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Original article

Morphological Character Study of Some Members of the Family Crassulaceae

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Abstract
The vegetative morphology and leaf epidermal morphology of ten species in the family Crassulaceae were studied with the motive of providing information for the characterization and improvement of their classification. The present study was carried out to investigate the epidermal structure and types of stomata on the lower surfaces of the leaves in the species. The species, namely *Aeonium decorum*, *Crassula capitella*, *Echeveria glauca*, *E. setosa*, *Graptopetalum paraguayense*, *Kalanchoe blossfeldiana*, *K. daigremontiana*, *K. fedtschenkoi*, *K. marmorata*, and *Sedum album*. The species studied have some morphological diagnostic characters unifying, such as succulent habitats, perennial herbs, erect stems, simple leaves, leaf type, and succulent leaf structure**.** The great majority of petiole detection of the leaf was sessile, and the mostly margin leaf were mostly entire. Types of stomata on the lower surfaces of the leaves in all species showed anisocytic stomata, while the leaf epidermis cells had straight, undulate, or rounded anticlinal walls, as well as trichomes were only observed in *Echeveria setosa*. The stomata shape was mostly elliptic, and the epidermal cell shapes were found to be polygonal or irregular on abaxial surfaces in the studied taxa. Thus, the above-mentioned morphological character features are of great taxonomic significance and support the taxonomic description in this family.
Keywords: Crassulaceae, Morphology, Leaf Shape, Epidermal Leaf, Stomata.

Introduction

Crassulaceae is one of the most important groups of succulents that are widely cultivated as ornamentals because their leaves are aggregated into colorful rosettes. Crassulaceae is a family of plants belonging to dicotyledons with succulent leaves and has Crassulacean Acid Metabolism (CAM) photosynthetic pathway, in which plants photosynthesize in the daytime and exchange gases during the cooler temperatures of the night [1]. It is also worldwide distributed; the family includes approximately 34-35 genera and over 1500 species. Crassulaceae includes no crop species, but numerous species are popular in horticulture as rocky gardens, green roofs, and indoor plants. The species of the family Crassulaceae are of succulent habit and typically leaf succulent herbaceous plants with flowers that are usually pentamerous and actinomorphic. Moreover, it has been demonstrated that many species easily hybridize both in wild and cultivated species. [1-5].

The most widely recognized classification of Crassulaceae is the Berger system based on morphological characters. According to this system, the Crassulaceae has six subfamilies, viz., Kalanchoideae, Cotyledonoideae, Sedoideae, Echeverioideae, Crassuloideae, and Sempervivoideae [6-7]. While Thorne (2000) recognized three subfamilies in Crassulaceae: Kalanchoideae, Crassuloideae, and Sedoideae [8]. The key taxonomic problems of this family concern generic and subgeneric classification of the subfamily Sedoideae Berger [9-10]. Jensen (1968) investigated the primary stem vasculature in 69 species of the Kalanchoideae, Cotyledonoideae, and Sedoideae [11].

Although the distribution of Crassulaceae is nearly worldwide and can be found in many parts of the world, it is mostly in the Northern Hemisphere and southern Africa, America, Asia, Europe, the Mediterranean region, the Himalayas, Brazil, and Madagascar. Typically, in dry or cold areas where water may be scarce, although a few are aquatic, in habitats where water is scarce, as a typical northern temperate element. Crassulaceae taxa are highly diverse in their appearance, but they are generally characterized by their succulent leaves and stems. These adaptations allow them to store water in arid environments, making them well-suited to hot and dry climates. Furthermore, the taxa usually grow in arid to semi-arid rocky and mountainous environments, while they are generally absent in true desert hot, arid habitats. Crassulaceae plants can tolerate a wide range of environmental conditions, from hot and dry deserts to cold and snowy mountains [12-16]. Crassulaceae species exhibit a wide range of growth habits, from tiny creeping plants to subshrubs and herbs. Some species grow as epiphytes, meaning that they grow on other plants. Also, some of the species are capable of reproducing vegetatively, producing new plants from stem or leaf cuttings. In general, the succulent and often colorful nature of Crassulaceae species makes them popular ornamental plants in gardens and landscapes around the world.

Crassulaceae are mainly perennial, rarely annual, or generally herbs, but there are some subshrubs and relatively few epiphytic that grow on the surface of plants or aquatic plants with succulent leaves. Stems are succulent, as may also be the underground or succulent roots, and may form rhizomes. Bulbils may form along the stem. In general, the leaf arrangement of Crassulaceae is alternate, opposite, or rarely whorled; the leaf is usually simple, stipules are absent, the leaf blade is entire or slightly incised, rarely lobed or

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imparipinnate, rarely compound pinnate or palmate, sessile or petiolate, leaves more or less flat to semi-terete, margin entire, sometimes toothed or lobed, the leaf is usually persistent, sometimes deciduous or caduceus, every so often dentate or more deeply incised, glabrous or tomentose [9,1,17].

The inflorescences are cymose, corymbiform, spiculate, racemose, paniculate, or sometimes reduced to a solitary flower; inflorescences are usually terminal or axillary. Flowers are usually bisexual, less commonly unisexual [18,19]. Flowers are often small, usually with five petals free or connate to a short to long corolla and arranged in clusters or cymes. They come in many colors, including orange, pink, red, yellow, or white, with sepals almost free or basally connate. Petals free or connate, stamens as many as petals, anthers are basifixed and open lengthwise. Gynoecium of three or more pistils; apocarpous to semi-apocarpous. Ovary is usually superior with terminal inconspicuous stigma, with carpels equal to the number of petals, usually free or almost so, sessile or sometimes stipitate, each forming a single locule, superior, free or almost so, basally with a small to conspicuous basal nectary scale, with few to many ovules [20,21,1].

Fruit is usually capsular with dehiscent follicles, and dry dehiscent. The seeds are small and smooth, generally with fleshy endosperm that is oily, scanty, or not developed; their surfaces have longitudinal ridges (costate) or papillate and usually brownish, fusiform, or ellipsoid; the embryo is straight [21-22]. Jafri and EL-Gadi (1981) recorded 17 species belonging to three genera in Libya, in addition to cultivated ornamental species of *Kalanchoe, Bryophyllum,* and *Echeveria*, often used as decorative plants in gardens or within the house [18]. Boulos (1999) recognized three genera comprising five species in Egypt [23]. Hooker (1886) recorded 40 taxa belonging to eight genera from the Indian subcontinent, of which two species were explored from Bangladeshi territory from Crassulaceae [24].

The classification of succulents is very difficult because the variability of most taxa is fairly large, and the phenomenon of succulence is often a condition for convergences. A number of taxa are used in horticulture, which tends to complicate their taxonomy (Uhl, 1963). The species in the Crassulaceae family grow in succulent forms. It's also hard to put them into groups within the Crassulaceae because many of the species can easily hybridize, both in the wild and in gardens, and some taxa of different species share traits with other taxa. The crassulaceae family was discussed by Schönland (1891) in Die Natürlichen Pflanzenfamilien of Engler and Prantle, giving the most definitive treatment until the revision by Berger (1930) [25-6].

Various authors have emphasized the use of morphological characters in taxonomy [26]. ‘t Hart (1995) treated various taxa that were formerly classified as Sedum in 32 segregate genera, utilizing a combined data set from morphology and molecular [5]. The leaves are the most morphologically diverse organ in angiosperms which can be used as valuable taxonomic tools and differentiation between species and families. There is little information about the macromorphological characters of some species in this family, and the available data involve only a few and more common species of the family. In the present study, the character features of the species are investigated to provide knowledge of essential taxonomic traits that would be helpful in solving some problems of the taxonomy of Crassulaceae and to add a new taxonomic contribution to help in the knowledge of the Crassulaceae.

Materials and methods

The present study is based on 10 species of Crassulaceae that were collected and examined from different phytogeographical regions and some botanical gardens of Libya (Table 1). The identification of species was authenticated based on previous information [27-18-23]. The macromorphological characters of the whole plant, *viz.* habit, stem, leaf, and flower, were studied and described directly from fresh specimens of the studied species or textbooks [18-28]. For light microscopical investigation (LM), lamina epidermal strips were prepared from fresh leaves of the remaining 10 studied species; then they were rinsed gently with running water, dried, and the epidermal layer was peeled and applied with domestic adhesive uniformly on the lower epidermis of the leaves. The epidermal strips were prepared by mechanically stripping [29]. (The photomicrographs were taken using a lamina, light, a microscope, and a digital camera. The magnification power was expressed by (x). Examination was achieved in the laboratory, Department of Biology, Faculty of Education, AL-Zawia University, Al-Zawia, Libya.

Table 1. Collecting data on the studied species of Crassulaceae.

No.	Species	Location
1	*Aeonium decorum* (Webb) Bolle.	C
2	*Crassula capitella* Thunb.	E
3	*Echeveria glauca* (Baker) E.Morren.	C
4	*E. setosa Rose* & Purpus	A
5	*Graptopetalum paraguayense* (N.E.Br.) E. Walther.	D
6	*Kalanchoe blossfeldiana* Poelln.	C
7	*K. daigremontiana* Raym. Hamet and H. Perrier- Ann.	A
8	*K. fedtschenkoi* Raym. Hamet and H. Perrier- Ann.	C
9	*K. marmorata* Baker.	F
10	*Sedum album* L	B

A: Western Mountain. B: Al-Zahra. C: Botanical Garden, Biology Department, Faculty of Education, Al-Zawia University. D: Surman. E: Green Mountain, Libya. F: Sobratah.

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Results and Discussion

The Crassulaceae is morphologically diverse in terms of shapes of leaves, leaf arrangement, anticlinal wall shape, stomata type, and epidermal cell shape. This morphological diversity is pivotal in systematics, and these characteristics have been employed in many species to solve some taxonomic issues at genera or family levels. The morphological characters are observable and frequently used in most taxonomic studies. In the present study, the obtained morphological data are presented in tables (2-3) and (Plate 1) to simplify deducing the most diagnostic characters.

All the species studied were perennial, erect, and succulent. The habit of the plants was sub-shrub in *Aeonium decorum* and *K. daigremontiana* or herbs in *Crassula capitella*, *Echeveria glauca, Echeveria setosa, Graptopetalum paraguayense*, *K. blossfeldiana*, *K. fedtschenkoi*, *K. marmorata,* and *Sedum album.* In the present study, the obtained data are in accordance with Jafri & EL-Gadi (1981), Theide & Eggli (2007), and Salim *et. al.* (2019) [18,1,30]. Thiede & Eggli (2007) stated that the plants of Crassulaceae have been described as subshrubs or herbs with succulent stems and leaves, and also these observations were in agreement with the previous work of Chernetskyy (2012) [1,18].

The results showed that the leaves of the studied species were simple in all the studied species, so the simple leaf type is a unifying character across the study, and these results were compared and agreed with Brito and Brito (1993), Medeiros *et al.* (2004) and Silva *et al.* (2006) [31-33]. Thiede & Eggli (2007) made a similar observation of the simple leaves of species in the family Crassulaceae. As well as this conclusion, in agreement with the work of Jain *et al.* (2008) and Walters (2011) [1,34,35].

The leaf's apex is obtuse in *Kalanchoe blossfeldiana, K. marmorata,* and *Sedum album,* or acute as in the other seven studied species. Akinsulire *et al.* (2018) used leaf apex as a classificatory character in a few species related to the family Combretaceae [36]. Margin is serrate in *Kalanchoe daigremontiana*, crenate in *K. fedtschenkoi* and *K. blossfeldiana,* or entire in *Aeonium decorum*, *Crassula capitella*, *Echeveria glauca*, *E. setosa, Graptopetalum paraguayense*, *K. marmorata,* and *Sedum album*. The leaf arrangements are alternate as in *Sedum album,* rosulate as in *Aeonium decorum, Echeveria glauca,* and *Graptopetalum paraguayens,* or opposite in the other six studied species. These findings were in accordance with those of Heywood (1978) and also with the previous work of Walters (2011), who confirmed the opposite or alternate leaf arrangement in members of the family Crassulaceae [35]. Besides, the present observations agreed with Olutayo and Arogundade (2022), who positioned all the present studied species as having opposite leaf arrangement, except *Bryophyllum delagoense* which has alternate leaf arrangement [37].

The leaves are petiolated as in *Kalanchoe blossfeldiana*, *K. fedtschenkoi,* and *K. daigremontiana,* or sessile as in the other seven studied species. This observation was previously recognized in some members of Crassulaceae by Walters (2011) [35]; besides, it is in accordance with both Chen *et al.* (2018) and Salim *et al.* (2019) [38-30]. The lamina shape is terete as in *Sedum album,* ovate as in *Kalanchoe blossfeldiana,* lanceolate as in *K. daigremontiana,* obovate as in *K. fedtschenkoi, K. marmorata, Echeveria glauca,* and *Graptopetalum paraguayense,* or oblanceolate as in *Echeveria setosa, Aeonium decorum,* and *Crassula capitella.* These data are in accordance with those of both Walters (2011) [35]. The leaf texture was hairy, as in *Echeveria setosa,* or glabrous in the other nine studied species. Two taxa of Crassulaceae, *Kalanchoe pinnata* and *K. crenata*, share characteristics related to leaf morphology, including succulent, decussate, and glabrous leaves, crenate margins, and ovate to elliptical leaf blades [39-40]. Hutchinson & Dalziel (1952) used the morphological features to characterize species in the Crassulaceae [41].

Lamina epidermal characters: The features of the epidermal stomatal type were similar among the abaxial leaf surfaces within the studied species. The leaf types of species studied were homostomatic, which means stomata were present on the abaxial surface of all study species, but trichomes were present just on *E. setosa* while absent in the other nine studied species. This finding was compatible with the work of Salim *et. al.* (2019) [30].

In all presently studied species, the stomata types are anisocytic with three to eight subsidiary epidermal cells that are arranged in mono- to polycyclic. This was agreed with the work of Anjoo & Kumar (2010) [40]. Also, this observation was previously recognized in some members of Crassulaceae by Duarte & Zaneti (2002) [47], besides, it was agreed with the results approved by Woo-Gyn & Min-Wung (1985) [42]. Chernetskyy (2012) recorded the anisocytic stomata in all the 35 studied Kalanchoe species [43]. Moreover, Chernetskyy and Weryszko-Chmielewska (2008) reported that the stomata are anisocytic, enclosed by three subsidiary epidermal cells of various volumes, one of which is considerably smaller than the others [44]. While Korn (1972) reported that stomata in some species of Crassulaceae were anisocytic with six subsidiary epidermal cells [45].

In the present study, the stomata shape was ovate as in Echeveria glauca, K. daigremontiana, and Kalanchoe blossfeldiana, or elliptic in the other seven studied species. Epidermal cell shape on the abaxial surface showed a polygonal appearance as in Aeonium decorum, Graptopetalum paraguayense, Kalanchoe daigremontiana, K. marmorata, and Sedum album, or an irregular one in the five other studied species. This distinction was agreed with the work of Kluge & Ting (1978) [46]. Besides, Woo-Gyn & Min-Wung (1985) suggested that the epidermal cell shape in some species of Crassulaceae was nearly always polygonal [42].

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Table 2. Macromorphological characters of the studied species of Crassulaceae.

Character Species	Duration	Habit	Stem		Leaf						
			Strength	Texture	Arrangement	Petiole detection	Shape	Margin	Apex	Composition	Texture
Aeonium decorum	Perennial	Sub-shrub	Erect	Glabrous	Rosulate	Sessile	Oblanceolate	Entire	Acute	Simple	Glabrous
Crassula capitella	//	Herb	//	//	Opposite	//	//	//	//	//	//
Echeveria glauca	//	//	//	//	Rosulate	//	Obovate	//	//	//	//
E. setosa	//	//	//	Hairy	Opposite	//	Oblanceolate	//	//	//	Hairy
Graptopetalum paraguayense	//	//	//	Glabrous	Rosulate	//	Obovate	//	//	//	Glabrous
Kalanchoe blossfeldiana	//	//	//	//	Opposite	petiolate	Ovate	Crenate	Obtuse	//	//
K. daigremontiana	//	Sub-shrub	//	//	//	//	Lanceolate	Serrate	Acute	//	//
K. fedtschenkoi	//	Herb	//	//	//	//	Obovate	Crenate	//	//	//
K. marmorata	//	//	//	//	//	Sessile	//	Entire	Obtuse	//	//
Sedum album	//	//	//	//	Alternate	//	Terete	//	//	//	//

Table 3. Lamina epidermal characters of the abaxial surfaces of the studied taxa by using light microscope

Character Species	Leaf surface	Epidermal cell shape	Anticlinal wall Shape	Stomata		Trichomes	Leaf type
				Type	Shape		
Aeonium decorum	Abaxial	Polygonal	Straight	Anisocytic	Elliptic	Absent	Homostomatic
Crassula capitella	//	Irregular	Slightly undulate	//	//	//	//
Echeveria glauca	//	//	Rounded	//	Ovate	//	//
E. setosa	//	//	Strongly undulate	//	Elliptic	Non-glandular	//
Graptopetalum paraguayense	//	Polygonal	Rounded	//	//	Absent	//
Kalanchoe blossfeldiana	//	Irregular	//	//	Ovate	//	//
K. daigremontiana	//	Polygonal	Straight	//	//	//	//
K. fedtschenkoi	//	Irregular	Strongly undulate	//	Elliptic	//	//
K. marmorata	//	Polygonal	Straight	//	//	//	//
Sedum album.	//	//	//	//	//	//	//

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The abaxial anticlinal wall was slightly undulate as in *Crassula capitella*, strongly undulate as in *E. setosa* and *K. fedtschenkoi,* rounded as in *Echeveria glauca, Kalanchoe blossfeldiana,* and *Graptopetalum paraguayense,* or straight as in the rest of the four studied species (Table 3 & Figure1). This state has also been reported for other species in Crassulaceae by Woo-Gyn & Min-Wung (1985) and Thiede & Eggli (2007) [42,1]. The morphological characters are employed in various aspects of plant taxonomy; therefore, this study clearly is useful for the identification of species and has taxonomic significance for the species of Crassulaceae. On the other hand, the diagnostic unifying characteristics are useful for the classification and identification of the species studied. The ten studied species have unified features in the morphological characters, such as perennial succulent, erect plants, simple leaf type, homostomatic leaf type, and stomata type. Finally, it is proposed to consider these results for future studies in this family and using as many samples and taxonomic tools as possible for the taxonomy of Crassulaceae.

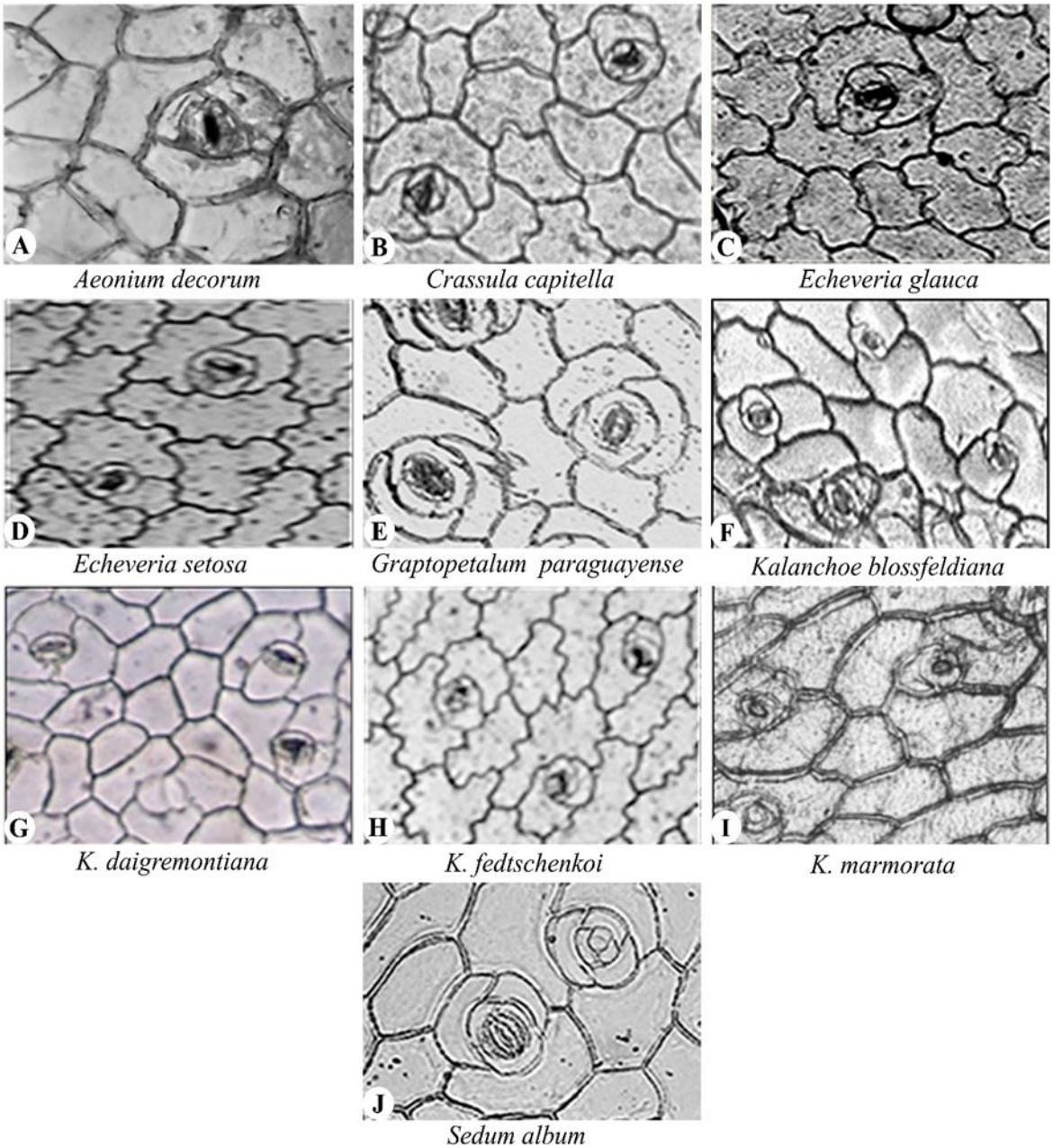

Figure 1. Major aspects of lamina epidermal characteristics (Light microscopy); *A. Anisocytic stomata with straight anticlinal wall; B. Anisocytic stomata with slightly undulate anticlinal wall; C. Anisocytic stomata with rounded anticlinal wall; D. Anisocytic stomata with strongly undulate anticlinal wall and eglandular, unicellular and unbranched trichomes; E. Anisocytic stomata with rounded anticlinal wall; F. Anisocytic stomata with rounded anticlinal wall; G. Anisocytic stomata with straight anticlinal wall; H. Anisocytic stomata with strongly undulate anticlinal wall; I. Anisocytic stomata with straight anticlinal wall.; j. Anisocytic stomata with straight anticlinal wall.*

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المستخلص

تمت دراسة وفحص بعض الصفات الشكلية الظاهرية ومورفولوجيا بشرة الورقة لعشرة أنواع من الفصيلة الكراسيولية (Crassulaceae) بهدف توفير معلومات لتوصيفها وتحسين تصنيفها. أجريت هذه الدراسة للتحقق في بنية البشرة وأنواع الثغور على البشرة السفلية للأوراق في هذه الأنواع. والأنواع هي Aeonium decorum، Crassula capitella، Echeveria glauca، E. setosa وGraptopetalum paraguayense، Kalanchoe blossfeldiana، K. daigremontiana، K. fedtschenkoi، K. marmorata وSedum album. الأنواع المدروسة لها بعض الصفات الظاهرية المورفولوجية الموحدة، مثل الطبيعة العصارية، الأعشاب المعمرة، السيقان المنتصبة، الأوراق البسيطة، نوع الورقة وبنية الورقة العصارية. وقد كانت الغالبية العظمى من الأوراق جالسة، وكانت حافة نصل الورقة في الغالب ملساء. وأظهرت أنواع الثغور على الأسطح السفلية للأوراق في جميع الأنواع ثغورا من النوع anisocytic، بينما كانت الجدر القطرية لخلايا السطح السفلي للورقة مستقيمة، متموجة أومنحنية، كما لوحظت الشعيرات فقط في نبات *Echeveria setosa*. وكان شكل الثغور أهليجياً في الغالب، ووجد أن أشكال خلايا البشرة مُضلعة (عديدة الزوايا) أو غير منتظمة على الأسطح السفلية للورقة في الأصناف المدروسة. وبالتالي، فإن السمات الشكلية المذكورة أعلاه ذات أهمية تصنيفية كبيرة، وتدعم الوصف التصنيفي لهذه العائلة.

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