SEED MORPHOLOGY, POLYPLOIDY AND THE EVOLUTIONARY HISTORY OF THE EPIPHYTIC CACTUS *RHIPSALIS BACCIFERA* (CACTACEAE)

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ABSTRACT

A SEM survey of seed, stem, stomata, and fruit characters was conducted to investigate patterns of infraspecific variability in *Rhipsalis baccifera*. New and Old World seeds were analyzed to assess the taxonomic value of their morphological features and the presence of gigas characters in polyploid *versus* diploid subspecies. The seeds are mussel-shaped and correspond to the *Rhipsalis*-type. Old World representatives have primarily oval seeds, whereas narrowly oval to oval seeds are more common in New World accessions. The seed coat is glossy, smooth, and without secondary sculpturing. The cell outline is slightly irregular with an overall elongate to rectangular shape. Cell size increases from the hilum-micropylar region to the apical portion of the seed. Seed and cell size increase with increasing level of polyploidization, its maximum expression occurring in polyploid African populations. It is likely that increase in seed size in the Old World is correlated with polyploid cytotypes. An increase in stomatal cell size is not evident with an increase in chromosome number, though stem and fruit size and the number of stomata are higher in the Old World polyploid subsp. *horrida*. The existence of smaller seeds in Paraguay and northern Argentina suggests that this South American region is the center of origin of *R. baccifera*, from where it radiated to North America and the Old World via eastern Brazil. We hypothesize that the extensive geographic distribution of *R. baccifera* in the New and Old Worlds has been possible due to reproductive strategies, progressive and recurrent cycles of polyploidy and dispersal events by migratory birds.

Key words: Cactaceae, gigas characters, polyploidy, *Rhipsalis*-type seed, *Rhipsalis baccifera*, seed morphology, SEM, stomata.

RESUMEN

Se realizó un estudio de microscopía electrónica de barrido (MEB) para analizar la morfología de semillas, estructura celular de la testa de semillas, tallo, estomas y frutos para investigar patrones de variabilidad infraespecífica en *Rhipsalis baccifera*, un miembro epífito tropical de la tribu Rhipsalideae (Cacteae). Esta especie es el único cactus que exhibe un patrón de distribución trasatlántica disjunta e incluye seis subespecies, de las cuales tres (subsp. *baccifera*, subsp. *hileibaiana*, y subsp. *shaferi*) se distribuyen en el Nuevo Mundo y tres (subsp.

erythrocarpa, subsp. *horrida* y subsp. *mauritiana*) en el Viejo Mundo. La distribución y evolución de este complejo taxonómico en los paleotrópicos está vinculada a sucesivos eventos de poliploidia, por lo que el número cromosómico varía de diploide a tretra- y octaploide. Debido a esto, semillas representativas del Viejo y Nuevo Mundo fueron comparadas para determinar el valor taxonómico de sus caracteres morfológicos y la presencia de gigantismo en subespecies poliploides. En general, las semillas de esta especie tienen la forma de mejillón y corresponden al tipo 'Rhipsalis'. Las subespecies del Viejo Mundo se caracterizan por poseer semillas de forma oval, mientras que los representativos del Nuevo Mundo tienen semillas con forma estrechamente oval a oval. El microrelieve externo de la semilla es lustroso, liso y sin estructuras secundarias. La forma de las células de la testa es ligeramente irregular y de manera general de forma alargada a rectangular. El tamaño de estas células aumenta de la región hilar del micrópilo hacia la región apical de la semilla. También es notorio que el tamaño de la semilla y las células de la testa aumenta de acuerdo al aumento del nivel de ploidía, con máxima expresión en las poblaciones poliploides de África. Es factible que el engrandecimiento del tamaño de la semilla esté estrechamente relacionado con citotipos poliploides. También se observó que no hay aumento en el tamaño de la célula del estoma con incremento en niveles de poliploidía. El tamaño del tallo, fruto y el número de estomas aumenta en las subespecies poliploides del Viejo Mundo. La presencia de semillas más pequeñas en el área de Paraguay y norte de Argentina sugiere que esta región de América del Sur es el centro de origen de *R. baccifera*, y que a partir de esta área la especie tuvo una radiación hacia América de Norte y el Viejo Mundo, a través del este de Brasil. Sugerimos que la extensa distribución geográfica de *R. baccifera* en ambos continentes ha sido posible debido a varios factores, incluyendo estrategias reproductivas (sexuales y asexuales, incluyendo viviparidad), incremento y progresión en número cromosómico con repetidos ciclos de poliploidía y dispersión por aves migratorias. No obstante, no es posible establecer una relación directa en el aumento del tamaño (gigantismo) de la diáspora en la familia, debido a que no se ha observado diferencia en el tamaño de semilla en especies diploides y poliploides de *Echinocereus* u otras especies de cactáceas. Investigaciones futuras comparando especies diploides y poliploides serán importantes para establecer patrones morfológicos más definidos.

Palabras clave: Cactaceae, caracteres gigantes, estoma, MEB, morfología, poliploide, *Rhipsalis baccifera*, tipo *Rhipsalis*, semilla.

INTRODUCTION

The genus *Rhipsalis* Gaertn. belongs to the Cactoideae, the largest and one of the most taxonomically complex of the four subfamilies recognized in the Cactaceae. *Rhipsalis* is included within the Rhipsalideae (sensu Barthlott and Taylor 1995; Anderson 2001), a tribe that is primarily distributed in South America. The circumscription of the genus has undergone several changes since Britton and Rose's early taxonomic treatment in 1923, in which they recognized 57 species. More recently, Barthlott and Taylor (1995) proposed 33 species in six subgenera. The existing taxonomic confusion and discrepancy in number of species between the two

treatments is based primarily on the close resemblance of *Rhipsalis* to *Lepismium* Pfeiff., another South American epiphytic cactus. While Britton and Rose treated *Rhipsalis* in a broader sense and *Lepismium* as a monotypic genus, Barthlott and Taylor segregated several *Rhipsalis* species and placed them within *Lepismium*, which consists of 14 species in their taxonomic treatment.

Regardless of the taxonomy, *Rhipsalis* is the largest genus in terms of number of species within the Rhipsalideae and, at the same time, the most widely distributed epiphytic cactus in the family. The genus is native to tropical America and it is distributed from northern Argentina and Uruguay to southern Mexico, southern Florida in the U.S., and the Caribbean Islands (Anderson 2001). It has a putative evolutionary center in southeastern Brazil (Barthlott 1983). The genus is characteristic of the epiphytic flora of the Neotropics, but *R. baccifera* (J.S. Muell.) Stearn is extensively distributed in the Paleotropics of the world, in particular central Africa and Madagascar (putative evolutionary secondary centers with neotenic polyploidy forms), Seychelles, Mauritius, and Sri Lanka. This species is, indeed, the only cactus exhibiting trans-Atlantic range disjunction, and according to Thorne (1973), it forms part of the 111 plant species that are mostly restricted to tropical South America and Africa, including Madagascar. It has been proposed that this species reached the Old World due to long-distance dispersal events (Barthlott 1983). However, Buxbaum (1980) suggests that humans traveling in ships from the East India Route as early as the 16th century (sailing first to Brazil before continuing their trip to the African west coast, around the Cape of Good Hope and on to India) may have taken *R. baccifera* with them. Once this plant reached Africa, birds may have contributed to further dispersal and the current extensive geographic radiation. The occurrences of this species are mainly along the old East India Route (D. Metzing, Universität Oldenburg, pers. com.).

Morphologically, *Rhipsalis baccifera* is quite different from its putative terrestrial Cactoideae ancestors, mainly due to the presence of dispersed areoles with minute, bristly spine-like structures (as opposed to large sclerified spines) and its pendulous epiphytic lifestyle in the humid tropics (fig. 1). In addition to ferns, orchids, aroids, and bromeliads, *R. baccifera* shares the tree canopies with *Lepismium cruciforme* (Vell.) Miq., *L. lorentzianum* (Griseb.) Barthlott and *R. floccosa* Salm-Dyck ex Pfeiff. in the Neotropics, especially in the tropical forests of South America (Cota-Sánchez, pers. obs.). Occasionally, this taxon grows on rocks as a lithophyte (Anthony 1949; Barthlott and Taylor 1995; Anderson 2001), and secondarily adapted terrestrial forms originating from epiphytic ancestors have been reported in Africa (Guillaumet 1972; Barthlott 1983). The stems are cylindric, terete and finely divided. The flowers are diurnal, small (less than 1 cm), whitish and insect pollinated. The fruits are fleshy, mucilaginous, and often contain numerous seeds.

The distribution of *Rhipsalis baccifera* in areas of the Old World has led to a taxonomically complex group. At present, six subspecies are provisionally recognized in Barthlott and Taylor's treatment (Barthlott and Taylor, 1995, page 63), in which they indicate the need of additional systematic studies in this taxonomically complex

species. Three of them (subsp. *baccifera*, subsp. *hileiabaiana* N.P. Taylor and Barthlott, and subsp. *shaferi* (Britton and Rose) Barthlott and N.P. Taylor) are distributed in the Americas. Another three subspecies (subsp. *erythrocarpa* (K. Schum.) Barthlott, subsp. *horrida* (Baker) Barthlott, and subsp. *mauritiana* (DC) Barthlott) have evolved in the Old World. In addition to geographic and distinguishing morphological features of this taxonomic complex, its evolutionary success and colonization of a large area of the Neo- and Paleotropics has involved changes at the chromosomal level.

The investigation of *Rhipsalis baccifera* is a fascinating endeavour, in part due to its unique distributional range in the Paleotropics in relation to other cacti, its neotropical relatives. Furthermore, this species is unique because it has undergone successive polyploidization events and its chromosome number varies from diploid ($2n = 2x = 22$) to tetraploid ($2n = 4x = 44$) to octaploid ($2n = 8x = 88$). The South American populations of *R. baccifera* in Brazil, Bolivia and Paraguay are mainly diploid, while those of Mexico, Costa Rica and the Caribbean are tetraploid (Barthlott 1976; Barthlott and Taylor 1995). Gadella *et al.* (1979) also reported the tetraploid condition for this species in Central America. Alternatively, in the Old World, tetra- and octaploid populations of *R. baccifera* are characteristic in Central Africa and Madagascar (Rowley 1978). In Madagascar, subsp. *horrida* has tetraploid and octaploid populations, while the eastern African populations of subsp. *erythrocarpa* and those of subsp. *mauritiana* from Africa, Comores, Seychelles, and Sri Lanka are tetraploid. The current trend is towards increasing chromosome number in populations from

South (diploid) to Central America and the Caribbean (tetraploid) to Africa, Madagascar, and Sri Lanka (tetraploid to octaploid) (Rowley 1978; Barthlott 1983). Overall, the diploid ancestral condition prevails in areas located near the putative center of origin in Brazil. This suggests that polyploidy in *R. baccifera* arose in neighboring areas from the proposed center of origin in South America and that subsequent duplication events were important in the radiation into new habitats.

Even though a few studies including seed morphology of *Rhipsalis* have been published (Barthlott 1974, 1984; Barthlott and Hunt 2000), the links between seed size and/or testa (outer integument of the seed coat) characters in relation to polyploidy are yet to be discussed. This paper specifically addresses what may arguably be a challenging aspect of polyploidy in plants: the existence of gigas characters in seeds and other organs. Since the evolution of gigas characters commonly associated with polyploid species has been insufficiently documented in the Cactaceae, we have conducted a Scanning Electron Microscope (SEM) survey of the testa in *R. baccifera* seeds to investigate patterns of infraspecific variation at the ultramorphological level in New and Old World representatives. Five out of the six subspecies were analyzed to: 1) document seed shape and size and microrelief features of testa and cell size variation, 2) determine whether seed characters can be used as taxonomic markers to support the infraspecific categories in Barthlott and Taylor's treatment, and 3) analyze different subspecies of *R. baccifera* along its distributional range to ascertain whether there is a correlation of seed characters and other plant parts (stem and fruit size,

and stomata frequency) with ploidy levels. We hypothesize that seeds from putative polyploid accessions will have apomorphic characters, i.e., larger seeds and larger cell size and bigger plant parts than diploid ancestral relatives. To test this idea, we investigated microrelief features of testa and cell size variation and the relationship between the increase in cell size with increase in polyploidization. We also compared quantitative traits of stem, fruit, the stomata complex and its epidermal cells between diploid and polyploid subspecies to determine whether these characters are linked to an increase in chromosome number. Finally, we discuss the taxonomic, ecological, and evolutionary significance of seed and fruit features, along with important traits such as morphology and reproductive strategies (vivipary) in the remarkable success and geographic radiation of this species.

MATERIAL AND METHODS

Seed material for analyses was obtained from herbarium specimens selected from a wide geographic range from areas of the New and Old Worlds and included representatives from 20 countries (13 in America and seven in the Old World), for a total of 37 accessions (table 1). Our taxonomic sampling includes five out of the six subspecies recognized by Barthlott and Taylor (1995), because the voucher specimens of the missing infraspecific taxon were infertile/sterile, poorly represented or absent in herbaria collections.

Due to the relevance of the proper identity of the plant material and the implications of importing and/or removing seeds from herbarium specimens, the sample size was limited in some accessions in order to preserve the integrity of the vouchers investigated. Depending on the specimen, the sample size ranged from 5 to 40 seeds. The seeds on individual fruits showed little difference among them, and whenever possible, they were randomly collected from the specimen/plant. In order to identify patterns of variation in seed size and shape, we measured the length and the width of at least five seeds per accession using a stereoscope with an ocular micrometer. Seed size was determined based on the longest dimension of the diaspore, and the seed shape was established based on the ratio between length and width according to Barthlott and Hunt (2000).

The seeds selected for SEM analysis were first washed with 50% ethanol, dried and cleaned for 45 s in an ultrasound chamber and then critical point dried for 4 h. Seeds were then affixed to the stubs and sputtered with gold with an Edwards S150B sputter coater following standard protocols. Four to six seeds per sample were examined, and one of them was photographed with a Philips SEM model 505. For comparative purposes, we took pictures of the entire seed at 78X and details of the seed coat and cell size/shape at 300X. Since cell size varies across the seed surface, for consistency we photographed the same portion (the center of the lateral surface) of each seed, with the exception of a few accessions where striation patterns were observed (see table 1). The terminology for seed morphology used here is based on that proposed for the Cactaceae by Voit (1979), Barthlott (1984), and Barthlott and Hunt (2000).

In order to investigate the relationship between organ and cell size in vegetative and reproductive characters, we compared li-

ving material of the diploid subsp. *baccifera* and the polyploid subsp. *horrida* growing in indoor collections of the Fairchild Tropical Botanic Garden (FTG), and the Montreal Botanic Garden (MT). Stem width, fruit size, number of seeds per fruit, number of stomata per square mm, and size of guard and subsidiary cells, were measured using either stereoscopic or light microscope.

Small portions (ca. 2 x 2 mm) of epidermis were removed from the stem and gently brushed with chloroform to remove waxes. The tissue was kept hydrated during microscopic examination. Measurements from macromorphological features of stem and fruit were made using a digital millimetric vernier caliper. Stem width measurements were taken from mature central branches of samples examined. At least ten (n = 10) stem measurements were taken in different portions of the subspecies under study to determine mean width of this structure (table 2). Similarly, mean length and width of at least ten fruits randomly selected of each species were estimated and the standard deviations calculated. For consistency, the same fruits were then dissected, and the number of seeds in each was determined (table 2). The measurements of micromorphological characters, such as the stomata cells (subsidiary and guard cells), were taken from epidermal stem tissue using an Axioplan light microscope. The observations of the entire stomata and individual cells were made in different portions of the plant, i.e., epidermis from different stem regions was subjected to the same observations. At least ten stomata and their cells were investigated for each accession. Subsidiary and guard cells were measured at 40X magnification using an ocular micrometer. Conversions were based on a calibration chart for this purpose. The number of stomata per mm2 was estimated at 10X magnification using an ocular micrometer. This estimate was based on the number of stomata found in a determined area followed by a cross multiplication calculation. The measurements obtained from seed, fruit, stem and stomata characters were used to calculate the mean and standard deviation for each sample (tables 1 and 2).

RESULTS

The overall seed shape of *Rhipsalis baccifera* is irregular and varies from oval (figs. 2A, C; 3A, C, E, G) to narrowly oval (table 1; figs. 2E, G). According to Voit (1979), this gross morphology belongs to the *Rhipsalis*-type (reviewed in Barthlott and Hunt, 2000) and describes seeds narrowly mussel-shaped, a seed shape that was confirmed in this survey (see figs. 2 and 3). The large majority of American accessions have narrowly oval seeds, while those of the Old World are primarily oval (table 1). In general, oval seeds have spherical or curved apical regions (figs. 2A, C; figs. 3A, C, E, G), whereas narrowly oval seeds from Brazil (fig. 2E) and Mexico (fig. 2G) have acute or pointed apices. This latter pattern was also observed in accessions from Argentina (Morrone & al. 1241, Vanni & al 3398), Costa Rica (Herrera 1780), Mexico (Ventura 19493, 20313, 22011), and Puerto Rico (Spetzman & al. 225) (pictures not shown). In addition, a conspicuous marginal crest was observed in subsp. *hileibaiana* (fig. 2E) and subsp. *baccifera* from Costa Rica (Herrera 1780), Cuba (Jack 5017), and Puerto Rico (Axelrod & Axelrod 2805, Miller & Taylor 6002, Taylor & Molano 8555, Axelrod & al. 1044) (table 1).

The external features and microrelief of

the seed coat of *Rhipsalis baccifera* are consistent throughout its range of distribution. Normally, the surface of the outer cell wall is black to black-brown, glossy, smooth, and lacks primary and secondary sculpture (see odd-numbered figures). The cell shape is elongate to rectangular (elongate-polygonal), the cell outline is slightly irregular, and only the angular portions (cell-wall junctions) of the outer periclinal cell walls are curved (figs. 2B, F, H; figs. 3B, D, F, H). Usually, cell size increases from the seed border (region of the testa adjacent to the hilum micropylar region) to the apical region (figs. 2A, C, E, G; figs. 3A, C, E, G). The anticlinal cell boundaries are shallowly to deeply channeled, straight (figs. 2F, H) to moderately undulated (figs. 2B; figs. 3D, F, H). In addition, the seeds from some accessions exhibit microcharacteristics not found elsewhere among the specimens investigated. The cell boundaries in seeds from Argentina (figs. 2B), Peru (figs. 2D), Seychelles (figs. 3B), and Costa Rica (Herrera 1780), Cuba (Jack 5017), Gabon (McPherson 16716), Jamaica (Gillis 8925), Mexico (Ventura 19493, 20313 & 22011), Puerto Rico (Spetzman & Díaz 225), and Venezuela (Trujillo & Jack 19545) (pictures not shown but see table 1) have a unique striation pattern, perpendicular to the cell outline. These striations are very localized in portions of the seed, and their taxonomic value and ecological significance is thus far unknown.

Individual seeds of *Rhipsalis baccifera* ranged from 0.86 to 1.48 mm in length and 0.38 to 0.88 mm in width (table 1). Lengthwise, these measurements correspond to seeds in the small- (0.9-1.1 mm) to medium-size (1.2-1.9 mm) categories proposed by Voit (1979). The smallest seeds (0.94-1.0 mm average) were observed in subsp. *shaferi* from Argentina (table 1; fig. 2A). These accessions, along with other specimens from Jamaica (MT 2745-1983) and Costa Rica (Herrera 1780), have the smallest seeds (0.38 to 0.49 mm). Nonetheless, the larger seeds are from South America, in accessions from Brazil (fig. 2E) and Venezuela (picture not shown) with seed length ranging from 1.24-1.32 mm and 1.23-1.37 mm, respectively (table 1). In the Old World, the seeds from the Democratic Republic of Congo (subsp. *horrida*) were not only the biggest but also the widest (table 1; fig. 3G). Larger seed coat cells were observed in samples from Africa, e.g., subsp. *horrida* and less evident in subsp. *mauritiana*, compared to cell size in American representatives (subsp. *baccifera* and subsp. *hileiabaiana*) (table 1; figs. 2B, D, F, H; figs. 3B, D, F, H).

A close check on polyploidy was also conducted by comparing measurements of the stem and the stomatal complex, including individual guard and subsidiary cells. The estimated mean values and standard deviations for the stem and fruit attributes investigated indicate that Old World polyploids (subsp. *horrida*) have more robust and wider (> 4 mm) stems with coarse bristles in the areoles than New World diploid (subsp. *baccifera*) relatives (< 4 mm width) (table 2; fig. 4). A similar condition was observed in the fruits and seeds. In our survey the fruits in the polyploid subsp. *horrida* are bigger (5.3-7.5 mm width and 6.3-7.5 mm length) in relation to diploid taxa with fruits ranging in average from 4.0-4.5 mm in width and 4.5-5.0 mm in length (table 2; fig. 4). Likewise, the number of seeds (1-15 seeds) per fruit is lower in the polyploid subspecies compared with higher number of seeds per fruit (15-28 seeds) in the diploid

accessions (table 2). There is also a slight increase in seed size in the accessions of subsp. *horrida* (table 2). Similarly, stomata distribution in the stem surface confirmed that its frequency is correlated with ploidy level since they are more abundant in Old World cytotypes. The number of stomata per mm^2 in the polyploid subsp. *horrida* is much higher (> 110) than in the putative diploid subsp. *baccifera* (< 80) (table 2). According to Eggli's (1984) classification of stomata in the Cactaceae, the stomata in *R. baccifera* correspond to the parallelocytic type, which is in agreement with our observations. In this type of stomata, a pair of guard cells surrounded by crescent-shaped subsidiary cells are situated parallel to the long axis of the guard cells of the epidermis of subsp. *baccifera* and subsp. *horrida* (fig. 5). The remainder of stomata/cell characters is less informative. Overall, the polyploid subsp. *horrida* has more robust, wider stems, larger fruits with thicker ovary walls, and fewer seeds than the diploid subsp. *baccifera* (table 2). In addition, the average seed number is about half in subsp. *horrida*, and the seeds are slightly larger than subsp. *baccifera* (table 2). It should be noted that statistical tests were not performed, in part due to the variable sample size. Our finding, nonetheless, provide new relevant information on polyploidy and organ size in plants despite the lack of formal tests.

DISCUSSION

The main features of *Rhipsalis* seeds, especially those concerning seed and topography of the seed coat, are common in Cactaceae. Seven main types of seed shape have been described in the subfamily (*Cereus*-type, *Rhipsalis*-type, *Arequipa*-type, *Notocactus*-type, *Thrixanthocereus*-type,

Astrophytum-type, and *Blossfeldia*-type), which have evolved from a Pereskioid-type ancestor with suborbicular-lenticular seed shape (Barthlott and Hunt, 2000). The *Rhipsalis*-type is characteristic of the members of the tribe Rhipsalideae (*Hatiora* Britton and Rose, *Lepismium*, *Rhipsalis* and *Schlumbergera* Lem.). Similarly, features of individual cells, cell boundaries, and cell sculpture observed in *Rhipsalis baccifera* are also found in other cacti. For example, the striation pattern (caused by cuticular folding crossing the cell boundaries) perpendicular to the cell outline is a basic common pattern in angiosperms (Barthlott and Hunt 2000) and appears to be widespread in the cactus family. Ongoing SEM studies of seed coat in other members of the Rhipsalideae, indicate that other taxa within this tribe share this characteristic striation pattern. The cell boundaries of the seed coat of *Lepismium lumbricoides* (Lem.) Barthlott, *R. mesembryanthemoides* Haw., and *R. teres* (Vell.) Steud. exhibit these striae (Cota-Sánchez and Bomfim-Patrício, unpub. data). A similar striation pattern has been observed outside the Rhipsalideae, in *Rebutia minuscula* K. Schum. (Trichocereeae) (Cota-Sánchez and Bomfim-Patrício, unpub. data). Moreover, Barthlott and Hunt (2000) illustrate examples resembling this pattern in *Rebutia marsoneri* Werderm. and *R. pygmaea* (R. E. Fr.) Britton & Rose, two *Rhipsalis* species (*R. pachyptera* Pfeiff. and *R. pentaptera* Pfeiff.), two *Espostoa Britton & Rose* species, and three Hylocereeae. Additional SEM studies may confirm whether these convergent features can be used as taxonomic characters.

Our study indicates that the external microrelief, including cell outline and shape, has limited taxonomic value at the infraspecific

level in *Rhipsalis baccifera*; nevertheless, seed and cell size tend to increase with levels of polyploidization, with its maximum expression in Old World populations, e.g., Kenya and Democratic Republic of Congo. Even though the consequences of an increase of genome polyploidization, which is interpreted as an ancient and recurrent process in angiosperm evolution (Adams and Wendel, 2005), and the resulting physiological and anatomical effects are not fully understood, an increase in organ and cell size with a subsequent decrease in cell surface:volume ratio in vegetative and reproductive structures of plants is often observed as a result of polyploidization (Stebbins 1971; Lewis 1979). In the cactus family, tetraploid populations of *R. baccifera* from the Ivory Coast have larger and more numerous fruits, and the number of colpi in pollen grains is higher compared to neotropical taxa (Barthlott 1983). The above characteristics and the general trend of increasing seed and cell size found in our survey suggest that gigas characters are associated with polyploidy in *R. baccifera*.

Other studies involving polyploid species and gigas characters in plants also demonstrate that tetraploid and hexaploid taxa have larger and heavier seeds while producing fewer flowers, fruits and seeds (Müntzing 1951; Bretagnolle and Lumaret 1995; Berkov 2001; Ramsey and Schemske 2002). Increasing seed size and weight with ploidy level has been reported in *Lotus* L. (Kelman and Forrester 1999) and *Gossypium* L. (Mehetre *et al.* 2003). On the other hand, studies of seed morphology in *Echinocereus* Engelm. reveal that, unlike *Rhipsalis baccifera* polyploids, gigas features are not correlated with increase in ploidy level in *Echinocereus*. For instance, seed size in tetraploid

species of *E. engelmannii* (Engelm.) Lem. and *E. triglochidiatus* Engelm. is similar to that of diploid species as is the cell size in diploid versus polyploid species (Cota-Sánchez and Bomfim-Patrício, unpub. data). Thus, a correspondence of increasing seed size with ploidy level cannot be generalized in the Cactaceae, and more studies to document this relationship are needed. Nonetheless, the repeated occurrence of increase in seed and cell size with ploidy in *R. baccifera* has been reinforced with the New and the Old World specimens. On these grounds and in a cautious judgment, we suggest a correlation of increasing seed and cell size with increase in ploidy level in this species. Since the investigation of seed characters from this perspective is still limited in the cactus family, the interpretation above should be considered bearing in mind that this study is the first attempt to interpret the correlation between ploidy level and gigas characters in the family. However, phenotypic differentiation between diploid and polyploidy cytotypes is likely the result of genotypic divergence and difference in gene dosage and evolutionary processes operating in these plants.

Early investigations involving diploid and polyploid plants have revealed that diploids can be distinguished based on the number of chloroplasts in guard cells. Dudley (1958) demonstrated that chloroplasts in guard cells are more abundant in tetraploid than in diploid lines of sugar beet. In addition, stomata tend to be smaller and higher in number in polyploid varieties of *Gossypium arboreum* L. (Mehetre *et al.* 2003). Our data are in agreement with this correlation of an increase in stomata number in the Old World polyploid *Rhipsalis baccifera* subsp. *horrida* (table 2). Overall, there

is an increase in stomata number with a decrease in cell surface:volume ratio and an increase in stem size (table 2). On the other hand, at the epidermal level, the cell size of the stomatal complex appears not to be associated with ploidy level, as demonstrated by relatively similar and/or overlapping size ranges in both diploid and polyploid subspecies. We attribute this variation in cell size to the stomata activity, since some guard cells exhibited different degrees of physiological activity as interpreted by the aperture of some stomata.

The existence of smaller seeds in South American accessions of *Rhipsalis baccifera* confirms that this area, in general, and southern Brazil, in particular, is one of the evolutionary centers of origin of the Rhipsalideae as first proposed by Loefgren (1915). A large number of species in the tribe occur in that area, and some have the most plesiomorphic morphological features, whereas taxa with apomorphic features have progressively differentiated in peripheral, distant areas. While we consider that the plesiomorphic seed condition in the family is represented by a Pereskioid ancestor, we believe, however, that regions of Paraguay and northern Argentina (not so far from eastern Brazil) are the putative center of origin of *R. baccifera*. The smallest seeds with smaller cell size are found in these areas, from which this species probably radiated northwards via the Central America land bridge and eastwards to Africa and the Caribbean via eastern Brazil. Apparently, this radiation was accompanied by an increase in seed size as evidenced by the larger seeds of subsp. *hileiabaiana* in northeastern Brazil as well as those from Venezuela, Costa Rica, Mexico, the Caribbean region and the Old World. It is noteworthy that the accessions from Brazil, Mexico, Venezuela, Costa Rica, and Puerto Rico have the largest seeds in the Americas (table 1; figs. 2E, G) and are found in warmer, more humid regions, while those from Argentina, Paraguay, and Peru have the smallest seeds in South America and are found in less mesic environments and at higher altitudes, with relatively colder regimes. We hypothesize that the putative origin of *R. baccifera* is in the aforementioned south-central region of South America, an area adjacent to the first center of diversity for Rhipsalideae, which is in agreement with Barthlott (1983).

In spite of the degree of infraspecific variability in seed size observed among the samples investigated, this variation is not taxonomically informative. We believe that this variability and the potential trend observed in increasing seed size in Old World accessions is the result of different ploidy levels of this species. It is also feasible that lower latitudes and proximity to the Equator are associated with the occurrence of larger seeds in *Rhipsalis baccifera* in accessions from northern Brazil and Venezuela. Murray *et al.* (2003) reported a trend of decreasing seed size with increasing latitude in seeds of *Glycine* L. Though a limited number of seed accessions from South American countries were examined, our data indicate that the smaller seeds of *R. baccifera* were found in South American specimens (northern Argentina and Peru), with an apparent trend in increasing seed size from the putative center of origin of the tribe in South America to Central and North America. A similar pattern occurs in seeds of the Central American and African accessions, in which the larger seeds were found in countries located closer to the equator. Further, seed size

variation in subsp. *baccifera* may be correlated with inherited polymorphism, or some accessions may represent tetraploid populations. Differences in seed size and shape at the infraspecific level have also been documented in *Kohlrauschia* Kunth (Caryophyllaceae) (Harper *et al.* 1970). Though no major distinguishing taxonomic features characterized the subspecies of *R. baccifera*, our data add to the body of existing information and provide insights into the ultramorphology of the seed and seed coat of this species.

The success and fast radiation of *Rhipsalis baccifera* in the Neo- and Paleotropics can also be attributed to its unique adaptability to withstand selective pressures and quickly establish in new areas with similar ecological conditions. The xeromorphic features and physiological adaptations to balance water loss coupled with the ability to reproduce both sexually and asexually are advantageous strategies to colonize the tree canopies. Interestingly, in greenhouse conditions, the seeds of *R. baccifera* germinate and grow successfully in a mixture of potting soil without a plant-host association (Cota-Sánchez, pers. obs.) and in shaded, wooded areas (S. Zona, Fairchild Tropical Garden, pers. com.). The ability of the seeds to germinate under these conditions indicates that true epiphytes find similar nutrients as those present in the humus and debris accumulated in the bark of trees, which has been observed in other strict epiphytic vascular plants (Benzing 1990). The germination of seeds of epiphytic species on an appropriate substrate independent from a host provides significant evidence of the overlapping requirements of the epiphytic and ground flora, further explaining the extensive

radiation of *R. baccifera* on ground substrates of the Old World.

Finally, vivipary, a rare event in angiosperms, has been documented in *Rhipsalis baccifera* subsp. *horrida* (Cota-Sánchez 2004) and interpreted as a mechanism for protecting the embryo and a specialized trait of evolutionary and biological significance providing new avenues for survival (Cota-Sánchez 2004; Cota-Sánchez *et al.*, 2007). The occurrence of this condition in this species aids in explaining its ample geographic distribution in the Old World. We believe that the viviparous trait confers fitness advantages by disseminating offspring in time and space in new areas of the host plant and other substrates of the relatively small (0.4 to 0.8 cm) viviparous seedlings of this species whenever plantlets land in sheltered microsites with optimal temperature and humidity (Cota-Sánchez 2004). In all, cactus vivipary is of significance in the radiation of this species. It increases the potential for colonization of new habitats, favors the survival and perpetuation of species in the stressed and driest conditions of the tree canopies via the formation of viable offspring, and facilitates germination, rapid rooting, and early seedling establishment.

Geologically speaking, the diversification of *Rhipsalis baccifera* into the Old continent must be a relatively recent migratory event, due to the Mid-Tertiary origin of the cactus family ca. 30 myabp (Hershkovitz and Zimmer 1997). Although we lack supporting evidence, we believe that the dispersal by birds is improbable because frugivorous birds are not able to cross the Atlantic Ocean from South America to Southern West Africa. The dispersal of this species into the Old

World is better explained by anthropogenic influence, as a consequence of the East India Route, as proposed by Buxbaum (1980). A subsequent fast radiation (resulting in high infraspecific diversity) into new areas, favored by ornitochory, facultative self-fertility, asexual reproduction, vivipary, and successive cycles of polyploidy could be a possible alternative hypothesis to intercontinental seed dispersal by birds. Singular storm events are less probable as a transport vector, because no tropical storms cross the ocean between the Paleotropical region of America and Africa in an easterly direction. Another possible explanation is trans-Atlantic dispersal by water, an idea that is in line with Renner (2004), who suggested that bidirectional trans-Atlantic dispersal of diasporas by water is more common than dispersal by wind or birds.

In conclusion, the conundrum of the dispersal history of *Rhipsalis baccifera* from the New to the Old World remains elusive. Despite the inferences reached thus far, further research is needed to document the different episodes and natural history of *R. baccifera*. The reconstruction of its biogeographic history based on age estimates is of particular interest to elucidate the dispersal direction and divergence time between the New and Old World populations, and to determine whether long-distance dispersal is indeed a legitimate biological process explaining the radiation of this enigmatic epiphytic cactus into the Old continent.

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Table 1. List of New World (NW) and Old World (OW) *Rhipsalis baccifera* accessions analyzed in this study. Asterisk (*) near the country of locality indicates accessions included in the figures. Plus symbol (+) indicates accessions with striation in cell boundaries, and triangle (σ) indicates presence of dorsal crest in the seeds. Measurements are in mm.

Taxon	Country, locality, date, voucher/collector name and number	Herbarium acronym	Seed shape	Sample size	Length mean and standard dev.	Width mean and standard dev.
NW Accessions: North, Central America & Caribbean						
subsp. *baccifera*	+, σ COSTA RICA. Alajuela, Laguna Las Camillas, Upala San José, 12 April 1988, *G. Herrera 1780*	ENCB	narrowly oval	10	1.14±0.11	0.52±0.03
subsp. *baccifera*	+, σ CUBA. Prov. Soledade. Arroyo Belmonte, 28 March 1927, *J. G. Jack 5017*	MO	narrowly oval	40	1.24±0.01	0.59±0.04
subsp. *baccifera*	DOMINICAN REPUBLIC. La Altagracia, Prov. North of Boca de Yuma, 14 July 1982, *J. Watson 1149*	FTG	oval	10	1.11±0.08	0.60±0.03
subsp. *baccifera*	DOMINICAN REPUBLIC. Prov. Perovia., N of Loma de La Valvacoa, North of the El Guineal Rural Area, 14 July 1982, *T. Zanoni et al. 21676*	JBSD	narrowly oval	10	1.14±0.04	0.58±0.05
subsp. *baccifera*	DOMINICAN REPUBLIC. Prov. Perovia, between Monte Negro and Quita Sueño, 22 Sept. 1983, *T. Zanoni et al. 27242*	JBSD	narrowly oval	16	1.19±0.11	0.59±0.07
subsp. *baccifera*	DOMINICAN REPUBLIC. Prov. Espaillat, N of the river Moca-Jamaro, 17 Feb. 1987, *T. Zanoni et al. 38160*	JBSD	narrowly oval	21	1.23±0.07	0.58±0.05
subsp. *baccifera*	JAMAICA. Charlton, St. Catherine, 29 Jan. 1963, *Adams, 12159*	UWI	oval	37	1.06±0.07	0.55±0.08
subsp. *baccifera*	JAMAICA. Montreal Botanical Garden, Living coll. Acc. No. MT 2745-1983	MT	oval	23	1.07±0.11	0.62±0.17

Table 1. Continuation.

Taxon	Country, locality, date, voucher/collector name and number	Herbarium acronym	Seed shape	Sample size	Length mean and standard dev.	Width mean and standard dev.
subsp. *baccifera*	+ MEXICO. Veracruz, Mpio. Atzalán, Santiago, 11 March 1982, *F. Ventura 19493*	ENCB	narrowly oval	10	1.20±0.05	0.59±0.04
subsp. *baccifera*	+ MEXICO. Veracruz, Mpio. Teocelo, La Barranca, 16 June 1983, *F. Ventura 20313*	ENCB	narrowly oval	10	1.23±0.05	0.57±0.03
subsp. *baccifera*	* MEXICO. Veracruz, Teocelo, Aug. 2002, *J. Meyrán 3502*	MEXU	narrowly oval	10	1.27±0.06	0.56±0.02
subsp. *baccifera*	+ MEXICO. Puebla., Mpio. Huaytamalco, Las Margaritas, 30 May 1986, *F. Ventura 22011*	ENCB	narrowly oval	10	1.18±0.07	0.57±0.06
subsp. *baccifera*	NICARAGUA Department of Esteli, North of Pie del Cerro Quiabú, 2 July 1982, *P. P. Moreno 16788*	MO	oval	22	1.32±0.06	0.72±0.03
subsp. *baccifera*	PANAMA. Barro Colorado Island, Canal Zone, 6 Feb. 1973, *P. Busey 312*	MO	oval	21	1.27±0.06	0.64±0.09
subsp. *baccifera*	σ PUERTO RICO. Rio Grande, SW Quebrada Grande, 25 Aug. 1991, *F. Axelrod 2805 & A. Axelrod*	UPRRP	narrowly oval	11	1.14±0.11	0.54±0.04
subsp. *baccifera*	σ PUERTO RICO. Mpio Cayey, on road to Farrallón, 9 Feb. 1991, *J. S. Miller & C. M. Taylor 6002*	UPRRP	narrowly oval	11	1.15±0.10	0.54±0.01
subsp. *baccifera*	σ PUERTO RICO. Mpio de Cayey, near Cercadillo, 8 Feb. 1989, *C. M. Taylor 8555 & B. Molano*	UPRRP	oval	10	1.16±0.02	0.60±0.03
subsp. *baccifera*	PUERTO RICO. Utuado, Barrio Limón, 9 July 1989, *F. Axelrod 1044, J. Ackerman & R. Calvo*	UPRRP	narrowly oval	10	1.17±0.08	0.55±0.05

Table 1. Continuation.

Taxon	Country, locality, date, voucher/collector name and number	Herbarium acronym	Seed shape	Sample size	Length mean and standard dev.	Width mean and standard dev.
subsp. *baccifera*	+ PUERTO RICO. E of Divisoria, between Barraquitas and Toro Negro, 1 Jan. 1967, *L.A. Spetzman 225 & J. D. Diaz Colon*	FTG	narrowly oval	9	1.24±0.01	0.54±0.04
South American Accessions						
subsp. *baccifera*	COLOMBIA. Prov. Valle, Mpio. San Pedro, 5 April 1986, *D. Gentry et al. 54088*	MO	narrowly oval	13	1.18±0.07	0.55±0.03
subsp. *baccifera*	*, + PERU. Dpto. Amazonas, Road Pedro Ruiz-Cachapoya, 11 March 1998, *H. van der Werff et al. 14685*	SI	oval	12	1.13±0.04	0.60±0.03
subsp. *baccifera*	+ VENEZUELA. Merida, before Quebrada de Cuervas, 27 July 1985, *B. Trujillo 19545 & M. P. Jack*	MO	narrowly oval	19	1.30±0.07	0.64±0.06
subsp. *hileibaiana*	*, σ BRAZIL. Bahia. Catolés de Cima, Mpio. Abaíra, Mata do Cignao, 16 Nov. 1992, *W. Ganev 1468*	SPF	narrowly oval	12	1.27±0.03	0.60±0.05
subsp. *hileibaiana*	σ BRAZIL. Bahia. Catolés de Cima, Mpio. Abaíra, Mata do Cignao, 28 Feb. 1992, *P. T Sano & T. Laessoe H52395*	SPF	narrowly oval	11	1.28±0.04	0.61±0.04
subsp. *shaferi*	*, + ARGENTINA. Prov. Corrientes, North of Corrientes, 28 Feb. 2002, *H. Cota-Sánchez s.n.*	SI	oval	15	1.00±0.12	0.52±0.03
subsp. *shaferi*	ARGENTINA. Prov. Misiones, near Iguazu, *R. Vanni et al. 3398*	SI	oval	5	1.06±0.15	0.53±0.04
subsp. *shaferi*	ARGENTINA. Prov. Misiones, Dept. Iguazu, Ruta Nacional 101, 13 Oct. 1996, *O. Morrone et al. 1241*	MO, SI	narrowly oval	28	0.96±0.10	0.44±0.06

Table 1. Continuation.

Taxon	Country, locality, date, voucher/collector name and number	Herbarium acronym	Seed shape	Sample size	Length mean and standard dev.	Width mean and standard dev.
OW Accessions:						
subsp. *horrida*	* DEMOCRATIC REPUBLIC OF CONGO 18 March 2003, *W. Barthlott 11831*	BONN	oval	13	1.44±0.04	0.76±0.12
subsp. *horrida*	+ GABON. Ogooue-Lolo, East of Lastourville, 27 Sept. 1996, *G. McPherson, 16716*	MO	oval	13	1.12±0.05	0.59±0.06
subsp. *horrida*	GABON. S of Medouneu, East of Abanga, 16 Nov. 1985, *A. M. Leeuwenberg 13570*	MO	narrowly oval	16	1.15±0.05	0.51±0.06
subsp. *horrida*	* MADAGASCAR. Montagne d'Ambre National Parc, June 1993, *S. Razafimandimbison s.n.*	TAN, UPS	oval	10	1.17±0.03	0.69±0.06
subsp. *horrida*	MADAGASCAR. Toliara Andohahela RN, June 1993, *B. Randriamanpionona 462*	MO	oval	10	1.20±0.05	0.67±0.01
subsp. *horrida*	MADAGASCAR. Montreal Botanical Garden. Living coll. *Acc. No. MT 2184-98*	MT	oval	15	1.26±0.07	0.65±0.05
subsp. *horrida*	TANZANIA. Kagera, 2 Oct. 1999, *L. Festo & W. Bayona 411*	MO	oval	11	1.07±0.06	0.54±0.04
subsp. *mauritiana*	KENYA. Ngangoa-Forest, Taita Hills, 18 March 2003, *W. Barthlott 04429*	BONN	oval	11	1.30±0.08	0.66±0.02
subsp. *mauritiana*	*, + SEYCHELLES. Mahé, La Misère, 18 March 1986, *W. Barthlott 13674*	BONN	oval	15	1.10±0.08	0.60±0.05
subsp. *mauritiana*	+ SRI LANKA. Prov. Sabaragamugua, Ratnapura District, 22 Oct. 1974, *G. Davidse 7909*	MO	narrowly oval	6	1.22±0.05	0.58±0.07

Table 2. A comparison of *Rhipsalis baccifera*'s diploid (subsp. *baccifera*) and polyploid (subsp. *horrida*) taxa on the basis of quantitative traits of stem, fruit, seed, and stomata cell characters. With the exception of seed number per fruit and number of stomata/mm², values given below represent the mean and the standard deviation for the character. Sample size (n = 10); n/a = not available.

Subspecies and Accession #	Ploidy level	Stem width (mm)	Fruit length (mm)	Fruit width (mm)	Seed number per fruit	Seed length (mm)	Seed width (mm)	Subsidiary cells length (μm)	Subsidiary cells width (μm)	Guard cells length (μm)	Guard cells width (μm)	Number of stomata per mm²
baccifera MT 2745-83	2x	2.6 ± 0.4	4.7 ± 0.7	4.5 ± 0.8	17-28	1.1 ± 0.1	0.6 ± 0.1	49.1 ± 5.9	15.5 ± 0.8	28.4 ± 4.1	25.0 ± 5.0	66
baccifera FTG 6847	2x	3.3 ± 0.2	n/a	n/a	n/a	n/a	n/a	48.5 ± 1.5	18.8 ± 3.7	32.0 ± 5.5	19.5 ± 0.5	79
baccifera FTG 3799	2x	3.0 ± 0.2	n/a	n/a	n/a	n/a	n/a	60.2 ± 4.8	32.7 ± 4.3	46.1 ± 3.9	29.3 ± 0.7	57
horrida MT 2184-95	4x, 8x	5.2 ± 0.4	6.4 ± 0.1	6.4 ± 1.1	1-15	1.3 ± 0.1	0.6 ± 0.1	50.3 ± 4.8	21.2 ± 3.9	41.3 ± 3.8	15.0 ± 0.0	114
horrida MT 3357-87	4x, 8x	4.7 ± 0.4	7.2 ± 0.3	6.7 ± 0.5	1-12	1.3 ± 0.1	0.7 ± 0.1	51.3 ± 3.8	15.9 ± 1.6	39.4 ± 5.6	18.8 ± 1.3	170

Fig. 1. *Rhipsalis baccifera* in fruit sharing the tree canopy with aroid and bromelioid taxa in northern Cartago, Costa Rica.
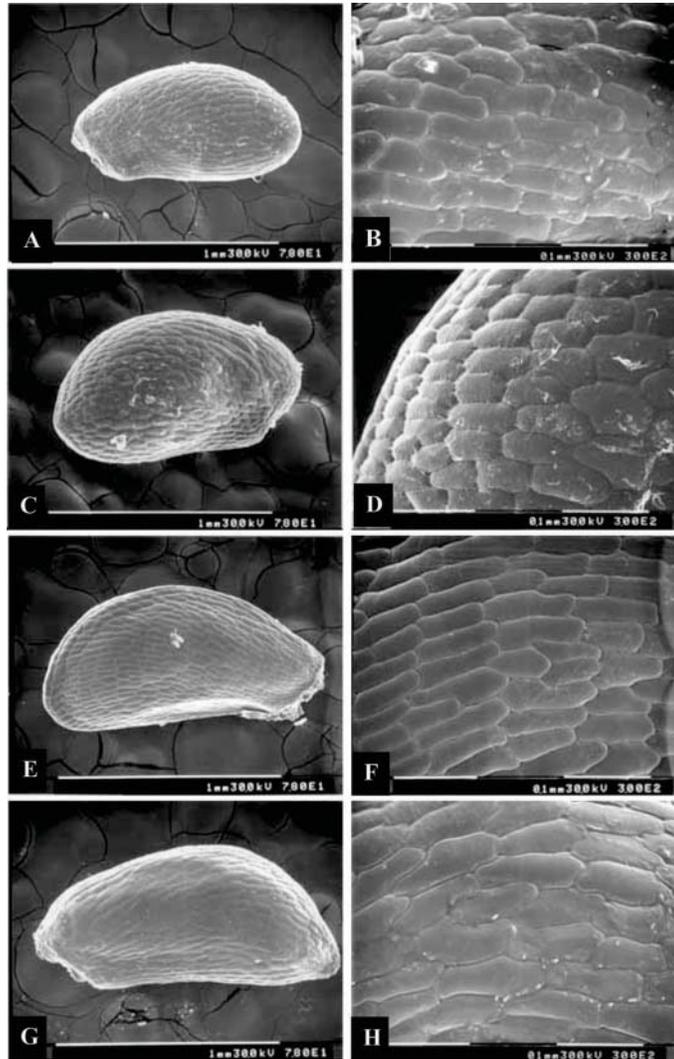
Fig. 2. Scanning electron photomicrographs from New World representative subspecies of *Rhipsalis baccifera* showing entire seed and testa detail. A. Oval seed in subsp. *shaferi* (Argentina). B. Testa detail subsp. *shaferi*. C. Oval seed in subsp. *baccifera* (Peru). D. Testa detail subsp. *baccifera*. E. Narrowly oval seed with conspicuous marginal crest in subsp. *hileiabaiana* (Brazil). F. Testa detail subsp. *hileiabaiana*. G. Narrowly oval seed in subsp. *baccifera* (Mexico). H. Testa detail subsp. *baccifera*. Scale bar in entire seeds = 1 mm; scale bar in testa details = 0.1 mm.
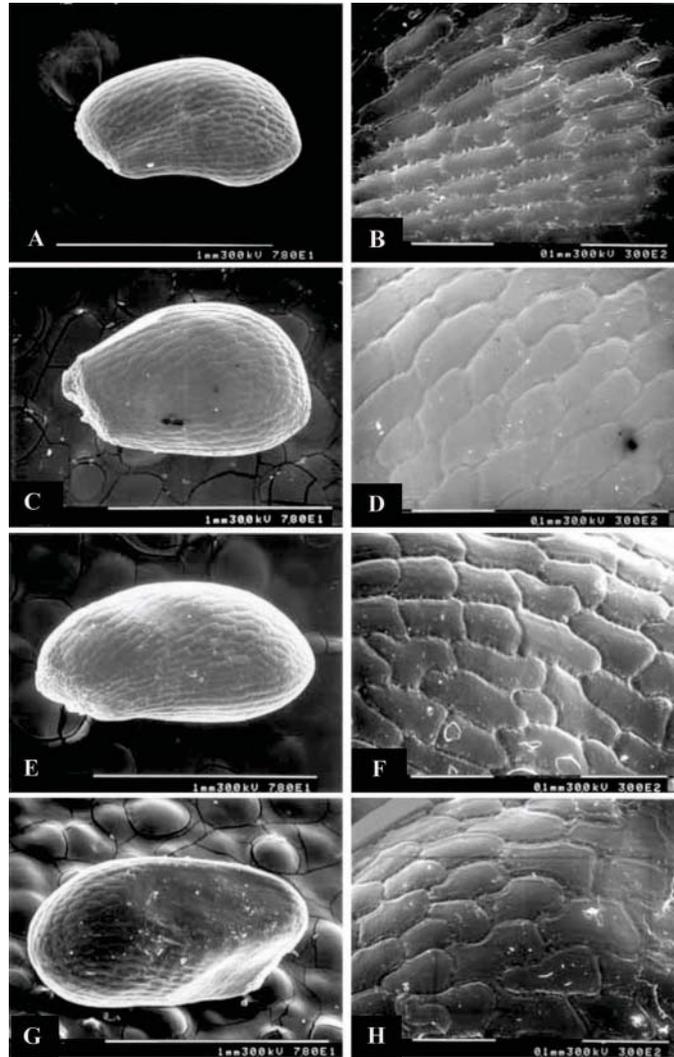
Fig. 3. Scanning electron photomicrographs from Old World representative subspecies of *Rhipsalis baccifera* showing entire oval seeds and testa detail. A. Entire seed subsp. *mauritiana* (Seychelles). B. Testa detail subsp. *mauritiana*. C. Entire seed subsp. *horrida* (Madagascar). D. Testa detail subsp. *horrida*. E. Entire seed subsp. *mauritiana* (Kenya). F. Testa detail subsp. *mauritiana*. G. Entire seed subsp. *horrida* (Democratic Republic of Congo). H. Testa detail subsp. *horrida*. Scale bar in entire seeds = 1 mm; scale bar in testa details = 0.1 mm.

Fig. 4. A comparison of diploid versus polyploidy subspecies of *Rhipsalis baccifera* showing putative stem and fruit gigas characters. A. American diploid subsp. *baccifera*. B. African polyploid subsp. *horrida*. Note difference in stem and fruit size. Scale bar in mm.
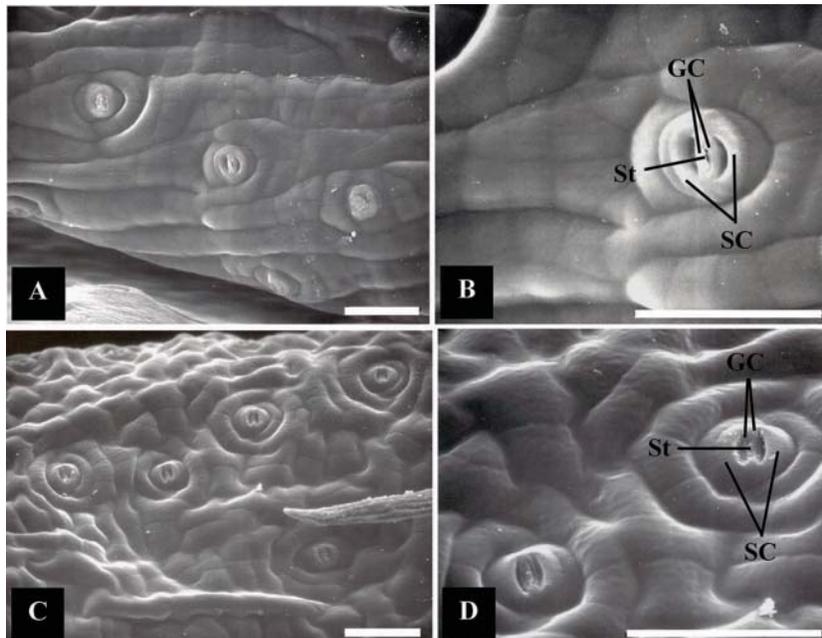
Fig. 5. Comparative pictures with scanning electron microscope showing the distribution and details of parallelocytic stomata in the stems of diploid and polyploid subspecies of *Rhipsalis baccifera*. A and B: general (186X) and close up (424X) view of the stomata in the diploid subsp. *baccifera*. C and D: general and close up view of the stomata in polyploid subsp. *horrida*. Note difference in stomata size and density in diploid versus polyploidy taxa. SC = subsidiary cells, GC = guard cells, and St = stomatal aperture. Scale bar = 0.1 mm.