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Taxonomic implications of seed morphology in *Melocactus* (*Cactaceae*) from Cuba

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Abstract: Although *Melocactus* is the second-most diverse cactus genus in Cuba, there is still no consensus regarding species circumscription. Seed morphology has not been used for classifying species in this group in Cuba, despite the taxonomically useful data obtained in some other genera of *Cactaceae*. In this study, seeds were evaluated for all *Melocactus* taxa known from Cuba, and seed morphological variability was analysed. Seed descriptions for each studied taxon (or localities) are presented here. We analysed 10 quantitative, and 19 qualitative characters in 50 seeds of 14 accessions. Seeds of the native species of *Melocactus* of Cuba are small to medium-sized, which place them among the smaller in the *Cactoideae*, and their shape is circular to broadly oval. Our results show that seven quantitative and six qualitative characters evaluated were suitable to distinguish among taxa. Such characters allowed establishing a general seed pattern for the *M. matanzanus* and *M. curvispinus* group, however, seeds in the *M. harlowii* group were less distinguishable using these characters. Colliculate relief characterized seeds of the *M. matanzanus* group with the testa border not expanded, while the *M. curvispinus* group seeds were identified by their ventro-apical keel. The most useful characters for species delimitation in the *M. curvispinus* group were seed length, and hilum-micropylar region characters. Significant characters that reveal differences within the *M. harlowii* group are lustre, seed relief and testa border expansion.

Key words: *Cactaceae*, Cuba, *Melocactus*, seed mass, seed morphology, taxonomy

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Introduction

Seed morphological characters have been considered significant traits for distinguishing among all taxonomic ranks in different plant families (Arroyo-Cosultchi & al. 2006; Ocampo 2013; Ocampo & Almeda 2013; Ullah & al. 2018; Dalavi & al. 2019). In *Cactaceae*, seed morphology has been used in the recognition of taxa in different genera, e.g. *Ferocactus* Britton & Rose (Taylor & Clarke 1983), *Pachycereus* (A. Berger) Britton & Rose (Arias & Terrazas 2004), *Stenocereus* (A. Berger) Ric-

cob. (Arroyo-Cosultchi & al. 2006) and *Neobuxbaumia* Backeb. (Arroyo-Cosultchi & al. 2007). In this regard, Barthlott & Hunt (2000) and Anderson (2001) considered that seed characters are not strongly influenced by the environment, which denotes strong genetic control (Barthlott 1981). However, the use of seed morphology for taxonomic purposes must be taken with caution, because several authors have found variation at the population level, e.g. *Melocactus* Link & Otto (Taylor 1991), *Crepis* L. (Imbert & al. 1997), *Aeschynomene* Hutch. (Zhang 1998), *Anigozanthos* Labill. (Tieu & al. 2001),

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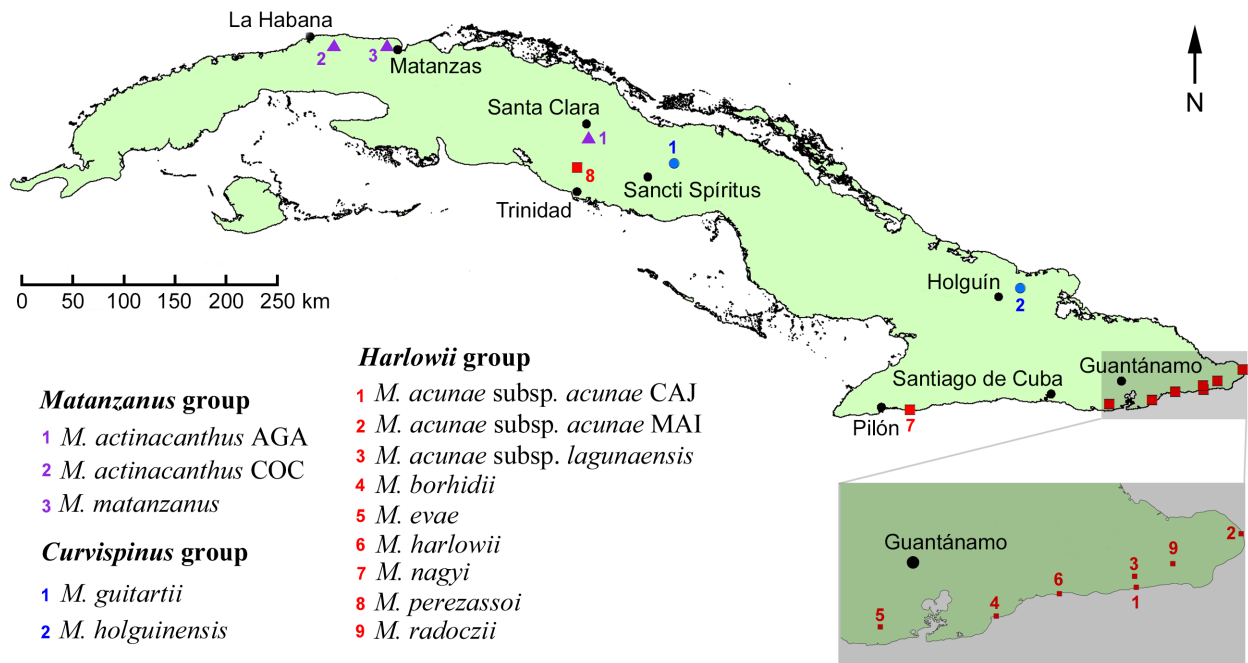


Fig. 1. *Melocactus* localities analysed for seed morphological variation in Cuba. – AGA = Sierra Alta de Agabama; CAJ = Cajobabo; COC = La Coca; MAI = Punta de Maisí.

Calophyllum L. (Sánchez & al. 2002), *Labiatae* (Pérez-García & al. 2003) and *Rhipsalis* Gaertn. (Cota-Sánchez & Bomfim-Patricio 2010). Despite this variation observed in seed morphology, subsequent molecular studies have confirmed the circumscription of taxa previously studied by the morphology of their seeds (see Tapia & al. 2017), and seed characters have been shown to delimit specific clades in *Cactaceae* (Barrios & al. 2020).

According to Barrios & al. (2015), *Melocactus* is among the *Cactaceae* with the most controversial taxonomy in Cuba. For this genus, a total of 38 accepted species have been described from Central and South America, with a further 91 names treated as synonyms, according to the checklist of Hunt (2016). *Melocactus*, commonly known as a melón espinoso, erizo or buche, are succulent plants with globose stems with a terminal cephalium from which the flowers and fruits emerge. The flowers are diurnal, without fragrance, usually pink or red, while the fruits are indehiscent, and the seeds are black and globose (Taylor 1991). The species number of *Melocactus* recognized in Cuba varies from three (Taylor 1991; Acevedo-Rodríguez & Strong 2012; Greuter & Rankin 2017) up to 11 (Lodé 2015). Other authors such as Mészáros (1976), Areces-Mallea (1993), Rodríguez (2005), Hunt & al. (2006), Guiggi (2010) and González-Torres & al. (2016) consider intermediate numbers. As a consensus, it is possible to establish three species groups, which correspond to the series recognized by Mészáros (1976), and species accepted by Taylor (1991) and Hunt & al. (2006). These groups are the *M. matanzanus*, *M. curvispinus* and *M. harlowii* groups (for authors of taxon names see Table 1).

Both the *Melocactus matanzanus* group and *M. curvispinus* group in Cuba each consist of two species. The members of both groups are simple and mainly globose-stemmed species, with dimensions that rarely exceed 10 cm without considering the cephalium, except in *M. holguinensis*, in which adult individuals generally exceed 12 cm and can reach 22 cm in height (Areces-Mallea 1976). However, it is in the *M. harlowii* group where the most species have been described: six species, one subspecies and one variety (Mészáros 1976; Areces-Mallea 1993). Members of the *M. harlowii* group have the most variable shapes within and among localities. There are individuals with single or articulated stems, with some of them forming very tight clusters (e.g. *M. evae*), with spines of different thickness, colour and length. In addition, distribution limits of the populations of this group are not well established, covering most of the coastal strip and adjacent hills from Pílon (Granma Province) to Punta de Maisí (Guantánamo Province) in eastern Cuba.

The main goal of this study was to evaluate the recognition of taxa proposed by Mészáros (1976) and Taylor (1991) through the analysis of the seed morphology of all *Melocactus* in Cuba. We hypothesize that there are seed characters that can be used to discriminate among Cuban *Melocactus* species.

Material and methods

Field collections and herbarium material

Mature seeds of 11 *Melocactus* species were collected between June 2016 and June 2018. Mature fruits of 10

Table 1. Taxa and locations included in seed micromorphology study of *Melocactus* in Cuba. Species groups indicated according to Mészáros (1976) and Taylor (1991) criteria consensus. * indicates fruits obtained from ex situ collections with parents from natural populations. Herbarium specimens deposited in Herbarium “Prof. Dr. Johannes Bisse” (HAJB), Jardín Botánico Nacional, Universidad de La Habana, Cuba (see Appendix 1).

Taxon	Province, locality in Cuba	Coll. date	Herbarium specimen	Protologue
<i>Melocactus matanzanus</i> León group				
<i>M. actinacanthus</i> Areces	Villa Clara, Sierra Alta de Agabama *	Jun 2018	–	Areces-Mallea 1976a
<i>M. actinacanthus</i> Areces	La Habana, La Coca	May 2016	<i>Barrios & González-Torres HFC 88802</i>	Areces-Mallea 1976a
<i>M. matanzanus</i> León	Matanzas, Tres Ceibas de Clavellinas	Feb 2018	<i>Majure & Barrios 7046</i>	León 1934
<i>Melocactus curvispinus</i> Pfeiff. group				
<i>M. guitartii</i> León	Sancti Spíritus, Peñón de Dagamal	Feb 2018	<i>Majure & al. 7039</i>	León 1934
<i>M. holguinensis</i> Areces	Holguín, La Palma *	Jan 2018	<i>Majure & al. 7030</i>	Areces-Mallea 1976b
<i>Melocactus harlowii</i> (Britton & Rose) Vaupel group				
<i>M. acunae</i> León subsp. <i>acunae</i>	Guantánamo, Cajobabo	Jan 2018	<i>Majure & al. 7027</i>	León 1934
<i>M. acunae</i> León subsp. <i>acunae</i>	Guantánamo, Punta de Maisí	Jan 2018	<i>Majure & al. 7025</i>	León 1934
<i>M. acunae</i> subsp. <i>lagunaensis</i> Mészáros	Guantánamo, between Vegueta and Vega Grande	Jan 2018	<i>Majure & al. 7021</i>	Mészáros 1976
<i>M. borhidii</i> Mészáros	Guantánamo, Tortuguilla	Jun 2016	<i>Barrios & al. HFC 88766</i>	Mészáros 1976
<i>M. evae</i> Mészáros	Guantánamo, Los Monitongos	Jan 2018	<i>Majure & al. 7028</i>	Mészáros 1976
<i>M. harlowii</i> (Britton & Rose) Vaupel	Guantánamo, Macambo	Jan 2018	<i>Majure & al. 7017</i>	Britton & Rose 1912
<i>M. nagyii</i> Mészáros	Granma, Salvial	Jun 2016	<i>Barrios & al. HFC 88502</i>	Mészáros 1976
<i>M. perezassoi</i> Areces	Villa Clara, Jibacoa	Feb 2018	<i>Majure & al. 7044</i>	Areces-Mallea 1993
<i>M. radoczii</i> Mészáros	Guantánamo, Guajimero *	Feb 2018	<i>Majure & Barrios 7050</i>	Mészáros 1976

individuals in each one of the 14 studied localities were randomly selected (Fig. 1, Table 1). We chose seeds without deformations and that were neither apparently sterile or broken; vouchers are listed in Appendix 1. In cases of the seeds obtained from private collections, it was confirmed that individuals had been collected in wild localities. Each locality was analysed as a taxon, the identity of which was determined according to the types and protologues of the names of the taxa. In *M. actinacanthus* and *M. acunae* subsp. *acunae*, it was possible to study two localities per taxon, which allowed us to evaluate possible intraspecific variation.

Stereomicroscopy and scanning electron microscopy

Seeds were examined by a stereoscopic light microscope (LM) Motic SMZ-168 and a scanning electron microscope (SEM) Hitachi Stereoscan Model Su1510 (10 kv voltage and secondary electron detector). For SEM, three seeds per taxon were randomly selected and washed in distilled water, TWENG detergent (10 drops), 4% sodium hypochlorite and five minutes of ultrasound. Later, they were fixed in an aluminium porta-sample with adhesive tape and coated with gold. This technique was carried out

at the Electron Microscopy Laboratory of the Instituto de Biología de la Universidad Nacional Autónoma de México. For each seed, SEM micrographs were taken in several views: lateral, frontal, apical, and cells of several regions: lateral, hilum-micropylar border, and apical. In the case of seeds of *Melocactus perezassoi*, it was not possible to take SEM micrographs, and their morphology was evaluated according to the photographs presented by Guiggi (2010) and observations made with LM; the seeds photographed by Guiggi (2010) and those used in this study came from the same population (the only known population for this taxon). Seed morphological characters were analysed using the micrographs obtained and observations in LM of 50 seeds per taxon (or per locality, when a taxon was sampled from more than one locality). Observations in LM allowed checking of all characters analysed in SEM.

Qualitative analysis

Seed morphological and microrelief characters were evaluated for each of the seed regions according to Barthlott & Hunt (2000). For general morphology, size based on length and seed shape according to length/breadth ratio was described. The colour, lustre, multicellular sculpture,

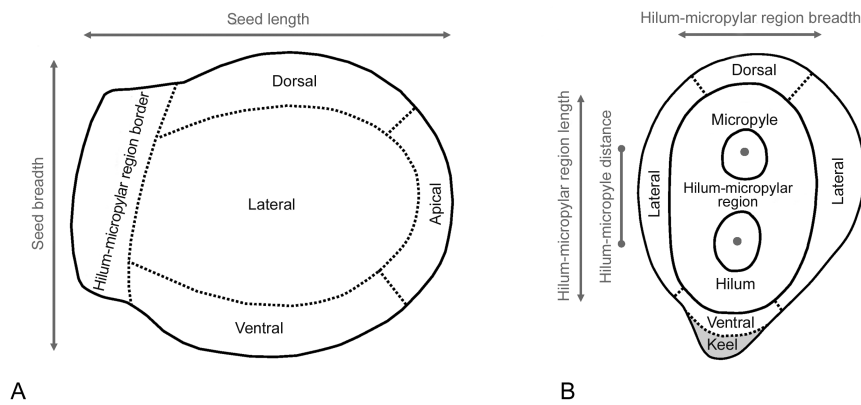


Fig. 2. *Melocactus* seed general scheme, regions and quantitative characters evaluated in lateral view (A) and frontal view (B).

relief, periphery differentiation degree, keel extension, cell size gradient near keel, and hilum-micropylar region were evaluated for the testa. For the seed regions (Fig. 2), we evaluated seed shape, periclinal wall relief, and relief and curvature of anticlinal cell boundaries. In some regions, specific characters were evaluated, such as microrelief in the lateral region and testa border expansion around the hilum-micropylar region border. In the hilum-micropylar region, the characters evaluated were seed shape, position relative to border, as well as hilum and micropyle configuration.

Most of the characters and character states evaluated were assumed according to Barthlott & Hunt (2000). For periclinal wall relief, modified states of Barthlott & Hunt (2000) were used: flat, low-convex and high-convex. Seed relief was evaluated according to the states proposed by Stearn (1983). Keel extension and cell size gradient near keel are described for the first time for *Melocactus* in the present study. Keel extension was evaluated in correspondence to the regions occupied by this structure (Fig. 2A). Cell size gradient toward keel was evaluated in the same way as cell size gradient toward hilum-micropylar region, as in Barthlott & Hunt (2000).

Quantitative analysis

The study of the dimensions was performed in 50 seeds randomly selected per accession. Photos were made in LM in lateral (Fig. 2A) and frontal (Fig. 2B) views, on millimetre paper that served as a scale. The quantitative variables measured in the AxioVision Rel. 4.8 program were: seed length and breadth in lateral view (Fig. 2A), as well as hilum-micropylar region length and breadth and hilum-micropyle distance in frontal view (Fig. 2B). From the respective length and breadth values, the length/breadth ratio of both, seed and hilum-micropylar region, were determined. The hilum-micropylar

region length/seed breadth ratio was also calculated.

The study of seed mass and testa mass was carried out from 50 seeds randomly selected per accession. The seed mass was evaluated in an analytical balance Sartorius (± 0.00001 g). Due to the similarity between the seed mass and the precision value of the balance, five seeds per replicate were randomly selected, for a total of ten replicates per taxon (or locality). In this way, the mass obtained for one seed was de-

termined as the division between five of the mass of each replicate. Prior to the weighing, the seeds were placed in an electric drying chamber for 17 hours at 105°C ($\pm 2^\circ\text{C}$) according to the ISTA (2007) standards to eliminate the water content. The dry mass of seed (DMS) was evaluated, and we then extracted the testa to determine the dry mass of testa (DMT). With these variables, the relative embryo mass (REM) was calculated according to Sánchez & al. (2002): $\text{REM} = (\text{DMS} - \text{DMT}) / \text{DMS}$.

Seed morphological similarity

A cluster analysis was performed based on quantitative non-correlated characters, and qualitative characters with discontinuity states and variation between taxa (Table 2). Quantitative characters were coded according to the proposal of Crisci & López-Armengol (1983) using average and standard deviation as two independent characters. In this way, a basic data matrix was made for 12 taxa (from 14 localities) and 20 characters, of them 14 multistate continuous quantitative, 4 bistate exclusive qualitative and 2 multistate qualitative characters with a logical sequence.

Statistical analysis

For quantitative characters, normality and variance homogeneity was checked by a Kolmogorov-Smirnov and Levene test. We performed three principal component

Table 2. Qualitative characters and encoded states to evaluate affinities among taxa of *Melocactus* in Cuba.

Character	Character states		
	1	2	3
Lustre	matte	glossy	
Keel extension	ventral	ventro-apical	
Seed relief	flat	coliculate	tuberculate
Hilum-micropylar region shape	oval	keyhole type	
Testa border expansion	constricted	not expanded	expanded
Microrelief	none	striate	

analyses (PCA) using Past 2.17 (Hammer & al. 2001) to explore the multivariate relationships and identify which characters were the ones that most contribute to distinguish among taxa or inter-locality variation in each *Melocactus* group. Differences between taxa were analysed by a non-parametric multivariate variance analysis (PERMANOVA) with pairwise comparisons. PERMANOVA was carried out in Past 2.17 and was considered statistically significant for $p < 0.05$. For each variable, a graph with mean and standard deviation was made. Among quantitative characters, we carried out a Spearman correlation test, which were considered statistically and biologically significant for $p < 0.05$ and $r > 0.7$, respectively. The basic data matrix was analysed by cluster analysis, as suggested by Crisci & López-Armengol (1983). The similarity matrix was generated with the Euclidean distance coefficient and a phenogram was produced with the method of unweighted pair-group average. All analyses were carried out in Statistica 8.0. The interpretation of the results focused at the level of the species group defined according to the consensus of the criteria of Mézaros (1976) and Taylor (1991).

Results

Variation in qualitative features

General morphology — Seeds of *Melocactus* in Cuba vary from small (0.81–1.19 mm) in *M. radoczii*, to medium-sized (1.20–1.63 mm) in *M. actinacanthus* (Sierra Alta de Agabama), *M. guitartii*, *M. matanzanus* and *M. perezassoi*. Variation in seed shape occurs within most species between circular to broadly oval, but broadly oval were exclusively found in *M. guitartii*, *M. harlowii* and the three localities of *M. acunae*.

Testa features — Testa was black in all species, glossy in *Melocactus acunae* subsp. *acunae* (Punta de Maisí), *M. borhidii*, *M. evae*, *M. harlowii*, *M. holguinensis* and *M. perezassoi*, but matte occurs in the remaining eight taxa evaluated. Multicellular sculptures are absent in Cuban *Melocactus*. Relief shows different patterns: flat in the *M. curvispinus* group (Fig. 3A, B), and almost half of the *M. harlowii* group: *M. acunae* subsp. *acunae* (Punta de Maisí; Fig. 4A), *M. borhidii* (Fig. 4C), *M. evae* (Fig. 3C) and *M. harlowii* (Fig. 4B). On the other hand, the relief is coliculate in the *M. matanzanus* group (Fig. 5A–C), *M. perezassoi* and *M. radoczii* (Fig. 3D) and tuberculate in *M. acunae* subsp. *acunae* (Cajobabo; Fig. 6A), *M. acunae* subsp. *lagunaensis* (Fig. 6B) and *M. nagyi* (Fig. 6C).

All taxa evaluated have a keel, which is exclusively ventral in the *Melocactus harlowii* and *M. matanzanus* groups, but ventro-apical in the *M. curvispinus* group. Cell size gradient near the hilum-micropylar region is abrupt, except in *M. actinacanthus* (Sierra Alta de Agabama; Fig. 5L), *M. acunae* subsp. *lagunaensis* (Fig. 6N), *M. evae* (Fig. 3S) and *M. harlowii* (Fig. 4N), in which

it is gradual. Cell size gradient near the keel is gradual, except in *M. acunae* subsp. *acunae* (Punta de Maisí), *M. acunae* subsp. *lagunaensis*, *M. matanzanus*, *M. nagyi* and *M. radoczii*.

Micromorphology of apical region — There is no variation in cell shape, all are elongate (Fig. 3U–X, 4P–R, 5O–Q, 6P–R). Periclinal wall relief is flat in *Melocactus acunae* subsp. *acunae* (Punta de Maisí), *M. borhidii* (Fig. 4R), the *M. curvispinus* group (Fig. 3U, V) and *M. evae* (Fig. 3W). Low-convex relief is present in the *M. matanzanus* group (Fig. 5O, P), although in *M. matanzanus* there are zones with high-convex periclinal walls (Fig. 5Q). Low-convex relief appears in *M. acunae* subsp. *acunae* (Punta de Maisí), *M. acunae* subsp. *lagunaensis* in which there are high-convex relief cells (Fig. 6Q), *M. harlowii* in which there are flat relief zones (Fig. 4Q), *M. perezassoi* and *M. radoczii* (Fig. 3X). Finally, high-convex relief appears in *M. acunae* subsp. *acunae* (Cajobabo; Fig. 6P) and *M. nagyi* (Fig. 6R). Anticlinal cell boundaries are generally straight with some irregularly curved areas.

Micromorphology of lateral region — Cells of the lateral region are elongate (Fig. 3N–P, 4J, K, 5I–K, 6J–L), isodiametric near the centre. In *Melocactus perezassoi*, toward the centre of the seed, there are elongated cells, and the isodiametric cells appear near the hilum-micropylar region border. Periclinal walls are flat, except in *M. acunae* subsp. *acunae* (Cajobabo; Fig. 6J), *M. acunae* subsp. *lagunaensis* (Fig. 6K), *M. nagyi* (Fig. 6L), *M. perezassoi* and *M. radoczii* (Fig. 3P), in which two states appear, flat and low-convex. Anticlinal cell boundaries are channelled and generally straight with some irregularly curved areas (Fig. 3M–P, 4J–L, 5I–K, 6J–L). The microrelief is striate, except in *M. evae*, which has no microrelief (Fig. 3O).

Micromorphology of ventral region — Cells in the ventral region are elongate with flat periclinal walls, except in *Melocactus acunae* subsp. *acunae* (Cajobabo), *M. acunae* subsp. *lagunaensis* and *M. nagyi*, which have low-convex periclinal walls. Anticlinal cell boundaries are channelled and straight.

Micromorphology of hilum-micropylar border — Hilum-micropylar border cells (Fig. 3Q–T, 4M–O, 5L–N, 6M–O) are isodiametric with flat periclinal walls. Anticlinal cell boundaries are channelled and straight. The hilum-micropylar region border is constricted in *Melocactus holguinensis* (Fig. 3B), expanded in *M. evae* (Fig. 3C), *M. perezassoi* and *M. radoczii* (Fig. 3D) and not expanded in the rest of the taxa studied.

Micromorphology of hilum-micropylar region — The hilum-micropylar region is oval except in *Melocactus guitartii* (Fig. 3E), which is a keyhole type. This region is

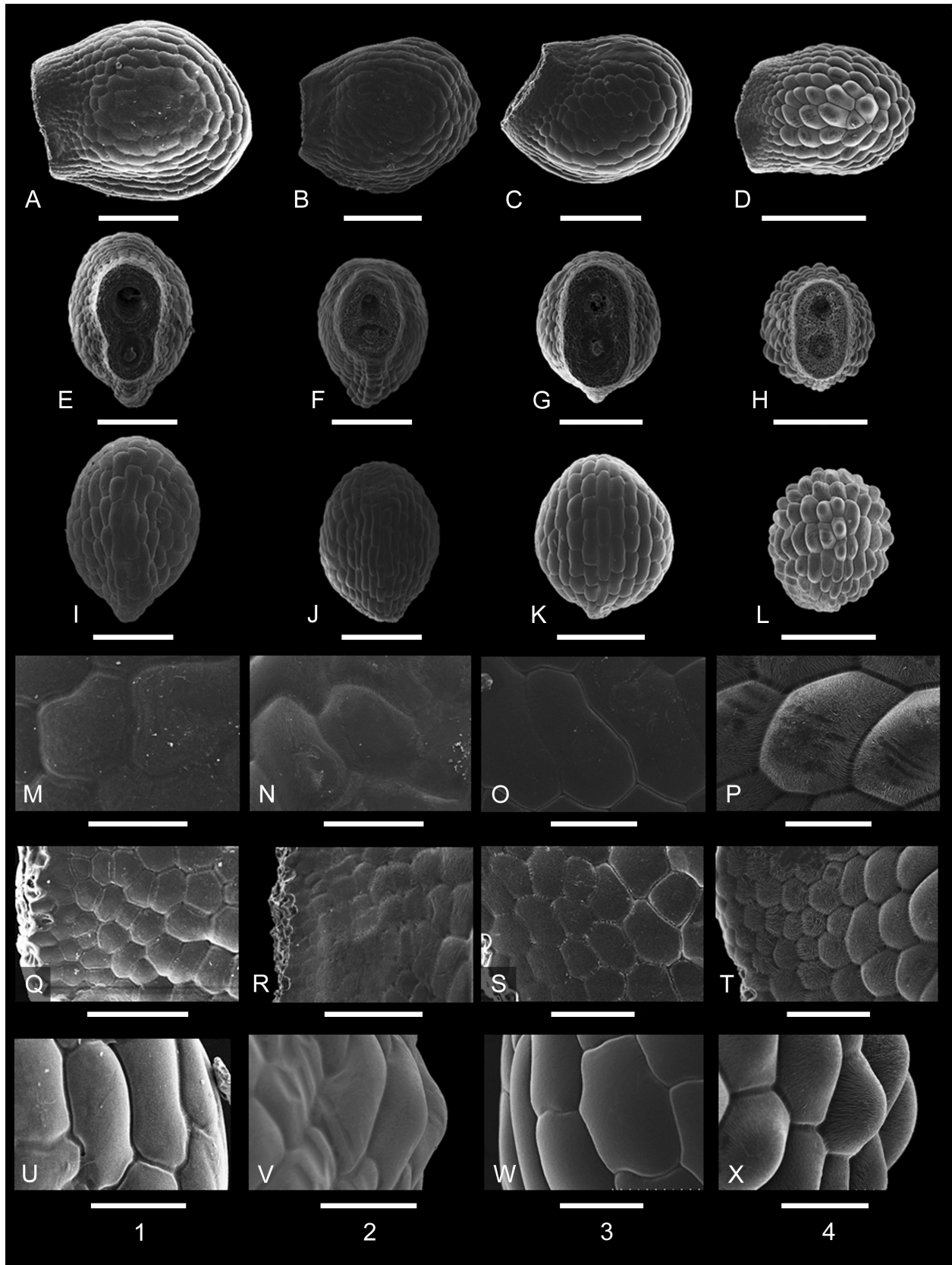


Fig. 3. Seeds of *Melocactus* in Cuba, scanning electron microscopy in lateral view (A–D), frontal view (E–H) and apical view (I–L), and cells of lateral region (M–P), hilum-micropylar border region (Q–T) and apical region (U–X). – Columns 1–4 correspond to taxa: *M. gutartii* (1); *M. holguinensis* (2); *M. evae* (3); *M. radoczii* (4). – Scale bars: A–L = 500 μ m; M–X = 100 μ m.

Table 3. Eigenvectors of principal component analysis (PCA) based on correlation matrix of seed dimensions of three *Melocactus* groups.

Character	<i>M. matanzanus</i> group		<i>M. curvispinus</i> group		<i>M. harlowii</i> group	
	axis 1	axis 2	axis 1	axis 2	axis 1	axis 2
Seed length (mm)	0.523	-0.0767	0.4066	0.04249	0.4473	-0.0488
Seed breadth (mm)	0.5122	-0.2707	0.2754	-0.4969	0.4268	-0.2849
Seed length/breadth ratio	-0.2012	0.4487	0.3238	0.5313	-0.0660	0.5404
Hilum-micropylar region length (mm)	0.4446	0.3554	0.4104	0.0234	0.4670	0.1472
Hilum-micropylar region breadth (mm)	0.3648	0.2963	-0.2036	0.4966	0.4155	-0.1304
Hilum-micropyle distance (mm)	0.2439	0.4328	0.3992	-0.0428	0.4308	0.1520
HMR length/breadth ratio	0.0150	0.0104	0.3877	-0.2377	-0.0450	0.4110
HMR length/seed breadth ratio	-0.1823	0.5639	0.3667	0.4044	0.1873	0.6275

superficial with respect to its border except in *M. acunae* subsp. *acunae* (both localities), *M. borhidii* and the *M. curvispinus* group, in which it is impressed. The hilum and micropyle form a group but are separated by sclerified tissue.

Variation in quantitative features

The PCA for the *Melocactus matanzanus* group showed overlap between taxa except for one point for the dimensions of both localities of *M. actinacanthus* that did not show overlap (Fig. 7A). The seed dimensions of *M. actinacanthus* from Sierra Alta de Agabama almost completely overlapped those of *M. matanzanus* (c. 80% of the smallest convex polygon), while the seeds of *M. actinacanthus* from La Coca were 38% of the smallest convex polygon. In this PCA, the first two components explained 71.84% of the variability. The characters that contributed the most to the first component were seed length, seed breadth and hilum-micropylar region length, while the second component was determined by hilum-micropylar region (HMR) length/seed breadth ratio, seed length/breadth ratio and hilum-micropyle distance (Table 3).

The PCA in the *Melocactus curvispinus* group showed both taxa well defined without interposition in the point cloud (Fig. 7B). In this PCA, the first two components

explained 87.31% of the variability. The characters that contributed the most to the first component were HMR length, seed length and hilum-micropyle distance, while the second component was determined by seed length/breadth ratio, seed breadth and hilum-micropylar region breadth (Table 3).

The PCA in the *Melocactus harlowii* group showed interposition between taxa, except in *M. perezassoi*, where all its points were well separated from the rest of the group (Fig. 7C). For both localities of *M. acunae* subsp. *acunae*, the seeds from Punta de Maisí coincide in 86% with respect to the smallest convex polygon of the points of the seeds from Cajobabo. In this PCA the first two components explained 76.72% of the variability. The characters that contributed the most to the first component were hilum-micropylar region length, seed length and hilum-micropyle distance, while the second component was determined by HMR length/seed breadth ratio, seed length/breadth ratio and HMR length/breadth ratio (Table 3).

Significant differences were found for all quantitative characters evaluated in *Melocactus* seeds (Table 4). In the *M. matanzanus* group, the seeds of three localities differ from each other with respect to seed length (Fig. 8A) and seed breadth (Fig. 8B), as well as the dry mass of seed (Fig. 9C), although were similar with respect to seed length/breadth ratio (Fig. 8C) and HMR length/breadth ratio (Fig. 9A), and relative embryo mass (Fig. 9D). *Melocactus actinacanthus* (Sierra Alta de Agabama) and *M. matanzanus* were similar to each other and different from *M. actinacanthus* (La Coca) with respect to hilum-micropyle distance (Fig. 8F) and to HMR length/seed breadth ratio (Fig. 9B). The two localities of *M. actinacanthus* were similar in terms of HMR length (Fig. 8D) and HMR breadth (Fig. 8E). On the other hand, *M. guitartii* and *M. holguinensis* differed in all quantitative variables evaluated (Fig. 8, 9). In the *M. harlowii* group, *M. perezassoi* had the greatest seed dimensions of the genus in Cuba (Fig. 8). On the other hand, *M. radoczii* had the smallest seeds for most of the analysed variables. The remaining taxa of the *M. harlowii* group had seeds with intermediate values for analysed variables (Fig. 8, 9).

Table 4. Quantitative characters measured in Cuban *Melocactus* with F values of non-parametric multivariate analyses of variances with pairwise comparisons test and associated probability (p). – HMR = hilum-micropylar region.

Quantitative character	F	p
Seed length	203.5	0.0001
Seed breadth	256.3	0.0001
Hilum-micropylar region length	221.7	0.0001
Hilum-micropylar region breadth	149.4	0.0001
Hilum-micropyle distance	149.6	0.0001
Seed length/breadth ratio	19.21	0.0001
HMR length/breadth ratio	66.79	0.0001
HMR length/seed breadth ratio	71.68	0.0001
Dry mass of seed	236.3	0.0001
Relative embryo mass	3.121	0.0004

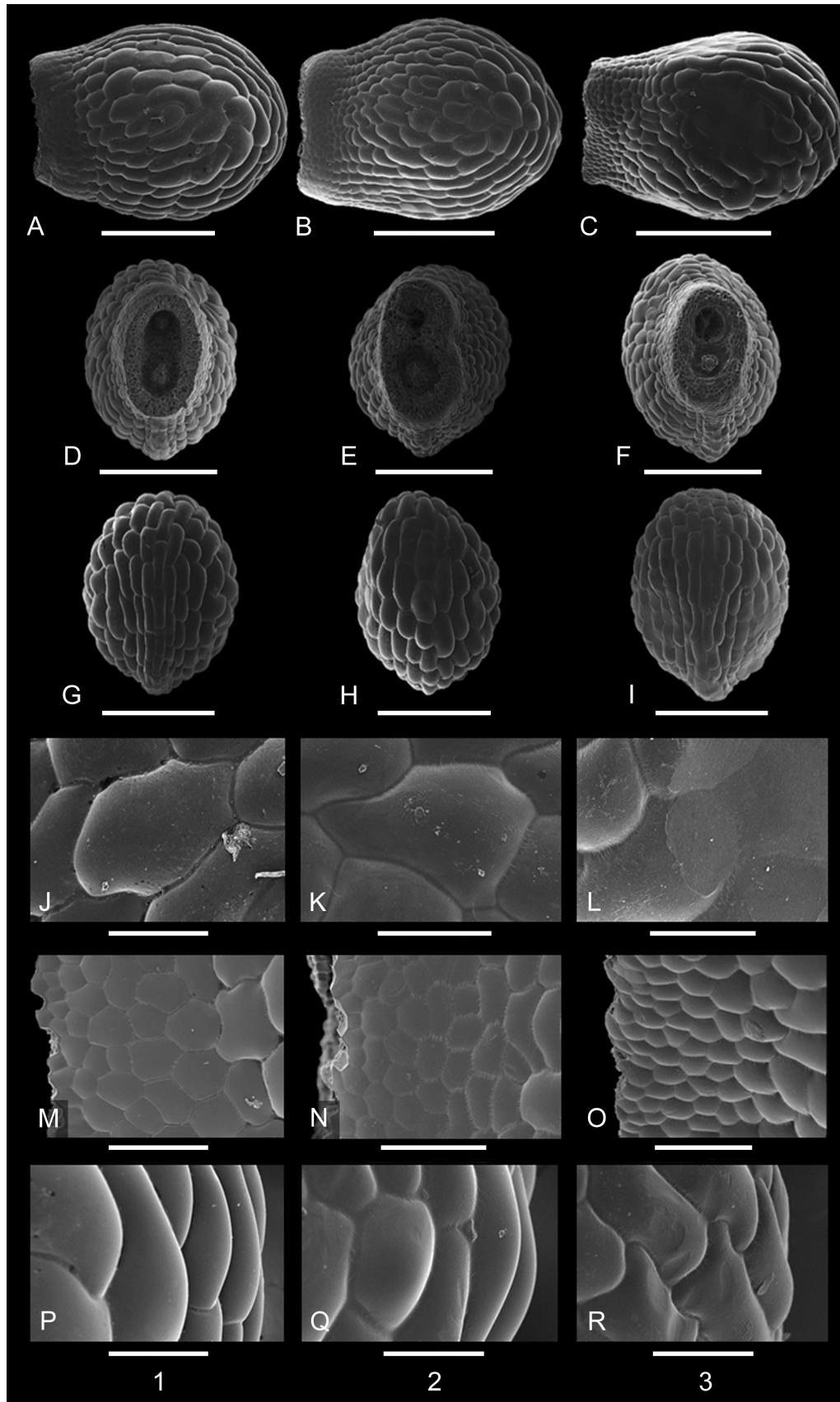


Fig. 4. Seeds of *Melocactus* in Cuba, scanning electron microscopy in lateral view (A–C), frontal view (D–F) and apical view (G–I), and cells of lateral region (J–L), hilum-micropylar border region (M–O) and apical region (P–R). – Columns 1–3 correspond to taxa: *M. acunae* subsp. *acunae* (Punta de Maisí) (1); *M. harlowii* (2); *M. borhidii* (3). – Scale bars: A–I = 500 μ m; J–R = 100 μ m.

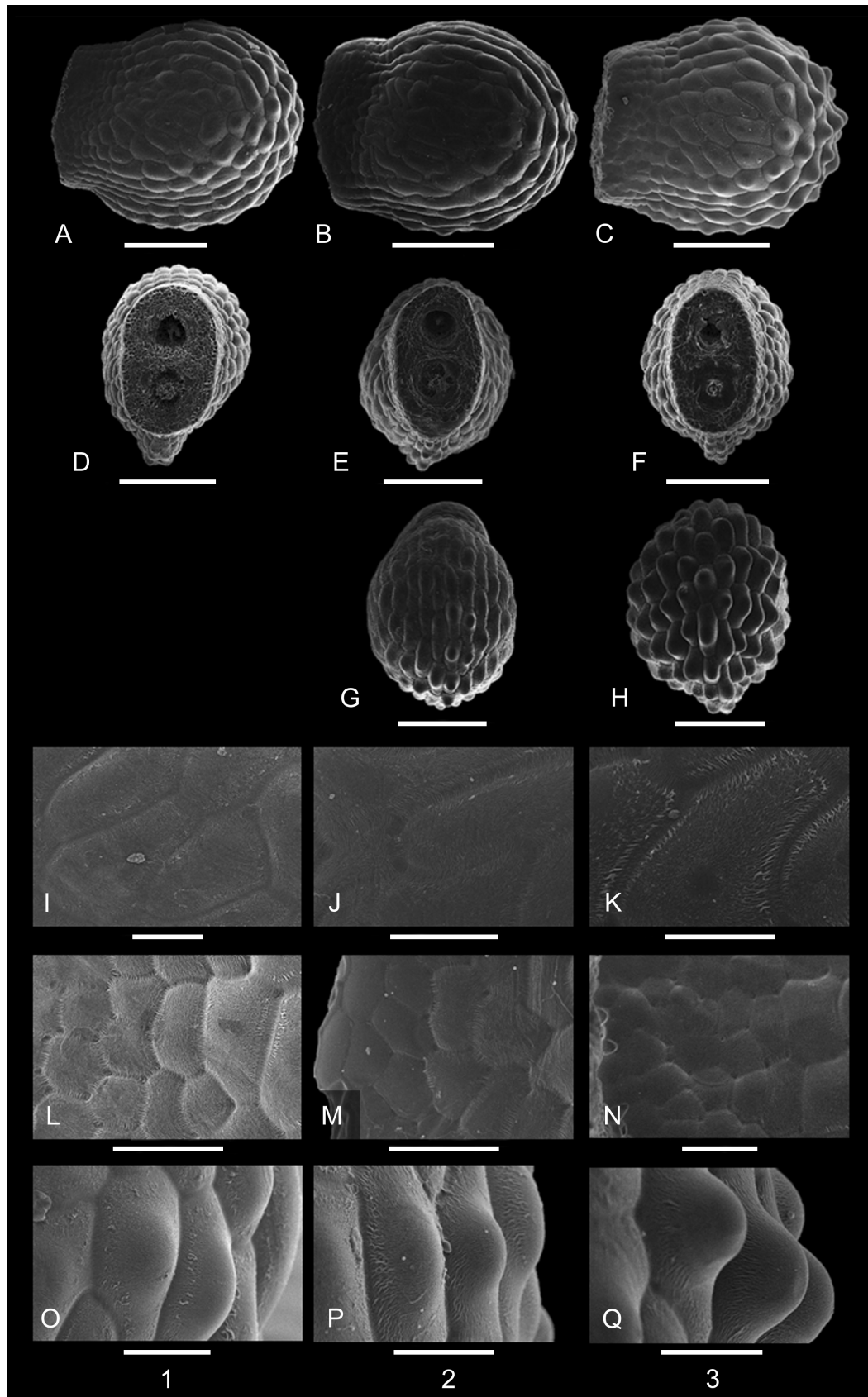


Fig. 5. Seeds of *Melocactus* in Cuba, scanning electron microscopy in lateral view (A–C), frontal view (D–F) and apical view (G–H), and cells of lateral region (I–K), hilum-micropylar border region (L–N) and apical region (O–Q). – Columns 1–3 correspond to taxa (or localities): *M. actinacanthus* (Sierra Alta de Agabama) (1); 2: *M. actinacanthus* (La Coca) (2); 3: *M. matanzanus* (3). – Scale bars: A–H = 500 μ m; I–Q = 100 μ m.

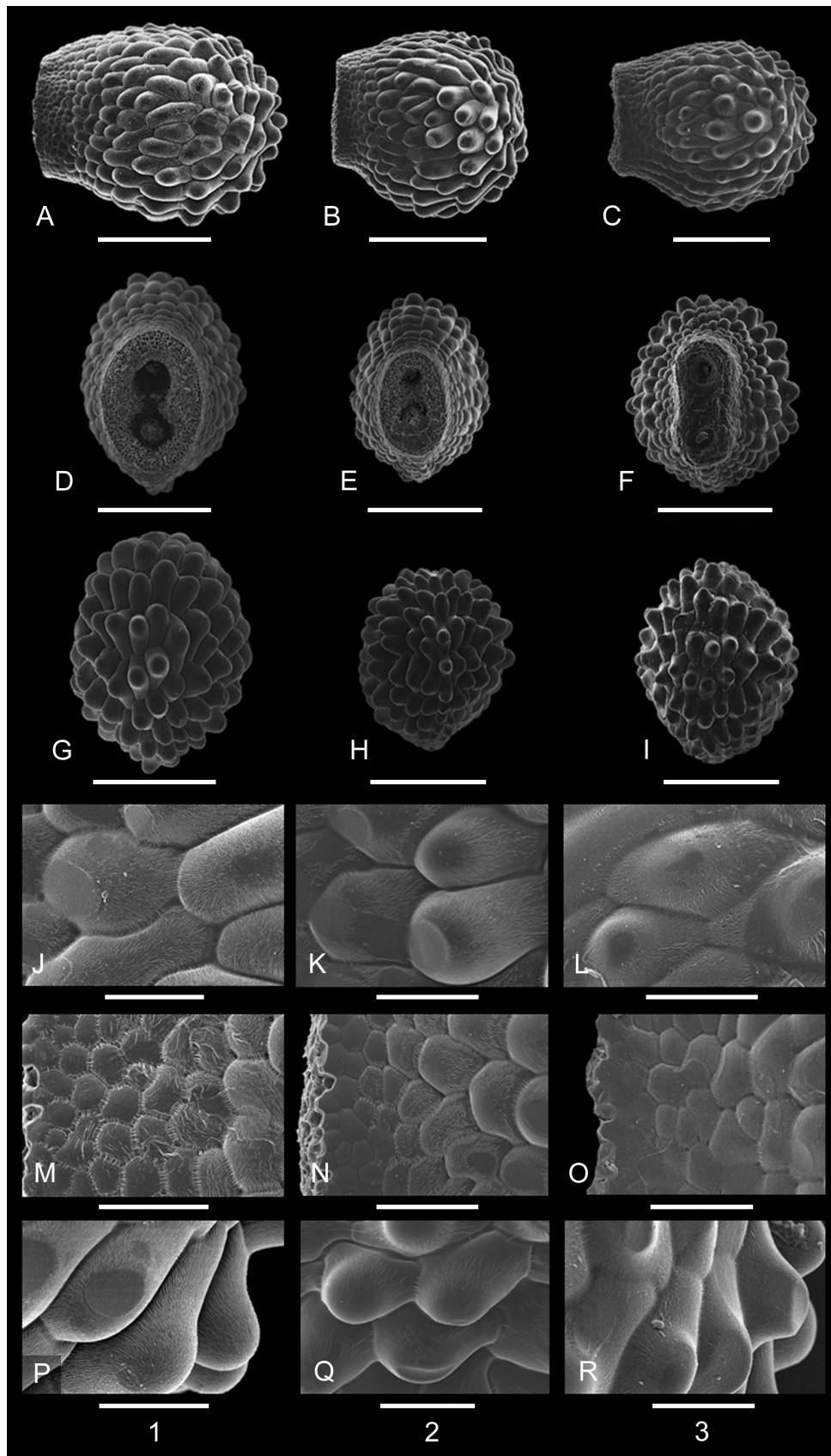


Fig. 6. Seeds of *Melocactus* in Cuba, scanning electron microscopy in lateral view (A–C), frontal view (D–F) and apical view (G–I), and cells of lateral region (J–L), hilum-micropylar border region (M–O) and apical region (P–R). – Columns 1–3 correspond to taxa: *M. acunae* subsp. *acunae* (Cajobabo) (1); *M. acunae* subsp. *lagunaensis* (2); *M. nagi* (3). – Scale bars: A–I = 500 μ m; J–R = 100 μ m.

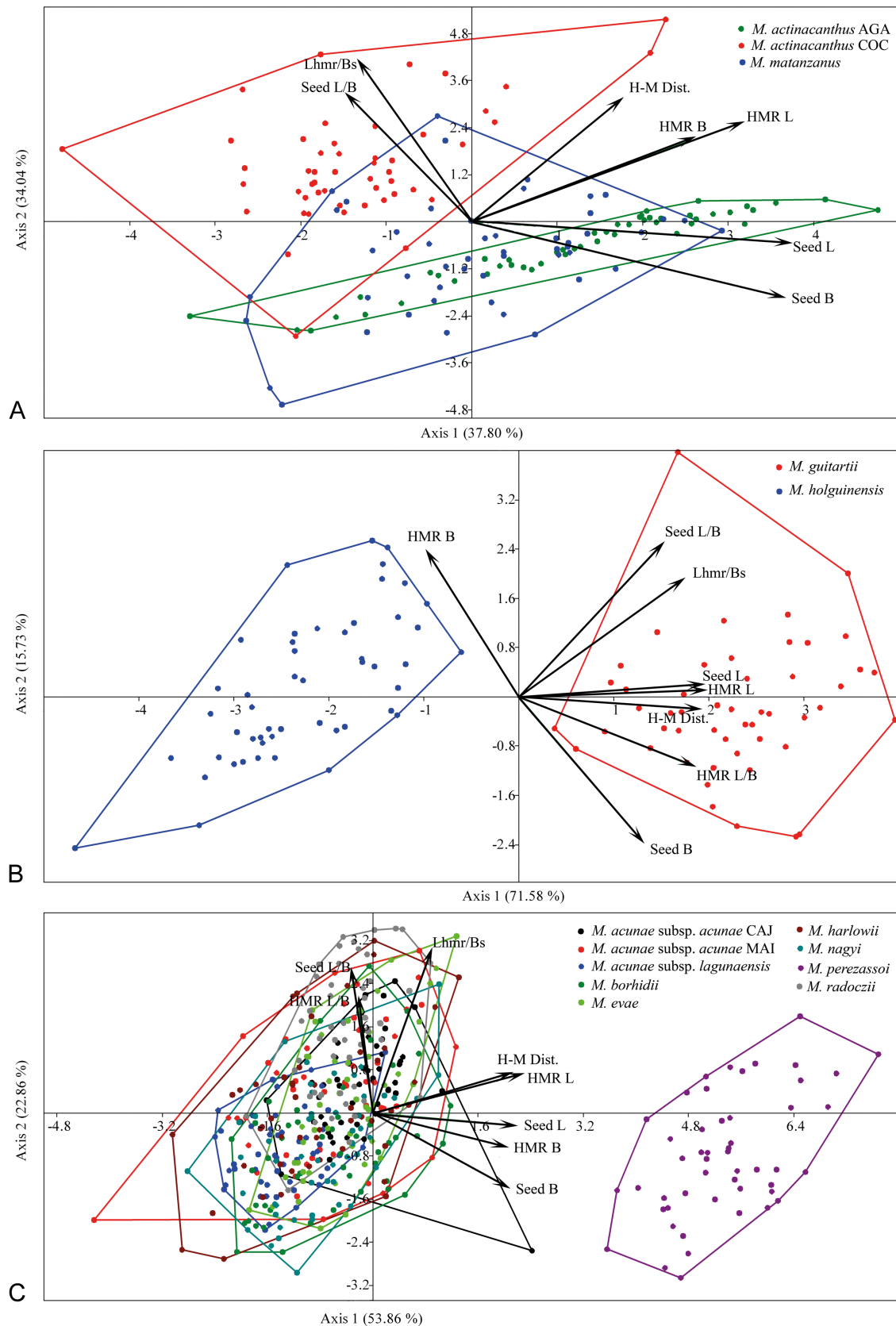


Fig. 7. Principal component analysis (PCA) based on seed dimensions of *Melocactus matanzanus* group (A), *M. curvispinus* group (B) and *M. harlowii* group (C). – Convex hull is smallest convex polygon containing all points of each taxon or locality. – Seed L = seed length; Seed B = seed breadth; Seed L/B = seed length/breadth ratio; HMR L = hilum-micropylar region length; HMR B = hilum-micropylar region breadth; H-M Dist. = hilum-micropyle distance; HMR L/B = HMR length/breadth ratio; Lhmr/Bs = HMR length/seed breadth ratio; localities: AGA = Sierra Alta de Agabama; CAJ = Cajobabo; COC = La Coca; MAI = Punta de Maisí.

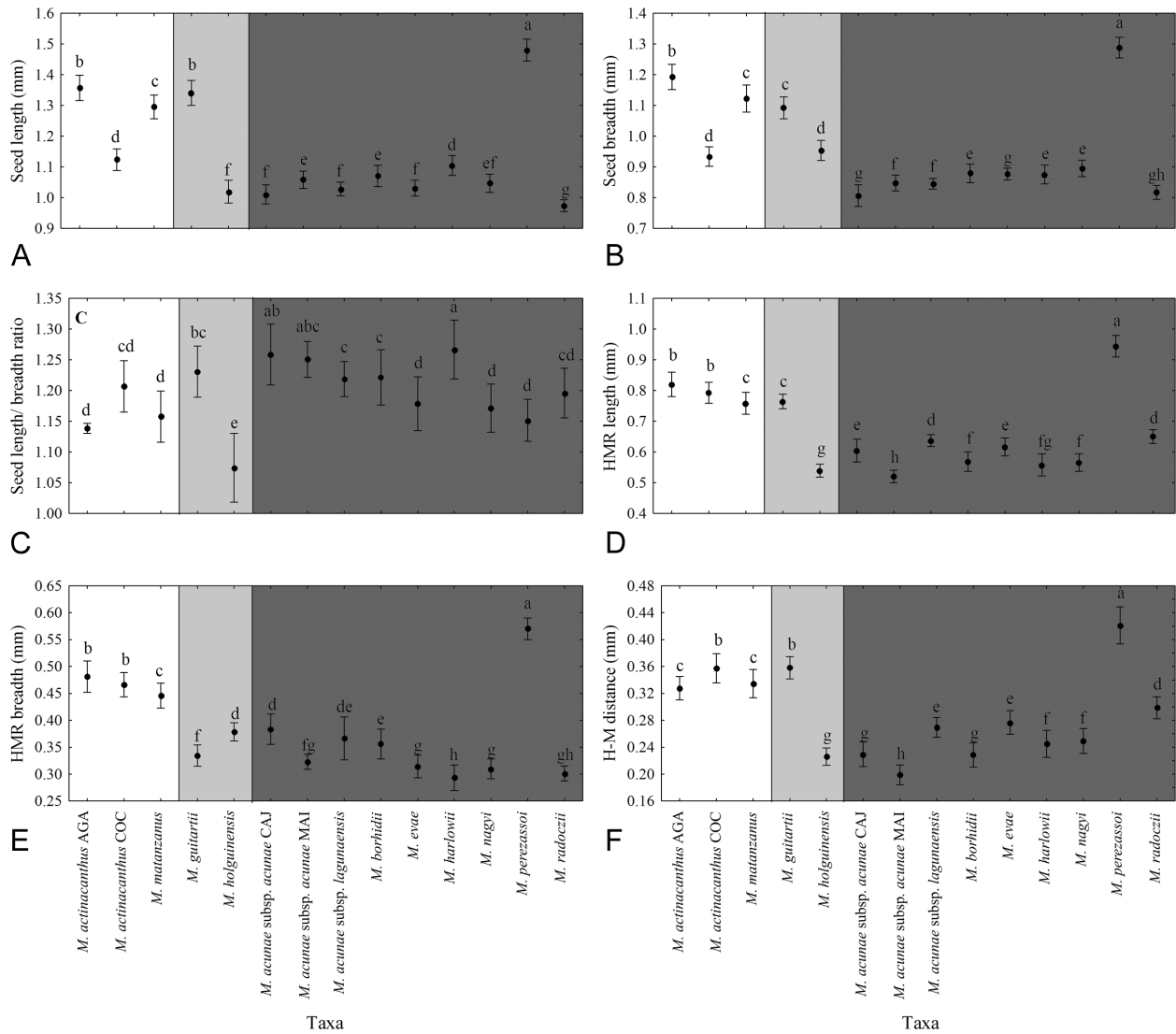


Fig. 8. Variation of length (A) and breadth (B) seeds, seed length/breadth ratio (C), length (D) and breadth (E) of HMR and hilum-micropyle distance (F) of *Melocactus* seeds in Cuba. – Black circle (●) indicates mean; lines extending above and below mean indicate standard deviation. Different letters represent statistically significant differences among taxa (or localities), according to non-parametric multivariate analyses of variances and pairwise comparisons for $p < 0.05$. – HMR = hilum-micropylar region; localities: AGA = Sierra Alta de Agabama; CAJ = Cajobabo; COC = La Coca; MAI = Punta de Maisí. – Shading refers to three groups: *M. matanzanus*, *M. curvispinus* and *M. harlowii* in this order.

Seed morphological similarity

The basic data matrix (from quantitative and qualitative characters) was formed by 20 characters (Table 5). The clustering pattern showed at the 2.1 level, two large, well-defined groups (Fig. 10). The separation at this level is given by keel extension. Species of the C group are separated from 1.9, because their seeds differ in lustre, hilum-micropylar region shape, testa border expansion and all quantitative variables. Also, from 1.9, the H3, H4 and H5 groups differ from the M, H1 and H2 groups by lustre of seeds.

At a cut-off of 1.5, *Melocactus perezassoi* is separated from the H4 and H5 groups (Fig. 10) by seed relief, testa border expansion and seed dimensions. *Melocactus evae* (H4 group) is differentiated from the H5 group at a distance of 1.45 by testa border expansion, microrelief,

seed length/breadth ratio and HMR length/seed breadth ratio. On the other hand, *M. radoczii* (H2 group) is separated from the group formed by H1 and M at a distance of 1.3 by testa border expansion, seed length and HMR length/breadth ratio. At a distance of 1.1, H1 differs from the M group by seed relief, seed and HMR length, hilum-micropyle distance, HMR length/seed breadth ratio and dry mass of seed.

There were three subgroups with high levels of seed morphological similarity, in which the Euclidean distance is less than 0.5. In the case of the H5 group, the taxa with the highest similarity are *Melocactus acunae* subsp. *acunae* (Punta de Maisí) and *M. borhidii*, which differ by a cut-off of 0.3 for seed length and its seed length/breadth ratio. Taxa of the M group are similar to each other—only

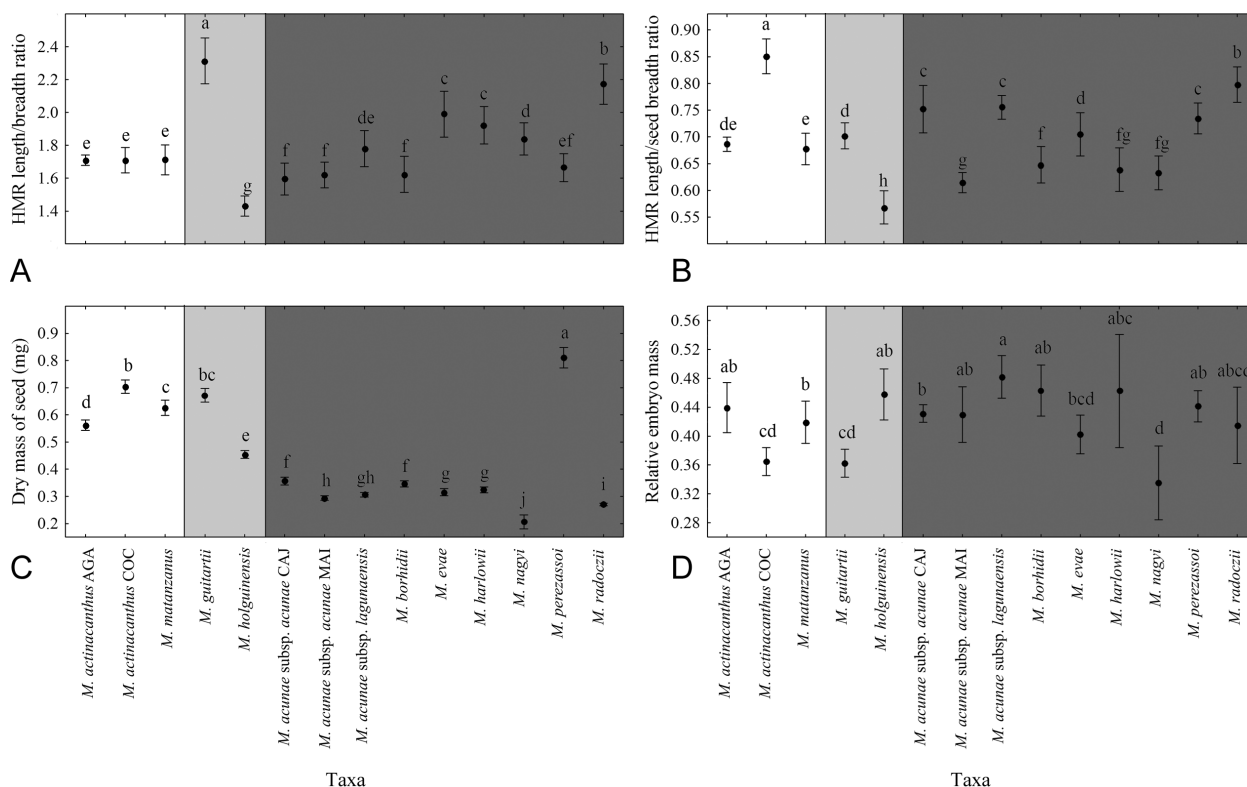


Fig. 9. Variation of HMR length/breadth ratio (A), HMR length/seed breadth ratio (B), dry mass (C) and relative embryo mass (D) of *Melocactus* seeds in Cuba. – Black circle (●) indicates mean; lines extending above and below mean indicate standard deviation. Different letters represent statistically significant differences among taxa (or localities), according to non-parametric multivariate analyses of variances and pairwise comparisons for $p < 0.05$. – HMR = hilum-micropylar region; localities: AGA = Sierra Alta de Agabama; CAJ = Cajobabo; COC = La Coca; MAI = Punta de Maisí. – Shading refers to three groups: *M. matanzanus*, *M. curvispinus* and *M. harlowii* in this order.

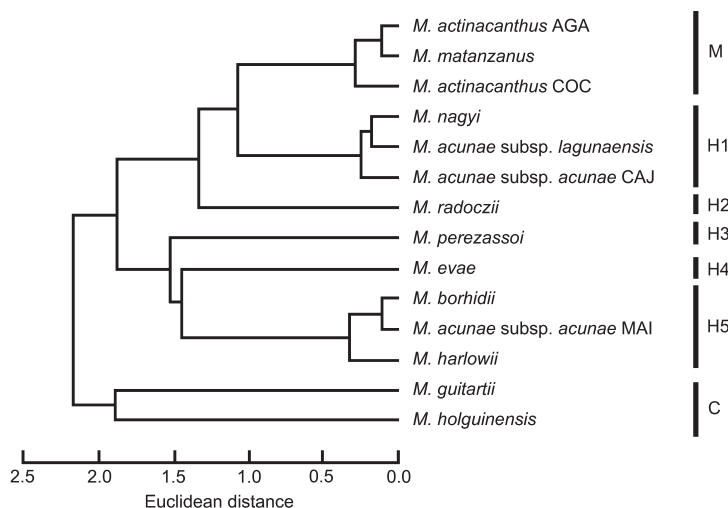


Fig. 10. Position of 12 taxa (from 14 localities) of *Melocactus* in Cuba, based on Euclidean distance matrix through unweighted pair-group average. – M = *M. matanzanus* group; H = *M. harlowii* group; H1–H5 = *M. harlowii* group sub-groups; C = *M. curvispinus* group; localities: AGA = Sierra Alta de Agabama; CAJ = Cajobabo; COC = La Coca; MAI = Punta de Maisí.

M. actinacanthus (La Coca) differs by a cut-off of 0.25 due to seed length, hilum-micropylar region length/seed breadth ratio and relative embryo mass. In the H1 group, *M. acunae* subsp. *lagunaensis* and *M. nagyi* (Fig. 10) differ by a distance of 0.25 due to HMR length/breadth ratio.

Discussion

Our results showed several seed characters with constant differences among localities and taxa (Table 5). *Melocactus* seeds in Cuba do not exceed 1.7 mm in length, a value lower than the maximum referred to this genus (2 mm, for *M. estevesii* P. J. Braun from Brazil) by Taylor (1991). Such dimensions placed the seeds of *Melocactus* among the smallest of the *Cactoideae*, together with other genera such as *Mammillaria* Haw., *Escobaria* Britton & Rose, *Pilosocereus* Byles & G. D. Rowley and *Rhipsalis* Gaertn., although with seeds slightly larger than *Aztekium* Boed., *Blossfeldia* Werderm., *Parodia* Speg. and *Strombocactus* Britton & Rose, which have the smallest seeds in the *Cactaceae* (Barthlott & Hunt 2000; Lodé 2015). It has been suggested that the decrease in seed length is

associated with greater water absorption capacity due to its greater area/mass ratio (Kikuzawa & Koyama 1999).

The HMR characters were evaluated for the first time for *Melocactus* in this study. However, in the monograph of Taylor (1991), micrographs are shown to

Table 5. Matrix of characters and codified states for cluster analysis of 12 taxa from 14 localities of *Melocactus* in Cuba. – AGA = *M. actinacanthus* (Sierra Alta de Agabama); COC = *M. actinacanthus* (La Coca); MAT = *M. matanzanus*; GUI = *M. guitartii*; HOL = *M. holguinensis*; CAJ = *M. acunae* subsp. *acunae* (Cajobabo); MAI = *M. acunae* subsp. *acunae* (Punta de Maisí); LAG = *M. acunae* subsp. *lagunaensis*; BOR = *M. borhidii*; EVA = *M. evae*; HAR = *M. harlowii*; NAG = *M. nagyi*; PER = *M. perezassoi*; RAD = *M. radoczii*. – Characters: Lu = lustre; Ke = keel extension; Sr = seed relief; Hs = hilum-micropylar region shape; Be = testa border expansion; M = microrelief; HMR = hilum-micropylar region; H-M Dist. = hilum-micropyle distance; Seed L/B = seed length/breadth ratio; HMR L/B = HMR length/breadth ratio; Lhmr/Bs = HMR length/seed breadth ratio; Emb. mass = relative embryo mass / 5. – We show mean and standard deviation (SD) for quantitative characters.

Taxon	Lu	Ke	Sr	Hs	Be	M	Seed length (mm)		HMR length (mm)		H-M Dist. (mm)	
							mean	SD	mean	SD	mean	SD
AGA	1	1	2	1	2	2	1.356	0.082	0.819	0.079	0.327	0.034
COC	1	1	2	1	2	2	1.123	0.070	0.792	0.068	0.357	0.044
MAT	1	1	2	1	2	2	1.294	0.077	0.758	0.071	0.334	0.042
GUI	1	2	1	2	2	2	1.340	0.082	0.764	0.047	0.358	0.033
HOL	2	2	1	1	1	2	1.019	0.075	0.944	0.070	0.421	0.055
CAJ	1	1	3	1	2	2	1.010	0.063	0.605	0.075	0.229	0.037
MAI	2	1	1	1	2	2	1.058	0.057	0.520	0.040	0.199	0.030
LAG	1	1	3	1	2	2	1.028	0.045	0.637	0.039	0.269	0.030
BOR	2	1	1	1	2	2	1.070	0.070	0.568	0.063	0.228	0.036
EVA	2	1	1	1	3	1	1.031	0.051	0.616	0.059	0.276	0.035
HAR	2	1	1	1	2	2	1.105	0.064	0.558	0.073	0.245	0.040
NAG	1	1	3	1	2	2	1.046	0.059	0.565	0.057	0.249	0.037
PER	2	1	2	1	3	2	1.480	0.072	0.539	0.043	0.226	0.026
RAD	1	1	2	1	3	2	0.974	0.039	0.650	0.044	0.298	0.032

Taxon	Seed L/B		HMR L/B		Lhmr/Bs		Emb. mass	
	mean	SD	mean	SD	mean	SD	mean	SD
AGA	1.142	0.100	1.716	0.180	0.687	0.066	0.083	0.012
COC	1.207	0.084	1.709	0.153	0.849	0.073	0.072	0.008
MAT	1.158	0.083	1.711	0.182	0.675	0.063	0.083	0.012
GUI	1.231	0.084	2.313	0.278	0.699	0.043	0.072	0.008
HOL	1.075	0.112	1.664	0.171	0.565	0.045	0.091	0.014
CAJ	1.259	0.099	1.594	0.193	0.749	0.093	0.086	0.005
MAI	1.251	0.058	1.619	0.157	0.613	0.047	0.085	0.015
LAG	1.219	0.057	1.779	0.219	0.754	0.046	0.096	0.012
BOR	1.221	0.090	1.622	0.220	0.646	0.072	0.092	0.014
EVA	1.178	0.088	1.989	0.277	0.702	0.067	0.080	0.011
HAR	1.267	0.095	1.922	0.228	0.637	0.083	0.092	0.031
NAG	1.171	0.079	1.838	0.196	0.631	0.064	0.067	0.020
PER	1.151	0.068	1.430	0.123	0.733	0.054	0.088	0.009
RAD	1.196	0.081	2.172	0.245	0.795	0.053	0.082	0.021

allow for the evaluation of some qualitative characters of the HMR. Hilum-micropylar region shape was identical in *Melocactus* in Cuba, except in *M. guitartii*. The peculiar keyhole-type shape present in this species is only observed in this genus for *M. curvispinus* (Taylor 1991). However, in other genera of *Cactoideae*, such as *Stenocereus* (Arroyo-Cosultchi & al. 2006), this form is more common.

The dry mass of seeds and relative embryo mass showed significant differences among some localities

and taxa (Fig. 9C, D). However, dry mass is an unused character for species delimitation and is generally evaluated in germination and dormancy studies (Sánchez & al. 2002; Luzuriaga & al. 2006), although its value is known in the evolution of angiosperms (Igea & al. 2017).

The seeds of *Melocactus* in Cuba possess a higher mass percentage to testa (Fig. 9C, D), which provides mechanical strength, might act as a mechanism for delaying germination (Daws & al. 2008) and gives protection to the seed. According to studies of Janzen (1969), Crawley

(1992) and Dalling & al. (2011), a greater investment in seed protection reduces the probability of predation and fungal attack to access reserves and the seed embryo. In *Cactoideae*, the role of the seed mass has not been clarified in germination, but it is known to affect the growth, survival and seedling shape (Sosa & al. 2014). The seeds with less mass have smaller embryos and a lower reserve of nutrients, which therefore results in smaller seedlings with lower tolerance to environmental stresses (Sosa & al. 2014).

Lustre showed discontinuity among evaluated localities, and its two states were stable in the taxa analysed. This character has been one of the least used by previous authors in other genera (see Arroyo-Cosultchi & al. 2006; Becerra & al. 2010; Parveen & al. 2015). In *Melocactus* it has been employed only by Barthlott & Hunt (2000) to describe *M. macracanthos* and by Guiggi (2010) when providing expanded descriptions for three species recognized for Cuba. According to Taylor (1991), the morphology of testa cells has been very useful in species group delimitation.

The ventral region of Cuban *Melocactus* seeds are differentiated from the lateral region, as noted in the micrographs presented by Taylor (1991) and in the study of Barthlott & Hunt (2000). However, analysis of this region of seeds in *Melocactus* had not been previously realized. The ventral region differentiation consists of the presence of a keel, the cells of which are minor compared to lateral cells, and its extension is variable. In all populations of *Melocactus* studied, there were seeds with a keel—this structure has been described for other genera that also belong to the tribe *Cereeae*, such as *Coleocephalocereus* Backeb. and *Stephanocereus* A. Berger (Barthlott & Hunt 2000). In *Melocactus*, seeds with ventral keel predominate, like in *Neobuxbaumia* (Arroyo-Cosultchi & al. 2007), and contrary to *Pilosocereus* (Franco-Estrada & al. 2014; Barrios pers. obs.) and *Stenocereus* (Arroyo-Cosultchi & al. 2006), where a dorsal keel (crest) predominates. The keel, being an external structure that occupies the ventral region, covers the cotyledons and probably could be related to dispersal by water currents, but its function has not been clarified.

Microrelief of the lateral region of *Melocactus* seeds in Cuba is one of the less variable characters; however, it allows differentiating *M. evae* from the rest of the taxa studied. Striate microrelief is a character state present in several genera of *Cereeae* (Barthlott & Hunt 2000) and is the most common state in *Melocactus*, with only six taxa with no microrelief reported by Taylor (1991).

Testa border varies between constricted, not expanded or expanded, and is related to HMR length/seed breadth ratio (Fig. 9B). Similarly, Arias & Terrazas (2004) found variation for this character in *Pachycereus*, unlike other genera such as *Neobuxbaumia* (Arroyo-Cosultchi & al. 2007) and *Stenocereus* (Arroyo-Cosultchi & al. 2006), in which states of this character remain constant. Of the populations evaluated in this study, only

Melocactus holguinensis has a constricted border. This character state is present in a few genera of *Cactoideae*, such as *Aztekium* Boed., *Leptocereus* (A. Berger) Britton & Rose, *Mammillaria*, *Neolloydia* Britton & Rose, *Obregonia* Fric ex A. Berger, *Ortegocactus* Alexander, *Peniocereus* (A. Berger) Britton & Rose, *Thelocactus* (K. Schum.) Britton & Rose and *Turbinicarpus* (Backeb.) Buxb. & Backeb., but not in all species (Barthlott & Hunt 2000).

Taxonomic implications of seed variability in Cuban *Melocactus*

Taylor (1991) argued that there is limited variation in seed size and morphology within the *Melocactus* taxa that he recognized for Central and South America. Our results allow us to recognize that several characters of the seed have discontinuous variation. Based on the taxonomic value of these characters, we argue for their importance in recognizing the species in *Melocactus* groups native to Cuba.

Melocactus matanzanus group

The three populations of the *Melocactus matanzanus* group in Cuba have seeds of similar morphology. The seed similarity in this case agrees with the taxonomic proposal of Taylor (1991), and followed by Hunt & al. (2006), Acevedo-Rodríguez & Strong (2012), Hunt (2016) and Greuter & Rankin (2017), who consider *M. actinacanthus* as synonymous with *M. matanzanus*. Vegetative, carpological and floral characters that sustain the similarity between these taxa are simple stems from 8 to 9 ribs, with flowers less than 2 cm and pink fruits (León 1934; Areces-Mallea 1976a). Guiggi (2010) recognized one species with two subspecies in this group. The main differences between *M. actinacanthus* and *M. matanzanus* according to Areces-Mallea (1976a) and Guiggi (2010) are diameter and height of the plant without the cephalium, cephalium diameter, number of seeds per fruit, flower length, radial spine number and the presence of a central spine only in *M. matanzanus*.

Seeds of two populations of *Melocactus actinacanthus* showed differences among seed dimensions (Fig. 7–9). However, point distributions of seed dimensions of *M. matanzanus* overlapped with both populations of *M. actinacanthus*. With respect to plant morphology, *M. actinacanthus* (La Coca) has intermediate characteristics between *M. matanzanus* and *M. actinacanthus* (Sierra Alta de Agabama) (Hernández & al. 2014). In addition, the common mentioned presence of a central spine in the individuals of *M. matanzanus* in its *locus classicus* is not a constant character (Barrios pers. obs.), because individuals without a central spine can be found. According to our results, we consider that there is insufficient evidence to separate the two entities into different species or sub-

species. Based on our seed morphological comparisons, we consider *M. actinacanthus* as synonymous with *M. matanzanus*.

Melocactus matanzanus seeds are similar to those of the *M. violaceus* group formed by *M. concinnus* Buining & Brederoo, *M. neryi* K. Schum., *M. salvadorensis* Werdermann and *M. violaceus* Pfeiff., as shown in the micrographs presented by Taylor (1991). Keel extension, testa border expansion, seed relief, microrelief and HMR shape are among the similar seed features of these species. Regarding seed length and breadth, there is overlap among intervals of *M. matanzanus* and similar species of the *M. violaceus* group. On the other hand, there are vegetative, carpological and floral characters that lend support for seed similarity among these species. The common characters among *M. matanzanus* and the related species of the *M. violaceus* group are the number of ribs, which varies to around 8 (León 1934; Taylor 1991), the same number of radial spines (León 1934; Taylor 1991) and cephalium length, which varies between 4–5 cm in *M. matanzanus* (León 1934), *M. cocinnus*, *M. neryi* and *M. violaceus*; however, this differs in *M. salvadorensis*, in which it exceeds 10 cm and can reach 15 cm (Taylor 1991). According to Taylor (1991), some species of the *M. violaceus* group crossed the Amazon and colonized part of northeastern South America and Cuba. Disjunct distributions among *M. matanzanus* and the *M. violaceus* group have been considered by Taylor (1991) as an example of long-distance dispersal, and birds are known to disperse the seeds of *Melocactus* (Casado & Soriano 2010).

Melocactus curvispinus group

Melocactus guitartii and *M. holguinensis* share only the presence of a ventro-apical keel in the seeds. Therefore, the seed characters sustain the recognition of two species, which agrees with Mészáros (1976), Areces-Mallea (1993), Rodríguez (2005) and González-Torres & al. (2016). When comparing the results of this study with the micrographs presented by Taylor (1991), seeds of *M. guitartii* are similar to those of *M. curvispinus* subsp. *curvispinus*, but not like those of *M. holguinensis*. Hilum-micropylar region shape, keel extension, seed relief, testa border expansion and seed length show a great similarity between the *M. curvispinus* and *M. guitartii* seeds referred to by Taylor (1991).

On the other hand, seed characters concur with the morphological similarities of stems, fruits and flowers found between *Melocactus curvispinus* subsp. *curvispinus* and *M. guitartii*. Therefore, this study supports the criterion of Taylor (1991), who considers that *M. guitartii* is synonymous with *M. curvispinus*. On the other hand, the seed morphology of *M. holguinensis* does not correspond to that of *M. curvispinus* subsp. *curvispinus* or *M. guitartii*. Taylor (1991) suggested that seeds of this group are characterized by their great length, which

is not the case in *M. holguinensis*. Therefore, in the *M. curvispinus* group, two taxa are recognized in Cuba, *M. curvispinus* subsp. *curvispinus* (= *M. guitartii*) and *M. holguinensis*.

Melocactus harlowii group

Morphology of seeds in the *Melocactus harlowii* group allows differentiation of three of its taxa, *M. evae*, *M. perezassoi* and *M. radoczii*. In the remaining localities, according to seed morphology, two groups are distinguished (H1 and H5; Fig. 10). The first group (H1) is formed by *M. acunae* subsp. *acunae* (Cajobabo), *M. acunae* subsp. *lagunaensis* and *M. nagyi*. The second group (H5) is formed by *M. acunae* subsp. *acunae* (Punta de Maisí), *M. borhidii* and *M. harlowii*. The definition of the taxonomic identity of the taxa formed in these two groups will need subsequent studies that integrate other aspects of the biology of these species, such as floral, carpological, karyological morphology and phylogenetics. Although Taylor (1991), Hunt & al. (2006), Acevedo-Rodríguez & Strong (2012), Hunt (2016) and Greuter & Rankin (2017) considered that the *M. harlowii* group represents a single species, our results show that within this group seed morphology is useful to recognize more than a single species.

Melocactus evae, *M. perezassoi* and *M. radoczii* differ from each other by seed relief, microrelief and seed dimensions. The recognition of these three taxa does not correspond to the delimitation proposed by Guiggi (2010), because he suggested *M. perezassoi* as a subspecies of *M. harlowii*, and *M. evae* and *M. radoczii* as synonymous with *M. harlowii* subsp. *harlowii*. According to Areces-Mallea (1993), the population of *M. perezassoi* seems to have been left as a remnant of an original population on coastal cliffs, after the regression of the sea. Seeds of *M. evae* and *M. radoczii* are more similar to *M. perezassoi* than to *M. harlowii*, due to their expanded testa border. The reasons why *M. perezassoi* can be recognized as an independent species within the *M. harlowii* group are geographic isolation and marked differences in terms of vegetative, carpological and floral characters (Areces-Mallea 1993), as well as the seed morphology analysed here. This species presents more separated areoles with a greater number of spines that are less robust, longer flowers with stigmatic lobes not exerted above the stamens, and long, clavate fruits (Areces-Mallea 1993). On the other hand, characters such as size and time of flower opening, as well as the habit of forming very tight clusters, distinguish *M. evae* from the rest of the *M. harlowii* group. *Melocactus radoczii*, despite possessing similar characteristics to *M. harlowii*, is among the species with the greatest number of spines per areole (up to 22 spines) (Barrios pers. obs.).

The H1 group formed by *Melocactus acunae* subsp. *acunae* (Cajobabo), *M. acunae* subsp. *lagunaensis* and *M. nagyi* is characterized by tuberculate seeds. The seeds

with tuberculate relief are present only in this group, which allows differentiating these populations from the rest of *M. harlowii* group. According to seed morphology, taxa belonging in this group show a marked difference with *M. harlowii* s.s., which contrasts with the taxonomic proposals of Taylor (1991), Areces-Mallea (1993), Rodríguez (2005), Hunt & al. (2006), Guigii (2010), Hunt (2016) and Greuter & Rankin (2017), which consider these taxa as synonymous with *M. harlowii*. Seeds that Taylor (1991) considered as *M. harlowii* are from a locality that is part of the distribution of *M. acunae* subsp. *acunae* (Mészáros 1976; Howard 1988); therefore, seed morphology of *M. harlowii* referred to by Taylor (1991) coincides with *M. acunae* within the *M. harlowii* group. Another species putatively related to *M. acunae* is *M. lemairei* (Monv. ex Lem.) Miq. ex Lem. from Hispaniola (Taylor 1991). *Melocactus lemairei* has seeds similar to those of *M. acunae* subsp. *acunae* (Cajobabo) and *M. acunae* subsp. *lagunaensis*, due to its tuberculate appearance, ventral keel, oval hilum-micropylar region and non-expanded border.

The H5 group formed by *Melocactus acunae* subsp. *acunae* (Punta de Maisí), *M. borhidii* and *M. harlowii* has in common lustrous seeds with flat relief, an unexpanded testa border and oval hilum-micropylar region. The seed morphology of these three taxa favours considering them as a single species, which does not correspond to the criterion of Mészáros (1976), which considered them as three species. However, other authors have included *M. borhidii* under the synonymy of *M. harlowii* (see Taylor 1991; Areces-Mallea 1993; Rodríguez 2005; Hunt & al. 2006; Guigii 2010; Hunt 2016; Greuter & Rankin 2017). The seeds in this group are similar to those of *M. intortus* (Mill.) Urb., because they have flat relief, striate microrelief, an unexpanded testa border and oval HMR. However, they differ by keel extension; due to the seeds of *M. intortus* having a ventro-apical keel (Taylor 1991).

One peculiarity in this study is the existence of different seed patterns for two evaluated localities of *Melocactus acunae* subsp. *acunae* (Fig. 10) according to seed dimensions (not distinguishable in PCA; Fig. 7C), which places them in different groups (H1 and H5). This result could be explained by the presence of inter-locality seed variation in *M. acunae* subsp. *acunae*, and this variation has been widely documented in plants (see Imbert & al. 1997; Zhang 1998; Tieu & al. 2001; Sánchez & al. 2002; Pérez-García & al. 2003), even in *Cactaceae* (see Taylor 1991; Cota-Sánchez & Bomfim-Patricio 2010; García-Beltrán & al. 2017). Morphological discontinuities are the basis of the phenetic species concept (Judd 1981; 2007), which allows the identification of populations with discontinuous morphological patterns as different species. In taxa such as *M. acunae* subsp. *acunae*, in which we observed inter-locality variation, it is not possible to apply such a concept, and the evaluation of other characters that complement this study will be necessary.

Based on our results, we propose the hypothesis that the distribution of *Melocactus* in eastern Cuba seems to be in response to several speciation processes. Parapatric speciation could be occurring in seven of the localities evaluated, because the localities are close to each other (Fig. 1), while allopatric speciation processes could have been responsible for the differentiation between *M. nagyii* and *M. perezassoi* (i.e. the rest of the *M. harlowii* group). An essential element in speciation in this group is dispersal and the fact that the fruit are readily consumed, and seeds could be easily dispersed. *Melocactus* seeds are characterized by being small to medium-sized and according to Matilla & al. (2005), dispersal probability increases in smaller seeds. In this sense, as hypothesized by Borhidi (1996) for succulents in the Cuban flora, it is possible that the ancestral populations of the *M. harlowii* group in eastern Cuba dispersed their seeds over long distances into different habitats, where geographic isolation caused by changes in vegetation distribution during the Pleistocene caused by climatic fluctuations favoured differences among localities through various speciation processes. In the future, an extensive phylogeographic study is required, to explain the historical processes responsible for the current geographic distributions, the possible gene flow between populations, but also as a comprehensive evaluation of the current circumscription of species.

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Appendix 1. Label data of herbarium specimens.

Melocactus actinacanthus, Cuba, La Habana, Campo Florido, loma al este de la cortina de la presa La Coca, 23°05.598'N, 82°07.040'W, 14 Jul 2011, *D. Barrios & L. R. González-Torres HFC 88802* (HAJB).

Melocactus acunae subsp. *acunae*, Cuba, Guantánamo, Imías, elevaciones que rodean la Playita de Cajobabo, 20°04.145'N, 74°29.020'W, 28 Jan 2018, *L. C. Majure, D. Barrios & E. Díaz 7027* (HAJB).

Melocactus acunae subsp. *acunae*, Cuba, Guantánamo, Maisí, a 200 metros al suroeste del faro, 20°14.560'N, 74°08.601'W, 17 m, 28 Jan 2018, *L. C. Majure, D. Barrios & E. Díaz 7025* (HAJB).

Melocactus acunae subsp. *lagunaensis*, Cuba, Guantánamo, Imías, escarpes del Viaducto la Farola, entre Veguita y Vega Grande, 20°06.266'N, 74°29.338'W, 28 Jan 2018, *L. C. Majure, D. Barrios & E. Díaz 7021* (HAJB).

Melocactus borhidii, Cuba, Guantánamo, San Antonio del Sur, Tortuguilla, 19°58.535'N, 74°56.297'W, 35 m, 20 Jun 2016, *D. Barrios, S. Arias & J. A. Acuña HFC 88766* (HAJB).

Melocactus evae, Cuba, Guantánamo, Hatibonico, Los Monitongos, justo al oeste de la Bahía de Guantánamo, 19°56.429'N, 75°18.885'W, 30 Jan 2018, *L. C. Majure, D. Barrios & E. Díaz 7028* (HAJB).

Melocactus gutartii, Cuba, Sancti Spíritus, Peñon de Dagamal, cercanía de la Reserva Florística Manejada Lebrije, 22°03'10"N, 79°08'00"W, 2 Feb 2018, *L. C. Majure, D. Barrios & E. Díaz 7039* (HAJB).

Melocactus harlowii, Cuba, Guantánamo, Imías, primera terraza de Macambo, 20°02.876'N, 74°44.016'W, 24 m, 27 Jan 2018, *L. C. Majure, D. Barrios & E. Díaz 7017* (HAJB).

Melocactus holguinensis, Cuba, Holguín, La Palma, a un costado del cementerio del pueblo Rafael Freyre, 21°01'47.165"N, 75°59'44.395"W, 70–90 m, 31 Jan 2018, *L. C. Majure, D. Barrios & E. Díaz 7030* (HAJB).

Melocactus matanzanus, Cuba, Matanzas, Tres Ceibas de Clavellinas, 23°05.629'N, 81°39.113'W, 53 m, 4 Feb 2018, *L. C. Majures & D. Barrios 7046* (HAJB).

Melocactus nagyi, Cuba, Granma, Pilón, El Salvial, alrededores de la Estación Ecológica El Macío, 27 Aug 2015, *D. Barrios, J. A. García, R. Verdecia & E. Palacio HFC 88502* (HAJB).

Melocactus perezassoi, Cuba, Villa Clara, cultivado en casa de Edgardo Díaz y colectado del farallón del Guanajo cercano al pueblo de Jibacoa, Manicaragua, 22°01'N, 79°59'W, 3 Feb 2018, *L. C. Majure, D. Barrios & E. Díaz 7044* (HAJB).

Melocactus radoczii, Cuba, Guantánamo, Maisí, en los márgenes del río Jauco cerca de Guajimero, al norte de La Tinta, 20°08.742'N, 74°21.916'W, 180 m, 5 Feb 2018, *L. C. Majure & D. Barrios 7050* (HAJB).

Appendix 2. Descriptions of seed morphology of 12 *Melocactus* taxa from 14 localities in Cuba.

Melocactus Link & Otto

Seeds small to medium-sized, 0.81–1.63 × 0.68–1.41 mm, circular or broadly oval. *Testa* black, matt or glossy, without multicellular sculpture, flat, coliculate or tuberculate, ventral or ventro-apical keel, cells gradually or abruptly smaller near keel and hilum-micropylar region. *Apical region* with elongate cells, periclinal walls flat, low-convex or high-convex, anticlinal cell boundaries channelled and straight or irregularly curved. *Lateral region* with elongate or isodiametric cells, periclinal walls flat, low-convex or high-convex, anticlinal cell boundaries channelled and straight or irregularly curved, microrelief flat or striate. *Ventral region* with elongate cells, periclinal walls flat, anticlinal cell boundaries channelled and straight. *Hilum-micropylar region border* with isodiametric cells, periclinal walls flat or low-convex, anticlinal cell boundaries channelled and straight, constricted, not expanded or expanded border. *Hilum-micropylar region* of 0.33–1.11 × 0.19–0.88 mm, oval or keyhole type, superficial or impressed, hilum and micropyle conjunct but separated 0.12–0.58 mm by sclerified tissue. Seeds of 0.14–0.88 mg and relative embryo mass of 0.14–0.76.

Melocactus actinacanthus Areces – Cuba, Villa Clara, Sierra Alta de Agabama (no herbarium specimen). – Fig. 5.1.

Seeds medium-sized, 1.15–1.52 × 0.96–1.41 mm, circular or broadly oval. *Testa* black matt, without multicellular sculpture, coliculate, ventral keel, cells gradually smaller near keel and hilum-micropylar region. *Apical region* with elongate cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight. *Lateral region* with elongate cells, periclinal walls flat, anticlinal cell boundaries channelled and straight, microrelief striate. *Ventral region* with elongate cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight. *Hilum-micropylar region border* with isodiametric cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight, not expanded border. *Hilum-micropylar region* of 0.63–0.97 × 0.31–0.57 mm, oval, superficial, hilum and micropyle conjunct but separated 0.26–0.39 mm by sclerified tissue. Seeds of 0.52–0.64 mg and relative embryo mass of 0.32–0.53.

Melocactus actinacanthus Areces – Cuba, La Habana, La Coca, *Barrios & González-Torres HFC 88802* (HAJB). – Fig. 5.2.

Seeds small to medium-sized, $0.91\text{--}1.29 \times 0.73\text{--}1.08$ mm, circular or broadly oval. *Testa* black matt, without multicellular sculpture, coliculate, ventral keel, cells gradually smaller near keel and abruptly smaller near hilum-micropylar region. *Apical region* with elongate cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight. *Lateral region* with elongate cells, periclinal walls flat, anticlinal cell boundaries channelled and irregularly curved, microrelief striate. *Ventral region* with elongate cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight. *Hilum-micropylar region border* with isodiametric cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight, not expanded border. *Hilum-micropylar region* of $0.67\text{--}1.05 \times 0.39\text{--}0.59$ mm, oval, superficial, hilum and micropyle conjunct but separated $0.28\text{--}0.51$ mm by sclerified tissue. Seeds of $0.64\text{--}0.78$ mg and relative embryo mass of $0.30\text{--}0.42$.

Melocactus acunae León subsp. *acunae* – Cuba, Guantánamo, *Cajobabo, Majure & al. 7027* (HAJB). – Fig. 6.1.

Seeds small or medium-sized, $0.88\text{--}1.15 \times 0.68\text{--}0.97$ mm, broadly oval. *Testa* black matt, without multicellular sculpture, tuberculate, ventral keel, cells gradually smaller near keel and abruptly smaller near hilum-micropylar region. *Apical region* with elongate cells, periclinal walls low-convex and high-convex, anticlinal cell boundaries channelled, straight and irregularly curved. *Lateral region* with elongate cells, periclinal walls flat and low-convex, anticlinal cell boundaries channelled, straight and irregularly curved, microrelief striate. *Ventral region* with elongate cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight. *Hilum-micropylar region border* with isodiametric cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight, not expanded border. *Hilum-micropylar region* of $0.33\text{--}0.74 \times 0.20\text{--}0.49$ mm, oval, impressed, hilum and micropyle conjunct but separated $0.12\text{--}0.31$ mm by sclerified tissue. Seeds of $0.32\text{--}0.40$ mg and relative embryo mass of $0.38\text{--}0.45$.

Melocactus acunae León subsp. *acunae* – Cuba, Guantánamo, Punta de Maisí, *Majure & al. 7025* (HAJB). – Fig. 4.1.

Seeds small or medium-sized, $0.93\text{--}1.18 \times 0.75\text{--}0.94$ mm, broadly oval. *Testa* black glossy, without multicellular sculpture, flat, ventral keel, cells abruptly smaller near keel and hilum-micropylar region. *Apical region* with elongate cells, periclinal walls flat, anticlinal cell boundaries channelled, straight and irregularly curved. *Lateral region* with elongate cells, periclinal walls flat, anticlinal cell boundaries channelled, straight and irregularly curved, microrelief striate. *Ventral region* with

elongate cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight. *Hilum-micropylar region border* with isodiametric cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight, not expanded border. *Hilum-micropylar region* of $0.46\text{--}0.65 \times 0.27\text{--}0.38$ mm, oval, impressed, hilum and micropyle conjunct but separated $0.12\text{--}0.27$ mm by sclerified tissue. Seeds of $0.28\text{--}0.32$ mg and relative embryo mass of $0.33\text{--}0.57$.

Melocactus acunae subsp. *lagunaensis* Mészáros – Cuba, between Veguita and Vega Grande, *Majure & al. 7021* (HAJB). – Fig. 6.2.

Seeds small or medium-sized, $0.95\text{--}1.15 \times 0.77\text{--}0.92$ mm, broadly oval. *Testa* black matt, without multicellular sculpture, tuberculate, ventral keel, cells abruptly smaller near keel and gradually smaller near hilum-micropylar region. *Apical region* with elongate cells, periclinal walls low-convex and high-convex, anticlinal cell boundaries channelled, straight and irregularly curved. *Lateral region* with elongate cells, periclinal walls flat and low-convex, anticlinal cell boundaries channelled and irregularly curved, microrelief striate. *Ventral region* with elongate cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight. *Hilum-micropylar region border* with isodiametric cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight, not expanded border. *Hilum-micropylar region* of $0.52\text{--}0.71 \times 0.29\text{--}0.88$ mm, oval, superficial, hilum and micropyle conjunct but separated $0.16\text{--}0.33$ mm by sclerified tissue. Seeds of $0.28\text{--}0.34$ mg and relative embryo mass of $0.40\text{--}0.56$.

Melocactus borhidii Mészáros – Cuba, Guantánamo, Tortuguilla, *Barrios & al. HFC 88766* (HAJB). – Fig. 4.3.

Seeds small or medium-sized, $0.94\text{--}1.23 \times 0.75\text{--}1.02$ mm, circular or broadly oval. *Testa* black glossy, without multicellular sculpture, flat, ventral keel, cells gradually smaller near keel and abruptly smaller near hilum-micropylar region. *Apical region* with elongate cells, periclinal walls flat, anticlinal cell boundaries channelled, straight and irregularly curved. *Lateral region* with elongate cells, periclinal walls flat, anticlinal cell boundaries channelled, straight and irregularly curved, microrelief striate. *Ventral region* with elongate cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight. *Hilum-micropylar region border* with isodiametric cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight, not expanded border. *Hilum-micropylar region* of $0.46\text{--}0.69 \times 0.23\text{--}0.48$ mm, oval, impressed, hilum and micropyle conjunct but separated $0.13\text{--}0.31$ mm by sclerified tissue. Seeds of $0.30\text{--}0.38$ mg and relative embryo mass of $0.33\text{--}0.59$.

Melocactus evae Mészáros – Cuba, Guantánamo, Los Monitongos, *Majure & al. 7028* (HAJB). – Fig. 3.3.

Seeds small or medium-sized, 0.93–1.16 × 0.78–0.98 mm, circular or broadly oval. *Testa* black glossy, without multicellular sculpture, flat, ventral keel, cells gradually smaller near keel and hilum-micropylar region. *Apical region* with elongate cells, periclinal walls flat, anticlinal cell boundaries channelled and straight. *Lateral region* with elongate cells, periclinal walls flat, anticlinal cell boundaries channelled, straight and irregularly curved, not microrelief. *Ventral region* with elongate cells, periclinal walls flat, anticlinal cell boundaries channelled and straight. *Hilum-micropylar region border* with isodiametric cells, periclinal walls flat, anticlinal cell boundaries channelled and straight, expanded border. *Hilum-micropylar region* of 0.49–0.78 × 0.22–0.41 mm, oval, superficial, hilum and micropyle conjunct but separated 0.20–0.35 mm by sclerified tissue. Seeds of 0.26–0.36 mg and relative embryo mass of 0.30–0.50.

Melocactus guitartii León – Cuba, Sancti Spíritus, Peñón de Dagamal, *Majure & al. 7039* (HAJB). – Fig. 3.1. Seeds medium-sized, 1.15–1.48 × 0.86–1.23 mm, broadly oval. *Testa* black matt, without multicellular sculpture, flat, ventro-apical keel, cells gradually smaller near keel and abruptly smaller near hilum-micropylar region. *Apical region* with elongate cells, periclinal walls flat, anticlinal cell boundaries channelled, straight and irregularly curved. *Lateral region* with elongate cells, periclinal walls flat, anticlinal cell boundaries channelled and irregularly curved, microrelief striate. *Ventral region* with elongate cells, periclinal walls flat, anticlinal cell boundaries channelled and straight. *Hilum-micropylar region border* with isodiametric cells, periclinal walls flat, anticlinal cell boundaries channelled and straight, not expanded border. *Hilum-micropylar region* of 0.68–0.90 × 0.23–0.41 mm, keyhole type, impressed, hilum and micropyle conjunct but separated 0.30–0.45 mm by sclerified tissue. Seeds of 0.60–0.78 mg and relative embryo mass of 0.32–0.45.

Melocactus harlowii (Britton & Rose) Vaupel – Cuba, Guantánamo, Macambo, *Majure & al. 7017* (HAJB). – Fig. 4.2.

Seeds small or medium-sized, 0.98–1.23 × 0.74–1.03 mm, broadly oval. *Testa* black glossy, without multicellular sculpture, flat, ventral keel, cells gradually smaller near keel and hilum-micropylar region. *Apical region* with elongate cells, periclinal walls flat and low-convex, anticlinal cell boundaries channelled, straight and irregularly curved. *Lateral region* with elongate cells, periclinal walls flat, anticlinal cell boundaries channelled, straight and irregularly curved, microrelief striate. *Ventral region* with elongate cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight. *Hilum-micropylar region border* with isodiametric cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight, not expanded border. *Hilum-micropylar region* of 0.37–0.73 × 0.19–0.43 mm, oval, superficial, hilum

and micropyle conjunct but separated 0.18–0.34 mm by sclerified tissue. Seeds of 0.28–0.34 mg and relative embryo mass of 0.21–0.76.

Melocactus holguinensis Areces – Cuba, Holguín, La Palma, *Majure & al. 7030* (HAJB). – Fig. 3.2.

Seeds small to medium-sized, 0.81–1.17 × 0.78–1.09 mm, circular or broadly oval. *Testa* black glossy, without multicellular sculpture, flat, ventro-apical keel, cells gradually smaller near keel and abruptly smaller near hilum-micropylar region. *Apical region* with elongate cells, periclinal walls flat, anticlinal cell boundaries channelled and straight. *Lateral region* with elongate cells, periclinal walls flat, anticlinal cell boundaries channelled and irregularly curved, microrelief striate. *Ventral region* with elongate cells, periclinal walls flat, anticlinal cell boundaries channelled and straight. *Hilum-micropylar region border* with isodiametric cells, periclinal walls flat, anticlinal cell boundaries channelled and straight, constricted border. *Hilum-micropylar region* of 0.42–0.62 × 0.30–0.44 mm, oval, impressed, hilum and micropyle conjunct but separated 0.17–0.28 mm by sclerified tissue. Seeds of 0.42–0.50 mg and relative embryo mass of 0.38–0.59.

Melocactus matanzanus León – Cuba, Matanzas, Tres Ceibas de Clavellinas, *Majure & Barrios 7046* (HAJB). – Fig. 5.3.

Seeds medium-sized, 1.12–1.44 × 0.95–1.32 mm, circular or broadly oval. *Testa* black matt, without multicellular sculpture, coliculate, ventral keel, cells abruptly smaller near keel and hilum-micropylar region. *Apical region* with elongate cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight. *Lateral region* with elongate cells, periclinal walls flat, anticlinal cell boundaries channelled and straight, microrelief striate. *Ventral region* with elongate cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight. *Hilum-micropylar region border* with isodiametric cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight, not expanded border. *Hilum-micropylar region* of 0.59–0.92 × 0.33–0.52 mm, oval, superficial, hilum and micropyle conjunct but separated 0.25–0.42 mm by sclerified tissue. Seeds of 0.54–0.70 mg and relative embryo mass of 0.29–0.50.

Melocactus nagyii Mészáros – Cuba, Granma, Salvial, *Barrios & al. HFC 88502* (HAJB). – Fig. 6.3.

Seeds small or medium-sized, 0.93–1.20 × 0.77–0.99 mm, circular or broadly oval. *Testa* black matt, without multicellular sculpture, tuberculate, ventral keel, cells abruptly smaller near keel and hilum-micropylar region. *Apical region* with elongate cells, periclinal walls low-convex and high-convex, anticlinal cell boundaries channelled and straight. *Lateral region* with elongate cells, periclinal walls flat and low-convex, anticlinal cell boundaries channelled and irregularly curved, microrelief striate. *Ventral*

region with elongate cells, periclinal walls low-convex and high-convex, anticlinal cell boundaries channelled and straight. *Hilum-micropylar region border* with isodiametric cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight, not expanded border. *Hilum-micropylar region* of 0.44–0.72 × 0.24–0.45 mm, oval, superficial, hilum and micropyle conjunct but separated 0.17–0.35 mm by sclerified tissue. Seeds of 0.14–0.28 mg and relative embryo mass of 0.14–0.46.

Melocactus perezassoi Areces – Cuba, Villa Clara, Jibacoa, *Majure & al.* 7044 (HAJB).

Seeds medium-sized, 1.32–1.63 × 1.13–1.41 mm, circular or broadly oval. *Testa* black glossy, without multicellular sculpture, coliculate, ventral keel, cells gradually smaller near keel and abruptly smaller near hilum-micropylar region. *Apical region* with elongate cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight. *Lateral region* with elongate and isodiametric cells, periclinal walls flat and low-convex, anticlinal cell boundaries channelled, straight and irregularly curved, microrelief striate. *Ventral region* with elongate cells, periclinal walls flat, anticlinal cell boundaries channelled and straight. *Hilum-micropylar region border* with isodiametric cells, periclinal walls flat, anticlinal cell

boundaries channelled and straight, constricted border. *Hilum-micropylar region* of 0.80–1.11 × 0.48–0.66 mm, oval, superficial, hilum and micropyle conjunct but separated 0.31–0.58 mm by sclerified tissue. Seeds of 0.62–0.88 mg and relative embryo mass of 0.35–0.50.

Melocactus radoczii Mészáros – Cuba, Guantánamo, Guajimero, *Majure & Barrios* 7050 (HAJB). – Fig. 3.4.

Seeds small, 0.88–1.07 × 0.74–0.94 mm, circular or broadly oval. *Testa* black matt, without multicellular sculpture, coliculate, ventral keel, cells abruptly smaller near keel and hilum-micropylar region. *Apical region* with elongate cells, periclinal walls low-convex, anticlinal cell boundaries channelled and straight. *Lateral region* with elongate cells, periclinal walls flat and low-convex, anticlinal cell boundaries channelled and straight, microrelief striate. *Ventral region* with elongate cells, periclinal walls flat, anticlinal cell boundaries channelled and straight. *Hilum-micropylar region border* with isodiametric cells, periclinal walls flat, anticlinal cell boundaries channelled and straight, expanded border. *Hilum-micropylar region* of 0.52–0.75 × 0.23–0.39 mm, oval, superficial, hilum and micropyle conjunct but separated 0.22–0.39 mm by sclerified tissue. Seeds of 0.26–0.28 mg and relative embryo mass of 0.23–0.57.

Willdenowia

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