

## Pollinic characterization of species of the botanic family Rutaceae Juss.

### Caracterização polínica de espécies da família botânica Rutaceae Juss.

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#### ABSTRACT

Species of the family Rutaceae Juss are used in the food and pharmaceutical industry, for their edible fruits and multiple medicinal properties. Aiming to complete the characterization of commercially important fruit species grown in Brazil, of melissopalynological interest, the following species were analyzed: *Citrus deliciosa* Ten., *C. grandis* (L.) Osbeck, *C. limettioides* Tan., *C. limon* (L.) Burm, *C. paradisi* Macfad., *Citrus x sinensis* var. *açucar* (L.) Osbeck x *Citrus sinensis* var. *sanguinea* (Engl.) Engl. and *Fortunella japonica* (Thunb.) Swingle. The pollen grains were prepared by acetolysis method for permanent slides. The pollen grains of all species showed up in monads, isopolars, with reticulate exine, prolate-spheroidal predominant form, mainly under circular and rectangular *ambitus*, radial and bilateral symmetry, average size, exine thickness from 1.68 to 3.20  $\mu\text{m}$ , number of apertures between 3 to 5. Variations in relation to characterization of the group may be related to changes due to random or purposeful crosses for selection and improvement of crop plants.

**Keywords:** Bee plant; citrus; pollen; slide collection.

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#### RESUMO

Espécies da família Rutaceae Juss. são utilizadas na indústria alimentícia e farmacológica, em virtude de seus frutos comestíveis e de suas múltiplas propriedades medicinais. Objetivando complementar a caracterização de espécies frutíferas, cultivadas, comercialmente importantes no Brasil e de interesse melissopalínológico, foram analisadas *Citrus deliciosa* Ten., *C. grandis* (L.) Osbeck, *C. limettioides* Tan., *C. limon* (L.) Burm, *C. paradisi* Macfad., *Citrus x sinensis* var. *açucar* (L.) Osbeck, *Citrus x sinensis* var. *sanguinea* (Engl.) Engl. e *Fortunella japonica* (Thunb.) Swingle. Os grãos de pólen foram preparados pelo método da acetólise para confecção de lâminas permanentes. Os grãos de pólen de todas as espécies analisadas mostraram-se em mônades, isopolares, com exina reticulada, forma predominante prolato-esferoidal, âmbito principalmente circular e quadrangular, simetria radial e bilateral, tamanho médio, espessura da exina entre 1,68 e 3,2  $\mu\text{m}$ , quantidade de aberturas entre 3 e 5. As variações em relação à caracterização do grupo podem estar relacionadas a mudanças ocorridas, aleatórias ou em função de cruzamentos visando a seleção e melhoramento de plantas de cultivo.

**Palavras-chave:** Cítricos; palinoteca; planta apícola; pólen.

## INTRODUCTION

The characterization of pollen grains is relevant in current plant palynology as it allows to add information to the botanical characteristics of the taxa, which can be useful in tracking pollen and the improvement of species, among other applications. According to Ferguson (1985), pollen grains can be studied to obtain comparative morphological data and also for understanding aspects of the reproductive

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systems, pollination and hybridization biology. In Brazil, Rutaceae Juss family makes up about 32 genera and about 150 species, the genus *Citrus* L., including oranges, lemons, limes and tangerines, being the most worthwhile (KOLLER, 1994). Barth (1980, 1982) stated that, in studies addressing palynological aspects of Rutaceae, the pollen type of this family is identified as 3-colporate, prolate, reticulated, features observed more frequently in the species and genera analyzed by that author, among them *Zanthoxylum* L., *Pilocarpus* Vahl, *Esenbeckia* Kunth and *Helietta* Tul., and considered the family euripollinic. Groppo *et al.* (2010), studying the genus *Hortia* Vand., proposed the taxon as stenopollinic, because of the great intramorphological similarity. Aiming to contribute to the pollen characterization of the family Rutaceae, fruit tree species, grown commercially and important in Brazil for their melissopalynological interest, were analyzed.

## MATERIAL AND METHODS

The pollen grains of seven *taxa* and two varieties of Rutaceae were analyzed: *Citrus deliciosa* Ten. (tangerine), *C. grandis* (L.) Osbeck (grapefruit), *C. limettioides* Tan. (sweet lime), *C. limon* (L.) Burm (lemon), *Citrus x paradisi* Macfad. (grapefruit), *Citrus sinensis* var. *açucar* (L.) Osbeck (sweet orange), *Citrus x sinensis* var. *sanguinea* (Engl.) Engl. (blood orange), *Fortunella japonica* (Thunb.) Swingle (kinkan). The Rutaceae *vouchers* were collected in Joinville city vicinity during the flowering months, herborized, identified with specialists and listed in the Herbarium of the LABEL (Bee Laboratory) of the University of Joinville Region (UNIVILLE). For pollen analysis, closed flower buds were collected and preserved in acetic acid. Anthers were macerated, centrifuged and pollen processed by acetolysis technique according to Erdtman (1952; 1960). Three to five permanent slides were mounted and the pollen grains were photographed in light microscope equipped with Dino-Eye Microscope Eye-Piece Camera, with observation at polar (P) and equatorial (E) views. Photomicrographs were performed under light microscopy, 20 grains for each species, to make measurements that were later submitted to statistical analysis (Dino Capture 2.0 software). The observations at polar (P) and equatorial (E) views were also made in scanning electron microscope (SEM). There were observed: polarity, *ambitus*, symmetry, size, shape, apertures, ornamentation and thickness of the exine, following the terminology of Barth and Melhem (1988) and Salgado-Labouriau (1971, 2007). The characterization of the form was done by calculating the ratio P/ E, according to Barth and Melhem (1988). For each sample, 20 measurements of the polar axis were carried out as well as 20 measures of the equatorial axis. The slides were numbered and listed in the slide collection of LABEL. Observations in scanning electron microscope (SEM model JEOL JSM-6701F) were performed at the University of the State of Santa Catarina (UDESC), the pollen being acetolyzed and dehydrated in 70% ethanol, placed on the SEM holders, allowed to dry and covered with gold by the metal vaporization.

## RESULTS AND DISCUSSION

Data related to the pollen grains are shown in Tables 1 and 2 and in Figures 1, 2 and 3.

The pollen grains of the studied species were all monads, isopolar and with perforated or reticulated exine (Table 2). The predominant form is prolate spheroidal (except *C. limettioides* and *Citrus x sinensis* var. *sanguinea*, with oblate spheroidal shape). The *ambitus* ranges from circular to square and from subcircular to quadrangular. The symmetry, between radial and bilateral. On average, all the grains have mean size (25-50  $\mu\text{m}$ ), exine thickness varying between 1.68 and 3.20  $\mu\text{m}$  (Table 1). In *Citrus sinensis* var. *açucar*, *Citrus x sinensis* var. *sanguinea* and *F. japonica*, four openings were viewed. In *C. large*, *C. delicious*, *C. limettioides*, *Citrus x paradisi* and *C. limon*, whose grains are dimorphic, there were visualized, respectively, 4-5 openings for the first four species and 3-4 openings for *C. limon*, in agreement for this latter species with Del Baño-Breis *et al.* (1993). Comparing the data obtained with those of Andres *et al.* (2001) for *C. delicious*, *C. grandis*, *C. sinensis* and *C. limon* (the latter without mention of variety), it was noted that for the polar and equatorial view, the grains analyzed in this work show higher grain measures and, for the equatorial view, too (except in *C. delicious*); in relation to the thickness of the exine, the data are consistent for *C. sinensis* and *C. grandis* and discordant for

*C. delicious* and *C. limon* with smaller and larger measures, respectively; for the ratio P/ E, the data are coincident or near due to the differences mentioned above and, for the apertures and ornamentation, the data are similar.

According to Erdtman (1960), the typical ancestral dicot type of pollen (currently referred to as Magnoliopsida) is 3-aperturado and Rutaceae family pollen grains are 3-6 colporate (CAMPBELL *et al.*, 2009), reflecting a conservative condition.

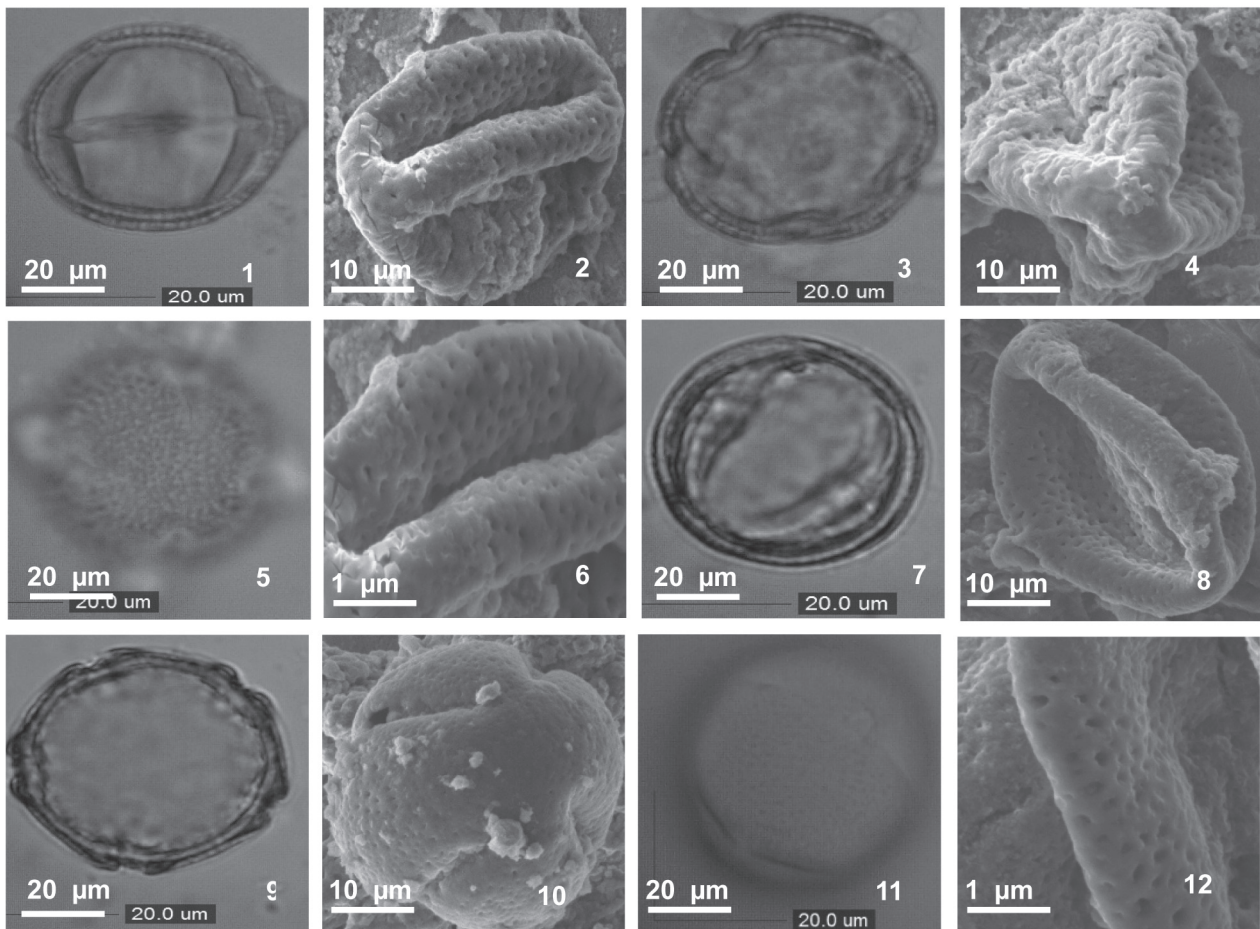
**Table 1** – Morphometric data of the pollen grains of the analyzed species of Rutaceae. Legend: P – polar axis ( $\mu\text{m}$ ); E – equatorial axis ( $\mu\text{m}$ ); P/E – ratio between the polar and equatorial axes that define the form of the pollen grain. The observed minimum and maximum measures are in brackets and, above these, is the arithmetic average.

N.	Botanic species	P	E	Exine	P/E	Form
1	<i>Citrus deliciosa</i> Ten.	30,45 (28,03-33,98)	29,89 (27,09-33,93)	1,68 (1,03-3,16)	1,01	prolate-spheroidal
2	<i>C. grandis</i> (L.) Osbeck	37,96 (33,99-42,68)	36,47 (32,12-40,93)	2,63 (1,63-3,45)	1,04	prolate-spheroidal
3	<i>C. limettioides</i> Tan.	36,07 (30,64-41,42)	36,81 (31,67-42,78)	2,85 (1,83-3,37)	0,98	oblate-spheroidal
4	<i>C. limon</i> (L.) Burm	39,71 (29,95-50,52)	35,29 (27,23-39,86)	3,2 (2,13-4,11)	1,12	prolate-spheroidal
5	<i>Citrus x paradisi</i> Macfad	36,82 (31,10-41,78)	34,51 (31,55-38,79)	2,65 (1,39-2,89)	1,06	prolate-spheroidal
6	<i>Citrus x sinensis</i> var. açucar (L.) Osbeck	30,03 (27,60-33,97)	28,63 (25,69-32,63)	2,88 (1,89-3,54)	1,04	prolate-spheroidal
7	<i>Citrus x sinensis</i> var. sanguinea (Engl.) Engl.	31,26 (26,66-34,75)	31,55 (27,88-35,70)	2,61 (1,73-3,26)	0,99	oblate-spheroidal
8	<i>Fortunella japonica</i> (Thunb.) Swingle	28,79 (25,39-37,37)	26,33 (22,93-29,74)	2,06 (1,81-3,50)	1,09	prolate-spheroidal

**Table 2** – Morphological characterization of the pollen grains of the analyzed species of Rutaceae.

N.	Botanic species	Unity	Âmbitus	Simmetry	Polarity	Ornamentation	Apertures
1	<i>Citrus deliciosa</i> Ten.	monad	circular and quadrangular	radial-bilateral	isopolar	perforated	4-5 colporate
2	<i>C. grandis</i> (L.) Osbeck	monad	circular and quadrangular	radial-bilateral	isopo-lar	perforated	4-5 colporate
3	<i>C. limettioides</i> Tan.	monad	subcircular	radial-bilateral	isopolar	reticulated	4-5 colporate
4	<i>C. limon</i> (L.) Burm	monad	circular and quadrangular	radial-bilateral	isopolar	reticulated	3-4 colporate
5	<i>Citrus x paradisi</i> Macfad	monad	subcircular	radial- bilateral	isopolar	perforated	4-5 colporate
6	<i>Citrus x sinensis</i> var. açucar (L.) Osbeck	monad	subcircular	bilateral	isopolar	perforated	4-colporate

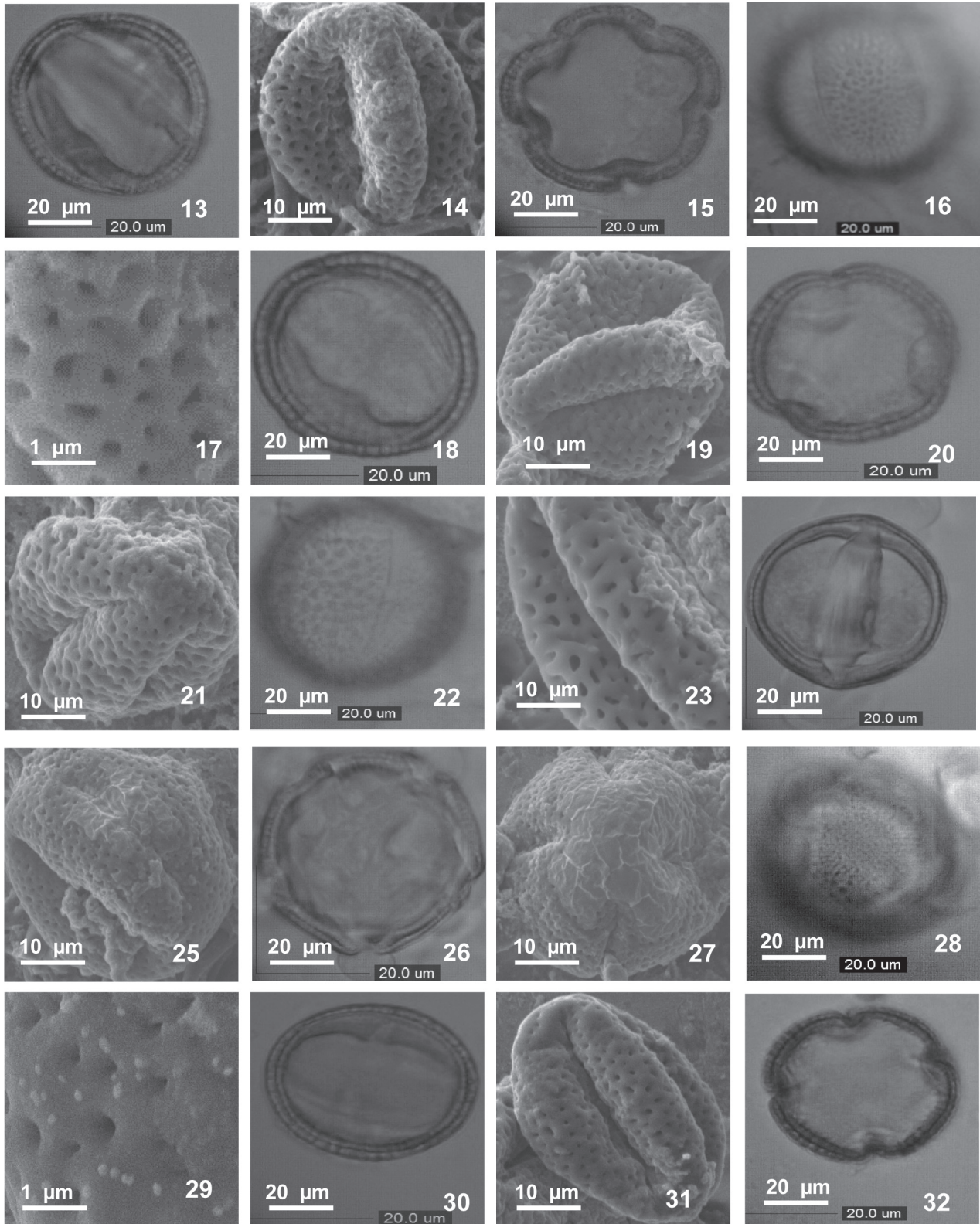
N.	Botanic species	Unity	Âmbitus	Simmetry	Polarity	Ornamentation	Apertures
7	<i>Citrus x sinensis</i> var. <i>sanguinea</i> (Engl.) Engl.	monad	circular-quadrangular	bilateral	isopolar	perforated	4-colporate
8	<i>Fortunella japonica</i> (Thunb.) Swingle	monad	quadrangular	bilateral	isopolar	reticulated	4-colporate



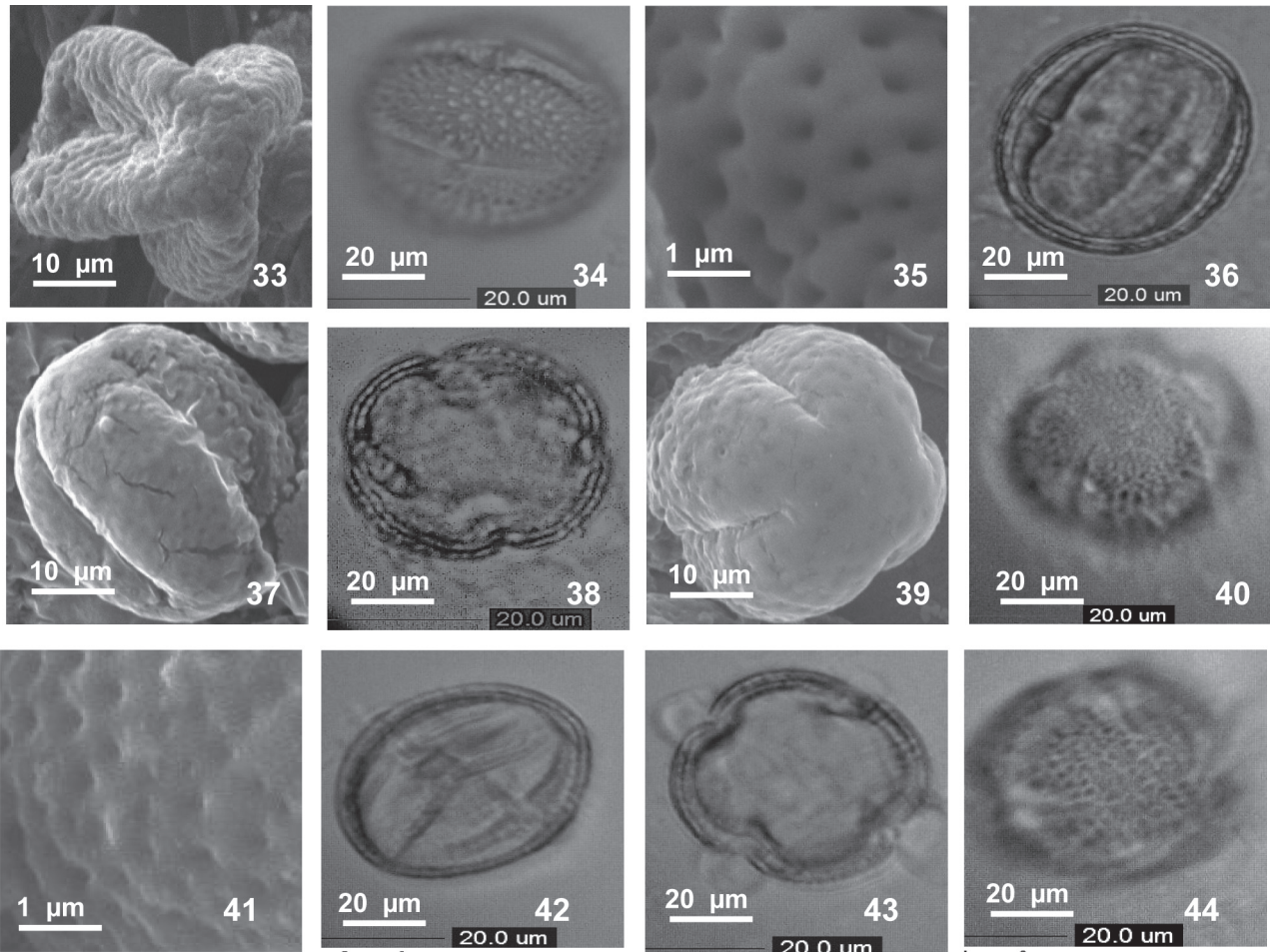
**Figure 1** – Pollen grains of the analyzed species of Rutaceae, in light microscope (LM) and in scanning electronic microscope (SEM) at equatorial view, polar view and showing the exine: *Citrus deliciosa* (LM 1, 3, 5; SEM 2, 4, 6); *Citrus grandis* (LM 7, 9, 11; SEM 8, 10, 12).

Barth (1980, 1982) as well as other authors, among which Dutra *et al.* (2013), claim that Rutaceae is a euripollinic family although Groppo *et al.* (2010) considered that some genera of Rutaceae have constant pollen morphology for their species and thus the family may be accepted as stenopollinic. On the other hand, Mabberley (1997) stated that the taxonomy of the citric fruits of *Citrus* genus is complicated by hybridity and apomixis, with many lines of stable hybrids which are granted the species status (which can reach 162), and considers that the members of current genus *Citrus* should in fact be accommodated in a maximum of four groups and three kinds of hybrids. The differences observed in this study – measures of grain and thickness of the exine – may reflect random and deliberate hybridizations occurred in the group, not effectively reported and included in the taxonomic reference, but that are evidenced in variations of pollen morphology.





**Figure 2** – Pollen grains of the analyzed species of Rutaceae, at light microscope (LM) and at scanning electronic microscope (SEM), at equatorial view, polar view and showing the exine: *Citrus limettioides* (LM 13, 15, 16; SEM 14, 17); *Citrus limon* (LM 18, 20, 22; SEM 19, 21, 23); *Citrus x. paradisi* (LM 24, 26, 28; SEM 25, 27, 29); *Citrus x. sinensis* var. *açucar* (LM 30, 32; SEM 31).



**Figure 3** – Pollen grains of the analyzed species of Rutaceae, at light microscope (LM) and at scanning electronic microscope (SEM), at equatorial view, polar view and showing the exine – *Citrus x sinensis* var. *açucar* (LM 34; (SEM 33, 36); *Citrus x sinensis* var. *sanguinea* (LM 36, 38, 40; SEM 37, 39, 41); *Fortunella japonica* (LM 42, 43, 44).

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