

Are there *Lacandonia* granules in *Taxodium mucronatum* ?

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Components of interphase cell nucleus have been studied since the last few decades. Currently compartmentalization of the nucleus is accepted as an important feature for the organization of nuclear processes and gene expression^{1,2}.

Contrary to animal cells, the complexity of the interphase plant cell nucleus is just beginning to emerge and so far not much is known about the compartmentalization of RNA-processing machineries³. In plants, heterochromatin arrangement could be chromocentric or reticulate; suggesting different conserved gene expression processes among great plant groups. Associated to reticulate plant nuclei a novel extranuclear RNP component named *Lacandonia* granules was described in the uncommon angiosperm *Lacandonia schismatica*^{4,5}.

Previous studies suggest that those RNPs have an important role in the mRNA processes, and they might be similar to structures described as Balbiani granules and perichromatin granules in insects and mammalian cells respectively. Besides *L. schismatica* such RNPs have been described in *Ginkgo biloba*, an early divergent gymnosperm, but not in lately divergent plants⁶. With the aim to determine their ubiquity we explored leaf nuclei in a lately divergent gymnosperm the: "Ahuehuete" (*Taxodium mucronatum*) by conventional TEM employing general and selective staining methods. Also a previous anatomical study was performed in order to identify leaf components, by bright-field microscopy and different illumination techniques.

Leaves and buds from young members and from the oldest living *Taxodium mucronatum* (2 000 years old) were subjected to TEM and anatomical procedures. TEM samples were fixed for 24 to 36 hours in 6% glutaraldehyde, buffered in PBS (pH 7.2). Some samples were postfixed in 1% osmium tetroxide overnight and subsequently dehydrated in a graded series of ethanol and embedded in an epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate, or with the EDTA procedure. Anatomical procedures employed FASSO as fixer and paraplast as the embedded medium.

Thick sections of the anatomical processed material by Nomarsky and polarization illumination revealed the abundance of calcium oxalate crystals in mesophyll cells and tannins in resiniferous channel cells. When stained with DAPI and analyzed in epifluorescence (NIKON E-800) and with the APOTOME system (Carl-Zeiss) microscope, leaf nuclei showed a reticulate pattern as observed in other gymnosperms. The same pattern could be observed by Atomic Force Microscopy (BioScope with a NanoScope IIIa controller) at 13.2 μm^2 scanning working in contact mode.

Ultrathin sections of *T. mucronatum* were contrasted with conventional uranyl acetate-lead citrate and analyzed in an electron microscope (JEOL 1010). The reticulate pattern and structures like LG were observed intermingled with chromatin bands. The EDTA procedure revealed the ribonucleoprotein nature of such elements. LG are present in nuclei of *T. mucronatum*, associated and intermingled with reticulated nuclei, which are similar in size, but not in number, to *L. schismatica*, with decreasing in number as mitosis progresses.

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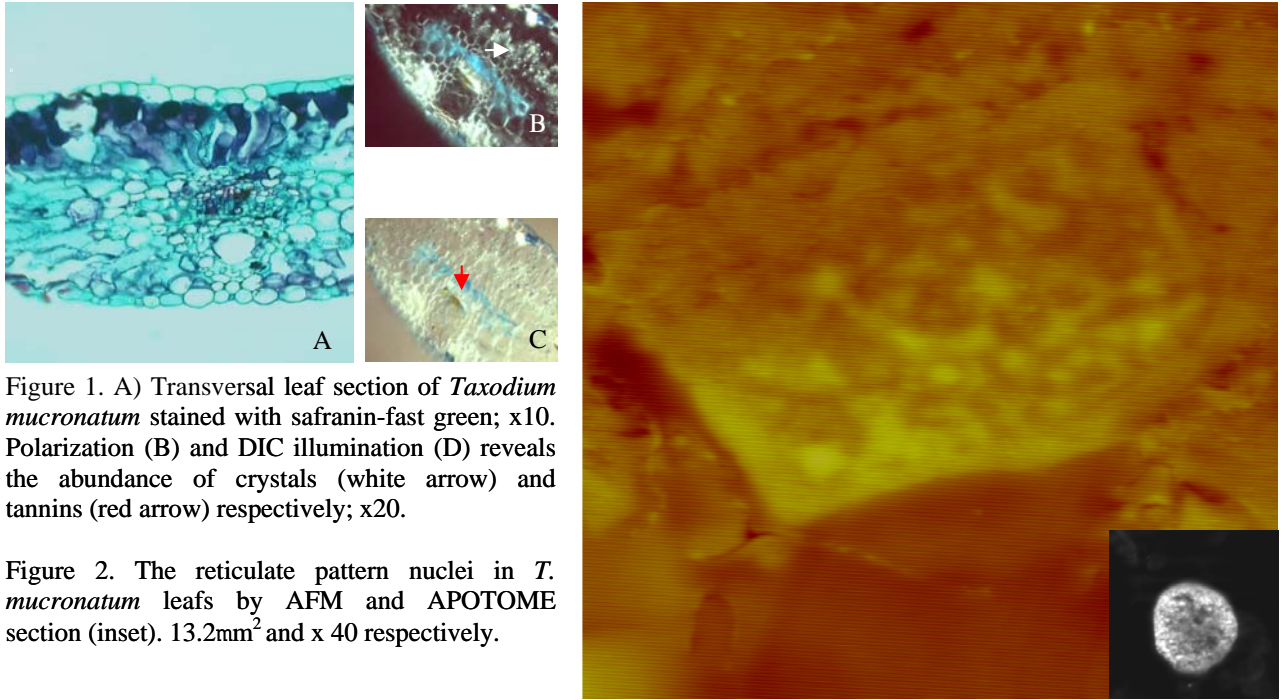


Figure 1. A) Transversal leaf section of *Taxodium mucronatum* stained with safranin-fast green; x10. Polarization (B) and DIC illumination (D) reveals the abundance of crystals (white arrow) and tannins (red arrow) respectively; x20.

Figure 2. The reticulate pattern nuclei in *T. mucronatum* leaves by AFM and APOTOME section (inset). 13.2mm² and x 40 respectively.

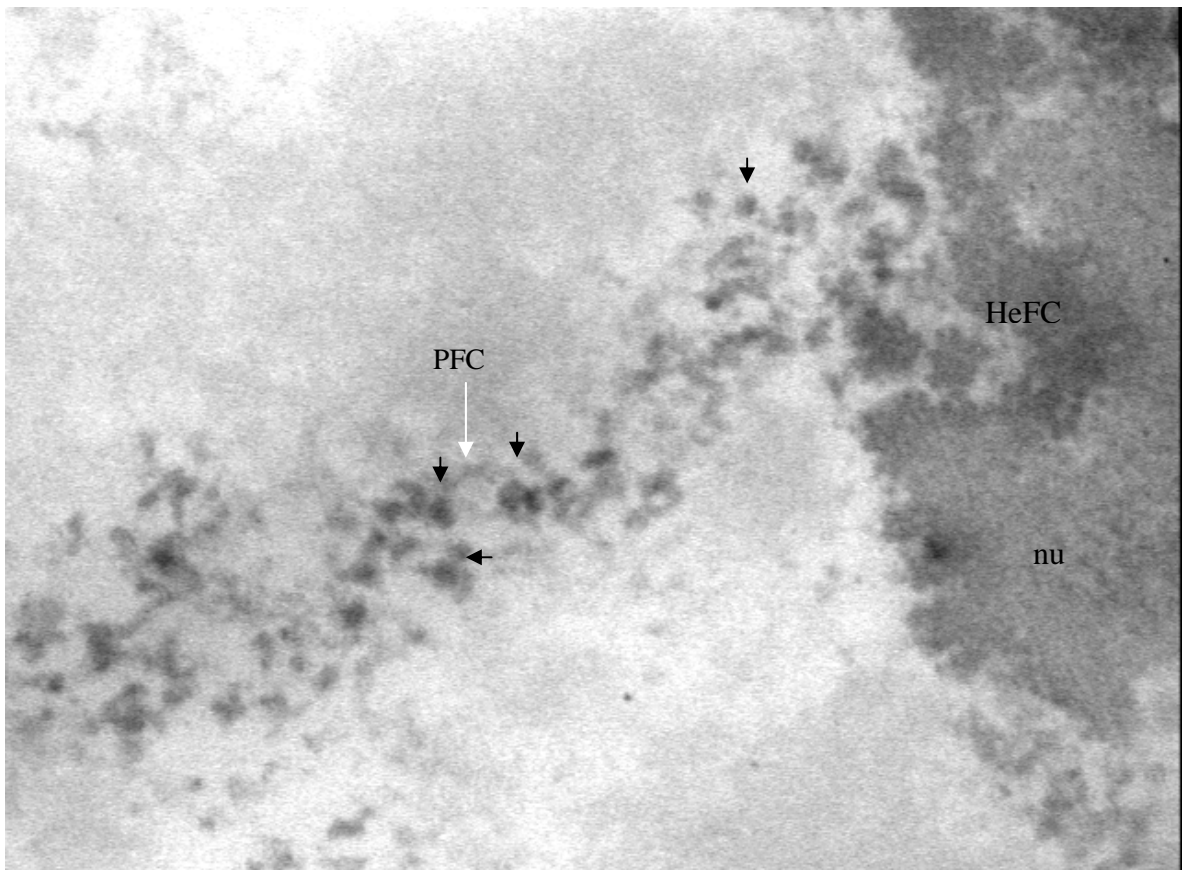


Figure 3. *T. mucronatum* nucleus stained with the EDTA procedure reveals the presence of LG, some interconnected with perichromatinian fibrils which emerge from the nucleolus. Nu, nucleolus; HeFC, heterogeneous fibrillar centers; perichromatinian fibrils (white arrow); LG (black arrows); x 60 000.