Identification of Mixed Tropical Hardwood (MTH) by characteristic morphological features – a contribution to species protection

Dissertation

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Contents

Abbreviations

APS	All pits similar in size and shape
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CC	Cell corner
CML	Compound middle lamella
DART TOFMS	Direct analysis in real time (DART) and time-of-flight mass spectrometry (TOFMS)
DBU	Deutsche Bundesstiftung Umwelt
DNA	Deoxyribonucleic acid
EU	European Union
EUTR	European Timber Regulation
FE-SEM	Field emission scanning electron microscope
GTTN	Global Timber Tracking Network
HolzSiG	Holzhandelssicherungsgesetz
IAWA	International Association of Wood Anatomists
ITS	Internal transcribed spacer
IVP	Intervessel pit
MDF	Medium density fiberboard
MTH	Mixed Tropical Hardwood
NGO	Non-governmental organization
PAP	Pit to axial parenchyma cell
PCR	Polymerase chain reaction
PF	Pit to fiber
PMV	Papierfabrikation und Mechanische Verfahrenstechnik
PT	Pit to tracheid
rDNA	ribosomal DNA
TI	Thünen Institute
UMSP	Universal microscope spectrophotometry
UV	Ultraviolet
VAS	Vessel-ray pit apparently simple
VRP	Vessel-ray pit

Abstract

This study deals with the investigation of 38 Asian timbers for identification purposes of their genera, thus making it possible to detect these timber genera in fibrous materials such as pulp and paper products as well as fiberboards.

Currently, the standard method for determining wood genera in fibrous materials is to compare the anatomy of the cells with references. The identification is made possible by the examination of certain cells of hardwood: the vessel elements. These have the most numerous and conspicuous characteristic morphological features which differ from genus to genus, thus enabling these genera to be differentiated. There are a number of atlases that list references to temperate woods, which are frequently used for pulp and paper. The most comprehensive sample is the "Fiber Atlas" by Ilvessalo-Pfäffli (1995).

The chief objective of the present investigation is to complement the hitherto existent fiber atlases with a whole new set of genera references, namely those of the Asian tree genera. Thus, it is now for the first time possible to determine these Asian tree genera in fibrous materials as well. Many of these belong to the group “Mixed Tropical Hardwood” (MTH). Particularly with regard to the increase in pulp production in Asia, and especially in Southeast Asia, the need has arisen to provide references for the indigenous Asian tree genera. The investigation procedure consisted of macerating each genus of a total of 35 genera (38 species). Each macerate was then stained and the vessel elements were separated out. Permanent microscope slides of the vessel elements were made and quantitative data on the vessel elements were collected. High-quality microphotographic images of each genus were taken and a selection of representative elements was compiled on one page. In addition, descriptions of the diagnostic features, including quantitative data, were noted. Of the respective genera, selected dimensions of the vessel elements and their intervessel pits as well as of the fibers were recorded. A significance analysis was carried out for the 23 species of the first publication (Helmling et al. 2016).

In addition, comparisons of the investigated genera within two families were made. For this study, four wood genera of the Anacardiaceae and six genera or subgenera of the Dipterocarpaceae were compared with each other. The compared genera of the Anacardiaceae are distinguishable, as a blind test showed. *Campnosperma* shows the biggest differences, while the other three genera *Gluta*, *Mangifera* and *Swintonia* are more similar. The investigated genera of the Dipterocarpaceae (*Dipterocarpus*, *Parashorea* and the four subgenera of *Shorea*: *Anthoshorea*, *Richetia*, *Rubroshorea* and *Shorea)* show great similarities and are hardly distinguishable in mixed samples. For the topochemical characterization, UV spectroscopic investigations were done. The lignin content of mechanical pulp, as compared with the lignin content of macerated tissue, was analyzed by investigating three genera (*Eucalyptus*, *Hevea* and *Shorea* subg. *Rubroshorea*). It could be demonstrated that, in contrast to mechanical pulp, macerated tissue contains nearly no residual lignin.

Furthermore, the variability of characteristic feature dimensions within a genus (*Gonystylus*) and a subgenus (*Shorea* subg. *Rubroshorea*) from different (geographical) origins was investigated. This investigation not only confirms the variability of the dimensions of characteristic morphological features within a genus, but also affirms the expediency of size classes as determined by the standards of the “International Association of Wood Anatomists” (IAWA).

The entire study contributes substantially to the implementation of the European Timber Regulation (EUTR), which was introduced in 2013 in order to thwart trade in illegally logged timber. With the “Atlas of Vessel Elements“ (Helmling et al. 2018) and the documented references therein, the inspectors authorized to implement the EUTR are provided with a means to effectively control pulp and paper products that are imported by market participants into the European Union (EU). This work equips the competent authorities – as well as the traders who carry out voluntary self-checks – with an array of data tools that extends to the vast domain of Asian timbers, thus now enabling their determination in pulp and paper.

Zusammenfassung

Innerhalb der vorliegenden Arbeit wurden 38 asiatische Hölzer untersucht, um zu ermöglichen, diese Gattungen in Fasermaterialien wie Zellstoffen und Papierprodukten sowie Faserplatten zu identifizieren.

Derzeit besteht die Standardmethode zur Bestimmung von Holzgattungen in Faserstoffen darin, die Anatomie der Zellen mit Referenzen zu vergleichen. Die Identifizierung wird durch die Untersuchung bestimmter Zellen im Laubholz möglich: der Gefäßelemente. Diese besitzen die zahlreichsten und auffälligsten charakteristischen morphologischen Merkmale, die sich von Gattung zu Gattung unterscheiden und eine Differenzierung dieser Gattungen ermöglichen. Es gibt bereits Atlanten mit Referenzen von Hölzern aus den gemäßigten Breiten, die häufig für Zellstoff und Papier verwendet werden. Umfangreichstes Beispiel ist der „Fiber Atlas“ von Ilvessalo-Pfäffli (1995).

Hauptziel der vorliegenden Untersuchung ist es, die bisher existierenden Faseratlanten um eine ganze Reihe neuer Gattungsreferenzen zu ergänzen, nämlich um die der asiatischen Baumgattungen. Damit ist es nun erstmals möglich, auch diese asiatischen Baumgattungen in Fasermaterialien zu bestimmen. Viele davon gehören zur Gruppe „Mixed Tropical Hardwood“ (MTH).
Vor dem Hintergrund des Anstiegs der Zellstoffproduktion in Asien, insbesondere in Südostasien, wurde es notwendig, Referenzen für die in Asien heimischen Baumgattungen zu schaffen.
Während der Untersuchungen wurden insgesamt 35 Gattungen (38 Arten) jeweils einzeln mazeriert und das erhaltene Mazerat angefärbt. Die Gefäßelemente wurden separiert, davon mikroskopische Dauerpräparate hergestellt und quantitative Daten über die Gefäßelemente erhoben. Hochwertige mikroskopische Aufnahmen der Gefäßelemente jeder Gattung wurden angefertigt und jeweils eine Auswahl repräsentativer Gefäßelemente auf einer Seite zusammengestellt. Darüber hinaus wurden Beschreibungen der diagnostischen Merkmale, einschließlich der quantitativen Daten, erstellt. Die quantitativen Daten beinhalten neben den Dimensionen der Gefäßelemente und ihrer Gefäßtüpfel auch die Dimensionen der Fasern der jeweiligen Gattung. Eine Signifikanzanalyse wurde an den 23 Gattungen der ersten Veröffentlichung durchgeführt (Helmling et al. 2016).

Zusätzlich wurde ein Vergleich der untersuchten Gattungen innerhalb von zwei Familien vorgenommen. Dafür wurden vier Gattungen der Anacardiaceae und sechs Gattungen bzw. Untergattungen der Dipterocarpaceae gegenübergestellt. Die verglichenen Gattungen der Anacardiaceae sind unterscheidbar, wie auch ein Blindtest zeigte. *Campnosperma* zeigt die größten Unterschiede, während die anderen drei Gattungen *Gluta*, *Mangifera* und *Swintonia* sich eher ähneln. Die untersuchten Gattungen der Dipterocarpaceae (*Dipterocarpus*, *Parashorea* und die Untergattungen von *Shorea*: *Anthoshorea*, *Richetia*, *Rubroshorea* und *Shorea*) zeigen große Ähnlichkeiten und sind in Mischproben kaum unterscheidbar.
Für die topochemische Charakterisierung wurden UV-spektroskopische Untersuchungen durchgeführt. Der Ligningehalt von mechanisch hergestelltem Holzschliff im Vergleich zu mazeriertem Gewebe wurde an drei Gattungen analysiert (*Eucalyptus*, *Hevea* und *Shorea* subg. *Rubroshorea*). Es konnte gezeigt werden, dass mazeriertes Gewebe im Gegensatz zu Holzschliff so gut wie keine Ligninrückstände mehr enthält.

Außerdem wurde die Variabilität der charakteristischen Strukturmerkmale in Bezug auf die Dimensionen innerhalb einer Gattung (*Gonystylus*) und einer Untergattung (*Shorea* subg. *Rubroshorea*) unterschiedlicher (geographischer) Herkunft untersucht. Diese Untersuchung zeigte einerseits die Variabilität der Dimensionen innerhalb einer Gattung, bestätigte aber auch den Sinn

festgelegter Größenklassen, wie sie in den Bestimmungsstandards der „International Association of Wood Anatomists“ (IAWA) vorliegen.

Die gesamte Arbeit trägt substanziell zur Umsetzung der Europäischen Holzhandelsverordnung (EUTR) bei, welche 2013 eingeführt wurde, um den Handel mit illegal eingeschlagenem Holz zu verhindern. Mit dem „Atlas of Vessel Elements“ (Helmling et al. 2018) und den darin dokumentierten Referenzen erhalten die zur Umsetzung der EUTR befugten Inspektoren ein Mittel zur wirksamen Kontrolle von Zellstoff- und Papierprodukten, die von Marktteilnehmern in die Europäische Union (EU) eingeführt werden. Diese Arbeit erweitert nun das Instrumentarium auf asiatische Hölzer und ermöglicht den zuständigen Behörden – wie auch den Händlern, die freiwillige Selbstkontrollen durchführen – deren Bestimmung in Zellstoff und Papier.

List of Publications

I. Helmling S, Olbrich A, Tepe L, Koch G (2016). Qualitative and quantitative characteristics of macerated vessels of 23 mixed tropical hardwood (MTH) species: a data collection for the identification of wood species in pulp and paper. Holzforsch 70(9):839-844, DOI: 10.1515/hf-2015-0195
(peer-reviewed)

II. Helmling S, Olbrich A, Heinz I, Koch G (2018). IAWA Atlas of Vessel Elements © 2018. IAWA Journal 39 (3):249–352 Leiden, The Netherlands, DOI: 10.1163/22941932-20180202
(peer-reviewed)

III. Odermatt J, Olbrich A, Helmling S, Wassink A (2017). Identifizierung von Mixed Tropical Hardwood (MTH) in Papier mittels chemo-taxonomischer und morphologischer Merkmale. Final report of the DBU project AZ 31759-31.

IV. Koch G, Haag V, Helmling S, Heinz I, Olbrich A (2017). Fasern im Fokus: Holzartenbestimmung von Faserplatten - Erfahrungen aus den Prüfungen im Kontext der EUTR. MDF Mag Co: 86–88

V. Sieburg-Rockel IJ, Koch G, Kaschuro S, Helmling S, Olbrich A (2019). Identifizierung von Holzarten in Spanplatten, Holztechnologie 60(3):5-9, Institut für Holztechnologie Dresden (ihd)

VI. Heinz I, Helmling S, Olbrich A, Koch G (2019). O-5: Identification of Asian timbers in pulp, paper and fiber boards. In: IAWA-IUFRO International Symposium: challenges and opportunities for updating wood identification; May 20–22, 2019, China, program & abstracts. pp. 5–6

VII. Schmitz N (editor), Beeckman H, Blanc-Jolivet C, Boeschoten L, Braga J W B, Cabezas J A, Chaix G, Crameri S, Degen B, Deklerck V, Espinoza E, Gasson P, Haag V, Helmling S, Horacek M, Koch G, Lancaster C, Lens F, Lowe A, Martínez-Jarquín S, Nowakowska J A, Olbrich A, Paredes-Villanueva K, Pastore T C M, Ramananantoandro T, Razafimahatratra A R, Ravindran P, Rees G, Soares L F, Tysklind N, Vlam M, Watkinson C, Wheeler E, Winkler R, Wiedenhoeft A C, Zemke V, Zuidema P (2020). A data analysis guide for wood identification. Overview of current practices for different methods. Global Timber Tracking Network, GTTN secretariat, European Forest Institute and Thünen Institute

1 Introduction

This work consists of two main chapters: The first chapter is the introduction, and the second chapter includes the results and the discussion. The first part of the introduction establishes the scientific connection between the two peer-reviewed publications in "Holzforschung" (2016) and in the "IAWA journal" (2018). The two publications lay the foundation for the topic "Identification of Mixed Tropical Hardwood (MTH) in paper by characteristic morphological features – a contribution to species protection".
This study is followed by supplements concerning the materials and the method as well as further investigations not included in the peer-reviewed articles.

The demand for paper products is increasing worldwide (Flosdorff et al. 2010, Williams 2014; FAO 2020). Accordingly, the production of pulp and paper has also been on the rise. In industrial countries, the rising consumption is partly due to the increasing extent of thriving online trade, where the purchased products are delivered in cardboard packaging material (Umweltbundesamt 2012). A further increase in paper consumption can be explained by the avoidance of plastic and the associated replacement by paper products.

In Germany, the total production of paper, cardboard and paperboard amounted to 20.4 million tons in the year 2019 (January – November; VDP Statistiken 2019). In comparison, the statistics of the year 2018 (January – November) in Germany showed that, while the production of graphic papers (−8.4 % to 6.6 million tons) and hygiene papers (−0.1 % to 1.4 million tons) declined, the production of cardboard packaging material stepped up by 0.6 % to 11.2 million tons. Within the same time periods, the production of the raw materials paper pulp and mechanical pulp in Germany decreased by 2.1 % to 2.1 million tons (VDP Statistiken 2019). In addition to its own production, Germany imported 11.6 million tons of paper and paper products in 2018 (FAO 2020).

For the production of pulp and paper, the producing companies need wood (or recycled paper). According to a study by the Thünen Institute for Forest Economy in 2009, between 103 million and 284 million cubic meters of raw wood were illegally logged. A study by Dieter et al. (2012) revealed that 7% to 17% of all globally logged timber originates from illegal sources. According to Nellemann and INTERPOL (2012) even 15% to 30% of the globally traded timber has been obtained illegally. The concern regarding the increasing proportion of illegally obtained material led to the enactment of several laws: the U.S. Lacey Act (USA, 1900/amended in 2008), the Illegal Logging Prohibition Act 2012 (Australia) and the European Timber Regulation (EUTR No. 995/2010, implemented in 2013). These laws and regulations aim to protect dwindling natural forests on our planet. The newest one, the EUTR of Europe, requires that all operators placing timber or timber products onto the EU market must declare the origin and species of their timber. They must fulfill their due diligence and are subject to control. In Germany, the controls of market participants are carried out by the "Bundesanstalt für Landwirtschaft und Ernährung" (BLE), or in English: Federal Office for Agriculture and Food. It is prohibited to place illegally harvested timber on the market. The German law which implements the EUTR in Germany is called the "Holzhandels-Sicherungs-Gesetz" (HolzSiG).

Paper products are usually made of pulp that originates from fast-growing plantation trees (e.g. *Eucalyptus, Populus, Pinus, Acacia*). These plantation trees often grow on sites that were once covered with forests, as can be seen in Brazil or Indonesia.

Figure 1 illustrates the locations of forests in the world (FAO 2001). Tropical rainforests are shown in violet. The largest tropical rainforest areas are located in South America, Central Africa and Southeast Asia. The timber of these primary forests also gets processed to pulp and paper or to fiberboards.

Particularly the tropical rain forests in Asia are potential suppliers of raw material for the pulp mills on account of the increasing pulp industry in Asia (Hirschberger et al. 2012, FAO 2015, Figure 2).

Due to the naturally occurring mixed genera in primary forests, the wood is declared as “Mixed Tropical Hardwood” (MTH). MTH originates in tropical primary forests with a high biodiversity of wood species (Voith 2004). By collecting wood chips from Indonesian paper mills, Greenpeace discovered that wood from tropical primary forests is being processed in local pulp mills (Greenpeace 2012). These wood chips included the protected wood species *Gonystylus* (Ramin). Ramin is protected by the regulations of the “Convention on International Trade in Endangered Species of Wild Fauna and Flora” (CITES). Until now, no effort has been made to determine the individual species of MTH wood and to control whether protected species such as *Gonystylus* (Ramin) have been processed to pulp.
The method for determining the species used for paper products will be explained in detail in chapter 1.1 (State of the art, p. 5).

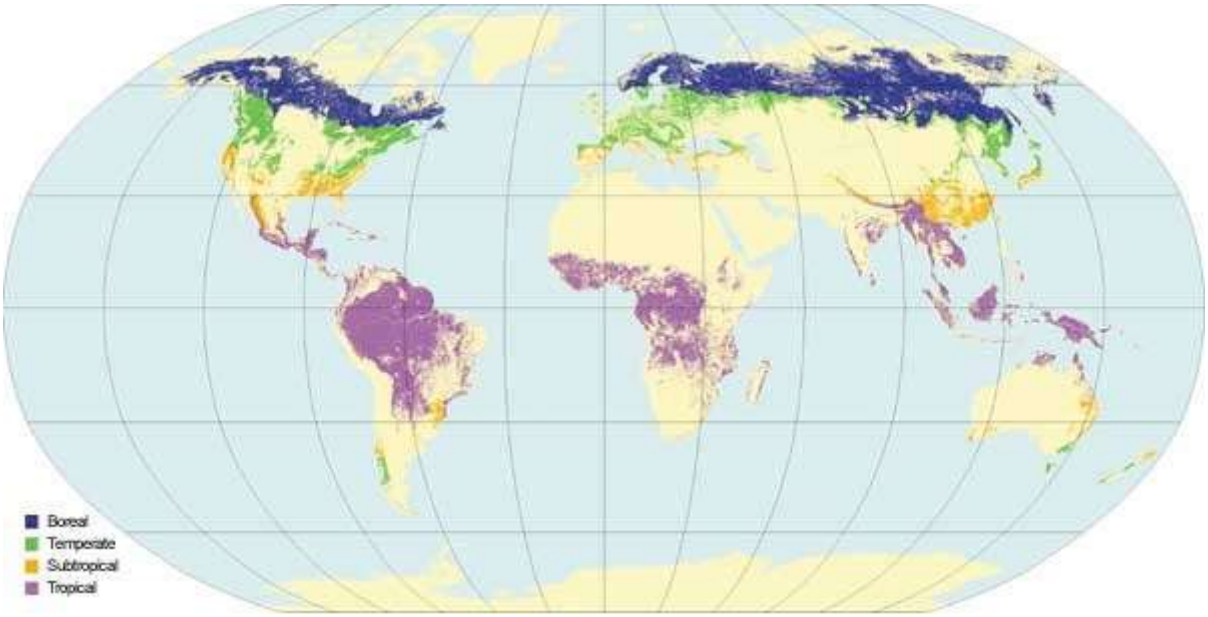


Figure 1 Locations of forests by region (www.fao.org/3/Y0900E/y0900e05.htm 01 Feb 2020)

In any case, evidently there is a need for a tool which enables the testing institutes and laboratories to control the contents of the produced material by order of the traders, consumers and competent authorities of each involved country. For instance, are the species found in the paper products identical to the species officially declared in the import documents? Have tree species protected by the regulations of CITES been used? In answer to these questions, the material must be investigated in order to determine the processed wood genera. The only investigation method established to date is by light microscopy.

For identification purposes, there is a need for a database that contains as many wood species as possible. The database is used as reference. The cell material that is viewed through a light microscope must be compared with the database. When this work was begun, the most extensive publicly available database was published as “Fiber Atlas” (Ilvessalo-Pfäffli 1995). This atlas mainly contains species that are often used for pulp and paper production, the species originating in the temperate zone (Europe and North America). A few other small atlases contain wood species usually

used for paper production (Carpenter and Leney 1952, Ezpeleta and Simon 1970, 1971, Harders-Steinhäuser 1974, Parham and Gray 1982). Until now, there were no fiber atlases containing references to tropical tree species. Since the production of pulp and paper in Asia is rapidly increasing, it has obviously become necessary to expand the existing database by those timbers indigenous to Asia.
This work serves to extend the existing references to the domain of Asian wood species, in order to enable their identification in pulp and paper products, as required by the EUTR.

The two publications contained in this work deal with the same topic: the identification of Mixed Tropical Hardwood (MTH) in pulp and paper products. Thus the various components of this work complement each other. The first publication, "Qualitative and quantitative characteristics of macerated vessels of 23 mixed tropical hardwood (MTH) species: a data collection for the identification of wood species in pulp and paper", published in "Holzforschung" (2016), deals with the materials and the specially developed method for the investigation of 23 species of MTH. A statistical evaluation is included. The second publication, "Atlas of Vessel Elements" (2018), contains a collection of 38 Asian timbers. These 38 timbers include the 23 species that were investigated in the first publication. The qualitative and quantitative characteristics of each species are described in detail and shown in photomicrographs. The photomicrographs in the Atlas were produced with a light microscope (Olympus, BX51, see 1.3.2). The field emission scanning electron microscope (FE-SEM) delivers images of the highest quality and detailed views of the vessel elements. However, this instrument is not available in every laboratory. Therefore, the light microscope was chosen, ensuring reproducibility of results and usability of the Atlas in every laboratory or testing institute. The scientist investigating a paper product of unknown composition can compare his/her own light microscope results with the images in the Atlas.

The Atlas contains an extensive introduction to the use of the Atlas, with exact descriptions of the characteristic morphological features. It provides the necessary skill set for the identification of the contained genera listed in the Atlas. The investigated species originated mainly in Southeast Asia. This region was chosen because particularly in Asia, the pulp industry is rapidly growing (Figure 2, Figure 3). A part of Figure 2 shows the example of Indonesia and the increasing production quantity of wood pulp. In the last 30 years, it has increased more than 80-fold (from 108,000 tons in 1988 to 8,679,335 tons in 2018).

In summary, the two publications, especially the second one, enable the detection of MTH in paper products and the identification (if present) of one of the 38 investigated genera listed in this Atlas. The objective of this work is to add a much-needed supplement of the tropical genera to the „Fiber Atlas" by Ilvessalo-Pfäffli (1995). Of course, it will become necessary in the future to further expand the existing database of references by many more genera.

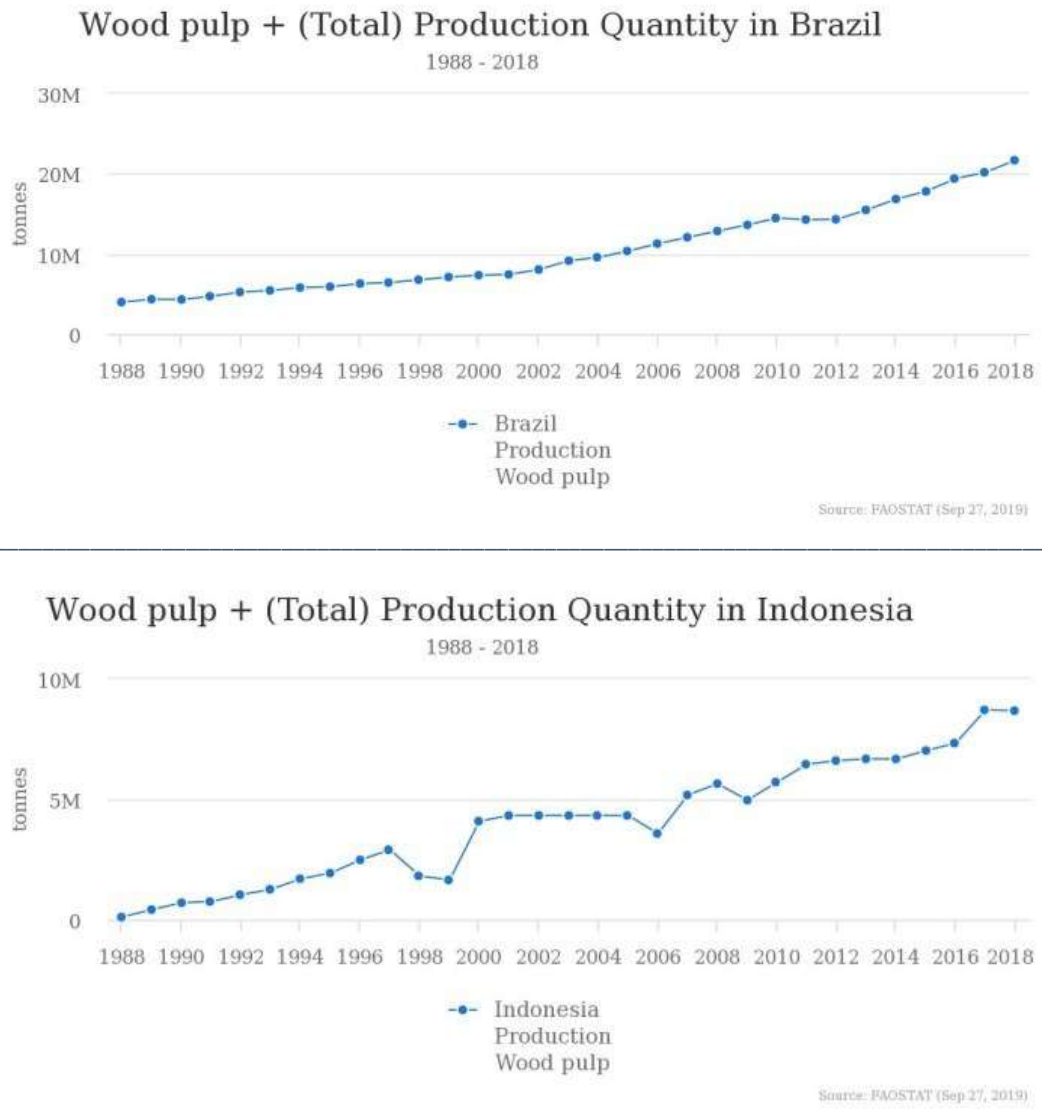


Figure 2 Wood pulp production quantity in Brazil and Indonesia (1988-2018) [FAOSTAT, Sep 27, 2019]

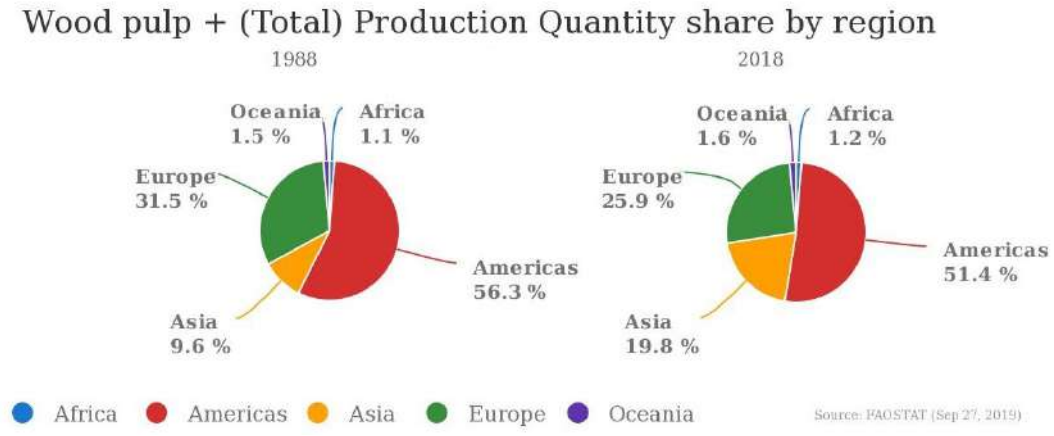


Figure 3 Regional Shares of the Total Worldwide Wood Pulp Production Quantity (1988 vs. 2018) [FAOSTAT, Sep 27, 2019]

1.1 State of the art

This chapter provides information on different approaches to methods of determining wood species in pulp and paper. The most suited and only established method today is to examine the morphology of certain cells – the vessel elements (see Identification of the genus).

In two consecutive projects of the "Deutsche Bundesstiftung Umwelt" (DBU) or in English: The German Federal Environmental Foundation, No. 29436/01 and No. 31759-31, a database of vessel elements of Asian tree species was created, as until then no database with information on these tree species had existed. The objective of the projects was to collect all pertinent data that would enable the identification of these Asian genera. Project partners were the University of Hamburg (Chemical Wood Technology, PD Dr. habil. J. Odermatt), the Thünen Institute of Wood Research (PD Dr. habil. G. Koch and Dr. A. Olbrich), the TU Darmstadt (PMV, Prof. Dr.-Ing. S. Schabel, Dr.-Ing. H.-J. Schaffrath), the ISEGA Research Company mbH (Aschaffenburg, Dr. R. Derra) and the EMSAT Services UG* (Hamburg, Dr. J. Odermatt). The results were published in two journals (both peer-reviewed): The first article was published in "Holzforschung" (2016); the second one was published in the "IAWA journal" (2018) as an "Atlas of Vessel Elements". The database that was developed in the two projects contains references to 38 newly researched tree species from Southeast Asia. In a third project (DBU No. 34295/01) the Atlas will be enlarged. Investigations of 20 more Asian tree species are planned, the results of which will be published in a second volume of the "Atlas of Vessel Elements". In the first volume of the Atlas, the collection of references is far from being complete, as the Atlas covers only a small species spectrum of Asian tree species. When tropical wood is found in paper, it often consists of a mixture of species, as the processed trees come from clear-cut natural forests. Hence the urgency of collecting as many species references as possible.

*EMSAT Services UG was project partner during the project no. 2 (DBU 31759-31) and afterwards of no. 3 (DBU no. 34295/01).

1.1.1 Identification of the genus

This section presents several methods for identifying the wood species or provenance of wood. The data analysis guide of the "Global Timber Tracking Network" (GTTN) gives a detailed overview of tools used to determine the provenance or wood species (Schmitz et al. 2020). In general, reference material is required for all of the following methods.

1.1.1.1 Identification by examining the morphology of certain cells – the vessel elements

For the identification of species in paper products, it is essential to investigate the processed material. Every hardwood species contains a certain cell type that forms water pipelines within the tree. These cells are called "vessel elements" and transport water from the roots through the trunk to the leaves (Figure 4). The vessel elements possess genus-specific morphological characters that, for the most part, vary greatly from genus to genus (Ilvessalo-Pfäffli 1995). Other commonly occurring cell types, such as fibers that support stabilization, look very similar to each other from species to species and are therefore not suited for the identification of the wood species.

By examining the vessel elements, it is possible to identify a genus – provided that a reference to this genus is stored in the database. The identification process is carried out by comparing the appearance of a vessel element, as seen through a light microscope, with pictures of references. The references are listed in atlases. Excellent fiber atlases that describe the vessel elements of tree species from the temperate zones of Europe and North America have been published, for example, by Ilvessalo-Pfäffli (1995) and Harders-Steinhäuser (1974). Certain characteristic features such as the opening of the vessel elements, the presence and shape of tails and the presence of helical thickenings and/or of tyloses, pit type, pit size and pit pattern, and the dimensions of the vessel

element itself, are decisive criteria for the identification of a genus. The characteristic structural features requisite for the identification of a genus are based on the “IAWA List of Microscopic Features for Hardwood Identification” (1989) and the "commented list of features for the determination of wood species" (Richter and Trockenbrodt 1999).

The identification process usually ends at the genus level. It is generally not possible to distinguish between different tree species in a common genus, as the features of their vessel elements are too similar to each other. As stated above, this identification process applies only to hardwood. Softwood does not possess vessel elements but tracheids. Tracheids have much fewer characteristic features and the differentiation between the genera is more limited.

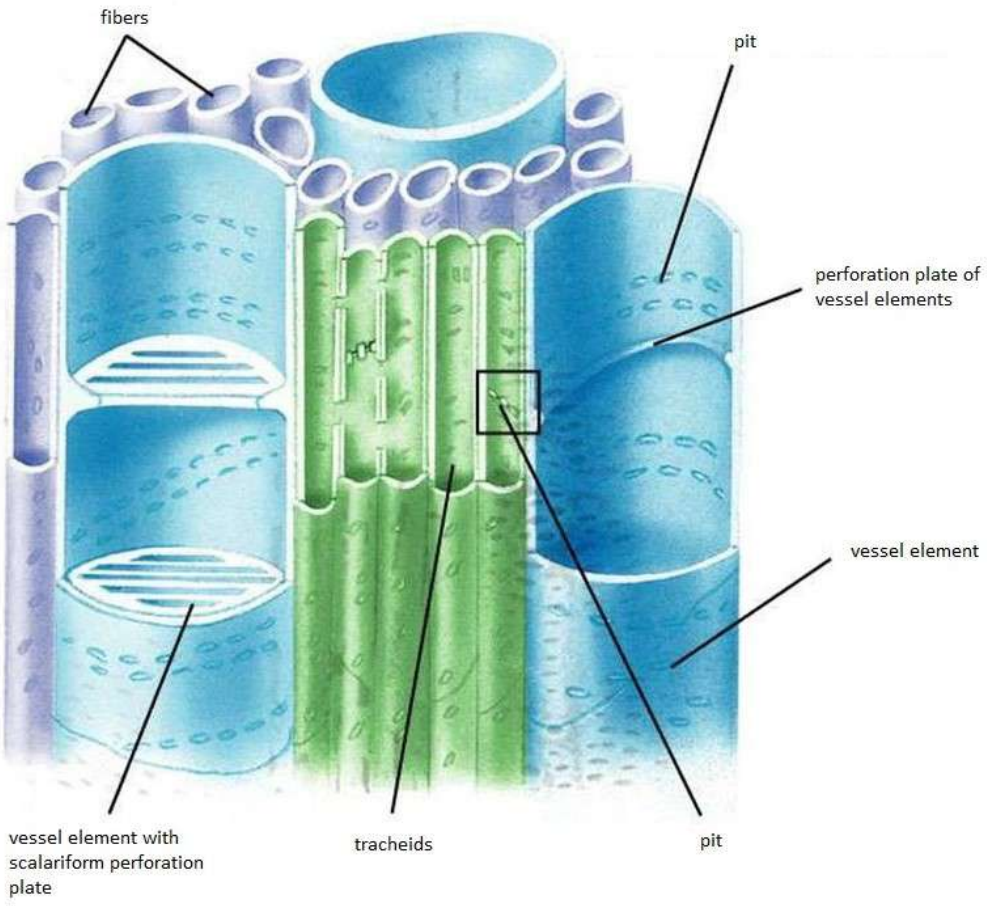


Figure 4 Structure of cell tissue of different cell types in hardwood (onlinesciencenotes.com/complex-permanent-tissues-in-plants-structure-types-and-functions 10th Aug 2019)

1.1.1.2 Identification of species by chemotaxonomic analyses

Extractives can also provide information about the species in pulp and paper products. During the production process of paper, many extractives get washed out, or they get changed by the use of chemicals, water and also thermal treatment. However, depending on the intensity of the pulping and bleaching procedures, some of the extractives do indeed remain in their original form. In bleached paper, residues of extractives can be found. These extractives are resinous and fatty acids, their metal salts and esters, fats, waxes, sterols, paraffins and oxidation-, condensation- and polymerization-products of these substances and, in the case of bleached pulps, chlorinated substances (Krause and Schempp 1991). Within the same DBU projects in which the results of this work were obtained, scientists of the University of Hamburg (PD Dr. Jürgen Odermatt et al.)

investigated the residual extractives in pulp. They were able to show that the quantity of the remaining extractives in paper products – after extraction, concentration and gas chromatography – suffices for obtaining significant chromatograms. For the identification of species in paper by means of chemotaxonomy, databases of the chromatograms of the different genera will be required. Also, this method still needs to be verified.

1.1.1.3 Identification of species in paper by determination of the deoxyribonucleic acid (DNA)

If the wood species is to be identified by DNA analysis, phylogenetic investigations at the molecular level are possible (De Filippis and Magel 1998). The DNA is the chemical carrier of genetic information (Raven et al. 2000). A decisive factor for successful DNA extraction is the input material. Materials such as leaves, needles and young branches are routinely being used for extracting the entire genomic DNA (Wischnewski 2014, Finkeldey et al. 2010). In contrast, DNA extraction from sapwood and heartwood is much more difficult, the reason being that these woods contain lower amounts of DNA (Wischnewski 2014). In addition, DNA extracted from sapwood and heartwood is of inferior quality because it degrades into smaller fragments during heartwood formation (Wischnewski 2014, Lindahl 1993). The ideal source of high-quality DNA is the cambium (Nowakowska 2011, Schmitz et al. 2020).

Wood possesses strong polymerase chain reaction (PCR) inhibitors, e.g. phenolic components, polysaccharides and proteins (Wischnewski 2014, Pandey et al. 1996). Numerous investigations have confirmed the suitability of the Internal transcribed spacer (ITS) region for identification purposes at the genus level or often even at the species level (Hansel et al. 2011). It has been shown that the rapidly evolving ITS region of ribosomal DNA (rDNA) is particularly well-suited for the construction of relationships, especially at the genus and species levels, and thus also for the identification of organisms (Magel 2008, Liston et al. 1996). The ITS region (located between 18S and 26S rDNA) consists of the ITS I- and ITS II sub-region, between which the conserved 5.8S rDNA is located (Magel 2008). The sequences of this region are very similar within genera of higher plants and are therefore easy to compare. Nevertheless, the sequences are variable enough to exhibit certain differences between species, as these non-coding regions usually change much faster than coding regions (Magel 2008). Due to their high replication number in the genome and their relatively small size of 565-700 base pairs (applies only to angiosperms), they are easy to amplify, but still have a sufficient length for phylogenetic studies (Liston et al. 1996). Because of the flanking conserved regions, they can easily be identified by using the primers (ITS 1 and ITS 4), which are universal for eukaryotes (White et al. 1990).

However, the DNA fragments in wood are further degraded by processing procedures such as drying or boiling, or merely by long-term storage (Wischnewski 2014, Abe et al. 2011).
In paper products, it is not possible to trace back the processed tree species by means of DNA analysis. The production of pulp involves treatment with chemicals as well as thermal treatment with temperatures above 100°C, which destroy the DNA.

1.1.1.4 Identification of origin by stable isotope analysis

The isotopic composition of biomass varies according to the geographical location, or, better said, each and every geographical location in the world hat its own characteristic isotopic make-up. The method of isotope analysis determines the proportions of isotopes of different chemical elements within a sample. Most chemical elements possess several isotopes. In isotope analyses, hydrogen, oxygen, carbon, nitrogen and sulphur are investigated. With a mass spectrometer, the isotopic composition can be determined very precisely if, for instance, a single sample of solid wood is analyzed. For this investigation, sapwood or heartwood has to be selected (Schmitz et al. 2020).

Sample materials other than wood, such as needles or leaves, are more suitable for the investigation of freshly cut trees (Horacek 2012).

Since during the paper production process pulps of different regions and species are mixed together, isotope analysis is not an adequate method for provenance identification. Furthermore, the isotope analysis method is complicated by the fact that in paper, additives like starch present a source of an additional isotope fingerprint – although there even is no individual isotopic fingerprint in the classical sense (Schmitz et al. 2020). As to the investigation of "Medium density fiberboard" (MDF): An isotope analysis is currently either not possible or extremely complicated and time-consuming (Schmitz et al. 2020).

1.1.1.5 Identification of origin by "Direct analysis in real time and time-of-flight mass spectrometry" (DART TOFMS)

Small chemical compounds taken from wood, such as extractives, can be examined by means of a mass spectrometer. DART TOFMS connects DART™ ionization with TOFMS (Espinoza et al. 2014). The DART TOFMS is currently the most developed method of mass spectrometry but is not regularly used for identification of origin purposes (Schmitz et al. 2020). According to Lancaster (2019), the signal was too low when testing the DART TOFMS for the identification of a pulp sample.

1.1.2 Cell types in pulp and paper

The materials that are processed for pulp and paper consist of plant cells and fillers. Kaolin or calcium carbonate, for example, can be added as fillers during processing (Weigl 1991). The plant cells are, for the most part, fibers. The fibers serve as stabilizing elements in the plants (Figure 5). All plant fibers are very similar in their appearance, regardless of the species, which renders them unsuited for the identification of individual genera. The remaining, non-fibrous plant cells are tracheids, parenchyma cells and vessel elements (Braun 1998, Raven et al. 2000, Nultsch 2001; Figure 5).

The tracheids have stabilizing and water-transporting functions. These elements are recognizable by the absence of openings (perforation plates) at the top or bottom and by their bordered pits.

The parenchyma cells store nutrients and are also pitted (simple pits, unbordered, along the sides of the cells). Tracheids and parenchyma cells are not sufficiently different from other cells to be of much assistance for identification purposes, but they can provide helpful clues.

The vessel elements transport water vertically from the roots through the trunk to the leaves (Figure 6). Therefore, they possess large openings at the top and at the bottom. This feature distinguishes the vessel elements from the tracheids. The vessel elements also possess pits of the newly introduced type APS ("all pits similar in size and shape") and type VAS ("vessel-ray pits apparently simple"). These two types are explained in detail in the "Atlas of Vessel Elements" (Helmling et al. 2018). Pits are little channels in the cell wall that connect to adjacent cells (Figure 7). These small pores are intended for the transfer of substances such as nutrients. Pits also are essential for the horizontal (as opposed to the vertical) transport of water in the plant. The vessel-ray pits, i.e. the pits connecting to adjacent wood rays, are particularly characteristic within a genus (Ilvessalo-Pfäffli 1995). They differ greatly in size and shape from genus to genus and can be (usually easily) recognized by their horizontal arrangement on the wall of the vessel element. In general, the pit sizes and shapes, the patterns of their intervessel pits, as well as the vessel-ray pits differ from genus to genus. In addition, the morphology of the vessel elements varies with regards to their dimensions, the kind of perforation plates, the presence of tyloses and helical thickenings, as well as the kinds of tails, if present. Hence the unique combination of these features within each genus makes it possible

to distinguish between different genera. As already stated before in 1.1.1, this is only valid for hardwood, since softwood does not possess any vessel elements.

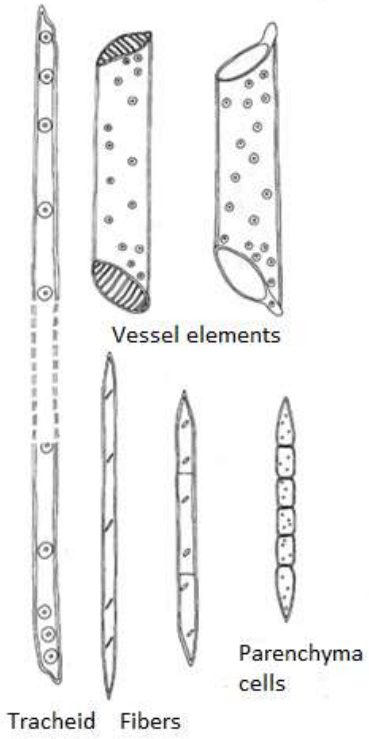


Figure 5 Basic shapes of cells that build the axially directed wood. Tracheid; Vessel elements with scalariform and simple perforation plates; Fibers, left unseptate and dead, right septate (often also unseptate) and alive. Parenchyma cells, subsequently septate (Braun, 1970)

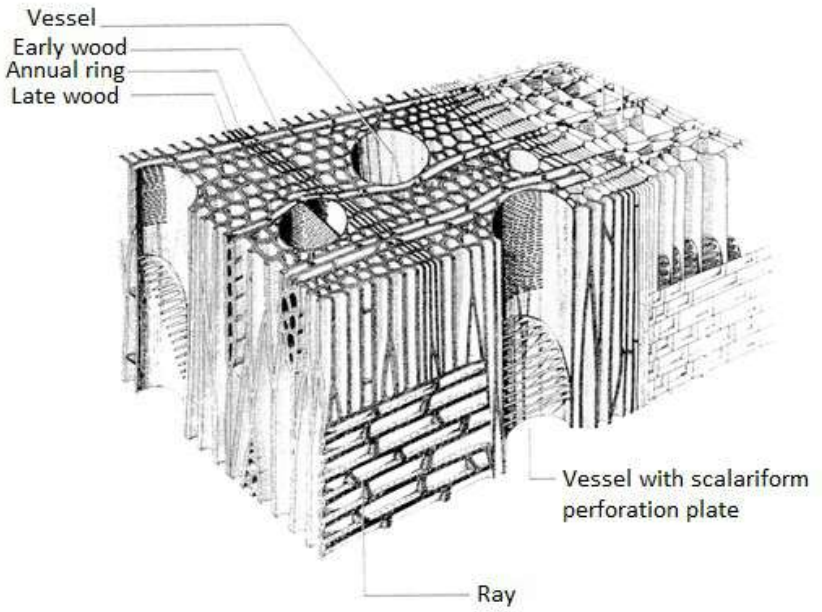


Figure 6 Hardwood. Detail of the wood of Betula on the border to the bark in three-dimensional view (Mägdefrau, 1951)

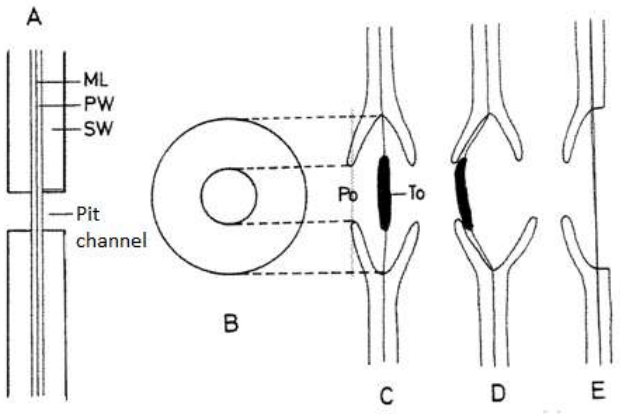


Figure 7 Schematic illustration of pits.
A: simple pit, unbordered; ML: Middle lamella; PW: Primary wall; SW: Secondary wall; B,C,D: Bordered pit between two tracheids in surface view (B) and in longitudinal section (C: with normal position of the torus, D: with position of the torus in case of one-sided pull); Po: Porus; To: Torus. E: unilaterally bordered pit between a tracheid (left) and a parenchyma cell (Braun, 1963)

Vestured pits

Pits can also be *vestured* (Figure 8). The possible function of vestured pits, which are bordered pits with protuberances from the secondary cell wall of the pit chamber, could be increased hydraulic resistance or minimized vulnerability to air seeding (Jansen et al. 2004). A correlation between the type of the perforation plate and vestured pits has been ascertained. All taxa with vestured pits possess simple perforation plates (Jansen et al. 2003). This characteristic feature is useful for the identification of wood species if the sample to be investigated is solid wood. In pulp and paper, it is generally not possible to detect the vestured pits anymore, due to the chemical treatment during processing, as well as to the dissolved lignin (regarding dissolved lignin, see also 1.3.5 and 2.2: Cellular UV microspectrophotometry). Vestures consist mainly of lignin, hemicellulose and a small amount of pectin, but not of cellulose (Jansen et al. 2000, Côté and Day 1962, Schmid 1965, Ohtani et al. 1984). That is the reason why – as opposed to helical thickenings - the vestures dissolve during maceration and are not detectable anymore (Scurfield and Silva 1970, Baird et al. 1974).

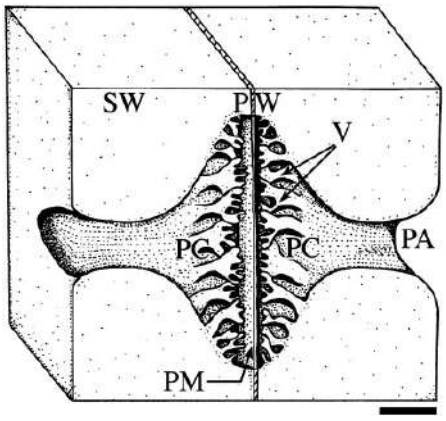


Figure 8 Diagram of a vestured pit pair in Flabellaria paniculata Cav. (Malpighiaceae) with overarching secondary cell wall (SW), vestures (V), pit chamber (PC), pit aperture (PA), pit membrane (PM), and primary cell wall + middle lamella (PW). (Bar = 2µm) (Jansen et al. 2004)

1.2 Material

The investigated species represent important trade species and commonly used species in paper products, as well as a CITES-protected species (*Gonystylus*) and two mangroves – in order to gain access to a first contact point of sensitive habitats. The selection of the taxa depended on the evaluation of about 2,150 official reports prepared at the Thünen Centre of Competence on the Origin of Timber, Hamburg, Germany, since the implementation of the EUTR (2013) in Germany. The species were chosen in cooperation with the two non-governmental organizations (NGOs), Greenpeace and Worldwide Fund for Nature (WWF). However, there was only one CITES-protected wood species selected, since these valuable woods are usually not processed into pulp. Monocots instead are commonly processed into pulp and paper; therefore *Dendrocalamus* and *Cocos* were included in the list of species to be examined. The other MTH species, although not CITES-listed, represent indicator species for the possible processing of primary forest. The material for the investigation was obtained from the scientific collection of the Thünen Institute of Wood Research (TI, Hamburg, Germany). The TI material originates mostly from natural forests (*Gonystylus*, *Shorea*). Some species originate from plantations (*Acacia*, *Hevea*, *Cocos*). 38 samples have been investigated (Table 1, p. 12). They can be assigned to 24 families and to 35 genera that mainly grow in Southeast Asia.

Further information about the chosen material can be found in the two publications Helmling et al. (2016) and Helmling et al. (2018).

The cladistics (Table 2, p.13) illustrates the relationships between the investigated genera. Most of the examined genera belong to different families. The investigated species of 24 families can be assigned to 15 different orders. Several representatives of the families of Anacardiaceae and Dipterocarpaceae were studied. Details and a complete comparison of the different genera within these two latter families can be found in chapter 1.3.6 (method) and in chapter 2.4 (results).

*Table 1 List of investigated species in alphabetical order of families (RBHw = vouchered material, * = commercial sample – genus verified).*

Family	Taxa/species	Trade name	RBHw No.
Acanthaceae	*Avicennia marina* (Forssk.) Vierh.	Api Api (mangrove)	24226
Altingiaceae	*Liquidambar formosana* Hance	Formosan sweet gum	23479
Anacardiaceae	*Campnosperma* sp.	Terentang	*
Anacardiaceae	*Gluta renghas* L.	Rengas	*
Anacardiaceae	*Mangifera* sp.	Mango	*
Anacardiaceae	*Swintonia* sp.	Merpauh	*
Aquifoliaceae	*Ilex triflora* var. *kanehirai* (Yamamoto) S. Y. Hu	Kecemang	10148
Arecaceae	*Cocos nucifera* L.	Coconut palm	*
Burseraceae	*Canarium* sp.	Kedondong	*
Calophyllaceae	*Calophyllum* sp.	Bintangor	*
Celastraceae	*Lophopetalum* sp.	Perupok	*
Dipterocarpaceae	*Shorea* subg. *Anthoshorea*	White Meranti	*
Dipterocarpaceae	*Shorea* subg. *Richetia*	Yellow Meranti	*
Dipterocarpaceae	*Shorea* subg. *Rubroshorea*	Dark/Light Red Meranti	*
Dipterocarpaceae	*Shorea* subg. *Shorea*	Bangkirai, Balau	*
Dipterocarpaceae	*Parashorea* sp.	Gerutu	*
Dipterocarpaceae	*Dipterocarpus* sp.	Keruing	*
Euphorbiaceae	*Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg.	Rubberwood	9880
Fabaceae-Caesalpinioideae	*Intsia* sp.	Merbau	*
Fabaceae-Caesalpinioideae	*Koompassia malaccensis* Maingay ex Benth.	Kempas	*
Fabaceae-Mimosoideae	*Acacia mangium* Willd.	Acacia	*
Fabaceae-Mimosoideae	*Albizia procera* (Roxb.) Benth.	White siris, Kokko	193
Fagaceae	*Castanopsis argentea* (Blume) A. DC.	Berangan	18490
Lauraceae	*Litsea resinosa* Bl.	Medang	15426
Malvaceae	*Durio* sp.	Durian	*
Malvaceae	*Heritiera* sp.	Mengkulang	*
Myrtaceae	*Eucalyptus globulus* Labill.	Eucalyptus	*
Myrtaceae	*Syzygium dyerianum* (King) Chantaran. & J. Parn.	Kelat	3854
Nyssaceae	*Nyssa javanica* (Blume) Wangerin	Tupelo, Nyssa	13989
Paulowniaceae	*Paulownia tomentosa* (Thunb.) Steud.	Paulownia	5531
Poaceae	*Dendrocalamus latiflorus* Munro	Bamboo	22667
Rhizophoraceae	*Rhizophora* sp.	Red Mangrove	24661
Sapotaceae	*Madhuca sericea* (Miq.) S. Moore	Bitis	*
Sapotaceae	*Palaquium* sp.	Nyatoh	*
Styracaceae	*Alniphyllum pterospermum* Matsum.	Mee Dong	10151
Tetrameristaceae	*Tetramerista glabra* Miq.	Punah	*
Theaceae	*Schima superba* Gardn. & Champ.	Samak, Puspa	24166
Thymelaeaceae	*Gonystylus* sp.	Ramin	*

Table 2 Cladistics of the investigated genera

Clade	Order	Family	Genus
Eudicotyledons	Aquifoliales	Aquifoliaceae	*Ilex*
	Celastrales	Celastraceae	*Lophopetalum*
	Cornales	Nyssaceae	*Nyssa*
	Ericales	Sapotaceae	*Madhuca*
			Palaquium
		Styracaceae	*Alniphyllum*
		Tetrameristaceae	*Tetramerista*
		Theaceae	*Schima*
	Fabales	Fabaceae-Caesalpinioideae	*Intsia*
			Koompassia
		Fabaceae-Mimosoideae	*Acacia*
			Albizia
	Fagales	Fagaceae	*Castanopsis*
	Lamiales	Acanthaceae	*Avicennia*
		Paulowniaceae	*Paulownia*
	Laurales	Lauraceae	*Litsea*
	Malpighiales	Calophyllaceae	*Calophyllum*
		Euphorbiaceae	*Hevea*
		Rhizophoraceae	*Rhizophora*
	Malvales	Dipterocarpaceae	*Dipterocarpus*
			Parashorea
			Shorea subg. *Anthoshorea*
			Shorea subg. *Richetia*
			Shorea subg. *Rubroshorea*
			Shorea subg. *Shorea*
		Malvaceae	*Durio*
			Heritiera
		Thymelaeaceae	*Gonystylus*
	Myrtales	Myrtaceae	*Eucalyptus*
			Syzygium
	Sapindales	Anacardiaceae	*Campnosperma*
			Gluta
			Mangifera
			Swintonia
		Burseraceae	*Canarium*
	Saxifragales	Altingiaceae	*Liquidambar*
Monocotyledons	Arecales	Arecaceae	*Cocos*
	Poales	Poaceae	*Dendrocalamus*

1.3 Methods

The basics of the methods used in this work are described in detail in the two attached publications Helmling et al. (2016) and Helmling et al. (2018). This following part provides additional information on the chosen methods.

1.3.1 Maceration and staining

For the determination of the cell type of the characteristic vessel elements, the solid wood tissue had to be dissolved, thereby causing the vessel elements to separate from the other cells. For this step, the wood had to be macerated.

Franklin (1945) developed a method for maceration by using equal parts of glacial acetic acid and a solution of H_2O_2. In this work, Franklin's method was chosen for maceration, with a 99% solution of glacial acetic acid and a 30% solution of H_2O_2.

The maceration process took place for about 48 hours at a temperature of approximately 60°C (Figure 9a). As soon as the samples were bleached, the test tubes were shaken in order to separate the cells (Figure 9b). Once the tissue was dissolved the maceration process was completed.

For the separation of the macerated tissue from the liquid chemicals, a filter of Bad Heilbrunner® Naturheilmittel GmbH & Co. KG turned out to be the most suitable one. In this filter, shaped like a small bag, the macerate was rinsed with tap water.

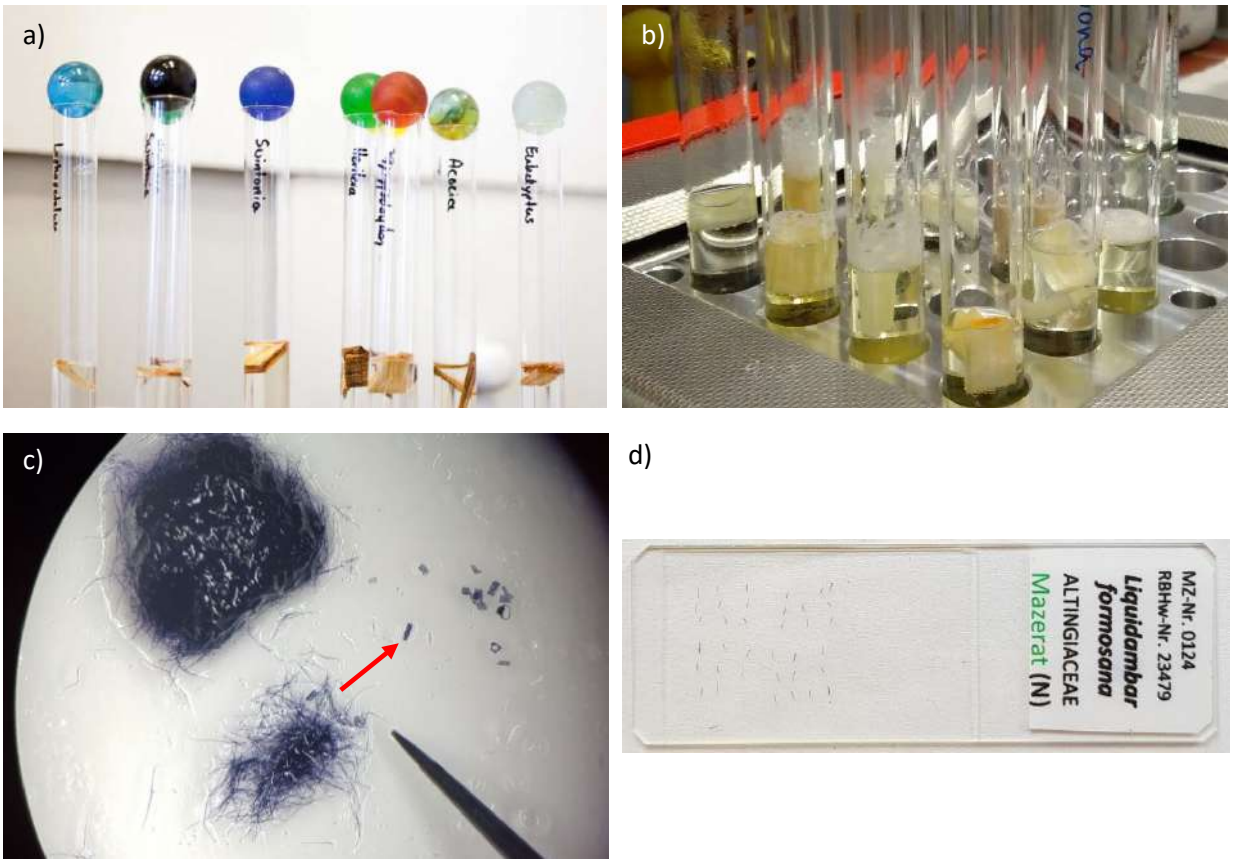


Figure 9 a) Beginning (samples are unbleached) and b) ending (samples are bleached) of a maceration of Asian timbers (photo a) Ilja Hendel); c) Separation of vessel elements (arrow) sorting them out of the stained tissue (nigrosin, 1%) under a reflected light microscope using a needle (arrow shows a separated vessel element); d) Permanent slide of embedded vessel elements of Liquidambar formosana (stained with nigrosin, 1%).

Next, the macerated tissue was stained with nigrosin (1%) (alcohol soluble, Alfa Aesar GmbH & Co. KG, Karlsruhe) (Figure 9c and d) or safranin (4%) (alcohol soluble, Chroma-Gesellschaft Schmid & Co. Stuttgart-Untertürckheim). Trypan blue (aqueous, 1%) was also briefly applied, but its use discontinued because of its carcinogenic properties.

The vessel elements were separated from the stained macerate under a reflected-light microscope with the aid of a dissecting needle and transferred to a microscope slide (Figure 9c). For each genus about 36 vessel elements were sorted out on the slides. The vessel elements were embedded in Euparal (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) for the production of permanent slides (Figure 9d). Further information about the chosen method for the maceration is included in Helmling et al. (2016).

1.3.2 Microscopy

In order to obtain average values, at least 25 vessel elements per genus must be measured (IAWA 1989). In this work, about 36 selected vessel elements of the stained material of all 38 species were investigated under the light microscope (Olympus BX 51, Olympus Deutschland GmbH, Hamburg, Germany; Helmling et al. 2016). All of them were measured (Cell*F, Olympus Soft Imaging Solutions GmbH, Münster, Germany; length and width of each vessel element; aperture and border of five intervessel pits per vessel element).

For the illustrations of the Atlas, micrographs of a smaller selection of vessel elements were taken in all focus levels (camera: Olympus DP 70, Olympus Deutschland GmbH, Hamburg, Germany). By taking six to eight (sometimes even more) pictures of all focus levels, a sharp image could be achieved (see 1.3.3). A total of more than 5000 pictures was taken.

Furthermore, the genera were investigated by using the FE-SEM (Quanta 250 FEG, FEI, Oregon, USA). The samples for the FE-SEM investigation were macerated by the above mentioned, tried-and-tested method of Franklin (p. 14). Then the unstained vessel elements were sorted out by needle and placed on an aluminium stub (diameter 13 mm) covered with 3M Scotch double-sided tape. Afterwards, they were put in a vacuum for sputter coating with gold to obtain a conductive surface (Sputter Coater SC 510, Bio-Rad, Quorum Technologies Ltd., East Sussex, UK, Target, Agar Scientific/Elektron Technology, Cambridge, UK). The pictures of the vessel elements were produced in a high vacuum with an acceleration voltage of 7 kV and a scan speed of 3 µs. The length and width of the vessel elements as well as the apertures and borders of the intervessel pits were measured in the same way as the samples under the light microscope: length and width of the vessel elements as well as aperture and border of the intervessel pits.

1.3.3 Illustrations in the “Atlas of Vessel Elements”

The pictures of the references in the database play the most important role in the identification of wood genera in paper products. They must be of the highest quality and should show the variety of the characteristic features within one genus (i.e., size/dimension, pit shape and pit formation). Therefore, one whole illustrated page was devoted to each genus in the “Atlas of Vessel Elements”: each page presenting a selection of representative vessel elements. Of particular importance for the identification procedure are the vessel-ray pits. These were therefore given special consideration during the selection of the vessel elements.

The free software “Picolay” by Heribert Cypionka (www.picolay.de) produces relatively sharp pictures by automatically stacking several micrographs of vessel elements in different focal levels. The software combines individual regions, each of which is optimally in focus, to create one sharp image from several images. Afterwards, the user can still manually correct parts of the image with

Adobe Photoshop. The individual vessel elements, which were selected for presentation in the Atlas were worked on with the Adobe Photoshop software. I.e., they were cut out along their shapes and improved in contrast and brightness.

1.3.4 Fiber dimensions

One part of the macerated tissue of each of the investigated genera (except for the monocots) was analyzed with the Kajaani FiberLab® fiber analyzer (Metso Automation, Field Systems Division, Kajaani, Finland), in order to determine the dimensions of the fibers (Tepe 2012, Helmling et al. 2018). The measurement results are listed in the Atlas (Helmling et al. 2018) in the corresponding individual description of each wood species. The fibers of the monocots were measured with the aid of the light microscope (Helmling et al. 2018).

1.3.5 Cellular UV microspectrophotometry

The investigations were implemented to study the impact of the maceration agents. The aim of the cellular UV microspectrophotometry was to make lignin visible in wood before and after maceration. By this method, the lignin content of chemically untreated wood can be compared with the lignin content of macerated wood of the same genus.

For the topochemical characterization of wood tissue, specifically concerning the distribution of lignin cellular UV microspectrophotometric investigations were undertaken. Three genera (*Eucalyptus, Hevea* and *Shorea* subg. *Rubroshorea*) were investigated by using a universal microspectrophotometer (UMSP 80, Zeiss Microscopy GmbH, Jena, Germany) (Figure 10). The measuring principle is that 1 µm thick semi-thin cuts are irradiated with monochromatic light in wavelengths ranging from 210 nm to 700 nm. A detector records the absorption of cell wall parts depending on the wavelength and determines extinction spectra. At the beginning of a series of measurements the system must be calibrated for the quantitative determination of the absorption values, which is done be determining a zero curve (transmission spectrum of the embedding medium) (Koch and Kleist 2001).

Preparation and embedding of the samples

Mechanical wood pulp of the three genera (*Eucalyptus, Hevea* and *Shorea* subg. *Rubroshorea*) was investigated. The material of the wood pulp of the three investigated genera derives from the master thesis of Saskia Poth (2015). For the production of the fibrous material, first wood chips were made. For defibering, the defibrator method with a previous steam treatment was used to soften the middle lamellas (defibrator product number: 674, Defibrator AB, Stockholm, Sweden). Subsequently, a refiner (Sprout-Waldron Division, Koppers Company Incorporation, Pennsylvania, USA; Deppe and Ernst 1996) was used to make the pulp. The pulp production was carried out in the Wood Chemistry Department of the Thünen Institute under the supervision of Dr. Othar Kordsachia (Poth 2015).

Macerated tissue, i.e. cell elements, of the same three genera *Eucalyptus, Hevea* and *Shorea* subg. *Rubroshorea* was investigated as well. Both the mechanical wood pulp and the macerated tissue were embedded in SPURR epoxy resin (Spurr 1969) to enable the production of semi-thin cuts. The samples were placed in the desiccator and the vacuum set for 10 minutes. They were then left in the vacuum for another 10 minutes. After venting the desiccator, the incoming air produced a negative pressure in the cell elements, which pulled the resin into the samples. The samples were shaken and the process was repeated. After every second run a resin change was made. This was done by removing the leftover resin with a glass pipette and filling new resin in. This procedure was repeated three to four times. The remaining resin was filled into moulds, in which the samples were embedded. To polymerise the resin, the moulds were heated in an oven overnight at 70°C.

For the production of the semi-thin cuts, a microtome (Ultracut E, C. Reichert Optische Werke AG, Vienna, Austria) with a diamond knife was used. For the cellular UV microspectrophotometry of the cell investigation, the samples were cut into 1 µm semi-thin sections. Two of the cuts were stained with toluidine blue. The staining enables a better overview of the samples' cross-sections when viewing them under the light microscope. The areas to be investigated at the UMSP were determined. Two other, uncolored cuts were fixed on quartz microscope slides with SPURR resin and water. They were then placed on a heating plate (90°C) so that the water could evaporate. A quartz cover glass was applied to the cut with embedding non-UV-absorbing glycerine (glycerine/water mixture nD = 1.46; Ehmcke et al. 2017). Immersion oil was added between the objective of the UMSP and the sample's cover glass. Then the area scanning analyses were begun (UMSP 80), with a wavelength of 278 nm (the absorption maximum of hardwood lignin).

Three measurements of mechanical wood pulp and of macerated tissue on each of the three genera *Eucalyptus*, *Hevea* and *Shorea* subg. *Rubroshorea* were done with the UMSP 80. The scan software APAMOS® (automatic photometric analysis of microscopic objects by scanning, Zeiss Microscopy GmbH, Jena, Germany) digitizes square fields of a local geometrical resolution of 0.25 µm x 0.25 µm and a photometrical resolution of 4096 greyscale levels (Koch and Kleist 2001, Ehmcke et al. 2016). To visualize the absorption, the greyscale levels were converted into 14 basic colors (Koch and Kleist 2001, Koch et al. 2003).

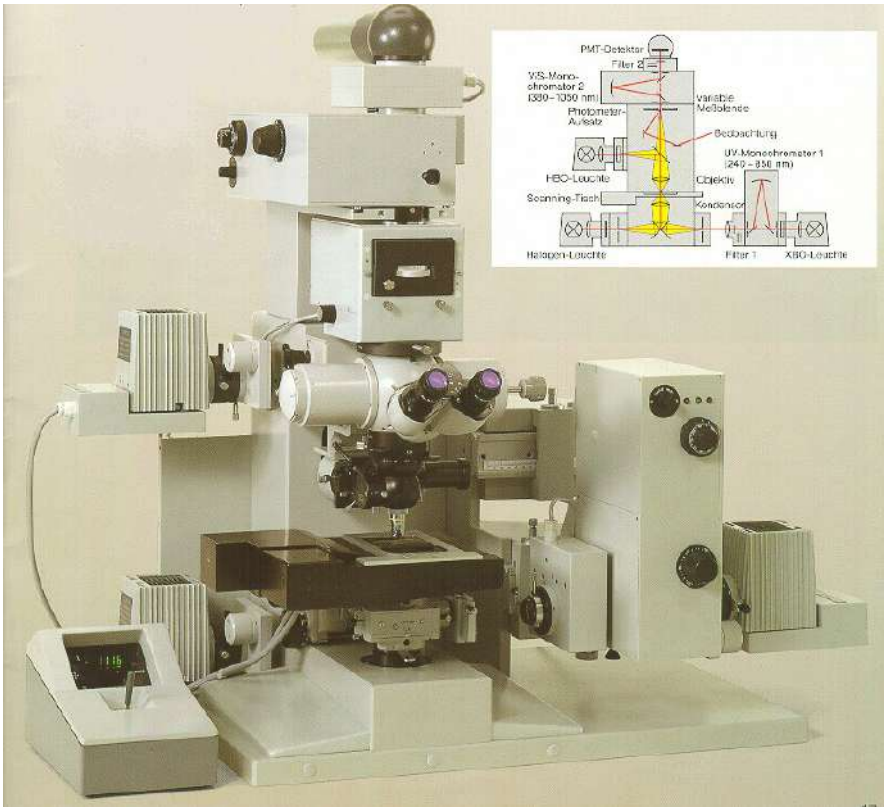


Figure 10 UMSP 80, Zeiss Microscopy GmbH, Oberkochen, Germany

1.3.6 Comparison of genera within one family (Anacardiaceae and Dipterocarpaceae)

There are two families with several genera on the list of the investigated species: the Anacardiaceae and the Dipterocarpaceae. This work deals with four genera of Anacardiaceae and three genera of Dipterocarpaceae, including four subgenera of the genus *Shorea* (Table 3). The genera/subgenera within one family were compared with each other. In pursuing the question of whether the genera within one family are distinguishable from one another, the differences and similarities were identified and listed.

Table 3 List of compared genera/subgenera of Anacardiaceae and Dipterocarpaceae

Family	**Taxa/species**	**Trade name**
Anacardiaceae	*Campnosperma* sp.	Terentang
	Gluta renghas L.	Rengas
	Mangifera sp.	Mango
	Swintonia sp.	Merpauh
Dipterocarpaceae	*Shorea* subg. *Anthoshorea*	White Meranti
	Shorea subg. *Richetia*	Yellow Meranti
	Shorea subg. *Rubroshorea*	Dark/Light Red Meranti
	Shorea subg. *Shorea*	Bangkirai, Balau
	Parashorea sp.	Gerutu
	Dipterocarpus sp.	Keruing

1.3.7 Variability of *Gonystylus* and *Shorea* subg. *Rubroshorea*

In order to gain further information about the morphological differences of samples originating in different places, the variability was investigated. For this investigation, the two genera *Gonystylus* and *Shorea* subg. *Rubroshorea* were chosen.

The vessel elements of these two genera were measured in the same tried-and-tested manner as the others (Figure 11). The samples of *Gonystylus* were collected from different carpentries in Germany, therefore their geographical origin is unknown. But since they were collected from various carpentries, it is likely that the samples originated in different regions. The ones of *Shorea* subg. *Rubroshorea* originate in different regions in Southeast Asia (mainly Indonesia and Malaysia, Table 4).

There were five samples of *Gonystylus* and seven samples of *Shorea* subg. *Rubroshorea*.

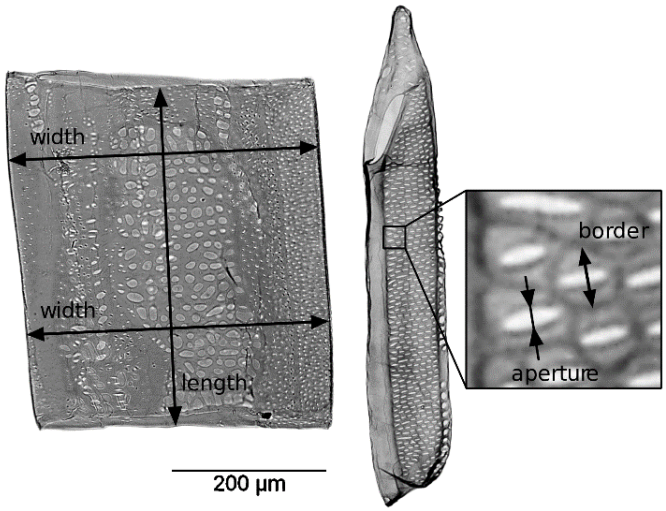


Figure 11 Kind of measurement of length and width of the vessel elements, apertures and borders of the intervessel pits (Helmling et al. 2018)

Table 4 list of species for the investigation of the variability

Sample naming	Genus	Origin
849-2		carpentry Steffen
849-3-A		carpentry Ratheiser
849-3-B	*Gonystylus* sp.	carpentry Ratheiser
849-3-C		carpentry Ratheiser
849-3-D		carpentry Ratheiser
839-1		Central Kalimantan
839-2		East Kalimantan
839-3		West Kalimantan
839-4	*Shorea* subg.	Mentawai Islands
839-5	*Rubroshorea*	Moluccas
839-6		West Malaysia
839-7		Sabah

1.3.8 Statistics

The vessel elements differ in size and shape. The quantitative data that were collected were used for exploring the question of whether it is possible to distinguish the genera solely by the distribution of their dimensions.

The verification of the distributions of the data with the software “SAS univariate” showed that the data do not show a normal distribution.

The Wilcoxon-Mann-Whitney test is distribution-independent and detects whether two distributions belong to the same basic unit. It shows whether the genera are significantly different or not. In this context, the measured values of 20 genera were compared with each other (Helmling et al. 2016). The values include the lengths and the widths of the vessel elements as well as of the intervessel pit borders and apertures.

In detail, the Wilcoxon-Mann-Whitney test is based on the ranking of the values of the measured samples of two measurements to be compared. The ranks (R) of the measured values are summarized in rank sums (U) for the corresponding species. [n] is the number of samples. First, for the null hypothesis H_0: $F_1(x) = F_2(x)$ and for the alternative hypothesis H_A: $F_1(x) \neq F_2(x)$ are defined. The level of significance is set at $\alpha = 5\%$ (Eckstein 2006, Rasch and Kubinger 2006, Pruscha 2006, Tepe 2012, Kuck 2014, Poth 2015).

Now the values of two measurements to be compared are first ranked. The ranking sum R of the respective species is then formed. Then the rank underruns U

$$U = n_1 \cdot n_2 + \frac{n_1 \cdot (n_1 + 1)}{2} - R_1 \qquad (1.1)$$

and the rank overruns U´

$$U' = n_1 \cdot n_2 + \frac{n_2 \cdot (n_2 + 1)}{2} - R_2 \qquad (1.2)$$

can be calculated.

In addition, the expected value μ_U

$$\mu_U = \frac{n_1 \cdot n_2}{2} \qquad (1.3)$$

is calculated, which represents half of all possible comparisons (i.e. U = U´) and the corresponding standard error σ_U.

$$\sigma_U = \sqrt{\frac{n_1 \cdot n_2 \cdot (n_1 + n_2 + 1)}{12}} \qquad (1.4)$$

Since the test variable z is discretely distributed in the Wilcoxon-Mann-Whitney test due to the ranking, the Yates correction, represented by the deduction term −0.5 in equation (1.5) must be taken into account in the calculation.

$$z = \frac{|U - \mu_U| - 0.5}{\sigma_U} \qquad (1.5)$$

The probabilities (p-values) are calculated using the standard normal distribution of z with μ = 1 and σ = 0. The null hypothesis is rejected if the p-value is greater than the significance level of α = 5% (Eckstein 2006, Rasch and Kubinger 2006, Pruscha 2006, Tepe 2012).

2 Results and discussion

In this work, a method was applied which is suitable for creating a database of tropical wood species, the aim being the creation of a tool for detecting tropical wood species in pulp and paper. The data of the investigated wood species were published in an Atlas with pictures and descriptions. The Atlas enables the identification of tropical wood genera in pulp and paper, which was not possible before and can be acclaimed as a great step forward towards global environmental protection.

Previously, other testing institutes referred to these tropical wood genera merely as the large group of “Mixed Tropical Hardwood” (MTH). By using the (so-called) exclusion method, this group of MTH was simply declared as “no known local woods from temperate zones; a mixture of different unknown species – therefore it must be tropical wood”.

The selection of the 38 investigated species was based on a group of wood genera that could potentially be found in paper, as well as on those wood genera that are economically relevant. Included is the CITES-protected wood genus *Gonystylus* (Ramin): When Greenpeace collected wood samples at a wood yard of a large Asian pulp company, most of the samples were Ramin (Greenpeace 2012). This tropical wood genus, highly relevant due to its protection status, is thus being processed into pulp and paper. Other CITES-protected species have not yet been investigated, as they are mostly valuable wood that is usually not processed into pulp. Only one individual per genus was examined, but its features were verified with slides and literature data from the databases deduced from studies of numerous vouchered specimens (Richter and Dallwitz 2000–onwards and InsideWood 2004–onwards).

However, the selection of the investigated species is far from being comprehensive and urgently requires an expansion of the species spectrum. With the help of the Atlas, wood species whose morphology corresponds to the characteristics presented in the Atlas can be identified in pulp and paper products. However, as long as there are still unknown and not yet investigated genera, the species determination cannot be unquestionably 100% correct. The determination can only be argued with, “The features of this species seem to coincide greatly with a certain genus in the Atlas, but one cannot be completely sure…”. But the more references are established, the more groups of wood genera will be discovered that are similar in the anatomy of their vessel elements – making it ever more difficult to be distinguishable from one another. Therefore there is a need for more intensive research.

Nevertheless, this work is a helpful contribution to the implementation of the EUTR, because the purpose of the implementation is to check whether the declarations of the traded articles are correct (and not to identify unknown species). The verification of market participants would not be possible without the references. The day-to-day business of the Thünen Centre of Competence on the Origin of Timber, Hamburg, Germany, shows that several products submitted for testing of the contents (fiberboards, paper products) contain wood species listed in the Atlas. About ten to twelve of the listed species have already been detected regularly in these products (e.g. *Hevea*, *Lophopetalum, Rhizophora* or *Schima* and *Liquidambar*). The products do not only consist of common plantation woods (e.g. *Acacia*, *Eucalyptus*, *Populus* and *Pinus*). In case the producer information on the species used for the production of a product includes a wood genus that is not yet available as a reference in the Atlas for pulp and paper, the large collection of wood samples in the Thünen Institute can be used to produce a corresponding macerate in a short time. Thus, a new reference can be created relatively quickly and the manufacturer's data can be thoroughly controlled and, as the case may be, validated. However, this new reference is then only usable as an overview on a microscope slide for checking the declaration. For the publication of this new reference, the vessel elements must be

sorted out (as described above), be photographed in several focal levels, be measured and be morphologically described in detail.

2.1 Key features for identification

The key features used in the descriptions of the individual wood species are:

- Shape of vessel elements drum-/barrel-/tube-shaped
- Tails with abrupt or gradual transition
- Perforation plates simple or scalariform and opening horizontal or inclined, narrowed or over the entire lumen
- Pit types alternate, opposite or scalariform
- Intervessel pit apertures circular, oval, slit-like, coalescent
- Type APS or type VAS
- Pits to axial parenchyma cells, to fibers, to tracheids
- Areas without any pits
- Tyloses
- Helical thickenings
- Dimensions of vessel elements (length, width, intervessel pit aperture and pit border (vertical))
- Fiber dimension

Features that are important for the identification of the wood genus are shown in Figure 12 (Helmling et al. 2016). The quantitative data were obtained by measuring the lengths and widths of the vessel elements and by measuring the borders and apertures of their intervessel pits (Figure 12a – corresponds to Figure 11). The different dimensions of the vessel elements of the investigated wood species are shown in Figure 12b. Different characteristic morphological features are shown in Figure 12c. Two of the most important features are the pit shapes and patterns and the type of the perforation plates. If vessel elements possess pits that are all similar in size and shape, they belong to the newly introduced type “APS”: **A**ll **P**its **S**imilar (Figure 13). If the vessel-ray pits and the intervessel pits differ, they are assigned to the type “VAS”: **V**essel-ray pits **A**pparently **S**imple (Figure 14). Despite belonging to type APS or VAS, species with scalariform perforation plates form another group because this feature is highly distinctive. Likewise, the arrangement of the pits in monocots is very distinctive (their pits are distributed very evenly over the entire vessel element walls) that they are assigned to a separate group as well.

Having defined these groups, the 38 investigated wood species in the Atlas were assigned to the following categories: 11 wood species belong to type APS, 19 wood species to type VAS, six wood species possess scalariform perforation plates and two are monocots.

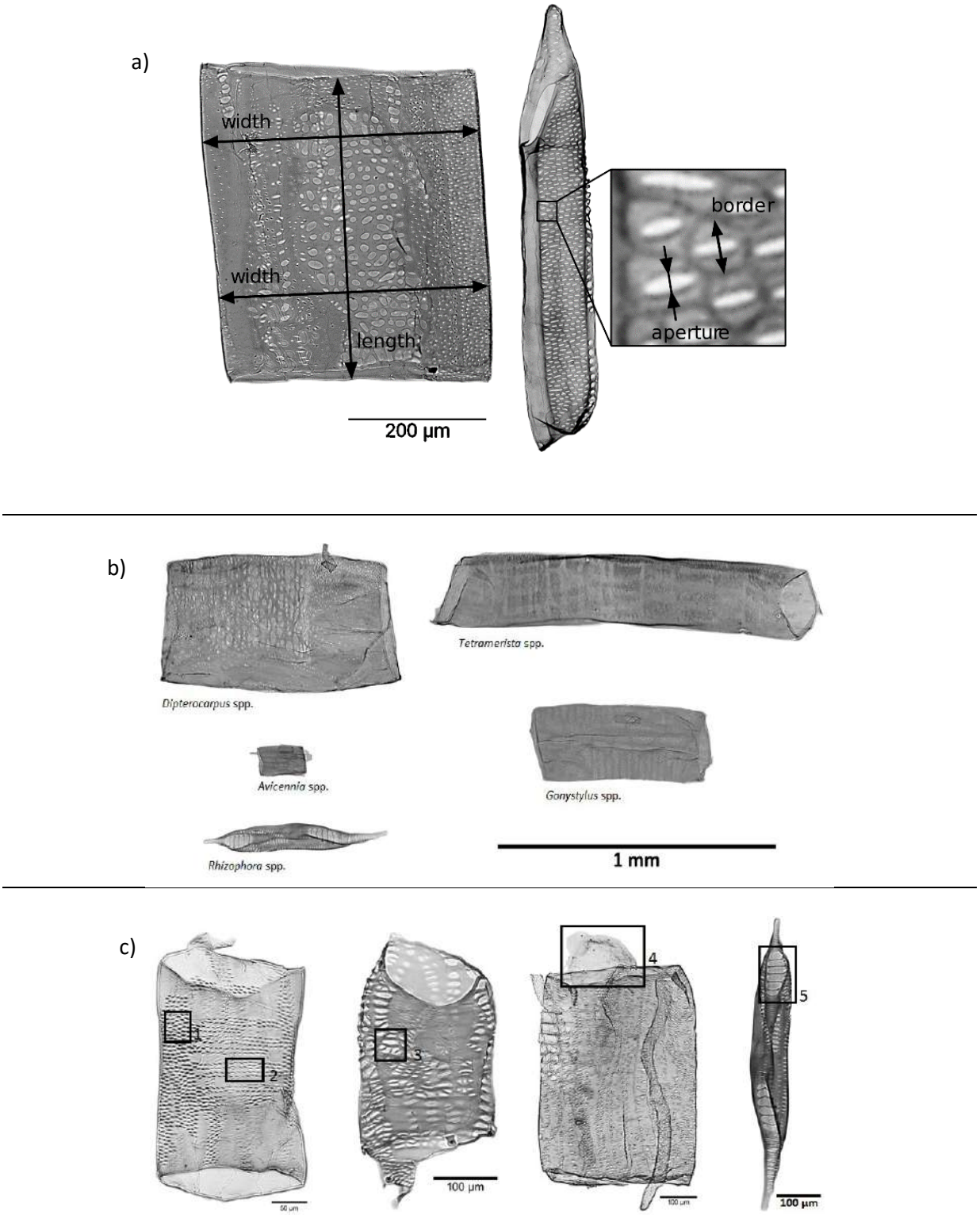


Figure 12 Acquisition of vessel element characters (a) Measurement on vessel elements (length, width, intervessel pit aperture and border) (b) Dimensions of vessel elements of different species (Dipterocarpus sp., Avicennia sp., Rhizophora sp., Tetramerista sp., Gonystylus sp.) (c) Different perforations (simple and scalariform) and different shapes, sizes and patterns of pits in vessel elements. Intervessel pits (1) and vessel-ray pits (2) on Acacia sp., increased vessel-ray pits on Mangifera sp. (3), tylosis (4) on Shorea subg. Anthoshorea, scalariform perforation (5) on Rhizophora sp.

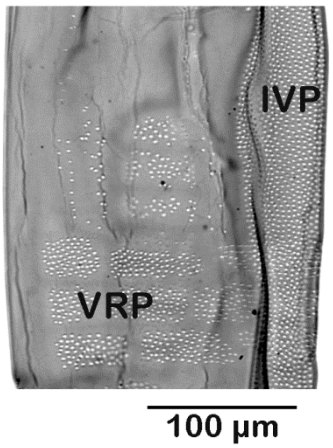


Figure 13 Type „APS“ – all pits similar. Similarity in size and shape of vessel-ray pits (VRP) and intervessel pits (IVP) (Helmling et al. 2018).

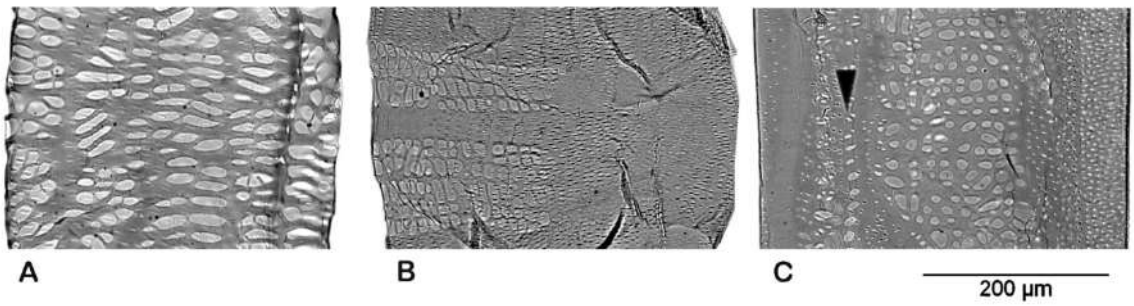


Figure 14 Type „VAS“ – vessel-ray pits apparently simple (Helmling et al. 2018)

2.2 Cellular UV microspectrophotometry

The lignin content in wood species varies from 20% to 30% (Koch and Schmitt 2003). This content is relevant for reactions with stains for application in light microscopy. Safranin, for instance, is often used to stain lignin (Bond et al. 2008). Astra-blue is used for the staining of cellulose in the absence of lignin (Srebotnik and Messner, 1994). The vessel elements investigated in this work were stained with safranin for light microscopy application to improve the visibility of their characteristic morphological features (1.3.1). This contradicts Franklin's statement (1945) that lignin is dissolved during maceration. Since the tissue could be stained with safranin, this led to the question whether all lignin is dissolved during maceration. Furthermore, the investigation of the lignin content leads to a more precise selection of possible staining agents. This, in turn, improves the methods for the identification of wood species.

According to Franklin (1945), lignin is dissolved during maceration with glacial acetic acid in combination with hydrogen peroxide. Scurfield and Silva (1970) also refer to these chemicals as a delignifying agent. In order to verify or disprove this statement and make the potential residual lignin visible after maceration, investigations were carried out with the use of the UMSP. UMSP is well-suited to study the delignification of wood during pulping processes (Schütt et al. 2013, Koch et al. 2003, Rehbein et al. 2010). UV spectrophotometry is the most useful method for the quantitative and qualitative analyses of lignin in solution. Lignin strongly absorbs ultraviolet light due to its aromatic nature (Lin 1992).

Mechanical wood pulp of three genera (*Eucalyptus, Hevea* and *Shorea* subg. *Rubroshorea*) was investigated by cellular UMSP. Macerated tissue of the same three genera was investigated as well, so that the lignin content of the mechanical wood pulp could be compared to the lignin content in macerated wood.

The following three pictures show the UV spectroscopic scanning profiles of the examined surfaces for the topochemical detection of lignin in mechanical wood pulp of the three genera *Eucalyptus*, *Hevea* and *Shorea* subg. *Rubroshorea* (Figure 15 to Figure 17).

All the samples were scanned with monochromatic UV-light at 278 nm (absorption maximum for hardwood lignin). The thresholds were set to the standard (min. 10% and max. 80%). The different colors show the intensities of the UV absorbance of the lignified cell walls. The UV microscopic scanning profiles of Figure 15 to Figure 17 show that despite mechanical treatment, the cell tissue and cell wall structure of all three genera are still intact and parts of coherent tissue are still distinctly visible. In addition, it becomes clear that lignin is still present in these tissues. The content of lignin is visualized by the different colors of the cell wall layers. The compound middle lamella (CML) and the cell corners (CC) possess the highest absorbance intensity which corresponds to the results of Koch and Kleist (2001) for untreated wood (Ehmcke 2016). There is an increase in the absorbance values from the S3 layer (innermost layer) to the area of the CML with abs_{278nm}. For *Eucalyptus* and *Hevea* the absorbance value increases from 0.09 (S3) to a maximum absorbance of 0.61 (CML/CC), for *Shorea* subg. *Rubroshorea* it increases from 0.06 (S3) to 0.83 (CC). The gradation from the CML to the S3 is clearly visible. The sections show the typical lignin distribution. A pit channel from cell to cell is also clearly visible in the scanning profile of *Hevea* (Figure 16, right cell, lower area). The highest lignin content is found in the cell corner of *Shorea* subg. *Rubroshorea* (Figure 17, yellow; UV absorption: 0.83). In the mechanical wood pulp of *Hevea* and *Eucalyptus*, a delignification with low absorbance values can be seen in the border areas of the cells shown. Here the water steam in the process of defibering has acted as a solvent (Schütt et al. 2013).

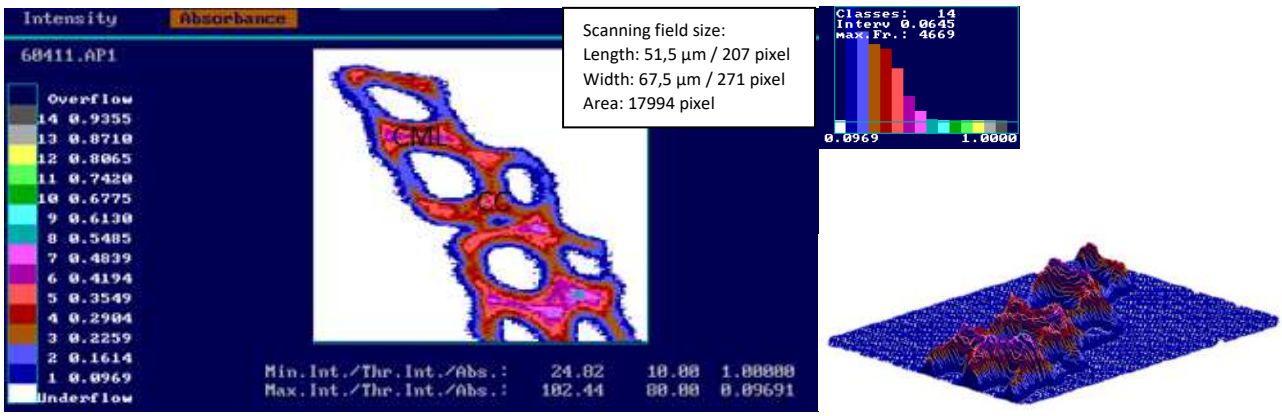


Figure 15 UV spectroscopic scanning profiles of fibrous tissue of mechanical wood pulp of Eucalyptus

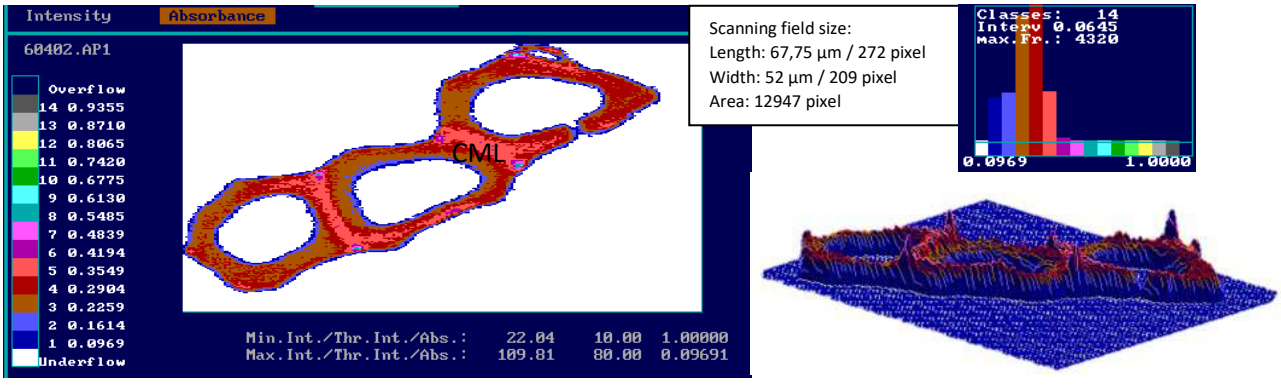


Figure 16 UV spectroscopic scanning profiles of fibrous tissue of mechanical wood pulp of Hevea

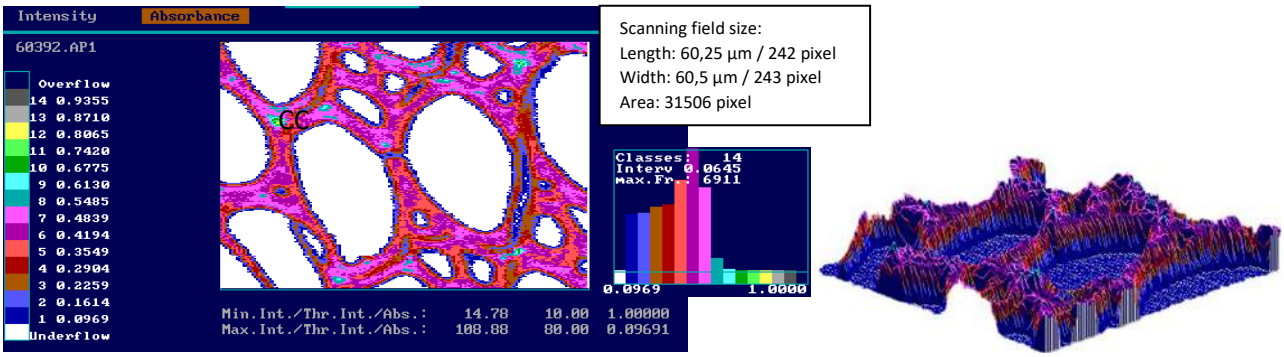


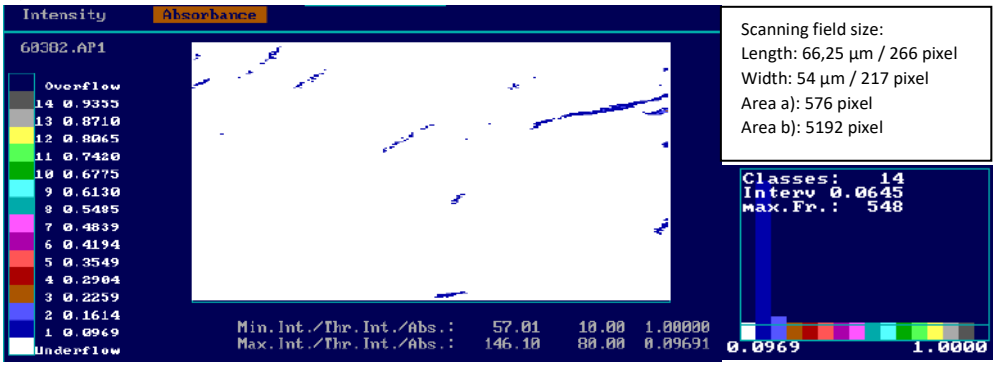
Figure 17 UV spectroscopic scanning profiles of fibrous tissue of mechanical wood pulp of Shorea subg. Rubroshorea

In the case of the investigated macerate, areas with individual cells are shown, usually fibers (Figure 18 to Figure 20). The figures Figure 18 to Figure 20 show the UV spectroscopic scanning profiles of the investigated areas for the topochemical detection of lignin in macerated tissue of *Eucalyptus*, *Hevea* and *Shorea* subg. *Rubroshorea:* false color rendering with the thresholds from min. 10% to max. 80% – the same as for the mechanical wood pulp (Figure 15 to Figure 17). The absorbance is so low that the results are white fields with sparsely arranged fibers - in the case of the three "a)-figures". Here, the absorbance values of *Eucalyptus* lie between 0.06 and 0.18, the ones of *Hevea* and *Shorea* subg. *Rubroshorea* lie between 0.07 and 0.33.

Afterwards, as can be seen in the three corresponding b)-figures, in order to intensify the visibility of the fibers and get a more detailed resolution, the thresholds were set to 40% (min.) to 92% (max.). With the changed thresholds, the absorbance values of *Eucalyptus* lie between 0.04 and 0.14, the ones of *Hevea* between 0.03 and 0.3 and the values of *Shorea* subg. *Rubroshorea* lie between 0.04 and 0.27. As already mentioned, the values are based on the same investigation as the first ones, only the visibility has been increased.

Both the first and even more the second analysis illustrate that the macerated samples contain almost no lignin anymore. This investigation confirmed Franklin's results (1945) that almost all lignin had been dissolved during maceration. The lignin has "vanished".

a)



b)

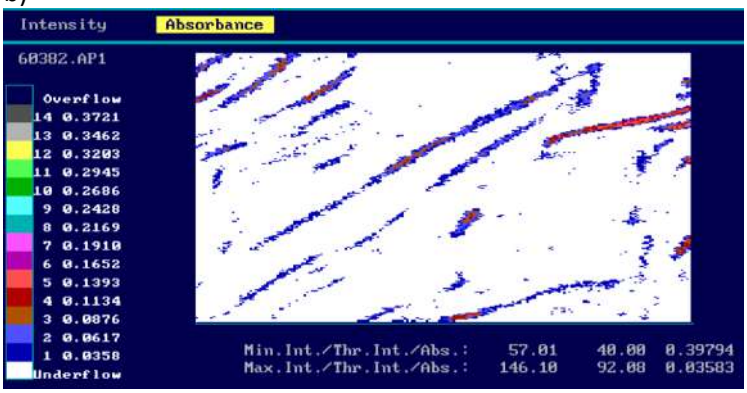
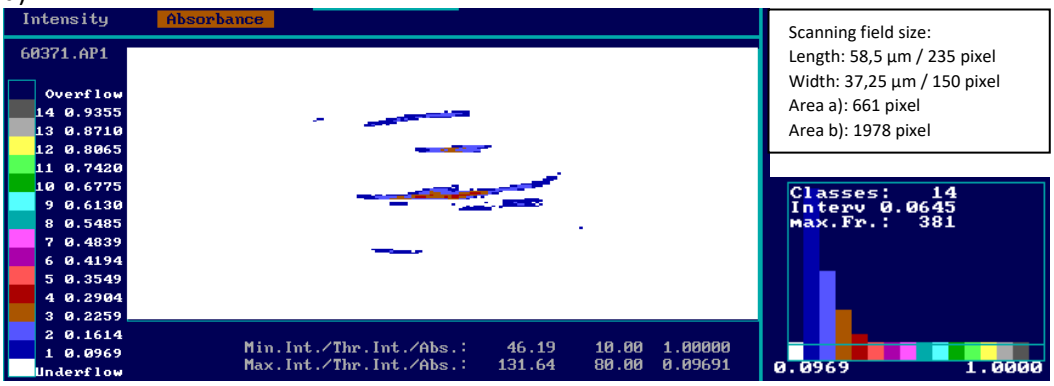


Figure 18 a) UV spectroscopic scanning profiles of macerated tissue of Eucalyptus (λ278nm). After maceration, there is nearly no lignin left in the samples. Thresholds: 10–80 %. b) Changed thresholds: 40–92%.

a)



b)

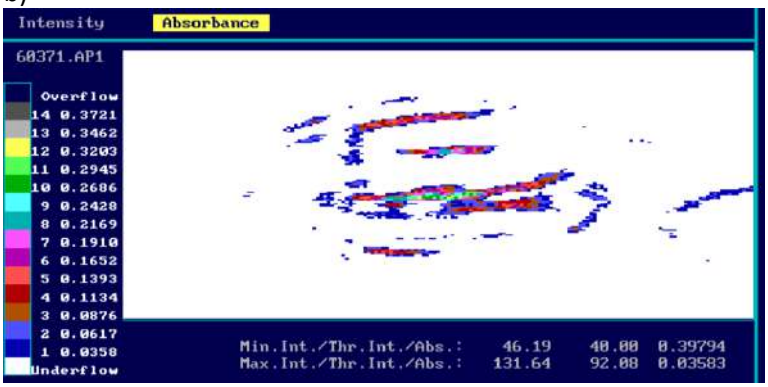
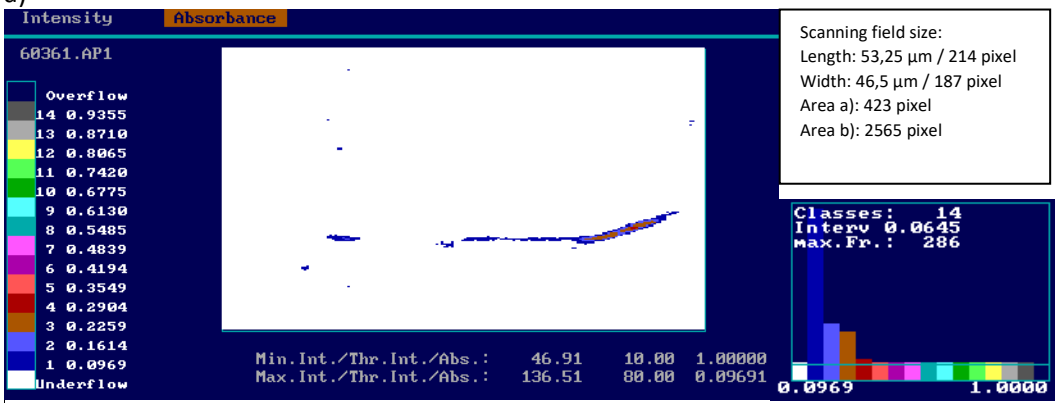


Figure 19 a) UV spectroscopic scanning profiles of macerated tissue of Hevea (λ278nm). Thresholds: 10–80 %. b) Changed thresholds: 40–92%.

a)



b)

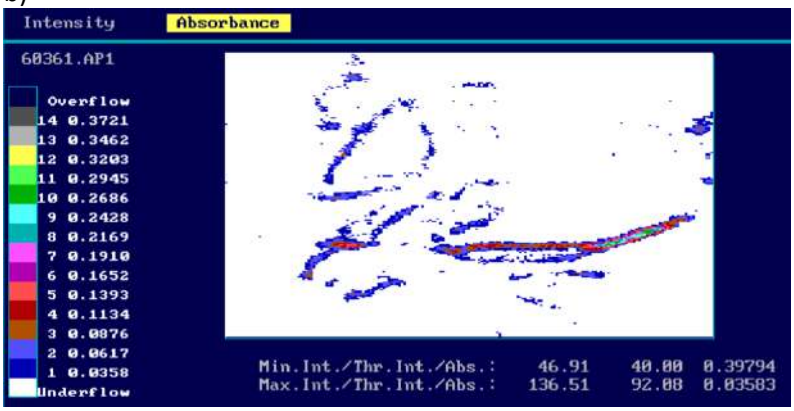


Figure 20 a) UV spectroscopic scanning profiles of macerated tissue of Shorea subg. *Rubroshorea (λ278nm). Thresholds: 10–80 %. b) Changed thresholds: 40–92%.*

2.3 Maceration, staining and microscopy

The method of Franklin (1945) turned out to be the most suitable one for the investigation. For the maceration, this method was chosen because it is relatively uncomplicated to handle and easy for other laboratories to reproduce. Franklin used equal parts of glacial acetic acid and solution of H_2O_2. In the past, further methods have been developed to macerate solid wood:

Solla (1879) wrote of long-known methods of artificially separating plant or wooden tissue and experimented a lot with these methods. He did tests with acetic acid, oxalic acid, tartaric acid, nitric acid, hydrochloric acid, sulphuric acid, chromic acid, Schultze's mixture, potassium hydroxide solution and chlorinated water in attempting to dissolve the middle lamella. Kisser (1926) found the mixture of "Schulze" (potassium chlorate and nitric acid) to be a good choice because it is not damaging to the cell walls. Spearin and Isenberg (1947) tested sodium chlorite acidified with acetic acid. According to Biermann (1996), it is the most gentle maceration technique. According to Panshin and de Zeeuw (1964) Schultze used concentrated nitric acid and a few crystals of potassium chlorate (0.5%). Sanderson (1994) recommended Schulze's fluid (0.5% potassium nitrate in concentrated nitric acid) or – if immediate fixation is desired – Jeffrey's fluid (10% (aq) nitric acid and 10% (aq) chromic acid in equal parts. One can assume that "Schulze" and "Schultze" are one and the same person – despite the different spellings and one deviation in the chemical used for the mixture of Schul(t)ze: Kisser (1926) and Panshin and de Zeeuw (1964) used potassium chlorate and nitric acid, while Sanderson (1994) wrote of potassium nitrate in concentrated nitric acid.

Generally, the maceration was estimated to take about 48 hours. Some wood samples (e.g. *Gonystylus*) needed one more day for maceration because the cells were not separated yet. Possibly it depends on the age of the chemical H_2O_2, but the tested wood species could also play a role. The maceration ends as soon as the wood is bleached. Shaking the test tubes causes the cells of the tissue to dissolve.

For the next step, the separation of the macerated tissue from the chemicals, different ways have been tried out:

Separation by the use of filter paper was tried, but this method is not recommended because of possible contamination by external fibers originating from the filter paper. A separation experiment with the use of centrifuges showed that centrifuges help to collect all the macerated cells together at one point but do not separate them from the chemicals. As already mentioned in "Maceration and staining", p. 14, the filter of Bad Heilbrunner® Naturheilmittel GmbH & Co. KG proved to be the most suitable device for separating the tissue from the chemicals.

The macerated material was placed under a reflected light microscope. Then the vessel elements to be examined were manually sorted out with a dissecting needle. Therefore, it cannot be excluded that an unrepresentative selection of vessel elements was obtained. Care was taken to sort out all vessel elements of a certain part of the samples but it is possible that small vessel elements were overlooked. From the previous literature research (Richter and Dallwitz 2000-onwards and InsideWood 2004-onwards), it was known which genera had only one size class of vessel elements and which had two. According to IAWA (1989), if two size classes are present, only the larger one is measured. In this work both size classes (if present) were measured.

As mentioned in the Atlas, the vessel element length was measured without the tail – according to "Hamburg convention". The IAWA (1989) recommends that the measurement includes the tail. But in solid wood the vessel element length is determined by cuts. Depending on the cut, the respective tail is by no means always visible, which speaks in favor of the practice in Hamburg. When measuring the diameter (width) of a vessel element, it is not possible to measure it tangentially or radially on

the macerate in a controlled or specific manner, as the vessel elements lie on the slides in various positions. Due to collapse and the flat lying on the slides, they are often wider than the corresponding literature values. For solid wood the procedures of these measurements are directed (Hamburg: radial; IAWA: tangential).

The staining with safranin (red) improved the contrast of the microphotographics of the vessel elements. The illustrations in the "Atlas of Vessel Elements" were planned as to be published in a black- and-white version. For this, the pictures of red stained vessel elements created good contrasts after conversion in the black-and-white pictures (Waitkus 2012).

The borders of the pits could not be seen well under the light microscope. Therefore they could not be measured well. Staining with nigrosin (black) or Alexander-Herzberg solution (zinc chloride iodine solution; Harders-Steinhäuser 1974) turned out to be the best choice for the visualization of the pits. That was important for the collection of quantitative data (measurements of the intervessel pit borders and apertures). Macerate that is stained and embedded in Alexander-Herzberg solution is not permanent – but helpful for measuring the pits of some wood species where nigrosin was not satisfactory. Trypan blue was tried for staining as well, but was not chosen because of its carcinogenic property. The reproducibility of these procedures for every laboratory or testing institute was always implicitly taken into consideration.

There have also been attempts to filter vessel elements out of pulp (Orblin et al. 2011). Large vessel elements are unwanted in the paper industry, due to disturbances during the inking of papers ("vessel picking") (Orblin et al. 2011). Some MTH-species possess large vessel elements, e.g. *Tetramerista*, *Shorea* and *Dipterocarpus*. They cause inkless spots on the printed surfaces (Orblin et al. 2011).
For the purpose of filtering out vessel elements, a drainage jar and the Bauer McNett Fiber Classifier (AB Lorentzen & Wettre, Stockholm, Sweden) were chosen. Several filters with different mesh sizes were used to sort out the cells by size, so that at the end of the filtering series theoretically one fraction should have contained more vessels than the others. However, the result was not satisfactory and the use of this method was discontinued. This is not to say that the method cannot be improved in time for use for fractionation.

As with the other discussed procedures, the reproducibility of the work with the microscope for every laboratory or testing institute in the world was implicitly considered as well. The pictures produced by the FE-SEM showed the characteristic details very well. Also the top and bottom or even the inner side (when the endings were inclined) of a vessel element could be seen very well and the intervessel pits could be measured (Figure 21).

In the latter case, it was even possible to compare the shapes of the pits on the inner wall with those on the outer wall. But there is a higher reproducibility of results, paired with a better practicability, when using a light microscope. Therefore the use of a light microscope was prioritized. Many of the pictures produced with the light microscope can be viewed in the "Atlas of Vessel Elements" (Helmling et al. 2018). Nevertheless, at the FE-SEM four genera were extensively examined for the bachelor thesis "Mikroskopische Charakterisierung der Gefäßelemente zur Identifizierung tropischer Nutzhölzer in Papier" (Microscopic characterization of vessel elements for the identification of tropical timber in paper) by Saskia Poth (2013).

At the beginning of the research with the light microscope, measurements were taken of both the intervessel pits and the vessel-ray pits. The measurements of the vessel-ray pits turned out to be inadequate for identification purposes because of a great variability and an unrepresentative, artificial deformation (due to preparation) of the larger, apparently simple vessel-ray pits. Thus, measurements of vessel-ray pits cannot be used as characteristics to help distinguish genera from

one another. Therefore, the taking of these measurements was not further pursued. The measurements of the intervessel pits, however, are useful for identification purposes (IAWA 1989). The values can therefore be compared with the values in the databases (Richter and Dallwitz 2000–onwards, InsideWood 2004–onwards) or with the values in other literature. The measurements of the intervessel pits help contribute towards the identification of a genus (IAWA 1989).

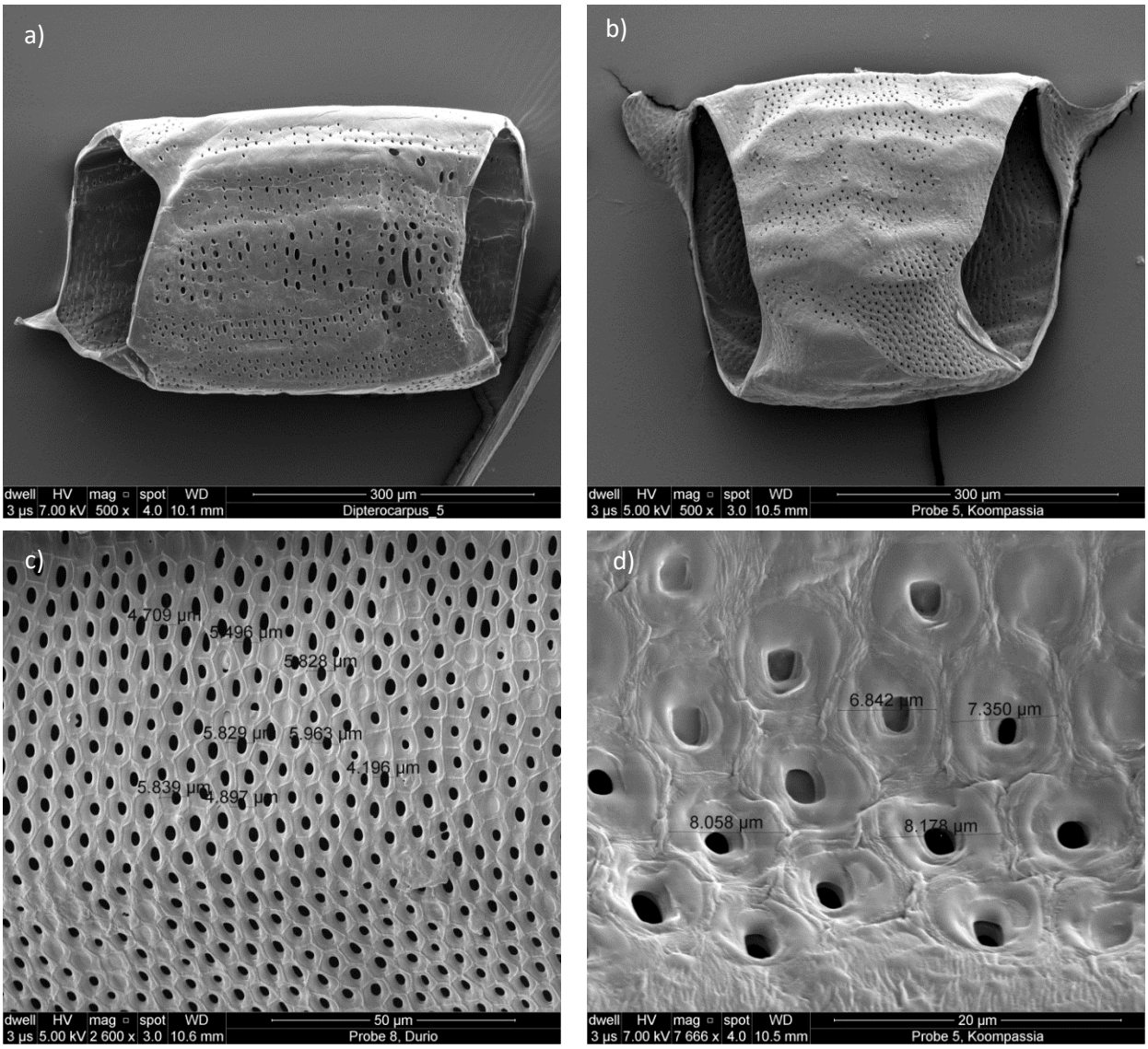


Figure 21 Investigations by using the FE-SEM (Vessel elements of a) Dipterocarpus and b) Koompassia; Measurements of intervessel pits of c) Durio and d) Koompassia

2.4 Comparison of species within one family

In the following comparative studies, the characteristic morphological features of four species of the Anacardiaceae and of six species of the Dipterocarpaceae, respectively, were compared to each other. Similarities and differences were identified. The here shown illustrations are taken from the Atlas (Helmling et al. 2018). The Atlas therefore contains enlarged images of these wood species, each shown on a separate page.

2.4.1 Anacardiaceae

The four Anacardiaceae are presented first: *Campnosperma* sp., Gluta *renghas* L., *Mangifera* sp. and *Swintonia* sp. (Figure 22).

With regard to the dimensions, it must be noted that *Campnosperma* sp. is the only one of the four investigated genera/species within the Anacardiaceae to have mainly tube-shaped vessel elements. They are very long and narrow. The other three genera/species, *Gluta renghas* L., *Mangifera* sp. and *Swintonia* sp., have vessel elements with similar dimension and are mainly barrel- or drum-shaped. The vessel elements of *Mangifera* sp. are the smallest ones. *Campnosperma* sp. also has large areas devoid of pits. This characteristic can be found only among the other genera in *Gluta renghas* L. The tails of the four genera/species are similar in that they are mostly short, though in exceptional cases long, with a gradual transition. The perforation plates of all four genera are simple. They are horizontal or slightly inclined. According to Richter and Dallwitz (2000), the perforation plates of *Campnosperma* sp. can also be scalariform with 6–36 bars.

The sizes of the intervessel pit borders differ (Table 5). The intervessel pits of *Gluta renghas* L. and *Swintonia* sp. are similar in size. The sizes of the intervessel pits of *Campnosperma* sp. and *Mangifera* sp. lie close together. All apertures of the intervessel pits are oval, except for *Campnosperma* sp.: Here they are oval to slit-like.

Table 5 Sizes of intervessel pit borders of the investigated Anacardiaceae

Genus	Size of intervessel pit borders
Campnosperma sp.	4–6–10 µm
Gluta renghas L.	6–11–15 µm
Mangifera sp.	3–7–12 µm
Swintonia sp.	6–10–16 µm

All the four genera/species belong to the type of VAS (**v**essel-ray pits are **a**pparently **s**imple). They differ from the intervessel pits (for further information about VAS, see p. 266 and p. 296 of the "Atlas of Vessel Elements", Helmling et al. 2018). The shapes of the vessel ray-pits vary from elongated to oval or circular to stretched. They all have in common that the vessel-ray pit openings are quite large (Table 6).

Table 6 Shape of vessel-ray pits of the investigated Anacardiaceae

Genus	Shape of vessel-ray pits
Campnosperma sp.	Large, apertures window-like; shape from elongated to oval or circular
Gluta renghas L.	Apertures oval or stretched (eye-shaped); in horizontal rows
Mangifera sp.	Apertures are isodiametric or stretched (eye-shaped); present on nearly every vessel element
Swintonia sp.	Very large, window-like

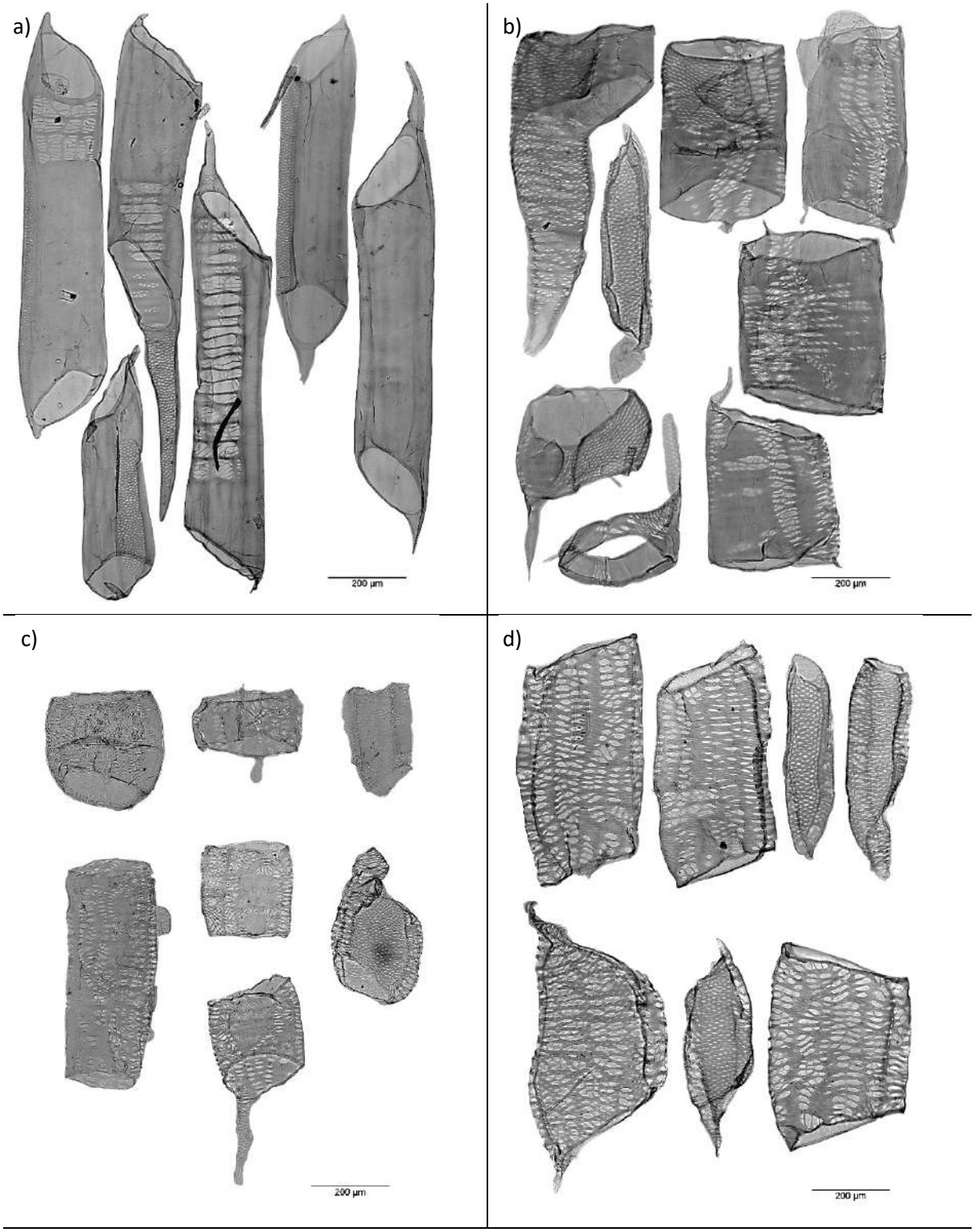


Figure 22 Illustrations of the investigated genera of the Anacardiaceae: a) Campnosperma sp. b) *Gluta renghas L. c) Mangifera sp*. d) *Swintonia sp*.

The quantitative data of the four genera/species are summarized in Table 7. The boxplots of Figure 23 give an overview of the dimensions of the four investigated Anacardiaceae. All of them except *Swintonia* sp. have small to large areas devoid of pits. All four of them possess tyloses. None of them possesses helical thickenings.

In case all of these four genera of Anacardiaceae are mixed together in a pulp or paper, it will be difficult to distinguish them from each other. The three genera/species *Gluta renghas* L., *Mangifera* sp. and *Swintonia* sp. are very similar in their appearance. If only one of the four genera/species is present, it may be possible to identify it, since in this case the greater number of vessel elements allow for a larger overall view. It is helpful to measure the intervessel pits. *Campnosperma* sp. is easier to distinguish from the other three genera/species due to its special appearance in dimension and type of pitting.

Table 7 Quantitative data of the four investigated Anacardiaceae

Genus	Vessel elements	Intervessel pit borders [µm]	Fibers (weighted averages)
Campnosperma sp.	(361–)646(–873) µm long, and (139–)192(–220) µm wide; l/w ratio 3.7	(4.0–)6.2(–9.6)	950 µm long, 24.5 µm wide. Fiber wall thickness 7.7 µm
Gluta renghas L.	(76–)361(–576) µm long, and (107–)265(–367) µm wide; l/w ratio 1.4	(6.4–)10.5(–14.6)	945 µm long, 20.9 µm wide. Fiber wall thickness 6.0 µm
Mangifera sp.	(75–)221(–550) µm long, and (126–)241(–385) µm wide; l/w ratio 0.9	(3.2–)7.4(–11.5)	700 µm long, 20.8 µm wide. Fiber wall thickness 5.8 µm
Swintonia sp.	(119–)396(–590) µm long, and (73–)194(–408) µm wide; l/w ratio 1.8	(6.1–)9.6(–16.1)	930 µm long, 23.5 µm wide. Fiber wall thickness 8.7 µm

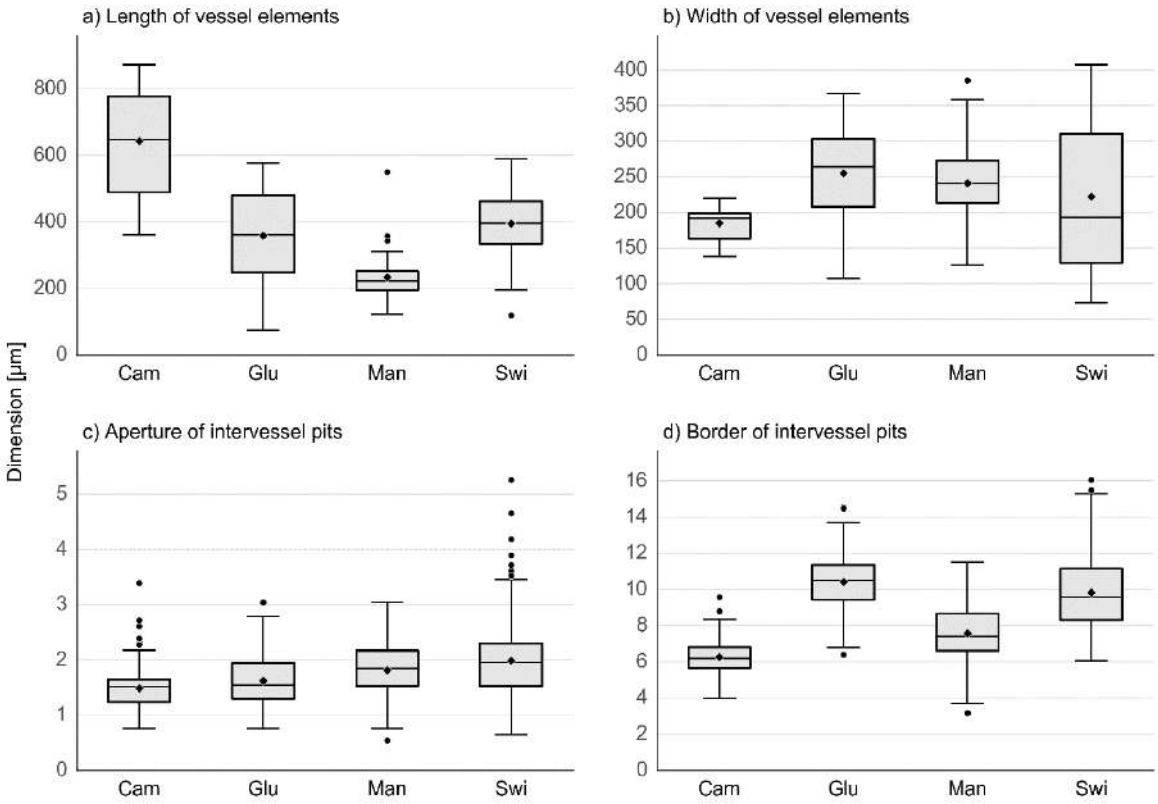


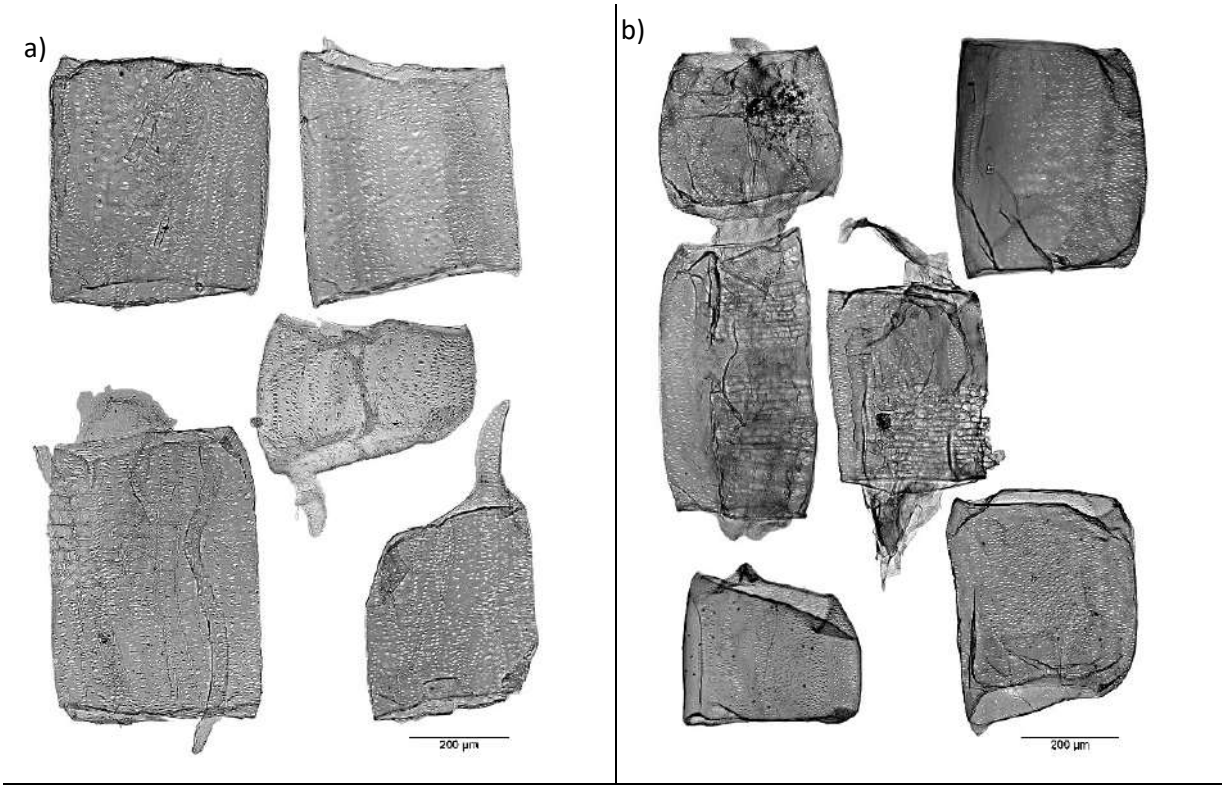
Figure 23 Boxplots of the four investigated genera of the family Anacardiaceae

2.4.2 Dipterocarpaceae

The following part consists of the comparison of the investigated genera of the Dipterocarpaceae.

The investigated genera of the Dipterocarpaceae are *Dipterocarpus* sp., *Parashorea* sp. and *Shorea* sp. The genus *Shorea* is classified in four subgenera: *Anthoshorea*, *Richetia*, *Rubroshorea* and *Shorea*. So three genera were investigated, and one genus of those three is divided into four subgenera.

The vessel element shapes of the six investigated genera and subgenera of the Dipterocarpaceae range from barrel- to drum-shaped. Only *Shorea* subg. *Rubroshorea* occasionally shows additional tube-shaped vessel elements (Figure 24). The tails are – if present – short with abrupt transitions (*Dipterocarpus*, *Parashorea*, *Anthoshorea*, *Rubroshorea*, *Shorea*), long with an abrupt transition (*Rubroshorea*), short with a gradual transition (*Richetia*), or – rarely – longer with gradual transitions (*Dipterocarpus*, *Anthoshorea*, *Shorea*). The tails can also be absent (*Dipterocarpus*, *Parashorea*). The perforation plates are always simple. They are virtually identical: extending across the entire lumen and horizontal. *Dipterocarpus* does not possess intervessel pits due to solitary vessels.



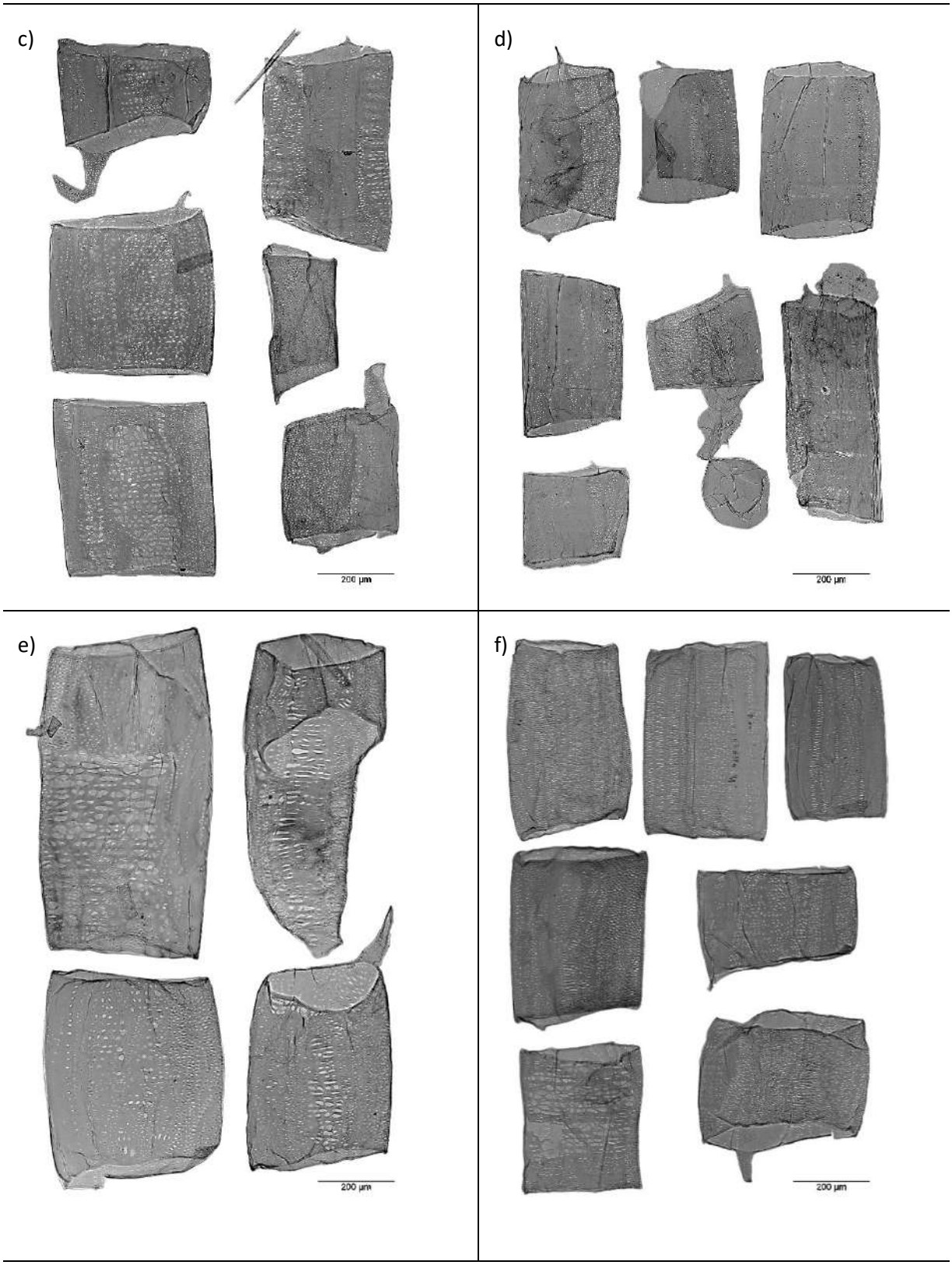


Figure 24 Illustrations of the investigated genera/subgenera of the Dipterocarpaceae a) Shorea subg. Anthoshorea b) Shorea subg. Richetia c) Shorea subg. Rubroshorea d) Shorea subg. Shorea e) Dipterocarpus sp. f) Parashorea sp.

As Table 8 shows, the range of intervessel pit sizes is small, being limited to very similar size classes. *Shorea* subg. *Rubroshorea* has the largest intervessel pits, all the others belong to size classes that lie very close together. Thus, this characteristic alone is not suitable for the differentiation of the individual genera and subgenera.

Table 8 Size of intervessel pit borders of the investigated Dipterocarpaceae

Genus	Size of intervessel pit borders
Dipterocarpus (pits to tracheids)	2-3-5 µm
Parashorea	2-4-7 µm
Shorea subg. *Anthoshorea*	2-5-8 µm
Shorea subg. *Richetia*	3-4-9 µm
Shorea subg. *Rubroshorea*	4-7-12 µm
Shorea subg. *Shorea*	3-5-9 µm

Table 9 Vessel-ray pits of the compared genera of Dipterocarpaceae

Genus	Shape of vessel-ray pits
Dipterocarpus (pits to tracheids)	Huge variety of size classes and shapes; larger pits gash-like, smaller ones circular or oval; cross-fields sometimes covering the entire vessel element wall
Parashorea	Rarely present; window-like or elongated
Shorea subg. *Anthoshorea*	window-like or sometimes elongated; apertures circular or sometimes elongated
Shorea subg. *Richetia*	Apertures window-like (rectangular) or oval; of variable size and shape
Shorea subg. *Rubroshorea*	Apertures window-like or elongated horizontally (gash-like); of variable size and shape
Shorea subg. *Shorea*	Apertures circular or oval, rather small

As with the investigated Anacardiaceae, all the six genera and subgenera belong to the type of VAS (**v**essel-ray pits are **a**pparently **s**imple). The vessel-ray pits differ from the intervessel pits or pits to tracheids.
The vessel-ray pits belong to a huge variety of sizes classes. They differ in shapes from gash-like to circular or oval to elongated (Table 9).

Areas devoid of pits are small (valid for all six genera) to large (valid for all genera except for *Shorea* subg. *Anthoshorea*). All six genera possess tyloses. None of them has helical thickenings.

The quantitative data of the six investigated sub-/genera of the Dipterocarpaceae are summarized in Table 10. The boxplots of Figure 25 give an overview of the dimensions of the six investigated Dipterocarpaceae.
The vessel elements of the four subgenera cannot be easily distinguished in pulp and paper. Their characteristic morphological features are very similar. Also, *Parashorea* is very difficult to be distinguished from the others. Only *Dipterocarpus* seems to be identifiable due to the unique feature of the size of the whole vessel element.

Nevertheless, the evaluation of the statistical analysis for significant differences showed that the (sub-)genera can be distinguished by their quantitative data (Table 11). Table 11 shows that only a few categories do not show significant differences after they have been compared with each other. *Dipterocarpus* is not included in the table, because in this genus intervessel pits do not exist due to vessel elements being almost exclusively solitary in intact wood. A table of 21 investigated species can be found in 2.6.

Despite all the difficulties in distinguishing, the results of the blind test of the projects mentioned below (2.7) were good when it came to identifying the genera investigated, including the genera of the Dipterocarpaceae. However, without doubt this requires experience and expertise.

Table 10 Quantitative data of the four investigated Dipterocarpaceae

Genus	Vessel elements	Intervessel pit borders [µm]	Fibers (weighted averages)
Dipterocarpus	(343–)601(–792) long and (294–)409(–480) wide; l/w ratio 1.4	Vessel to tracheid pit borders (2.3–)3.3(–5.2)	1390 long, 26.5 wide. Fiber wall thickness 6.1 µm
Parashorea	(140–)425(–568) long, and (205–)369(–453) wide; l/w ratio 1.1	(1.7–)3.8(–7.2)	1145 long, 19.9 wide. Fiber wall thickness 3.6 µm
Shorea subg. *Anthoshorea*	(244–)453(–645) long, and (307–)407(–461) wide; l/w ratio 1.1	(2.3–)4.9(–7.7)	1460 long, 21.8 wide. Fiber wall thickness 3.7 µm
Shorea subg. *Richetia*	(206–)317(–533) long, and (251–)344(–413) wide; l/w ratio 0.9	(2.6–)4.4(–9.3)	895 long, 22.0 wide. Fiber wall thickness 5.2 µm
Shorea subg. *Rubroshorea*	(188–)376(–573) long, and (139–)312(–421) wide; l/w ratio 1.2	(3.7–)6.6(–12.2)	1230 long, 21.0 wide. Fiber wall thickness 4.3 µm
Shorea subg. *Shorea*	(135–)312(–568) long, and (161–)279(–333) wide; l/w ratio 1.2	(2.6–)4.7(–9.4)	905 long, 18.2 wide. Fiber wall thickness 4.7 µm

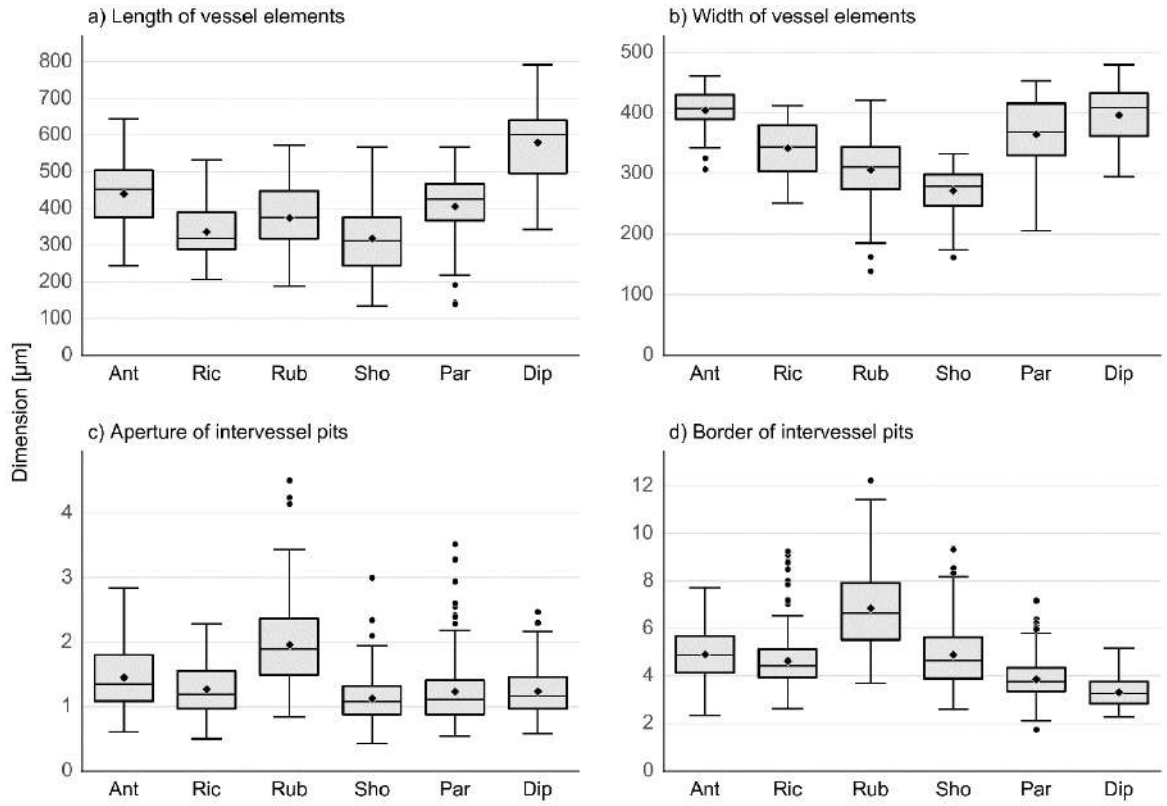


Figure 25 Boxplots of the six investigated sub-/genera of the family Dipterocarpaceae

Table 11 Significance of accordance of two distributions showing five (sub-)genera of the Dipterocarpaceae. The abbreviations result from the first three letters of the investigated genera mentioned in Table 1, p 12.Italic values indicate no significant difference. Level of significance: 5%.

	Sho-Ant	Sho-Ric	Sho-Rub	Sho-Sho	
Par	*0.089*	0.000	0.030	0.000	Length
	0.000	0.002	0.000	0.000	Width
	0.000	0.004	0.000	*0.138*	Aperture
	0.000	0.000	0.000	0.000	Border
Sho-Ant		0.000	0.003	0.000	Length
		0.000	0.000	0.000	Width
		0.040	0.000	0.000	Aperture
		0.241	0.000	0.002	Border
Sho-Ric			0.018	*0.182*	Length
			0.000	0.000	Width
			0.000	0.034	Aperture
			0.000	0.000	Border
Sho-Rub				0.004	Length
				0.000	Width
				0.000	Aperture
				0.000	Border

2.5 Variability of *Gonystylus* and *Shorea* subg. *Rubroshorea*

The study of the variability was a work package of the DBU project "AZ 31759". The results can therefore also be found in the final report of this project (www.dbu.de).

For the general descriptions of the characteristics of the different investigated genera the sizes of the vessel elements as well as of the apertures and borders of the intervessel pits were measured. The measurements had been carried out on only one tree/one sample per genus. For the investigation of the variability, five samples of *Gonystylus* and seven samples of *Shorea* subg. *Rubroshorea* were investigated.

These two genera were chosen because the tree species of the family Dipterocarpaceae, to which the subgenus *Rubroshorea* belongs, are very different with almost 200 species and represent a tree species family that is a dominant constituent of the tropical rain forests of Southeast Asia (Bansal et al. 2019). *Gonystylus* spp. (Ramin) was chosen because of its special protection status (CITES II). As already mentioned above, when Greenpeace collected wood samples at a wood yard of a large Asian pulp company, 46 out of 59 samples were identified as Ramin (Greenpeace 2012). Therefore, *Gonystylus* is highly relevant due to its protection status.

Only macerated material was investigated. The vessel elements of the pulp were unfortunately so damaged that they could not be measured. Five different macerated specimen of the CITES-protected genus *Gonystylus* (849-2, 839-3-A, B, C and D), as seen through a light microscope, were compared with the corresponding references published in the "Atlas of Vessel Elements". In all of the samples, the structural features of the vessel elements coincide exactly with the vessel element features as described in the corresponding references in the Atlas. The structural features that coincide with the references include: simple perforation plates, alternating intervessel pits, vessel-ray pits that are identical in size and shape to the intervessel pits, and arrangement of the pits to axial parenchyma cells.

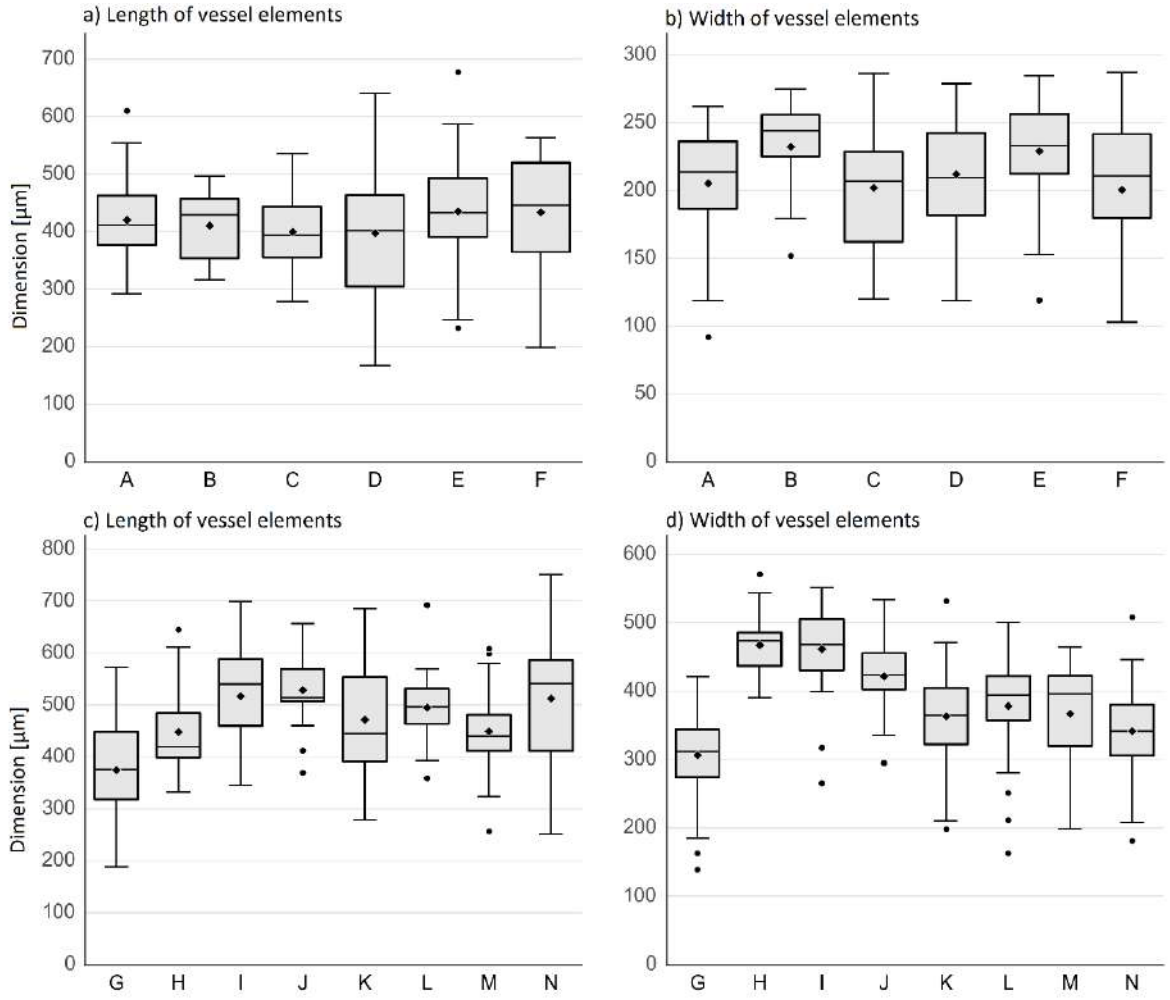


Figure 26 Variability: Length and width of vessel elements of Gonystylus (a and b) and of Shorea subg. Rubroshorea (c and d)
A – F: Gonystylus: A) macerate; B) 849-2; C) 849-3-A; D) 849-3-B; E) 849-3-C; F) 849-3-D
G – N: Shorea subg. Rubroshorea: G) macerate; H) Central Kalimantan; I) East Kalimantan; J) West Kalimantan; K) Mentawi Islands; L) Moluccas; M) Sabah; N) West Malaysia

For the woods of the genus *Shorea*, macerates of wood of the subgenus *Rubroshorea* from seven different origins were compared with the references. Here, too, the structural features of the vessel elements of the specimens are completely identical to the references: simple perforation plate, alternating intervessel pits, apparently simple vessel-ray pits, which are very variable in size and shape, pits to paratracheal tracheids. On average, the lengths and widths of 30-35 vessel elements, as well as five intervessel pits per vessel element were measured. A sum of 351 measurements of the lengths and widths of the vessel elements and 2041 measurements of the intervessel pits were carried out. In total, 4784 measurements were made (Figure 26).

When the collected vessel element data of the different macerates of the two genera *Gonystylus* and *Shorea* subg. *Rubroshorea* were analyzed together with the references (Figure 26), it became apparent that the parameter of the vessel element width (Figure 26, d), H–N) in the samples of *Shorea* subg. *Rubroshorea* partly shows remarkably higher values in comparison to the reference (Figure 26, d) G). Since the vessel elements of the genus *Shorea* have large diameters and show areas of cross fields with large, apparently simple vessel-ray pits, their dimensions are not as constant as in most single cells. It is possible that due to differences in preparation (they were prepared later than the reference sample), the vessel elements in the variability samples lay flatter on the slides than in the reference sample and thus appeared wider under the transmitted light microscope (e.g. due to a different amount of the embedding agent Euparal or due to cell collapse after dry falling).

In general, the measured values of both investigated genera or subgenera show a considerable variability. Since the variability within a single sample is already large, the metric data are only used in the form of size classes when wood species are to be identified (IAWA 1989). Furthermore, it is also known that the diameter of the vessel elements is influenced by environmental factors, and that the dimension is dependent on the location, e.g. on the concentration of potassium in the soil (Fromm 2013). The identification process of unknown fibrous materials is further hindered by the fact that they are usually mixtures of different wood species. A statistical evaluation would therefore not make sense. Instead, the measured values are used to identify unknown samples in order to determine whether a vessel element that corresponds to the structural features of a particular species does not deviate from the size class of the references.

Against this background, the deviations of the measured values of the investigated macerates of the two (sub-)genera show on the one hand the variability of the dimensions of the vessel elements of a genus. On the other hand, the deviations confirm that the measured values of the individual samples sufficiently reflect the genera for the application in broad size classes.

In summary, it can be stated that the characteristic morphological features visible on the vessel elements and described in the Atlas confirmed to be consistent within the genus. This is in accordance with the experiences from the wood species identification on the basis of the standardized structural features in the IAWA determination key.

2.6 Statistics

The verification of the significance of the data for the differentiation of two species led to 210 combinations of 21 wood species that were compared in terms of length and width of their vessel elements, and diameter of their intervessel pit apertures and borders. Aperture and border sizes of the pits of *Cocos nucifera* are missing in the data because scalariform pits can be deformed in macerations or pulp. The statistical evaluation resulted in significant and non-significant differences. The Wilcoxon-Mann-Whitney test showed that in almost every case of comparison at least two of the four parameters (lengths and widths of the vessel elements and apertures and borders of the intervessel pits) displayed significant differences. 69% of the combinations showed significant differences for all four parameters. 21% showed significant differences in three of four and 8% in two of four parameters. Only 1% showed significant differences in only one parameter (Table 12).

This means that genera can be identified by their quantitative data. Even the subgenera of *Shorea* were distinguishable in this way. This is hardly possible with the conventional method, the visual evaluation of recognizable characteristic morphological features.

For genera identification by their quantitative data they must be available in monofraction samples and not in composite samples. For paper products, pulps are usually mixtures consisting of different genera. Thus, in practice, it almost never occurs that paper is produced from only one genus. Statistical analysis using the Wilcoxon-Mann-Whitney test is therefore not relevant for the identification of wood species in practice.

The results of the statistical analysis are published in Helmling et al. (2016). The abbreviations in Table 12 result from the first three letters of the investigated genera mentioned in Table 1 (p. 12).

Table 12 Significance of accordance of two distributions showing 21 species. The abbreviations result from the first three letters of the investigated genera mentioned in Table 1, p 12.Italic values indicate no significant difference. Level of significance: 5%.

	Avi	Coc	Den	Dur	Euc	Gon	Her	Int	Koo	Lop	Man	Pal	Par	Sho-Ant	Sho-Ric	Sho-Rub	Sho-Sho	Rhi	Swi	Tet	
	0.000	0.000	0.000	0.000	0.001	0.000	*0.420*	0.001	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	Length
Aca	0.000	0.000	0.002	0.000	*0.199*	0.001	0.000	0.000	0.000	0.046	*0.077*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	*0.398*	0.000	Width
	0.000		0.000	0.000	0.000	0.000	*0.492*	0.000	0.000	0.000	0.000	0.050	0.001	0.023	*0.432*	0.000	0.020	0.000	0.000	0.000	Aperture
	0.000		0.000	0.000	*0.081*	0.000	0.000	*0.163*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.003	0.000	0.000	Border
		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	Length
Avi		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	Width
			0.132	0.000	0.000	0.027	0.000	0.000	0.000	*0.470*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	Aperture
			0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	Border
			0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	Length
Coc			0.004	*0.334*	0.000	0.000	*0.175*	0.003	0.015	0.000	0.000	*0.134*	0.001	0.000	0.020	*0.473*	0.004	0.000	0.002	*0.174*	Width
				0.028	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	*0.194*	0.000	0.000	Length
Den				0.000	0.027	0.000	0.000	0.000	0.000	0.000	0.043	0.034	0.000	0.000	0.000	0.000	*0.289*	0.000	0.027	0.019	Width
				0.000	0.000	*0.304*	0.000	0.000	0.000	*0.113*	0.000	0.000	0.026	0.000	0.000	0.000	0.002	0.000	0.000	0.000	Aperture
				0.000	0.000	*0.098*	0.000	0.000	0.000	*0.393*	0.000	0.000	0.028	0.000	0.000	0.000	0.000	0.000	0.000	0.001	Border
					0.000	0.001	0.000	0.000	0.007	*0.053*	0.000	0.000	0.001	0.009	0.000	0.000	0.000	*0.206*	0.000	0.000	Length
Dur					0.000	0.000	*0.363*	0.005	0.029	0.000	0.000	0.027	0.001	0.000	0.034	*0.207*	0.000	0.000	0.000	0.039	Width
					0.000	0.000	0.000	0.000	0.025	0.000	0.039	0.000	0.000	0.000	0.000	*0.227*	0.000	0.000	0.002	0.000	Aperture
					0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	*0.057*	0.000	*0.174*	0.000	0.000	Border
						0.000	0.001	0.389	0.000	0.000	0.000	0.000	0.000	0.000	0.159	0.005	0.461	0.000	0.001	0.000	Length
Euc						0.000	0.000	0.000	0.000	0.003	0.375	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.106	0.000	Width
						0.000	0.000	0.076	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000	0.000	Aperture
						0.000	0.073	0.292	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.212	0.000	0.000	0.000	Border
							0.000	0.000	*0.141*	0.042	0.000	0.000	*0.481*	*0.113*	0.000	0.015	0.000	0.000	*0.192*	0.000	Length
Gon							0.000	0.000	0.000	*0.205*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	*0.323*	0.000	Width
							0.000	0.000	0.000	0.016	0.000	0.000	*0.061*	0.000	0.000	0.000	0.005	0.000	0.000	0.000	Aperture
							0.000	0.000	0.000	*0.138*	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.013	Border
								0.003	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	Length
Her								0.002	0.008	0.000	0.000	*0.009*	0.003	0.000	0.196	0.044	0.000	0.000	0.000	0.018	Width
								0.001	0.000	0.000	0.000	*0.064*	0.001	0.036	*0.489*	0.000	0.021	0.000	0.000	0.000	Aperture
								0.009	0.000	0.000	0.000	0.021	0.000	0.004	0.000	0.000	*0.350*	0.000	0.000	0.000	Border
									0.000	0.000	0.000	0.000	0.001	0.000	*0.307*	0.024	*0.348*	0.000	0.003	0.000	Length
Int									*0.474*	0.000	0.000	0.000	*0.131*	*0.086*	0.002	0.000	0.000	0.000	0.000	0.000	Width
									0.000	0.000	0.000	0.000	0.000	*0.136*	0.004	0.000	0.000	0.000	0.000	0.000	Aperture
									0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	*0.070*	0.000	0.000	0.000	Border
										0.276	0.000	0.000	*0.136*	*0.484*	0.000	0.002	0.000	0.000	0.046	0.000	Length
Koo										0.000	0.000	0.000	*0.158*	*0.144*	0.013	0.000	0.000	0.000	0.000	0.000	Width
										0.000	*0.496*	0.000	0.000	0.000	0.000	*0.223*	0.000	0.000	*0.092*	0.000	Aperture
										0.000	*0.169*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	Border
											0.000	0.000	0.034	*0.285*	0.000	0.001	0.000	0.005	0.014	0.000	Length
Lop											0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	*0.375*	0.000	Width
											0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	Aperture
											0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.001	Border
												0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	Length
Man												0.000	0.000	0.000	0.000	0.000	0.000	0.000	*0.151*	0.001	Width
												0.000	0.000	0.000	0.000	*0.321*	0.000	0.000	*0.062*	0.000	Aperture
												0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	Border
													0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	Length
Pal													0.000	0.000	0.000	*0.069*	0.045	0.000	0.003	*0.403*	Width
													0.013	0.000	*0.070*	0.000	*0.256*	0.000	0.000	0.000	Aperture
													0.000	*0.118*	0.029	0.000	0.011	0.000	0.000	0.000	Border
														0.089	0.000	0.030	0.000	0.000	*0.260*	0.000	Length
Par														0.000	0.002	0.000	0.000	0.000	0.000	0.000	Width
														0.000	0.004	0.000	*0.138*	0.000	0.000	0.000	Aperture
														0.000	0.000	0.000	0.000	0.000	0.000	0.000	Border
															0.000	0.003	0.000	0.000	0.045	0.000	Length
Sho-Ant															0.000	0.000	0.000	0.000	0.000	0.000	Width
															0.040	0.000	0.000	0.000	0.000	0.000	Aperture
															0.241	0.000	0.002	0.000	0.000	0.000	Border
																0.018	*0.182*	0.000	0.002	0.000	Length
Sho-Ric																0.000	0.000	0.000	0.000	0.000	Width
																0.000	0.034	0.000	0.000	0.000	Aperture
																0.000	0.000	0.000	0.000	0.000	Border
																	0.004	0.000	*0.127*	0.000	Length
Sho-Rub																	0.000	0.000	*0.155*	0.000	Width
																	0.000	0.000	0.041	0.000	Aperture
																	0.000	0.006	0.000	0.000	Border
																		0.000	0.000	0.000	Length
Sho-Sho																		0.000	0.006	*0.076*	Width
																		0.000	0.000	0.000	Aperture
																		0.000	0.000	0.000	Border
																			0.000	0.000	Length
Rhi																			0.000	0.000	Width
																			0.000	0.000	Aperture
																			0.000	0.000	Border
																				0.000	Length
Swi																				0.001	Width
																				0.000	Aperture
																				0.000	Border

2.7 Blind test and applicability

During a blind test, test sheets of MTH – from 31 unnamed Asian wood species – were produced by an external institute. The sheets were mixed with beech pulp (standard pulp) and blind-tested for the analysis of their composition. All project partners of the above mentioned DBU-projects (PMV, TI and ISEGA) each received 15 test sheets of unknown composition and were requested to determine the genera of wood contained in them. If – as in this case – fibrous material is examined that was not "gently" macerated, but instead went through all the processing steps of cooking and bleaching and was made into paper, many of the vessel elements are torn to pieces or folded. In addition, both industrially produced paper and test sheets contain mixtures of several genera, which means that the identification procedure is quite challenging. The test sheets of the blind test were used to make microscope slides for light microscopy. For every sheet, the project partners had to determine which of the 32 pulps were contained. The project partners were also asked to comment on which of the identification results they considered to be rather uncertain, i.e. which they would note in a real report as "not clearly identifiable". The evaluations of the extensive blind test showed that PMV, TI and ISEGA with rates of 74%, 92% and 96%, respectively, were able to make correct decisions. In comparison to the blind test of the previous project (86%, 90% and 86%, respectively), two out of three partners were able to significantly improve their rates, although the second blind test contained more species (32 instead of 23), thus increasing the possibility of mistaken identification.

In the self-assessment, the partners from PMV and TI emphasized that the differentiation of the subgenera of the genus *Shorea* was considered to be much more difficult, due to their great similarities among each other, and that there was the possibility of mistaken identification with two other genera (*Dipterocarpus* and *Parashorea*) of the family Dipterocarpaceae (see also 2.4.2). This self-assessment proved to be accurate, since an evaluation of the identification of the six pulps of the Dipterocarpaceae led to a lower hit rate (63%, 82% and 93% correct decisions, respectively) for all participants as compared to the entire study. The high identification rate of the ISEGA showed, however, that the selected wood species from the Dipterocarpaceae can be differentiated with high microscopic effort (15-20 slides were analyzed per blind test sheet). Another group of three not closely related genera (*Gonystylus*, *Lophopetalum* and *Durio*) coincidentally possesses vessel elements with very similar anatomies. The TI classified this group with a high possibility of mistaken identification. Since the genus *Gonystylus*, which is under CITES protection, belongs to this group, this fact is of a particular relevance. The rate of correct identification of wood species in the 15 test sheets was, with 67%, 100% and 100%, respectively, fortunately completely without errors in two project partners.

It must now be taken into account that real samples may contain further wood species for which no references are available yet and which may also show a high similarity to *Gonystylus*. Therefore, the development of chemotaxonomy as an additional or complementary method, since the method is independent of cell anatomy (see also 1.1.1), is of great importance.

The good results of the blind test are a proof of the applicability of the elaborately compiled references. It could be shown that the described and illustrated morphological features in the "Atlas of Vessel Elements" (Helmling et al. 2018) can be successfully used for the determination of wood genera in paper. These points render the Atlas an indispensable reference for practical use (in every laboratory in the world). Further information on the blind test can be found in the respective final reports of the projects (www.dbu.de).

2.8 Conclusion

The identification of internationally traded wood and wood products is essential to enforcing the legal utilization of wood, especially in pulp and paper, which is traded extensively. The identification of certain individual cell elements, the vessel elements, requires standardized morphological descriptions with high-quality images. These descriptions have now been made available in the form of an “Atlas of Vessel Elements” by Helmling et al. (2018), of the leading tropical timber species originating in Asia. Since the fiber components of pulp and paper are subject to the strict control of the EUTR, the Thünen Centre of Competence on the Origin of Timber, Hamburg, Germany, receives samples every day that must be examined in order to control and verify the declarations of trading companies and competent authorities. In this context, earlier versions of the content of this work successfully proved to be a useful, integral part of the identification processes.

Although the work has been expanded to its present extent, it admittedly has its shortcomings, such as the yet limited number of described taxa, as well as insufficient sampling for evaluating the full range of variations in vessel element morphology. The latter applies especially to the species, subgenus and genus levels.

However, the verifiable results of a blind test clearly demonstrated the applicability of the Atlas and the practical usefulness of the collected qualitative data. Thus, this work can be appreciated as a valuable aid in wood identification in pulp and paper, as well as as a supplement to the existing fiber atlases, in particular to the fiber atlas by Ilvessalo-Pfäffli (1995), which contains descriptions of tree species mainly used in pulp and paper production. Future work will be necessary to continuously extend the database with descriptions of further unknown or lesser-known species. By displaying the considerable variety of vessel elements in various Asian timbers, the results of this work will be of substantiable assistance in identifying tropical and temperate wood genera in pulp, paper, and fiber boards. If this work will succeed in helping to preserve protected tree species, then its purpose shall be more than fulfilled.

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4 List of figures and tables

5 Publications: Explanations on the own shares of the submitted work

No.	Authors and title	Own shares
I	Helmling S, Olbrich A, Tepe L, Koch G (2016) Qualitative and quantitative characteristics of macerated vessels of 23 mixed tropical hardwood (MTH) species: a data collection for the identification of wood species in pulp and paper. Holzforsch 70(9):839–844, DOI: 10.1515/hf-2015-0195 **(peer-reviewed)**	Complete conception of the work. Literature research. Test planning and execution and data evaluation partly in cooperation with the co-authors. The co-authors took part in the discussion. Complete writing of the first version of the manuscript.
II	Helmling S, Olbrich A, Heinz I, Koch G (2018) IAWA Atlas of Vessel Elements © 2018. IAWA Journal 39 (3):249–352 Leiden, The Netherlands, DOI: 10.1163/22941932-20180202 **(peer-reviewed)**	Complete conception of the work. Literature research. Test planning in cooperation with the co-authors, mainly with Andrea Olbrich. Complete execution and data evaluation. Research work was supported by the theses of Arne Kuck, Saskia Poth and Lena Tepe (M.Sc. of Wood Science, University of Hamburg). The co-authors took part in the discussion. Complete writing of the first version of the manuscript.
III	Odermatt J, Olbrich A, Helmling S, Wassink A (2017). Identifizierung von Mixed Tropical Hardwood (MTH) in Papier mittels chemo-taxonomischer und morphologischer Merkmale. Final report of the DBU project AZ 31759-31.	Participation in the parts on microscopic investigations. Creation of images. Participation in discussion of the results and the publication-ready presentation of the work.
IV	Koch G, Haag V, Helmling S, Heinz I, Olbrich A (2017) Fasern im Fokus: Holzartenbestimmung von Faserplatten – Erfahrungen aus den Prüfungen im Kontext der EUTR. MDF Mag Co: 86–88	Participation in discussion of the results and the publication-ready presentation of the work.
V	Sieburg-Rockel IJ, Koch G, Kaschuro S, Helmling S, Olbrich A (2019) Identifizierung von Holzarten in Spanplatten, Holztechnologie 60(3):5–9, Institut für Holztechnologie Dresden (ihd)	Participation in discussion of the results and the publication-ready presentation of the work.
VI	Heinz I, Helmling S, Olbrich A, Koch G (2019) O-5: Identification of Asian timbers in pulp, paper and fiber boards. In: IAWA-IUFRO International Symposium: challenges and opportunities for updating wood identification; May 20–22, 2019, China, program & abstracts. pp. 5–6	Participation in discussion of the results and the publication-ready presentation of the work.

VII	Schmitz N (editor), Beeckman H, Blanc-Jolivet C, Boeschoten L, Braga J W B, Cabezas J A, Chaix G, Crameri S, Degen B, Deklerck V, Espinoza E, Gasson P, Haag V, Helmling S, Horacek M, Koch G, Lancaster C, Lens F, Lowe A, Martínez-Jarquín S, Nowakowska J A, Olbrich A, Paredes-Villanueva K, Pastore T C M, Ramananantoandro T, Razafimahatratra A R, Ravindran P, Rees G, Soares L F, Tysklind N, Vlam M, Watkinson C, Wheeler E, Winkler R, Wiedenhoeft A C, Zemke V, Zuidema P (2020). A data analysis guide for wood identification. Overview of current practices for different methods. Global Timber Tracking Network, GTTN secretariat, European Forest Institute and Thünen Institute.	Participation in the chapter “Data analysis for taxon identification of pulp, paper and fibreboard”; discussion of the results and the publication-ready presentation of the work.

Prof. Dr. E. Magel

Eidesstattliche Versicherung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

Stephanie Helmling

Publication I

Helmling S, Olbrich A, Tepe L, Koch G (2016)

Qualitative and quantitative characteristics of macerated vessels of 23 mixed tropical hardwood (MTH) species: a data collection for the identification of wood species in pulp and paper

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DOI: 10.1515/hf-2015-0195

(peer-reviewed)

Stephanie Helmling*, Andrea Olbrich, Lena Tepe and Gerald Koch

Qualitative and quantitative characteristics of macerated vessels of 23 mixed tropical hardwood (MTH) species: a data collection for the identification of wood species in pulp and paper

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Abstract: The identification of wood species in paper products is highly important for the enforcement of the newly established timber regulations regarding the control of illegal logging. In the context of European Timber Regulation (EUTR), in the present paper a database has been established containing reference samples and anatomical descriptions for the identification of 23 tropical timbers from Southeast Asia in pulp and paper products. The vessel elements and fibers of these mixed tropical hardwoods (MTH) were characterized by light microscopy. The woods in focus were macerated, embedded and compared visually and statistically. The collected microscopic data and images are helpful for the identification of the wood species used for paper production.

Keywords: European Timber Regulation (EUTR), fiber atlas, identification of wood species in paper, intervessel pitting, maceration, mixed tropical hardwoods (MTH), vessels and vessel elements, vessel-ray pitting

Introduction

The fiber components of pulp and paper are subjected to the strict control of the European Timber Regulation (EUTR, No. 995/2010, implemented in March 2013), according to which both the producer of wood products and traders are responsible for the wood species applied (Koch et al. 2015). The key purpose of EUTR is to minimize the utilization of illegally logged timber or products derived from it on the EU market. Thus pertinent details of the product are needed, which make possible the rapid identification of wood species and their origin (Dormontt et al. 2015, Koch and Schmitt 2015).

Tropical tree species (among others mangrove trees) are frequently used for the increasing pulp production in Southeast Asia and China (Williams 2014, FAO 2015). It cannot be excluded that tropical timbers from natural forests with protected tree species are also processed (Greenpeace 2012). Their identification in pulp and paper requires a standardized morphological description of the individual wood cells in the products. In detail, individual vessel elements are best suited for microscopic identification based on typical features such as structure of perforation plates, helical thickenings, and arrangement of vessel-ray pits (Core et al. 1979, Carlquist 2001). Such characteristics for the most important species grown in the temperate zone and in plantations are well described in the fiber atlases of Harders-Steinhäuser (1974) and of Ilvessalo-Pfäffli (1995), which are also suitable for wood identification in pulp and paper. However, no such database is available for tropical timbers used for pulp and paper production. More than a hundred anatomical characteristics are available for the identification of solid softwoods and hardwoods (IAWA 1989, IAWA 2004). Compared to solid wood, there are essentially less microscopically relevant key characteristics of macerated tissues (Gasson 2011). For the determination of wood species in pulp and paper, a reliable database is still not yet available. Even if species-relevant details are known from solid wood, their identification in pulp and paper is more difficult because of the complex mixture of fiber and vessel elements from various wood species that suffered mechanical defects during the pulp production (Koch et al. 2015). Moreover, the anatomically relevant vessels are scarce in such mixtures.

The aim of the present paper is the establishment of a fiber atlas focusing on tropical species from Southeast

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Asia. The first step to this goal is the description of intact vessel elements in solid wood. Then, the quality of the key features of the damaged vessels in pulp and paper has to be investigated. The expectation is that some features will be suitable for the identification of tropical woods from illegal logging.

Materials and methods

Wood from 20 different genera representing 14 families from Southeast Asia was chosen and prepared for maceration (Table 1). The genera were chosen in cooperation with Greenpeace and represent some of the principal timbers currently traded in Southeast Asia. For instance, four subgenera of *Shorea*, as well as two monocots *(Cocos* sp. and *Dendrocalamus* sp.) are included in the list. Authenticated specimens of the wood collection of the Thünen Institute served as reference material.

The samples were prepared and macerated according to the method of Franklin (1945). Maceration is a gentle method to dismantle the cell structure by dissolving the lignin without destroying the cells, in contrast to the defibration process in paper production in the course of which heavy mechanical and chemical stress is imposed on both fiber and vessel elements. For maceration, the samples ($1\times1\times1$ cm^3) of each species were split into 1 mm thick slivers in the radial direction to obtain a representative selection of vessel elements from successive growth increments. The samples were subsequently boiled in distilled water. A solution of equal parts of glacial acetic acid (99%) (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) and 30% solution of H_2O_2 (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) was filled into test tubes and two to three wood slivers were dropped in the reagent (Rotilabo block-thermostat H 250, Carl Roth GmbH & Co. KG). The tubes covered by marbles were then heated to about 60°C for 48 h, so that the emerging steam condensed on the marble surface and the gas could escape. The maceration ended as soon as the samples were bleached. The woody tissue was broken up, and the individual elements were separated by shaking the test tubes. The resulting suspension was rinsed with tap water and kept in a plastic filter bag in ethanol (96%).

For better contrast, a part of the pulp was stained with safranin (4%) (alcohol soluble, Chroma-Gesellschaft Schmid & Co. Stuttgart-Untertürckheim), another part with nigrosin (1%) (alcohol soluble, Alfa Aesar GmbH & Co. KG, Karlsruhe). The vessel elements of the pulp were then selected by needle, placed on microscope slides, and embedded in Euparal (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) to store them permanently (Figure 1). The microscope slides were placed under a flue to evaporate for 1 day and then cured at 50°C for 10 days.

Qualitative parameters and their quantification: The qualitative parameters were evaluated visually, i.e. the comparative shape of the vessel elements, the appendices, perforation plates, presence of tyloses, arrangement of the intervessel pits, vessel-ray pits, and pits to parenchyma cells and fibers. The vessel element dimensions were determined by measuring the length and width including the length/width ratio based on vessel elements stained with safranin. The intervessel pits were measured by quantifying the vertical diameter of their apertures and borders (Figure 2a) based on vessel elements stained with nigrosin. Measurements were taken of at least 36 vessel elements of each species (software: cell^F, Olympus Soft Imaging Solutions GmbH, Münster, Germany).

Table 1: List of investigated species.

Family	Species	Trade name
Acanthaceae	*Avicennia marina* (Forssk.) Vierh.	Api Api (Mangrove)
Anacardiaceae	*Mangifera* sp.	Mango
Anacardiaceae	*Swintonia* sp.	Merpauh
Arecaceae	*Cocos nucifera* L.	Coconut Palm
Calophyllaceae	*Calophyllum* sp.	Bintangor
Celastraceae	*Lophopetalum* sp.	Perupok
Dipterocarpaceae	*Shorea* subg. *Rubroshorea*	Red Meranti
Dipterocarpaceae	*Shorea* subg. *Anthoshorea*	White Meranti
Dipterocarpaceae	*Shorea* subg. *Shorea*	Bangkirai, Balau
Dipterocarpaceae	*Shorea* subg. *Richetia*	Yellow Meranti
Dipterocarpaceae	*Parashorea* sp.	Gerutu
Dipterocarpaceae	*Dipterocarpus* sp.	Keruing
Fabaceae-Caesalpinioideae	*Intsia* sp.	Merbau
Fabaceae-Caesalpinioideae	*Koompassia malaccensis* Maingay ex Benth.	Kempas
Fabaceae-Mimosoideae	*Acacia mangium* Willd.	Acacia
Malvaceae	*Durio* sp.	Durian
Malvaceae	*Heritiera* sp.	Mengkulang
Myrtaceae	*Eucalyptus globulus* Labill.	Eucalyptus
Poaceae	*Dendrocalamus latiflorus* Munro	Bamboo
Rhizophoraceae	*Rhizophora* sp.	Red Mangrove
Sapotaceae	*Palaquium* sp.	Nyatoh
Tetrameristaceae	*Tetramerista glabra* Miq.	Punah
Thymelaeaceae	*Gonystylus* sp.	Ramin

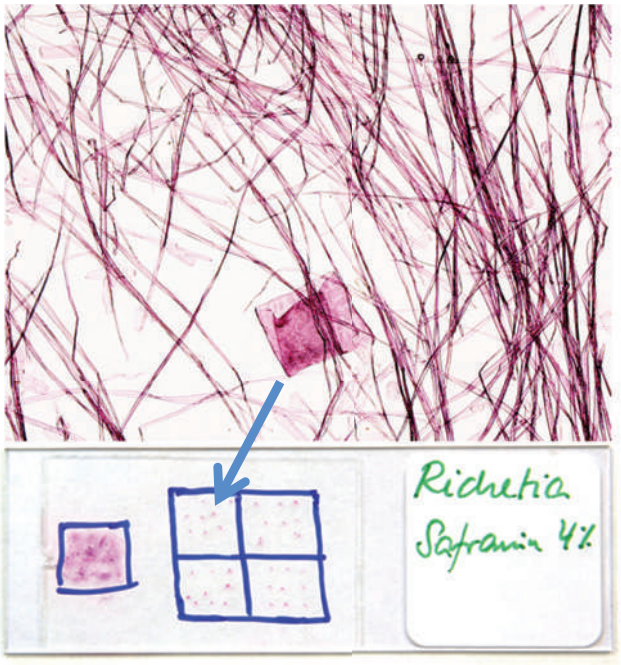

Figure 1: Macerated fibers and vessel elements, put on a microscope slide.

Statistical analysis: The recorded data of vessel elements and pits were statistically analyzed in SAS UNIVARIATE (SAS Institute Inc., USA). During analysis, it became evident that several attributes are not normally distributed. Because of the different types of deviation, the Wilcoxon-Mann-Whitney test was applied, i.e. a statistical analysis independent from data distribution. The length and width of the vessel elements as well as the diameter of the apertures and borders of the intervessel pits of all species were compared in terms of significance of differences (level of significance: 5%).

Results and discussion

The vessel elements and intervessel pits of the wood species were measured, analyzed, and compared to each other based on statistically evaluated data in terms of length, width, pit diameter of aperture and border, and visual impression concerning shapes and patterns. The results show that the evaluation of vessel dimensions allows the identification of the species in paper made of pure material (only one species applied, i.e. monofraction). This, however, was not possible when the mixture consisted of several species, as is common practice in the paper-producing industry. In paper, the vessel elements sometimes are ripped or folded that impedes the measurement of the vessel dimensions. In the case of paper, therefore, species identification relies on the qualitative analysis of vessel elements by means of the visual impression and comparison with references in the fiber atlas

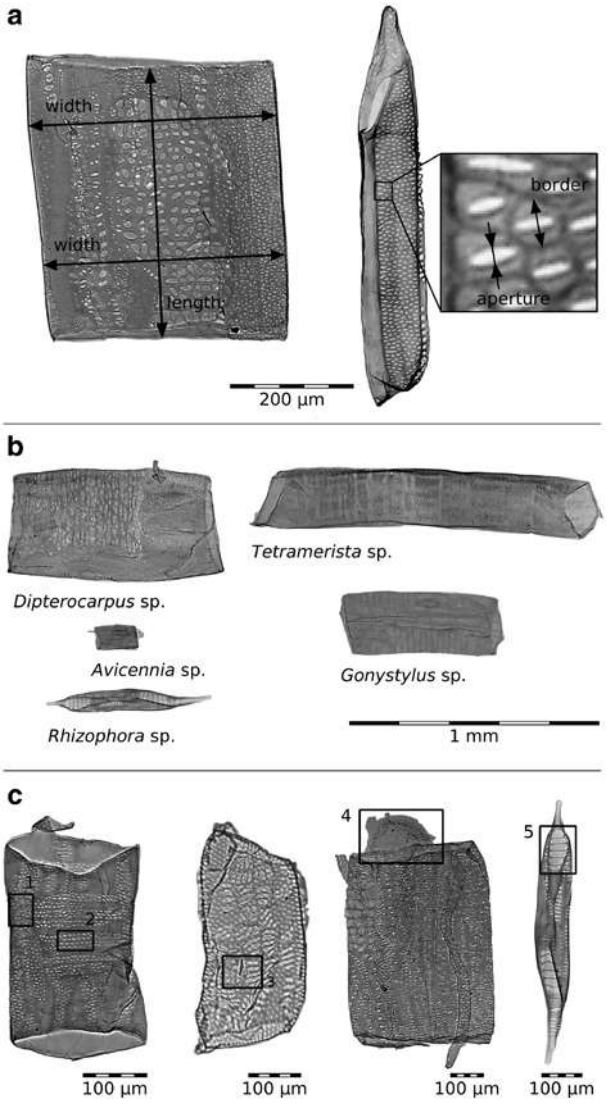

Figure 2: Acquisition of vessel element characters (a) Measurement on vessel elements (length, width, intervessel pit aperture and border) (b) Dimensions of vessel elements of different species (*Dipterocarpus* sp., *Avicennia* sp., *Rhizophora* sp., *Tetramerista* sp., *Gonystylus* sp.) (c) Different perforations (simple and scalariform) and different shapes, sizes and patterns of pits in vessel elements. Intervessel pits (1) and vessel-ray pits (2) on *Acacia* sp., increased vessel-ray pits on *Mangifera* sp. (3), tylosis (4) on *Shorea* subg. *Anthoshorea*, scalariform perforation (5) on *Rhizophora* sp.

(Ilvessalo-Pfäffli 1995) or micrographic atlases of Southeast Asian timber (Hayashi et al. 1973, Ogata et al. 2008).

Shapes and patterns

The shapes and patterns of the vessel elements and their pits were visually compared. As seen in Figure 2b, the vessel elements vary greatly in size. They possess

divergent dimensions and shapes: some of them are slim and elongated (*Tetramerista* sp.), others are very small and short (*Avicennia* sp.). Some species show appendices that differ in length, width, and form. The appendices of *Gonystylus* sp., for example, are short and the transition is abrupt while *Rhizophora* sp. has long appendices with a smooth transition. The shape and formation of the pits are also very different (Figure 2c). The pit arrangements are in relation to the pit type: the intervessel pits of some species are arranged in vertical lines or in fields; vessel-ray pits appear horizontal in long lines (according to the rays). While the pits to longitudinal parenchyma cells (Figure 2c-1) and the vessel-ray pits (Figure 2c-2) of *Acacia* sp. resemble each other, they can still be differentiated due to their arrangement in vertical rather than horizontal lines. Furthermore, pit size and shape can be indicative of the genus: the vessel-ray pits of *Mangifera* sp., for example, appear bigger, elongated, and wider (Figure 2c-3) than those of *Acacia* sp. (Figure 2c-2) and possess reduced or apparently simple borders. It is possible to determine the adjacent cells, such as a tracheid or a parenchyma cell, by looking at the pit arrangement.

If wood species generate tyloses, like *Shorea* subg. *Anthoshorea* (Figure 2c-4), the tyloses are kept after maceration and are a helpful feature for identification. The perforation of vessel elements can be simple (*Acacia* sp., *Mangifera* sp., *Shorea* subg. *Anthoshorea*) or scalariform (Figure 2c-5, *Rhizophora* sp.).

Results of quantitative measurements

In Figure 3, the boxplots of 10 selected species show the distribution of the measurements of the parameters length and width of the vessel elements as well as the diameter of the apertures and borders of intervessel pits. The boxplots of one parameter have mostly a similar appearance with overlaps and some deviations from the main field. Species belonging to the genus *Tetramerista* have the longest and widest vessel elements but the smallest intervessel pit apertures. This is a useful combination well-suited for identification, even if the distribution of the width is

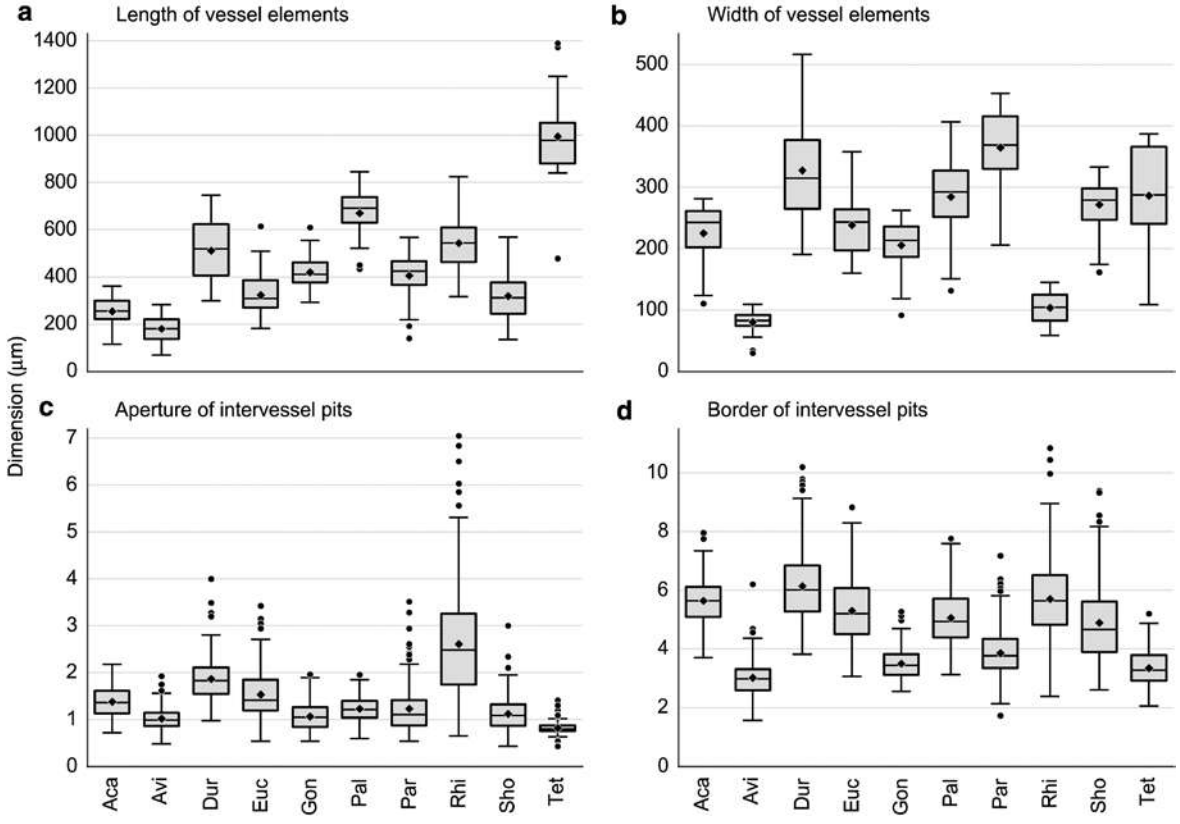

Figure 3: Variation of quantitative data of *Acacia* sp. (Aca), *Avicennia* sp. (Avi), *Durio* sp. (Dur), *Eucalyptus* sp. (Euc), *Gonystylus* sp. (Gon), *Palaquium* sp. (Pal), *Parashorea* sp. (Par), *Rhizophora* sp. (Rhi), *Shorea* subg. *Shorea* (Sho), *Tetramerista* sp. (Tet) in (a) length of vessel elements (b) width of vessel elements (c) diameter of apertures of intervessel pits (d) diameter of borders of intervessel pits.

broad. The latter is overlapping in a wide field apart from the genera *Avicennia* and *Rhizophora*. *Avicennia* sp. is very small on the whole, which has the shortest and the most slender vessel elements.

The boxplots of the intervessel pit apertures show a very similar distribution with a clear interquartile range; only *Tetramerista* sp. deviates with a very small interquartile range. The scalariform intervessel pits of *Rhizophora* sp. have the widest and *Tetramerista* sp. the smallest intervessel pit apertures. The size of the borders of the intervessel pits corresponds to the distribution of the apertures. As expected, the diameters of the borders are slightly larger than those of the apertures. *Durio* sp. and *Rhizophora* sp. have the largest borders, *Avicennia* sp. has the smallest.

As visible in the boxplots, the species lie close to each other in almost every case and mostly are overlapping. The quantitative data of the vessel elements are not sufficient for species identification, but they are a helpful addendum in the fiber atlas and complement the qualitative characteristics for species identification (Richter and Dallwitz 2000). They provide an indication for the final specific attribution of vessel elements in mixed pulps and papers and constitute a possibility to confirm the prior findings.

Table 2: Significance of accordance of two distributions showing the example of five species [*Dendrocalamus* sp. (Den), *Heritiera* sp. (Her), *Intsia* sp. (Int), *Shorea* subg. *Shorea* (Sho-Sho), *Acacia* sp. (Aca)].

			Species	
Species	**Den**	**Her**	**Int**	**Sho-Sho**
Aca				
Length	0.000	*0.420*	0.001	0.001
Width	0.002	0.000	0.000	0.000
Aperture	0.000	*0.492*	0.000	0.020
Border	0.000	0.000	*0.163*	0.012
Den				
Length		0.000	0.000	0.000
Width		0.000	0.002	*0.289*
Aperture		0.000	0.000	0.000
Border		0.000	0.000	0.000
Her				
Length			0.003	0.002
Width			0.002	0.000
Aperture			0.001	0.021
Border			0.009	*0.350*
Int				
Length				*0.348*
Width				0.000
Aperture				0.000
Border				*0.070*

Italic values indicate no significant difference.

The verification of the significance of the data for the differentiation of two species led to 190 combinations of 20 wood species that are compared in terms of length and width of vessel elements, and diameter of intervessel pit apertures and of borders. The statistical evaluation according to the Wilcoxon-Mann-Whitney test resulted in significant and non-significant differences (always between two species to compare): 69% of the combinations showed significant differences for all four parameters, 21% in three of four and 8% in two of four parameters. Only 1% show significant differences in only one parameter. In almost every case, at least two of the four parameters display significant differences (Table 2). This method allows for the differentiation between the subgenera of *Shorea*, which cannot be achieved reliably by the commonly used visual evaluation. This way of determination of species based on quantitative measurements and evaluated by statistical analysis is useful for monofraction samples, i.e. if only one wood was used for pulping and paper making.

Until now, the database contains the results of 36 vessel elements for each species that originate from a single wood sample from the investigated species. The variability is not yet given and will be the subject of future research.

Conclusion

If the pulp or paper is composed of only one wood species, the wood can be identified at least to the generic level. The species assignment is more difficult in case of the presence of several wood species. A statistical analysis is nearly not possible because there must be at least 25 vessel elements per species to make a safe conclusion about the average dimensions of the vessel elements and their pits (IAWA 1989). Nevertheless, a new criterion is established based on the size distribution of the vessel elements that complements already-existing features for wood identification. For the differentiation of individual species in paper, qualitative data are more important because of the inhomogeneity of the material. Based on a few parts of vessel elements, the statistical data are not reliable, but they give some hints to some species.

Acknowledgments: This work was funded by the German Environmental Foundation (DBU) (Grant/Award Number: "AZ-29436") in connection with the project "Identification of Mixed Tropical Hardwood (MTH) in paper – a contribution to species protection".

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Publication II

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DOI: 10.1163/22941932-20180202

(peer-reviewed)

ATLAS OF VESSEL ELEMENTS

Identification of Asian Timbers

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Contents

PREFACE

Pulp production is increasing worldwide, especially in Southeast Asia and China (WWF 2009; Williams 2014; FAO 2015), and the use of tropical timbers from natural forests is not uncommon (Greenpeace 2012). To ensure that no protected timbers are used, those detected in pulp and paper products must be identified.

Individual vessel elements are best suited for the microscopic identification of wood species in those products on the basis of typical morphological features such as type of perforation plates, presence *vs*. absence of helical thickenings, and type and arrangement of vessel-ray pits (Core *et al*. 1979; Carlquist 2001).

To allow identification, a standardized morphological description of these features is required for commonly used timber. The fiber atlases by Carpenter and Leney (1952), Ezpeleta and Simon (1970, 1971), Harders-Steinhäuser (1974), Parham and Gray (1982) and Ilvessalo-Pfäffli (1995) describe these characteristics for the main species grown in the temperate zone and in plantations. There is, however, no corresponding database available for important Asian timbers.

In recent years, the rapid and reliable identification of wood species and their origin has become necessary (*e.g*. Dormontt *et al*. 2015; Koch & Schmitt 2015; Koch *et al*. 2015), owing to the following laws that have recently come into effect: the U.S. Lacey Act (USA, 1900/amended in 2008), the Illegal Logging Prohibition Act 2012 (Australia), and the European Timber Regulation (EUTR, No. 995/2010. implemented in 2013). Besides other timber products, these laws subject the fiber components of pulp and paper to strict controls, aiming to minimize the trade and use of illegally logged timber or products derived from it. Both producers and traders of wood products are responsible for the correct declaration of the species and origin (Koch & Schmitt 2015).

To support the request for due diligence, our team from the University of Hamburg and the Thünen Institute (Hamburg, Germany) developed this atlas of vessel elements for the identification of Asian timbers, with funding from the German Environmental Foundation (Deutsche Bundesstiftung Umwelt, DBU). The vessel atlas describes 38 tropical and temperate Asian timbers, known for their potential in utilization of pulp and paper, including for the first time also lesser known species and the monocots Bamboo and Coconut palm. Each species is illustrated with high-quality photomicrographs at standard magnification.

Important diagnostic features are highlighted in the text and figures for easy identification. The taxonomy of the individual species is described in detail in the PROSEA Timber volumes (Soerianegara *et al*. 1993; Lemmens *et al*. 1995; Sosef *et al*. 1998). The selection of the taxa depends on the evaluation of about 2,150 official reports prepared at the Thünen Centre of Competence on the Origin of Timber since the implementation of the EUTR (2013) in Germany. Based on this experience, the atlas includes the commonly used timbers regularly identified in fiber boards, pulp, and paper from Asia, *e.g*. *Liquidambar*, *Nyssa* and *Schima*. Furthermore, taxa with wide distribution and high economic potential, *e.g*. of the families Dipterocarpaceae, Sapotaceae, etc. are considered (Helmling *et al*. 2016).

DOI 10.1163/22941932-20180202

Reference materials for the morphological description of the vessel elements are provided from vouchered specimens of the scientific wood collection (RBHw) of the Thünen Institute. Furthermore, well-documented assortments of mono-fractioned pulp (each pulp consisting of specimens belonging to a single genus) were produced separately to study the influence of mechanical impact on the morphology of carefully macerated vessel elements (not damaged) and mechanically pulped fibers (partly damaged). Paper sheets of unknown mixtures (blind test) were also produced and microscopically analyzed to assess the applicability of the information contained in the atlas. The verifiable results of the blind tests clearly demonstrated the usefulness of the atlas.

Based on this experience, the atlas should be understood as an aid to wood identification in pulp and paper, to be used as a supplement to the existing fiber atlases, in particular the atlas by Ilvessalo-Pfäffli (1995) with descriptions of tree species mainly used in the pulp and paper production.

We trust that by displaying the considerable variety of vessel elements in Asian timbers, this atlas will assist in identifying tropical and temperate woody genera in pulp, paper, and fiber board. We also hope that it may help to preserve protected tree species.

Stephanie Helmling, Andrea Olbrich, Immo Heinz, Gerald Koch
Hamburg, Germany 2018

HOW TO USE THE VESSEL ELEMENT ATLAS

This atlas contains the description of individual wood species, but the features may also be valid for the respective genus as the similarity within genera is often very high.

The identification of the material used in pulp, paper and fiber boards is a challenging task due to the missing tissue structure. The separated wood fibers offer less information than solid wood tissue. Furthermore, pulp often consists of a mixture of different wood species which makes identification even more difficult.

The wood identification also depends on the method of pulp production. If the pulp was strongly refined, the limits of wood identification have been reached.

It is possible to determine the family and often the genus. Individual species within a genus are too similar to distinguish them. To identify a timber the sample of pulp and paper products or fiber boards must be compared with the pictures of this atlas. In the case of having identified, *e.g.*, *Eucalyptus globulus*, according to the similarity of investigated vessel elements with the pictures in this atlas, one can only conclude that the cells have highest similarity with cells of *Eucalyptus* spp.

The standard species for the production of pulp and paper from temperate zones are well described in the atlases of Parham and Gray (1982) and Ilvessalo-Pfäffli (1995). The present atlas deals with a selection of Asian timbers. For the identification of wood species in pulp and paper of unknown provenance it makes sense to use these atlases together.

PREPARATION OF PULP SAMPLES FOR IDENTIFICATION WITH THE LIGHT MICROSCOPE

Paper

To prepare the samples for investigation, a piece of paper (1.5 cm^2) is put into a test tube filled with distilled water to soften the paper. After a few minutes the pulp mass is rolled to a ball between the fingers and placed in a tube with water again. By shaking the test tube the fibers are individualized and the fiber suspension is poured on a filter. Part of the material is then placed on 2 or 3 microscopic slides. Staining of the fibrous mix is done with two drops of Alexander and one drop of Herzberg solutions. Alexander solution (Merck KGaA, Darmstadt, Germany) consists of calcium nitrate tetrahydrate, Herzberg solution (Harders-Steinhäuser 1974) of zinc chloride and potassium iodide (Merck KGaA, Darmstadt, Germany). Cover glasses are placed on the stained pulp, and slides should be investigated immediately, since the staining is not permanent. After this process untreated cells with a high amount of lignin (mechanical pulp) are stained yellow, delignified cells (chemical pulp) are greyish blue (softwoods) or blue (hardwoods).

Medium Density Fiber board (MDF)

To prepare MDF samples, a piece of the material is boiled in water and the resulting pulp placed on a filter. For better contrast, the pulp is stained with nigrosin (1%) (alcohol soluble, Alfa Aesar GmbH & Co. KG, Karlsruhe). After 10–15 minutes the material is rinsed with de-ionised water and mounted in glycerine (86%, Carl Roth GmbH & Co. KG, Karlsruhe, Germany) on microscope slides.

Recommendations

Since there are many more fibers than vessel elements in the pulp it is important to obtain a representative range of vessel elements of the used species. Therefore, it is recommended to investigate enough material which usually requires observation of several microscope slides.

For the production of permanent slides it is useful to increase the contrast by staining the samples with nigrosin (1%, alcohol soluble, Alfa Aesar GmbH & Co. KG, Karlsruhe) or safranine (4%, alcohol soluble, Chroma-Gesellschaft Schmid & Co. Stuttgart-Untertürckheim). The stained vessel elements of the pulp are selected by needle, placed on microscope slides and mounted in Euparal (Carl Roth GmbH & Co. KG, Karlsruhe, Germany).

MATERIALS AND METHODS

This atlas contains 38 wood species that mainly originate from Southeast Asia (see Table 1). We selected commonly traded timbers, widely used plantation species, and species from endangered habitats. However, it should be stressed that the selection is far from comprehensive, and that other tree species than those treated here may be present in pulp and paper or MDF from Asia.

Authenticated specimens of the wood collection of the Thünen Institute (RBHw, Hamburg, Germany) served as reference material. The descriptions are generally based on one specimen per species only, but features were verified with slides and literature data from the databases in Hamburg and InsideWood (2004-onwards).

The selection of the material and the method (maceration according to Franklin, 1945) are also described by Helmling *et al*. (2016). Microscopy and staining are included.

The botanical names were taken from:
Catalogue of life (http://www.catalogueoflife.org/col/search).
Flora of China (www.efloras.org).
Grin (https://npgsweb.ars-grin.gov).
Mabberley (2008).

The trade names were taken from:
Brink M & Escobin RP (2003).
Flora of China (www.efloras.org).
Hayashi (1973), Wong (2002), and Jiang (2013).
Lemmens RHMJ, Soerianegara I & Wong WC (1995).
Richter HG & Dallwitz MJ (2000 onwards).
Soerianegara I & Lemmens RHMJ (1993).
Sosef MSM, Hong LT & Prawirohatmodjo S (1998).

The codes in the taxon descriptions refer to DIN EN 13556:2003.

The information on geographic distribution of the wood species was extracted from:
Catalogue of life (http://www.catalogueoflife.org/col/search).
Grin (https://npgsweb.ars-grin.gov).
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Richter HG & Dallwitz MJ (2000 onwards).
Soerianegara I & Lemmens RHMJ (1993).
Sosef MSM, Hong LT & Prawirohatmodjo S (1998).

The investigated characters of all species were compared with data in literature:
Brink M & Escobin RP (2003).
InsideWood (http://insidewood.lib.ncsu.edu/search).
Lemmens RHMJ, Soerianegara I & Wong WC (1995).
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Table 1. List of investigated species in alphabetical order of families (RBHw = vouchered material, ∗ = commercial sample – genus verified).

Family	Taxa / species	Trade name	RBHw No.
Acanthaceae	*Avicennia marina* (Forssk.) Vierh.	Api-Api (mangrove)	24226
Altingiaceae	*Liquidambar formosana* Hance	Formosan sweet gum	23479
Anacardiaceae	*Campnosperma* sp.	Terentang	∗
Anacardiaceae	*Gluta renghas* L.	Rengas	∗
Anacardiaceae	*Mangifera* sp.	Mango	∗
Anacardiaceae	*Swintonia* sp.	Merpauh	∗
Aquifoliaceae	*Ilex triflora* var. *kanehirai* (Yamamoto) S.Y. Hu	Kecemang	10148
Arecaceae	*Cocos nucifera* L.	Coconut palm	∗
Burseraceae	*Canarium* sp.	Kedondong	∗
Calophyllaceae	*Calophyllum* sp.	Bintangor	∗
Celastraceae	*Lophopetalum* sp.	Perupok	∗
Dipterocarpaceae	*Shorea* subg. *Anthoshorea*	White Meranti	∗
Dipterocarpaceae	*Shorea* subg. *Richetia*	Yellow Meranti	∗
Dipterocarpaceae	*Shorea* subg. *Rubroshorea*	Dark / Light Red Meranti	∗
Dipterocarpaceae	*Shorea* subg. *Shorea*	Bangkirai, Balau	∗
Dipterocarpaceae	*Parashorea* sp.	Gerutu	∗
Dipterocarpaceae	*Dipterocarpus* sp.	Keruing	∗
Euphorbiaceae	*Hevea brasiliensis* (Willd. *ex* A.Juss.) Müll. Arg.	Rubberwood	9880
Fabaceae-Caesalpinioideae	*Intsia* sp.	Merbau	∗
Fabaceae-Caesalpinioideae	*Koompassia malaccensis* Maingay *ex* Benth.	Kempas	∗
Fabaceae-Mimosoideae	*Acacia mangium* Willd.	Acacia	∗
Fabaceae-Mimosoideae	*Albizia procera* (Roxb.) Benth.	White siris, Kokko	193
Fagaceae	*Castanopsis argentea* (Blume) A. DC.	Berangan	18490
Lauraceae	*Litsea resinosa* Blume	Medang	15426
Malvaceae	*Durio* sp.	Durian	∗
Malvaceae	*Heritiera* sp.	Mengkulang	∗
Myrtaceae	*Eucalyptus globulus* Labill.	Eucalyptus	∗
Myrtaceae	*Syzygium dyerianum* (King) Chantaran. & J. Parn.	Kelat	3854
Nyssaceae	*Nyssa javanica* (Blume) Wangerin	Tupelo, Nyssa	13989
Paulowniaceae	*Paulownia tomentosa* (Thunb.) Steud.	Paulownia	5531
Poaceae	*Dendrocalamus latiflorus* Munro	Bamboo	22667
Rhizophoraceae	*Rhizophora* sp.	Red Mangrove	24661
Sapotaceae	*Madhuca sericea* (Miq.) S. Moore	Bitis	∗
Sapotaceae	*Palaquium* sp.	Nyatoh	∗
Styracaceae	*Alniphyllum pterospermum* Matsum.	Mee Dong	10151
Tetrameristaceae	*Tetramerista glabra* Miq.	Punah	∗
Theaceae	*Schima superba* Gardn. & Champ.	Samak, Puspa	24166
Thymelaeaceae	*Gonystylus* sp.	Ramin	∗

INTRODUCTION TO KEY FEATURES

The following section presents the main characters used in the descriptions of the individual wood species.

Shape of the vessel elements

The vessel elements are basically tubes that can be divided into three types. The shape of the vessel body (not considering the tails) resembles that of a "drum", a "barrel", or a "tube", terms used in the descriptions of the wood species. Some species possess vessel elements of one type, others vessel elements of various types.

If the ratio of length to width is ≤ 1 the vessel element is designated drum-shaped (Fig. 1A).

If the ratio ranges from 1 to 2, the vessel element is designated barrel-shaped (Fig. 1B).

If the ratio is ≥ 2, the vessel element is designated tube-shaped (Fig. 1C).

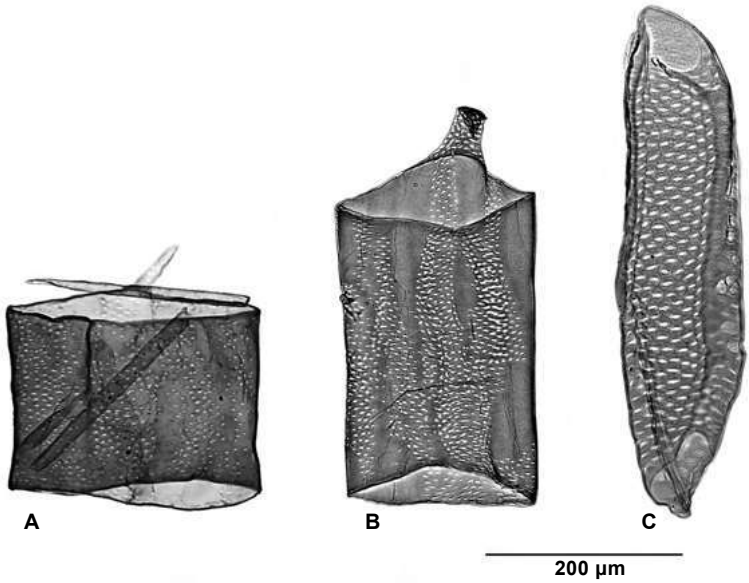


Figure 1. Shape of the vessel elements.
A: drum-shaped (*Albizia procera*, ratio length/width = 0.8. – B: barrel-shaped (*Acacia mangium*, ratio l/w = 1.7. – C: tube-shaped (*Swintonia* sp., ratio l/w = 3.5).

Tails

The vessel elements frequently possess tails at the top and/or the bottom. The transition from the more or less cylindrical vessel element body to the tail can be abrupt (Fig. 2A) or gradual (Fig. 2B).

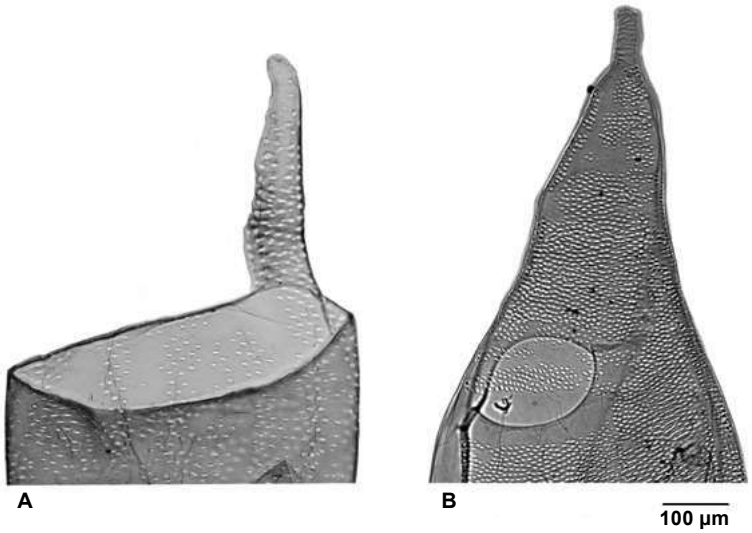


Figure 2. Tails.
A: with abrupt transition, *Albizia procera*. – B: with gradual transition and laterally positioned perforation plate, *Lophopetalum* sp.

Perforation plates

Perforation plates are mainly located at both ends of the vessel elements, sometimes positioned laterally (Fig. 2B).

The perforation plates can be simple (a perforation with a single circular or elliptical opening, Fig. 3A, C and D), or scalariform (a perforation plate with parallel openings separated by one to many mainly unbranched bars, Fig. 3B). The opening can comprise the entire lumen (Fig. 3A–C) or can be narrowed (of a lesser diameter, adapted to the vessel end taper, Fig. 3D).

Perforations can be horizontal (extending over the entire diameter and more or less perpendicular to the longitudinal axis (Fig. 3A) or inclined and elongated (Fig. 3E and F). The shape of vessel elements with inclined endings reminds of a parallelogram (inclination at vessel tips parallel, Fig. 3E) or a trapezium (inclination of vessel ends in opposite directions, Fig. 3F). Both conditions often occur within the same species.

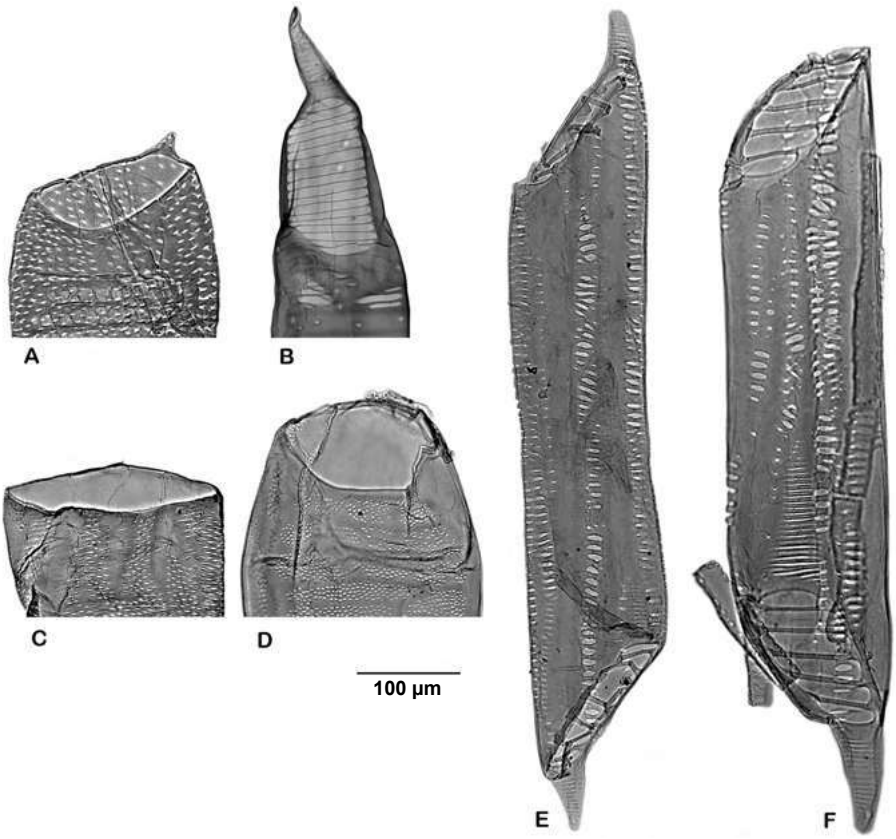


Figure 3. Perforation plates as defined in this atlas.
A: simple: *Eucalyptus globulus*. – B: scalariform: *Liquidambar formosana*. – C: opening horizontal, extending over the entire lumen: *Acacia mangium*. – D: narrowed: *Lophopetalum* sp. – E: inclined, parallelogram: *Rhizophora* sp. – F: inclined, trapezium: *Rhizophora* sp.

Pit types

The different pits constitute an important feature for wood identification. The fiber atlas of Ilvessalo-Pfäffli (1995) includes descriptions of the different pit types and their arrangements on vessel elements. It should be noted that the pit types are sometimes difficult to distinguish.

Intervessel pits are the connections between adjacent vessel elements. They can be recognized due to their arrangement in large fields. The arrangement can be alternate (arranged in diagonal rows), opposite (arranged in short to long horizontal rows, *i.e.*, rows orientated transversely across the length of the vessel) or scalariform (elongated or linear intervessel pits arranged in a ladder-like series, Fig. 4; IAWA Committee 1989). Within one species they have the same size range, a combination of features important for the identification of the timbers. The pit apertures can be circular to oval (Fig. 5A), slit-like (Fig. 5B) or coalescent (Fig. 5C, connecting several pits across their borders).

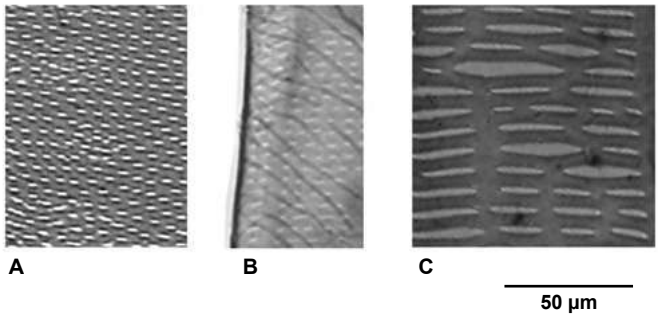


Figure 4. Arrangement of intervessel pits.
A: alternate, *Lophopetalum* sp. – B: opposite, *Ilex triflora*. – C: scalariform, *Nyssa javanica*.

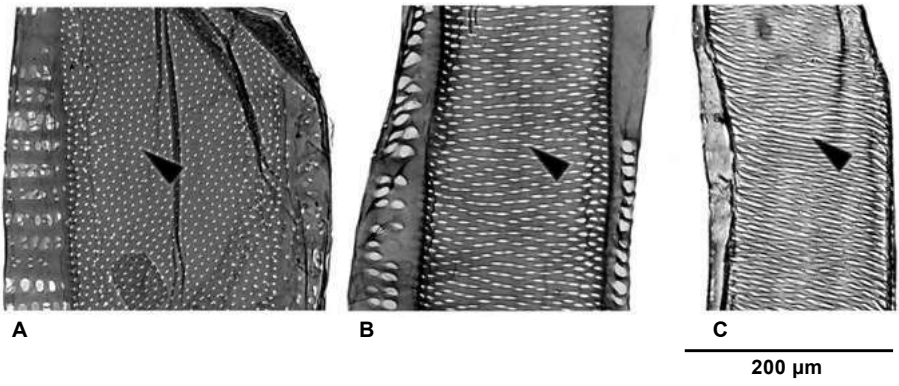


Figure 5. Intervessel pit apertures.
A: circular to oval, *Syzygium dyerianum*. – B: slit-like, *Canarium* sp. – C: coalescent, *Hevea brasiliensis*.

Vessel-ray pits connect vessel elements with ray parenchyma cells. They are arranged in horizontal rows, often several rows per ray cell. Vessel-ray pits are either similar in size and shape to intervessel pits or different. The species group possessing similar pits is denominated **APS** (**A**ll **P**its **S**imilar in size and shape) (see p. 272, Hardwoods with simple perforations).

The group of species with vessel-ray pits differing in appearance and morphology from intervessel pits is denominated VAS in this atlas (see p. 296 in the introductory comments to the species group **VAS** (**V**essel-ray pits **A**pparently **S**imple)). They have much reduced borders, the apertures are elongated or window-like, small or large, circular or angular, extended horizontally ('gash-like') or vertically ('palisade'). Various combinations of vessel-ray pit features may occur within one species (Fig. 6 A–C).

Pits to axial parenchyma cells are the connections between vessel elements and contact axial parenchyma cells. They usually resemble vessel-ray parenchyma pits and often form brick-like pit fields reflecting the outline of the respective parenchyma cells (Fig. 6 A and D).

Pits to fibers are the connection between vessel elements and fibers. They are arranged in single vertical rows (Fig. 6 A) and are rarely present.

Pits to tracheids connect vessel elements with tracheids. They are usually similar to intervessel pits in size and shape and are arranged in one to several, sinuous or straight vertical strips (Fig. 6 A and C).

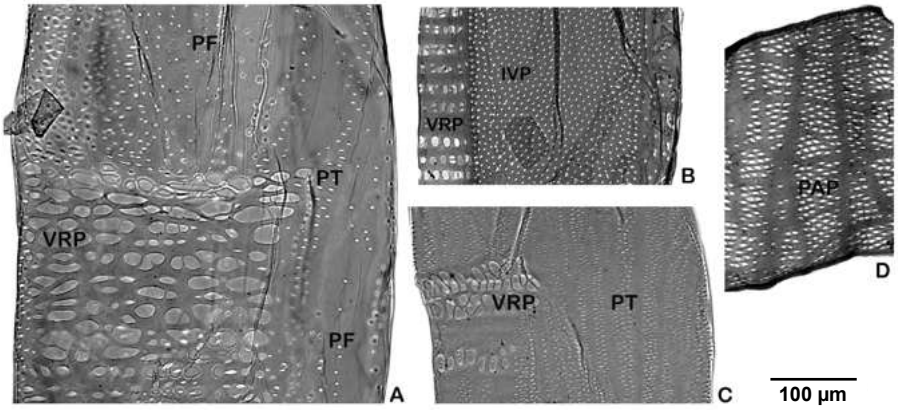


Figure 6. Pit type.
Vessel-ray pits (VRP), pits to axial parenchyma cells (PAP), pits to fibers (PF), pits to tracheids (PT), intervessel pits (IVP).
A: *Dipterocarpus* sp. – B: *Syzygium dyerianum*. – C: *Calophyllum* sp. – D: *Paulownia tomentosa*.

Areas without any pits

Some vessel elements possess very large (Fig. 7A) or large areas without any pits (Fig. 7, arrow in B), some have small areas (Fig. 7, arrowhead in B). Areas devoid of pits may also be absent (Fig. 7C).

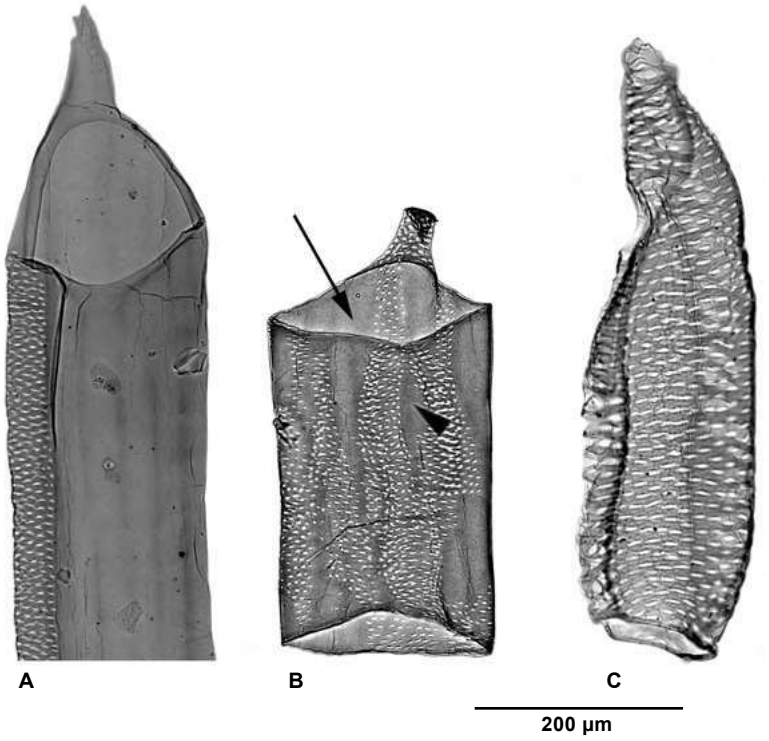


Figure 7. Areas without any pits.
A: very large, *Campnosperma* sp. – B: large (arrow), small (arrowhead), *Acacia* sp. – C: absent, *Swintonia* sp.

Tyloses

Certain wood species generate tyloses in the heartwood (Fig. 8A), less frequently also in the sapwood. Tyloses are outgrowths from an adjacent ray or axial parenchyma cell through a pit in a vessel wall, partially or completely blocking the vessel lumen. They constitute a feature which should only be used in a positive sense, *i.e.* if present. If they are absent the material studied may consist entirely of sapwood of a species capable of forming tyloses in the heartwood, or else the species in question does not produce tyloses. It is also possible that tyloses may be removed from the vessel elements during the pulping process.

Certain wood species can also contain tyloses due to compartmentalisation of damage in the tree after wounding (Schmitt & Liese 1994 and Liese 1995; Dujesiefken & Liese 2008) when normal wood does not form tyloses. Normal sapwood may also contain tyloses in embolized vessels (De Micco *et al*. 2016).

Helical thickenings

Some wood species possess helical thickenings (ridges on the inner face of the vessel element wall in a roughly helical pattern) throughout the vessels or restricted to the tails (IAWA Committee 1989). Helical thickenings are not removed during pulping (Fig. 8B and C).

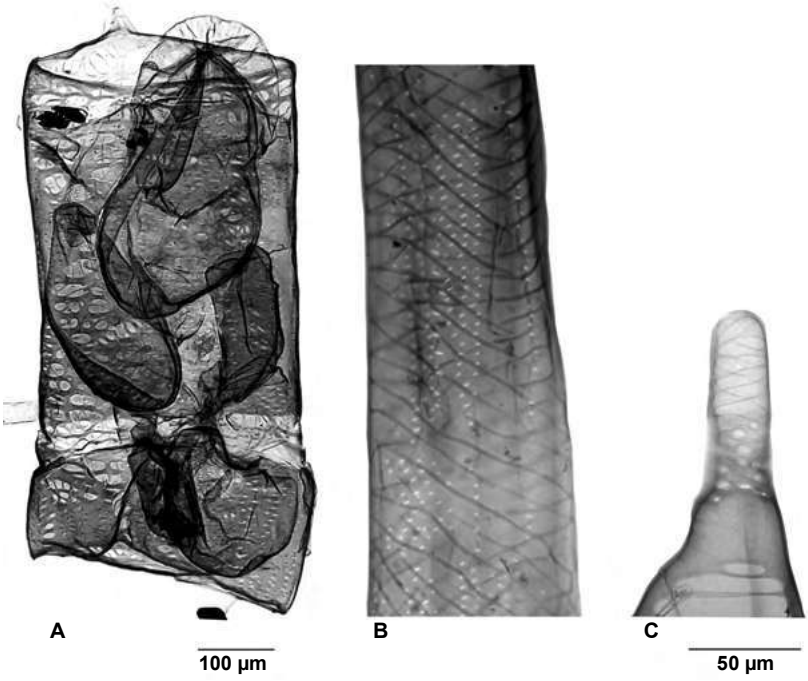


Figure 8. Tyloses and helical thickenings.
A: tyloses, *Castanopsis* sp. – B: helical thickenings in vessel elements, *Ilex triflora* var. *kanehirai*. – C: helical thickenings in vessel element tails, *Liquidambar formosana*; scale 50 µm is valid for B and C.

Dimensions of vessel elements

The dimensions of the vessel elements were determined as illustrated in Figure 9 (software: cellSens, Olympus Soft Imaging Solutions GmbH, Münster, Germany). At least 36 vessel elements of each species were measured (length, width [µm]).

Element length was measured excluding the tails, according to conventions maintained in Hamburg, despite international recommendations to include the tails in the length measurements (IAWA Committee 1989). In pulp, we often found the tails to be damaged. Vessel width in macerations probably corresponds most closely to radial vessel diameter. Note that our quantitative data for vessel width are often substantially higher than vessel diameters reported in the wood anatomical literature. This may be due to the flattening of macerated vessels in microscopic slides. Furthermore, the vertical dimensions [µm] of pit apertures and borders were determined.

Vessel and pit dimensions are helpful when identifying an unknown species. They must be seen as guidelines, not as absolute values due to the variability of the species. Please note that the vertical dimensions of the pit borders may deviate from horizontal diameters given in most wood anatomical descriptions in the literature (of alternate pits only; cf. IAWA Committee 1989 and InsideWood 2004-onwards).

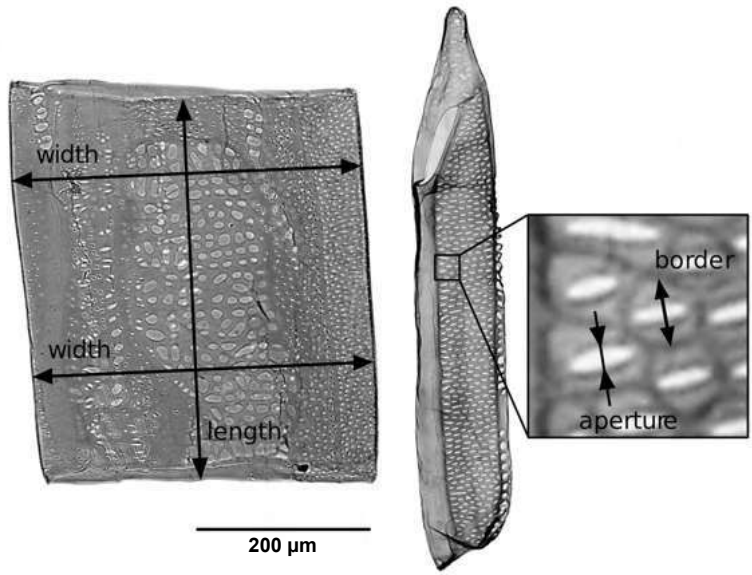


Figure 9. Measurement of vessel elements (length, width, vertical intervessel pit aperture and pit border size [µm]).

Fiber dimensions

The dimensions of the pulp fibers of Hardwoods were characterized morphologically with a Kajaani FiberLab® fiber analyzer. The fiber analyzer determines fiber length, fiber curl, fiber kinks, fibrillation, fractionation, and other parameters. The three important fiber characteristics length and width as well as cell wall thickness are presented in this atlas. All dimensions represent the weighted average (not the arithmetic average that is also calculated) because cell fragments, parenchyma cells and vessel elements in the pulp influence the measurement results. The length weighted average tends to reduce this influence (Nygård 2006).

Arithmetic average fiber length [mm]: $$L(n) = \frac{\sum (n_i \cdot l_i)}{\sum n_i}$$

Length weighted fiber length [mm]: $$L(l) = \frac{\sum (n_i \cdot l_i^2)}{\sum n_i l_i}$$

l_i = average length in class i (i = 1 ... 152)
n_i = number of fibers in class i

Since for *Dendrocalamus latiflorus* (Bamboo) the data obtained with the fiber analyzer were much lower than what we observed in the microscopes, the algorithm for length weighted average of fiber length apparently does not sufficiently reduce the influence of parenchyma cells and cell fragments. Therefore the length of fibers of Monocots was determined by measuring 90 fibers in the light microscope (software: cellSens, Olympus Soft Imaging Solutions GmbH, Münster, Germany).

DESCRIPTIONS OF WOODY ANGIOSPERMS OF TROPICAL AND TEMPERATE ASIA

The following pages contain the descriptions and accompanying images of 38 species. For quicker orientation, the taxa are roughly divided into three groups and two subgroups in the following order:

Monocots: without vessel-ray pits; with axially oriented pit fields and lines void of pits (p. 267)

Hardwood species with (mainly) simple perforation plates (p. 272)
APS: **A**ll **P**its **S**imilar (in size and shape) (p. 272)
VAS: **V**essel-ray pits **A**pparently **S**imple (p. 296)

Hardwood species with exclusively scalariform perforation plates (p. 336)

The descriptions start with two monocots, Bamboo and Coconut palm, followed by the group of Hardwoods with simple perforations and with two subgroups:

"APS" (All Pits Similar). Species summarized in this subgroup possess the common feature of vessel-ray and vessel-axial parenchyma pits similar to intervessel pits. The similarity refers to the size and shape of the pits.

"VAS" (Vessel-ray pits Apparently Simple). The pits of vessel elements connecting to parenchyma cells – like vessel-ray pits or pits to axial parenchyma cells – are characterized by large apertures and their borders are much reduced and not or hardly visible under the light microscope. They differ in size and shape from the intervessel pits or pits to tracheids or fibers.

The second Hardwood group contains wood species with all perforation plates scalariform. It is also possible to distinguish these species into APS and VAS but since there are only six species in this group, such a fine subdivision was deemed unnecessary.

At the beginning of each chapter of the groups and subgroups introductory comments are given about the main features characteristic of the different wood species in these groups.

Monocots
introductory comments

This chapter contains two monocots: *Dendrocalamus latiflorus* (Bamboo) and *Cocos nucifera* (Coconut palm). They are included in this atlas because the material is expected to appear in paper products as an easily available resource from plantations.

As *Dendrocalamus latiflorus* is a grass and not a tree species it produces only a primary shoot without secondary growth (Liese 1998). There are no rays so the vascular bundles do not possess any vessel-ray pits. The vessel pits existing in vascular bundles connect with parenchyma cells or fibers.

Cocos nucifera is, like bamboo, a monocot. There is no secondary growth. The vascular bundles include very long vessel elements. They are by far the longest included in this atlas (Fig. 10a). The vessel pits present in the vascular bundles connect with adjacent parenchyma cells or fibers or with other metaxylem vessels.

Figure 10 shows the variation of vessel element length and width of the two monocots as well as the diameter of the apertures and borders of intervessel pits. The length of the vessel elements shows a significant difference whereas the width is similar and overlapping.

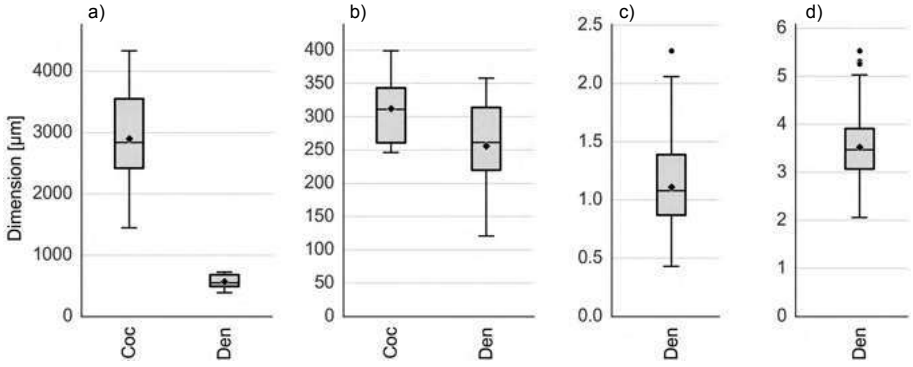
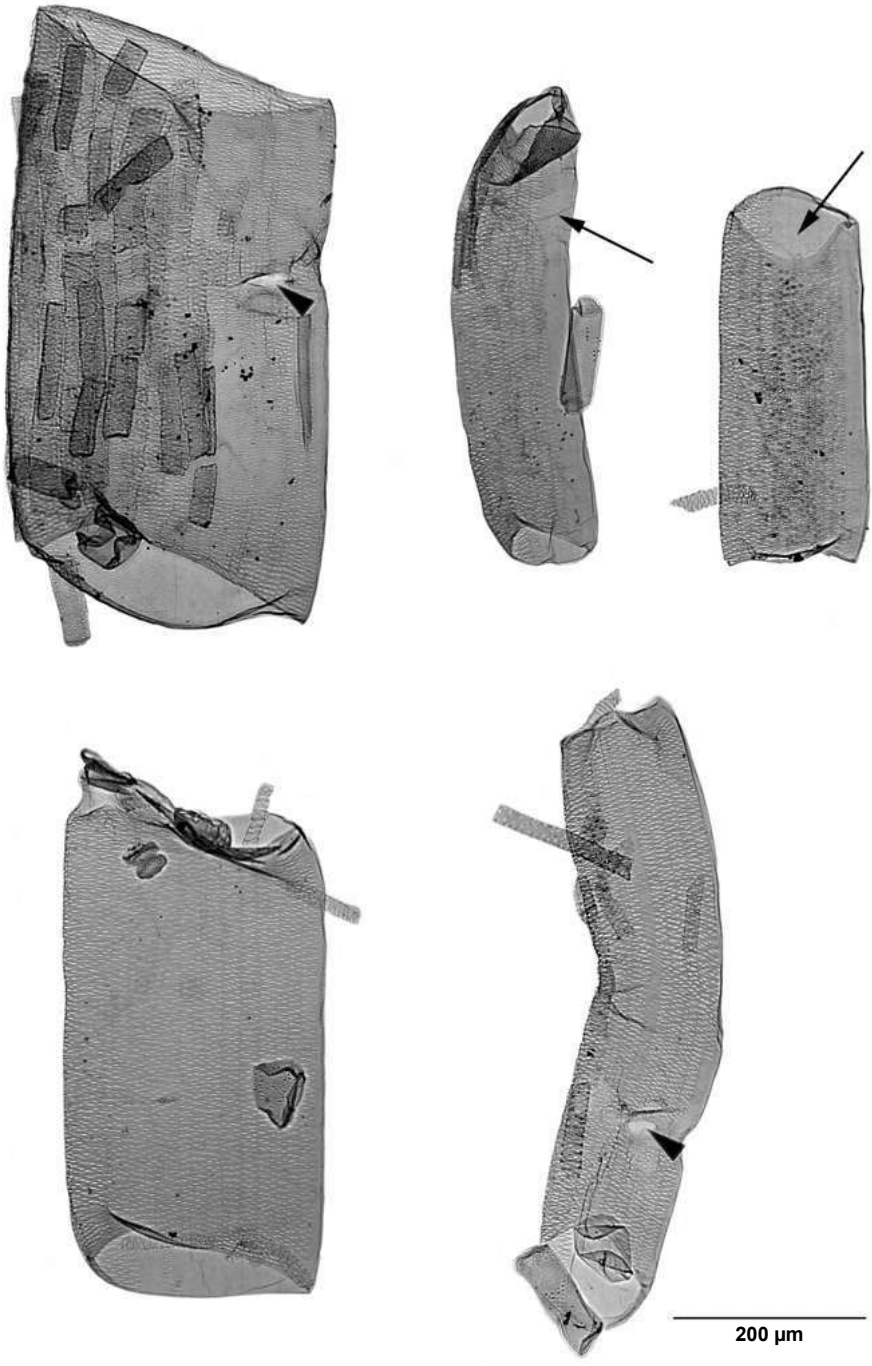


Figure 10. Boxplots of *Cocos nucifera* (Coc) and *Dendrocalamus latiflorus* (Den).
a) length and b) width of the vessel elements, c) diameter of pit apertures and d) borders; aperture and border sizes of the pits of *Cocos nucifera* are missing in this figure because scalariform pits can be deformed in macerations or pulp (p. 271).



Dendrocalamus latiflorus

Dendrocalamus latiflorus Munro (Poaceae)

Trade names: bamboo, Taiwan giant bamboo.
DIN EN 13556 : 2003 code: not listed.
CITES regulations: not protected.
Geographic distribution: from Myanmar to Southern China and Taiwan; cultivated in India, Thailand, Vietnam, Philippines and Japan.

Dendrocalamus latiflorus, a monocot; culms consist of parenchyma cells (about 50 %), fibers (about 40 %) and conducting tissue (about 10 %, vessel elements included) (Liese 1997).

Vessel elements: rather long (about 550 μm), of average width (about 260 μm), either tube-shaped (those with a smaller diameter) or barrel-shaped (those with a larger diameter).

Tails: absent.

Perforation plates: simple, extending over the entire lumen; slightly inclined or horizontal.

Pits to parenchyma cells: alternate; 2–4–6 μm; present in large fields over a wide area; apertures slit-like.

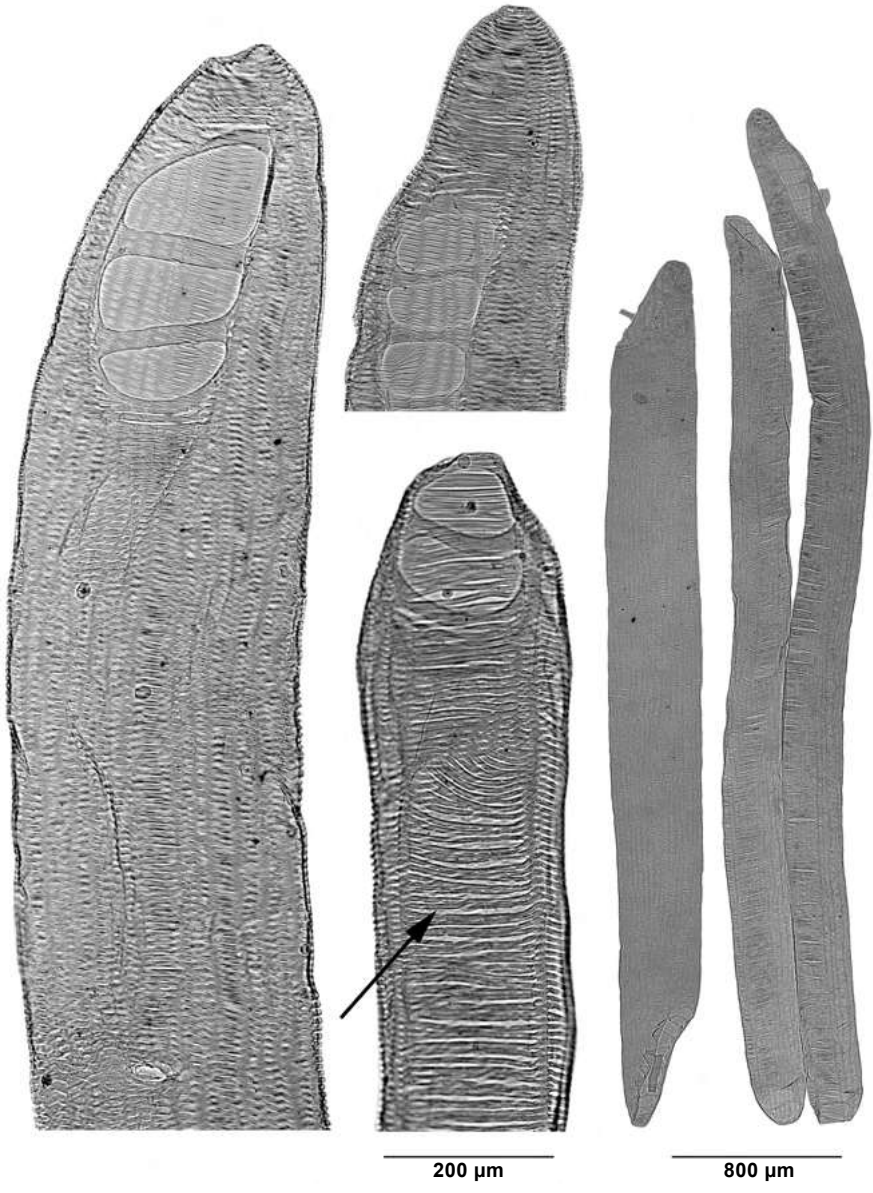
Areas without any pits: in the cell wall areas that border fiber sheaths, oriented vertically (arrows).

Tyloses: present.

Helical thickenings: absent.

Notes on identification: Sometimes the vessel elements of *Dendrocalamus* spp. reveal small lateral holes due to vessel branching at the internodes (arrowheads). The vessel elements possess a similar appearance as those of wheat, corn and sugar cane with the maximum vessel element length of wheat: 1.0 mm; corn: 0.6 mm; sugar cane: 2.1 mm (according to Ilvessalo-Pfäffli 1995).

Quantitative data:
Vessel elements (388–)546(–723) μm long, and (121–)261(–358) μm wide; l/w ratio 2.3.
Fibers (487–)1262(–2575) μm long. Fiber diameter and fiber wall thickness very variable.



Cocos nucifera

Cocos nucifera L. (Arecaceae)

Trade name: coconut palm.
DIN EN 13556:2003 code: not listed.
CITES regulations: not protected.
Geographic distribution: Tropics worldwide. Widely cultivated.

Cocos nucifera, a palm tree, is a monocot. The stems are built up of vascular bundles (vessels, fibers, parenchyma, phloem) embedded in ground tissue parenchyma.

Vessel elements: very long (about 2840 μm, width about 310 μm), much longer than vessel elements in dicotyledons, tube-shaped.

Tails: absent.

Perforation plates: scalariform with few and very thick bars (2–4 bars), inclined.

Intervessel pits: scalariform (arrow), with slit-like apertures.

Pits to parenchyma cells: apertures oval to slit-like.

Areas without any pits: absent.

Tyloses and helical thickenings: absent.

Quantitative data:
Vessel elements (1447–)2840(–4334) μm long and (246–)311(–399) μm wide; l/w ratio 8.4 (contrary to the descriptions of the other species, the apertures and borders of the pits of *Cocos nucifera* are missing in the data because the apertures can be deformed in macerations or pulp).
Fibers (1151–)2646(–4358) μm long. Fiber diameter and fiber wall thickness very variable.

Hardwoods with simple perforations

"**APS**" (**A**ll **P**its **S**imilar in size and shape) – introductory comments

The eleven wood species described in the following chapter possess the common feature of similar pits. All pit types in the vessel walls are similar in size and shape. The vessel-ray and vessel-axial parenchyma pits have the same appearance and size as the intervessel pits but their grouping may be different. In many cases the vessel-ray pits can be detected due to their specific arrangement in horizontally oriented pit areas separated by pitless zones (due to cell walls perpendicular to the vessel wall), *e.g. Acacia* sp., while the intervessel pits are arranged in continuous fields or areas (Figure 11).

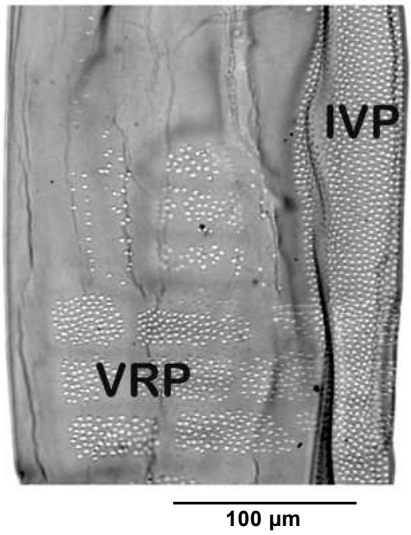


Figure 11. Similarity in size and shape of vessel-ray pits (VRP) and intervessel pits (IVP), *Lophopetalum* sp.

Figure 12 shows the variation of the vessel element parameters length and width as well as the diameter of the apertures and borders of intervessel pits. The boxplots show how variable the dimensions of the different species can be.

Tetramerista glabra possesses the longest vessel elements in this group. At the same time it reveals the smallest pit apertures. This is a useful combination well-suited for identification and illustrates the potential importance of the quantitative data. However, more specimens per species should be measured for using the quantitative features as robust diagnostic characters.

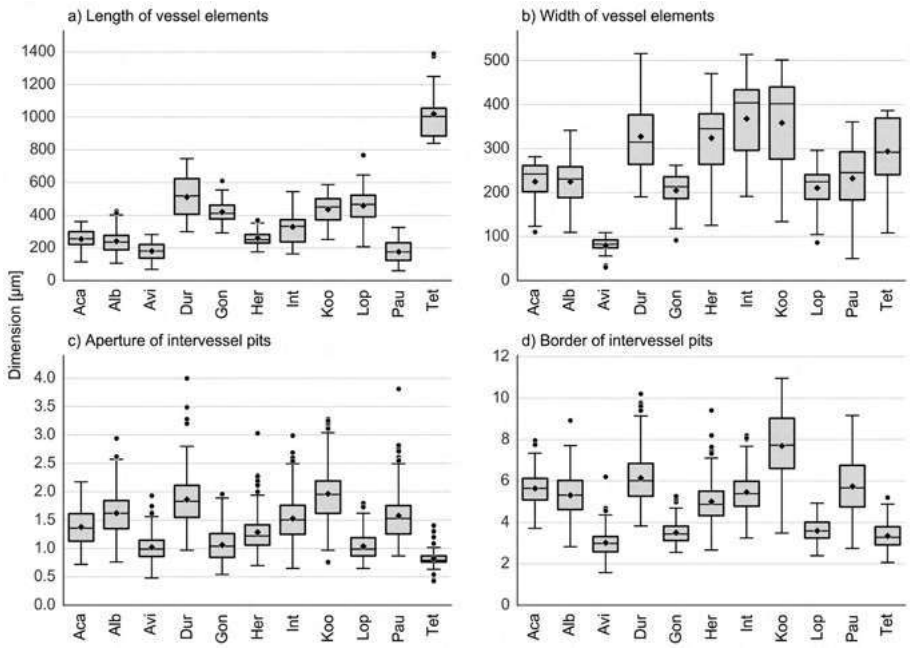
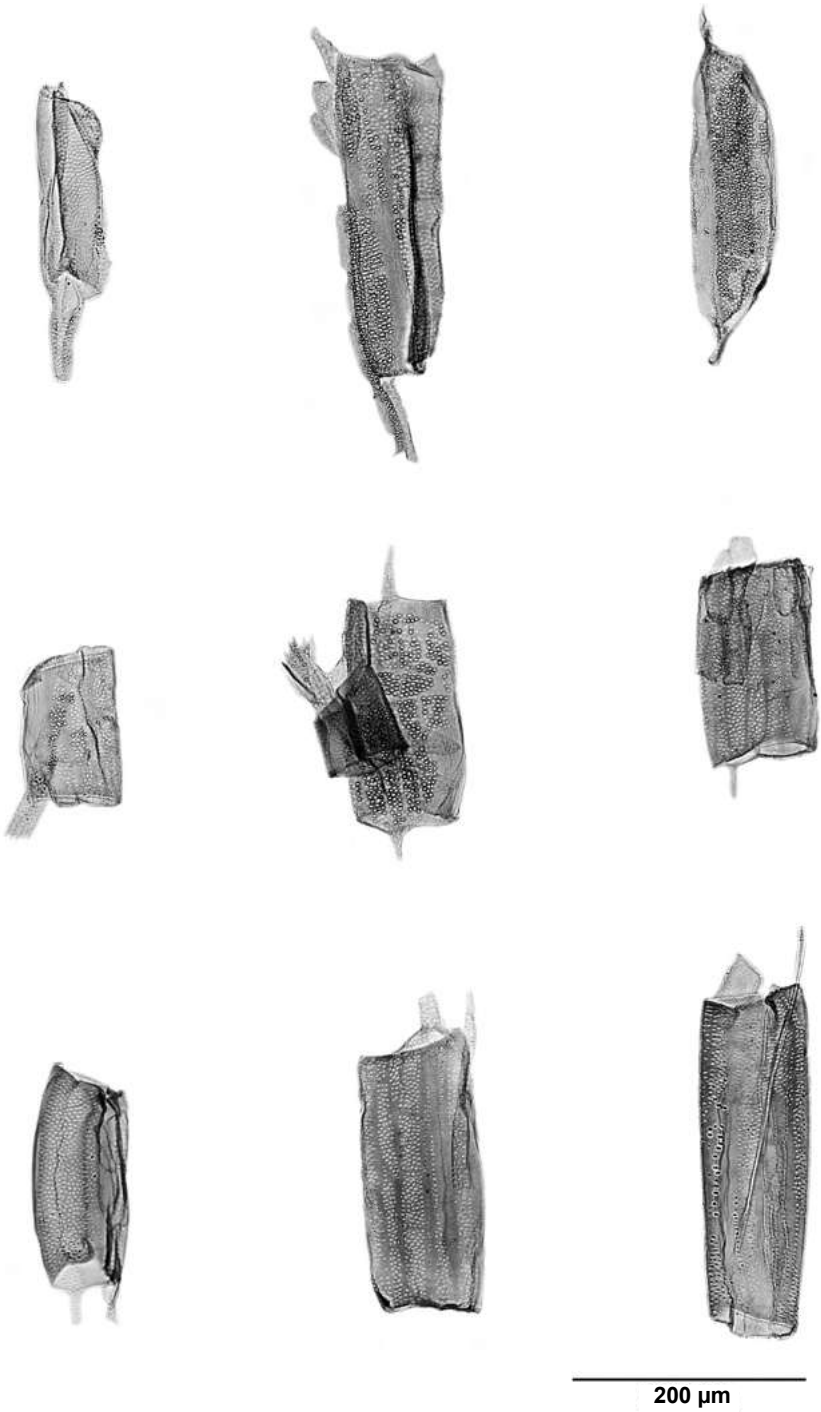


Figure 12. Boxplots of 11 wood genera/species: length and width of the vessel elements, diameter of pit apertures and borders.
Aca = *Acacia mangium*; Alb = *Albizia procera*; Avi = *Avicennia marina*; Dur = *Durio* sp.; Gon = *Gonystylus* sp.; Her = *Heritiera* sp.; Int = *Intsia* sp.; Koo = *Koompassia malaccensis*; Lop = *Lophopetalum* sp.; Pau = *Paulownia tomentosa*; Tet = *Tetramerista glabra*.



Avicennia marina

Avicennia marina (Forssk.) Vierh. (Acanthaceae)

Trade name: api-api.
DIN EN 13556:2003 code: not listed.
CITES regulations: not protected.
Geographic distribution: throughout the Tropics.

Avicennia is a genus of mangroves.

Vessel elements: very small (length about 180 μm, width 80 μm), mainly barrel-shaped.

Tails: often short with abrupt transition.

Perforation plates: simple, extending over the entire lumen; horizontal or inclined.

Intervessel pits: alternate; vertical diameter 2–3–6 μm; present over a wide area and in the tails.

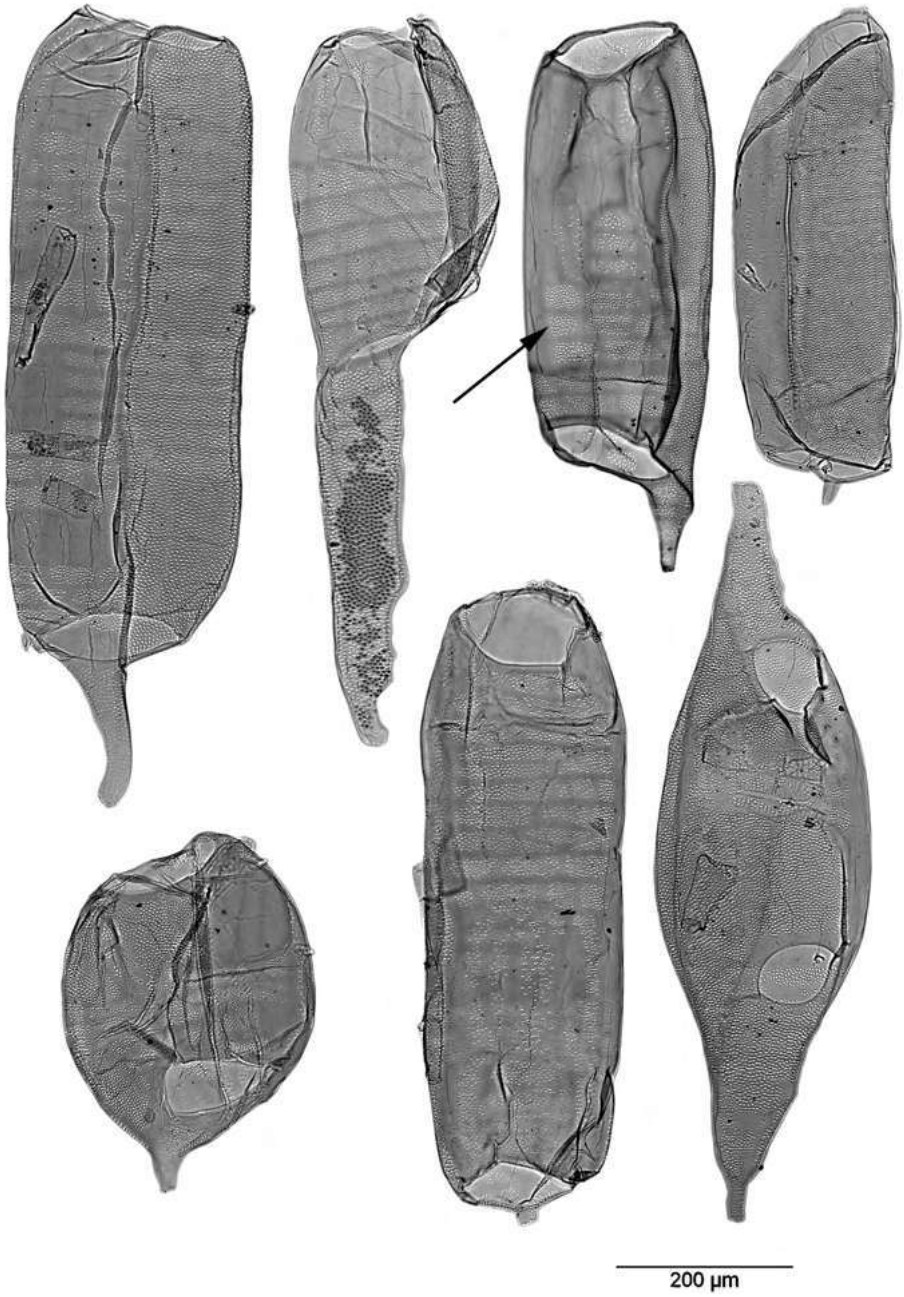
Vessel-ray pits: APS; rarely present; in horizontal rows (3–5 rows per ray cell); oval apertures.

Pits to axial parenchyma cells: numerous; in longitudinal bands or blocks comprising 3–5 rows of pits.

Areas without any pits: present, small.

Tyloses and helical thickenings: absent.

Quantitative data:
Vessel elements (69–)180(–282) μm long, and (30–)83(–109) μm wide; l/w ratio 2.3.
Intervessel pit borders (1.6–)3.0(–6.2) μm in vertical diameter; pit apertures (0.5–)1.0(–1.9) μm.
Fibers 810 μm long, 16.8 μm wide. Fiber wall thickness 6.3 μm (weighted averages).



Lophopetalum sp.

***Lophopetalum* sp.** (Celastraceae)

Trade names: perupok, perupuk (MY, ID); adau, dual (BN); medang kerupuk, pasana (ID); abuab (PH); taung-yemaré (MM); phuamphrao, dimi, samet-thung (TH).
DIN EN 13556 : 2003 code: not listed.
CITES regulations: not protected.
Geographic distribution: Eastern Pakistan, Western India, Indochina and throughout Malesia; main centers of endemism are Borneo and New Guinea.

Vessel elements: average length about 470 μm, width about 230 μm; mostly rather slim (tube-shaped), sometimes shorter and "drop-shaped".

Tails: short to very long, with abrupt or gradual transition.

Perforation plates: simple; in tapering end walls; sometimes positioned laterally.

Intervessel pits: alternate; vertical diameter 2–4–5 μm; present in large fields covering a wide area; apertures oval.

Vessel-ray pits: APS; often present, in long horizontal rows (6–8 rows per ray cell) separated by well-defined pitless zones (arrow).

Pits to axial parenchyma cells: present, arranged in sinuous vertical bands.

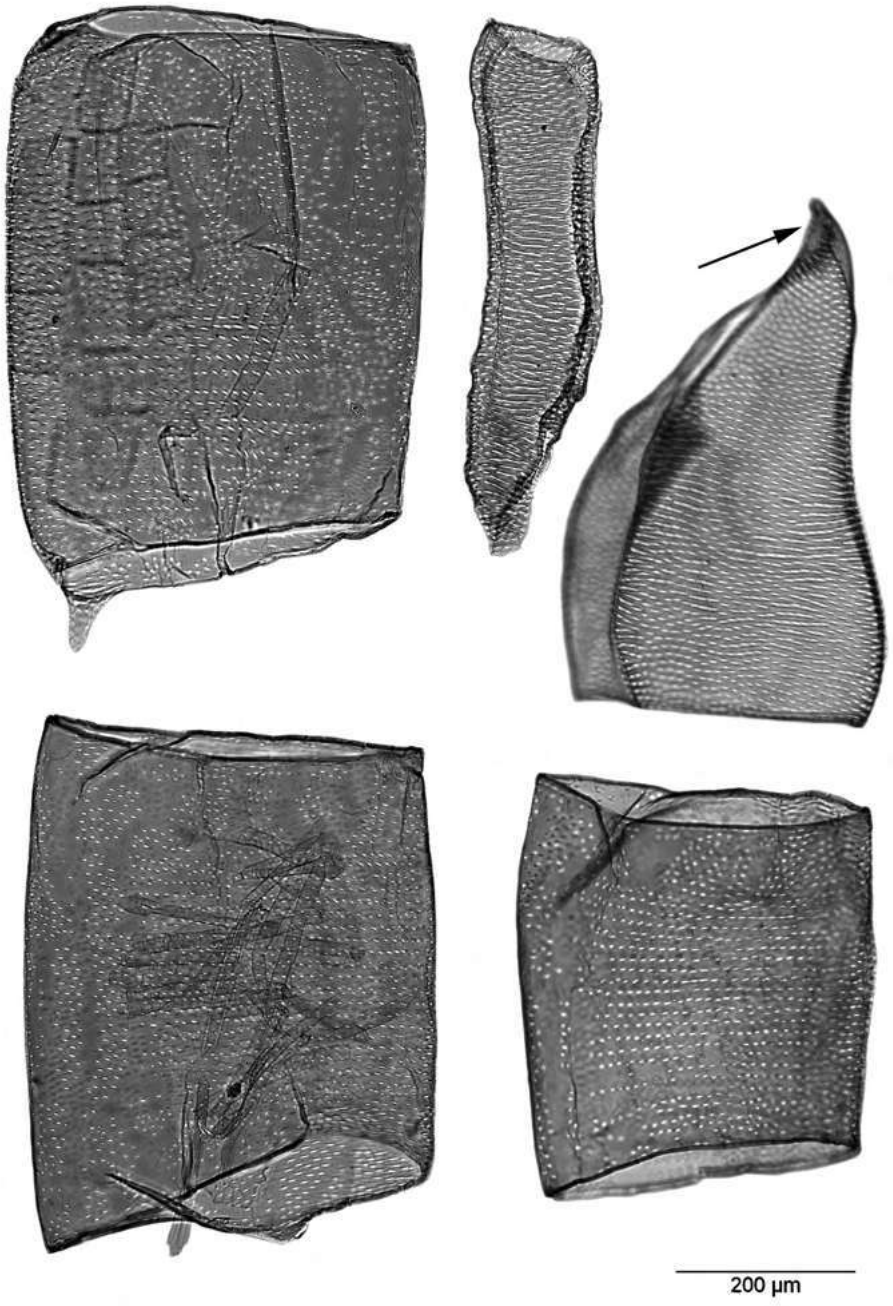
Pits to fibers: rarely present in single vertical rows.

Areas without any pits: sometimes present; large.

Tyloses and helical thickenings: absent.

Notes on identification: The vessel elements of *Lophopetalum* spp. are similar to those of *Gonystylus* spp. and *Durio* spp. (see *Gonystylus* sp., p. 294).

Quantitative data:
Vessel elements (207–)467(–767) μm long, and (87–)225(–296) μm wide; l/w ratio 2.2.
Intervessel pit borders (2.4–)3.6(–4.9) μm in vertical diameter; pit apertures (0.7–)1.0(–1.8) μm.
Fibers 980 μm long, 25.3 μm wide. Fiber wall thickness 7.2 μm (weighted averages).



Koompassia malaccensis

Koompassia malaccensis Maingay *ex* Benth. (Fabaceae–Caesalpinioideae)

Trade names: kempas (MY, ID, MY-swk, GB, NL); hampas, pah, mengris, impas, toemaling, garis, ajam (ID).
DIN EN 13556:2003 code: KOML.
CITES regulations: not protected.
Geographic distribution: Southern Thailand and Malesia (except Philippines).

Vessel elements: two size classes (length about 450 μm, width about 400 μm), barrel-shaped (those with a larger diameter) or tube-shaped (those with a smaller diameter).

Tails: small with abrupt transition, or absent.

Perforation plates: simple; extending over the entire lumen; horizontal, rarely inclined and pointed (arrow).

Intervessel pits: alternate; vertical diameter 4–8–11 μm; present in fields, sometimes over the whole area; apertures slit-like, sometimes coalescent.

Vessel-ray pits: APS; numerous, in horizontal pattern (1–2 rows per ray cell).

Pits to axial parenchyma cells: in vertically oriented blocks.

Areas without any pits: regularly present; small.

Tyloses and helical thickenings: absent.

Quantitative data:
Vessel elements (251–)450(–588) μm long, and (134–) 402(–502) μm wide; l/w ratio 1.2.
Intervessel pit borders (3.5–)7.7(–11.0) μm in vertical diameter; pit apertures (0.8–) 2.0(–3.3) μm.
Fibers 1310 μm long, 21.7 μm wide. Fiber wall thickness 4.9 μm (weighted averages).

Intsia sp.

***Intsia* sp.** (Fabaceae–Caesalpinioideae)

Trade names: merbau (MY); Malacca teak, mirabow, Moluccan ironwood (GB); ipil, kayu besi (ID); kwila, bendora (PG); ipil, ipil laut, malaipil (PH); tat-takun (MM); krakas prak (KH); lumpho, lumpho thale (TH); hintzy (MG).
DIN EN 13556-2003 code: INXX (main species are *I. bijuga* and *I. palembanica*).
CITES regulations: not protected.
Geographic distribution: Indochina, Indomalesia, Pacific Islands, Australia, Madagascar, East Africa.

Vessel elements: rather short, but wide (length about 330 μm, width about 400 μm); often drum-shaped (those with a larger diameter), complemented by some barrel-shaped elements (those with a smaller diameter).

Tails: rather short with abrupt transition.

Perforation plates: simple, extending over the entire lumen; rarely positioned laterally; horizontal.

Intervessel pits: alternate; vertical diameter 3–5–8 μm; present over a wide area and in the tails; apertures oval.

Vessel-ray pits: APS; in 3–5 horizontal rows.

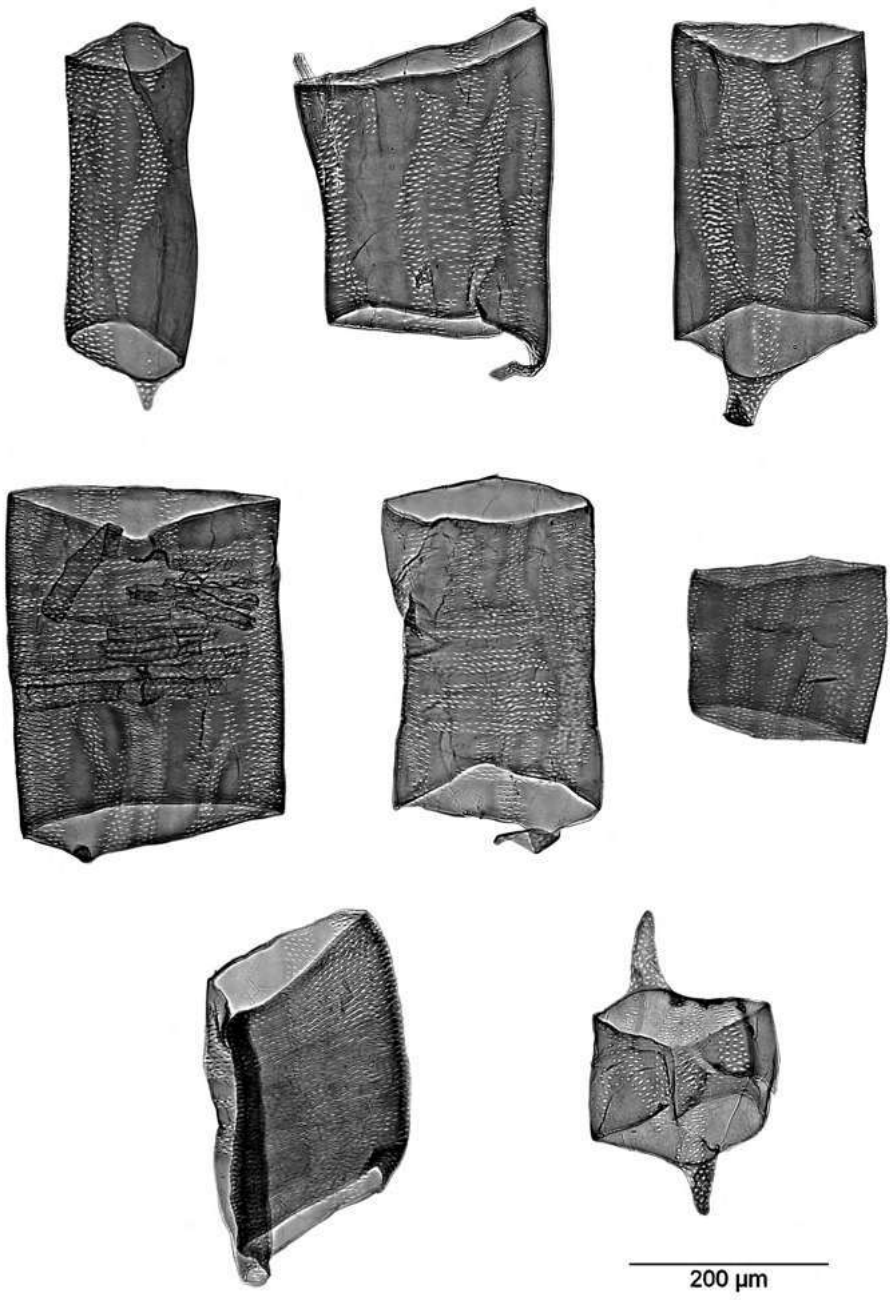
Pits to parenchyma cells: blocks arranged in sinuous longitudinal bands mirroring the parenchyma strands.

Areas without any pits: regularly present; small.

Tyloses and helical thickenings: absent.

Notes on identification: The intervessel pitting is similar to that of *Acacia* spp. but the vessel elements of *Intsia* spp. are larger in diameter.

Quantitative data:
Vessel elements (164–)333(–544) μm long, and (191–)405(–514) μm wide; l/w ratio 0.9.
Intervessel pit borders (3.2–)5.4(–8.2) μm in vertical diameter; pit apertures (0.7–)1.5(–3.0) μm.
Fibers 1590 μm long, 21.9 μm wide. Fiber wall thickness 5.3 μm (weighted averages).



Acacia mangium

Acacia mangium Willd. (Fabaceae–Mimosoideae)

Trade names: acasia, mangium (ID, MY).
DIN EN 13556:2003 code: not listed.
CITES regulations: not protected.
Geographic distribution: native to Australia and the Moluccan Islands; introduced in many countries, most notably in Asia (Bangladesh, China, Indonesia, Laos, Malaysia, Nepal, Philippines, Thailand, Vietnam) but also in Brazil, Cameroon, Costa Rica, Hawaii.

Acacia mangium plantations provide solid wood and short fiber raw material for the pulp and paper industry.

Vessel elements: rather small (length about 260 μm, width about 240 μm), often elongated (barrel-shaped), sometimes shorter (drum-shaped).

Tails: mostly short with abrupt transition.

Perforation plates: simple, extending over the entire lumen; horizontal or slightly inclined.

Intervessel pits: pits alternate; vertical diameter 4–6–8 μm; rather scarce; apertures oval to slit-like.

Vessel-ray pits: APS; arranged in 2–3 horizontal rows per ray cell, separated by spaces free of pits.

Pits to axial parenchyma: frequent blocks, arranged in sinuous longitudinal rows mirroring the parenchyma strands.

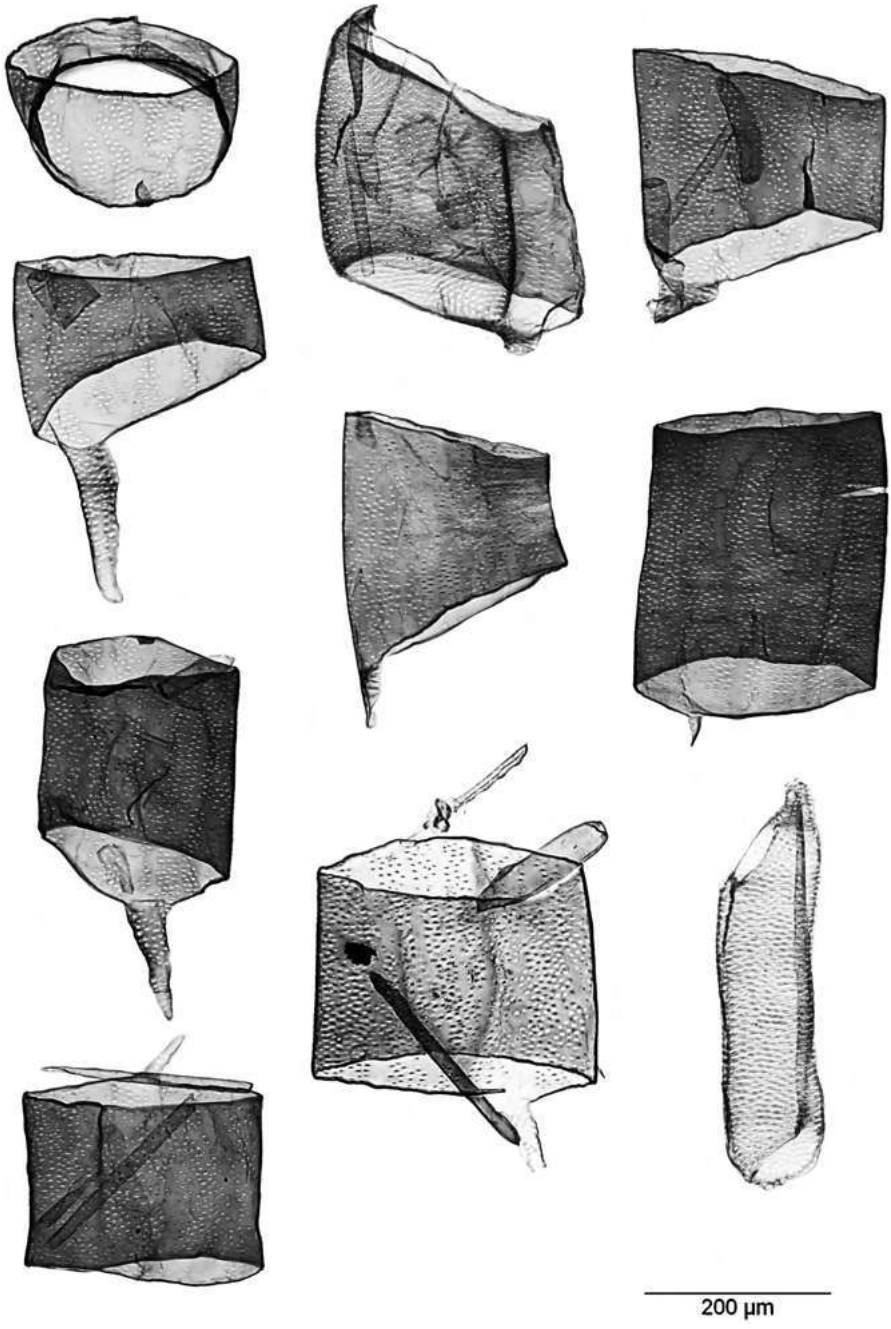
Areas without any pits: regularly present; small to large.

Tyloses and helical thickenings: absent.

Notes on identification: Vessel elements of *Acacia* spp. are similar in appearance to *Albizia* spp. (same family). The tails of *Acacia* spp. are shorter than those of *Albizia*.

The intervessel pits are rather scarce whereas the ones of *Albizia* spp. are regularly present.

Quantitative data:
Vessel elements (114–)256(–361) μm long, and (111–)243(–282) μm wide; l/w ratio 1.0.
Intervessel pit borders (3.7–)5.6(–8.0) μm in vertical diameter; pit apertures (0.7–)1.4(–2.2) μm.
Fibers 830 μm long, 17.9 μm wide. Fiber wall thickness 4.3 μm (weighted averages).



Albizia procera

Albizia procera (Roxb.) Benth. (Fabaceae–Mimosoideae)

Trade names: tall albizia (GB); ki hiyang, wangkal, weru (ID); brown albizia (PG); akleng parang (PH); kokko-sit, sit, sitpen (MM); suan, thing thon (TH); muong xanh (VN); karangro, karak, baro, dun-siris, gurar (IN); oriang (MY); seto siris (NP); tramkang (KH); rain siris (AU).
DIN EN 13556:2003 code: AZXX (*Albizia* spp.).
CITES regulations: not protected.
Geographic distribution: China, Indochina, Indomalesia, Pacific Islands, Australia.

Vessel elements: quite short (about 240 μm, width about 230 μm); mainly drum-shaped (with larger diameter), sometimes tube and barrel-shaped (with smaller diameter).

Tails: sometimes short to mostly rather long, with abrupt transition.

Perforation plates: simple, extending over the entire lumen; horizontal to slightly inclined.

Intervessel pits: alternate; vertical diameter 3–5–9 μm; arranged in large fields, also in the tails; apertures circular to oval.

Vessel-ray pits: APS; similar to intervessel pits.

Pits to axial parenchyma cells: present in blocks (2–4 rows of pits wide) forming vertical strips.

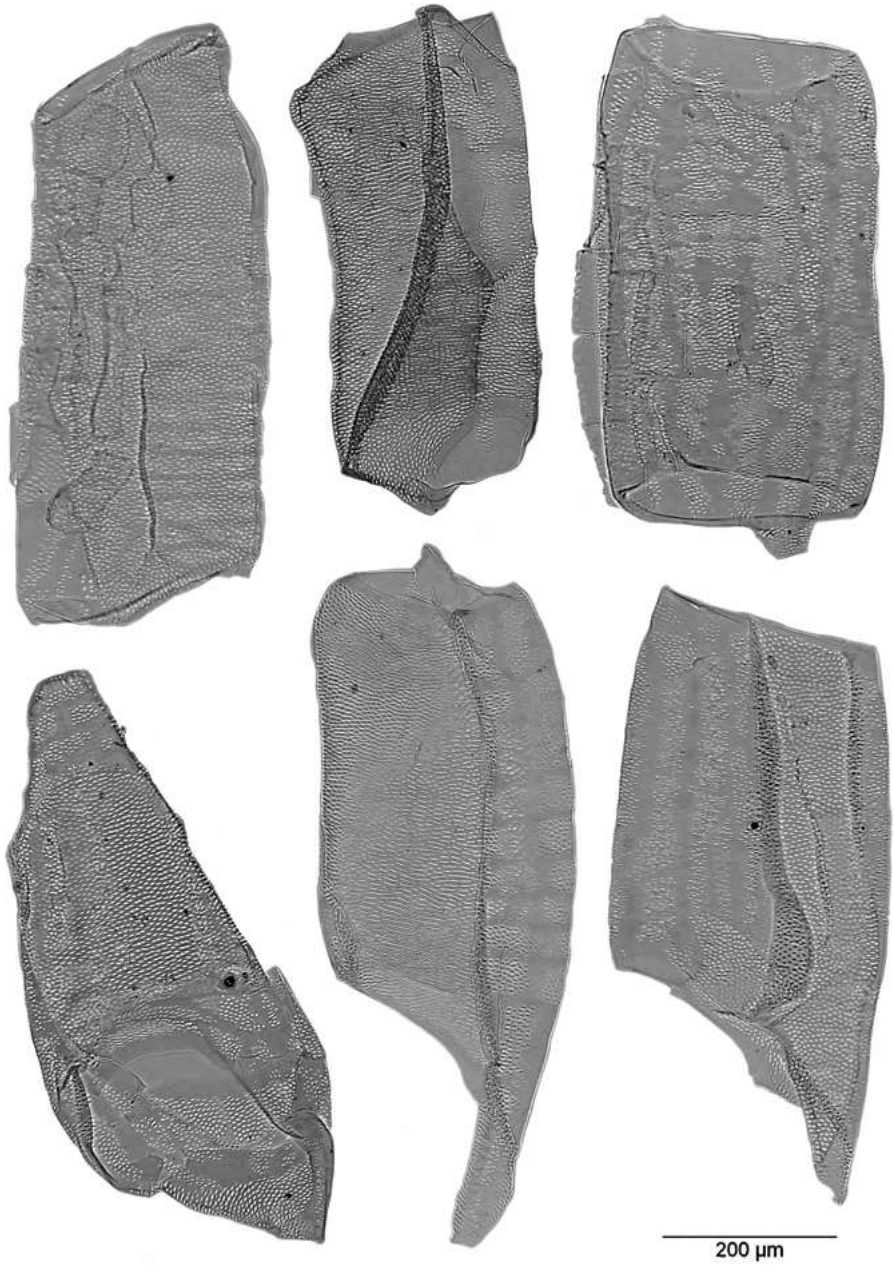
Pits to fibers: rarely present; arranged in single vertical rows; rather small.

Areas without any pits: regularly present; small.

Tyloses and helical thickenings: absent.

Notes on identification: Vessel elements of *Albizia* spp. are similar to those of *Acacia* spp. in their appearance (same family, p. 282).

Quantitative data:
Vessel elements (106–)235(–426) μm long, and (110–)231(–342) μm wide; l/w ratio 1.0.
Intervessel pit borders (2.8–)5.3(–8.9) μm in vertical diameter; pit apertures (0.8–)1.6(–2.9) μm.
Fibers 890 μm long, 23.2 μm wide. Fiber wall thickness 2.3 μm (weighted averages).



Durio sp.

***Durio* sp.** (Malvaceae)

Trade names: durian (DE, ID, MY); punggai, durian isa (MY).
DIN EN 13556:2003 code: DUXX.
CITES regulations: not protected.
Geographic distribution: Indochina, Indomalesia.

Durio spp. is cultivated as plantation tree for fruit production. The wood is used for furniture or ply-wood.

Vessel elements: rather large (length about 520 μm, width about 320 μm), mainly barrel-shaped.

Tails: short with abrupt transition, sometimes longer with gradual transition.

Perforation plates: simple, extending over the entire lumen; horizontal or inclined.

Intervessel pits: alternate; vertical diameter 4–6–10 μm; numerous; present over a wide area and in the tails; pit apertures oval to slit-like.

Vessel-ray pits: APS; cross-fields arranged in neat horizontal series with 3–6 rows of pits per ray cell.

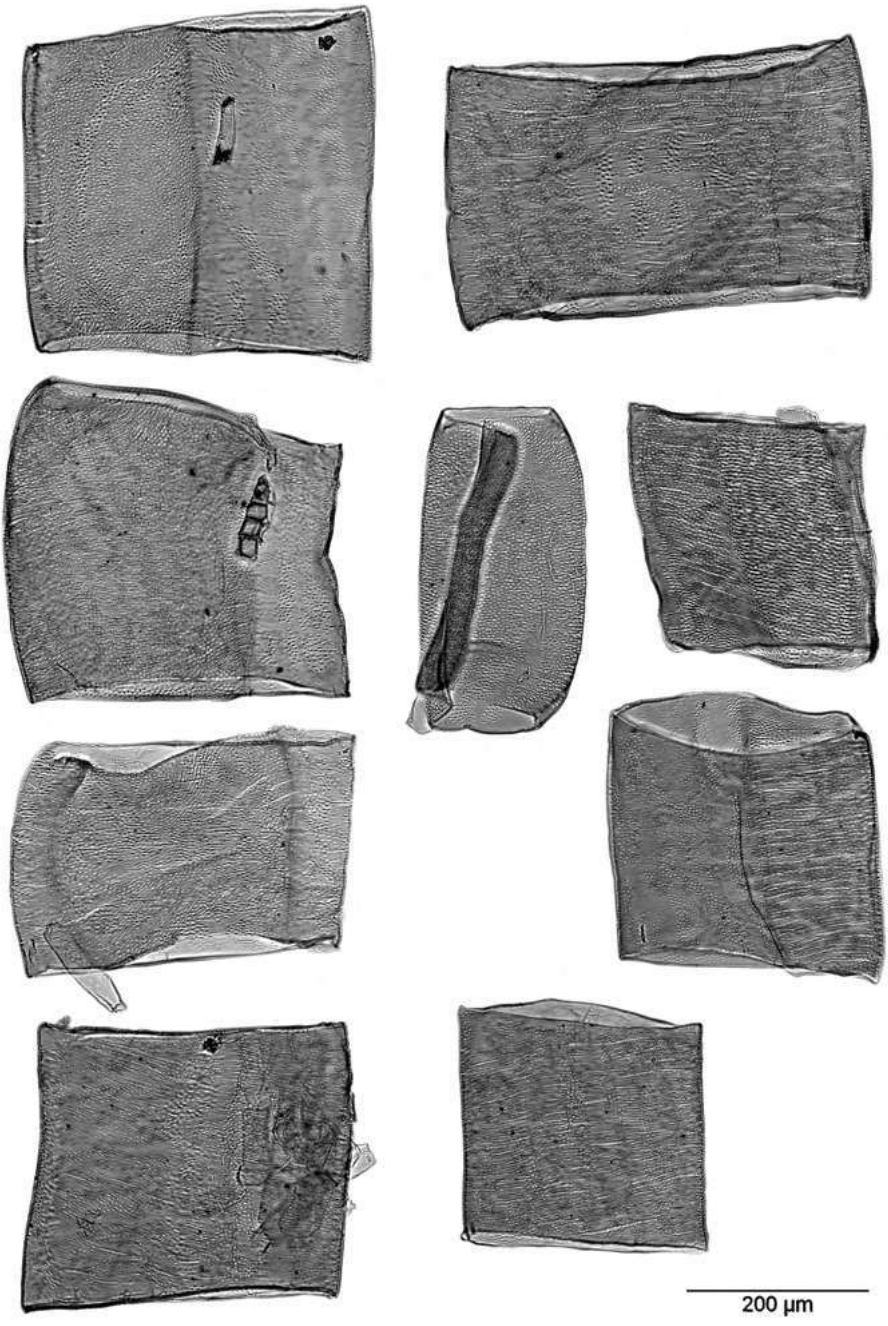
Pits to axial parenchyma cells: cross-fields arranged in longitudinal series (3–5 pits wide) of mostly irregular outline; apertures oval to slit-like.

Areas without any pits: regularly present; small.

Tyloses and helical thickenings: absent.

Notes on identification: Vessel elements of *Durio* spp. are similar in appearance to those of *Gonystylus* spp. and *Lophopetalum* spp. (see *Gonystylus* sp., p. 294).

Quantitative data:
Vessel elements (299–)519(–746) μm long, and (191–)315(–516) μm wide; l/w ratio 1.6.
Intervessel pit borders (3.8–)6.0(–10.2) μm in vertical diameter; pit apertures (1.0–)1.8(–4.0) μm.
Fibers 1375 μm long, 29.4 μm wide. Fiber wall thickness 8.0 μm (weighted averages).



Heritiera sp.

***Heritiera* sp.** (Malvaceae)

Trade names: mengkulang (MY); palapi, teraling, tarrietia (ID); huynh (VN); dong chem, sempong, sonloc (KH); lumbayau (PH); may nhom pa (LA), kanzo (MM).
DIN EN 13556:2003 code: HEXM.
CITES regulations: not protected.
Geographic distribution: Tropical Africa, Southern Asia from India to New Guinea, Micronesia and tropical Australia.

Vessel elements: rather short and wide (length about 250 μm, width about 350 μm); mainly drum-shaped, sometimes barrel-shaped.

Tails: rarely present; long with gradual transition; pitted.

Perforation plates: simple, extending over the entire lumen; horizontal.

Intervessel pits: alternate; vertical diameter 3–5–9 μm; present in large fields over a wide area.

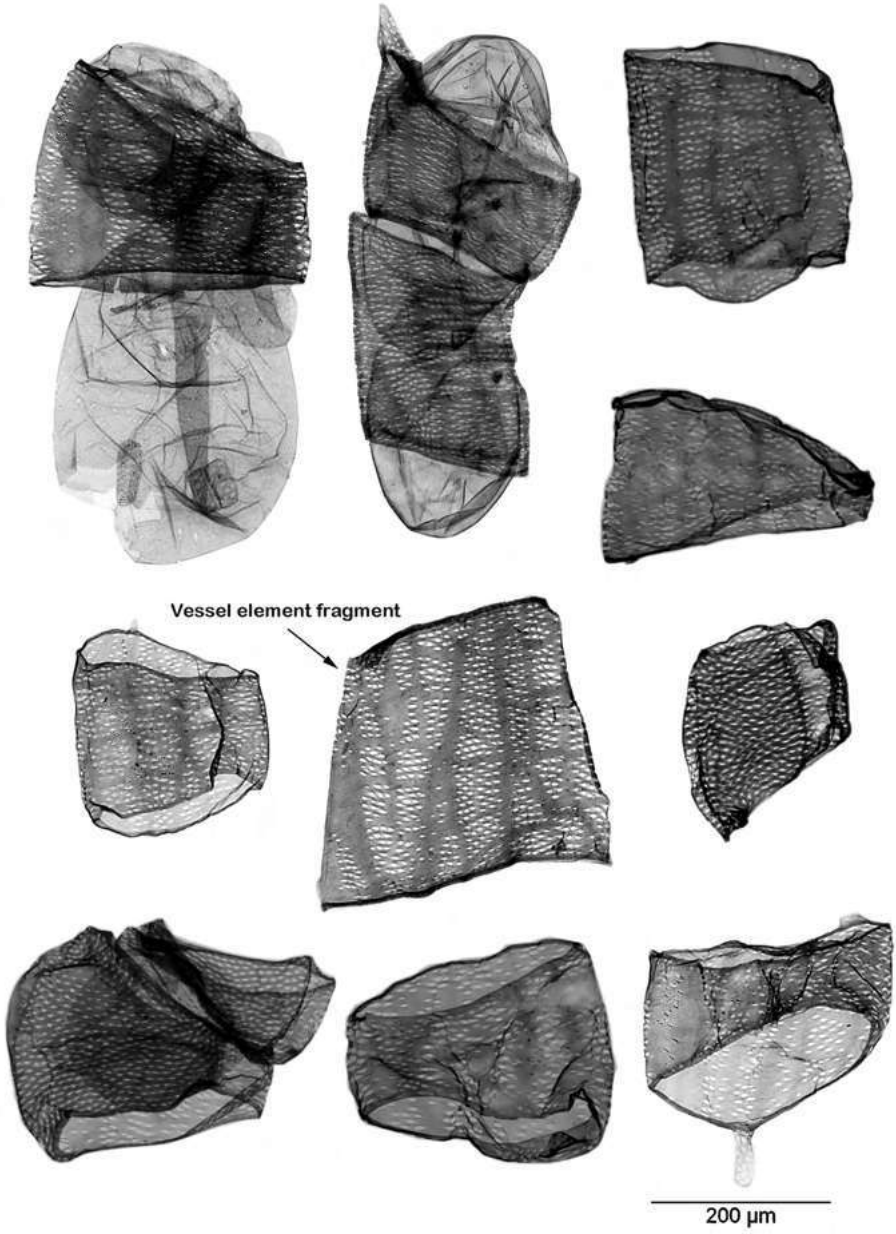
Vessel-ray pits and pits to parenchyma cells: APS; cross-fields aligned in horizontal (vessel-ray pits) or vertical (pits to parenchyma cells) series, similar to those of *Tetramerista* sp.; apertures slit-like, in centre of the cross-fields sometimes coalescent.

Areas without any pits: if present, very small.

Tyloses and helical thickenings: absent.

Notes on identification: see *Tetramerista glabra* (p. 292).

Quantitative data:
Vessel elements (175–)250(–369) μm long, and (126–)345(–471) μm wide; l/w ratio 0.8.
Intervessel pit borders (2.7–)4.9(–9.4) μm in vertical diameter; pit apertures (0.7–)1.2(–3.0) μm.
Fibers 1290 μm long, 21.0 μm wide. Fiber wall thickness 5.1 μm (weighted averages).



Paulownia tomentosa

Paulownia tomentosa (Thunb.) Steud. (Paulowniaceae)

Trade names: kiri, shima-giri (JP); mao pao tong (CN); paulownia impérial (FR); empresstree (GB, US); Chinesischer Blauglockenbaum (DE).
DIN EN 13556:2003 code: not listed.
CITES regulations: not protected.
Geographic distribution: Temperate Asia. Cultivated worldwide in regions with a temperate or subtropical climate.

Vessel elements: rather short and wide (length about 180 μm, width about 250 μm); mainly drum-shaped (those with a larger diameter, earlywood vessel elements), sometimes barrel-shaped (those with a smaller diameter, latewood vessel elements).

Arrow: One vessel element was cut in half to improve the presentation of the characteristics (pit arrangement).

Tails: rare, short with abrupt transition.

Perforation plates: simple, extending over the entire lumen; horizontal or inclined.

Intervessel pits: alternate; vertical diameter 3–6–9 μm; distributed over a wide area on the entire vessel element wall; apertures oval.

Vessel-ray pits: APS; cross-fields in horizontal series of mostly two rows of pits; pits occasionally with large apertures resembling simple pits.

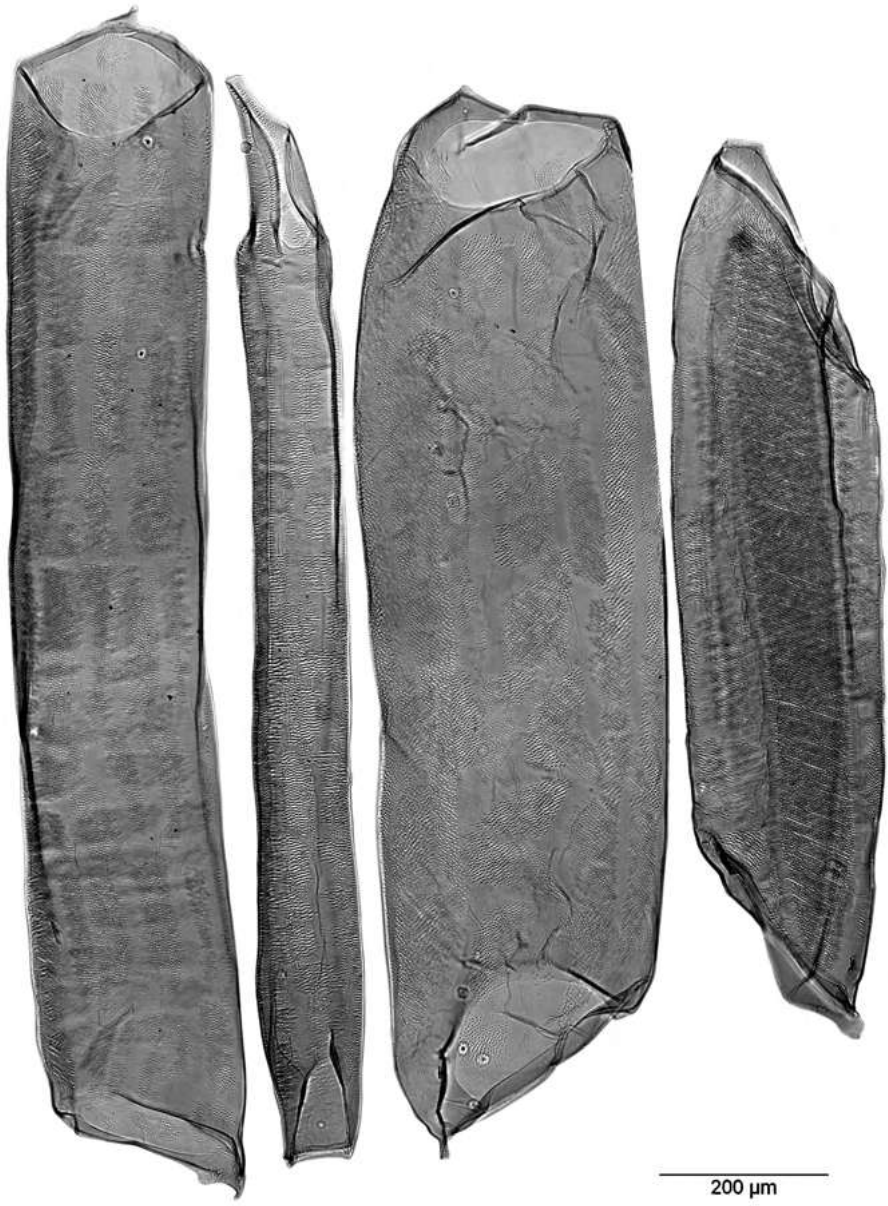
Pits to axial parenchyma cells: numerous, sometimes larger than the intervessel pits; cross-fields in neatly vertical series.

Areas without any pits: regularly present; small.

Tyloses: present.

Helical thickenings: absent.

Quantitative data:
Vessel elements (60–)175(–325) μm long, and (50–)245(–361) μm wide; l/w ratio 0.7.
Intervessel pit borders (2.7–)5.7(–9.2) μm in vertical diameter; pit apertures (0.9–)1.5(–3.8) μm.
Fibers 560 μm long, 25.8 μm wide. Fiber wall thickness 3.0 μm (weighted averages).



Tetramerista glabra

Tetramerista glabra Miq. (Tetrameristaceae)

Trade names: punah (MY); entuyut (MY-swk); tuyut (MY-sab); punak (ID).
DIN EN 13556:2003 code: not listed.
CITES regulations: not protected.
Geographic distribution: Western Indomalesia.

Vessel elements: very long (about 980 µm), width varies from slim to quite wide (average about 290 µm); mainly tube-shaped.

Tails: short with abrupt transition or longer with gradual transition.

Perforation plates: simple, often extending over the entire lumen; inclined.

Intervessel pits: alternate; very small; vertical diameter 2–3–5 µm; covering large vessel wall areas; apertures circular to oval; inner pit apertures slit-like and coalescent: forming diagonal striations (similar to *Heritiera* sp., p. 288).

Vessel-ray pits: APS; in horizontal series of small cross-fields, pits with oval apertures.

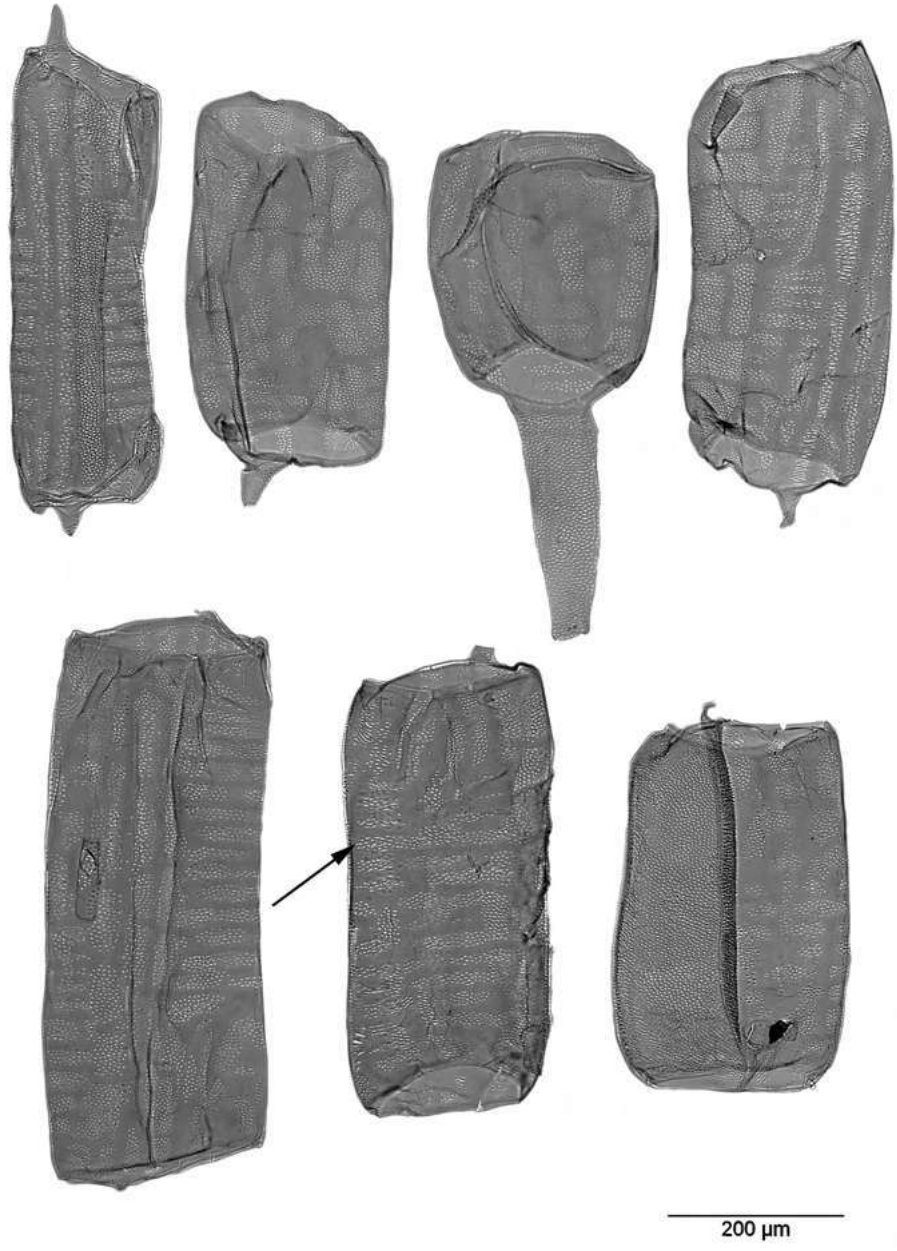
Pits to axial parenchyma cells: in vertical series of elongated cross-fields separated by pit-free lines; inner pit apertures slit-like, partly coalescent, connecting 2–4 pits.

Areas without any pits: regularly present; rather small to sometimes large.

Tyloses and helical thickenings: absent.

Notes on identification: The pit shape of *Tetramerista* spp. is similar to that of *Heritiera* spp. (p. 288), but the vessel elements are longer.

Quantitative data:
Vessel elements (479–)978(–1390) µm long, and (108–)288(–387) µm wide; l/w ratio 3.5.
Intervessel pit borders (2.1–)3.3(–5.2) µm in vertical diameter; pit apertures (0.4–)0.8(–1.4) µm.
Fibers 895 µm long, 30.2 µm wide. Fiber wall thickness 7.0 µm (weighted averages).



Gonystylus sp.

***Gonystylus* sp.** (Thymelaeaceae)

Trade name: ramin (DE, GB, MY, ID).
DIN EN 13556 : 2003 code: GYBN (*G. bancanus* (Miq.) Kurz).
CITES regulations: protected (Annex II).
Geographic distribution: Indomalesia and West Pacific islands (Fiji).
The wood was often used for furniture, broomsticks and moldings.

Vessel elements: of medium size or large (length 410 μm, width 210 μm); often elongated (barrel-shaped), rarely drum-shaped.

Tails: often short and rarely long; both with abrupt transition.

Perforation plates: simple, mostly extending over the entire lumen; horizontal or slightly inclined and narrowed.

Intervessel pits: alternate; vertical diameter 3–5 μm; present over a wide area and in the tails.

Vessel-ray pits: APS; cross-fields in horizontal series (3–6 pit rows per ray cell); apertures oval, sometimes slit-like.

Pits to axial parenchyma cells: cross-fields arranged in vertical series, separated by pitless strips.

Areas without any pits: regularly present; rather small, sometimes larger.

Tyloses and helical thickenings: absent.

Notes on identification: Vessel elements of *Gonystylus* spp. are similar in their appearance to those of *Durio* spp. and *Lophopetalum* spp. Corners of cross-fields of vessel-ray pits often rounded (arrow) as pits in the corners are often missing (*Lophopetalum* spp.: cross-fields rectangular, "corner pits" present, p. 276).

Quantitative data:
Vessel elements (292–)412(–607) μm long, and (92–)214(–262) μm wide; l/w ratio 2.0.
Intervessel pit borders (2.6–)3.4(–5.3) μm in vertical diameter; pit apertures (0.5–)1.0(–2.0) μm.
Fibers 1160 μm long, 29.0 μm wide. Fiber wall thickness 7.4 μm (weighted averages).

"**VAS**" (**V**essel-ray pits **A**pparently **S**imple) introductory comments

In the 19 genera and subgenera belonging to this group the vessel-ray pits differ in type and shape from the intervessel pits. The borders of the vessel-ray pits are not or hardly visible under the light microscope – and pits are called "apparently simple", represented by the acronym "VAS" (Vessel-ray pits Apparently Simple). Due to the different shapes of the pit apertures they can be subdivided into three groups. Figure 13 shows three different types of pit shapes and arrangement.

The numerous vessel-ray pits and pits to axial parenchyma cells of Anacardiaceae have a remarkable appearance. The apertures are elongated and resemble eyes (Fig. 13A).

Reticulate vessel-ray pits are common in the species of *Canarium*, *Calophyllum*, *Hevea brasiliensis*, *Castanopsis argentea*, *Eucalyptus globulus*, *Madhuca sericea*, and *Syzygium dyerianum*. The vessel-ray pits are arranged in horizontal rows along the rays and resemble a net-like structure (Fig. 13B).

Furthermore, this group also includes the important subgenera of *Shorea* and two further genera of the Dipterocarpaceae. The vessel elements are mainly barrel-shaped.

Based on practical experience, it is possible to identify the vessel elements of the genus *Shorea* but it is impossible to separate the individual subgenera. Considering the family as a whole, there is a high risk to confuse the economically important genera, *e.g*. *Shorea*, *Parashorea*, and *Dipterocarpus*. However, the appearance of the simple vessel-ray pits and the pits to axial parenchyma cells of *Shorea* differ remarkably in size and shape (Fig. 13C).

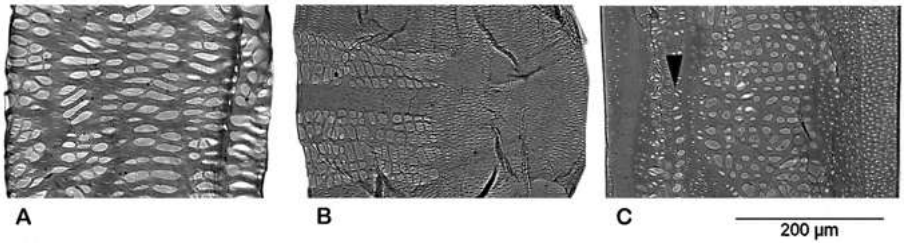


Figure 13. – A: gash-like vessel-ray pit apertures, *Swintonia* sp. – B: reticulate (and partly palisade) vessel-ray pits, *Calophyllum* sp. – C: diversity of pit sizes and shapes: vessel-ray pits and pits to axial parenchyma cells (arrowhead), *Shorea* subg. *Rubroshorea*.

Figure 14 shows the variation of vessel element length and width as well as the vertical diameter of the apertures and borders of intervessel pits. The boxplots of the length (Fig. 14a), width (Fig. 14b) and the borders of the intervessel pits (Fig. 14d) show how variable the dimensions of the different species can be. The dimensions of the apertures of the intervessel pits (Fig. 14c) lie close together, with some deviations (*e.g. Calophyllum* sp., *Madhuca sericea*, and *Canarium* sp.).

Mangifera sp. possesses the shortest vessel elements in this group (Fig. 14a) whereas the width of *Mangifera* sp. is average (Fig. 14b). *Syzygium dyerianum* reveals the longest vessel elements (Fig. 14a) whereas the width is similar to that of *Mangifera* sp. (Fig. 14b).

Because we only studied one sample per genus or species, these differences must be tested with much broader sampling.

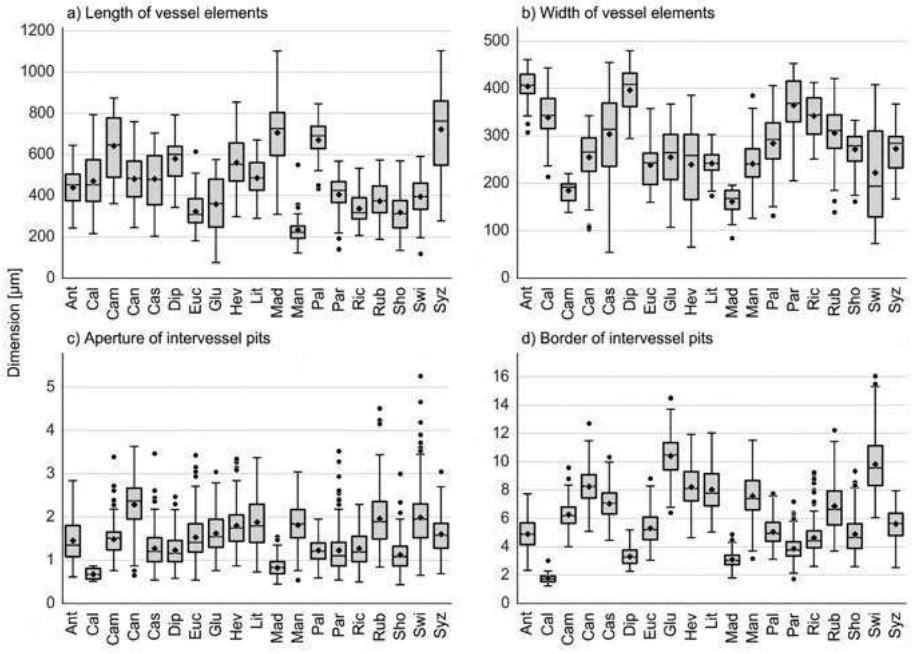
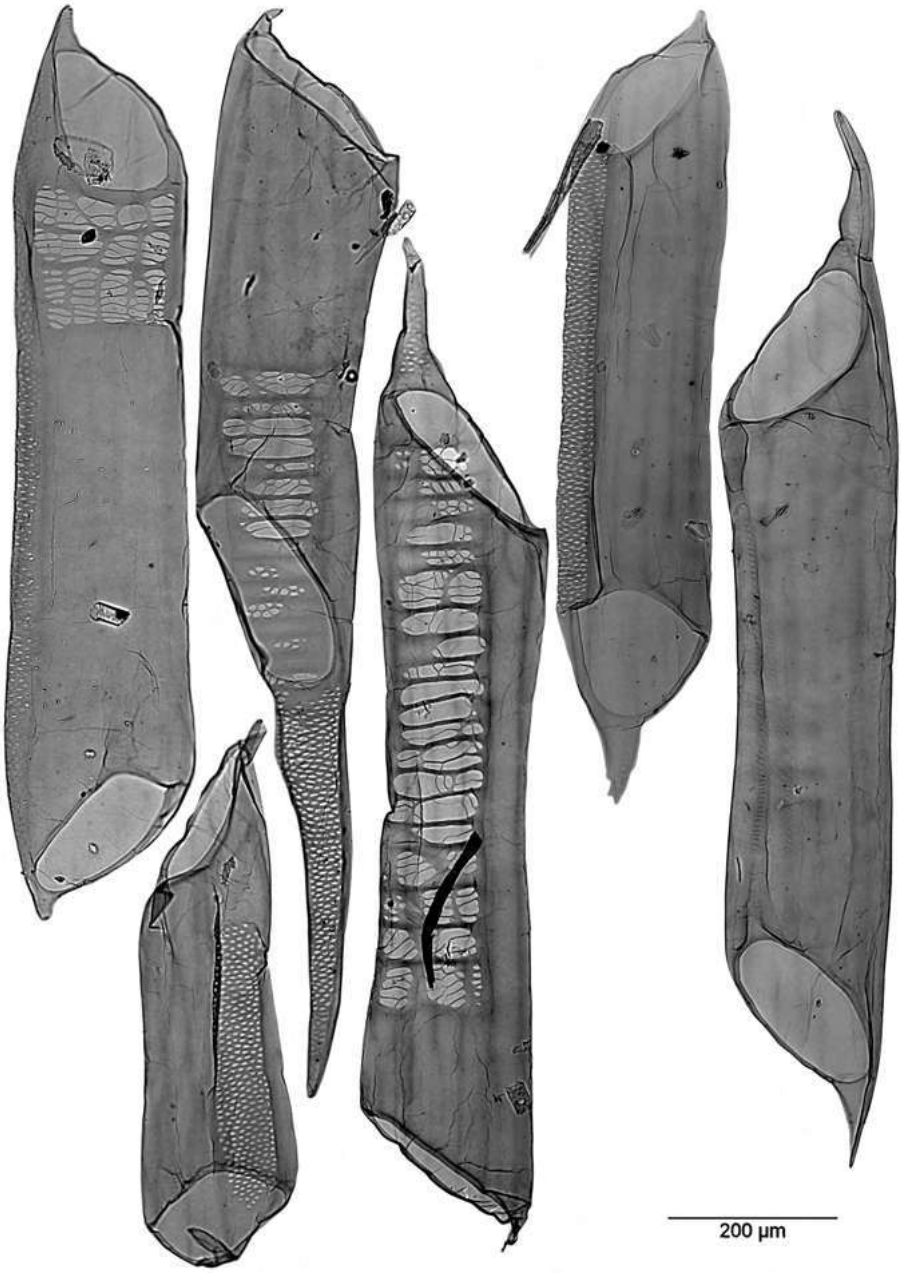


Figure 14. Boxplots of 19 wood genera/species (length and width of the vessel elements, diameter of pit apertures and borders).

Ant = *Shorea* subg. *Anthoshorea*; Cal = *Calophyllum* sp.; Cam = *Campnosperma* sp.; Can = *Canarium* sp.; Cas = *Castanopsis argentea*; Dip = *Dipterocarpus* sp.; Euc = *Eucalyptus* sp.; Glu = *Gluta renghas*; Hev = *Hevea brasiliensis*; Lit = *Litsea resinosa*; Mad = *Madhuca sericea*; Man = *Mangifera* sp.; Pal = *Palaquium* sp.; Par = *Parashorea* sp.; Ric = *Shorea* subg. *Richetia*; Rub = *Shorea* subg. *Rubroshorea*; Sho = *Shorea* subg. *Shorea*; Swi = *Swintonia* sp.; Syz = *Syzygium dyerianum*.



Campnosperma sp.

Campnosperma **sp.** (Anacardiaceae)

Trade names: terentang, kelinting, melumut, serentang (MY); pauh lebi, tumbus (ID); campnosperma (PG); nangpron, huasum sangtrang (TH); nisperillo, orey, sajo (PA).

DIN EN 13556:2003 code: not listed.

CITES regulations: not protected.

Geographic distribution: South and Central America, Madagascar, Seychelles, Sri Lanka, the whole Southeast Asia, Micronesia and Melanesia.

Vessel elements: long and slim (tube-shaped), length about 650 μm, width about 190 μm.

Tails: either short or long with gradual transition; larger tails often covered with pits.

Perforation plates: simple and more rarely scalariform (with 6–36 bars; Richter & Dallwitz 2000); extending over the entire lumen; inclined (parallelogram or trapezium).

Intervessel pits: alternate; vertical diameter 4–6–10 μm; present over a wide area; apertures oval to slit-like.

Vessel-ray pits: VAS; large; apertures window-like, sometimes subdivided into smaller units but still large; shape varies from elongated to oval or circular.

Pits to fibers: rarely present, arranged in short single vertical rows.

Areas without any pits: regularly present; very large.

Tyloses: present.

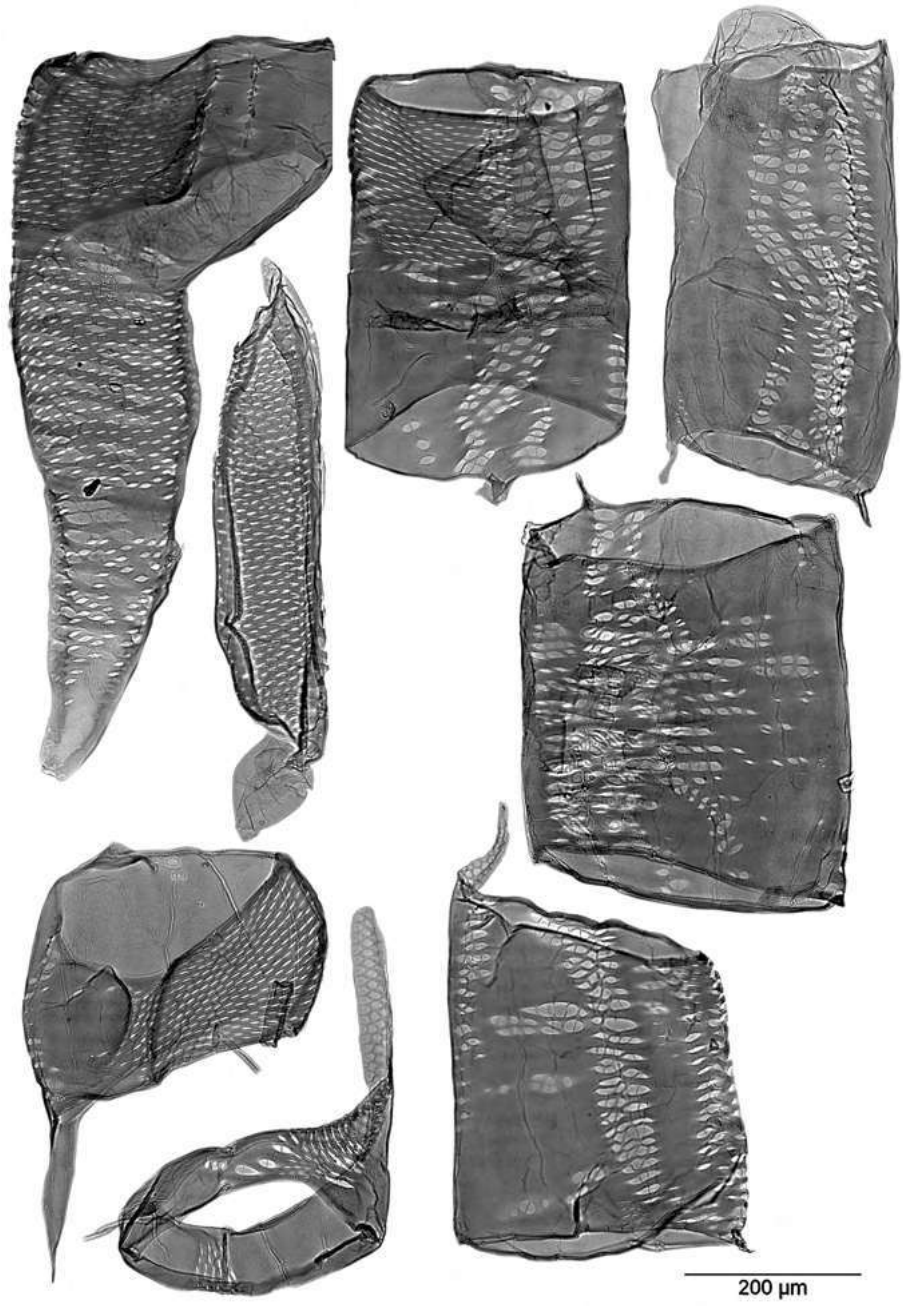
Helical thickenings: absent.

Quantitative data:

Vessel elements (361–)646(–873) μm long, and (139–)192(–220) μm wide; l/w ratio 3.7.

Intervessel pit borders (4.0–)6.2(–9.6) μm in vertical diameter; pit apertures (0.8–)1.5(–3.4) μm.

Fibers 950 μm long, 24.5 μm wide. Fiber wall thickness 7.7 μm (weighted averages).



Gluta renghas

Gluta renghas L. (Anacardiaceae)

Trade names: rengas, rengas kerbau jalang (MY); rengas tembaga (ID); hekakoro (PG); thayet-thitsi (MM); kroeul (KH); rakban (TH).
DIN EN 13556 : 2003 code: not listed.
CITES regulations: not protected.
Geographic distribution: Indochina to Indomalesia (one species in Madagascar).

Vessel elements: rather large, some very short (length about 360 μm, width about 270 μm); barrel or drum-shaped.

Tails: either short with abrupt transition or very long with gradual transition.

Perforation plates: simple, extending over the entire lumen; horizontal or slightly inclined.

Intervessel pits: alternate; vertical diameter 6–11–15 μm; present over a wide area; apertures oval.

Vessel-ray pits: VAS; arranged in horizontal rows; similar to pits to axial parenchyma cells.

Pits to axial parenchyma cells: similar to vessel-ray pits in size and shape; arranged in vertical series.

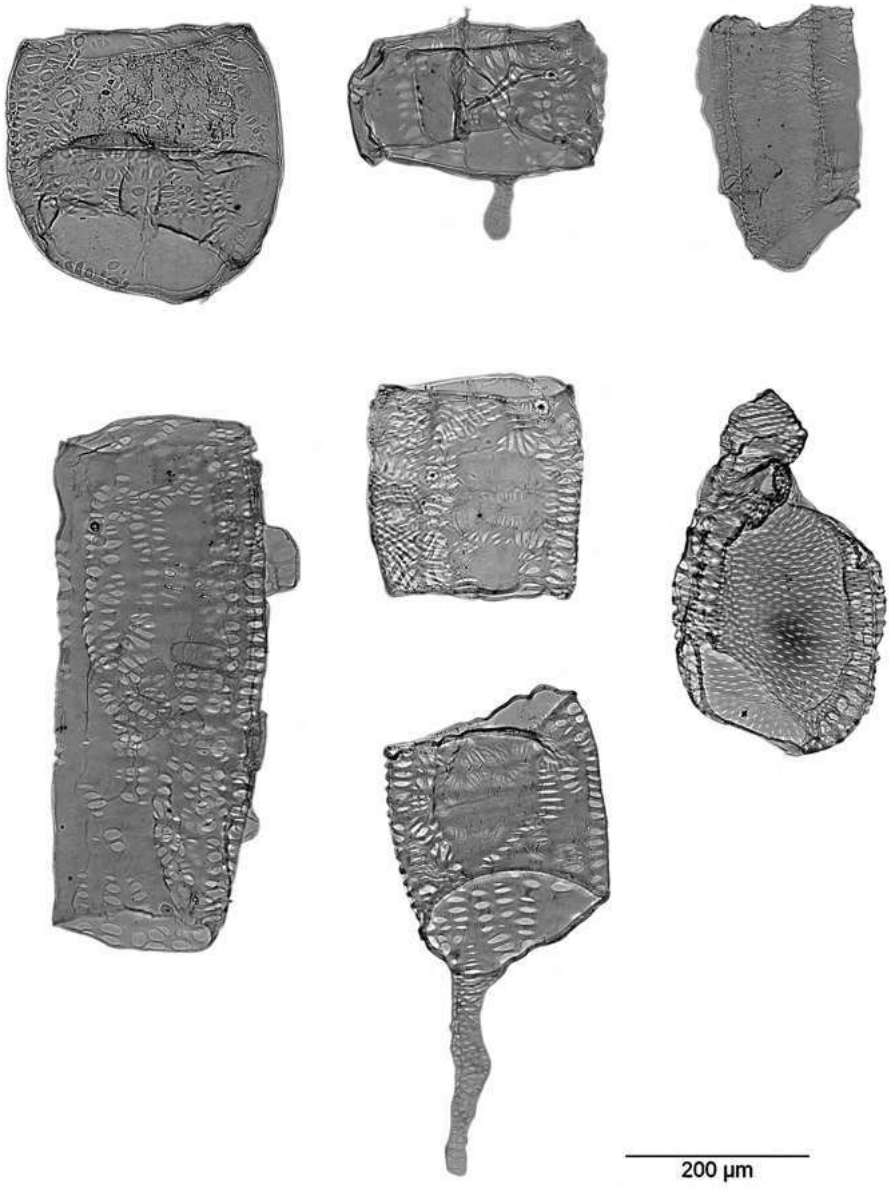
Areas without any pits: regularly present; large to very large.

Tyloses: present.

Helical thickenings: absent.

Notes on identification: The vessel elements of *Gluta renghas* are similar to those of *Mangifera* spp. and *Swintonia* spp. (see *Mangifera* sp., p. 302).

Quantitative data:
Vessel elements (76–)361(–576) μm long, and (107–)265(–367) μm wide; l/w ratio 1.4.
Intervessel pit borders (6.4–)10.5(–14.6) μm in vertical diameter; pit apertures (0.8–)1.5(–3.0) μm.
Fibers 945 μm long, 20.9 μm wide. Fiber wall thickness 6.0 μm (weighted averages).



Mangifera sp.

Mangifera sp. (Anacardiaceae)

Trade names: machang, tiger wood (MY), mango (IN).
DIN EN 13556:2003 code: not listed.
CITES regulations: not protected.
Geographic distribution: India, Pakistan, Sri Lanka, Indochina, Indomalesia.
Mangifera sp. is cultivated as a fruit tree. The wood is commonly used for furniture and plywood cores.

Vessel elements: rather short (length about 220 µm, width about 250 µm), often drum-shaped, sometimes elongated (tube-shaped).

Tails: often long with abrupt transition or short with gradual transition.

Perforation plates: simple, extending over the entire lumen; horizontal or slightly inclined.

Intervessel pits: pits alternate; vertical diameter 3–7–12 µm; present over a wide area and in the tails; apertures oval.

Vessel-ray pits: VAS; isodiametric or stretched (eye-shaped); in one ray cell all the pits are of the same type; present on nearly every vessel element.

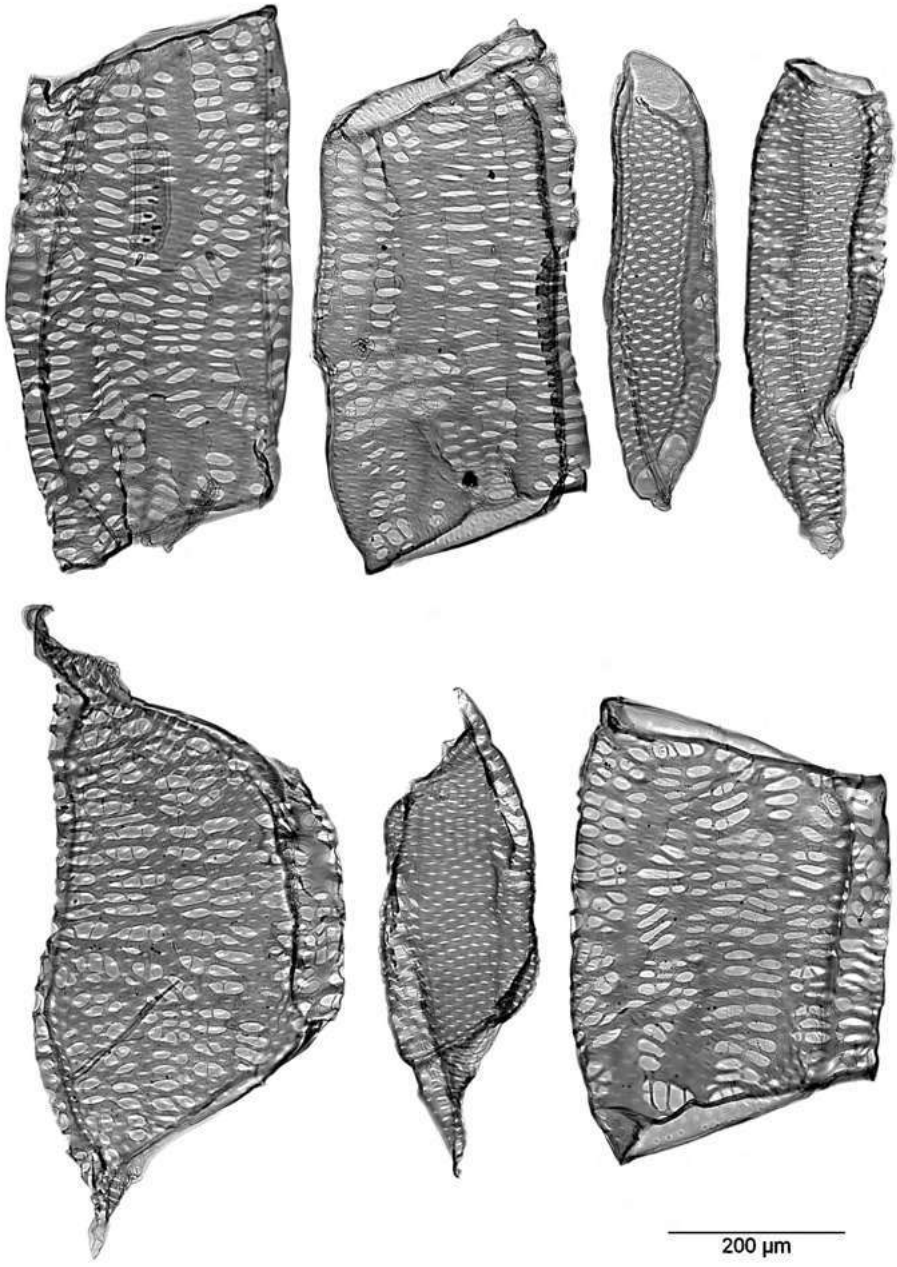
Areas without any pits: regularly present; small to large.

Tyloses: present, thin-walled.

Helical thickenings: absent.

Notes on identification: The vessel elements of *Mangifera* spp. are similar to those of *Gluta renghas* spp. and *Swintonia* spp. *Mangifera* sp. has the smallest vessel elements with small intervessel pits. *Swintonia* sp. is characterized by the largest intervessel pits. The three genera show very similar pit features.

Quantitative data:
Vessel elements (75–)221(–550) µm long, and (126–)241(–385) µm wide; l/w ratio 0.9.
Intervessel pit borders (3.2–)7.4(–11.5) µm in vertical diameter; pit apertures (0.5–)1.8(–3.0) µm.
Fibers 700 µm long, 20.8 µm wide. Fiber wall thickness 5.8 µm (weighted averages).



Swintonia sp.

***Swintonia* sp.** (Anacardiaceae)

Trade names: merpauh, selan (MY-swk); kaluis, lomarau (PH); civit, taung-thayet (MM); muom (KH, VN).
DIN EN 13556 : 2003 code: not listed.
CITES regulations: not protected.
Geographic distribution: Bangladesh, Indochina to Indomalesia.

Vessel elements: of average size (length about 400 µm, width about 190 µm); mainly barrel-shaped (those with a larger diameter), sometimes tube-shaped (those with a smaller diameter); width varies from average to slim dimensions.

Tails: if present often short with gradual transition.

Perforation plates: simple, extending over the entire lumen; inclined (trapezium or parallelogram).

Intervessel pits: alternate; quite large; vertical diameter 6–10–16 µm; present over a wide area and in the tails; apertures oval.

Vessel-ray pits: VAS; very large, window-like arranged in 2–3 rows per cross-field.

Pits to axial parenchyma cells: elongate, often present over the entire vessel element wall.

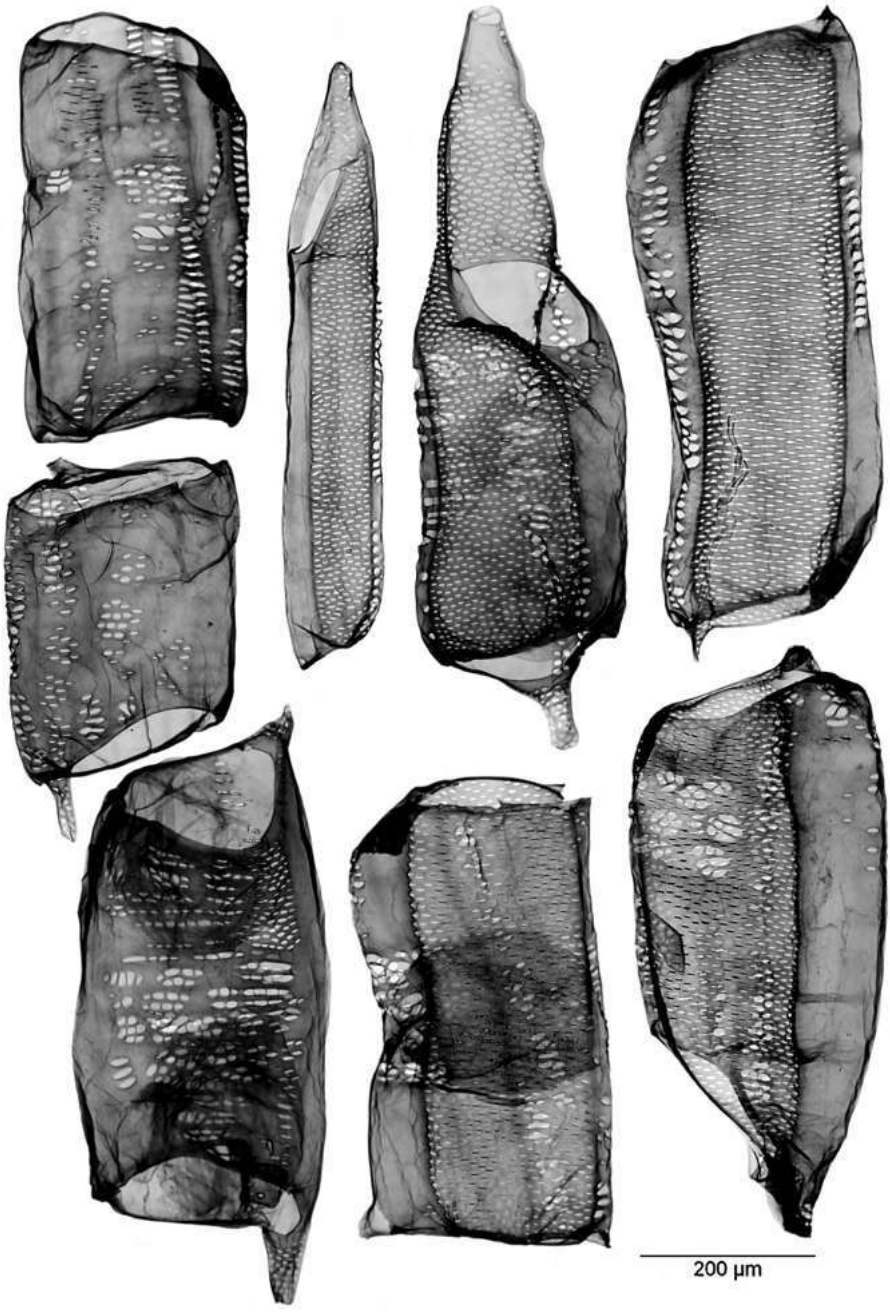
Areas without any pits: if present, very small.

Tyloses: present.

Helical thickenings: absent.

Notes on identification: The vessel elements of *Swintonia* spp. are similar to those of *Gluta renghas* spp. and *Mangifera* spp. (see *Mangifera* sp., p. 302).

Quantitative data:
Vessel elements (119–)396(–590) µm long, and (73–)194(–408) µm wide; l/w ratio 1.8.
Intervessel pit borders (6.1–)9.6(–16.1) µm in vertical diameter; pit apertures (0.7–)2.0(–5.3) µm.
Fibers 930 µm long, 23.5 µm wide. Fiber wall thickness 8.7 µm (weighted averages).



Canarium sp.

***Canarium* sp.** (Burseraceae)

Trade names: kedondong, kenari, kerantai (ID); kerantai, upi, seladah (MY); upi (BN, MY-swk); canarium, galip (PG); pili, pilingliitan, pagsahingin (PH); makoem (TH); tram (VN).

DIN EN 13556 : 2003 code: CNXX.

CITES regulations: not protected.

Geographic distribution: "Old world tropics" from tropical Africa to tropical Asia, northern Australia and the Pacific.

Vessel elements: rather large (length about 480 μm, width about 270 μm); mainly barrel-shaped (those with a larger diameter), sometimes tube-shaped (those with a smaller diameter).

Tails: if present, rather short with abrupt transition or very long with gradual transition.

Perforation plates: simple, often extending over the entire lumen; horizontal or inclined.

Intervessel pits: alternate; vertical diameter 5–8–13 μm; often present over a wide area covering the entire vessel element wall.

Vessel-ray pits: VAS; cross-fields arranged in horizontal series, with one or several rows of pits variable in size.

Pits to fibers: rarely present, arranged in single vertical rows.

Areas without any pits: present on radial faces; large to very large.

Tyloses: present.

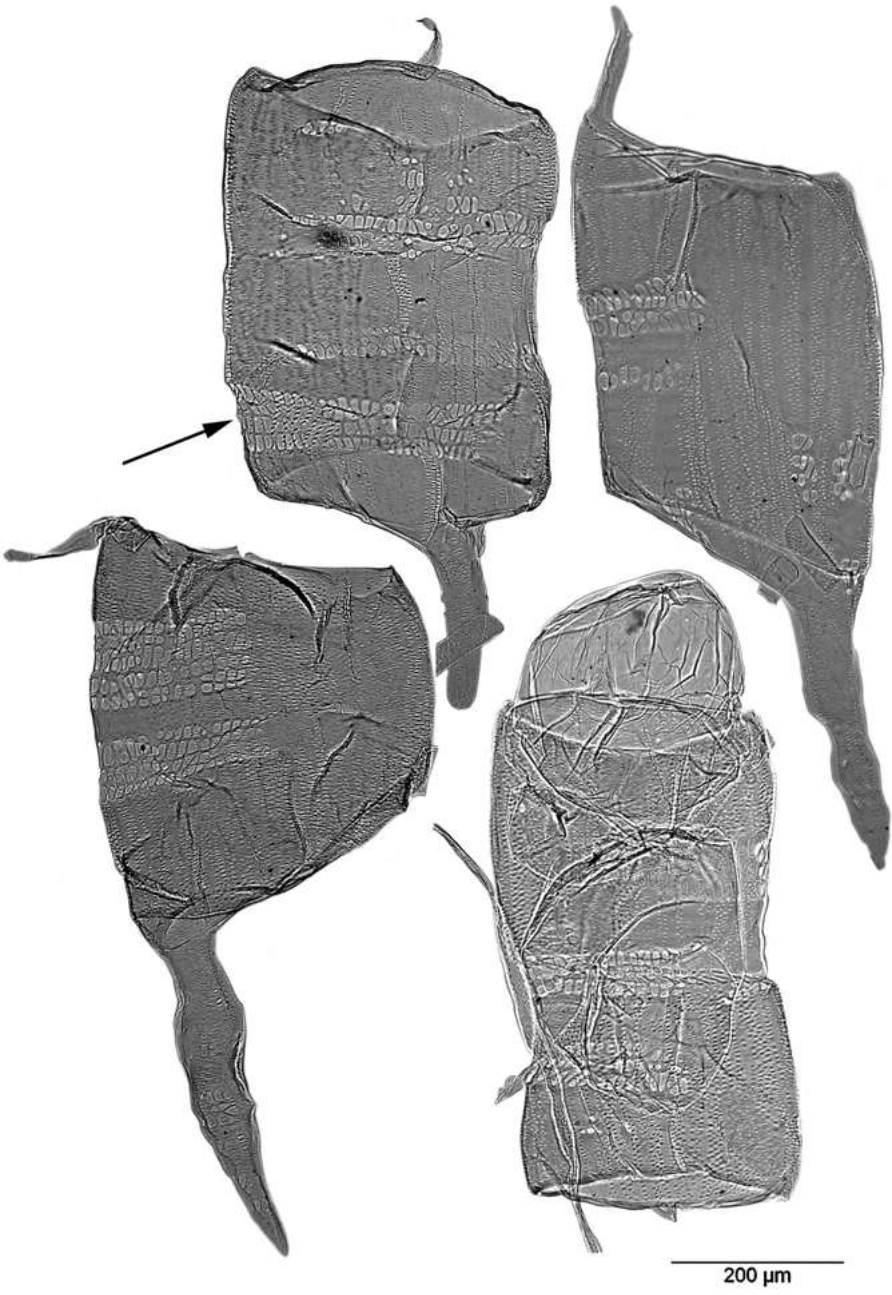
Helical thickenings: absent.

Quantitative data:

Vessel elements (246–)484(–759) μm long, and (103–)266(–343) μm wide; l/w ratio 1.9.

Intervessel pit borders (5.1–)8.3(–12.7) μm in vertical diameter; pit apertures (0.7–)2.4(–3.6) μm.

Fibers 920 μm long, 24.7 μm wide. Fiber wall thickness 4.2 μm (weighted averages).



Calophyllum sp.

***Calophyllum* sp.** (Calophyllaceae)

Trade names: bintangor (MY, DE); bitaog, kalofilum, kamdeb, tamanu, bakokol, entangor, mentangor (MY); ponnyet, tharapi (MM); domba-gassa (LK); bansangal, vutalau, zarumayen (PH); vintanina (MG); palo maría (PH = *C. inophyllum*), Alexandrien laurel (IN = *C. inophyllum*).

DIN EN 13556:2003 code: CLXX.

CITES regulations: not protected.

Geographic distribution: Indomalesia, Micronesia, Melanesia and northern Australia, also in Central and South America, Madagascar and surrounding islands.

Vessel elements: rather large (length about 450 μm, width about 340 μm); elongated (barrel-shaped) or shorter (drum-shaped).

Tails: long with abrupt transition, sometimes shorter.

Perforation plates: simple, extending over the entire lumen; horizontal or slightly inclined.

Intervessel pits: absent (due to vessels being almost exclusively solitary in the intact wood).

Vessel-ray pits: VAS; window-like (angled to oval), cross-fields with 2–5 horizontal rows of pits arranged in a reticulate pattern (arrow).

Pits to tracheids: numerous; in 2–3-seriate bands; vertical diameter 1–2–3 μm; apertures oval to slit-like; pits alternate.

Areas without any pits: if present, small.

Tyloses: present.

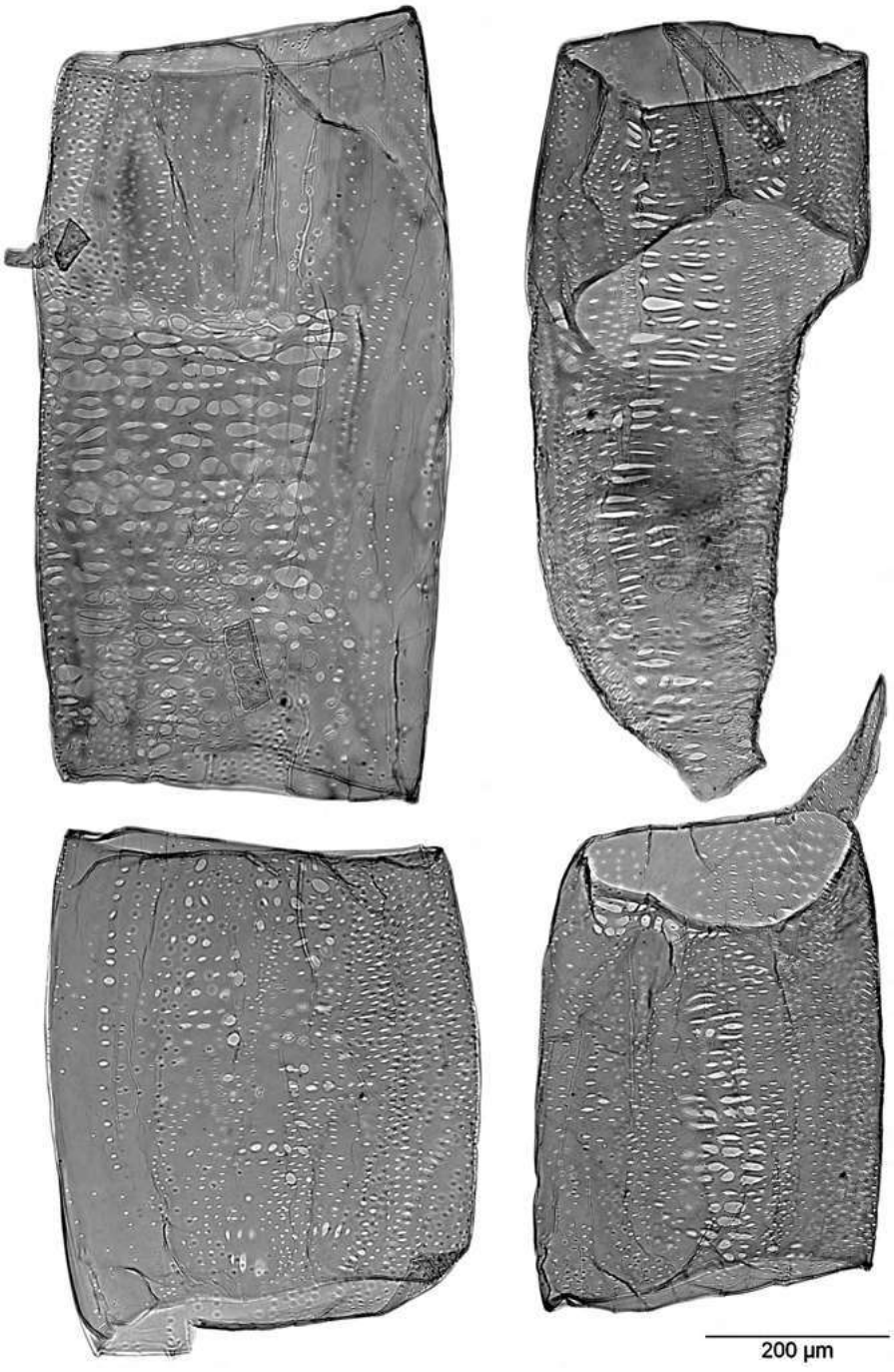
Helical thickenings: absent.

Quantitative data:

Vessel elements (216–)453(–794) μm long, and (214–)343(–443) μm wide; l/w ratio 1.3.

Vessel to tracheid pit borders (1.2–)1.7(–3.0) μm in vertical diameter; pit apertures (0.5–)0.7(-0.9) μm.

Fibers 1120 μm long, 24.4 μm wide. Fiber wall thickness 7.2 μm (weighted averages).



Dipterocarpus sp.

Dipterocarpus **sp.** (Dipterocarpaceae)

Trade names: keruing (ID, MY, DE); yang (FR, TH, VN); gurjun (IN, MM, LK); dau (VN, FR); white kanyin, kanyin-byu (MM); choeuteal (KH); nhang (LA); keroewing (NL); yang hin, yang na (TH); dzao long (VN).
DIN EN 13556:2003 code: DPXX.
CITES regulations: not protected.

Geographic distribution: India, Pakistan, Sri Lanka, Indochina and Western Indomalesia.

Vessel elements: rather large (length about 600 μm, width about 410 μm); elongated (barrel-shaped), rarely shorter (drum-shaped).

Tails: short with abrupt transition, long with gradual transition or absent.

Perforation plates: simple, extending over the entire lumen; horizontal or slightly inclined.

Intervessel pits: absent (due to vessels being almost exclusively solitary in intact wood).

Vessel-ray pits: VAS; huge variety of size classes and shapes; the larger pits gash-like, the smaller ones circular or oval; cross-fields sometimes covering the entire vessel element wall.

Pits to tracheids: alternate; vertical diameter 2–3–5 μm; arranged in sinuous strips; sometimes broadened in horizontal direction.

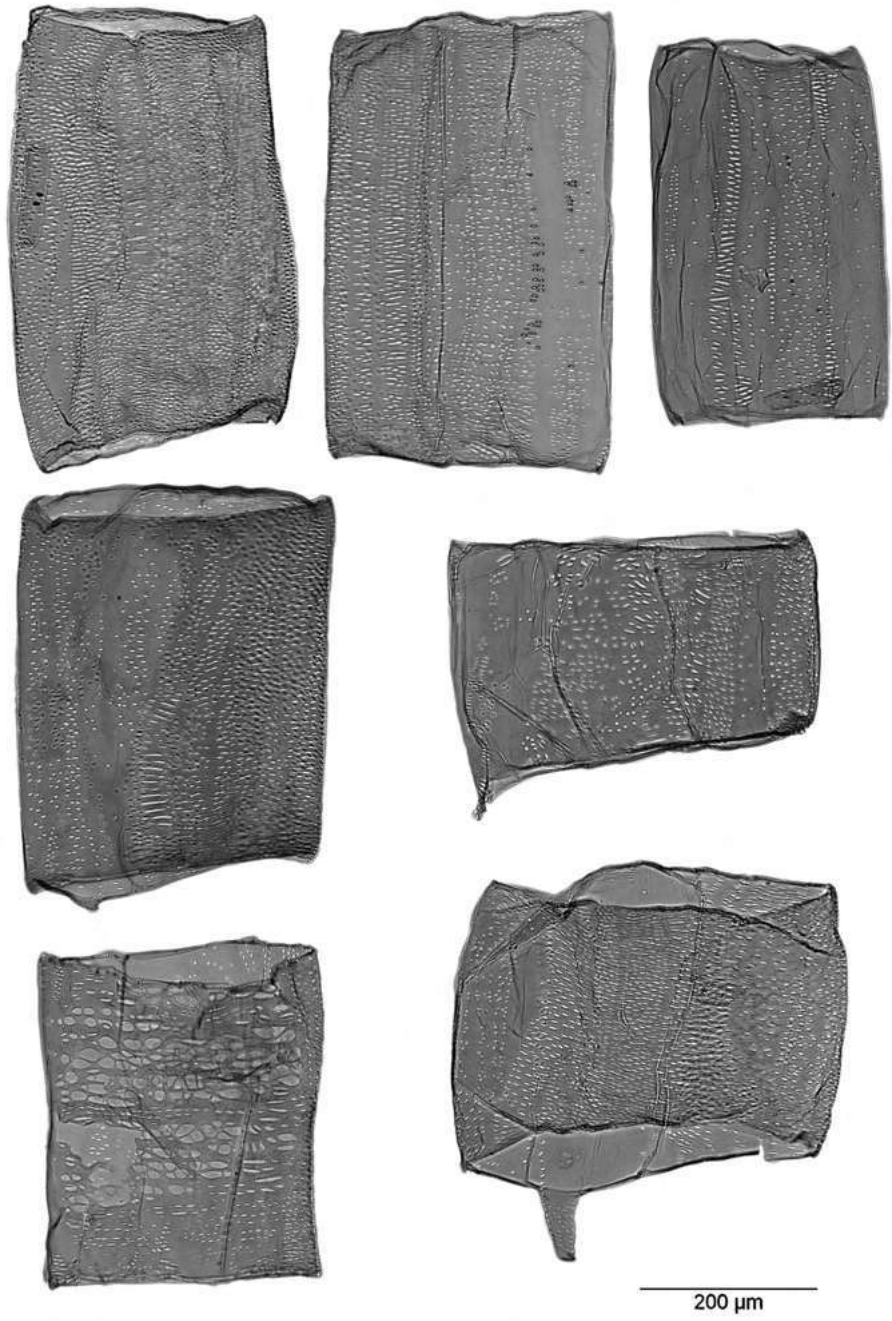
Pits to fibers: present in single vertical rows.

Areas without any pits: regularly present; small to large.

Tyloses: present.

Helical thickenings: absent.

Quantitative data:
Vessel elements (343–)601(–792) μm long, and (294–)409(–480) μm wide; l/w ratio 1.4.
Vessel to tracheid pit borders (2.3–)3.3(–5.2) μm in vertical diameter; pit apertures (0.6–)1.2(–2.5) μm.
Fibers 1390 μm long, 26.5 μm wide. Fiber wall thickness 6.1 μm (weighted averages).



Parashorea sp.

***Parashorea* sp.** (Dipterocarpaceae)

Trade names: light wood (0.4–0.6 g/cm^3): white seraya, white lauan (DE); white seraya, urat mata (MY-sab); seraya puteh (MY-swk); white lauan, bagtikan (PH); pendan (ID). — heavy wood (0.6–0.8 g/cm^3): heavy white seraya, urat mata batu (MY-sab); gerutu, meranti gerutu (MY); khai kheo, khiansai (TH); thinkadu, tavoy wood (MM); cho chi (VN).
DIN EN 13556:2003 code: PHWS/PHMG.
CITES regulations: not protected.
Geographic distribution: Indochina, Indomalesia.

Vessel elements: rather short and wide (length about 430 μm, width about 370 μm), barrel or drum-shaped, shape well-defined with almost rectangular outlines.

Tails: mostly absent, few long or short tails present with abrupt transition.

Perforation plates: simple, extending over the entire lumen; horizontal.

Intervessel pits: alternate; vertical diameter 2–4–7 μm; if present, in large fields over a wide area; apertures oval or sometimes elongated.

Vessel-ray pits: VAS; rarely present; pits window-like or elongated.

Pits to tracheids: in vertical strips (3–5 pit rows), apertures oval to slit-like or gash-like.

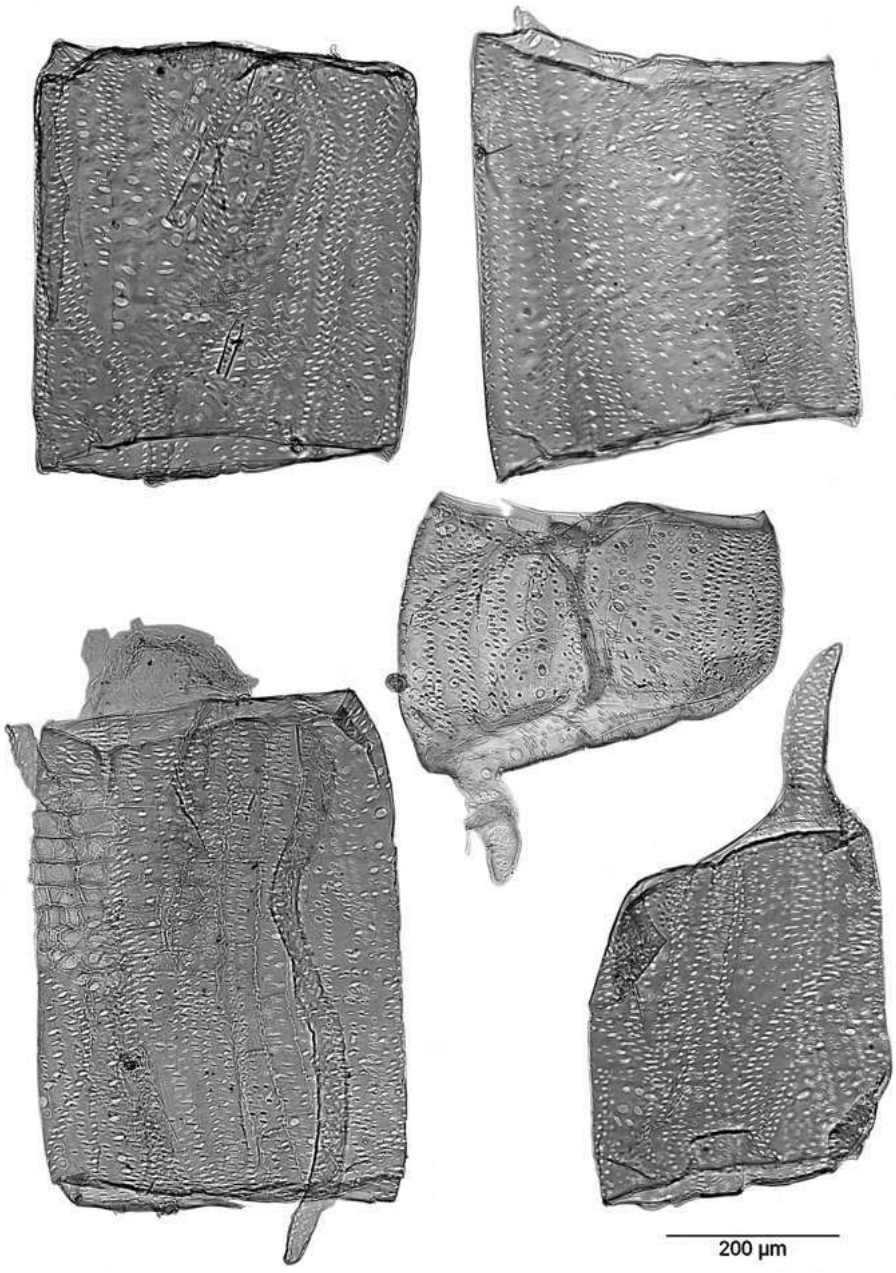
Pits to fibers: present in single vertical rows.

Areas without any pits: regularly present; small to sometimes large.

Tyloses: present.

Helical thickenings: absent.

Quantitative data:
Vessel elements (140–)425(–568) μm long, and (205–)369(–453) μm wide; l/w ratio 1.1.
Intervessel pit borders (1.7–)3.8(–7.2) μm in vertical diameter; pit apertures (0.5–)1.1(–3.5) μm.
Fibers 1145 μm long, 19.9 μm wide. Fiber wall thickness 3.6 μm (weighted averages).



Shorea subg. *Anthoshorea*

Shorea subg. *Anthoshorea* (Dipterocarpaceae)

Trade names: white meranti (MY, ID); meranti putih, kayu tahan (ID); melapi (MY-sab, ID-kal); white lauan (PH); luombor (KH); khiem kha norng, takhian-sai, chai (TH); bo-bo (VN).
DIN EN 13556:2003 code: SHWM.
CITES regulations: not protected.
Geographic distribution: Indochina, Indomalesia.

Vessel elements: average length (about 450 μm), quite wide (410 μm), often barrel-shaped and sometimes drum-shaped.

Tails: if present, rather short with abrupt transition or longer with gradual transition.

Perforation plates: simple, extending over the entire lumen; horizontal.

Intervessel pits: alternate; vertical diameter 2–5–8 μm; apertures oval.

Vessel-ray pits: VAS; window-like, sometimes elongated; in alternately or separately arranged rows; apertures circular, sometimes elongated.

Pits to axial parenchyma cells: similar in size and shape to vessel-ray pits.

Pits to tracheids: alternate; similar to intervessel pits and pits to fibers, present in sinuous or straight vertical strips of 1–2 pits.

Pits to fibers: present in single vertical rows.

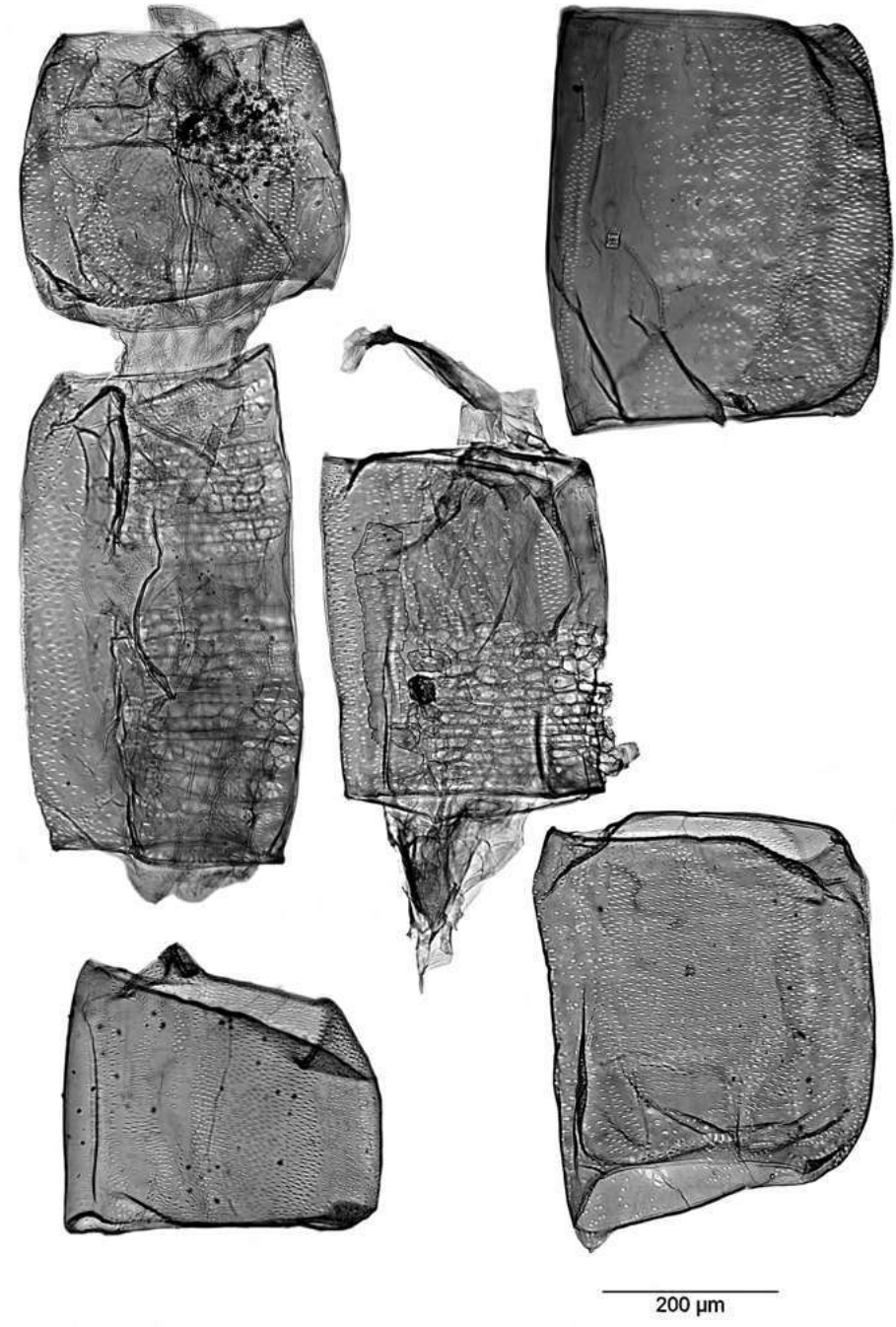
Areas without any pits: if present, rather small.

Tyloses: present.

Helical thickenings: absent.

Notes on identification: The subgenera of *Shorea* cannot be separated by means of vessel element features as they are of similar appearance.

Quantitative data:
Vessel elements (244–)453(–645) μm long, and (307–)407(–461) μm wide; l/w ratio 1.1.
Intervessel pit borders (2.3–)4.9(–7.7) μm in vertical diameter; pit apertures (0.6–)1.4(–2.8) μm.
Fibers 1460 μm long, 21.8 μm wide. Fiber wall thickness 3.7 μm (weighted averages).



Shorea subg. *Richetia*

Shorea subg. *Richetia* (Dipterocarpaceae)

Trade names: yellow meranti (MY); yellow seraya (MY-sab); yellow lauan (PH); meranti kuning (ID); meranti damar hitam (MY); selangan kuning, selangan kacha (MY-sab).
DIN EN 13556:2003 code: SHYM.
CITES regulations: not protected.
Geographic distribution: Indomalesia.

Vessel elements: rather short and wide (length about 320 μm, width about 340 μm), drum or barrel-shaped.

Tails: if present, short with gradual transition.

Perforation plates: simple, extending over the entire lumen; horizontal.

Intervessel pits: alternate; vertical diameter 3–4–9 μm; covering large fields over a wide area; apertures oval to slit-like.

Vessel-ray pits: VAS; apertures window-like (rectangular) or oval; of variable size and shape; arranged in one to several rows per ray cell.

Pits to tracheids: forming long vertical strips (3–5 pits wide); apertures elongated.

Pits to fibers: present in single vertical rows.

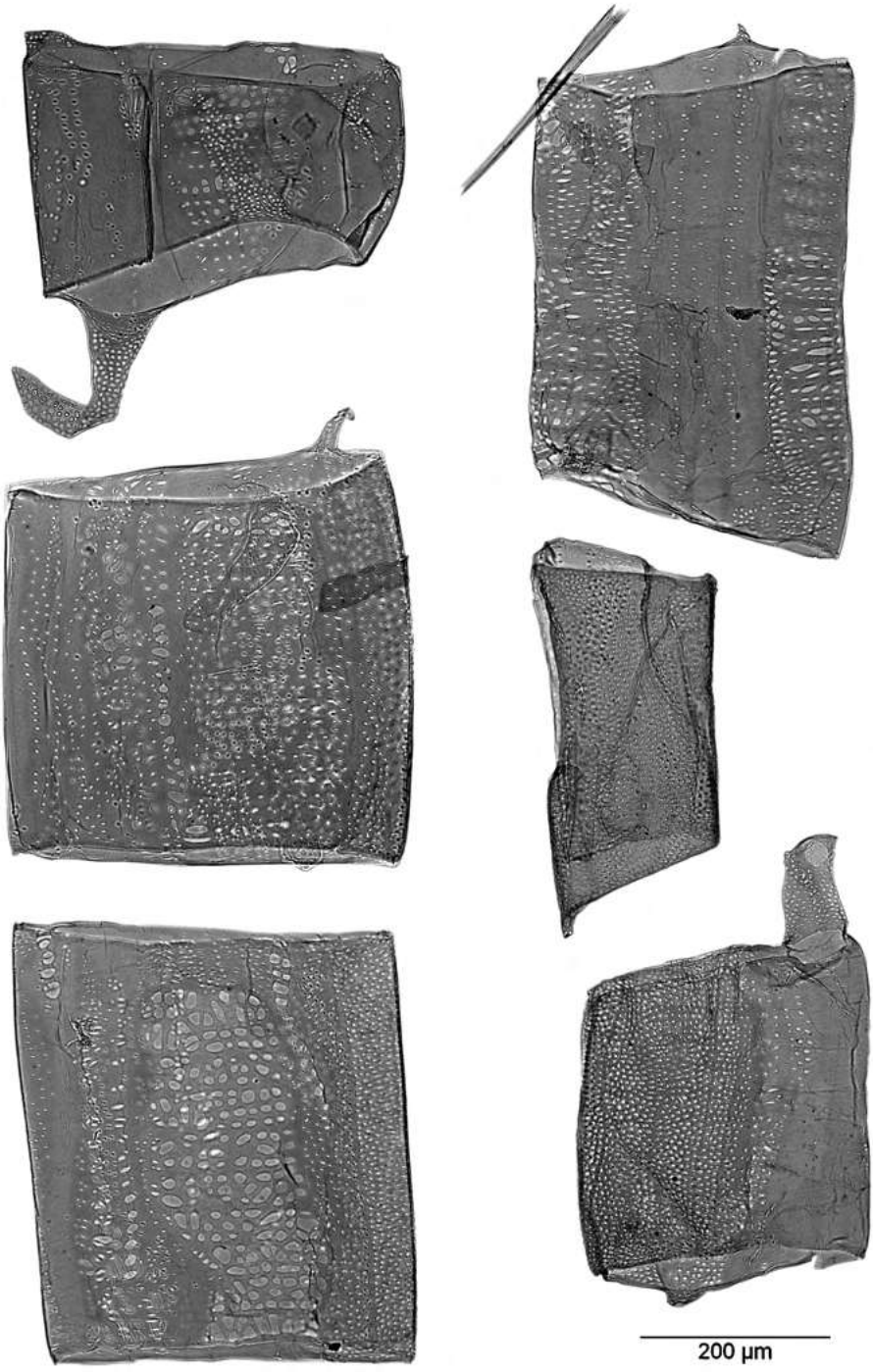
Areas without any pits: if present, small to large.

Tyloses: often present.

Helical thickenings: absent.

Notes on identification: See *Shorea* subg. *Anthoshorea* (p. 314).

Quantitative data:
Vessel elements (206–)317(–533) μm long, and (251–)344(–413) μm wide; l/w ratio 0.9.
Intervessel pit borders (2.6–)4.4(–9.3) μm in vertical diameter; pit apertures (0.5–)1.2(–2.3) μm.
Fibers 895 μm long, 22.0 μm wide. Fiber wall thickness 5.2 μm (weighted averages).



Shorea subg. *Rubroshorea*

Shorea subg. *Rubroshorea* (Dipterocarpaceae)

Trade names: dark/light red meranti, seraya, lauan (DE); kawang, seraya bunga, red seraya (MY-sab); red lauan (PH); meranti merah (ID).
DIN EN 13556:2003 code: SHDR/SHLR.
CITES regulations: not protected.
Geographic distribution: Indomalesia.

Vessel elements: rather short and wide (length about 380 μm, width about 310 μm); mainly barrel- or drum-shaped (those with a larger diameter), sometimes tube-shaped (those with a smaller diameter).

Tails: if present, long or short with abrupt transition.

Perforation plates: simple, extending over the entire lumen; horizontal.

Intervessel pits: alternate; vertical diameter 4–7–12 μm; present in large fields over a wide area; apertures oval.

Vessel-ray pits: VAS; apertures window-like (more or less circular) or elongated horizontally (gash-like); of variable size and shape; in several rows per ray cell.

Pits to axial parenchyma cells: similar to vessel-ray pits; in narrow vertical bands.

Pits to tracheids: arranged in wide vertical strips; 1–4 pit rows wide.

Pits to fibers: present in single vertical rows.

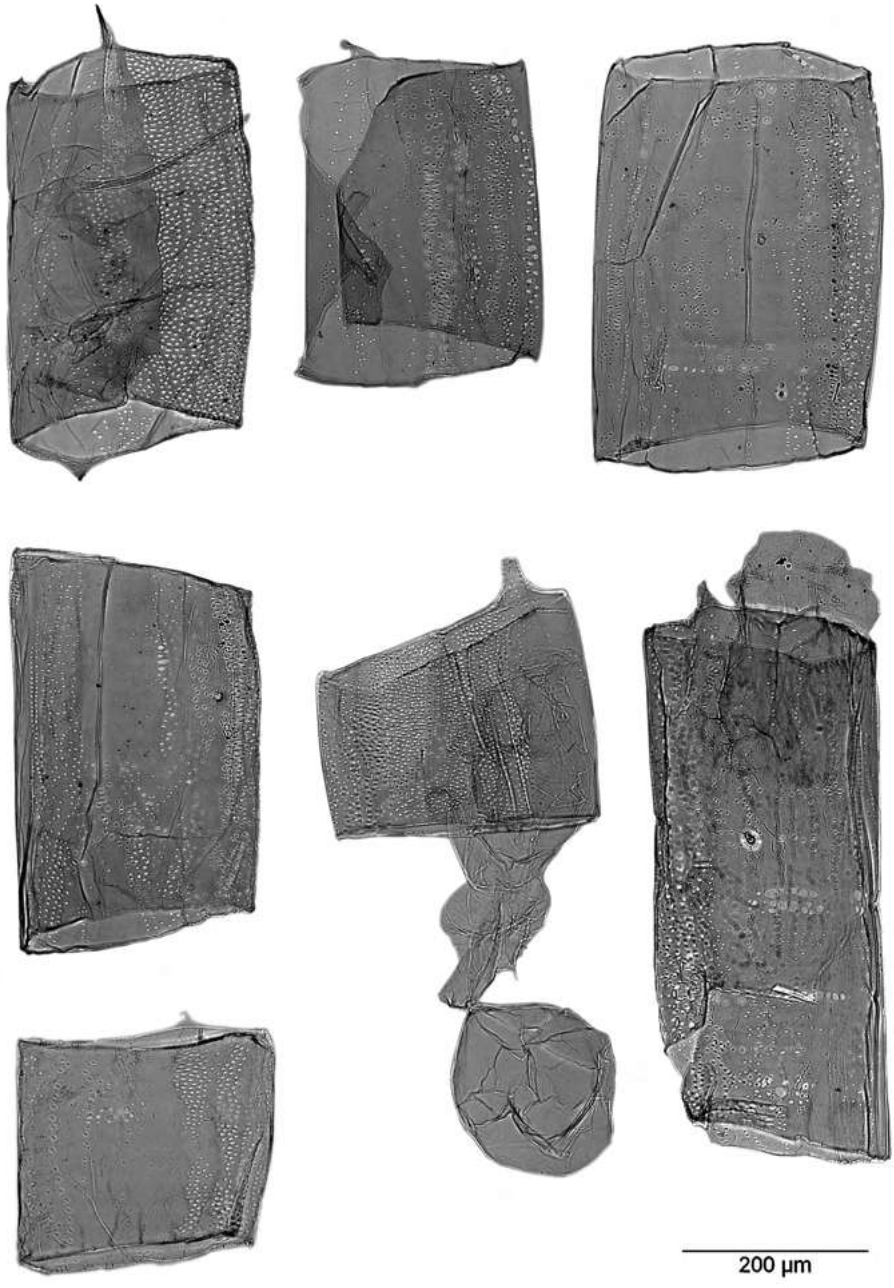
Areas without any pits: regularly present; small or large.

Tyloses: often present.

Helical thickenings: absent.

Notes on identification: See *Shorea* subg. *Anthoshorea* (p. 314).

Quantitative data:
Vessel elements (188–)376(–573) μm long, and (139–)312(–421) μm wide; l/w ratio 1.2.
Intervessel pit borders (3.7–) 6.6(–12.2) μm in vertical diameter; pit apertures (0.8–) 1.9(–4.5) μm.
Fibers 1230 μm long, 21.0 μm wide. Fiber wall thickness 4.3 μm (weighted averages).



Shorea subg. *Shorea*

Shorea* subg. *Shorea (Dipterocarpaceae)

Trade names: balau, selangan batu no. 1 – no. 2 (MY, DE), selangan batu tatuk, balau bukit, hitam, kumus hitam, laut, laut merah, sengkawang, damar laut (MY), balau bunga, bangkirai, semantok lungkik (ID), yakal (PH), teng (TH), sal (IN).
DIN EN 13556 : 2003 code: SHRB.
CITES regulations: not protected.
Geographic distribution: India, Pakistan, Sri Lanka, Indochina, Indomalesia.

Vessel elements: rather short (length about 310 μm, shorter than the other subgenera of *Shorea*, width about 280 μm); mostly barrel-shaped, sometimes drum-shaped.

Tails: rather short with abrupt transition, rarely longer with gradual transition.

Perforation plates: simple, extending over the entire lumen; horizontal.

Intervessel pits: alternate; vertical diameter 3–5–9 μm; wide fields; apertures oval.

Vessel-ray pits: VAS; apertures circular or oval, rather small; in horizontal rows (1–3 rows per ray cell).

Pits to axial parenchyma cells: similar to vessel-ray pits but somewhat larger; arranged in vertical bands.

Pits to fibers: present in long single vertical rows.

Pits to tracheids: similar to intervessel pits; in vertical strips, 3–5 pits wide.

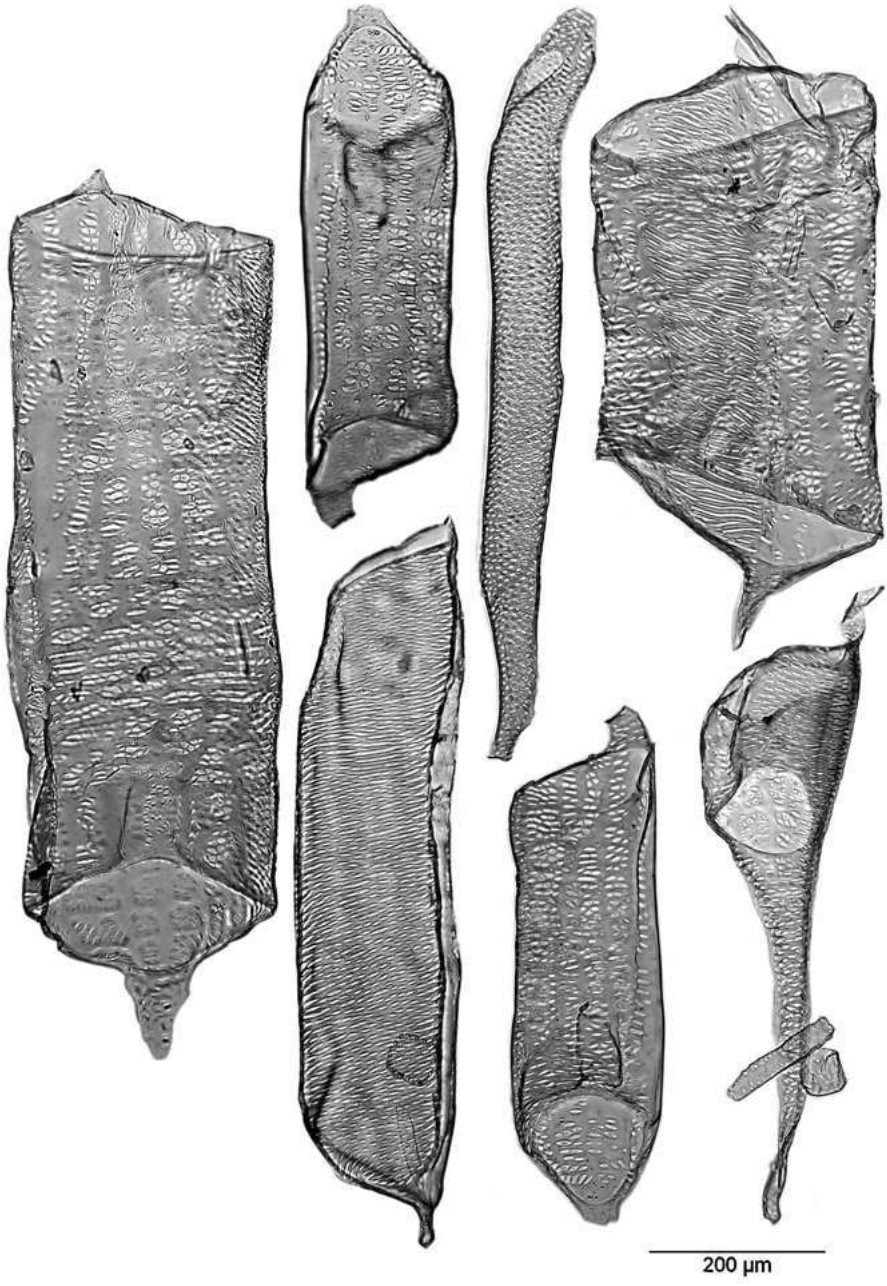
Areas without any pits: regularly present; small or large.

Tyloses: often present.

Helical thickenings: absent.

Notes on identification: See *Shorea* subg. *Anthoshorea* (p. 314).

Quantitative data:
Vessel elements (135–)312(–568) μm long, and (161–)279(–333) μm wide; l/w ratio 1.2.
Intervessel pit borders (2.6–)4.7(–9.4) μm in vertical diameter; pit apertures (0.4–)1.1(–3.0) μm.
Fibers 905 μm long, 18.2 μm wide. Fiber wall thickness 4.7 μm (weighted averages).



Hevea brasiliensis

Hevea brasiliensis (Willd. *ex* A. Juss.) Müll. Arg. (Euphorbiaceae)

Trade names: rubberwood; hevea (GB, DE, MY, TH, IN); Gummibaum (DE); seringa, seringeira (BR); kayu karet (ID); arbol de caucho (sAM).
DIN EN 13556 : 2003 code: HVBR.
CITES regulations: not protected.
Geographic distribution: native to tropical South America, widely cultivated in Indomalesia and Africa.

Vessel elements: rather long and often slim (length about 550 μm, width about 260 μm); mainly tube-shaped (those with a smaller diameter), sometimes barrel-shaped (those with a larger diameter).

Tails: short with abrupt transition or very long with gradual transition.

Perforation plates: simple, extending over the entire lumen; inclined or transverse.

Intervessel pits: alternate; vertical diameter 5–8–12 μm; numerous; apertures slit-like, often coalescent; sometimes out of center (irregular appearance).

Vessel-ray pits: VAS; rarely present; window-like (rectangular) or elongated horizontally (gash-like).

Pits to axial parenchyma cells: similar to vessel-ray pits in size and shape; cross-fields well-defined.

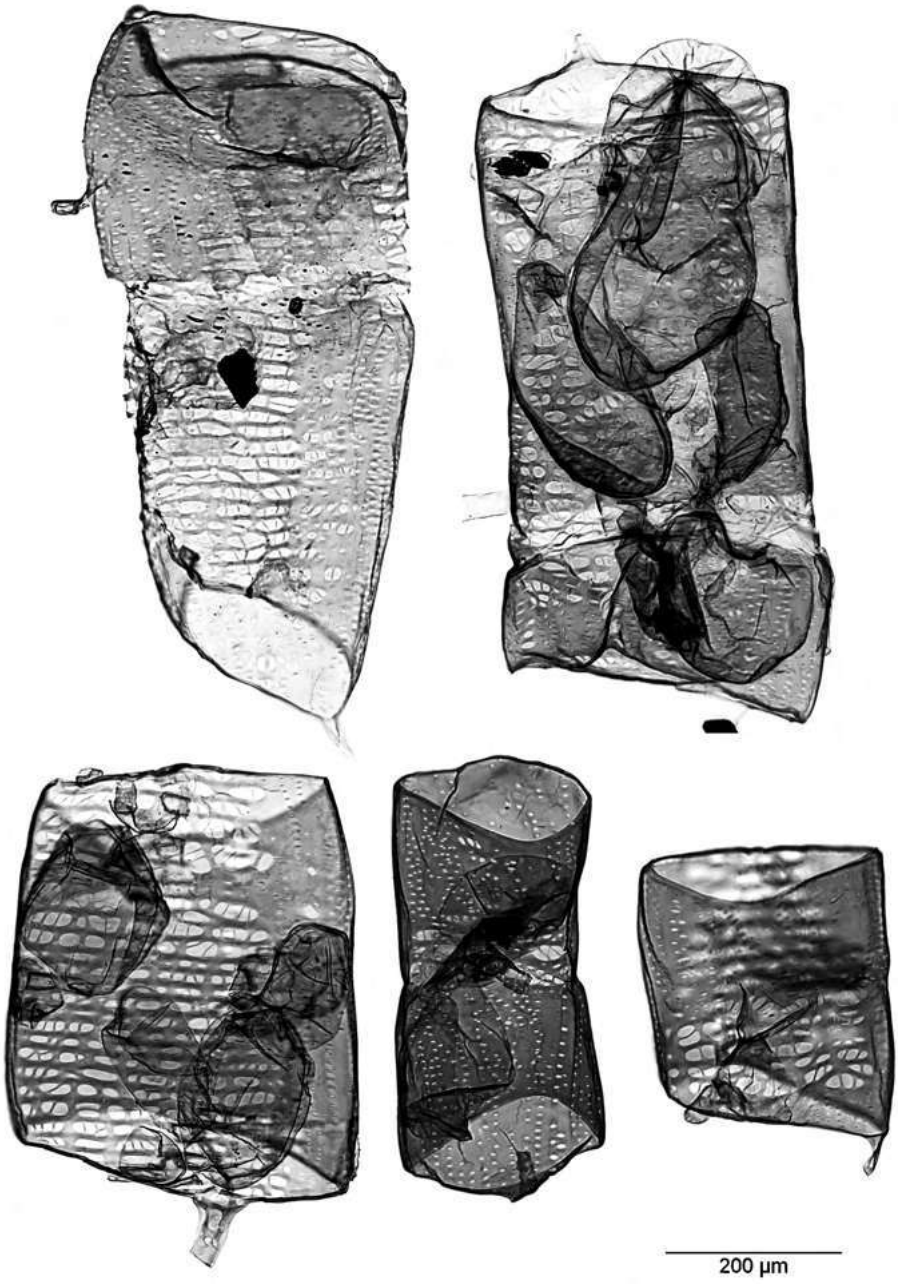
Pits to fibers: present in single vertical rows.

Areas without any pits: regularly present; small.

Tyloses: present.

Helical thickenings: absent.

Quantitative data:
Vessel elements (298–)551(–854) μm long, and (66–)259(–386) μm wide; l/w ratio 2.2.
Intervessel pit borders (4.6–)8.1(–11.9) μm in vertical diameter; pit apertures (0.9–)1.7(–3.3) μm.
Fibers 1190 μm long, 24.4 μm wide. Fiber wall thickness 3.1 μm (weighted averages).



Castanopsis argentea

Castanopsis argentea (Blume) A. DC. (Fagaceae)

Trade names: berangan, saninten, New Guinea oak (PG); Malayan chestnut, jertek tangga kata (MY); Philippine chestnut (PH); katia (MM); ko (LA, TH); ko-nam (TH).

DIN EN 13556:2003 code: not listed.

CITES regulations: not protected.

Geographic distribution: Sumatra and Java. (*Castanopsis* spp.: India, Pakistan, Sri Lanka, Indochina, Indomalesia).

Vessel elements: rather large and wide (length about 480 μm, width about 310 μm); mainly barrel-shaped (those with a larger diameter), sometimes tube-shaped (those with a smaller diameter).

Tails: short with abrupt transition.

Perforation plates: simple, extending over the entire lumen; horizontal or slightly inclined.

Intervessel pits: absent (due to almost exclusively solitary vessels in intact wood).

Vessel-ray pits: VAS; forming remarkably extensive pitted areas with single, horizontal rows of large to sometimes smaller pits (two size classes).

Pits to fibers: present in single vertical rows.

Pits to tracheids: numerous, arranged in vertical sinuous strips (2–3 pits wide), apertures oval; vertical diameter 5–7–10 μm.

Areas without any pits: regularly present; small to large.

Tyloses: present.

Helical thickenings: absent.

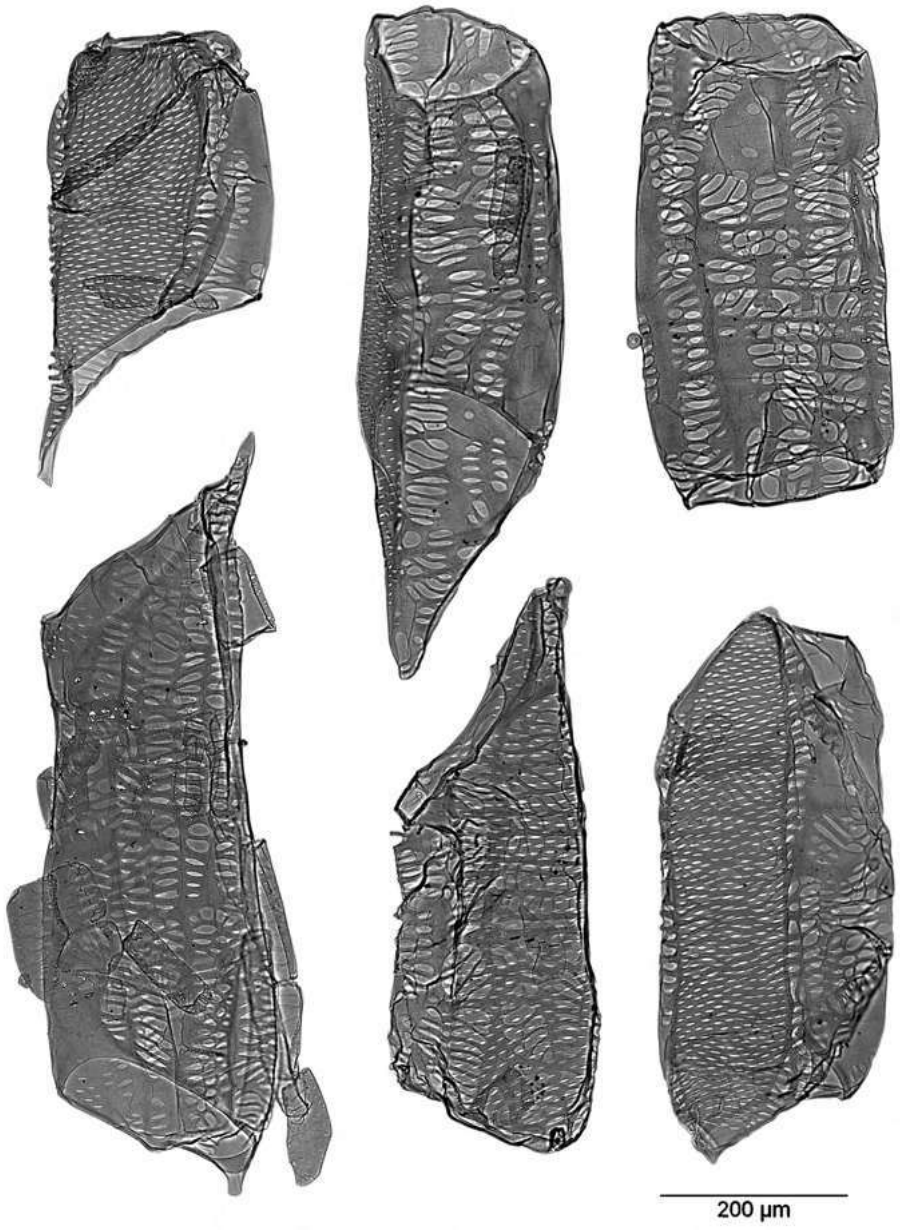
Notes on identification: *Castanopsis* spp. are similar to *Lithocarpus* spp. and *Castanea* spp. (not treated in this atlas).

Quantitative data:

Vessel elements (204–)479(–704) μm long, and (55–)314(–455) μm wide; l/w ratio 1.6.

Vessel to tracheid pit borders (4.5–)7.1(–10.3) μm in vertical diameter; pit apertures (0.5–)1.2(–3.5) μm.

Fibers 1160 μm long, 22.7 μm wide. Fiber wall thickness 2.8 μm (weighted averages).



Litsea resinosa

Litsea resinosa Blume (Lauraceae)

Trade name: medang (ID, MY).
DIN EN 13556:2003 code: not listed.
CITES regulations: not protected.
Geographic distribution: Java, Borneo, Peninsular Malaysia.

Vessel elements: rather long (length about 490 μm, width about 240 μm); mainly barrel-shaped.

Tails: long or short with gradual transition.

Perforation plates: simple; extending over the entire lumen; inclined (parallelogram or trapezium-shaped) to horizontal. Sometimes with tapering end walls.

Intervessel pits: alternate; vertical diameter 5–8–12 μm; covering wide areas and the tails; apertures slit-like.

Vessel-ray pits: VAS; quite large, window-like, circular or elongated horizontally (gash-like), sometimes vertically (palisade).

Pits to axial parenchyma cells: similar to vessel-ray pits in size and shape.

Pits to fibers: present in longitudinal single rows.

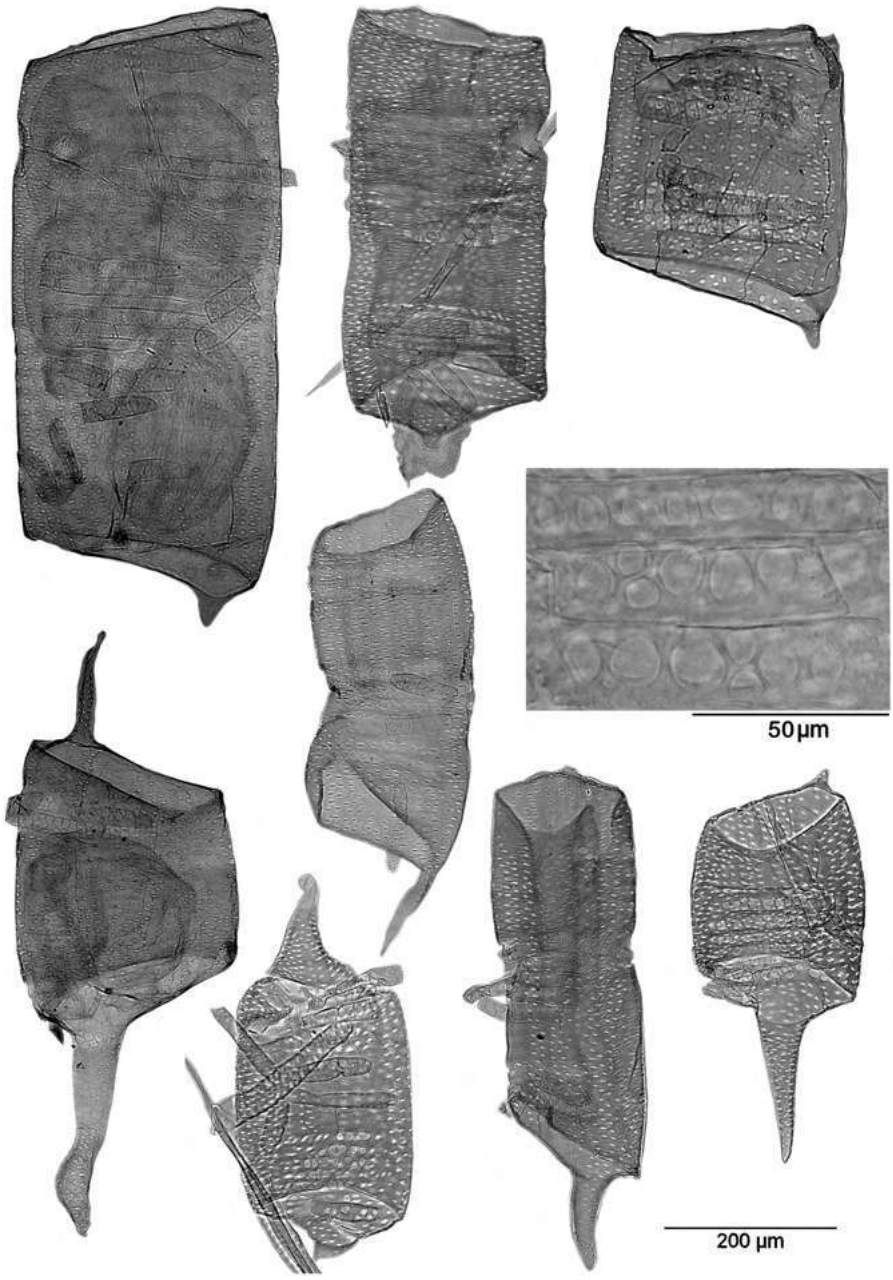
Areas without any pits: regularly present; small to large.

Tyloses: present.

Helical thickenings: absent.

Notes on identification: vessel element features of this species resemble those of some Anacardiaceae but vessel-ray pits are larger and arranged in more well-defined blocks.

Quantitative data:
Vessel elements (290–)486(–671) μm long, and (173–)242(–303) μm wide; l/w ratio 2.0.
Intervessel pit borders (5.0–)7.8(–12.0) μm in vertical diameter; pit apertures (0.7–)1.8(–3.4) μm.
Fibers 1055 μm long, 26.3 μm wide. Fiber wall thickness 6.7 μm (weighted averages).



Eucalyptus globulus

Eucalyptus globulus Labill. (Myrtaceae)

Trade names: blue gum, Tasmanian blue-gum, southern blue-gum (AU).
DIN EN 13556:2003 code: EUGL.
CITES regulations: not protected.
Geographic distribution: native to Australia; introduced in many countries, most notably in the Mediterranean, North Africa, Southwest Asia, South Africa, North America, Temperate South America.

Eucalyptus spp. plantations provide short fiber raw material for the pulp and paper industry.

Vessel elements: slim (length about 310 μm, width about 240 μm); barrel or tube-shaped.

Tails: long with gradual or abrupt transition or short with abrupt transition.

Perforation plates: simple, extending over the entire lumen; horizontal or slightly inclined.

Intervessel pits: very rarely present; alternate; vertical diameter 3–5–9 μm; arranged in fields; present in the tails; similar in size and shape to pits to tracheids; apertures slit-like.

Vessel-ray pits: VAS; large apertures circular to oval (see detail in box); sometimes with adhering parenchyma cells.

Pits to tracheids: in vertical strips 2–3 pits wide.

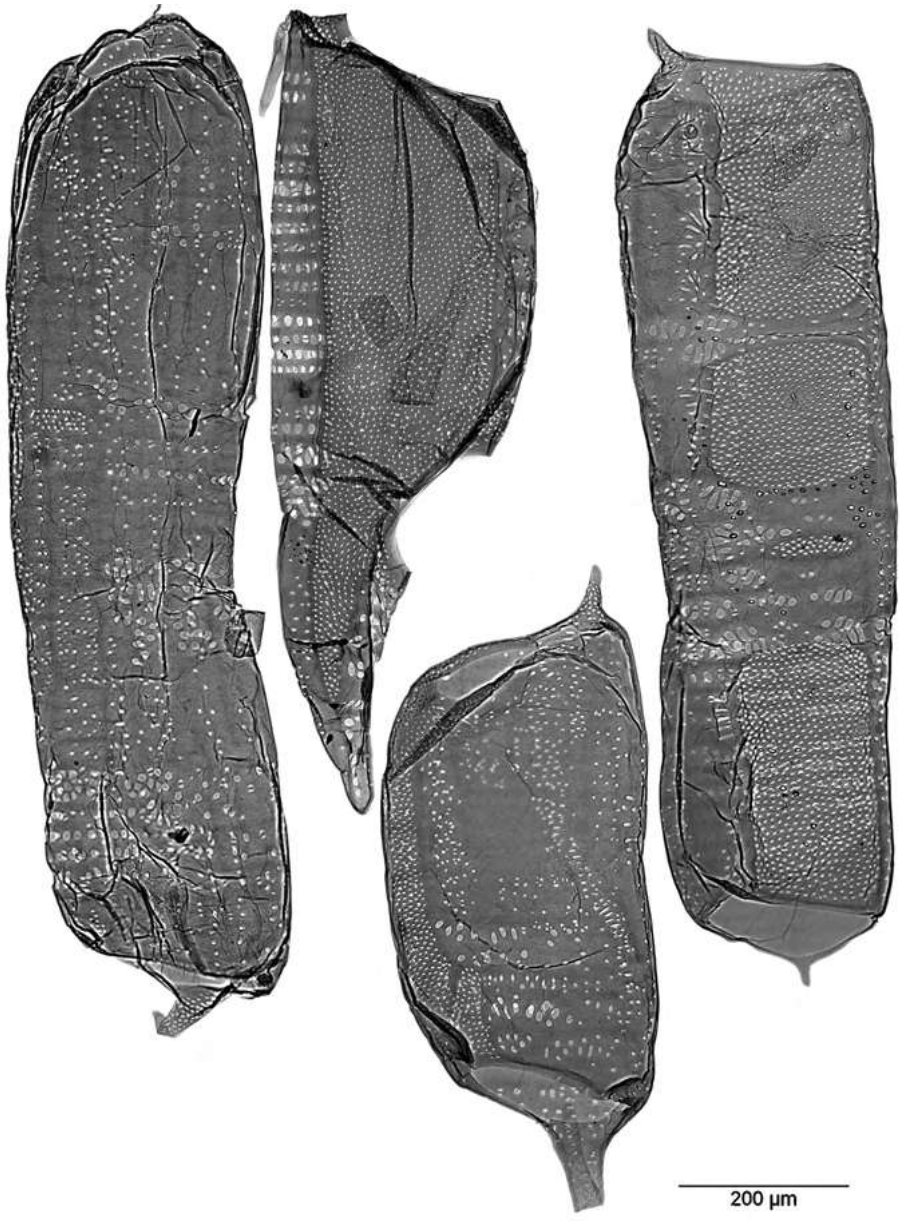
Pits to fibers: regularly present in single vertical rows.

Areas without any pits: if present, rather small.

Tyloses: present.

Helical thickenings: absent.

Quantitative data:
Vessel elements (182–)309(–615) μm long, and (160–)243(–358) μm wide; l/w ratio 1.4.
Intervessel pit borders (3.1–)5.2(–8.8) μm in vertical diameter; pit apertures (0.6–)1.4(–3.4) μm.
Fibers 800 μm long, 18.9 μm wide. Fiber wall thickness 6.0 μm (weighted averages).



Syzygium dyerianum

Syzygium dyerianum (King) Chantaran. & J. Parn. (Myrtaceae)

Trade name: kelat (MY).
DIN EN 13556:2003 code: not listed.
CITES regulations: not protected.
Geographic distribution: Thailand, Peninsular Malaysia.

Vessel elements: quite large and elongated (length about 760 μm, width about 290 μm); often tube-like, sometimes barrel-shaped.

Tails: long with gradual transition or short with abrupt transition.

Perforation plates: simple; slightly narrowed, slightly inclined or horizontal.

Intervessel pits: alternate; vertical diameter 3–6–8 μm; forming fields covering a wide area; also in the tails; apertures circular to oval.

Vessel-ray pits: VAS; distributed over a great part of the vessel element wall; arranged in exact horizontal rows; window-like circular to oval apertures or extended diagonally, giving rise to a reticulate pattern in cross fields.

Pits to axial parenchyma cells: in vertically oriented blocks, with circular apertures.

Areas without any pits: regularly present; small to large.

Tyloses: present.

Helical thickenings: absent.

Notes on identification: the vessel element features are in part similar to those observed in species of Dipterocarpaceae.

Quantitative data:
Vessel elements (278–)763(–1104) μm long, and (167–)285(–367) μm wide; l/w ratio 2.7.
Intervessel pit borders (2.5–)5.7(–8.0) μm in vertical diameter; pit apertures (0.7–)1.6(–3.1) μm.
Fibers 1345 μm long, 28.8 μm wide. Fiber wall thickness 8.8 μm (weighted averages).

Madhuca sericea

Madhuca sericea (Miq.) S. Moore (Sapotaceae)

Trade names: bitis, nyatoh batu (MY, ID).
DIN EN 13556 : 2003 code: MDXX (*Madhuca* spp.).
CITES regulations: not protected.
Geographic distribution: Peninsular Malaysia, Sumatra, Borneo. (*Madhuca* spp: India, Pakistan, Sri Lanka, Indochina, Indomalesia, Pacific Islands).

Vessel elements: long and slim (length about 730 μm, width about 170 μm); tube-shaped.

Tails: very long with gradual transition or short with abrupt or gradual transition.

Perforation plates: simple, often extending over the entire lumen; inclined.

Intervessel pits: alternate; vertical diameter 2–3–5 μm; numerous; large fields covering a wide area of the vessel element wall; also in the tails; apertures oval.

Vessel-ray pits: VAS; apertures window-like, circular to oval to elongated horizontally (gash-like); irregularly shaped.

Pits to axial parenchyma cells: similar to vessel-ray pits in size and shape.

Pits to tracheids: if present, in longitudinal well-defined strips (6–8 pits wide); similar to intervessel pits.

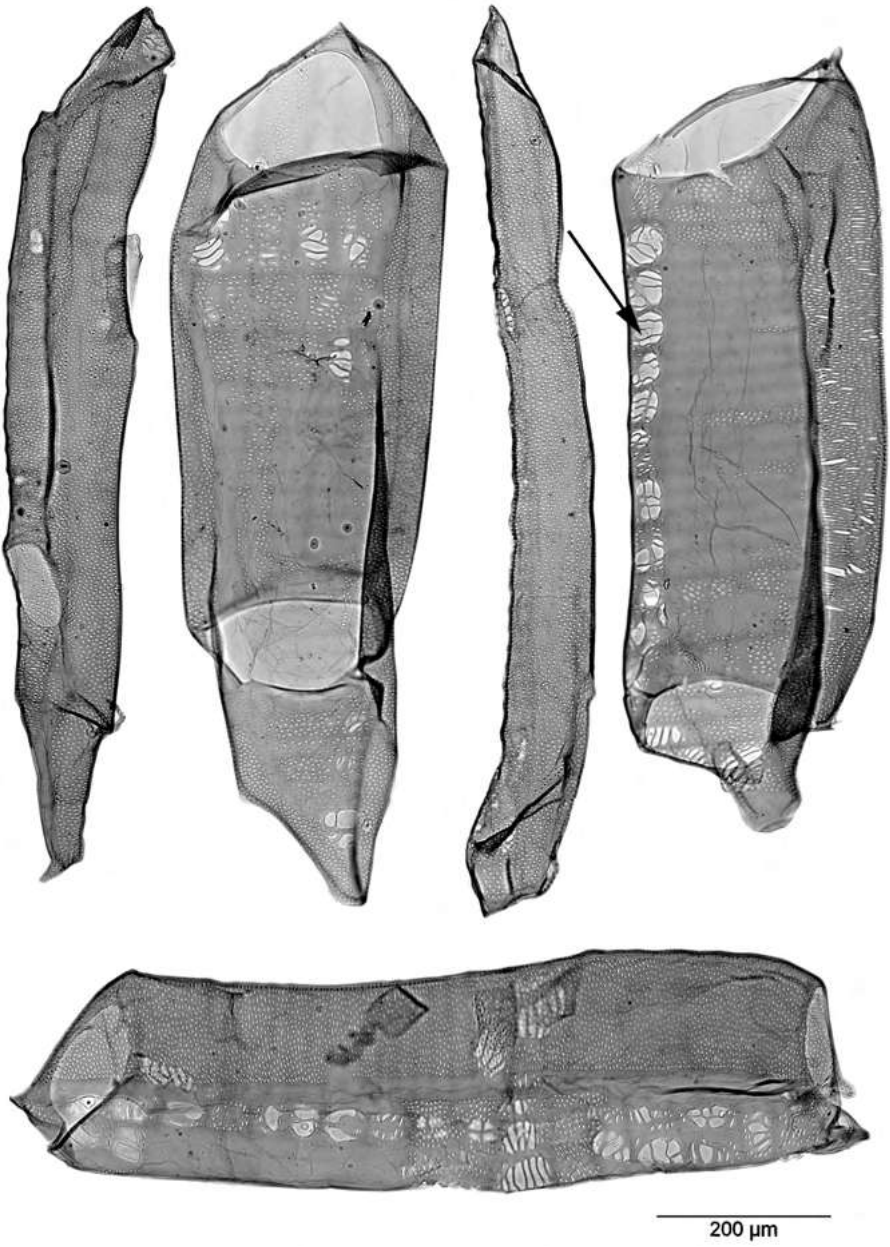
Pits to fibers: present in longitudinal single rows.

Areas without any pits: regularly present; large to very large.

Tyloses: often present.

Helical thickenings: absent.

Quantitative data:
Vessel elements (310–)725(–1103) μm long, and (84–)168(–196) μm wide; l/w ratio 4.2.
Intervessel pit borders (1.8–)3.0(–4.9) μm in vertical diameter; pit apertures (0.5–)0.8(–1.5) μm.
Fibers 1485 μm long, 27.5 μm wide. Fiber wall thickness 7.7 μm (weighted averages).



Palaquium sp.

***Palaquium* sp.** (Sapotaceae)

Trade names: nyatoh (MY, ID, DE); chay (VN); pencil cedar, red planchonella (PG); pali (IN); nato (PH); kha-nunnok (TH); riam jangka (MY-swk); hangkang, balam teruing puteh, balam masin, kayu tanjung hutan, mayang, taban (MY, ID); moordooke (AU).

DIN EN 13556:2003 code: PPXX.

CITES regulations: not protected.

Geographic distribution: India, Sri Lanka, South China, Indochina, Indomalesia and Pacific Islands.

Vessel elements: large (length about 690 μm, width 290 μm); elongated: those with a smaller diameter are tube-shaped; those with a larger diameter are barrel-shaped.

Tails: short to very long, with gradual transition.

Perforation plates: simple or very rarely scalariform (with 3–10 bars, Richter & Dallwitz 2000); extending over the entire lumen; inclined; few positioned laterally.

Intervessel pits: regularly present; alternate; vertical diameter 3–5–8 μm; large areas with numerous intervessel pits; apertures circular to oval.

Vessel-ray pits: VAS; in two size classes: larger ones mostly elongated, in groups of 4–8 pits per ray cell (arrow); smaller ones similar to intervessel pits, cross-fields with 3–5 pit rows; apertures oval.

Areas without any pits: regularly present; large to very large.

Tyloses: present.

Helical thickenings: absent.

Quantitative data:

Vessel elements (434–)691(–846) μm long, and (132–)292(–406) μm wide; l/w ratio 2.4.

Intervessel pit borders (3.1–)4.9(–7.8) μm in vertical diameter; pit apertures (0.6–)1.2(–ß2.0) μm.

Fibers 1900 μm long, 27.5 μm wide. Fiber wall thickness 6.6 μm (weighted averages).

Hardwoods with scalariform perforation plates
introductory comments

This chapter deals with six wood species that possess exclusively scalariform perforation plates. The monocot *Cocos nucifera* (p. 270) also has scalariform perforations. The bars can be thick or thin and differ in number (Fig. 15). These genera could also be included in the groups of APS (p. 272) and VAS (p. 296) but the highly diagnostic feature of the scalariform perforation plates justifies assigning them to a separate group.

There are some genera in the VAS group (p. 296) that, according to literature reports, occasionally have scalariform perforation plates (*Campnosperma* sp. (p. 298) and *Palaquium* sp. (p. 334)).

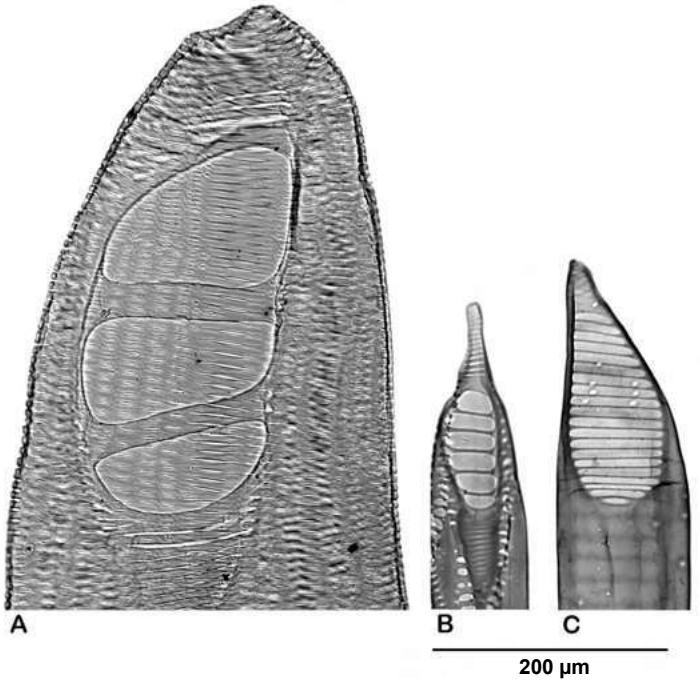


Figure 15. Scalariform perforation plates.
A: 2–4 thick bars, *Cocos nucifera*. – B: 3–10 thin bars, *Rhizophora* sp. – C: 15–30 thin bars, *Liquidambar formosana*.

Figure 16 shows the variation of vessel element length and width as well as the diameter of the apertures and borders of intervessel pits. The boxplots of the length show similar dimensions for *Alniphyllum pterospermum*, *Ilex triflora*, *Liquidambar formosana* and *Schima superba* (Fig. 16a) whereas the values of the width vary (Fig. 16b). *Nyssa javanica* possesses the longest and widest vessel elements and *Rhizophora* sp. the shortest ones. The apertures of intervessel pits of all six species are of similar size with small deviations, the vertical diameters of the pit borders are correspondingly larger.

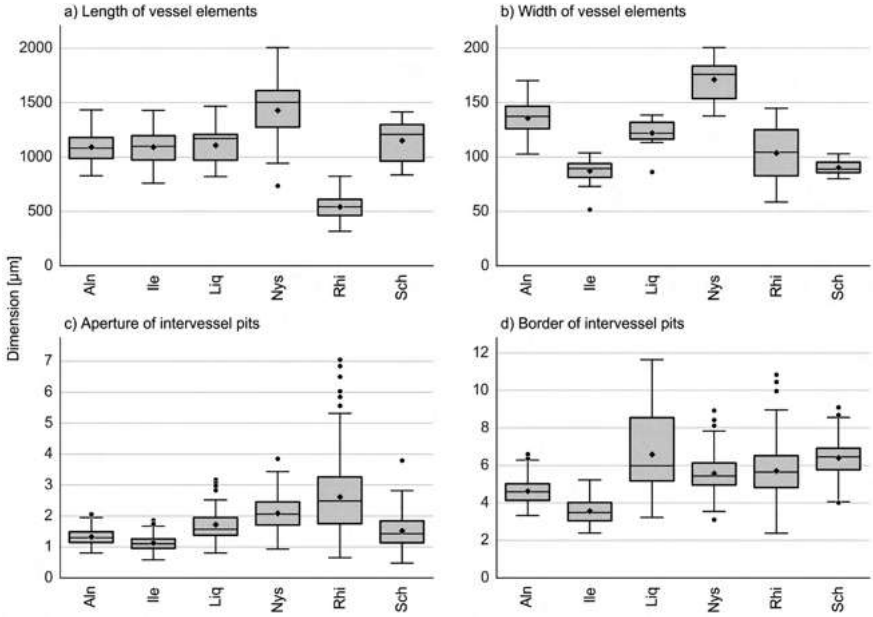


Figure 16. Boxplots of six wood genera/species (length and width of the vessel elements, diameter of pit apertures and borders):
Aln = *Alniphyllum pterospermum*; Ile = *Ilex triflora* var. *kanehirai*; Liq = *Liquidambar formosana*; Nys = *Nyssa javanica*; Rhi = *Rhizophora* sp.; Sch = *Schima superba*.

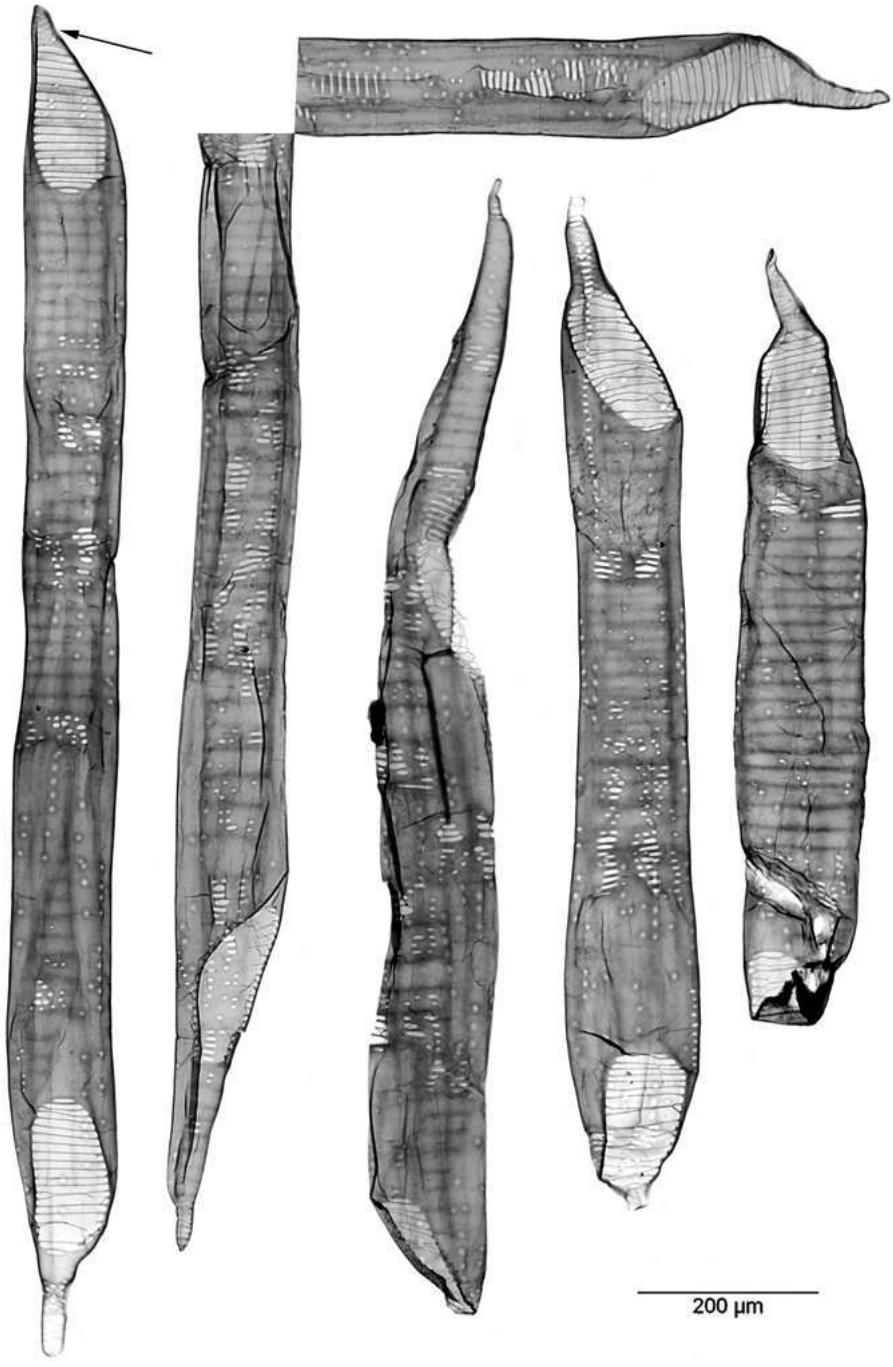
Table 2, a comparison of the eight species with scalariform perforation plates, shows similarities and major differences.

Vessel elements of *Nyssa* spp. are similar to those of *Liriodendron* spp. and *Alniphyllum* spp. *Nyssa* spp. have more numerous thin bars and opposite intervessel pits that distinguish them from *Liquidambar* spp. and *Schima* spp. (see notes on identifications at the end of the individual species descriptions). *Rhizophora* stands out with very extensive scalariform intervessel pits.

Table 2. Species with scalariform perforation plates.

Species	Length of vessel elements (µm)	Number of bars	Intervessel pits	Size of intervessel pits (µm)	Pits to ray parenchyma cells
Alniphyllum	830–1080–1430	8–15	Opposite/alternate	3–5–7	APS
Cocos	1450–2840–4330	2–4	Scalariform	–	–
Ilex	760–1100–1430	20–40	Opposite	2–4–5	APS
Liquidambar	820–1170–1470	15–30	Scalariform	3–6–12	VAS
*Liriodendron**	–	3–5–10	Scalariform/opposite	10–11	VAS
Nyssa	740–1500–2000	20–30(–50)	Opposite/scalariform	3–5–9	VAS
Rhizophora	320–540–820	3–10	Scalariform	2–6–11	VAS
Schima	840–1210–1410	5–15	Scalariform	4–7–9	VAS

**Liriodendron* is not treated in this atlas but is commonly used for pulp and paper from North America and temperate China (data: Richter & Dallwitz 2000).



Liquidambar formosana

Liquidambar formosana Hance (Altingiaceae)

Trade names: Chinese sweet gum, Formosan sweet gum (US), feng xiang shu (CN).
DIN EN 13556:2003 code: LQST (*Liquidambar styraciflua* L.).
CITES regulations: not protected.
Geographic distribution: China and Indochina. (*Liquidambar* spp.: temperate Asia (*L. formosana*), North America, Mexico and Central America).

Vessel elements: quite long and slim (length about 1170 μm, width about 120 μm), tube-shaped.

Tails: short or long, with gradual transition.

Perforation plates: scalariform (15–30 thin bars); extending over the entire lumen; inclined.

Intervessel pits: rarely present; vertical diameter 3–6–12 μm; scalariform.

Vessel-ray pits: VAS; oval or elongated horizontally (gash-like); giving rise to a scalariform pattern in the cross-fields.

Pits to fibers: often present in single vertical rows.

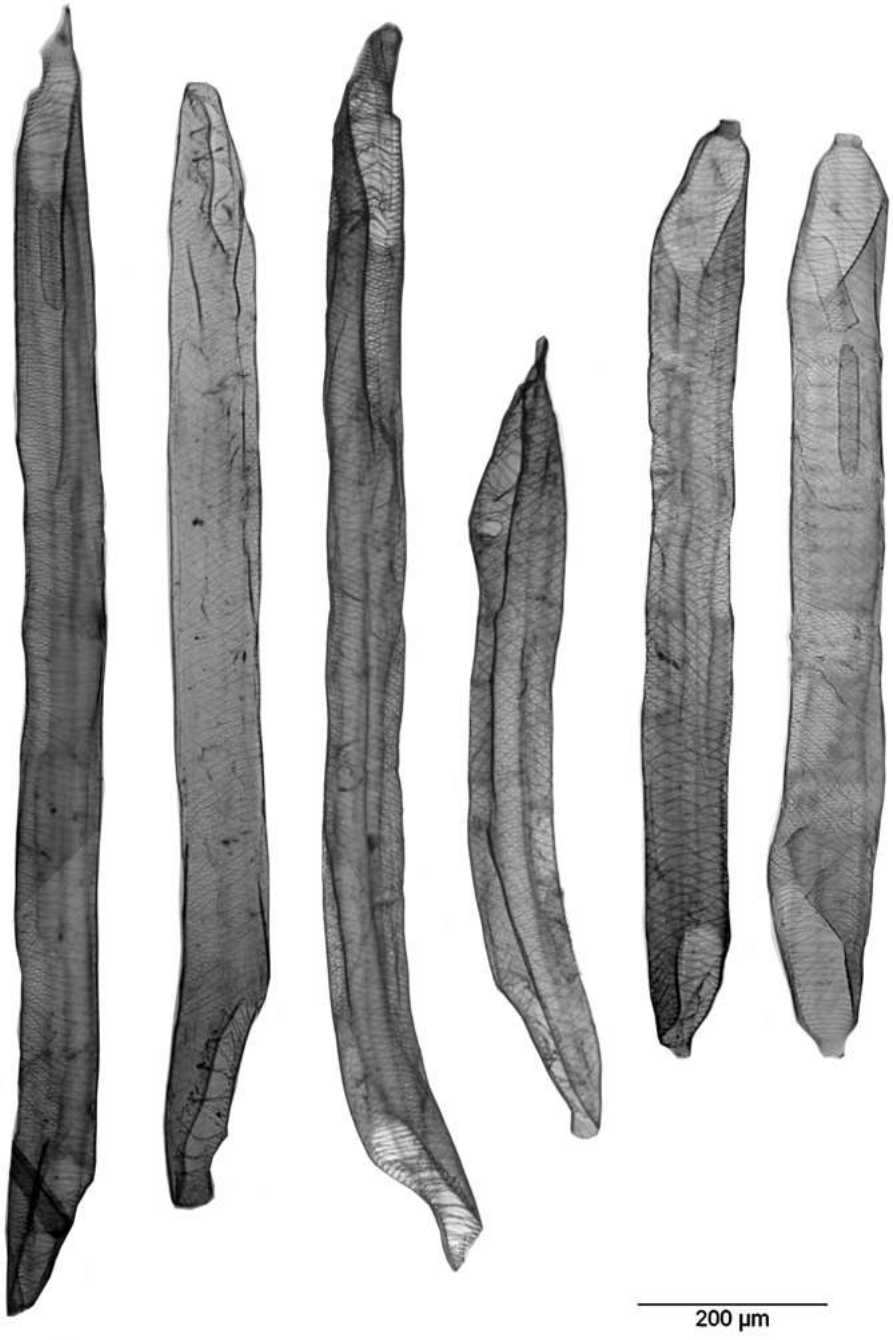
Areas without any pits: regularly present; large.

Tyloses: present.

Helical thickenings: sometimes present at the end of the tails (arrow).

Notes on identification: Vessel elements of *Liquidambar* spp. are similar to those of *Schima* spp. but the number of bars differs (*Liquidambar* spp. 15–30 bars, *Schima* spp. 5–15 bars). They cannot be easily distinguished in mixed pulp.

Quantitative data:
Vessel elements (822–)1172(–1467) μm long, and (86–)122(–138) μm wide; l/w ratio 9.4.
Intervessel pit borders (3.2–)6.0(–11.6) μm in vertical diameter; pit apertures (0.8–)1.6(–3.2) μm.
Fibers 1720 μm long, 27.3 μm wide. Fiber wall thickness 2.8 μm (weighted averages).



Ilex triflora var. *kanehirai*

Ilex triflora var. ***kanehirai*** (Yamamoto) S.Y. Hu (Aquifoliaceae)

Trade names: san hua dong qing, yuan bian zhong (CN), kecemang (ID).
DIN EN 13556:2003 code: not listed.
CITES regulations: not protected.
Geographic distribution: China, Taiwan; The genus *Ilex* is almost cosmopolitan and occurs throughout tropical, subtropical and temperate regions of the world.

Vessel elements: quite long and slim (length about 1100 μm, width about 90 μm); tube-shaped.

Tails: long or short with gradual transition.

Perforation plates: scalariform (20–40 thin bars); extending over the entire lumen; inclined.

Intervessel pits: opposite; occasionally merging into a scalariform pattern; vertical diameter 2–4–5 μm; oval to almost rectangular; sometimes covering a wide area of the vessel element wall.

Vessel-ray pits: APS; rarely present; arranged in blocks of several horizontally oriented, elongated pits (gash-like).

Pits to fibers: in single vertical rows.

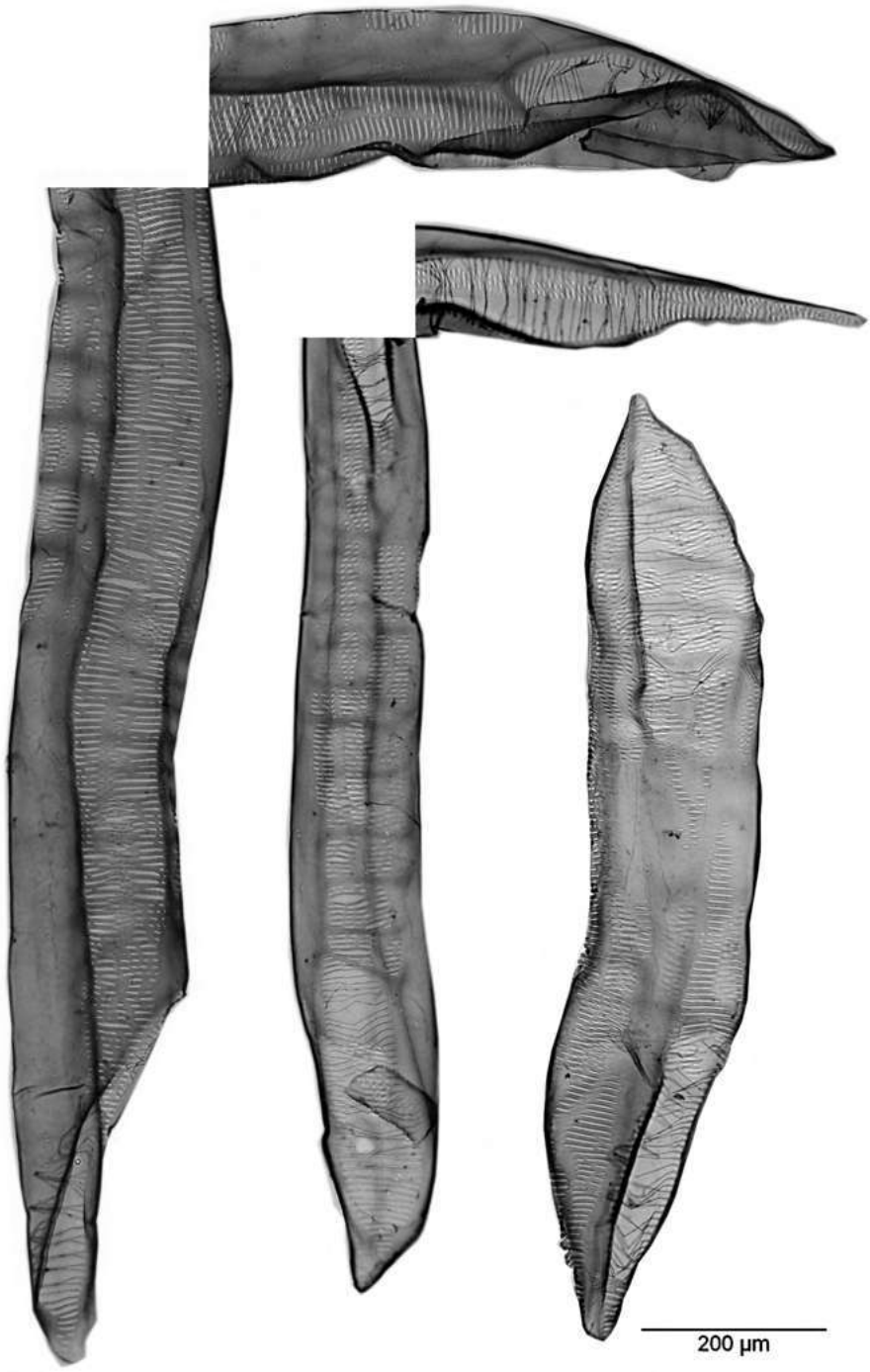
Areas without any pits: regularly present; very large.

Tyloses: absent.

Helical thickenings: present in vessel elements, possibly also in fibers.

Notes on identification: In pulp and paper, the vessel elements of *Ilex* spp. are often accompanied by fibers with helical thickenings if they originate from subtropical or temperate regions. Most tropical species (like *I. cymosa*) do not have helical thickenings (Baas 1973).

Quantitative data:
Vessel elements (759–)1098(–1430) μm long, and (52–)89(–104) μm wide; l/w ratio 12.4.
Intervessel pit borders (2.4–)3.5(–5.2) μm in vertical diameter; pit apertures (0.6–)1.1(–1.9) μm.
Fibers 1290 μm long, 34.4 μm wide. Fiber wall thickness 4.1 μm (weighted averages).



Nyssa javanica

Nyssa javanica (Blume) Wangerin (Nyssaceae)

Trade names: kalay, hirun, chilauni (IN); thang khok, khai pla, khueng khak, mueat khon khao (TH); kirung, hirung, wuru gading, kapi dengkung (ID); terang bulu (MY); theun (LA). Similar species occur in North America.
DIN EN 13556:2003 code: NYAQ (*Nyssa aquatica*).
CITES regulations: not protected.
Geographic distribution: India, S-China, Malaysia, Indonesia, Indochina.

Vessel elements: very long and slim (length about 1500 μm, width about 180 μm); tube-shaped.

Tails: long with gradual transition.

Perforation plates: scalariform (20–30(–50) thin bars); bars sometimes branched; extending over the entire lumen; inclined.

Intervessel pits: opposite and scalariform; vertical diameter 3–5–9 μm; more or less rectangular or elongated horizontally.

Vessel-ray pits: VAS; oval or elongated horizontally.

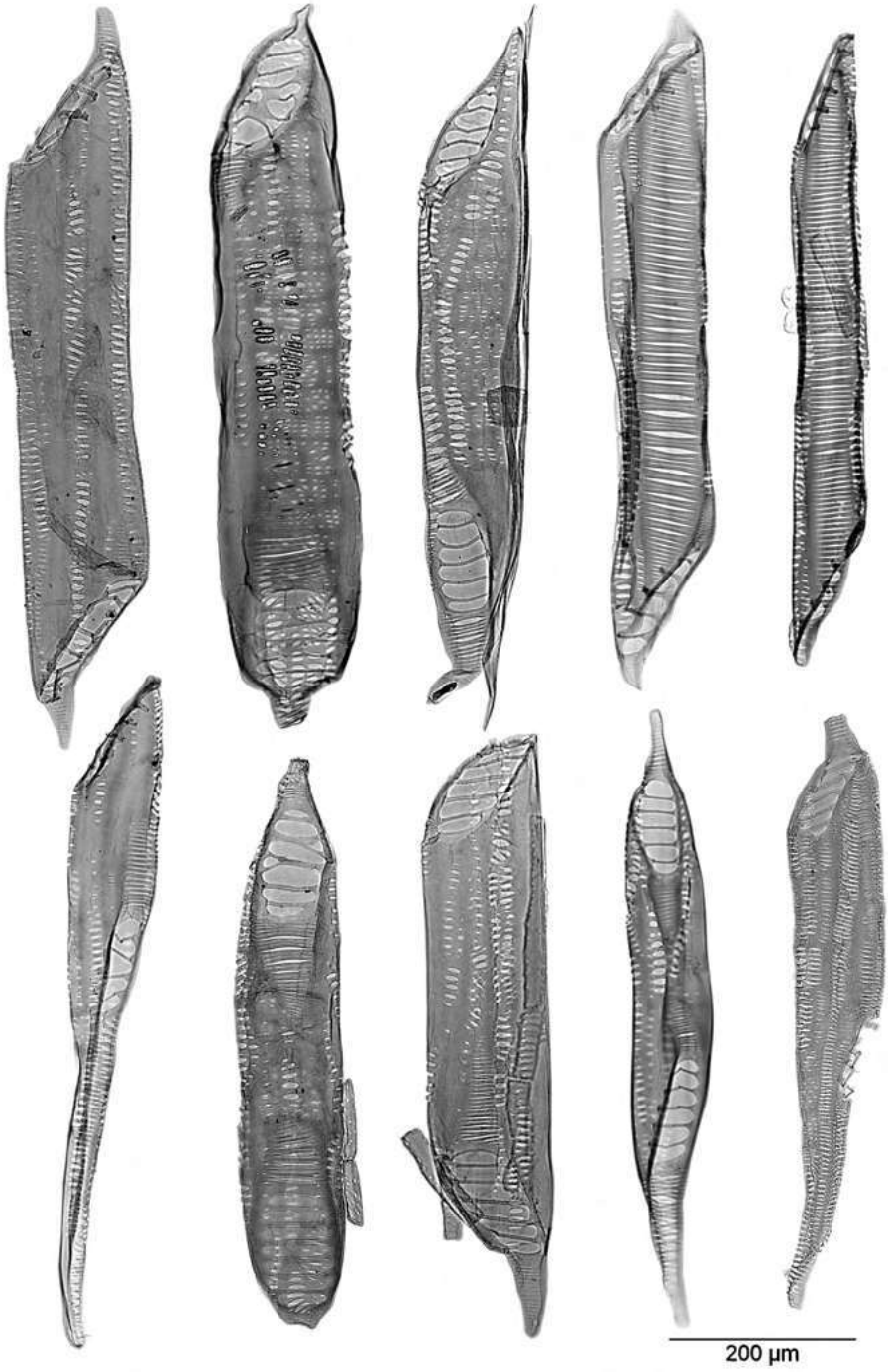
Pits to fibers: circular to oval in single vertical rows.

Areas without any pits: regularly present; large to very large.

Tyloses and helical thickenings: absent.

Notes on identification: Vessel elements of *Nyssa* spp. are similar to those of *Liriodendron* spp. and *Alniphyllum* spp. The pits to ray parenchyma cells differ (*Nyssa* spp. and *Liriodendron* spp. = VAS type, *Alniphyllum* spp. = APS type).
Nyssa spp. and *Liriodendron* spp. possess more numerous thin bars and opposite intervessel pits that distinguish them from *Liquidambar* spp. and *Schima* spp.

Quantitative data:
Vessel elements (736–)1502(–2006) μm long, and (137–)176(–200) μm wide; l/w ratio 8.7.
Intervessel pit borders (3.1–)5.4(–8.9) μm in vertical diameter; pit apertures (0.9–)2.1(–3.9) μm.
Fibers 1350 μm long, 27.0 μm wide. Fiber wall thickness 3.0 μm (weighted averages).



Rhizophora sp.

Rhizophora sp. (Rhizophoraceae)

Trade name: red mangrove.
DIN EN 13556:2003 code: not listed.
CITES regulations: not protected.
Geographic distribution: *Rhizophora* is a pantropical mangrove genus, also widely distributed in the Asia-Pacific region.

Vessel elements: quite long and slim (length about 540 μm, width about 100 μm); tube-shaped.

Tails: long or short with gradual transition, covered with pits.

Perforation plates: scalariform (3–10 thin bars); bars can be branched; extending over the entire lumen; inclined (parallelogram or trapezium).

Intervessel pits: scalariform; vertical diameter 2–6–11 μm.

Vessel-ray pits: VAS; oval to rectangular, sometimes opposite.

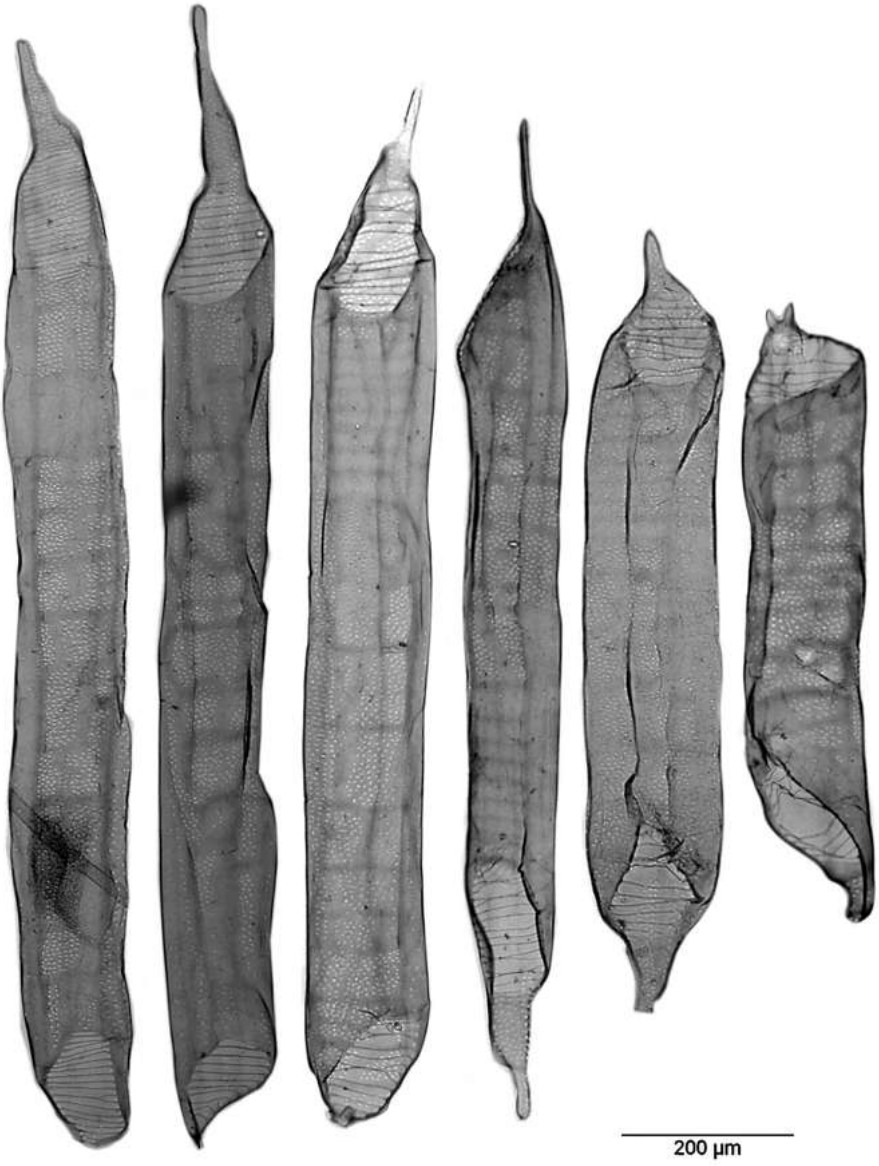
Pits to axial parenchyma cells: oval to gash-like; in vertical bands.

Areas without any pits: regularly present; large.

Tyloses: present.

Helical thickenings: absent.

Quantitative data:
Vessel elements (317–)543(–824) μm long, and (59–)104(–145) μm wide; l/w ratio 5.3.
Intervessel pit borders (2.4–)5.6(–10.8) μm in vertical diameter; pit apertures (0.7–)2.5(–7.1) μm.
Fibers 1065 μm long, 26.4 μm wide. Fiber wall thickness 5.9 μm (weighted averages).



Alniphyllum pterospermum

Alniphyllum pterospermum Matsum. (Styracaceae)

Trade names: chi-yang-ye (CN); mee dong (LA).
DIN EN 13556:2003 code: not listed.
CITES regulations: not protected.
Geographic distribution: China, Taiwan, Laos, Vietnam.

Vessel elements: rather long and slim (length about 1080 μm, width about 140 μm); tube-shaped.

Tails: mostly long with gradual transition, or short.

Perforation plates: scalariform (8–15 bars); bars can be branched; extending over the entire lumen; inclined.

Intervessel pits: alternate or opposite; vertical diameter 3–5–7 μm.

Vessel-ray pits: APS; similar to intervessel pits; forming well-defined blocks of a shape corresponding to the respective ray cells.

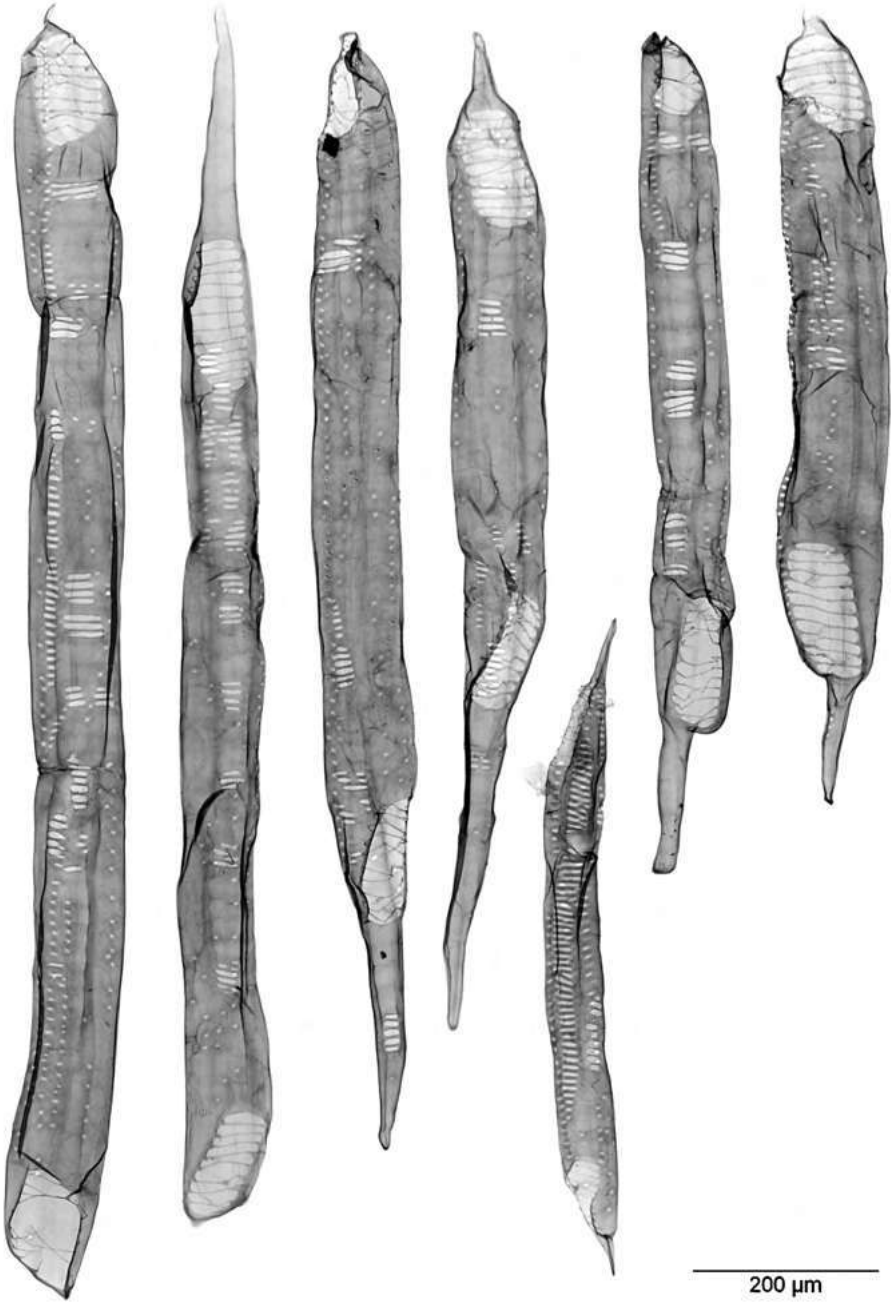
Pits to fibers: present in single vertical rows.

Areas without any pits: regularly present; large.

Tyloses and helical thickenings: absent.

Notes on identification: Vessel elements of *Alniphyllum* spp. are similar to those of *Nyssa* spp. (see *Nyssa javanica*, p. 342).

Quantitative data:
Vessel elements (828–)1081(–1433) μm long, and (103–)137(–170) μm wide; l/w ratio 8.1.
Intervessel pit borders (3.3–)4.6(–6.6) μm in vertical diameter; pit apertures (0.8–)1.3(–2.1) μm.
Fibers 1440 μm long, 35.7 μm wide. Fiber wall thickness 3.7 μm (weighted averages).



Schima superba

Schima superba Gardn. & Champ. (Theaceae)

Trade names: puspa, seru (ID); medang gatal, samak (MY); Chinese guger tree (TW, US); laukya (MM); gaobei, huazi (CN); needlewood (GB); chilauni (IN); thalo, champa dong (TH).
DIN EN 13556:2003 code: not listed.
CITES regulations: not protected.
Geographic distribution: China, Taiwan, Ryukyu Islands. The genus *Schima* also occurs in tropical Malaysia and Indonesia.

Vessel elements: long and slim (length about 1210 μm, width about 90 μm); tube-shaped.

Tails: long with gradual transition.

Perforation plates: scalariform (5–15 bars); bars can be branched; extending over the entire lumen; inclined.

Intervessel pits: rarely present; vertical diameter 4–7–9 μm; scalariform; forming vertical series of horizontally elongated pits.

Vessel-ray pits: VAS; two size classes; oval or elongated horizontally (gash-like) arranged in short to long horizontal rows (opposite) or in a ladder-like series (scalariform).

Pits to fibers: circular, often present in single vertical rows.

Areas without any pits: regularly present; large.

Tyloses: present.

Helical thickenings: may be present at the end of the tails.

Notes on identification: Vessel elements of *Schima* spp. are similar to those of *Liquidambar* spp. (see *Liquidambar formosana*, p. 338).

Quantitative data:
Vessel elements (836–)1208(–1414) μm long, and (80–)89(–103) μm wide; l/w ratio 13.6.
Intervessel pit borders (4.0–)6.5(–9.1) μm in vertical diameter; pit apertures (0.5–)1.4(–3.8) μm.
Fibers 1350 μm long, 30.5 μm wide. Fiber wall thickness 3.3 μm (weighted averages).

CONCLUSION

The identification of internationally traded wood and wood products is of prime importance in enforcing the legal utilization of wood, especially in pulp and paper which is traded on the largest scale. The identification of individual cell elements (vessels) requires a standardized morphological description with high quality images which is now available for the most important tropical timber species from Asia. Since the fiber components of pulp and paper are subjected to the strict control of the European Timber Regulation (EUTR) from March 2013 onwards, we daily receive samples for checking the declarations from trading companies and competent authorities. In this context, we successfully tested earlier versions of this vessel atlas as an integrated part of our identifications. However, it is obvious that the present Atlas still has weaknesses such as: 1) limited number of taxa included; 2) insufficient sampling to evaluate the full range of variation in vessel element morphology, especially for quantitative parameters at both the species, subgenus and genus level. Future work is necessary to continuously extend the database by describing further unknown or lesser known species.

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ABBREVIATIONS

APS - all pits similar in size and shape
EUTR - European Timber Regulation
IVP - intervessel pit
MTH - Mixed Tropical Hardwood
PAP - pit to axial parenchyma cell
PF - pit to fiber
PT - pit to tracheid
VAS – vessel-ray pit apparently simple
VRP - vessel-ray pit

Codes of two capital letters are according to ISO 3166 (except BN)

AU = Australia
BN = Borneo
BR = Brazil
CN = China
DE = Germany
FR = France
GB = Great Britain
ID = Indonesia
ID-kal = Indonesia-Kalimantan
IN = India
JP = Japan
KH = Cambodia
LA = Laos
LK = Sri Lanka
MG = Madagascar
MM = Myanmar (Burma)
MY = Malaysia
MY-sab = Malaysia-Sabah
MY-swk = Malaysia-Sarawak
NL = Netherlands
NP = Nepal
PA = Panama
PG = Papua New Guinea
PH = Philippines
sAM = South Americas
TH = Thailand
TW = Taiwan
US = United States of America
VN = Vietnam

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Identifizierung von Mixed Tropical Hardwood (MTH) in Papier mittels chemo-taxonomischer und morphologischer Merkmale

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Identifizierung von Mixed Tropical Hardwood (MTH) in Papier mittels chemotaxonomischer und morphologischer Merkmale

Abschlussbericht

zum

DBU-Projekt AZ 31759-31

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Abkürzungsverzeichnis

AMDIS	Automated Mass Spectral Deconvolution and Identification System
ASE	Accelerated Solvent Extraction
atro	absolut trocken
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
EIC	extracted ion chromatogram
FE-SEM	Field emission scanning electron microscope (Feldemissions-Rasterelektronenmikroskop)
fSI	forward similarity index
EUTR	EU-Holzhandelsverordnung (European Timber Regulation)
GCxGC	zweidimensionale Gaschromatographie
HolzSIG	Holzhandelssicherungsgesetz
HRTOF	high resolution time of flight mass spectrometry
INT SUM	integration-summation parameter
IAWA	International Association of Wood Anatomists
iSI	intercept similarity index
MQ	match quality
MTH	Mixed Tropical Hardwood
m/z	Massenzahl = Massenspur in einem Massenspektrum
PCA	principal component analysis
rSI	reverse similarity index
RT	Retention time
SPE	solid phase extraction
uSI	union similarity index
TD-GC/MS	Thermodesorption-Gaschromatographie/Massenspektroskopie
UCM	undissolved complex mixture

1. Zusammenfassung

Das Ziel des bearbeiteten Projekts ist die Verbesserung der Identifizierung der für die Produktion von Papieren verwendeten Holzarten. Das Projekt unterstützt direkt die Umsetzung der EU-Holzhandelsverordnung (EUTR) und damit die Verhinderung des Handels mit illegal eingeschlagenem Holz. Während die Identifizierung der für die Papierherstellung verwendeten Holzarten der gemäßigten Zone Europas und Nordamerikas schon lange vor dem Projektbeginn etabliert war (Carpenter 1931), wurden in der vorliegenden Untersuchung die Grundlagen geschaffen, auch ausgewählte „mixed tropical hardwoods“, also Laubhölzer, deren natürliche Verbreitungsgebiete in tropischen Regionen liegen, zu ermöglichen.

Erste Grundlagen wurden in dem Vorläuferprojekt (DBU-Projekt AZ-29436) gelegt. Es wurden zwei unterschiedliche Techniken eingesetzt, die Mikroskopie zum Erkennen anatomischer Unterschiede und die Chemotaxonomie, die Unterschiede im Metabolom der Holzfasern ausnutzt. Während die Mikroskopie für die Identifizierung von Massivholz aber auch von Holzfasern etabliert ist, lagen für den chemotaxonomischen Ansatz lediglich wenige, erste Versuche vor.

Die im Vorläuferprojekt begonnene Herstellung von Referenzmaterialien wurde fortgesetzt, sodass jetzt 31 sortenreine, gebleichte Zellstoffe und 38 Mazerate relevanter, asiatischer Hölzer hergestellt und untersucht wurden. Dazu gehört die Gattung *Gonystylus spp*. (Ramin), die nach dem Washingtoner Artenschutzübereinkommen unter besonderem Schutz steht (CITES II).

Ein wichtiges, grundlegendes Ergebnis ist die Erstellung und Veröffentlichung der anatomischen Referenzen in Form des „Atlas of vessel elements“ (Helmling et al. 2018). Neben einführenden Erklärungen zur mikroskopisch-anatomischen Identifizierung von Laubholzfaserstoffen an den Gefäßelementen, werden die charakteristischen Strukturmerkmale der einzelnen Gattungen/Untergattungen eingehend beschrieben und mit hervorragenden, mikroskopischen Bildern belegt. Damit wurden die Grundlagen geschaffen, diese Gattungen zu identifizieren.

Durch ergänzende, feinstrukturelle Untersuchungen mittels Rasterelektronenmikroskopie wurden weitere anatomische Merkmale gesucht und gefunden.

Für die Chemotaxonomie musste ein hoher Aufwand im Bereich der Methodenentwicklung geleistet werden. Bei der Probenvorbereitung wurde nach einer günstigen Kombination aus Probenzerkleinerung und Extraktion gesucht. Die Mahlung unter Kryobedingungen und eine anschließende Soxtherm-Extraktion wurden als günstig erachtet. Bei der anschließenden Analytik der Extrakte wurden die Bedingungen der TD-GC/MS an die komplexen Extraktmischungen angepasst. Es zeigte sich, dass das Trennvermögen der 1D-GC in vielen Fällen nicht ausreicht, weshalb zusätzliche Techniken wie Vortrennung mittels SPE und GCxGC-Messungen getestet wurden. Während bei Fragen zur natürlichen Variabilität und Herkunftsfragen multivariate Auswertemethoden zum Einsatz kamen, insbesondere die PCA, wurde für die Identifizierung in Papier ein Datenbankansatz eingesetzt, der hilft, die hohe Anzahl an anfallenden GC/MS-Daten zu bewältigen. Verschiedene Softwarepakete wurden hierfür getestet. Die Software „Openchrom“ wurde als geeignet erachtet. Die Software ist relativ neu und noch unter intensiver Entwicklung.

Als Nebenprojekt wurden verschiedene Ansätze verfolgt, die Gattung *Gonystylus spp.*in industriell hergestelltem MTH zu identifizieren und zu quantifizieren. Dies ist chemotaxonomisch bisher noch nicht zufriedenstellend gelungen.

Ein weiteres, wichtiges Teilprojekt sind erste Untersuchungen zur natürlichen Variabilität. Da für die chemotaxonomischen Untersuchungen mit hohem Aufwand sortenreine Zellstoffe produziert werden mussten,

stand jeweils nur eine Probe als Referenz zur Verfügung. Für die Entwicklung der Methode ist es jedoch unerlässlich die Aussagekraft der ermittelten charakteristischen, chemotaxonomischen Informationen auf ihre Varianz innerhalb der Gattung zu prüfen. In dieser ersten Untersuchung zur natürlichen Variabilität wurden 6 verschiedene Proben der Gattung *Gonystylus spp.* und 14 Proben der *Shorea* Untergattung *Rubroshorea* mikroskopisch und chemotaxonomisch untersucht. Da im Falle der *Rubroshorea*-Proben auch das Herkunftsgebiet bekannt war, konnte auch dieser Aspekt untersucht werden. Während sich die entscheidenden mikroskopischen Merkmale im Falle der *Gonystylus*- und *Rubroshorea*-Proben als stabil erwiesen, wurden deutliche systematische Unterschiede in der Extraktstoffzusammensetzung der untersuchten Proben gefunden, die vielleicht in Zukunft für tiefergehenden Identifizierungen genutzt werden können, die aktuellen Identifizierungen aber erst einmal erschweren.

Abgeschlossen wurde das Projekt mit einem Blindversuch. Der Hochschule München wurden die vorliegenden 31 Referenzzellstoffe zu Verfügung gestellt mit der Aufgabe, 15 Prüfblätter unbekannter Zusammensetzungen herzustellen. Die Zusammensetzungen wurde von den Partnerlaboren der TU Darmstadt und der ISEGA sowie dem TI mittels Mikroskopie auf Basis des Gefäßatlases ermittelt. Auf eine umfassende chemotaxonomische Bestimmung wurde verzichtet, da der Entwicklungsstand der Methodik als noch nicht ausreichend erachtet wurde. Im Rahmen des Blindtest waren 32x15=480 Entscheidungen zu treffen. Es wurde von den drei Instituten eine hervorragende Trefferquote von 74%, 92% und 96% erreicht. Für die im Fokus der Untersuchung stehende Gattung *Gonystylus* und die mikroskopisch-anatomisch ähnlichsten Gattungen *Lophopetalum* und *Durio* wurde eine Quote von 67%, 100% und 100% erzielt. Für das Projekt und insbesondere für den Faseratlas spricht, dass die besten Werte von einem „externen“ Institut erzielt wurden, das in die Herstellung und Untersuchung der Referenzstoffe nicht involviert war, und dem lediglich der Gefäßatlas als Grundlage für die Identifizierungen zur Verfügung stand.

So erfreulich die Mikroskopieergebnisse auch sind, muss berücksichtigt werden, dass in realen Proben größere Variationen, insbesondere durch weitere Holzarten enthalten sein können, für die noch keine Referenzen vorliegen und die auch eine hohe Ähnlichkeit zu z.B. *Gonystylus* spp. aufweisen könnten. Aus diesem Grund und um möglichst gerichtsfeste Aussagen zu erzielen, ist die Entwicklung einer von der Anatomie unabhängigen Methode wie der Chemotaxonomie von großer Bedeutung und sollte zusammen mit der Untersuchung zusätzlicher Referenzmaterialien unbedingt weitergeführt werden.

2. Einleitung/Motivation

Die Bedeutung von Wäldern für die Umwelt ist Thema vieler Veröffentlichungen. So wird die Klimawirkung sehr eindrücklich von Di Lallo et al. 2017 beschrieben: „….Deforestation and forest degradation are the largest anthropogenic sources of CO_2 emissions into the atmosphere“ und „Tropical forests are the cornerstones of climate change mitigation—they sequester more carbon at faster rates than temperate and boreal forests“. Ein anderer wichtiger Umweltaspekt ist der Artenreichtum tropischer Wälder. Insbesondere durch Berichte zu der aktuell höheren Auslöschungsrate im historischen Vergleich, in denen vom 6. Massensterben (mehr als 75% der Arten sind vom Aussterben bedroht) innerhalb der letzten 540 Millionen Jahre der Erdgeschichte geschrieben wird (Barnosky et al. 2011; Mouillot et al. 2013), findet sich das Thema auch in vielen Massenmedien (Stein 2014; Boeing 2017). (Gardner et al. 2009) schreiben, dass das „…Tropical forest ecosystems host at least two-thirds of the Earths terrestrial biodiversity…” und weiter, dass “…the combined influence of persistently high rates of deforestation and forest degradation (FAO 2006), over-harvesting, invasive species and global environmental change threatens to make tropical forests the epicentre of current and future extinctions”. „The Plant List“, eine Arbeitsliste aller bekannten Pflanzenarten, enthält 350.699 Arten aus 17.020 Gattungen, beziehungsweise 642 Familien (Anonymous 2013). Steege et al. 2016 ordnen beispielsweise dem Amazonasregenwald 11676 Baumarten aus 1225 Gattungen und 140 Familien zu. Folgerichtig wurden auf der 17. Vertragsstaatenkonferenz des Washingtoner Artenschutzabkommens (CITES) im Oktober 2016 weitere wichtige Baumarten in den Annex II bzw. (EU) Anhang B aufgenommen. Ferner gab es für bereits geschützte Holzarten Änderungen in der Fußnotenregelung. (Koch und Haag 2017). War bisher lediglich die Art Dalbergia nigra als bedrohte Art gelistet (Anhang A), so wurden bei der jüngsten Konferenz alle 250 Dalbergiaarten in den Anhang B aufgenommen.

Eine zentrale Rolle bei der Umwandlung von Tropenwald und den damit induzierten Umweltwirkungen wird dem illegalen Holzeinschlag zugeordnet. Die Daten von Lawson 2014 in seinem Bericht für „Forest Trends“ belegen das Ausmaß illegaler Entwaldung. Danach erfolgte im Zeitraum von 2000-2012 49% der Umwandlung von tropischen Waldgebieten in Agrarflächen illegal. Infolge dessen ist auch die Nutzung von auf diesen Flächen gewonnenen Holzes illegal. Die Problematik des illegalen Holzeinschlags und die Schutzwürdigkeit der Tropenwälder sind erkannt und spiegeln sich in der Bildung entsprechender Organisationen und Umweltschutzprogrammen auf höchster Ebene wieder. Beispiele sind der Weltrat für biologische Vielfalt (IPBES), das 2005 aus dem Rahmenübereinkommen der Vereinten Nationen über Klimaänderungen (UNFCCC) hervorgegangene REED+-Konzept (Reducing Emissions from Deforestation and forest Degradation), CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) oder dem Aktionsplan FLEGT (Forest Law Enforcement, Governance and Trade).

Der FLEGT-Aktionsplan wird über EU- Verordnungen und das EUTR (europäische Holzhandelssicherungsgesetzt) sowie das deutsche Holzhandelssicherungsgesetz in europäisches und nationales Recht umgesetzt. Danach sind die Marktteilnehmer verpflichtet, definierten „Sorgfaltspflichten“ beim Handel mit Holz nachzukommen. Diese Sorgfaltspflichten umfassen insbesondere Angaben zu den zur Herstellung der Produkte verwendeten Holzarten sowie deren Herkünfte und sind in einem 25-seitigen Leitfaden näher erläutert (Anonymous 2016b). Problematisch ist die sich aus den Sorgfaltspflichten ergebende Rückverfolgbarkeit (zur Risikobewertung durch Informationen zu den Lieferanten und allgemeine Informationen zu den Hintergründen zur Bewertung der produktspezifischen Informationen) beim Vorliegen sogenannter „komplexer Lieferketten“. Komplexe Lieferketten liegen vor, wenn „Holz mehrerer Arten oder aus mehreren Quellen für das betreffende Erzeugnis verwendet wurde“ und/oder „die Zahl der Verarbeiter und Vermittler zwischen dem Ort des Holzeinschlags und dem Marktteilnehmer hoch ist“ (Anonymous 2016b). Dies ist sehr häufig bei Papierprodukten gegeben, bei denen mit Fasermischungen gearbeitet wird oder bei denen „Mixed tropical hardwood (MTH)“ eingesetzt wird. Zwar

fallen nicht alle Papiererzeugnisse unter das EUTR, aber mit den Verpackungspapieren, wenn sie als eigenständiges Erzeugnis eingeführt wurden, unterliegt eine der großen Sortengruppen aus dem Bereich der Papiererzeugung der EUTR (Anonymous 2016b).

Zudem muss die Bedeutung der Papierproduktion für die Holzverwendung hervorgehoben werden. Mit einer Produktion von ca. 400 Mio. t/J bilden die Papier-, Karton- und Pappenprodukte eine wichtige Produktgruppe in der Forst-Holz-Papier-Kette (Anonymous 2016a). In einer Auswertung der UN Comtrade Daten, um den Holzhandel von und mit China zu beleuchten, wird der Umsatz mit Papierhalb- und Papierfertigwaren mit einem dreimal höheren Wert angegeben als der Umsatz mit Holzhalb- und Holzfertigwaren (Dieter und Jochem 2017). Mit einem Weltmarktanteil von 15% beim Import von Papierhalbwaren und 18% beim Export von Papierfertigwaren ist China der bedeutendste Marktteilnehmer. Da China gleichzeitig wichtiger Handelspartner eines der „hot spots“ des illegalen Holzeinschlags, dem Malayischen Archipels, ist, ergeben sich nach den EUTR erhöhte Anforderungen bei der Risikobewertung beim Inverkehrbringen relevanter Holzerzeugnisse.

Um die oben geschilderten Umweltschutzmaßnahmen durchsetzen zu können, werden Methoden benötigt, um auch in den verschiedenen Papiersorten eine Artidentifizierung der für die Herstellung verwendeten Holzarten vornehmen zu können, obwohl diese in einem stark modifizierten Zustand vorliegen. Die Anforderungen an solche Methoden werden durch die Betrachtung der Bedingungen bei der Papier- bzw. Zellstoffherstellung deutlich.

Eine „Statistische Tonne“ Papier besteht aus: Fasern, Füllstoffen, Hilfsstoffen (Kleemann 2006). Die Fasern für die 3000 verschiedenen Papiersorten (Reinhold 2015) werden zu 80-85% aus Holz und zu 20-15% aus Einjahrespflanzen gewonnen. Es werden ca. 200.000.000t Primärfasen pro Jahr für die weltweite Papierproduktion benötigt. Damit gehört Zellstoff zu den mengenmäßig wichtigsten Massengütern. Ca. 20-25% der benötigten Fasern werden mechanisch hergestellt, 75-80% werden in den verschiedenen Zellstoffherstellungsverfahren gewonnen. Das Holz wird zu Hackschnitzeln (Höhe/Breite/Dicke - ca. 30/30/5mm) zerkleinert, um eine möglichst gleichmäßige Penetration mit den Aufschlusschemikalien zu gewährleisten. Diese Chemikalien wirken dann ca. 2h bei 140-170°C und sehr niedrigen oder extrem hohen pH-Werten auf das Holz ein mit dem Ziel, das in Anteilen von 20-35% in den verschiedenen Holzarten vorhandene Lignin möglichst selektiv zu entfernen. Die Mittellamelle, die Schicht zwischen den Holzzellen, wird hierbei aufgelöst, sodass geringe Scherkräfte ausreichen, um den Holzverbund aufzulösen und die einzelnen Holzzellen zu erhalten. Da die Delignifizierung nach dem Holzaufschluss für hochweiße Qualitäten nicht ausreicht, schließt sich nach dem Holzaufschluss in den meisten Fällen eine mehrstufige Bleichsequenz mit unterschiedlichen Bleichchemikalien bei unterschiedlichen Bedingungen (Temperatur, pH-Wert, Chemikalienkonzentrationen, Zeit und Konsistenz) an. Über den Zellstoffherstellungsprozess gehen ca. 50% der Holzsubstanz verloren. Nach dieser intensiven Behandlung sind neben den Hauptkomponenten Lignin und Hemicellulosen auch akzessorischen Bestanteile sowie die vorhandene DNA und RNA entfernt, beziehungsweise denaturiert, weshalb Methoden der Gentechnik zur Artidentifizierung entsprechend geringe Chancen unterstellt werden. Erste Extraktionsversuche an gebleichten Zellstoffen zeigten jedoch, dass nicht alle sekundären Pflanzenstoffe entfernt werden und die verbleibenden Extraktstoffe systematische Unterschiede zeigen, die auf die verwendeten Rohstoffe zurückzuführen sind.

Die sichere Identifikation der für die Papierherstellung verwendeten Holzarten ist für den Umwelt- und Artenschutz von großer Bedeutung. Weder Behörden noch Verbraucher können auf den Einsatz problematischer Holzarten im Papier reagieren, solange diese nicht zweifelsfrei nachgewiesen werden können. Studien im Auftrag

des WWF Deutschland 2010 (Flosdorff et al. 2010) und (Hirschberger et al. 2012) legen nahe, dass Hölzer aus tropischen Naturwäldern für die Herstellung von Druckerzeugnissen, z.B. von Kinderbüchern, eingesetzt werden. Recherchen von Greenpeace International ergaben, dass Tropenhölzer, unter anderem auch CITES geschützte Arten, in Prozessen zur Papier- und Zellstoffproduktion eingesetzt werden. Das Problem wird durch den weltweit zunehmenden Bedarf an Zellstoff für Verpackungsmaterial verschärft. Laut einer Studie des Thünen-Instituts für Forstökonomie wurden im Jahr 2009 zwischen 103 und 284 Millionen Kubikmeter Rohholz illegal eingeschlagen. 7 bis 17 Prozent des gesamten globalen Einschlags stammen aus illegalen Quellen und stellen somit eine erhebliche Größenordnung dar (Dieter et al. 2012).

Die Ermittlung der für die Papierherstellung verwendeten Holzarten ist heute nur durch vergleichende Fasermikroskopie für konventionelle Zellstoffe mit Hilfe von Referenzfasern möglich. Tropische Holzarten konnten mangels Referenzmaterialien und Erfassung ihrer spezifischen Merkmale bisher nicht eindeutig identifiziert werden. Eine eindeutige Identifikation der Fasern erfordert daher die Bereitstellung von belegtem Referenzmaterial und die Bestimmung geeigneter Identifizierungsmerkmale. Dieses fehlte bislang für MTH-Arten. Verschärft wird die Situation in Zukunft durch die verstärkte Nutzung der sogenannten „lesser known species“. Dadurch wird der Nutzungsdruck auf bisher nicht oder weniger stark von illegaler Holznutzung betroffene Regionen erhöht und es erhöht sich die Anzahl der Holzarten in den verschiedenen Holzprodukten.

3. Zielsetzung

Das Ziel des Projektes ist es, chemische und weitere morphologische Merkmale für die 28 im Vorgängerprojekt untersuchten Gattungen/Subgattungen sowie für 5-10 weitere, als wichtig erachtete Gattungen, herauszuarbeiten und deren Identifizierungspotenzial zu bewerten. Beide Ansätze der Faseridentifizierung, chemisch und morphologisch, sollen verfolgt und kombiniert werden, um zuverlässigere Identifizierungen zu ermöglichen. Dadurch ergeben sich die folgenden Teilziele:

a) Die Erweiterung der Untersuchungen um 5-10 relevante Gattungen.
b) Die Verbesserung der Probenvorbereitung, Analytik und Datenauswertung zur möglichst vollständigen Erfassung aller chemotaxonomisch relevanten Informationen, die aus den Zellstoffen extrahiert und für Identifizierungsprozesse zur Verfügung gestellt werden sollen.
c) Die lichtmikroskopische Untersuchung der weiteren, im Vergleich zu dem Vorläuferprojekt neu aufgenommenen Gattungen und Erfassung morphologischer Merkmale aller Gattungen/Subgattungen im Feldemissions-Rasterelektronenmikroskop (FE-SEM) sowie die methodische Weiterentwicklung der lichtmikroskopischen Faseranalyse mit einer entsprechenden Erweiterung des im Vorgängerprojekt erstellten Faseratlasses.
d) Evaluierung der systematischen Unterschiede zwischen Gattungen unter Berücksichtigung der natürlichen Variabilität innerhalb einer Gattung.

4. Ergebnisse

Die erforderlichen Maßnahmen sind in neun Arbeitspakete untergliedert und wurden von den Projektteilnehmern teils nacheinander, teils parallel bearbeitet. Der erreichte Stand wird im Folgenden dargestellt.

4.1. Materialbeschaffung und Herstellung der Referenzproben (Zellstoffe und Mazerate) - AP 1

Die bereits im Vorgängerprojekt erstellte Sammlung an industrienah hergestellten, sortenreinen Referenzzellstoffen und –mazeraten (vgl. Tabelle 1) wurde im laufenden Projekt weiter ausgebaut. Neues Probenmaterial wurde zum einen unter dem Aspekt der Erweiterung der Sammlung relevanter Gattungen gesucht und zum anderen für die Evaluierung der natürlichen Variabilität eingesetzt. Für jede Holzprobe wurde die Richtigkeit der Gattung mit Hilfe der herbarbelegten Sammlung des Thünen-Instituts geprüft.

Tabelle 1: Ausgewählte Gattungen des Vorgänger-Projekts

	Gattung	Handelsname	Familie	Mazerat	Zellstoff
1	*Acacia* spp.	Mangium	Fabacaceae	X	X
2	*Avicennia* spp.	Api Api	Acanthaceae	X	X
3	*Calophyllum* spp.	Bintangor	Calophyllaceae	X	X
4	*Cocos nucifera*	Kokospalme	Arecaceae	X	X
5	*Dendrocalamus* spp.	Bambus	Poaceae	X	X
6	*Dipterocarpus* spp.	Keruing	Dipterocarpaceae	X	X
7	*Durio* spp.	Durian	Malvaceae	X	X
8	*Eucalyptus* spp.	Eukalyptus	Myrtaceae	X	X
9	*Gonystylus* spp.	Ramin	Thymelaeaceae	X	X
10	*Heritiera* spp.	Mengkulang	Malvaceae	X	X
11	*Intsia* spp.	Merbau	Fabaceae	X	X
12	*Koompassia* spp.	Kempas	Fabaceae	X	X
13	*Lophopetalum* spp.	Perupok	Celastraceae	X	X
14	*Mangifera* spp.	Mango	Anacardiaceae	X	X
15	*Palaquium* spp.	Nyatoh	Sapotaceae	X	X
16	*Rhizophora* spp.	Rote Mangrove	Rhizophoraceae	X	X
17	*Shorea* subg. *Rubroshorea*	Rotes Meranti	Dipterocarpaceae	X	X
18	*Shorea* subg. *Anthoshorea*	Weißes Meranti	Dipterocarpaceae	X	X
19	*Shorea* subg. *Shorea*	Bangkirai	Dipterocarpaceae	X	X
20	*Shorea* subg. *Richetia*	Gelbes Meranti	Dipterocarpaceae	X	X
21	*Swintonia* spp.	Merpauh	Anacardiaceae	X	X
22	*Tetramerista* spp.	Punah	Tetrameristaceae	X	X
23	*Campnosperma* sp.	Terentang-Asia	Anacardiaceae	X	
24	*Eugenia* (*Syzygium*) sp.	Kelat	Myrtaceae	X	
25	*Gluta* sp.	Rengas	Anacardiaceae	X	
26	*Litsea* sp.	Medang	Lauraceae	X	
27	*Madhuca* sp.	Bitis	Sapotaceae	X	
28	*Parashorea* sp.	Gerutu	Dipterocarpaceae	X	

4.1.1. Erweiterung der Sammlung mit neuen relevanten Gattungen.

Die Liste der im Vorgänger-Projekt untersuchten Hölzer (Tabelle 1) aus dem südostasiatischen Raum wurde in diesem Projekt um 11 Gattungen erweitert, die ein hohes Potenzial für die Herstellung von Zellstoff und Papier aufweisen (Tabelle 2). Die Auswahl der Hölzer erfolgte aufgrund der großräumigen Verbreitung der Gattungen und dementsprechend guter Beschaffungsmöglichkeiten für die Zellstoffindustrie. Die Hölzer stammen z.T. aus Plantagen (*Hevea brasiliensis*, *Paulownia* spp.) oder sind Nebenprodukte aus Kuppelproduktionen, die als Zellstoff Verwendung finden.

Die Beschaffung des Materials für die Herstellung der Mazerate (Faservereinzelung durch Auflösen des Gewebeverbunds) als belegtes Vergleichsmaterial konnte durch die Bereitstellung von Proben aus der herbarbelegten Sammlung des Thünen-Instituts sichergestellt werden. Die Mazerate liegen daher für alle 11 Gattungen vor. Die Materialbeschaffung von weiteren 1-2 kg Holz je Gattung für die chemotaxonomischen Untersuchungen erwies sich jedoch als schwierig. Aufgrund unsachgemäßer Lagerung während des Transports war eine aus Vietnam erhaltene Probe bei Ankunft in Hamburg mikrobiell befallen und ist somit nicht für chemotaxonomische Untersuchungen zu verwenden. Eine weitere von dort erhaltene Probe erwies sich nach der Holzartenbestimmung als eine nicht angeforderte Gattung, wodurch die Probleme einer genauen Bestimmung am Herkunftsort sichtbar werden. Neun Gattungen konnten in ausreichender Menge beschafft und zu Zellstoff verarbeitet werden.

Tabelle 2: Ausgewählte, neu in die Untersuchung aufgenommene Gattungen

	Gattung	**Handelsname**	**Familie**	**Mazerat**	**Zellstoff**
1	Liquidambar formosana	Red gum	ALTINGIACEAE	X	
2	Canarium spp.	Kedondong	BURSERACEAE	X	X
3	Hevea brasiliensis	Rubberwood	EUPHORBIACEAE	X	X
4	Albizia spp.	White siris, kokko	FABACEAE-MIMOSOIDEAE	X	
5	Castanopsis spp.	Berangan	FAGACEAE	X	X
6	Paulownia spp	Paulownia	PAULOWNIACEAE	X	X
7	Schima spp.	Samak, puspa	THEACEAE	X	X
8	Parashorea spp.	Gerutu	DIPTEROCARPACEAE		X
9	Alniphyllum spp.	Mee Dong	STYRACACEAE	X	X
10	Ilex spp.	Kecemang	AQUIFOLIACEAE	X	X
11	Nyssa spp.	Tupelo, Nyssa	NYSSACEAE	X	X

Das neue Material wurde nach der bewährten Methode mazeriert. Gleichzeitig wurden vollgebleichte Referenzzellstoffe hergestellt, an denen chemotaxonomische und vergleichende morphologische Untersuchungen vorgenommen werden können.

4.1.2. Proben zur Evaluierung der natürlichen Variabilität auf Gattungsebene

Die natürliche Variabilität der chemischen und morphologischen Merkmale innerhalb einer Gattung wurde anhand der Gattungen *Gonystylus spp*. und *Shorea subg*. *Rubroshorea* überprüft. Für diese beiden Gattungen konnten bisher sechs bzw. 14 Proben beschafft werden, vgl. Tabelle 1. Für die *Gonystylus spp.*-Proben liegen uns leider keine genaueren Informationen zur Herkunft vor, sie konnten als Kanteln oder Profilleisten von verschiedenen holzverarbeitenden Betrieben besorgt werden. Die Proben der Subgattung *Rubroshorea* können dagegen einzelnen Regionen Südostasiens zugeordnet werden.

Tabelle 3: Proben zur Evaluierung der Variabilität

Probe	Gattung	Herkunft	gekocht	gebleicht
849-1	Gonystylus spp.	Schreinerei Olbrich	X	X
849-2	Gonystylus spp.	Schreinerei Steffen	X	X
849-3-A	Gonystylus spp.	Schreinerei Ratheiser	X	X
849-3-B	Gonystylus spp.	Schreinerei Ratheiser	X	X
849-3-C	Gonystylus spp.	Schreinerei Ratheiser	X	X
849-3-D	Gonystylus spp.	Schreinerei Ratheiser	X	X
839-1	Rubroshorea	Central Kalimantan	X	X
839-2	Rubroshorea	East Kalimantan	X	X
839-3	Rubroshorea	West Kalimantan	X	X
839-4	Rubroshorea	Mentawai Islands	X	X
839-5	Rubroshorea	Molukken	X	X
839-6	Rubroshorea	West Malaysia	X	X
839-7	Rubroshorea	Sabah	X	X
839-8	Rubroshorea	West Kalimantan	X	X
839-9	Rubroshorea	East Kalimantan	X	X
839-10	Rubroshorea	West Malaysia	X	X
839-11	Rubroshorea	Mentawai Islands	X	X
839-12	Rubroshorea	Sabah	X	X
839-13	Rubroshorea	Molukken	X	X
839-14	Rubroshorea	Central Kalimantan	X	X

4.5. Lichtmikroskopische Untersuchung an neu aufgenommenen Arten (AP 5)

Im Rahmen des Arbeitspakets 5 wurden die diagnostischen Strukturmerkmale der Gefäßelemente für die Hölzer der neu ausgewählten zehn Gattungen anhand der mazerierten, isolierten Gefäßelemente untersucht und ihre quantitativen Merkmale histometrisch bestimmt.

Von den Hölzern der ausgewählten Gattungen wurden dafür aus Mazeraten gefärbte Dauerpräparate erstellt und im Anschluss lichtmikroskopische Bilder von jeweils mindestens 36 Gefäßelementen aufgenommen. Die Bilder der Gefäßelemente wurden auf ihre qualitativen Merkmale hin untersucht und systematisch ausgewertet. Dies beinhaltet die Beschreibung der Form der Gefäßelemente und des Fortsatzes, die Art der Gefäßdurchbrechung, das Vorhandensein von Thyllen sowie die Anordnung und Form der Gefäß- und Kreuzungsfeldtüpfel.

Für jede der 38 Gattung/Untergattungen des gesamten Projekts wurde eine Bildtafel mit ausgewählten Gefäßelementen erstellt, um die natürliche Variationsbreite der Gefäßelemente einer Gattung zu dokumentieren. Die Auswahl ermöglicht einen Überblick über die Ausprägung der qualitativen Merkmale (v.a. Art und Größe der Tüpfel), die der Identifizierung dienen (**Abbildung 26**).

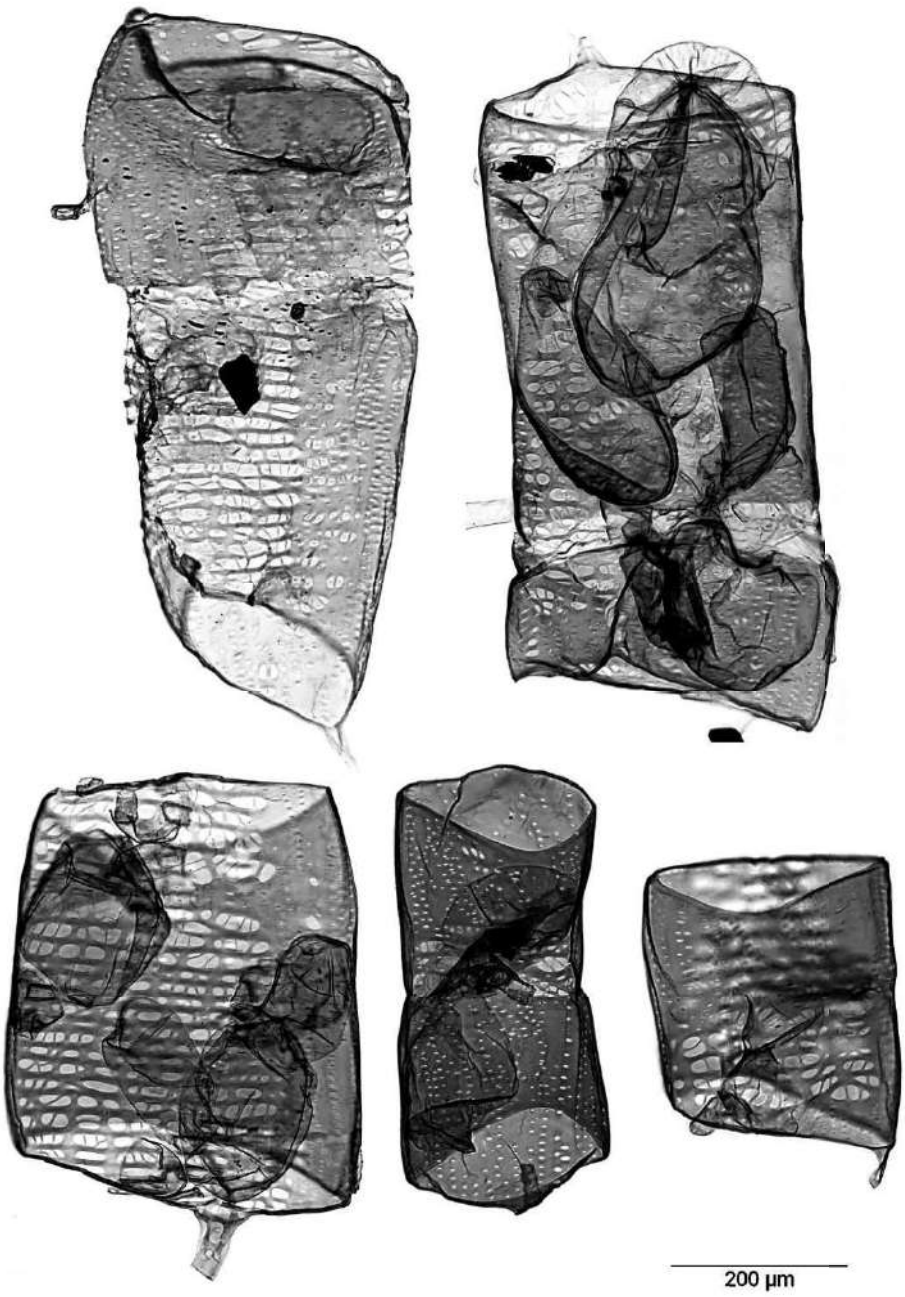


Abbildung 26: Bildtafel von Gefäßelementen der Holzart *Castanopsis argentea*

Die Gefäßelemente wurden wie in Abbildung 27 dargestellt vermessen (Länge, zweimal der Durchmesser, Hof- und Aperturdurchmesser der Gefäßtüpfel in axialer Richtung).

Neu aufgenommen in die quantitative Merkmalsliste wurde die Gefäßtüpfelhofgröße. Im ersten Projektzeitraum konnten die Höfe nicht vermessen werden, da in den mit Safranin gefärbten Proben die Höfe nicht klar erkennbar waren. Schon für die Holzartenidentifizierung am Holz konnte gezeigt werden, dass die Größe der Gefäßtüpfelhöfe ein aussagekräftiges Merkmal darstellt (Richter und Dallwitz, 2000). Während die Größe der Apertur der Gefäßtüpfel nicht zur Identifizierung verwendet wird und folglich bisher nicht in der Literatur beschrieben wurde. Im zweiten Projektzeitraum wurde

deshalb nach geeigneten Färbemethoden gesucht, mit denen der Hof klar erkennbar wird. Alle 38 Mazerate wurden dann zusätzlich mit Nigrosin und mit der Alexander-Herzberg-Färbung angefärbt. Die Alexander-Herzberg-Färbung kann zwar nicht konserviert werden, ermöglicht aber eine besonders deutliche Differenzierung von Hof und Apertur. Die Erfassung von Hof- und Aperturdurchmesser der Gefäßtüpfel stellt eine entscheidende Verbesserung der Daten im Vergleich zur Vorgängerstudie dar.

Die Ergebnisse der Vermessung des Durchmessers der Kreuzungsfeldtüpfelapertur im Vorgängerprojekt erwiesen sich aufgrund ihrer Variationsbreite als unzureichend aussagekräftig für die Holzartenidentifizierung. Daher wurde in diesem Projekt auf ihre Erhebung verzichtet.

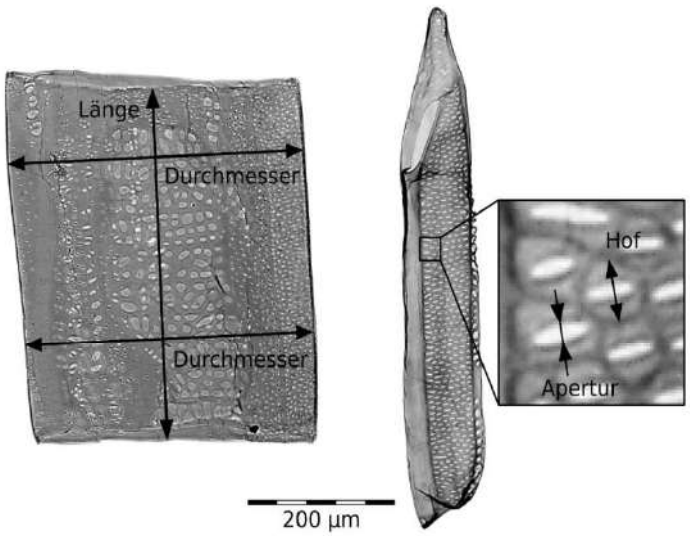


Abbildung 27: Darstellung und Datenerhebung der quantitativen Merkmale durch Vermessen der Länge und des Durchmessers der Gefäßelemente sowie der Gefäßtüpfelapertur und des –hofs (am Beispiel von *Shorea* subg. *Rubroshorea* (linke Abbildung) und *Canarium* cf. *littorale* (rechte Abbildung)

Zum Abschluss des Projekts wurden die grundlegenden Arbeiten zur Charakterisierung der anatomischen Strukturmerkmale in Form eines umfangreichen, 108 Seiten umfassenden Gefäßatlas mit dem Titel „Atlas of vessel elements – identification of Asian timbers“ ausgearbeitet, beim IAWA Journal zur Veröffentlichung eingereicht und von den Gutachtern akzeptiert. Der Gefäßatlas wird Anfang 2018 online und in gedruckter Form im IAWA Journal 39 (3) veröffentlicht.

Im „Atlas of vessel elements“ wird einführend beschrieben, anhand welcher Merkmale die untersuchten Hölzer an ihren Gefäßen in Faserstoffen wie Papier und Faserplatten diagnostiziert werden. Die Beschreibungen der einzelnen Gattungen/Untergattungen sind für eine einfachere Orientierung in vier Gruppen mit grob ähnlichen Strukturmerkmalen unterteilt. Jede Gattung wird auf einer Veröffentlichungsseite detailliert beschrieben (**Abbildung 28**). Klar strukturiert sind alle morphologischen Merkmale aufgelistet (z.B. Art der Gefäßelementenddurchbrechung, Form des Gefäßelementes und des Fortsatzes, Anordnung und Erscheinungsbild der verschiedenen Tüpfel und Messwerte der Gefäßelemente und deren Gefäßtüpfel). Des Weiteren werden wichtige Informationen zu Handelsnamen, geographischen Verbreitungsgebieten und der DIN EN 13556-2003 Code aufgelistet. Auf der jeweils gegenüberliegenden Seite zeigt eine Bildtafel vier bis zehn repräsentative

Gefäßelemente zum Vergleich der natürlichen Variationsbreite in standardisierter Vergrößerung (**Abbildung 28**). Neben den beschriebenen Strukturmerkmalen, sind die Form der verschiedenen Tüpfel, ihre Anordnung und Ausrichtung für die Gattungen sehr charakteristisch. Die hochwertigen mikroskopischen Aufnahmen stellen daher eine grundlegende Referenz für die Charakterisierung und Bestimmung der individuellen Gefäßelemente dar. Der genaue Abgleich einer unbekannten Probe mit den Abbildungen ist deshalb essentiell für eine Identifizierung. Aus diesem Grund wurde auf die Qualität und Aussagekraft der Abbildungen besonderer Wert gelegt. Dies wurde auch von den Gutachtern des IAWA Journals bestätigt.

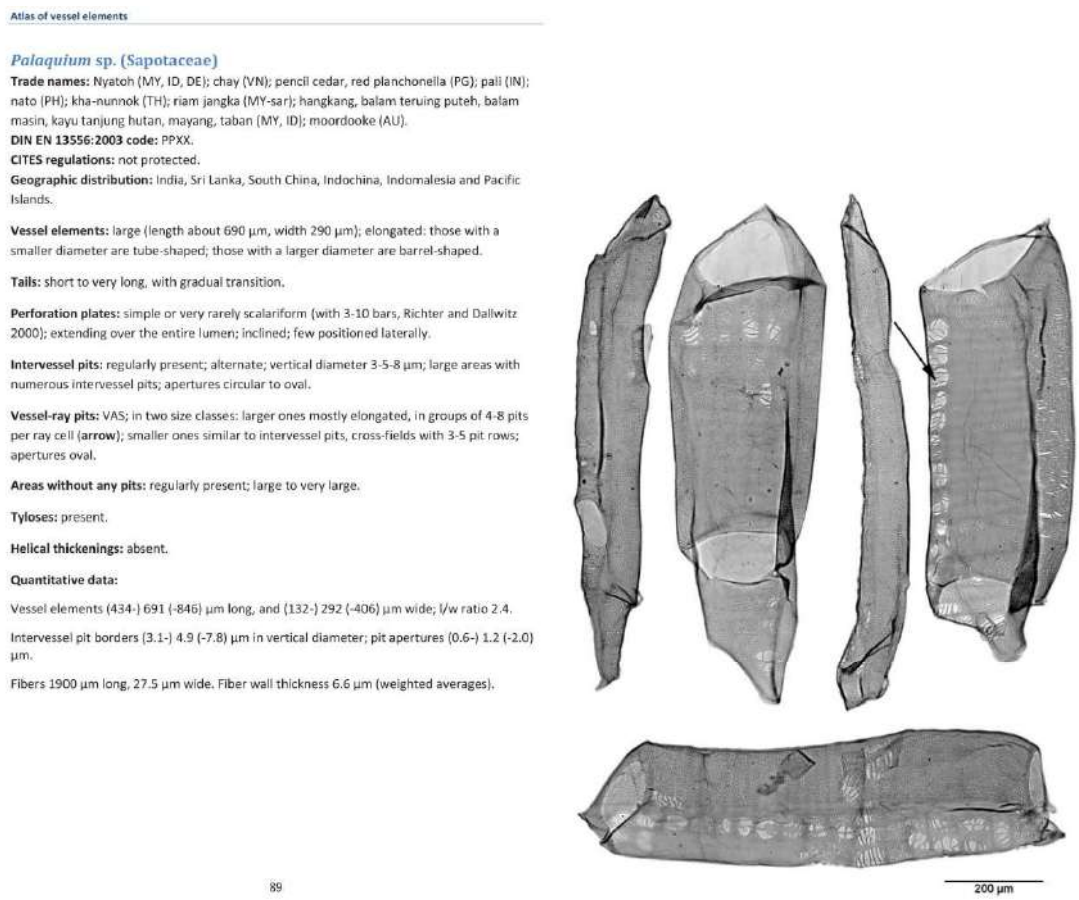


Abbildung 28: Darstellung der Referenzen im „Altas of vessel elements“ am Beispiel von *Palaquium* sp.

Mit dem IAWA Journal wird der Atlas in der international anerkanntesten, englischsprachigen Zeitschrift für Holzanatomie veröffentlicht. Die Publikation als im Internet frei zugängliche Version ermöglicht es allen Prüfinstituten weltweit, die Referenzen für die Überprüfung von Papier und Faserplatten anzuwenden. Der Atlas ist damit eine direkte Unterstützung bei der Überprüfung der Holzquellen für den internationalen Papierhandel.

4.6. Elektronenmikroskopische Untersuchung (FE-SEM) (AP 6)

Die im vorliegenden Bericht unter Kap. 4.5, 4.7.2 und 4.8.1 beschriebenen Strukturmerkmale sind als „diagnostische“ Merkmale international etabliert und von der IAWA in Form einer Merkmalsliste veröffentlicht (IAWA Committee, 1989), die als Grundlage für die anatomische Bestimmung von individuellen Arten, Gattungen oder Familien dient. Da die Kombination dieser Strukturmerkmale für die Identifizierung des Holzgewebes in den drei anatomischen Richtungen (transversal, radial und tangential) angewendet wird, bleiben nur wenige Merkmale, die an den vereinzelten Gefäßelementen eines Papiers analysiert werden können. Um die Genauigkeit der Beschreibung der untersuchten Referenzen zu erhöhen und damit die Identifizierung über die Anatomie zu vereinfachen bzw. zu verbessern, wurden ergänzende Untersuchungen der Referenzen mit dem Feldemissions-Rasterelektronenmikroskop (FE-SEM) bei wesentlich höherer Auflösung durchgeführt, um einzelne Strukturmerkmale besser darstellen oder für eine diagnostische Differenzierung nutzen zu können.

Zunächst wurden von 35 Gattungen isolierte Gefäßelemente aus mazeriertem Referenzmaterial und für insgesamt 28 Gattungen/Untergattungen auch aus dem Zellstoff für die Untersuchung im FE-SEM präpariert. Von 28 Gattungen/Untergattungen wurden von den Mazerat- und Zellstoffproben standardisierte Übersichts- und Detailaufnahmen angefertigt. Da die Gefäßelemente im Mazerat wesentlich besser erhalten sind (**Abbildung 29**, links), können an diesen Proben alle Details der charakteristischen Strukturmerkmale am besten untersucht werden. Der Vergleich mit den Gefäßelementen des Zellstoffs ist von großer Bedeutung, um abschätzen zu können, welche Merkmale an den Gefäßelementen des Zellstoffs durch die starken chemischen und mechanischen Einwirkungen bei der Zellstoffherstellung beeinträchtigt bzw. verändert werden (**Abbildung 29**, rechts).

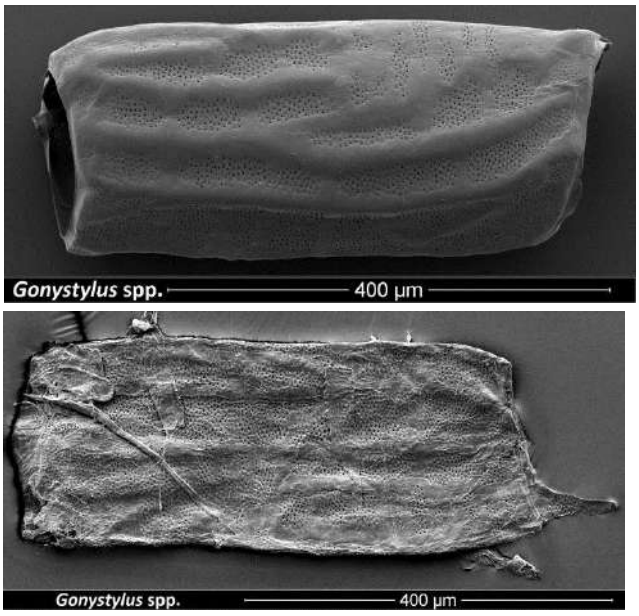


Abbildung 29: Übersichtsaufnahmen der Gefäßelemente von *Gonystylus*. Links: Mazerat; Rechts: Zellstoff

Die Ergebnisse der FE-SEM-Analysen zeigen, dass mit Hilfe der hochauflösenden Technik zusätzliche bzw. besser abgrenzbare Strukturmerkmale identifiziert werden können. Ein Beispiel sind die Ränder an den Gefäßenddurchbrechungen isolierter Gefäßelemente. Je nach Gattung liegt ein breiter, schmaler oder kein nach innen liegender Rand vor. Zum Teil kann ein solcher Rand auch Tüpfel aufweisen (**Abbildung 30**).

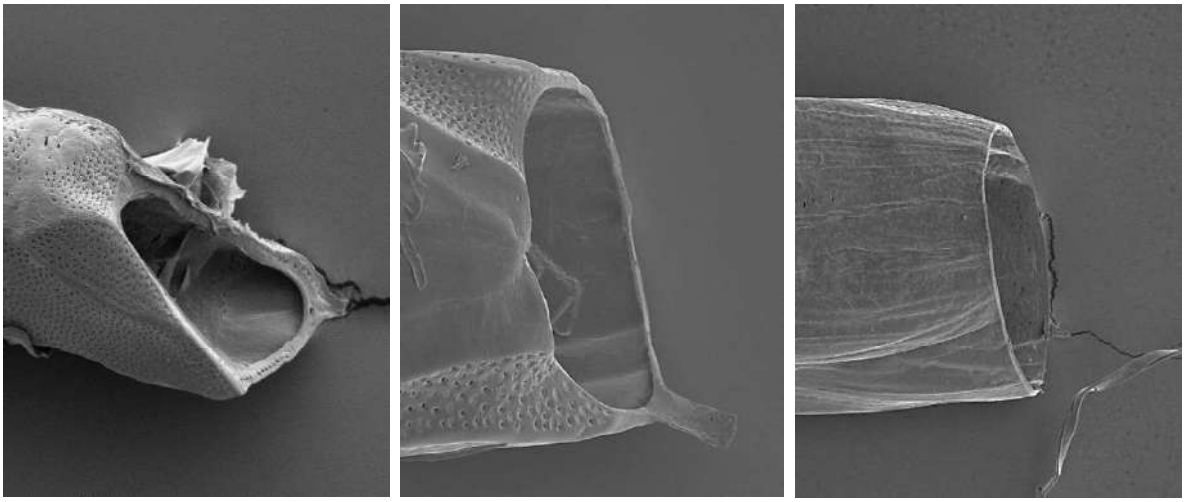


Abbildung 30: Gefäßelemente mit unterschiedlichen Rändern der Gefäßelementenddurchbrechung
Links: Gefäßelementenddurchbrechung mit breitem, nach innen liegendem Rand mit Tüpfeln der Gattung *Palaquium*; Mitte: Gefäßelementenddurchbrechung mit schmalem, nach innen liegendem Rand der Gattung *Shorea* subg. *Rubroshorea*; Rechts: Gefäßelementenddurchbrechung ohne Rand der Gattung *Dipterocapus*

Zusätzliche diagnostische Informationen über die Tüpfel können aus der Gefäßinnenansicht abgeleitet werden. Schon im Vorfeld der Studie war bekannt, dass die innere Apertur der Tüpfel zum Teil rund, schlitzförmig oder auch erweitert sein kann (eine mehrere Tüpfel einschließende innere Einkerbung der Zellwand) (**Abbildung 31**). Diese erweiterte innere Apertur ist z.B. bei der Art *Tetramerista glabra* so stark ausgeprägt, dass sie auch im Lichtmikroskop problemlos sichtbar ist. Das Vorkommen dieses speziellen Strukturmerkmals wurde nachträglich an belegten Schnittpräparaten aus der wissenschaftlichen Holzsammlung des Thünen-Instituts überprüft und konnte für alle vier dokumentierten Präparate der Art *Tetramerista glabra* eindeutig bestätigt werden Es ist deshalb ein geeignetes Strukturmerkmal, das in die von der IAWA ausgeführten Merkmalsliste für die Holzartenbestimmung aufgenommen werden sollte und auch für die anatomische Beschreibung von anderen Gattungen angewendet werden kann.

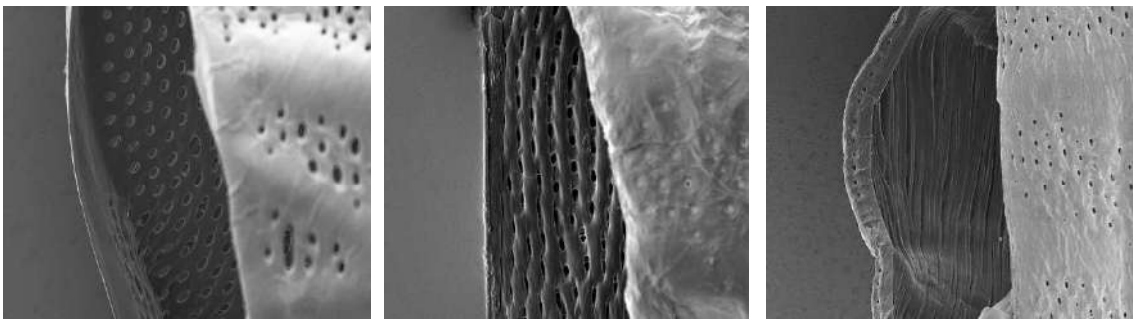


Abbildung 31: Gefäßinnenansicht mit ovaler Tüpfelform der Gattung *Durio* (links), mit ovalen Tüpfeln mit schlitzartiger, erweiterter Apertur der Art *Albizia procera* (Mitte) und mit lamellenartiger, erweiterter Apertur der Gattung *Heritiera* (rechts)

Neben den qualitativen Merkmalen konnten die quantitativen Merkmale wesentlich genauer vermessen werden. Dies gilt vor allem für die Höfe und Aperturen der Tüpfel.

4.7. Evaluierung der natürlichen Variabilität auf Gattungsebene (AP 7)

Die natürliche Variabilität wurde beispielhaft an zwei Gattungen untersucht, an *Rubroshorea* und *Gonystulus*. Diese beiden Gattungen wurden gewählt, weil die Baumarten aus der Familie der *Dipterocarpaceae*, zu der die Untergattung *Rubroshorea* gehört, mit ca. 200 Arten sehr vielfältig ist und die am häufigsten vorkommende Baumartenfamilie Südostasiens darstellt. *Gonystylus spp*. wurde aufgrund des besonderen Schutzstatus gewählt. Die für diese Untersuchung zur Verfügung stehenden Proben sind in Tabelle 1 gelistet.

4.7.1. Chemotaxonomie

4.7.1.1. Rubroshorea

Es lagen 14 Proben aus 7 verschiedenen Regionen Süd-Ostasiens vor (Abbildung 32). Aus jeder Region wurden je zwei Proben zu gebleichten Zellstoffe verarbeitet. Diese Zellstoffe wurden nach der in AP 2 optimierten Methoden extrahiert und jeder Extrakt wurde nach der ebenfalls in AP 2 optimierten TD-GC/MS Methode als Vierfachfachbestimmung analysiert.

Abbildung 32: Herkunft der untersuchten Rubroshoreaproben

Ein Chromatogramm aus dieser Messserie ist in Abbildung 33 beispielhaft aufgeführt.

Da zu den untersuchten Proben keine zusätzlichen Informationen verfügbar sind, gibt es keine Hinweise, ob die Unterschiede evtl. durch das Vorliegen unterschiedlicher Arten oder unterschiedlicher Herkünfte verursacht werden. Interessant ist allerdings, dass die Proben sich so klar gruppieren. Dies spricht für systematische Unterschiede innerhalb der Gattung *Gonystylus spp.*. Um für Identifizierungen geeignete Marker zu finden, die über alle *Gonystylus spp*-Arten hinweg gültig sind, muss idealerweise ein Datensatz untersucht werden, der die möglichen Variationen abdeckt. Alternativ kann auch mit mehreren Markern gearbeitet werden, die jeweils eine Teilgruppe innerhalb der Gattung *Gonystylus spp.* abdecken. Für die in dem vorliegenden Projekt anvisierte Quantifizierung von *Gonystylus spp*. in Papier ergeben sich aus dieser Erkenntnis Vor- und Nachteile. Markerverbindungen müssen zusätzlich auf die Gültigkeit für die jeweiligen Untergruppen der Gattung *Gonystylus spp*. geprüft werden, eröffnen aber auch die Möglichkeit auf über die Gattung hinausgehende Identifizierungen.

Eine erste Betrachtung der Chromatogramme zeigt eine hohe qualitative Übereinstimmung der Chromatogramme der verschiedenen *Gonystylus spp*.-Proben. Es konnten in einer ersten Durchsicht unter den verwendeten Bedingungen keine Verbindungen gefunden werden, die für die jeweiligen Proben spezifisch wären. Die Unterschiede zwischen den Proben werden durch die relativen Unterschiede der Signalintensitäten, also der mengenmäßigen Zusammensetzung der Extrakte, verursacht, die durch die multivariate Auswertung mittels PCA veranschaulicht werden können. Allerdings können in dem Chromatogramm in Abbildung 38, trotz der optimierten Trennbedingungen, die sogenannten „Alkanberge“ oder „UCM-unresolved complex mixtures“ erkannt werden. Wie vielfältig die Zusammensetzung dieser UCMs sein kann, wurde von (Kuhnert 2011) beschrieben, der den Thearubigenhügel bei Schwarzem Tee untersuchte, dem er in einer ersten Schätzung mit bis zu 30000 Einzelverbindungen eine ähnliche Komplexität wie der Zusammensetzung von Erdöl unterstellte.

4.7.2. Anatomie

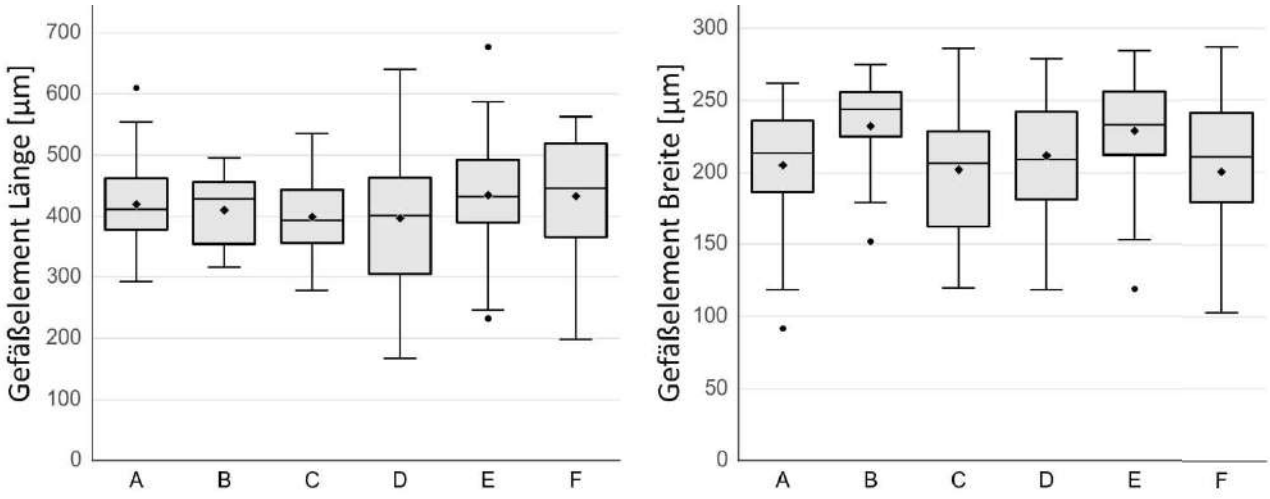
Fünf verschiedene Hölzer der nach CITES geschützten Gattung *Gonystylus* (849-2, 839-3-A, B, C und D) wurden als Mazerat mit den im „Atlas of vessel elements“ veröffentlichten Referenzen lichtmikroskopisch verglichen. Alle zeigen eine exakte Übereinstimmung der Strukturmerkmale der Gefäßelemente (einfache Gefäßenddurchbrechung, wechselständige Gefäßtüpfel, Kreuzungsfeldtüpfel, die in Größe und Form mit den Gefäßtüpfeln übereinstimmen, Anordnung der Tüpfel zu axialen und radialen Parenchymzellen).

Für die Hölzer der umfangreichen Gattung *Shorea* (über 100 Arten) konnten Mazerate aus Holz der Untergattung *Rubroshorea* aus sieben verschiedenen Herkünften mit den Referenzen verglichen werden. Auch hier stimmen die Strukturmerkmale der Gefäßelemente der Proben vollkommen überein (einfache Gefäßenddurchbrechung, wechselständige Gefäßtüpfel, scheinbar einfache Kreuzungsfeldtüpfel, die in ihrer Größe und Form sehr variabel sind, Tüpfel zu paratrachealen Tracheiden).

Analysiert man die Messdaten der Gefäßelemente der verschiedenen Mazerate der beiden Gattungen zusammen mit den Referenzen (**Abbildung 41**), so ist auffällig, dass vor allem der Parameter Gefäßelementbreite bei den Proben von *Shorea* subg. *Rubroshorea* höhere Werte aufweist im Vergleich zu der Referenz. Da die Gefäßelemente der Gattung *Shorea* sehr breit sind und ihre Kreuzungsfelder mit ihren großen, scheinbar einfachen Kreuzungsfeldtüpfeln eher einem Netz als einer Wand gleichen, sind sie besonders instabil. Es ist möglich, dass leichte Unterschiede bei der Präparation (die Referenz- und Varianzproben wurden von unterschiedlichen Personen präpariert) dazu führten, dass die Röhren der Gefäßelemente bei den Varianzproben abgeflachter auf dem Objektträger lagen als bei der Referenzprobe und damit im Durchlichtmikroskop breiter erschienen.

Insgesamt zeigen die Messwerte eine z.T. erhebliche Varianz. Da jedoch allein die Varianz innerhalb einer Probe groß ist und zudem bekannt ist, dass der Durchmesser der Gefäßelemente durch Umweltfaktoren beeinflusst wird, verwendet man bei der Holzartenerkennung am Holz schon aus der jahrzehntelangen Erfahrung die metrischen Daten immer nur in Form von Größenklassen. Für viele Gattungen ist beschrieben, dass deren Gefäßgrößen von Art zu Art tendenziell unterschiedlich sind. Bei der Identifizierung von unbekannten Faserstoffen kommt erschwerend hinzu, dass es sich meist um Mischpräparate unterschiedlichster Hölzer handelt. Eine statistische Auswertung schließt sich deshalb aus. Stattdessen werden die Messwerte bei der Identifizierung von unbekannten Proben zusätzlich eingesetzt, um abzugleichen, ob ein Gefäßelement, das von den Strukturmerkmalen einer bestimmten Gattung entspricht, auch von den Messwerten nicht von der Größenklasse der Referenzen abweicht.

Vor diesem Hintergrund zeigen die leichten Abweichungen der Messwerte der untersuchten Zellstoffe der beiden Gattungen zwar einerseits die Varianz der Ausmaße der Gefäßelemente einer Gattung und bestätigen andererseits, dass auch schon die Messwerte der einzelnen Proben die Gattungen für die Anwendung in groben Größenklassen ausreichend wiederspiegeln.



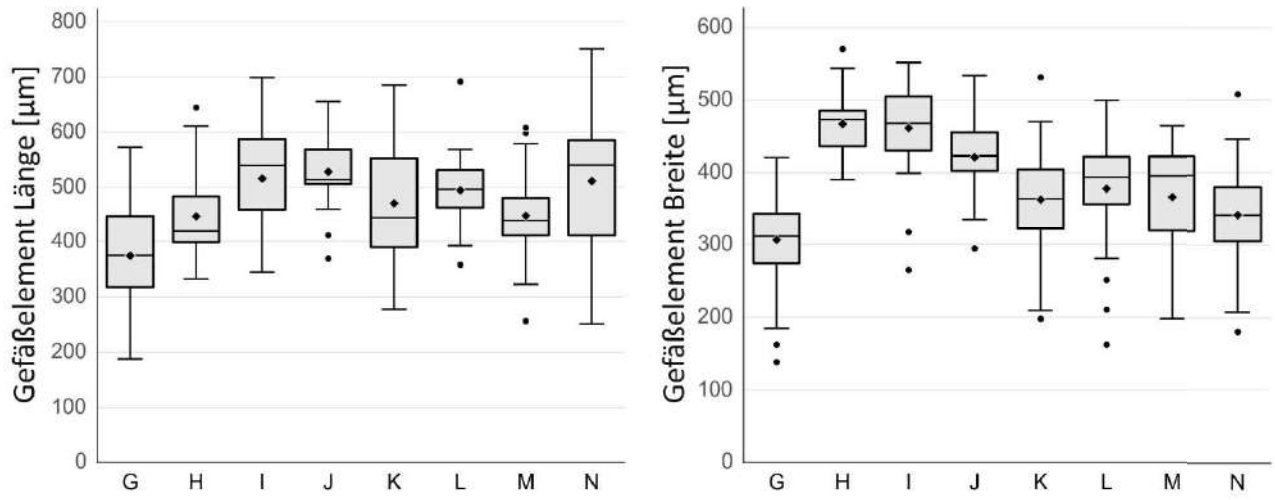


Abbildung 41: Länge und Breite der Gefäßelemente der Varianzproben

(**A) – (F): Gattung *Gonystylus*:**

(A) Referenz; (B) 849-2; (C) 849-3-A; (D) 849-3-B; (E) 849-3-C (F) 849-3-D

(G) – (N): Untergattung *Shorea* subg. *Rubroshorea*

(G) Referenz; (H) Central Kalimantan; (I) East Kalimantan; (J) West Kalimantan; (K) Mentawi Islands (L) Molukken;

(M) Sabah; (N) West Malaysia

Zusammenfassend lässt sich feststellen, dass die an den Gefäßelementen sichtbaren und im Atlas beschriebenen Strukturmerkmale als konsistent innerhalb der Gattung angenommen und bestätigt werden können. Dies ist in Übereinstimmung mit den Erfahrungen aus der Holzartenidentifizierung von Massivholz anhand der standardisierten Strukturmerkmale im IAWA Bestimmungsschlüssel. Der „Atlas of vessel elements“ kann damit als Referenz für die dargestellten Gattungen angesehen werden.

4.8. Anwendung auf Praxisbeispiele (AP 8)

Wie im Vorläuferprojekt wurde auch in diesem Projekt ein Blindtest durchgeführt. Hierzu wurden die auch für die Erstellung der Datenbank verwendeten sortenreinen Zellstoffe (s. Tabelle 10 im Anhang) der Hochschule München, Fr. Prof. Dr. Helga Zollner-Croll, von der Fakultät Papier und Verpackung, zur Verfügung gestellt. Aus diesen Zellstoffen wurden von der Hochschule München Prüfblätter definierter Zusammensetzung hergestellt, ohne den beteiligten Arbeitsgruppen die Zusammensetzung mitzuteilen. Die Zusammensetzung der Prüfblätter sollte von den beteiligten Arbeitsgruppen erkannt werden.

Die Hochschule München wurde gebeten, die in der Tabelle 11 im Anhang aufgeführten Eckpunkte einzuhalten. Den beteiligten Instituten, Technische Hochschule Darmstadt und der ISEGA wurden die Eckpunkte ebenfalls mitgeteilt. Zudem erhielten die beiden beteiligten Institute mit der Übersendung der Prüfblätter eine vollständige Arbeitsfassung des in diesem und dem Vorläuferprojekt erstellten

Gefäßatlases (Helmling et al. 2017), mit dessen Hilfe die Identifizierungen der Blindproben durchgeführt werden können.

Da mit der Chemotaxonomie eine von der Mikroskopie vollständig unabhängige Methode eingesetzt wurde, entschieden sich die Verfasser für eine entsprechend getrennte Darstellung dieses Untersuchungsabschnittes.

4.8.1. Lichtmikroskopische Untersuchung der Blindproben

Die Projektteilnehmer haben aus den erhaltenen 15 Prüfblättern angefärbte, mikroskopische Präparate erstellt und im Lichtmikroskop untersucht. Für alle Prüfblätter mussten die Projektteilnehmer festlegen, welche der 32 Zellstoffe (31 aus asiatischen Hölzern plus einen Buchenzellstoff) enthalten sind. Zusätzlich sollten die Teilnehmer für sich einschätzen, welche Ergebnisse der Identifizierungen sie eher als unsicher ansehen – die sie also in einem realen Gutachten entsprechend als nicht „sicher identifizierbar“ vermerken würden.

Für die Auswertung der Prüfblätter mussten je Prüfblatt 32 Entscheidungen, ob der Zellstoff enthalten ist oder nicht, getroffen werden. Daraus ergeben sich insgesamt 480 Entscheidungen. **Die Auswertungen des umfangreichen Blindtests haben ergeben, dass von den Teilnehmer PMV, TI und ISEGA 74, 92 und 96 % richtige Entscheidungen erzielt werden konnten.** Im Vergleich zum Blindtest des Vorläuferprojekts (86, 90 und 86 %) konnten zwei von drei Teilnehmern die Quote signifikant verbessern, obwohl im zweiten Blindtest mehr Zellstoffe (32 statt 23) enthalten waren und damit die Verwechslungsgefahr stieg.

Bei der Selbsteinschätzung haben die Teilnehmer von PMV und TI hervorgehoben, dass eine Differenzierung der Untergattungen der Gattung *Shorea* - aufgrund ihrer hohen Ähnlichkeiten untereinander - als deutlich schwerer angesehen wird und auch eine Verwechslungsgefahr mit den beiden anderen Gattungen (*Dipterocarpus* und *Parashorea*) aus der Familie der DIPTEROCARPACEAE besteht. Diese Selbsteinschätzung trifft zu, da eine Auswertung der Identifizierung der sechs Zellstoffe der DIPTEROCARPACEAE im Vergleich zur gesamten Studie bei allen Teilnehmern zu einer geringeren Trefferquote führte (63, 82 und 93 % richtige Entscheidungen). Die hohe Bestimmungsquote der ISEGA zeigt jedoch, dass die ausgewählten Hölzer aus der Familie der DIPTEROCARPACEAE mit sehr hohem mikroskopischem Aufwand (hier wurden je Blindtestprobe 15 – 20 Präparate analysiert) sehr gut differenziert werden können.

Eine weitere Gruppe von drei nicht eng verwandten Gattungen (*Gonystylus*, *Lophopetalum* und *Durio*) - aber mit sehr ähnlicher Anatomie der Gefäßelemente - wurde vom Thünen-Institut mit hohem Verwechslungsrisiko eingestuft. Da zu dieser Gruppe die unter CITES-Schutz stehende Gattung *Gonystylus* zählt, ist dies von besonderer Relevanz. Umso erfreulicher ist, dass die Quote der korrekten Identifizierungen in den 15 Blindproben mit 67, 100 und 100 % bei zwei Projektpartnern fehlerfrei erfolgte.

Bei der abschließenden Betrachtung muss berücksichtigt werden, dass in realen Proben weitere Holzarten enthalten sein können, für die noch keine Referenzen vorliegen und die auch eine hohe

Ähnlichkeit zu *Gonystylus spp*. aufweisen könnten. Deshalb ist die Entwicklung der von der Anatomie unabhängigen Chemotaxonomie als zusätzliche bzw. ergänzende Methode von großer Bedeutung. Die guten bis sehr guten Ergebnisse des Blindtests zeigen jedoch eindeutig, dass die im Faseratlas beschriebenen und illustrierten anatomischen Strukturmerkmale erfolgreich für die Bestimmung der Hölzer in Papier verwendet werden können und ein „unverzichtbares“ Referenzmaterial für die Praxis darstellen.

4.8.2. Identifizierung mittels Chemotaxonomie

Wie eine Identifizierung mittels Chemotaxonomie aussehen könnte, soll in dem folgenden Abschnitt beispielhaft gezeigt werden. Da der Datenbankansatz noch in einer frühen Entwicklungsphase ist und noch von etlichen Unzulänglichkeiten geprägt ist, wurde vorerst darauf verzichtet, die bestehende Datenbank auf alle Blindproben anzuwenden, Stattdessen werden in dem folgenden Abschnitt die Möglichkeiten des Datenbankansatzes beispielhaft aufgezeigt.

Es können verschiedene Vergleiche mit Hilfe der in Kap. 4.4.1 erstellten Datenbank durchgeführt werden (Abbildung 43). Der für die Fragestellung im vorliegenden Projekt sich anbietende Vergleich ist der in Abbildung 42 dargestellte Ablauf, der dem Vorgehen bei der Berechnung des fSI entspricht. Jede in der Analysenprobe detektierte Verbindung wird gegen die Datenbank geprüft und es wird festgehalten, ob eine Übereinstimmung mit einer in der Datenbank abgelegten Verbindung gegeben ist. Das Ergebnis dieses Vergleichs wird ausgewertet und in Form einer Tabelle dargestellt, die im besten Falle die qualitative und quantitative Zusammensetzung der untersuchten Mischung wiedergibt.

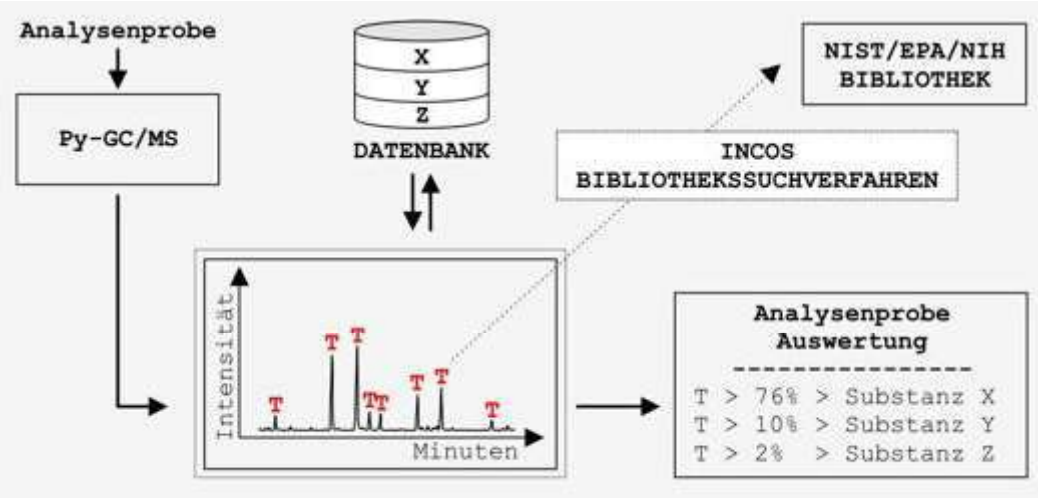


Abbildung 42: Schema der Datenbankabfrage einer zu untersuchenden Probe

Diese theoretische Verfahrensweise stellt sich in der praktischen Anwendung folgendermaßen dar. Die in Form von Prüfblättern vorliegenden Blindproben werden wie in Kap. 4.2 beschrieben aufbereitet und in einer Dreifachbestimmung analysiert. Die Datenvorbehandlung erfolgte wie in Kap. 4.30 beschrieben. Die sich ergebenden GC/MS-Datensätze einer Probe werden übereinandergelegt, um jeweils einen repräsentativen GC/MS-Datensatz für je eine Blindprobe auszuwählen und gegen die in Kap. 4.4.1 erstellte Datenbank zu testen.

5. Öffentlichkeitsarbeit/Veröffentlichungen/Vorträge

5.1. Öffentlichkeitsarbeit

Das Projekt wurde bei der „Nacht des Wissens“ 2013, 2015 und 2017 in Hamburg sowie auf der Grünen Woche 2017 in Berlin und der Ligna 2015 und 2017 in Hannover vorgestellt.

Während des Branchentags des GD Holz in Köln wurde das Projekt am Stand des Thünen-Instituts/Universität Hamburg thematisiert.

5.2. Veröffentlichungen

Helmling S, Olbrich A, Heinz I, Koch G (2018) Atlas of vessel elements – identification of Asian timbers. IAWA Journal 39 (3)

Koch G, Haag V, Helmling S, Heinz I, Olbrich A (2017) Fasern im Fokus: Holzartenbestimmung von Faserplatten - Erfahrungen aus den Prüfungen im Kontext der EUTR. MDF Mag Co: 86-88

Helmling S, Olbrich A, Tepe L, Koch G (2016) Qualitative and quantitative characteristics of macerated vessels of 23 mixed tropical hardwood (MTH) species: a data collection for the identification of wood species in pulp and paper. Holzforschung 70: 839-844

Koch, G., Haag, V, Heinz, I., Richter, H.G. (2016): Die Europäische Holzhandelsverordnung (EUTR). Anforderungen an die Holzartenbestimmung in der Praxis. Holztechnologie 57(1), 5-11.

5.3. Vorträge

Olbrich A (2017) Wood identification. BRC Timber Workshop, London, UK

Olbrich A, Heinz I (2017) CITES-geschützte Hölzer in Zellstoff und Papier? Aufbau einer Datenbank (Faseratlas) für die Bestimmungen von tropischen Hölzern in Papier. Informationsveranstaltung über Anforderungen und Auswirkungen der neuen CITES-Listungen wichtiger Wirtschaftsbaumarten für die Holzverwendung und den Holzhandel – BfN und Thünen Institut, Hamburg

Olbrich A, Koch G (2017) Identifizierung von Tropenhölzern in Papier mit holzanatomischen Methoden. Workshop - Illegalen Holzeinschlag eindämmen DBU, Osnabrück

Odermatt J, Wassink A (2017) Identifizierung von Tropenhölzern in Papier mit chemischen Methoden. Workshop - Illegalen Holzeinschlag eindämmen DBU, Osnabrück

Olbrich A (2017) Wood identification. WWF GFTN-UK meeting, London, UK

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Olbrich A, Helmling S, Tepe L, Poth S, Kuck A, Koch G (2015) Identifizierung des CITES geschützten Ramins in Papier und Faserplatten. Erkennung und Bestimmung von CITES-Hölzern im Handel – BfN und Thünen Institut, Hamburg

Olbrich A, Wassink A (2015) Identifizierung von Tropenhölzern in Papier und Faserplatten. 3. Dresdener Holzanatomisches Kolloquium (ihd), Dresden

Olbrich A, Helmling S, Tepe L, Poth S, Kuck A, Koch G (2014) Identifizierung von „Mixed Tropical Hardwood (MTH)“ in Papier – ein Beitrag zum Artenschutz. Abschluss-Meeting zum DBU Projekt Az. 29436, Osanbrück

Olbrich A (2014) Identification of Mixed Tropical Hardwood in Pulp and Paper. Chatham House - Pulp and Paper Workshop, Teddington, UK

6. Fazit/Ausblick

Im vorliegenden Projekt wurden zwei verschiedene Ansätze zur Identifizierung von MTHs in Papier verfolgt, die Chemotaxonomie und die Anatomie mittels Mikroskopie.

Die Synergien, die sich durch die Anwendung der beiden voneinander unabhängigen Methoden ergeben, können noch nicht genutzt werden, was auf den Nachholbedarf der Chemotaxonomie im Hinblick auf die generelle Methodenentwicklung zurückgeführt werden muss.

Im vorliegenden Projekt wurde im Bereich der Chemotaxonomie eine geeignete Kombination aus: Probenvorbereitung, Extraktion und 1D-GC-Charakterisierung der erhaltenen Extrakte aus gebleichten Zellstoffen/Papier erarbeitet. Es zeigte sich, dass das Auflösungsvermögen der 1D-GC teilweise nicht ausreicht, die Vielzahl an noch auffindbaren, sekundären Pflanzenstoffen vollständig aufzutrennen. Erste Versuche durch verbesserte Fraktionierung mittels SPE oder durch besser auflösende Chromatographie, mittels 2D-GC, versprechen einen noch höheren Informationsgehalt und damit eine bessere Basis zum Auffinden von systematischen Unterschieden zwischen den Gattungen, die dann für Identifizierungen genutzt werden können (s. Kap. 4.2.2 und 4.2.3.2).

Aber auch die durch 1D-GC gewonnene Informationsmenge ist schon sehr hoch (30 bis 300 Einzelverbindungen/Extrakt). Die Verarbeitung der sich in Form der GC/MS-Datensätze ergebenden Informationsmenge ist nur durch geeignete rechnergestützte Auswertung denkbar. Die Prüfung spezifischer Auswertsoftware für die GC/MS-Datensätze, offenbarte jedoch noch Mängel (s. Kap. 4.3.1). Diese Mängel sind von den Softwareentwicklern registriert und werden in Kürze abgestellt. Erst mit einem geeigneten Datenbankansatz ist eine systematische Auswertung der verfügbaren Informationen zielführend, weshalb auf eine Auswertung der Blindproben mittels Chemotaxonomie (s. Kap. 4.8) zum jetzigen Zeitpunkt verzichtet wurde.

Die verschiedenen PCA-Auswertungen, z. B. in Kap. 4.7.1, in denen die Herkünfte verschiedener *Rubroshorea*-Arten im Ansatz unterschieden werden konnten, lassen die Möglichkeiten von Identifizierungen durch die Chemotaxonomie erahnen. Die Ergebnisse belegen das Potential der

Chemotaxonomie, über die Gattungsebene hinaus Identifizierungen zu erzielen. Die systematischen Unterschiede sind auf die „Fingerprint“-Eigenschaften des chromatographisch ermittelten Extraktstoffprofils zurückzuführen. Dieser Ansatz wird in vielen Bereichen für Identifizierungen oder Qualitätsfragen genutzt. Beispiele dafür sind die Charakterisierung der Qualität chinesischer Heilmittel, die aus Extrakten entsprechender Heilpflanzen stammen (Liang et al. 2010), der Herkunft und Qualität verschiedener Weine (Zea et al. 2007) oder auch der Charakterisierung von Rohöl (Wei et al. 2017). Ob solche „Fingerprints“ auch für Fragen der Rückverfolgbarkeit durch die Charakterisierung/Identifizierung einzelner Papier- oder Zellstofflieferungen im Zusammenhang mit den aus dem EUTR resultierenden Sorgfaltspflichten genutzt werden können (s. Kap. 2), ist eine weitere interessante Fragestellung. Gleichzeitig zeigen die Ergebnisse zur Variabilität innerhalb der Gattung allerdings auch, dass die Extraktstoffzusammensetzung deutlich stärker von diesen Variationen beeinflusst wird als die anatomischen Merkmale (Kap. 4.7.)

Diese Variationen innerhalb der Gattung sind vermutlich ein wichtiger Grund, weshalb die Quantifizierung von *Gonystylus spp*. in Papier nur in Einzelfällen gelingt, aber bisher nicht mit einer für alle Fälle geltenden chemotaxonomischen Methodik. Im Einzelfall konnte eine Quantifizierung auch bei niedrigen, einstelligen Prozentanteilen von *Gonystylus*-Zellstoff in Mischungen mit industriell hergestelltem MTH-Zellstoff erreicht werden (s. Kap. 4.4.2).

Wie sich diese gattungsimmanenten Variationen bei weiteren Untersuchungen auswirken ist eine spannende Fragestellung. Weitere Untersuchungen werden zeigen, ob eine verbesserte Auswertung allein oder auch in Kombination mit einer verbesserten Auflösung zu universellen Gattungsmarkern führen. Hierdurch würde nicht nur die Informationsnutzung verbessert, sondern auch die Informationsmenge gesteigert. Dadurch wären trotz der gattungseigenen Variationen vielleicht sogar art- oder herkunftsspezifische Unterschiede erkennbar, was über die Gattungsebene hinausreichende Identifizierungen ermöglichen würde. Diese Informationen könnten dann wie weiter oben schon beschrieben für die Erfüllung der Sorgfaltspflichten, die sich aus dem EUTR und HolzSIG ergeben, genutzt werden.

Methodisch wird die Reproduzierbarkeit ein wichtiger Aspekt in den weiteren Untersuchungen sein. Bisher lag der Focus noch nicht auf der Reproduzierbarkeit, da zuerst der generelle Ablauf der einzelnen Arbeitsschritte der Methodik festgelegt werden musste. Verschiedene Aspekte müssen in diesem Zusammenhang untersucht werden. Als Bestandteil einer Validierung muss die Reproduzierbarkeit der Methodik an sich betrachtet und gegebenenfalls verbessert werden (Becerra und Odermatt 2012). Für die anvisierten Anwendungen auf industriell hergestellte Papiere müssen Einflüsse durch die anderen Papierkomponenten, wie Füllstoffe, Additive und Recyclingfasern, berücksichtigt werden.

Im Bereich der Anatomie wurden die anatomisch-strukturellen Referenzen zur Identifizierung von 38 Gattungen/Untergattungen asiatischer Hölzer in Papier grundlegend ausgearbeitet, dokumentiert und werden als „Atlas of vessel elements - Identification of Asian Timbers“ im IAWA Journal in 2018 veröffentlicht.

Der im Projekt durchgeführte Blindtest zeigt eindeutig, dass unbekannte Proben mit den somit für die Öffentlichkeit zugänglichen Referenzen verglichen werden und damit identifiziert werden können. Bei der Identifikation kann man jedoch „nur“ mit der „besten“ Übereinstimmung zu den bestehenden Referenzen argumentieren. Für einen gerichtsfesten Beweis, dass beispielsweise ein Papier unter anderem Holz der unter CITES-Schutz stehenden Gattung *Gonystylus* enthält, ist deshalb unerlässlich, eine von der Anatomie unabhängige Methode zu etablieren.

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Publication IV

Koch G, Haag V, Helmling S, Heinz I, Olbrich A (2017)

Fasern im Fokus: Holzartenbestimmung von Faserplatten – Erfahrungen aus den Prüfungen im Kontext der EUTR

MDF Mag Co: 86–88

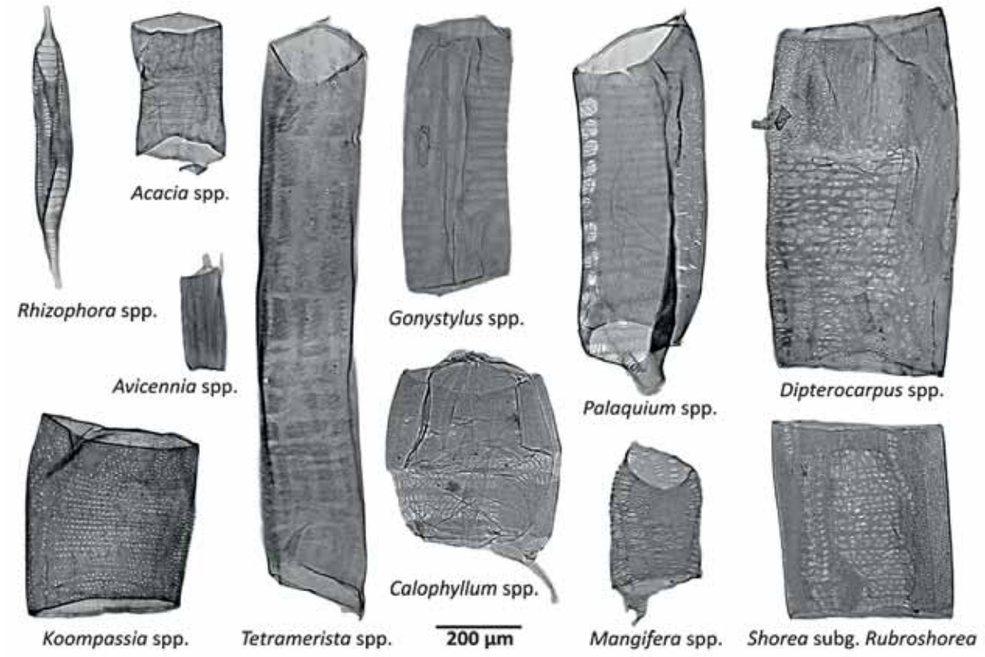

Abb. 2: Bildtafel mit einzelnen Gefäßelementen wichtiger Wirtschaftsbaumarten aus Asien (Referenzen für die Bestimmung von Hölzern in Faserplatten, Zellstoff und Papier)

Fasern im Fokus

Holzartenbestimmung von Faserplatten – Erfahrungen aus den Prüfungen im Kontext der EUTR

Von PD Dr. Gerald Koch, Volker Haag, Stephanie Helmling, Dr. Immo Heinz und Dr. Andrea Olbrich, Thünen-Institut für Holzforschung, Hamburg

Seit Inkrafttreten der Europäischen Holzhandelsverordnung (EUTR) im März 2013 werden am Thünen-Kompetenzzentrum Holzherkünfte zunehmend Produkte aus Faserplatten (MDF) analysiert, um die darin enthaltenen Hölzer – gemäß den geforderten Sorgfaltspflichten – zu bestimmen bzw. zu überprüfen. In der Ausgabe des MDF-Magazins 2015 wurde bereits ausführlich über die Hintergründe (gesetzliche Anforderungen), analytischen Methoden und ersten Erfahrungen aus den Prüfungen unter dem Schlagwort „Pflicht-Bewusstsein“ berichtet. Diese für die Hersteller, Händler und Verbraucher wichtigen Erkenntnisse können nun nach zwei weiteren Jahren intensiver Prüfungen und Forschungsaktivitäten erweitert bzw. präzisiert werden.

Die zunehmenden Anfragen für die Holzartenbestimmung von Faserplatten kommen vor allem aus dem Bereich der Handelsunternehmen, die Holzprodukte mit Bauteilen aus MDF in die EU einführen (ca. 250 Prüfaufträge im Zeitraum Jan. 2016 bis Aug. 2017). Es handelt sich dabei im Wesentlichen um

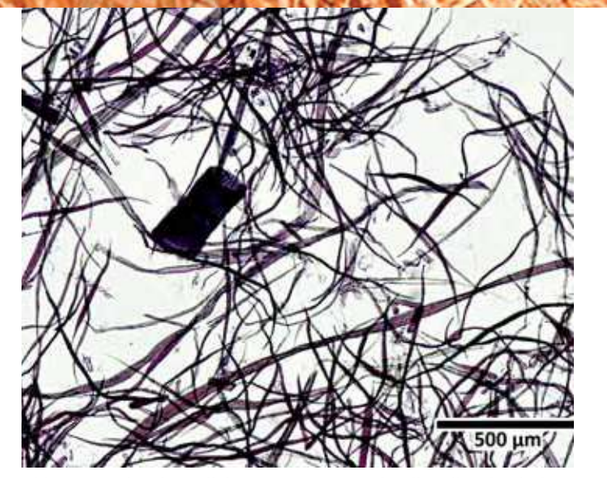

Abb. 1: Vereinzelte Zellelemente (Mazerat) für die mikroskopische Bestimmung der Hölzer. Das Mazerat enthält eine Mischung aus Nadelholz-Tracheiden und Laubholz-Gefäßen (Fotos: Thünen-Kompetenzzentrum)

Botanische Gattung / Familie	Handelsname
Acacia mangium / FABACEAE-MIMOSOIDEAE	Akazie / akasia
Acer spp. / SAPINDACEAE	Ahorn / maple
Alniphyllum spp. / STYRACACEAE	Fortunes China-bells
Alnus spp. / BETULACEAE	Erle / alder
Betula spp. / BETULACEAE	Birke / birch
Eucalyptus spp. / MYRTACEAE	Eukalyptus / eucalypt
Fagus sylvatica / FAGACEAE	Buche / beech
Liquidambar spp. / HAMAMELIDACEAE	Amberbaum / Sweet gum
Liriodendron spp. / MAGNOLIACEAE	Tulpenbaum / Tulip wood
Magnolia spp. / MAGNOLIACEAE	Magnolie / magnolia
Nyssa spp. / NYSSACEAE	Chinese tupelo
Picea spp. / PINACEAE	Fichte / spruce
Pinus spp. / PINACEAE	Kiefer / pine
Populus spp. / SALICACEAE	Pappel / poplar
Pseudotsuga spp. / PINACEAE	Douglasie / Douglas-fir
Schima spp. / THEACEAE	Schima / samak
Tilia spp. / MALVACEAE	Linde / lime

Tabelle 1: Auflistung der regelmäßig in Span- und Faserplatten bestimmten Hölzer aus asiatischer Produktion

Familie	Gattung/Art	Handelsnamen
Acanthaceae	Avicennia marina	Api Api (mangrove)
Altingiaceae	Liquidambar formosana	Formosan sweet gum
Anacardiaceae	Campnosperma sp.	Terentang
Anacardiaceae	Gluta renghas	Rengas
Anacardiaceae	Mangifera sp.	Mango
Anacardiaceae	Swintonia sp.	Merpauh
Aquifoliaceae	Ilex triflora var. kanehirai	Kecemang
Arecaceae	Cocos nucifera	Coconut palm
Burseraceae	Canarium sp.	Kedondong
Calophyllaceae	Calophyllum sp.	Bintangor
Celastraceae	Lophopetalum sp.	Perupok
Dipterocarpaceae	Shorea subg. Anthoshorea	White Meranti
Dipterocarpaceae	Shorea subg. Richetia	Yellow Meranti
Dipterocarpaceae	Shorea subg. Rubroshorea	Dark/Light Red Meranti
Dipterocarpaceae	Shorea subg. Shorea	Bangkirai, Balau
Dipterocarpaceae	Parashorea sp.	Gerutu
Dipterocarpaceae	Dipterocarpus sp.	Keruing
Euphorbiaceae	Hevea brasiliensis	Rubberwood
Fabaceae-Caesalpinioideae	Intsia sp.	Merbau
Fabaceae-Caesalpinioideae	Koompassia malaccensis	Kempas
Fabaceae-Mimosoideae	Acacia mangium	Acacia
Fabaceae-Mimosoideae	Albizia procera	White siris, Kokko
Fagaceae	Castanopsis argentea	Berangan
Lauraceae	Litsea resinosa	Medang
Malvaceae	Durio sp.	Durian
Malvaceae	Heritiera sp.	Mengkulang
Myrtaceae	Eucalyptus globulus	Eucalyptus
Myrtaceae	Syzygium dyerianum	Kelat
Nyssaceae	Nyssa javanica	Tupelo, Nyssa
Paulowniaceae	Paulownia tomentosa	Paulownia
Poaceae	Dendrocalamus latiflorus	Bamboo
Rhizophoraceae	Rhizophora sp.	Red Mangrove
Sapotaceae	Madhuca sericea	Bitis
Sapotaceae	Palaquium sp.	Nyatoh
Styracaceae	Alniphyllum pterospermum	Mee Dong
Tetrameristaceae	Tetramerista glabra	Punah
Theaceae	Schima superba	Samak, Puspa
Thymelaeaceae	Gonystylus sp.	Ramin

Tabelle 2: Auflistung der individuellen Arten im „Atlas of Vessel Elements – Identification of Asian Timbers“

Möbelbauteile und Rahmen, die vollständig der EUTR unterliegen. Gleichzeitig erhält das Komptenzzentrum vermehrt Prüfmuster, die von den Inspekteuren/-innen der Bundesanstalt für Landwirtschaft und Ernährung (BLE) im Rahmen staatlicher Kontrollen entnommen werden. Nachdem die Prüfungen durch die BLE in den Jahren 2013 bis 2016 vorrangig auf Massivhölzer, v.a. Schnitthölzer aus tropischen Regionen und Russland ausgerichtet waren, werden ab 2017 schwerpunktmäßig Faserplatten und Papierprodukte kontrolliert (ca. 30 Prüfaufträge für MDF in 2017). Faserplatten werden zudem zahlreich zu Produkten verarbeitet und importiert, die bisher (noch) nicht der EUTR unterliegen. Hierzu zählen v. a. Bauteile von Kinderspielzeugen und Deko-Artikel, die ebenfalls regelmäßig zur Analyse an das Kompetenzzentrum gesendet werden, um die angegebenen Deklarationen aus handelsrechtlichen Gründen zu prüfen (ca. 150 Prüfaufträge im Zeitraum Jan. 2016 bis Aug. 2017).

Mischungen von bis zu zehn verschiedenen Hölzern

Auf der Grundlage dieser umfangreichen Prüfungen (ca. 560 Einzelproben in 2016 und 2017) können detaillierte Informationen über die in den Faserplatten verwendeten Hölzer und Deklarationen bereitgestellt werden. Die untersuchten Warenmuster, die hauptsächlich aus asiatischer Produktion stammen, enthalten i. d. R. unterschiedliche Mischungen

von bis zu zehn verschiedenen Hölzern (siehe Tab. 1), die weitestgehend mit den bereits 2015 im MDF-Magazin veröffentlichten Arten übereinstimmen. Im Detail lassen sich die analysierten Hölzer in drei Gruppen unterscheiden:

1. Nadelhölzer aus der Familie der PINACEAE (Kiefer, Fichte, Douglasie, Lärche) und zusätzlich Chin. Spießtanne (= Cunninghamia aus der Familie der CUPRESSACEAE).
2. Laubhölzer aus temperierten Verbreitungsgebieten der Familien BETULACEAE, FAGACEAE, MALVACEAE, SALICACEAE, etc. (s. Tab. 1). Hierzu zählen auch Hölzer der Gattungen Liquidambar, Magnolia, Nyssa und Schima, die im temperierten Asien weit verbreitet sind und regelmäßig in den Faserplatten vorkommen.
3. Plantagenhölzer aus den Familien der MYRTACEAE und FABACEAE-MIMOSOIDEAE, wobei es sich hauptsächlich um Eucalyptus spp. und Acacia mangium handelt. Zu den Plantagenhölzern zählen aber auch die schnellwachsenden Kiefern, v. a. Pinus radiata aus der Familie der PINACEAE.

Faseratlas als Bestimmungsschlüssel asiatischer Hölzer

In den untersuchten Proben können bisher keine signifikanten Sortimente oder Beimischungen von „klassischen“ tropischen Baumarten, z. B. aus den Familien der DIPTEROCARPACEAE (Meranti) oder SAPOTACEAE (Nyatoh) nachgewiesen werden, wie sie z. B. regelmäßig in Sperrhölzern vorkommen (vgl. MDF-Magazin 2015). Dennoch können in einzelnen Proben immer wieder Zellelemente (Gefäßtypen) detektiert werden, die sich nicht den oben aufgelisteten Hölzern zuordnen lassen. Für die Identifizierung dieser Arten wurde in den letzten beiden Jahren intensiv an der Erstellung bzw. Erweiterung eines Bestimmungsschlüssels (Faseratlas) für Hölzer aus Asien gearbeitet, der aktuell die wichtigsten 38 Wirtschaftsbaumarten (inkl. Tropenhölzer) aus Asien umfasst (s. Tab. 2).
Mit Hilfe dieser speziellen Referenzen und der bereits vorliegenden Fachliteratur (Fiber Atlas – Identification of Papermaking Fibers; Ilvessalo-Pfäffli, M.-S., 1995) lassen sich die in den Faserplatten sowie in Zellstoff und Papier enthaltenen Hölzer routinemäßig am Kompetenzzentrum bestimmen und mit den angegebenen Deklarationen vergleichen. Die Auswertung der von der BLE eingereichten Prüfaufträge zeigt (aktuell) die großen Schwierigkeiten bzw. Anforderungen in Bezug auf eine eindeutige Deklaration der in Faserplatten (MDF) enthaltenen Hölzer. In den meisten Fällen

Abb. 4: Mikroskopie von Zellelementen einer Faserplatte (MDF)

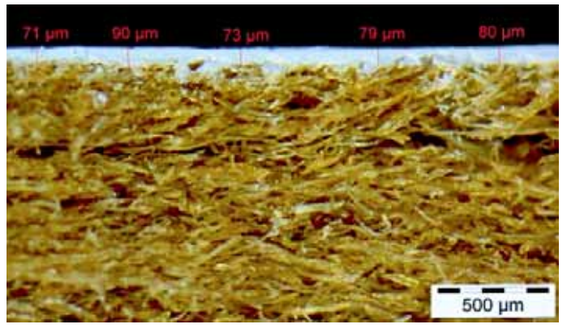

Abb. 3: Auflichtaufnahme einer Faserplatte mit Vermessung der aufgetragenen Lackschicht

werden in den Begleitdokumenten keine oder nur unzureichende Angaben zu den verwendeten Hölzern gemacht, so z. B. nur Angaben zu einzelnen Taxa wie „eucalypt“ oder „pine“, wogegen sich in den untersuchten Proben i. d. R. Mischungen von bis zu zehn Arten nachweisen lassen. In anderen Fällen werden Listen mit über 50 (!) individuellen Arten von Acer negundo bis Ulmus rubra (Laubhölzer) und Abies fraseri bis Tsuga caroliniana (Na-delhölzer) aufgeführt, die in Nordamerika, Mitteleuropa und im temperierten Asien verbreitet sind und „theoretisch“ in den Faserplatten vorkommen könnten; gemäß der Intention „es wird schon die richtige Holzart dabei sein“. Diese Art der Sorgfaltspflicht-Erfüllung setzt aber voraus, dass für jede individuelle Art ein Nachweis (Zertifikat) der legalen Herkunft vorgelegt werden müsste. Die Anzahl der untersuchten Faserplatten, die nur eine Holzart, z. B. ausschließlich Eukalyptus, Pappel oder Kiefer enthalten, ist sehr selten. In diesen Fällen stimmen die Ergebnisse der mikroskopischen Bestimmungen mit den angegebenen Deklarationen überein. Bei der überwiegenden Anzahl der analysierten Proben handelt es sich aber um die bereits erwähnten Mischungen der aufgelisteten Hölzer (s. Tab. 1), so dass die Hersteller und Importeure die Angaben zu den Deklarationen durch regelmäßige Kontrollen und Überprüfungen der Zertifikate präzisieren bzw. optimieren sollten.

Gefäßzellendiagnostik identifiziert die Holzarten

Aus den bisherigen Erfahrungen und Entwicklungen lässt sich zudem ableiten bzw. erwarten, dass die Produktionsstätten weiter nach Asien verlagert und zunehmend Hölzer aus dem asiatischen Verbreitungsgebiet verwendet werden. Daher wurde am Thünen-Kompetenzzentrum Holzherkünfte mit finanzieller Förderung durch die Deutsche Bundesstiftung Umwelt (DBU) in den letzten beiden Jahren intensiv an der Erstellung eines „Atlas of Vessel Elements – Identification of Asian Timbers“ gearbeitet, der sowohl für die Bestimmung von Zellelementen in Faserplatten (MDF) als auch für die Identifizierung von Zellstoff und Papier verwendet werden kann. Der Faseratlas, der aktuell zur Veröffentlichung im IAWA-Journal (International Association of Wood Anatomists) eingereicht wurde, umfasst eine detaillierte Beschreibung und bildliche Dokumentation der Strukturmerkmale von insgesamt 38 botanischen Taxa (s. Tab. 2) aus Asien. Die aufgeführten Arten besitzen ein hohes wirtschaftliches Potential und können, wie bereits am Beispiel von Liquidambar, Ilex oder Nyssa, regelmäßig in Faserplatten nachgewiesen werden.
Die Bestimmung bzw. Unterscheidung dieser Laubholzarten erfolgt ausschließlich anhand von individuellen Strukturmerkmalen der vereinzelten (= mazerierten) Gefäßzellen (Abb. 1 und 2), die einführend im Faseratlas beschrieben und für jede individuelle Art anatomisch charakterisiert sowie mit hochauflösenden Bildern dokumentiert werden. Als wichtige anatomische Bestimmungsmerkmale werden dabei die Form, Größe und Anordnung der Gefäßtüpfel (= Aussparungen zum Stoffaustausch) verwendet. Weiterhin haben die Art der Gefäßdurchbrechungen und z. B. das Vorkommen von spiraligen Verdickungsleisten eine hohe diagnostische Bedeutung. Abb. 2 zeigt eine Bildtafel zur anatomischen Unterscheidung und Bestimmung individueller Arten mit charakteristischen Strukturmerkmalen. Die Erkennung und Differenzierung dieser Merkmale erfordert eine große mikroskopische Erfahrung und spezifische wissenschaftliche Expertise, die derzeit nur von wenigen Instituten weltweit durchgeführt bzw. angeboten werden kann. Der neu erstellte Faseratlas bietet dafür eine unverzichtbare Hilfe, um die Bestimmung der Hölzer in Faserplatten (Abb. 3 und 4), Zellstoff und Papier zu ermöglichen. Näheres:
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Publication V

Sieburg-Rockel IJ, Koch G, Kaschuro S, Helmling S, Olbrich A (2019)

Identifizierung von Holzarten in Spanplatten

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Identifizierung von Holzarten in Spanplatten

Jördis Sieburg-Rockel, Gerald Koch, Sergej Kaschuro, Stephanie Helmling, Andrea Olbrich

In die EU eingeführte Hölzer und Holzprodukte (Spanplatten eingeschlossen) unterliegen seit 2013 der Holzhandelsverordnung (*EUTR,* 2010). Im Hinblick auf die korrekte und vollständige Deklaration werden am Thünen-Kompetenzzentrum Holzherkünfte erstmalig die Holzartenzusammensetzungen in Spanplatten grundlegend untersucht. Die anatomische Bestimmung der Späne in den Deck- und Mittelschichten ist aufgrund der unterschiedlichen Spangrößen und -geometrie deutlich schwieriger im Vergleich zur Bestimmung von Massivholzproben und erfordert einen hohen präparativen Aufwand. Die bisherigen Ergebnisse der mikroskopischen Untersuchungen zeigen, dass die Holzartenzusammensetzungen zumeist den regionalen Herkünften der Hölzer bzw. Produktionsstätten entsprechen. Die identifizierten Hölzer können in fünf Gruppen – Nadelhölzer, Laubhölzer (temperiert), Laubhölzer (asiatisch), Plantagenhölzer und Recyclinghölzer – unterteilt werden. Auf der Grundlage dieser Ergebnisse werden die Referenzen für die Bestimmung der Hölzer in Spanplatten, gemäß den Anforderungen der *EUTR* (2010), maßgeblich erweitert.

Schlüsselwörter: Spanplatten, Holzartenbestimmung, EUTR

Einleitung

Die Artenvielfalt in Holzwerkstoffen, wie z. B. Sperrholz, Faserplatten und Spanplatten aber auch Papieren, wird immer größer. Einerseits werden aufgrund der wachsenden Nachfrage und globalisierter Märkte immer mehr Austauschhölzer mobilisiert. Andererseits werden aus ökonomischen Gründen und zur stofflichen Weiternutzung des wertvollen Rohstoffes Holz – primär in Spanplatten – vermehrt Recyclinghölzer eingemischt. Der Anteil an Altholz, der in Europa produzierten Spanplatten, liegt im Schnitt bei 31 %. In China beträgt der Anteil an Recyclingholz nach den letzten Erhebungen hingegen nur 1 % (*EPF,* 2017) und ist damit noch deutlich steigerungsfähig.

Von großer Bedeutung sind diese Entwicklungen in Bezug auf die Umsetzung der Europäischen Holzhandelsverordnung (*EUTR,* 2010), die im März 2013 in Kraft getreten ist, um den Import und Handel mit illegal eingeschlagenem Holz in die EU zu verhindern. Danach müssen im Rahmen der Sorgfaltspflicht die in den eingeführten Produkten enthaltenen Hölzer mit botanischem Namen benannt und deren Herkunft aus glaubhaft legalen Quellen dokumentiert sein. In Deutschland werden die Prüfmuster aus den staatlichen Kontrollen der zuständigen Bundesanstalt für Landwirtschaft und Ernährung (BLE) am Thünen-Kompetenzzentrum Holzherkünfte untersucht (*Koch et al.,* 2016). Das Kompetenzzentrum hat sich zu einer der europaweit führenden Anlaufstellen für Behörden, Holzhandel, Verbraucher und Verbände bei Fragen des Art- und Herkunftsnachweises von Holz und Holzprodukten entwickelt. Besonders die Zahl der Gutachten zur anatomischen Bestimmung der Holzarten nimmt seitdem jährlich um 25 % bis 30 % zu. Neben den Massivhölzern werden zunehmend Produkte und Bauteile aus Holzwerkstoffen und Papier analysiert (*Koch et al.,* 2017). Die Deklaration der in Europa hergestellten Spanplatten umfasst in der Regel eine Auflistung der gesamten heimischen Wirtschaftsbaumarten, hinzu kommt der nicht näher definierte Altholzanteil. Für in Asien gefertigte Platten gibt es zumeist keine bzw. nur unvollständige Deklarationen. Da das Einzugsgebiet für einen rentablen Holzeinkauf zur Herstellung von Spanplatten zwischen 100 km und 200 km in Mitteleuropa liegt, kann von einer bestimmten räumlich begrenzten Artenvielfallt ausgegangen werden, wenn der Produktionsstandort bekannt ist.

Material und Methoden

Bei der überwiegenden Anzahl der untersuchten Spanplatten handelt es sich um Muster aus dem Holzhandel, die zu einer Prüfung an das Thünen-Kompetenzzentrum eingeschickt wurden. Sie bilden die Grundlage der erstmalig systematisch

Abb. 1: Anfertigung von Handschnitten
Fig. 1: The production of hand cuts

untersuchten Holzartenzusammensetzung in Spanplatten, hergestellt in Europa und Asien. Exemplarisch werden drei repräsentative Plattentypen vorgestellt. Eine Spanplatte aus einer europäischen Produktion und zwei Platten aus asiatischen Produktionslinien.
Die anatomischen Holzartenbestimmungen erfolgen in einer Kombination aus zwei bewährten Methoden:

1. Für die Bestimmung der gröberen Spanfraktionen aus der Mittelschicht der Platte werden von diesen, wie für die Analyse von Massivhölzern, mikroskopische Schnitte in den drei anatomischen Richtungen des Holzes angefertigt. Die Späne werden nach der Vereinzelung durch Aufkochen in Leitungswasser mit einer handelsüblichen Rasierklinge unter dem Auflichtmikroskop von Hand geschnitten (Abb. 1). Diese Schnitte in Quer-, Radial- und Tangentialrichtung des Holzes enthalten die charakteristischen mikroskopischen Strukturmerkmale. Trotz der vergleichsweise kleinen Dimensionen, können die bis zu 100 Merkmale des Gewebes untersucht bzw. beschrieben werden. Um die Variabilität (möglicher) unterschiedlicher Arten erfassen zu können, werden die Querschnitte der Platten zunächst makroskopisch begutachtet und für jede Spanplatte bis zu zehn ausgewählte Späne einzeln geschnitten, präpariert und lichtmikroskopisch untersucht. Da die Querschnittsflächen der Spanfraktionen (Abb. 2) zumeist kleiner als 1 mm² sind, ist ihre anatomische Bestimmung im Vergleich zu Massivholzproben deutlich erschwert.
2. Die feineren Deckschichten der Spanplatten werden analog zur Analyse von Faserplatten zunächst separiert, angefärbt und dann lichtmikroskopisch untersucht (*Koch et al.,* 2017). An den vereinzelten Zellelementen und Faserbündel lassen sich die wichtigen Strukturmerkmale zur Unterscheidung von Nadelholztracheiden und Laubholzgefäßen sicher differenzieren (abgrenzen). Insgesamt können im Vergleich zur Massivholzbestimmung deutlich weniger Strukturmerkmale verwendet werden, die in den meisten Fällen aber ausreichend sind, um die Hölzer auf Gattungsebene über den direkten Abgleich mit bildlich dokumentierten Referenzen zu identifizieren. Für die Bestimmung der Hölzer Europas und Nordamerikas liegt bereits seit Jahren das Standardwerk „Fiber Atlas – Identification of Papermaking Fibers" von *Ilvessalo-Pfäffli* (1995) vor. Als Referenz für die Bestimmung asiatischer Wirtschaftsbaumarten wurde am Thünen-Kompetenzzentrum Holzherkünfte der „Atlas of Vessel Elements – Identification of Asian Timbers" erstellt und aktuell im IAWA-Journal veröffentlicht (*Helmling et al.,* 2018).
Enthält die Deckschicht einer Spanplatte mehrere Zelllagen dicke Gewebeverbünde, besteht die Möglichkeit, die Späne zusätzlich zu mazerieren, d. h. den Zellverbund aufzulösen. Dies erfolgt entsprechend der Methode von *Franklin* (1945) in einer Lösung, bestehend zu gleichen Teilen aus Essigsäure (99 %) und Wasserstoffperoxid (30 %) unter Einwirkung von Hitze (60 °C), die speziell für die Gefäßanalyse auch von *Helmling et al.* (2016) beschrieben wird. Die vereinzelten Zellen werden anhand ihrer individuellen Strukturmerkmale, wie oben für die Faserstoffe beschrieben, mikroskopisch identifiziert.

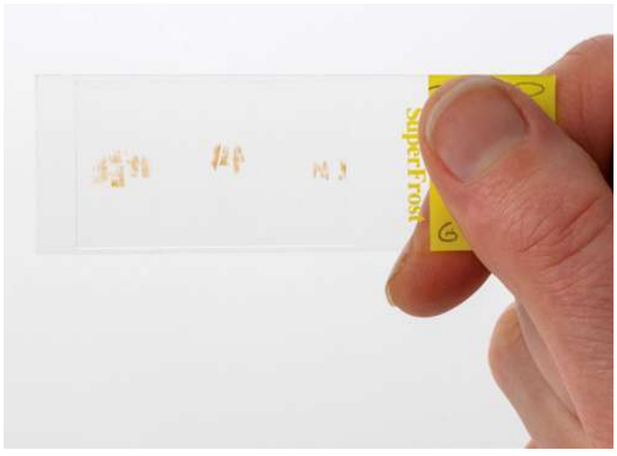

Abb. 2: Objektträger mit mikroskopischen Schnitten eines Spans in den drei anatomischen Richtungen
Fig. 2: Slide with microscopic sections of a chip in the anatomical directions

Ergebnisse

Abb. 3a zeigt die Querschnittsfläche der ausgewählten Spanplatte aus europäischer Produktion. Mit Hilfe der beschriebenen mikroskopischen Verfahren können die separierten Spanfraktionen eindeutig bestimmt und den folgenden Gattungen/Arten Fichte, Kiefer, Esche, Buche und Ahorn zugeordnet werden. Die Mischung aus Laub- und Nadelhölzern ist dabei nicht ungewöhnlich. In der Makroaufnahme (Abb. 3b) sind die Querschnittsflächen eines Nadelholzes, aber auch von Buche, Esche und Ahorn bereits erkennbar.
Abb. 4a zeigt den Querschnitt einer Platte, die vollständig

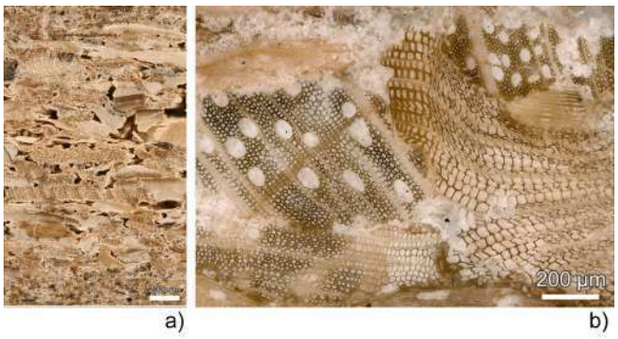

Abb. 3: a) Querschnittsfläche einer Spanplatte aus europäischer Produktion; b) Querschnittsflächen eines Nadelholzes, Buche, Esche und Ahorn in der Deckschicht der Spanplatte
Fig. 3: a) Cross-sectional area of a particle board from European production; b) Cross-sectional areas of coniferous wood, beech, ash and maple in the surface layer of the particle board

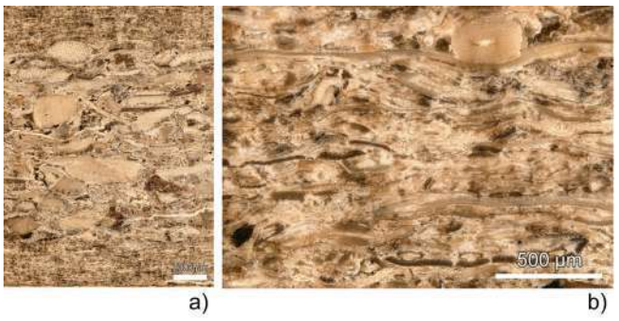

Abb. 5: a) Querschnitt einer Spanplatte aus Asien mit verschiedenen Hölzern in der Mittelschicht und einer Deckschicht aus Gräsern (Monokotyledonen); b) Detailaufnahme der Deckschicht
Fig. 5: a) Cross-section of a particleboard from Asia with various types of wood in the middle layer and a surface layer of grasses (monocotyledons); b) Detailed view of the surface layer

Spanfraktionen asiatischer Laubhölzer enthält. Die mikroskopischen Analysen haben als „bemerkenswertes" Ergebnis ergeben, dass die Strukturmerkmale einzelner Späne beste Übereinstimmung mit denen des chinesischen Zierstrauches (*Loropetalum chinense*) zeigen. Die Verwendung dieser – für den europäischen Verarbeiter – eher unbekannten Art, ist aber in Bezug auf die geographische Herkunft nicht ungewöhnlich, da sie im temperierten Asien natürlich verbreitet ist. Weiterhin können in der asiatischen Platte Hölzer der Gattungen Pulai, Rubberwood, Melia, Amour cork tree und Ficus (Abb. 4b) mikroskopisch bestimmt werden.
Abb. 5a zeigt den Querschnitt einer Platte, deren Deckschicht – als Besonderheit – vornehmlich aus Gräsern (Monokotyledonen) der Familie der POACEAE besteht. Ein eindeutiges diagnostisches Merkmal sind die typischen Leitbündel in den Querschnittsflächen (Abb. 5b). Zusätzlich weist das untersuchte Muster eine außergewöhnlich vielfältige Mischung unterschiedlicher asiatischer Laub- und Nadelhölzer auf. Im Detail konnten die folgenden Hölzer bestimmt werden:

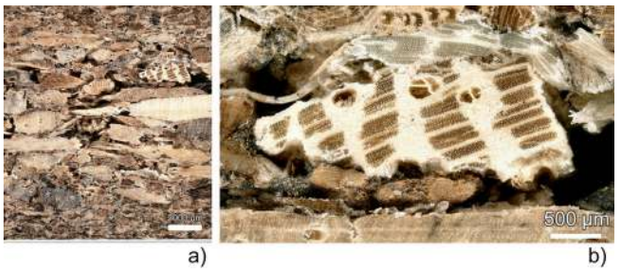

Abb. 4: a) Querschnitt einer Platte, vollständig aus asiatischen Laubhölzern; b) Querschnitt eines Spans aus der Mittelschicht, der größte Übereinstimmung mit Ficus spp. (MORACEAE) zeigt
Fig. 4: a) Cross-section of a panel, made entirely of asian hardwoods; b) Cross-section of a chip from the middle layer which shows the best match with Ficus spp. (MORACEAE)

- Rasamala,
- Celtis,
- chinesische Spießtanne,
- Pappel,
- Kiefer,
- Flügelnuss,
- Eiche,
- Toona,
- eine ULMACEAE und
- eine JUGLANDACEAE.

Grundsätzlich haben die anatomischen Untersuchungen der Spanplatten bisher gezeigt, dass die Holzartenzusammensetzungen der Deck- und Mittelschichtspäne zumeist übereinstimmen, mit Ausnahme der Platte mit Deckschichten aus Monokotyledonen.
In Tab. 1 sind die anatomisch bestimmten Hölzer aufgelistet. Die analysierten Hölzer lassen sich in folgende Gruppen aufteilen:

- Nadelhölzer aus der Familie der PINACEAE (Hierzu zählen Kiefer, Fichte, Lärche, Douglasie und Tanne),
- Laubhölzer aus temperierten Verbreitungsgebieten (Eiche, Buche (FAGACEAE), Birke, Erle (BETULACEAE), Ahorn (SAPINDACEAE) und Esche (OLEACEAE),
- Laubhölzer aus asiatischen Verbreitungsgebieten (z. B. Canarium (BURSERACEAE), Medang (LAURACEAE), Liquidambar (ALTINGIACEAE) oder Machang (ANACARDIACEAE), die für Spanplatten aus asiatischer Produktion bestimmt wurden),
- weltweit in Plantagen angebaute Laub- und Nadelhölzer (z. B. Eukalyptus (MYRTACEAE) und Hevea (EUPHORBIACEAE) oder *Pinus radiata* (PINACEAE),
- Recyclinghölzer – Altholz (Späne mit Farbanhaftungen und kreuzverleimte Späne (z. B. aus Pappel), wahrscheinlich aus Sperrhölzern oder einzelne tropische Baumarten in einer sonst homogen europäischen Mischung von Hölzern (z. B. Limba und Afzelia in einer Mischung mit Kiefer, Fichte und Buche)).

Exakte quantitative Aussagen zu Anteilen bestimmter Holz-

Tab. 1: Liste der bisher identifizierten Hölzer in Spanplatten
Tab. 1: List of identified woods in particleboards so far

Botanische Gattung/Art	Handelsname	Familie
Abies spp.	Tanne	PINACEAE
Acer spp.	Ahorn	SAPINDACEAE
Afzelia spp.	Afzelia	FABACEAE-CAESALPINIOIDEAE
Alnus spp.	Erle	BETULACEAE
Alstonia spp.	Pulai	APOCYNACEAE
Altingia spp.	Rasamala	ALTINGIACEAE (HAMAMELIDACEAE)
Betula spp.	Birke	BETULACEAE
Canarium spp.	Canarium	BURSERACEAE
Castanea spp.	Edelkastanie	FAGACEAE
Celtis spp.	Celtis	CANNABACEAE (ULMACEAE)
Cinnamomum spp.	Medang, cinamon	LAURACEAE
Cunninghamia spp.	Chin. Spießtanne	CUPRESSACEAE
Cupressus spp.	Zypresse	CUPRESSACEAE
Eucalyptus spp.	Eukalyptus	MYRTACEAE
Fagus sylvatica	Buche	FAGACEAE
Falcataria moluccana	Jeungjing	FABACEAE-MIMOSOIDEAE
Fraxinus spp.	Esche	OLEACEAE
Hevea brasiliensis	Rubberwood	EUPHORBIACEAE
Juglans spp.	Nußbaum	JUGLANDACEAE
Larix spp.	Lärche	PINACEAE
Liquidambar spp.	Red gum, sweet gum	ALTINGIACEAE
Loropetalum chinense	Chinese fringe flower	HAMAMELIDACEAE
Mangifera indica	Machang	ANACARDIACEAE
Melia azedarach	Paraiso	MELIACEAE
Paulownia tomentosa	Blauglockenbaum	PAULOWNIACEAE
Phellodendron spp.	Amur cork tree	RUTACEAE
Picea spp.	Fichte	PINACEAE
Pinus spp.	Kiefer	PINACEAE
Monokotyledonen	Gras, Reis, Bambus	POACEAE
Pometia pinata	Kasai	SAPINDACEAE
Populus spp.	Pappel	SALICACEAE
Pseudotsuga menzesii	Douglasie	PINACEAE
Pterocarya spp.	Flügelnuss	JUGLANDACEAE
Quercus spp.	Eiche	FAGACEAE
Shorea spp., subg. *Rubroshorea*	Red Balau	DIPTEROCARPACEAE
Terminalia superba	Limba	COMBRETACEAE
Toona spp.	Kalantas	MELIACEAE

arten in den Platten können zumeist nicht gemacht werden. Die hierfür erforderliche Analyse und Auszählung aller Späne pro definierter Fläche oder Raumgröße ist sehr aufwendig und kann innerhalb einer Platte variieren.

Fazit

Die Bestimmung der Holzartenzusammensetzung in Spanplatten ist aufgrund der unterschiedlichen Spangröße und -geometrie sowie der Vielfalt der verarbeiteten Hölzer (speziell Sortimente aus Asien) eine besondere Herausforderung. Nicht nur in Bezug auf die mikroskopische Bestimmung, die eine große Erfahrung und wissenschaftliche Expertise erfordert, sondern auch unter Berücksichtigung der aufwendigen Probenvorbereitung. Die Holzanatomie ist für die Bestimmung der Hölzer in Spanplatten derzeit die einzige praktikable Methode. Genetische Verfahren können aufgrund der thermisch-bedingten Veränderungen des Holzgewebes, der kleinen Partikelgrößen und -mischungen nicht verwendet werden.
Die holzanatomische Expertise wird daher zunehmend nachgefragt, nicht zuletzt auch infolge der Altholzverwertung, die zukünftig eine noch größere Artenvielfalt in den Spanplatten erwarten lässt. Für die Einhaltung und Umsetzung der *EUTR* (2010) ist es deshalb umso wichtiger, die Referenzen kontinuierlich zu erweitern. In diesem Zusammenhang stellt sich auch die Frage, wie sich die größere Variabilität in der Holzartenzusammensetzung auf die Produkteigenschaften auswirkt.

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ABSTRACT

Identification of wood species in particle boards

Wood and wood products (including particle board) imported into the EU have been subject to the EU Timber Trade Regulation (EUTR, 2010) since 2013. With regard to the requirements of a clear declaration, the wood compositions in particleboards are being investigated for the first time at the Thünen Centre of Competence on the Origin of Timber. Due to the different sizes and geometry, the anatomical identification of particles in the top and middle layers is considerably more difficult as compared to solid wood samples and requires a high methodological approach. The previous results of the microscopic studies show that the compositions of the identified botanical taxa mostly correspond to the regional origins of the woods or production sites. The identified woods can be divided into five groups – softwood, hardwood (tempered), hardwood (Asia), plantation wood and recycled wood. Based on these results, the references for the determination of woods in particleboards are significantly extended according to the requirements of EUTR (2010).

Keywords: *Particleboards, identification of wood species, EUTR*

für Holzforschung, Leuschnerstraße 91c, 21031 Hamburg, in der Abteilung Qualität von Holz und Holzprodukten. Hier forscht sie im Bereich der mikroskopischen Bestimmung international gehandelter Hölzer (Schwerpunkt: Holzwerkstoffe/Spanplatte) und spektroskopischen Analyse zur Charakterisierung der Materialeigenschaften. Zuvor war sie in der Holzartenbestimmung an Kunstwerken und mit der Erstellung von Datenblättern handelsrelevanten Hölzern tätig.
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Sergej Kaschuro ist biologisch-technischer Assistent. Er ist seit 2011 am Thünen-Institut für Holzforschung für Präparation und bildgebende Verfahren verantwortlich.

Dipl.- Holzwirtin Stephanie Helmling absolvierte nach ihrer Ausbildung zur Orgelbauerin in Bonn das Studium der Holzwirtschaft an der Universität Hamburg. Seit 2011 ist sie als wissenschaftliche Mitarbeiterin am Thünen-Institut für Holzforschung und an der Universität Hamburg tätig und baut im Rahmen ihrer Promotion Referenzen tropischer Hölzer zur Faseranalyse in Zellstoff und Papier auf.

Dr. Andrea Olbrich (Dipl.-Biol.) hat sich während des Studiums an der Georg-August-Universität Göttingen und der Promotion an der Ruprecht-Karls-Universität Heidelberg sich auf Botanik und Mikroskopie spezialisiert. Mit ihrer anschließenden Tätigkeit am Institut für Forstbotanik der Universität Göttingen fokussierte sie sich auf die Holzanatomie und ist heute als wissenschaftliche Mitarbeiterin des Thünen-Instituts für Holzforschung für das Thünen-Kompetenzzentrum für Holzherkünfte die Ansprechpartnerin für die Holzartenbestimmung in Fasermaterialien.

Publication VI

Heinz I, Helmling S, Olbrich A, Koch G (2019) O-5:

Identification of Asian timbers in pulp, paper and fiber boards

IAWA-IUFRO International Symposium: challenges and opportunities for updating wood identification; May 20–22, 2019, China, program & abstracts. pp. 5–6

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Challenges and Opportunities for Updating Wood Identification

IAWA-IUFRO International Symposium 2019

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O-5: Identification of Asian Timbers in Pulp, Paper and Fiber Boards

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Abstract

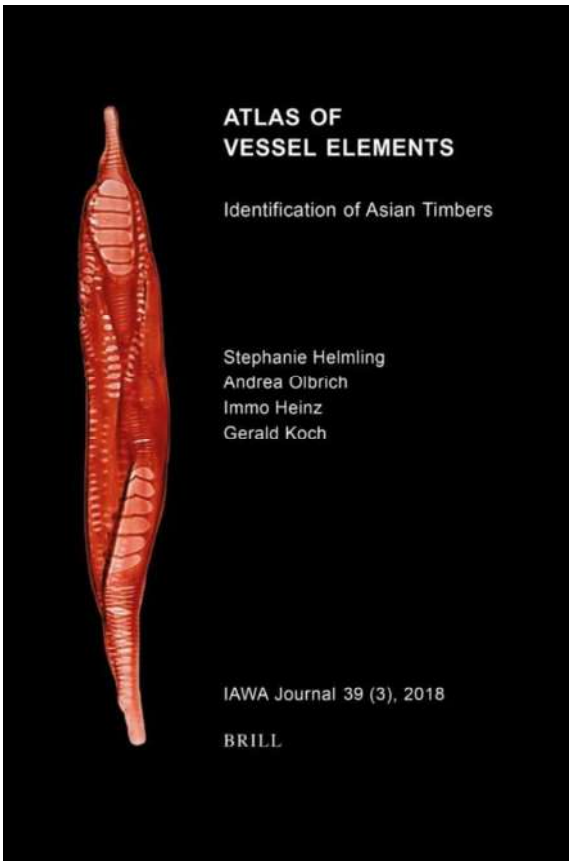
The identification of timbers used in pulp and paper is becoming increasingly important for the implementation of national and international legislations that forbids the use of illegally logged or endangered species, e.g., European Timber Regulation (EUTR) and CITES policies.

Wood anatomy currently provides the "exclusive" method for the identification of pulp and paper components as well as those of fiber boards which are also subject to the controls of the EUTR. In comparison to the microscopic identification of solid wood blocks, the number of usable microscopic features is severely reduced in the macerated tissue of pulp and paper. In detail, the separated vessel elements provide the best information for a microscopic identification based on typical features like perforation plates, presence of helical thickenings and shape and arrangement of vessel-ray pits, e.g.

These individual morphological information are already described in the "Fiber Atlas - Identification of Papermaking Fibers" including the wood anatomical characterization of the most important temperate and plantation-grown species used for pulp and paper production (Ilvessalo-Pfäffli 1995). However, the increasing pulp production in Asia involves the frequent use of tree species from these regions and requires a defined morphological description of the tissues.

In order to enable the essential identification of Asian timbers used in pulp, paper, and fiber board production, the morphological characteristics of vessels in macerated material of 38 important timbers distributed in Asia are recently studied by a team of authors from the Thünen Centre of Competence on the Origin of Timber and published (open access) as special edition "Atlas of vessel elements - Identification of Asian Timbers" in the IAWA Journal issue 39 (3).

(http://booksandjournals.brillonline.com/content/journals/10.1163/22941932-20180202).



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A data analysis guide for wood identification. Overview of current practices for different methods

Global Timber Tracking Network, GTTN secretariat, European Forest Institute and Thünen Institute

Reduced to relevant chapters: p. 1–2, 4–6, 13, 34–42.

Overview of current practices in data analysis for wood identification

A guide for the different timber tracking methods

June 2020

Editor:
Nele Schmitz

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*Names are listed in alphabetical order.

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Table of Contents

1. Wood anatomy

Definition wood anatomical reference data: descriptions and/or illustrations of the macroscopic and/or microscopic features of the wood, preferably covering many samples from all major woody lineages.

Authors: Hans Beeckman, Peter Gasson, Volker Haag, Stephanie Helmling, Gerald Koch, Frederic Lens, Andrea Olbrich, Prabu Ravindran, Elisabeth Wheeler, Alex C. Wiedenhoeft, Valentina Th. Zemke

*Authors are in alphabetical order.

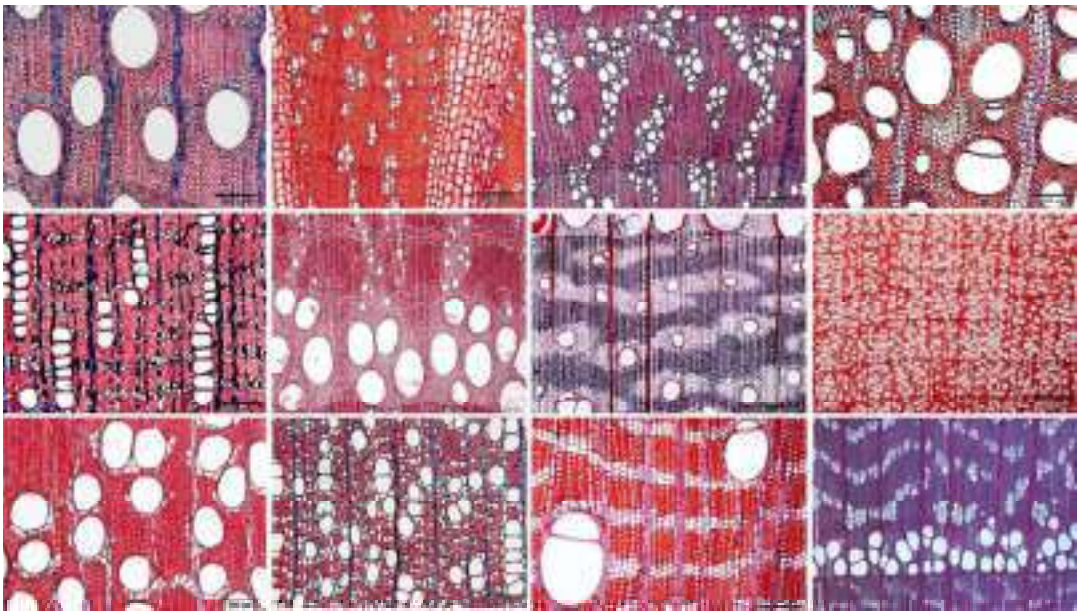


Fig. 1: Microsections showing the variety of the anatomy of hardwoods

1.3 DATA ANALYSIS FOR TAXON IDENTIFICATION OF PULP, PAPER AND FIBREBOARD

1.3.1 DEVELOPMENT OF VESSEL ELEMENT REFERENCE DATA

In most cases, pulp, paper, and fibreboard are made of more than one timber species. Maceration of the pulp, paper or fibreboard hence gives isolated cells of a mixture of wood species. In fibre material made of hardwood the vessel elements are the cell type with the most structural characteristics. Therefore, **high-value descriptions** and **micrographic illustrations** of these cells are needed as references[16].

1.3.1.1 PREPARATION OF REFERENCE SLIDES

For the preparation of slides that can be stored permanently as references:

- ☑ **Take splits** of 1 cm length, 1 mm thickness and 1 cm in the radial direction to obtain a representative selection of vessel elements from successive growth increments.
- ☑ **Boil** the splits in distilled water and then **macerate** them in a solution of equal parts of glacial acetic acid (99%) and H_2O_2 (30%) at 60°C for 48h following the method of Franklin (1945)[17]. The woody tissue is broken up.
- ☑ **Separate the individual cell elements** by shaking the test tubes.
- ☑ In a plastic filter, **rinse** the resulting suspension with tap water and **stain** with Nigrosin (1%) (alcohol soluble).
- ☑ **Select the vessel elements** from the other cells in the pulp by needle using a reflected light microscope, place them on microscope slides, and embed in Euparal. Place the microscope slides under a flue to evaporate for 1 day and then heat at 50°C for 10 days.

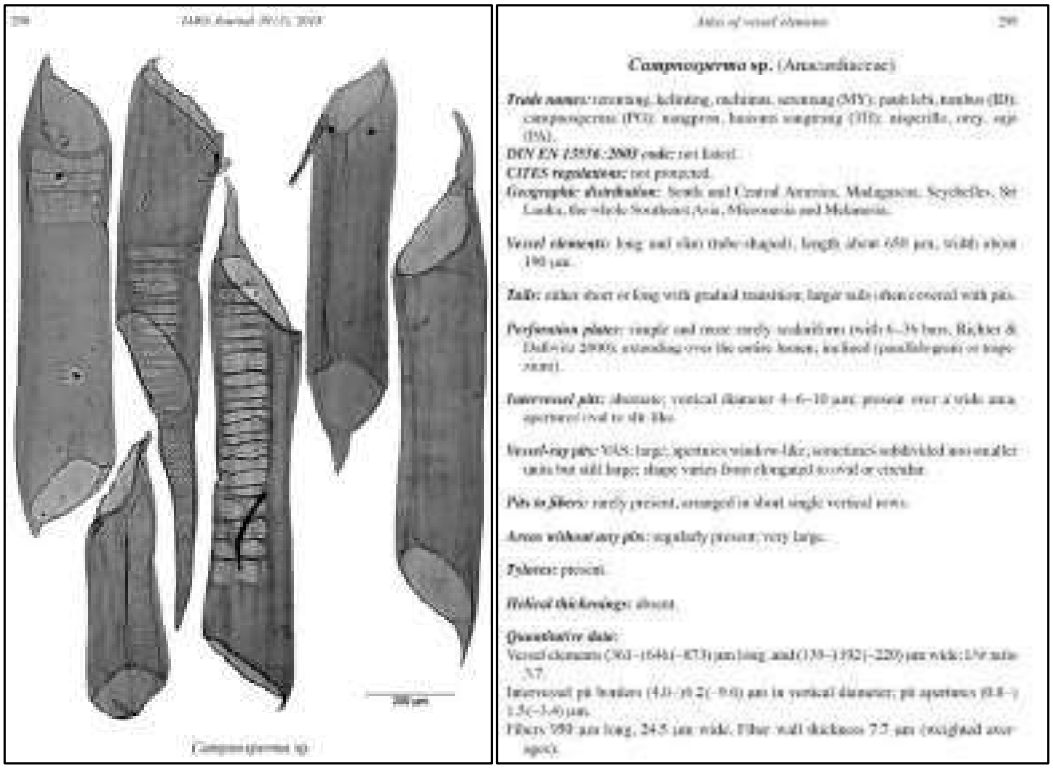
1.3.1.2 PUBLISHING REFERENCES

To make the references available for all testing institutes worldwide a scientific publication is essential. Therefore, reference slides have to be analysed (for an example see Helmling *et al.* 2018) and documented with **micrographs using a**

[16] See also this presentation on the need for vessel atlases.

[17] For a detailed description see Helmling *et al.* (2016).

transmitted light microscope (bright field microscope) with measuring software[18] (or eyepiece grid or drawing tube with self-made calibration units). The observed structural characteristics should then be **verified with literature data** from various sources (such as *InsideWood*).



***Campnosperma* sp.** (Anacardiaceae)

Trade names: [illegible]

DIN EN 13556: 2003 code: not listed.

CITES regulations: not protected.

Geographic distribution: South and Central America, Madagascar, Seychelles, Sri Lanka, the whole Southeast Asia, Micronesia and Melanesia.

Vessel elements: long and slim (tube-shaped), length about 650 µm, width about 190 µm.

Tails: either short or long with gradual transition; larger tails often covered with pits.

Perforation plates: simple and more rarely scalariform (with 6–36 bars, Richter & Dallwitz 2000); extending over the entire lumen; inclined (parallelogram or trapezoid).

Intervessel pits: alternate; vertical diameter 4–6–10 µm; present over a wide area; aperture oval to slit-like.

Vessel-ray pits: VRS: large; apertures window-like; sometimes subdivided into smaller units but still large; shape varies from elongated to oval or circular.

Pits to fibers: rarely present, arranged in short single vertical rows.

Areas without any pits: regularly present; very large.

Tyloses: present.

Helical thickenings: absent.

Quantitative data:

Vessel elements (363–(646)–873) µm long and (139–(192)–220) µm wide; 1/mm² ratio: 3.7.

Intervessel pit borders (4.0–(6.2)–(–9.6)) µm in vertical diameter; pit apertures (0.8–(1.5)–(–3.4)) µm.

Fibers 950 µm long, 24.5 µm wide. Fiber wall thickness 7.7 µm (weighted average).

Fig. 7: Example of a reference for wood identification of pulp, paper and fibreboard showing *Campnosperma* sp. Photomicrographs of the vessel elements are produced with a light microscope. Each image of a vessel element is a superposition of up to 10 micrographs taken at different focus points with a light microscope (Olympus, BX51) (from Helmling *et al.* 2018).

[18] *E.g.* ImageJ is an open source software.

1.3.2 ANALYSIS OF TEST SAMPLES FROM FIBRE MATERIAL

CAUTION: Since it is common practice in industry to produce pulp, paper, and fibreboard of a **mixture of timber species** it is important to obtain a representative range of vessel elements and to identify each of them separately. Therefore, it is recommended to prepare and investigate enough material, which usually requires several microscopic slides.

1.3.2.1 PREPARATION OF FIBRE SAMPLES FOR IDENTIFICATION

To prepare the samples for investigation **fibres are separated into individual cells and stained**.

Paper

A piece of paper (1.5 cm^2) is put into a test tube filled with **distilled water to soften** the paper. After a few minutes, the pulp mass is rolled to a ball between the fingers and placed in a tube with water again. The test tube is shaken, and the fibre suspension poured on a filter.

Part of the material is then placed on 2 or 3 microscope slides. Staining of the fibrous mix is done with two drops of **Alexander and** one drop of **Herzberg solutions**[19], which allow differentiation between chemical pulp (violet) and mechanical wood pulp (yellow). Cover glasses are placed on the stained pulp, and slides should be investigated immediately, since the staining is not permanent. After this process cells with a high amount of lignin (mechanical pulp) are stained yellow, de-lignified cells (chemical pulp) are greyish blue (softwoods) or blue (hardwoods).

Fibreboard

A piece of the material is **boiled in water** and the resulting pulp placed on a filter.

For better contrast, the pulp is stained with **Nigrosin** (1%, alcohol soluble). After 10-15 minutes the material is rinsed with de-ionised water and mounted in glycerine (70-90%) on microscope slides.

[19] Preparation of the solutions is described in Harders-Steinhäuser (1974). Alexander solution consists of calcium nitrate tetrahydrate, Herzberg solution of zinc chloride and potassium iodide (Merck KGaA, Darmstadt, Germany).

1.3.2.2 COMPARING VESSEL ELEMENTS OF TEST SAMPLE WITH REFERENCE DATA

Restrict the list of potential candidates for identification of the unknown sample by looking at the main anatomical characteristics:

- Type of perforation plate
- Size of intervessel pits
- Vessel-ray pits[20]
- Helical thickenings
- Tyloses
- Vessel element length, width, and ratio of length to width

Compare the appearance of vessel elements to make a clear match with the reference. All the details of interest - like arrangement of the different pit types and shape of the pits - can only be provided by physical references or high-quality micrographs. A well-trained person with practice can directly distinguish the different commonly used timbers. In general, the anatomy of vessel elements of the timber species within a genus or in some cases within a family is so similar, that the identification of timbers in pulp, paper and fibreboard is restricted to genus or family level.

[20] Vessel-ray pits are either similar in size and shape to intervessel pits (All Pits Similar, APS) or different (Vessel-ray pits Apparently Simple, VAS) (Helmling *et al.* 2018).

1.3.3 STRENGTHS & LIMITATIONS

Strengths

Wood anatomy currently provides the only established method for the **identification of pulp, paper, and fibreboard components**, which are also subject to the controls of timber regulations.

The microscopic analysis of fibre material can identify cells, which fully correspond to scientifically described fibre references of timbers **up to family or genus level**. It can be substantiated if a declaration of the incorporated timbers in fibre materials is correct or if there are additional timbers represented.

Limitations

The number of useable microscopic features for identification is severely reduced in the macerated tissue of pulp, paper, and fibreboard, in comparison to the microscopic identification of solid wood blocks. The few anatomical characteristics of an unknown vessel element lead to many possible candidates. Therefore, **expertise is crucial** since the appearance of the unknown vessel element has to be recognized and matched with the reference material.

Fibre material is mostly made up of multiple timbers complicating their identification.

Identification to species level is not possible for pulp, paper, and fibreboard.

Scientifically described fibre references are still limited, especially for tropical timbers.

1.4 KEY LITERATURE FOR WOOD ANATOMICAL DATA ANALYSIS

BOX 5: NON-EXHAUSTIVE OVERVIEW OF PRINTED WOOD ANATOMICAL REFERENCE ATLASES

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