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Leaf epidermal structure in the dwarf succulent genus *Conophytum* N.E. Br. (Aizoaceae)

A.J. Young¹, L. Pulido Suarez², M. Kapralov¹ & M.R. Opel³

1. School of Natural Sciences and Psychology, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF UK. (email: a.j.young@ljmu.ac.uk)

2. Departamento de Bioquímica, Microbiología, Biología Celular y Genética. Facultad de Farmacia, Universidad de La Laguna. Avenida Astrofísico Francisco Sánchez, s/n. 38200, Spain.

3. Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Ct 06269 USA.

Summary: The epidermal structure of 66 species and subspecies of the dwarf succulent genus *Conophytum* N.E. Br. was examined using scanning electron microscopy. Taxa within this genus possess a number of adaptations to their arid environment including sunken stomata, a prominent wax layer and trichomes. The range of epidermal morphologies present in this genus is described and the use of these to inform infrageneric classification in *Conophytum* is discussed. In many cases the epidermis of closely related species is similar but this is not always the case across the 16 sections that comprise the genus. Whilst this study confirms the assignment of several recently described taxa to existing sections it suggests that the infrageneric classification of *Conophytum* needs to be re-evaluated.

Zusammenfassung: Die Epidermisstruktur von 66 Arten und Unterarten der Zwergsucculentengattung *Conophytum* N. E. Br. wurde rasterelektronenmikroskopisch untersucht. Die Taxa dieser Gattung besitzen eine Reihe von Anpassungen an ihre trockene Umwelt wie eingesenkte Spaltöffnungen, eine deutliche Wachsschicht und Trichome. Die in dieser Gattung vorhandene morphologische Vielfalt der Epidermen wird beschrieben und deren Nutzen für die infragenerische Klassifikation von *Conophytum* diskutiert. In vielen Fällen ähneln sich die Epidermen nah verwandter Arten, jedoch ist das in den 16 Sektionen, die diese Gattung umfasst, nicht immer der Fall. Während diese Studie die Zuordnung von mehreren kürzlich beschriebenen Taxa zu bestehenden Sektionen bestätigt, zeigt sie gleichzeitig, dass die infragenerische Gliederung von *Conophytum* neu bewertet werden muss.

Keywords: Aizoaceae, *Conophytum*, epidermis, scanning electron microscopy, succulent

Introduction

The dwarf succulent genus *Conophytum* displays a remarkably high degree of diversity (165 species and subspecies are recognised, Hammer & Young, 2017), especially considering it is the result of a very recent radiation event of the family Aizoaceae (Klak *et al.*, 2004; Valente *et al.*, 2014). The genus is strongly associated with the winter-rainfall region of south Africa and Namibia, and especially the Succulent Karoo biome. More than 93% of *Conophytum* taxa are recorded from this biome alone and 60% of taxa are endemic to the biome. Many of the remaining taxa are located on the immediate fringes of this biome (in the Fynbos, Desert and Nama Karoo biomes) and often in the transitional area at the boundary of winter- and summer-rainfall areas (Young & Desmet, 2016). The Succulent Karoo is characterised by a high degree of floral endemism, especially in dwarf leaf-succulents (Driver *et al.*, 2003; Mucina *et al.*, 2006). Such endemism is reflective of the high speciation seen in the genus and may be very highly localised (e.g., many taxa are only recorded from a single hill). This is a strong characteristic in *Conophytum* with approximately a quarter of all taxa regarded as point-endemics. Such localised endemism may be dependent upon specific tolerance to the niche environmental conditions these plants experience.

Conophytum taxa display several adaptations to the arid and semi-arid environments they inhabit. In addition to CAM-photosynthesis (Crassulacean Acid Metabolism) and the miniaturisation of the growth form, the morphology of the leaf epidermis may show several characteristics that minimise water loss (e.g., the presence of trichomes, bladder cells, sunken stomata, a prominent wax layer). Some of these morphological features are found across other members of the Aizoaceae (Ihlenfeldt & Hartmann, 1982).

Differences in epidermal structure across the 16 sections that comprise the genus were highlighted by Opel (2002, 2004). Since those earlier studies, several new *Conophytum* taxa have been discovered and subsequently described. The aim of the current study was to examine the epidermal structure of the remaining taxa in the genus, including those described since the original work.

Materials and Methods

Plant material

A total of 66 individual species and subspecies representing 14 of the 16 defined sections (Hammer & Young, 2017) of the genus *Conophytum* were studied (Table 1). Combined with the earlier work of Opel (2002, 2004) this provides a comprehensive analysis of the epidermal structure of members of this succulent genus. Opel (2002, 2004) examined all the taxa in Sections Batrachia and Subfenestrata and no new observations are made here.

Scanning Electron Microscopy

Thin slices of epidermal material were removed from the upper sides of the plant bodies (connate leaf pairs) using a fresh razor blade. A similar area of tissue was therefore sampled across all species examined and comparable to that used by Opel (2002, 2004). The tissues were immediately fixed in 100% methanol for 30 min, then transferred to 100% dry ethanol for a further 30 min. The tissues were dehydrated further in 100% dry acetone for 30 min, followed by at least 30 min in 100% dry acetone. Comparison with samples prepared by cryo-preservation (i.e., avoiding exposure to organic solvents) showed that the dehydration procedure used in this study retained the structure and integrity of succulent tissues and the epicuticular wax layer. Critical point drying was performed using an Emitech K850 dryer (Quorum Tech Ltd., East Grinstead, U.K.). Samples were coated with gold using a Emitech K550X sputter coater (Quorum Tech Ltd., East Grinstead, U.K.). Coated epidermal tissues were viewed on a FEI Quanta 200 microscope (ThermoFischer Scientific) at 10 or 15kv.

Results and discussion

The morphological features of individual *Conophytum* taxa are described on the basis of the sectional classification (Hammer, 2002; Hammer and Young, 2017). For selected taxa the dimensions of individual stomatal guard cells were determined (Table 2). The length of the guard cells ranged from 15.6µm (*C. bachelorum*, section Wettsteinia) to 28.3µm (*C. flavum* subsp. *novicium* var. *kosiesense* nom. prov., section Wettsteinia) but did

not reveal any taxonomic relationship, nor was it related to stomatal density (per unit area of leaf) or geographic distribution (not shown).

Barbata (Figures 1A–B).

C. depressum subsp. *perdurans* (1A) and *C. stephanii* subsp. *stephanii* (1B)

The observations for these two subspecies align with the observations made by Opel (2002, 2004) for other taxa in this section. Epidermal cells are a simple hexagonal structure; bladder cells are absent; trichomes are very long for the genus at up 300µm; subsidiary cells are glabrous and nested. The epicuticular wax layer is not prominent in either subspecies examined.

Biloba (Figure 1C).

C. velutinum subsp. *polyandrum*

This subspecies possesses a prominent globular epicuticular wax layer typical of the section but different to the powdery wax layer seen in *C. velutinum* subsp. *velutinum* (Figure 1C, c.f. Figures 3–16, Opel 2002), though this may be a reflection of different growth conditions; papillae are present.

Cataphracta (Figure 1D).

C. calculus subsp. *vanzyliei*

The main characteristic of this nocturnal flowering taxon is the presence of sunken stomata (relatively rare in the genus) with two, small, subsidiary cells; epidermal cells are polygonal and may possess poorly developed papillae.

Cheshire-Feles (Figures 1E–F)

C. maughanii subsp. *armeniaceum* (1E) and subsp. *latum* (1F)

Both subspecies are characterised by the presence of glabrous bladder cells. Papillae and trichomes are absent which differentiates them from *C. maughanii* subsp. *maughanii* (Opel, 2002), although truly glabrous taxa exist within this section. A thin epicuticular wax coating is seen in *C. maughanii* subsp. *latum* (Figure 1F).

Conophytum (Figures 2A–F)

C. obcordellum subsp. *rolfii* (2A) and subsp. *stenandrum* (2B); *C. piluliforme* subsp. *edwardii* (2C); *C. truncatum* subsp. *viridicatum* (2D); *C. uviforme* subsp. *decoratum* (2E) and subsp. *subincanum* (2F).

In common with *C. obcordellum* subsp. *ob-*

cordellum (Opel, 2002) the epidermal cells of both subsp. *rolfii* (Figure 2A) and subsp. *stenandrum* (Figure 2B) possess interlocking corners, the stomata are superficial with three nested subsidiary cells. But in contrast to subsp. *obcordellum* the cells of both subsp. *rolfii* and subsp. *stenandrum* have short trichomes, although there are patches of the epidermis which are glabrous.

The epidermal cells of *C. piluliforme* subsp. *edwardii* possess papillae whilst those of *C. truncatum* subsp. *viridicatum* (Figure 2D) have short trichomes. The epicuticular wax layer in the latter is very prominent. The stomata have three subsidiary cells in *C. piluliforme* subsp. *edwardii* (Figure 2C) but only two are evident in *C. truncatum* subsp. *viridicatum* which also has raised stomata (unusual in the section), in common with *C. truncatum* subsp. *truncatum* (Opel, 2002). Both *C. uviforme* subsp. *decoratum* (Figure 2E) and subsp. *subincanum* (Figure 2F) also possess raised stomata, in contrast to *C. uviforme* subsp. *uviforme*. The epidermal cells of these two taxa possess papillae and a prominent wax layer.

Costata (Figure 3A)

C. angelicae subsp. *tetragonum*

The epidermis of *C. angelicae* subsp. *tetragonum* (Figure 3A) differs from that of subsp. *angelicae* (Opel, 2002, 2004) in that the cells lack interlocking corners. Cells have blunt papillae and the superficial stomata are surrounded by three glabrous subsidiary cells.

Cylindrata (Figures 3B–F)

C. buysianum subsp. *buisianum* (3B) and subsp. *politum* (3C); *C. roodiae* subsp. *corrugatum* (3D) and subsp. *sanguineum* (3E); *C. youngii* (3F)

In *C. buysianum* subsp. *buisianum* (Figure 3B) and subsp. *politum* (Figure 3C) epidermal cells may possess interlocking corners, but this is a less pronounced feature than in other members of the section. In subsp. *buisianum* papillae are generally absent and may be confined to the tips of the plant body. In contrast, subsp. *politum* is covered with short trichomes (to 15µm). A smooth wax layer is present in both taxa. Stomata are superficial with three subsidiary cells.

The epidermal morphology of two subspecies of *C. roodiae* examined here differ. The epidermis of *C. roodiae* subsp. *sanguineum* (Figure 3E) closely resembles that of subsp. *cylindratum* (recently restored to full species status; Hammer and Young, 2017) – possessing cells with undulate margins and interlocking corners. By contrast the epidermis of subsp. *corrugatum* (Figure 3D) more

closely resembles that of *C. buysianum* than *C. roodiae*, lacking as it does cells with undulate margins. Given other morphological similarities between subsp. *corrugatum* and subsp. *roodiae* (these taxa are readily confused in habitat) this is an unexpected observation. In *C. roodiae* the stomata are superficial and possess three subsidiary cells.

C. youngii (Figure 3F) has epidermal cells with undulate margins and interlocking corners like most taxa in the section. However, the minute ridges on the cells — cuticular folds — are extremely rare in the genus, with similar structures found only in two other species, both in section *Cylindrata*: *C. khamiesbergense* and, especially, *C. rugosum* (Opel, 2002). On this basis the original assignment of this new taxon to section *Cylindrata*, with *C. rugosum* as its closest relative, appears to be correct (Rodgerson, 2012).

Herreanthus (Figures 4A–C)

C. danielli (4A); *C. marginatum* subsp. *karamoepense* (4B) and subsp. *littlewoodii* (4C)

The epidermis of *C. danielli* (Figure 4A) most closely resembles *C. blandum* (Opel, 2002, 2004) rather than the other species in this section (*C. herreanthus*, *C. marginatum* and *C. regale*). The surface is covered by a dense layer of wax granules; cells are polygonal and possess long (to 100µm) trichomes; stomata are superficial with four glabrous subsidiary cells. Both subspecies of *C. marginatum* examined here (Figures 4B, C) share features with *C. herreanthus* and also possess a dense epicuticular granular wax layer. Both subsp. *karamoepense* (Figure 4B) and subsp. *littlewoodii* (Figure 4C) have part-sunken stomata with two subsidiary cells that possess papillae. In subsp. *karamoepense* parts of the epidermis may be glabrous.

Minuscula (Figures 4D–F; 5A–F; 6A–F; 7A–F)

C. antonii (4D); *C. auriflorum* subsp. *turbini-forme* (4E); *C. brunneum* (4F); *C. bruynsii* (5A); *C. cubicum* (5B); *C. ectypum* subsp. *brownii* (5C) and subsp. *cruciatum* (5D); subsp. *ignavum* (5E) and subsp. *sulcatum* (5F); *C. hanae* (6A); *C. hyracis* (6B); *C. irmae* (6C); *C. longibracteatum* (6D); *C. minusculum* subsp. *aestiflorens* (6E); *C. mirabile* (6F); *C. pium* (7A); *C. swanepoelianum* subsp. *proliferans* (7B); *C. tantillum* subsp. *amicorum* (7C), subsp. *heleniae* (7D), subsp. *eenkokerense* (7E) and subsp. *lindenianum* (7F)

On the basis of its epidermal characteristics, especially cell shape, Opel (2002, 2004) divided this large section into two broad geographical

groups – representing southern and northern forms (the epidermis of northern forms may also resemble that seen in some taxa in section Biloba and Wettsteinia – see below). In northern taxa, the cells are typically hexagonal and this was generally observed here (e.g., in *C. cubicum* Figure 5B) but this cell shape was also observed in some taxa whose geographical range is restricted to more southern latitudes (e.g., *C. hyracis* from the Knersvlakte). Similarly, species whose epidermal cells have distinct undulate margins (e.g., in *C. ectypeum*) and/or interlocking corners (e.g., *C. irmae* Figure 6C, *C. tantillum* subsp. *lindenianum* Figure 7F), as was considered typical of southern Minuscula species, can be found in the northern part of the distribution range of the genus. The cell shape in *C. hanae* (Figure 6A) appears to be intermediate between these types. Indeed, once the additional 21 taxa studied here are considered alongside those taxa originally examined by Opel (2002, 2004), the north-south distinction based on cell shape *per se* becomes less clear cut. The presence of prominent blunt papillae or trichomes is more frequent in the northernmost species whilst southern species are mostly glabrous or with only very faint papillae (e.g. *C. brunneum* Figure 4F).

The epidermal cells of *C. auriflorum* subsp. *turbiniiforme* (Figure 4E) and subsp. *auriflorum* are of different shape: in the former the cells have undulating margins and interlocking corners compared to hexagonal cells seen in the latter (Opel, 2002, 2004). Pollen exine structure in these two taxa also differs (unpublished). Taken together this suggests that these may be better treated as separate species.

A granulated epicuticular wax layer is seen across the section (with the exception of *C. mirabile*) and in many species is very prominent (notably in northern taxa such as *C. cubicum*). Stomata are superficial and possess two or three subsidiary cells which may be nested or parallel.

Within this large section a number of taxa stand out in terms of their epidermal morphology. For example, the elongated epidermal cells of *C. minusculum* subsp. *aestiflorens* make it one of the most distinctive taxa. *C. mirabile* differs from other taxa examined in this section by the presence of long (ca. 500µm) trichomes. All other Minuscula examined had papillae (often faint) or were glabrous (e.g., *C. pium*).

The classification of some recently described species (*C. pium*, *C. hyracis* and *C. antonii*; Hammer, 2009) as Minuscula (largely on the basis of flower structure) is supported by their epidermal characteristics. This large section of taxa displays a wide range of structural features

Ophthalmophyllum (Figures 8A–B)

C. concordans (8A); *C. devium* subsp. *stiriferum* (8B)

Both of these taxa share some common features of the epidermal structure reported by Opel (2002) for other members of section Ophthalmophyllum. The epidermis of *C. concordans* (Figure 8A) is almost identical to that of *C. caroli*; cells are papillate (or with short trichomes); bladder cells are prominent; stomata are superficial with three subsidiary cells. The bladder cells of *C. devium* subsp. *stiriferum* (Figure 8B) are arguably its most distinctive feature. However, the epidermal cells have fully undulate margins with interlocking corners – a feature unique within the section (other species in the section have interlocking corners only). It also differs from subsp. *devium* in that the three nested subsidiary cells that surround the superficial stomata are glabrous.

Pellucida (Figures 8C–F; 9A–B)

C. arthurolfago (8C); *C. lithopsoides* subsp. *bo reale* (8D), subsp. *koubergense* (8E); *C. pellucidum* subsp. *cupreatum* (8F), subsp. *cupreatum* var. *terrestre* (9A) and subsp. *saueri* (9B)

All of these taxa possess papillae, but the subsidiary cells are typically glabrous (except for *C. pellucidum* subsp. *cupreatum* and var. *terrestre*, Figure 9A). Three subsidiary cells around the superficial stomata are common, but four (occasionally five) are seen in *C. arthurolfago* (Figure 8C). The epidermis of *C. pellucidum* subsp. *cupreatum* var. *terrestre* is unique in that it possesses rows of longer trichomes. These can be seen by the naked eye and give some populations of var. *terrestre* its characteristic ‘icicle’ sides (although this feature did not form part of the formal description). Whilst the cells of *C. arthurolfago* and the two subspecies of *C. lithopsoides* are polygonal (Figures 8D, E), those of *C. pellucidum* subsp. *cupreatum* and subsp. *saueri* (Figure 9B) possess interlocking corners and in the case of var. *terrestre* the cells have undulate margins.

Saxetana (Figures 9C–D)

C. klinghardtense subsp. *baradii* (9C); *C. quaesitum* subsp. *densipunctum* (9D)

The hexagonal epidermal cells of both these taxa are covered by a dense granular wax layer. A prominent feature of *C. klinghardtense* subsp. *baradii* (Figure 9C) is the covering by medium length (ca. 50µm) appressed trichomes. Unlike subsp. *klinghardtense* (raised stomata) stomata in subsp. *baradii* are superficial and the pair of sub-

subsidiary cells lack papillae. The stomata more closely resemble those seen in *C. quaesitum* subsp. *quaesitum* in which the stomata may be half-sunken (Opel, 2002). A similar, albeit less pronounced, feature is seen in *C. quaesitum* subsp. *densipunctum* (Figure 9D). The subsidiary cells of the stomata of this taxon often feature a prominent appressed trichome.

Verrucosa (Figure 9E)
C. hermarium

The undulate margins of the glabrous (occasionally with short papillae) epidermal cells of *C. hermarium* are also seen in *C. vanheerdei* (Opel, 2002). The superficial stomata with three subsidiary cells are typical of the section.

Wettsteinia (Figures 9F; 10A–F; 11A–F)

C. bachelorum (9F); *C. chrisolum* (10A); *C. confusum* (10B); *C. crateriforme* (10C); *C. francoiseae* (10D); *C. jucundum* subsp. *fragile* (10E), subsp. *marlothii* (10F) and subsp. *ruschii* (11A); *C. obscurum* subsp. *sponsaliorum* (11B) and subsp. *vitreopapillum* (11C); *C. smaleorum* (11D); *C. taylorianum* subsp. *ernianum* (11E) and subsp. *rosynense* (11F)

Across the section, epidermal cells are typically polygonal (in *C. obscurum* subsp. *vitreopapillum* the cells have interlocking corners) and possess papillae. The blunt papillae are often very faint and may even be absent in patches across the epidermis. Two subsidiary cells are usually present in taxa right across the section, occasionally three (e.g., *C. smaleorum* Figure 11D, *C. obscurum* subsp. *sponsaliorum* Figure 11B). The epicuticular granular wax layer is often very prominent (notably in *C. obscurum* subsp. *sponsaliorum*).

The stomata of taxa in this section are generally superficial (as in the majority of *Conophytum* species) but in *C. jucundum* subsp. *marlothii* and subsp. *ruschii* the stomata are sunken and closely resemble the epidermis of subsp. *jucundum* (c.f. Figures 3–17 in Opel, 2002). However, in *C. jucundum* subsp. *fragile* the stomata are superficial (Figure 10E). The two subsidiary cells of all three subspecies of *C. jucundum* are relatively unusual in that they possess papillae or trichomes (in the case of subsp. *ruschii*). Sunken stomata are also found in several other populations of *C. jucundum* (not shown) and in other taxa in this section, notably *C. francoiseae* (Figure 10D), another Richtersveld endemic.

When *C. crateriforme* was recently described it was thought to be more closely related to *C.*

francoiseae rather than *C. jucundum* (Young *et al.*, 2015a). The epidermal structures seen here suggest that it has affinity to neither as it lacks the sunken stomata seen in both of these, possessing superficial stomata instead (Figure 10C). Another recently described species, *C. smaleorum* (Figure 11D), was also assigned to section *Wettsteinia* (Rodgers & Young, 2013) and, although it possesses three subsidiary cells around its superficial stomata rather than the usual two, it fits well into this section.

The cell size of *C. jucundum* subsp. *jucundum* has been reported as atypically small for the section (ca. 25µm diameter). In the present study *C. francoiseae* was similarly sized but the dimensions of other subspecies of *C. jucundum* were found to be more typical at 50µm.

Because of a lack of material in cultivation at that time, Opel (2002, 2004) was only able to examine fragmentary air-dried material for *C. bachelorum* and, on that basis, determined that the epidermis was glabrous (and similar to *C. chrisocruxum*). The rediscovery of *C. bachelorum* in habitat has permitted analysis of fresh leaves. This revealed the presence of faint (low) papillae rather than a completely glabrous surface. It also confirmed that the stomata are superficial (Figure 9F). *C. confusum* (Young *et al.*, 2015b) has an epidermal morphology very similar to that of *C. bachelorum* (c.f. Figures 10B & 9F). *C. chrisolum* also possesses low, blunt, papillae, although areas of the epidermis are completely glabrous (Figure 10A). By contrast, the epidermis of *C. chrisocruxum* lacks papillae (Opel 2002, 2004). Based on differences in their phenology (seasonality of flowering), these four species can be placed into two pairs: *C. bachelorum* and *C. chrisocruxum* that flower in the austral spring, whilst *C. confusum* and *C. chrisolum* flower in the austral autumn.

Overall, the epidermal structure of *Conophytum* shows several morphological adaptations to the arid environment in which the plants live. Table 3 provides a summary of the main epidermal features identified by this study and that conducted by Opel (2002, 2004) across the 16 infrageneric sections that comprise the genus. Xerophytic adaptations seen in *Conophytum* include bladder cells (rare across the genus), trichomes (which vary considerably in length ranging from short papillae to long hairs), a wax layer (typically granular) and sunken stomata (again rare, see below). Such features may be a useful tool in infrageneric classification but usually only when combined with other morphological features (e.g. flower structure) and molecular phylogenies (Powell, 2016).

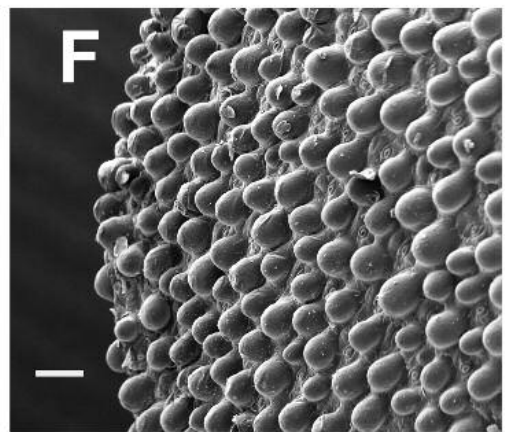
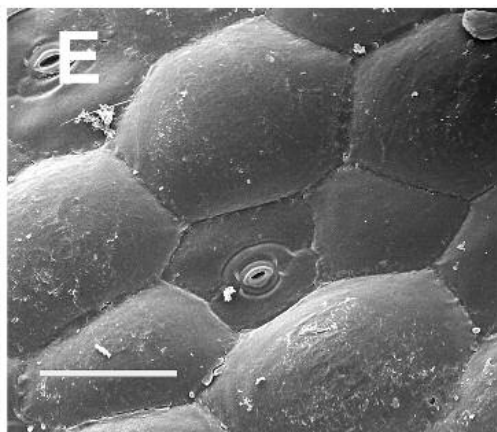
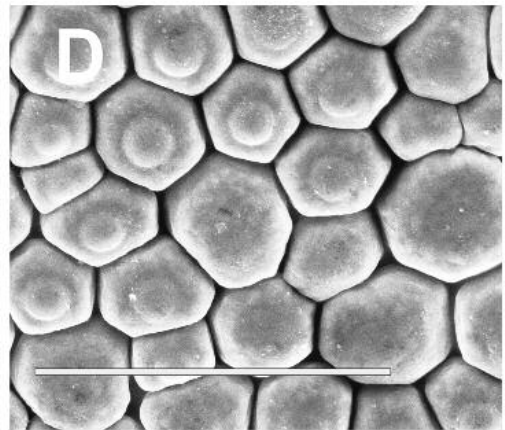
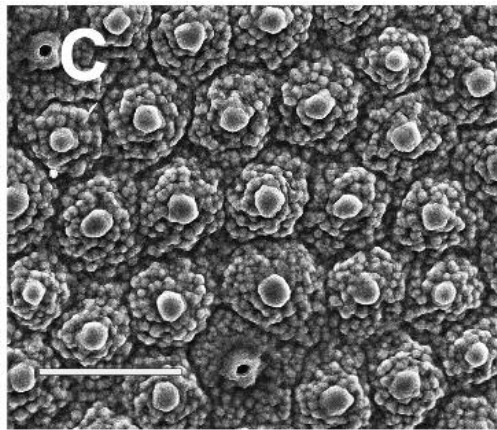
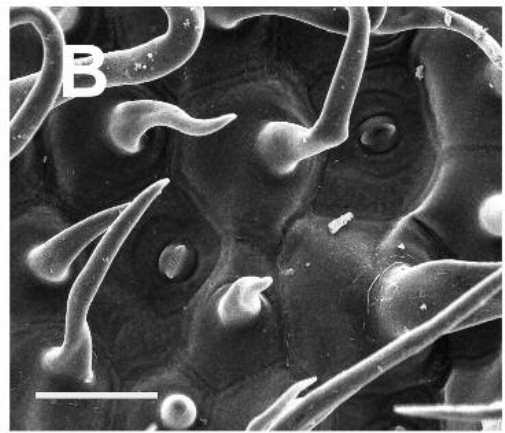
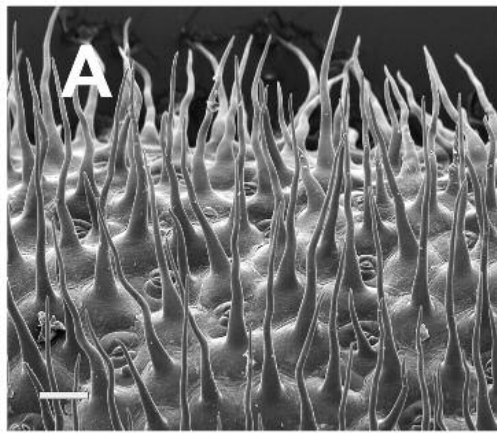


Figure 1. (A) *C. depressum* subsp. *perdurans*; (B) *C. stephanii* subsp. *stephanii*; (C) *C. velutinum* subsp. *polyandrum*; (D) *C. calculus* subsp. *vanzylii*; (E) *C. maughanii* subsp. *armeniacum*; (F) *C. maughanii* subsp. *latum*. Scale bar = 100µm

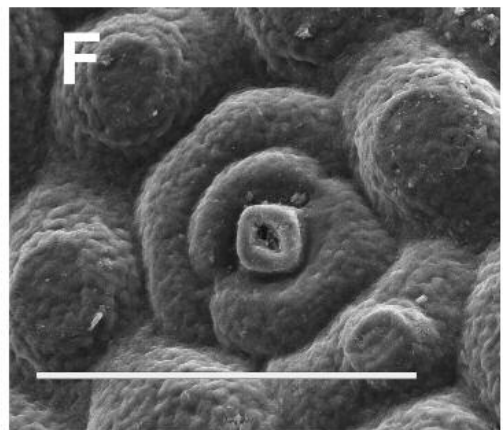
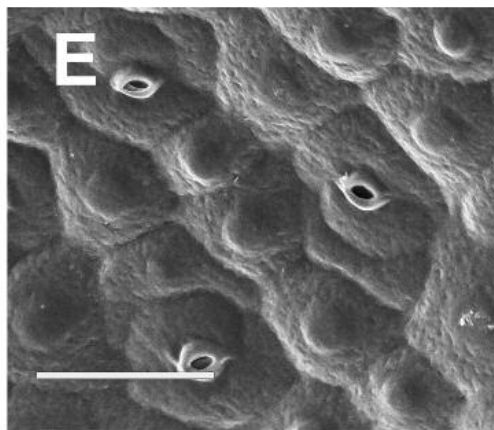
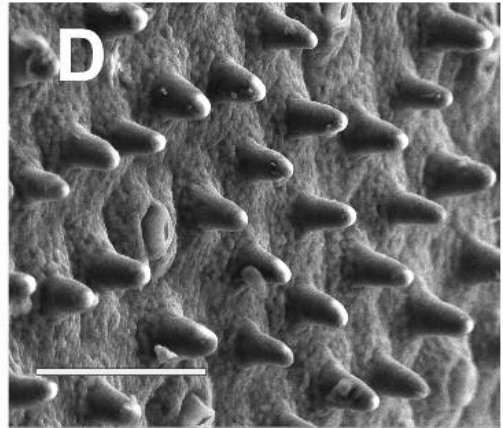
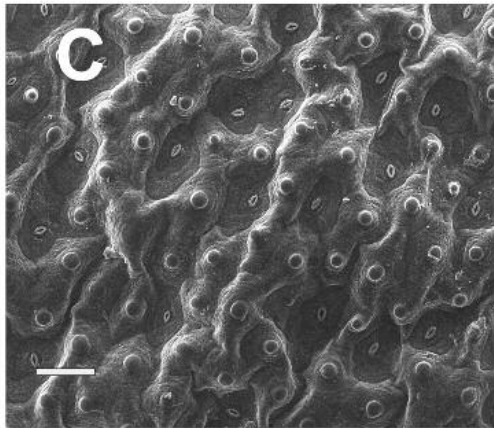
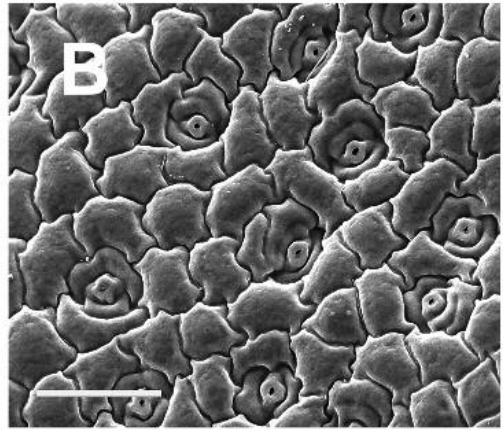
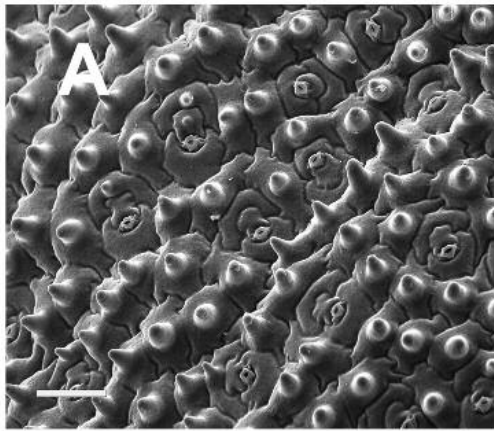


Figure 2. (A) *C. obcordellum* subsp. *rolfii* (B) *C. obcordellum* subsp. *stenandrum*; (C) *C. piluliforme* subsp. *edwardii*; (D) *C. truncatum* subsp. *viridicatum*; (E) *C. uviforme* subsp. *decoratum*; (F) *C. uviforme* subsp. *subincanum*. Scale bar = 100µm

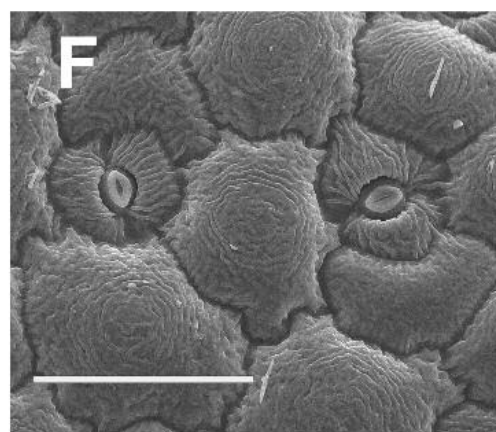
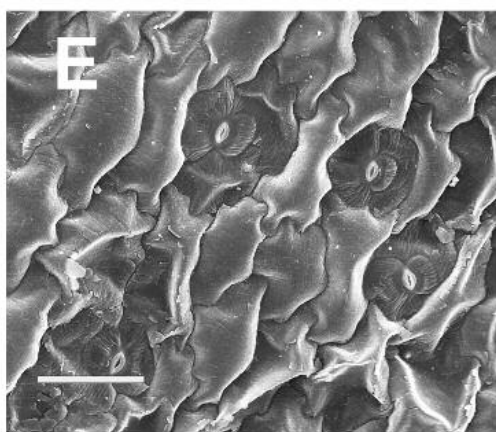
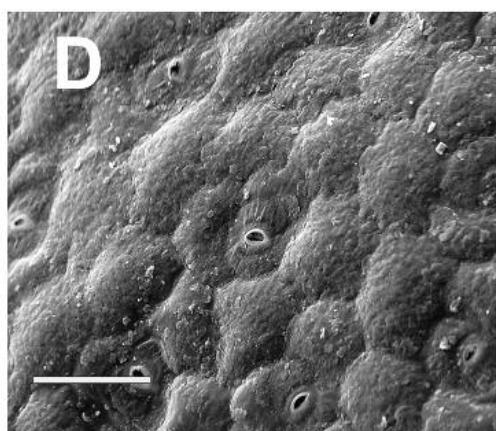
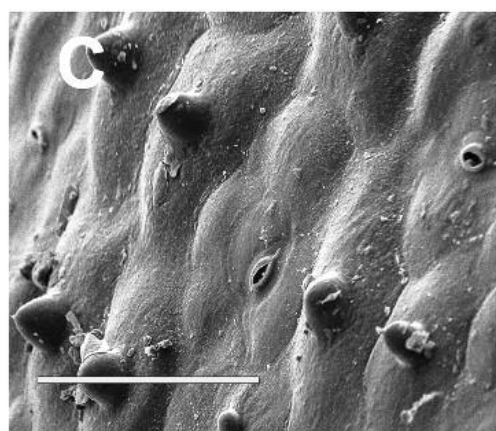
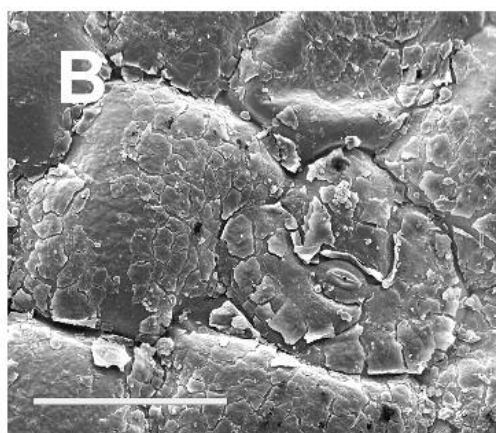
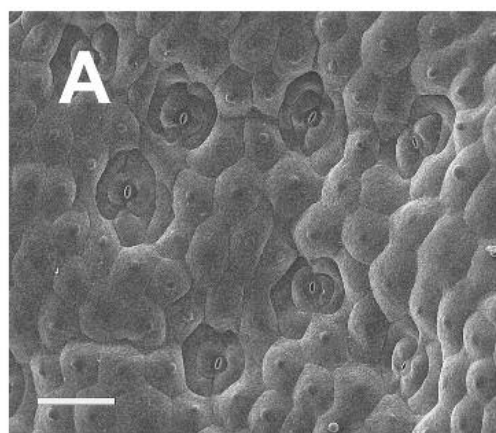


Figure 3. (A) *C. angelicae* subsp. *tetragonum*; (b) *C. buysianum* subsp. *buisianum*; (C) *C. buysianum* subsp. *politum*; (D) *C. roodiae* subsp. *corrugatum*; (E) *C. roodiae* subsp. *sanguineum*; (F) *C. youngii*. Scale bar = 100µm

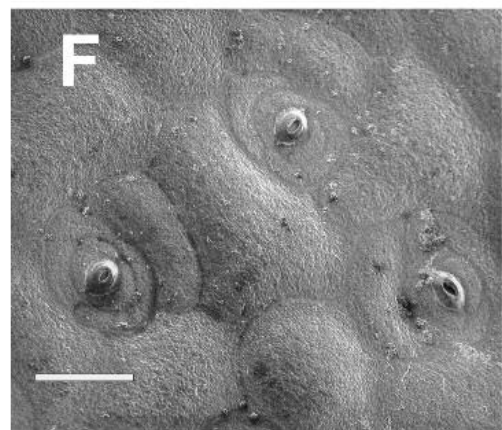
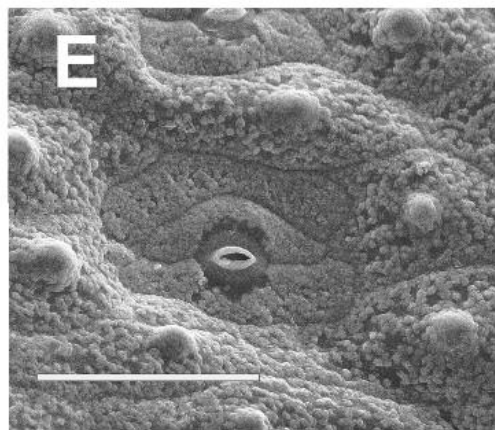
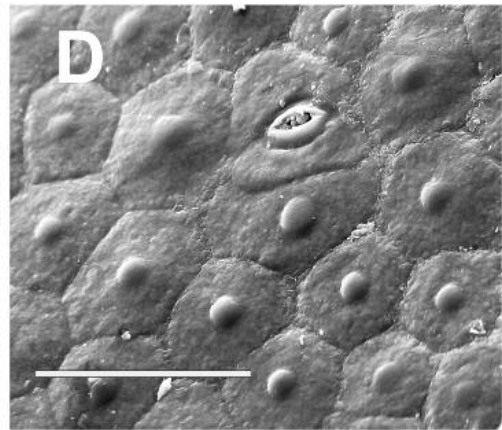
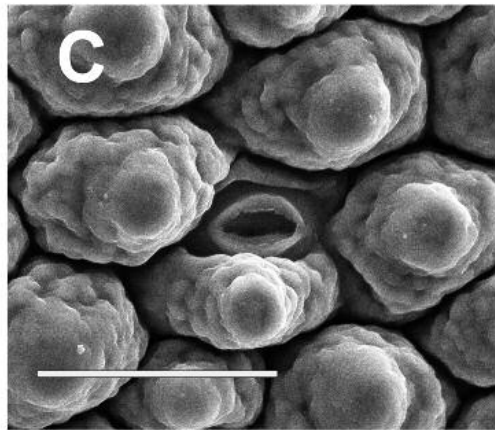
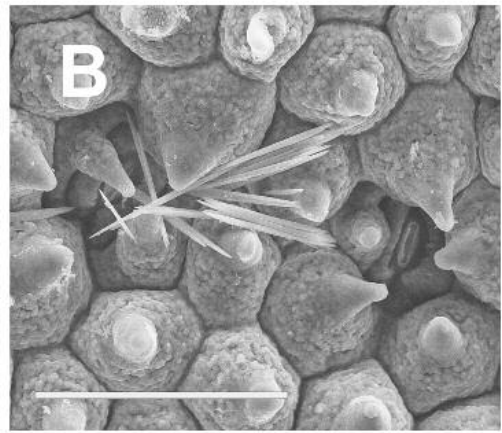
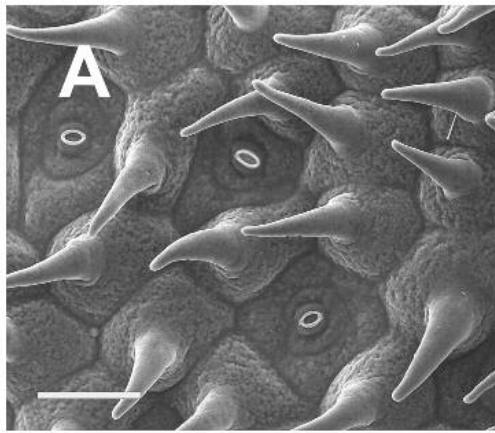


Figure 4. (A) *C. danielli*; (B) *C. marginatum* subsp. *Karamoepense* (stray raphide crystals released from internal tissues during sample preparation); (C) *C. marginatum* subsp. *littlewoodii*; (D) *C. antonii*; (E) *C. auriflorum* subsp. *turbiniforme*; (F) *C. brunneum*. Scale bar = 100µm, except for (C) = 50µm.

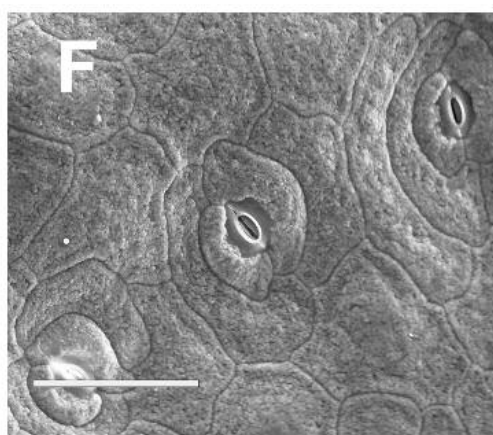
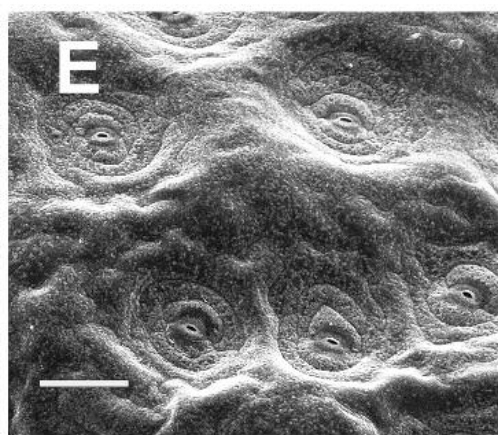
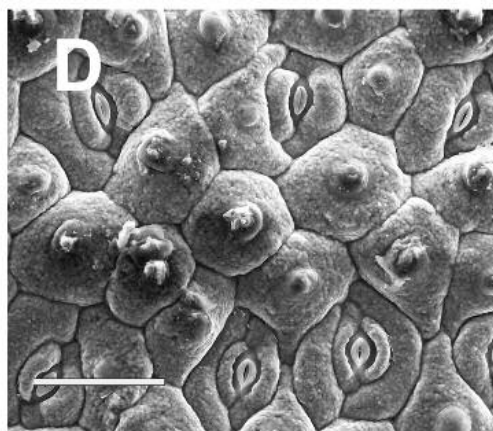
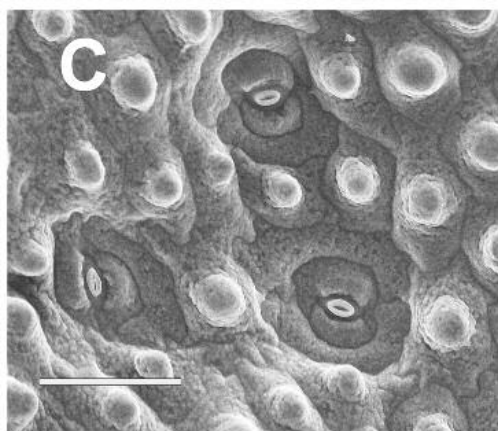
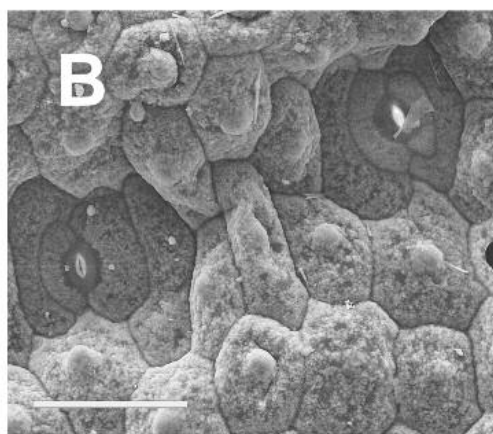
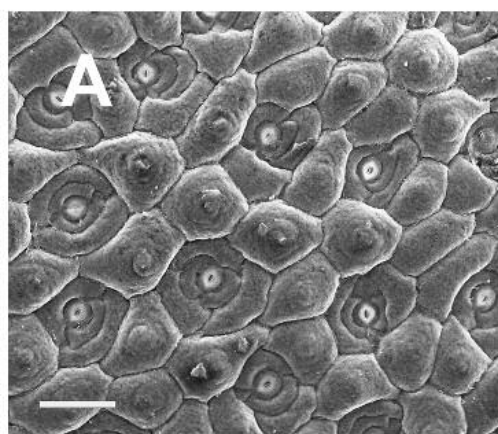


Figure 5. (A) *C. bruynsii*; (B) *C. cubicum*; (C) *C. ectypum* subsp. *brownii*; (D) *C. ectypum* subsp. *cruciatum*; (E) *C. ectypum* subsp. *ignavum*; (F) *C. ectypum* subsp. *sulcatum*. Scale bar = 100µm

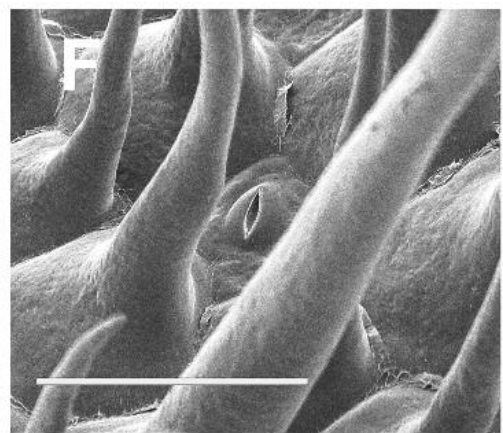
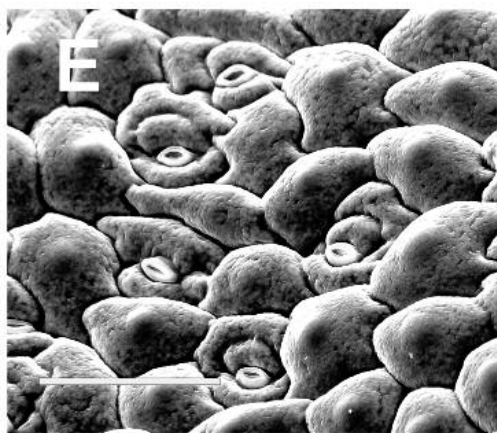
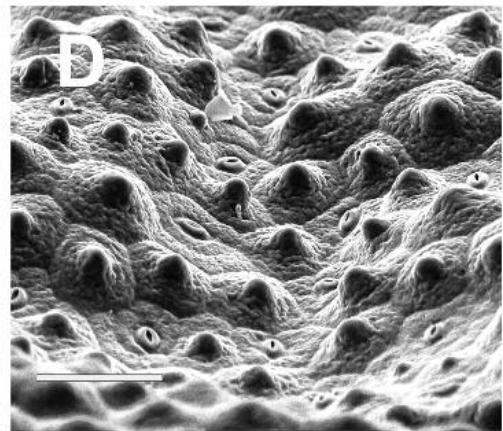
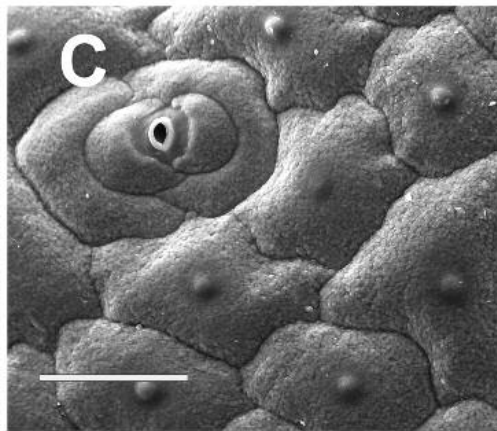
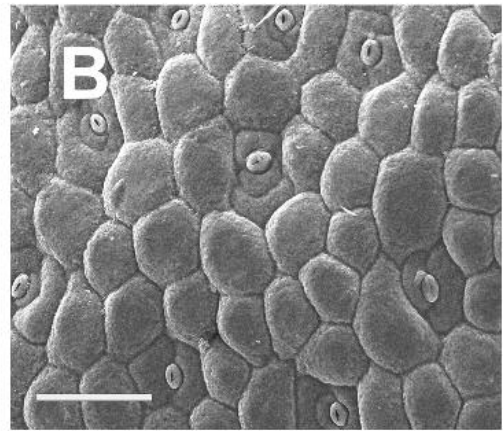
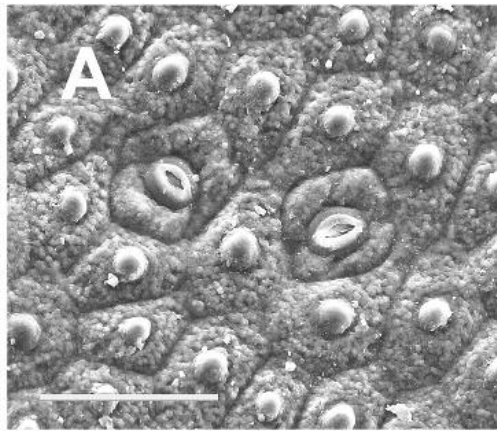


Figure 6. (A) *C. hanae*; (B) *C. hyracis*; (C) *C. irmae*; (D) *C. longibracteatum*; (E) *C. minusculum* subsp. *aestiflorens*; (F) *C. mirabile*. Scale bar = 100µm

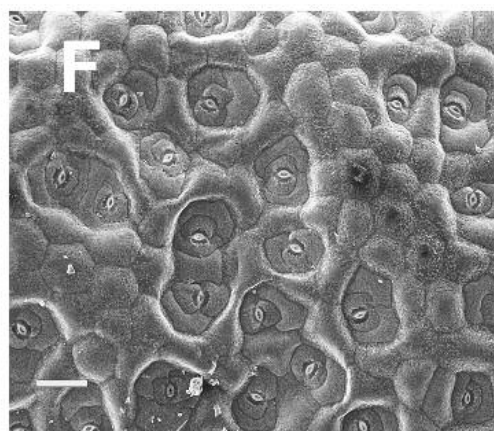
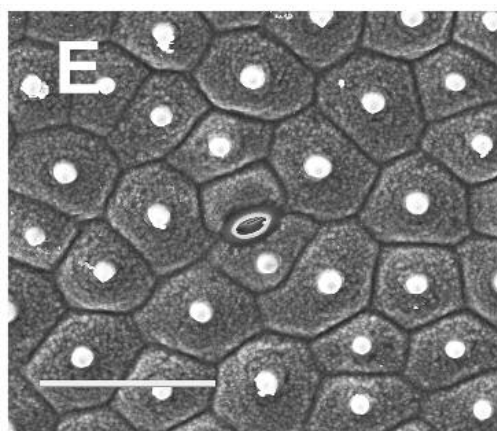
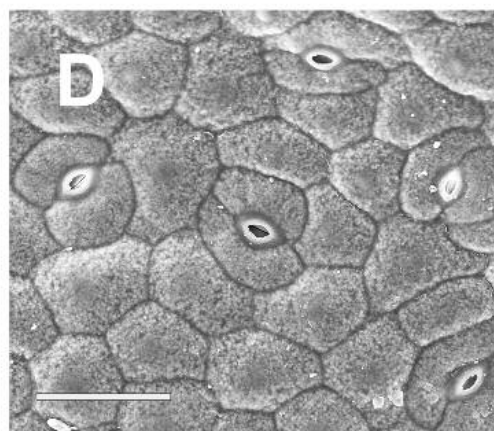
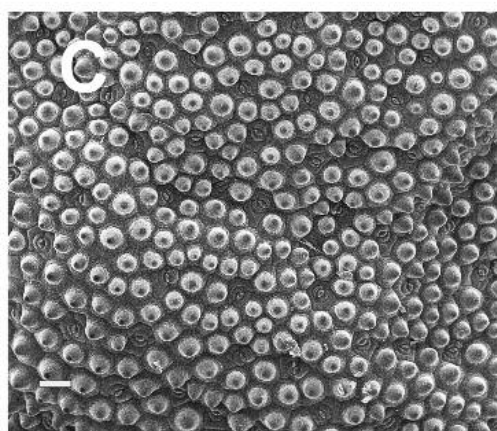
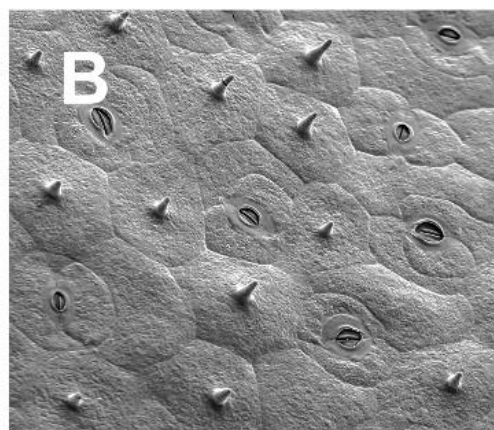
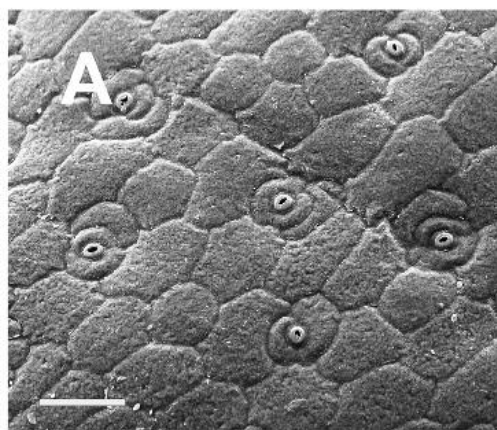


Figure 7. (A) *C. pium*; (B) *C. swanepoelianum* subsp. *proliferans*; (C) *C. tantillum* subsp. *amicorum*; (D) *C. tantillum* subsp. *heleniae*; (E) *C. tantillum* subsp. *eenkokerense*; *C. tantillum* subsp. *lindenianum*. Scale bar = 100µm

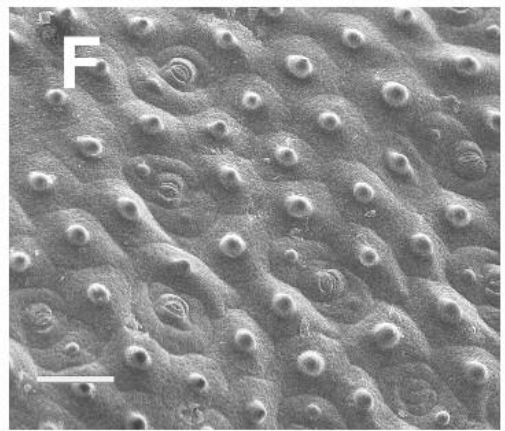
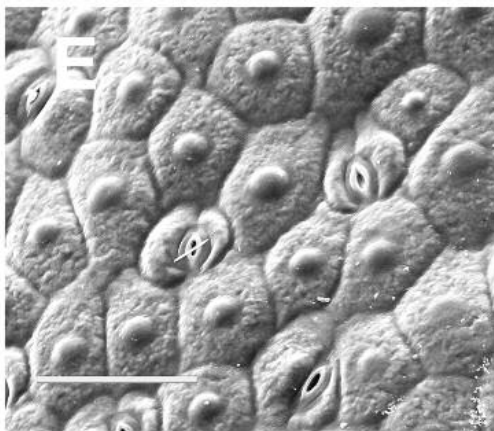
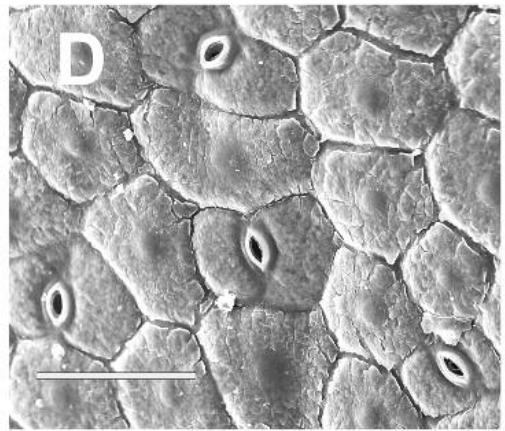
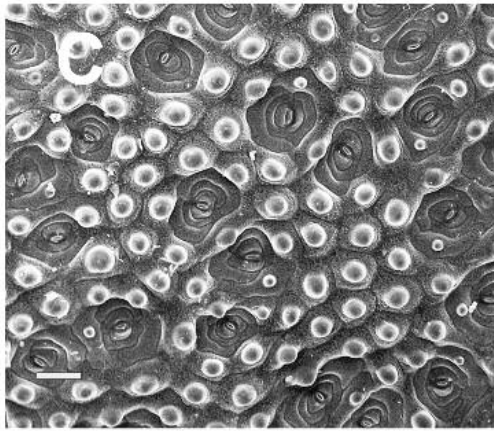
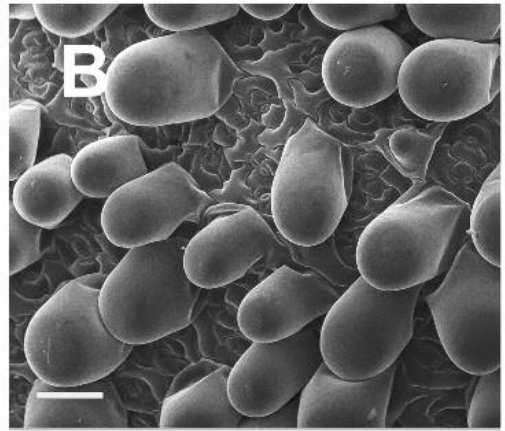
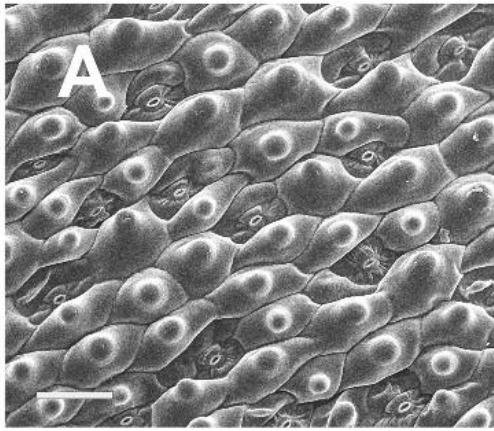


Figure 8. (A) *C. concordans*; (B) *C. devium* subsp. *stiiriferum*; (C) *C. arthurolfago*; (D) *C. lithopsoides* subsp. *boreale*; (E) *C. lithopsoides* subsp. *koubergense*; (F) *C. pellucidum* subsp. *cupreatum*. Scale bar = 100µm

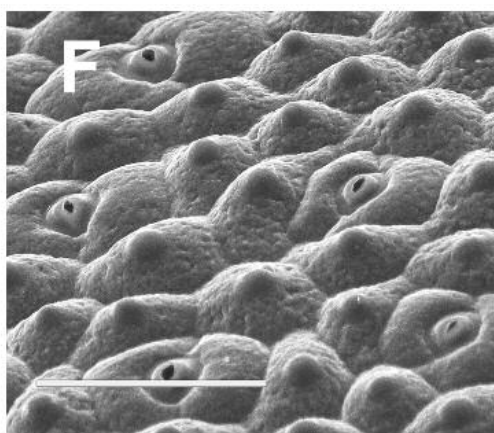
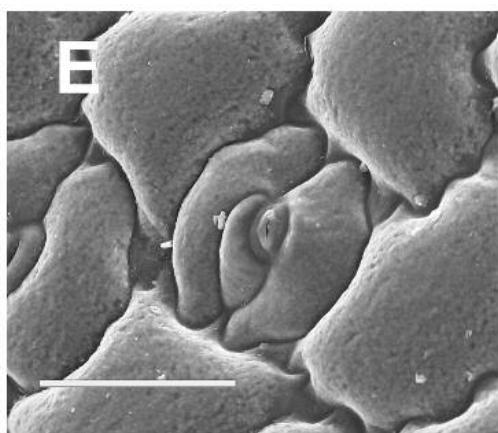
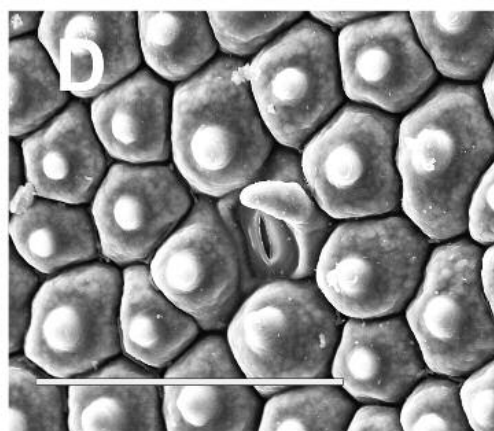
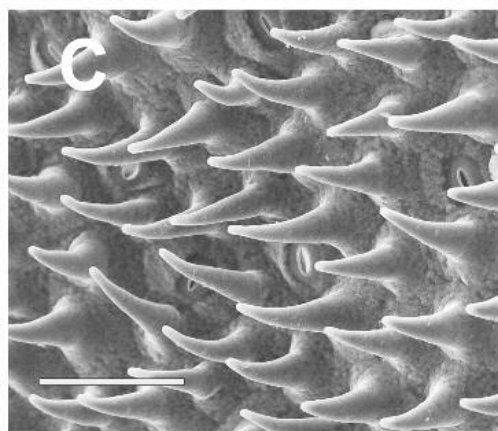
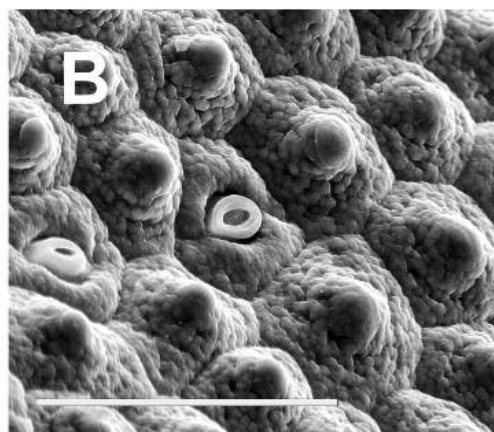
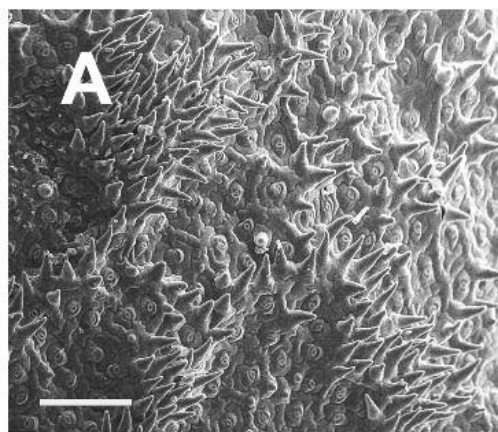


Figure 9. (A) *C. pellucidum* subsp. *cupreatum* var. *terrestre*; (B) *C. pellucidum* subsp. *saueri*; (C) *C. klinghardtense* subsp. *baradii*; (D) *C. quaesitum* subsp. *densipunctum*; (E) *C. hermarium*; (F) *C. bachelorum*. Scale bar = 100µm

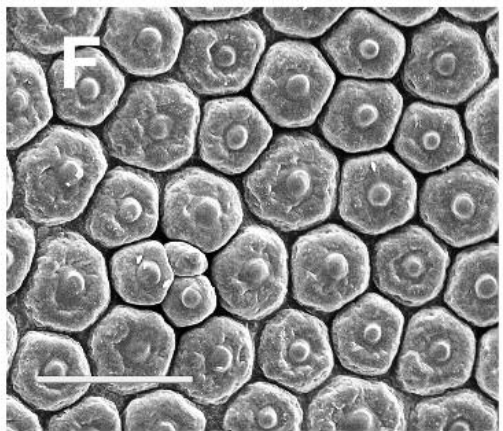
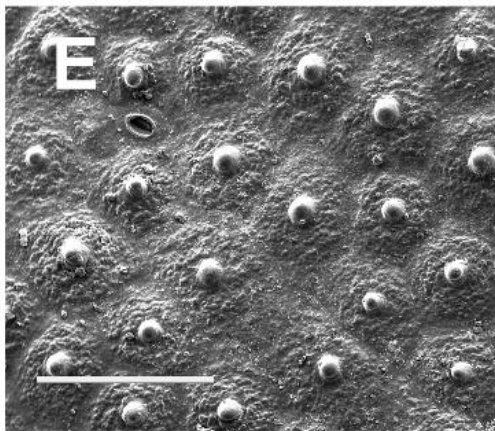
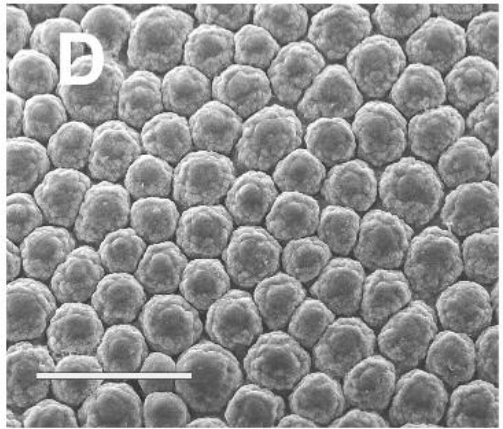
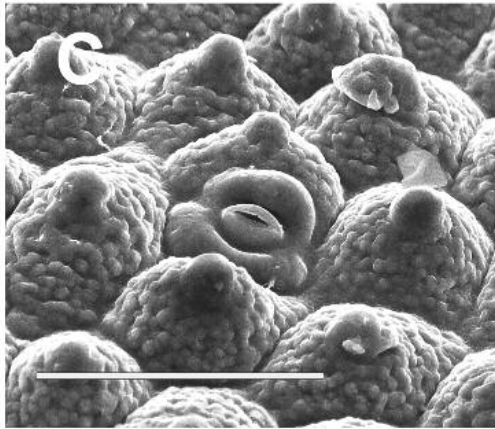
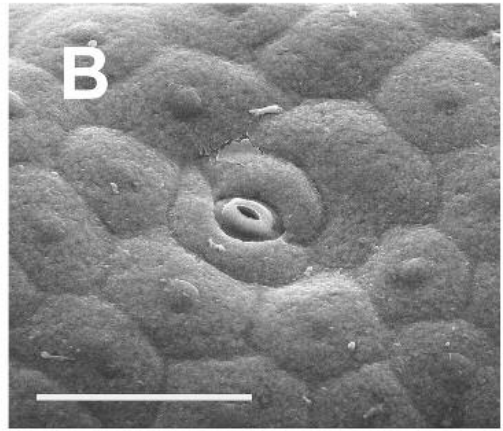
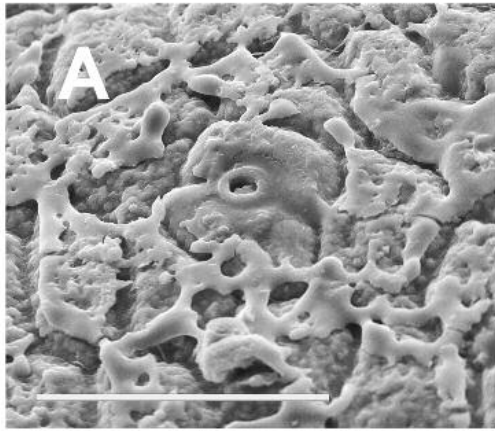


Figure 10. (A) *C. chrisolum*; (B) *C. confusum*; (C) *C. crateriforme*; (D) *C. francoiseae*; (E) *C. jucundum* subsp. *fragile*; (F) *C. jucundum* subsp. *marlothii*. Scale bar = 100µm

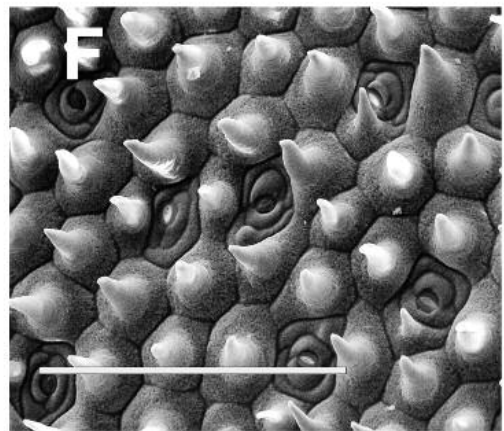
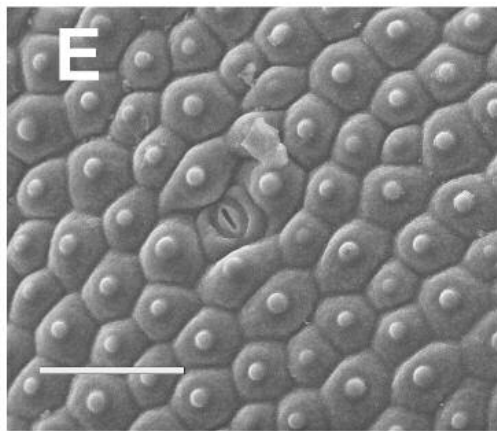
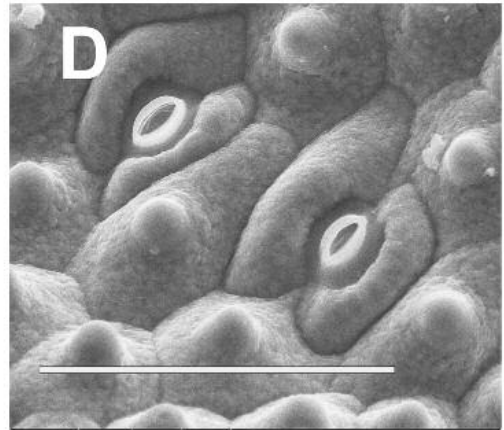
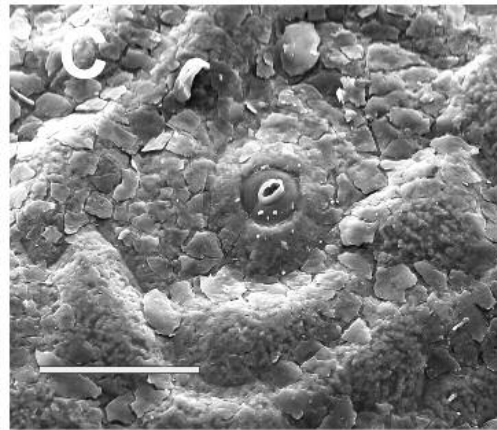
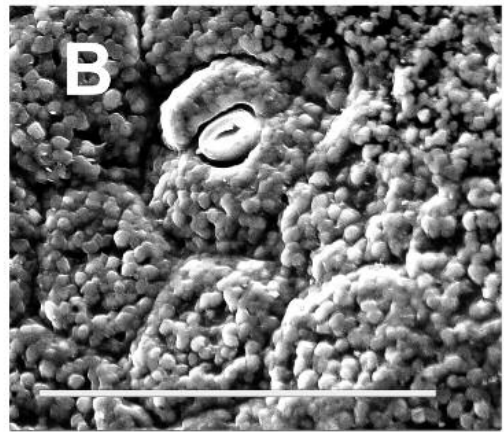
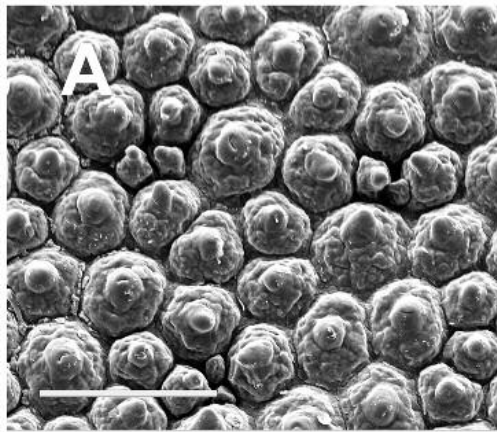


Figure 11. (A) *C. jucundum* subsp. *ruschii*; (B) *C. obscurum* subsp. *sponsaliorum*; (C) *C. obscurum* subsp. *vitreopapillum*; (D) *C. smaleorum*; (E) *C. taylorianum* subsp. *ernianum*; (F) *C. taylorianum* subsp. *rosy-nense*. Scale bar = 100µm

Table 1. List of *Conophytum* taxa examined in this study (organised by infrageneric section).

Species	Subspecies	Section	Sample
<i>C. depressum</i>	subsp. <i>perdurans</i>	Barbata	CR1563
<i>C. stephanii</i>	subsp. <i>stephanii</i>	Barbata	LAV26767A
<i>C. velutinum</i>	subsp. <i>polyandrum</i>	Biloba	MG1446.2
<i>C. calculus</i>	subsp. <i>vanzyliei</i>	Cataphracta	B&H2039
<i>C. maughanii</i>	subsp. <i>armeniacum</i>	Cheshire-Feles	SHsn
<i>C. maughanii</i>	subsp. <i>latum</i>	Cheshire-Feles	CM362
<i>C. subterraneum</i>		Cheshire-Feles	PGD3186
<i>C. obcordellum</i>	subsp. <i>rolfii</i>	Conophytum	CR1003
<i>C. obcordellum</i>	subsp. <i>stenandrum</i>	Conophytum	SHsn
<i>C. piluliforme</i>	subsp. <i>edwardii</i>	Conophytum	DT705
<i>C. truncatum</i>	subsp. <i>viridicatum</i>	Conophytum	LAV27974
<i>C. uviforme</i>	subsp. <i>decoratum</i>	Conophytum	PAVsn
<i>C. uviforme</i>	subsp. <i>subincanum</i>	Conophytum	SB885
<i>C. angelicae</i>	subsp. <i>tetragonum</i>	Costata	MG1440.65
<i>C. buysianum</i>	subsp. <i>buysianum</i>	Cylindrata	CR1462
<i>C. buysianum</i>	subsp. <i>politum</i>	Cylindrata	NBG0256067
<i>C. roodiae</i>	subsp. <i>corrugatum</i>	Cylindrata	TS617
<i>C. roodiae</i>	subsp. <i>sanguineum</i>	Cylindrata	SH1438
<i>C. youngii</i>		Cylindrata	ADH4778
<i>C. danielii</i>		Herreanthus	TJ0003
<i>C. marginatum</i>	subsp. <i>karamoepense</i>	Herreanthus	ARM950D
<i>C. marginatum</i>	subsp. <i>littlewoodii</i>	Herreanthus	ARM714B
<i>C. antonii</i>		Minuscula	CR1457
<i>C. auriflorum</i>	subsp. <i>turbiniiforme</i>	Minuscula	B&H2324
<i>C. brunneum</i>		Minuscula	SHsn
<i>C. bruynsii</i>		Minuscula	CR1418
<i>C. cubicum</i>		Minuscula	CR1484A
<i>C. ectypum</i>	subsp. <i>brownii</i>	Minuscula	TS478
<i>C. ectypum</i>	subsp. <i>cruciatum</i>	Minuscula	SB791
<i>C. ectypum</i>	subsp. <i>ignavum</i>	Minuscula	CR1384
<i>C. ectypum</i>	subsp. <i>sulcatum</i>	Minuscula	TS468
<i>C. hanae</i>		Minuscula	PAVsn
<i>C. hyracis</i>		Minuscula	<i>Helme, Hammer & Harrower 2730</i>
<i>C. irmae</i>		Minuscula	B&H2318

Species	Subspecies	Section	Sample
<i>C. longibracteatum</i>		Minuscula	PVB6749
<i>C. minusculum</i>	subsp. <i>aestiflorens</i>	Minuscula	ARM460B
<i>C. mirabile</i>		Minuscula	PGD2335
<i>C. pium</i>		Minuscula	CR1417
<i>C. swanepoelianum</i>	subsp. <i>proliferans</i>	Minuscula	ARM382
<i>C. tantillum</i>	subsp. <i>amicorum</i>	Minuscula	PVB9204/A
<i>C. tantillum</i>	subsp. <i>eenkokerense</i>	Minuscula	CR1278
<i>C. tantillum</i>	subsp. <i>heleniae</i>	Minuscula	RR1635
<i>C. tantillum</i>	subsp. <i>lindenianum</i>	Minuscula	TS447
<i>C. concordans</i>		Ophthalmophyllum	SH2067
<i>C. devium</i>	subsp. <i>stiriiferum</i>	Ophthalmophyllum	CR1340
<i>C. arthurolfago</i>		Pellucida	SH2065
<i>C. lithopsoides</i>	subsp. <i>boreale</i>	Pellucida	Clonotype
<i>C. lithopsoides</i>	subsp. <i>koubergense</i>	Pellucida	CR1788
<i>C. pellucidum</i>	subsp. <i>cupreatum</i>	Pellucida	LAV30215
<i>C. pellucidum</i>	subsp. <i>cupreatum</i> var. <i>terrestre</i>	Pellucida	SB1493
<i>C. pellucidum</i>	subsp. <i>saueri</i>	Pellucida	<i>N. Sauer</i> 89/0401
<i>C. klinghardtense</i>	subsp. <i>baradii</i>	Saxetana	SH906
<i>C. quaesitum</i>	subsp. <i>densipunctum</i>	Saxetana	Clonotype
<i>C. hermarium</i>		Verrucosa	SH565
<i>C. bachelorum</i>		Wettsteinia	PD3636
<i>C. chrisolum</i>		Wettsteinia	CR1266
<i>C. confusum</i>		Wettsteinia	ADH4777
<i>C. crateriforme</i>		Wettsteinia	ADH1503
<i>C. francoiseae</i>		Wettsteinia	ARM914
<i>C. jucundum</i>	subsp. <i>fragile</i>	Wettsteinia	SH617
<i>C. jucundum</i>	subsp. <i>marlothii</i>	Wettsteinia	LAV27906
<i>C. jucundum</i>	subsp. <i>ruschii</i>	Wettsteinia	DT7803
<i>C. obscurum</i>	subsp. <i>sponsaliorum</i>	Wettsteinia	SH1457
<i>C. obscurum</i>	subsp. <i>vitreopapillum</i>	Wettsteinia	CR1152
<i>C. smaleorum</i>		Wettsteinia	ADH4146.2
<i>C. taylorianum</i>	subsp. <i>ernianum</i>	Wettsteinia	MG1456.2
<i>C. taylorianum</i>	subsp. <i>rosynense</i>	Wettsteinia	EVJ118

Table 2. Stomatal guard cell dimensions in the genus *Conophytum* (organised by Section).

Species	Guard cell length (µm) n= 25, mean (± SE)	Section
<i>C. stephanii</i> subsp. <i>stephanii</i>	26.64 (0.60)	Barbata
<i>C. velutinum</i> subsp. <i>polyandrum</i>	22.30 (0.36)	Biloba
<i>C. piluliforme</i> subsp. <i>edwardii</i>	22.16 (0.50)	Conophytum
<i>C. truncatum</i> subsp. <i>viridicatum</i>	26.11 (0.56)	Conophytum
<i>C. uviforme</i> subsp. <i>decoratum</i>	23.95 (0.62)	Conophytum
<i>C. uviforme</i> subsp. <i>subincanum</i>	21.25 (0.47)	Conophytum
<i>C. angelicae</i> subsp. <i>tetragonum</i>	16.75 (0.29)	Costata
<i>C. rugosum</i>	19.37 (0.37)	Cylindrata
<i>C. youngii</i>	17.26 (0.37)	Cylindrata
<i>C. danielii</i>	24.53 (0.38)	Herreanthus
<i>C. marginatum</i> subsp. <i>karamoepense</i>	17.89 (0.44)	Herreanthus
<i>C. tantillum</i> subsp. <i>lindenianum</i>	23.44 (0.32)	Minuscula
<i>C. auriflorum</i> subsp. <i>turbiniiforme</i>	23.01 (0.45)	Minuscula
<i>C. cubicum</i>	16.68 (0.50)	Minuscula
<i>C. ectypum</i> subsp. <i>brownii</i>	18.70 (0.36)	Minuscula
<i>C. hanae</i>	26.75 (0.62)	Minuscula
<i>C. hyracis</i>	20.96 (0.26)	Minuscula
<i>C. longibracteatum</i>	23.59 (0.51)	Minuscula
<i>C. minusculum</i> subsp. <i>aestiflorens</i>	21.94 (0.38)	Minuscula
<i>C. mirabile</i>	23.96 (0.64)	Minuscula
<i>C. devium</i> subsp. <i>stiriiferum</i>	17.64 (0.32)	Ophthalmophyllum
<i>C. verrucosum</i>	23.08 (0.34)	Ophthalmophyllum
<i>C. pellicidum</i> subsp. <i>cupreatum</i>	22.90 (0.59)	Pellucida
<i>C. pellucidum</i> subsp. <i>saueri</i>	18.25 (0.35)	Pellucida
<i>C. klinghardtense</i> subsp. <i>baradii</i>	19.89 (0.34)	Saxetana
<i>C. bachelorum</i>	15.62 (0.43)	Wettsteinia
<i>C. chrisocruxum</i>	19.19 (0.31)	Wettsteinia
<i>C. chrisolum</i>	21.71 (0.35)	Wettsteinia
<i>C. confusum</i>	25.09 (0.34)	Wettsteinia
<i>C. crateriforme</i>	20.71 (0.32)	Wettsteinia
<i>C. flavum</i> (<i>ornatum</i>)	22.95 (0.41)	Wettsteinia
<i>C. flavum</i> (<i>tetracarpum</i>)	25.38 (0.37)	Wettsteinia
<i>C. flavum</i> subsp. <i>novicium</i> var. <i>novicium</i> nom. prov.	25.33 (0.29)	Wettsteinia
<i>C. flavum</i> subsp. <i>novicium</i> var. <i>kosiesense</i> nom. prov.	28.29 (0.41)	Wettsteinia
<i>C. obscurum</i> subsp. <i>sponsaliorum</i>	16.84 (0.21)	Wettsteinia
<i>C. smaelorum</i>	17.54 (0.30)	Wettsteinia
<i>C. taylorianum</i> subsp. <i>ernianum</i>	15.92 (0.52)	Wettsteinia
<i>C. taylorianum</i> subsp. <i>rosynense</i>	18.45 (0.33)	Wettsteinia

In most species studied the structure of the epidermis generally confirms the assignment of individual taxa by Hammer (2002) and more recently by others to the 16 infrageneric sections. However, few sections possess a unique set of epidermal characteristics and many taxa right across the genus, in different sections, share at least some of the same features in common. In other sections (notably *Minuscula*, *Saxetana* and *Wettsteinia*) there is often considerable variation in their epidermal morphology and further refinement of these sections may be warranted. For example, the presence of sunken stomata is relatively rare in *Conophytum*, being restricted to several taxa in section *Wettsteinia* (e.g., *C. francoiseae* and *C. jucundum*), *C. herreanthus* (section *Herreanthus*) and all taxa in sections *Batrachia* and *Cataphracta*. These taxa are all found in the northern part of the distribution range of *Conophytum*, mainly within (but not restricted to) the Richtersveld bioregion of the Succulent Karoo Biome, an area characterised by extremely low rainfall. Sunken stomata are found in some species of several related genera, e.g., *Cheiridopsis acuminata*, *Ch. pilosula*, *Ch. ponderosa* and *Odontophorus angustifolius*, all of which are found in the same broad geographical area as those *Conophytum* with sunken stomata (Powell *et al.*, 2017). Biogeography alone does not fully explain the presence or absence of features such as sunken stomata as many other *Conophytum* taxa that lack these are found in the Richtersveld. A common feature for most species that possess sunken stomata is that they typically grow fully exposed to the elements in very open, sunny microhabitats. Conversely, all of the buried or partially buried window-plant *Conophytums* with leaves mostly protected by the soil, have superficial stomata, despite also growing in the more arid parts of the range of the genus and mostly in very sunny, exposed niches. We might also predict that the presence of long trichomes (e.g., in *C. stephanii* and *C. mirabile*) suggests an adaptation that traps moisture from advective fogs. However, we do not have sufficient data on the local environmental conditions that each *Conophytum* species experiences, bearing in mind that they are often highly localised, niche-dwellers.

This study suggests that the species ranking of some other *Conophytum* taxa should be re-examined, e.g., *C. auriflorum* subsp. *auriflorum* and subsp. *turbiniforme* and the two subspecies of *C. maughanii* examined here possess different epidermal morphologies, perhaps to a degree not expected at the intraspecific level. Similarly, whilst some features are shared there is some variation

in epidermal structure across those six subspecies currently assigned to *C. tantillum* (Figures 7B–F; Opel, 2002, 2004). In 2002 Hammer acknowledged that a reassessment of some of these subspecies of *C. tantillum* is needed. The observations here would support such a reassessment.

Conclusions

A recent phylogenetic study by Powell (2016) has determined that some morphological characteristics including the presence, absence and type of epidermal papillae (trichomes) have evolved several times across the *Conophytum*-clade and the majority of clades in *Conophytum* included a range of epidermal characters. So, whilst the epidermal morphology of *Conophytum* provides useful information concerning putative relationships between individual taxa (to subspecies level) it should not, on its own, be used to describe all the infrageneric sections across the genus. The present study, when combined with the results of the earlier studies by Opel (2002, 2004), provides a complete survey of the epidermal structure of all currently recognised *Conophytum* species and subspecies. Our observations suggest that the infrageneric classification of *Conophytum* should be re-evaluated, especially for some of the larger sections.

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