Acta Biológica Catarinense
2017 Jul-Set;4(2):68-76

Palynological characterization of species of Verbenaceae J. St.-Hil. and Lamiaceae Martinov (Lamiales Bromhead)

Caracterização palinologia de especies de Verbenaceae J. St.-Hil. E Lamiaceae Martinov

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ABSTRACT
Clerodendrum splendens A. Chev., *Clerodendrum* x *speciosum* Tiej. & Binn, *Clerodendrum thomsonae* Balf. F., *Clerodendrum ugandense* L., *Congea tomentosa* Roxb., *Duranta erecta* L., *Petrea volubilis* L. and *Petrea volubilis* f. *albiflora* (Standl.) Standl. pollen grains were acetolyzed, photographed and measured under light microscopy and scanning electron microscopy. Values presented are averages in micrometers. Grains are monads, radially symmetrical, isopolar, large (*C. ugandense*, very large, *C. tomentosa*, small-medium and *D. erecta*, medium),tricolpate (*P. volubilis* f. *albiflora*, dimorphic grains with 3-4 *colpus*). *Ambitus* is circular (*C. tomentosa* and *D. erecta*, sub-circular, *P. volubilis* , triangular, *P. volubilis f. albiflora*, triangular-quadrangular). The form is oblate-spheroidal (*C. splendens, C.* x *speciosum, C. ugandense*), prolate-spheroidal (*C. thomsonae*), prolate (*C. tomentosa*), suboblate (*D. erecta*) and oblate (*P. volubilis, P. volubilis* f. *albiflora*). Exine thickness is in *C. splendens* 4,28, *C.* x *speciosum* 4,19, *C. ugandense* 4,33, *C. thomsonae* 4,18, *C. tomentosa* 1,4, *D. erecta* 1,55, *P. volubilis* 2,49, *P. volubilis f. albiflora* 2,68. Ornamentation is micro-echinate (*C. splendens, C.* x *speciosum, C. thomsonae*), echinate (*C. ugandense*), reticulate (*C. tomentosa*), psilate *(D. erecta, P. volubilis, P. volubulis* f. *albiflora*). *Duranta* and *Petrea* are close to Verbenaceae pattern, *Congea* to Lamiaceae and *Clerodendrum* loosely to Lamiaceae.
Keywords: *Clerodendrum*; *Congea*; *Duranta*; *Petrea*.

Resumo: Grãos de pólen de *Clerodendrum splendens* A. Chev., *Clerodendrum* x *speciosum* Tiej. & Binn, *Clerodendrum thomsonae* Balf. F., *Clerodendrum ugandense* L., *Congea tomentosa* Roxb., *Duranta erecta* L., *Petrea volubilis* f. *albiflora* (Standl.) Standl., *Petrea volubilis* L. foram acetolisados, fotografados, medidos ao microscópio de luz e microscópio eletrônico de varredura. Os valores são médias em micrômetros. Os grãos são mônades, radialmente simétricos, isopolares, grandes (*C. ugandense* – muito grande, *C. tomentosa* – pequeno-médio e *D. erecta* – médio), tricolpados (*P. volubilis* f. *albiflora* – grãos dimórficos 3-4 colpos). Âmbito circular (*C. tomentosa* e *D. erecta* – subcircular, *P. volubilis* – triangular, *P. volubilis* f. *albiflora* – triangular-quadrangular). Formas oblato-esferoidal (*C. splendens*, *C.* x *speciosum*, *C. ugandense*), prolato-esferoidal (*C. thomsonae*), prolato (*C. tomentosa*), suboblato (*D. erecta*) e oblato (*P. volubilis*, *P. volubilis* f. *albiflora*). Exina (espessura) em *C. splendens* 4,28, *C.* x *speciosum* 4,19, *C. ugandense* 4,33, *C. thomsonae* 4,18, *C. tomentosa* 1,4, *D. erecta* 1,55, *P. volubilis* 2,49, *P. volubilis* f. *albiflora* 2,68. Ornamentação microequinada (*C. splendens*, *C.* x *speciosum*, *C. thomsonae*), equinada (*C. ugandense*), reticulada (*C. tomentosa*), psilada (*D. erecta*, *P. volubilis*, *P. volubulis* f. *albiflora*). *Duranta* e *Petrea* estão próximos ao padrão de Verbenaceae, *Congea* de Lamiaceae e *Clerodendrum*, menos intensamente, de Lamiaceae.
Palavras-chave: *Clerodendron*; *Congea*; *Duranta*; *Petrea*.

Recebido em: 31 jul. 2017
Aceito em: 23 out. 2017

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INTRODUCTION

Verbenaceae is characterized mainly by the arbustive or arboreal habit, but also herbaceous and lianas, producer of essential oils, having pantropical and mainly neotropical distribution and the family has importance as many species are used as ornamental, aromatic and forage resource (ATKINS, 2004).

In terms of floral definition, Verbenaceae shows zygomorphic flowers, didinamous androecius, two carpels, ovary usually with four lobules with terminal or subterminal style, and drupa or schizocarpic fruits (MOLDENKE, 1965). Verbenaceae initially included about 3000 species in 90 genera and, in Brazil, about 300 species in 16 genera (SALIMENA & MULGURA, 2015).

A close relationship between the Lamiaceae and Verbenaceae has long be recognized as the two families share opposite leaves, zygomorphic flowers and a bicarpellate gynoecium that, by the formation of false partitions, develops into four uniovulate locules (CRONQUIST, 1981). The Labiatae generally are distinguished from the Verbenaceae by a deeply four-lobed ovary with a gynobasic style, whereas most Verbenaceae have an unlobed ovary with a terminal style (WAGSTAFF & OLMSTEAD, 1997). However, *taxa* with an intermediate morphology exist in both families and the boundary between the two families is somewhat arbitrary (WAGSTAFF *et al.*, 1998).

Lamiaceae is a *taxa* of considerable economic importance, containing several timber trees, many species of horticultural value, culinary herbs, perfumery essences, medicinal plants and divers nectar-producing species which procure a honey of high quality (HARLEY, 2012).

There are different proposed classifications for several genera and species in Verbenaceae and in Lamiaceae aiming to solve taxonomic delimitation (SANDERS, 2001). As Palynology is considered currently a useful tool to discriminate between closely related species (PERVEEN & QAISER, 2007), this work aimed to contribute to the palynological characterization of species of the families Verbenaceae and Lamiaceae.

MATERIAL AND METHODS

Pollen grains of the following species of Verbenaceae were studied: *Congea tomentosa* Roxb., *Duranta erecta* L., *Petrea volubilis* L., *Petrea volubilis* fo. *albiflora* (Standl.) Stanndl. as well as these of Lamiaceae*: Clerodendrum splendens* A. Chev., *Clerodendrum* x *speciosum* Tiej.& Binn, *Clerodendrum thomsonae* Balf. F. and *Clerodendrum ugandense* L. Comparison materials were not used but only vouchers from the direct samplings.

Samplings were collected in the municipalities of Joinville, state of Santa Catarina and of Alambari, state of São Paulo, during the blooming months, herborized (exsicates), identified with the help of specialists, conserved and registered at the Herbarium Label (Bee Laboratory) of the University of the Region of Joinville (Univille).

Closed floral buds were conserved in glass bottles, containing glacial acetic acid, pure for analysis (P.A.), the botanic identification and the sampling date. Bottles were sealed with rubber covers until the moment of preparation of the glass slides.

For the analysis of the pollinic material, the anthers of the floral buds were taken off and torn for the liberation of the pollen grains that were then submitted to the acetolysis method (ERDTMAN, 1952). Recommendations of Salgado-Laboriau (1973) were followed with the utilization of Kisser's gelatin, closing the slides with paraffin as well as taking photographs.

Measures of the pollen grains were taken in at most seven days, from the pollen slides, observed on the light microscope. Measures are presented in micrometers.

The pollen grains of each species were photographed 25 times in polar view (PD), the same in equatorial view (ED), ten times in equatorial diameter in polar view (EDPV) and the apocolpus side (AS) was also measured, when possible. We calculated the arithmetic mean, the standard deviation and the confidence interval of the measures.

The characterization of the form of pollen grains of the species was performed by calculating the ratio P/E (ERDTMAN, 1960) as well as the AS/ EDPV the size of the polar area (FAEGRI *et al.*, 1992).

For each analyzed species, in relation to the size of the pollen grain, we verified, in both views, the maximum size, the minimum size, the average size and the standard deviation. The number and type of apertures was observed as well as the ornamentation of the exine.

All features were registered and described according to the terminologies of Barth & Melhem (1988) and Punt *et al.* (2007). The observations took place under a light microscope Bioval with equipment Dino-Eye Microscope Eye-Piece Camera, associated with the software DinoCapture 2.0 at the University of the Region of Joinville, and scanning electron microscope (SEM) of the University of the State of Santa Catarina (Udesc). Pollen slides were numbered and deposited at the pollen reference slides collection of Label-Bee Laboratory, at the University of the Region of Joinville. Measures were submitted to statistical analysis on Microsoft Excel. Results of all measures and observations are presented in tables (1, 2 and 3) and figure 1.

RESULTS

All pollen grains are in monads, with radial simmetry and isopolars.

The size is large (except *Clerodendrum ugandense,* very large and *Congea tomentosa* and *Duranta erecta*, medium).

Most pollen grains show circular ambitus (except *D. erecta,* sub-circular, *Petrea volubilis*, triangular and *Petrea volubilis f. albiflora,* triangular to quadrangular).

In terms of apertures, all pollen grains are tricolpate, with dimorphic grains in *Petrea volubilis* f. *albiflora* (3-4 colpus). This species appears to have demicolpus, however, the pollen grains were rarely found in equatorial view with the apertures well-positioned.

Considering the average of the greater measure, the form is oblate-spheroidal (*C. splendens*, *C.* x *speciosum*, *C. ugandense*), prolate-spheroidal (*C. thomsonae)*, prolate (*Congea tomentosa)*, suboblate (*Duranta erecta)* and oblate (*Petrea volubilis*, *P. volubilis* f. *albiflora)*.

The average exine is thicker in *C. splendens* (4,28), *C.* x *speciosum* (4,19), *C. thomsonae* (4,18), *C. ugandense* (4,33) and thinner in *C. tomentosa* (1,4), *D. erecta* (1,55), *P. volubilis* (2,49), *P. volubilis* f. *albiflora* (2,68).

Table 1 – Morphometric data of the pollen grains of the analysed species, in (µm). Legend: $\bar{x} \pm s_{\bar{x}}$: arithmetic mean ± standard deviation; CI: confidence interval; P/E: ratio between mean of polar diameter/equatorial diameter values.

Species	Polar diameter			Equatorial diameter				
	Range	$\bar{x} \pm s_{\bar{x}}$	CI 95%	Range	$\bar{x} \pm s_{\bar{x}}$	CI 95%	P/E	Form
Clerodendrum splendens	58,01-68,59	63,4±3,06	62,64-64,15	59,92-76,18	69,48±3,39	68,68-70,27	0,91	Oblate-spheroidal
Clerodendrum x *speciosum*	58,86-78,91	66,26±4,92	65,07-67,44	60,58-74,16	69,29±2,7	68,65-69,92	0,95	Oblate-spheroidal
Clerodendrum thomsonae	63,82-81,75	73,12±4,92	71,99-74,24	54,65-75,83	69,21±4,94	68,04-70,37	1,05	Prolate-spheroidal
Clerodendrum ugandense	81,28-114,76	98±7,29	96,54-99,44	96,92-113,35	103,84±4,3	103-104,67	0,94	Oblate-spheroidal
Congea tomentosa	31,9-50,1	43,2±4,88	41,74-44,65	21,95-32,14	26,92±2,4	26-27,83	1,6	Prolate
Durancta erecta	26,2-34,85	31,24±2,1	30,5-31,97	34,2-41,7	38,12±1,96	37,49-38,74	0,81	Suboblate
Petrea volubilis	24,25-46,64	35,74±5,11	34,04-37,43	44,30-63,65	56,07±4,66	54,84-57,29	0,63	Oblate
Petrea volubilis f. *albiflora*	31,76-57,66	43,22±10,11	46,66-52,33	50,75-69,45	58,87±4,86	56,07-60,05	0,73	Oblate

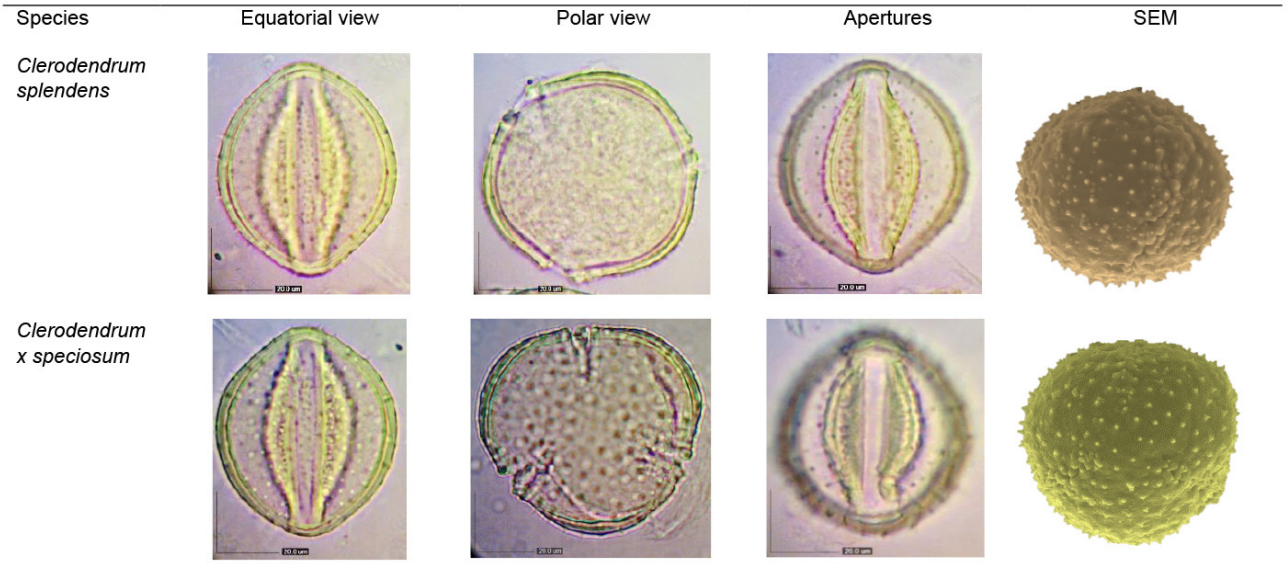
Table 2 – Measurements (µm) of pollen grains in polar view (n=10) and equatorial view (n=25). EDPV = equatorial diameter in polar view; ($\bar{x}$) arithmetic mean.

Species	EDPV		Colpus		Apocolpus side		Polar Area Index
	Range	$\bar{x}$	Length	Width	Range	$\bar{x}$	
Clerodendrum splendens	57,41-75,5	66,26	64,57	10,7	22,11-37,34	31,26	0,330
Clerodendrum x *speciosum*	51,92-72,93	65,21	60,35	8,92	25,04-34,86	29,45	0,355
Clerodendrum thomsonae	67,95-87,84	77,53	42,44	2,64	21,45-44,85	32,44	0,350
Clerodendrum ugandense	110,26-120,17	115,93	-	-	47,85-70,51	55,95	0,441
Congea tomentosa	24,96-42,11	34,71	28,08	2,56	34,45-44,76	40,31	0,772
Durancta erecta	30,38-40,71	35,27	23,52	2,29	21,01-31,04	27,59	0,703
Petrea volubilis	45,01-68,5	55,6	–	–	39,51-59,38	49,8	0,786
Petrea volubilis f. *albiflora*	36,4-65,63	55,62	–	–	34,45-44,76	40,4	0,772

The ornamentation of the exine is micro-echinate (*C. splendens, C.* x *speciosum*, *C. thomsonae*), echinate (*C. ugandense*), reticulate (*C. tomentosa*), and psilate (*D. erecta*, *P. volubilis*, *P. volubilis* f. *albiflora*).

Table 3 – Morphometric data of the pollen grains of the analysed species.

Species	Size	Ambitus	Exine ornamentation
Clerodendrum splendens	Large	Circular	Microechinate
Clerodendrum x *speciosum*	Large	Circular	Microechinate
Clerodendrum thomsonae	Large	Circular	Microechinate
Clerodendrum ugandense	Very large	Circular	echinate
Congea tomentosa	Small-Medium	Sub-circular	Reticulate
Durancta erecta	Medium	Sub-circular	Psilate
Petrea volubilis	Medium-large	Triangular	Psilate
Petrea volubilis f. *albiflora*	Medium-large	Triangular-quadrangular	Psilate



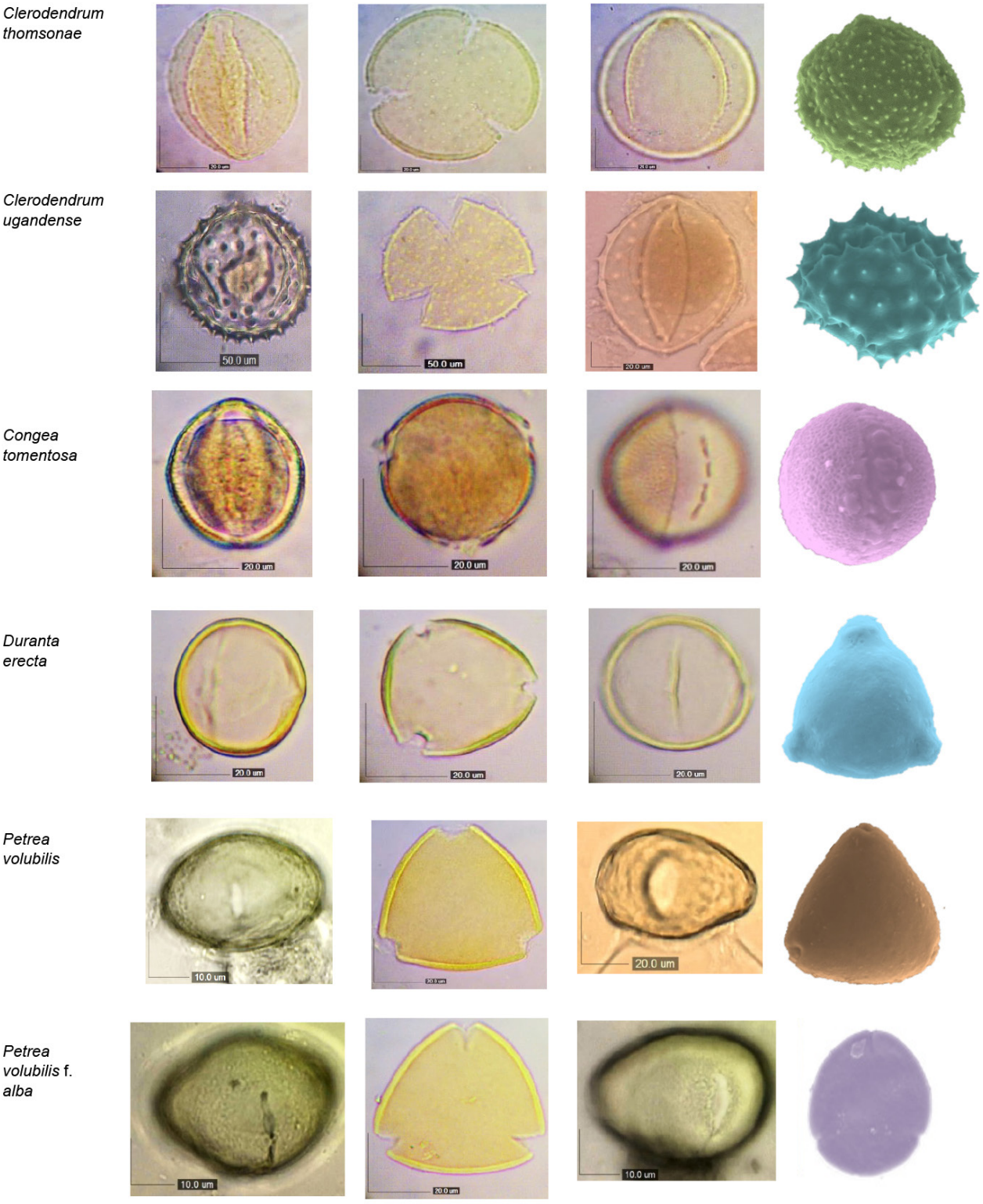


Figure 1 – Pollen grains of the analyzed species in light microscope (LM) and scanning electronic microscope (SEM) (The images at SEM were color graded).

DISCUSSION

For *Duranta erecta*, Raj (1983) observed different values for the measures: 24-27 µm for the polar axis and 29-31 µm for the equatorial diameter. Sousa *et al.* (2011), for the same species, report also some aspects that are different from those described in the present study: prolate-spheroidal form, tricolporate, equatorial diameter (E) of 17.66 µm and polar axis (P) of 18.80 µm, exine around 3.48 µm, triangular *amb* (with intraspecific variation) and a psilate-perfurate exine ornamentation. Halbritter (2016), in turn, mentions the following for the species: size 26-50 µm, oblate form, triangular *amb*, tricolporate grains, with the annotation that the shape (hydrated) can vary from oblate to spheroidal. All authors considered *D. erecta* as Verbenaceae. In spite of the differences observed between the cited studies and the present one, the pattern displayed is correlable to Verbenaceae. According to Bhowmik & Datta (2012), there is the possibility that the ecological and geographical variations in the pollen morphology could be an indication of the genetic impact of the environment on the plant.

About *Congea*, Erdtman (1986) described pollen grains of *C. villosa* (mentioned as belonging to Symphoremoideae, formerly a subfamily of Verbenaceae) as 3-colporate (*colpi* more or less irregularly 3-4 orate), subprolate (about 35 x 30 µm), sexine as thick as nexine, reticulate (*muri* simpli-dupli baculate), *colpi* membranes psilate, *ora* lalongate, not confined to the *colpi* membranes but forming more or less deep cuts in the adjoinning mesocolpial exine. Rahman (1962), in turn, in a study about pollen of Lamiaceae, described the grains of *Congea tomentosa* as 3-zonicolporate, subprolate (39 x 31 µm; range 35-42 x 28 x 32 µm), with circular *amb*, ectocolpium 4-2, endoapertures 3-5, exine 2.1 µm thick, with areolate ornamentation, stressing that colporate sporomorphs are a rarity among angiosperms, the common situation being two endoapertures on every *colpus* in some species and precised that *C. villosa* has 3-4 endoapertures. These data are close from those of the present study and show a pollen pattern of similar to that of Lamiaceae, *s.l. Congea* was placed, by Moldenke (1973), in Symphorematoideae. This subfamily was recognized, afterwards, as a *taxon* of Lamiaceae by Harley (2012), that distinguished seven subfamilies within the family Lamiaceae (Symphorematoideae, Viticoideae, Ajugoideae, Prostantheroideae, Scutellarioideae, Lamioideae and Nepetoideae), after studies done by Wagstaff *et al*. (1998), where these last authors showed *Congea tomentosa* nested within Labiatae *s. l*. and as sister group to subfamily Nepetoideae, based on ndhF and combined analysis, although Bendiksby *et al.* (2011) showed *Congea* as sister to a clade of Viticoid *genera*.

Many publications refer to the genus *Clerodendrum* as *Clerodendron* but the first form is the official spelling (Kitajima *et al*., 2008). Halbritter & Buchner (2011), Halbritter & Buchner (2016) and Halbritter (2016) studied *Clerodendrum paniculatum*, *C. thomsonae* and *C. thrichotomum*, respectively, all mentioned as Lamiaceae, and describe, in general terms, grains of large-medium size, 3-6 colpate, with circular *amb*, iso or heteropolar, with spheroidal shape (=form) and echinate-microechinate perforate exine. Lovly & Merlee Teresa (2016) described *C. inerme* (with no indication of family) grains with polar length 50.78 µm, equatorial diameter 49.68 µm, P/E ratio 1, shape prolate-spheroidal, *amb* circular, tricolporate with *apoporium*, exine thickness 0.24 µm, exine ornamentation echinate, exine tectate and columellate. *C. buchainii*, *C. floribundum*, *C. inerme* and *C. tomentosum*, all mentioned as Verbenaceae (AUSTRALASIAN, n.d.a, n.d.b, n.d.c, n.d.d) are reported as 3-colpate, with echinate exine, circular *amb*, form spheroidal/ subprolate/subprolate/subprolate, polar length 46-51-78,3-63 µm x equatorial diameter 46-61-69,1-77 µm, exine 2,4-3-3. The slight differences observed in the present study in *C.* x *speciosum* are due to the variability in pollen morphology, including size deviation and morphological changes, that can occur associated with hybrids among angiosperms groups (CHATURVEDI *et al.*, 2000). Based on cladistic analysis of the chloroplast DNA and internal transcriber spacer sequences (STEANE *et al.*, 2004) and other studies (SIVADAS & SREELEKHA, 2011), the genus *Clerodendrum*, originally considered as a member of the family Verbenaceae, is now considered as a member of the family Lamiaceae. Palynological data obtained by the above mentioned authors are close from those of the present study. However, these patterns fit loosely those of Lamiaceae pollen grains described by Erdtman (1945).

According to Halbritter & Buchner (2016), *Petrea volubilis* (mentioned as Verbenaceae) pollen grains are monads, large, angulaperturate, 3-colpate (with *brevicolpus*), isopolar, oblate, *amb* triangular,

with perforate exine and Ubisch bodies. Ghosh & Mandal (2016) describe *Petrea volubilis* (mentionned as Verbenaceae) grains as tricolporate, with exine microreticulate, measuring 36.30 x 22.27 µm, prolate, with *amb* triangular-obtuse-convex. The fact that there are triangular ou quadrangular *ambitus* in *P. volubilis* f. *albiflora* reflects the variation in the number of apertures and the slight differences observed between *P. volubilis* and *P. volubilis* f. *albiflora*, in the present work, are due probably to the variability of hybrids (CHATURVEDI *et al.*, 2000). The characterization realized by the first author is similar to that obtained in this study but the second ones mention prolate grains. The first pattern verges on the general form of Verbenaceae.

Some of the studied species of this work were already verified by others, as mentioned. The observed differences in the results (mainly about measures, exine thickness and ornamentation) may be due to differences in the treatment and fixation process.

An alternative classification of the Lamiaceae, based on the palynological characters, was proposed by Erdtman (1945) who stated that Lamiaceae could be split into two subfamilies: Lamioideae having tricolpate pollen shed in a two-celled stage and Nepetoideae having hexacolpate pollen shed in a three-celled stage. Following this system, the species of Lamiaceae studied in this work are in Lamioideae. Sanders (2001) reports that, after cladistic reevaluation of the phenetic analysis of the pollen characters related by Raj (1983), the pollen of Verbenaceae *sensu strictu* can be described as suboblate to prolate, radially symmetric, generally tricolporate (triporate or tricolpate in some *genera* ou rarely with four-five independent apertures), the *colpi* generally long, narrow, with endings tapering, equatorially constricted, with a thickened margin, *ora* generally elongate, in straight angles with the *colpi* (lalongate) and exine tectate-perforate, with *supratectum* elements missing, except in a few *genera*. However, Verbenaceae is an eurypalynous family and shows an array of pollen types differing in nature, number and complexity of aperture and exine ornamentation, these variations being of considerable systematic value (ATKINS, 2004). As a close relationship seems to exist between the Lamiaceae and Verbenaceae, *taxa* with an intermediate palynological morphology are seemly to exist in both families. More studies are necessary to understand the patterns.

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