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SEM and TEM Cross-section films Study of *Chrysanthemum leucanthemum* (Asteraceae) Pollen from Costa Rica

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ABSTRACT *Chrysanthemum leucanthemum* L. = *Leucanthemum vulgare* is a plant commonly known as Marguerite in Latin America, native to Eurasia, naturalized in the temperate part of north America, in Mexico, Guatemala and Costa Rica. This plant belongs to Anthemideae Tribe, Asteroideae subfamily, Asteraceae or Compositae family. It has a very unstable nomenclature since there is no consensus among taxonomists about its appropriate classification [1,2]. Willis [2] reported the genus *Chrysanthemum* comprising of about of 200 species. All are insect pollinated. Palynology can play an important role in the formation of natural groups and help in the assessment of taxonomical relationship between species. Studies by Scanning Electron Microscopy (SEM) about pollen grains of Anthemideae have revealed ultrastructure exine variation [3]. For example, exine stratification of Anthemideae taxa is qualitatively identical except for *Ursinia* (grains essentially lack basal columellae) and the *Artemisia* group (branches of basal columellae are complex and interwoven). Then, *Artemisia* presents complex branches of basal columellae and a doble tectum (synapomorphy) and plesiomorphic of Anthemideae [4]. This work has for objective to reveal of fine scale exine stratification by means of two related high-resolution techniques (TEM and SEM). Anthers of *Leucanthemum* were subjected to Erdman acetolysis technique [5] which removes the cellular contents and the cellulose wall (intine) of the pollen. Later the pollen grains were processed by the standard methods described to TEM. Cross thin and thick films were gotten by ultramicrotome, and only the first were studied by TEM. After for the SEM study, epoxy resin of thick cross sections was removed using a solution of absolute alcohol and sodium hydroxide. Finally, the samples were prepared by the conventional technique for SEM. The pollen characters studied were size, shape, apertural type and exine constitution. Pollen grains show considerable variation in pollen size from 37 mm to 55 mm. They had subspheroidal shape, 3-colpate exine, tectum micro-reticulate, micro-perforate, with spines. The palynological results from TEM and SEM indicate that their ultrastructure exine has some typical features congruent with their taxonomic status in Asteraceae, i.e., Anthemoid pollen pattern (Figure 1a-b). That is an infratectum comprising two layers of robust columellae, separated by a spongy internal tectum. This study of pollen shows that the information can be useful to know more about ultrastructure of one more species (*Leuchanthemum*), from the Anthemideae group.

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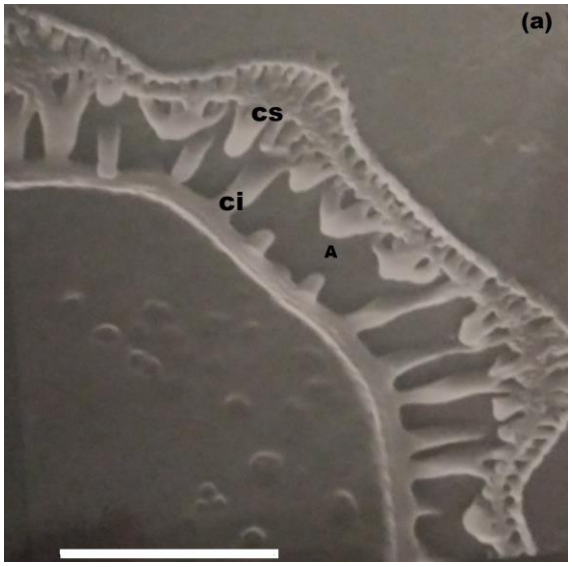

Figure 1. SEM micrography, from cross section pollen of *Leucanthemum*. Image show infratectal (ci) and supratectal (cs) columellae, typic of their taxonomic placements in Asteraceae.

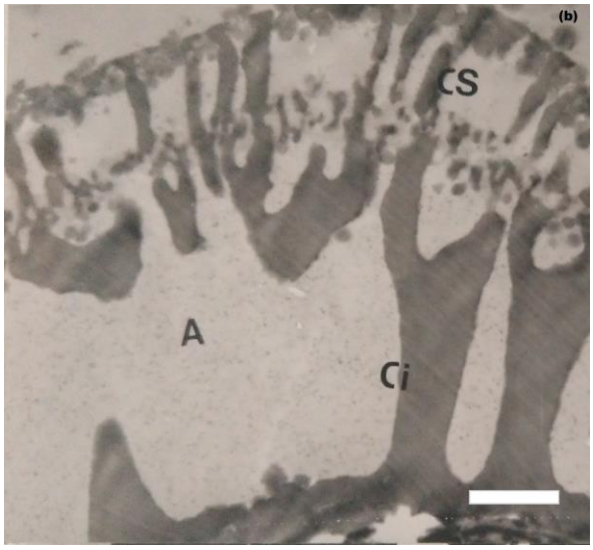

Figure 2. TEM micrography, from cross section pollen of *Leucanthemum*. It shows the same pattern in the ultrastructure of exine as in the SEM image.

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