ORIGINAL ARTICLE

New insights into the pollen morphology of the genus *Mutisia* (Asteraceae, Mutisieae)

M. C. Tellería · L. Katinas

Abstract The pollen morphology from 67 collections representing 52 species of *Mutisia* was analyzed by means of light, scanning electron and transmission electron microscopy. Pollen of *Mutisia* is comparable to that of the more widespread type found in Mutisiinae. The pollen grains of the genus are characterized by the spheroidal-subprolate to prolate shape, large size, tricolporate aperture, with mesoaperture, exine Mutisia type, with sculpturing microechinate to rugulate or microechinate-rugulate. This basic pollen type is very variable in size, endoaperture shape, and exine thickness of the pollen grains. A new type of arrangement of columellae was observed in pollen of *Mutisia* section *Mutisia* (e.g., *M. stuebelli*, *M. microphylla*, and *M. sodiroi*). It is suggested that it might have harmo-megathic significance. The pollen morphology and polymorphism are discussed with regard to functional mechanisms and possible evolutionary significance.

Keywords *Mutisia* · LM · SEM · TEM ·
Pollen polymorphism · *Chaetanthera* · South America

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Introduction

Mutisia L. f. (Asteraceae, Mutisieae) comprises 63 species endemic to South America (Cabrera 1965). In addition to the fact that *Mutisia* is the type genus of Mutisieae, it is one of the largest genera of the tribe in terms of number of species (Katinas et al. 2008). It is characterized by the climbing habit of most species and its solitary showy, commonly bird-pollinated florets (Sazima and Machado 1983). The major concentration of species occurs in the Andes, from Colombia to southern Chile and Argentina, and 11 species inhabit southern Brazil, Paraguay, Uruguay, and northeastern Argentina. The species of *Mutisia* grow in very distinct habitats such as at different latitudes of the Andes (yungas, lomas formation, montane forests, sub-Antarctic forests, paramo, Altoandean vegetation), tropical forests in southern Brazil and adjacent areas, the Puna or Altiplano, arid areas of Patagonia in southern Chile and Argentina, and in the mediterranean area of central Chile. The altitudinal range for the genus starts at sea level rising to about 4,600 m. Several species (e.g., *M. clematis*, *M. decurrens*, *M. ledifolia*, *M. subulata*) have considerable chromosomal and morphological variability, and there are reports of hybrid species and polyploids (diploids and tetraploids) in the genus (Cabrera 1965). *Mutisia* is subdivided into six sections: *Fruticosa*, *Guariruma*, *Holophyllum*, *Isantha*, *Mutisia*, and *Ovata* (Cabrera 1965) on the basis of habit, leaf, and capitulum features. Cabrera (1965) hypothesized an evolutionary scheme of the sections of *Mutisia* where tetraploid and trailing plants with winged stems, compound leaves with tendrils, big capitula, phyllaries of the involucre without apical appendage, and dimorphic florets are evolved features in the genus. In Cabrera's scheme, *Mutisia* sections *Isantha*, *Holophyllum*, and *Fruticosa* would be the most

primitive giving rise to the more evolved sections *Guariruma*, *Mutisia*, and *Ovata*.

The morphological features of pollen grains of *Mutisia* have been studied by several authors (Wodehouse 1929; Stix 1960; Hansen 1991; Tellería and Forcone 2000; Tellería et al. 2003; Tellería and Katinas 2004; Zao et al. 2006; Katinas et al. 2008). However, only Cabrera (1965) and Parra and Marticorena (1972) have reported the size variability of pollen grains in this genus. Cabrera (1965) attributed this variation to the ploidy levels of the genus.

Pollen features with adaptive value have supported infrageneric classification in other genera of the tribe; this is the case for the large Andean genus *Chaetanthera* (43 species) (Tellería and Katinas 2004). Two pollen types together with other morphological features allow two groups of subgenera in *Chaetanthera* to be distinguished; these features in turn may be related either to the low temperatures or to the dry environments where each of the groups grows. Therefore, we wonder if pollen morphology in *Mutisia*, another Andean-centered genus but with different morphology and ploidy levels also supports its infrageneric taxonomy and has some adaptive value. It would be particularly interesting to find out if there are different pollen strategies in the genera of Mutisieae.

The aims of the present study were to (1) provide a more extensive knowledge of pollen morphology for this genus, based on observations from light microscopy (LM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM), (2) analyze the distribution of pollen polymorphism within the species of the genus with regard to the infrageneric classification of Cabrera (1965), and (3) make suggestions about the possible significance of the pollen polymorphism when compared with nonpolymorphic genera of the tribe.

Materials and methods

The following herbaria loaned specimens for palynological review of *Mutisia*: BAF, F, LP, SI, and WIS (Holmgren et al. 1990). Depending upon the availability of material, two specimens for each species were sampled. Pollen from 67 specimens of 52 species (see "Appendix") was acetolyzed in the standard way, chlorinated, and mounted in glycerol jelly (Erdtman 1960). Whenever possible, the polar (P) and equatorial (E) diameters of 25 pollen grains from each specimen were measured, and the average and standard deviations were calculated. Only fully intact pollen grains were measured. The P/E ratio was calculated for each specimen. The measurements of exine thickness are based on 15 pollen grains. The ratio ectosexine/endosexine was estimated.

For SEM, acetolyzed pollen grains were suspended in 90% ethanol and mounted on stubs and examined using a JEOL JSM T-100 SEM. For TEM, the fresh pollen grains were fixed in 1.5% glutaraldehyde and then in buffered 2% Os O_4 for 2 h at room temperature. After that, they were washed for 30 min in distilled water and dehydrated in an ethanol series and finally embedded in acetone–Spurr 3:1 for 6 h and acetone–Spurr 1:1 for 16 h and twice in Spurr for 24 h. Ultrathin sections were mounted in single grids and stained with lead citrate (1 min) and uranyl acetate (10 min). The examinations were made with a Zeiss T-109 TEM at the Instituto de Biología Celular, Universidad de Buenos Aires.

The terminology in general follows Punt et al. (2007); the characterization of Mutisia exine type was taken from Katinas et al. (2008) who basically expanded the characterization of Stix (1960). The Mutisia exine type is scabrate, verrucate, microechinate or echinate with short spines (usually less than 2 μm), with tectum imperforate or scarcely perforate. The range of exine thickness is broad (4–18 μm). The ectosexine is equal to, thinner than, or thicker than endosexine and is slightly columellate or with compact aspect. The endosexine has stout and ramified columellae, with the two layers separated by an inconspicuous or conspicuous internal tectum. The ectosexine/endosexine ratio is variable: 1:1; 1:2; 1:2.5; 1.5:1; 2:1; 2.5:1. The size classification was taken from Erdtman (1969). The term polymorphism was used in the sense of Borsch and Wilde (2000) to reflect the overall range of variability in the pollen grains (e.g., size, exine thickness), including discrete characters and gradual differences.

Results

General description of the pollen of the genus *Mutisia*

Pollen grains are radially symmetrical and isopolar, spheroidal-subprolate, subprolate-prolate or prolate, elliptic to subrectangular outline in equatorial view, and circular to triangular outline in polar view (Fig. 1a–e).

Pollen grains have a compound apertural system, they are tricolporate but each aperture comprises an ecto-, meso-, and endoaperture, each of which is situated in different layers of the pollen wall (Fig. 1f). In the ectoaperture, the colpi are long, the ends are usually acute, and the colpus membrane is psilate or scarcely microgranulate. Sometimes the ends of the colpi are united, forming a parasyncolpus. The colpi are a thinning of the sexine. The endoaperture is lalongate or fused to an equatorial endocingulum with many intermediate stages (Fig. 2; Fig. 3c–g). The endoaperture is a thinning of nexine. The mesoaperture

Fig. 1 SEM micrographs of whole pollen grains of *Mutisia*. **a**, **b** *M. grandiflora*. **a** Equatorial view of subrectangular grain. **b** Polar view. **c** *M. clematis*. Equatorial and subpolar view. **d** *M. campanulata*. Equatorial view of elliptic grain. **e**, **f** *M. pulchella*. **e** Equatorial view. **f** Detail of compound aperture: ectoaperture (*A*), mesoaperture (*B*), endoaperture (*C*). *Scale bars*: **a–e** = 10 μm, **f** = 5 μm
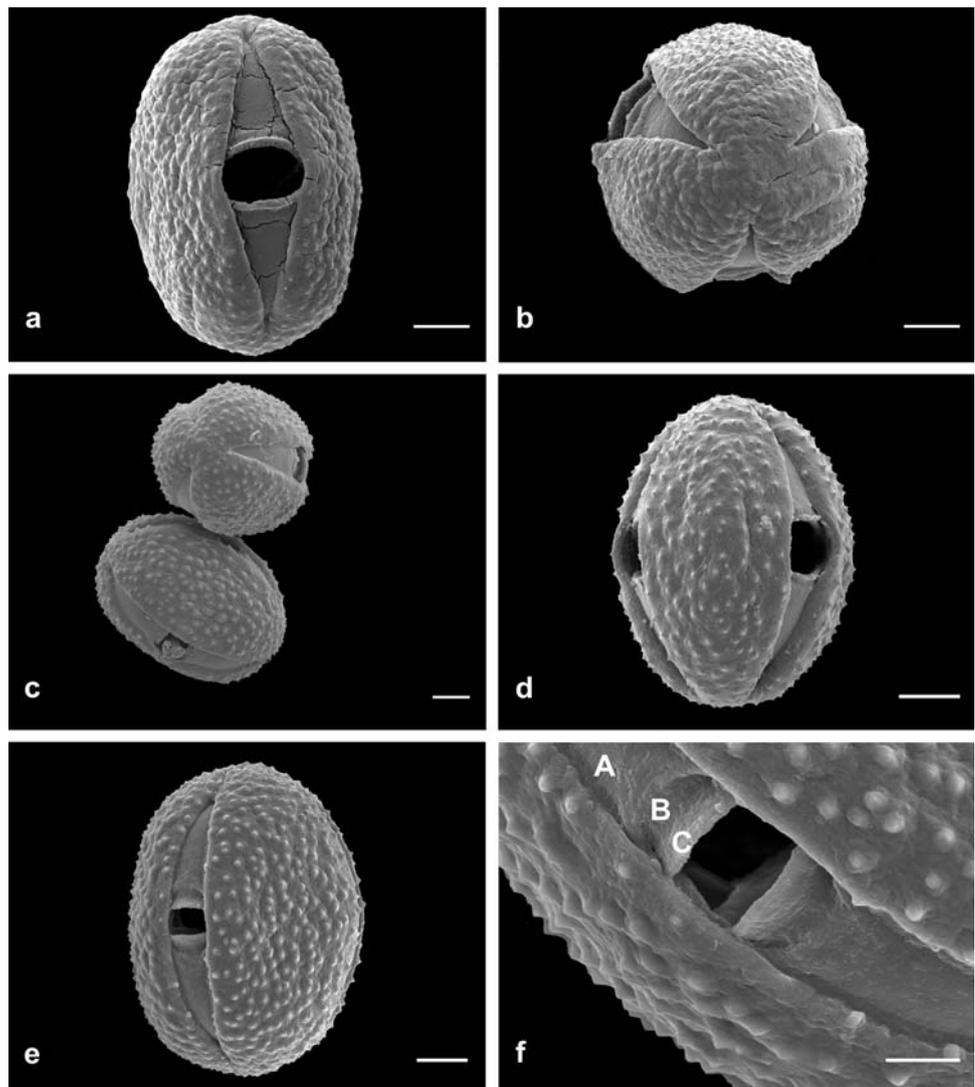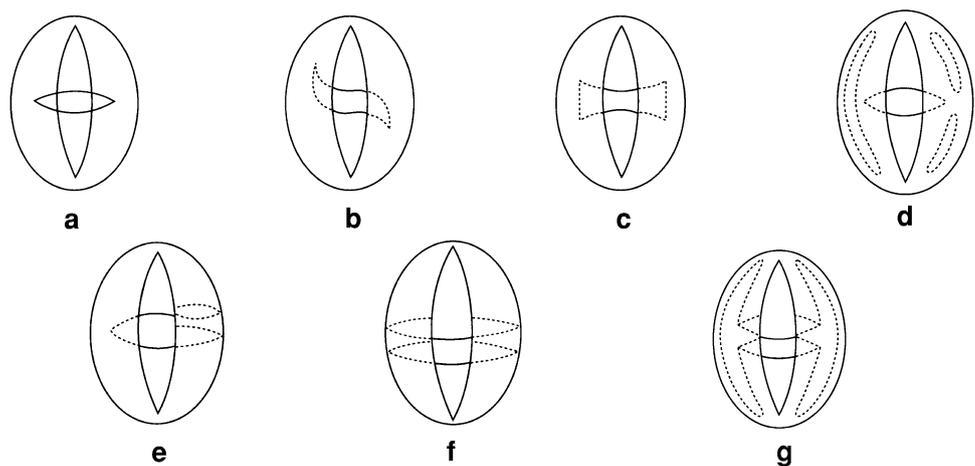

Fig. 2 Schemes illustrating different variants of endoapertures within the *Mutisia* genus. **a** "Regular type." **b** With horns. **c** With ramified ends. **d** Endocolpus not connected to the endoaperture. **e** Incomplete endocingulum. **f** Complete endocingulum. **g** Endocingulum with long extensions in the mesocolpium

is generally conspicuous, it appears as a thin area involving the inner layer of the sexine and the outer layer of the nexine (Fig. 3a, b).

Pollen exine is tectate, the tectum is imperforate or scarcely perforate, with rugulate-microechinate or microechinate surface (Fig. 1a, d). The exine can be
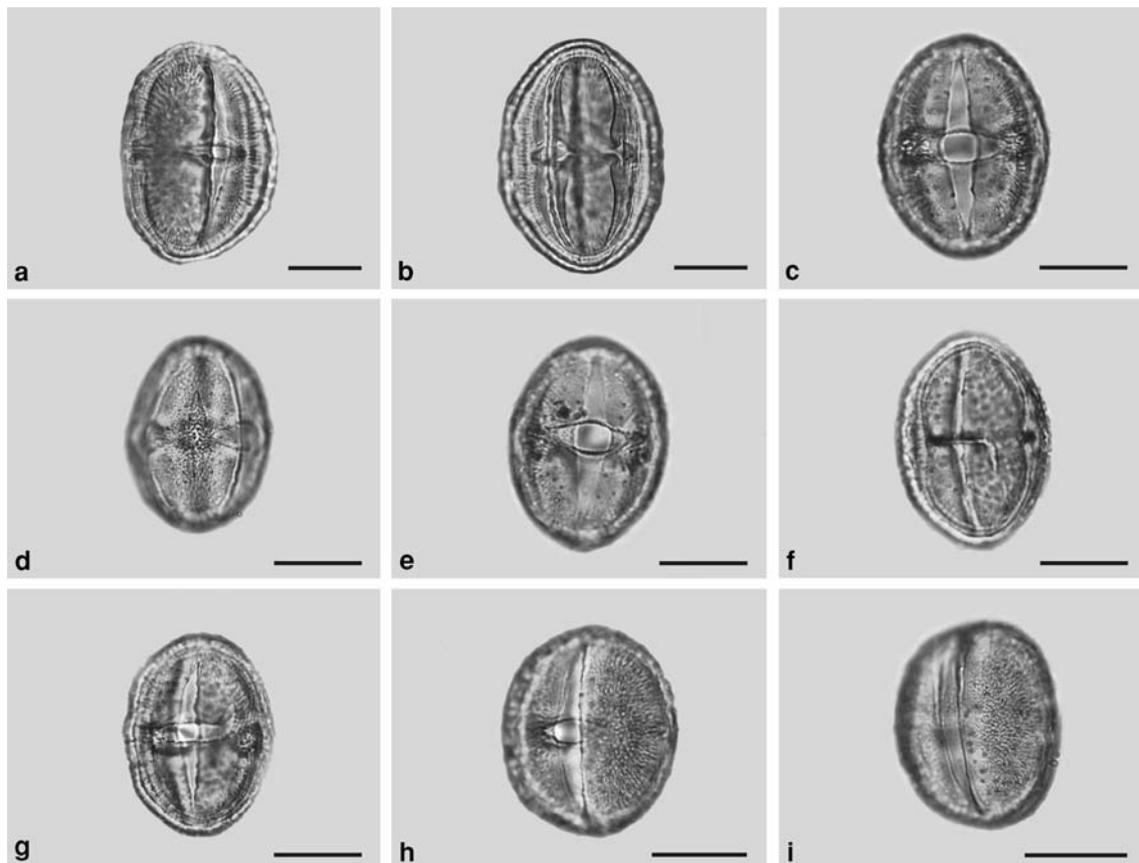
Fig. 3 LM micrographs of equatorial view of *Mutisia* pollen showing apertures. **a**, **b** *M. cana*. **a** Intermediate focus showing the compound aperture with mesoaperture. **b** Intermediate focus showing the costae. **c–g** Different variants of endoapertures. **c–e** *M. campanulata*. **c** Cingulated. **d** Endocingulum with extensions in the mesocolpium. **e** With ramified ends. **f**, **g** With horns. **f** *M. sodiroi*. **g** *M. microphylla*. **h**, **i** *M. brachyantha*. **h** Colporate grain. **i** Colpate grain with undeveloped endoaperture. *Scale bars* 30 µm

thickened at the equator, from 4 to 21 µm thick (e.g., *M. acerosa*, *M. comptoniaefolia*, *M. decurrens*, *M. homoeantha*) (Fig. 4a–e) or uniformly thickened in complete grain (e.g., *M. clematis*, *M. grandiflora*, *M. oligodon*) (Fig. 4f–l). Sexine consists of ectosexine with compact (Fig. 4a) or finely columellate aspect (Fig. 4f), endosexine with stout or thin ramified columellae, both layers separated by an internal tectum (Fig. 5a, b). The nexine is thickened towards the endoapertures forming costae (e.g., Fig. 3d). Pollen grains with thicker exine exhibit endosexine with stout columellae (Fig. 4a, b); in contrast, pollen grains with thin exine have the endosexine with thin columellae (Fig. 4f–l). The columellae are commonly almost perpendicular to the nexine layer (Fig. 4a, c; Fig. 5a), however, they are evidently inclined in *M. microphylla*, *M. sodiroi*, and *M. stuebelii* (Fig. 4h–l; Fig. 5c). The inclined arrangement of columellae starts in the equatorial plane (Fig. 5c). Note that both pollen types, tricolporate and tricolpate, were found in a single specimen. In these cases, the proportion of tricolpate

grains was always insignificant with relation to tricolporate grains [e.g., *M. andersonii*, *M. brachyantha* (Fig. 3h, i), *M. comptoniaefolia*, *M. hamata*, *M. lanata*, *M. mathewsii*, *M. oligodon*, *M. retrorsa*, and *M. spectabilis*].

Under SEM, the tectum surface is scarcely perforated. The inner surface of the nexine presents artificial endocracks (an irregular groove occurring in the inner surface of the nexine) (Fig. 5d).

Under TEM, pollen of different species of *Mutisia* shares the exine-type stratification. The endexine appears as a dark-colored layer surrounding the intine. The ectexine is composed of endoectexine and ectoectexine; both layers are separated by a thick internal tectum. The endoectexine has large columellae that are attached to a continuous foot layer; within the columellae there is a nonhomogeneous material that might be pollenkitt. The ectoectexine has thin and unramified columellae. The tectum is scarcely perforated, and the microspines have a small apical channel (Fig. 5b).
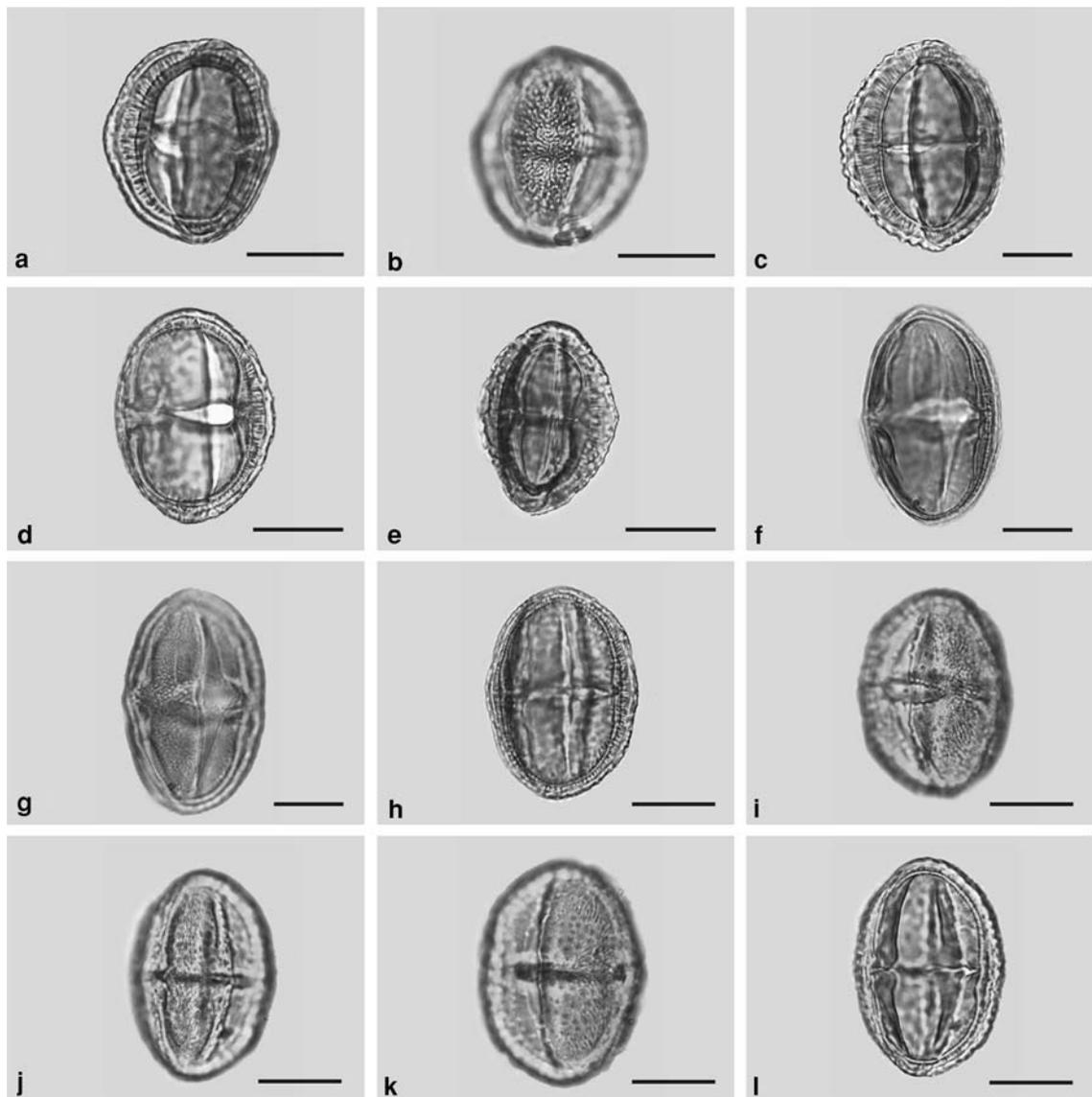
Fig. 4 LM micrographs of equatorial view of pollen of *Mutisia* showing exine features. **a, b** *M. homoeantha.* **a** Optical section showing the thick exine with the surface slightly rugulate. **b** Intermediate focus showing thick columellae in section. **c** *M. decumbens.* Optical section of thick exine. **d, e** *M. campanulata.* Pollen grains with different exine thickness. **f, g** *M. grandiflora.* **f** Optical section. **g** Intermediate focus, note the delicate columellae in section. **h–l** Pollen grains with inclined columellae. **h, i** *M. microphylla.* **h** Optical section. **i** Intermediate focus. **j–l** *M. sodiroi.* **j, k** Intermediate focus, note the inclined columellae that appear as thin and short bars. **l** Optical section. *Scale bars* 30 μm
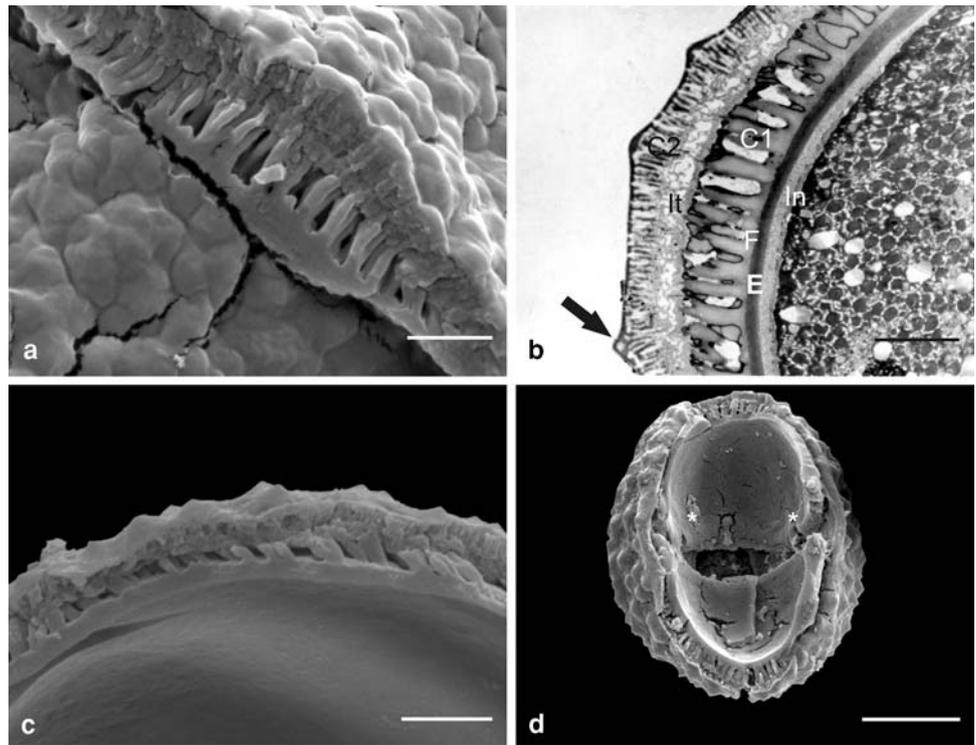
Pollen polymorphism

Pollen polymorphism in *Mutisia* is represented by strong variation in (1) size, (2) endoaperture features, and (3) exine thickness. These variations occur in different specimens of a single species, and frequently in the same specimen. As a rule, distinction of "deviant forms" from "regular or common forms" cannot be made because the variation is a continuous range. On the other hand, some specimens also produce a variable proportion of "aberrant grains" (those obviously suffering from irregular defects in ontogenetic processes). The aberrant grains are usually spheroidal (in inaperturate or bi-aperturate grains), or asymmetric with pyramidal or cuboidal shape (in 3- or 4-aperturate grains). However, in such specimens, a variable number of "regular" grains could be recognized (e.g., *M. coccinea* var. *dealbata*, *M. hamata*, *M. homoeantha*, *M. speciosa*, and *M. spinosa*) (Fig. 6a–c).

Size

Pollen grains range from large to very large (*P*: 46–109 μm and *E*: 32–83 μm); only *M. ledifolia* has medium-sized grains (Table 1). The great variation in the pollen size is

Fig. 5 Detail of exine of pollen of *Mutisia*. **a** *M. grandiflora*. Detail of exine structure and tectum surface with SEM. **b** *M. spinosa*. TEM detail of exine section through nonapertural nexine showing intine (*In*), endexine (*E*), foot layer (*F*), endoectexine (*C1*), internal tectum (*It*), ectoectexine (*C2*) and apical channel in the microspine (*arrow*). **c** *M. sodiroi*. Exine structure showing the inclined columellae of endosexine. **d** *M. hamata*. Half pollen grains showing artificial endocracks on the nexine surface and endoaperture with extensions (*asterisks*). *Scale bars* **a**, **c** = 2 μm, **b** = 7 μm, **d** = 10 μm

apparent from the large standard deviation. There is also variation between different samples from a single species (Figs. 7, 8). Differences of the polar axis length were particularly strong in *M. sinuata* (e.g., specimen Fabris and Marchionni 2344 LP) (Fig. 9) and *M. vicia*.

Endoaperture

Several variants of endoaperture, from lalongate to cingulated, sometimes with free endocolpi in the mesocolpium, can be found in a single specimen. Some variants of endoapertures, such as with horns, with ramified ends, with incomplete and complete endocingulum, and with endocingulum with long extensions in the mesocolpium, were recognized (Fig. 2). It should be noted that several variants of endoapertures were found in the same specimen.

Exine

The exine variations are evident in the level of thickness. The exine thickness can differ greatly in grains of a single specimen. Some grains have the exine equally, or slightly, thickened in the complete grain, and in this case the internal and external outline is more or less similar. Other grains are strongly thickened at the equator, with dumbbell-shaped outline (e.g., *M. campanulata* Fig. 4d, e; *M. involucrata*; and *M. oligodon*). If the exine is thickened at the equatorial level, the polar outline is angular, and in such case the extremes of colpi are frequently united in a parasyncolpus. If the exine thickness is more or less similar in the complete grain, the polar outline is circular. Between these two extremes, triangular and circular polar outline, there are many intermediate variants.

Discussion

The palynological study of most species of *Mutisia* carried out in this work allowed us to characterize pollen morphology at the generic level, recognize their polymorphic features, detect the presence of polymorphism in the different sections of the genus, and hypothesize about its origin and significance.

Pollen morphology and polymorphism and their possible significance

Pollen of *Mutisia* is comparable to that of the most widespread type of pollen found in Mutisiinae (Katinas et al. 2008). The constancy of the following set features: subprolate to prolate shape, large size, tricolporate aperture with mesoaperture, Mutisia exine type, and microechinate to rugulate sculpturing are useful indicators of the genera of the *Mutisia* group (i.e., *Brachyclados*, *Chaetanthera*, *Lulia* and *Pachylaena*; Tellería and Katinas 2004). As a deviation from this basic type, substantial intraspecific variation was found in several important characters, such as the size of pollen, the shape of the endoapertures, and the
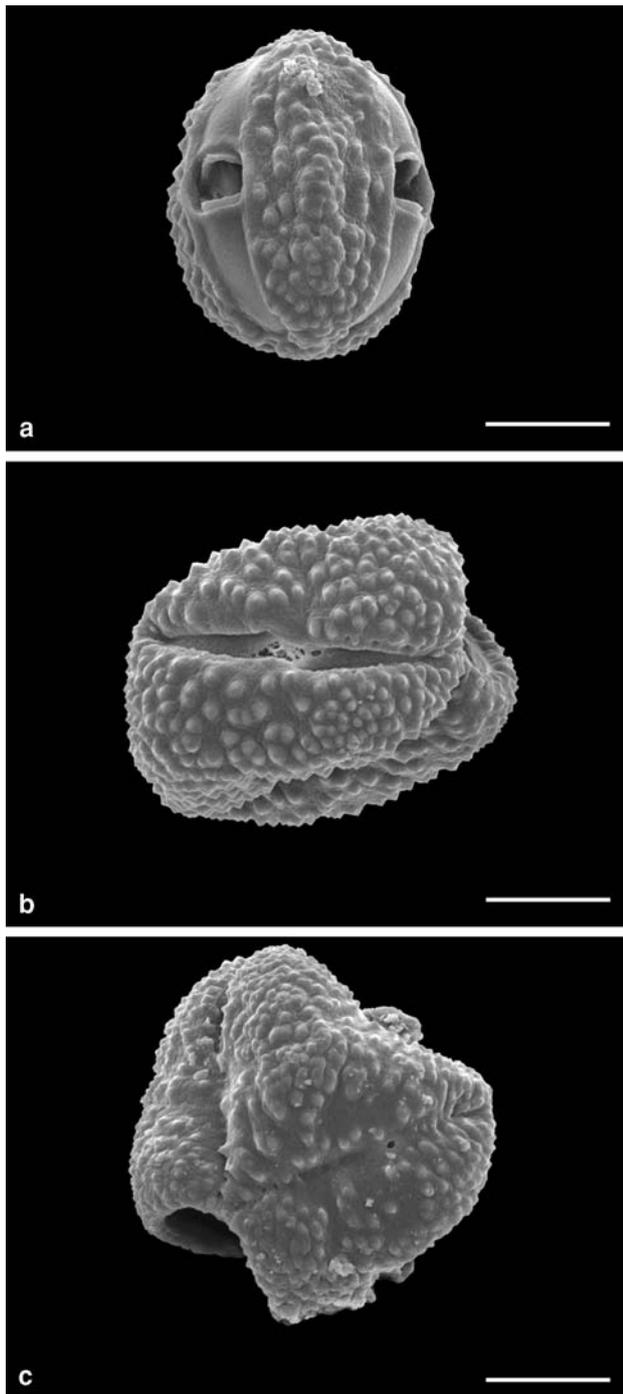
Fig. 6 Regular and aberrant grains of *M. spinosa*. **a** Regular grain in equatorial view. **b**, **c** Different types of aberrant grains. *Scale bars* 10 μm

exine thickness of the pollen grains. Only the size variation of the pollen has been reported in previous studies of the genus (Cabrera 1965; Parra and Marticorena 1972). The production of grains with a great variation in size does not characterize species given that different specimens of the same species can produce grains with a different range of

sizes (Figs. 7, 8). The existence of a correlation between ploidy levels and pollen variability is today under discussion, but some authors who work on different genera of angiosperms have presented evidence that pollen-size polymorphism is a consequence of polyploidy (e.g., Bronckers 1963; Mignot et al. 1994; Nadot et al. 2000). On the other hand, experimental studies carried out in Asteraceae have shown that the breeding system of a species is likely to be intimately connected with the production of polymorphic pollen (e.g., *Cichorium intybus*: Stainier et al. 1989; *Polymnia laevigata*: Fisher and Wells 1962; *Stevia*: King and Robinson 1967, 1968; *Sonchus* and *Reichardia*: Mejías and Diez 1993; Diez et al. 1999). Size polymorphism in pollen of *Mutisia* might corroborate this last statement since some species are believed to hybridize with each other fairly extensively (e.g., *M. acuminata* var. *paucijuga* × *M. vicia* var. *glabra*, *M. kurtzii* var. *anomala* × *M. acuminata* var. *paucijuga*, *M. orbignyana* × *M. acuminata* var. *hirsuta*; Cabrera 1965).

In the subtribe Mutisiinae, the pollen of *Mutisia* appears as remarkably large in comparison with that of allied genera (Tellería and Katinas 2004). This particularity might be related to the large size of corollas of *Mutisia*; for instance in *M. grandiflora,* corollas can reach ca. 14 cm in the marginal flowers with exserted anthers and styles. Coincidently, *M. grandiflora* is one of the species that has larger pollen grains, the polar diameter reaching 109 μm in length (Table 1). It has been suggested that in Asteraceae larger pollen grains are most likely to contain enough stored energy to support tube growth through longer styles, whereas smaller grains appear to be sufficient for pollen tube growth through shorter styles (Torres 2000). Therefore, the large pollen size in *Mutisia* might have selective value in relation to the post-pollination process.

The common aperture of *Mutisia* pollen is the tricolporate type, with long colpi and lalongate endoapertures. Besides this "standard" type of endoaperture, some specimens show a range of transient conditions where the lalongate shape is gradually transformed into a complete and broad endocingulum. In turn, the endocingulum is frequently connected with a variable number of short endocolpi. According to El-Ghazali (1990), the endoaperture type can be useful for characterizing certain taxa; however, this does not occur in *Mutisia*. This variation of endoapertures is shared with other genera of Mutisieae sensu lato (i.e., Guayana Highland-centered genera and the *Gochnatia* complex; Tellería 2008).

The high variability of pollen size and endoaperture types stands in contrast to the more conserved nature of exine type, because the pollen of all species of *Mutisia* has the Mutisia exine type. According to Halbritter and Hesse (2004), the pollen of most Asteraceae has a very rigid wall, making harmomegathic movements difficult. Pollen of

Table 1 Pollen of examined *Mutisia* species arranged by section, showing measurements of polar (P) and equatorial (E) diameters with the average (X) and standard deviation (δ), P/E ratio, exine thickness at the poles (P) and equator (E), and the ectosexine/endosexine (Ec/En) ratio

Section	Species[a]	P (μm)	X (δ)	E (μm)	X (δ)	P/E	Exine (P)	Exine (E)	Ratio Ec/En
Fruticosa	*M. comptoniaefolia*	66–93	83 (6)	44–81	64 (7)	Subprolate	9–10	10–18	1:1.5; 1:2
	M. homoeantha	57–63	61 (2)	48–61	53 (3)	Spheroidal-subprolate	6–7	11–14	1:1–3
	M. ledifolia	44–59	54 (3)	38–50	45 (2)	Spheroidal-subprolate	6–9	6–11	1:1; 1:2.5
Guariruma	*M. alata*	67–90	84 (5)	53–71	65 (4)	Subprolate	6–8	9–11	1:1
	M. andersonii	60–71	65 (3)	43–55	49 (4)	Subprolate	5–6	7–8	1:1
	M. arequipensis	63–70	67 (3)	44–63	51 (9)	Subprolate	6–8	10–12	1:1.5
	M. cochabambensis[1]	60–67	64 (2)	48–63	52 (3)	Subprolate	5–7	5–10	1.5:1; 1:1
	M. cochabambensis[2]	51–71	60 (6)	40–58	47 (6)	Subprolate	6–8	9–11	1.5:1; 1:1
	M. decurrens[3]	64–92	80 (7)	57–74	65 (4)	Subprolate-prolate	6–10	9–15	1:2; 1:2.5
	M. decurrens[4]	72–95	84 (5)	59–71	66 (3)	Subprolate	8–11	16–21	1:2
	M. hamata[b]	55–71	62 (3)	46–53	50 (2)	Subprolate-prolate	6–8	10–18	1:1
	M. lanigera	67–78	73 (3)	54–58	57 (1)	Subprolate	7	10–12	1:1; 1.5:1
	M. mandoniana	67–85	72 (4)	45–60	53 (4)	Subprolate-prolate	7–8	9–14	1:1
	M. retrorsa[b]	56–70	65 (4)	44–58	53 (4)	Subprolate	6–9	9–13	1:1.5; 1:1
	M. saltensis	68–80	73 (7)	42–52	47 (5)	Prolate	7	10	1:1; 1:1.5
	M. sinuata[5]	53–100	66 (16)	32–65	46 (9)	Subprolate-prolate	6–9	8–16	2:1; 1:1
	M. sinuata[6]	59–85	72 (5)	45–63	55 (4)	Subprolate	6–9	10–14	1:2; 1:3
	M. spectabilis	60–83	72 (6)	41–58	53 (4)	Subprolate- prolate	5–7	6–11	1:1; 1:1.5
	M. subspinosa	56–75	64 (4)	43–56	50 (4)	Subprolate-prolate	5–8	9–14	1:1; 1:2
Holophyllum	*M. acerosa*	57–67	60 (3)	47–52	49 (2)	Subprolate	5–6	9–12	1:1
	M. friesiana	62–87	74 (7)	51–71	58 (5)	Subprolate-prolate	5–8	9–14.5	1:1
	M. linearifolia	58–69	65 (4)	53–58	55 (3)	Spheroidal-subprolate	7–9	10–13	1:2.5
	M. mathewsii	62–70	66 (2)	44–53	48 (2)	Subprolate-prolate	6–9	9–15	1:1; 1.5:1
	M. subulata[7]	69–107	83 (9)	54–79	63 (8)	Subprolate-prolate	6–12	9–21	1:1; 3:1
	M. subulata[8]	77–93	85 (4)	60–73	66 (3)	Subprolate-prolate	7–10	14–18	1:1; 1:1.5
Isantha	*M. kurtzii*[9]	64–81	72 (4)	48–59	55 (2)	Subprolate-prolate	7–9	10–11	1: 0.5–1
	M. kurtzii[10]	64–94	77 (8)	46–62	54 (5)	Prolate	7	13	1:3
	M. linifolia	63–75	68 (5)	42–53	48 (4)	Prolate	7	7–10	1:1; 1:1.5; 1.5:1
	M. orbignyana[11]	70–82	70 (4)	54–67	61 (3)	Subprolate-prolate	9–14.5	12–18	1:1; 1.5:1
	M. orbignyana[12]	71–90	82 (4)	54–90	63 (3)	Subprolate-prolate	14–15	13–19	1:1

Table 1 continued

Section	Species[a]	P (μm)	X (δ)	E (μm)	X (δ)	P/E	Exine (P)	Exine (E)	Ratio Ec/En
Mutisia	M. acuminata	66–78	71 (3)	49–58	53 (2)	Subprolate-prolate	5–7	9–10	1:1
	M. campanulata	58–77	71 (4)	43–62	54 (5)	Spheroidal to prolate	7–10	7–12	1:1.5
	M. clematis	66–73	69 (2)	56–66	52 (2)	Subprolate-prolate	4–5	6–9	0.5:1; 1:1; 1.5:1
	M. coccinea	46–88	60 (16)	35–69	48 (12)	Subprolate	4–6	6–12	1:1; 1:1.5
	M. grandiflora[13]	69–99	88 (8)	47–62	56 (4)	Prolate	6–9	7–10	2:1
	M. grandiflora[14]	82–109	94 (8)	51–73	65 (7)	Prolate	5–9	10–15	1:1; 1.5:1
	M. hamata[b]	55–71	62 (3)	46–53	50 (2)	Subprolate-prolate	6–8	10–18	1:1
	M. intermedia	79–90	85 (3)	49–67	59 (5)	Prolate-perprolate	7–9	8–11	1.5:1
	M. lanata[15]	54–64	60 (3)	42–49	44 (3)	Subprolate	5	10	1:1; 1.5:1
	M. lanata[16]	62–83	71 (5)	42–51	47 (2)	Prolate	5–7	7–10	1:5; 1.5:1
	M. lehmannii[17]	73–102	90 (9)	44–67	58 (7)	Subprolate-prolate	5–8	8–13	1:1; 1.5:1; 2:1
	M. lehmannii[18]	92–109	98 (4)	59–69	64 (3)	Prolate	6–7	10–11	1.5:1
	M. microphylla[19]	67–83	73 (4)	46–60	55 (3)	Prolate	6–8	7–10	1.5:1; 2:1
	M. microphylla[20]	58–88	72 (9)	40–60	51 (6)	Prolate	5–9	7–16	1:1; 1.5:1; 1:2; 2:1
	M. pulcherrima	72–109	98 (7)	66–83	75 (4)	Subprolate-prolate	8–11	10–14.5	1:1
	M. retrorsa[b]	56–70	65 (4)	44–58	53 (4)	Subprolate	6–9	9–13	1:1.5; 1:1
	M. rimbachii	64–70	67 (2)	42–47	46 (3)	Prolate	6–9	10–12	1:1
	M. sodiroi[21]	63–78	71 (4)	44–54	49 (2)	Prolate	4–5	7–10	1.5:1; 2:1
	M. sodiroi[22]	79–86	83 (3)	60–65	62 (2)	Subprolate	7–9	11–12	1.5:1; 1:1
	M. speciosa[23]	69–83	77 (4)	47–54	51 (2)	Prolate	6–10	6–9	1:2; 1:3
	M. speciosa[24]	54–74	66 (4)	46–57	50 (2)	Subprolate	6–8	8–10	1:1
	M. spectabilis	60–83	72 (6)	41–58	53 (4)	Prolate	5–7	6–11	1:1; 1:1.5
	M. stuebelii	67–92	75 (6)	51–62	55 (3)	Subprolate-prolate	4–7	4–9	1:1
	M. venusta	81–102	88 (5)	48–68	58 (4)	Prolate	9–11	11–18	1:1
	M. vicia	73–99	88 (8)	50–62	57 (5)	Subprolate-prolate	8–9	12–15.5	1:1
	M. wurdackii	73–88	81 (3)	50–63	57 (3)	Subprolate-prolate	6–9	9–14.5	1:1.5; 1:1
Ovata	M. brachyantha	52–68	63 (4)	44–58	52 (3)	Subprolate	5–7	9–10	1:1
	M. cana	82–102	91 (6)	57–69	63 (4)	Prolate	7–8	11–17	1:1.5
	M. ilicifolia	72–87	81 (3)	57–71	64 (4)	Subprolate	5–8	9–14	1:1; 1:1.5
	M. involucrata	63–82	73 (5)	44–66	57 (5)	Subprolate	9–13.5	12–20	1:1; 1:1.5; 1.5:1
	M. latifolia	60–72	66 (3)	45–60	49 (3)	Subprolate	6–9	9–12	1:1

Table 1 continued

Section	Species[a]	P (μm)	X (δ)	E (μm)	X (δ)	P/E	Exine (P)	Exine (E)	Ratio Ec/En
	M. macrophylla[25]	71–98	80 (6)	49–63	59 (3)	Subprolate-prolate	7–8	10–12	1:1
	M. macrophylla[26]	73–87	83 (6)	52–64	60 (5)	Prolate	8–10	10–13	1:1
	M. oligodon[27]	58–74	65 (4)	48–60	53 (3)	Subprolate	6–8	8–11	1:1.5
	M. oligodon[28]	50–75	64 (9)	45–58	51 (4)	Spheroidal-subprolate	5–4	5–10	1:1; 1.5:1
	M. spinosa[29]	54–72	61 (4)	44–53	49 (2)	Subprolate-prolate	5–7	7–12	1:1; 1:1.5
	M. spinosa[30]	71–85	77 (3)	51–62	57 (3)	Subprolate-prolate	7–8	11–15	1:2; 1:1

[a] Superscript numbers refer to collecting data (see "Appendix")

[b] Species placed in two sections

Mutisia also falls within this generalization since most of the specimens analyzed have a predominantly thick exine (Table 1) with a rigid appearance. However, the rigidity of the exine seems to be compensated by the endoapertures, which provide areas of more flexibility, thus allowing expansion during hydration (Blackmore and Barnes 1986; Furness et al. 2007).

Additionally, the exine with inclined columellae in the pollen of some species of *Mutisia* might also have harmomegathic significance. In fact, this particular arrangement of columellae delimits a weak area in the equatorial region that would increase the plasticity of the pollen wall during hydration. The exine with inclined columellae is common in pollen from species of the section *Mutisia* that share their geographical area of distribution: *M. stuebelli* in Colombia, *M. microphylla* in Ecuador, and *M. sodiroi* in both countries. This appears to suggest a high degree of experimentation prior to evolutionary canalization of some key morphological features, as suggested for the pollen of some eudicots (Furness et al. 2007).

Evolutionary considerations

In an evolutionary context, the comparison between the pollen morphology of *Mutisia* and that of *Chaetanthera*, another Andean-centered genus of Mutisieae, provides interesting data. Thus, a possible relationship among environmental heterogeneity, hybridization, polyploidy, and pollen polymorphism can be suggested.

Species of *Chaetanthera* inhabit two main habitats: the high elevations of the Puna and Altoandean provinces and the lower places of the central Chilean province (Cabrera and Willink 1980). There are no reports of hybridization and polyploidy in *Chaetanthera*. In this genus, two well-defined pollen types were recognized: type I, which defines two subgenera, has thinner exine, is equally thickened in the whole grain, has subrectangular shape, and medium size; and type II, which defines five subgenera, has a very thickened exine at the equatorial level, dumbbell-shaped nexine outline, elliptic shape, and large size (Tellería and Katinas 2004). The pollen of *Chaetanthera* seems to be adapted to dry and cold environments, with a clear correspondence between its two palynological types and the infrageneric categories (Tellería and Katinas 2004).

As regards *Mutisia,* according to Cabrera (1965) the center of origin is the Yungas, a relatively young formation (Vélez et al. 2003). The Yungas comprises the tropical forests at the eastern slopes of the Andes of southern Peru, Bolivia, and northwestern Argentina. In this extensive region, *Mutisia* occupies heterogeneous habitats, ranging from semideserts to subtropical rain forests. Unlike *Chaetanthera*, in *Mutisia* the hybridization might be the underlying common denominator that promotes the

Fig. 7 Comparison of the polar diameters from different specimens of individual species cited in Table 1 and the "Appendix"
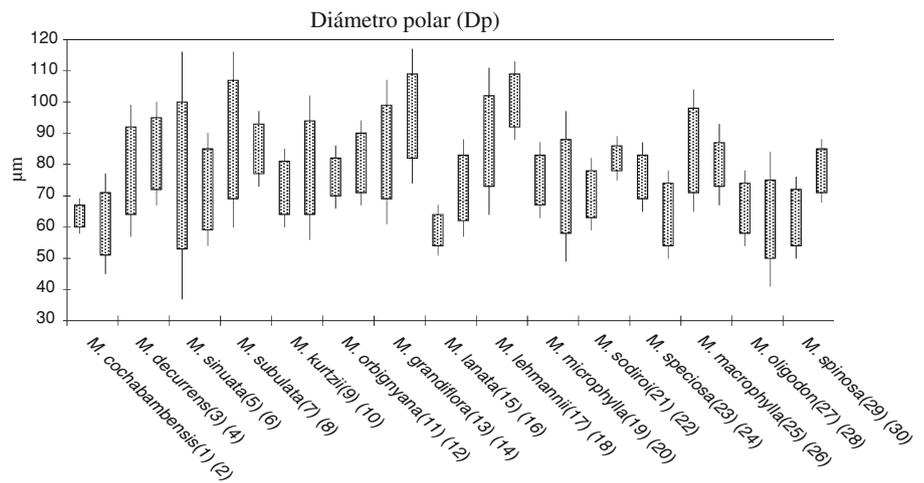

Diámetro polar (Dp)

Fig. 8 Comparison of the equatorial diameters from different specimens of individual species cited in Table 1 and the "Appendix"
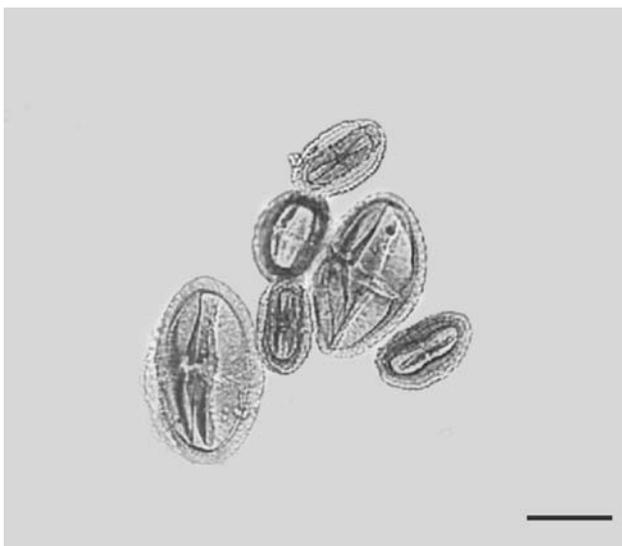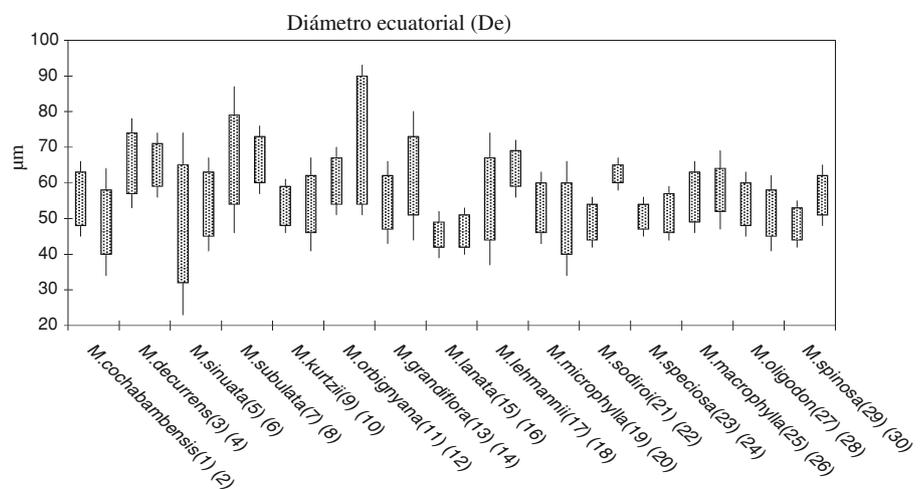

Diámetro ecuatorial (De)

Fig. 9 *M. sinuata*. General view of pollen grains with different sizes from specimen Fabris & Marchionni 2344 (LP). *Scale bars* 45 μm

coexistence of different pollen variants that could be advantageous in unpredictable conditions (e.g., changing environmental conditions, the presence of pollinators). Thus, selective forces might favor either one variant or the other as suggested for other species (e.g., Nadot et al. 2000).

Conclusions

The LM examinations revealed the variability of some inner features of pollen grains of *Mutisia* such as the exine structure and the endoaperture shape; the SEM showed the little variability in sculptural features; whereas the TEM revealed the presence of apical channels inside the small microspines, a condition which is not apparent in LM and SEM. The study of most species of *Mutisia* showed that the pollen has a basic morphology shared by all species but which is also very polymorphic both within and among

species. Therefore, pollen morphology is not helpful to support Cabrera's (1965) infrageneric classification of *Mutisia*. Hybridization represents a major evolutionary force for most plants (e.g., Wang and Cruzan 1998), and in *Mutisia* this process might be reflected in its evident pollen polymorphism. Some pollen features such as large size, exine with inclined columellae, and endoaperture variants, might be viewed as advantageous features for post-pollination and harmomegathic mechanisms.

Appendix

Voucher material examined for the study of pollen. *Superscripts* refer to Table 1.

Fruticosa section. *M. comptoniaefolia* Rusby: Krach 7178 (SI). *M. homoeantha* Wedd.: Meyer 17565 (LP). *M. ledifolia* Decaisne: Cabrera 9438 (LP).

Guariruma section. *M. alata* Hieron.: López et al. 6719 (LP). *M. andersonii* Sodiro: Scolnik 1532 (LP). *M. arequipensis* Cabrera: Treacy 840, 829 (WIS). *M. cochabambensis* Hieron.: [1]Zamaloa 2033 (LP); [2]Cañigueral 11 (LP). *M. decurrens* Cav.: [3]Grüner 132 (LP); [4]Soriano 4294 (LP). *M. hamata* Reiche: Cabrera et al. 22495 (LP). *M. lanigera* Wedd.: Riccardi and Marticorena 25468 (LP). *M. mandoniana* Wedd.: Sodiro 4549 (SI). *M. retrorsa* Cav.: Vignati 420 (LP). *M. saltensis* Cabrera: Cabrera et al. 25519 (LP). *M. sinuata* Cav.: [5]Fabris and Marchionni 2344 (LP); [6]Fabris 1221 (LP). *M. spectabilis* Phil.: Jiles 1834 (LP). *M. subspinosa* Cav.: Ruiz Leal 1051 (LP); Villavicencio, O'Donell 1331 (LP).

Holophyllum Section. *M. acerosa* Poepp.: Cabrera 3463 (LP). *M. friesiana* Cabrera: Cabrera et al. 22501 (LP). *M. linearifolia* Cav.: Marticorena and Matthei 947 (LP). *M. mathewsii* var. *anomala* Cabrera: Macbride and Featherstone 907 (LP). *M. subulata* R. et P.: [7]Jiles 4189, [8]Jiles 2586 (LP).

Isantha Section. *M. kurtzii* R. E. Fries: [9]Fabris et al. 4082 (LP); [10]Cabrera 9001 (LP).

M. linifolia Hook.: Dawson and Pujals 1611 (LP). *M. orbignyana* Wedd.: [11]Isern 394 (LP); [12]Meyer et al. 20888 (LP).

Mutisia Section. *M. acuminata* var. *paucijuga* (Gris.) Cabrera: Cabrera et al. 13894 (LP). *M. campanulata* Less.: Hatschbach 4058 (LP). *M. clematis* L.: Fosberg 22294 (LP). *M. coccinea* St. Hil.: Krapovickas et al. 22993 (LP).

M. grandiflora Humb. et Bonpl.: [13]Acosta Solís 5442 (F); [14]Cuatrecasas 20917 (F). *M. hamata* Reiche: Cabrera et al. 22495 (LP). *M. intermedia* Hieron.: Sodiro (BAF). *M. lanata* R. et P.: [15]Scolnik and Luti 519 (LP); [16]Weddell 2314 (LP). *M. lehmannii* Hieron: [17]Jaramillo 5415 (F); [18]Dorr and Valdespino 6382 (F). *M. microphylla* Willd. ex DC.: [19]Firmin 524 (F); [20]Romoleroux 297 (F). *M. pulcherrima* Muschl.: Sagástegui 7469 (LP). *M. retrorsa* Cav.: Vignati 420 (LP). *M. rimbachii* Sodiro ex Harris: Villacrés 234 (F). *M. sodiroi* Hieron.: [21]Sodiro (BAF); [22]Fosberg 21188 (F). *M. speciosa* Ait.: [23]Grüner 1077 (LP); [24]Rodríguez 1265 (LP). *M. spectabilis* Phil.: Jiles 1834 (LP). *M. stuebelii* Hieron.: Cuatrecasas 19156 (FM). *M. subulata* R. et P.: Jiles 2586 (LP). *M. venusta* Blake: Vargas 4420 (LP). *M. vicia* Koster: without leg. no. 2256 (LP). *M. wurdackii* Cabrera: López et al. 4303 (LP).

Ovata Section. *M. brachyantha* Phil.: Wederman 541 (LP). *M. cana* Poepp. et Endl.: Jiles 2710. *M. ilicifolia* Phil. var. *decandolleana* (Phil.) Cabrera: Pisano et al. 1665 (LP), Jiles 1871 (LP). *M. involucrata* Phil.: Barros 3804 (LP). *M. latifolia* D. Don: Jiles 3139 (LP). *M. macrophylla* Phil.: [25]Barros 7552; [26]Barros 1772 (LP). *M. oligodon* Poepp. et Endl.: [27]Cabrera 6090 (LP); [28]Ledezma 650 (LP). *M. spinosa* Ruiz et Pav.: [29]Landrum 4378 (LP); [30]Hollermayer 725 (LP).

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