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Research article

Anatomical adaptations of four *Crassula* species to water availability

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The genus *Crassula* contains a number of highly adaptable species, which can inhabit a wide range of environments. This investigation aimed to examine whether there are any differences in the anatomical adaptations in relation to water availability of four species of *Crassula*: the New Zealand pygmy weed, *Crassula helmsii* (T Kirk) Cockayne (from an aquatic habitat); the fairy crassula: *Crassula multicava* Lemaire ssp. *multicava* (from a subtropical habitat); the jade plant, *Crassula ovata* (Miller) Druce; and the anteelplakkie, *Crassula socialis* Schönland (both from semi-arid habitats). Plants were grown in a greenhouse and the anatomical features of stems and leaves were examined using light microscopy. Plant material was sectioned by hand and sections were stained with Toluidine blue O. Cuticle thicknesses were measured by treating sections with Sudan black B. Stomatal and hydathode densities on leaves and stems were measured using epidermal peels. Two measures of leaf succulence were used: degree of succulence and succulence quotient. The aquatic species *C. helmsii* had significantly fewer features associated with conserving water, including the thinnest cuticles on the adaxial leaf ($P < 0.001$) and abaxial leaf ($P < 0.001$). In contrast, the semi-arid species *C. ovata* had significantly the highest hydathodes on adaxial leaf surfaces ($P < 0.001$). *Crassula ovata* also had significantly the highest degree of succulence ($P < 0.001$), while *C. socialis* had the highest succulence quotient. The subtropical species, *C. multicava*, had significantly the thickest cuticles on adaxial leaf ($P < 0.001$) and stem ($P < 0.001$). *Crassula* species from arid environments had significantly more water conserving anatomical features, such as reduced stomatal densities, than those from less arid environments. However, all species studied possessed varying degrees of similar anatomical features. These features make *Crassula* a highly adaptable genus able to inhabit a wide range of environments.

Key words: *Crassula*, anatomy, adaptation, water availability, succulence, xerophyte.

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Introduction

The unique physical and chemical properties of water lend to it a diverse range of biological roles and make it essential in the structure and function of plants.[1,2] Water is involved in maintaining the structure of nucleic acids,[3] proteins[4] and polysaccharides.[5] It is also a solvent to a variety of organic compounds, ions and small molecules,[6] which accounts for the number of compounds dissolved within cells.[7] By acting as a solvent, water is able to transport compounds through the plant,[6] including sucrose, one of the major products of photosynthesis.[8] Water is also a reactant in photosynthesis,[9] a coolant[10] and is a component of the plant body, making up 90% of the fresh weight of herbaceous plants and 70% of the fresh weight of woody plants.[6] It is, therefore, important for plants to be able to maintain water at adequate levels for survival.

Semi-arid environments are characterized by low rainfall, usually between 250 and 500 mm per year,[11] and are prone to droughts. Droughts are one of the most severe types of plant stress and can significantly reduce plant productivity by causing desiccation, wilting,[12] leaf abscission[13] and eventually death.[14] However, many species have adapted to survive in such habitats and endure periods of drought,[15] leading to the high richness in plant morphology and growth forms often found in arid areas.[16]

Succulence is an adaptation to arid environments commonly found in Crassulacean acid metabolism (CAM)

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plants, including most *Crassula* species.[17] Succulent organs store water and increase the amount of available water.[18] In most plant cells the vacuole occupies 70–80% of the cell's volume, but in succulents this is around 90%.[9] The weight of succulent organs often necessitates either a strong woody stem for support or a compact growth form.[18] Quotients have been developed to quantify succulence and one of the earliest attempts involved dividing the surface area of a leaf by its water content to give amount of water in grams per square decimetre (dm^2) of a leaf.[19] More recent quotients have been developed, including a quotient that gives grams of water per gram of organic matter, which allows an understanding of how much energy a succulent uses to store water.[18]

Succulent plants in arid habitats often possess xeromorphic epidermides, which limit transpiration by having thickened cuticles.[15,18] Other adaptations include lower densities of stomata than mesophytes.[20] Additionally, many *Crassula* species have developed specialized pores named hydathodes, which are similar to stomata in appearance, though often two to three times larger.[18] Hydathodes are usually connected to the vascular tissue,[21] and it has been suggested that hydathodes may be able to take up moisture deposited on leaves by dew or fog.[22,23]

Flooding is another serious plant stress and is a large contributor to plant mortality as it lowers oxygen levels.[9,24] However, some plants have adapted to live in aquatic habitats such as lakes and ponds, including one species of *Crassula*.[25] Some aquatic plants can have a role in maintaining water quality and providing other organisms with food and habitats.[26,27]

Most aquatic plants have little need to conserve water and xeromorphic traits are usually absent.[15] Aquatic plants generally have much thinner cuticles than terrestrial plants, which can be up to three times more permeable.[28,29]

There have been very few anatomical studies of members of the Crassulaceae in the past, possibly due to the difficulties presented by these plants such as thin cell walls and large vacuoles.[21] However, a knowledge of anatomy is very important in achieving a full understanding of plant functions, systematics and development.[15] *Crassula* species have evolved to survive in a wide variety of habitats and possess a range of anatomical adaptations.[18] As *Crassula* contributes significantly to succulent plant diversity, knowledge about their adaptations and evolution is important and may aid in preserving that diversity.

The species investigated were as follows: the New Zealand pygmyweed, *Crassula helmsii* (T Kirk) Cockayne; the fairy Crassula, *Crassula multicava* Lemaire ssp. *multicava*; the jade plant, *Crassula ovata* (Miller) Druce and the 'anteelplakkie', *Crassula socialis* Schönland. *Crassula helmsii* is the only aquatic member of the Crassulaceae.[25] It originates from the edges of water bodies and around cliffs of coastal areas in South Island, New Zealand,[30] and is an aggressive invasive in Europe.[31,32] There have been numerous ecological investigations of this species,[31,32] however, this investigation is novel in examining the anatomy of *C. helmsii*. *Crassula multicava* is a succulent plant that inhabits shaded areas along the subtropical coast of South Africa.[33] It grows along estuaries on rocky cliff edges and its natural habitat experiences high rainfall (about 800–1000 mm per year) and a high maximum temperature of 41°C. *Crassula ovata* is also a succulent;[34] however, it inhabits the Eastern Cape of South Africa, which experiences more droughts and a more unpredictable pattern of rainfall (typical rainfall around 250–550 mm per year) with a maximum temperature of 40°C.[33] *Crassula ovata* generally forms small shrubs or trees, and has thick ovate-shaped leaves.[35] *Crassula socialis* is a rarely studied species, which also inhabits the Eastern Cape, but lives along the coast on cliff faces and has a compact growth form.[33]

The aim of this investigation was to discover whether species of *Crassula* from different environments have differences in anatomy, which could be related to water availability. Anatomical features were described using light microscopy and staining techniques. Cuticle thicknesses, stomata and hydathode densities were quantified. In addition, succulence quotients were used to compare leaf succulence. The results were statistically analysed and differences related to adaptation in different environments. The hypothesis tested was that *Crassula* species originating from more arid environments have more water retention features than those from aquatic environments. Therefore, it is expected that thicker cuticles, thicker epidermides, higher succulence, lower stomatal densities and higher hydathode densities will be found in the most arid species.

Materials and methods

During September 2009 48 cuttings of *C. helmsii* (T Kirk) Cockayne, *C. multicava* Lemaire ssp. *multicava*, *C. ovata* (Miller) Druce and *C. socialis* Schönland were taken from four original plants and placed in a half perlite and half compost mixture (which was kept waterlogged for *C. helmsii*). The cuttings were left to grow in the University greenhouse for ~5 months before sectioning. The plants were kept under ambient conditions in the greenhouse and no specific lighting or temperature regimes were used. These conditions were unlikely to effect the development of any of the species in a way which would affect the results.

Sectioning techniques

A hand-sectioning technique[36] was used to produce fresh sections, a method which allows the observation of living cells and avoids the distortion caused to cells when fixed with chemicals.[37] Cross sections were made by cutting at right angles to the longitudinal direction of the stem or leaf. Sections around 0.25–0.5 mm thick were chosen for

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viewing under the microscope. Plant material and single-edged razor blades were kept moist with distilled water and the cut material was stored in distilled water while further sections were made.

Microscopy

A Nikon LABOPHOT-2 light microscope was used in this investigation and photomicrographs were taken using a Nikon COOLPIX 4500 Digital Camera. Images were cropped and labelled using Microsoft Word drawing tools.

Cells with red or pink contents were identified as tannin cells based on previous anatomical studies on *Crassula* species.[18,21] Toluidine blue O (TBO) pH 4.0 was used to differentially stain tissues.[36] Sectioned material was rinsed in distilled water and then placed in TBO. Sections were then removed from the stain after 5 min for *C. helmsii*, 20 min for *C. socialis* and 30 min for *C. multicava* and *C. ovata*. The stained sections were rinsed with distilled water so that excess stain was removed. Sudan Black B, a lysochrome dye which binds to lipids,[38] was used to demonstrate cuticles so that cuticle thickness could be measured. Sections were placed in industrial methylated spirits (IMS) for a few seconds, then placed in a 100 ml solution of 70% IMS and 0.07 g of Sudan Black B for 5 min. They were then rinsed with 50% ethanol and mounted in glycerine.[39] Photomicrographs were made of sections and cuticle thickness was then measured on screen against the scale and replicated 20 times with stem, abaxial and adaxial cuticles from different cuttings.

Stomata and hydathodes

Epidermal peels were taken from stems and the adaxial and abaxial leaf surfaces of each species using forceps[40] and mounted in distilled water for viewing and photomicrography. Stomata and hydathodes were counted per square millimetre area. Thirty replicates were made for each organ and hydathodes were distinguished from stomata by the presence of larger guard cells with surrounding tannin cells.[18]

Succulence

Degree of succulence considers the amount of water stored in a leaf in relation to the leaf surface area, giving the mass of water in grams per squared decimetre in a leaf:[19]

$$\text{Degree of succulence} = \frac{\text{Water in g at full hydration}}{\text{Surface area of the organ (dm}^2\text{)}}$$

Ten leaves of each species were taken from different plants and the fresh weights were recorded immediately. The surface area of the leaves was measured by placing them on graph paper and counting the number of square millimetre covered,[41] which was then converted into square decimetre. Water was then removed from the leaves by placing them in an oven at 95°C for 48 h. The dried leaves were weighed and this weight was subtracted from the fresh weight to give water in grams at full hydration. The values obtained were used to calculate the degree of succulence.

Leaf succulence was also measured using a succulence quotient that measures the amount of water a leaf can store at the expense of 1 g of organic matter and the formula for this quotient was as follows:[18]

$$\text{Succulence quotient} = \frac{\text{water in g at full hydration/leaf area in dm}^2}{(\text{dry matter} - \text{ash})\text{ in g/leaf area in dm}^2}$$

Leaves were weighed and their surface area was measured in square decimetre using graph paper. The leaves were then placed in an oven at 95°C for 2 days to remove water and reweighed to obtain the dry weight. Water content was calculated by subtracting dry weight from fresh weight. The dried leaves were then placed in furnace at 500°C for 6 h so that ash weight could be recorded. Succulence quotient values were calculated using the succulence quotient formula. The same leaves were used to calculate both the degree of succulence and succulence quotient.

Statistical analysis

Data analysis was carried out using Minitab Version 15.0 and Microsoft Excel 2003. One-way analysis of variance with Tukey's pair-wise comparisons was used to test for significant differences in the adaxial and abaxial leaf cuticle thicknesses, stem cuticle thicknesses, adaxial and abaxial stomatal densities, adaxial and abaxial hydathode densities, degrees of succulence and succulence quotients of all species. Paired two sample *t*-tests were used to determine any significant difference between thicknesses of adaxial and abaxial cuticles in each species and any significant difference in stem stomatal density between *C. helmsii* and *C. multicava*.

Results

Habit

All four species grew very quickly and *C. helmsii* grew at the fastest rate. The leaves of *C. helmsii* were of small size and pale pink and green in colour. *Crassula multicava* and *C. ovata* both had tree-like growth forms and thick, succulent leaves. However, *C. ovata* possessed a woody stem and bark, while *C. multicava* had a more flexible stem and lacked bark. *Crassula socialis* differed from the other species by having shortened internodes and a compact growth form. Its leaves were imbricated and organized into rosettes, and possessed a noticeable covering of trichomes along the margins.

General anatomy

The stems of all species except *C. ovata* had uniseriate epidermides while *C. ovata* possessed a periderm (Table 1;

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Table 1. General stem anatomy of each species: thickness of epidermides; presence of trichomes, stomata and periderms; presence and location of air spaces; presence of secondary thickening

Species	Epidermis	Trichomes	Stomata	Periderm	Air spaces	Secondary thickening
C. helmsii	Uniseriate	Absent	Present	Absent	In cortex	Absent
C. multicava	Uniseriate	Absent	Present	Absent	Absent	Present
C. ovata	Absent	Absent	Absent	Present	Absent	Present
C. socialis	Uniseriate	Absent	Absent	Absent	In cortex	Present

Fig. 1A). All of the species lacked stem trichomes and were glabrous. Stomata were present on the stems of *C. helmsii* and *C. multicava* but absent in *C. ovata* and *C. socialis*. Both *C. helmsii* and *C. Socialis* possessed air spaces within the cortical tissue (Fig. 1B). Secondary thickening was present in *C. multicava*, *C. ovata* and *C. socialis* (Fig. 1C). Amyloplasts and tannin cells were present in all species (Table 2; Fig. 1D). Chloroplasts were found in the cortical cells of *C. helmsii*, *C. multicava* and *C. ovata* but absent in *C. socialis* stems.

The leaves of all species had uniseriate epidermides and all species had glabrous leaves, except *C. socialis*, which had trichomes on the leaf margins (Table 3; Fig. 2A). Stomata (Fig. 2B) were not sunken and all four species were amphistomatic (stomata were present on both leaf surfaces). Hydathodes also occurred (Fig. 2B and C) on both leaf surfaces except in *C. helmsii*, where hydathodes were present on the abaxial surface only. Air spaces were present in the mesophyll of *C. helmsii* and *C. socialis*. The vascular tissue of *C. socialis* was not clearly visible, even after staining with TBO. *Crassula helmsii*, *C. multicava* and *C. ovata* had vascular bundles scattered throughout the leaves (Fig. 2D). Amyloplasts, chloroplasts and tannin cells were found in all species (Table 2).

Crassula multicava had the thickest adaxial leaf cuticle (Fig. 3A) ($P < 0.001$, $F = 28.4$, d.f. $= 76$), while *C. helmsii* had the thinnest adaxial cuticle ($P < 0.001$, $F = 28.4$, d.f. $= 76$). The adaxial cuticle of *C. multicava* was almost

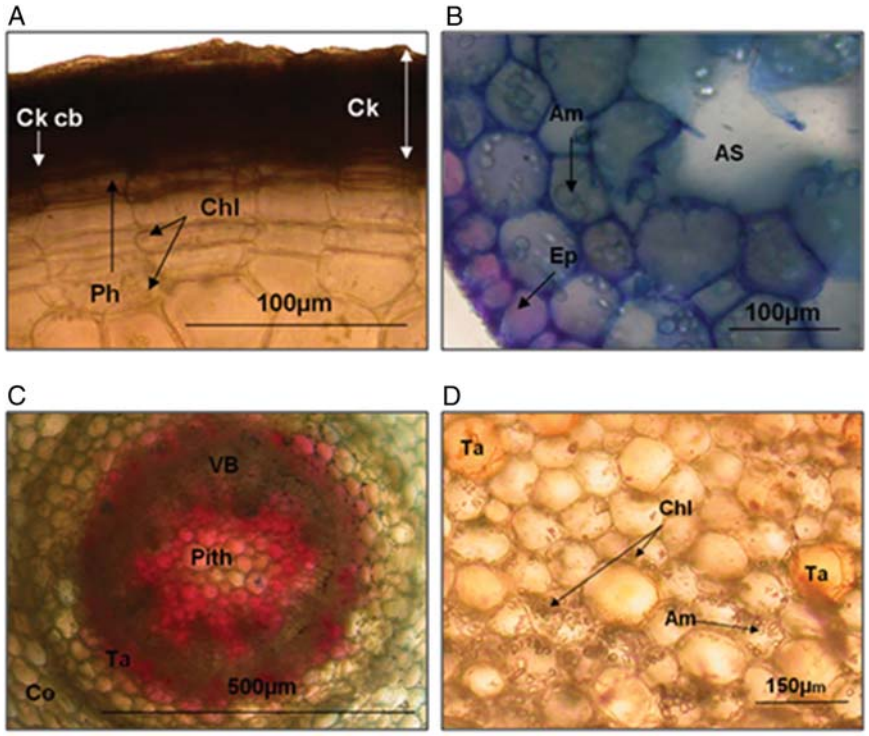

Figure 1. Photomicrographs of stem anatomy for each species studied. (**A**) *Crassula ovata* cross section (unstained) showing a periderm made up of cork (Ck), cork cambium (Ck cb) and phellum (Ph) with chloroplast (Chl) containing cells beneath. (**B**) *C. helmsii* cross section (stained with TBO) showing a uniseriate epidermis (Ep), amyloplasts (Am) and air spaces (AS). (**C**) *C. socialis* cross section (stained with TBO) showing pith, vascular bundles (VB), tannin containing cells (Ta) and part of the cortex (Co). (**D**) *C. multicava* cross section (unstained) showing cortex with tannin containing cells (Ta), chloroplasts (Chl) and amyloplasts (Am).

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Table 2. Cell contents of stems and leaves in each species: presence and location of choroplasts and amyloplasts; presence and location of tannin containing cells

Organ	Species	Chloroplasts	Amyloplasts	Tannin
Stem	*C. helmsii*	Cortex	Cortex	Ep, Vasc. Tissue
	C. multicava	Cortex	Cortex, pith	Cortex
	C. ovata	Cortex	Cortex, pith	Cortex
	C. socialis	Absent	Cortex, pith	Pith
Leaf	*C. helmsii*	Mesophyll, GC	Present	Ep, Hy
	C. multicava	Mesophyll, GC	Present	Ep, Hy, Margins
	C. ovata	Mesophyll, GC	Present	Ep, Hy, Margins
	C. socialis	Mesophyll, GC	Present	Hy, margins

Ep, epidermis; Vasc, vascular; GC, guard cells; Hy, hydathodes.

twice as thick as that of *C. ovata* and *C. socialis*. There was no significant difference in adaxial cuticle thickness between *C. ovata* and *C. socialis*. *Crassula multicava* also possessed the thickest abaxial cuticles, and both *C. multicava* and *C. ovata* had significantly thicker abaxial cuticles than *C. helmsii* and *C. socialis* ($P < 0.001$, $F = 17.03$, d.f. = 3). However, there was no significant difference in abaxial cuticle thicknesses of *C. helmsii* and *C. socialis*. In all species, the adaxial cuticles were thicker than the abaxial cuticles, and this difference was significant in *C. multicava* ($P = 0.01$, $t = 2.59$, d.f. = 38, 95% CI = 0.321 μm, 3.029 μm) and *C. socialis* ($P < 0.001$, $t = 6.36$, d.f. = 38, 95% CI = 1.643 μm, 3.257 μm).

The stem cuticle of *C. multicava* was around 3.5 times thicker than those of *C. helmsii* and *C. socialis* ($P < 0.001$, $F = 41.16$, d.f. = 57), however, there was no significant difference between the cuticle thicknesses of *C. helmsii* and *C. socialis*. The stems of *C. ovata* possessed a periderm and no cuticle.

Stomata and hydathodes

Crassula helmsii had the greatest adaxial leaf stomatal density with an average of 20 stomata per mm^2 (Fig. 3B), this was significantly higher than that in the other species ($P < 0.001$, $F = 181.68$, d.f. = 116). No significant differences were found in the adaxial stomatal densities of *C. multicava*, *C. ovata* and *C. socialis*. *Crassula helmsii* had significantly the highest abaxial stomatal density at over 13 per mm^2 ($P < 0.001$, $F = 84.02$, d.f. = 116). *Crassula multicava* had an average of ~6 stomata per mm^2, which was significantly higher than that of *C. ovata* and *C. socialis*. Stomata were only found on the stems of *C. helmsii* and *C. multicava* and the average number of stomata were low in both species, at <1 per mm^2. There was no significance difference in stomatal densities of the stems of these species ($P = 0.16$, $t = 1.43$, d.f. = 58, 95% CI = −0.142, 0.810).

Hydathodes were only found on leaves and the average number was <1 per mm^2 in all species (Fig. 3C). *Crassula ovata* had the highest number of adaxial hydathodes at 0.8 hydathodes per mm^2 ($P < 0.001$, $F = 12.26$, d.f. = 87). There was no significant difference in abaxial hydathode densities between any of the species.

Succulence

When measuring succulence by degree of succulence,[19] *C. ovata* was the most succulent species, containing an average 3 g of water per square decimetre in contrast to *C. socialis* with 1 g, *C. multicava* with 1.5 g and *C. helmsii* with 0.1 g (Fig. 4A) ($P < 0.001$, $F = 124.37$, d.f. = 36).

Using the succulence quotient,[18] the amount of water that can be stored per gram of organic matter was calculated. *Crassula socialis* was the most succulent species, followed by *C. ovata*, *C. multicava* and *C. helmsii* (Fig. 4B). On average a leaf from *C. socialis* could store 10 g more water per gram of organic matter than *C. ovata*. The differences between *C. ovata* and *C. socialis* were not significant. However, *C. socialis* could store significantly more water per gram of organic matter than *C. multicava* and *C. helmsii* ($P < 0.001$, $F = 8.80$, d.f. = 36).

Discussion

Crassula species share many anatomical features but, as expected, a greater number of water-saving features were found in those from arid environments. All species had uniseriate leaf epidermides but arid species were found to have the thickest cuticles. Secondary thickening was found only

Table 3. General leaf anatomy of each species: thickness of epidermides; presence of trichomes; presence and location of stomata and hydathodes; presence and location of air spaces; distribution of vascular tissue

Species	Epidermis	Trichomes	Stomata	Hydathodes	Air spaces	Vascular tissue
C. helmsii	Uniseriate	Absent	Ab and Ad	Ab	Mesophyll	Scattered
C. multicava	Uniseriate	Absent	Ab and Ad	Ab and Ad	Absent	Scattered
C. ovata	Uniseriate	Absent	Ab and Ad	Ab and Ad	Absent	Scattered
C. socialis	Uniseriate	Present	Ab and Ad	Ab and Ad	Mesophyll	Unclear

Ab, abaxial; Ad, adaxial.

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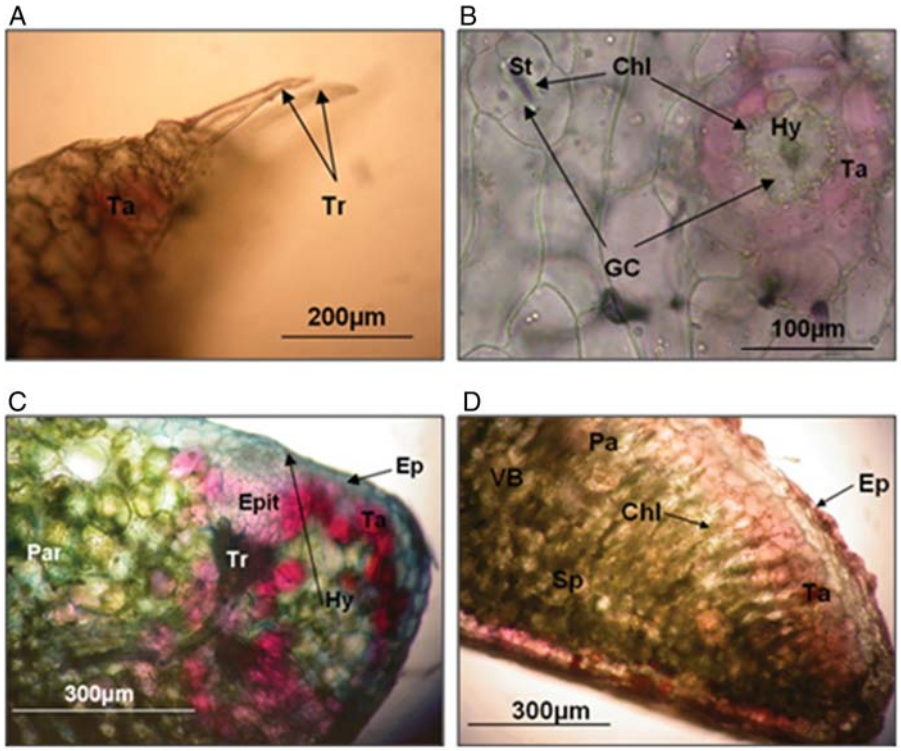

Figure 2. Photomicrographs of leaf anatomy for each species studied. (**A**) *C. socialis* cross section (unstained) showing the leaf margin with a trichome (Tr) and tannin containing cells (Ta). (**B**) *C. helmsii* abaxial epidermal peel showing a stoma (St) and hydathode (Hy) with guard cells (GC) containing chloroplasts (Chl). The hydathode is surrounded by tannin cells (Ta). (**C**) *C. ovata* cross section of leaf margin (stained with TBO) showing parenchyma tissue (Par) and a uniseriate epidermis (Ep). A hydathode (Hy) is apparent with epithem (Epit) and tracheary elements (Tr) surrounded by tannin containing cells (Ta). (**D**) *C. multicava* cross section of leaf margin (unstained) showing palisade (Pa) and spongy (Sp) mesophyll and a vascular bundle (VB). Chloroplasts (Chl) and tannin cells (Ta) are present, along with a uniseriate epidermis (Ep).

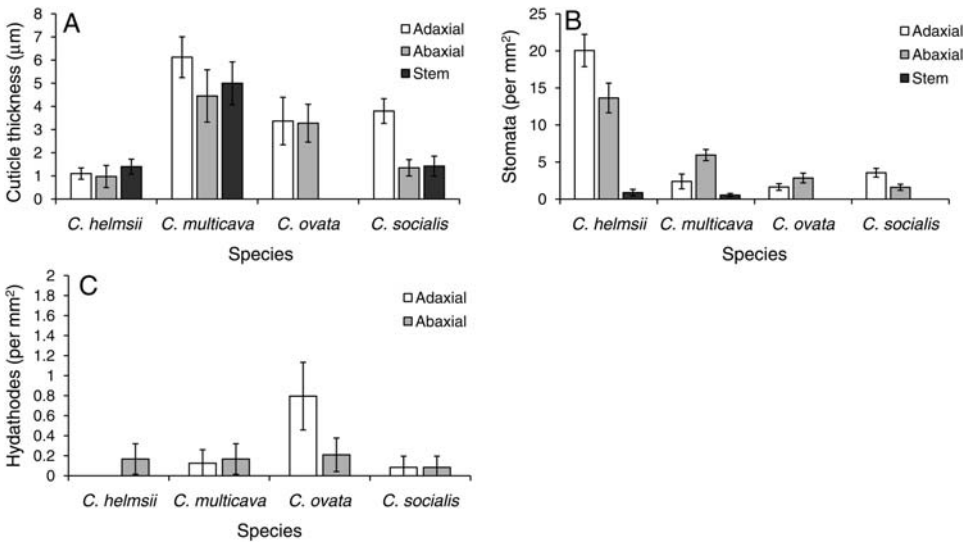

Figure 3. Measurements of leaf and stem anatomical features in the species studied. (**A**) Mean thickness of adaxial, abaxial and stem cuticles in micrometre (error bars = 95% confidence intervals). (**B**) mean number of stomata per mm^2 on adaxial, abaxial and stem epidermal peels (error bars = 95% confidence intervals). (**C**) mean number of hydathodes per mm^2 on adaxial and abaxial epidermal peels (error bars = 95% confidence intervals).

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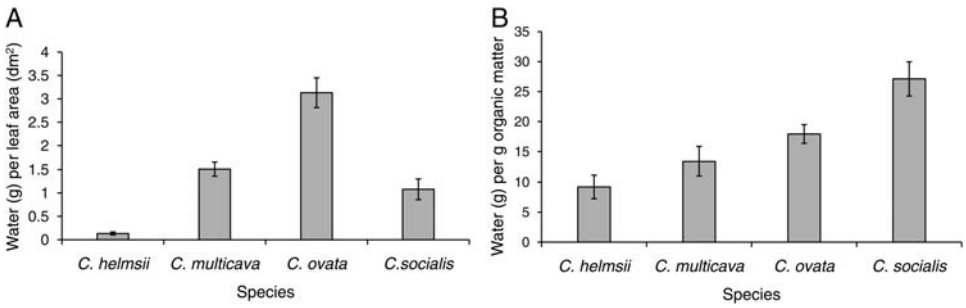

Figure 4. Leaf succulence measurements of the species studied. (**A**) the degree of succulence in grams of water per leaf area (dm^2) (error bars = 95% confidence intervals). (**B**) the succulence quotient in grams of water per gram of organic matter (error bars = 95% confidence intervals).

in the arid species. There were no major differences in possession of chloroplasts, amylpolasts and tannin-containing cells between arid and the aquatic species but only *C. socialis* possessed trichomes. Each species was amphistomatic, with low stomatal densities but the arid species had the lowest stomatal densities and highest hydathode densities. Arid species were also the most succulent.

Previous studies have found that multiple epidermides are not always present in succulent species,[18] possibly because many succulents can increase water use efficiency through CAM.[35] All species investigated were found to have uniseriate leaf epidermides and uniseriate stem epidermides except *C. ovata* (which had a periderm).

Cuticles provide a barrier against water loss.[42] The adaxial surface of leaves is usually more exposed to desiccation than the abaxial surface and, therefore, often has thicker cuticles.[43] This was observed in the four investigated species. *Crassula helmsii* had the thinnest leaf cuticles as it is at lower risk of desiccation.[9] *Crassula multicava*, from the subtropical coast, was found to have the thickest cuticles. Plant cuticles have other functions besides the prevention of water loss such as protection from fungi,[44] which may be important in a moist subtropical environment.

Secondary thickening was present in the stems of *C. multicava* and *C. ovata* and it is likely to have an important role in supporting the weight of their succulent leaves.[18] Neither *C. helmsii* nor *C. socialis* should require secondary thickening for support, as *C. helmsii* is an aquatic species and can be supported by water,[45] while *C. socialis* has a compact growth form.[18] As expected there was no evidence of secondary thickening in *C. helmsii*. However, secondary thickening was observed in *C. socialis*, suggesting that this trait has other benefits to *Crassula* species in addition to providing support for succulent leaves. *Crassula ovata* was found to possess a periderm, which can act as a barrier to water loss.[46] The periderm replaces the epidermis during secondary thickening, and is made up of cork, cork cambium and phelloderm.[17] Cork cell walls contain suberin, which is hydrophobic and provides additional protection from desiccation.[47] The lack of periderm in *C. multicava* and *C. socialis* was probably due to these plants being in an early stage of development in this investigation.

Chloroplasts were present in the mesophyll and guard cells of leaves in all species, and in the cortex of stems in all species except *C. socialis*. The presence of amyloplasts within stems and leaves indicates that the plants are photosynthesizing and storing the products as starch.[48] *Crassula socialis* lacked chloroplasts within stems, possibly because its compact growth form keeps stems from being exposed to light and prevents chlorophyll development. Although *C. ovata* possessed a thick periderm, the first few layers of cells beneath contained large numbers of chloroplasts indicating the presence of lenticels for gas exchange to allow photosynthesis. This is similar to other Crassulaceae species, which can carry out photosynthesis within stems.[49] Aerenchyma and intercellular air spaces were present in *C. helmsii* and would allow the exchange of gases and prevent anoxia when submerged.[9] Intercellular air spaces also allow respiration in tissues of many plants,[50] and small air spaces were observed in *C. socialis*.

Trichomes were present on the leaf margins on *C. socialis*, which was the only species in which trichomes were observed. An important feature of arid environments are high levels of ultraviolet-B radiation that can damage cells in leaves.[50] Trichomes can protect leaves from this damage by reflecting solar radiation[48] and can also increase the boundary layer of leaves and reduce the rate of transpiration.

Tannin containing cells were present in the stems and leaves of all the species investigated, and are a common feature of many succulent species.[18] This investigation found the greatest densities of tannin cells in the margins of leaves and around hydathodes in *C. ovata* and *C. multicava*. The distribution of tannin cells in *C. ovata* was similar to previous studies of this species.[21] In some Crassulaceae species, tannin cells are thought to prevent damage from excessive incoming solar radiation in high-light environments.[51] In this way, tannins may protect *C. ovata* from the high radiation in the Eastern Cape in the same way as trichomes protect *C. socialis*, which is from the same region. In contrast, *C. multicava* often grows in

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shade and in a less arid habitat.[33] Chemical analyses of wild *C. multicava* have found large amounts of condensed tannins,[52] correlating with the large number of tannin cells observed within *C. multicava* in this investigation. In *C. multicava* tannins may be useful in discouraging herbivory by inhibiting digestive enzymes of animals, which forage succulent plants.[53,54]

All species were amphistomatic and had low stomatal densities, which agrees with other studies on *Crassula* species.[55] *Crassula helmsii* had significantly higher densities of leaf stomata than other species, on average 20 per mm^2 on the adaxial surface and 13 per mm^2 on the abaxial surface. As *C. helmsii* grows near or in water this species can have a greater number of stomata on the above-water parts without risk of desiccation. *Crassula multicava* had an average of 5 stomata per mm^2 on the abaxial surface, which was significantly higher than that of *C. ovata* and *C. socialis*, possibly because it originates from a less arid environment. *Crassula helmsii* and *C. multicava* were also the only species with stem stomata, although the density was <1 stoma per mm^2. There was a large amount of variation in stem stomata density and no significant difference between the two species. Succulents in general have been found to have low densities of stomata, for example 15–35 stomata per mm^2 of leaf area compared with 200–600 per mm^2 in tropical forest trees, as well as having one of the lowest transpiration rates of all plant types.[56] *Crassula ovata* and *C. socialis* were from the most arid habitats of the species studied, and, therefore, may have a greater need to conserve water by reducing the number of stomata on leaves and lacking stomata on stems.

Hydathodes were present on the leaves of all the investigated species and the average density of hydathodes was low at <1 per mm^2. *Crassula ovata* had significantly more adaxial hydathodes per mm^2 than the other species. However, the presence of hydathodes on all the investigated species is consistent with other studies, which have found that most *Crassula* species possess hydathodes.[18] These features may be important in absorbing water in arid habitats from coastal fog.[18] The hydathodes were found scattered across the entire lamina of the leaves and this is often the case in many Crassulaceae species where any minor vein may terminate into a hydathode on the leaf surface.[57]

There were differences in the level of succulence depending on the quotient used. With degree of succulence (water gram per square decimetre), the order from most succulent to least succulent was *C. ovata* > *C. multicava* > *C. socialis* > *C. helmsii*. With succulence quotient (water gram per organic matter gram), the order was *C. socialis* > *C. ovata* > *C. multicava* > *C. helmsii*. The differences in results reflect that there is no real consensus on how succulence is defined and measured, and that degree of succulence and the succulence quotient measure different things. However, these quotients can both be used to understand different aspects of water storage in leaves, as they show that *C. ovata* and *C. multicava* have the greatest water content per surface area and have thicker leaves, but do not store as much water at the expense of organic matter as *C. socialis*. This indicates that *C. socialis* expends less energy on water storage and is also likely to be faster growing than the other species.[18] The amount of succulence is greater in species that are from more arid environments.

Conclusion

It was found that *Crassula* species show a number of anatomical differences based on habitat of origin, with arid species possessing the most features used to conserve water. The aquatic species, *C. helmsii*, had less water-saving features than *C. multicava*, *C. ovata* and *C. socialis*. The most succulent species, *C. ovata* and *C. socialis*, originated from the driest habitats. *Crassula ovata* also had the highest numbers of hydathodes of all the species studied. All species shared traits such as hydathodes, tannin cells and succulence, regardless of whether they originate from aquatic or arid environments. These traits seem to have multiple uses depending on the species and environment. This is what makes *Crassula* a successful and highly adaptable genus able to inhabit different environments around the world, many of which are extreme.

The results add to knowledge gained from the small number of previous anatomical studies on *Crassula*, confirming that these plants are exceptionally adaptable and have evolved a number of unique anatomical features. This study was novel in looking at the anatomy of lesser known species such as *C. socialis*. Future studies can build upon this by examining other species within *Crassula* and the Crassulaceae, as it is important to gain a better understanding of the evolution of these rarely studied and adaptable plants.

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