ATOMIC FORCE MICROSCOPY OBSERVATIONS OF *Lacandonia granules*.

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Lacandonia schismatica E. Martínez & C. H. Ramos (Lacandoniaceae: Triuridales) is an endemic and rare plant with the sex organs spatially inverted [1]. Interestingly, within the cell nucleus, the chromatin is reticulated and it is associated to a novel structure named *Lacandonia* granules, a very abundant ribonucleoprotein particle showing similarities to perichromatin and Balbiani ring granules present in cells of mammals and insects, respectively [2]. It is suggested that both structures participate in the metabolism of the RNA, in phenomena of transport and/or storage of mRNA [3]. Although these particles have been observed in several groups of plants as *Ginkgo biloba* E. Kaempfer (Ginkgoaceae: Ginkgoales), their fine structure has not been observed with sufficient resolution. Previous studies demonstrate that the cellular and nuclear structure of *Lacandonia schismatica* is susceptible of being analyzed with atomic force microscopy [4]. The goal of this work is to visualize and to analyze the nanometric structure and organization of Lacandonia granules "*in situ*" by atomic force microscopy, using techniques to prepare biological samples for transmission electron microscopy. Samples of organs of 1 mm^3 approximately were fixed during 2 hours at room temperature in a mixture 6% glutaraldehyde and 4% paraformaldehyde buffered in PBS 0.16 M at pH 7.2. Postfixation was done in 1% OsO_4 overnight. Samples were subsequently dehydrated in a graded series of ethanol and embedded in an epoxy resin (glycide ether 10, Merck). 0.2 µm thickness semithin sections were placed on glass slides and examined with an atomic force microscope Autoprobe CP Research (ThermoMicroscopes) in the contact mode and intermittent contact (IC) also called tapping mode. A scan rate of 1-2 hertz and a force constant 10 nN were applied. A silicon tip, with radius of curvature <10 nm and scanner actuators of 10µm and 5µm were used. Using the contact mode, it was possible to observe some granular structures inside the interchromatin space (Fig.1a) and in the periphery of the perichromatin space that may correspond to Lacandonia granules. They are irregular in shape. Some of them are observed with spherical shape, maybe due to the level of the section obtained and to the topography that they presented at the moment of the scanning, making measurements of average of heights, in the area where they are appreciated in great quantity of granules they indicate us that they are in the range reported in the literature [2], besides observing the great variety of ribonucleoproteins molecules that are in the area mentioned previously (Fig.1b). In the mode IC, in which the separation of the tip is some dozens of angstroms, the granules are appreciated in certain depth with more definition (Fig.1c), until being able to observe a heap of these structures as they have been observed by electron microscopy, but with better vertical resolution (Fig.1d), what allows us to obtain three-dimensional images for the observation of fine details of the structure. This will allow us to advance in the knowledge of their cellular biology. We thanks DGAPA-UNAM IN-221202.

References

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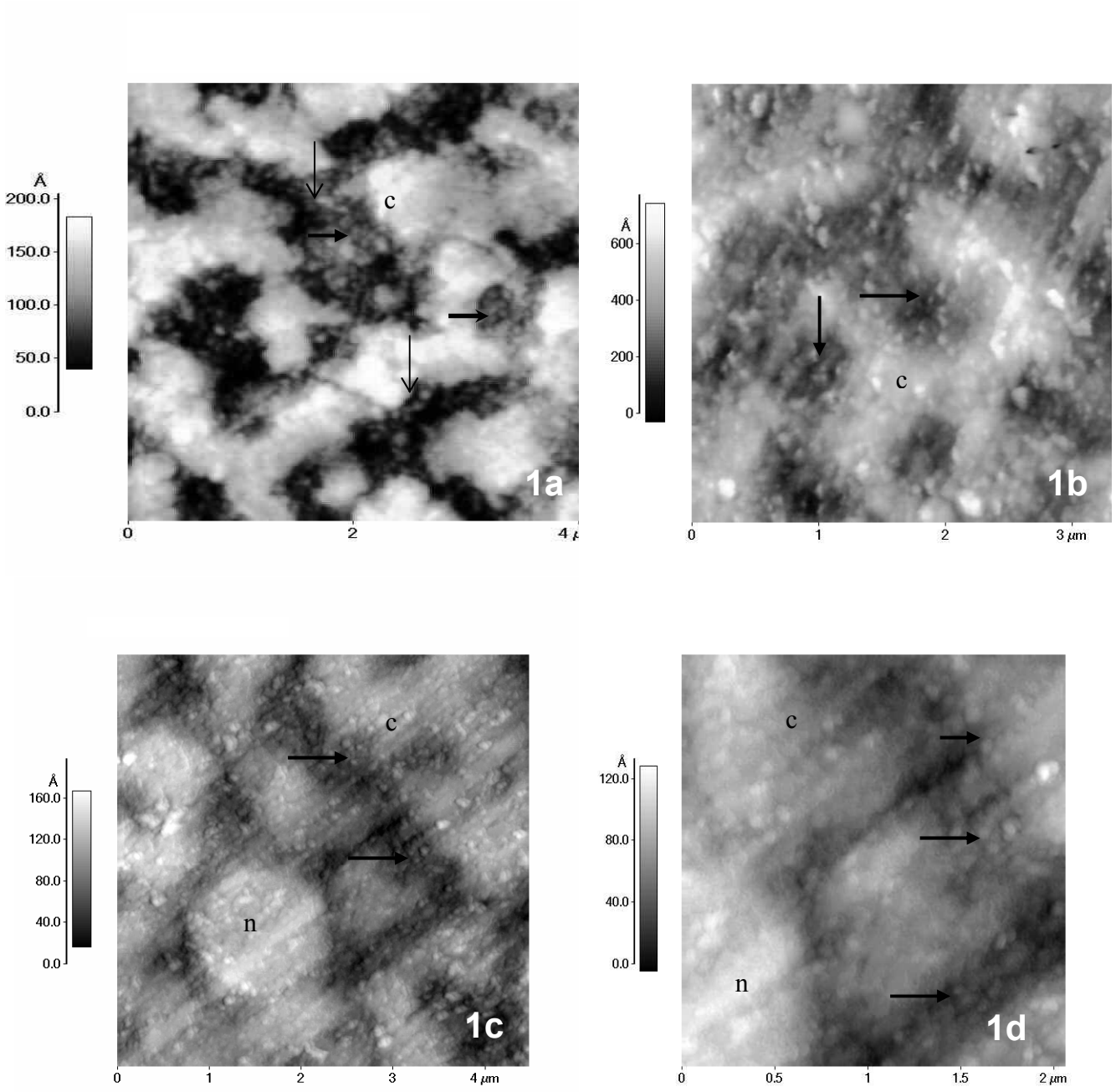

Figure 1. *L. schismatica* nuclei by atomic force microscopy. c: chromatin, n: nucleolus, large arrows, Lacandonia granules, thin arrows, interchromatin space.