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A comparative ultrastructural study of the pollen of *Linum burkartii* and *L. usitatissimum* (Linaceae)[1,2]

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LATTAR, E. C. (Instituto de Botánica del Nordeste, UNNE-CONICET, Corrientes, Argentina), B. GALATI (Cátedra de Botánica, Facultad de AgronomÍa, Universidad de Buenos Aires, Buenos Aires), S. PIRE (Centro de EcologÍa Aplicada del Litoral, CECOAL-CONICET, Corrientes, Argentina), AND M. FERRUCCI (Instituto de Botánica del Nordeste, UNNE-CONICET, Corrientes, Argentina). A comparative ultrastructural study of the pollen of *Linum burkartii* and *L. usitatissimum* (Linaceae). J. Torrey Bot. Soc. 139: 113–117. 2012.—We report a detailed study of the pollen wall structure in *Linum burkartii* and *L. usitatissimum* (Linaceae). This study was carried out using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Results confirm that the exine of the species studied is formed by: the ectexine constituted by clavae, gemmae, and a loose basal layer, a thin endexine and a fibrilar intine. These observations constitute the first report on the ultrastructure of pollen in the Linaceae that characterizes the species here studied and expands the palynological knowledge of this family.

Key words: *Linum burkartii*, *Linum usitatissimum*, Linaceae, pollen, spongy basal layer.

Linum L., a genus that comprises about 180 species, is considered one of the most diverse in terms of number of species within the family (McDill et al. 2009). There are several contributions that treat pollen morphology of the Linaceae. Saad (1961a, b, 1962) and Erdtman (1966) studied the pollen grains using only light microscopy. Dulberger (1981), Punt and Den Breejen (1981), Candau (1987) and Lattar et al. (2012), conducted their studies using light microscopy and scanning electron microscopy. In these contributions, the morphology and sculpture of the pollen of most species of the Linaceae were studied in detail. However, the controversy over the interpretation of the pollen wall structure between the different authors has not been resolved, and there is no uniformity of criteria in the delimitation of the ectexine and the endexine.

The aim of the present work was to analyze the ultrastructure of pollen grains of *Linum burkartii* Mildner and *L. usitatissimum* L. using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) in order to obtain a more exact interpretation of the exine. These results represent the first contribution to the study of the pollen ultrastructure in the Linaceae and expand the palynological knowledge of the family.

Materials and methods. Samples of *Linum burkartii* and *L. usitatissimum,* collected from Entre Ríos Province (Argentina), were fixed in FAA (formalin, alcohol, acetic acid). For scanning electron microscopy (SEM), anthers fixed in FAA were transferred to 100% acetone and then air-dried. For light microscope, anthers were embedded in paraffin, sections (10–12 μm thick) were transferred to xylene for 10 min and air-dried (Rosenfeldt and Galati 2007).

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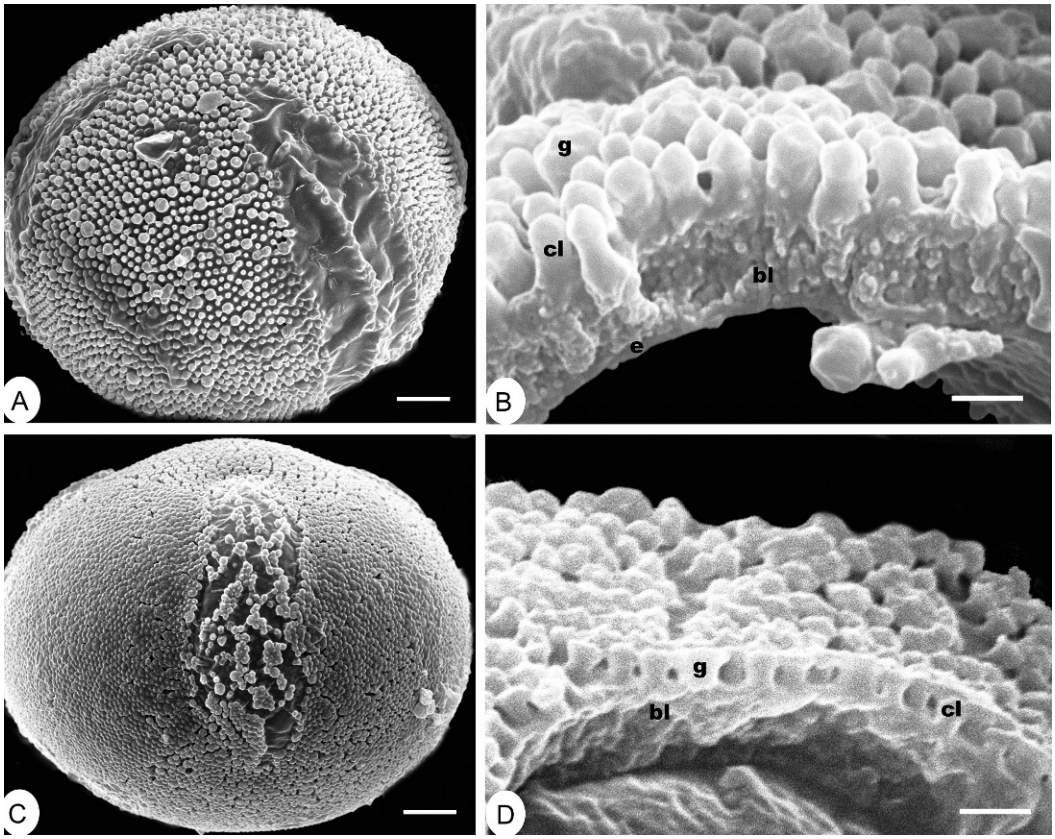

FIG. 1. SEM micrographs of pollen grains of *Linum* species. Abbreviations: c: clava; g: gemma; bl: basal layer; e: endexine. *Linum burkartii*: **A.** equatorial view showing the colpi and ornamentation of ectexine; B. detail of pollen wall section; *L. usitatissimum:* **C.** equatorial view showing the colpate condition and ornamentation of ectexine; **D.** detail of the pollen wall. Scale bars: **A**; **C** = 10 μm; **B**; **D** = 2 μm.

For transmission electron microscopy (TEM), material was fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.2) for 2 h and post-fixed in 1.5% O_sO_4 at 2 °C in the same buffer for 3 h. Then, the material was dehydrated in an ascending acetone series and embedded in Spurr resin. Ultrathin sections were made on a Reichert-Jung ultramicrotome, stained with uranyl acetate and lead citrate (O' Brien and McCully 1981), observed and photographed in a Philips EM 301 TEM.

The palynological terminology used in this work is in accordance with Hesse et al. (2009).

Specimens investigated. *Linum burkartii* Mildner. ARGENTINA, Entre Ríos: Dept. La Paz, Lattar 14 (CTES). *Linum usitatissimum* L. ARGENTINA, Entre Ríos: Dept. La Paz, Lattar et al. 15 (CTES).

Results. SEM. The pollen grains are 3-colpate in both species (Fig. 1, A, C). The exine of the pollen grains of *Linum burkartii* and *Linum usitatissimum* consists of an ectexine formed by sculptural elements (clava and gemma) and a spongy basal layer, and a thin, continuous and compact endexine (Fig. 1, B, D).

TEM. *Linum burkartii.* The pollen wall is composed of exine (ectexine and endexine) and intine. The ectexine is formed by a basal layer, and sculptural elements, gemmae and clavae. The gemma (0.5–0.8 μm in height and 0.5–0.7 μm in diameter) and clava (1.08 μm in height and 1.2–1.84 μm in diameter) have compact spongy structure. The basal layer presents a loose spongy structure (1.3 μm thick). The endexine is thin (100 nm thick), (Fig. 2A). The intine is fibrillar and thin (0.5 μm thick). Pollen-kitt is absent. Inside the pollen grain, the vegetative cell cytoplasm

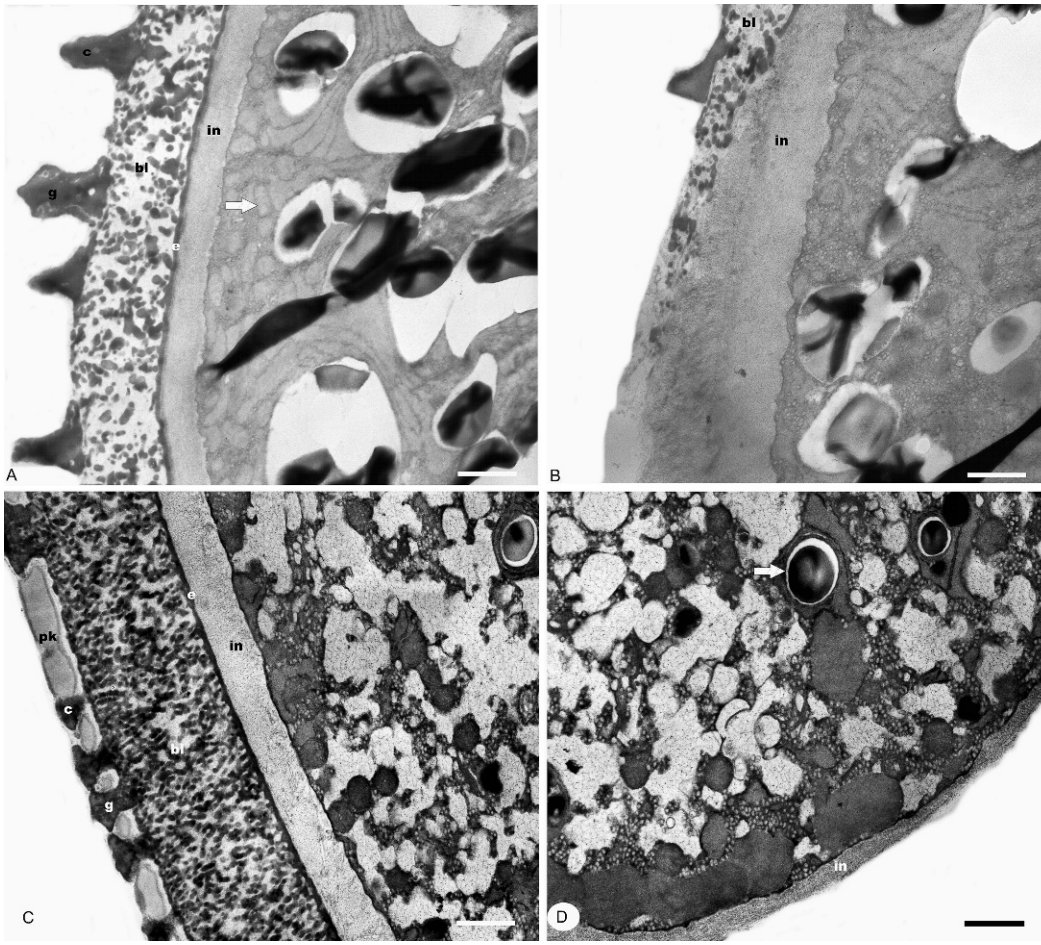

FIG. 2. TEM micrographs of pollen wall of *Linum* species. Abbreviations: c: clava; g: gemma; bl: basal layer; e: endexine; in: intine; pk: pollen kitt. *L. burkartii*: **A.** section through wall showing sculptural elements (clava and gemma), basal layer, endexine and intine; **B.** detail of the aperture, the basal layer remains intact and the sculptural elements become smaller. Arrow shows starch grains; *L. usitatissimum:* **C.** section through wall showing sculptural elements (clava and gemma), basal layer, endexine and intine, presence of pollen kitt; **D.** region of the aperture, the exine and the basal layer disappear leaving only intine. Arrow shows abundant oils. Scale bars: **A–D** = 1 μm.

shows small vesicles and abundant starch grains. In the region of the aperture, both the ectexine and the endexine are absent, leaving only the thickened intine forming an oncus (Fig. 2B).

Linum usitatissimum. The pollen wall is composed of exine (ectexine and endexine) and intine. The ectexine is formed by sculptural elements, gemmae and clavae, and a basal layer. The gemmae (0.4–0.5 μm in height and 0.4–0.6 μm in diameter) and the clavae (0.8–1.17 μm in height and 0.9–1.05 μm in diameter) have compact spongy structure. The basal layer possesses a loose spongy structure (1.7 μm thick). The endexine is thin (100 nm thick). The intine is fibrillar and thin (0.7 μm thick). Pollen-kitt is present (Fig. 2C). Inside the mature pollen grain, the vegetative cell cytoplasm shows abundant vesicles, mitochondria and oils; a small number of starch grains is also observed. In the region of the aperture, the ectexine and the endexine are reduced to some isolated processes and the intine is thinned (Fig. 2D).

Discussion. This is the first report on the analysis of the exine ultrastructure in *Linum burkartii*, a species endemic to the Province of Entre Ríos (Argentina), as well as in *Linum usitatissimum*, a cultivated species native to

Europe. This paper confirms that the exine is formed by sculptural elements, gemmae and clavae, a basal layer and a thin endexine. Both the sculptural elements and the basal layer have spongy structure and form the ectexine. Other interpretations of the exine structure in species of *Linum* were suggested by Saad (1961 a, b 1962), who described the exine as consisting only of sculptural elements (capitate bacula and clavae), and lacking a basal layer.

A controversial point in the study of Linaceae's pollen is the presence or absence of a tectum in *Linum* species. This has led many authors to make different interpretations. Erdtman (1966) analyzed the pollen grains of some species of *Linum*, including *L. usitatissimum*, using the light optical microscope and interpreted the exine as formed by verrucae of different sizes, but made no reference to the presence or absence of a tectum. Dulberger (1981) studied the exine sculpture in three distylous species of *Linum* and found dimorphism in the sculptural processes of the pollen. However, like Erdtman (1966), this author made no reference to the presence or absence of a tectum. Candau (1987) interpreted *L. usitatissimum* as having a well-developed tectum, with more or less regular elements densely arranged. Punt and Den Breejen (1981) studied European populations of *L. usitatissimum* and interpreted the sexine as tectate-scabrate and the tectum with the heads of the sculptural elements very closed space and possibly overlapped or fused.

Finally, in a recent study on the morphological and morphometric features of pollen of Linaceae species from Argentina, it was interpreted that the tectum is absent in most of them, except for *Linum usitatissimum* where the heads of the sculptural elements are fused only occasionally (Lattar et al. 2012).

The results obtained by TEM show that in *Linum burkartii* the tectum is absent, whereas in *L. usitatissimum* the tectum is discontinuous due to partial fusion of the heads of the sculptural elements. This fusion would indicate the disappearance of the tectum in Linaceae, since in most of the species studied, the tectum is absent. Studies of these two species by TEM also confirmed differences between them: *L. burkartii* has a thinner basal layer and intine then *L. usitatissimum*; in *L. burkartii* the reserve substance of the pollen grains is starch, in contrast with a considerable amount of oil in *L. usitatissimum*. In the region of the apertures, both the ectexine and the endexine in *L. burkartii* are absent, leaving only a thickened intine forming an oncus, which is normally found in the pollen grains of most studied species (Hyde 1955). In *L. usitatissimum* the intine is thin in the apertural region, a characteristic not commonly observed within Spermatophyta (Lernsten 2004). Usually, the typical wall aperture of angiosperm pollen is characterized by the reduction of exinous structures beneath the aperture or by a deviant exine, and a thick, often bilayered intine (Hesse et al. 2009).

The type of reserve substance could be related to the effective pollinators or pollination mode in each of the species studied. Starch-rich pollen is associated with pollination by wind, birds, and lepidopterans (moths and butterflies), while lipid-rich pollen is associated with bee and fly pollination (Lernsten 2004).

The observations made on the ultrastructure of the pollen grains wall of *Linum burkartii* and *L. usitatissimum* represent the first contribution to a clear and comprehensible interpretation of the exine. It would be interesting to extend this type of study to other species of *Linum* in order to verify if there is variability in the thickness of the basal layer between American and Eurasian species, and clarify the development of the tectum in others species of *Linum*. These palynological data are important to better understand the phylogeny of the genus which is so far considered as non-monophyletic (McDill et al. 2009).

Conclusions. The results obtained with TEM, show that *Linum burkartii* and *L. usitatissimum* shared an exine formed by the ectexine, constituted by gemmae and clavae, and a loose spongy basal layer, a thin endexine, and a fibrillar intine. In addition, it is confirmed that in *L. burkartii* the tectum is absent, whereas in *L. usitatissimum* the tectum is discontinuous due to partial fusion of the heads of the sculptural elements. This latter feature would indicate a progressive reduction of the tectum in Linaceae.

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