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THE TAXONOMIC SIGNIFICANCE OF LEAF EPIDERMAL MICROMORPHOLOGICAL CHARACTERS IN DISTINGUISHING 43 SPECIES OF *ALLIUM* L. (AMARYLLIDACEAE) FROM CENTRAL ASIA

CHEN-YI LIN[1,2] AND DUN-YAN TAN[1*]

[1]Key Laboratory of Western Arid Region Grassland Resources and Ecology, Ministry of Education, China; Xinjiang Key Laboratory of Grassland Resources and Ecology; College of Pratacultural and Environmental Science, Xinjiang Agricultural University, Ürümqi 830052, China
[2]College of Forestry and Horticulture, Xinjiang Agricultural University, Ürümqi 830052, China
**Corresponding authors e-mail: tandunyan@163.com*

Abstract

The genus *Allium* is comprised of more than 800 species, and although previous studies have been useful in identifying the species, there is a paucity of easy-to-observe morphological characters with which to distinguish them. Thus, we determined the micromorphological characteristics of the leaf epidermis of 43 species of *Allium* from Central Asia using light microscopy and evaluated their taxonomic significance. Our study examined variability in epidermal cell shape and size and the stomatal apparatus. The stomatal apparatus is ellipsoid, anomocytic and amphistomatic. The shape (rectangular or rhomboid) of epidermal cells, pattern (straight or arched) of anticlinal walls, and stomatal index are stable within a species, while there are differences among species that allow for species delimitation. Based on the shape and pattern of anticlinal walls of leaf epidermal cells, the 43 sampled species could be divided into three distinct types of epidermal cells: type 1, rhomboid cell shape and straight anticlinal walls; type 2, rhomboid cell shape and arched anticlinal walls; and type 3, rectangular cell shape and straight anticlinal walls. These leaf epidermal micromorphological characters prove to be the taxonomic significance in distinguishing and delimitating species in Allium.

Key words: *Allium*, Leaf epidermal cell, Micromorphological characters, Taxonomic significance.

Introduction

Allium L. is a widely distributed genus comprised of more than 800 species that occurs mainly in seasonally dry regions in the Northern Hemisphere, including Europe, Asia and North America, and in South Africa (Stearn, 1980; Xu & Kamelin, 2000; Friesen *et al*., 2006; Fritsch *et al*., 2010). Central Asia is the main center of distribution and diversity of this genus, and it is rich in species of *Allium* (Fritsch & Friesen, 2002; Friesen *et al*., 2006).

Based on morphological characters, the traditional taxonomic system placed *Allium* in the Liliaceae (Krause, 1930). However, modern taxonomic treatments have placed the genus in the Alliaceae (Dahlgren *et al*., 1985; Hanelt *et al*., 1992; Takhtajan, 1997; APG II, 2003) or in the Amaryllidaceae (APG III, 2009) based on morphological, anatomical, cytological and molecular data. Infrageneric classification of the genus *Allium* is problematic because of proliferation of synonyms and lack of good and consistent taxonomic characters, resulting in disagreements on which ones to use for taxon boundaries (Xu, 1980; Wu *et al*., 2003). Several characters have been used in taxonomic classification of *Allium*, and some of them have proved to be useful at the subgeneric and sectional levels. Vvedensky (1935), Xu (1980) and Stearn (1980) divided the species of *Allium* from Russia, China and Europe into 10, 9 and 13 sections, respectively, based on morphological characters of the rhizome, bulb, leaf and flower. Hanelt *et al*. (1992) divided the genus into six subgenera and 50 sections and Friesen *et al*. (2006) and Nguyen *et al*. (2008) into 15 subgenera and 56 sections using information on anatomy, cytogeography and DNA ITS sequences.

Morphological characters of leaf epidermal cells, i.e. shape of anticlinal walls and the distribution, density and type of stomata and trichomes, are of potential taxonomic significance and have been widely used for the classification of taxa in various plant families (Stace, 1984; Baranova, 1992; Ahmad *et al*., 2010; Nurit-Silva & De Fátima Agra, 2011; Al-Edany & Al-Saadi, 2012). For example, in Lycoris (Amaryllidaceae) the shape of cells and distribution of stomata were used for species identification (Deng & Zhou, 2005), and in Smilacina (Liliaceae) stomatal and other epidermal features can be used to distinguish species (Tang *et al*., 2007). Krahulec (1980), Tanker & Kurucu (1981), Fritsch (1988), Uysal (1999), Choi & Oh (2011) found that characters of leaf anatomy in *Allium* taxa as well as papillae, laticifers and vascular bundles were important characters in relation to taxonomy. Gregory (1996) divided 22 species of sect. *Allium* into two distinct groups based on number of stomata, height of guard cells and vascular bundles arrangement. Yousaf *et al*. (2008) documented variation in size and shape of stomatal cells, trichomes, silica bodies and dimensions of nonstomatal cells of 18 species of *Allium* from Pakistan. Thus, various authors have concluded that leaf epidermal anatomy can provide a significant tool for resolution of the taxonomic confusion of *Allium* species.

We tested the hypothesis that characteristics of leaf epidermal cells of *Allium* species have strong taxonomic significance. Forty-three species of *Allium* were collected in Xinjiang Province of northwest China, one of the diversity centers of this genus in Central Asia. The material was used to answer the following questions. (1) Which leaf epidermal characters are stable within a species, and which differ between species? (2) Do leaf epidermal characters provide useful information for a better understanding of both infrageneric and species delimitation?

Materials and Methods

Leaf material was collected from living plants of the 43 *Allium* species in the field or from dried specimens in the herbaria of Xinjiang Agriculture University (XJA); Xinjiang Ecology and Geography Research Institute, Chinese Academy of Sciences (XJBI); Xinjiang University (XJUG); and Shihezi University (SHI). We followed Xu & Kamelin (2000) and The Plant List (2013) for identification of these species. A list of voucher specimens used in this study is given in Table 1. One fully expanded leaf was taken from the middle of the stem of five mature individuals of each species. Further, the middle portion of each sampled leaf was used for the micromorphological observations, following Deng & Zhou (2005). Samples of leaf epidermis from living plants were fixed immediately after collection in formalin acetic acid for a minimum of 24 h and then submerged and washed with distilled water for 2 h (Yousaf *et al*., 2008). Leaves from herbarium specimens were fully rehydrated, following Nurit-Silva & De Fátima Agra (2011). Portions of leaf epidermis from living or herbarium material were stained with a solution of 1% safranin (in 50% ethanol) before they were mounted on slides with Canada balsam. The preparation procedure followed that of Ellis (1979). A minimum of five slides was prepared for each section of an individual leaf.

Leaf epidermis cells were examined using an Olympus BH-2 light microscope. Descriptive terminology of the leaf epidermal cells follows Dilcher (1974), Charlton (1988) and Croxdale (1998, 2000), and trichome description and classification follow Theobald *et al*. (1979). The following characteristics were described/determined: (1) shape and pattern of the anticlinal walls of epidermal cells: rhomboid, rectangular, straight and arched; (2) length and width of epidermal cells; (3) density of epidermal cells; (4) stomatal apparatus: guard cell length and width and (5) stomatal index (I) using the formula:

$$I = [S/ (E+S)] \times 100$$

where S is number of stomata per unit area (mm2) and E the number of epidermal cells in the same area.

Results

Epidermal cells: Under light microscopy, epidermal cells and stomatal apparatus characters occurred consistently on both adaxial and abaxial blade surfaces from the center section of each leaf of each *Allium* species. That is, within a species, the shape of the epidermal cells was the same on the adaxial and abaxial side of the leaf. Thus, only characteristics of the adaxial leaf epidermis for the 43 species of *Allium* are summarized in Table 2 and Figs. 1-43. All *Allium* species examined had long epidermal cells, and the long axis of the cells was parallel with the veins. Thirty species had rhomboid cells and 13 rectangular cells (Table 2).

Epidermal cells of 36 species had straight anticlinal walls, and the remaining five, *A. altaicum* (Fig. 26), *A. fistulosum* (Fig. 27), *A. cepa* (Fig. 28), *A. galanthum* (Fig. 29) and *A. schoenoprasum* (Fig. 32) arched walls. The pattern of the anticlinal walls of epidermal cells was stable within a species, but there are differences between species. Cell size varied greatly between species. The largest cells, in *A. porrum* (Fig. 39), were 589.24 µm in length and 37.57 µm in width, while the smallest ones, in *A. atrosanguineum* (Fig. 31), were 141.21 µm in length and 29.74 µm in width. Average cell length ranged from 589.24 µm (*A. porrum*) to 141.21 µm (*A. atrosanguineum*), and average cell width from 56.67 µm (*A. roborowskianum*) to 13.62 µm (*A. tekesicola*).

All 43 species had long epidermal cells, and the following ones also had short epidermal cells that varied in length and width, respectively: 42.53 and 23.42 µm, *A. obliquum* (Fig. 17); 74.7 and 21.4 µm, *A. tuberosum* (Fig. 1); 44.0 and 17.8 µm, *A. hymenorrhizum* (Fig. 18); 37.0 and 20.8 µm, *A. strictum* (Fig. 5); 53.2 and 22.1 µm, *A. pallasii* (Fig. 36); 135.8 and 22.9 µm, *A. caeruleum* (Fig. 37); 68.5 and 23.4 µm, *A. ceasium* (Fig. 38) and 67.7 and 16.2 µm, *A. delicatulum* (Fig. 34). Thus, long and short epidermal cells as opposed to having only long epidermal cells are distinctive features with taxonomic significance.

Average density of epidermal cells differed among the 43 species examined and ranged from 33.66 / mm^2 (*A. roborowskianum*) to 310.91/ mm^2 (*A. tekesicola)*.

Stomata: Stomata in the 43 *Allium* species occurred on both adaxial and abaxial surfaces of the leaf, and they were located between the veins in linear aggregations in longitudinal cell profile. Orientation of the guard cells was parallel with the long axis of the leaf blade. The stomatal apparatus is ellipsoid and consists of guard cells only.

The stomatal apparatus varied in size among the 43 taxa. Average stomata size ranged from 26.13 µm in length and 17.92 µm in width in *A. tekesicola* (Fig. 8) to 68.56 µm in length and 49.50 µm in width in *A. przewalskianum* (Fig. 7). The stomatal index ranged from 59.62 % in *A. semenovii* (Fig. 30) with the stomata being densely distributed to 28.28 % in *A. atrosanguineum* (Fig. 31) with the stomata being sparsely distributed.

Trichomes: Trichomes were found only in *A. grisellum* (Fig. 33), and there were several trichomes on both the adaxial and abaxial surfaces of the leaf. These trichomes were straight, multicellular without branches, papillae and tuberculate, and gradually narrowed from the flattened basal part to the tip.

Table 1. Species from Xinjiang, China, used for taxon sampling. The arrangement of taxa follows Xu and Kamelin (2000).

Taxa	Collection locality	Voucher (Herbarium)	Kind of material used
A. tuberosum Rottler ex Sprenge.	Urumqi, Xinjiang, China	D.Y. Tan 367 (XJA)	L
A. ramosum L.	Nileke, Xinjiang, China	Unknown 741254 (XJA)	H
A. oreoprasum Schrenk	Urumqi, Xinjiang, China	D.Y. Tan 0322 (XJA)	L
A. lineare L.	Fukang, Xinjiang, China	J..M. Xu 00054704 (XJBI)	H
A. strictum Schrad.	Altay, Xinjiang, China	D.Y. Tan 0276 (XJA)	L
A. flavidum Ledeb.	Fuhai, Xinjiang, China	W.S. Zhang 807-2 (XJUG)	H
A. przewalskianum Regel	Urumqi, Xinjiang, China	D.Y. Tan 010713 (XJA)	L
A. tekesicola Regel	Nileke, Xinjiang, China	Z.C. Zhang 070813 (XJA)	H
A. mongolicum Regel	Urumqi, Xinjiang, China	C.Y. Lin 070507 (XJA)	L
A. caespitosum Siev. ex Bong. & C.A. Mey.	Altay, Xinjiang, China	G.J. Liu 00054487 (XJBI)	H
A. polyrhizum Turcz. ex Regel	Tuoli, Xinjiang, China	D.Y. Tan 020352 (XJA)	L
A. bidentatum Fisch. ex Prokh. & Ikonn.-Gal.	Balikun, Xinjiang, China	Z.L. Wang 87-00135 (XJUG)	H
A. anisopodium Ledeb.	Habahe, Xinjiang, China	J..M. Xu 00054464 (XJBI)	H
A. weschniakowii Regel	Bole, Xinjiang, China	N.R. Cui 820348 (XJA)	H
A. senescens L.	Urumqi, Xinjiang, China	D.Y. Tan 03611 (XJA)	L
A. nutans L.	Buerjin, Xinjiang, China	D.Y. Tan 0722 (XJA)	L
A. obliquum L.	Fuyun, Xinjiang, China	D.Y. Tan 020707 (XJA)	L
A. hymenorhizum Ledeb.	Nileke, Xinjiang, China	Z.C. Zhang 070817 (XJA)	L
A. carolinianum DC	Urumqi, Xinjiang, China	D.Y. Tan 0076 (XJA)	L
A. platyspathum Schrenk	Kanasi, Xinjiang, China	P. Liu 801295 (XJUG)	H
A. saxatile M. Bieb.	Urumqi, Xinjiang, China	D.Y. Tan 070516 (XJA)	L
A. caricoides Regel	Hejing, Xinjiang, China	D.Y. Tan 010817 (XJA)	L
A. tianschanicum Rupr.	Nileke, Xinjiang, China	Z.C. Zhang 070814 (XJA)	H
A. setifolium Schrenk	Urumqi, Xinjiang, China	D.Y. Tan 070428 (XJA)	L
A. subtilissimum Ledeb.	Urumqi, Xinjiang, China	D.Y. Tan 070822 (XJA)	L
A. semenovii Regel	Shawan, Xinjiang, China	G.J. Liu 00054970 (XJBI)	H
A. altaicum Pall.	Fuyun, Xinjiang, China	Z.L. Wang 0028 (XJUG)	H
A. fistulosum L.	Urumqi, Xinjiang, China	C.Y. Lin 070911 (XJA)	L
A. atrosanguineum Schrenk	Yili, Xinjiang, China	Y. Zhang 070509 (XJA)	L
A. schoenoprasum L.	Buerjin, Xinjiang, China	D.Y. Tan 00762 (XJA)	L
A. cepa L.	Urumqi, Xinjiang, China	C.Y. Lin 070913 (XJA)	L
A. galanthum Kar. & Kir.	Tacheng, Xinjiang, China	D.Y. Tan 02054 (XJA)	L
A. grisellum J. M. Xu	Bole, Xinjiang, China	D.Y. Tan 9954 (XJA)	L
A. delicatulum Siev. ex Schult. & Schult. f.	Emin, Xinjiang, China	D.Y. Tan 2486 (XJA)	L
A. glomeratum Prokh.	Xinyuan, Xinjiang, China	G.J. Liu 00054640 (XJBI)	H
A. pallasii Murray	Urumqi, Xinjiang, China	D.Y. Tan 135 (XJA)	L
A. caeruleum Pall.	Manasi, Xinjiang, China	D.Y. Tan 86-23 (XJA)	L
A. ceasium Schrenk	Emin, Xinjiang, China	D.Y. Tan 0053 (XJA)	L
A. porrum L.	Urumqi, Xinjiang, China	C.Y. Lin 070929 (XJA)	L
A. sativum L.	Urumqi, Xinjiang, China	C.Y. Lin 070923 (XJA)	L
A. roborowskianum Regel	Altay, Xinjiang, China	D.Y. Tan 0472 (XJA)	L
A. robustum Kar. & Kir.	Yumin, Xinjiang, China	D.Y. Tan 00924 (XJA)	L
A. fetisowii Regel	Yumin, Xinjiang, China	D.Y. Tan 09213 (XJA)	L

L: Leaf material was collected from living plants in the field; H: Leaf material was collected from herbarium specimens

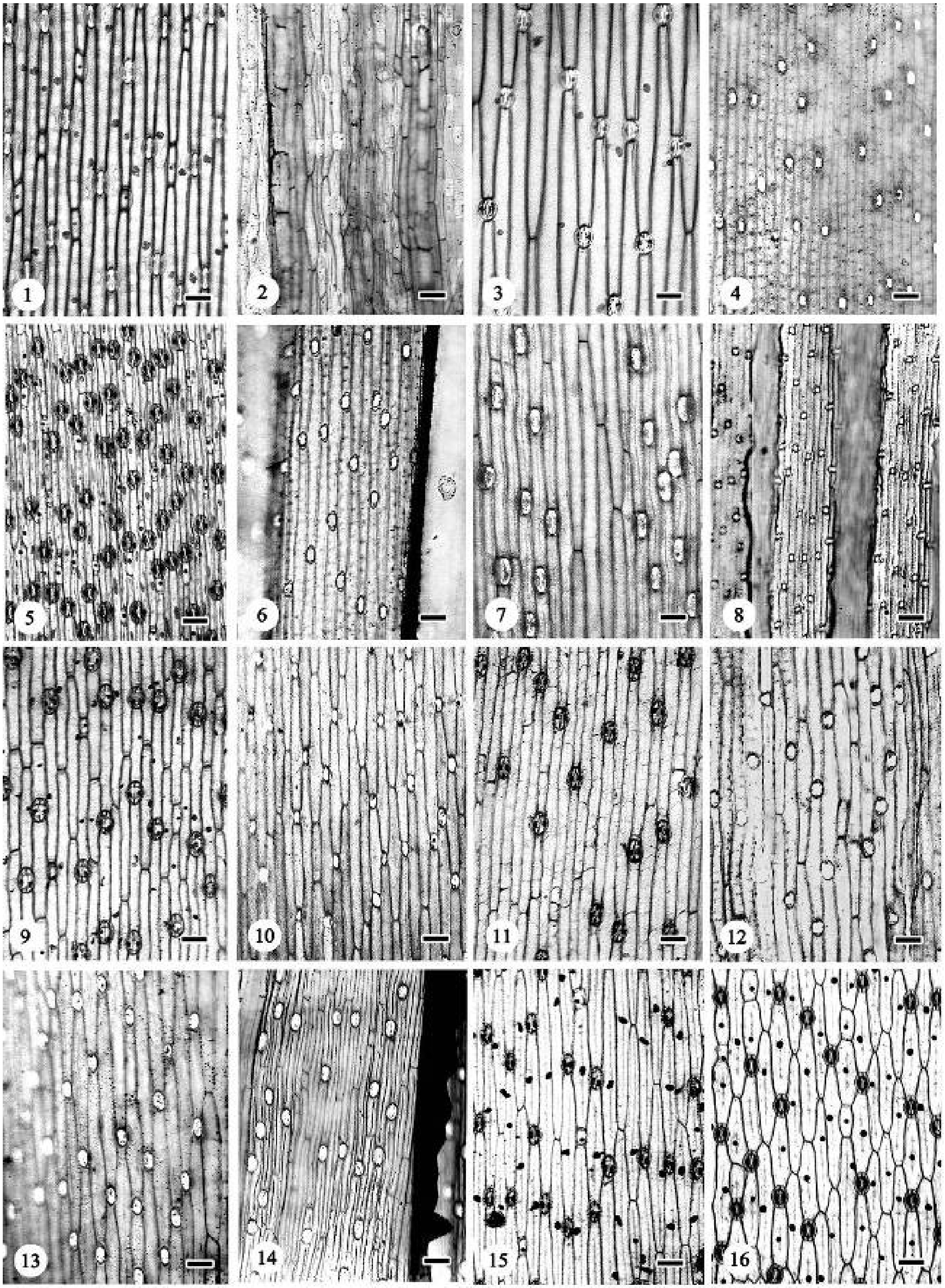
Table 2. Characteristics of adaxial epidermal cells of leaves of 43 species of *Allium* from Xinjiang under light microscopy.

Taxa	Shape	P.A.W.	Epidermal cell			Stomatal apparatus			Fig.
			Length (μm)	width (μm)	D.E.C. (number/mm^2)	Length (μm)	width (μm)	S.I	
A. tuberosum	Rho	Str	543.64±21.73 (370.0-779.6)	31.99±0.53 (27.2-41.0)	70.90±2.12 (54.4-87.5)	68.6±0.60 (65.3-76.5)	41.61±0.40 (37.6-45.6)	41.04±1.23 (31.58-48.27)	1
A. ramosum	Rho	Str	185.20±11.21 (115.6-283.9)	19.24±0.73 (13.2-28.1)	280.72±13.96 (218.9-342.7)	36.82±0.83 (29.7-42.5)	25.50±0.97 (16.5-31.8)	37.02±1.52 (29.23-41.82)	2
A. oreoprasum	Rho	Str	569.54±27.78 (316.5-798.5)	29.83±0.92 (24.25-35.09)	37.22±1.03 (26.95-43.95)	48.85±0.53 (43.6-54.1)	39.20±0.63 (32.8-45.5)	46.05±0.22 (44.30-48.53)	3
A. lineare	Rho	Str	364.60±18.69 (187.1-509.0)	21.20±0.63 (17.0-28.0)	106.65±2.34 (87.3-120.6)	43.92±0.83 (38.8-52.9)	22.10±0.52 (18.2-27.8)	48.23±0.49 (35.8-52.5)	4
A. strictum	Rho	Str	243.33±10.61 (125.3-344.1)	20.51±0.50 (15.3-26.5)	212.22±4.69 (179.7-248.9)	48.63±0.39 (45.3-54.1)	38.33±0.44 (34.0-41.8)	42.06±0.71 (36.36-47.92)	5
A. flavidum	Rho	Str	360.00±21.18 (151.3-521.7)	22.51±0.53 (16.7-26.4)	100.23±0.83 (95.2-105.9)	54.85±0.51 (52.4-62.6)	36.40±0.38 (34.0-40.6)	50.28±0.14 (49.43-50.99)	6
A. przewalskianum	Rec	Str	393.56±16.38 (280.4-651.7)	36.52±0.79 (28.5-44.4)	51.00±0.80 (43.8-57.9)	68.56±0.67 (61.2-74.8)	49.50±1.36 (23.7-57.0)	37.96±0.55 (33.33-42.86)	7
A. tekesicola	Rec	Str	212.27±8.54 (112.5-282.5)	13.62±0.30 (11.2-18.2)	310.91±4.92 (277.7-348.6)	26.13±0.63 (22.1-30.2)	17.92±0.45 (15.1-20.8)	50.00±0.15 (42.85-53.27)	8
A. mongolicum	Rec	Str	352.33±14.72 (198.7-506.1)	24.15±0.60 (18.3-32.2)	109.54±1.89 (93.6-124.0)	52.55±0.61 (45.5-58.4)	33.03±0.57 (27.0-37.8)	37.41±0.14 (29.97-44.84)	9
A. caespitosum	Rho	Str	281.73±12.94 (148.1-450.8)	23.82±0.69 (17.4-30.5)	142.03±5.65 (108.2-176.1)	45.27±1.29 (35.4-54.6)	31.81±1.74 (21.4-50.1)	44.34±1.52 (35.84-53.08)	10
A. polyrhizum	Rho	Str	377.06±24.59 (264.8-620.5)	21.21±0.40 (16.52-24.7)	83.89±2.70 (67.9-98.0)	46.07±0.53 (42.6-50.9)	34.72±0.26 (32.6-36.8)	42.76±0.40 (40.35-44.58)	11
A. bidentatum	Rec	Str	246.30±10.69 (142.5-361.9)	23.8±0.68 (17.0-28.9)	120.03±1.41 (109.3-139.4)	40.21±1.60 (29.0-51.8)	27.72±1.80 (17.4-45.3)	42.30±0.02 (40.02-42.37)	12
A. anisopodium	Rho	Str	302.45±9.28 (256.8-465.2)	25.40±1.09 (17.8-36.3)	145.07±5.08 (117.9-183.7)	46.17±0.71 (40.5-53.8)	30.07±0.42 (24.0-33.8)	41.74±0.75 (36.99-45.97)	13
A. weschniakowii	Rho	Str	203.30±8.08 (132.7-345.0)	20.03±0.33 (16.0-25.1)	119.79±2.68 (109.3-132.4)	36.2±0.42 (32.3-38.8)	22.80±0.28 (20.1-25.6)	40.78±0.52 (35.65-42.37)	14
A. senescens	Rho	Str	296.63±12.53 (174.8-435.9)	27.71±0.58 (22.30-35.4)	85.83±2.27 (72.9-99.8)	48.07±0.64 (40.1-53.7)	32.63±0.33 (28.1-34.9)	40.75±0.26 (39.02-41.98)	15
A. nutans	Rho	Str	210.00±10.55 (151.10-284.52)	29.36±0.59 (25.88-36.30)	109.68±4.82 (81.9-136.89)	47.46±0.32 (43.9-48.9)	32.51±0.21 (30.8-35.1)	29.34±0.49 (25.93-32.92)	16
A. obliquum	Rho	Str	257.98±14.44 (147.1-363.4)	28.71±0.80 (21.00-34.38)	216.03±8.35 (172.6-263.0)	43.34±0.43 (39.5-46.5)	36.50±0.66. (32.8-42.0)	37.94±1.56 (30.2-38.0)	17
A. hymenorhizum	Rho	Str	225.08±14.03 (111.6-365.8	17.43±0.53 (14.3-25.3)	223.4±7.19 (185.0-263.4)	35.55±0.51 (32.1-38.9)	25.14±0.49 (21.4-30.3)	40.79±0.12 (36.63-44.90)	18
A. carolinianum	Rho	Str	244.60±16.14 (147.4-313.1)	28.46±0.70 (21.0-35.6)	115,83±4.50 (101.9-125.9)	40.62±0.33 (38.8-43.2)	33.30±0.36 (30.4-35.9)	44.58±0.37 (43.93-46.03)	19
A. platyspathum	Rec	Str	364.00±22.18 (184.1-629.4)	20.33±0.38 (16.0-29.2)	66.04±2.30 (56.0-79.4)	39.50±0.88 (27.2-49.7)	23.82±0.57 (18.8-33.1)	47.53±1.53 (38.87-55.55)	20
A. saxatile	Rec	Str	345.15±14.16 (216.3-427.8)	17.90±0.39 (13.6-23.2)	114.86±1.66 (104.0-123.8)	46.66±0.29 (42.7-52.8)	34.23±0.14 (32.0-40.7)	48.60±0.10 (48.02-49.17)	21
A. caricoides	Rec	Str	444.04±19.81 (290.0-658.5)	27.50±0.74 (21.0-37.0)	63.35±0.93 (56.9-74.2)	51.01±0.41 (47.6-56.8)	39.28±0.39 (33.3-42.9)	49.00±0.46 (46.67-54.17)	22

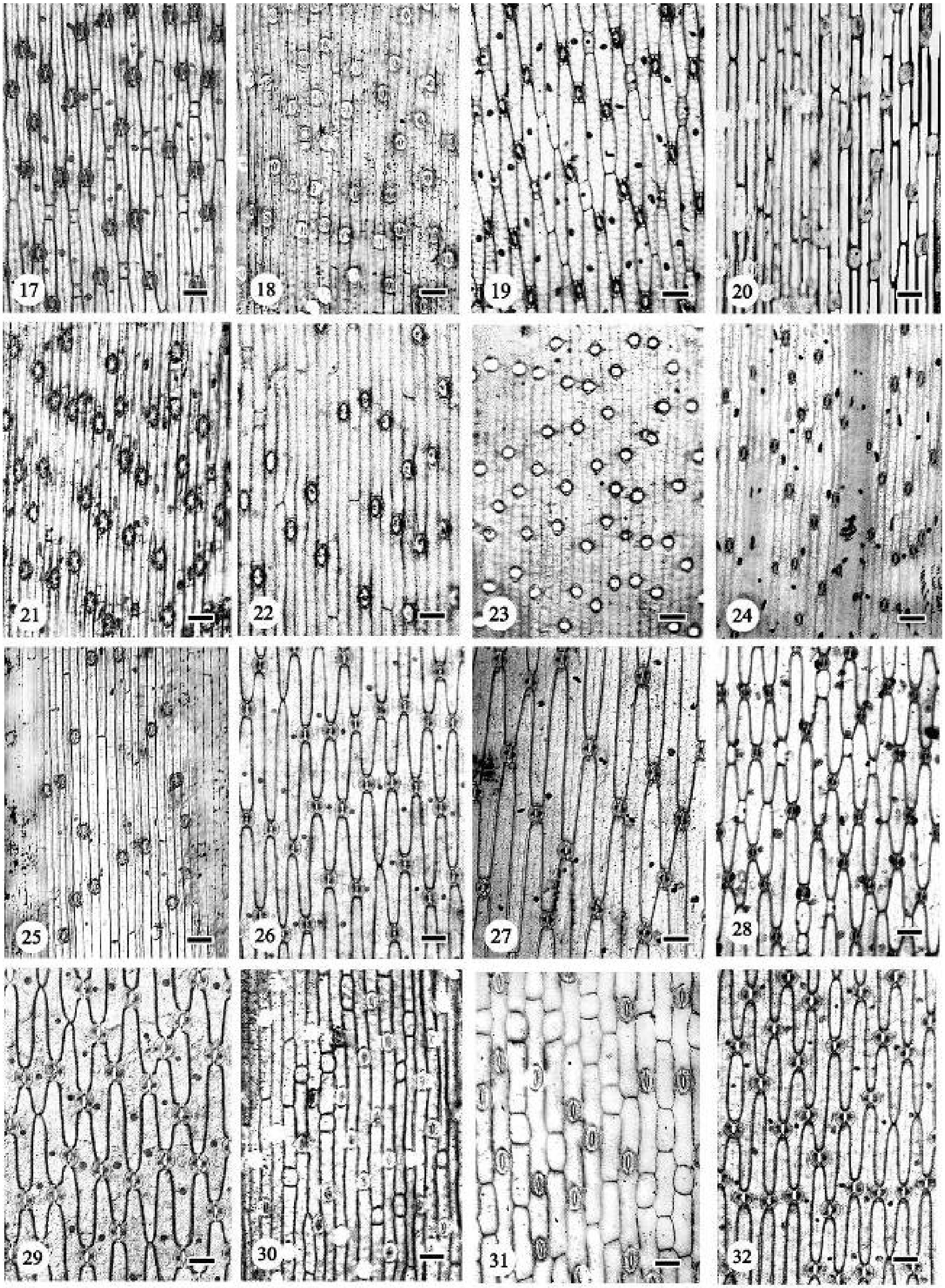
Table 2. (Cont'd.).

Taxa	Shape	P.A.W.	Epidermal cell			Stomatal apparatus			Fig.
			Length (µm)	width (µm)	D.E.C. (number/mm^2)	Length (µm)	width (µm)	S.I	
A. tianschanicum	Rho	Str	322.65±15.50 (225.3-543.1)	18.78±0.38 (15.9-23.3)	124.71±1.49 (116.2-139.8)	53.79±0.50 (49.0-57.8)	35.10±0.51 (32.6-40.0)	50.37±0.40 (47.38-51.47)	23
A. setifolium	Rho	Str	296.40±16.40 (148.7-475.7)	17.58±0.37 (14.0-23.0)	151.34±1.66 (143.0-159.7)	30.98±0.47 (27.9-36.1)	22. 39±0.37 (20.1-25.7)	43.64±0.32 (42.06-45.21)	24
A. subtilissimum	Rec	Str	400.91±13.54 (282.1-502.5)	16.53±0.36 (13.0-19.7)	115.80±2,45 (96.7-127.9)	39.29±0.49 (35.1-42.7)	32.37±0.30 (30.0-35.4)	45.42±0.73 (41.82-49.02)	25
A. altaicum	Rho	Arc	461.33±17.09 (368.2-613.2)	30.56±0.61 (26.1-34.4)	57.83±2.47 (44.7-79.2)	45.23±0.26 (43.8-51.6)	42.75±0.30 (41.0-44.9)	49.94±1.0 (44.0-55.6)	26
A. fistulosum	Rho	Arc	370.60±16.87 (210.6-546.7)	34.24±0.69 (27.5-40.4)	67.03±1.91 (56.5-75.9)	42.50±0.64 (38.2-46.6)	34.20±0.48 (30.8-37.1)	49.18±0.35 (46.84-51.28)	27
A. cepa	Rho	Arc	255.10±12.84 (118.6-387.3)	33.74±0.77 (28.2-52.8)	91.73±1.17 (85.9-97.9)	38.80±0.65 (35.1-42.1)	30.07±0.40 (25,5-31.9)	48.43±0.15 (47.49-49.22)	28
A. galanthum	Rho	Arc	267.02±14.45 (169.3-475.2)	41.01±0.72 (34.0-46.9)	57.30±1.34 (50.6-67.1)	50.47±0.68 (43.5-53.9)	46.80±0.41 (44.0-49.4)	43.67±0.59 (38.30-47.37)	29
A. semenovii	Rec	Str	184.22±8.72 (117.3-265.9)	24.87±0.46 (21.4-28.9)	180.24±2.45 (147.8-200.5)	39.50±0.86 (31.8-45.7)	28.40±0.46 (25.6-32.1)	59.62±0.20 (48.37-62.58)	30
A. atrosanguineum	Rec	Str	141.21±7.51 (84.4-221.7)	29.74±1.69 (20.4-42.8)	242.99±3.03 (227.1-265.75)	47.74±0.72 (41.7-54.0)	36.50± 0.79 (30.0-43.5)	28.28±0.29 (24.5-29.87)	31
A. schoenoprasum	Rho	Arc	494.85±17.42 (338.3-708.2)	33.15±0.73 (26.0-42.5)	55.72±2.21 (41.3-69.9)	49.22±0.70 (44.5-57.5)	45.87±0.50 (40.2-49.6)	50.81±0.31 (48.57-53.13)	32
A. grisellum	Rec	Str	309.17±11.67 (208.4-390.1)	19.66±0.36 (17.1-23.1)	86.65±3.17 (67.4-105.7)	47.35±0.49 (42.8-50.6)	39.97±0.53 (33.6-44.1)	32.20±1.23 (25.71-40.74)	33
A. delicatulum	Rho	Str	420.14±14.11 (313.4-577.0)	30.91±0.98 (23.4-39.9)	98.43±3.12 (65.6-116.5)	54.79±0.63 (50.3-61.2)	34.12±0.52 (30.2-38.2)	42.22±0.73 (38.00-48.33)	34
A. glomeratum	Rec	Str	381.8±18.70 (218.1-577.2	16.51±0.39 (12.5-23.4)	136. 19±2.15 (123.5-149.5)	26.95±0.47 (22.9-29.7)	16.19±0.51 (13.3-21.5)	54.67±0.41 (52.38-56.92)	35
A. pallasii	Rec	Str	483.50±17.75 (286.6-616.2)	25.22±0.67 (17.5.0-30.4)	95.30±3.01 (77.4-127.1)	49.27±0.96 (43.3-62.4)	35.13±1.10 (25.2-41.5)	44.33±0.72 (38.33-47.06)	36
A. caeruleum	Rho	Str	503.04±21.21 (249.8-649.0)	29.04±1.11 (23.0-39.6)	64.64±1.28 (54.3-72.2)	47.89±0.79 (38.8-54.6)	38.18±0.76 (31.3-44.2)	47.08±0.54 (42.5-51.4)	37
A. ceasium	Rho	Str	495.56±17.41 (303.7-679.4)	27.27±0.66 (22.8-34.0)	61.48±1.33 (49.48-74.18)	64.55±0.66 (58.9-71.5)	42.39±0.36 (39.3-45.5)	51.24±0.88 (43.9-57.14)	38
A. porrum	Rho	Str	589.24±28.37 (284.5-799.8)	37.57±1.48 (23.0-53.4)	43.47±1.20 (36.9-49.7)	47.58±0.61 (42.4-52.2)	32.72±0.42 (30.7-37.8)	51.13±0.28 (48.98-53.00)	39
A. sativum	Rho	Str	349.89±11.82 (264.9-507.4)	40.10±0.99 (25.8-49.0)	67.58±0.71 (63.6-71.9)	49.80±0.58 (46.1-58.1)	36.43±0.44 (33.2-40.4)	49.75±0.34 (49.23-50.38)	40
A. roborowskianum	Rho	Str	495.51±18.00 (376.9-772.5)	56.67±1.19 (44.8-66.6)	33.66±0.38 (30.9-37.0)	57.63±0.48 (51.9-62.7)	47.19±0.52 (41.7-52.8)	50.08±0.27 (40.31-51.91)	41
A. robustum	Rho	Str	291.61±12.33 (189.2-515.1)	35.22±1.05 (27.0-43.9)	85.84±1.01 (75.0-92.1)	50.33±0.40 (47.7-55.3)	46.61±1.74 (40.5-76.2)	44.95±0.38 (41.46-47.73)	42
A. fetisowii	Rho	Str	403.86±12.58 (217.0-563.7)	35.60±1.26 (22.5-45.7)	58.42±0.72 (52.1-64.7)	55.24±0.65 (46.4-60.8)	41.93±0.31 (32.3-44.6)	41.31±0.78 (35.5-48.4)	43

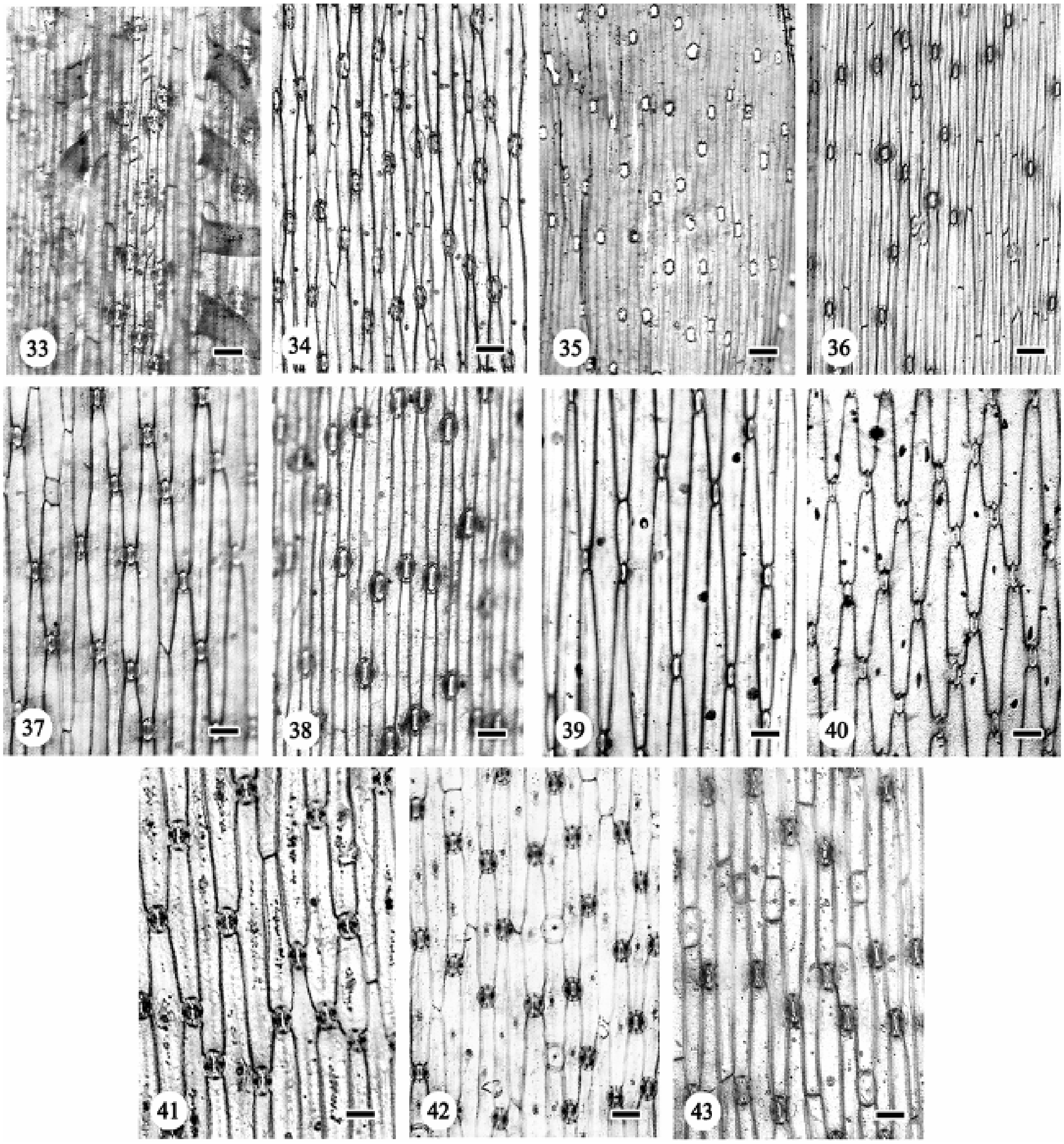
Data are presented as mean ± standard error and measurement minimum and maximum are given in parentheses. Rec: Rectangular; Rho: Rhomboid; Str: Straight; Arc: Arched; P.A.W.: Pattern of anticlinal walls; D.E.C.: Density of epidermal cell; S.I.: Stomatal index



Figs. 1-16. Characteristics of adaxial epidermal cells in *Allium*. Scale bar = 40 µm.
1. *A. tuberosum*, 2. *A. ramosum*, 3. *A. oreoprasum*, 4. *A. lineare,* 5. *A. strictum*, 6. *A. flavidum,* 7. *A. przewalskianum*, 8. *A. tekesicola*, 9. *A. mongolicum*, 10. *A. caespitosum*, 11. *A. polyrhizum*, 12. *A. Bidentatum*, 13. *A. anisopodium*, 14. *A. weschniakowii*, 15. *A. senescens*, 16. *A. nutans*



Figs. 17-32. Characteristics of adaxial epidermal cells in *Allium*. Scale bar = 40 µm.
17. ***A. obliquum***, 18. ***A. hymenorhizum***, 19. ***A. carolinianum,*** 20. ***A. platyspathum***, 21. ***A. saxatile***, 22. ***A. caricoides***, 23. ***A. tianschanicum,*** 24. ***A. setifolium***, 25. ***A. subtilissimum***, 26. ***A. altaicum***, 27. ***A. fistulosum,*** 28. ***A. cepa***, 29. ***A. galanthum***, 30. ***A. semenovii***, 31. ***A. atrosanguineum***, 32. ***A. schoenoprasum***.



Figs. 33-43. Characteristics of adaxial epidermal cells in *Allium*. Scale bar = 40 μm.
33. *A. grisellum*, 34. *A. delicatulum*, 35. *A. glomeratum*, 36. *A. Pallasii*, 37. *A. caeruleum*, 38. *A. ceasium*, 39. *A. porrum*, 40. *A. sativum*, 41. *A. roborowskianum*, 42. *A. robustum*, 43. *A. fetisowii*

Discussion

Leaf epidermal cells of *Allium* are usually rectangular to linear in shape, with straight anticlinal walls, and the stomatal apparatus is amphistomatic and anomocytic with stomata being more or less raised from the epidermal surface (Choi *et al*., 2004; Choi & Cota-Sanchez, 2010), and the taxonomic significance of these characters was recognized by Krahulec (1980), Tanker & Kurucu (1981) and Uysal (1999). Our observations on leaf epidermal cells of 43 *Allium* species showed that each species had its own unique combination of morphological features, including shape, size and density of cells; pattern of anticlinal walls; size of guard cells; and stomatal index. The shape of epidermal cells, pattern of anticlinal walls and stomatal index are stable within a taxon, but these characters differ among species and thus are useful for delimitation of species.

Gregory (1996) regarded the width and length of guard cells in 22 species of *Allium* sect. *Allium* to be of significant taxonomic value and thus divided this section into two subsections: *Oenoprason* and *Scordoprason* based on guard cells being about half the width of epidermal cells and shorter than half the length of the epidermal cells, respectively. However, the width and length of guard cells in the 43 species we studied did not allow us to divide the species into sections or groups. Anomocytic stomata apparatus appears to be a consistent character for *Allium*, i. e. the stomatal index is stable within a species but can differ between species.

Trichomes have important morphological/taxonomic value in *Allium*. Yousaf *et al*. (2008) found that trichomes were present in eight of 20 species of *Allium* from Pakistan, i.e. *A. barszczewksi*, *A. borszczowii*, *A. micranthum*, *A. lamondae*, *A. miserbile*, *A. longicollum*, *A. gilli* and *A. dolichostylum*, and they could be used for the resolution of taxonomic confusion of these species. However, in our study trichomes were found on the leaf epidermis of only *A. grisellum*. This character previously has not been recorded for *Allium* in the Flora of China (Xu & Kamelin, 2000).

Epidermal characters can provide useful information for a better infrageneric resolution of Allium. As a result, some taxonomic information at the species level can be obtained from four main qualitative characters: rectangular vs. rhomboid shape of leaf epidermal cells and straight vs. arched pattern of the anticlinal walls. Our 43 study species can be divided into three distinct types based on shape and pattern of anticlinal walls of leaf epidermal cells: type 1, rhomboid cell shape and straight anticlinal walls, i.e., *A. tuberosum*, *A. ramosum*, *A. oreoprasum*, *A. lineare*, *A. strictum*, *A. flavidum*, *A. caespitosum*, *A. polyrrhizum, A. anisopodium*, *A. weschniakowii*, *A. senescens*, *A. nutans*, *A. obliquum*, *A. hymenorrhizum*, *A. carolinianum*, *A. tianschanicum*, *A. setifolium*, *A. delicatulum*, *A. caeruleum*, *A. ceasium*, *A. porrum*, *A. sativum*, *A. roborowskianum*, *A. robustum*, *A. fetissowii*; type 2, rhomboid cell shape and arched anticlinal walls, i.e., *A. altaicum*, *A. fistulosum*, *A. cepa*, *A. galanthum*, *A. schoenoprasum*; and type 3, rectangular cell shape and straight anticlinal walls, i.e., *A. przewalskianum*, *A. tekesicola*, *A. mongolicum*, *A. bidentatum*, *A. platyspathum*, *A. saxatile*, *A. caricoides*, *A. subtilissimum, A. semenovii*, *A. atrosanguineum*, *A. grisellum*, *A. glomeratum*, *A. pallasii*.

Using the Allium classification system of Vvedensky (1935) and Xu (1980), the 43 studied species belong to six sections (*Rhiziridium*, *Haplostemon*, *Porrum*, *Schoenoprasum*, *Molium*, *Cepa*) based on morphological characters; however, these species belong to three types based on leaf epidermis characters of their. Type 1 includes sections *Rhiziridium* (17 species), *Haplostemon* (3 species), *Porrum* (2 species) and *Molium* (3 species); type 2 sections *Cepa* (2 species) and *Schoenoprasum* (3 species); and type 3 sections *Rhiziridium* (9 species), *Schoenoprasum* (1 species) and *Haplostemon* (3 species). Thus, our type 1 includes species from four sections, type 2 species from four sections, and type 3 species from three sections. In the *Allium* classification systems of Friesen *et al*. (2006) and of Li *et al*. (2010), which are based on analyses of DNA ITS sequences, our study species can be divided into six subgenera and 14 sections (Friesen *et al*., 2006) and seven subgenera and 20 sections (Li *et al*., 2010), respectively. Type 1 includes subgenera *Butomissa* (3 species), *Reticulatobulbosa* (3 species), *Rhizirideum* (5 species), *Polyprason* (5 species), *Allium* (5 species) and *Melanocrommyum* (3 species); type 2 sections *Cepa* (5 species); and type 3 sections *Rhizirideum* (3 species), *Reticulatobulbosa* (1 species), *Polyprason* (4 species), *Cepa* (3 species) and *Allium* (2 species). Thus, type 1 in our study includes species from six subgenera, type 2 species from one subgenus and type 3 species from five subgenera. All 43 species in our study belong to different sections based on morphological characters and to different subgenera based on DNA characters. However, leaf micromorphological characters do not completely support the sectional and subgeneric classification of Allium based on morphological and DNA molecular studies.

Some morphological leaf epidermal characters of *Allium* can be modified by environmental conditions. For example, leaves hairy, xerophytic stomata (Uysal, 1999), and papillae's shape shows the taxonomic and ecological relationships of the plant (Krahulec, 1980). These characters may be adaptations to local habitat factors. Also, convergent evolution can lead to similarity of leaf epidermal pattern characters in taxonomically different species, and such a case was reported by Krahulec (1980). Further, many other micromorphological characters of the seed coat, pollen grains, bulb coats and floral structures are diagnostically important and commonly used in species descriptions and have greatly improved the taxonomy of the genus (Namin *et al*., 2009a, 2009b; Choi & Oh, 2011; Choi *et al*., 2011, 2012; Celep *et al*., 2012; Rola, 2014).

In our study, the main differences in leaf epidermal micromorphological characters were at the species level; specific characters for only a few subgenera were identified. Thus, we conclude that these characters are useful in helping to distinguish species of *Allium*. Consequently, leaf epidermal micromorphological traits need to be considered along with information from studies of morphology and anatomy, palynology, cytology and molecular biology in the on-going attempt to obtain a full understanding the taxonomy, systematic position and phylogenetic relationships of *Allium* species.

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