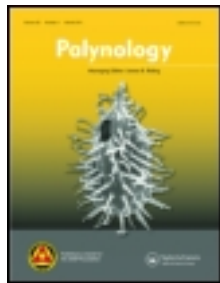


This article was downloaded by: [Gisela M. Via do Pico]

On: 26 February 2013, At: 03:39

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Palynology

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/tpal20>

Pollen morphology and implications for the taxonomy of the genus *Chrysolaena* (Vernonieae, Asteraceae)

Gisela M. Via do Pico^a & Massimiliano Dematteis^a

^a Instituto de Botánica del Nordeste (UNNE-CONICET), Casilla de Correo 209, CP 3400, Corrientes, Argentina

Accepted author version posted online: 19 Nov 2012. Version of record first published: 26 Feb 2013.

To cite this article: Gisela M. Via do Pico & Massimiliano Dematteis (2013): Pollen morphology and implications for the taxonomy of the genus *Chrysolaena* (Vernonieae, Asteraceae), *Palynology*, DOI:10.1080/01916122.2012.749309

To link to this article: <http://dx.doi.org/10.1080/01916122.2012.749309>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.tandfonline.com/page/terms-and-conditions>

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Pollen morphology and implications for the taxonomy of the genus *Chrysolaena* (Vernonieae, Asteraceae)

Gisela M. Via do Pico* and Massimiliano Dematteis

Instituto de Botánica del Nordeste (UNNE-CONICET), Casilla de Correo 209, CP 3400 Corrientes, Argentina

This study aims at finding new morphological characters to evaluate the taxonomic position of species in the genus *Chrysolaena* (Vernonieae, Asteraceae) as suggested by the current classification of tribe Vernonieae. We have characterized the morphology of pollen grains in all 18 species of *Chrysolaena*. Results show that all the studied species possess pollen grains of type “C”, which is therefore characteristic of the genus. This pollen type is 3-colporate, echinolophate, oblate-spheroidal, with a total of 29 lacunae, including the polar lacuna, but lacking the equatorial lacuna. *Chrysolaena* is considered stenopalynous due to the fact that the pollen type in most of the species is similar. Nevertheless, variation in the size of pollen grains allowed us to identify three groups of taxa: small, medium-sized and large. Pollen morphology is a useful taxonomic marker, in addition to other morphological and molecular characters defining the genus.

Keywords: pollen morphology; taxonomy; *Chrysolaena*; Vernonieae; Asteraceae

1. Introduction

The tribe Vernonieae Cass. is one of the largest groups of Compositae (Asteraceae), with around 1500 species recorded in America, Asia and Africa. A total of 126 genera distributed in 21 subtribes are currently recognized in the tribe, 15 of which, including about 70 genera and approximately 600–700 species, occur in the New World (Keeley & Robinson 2009). The Tribe Vernonieae is considered one of the biologically and taxonomically most complex groups within the family (Keeley et al. 2007). Relationships among species are unclear making it difficult to organize and build a phylogenetic classification (Jones 1979).

The Subtribe Lepidaploinae S.C. Keeley & H. Rob. is one of the recently circumscribed subtribes, including 12 genera, five of them monotypic, and about 320 species that mainly occur in the Western Hemisphere (Keeley & Robinson 2009). Several of these 12 genera are segregates, such as *Lessingianthus* H. Rob., *Lepidaploa* (Cass.) Cass. and *Chrysolaena* H. Rob., which were previously included in the diverse genus *Vernonia* Schreb.

Chrysolaena was described by Robinson (1988), including species of *Vernonia* subsection *Flexuosae* (Cabrera 1944). Although two of the features used to recognize this genus are the apical anther appendages and the base of the style, Robinson (1988) placed some species with these appendages, such as *Vernonia desertorum* Mart. ex DC. and *V. simplex* Less., in

Lessingianthus subgenus *Oligocephalus* H. Rob. A recent contribution by Dematteis (2007) redefined *Chrysolaena* by incorporating *Lessingianthus* subgenus *Oligocephalus* and described *C. guaranitica* Dematt., a new species endemic to eastern Paraguay. The most recent taxonomic revision of *Chrysolaena* (Dematteis 2009) comprises all 18 currently recognized species, including two new species and new combinations of taxa previously placed in *Vernonia*.

Chrysolaena is characterized by the presence of a sericeous or velutinous indumentum, anthers with an apical glandular appendage, styles without a basal node and glandular cypselas (Robinson 1988; Dematteis 2009). Other distinctive features are the pollen morphology and chromosome number. The latter is probably one of the most important features to separate *Chrysolaena* from the remaining genera of Vernonieae, because it is the only known American genus with the base number $x = 10$ (Dematteis 2007). Most of the New World genera have base numbers ranging from $x = 14$ to $x = 17$, which in some cases are specific to genera or sections.

The majority of *Chrysolaena* species are distributed throughout central South America, with the main center of diversification of the genus being in southern Brazil and northeastern Argentina. However, the distribution area of the genus also includes a part of Peru, the Amazonian region of Brazil, and the province of Buenos Aires in Argentina (Dematteis

*Corresponding author. Email: gisela_viadopico@hotmail.com

2009). Most species have a wide geographical distribution, whereas only few are endemic; for example, *Chrysolaena nicolackii* H. Rob. from southern Brazil, and *C. candelabrum* (Chodat) Dematt., and *C. guaranitica* from Paraguay.

The pollen morphology of Vernoniae is highly variable and has been widely used in taxonomic treatments at the generic and subtribal level (Kingham 1976; Keeley & Jones 1977). Steetz (1864) first described pollen morphology in Vernoniae. Later, Wodehouse (1928) studied pollen surface morphology in North American *Vernonia* species and recognized two basic forms: echinolophate and subechinolophate. Pollen grains varied in size and showed exine sculpturing, such as lacunae, germinal furrows, spines, other protuberances, and ridges or crests. Jones (1970) was the first to examine *Vernonia* pollen with scanning electron microscopy, describing pollen morphology in about 500 species in a series of studies as a single author (Jones 1970, 1979, 1981) and other studies with collaborators (Keeley & Jones 1977, 1979). Kingham (1976) recognized six types of pollen grains (I–VI) studying 85 species of Vernoniae from tropical Africa. Based on the polar orientation, Keeley and Jones (1977) first recognized pollen types A–C, and then additionally types D–F (Keeley & Jones 1979). Robinson (1990, 1992) described the remaining four of the 10 currently recognized pollen types in Vernoniae (A–J). These studies not only described pollen types, but also discussed the variability and taxonomic value of pollen characteristics in Vernoniae. Pollen characteristics, style base, and anther appendages are widely used to delimit natural groups in the tribe. Pollen morphology of *Chrysolaena* has previously been investigated by some authors (Keeley & Jones 1977; Jones 1979; Dematteis & Pire 2008; Dematteis 2009; Mendonça et al. 2007, 2010), but they mainly focused on differences among genera in the tribe and considered only a few species of *Chrysolaena*.

In this study, we characterize pollen morphology in all species of *Chrysolaena*, in order to provide new information in support of taxonomic classifications proposed for the tribe.

2. Materials and methods

For all 18 species studied, pollen samples were obtained from the anthers of flower buds of herbarium specimens at the Instituto de Botânica del Nordeste (CTES). Species studied and voucher specimen data are listed in Table 1.

The pollen grains were acetolyzed according to the methodology of Erdtman (1960). For light microscopy (LM), pollen grains were mounted on glass slides using glycerine jelly and subsequently examined with a Zeiss

Axioplan light microscope. Permanent preparations were deposited at the Palynological Laboratory of the Universidad Nacional del Nordeste (PAL-CTES).

The following pollen measurements were taken for at least 30 grains per sample: polar axis (P), equatorial diameter (E), exine thickness, lacuna diameter, spine length, colpus length, and pore diameter.

For analyses with the scanning electron microscope (SEM), acetolyzed pollen grains were first washed in alcohol 96% and absolute alcohol (100%), then plated with gold-palladium and examined with a JEOL 5801 LV microscope. The terminology of Keeley and Jones (1979) and Punt et al. (2007) was used to describe the pollen morphology.

For morphometric analyses, a scatter diagram that considered the average values of the equatorial diameter and the polar axis of the pollen grains was used. A basic matrix based on seven pollen characters (Table 3) was compiled with the values for each species. The qualitative variable (SP: spine presence) represents codes for character states observed in each species, and was transformed into a “dummy” variable by transforming the possible states of each variable into a variable with the two states ‘presence’ and ‘absence’. Using the data matrix mentioned above, a Principal Component Analysis (PCA) was carried out to evaluate the contribution of each pollen parameter to species affiliation. In order to estimate the degree of variation and statistical significance of palynological character sets, averages were compared by the multivariate analysis of variance (MANOVA). For this analysis only, the variables with the highest values of eigenvectors resulting from PCA were considered (equatorial diameter, polar axis, and spine length). This means that the qualitative variable “presence of spines” (SP) was excluded from this analysis. Statistical analyses were carried out with Infostat software, version 2011 (Di Rienzo et al. 2011).

3. Results

The measurements of pollen grain characteristics in *Chrysolaena* species investigated in this study are listed in Table 2.

3.1. General pollen morphology

The pollen grains were radially symmetric, oblate-spheroidal (P/E, from 0.86 to 0.99), 3-colporate, echinolophate. Values of E ranged between 36.72 and 61.20 μm , and of P between 35.36 and 68.00 μm . Lacunae are surrounded by lophae and are more or less regular in outline, displaying various forms (pentagonal, hexagonal, and circular). There are 29 lacunae in total, 3 poral, 6 abporal, 12 paraporal, 6

Table 1. List of *Chrysolepta* specimens examined in this study.

Species	Location and Voucher specimens
<i>C. campestris</i> (DC.) Dematt.	Brazil, Minas Gerais: Entrance to Curvelho-Diamantina, km 108, neighborhood entrance, rocky field. R Mello Silva et al. 8708 (CTES).
<i>C. candelabrum</i> (Chodat) Dematt.	Paraguay, Caaguazú: 32 km N of Caaguazú. Road to Yhú, in Cerrado. Krapovickas et al. 45761 (CTES).
<i>C. cognata</i> (Less.) Dematt.	Argentina, Misiones: General Manuel Belgrano, Campina de Americo. Cemetery. Dematteis et al. 3053 (CTES).
“	Argentina, Misiones: San Ignacio. House of Horacio Quiroga. 27°15' 4''S-55°33'2''W. 68 m.a.m.s.l. Dematteis et al. 3040 (CTES).
“	Argentina, Misiones: General Manuel Belgrano. 4 km E of Dos Hermanas. Road to Bernardo de Irigoyen. On Route 17. 26°17'15''S-53°43'42''W. 771 m.a.m.s.l. Via do Pico et al. 32 (CTES).
“	Argentina, Corrientes: San Roque. 12 km N of San Roque. Route 12. 28°27'51''S-58°43'16''W. 72 m.a.m.s.l. Via do Pico et al. 6 (CTES).
“	Brazil, Rio Grande do Sul: Itaará, field. Deble & Oliveira s.n. Paraná. Campo Mourao. Edge of road. Krapovickas & Cristóbal 46948 (CTES).
<i>C. cordifolia</i> Dematt.	Argentina, Corrientes: Ituzaingó. 9 km of Route 12, road to San Carlos. Krapovickas et al. 24905 (CTES).
“	Argentina, Misiones: General Manuel Belgrano. Campina de Americo. Cemetery. Keller 3975 (CTES Holotype).
<i>C. cristobaliana</i> Dematt.	Brazil, Mato Grosso do Sul: (Mun. Bonito) Highway Guia Lopes da Laguna, road to Bonito, River Miranda. Hatschbach et al. 74423 (CTES).
“	Brazil, Mato Grosso do Sul: 6 km of Campo Grande, closed road to Sidrolândia. Krapovickas & Cristóbal 34544 (CTES Holotype).
<i>C. desertorum</i> (Mart. ex DC.) Dematt.	Brazil, Minas Gerais: São Thomé das Letras. Serra do Cantagalo. Rocky field. Rossi et al. 5840 (CTES).
<i>C. dusenii</i> (Malme) Dematt.	Brazil, São Paulo: Mun. Itararé Fazenda Espinho. Souza et al. 4688 (CTES).
<i>C. flexuosa</i> (Sims) H. Rob.	Argentina, Corrientes: San Roque. 12 km N of San Roque. Route 12. 28°27'51''S-58°43'16''W. 72 m.a.m.s.l. Via do Pico et al. 5 (CTES).
<i>C. guaranitica</i> Dematt.	Paraguay, Caaguazú: 2 km E of Caaguazú. Route 2. Krapovickas et al. 12524 (CTES Holotype).
<i>C. lithospermifolia</i> (Hieron.) H. Rob.	Argentina, Corrientes: Saladas. Creek San Lorenzo. 28°06'50''S-58°46'13''W. 53 m.a.m.s.l. Via do Pico et al. 3 (CTES).
<i>C. nicolackii</i> H. Rob.	Brazil, Paraná: Mun. Campo Largo, Serra São Luiz do Puruña. Cordeiro & Nicolack 682 (CTES Isotype).
“	Brazil, Paraná: Mun. Ponta Grossa. Cachoeira da Mariquinha. Silva et al. 6214 (CTES).
“	Brazil, Paraná: Mun. Palmeira. River Tinagi. Field. Silva et al. 5533 (CTES).
<i>C. obovata</i> (Less.) Dematt.	Bolivia, Santa Cruz: Ñuño de Chávez. 3 km NW of San Javier, road to tannery. 16°16'38''S-62°32'1''W. 518 m.a.m.s.l. Dematteis et al. 3475 (CTES).
<i>C. oligophylla</i> (Vell.) H. Rob.	Brazil, Paraná: Mun. Jaguariaiva. Fazenda Caçador da Boa Vista, Morro Azul. Barboza et al. 1729 (CTES).
<i>C. platensis</i> (Spreng.) H. Rob.	Argentina, Misiones: General Manuel Belgrano. 22 km S of Dos Hermanas. 25°18'33''S-53°45'33''W. 768 m.a.m.s.l. Dematteis et al. 3083 (CTES).
“	Argentina, Misiones: General Manuel Belgrano. N of Dos Hermanas. 26°16'47''S-53°46'02''W. Keller & Ritter 4989 (CTES).
<i>C. propinqua</i> (Hieron.) H. Rob.	Argentina, Corrientes: Santo Tomé. 12,7 km S of Virasoro, road to Santo Tomé. 28°08'42''S-56°03'57''W. 127 m.a.m.s.l. Via do Pico et al. 20 (CTES).
<i>C. sceptrum</i> (Chodat) Dematt.	Paraguay, Amambay: 41 km S of Bella Vista, road to River Aquidabán by route 3. Dematteis et al. 909 (CTES).
“	Paraguay, Concepción: 6 km W of Puentesío, road to San Carlos del Apa. Dematteis et al. 3307 (CTES).
<i>C. simplex</i> (Less.) Dematt.	Brazil, Minas Gerais: Mun. Gouveia. Serra do Espinhaço, highway to Barão do Guaçuí. 1300 m height. Hatschbach et al. 69630 (CTES).
“	Brazil, Minas Gerais: São Sebastião da Vitória. UFIF & Embraoa 23053 (CTES).
“	Brazil, Minas Gerais: Mun. Gouveia. Serra do Espinhaço. Hatschbach 27322 (CTES).
“	Bolivia, Santa Cruz: Prov. Chiquitos. Mountain Mutún. 7 km NE of airstrip of the mining camp (25 km S of Puerto Suárez). 19°11,4'S-57°52,7'W. 730 m height. Vargas et al. 3247 (CTES).
<i>C. verbascifolia</i> (Less.) H. Rob.	Argentina, Corrientes: Ituzaingó. 1,8km S of San Carlos. 27°45'28''S-55°54'24''W. 87 m.a.m.s.l. Via do Pico et al. 44 (CTES).

interpolar and 2 polar. Rarely, there are four or only one polar lacunae (two at each pole or in only one of the two poles, respectively; Plate 1, figures 1 and 2), or

there are equatorial lacunae (Plate 1, figure 14). Polar lacunae measure between 5.44 and 16.32 μm , and are triangular to slightly pentagonal (Plate 2, figures 2, 5,

Table 2. Summary of pollen measurements in *Chrysolaena*.

Species & voucher	P	E	P/E	Colpus length	Pore diameter	Exine thickness	Spine length	Polar lacuna diameter
<i>C. campestris</i> 8708	40.8 (45.2) 50.32	47.6 (50.18) 54.4	0.91	35.36 (39.12) 44.88	6.8–9.52 x 6.8–10.9	5.44 (7.16) 8.16	2.72 (3.11) 4.08	8.16 (10.74) 13.6
<i>C. candellabrum</i> 45761	43.52 (46.87) 50.32	47.6 (50.27) 53.04	0.93	40.8 (44.83) 47.6	8.16–10.88 x 8.16–10.88	5.44 (6.57) 6.8	2.72 (3.01) 4.08	9.52 (11.65) 13.6
<i>C. cognata</i> 6	40.8 (44.56) 47.6	46.24 (51.5) 55.76	0.86	32.64 (35.9) 38.08	5.44–10.88 x 8.16–12.24	5.44 (6.71) 8.16	2.72 (2.83) 3.4	9.52 (11.06) 14.96
<i>C. cordifolia</i> 24905	43.52 (46.47) 50.32	46.24 (49.14) 51.68	0.95	38.08 (41.21) 43.52	6.8–9.52 x 6.8–9.52	5.44 (7.25) 8.16	2.04 (2.63) 2.72	8.16 (10.93) 13.6
<i>C. cristobaliana</i> 34544	44.88 (54.45) 61.2	44.88 (59.21) 68	0.92	38.08 (47.15) 54.4	6.8–10.9 x 6.8–14.96	5.44 (6.98) 8.16	2.72 (3.65) 4.08	5.44 (9.65) 4.96
<i>C. desertorum</i> 5840	38.08 (45.7) 48.96	47.6 (50.32) 54.4	0.91	36.72 (39.03) 43.52	8.16–10.88 x 6.8–12.24	6.8 (7.25) 8.16	2.72 (3.20) 4.08	8.16 (10.56) 14.96
<i>C. dusenii</i> 4688	47.6 (48.82) 53.04	57.12 (59.75) 62.56	0.83	42.16 (45.65) 48.96	8.16–12.2 x 6.8–12.24	6.8 (7.84) 9.52	2.72 (2.97) 4.08	6.8 (8.93) 12.24
<i>C. flexuosa</i> 5	39.44 (45.83) 51.68	46.24 (51.54) 55.76	0.89	32.64 (35.95) 39.44	6.80–10.88 x 6.80–13.6	6.8 (7.43) 9.52	2.72 (3.10) 4.08	8.16 (11.42) 14.96
<i>C. guaranitica</i> 12524	42.16 (46.51) 50.32	43.52 (47.24) 50.32	0.98	38.08 (42.02) 46.24	6.8–9.52 x 6.8–9.52	5.44 (6.3) 6.8	2.72 (3.04) 4.08	9.52 (12.19) 16.32
<i>C. lithospermifolia</i> 3	38.08 (41.43) 46.24	35.36 (43.75) 46.24	0.95	29.90 (33.10) 36.70	5.44–9.52 x 5.44–10.90	5.44 (5.67) 6.8	1.36 (2.68) 2.85	6.8 (10.11) 13.6
<i>C. nicolacii</i> 682	46.24 (49.32) 51.68	50.32 (54.26) 58.48	0.91	43.52 (47.60) 50.32	6.8–10.88 x 6.8–10.88	6.8 (7.34) 9.52	2.72 (3.24) 4.08	9.52 (11.56) 14.96
<i>C. nicolacii</i> 5533	46.24 (52.04) 57.12	54.4 (58.21) 61.2	0.89	46.24 (49.96) 57.12	9.52–13.6 x 6.8–12.24	6.8 (7.98) 9.52	1.36 (2.45) 2.72	8.16 (12.33) 16.32
<i>C. obovata</i> 3475	38.08 (44.93) 50.32	44.88 (48.01) 50.32	0.94	32.64 (38.54) 43.52	6.8–12.24 x 8.16–10.90	5.44 (6.48) 8.16	2.04 (2.72) 3.4	6.8 (9.97) 12.24
<i>C. oligophylla</i> 1729	47.6 (53.54) 57.11	53.04 (60.02) 68	0.89	40.80 (45.02) 50.32	8.16–12.24 x 8.16–13.6	6.8 (7.8) 9.52	2.72 (4.08) 5.44	8.16 (11.38) 14.96
<i>C. platenis</i> 3083	36.72 (37.4) 38.08	40.8 (42.84) 44.88	0.87	39.44 (42.11) 44.88	8.16–9.52 x 6.80–8.16	5.44 (6.12) 6.8	2.72 (3.4) 4.08	8.16 (8.84) 9.52
<i>C. platenis</i> 4989	36.72 (38.76) 42.16	40.8 (44.06) 47.6	0.88	35.36 (38.44) 40.8	6.8–9.52 x 6.8–9.52	5.44 (5.89) 6.8	2.04 (3.72) 4.08	6.8 (10.34) 12.24
<i>C. propinqua</i> 20	39.44 (43.57) 47.6	42.16 (47.15) 51.68	0.92	34.00 (36.86) 40.80	6.80–9.52 x 6.80–9.52	6.8 (8.29) 9.52	2.04 (2.85) 3.4	8.16 (10.79) 13.6
<i>C. sceptra</i> 909	43.52 (48.01) 53.04	48.96 (51.63) 55.76	0.93	36.36 (38.44) 42.16	8.16–12.24 x 6.8–10.9	5.44 (7.28) 9.52	2.72 (3.55) 4.08	9.52 (11.7) 14.96
<i>C. simplex</i> 27322	47.6 (51.54) 54.4	48.96 (51.77) 55.77	0.99	40.8 (44.20) 47.6	6.8–12.24 x 6.8–12.24	5.44 (6.4) 6.8	1.36 (1.41) 2.04	6.8 (13.01) 14.96
<i>C. simplex</i> 69630	43.52 (45.33) 47.6	40.8 (45.79) 48.96	0.99	40.8 (43.29) 46.24	6.8–9.52 x 6.8–10.88	5.44 (6.16) 6.8	2.04 (2.69) 4.08	9.52 (11.33) 13.6
<i>C. simplex</i> 23053	44.88 (45.97) 47.6	44.88 (46.51) 48.96	0.99	42.16 (44.06) 46.24	8.16–9.52 x 6.8–9.52	5.44 (6.8) 8.16	2.72 (2.99) 3.4	10.88 (11.15) 12.24
<i>C. verbascifolia</i> 44	42.16 (45.51) 48.96	46.24 (48.91) 54.4	0.93	36.72 (39.98) 42.16	6.80–12.20 x 8.16–10.90	5.44 (6.71) 8.16	2.72 (3.22) 4.08	5.44 (8.57) 9.52

P – polar axis, E – equatorial diameter, P/E – ratio; P, E, colpus length, pore diameter, exine thickness, spine length and polar lacunae diameter – all in μm .

Table 3. List of palynological characters used in the statistical analyses of *Chrysolaena* species.

	Character
1	Polar axis (μm)
2	Equatorial diameter (μm)
3	Exine thickness (μm)
4	Spines length (μm)
5	Lacunae diameter (μm)
6	Colpus length (μm)
7	Spines
	0=presence
	1=absence

and 7; Plate 3, figures 1 and 4). The colpi are visible in polar view (Plate 2, figure 5) and relatively long, measuring 32.64 to 57.12 μm . They interrupt the lophae that separate the poral lacuna from the abporal lacunae. The pori are circular and lalongate in most species, and lolongate in the remaining species (Plate 2, figure 6; Plate 3, figure 6). The exine excluding the spines is 5.44 to 9.52 μm thick, with the sexine thicker than the nexine. The tectum is discontinuous, densely microperforate, usually with acute spines linearly disposed on the lophae (Plate 2, figure 8; Plate 3, figure 3), and rarely with verrucae (Plate 3, figures 4–6) of 1.36–2.04 μm in diameter (in *C. simplex*). The spine length varies from 1.36 μm in *C. simplex* (N° 27322) to 5.44 μm in *C. oligophylla* (N° 1729).

Key to the species of *Chrysolaena* based on pollen morphology

- A. Pollen grains with warts.
 - 1a. *C. simplex* 27322
- A'. Pollen grains with spines.
 - B. Polar axis 37.4–45.97 μm .
 - C. Equatorial diameter 42.84–46.51 μm .
 - D. Colpus 33.1–38.44 μm long.
 - E. Spines 1.36–2.85 μm long.
2. *C. lithospermifolia*
 - E'. Spines 2.04–4.08 μm long.
 - 3a. *C. platenis* 4989
 - D'. Colpus 42.11–44.06 μm long.
 - F. Polar lacunae > 11 μm in diameter.
 - 1b. *C. simplex* 23053–69630
 - F'. Polar lacunae < 11 μm in diameter.
 - 3b. *C. platenis* 3083
- C'. Equatorial diameter 47.15–51.54 μm .
- G. Colpus 35.9–36.86 μm long.
- H. Exine < 7 μm thick.
4. *C. cognata*
- H'. Exine > 7 μm thick.
- I. Spines > 3 μm long
5. *C. flexuosa*
- I'. Spines < 3 μm long

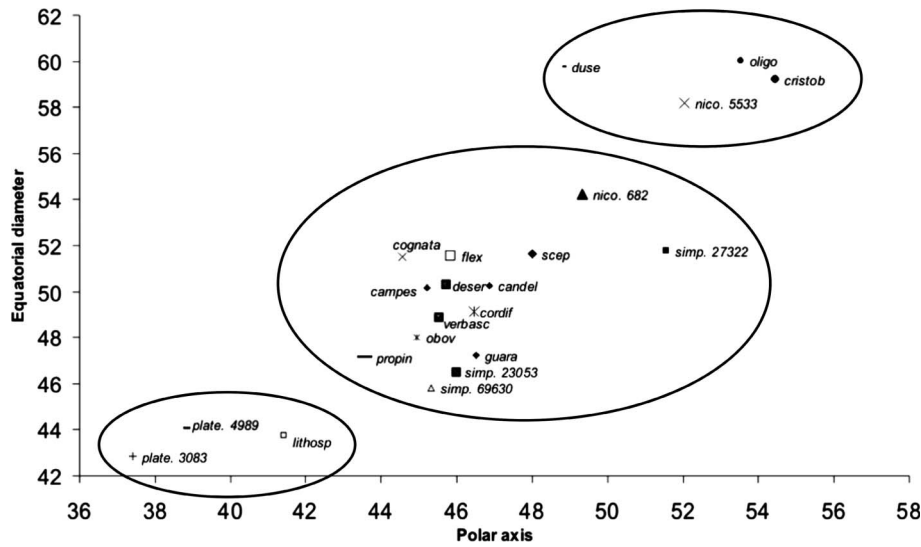


Figure 1. Dispersion diagram showing the relationship between polar axis and equatorial diameter (μm). Circles indicate three groups of species differentiated by pollen size. Abbreviations: *oligo* – *Chrysoleaena oligophylla*; *cristo* – *C. critobaliana*; *duse* – *C. dusenii*; *nico* – *C. nicolackii*; *simp* – *C. simplex*; *flex* – *C. flexuosa*; *scep* – *C. sceptrum*; *campes* – *C. campestris*; *deser* – *C. desertorum*; *candel* – *C. candelabrum*; *verbasc* – *C. verbascifolia*; *obov* – *C. obovata*; *guara* – *C. guaranitica*; *propin* – *C. propinqua*; *lithosp* – *C. lithospermifolia*; *plate* – *C. platensis*.

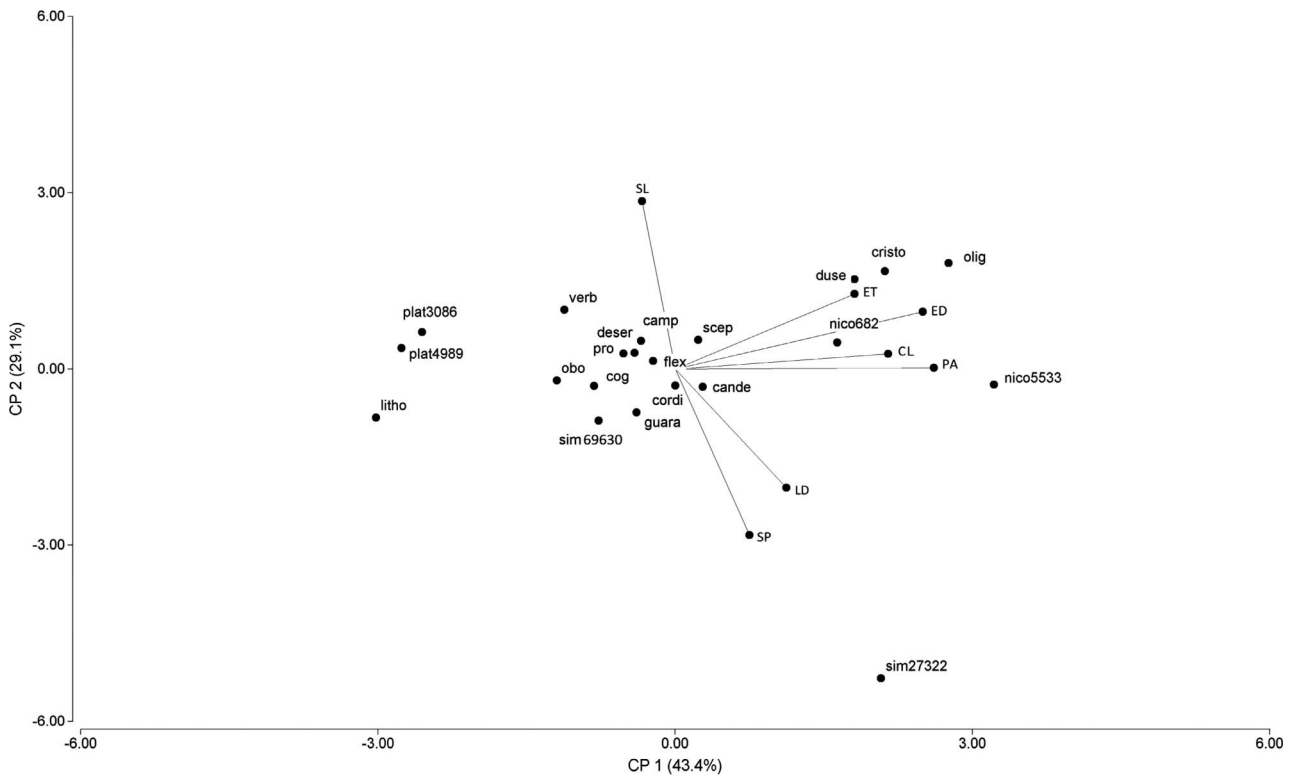


Figure 2. Principal component analysis of *Chrysoleaena* species. Abbreviations: *olig* – *Chrysoleaena oligophylla*; *cristo* – *C. critobaliana*; *duse* – *C. dusenii*; *nico* – *C. nicolackii*; *simp* – *C. simplex*; *flex* – *C. flexuosa*; *scep* – *C. sceptrum*; *camp* – *C. campestris*; *deser* – *C. desertorum*; *cande* – *C. candelabrum*; *verb* – *C. verbascifolia*; *obo* – *C. obovata*; *guara* – *C. guaranitica*; *pro* – *C. propinqua*; *litho* – *C. lithospermifolia*; *plat* – *C. platensis*. CP – component; ED – equatorial diameter; PA – polar axis; CL – colpus length; LD – lacunae diameter; SL – spine length; SP – spine presence; ET – exine thickness.

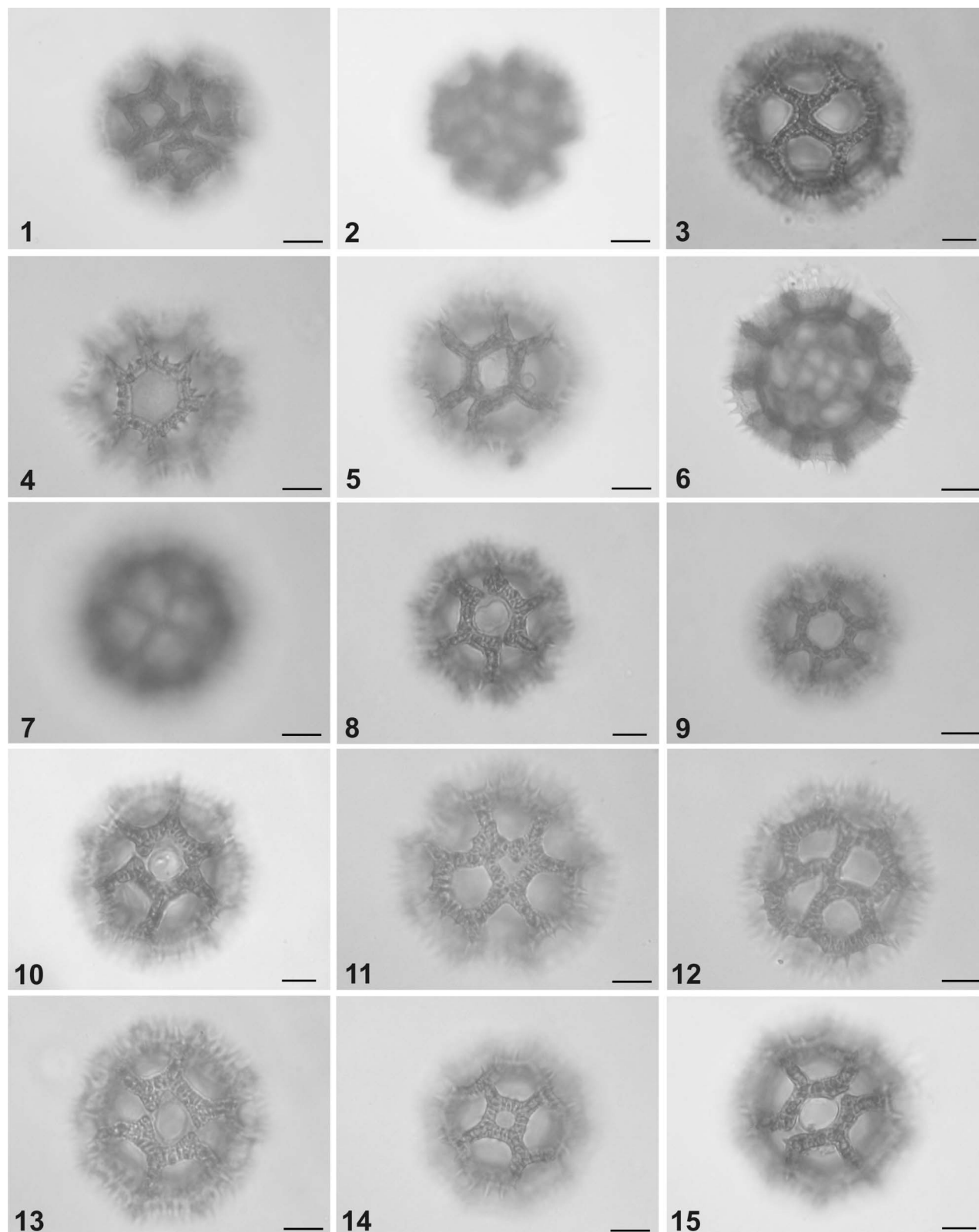


Plate 1. Pollen of *Chrysolaena* (LM). Figures 1–2. *C. dusenii*: Figure 1. Polar view, high focus; (2) polar view, low focus; (3) *C. flexuosa*, equatorial view, mesocolpium; Figures 4–7. *C. guaranitica*: (4) Polar view; (5) Equatorial view, high focus; (6) Equatorial view, mid focus; (7) Equatorial view, low focus; (8) *C. lithospermifolia*, equatorial view showing aperture; (9) *C. platensis*, polar view; (10) *C. propinqua*, equatorial view; Figures 11–13. *C. oligophylla*: (11) Polar view; (12) Equatorial view, mesocolpium; (13) Equatorial view; (14) *C. candelabrum*, atypical equatorial view showing equatorial lacuna; (15) *C. verbascifolia*, equatorial view. Scale bars – 10 μm .

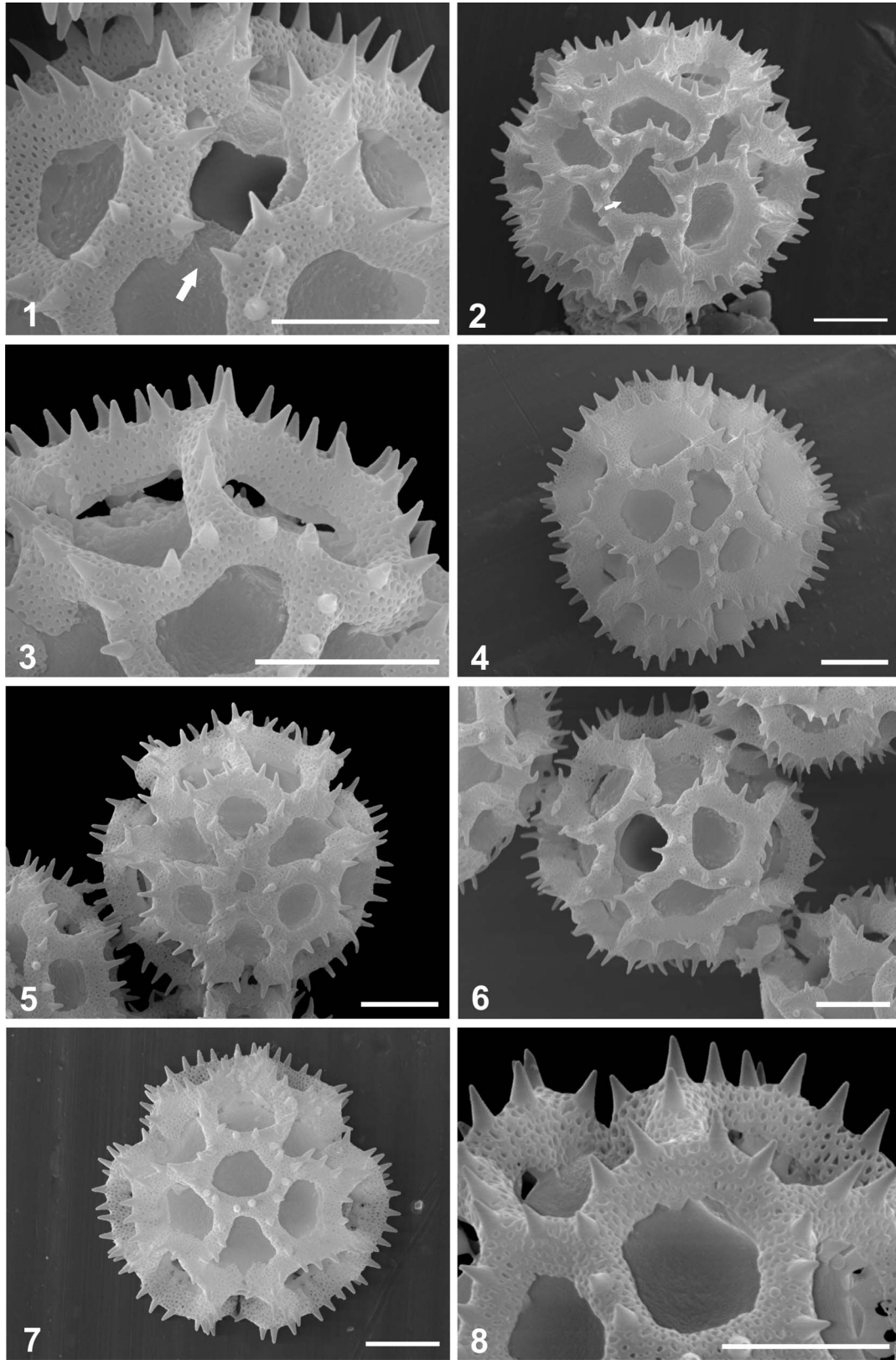


Plate 2. Pollen of *Chrysolaena* (SEM). Figure 1. *C. campestris*, close up of the colpus area with pore; note the colpus interruption to lophate ridge (arrow); Figure 2. *C. candelabrum*, polar view showing polar lacuna (arrow); Figure 3. *C. cognata*, detail of microperforate tectum and spines; Figure 4. *C. cordifolia*, polar view showing polar lacuna; Figure 5. *C. cristobaliana*, polar view; Figure 6. *C. desertorum*, equatorial view; Figure 7. *C. obovata*, polar view showing the colpi; Figure 8. *C. sceptrum*, detail of lacunae and spines. Scale bars – 10 μ m.

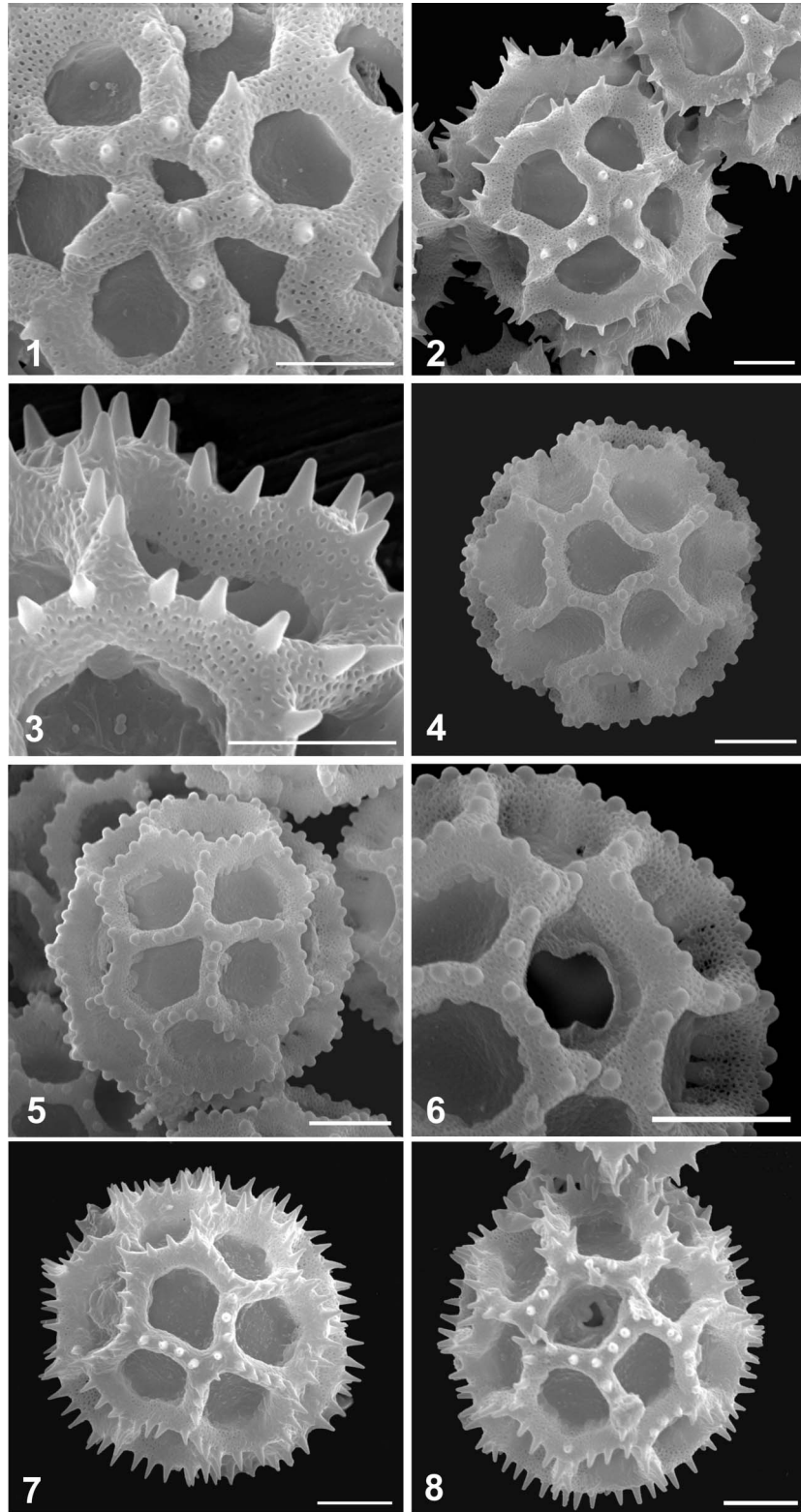


Plate 3. Pollen of *Chrysoalaena* (SEM). Figures 1–2. *C. duseinii*: (1) Close up of polar view; (2) Equatorial view, mesocolpium, showing the 4 intercolpar lacunae and the absence of equatorial lacuna; (3) *C. nicolackii*. Cordeiro & Nicolack 682, detail of spines; Figures 4–6. *C. simplex*. Hatschbach 27322: (4) Polar view showing polar lacuna; (5) Equatorial view, mesocolpium; (6) Close up, perimeter of equatorial view showing porus and verrucae; Figures 7–8. *C. simplex*. Hatschbach et al. 69630: (7) Equatorial view, mesocolpium; (8) Equatorial view. Scale bars – 10 μm .

6. *C. propinqua*
 G'. Colpus 38.54–39.98 μm long.
 J. Exine <7 μm thick.
 K. Spines > 3 μm long.
7. *C. verbascifolia*
 K'. Spines <3 μm long.
8. *C. obovata*
 J'. Exine > 7 μm thick.
 L. Polar lacunae 10.74 μm in average diameter.
9. *C. campestris*
 L'. Polar lacunae 10.56 μm in average diameter.
10. *C. desertorum*
 B'. Polar axis 46.47–54.45 μm .
 M. Equatorial diameter 47.24–54.26 μm .
 N. Colpus 38.44–42.02 μm long.
 O. Spines <3 μm long.
11. *C. cordifolia*
 O'. Spines > 3 μm long.
 P. Exine <7 μm thick.
12. *C. guaranitica*
 P'. Exine > 7 μm thick.
13. *C. sceptrum*
 N'. Colpus 44.83–47.60 μm long.
 Q. Exine > 7 μm thick.
14. *C. candelabrum*
 Q'. Exine <7 μm thick.
- 15a. *C. nicolackii* 682
 M'. Equatorial diameter 58.21–60.02 μm .
 R. Colpus 45.02–45.65 μm long.
 S. Exine <3.5 μm thick.
16. *C. dusenii*
 S'. Exine > 3.5 μm thick.
17. *C. oligophylla*
 R'. Colpus 47.15–49.96 μm long.
 T. Exine 5.44 to 8.16 μm thick.
18. *C. cristobaliana*
 T'. Exine thickness 6.8 (7.25) 8.16 μm .
- 15b. *C. nicolackii* 5533

3.2. Statistical analyses

In the dispersion diagram (Figure 1), which relates the polar axis to the equatorial diameter, three more or less definite groups differing in pollen size can be identified. Group 1 includes two species, *C. platensis* and *C. lithospermifolia*, which possess the smallest pollen grains (P: 37.4–41.43 μm , E: 42.84–44.06 μm). Group 2 includes thirteen species, *C. campestris*, *C. candelabrum*, *C. cognata*, *C. cordifolia*, *C. desertorum*, *C. flexuosa*, *C. guaranitica*, *C. nicolackii* N° 682, *C. obovata*, *C. propinqua*, *C. sceptrum*, *C. simplex*, and *C. verbascifolia*, which all possess intermediate-sized pollen grains (P: 43.57–51.54 μm , E: 45.79–54.26 μm). Finally, Group 3 includes four species, *C. dusenii*, *C. nicolackii* N° 5533, *C. oligophylla*, and *C. cristobaliana*,

which possess the largest pollen grains (P: 48.82–54.45 μm , E: 58.21–60.02 μm).

The Principal Component Analysis of pollen characters showed that 72.5% of the pollen morphological variation can be explained by the first two principal components, PC I–II (Figure 2). PC I explains 43.4% of the total variation, while PC II explains 29.1%. Eigenvectors resulting from the ordination analysis indicate that the equatorial diameter, polar axis, exine thickness, and colpus length are strongly positively correlated with PC I, the spine length correlates positively with PC II, whereas the presence of spines and lacunae diameter correlate negatively with PC II. The scatter plot of the PC I and PC II components shows three more or less defined groups of species. Isolation of the population of *C. simplex* N° 27322 from the rest is explained by the absence of spines.

The hypothesis of no differences among pollen in *Chrysolaena* species was tested using MANOVA. The analysis yielded a WILKS' $\lambda=0.06$; $F=18.21$, $p<0.0001$, which means that pollen grain morphology is significantly different among species.

4. Discussion

Chrysolaena resulted from the segregation of different sections and subsections of *Vernonia* to new genera (Robinson 1988). This segregation was based on different features, including vegetative morphology, chromosome number, chemical composition, and especially the variation in pollen morphology among the different genera. In this study, we show that *Chrysolaena* has pollen grains of type "C", as described by Keeley and Jones (1979), and previously designated as type "*Vernonia cognata*" by Stix (1960). Our results support the conclusion of Robinson (1988) that this type of pollen characterizes the entire genus.

This is the first study describing pollen morphology in all species of the genus *Chrysolaena*. Our findings are consistent with reports by Mendonça et al. (2007) on *C. lithospermifolia*, *C. platensis*, *C. oligophylla*, and *C. obovata* [sub nom. *C. herbacea* (Vell.) H. Rob.], and agree with work by Dematteis & Pire (2008) on *C. propinqua*, and Mendonça et al. (2010) on *C. desertorum* and *C. simplex* (sub nom. *Lessingianthus*).

Chrysolaena species are palynologically homogeneous with respect to pollen type, shape (oblate-spheroidal), and number of apertures (3-colporate). They differ in grain size, exine thickness, spine length, and diameter of the polar lacuna (Table 2). Based on these latter quantitative pollen traits, it was possible to compile a taxonomic key to all species. The large infraspecific variation in *C. platensis* and *C. nicolackii* led to the separation of specimens from different populations in the key.

Species were also grouped according to grain size as suggested by the dispersion diagram of the relationship P/E (see Figure 2). However, some populations appear separated from other samples of the same species. For example, in *C. nicolackii*, one population is included in Group 2 (medium pollen grains), while the other is in Group 3 (large pollen grains). According to the Erdtman (1966) classification of pollen size, pollen grains of *Chrysolaena* are considered medium (25–50 μm) to large (50–100 μm).

Chrysolaena simplex N° 27322 is the only taxon that differs in wall ornamentation. Instead of the acute spines characterizing all other species (Plate 3, figures 4–6), it displays elements that – according to their length and morphology – can be classified as verrucae (Punt et al. 2007). Verrucae are wart-like sexine elements that are more than 1 μm wide, wider than high, and are not constricted at the base. However, the other two populations of *C. simplex* studied possess spines on the tectum, as in Dematteis (2009) and Mendonça et al. (2010).

Two basic forms of pollen grains, echinolophate and subechinolophate, have been recognized in North American Vernoniae by Wodehouse (1928). Several authors (e.g. Jones 1970; Kingham 1976) suggested that there is an evolutionary trend from the “primitive” echinate or subechinolophate to the “advanced” psilolophate and echinolophate pollen grains. The psilolophate form would result from a gradual reduction of the spines. This form of pollen grain is observed, for example, in *Lessingianthus chamaepeuces* (Sch. Bip. ex Baker) H. Rob., characterized by pollen type “B” (Angulo & Dematteis 2010), and *Mesanthophora brunneri* H. Rob., with pollen type “E” (Dematteis & Pire 2008). This paper reports for the first time the occurrence of intraspecific polymorphism as a result of the variation in the wall ornamentation in *C. simplex*. The ornamentation found in *C. simplex* have not been observed before in *Chrysolaena*, in which spines or spinules are the most common ornamentation (Punt et al. (2007), who refer to them as sharp objects). Wart-like structures similar to those of *C. simplex* and called “short spinules” occur in species of other Vernoniae genera such as *Elephantopus* L., *Pseudoelephantopus* Rohr., and *Rolandra* Rottb., which have pollen type “F”, and *Sparganophorus* Boehm. with pollen type “J” (Robinson 1992). However, compared to *C. simplex*, pollen grains in these species are triplicate and differ in the disposition of the lacunae. The original description of pollen type “C” refers to spines or projections of the wall, and therefore does not exclude the unique pollen morphology observed in *C. simplex*.

Most studies in the Vernoniae have shown that different groups share not only similar pollen types, but also morphological and cytological characters (such as

number of chromosomes). However, differences in the interpretation of results and the various analytical approaches used make it difficult to compare results across studies. Also the description of new species and the continuous evolution of the group hamper resolution of phylogenetic relationships in the Vernoniae.

Pollen type “C” is found within several genera of Vernoniae such as *Eirmocephala* H. Rob. and *Lepidaploa*. In the New World, this pollen type is characteristic of the genera *Stenocephalum* Sch. Bip. and *Chrysolaena* (Robinson 1999), although conspicuous morphological features differentiate them from one another.

Depending on the shared features considered, *Chrysolaena* appears to be phylogenetically more closely related to either *Lessingianthus* or *Lepidaploa*. With respect to pollen morphology, *Chrysolaena* appears to be more closely related to *Lepidaploa*. This relationship is further supported by the presence of glands on anther appendages in some species of *Lepidaploa* (Robinson 1990), and also by molecular studies (Keeley et al. 2007), in which *Lessingianthus* appears sister to *Chrysolaena* and *Lepidaploa*.

Lessingianthus (pollen type “B”) is generally characterized by possessing larger pollen than the remaining species of the tribe. Pollen size is often influenced by internal and external factors (genetic and environmental). In many cases, species show a positive correlation between pollen size and chromosome number or ploidy level. For instance, this correlation appears to exist in *Lessingianthus*, since it includes the greatest proportion of polyploid entities and the highest ploidy levels (i.e. octoploids and decaploids) observed in tribe Vernoniae (Dematteis 2002; Angulo & Dematteis 2012). Although in *Chrysolaena*, cytological information is still incomplete, and chromosome numbers are still unknown in some species, the base number in the genus is $x=10$, with chromosome numbers ranging from $2n=20$ to $2n=80$ and different ploidy levels or cytotypes observed (Ruas et al. 1991; Dematteis 1997, 1998, 2002, 2007, 2009; Angulo & Dematteis 2009a, 2009b; Via do Pico & Dematteis 2012). Both diploid and tetraploid populations were found in *C. flexuosa*, *C. propinqua* and *C. lithospermifolia*, whereas only diploid populations were found in *C. verbascifolia*, only octoploid populations in *C. sceptrum*, and one tetraploid population in *C. simplex*. *Chrysolaena cognata* and *C. platensis* display the greatest variation in ploidy levels with diploid, tetraploid, hexaploid and octoploid populations, and even odd polyploids in *C. cognata* (Galiano & Hunziker 1987; Ruas et al. 1991; Dematteis 1997a, 2002, 2009; Angulo & Dematteis 2009b; Via do Pico & Dematteis 2012). Considering our measurements of pollen size and these published counts we observe no correlation between ploidy level and pollen

size: pollen grains of diploid taxa are similar in size to those of polyploid species. This lack of correlation was observed also in *Vernonanthura* H. Rob., another genus of the tribe Vernonieae segregated from *Vernonia*. As in *Chrysolaena*, all species in *Vernonanthura* have very similar pollen grains, and pollen of some species can only be distinguished from that of others based on the length and morphology of the spines (Vega & Dematteis 2011).

Principal Component Analysis of pollen data recognizes three groups of species within *Chrysolaena* (Figure 2). Firstly, the polar axis and equatorial diameter and secondly, the length and presence of spines contribute to the discrimination of taxa. The groupings observed were consistent with the dispersion diagram showing the relationship P/E (Figure 1).

Although the morphology of pollen grains is very similar among *Chrysolaena* species, MANOVA results were significant. Palynological studies in *Lessingianthus* (Angulo & Dematteis 2010) and *Stemodia* L. (Plantaginaceae) (Sosa et al. 2012) also show that the implementation of quantitative trait analyses and multivariate statistical analyses is a useful approach when species display very similar characters.

5. Conclusions

The pollen grain type “C” that characterizes *Chrysolaena* was found in all specimens analyzed. Based on our results, *Chrysolaena* is considered a stenopalynous genus. However, using quantitative traits it was possible to build a taxonomic key to separate species, and statistical analyses of pollen grain characteristics allowed us to recognize three more or less definite groups of species. Despite similarity in pollen type, multivariate analyses support the use of quantitative pollen data as good taxonomic characters. Finally, pollen morphology constitutes a good taxonomic marker, in addition to other morphological and molecular features that characterize *Chrysolaena*.

Acknowledgements

We thank B. Marazzi for assisting with the English. This work was supported by greatly appreciated grants from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

Author biographies

GISELA MARIEL VIA DO PICO is a Research Fellow at the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Botánica del Nordeste, Corrientes, Argentina. She graduated from the Universidad Nacional del Nordeste with an MA in Biology in 2010. She is currently Assistant Professor of Cell and General Biology at the Universidad Nacional del Nordeste. She is a postgraduate student of the Universidad Nacional de Córdoba,

Argentina. Her research focuses on the morphology, anatomy, cytology, palynology and taxonomy of *Chrysolaena* (Asteraceae).

MASSIMILIANO DEMATTEIS is a Senior Researcher at the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Botánica del Nordeste, Corrientes, Argentina. He graduated from the Universidad Nacional de Misiones with an MA in Genetics in 1994 and a BD from the Universidad Nacional de Córdoba in 2000. He was Assistant Professor of Genetics from 1995–1998 and Associate Professor of Cell and General Biology at the Universidad Nacional del Nordeste from 2003–2010. He is currently Professor of Cell and Molecular Biology and President of the Botanical Society of Argentina. His research currently focuses on the morphology, anatomy, palynology and taxonomy of the plant family Asteraceae, including the tribes Eupatorieae and Vernonieae from the New World.

References

- Angulo MB, Dematteis M. 2009a. Caryological analysis of South American species of *Vernonia* (Vernonieae, Asteraceae). *Plant Biosys.* 143(1): 20–24.
- Angulo MB, Dematteis M. 2009b. Karyotype analysis in eight species of *Vernonia* (Vernonieae, Asteraceae) from South America. *Caryologia* 62(2): 81–88.
- Angulo MB, Dematteis M. 2010. Pollen morphology of the South American genus *Lessingianthus* (Vernonieae, Asteraceae) and its taxonomic implications. *Grana* 49: 12–25.
- Angulo MB, Dematteis M. 2012. Cytotaxonomy of some species of the South American genus *Lessingianthus* (Asteraceae, Vernonieae). *Plant System Evol.* 298(2): 277–285.
- Cabrera AL. 1944. Vernoneias Argentinas (Compositae). *Darwiniana* 6: 265–379.
- Dematteis M. 1997. Cromosomas en *Vernonia platensis* y especies afines (Asteraceae). *Bonplandia* 9(3–4): 259–264.
- Dematteis M. 1998. Chromosome studies on *Vernonia flexuosa* and *V. lithospermifolia*. *Compos Newslett.* 32: 10–16.
- Dematteis M. 2002. Cytotaxonomic analysis of South American species of *Vernonia* (Vernonieae: Asteraceae). *Bot J Linn Soc.* 139(4): 401–408.
- Dematteis M. 2007. Taxonomic notes on the genus *Chrysolaena* (Vernonieae, Asteraceae), including a new species endemic to Paraguay. *Annal Botan Fenn.* 44(1): 56–64.
- Dematteis M. 2009. Revisión taxonómica del género sudamericano *Chrysolaena* (Vernonieae, Asteraceae). *Bol Soc Arg Botán.* 44(1–2): 103–170.
- Dematteis M, Pire SM. 2008. Pollen morphology of some species of *Vernonia sensu lato* (Vernonieae, Asteraceae) from Argentina and Paraguay. *Grana* 47: 117–129.
- Di Rienzo JA, Casanoves F, Balzarini MG, González L., Tablada M, Robledo CW. InfoStat versión 2011. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. Available from: <http://www.infostat.com.ar>.
- Erdtman G. 1960. The acetolysis method. A revised description. *Svensk Botanisk Tidskrift* 54: 561–564.
- Erdtman G. 1966. Pollen morphology and plant taxonomy. Angiosperms: An introduction to Palynology. New York: Hafner Publishing Company.
- Galiano NG, Hunziker JH. 1987. Estudios cariológicos en Compositae IV. Vernonieae y Eupatorieae. *Darwiniana* 28(1–4): 1–8.

- Jones SB. 1970. Scanning electron microscopy of pollen as an aid to the systematics of *Vernonia* (Compositae). Bull Torrey Bot Club 97: 325–335.
- Jones SB. 1979. Chromosome numbers of Vernoniaeae (Compositae). Bull Torrey Bot Club 106: 79–84.
- Jones SB. 1981. Synoptic classification and pollen morphology of *Vernonia* (Compositae: Vernoniaeae) in the Old World. Rhodora 83: 59–75.
- Keeley SC, Jones SB. 1977. Taxonomic implications from external pollen morphology to *Vernonia* (Compositae) in the West Indies. Am J Bot. 64: 576–584.
- Keeley SC, Jones SB. 1979. Distribution of the pollen types in *Vernonia* (Vernoniaeae: Asteraceae). System Bot. 4: 195–202.
- Keeley SC, Robinson H. 2009. Vernoniaeae. In: Funk VA, Susanna A, Stuessy TF, Bayer RJ, editors. Systematics, evolution and biogeography of Compositae, Vienna: International Association for Plant Taxonomists; p. 439–469.
- Keeley SC, Forsman ZH, Chan R. 2007. A phylogeny of the “evil tribe” (Vernoniaeae: Compositae) reveals Old/New World long distance dispersal: Support from separate and combined congruent datasets (trnL-F, ndhF, ITS). Mol Phylogen Evol. 44: 89–103.
- Kingham DL. 1976. A study of the pollen morphology of tropical African and certain other Vernoniaeae (Compositae). Kew Bull. 31: 9–26.
- Mendonça CBF, Albuquerque de Souza M, Gonçalves-Esteves V, Esteves RL. 2007. Palinotaxonomia de espécies de *Chrysolaena* H. Rob., *Echinocoryne* H. Rob. e *Stenocephalum* Sch. Bip. (Vernoniaeae-Compositae) ocorrentes no sudeste do Brasil. Acta Bot Brasil. 21(3): 627–639.
- Mendonça CBF, Carrijo TT, Esteves RL, Gonçalves-Esteves V. 2010. *Lessingianthus* H. Rob. (Vernoniaeae-Asteraceae): Generic and infrageneric relationships based on pollen morphology. Nordic J Bot. 28: 1–10.
- Punt W, Hoen PP, Blackmore S, Nilsson S, Le Thomas A. 2007. Glossary of pollen and spore terminology. Rev Palaeobot Palynol. 143(1–2): 1–81.
- Robinson H. 1988. Studies in the *Lepidaploa* complex (Vernoniaeae: Asteraceae). V. The new genus *Chrysolaena*. Proc Biol Soc Washington 100(4): 952–958.
- Robinson H. 1990. Studies in the *Lepidaploa* complex (Vernoniaeae: Asteraceae). VII. The genus *Lepidaploa*. Proc Biol Soc Washington 103(2): 464–498.
- Robinson H. 1992. The Asteraceae of the Guianas, III: Vernoniaeae and restoration of the genus *Xiphochaeta*. Rhodora 94: 348–361.
- Robinson H. 1999. Generic and subtribal classification of American Vernoniaeae. Smithson Contrib Bot. 89: 1–116.
- Ruas PM, Ruas CF, Vieira AOS, Matzenbacher NI, Martins NS. 1991. Cytogenetics of genus *Vernonia* Schreber (Compositae). Cytologia 56: 239–247.
- Sosa MM, Panseri AF, Salgado CR. 2012. Pollen morphology of eight species of *Stemodia* (Plantaginaceae) from South America. Palynology 36: 1–9.
- Steetz J. 1864. Crystalpollen und Ambassa. In Peters WCH, editor. Naturwissenschaftliche Reise nach Mossambique auf Befehl seiner Majestüats des Künionigs Friedrich Wilhelm. IV. Vol. 6, Botanik, P. 2. Berlin: G. Reimer; p. 363–364.
- Stix E. 1960. Pollenmorphologische Untersuchungen an Compositen. Grana Palynol. 2: 41–104.
- Vega AJ, Dematteis M. 2011. Pollen morphology of some species of *Vernonanthura* (Asteraceae, Vernoniaeae) from southern South America. Palynology 35: 94–102.
- Via do Pico GM, Dematteis, M. 2012. Chromosome number, meiotic behavior and pollen fertility of six species of *Chrysolaena* (Vernoniaeae, Asteraceae). Caryologia 65(3): 176–181.
- Wodehouse RP. 1928. Phylogenetic value of pollen grain characters. Annal Bot. 168: 891–934.