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Solving some ambiguities using micromorphological traits in some species of *Campanula* in the north and northwest of Iran

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Soheila Abdollahfard: PhD Student, Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

Fariba Sharifnia✉: Associate Prof., Department of Biology, North Tehran Branch, Islamic Azad University, Tehran, Iran (sharifnia2021@gmail.com)

Sara Saadatmand: Associate Prof., Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

Mostafa Assadi: Research Prof., Research Institute of Forests and Rangelands, Agricultural Research Education and Extension Organization (AREEO), P.O. Box 13185-116, Tehran, Iran

Iraj Mehregan: Associate Prof., Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

Abstract

In the present research, scanning electron microscope (SEM) was employed to observe the outer perianth (sepal) of 14 species of *Campanula* (*Campanulaceae*) in the north and northwest of Iran through micromorphological studies. Hence, by a precise observation, the perianths were examined in terms of hairiness, hair shape, hair density and epidermal pattern, and then a table of traits was prepared. The species were further categorized into two groups: hairy and hairless; in terms of hairiness, into five groups: stellate, strigose, villouse, crispate, and hooked; and in terms of hair variations into five groups: striate, rugose, cup-shaped, papillate; and striate-papillate. According to the results, the species complex *C. reuteriana* and *C. propinqua* exhibit differences in terms of the hair type, perianth, as well as epidermal pattern of the perianth. Furthermore, based on the results of this research, the micromorphological studies of the epidermal pattern of perianth confirmed the separation of species complex *C. trachelium* from *C. latifolia*. In general, the present study revealed that, species are differentiated and identified via their micromorphological features.

Keywords: *Campanulaceae*, *C. propinqua*, *C. reuteriana*, perianth epidermal pattern, SEM

رفع برخی ابهامات با استفاده از صفات ریزریخت‌شناسی در برخی گونه‌های *Campanula* در شمال و شمال‌غرب ایران*

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سهیلا عبداله‌فرد: دانشجوی دکتری گروه زیست‌شناسی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران

فریبا شریف‌نیا✉: دانشیار گروه زیست‌شناسی، واحد تهران شمال، دانشگاه آزاد اسلامی، تهران، ایران (sharifnia2021@gmail.com)

سارا سعادتمند: دانشیار گروه زیست‌شناسی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران

مصطفی اسدی: استاد پژوهش مؤسسه تحقیقات جنگل‌ها و مراتع کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی، صندوق پستی ۱۱۶-۱۳۱۸۵، تهران، ایران

ایرج مهرگان: دانشیار گروه زیست‌شناسی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران

خلاصه

در این پژوهش، گلپوش خارجی (کاسبرگ) ۱۴ گونه از جنس *Campanula* (گل‌استکانیان) با میکروسکوپ الکترونی نگاره (SEM) در شمال و شمال‌غرب ایران مورد مطالعات ریزریخت‌شناسی قرارگرفت. در مشاهدات مورد نظر، گلپوش‌ها از نظر وضعیت، شکل و تراکم کرک‌ها و همچنین الگوی اپیدرمی گلپوش بررسی و جدول صفات تهیه گردید. همچنین، گونه‌ها از نظر وضعیت کرک به دو گروه کرک‌آلود و بدون کرک و در رابطه با تنوع کرک به پنج گروه ستاره‌ای (stellate)، خمیده و زبر (strigose)، کرک بلند (villouse)، مجعد (crispate) و قلابی (hooked) و نیز براساس الگوی اپیدرمی گلپوش به پنج گروه مخطط (striate)، چروکیده (rugose)، فنجانی شکل (cup-shaped)، دارای برجستگی (papillate) و مخطط دارای برجستگی (striate-papillate) دسته‌بندی شدند. طبق نتایج به دست آمده در این بررسی، کمپلکس گونه‌ای *C. reuteriana* و *C. propinqua* تفاوت‌هایی را از نظر نوع کرک گلپوش و الگوی اپیدرمی گلپوش نشان دادند. همچنین در مطالعات ریزریخت‌شناسی الگوی اپیدرمی گلپوش، جدایی کمپلکس گونه‌ای *C. trachelium* از *C. latifolia* بر اساس نتایج به دست آمده در این تحقیق تایید می‌شود. به طورکلی، مطالعه حاضر نشان داد که گونه‌ها با صفات ریزریخت‌شناسی خود مشخص و متمایز می‌شوند.

واژه‌های کلیدی: الگوی اپیدرمی گلپوش، گل‌استکانیان، میکروسکوپ الکترونی نگاره، *C. propinqua*،*C. reuteriana*

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Introduction

The *Campanulaceae* Jussieu is a well-known family comprising 84 genera and nearly 2400 species (Lammers 2007). The family shows a cosmopolitan distribution i.e., a wide array of habitats from tropical rainforests to tundra (Antonelli 2008). Within the genus *Campanula* L., about 580–600 species are currently known word-widely (Mansion *et al*. 2012). *Campanula* is represented by annual and perennial species occurring in different types of habitats such as grasslands, garrigue, meadows, woodlands, and rupestrian (Kovačić 2004, Roquet *et al*. 2008, Bogdanović *et al.* 2014) mostly distributed in the northern hemisphere. About 250 species are so far known in the Mediterranean area (Park *et al*. 2006), that can be considered as one of the principal centers of its diversity (Myers *et al*. 2000). According to the literature, the genus shows remarkable variability in the habit, leaf as well as flower morphology (Roquet *et al*. 2008), carpology (Kolakovsky 1986), seed micromorphology (Akcin 2009, Alçitepe 2010), palynology (Dunbar 1975), and karyology (Kovanda 1970), making the natural hierarchical arrangement of the genus as a challenging issue. Iran with about 44 species and 11 endemics classified in five subgenera and 10 sections (Aghabeygi & Jalilian 2010, Rechinger & Schimann-Czeika 1965), is an important diversification center of this genus.

The taxonomic history of *Campanula* dates back to Linnaeus (1753) who described 34 species, of which eight species occur in Iran. De Candolle (1830) published the first inclusive monograph of the family, including *Campanula* along with 20 other genera. Boissier (1875) recorded 125 species of *Campanula* in Flora Orientalis, of which 19 are known from Iran.

Since most morphological characters are highly plastic and not suitable to delineate natural groups (Roquet *et al*. 2008), the taxonomic treatment of *Campanula* is rather complex. There have been several attempts at the classification (De Candolle 1830), but none of them have been confirmed by any phylogenetic studies available to date (Cellinese *et al.* 2009, Haberle *et al*. 2009, Mansion *et al*. 2012). In the genus *Campanula*, morphological and molecular results are often in conflict, but some small groups are morphologically and karyologically well-differentiated, forming distinct and well-supported clades in phylogenetic trees, suggesting their monophyletic origin (Park *et al*. 2006, Mansion *et al.* 2012, Lakusˇic´ *et al*. 2013). Molecular phylogenetic studies have revealed that, *Campanula*, as currently delimited: (1) is not monophyletic, (2) has unresolved phylogenetic relationships with several closely related genera, and (3) the majority of *Campanula* species constitute two main groups named as the *Campanula* s.str. clade and the *Rapunculus* clade (Eddie *et al*. 2003, Park *et al.* 2006, Roquet *et al.* 2008, Mansion *et al*. 2012, Lakušić *et al.* 2013). However, *Campanula* presents a great variety of basic chromosome numbers, even within the taxa of the Mediterranean basin alone. The most common number i.e., x = 17, has been found in some *Campanulaceae* not closely related to *Campanula*, such as *Canarina* L., *Nesocodon* M. Thulin, and *Ostrowskia* Regel. Several chromosome numbers have been suggested as ancestral numbers for the genus or the family (e.g., x = 7 by Raven 1975, x = 8 by Contandriopoulos 1984). Pollen studies in *Campanula* made by Dunbar (1975) suggested a relationship between a change in pollen ornamentation from the ridges to finger-like structures and the reduction of the inflorescence. In recent years, however, micro-morphological studies have attracted special attention, as better details of external traits may be observed by scanning electron microscopy (20 times more than optical microscopy) (Barani *et al.* 2016).

Trichome is one of the key traits in micromorphology used by botanists, and sometimes it is even considered a determining factor in plant identification (Ghahremaninejad 2004, Zeraatkar *et al.* 2022), using the trichome traits is of paramount importance in classification. Since some plant families are easily identified by their trichome types, therefore, trichomes are used in the classification of genera and species as well as in the analysis of intraspecific hybrids

(Metcalfe & Chalk 1950, Sharifnia & Behzadi Shakib 2012, Mirzadeh *et al*. 2015).

The present study examined some species of the genus *Campanula* (14 species) from the northern and northwestern provinces of Iran which is rich in terms of plant diversity. The type of trichomes on the outer surface of the external perianth (sepals) was examined by scanning electron microscope (SEM) (model Phylips-XL30 ESEM). The perianths were assessed due to the trichomes and the trichome morphology, as well as epidermal pattern morphology was compared based on their differences. The present study, therefore, aims to investigate the taxonomic significance of micro-morphological characteristics used for species identification and delimitation in the Iranian *Campanula* genus.

Scanning electron microscopy (SEM) has been proved as a reliable technique for observations of the surface morphology of plant materials, primarily due to the improved depth of field and high resolution, which are unobtainable with the light microscope. This tool along with light microscope (LM) allows the use of more micromorphological data in taxonomic and morpho-genetic studies of many plant families. So far, no similar study was performed on the micromorphology of sepals of *Campanula*. Thus, the present study has been outlined to shed more light on the micromorphology of the perianth of the genus focusing on some Iranian species which may provide some evidences useful in the systematics of this genus.The close patterns of epidermal sepal in the angiospermae have important characteristics to identify the species (Christensen & Hansen 1998). In the present study, the micromorphological features of sepals were studied for the better identification providing more detailed information for all 14 *Campanula* taxa.

Materials and Methods

This study was carried out based on the herbarium specimens available in the herbarium of the Ministry of Jihad-e-Agriculture (IRAN), and Science and Research Branch (IAUH) and North Tehran Branch (IAUNT) of Islamic Azad University (Tehran, Iran) (Table 1).The list of samples to investigate the micromorphology of perianth epidermal pattern is shown in table 2.

For the present study, the outer part of the perianth (sepal) was glued on the aluminum bases using double-sided adhesive and covered with a thin layer of gold after freezing by a coating machine. The samples were observed by scanning electron microscope (SEM) (model Phylips-XL30 ESEM).

Each sample was photographed under three magnifications of x50, x100, and x200. In the micro-morphological studies of the epidermal pattern of sepals, some traits such as the presence and absence of trichome, the trichome density, the trichome morphology, and the type of epidermal pattern of the perianth were examined (Table 2). In order to compare the species, a separate table was prepared (Table 2). Christensen & Hansen (1998) showed that, angiosperms have distinct perianth epidermal patterns that are solely governed by genetics and not at all by environmental factors. The terminology used in the present study, is based on previously published studies by Christensen & Hansen (1998).

Table 1. *Campanula* species and their localities and voucher numbers

No.	Taxon	Locality, collector & voucher No.
1	*C. latifolia* L.	Gilan prov.: Talesh, nav village to Khaheh-sara (Cheraghi Nav IAUNT-13982)
2	*C. rapunculoides* L.	Gilan prov.: Talesh, nav village (Cheraghi Nav IAUNT-13974)
3	*C. glomerata* L.	Gilan prov.: Talesh , nav village (Cheraghi Nav IAUNT-13980)
4	*C. involucrata* Auch. ex DC.	Azarbaijan prov.: Azar-shahr (Mehdi Poor IAUNT-16367)
5	*C. lactiflora* M.B.	Gilan prov.: Talesh, nav village to Khaneh-sara (Cheraghi Nav IAUNT-13979)
6	*C. odontosepala* Boiss.	Gilan prov.: Talesh, nav village (Cheraghi Nav IAUNT-13976)
7	*C. propinqua* Fisch. & Mey.	Azarbaijan prov.: Salmas to Urmia, 60km to Ghoshchi (Delghandi & Abbasi IRAN-36465)
8	*C. lambertiana* (DC.) Rech.f.	Gilan prov.: Talesh, nav village to Charso(Cheraghi Nav IAUNT-13978)
9	*C. reuteriana* Boiss. & Bal.	Gilan prov.: Jirandeh, Dosalan to Damash (Parsi & Saadati IAUH-000014199)
10	*C. savalanica* Fedor.	Ardebil prov.: Sabalan (Mozaffariyan IAUH-03267)
11	*C. sclerotricha* Boiss.	Gilan prov.: Jirandeh, near Damash (Parsi & Saadati IAUH-000014200)
12	*C. stevenii* M.B.	Gilan prov.: Talesh, nav village (Cheraghi Nav IAUNT-13973)
13	*C. stricta* L.	Gilan prov.:Talesh to Charsoo (Cheraghi Nav IAUNT-13977)
14	*C. trachelium* L.	Ardebil prov.: Between Ardebil & Astara (Khansari IAUH-03264)

IAUH: Herbarium of Islamic Azad University, Science and Research Branch, IAUNT: herbarium of Islamic Azad University, North Tehran Branch & IRAN: Herbarium of the Ministry of Jihad-e-Agriculture (IRAN), Iranian Research Institute of Plant Protection

Results

The micromorphological results of sepals are given in figures 1–5 and table 2.In general, in the study of the micromorphology of the sepals in this genus; the species were classified into two categories hairy and non-hairy. Among the studied samples, *C. stevenii* (Fig. 3 L1-L3) and *C. latifolia* (Fig. 1 A1-A3) were hairless while other specimens were hairy. In terms of hair density in hairy species, six species of *C. propinqua*, *C. reuteriana*, *C. savalanica* (Fig. 2 G1-G3, I1-I3 & J1-J3), *C. glomerata*, *C. stricta*, and *C. trachelium* (Figs 1 C1-C3 & 3 M1-M3, N1-N3) with high density and other species with low density were observed. In addition, hair shape trait was investigated and five shapes: 1. Long hair (villouse) in species *C. lactiflora* (Fig. 1 E1-E3), *C. savalanica* (Fig. 2 J1-J3), *C. sclerotricha* (Fig. 3 K1-K3) and *C. stricta* (Fig. 3 M1-M3), 2. Curved hairs with ridges on the surface (strigose) in *C. rapunculoides* (Fig. 1 B1-B3), *C. glomerata* (Fig. 1C1-C3), *C. odontosepala* (Fig. 2 F1-F3), *C. trachelium* (Fig. 3 N1-N3) and *C. involucrata* (Fig. 1 D1-D3), 3. Hooked hair in *C. propinqua* (Fig. 2 G1-G3), 4. Crispate hair in *C. reuteriana* (Fig. 2 I1-I3), and 5. Stellate hair in *C. lambertiana* (Fig. 2 H1-H3) (Table 2) were observed.

Based on the epidermal pattern of sepals, five following types have been observed: Type 1. With protrusions (papillate) in *C. glomerata*, *C. involucrata*, *C. lactiflora* (Fig. 1 C1-C3, D1-D3 & E1-E3), *C. reuteriana* (Fig. 2 I1-I3), *C. sclerotricha* (Fig. 3 K1-K3), *C. trachelium* (Fig. 3 N1-N3), *C. stricta* (Fig. 3 M1-M3), and *C. stevenii* (Fig. 3 L1-L3), Type 2. Wrinkled (rugose) in *C. rapunculoides* (Fig. 1 B1-B3), and *C. odontosepala* (Fig. 2 F1-F3), Type 3. Cup-shaped in *C. latifolia* species (Fig. 1 A1-A3), Type 4. Striate in *C. propinqua* (Fig. 2 G1-G3), *C. savalanica* (Fig. 2 J1-J3), and Type 5. Striated-papillate in *C. lambertiana* (Fig. 2 H1-H3) (Table 2). Thus with these traits, we could solve the interspecies differences of some species which already had some taxonomical ambiguities. According to these traits, an identification key for the *Campanula* species was also prepared (see identification key) which is based on the trichome shape, trichome density, and epidermal pattern of the perianth (sepal).

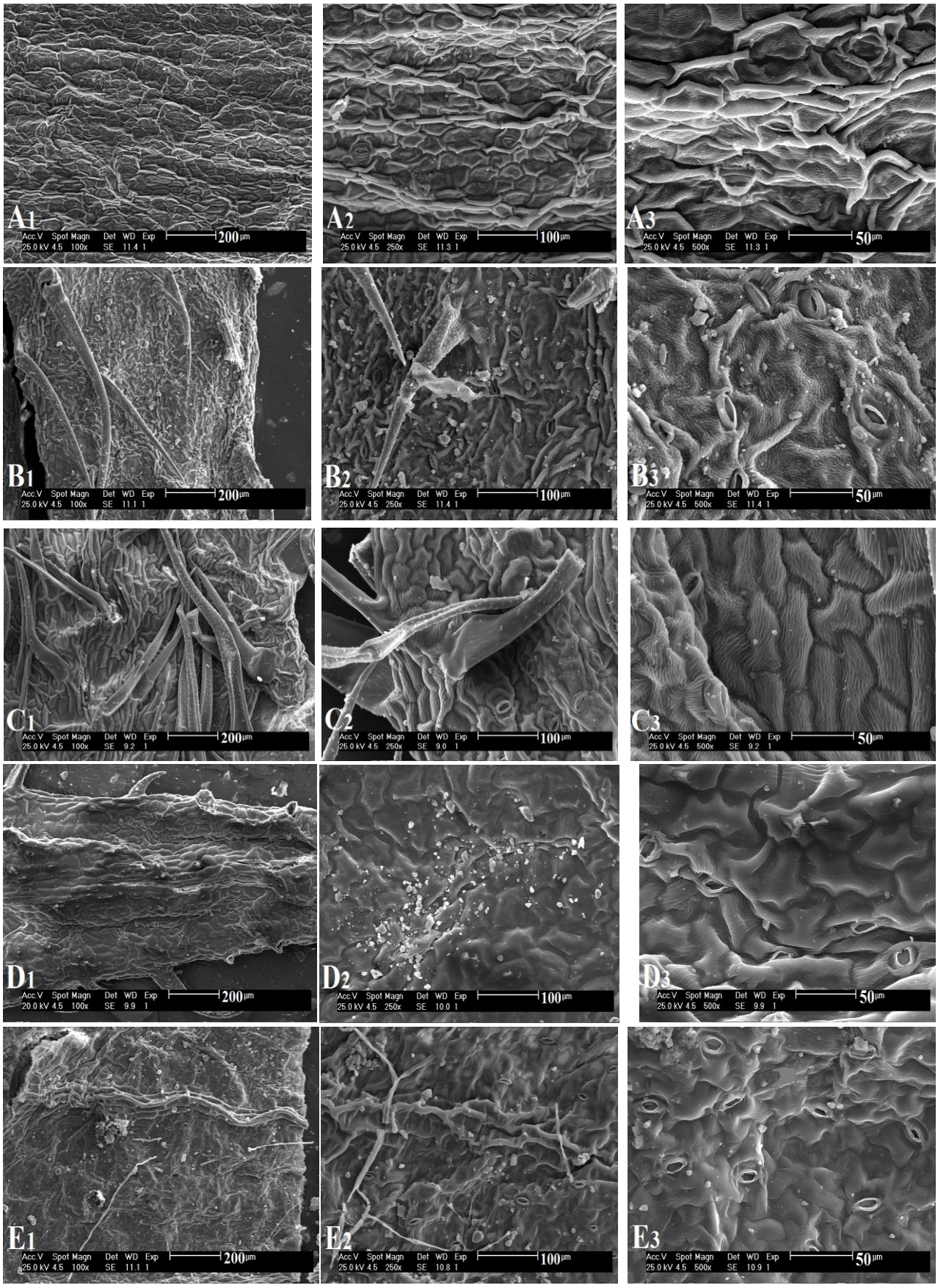

Fig. 1. SEM photographs showing sepal hair found in *Campanula*: A1-A3. *C. latifolia*, B1-B3. *C. rapunculoides*, C1-C3. *C. glomerata*, D1-D3. *C. involucrata*, E1-E3. *C. lactiflora*.

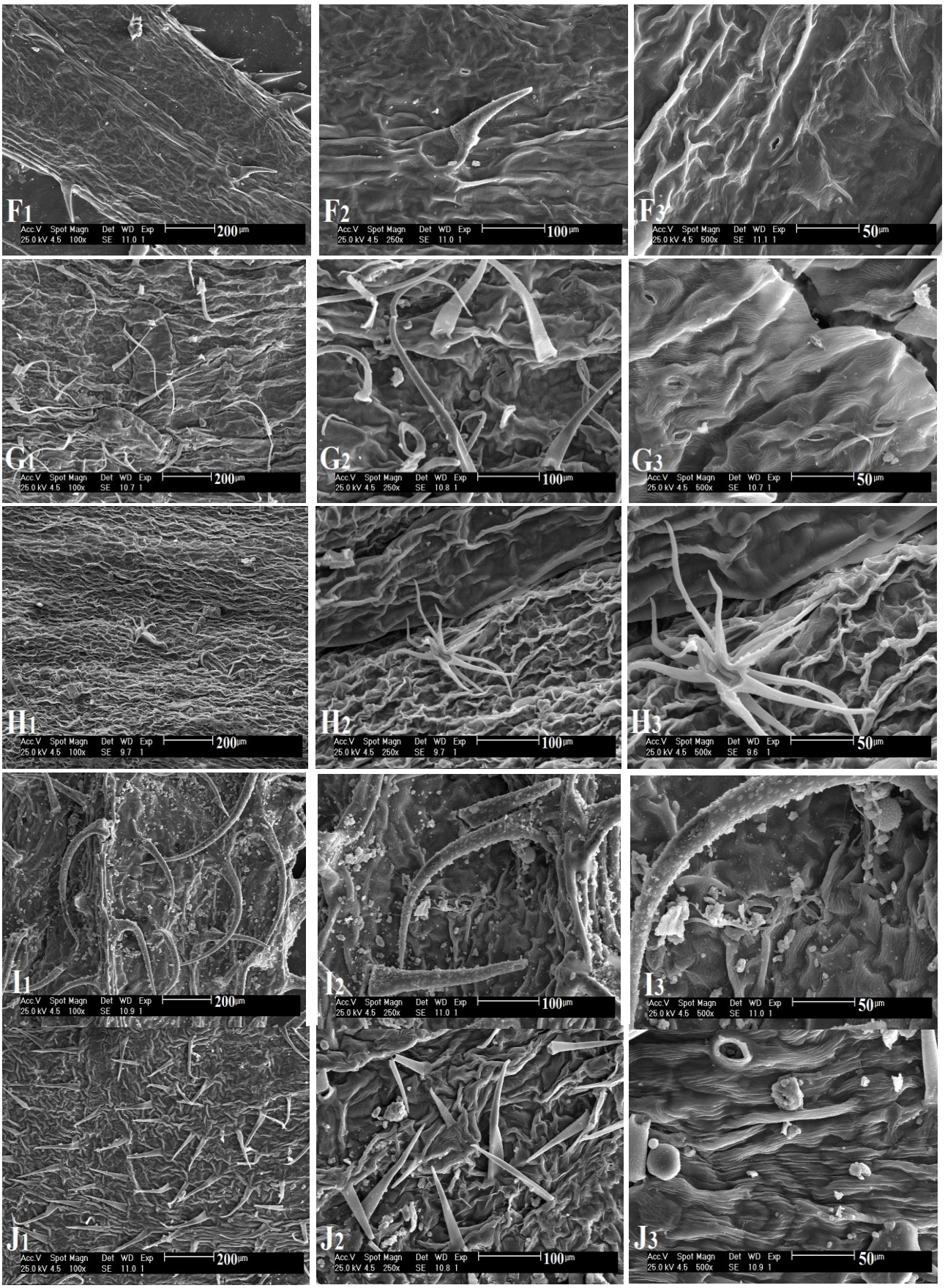

Fig. 2. SEM photographs showing sepal hair found in *Campanula*: F1-F3. *C. odontosepala*, G1-G3. *C. propinqua*, H1-H3. *C. lambertiana*, I1-I3. *C. reuteriana*, J1-J3. *C. savalanica*.

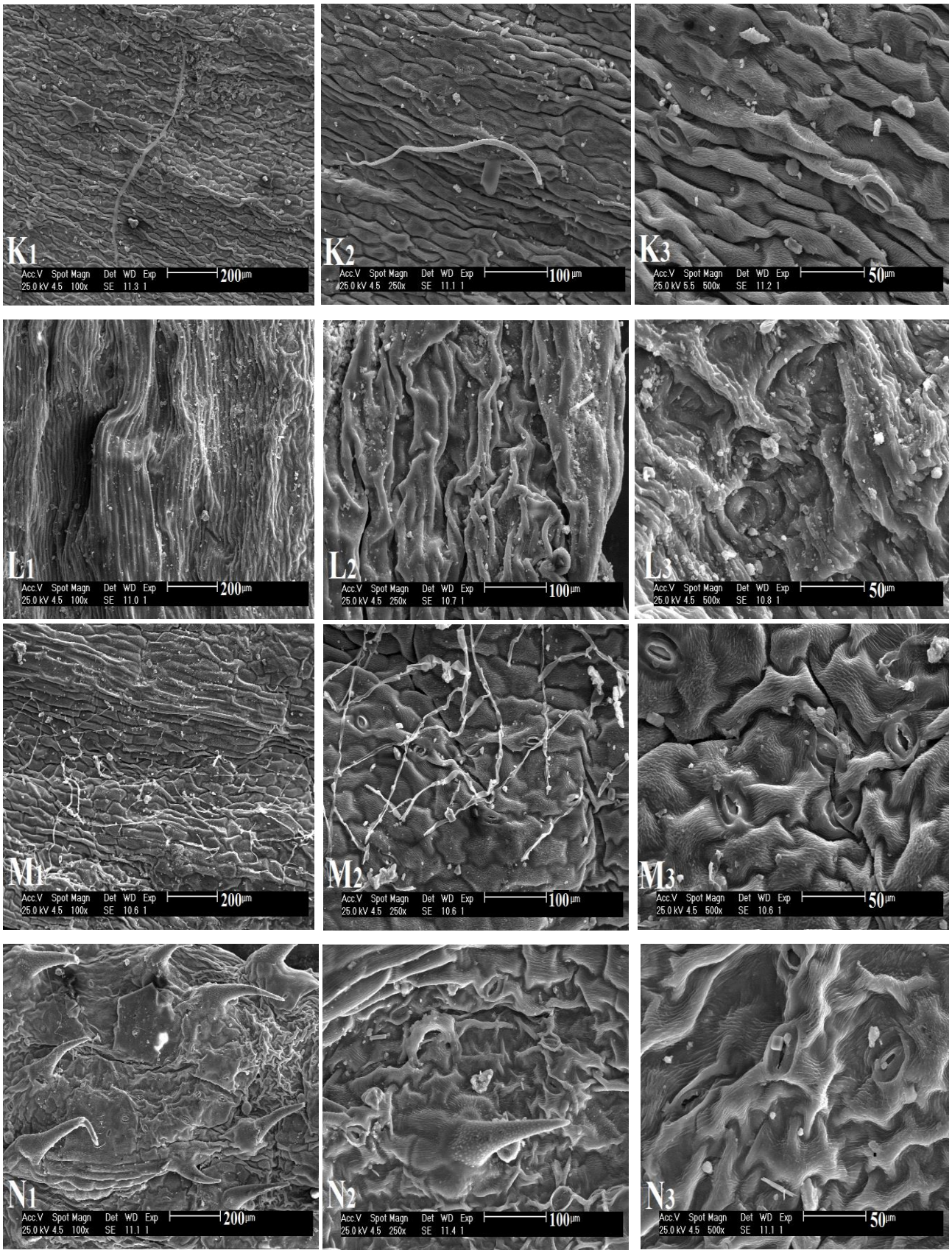

Fig. 3. SEM photographs showing sepal hair found in *Campanula*: K1-K3. *C. sclerotricha*, L1-L3. *C. stevenii*, M1-M3. *C. stricta*, N1-N3. *C. trachelium*.

Table 2. Distribution of sepal hair characteristics in studied species

No.	Taxon	Sepal epidermal pattern	Presence or absence of hair	Shape of trichome	Density of trichome
1	*C. latifolia*	Cup-shaped	Hairless	-	-
2	*C. rapunculoides*	Rugose	Hairy	Strigose	Lax
3	*C. glomerata*	Papillate	Hairy	Strigose	Dense
4	*C. involucrata*	Papillate	Hairy	Strigose	Lax
5	*C. lactiflora*	Papillate	Hairy	Villouse	Lax
6	*C. odontosepala*	Rugose	Hairy	Strigose	Lax
7	*C. propinqua*	Striate	Hairy	Hooked	Dense
8	*C. lambertiana*	Striate-papillate	Hairy	Stellate	Lax
9	*C. reuteriana*	Papillate	Hairy	Crispate	Dense
10	*C. savalanica*	Striate	Hairy	Villouse	Dense
11	*C. sclerotricha*	Papillate	Hairy	Villouse	Lax
12	*C. stevenii*	Papillate	Hairless	-	-
13	*C. stricta*	Papillate	Hairy	Villouse	Dense
14	*C. trachelium*	Papillate	Hairy	Strigose	Dense

Identification key based on micromorphological traits for some studied species of *Campanula*

1. Epidermal pattern cup-shaped ..…….……..……... *C. latifolia*

- Epidermal pattern non-cup-shaped ……..……..…….…..…... 2

2. Epidermal pattern rugose .……...… 3

- Epidermal pattern non-rugose ……...…….……..… 4

3. Sepal trichomes dense .……...…….…… *C. glomerata*

- Sepal trichomes low and non-dense ……..…..……...….. *C. involucrata*

4. Epidermal pattern striate ……...…….. 5

- Epidermal pattern non-striate ……..……...…….…..... 6

5. Epidermal pattern striate-papillate ……...… *C. lambertiana*

- Epidermal pattern striate ……...……...……..… 7

6. Trichomes villouse ……...…….…..... *C. savalanica*

- Trichomes hooked ……...…. .*C. propinqua*

7. Epidermal pattern and villouse trichomes papillate ……..……..…. 8

- Epidermal pattern and non-villouse trichomes papillate ……..……...… 10

8. Trichomes dense ……..…….……9

- Trichomes dense and villouse ……..…... *C. stricta*

9. Trichomes with relatively wide basal-cell ……..…….. *C. lactiflora*

- Trichomes without relatively wide basal-cell ……..... *C. sclerotricha*

10. Epidermal pattern non-trichomes papillate …… *C. stevenii*

- Epidermal pattern trichomes papillate ……..……... 11

11. Trichomes stellate ……... *C. lambertiana*

- Trichomes dense-strigose ……..……..... 14

12. Trichomes crispate ……..... *C. reuteriana*

- Trichomes strigose ……...……..…….. *C. trachelium*

Discussion

In the present study, 14 species of *Campanula* were taxonomically studied based on micro-morphological characteristics. The micromorphological characteristics were studied under scanning electron microscope (SEM). Different characteristics studied here, therefore, provided some significant information for the correct identification and species delimitation. Such features were not changed with the environmental stress and possessed valuable information for the taxonomic as well as the physiological characteristics. Some of these features were used as diagnostic characters in the family, subfamily, tribe, genus, and species (Verhoeven & Venter 1992, Aedo 2017, Noroozi *et al*. 2021). The character studied here is in agreement with the micromorphological work of previous works (Park *et al.* 2006, Mansion *et al*. 2012 & Lakusˇic´ *et al*. 2013).

In addition, in the identifications and morphological studies, the closeness and similarity of *C. trachelium* and *C. latifolia* species were evident so that, these species both had dark blue flowers, hanging, and ovoid capsules. In general, despite the great morphological similarity, *C. trachelium* and *C. latifolia* species showed differences on the basis of the perianth epidermal pattern (Rechinger & Schimann-Czeika 1965, Aghabeygi & Jalilian 2010). The epidermal pattern of the sepal in *C. latifolia* species was cup-shaped, while in *C. trachelium* it was papillate.

Among the studied species, despite the closeness of *C. propinqua* and *C. reuteriana* species, based on morphological examination and information available (Damboldt 1978, Aghabeygi & Jalilian 2010), some complex and close species were also observed. The epidermal pattern of the flower cap was striate in *C. propinqua* and papillate in *C. reuteriana*, based on the electron microscope research, which demonstrated that, these species both varied in terms of kind of the hairs. Due to the epidermal pattern of perianth, which is completely under genetic control, it was proved that, these two species are separate, which is in agreement with Flora Iranica (Rechinger & Schimann-Czeika 1965).

Although, the micromorphological examination of north and northwest *Campanula* species cannot be effective at the level of the section, it can be quite effective to solve the taxonomy of adjacent species. Two species i.e., *C. glomerata* and *C. involucrata* were, therefore, placed in the same section due to their similarity in the vegetative period (both perennial). Furthermore, in studying the micromorphology of sepal, both species were strigose due to hair type and papillate in terms of the perianth pattern (Aghabeygi & Jalilian 2010).

Furthermore, Serposhan (2014) confirmed the taxonomic importance of achene and trichome traits based on the matching of molecular data and micro-morphological studies. Salmaki *et al*. (2009) studied the species of the genus *Stachys* L. in Iran in terms of the trichome micromorphology. They stated that, this trait is more useful in separating species within a section than in separating larger groups such as sections. In the flora of Iran (Aghabeygi & Jalilian 2010), it was mentioned that, the *C. sclerotricha* is a species close to *C. glomerata*, but the results of perianth epidermal pattern confirmed the separation of these species. Eshratifar *et al*. (2011) studied the achene and leaf hairs of the genus *Teucrium* and found that, these traits have systematic value which are consistent with the classifications of the genus. Osman (2012) stated that, the stem anatomical characteristics, the morphology of pollen, and the types of hairs are useful to separate the species of *Ballota* L. The above-mentioned studies, therefore, showed the priority and importance of morphological and micromorphological investigations for many genera of the *Lamiaceae* family as well. Barani *et al*. (2015) studied the epidermal pattern and perianth hair variations in *Delphinium* L. species in Iran. In addition, Shojaei *et al.* (2020) examined the micromorphological traits of achene, leaves, and perianth of both Iranian species of *Anemone* L. and *Pulsatilla* Mill.

The present investigation is, therefore, the first study, which describes the micromorphology of different parts i.e., the sepals of 14 *Campanula* taxa from Iran.

These characters along with scanning electron microscopy, consider as a reliable source of taxonomic information that help in species delimitation and correct identification. The characters considered here are found useful for the taxonomic delimitation of almost all taxa studied at the generic level. Pollen morphology may also be useful in the taxonomy of *Campanula* at various levels. Such data to some extent provide evidence of species delimitation as well as circumscription of subgeneric taxa in the *Campanula* genus (Khansari *et al.* 2012).

Conclusion

By the help of all traits mentioned above, authors of the paper tried to solve the interspecies differences of some species that had taxonomical ambiguities. For example, in the flora of Turkey (Damboldt 1978), the two species of *C. propinqua* and *C. reuteriana* are synonymous but electron microscope studies revealed the difference between the two both in terms of the hair type and the epidermal pattern of the perianth.

It is finally concluded that, the pattern of sepals is a useful character to distinguish *Campanula* at its species level. These results showed that, the abaxial surface of sepal ornamentation, could divide the species of this genus in five groups of papillate, cup-shaped, rugose, striate, and striate-papillate.

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