Plant Systematics and Evolution (2018) 304:981–993
https://doi.org/10.1007/s00606-018-1526-z

ORIGINAL ARTICLE

Pollen morphology in Brazilian species of Gloxiniinae (Gesneriaceae): variation in apertures and pattern of ornamentation

Cintia N. de Souza[1] · Andréa O. de Araujo[2] · Alain Chautems[3] · Maria A. V. da Cruz-Barros[4] · Eduardo C. Gasparino[1]

Received: 20 February 2018 / Accepted: 4 June 2018 / Published online: 30 June 2018
© Springer-Verlag GmbH Austria, part of Springer Nature 2018

Abstract

Gloxiniinae comprises herbs and subshrubs usually growing on rocky outcrops, rocky fields or gallery forests. For Gesneriaceae in some cases variations in pollen morphology allow the differentiation of genera and species. We studied the pollen grains of ten Brazilian species of Gloxiniinae (Gesneriaceae), including the genera *Chautemsia*, *Gloxinia*, *Goyazia*, *Mandirola* and *Seemannia* with the purposes of describing the pollen morphology of these genera, and contributing to the taxonomic characterization of the group and a better delimitation of genera and species of Gesneriaceae. The pollen grains were acetolyzed, measured, photographed and described qualitatively. Quantitative data were analyzed through descriptive and multivariate statistics. Non-acetolyzed pollen grains were observed in SEM and TEM in order to provide more details of the ornamentation and the structure of the exine. The analyzed species differ as to the pollen amb, shape, length, width and ends of colpi, presence or absence of margo, type of endoaperture and exine ornamentation. We found circular, subcircular and subtriangular amb, oblate spheroidal and subprolate shape, long or very long colpi, narrow, wide or very wide, with or without margo, lalongate or lolongate endoaperture in microreticulate or reticulate pollen grains. The reticulate ornamentation appears in the *Gloxinia* species' pollen grains, and lolongate endoapertures represent the pattern for the subtribe pollen grains except in *Chautemsia*. The pollen grain metrics examined by principal component analysis allow generic distinction. We conclude that qualitative and quantitative pollen data help to delimit the genera and species of Gloxiniinae.

Keywords Endoaperture · Exine · Gesnerieae · Multivariate analysis · Palynotaxonomy · Pollen grains

Handling editor: Ricarda Riina.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00606-018-1526-z) contains supplementary material, which is available to authorized users.

✉ Cintia N. de Souza
cintiasouza011@gmail.com

Andréa O. de Araujo
andrea.ufabc@gmail.com

Alain Chautems
alain.chautems@ville-ge.ch

Maria A. V. da Cruz-Barros
mcruzbarros@gmail.com

Eduardo C. Gasparino
ecgasparino@fcav.unesp.br

1 Departamento de Biologia Aplicada à Agropecuária, Faculdade de Ciências Agrárias e Veterinárias (FCAV), UNESP—Univ. Estadual Paulista, Via de Acesso Prof. Paulo Donato Castellane, s/n, Jaboticabal, SP 14884-900, Brazil

2 Centro de Ciências Naturais e Humanas, UFABC – Univ. Federal do ABC, Rua São Paulo, s/nº - Jardim Antares, São Bernardo do Campo, SP 09606-070, Brazil

3 Laboratoire de Systématique Végétale et Biodiversité, Conservatoire et Jardin botaniques de la Ville de Genève & Université de Genève, CP 71, 1292 Chambésy, Geneva, Switzerland

4 Instituto de Botânica, Núcleo de Pesquisas em Palinologia, Av. Miguel Stéfano, 3687 Água Funda, São Paulo, SP 04045-972, Brazil

Introduction

Gesneriaceae Rich & Juss. ex DC. includes some 147–148 genera and 3260–3435 species, with a wide distribution in the tropical regions and few representatives in the temperate regions. The family centers of diversity are located in northwestern South America (Colombia to Ecuador) and also in southeastern Brazil (Chautems 1991; Souza and Lorenzi 2012; Weber 2004; Weber et al. 2013). All Neotropical species are included in the subfamily Gesnerioideae, where we find the tribe Gesnerieae and the subtribes Gesneriinae, Gloxiniinae, Columneinae, Sphaerorrhizinae and Ligeriinae (Weber et al. 2013).

The Gloxiniinae is composed of 21 genera and 160 species, comprising the genera previously treated in the traditional Gloxinieae tribe (sensu Roalson et al. 2005a, b; Wiehler 1983). In Brazil, Gloxiniinae is represented by 15 species, distributed in eight genera, three of them are endemic to the country. These species are mostly found on rocky outcrops, rocky fields or gallery forests in the Cerrado biome, growing as perennial herbs or subshrubs, with scaly rhizomes, leaves opposite and thin, flowers mostly purple, sometimes white or red, and dry or fleshy capsule (Araujo 2007; Weber 2004; Weber et al. 2013).

Molecular studies such as that conducted by Smith (1996, 2000), Smith et al. (1997), Citerne et al. (2000), Zimmer et al. (2002), Mayer et al. (2003), Perret et al. (2003), Roalson et al. (2005a, b) and Clark et al. (2006, 2012) discussed the monophyly of groups within Gesneriaceae and reconsidered the placement of some species, genera or even tribes.

Roalson et al. (2005a) and Zimmer et al. (2002) based on phylogenetic analysis suggested that the genera in Gloxiniinae were not monophyletic, especially *Gloxinia* L'Hér, thus pointing to the need for reconsidering the taxonomy of the group (Araujo et al. 2012). So the genera *Gloxinia*, *Goyazia* Taub., *Mandirola* Decne. and *Seemannia* Regel were reviewed and reorganized by Roalson et al. (2005a). In addition, the studies of Boggan et al. (2008) and Araujo et al. (2010a, b) added new descriptions of genera and species, contributing to a clearer taxonomic characterization of the subtribe.

The pollen morphology of some Gloxiniinae species was analyzed by Howard (1975), Williams (1978), Filice et al. (1981), Xifreda (1996) and Gasparino (2008). Gasparino et al. (2010) summarized some palynological studies of Gesneriaceae, emphasizing the importance of pollen morphology for taxa circumscription. Besides the recently developed studies based on molecular characters, pollen morphology studies represent an additional tool for improving delimitation of genera and species in Gesneriaceae, as advocated by Fritze and Williams (1988) and Gasparino et al. (2011).

In this context, this study intends to characterize the pollen grains in Brazilian species of Gloxiniinae (Gesneriaceae) with the purpose of describing the pollen morphology of these genera, contributing to the taxonomic characterization of the group and a better delimitation of genera and species.

Materials and methods

We analyzed the pollen grains of ten Brazilian species of Gloxiniinae (Gesneriaceae): *Chautemsia* A.O.Araujo & V.C.Souza (*Chautemsia calcicola* A.O.Araujo & V.C.Souza), *Gloxinia* L'Hér. (*Gloxinia alterniflora* A.O.Araujo & Chautems; *G. erinoide*s (DC.) Roalson & Boggan; *G. perennis* Fritsch), *Goyazia* Taub. (*Goyazia petraea* (S.M.Phillips) Wiehler; *G. rupicola* Taub.), *Mandirola* Decne. (*Mandirola hirsuta* (DC.) A.O.Araujo & Chautems; *M. multiflora* (Gardner) Decne.; *Mandirola rupestris* (Gardner) Roalson & Boggan) and *Seemannia* Regel (*Seemannia sylvatica* (Kunth) Hanst.) (Fig. 1). However, only the ten species mentioned above were studied in the present study, because for the other five species belonging to the Gloxiniinae subtribe: *Diastema* Benth. (*Diastema racemiferum* Benth.); *Goyazia* Taub. (*Goyazia villosa* (Gardner) R.A. Howard); *Monopyle* Moritz ex Benth. & Hook.f. (*Monopyle reflexa* (Rusby) Roalson & Boggan); *Phinaea* Benth. (*Phinaea albolineata* (Hook.) Benth. ex Hemsl.); and *Seemannia* Regel (*Seemannia purpurascens* Rusby) there were not available material. For the pollen morphology analysis, we used at least two flowers of each specimen, and for each studied species, a "standard" specimen was chosen for presentation of the descriptions and illustrations. These standard specimens are indicated by asterisks in the description of the material used (Online Resource 1). The pollen material was obtained from flowers in anthesis from exsiccates or in alcohol 70% deposited in the herbaria ESA, G, HUFABC, SP and UEC (acronyms according to Thiers (2016), continuously updated).

In light microscopy analysis, pollen grains were acetolyzed according to Erdtman (1960), with modifications proposed by Melhem et al. (2003). We measured the diameters in polar and equatorial views ($n = 25$) and pollen aperture and exine thickness ($n = 10$) up to 7 days after their preparation (Melhem and Matos 1972; Salgado-Labouriau 1973), and the slides were deposited in the pollen reference collection of the Plant Morphology and Palynology Laboratory, Departamento de Biologia aplicada à Agropecuária, Universidade Estadual Paulista, Unesp, Jaboticabal, Brazil. For scanning electron microscopy analysis (SEM), non-acetolyzed pollen grains were prepared according to Melhem et al. (2003), and for transmission electron microscopy analysis (TEM), we followed the protocols of Haddad et al. (1998) and Sabatini et al. (1963).

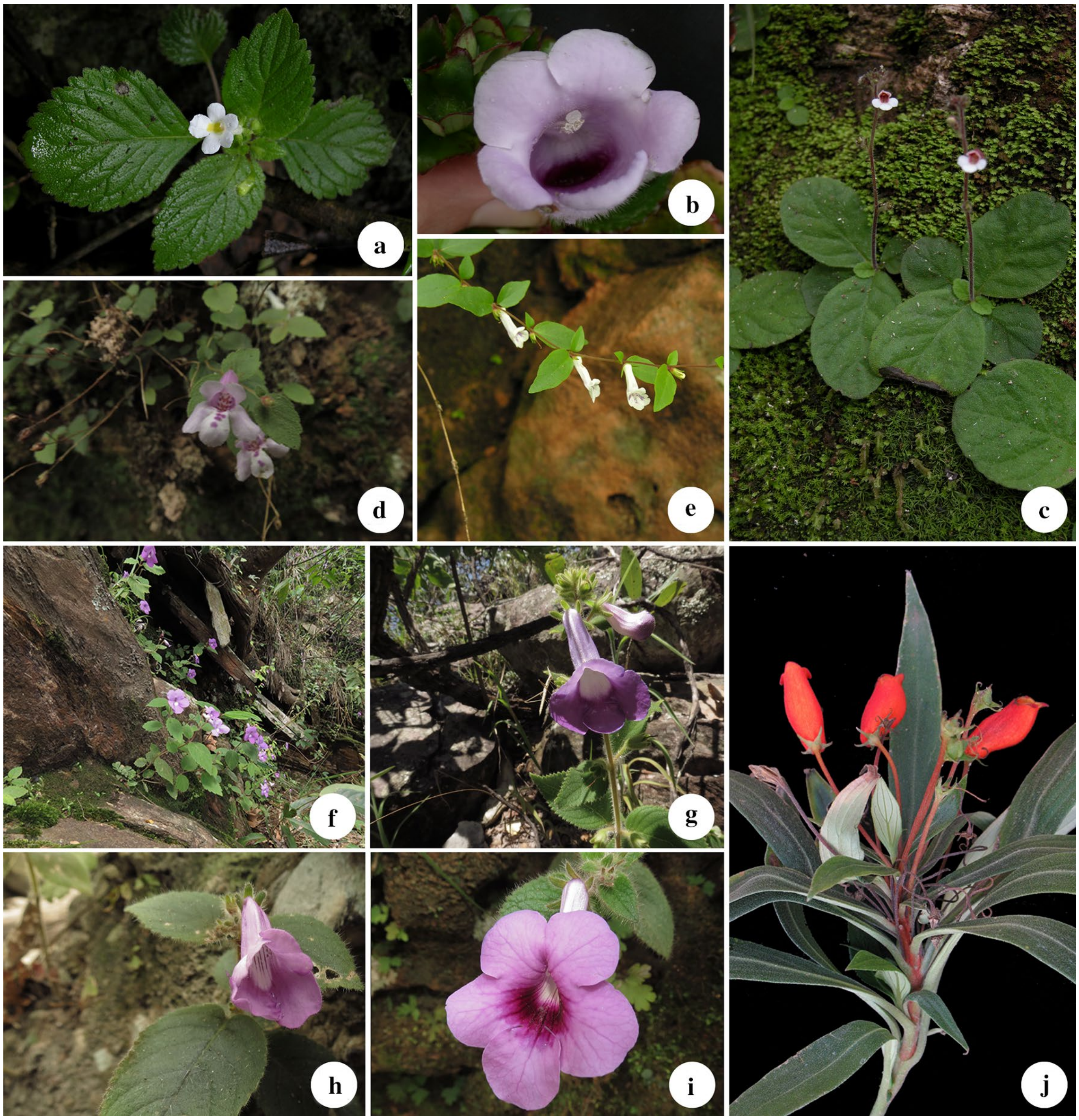

Fig. 1 Overview of the morphological diversity of Brazilian species of Gloxiniinae. **a** *Chautemsia calcicola* (*Araujo* et al. *500*). **b** *Gloxinia perennis* (*Araujo* et al. *468*). **c** *Gloxinia erinoides* (*Araujo* et al. *501*). **d** *Goyazia petraea* (*Araujo* et al. *1065-24*). **e** *Goyazia rupicola* (*Araujo* et al. *545*). **f** *Mandirola hirsuta* (*Araujo* et al. *1139*). **g–h** *Mandirola multiflora* (*Araujo* et al. *1111*; *Araujo* et al. *1128*). **i** *Mandirola rupestris* (*Araujo* et al. *1104*). **j** *Seemannia sylvatica* (*Araujo* et al. *750*)

Means (x), standard deviation (sx), standard error (s), 95% confidence intervals (CI), coefficient of variability (V) and range (R) were calculated based on pollen grains diameter measurements according to Vieira (2011) and Zar (1999). The mean values between each pollen diameter were compared through the analysis of the confidence interval, presenting the data in graphs, generated from the statistical package MINITAB 10.3 for Windows (2003). A Tukey's test was run, and results were represented by horizontal lines at the top of confidence intervals graphs, joining values that were not significantly different.

To verify if the quantitative data of the pollen grains allowed species grouping, a principal component analysis (PCA) was performed using the programs Fitopac (Shepherd 1996) and PC-ORD version 5 (McCune and Mefford 2011). For this analysis, we used the following metric variables: length of colpus (CLEN), width of colpus (CWID), equatorial diameter in equatorial view (EDEV), equatorial diameter in polar view (EDPV), polar diameter in equatorial view (PDEV), length of endoaperture (ELEN), width of endoaperture (EWID), polar area index (PAI), exine (EXIN), nexine (NEXI), sexine (SEXI), tectum (TECT) and shape (SHAP).

Photomicrographs were taken with a light microscope Bel Photonics for LM photos, a JEOL JSM 5410 and a PHILIPS Serie XL 20, S/W, ver. 5.21 scanning electron microscope for the SEM images, and a JEOL JEM 1010 and a PHILIPS EM 208 transmission electron microscope for the TEM images. Pollen description and terminology follow Punt et al. (2007) glossary, and Faegri and Iversen (1966) for the polar area index, and Gasparino et al. (2013) for width index of colpi.

Results

General description

The pollen grains of Gloxiniinae studied species are monads, small to medium, isopolar, with circular, subcircular or subtriangular amb, suboblate, oblate spheroidal to subprolate, 3-colporate, with long to very long colpi, narrow, wide or very wide, rounded or tapered at the polar ends, with or without margo, lalongate or lolongate endoaperture, sometimes not evident. The exine is microreticulate or reticulate, homobrochate or heterobrochate; the sexine is thicker than the nexine (Figs. 2, 3, 4, Tables 1, 2, 3).

Pollen unit, size, amb and shape

All species have pollen grains in monads. Pollen grains are small or medium (Table 1), with size varying from 17.02 µm in *Mandirola hirsuta* to 25.00 µm in *Chautemsia calcicola* (polar diameter in equatorial view) and from 18.50 µm in *Mandirola rupestris* to 27.83 µm in *Chautemsia calcicola* (equatorial diameter in equatorial view—Table 2).

The amb of pollen grains are in circular *Chautemsia calcicola* (Fig. 2a), *Gloxinia erinoides* (Fig. 2e), *Goyazia petraea* (Fig. 2i), *G. rupicola* (Fig. 2k), *Mandirola hirsuta* (Fig. 2m) and *Seemannia sylvatica* (Fig. 2s), subcircular in *Gloxinia perennis* (Fig. 2g) and *Mandirola rupestris* (Fig. 2q) and subtriangular in *Gloxinia alterniflora* (Fig. 2c) and *Mandirola multiflora* (Fig. 2o).

Regarding shape, the analyzed species present pollen grains suboblate in *Gloxinia alterniflora* (Fig. 2d) and *G. erinoides* (Fig. 2f), oblate spheroidal in *Chautemsia calcicola* (Fig. 2b), *Gloxinia perennis* (Fig. 2h), *Goyazia petraea* (Fig. 2j), *G. rupicola* (Fig. 2l), *Mandirola hirsuta* (Fig. 2n), *M. multiflora* (Fig. 2p) and *Seemannia sylvatica* (Fig. 2t) or subprolate in *Mandirola rupestris* (Fig. 2r). However, variations within the samples of each species were observed; for this reason we present the variation and the predominant type for the shape of the pollen grains (Table 1).

Aperture, exine ornamentation and structure

The pollen grains of all species are 3-colporate, with long colpi (*Gloxinia alterniflora*, Table 3) or very long colpi, narrow (in *Chautemsia calcicola*, Fig. 3a and *Mandirola rupestris*, Fig. 3q), wide (in *Goyazia rupicola*, Fig. 3k and *Mandirola hirsuta*, Fig. 3m) or very wide (the other species, Table 3), with margo or not (*Chautemsia calcicola*, Fig. 2a), rounded at the ends (in *Gloxinia* species, *Mandirola hirsuta*, *M. rupestris* and *Seemannia sylvatica,* Fig. 2c, e, g, m, q, s) or tapered at the ends (in *Chautemsia calcicola, Goyazia petraea*, *G. rupicola* and *Mandirola multiflora* Fig. 2a, i, k, o). The endoaperture is lalongate in *Chautemsia calcicola* (length = 1.8 µm and wide = 5.4 µm), while the other species have lolongate endoapertures in the pollen grains, sometimes with ornamentation in endoaperture (Fig. 3, Table 3).

The pollen grains present microreticulate exine in *Chautemsia* (Fig. 3b–b'), *Goyazia* (Figs. 3j–j', l–l', 4f–h), *Mandirola* (Fig. 3n–n', p–p', r–r', 4i) and *Seemannia* (Fig. 3t–t', 4j–k), and reticulate in *Gloxinia* (Figs. 3d–d', f–f', h–h', 4a–d), sometimes with ornamentation in lumina. The exine thickness ranged from 1.27 to 2.36 µm, 0.99 to 1.99 µm (sexine) and 0.29 to 0.37 (nexine—Table 3). *Gloxinia erinoides* has pollen grains with homobrochate reticulum (Fig. 3f-f', Fig. 4a-b), while in *G. alterniflora* and *G. perennis*, the reticula of the pollen grains are heterobrochate (Figs. 3d–d', h–h', 4c–d).

The exine structure was analyzed in *Gloxinia perennis* and *Seemannia sylvatica* (Fig. 4e–l). The TEM observations show that the tectum is continuous and slightly wavy in *G. perennis* (Fig. 4e) and compact, discontinuous and as thick as the nexine in *S. sylvatica* (Fig. 4l). The infratectal layer presents long columellae with different diameters and close to each other; sometimes, the columellae are incomplete and generally thicker near the foot layer. We can see a clear distinction between the foot layer and the endexine in *S. sylvatica* (Fig. 4l). The foot layer is wavy in the region in contact with the columellae in *S. sylvatica*, while in *G. perennis* the foot layer is more linear. The endexine is thin and lamellate, and the intine is thick in both species.

Analysis of quantitative data

When we analyze the mean and confidence interval of pollen grains diameters among species (Fig. 5a–b), it was observed that *Mandirola hirsuta*, *Goyazia petraea*, *Gloxinia erinoides*, *Goyazia rupicola*, *Mandirola multiflora* and *Gloxinia alterniflora* share the lowest values of polar diameter in equatorial view (PDEV) and present a continuous group. *Gloxinia perennis* and *Seemannia sylvatica* have intermediate sizes (Fig. 5a), and the highest values were observed in the pollen grains of *Mandirola rupestris* and *Chautemsia calcicola* (Fig. 5a).

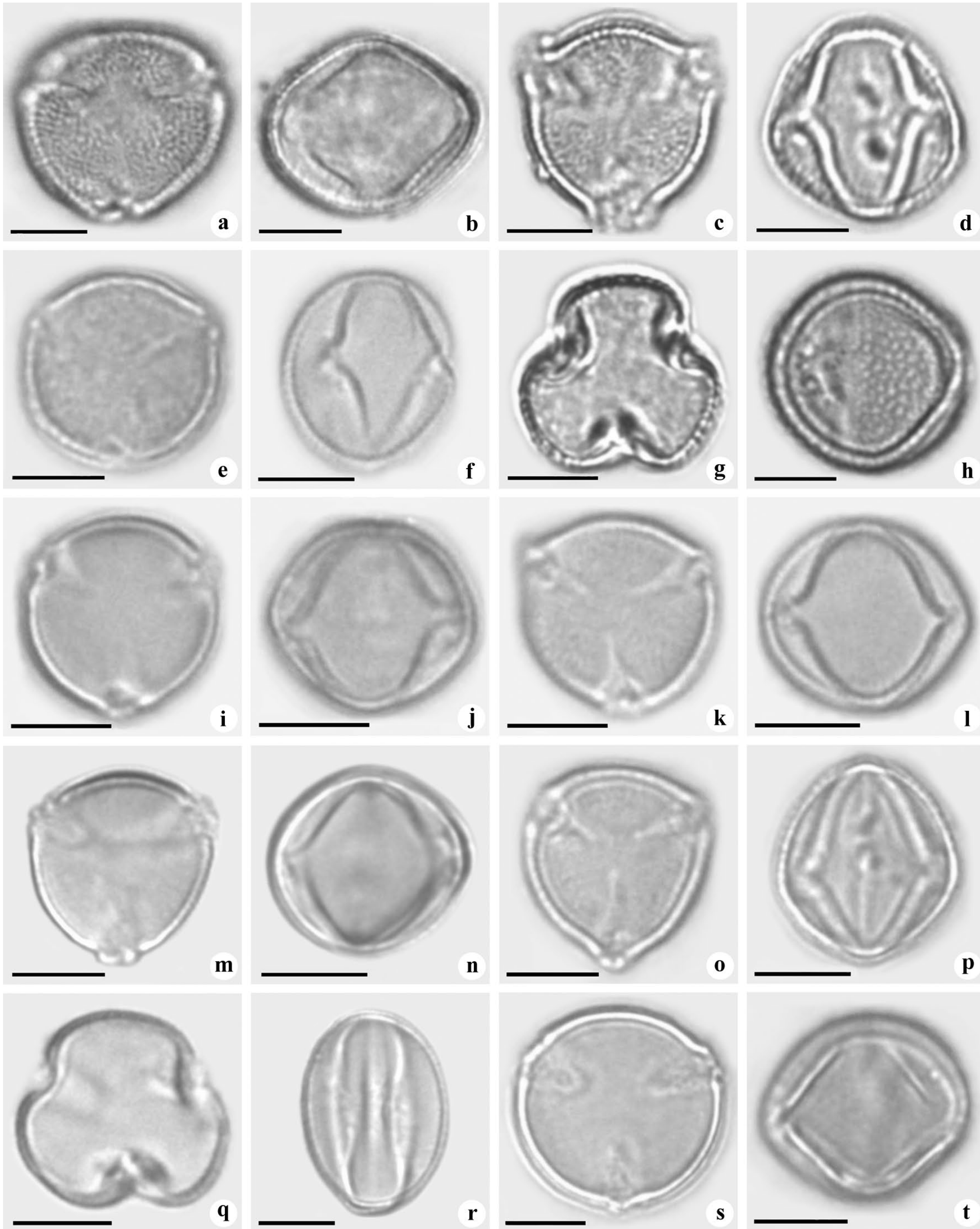

Fig. 2 Photomicrographs of the pollen grains of Brazilian species of Gloxiniinae. **a, b** *Chautemsia calcicola*. **c, d** *Gloxinia alterniflora*. **e–f** *Gloxinia erinoides*. **g, h** *Gloxinia perennis*. **i, j** *Goyazia petraea*. **k, l** *Goyazia rupicola*. **m–n** *Mandirola hirsuta*. **o, p** *Mandirola multiflora*. **q, r** *Mandirola rupestris*. **s, t** *Seemannia sylvatica*. **a**, **c**, **e**, **g**, **i**, **k**, **m**, **o**, **q** and **s**—polar view with emphasis on the ambit; **b**, **d**, **f**, **h**, **j**, **l**, **n**, **p**, **r** and **t**—equatorial view with emphasis on the shape. Scales = 10 µm

For the equatorial diameter in equatorial view (EDEV) (Fig. 5b), pollen grains of *Mandirola rupestris*, *M. hirsuta*, *Goyazia petraea*, *Mandirola multiflora*, *Goyazia rupicola*, *Gloxinia perennis*, *G. erinoides* and *G. alterniflora* form a large group sharing the smallest values for this dimension. *Seemannia sylvatica* presents intermediate values between

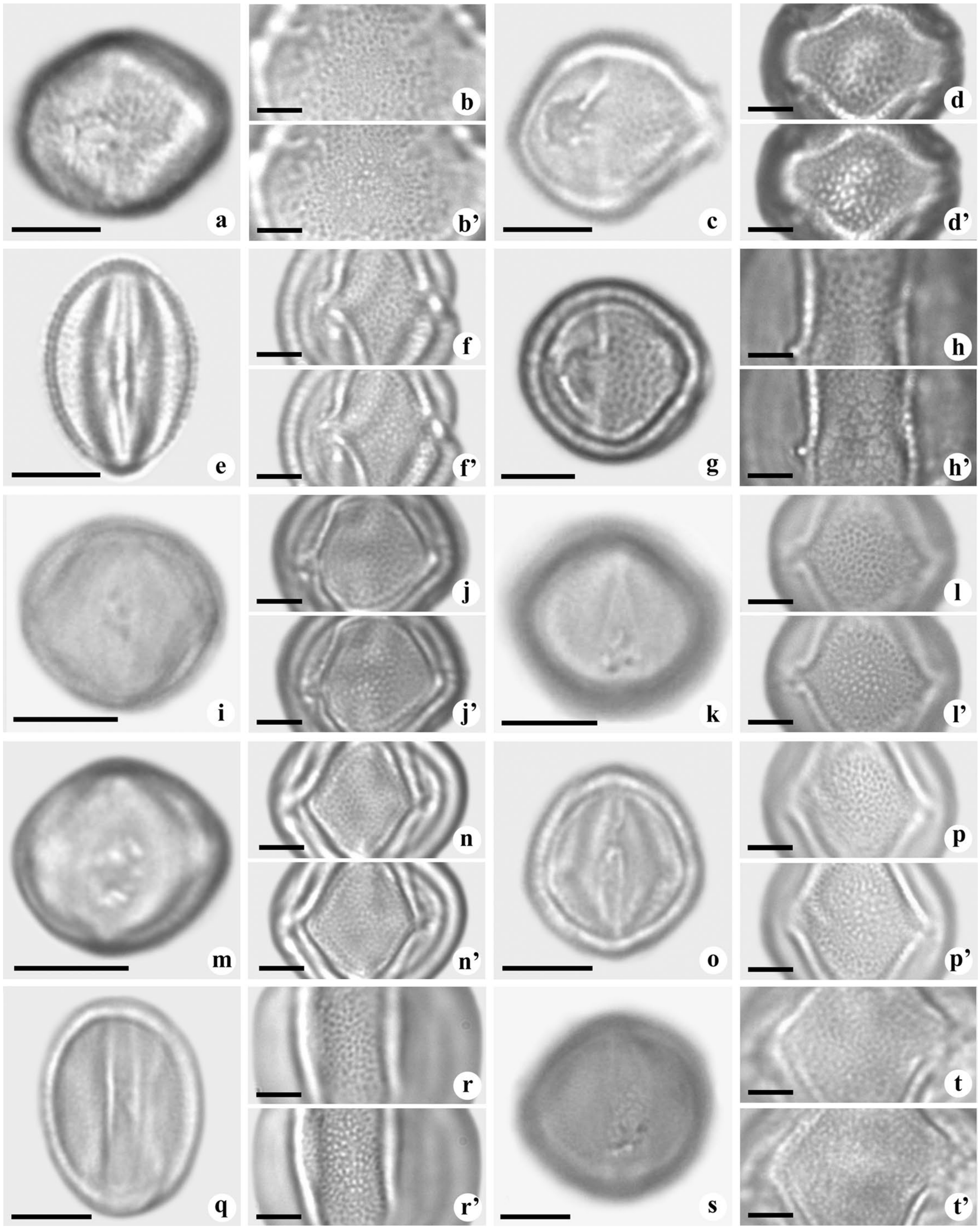

Fig. 3 Photomicrographs of the pollen grains of Brazilian species of Gloxiniinae. **a, b'** *Chautemsia calcicola*. **c, d'** *Gloxinia alterniflora*. **e, f'** *Gloxinia erinoides*. **g, h'** *Gloxinia perennis*. **i, j'** *Goyazia petraea*. **k, l'** *Goyazia rupicola*. **m, n'** *Mandirola hirsuta*. **o, p'** *Mandirola multiflora*. **q, r'** *Mandirola rupestris*. **s, t'** *Seemannia sylvatica*. **a**, **c**, **e**, **g**, **i**, **k**, **m**, **o**, **q** and **s**—equatorial view with emphasis on the apertures (Scales = 10 µm); **b-b'**, **d-d'**, **f-f'**, **h–h'**, **j-j'**, **l-l'**, **n–n'**, **p-p'**, **r-r'** and **t-t'**—ornamentation in high and low focus (Scales = 5 µm)

the analyzed species and, finally, *Chautemsia calcicola* was the species that presented the largest pollen grains, based on EDEV (Fig. 5b).

We can make some considerations regarding the measurements of pollen grain diameters: (a) there is a variation in the diameter measurements between the *Gloxinia* species; in the polar diameter, *G. erinoides* and *G. alterniflora* are separated from *G. perennis* because this species has larger diameters (Fig. 5a); however, in the equatorial diameter *Gloxinia* species are in the same group (Fig. 5b). (b) *Mandirola rupestris* appears next to *Chautemsia calcicola*, with higher values of polar diameters, away from the other *Mandirola* species (Fig. 5a); for equatorial diameters these differences were not observed, and *Mandirola* species share similar values (Fig. 5b).

Relationships among the Gloxiniinae studied species based on quantitative data were explored using PCA analysis (Fig. 6). This analysis explained 85.46% of total data variance. The first axis of the principal component analysis was the most significant for the species ordination and explained 59.36% of the total variability of the analyzed data. On this axis, *Chautemsia calcicola* was placed on the negative side of the axis 1, distant from the other species mainly due to the high values of width of endoaperture and sexine thickness (Fig. 6; Table 4). Also on the negative side of the axis, we can notice the placement of *Mandirola rupestris* and *Seemannia sylvatica* that were separated from the others by the aperture and PAI measurements (Fig. 6; Table 4).

The second component explained 26.10% of the variability of the data, presenting the lowest values of the metric variables related to axis 2; the species *Goyazia petraea*, *G. rupicola* and *Mandirola multiflora* appear on the negative side of this axis. On the positive side of axis 2, we can observe that the species of *Gloxinia* (*G. alterniflora*, *G. erinoides* and *G. perennis*) and *Mandirola hirsuta* were grouped due to nearby values for width of colpi and width of endoaperture (Fig. 6).

Discussion

Pollen grains of Brazilian Gloxiniinae species were previously studied by Howard (1975), Williams (1978), Filice et al. (1981), Xifreda (1996) and Gasparino (2008). For the recently described *Chautemsia calcicola* A.O.Araujo & V.C.Souza and *Gloxinia alterniflora* A.O.Araujo & Chautems (Araujo et al. 2010a,b), the present study produced the first palynological data.

Araujo (2007), Perret et al. (2013) and Roalson et al. (2005a) established that *Mandirola* and *Goyazia* were closely related genera, based on morphological and molecular data. According to Araujo (2007) and Araujo et al. (2010b), *Mandirola* is polyphyletic by the circumscription of Roalson et al. (2005a), and the species recognized in *Goyazia* should be included in *Mandirola*.

Howard (1975) observed prolate spheroidal to prolate pollen grains, tricolpate, long colpi with granulate membrane, and reticulate exine in *Goyazia villosa* (Gardner) R.A. Howard. In our results, we found tricolporate and microreticulate pollen grains for *Goyazia*. The absence of endoaperture and reticulated ornamentation, as described by Howard (1975), are pollen characters that corroborate the inclusion of *G. villosa* in *Phinaea* as suggested by Boggan et al. (2008).

Gasparino (2008) described *Mandirola multiflora* (treated by the author as *M. hirsuta*), *Goyazia petraea* and *G. rupicola* (under *Mandirola*, following Araujo 2007), and he defined the pollen grains as prolate spheroidal to subprolate, tricolporate and microreticulate. Our data corroborate the study of Gasparino (2008) regarding the presence of endoaperture and the microreticulate ornamentation, and diverge as to the pollen grains shape.

The *Mandirola* and *Goyazia* species analyzed in this study present variations regarding the amb, shape, width and ends of colpi. However, we observed that these variations also occur within the samples for each species studied, which turn their discrimination at the species level difficult. Thus, the close relationship between genera can be observed by the qualitative and the quantitative data of the pollen grains which confirms the literature data.

Williams (1978) analyzed the pollen morphology of some species of the Gloxiniinae, among them is *Gloxinia perennis* (L.) Fritsch, and described the pollen grains of the tribe as 3-colpate or 3-colporate, prolate spheroidal to prolate. *Gloxinia erinoides* treated within of *Koellikeria* was studied by Filice et al. (1981) that described small pollen grains, subprolate, tricolporate, with long colpi and microreticulate ornamentation. Gasparino (2008) produced data on *Gloxina erinoides* and *G. perennis* that show that the pollen grains are small, oblate spheroidal, 3-colporate, with long colpi, rounded at the ends and reticulate exine. For the species analyzed here, our data diverge from Williams (1978) as to shape, from Filice et al. (1981) as to shape and ornamentation, but are similar to the data described by Gasparino (2008).

The studied species of *Gloxinia* have details on the pollen grains that help in their identification, such as amb, shape and variations in ornamentation (homobrocate or heterobrocate reticulum); however, considering all the Gloxiniinae studied species the pollen grains of *Gloxinia* are distinct and can be discriminated from the other genera by the reticulate ornamentation (as observed in ancestral pollen morphology reconstruction). Therefore, the pollen ornamentation is an important character for the taxonomy of the genus.

Xifreda (1996) studied the pollen grains of *Seemannia gymnostoma* (Griseb.) Toursark. and *S. nematanthodes* (Kuntze) Schum., both treated by the author within *Gloxinia*. Based on scanning electron microscopy (SEM) analysis, pollen grains present reticulate-perforate ornamentation

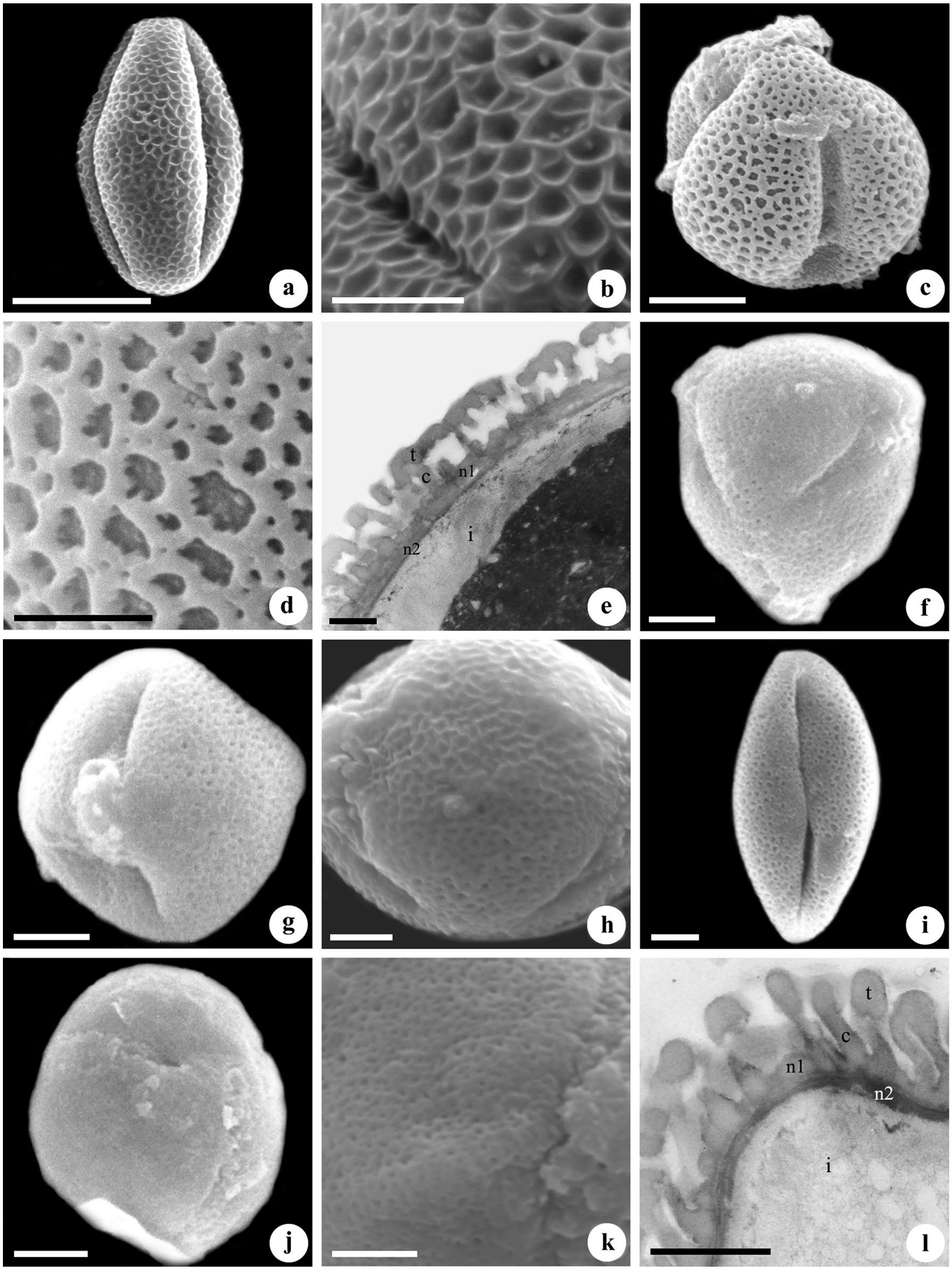

Fig. 4 Electron photomicrographs of the pollen grains of *Gloxinia*, *Goyazia*, *Mandirola* and *Seemannia*. **a, b** *Gloxinia erinoides*. **a** Equatorial view (SEM). **b** Ornamentation (SEM). **c–e** *Gloxinia perennis*. **c** Polar view (SEM). **d** Ornamentation (SEM). **e** Section through pollen wall (TEM). **f–h** *Goyazia rupicola*. **f** Polar view (SEM). **g** Equatorial view (SEM). **h** Ornamentation (SEM). **i** *Mandirola multiflora*. **i** Equatorial view (SEM). **j–l** *Seemannia sylvatica*. **j** Polar view (SEM). **k** Ornamentation. **l** Section through pollen wall (TEM). (*t* tectum, *c* columella, *n1* nexine 1, *n2* nexine 2, *i* intine). Scales: **e** = 500 nm; **l** = 750 nm; **b**, **d**, **h** and **k** = 2 µm; **c**, **f**, **g**, **i**, and **j** = 5 µm; **a** = 10 µm

Table 1 Morphological characterization of Brazilian Gloxiniinae pollen grains

Species	Size	Amb	P/E	Shape (shape variation)	PAI	WCI	Endoaperture	Exine
Chautemsia calcicola	S-M	Circular	0.90	OS* (SO, SP, PS)	0.15	9.97	LA	Microreticulate
Gloxinia alterniflora	S	Subtriangular	0.87	SO (OS*, SP)	0.31	4.11	LO	Reticulate
Gloxinia erinoides	S	Circular	0.84	SO (OS*)	0.12	4.07	LO	Reticulate
Gloxinia perennis	S	Subcircular	0.99	OS* (SO, PS, SP)	0.24	3.99	LO	Reticulate
Goyazia petraea	S	Circular	0.88	OS* (SO, PS, SP)	0.13	4.00	LO	Microreticulate
Goyazia rupicola	S	Circular	0.88	OS* (SO, PS)	0.13	6.52	LO	Microreticulate
Mandirola hirsuta	S	Circular	0.89	OS* (SO, PS)	0.15	5.48	LO	Microreticulate
Mandirola multiflora	S	Subtriangular	0.90	OS* (SO, PS)	0.12	4.51	LO	Microreticulate
Mandirola rupestris	S	Subcircular	1.28	SP (P*, PS)	0.21	8.44	LO	Microreticulate
Seemannia sylvatica	S-M	Circular	0.89	OS* (SO)	0.21	4.66	LO	Microreticulate

S small, *M* medium, *P/E* ratio between polar and equatorial diameter, *SO* suboblate, *OS* oblate spheroidal, *PS* prolate spheroidal, *P* prolate, *SP* subprolate, * predominant shape, *PAI* polar area index, *WCI* width colpus index, *LA* lalongate, *LO* lolongate

Table 2 Quantitative data of Brazilian Gloxiniinae pollen grains

Species	(R) $x \pm s_x$	s	CI	V
Equatorial diameter in polar view				
Chautemsia calcicola	(20.00–32.50) 27.50 ± 0.62	3.10	26.12–28.68	11.32
Gloxinia alterniflora	(17.95–23.08) 20.51 ± 0.15	0.74	20.21–20.82	3.61
*Gloxinia erinoides**	(17.95–20.51) 20.19 ± 0.32	0.91	19.44–20.95	4.49
Gloxinia perennis	(17.95–23.08) 20.92 ± 0.29	1.29	20.30–21.54	6.15
Goyazia petraea	(17.95–20.51) 19.69 ± 0.24	1.22	19.19–20.19	6.20
Goyazia rupicola	(17.95–20.51) 19.38 ± 0.26	1.30	18.85–19.92	6.70
Mandirola hirsuta	(17.95–20.51) 18.77 ± 0.24	1.22	18.27–19.27	6.50
Mandirola multiflora	(17.95–20.51) 19.49 ± 0.26	1.28	18.96–20.01	6.58
*Mandirola rupestris**	(17.50–20.00) 19.17 ± 0.83	1.44	15.58–22.75	7.53
Seemannia sylvatica	(23.08–25.64) 24.31 ± 0.26	1.31	23.77–24.85	5.38
Polar diameter in equatorial view				
Chautemsia calcicola	(20.00–27.50) 25.00 ± 0.38	1.85	24.20–25.80	7.39
Gloxinia alterniflora	(15.38–20.51) 18.36 ± 0.24	1.21	17.86–18.86	6.60
Gloxinia erinoides	(15.38–23.08) 17.56 ± 0.43	1.91	16.67–18.46	10.88
Gloxinia perennis	(17.95–25.64) 20.35 ± 0.60	2.38	19.08–21.62	11.70
Goyazia petraea	(15.38–20.51) 17.23 ± 0.28	1.39	16.66–17.80	8.06
Goyazia rupicola	(15.38–17.95) 17.74 ± 0.14	0.71	17.45–18.04	4.00
Mandirola hirsuta	(15.38–17.95) 17.02 ± 0.25	1.26	16.51–17.54	7.38
Mandirola multiflora	(15.38–20.51) 17.74 ± 0.25	1.26	17.22–18.26	7.13
Mandirola rupestris	(20.00–25.00) 23.70 ± 0.36	1.79	22.96–24.44	7.53
Seemannia sylvatica	(20.51–23.08) 22.27 ± 0.28	1.22	21.68–22.86	5.50
Equatorial diameter in equatorial view				
Chautemsia calcicola	(20.00–32.50) 27.83 ± 0.67	3.22	26.43–29.22	11.59
Gloxinia alterniflora	(17.95–23.08) 21.13 ± 0.27	1.34	20.58–21.68	6.34
Gloxinia erinoides	(17.95–25.64) 20.90 ± 0.34	1.51	20.19–21.60	7.20
Gloxinia perennis	(17.95–25.64) 20.51 ± 0.52	2.09	19.40–21.63	10.21
Goyazia petraea	(17.95–20.51) 19.49 ± 0.26	1.28	18.96–20.01	6.58
Goyazia rupicola	(17.95–20.51) 20.10 ± 0.19	0.96	19.71–20.50	4.77
Mandirola hirsuta	(17.95–20.51) 19.18 ± 0.26	1.31	18.64–19.72	6.82
Mandirola multiflora	(17.95–20.51) 19.69 ± 0.24	1.22	19.19–20.19	6.20
Mandirola rupestris	(15.00–22.50) 18.50 ± 0.35	1.77	17.77–19.23	9.56
Seemannia sylvatica	(23.08–25.64) 25.10 ± 0.25	1.07	24.58–25.62	4.28

R range, *x* mean (μm), *sx* standard deviation (μm), *s* standard error (μm), *CI* confidential interval in 95% (μm), *V* coefficient of variability (%), * $n \leq 25$

Table 3 Measures (μm) of Brazilian Gloxiniinae pollen grains, apertures and exine. $n \leq 10$

Species	Colpus		Endoaperture		Exine	Sexine	Nexine	Tectum
	Length	Width	Length	Width				
Chautemsia calcicola	19.59	2.79	1.81	5.40	2.36	1.99	0.37	0.37
Gloxinia alterniflora	13.90	3.50	5.32	3.70	1.82	1.53	0.29	0.22
Gloxinia erinoides	13.90	3.50	5.32	3.70	1.27	0.99	0.29	0.22
Gloxinia perennis	13.90	3.50	5.32	3.70	1.77	1.49	0.29	0.22
Goyazia petraea	12.79	3.32	4.43	2.78	1.38	1.10	0.29	0.22
Goyazia rupicola	12.79	2.10	4.61	2.22	1.38	1.10	0.29	0.22
Mandirola hirsuta	12.52	3.50	5.15	3.70	1.39	1.10	0.29	0.22
Mandirola multiflora	13.90	2.97	4.79	2.96	1.38	1.10	0.29	0.22
Mandirola rupestris	16.68	2.19	3.52	2.13	1.82	1.52	0.30	0.24
Seemannia sylvatica	15.02	3.67	5.67	3.70	1.77	1.49	0.29	0.22

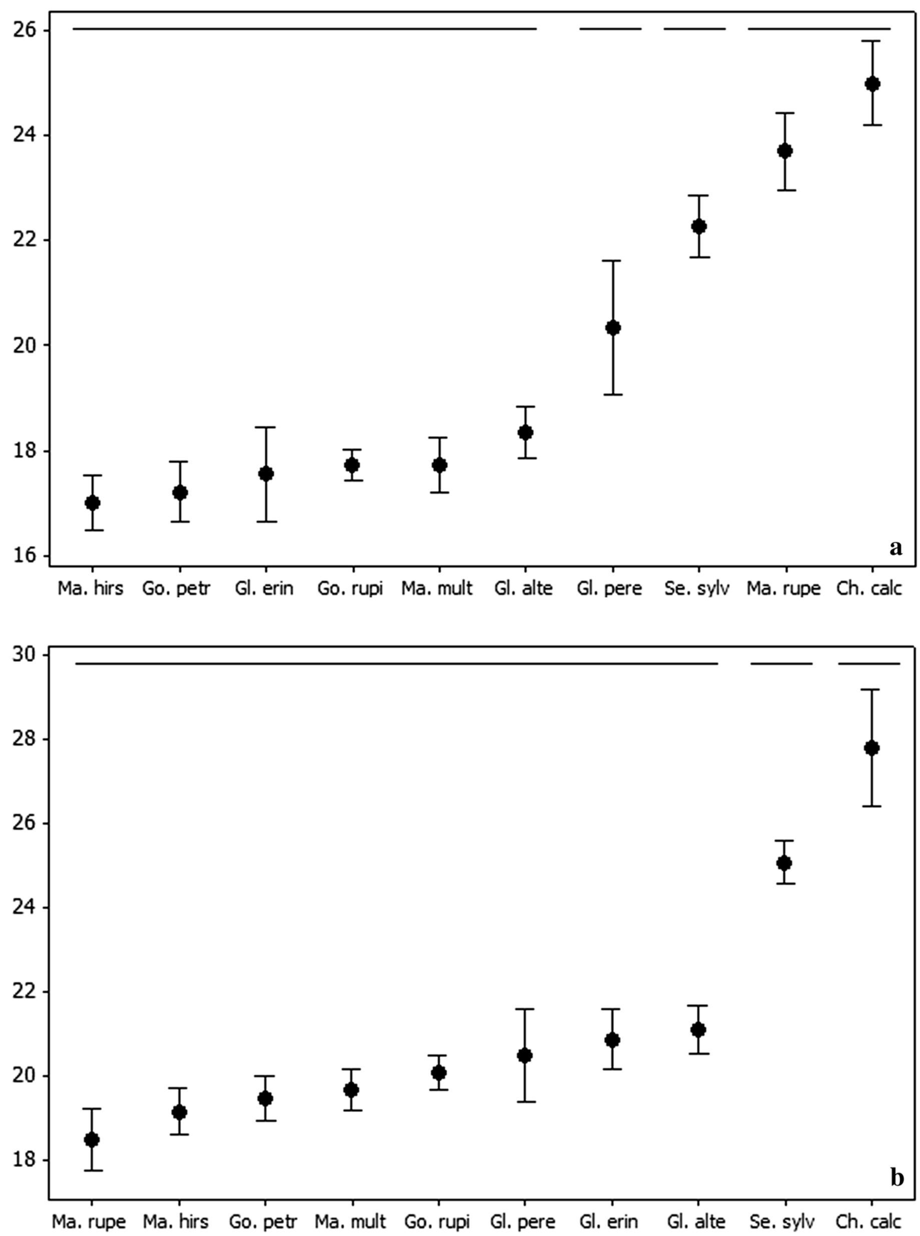

Fig. 5 Representation of confidence interval of mean in 95% of the pollen grains of Brazilian species of Gloxiniinae. **a** Polar diameter in equatorial view. **b** Equatorial diameter in equatorial view. The higher and lower boundaries show the confidence interval; the average circle shows the arithmetic mean. The horizontal lines at the top represent the result of the Tukey's test. Ch. calc = *Chautemsia calcicola*, Gl. alte = *Gloxinia alterniflora*, Gl. erin = *Gloxinia erinoides*, Gl. pere = *Gloxinia perennis*, Go. petr = *Goyazia petraea*, Go. rupi = *Goyazia rupicola*, Ma. hirs = *Mandirola hirsuta*, Ma. mult = *Mandirola multiflora*, Ma. rupe = *Mandirola rupestris*, Se. sylv = *Seemannia sylvatica*

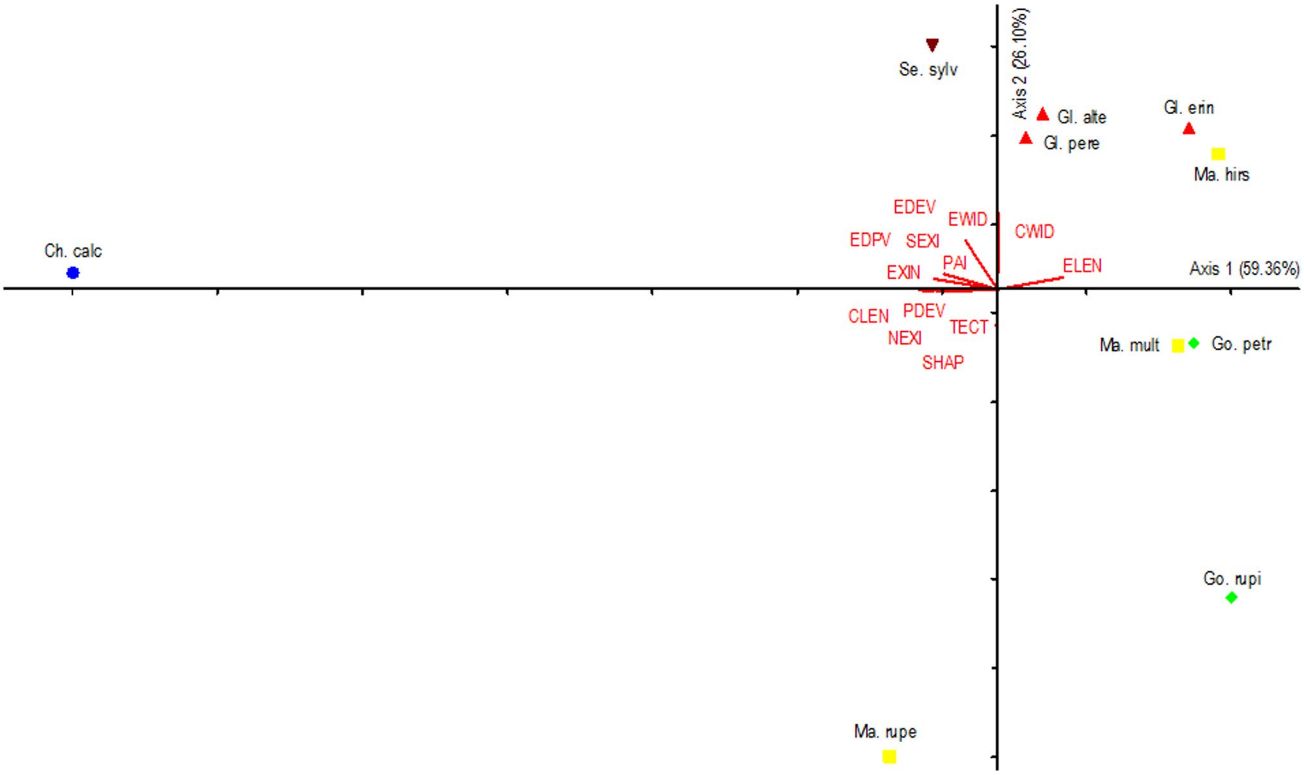

Fig. 6 Principal component analysis performed with the pollen metrical variables from Brazilian species of Gloxiniinae. Ch. calc = *Chautemsia calcicola*, Gl. alte = *Gloxinia alterniflora*, Gl. erin = *Gloxinia erinoides*, Gl. pere = *Gloxinia perennis*, Go. petr = *Goyazia petraea*, Go. rupi = *Goyazia rupicola*, Ma. hirs = *Mandirola hirsuta*, Ma. mult = *Mandirola multiflora*, Ma. rupe = *Mandirola rupestris*, Se. sylv = *Seemannia sylvatica*

with angular lumina in *S. gymnostoma* or circular lumina in *S. nematanthodes*. Gasparino (2008) analyzed the pollen morphology of two other species of *Seemannia* Regel, *S. purpurascens* Rusby and *S. sylvatica* (Kunth) Hanst. He described small pollen grains, with circular amb, prolate spheroidal, 3-colporate, with rounded ends and microreticulate exine. In the present study, our pollen data are similar to what Gasparino (2008) observed, except for the shape that we describe here as oblate spheroidal. Our data differ from Xifreda (1996) regarding the ornamentation for the genus, since we observed microreticulate pollen grains for *Seemannia*.

Although the pollen morphology has a small variation in the Gloxiniinae analyzed species, as already reported by Williams (1978) for Gesnerieae, differences in amb, shape, length and width of colpi, presence or absence of margo and type of endoaperture were observed. The reticulate ornamentation appears in the *Gloxinia* species, whereas *Chautemsia*, *Goyazia*, *Mandirola* and *Seemannia* have microreticulate pollen grains. Lolongate endoapertures represent the pattern for the subtribe pollen grains except in *Chautemsia* with lalongate pollen grains; therefore, this is a pollen characteristic for the genus. The ordering of species using quantitative data made by the PCA separated *Chautemsia calcicola* from the other species based mainly on the width of endoapertures and the thickness of sexine. For the species of *Mandirola* and *Goyazia,* there is a remarkable similarity of the quantitative and qualitative data of the pollen grains.

Table 4 Pearson and Kendall correlation coefficients for pollen grain metric variable of the first and the second axis of principal component analysis (PCA) ordination in Brazilian species of Gloxiniinae

Variables	Principal components	
	Axis 1	Axis 2
EDPV	−0.2668	0.1636
PDEV	−0.3038	−0.0984
EDEV	−0.2578	0.2080
CLEN	−0.3205	−0.0816
CWID	0.0642	0.5625
ELEN	0.5972	0.3917
EWID	−0.3434	0.6430
EXIN	−0.2915	0.0186
SEXI	−0.3027	0.0245
NEXI	−0.0506	−0.0074
TECT	−0.0926	−0.0110
SHAP	−0.0260	−0.1630
PAI	−0.0154	0.0514

Acknowledgements The authors thank the curators of Herbaria ESA, G, HUFABC, SP and UEC for kindly allowing pollen sampling from herbarium sheets and Mauro Peixoto for providing some samples of the pollen materials for the present study. C. N. Souza is funded by a FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) scholarship (2015/02707-0). A.O. Araujo is funded by a FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) research Grant (2011/20269-9).

Compliance with ethical standards

Conflict of interest The authors declared that they have no conflict of interest.

Information on Electronic Supplementary Material

Online Resource 1. Examined Material.

References

Araujo AO (2007) Estudos taxonômicos em *Gloxinia* L´Hér. sensu lato (Gesneriaceae). PhD thesis, Universidade de São Paulo, São Paulo

Araujo AO, Souza VC, Chautems A (2010a) Estudos taxonômicos em *Gloxinia s.l.* (Gesneriaceae)—I: uma nova espécie do Mato Grosso do Sul, Brasil. Rodriguésia 61:S67–S72. https://doi.org/10.1590/2175-7860201061135

Araujo AO, Souza VC, Perret M (2010b) *Chautemsia calcicola*: a new genus and species of Gloxinieae (Gesneriaceae) from Minas Gerais, Brazil. Taxon 59:203–208. http://www.jstor.org/stable/27757063

Araujo AO, Souza VC, Chautems A (2012) Estudos taxonômicos em Gloxinieae (Gesneriaceae)—notas nomenclaturais. Acta Bot Brasil 26:891–900. https://doi.org/10.1590/S0102-33062012000400018

Boggan JK, Roalson EH, Skog LE (2008) A review of the Neotropical genera *Amalophyllon*, *Niphaea* and *Phinaea* (Gesneriaceae—Gloxinieae). Selbyana 29:157–176. https://doi.org/10.2307/41760330

Chautems A (1991) A família Gesneriaceae na região cacaueira do Brasil. Revista Brasil Bot 14:51–59

Citerne HL, Moeller M, Cronk QCB (2000) Diversity of cycloidea-like genes in Gesneriaceae in relation to floral symmetry. Ann Bot (Oxford) 86:167–176. https://doi.org/10.1006/anbo.2000.1178

Clark JL, Herendeen PS, Skog LE, Zimmer EA (2006) Phylogenetic relationships and generic boundaries in the Episcieae (Gesneriaceae) inferred from nuclear, chloroplast, and morphological data. Taxon 55:313–336. https://doi.org/10.2307/25065580

Clark JL, Funke MM, Duffy AM, Smith JF (2012) Phylogeny of a neotropical clade in the Gesneriaceae: more tales of convergent evolution. Int J Pl Sci 173:894–916. https://doi.org/10.1086/667229

Erdtman G (1960) The acetolysis method. A revised description. Svensk Bot Tidskr 54:561–564

Faegri G, Iversen J (1966) Textbook of modern pollen analysis, 2nd edn. Scandinavian University Books, Copenhagen

Filice MAC, Sanchis AM, Villar LM (1981) Granos de pólen de las Gesneriaceae de la Argentina. Comun Mus Argent Ci Nat "Bernardino Rivadavia" 2:77–89

Fritze KJ, Williams NH (1988) The taxonomic significance of pollen morphology in the *Columnea* alliance (Gesneriaceae: Gesnerioideae). Ann Missouri Bot Gard 75:168–191. https://doi.org/10.2307/2399471

Gasparino EC (2008) Palinotaxonomia de espécies brasileiras de Gesneriaceae, com ênfase nas ocorrentes no Estado de São Paulo. PhD thesis, Instituto de Botânica, São Paulo

Gasparino EC, Cruz-Barros MAV, Chautems A (2010) Palinotaxonomia de Gesneriaceae, com ênfase nas espécies brasileiras. In: Absy ML, Matos FDA, Amaral IL (eds) Diversidade Vegetal Brasileira: Conhecimento, Conservação e Uso. Sociedade Botânica do Brasil, Manaus, pp 466–471

Gasparino EC, Cruz-Barros MAV, Chautems A, Galleti SR (2011) Palinotaxonomia de *Besleria* L. e *Napeanthus* Gardn. (Beslerieae/Napeantheae—Gesneriaceae) com ênfase nas espécies ocorrentes no Estado de São Paulo. Revista Brasil Bot 34:321–333. https://doi.org/10.1590/S0100-84042011000300007

Gasparino EC, Cruz-Barros MAV, Chautems A (2013) Pollen Morphology in Brazilian species of *Codonanthe* (Mart.) Hanst. and *Nematanthus* Schrader (Gesneriaceae). Grana 52:258–274. https://doi.org/10.1080/00173134.2013.826274

Haddad A, Sesso A, Attias M, Farina M, Meirelles MN, Siveira M, Benchimol M, Soares MJ, Barth OM, Machado RD, Souto-Padrón T, Souza W (1998) Técnicas básicas de microscopia eletrônica aplicadas às Ciências Biológicas. Sociedade Brasileira de Microscopia, Rio de Janeiro

Howard RA (1975) The genus *Anetanthus* (Gesneriaceae). J Arnold Arbor 56:364–368

Mayer V, Moeller M, Perret M, Weber A (2003) Phylogenetic position and generic differentiation of Epithemateae (Gesneriaceae) inferred from plastid DNA sequence data. Amer J Bot 90:321–329. https://doi.org/10.3732/ajb.90.2.321

McCune B, Mefford, MJ (2011) PC-ORD. Multivariate analysis of ecological data (Version 5). MjM Software, Gleneden Beach

Melhem TS, Matos MER (1972) Variabilidade de forma dos grãos de pólen de *Eriope crassipes* Benth. Labiatae. Hoehnea 2:1–10

Melhem TS, Cruz-Barros MAV, Corrêa AMS, Makino-Watanabe H, Silvestre-Capelato MSF, Golçalves-Esteves VL (2003) Variabilidade polínica em plantas de Campos do Jordão (São Paulo, Brasil). Bol Inst Bot (São Paulo) 16:1–104

Minitab (2003) Minitab for windows. (Version 10.3). Minitab Inc, State College

Perret M, Chautems A, Spichiger R, Kite G, Savolainen V (2003) Systematic and evolution of tribe Sinningieae (Gesneriaceae): evidence from phylogenetic analyses of six plastid DNA regions and nuclear *ncp*GS. Amer J Bot 90:445–460. https://doi.org/10.3732/ajb.90.3.445

Punt W, Hoen PP, Blackmore S, Nilsson S, Le Thomas A (2007) Glossary of pollen and spore terminology. Rev Palaeobot Palynol 143:1–81. https://doi.org/10.1016/j.revpalbo.2006.06.008

Roalson EH, Boggan JK, Skog LE (2005a) Reorganization of tribal and generic boundaries in the Gloxinieae (Gesneriaceae: Gesnerioideae) and the description of new tribe in the Gesnerioideae Sphaerorrhizeae. Selbyana 25: 225-238. http://www.jstor.org/stable/41760465

Roalson EH, Boggan JK, Skog LE, Zimmer EA (2005b) Untangling Gloxinieae (Gesneriaceae). I. Phylogenetic patterns and generic boundaries inferred from nuclear, chloroplast, and morphological cladistic datasets. Taxon 54: 389-410. doi: http://www.jstor.org/stable/25065368

Sabatini DD, Bensch K, Barrnett RJ (1963) Cytochemistry and electron microscopy: the preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J Cell Biol 17:19–58

Salgado-Labouriau ML (1973) Contribuição à Palinologia dos Cerrados. Academia Brasileira de Ciências, Rio de Janeiro

Shepherd GJ (1996) Fitopac 1: manual do usuário. Universidade Estadual de Campinas, Brazil, Departamento de Botânica, Campinas

Smith JF (1996) Tribal relationships within Gesneriaceae: a cladistic analysis of morphological data. Syst Bot 21:497–513. https://doi.org/10.2307/2419611

Smith JF (2000) Phylogenetic resolution within the tribe Episcieae (Gesneriaceae): congruence of ITS and *ndh*F sequences from parsimony and maximum-likelihood analyses. Amer J Bot 87:883–897

Smith JF, Wolfram JC, Brown KD, Carrol CL, Denton DS (1997) Tribal relationships in the Gesneriaceae: evidence from DNA sequences of the chloroplast gene ndhF. Ann Missouri Bot Gard 84:50–66. https://doi.org/10.2307/2399953

Souza VC, Lorenzi H (2012) Botânica Sistemática: guia ilustrado para identificação das famílias de Fanerógamas nativas e exóticas no Brasil, baseado em APG III, 3ª edn. Plantarum, Nova Odessa

Thiers B (2016) Index herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual herbarium, Bronx. Available at: http://sweetgum.nybg.org/ih/

Vieira S (2011) Introdução à Bioestatística, 4ª edn. Elsevier, Rio de Janeiro

Weber A (2004) Gesneriaceae. In: Kubitzki K, Kadereit JW (eds) The families and genera of vascular plants, vol. 7. Springer, Berlin, pp 63–158

Weber A, Clark JL, Moeller M (2013) A new formal classification of Gesneriaceae. Selbyana 31:68–94

Wiehler H (1983) A synopsis of the neotropical Gesneriaceae. Selbyana 6:1–219

Williams NH (1978) Pollen structure and the systematics of the neotropical Gesneriaceae. Selbyana 2:310–322. http://www.jstor.org/stable/41759480

Xifreda CC (1996) Citas nuevas o críticas para la flora Argentina II: *Gloxinia gymnostoma* y *G. nematanthodes* (Gesneriaceae). Darwiniana 34:383–388

Zar JH (1999) Biostatistical analysis, 4th edn. Prentice Hall, New Jersey

Zimmer EA, Roalson EH, Skog LE, Boggan JK, Idnurm A (2002) Phylogenetic relationships in the Gesnerioideae (Gesneriaceae) based on nrDNA ITS and cpDNA *trn*L-F and *trn*E-T spacer region sequences. Amer J Bot 89:296–311. https://doi.org/10.3732/ajb.89.2.296