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Plant anatomy against crime: *Araucaria angustifolia* wood sawdust identification

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Resumo

Equipes do Instituto de Criminalística de São Paulo recebem motosserras envolvidas em investigações de desmatamento em áreas de preservação. Objetivamos verificar se a partir da serragem coletada nas motosserras com fragmentos de lenho e casca é possível diferenciar materiais provenientes de *Araucaria angustifolia* (nativa), *Pinus* spp. e *Eucalyptus* spp. (exóticas). Tal conhecimento é crucial para o esclarecimento de crimes ambientais, particularmente onde ocorre *A. angustifolia*, espécie sob risco de extinção. Obtivemos amostras de troncos de árvores adultas de *A. angustifolia*, *Pinus* spp., *Eucalyptus* spp., e *Corymbia citriodora*. Nossas amostras de lenho e casca são materiais remanescentes de outros estudos do grupo no Instituto Florestal (material referência). Adicionalmente, com a motosserra obtivemos serragens de *A. angustifolia*, *Pinus caribaea e Eucalyptus grandis*, com códigos para não visualizar as espécies investigadas. Preparamos todas as amostras com base na metodologia padrão para maceração da madeira e casca. Então, preparamos lâminas histológicas e analisamos comparativamente diversas lâminas no microscópio de luz. Confirmamos pela análise de serragem de motosserras que é possível obter fragmentos de células que contribuem para separar os gêneros de plantas estudados. Por exemplo, as paredes das traqueídes de *A. angustifolia* possuem pontoações poligonais, com duas ou mais pontoações alternas, enquanto que em *Pinus* spp., há pontoações circulares, geralmente com pontoações unisseriadas nas paredes radiais, e pontoações opostas se houver mais de uma linha. Além disso, apenas em *Eucalyptus* spp. foram encontrados elementos de vaso e fibras nos fragmentos de madeira. Também foram encontradas células da casca e de tecidos imaturos que colaboram na investigação.

Palavras-Chave: Anatomia Vegetal; Crimes Ambientais; Fragmentos de Madeira e Casca; Serragem; Araucária.

Abstract

Forensic Experts from São Paulo Criminalistics Institute received chainsaws allegedly involved in deforestation of preservation areas. Here, we aimed to examine wood and bark sawdust from chainsaws to determine the potential of differentiating materials from *Araucaria angustifolia* (native), *Pinus* spp. and *Eucalyptus* spp. (exotic species in Brazil). Such a knowledge is crucial to elucidate environmental crimes, particularly where *A. angustifolia* occurs as it is a species at risk of extinction. We obtained samples of adult tree trunks from *A. angustifolia*, *Pinus* spp., *Eucalyptus* spp., and *Corymbia citriodora*. Our reference samples of wood and bark came from material used in previous studies run in the Instituto Florestal. We collected sawdust samples of *A. angustifolia*, *Pinus caribaea* and *Eucalyptus grandis* and tagged with codes to prevent bias. We prepared all samples based on the standard methodology for wood and bark maceration. Then, we prepared histological slides and comparatively analyzed several slides under light microscopy. Results showed that it is possible to obtain cell fragments to discriminate between the study genera. For example, tracheids on the cell walls of *A. angustifolia* have polygonal pits with two or more alternating pits, while on *Pinus* spp., circular pits are observed, usually with uniseriate pits on the radial walls and opposing pits in the case of more than one row. Moreover, vessel elements and fibres can only be found in the wood fragments of *Eucalyptus* spp. Bark cells and cells from immature tissues also serve as corroborative evidence.

Keywords: Plant Anatomy; Environmental Crimes; Wood and Bark Fragments; Sawdust; Araucaria.

1. INTRODUCTION

Plant anatomy is the science dedicated to the study of cellular structures and organization of plant tissues and organs [1-3], and it is an excellent tool to identify timber.

Wood consists of a tissue called secondary xylem related to growth in thickness. It consists of tracheary elements, which are cells that comprise the water-conducting system of plants and can provide support, fibres or supporting cells, and parenchymal cells for storage. The diversity found in cell types and tissue organization allows for the recognition of genera. In most cases, this is enough to identify plant species involved in environmental crimes like deforestation and wood smuggling. Especially, for commercial timber traded in Brazil, extensive literature and online databases provide identification support [4-6]. However, traditional macroscopic and microscopic analysis for timber identification is mostly supported by characters observed in transversal and longitudinal sections of wood, which is applicable for solid wood, wood chips, and manufactured objects. However, wood sawdust, the powder or dust formed by grinding and sieving wood, requires a different approach. More specifically, because many different trees, possibly from many different species, are likely to be present, sawdust cannot be individually processed [7]. This means that wood sawdust found attached to chainsaws must be analyzed as macerated tissue in order to identify specific cells of tree species in dispute as long as such cells are still recognizable and capable of individualization. This approach is obviously faster and more cost-effective compared to molecular biology, which can be hindered by cell degradation.

Araucaria angustifolia (Bertol.) Kuntze (Araucariaceae Family – Gymnosperm) occurs mainly in southern and southeastern Brazil also in Paraguay and Argentina [8]. It is such a key species to the ecosystem that sites in the Atlantic Forest are named “Araucaria Forest”. However, in 1992, *Araucaria angustifolia* entered the Official List of Species of the Brazilian Flora Threatened with Extinction organized by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA), and nowadays its original coverage is about only 3% [9], owing to extensive exploitation with no concern for its preservation or its sustainability in relation to extractive processes [10].

In order to strengthen justice in crimes against key species of Brazilian vegetation, this work aimed to employ methods of plant anatomy to verify the presence of *Araucaria angustifolia* cells in its wood sawdust and compare it against sawdust of other major sample sources, including *Pinus* and *Eucalyptus*.

2. MATERIAL AND METHODS

In order to characterize *Araucaria angustifolia* and distinguish it from other introduced exotic genera into Brazil, we elected *Pinus caribaea* Morelet (Family Pinaceae - Gymnosperm) and *Eucalyptus grandis* W.Hill ex Maiden (Family Myrtaceae - Angiosperm) to respectively represent the genera *Pinus* and the *Eucalyptus-Corymbia* complex as each one comprises species sharing similar wood anatomy.

Our reference samples were divided into two major groups of each species analyzed. The first is macerated reference, consisting of trunk segments with 2 cm^3 reduced to 0.5 cm thick sticks. The second is wood sawdust reference, consisting in 1 cm^3 of wood sawdust collected from chainsaw debris while the first sample group was cut, thus from the same trunk. Both groups of samples were prepared according to the modified Franklin method (Berlyn and Miksche 1976) to achieve cell dissociation and stained with alcoholic safranin. Slides were mounted with a solution of water and glycerin (1:1). Also, sawdust of the three species were mixed and slides were mounted.

We applied this cell dissociation method to two abandoned chainsaws seized at sites where *A. angustifolia* occurs. These two independent wood sawdust samples were collected from different points on two different chainsaws seized in the largest area of preserved Atlantic Forest in the metropolitan region of São Paulo city. They are hereinafter termed Case Study Sample no. 1 and Sample no. 2 (CSS 1 and 2). Five slides of each were analyzed.

All measurements were obtained using an Olympus CX 31 microscope equipped with a digital camera (Olympus Evolt E 330) and image analyzer software (Image-Pro Plus 6.3).

Analysis consisted of description and caracterization of lignified and suberized cells following terminology adopted by IAWA [5,6].

3. RESULTS

In general *A. angustifolia*, *Pinus* spp., and *Eucalyptus* spp. could be distinguished from each other, and such differences could be detected through sawdust microscopical analysis. For example, *A. angustifolia* and *Pinus* spp. present tracheids, and *Eucalyptus* spp. present vessel elements and fibres. Figure 1 shows schematic illustration of different cell types, including vessels, fibres, and tracheids, placed on a scale that indicates size differences. Additionally, according to Richter *et al*. [6], uniseriate pitting in radial tracheid walls is the most common condition in coniferous woods (Pinaceae), while pitting in earlywood tracheids

is often biseriate. Typically, tri- or more seriate pitting occurs in Araucariaceae.

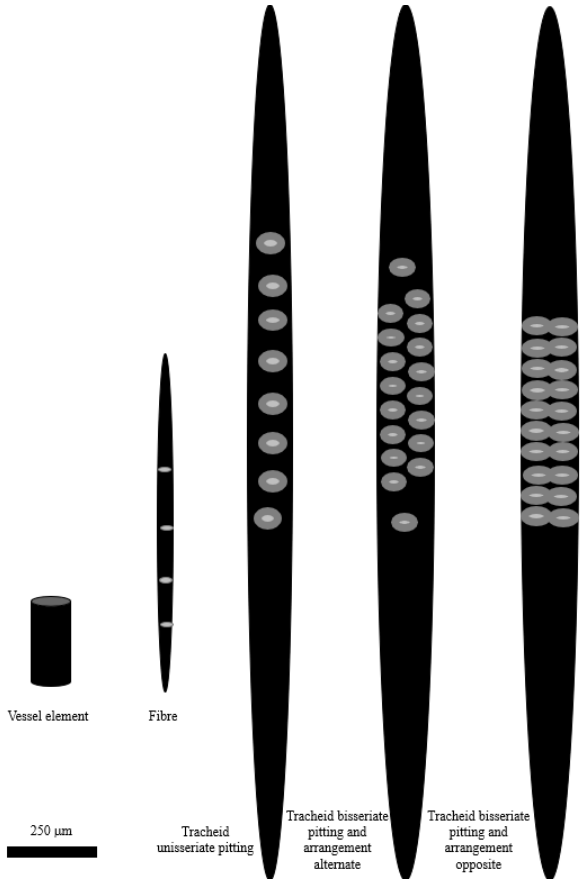


Figure 1. Schematic illustration of different wood cells: vessel element, fibre, and tracheids according to their size proportions.

The informative and diagnostic elements for species determination in macerated reference samples are as follows:

i. *Araucaria angustifolia*

Wood – Long tracheids (over 5000 micrometers), polygonal bordered pit outline, with two or more seriate and alternate pitting in radial walls (Figures 2a-c).
Bark - Branched sclereids (Figure 2d).

ii. *Pinus caribaea*

Wood – Long tracheids (over 5000 micrometers), circular bordered pit outline, usually uniseriate and pitting in radial walls (Figures 3a-b), but opposed pitting if more than one row.

Bark - Stone cells with puzzle-piece shape (Figure 3c).

iii. *Eucalyptus grandis*

Wood – Wide and short vessel elements with simple perforation plate, intervessel alternate pits (Figure 4a); Fibres with simple pits (Figures 4b-c).

Bark - wood-like fibres and branched sclereids (Figure 4c).

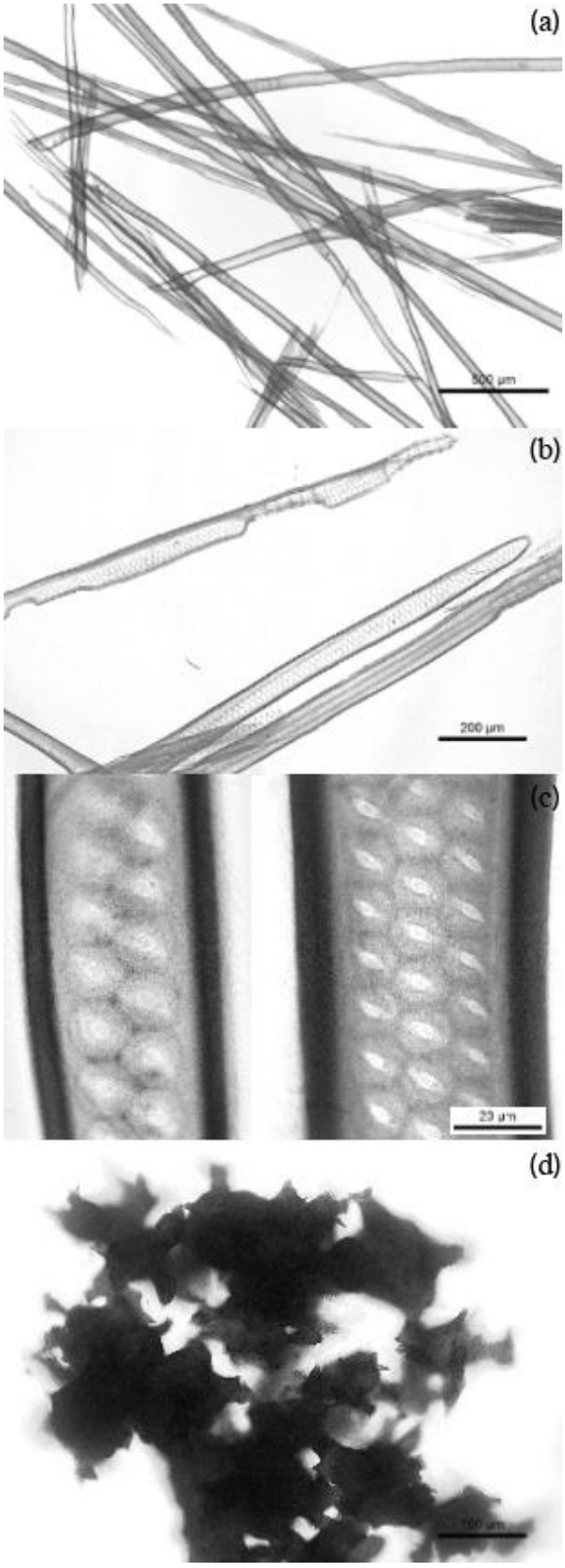


Figure 2. *Araucaria angustifolia*. a-b. Tracheids in macerated sample. c. Tracheids with two and three rows of pits. d. Branched sclereids from the bark.

The wood sawdust reference samples presented cells corresponding to the macerated reference samples of respective species, both wood and bark. Tracheary elements with ring-like and spiral thickenings were also found in *Pinus caribaea*. In mixed slides, species could also be distinguished between the study genera.

The wood sawdust from CSS1 presented tracheids with uniseriate pitting, circular pitting outline and opposite arrangement, compatible with the gymnosperm group, including *Pinus* species. The wood sawdust from CSS2 presented fibres and vessel elements with simple perforation plate and alternate pitting, compatible with the angiosperm group, including the *Eucalyptus-Corymbia* complex. Tracheids of *A. angustifolia* were not found in samples from CSS1 and CSS2.

Bark cells were observed in both samples. CSS1 presented stone cells typical of *Pinus*. CSS2 presented sclereids with appendages and crystalline series compatible with the *Eucalyptus-Corymbia* complex. As compatibily does not infer identification, species were not identified in either sample.

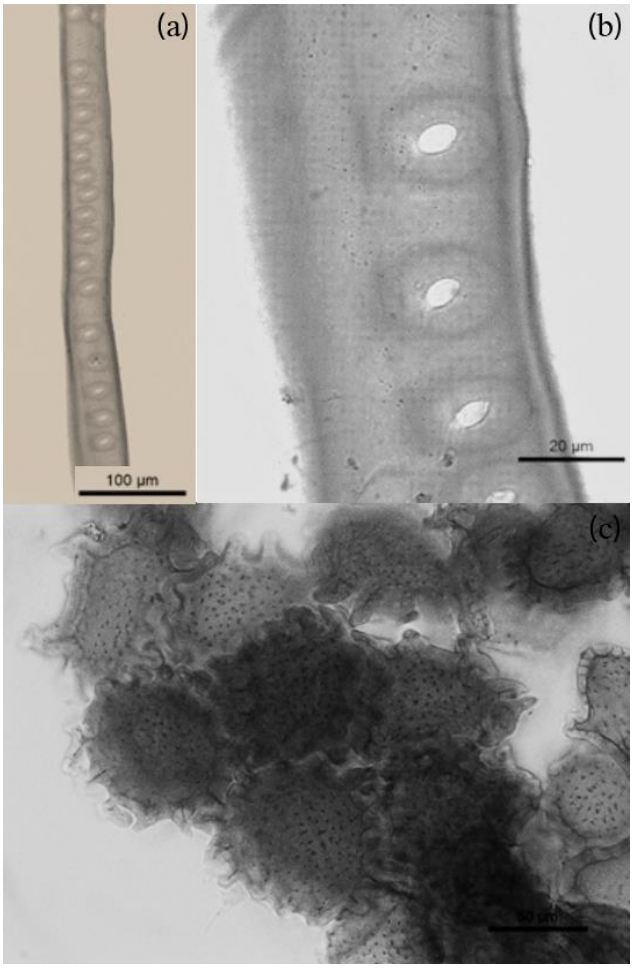
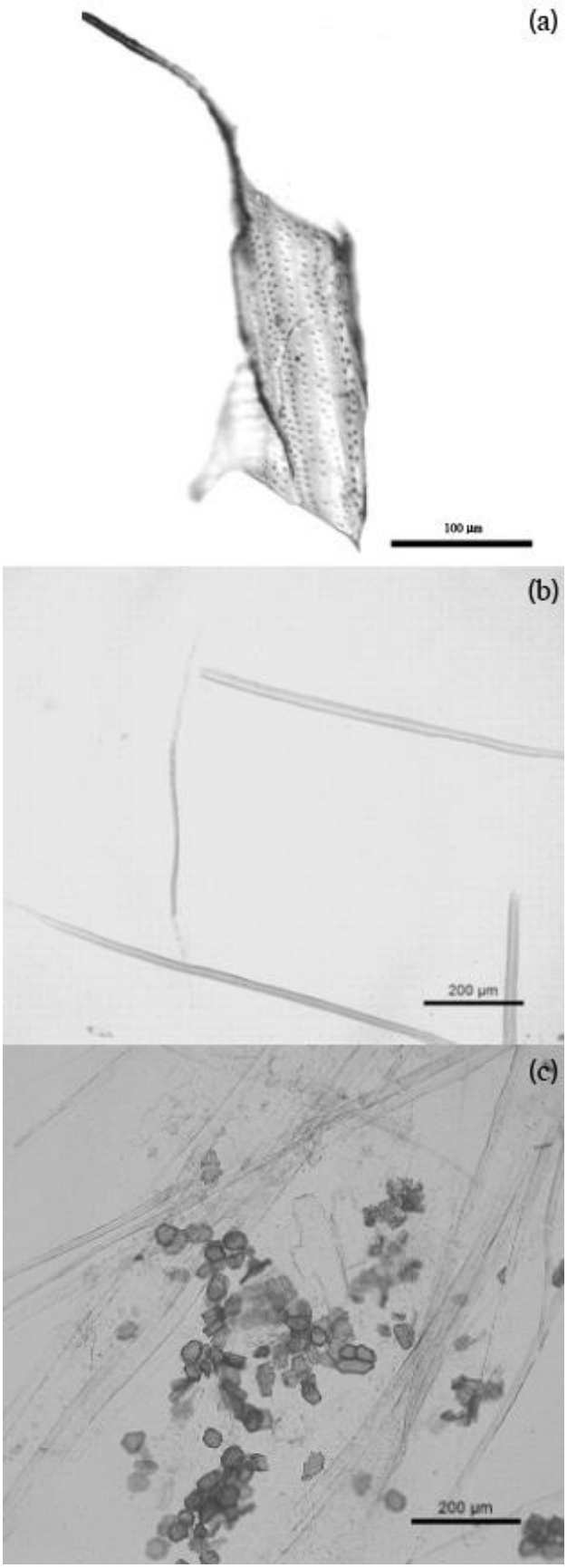


Figure 3. *Pinus caribaea*. a-b. Tracheid with one row of pits. c. Stone cells from bark.



Figures 4. *Eucalyptus grandis*. a Vessel element. b. Fibre fragments. c. Sclereids and fibres from bark.

4. DISCUSSION

Wood sawdust contains cells and cell fragments and sawing does not destroy cells or alter them in any way that would lead to confusion over the cells of other forestry cells.

To obtain reliable results, analysts must consider the complexity of wood sawdust samples, in which one can find cells from different species and even mixtures of tissues of such as wood (secondary xylem) and bark (periderm and secondary phloem).

Therefore, to verify the presence of *A. angustifolia* in a wood sawdust sample, two steps must be followed. First, characterize all cell types in the sample, such as water-conducting cells (tracheids and vessel elements), fibres, sclereids, and suberified cells, since the presence of wood and bark cells suggests contact with the trunk. Also, ring-thickening tracheary elements from primary tissue cells, such as nodes and young branches, can be found.

Tracheids occur in gymnosperms and are long, thin and imperforate, while vessel elements, from angiosperms, are shorter, wider, and have perforation plates. Fibres are also long and narrow, and they are easily distinguishable by their simple intervessel pits, while conducting cells have bordered pits.

Araucaria angustifolia identification relies on tracheid analysis since the presence of branched sclereids is not exclusive to its bark. Branched sclereids also occur in species with phylogenetic and distant affinities [11].

Second, if tracheids are found in a sample, then characterize the bordered intervessel pits on the radial walls, which are exclusive to *Araucaria*, *Agathis* and *Wollemia*, the three genera of the Araucariaceae family, and so they can, in turn, be distinguished from other gymnosperms. They must be in two or more rows, in alternating arrangement and show polygonal outline.

A. angustifolia is the only species of Araucariaceae family in Brazil and cultivation of other species of this genera is very restricted (for instance, *Araucaria cunninghamii* at the ecological station - ESALQ / USP). Therefore, Araucariaceae family diagnosis supports *A. angustifolia* identification.

When applying the technique herein discussed in border areas in southern Brazil, it should be noted the distinction among genera and species of the Araucariaceae family in neighboring countries can be achieved through measuring different characters of tracheids and comparing with data from specific bibliography [12].

5. CONCLUSION

By sawing analysis of chainsaws, we have confirmed that it is possible to obtain cell fragments allowing investigators to separate the genera of plants studied. In addition, evidence of contact with the trunk can be achieved, providing relevant information to investigators.

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