# Hippeastrum species in areas of restinga in the state of Rio de Janeiro, Brazil: pollen characters

Renata Suzano Candido<sup>1</sup>, Ana Carolina da Silva Fourny<sup>2</sup>, Vania Gonçalves-Esteves<sup>2,3</sup> and Rosana Conrado Lopes<sup>1</sup>

Received: 18 December, 2012. Accepted: 22 May, 2013

#### **ABSTRACT**

The order Asparagales comprises 14 families, five of which occur in Brazil. Amaryllidaceae is a family of economic relevance and includes numerous ornamental genera. The genus *Hippeastrum* is widely distributed in Brazil and comprises 34 species, 11 of which occur in areas of *restinga* (coastal woodland) and Atlantic Forest in the state of Rio de Janeiro. The morphology of *Hippeastrum* has not been extensively studied in Brazil, where only a few systematic floristic surveys have been carried out with native species. In field studies and reviews of herbarium collections, we identified five *Hippeastrum* species occurring in areas of *restinga* in the state of Rio de Janeiro. The five species identified could be distinguished according to the following palynological characteristics: pollen grain size, polarity, pollen units, shape, aperture (number and type), exine sculpture, colpus length and pattern of sexine ornamentation. Of the five species identified, *Hippeastrum aulicum* Herb. and *H. glaucescens* (Ker Gawl.) Herb. were not identified in the field. Among the three species that were found in the field, *H. striatum* had the widest distribution in the study area, whereas the distribution of *H. reticulatum* was restricted to a single area of *restinga*, in the Jaconé district of the municipality of Saquarema. Through palynological examinations of specimens from herbaria in the state of Rio de Janeiro, we were able to confirm the identity of all five species of *Hippeastrum* studied. Our data represent a relevant contribution to increasing knowledge of this plant group in the region and will aid in future conservation efforts.

Key words: Amaryllidaceae, Asparagales, conservation, pollen

# Introduction

The order Asparagales comprises 14 families, five of which occur in Brazil: Amaryllidaceae, Asparagaceae, Hypoxidaceae, Iridaceae, and Orchidaceae (APG III 2009). Amaryllidaceae is widely distributed in temperate and tropical areas worldwide. The family comprises three subfamilies, 73 genera, and 1605 species (APG III 2009). In Brazil, there are 14 recognized genera, comprising 134 native species (Dutilh & Oliveira 2012) in 14 tribes. The genus Hippeastrum belongs to the tribe Hippeastreae (Meerow et al. 2000). Hippeastrum is widely distributed in Brazil, with 34 species, 11 of which occur in areas of restinga (coastal woodland) and Atlantic Forest in the state of Rio de Janeiro (Dutilh & Oliveira 2012).

Amaryllidaceae is a family of economic relevance, including ornamental genera that occur in Brazil, such as *Hippeastrum* Herb. (Dutilh & Oliveira 2012). The morphology of *Hippeastrum* has not been extensively studied in Brazil, only a few systematic floristic surveys having been undertaken with native species (Dutilh 1987, 1996, 2005; Amaral 2007; Alves-Araújo *et al.* 2009). Information about

the morphology and taxonomy of the genus in the state of Rio de Janeiro is still scarce, citations in lists of local floras representing the only records currently available (Araujo 2000; Alves 2001; Argôlo 2001; Braga 2004; Menezes *et al.* 2005; Bocayuva *et al.* 2006).

Restingas constitute one of the component habitats of the Atlantic Forest biome, a known biodiversity "hotspot" (Myers et al. 2000). Although restinga habitats were once distributed along most of the coastline of the state of Rio de Janeiro, they have been diminishing at an alarming rate (Rocha et al. 2007) due to the removal of native vegetation for human occupation, the replacement of substrates, the displacement of native vegetation by exotic plant species and the selective extraction of plants with economic value, such as bromeliads, orchids and clusias (Rocha et al. 2007).

In view of the lack of studies focusing on Amaryllidaceae and the genus *Hippeastrum* in areas of *restinga* within the state of Rio de Janeiro and the increasing degradation of the original flora, we sought to determine the number of species of this genus existing in those areas. Although many collections of *Hippeastrum* have been stored in herbaria in the state of Rio de Janeiro during the last century,

¹ Universidade Federal do Rio de Janeiro, Instituto de Biologia, Departamento de Botânica, Rio de Janeiro, RJ, Brazil

<sup>&</sup>lt;sup>2</sup> Universidade Federal do Rio de Janeiro, Museu Nacional, Departamento de Botânica, Rio de Janeiro, RJ, Brazil

<sup>&</sup>lt;sup>3</sup> Author for correspondence: esteves.vr@gmail.com

the preparation of this material often obscures the corona, which is important for species delimitation. We therefore analyzed palynological characteristics relevant to the classification of *Hippeastrum* species, in order to expand our understanding of this genus in the region and to aid future conservation efforts.

## Materials and methods

We conducted regular excursions over a two-year period (from January 2008 to January 2010) in areas of *restinga* in the state of Rio de Janeiro, observing *Hippeastrum* species in their natural habitats, determining their geographic distribution, investigating their growth habits, as well as identifying intrapopulation and interpopulation variation. Specimens were identified, photographed and prepared with traditional techniques (Fidalgo & Bononi 1989). The specimens were then deposited in the Herbarium of the Biology Institute of the Federal University of Rio de Janeiro (code, RFA). Duplicates were also provided to the Herbarium of the Botany Department of the National Museum of the Federal University of Rio de Janeiro (code, R).

For palynological studies, we collected flowers or flower buds of *Hippeastrum* species occurring in the study area from established collections in herbaria in the state of Rio de Janeiro (Appendix 1). The slides prepared during this study were deposited in the Palynology Laboratory of the Botany Department of the National Museum of the Federal University of Rio de Janeiro.

To prepare specimens for analysis under light microscopy (LM), we performed acetolysis with 60% lactic acid (Raynal & Raynal 1971), because the pollen grains showed low resistance to traditional acetolysis. Photomicrographs of pollen grains were obtained with a digital camera (Cybershot DSC-W7; Sony, Tokyo, Japan) coupled to a microscope (Axiostar Plus; Carl Zeiss, Oberkochen, Germany).

For scanning electron microscopy (SEM) analyses, non-acetolyzed pollen grains were spread onto the surface of double-sided carbon tape on aluminum supports. The samples were then transferred to a vacuum chamber, sputter-coated with a thin layer of palladium gold (ca. 2 nm thick) and subsequently observed using a Zeiss scanning electron microscope (SEM) in the Laboratory of Cellular Ultrastructure at the Biophysics Institute of the Federal University of Rio de Janeiro, with a Zeiss scanning electron microscope (DS M960; Carl Zeiss), and in the Laboratory of the Invertebrate Department of the National Museum of the Federal University of Rio de Janeiro, with a JEOL scanning electron microscope (JSM-6390LV; JEOL, Tokyo, Japan). A pollen key was prepared based on the features observed under LM and SEM.

Pollen grain measurements of standard specimens were taken under LM with an ocular micrometer. The largest and smallest equatorial diameters of 25 pollen grains were measured in polar views. Polar and equatorial diameters of ten pollen grains were measured in equatorial views, and ten measurements were made to determine the dimensions of colpi and exine layers. When possible, ten measurements were also made of the lumen of the reticulum (ornamentation). The terminology used in describing pollen shape, size, morphology and ornamentation was based on Punt *et al.* (2007).

Four specimens of each species were analyzed and compared when possible; one specimen of each species was chosen for statistical treatment and illustration (Appendix 1). To confirm the stability of the reference material data (Mendonça *et al.* 2010; Carrijo *et al.* 2011, Lopes *et al.* 2013), hereafter referred to as comparison material, ten of the same types of measurements were made of pollen grains from another collection.

# Results

In areas of *restingas* in the state of Rio de Janeiro, the genus *Hippeastrum* is represented by five species (Fig. 1), specimens of which are on deposit in herbaria collections: *Hippeastrum aulicum* Herb., *H. glaucescens* (Ker Gawl.) Herb., *H. puniceum* (Lam.) Kuntze, *H. reticulatum* Herb., and *H. striatum* (Lam.) H. E. Moore. Of those five species, only the last three were found in the field during the present study; the first two being represented only by herbarium specimens.

#### Size

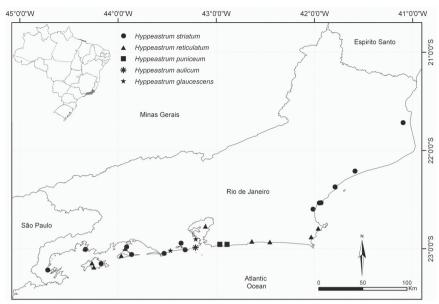
The diameters of pollen grains of the five species evaluated, in polar view and equatorial view, are shown in Tab. 1 As can be seen, the diameters of *Hippeastrum aulicum* pollen grains were very large (100-110  $\mu$ m), whereas those of the remaining taxa were classified as large (57.5-77.5  $\mu$ m).

### Morphology

All of the species analyzed here had heteropolar pollen grains (Fig. 6, 13, 20, 27 and 34) in monads, elliptical in polar view (Fig. 9, 16, 23 and 30); in equatorial view, the pollen grains were occasionally concave-convex, as in the case of *Hippeastrum aulicum* (Fig. 6), or slightly plane-convex, as in the cases of *H. puniceum* (Fig. 20), *H. reticulatum* (Fig. 27), *H. glaucescens* (Fig. 13) and *H. striatum* (Fig. 34).

## **Apertures**

The pollen grains were monocolpate (Fig. 7, 14, 21, 28 and 35), with long, wide colpi (Table 2). The colpi were found o have pointed or rounded edges (Fig. 7, 14, 21, 28 and 35). The colpi were longest in *Hippeastrum aulicum* (ca. 101  $\mu$ m) and shortest in *H. reticulatum* (ca. 41  $\mu$ m), whereas they were widest in *H. striatum* (ca. 20.5  $\mu$ m) and narrowest in *H. aulicum* (ca. 8.2  $\mu$ m).



**Figure 1.** Distribution of *Hippeastrum* species in areas of *restinga* (coastal woodland) in the state of Rio de Janeiro, Brazil, based on the specimens examined.

**Table 1**. Diameters of pollen grains of five species of *Hippeastrum*, in polar and equatorial views. Samples from areas of *restinga* (coastal woodland) in the state of Rio de Janeiro, Brazil.

	Polar view					Equatorial view $(n = 10 \text{ per species})$				
	(n = 25 per species)									
Species	Largest equatorial diameter			Smallest equatorial diameter			Polar diameter		Equatorial diameter	
	(μm)			(µm)			(µm)		(µm)	
	Range	$\overline{x} \pm s_x$	95% CI	Range	$\bar{x} \pm s_x$	95% CI	Range	$\overline{\mathbf{x}}$	Range	$\overline{\mathbf{x}}$
H. aulicum }(Ker-Gawl.) Herb.	100.0- 110.0	104.7±0.6	103.5- 105.9	50.0-60.0	53.0±0.6	51.8-54.2	50.0-57.5	54.7	105.0- 110.0	106.7
H. glaucescens (Ker Gawl.) Herb.	72.5-77.5	74.3±0.4	73.5-75.1	45.0-50.0	47.4±0.4	46.6-48.2	42.5-47.5	45.7	70.0-75.0	72.7
H. puniceum (Lam.) Kuntze.	75.0-82.5	79.3±0.5	78.3-80.3	32.5-42.5	36.9±0.6	35.7-38.1	32.5-37.5	35.7	70.0-80.0	73.6
H. reticulatum (L'Hér) Herb.	62.5-70.0	65.5±0.4	64.7-66.3	40.0-46.2	42.2±0.4	41.4-43.0	37.5-42.5	40.0	65.0-70.0	67.4
H. striatum (Lam.) H. E. Moore	57.5-62.5	60.6±0.3	60.0-61.2	40.0-43.7	41.8±0.3	41.2-42.4	32.5-40.0	37.5	60.0-65.0	62.0

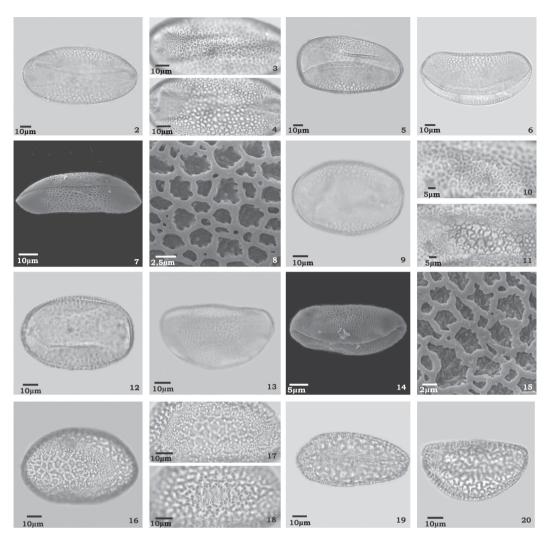
 $<sup>\</sup>overline{x} \pm sx$  – arithmetic mean  $\pm$  standard deviation.

**Table 2.** Polar and equatorial diameters of pollen grains of five *Hippeastrum* species (n = 10 per species), in equatorial view. Samples from areas of *restinga* (coastal woodland) in the state of Rio de Janeiro, Brazil.

	Polar dia	ameter	Equatorial diameter (μm)		
Species	(μπ	1)			
	Range	$\overline{\mathbf{x}}$	Range	$\overline{\mathbf{x}}$	
H. aulicum	50.0-57.5	54.7	105.0-110.0	106.7	
H. glaucescens	42.5-47.5	45.7	70.0-75.0	72.7	
H. puniceum	32.5-37.5	35.7	70.0-80.0	73.6	
H. reticulatum	37.5-42.5	40.0	65.0-70.0	67.4	
H. striatum	32.5-40.0	37.5	60.0-65.0	62.0	

 $<sup>\</sup>overline{x}$  – arithmetic mean.

Acta bot. bras. 27(4): 661-668. 2013.



Figures 2-20. Photomicrographs of *Hippeastrum* pollen grains: 2-8 (*H. aulicum*): 2-4, polar view (LM); 5, cross section (LM); 6, equatorial view (LM); 7, aperture (SEM); 8, surface detail (SEM)—9-15 (*H. psittacinum*): 9-11, polar view (LM); 12, cross section (LM); 13, equatorial view (LM); 14, aperture (SEM); 15, surface detail (SEM)—16-22 (*H. puniceum*): 16-18, polar view (LM); 19, cross section (LM); 20, equatorial view (LM).

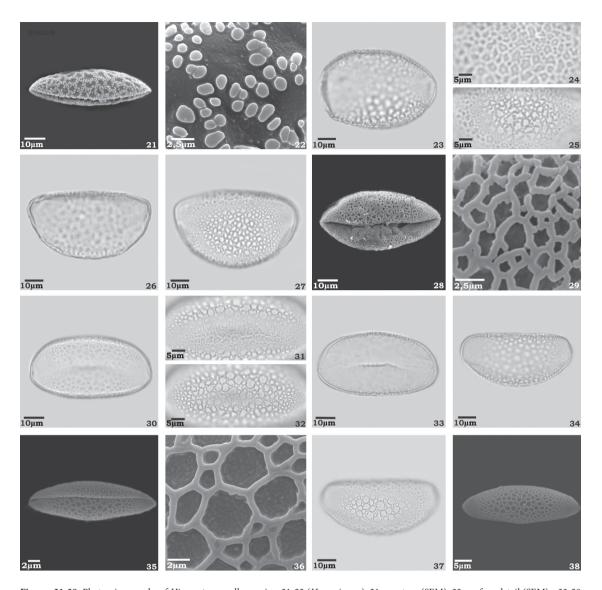
#### Exine ornamentation

Most of the *Hippeastrum* species evaluated showed sexine semitectate, reticulate heterobrochate and sparse perforations, with narrower lumina surrounding the larger ones. Intectate and retipilate exines were seen only in *H. puniceum* (Fig. 16, 17, 18, 21 and 22). The pila were round, of different diameters and arranged in double rows in an approximately reticulate pattern. The diameters of the "lumina" decreased at the ends of the pollen grains, which were sparsely scabrous (Fig. 22).

The diameter of the lumina decreased at the ends of the apertures in *Hippeastrum aulicum* (Fig. 7), *H reticulatum* (Fig. 28) and *H. striatum* (Fig. 35 and 38), as well as in the median aperture region of *H. reticulatum* (Fig. 28). Under SEM, the columellae of *H. aulicum* are clearly visible with straight muri (Fig. 8); in *H. glaucescens*, the columellae were hardly visible and the muri were sinuous,

with scattered granules within the lumen (Fig. 15); in *H. reticulatum*, the columellae were hardly visible and the muri were straight, with scattered interruptions, whereas the columellae were hardly visible and the lumina showed no granulation (Fig. 29). In *H. striatum*, the muri were straight and were almost at the level of the lumen, which was densely scabrous (Fig. 36).

Among the reticulate species studied, lumen diameters were largest in *Hippeastrum striatum* (ca. 4.9  $\mu$ m). Among all of the species studied, lumen diameters were largest in *H. puniceum* (ca. 5.9  $\mu$ m) and smallest in *H. reticulatum* (ca. 2.4  $\mu$ m). The largest lumina were found in the central region of the pollen grain in all of the species, lumen diameters decreasing toward the ends (Fig. 7, 14, 21, 28, 35, 37 and 38). In *H. aulicum* and *H. glaucescens*, the sexine was as thick as the nexine and thicker than in the other species (Table 3). The thickest sexine was seen in *H. reticulatum* (1.6  $\mu$ m).



**Figures 21-38.** Photomicrographs of *Hippeastrum* pollen grains: 21-22 (*H. puniceum*): 21, aperture (SEM); 22, surface detail (SEM)—23-29 (*H. reticulatum*): 23-25, polar view (LM); 26, cross section (LM); 27, equatorial view (LM); 28, aperture (SEM); 29, surface detail (SEM)—30-38 (*H. striatum*): 30-32, polar view (LM); 33, cross section (LM); 34, equatorial view (LM); 35, aperture (SEM); 36, surface detail (SEM); 37, surface (LM); 38, surface (SEM).

On the basis of our findings, we prepared an analytical key for classifying the different pollen types found in *Hippeastrum* species occurring in areas of restinga in the state of Rio de Janeiro:

- 1. Sexine semitectate, reticulate

  - 2. Colpus < 71  $\mu m$  in length, sexine with columellae hardly visible under SEM

    - 3. Colpus < 52 µm in length

All of the species analyzed could therefore be categorized by their pollen characters.

Acta bot. bras. 27(4): 661-668. 2013.

**Table 3.** Means of the measurements of the colpi, exine layers and lumina of pollen grains of five species of *Hippeastrum* (n = 10 per species). Samples from areas of *restinga* (coastal woodland) in the state of Rio de Janeiro, Brazil.

	Col	pus	I	Lumen			
Species	Length	Width	Exine	Sexine	Nexine	Diameter	
	(µm)	(µm)	(µm)	(µm)	(µm)	$(\mu m)$	
H. aulicum	101.2	8.2	2.0	1.0	1.0	4.5	
H. glaucescens	70.0	7.5	2.0	1.0	1.0	4.6	
Н. рипісеит	70.5	10.0	2.4	1.4	1.0	5.9	
H. reticulatum	41.0	9.7	2.6	1.6	1.0	2.4	
H. striatum	51.3	20.5	2.2	1.2	1.0	4.9	

# Discussion

Our results indicate that, of the five species studied, two (Hippeastrum aulicum and H. glaucescens) are no longer found in their natural environment, probably due to the ornamental value and predatory harvesting of these species. There is considerable taxonomic confusion surrounding the genera Hippeastrum and Amaryllis. However, in an early study, (Herbert 1821) posited that all Amaryllidaceae species occurring in the Americas should be classified as belonging to the genus Hippeastrum, and that all those occurring in Africa should be classified as belonging to the genus Amaryllis. Even today we find erroneous citations of Amaryllis in Brazil, as reported in Zamith & Scarano (2004), who asserted that the genus Amaryllis could be used in order to restore restinga vegetation in the state of Rio de Janeiro. The genus cited by Zamith & Scarano (2004) was most likely *Hippeastrum* and not *Amaryllis*.

Among the species evaluated in the present study, Hippeastrum striatum had the widest distribution in restinga vegetation in the state of Rio de Janeiro, whereas the species H. reticulatum was found in only a single area of restinga, in the Jaconé district of the municipality of Saquarema. This finding is worrisome, because it shows that H. reticulatum is vulnerable to local extinction. Due to the difficulty in finding some of these species in the field, we were obliged to use the specimens deposited in herbaria in the state of Rio de Janeiro as sources for palynological studies. This fact showed how the species are vulnerable in this environment. The sizes of the pollen grains of Hippeastrum varied from large to very large, corroborating the results reported by Alves-Araújo et al. (2007); a number of previous studies have reported that, in species belonging to the family Amaryllidaceae, pollen grains are large (Erdtman 1952; Walker & Doyle 1975; Zavada 1983; Roubik & Moreno 1991).

All five of the species analyzed here had heteropolar pollen in monads, a finding that has not been previously described in the literature. As previously reported for Amaryllidaceae (Erdtman 1952; Walker & Doyle 1975; Zavada 1983), we found that all five species showed elliptical pollen grains in polar view. In equatorial view, *H. aulicum* showed

concavo-convex grains, whereas the remaining species showed plano-convex grains.

We found that the apertures of the pollen grains evaluated were monocolpate. The colpi were long and wide, with sharp or rounded ends. This is the most common aperture type among the monocots; within the Asparagales clade, most species have monocolpate pollen grains (Pennet *et al.* 2005). A recent study confirmed this aperture type in Asparagaceae (Lopes *et al.* 2013). However, previous studies of Asparagaceae have described bicolpate pollen grains (Dahlgren *et al.* 1985; Erdtman 1952), with narrow colpi (Roubik & Moreno 1991). Other authors have made observations similar to ours (Alves-Araújo *et al.* 2007). The fact that Dahlgren *et al.* (1985) and Erdtman (1952) both examined the genus *Amaryllis* could explain the apparently conflicting results.

We found that the sexine of *H. puniceum* was intectate, differing from the semitectate sexine observed in the other four species evaluated. Intectate sexines have also been reported for the genus *Amaryllis* (Roubik & Moreno 1991), whereas semitectate sexines have been described for the family Amaryllidaceae (Erdtman 1952; Walker & Doyle 1975; Zavada 1983).

Although we found the ornamentation pattern of *H. puniceum* to be retipilate, that of the other species was reticulate heterobrochate with perforations—the only type of ornamentation previously described for the family (Erdtman 1952; Walker & Doyle 1975; Zavada 1983; Roubik & Moreno 1991; Alves-Araújo *et al.* 2007).

# Acknowledgments

We are grateful to the staff of the Laboratory of Cellular Ultrastructure at the Biophysics Institute of the *Universidade Federal do Rio de Janeiro* (UFRJ, Federal University of Rio de Janeiro), specifically to Noêmia R. Gonçalves, for her technical assistance with the SEM. We also thank Roy R. Funch for editing the English-language version of the manuscript. This study received financial support from the Brazilian *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq, National Council for Scientific and

Technological Development; fellowship grants to RSC and VCE) and from the *Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro* (FAPERJ, Foundation for the Support of Research in the State of Rio de Janeiro).

# References

- Aguiar, A.C.A.; Gonçalves, V.M.; Gonçalves-Esteves, V. & Yamamoto, K. 2008. Palynotaxonomy of Brazilian species of *Polygala*. **Botanical Journal of the Linnean Society 157**: 609-619.
- Alves, R.J.V. 2001. Amaryllidaceae. Pp. 24-25. In: Costa, A.F. & Dias, I.C.A. (Eds.). Flora do Parque Nacional da Restinga de Jurubatiba e arredores, Rio de Janeiro, Brasil: listagem, florística e fitogeografia. Rio de Janeiro, Museu Nacional.
- Alves-Araújo, A.; Alves, M. & Santos, F.A.R. 2007. Caracterização palinológica das espécies de Amaryllidaceae sensu stricto ocorrentes no nordeste brasileiro. **Acta Botanica Brasilica 21**(4): 967-976.
- Alves-Araújo, A.; Dutilh, J.A.H. & Alves, M. 2009. Amaryllidaceae s.s. e Alliaceae s.s. no nordeste brasileiro. **Rodriguesia 60**(2): 311-331.
- Amaral, A.C. 2007. Amaryllidaceae Jaume St.-Hil.: Levantamento das espécies do Distrito Federal, Brasil, e estudos de multiplicação in vitro. M.Sc. Dissertation, Universidade de Brasília, Brasil.
- APG III. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. **Botanical Journal of the Linnean Society 161**: 105-121.
- Araujo, D.S.D. 2000. Análise Florística e fitogeográfica das Restingas do Estado do Rio de Janeiro. Tese de doutorado, Universidade Federal do Rio de Janeiro, Brasil.
- Argôlo, A.M. 2001. Levantamento florístico, caracterização fisionômica e comparação da restinga de Grumari, RJ, com outras restingas do Estado do Rio de Janeiro. M.Sc. Dissertation, Universidade Federal do Rio de Janeiro, Brasil.
- Bocayuva, M.; Zaldini, C.A.; Cardoso, L.T.J.; Dias-melo, R.; Leitman, P.; Silva, B.R.; Braga, J.M.A. & Forzza, R.C. 2006. Parque Natural Municipal da Prainha, Rio de Janeiro, Brasil: Monocotiledôneas da Prainha. http://fm2.fieldmuseum.org/plantguides/guide\_pdfs/192%20Prainhamonoc%202.0.pdf (27 September 2012).
- Braga, H.N. 2004. **Levantamento das espécies fanerogâmicas da Praia Virgem, Município de Rio das Ostras, RJ**. M.Sc. Dissertation, Universidade Federal do Rio de Janeiro, Brasil.
- Carrijo, T.T.; Fourny, A.C.S.; Freitas, M.F.; Gonçalves-Esteves, V. & Peixoto, A.L. 2011. Insights on Ardisia and Stylogyne (Myrsinoideae—Primulaceae) based on pollen morphology. Plant Systematic and Evolution 297: 261-269.
- Dahlgren, R.M.T.; Cliford, H.T. & Yeo, P.F. 1985. The families of the monocotyledons. Berlin, Springer–Verlag.
- Dutilh, J.H.A. 1987. Investigações citotaxonomicas em populações brasileiras de *Hippeastrum* Herb. M.Sc. Dissertation, Universidade Estadual de Campinas, Brasil.
- Dutilh, J.H.A. 1996. Biossistemática de quatro espécies de Hippeastrum

- **Herb.** (Amaryllidaceae). D.Sc. Dissertation, Universidade Estadual de Campinas, Brasil.
- Dutilh, J.H.A. 2005. Amaryllidaceae. In: Wanderley, M.G.L.; Shepherd, G.J.; Melhem, T.S. & Giulietti, A.M. (Eds.). Flora Fanerogâmica do Estado de São Paulo. São Paulo, Instituto de Botânica.
- Dutilh, J.H.A. & Oliveira, R.S. 2012. Amaryllidaceae. http://floradobrasil.jbrj.gov.br/2012/FB004354 (27 September 2012).
- Erdtman, G. 1952. Pollen morphology and plant taxonomy Angiosperms. Upsala, Almqvist e Wiksell.
- Fidalgo, O. & Bononi, V.L.R. 1989. Técnicas de coleta, preservação e herborização de material botânico. São Paulo, Instituto de Botânica.
- Herbert, W.H. 1821. Hippeastrum stylosum: Long-styled Knight's-star Lily. Bot. Mag. 49: 2278.
- Lopes, R.C.; Andreata, R.H.P.; Cartaxo-Pinto, S.; Trovo, M. & Gonçalves-Esteves, V. 2012. Pollen morphology and wall structure of neotropical species of *Herrerria* and *Clara* (Asparagaceae – Agavoideae) and its taxonomic implications. Plant Systematic and Evolution 299: 25-34.
- Meerow, A.W.; Guy, C.L.; Li, Q.B. & Yang, S.L. 2000. Phylogeny of the American Amaryllidaceae based on nrDNA ITS sequences. **Systematic Botany 25**(4): 708-726.
- Mendonça, C.B.F.; Carrijo, T.T.; Esteves, R.L. & Gonçalves-Esteves, V. 2010. Lessingianthus H. Rob. (Vernonieae-Asteraceae): generic and infrageneric relationships based on pollen morphology. Nordic Journal of Botany 28: 1-10.
- Menezes, L.F.T.; Peixoto, A.L. & Araújo, D.S.D. 2005. **História Natural da Marambaia**. Rio de Janeiro: UFRRJ.
- Myers, N.; Mittermeier, R.A.; Mittermeier, C.G.; Fonseca, G.A. & Kent, J. 2000. Biodiversity hotspots for conservation priorities. Nature 403(24): 853-858.
- Penet, L.; Nadot, S.; Ressayre, A.; Forchioni, A.; Dreyer, L. & Gouyon, P.H. 2005. Multiple developmental pathways leading to a single morph: monosulcate pollen (Examples from the Asparagales). Annals of Botany 95: 331-343.
- Punt, W.; Hoen, P.P.; Blackmore, S.; Nilsson, S. & Le Thomas, A. 2007. Glossary of pollen and spore terminology. Review of Palaeobotany & Palynology 143: 1-81.
- Raynal, A. & Raynal, J. 1971. Une technique de preparation des grains de pollen fragilis. Adansonia 11(1): 77-79.
- Rocha, C.F.D.; Bergallo, H.G.; Van Sluys, M.; Alves, M.A.S. & Jamel, C.E. 2007. The remnants of restinga habitats in the Brazilian Atlantic Forest of Rio de Janeiro state, Brazil: habitat loss and risk of disappearance. Brazilian Journal of Biology 67(2): 263-273.
- Roubik, D.W. & Moreno, J.E. 1991. Pollen and Spores of Barro Colorado Island. Systematic Botany Monographs 36: 1-268.
- Walker, J.W. & Doyle, J.A. 1975. The bases of angiosperm phylogeny: Palynology. Annals of the Missouri Botanical Garden 62: 664-723.
- Zamith, L.R. & Scarano, F.R. 2004. Produção de mudas de espécies das Restingas do município do Rio de Janeiro, RJ, Brasil. Acta Botanica Brasilica 18(1): 161-176.
- Zavada, M.S. 1983. Comparative morphology of monocot pollen and evolutionary trends of apertures and wall structures. Botanical Review 49(4): 331-379.

Online version: www.scielo.br/abb and http://www.botanica.org.br/acta/ojs

#### Appendix 1

#### Specimens examined

Hippeastrum aulicum: BRASIL. Rio de Janeiro: Praia de Ipanema, VIII/1913, fl. fr., F.C. Hoehne\* 141 (R).

Hippeastrum glaucescens: BRASIL. Rio de Janeiro: Rio de Janeiro, Reserva Biológica de Jacarepaguá, 24/VIII/1965, fl., H.E. Strang 659 (GUA); Recreio dos Bandeirantes, 13/IX/1968, fl., M. Honorina\* 50 (R).

Hippeastrum puniceum: BRASIL. Rio de Janeiro: Praia da Barra, 25/IX/2008, fl., R.S. Candido et al.\* 105 (R); Restinga de Itaipuaçu, 31/V/2007, fl., R.C. Lopes 304 (RFA)

Hippeastrum reticulatum: BRASIL. Rio de Janeiro: Angra dos Reis, Ilha Grande, Abraão, 8/VIII/1995, fl. fr., C.A.L. de Oliveira 1042 (GUA); Ilha Grande, trilha para Parnaióca, 20/IV/2001, fl., F. Pinheiro et al. 783 (HB); Cabo Frio, Fazenda José Gonçalves, 22/X/1997, fl., A. Lobão 273 (RB); Estrada antiga para Búzios, 28/VI/1995, fr., D. Araujo et al. 10313 (GUA); Mangaratiba, Marambaia, Praia da Gaeta, 23/XII/2004, fl., L.F.T. Menezes et al. 1219 (RBR); Rio de Janeiro, Sepetiba, Bahia de Sepetiba, Ilha Furtada, 10/II/1969, fl., D. Sucre\* 4723 (RB); Ilha de Paquetá, Morro da Imbuca, 24/IV/1952, fl., E. Pereira 678 (RB); Saquarema, Reserva Ecológica de Jacarepiá, 25/IV/1991, fl., D. Araujo 9386 (GUA); Jaconé, Sambaqui de Jaconé, 5/IV/2000, fl., C. Farney 4040 (RB); Jaconé, 14/VIII/2008, fr., R.S. Candido 73 (RFA). Hippeastrum striatum: BRASIL. Rio de Janeiro: Angra dos Reis, Ilha Grande, Vila Dois Rios, 23/III/2000, fl., F. Pinheiro et al. 393 (HB); Colégio Naval, 27/XI/2008, fl., R.S. Candido 115 et al. (R); Carapebus, Restinga de Carapebus, 29/VIII/2008, fl., R.S. Candido et al. 96 (RFA); Restinga de Carapebus, 29/VIII/2008, fl., R.S. Candido et al. 98 (RFA); Casimiro De Abreu, Distrito de Barra de São João, s.d., F. Segadas Vianna s.n. (R 205743); Macaé, Lagoa Comprida, 23/VIII/1982, fl., D. Araujo et al. 5151 (GUA); Mangaratiba, Restinga da Marambaia, Praia da Gaeta, 11/VI/2004, fl., L.F.T. Menezes et al. 1141 (RBR); Paraty; Praia do Sono, 12/XII/2008, fl., R.S. Candido et al. 136 (RFA); Praia do Sono, 12/XII/2008, fl., R.S. Candido et al. 136 (RFA); Restinga da Praia das Areias Negras, 28/VIII/2008, fl., R.S. Candido 87 (R); Restinga da Praia das Areias Negras, 28/VIII/2008, fl., R.S. Candido et al. 80 (RFA); Restinga da Praia Virgem, 28/VIII/2008, fl., R.S. Candido et al. 90 (R). Rio de Janeiro, Sepetiba, Bahia de Sepetiba, Ilha Furtada, 24/VIII/1968, fl., D. Sucre 3596 (RB); Restinga da Praia Virgem, 28/VIII/2008, fl., R.S. Candido et al. 102 (RFA); 10/X/2007, fl., R.S. Candido et al. 148 (R); São João da

#### Additional specimens examined

Hippeastrum aulicum: BRASIL. Paraná: Ipiranga, Serra do Mar, 05/IV/1904, fl., P. Dusén\* 4368 (R); Rio de Janeiro: Teresópolis, Serra dos Órgãos, 12/I/1960, fl., M.R. Rodrigues s/nº (R 209581). Hippeastrum puniceum: BRASIL. Rio de Janeiro: Campos dos Goytacazes, Rodovia Campos-Farol, 18/IX/2008, fl., R.S. Candido et al. 104 (RFA); São Paulo: São Paulo, Estreito, 05/XI/1997, fl., W.M. Ferreira 1461 (SPSF); Minas Gerais: Sete Lagoas, 10/X/1957, fl., E.P. Heringer s.n. (HB 31629). Hippeastrum striatum (Lam.) H. E. Moore: BRASIL. Rio de Janeiro: Campos dos Goytacazes, Rio Preto, 30/VII/1947, fl., L.A. Parma s.n. (RB 60751).

R – Herbarium of the Botany Department of the National Museum of the Federal University of Rio de Janeiro; GUA –Alberto Castellanos Herbarium of the Foundation for Environmental Engineering in the State of Rio de Janeiro; RFA – Herbarium of the Biology Institute of the Federal University of Rio de Janeiro; HB – Bradeanum Herbarium (Rio de Janeiro); RB – Herbarium of the Rio de Janeiro Botanical Garden; RBR – Herbarium of the Botany Department of the Federal Rural University of Rio de Janeiro; SPSF – Dom Bento José Pickel Herbarium of the São Paulo State Forestry Institute.

\*Specimen chosen for statistical treatment and illustration.