CELL PATTERN AND ULTRASCULPTURE OF BULB TUNICS OF SELECTED ALLIUM SPECIES (AMARYLLIDACEAE), AND THEIR DIAGNOSTIC VALUE

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Cell pattern and ultrasculpture were examined by light and scanning electron microscopy in bulb tunics of 46 Allium species to determine the diagnostic value of micromorphological characters. The study examined the diversity of these characters, evaluated their usefulness at different taxonomic levels (species, section, subgenus), and considered the results in relation to the recent intrageneric classification of the genus. Detailed characteristics are provided for the investigated species, and taxa showing the presence of calcium oxalate crystals in bulb tunic cells are indicated. The results suggest that several bulb tunic characters are of taxonomic significance in Allium as their variation between specimens of the same species was negligible; they can be useful elements of species descriptions and determination keys.

Allium subgenus Allium shows considerable variation of bulb tunic ultrasculpture and hexagonal or elongated cell patterns. Differences in ultrasculpture are sufficient to distinguish species within the Amerallium subgenus. Three subgenera (Anquitum, Botomissa, Reticulatobulbosa) are characterized by fibrous tunics with reticulate ultrasculpture. Rectangular to elliptic cells with thick walls, giving the bulb tunic an almost perforated structure, are characteristic for Allium subgenus Cepa. No specific pattern was found for Allium subgenus Melanocrommyum and Polyprason. The only representative of subgenus Microscordum (Allium monanthum) showed distinct herringbone ultrasculpture. The bulbs of Allium subgenus Rhizirideum representatives can be distinguished by their linear ultrasculpture following the long axis of the elongated cells. Allium bulb tunic ultrasculpture and cell pattern show some degree of variability. These characters are of potential use in taxonomic delimitation, species determination and further study of the relationships between species, particularly in members of subgenus Amerallium.

Key words: Allium, bulb tunic, calcium oxalate crystals, micromorphology, SEM, taxonomy, ultrasculpture.

INTRODUCTION

The genus Allium L. includes over 800 species, 15 subgenera and more than 70 sections (Friesen et al., 2006; Fritsch et al., 2010) of herbaceous perennial plants with natural distributions in the Northern Hemisphere (Stearn, 1978, 1980; Friesen, 1988; Friesen et al., 2006; Fritsch and Abbasi, 2009). The genus Allium was previously classified as a member of the Lilliaceae family and then Alliaceae, subfamily Allioideae Herb. (Fay and Chase, 1996). Recently Allium has been placed within Amaryllidaceae (APG III, 2009). The genus is characterized by the presence of bulbs enclosed in membranous (sometimes becoming fibrous) tunics, free or almost-free tepals and often a subgynobasic style (Friesen et al., 2006). The diversity of Allium is greatest in Southwest and Central Asia and the Mediterranean region, and there is a second smaller center of diversity in North America (McNeal, 1992; Friesen et al., 2006; Nguyen et al., 2008).

Several criteria have been used in taxonomic classification of Allium. Some characters have proved useful at subgeneric and sectional levels, including the structure and shape of rhizome and bulb, and anatomical features of root, leaf, scape, ovary, septal nectaries and seed (De Wilde-Duyfjes, 1976; Fritsch, 1992, 1993; Kruse, 1992; McNeal, 1992; Friesen et al., 2006; Gurushidze et al., 2008; Nguyen et al., 2008; Choi and Cota-Sánchez, 2010; Celep et al., 2012). Many other characters are diagnostically important and commonly used in species descriptions. Scanning electron microscopy (SEM) has greatly improved the taxonomy of the genus.
facilitating studies of the ultrasculpture and ornamentation of the seed coat (Pastor, 1981; Kruse, 1984, 1992; Češmedžiev and Terzijski, 1997; Fritsch et al., 2006; Neshati and Fritsch, 2009; Celep et al., 2012; Choi et al., 2012), pollen grains (Güler and Pehlivan, 2006; Namin et al., 2009), bulb coats (De Wilde-Duyfjes, 1976; McNeal, 1982, 1992; Choi and Oh, 2011), floral structures (Choi et al., 2011) and leaf epidermal cells (Garbari et al., 1979; Krahulec, 1980; Choi et al., 2004; Choi and Oh, 2011).

The bulb plays an important role in species identification within the genus Allium. Bulb structure, shape and size and especially the texture of the outer bulb tunic play a great part in species diagnosis (De Wilde-Duyfjes, 1976; McNeal, 1982; Speta, 1984; Pastor and Valdès, 1986; McNeal and Jacobsen, 2002). Bulb tunic ornamentation has been used to elucidate the relationships among North American species (McNeal, 1992) and East Asian species (Choi and Oh, 2011). The bulb tunic develops through different lignification of the inner and radial cell walls of the adaxial epidermis of the uppermost foliage leaf (McNeal and Ownbey, 1973). Depending on the lignification processes, two bulb coat types have been distinguished (fibrous-reticulate, smooth nonreticulate), and the usefulness of tunic cell pattern and shape in distinguishing particular species has been noted (McNeal, 1992). Nevertheless, the use of bulb tunic ornamentation and cell pattern for identifying Allium species is rarely considered, especially in reference to comprehensive classification of the genus.

What is the relationship between micromorphology and the molecular data used to reconstruct Allium phylogeny? The bulb tunic ornamentation of some species has not been investigated previously. In this work I examine these microstructures in order to assess their usefulness for the classification of Allium proposed by Friesen et al. (2006), and specifically, to study the diversity of bulb tunic cell pattern and ultrasculpture in selected Allium species, to assess the diagnostic value of these characters at different taxonomic levels (species, section, subgenus), and to consider the results in relation to the recent infrageneric classification.

MATERIALS AND METHODS

The ultrasculpture of the bulb tunics of 46 selected Allium species representing 10 subgenera and 21 sections was examined by scanning electron microscopy (SEM) (JEOL JSM-5410 and NORAN Vantage) at 15 kV accelerating voltage. The samples were sputter-coated with gold (for details see Rola, 2012). The observations used 92 herbarium specimens (two of each species when available, two samples from one specimen when not). The specimens are housed in KRA (Jagiellonian University, Cracow). When possible the specimens were selected from different parts of the species’ distribution range. Next, epidermal peels of the inner surface of the bulb tunic of each species (except for species with typical reticulate or crustaceous outer tunics) were examined by light microscopy (LM) to observe their cell shape patterns. Table 1 lists the examined species together with herbarium collection data (when labels were legible). The terminology describing bulb tunic ultrasculpture follows De Wilde-Duyfjes (1976) and McNeal (1992).

RESULTS AND DISCUSSION

The diversity of bulb tunic texture, ultrasculpture and cell patterns of the studied Allium species are presented in Figures 1–7 and Table 1. In most cases the texture of bulb tunics was membranous or papery (Tab. 1). In some it was fibrous, and the fibers were more or less parallel (e.g., A. moschatum, Fig. 4a) or formed a reticulate structure (taxa of 3 subgenera: Reticulatobulbosa, Butomissa and Anguinitum, Figs. 2h,i, 3a, 4b–g). In some species, mainly from subgenus Amerallium, the structure of outer tunics was chartaceous or crustaceous with distinctive ultrasculpture visible under SEM (e.g., Fig. 2d–f). These characters of the outer bulb tunic apparently are of high diagnostic value. I determined some species-specific and subgenus-specific characters for which variation between specimens representing the same species is negligible; specimens from different geographical areas (Tab. 1) showed uniform outer bulb tunic ultrasculpture and cell pattern. These characters are stable and therefore can be successfully used in species descriptions and determination keys.

Some taxa had numerous calcium oxalate crystals visible by LM, or in the form of verrucae under SEM (Tab. 1). The crystals are actually intracellular, embedded in the cell mesophyll (Prychid and Rudall, 1999); that is why only their shapes were visible by SEM. This study supports Jaccard and Frey's (1928) suggestion that crystal types are useful for determining some species but not for the whole systematics of the genus. In contrast, Chartschenko (1932) distinguished 8 types and forms of calcium oxalate crystals in Allium and remarked that they play an important role in phyllogenetic systematics. However, his classification of certain species differs from the recent infrageneric classification (Friesen et al., 2006). I found calcium oxalate crystals in the outer bulb tunics of 23 of the examined species (Tab. 1), but no specific types of oxalate crystals were characteristic of subgenera or sections.

In the present work the main differences in bulb tunic ultrasculpture and cell pattern were at species level: specific characters for only a few subgenera were identified. Convergent evolution can lead to sim-
TABLE 1. Main characteristics of outer bulb tunics of examined *Allium* species, with herbarium data. Subgenera and sections follow Friesen et al. (2006) and Nguyen et al. (2008)

| Species | Subgenus/section | Character of outer bulb tunic | Utrasculture of outer bulb tunic | Cell pattern of inner surface of outer bulb tunic | Herbarium data |
|---------|------------------|-------------------------------|----------------------------------|-----------------------------------------------|----------------|
| *Allium acutiflorum* Loisel. | *Allium/Allium* | membranous to coriaceous | striate | hexagonal cells with calcium oxalate crystals | France: Montagne de la Gardiolle near Mireval (KRA 0284803) |
| *Allium ampeloprasum* L. | *Allium/Allium* | papery, slightly fibrous | smooth | elongated spindly cells with calcium oxalate crystals | Croatia: Vodice (KRA 0307370) |
| | | | | | Italy: Sardini, Capo Caccia (KRA 0323544) |
| | | | | | Italy: Ligurian Alps, Madonna di Palara (KRA 0326740) |
| *Allium atroviiaceum* Boiss. | *Allium/Allium* | membranous to fibrous | smooth | transversely elongated cells with calcium oxalate crystals | China: Tian Shan (KRA 0205190) |
| *Allium polyanthum* Schult. & Schult.f. | *Allium/Allium* | membranous | smooth to slightly striate | rectangular/hexagonal transversely elongated cells with calcium oxalate crystals | KRA 0205452 |
| *Allium scorodoprasum* subsp. *rotundum* (L.) Stearn | *Allium/Allium* | coriaceous | pattern of hexagonal cells | hexagonal cells with calcium oxalate crystals | Poland: Niecka Niżniańska basin, Busko Zdrój (KRA 0358174) |
| | | | | | Poland: Płaskowyż Prosowicki plateau, Piotrowice Wielkie (KRA 0304490) |
| | | | | | Croatia: between Vodice and Sibenik, along Krk river (KRA 0307366) |
| *Allium scorodoprasum* L. subsp. *scoorodoprasum* | *Allium/Allium* | membranous to subcoriaceous | striate | hexagonal cells and calcium oxalate crystals | Poland: Kotlina Sandomierska valley, Przychojec vill. (KRA 0361634) |
| *Allium sphaerocephalon* L. | *Allium/Allium* | membranous or coriaceous | pattern of hexagonal cells | hexagonal cells and calcium oxalate crystals | Austria: Baden, Kalvarienburg Mt. (KRA 205604) |
| | | | | | Austria: Burgenland, Hackerberg (KRA 0287168) |
| | | | | | Italy: Ligurian Alps, Pigna vill. (KRA 0326747) |
| *Allium vineale* L. | *Allium/Allium* | papyraceous | striate with verrucose ribs | elongated cells | Poland: Beskid Makowski Mts, Plebanska Góra Mt. near Myślenice (KRA 0257912) |
| | | | | | Poland: Wyżyna Kielecka upland, Eustachów vill. (KRA 0273547) |
| *Allium carinatum* L. | *Allium/ Codonoprasum* | membranous to papery | pattern of hexagonal cells | hexagonal cells with calcium oxalate crystals | Slovenia: Ilirska Bistrica near Snežnik Mt. (KRA 0307361) |
| | | | | | Czech Republic: Bile Karpaty Mts, Nová Lhota vill. (KRA 205290) |
| | | | | | Italy: Dolomites Mts, Magasa vill. (KRA 0326743) |
TABLE 1. Cont.

| Species                  | Subgenus/section      | Character of outer bulb tunic | Ultrasculpture of outer bulb tunic | Cell pattern of inner surface of outer bulb tunic | Herbarium data                                                                 |
|--------------------------|-----------------------|-------------------------------|-----------------------------------|-----------------------------------------------|--------------------------------------------------------------------------------|
| *Allium flavum* L.       | *Allium* / *Codonoprasum* | membranous                    | striate with smooth ribs          | elongated cells with calcium oxalate crystals  | Czech Republic: Moravia, Mohelno (KRA 0376156, KRA 0376195) Hungary: Heves (KRA 0360772) |
| *Allium melanantherum* Pančić | *Allium* / *Codonoprasum* | membranous                    | smooth                            | elongated cells with calcium oxalate crystals  | Bulgaria: Tintjava (KRA 118239)                                                   |
| *Allium oleraceum* L.    | *Allium* / *Codonoprasum* | membranous                    | striate with striped ribs         | elongated cells with calcium oxalate crystals  | Poland: Wyżyna Kielecka upland, Kolonia Kuźnia vill., (KRA 0321688) Poland: Płaskowyż Prosowicki plateau, Gruszów vill. (KRA 0306534) Bulgaria: Karlovo vill. (KRA 0239207) |
| *Allium paniculatum* L.  | *Allium* / *Codonoprasum* | membranous to papery          | pattern of hexagonal cells        | hexagonal cells with calcium oxalate crystals  | Hungary: Krassó-Szörény County (KRA 060752, KRA 060754)                         |
| *Allium stamineum* Boiss. | *Allium* / *Codonoprasum* | membranous to papery          | smooth with pattern of oxalate crystals | hexagonal cells with calcium oxalate crystals  | Israel: Carmel Mt. near Haifa (KRA 0205636)                                      |
| *Allium ursinum* L.      | *Amerallium* / *Arctoprasum* | papery                        | striate with pattern of elongated deltoid cells | deltoid/rectangular elongated cells with calcium oxalate crystals | Poland: Beskid Niski Mts, Krempna vill. (KRA 364311) Poland: Pogórze Śląskie foothills, Rudzica/Landek (KRA 0293825) |
| *Allium pendulinum* Ten. | *Amerallium* / *Briseis* | membranous                    | sinuate with curved cells         | irregular cells with sinuate walls            | Italy: Sicily, Madonie, Vallone Pomieri (KRA 0359983) Italy: Lucania, Pignola vill. (KRA 0205450) |
| *Allium triquetrum* L.   | *Amerallium* / *Briseis* | membranous                    | smooth                            | rhomboid/deltoid cells                        | Italy: Ostriconi (KRA 0352500) Italy: Sardinia, Giara di Gesturi plateau (KRA 0323540) |
| *Allium chamaemoly* L.   | *Amerallium* / *Chamaeprason* | chartaceous                   | faveolate with rhomboidal pattern | rhomboidal cells                              | Italy: Province of Genoa (KRA 0205299)                                            |
| *Allium moly* L.         | *Amerallium* / *Mollium* | chartaceous                   | conspicuously rugose-sinuate      | irregular cells with thick cell walls         | KRA 0205127                                                                      |
| *Allium roseum* L.       | *Amerallium* / *Mollium* | crustaceous                   | faveolate with the pattern of rectangular cells and thick walls with semilunar, tubercular appendages | pattern with specific rectangular cells        | Italy: Corsica, Ostraconi (KRA 0352503) Italy: Calabria, Craco vill. (KRA 0314227, KRA 03144229) |
| *Allium subhirsutum* L.  | *Amerallium* / *Mollium* | membranous                    | smooth with pattern of irregular cells with sinuous walls | irregular cells with sinuous walls            | Italy: Sardinia, Caprera (KRA 0386812) Greece: Peloponnese, Kalamaki near Mili (KRA 0326991) |
| Species                | Subgenus/ section | Character of outer bulb tunic | Ultrasculpture of outer bulb tunic | Cell pattern of inner surface of outer bulb tunic | Herbarium data                                                                 |
|------------------------|-------------------|------------------------------|-----------------------------------|---------------------------------------------------|-------------------------------------------------------------------------------|
| Allium tricoecum Sol. | Anguinum/ Anguinum| fibrous                      | reticulate                        | elongated cells                                   | KRA 0290518                                                                  |
| Allium victorialis L. | Anguinum/ Anguinum| fibrous                      | reticulate                        | elongated cells                                   | Poland: Wyżyna Kieiecka upland, Lipie forestry near Starachowice (KRA 0322064) |
|                        |                   |                              |                                   |                                                   | Poland: Bieszczady Mts, Szeroki Wierch Mt. (KRA 0377320)                       |
| Allium ramosum L.      | Butomissa/ Butomissa| reticulate to subreticulate  | reticulate                        | elongated cells                                   | China: Shandong Province, Shilaizhen (KRA 0346761)                           |
| Allium cepa L.         | Cepa/ Cepa        | chartaceous                  | pattern of rectangular cells      | rectangular/hexagonal cells with thick walls and calcium oxalate crystals | Poland: Wysoka Nowa vill. near Szyslowiec (KRA 0320763)                        |
|                        |                   |                              |                                   |                                                   | Poland: Plaskowyąż, Proszowicki plateau, Gniazdowice vill. (KRA 0306552)       |
| Allium fistulosum L.   | Cepa/ Cepa        | chartaceous                  | pattern of rectangular cells      | rectangular cell with thick walls and calcium oxalate crystals | Poland: Niziną Śląska lowland, Kluczbork (KRA 0137200, KRA 030864)           |
| Allium sibiricum L.    | Cepa/ Shoeneoprasum| papyraceous to subcoriaceous | pattern of rectangular cells      | rectangular cell with thick walls and calcium oxalate crystals | Poland: Beskid Żywiecki Mts, Pileko Mt., Hała Cebula (KRA 109402) Czech Republic: Moravia, Jesenik vill. (KRA 0205617) |
| Allium schoenoprasum L.| Cepa/ Shoeneoprasum| papyraceous to chartaceous   | pattern of elliptical cells       | rectangular to elliptical cells with thick cell walls and calcium oxalate crystals | Poland: Skarżysko-Kamienna (KRA 0361634) Poland: Zła Wieś vill. near Świecie (KRA 0369366) |
| Allium atropurpureum   | Melanoecrommyum/  | membranous                   | striate                           | rectangular elongated cells with calcium oxalate crystals | Romania: Banatus, Timis-Torontal (KRA 0205188) Romania: Temes, Vinga (KRA 459521) |
| Waldst. & Kit.         | Melanoecrommyum   |                              |                                   |                                                   |                                                                               |
| Allium nigrum L.       | Melanoecrommyum/  | membranous to chartaceous    | cobwebby                          | reticulate pattern                                | Italy: Sardinia, Su Nuraxi near Barumini (KRA 0386771)                       |
|                        | Melanoecrommyum   |                              |                                   |                                                   |                                                                               |
| Allium monanthum       | Microscordum/     | papery                        | herringbone                       | herringbone pattern                               | Japan: Honshu (KRA 0311580)                                                  |
| Maxim.                 | Microscordum      |                              |                                   |                                                   |                                                                               |
| Allium suaveolens Jacq.| Polyprason/       | papery                        | pattern of rectangular/hexagonal cells | rectangular/hexagonal cells with calcium oxalate crystals | Hungary: Veszprém vill. (KRAM 427744) Austria: near Vienna (KRA 079950)       |
|                        | Doghestanica      |                              |                                   |                                                   |                                                                               |
| Allium saxatile M.Bieb.| Polyprason/       | coriaceous                    | smooth                            | hexagonal cells with calcium oxalate crystals in the form of fine grains typically deposited by gravity on bottom wall of cell | Bosnia and Herzegovina: Trebinje (KRA 0278277) Azerbaijan: Quba (KRA 0205468) |
TABLE 1. Cont.

| Species               | Subgenus/section            | Character of outer bulb tunic | Ultrasculpture of outer bulb tunic | Cell pattern of inner surface of outer bulb tunic | Herbarium data                      |
|-----------------------|-----------------------------|-------------------------------|-------------------------------------|--------------------------------------------------|-------------------------------------|
| Allium oreophilum      | Polyprason/                 | papery                        | smooth                              | hexagonal to rectangular cells with calcium oxide crystals | Uzbekistan: Tashkent distr. (KRA 0205438) |
| C.A.Mey.              | Polyprason                  |                               |                                     |                                                  |                                     |
| Allium moschatum L.    | Polyprason/ Scorodon        | fibrous                       | reticulate                          | elongated cells                                  | Hungary: Budapest, Blockenberg Mt. (KRA 0205389, KRA 0205931) |
| Vedd.                 |                             |                               |                                     |                                                  |                                     |
| Allium inconspicuum    | Reticulatobulbosass/ Campanulata | fibrous                       | reticulate                          | elongated cells                                  | Uzbekistan: Tashkent distr. (KRA 0205235, KRA 0205234) |
| Vedd.                 |                             |                               |                                     |                                                  |                                     |
| Allium triechyscordum  | Reticulatobulbosass/ Flaviscordum | fibrous                       | reticulate                          | elongated cells                                  | KRA 0205305                         |
| Lede.                 |                             |                               |                                     |                                                  |                                     |
| Allium flavidum        | Reticulatobulbosass/        | fibrous                       | reticulate                          | elongated cells                                  | KRA 0205172                         |
| Lede.                 | Reticulatobulbosass/        |                               |                                     |                                                  |                                     |
| Allium lineare L.      | Reticulatobulbosass/        | fibrous                       | reticulate                          | elongated cells                                  | Ukraine: Lugusjkiensis (KRA 060756)  |
| Willd. ex Schult.f.    | Reticulatobulbosass/        |                               |                                     |                                                  |                                     |
| Allium strictum        | Reticulatobulbosass/        | fibrous                       | reticulate                          | elongated cells                                  | Russia: Kamchatka Peninsula (KRA 0205635) |
| Schrad.               | Reticulatobulbosass/        |                               |                                     |                                                  |                                     |
| Allium ochroleucum     | Rhizirideum/                | fibrous                       | smooth to striate                   | rectangular elongated cells                       | Slovakia: Veläk Fatra Mts, Drjenok (KRA 0205314) |
| Waldst. & Kit.         | Oreiprason                  |                               |                                     |                                                  | Austria: Kärnten, Kanalthal (KRA 0205317) |
| Allium albidum         | Rhizirideum/                | membranous                    | striate                             | rectangular elongated cells with calcium oxide crystals | Armenia: Sevan (KRA 062845)         |
| Fisch.                | Rhizirideum                 |                               |                                     |                                                  | (KRA 0204937)                       |
| Allium angulosum L.    | Rhizirideum/                | membranous                    | striate                             | rectangular / hexagonal elongated cells           | Poland: Haranow vill. near Busko-Zdroj (KRA 0407262) |
|                       | Rhizirideum                 |                               |                                     |                                                  | Poland: Kotlina Sandomierska valley, Sieniawa vill. (KRA 0333962) |
| Allium flavescens      | Rhizirideum/                | subcoriaceeous                | striate                             | rectangular / hexagonal elongated cells           | Romania: (KRA 0205140)              |
| Besser                | Rhizirideum                 |                               |                                     |                                                  |                                     |
| Allium senescens L.    | Rhizirideum/                | membranous                    | striate                             | rectangular / hexagonal elongated cells           | Hungary: Pest, Pilisszent (KRA0205383) |
|                       | Rhizirideum                 |                               |                                     |                                                  | Slovakia: Prielom Hornádu (KRA 0205387)          |

Similarity of characters in taxonomically different species, and sometimes there is a great variation of ultrasculpture within subgenera. Such a case was reported for leaf epidermal pattern (Krahulec 1977, 1980). Some bulb tunic characters might also be modified by environmental conditions, and some structures may be
adaptsions to local habitat factors (Krahulec, 1980; McNeal and Ownbey, 1982; McNeal, 1992).

Following are findings for particular subgenera.

*Allium* subgenus *Allium*

The species referred to this subgenus were characterized by considerable variation. Bulb tunic ultrasculpture was striate with distinct ribs (Fig. 1a,h,j,l), hexagonal (Fig. 1e,g,i,m) or smooth to reticulate (Fig. 1b–d,k,n). Eight taxa of this subgenus had hexagonal cells with numerous or less numerous calcium oxalate crystals (Figs. 5a,d,e–g,i, 6a,b). The other species had elongated cells. Calcium oxalate crystals were fairly common in the bulb tunic of members of this subgenus (Tab. 1; compare with data on crystals in epidermal tissue in Mathew, 1996). Only *A. vineale* showed no crystals. The inner surface of the outer

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**Fig. 1.** Ultrasculpture of outer bulb tunic (inner surface) of examined *Allium* species. (a) *Allium acutiflorum*, (b) *A. ampeloprasum*, (c) *A. atroviolaceum*, (d) *A. polyanthum*, (e) *A. scorodoprasum* subsp. *rotundum*, (f) *A. scorodoprasum* subsp. *scorodoprasum*, (g) *A. sphaerocephalon*, (h) *A. vineale*, (i) *A. carinatum*, (j) *A. flavum*, (k) *A. melananthemum*, (l) *A. oleraceum*, (m) *A. paniculatum*, (n) *A. stamineum*. Bar = 50 μm.
Cell pattern and ultrasculpture of Allium bulb tunics

The bulb tunic of *A. ampeloprasum* was composed of elongated cells with numerous oxalate crystals (Fig. 5b); in contrast, De Wilde-Duyfjes (1976) observed hexagonal cells in African specimens. My study showed subtle differences between the outer bulb tunics of *A. ampeloprasum* and *A. atrovioleum*. The latter differs in having distinct reticulate fibrous outer tunics with prominent ribbing (Fig. 5c), which are absent in *A. ampeloprasum* (Fig. 5b) (see also descriptions in Vvedensky, 1935; Wendelbo, 1985). *Allium sphaerocephalon* showed two types of ultrasculpture and cell pattern depending on bulb tunic texture. In membranous bulb tunics, the cell pattern was hexagonal to rectangular with numerous oxalate crystals (Fig. 5g); more coriaceous bulb tunics had striate ultrasculpture with rectangular elongated cells. Similar observations were made by De Wilde-Duyfjes (1976), who presented three types of bulb tunic cell pattern. *Allium vineale* bulb tunics showed a characteristic ribbed structure with elongated cells, as also found by De Wilde-Duyfjes (1976) and McNeal and Jacobsen (2002), but high magnification also revealed papillary structure of the ribs (Fig. 1h). *Allium oleraceum* had distinct ultrasculpture with longitudinal ribs (compare with illustration in Aedo, 2013), with additional ultrasculptural details: ribs covered with spiral protrusions (Fig. 1i). The outer bulb tunic of *A. paniculatum* was membranous, with hexagonal cells (Fig. 6a), similar to the description from De Wilde-Duyfjes (1976), who reported, however, that the outermost bulb tunic leaf had longitudinal ribs, not observed here. *Allium stamineum* had outer bulb tunics without nerves, consistent with the description from Vvedensky (1935) but contradicting Wendelbo (1985); its numerous calcium oxalate crystals (Fig. 6b) gave it a specific ultrasculpture visible by SEM (Fig. 1n).

*Allium* subgenus *Amerallium*

This subgenus showed the most distinctive structures, with great diversity of outer bulb tunic ultrasculpture.
Species-specific differences were recorded and certain patterns were characteristic for particular species. The representative of section Arctoprasum – A. ursinum – showed a distinct type of cell pattern with elongated cells (Figs. 2a, 6c). A similar cell arrangement was observed in other members of subgenus Amerallium from North America, for example A. lemmonii S.Watson (McNeal, 1992) and A. punctum L.F. Hend. (McNeal and Jacobsen, 2002), but in those cases the ultrasculpture showed thick lignified cell walls (McNeal, 1992). Allium pendulinum had curved cells with sinuate cell walls (Figs. 2b, 6d), with ultrasculpture somewhat resembling that reported by McNeal (1992) for North American taxa of this subgenus: A. tribracteatum Torr. and A. nevadense S. Watson. Allium triquetrum did not have distinct ultrasculpture and its cell pattern consisted of roughly regular parallelograms (Fig. 6e). Similar observations were made by Aedo (2013). Allium chamaemoly had characteristic rhomboidal ultrasculpture (Fig. 2d) visible even at low magnification, as also reported by Aedo (2013). The pattern in A. moly was similar to A. pendulinum but the walls were much thicker (Figs. 2e, 6f). In A. roseum the inner layer of the bulb coat has been described as pitted and sclerified (Coste and Flahault, 1906; De Wilde-Duyfjes, 1976; Pastor and Valdés, 1983; Kollmann, 1984). Previous reports indicated finely sinuate cell walls (De Wilde-Duyfjes, 1976; in my study additional ultrasculptural details were visible (Fig. 2f). In fact the pitted structure of outer bulb tunics results from semilunar tubercular appendages clearly visible under SEM (Fig. 2f). All examined specimens of A. subhirsutum had sinuous cell walls in which no two cells appeared to have the same shape (Figs. 2g, 6g). Similar observations were made by De Wilde-Duyfjes (1976) but she found that three subspecies differ in the bulb tunic cell pattern; two of them, A. subhirsutum subsp. subvillosum and subsp. spathaceum, proved to have sinuous cells.

Fig. 3. Ultrasculpture of outer bulb tunic (inner surface) of examined Allium species. (a) Allium ramosum, (b) A. cepa, (c) A. fistulosum, (d) A. sibiricum, (e1, e2) A. schoenoprasum, (f) A. atropurpureum, (g) A. nigrum, (h) A. monanthum, (i) A. suaveolens, (j) A. saxatile, (k) A. oreophilum. Bar = 50 μm.
Species of this subgenus are distributed mainly in the Mediterranean area, but a number of species with similar characters occur in North America (Badr and Elkington, 1978). The bulb tunic ultrasculpture of North American representatives of subgenus *Amerallium* has been examined and deemed valuable in elucidating relationships among the North American species (McNeal, 1992). The native North American taxa of subgenus *Amerallium* were found to be a well-supported monophyletic clade, sister to the Old World taxa (Nguyen et al., 2008). Scape anatomical characters have also indicated close relationships between Old World and New World species of subgenus *Amerallium* (Fritsch, 1993). The species examined during my study showed great variation of bulb tunic ultrasculpture and certain patterns proved to be characteristic for particular species. The ultrasculpture pattern of North American representatives has also been found to be species-specific (McNeal and Ownbey, 1982; McNeal, 1992; McNeal and Jacobsen, 2002), while a special type of leaf anatomy, scape anatomical structures and hypodermal laticifers in bulb scales are constant and characteristic for the whole subgenus (Huang and Sterling, 1970; Fritsch, 1988, 1993).

*Allium* subgenus *Anguinum*

*Allium victorialis* and *A. tricoccum* were characterized by fibrous and reticulate tunicas (Fig. 2h,i). The bulb tunic creates a specific reticulum structure consisting of elongated cells. This result confirms previously published reports (e.g., Pastor and Valdés, 1983; Xu and Kamelin, 2000; Aedo, 2013). A similar arrangement has been observed among other species from Korea and northeastern China representing the same sister group of species, belonging to subgenus *Anguinum*: *A. microdictyon* Prokh. and *A. ochotense* Prokh. (Choi and Oh, 2011).
Fig. 5. Cell pattern of outer bulb tunic (inner surface) of selected Allium species. (a) Allium acutiflorum, (b) A. ampeloprasum, (c) A. atroviolaceum, (d) A. polyanthum, (e) A. scorodoprasum subsp. rotundum, (f) A. scorodoprasum subsp. scorodoprasum, (g) A. sphaerocephalon, (h) A. vineale, (i) A. carinatum, (j) A. flavum, (k) A. melanatherum, (l) A. oleraceum. Bar = 100 μm.

Fig. 6. Cell pattern of outer bulb tunic (inner surface) of selected Allium species. (a) Allium paniculatum, (b) A. stamineum, (c) A. ursinum, (d) A. pendulinum, (e) A. triquetrum, (f) A. moly, (g) A. subhirsutum, (h) A. cepa, (i) A. fistulosum, (j) A. sibiricum, (k) A. schoenoprasum, (l) A. atropurpureum. Bar = 100 μm.
Cell pattern and ultrasculpture of Allium bulb tunics

Allium subgenus Butomissa

Allium ramosum, the only representative of this subgenus examined in this study, is easily distinguished by its characteristic fibrous and reticulate tunics (Fig. 3a) consisting of elongated cells, consistent with results from Choi and Oh (2011).

Allium subgenus Cepa

A pattern of rectangular to elliptic cells was recorded (Fig. 6h–k). The sculpture was quite distinct and consisted of cells with thick walls, giving the bulb tunic an almost perforated structure (Figs. 3b–e, 6h–k). Calcium oxalate crystals were very common components of such ultrasculpture in this subgenus. The calcium oxalate was crystallized in the form of regular pyramidal prisms (Fig. 6h,i) or such prisms with blunt ends (Fig. 6j). Such crystals correspond respectively to the cepa and schoenoprasum types described by Chartschenko (1932).

Allium subgenus Melanocrommyum

No specific pattern was identified for this subgenus. Allium atropurpureum showed striate ultrasculpture with cells remarkably elongated (Figs. 3f, 6l). In contrast, A. nigrum develops a specific network on the bulb tunic surface (Figs. 3g, 7a) and consequently a specific ultrasculpture visible by SEM. Such structure has not been observed previously; Aedo (2013) reported striate ultrasculpture with elongated spindle-shaped cells.

Allium subgenus Microscordum

Allium monanthum showed a very distinct herringbone pattern of ultrasculpture (Fig. 3h), as also given by Choi and Oh (2011), but such a pattern cannot be regarded as specific to this subgenus. Some North American taxa of subgenus Amerallium (e.g., A. crispum Greene, A. bolanderi S. Watson, A. serra McNeal & Ownbey) also have
such bulb tunic ultrasculpture (McNeal, 1992; McNeal and Jacobsen, 2002; Nguyen et al., 2008). In this context, note that subgenus Microscordum is most closely related to subgenus Amerallium (Friesen et al., 2006).

**Allium subgenus Polyprason**

The examined taxa did not present distinct ultrasculpture under SEM. Hexagonal to rectangular cell patterns were recorded (Fig. 7b–d). *Allium moschatum* was characterized by a bulb tunic which breaks into strips (Fig. 4a) and consequently it resembles reticulate ultrasculpture. *Allium saxatile* had characteristic hexagonal cells with calcium oxalate crystals in the form of fine crystal grains typically deposited by gravity on the bottom wall of the cell (Fig. 7c). This corresponds to the *globosum* type of calcium oxalate crystals described byChartschenko (1932).

**Allium subgenus Reticulatobulbosa**

The bulb tunics are fibrous with reticulate ultrasculpture and a linear arrangement of cells (Fig. 4b–g). An almost similar appearance was described for species of the same section, *Reticulatobulbosa*: *A. koreanum* H.J. Choi & B.U. Oh and *A. splendens* Willd. ex Schult.f. (Choi and Oh, 2011).

**Allium subgenus Rhitridioides**

The taxa show characteristic linear ultrasculpture under SEM. Hexagonal to rectangular cell walls (Figs. 4h–l, 7e–i). This pattern proved to be specific to this subgenus. Krahulec (1980) found that leaf epidermal anatomy is also uniform in this subgenus.

**CONCLUSION**

The ultrasculpture and cell pattern of the outer bulb tunic are generally differentiated within particular evolutionary lines of *Allium*. Although those characters do not directly indicate basal or advanced evolutionary levels, variation of ultrasculpture is sufficient to distinguish species within subgenus Amerallium, and characters of ultrasculpture or cell shape are diagnostic for some subgenera. The results indicate that *Allium* bulb tunic ultrasculpture is a useful character for taxonomic delimitation and species determination. It should help in analysis of the relationships between species of the first evolutionary line (Friesen et al., 2006), particularly in representatives of subgenus Amerallium.

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