

Pollen morphology of *Oxalis* species from Buenos Aires province (Argentina)

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ABSTRACT: Pollen morphology and the degree of pollen variability within nine species and two varieties of *Oxalis* species from Buenos Aires Province (Argentina) were studied using light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Oxalis pollen grain is generally 3-colpate and the shape is prolate spheroidal, oblate spheroidal or spheroidal. The exine is microreticulate. The brochi are circular to polygonal; brochi sizes decrease near the colpi. The colpus membrane observed in unacetolysed material, is crustate or granular. The colpus length varies within a species; the largest grains are produced in stamens with long filaments and the smallest ones in stamens with short filaments.

Four pollen types are distinguished by means of LM, SEM and TEM. They are recognized mostly on the basis of their colpus membrane structure.

In conclusion, the morphological similarity among *Oxalis* species is also reflected in their pollen morphology.

Introduction

The genus *Oxalis* L. comprises about 800 cosmopolitan species.

Previous studies of pollen morphology in this genus are meagre and they were conducted using only light microscopy (Erdtman, 1952; Huynh, 1969 a,b) or SEM (Cerceanu, 1995). Most of these reports analysed the formation of aberrant pollen. According to the apertures number and position, seven categories of grain aberrations and two succesiform series were identified by Dreyer and Van Wyk (1998) in Southern African *Oxalis*. Ghosh and Verma (1985) recognized three types

of pollen in *O. corymbosa* DC. and *O. dehradunensis* Raizada, and two types in *O. corniculata* Linn. Pollen morphology of species of this genus from Pakistan and Taiwan (Yueyang Lake) was studied by Perveen and Kaiser (2003) and Wang and Chen (2001), respectively.

The aim of the present study was to provide detailed pollen morphological and structural descriptions and to analyse the degree of pollen variability within *Oxalis* species from Buenos Aires Province (Argentina) using LM, SEM and TEM.

Materials and Methods

This study is based on materials of nine species and two varieties of *Oxalis* collected from Buenos Aires Province and Buenos Aires city, Argentina. The taxa are: *O. articulata* Savigny (SI 27974); *O. lasiopetala* Zuccarini (SI 1673); *O. floribunda* Lehmann (SI 28745);

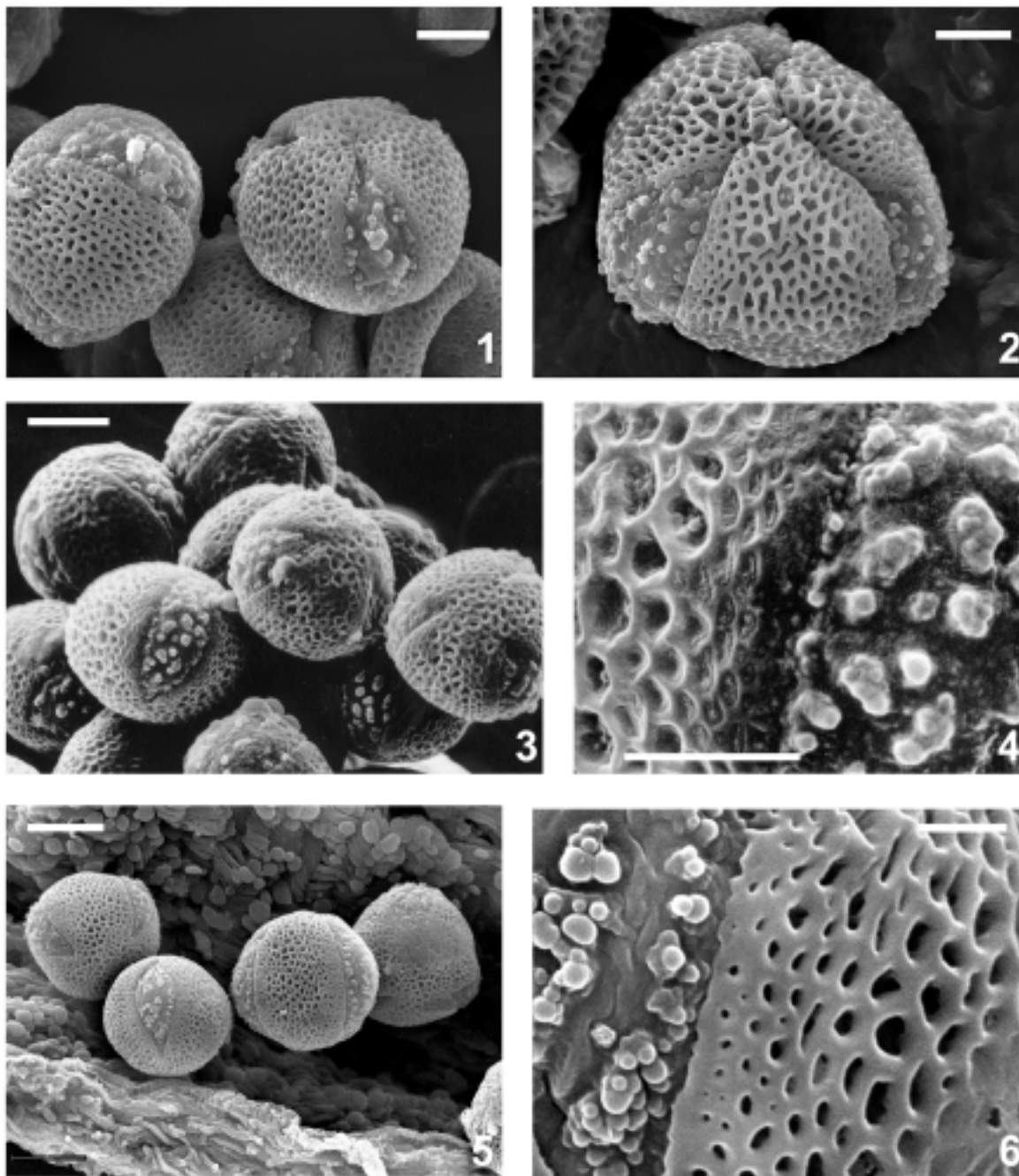
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O. paludosa Saint Hilaire (SI 1764); *O. conorrhiza* Jacqui (SI 2075); *O. corniculata* L. var. *atropurpurea* Planchon (CTES 43177); *O. corniculata* L. var. *corniculata* (SI 555); *O. hispidula* Zuccarini (SI 1345); *O. perdicaria* Bertero (SI 20045); *O. debilis* Humboldt, Bonpland et Kunth (SI 2015); *O. niederleinii* Knuth (SI 1042).

For scanning electron microscopy (SEM), anthers fixed in FAA (formalin, alcohol, acetic acid) were transferred to 100° acetone and then, air dried. For exine sections, anthers were embedded in paraffin wax. Sections of 3 µm thick were transferred to xilene for ten min and air dried.



FIGURES 1-6. Type I. SEM photographs of pollen grains of *Oxalis* species. Figs. 1-2. *O. floribunda*: Fig. 1. Polar and equatorial views, Fig. 2. Detail of polar view. Figs. 3-4. *O. articulata*: Fig. 3. Polar and equatorial views, Fig. 4. Detail of colpus and exine. Figs. 5-6. *O. lasiopetala*: Fig. 5. Polar and equatorial views, Fig. 6. Detail of colpus and exine. Scale bars: Figs. 1, 2=5µm; Fig. 3=10µm; Fig. 4= 5 µm; Fig. 5=10µm and Fig. 6=1,5µm.

The sputtering treatment was made with gold-palladium for 3 min. Scanning micrographs were taken with a Philips XL 30 microscope.

For transmission electron microscopy (TEM) studies, the material was pre-fixed in 2,5% glutaraldehyde in phosphate buffer (pH 7,2) for 2 h and post-fixed in OsO_4 at 2°C in the same buffer for 3 h. Then, it was dehydrated in ethanol series and embedded in Spurr's resin. Fine sections were made on a Sorvall ultramicrotome, stained with uranyl acetate and lead citrate (O'Brien and Mc Cully, 1981). The sections were observed and photographed in a JEOL - JEM 1200 EX II TEM at 85.0 Kv.

The palynological terminology used in this work is in accordance with Punt *et al.* (1994). Unacetolysed

pollen grains from mature but not dehiscent anthers of 10 flowers were mounted in glycerine and the pollen diameter was measured using a light microscope (LM) with a calibrated ocular micrometer.

Results

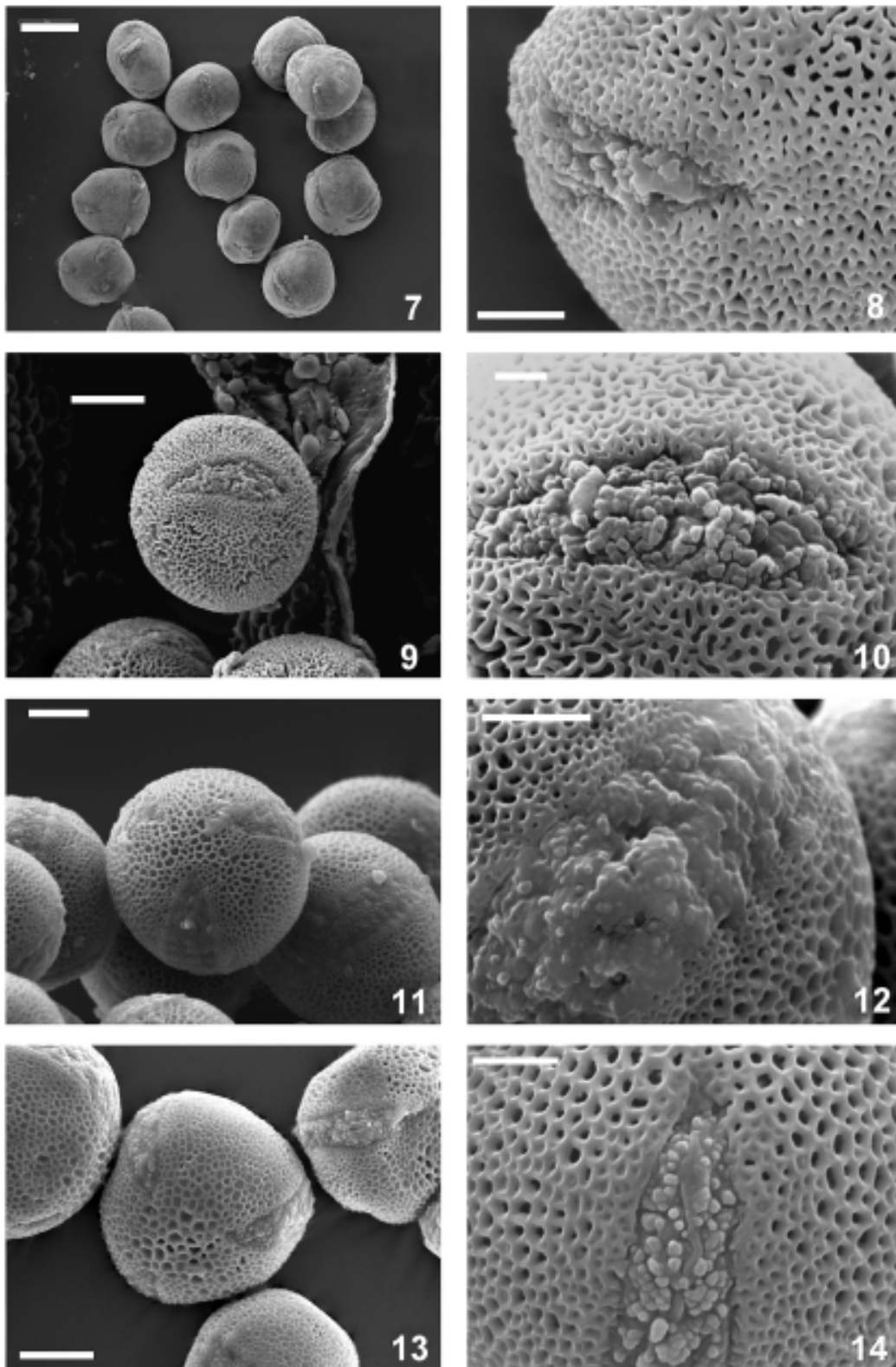
General morphology and structure

Oxalis pollen grain is generally 3-colpate and the shape is prolate spheroidal, oblate spheroidal or spheroidal. Amb rounded or triangular. The exine is microreticulate (Figs. 1-22). The brochi are circular to polygonal. The brochi sizes decrease near the colpi (Figs.

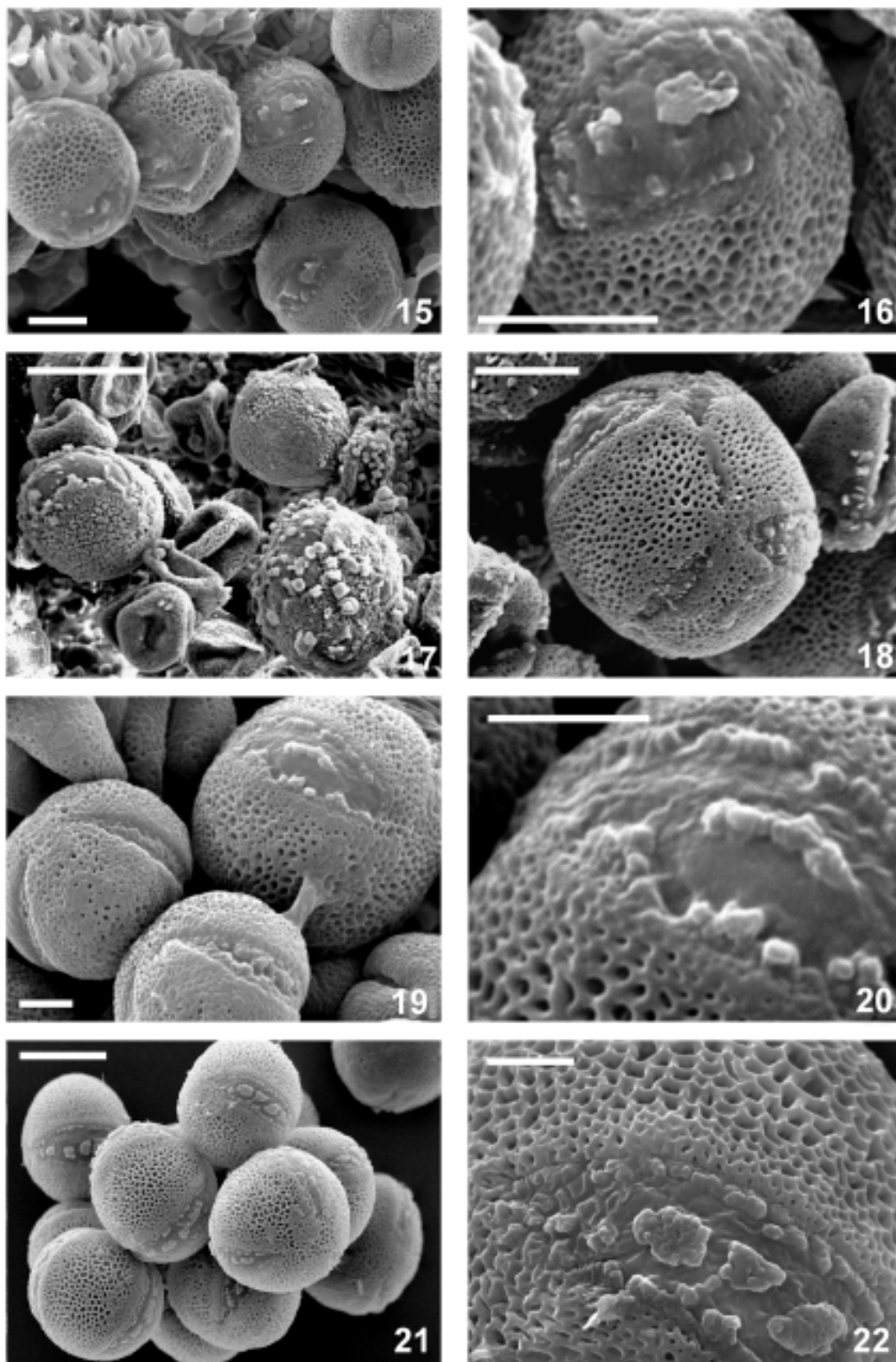
TABLE 1.

Characters in *Oxalis* species pollen

Species	Pollen grain size (μm)				Shape P/E P: polar diameter, E: equatorial diameter		Exine		Pollen Type (Sub-spheroidal)	
	Long Filament		Short Filament		Long Filament	Short Filament	Thickness (μm)	Structure	Long Filament	Short Filament
	P	E	P	E						
<i>O. articulata</i>	25.5	25.80	19.15	18.62	0.99	1.02	1.62-2.00	Semitectate Microreticulate	Spheroidal	Spheroidal
<i>O. floribunda</i>	33.25	35.91	23.14	18.62	0.92	0.88	0.90-1.10	Semitectate Microreticulate	Oblate Spheroidal	Oblate Spheroidal
<i>O. lasiopetala</i>	21.28	24.20	24.87	25.80	0.88	0.96	1.10-1.40	Semitectate Microreticulate	Oblate Spheroidal	Oblate Spheroidal
<i>O. paludosa</i>	27.53	35.11	27.26	29.26	0.78	0.93	1.20-1.60	Semitectate Microreticulate	Sub-oblate	Oblate Spheroidal
<i>O. conorrizha</i>	33.78	37.24	33.38	29.65	0.90	1.12	2.50-3.20	Semitectate Microreticulate	Oblate Spheroidal	Prolate Spheroidal
<i>O. corniculata</i>	34.98	36.84	36.17	38.57	0.88	0.93	1.50-1.83	Semitectate Microreticulate	Oblate Spheroidal	Oblate Spheroidal
<i>O. perdicaria</i>	29.79	31.12	23.00	24.07	0.96	0.96	1.33-1.66	Semitectate Microreticulate	Oblate Spheroidal	Oblate Spheroidal
<i>O. debilis</i>	29.12	28.86	29.65	24.47	1.00	1.21	0.80-1.60	Semitectate Microreticulate	Spheroidal	Sub-prolate
<i>O. hispidula</i>	36.84	40.43	35.64	33.11	0.91	1.07	3.16-3.50	Semitectate Microreticulate	Oblate Spheroidal	Prolate Spheroidal
<i>O. niederleinii</i>	48.81	49.60	41.23	45.48	0.98	0.91	1.50-1.83	Semitectate Microreticulate	Oblate Spheroidal or Spheroidal	Oblate Spheroidal



FIGURES 7-14. Type II. SEM photographs of pollen grains of *Oxalis* species. Figs. 7-8. *O. corniculata* var. *atropurpurea*: Fig. 7. Polar and equatorial views, Fig. 8. Detail of colpus and exine. Figs. 9-10. *O. corniculata* var. *corniculata*: Fig. 9. Equatorial view, Fig. 10. Detail of colpus and exine. Figs. 11-12. *O. conorrhiza*: Fig. 11. Polar and equatorial views, Fig. 12. Detail of colpus and exine. Figs. 13-14. *O. paludosa*: Fig. 13. Polar and equatorial views, Fig. 14. Detail of colpus and exine. Scale bars: Fig. 7=20 μ m; Fig. 8=4 μ m; Fig. 9=10 μ m; Fig. 10=2 μ m ; Fig. 11= 10 μ m; Fig. 12=05 μ m; Fig. 13=10 μ m and Fig. 14=4 μ m.



FIGURES 15-22. Types III and IV. SEM photographs of pollen grains of *Oxalis* species. Figs. 15-16. *O. perdicaria*: Fig. 15. Polar and equatorial views, Fig. 16. Detail of colpus and exine. Figs. 17-18. *O. debilis*: Fig. 17. Aberrant pollen grains, Fig. 18. Detail of a probable viable pollen grain. Figs. 19-20. *O. hispidula*: Fig. 19. Polar and equatorial views, Fig. 20. Detail of colpus and exine. Figs. 21-22. *O. niederleinii*: Fig. 21. Polar and equatorial views, Fig. 22. Detail of colpus and exine. Scale bars: Figs. 15, 16=10 μ m; Fig. 17=25 μ m; Fig. 18=10 μ m; Figs. 19, 20=4 μ m; Fig. 21=20 μ m and Fig. 22=4 μ m.

2,4,6,8,10,12,14,16,18,20,22). The colpus membrane is granular. The colpus length varies within a species. The largest grains are produced in stamens with long filament and the smallest ones in stamens with short filament (Table 1).

Four pollen types are distinguished by means of LM, SEM and TEM. They are recognised mostly on the basis of colpus membrane ornamentation :

Key to pollen types

- A Colpus membrane with irregularly scattered granules.....Type II
- AA Colpus membrane with granules arranged in a row on the margin.
- B Granules assembled in clusters irregularly scattered on the colpus membrane.....Type I
- BB Granules assembled in clusters aligned on the colpus membrane center.....C
- C Small clusters more or less aligned on the colpus membrane centerType III
- CC Conspicuous clusters extensively aligned on the colpus membrane center.....Type IV

Type I:

Oblate spheroidal or spheroidal pollen grain; 19.15-33.25 x 18.62-35.91 μm . (Figs. 1-6).

Colpus membrane granular. Small granules arranged in an irregular file on the colpus membrane margin. Granules more or less assembled in clusters irregularly scattered on the colpus membrane.

Exine 1.1-2 μm thick. Intine thinner than exine. Foot layer very thin to absent. Columellae variable in height, 0.30-1.87 μm . Endexine continuous but irregular in thickness (Figs. 23-25).

Taxa included: *O. articulata* Savigny, *O. lasiopetala* Zuccarini, *O. floribunda* Lehmann.

Type II:

Pollen grains oblate spheroidal to suboblate, rarely prolate-spheroidal; 27.26-36.17 x 29.26-38.57 μm ; rarely 6-colpate (*O. corniculata* var. *purpurea*) (Figs. 7-14).

Colpus membrane with granules irregularly and densely scattered.

Exine 1.2-3.2 μm thick. Intine almost as thick as exine or thicker. Foot layer thick as tectum, granular in contact with the intine in some cases (*O. corniculata*) (Fig. 26). Columellae variable in height, 0.2-1.2 μm . Endexine discontinuous to absent (Figs. 26-29).

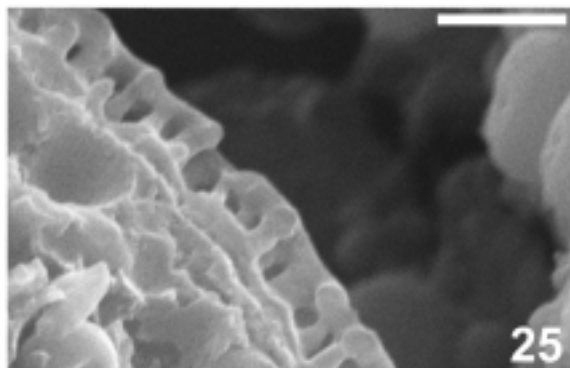
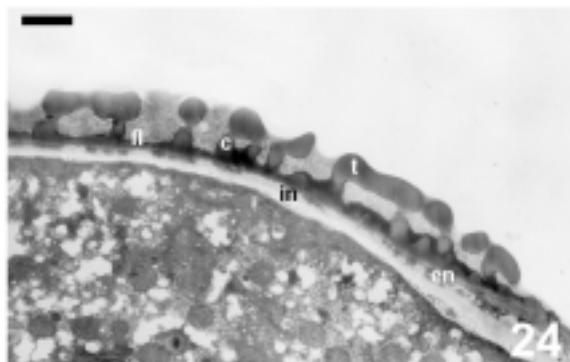
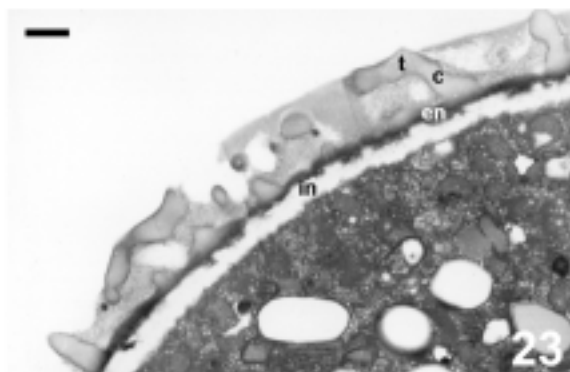
Taxa included: *O. paludosa* Saint Hilaire, *O. conorrhiza* Jacquin, *O. corniculata* L. var. *atropurpurea* Planchon and *O. corniculata* L. var. *corniculata*.

Type III:

Pollen grain oblate spheroidal to prolate spheroidal, rarely subprolate; 23-36.84 x 24.07-40.43 μm . (Figs. 15-20).

Colpus membrane with granules arranged in a file on the colpus margin and assembled in central small clusters (Figs. 16, 18, 20).

Exine 0.8-3.5 μm thick. Intine almost as thick as exine. Foot layer thinner than tectum. Columellae vari-



FIGURES 23-25. Type I. TEM photographs of pollen wall of *Oxalis* species. Section through wall showing tectum, columellae, foot layer, darkly stained endexine and intine white. Fig. 23. *O. articulata*. Fig. 24. *O. lasiopetala*. Fig. 25. SEM photograph of pollen wall section of *O. articulata*. c= columellae; en= endexine; in= intine; ft= foot layer; t= tectum. Scale bars: Figs. 23,24=1 μm and Fig. 25=1,5 μm .

able in height 0.28-1.66 μm . Endexine very thin and discontinuous (Figs. 30-32, 34).

Taxa included: *O. hispidula* Zuccarini, *O. perdicaria* Bertero, *O. debilis* Humboldt, Bonpland et Kunth.

In *Oxalis debilis* (Figs. 17-18) most of the pollen grain are anomalous or abortive. Pollen grains of this species are 8-12 colpate (Fig. 18).

Type IV:

Pollen grains oblate spheroidal to spheroidal, rarely subprolate; 41.23-48.8 x 45.48-49.6 μm . Amb rounded to triangular (Figs. 21-22).

Colpus membrane with granules arranged in a row on the colpus margin and assembled in central conspicuous clusters (Fig. 22).

Exine 1.50-1.83 μm thick. Intine almost as thick as exine. Foot layer thinner than tectum. Columellae very straight, arranged equally distant, variable in height 0.66-

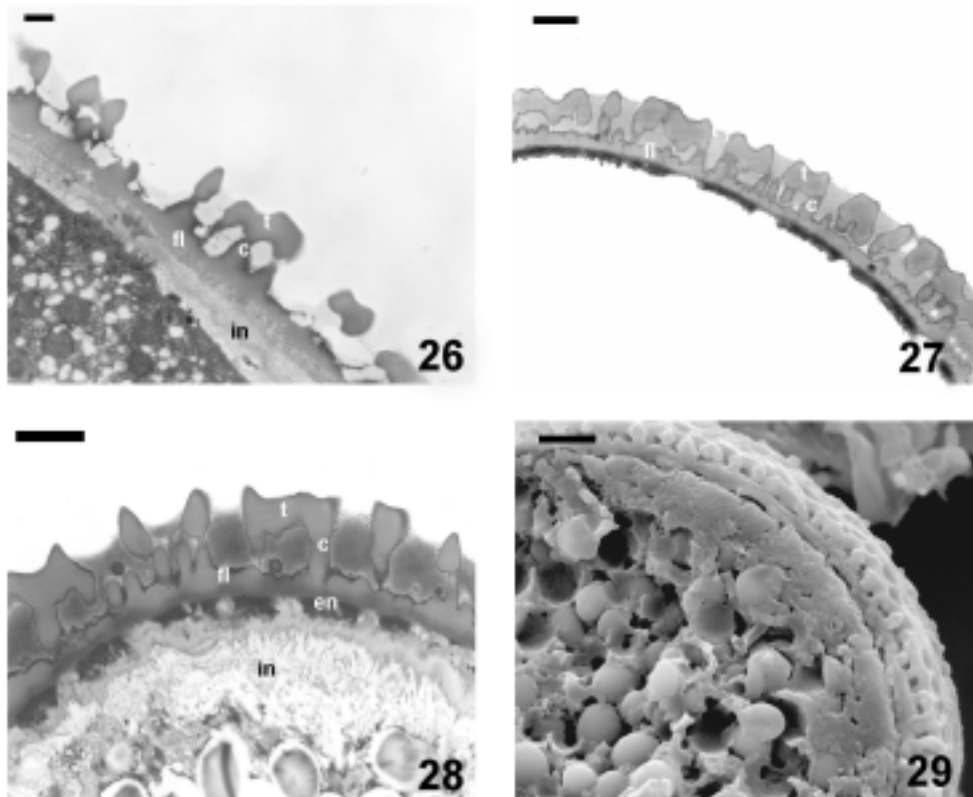
0.75 μm . Endexine very thin and discontinuous (Figs. 33, 35).

Taxa included: *O. niederleinii* Knuth.

Discussion

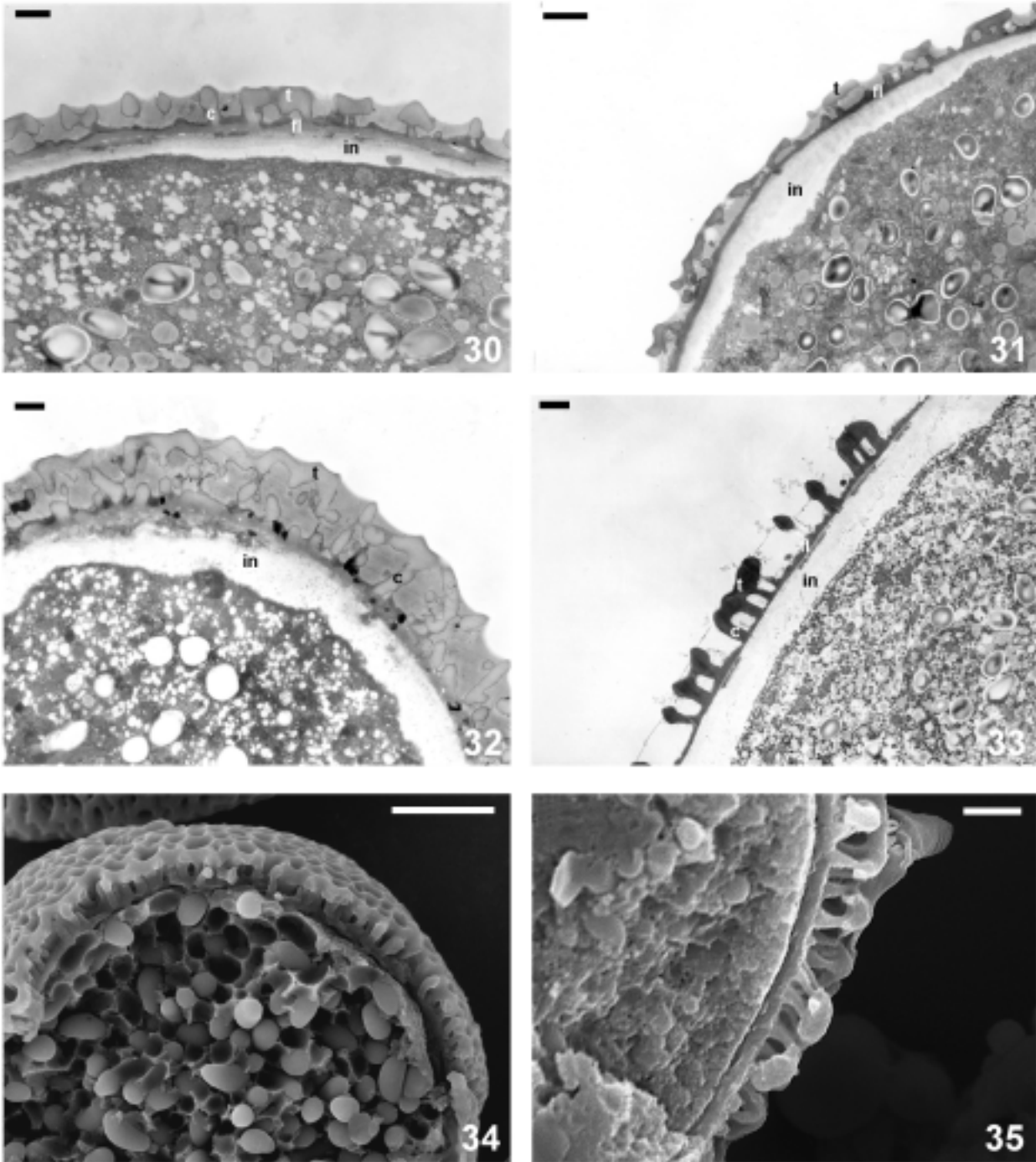
In the present study, four pollen types have been distinguished mainly on the basis of the colpus membrane ornamentation.

According to Huynh (1969a), pollen grains from 424 species of *Oxalis* can be ranged into 13 pollen-types. The species studied in this paper could be referred to the group A of the Huynh's classification according to the general morphology of the pollen grain. This author established types within each group; however, he didn't distinguish between the pollen of the long and short stamen filaments, and the sizes are too variable. For this reason, the pollen studied can not be referred to none of the Huynh's types.



FIGURES 26-28. Type II. TEM photographs of pollen grain wall of *Oxalis* species. Section through the wall showing tectum, columellae, foot layer, darkly stained endexine and intine white. Fig. 26. *O. corniculata* var. *corniculata*. Fig. 27. *O. paludosa*. Fig. 28. *O. conorrhiza*. Fig. 29. SEM photograph of pollen wall section of *O. conorrhiza*. c= columellae; en= endexine; in= intine; ft= foot layer; t= tectum.

Scale bars: Fig. 26= 0,5 μm ; Fig. 27= 1 μm ; Fig. 28= 1,5 μm and Fig. 29= 2 μm .



FIGURES 30-35. Types III and IV. TEM photographs of pollen grains of *Oxalis* species. Section through the wall showing tectum, columellae, foot layer, darkly stained endexine and intine white. Fig. 30. *O. perdicaria*. Fig. 31. *O. debilis*. Fig. 32. *O. hispidula*. Fig. 33. *O. niederleinii*. Figs. 34-35. SEM photographs of pollen wall section of *Oxalis* species. Fig. 34. *O. hispidula*. Fig. 35. *O. niederleinii*.
c= columellae; in= intine; ft= foot layer; t= tectum.

Scale Bars: Fig. 30= 1 μ m; Fig. 31= 2 μ m; Figs. 32, 33= 1 μ m; Fig. 34= 5 μ m and Fig. 35= 2 μ m.

The pollen grain wall section is another feature with diagnostic value in the genus *Oxalis*. In the type I, the exine is thin and the foot layer is from extremely thin to absent (Figs. 23-25). The endexine is continuous. In types II, III and IV, the exine is thick and the endexine is discontinuous or absent (Figs. 26-35). However, the foot layer is thicker in the type II (Figs. 26-29) than in types III and IV (Figs. 30-32). Type IV is differentiated from type III by the presence of straight columellae that are arranged equally distant (Fig. 33).

Anormal pollen grains with a variable number of apertures have been observed in *O. corniculata* var *atropurpurea* and *O. debilis* (Figs. 7, 17-18).

The occurrence of aberrant pollen in the genus *Oxalis* has been reported previously by Dreyer and Van Wyk (1998). These authors showed that the aberrations always affect the number and arrangement of the colpi, but never alter the exine structure of the grain.

Dreyer and Van Wyk (op.cit) identified 7 types or categories of pollen in the genus *Oxalis*, based on aperture number and arrangement. Some individual grains of *O. corniculata* var. *atropurpurea* could be referred to the category C (6-pantocolpate) (Fig. 7). In *O. corniculata* var. *atropurpurea* two types of pollen are observed; one type is 3-colpate and the other one is 6-pantocolpate. In concordance with these observations, Ghosh and Verma (1985) found two types of pollen in this species.

The majority of *O. debilis* pollen grains are aberrant, with numerous variable forms, and cannot be referred to any category identified by Dreyer and Van Wyk (1998) (Figs. 17-18). This fact might be related to the absence of seed production in this species (Jehlík, 1995).

In a previous study on orbicule morphology in *Oxalis* species from Buenos Aires Province (Rosenfeldt and Galati, in press), four types were distinguished. The taxa included in each of these orbicule types are in concordance with the ones present in pollen types identified in this work.

The taxa included in the Type I belong to the Section *Articulatae*, the ones of the Types II and IV are in the Section *Corniculatae* and the Type III agrees with the Section *Ionoxalis* (Lourteig, 1983, 2000).

Our results indicate that the close morphological similarity among *Oxalis* species is also reflected in their pollen and orbicules.

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