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ORIGINAL ARTICLE

Pollen morphology and evolution in *Astraea* (Euphorbiaceae: Crotoneae)

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Abstract

Astraea is a genus with 13 species, widely distributed throughout Neotropics and especially diverse in Brazil. The genus is currently placed in Crotoneae as sister to *Acidocroton.* This work aimed to characterize the pollen of *Astraea* as a contribution to the taxonomy and to understand the evolution of pollen traits between *Astraea* and *Acidocroton*. Pollen grains of 12 species of *Astraea* and three of *Acidocroton* were gathered from herbarium specimens, acetolyzed, measured, photographed and described under light microscope and scanning electron microscopy. Our results demonstrate that *Astraea* is a stenopolinic (stenopalynous) genus with pollen grains that are apolar, spherical, medium to large, inaperturate, showing a *Croton* pattern exine with sexine thicker than nexine, and with rosettes having clavae or pila in their lumen. The rosettes consist of 5-7(-8) pila that can be rounded to triangular with psilate to plicate surface. Pollen features did not reveal synapomorphies for *Astraea*, *Acidocroton* or even for the *Astraea* + *Acidocroton* clade. Several pollen features evolved independently among recent lineages of *Astraea*, and we interpreted these evolutionary shifts as adaptations to new habitats. This work consolidates the palynological knowledge of *Astraea* and *Acidocroton* and will contribute to future palynological and systematic studies in Euphorbiaceae.

Keywords *Acidocroton* · *Croton* pattern pollen · Palynotaxonomy

Introduction

Astraea Klotzsch is a genus in Euphorbiaceae represented by 13 species that are found in the Neotropics and Tropical West Africa, with its greatest diversity in eastern Brazil (Silva et al. 2020). Weedy species, such as *A. lobata* (L.) Klotzsch and *A. trilobata* (Forssk.) O.L.M.Silva & Cordeiro, are found in the Old World (Silva et al. 2019). Species of *Astraea* may be recognized by their mostly deeply lobed or partite leaves, spiciform thyrses, staminate flowers with petals bearing moniliform trichomes at the base and stamens incurved in bud, pistillate flowers with slender, cylindrical multifid styles and mostly tetrangular seeds in cross section (Silva et al. 2020).

The genus *Astraea* is currently placed in tribe Crotoneae, subfamily Crotonoideae (Wurdack et al. 2005; Berry et al. 2005; Webster 2014), the most species rich lineage of Euphorbiaceae in the Neotropics (Ulloa-Ulloa et al. 2017). In spite of being traditionally included as a section of the giant *Croton* L. (Baillon 1858; Webster 1993), molecular data place *Astraea* as sister to *Acidocroton* Griseb. (Berry et al. 2005; Silva et al. 2020), found in Antilles, Central America, and northern South America. Berry et al. (2005) showed that the *Astraea-Acidocroton* clade emerged sister to the hyperdiverse *Brasiliocroton-Croton* clade, but a recent phylogeny recovered *Croton* sister to the *Astraea-Acidocroton* clade (Silva et al. 2020). The close relationship between *Astraea* and *Acidocroton* is surprising since the latter has distinctive xerophytic features in Crotoneae, such as stipules transformed in spines, leaves mostly arranged in short shoots

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(brachiblasts) and glomeruliform inflorescences (Webster 2014).

Pollen data have long been used in the classification of Euphorbiaceae (Punt 1962; Webster 1975, 1994, 2014). Crotoneae pollen has been studied extensively, but most works focused only on *Croton* (Erdtman 1952; Punt 1962; Nowicke 1994; Lobreau-Callen and Cervera 1997; Souza et al. 2016, 2019). For *Astraea*, only two works include descriptions of pollen grains (Oliveira and Santos 2000, Carreira and Barth 2003), whereas for *Acidocroton* no pollen data exist.

Taxonomic changes were proposed recently for *Astraea* including the re-delimitation of *A. lobata* (Silva et al. 2019), recognition of distinct species based on former varieties of *Croton lobatus* (Silva and Cordeiro 2017, Silva et al. 2019) and revision of the delimitation of some species, such as *A. paulina* and *A. cincta* (Silva et al. 2017, 2019). The genus is monophyletic and consists of three main clades (Silva et al. 2020). As palynological data are scarce for the genus, we aimed to characterize the pollen grains of *Astraea*, including a few representatives of *Acidocroton*, as a contribution to their taxonomy, to identify synapomorphies for both clades, and finally to infer the evolution of pollen traits within *Astraea* using the most recent phylogeny.

Materials and methods

Taxon sampling

Samples of 40 specimens from 12 of the 13 species of *Astraea*, plus five specimens from three of the 12 species of *Acidocroton* (Table 1) were gathered from collections deposited in ALCB, CEN, COL, G, HUEFS, RB, SP, SPF, MBM, MO, UB, US and VIC.

Light (LM) and scanning electronic microscopy (SEM)

For LM, pollen grains were submitted to acetolysis following Erdtman (1960). From five of the mounted slides, one was stained with safranin. Slides were identified and deposited in the pollen library at Plant Micromorphology Laboratory of the Universidade Federal de Feira de Santana, Bahia, Brazil.

For SEM, acetolyzed pollen grains were washed and dehydrated in ascending hydroethanolic series (50, 70, 90 and 100%), for 10 min at each step. A drop of absolute ethanol containing pollen grains was directly applied to a stub and, after complete drying, the sample was metalized with high-vacuum gold evaporation. Samples were then qualitatively analyzed, and images were obtained using a Quanta 250 microscope (FEI Company) at the Electronic Microscopy Center in the Universidade Estadual de Santa Cruz (UESC) and a JEOL 6390LV microscope at the Plataforma de Microscopia Eletrônica in Centro de Pesquisas Gonçalo Moniz (FIOCRUZ).

Pollen grains characterization

Pollen grains were described regarding size, shape, polarity, apertures, ornamentations and exine sculpture, adopting the nomenclature of Punt et al. (2007) and Hesse et al. (2009). The size classes follow Erdtman (1952). The measures were taken randomly in 25 pollen grains for each specimen, distributed in, at least, five slides. Exine thickness and diameter of pila, rosettes and the central space within rosettes were measured in 10 pollen grains from each specimen as illustrated by Souza et al. (2016).

Quantitative data were treated statistically, with arithmetic mean, sample and mean standard deviation, coefficient of variability, 95% confidence interval and variation range for measures taken from 25 pollen grains for each specimen. For exine thickness and diameter of pila, rosettes and the central space within rosettes we only calculated the arithmetic mean.

Ancestral states reconstruction

We employed the phylogeny of *Astraea* resulted from Bayesian inference based on ITS sequences from all Crotoneae genera from Silva et al. (2020), deposited in TreeBase (study ID 25606). As our study focuses on *Astraea*, we used *Acidocroton* as outgroup, and the remaining genera of Crotoneae were pruned from the tree using the drop.tip function from the *ape* package (Paradis and Schliep 2019) in R (R Core Team 2020). Six palynological characters (Table 2) were selected and their states coded and treated as unordered. Reconstructions of ancestral states were then performed using maximum likelihood, with one-parameter Markov k-state (Mk1) model, as implemented in Mesquite v. 3.2 (Maddison and Maddison 2009).

Results

Pollen grains characterization

Astraea

Pollen grains of *Astraea* are monads, apolar, spherical, inaperturate, with *Croton* pattern exine, and sexine thicker than the nexine (Figs. 1, 2, 3, 4). The predominant size is medium (Tables 3 and 4, Figs. 5 and 6), but large pollen grains are also observed exclusively in *A. cincta* (Müll.Arg.) Caruzo and Cordeiro. In *Astraea*, the rosette has 5-7(-8) pila with subcircular to subtriangular heads,

Table 1 Examined material of the species of *Acidocroton* and *Astraea* used in this study (Euphorbiaceae)

Species	Locality	Voucher
Acidocroton gentryi Fern.Alonso & R.Jaram	Colombia, Cundinamarca: Narinõ	*R. Jaramillo-Mejía 8285* (COL)
	Colombia, Cundinamarca: Narinõ	*A. Férnandez-Peréz 10688* (COL)
	Colombia, Tolima: Coello	*J. Cabezas* et al. *89* (COL)
Acidocroton oligostemon Urb.	Cuba, Santiago, El Morro	*B. Clemente 2473* (NMHN)
Acidocroton spinosus (Standl.) G.L.Webster	México, Jalisco: Chamela	*A.L. Gentryi 74385* (MO)
Astraea cincta (Müll.Arg.) Caruzo & Cordeiro	Brazil, Mato Grosso do Sul: Campo Grande	*F.C. Hoehene s.n* (SP 35784)
	Brazil, Mato Grosso do Sul: Corguinho	*O.L.M. Silva 238* (SP)
	Brazil, Minas Gerais: Joaquim Felício	*O.L.M. Silva 263* (SP)
	Brazil, Goiás: Caiapônia	*Sidney 1532* (UB)
	Brazil, Minas Gerais: Morada Nova de Minas Gerais	*R.F. Vieira 946* (CEN)
Astraea comosa (Müll.Arg.) B.W.van Ee	Brazil, Minas Gerais: Diamantina	*J.R.P. Pirani 3961* (SPF)
	Brazil, Minas Gerais: Joaquim Felício	*O.L.M. Silva 266* (SP)
	Brazil, Minas Gerais: Serro	*I. Cordeiro 3047* (SP)
Astraea digitata (Müll.Arg.) O.L.M.Silva & Cordeiro	Brazil, Bahia: São Francisco do Conde	*M.L. Guedes s.n* (ALCB 27934)
	Brazil, Bahia: Ilhéus	*J.L. Haje 1118* (CEPEC)
	Brazil, Bahia: Ilhéus	*O.L.M. Silva 228 (SP)*
Astraea gracilis (Müll.Arg.) O.L.M.Silva & Cordeiro	Brazil, Espírito Santo: Nova Venécia	*O.L.M. Silva 283 (SP)*
	Brazil, Bahia: Ilhéus	*O.L.M. Silva 224* (SP)
	Brazil, Bahia: Itacaré	*O.L.M. Silva 222* (VIC)
Astraea jatropha (Müll.Arg.) B.W.van Ee	Brazil, São Paulo: Arujá	*O.L.M. Silva 139* (SP)
	Brazil, São Paulo: Campo Limpo Paulista	*S.L. Jung 32* (SP)
	Brazil, Poços de Caldas, MG	*O.L.M. Silva 250* (SP)
Astraea klotzschii Didr.	Brazil, Rio de Janeiro: Maricá	*I. Cordeiro 3384* (SP)
	Brazil, Bahia: Ilhéus	*O.L.M. Silva 215* (SP)
Astraea lobata (L.) Klotzsch	Brazil, Minas Gerais: Três Lagoas	*O.L.M. Silva 121* (SP)
	Brazil, Rio Grande do Sul: Santo Antônio das Missões	*E. Melo 7749* (SP)
	Ecuador, Manabi: Bahia de Ceraquez	*C. Ceron 6716* (G)
	Argentina, Corrientes: Ituzaingó	*S.G. Tressens 3005* (MO)
	Brazil, Mato Grosso: Rondonópolis	*L.B. Mota 26* (SPF)
	Brazil, Mato Grosso do Sul: Taquarussu	*M.C. Souza 2546* (HUEM)
Astraea macroura (Mart. ex Colla) P.L.R. Moraes, De Smedt & Guglielmone	Brazil, Espírito Santo: Linhares	*D.A. Folli 1974* (SP)
Astraea paulina Didr.	Brazil, São Paulo: Botucatu	*O.L.M. Silva 213* (SP)
	Brazil, Bahia: Mucugê	*O.L.M. Silva 188* (SP)
	Bolivia, Santa Cruz: Santiago de Chiquitos	*O. L. M. Silva 206* (SP)
Astraea praetervisa (Müll.Arg.) P.E.Berry	Brazil, Bahia: Ilhéus	*W. Thomas 9709* (SP)
	Brazil, Bahia: Ilhéus	*I.R. Pirani 2938* (SPF)
	Brazil, Bahia: Ilhéus	*S.C. Santana 1123* (RB)
Astraea subcomosa (Müll.Arg.) Caruzo	Brazil, Minas Gerais: Serranópolis de Minas	*E. Barbosa 3808* (MBM)
	Brazil, Minas Gerais: Grão Mogol	*A. Freire-Fierro in CFCR12700* (SP)
	Brazil, Bahia: Rio de Contas	*L.R. Lima 261* (SPF)
Astraea surinamensis (Miq.) O.L.M.Silva & Cordeiro	Brazil, Goiás: Goiânia	*O.L.M. Silva 115* (SP)
	Brazil, Maranhão: Porto Franco	*I. Cordeiro 3266A* (SP)
	Brazil, Bahia: Ibotirama	*I. Paula-Souza 9349* (SP)

Table 2 Pollen characters reconstructed for *Acidocroton* and *Astraea* (Euphorbiaceae) in this study and coding of their respective states

Character	States and coding
1. Pollen size (µm)	30–40 (0), 41–50 (1), 51–60 (2)
2. Pilum head shape	Subtriangular (0), subcircular (1), circular (2)
3. Pilum surface	Plicate (0), psilate (1)
4. Distribution of sexine elements in the lumen of the rosettes	Sparse (0), dense (1)
5. Disposition of the sexine elements in the lumen of the rosettes	Spaced, surrounding the base of the pilum (0), aggregate in the central region of the rosette (1)
6. Sexine thickness (µm)	Up to 2.9 (0), 3.0–4.0 (1), more than 4.0 (2)

which lumen has sexine elements. In *A. cincta* (Fig. 1c) and *A. surinamensis* (Miq.) O.L.M.Silva & Cordeiro (Fig. 4l), the lumen of the rosettes is reduced.

Pilum surface varies from psilate in *Astraea comosa* (Müll.Arg.) B.W. van Ee (Fig. 4c), *A. klotzschii* Didr. (Figure 4e), *A. paulina* Didr. (Fig. 4i) and *A. subcomosa* (Müll.Arg.) Caruzo (Fig. 4k), to plicate in the remaining species of *Astraea* (Fig. 4a–b, d, f–h, j, l). In the later, plicae show different levels of folding density (number) and deepness of the pilum grooves (Fig. 4). Only in *A. cincta* and *A. digitata* (Müll.Arg.) O.L.M.Silva & Cordeiro, we observed more than 10 plicae per pilum. As for deepness, only in *A. cincta* and *A. praetervisa* (Müll.Arg.) P.E.Berry, shallow folding is observed. Overall, pila with shallow plicae show more foldings than those with deep plicae: a minimum of eight foldings in deep plicae and a maximum of 13 in shallow plicae.

The sexine elements of the rosette lumen are clavae or pila (Figs. 1, 2, 3, 4). Almost all species have only one of them (Fig. 3d; Table 3), while in *A. lobata* and *A. comosa,* have both (Fig. 3g). The distribution of clavae and pila vary from dense in *A. gracilis* (Müll.Arg.) O.L.M.Silva & Cordeiro, *A. macroura* (Mart. ex Colla) P.L.R.Moraes, De Smedt & Guglielmone (Fig. 4g), *A. jatropha* (Müll. Arg.) B.W.van Ee (Fig. 4h), *A. praetervisa* (Fig. 4j) and *A. subcomosa* (Fig. 4k) to slightly sparse in the remaining species.

In the lumen of rosettes, clavae and pila are sparse surrounding the base of the pilum in most species (Fig. 4c, g, k) or aggregated in the central region of the lumen in *A. gracilis*, *A. jatropha* and *A. praetervisa* (Fig. 4h, j). Finally, the sexine is thicker than the nexine in all species we analyzed (Table 3), with the thinnest in *A. digitata* (2.7 µm) and the thickest in *A. cincta* (4.0 µm).

Acidocroton

Pollen grains of *Acidocroton* are monads, apolar, spherical, inaperturate, with *Croton* pattern exine and sexine thicker than the nexine (Figs. 3a–c, 4a). Species of *Acidocroton* have medium pollen grains with diameter varying from 33.8 µm in *A. gentryi* Fern.Alonso & R.Jaram to 44.1 µm in *A. oligostemon* Urb. (Table 4, Fig. 5). The rosette is composed by 5-7 triangular pila, or subtriangular in *A. gentryi* and *A. oligostemon*. In *A. gentryi,* pilum surface is plicate, pila with deep plicae. The rosettes have a well delimited lumen with clavate sparsely distributed surrounding the base of the pilum in *A. gentry* to aggregate in the central region of the lumen in the other species.

Ancestral states reconstruction

Our ancestral state reconstruction (Fig. 7) indicates that the putative ancestor of *Astraea* most likely had medium pollen grains bearing subcircular pila and spaced sexine elements in the lumen of the rosettes surrounding the base of the pilum and with sexine of 3–4 µm. For the pilum surface, our reconstruction could not recover a most likely state between plicate and psilate.

Regarding pollen grains size, we recovered transitions to slightly larger pollen grains (from less than 40 µm to up to 46 µm) only in species of clade C (*A. comosa, A. jatropha, A. klotzschii, A. paulina* and *A. subcomosa*) and to more than 50 µm in *A. cincta*. Most putative ancestors in *Astraea* were inferred as having subcircular pilum head, with a shift to subtriangular ones in the putative ancestor of clade C2, which could represent a synapomorphy for this group, although *A. digitata* has also subcircular pilum head. In this scenario, the subtriangular pilum head, observed also in *A. macroura*, has an independent origin of this feature in C1, and circular pilum head, observed only in *A. comosa* and *A. subcomosa*, is restricted to C1. Nevertheless, the psilate pilum surface evolved separately in C1 (*A. subcomosa* and *A. comosa*) and C2 (*A. paulina* and *A. klotzschii*).

While most putative ancestors in *Astraea* were inferred as having sparse sexine elements in the lumen of the rosette, for clade C1 we inferred a putative ancestor with dense sexine elements in the lumen of the rosette. This shift could be interpreted as a synapomorphy for C1, with a reversion to sparse sexine elements in *A. comosa*. Regarding the disposition of such sexine elements, independent shifts from spaced, surrounding the base of the pilum, to aggregate in the central region of the rosette, were inferred in both C1 (*A. jatropha* and *A. praetervisa*) and C2 (*A. gracilis*). Finally, for sexine thickness, we inferred independent reductions (to up to 2.9 µm) in *A. lobata* and both C1 (*A. praetervisa*) and C2 (*A. digitata* and *A. gracilis*), with the sexine with more than

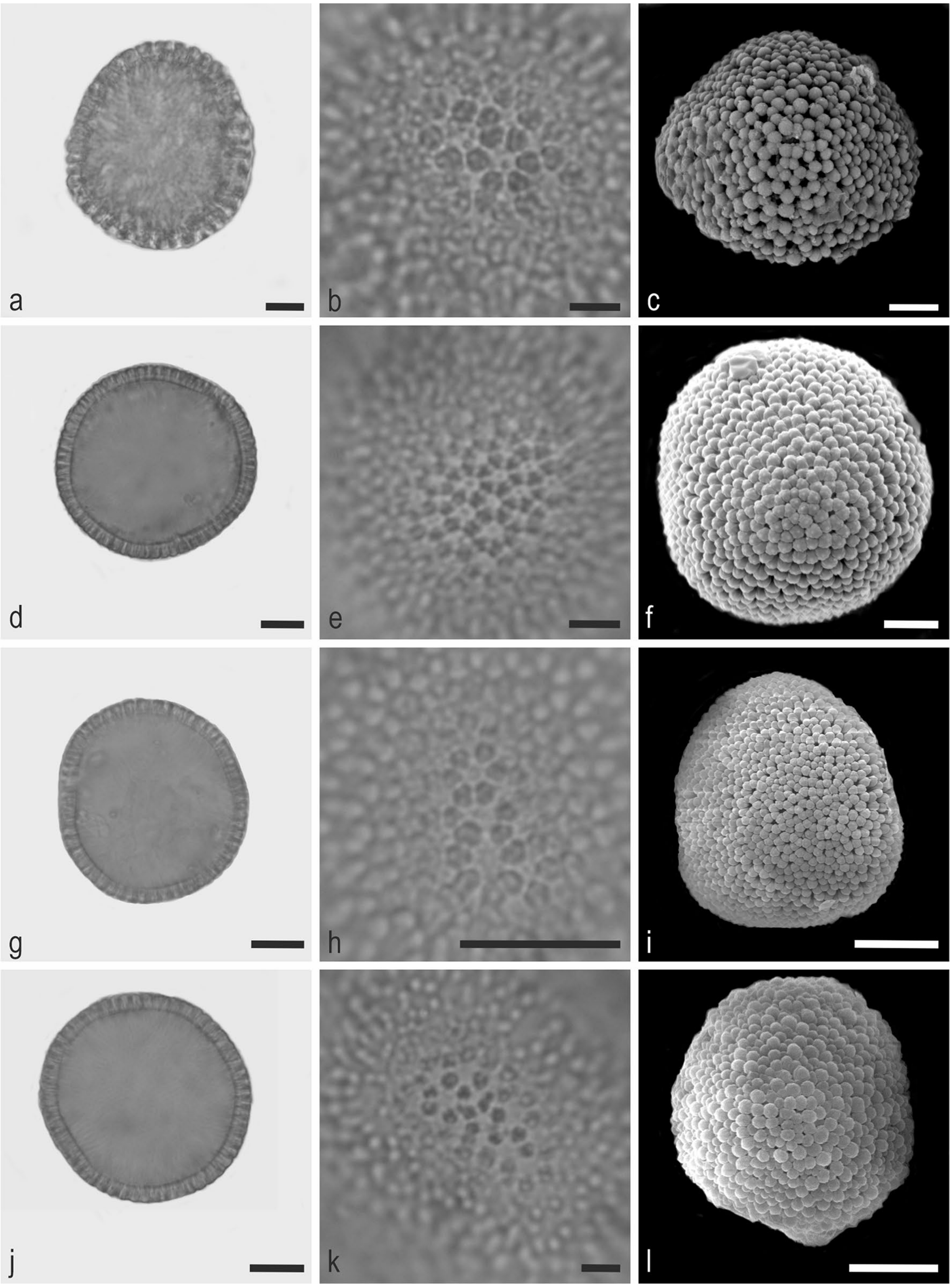

Fig. 1 Pollen grains of species of *Astraea* (Euphorbiaceae). *Astraea cincta*: **a** optical section; **b** detail of the surface; **c** surface (SEM); *A. lobata*: **d** optical section; **e** detail of the surface; **f** surface (SEM); *A. macroura*: **g** optical section; **h** detail of the surface; **i** surface (SEM); *A. surinamensis*: **j** optical section; **k** detail of the surface; **l** surface (SEM). Scale bars 5 µm (**b**, **e**, **h**, **k**), 10 µm (**a**, **c**–**d**, **f**–**g**, **i**–**j**, **l**)

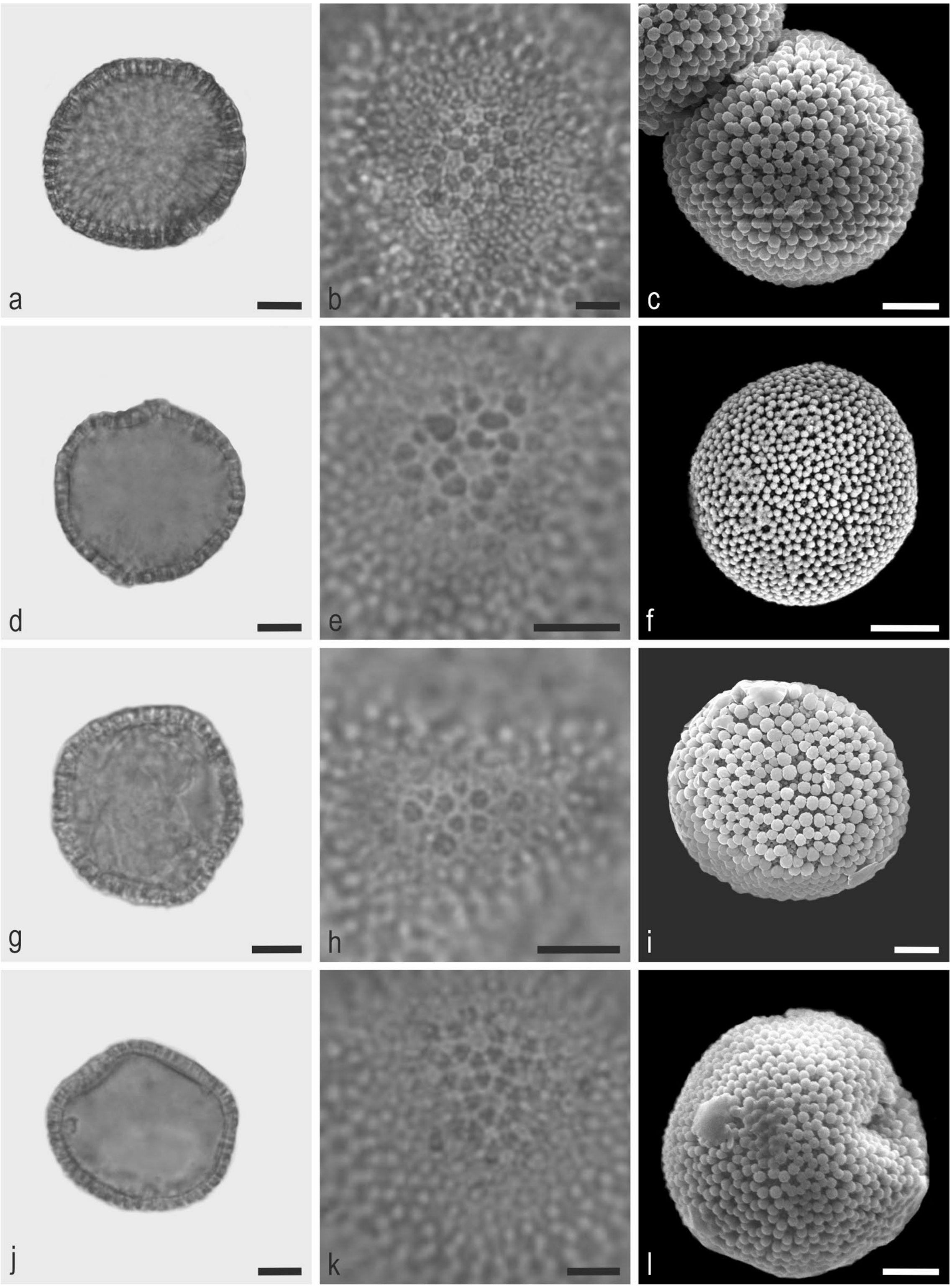

Fig. 2 Pollen grains of species of *Astraea* (Euphorbiaceae). *Astraea comosa*: **a** optical section; **b** detail of the surface; **c** surface (SEM); *A. digitata*: **d** optical section; **e** detail of the surface; **f** surface (SEM); *A. jatropha*: **g** optical section; **h** detail of the surface; **i** surface (SEM); *A. paulina*: **j** optical section; **k** detail of the surface; **l** surface (SEM). Scale bars 5 µm (**b**, **e**, **h**, **k**) 10 µm (**a**, **c**–**d**, **f**–**g**, **i**–**j**, **l**)

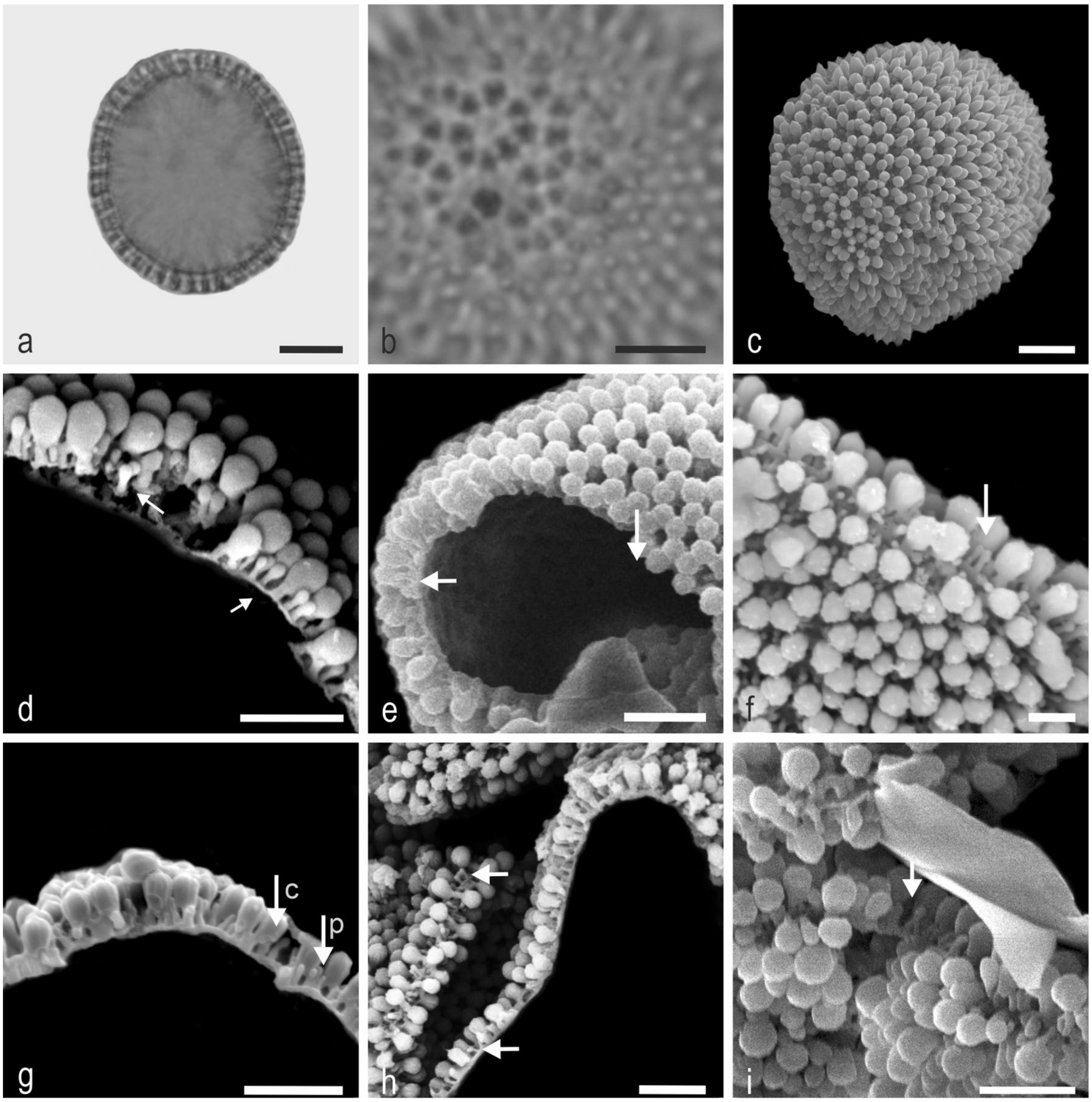

Fig. 3 Pollen grains of species of *Acidocroton* and *Astraea* (Euphorbiaceae). *Acidocroton gentryi*: **a** optical section; **b** detail of the surface; **c** surface (SEM); *Astraea cincta*: **d** detail of exine structure (SEM), arrow showing clavae; *Astraea comosa*: **e** detail of the surface (SEM); *Astraea gracilis*: **f** detail of exine structure (SEM); *Astraea lobata*: **g** detail of the surface (SEM); *Astraea praetervisa*: **h** detail of the surface (SEM); *Astraea surinamensis*: **i** detail of the surface (SEM). c, clava; p, pilum; arrows indicate sexine elements in the lumen of the rosettes. Scale bars 5 μm (**b**–**i**), 10 μm (**a**)

4.0 μm, as well as the pollen larger than 50 μm, representing autapomorphies of *A. cincta*.

Discussion

The work of Carreira and Barth (2003) described the pollen grains of *Astraea paulina* (as *Croton lobatus* in their work) as slightly large (48–53 μm), while Oliveira and Santos (2000) described the pollen of *A. surinamensis* (as *C. lobatus* in their work) similar to what we observed. Our analyzes showed that the majority of *Astraea* pollen grains are in the 40–50 μm range. The smallest diameter was observed in *Acidocroton gentryi* (33.8 μm), and *Astraea cincta* has the largest pollen grains (51.9 μm). Although we could not identify any synapomorphy for the clade *Astraea* + *Acidocroton,* our work confirmed that spheroidal and inaperturate pollen grains with *Croton* pattern ornamentation are shared among all representatives of Crotoneae following the circumscription of Wurdack et al. (2005). The presence of sexine elements in the lumen of the rosettes, as well as having the sexine thicker than the nexine, are features widely present in

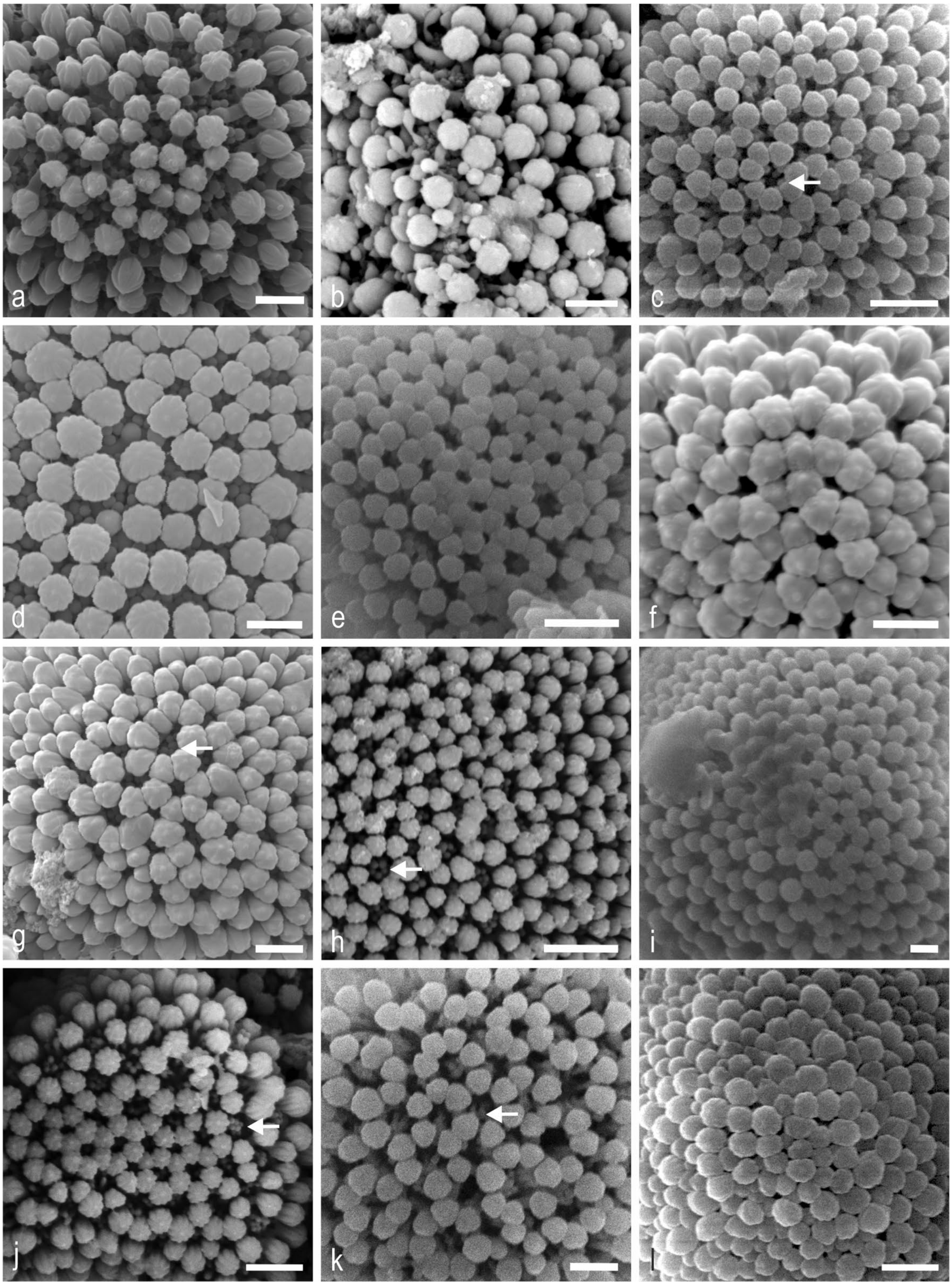

Fig. 4 SEM details of the surface of pollen grains of species of *Acidocroton* and *Astraea* (Euphorbiaceae). **a** *Acidocroton gentryi*; **b** *Astraea cincta*; **c** *Astraea comosa*; **d** *Astraea digitata*; **e** *Astraea klotzschii*; **f** *Astraea lobata*; **g** *Astraea macroura*; **h** *Astraea jatropha*; **i** *Astraea paulina*; **j** *Astraea praetervisa*; **k** *Astraea subcomosa*; **l** *Astraea surinamensis*. Scale bars 2 µm (**c**, **e**–**f**, **h**–**l**), 5 µm (**a**, **b**, **d**, **g**)

Table 3 Pollen characters in species of *Acidocroton* and *Astraea* (Euphorbiaceae) included in this study

Species	Size	Diameter	*Croton* pattern exine									S/N	Exine thickness
			Pilum head shape	Pilum surface	Pilum head foldings	Pilum grooves	Pilum number	Center of the rosette	Sexine elements in the lumen of the rosette				
									Type	Distribution	Disposition		
Astraea A													
A. cincta	L	51.9	SC-ST	Plicate	13	Shallow	5-7-(8)	WD-R	Cl	Sparse	SP	S > N	4.1
A. lobata	M	45.4	SC-ST	Plicate	8	Deep	5-7	WD	P, Cl	Sparse	SP	S > N	3.2
Astraea B													
A. surinamensis	M	45.2	ST	Plicate	8	Shallow	5-7	WD-R	Cl	Sparse	SP	S > N	3.1
Astraea C1													
A. comosa	M	45.5	C	Psilate	–	–	5-8	WD	P, Cl	Sparse	SP	S > N	3.2
A. macroura	M	35.8	SC	Plicate	7	shallow	5-8	WD	Cl	Dense	SP	S > N	3.0
A. jatropha	M	43.0	ST	Plicate	10	shallow	5-7	WD	Cl	Dense	AG	S > N	3.0
A. praetervisa	M	38.3	ST	Plicate	10	deep	5-7	WD	Cl	Dense	AG	S > N	2.9
A. subcomosa	M	48.0	C	Psilate	–	–	5-7	WD	Cl	Dense	SP	S > N	3.7
Astraea C2													
A. digitata	M	38.2	SC-ST	Plicate	13	Shallow	5-7	WD	Cl	Sparse	SP	S > N	2.7
A. gracilis	M	40.6	SC	Plicate	9	Shallow	5-7	WD	Cl	Dense	AG	S > N	2.8
A. klotzschii	M	45.1	SC	Psilate	–	–	5-8	WD	Cl	Sparse	SP	S > N	3.2
A. paulina	M	44.4	SC	Psilate	–	–	5-8	WD	Cl	Sparse	SP	S > N	3.3
Acidocroton													
Ac. gentry	M	33.8	ST	Plicate	7	Deep	5, 7	WD	Cl	Sparse	SP	S > N	3.0
Ac. oligostemon	M	44.1	ST	–	–	–	5, 7	WD	–	Sparse	AG[a]	S > N	3.8
Ac. spinosus	M	40.1	T	–	–	–	5, 7	WD	–	Sparse	AG[a]	S > N	3.0

L large; *M* medium sized; *C* circular; *SC* subcircular; *ST* subtriangular; *T* triangular; *S* sexine; *N* nexine; *R* reduced; *WD* well delimited; *Cl* clava; *P* pilum; *SP* spaced, surrounding the base of the pilum; *AG* aggregated in the central region of the rosette

[a] Based only on light microscopy

Table 4 Pollen characters in species of *Acidocroton* and *Astraea* (Euphorbiaceae) included in this study

Species	D		DR	DPi	DCSR	Sex	Nex	Exine
	$\bar{x} \pm S\bar{x}$	Fv						
Astraea A								
Astraea cincta								
F.C. Hoehene s.n (SP 35784)	52.4±0.14	50.0–57.5	6.8	1.7	2.7	2.7	1.0	3.7
O.L.M. Silva 238 (SP)	50.1±0.19	45.0–55.0	4.9	1.0	1.7	3.0	1.0	4.0
Sidney 1532 (UB)	50.4±0.40	47.5–55.5	5.2	1.4	1.6	3.1	1.0	4.1
O.L.M. Silva 263 (SP)	52.5±0.17	50.0–55.0	5.4	1.4	2.75	3.3	1.0	4.3
R.F. Vieira 946 (CEN)	54.3±0.21	50.0–57.5	7.0	2.0	3.0	3.6	1.0	4.6
Astraea lobata								
O.L.M. Silva 121 (SP)	42.5±0.11	42.5–50.0	4.0	1.0	1.75	2.0	1.0	3.0
L.B. Mota 26 (SPF)	48.5±0.14	45.0–50.0	3.75	1.0	1.75	2.1	1.0	3.1
C. Ceron 6716 (G)	41.1±0.16	37.5–42.5	4.3	1.0	2.2	2.4	1.0	3.4
S.G. Tressens 3005 (MO)	49.3±0.10	47.5–52.5	4.6	1.0	2.1	2.7	1.0	3.7
E. Melo 7749 (SP)	50.0±0.08	47.5–52.5	2.8	1.3	1.0	2.1	1.0	3.1
M.C. Souza 2546 (HUEM)	41.4±0.13	40.0–45.0	3.9	1.0	1.9	2.0	1.0	3.0
Astraea B								
Astraea surinamensis								
O.L.M. Silva 115 (SP)	47.1±0.14	45.0–50.0	4.0	1.0	2.0	2.2	1.0	3.2
I. Cordeiro 3266A (SP)	44.9±0.18	40.0–50.0	4.0	1.0	2.2	2.2	1.1	3.3
I. Paula-Souza 9349 (SP)	43.6±0.20	40.0–47.5	3.9	1.0	1.9	2.0	1.0	3.0
Astraea C1								
Astraea comosa								
J.R.P. Pirani 3961 (SPF)	46.1±0.17	42.5–50.0	4.4	1.0	1.7	2.8	1.0	3.8
O.L.M. Silva 266 (SP)	44.1±0.17	42.5–50.0	4.0	1.0	1.9	2.0	1.0	3.0
I. Cordeiro 3047 (SP)	46.4±0.20	42.5–50.0	4.3	1.0	1.7	2.0	1.0	3.0
Astraea macroura								
D.A. Folli 1974 (SP)	35.8±0.13	32.5–37.5	2.1	0.75	1.0	2.0	1.0	3.0
Astraea jatropha								
O.L.M. Silva 139 (SP)	46.6±0.16	45.0–50.0	4.3	1.0	1.85	2.0	1.0	3.0
S.L. Jung 32 (SP)	43.0±0.15	40.0–45.0	4.0	1.0	2.2	2.2	1.0	3.2
O.L.M. Silva 250 (SP)	39.5±0.12	37.5–42.5						
Astraea praetervisa								
W. Thomas 9709 (SP)	39.0±0.15	37.5–42.5	3.3	1.0	1.1	2.0	1.0	3.0
I.R. Pirani 2938 (SPF)	44.5±0.12	42.5–47.5	3.7	1.0	1.6	2.0	1.0	3.0
S.C. Santana 1123 (RB)	31.4±0.20	27.5–35.0	3.5	1.0	1.6	2.0	0.9	2.9
Astraea subcomosa								
A. Freire-Fiera 12700 (SP)	48.1±0.16	45.0–52.5	5.0	1.0	1.9	3.6	1.0	4.6
L.R. Lima 261 (SPF)	48.4±0.22	45.0–52.5	5.2	1.1	2.7	2.0	1.0	3.0
E. Barbosa 3808 (MBM)	38.7±0.14	35.0–42.5	3.2	1.0	1.0	2.0	1.0	3.0
Astraea C2								
Astraea digitata								
M.L. Guedes s.n (ALCB 27934)	39.8±0.16	37.5–42.5	3.1	1.0	1.5	2.0	1.0	3.0
J.L. Haje 1118 (CEPEC)	34.5±0.18	30.0–37.5	2.2	0.75	1.0	1.1	1.0	2.1
O.L.M. Silva 228 (SP)	40.4±0.16	37.5–42.5	3.6	1.0	1.65	2.0	1.0	3.0
Astraea gracilis								
O.L.M. Silva 283 (SP)	42.5±0.16	37.5–45.0	3.9	1.0	1.9	2.0	1.0	3.0
O.L.M. Silva 224 (SP)	39.0±0.12	35.0–42.5	3.7	1.0	1.6	2.0	1.0	3.0
O.L.M. Silva 222 (VIC)	40.3±0.13	37.5–42.5	3.2	1.0	1.2	1.6	1.0	2.6
Astraea klotzschii								
I. Cordeiro 3384 (SP)	40.5±0.15	37.5–42.5	3.1	1.0	1.3	2.0	1.0	3.0

Table 4 (continued)

Species	D		DR	DPi	DCSR	Sex	Nex	Exine
	$\bar{x} \pm S\bar{x}$	Fv						
O.L.M. Silva 215 (SP)	49.7 ± 0.12	47.5–52.5	4.9	1.0	2.0	2.5	1.0	3.5
Astraea paulina								
O.L.M. Silva 213 (SP)	41.3 ± 0.18	37.5–45.0	3.5	1.0	1.2	2.2	1.0	3.2
O.L.M. Silva 188 (SP)	47.7 ± 0.18	45.0–50.0	4.2	1.0	1.9	2.7	1.0	3.7
O.L.M. Silva 206 (SP)	44.2 ± 0.18	40.0–50.0	4.1	1.1	1.9	2.0	1.0	3.0
Acidocroton								
Acidocroton gentryi								
R. Jaramillo-Mejía 8285 (COL)	30.1 ± 0.12	27.5–32.5	3.2	1.0	1.2	2.0	1.0	3.0
A. Férnandez-Peréz 10688 (COL)	35.4 ± 0.13	32.5–37.5	3.6	1.0	1.6	2.0	1.0	3.0
J. Cabezas et al. 89 (COL)	32.3 ± 0.40	30.0–32.5	3.8	1.0	1.9	1.7	1.0	2.7
Acidocroton oligostemon								
B. Clemente 2473 (NMNH)	44.1 ± 0.15	40.0–47.5	4.3	1.0	2.2	2.8	1.0	3.8
Acidocroton spinosus								
A.L. Gentryi 74385 (MO)	40.1[a]	37.5–42.5	3.8	1.0	1.8	2.0	1.0	3.0

A, B, C1 and C2, clades and subclades of *Astraea* molecular phylogeny according to Silva et al. (2020)

D pollen grain diameter; *DR* diameter of the pilum rosettes; *DPi* diameter of the pila; *DCSR* diameter of the central space of the rosettes; *Sex* Sexine; *Nex* Nexine

[a] $n < 25$ measurements for pollen grain diameter; measurements in µm and indices in absolute numbers

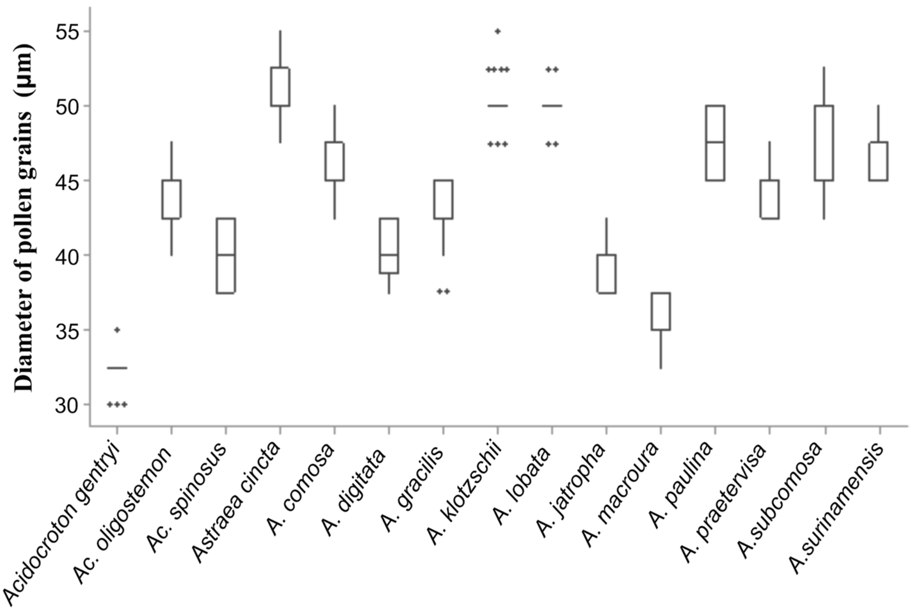

Fig. 5 Boxplot graph of the distribution of the variable diameter of pollen grains of *Acidocroton* and *Astraea* (Euphorbiaceae). The horizontal bar inside the rectangle is the median, the rectangle shows 50% of interquartile, the ends show the amplitude variation, and the black circles correspond to the outlier

Croton, *Brasiliocroton* and *Sagotia* (Erdtman 1952; Thanikaimoni et al. 1984; Nowicke 1994; Lima et al. 2007; Souza et al. 2016, 2019) and now extended to *Acidocroton* and *Astraea* because of our study.

The morphology of the pilum surface of the rosette is a character of taxonomic value as demonstrated by Thanikaimoni et al. (1984), Nowicke (1994), Carreira et al. (1996), Lobreau-Callen and Cervera (1997), Souza et al. (2016), Ren-Yong et al. (2018) and Souza et al. (2019). Carreira et al. (1996), who studied the pollen morphology of several lianescent species of *Croton*, verified the presence of pilum with a striate and psilate surface. Ren-Yong et al. (2018) showed that the *Trigonostemon* (Euphorbiaceae) species differ from other genera (*Dimorphocalyx*, *Ostodes* and *Tritaxis*) by the absence of striate ornamentation on the subunits (pila). Souza et al. (2019) showed that the species of the genus *Brasiliocroton* have pila with variation in the surface of ornamentation, *Brasiliocroton mamoninha* P.E.Berry & Cordeiro has pila > 5-plicate and pointed apex, whereas *B. muricatus* Riina & Cordeiro had pila < 5-Plicate and rounded

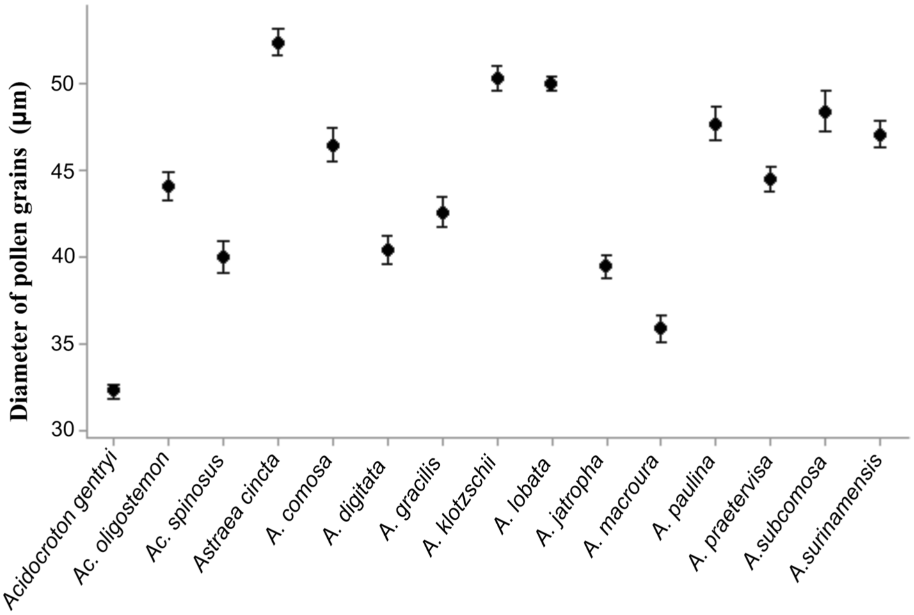

Fig. 6 Graphic representation of 95% confidence interval for the mean diameter of the pollen grains of the *Acidocroton* and *Astraea* species (Euphorbiaceae). Circles represent the means, and vertical lines represent the range of variation. *Individual standard deviations were used to calculate the intervals

apex, thus contributing to the taxonomic circumscription of these taxa. For Thanikaimoni et al. (1984), the ornamentation of sculptural elements (pilum) is a diversification of the *Croton* pattern and may also be associated with pollination. In *Astraea*, however, we observed only psilate and plicate pila.

Most pollen characters analyzed are conserved in *Astraea*, with shifts concentrated within clades and evolving independently, a pattern also documented for monocots and basal angiosperms (Lu et al. 2015, Zhang et al. 2017). Silva et al. (2020) inferred recent divergences (from 10 Ma onward) within clades A, B, C1 and C2 of *Astraea*, most likely influenced by the colonization of humid areas, as, for example, in clade C1 we find species restricted to dry (*A. comosa* and *A. subcomosa* in *campo rupestres*) and humid areas (*A. macroura* and *A. praetervisa* in seashore vegetation). Therefore, shifts in pollen features may also be linked to the colonization of new habitats along the evolutionary history of *Astraea*.

Psilate pila are inferred as derived states (Fig. 7) in two distinct lineages: *Astraea comosa* and *A. subcomosa* in C1 and *A. klotzschii* and *A. paulina* in C2. These species with psilate pila are the only ones found in *campos* rupestres (Silva et al. 2020): *A. comosa* is restricted to the Meridional Espinhaço Range, while *A. subcomosa* is restricted to the Septentrional Espinhaço Range and Chapada Diamantina, *A. paulina* is found along the Espinhaço Range, but also in gallery forests in the Cerrado domain, and *A. klotzschii* is mainly found in seashore vegetation in eastern Brazil, but with a few collections from Chapada Diamantina.

Astraea cincta is unique in the genus due to its xylopodiferous underground system, cartilaginous leaf margin and rounded seeds (Silva et al. 2019). Pollen data provided additional unique features for this species: the largest pollen grains and the thickest exine (Fig. 7; Table 3). Such features seem to be related to preventing water loss during pollen dispersal as Nepi et al. (2001) have suggested for many groups of angiosperms. In fact, *A. cincta* is found in seasonally dry open vegetation in central Brazil and eastern Bolivia (Silva et al. 2019). Other factors such as transport conditions and level of polyploidy may reflect directly in pollen size (Muller 1979). However, there are no data available in the literature for cytotaxonomy and pollination system involving *Astraea* species, except the chromosome number of *A. lobata* (Miller and Webster 1966).

Medium pollen grains are observed in the remaining species of *Astraea*. However, most species restricted to the Atlantic rain Forest domain (namely, *A. gracilis*, *A. digitata*, *A. macroura* and *A. praetervisa*) have slightly smaller pollen grains (35.8–40.6 µm vs 43–48 µm in the remaining species with medium pollen grains), indicating that smaller pollen grains in *Astraea* may be associated with humid areas in both C1 and C2. In C2, *A. gracilis* and *A. digitata* have congruent patterns also in pilum surface and exine thickness, when compared to their sister species (*A. paulina* and *A. klotzschii*). The occurrence of *Astraea paulina* in the Cerrado domain suggests an association of plicate pilum surface and thinner exine with more humid habitats. Although *A. klotzschii* is also in the Atlantic Forest, it occurs in a different habitat (mostly restricted to seashore vegetation) from *A. gracilis* and *A. digitata* (borders of humid forests).

Finally, in support of the segregation of *Astraea digitata*, *A. gracilis, A. jatropha* and *A. surinamensis* from *A. lobata*, our data show that only *A. lobata* has both pilum and clava as sexine elements inside the lumen of its rosettes, while the remaining species have strictly clavate sexine elements.

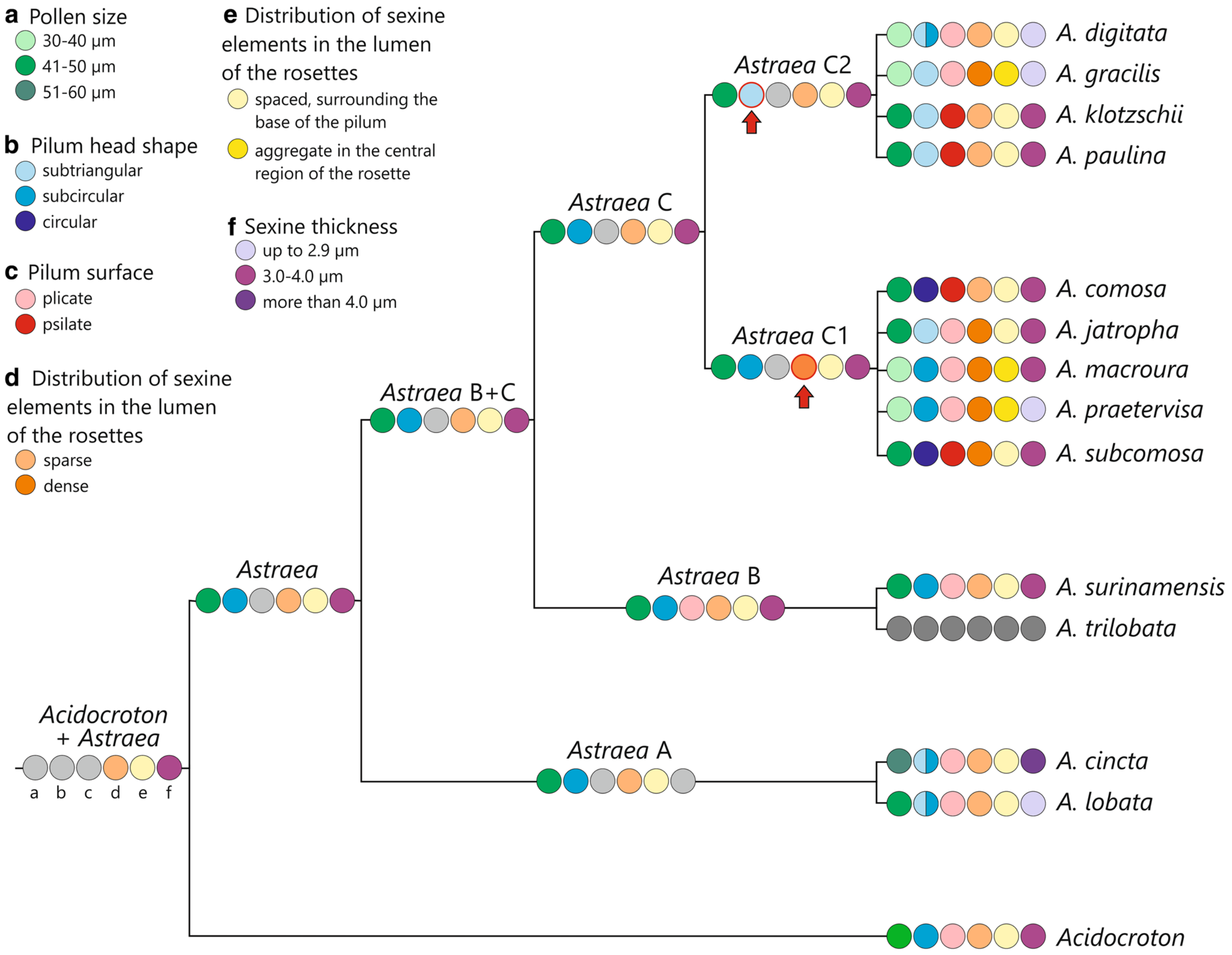

Fig. 7 Inferred ancestral state reconstruction of *Acidocroton* and *Astraea* (Euphorbiaceae) for pollen size, pilum head shape, pilum surface, distribution and disposition of sexine elements in the lumen of the rosettes and sexine thickness using maximum likelihood over the ITS tree from Silva et al. (2020). Color codes for character states are shown on the upper left; gray represents unknown or inconclusive state reconstruction. Character state shifts in putative ancestors are indicated with a red arrow

Also, *A. jatropha* and *A. digitata* have a high number of pilum head folding (10 and 13, respectively, vs. eight in *A. lobata*), while *A. digitata* and *A. gracilis* have smaller pollen grains (38.2 and 40.6 µm, respectively, vs. 45.4 µm in *A. lobata*) with slightly thinner sexine (2.7 and 2.8 µm, respectively, vs. 3.2 µm in *A. lobata*). Pollen grains of *A. surinamensis*, however, are very similar to those of *A. lobata*.

Conclusions

With an almost complete sampling of *Astraea*, we found little variation in pollen morphology, confirming its stenopalynous nature. Our pollen data support the placement of *Astraea* in Crotoneae based on shared features such as apolar, spheroidal, inaperturate pollen grains with *Croton* pattern exine. Shifts to less conserved pollen features in the most recent diverging clade of *Astraea* may be associated with the colonization of new habitats. Distinct pollen features among species within that clade also suggest this pattern. Although we did not identify any synapomorphy, neither for *Astraea* nor for the *Astraea* + *Acidocroton* clade, our results expand the palynological data for Crotoneae and will contribute to future taxonomic and phylogenetic studies in this group.

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Compliance with ethical standards

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

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