



Recent research and practice on wood identification in China towards promoting legal logging and timber trade: combining wood anatomy, genetics and chemical methods

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UW, Seattle, USA



Outline


- ❑ **1. Background**
- ❑ **2. Genetic method for wood ID**
- ❑ **3. Combined methods for wood ID**
- ❑ **4. Network development of wood ID**



1. Background



CITES -- timber species (tropical timber)

CITES CoP	Total Number of Timber Species in CITES Appendix	CITES Appendix for Timber Species		
		I	II	III
2010, 15 CoP	111	7	94	10
2013, 16 CoP	247	7	231 New-added: <i>Dalbergia cochinchinensis</i> , <i>D. granadillo</i> , <i>Osyris lanceolata</i> and 48 <i>D. spp.</i> and 84 <i>Diospyros spp.</i> (populations of Madagascar) From III to II: <i>Dalbergia retusa</i> , <i>D. stevensonii</i>	9
2016, 17 CoP	~ 503 	7	~ 486 New-added: most of <i>Dalbergia spp.</i> , <i>Guibourtia tessmannii</i> , <i>G. demeusei</i> and <i>G. pellegriniana</i> From III to II: <i>Pterocarpus erinaceus</i> , etc.	10

1. Background

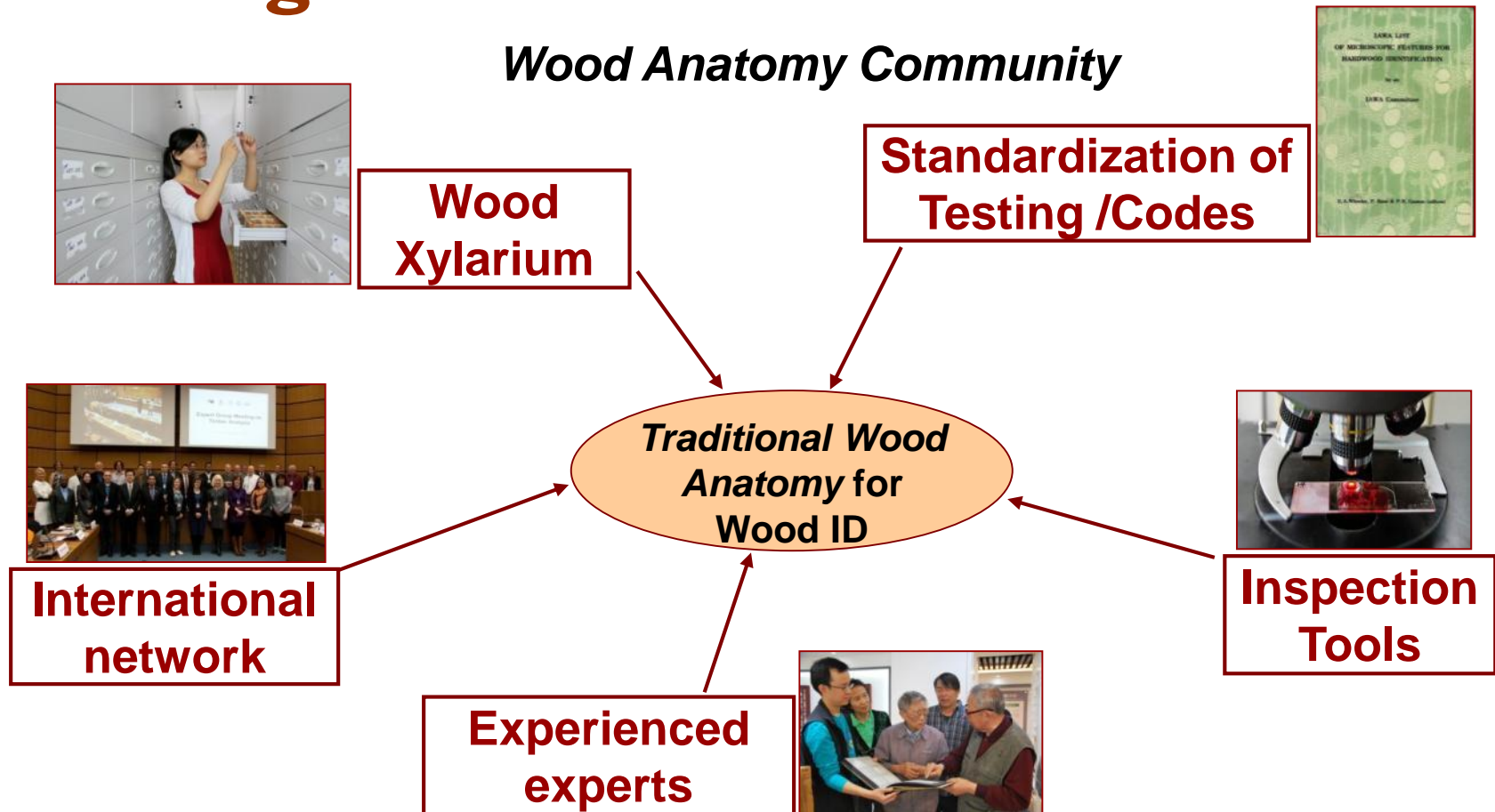
International / Regional Timber Trade Convention or Law

- CITES
- September 2010, The Lacey Act Amendment, USA
- March 2013, European Union Timber Regulation, EUTR, EU
- November 2014, Australian Timber Regulation, Australia
- September 2006, Chinese Regulations on Administration of Import and Export of Endangered Wild Animals and Plants, China



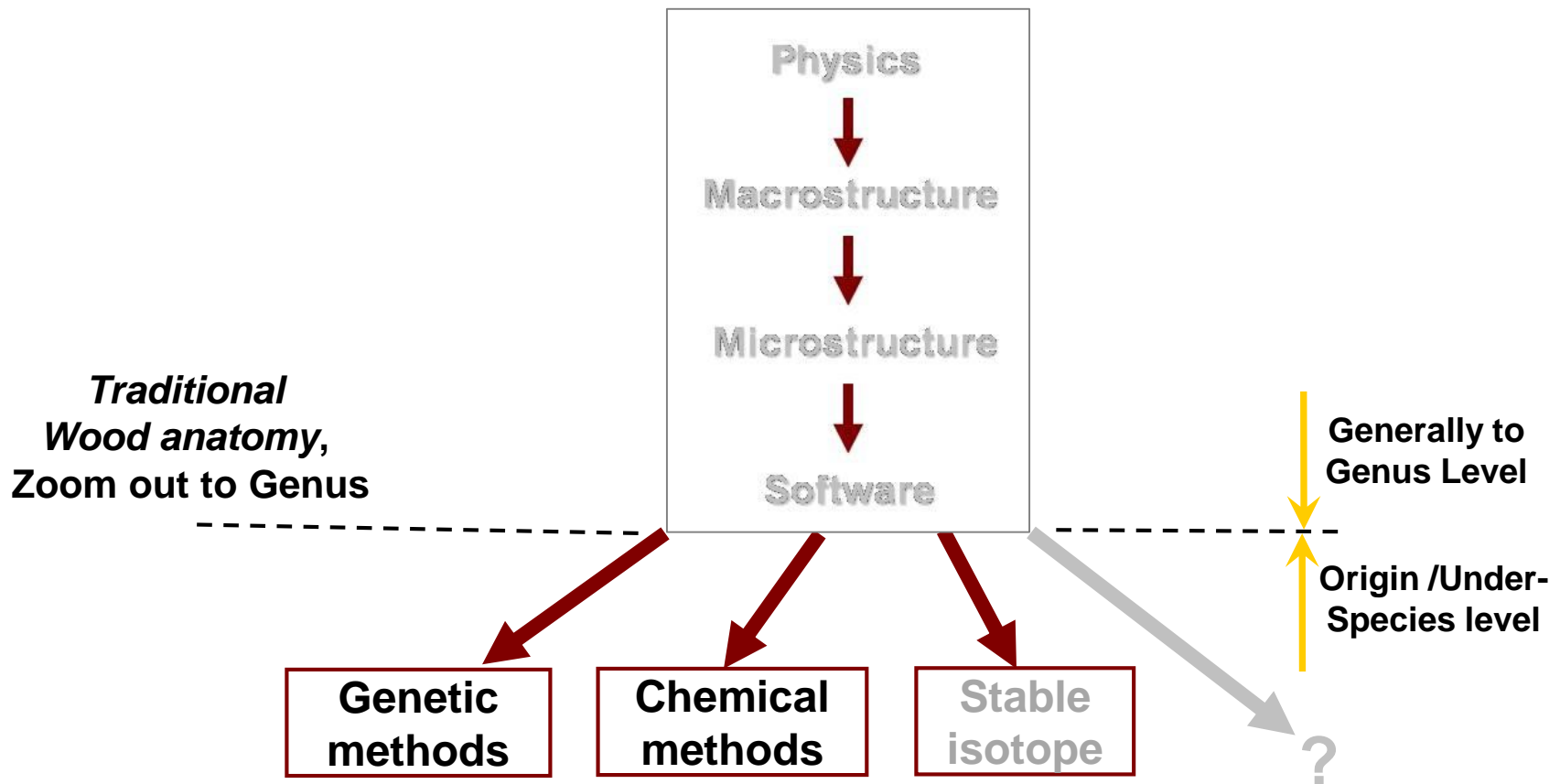
1. Background

Wood Anatomy Community



IAWA (International **A**ssociation of **W**ood **A**natomists), founded in 1931

1. Background



Resolution of Wood Identification?



Outline

- 1. Background
- **2. Genetic method for wood ID**
- 3. Combined methods for wood ID
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2. Genetic Methods for Wood ID

2.1 Recent update on *Dalbergia* spp.

Materials

9 species of *Dalbergia*, 50 vouchered samples

1. *Dalbergia odorifera* (China, CITES II)
2. *Dalbergia cochinchinensis* (Asia, CITES II)
3. *Dalbergia latifolia* (Asia, CITES II)
4. *Dalbergia retusa* (Central America, CITES II)

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DNA extraction from dried heartwood
...success rate of PCR amplification

2. Genetic Methods for Wood ID

2.1 Recent update on *Dalbergia* spp.

Chloroplast DNA

--*matK*

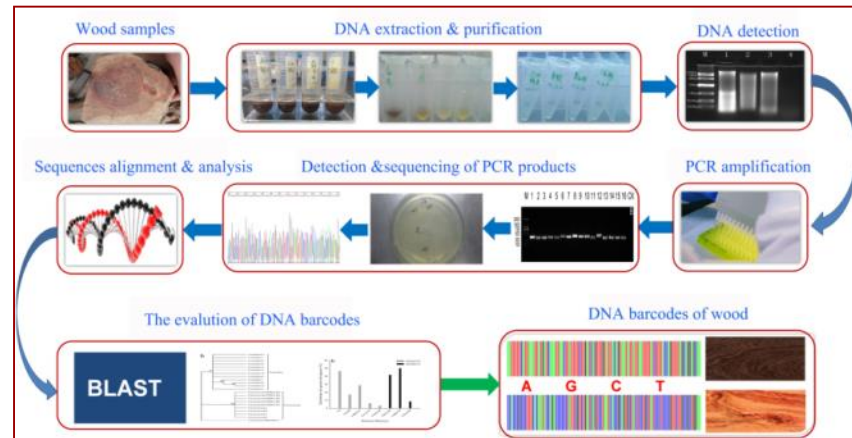
--*trnL* (>90%)

--*trnV-trnM*

--*trnC-petN* (>90%)

--*trnH-psbA*

--*trnS-trnG*



Nuclear DNA

--ITS2 (>90%)

301 newly sequences to GenBank

Three DNA analysis methods:

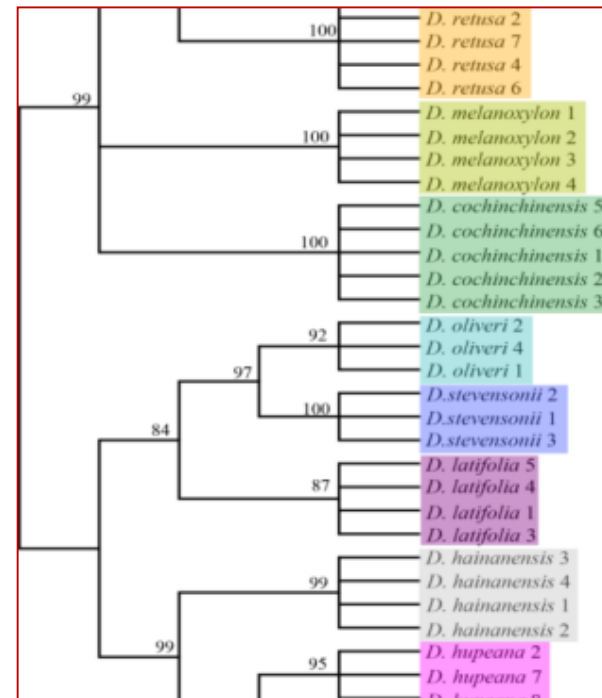
Character-based / TAXONDNA / NJ tree

2. Genetic Methods for Wood ID

2.1 Recent update on *Dalbergia* spp.

9 *Dalbergia* species:

ITS2+trnH-psbA.



ITS2+trnH-psbA

Three DNA analysis methods:

Character-based / TAXONDNA / NJ tree

2. Genetic Methods for Wood ID

2.2 Recent update on *Pterocarpus* spp.

Materials

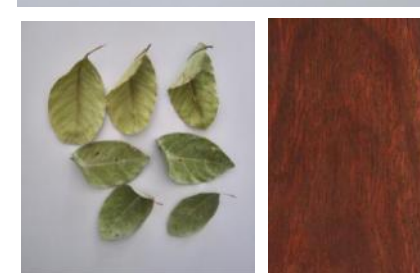
7 species of *Pterocarpus*, 42 vouched samples

1. *Pterocarpus angolensis* (Africa)
2. *Pterocarpus erinaceus* (Africa, CITES II)
3. *Pterocarpus macrocarpus* (Asia)
4. *Pterocarpus santalinus* (Asia, CITES II)
5. *Pterocarpus tinctorius* (Africa)

.....

DNA extraction from dried heartwood

...success rate of PCR amplification



2. Genetic Methods for Wood ID

2.2 Recent update on *Pterocarpus* spp.



Chloroplast DNA

--*rbcL*

--*matK*

--*ndhF-rpl32*

	10	20	30	40	50	60
<i>P. santalinus</i>	AGGAATTTGTTTATTGGACCATTTTAAC	TTTGACTTTGCAATATTAGAAGTATTGTGCA				
<i>P. tinctorius</i>
	70	80	90	100	110	120
<i>P. santalinus</i>	GAGATTCAGAATAATAGAAAATTCTATT	TATATGTTCACTCTTTTTATTA-----	ACT			
<i>P. tinctorius</i>
	130	140	150	160	170	
<i>P. santalinus</i>	GAAGCCTTTRCAAAAGAGATAAACC	GCAGTAAGAACAANAACCTCATTACGAATC				
<i>P. tinctorius</i>

Nuclear DNA

--ITS2

Pterocarpus santalinus/P. tinctorius

Three DNA analysis methods:

Character-based / TAXONDNA / NJ tree



Outline

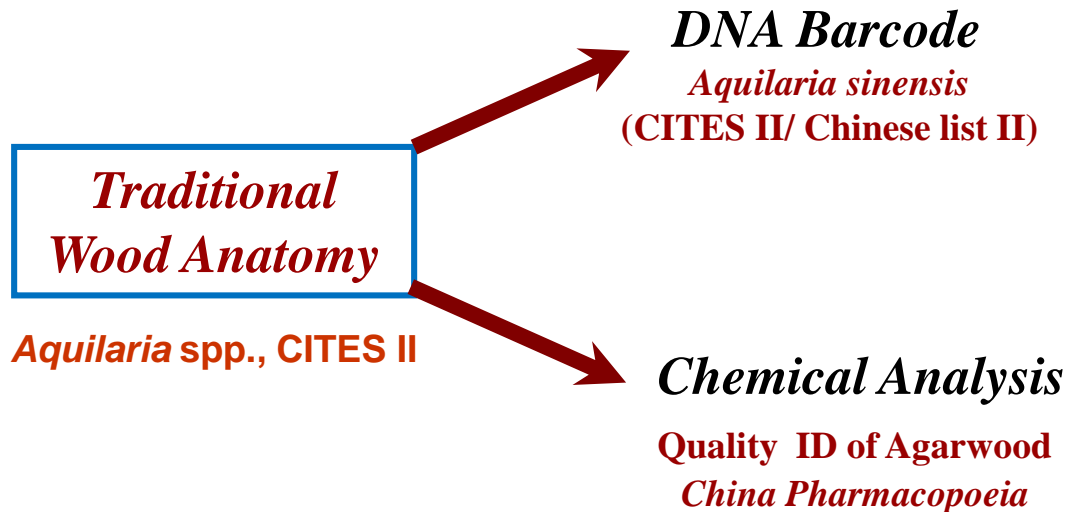
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3. Combined Methods for Wood ID: Agarwood

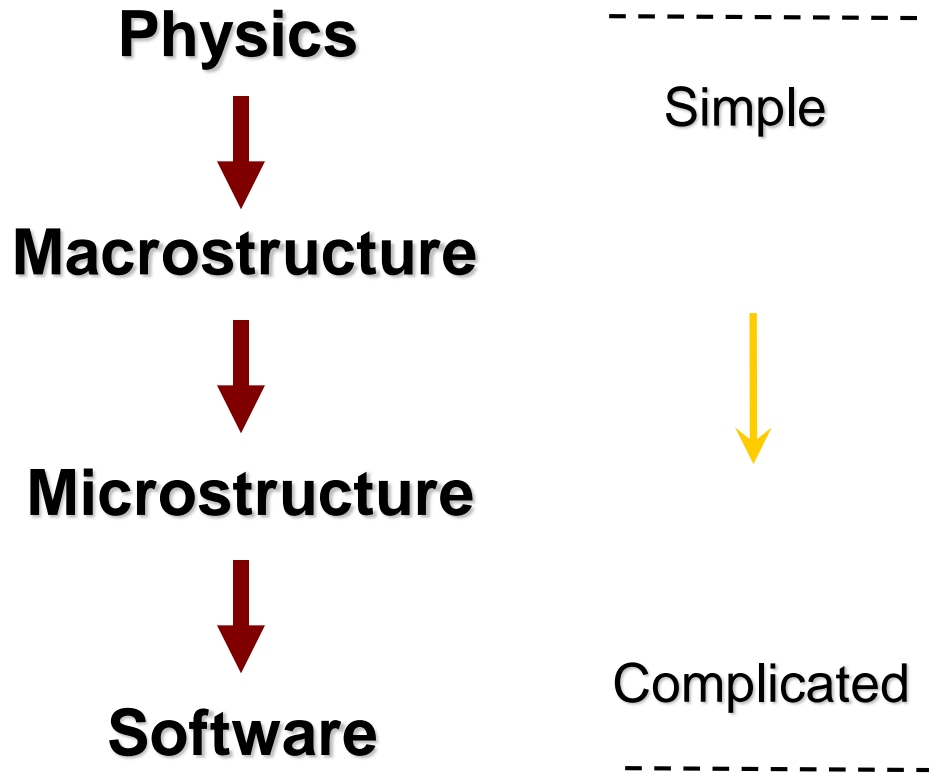
Agarwood / *Aquilaria* spp.

- **Agarwood**,
xylem and its inclusions (**resin**), in *Aquilaria* tree



3. Combined Methods for Wood ID: Agarwood

3.1 Traditional Wood Anatomy

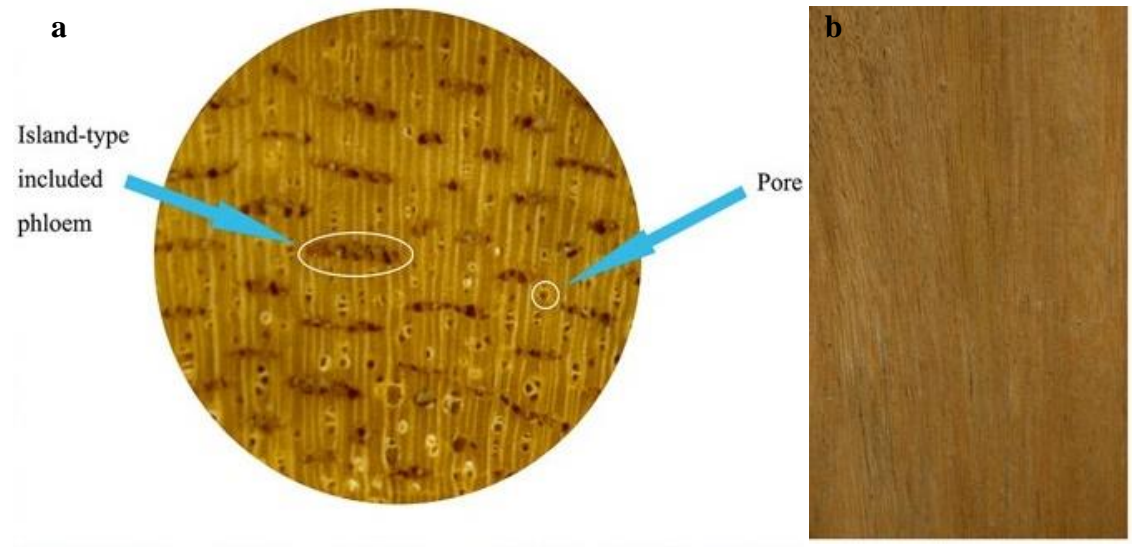
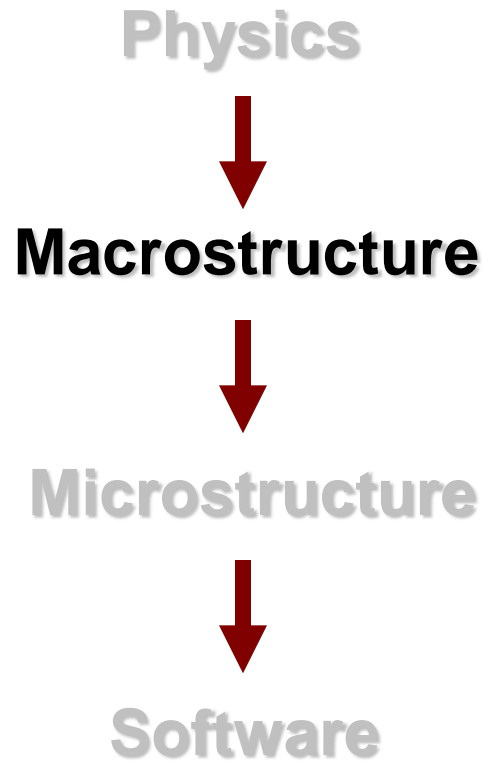


Color, Odor and Density

Aquilaria spp., CITES II

3. Combined Methods for Wood ID: Agarwood

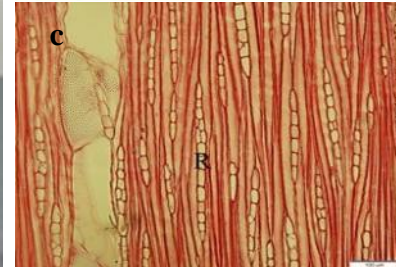
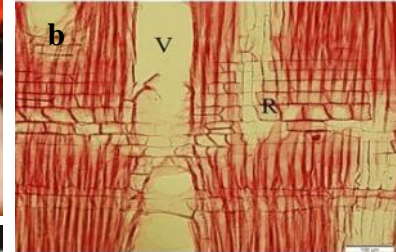
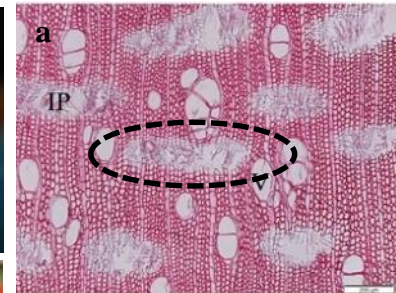
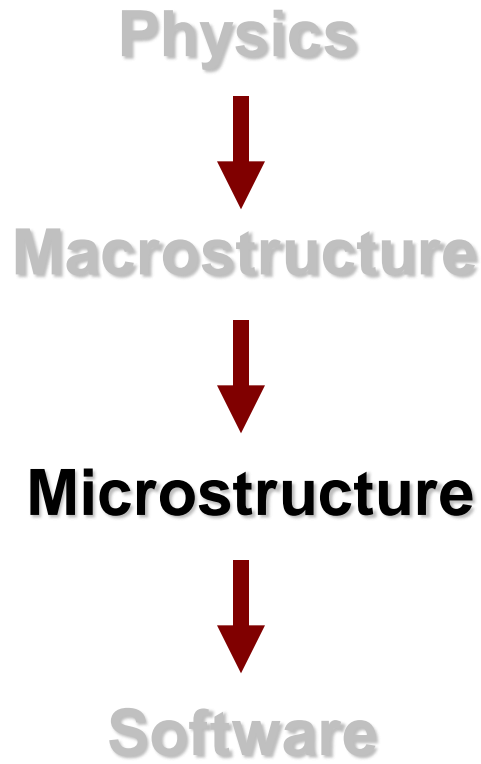
3.1 Traditional Wood Anatomy



Aquilaria spp., CITES II

3. Combined Methods for Wood ID: Agarwood

3.1 Traditional Wood Anatomy



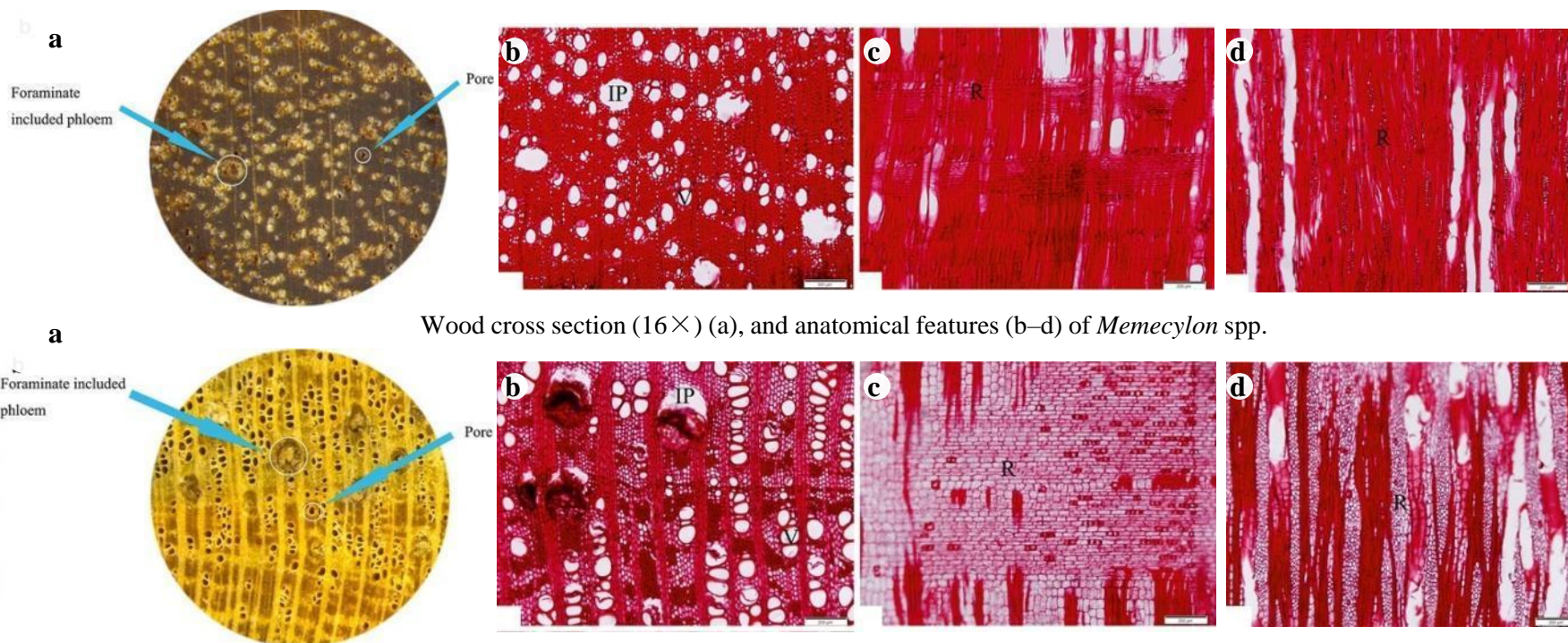
Aquilaria spp., CITES II

3. Combined Methods for Wood ID: Agarwood

3.1 Traditional Wood Anatomy

Look-alike species

- *Memecylon* (Melastomataceae) / *Strychnos* (Loganiaceae)



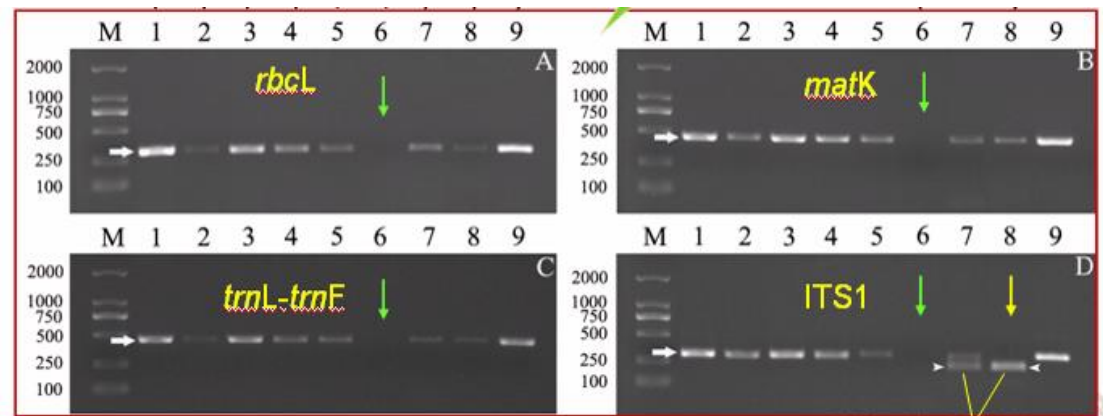
3. Combined Methods for Wood ID: Agarwood

3.2 DNA Barcode

- Wood powder
- DNA extraction
- DNA assessment



Aquilaria sinensis
(CITES II/Chinese list II)



PCR amplification

4. Genetic Methods for Wood ID

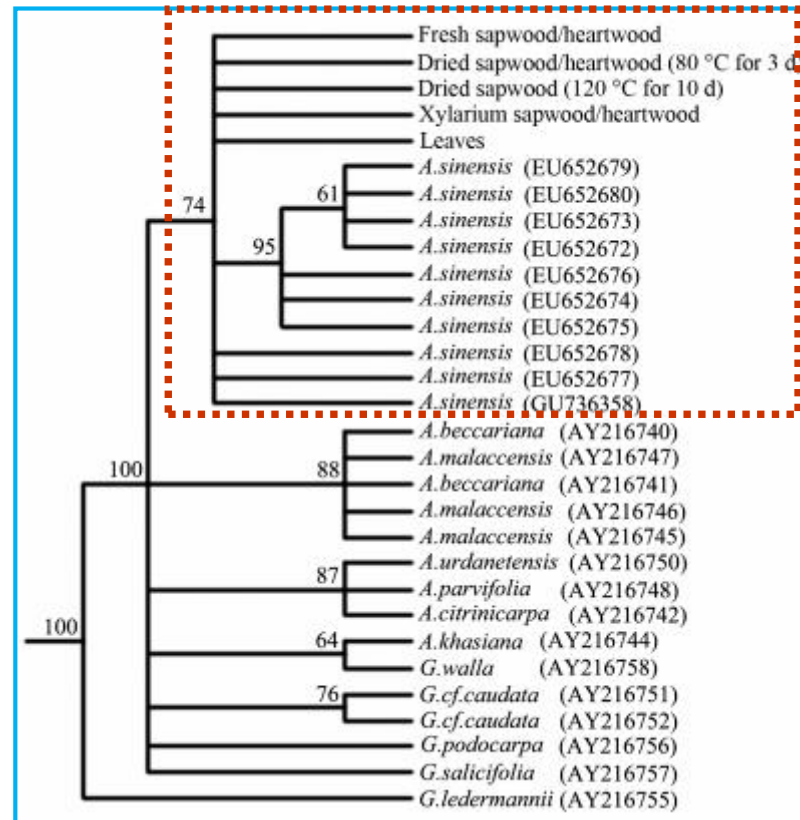
3.2 DNA Barcode



Aquilaria sinensis
(CITES II/Chinese list II)

Phylogenetic tree
Chloroplast DNA
trnL-trnF

7 Aquilaria spp./5 Gyrinops spp.



4. Genetic Methods for Wood ID

3.2 DNA Barcode



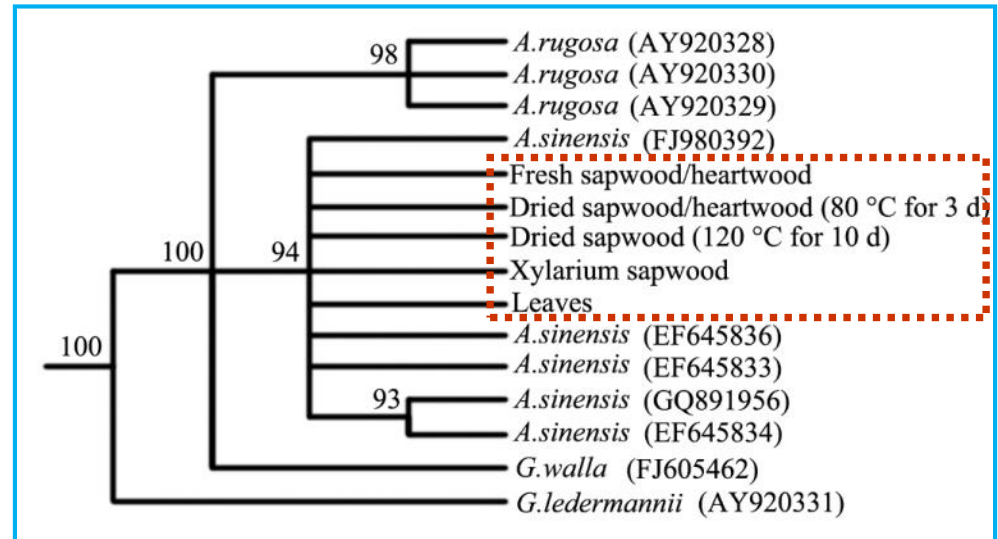
Phylogenetic tree

Nuclear DNA

ITS1

Aquilaria sinensis
(CITES II/Chinese list II)

3 *Aquilaria* spp./2 *Gyrinops* spp.



3. Combined Methods for Wood ID: Agarwood

3.3 *Chemical analysis*

- **166 Agarwood samples** (Chinese Academy of Forestry, Chinese Academy of Medical Sciences, Chinese Academy of Tropical Agricultural Sciences and Beijing Tian-yi-li-hua Institute of Agarwood etc).

-- **ethanol extractive: $\geq 10.0\%$**

- hot-dipping method
- not less than 10.0 % (China Pharmacopoeia)

-- **a) Color reaction**

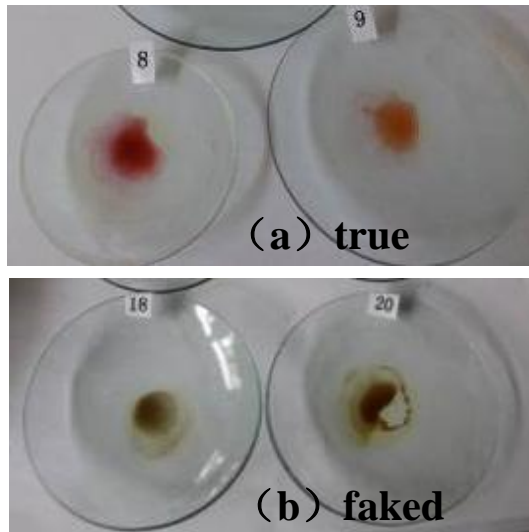
b) Thin-layer chromatography (TLC)

c) High performance liquid chromatography (HPLC)

3. Combined Methods for Wood ID: Agarwood

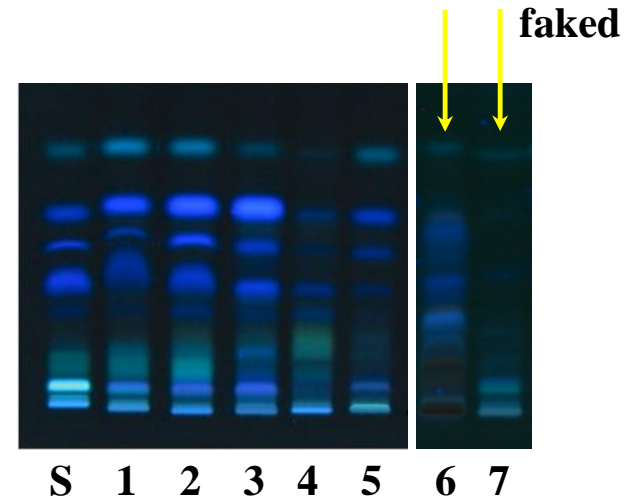
3.3 Chemical analysis

a) Color reaction



Cherry red, vanillin reagent

b) Thin-layer chromatography (TLC)



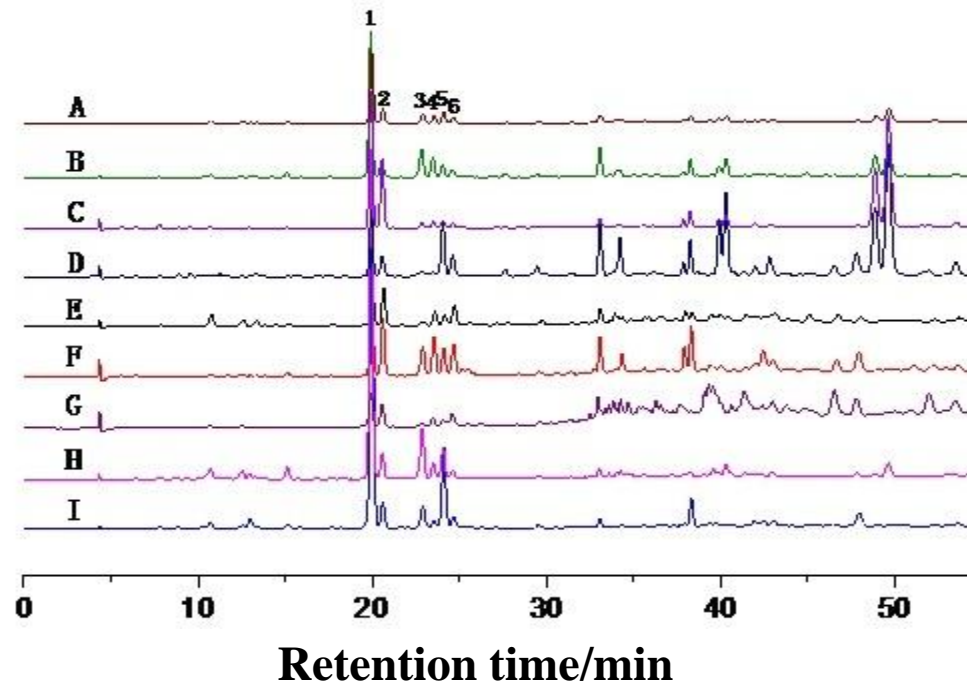
fluorescent spots color and location

Note: **S-standard reference sample**; 1-Cambodia; 2-Hainan, China; 3-Vietnam; 4-Indoesia; 5-Vietnam

3. Combined Methods for Wood ID: Agarwood

3.3 Chemical analysis

c) High performance liquid chromatography (HPLC)

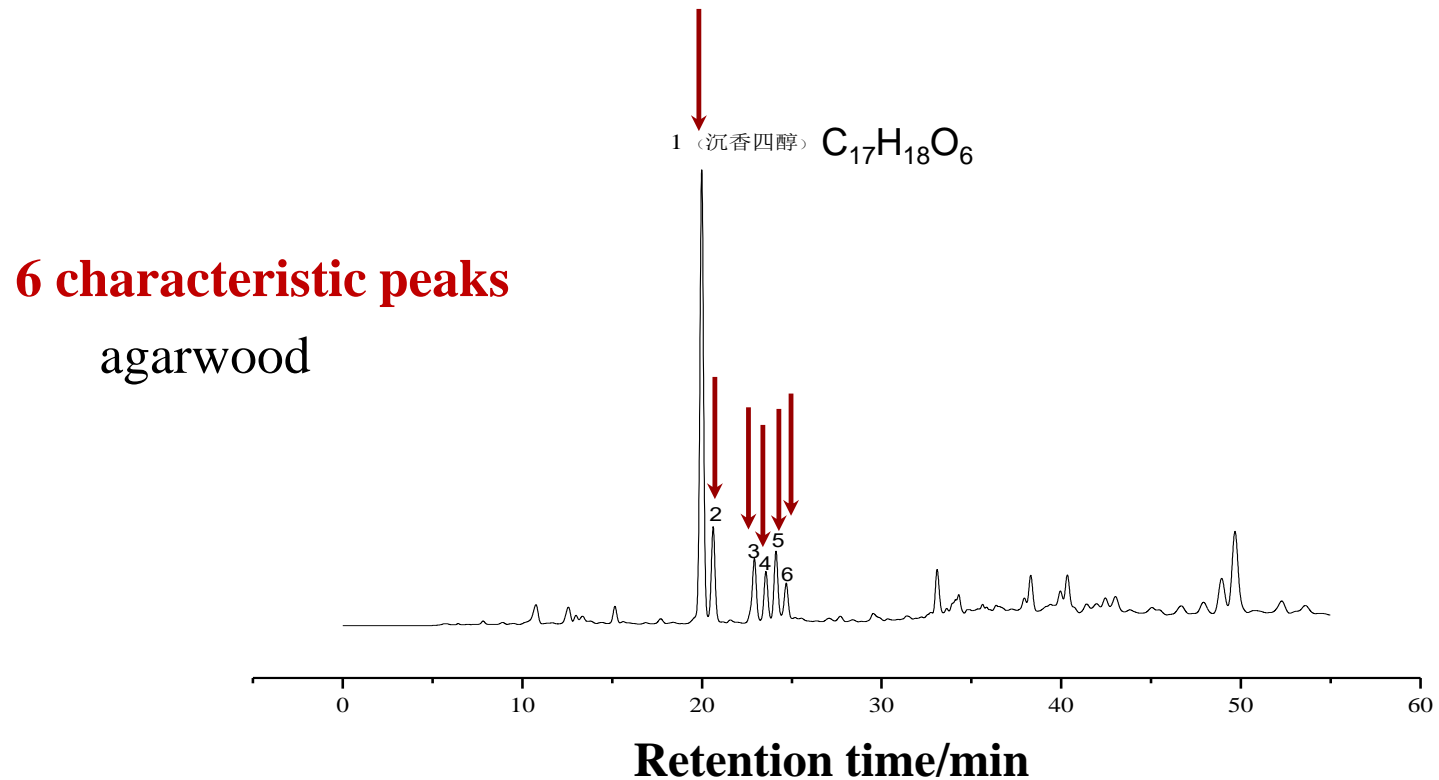


Note: **A-Standard reference sample**; B-Hainan, China; C,D- Guangdong, China; E-Hainan, China; F-Hongkong, China; G-Laos; H-Malaysia; I-Vietnam

3. Combined Methods for Wood ID: Agarwood

3.3 Chemical analysis

c) High performance liquid chromatography (HPLC)



3. Combined Methods for Wood ID: Agarwood

- National Forestry Standard: *Agarwood* (Project No. 2014-LY-071)

Testing Items		Requirements		
检验项目		要求		
沉香的木质部构造特征	宏观构造特征	应符合附录A沉香属树种的木质部宏观构造特征。		Traditional Wood Anatomy
	微观构造特征	应符合附录A沉香属树种的木质部微观构造特征。		
沉香的分分泌物特征化学成分	乙醇提取物	≥10.0%		Chemical Analysis
	显色反应	樱红色或紫堇色或浅红色或浅紫色，不应呈无色或浅黄色。		
	薄层色谱	在与对照样色谱相应的位置上，显相同颜色的荧光斑点。		
	高效液相特征图谱	满足其中任一条件	应呈现图1所示的8个特征峰，并应与对照样色谱峰中的8个特征峰相对应，其中峰2应与对照样峰2的保留时间相一致。 应呈现图2所示的8个特征峰，并应与对照样色谱峰中的8个特征峰相对应，其中峰1应与对照样峰1的保留时间相一致。	

Ethanol Extractive

Colour Reaction

TLC

HPLC





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- **4. Network development of wood ID**




4. Network for Wood ID

**“China-USA Workshop on Wood Identification and Wood Tracking System”
July 27-28, 2009, Beijing, China**

Introduction to the Research on Wood Anatomy in CRIWI

Yin Yafang

On behalf of
Department of Wood Anatomy and Utilization
Research Institute of Wood Industry (CRIWI)
Chinese Academy of Forestry (CAF)



July 27-28, 2009
Beijing, China

Wood identification technologies and training and wood tracking for the China-USA workshop



Alex C. Wiedenhoft, Ph.D.
Center for Wood Anatomy Research
USFS Forest Products Laboratory
July 27-28, 2009, Beijing

“Rebirth” for wood anatomy in China!

4. Network for Wood ID



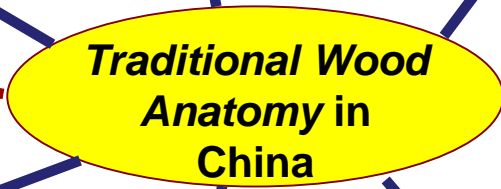
2010-, CITES



2011-, ID Manual



2011-, WoodID Training Course



2012-, Genetic WoodID



2014-, IAWA-China Group



2014-, New Wood Collection



2013, WoodID Workshop

New “start point” of Wood Anatomy in CAF, China, from 2010

4. Network for Wood ID

Suggestions and Prospects

1) *Reemphasizing Traditional Wood Anatomy and Wood Collections*

**CITES CoP17, Decisions 17.166–17.169:
Identification (timber) (December, 2016)**

Parties are encouraged to expand or create and maintain scientific reference collections essentialto identify CITES-listed tree species,



Wood Collection, FPL

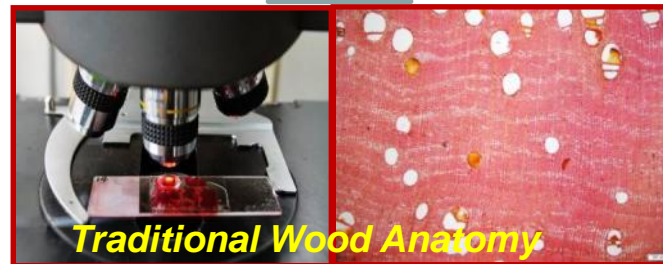


Wood Collection, CAF

4. Network for Wood ID

Suggestions and Prospects

- 2) *Reconciling Traditional Wood Anatomy with New Concept Technologies.*



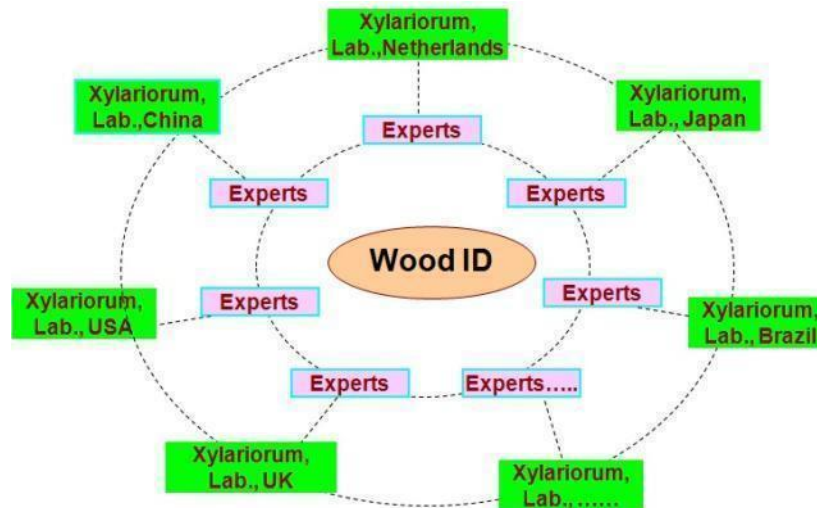
4. Network for Wood ID

Suggestions and Prospects

3) *Reshaping Network on Innovating of Wood Science Community*



IAWA-WRAITH Group
IAWA-China Group



GTTN /
GTTN //

IAWA (**I**nternational **A**ssociation of **W**ood **A**natomists), founded in 1931

Acknowledgements



- International Program, Forest Service (FS), USDA, US
- World Resources Institute (WRI), US
- State Forestry Administration (SFA), China



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- Dr. Alex Wiedenhoft, Forest Products Laboratory (FPL), US
- Dr. John Hermanson, Forest Products Laboratory (FPL), US



- Ms. Shelley Gardner, Forest Service (FS), USDA, US
- Dr. Meaghan Parker, World Resources Institute (WRI), US
- Ms. Julie Hundersmarck, Forest Service (FS), USDA, US

China

America

Brazil

More

***International Cooperation
is Really Needed !***



*Thanks for Your
Comments !*



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