Fibre length

Fibers obtained from maceration were observed using an image analyzer. The line tool was used to measure the fibre length. The images were taken in 10X. It was measured in micrometers (µm).

3.3.3. Analysis of physical properties:

Physical properties like moisture content (MC), specific gravity (SPG), and shrinkage (radial and tangential) can be analyzed in the wood anatomical studies. In this study, the specific gravity of the wood specimens was analyzed along with the Moisture content of the species.

3.3.3.1 Specific gravity

The specific Gravity of wood is the ratio between the oven-dry weight of the wood and the weight of an equal volume of water. For the analysis, wood specimens of 3x3x3 cm dimensions were taken. The samples were soaked in water in order to achieve complete saturation. The saturated samples were dipped in a beaker containing water by using a needle to obtain the volume. After recording the volume, the samples were kept for drying in a hot air oven for approximately 48 hrs. The oven-dry weight of the samples was recorded. Specific Gravity was determined by using the formula:

Specific gravity = oven-dry weight / green volume.

The volume of water displaced by water-soaked wood Specific gravity is the density of water at normal room temperature which is 1g/cc.

3.4. IDENTIFICATION PROCEDURES

One of the difficult tasks in wood identification is knowing to what level (family, genus, species group, or species) wood can be identified and when to be satisfied with an identification. Isolated pieces of wood usually cannot be identified to species, and often not to a single genus. There are no all-purpose rules. The level to which identification can be done varies within and between families. Identification, by comparison, is one of the most frequently used methods of identification and is the basis of most natural history field guides (Pankhurst, 1978). Wood anatomical atlases are the equivalent of such field guides. A particularly valuable atlas is the CSIRO Hardwood Atlas (Ilic, 1991) that has photographs (no text descriptions) of some 1800 species (cross, radial, and tangential sections are illustrated) and were intended to be a portable wood anatomical slide collection.

3.4.1. Dichotomous keys:

Dichotomous keys present a series of paired contrasting choices, with one or more features used at each dichotomy/couplet. At each couplet, one of the two statements is chosen as applying to the unknown. The key user is directed to another

couplet, and this process is continued until finally reaching a name (which for wood could be a species, species group, genus, group of genera, or family). The starting point and sequence in which features are used are predetermined by the author of the dichotomous key. The unknown must match every characteristic of the taxon as defined in the key and there is usually only one path to one's identity.

Dichotomous keys direct the observer to look for the features the key constructor considered, useful for distinguishing features; a well-constructed dichotomous key can quickly lead to identification. Dichotomous keys are useful for unknown species for which there is a small number of possible matches and for material with all features. They are particularly useful as regional works, and for commercially important woods, and for woods of a particular family or genus.

3.4.2. Computer-aided wood identification:

In this study, two major websites were used for the identification processes. They were "Inside wood" (http://insidewood.lib.ncsu.edu/search) and "Timbers of Kerala" (http://trees.kau.edu/index.php).

For describing microscopic features, the IAWA list of microscopic features for hardwood identification was made of. A handbook of lesser-known timbers by Bhat *et al*. (2007) was also referred. The number of features in the IAWA list (163 anatomical and 58 miscellaneous features) exceeds the number that could be accommodated on marginally perforated cards and allows a more complete description of woods, which enables to distinguish a greater number of species.

So, the collected wood samples were authenticated using the above-mentioned methods and were taken to the Biotechnology Laboratory under the Department of Biotechnology of the Kerala Forest Research Institute, Peechi where the extraction, amplification, and sequencing of DNA were carried out in order to develop the DNA Barcode based database.

3.5. MOLECULAR METHOD

3.5.1 Instruments used

- Cold centrifuge
- Electronic balance
- Gel electrophoresis unit
- Gel documentation system
- pH meter
- Nanodrop 1000
- Thermal cycler - 200
- UV transilluminator
- Water bath

3.5.2. Isolation of DNA by CTAB method

3.5.2.1 Materials

- Wood and leaf samples
- Liquid Nitrogen
- CTAB extraction buffer
    1. 2% CTAB
    2. 0.7M NaCl
    3. 0.05M EDTA
    4. 100 Mm Tris-HCl
    5. Distilled water
    6. RNase
    7. Chloroform: isoamyl alcohol
    8. 70% ethanol
    9. Sterile distilled water

3.5.2.2 Procedure

A hundred milligrams of tissue were grounded to a fine powder with liquid nitrogen using mortar and pestle. 1.5 ml of pre-heated 2 percent CTAB buffer was added. The slurry was poured into a 1.5 ml microcentrifuge tube and 10 µl RNase was added. The tube was incubated for 30 minutes at 65◦ C in a water bath. Gentle inversion was given in every 5 minutes. After incubation, an equal volume of chloroform: iso-amyl alcohol (24:1) was added and centrifuged at 5,000 rpm for 10 minutes. The supernatant was collected and transferred to a new microcentrifuge tube. An equal amount of chloroform: isoamyl alcohol (24:1) was again added and centrifuged at 5,000 rpm for 10 minutes. The supernatant was collected and an equal volume of chloroform: isoamyl alcohol was again added. Centrifugation was performed at 5,000 rpm for 10 minutes. The supernatant was transferred into a new microcentrifuge tube and two-volume of chilled ethanol was added. Then centrifugation was performed at 5,000 rpm for 5 minutes. Ethanol was decanted. DNA pellet was air-dried and dissolved in 50µl sterile double distilled water.

3.5.3. DNA Isolation by the kit method

3.5.3.1 Materials

- Wood and leaf samples
- Liquid nitrogen
- Buffer AP1
- RNase A
- Buffer P3
- Buffer AW1
- Buffer AW2
- Buffer AE
- Distilled nuclease-free water

3.5.3.2 Procedure

100 mg of tissue sample was weighed and powdered using liquid nitrogen in a mortar and pestle, 400 µl buffer AP1 and 4µl RNase A were then added. Vortexed and incubated for 10 minutes at 65℃. The tubes were inverted 2-3 times during incubation. 130µl buffer P3 was added, mixed, and incubated for 5 minutes on ice. The lysate was centrifuged for 5minutes at 14,000 rpm and was pipetted into a QIAshredder spin column placed in a 2ml collection tube. Centrifugation was performed for 2 minutes at 14,000 rpm. The flow-through was transferred into a new tube without disturbing the pellet if present. 1.5 volume of buffer AW1 was added and mixed by pipetting. Then 650 µl of the mixture was transferred into a DNeasy Mini spin column placed in a 2 ml collection tube. Centrifugation was done for 1minute at 8,000rpm and the flow-through was discarded. This step was repeated with the remaining sample. The spin column was placed into a new 2 ml collection tube and 500 µl Buffer AW2 was added. Centrifugation was carried out for 1minute at 8,000 rpm and the flow-through was discarded. 500µl buffer AW2 was again added and centrifuged for 2minute at 14,000rpm. The spin-column was removed from the collection tube carefully so that the column does not come into contact with the flow-through. The spin column was transferred to a new 1.5 ml or 2ml microcentrifuge tube. Then 100µl buffer AE was added for elution. The tube was incubated at room temperature (15-25℃) for 5 minutes and centrifuged for 1minute at 8,000rpm. Then the step above was repeated for proper elution. The DNA was quantified in the Nanodrop spectrophotometer (Thermoscientific Nanodrop 1000). The DNA sample was stored at 20℃ for downstream use.

3.5.4. Agarose gel electrophoresis

3.5.4.1 Materials

- DNA sample
- Agarose
- 1X TBE buffer
- Ethidium bromide

- Gel loading Buffer
- Electrophoretic apparatus

3.5.4.2 Procedure

1.5 gm of agarose was added to 80 ml 1X TBE buffer in a conical flask. The mixture was boiled until all the agarose was melted.1 µl ethidium bromide was added to the molten agarose. The agarose was poured into a clean gel casting tray, an appropriate comb was inserted and left for solidification. After solidification, the comb was removed carefully and the gel was placed into the electrophoretic apparatus containing running buffer. 4 µl of DNA sample and 2 µl of gel loading dye were mixed together and loaded into the wells. Then electrophoresis was carried out at a constant voltage (50 V) until the bromophenol blue front has migrated to the bottom of the gel. DNA was visualized using a Syngene gel documentation system.

3.5.5 Polymerase chain reaction

3.5.5.1 Materials

- 10 X Taq enzyme buffer
- dNTPs
- Forward primer
- Reverse primer
- Distilled water
- Taq polymerase
- Template DNA

3.5.5.2 Procedure

PCR reaction mix (20 µl) was prepared by the addition of template DNA extracted, 200 mM dNTPS, 20 pM/ 0.2 µl forward and reverse primer, 2U of Taq DNA polymerase, and 10X Taq buffer.PCR reaction consisted of an initial denaturation at 94℃ for 5 minutes followed by repeated cycles of denaturation at

94 ℃ reactions, primer annealing at respective annealing temperature for 1 minute and cycle extension at 72℃ for 10 minutes. The annealing temperatures of *trnH* and *rbcL* are the same whereas the annealing temperature of *matK* and *ITS* are different (Table 2). PCR amplifications were performed in a thermal cycler and the products were electrophoresed in an agarose gel and visualized under the gel documentation system.

Table 2. Barcoding primers used and PCR reaction conditions

Barcode region	**Primer**	**Primer sequence 5'-3' (Reference)**	**Annealing temperature**
rbcL	1F 724R	ATGTCACCACAAACAGAAAC TCGCATGTACCTGCAGTAGC (Kress *et al.*, 2005)	60° C – 40 s
matK	472F 1248R	CCCRTYCATCTGGAAATCTTGGTT GCTRTRATAATGAGAAAGATTTCTG C (Yu *et al*., 2011)	60° C – 40 s
psbA-trnH	trnH psbA	GTWATGCAYGAACGTAATGCTC CGCGCATGGTGGATTCACAATCC (Kress *et al.*, 2005)	59° C – 50 s

3.5.6. Elution of PCR products

3.5.6.1 Materials

- NT 1 buffer
- NT 3 buffer
- NE elution buffer

3.5.6.2 Procedure

PCR products were electrophoresed and viewed under UV transilluminator. The appropriate bands were excised and transferred into microcentrifuge tubes for further elution. To, 1. volume of sample, 2. volumes of NT1 buffer solution was added and the tubes were incubated for 5 minutes at 55^0 C in a water bath. The mixture was transferred into a Nucleospin Gel and PCR Cleanup column and centrifuged at 11,000 rpm for 30 seconds. After centrifugation, the flow-through was discarded and the column was placed back into the collection tube. 700 µl NT3 buffer was added to the column and centrifuged again at 11,000 rpm for 30 seconds. The above process was repeated and the column was air-dried for the complete removal of NT3 buffer. 15 µl NE buffer was added and centrifuged at 11,000 rpm for 1minute. The above step was repeated for complete elution. The Eluted PCR product was stored at -20^0 C for downstream processing.

3.5.7. Sequencing

The sequencing was done at Scigenome Labs Pvt. Ltd. Kakkanadu, Kochi, using an ABI 3730Xl sequencer (ThermoFisher Scientific, USA). The sequencing method adopted was Sanger Sequencing (Sanger *et al*., 1977).

RESULTS

RESULTS

The study titled “Integration of DNA barcoding and wood anatomy for the identification of selected timbers” has two objectives; the first one was to authenticate the collected wood samples. The authentication of the wood samples was done by comparing the (i) general features like colour and odour, (ii) physical properties such as moisture content and specific gravity, and (iii) micro-anatomical properties such as the number and distribution of vessels, rays, etc., with standard references such as Inside Wood database and Timbers of Kerala database. In the second objective, wood samples were also used for the development of the DNA barcode database as a supplementary tool for the precise identification of the wood species.

4.1. DESCRIPTION OF GENERAL FEATURES AND ANATOMY (HARDWOOD SPECIES)

4.1.1. *Acacia mangium* Will.

Trade name: Mangium

Local: Mangium

Family: Leguminosae

4.1.1.1 General features

Heartwood and sapwood are distinct by its yellowish-brown heartwood and creamy white sapwood. It has a coarse texture with straight grain and showed a specific gravity of 0.546.

4.1.1.2 Anatomical features

Vessel diameter ranges from 190 µm - 320 µm with a mean of 226.5µm.

Vessel frequency ranges from 12 numbers/mm^2 - 20 numbers/mm^2 with a mean of 16 numbers/mm^2.

Ray height ranges from 130 µm - 540 µm with a mean of 340.2 µm.

Ray frequency ranges from 7 numbers/mm - 10 numbers/mm with a mean of 9 numbers/mm.

Fibre length ranges from 1100 µm - 1400 µm with a mean of 1230µm.

4.1. 2. *Bridelia retusa* Spreng.

Trade name: Kasi

Local: Mulluvenga, Kaini

Family: Euphorbiaceae

4.1.2.1 General features

Heartwood and sapwood are distinct by their olive-brown heartwood and greyish white sapwood. The texture is observed to be fine to medium with shallowly or deeply interlocked grain. The growth rings were distinct and showed a specific gravity of 0.614.

4.1.2.2 Anatomical features

Vessel diameter ranges from 120 µm - 200 µm with a mean of 143.5µm.

Vessel frequency ranges from 6 numbers/ mm^2 - 12 numbers/ mm^2 with a mean of 9 numbers/ $mm^{2.}$

Ray height ranges from 910 µm -1520 µm with a mean of 1170 µm.

Ray frequency ranges from 5 numbers/ mm- 8 numbers/ mm with a mean of 6 numbers/mm

Fibre length ranges from 1100 µm - 1400 µm with a mean of 1097.9 µm.

4.1.3. *Melia dubia* Cav.

Trade name: Malabar Neem

Local: Kattu-veppu, Mala-veppu.

Family: Meliaceae

4.1.3.1 General features

Heartwood and sapwood distinction is clear with light red heartwood and grey or pinkish-white sapwood with a yellow cast. The wood is having a coarse texture with a straight grain. The growth rings were distinct and the wood showed a specific gravity of 0.537.

4.1.3.2 Anatomical features

Vessel diameter ranges from 120 µm - 200 µm with a mean of 132.7µm.

Vessel frequency ranges from 2 numbers/mm^2 - 9 numbers/mm^2 with a mean of 7 numbers/$mm^{2.}$

Ray height ranges from 530 µm - 866 µm with a mean of 645.3 µm.

Ray frequency ranges from 1 number/ mm - 5 nos/mm with a mean of 2 numbers/ mm

Fibre length ranges from 1100 µm - 1400 µm with a mean of 1190µm.

4.1.4. *Pterocarpus santalinus* L.f

Trade name: Red sanders

Local: Rakthachandanam

Family: Leguminosae

4.1.4.1 General features

The heartwood and sapwood are sharply demarcated. The heartwood is dark orange-red in colour whereas sapwood is yellowish-white. The growth rings are barely visible through hand lenses after sanding properly. The wood is having a fine texture with interlocked to wavy grain and with a specific gravity of 0.960.

4.1.4.2 Anatomical features

Vessel diameter ranges from 270 µm - 350 µm with a mean of 310.9µm.

Vessel frequency ranges from 2 numbers/ mm^2 - 6 numbers/ mm^2 with a mean of 3 numbers/$mm^{2.}$

Ray height ranges from 110 µm - 170 µm with a mean of 133.7 µm.

Ray frequency ranges from 9 numbers/ mm - 15 numbers/ mm with a mean of 11 numbers/ mm.

Fibre length ranges from 1100 µm - 1200 µm with a mean of 1111 µm.

4.1.5. *Santalum album* Linn.

Trade name: Sandalwood

Local: Chandanam

Family: Santalaceae

4.1.5.1 General features

There was a clear sapwood-heartwood distinction, the sapwood was whitish where the heartwood was yellowish in colour. Growth rings were distinct with straight grain and fine texture. The specific gravity was observed as 0.987.

4.1.5.2 Anatomical features

Vessel diameter ranges from 65 µm - 70 µm with a mean of 66.8µm.

Vessel frequency ranges from 6 numbers/ mm^2 - 12 numbers/ mm^2 with a mean of 8 numbers/ $mm^{2.}$

Ray height ranges from 250 µm - 300 µm with a mean of 272.4 µm.

Ray frequency ranges from 6 numbers/ mm - 9 numbers/ mm with a mean of 8 numbers/ mm

Fibre length ranges from 1200 µm - 2100 µm with a mean of 1431µm.

4.1.6. *Shorea robusta* Roth

Trade name: Red Balau

Local: Sal Tree

Family: Dipterocarpaceae

4.1.6.1 General features

Heartwood and sapwood showed a clear distinction. The heartwood was having brown colour whereas sapwood was white or pale white. The growth rings were indistinct with moderately coarse-textured and interlocked grain. The sample showed a specific gravity of 0.628.

4.1.6.2 Anatomical features

Vessel diameter ranges from 100 µm - 300 µm with a mean of 220.7 µm.

Vessel frequency ranges from 9 numbers/ mm^2 - 15 numbers/ mm^2 with a mean of 12 nos/ $mm^{2.}$

Ray height ranges from 630 µm - 925 µm with a mean of 766.8 µm.

Ray frequency ranges from 2 numbers/ mm - 5 numbers/ mm with a mean of 3 numbers/ mm

Fibre length ranges from 800 µm - 1500 µm with a mean of 1140.5µm.

An overview of the microanatomical properties of the samples under study is given in Table 3.

Table 3. Overview of microanatomical properties of the different wood samples

Sl. No.	**Name of the tree**	**Vessel diameter (µm)**	**Vessel frequency (no mm^{-2})**	**Ray height (µm)**	**Ray frequency (no mm^{-1})**	**Fiber length (µm)**	**Specific gravity**
1	*Acacia mangium*	226.5	16	340.2	9	1230	0.546
2	*Bridelia retusa*	143.5	9	1170	6	1097.9	0.614
3	*Melia dubia*	132.7	7	645.3	2	1190	0.537
4	*Pterocarpus santalinus*	310.9	3	133.7	15	1111	0.960
5	*Santalum album*	66.8	8	272.4	8	1431	0.987
6	*Shorea robusta*	220.7	12	766.8	3	1140.5	0.628

The highest specific gravity was found in the *Pterocarpus santalinus* and *Santalum album.* The lowest specific gravity was estimated for *Bridelia retusa* and *Melia dubia.*

The highest vessel frequency was for *Acacia mangium* and the lowest for *Pterocarpus santalinus.* The highest vessel diameter was observed in *Pterocarpus santalinus* and the lowest for the *Santalum album***.**

The ray frequency was highest for *Pterocarpus santalinus* while it was lowest for *Melia dubia* and in the case of ray width, *Shorea robusta* showed the

highest value and *Bridelia retusa* showed the lowest value. The maximum fibre length was found for the *Santalum album* and minimum for *Bridelia retusa.*

4.2. ANALYSIS OF VARIATION (ANOVA) AND POST HOC ANALYSIS

Analysis of variation (ANOVA) was conducted for each character with different species combinations, to check whether these characters are significant in distinguishing the species considered for this study. The ANOVA tables and the results of multiple pairwise comparison analysis of every species for each character are given under separate headings.

4.2.1.Vessel Diameter

Table 4. One way ANOVA of all species for Vessel Diameter.

	Degrees of freedom	**Sum of squares**	**Mean sum of squares**	**F value**	**Pr(>F)**
Species	5	186320	37264	45.36	$1.86e^{-11}$ ***
Error	24	19718	822		

As the '*p*' value is less than the significance level 0.05, we can conclude that there is much significant difference in the mean value of vessel diameter for each species considered in this study. The table below shows the results of multiple pairwise-comparison analysis of every species for vessel diameter using the Tukey Honest Significant Differences test in RStudio (Table 5).

Table 5. Multiple pairwise-comparison analysis of each species combination for vessel diameter.

Name of the species Combination	**The difference in Mean Vessel Diameter**	**Corrected "*p*" Value**
Bridelia retusa - Acacia mangium	82.94	0.0015224**
Melia dubia - Acacia mangium	93.80	0.0003463**
Pterocarpus santalinus - Acacia mangium	84.46	0.0012380**
Santalum album - Acacia mangium	159.64	0.0000001**
Shorea robusta - Acacia mangium	5.74	0.9995166
Melia dubia - Bridelia retusa	10.86	0.9900962
Pterocarpus santalinus - Bridelia retusa	167.40	0.0000000**
Santalum album - Bridelia retusa	76.70	0.0035410**
Shorea robusta - Bridelia retusa	77.20	0.0033107**
Pterocarpus santalinus - Melia dubia	178.26	0.0000000**
Santalum album - Melia dubia	65.84	0.0148340*
Shorea robusta - Melia dubia	88.06	0.0007579**
Santalum album - Pterocarpus santalinus	244.10	0.0000000**
Shorea robusta - Pterocarpus santalinus	90.20	0.0005659**
Shorea robusta - Santalum album	153.90	0.0000002**

From the above table, the adjusted *p* obtained in the post hoc analysis is very much significant for many of the species combinations. The species combination of *Santalum album - Acacia mangium, Pterocarpus santalinus - Bridelia retusa, Santalum album - Pterocarpus santalinus, Shorea robusta - Santalum album* are very much distinguishable using the character vessel diameter. The table shows that the difference in the mean diameter of *Shorea robusta* to *Acacia mangium*, and *Melia dubia* to *Bridelia retusa* are not significant.

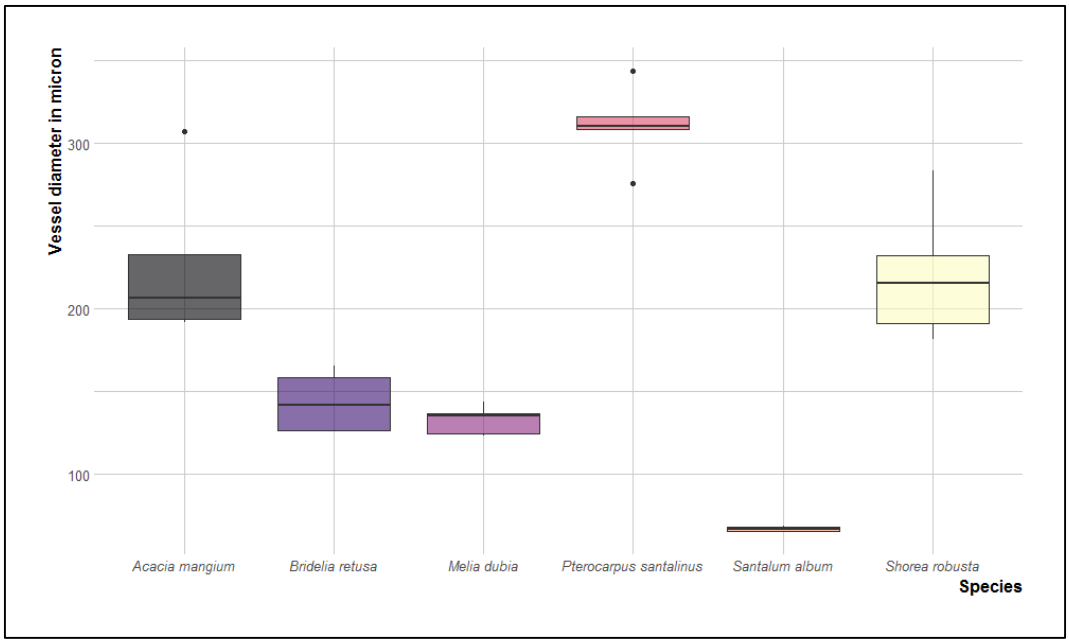

Figure 1. The comparison of mean vessel diameter for different species.

4.2.2.Vessel Frequency

Table 6. One way ANOVA of all species for Vessel.Frequency.

	Degrees of freedom	**Sum of squares**	**Mean sum of squares**	**F value**	**Pr(>F)**
Species	5	534.2	106.83	32.54	$6.11e^{-11}$ ***
Error	24	78.8	3.28		

As the '*p*' value is less than the significance level 0.05, we can conclude that there is much significant difference in the mean value of vessel frequency for species considered in this study. The table below shows the results of multiple pairwise-comparison analysis of every species for vessel frequency (Table 7).

Table 7. Multiple pairwise-comparison analysis of each species combination for vessel frequency.

Name of the species Combination	**The difference in Mean Vessel Frequency**	**Corrected "*p*" Value**
Bridelia retusa - Acacia mangium	7.2	0.0000231**
Melia dubia - Acacia mangium	10.2	0.0000001**
Pterocarpus santalinus - Acacia mangium	13.2	0.0000000**
Santalum album - Acacia mangium	8.2	0.0000030**
Shorea robusta - Acacia mangium	4.2	0.0137341*
Melia dubia - Bridelia retusa	3.0	0.1313664
Pterocarpus santalinus - Bridelia retusa	6.0	0.0002976**
Santalum album - Bridelia retusa	1.0	0.9494714
Shorea robusta - Bridelia retusa	3.0	0.1313664
Pterocarpus santalinus - Melia dubia	3.0	0.1313664
Santalum album - Melia dubia	2.0	0.5172528
Shorea robusta - Melia dubia	6.0	0.0002976**
Santalum album - Pterocarpus santalinus	5.0	0.0025648**
Shorea robusta - Pterocarpus santalinus	9.0	0.0000006**
Shorea robusta - Santalum album	4.0	0.0205808*

From the above table, the adjusted *p* obtained in the post hoc analysis is very much significant for most of the species combination. The species combinations which are much distinguishable using the character vessel frequency include *Santalum album - Acacia mangium, Pterocarpus santalinus - Acacia mangium, Melia dubia - Acacia mangium, Shorea robusta - Pterocarpus santalinus* since its '*p*' values are very much less than the significant value. The species pairs *Santalum album* from *Melia dubia, Santalum album* from *Bridelia retusa, Melia dubia* from *Bridelia retusa, Shorea robusta* from *Bridelia retusa* and *Pterocarpus santalinus* from *Melia dubia,* the p- values didn't show significant difference.

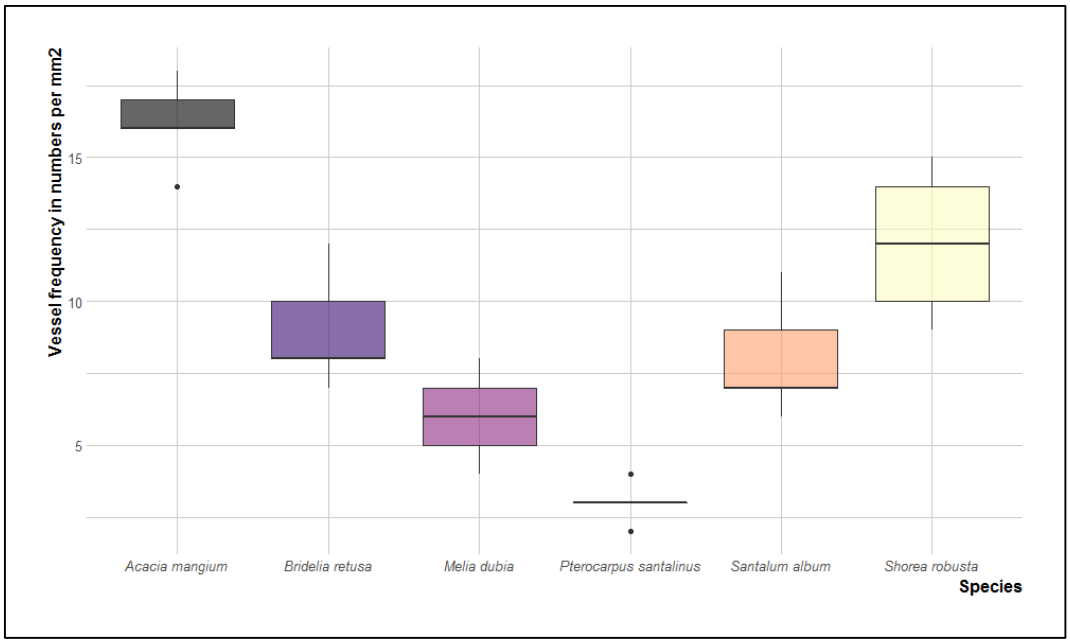

Figure 2. The comparison of mean vessel frequency for different species.

4.2.3. Ray Height

Table 8. One way ANOVA of all species for Ray Height.

	Degrees of freedom	**Sum of squares**	**Mean sum of squares**	**F value**	**Pr(>F)**
Species	5	3674448	734890	59.62	$9.54e^{-11}$ ***
Error	24	295816	12326		

As the '*p*' value is less than the significance level 0.05, we can conclude that there is much significant difference in the mean value of ray height for species considered in this study. The table below shows the results of multiple pairwise-comparison analysis of every species for ray height (Table 9).

Table 9. Multiple pairwise-comparison analysis of each species combination for ray height.

Name of the species Combination	**The difference in Mean Ray Height**	**Corrected "*p*" Value**
Bridelia retusa - Acacia mangium	829.94	0.0000000**
Melia dubia - Acacia mangium	305.12	0.002677**
Pterocarpus santalinus - Acacia mangium	206.48	0.068899
Santalum album - Acacia mangium	67.78	0.924427
Shorea robusta - Acacia mangium	426.62	0.000038**
Melia dubia - Bridelia retusa	524.82	0.0000014**
Pterocarpus santalinus - Bridelia retusa	1036.42	0.000000**
Santalum album - Bridelia retusa	897.72	0.000000**
Shorea robusta - Bridelia retusa	403.32	0.0000852**
Pterocarpus santalinus - Melia dubia	511.6	0.0000022**
Santalum album - Melia dubia	372.9	0.000247**
Shorea robusta - Melia dubia	121.5	0.526252
Santalum album - Pterocarpus santalinus	138.7	0.384448
Shorea robusta - Pterocarpus santalinus	633.1	0.0000001**
Shorea robusta - Santalum album	494.4	0.0000039**

The character ray height can be used as a significant anatomical parameter for distinguishing most species considered for the study, but it doesn't show a significant variation for *Santalum album* from *Bridelia retusa, Santalum album* from *Acacia mangium, Shorea robusta* from *Melia dubia,* and *Santalum album* from *Acacia mangium*.

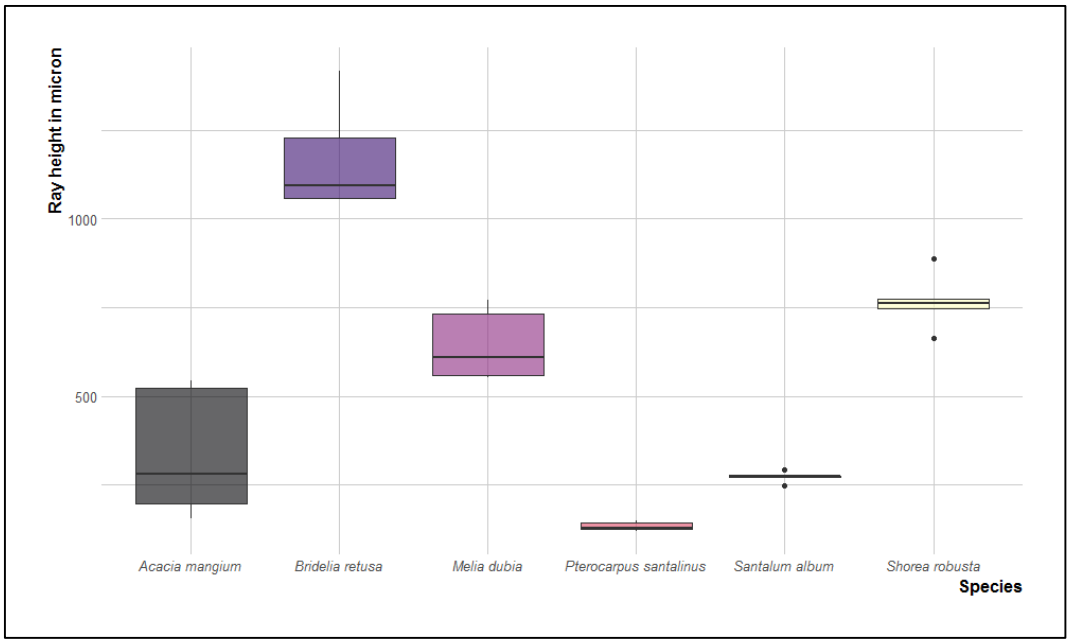

Figure 3. The difference in mean ray height for different species

4.2.4.Ray Frequency

Table 10. One way ANOVA of all species for Ray Frequency

	Degrees of freedom	**Sum of squares**	**Mean sum of squares**	**F value**	**Pr(>F)**
Species	5	307.5	61.50	52.71	$3.67e^{-11}$ ***
Error	24	28.0	1.17		

From the ANOVA table, we can conclude that there is much significant difference in the mean value of ray frequency for the species considered in this study. The table below shows the results of multiple pairwise-comparison analysis of every species for ray frequency (Table 11).

Table 11. Multiple pairwise-comparison analysis of each species combination for ray frequency.

Name of the species Combination	**The difference in Mean Ray Frequency**	**Corrected "*p*" Value**
Bridelia retusa - Acacia mangium	3	0.0023913**
Melia dubia - Acacia mangium	7	0.0000000**
Pterocarpus santalinus - Acacia mangium	2	0.0707799
Santalum album - Acacia mangium	1	0.6892013
Shorea robusta - Acacia mangium	6	0.0000001**
Melia dubia - Bridelia retusa	4	0.0000649**
Pterocarpus santalinus - Bridelia retusa	5	0.000002**
Santalum album - Bridelia retusa	2	0.0707799
Shorea robusta - Bridelia retusa	3	0.0023913**
Pterocarpus santalinus - Melia dubia	9	0.0000000**
Santalum album - Melia dubia	6	0.0000001**
Shorea robusta - Melia dubia	1	0.6892013
Santalum album - Pterocarpus santalinus	3	0.0023913**
Shorea robusta - Pterocarpus santalinus	8	0.0000000**
Shorea robusta - Santalum album	5	0.000002**

The character ray frequency can be used as a significant anatomical parameter for distinguishing most species considered for the study, except for distinguishing *Santalum album* from *Acacia mangium* and *Shorea robusta* from *Melia dubia,* since there is no significant difference in the mean values for this species.

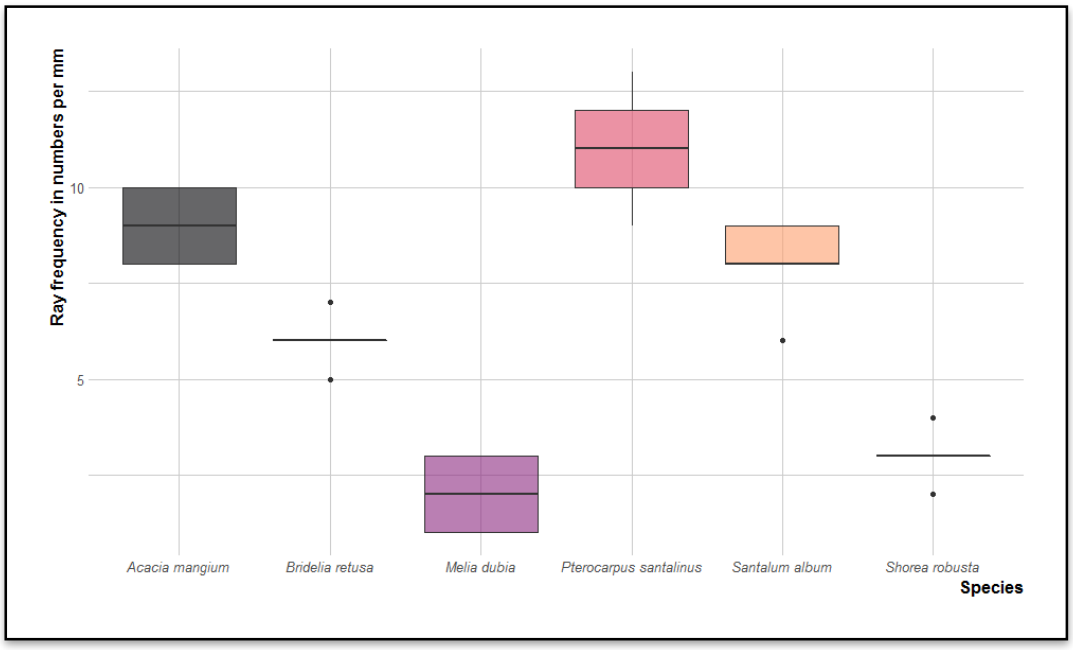

Figure 4. The difference in mean ray frequency for different species

4.2.5. Fiber Length

Table 12. One way ANOVA of all species for fiber length

	Degrees of freedom	**Sum of squares**	**Mean sum of squares**	**F value**	**Pr(>F)**
Species	5	380810	76162	2.702	$0.0449e^{-11}$ *
Error	24				

The ANOVA table concludes that fiber length alone could not distinguish many species considered for this study. The p-value in the table denotes a less significant difference in the mean fiber length of species under study. The table below shows the results of multiple pairwise-comparison analysis of every species for fiber length (Table 13).

Table 13. Multiple pairwise-comparison analysis of each species combination for fiber length.

Name of the species Combination	**The difference in Mean Fiber Length**	**Corrected "*p*" Value**
Bridelia retusa - Acacia mangium	132.56	0.808869
Melia dubia - Acacia mangium	39.96	0.998885
Pterocarpus santalinus - Acacia mangium	119.7	0.865226
Santalum album - Acacia mangium	200.22	0.434332
Shorea robusta - Acacia mangium	89.98	0.955193
Melia dubia - Bridelia retusa	92.6	0.949597
Pterocarpus santalinus - Bridelia retusa	12.86	0.999996
Santalum album - Bridelia retusa	332.78	0.045657*
Shorea robusta - Bridelia retusa	42.58	0.998487
Pterocarpus santalinus - Melia dubia	79.74	0.973068
Santalum album - Melia dubia	240.18	0.247912
Shorea robusta - Melia dubia	50.02	0.996747
Santalum album - Pterocarpus santalinus	319.92	0.059199
Shorea robusta - Pterocarpus santalinus	29.72	0.999735
Shorea robusta - Santalum album	-290.2	0.10505

The pair-wise comparison of mean fiber length doesn't showed significant variation for the studied species except for *Santalum album – Bridelia retusa* combination.

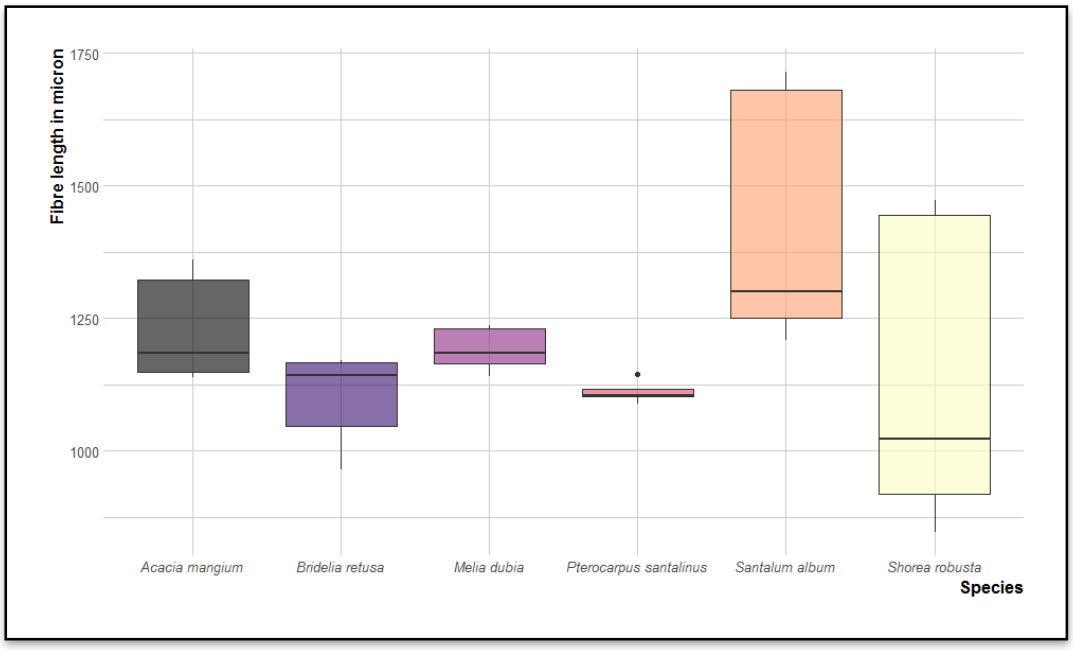

Figure 5. The difference in mean fiber length values for different species

The above-mentioned characters in combination could be used in identifying the wood species. The mean values and the range of each character for different species could be compared with existing databases and could be discussed in the next chapter.

4.3. DESCRIPTION OF GENERAL FEATURES AND ANATOMY (SOFTWOOD SPECIES).

4.3.1. *Thuja plicata* Donn

Trade name: Cedar Wood

Local: Western Cedar

Family: Cupressaceae

4.3.1.1 General features

The heartwood and sapwood were indistinct. The cross-section has a red-brown colour with a sweet aroma. The resin canals were absent. The timber showed a tight straight grain with a smooth texture. Intercellular spaces throughout the

wood were absent. The growth ring boundaries were distinct and the specific gravity of the sample was estimated as 0.344.

4.3.1.2 Anatomical features

Tracheid length ranges from 1400 µm - 3100 µm with a mean of 1734.6 µm.

Tracheid width ranges from 11 µm – 30 µm with a mean of 21 µm.

Ray height ranges from 325 µm - 655 µm with a mean of 432 µm.

Ray frequency ranges from 5 numbers/mm - 12 numbers/mm with a mean of 8 numbers/mm

An overview of the microanatomical properties of the sample (softwood) understudy is given in Table 14.

Table 14. Overview of microanatomical properties of the softwood samples

Sl. No.	**Name of the tree**	**Tracheid length (µm)**	**Tracheid width (µm)**	**Ray height (µm)**	**Ray frequency (no mm^{-2})**	**Specific gravity**
1	*Thuja plicata*	226.47	16	340	9	0.344

4.4. DNA ISOLATION

DNA was isolated from wood samples of *Acacia mangium, Bridelia retusa, Melia dubia, Pterocarpus santalinus, Santalum album, Shorea robusta,* and from the wood sample of *Thuja plicata* using the modified CTAB method and QIAGEN –Dneasy kit method. Modified CTAB method could yield intact DNA for all the species except *Thuja plicata,* but the Dneasy plant mini kit (QIAGEN) with some in house modifications could yield much better results.

The success rates of each protocol (CTAB and DNeasy Plant Mini Kit) for each species are shown in the table below (Table 15).

Table 15. The success rates of CTAB and DNeasy protocols for each species

Name of the species	**Isolation with CTAB protocol (%)**	**Isolation with DNeasy Plant Mini Kit protocol (%)**
Acacia mangium	60	80
Bridelia retusa	40	60
Melia dubia	40	70
Pterocarpus santalinus	50	60
Santalum album	60	70
Shorea robusta	50	80
Thuja plicata	-	60

The isolated DNA was resolved in order to check the quality and quantity of DNA. (Plate 2).

4.4. POLYMERISE CHAIN REACTION (PCR)

The extracted DNA of all species was subjected to Polymerase Chain Reaction with primers of *rbcl*, *matK* and *trnH-psbA.* All the species under study showed consistent amplification for the gene region *matK*. The PCR products were separated using 1.5 percent agarose for visualization and documented using gel documentation and analysis system (plates 3 and 4) The PCR products were scaled up to 40 µl. The eluted products were subjected to Sanger's dideoxy sequencing.

4.5. SEQUENCE ANALYSIS

The lengths of the sequences were 750bp, 700bp, 400bp, for *rbcL, matK, trnH-psbA,* respectively**.** The *matK* sequence of each species was manually edited and multiple sequence alignment was performed in Mega 7(Fig.6-12). Homology searches of all the sequences obtained from the selected timbers were performed using BLAST to confirm the identity of sequences.

Edited sequences were submitted to NCBI GenBank (http://www.ncbi.nlm.nih.gov/genbank/) as well as the BOLD public domain (http://www.boldsystems.org/) with group submission ID: 2381618.

The DNA barcode database could serve as a supplementary tool in wood identification. DNeasy plant mini kit with some in-house modifications could yield enough quantity of intact pure DNA. Among the three gene sites considered for the study *viz. rbcL, matK and tnrH-psbA,* the *matK* region showed the highest rate of amplification and quality sequence with an average sequence length of 700 bp for all species under study.

An overview of the DNA barcode sequences of the samples under study is given in Table 16.

Table 16. An overview of the DNA barcode sequences of the samples under study.

Sl. No.	Name of the tree	SEQUENCES (matK)
1	***Shorea robusta***	CCTCCTCTTTGCATTTATTACGGTTCCTTCTCTACAAGTATTGTAATTTTAAGAGTCTTATTACTCCAAAGAAATCCCCCTTTTTTTTGAATCCAAGATTGTTCTT GTTCCTATATAATTCTTATGTATGTGAATACGAATCCATTTTCCTTTTTCTCCGTAACCAATCCTCTCATTTACGATCAACTGCTTCTGGAGTCTTTCTTGAACGA ATCCATTTCTATCGAAAAATAGAGCATCTAGTAGAAGTTTTGGCTATGATTTTCAGGCTAACCTATGTTTGTTCAAGGATCCTTTCATACATTTTGTTAGATATC AAAGAAAATCAATTCTTTCTTCCAAGGATACGCCTCTTCTGATGAATAAGTGGAAATTTTACTTTGTCGATTTATGGCAATATTATTTTTACATGTGGTCTCAAT CAGGAAGGATACGGATCCGGATAAACCAATTATCAAAAAATTCTCTCGATTTTCTGGGTTATCGTTCAAGTATGCGATTAGATTCTTTAGTGGTACGGAGTCAA ATGCTAGAAAACTCATTTATAGTAGATAATGTTATGAAGAAGCTGGAGACAAAAATTCCAATTCTAGCCTTGATTGGATCATTGTCCAAAGCAAAATTGTGTA ACGCAATAGGACATCCCATTAGTAAGCCGATCTGGACCGACTCATCAGATTCTGATATTATTGATCGTTTTGTGCGTATATGCAGAAATCTTTCTCATTATTAC AGTGGATCCTCAAAAAAAAAAAATTTGTATCGAATCAAATATATACTT
2	***Acacia mangium***	CGATACTGGGTGAAAGATGCCTCCTCCTTTCATTTATTAAGGCTCTTTCTTTATGAGTATTGTAATTGGAATAGTCTTATTACTCCAAAAAAAAGGATTTCTACT TTTTCAAAAAGTAATCCAAGATTTTTCCTGTTCCTATATAATTTTTATGTATGTGAATACGAATCCATCTTTCTTTTTCTCCGTAACAAATCTTCTTATTTACGAT TAACATCTTCTGGAGTCTTTTTTGAACGAATCTATTTCTATGCAAAAATAGAACATTTTGTAGAAGTCTTTGATAAGGATTTTCCGTCCACCCTATGGTTCTTCA AGGACCCTTTCATTCATTATGTTAGATATCAAGGAAAATCCATTCTAGTTTCAAAGAATACGCCCTTTTTGATGAAAAAATGGAAATACTATCTTATCCATTTA TGGCAATGTCATTTTTTTGTTTGGTCTCAACCAGGAAAGATCCATATAAACCAATTATCCGAGCATTCATTTTACTTTTTGGGCTATTTTTCAAATGTGCGGCTA AATCCTTCAGTGGTACGGAGTCAAATGTTGGAAAAGTCATTTATAATGGAAAATCTTATGAAAAAGCTTGATACAATAATTCCAATTATTCCTCTAATTAGATC ATTGGCTAAAGCAAATTTTTGTAATGTATTAGGACATCCCATTAGTAAGCCGGTCTGGGCCGATTCATCCGATTTTGATATTATTGAGCGATTTTTGCAGATAT GCAGAGATCTCTCTCATTATTACAACGGATCCTCAAAAAAAAAGAGTTTGTATCGAATCAAATATATACTTCGGCTTTCTTGTATT
3	***Santalum album***	TAATTCTCATGTCCGTGAATACGAATCCATTTTCATTTTTCTCCGTAACCAATCTTCTCATTTACGATCAAGATCTTTTGGAACCCTTCTTGAGCGAATATATTTA CATGAAAAAATAGAACATATCGTGGATATGTTTACTAAGGATTTTCAGGCCATTCCATGGTTGTTAAAGGATCCTTTCATTCATTATGTTAGGTATCAAGGAAA AGTAATTCTGGCTTCAAAAGGGACGCCTCTCTGATGAATAAATGGAAATATTATCTTTCCAATTTCTGGCAATGTCGTTTTTATGTGTGGTCTCAACCAGGAAG GATCCATATAAACCAATTATCCAACCATTCCCTAGACTTTCTAGGCTATCTTTCAAGTGTACAAATAAATCCTTCCGTGGTGCGTAGTCAAATGCTAAAAAATT CATTTATAATAGATAATGGTATTAAGAAGTTTGATACCACAGTTCCAATTATTCTTTTGATTGGATCATTCGCTAAAGCAAAATTTTGTAACGTCTTAGGACATC CCATTAGTAAGTCGGTCTGGGCTGATTTAGCGGATTCTGAGATTATTGACCGATTTTGGCGTATATGCAGAAATCTTTCTCATTATCACAGCGGATCCTC

4	***Pterocarpus santalinus***	TGTATGTGAACACGAATCCATCTTCCTTTTTCTACGTAAGAAATCCTCTCATTTACGATTAAACTCTTATAGCGTTCTTTTTGAGCGAATCTATTTCTATGCAAA AATCGAACATCTTGTGGAAGTCTTTTCTAAAAATTTTTCGTCTACCTTATCCTTCTTCAAGGATCCTTTGATTCATTATGTTAGATATCAAGGAAAATCCATTCT GGCTTCAAAGAATGCGCCTCTTTCGATGAATAAATGGAAATACTATCTCATCTATTTCTGGCAATGTCATTTTGATGTTTGGTCTCAACCAGGAACGATCCATA TAAACCAATTATTATCCGAGCATTCATTTAACTTTTTTTGGGGGGGTTATCTTTCAAATGTGCGGCTAAATTTTTCAGTGGTACGGAGTCAAATGCTAGAAAATT CATTTCTAATCGAAATTGTTATGAAAAAACTTGATACAATAGTTCCAATTATTCCTTTAATTAGATCTTTGGCTAAAGCGAAATTTTGTAATATATTAGGGCATC CCATTAGTAAGCCCGTTTGGACCGATTCATCCG
5	***Bridelia retusa***	TTTTTCCAAAGAACTCTATTTCCATTTTTTCAAAAAGTAATCCAAGATTATTCTTGTTTCTATATAATTATCATGTATATGAATACGAATCCATCTTCTTTTTTCT CCGTAACCAATCCTTTCATTTACAATCAACATTTTTTCGGACTTTTGTTGAGCGAATATATTTCTATGGAAAAATAGAACATTTTGTGGAAGTCTTTGCTAATGA TTTTCAGGTCATTCTCTGGTTGTTCAAGGATCCTTTCATACATTATGTTAGATATCAAGGAAAGTCGATTCTGGTTTCAAAGGATACACCTTTTCTAATTAAAAA ATGGAAAAGTTACCTTGTCAATTTATGTCAATGTCATTTTGATCTGTGGTTTCAACCAAGAAAAATCTATATAACCCCATTATCGAAGCATTCTATCCACTTTTT AGGTTATCTTTCAAATCTACGACTCAGTCTTTTAGTGGTACGGAGTCAAATGTTGGAAATTCTTTTTTAATAGATAATACTATGAGGAAAGTCGATGGAATAAT TCCAATTATTCCTTTGATTCGTTCATTAGCAAAAACGCAATTTTGTAATGCAATTGGAGATCCTATTAGTAAATCTATTTGGGCTGATTCATCGGATTCCGATAT TATCAACCGATTTGTACGCATATGCAGAAATTTTTCTCATTATTATAGTGGGTCCTCAAAAAAAAGAGTTTGTATCGAATCAAATATATAC
6	***Melia dubia***	CATTTAAATTATGTGTTAGATGGACTAATACCCCACCCCATTCGCCCTGAAATATTGGTTCAACTCCTTCGCTACTGGGTAAAAGACGCCTCTTCTTTACATTTA TTACGGTTCTTTCTCACGAGCATTTTAATTTGAATTGGAATAGTCTTTTTAGTTCAAAGAACTCCATTTCCATTTTTTCAAACAGGAATTCAAGACTATTATTGTT TTTATATAATTCTTATGTATATGAATATGAATCCGTTTTCTTTGTTCTCTGTAACCAATTTTCTCATTTACGATCAACATCCTCTGGAGTCCTTGTTGAACGAATA GATTTCTATAGAAAAGTCGACGATCTTGTCGAAGTCTTTACTAATGATTTTCAGGACATCTTATGTTTGTTCAAGGATCCTTTTATGCATTATGTTAGATATCAA GGAAAATTCATTCTGGCTTCAAAAGATACGCCTCTTCTGATGAAGAAATGGAAATATTATCTTGTCAATTTATGGCAATGGCATTTTCACGTGTGGTCTCAACC AGGAATGTTTCATATAAACCACTTAGCCAAGCACTCTATCAACTTTTTGGGCTATCATTCCAGTGTACGACTAAATCCTTTGGTGGTACGGAGTCAACTGCTAG AAAATTCATTTCTAATAGATAATACTATGAAGAAGGTCGATCCAATCGTTCCAATTAGTCATCTGATTGGATCATTGACTAAGGCACGGTTTTGTAATCCATTA GGGCATCCCATCAGTAAGCCGACCTGGGCCGATTCCGCGGATTCTCATATTATCGGCCGATTTGTGCGTATATGCAGAAATCTTTCTCATTATTACAGCGGATC CTCAAAAAAAAAAGGTTTGTATCG
7	***Thuja plicata-***	ATGGATGAATTCCAAAGAAATTCAAACAAACATCGATCTTGGCAACAATTCTTTTTATATCCGCTTTTTTTTCGGGAAGATCTTTACGCAATTGCTCATGATCAT CATTTAGATAGATCTGGTTCCTCCGAACCAACGGAAATTTTATTTTCTCATTTTTTTAGTTTCCTAACTGTAAAACGTTCAATACGTCGGATACGTAAACAGAAT AAATCAATTAGTTTATTTGGGAATTCCGATTCAAATAAATTGATTGAATACAATAAGAATTTTTCTTTTAAATCGATGCTAGAAGGTTTTACAATTGTCTTGGA AGTTTCGATCGCAATGCGATCAAAACATTTCATAAAAGGAATGGATGGATGGAATAGTCTCCGATCCATCCATTGCATATTTCCTTTTATGGAGGATAAATTAC CACATTCCAATTATATATCAGATATACGAGTGCCCTACTCAATTCATCCGGAAATTTTGGTTCGAATTTTTCGTCGCTGGATCCGAGATGTTCCTTCTTTGCATT TATTACGATTGATTCTCCATGAATGGAAAAATAGTTTTAGTCAAGAAAATTTGGAGAAAGTTCTGATTACACAGAGGGGAAATACGAGGTTCTCCCTGTTCCTT TGGAATTCTTATGTGTATGAATGCGAATCGTTTTTAATTCCTCTTATTAAACGATTTTTTAATCCACAATCATTGTTATATGGATCTTTTCCCGATCGAACTCATT TTGAGAAAAAGATAAAAGATATTGTTATATTTCCTCTTCAGAAAATTTCAACAAAAAAGATCTGGTTGTTGAAGGATTCTTT

DISCUSSION

DISCUSSION

The results obtained from the study titled "Integration of DNA barcoding and wood anatomy for the identification of selected timbers" are discussed in this chapter.

The present study focuses on seven species of commercially important timbers that are currently used in Kerala. The study aimed at authenticating the selected timber species whose specimens were collected from different timber traders and sawmills across Kerala and to develop a DNA barcode database for supplementing the identification process.

The anatomical and physical parameters aided in the identification of wood samples were compared with the available database. DNA extraction, PCR amplification, and sequencing of the barcode were also done with modified protocols.

5.1. PHYSICAL PROPERTIES

Wood properties vary greatly within a tree and from tree to tree. Wood property variation patterns that arise from apical or cambial aging and positional effects of the crown are regarded as intrinsic. External factors such as environment, site conditions, and silvicultural treatments also have impacts on regular patterns of wood variation and these are regarded as extrinsic. Wood properties vary within the position in a tree and with the age at which the growth sheath is formed. Therefore, systematic radial and axial patterns of wood property variations can be identified (Amarasekera and Denne, 2002).

5.1.1. Specific gravity

Specific gravity may be the most widely studied property of wood. It is a function of the proportion of cell wall materials versus cellular voids. Many authors identify specific gravity as a key wood property in forest products because it has a major effect on the yield and quality of both fibrous and solid wood products (Bhat, 1985; Haslett and Young, 1990). As such, specific gravity is often considered as a

measure of wood quality (Zobel and Van Buitjenen, 1989; Woodcock and Shier, 2002). Numerous authors have classified timber species based on the specific gravity as it is the single best index that can be easily measured to predict the strength properties of wood (Bhat, 1985; Ruwanpathiranal *et al*., 1996).

The present study also validates that the wood specific gravity differed significantly between species. The highest specific gravity was found in the *Pterocarpus santalinus* and *Santalum album.* The lowest specific gravity was for *Bridelia retusa* and *Melia dubia.*

Nazma *et al.* (1981) found the mean value of specific gravity of *Santalum album* as 0.945 in India. The specific gravity of the *Santalum album* in this study was 0.987, which is really comparable with previous studies.

The slow growth of *Pterocarpus santalinus*, results in very heavy, dense heartwood, with air-dry specific gravity ranges of 0.87–1.2 (Gupta and Uniyal, 2003) and 1.04–1.15 (Chauhan and Rao, 2003). The present study also came up with a specific gravity ranges from 0.85-1.25 with an average of 0.960.

Cannel (1984) reported the wood specific gravity of *Shorea robusta* as 0.59. Studies on the specific gravity of 15 species of *Shorea* varied from 0.21-0.71 (Suzuki, 1999). Chaturvedi *et al*. (2010) reported that the specific gravity of *Shorea robusta* ranges from 0.46-0.73. The current study showed a relatable specific gravity value of 0.628.

In a study conducted on the behavior of five tree species in compression, the specific gravity of *Thuja plicata* was reported to be 0.32 (Ellis and Steiner, 2002). Miles and Smith (2009) reported that the specific gravity of the genus *Thuja* ranges from 0.28-0.40 and the specific gravity of *Thuja plicata* ranges from 0.30-0.40. The mean specific gravity of *Thuja plicata* according to this study was 0.344.

The range of minimum to maximum values of specific gravity for *Melia dubia* was 0.46–0.51 for a total GBH variation from 38 to 92 cm (Sharma *et al*, 2012**).** Saravanan *et al***.** (2014) reported that the wood specific gravity of *Melia*

dubia aged three, four and five years as 0.45, 047 and 0.60 respectively. This study also had a comparable result of a specific gravity range with a mean of 0.537.

In a study conducted to classify tropical timbers for kiln schedules, the specific gravity of *Bridelia retusa* was found to be 0.499 (Hidayat and Simpson, 1994). Shanavas and Kumar (2003) reported that the sapwood and heartwood specific gravities of *Bridelia retusa* were 0.696 and 0.723 respectively with a mean of 0. 620. The result of the present study also showed a comparable value for specific gravity of 0.614 as reported earlier.

Acacia mangium shows significant variations in the value of specific gravity with a change in provenance and site of sample collected, the specific gravity values obtained from Malaysia and Indonesia were lower when compared to the value obtained from Thailand and were 0.42, 0.47 & 0.55 respectively (Sahri *et al.*, 1998). Shanavas and Kumar (2003) reported that the sapwood and heartwood specific gravities of *Acacia mangium* were 0.42 and 0.49 respectively with a mean of 0. 516. The current study also had a comparable value for specific gravity (0.44).

5.2. ANATOMICAL PROPERTIES (HARDWOOD SPECIES)

Anatomy forms a strong basis for the identification of species. It is found that the transverse section of wood under magnification serves in the majority of cases as sure means of identification (Howard, 1941b).

5.2.1. Vessel morphology

Rao *et al*. (2003) studied the radial variation in anatomical properties of plantation-grown *Tecomella undulata*. They found that vessel frequency, vessel diameter and percentage of solitary vessels were interrelated and significantly varied from pith to the periphery. It was observed that the diameter of earlywood vessels increased along with cambial age whereas, the frequency of vessels decreased (Helinska and Fabisiak, 1999). So that slight variations in the observations of the current study can be of the above-described factors. The highest vessel frequency is for the *Acacia mangium* and the

lowest for *Pterocarpus santalinus.* While comparing the measurements of the current study with the measurements of the available databases, the vessel frequencies were almost similar and the species' were identified based on the results. The highest vessel diameter is found in *Pterocarpus santalinus* and the lowest for the *Santalum album***.**

The multiple pairwise-comparison analysis of every species for vessel diameter using Tukey Honest Significant Differences test in RStudio reveals that the species combinations of *Santalum album - Acacia mangium, Pterocarpus santalinus - Bridelia retusa, Santalum album - Pterocarpus santalinus, Shorea robusta - Santalum album* are very much distinguishable using the character vessel diameter.

The species combinations which are very much distinguishable using the character vessel frequency include *Santalum album - Acacia mangium, Pterocarpus santalinus - Acacia mangium, Melia dubia - Acacia mangium, Shorea robusta – Pterocarpus santalinus* since its '*p*' values are much less than the significant value. It is difficult to distinguish *Santalum album* from *Melia dubia, Santalum album* from *Bridelia retusa, Melia dubia* from *Bridelia retusa, Shorea robusta* from *Bridelia retusa* and *Pterocarpus santalinus* from *Melia dubia* since the '*p'* values of these combinations does not show a significant difference.

The comparison between the data obtained from the current study and the databases available are presented in the Tables below. The vessel frequency is given in Table 17 and the vessel diameter is given in Table 18.

Table 17. The comparison of vessel frequency between the current study and databases

Name of the tree	**Measurements from Inside wood. (no mm^{-2})**	**Measurements from Timbers of Kerala (no mm^{-2})**	**Measurements from the current study (no mm^{-2})**
Acacia mangium	5-20	12-18	16
Bridelia retusa	5-20	-	9
Melia dubia	<=9	5-10	7
Pterocarpus santalinus	<=5	3-5	3
Santalum album	5-20	8-12	8
Shorea robusta	5-20	10-15	12

Table 18. The comparison of vessel diameter between the current study and databases

Name of the tree	**Measurements from Inside wood. (µm)**	**Measurements from timbers of Kerala (µm)**	**Measurements from the current study (µm)**
Acacia mangium	100 - 200	170 - 280	226.47
Bridelia retusa	100 - 200	120 - 200	143.53
Melia dubia	100 - 200	-	132.64
Pterocarpus santalinus	100 - 200	70 - 320	310.93
Santalum album	50 - 100	55 - 70	66.84
Shorea robusta	100 - 200	200 - 350	220.74

5.2.2. Ray morphology

Wood properties such as ray characteristics are used to evaluate the suitability of wood for a particular application. Differences in ray properties are observed between species, sites, trees, and within the same tree. Ray tissue constitutes an average of 17 % of hardwood xylem, and sometimes it may reach more than 30 % (Haygreen and Bower 1982). Various studies (Liese *et al*., 1975; Eckstein *et al*., 1974; Grill *et al*., 1979; Schneider and Halbwacks, 1989) have found that the number of rays was significantly greater and the rays were shorter in height in conditions with increasing environmental stress. Higher rays were observed in species of more mesic habitat (Barajas- Morales, 1985) and a trend towards lower rays was observed in species of drier regions (Lens *et al*., 2004).

The ray frequency is highest for *Pterocarpus santalinus* and lowest for *Melia dubia* and in the case of ray hight, *Shorea robusta* showed the highest value and *Bridelia retusa showed* the lowest value.

The character ray frequency can be used as a significant anatomical parameter for distinguishing most species considered for the study, except for distinguishing *Santalum album* from *Acacia mangium* and *Shorea robusta* from *Melia dubia,* since there is no significant difference in the mean values for this species.

The character ray height can be used as a significant anatomical parameter for distinguishing most species considered for the study, but it cannot be used in distinguishing *Santalum album* from *Bridelia retusa, Santalum album* from *Acacia mangium, Shorea robusta* from *Melia dubia,* and *Santalum album* from *Acacia mangium* since there is no significant difference in the mean values.

The comparison between the data obtained from the current study and the databases available are presented in the Tables below. The ray frequency is given in Table 19 and the ray width is given in Table 20.

Table 19. The comparison of ray frequency between the current study and databases.

Name of the tree	**Measurements from inside wood. (no mm^{-1})**	**Measurements of Timbers from Kerala (no mm^{-1})**	**Measurements from the current study (no mm^{-1})**
Acacia mangium	4 - 12	9-12	9
Bridelia retusa	4 - 12	5-7	6
Melia dubia	<= 4	-	2
Pterocarpus santalinus	>= 12	13-16	15
Santalum album	4 - 12	6 - 9	8
Shorea robusta	4 - 12	2-5	3

Table 20. The comparison of ray height between the current study and databases.

Name of the tree	**Measurements of inside wood. (mm)**	**Measurements of Timbers of Kerala (µm)**	**Measurements of the current study (µm)**
Acacia mangium	<= 1 mm	250 - 390	340
Bridelia retusa	>= 1 mm	600 - 1690 (1070)	1170
Melia dubia	<= 1 mm		646
Pterocarpus santalinus	<= 1 mm	140 - 215 (160)	133
Santalum album	<= 1 mm	200 - 420 (295)	272
Shorea robusta	=> 1 mm	650 - 1090 (785)	766

5.2.3. Fiber morphology

The macerated fiber characteristics can be very helpful in the identification of small to large wood samples and various type of composites, which may not provide enough of all the surfaces necessary to reveal diagnostic characteristics (Wheeler and Baas, 1998) as well as sawdust (Tsoumis, 1985), wood fragments (Hoadley, 1990), veneers, small pieces of historical and archaeological wood (Jashemski, 1990; Safdari *et al*., 2008), decayed, stained, and finished wood and wood with preservatives which would not have the original colour and natural texture.

In the present study, the fibers of the six hardwood species were macerated according to the standard protocols and the mean fiber lengths were observed and compared with the available references in order to authenticate the species under study. *Santalum album* showed the highest mean fiber length while *Melia dubia, Pterocarpus santalinus,* and *Shorea robusta* did not show many differences. *Bridelia retusa* showed the least value for mean fiber length.

The mean fiber length alone could not be used as an anatomical parameter in wood species identification. It is clear that the mean fiber length of most of the sp[ecies combination used in this study is overlapped and the adjusted '*p'* value is having higher values than the significant value.

While comparing with the data obtained from the current study and standard references such as the Inside Wood database. The comparison between the data obtained from the current study and the database available is given in the table below (Table. 21).

Table 21. The comparison of mean fiber length between the current study and databases.

Name of the tree	Measurements of Inside wood (µm)	Measurements of the current study (µm)
Acacia mangium	900 - 1600	1230.44
Bridelia retusa	900 - 1600	1097.88
Melia dubia	<= 900 900-1600 >= 1600	1190.48
Pterocarpus santalinus	900 - 1600	1110.74
Santalum album	<= 900 900-1600	1430.67
Shorea robusta	900 - 1600	1140.47

5.3. ANATOMICAL PROPERTIES (SOFTWOOD SPECIES)

Softwoods are considered to have a much simpler structure than hardwoods and are comprised of a limited number and more uniform cell types, which comprises of longitudinal tracheid, which help in conduction and support, parenchyma cells, aided in storage and ray tracheid, helps in lateral conduction. According to the IAWA list of microscopic features for softwood identification, the key features help in distinguishing the softwood includes the morphology of tracheid, axial parenchyma, ray composition, intercellular canals, and mineral inclusions (IAWA Committee, 2004).

5.3.1. Tracheid Length

Anatomical comparison studies of North American Eastern, Southern, and Western Cedar Wood revealed the very small tracheid length of western cedarwood

(Eom *et al.*, 2008). According to the IAWA list of microscopic features for softwood identification, short tracheid length refers to length ranges below 3000 µm and was a characteristic feature of Cupressaceae family member (IAWA Committee, 2004).

Observations in the current study also indicated a comparable tracheid length ranging from 1400 µm - 3100 µm with a mean of 1734.64 µm, which is much comparable with the existing data.

5.3.2. Tracheid Width

Tracheids are arranged in orderly, neat rows within the trunk, and are longitudinal (they run lengthwise with the trunk). Tracheid can vary in diameter depending on the species, and because they account for over 90 % of the cells, their diameter greatly influences the overall texture and feel of the wood. Eric Meier stated that the tracheid width of *Thuja plicata* was very small usually below 25 µm., which resulted in its smooth grain (Meier, 2105).

In this study also, *Thuja plicata* showed a tracheid width ranging from 11 µm to 30 µm with a mean value of 21 µm and smooth texture.

The comparison between the data obtained from the current study and the databases available are given (Table 22).

Table 22. The comparison of measurements between the current study and previous studies.

Anatomical character	**Measurements in previous studies (µm)**	**Measurements of the current study (µm)**
Tracheid length	800 – 1600 (Eom *et al*, 2008) < 3000 (IAWA Committee, 2004)	1400 - 3100 (1734.64)
Tracheid width	< 25	11 – 30 (21)

5.4. DNA ISOLATION

The current study also aims at developing a DNA barcode database for the species whose identity was ascertained using the above discussed physical and anatomical properties. The major constraint in achieving the second goal was the isolation of good quality DNA from dried and processed wood samples.

The assumption of getting high quality and quantity DNA gets often violated when wood or wood products are the sources of DNA, and only short DNA barcodes can be reliably amplified (Tang *et al*., 2011; Jiao *et al*., 2012, 2014; Yu *et al.*, 2016). Recent studies showed an increasing interest in DNA methods to verify species identity and origin of internationally traded wood to combat illegal logging (Lowe and Cross, 2011; Lowe *et al*., 2016). Several studies have proved the ability of barcoding in effectively discriminating important timber species, such as agarwood (*Aquilaria sp.*) (Jiao *et al*., 2014; Lee *et al*., 2016), oak (*Quercus*) (Deguilloux and Petit, 2004), mahogany (*Swietenia*) (Degen *et al*., 2013),

Dalbergia species (Hartvig *et al*., 2015; Hassold *et al.*, 2016; Yu *et al*., 2016; 2017), sandalwood (*Santalum album*) (Dev *et al*., 2014).

Although various standard procedures are available in different pieces of literature regarding the successful isolation of DNA, the species under consideration and the age of wood samples may affect the success rate of getting intact DNA. Modifications in the standard isolation procedure for getting better results are also practiced. DNeasy Plant Mini Kit yielded the maximum quantity of DNA for the studied wood species in the current study. The efficiency of kit based DNA extraction over standard protocol was earlier demonstrated in several studies (Tnah *et al*., 2011). The wood samples of *Acacia mangium* and *Santalum album* yielded a considerable amount of DNA using the CTAB protocol, while the wood samples of *Thuja plicata* failed to yield quality DNA after isolation with CTAB protocol.

It was very hard to get intact DNA from the wood specimens of *Thuja plicata*. Being a softwood species, the polyphenolic contents, and the chemicals used in treating the timber negatively affected the rate of successful DNA isolation. The use of PVP (polyvinyl pyrrolidone) during the cell lysis stage resulted in an increased success rate of DNA extraction. PVP forms a complex with polyphenols through hydrogen bonding, allowing them to be separated from the DNA, and reducing levels of polyphenol in the product (Maliyakal, 1992; John, 1992). Wood samples soaked in water for 3-5 days followed by increasing the incubation time after sample treatment with lysis buffer in cold temperatures, showed a high yield of intact DNA while using the DNeasy Plant Mini Kit.

5.5. POLYMERISE CHAIN REACTION (PCR)

Standardized amplification procedures for specific gene regions are available for different species. According to different studies, seven standard candidate plastid DNA regions (*atpF-atpH* spacer, *matK* gene, *rbc*L gene, *rpoB* gene, *rpoC1* gene, *psbK–psbI* spacer, and *trnH–psbA* spacer) have species-level discrimination capabilities. Based on the assessments of recoverability, sequence

quality, and levels of species discrimination, Hollingsworth *et al.* (2011), recommend the 2-locus combination of *rbcL+matK* as the plant barcode. This core 2-locus barcode can provide a universal framework for the routine use of DNA sequence data to identify specimens and contribute toward the discovery of overlooked species of land plants (Hollingsworth *et al*., 2011).

In this study, barcode gene regions considered were the *rbcL* gene, *matK* gene and *trnH–psbA* spacer. Gene region *matK* showed the highest rate of amplification and quality sequence with an average length of 700 bp for all species under study. A comparison of gene regions tested for suitability and recommendations of DNA regions for barcoding is given below (Table 23).

Table 23. DNA segments tested for their suitability and recommendations of DNA markers for barcoding in land plants

DNA segment tested for suitability	**Proposed/recommended**	**Reference**
nrITS, rbcL, trnH–psbA	*nrITS and trnH–psbA*	Kress *et al*., 2005
ITS1, accD, ndhJ, matK, trnH-psbA, rbcL, rpoB, rpoC1, ycf5	*rbcL and trnH–psbA*	Kress *et al*., 2007
nrITS, accD, ndhJ, matK, trnH-psbA, rpoB, rpoC1, ycf5	*NrITS*	Sass *et al*., 2007
accD, matK, trnH–psbA, rbcL, rpoB, rpoC1	*matK and trnH–psbA*	Newmaster *et al*., 2009
matK, trnH-psbA, psbK-psbI, atpF-atpH	*matK or matK + trnH–psbA + psbK–psbI*	Lahaye *et al.*, 2008

accD, matK, trnH-psbA, rbcL, rpoB, rpoC1, ycf5, ndhJ	*matK or matK + trnH–psbA*	Lahaye *et al*., 2008
matK, rbcL, rpoB, rpoC1, trnH-psbA	*matK*	Starr *et al*., 2009
tpF–atpH, matK, rbcL, rpoB, rpoC1, psbK–psbI, trnH–psbA	*rbcL + matK*	Hollingsworth *et al*., 2009b
rbcL, matK, trnH–psbA	*matK*	Present study

SUMMARY

SUMMARY

The study entitled 'Integration of DNA barcoding and wood anatomy for the identification of selected timbers' was carried out for creating a DNA barcode database integrated with the anatomical characters of selected timbers that are currently traded in Kerala. The study also aims at confirming the identity of samples collected from timber merchants across Kerala by comparing the (i) general features like colour and odour, (ii) physical properties such as moisture content and specific gravity, and (iii) micro-anatomical properties such as the number and distribution of vessels, rays etc., with standard references such as Inside Wood database and Timbers of Kerala database. The results obtained from this study are summarized in this chapter.

1. Seven selected timber species that are regularly traded in Kerala were selected for the present study *viz*, Indian Sal (*Shorea robusta* Gaertn), Mangium (*Acacia mangium* Willd), Indian sandalwood (*Santalum album* Linn), Western red cedar (*Thuja plicata* Donn), Red sanders (*Pterocarpus santalinus* L.F), Mulluvenga (*Bridelia retusa* Spreng) and Malabar neem (*Melia dubia* Cav).
2. For the selected timber species, the wood specific gravity differed significantly between species. The highest specific gravity was found in the *Pterocarpus santalinus* L.F and *Santalum album* Linn. The lowest specific gravity was for *Bridelia retusa* Spreng and *Melia dubia* Cav.
3. Vessel frequency was higher for the *Acacia mangium* Willd and lowest for *Pterocarpus santalinus* L.F. The highest vessel diameter was found in *Pterocarpus santalinus* L.F and the lowest for the *Santalum album* Linn.
4. Ray frequency is highest for *Pterocarpus santalinus* L.F and lowest for *Melia dubia* Cav and in the case of ray width, *Shorea robusta* Gaertn showed the highest value and *Bridelia retusa* Spreng the lowest value.

5. *Santalum album* Linn showed the highest mean fiber length while *Melia dubia* Cav*, Pterocarpus santalinus* L.F*, and Shorea robusta* Gaertn did not show many differences. *Bridelia retusa* Spreng showed the least value for mean fiber length.
6. *Thuja plicata* Donn showed a tracheid width ranging from 11 µm to 30 µm with a mean value of 21 µm and smooth texture.
7. Each species showed clear distinctions in the amount of quality DNA obtained during isolation with different methods and modifications, the wood samples of *Acacia mangium* Willd and *Santalum album* Linn yielded a considerable amount of DNA using the CTAB protocol, while the wood samples of *Thuja plicata* Donn failed to yield quality DNA after isolation with CTAB protocol.
8. DNeasy Plant Mini Kit with some in-house modifications yielded the maximum quantity of DNA for all the wood species in the current study.
9. Wood samples soaked in water for 3-5 days followed by increasing the incubation time after sample treatment with lysis buffer in cold temperatures, showed a high yield of intact DNA.
10. Gene regions considered for the study were the *rbcL* gene, *matK* gene and *trnH–psbA* spacer. Gene region *matK* showed the highest rate of amplification and quality sequence with an average length of 700 bp for all species under study.
11. The obtained sequences were submitted to NCBI GenBank (http://www.ncbi.nlm.nih.gov/genbank/) with group submission ID: **2381618.**

REFERENCES

REFERENCES

Alves, E.S. and Angyalossy-Alfonso, V. 2002. Ecological trends in the wood anatomy of some Brazilian species. 2. Axial parenchyma, rays and fibers. *IAWA J*., 23: 391–418.

Amarasekara, H. and Denne, M.P. 2002. Effects of crown size on wood characteristics of Corsican pine in relation to definitions of juvenile wood, crown formed wood and core wood. *Forestry*, 75(1): 51-61.

Anoop, E.V., Jijeesh, C.M., Sindhumathi, C.R., and Jayasree, C.E. 2014. Wood physical, anatomical and mechanical properties of big leaf mahogany (*Swietenia macrophylla* Roxb.) a potential exotic for South India. *Res. J. Agric. For. Sci.*, 2: 7-13.

Asia Forest Partnership. 2005. Developing minimum standards of legality, timber tracking and chain of custody systems, verification systems among Asia Forest Partnership (AFP) partners. Work plan on legality, prepared by the Global For. Services (GFS) under the coordination of the Center for International For. Research (CIFOR)

Asif, M.J. and Cannon, C.H. 2005. DNA extraction from processed wood: a case study for the identification of an endangered timber species (*Gonystylus bancanus*). *Plant Mol. Biol. Rep.*, 23(2): 185-192.

Austerlitz, F., David, O., Schaeffer, B., Bleakley, K., Olteanu, M., Leblois, R., Veuille, M., and Laredo, C. 2009. DNA barcode analysis: a comparison of phylogenetic and statistical classification methods. *BMC Bioinformat.*, 10(14): S10.

Baas P., Werker, E., and Fahn, A. 1983. Some ecological trends in vessel characters. *IAWA Bull*., 4: 141-159.

Baker, C.S. 2008. A truer measure of the market: the molecular ecology of fisheries and wildlife trade. *Mol. Ecol.*, 17(18): 3985-3998.

Baker, C.S., Steel, D., Choi, Y., Lee, H., Kim, K.S., Choi, S.K., Ma, Y.U., Hambleton, C., Psihoyos, L., Brownell, R.L., and Funahashi, N. 2010. Genetic evidence of illegal

trade in protected whales links Japan with the US and South Korea. *Biol. Lett.*, 6(5): 647-650.

Barajas-Morales, J. 1985. Wood structural differences between trees of two tropical forests in Mexico. *IAWA Bull.*, 6: 355–364.

Barns, S.M., Delwiche, C.F., Palmer, J.D., and Pace, N.R. 1996. Perspectives on archaeal diversity, thermophily and monophyly from environmental rRNA sequences. In: *Proceedings of the National Academy of Sciences*, 93(17): 9188-9193.

Bertolazzi, P., Felici, G., and Weitschek, E. 2009. Learning to classify species with barcodes. *BMC Bioinformat.*, 10(S14): S7.

Bhadury, P., Austen, M.C., Bilton, D.T., Lambshead, P.J.D., Rogers, A.D., and Smerdon, G.R. 2006. Development and evaluation of a DNA-barcoding approach for the rapid identification of nematodes. *Mar. Ecol. Prog. Ser.*, 320: 1-9.

Bhagawat, P., Uppal, M., and Choi, G. 2005. FPGA based implementation of decoder for array low-density parity-check codes. In: *Proceedings.(ICASSP'05). IEEE International Conference on Acoustics, Speech, and Signal Processing, March 2005*, 5: v-29.

Bhat, K.M. 1985. Properties of selected less-known tropical hardwood. *J. Indian Acad. Wood Sci.*, 16(1): 26-35.

Bhat, K.M., Thulasidas, P.K., and Hussain, K.H. 2007. *A Hand Book of Lesser Known Timbers*. KFRI Research Report No. 304.

Bhat, K.V. 1994. *Physical and Anatomical Charateristic of Wood of Some Less-knownTree Species of Kerala.* KFRI Research Report 96.

Bond, B. 1914. Wood Identification for Hardwood and Softwood Species Native to Tennessee. [PhD Thesis]. The University of Tennessee, Institute of Agriculture, U.S. Department of Agriculture. 268p.

Bravo, S. 2010. Anatomical changes induced by fire-damaged cambium in two native tree species of the chaco region, Argentina. *IAWA J.*, 31 (3): 283–292.

Brodersen, C.R., Mc Elrone, A.J., Choat, B., Matthews, M.A., and Shackel, K.A. 2010. The dynamics of embolism repair in xylem: In vivo visualizations using high resolution computed tomography. *Plant Physiol*., 154: 1088–1095.

Brunner, M., Kucera, L.J., and Zurcher, E. 1994. *Major Timber Trees of Guyana - A Lens Key*. Tropenbos Series 10. The Tropenbos Foundation, Wageningen & Swiss Federal Institute of Technology, Zurich. 568p.

Cannel, M.G.R. 1984. Woody biomass of forest stands. *For. Ecol. Manag.,* 8: 299–312.

Cano-Gauci, D.F. and Sarkar, B. 1996. Reversible zinc exchange between metallothionein and the estrogen receptor zinc finger. *FEBS Lett.*, 386(1): 1-4.

Carlquist, S. 1975. *Ecological Strategies of Xylem Evolution*. University of California Press, Berkeley, 259p.

Carlquist, S. 1988. *Comparative Wood Anatomy. Systematic and Evolutionary Aspects of Dicotyledon Woods*. Springer, Berlin. 324p.

Carlquist, S. 1989. Anatomy of vine and liana stems: A review and synthesis. In: Putz, F.E. and Mooney, H.A. (eds). *The Biology of Vines*. Cambridge University Press, Cambridge, UK. pp. 53–71.

Chakraborty, A., Sakai, M., and Iwatsuki, Y. 2006. Museum fish specimens and molecular taxonomy: a comparative study on DNA extraction protocols and preservation techniques. *J. Appl. Icthyol.*, 22(2): 160-166.

Chalk, L. 1955. Ray volumes in hardwoods. *Trop. Woods*, 101: 1-10.

Chang, C.H., Lin, H.Y., Ren, Q., Lin, Y.S., and Shao, K.T. 2016. DNA barcode identification of fish products in Taiwan: Government-commissioned authentication cases. *Food Control*, 66: 38-43.

Chase, J.M. 2005. Towards a really unified theory for metacommunities. *Funct. Ecol.*, 19(1): 182-186.

Chase, M.W. and Albert, V.A. 1998. A perspective on the contribution of plastid rbcL DNA sequences to angiosperm phylogenetics. In: *Molecular Systematics of Plants II* . Springer, Boston, MA, pp. 488-507.

Chase, M.W., Cowan, R.S., Hollingsworth, P.M., van den Berg, C., Madrinan, S., Petersen, G., Seberg, O., Jorgsensen, T., Cameron, K.M., Carine, M., and Pedersen, N. 2007. A proposal for a standardised protocol to barcode all land plants. *Taxon*, 56(2): 295-299.

Chase, T.D., Johnson Controls Technology Co, 2007. Hvac control with programmed run-test sequence. U.S. Patent Application 11/678,172.

Chatigny, M.E. 2000. The extraction of DNA from formalin-fixed, ethanol-preserved reptile and amphibian tissue. *Herpetol. Rev.*, 31(2): 86.

Chaturvedi, R.K., Raghubanshi, A.S., and Singh, J.S. 2010. Non-destructive estimation of tree biomass by using wood specific gravity in the estimator. *Natl. Acad. Sci. Lett.*, 33: 133-138.

Chauhan, L. and Rao, R.V. 2003. *Wood Anatomy of the Legumes of India. Their Identification, Properties and Uses*. Bishen Singh Mahendra Pal Singh, Dehra Dun, India. 296p.

Chimelo, J.P. and Mattos-Filho, A. 1988. Preliminary wood structure observations of five hardwood species from different sites in Brazil. In: *Annals of 5th Conference of International Union of Forestry Research Organization (IUFRO*), *Sao Paulo–Brazil II*: p. 100.

Chong, S.H. 1992. *Studies on the Wood Properties of Lesser Known Species Grown in Indonesia. Wood Properties of Kereta, Kenari, Bitanagur, Giam and Kempas*. Research report of the Forestry Research Institute. Seoul. 46: 73-93.

Chowdhury, K.A. 1945. *The Identification of Burma Commercial Timbers*. Ind. For. Rec. Forest Research Institute, DehraDun. 26p.

Chowdhury, K.A. and Gosh, S.S. 1958. *Indian Woods, Their Identification, Properties and Uses. Vol I.* Manager of Publications, Delhi, 304p.

Chu, M., Vishwanath, K., Klose, A.D., and Dehghani, H. 2009. Light transport in biological tissue using three-dimensional frequency-domain simplified spherical harmonics equations. *Phys. Med. Biol.*, 54(8): 2493.

Chudnoff, M. 1984. *Tropical Timbers of the World*. United States Department of Agriculture. Agriculture Handbook Number 607. 464p.

Coley, P.D. and Barone, J.A. 1996. Herbivory and plant defenses in tropical forests. *Ann. Rev. Ecol. Syst.*, 27(1): 305-335.

Couvreur, T.L., Chatrou, L.W., Sosef, M.S., and Richardson, J.E. 2008. Molecular phylogenetics reveal multiple tertiary vicariance origins of the African rain forest trees. *BMC Biol.*, 6(1): 54.

Csaikl, U.M., Bastian, H., Brettschneider, R., Gauch, S., Meir, A., Schauerte, M., Scholz, F., Sperisen, C., Vornam, B., and Ziegenhagen, B. 1998. Comparative analysis of different DNA extraction protocols: a fast, universal maxi-preparation of high quality plant DNA for genetic evaluation and phylogenetic studies. *Plant Mol. Biol. Rep.*, 16(1); 69-86.

De Filippis, L. and Magel, E., 1998. Differences in genomic DNA extracted from bark and from wood of different zones in Robinia trees using RAPD-PCR. *Trees,* 12(6): 377-384.

Degen, B., Ward, S.E., Lemes, M.R., Navarro, C., Cavers, S., and Sebbenn, A.M. 2013. Verifying the geographic origin of mahogany (Swietenia macrophylla King) with DNA-fingerprints. *Forensic Sci. Int-Gen.*, 7(1): 55-62.

Deguilloux, M. F., Pemonge, M., and Petit, R. 2002. Novel perspectives in wood certification and forensics: dry wood as a source of DNA. In: *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 269(1495): 1039-1046.

Deguilloux, M.F., Dumolin-Lapegue, S., Gielly, L., Grivet, D., and Petit, R.J. 2003. A set of primers for the amplification of chloroplast microsatellites in Quercus. *Mol. Ecol. Notes*, 3(1): 24-27.

Deguilloux, M.F., Pemonge, M.H., and Petit, R.J. 2004. DNA-based control of oak wood geographic origin in the context of the cooperage industry. *Ann. For. Sci.*, 61(1): 97-104.

Deguilloux, M.F., Pemonge, M.H., Bertel, L., Kremer, A., and Petit, R.J. 2003. Checking the geographical origin of oak wood: molecular and statistical tools. *Mol. Ecol.*, 12(6): 1629-1636.

Dev, S.A., Anoop, B.S., Anoja, K., Udayan, P.S., and Muralidharan, E.M. 2015. Species discrimination through DNA barcoding in the genus *Salacia* of the Western Ghats in India. *Nordic J. Bot.*, 33(6): 722-728.

Dev, S.A., Muralidharan, E.M., Sujanapal, P., and Balasundaran, M. 2014. Identification of market adulterants in East Indian sandalwood using DNA barcoding. *Ann. For. Sci.*, 71(4): 517-522.

Dickison W. C. 2000. *Integrative Plant Anatomy.* Academic press, USA, 265p.

Doyle, J.J. and Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.*, 19(1): 11-15.

Dumolin, L.S., Pemonge, M.H., Gielly, L., Taberlet, P., and Petit, R.J. 1999. Amplification of oak DNA from ancient and modern wood. *Mol. Ecol.*, 8(12): 2137-2140.

Dunisch O. and Puls, J. 2003. Changes in content of reserve materials in an evergreen, a semi deciduous, and a deciduous Meliaceae species from the Amazon. *J. Appl. Bot*., 77: 10–16

Ebach, M.C. and Holdrege, C. 2005. DNA barcoding is no substitute for taxonomy. *Nature*, 434(7034): 697-697.

Eckstein D., Frisse, E., and Liese, W. 1974. Wood anatomical investigations on environmentally damaged street trees in downtown Hamburg. *Eur. J. For. Path.*, 4: 232-244.

Edlin, H. L. 1977. *What Wood is That? A Manual of Wood Identification*. Stobart and son Limited. London, 160p.

Ellis, S. and Steiner, P. 2002. The behaviour of five wood species in compression. *IAWA J.*, 23(2): 201-211.

Eom, Y.G., Kwon, O., Hanna, R.B., and Meyer, R.W. 2008. Anatomical comparison of North American eastern, southern, and western redcedar wood. *J. Korean Wood Sci. Technol.*, 36(2): 9-20.

Esteban, L. G. and De Palacios, P. 2009. Comparative wood anatomy in Abietoideae (Pinaceae). *Bot. J. Linn. Soc.*,160: 184–196.

Evans, K.M., Wortley, A.H., and Mann, D.G. 2007. An assessment of potential diatom "barcode" genes (cox1, rbcL, 18S and ITS rDNA) and their effectiveness in determining relationships in Sellaphora (Bacillariophyta). *Protist*, 158(3): 349-364.

Evershed, R.P., Bland, H.A., van Bergen, P.F., Carter, J.F., Horton, M.C., and Rowley-Conwy, P.A. 1997. Volatile compounds in archaeological plant remains and the Maillard reaction during decay of organic matter. *Science*, 278(5337): 432-433.

Farmer R. H. 1972. *Handbook of Hardwoods*. Her Majesty's Stationery Office. London, 243p.

Fazekas, A.J., Burgess, K.S., Kesanakurti, P.R., Graham, S.W., Newmaster, S.G., Husband, B.C., Percy, D.M., Hajibabaei, M., and Barrett, S.C. 2008. Multiple multilocus DNA barcodes from the plastid genome discriminate plant species equally well. *PLoS One*, 3(7): e2802.

Fazekas, A.J., Kesanakurti, P.R., Burgess, K.S., Percy, D.M., Graham, S.W., Barrett, S.C., Newmaster, S.G., Hajibabaei, M., and Husband, B.C. 2009. Are plant species

inherently harder to discriminate than animal species using DNA barcoding markers. *Mol. Ecol. Resour.*, 9: 130-139.

Fichtler, E. and Worbes, M. 2012. Wood anatomical variables in tropical trees and their relation to site conditions and individual tree morphology. *IAWA J.*, 33 (2): 119–140.

Floyd, R.M., Wilson, J.J. and Hebert, P.D., 2009. DNA barcodes and insect biodiversity. *Insect Biodiversity: Science and Society. Oxford: Blackwell Publishing*, pp.417-431.

Fonti, P., Arx, G.V., Garcia-Gonzalez, I., Eilmann, B., Sass-Klaassen, U., Gartner, H., and Eckstein, D. 2010. Studying global change through investigation of the plastic responses of xylem anatomy in tree rings. *New Phytol*. 185: 42–53

Ford, C.S., Ayres, K.L., Toomey, N., Haider, N., Stahl, V.A.J., Kelly, L.J., Wikstrom, N., Hollingsworth, P.M., Duff, R.J., Hoot, S.B., and Cowan, R.S. 2009. Selection of candidate coding DNA barcoding regions for use on land plants. *Bot. J. Linn. Soc.*, 159(1): 1-11.

France, R.L. 1996. Carbon-13 conundrums: limitations and cautions in the use of stable isotope analysis in stream ecotonal research. *Can. J. Fish. Aquat. Sci.*, 53(8): 1916-1919.

Fredricks, D.N., Fiedler, T.L., and Marrazzo, J.M. 2005. Molecular identification of bacteria associated with bacterial vaginosis. *New Engl. J. Med.,* 353(18): 1899-1911.

Friar, E.A. 2005. Isolation of DNA from plants with large amounts of secondary metabolites. In: Zimmer, E.A. and Roalson, E.H. (eds) *Methods in Enzymology: Producing the Biochemical Data.* Academic Press, pp. 1-12.

Gamble J. S. 1922. *A Manual of Indian Timbers*. [2nd Ed.]. Sampson Low, Marston and India Ltd. London. 578p.

Gasson, P., Baas, P., and Wheeler, E. 2011 . Wood Anatomy Of CITES-Listed Tree Species *IAWA J.*, 32 (2): 155–198.

Gasson, P., Miller, R., Stekel, D.J., Whinder, F., and Zieminska, K. 2010. Wood identification of Dalbergia nigra (CITES Appendix I) using quantitative wood anatomy, principal components analysis and naive Bayes classification. *Ann. Bot.*, 105: 45-56.

Gathier, G., van der Niet, T., Peelen, T., van Vugt, R.R., Eurlings, M.C., and Gravendeel, B. 2013. Forensic identification of CITES protected slimming cactus (Hoodia) using DNA barcoding. *J. Forensic Sci.*, 58(6): 1467-1471.

Ghosh S. S. 1960. Identification of timbers with special reference to the perforated card key. *Timber Trade*, 2: 1-13.

Gilbert, M.T.P., Moore, W., Melchior, L., and Worobey, M. 2007. DNA extraction from dry museum beetles without conferring external morphological damage. *PLoS One*, 2(3): e272.

Gonzalez, M.A., Baraloto, C., Engel, J., Mori, S.A., Petronelli, P., Riera, B., Roger, A., Thebaud, C., and Chave, J. 2009. Identification of Amazonian trees with DNA barcodes. *PLoS One*, 4(10): e7483.

Greenstone, M.H., Rowley, D.L., Heimbach, U., Lundgren, J.G., Pfannenstiel, R.S., and Rehner, S.A. 2005. Barcoding generalist predators by polymerase chain reaction: carabids and spiders. *Mol. Ecol.*, 14(10): 3247-3266.

Grill D., Liegl, E., and Windisch, E. 1979. Wood anatomical investigations on trees polluted with exhaust gas. *Phytopath*., 94: 335-342.

Gugerli, F., Parducci, L., and Petit, R.J. 2005. Ancient plant DNA: review and prospects. *New Phytol.*, 166(2): 409-418.

Gupta, A. 2016. Variations in wood anatomy of hardwood species collected from the coal mines of Jharkhand. [PhD Thesis]. Forest Research Institute (Deemed) University, Dehra Dun, Uttarakhand. 328p.

Gupta, S. and Uniyal, B.M. 2003. Indian Woods-their Medicinal Importance and Identification. *Indian For.*, 129(10): 1225-1240.

Hacke U. G., Jacobsen, A. L., and Pratt, R. B. 2009. Xylem function of arid-land shrubs from California, USA: an ecological and evolutionary analysis. *Plant Cell Environ.*, 32: 1324–1333.

Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W., and Hebert, P.D. 2006. DNA barcodes distinguish species of tropical Lepidoptera. In: *Proceedings of the National Academy of Sciences*, 103(4): 968-971.

Hajibabaei, M., Singer, G.A., Hebert, P.D., and Hickey, D.A. 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends Genet.*, 23(4): 167-172.

Halbwachs, G. and Kisser, J. 1967. Dwarfism in spruce and birch caused by smoke immissions. *Cbl. Ges. Forstwes.*, 84: 156-173.

Halos, L., Jamal, T., Vial, L., Maillard, R., Suau, A., Le Menach, A., Boulouis, H.J., and Vayssier-Taussat, M. 2004. Determination of an efficient and reliable method for DNA extraction from ticks. *Vet. Res.*, 35(6): 709-713.

Harikrishnan, M. 1960. *Twenty-five Important Commercial Timbers of Madras State with a Key to Their Identification*. Special paper submitted for the diploma in Forestry. Forest Research Institute, Dehra Dun. 58p.

Hartvig, I., Czako, M., Kjaer, E.D., Nielsen, L.R., and Theilade, I. 2015. The use of DNA barcoding in identification and conservation of rosewood (Dalbergia spp.). *PLoS One*, 10(9): e0138231.

Haslett, A.N. and Young, G.D. 1990. Plantation grown tropical timbers 1. Wood property and processing evaluation procedures to improve usage. *J. Trop. For. Sci.* , 1: 131-139.

Hassold, S., Lowry, P.P., Bauert, M.R., Razafintsalama, A., Ramamonjisoa, L., and Widmer, A. 2016. DNA barcoding of malagasy rosewoods: towards a molecular identification of CITES-listed Dalbergia species. *PLoS One*, 11(6): e0157881.

Haygreen J. G. and Bower J. L. 1982. *Forest Products and Wood Science.* Iowa State University Press. Ames, IA. 452p.

Hebert, P.D. and Gregory, T.R. 2005. The promise of DNA barcoding for taxonomy. *System. Biol.*, 54(5): 852-859.

Hebert, P.D., Cywinska, A., Ball, S.L., and Dewaard, J.R. 2003. Biological identifications through DNA barcodes. In: *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(1512): 313-321.

Hebert, P.D., Stoeckle, M.Y., Zemlak, T.S., and Francis, C.M. 2004. Identification of birds through DNA barcodes. *PLoS Biol.*, 2(10): e312.

Helinska, R. L. and Fabisiak, E. 1999. Radial variation of earlywood vessel lumen diameter as an indicator of the juvenile growth period in ash (Fraxinus excelsior L.). *Holz Roh. Werkst.*, 57(4): 283-286.

Hidayat, S. and Simpson, W.T. 1994. *Use of Green Moisture Content and Basic Specific Gravity to Group Tropical Woods for Kiln Drying*. Res. Note FPL-RN-0263. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. 39 p.

Hidayat, W., Kim, Y.K., Jeon, W.S., Lee, J.A., Kim, A.R., Park, S.H., Maail, R.S., and Kim, N.H. 2017. Qualitative and Quantitative Anatomical Characteristics of Four Tropical Wood Species from Moluccas, Indonesia. *J. Korean Wood Sci. Technol*., 45(4): 369-381.

Hill, C.A. and Gutierrez, J.A. 2003. A method for extraction and analysis of high quality genomic DNA from ixodid ticks. *Med. Vet. Entomol.*, 17(2): 224-227.

Hoadley, R. B. 1990. *IdentifyingWood: Accurate Results with Simple Tools*. Tauton Press, Newton. CT. 223p

Hollingsworth, M.L., Andra Clark, A.L.E.X., Forrest, L.L., Richardson, J., Pennington, R.T., Long, D.G., Cowan, R., Chase, M.W., Gaudeul, M., and Hollingsworth, P.M. 2009. Selecting barcoding loci for plants: evaluation of seven candidate loci with species- level sampling in three divergent groups of land plants. *Mol. Ecol. Resour.*, 9(2): 439-457.

Hollingsworth, P.M., Forrest, L.L., Spouge, J.L., Hajibabaei, M., Ratnasingham, S., van der Bank, M., Chase, M.W., Cowan, R.S., Erickson, D.L., and Fazekas, A.J. 2009. A DNA barcode for land plants. In: *Proceedings of the National Academy of Sciences*, 106(31): 12794-12797.

Hollingsworth, P.M., Graham, S.W., and Little, D.P. 2011. Choosing and using a plant DNA barcode. *PLoS One*, 6(5): e19254

Howard A. L. 1941a. *Studies of the Identification of Timbers*. International Book Distributors, Dehra Dun, India, 110p.

Howard, R.A. 1941b. The comparative morphology of the Icacinaceae. I. Anatomy of the node and internode. *J. Arn. Arb*., 22:125-132.

Ilic, J. 1991. *CSIRO Atlas of Hardwoods*. Springer-Verlag: Berlin, 525p.

International Association of Wood Anatomists Committee, 2004. IAWA list of microscopic features for softwood identification. *IAWA J.*, *25*(1): 1-70.

Iyengar, A. 2014. Forensic DNA analysis for animal protection and biodiversity conservation: a review. *J. Nature Conserv.*, 22(3): 195-205.

Izumoto, Y. and Hayashi, S. 1990. Identification system of wood assisted by microcomputer II. *Mem. Osaka Kyoiku Univ. sero III*, 39 (1): 87-102.

Jashemski, W.F. 1990. Evidence of flora and fauna in the gardens and cultivated land destroyed by Vesuvius in A.D. 79. In: Lockley, M.G. and Rice, A. (eds), *Volcanism and Fossil Biotas*. Boulder, Colorado, Geological Society of America, Special Paper. pp.113-153.

Jayeola, A.A., Aworinde, D.O., and Folorunso, A.E. 2009. Use of wood characters in the identification of selected timber species in Nigeria. *Not. Bot. Horti. Agrobot.* , 37(2): 28-32.

Jiao, L., Yin, Y., Cheng, Y., and Jiang, X. 2014. DNA barcoding for identification of the endangered species *Aquilaria sinensis*: comparison of data from heated or aged wood samples. *Holzforschung*, 68(4): 487-494.

Jiao, L., Yin, Y., Xiao, F., Sun, Q., Song, K., and Jiang, X. 2012. Comparative analysis of two DNA extraction protocols from fresh and dried wood of *Cunninghamia lanceolata* (Taxodiaceae). *IAWA J.*, 33(4): 441-456.

Jiao, X., Li, Y., Niu, J., Xie, X., Wang, X., and Tang, B. 2018. Small-molecule fluorescent probes for imaging and detection of reactive oxygen, nitrogen, and sulfur species in biological systems. *Anal. Chem.*, 90(1): 533-555.

John, M.E. 1992. An efficient method for isolation of RNA and DNA from plants containing polyphenolics. *Nucleic Acids Res.*, 20(9): 2381.

Kartusch, B. and Halbwachs, G. 1985. Wood anatomical investigations on specimens of Alnus glutinosa that have been exposed to different pollution levels. *Ang. Bot.*, 59: 249-260.

Kress, K.P. and Wolf, P., Airbus Defence and Space GmbH, 2005. Evaluation of damage to structures under test using ultrasound. U.S. Patent Application 10/860,047.

Kress, W.J. and Erickson, D.L. 2007. A two-locus global DNA barcode for land plants: the coding rbcL gene complements the non-coding trnH-psbA spacer region. *PLoS One*, 2(6): e508.

Kuksa, P. and Pavlovic, V. 2009. Efficient alignment-free DNA barcode analytics. *BMC Bioinformat.*, *10*(S14): S9.

Kuroda, K. 1987. Hardwood identification using a microcomputer and IAWA codes. *IAWA Bull.*, 8: 69-77.

Kywe, U.T. and Soe, K. 1983. *The Morphological and Anatomical Characteristics of (25) Commercial Burmese Timbers* (Part 1), Then Kywe forest research Institute and Rangoon University, 104p.

Lahaye, R., Van der Bank, M., Bogarin, D., Warner, J., Pupulin, F., Gigot, G., Maurin, O., Duthoit, S., Barraclough, T.G., and Savolainen, V. 2008. DNA barcoding the floras of biodiversity hotspots. In: *Proceedings of the National Academy of Sciences*, 105(8): 2923-2928.

Lee, A.B. and Cooper, T.A. 1995. Improved direct PCR screen for bacterial colonies: wooden toothpicks inhibit PCR amplification. *Biotechniques*, 18: 225-226.

Lee, H.Y., Lee, S.D., and Shin, K.J. 2016. Forensic DNA methylation profiling from evidence material for investigative leads. *BMB Rep.*, 49(7): 359.

Lens, F., Luteyn, J.L., Smets, E., and Jansen, S. 2004. Ecological trends in the wood anatomy of Vaccinioideae (Ericaceae sl). *Flora-Morphology, Distribution, Funct. Ecol. Plants*, 199(4): 309-319.

Lens, F., Sperry, J. S., Christman, M. A., Choat, B., Rabaey, D., and Jansen, S. 2011. Testing hypotheses that link wood anatomy to cavitation resistance and hydraulic conductivity in the genus Acer. *New Phytol*., 190: 709–723.

Liang, C.B., Baas, P., Wheeler, E.A., and Shuming, W. 1993. Wood anatomy of trees and shrubs from China. VI. Magnoliaceae. *IAWA J.*, 14(4): 391-412.

Liese, W., Schneider, M., and Eckstein, D. 1975. Histometrische Untersuchungen am Holz einer rauchgeschaÈdigten Fichte. *Eur. J. For. Path*., 5: 152-161.

Linacre, A. and Tobe, S.S. 2011. An overview to the investigative approach to species testing in wildlife forensic science. *Invest. Genet.*, 2(1): 2.

Linder, E.V. 2008. Mapping the cosmological expansion. *Rep. Prog. Phys.*, 71(5): p.056901.

Longui E.L., Romeiro, D., Testoni, L.N., Aguiar, O.T.D., Filho, R.C., Lima, I.L.D., and Florsheim, S.M.B. 2012. Water deficit affects wood vessels of Croton floribundus

Spreng. in different vegetation types, Sao Paulo State, Brazil. *Hoehnea*., 39(1): 113-123.

Lowe, A. 2007. Can we use DNA to identify the geographic origin of tropical timber. In: *Proceedings of the international workshop Fingerprinting methods for the identification of timber origins, October*, pp. 8-9.

Lowe, A.J. and Cross, H.B. 2011. The Applicat ion of DNA methods to Timber Tracking and Origin Verification. *IAWA J.*, 32(2): 251-262.

Lowe, A.J., Dormontt, E.E., Bowie, M.J., Degen, B., Gardner, S., Thomas, D., Clarke, C., Rimbawanto, A., Wiedenhoeft, A., Yin, Y., and Sasaki, N. 2016. Opportunities for improved transparency in the timber trade through scientific verification. *BioScience*, 66(11): 990-998.

Lu, Y., Hu, R., Zhu, G., Zhang, X., Mei, L., Liu, Q., Qiu, L., Wu, C., and Tan, W. 2015. Preparation and biomedical applications of programmable and multifunctional DNA nanoflowers. *Nat. Protoc.*, 10(10): 1508.

Luchi A. E., Silva L. C. P., and Moraes M. A. 2005. Anatomia comparada do lenho de Xylopia aromatica (Lam.) Mart. em areas de cerrado e de plantacao de Pinus elliottii Engelm. *Braz. J. Bot*., 28: 809–820.

Lushington A. W. 1919. *Nature and uses of Madras Timbers*. S.P.C.K. Press, Madras, 358p.

Maiti, R., Rodriguez, H.G., Para, A.C., Kumari, C.H.A., and Sarkar N. C. 2016. A comparative wood anatomy of 15 woody species in North-eastern Mexico. *Forest Res.*, 5: 166.

Maliyakal, E.J. 1992. An efficient method for isolation of RNA and DNA from plants containing polyphenolics. *Nucleic Acid Res.,* 20:2381.

Meier, E.W. 2015. *Identifying and Using Hundreds of Woods Worldwide*. Wood database. 272p.

Meier, R., Shiyang, K., Vaidya, G., and Ng, P.K. 2006. DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. *System. Biol.*, 55(5): 715-728.

Menon P. K. B. 1959. The wood anatomy of Malayan timbers: commercial timbers 3. Light hardwoods 2. Medium hardwoods. *Res. Pamph. For. Res. Inst. Kepong*., 27: 30-37.

Menon P. K. B. 1971. The anatomy and identification of Malaysian Hardwoods. *Malayan For. Rec.,* 27: 124.

Menon, P. K. B. 1955. The wood anatomy of Malayan timbers: commercial timbers 1. Heavy hardwoods 2. Medium hardwoods. *Res. Pamph. For. Res. Inst. Kepong*., 18: 19-29.

Meyer, C.P. and Paulay, G. 2005. DNA barcoding: error rates based on comprehensive sampling. *PLoS Biol.*, 3(12): e422.

Miles, A. 1978. *Photomicrographs of World Woods*. Building Research Establishment. Her Majesty's Stationery office, London, 233p.

Miles, P.D. and Smith, W.B. 2009. *Specific Gravity and Other Properties of Wood and Bark for 156 Tree Species Found in North America* (Vol. 38). US Department of Agriculture, Forest Service, Northern Research Station. 39p.

Miller, R.B. and Detienne, P. 2001. *Major Timber Trees of Guyana Wood Anatomy*. Tropenbos International Wageningen, the Netherlands, 226p.

Monteoliva, S. and Olivera, H. 1994. Introduction to the use of computer systems for wood identification in Argentina. *IAWA J.* 15: 329 (Abstract).

Moritz, C. and Cicero, C. 2004. DNA barcoding: promise and pitfalls. *PLoS Biol.*, 2(10): e354.

Mtambo, J., Van Bortel, W., Madder, M., Roelants, P., and Backeljau, T. 2006. Comparison of preservation methods of Rhipicephalus appendiculatus (Acari:

Ixodidae) for reliable DNA amplification by PCR. *Exp. Appl. Acarol.*, 38(2-3): 189-199.

Muellner, A.N., Schaefer, H., and Lahaye, R. 2011. Evaluation of candidate DNA barcoding loci for economically important timber species of the mahogany family (Meliaceae). *Mol. Ecol. Resour.*, 11(3): 450-460.

Muller, F.M.C., Werner, K.E., Kasai, M., Francesconi, A., Chanock, S.J., and Walsh, T.J. 1998. Rapid extraction of genomic DNA from medically important yeasts and filamentous fungi by high-speed cell disruption. *J. Clin. Microbiol.*, 36(6): 1625-1629.

Murray, M.G. and Thompson, W.F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.*, 8(19): 4321-4326.

Nazma, P., Ganapathy, P.M., Sasidharan, N., Bhat, K.M., and Gnanaharan, R. 1981. *A Handbook of Kerala Timbers*. *KFRI Research Report No. 9*. 260p.

Newman, M.F., Burgess, P.F., and Whitemore, T.C. 1996. *Manual of Dipterocarp Forests. Anisoptera, Parashorea, Shorea*. Royal Botanical Garden. Edinburgh, 156p.

Newmaster, S. G. and Raghupathy, S. 2009. Testing plant barcoding in a sister species complex of pantropical Acacia (Mimosoideae, Fabaceae). *Mol. Ecol. Res*., 9 (Suppl. 1): 172–180.

Newmaster, S.G., Fazekas, A.J., Steeves, R.A.D., and Janovec, J. 2008. Testing candidate plant barcode regions in the Myristicaceae. *Mol. Ecol. Resour.*, 8(3): 480-490.

Nishiguchi, M., Yoshida, K., Sumizono, T., and Tazaki, K. 2002. A receptor-like protein kinase with a lectin-like domain from lombardy poplar: gene expression in response to wounding and characterization of phosphorylation activity. *Mol. Genet. Genom.*, 267(4): 506-514.

Novruzova, Z. A. 1972. The effect of ecological conditions on relation of ring width to solid matter in trees and shrubs. *For. Abstr.*, 34: 4200.

Palmer, J.D., 1992. Mitochondrial DNA in plant systematics: applications and limitations. In: *Molecular Systematics of Plants.* Springer, Boston, MA, pp. 36-49.

Pande, P.K. 2012. Status of anatomy and physical properties of wood in poplars. *For. Bull.*, 12(1): 223-226.

Pankhurst, R.J. 1978. *Biological Identification. The Principles and Practice of Identification Methods in Biology*. Edward Arnold (Publishers) Ltd., 104p.

Pearson R. S. and H. P. Brown. 1932. *Commercial Timbers of India.* [Vol 1]. Government of India, Calcutta. 548p.

Pirttila, A.M., Hirsikorpi, M., Kamarainen, T., Jaakola, L., and Hohtola, A. 2001. DNA isolation methods for medicinal and aromatic plants. *Plant Mol. Biol. Rep.*, 19(3): 273-273.

Poinar, H.N., Hofreiter, M., Spaulding, W.G., Martin, P.S., Stankiewicz, B.A., Bland, H., Evershed, R.P., Possnert, G. and Paabo, S. 1998. Molecular coproscopy: dung and diet of the extinct ground sloth Nothrotheriops shastensis. *Science*, 281(5375): 402-406.

Poorter, L., Mc Donald, I., Alarcon, A., Fichtler, E., Licona, J. C., Pena-Claros, M., Sterck, F., Villegas, Z., and Sass-Klaassen, U. 2009. The importance of wood traits and hydraulic conductance for the performance and life history strategies of 42 rainforest tree species. *New Phytol.*, 185: 481– 492.

Porco, D., Rougerie, R., Deharveng, L., and Hebert, P. 2010. Coupling non destructive DNA extraction and voucher retrieval for small soft bodied Arthropods in a high throughput context: The example of Collembola. *Mol. Ecol. Resour.*, 10(6): 942-945.

Purkayastha, S.K. 1982. *Indian Woods, Their Identification, Properties and Uses*. [Vol IV]. Myrtaceae to Symplococaceae. Controller of publications, New Delhi. 172p.

Purkayastha, S.K., Juneja, K.B.S., and Husain K. S. M. 1976. *Anatomy of More Important Andaman Commercial Timbers (With Notes on Their Supply, Properties and Use).* Ind. For. Rec. Vol 1 & 2. Forest Research Institute Press, DehraDun, 42p.

Rachmayanti, Y., Leinemann, L., Gailing, O., and Finkeldey, R. 2006. Extraction, amplification and characterization of wood DNA from Dipterocarpaceae. *Plant Mol. Biol. Rep.*, 24(1): 45-55.

Rachmayanti, Y., Leinemann, L., Gailing, O., and Finkeldey, R. 2009. DNA from processed and unprocessed wood: factors influencing the isolation success. *Forensic Sci. Int-Gen.*, 3(3)l: 185-192.

Rajput, K.S., Rao, K.S., and Kim, Y.S. 2008. Cambial activity and wood anatomy in Prosopis spicigera (Mimosaceae) affected by combined air pollutants. *IAWA J.*, 29 (2): 209–219.

Rao, K.R. 1963. *Indian Woods, Their Identification, Properties and Uses.* [Vol II]. Manager of publications, Delhi. 386p.

Rao, K.R. and Juneja, K.B.S. 1971. *A Handbook for Field Identification of Fifty Important Timbers of India*. Manager of Publication, New Delhi, India, 123p.

Rao, K.R. and Purkayastha, S.K. 1972. *Indian Woods, Their Identification, Properties and Uses*. [Vol III]. Leguminosae to Combretacae. Manager of Publications, New Delhi, 262p.

Rao, R.V, Sujatha, M., Hemavathi, T.R. 2003. Radial variation in anatomical properties of plantation grown Tecomella undulata. *J. Trop. Forest Prod.*, 9(1–2):69–76.

Ratnasingham, S. and Hebert, P.D., 2007. BOLD: The Barcode of Life Data System (http://www. barcodinglife. org). *Mol. Ecol. Notes*, 7(3): 355-364.

Rendle, B.J. 1970. *World Timbers*. [Vol III]. Asia, Australia and New Zealand. University of Toronto Press. London. 168p.

Ribeiro, R.A. and Lovato, M.B. 2007. Comparative analysis of different DNA extraction protocols in fresh and herbarium specimens of the genus Dalbergia. *Genet. Mol. Res*, 6(1): 173-187.

Richardson, T.J., Shokrollahi, M.A., and Urbanke, R.L. 2001. Design of capacity-approaching irregular low-density parity-check codes. *IEEE T. Inform. Theory*, 47(2): 619-637.

Richter H.G. and Trockenbrodt, M. 1996. Computer-aided wood identification with DELTA / INTKEY - a demonstration. *IAWA J.*, 17: 262.

Richter, H. G., Grosser, D., Heinz, I., and Gasson, P. E. 2004. IAWA List of Microscopic Features for Softwood Identification. *IAWA J.,* 25 (1): 1–70.

Richter, H.G. and Trockenbrodt, M. 1993. IAWA list of features for hardwood identification adapted to the DELTA system. Federal Research Centre for Forestry and Forest Products, Hamburg.

Richter, H.G., Grosser, D., Heinz, I., and Gasson, P.E. (eds). 2004. IAWA list of microscopic features for softwood identification. *IAWA J.*, 25(1): 1-70.

Robba, L., Russell, S.J., Barker, G.L., and Brodie, J. 2006. Assessing the use of the mitochondrial cox1 marker for use in DNA barcoding of red algae (Rhodophyta). *Am. J. Bot.*, 93(8): 1101-1108.

Rohland, N., Siedel, H., and Hofreiter, M. 2004. Nondestructive DNA extraction method for mitochondrial DNA analyses of museum specimens. *Biotechniques*, 36(5): 814-821.

Ruwanpathiranal, N.D., Amarasekera, H.S., and de Silva, M.P. 1996. Variation of *Pinus caribaea* wood density with height in tree and distance from pith in different site classes. In: *Proceedings of the Second Annual Symposium on Management and Sustainable Utilization of Forest Resources*, pp.49-57.

Safdari, V., Ahmed, M., Palmer, J., and Baing, M.B. 2008. Identification of Iranian commercial wood with hand lens. *Pak. J. Bot.,* 40: 1851-1864.

Sahoo, S., Anoop, E.V., Vidyasagaran, K., Kunhamu, T.K., and Santhoshkumar, A.V. 2017. Vessel morphology variation and ecoanatomical properties of anjily (Artocarpus hirsutus Lam.) wood grown in different agro-climatic zones of Thrissur, Kerala. J. Trop. Agric., 55(1) :40-44.

Sahri, M.H., Ashaari, Z., Kader, R.A., and Mohmod, A.L. 1998. Physical and Mechanical Properties of *Acacia mangium* and *Acacia auriculiformis* from different Provenances. *Tropical*, *21*(2): 73.

Sands, C.J., McInnes, S.J., Marley, N.J., Goodall-Copestake, W.P., Convey, P., and Linse, K. 2008. Phylum Tardigrada: an "individual" approach. *Cladistics*, 24(6): 861-871.

Sanger, F., Nicklen, S., and Coulson, A.R. 1977. DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences,* 74(12): 5463-5467.

Santini, N.S., Schmitz, N., and Lovelock, C.E. 2012. Variation in wood density and anatomy in a widespread mangrove species. *Trees*, 26(5): 1555-1563.

Santos, M.C., Saito, C.P., and Line, S.R. 2008. Extraction of genomic DNA from paraffin-embedded tissue sections of human fetuses fixed and stored in formalin for long periods. *Pathol. Res. Pract.*, 204(9): 633-636.

Saravanan, V., Parthiban, K.T., Thiruneraiselvan, S., Kumar, P., Vennila, S., and Kanna, S.U. 2014. Comparative study of wood physical and mechanical properties of Melia dubia with Tectona grandis at different Age Gradation. *Res. J. Rec. Sci.*,3: 256-263.

Sarkar, I.N., Planet, P.J., and Desalle, R.O.B. 2008. CAOS software for use in character-based DNA barcoding. *Mol. Ecol. Resour.*, 8(6): 1256-1259.

Sass, C., Little, D. P., Stevenson, D. W., and Specht, C. D. 2007. DNA barcoding in the Cucadales: testing the potential of proposed barcoding markers for species indentification of cycades. *PLoS One*, 2: e1154.

Savolainen, V., Cowan, R.S., Vogler, A.P., Roderick, G.K., and Lane, R. 2005. Towards writing the encyclopaedia of life: an introduction to DNA barcoding.*Philos. T. R. Soc. B.*, 360(1462): 1805-1811.

Saxena, V. and Gupta, S. 2011. Wood Anatomy of Family Salvadoraceae from the Indian Subcontinent with Special Reference to the Ultrastructure of the Vessel Wall. *Aliso*, 29(1): 59–63.

Schander, C. and Kenneth, H.M. 2003. DNA, PCR and formalinized animal tissue–a short review and protocols. *Org. Divers. Evol.*, 3(3): 195-205.

Schill, R.O. 2007. Comparison of different protocols for DNA preparation and PCR amplification of mitochondrial genes of tardigrades. *J. Limnology*, 66(1s): 164-170.

Schindel, D.E. and Miller, S.E. 2005. DNA barcoding a useful tool for taxonomists. *Nature*, 435(7038): 17-18.

Schizas, N.V., Street, G.T., Coull, B.C., Chandler, G.T., and Quattro, J.M. 1997. An efficient DNA extraction method for small metazoans. *Mol. Mar. Biol. Biotech.*, 6(4): 381.

Schneider, M. and Halbwachs, G. 1989. Anatomical and morphological investigations into the regenerative ability of a spruce damaged by fluorine emissions. *Eur. J. For. Path*., 19: 29-46.

Seberg, O. and Petersen, G. 2009. How many loci does it take to DNA barcode a crocus?. *PLoS One*, 4(2): e4598.

Seifert, K.A., Samson, R.A., dewaard, J.R., Houbraken, J., Levesque, C.A., Moncalvo, J.M., Louis-Seize, G., and Hebert, P.D. 2007. Prospects for fungus identification using CO1 DNA barcodes, with Penicillium as a test case. In: *Proceedings of the National Academy of Sciences*, 104(10): 3901-3906.

Shanavas, A. and Kumar, B.M. 2003. Fuelwood characteristics of tree species in homegardens of Kerala, India. *Agrofor. Syst.*, *58*(1): 11-24.

Sharma, M. R. 1964. Morphological and anatomical investigations on Artocarpus sp. *J. Indian Acad. Wood Sci.,*: 256-287

Sharma, S.K., Shukla, S.R., Sujatha, M., Shashikala, S., and Kumar, P. 2012. Assessment of certain wood quality parameters of selected genotypes of *Melia dubia* cav. Grown in a seedling seed orchard. *J. Indian Acad. Wood Sci.*, *9*(2): 165-169.

Shaw, J., Lickey, E.B., Schilling, E.E., and Small, R.L. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *Am. J. Bot.*, 94(3): 275-288.

Shepard, K.A., Gerber, A.P., Jambhekar, A., Takizawa, P.A., Brown, P.O., Herschlag, D., DeRisi, J.L., and Vale, R.D. 2003. Widespread cytoplasmic mRNA transport in yeast: identification of 22 bud-localized transcripts using DNA microarray analysis. In: *Proceedings of the National Academy of Sciences*, 100(20): 11429-11434.

Shepherd, R., Reader, S., and Falshaw, A. 1997. Chitosan functional properties. *Glycoconjuate J.*, 14: 535–542.

Singh, M. K., Sharma, M. B., and Sharma, C. L. 2013. Wood anatomical variations in some Terminalia species of Assam. *Int. J. Bot. Res.*, 3(2): 13-18.

Skujienė, G. and Soroka, M. 2003. A comparison of different DNA extraction methods for slugs (Mollusca: Pulmonata). *Ekologija*, 1: 12-16.

Speirs, A., McConnachie, G., and Lowe, A.J. 2009. Chloroplast DNA from 16th century waterlogged oak in a marine environment: initial steps in sourcing the Mary Rose timbers. In: Haslam, M., Robertson, G., Crowther, A., Nugent, S., and Kirkwood, L. (eds), *Archaeological Science Under A Microscope: Studies in Residue and DNA Analysis in Honour of Tom Loy*, Terra australis, pp. 165–179.

Sperry J. S. 2003. Evolution of water transport and xylem structure. *Int. J. Plant Sci*., 164: S115-S127.

Sperry J. S. and Hacke, U.G. 2004. Analysis of circular bordered pit function. Angiosperm vessels with homogenous pit membranes. *Am. J. Bot.* 91(3): 369–385.

Starr, J.R., Naczi, R.F., and Chouinard, B.N. 2009. Plant DNA barcodes and species resolution in sedges (Carex, Cyperaceae). *Mol. Ecol. Resour.*, 9: 151-163.

Suzuki, E. 1999. Diversity in specific gravity and water content of wood among Bornean tropical rainforest trees. *Ecol. Res.*, 14(3): 211-224.

Taberlet, P., Coissac, E., Pompanon, F., Gielly, L., Miquel, C., Valentini, A., Vermat, T., Corthier, G., Brochmann, C., and Willerslev, E. 2007. Power and limitations of the chloroplast trn L (UAA) intron for plant DNA barcoding. *Nucleic Acids Res.*, 35(3): e14-e14.

Tang, X., Zhao, G., and Ping, L. 2011 Wood identification with PCR targeting noncoding chloroplast DNA. *Plant. Mol. Biol.*, 77(6):609–617.

Tani, N., Tsumura, Y. and Sato, H. 2003. Nuclear gene sequences and DNA variation of Cryptomeria japonica samples from the postglacial period. *Mol. Ecol.*, 12(4): 859-868.

Thomson, J.A. 2002. An improved non-cryogenic transport and storage preservative facilitating DNA extraction from 'difficult'plants collected at remote sites. *Telopea*, 9(4): 755-760.

Titmuss, F. H. 1971. *Commercial Timbers of the World*. The Technical Press Limited. London, 366p.

Tnah, L.H., Lee, C.T., Lee, S.L., Ng, K.K.S., Ng, C.H., and Hwang, S.S. 2011. Microsatellite markers of an important medicinal plant, Eurycoma longifolia (Simaroubaceae), for DNA profiling. *Am. J. Bot.*, 98(5): e130-e132.

Tnah, L.H., Lee, S.L., Ng, K.K., Tani, N., Bhassu, S., and Othman, R.Y. 2009. Geographical traceability of an important tropical timber (*Neobalanocarpus heimii*) inferred from chloroplast DNA. *For. Ecol. Manag.*, 258(9): 1918-1923.

Tnah, L.H., Lee, S.L., Ng, K.K.S., Bhassu, S., and Othman, R.Y. 2012. DNA extraction from dry wood of Neobalanocarpus heimii (Dipterocarpaceae) for forensic DNA profiling and timber tracking. *Wood Sci. Technol.*, 46(5): 813-825.

Tnah, L.H., Lee, S.L., Ng, K.K.S., Faridah, Q.Z., and Faridah-Hanum, I. 2010. Forensic DNA profiling of tropical timber species in Peninsular Malaysia. *For. Ecol. Manag.*, 259(8): 1436-1446.

Tochigi, T., Shiokura, T., Lantican, C. B., Salud, C. C., and Madamba, C. B. 1984. Computer assisted tropical wood identification (CATWI). In: *Proc. Pacific Regional Wood Anatorny Conference*, Tsukuba, Japan: 174-176.

Trotter, H. 1929. *The Common Commercial Timbers of India and Their Uses; Description of Common Indian Woods*. Culcutta Government of India Central Publication Branch, 126p.

Trotter, H. 1959. *The Common Commercial Timbers of India and their Uses*. Controller of Publications, New Delhi, 296p.

Troup, R. S. 1909. *Indian Woods and Their Uses*. Soni Reprint Agency. New Delhi, 273p.

Tsoumis, G. 1985. Identification of European Conifers from Sawdust In: Kucera, L. J. (ed.) *Xylorama.* pp.198-203

Tyree, M.T, Davis, S.D., and Cochard, H. 1994. Biophysical perspectives of xylem evolution: is there a tradeoff of hydraulic efficiency for vulnerability to dysfunction. *IAWA J*., 15(4): 335–360.

Tzschucke, C.C., Markert, C., Bannwarth, W., Roller, S., Hebel, A., and Haag, R. 2002. Modern separation techniques for the efficient workup in organic synthesis. *Angew. Chem. Int. Edit.*, 41(21): 3964-4000.

Van Moorsel, C.H., Dijkstra, E.G., and Gittenberger, E. 2000. Molecular evidence for repetitive parallel evolution of shell structure in Clausiliidae (Gastropoda, Pulmonata). *Mol. Phylogenet. Evol.*, 17(2): 200-208.

Vijayan, K. and Tsou, C.H. 2010. DNA barcoding in plants: taxonomy in a new perspective. *Curr. Sci.*: 1530-1541.

Wallich, N. 1832. *Catalogue of Indian Woods*. Transactions of the Society, Instituted at London, for the Encouragement of Arts, Manufactures, and Commerce, (1831-1832): 439-481.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., and Hebert, P.D. 2005. DNA barcoding Australia's fish species. *Philos. T. R. Soc. B.*, 360(1462): 1847-1852.

Wheeler, E. A. and Baas, P. 1998. Wood Identification -A Review. *IAWA J.*, 19 (3): 241-264.

Wheeler, E.A., Baas, P., and Gasson, P.E. 1989. IAWA List of Microscopic Features for Hardwood Identification. *IAWA Bull.*, 10(3): 219-332

Wheeler, E.A., Baas, P., and Rodgers, S. 2007. Variations in dicot wood anatomy: A Global Analysis Based on the Insidewood Database. *IAWA J.*, 28 (3): 229–258.

Wheeler, J. K., Sperry, J., Hacke, U.G., and Hoang, N. 2005. Inter-vessel pitting and cavitation in woody Rosaceae and other vesselled plants: a basis for a safety versus efficiency trade-off in xylem transport. *Plant Cell Environ.*, 28: 800–812.

Will, K.W., Mishler, B.D., and Wheeler, Q.D. 2005. The perils of DNA barcoding and the need for integrative taxonomy. *System. Biol.*, 54(5): 844-851.

Wimmer, R. 2002. Wood anatomical features in tree-rings as indicators of environmental change. *Dendrochronologia*, 20/1-2: 21-36.

Winnepenninckx, B., Backeljau, T., and De Wachter, R. 1993. Complete small ribosomal subunit RNA sequence of the chiton Acanthopleura japonica (Lischke, 1873) (Mollusca, Polyplacophora). *Nucleic Acids Res.*, 21(7): 1670.

Woodcock, D. and Shier, A. 2002. Wood specific gravity and its radial variations: the many ways to make a tree. *Trees*, 16(6): 437-443.

Yan, L.J., Liu, J., Möller, M., Zhang, L., Zhang, X.M., Li, D.Z., and Gao, L.M. 2015. DNA barcoding of Rhododendron (Ericaceae), the largest Chinese plant genus in biodiversity hotspots of the Himalaya–Hengduan Mountains. *Mol. Ecol. Resour.*, 15(4): 932-944.

Yang, J. and Cheng, F. 1990. Microcomputer-assisted wood identification system WIP-89. *J. Beijing For. Univ*., 12 (4): 88-94.

Yu, J., Xue, J.H., and Zhou, S.L. 2011. New universal matK primers for DNA barcoding angiosperms. *J. System. Evol.*, *49*(3): 176-181.

Yu, M., Jiao, L., Guo, J., Wiedenhoeft, A.C., He, T., Jiang, X., and Yin, Y. 2017. DNA barcoding of vouchered xylarium wood specimens of nine endangered *Dalbergia* species. *Planta*, 246(6): 1165-1176.

Yu, M., Liu, K., Zhou, L., Zhao, L., and Liu, S. 2016. Testing three proposed DNA barcodes for the wood identification of *Dalbergia odorifera* T. Chen and *Dalbergia tonkinensis* Prain. *Holzforschung,* 70(2):127–136.

Zang, Y., Lei, J., Hao, Q., and Ju, H. 2016. CdS/MoS2 heterojunction-based photoelectrochemical DNA biosensor via enhanced chemiluminescence excitation. *Biosens. Bioelectron.*, 77: 557-564.

Zemlak, T.S., Ward, R.D., Connell, A.D., Holmes, B.H., and Hebert, P.D. 2009. DNA barcoding reveals overlooked marine fishes. *Mol. Ecol. Resour.*, 9: 237-242.

Zhang, D., Yang, Y., Castlebury, L.A., and Cerniglia, C.E. 1996. A method for the large scale isolation of high transformation efficiency fungal genomic DNA. *FEMS MicroBiol. Lett.*, 145(2): 261-265.

Zhang, Q. C., Cheng, F., and Lian, Y.H. 1986. Microcomputer identification of hardwood species. *Scientia Silvae Sin*., 22: 213-217.

Zheng, G.X., Lau, B.T., Schnall-Levin, M., Jarosz, M., Bell, J.M., Hindson, C.M., Kyriazopoulou-Panagiotopoulou, S., Masquelier, D.A., Merrill, L., Terry, J.M., and Mudivarti, P.A. 2016. Haplotyping germline and cancer genomes with high-throughput linked-read sequencing. *Nat. Biotechnol.*, 34(3): 303.

Zimmermann, M. H. 1983. Xylem structure and the ascent of sap. Springer- Verlag, New York. 289p.

Zobel, B.J. and Van Buijtenen, J.P. 1989. *Wood Variations: Its Causes and Control*. Berlin, Springer-Verlag, 363p.

INTEGRATION OF DNA BARCODING AND WOOD ANATOMY FOR THE IDENTIFICATION OF SELECTED TIMBERS

By

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ABSTRACT OF THE THESIS

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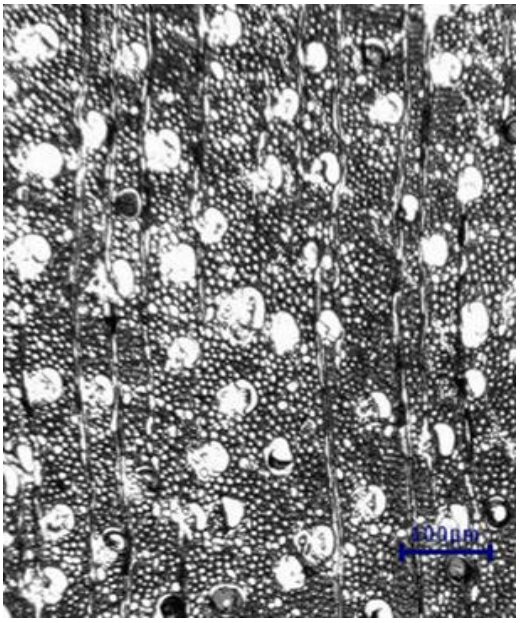
ABSTRACT

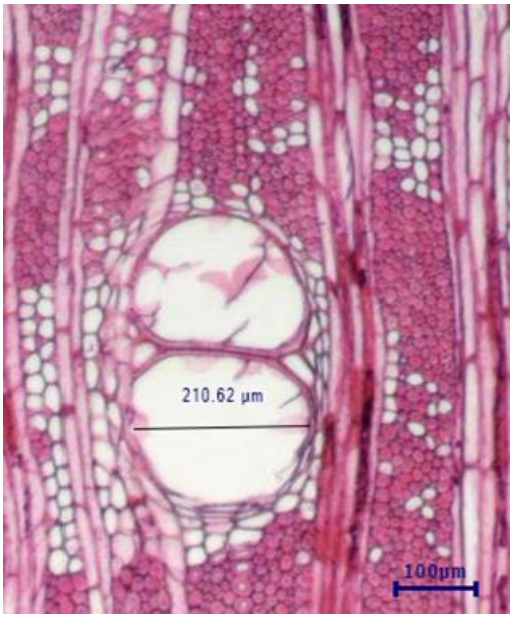
In the field of wood marketing, the accurate identification of species has greater importance. Traditional methods of wood identification are based on physical characters and anatomical features of the wood species concerned. But they have some major limitations which could be overcome by the integration of DNA barcoding with traditional wood taxonomy methods. This study was conducted to confirm the authenticity of certain wood samples based on their physical and anatomical characters and to create a DNA Barcode database of seven selected timber species that are regularly traded in Kerala. The wood samples of seven tree species viz., Indian Sal (*Shorea robusta* Gaertn), Mangium (*Acacia mangium* Willd), Indian sandalwood (*Santalum album* Linn), Western red cedar (*Thuja plicata* Donn), Red sanders (*Pterocarpus santalinus* L.F), Mulluvenga (*Bridelia retusa* Spreng) and Malabar neem (*Melia dubia* Cav) were collected from sawmills and timber traders across Kerala and their anatomical and physical properties were studied in detail. The identity of samples was confirmed using computer-aided wood identification software in addition to the use of dichotomous keys. The wood samples were then used for DNA isolation, amplification and sequencing.

The detailed study on general features such as colour and odour, physical properties like moisture content and specific gravity and micro-anatomical properties such as the number and distribution of vessels and rays was appropriate to confirm the identity of species as the properties were highly comparable with the existing databases. In further procedures, the DNeasy Plant Mini Kit (Qiagen) with some in house modifications could yield the maximum quantity of DNA for the studied wood species in the current study. Also the samples which are soaked in water yielded maximum amount of DNA even if it was treated with wood chemicals during transits for longevity. PCR amplifications were carried out using COBOL Plant Working Group (2009) recommended universal primers for *rbcL*, *matK*, and *trnH-psbA*, from which the *matK* region showed reasonable amplification.

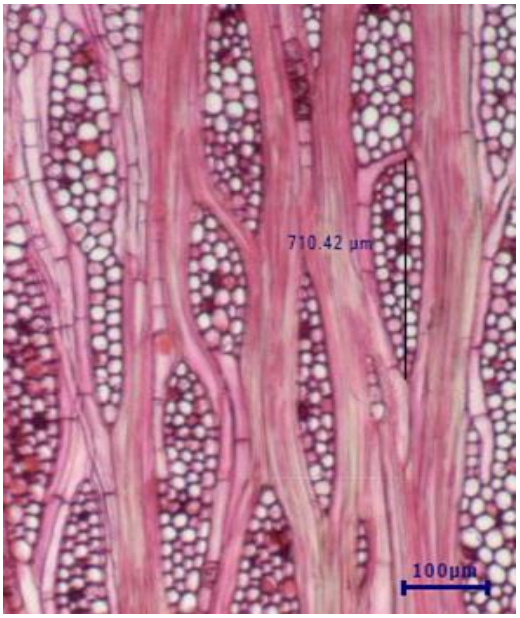
The results indicated that the DNA from wood tissues of the studied species could be successfully amplified. The DNA sequences from the wood tissues notably matched with the sequences of respective species deposited in the NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/) as well as BOLD public domain (https://www.boldsystems.org/). Consequently, the study results proved that the DNA barcoding technology in combination with the physical and anatomical characters are highly desirable for accurate species-level identification of wood samples.



1a



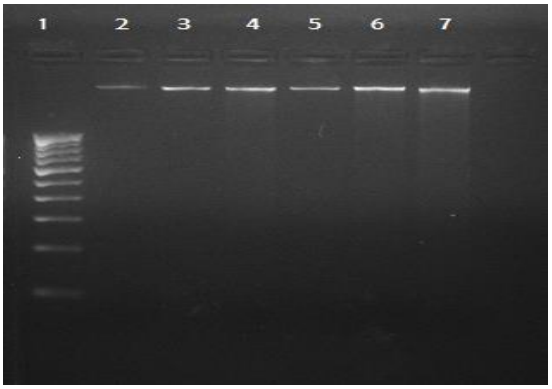
1b



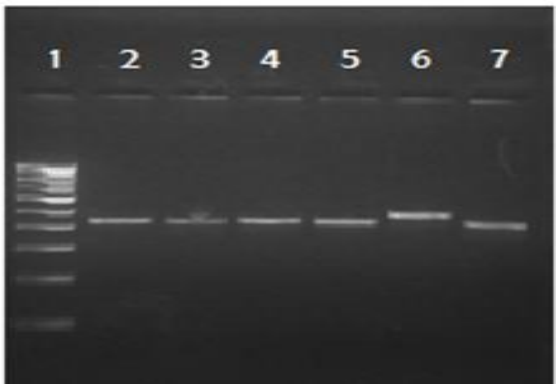
1c

1d

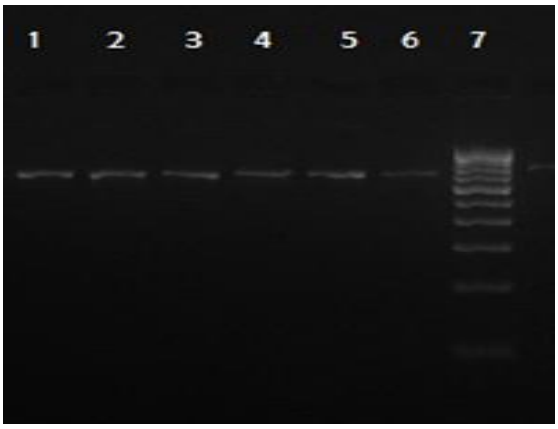
Plate 1. Anatomical features of wood samples. a. TS (4X) *Santalum album*. b. TS (10 X) *Shorea robusta.* c. TLS (10X) *Shorea robusta.* d. Fibre (Maceration, 40X) *Bridelia retusa.*



2a



2b



2c

Plate 2. Gel electrophoresis. a. Isolation of DNA. lane 1 100bp ladder, lane 2-7 DNA. b. PCR products of *trnH-psbA*. lane 1-100bp ladder, lane 2-7 (2) *Acacia mangium* (3) *Bridelia retusa* (4) *Melia dubia* (5) *Pterocarpus santalinus* (6) *Santalum album* (7) *Shorea robusta*.c. PCR products of *matK* gene lane 1-7 (1) *Acacia mangium* (2) *Bridelia retusa* (3) *Melia dubia* (4) *Pterocarpus santalinus* (5) *Santalum album* (6) *Thuja plicata* and lane (7) 100bp ladder.

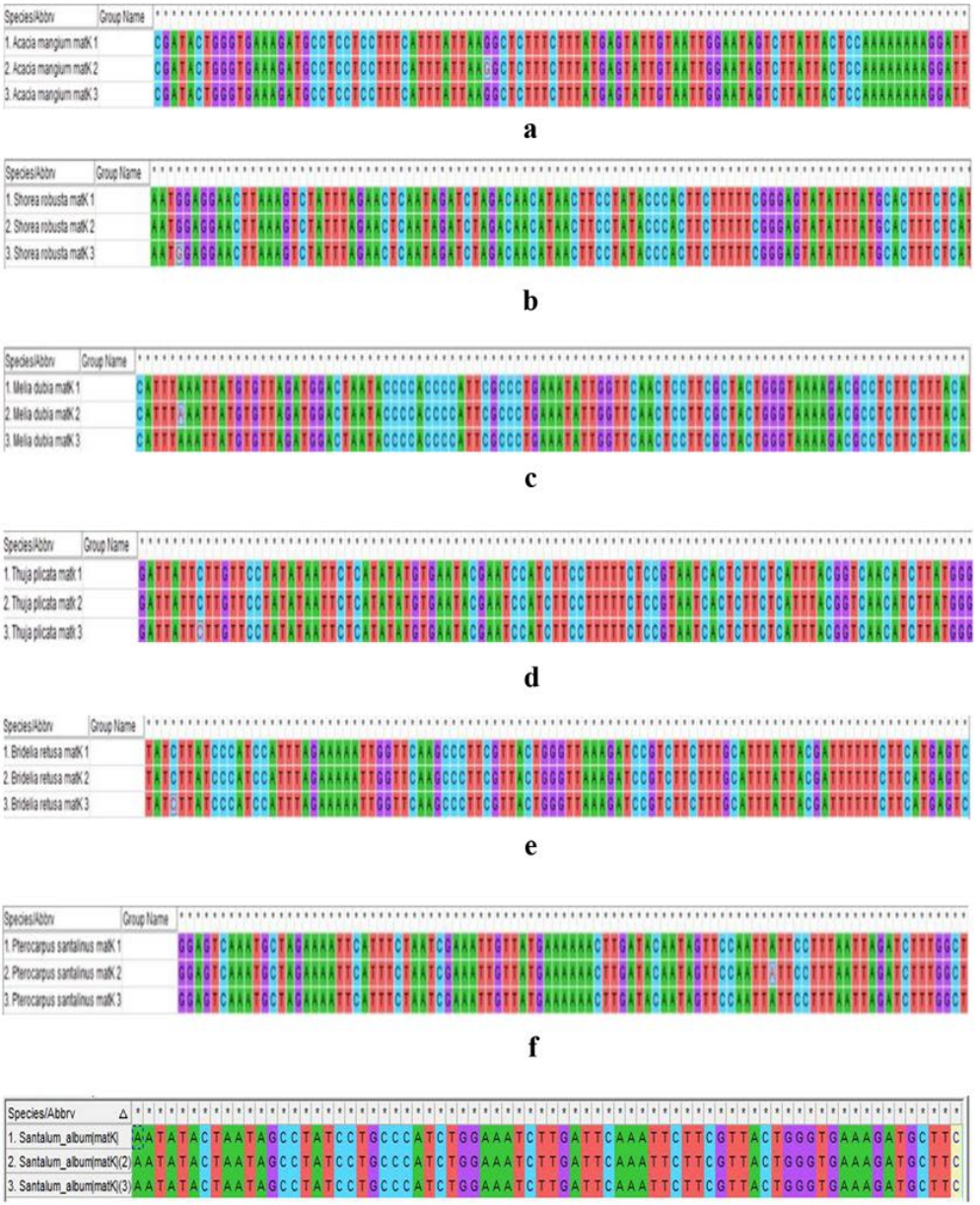


Plate 3. Multiple sequence alignment of different species. a. *Acacia mangium (matK)* b. *Shorea robusta (matK)* c. *Melia dubia (matK)* d. *Thuja plicata (matK) (matK)* e. f. *Pterocarpus santalinus Santalum album (matK)*

സംഗ്രഹം

മരങ്ങളുടെ കൃത്യവും സൂക്ഷ്മമവുമായ തിരിച്ചറിയൽ തടിവിപണിയിൽ ഏറെ പ്രാധാന്യം അർഹിക്കുന്നു. പരമ്പരാഗതമായ തടി തിരിച്ചറിയൽ രീതികൾ ഓരോ തടി ഇനത്തിൻറെയും ഭൗതികഗുണങ്ങളും അന്തർഘടനയും ഉപയോഗിച്ചിട്ടുള്ളതാണ്. എന്നാൽ ഇത്തരം പരമ്പരാഗത രീതികൾക്ക് ചില പ്രധാന പോരായ്മകൾ ഉണ്ട്. ഈ പോരായ്മകൾ തരണം ചെയ്യാൻ പരമ്പരാഗത രീതികൾക്ക് ഒപ്പം ഡി. എൻ. എ ബാർകോഡ് എന്ന സാങ്കേതിക വിദ്യ കൂടി സമാകലനം ചെയ്ത് ഉപയോഗിക്കാവുന്നതാണ്. കേരളത്തിൽ പതിവായി വാണിജ്യം നടത്തിവരുന്ന ചില തടികളുടെ പ്രാമാണ്യം അവയുടെ ഭൗതികഗുണങ്ങളും അന്തർഘടനയും ഉപയോഗിച്ച് തെളിയിക്കുന്നതിനും അവയ്ക്ക് ഒരു ഡി. എൻ. എ ബാർകോഡ് വിവരസങ്കലനം സൃഷ്ടിക്കുന്നതിനും ആണ് ഈ പഠനം നടത്തിയത്. കേരളത്തിൽ വിൽക്കപ്പെടുന്ന ഏഴ് വിവിധ ഇനം മരങ്ങളുടെ തടികഷ്ണങ്ങൾ കേരളത്തിലെ ഈർച്ച മില്ലുകളിൽ നിന്നും മറ്റു തടി വ്യാപാരികളിൽ നിന്നും ശേഖരിക്കുകയായിരുന്നു പഠനത്തിൻറെ ആദ്യ പടി. ഇത്തരത്തിൽ സാൽ, മാഞ്ചിയം, ചന്ദനം, റെഡ് സെഡാർ, രക്തചന്ദനം, മുള്ളുവേങ്ങ, മലവേപ്പ് എന്നീ മരങ്ങളുടെ ഭൗതികഗുണങ്ങളും അന്തർഘടനയും വിശദമായി പഠിച്ചു. കമ്പ്യൂട്ടർ അധിഷ്ഠിത തടി തിരിച്ചറിയൽ രീതികൾ ഉപയോഗിച്ച് അവയുടെ ഏകതാനത ഉറപ്പു വരുത്തി. അതിനു ശേഷം അതേ തടി

കഷ്ണങ്ങളിൽ നിന്ന് ഡി. എൻ. എ വേർതിരിച്ചെടുത്ത് വികസിപ്പിച്ച് അവയിലെ സീകൻസുകൾ തിരിച്ചറിഞ്ഞു.

തടിയുടെ പൊതു പ്രത്യേകതകൾ ആയ നിറം, മണം, ഭൗതിക ഗുണങ്ങൾ ആയ ഈർപ്പത്തിൻറെ അളവ്, നിർദിഷ്ട ഗുരുത്വകർഷണം, സൂക്ഷ്മ അന്തർഘടനാ സവിശേഷതകൾ ആയ വെസ്സലുകളുടെയും റേകളുടെയും എണ്ണവും വിതരണവും തുടങ്ങിയ ഘടകങ്ങൾ തടിയുടെ ഇനം സ്ഥിരീകരിക്കാൻ ഏറെ അനുയോജ്യമാണ് എന്ന് പഠനത്തിലൂടെ തിരിച്ചറിഞ്ഞു. തുടർന്നുള്ള പ്രവർത്തനങ്ങളിൽ ചില പരിണാമങ്ങൾ വരുത്തിയ ഡിഎന്നേസി പ്ലാൻറ് മിനി കിറ്റ് ഉപയോഗിച്ചപ്പോൾ പരമാവധി അളവിൽ ഡി. എൻ. എ വേർതിരിച്ചെടുക്കാൻ സാധിച്ചു. മാത്രമല്ല വെള്ളത്തിൽ കുതിർത്തി വച്ച മരകഷ്ണങ്ങൾ അവ മറ്റു രാസവസ്തുക്കൾ കൊണ്ട് പരിചരിച്ചതാണെങ്കിൽ കൂടി കൂടുതൽ ഡി. എൻ. എ ഉത്പാദിപ്പിച്ചു. COBOL സസ്യപഠന വിഭാഗത്തിൻറെ 2009ലെ നിർദ്ദേശങ്ങൾക്ക് അനുസൃതമായി rbcL, matK, trnH-psbA എന്നീ പ്രൈമറുകളിൽ PCR ഉപയോഗിച്ച് ഡി. എൻ. എ വികസിപ്പിച്ചപ്പോൾ matK എന്ന ജീൻ മണ്ഡലത്തിൽ ഏറ്റവും ഉയർന്ന തോതിൽ ആംപ്ലിഫിക്കേഷൻ കാണിച്ചു.

പ്രസ്തുത പഠനത്തിൽ ഉപയോഗിച്ച എല്ലാ തടിഇനങ്ങളിൽ നിന്നും ഡി. എൻ. എ വികസിപ്പിക്കാൻ വിജയകരമായി സാധിച്ചു എന്ന് പഠനഫലങ്ങൾ വിലയിരുത്തുമ്പോൾ മനസിലാക്കാം. തടികലകളിൽ നിന്നുള്ള ഡി. എൻ. എയുടെ സീകൻസുകൾ NCBI ജനിതക ബാങ്കിലെയും BOLD പൊതു ഡൊമൈനിലേയും ഇതേ

വ്യക്ഷഇനങ്ങളുടെ സീകവൻസുകളുമായി ശ്രദ്ധേയമായ അനുരൂപത പ്രകടിപ്പിക്കുന്നുണ്ട്. മാത്രമല്ല ഡി. എൻ. എ ബാർകോഡിങ് എന്ന സാങ്കേതിക വിദ്യ മറ്റു പരമ്പരാഗത തടി തിരിച്ചറിയൽ രീതികളുടെ കൂടെ ഉപയോഗിക്കുന്നത് സൂക്ഷ്മമായ സ്പീഷീസ് തിരിച്ചറിയലിന് ഉചിതമാണെന്ന് പഠനഫലങ്ങൾ വ്യക്തമാക്കുന്നു.