

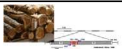
## Use of DNA-markers for rapid identification of CITES-listed timber species

Prof. Dr. Elisabeth Magel

Wood Biology, Institute of Wood Science, University of Hamburg

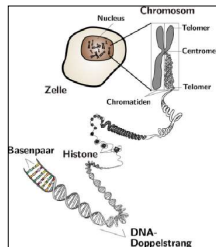


- Definitions
- Nuclear DNA and genetic fingerprinting
- rDNA and the ITS – region
- ITS – region and molecular wood identification

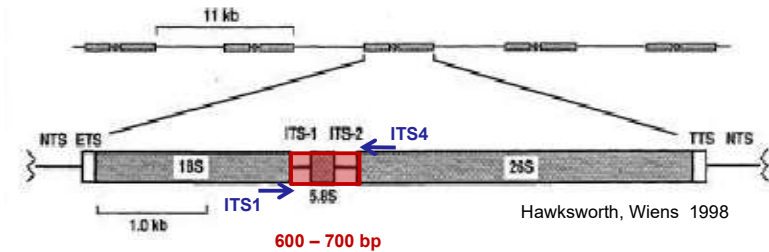


**DNA = DeoxyriboNucleic Acid**

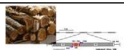
- coding and expressing of genetic information
- includes both coding region (the genes) and non-coding sequences
- stores the hereditary information
- sequence of nucleotides A, T, C, G = DNA-sequence
- **nucleus**, mitochondria, plastids



## DNA-marker and genetic fingerprinting: ITS (Internal Transcribed Spacer) of the ribosomal DNA (rDNA):

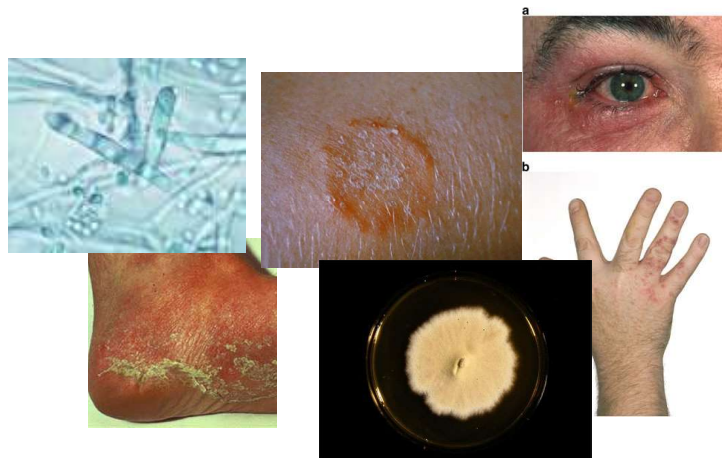


between 18S and 26S rDNA, encloses 5.8S rDNA (coding for ribosome subunits)  
comprises the non-coding variable ITS-1 and ITS-2, about 700 bp  
up to 30000 copies  
PCR amplification by using ITS1 and ITS4 (universal primers)  
rapidly evolving (closely related species and at the subspecies level)  
species specificity of the ITS-sequence

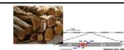


### The ITS – region:

..... Identification of human pathogenic fungi



images.google.de/images?gbv=2&hl=de&q=trichophyton&btnG=Bilder-Suche

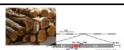


### The ITS – region:

..... Detection of truffle species in processed food products (Karkouri, et al. 2007, Applied and Environmental Microbiology)

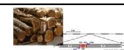
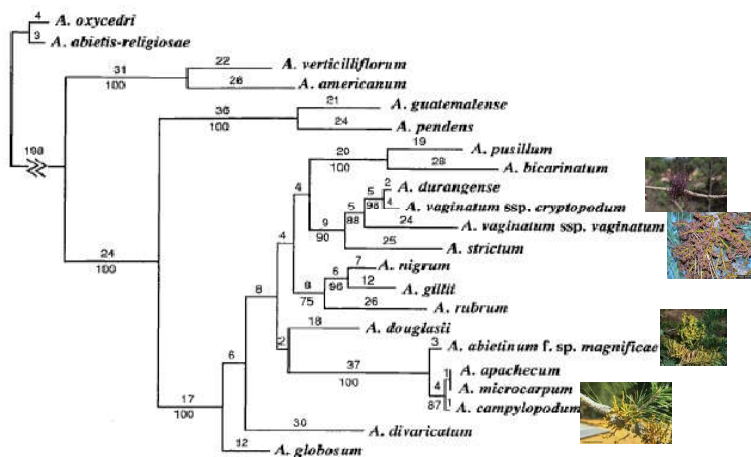


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### The ITS – region:

..... Construction of phylogenetic trees (Arceuthobium; Hawksworth, Wiens, Dwarf Mistletoes: Biology, Pathology, and Systematics, 1998)

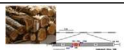


### The ITS – region:

..... Elucidating the relationships of genera (Corymbia, Angophora und Eucalyptus, Ladiges, Udovicic 2000, Australian Systematic Botany 13)



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## Use of DNA-markers for rapid identification of CITES-listed timber species

Prof. Dr. Elisabeth Magel, Wood Biology, Institute of Wood Science, University of Hamburg



### The ITS – region:

..... Diagnosis of wood and tree fungi (ITS rDNA database; Prof. Dr. O. Schmidt, Dr. T. Huckfeldt, U. Moreth 2008, Molekulare Untersuchungen an Hausfäulepilzen, Zeitschrift für Mykologie; Ribosomal DNA Intergenic Spacer Of Indoor Wood-Decay Fungi, Holzforschung)



Coniophora vaginata, Sara Blinnig, © www.nature.com



Aphylloanthrodia, Sara Blinnig, © www.nature.com



www.ib-rauch.de



www.aebg.gov.au/fungi/images/011

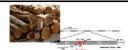


www.botany.ut.ee/BOI\_tudengile/Mykoloogia/Pilt\_12\_nw.jpg



http://www.vti.de

/www.holzfragen.de/bilder/brauner\_kellerschwamm\_24. Coniophora



## Use of DNA-markers for rapid identification of CITES-listed timber species

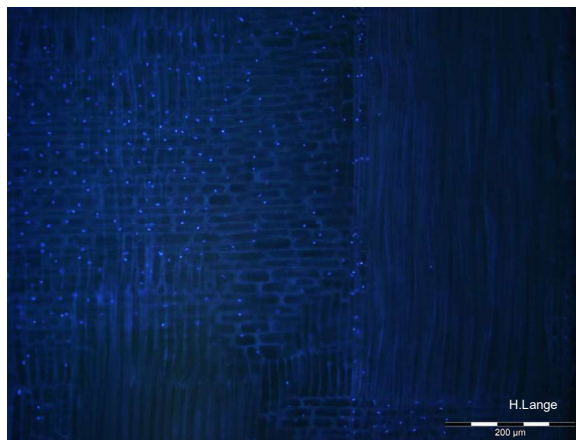
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Identification of nuclei and DNA in the wood by DAPI (4',6-Diamidino-2-phenylindol) staining (*Robinia pseudoacacia*)

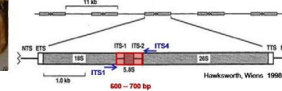


http://delta-intkey.com/wood/images/teak-s12.jpg



H.Lange

200 µm



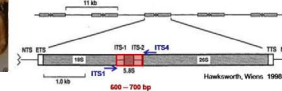
## Use of DNA-markers (ITS) for rapid identification of CITES-listed timber species

### Detection of DNA in wood

Selection wood species

Species specific sequence of the ITS-region – database

Species specific primer



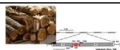
## Use of DNA-markers (ITS) for rapid identification of CITES-listed timber species

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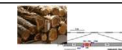
Species specific sequence of the ITS-region – database

Species specific primer



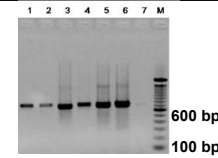
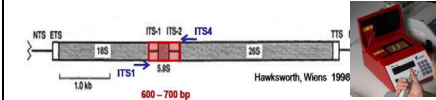
Wood species :

	Cites - listed	Substitutional traded timber
Mahagoni	<i>Swietenia macrophylla</i> <i>S. mahagoni</i> <i>S. humilis</i>	<i>Khaya ivorensis</i> <i>Entandrophragma cylindricum</i> (Sapeli) <i>E. utile</i> (Sipo) <i>Carapa guianensis</i>
Ramin	<i>Gonystylus bancanus</i>	<i>Terminalia superba</i> <i>Pterygota spp.</i> <i>Dyera costulata</i>
Cedro	<i>Cedrela odorata</i>	<i>Toona sureni</i>
Palisander	<i>Dalbergia nigra</i>	<i>D. spruceana</i> <i>D. latifolia</i>
Merbau	<i>Intsia bijuga</i>	<i>Azelia bipindensis</i> <i>A. africana</i> <i>A. pachyloba</i> <i>A. bella</i>



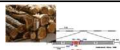
Methods:

1. Homogenisation of wood

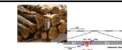
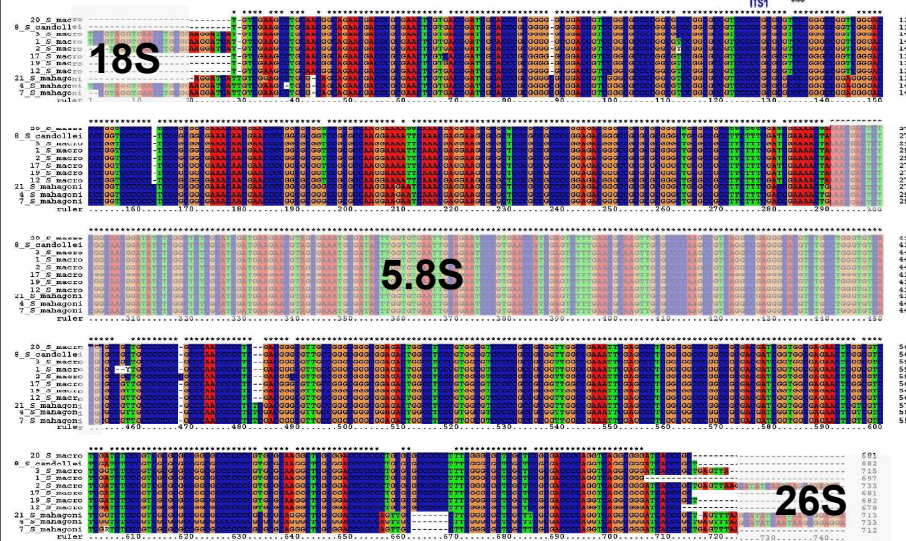
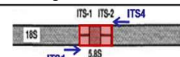


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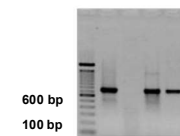
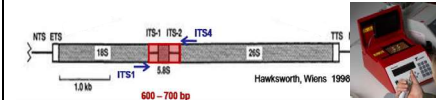
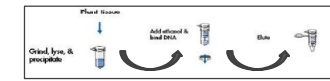
ITS-sequence of *S. macrophylla*, *S. mahagoni*



Methods: **Taxon primer**

6 – 8 h

1. Homogenisation of wood

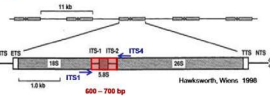


2. DNA-extraction

3. Amplification of the ITS-region  
(PCR = PolymeraseChain Reaction;  
primers: **tax for / tax rev**)

4. Detection

yes / no



## Use of DNA-markers (ITS) for rapid identification of CITES-listed timber species

Detection of DNA in wood

Selection wood species

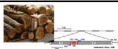
Species specific sequence of the ITS-region – database

Species specific primer (taxon primer, tax for / tax rev)

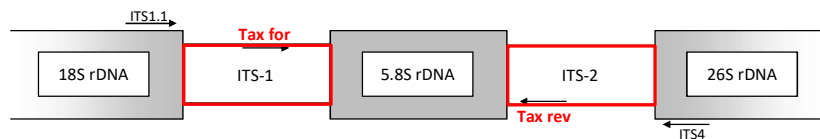


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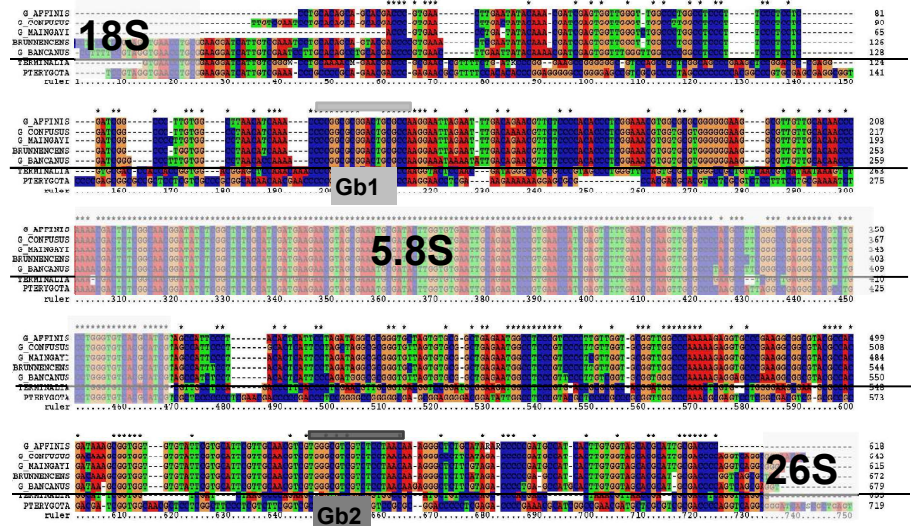
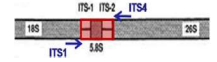


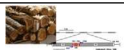
## Molecular wood identification – Design of Taxon primer



### ITS-sequence of *Gonystylus*, *Pterygota* and *Terminalia*

*Gonystylus* taxon-primer (Gb1, Gb2, 358 bp)

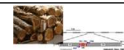
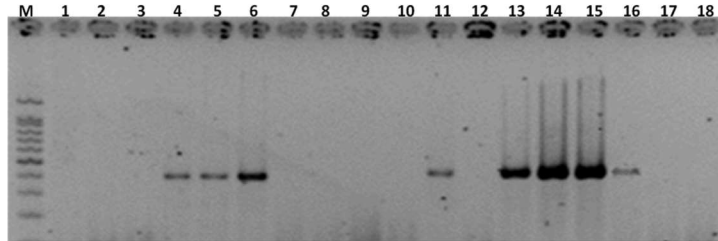




### Identification of Ramin by using species specific primers (Gb1, Gb2) for the ITS region of *Gonystylus* (length of fragment 358 bp) yes / no

(Methods: homogenisation of wood – DNA-extraction – PCR – documentation)

Which samples are Ramin? **4, 5, 6, 11, 13, 14, 15, 16**



### Your practical work:

#### Identification of members of mahogany family by using taxon primers

*Entandrophragma cylindricum*

*Khaya* spp.

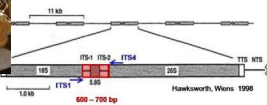
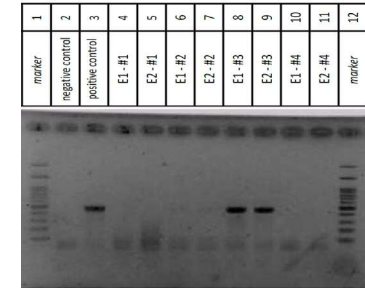
*Swietenia mahagoni*

*Swietenia macrophylla*



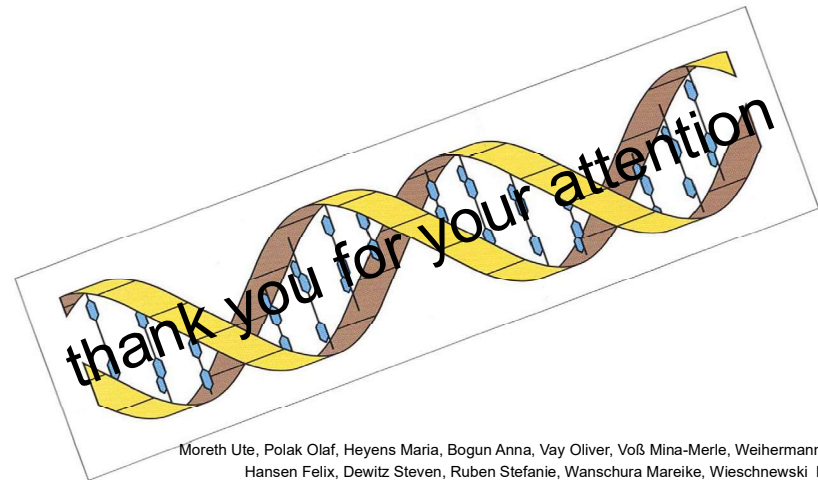
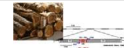
#### Methods:

homogenisation of wood  
DNA-extraction  
PCR  
documentation



### Use of DNA-markers for rapid identification of CITES-listed timber species (ITS - Region)

- ✓ Identification of individual species of one genera (e.g. *Swietenia*)
- ✓ Identification of species of different genera (*Swietenia* and *Khaya*; *Gonystylus*, *Terminalia* and *Pterygota*)
- ✓ Species specific primers (taxon primer) for identification of CITES-listed and trade timber species
- ✓ Revision of species identification (*S. candollei*)
- ✓ ..... additional element for identification of the (geographical) origin (*S. macrophylla*)



Moreth Ute, Polak Olaf, Heyens Maria, Bogun Anna, Vay Oliver, Voß Mina-Merle, Weihermann Camilla, Hansen Felix, Dewitz Steven, Ruben Stefanie, Wanschura Mareike, Wieschnewski Nico, NN, Dr. Rob Ogden, Tepnel Manchester, PD Dr. G. Koch Holzsammlung vTI, PD Dr. B. Degen, Inst. II Forstgenetik, Hr. Rühmann, Hr. Csala Gewächshaus vTI, Furnierwerk Winsen, Theodor Nagel, Bot. Gärten Deutschlands (Jena, Halle, Osnabrück, Tübingen, ...)