Plant Syst Evol (2012) 298:695–701
DOI 10.1007/s00606-011-0579-z

ORIGINAL ARTICLE

Palynological study of the Venezuelan species of the genus *Hymenocallis* (Amaryllidaceae)

Joan Martín · María B. Raymúndez · Joan Vallès · Teresa Garnatje · Elena Raimúndez

Received: 12 September 2011 / Accepted: 5 December 2011 / Published online: 12 January 2012
© Springer-Verlag 2012

Abstract Pollen morphology and exine structure of the seven *Hymenocallis* Salisb. species present in Venezuela were investigated using scanning (SEM) and transmission (TEM) electron microscopy. Pollen grains of all species are monosulcate, heteropolar, with bilateral symmetry, oblate to peroblate, heterobrochate, semitectate-columellate, possess unequal tectal surfaces, and are clavate or baculate when viewed with TEM. They present two equatorial apexes. When observed under SEM their surfaces appear as granulate; however, it is evident from TEM that they are in fact constituted by pila. All the species possess very large pollen (ranging from 64 to 85 μm and from 125 to 155 μm for polar and equatorial axes, respectively), and no relationship was evident between pollen size and chromosome number. All the taxa are also very similar in relation to their pollen outline, ornamentation and internal structure, thus indicating that the genus is accurately delimited from the palynological point of view and that discrete characteristics can only be used in some cases to delimit individual species.

Keywords Equatorial apexes · *Hymenocallis* · Neotropics · Pollen grain structure · Pollen morphology · SEM · TEM

J. Martín · J. Vallès (✉)
Laboratori de Botànica, Facultat de Farmàcia, Universitat de Barcelona, Av. Joan XXIII s.n., 08028 Barcelona, Catalonia, Spain
e-mail: joanvalles@ub.edu

J. Martín
e-mail: jomartinv@ub.edu

M. B. Raymúndez
Laboratorio de Biosistemática y Citogenética Vegetal, Facultad de Ciencias, Centro de Botánica Tropical, Instituto de Biología Experimental, Universidad Central de Venezuela, Apartado 47114, Caracas 1041-A, Venezuela
e-mail: maria.raymundez@ciens.ucv.ve

T. Garnatje
Institut Botànic de Barcelona (IBB-CSIC-ICUB), Passeig del Migdia s.n., Parc de Montjuïc, 08038 Barcelona, Catalonia, Spain
e-mail: tgarnatje@ibb.csic.es

E. Raimúndez
Laboratorio de Biología Reproductiva de Angiospermas, División de Ciencias Biológicas, Departamento de Biología de Organismos, Universidad Simón Bolívar, Valle de Sartenejas, Baruta, Apartado 89000, Caracas 1080-A, Venezuela

Introduction

Hymenocallis Salisb. is a neotropical genus of geophytes belonging to family Amaryllidaceae. They were formerly known as the American representatives of the Old World genus *Pancratium* L. (Sealy 1954; Meerow et al. 2002). *Hymenocallis* was segregated as a distinct genus by Salisbury (1812), based mainly on the marked differences between the black, dry and compressed seeds with a phytomelan layer of *Pancratium,* and the almost ovoid, green, fleshy and mostly viviparous seeds of *Hymenocallis*. Together, *Ismene* Salisb., *Leptochiton* Sealy and *Hymenocallis* form the tribe Hymenocallideae (sensu Meerow et al. 2002). They are commonly named "spider lilies" because of the beautiful staminal membrane surrounded by long and narrow tepals that characterise their flowers.

Despite its putative Andean origin (Meerow et al. 1999, 2000), Mexico represents the main centre of diversity in North America, followed by the southeastern United States (Meerow et al. 2002). Venezuela seems to be the main centre of diversity in northern South America (Raymúndez

1997; Raymúndez et al. 2005). *Hymenocallis* is a well known but taxonomically complicated genus (Sealy 1954; Traub 1962, 1980; Meerow and Dehgan 1985) due to the poor preservation of the stereomorphic floral characters on herbarium *exsiccata*, the low number of collections and duplicates, and the frequent absence of precise details about geographic distribution and inter and intraspecific variation. It is important to mention that a great number of taxa bloom during a very short period of time once per year and that some species have low population numbers, further complicating an already challenging study (Sealy 1954; Traub 1962; Raymúndez et al. 2005).

In Venezuela, the genus comprises seven species (Raymúndez 1997; Campbell 2008), with four of them considered as endemics [*H. bolivariana* Traub, *H. lobata* Klotzcsh, *H. guianensis* Herb. and *H. venezuelensis* Traub), one considered native to northern South America (*H. tubiflora* Herb.), one widespread from Central to South America (*H. littoralis* (Jacq.) Salisb.) and one introduced from the Caribbean Islands but naturalised everywhere (*H. caribaea* (L.) Herb.)]. All of them belong to *Hymenocallis* sect. *Hymenocallis*, as considered by Traub (1980), and to *Hymenocallis* sensu Meerow et al. (2002).

Aside from a few palynological studies comprising some widespread representatives of the genus (quoted later), available information about pollen from South American *Hymenocallis* species is very scarce. Nothing about the pollen of Venezuelan endemics or South American petiolated species has been published. Meerow and Dehgan (1985, 1988) were the first to focus solely on *Hymenocallis* and other amaryllidaceous relatives formerly included in the genus. Using scanning electron microscopy, they examined the pollen grains of *H. quitoensis* (Herb.) Sealy [*Leptochiton quitoensis* (Herb.) Sealy], *H. narcissiflora* (Jacq.) Macbr. (*Ismene narcissiflora* Jacq.), *H. amancaes* (Ruiz and Pav.) Nichols. [*Ismene amancaes* (Ruiz and Pav.) Herb.], *H. horsmannii* Baker [*H. glauca* (Zucc.) Roem.], *H. caroliniana* (L.) Herb., *H. morrisonii* (Vargas) Traub [*Ismene morrisonii* (Vargas) Gereau and Meerow], *H. longipetala* (Herb.) Macbr. [*Ismene longipetala* (Lindl.) Meerow] and *H. latifolia* (Mill.) Roem. As described above, only three of them [*H. latifolia*, *H. caroliniana* and *H. horsmannii* (*H. glauca*)] are currently included in the genus *Hymenocallis* (Topicos 2011). The remainder have been transferred to the related genera *Ismene* and *Leptochiton*. Actually, none of the three *Hymenocallis* species studied by Meerow and Dehgan (1985, 1988) grow in South America, but rather in Mexico or southern North America. Only *H. quitoensis* has previously been studied using both scanning and transmission electron microscopy techniques, but it is now considered, under the name *Leptochiton quitoensis*, as belonging to a closely related genus from tribe Hymenocallideae (Meerow and Dehgan 1985, 1988).

Other researchers have previously studied palynological features from a few *Hymenocallis* species: Erdtman (1966) considered both *H. speciosa* (Salisb.) Salisb. and *H. declinata* (Jacq.) Sweet, in his pollen referential compendium, Huang (1972) describes *H. speciosa* in his extensive work about the plants of Taiwan, Ravikumar and Nair (1982) produced a detailed study about pollen grains of *H. littoralis* (under its synonym *H. pedalis* Herb.), while Roubik and Moreno (1991) briefly mention the presence of pollen from *H. pedalis* Herb. (*H. littoralis*) at Barro Colorado Island (Panama). Consequently, with the scarcity of information about pollen of almost all the species of the genus *Hymenocallis*, this article aims to characterise the pollen morphology, morphometry and ultrastructure of South American, and particularly Venezuelan representatives of *Hymenocallis* using the techniques of light microscopy as well as scanning and transmission electron microscopy.

Materials and methods

Light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) studies were carried out for the seven *Hymenocallis* species growing in Venezuela. Other samples were dissected from blooming flowers cultivated under greenhouse conditions at the Instituto de Biología Experimental, Universidad Central de Venezuela. Vouchers of each species were deposited at the "Herbario Nacional de Venezuela" (VEN). Table 1 shows the names, collectors, origin and general distribution of the samples under study. We have used one population per species, although most of them are widely distributed in the geographical area considered, because pollen grain morphology is homogeneous enough throughout the genus (see "Results and discussion").

Anthers were placed over an acetonitrile filter on a glass slide support, dehydrated with two drops of 99% ethanol and dissected with a lancet to expose pollen. The micromethod of Avetisian (1950) was followed to prepare acetolysed pollen for OM, and pollen grains were observed using a Visopan apparatus (Reichert, Austria). For SEM, pollen grains were treated applying critical point drying, and observed and photographed in a Zeiss DSM 940 A microscope at 15 kV. For TEM study, non-acetolysed pollen samples were fixed in 2% w/v paraformaldehyde/2.5% v/v glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Postfixation was performed in OsO_4 1% w/v + $K_3Fe(CN)_6$ 0.8% w/v in the same buffer. Samples were dehydrated in acetone, embedded in Spurr′s resin and cut to 60 nm using an Ultracut-E microtome. Slices were examined and photographed under a Hitachi H-800 MT instrument. Light microscopy observations were performed at

Table 1 Names, collectors, provenance and general distribution of the species considered for this study

Taxa	Collectors and localities	General distribution
Hymenocallis bolivariana Traub	*M.B. Raymúndez* (500), E. Raimúndez, A. Mondragón and M. Escala. Venezuela. Between Píritu and Araure, edo. Portuguesa. Near to a stopped spout, beside the National Road	Venezuela (endemic from Venezuelan Llanos and Guayana)
Hymenocallis caribaea (L.) Herb.	*M.B. Raymúndez* (511). Venezuela. Caracas, Dtto. Capital. In front of Facultad de Ciencias, Universidad Central de Venezuela	Caribbean Islands; Venezuela (naturalized)
Hymenocallis guianensis Herb.	*M.B. Raymúndez* (356), F.J. Reimúndez and A. Mondragón. Venezuela. Near the border of Río Grande, just before the Reserva Forestal de Imataca, edo. Bolívar, near the limits with Delta Amacuro. At the understory of a river forest	Venezuela (endemic from Guayana and Orinoco delta)
Hymenocallis littoralis (Jacq.) Salisb.	*M.B. Raymúndez* (*s.n.*), E. Raimúndez and L. Camero. Venezuela. Caño Negro, between El Vigía and Santa Bárbara, Sur del Lago de Maracaibo, edo. Zulia. At the border of a spout, near a "platanal" (banana sowing field)	Central to South America (from Costa Rica and Panamá to Venezuela, Brazil, Ecuador and Perú)
Hymenocallis lobata Klotzsch	*M.B. Raymúndez* (*s.n*), B. Manara and L. Ruíz. Venezuela. Surroundings of Los Pijiguaos, edo. Bolívar. At a seasonal wet savanna	Venezuela (endemic from wet savannas at the northern Venezuelan Guayana)
Hymenocallis tubiflora Herb.	*M.B. Raymúndez* (281), E. Raimúndez, G. Linero and B. Vera. Venezuela. Caripe, edo. Monagas. Understory of the forest that surrounds the town	Northern Venezuela, Trinidad and Tobago, French Guiana, Brazil
Hymenocallis venezuelensis Traub	*M.B. Raymúndez* (*s.n.*), A. Fernández, R. Gonto and J.A. González. Venezuela. Near Hato Piñero, edo. Cojedes. Upper limit of a flooded savanna	Venezuela (endemic from flooded savanas, "bajíos", at the Venezuelan Llanos)

All the *exsiccata* were obtained from the Herbario Nacional de Venezuela (VEN)

the Laboratori de Botànica, Facultat de Farmàcia, Universitat de Barcelona, and electron microscopy procedures were carried out at the Centres Científics i Tecnològics, Universitat de Barcelona. Morphological descriptions of pollen grains were performed following the shape and sculpturing classification of Hesse et al. (2009).

Mean values of polar (*P*) and equatorial (*E*) axes for each of the seven species under study were based on measurements, using LM, of eight fully developed pollen grains. Generalised linear models (GLM) were used to test the mean differences in *P*, *E* and *P*/*E* values among taxa. These tests were conducted with Stata 10.0 (Stata Corp., College Station, TX, USA).

Table 2 Polar (*P*) and equatorial (*E*) lengths of pollen grains of the seven species of *Hymenocallis* (mean ± SD, μm) ($N = 8$ for all measurements)

Taxon	P^a	E^a	*P*/*E*
H. bolivariana	64.26 ± 8.46	134.36 ± 9.48	0.48
H. caribaea	83.71 ± 3.74	135.59 ± 7.44	0.62
H. guianensis	73.57 ± 5.55	130.39 ± 2.27	0.56
H. littoralis	77.27 ± 12.85	130.77 ± 5.65	0.59
H. lobata	73.49 ± 10.80	125.36 ± 11.38	0.59
H. tubiflora	84.82 ± 9.83	141.67 ± 3.22	0.60
H. venezuelensis	65.08 ± 1.34	155.01 ± 1.52	0.42

[a] Values are mean ± SD

Results and discussion

Mean values of polar (*P*) and equatorial (*E*) pollen grain axes for each taxon are given in Table 2. Figure 1 presents SEM pictures of the pollen grains of all the studied taxa and Fig. 2 TEM pictures of one representative (*H. caribaea*). Figure 3 contains the box-and-whisker plots for the P/E ratio of the species considered.

The seven species possess monosulcate and heteropolar pollen, with bilateral symmetry. It is oblate to peroblate, heterobrochate with an occasionally slightly interrupted wide curvaceous-tortuous reticulum and an extensive lumen. Additionally, the pollen grains have a reticulum cristatum more or less apparent depending on the species. This reticulum is common to all studied species, but it is remarkable that it is much denser in *H. littoralis* than in the other Venezuelan taxa of the genus. Pollen grains are semitectate-columellate, with unequal tectal surface, clavate or baculate from TEM views (Fig. 2a–d). Pollen grains also display two equatorial apexes, which appear as granulate surfaces under SEM, but are evidently constituted by pila when observed with TEM. We agree with Ravikumar and Nair (1982) and Alves-Araujo et al. (2007) on the general morphology and ornamentation of pollen grains of *Hymenocallis*. The studies undertaken by these authors focused only on *H. littoralis* and *H. pedalis*, but the

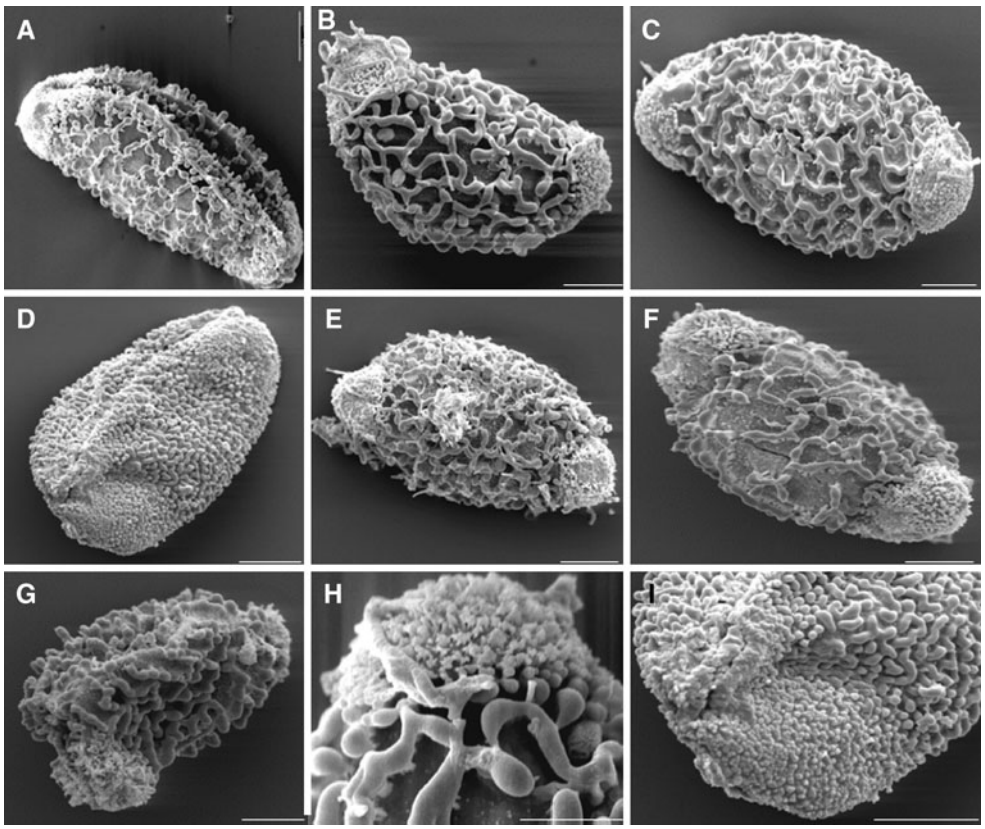

Fig. 1 Scanning electron microscope (SEM) images of the seven *Hymenocallis* species studied. **a** *H. bolivariana.* **b** *H. caribaea*. **c** *H. guianensis*. **d** *H. littoralis*. **e** *H. lobata*. **f** *H. tubiflora*. **g** *H. venezuelensis. Scale bars* 20 µm. **h** Detail of the changing exine pattern between an equatorial apex and the middle zone on an *H. bolivariana* pollen grain. *Scale bar* 15 µm. **i** Equatorial apex surface, detailed view. *Scale bar* 10 µm

addition of our data confirms the prevalence of these traits to a larger extent in the genus.

Under TEM, a group of bacula (columellae) is seen in a thin exine foot layer, in the inner part of which endexine lamellae are observed. Depending on the nature of the slice, discontinuous portions of the tectum are viewed, which reach to be transformed in clavae, parietal (of the lateral wall) or terminal (of the apexes) (Fig. 2a–d). Figure 2c shows the columellae on a fine exine layer. The columellae, although coming from foot layer, appear to be embedded in an amorphous mass and are not randomly distributed, but exhibit different degrees of inclination (Fig. 2d); this figure shows both entire columellae and fragments. The pollen wall structure is described and illustrated here for *H. caribaea*, but we have also studied it in *H. venezuelensis* (data not shown). The latter species shows the same structure as the former, and this organisation is also coincidental with that presented by Meerow and Dehgan (1985) for *H. quitoensis* (see next paragraph), suggesting a common pattern in the genus.

In the detailed views of the equatorial apex using SEM (Fig. 1h, i), no bacula appear, but instead clava-like pins, very close among them. Under increased magnification, the clavae show a structure with crystalline appearance. When the exine structure of *H. caribaea* is compared with that of another *Hymenocallis* species studied by Meerow and Dehgan (1985) (*H. quitoensis* = *I. quitoensis*), large similarities can be observed, despite these authors' description of the exine structure at the apexes as "baculate, gemmate or scabrate" and not constituted by clavae, as we observed on *H. caribaea*. As stated above, TEM microphotographs also show a very similar morphology for the two species, both at the main reticulum and at the equatorial apexes.

Describing the pollen of *H. quitoensis,* Meerow and Dehgan (1985) named the equatorial ends "auriculae". That term is only applicable to the spores' structure (Potonié and Kremp 1955). Instead of "auricula" we propose to use the term "apex", which better describes the precise location of these structures in *Hymenocallis* pollen grains. Ravikumar and Nair (1982) used the term "cappus" referring to the changing pattern of pollen grains of *H. littoralis* at the equatorial extremes, but Alves-Araujo et al. (2007) suggested the term "calotte" to describe them. Given the analogy to the polar calottes of some planets, we

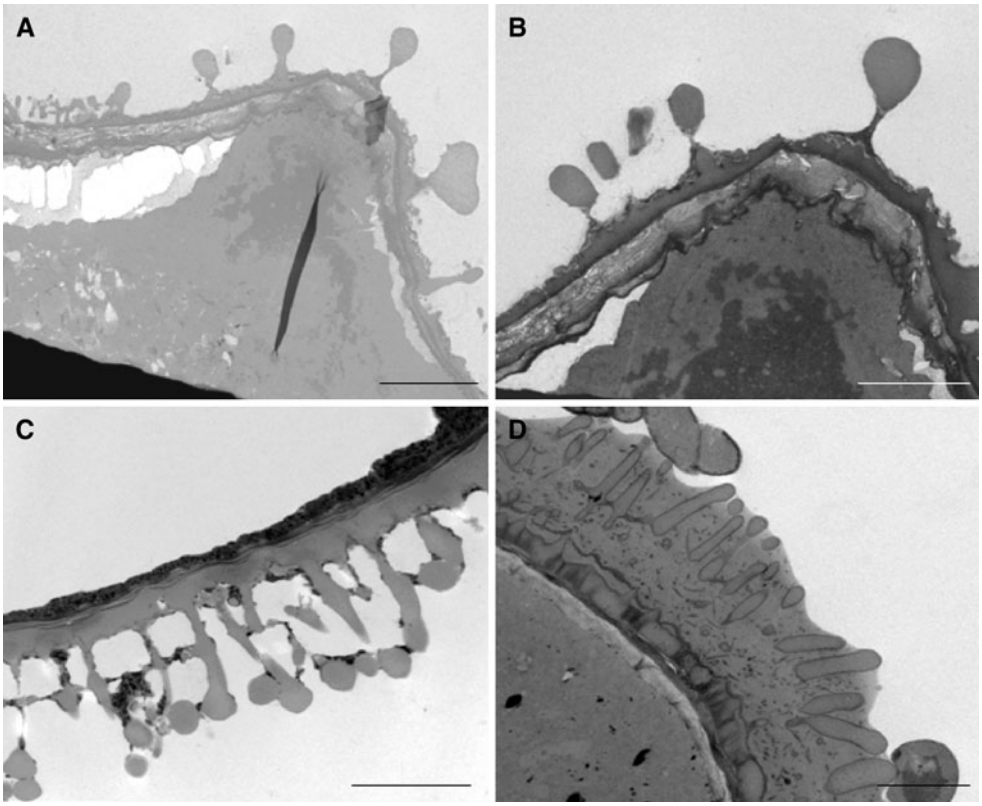

Fig. 2 Transmission electron microscope (TEM) images of *Hymenocallis caribaea*. **a–b** Cross section of the main reticulum. *Scale bars* 5 μm. **c** Cross section of the exine at the equatorial apex level. **d** Cross section of columellae, foot layer and a portion of cytoplasm. *Scale bars* 2 μm

find this term in principle adequate from a descriptive point of view, but it cannot be used since, although it appears in some works, it is not a palynological term (M. Hesse, personal communication).

Significant differences in P ($p = 0.0000$), E ($p = 0.0000$) and P/E ($p = 0.0000$) ratio values have been found between species. The test shows that *H. bolivariana* and *H. venezuelensis*, both endemic to Venezuela, present means of P/E ratio significantly different to those of the remaining taxa (Fig. 3). The P/E ratio in *H. venezuelensis* is the smallest in all the studied taxa and is very constant, whilst that of *H. bolivariana* is also small and more variable. One of the most widely distributed taxa among those considered, *H. caribaea*, also shows a quite constant P/E ratio, and it is only separable from *H. guianensis* ($p = 0.0443$).

According to *P* and *E* mean values, all the species have very large-sized pollen, following the classification of Walker and Doyle (1975). Even though pollen size was not able to separate species of *Hymenocallis*, comparing its pollen with the rest of the genera of the family (especially the American ones), its bigger volume could be related to its putative tetraploid Andean origin (Meerow et al. 1999, 2000), with *Hymenocallis* having the largest pollen of all the Andean clade (Meerow and Dehgan 1985, 1988).

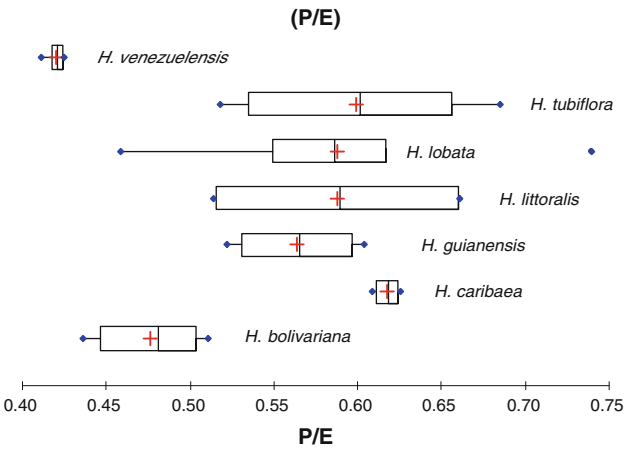

Fig. 3 Box-and-whisker plots for the P/E ratio of the studied species

Hymenocallis shows one of the widest ranges of chromosome numbers within the family (Meerow 1984), but no relationship could be established, either between pollen size and ploidy level, or between pollen size and nuclear DNA C-value (results not shown), since Venezuelan species range from $2n = 24$ to $2n = 112$, with $2n = 46$ being the most common number (Raymúndez 1997; Raymúndez et al. in prep.).

Our results about the general characteristics and ornamentation pattern of *Hymenocallis* pollen grains also agree with those of Erdtman (1966) and Huang (1972) for *H. speciosa* and *H. declinata* (*H. caribaea*), with those of Meerow and Dehgan (1985) for *H. caroliniana* and *H. horsmannii*, and with those of Meerow and Dehgan (1988) for *H. latifolia*. Likewise, some kind of equatorial exine polymorphism could also be seen in other genera belonging to the phylogenetically related Andean clade of the Amaryllidaceae. Within tribe Hymenocallideae, *Ismene* and *Leptochiton* seem to retain the same extremely different pattern of *Hymenocallis s.s.,* forming equatorial apexes, when comparing our present results with those of Meerow and Dehgan (1985, 1988) for *L. quitoensis* (*H. quitoensis*), *I. amancaes* (*H. amancaes*) and *I. narcissiflora* (*H. narcissiflora*). Nevertheless, diagnostic pollen dimorphism at the apexes is not equally retained for all members of the tribe, since two *Ismene* species differ at the meridional facies from a tectate-perforate apex on *I. longipetala* (*H. longipetala*) to an extreme reduction of the coarseness of the exine reticulum, but without apexes, on *I. morrisonii* (*H. morrisonii*) (Meerow and Dehgan 1985). Additional studies about fine exine structure for more members of tribe Hymenocallideae are needed.

Within the Andean clade, but outside of tribe Hymenocallideae, *Eucharis* Planch. and Lind., *Caliphruria* Herb., *Urceolina* Rchb. (tribe Eucharideae), *Pamianthe* Stapf. and *Paramongaia* Velarde (tribe Stenomesseae) develop an exine pattern with a more or less broad reticulum at the centre of the pollen grain and a narrow one at the poles, sometimes turning into an extremely close pattern at the meridional facies of the grain, but without developing the equatorial apexes. Out of the Andean clade, the amaryllidaceous genus *Hippeastrum* Herb. and even the Old World *Pancratium* also present a reticulate dimorphic exine (Meerow and Dehgan 1988; Alves-Araujo et al. 2007).

Pollen morphology must be seen as the consequence of two main forces; the phylogenetic environment and the pollen function related to reproductive events. Walker and Doyle (1975) suggest that family Amaryllidaceae shares plesiomorphic pollen characters with Liliaceae *s.l.*, which can be considered as ancestral for the angiosperms in comparison with other monocot families. The reticulate exine and its extreme dimorphic pattern are considered plesiomorphic conditions (even if the exine dimorphism results a diagnostic character for the genus), whereas pollen size is a synapomorphic feature (Meerow and Dehgan 1985, 1988; Meerow et al. 2002).

The second force determining pollen morphology is the pollen-pollinator and pollen-flower relationship. In this sense, pollen exine ornamentation and pollen size are the centre of interest relating them with flower morphology, especially with stigma volume and style length among related species (Cruden and Lyon 1985; Cruden 2009). The fragrant, greenish-white flowers of *Hymenocallis* are characterised by a long, narrow perigonium tube and six long stamens connected by a membranous white corona, specialised for hawkmoth (Sphingidae) pollination, being visited at the evening or during the early night (MB Raymúndez, personal observation). Venezuelan *Hymenocallis* species exhibit a wide range of style lengths, ranging from 8.2 cm (*H. lobata* and *H. venezuelensis*) up to 22.5 cm (*H. guianensis*) (Raymúndez 1997), but no relationship is evident between pollen size and style length, as would be expected for a phylogenetically closely related group, because of the superposed intervals observed for *P* and *E* measurements among Venezuelan species. As well as other pollen attributes, a reticulate exine represents another general symplesiomorphy shared with almost all of the rest of the family and a great number of monocotyledons. Further studies utilising broad population samples are necessary to determine relationships between pollen size and ploidy level and/or pollen size and floral traits (i.e. stigma depth, pollen volume) when considering reproductive behaviour related to the evolution of pollen features in *Hymenocallis*.

Concluding remarks

All *Hymenocallis* species studied are very similar to each other in relation to their pollen outline, ornamentation and internal structure, implying that the genus is perfectly delimited from a palynological point of view. This is supported by the coincidence of the pattern described in this article with that of the few other taxa of the genus investigated to date from a palynological point of view (Meerow and Dehgan 1985). Discrete features cannot be used to characterise all the individual species. There are sometimes slight differences in the coarseness and continuity of the reticulum walls and in the density of this reticulum between the species, but they are not different enough to separate and identify any of them. As commented above, pollen grain morphometry allows us to distinguish only a limited number of taxa. Despite this limitation, it is interesting to remark that *H. bolivariana* and *H. venezuelensis*, the two Venezuelan endemic species of the genus, can be separated with these data of the remaining taxa.

Acknowledgments We remember with sympathy the late Ramon Fontarnau (Universitat de Barcelona), who frequently helped us with the use of the scanning electron microscope, and we also acknowledge the assistance with electron microscopy provided by Ana Domínguez and Núria Cortadellas (Universitat de Barcelona). Dion Devey (Royal Botanic Gardens, Kew) is thanked for reading the text

and correcting the English and Miguel Ángel Canela (Universidad de Navarra) for the assistance with statistics. Two anonymous referees and the associate editor Michael Hesse are thanked for their advice, which has been very useful for improving the manuscript. This work was subsidized by MICINN-Spanish Government (projects CGL2007-64839-C02-01 and 02, CGL2010-22234-C02-01 and 02/BOS) and GReB (Grup de Recerca en Biodiversitat i Biosistemàtica vegetals; Generalitat de Catalunya, project 2009SGR439).

References

Alves-Araujo A, Ribeiro dos Santos FA, Alves M (2007) Caracterização palinológica de espécies de Amaryllidaceae sensu *stricto* ocorrentes no nordeste brasileiro. Acta Bot Bras 21:967–976

Avetisian EM (1950) Simplified method of pollen preparation by acetolysis [in Russian]. Zhurn Russk Bot Obsc Akad Nauk SSSR 35:385

Campbell L (2008) Amaryllidaceae. In: Hokche O, Berry P, Huber O (eds) Nuevo catálogo de la flora vascular de Venezuela. Fundación Instituto Botánico de Venezuela Dr. Tobías Lasser, Caracas, Venezuela, pp 676–677

Cruden RW (2009) Pollen grain size, stigma depth and style length: the relationships revisited. Plant Syst Evol 278:223–238

Cruden RW, Lyon DL (1985) Correlations among stigma depth, style length, and pollen grain size: Do they reflect function or phylogeny? Bot Gaz 146:143–149

Erdtman G (1966) Pollen morphology and plant taxonomy–angiosperms: an introduction to the study of pollen grains and spores. Hafner Publishing Company, New York

Hesse M, Halbritter H, Zetter R, Weber M, Buchner R, Frosch-Radivo A, Ulrich S (2009) Pollen terminology. An illustrated handbook, Springer-Heidelberg

Huang TC (1972) Pollen flora of Taiwan. National Taiwan University Botany Department Press, Taipei

Meerow A (1984) Karyotype evolution in the Amaryllidaceae. Herbertia 40:139–154

Meerow AW, Dehgan B (1985) The auriculate pollen grain of *Hymenocallis quitoensis* Herb. (Amaryllidaceae) and its systematic implications. Am J Bot 72:540–547

Meerow AW, Dehgan B (1988) Pollen morphology of the Eucharidae (Amaryllidaceae). Am J Bot 75:1857–1870

Meerow AW, Fay MF, Guy CL, Li QB, Zaman FQ, Chase MW (1999) Systematics of Amaryllidaceae based on cladistic analysis of plastid *rbc*L and *trn*L-F sequence data. Am J Bot 86:1325–1345

Meerow AW, Guy CL, Li QB, Yang SL (2000) Phylogeny of the American Amaryllidaceae based on nrDNA ITS sequences. Syst Bot 25:708–726

Meerow AW, Guy CL, Li QB, Clayton JR (2002) Phylogeny of the tribe Hymenocallideae (Amaryllidaceae) based on morphology and molecular characters. Ann Mo Bot Gard 89:400–413

Potonié R, Kremp GOW (1955) Die Sporae dispersae des Rhurkarbons ihre Morphographie und Stratigraphie mit Ausblicken auf Auten anderer Gebiete und Zeitabschnitte. Palaeontographica 98:1–136 (Abt. B)

Ravikumar C, Nair PKK (1982) Pollen morphological analysis of *Hymenocallis littoralis* Salisb. and *H. pedalis*. SEM and light microscopic study. J Palynol 18:43–54

Raymúndez MB (1997) Estudios citogenéticos, embriológicos y morfoanatómicos en especies venezolanas del género *Hymenocallis* Salisb. (Amaryllidaceae). Tesis Doctoral. Facultad de Ciencias. Universidad Central de Venezuela, Caracas

Raymúndez MB, Escala M, Xena de Enrech N (2005) Morfoanatomía foliar en la delimitación de especies del género *Hymenocallis* Salisb. (Amaryllidaceae J. St.-Hil.) presentes en Venezuela. Acta Bot Venez 28:301–319

Roubik DW, Moreno JE (1991) Pollen and spores of Barro Colorado Island. Monographs in Systematic Botany, vol 36. Missouri Botanical Garden, St. Louis

Salisbury J (1812) *Hymenocallis*. Trans Hort Soc London 1:338

Sealy JR (1954) Review of the genus *Hymenocallis*. Kew Bull 2:201–240

Traub HP (1962) Key to the subgenera, alliances and species of *Hymenocallis*. Pl Life 18:55–72

Traub HP (1980) Sections and alliances, genus *Hymenocallis* Salisb. Pl Life 36:46–49

Tropicos on line: http://www.tropicos.org/. Accessed March 2011

Walker JW, Doyle JA (1975) The bases of angiosperm phylogeny: palynology. Ann Mo Bot Gard 62:664–723