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ON THE TAXONOMY OF *CACTACEAE* JUSS BY THE EVIDENCE OF SEED MICROMORPHOLOGY AND SDS-PAGE ANALYSIS

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ABSTRACT *Numerical classification of 16 taxa of Cactaceae was studied using combination of micromorphological characters of seeds (using L.M and SEM) and SDS-PAGE analysis. Aspects of seed micromorphology and seed protein variation as defined were recorded and scored comparatively for the OTU's into a data matrix. Phenetic relationships of these taxa were established based on UPGMA-clustering method by using Jaccard coefficient of the NTSYS-pc 2.2 program. The results were compatible with the traditional relationships of some taxa as the split-off of Opuntia humifusa and Astrophytum myriostigma, at separate lines, these results are compatible with their placement in tribes Opuntieae (subfamily Opuntioideae) and Cacteae (Subfamily cactoideae) respectively, at the time, the placement of three taxa Pseudorhipsalis ramulosa, Rhipsalis baccifera Accession 1, and Rhipsalis baccifera Accession 2 together, the clustering of Hylocereus triangularis and Neobuxbaumia euphorbioides together at a unique tribe Phyllocacteae. The findings contradict in a number of cases the traditional studies, as the grouping of Trichocereus vasquezii with the two represents of genus Parodia despite of their placement in different tribes.*

KEYWORDS: Cactaceae, SDS-PAGE, Seed micromorpgology, SEM

INTRODUCTION

The Cactaceae are an exciting and problematic group of plants because of their varied morphology, succulence, and their showy flowers (Barthlott and Hunt 1993). Cactaceae comprise some 1870 species in 130 genera (Nyffeler and Eggli 2010b) and are estimated to have distributed about 30 million years ago (Hershkovitz and Zimmer 1997).

The Cactaceae are diversified group of warm and arid areas of the New World, plants are adapted to many different habitats, including bare, hot deserts, sandy coastal stretches, scrublands, dry deciduous forests, high alpine steppes, and even tropical rain forests (Barthlott and Hunt 1993). Centers of diversity are the arid regions of North and South America, notably the southwestern United States and Mexico, East Brazil, and the eastern and western slopes of the South American Andes. Only a single epiphytic species, *Rhipsalis baccifera* (J. S. Muell.) Stearn, extends naturally to southern Africa, because of the sticky small fruits which presumably were carried across the Atlantic Ocean by birds. (Barthlott 1983; Barthlott and Taylor 1995). Cacti grow at altitudes from below sea level to over 4,500 m in the Andes; and in climates having no measurable rainfall to more than 500 cm of annual precipitation.

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The Cactaceae are a morphologically very diverse family. They have evolved a variety of growth-forms ranging from tree-like, large columnar forms to shrubby forms or succulent climbers and to small globular forms, and many are grown today as pot plants for their unusual habits and large, showy flowers.

The unusual vegetative morphology is the result of the following major modifications of the general structure of a perennial dicotyledonous flowering plant (Goebel 1889; Rauh 1979): (1) the leaves are highly reduced or lost, (2) the stems remain green and photosynthetically active for several years with retarded bark formation, (3) cortex and pith are transformed into a succulent water-storage tissue, (4) short side-branches are modified into clusters of spines called areoles, and (5) branching is often highly reduced.

Due to their highly modified vegetative and floral morphology, taxonomists generally regarded the cacti as a very distinct group and placed it in its own order, Cactales (Opuntiales according to Engler 1892; e.g., Hutchinson 1973; Benson 1979). There was disagreement about the closest relatives of the cacti until studies of embryology (e.g., Schnarf 1931), plant pigment chemistry (e.g. Mabry *et al* 1963), and sieve-element plastids (e.g., Behnke 1972) suggested a close relationship of the family Cactaceae to the core Caryophyllales. Molecular studies have confirmed this inference and have identified a distinct clade consisting of Portulacaceae, Basellaceae, Cactaceae, and Didieraceae (e.g., Manhart and Rettig 1994). Additionally, recent studies based on increased taxon sampling have suggested that the three latter families are in fact nested in paraphyletic Portulacaceae (Hershkovitz and Zimmer 1997; Applequist and Wallace 1999).

The recent studies reported that it is part of a clade that contains most of the succulent families of the order Caryophyllales: Cactaceae, Anacampserotaceae, Basellaceae, Didiereaceae, Halophytaceae, Montiaceae, Portulacaceae, and Talinaceae (Cuénoud *et al*. 2002 and Schäferhoff *et al*. 2009). The sister group of the Cactaceae is the former Portulacaceae tribe Anacampseroteae, now separated as an own family Anacampserotaceae (Nyffeler 2007; Nyffeler and Eggli 2010a).

The establishment of taxonomic units within the Cactaceae has been always difficult and controversial. Beginning with Schumann's (1899) first comprehensive monograph of the family, many classification systems have been proposed in the last centuries (Backeberg 1958-1962; Britton and Rose 1919-1923; Buxbaum 1962, Gibson and Nobel 1986; Barthlott 1988; Barthlott and Hunt 1993). These classifications were often rather subjective and therefore largely incompatible with each other.

The family Cactaceae is generally classified into three subfamilies: Pereskioideae, Opuntioideae, and Cactoideae (Schumann 1899a and Barthlott and Hunt 1993). Recently, however, it was suggested that *Maihuenia* (F. A. C. Weber) K. Schum., traditionally placed with *Pereskia* in the subfamily Pereskioideae, should be considered as a subfamily of its own (Wallace 1995a and Anderson 2001). Indeed, *Maihuenia* and *Pereskia* have been placed together essentially because they lack distinct synapomorphies. The subfamily Opuntioideae is characterized by the bony aril of the seeds and the presence of glochids (barbed hairs) in the areoles, while the subfamily Cactoideae is distinct in its lack of leaves (with a few exceptions, e.g., *Corryocactus brevistylus* (K. Schum.) Britton and Rose), the characteristic hilum-micropylar region of the seeds (Barthlott and Voit 1979), and an intron loss in the

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chloroplast gene rpoc1 (Wallace and Cota 1995). Metzing and Kiesling (2008) summarize early (pre-DNA) studies in the family, and include reproductions of some remarkable evolutionary trees. For a recent classification of the whole family, four subfamilies, eight tribes (two for Opuntioideae, six for Cactoideae), and six subtribes (for Cactoideae) were suggested by Nyffeler and Eggli (2010a)

The present study aims at studying the relationships within Cactaceae using seed micromorphology as well as protein analysis and comparing results with what already known about the family at the generic and subgenric levels.

MATERIAL AND METHODS

Taxon Sample

The study included 16 Operational Taxonomic Units (OTU's), representing ten genera of Cactaceae. The genus *Harrisia* is represented by 2 species: *H. Pomanensis* (F.A.C. Weber) Britton and Rose (with 3 accessions) and *H. Tortuosa* (Otto and A. Dietr.) Britton and Rose. Genus *Parodia* is represented also by two species, *P. Leninghausii* (K. Schum) F.H. Brandt and *P. Schumanniana* subsp. *Claviceps* (F. Ritter) Hofacker. The genus *Rhipsalis* is represented by two species *R. Baccifera* (J.S.Muell) stearn (with 2 accessions) and *R. Micrantha* (Kunth) DC. The remaining genera are represented by single species for each one. A list of taxa and collection data of the specimens representing them is given in Appendix (1).

Seed Scanning Technique

Seeds of taxa were collected from Botanical Garden, Berlin-Dahlem at 2010 (Appendix 1); seeds were examined by light microscope (L.M.) for the study of external morphology of seeds. For SEM studies, six mature seeds from each taxon were selected. The seeds were mounted on SEM stubs, using double sided cellotape, coated with gold, palladium in vacuum evaporator, examined and photographed in a JEOL JSM 5400 LV scanning electron microscope which operated at accelerated voltage of 15 KV, at electron microscopy unit, Assiut University, Egypt. Since testa cell morphology varies depending on the region examined, close-up views were always taken from the lateral region of the seed (Barthlott and Voit 1979). Terminology concerning the description of outer seed pattern follows Barthlott and Hunt (2000).

SDS-protein analysis technique.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed for total proteins of the sixteen OTU's of Cactaceae according to the method of Laemmli (1970), as modified by Studier (1973). Protein extraction was conducted by mixing 0.03 g of seeds with an equal weight of pure, clean, sterile fine sand. The seeds were then ground to fine powder using a mortar and pestle and homogenized with 1M Tris-hcl buffer, ph 8.8 in clean eppendorf tube and left in refrigerator overnight. Then centrifuged at 5000 rpm for 10 min. Then a volume of 25 µl protein extract was added to 10 µl of treatment buffer. Then 25 µl of each sample was loaded in the gel. After electrophoresis, the gel was stained by comassie brilliant blue. The gel was destained after the appearance of bands and photographed. All gels were scanned and analyzed.

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Table 1. Placement of the studied genera in some selected classification systems

No.	Genus	Endler and Buxbaum (1974)	Gibson and Nobel (1986)	Barthlott and Hunt (1993)	Anderson (2001) and Nyffeler and Eggli (2002)	Nyffelerand Eggli (2010b)
1	Astrophytum	Notocacteae	Cacteae	Cacteae	Cacteae	Subfamily cactoideae Tribe Cacteae
2	Echinopsis	Trichocereeae	Trichocereeae	Trichocereeae	Trichocereeae	Tribe cereeae subtribe: Trichocereinaeae
3	Harrisia	Hylocereeae	Hylocereeae	Echinocereeae	Trichocereeae	Tribe cereeae subtribe: Trichocereinaeae
4	Hylocereus	Hylocereeae	Hylocereeae	Hylocereeae	Hylocereeae	Tribe phyllocactaceae sub-tribe: Hylocereinae
5	Neobuxbaumia	Pachycereinae	Pachycereinae	Pachycereinae	Pachycereinae	Tribe phyllocactaceae subtribe: Echinocereinae
6	Opuntia	Opuntioideae	Opuntioideae	Opuntioideae	Opuntioideae	Subfamily Opuntioideae Tribe Opuntieae
7	Parodia	Notocacteae	Notocacteae	Notocacteae	Notocacteae	Tribe Notocacteae
8	Pseudorhipsalis	Hylocereeae	Notocacteae	Hylocereeae	Hylocereeae	Tribe Rhipsalideae
9	Rhipsalis	Hylocereeae	Notocacteae	Rhipsalideae	Rhipsalideae	Tribe Rhipsalideae
10	Trichocereus	Hylocereeae	Hylocereeae	Echinocereeae	Trichocereeae	Tribe cereeae subtribe: Trichocereinaeae

Data analysis

Aspects of seed morphology and seed protein variation as defined in Appendix (2) are recorded and scored comparatively for the OTU's into a data matrix. In preparing the raw data matrix, multistate characters were transformed into two-state characters in coding and their presence or absence was coded 1 and 0 respectively (Appendix 2). The program NTSYS-pc 2.2 (Rohlf 2005) was used in the data analysis as follows: the raw data matrix was standardized with STAND module; similarity matrix was generated by SIMQUAL module based on Jaccard coefficient. Clustering was performed using unweighted pair-group method with arithmetic average (UPGMA) and represented in phenogram (tree), there are three phenograms the first is made using micromorphological data, the second is prepared using SDS-PAGE analysis data and the third is resulted from the combination of micromorphological and biochemical data. The distortion between each tree and its related distance matrix (Rohlf and Sokal 1981) was evaluated by computing the tree's cophenetic (ultramatric) value matrix using COPH and comparing them using MXCOMP modules.

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RESULTS AND DISCUSSION

Micromorphological Data

External seed morphology*:* Seeds are narrowly ovoid only in *Hylocereus triangularis* (7); oblong-ovoid in two species: *Echinopsis mirabilis* (2) and *Rhipsalis micrantha* (15); elliptic only in *Astrophytum myriostigma* (1); rounded only in *Opuntia humifusa* (9), and the remaining OTU's have ovoid seeds (Fig 1). Seed color varies in the studied taxa. It was brown only in *Neobuxbaumia euphorbioides* (8); yellow in 4 taxa and black in 11 taxa (Appendix 2). The seed surface of 10 investigated OTU's is matte, the semi-matte and glossy surface are observed equally in the other 6 taxa (Appendix 2); 9 taxa have small seed length (0.8mm-1.9mm); the remaining 7 OTU's showed long seed length (2.7mm-7.5mm); at the same time 11 OTU's of the examined taxa have small seed width (0.05mm-1.9mm), only 5 OTU's have seed width (2mm-4.5mm) (Appendix 2).

Four patterns of overall seed coat are recognized: reticulate in *Parodia leninghausii* (10), *Parodia schumanniana* subsp. *Claviceps* (11), *Rhipsalis micrantha* (15) and *Trichocereus vasquezii* (16); rugose in *Opuntia humifusa* (9), *Pseudorhipsalis ramulosa* (12), *Rhipsalis baccifera* Accession 1 (13) and *Rhipsalis baccifera* Accession 2 (14); smooth only in *Hylocereus triangularis* (7) and *Neobuxbaumia euphorbioides* (8); and granulate in the remaining 6 OTU's (Appendix 2, Fig1).

Testa cell pattern: The testa cells are variable in size that appeared gradually smaller towards hilum in three OTU's: *Harrisia pomanensis* Accession 1 (3), *Harrisia pomanensis* Accession 2 (4) and *Trichocereus vasquezii* (16); but appeared abruptly smaller towards hilum in another three OTU's: *Parodia leninghausii* (10), *Parodia schumanniana* subsp. *Claviceps* (11) and *Rhipsalis micrantha* (15); the remaining taxa appeared have a uniform testa cells (Appendix 2).

Testa cells have angular shape in 7 taxa; only 2 OTU's: *Echinopsis mirabilis* (2) and *Harrisia tortuosa* (6) have cells with rounded shape (Fig 2); Both *Astrophytum myriostigma* (1) and *Harrisia pomanensis* Accession 1 (3) have angular to rounded testa cells; but the other 5 taxa represent a variable testa cell shapes (Appendix 2, Fig2).

Table 2. The molecular weights of protein bands extracted in Tris-HCl buffer of the 16 studied taxa of Cactaceae

Rf	Mw.	Taxa															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0.01	142	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
0.03	137	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0
0.28	**65**	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
0.73	29	1	1	1	1	1	1	0	0	0	0	0	0	1	1	1	0
0.75	27.7	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0
0.76	27.1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	0	0
0.88	21	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
0.90	20	1	1	1	1	1	1	1	1	1	0	0	0	0	1	1	0
0.92	18.5	0	0	1	1	1	1	0	1	1	0	0	0	0	1	1	0

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0.94	**17**	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
0.97	**15.5**	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

Anticlinal boundaries are shallowly channeled in *Hylocereus triangularis* (7), *Parodia leninghausii* (10) and *Parodia schumanniana* subsp. *Claviceps* (11), and deeply channeled in 8 OTU's of the selected taxa (Appendix 2, Fig 2). In addition, *Neobuxbaumia euphorbioides* (8) and *Trichocereus vasquezii* (16) are unique in having raised anticlinal wall borders in contrast to the other examined taxa, at the same time, anticlinal walls are not obvious or may at the same levels with pereclinal walls in 3 OTU's: *Opuntia humifusa* (9), *Pseudorhipsalis ramulosa* (12) and *Rhipsalis micrantha* (15) (Fig 2).

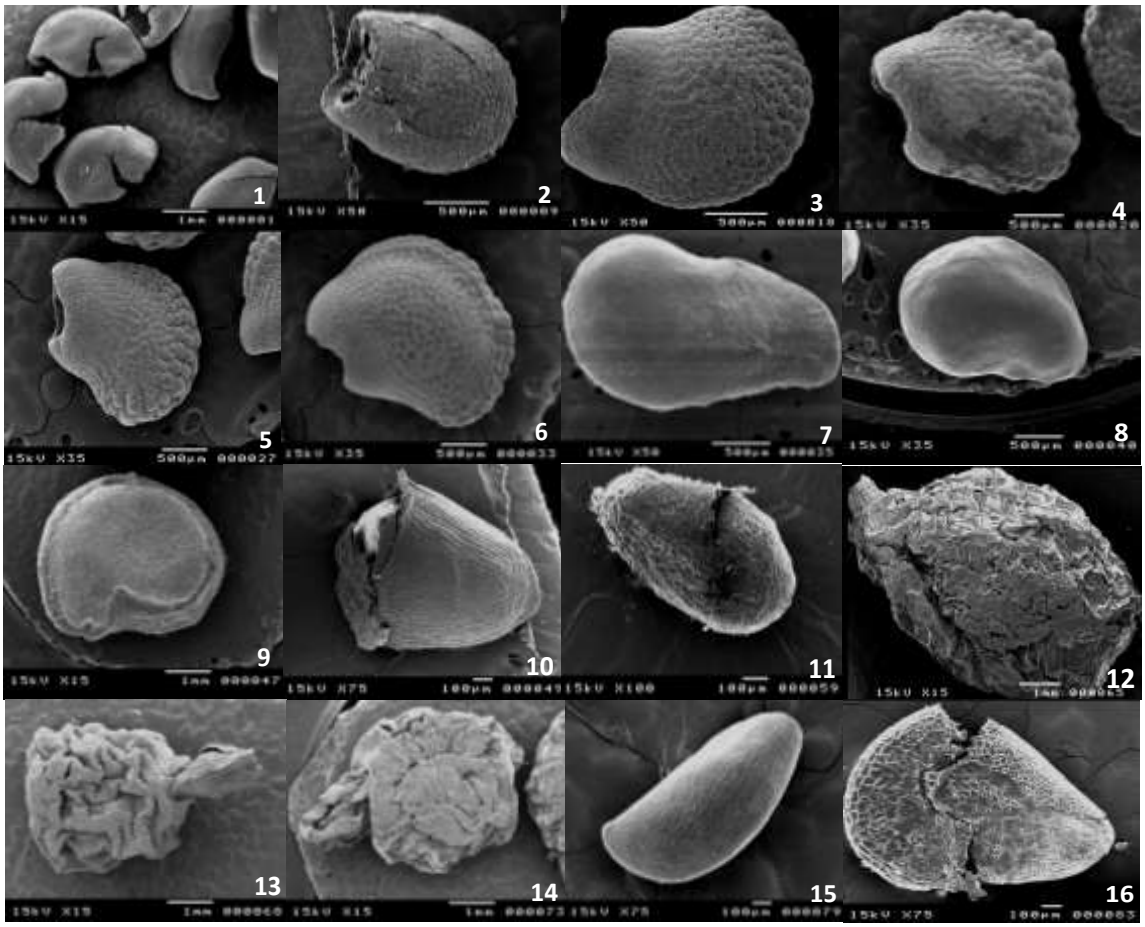

Figure 1. SEM micrographs of mature seeds of the 16 taxa representing the Cactaceae in this study, showing the characters of external seed morphology and testa cell pattern, numbers refereeing to the taxa (Appendix 1)

Sculpture of pereclinal walls: The pereclinal walls are flat in 6 taxa (Appendix 2); 8 taxa have concave pereclinal walls; but only 2 OTU's: *Neobuxbaumia euphorbioides* (8) and *Trichocereus vasquezii* (16) appeared with convex pereclinal walls. *Astrophytum myriostigma* appeared with granulate texture of pereclinal walls; 3 taxa: *Opuntia humifusa* (9), *Rhipsalis baccifera* Accession 1 (13) and *Rhipsalis baccifera* Accession 2 (14) have a ribbed texture of pereclinal walls. in addition, the microreticulate texture appeared in five taxa (Appendix 2);

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the pereclinal walls texture was striated in 3 OTU's: *Hylocereus triangularis* (7), *Parodia schumanniana* subsp. *Claviceps* (11) and *Trichocereus vasquezii* (16) (Fig 2); at the same time, pereclinal walls are smooth in 3 taxa: *Neobuxbaumia euphorbioides* (8), *Pseudorhipsalis ramulosa* (12) and *Rhipsalis micrantha* (15); while *Parodia leninghausii* (10) showed a variable pereclinal wall texture. 10 OTU's are characterized by the presence of microrelief on their seed testa, while the remaining 6 taxa have no (Appendix 2, Fig 2).

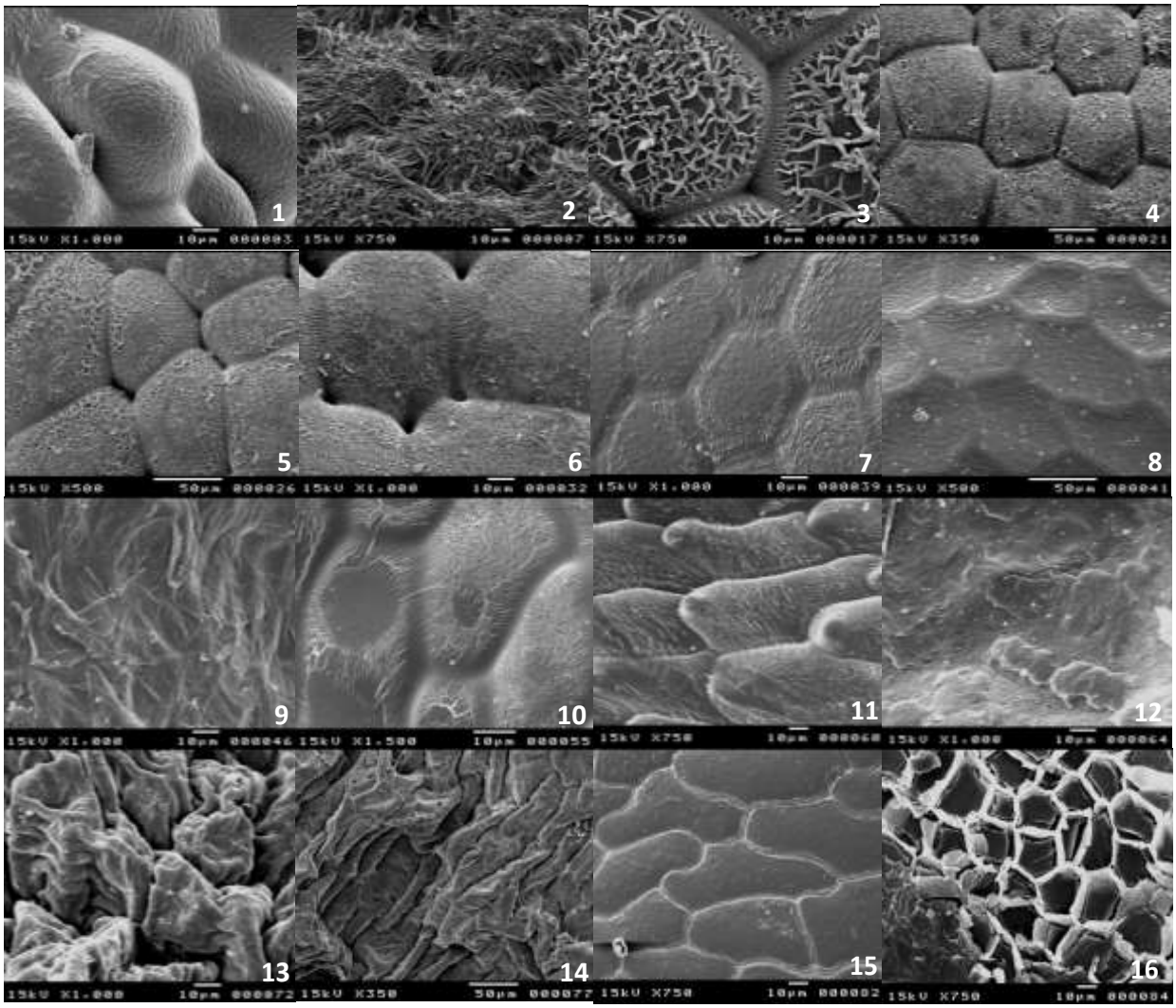

Figure 2. SEM micrographs of cell shape, anticlinal and pereclinal characters of mature seeds of the 16 taxa representing the Cactaceae in this study, numbers refereeing to the taxa (Appendix 1)

Biochemical data "SDS- PAGE Protein characters"

Patterns of protein bands were used as biochemical markers for the classification of certain taxa of cactaceae. The resulted profile of SDS-PAGE showed eleven bands distributed in all taxa, the molecular weights of the recorded bands ranged from 142 to 15.5 KDa (Table 2). Only three unique bands have been observed in three taxa: *Neobuxbaumia euphorbioides* (8)

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have one positive specific band with molecular weight of 65 KDa, *Opuntia humifusa* (9) have one positive specific band with molecular weight of 15.5 KDa, and *Parodia leninghausii* (10) have the third positive specific band with molecular weight of 17 KDa. These bands could be considered as specific markers for distinguishing these taxa from the others, the other bands are polymorphic.

Phenetic analysis

Figure (3) shows the UPGMA phenogram based on the analysis of 43 seed micromorphological characters listed in Appendix (2) and recorded comparatively for 16 taxa belonging to 10 genera of family Cactaceae at the similarity level of 0.14.

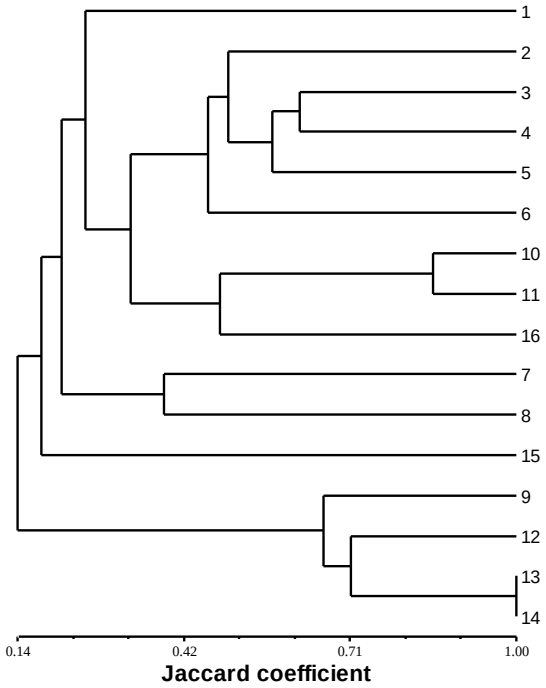

Figure 3. UPGMA phenogram based on the cactaceae seed morphology

The UPGMA phenogram obtained from SDS-PAGE analysis of the sixteen taxa of Cactaceae is displayed in figure (4). The UPGMA method was used to calculate the similarity coefficient among the studied taxa, based on existence of the bands (presence or absence) (Table 2). Figure (5) displays the UPGMA phenogram based on the analysis of 43 seed micromorphological characters combined with the data obtained from the SDS-PAGE analysis of the sixteen taxa of Cactaceae.

As displayed in Figure 5, the first level of the phenogram separates three taxa: *Parodia leninghausii* (10), *Parodia schumanniana* subsp. *Claviceps* (11) and *Trichocereus vasquezii* (16) at the similarity level of 0.14, because of black, ovoid, mutte seeds, angular testa cell

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shape and presence of microrelief. On the other hand, the both taxa *Parodia leninghausii* and *Parodia schumanniana* subsp. *Claviceps* is more related to each other and clustered at the similarity level of 0.49 because of sharing the both polymorphic bands with molecular weights 137 and 27.7 KDa, This result supports their placement under tribe Notocacteae, while the third one "i.e. *Trichocereus vasquezii*" belongs to other different tribe (Endler and Buxbaum 1974; Gibson and Nobel 1986; Barthlott and Hunt 1993; Anderson 2001; Nyffeler and Eggli 2002 and Nyffeler and Eggli 2010b) (Table 1).

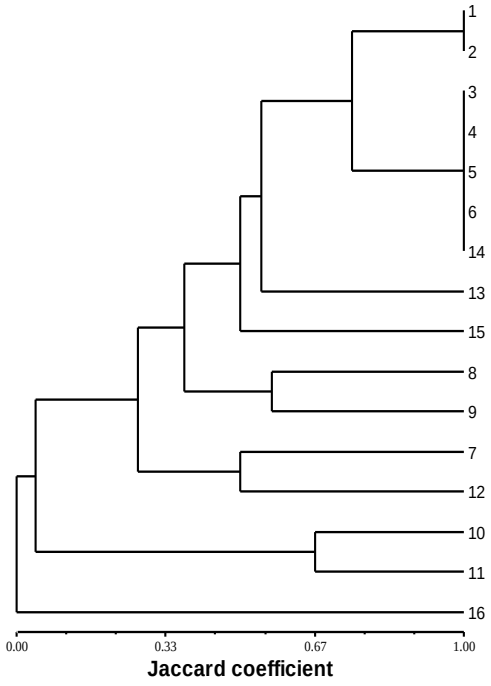

Figure 4. UPGMA phenogram based on the SDS-PAGE data

The second level of the phenogram separates four OTU's: *Opuntia humifusa* (9), *Pseudorhipsalis ramulosa* (12), *Rhipsalis baccifera* Accession 1 (13), and *Rhipsalis baccifera* Accession 2 (14) at the similarity level of 0.18, on the basis of having yellow matte seeds, large seeds in length and width (2-7.5mm) and (2-4.5mm) respectively, uniform testa, variable seed shape, irregular thickness of anticlinal walls, and flat pereclinal walls in addition to sharing the polymorphic band with molecular weight 27.1 KDa. Furthermore, *Opuntia humifusa* (9) split off from the other three taxa at the similarity level of 0.54 because of rounded seed shape and the presence of microrelief, as well as the bands with molecular weights 21 and 15.5 KDa, which supports its placement in unique tribe Opuntieae, subfamily Opuntioideae (Endler and Buxbaum 1974; Gibson and Nobel 1986; Barthlott and Hunt 1993; Anderson 2001; Nyffeler and Eggli 2002 and Nyffeler and Eggli 2010b) at the same time, the remaining 3 taxa are clearly clustered together at the similarity level of 0.57, which supports their placement in tribe Rhipsalideae (Nyffeler and Eggli 2010b) (Table1).

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At the third level of the hierarchy, *Rhipsalis micrantha* (15) splits off separately at the similarity level of 0.20 due to oblong-ovoid, semi-matte seeds, testa cells are abruptly smaller near hilum and anticlinal walls are at the same level with pereclinal walls, this taxon splits by the same way in both phenograms resulted from seed micromorphological characters and SDS-PAGE analysis data (see Fig 3 and Fig 4)). At the fourth level of the phenogram, 8 taxa are clustered together at the similarity level of 0.25 (Fig 5), this cluster divided into 2 subgroups (a) and (b) at the similarity levels of 0.28 and 0.41 respectively. The subgroup (a) consists of three taxa: *Harrisia pomanensis* Accession 3 (5), *Neobuxbaumia euphorbioides* (8) and *Hylocereus triangularis* (7) this is may due to sharing the long narrow seeds with length (2-7.5mm) and width (0.05- 1.9 mm), uniform cell coat pattern, angular testa cell

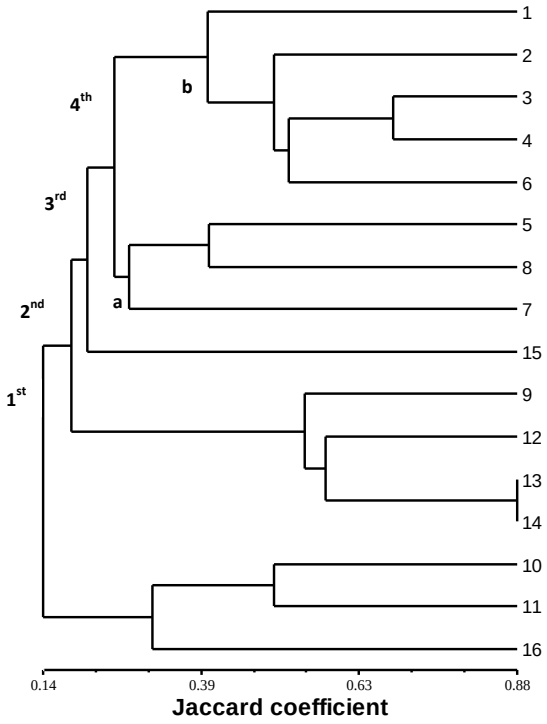

Figure 5. UPGMA phenogram based on the analysis of 43 seed micromorphological characters combined with the data obtained from the SDS-PAGE analysis of the sixteen taxa of Cactaceae.

shape, thick anticlinal walls, in addition to polymorphic bands with molecular weights 27.1 and 20 KDa. On the other hand, the both taxa *Harrisia pomanensis* Accession 3 and *Neobuxbaumia euphorbioides* uncommonly appeared more related to each other and clustered at the similarity level of 0.41, because sharing the band with molecular weight 18.5 KDa. This result is not compatible with the previous works which placed them in two different tribes, while the third one "i.e. *Hylocereus triangularis*" belongs to other different tribe (Endler and Buxbaum 1974; Gibson and Nobel 1986; Barthlott and Hunt 1993; Anderson 2001; Nyffeler and Eggli 2002 and Nyffeler and Eggli 2010b) (Table 1).

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The subgroup (b) comprises the remaining 5 taxa: *Astrophytum myriostigma* (1), *Echinopsis mirabilis* (2), *Harrisia pomanensis* Accession 1 (3), *Harrisia pomanensis* Accession 2 (4), and *Harrisia tortuosa* (6) due mainly to their sharing the black seeds, granulate pattern of seed coat, deeply channeled anticlinal walls, and concave, straighted pereclinal walls, in addition to bands with molecular weights 29, 27.1 and 20 KDa. At the same time, *Astrophytum myriostigma* splits off in far distance from the others because of glossy seeds; granulate pereclinal walls, and the absence of microrelief. This result explains its placement under the unique subfamily Cactoideae, tribe Cacteae (Gibson and Nobel 1986; Barthlott and Hunt 1993; Anderson 2001; Nyffeler and Eggli 2002 and Nyffeler and Eggli 2010b) (Table 1). On the hand, *Echinopsis mirabilis* splits of in separate line at the similarity level of 0.49, but in near distance with the other three taxa, this result supports their placements within subtribe Trichocereinaceae (Anderson 2001; Nyffeler and Eggli 2002 and Nyffeler and Eggli 2010b) (Table1). Furthermore, the taxa numbers 3 and 4 represent two accessions of the *Harrisia pomanensis* species which explains the close relationship to each other at the similarity level of 0.68. The pure clustering of the three taxa numbers 3, 4 and 6 is compatible with the placement of genus *Harrisia* under different tribes than Trichocereinaceae by the results of Endler and Buxbaum (1974); Gibson and Nobel (1986) and Barthlott and Hunt (1993) (Table 1).

In summary, Seed micromorphology and biochemical characters, perhaps in combination, seem to be used at the first time in studying the classification of Cactaceae. The inclusion of these characters in a numerical analysis are useful in understand the relationships within Cactaceae. The findings revealed by this analysis conflict in a number of cases with traditional, well-established ideas about relationships within this family, but they also provide strong evidence to resolve various old debates about the placement of certain enigmatic taxa.

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APPENDIXES

Appendix 1. The collection data of the studied taxa as kept in The Botanic Garden, Berlin-Dahlem

No.	Taxa	Collection data
1	*Astrophytum myriostigma* Lem.	XX-0-B-0327074
2	*Echinopsis mirabilis* Speg.	XX-0-B-1274174
3	*Harrisia pomanensis* (F.A.C. Weber) Britton and Rose (Accession 1)	PY-0-B-2360685: Boqueron, Estigarribia, leg. Ahlgrimm 339
4	*Harrisia pomanensis* (F.A.C. Weber) Britton and Rose (Accession 2)	AR-0-B-1428794: Prov. Catamarca, Dept. Capayán, E Huillapima, 50m leg. Leuenberger and al. 4362
5	*Harrisia pomanensis* (F.A.C. Weber) Britton and Rose (Accession 3)	AR-0-B-1603786: Prov. La Rioja, Dept. General mOcampo, Milagro, 400m, leg. Leuenberger 3608
6	*Harrisia tortuosa* (Otto and A. Dietr.) Brittonand Rose	XX-0-B-0458674: (Bot. Garten Liège)
7	*Hylocereus triangularis* (L.) Britton and Rose	XX-0-B-0149674
8	*Neobuxbaumia euphorbioides* (Haw.) Buxb.	XX-0-B-1085774
9	*Opuntia humifusa* (Raf.) Raf.	US-0-B-1140979: Georgia (Bot. Garten Halle)
10	*Parodia leninghausii* (K.Schum.) F.H.Brandt	XX-0-B-1553074
11	*Parodia schumanniana* subsp. *Claviceps* (F.Ritter) Hofacker	XX-0-B-0700186: (Bot. Garten Linz)
12	*Pseudorhipsalis ramulosa* (Salm-Dyck)	EC-0-B-1530892: (Aarhus Botanical

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	Barthlott	Institute)
13	*Rhipsalis baccifera* (J.S. Muell.) Stearn (Accession 1)	GF-0-B-2010187: Saül, 206m, leg. Freiberg 309
14	*Rhipsalis baccifera* (J.S.Muell.) Stearn (Accession 2)	IN-0-B-1870488: Westbengalen, S de Krishnanagar (Bot. Garten Lüttich)
15	*Rhipsalis micrantha* (Kunth) DC.	XX-0-B-2660390: (Bot. Garten Leipzig)
16	*Trichocereus vasquezii* Rausch	BO-0-B-2011982: Tarija, leg. Rausch 619 (Bot. Garten Wien, Schönbrunn)

Code of Countries of origin: XX origin unknown; PY Paraguay; AR Argentinien; US USA; EC Ekuador; GF Französisch Guayana; IN Indien; BO Bolivien

Appendix 2. Data matrix based on 43 micromorphological character states of seeds of 16 taxa of Cactaceae, taxa numbers as defined in Appendix (1)

Characters	**No.**	**Character States/ Taxa No.**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Seed shape	1	Ovoid	0	0	1	1	1	1	0	1	0	1	1	1	1	1	0	1
	2	Narrowly ovoid	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	3	Oblong-ovoid	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	4	Elliptic	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5	Rounded	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Seed Color	6	Black	1	1	1	1	1	1	1	0	0	1	1	0	0	0	1	1
	7	Brown	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	8	Yellow	0	0	0	0	0	0	0	0	1	0	0	1	1	1	0	0
Seed luster	9	Matte	0	1	1	0	1	0	0	0	1	1	1	1	1	1	0	1
	10	Semi-matte	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0
	11	Glossy	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Seed length	12	0.8-1.9 mm	0	1	1	1	0	0	0	0	0	1	1	0	0	0	1	1
	13	2 – 7.5 mm	1	0	0	0	1	1	1	1	1	0	0	1	1	1	0	0
Seed width	14	0.05 – 1.9 mm	1	1	1	1	1	0	1	1	0	1	1	0	0	0	1	1
	15	2 – 4.5 mm	0	0	0	0	0	1	0	0	1	0	0	1	1	1	0	0
Over all seed coat pattern	16	Reticulate	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1
	17	Rugose	0	0	0	0	0	0	0	0	1	0	0	1	1	1	0	0
	18	Smooth	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
	19	Granulate	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
Homogeneity of testa "testa cell pattern"	20	Uniform	1	1	0	0	1	1	1	1	1	0	0	1	1	1	0	0
	21	Gradually smaller toward the hilum	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1
	22	Abruptly	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0

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		smaller near hilum																
Cell shape	23	Angular	0	0	0	1	1	0	1	1	0	1	1	0	0	0	0	1
	24	Rounded	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	25	Angular to rounded	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	26	Variable	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	0
Anticlinal walls "shape"	27	Slightly undulated "shallowly channeled"	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0
	28	Undulated "deeply channeled"	1	1	1	1	1	1	0	0	0	0	0	0	1	1	0	0
	29	Raised	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
	30	At the same level	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0
Anticlinal walls "thickness"	31	Thick	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	1
	32	Thin	0	0	1	0	0	1	0	0	0	1	1	0	0	0	1	0
	33	Irregular	1	1	0	0	0	0	0	0	1	0	0	1	1	1	0	0
Periclinal walls "level"	34	± flat	0	0	0	0	0	0	1	0	1	0	0	1	1	1	1	0
	35	Concave	1	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0
	36	Convex	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Periclinal walls "texture"	37	Granulate	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	38	Ribbed	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0
	39	Microreticulate	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
	40	Striated	1	1	1	1	1	1	1	0	0	0	1	0	0	0	0	1
	41	Smooth	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0
	42	Variable	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Microrelief	43	Presence	0	1	1	1	1	1	1	0	1	1	1	0	0	0	0	1

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