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Morphology of the seed coat of *Oxalis* spp. from Buenos Aires Province (Argentina)

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Abstract: The aim of the present study is to provide detailed morphological descriptions of the seed coat in 10 species of *Oxalis* from Buenos Aires Province, Argentina, using light microscopy and scanning electron microscopy. The seed is ovate and brown, and its surface is smooth. *Oxalis* has a mechanism of spermobolia by which a part of the seed coat is detached by the rupture of cells, leaving exposed a layer of opened cells, generally with oxalate crystals. This new coat surface has different characteristics in the species studied in this work. Three types are recognized, mostly on the basis of anatomical and ultrastructural characteristics of the seed coat. The species grouped in each of the 3 types of seeds belong to different sections of the genus; thus, the seed morphology could be of taxonomic value for the genus *Oxalis*.

Key words: Seed, *Oxalis*, spermobolia, seed coat, classification

1. Introduction

The cosmopolitan genus *Oxalis* L. comprises about 800 species. Previous studies on seed morphology in this genus are meager (Bouman, 1974; Corner, 1976; Boesewinkel, 1988; Werker, 1997) and most of them are related to the mechanism of self-dispersion or spermobolia characteristics of the genus *Oxalis* (Bouman, 1974; Schoenberg, 1985; Werker, 1997).

When the seed matures, the outer layer of the seminal cover is detached violently due to the action of opposing forces within the seed coat. The rupture is attributed to the interplay of forces between a thick elastic cuticular membrane and subepidermal parenchymatic cells, which become highly turgid due to the degradation of starch immediately after maturation of the seed (Schoenberg, 1985).

Seed characters have often been used for taxonomic and phylogenetic studies in several genera and families of angiosperms (Barthlott, 1981, 1984; Takahashi, 1993; Akbari and Azizian, 2006; Fagúndez and Izco, 2011; Bona, 2013; Mostafavi et al., 2013). Corner (1976) and Kumar and Sing (1990) noted the importance of the morphology of the seed coat to solve problems of classification and phylogenetic considerations. However, there are no detailed studies on the morphology and anatomy of the seminal cover of different species of the genus *Oxalis* so far.

A general revision of the genus *Oxalis* was carried out by Lourteig (2000). Oberlander et al. (2011) studied the molecular phylogenetics and origins of the southern African species of this genus. The southern American *Oxalis* lineage is systematically poorly understood so far. The American bulb-bearing *Oxalis* taxa included in the sections *Ionoxalis* Small, *Pseudobulbosae* Norlind, *Palmatifoliae* DC., and *Articulatae* Knuth. have been recently studied (Gardner et al., 2012). These authors concluded that the bulb-bearing *Oxalis* comprise 2 clades: the first distributed in SE South America and the other in the Andes and North America.

In the present study, the anatomy and the ultrastructure of the seed coat of 10 species of *Oxalis* included in 3 different sections of the genus (*Articulatae*, *Corniculatae* DC., and *Ionoxalis*) were examined. The selected species are all native from Buenos Aires Province (Argentina). The aim of this study is to contribute relevant data for future morphologic phylogenies that help to clarify problems of the generic taxonomy.

2. Materials and methods

The seeds of the studied species were collected from natural populations growing in the field. Voucher specimens of the species, listed in an alphabetic order, were deposited in Darwinion Institute Herbarium (SI), San Isidro, Buenos Aires Province, Argentina.

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Oxalis articulata Savigny: Argentina. Ciudad Autónoma de Buenos Aires. Ciudad Universitaria. Nuñez. 10/2000. Rosenfeldt, S.1.

Oxalis conorrhiza Jacq.: Argentina. Córdoba. Colón. Río Ceballos. 1/2003. Rosenfeldt, S. 4; Argentina. Ciudad Autónoma de Buenos Aires. Ciudad Universitaria. 2/2004. Rosenfeldt, S. 8.

Oxalis corniculata L. var. ***corniculata***: Argentina. Ciudad Autónoma de Buenos Aires. Ciudad Universitaria. 10/2003. Rosenfeldt, S. 10.

Oxalis corniculata L. var. ***atropurpurea*** Planch.: Argentina. Buenos Aires. Escobar. 10/2004. Rosenfeldt, S. 15.

Oxalis floribunda Lehm.: Argentina. Entre Ríos. Concepción. 11/2003. Rosenfeldt, S. 12.

Oxalis hispidula Zucc.: Argentina. Ciudad Autónoma de Buenos Aires. Ciudad Universitaria. Nuñez. 5/2001. Rosenfeldt, S. 2.

Oxalis lasiopetala Zucc.: Argentina. Entre Ríos. Concordia. Parque San Carlos. 11/2003. Rosenfeldt, S. 6.

Oxalis niederleinii R.Knuth.: Argentina. Entre Ríos. Concordia. Parque San Carlos. 11/2004. Rosenfeldt, S. 9; Entre Ríos. Concordia. 11/2002. Rosenfeldt S. 3.

Oxalis paludosa A.St.-Hil.: Argentina. Entre Ríos. Concordia. Parque San Carlos. 11/2003. Rosenfeldt, S. 7.

Oxalis perdicaria Bertero: Argentina. Buenos Aires. Zárate-Campana. 4/2006. Rosenfeldt, S. 14.

Oxalis refracta A.St.-Hil.: Argentina. Buenos Aires. Isla Martín García. 12/2005. Rosenfeldt, S. 11.

For scanning electron microscopy (SEM), seeds from herbarium material were transferred to 100% acetone and then air-dried. The sputtering treatment was done with gold palladium for 3 min. Scanning micrographs were taken with a Philips XL 30.

For light microscopy, the seeds were prefixed overnight in 2.5% glutaraldehyde in phosphate buffer (pH 7.2) and then postfixed in 1.5% OsO_4 at 2 °C in the same buffer for 3 h. Following dehydration in acetone series, the material was embedded in Spurr's resin. Sections of 1 µm were stained with toluidine blue and observed with a Wild M20 microscope. The photomicrographs were taken with a Nikon microscope.

Seed sections were stained with Sudan Black B (Pearse, 1961) to localize lipids, periodic acid–Schiff (Jensen, 1962) to find insoluble polysaccharides, and Ruthenium red (Jensen, 1962) for pectins.

3. Results

The ovule is anatropous and bitegmic, and the micropyle is formed by both integuments.

Both integuments are composed of 3 to 5 layers of cells. The outer tangential wall of the outer epidermis cells is markedly thickened and the cuticle is relatively conspicuous.

Considering the anatomical characteristics of the testa and the tegmen, as well as the ultrastructure of the mature seed surface, 3 different types of seeds were identified.

3.1. Type I (*O. articulata*, *O. lasiopetala*, *O. floribunda*) (Figures 1–5)

3.1.1. Testa (outer integument)

The testa develops from localized cell divisions in the subepidermal layer of the outer integument after the ovule is fertilized. Therefore, this integument has a varying thickness in different areas in terms of number of cell layers. These divisions cause the future rumination of the mature seed (Figure 1).

In the mature seed, the cuticle of the outer epidermis of the testa (exotesta) presents a significant thickness compared to previous stages. Cells of the epidermis and subepidermal parenchymatic layers (mesotesta) have thin walls and are highly vacuolated. The cells of the inner epidermis of the testa (endotesta) have convex tangential walls toward the parenchymatic tissue of the mesotesta. A pectic homogeneous substance is observed within the endotesta cells, occupying 50% or more of its volume. Some of these cells have cubic crystals of calcium oxalate within the pectic matrix (Figure 1).

3.1.2. Tegmen (inner integument)

The cells of the outer epidermis of tegmen (exotegmen) develop lignified secondary walls and present a reduced lumen. These cells are elongated with more or less sharp ends in longitudinal sections of the seed. In cross-section, they are elliptic or isodiametric (Figure 1). The remaining layers of the inner integument of the ovule degenerate and only remains of them can be observed.

3.1.3. Outer surface

The seed is ovate and uniformly brown, and its surface is smooth. The outer layers of the seed coat are separated violently upon the seed's maturation. The removed layers are: exotesta, mesotesta, and the outer tangential wall of the endotesta cells (Figure 2). After separation of these layers of the seed coat, the seed presents crests and depressions with a new surface formed by endotesta cells lacking the outer tangential cell wall and containing a pectic matrix. This pectic deposit is observed with SEM in *O. articulata* (Figure 3) and *O. floribunda* (Figure 4) as a solid central matrix with trabeculae that join the central matrix with the radial walls of the cell. In *O. articulata* and *O. lasiopetala* the central matrix is not completely solid, and it has microspicules on its surface (Figures 3 and 5).

Some areas of the seed coat depressions have cells lacking the compact pectic deposit. These cells possess crystals of calcium oxalate only in *O. lasiopetala* (Figure 5).

3.2. Type II (*O. corniculata* L. var. *corniculata* and var. *atropurpurea*, *O. paludosa*, *O. conorrhiza*, *O. niederleinii*, and *O. refracta*) (Figures 6–9)

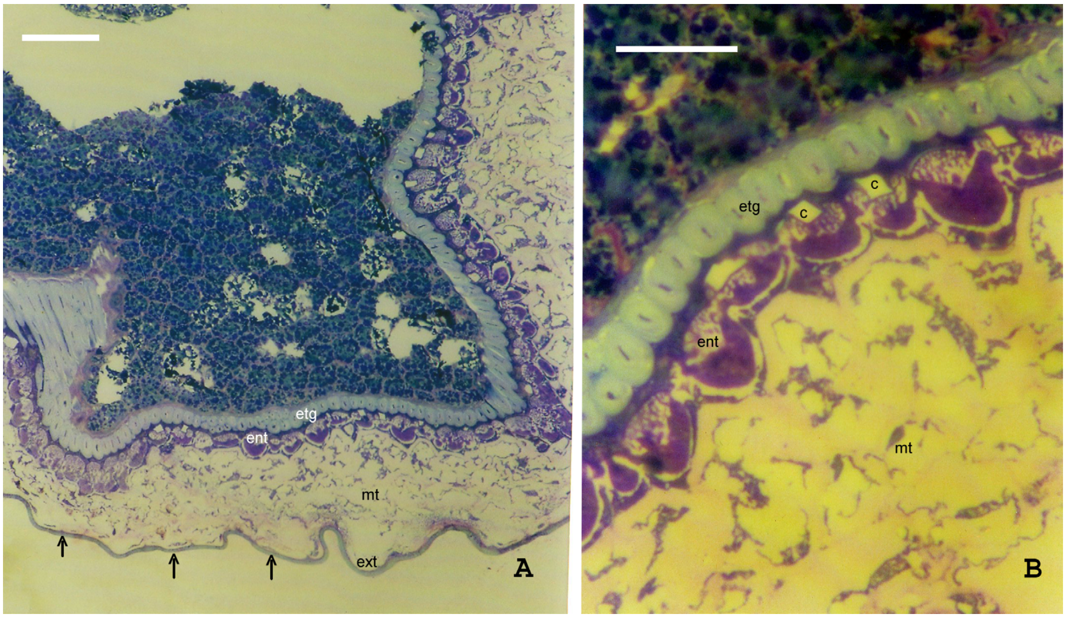

Figure 1. *Oxalis articulata*. Transversal sections of young seed observed with light microscope. A- General aspect (arrows showing cuticle), B- detail. Scale bars: A, B = 1 µm. Abbreviations: ext: exotesta; mt: mesotesta; ent: endotesta; etg: exotegmen; c: calcium oxalate crystals.

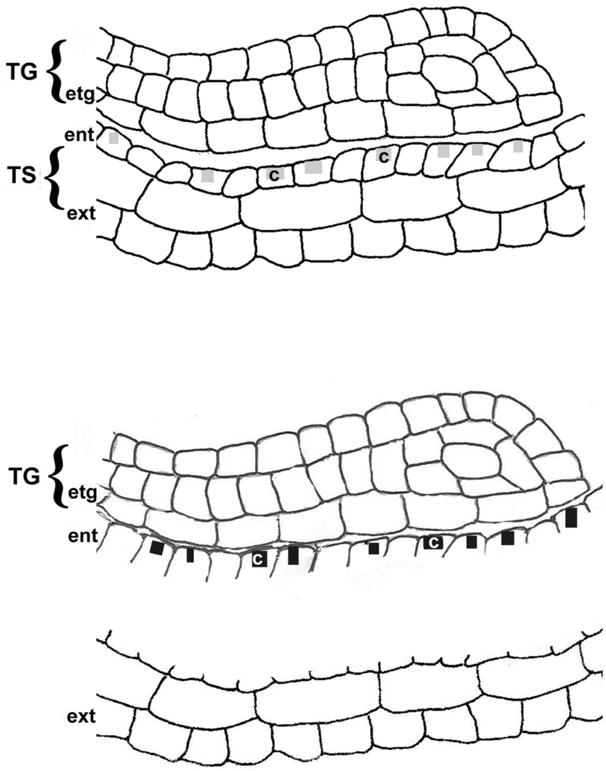

Figure 2. Model explaining the autochory. Abbreviations: TS: testa; TG: tegmen; ext: exotesta; ent: endotesta; etg: exotegmen; c: calcium oxalate crystals.

3.2.1. Testa

The testa develops from localized cell divisions in the subepidermal layer of the outer integument after the ovule is fertilized. These localized subepidermal cells increase their volume while the others remain thin in transection. This causes different thicknesses in the testa and hence the future rumination of the mature seed. In cross-section, in areas of reduced thickness of the coat, the endosperm of the seed is introduced substantially (Figure 6).

In the mature seed, the cuticle of the exotesta is remarkably thicker compared to previous stages (Figure 6). Cells of the mesotesta and endotesta have thin walls and are highly vacuolated.

The cells of endotesta have straight inner tangential walls toward the subepidermal tissue, unlike the type previously described. Within each of these cells, a cubic crystal of calcium oxalate can be observed, which occupies much of its volume (Figure 6).

3.2.2. Tegmen

The cells of the exotegmen develop lignified secondary walls, and the lumen is not reduced as in Type I (Figure 6). These cells are elongated with more or less sharp ends in longitudinal sections of the seed. In cross-section, they are elliptic. The remaining layers of the inner integument of the ovule degenerate and only remains of them can be observed.

3.2.3. Outer surface

The layers that are separated violently upon the seed's maturity are the same as in Type I: exotesta, mesotesta, and

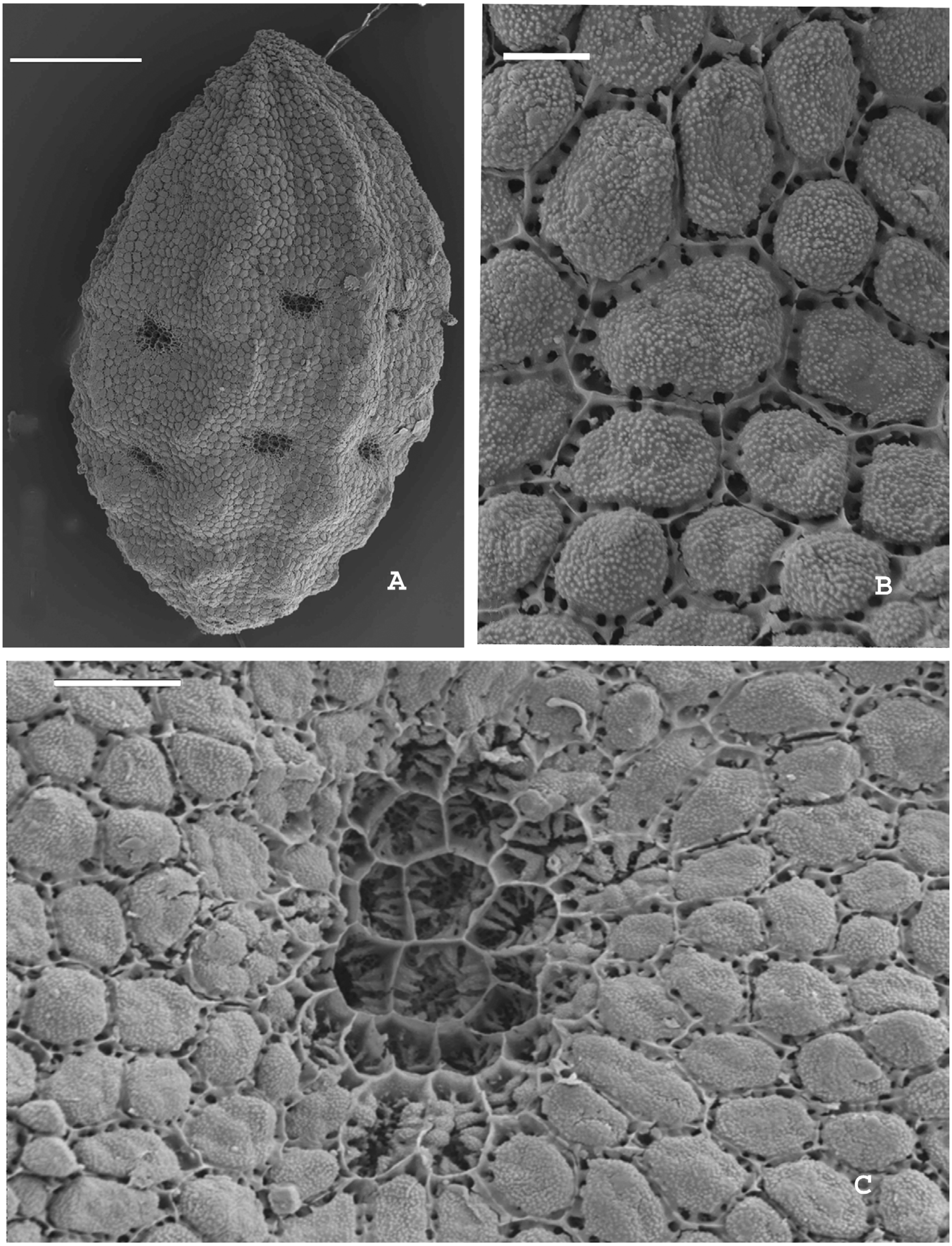

Figure 3. SEM images of mature seed surface. Type I. *O. articulata*. A- General aspect, B and C- seminal surface (endotesta) of removed outer layers, B- detail of cells with pectic substances, C- detail of an area of depression with cells without central pectic matrix. Scale bars: A = 200 µm; B = 10 µm; C = 20 µm.

the outer tangential wall of the endotesta. After separation of these layers, the seed has a new surface formed by endotesta cells lacking the outer tangential cell wall and containing a cubic crystal (Figures 7–9). The inner surface of these cells is smooth in some of them and microspiculate in others (Figure 8).

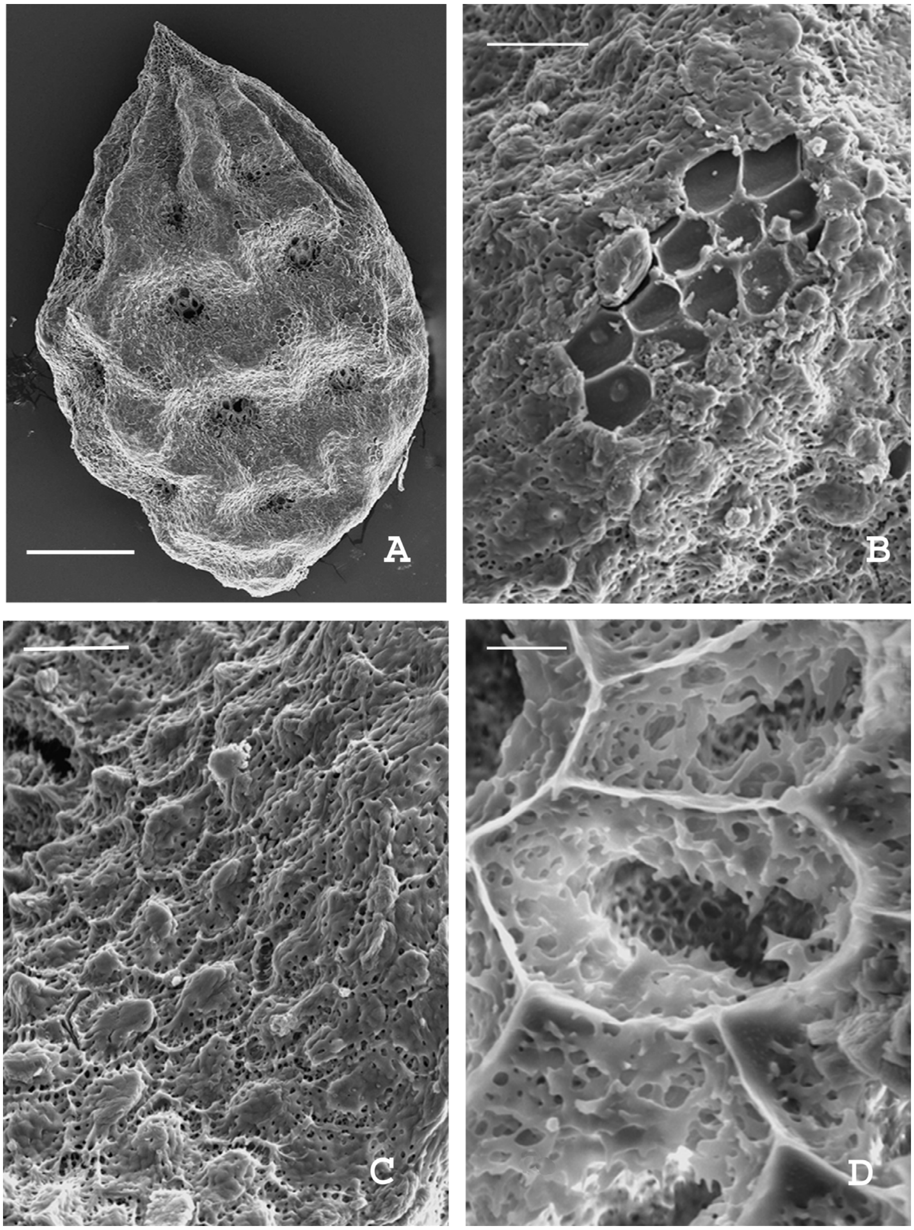

Figure 4. SEM images of mature seed surface. Type I. *O. floribunda*. A- General aspect; B, C, and D- details of seminal surfaces (endotesta) of removed outer layers; B- detail of crest cells; C and D- detail of depression cells. Scale bars: A = 200 µm; B, C = 20 µm; D = 0.5 µm.

Some areas of the seed coat have cells lacking crystals. Peripheral cells to these areas have small crystals. The size of the crystals increases gradually in more distant cells to these areas (Figures 7 and 8).

3.3. Type III (*O. hispidula*, *O. perdicaria*) (Figures 10 and 11)

3.3.1. Testa

The testa develops from localized cell divisions in the subepidermal layer of the outer integument after the ovule is fertilized. Therefore, this integument has a varying thickness in different areas in terms of number of cell layers, causing the future rumination of the mature seed.

The cuticle of the outer epidermis of the testa is rather thick. Cells of the epidermis and subepidermal parenchymatic layers have thin walls and are highly vacuolated.

In the mature seed, the cells of the endotesta have straight inner tangential walls towards the mesotesta. A pectic deposit occupies 50% or more of the cellular volume

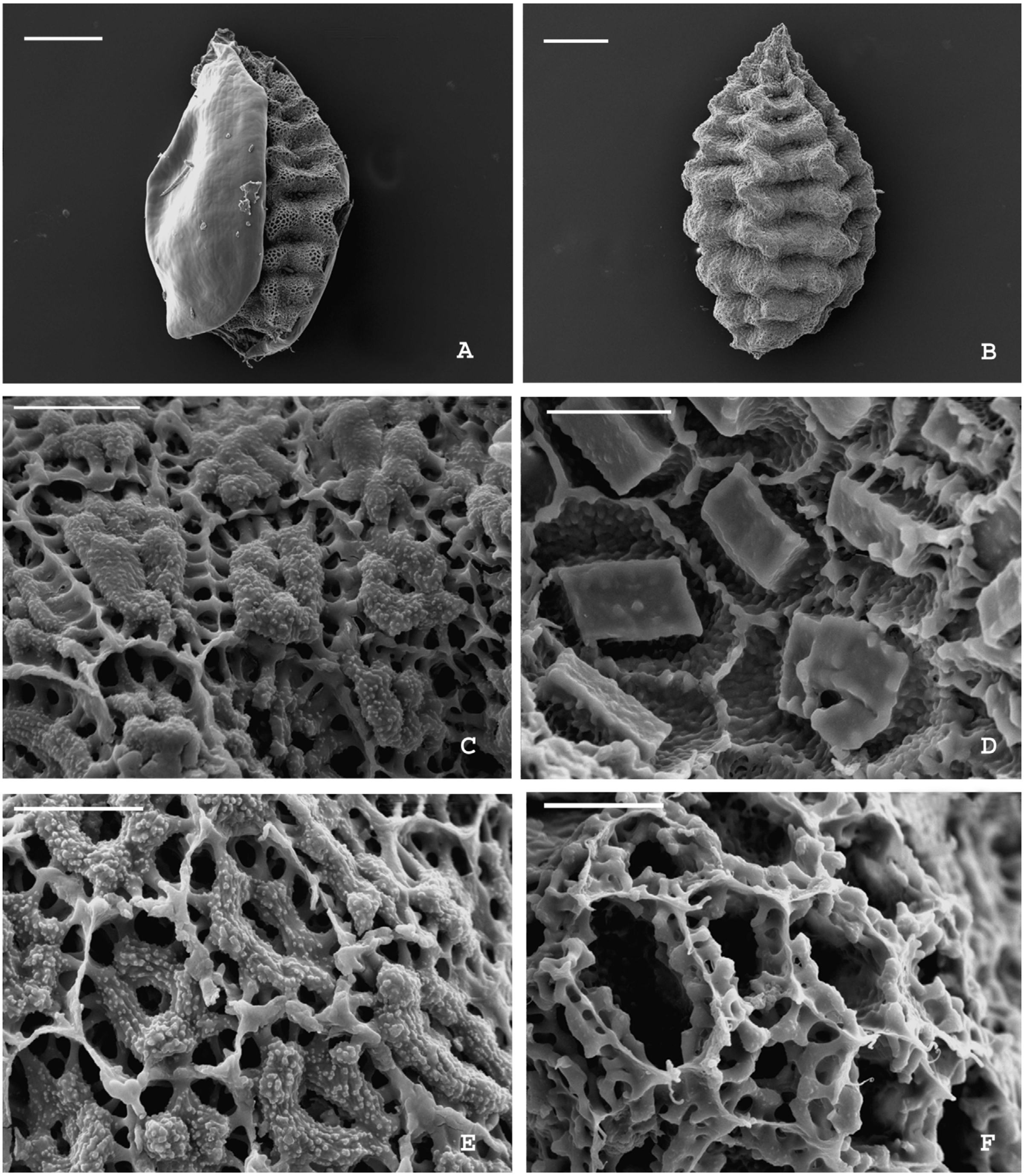

Figure 5. SEM images of mature seed surface. Type I. *O. lasiopetala.* A- General aspect showing the separation of the seed coat in 2 layers; B- seed coat with removed outer layers; C, D, E, and F- detail of various areas of the seminal surface (endotesta); C- cells between the crest and the depression; D- cells of the depression; E and F- cells of the crest. Scale bars: A, B = 20 µm; C–F = 10 µm.

of these endotesta cells. This deposit is more or less loose and irregular. None of these cells have calcium oxalate crystals (Figure 10).

3.3.2. Tegmen

The cells of the exotegmen develop lignified secondary walls (Figure 10). These cells are elongated with more or less sharp ends visible in longitudinal sections of the

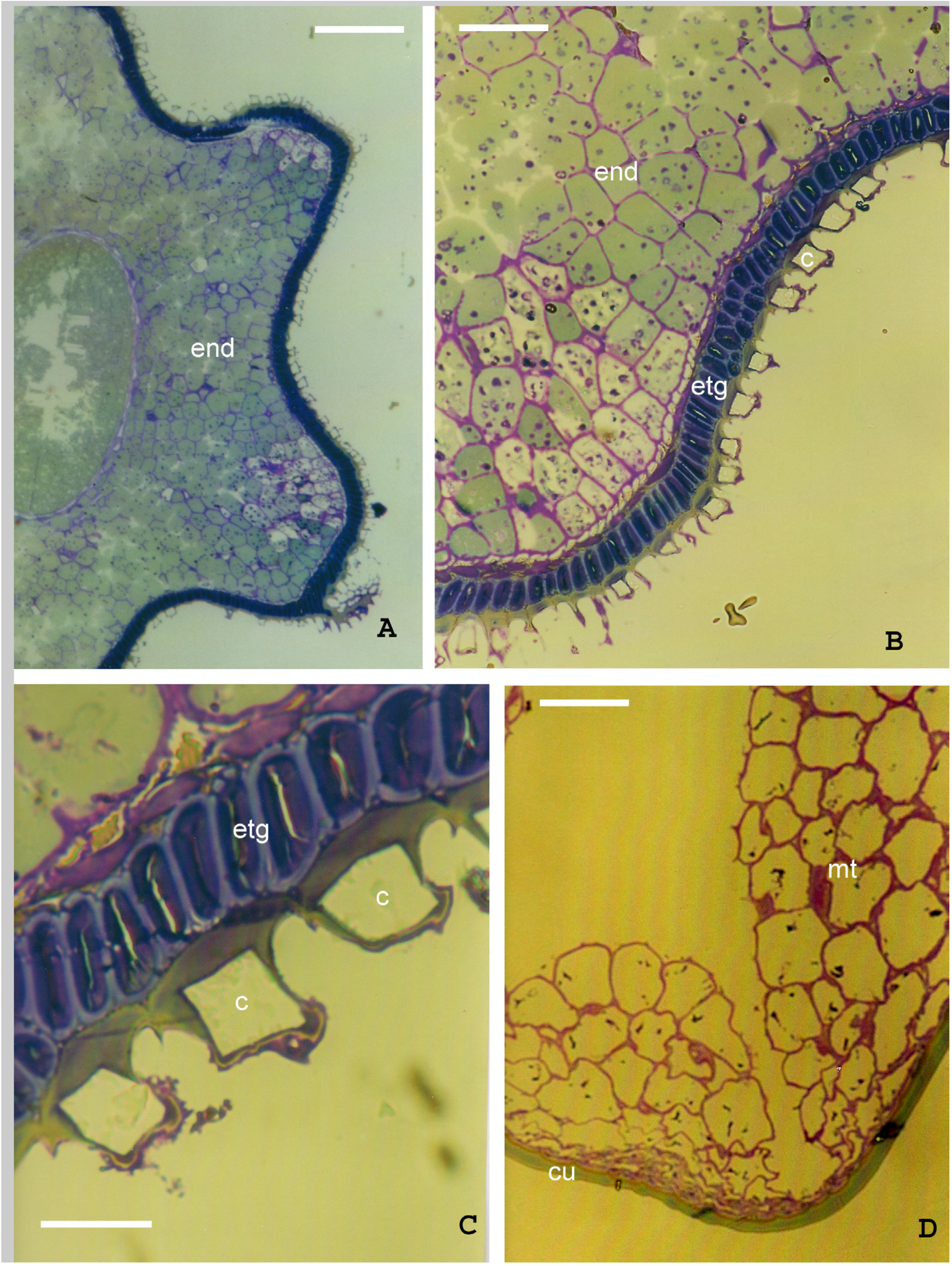

Figure 6. Seed transversal sections observed with light microscope. *O. niederleinii*. A- General aspect of seed without the outer layers of the testa, B and C- details of A, D- detail of the outer layers of the testa (exo- and mesotesta). Abbreviations: mt: mesotesta; end: endosperm; etg: exotegmen; c: calcium oxalate crystals; cu: cuticle. Scale bars: A, B, D = 1 µm; C = 5 µm.

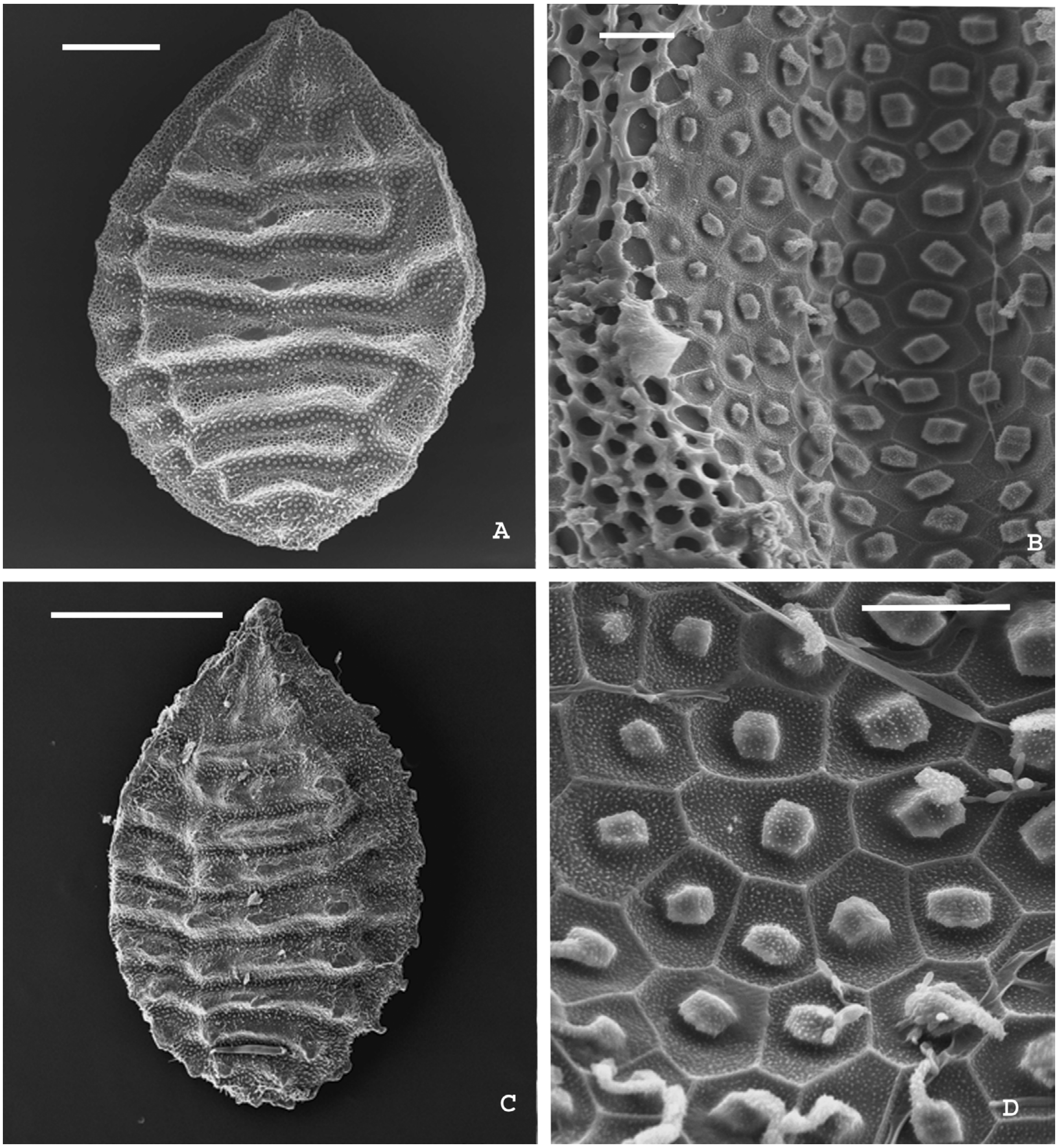

Figure 7. SEM images of mature seed surface. Type II. *O. corniculata*. A and B- *O. corniculata* var. *corniculata*, A- general aspect, B- detail of seminal surface (endotesta), C and D- *O. corniculata* var. *atropurpurea*, C- general aspect, D- detail of seminal surface (endotesta). Scale bars: A = 200 µm; B and D = 20 µm; C = 500 µm.

seed. In cross-section, they show approximately circular contours (Figure 10). The remaining layers of the inner integument of the ovule degenerate and only remains of them can be observed.

3.3.3. Outer surface

The same layers as in the previous types are separated violently upon maturation of the seed. After separation of these layers of the seed coat, the new surface of the seed is formed by cells lacking the outer tangential cell wall and containing a granular pectic deposit (Figure 11).

4. Discussion

Seeds of the 10 species of *Oxalis* studied in this work were grouped into 3 distinct types. These types were established according to the following characters: the presence or absence of calcium oxalate crystals in the endotesta, pectic deposit morphology of endotesta cells, and characters of the exotegmen fibers. The 3 seed types coincide with the sectional classification of the genus proposed by Lourteig (2000) (Table).

Bahadur et al. (1983) studied the seed morphology of 5 species of *Oxalis*; 2 of them belonged to the section *Corniculatae*, while the others belonged to other sections

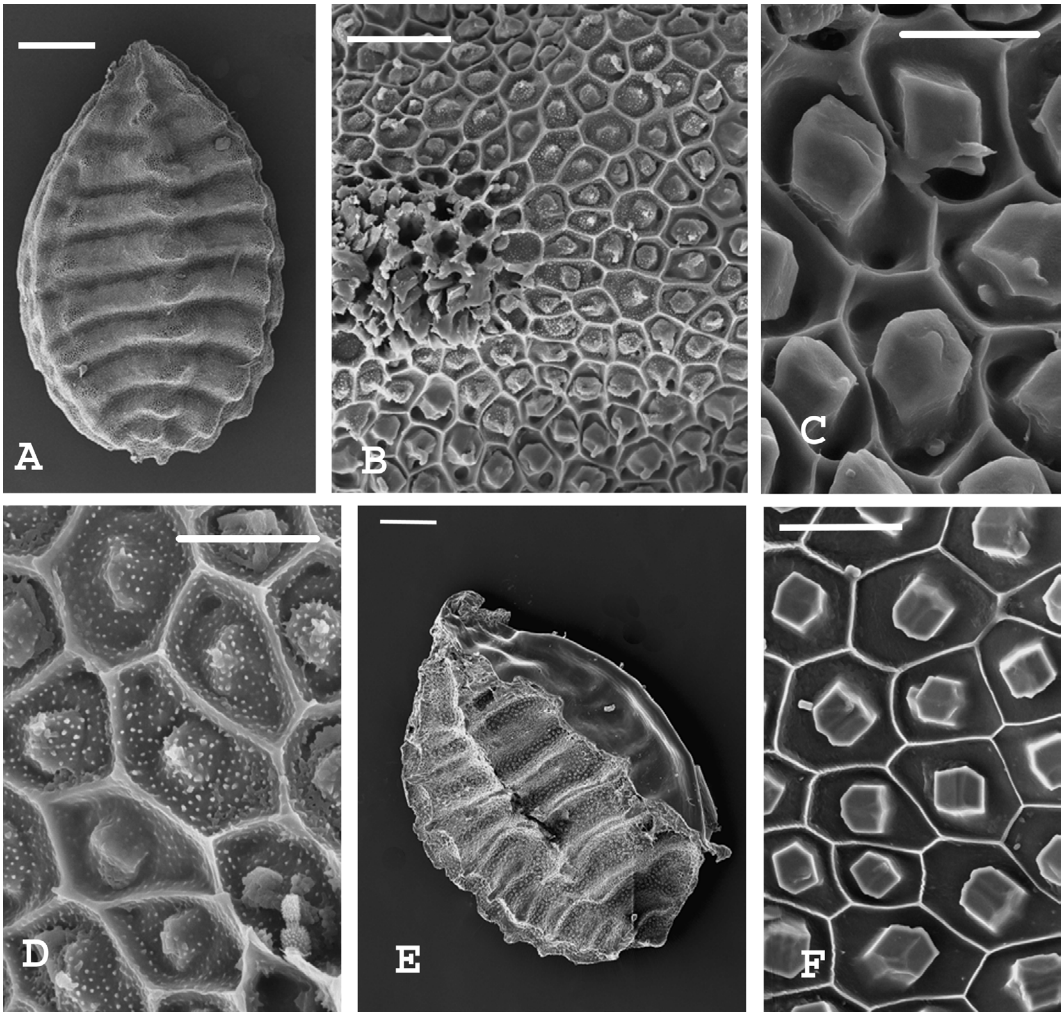

Figure 8. SEM images of mature seed surface. Type II. A, B, C, and D- *O. paludosa*; A- general aspect; B, C, and D- detail of various seminal surface areas (endotesta); B- cells of the crest and the depression; C- cells of the depression; D- cells of the crest; E and F- *O. conorrhiza*; E- general aspect of the seed with remains of outer layers of the testa; F- detail of cells of the crest (endotesta). Scale bars: A = 500 µm; B, F = 20 µm; C, D = 10 µm; E = 200 µm.

that were not studied in this work. The species of the section *Corniculatae* studied by these authors have a seed morphology matching the characteristics of the Type II seeds described in this study. The aforementioned publication and our own results present preliminary data of *Oxalis* seed coat morphology. More species from different sections must be studied to consider the taxonomic value of the characteristics of the seed coat.

All seeds of *Oxalis* are ruminate. This rumination is caused by localized cell divisions in the subepidermal layers of the outer integument after fertilization, generating the localized intrusion of the seminal cover towards the endosperm. According to the classification of types of rumination given by Periasamy (1962), the *Oxalis* seed corresponds to the *Annona* type and not to the *Passiflora* type as previously described by Werker (1997). In the *Passiflora* type, the rumination is a consequence of the radial extension of a few cells of the ovule integuments.

The seeds of *Oxalis* are exotegmic. This means that the outer epidermis cells of the tegmen develop mechanical characters. These cells correspond to the fibrous type according to Boesewinkel and Bouman (1984), with cells elongated in the direction of the longitudinal axis of the seed.

The endotesta cells have cubic crystals of calcium oxalate in all species except those belonging to Type III, *O. hispidula* and *O. perdicaria*. This is the first report on the absence of calcium oxalate crystals in seeds of species of the family Oxalidaceae.

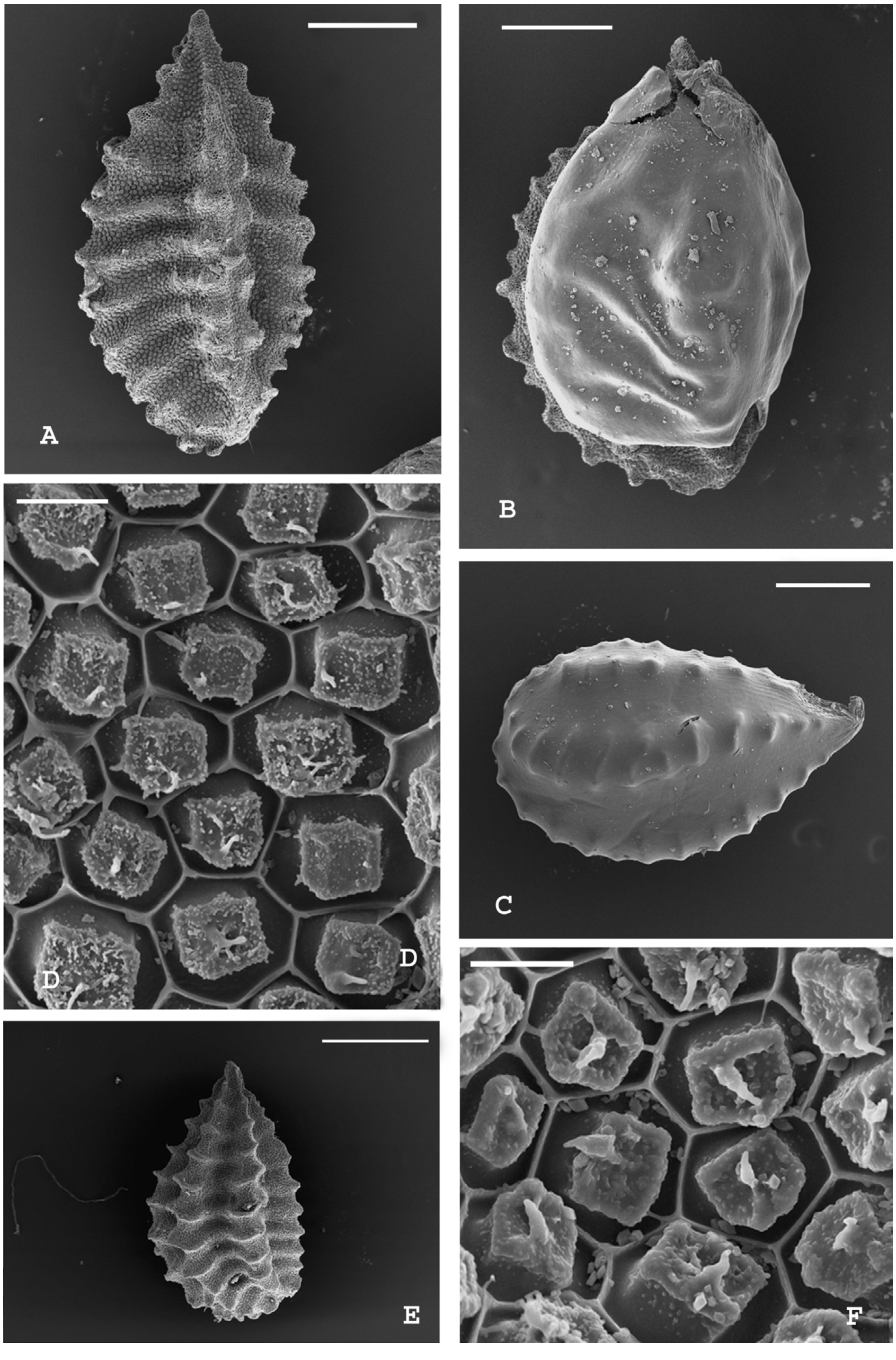

Figure 9. SEM images of mature seed surface. Type III. A, B, C, and D- *O. niederleinii*; A- general aspect of seed without the outer layers of the testa; B- general aspect showing the detachment of the outer layers of the seed coat; C- general aspect of the seed before of the detachment of the outer layers of the seed coat; D- detail of seminal surface (endotesta); E and F- *O. refracta*; E- general aspect of seed without the outer layers of the testa; F- detail of seminal surface (endotesta). Scale bars: A, B, C = 500 µm; D, F = 20 µm; E = 1000 µm.

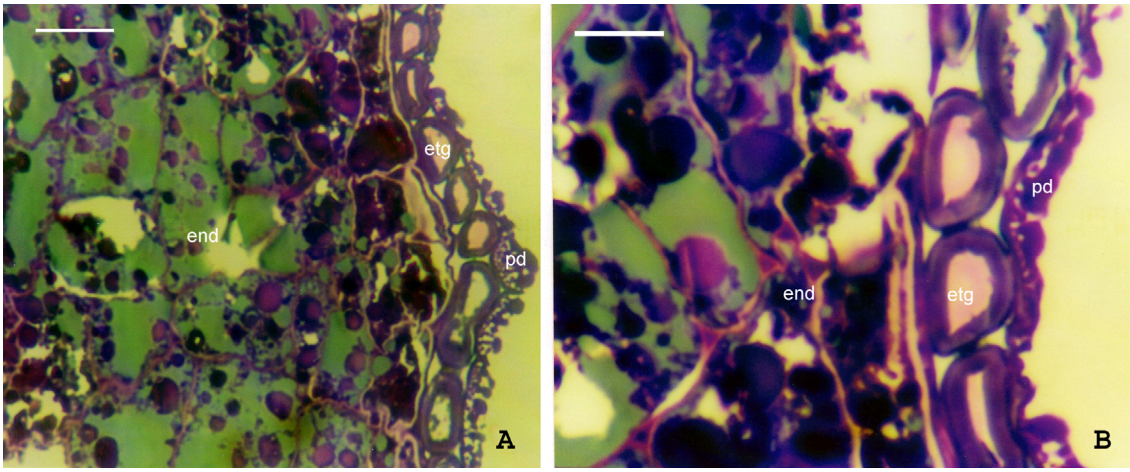

Figure 10. *O. hispidula*. Seed transversal sections observed with light microscope. A- General aspect, B- detail. Abbreviations: pd: pectic deposit; etg: exotegmen; end: endosperm. Scale bars: A, B = 1 µm.

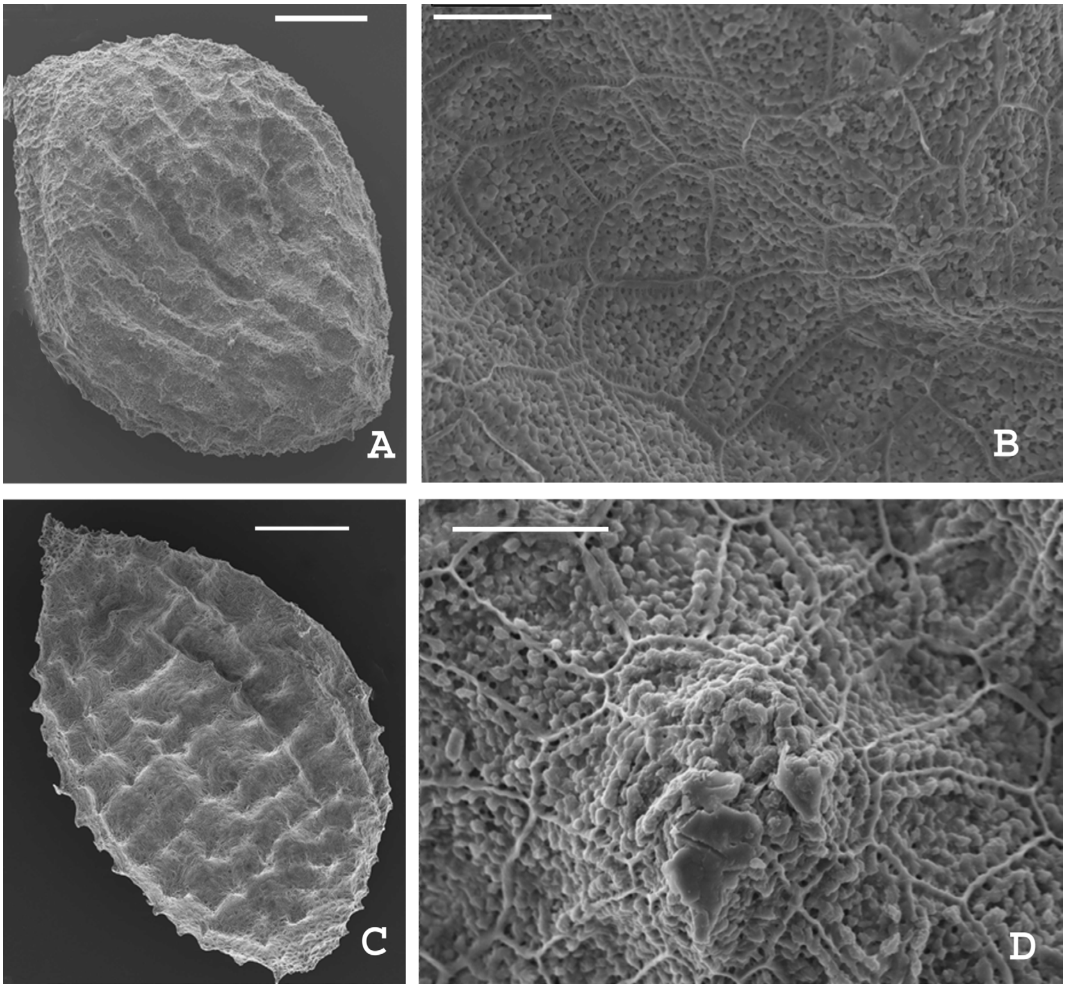

Figure 11. SEM images of mature seed surface. Type III. A and B- *O. perdicaria*, A- general aspect, B- detail of seminal surface (endotesta), C and D- *O. hispidula*, C- general aspect, D- detail of seminal surface (endotesta). Scale bars: A, C = 200 µm; B, D = 20 µm.

Table. Types of *Oxalis* seed.

Type	Ovule		Seed					Section and taxa
	Number of layers of the integuments		Testa			Tegmen	Mature seed surface	
	Outer	Inner	Localized Subepidermal cell divisions (mesotesta)	Exotesta	Endotesta	Exotegmen		
I	3–5	5	Yes	Cells with thin walls and highly vacuolated. Thick cuticle.	Compact pectic substance into the cells, occupying 50% or more of their volume. Convex inner tangential wall. Occasional presence of 1 or more cubic crystals of calcium oxalate per cell.	Elongated cells in longitudinal section and isodiametric in transversal section with reduced lumen. Secondary walls highly lignified.	More or less solid deposit of pectic material with microspicules on its surface. Occasional presence of cubic crystals of calcium oxalate.	Articulatae *O. articulata*, *O. lasiopetala*, *O. floribunda*.
II	3–4	3–4	Yes	Cells with thin walls and highly vacuolated. Thick cuticle.	Straight inner tangential wall. Presence of cubic crystals of calcium oxalate.	Elongated cells in longitudinal section and isodiametric in transversal section with more or less reduced lumen. Secondary walls highly lignified, sometimes differentiate in 2 strata.	Cubic crystals of calcium oxalate sometimes embedded in a matrix of pectic material.	Corniculatae *O. paludosa*, *O. conorrhiza*, *O. corniculata* L. var. *corniculata*, *O. corniculata* L. var. *atropurpurea*, *O. niederleinii*, *O. refracta*.
III	3–4	3–4	Yes	Cells with thin walls and highly vacuolated. Thick cuticle.	Loose pectic substance into the cells, occupying 50% or more of their volume. Straight inner tangential wall. Without crystals of calcium oxalate.	Elongated cells in longitudinal section and circular contour in transversal section. Secondary walls highly lignified.	Granular deposit of pectic material. No crystals of calcium oxalate.	Ionoxalis *O. hispidula*, *O. perdicaria*.

The presence of crystals in the seed coat is fairly common (Werker, 1997). In the genus *Oxalis*, these crystals are located in the endotesta. However, it appears that the more common location is in the exotesta or the mesotesta (Werker, 1997). According to Bouman (1974, in Werker, 1997) the role of the crystals is related to the separation into 2 layers of the seed coat at the time of seed dispersal. This separation occurs at the endotesta by the rupture of the outer tangential wall of their crystalliferous cells. However, several species have no crystals in some of these cells, and, further, the endotesta cells lack crystals in all species belonging to Type III. Moreover, in some species, like *O. floribunda*, the crystals are smaller and do not occupy the entire cell lumen. In all these cases, a dense pectic deposit occupies most of the cell volume. This substance could replace the crystals in their mechanical role. The rigid pectates in the dry seed could act as the crystals allowing the separation into 2 layers of the seed coat by rupture of the outer tangential wall of the endotesta cells.

The seeds are dispersed by autochory. *Oxalis* is one of the few genera of angiosperms in which this mechanism involves only the seed. Upon the seed's maturation, the parenchyma cells of the testa markedly increase their volume, generating a tension that cannot be compensated by a thick and elastic cuticular membrane. Excessive stretching of the latter, which finally returns to its original state, results in the violent rupture and separation of the outer layers of the testa. This very special mechanism for seed dispersal of *Oxalis* was newly described by Schoenberg (1985) as spermobolia. In the present paper, the anatomical details for understanding this mechanism of dispersion were described for 10 species of the genus. Their spermobolia was considered a unique case, as is so far known, in which the seed is deprived of a part of its cover in order to disperse the remaining seminal unit containing the embryo.

Acknowledgments
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