
Allergenic proteins in *Urtica dioica*, a member of the Urticaceae allergenic family

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Background: Allergy to the pollen of flowering plant species significantly affects the health of people in many parts of the world. Pollens of related genera usually share common antigens and are often, but not always, cross-reactive. Several studies have shown that *Parietaria* pollen is one of the most common causes of pollinosis in the Mediterranean area, whereas *Urtica* has no allergenic significance.

Objectives: To report on the localization of *Parietaria judaica* major allergen in *Urtica dioica* pollen grains and on the detection of allergenic proteins in *U dioica* pollen grains during the hydration-activation process.

Methods: A combination of transmission electron microscopy and immunocytochemical methods was used to locate allergenic proteins in *U dioica* pollen grains after different periods of hydration-activation using the anti-Par j 1 (4.1.3.) monoclonal antibody and serum samples from allergic patients.

Results: No significant labeling was noted for Par j 1 allergen after 10, 15, and 20 minutes in the walls and cytoplasm. Slight labeling was observed for allergic proteins in the walls of *U dioica* after 10 minutes of hydration, and no significant labeling was found after 15 and 20 minutes of hydration.

Conclusions: Immunocytochemical methods confirmed the absence of cross-reactivity between 2 related genera, *Parietaria* and *Urtica*, and the lowest allergenic potential of *U dioica*.

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INTRODUCTION

Pollen, the male gametophyte of flowering plant species, is one of the most predominant sources of environmental allergens and a significant cause of allergic diseases. Mature pollen grains have various types of proteins at the time of anther dehiscence; some of these are glycoproteins with a low molecular weight and are easily diffused when they come into contact with a wet medium.^{1,2} Most of these proteins are pollen allergens capable of inducing IgE-mediated type I allergy in humans.

In recent years, pollen measurements have been performed in several countries to obtain information on daily allergenic pollen counts and to forecast airborne pollen concentrations to understand the relation to pollen allergy symptoms. With up to 20% of the population in industrialized countries experiencing pollen allergy, much research has been conducted to identify allergenic pollen species and the allergy-causing components present in their pollen.³

Urticaceae is an allergen family frequently encountered in pollen calendars because its members occur widely in nitrogen-rich soils and are, therefore, common in cultivated areas or on abandoned land close to human habitation.⁴ Two genera

are of major significance in airborne pollen studies: *Parietaria* and *Urtica*. Because the pollen grains of these 2 species are too similar to be distinguished morphologically from each other under light microscopy, and because both species have similar growing periods and almost simultaneous flowering times, together they figure as Urticaceae in pollen calendars.⁴ However, *Parietaria judaica* is considered to cause numerous allergies in the Mediterranean region,⁵ whereas *Urtica dioica* pollen displays little allergenic activity,⁴ although some cases have been reported in the United States.^{6–8} Traditionally, *U dioica* has been identified as an allergen source that induces urticaria and dermatitis,^{9,10} but no distinct allergenic protein has been identified.⁹

Previous studies¹¹ have successfully identified the major allergen of *P judaica*, Par j 1, a lipid transfer protein (LTP),^{12–14} and located the allergenic proteins of *P judaica* in pollen grains of this species.¹⁵ The aim of the present research was to locate these same proteins in *U dioica* pollen grains to establish which proteins are common to these 2 species because LTPs have been described as panallergens.^{16–18} To achieve this aim, we compared the labeling of *U dioica* pollen grains with 10-, 15-, and 20-minute hydrated pollen based in previous observations in *P judaica*.^{11,15}

MATERIALS AND METHODS

Plants Materials

Pollen from mature anthers of *U dioica* was collected in the area surrounding Barcelona (Spain) each spring in 2000 to 2003. The pollen samples were used fresh, without any storage. The observations were made in mature pollen and in

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hydrated and activated pollen in the germination medium. We wanted to establish a comparative hydration sequence because most biological fixatives change hydration status rapidly.

Hydration and Activation

Hydration and activation of mature pollen grains were induced in vitro after sprinkling the grains onto 0.7% agar germination medium supplemented with 0.35M sucrose, calcium chloride (300 mg/L), and boric acid (100 mg/L) for 10, 15, and 20 minutes at room temperature to generate humidity and temperature conditions similar to the receptive stigma. After these times, the process was interrupted, and pieces of agar with pollen grains were cut and fixed.

Transmission Electron Microscopy

A conventional protocol¹⁹ was used to obtain good structural preservation and good antibody-binding capacity. For immunogold labeling, anti-Par j 1 (4.1.3.) monoclonal antibody (ALK-Abelló A/S, Madrid, Spain)²⁰ and serum samples from allergic patients (El Bierzo Hospital, Ponferrada [León], Spain) were used. Radioallergosorbent testing (RAST) of these serum samples disclosed class 2 specific IgE positivity to *Parietaria* and *Urtica* extracts (0.7–3.5 Phadebas RAST units per milliliter). Serum samples from nonallergic individuals were used for control purposes. Ultrathin sections were incubated following a protocol previously described by Castells et al²¹ for polyclonal antibodies and by Suárez-Cervera et al¹⁹ for monoclonal antibody. Sections were stained with uranyl acetate and lead citrate and were observed using microscopes (EM 301; Philips, Eindhoven, the Netherlands, and model 1010; JEOL, Tokyo, Japan).

RESULTS

Pollen grains of *U dioica* are spherical, 3 pored, and bicellular. They contain a small generative cell and a large vegetative one. The wall is formed by a thin outer layer (exine) and an inner layer (intine) that is thin in the nonapertural region but thickens below the apertures to form a convex structure (oncus) (Fig 1). In the cytoplasm, vacuoles, lipid bodies, mitochondria, endoplasmic reticulum, dictyosomes, and ribosomes were observed (Fig 1).

Immunolocalization of Par j 1

After 10 minutes of hydration, isolated and nonsignificant epitopes of the Par j 1 protein were located in the nonapertural (Fig 2A) and apertural pollen walls, especially in the oncus (Fig 2B). Very few proteins recognized by Par j 1 (4.1.3.) monoclonal antibody were found in cytoplasm lipid bodies, vacuoles, endoplasmic reticulum, or dictyosomes (Fig 2A). After 15 and 20 minutes, no significant labeling was observed in the wall or cytoplasm (Fig 2C, D).

Immunolocalization Allergenic Proteins in Patient Serum Samples

After 10 minutes of hydration, slight labeling was observed in the nonapertural (Fig 3A) and apertural (Fig 3B) pollen walls (exine and intine), especially in the hydrated intine at the porus level (oncus) (Fig 3B). Very few allergenic proteins were released from the pollen grains in the exudates (Fig 3A). The cytoplasm displayed no significant labeling (Fig 3A, B).

In pollen grains hydrated for 15 and 20 minutes, no significant labeling was found in the wall or cytoplasm (Fig 3C, D). No significant labeling was observed in any of the controls (Fig 1B).

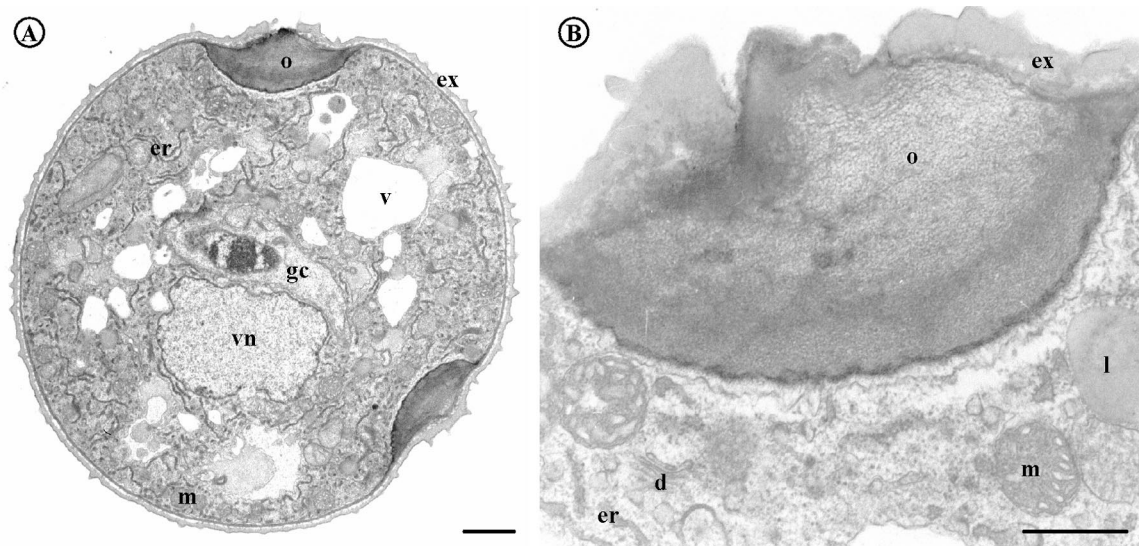


Figure 1. Immunolocalization of allergenic proteins in *Urtica dioica* pollen grains after 10 minutes of hydration. A, In this control the samples were not incubated with anti-Par j 1 (4.1.3.) monoclonal antibody. Cross-section of entire pollen grain. Vegetative cell with numerous vacuoles (v), mitochondria (m), endoplasmic reticulum (er), and a large vegetative nucleus (vn) close to the small generative cell (gc). Wall with thin outer layer (exine [ex]) and thickened inner layer (intine) under the apertures forming an oncus (o). Bar = 1 μ m. B, In this control the samples were not incubated with serum sample from an allergic patient. Apertural region. No labeling is observed in the exine, oncus, or cytoplasmic organelles. Bar = 0.5 μ m. d indicates dictyosomes; l, lipid bodies.

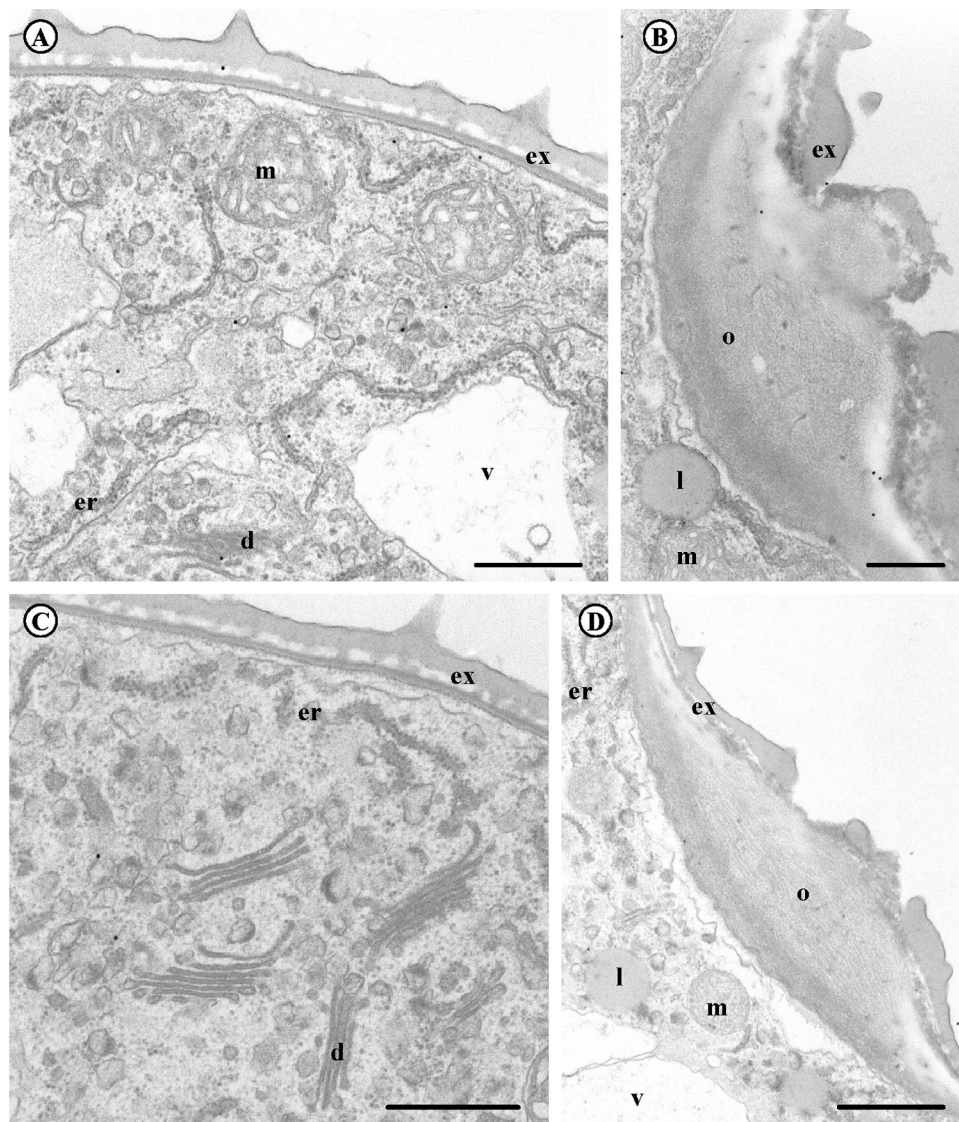


Figure 2. Immunolocalization of Par j 1 (the major allergen of *Parietaria judaica* pollen) in *Urtica dioica* pollen grains. After 10 minutes of hydration, some proteins were observed in the nonapertural wall (A) and in the oncus (o) (B). In the cytoplasm, very few proteins recognized by Par j 1 (4.1.3.) monoclonal antibody were found in endoplasmic reticulum (er) or dictyosomes (d). After 15 minutes (C) and 20 minutes (D), no significant labeling was observed in the wall or cytoplasm. ex indicates exine; l, lipid bodies; m, mitochondria; v, vacuoles. Bars = 0.5 μm .

DISCUSSION

In natural conditions, proteins are released in the stigma because pollen grains absorb water and release low-molecular-weight proteins on contact with stigmatic secretion. As a result, pollen-stigma adhesion²² and pollen-stigma recognition take place.^{23–25} If these processes occur in the mucosa of sensitive individuals, the released proteins act as allergens and prompt allergic sensitization by reacting with human antibodies.²⁶ However, the proteins released during pollen-stigma recognition are not all allergenic. The allergenic molecules contained in the pollen are usually a complex mixture of various proteins differing in either specific IgE binding or

biochemical proteins.²⁷ In allergenic individuals, approximately 10 or 12 of the 40 proteins released are recognized by IgE.^{28,29} Furthermore, the content of these proteins in the pollen grains is unstable, displaying variation in relationship to the progress of the germination; hydration time plays an important role in labeling intensity.^{11,21,22}

Urtica belongs to the Urticaceae family, as does the allergenically important genus *Parietaria*. In plants with a close taxonomic relationship, pollen proteins probably have a homologous sequence.³⁰ Extensive cross-reactivity among different species and genera of the same family could be expected.³¹ This is certainly the case in the *Parietaria* genus;

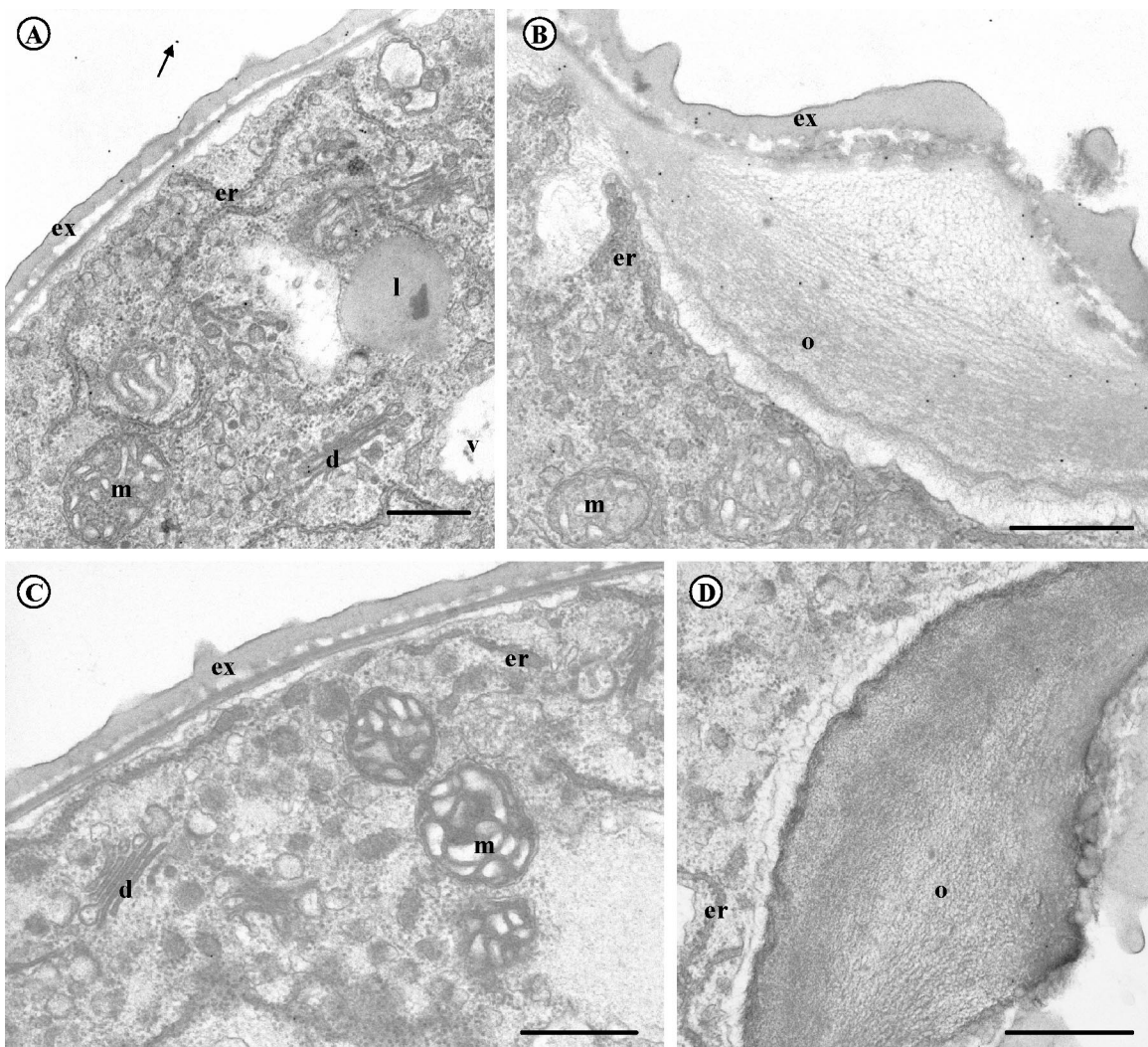


Figure 3. Immunolocalization of allergenic proteins in *Urtica dioica* pollen grains using serum samples from allergic patients displaying specific IgE to *Parietaria* and *Urtica*. After 10 minutes of hydration, slight labeling was observed in the nonapertural (A) and apertural (B) pollen walls. Very few allergenic proteins were released from pollen grain in exudates (arrow). In the cytoplasm, there was no significant labeling. In pollen grains hydrated for 15 minutes (C) and 20 minutes (D), no significant labeling was found in the wall or cytoplasm. d indicates dictyosomes; er, endoplasmic reticulum; ex, exine; l, lipid bodies; m, mitochondria; o, oncus; v, vacuoles. Bars = 0.5 μ m.

several species, including *P judaica*, *Parietaria officinalis*, *Parietaria mauritanica*, and *Parietaria lusitanica*, display a cross-reactive response to Par j 1 antibodies.^{14,32,33} However, there generally seems to be little cross-reactivity of *Parietaria* with other genera belonging to the same family. Par j 1 has not been detected by enzyme-linked immunosorbent assay in *U dioica*.³³ Furthermore, RAST inhibition studies have revealed the absence of cross-reactivity between *Parietaria* and *Urtica*.^{32,34–36}

The major allergen of *P judaica*, Par j 1, is a member of the LTP family.^{12–14} The ubiquitous distribution of the LTP family in plants³⁷ has led to this protein family being described as novel plant panallergens.^{16–18} Various roles have been sug-

gested for plant LTPs in cutin formation, embryogenesis, defense reactions against phytopathogens, symbiosis, and adaptation to various environmental conditions.^{37,38} LTPs have recently been located in pollen grains in the organelles of the cytoplasm and in the lipid bodies associated with vacuoles.¹¹ These allergens are mostly involved in allergies to plant foods^{16,17} and may be responsible for cross-reactions among fruits or pollens, which is frequent in other plant panallergens.³⁹ Nevertheless, LTPs are also considered to be involved in food-allergic reactions with no associated pollenosis.^{40–42} In addition, significant differences in specific IgE-binding capacities have been observed among members of the LTP family in plants.³⁹

In the present study, *U dioica* pollen grains did not react with the antibodies against LTPs of *P judaica*, indicating low similarity between the pollen LTPs of the 2 related genera. Previous studies have shown the localization of LTPs in the cytoplasm and apertural wall of *P judaica*, and abundant labeling is observed at 10 minutes (Fig 4) and 15 minutes of hydration, whereas labeling decreases at 20 minutes.¹¹ The lack of labeling in *U dioica* suggests that *P judaica* LTPs are genus-specific proteins crucial in the allergic process and confirms the nonexistence of cross-reaction between *P judaica* and *U dioica* pollen grains by means of immunocytochemical methods, although LTPs have been described as panallergens.^{16–18}

The proteins recognized by the IgE antibodies in serum samples from allergic patients in *U dioica* pollen grains are less abundant than those observed in *P judaica* pollen grains,¹⁵ in which abundant gold particles are observed after 10 minutes (Fig 5), 15 minutes, and 20 minutes of hydration; in this case, this result showed similar labeling intensity during the first 20 minutes of hydration.¹⁵ This could indicate that *U dioica* releases fewer allergenic proteins than *P judaica* during the fertilization processes and agrees with the lowest allergenic potential of *U dioica* to induce respiratory diseases.³⁵

Allergen cellular sites in the pollen cytoplasm have been identified by means of immunocytochemical studies in different pollen species.^{11,21,22,43,44} Nevertheless, the exine and intine pollen walls at the apertural and nonapertural regions are a physical route available so that allergen molecules can be secreted to the surface during pollen hydration. The structure of the Urticaceae apertural wall, which is formed by a developed intine (oncus), is considered an adaptation to pollen tube growth.^{26,45} During the activation process, the aper-

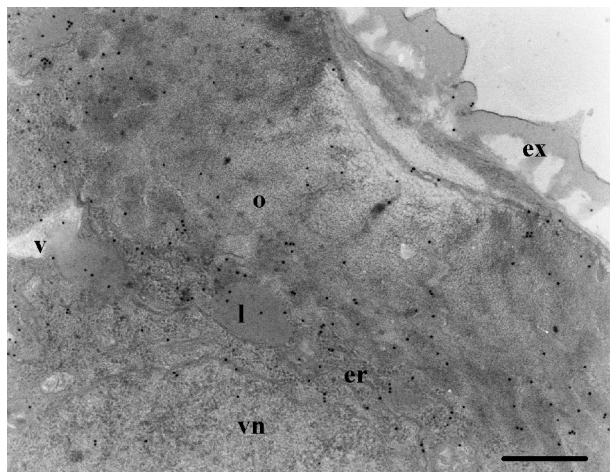


Figure 4. Immunolocalization of Par j 1 in *Parietaria judaica* pollen grains after 10 minutes of hydration. Gold particles were seen in the cytoplasm (endoplasmic reticulum [er], lipid bodies [l], and vacuoles [v]) and in the intine wall (oncus [o]). ex indicates exine; vn, vegetative nucleus. Bar = 0.5 μ m. Reprinted with permission from Vega-Maray et al.¹¹

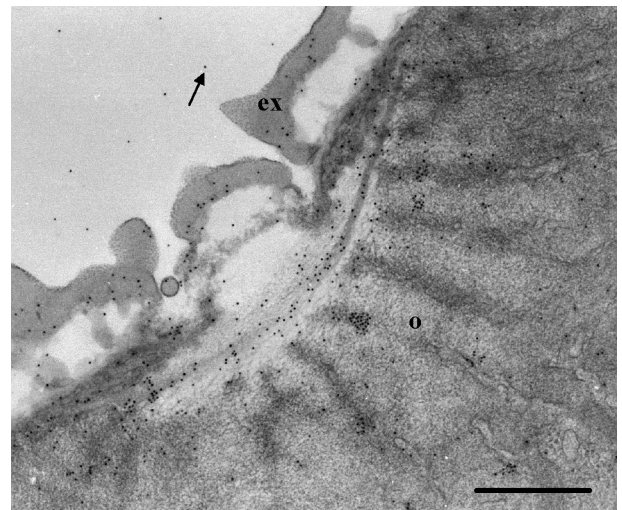


Figure 5. Immunolocalization of allergenic proteins in *Parietaria judaica* pollen grains using serum samples from allergic patients displaying specific IgE to *Parietaria* and *Urtica* after 10 minutes of hydration. Abundant labeling was observed in the apertural wall (exine [ex] and intine) and in the material exuded by the pollen grain. o indicates oncus; arrow, exudates. Bar = 0.5 μ m. Reprinted with permission from Vega-Maray et al.¹⁵

tural pectin fraction of the intine undergoes rapid dilation and gelation, facilitating soluble protein diffusion through this region.^{19,46} So, the immunolabeling located in the apertural and nonapertural pollen walls after 10 minutes of hydration with the patient's sera is consistent with the role of the exine and intine in the cell.

CONCLUSION

Par j 1, the major allergen of the *P judaica* pollen grain, does not seem to be present in *U dioica* pollen, although the 2 species belong to the same family (Urticaceae) and are morphologically similar. This study confirms the nonexistence of cross-reactive antigens between *P judaica* and *U dioica* pollen grains. The proteins recognized by the IgE antibodies in serum samples from allergic patients in *U dioica* pollen grains are less abundant than those observed in *P judaica* pollen, and this agrees with the lowest allergenic potential of *U dioica* to induce respiratory diseases.

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