KSÜ Tarım ve Doğa Derg 28 (2), 380-402, 2025
KSU J. Agric Nat 28 (2), 380-402, 2025
DOI: 10.18016/ksutarimdoga.vi.1589750

A Scanning Electron Microscope Study of Vegetative Parts of the Genus *Vincetoxicum* in Türkiye

Seher GÜVEN [1]✉, Selin Meral DOĞAN [2]
[1]Department of Biology, Faculty of Sciences and Arts, Recep Tayyip Erdogan University, Rize, Türkiye, [2]Republic of Türkiye Ministry of Health, Çayeli İshakoğlu State Hospital, Biochemistry Laboratory, Rize, Türkiye
[1]https://orcid.org/0000-0001-8883-8489, [2]https://orcid.org/0009-0009-6051-5093
✉: seher.cakmak@erdogan.edu.tr

ABSTRACT

In Türkiye, *Vincetoxicum* is represented by 11 taxa belonging to nine species, three of which (*V. coskuncelebiana* S.Makbul & S.Güven, *V. fuscatum* (Hornem.) subsp. *boissieri* (Kusn.) Browicz, and *V. parviflorum* Decne) are endemics. In this study, the micro-morphology of the stem, petiole, and lamina of all Turkish *Vincetoxicum* taxa was examined using a scanning electron microscope (SEM) for the first time. Micro-morphological observations were carried out on herbarium samples belonging to 21 populations from various habitats. A detailed micro-morphological description of the stem, petiole, and lamina, supported by SEM micrographs, was created for each examined taxon. It was determined that the micro-morphological characteristics such as the pattern of pubescence, the distribution and density of trichomes on the stem, petiole, and lamina, and the position of stomata on both sides of the leaf contributed to the distinction of the Turkish *Vincetoxicum* taxa. On the other hand, no difference was observed among the examined taxa in terms of the appearance of epicuticular wax, and shape and concavity/convexity properties of epidermal cells on stem, petiole, and lamina.

Botany

Research Article

Article History
Received : 23.11.2024
Accepted : 28.01.2025

Keywords
Apocynaceae
Leaf
SEM
Stem
Vincetoxicum

Türkiye *Vincetoxicum* Cinsinin Vejetatif Kısımlarının Taramalı Elektron Mikroskobu İle İncelenmesi

ÖZET

Vincetoxicum, Türkiye'de üçü endemik (*V. coskuncelebiana* S.Makbul & S.Güven, *V. fuscatum* (Hornem.) subsp. *boissieri* (Kusn.) Browicz ve *V. parviflorum* Decne) olmak üzere dokuz türe ait 11 takson ile temsil edilmektedir. Bu çalışmada, tüm Türkiye *Vincetoxicum* taksonlarının gövde, petiyol ve lamina mikro-morfolojisi taramalı elektron mikroskobu (SEM) kullanılarak ilk kez taksonomik yönden değerlendirilmiştir. Mikro-morfolojik incelemeler, farklı habitatlardan toplanan 21 populasyona ait herbaryum örnekleri üzerinde gerçekleştirilmiştir. İncelenen her takson için SEM fotoğraflarıyla desteklenen ayrıntılı birer gövde, petiyol ve lamina mikro-morfolojik betimi oluşturulmuştur. Türkiye *Vincetoxicum* taksonlarının ayrımında; gövde, petiyol ve lamina yüzeylerindeki tüylenme çeşidi, tüy dağılımı ve yoğunluğu ile yaprağın her iki yüzeyindeki stoma durumu gibi mikromorfolojik karakterlerin önemli rol oynadığı belirlenmiştir. Diğer taraftan, incelenen taksonlar arasında gövde, yaprak sapı ve lamina üzerindeki epikütikular mumun görünümü, epidermis hücrelerinin şekli ve içbükeylik/dışbükeylik özellikleri bakımından bir fark görülmemiştir.

Botanik

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 23.11.2024
Kabul Tarihi : 28.01.2025

Anahtar Kelimeler
Apocynaceae
Yaprak
SEM
Gövde
Vincetoxicum

Atıf İçin: Güven, S., & Doğan, S. M., (2025). Türkiye *Vincetoxicum* Cinsinin Vejetatif Kısımlarının Taramalı Elektron Mikroskobu İle İncelenmesi. *KSÜ Tarım ve Doğa Derg 28* (2), 380-402. DOI: 10.18016/ksutarimdoga.vi.1589750.

To Cite: Güven, S., & Doğan, S. M., (2025). A Scanning Electron Microscope Study of Vegetative Parts of the Genus Vincetoxicum in Türkiye. *KSU J. Agric Nat 28* (2), 380-402. DOI: 10.18016/ksutarimdoga.vi.1589750.

INTRODUCTION

Vincetoxicum Wolf s.str., one of the taxonomically complex genera of the subfamily Asclepiadoideae (Apocynaceae), has attracted the attention of many taxonomists for a long time. Recently, based on extensive morphological data supported by molecular evidence, Liede-Schumann et al. (2012) expanded *Vincetoxicum* by transferring the members of the seven genera in the subtribe Tylophorinae (*Biondia* Schltr., *Blyttia* Arn., *Diplostigma* K. Schum., *Goydera* Liede, *Pleurostelma* Baill., *Rhyncharrhena* F. Muell., *Tylophora* R. Br.) into the genus. More recent taxonomic revisions contributing to the systematics of the genus were carried out on a large group of taxa including many new regional endemic species, new combinations, new names, and typifications (Liede-Schumann & Meve, 2018; Shah et al., 2021). The current *Vincetoxicum* s.l. comprises approximately 300 natural species distributed throughout the Far East, Africa, the Mediterranean, Caucasus, southern Russia, and Europe (Liede-Schumann et al., 2016; Liede-Schumann & Meve, 2018), and some introduced species in North America (Sheeley & Raynal, 1996).

Vincetoxicum is a difficult genus due to genetic and morphological variations observed among closely related taxa (Browicz, 1978). These variations lead to difficulties in the identification of *Vincetoxicum* species based solely on traditional (phenetic) characters. Therefore, more reliable characteristics need to be revised by using different methods and techniques in the distinction of *Vincetoxicum* taxa. In order to contribute to the systematics of the genus, to date, numerous taxonomical studies based on anatomical (Metcalfe & Chalk, 1950), palynological (Fishbein, 2001; Yamashiro et al., 2008; Verhoeven & Venter, 2001; Sinha & Mondal, 2011; Sreenath et al., 2012; Feng et al., 2012; Chang et al., 2012; Shah & Ahmad, 2014; Yaseen & Perveen, 2014), cytological (Yamashiro et al., 2002), and molecular phylogenetic data (Civeyrel et al., 1998; Liede & Täuber, 2002; Liede et al., 2002; Sennblad & Bremer, 2002; Yamashiro et al., 2004; Liede-Schumann et al., 2012; Liede-Schumann et al., 2016) have been performed on the members of *Vincetoxicum*, excluding Türkiye.

In Türkiye, the genus *Vincetoxicum* is represented by nine species and 11 taxa, three of which (*V. coskuncelebiana* S.Makbul & S.Güven, *V. fuscatum* (Hornem.) subsp. *boissieri* (Kusn.) Browicz, and *V. parviflorum* Decne) are endemics (Güner, 2012; Güven et al., 2021a,b; Makbul & Güven, 2022). The Turkish *Vicetoxicum* is composed of perennial, herbaceous plants growing in dry river valleys, open rocky slopes, steppes, and mountain slopes to shrublands and *Quercus*-dominated forests (Browicz, 1978; Güven, 2017). The detailed morphological properties including color, shape, and indumentum of corolla, size, and shape of corona and gynostegium, and indumentum of stem and leaf were examined using a stereomicroscope for the genus *Vincetoxicum* in Türkiye (Güven, 2017; Güven et al., 2021a). Additionally, the seed micro-morphological (İlçim et al., 2010; Güven et al., 2021a), palynological (Güven et al., 2015, 2021a), cytological and molecular characteristics (Güven et al., 2019) of the Turkish *Vincetoxicum* taxa were also examined comparatively. Although palynological characters such as corpusculum shape (ovate or oblong), pollinium shape (elliptic, clavate, ovate or obovate) and surface ornamentation (rugulate or gemmate) supported the separation of the taxa at the species level (Güven et al., 2015, 2021a), cytological and molecular data (Güven et al., 2019), and seed micro-morphological features (İlçim et al., 2010; Güven et al., 2021a) provided only limited information for the genus.

Micro-morphological properties of vegetative organs including size and shape of epidermal cells, cell wall undulations, epicuticular wax structure, stomatal morphology, patterning, and trichome morphology were very valuable in angiosperm taxonomy (Behnke & Barthlott, 1983; Stace et al., 1984; Sivarajan, 1991; Barthlott, 1994; Theisen & Barthlott, 1994; Barthlott et al., 1998; Carpenter, 2005; Tomaszewski & Zieliński, 2014). However, there is a very limited number of studies on the leaf and stem micro-morphology of Apocynaceae taxa (Joubert, 2007; Xin-sheng, 2010; Carvalho et al., 2017; Bashir et al., 2020; El-Taher, et al, 2020; Medina et al., 2021; Abeysinghe & Scharaschkin, 2022; Ridzuan & Kalu, 2023; Malaspina et al., 2024) including a few numbers of *Vincetoxicum* (Shah et al., 2018). Although stem and leaf indumentum have been considered diagnostic characters within the *Vincetoxicum*, information regarding the vegetative micro-morphological features of the related species is still lacking for the members of Turkish *Vincetoxium.* Therefore, the present study aimed: (1) to document and illustrate a detailed description of the micro-morphology of stem, petiole, and lamina for each Turkish *Vincetoxicum* species using scanning electron microscopy (SEM), (2) to evaluate the taxonomic or diagnostic importance of stem, petiole, and lamina micro-morphological characteristics among the members of *Vincetoxicum* in Türkiye.

MATERIAL and METHOD

Specimens

Micro-morphological observations were carried out on plant samples deposited at the Herbarium of Biology (RUB) at Recep Tayyip Erdogan University. The present study included individuals from 21 different populations,

representing 11 taxa of *Vincetoxicum*, and detailed locality information and distribution in Türkiye of the investigated taxa is listed in Table 1 and Figure 1, respectively.

Scanning electron microscopy

Micro-morphological examinations were carried out on five individuals per population for each examined *Vincetoxicum* taxon, excluding *V. funebre* (10 individuals were used from one population). Only healthy individuals without visible damage or contamination, were selected, and stem parts between the third and fourth nodes of the plant from the base, and fully expanded leaves from the third or fourth nodes were randomly sampled. For each of 10 individuals per 11 examined taxon, small pieces (5 x 5 mm) were cut out from the stem, petiole, and lamina separately, and all pieces were passed through an ethanol series (60-80%) to remove the debris present on the surface. For SEM studies, the cleaned stem petiole and lamina samples were air-dried under ambient conditions and then were transferred on aluminum stubs, coated with gold for 4 min in a sputter-coater and observed by a JEOL-JSM 6610 microscope at the Central Research Laboratories of Recep Tayyip Erdogan University. In this study, SEM examinations were carried out following Güzel (2021) and Aybeke (2022), and we classified the stomatal type following Dilcher (1974). A detailed micro-morphological description of the stem, petiole, and lamina, supported by SEM micrographs, was created for each examined taxon. However, since no differences could be detected between individuals belonging to different populations of each *Vincetoxicum* taxon employing stem, petiole, and lamina micro-morphology, SEM micrographs obtained from only one locality were used for the description of each taxon. The micro-morphological terminology followed that of Barthlott et al. (1998), Stearn (1992), Koch et al. (2008), and Prüm et al. (2012).

Table 1 Locality informations of the investigated taxa
Çizelge 1. İncelenen taksonların lokalite bilgileri

Taxa (*Taksonlar*)	No	Locality (*Lokalite*)	Collector number (*Toplayıcı numarası*)
Vincetoxicum canescens (Willd.) Decne subsp. *canescens*	1	Erzincan: Erzincan to Kemah	S. Güven 36 & S. Makbul (RUB)
	2	Adana: Pozantı	S. Güven 90 & S. Makbul (RUB)
Vincetoxicum canescens (Willd.) Decne subsp. *pedunculata* Browicz	3	Manisa: Kula	S. Güven 51 & S. Makbul (RUB)
	4	Muğla: Yılanlı Mountain	S. Güven 56 & S. Makbul (RUB)
Vincetoxicum coskuncelebiana S. Makbul & S.Güven	5	Ardahan: Çıldır, Taşbaşı Village	S. Güven 69 & S. Makbul (RUB)
	6	Ardahan: Çıldır	S. Güven 127 & S. Makbul
Vincetoxicum funebre Boiss. & Kotschy	7	Ardahan: Posof	S. Güven 126 & S. Makbul (RUB)
Vincetoxicum fuscatum (Hornem.) subsp. *fuscatum*	8	Niğde: Ulukışla	S. Güven 93 & S. Makbul (RUB)
	9	İstanbul: Paşaköy	S. Güven 22 & S. Makbul (RUB)
Vincetoxicum fuscatum (Hornem.) subsp. *boissieri* (Kusn.) Browicz	10	Erzincan: Erzincan to Sakaltutan	S. Güven 35 & S. Makbul (RUB)
	11	Bayburt: Değirmencik Village	S. Güven 73 & S. Makbul (RUB)
Vincetoxicum hirundinaria Medicus subsp. *hirundinaria*	12	Kırklareli: Demirköy	S. Güven 28 & S. Makbul (RUB)
	13	Kırklareli: Vize, Kızılağaç	S. Güven 19 & S. Makbul (RUB)
Vincetoxicum parviflorum Decne	14	Tunceli: Ovacık, Karagöl	S. Güven 80 & S. Makbul (RUB)
	15	Kayseri: Yahyalı	S. Güven 95 & S. Makbul (RUB)
Vincetoxicum scandens Somm. & Lev.	16	Trabzon: Çaykara	S. Güven 169 & S. Makbul (RUB)
	17	Rize: Çamlıkköy	S. Güven 30 & S. Makbul (RUB)
Vincetoxicum speciosum Boiss. & Spruner	18	Tekirdağ: Saray	S. Güven 20 & S. Makbul (RUB)
	19	İstanbul: Çatalca	S. Güven 132 & S. Makbul (RUB)
Vincetoxicum tmoleum Boiss.	20	Ankara: Kızılcahamam	S. Güven 61 & S. Makbul (RUB)
	21	Erzurum: Oltu	S. Güven 72 & S. Makbul (RUB)

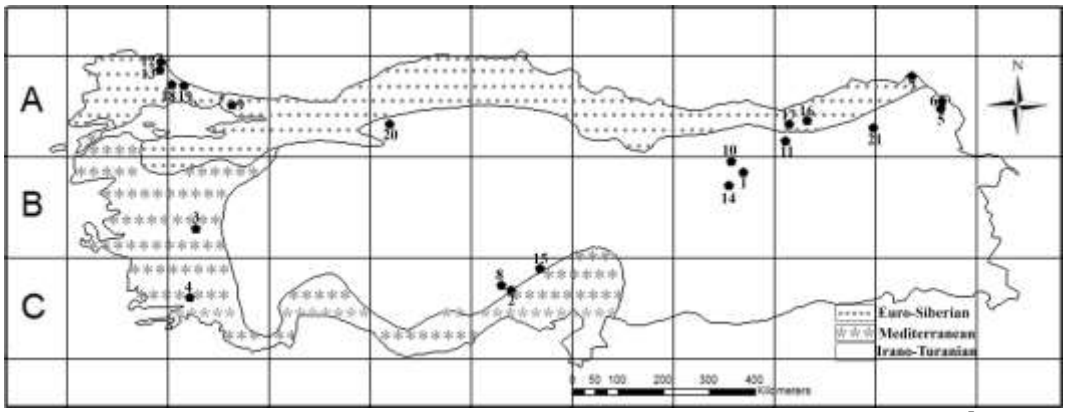

Figure 1. Distribution map of the examined taxa according to phytogeographic regions [The map of Türkiye according to the grid system of P. H. Davis (1965); refer to Table 1 for explanation of accession no).

Şekil 1. İncelenen taksonların fitocoğrafik bölgelere göre dağılım haritası [P. H. Davis (1965)'de kareleme sistemine göre Türkiye haritası; örnek numarasının açıklaması için Tablo 1'e bakınız).

RESULTS

***Vincetoxicum canescens* (Willd.) Decne subsp. *canescens*:** Stem and petiole surfaces with densely canescent-tomentose indumenta all around, consisting of non-glandular, grayish white, matted, long wool-like trichomes with verrucose ornamentation, epicuticular wax smooth (Figure 2a-f). Leaf; amphistomatic with anomocytic stomata, adaxial and abaxial surfaces with densely canescent-tomentose indumenta all around, consisting of nonglandular, grayish white, matted, long wool-like trichomes with verrucose ornamentation; epidermal cells on both sides polygonal, periclinal cell wall convex, anticlinal cell wall channeled and straight, epicuticular wax striate (Figure 2g-l).

***Vincetoxicum canescens* (Willd.) Decne subsp. *pedunculata* Browicz:** Stem and petiole surfaces with densely canescent-tomentose indumenta all around, consisting of non-glandular, grayish white, matted, long wool-like trichomes with verrucose ornamentation, epicuticular wax smooth (Figure 3a-f). Leaf; amphistomatic with anomocytic stomata, adaxial and abaxial surfaces with densely canescent-tomentose indumenta all around, consisting of nonglandular, grayish white, matted, long wool-like trichomes with verrucose ornamentation; epidermal cells on both sides polygonal, periclinal cell wall convex, anticlinal cell wall channeled and straight, epicuticular wax striate (Figure 3g-l).

***Vincetoxicum coskuncelebiana* S.Makbul & S.Güven:** Stem surface with a crisped indument along one side (uniserially) (Figure 4a-c) and petiole surface with a sparsely crisped indument all around (Figure 4d-f), consisting of non-glandular, short curly trichomes with verrucose ornamentation, epicuticular wax smooth on both stem and petiole (Figure 4a-f). Leaf; hypostomatic with anomocytic stomata, adaxial and abaxial surfaces with crisped indumenta along margins and veins, consisting of nonglandular, short curly trichomes with verrucose ornamentation; epidermal cells on both sides polygonal or irregularly shaped, periclinal cell wall convex or concave, anticlinal cell wall channelled, straight to slightly undulate, epicuticular wax striate (Figure 4g-l).

***Vincetoxicum funebre* Boiss. & Kotschy:** Stem surface with a crisped indument along two sides (biserial) (Figure 5a-c) and petiole surface with a densely crisped indument all around (Figure 5d-f), consisting of non-glandular, short curly trichomes with verrucose ornamentation, epicuticular wax smooth on both stem and petiole (Figure 5a-f). Leaf; hypostomatic with anomocytic stomata, adaxial and abaxial surfaces with crisped indumenta along margins and veins, consisting of nonglandular, short curly trichomes with verrucose ornamentation; epidermal cells on both sides polygonal or irregularly shaped, periclinal cell wall convex or concave, anticlinal cell wall channelled, straight to slightly undulate, epicuticular wax striate (Figure 5g-l).

***Vincetoxicum fuscatum* (Hornem.) subsp. *fuscatum*:** Stem surface with a crisped indument along two sides (biserial) (Figure 6a-c) and petiole surface with a sparsely crisped indument all around (Figure 6d-f), consisting of non-glandular, short curly trichomes with verrucose ornamentation, epicuticular wax smooth on both stem and petiole (Figure 6a-f). Leaf; hypostomatic with anomocytic stomata, adaxial and abaxial surfaces with crisped indumenta along margins and veins, consisting of non-glandular, short curly trichomes with verrucose ornamentation; epidermal cells on both sides polygonal or irregularly shaped, periclinal cell wall convex, anticlinal cell wall channelled, straight to slightly undulate, epicuticular wax striate (Figure 6g-l).

***Vincetoxicum fuscatum* (Hornem.) subsp. *boissieri* (Kusn.) Browicz:** Stem surface with a crisped indument along two sides (biserially) (Figure 7a-c) and petiole surface with a sparsely crisped indument all around (Figure 7d-f),

consisting of non-glandular, short curly trichomes with verrucose ornamentation, epicuticular wax smooth on both stem and petiole (Figure 7a-f). Leaf; hypostomatic with anomocytic stomata, adaxial and abaxial surfaces with crisped indumenta along margins and veins, consisting of non-glandular, short curly trichomes with verrucose ornamentation; epidermal cells on both sides polygonal or irregularly shaped, periclinal cell wall convex, anticlinal cell wall channelled, straight to slightly undulate, epicuticular wax striate (Figure 7g-l).

Vincetoxicum hirundinaria* Medicus subsp. *hirundinaria: Stem surface with a crisped indument along two sides (biserially) (Figure 8a-c) and petiole surface with a sparsely crisped indument all around (Figure 8d-f), consisting of non-glandular, short curly trichomes with verrucose ornamentation, epicuticular wax smooth on both stem and petiole (Figure 8a-f). Leaf; hypostomatic with anomocytic stomata, adaxial and abaxial surfaces with crisped indumenta along margins and veins, consisting of non-glandular, short curly trichomes with verrucose ornamentation; epidermal cells on both sides polygonal or irregularly shaped, periclinal cell wall convex, anticlinal cell wall channelled, straight to slightly undulate, epicuticular wax striate (Figure 8g-l).

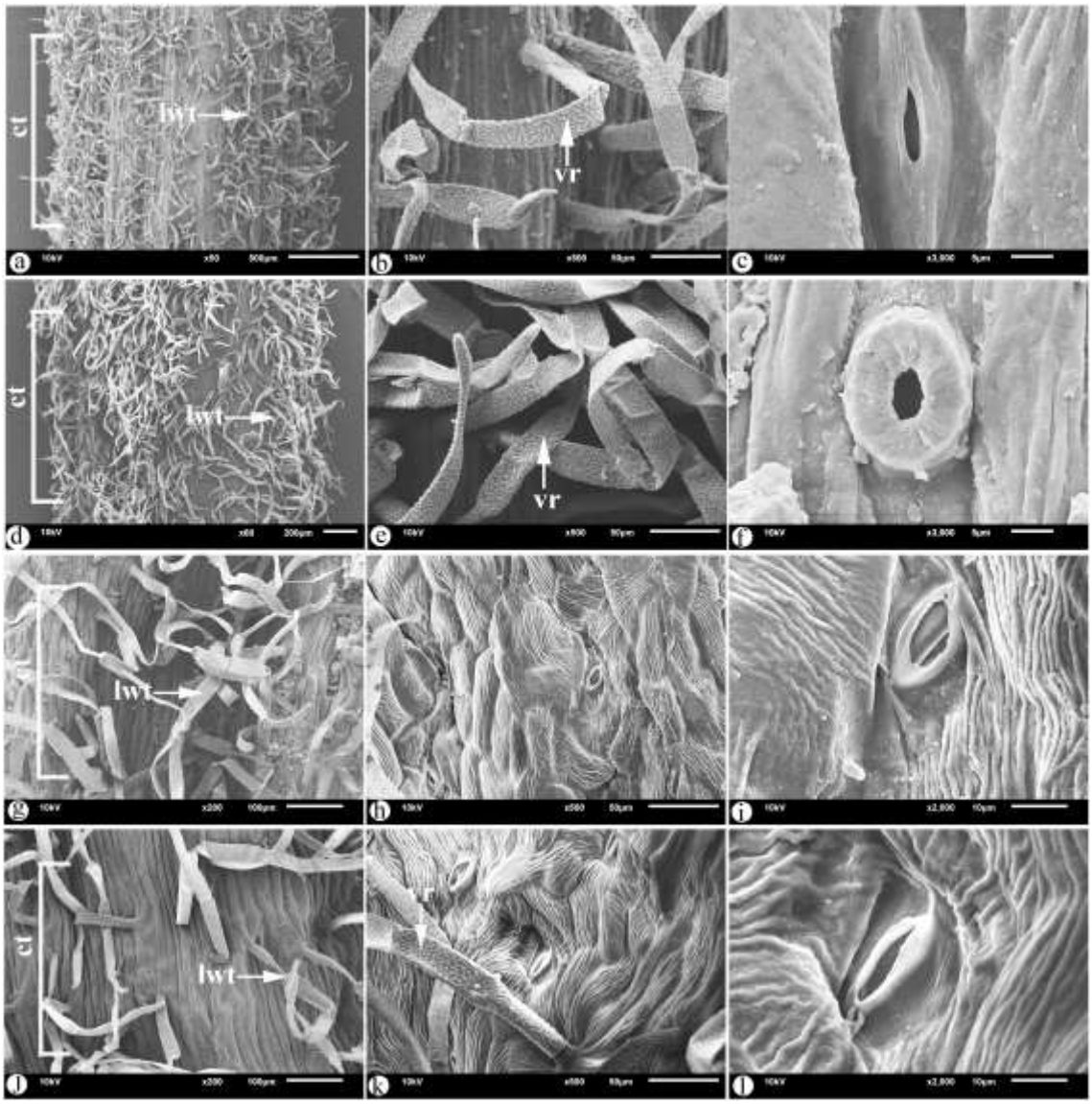

Figure 2. Scanning electron micrographs of stem, petiole and lamina surfaces of *Vincetoxicum canescens* subsp. *canescens* (S.Güven 90 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, ct: canescent-tomentose indumentum (all around), lwt: long wool-like trichome, vr: verrucose trichome ornamentation.

Şekil 2. *Vincetoxicum canescens* subsp. *canescens* (S.Güven 90 & S.Makbul)'in gövde, petiyol ve yaprak yüzeylerine ait taramalı elektron mikroskobu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, ct: canescent-tomentose tüy örtüsü (tüm yüzey boyunca), lwt: uzun yünsü tüy, vr: verrucose tüy ornamentasyonu.

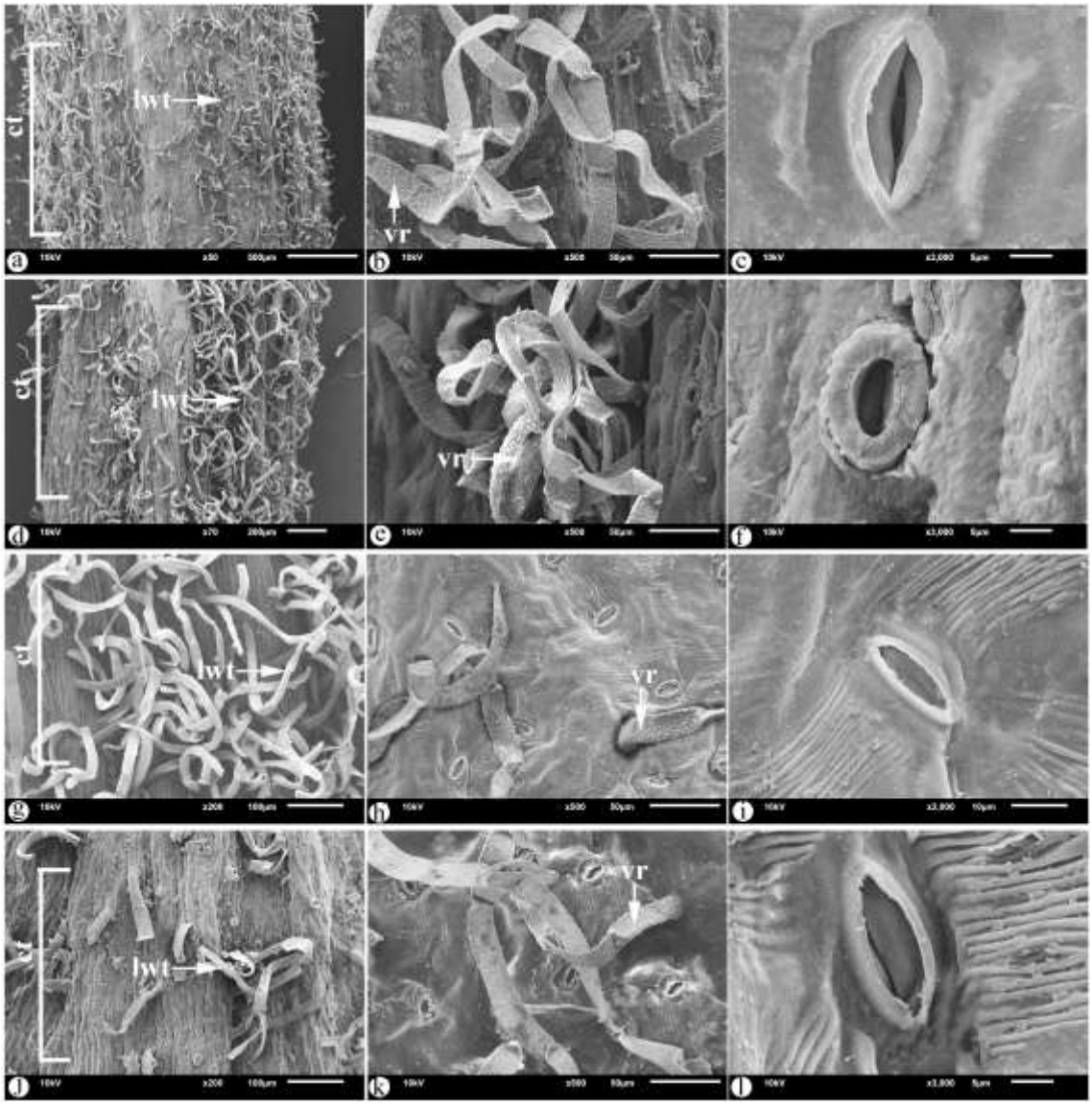

Figure 3. Scanning electron micrographs of stem, petiole and lamina surfaces of *Vincetoxicum canescens* subsp. *pedunculata* (S.Güven 56 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, ct: canescent-tomentose indumentum (all around), lwt: long wool-like trichome, vr: verrucose trichome ornamentation.

Şekil 3. *Vincetoxicum canescens* subsp. *pedunculata* (S.Güven 56 & S.Makbul)'nın gövde, petiyol ve lamina yüzeylerine ait taramalı elektron mikroskobu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, ct: canescent-tomentose tüy örtüsü (tüm yüzey boyunca), lwt: uzun yünsü tüy, vr: verrucose tüy ornamentasyonu.

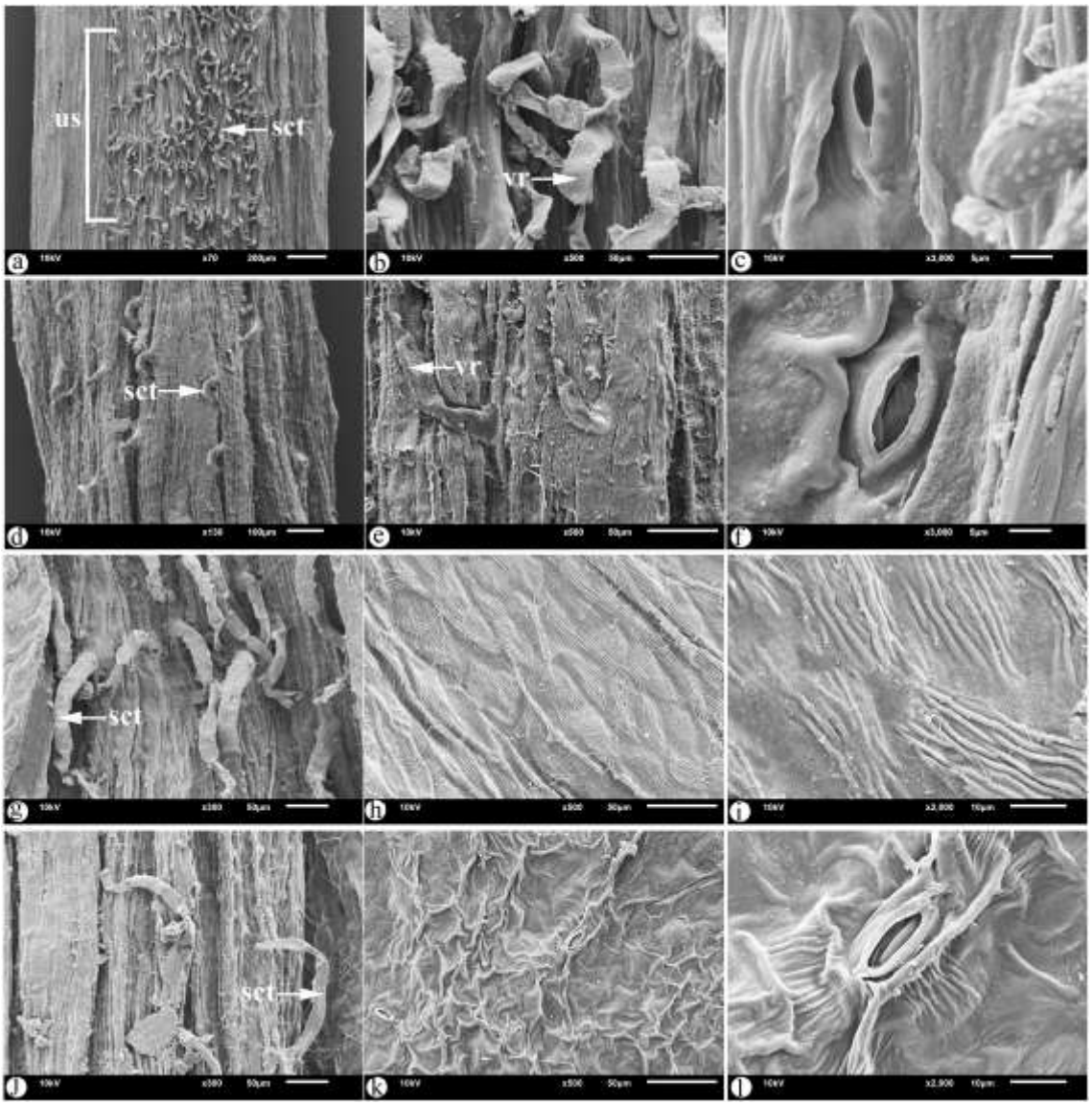

Figure 4. Scanning electron micrographs of stem, petiole, and lamina surfaces of *Vincetoxicum coskuncelebiana* (S.Güven 127 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, sct: short curly trichome, us: hairy along one side (uniserially), vr: verrucose trichome ornamentation.

Şekil 4. *Vincetoxicum coskuncelebiana* (S.Güven 127 & S.Makbul)'nın gövde, petiyol ve lamina yüzeylerine ait taramalı elektron mikroskobu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, sct: kısa kıvrık tüy, us: bir taraf boyunca tüylü (uniserially), vr: verrucose tüy ornamentasyonu.

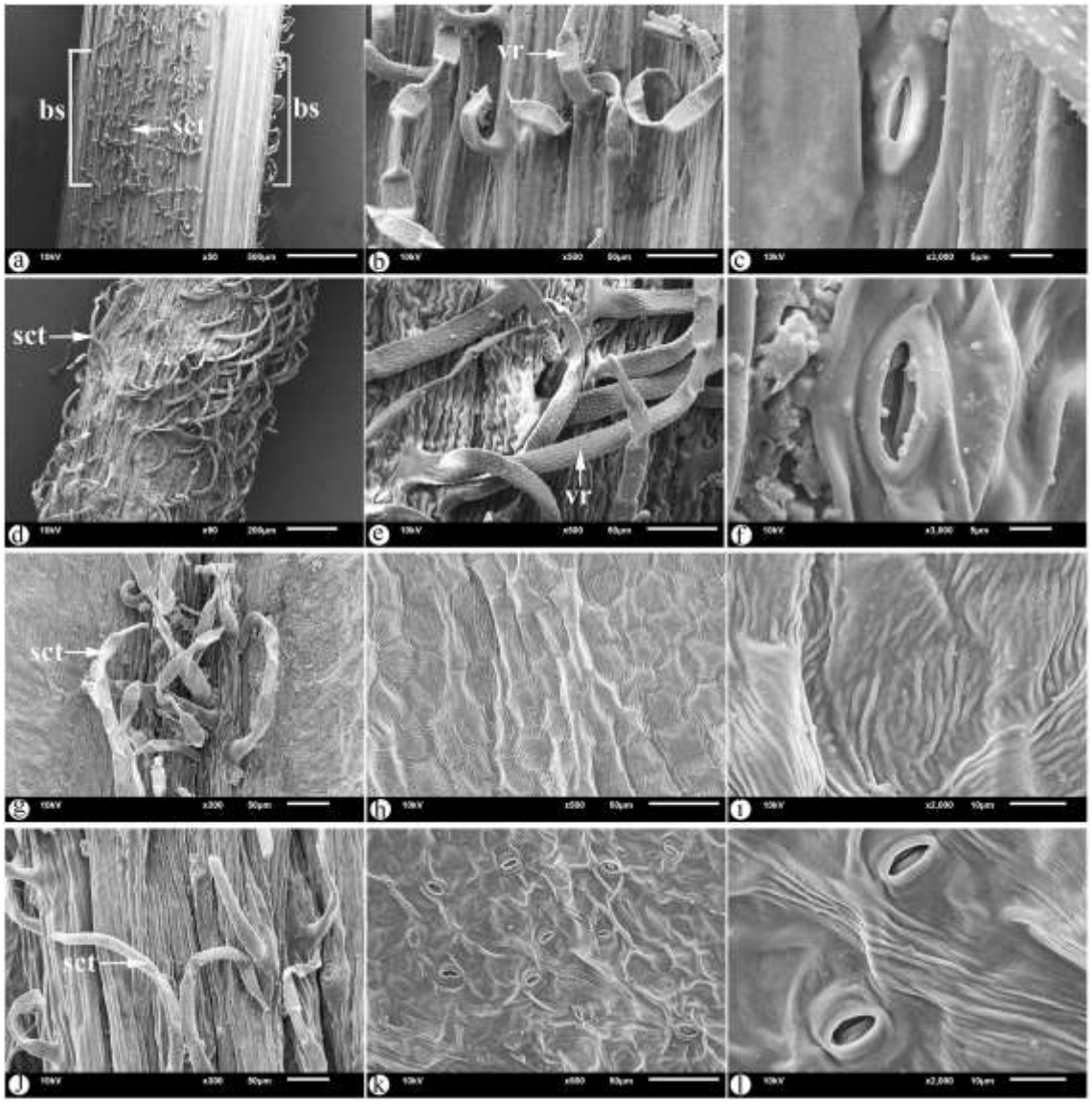

Figure 5. Scanning electron micrographs of stem, petiole and lamina surfaces of *Vincetoxicum funebre* (S.Güven 126 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, bs: hairy along two sides (biserially), sct: short curly trichome, vr: verrucose trichome ornamentation.

Şekil 5. *Vincetoxicum funebre* (S.Güven 126 & S.Makbul)'nin gövde, petiyol ve lamina yüzeylerine ait taramalı elektron mikroskobu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, bs: iki taraf boyunca tüylü (biserially), sct: kısa kıvrık tüy, vr: verrucose tüy ornamentasyonu.

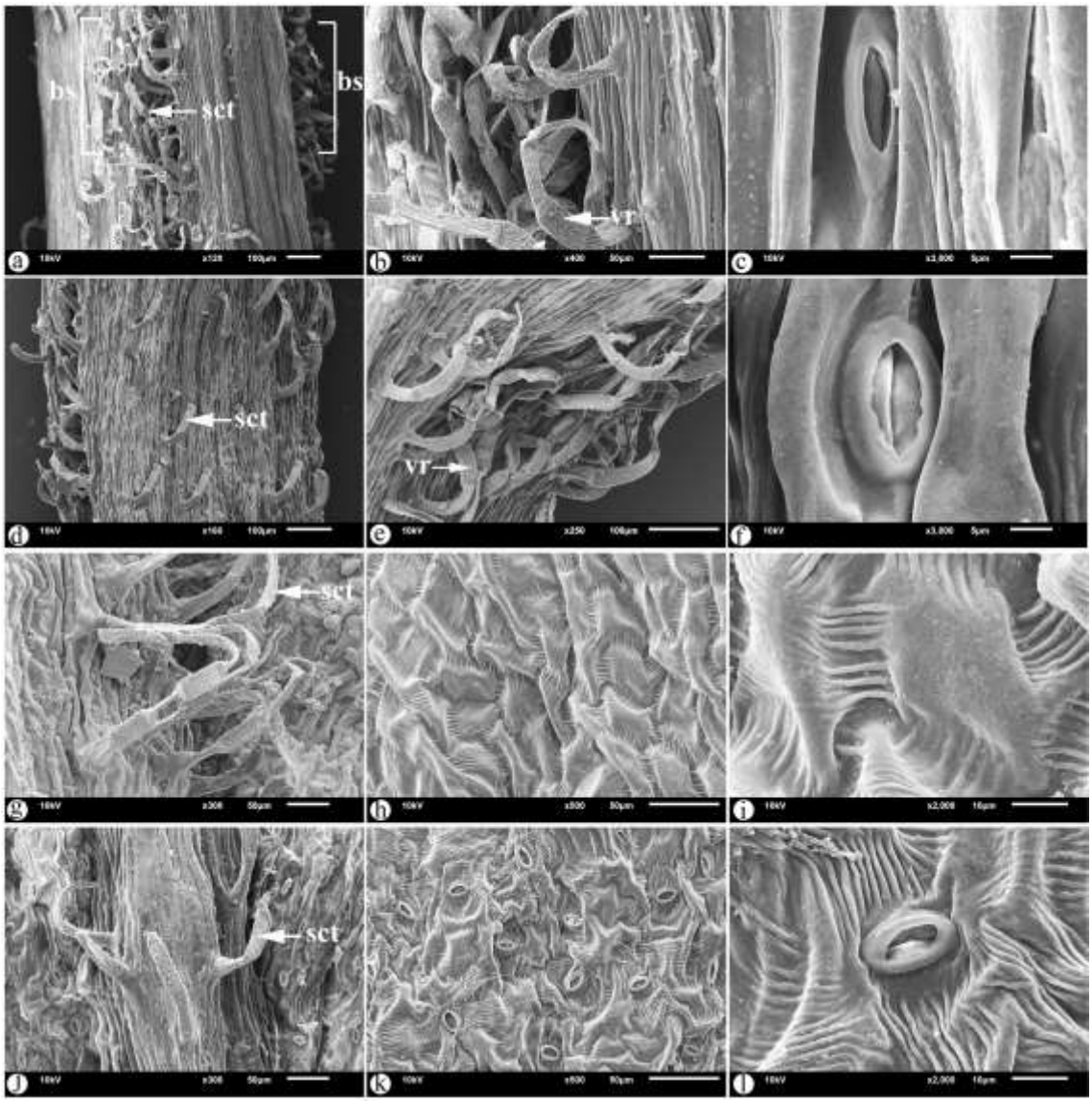

Figure 6. Scanning electron micrographs of stem, petiole and lamina surfaces of *Vincetoxicum fuscatum* subsp. *fuscatum* (S.Güven 22 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, bs: hairy along two sides (biserially), sct: short curly trichome, vr: verrucose trichome ornamentation.

Şekil 6. *Vincetoxicum fuscatum* subsp. *fuscatum* (S.Güven 22 & S.Makbul)'un gövde, petiyol ve lamina yüzeylerine ait taramalı elektron mikroskobu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, bs: iki taraf boyunca tüylü (biserially), sct: kısa kıvrık tüy, vr: verrucose tüy ornamentasyonu.

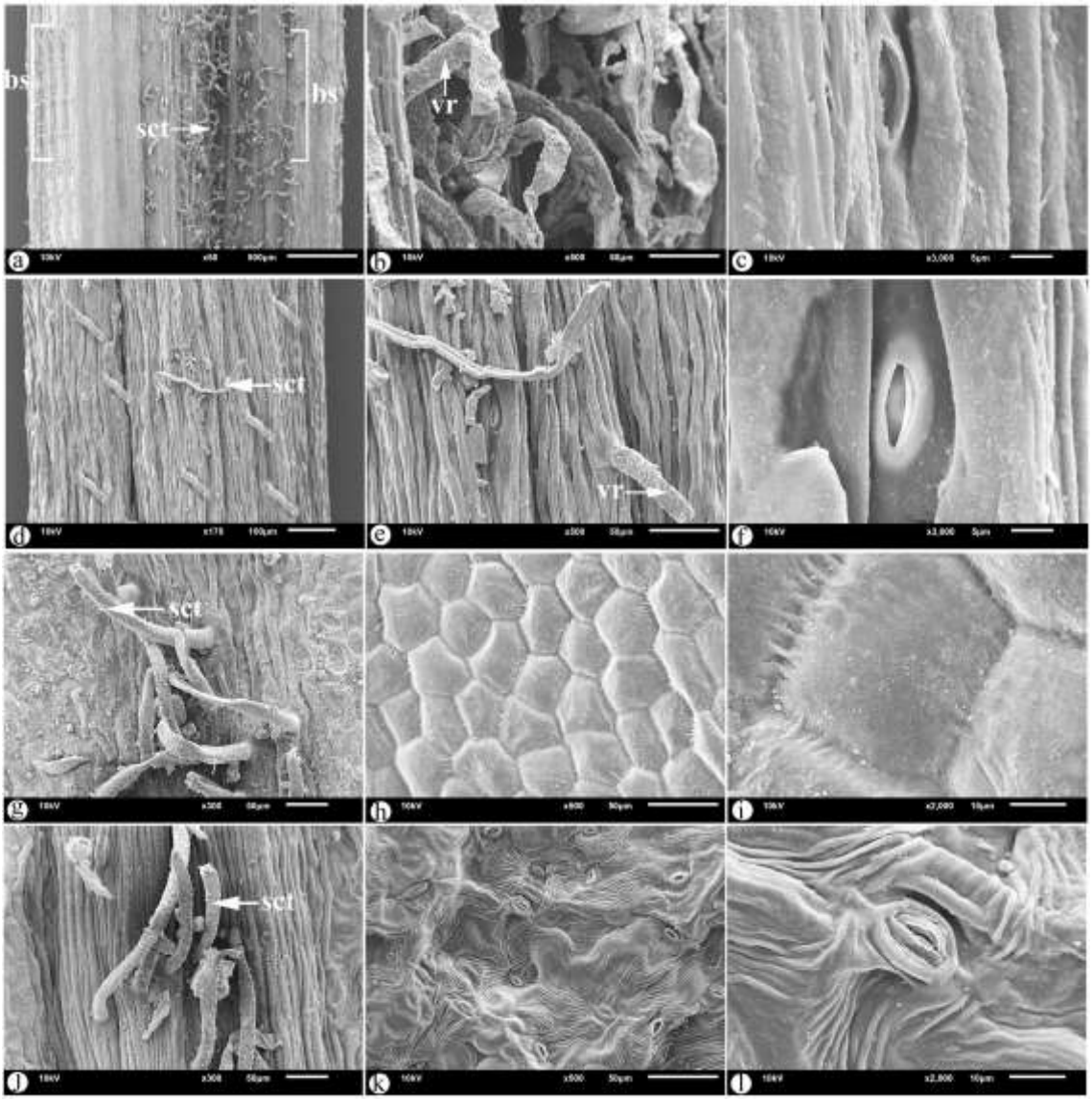

Figure 7. Scanning electron micrographs of stem, petiole and lamina surfaces *Vincetoxicum fuscatum* subsp. *boissieri* (S.Güven 73 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, bs: hairy along two sides (biserially), sct: short curly trichome, vr: verrucose trichome ornamentation.

Şekil 7. *Vincetoxicum fuscatum* subsp. *boissieri* (S.Güven 73 & S.Makbul)'nin gövde, petiyol ve lamina yüzeylerine ait taramalı elektron mikroskobu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, bs: iki taraf boyunca tüylü (biserially), crt: kısa kıvrık tüy, vr: verrucose tüy ornamentasyonu.

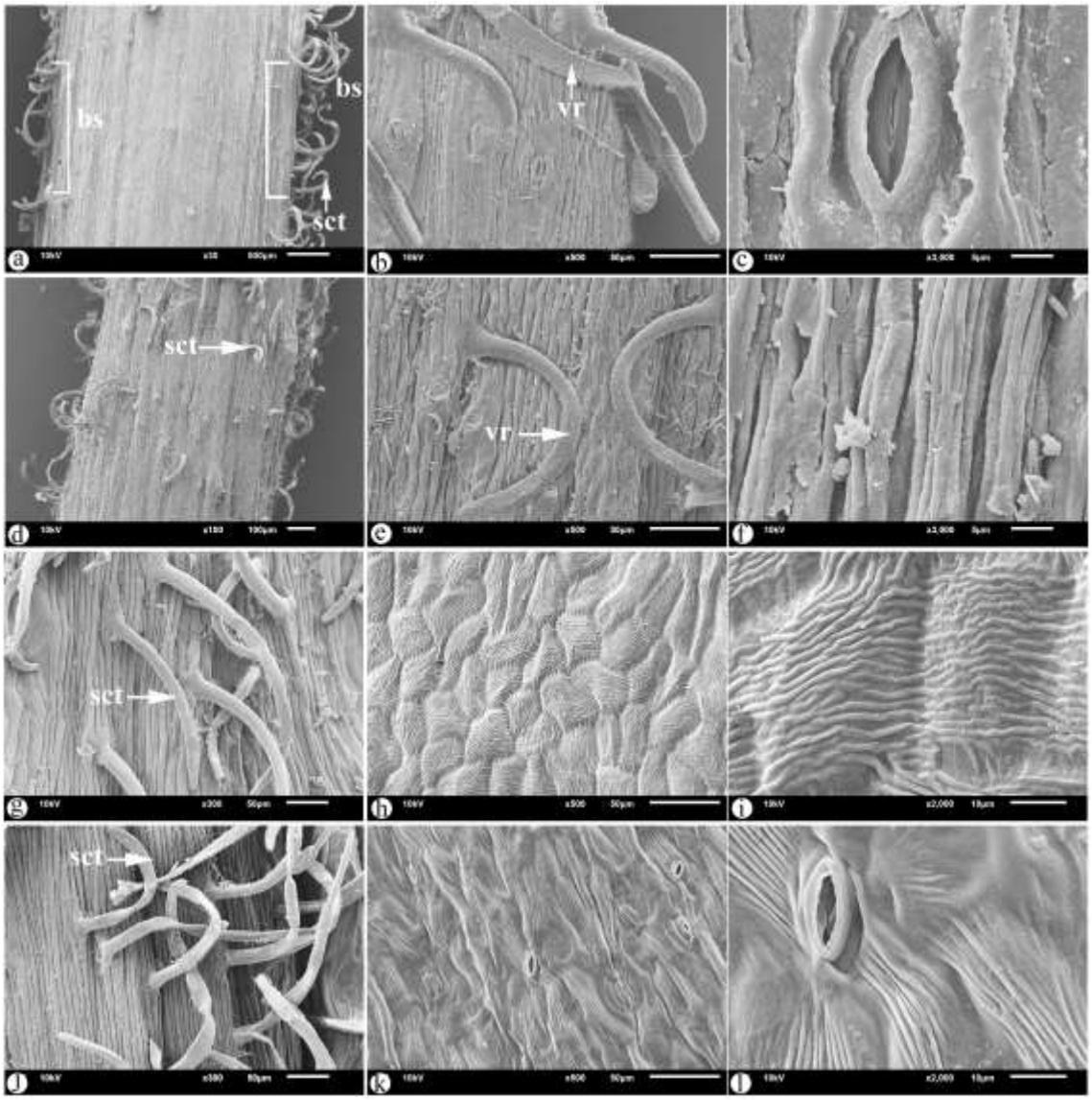

Figure 8. Scanning electron micrographs of stem, petiole and lamina surfaces of *Vincetoxicum hirundinaria* subsp. *hirundinaria* (S.Güven 19 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, bs: hairy along two sides (biserially), sct: short curly trichome, vr: verrucose trichome ornamentation.

Şekil 8. *Vincetoxicum hirundinaria* subsp. *hirundinaria* (S.Güven 19 & S.Makbul)'nın gövde, petiyol ve lamina yüzeylerine ait taramalı elektron mikroskobu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, bs: iki taraf boyunca tüylü (biserially), sct: kısa kıvrık tüy, vr: verrucose tüy ornamentasyonu.

Vincetoxicum parviflorum Decne: Stem and petiole surfaces with sparsely crisped indumenta all around, consisting of non-glandular, short curly trichomes with verrucose ornamentation, epicuticular wax smooth (Figure 9a-f). Leaf; amphistomatic with anomocytic stomata, adaxial and abaxial surfaces with sparsely crisped indumenta all around, consisting of non-glandular, short curly trichomes with verrucose ornamentation; epidermal cells on both sides polygonal, periclinal cell wall convex, anticlinal cell wall channelled, straight, epicuticular wax striate (Figure 9g-l).

Vincetoxicum scandens Somm. & Lev.: Stem surface with a crisped indument along one side (uniserially) (Figure 10a-c) and petiole surface with a sparsely crisped indument all around (Figure 10d-f), consisting of non-glandular, short curly trichomes with verrucose ornamentation, epicuticular wax smooth on both stem and petiole (Figure 10a-f). Leaf; hypostomatic with anomocytic stomata, adaxial and abaxial surfaces with crisped indumenta along margins and veins, consisting of nonglandular, short curly trichomes with verrucose ornamentation; epidermal cells on both sides polygonal or irregularly shaped, periclinal cell wall convex or concave, anticlinal cell wall channelled, straight to slightly undulate, epicuticular wax striate (Figure 10g-l).

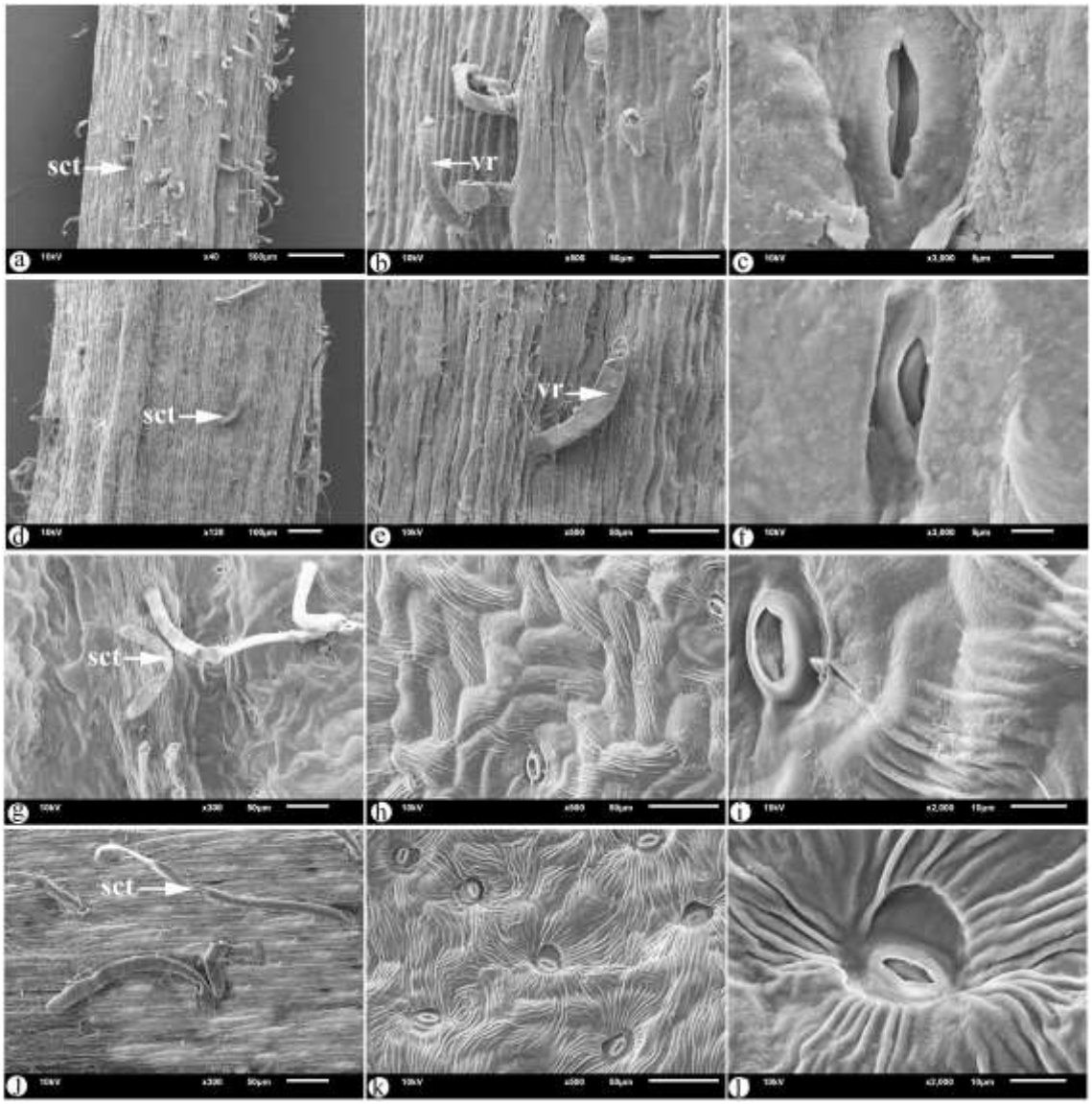

Figure 9. Scanning electron micrographs of stem, petiole and lamina surfaces of *Vincetoxicum parviflorum* (S.Güven 95 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, sct: short curly trichome, vr: verrucose trichome ornamentation.

Şekil 9. *Vincetoxicum parviflorum* (S.Güven 95 & S.Makbul)'un gövde, petiyol ve lamina yüzeylerine ait taramalı elektron mikroskobu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, sct: kısa kıvrık tüy, vr: verrucose tüy ornamentasyonu.

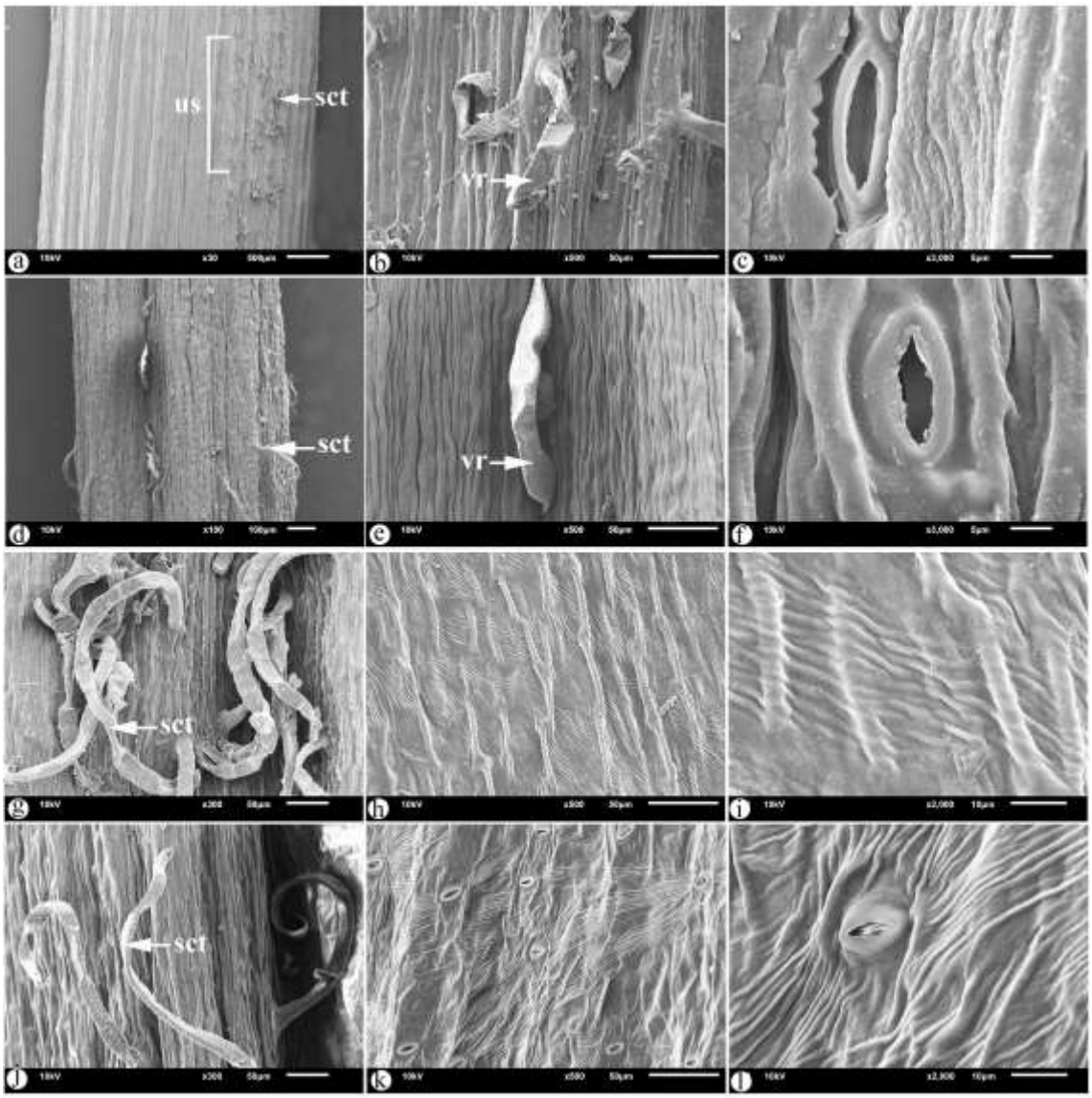

Figure 10. Scanning electron micrographs of stem, petiole and lamina surfaces of *Vincetoxicum scandens* (S.Güven 169 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, sct: short curly trichome, us: hairy along one side (uniserially), vr: verrucose trichome ornamentation.

Şekil 10. *Vincetoxicum scandens* (S.Güven 169 & S.Makbul)'in gövde, petiyol ve lamina yüzeylerine ait taramalı elektron mikroskobu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, sct: kısa kıvrık tüy, us: bir taraf boyunca tüylü (uniserially), vr: verrucose tüy ornamentasyonu.

Vincetoxicum speciosum Boiss. & Spruner: Stem and petiole surfaces with densely velutinous-lanate indumenta all around, consisting of non-glandular, long, wooly, curled or wavy trichomes with verrucose ornamentation, epicuticular wax smooth (Figure 11a-f). Leaf; hypostomatic with anomocytic stomata, adaxial and abaxial surfaces with densely velutinous-lanate indumenta all around, consisting of non-glandular, long, wooly, curled or wavy trichomes with verrucose ornamentation; epidermal cells on both sides polygonal or irregularly shaped, periclinal cell wall convex or concave, anticlinal cell wall channeled, straight to slightly undulate, epicuticular wax striate (Figure 11g-l).

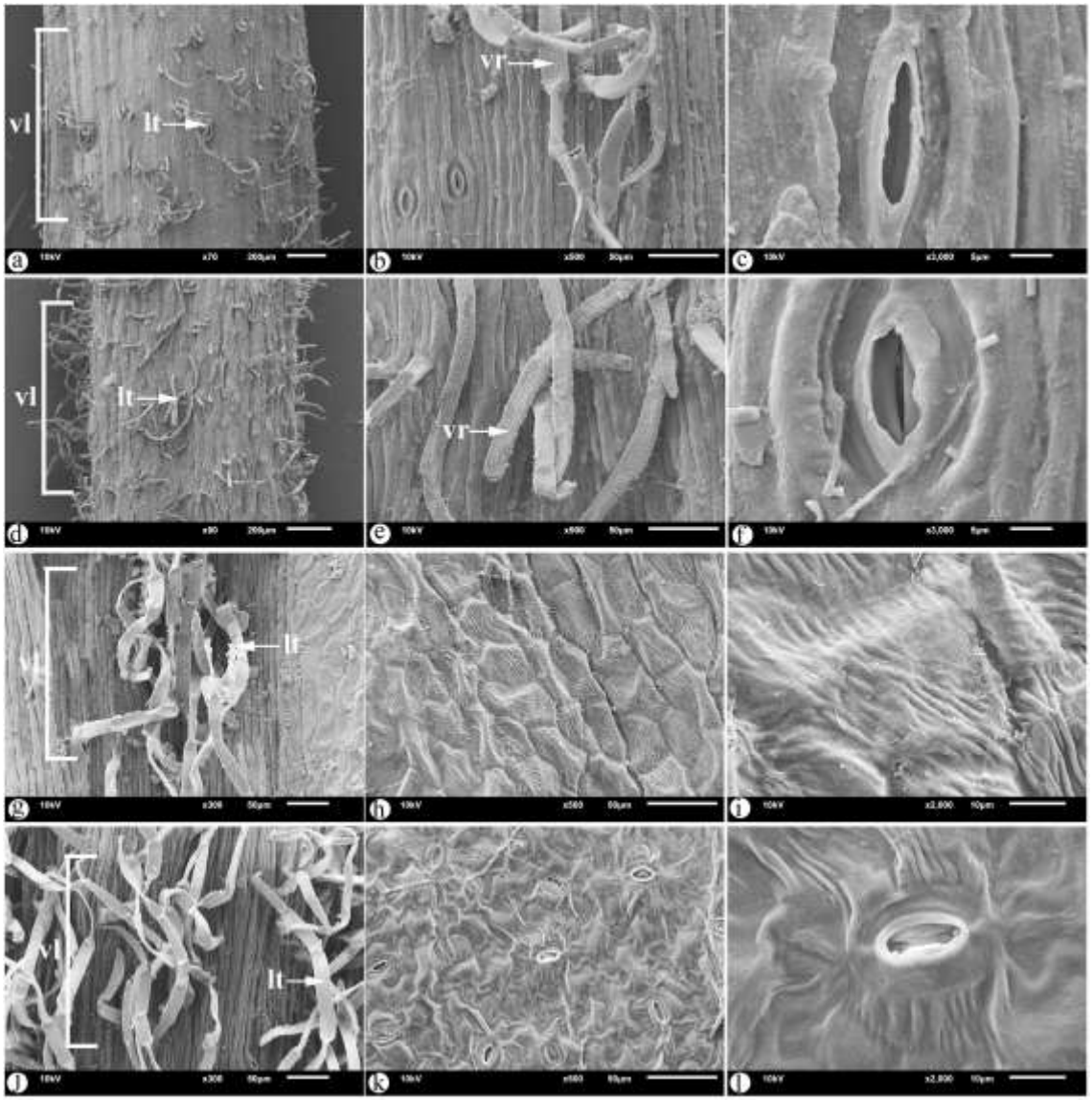

Figure 11. Scanning electron micrographs of stem, petiole and lamina surfaces of *Vincetoxicum speciosum* (S.Güven 132 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, lt: lanate trichome, vl: velutinous-lanate indument (all around), vr: verrucose trichome ornamentation.

Şekil 11. *Vincetoxicum speciosum* (S.Güven 132 & S.Makbul)'un gövde, petiyol ve lamina yüzeylerine ait taramalı elektron mikroskobu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, lt: lanat tüy, vl: velutinous-lanate tüy örtüsü (tüm yüzey boyunca), vr: verrucose tüy ornamentasyonu.

Vincetoxicum tmoleum Boiss.: Stem and petiole surfaces with densely crisped indumenta all around, consisting of non-glandular, short curly trichomes with verrucose ornamentation, epicuticular wax smooth (Figure 12a-f). Leaf; amphistomatic with anomocytic stomata, adaxial and abaxial surfaces with densely crisped indumenta all around, consisting of nonglandular, short curly trichomes with verrucose ornamentation; epidermal cells on both sides polygonal or irregularly shaped, periclinal cell wall convex, anticlinal cell wall channelled, straight to slightly undulate, epicuticular wax striate (Figure 12g-l).

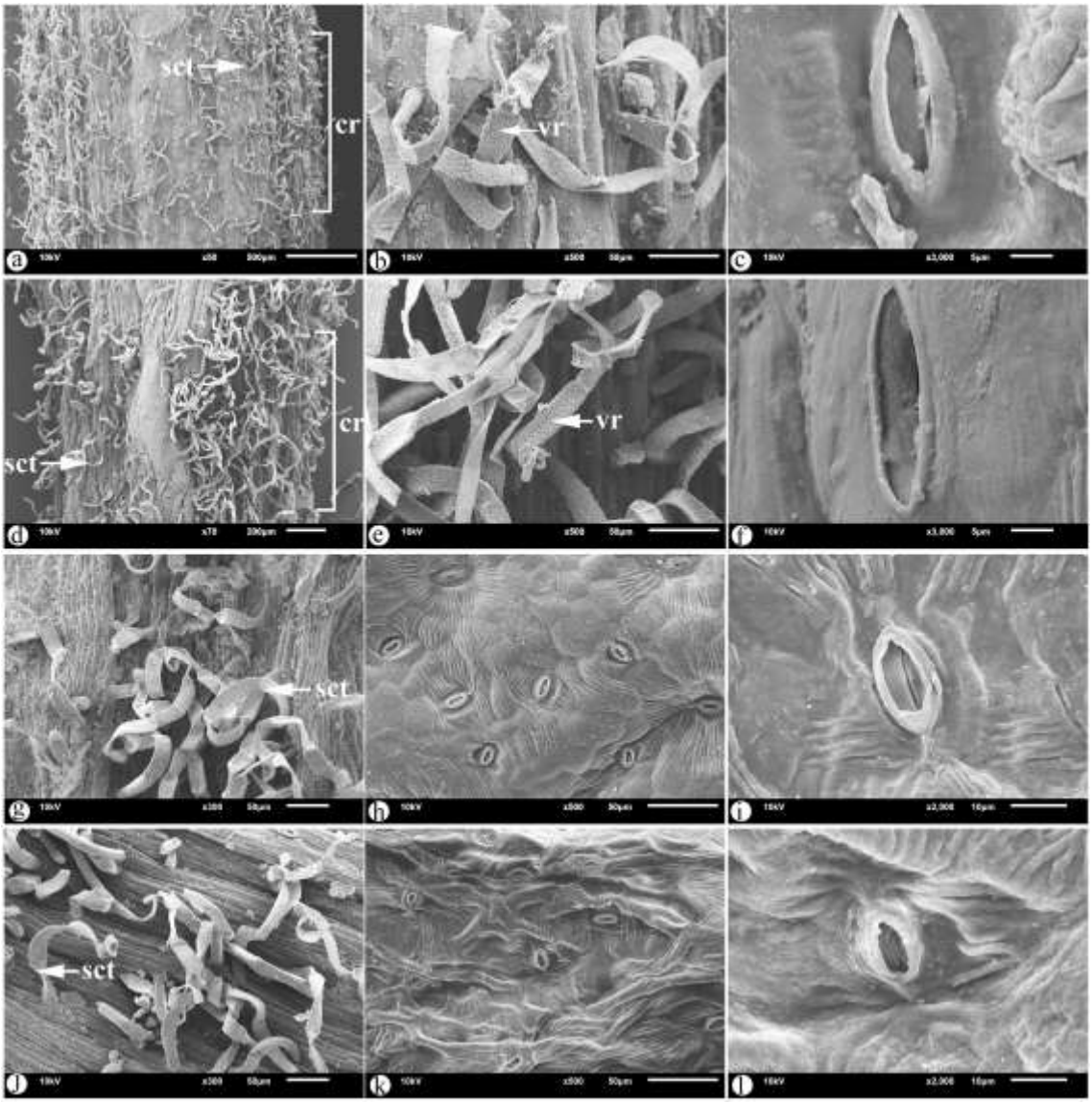

Figure 12. Scanning electron micrographs of stem, petiole and lamina surfaces of *Vincetoxicum tmoleum* (S.Güven 61 & S.Makbul) a-c. Stem, d-f. Petiole, g-i. Adaxial surface of the lamina, j-l. Abaxial surface of the lamina, cr: crisped indument (all around), sct: short curly trichome, vr: verrucose trichome ornamentation.

Şekil 12. *Vincetoxicum tmoleum* (S.Güven 61 & S.Makbul)'un gövde, petiyol ve lamina yüzeylerine ait taramalı elektron mikroskobu görüntüleri a-c. Gövde, d-f. Petiyol, g-i. Lamina üst yüzey, j-l. Lamina alt yüzey, sct: kısa kıvrık tüy, cr: kısa kıvrık tüy örtüsü (tüm yüzey boyunca), vr: verrucose tüy ornamentasyonu.

DISCUSSION

The present study is the first comprehensive report regarding the surface micro-morphology of vegetative organs of all the genus *Vincetoxicum* in Türkiye. Detailed surface feature of stem, petiole and lamina based on SEM analyzes played an important role in identification and classification of flowering plants (Tomaszewski & Zieliński, 2014). In this study, 11 *Vincetoxicum* taxa were compared on the basis of micro-morphological characteristics of vegetative parts such as stem, petiole and lamina. The most important distinguishing micro-morphological characters for Turkish *Vincetoxicum* species were determined as the distribution of stomata on leaves, and intumentum properties of stem, petiole and lamina, such as the pattern of pubescence, and the distribution and density of trichomes. On the other hand, no difference was observed among the examined taxa in terms of appearance of epicuticular wax, and shape and concavity/convexity properties of epidermal cells on stem, petiole and lamina (Figures 2-12). All the distinguishing micro-morphological characteristics were compared in both Appendix and Figure 13.

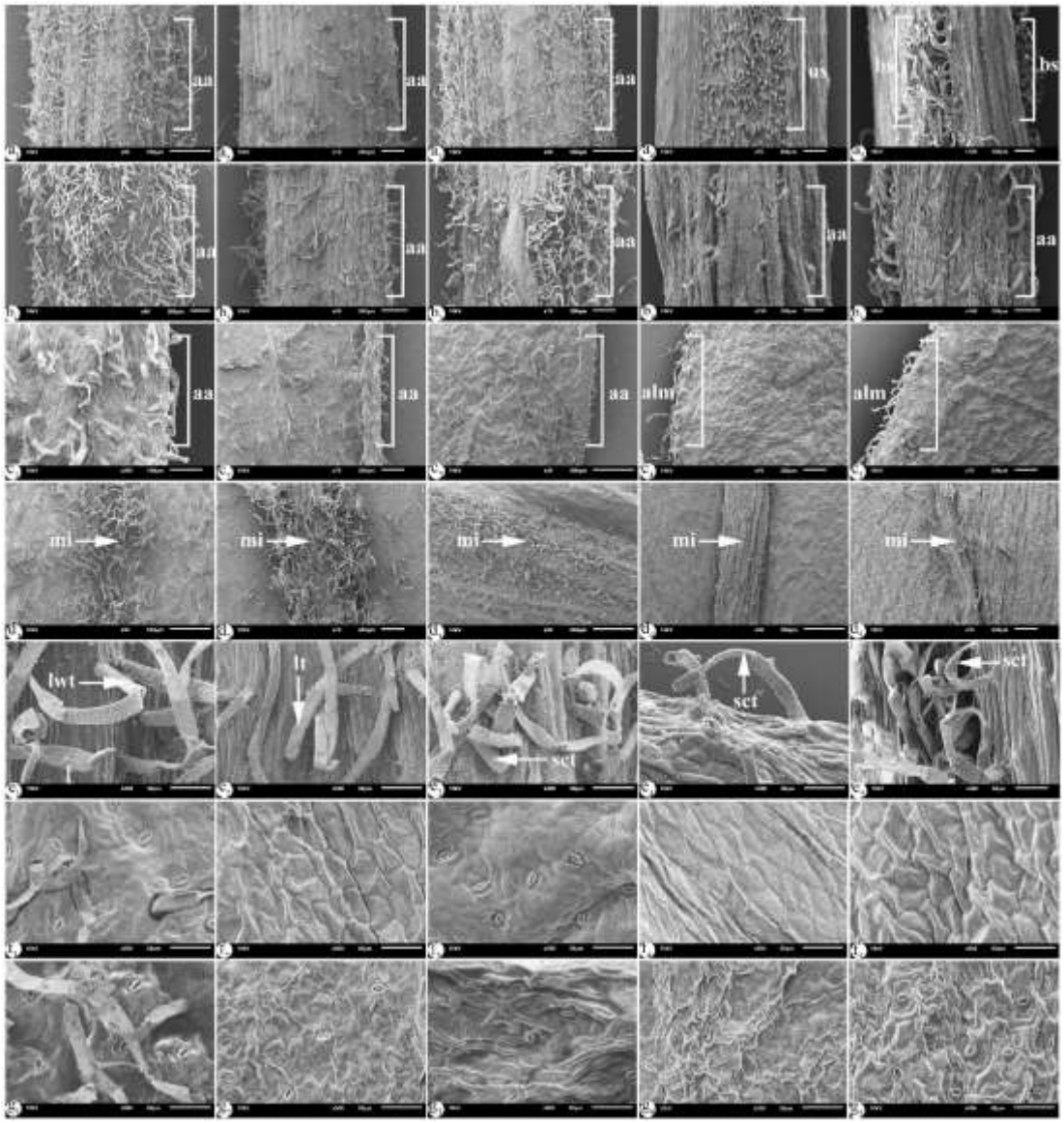

Figure 13. Distinguishing mico-morphological characteristics of stem, petiole, and lamina of Turkish *Vincetoxicum* taxa a. Distribution of indumentum on stem: a_1-a_3: densely hairy all around (aa), a_4: sparcely hairy along one side-uniserially (us), a_5: sparcely hairy along two sides-biserially (bs); b. Distribution and density of indumentum on petiole: b_1-b_3: densely hairy all around (aa), b_4-b_5: sparsely hairy all around (aa), c. Distribution of indumentum on lamina and leaf margin: c_1-c_3 densely hairy all around, c_4-c_5: sparcely hairy along margins and veins (alm), d. Distribution of indumentum on lamina and midrib (mi): d_1-d_3: densely hairy all around, d_4-d_5: sparcely hairy along midrib, e. The pattern of pubescence on stem, petiole, and lamina e_1. grayish white, matted, long wool-like trichome (lwt), e_2. lanate trichome (lt), e_3-e_5. short curly trichome (sct); f. Adaxial surface of lamina, f_1 and f_3: amohistomatic, f_2, f_4 and f_5: hipostomatic, g. Abaxial surface of lamina. 1. *Vincetoxicum canescens* subsp. *canescens*, 2. *V. speciosum*, 3. *V. tmoleum*, 4. *V. coscuncelebiana*, 5. *V. fuscatum* subsp. *fuscatum*

Şekil 13. Türkiye *Vincetoxicum* taksonlarının gövde, yaprak sapı ve laminasına ait ayırdedici mikromorfolojik özellikleri. a. Gövde üzerindeki tüy dağılımı: a_1-a_3: tüm yüzey boyunca yoğun tüylü (aa), a_4: bir yüzey boyunca seyrek tüylü-tek sıralı (us), a_5: iki yüzey boyunca seyrek tüylü-iki sıralı (bs); b. Yaprak sapı üzerindeki tüy dağılımı ve yoğunluğu: b_1-b_3: tüm yüzey boyunca yoğun tüylü (aa), b_4-b_5: tüm yüzey boyunca seyrek tüylü (aa), c. Yaprak laminası ve kenarı üzerindeki tüy dağılımı: c_1-c_3 tüm yüzey boyunca yoğun tüylü, c_4-c_5: kenarlar ve damarlar boyunca seyrek tüylü (alm), d. Lamina ve orta damar üzerindeki tüy dağılımı (mi): d_1-d_3: tüm yüzey boyunca yoğun tüylü, d_4-d_5: orta damar boyunca seyrek tüylü, e. Gövde, yaprak sapı ve lamina üzerindeki tüylenme deseni e_1. gri-beyaz, karışık, uzun yünsü tüy (lwt), e_2. lanat tüy (lt), e_3-e_5. kısa kıvrık tüy (sct); f. Laminanın adaksiyal yüzeyi, f_1 ve f_3: amfistomatik, f_2, f_4 ve f_5: hipostomatik, g. Laminanın abaksiyal yüzeyi. 1. *Vincetoxicum canescens* subsp. *canescens*, 2. *V. speciosum*, 3. *V. tmoleum*, 4. *V. coscuncelebiana*, 5. *V. fuscatum* subsp. *fuscatum*

The present investigations revealed that pubescence properties could be useful diagnostic characteristics for Turkish *Vincetoxicum*. The stem, petiole, and lamina indumentum of the examined taxa, composed of multicellular non-glandular trichomes, could clearly be divided into three groups based on the pattern of pubescence; (1) canescent-tomentose with grayish white, matted, long wool-like trichomes in *V. canescens*, (2) velutinous-lanate with long, wooly, curled or wavy trichomes in *V. speciosum*, and (3) crisped hairy with short curly trichomes in the remaining taxa (Appendix, Figure 13). It was reported that within the Apocynaceae, trichome types, ranging from unicellular to multicellular, glandular to non-glandular, showed a wide variation at both the genus and species levels. In addition, some of the Apocynaceae taxa have only simple non-glandular hairs, while some have both simple and glandular hairs (Gabr et al., 2015; El-Taher, et al, 2020; Medina et al., 2021). The trichome distribution on the stem, detected as another diagnostic character, was present along only one side (uniserially) in *V. coskuncelebiana* (Figure 4a) and *V. scandens* (Figure 10a), along two sides (biserially) in *V. funebre* (Figure 5a), *V. fuscatum* (Figure 6a and Figure 7a) and *V. hirundinaria* (Figure 8a), and along the entire surface in *V. canescens* (Figure 2a and Figure 3a), *V. parviflorum* (Figure 9a), *V. speciosum* (Figure 11a) and *V. tmoleum* (Figure 12a). Consistent with the stem indumentum, the trichomes were present along the entire surface on both adaxial and abaxial sides of the leaf in the taxa of *V. canescens* (Figure 2g-l and Figure 3g-l), *V. parviflorum* (Figure 9g-l), *V. speciosum* (Figure 11g-l) and *V. tmoleum* (Figure 12g-l). On the other hand, the leaves of the remaining examined taxa were hairy only along the veins and margins (Figure 13c_4-c_5 and d_4-d_5). The obtained SEM data relating to the pattern of pubescence was compatible with the previous light microscopy-based morphological studies on the genus (Pobedimova, 1952; Grossheim, 1967; Rechinger, 1970; Markgraf, 1972; Browicz, 1978; Güven et al., 2021a). The trichome ornamentation was determined as verrucose in all the investigated taxa. Consistent with our data, Liede (1996) described the trichome ornamentation as verrucose on the corolla surface of *Cynanchum* and *Vincetoxicum* taxa. Trichome surface features such as presence/absence and type of ornamentation (papillate, verrucose) were used as distinguishing characteristics for various plant groups in recent years (Martin & Juniper, 1970; Theobald et al. 1979; Freire et al., 2005). However, this character did not provide any distinction between the examined taxa.

Studies on the Apocynaceae family revealed that the distribution of stomata on the leaf contributed to the distinction of taxa at the genus and species levels (Omino, 1996; Jaleel et al., 2009; Abere & Onwukaeme, 2012). Among the studied *Vincetoxicum*, leaves were amphistomatic in *V. canescens* (Figure 2g-l and Figure 3g-l), *V. parviflorum* (Figure 9g-l), and *V. tmoleum* (Figure 12g-l) and hypostomatic in the remaining ones (Appendix). Recent micro-morphological studies on different Apocynaceae taxa also revealed the presence of amphistomatic and hypostomatic leaves in the family (Metcalfe & Chalk, 1950; Joubert, 2007; Xin-sheng, 2010; Medina et al., 2021). However, this character did not sufficiently support the distinction of the examined taxa at the species level.

Leaves and stems of many plant species are covered with a whitish coating proper, composed of epicuticular waxes, external to the cuticle. Micro-morphological investigations carried out that the epicuticular wax had a crystallized or semi-crystallized structure and the outer cellular wall could have a smooth surface structure or could have crusts, platelets, granules, tubules exhibiting various ornamentations such as striate, reticulate, or micro-papillose (Barthlott et al., 1998; Tomaszewski & Zieliński, 2014) In the present study, similarities and dissimilarities between the wax structures on the stem, petiole and both sides of leaf were examined using a SEM. The epicuticular wax layer was determined as smooth on the stem and petiole and striate on the leaf surface. Additionally, a stronger similarity was observed between adaxial and abaxial leaf surfaces for all the investigated taxa. Stem, petiole, and leaf epicuticular wax structures did not provide any distinction between the examined taxa. On the other hand, the major micro-morphological difference between *Vincetoxicum stocksii* Ali & Khatoon and *V. luridum* Stocks ex S.A. Shah was determined as the presence of epicuticular wax in the form of threads on the abaxial surface of *V. stocksii* and their absence in *V. luridum* (Shah et al., 2018). In parallel with our data, it has been determined that the epicuticular wax layer of the leaf is striated in different Apocynaceae members (Joubert, 2007; Xin-sheng, 2010; Bashir et al., 2020; Medina et al., 2021).

Among the studied taxa, leaf epidermal cells were found to be polygonal or irregularly shaped with convex or concave periclinal walls, and channelled, straight to slightly undulate anticlinal walls. Similar epidermal micro-morphological features were previously reported for taxa of *Cynanchum* (Xin-sheng, 2010) and *Cryptolepis* (Joubert, 2007). However, these characters did not provide sufficient data for the delimitation of the studied *Vincetoxicum* taxa.

The taxonomic relationships of the Turkish *Vincetoxicum* were previously evaluated in the light of palynological (Güven et al., 2015), cytological, and molecular (Güven et al., 2019, 2021a) data. The studied 11 *Vincetoxicum* taxa clustered under five separate groups in the phylogenetic trees inferred from ITS sequences (Güven et al., 2021a). Of these groups, the first one included *V. parviflorum*, the second *V. fuscatum*, the third *V. tmoleum* and *V. canescens*, the fourth *V. speciosum*, and the fifth *V. coskuncelebiana* (=*V. anatolicum*), *V. funebre*, *V. scandens*, and *V. hirundinaria* members (Güven et al., 2021a). The present micro-morphological properties of vegetative parts of the investigated taxa appeared to be partially compatible with their previous phylogenetic analysis. *V. canescens,*

V. tmoleum, V. parviflorum, and *V. speciosum* have amphistomatic leaves (excluding *V. speciosum* with hypostomatic leaves) and a dense pubescence all around on stem, petiole and lamina. The remaining examined taxa of *Vincetoxicum* are characterized by hypostomatic leaves hairy along veins and margins, and stems uniserially (*V. coskuncelebiana* and *V. scandens*) or biserially (*V. funebre, V. fuscatum* and *V. hirundinaria*) hairy (Appendix).

Browicz (1978) stated that, since *V. parviflorum* was morphologically close to *V. fuscatum* taxa, it could be a subspecies or variety of *V. fuscatum*. However, detailed taxonomic studies on the genus determined that these two taxa showed significant differences. While *V. parviflorum* was characterized with free and apically erect corona segments, sparcely crisped hairy follicles, elliptical pollinia (Güven et al., 2021a), and a somatic chromosome number of 2n=22 (Güven et al., 2019), *V. fuscatum* taxa had partly fused and apically curved corona segments, glabrous follicle, clavate pollinia (Güven et al., 2021a), and 2n=44 somatic chromosome number (Güven et al., 2019). These two species also clustered under different branches in the previous palynological (Güven et al., 2015) and molecular phylogenetic analyses (Güven et al., 2019, 2021a). The present micro-morphological data also supported the separation of these two species. In the examined *V. fuscatum* taxa, the thichomes are distributed biserially on the stem, and along the veins and margins on the leaves, and the leaves are hypostomatic. *V. parviflorum* is easily separated from *V. fuscatum* taxa with its leaves including stomata on both sides and stems and leaves covered with hairs along the entire surface. *V. fuscatum* subsp. *boissieri* and *V. fuscatum* subsp. *fuscatum*, two morphologically similar subspecies distinguished only by corolla indumentum (Browicz, 1978; Güven 2017), were also reported to be similar in terms of palynological (Güven et al., 2015), cytological and molecular (Güven et al., 2019, 2021a) data. Similarly, there is no difference between these two subspecies in terms of stem, petiole and lamina micro-morphology.

Molecular analyses were carried out that *V. tmoleum* and *V. canescens* taxa were closely related (Liede-Schumannn et al., 2016; Güven et al., 2019, 2021a). These two species are characterized by similar yellow or greenish yellow, campanulate corolla with dense indument on the adaxial surface and free corona segments (Güven et al., 2021a), ovate pollinia, rugulate pollen surface, and ovate corpusculum (Güven et al., 2015), and 2n=22 somatic chromosome number (Güven et al., 2019). On the other hand, *V. tmoleum* and *V. canescens* taxa exhibit quite different features in terms of stem and fruit morphology. *V. canescens* taxa, characterized by their decumbent stems and ovoid fruits, can be easily distinguished from *V. tmoleum* which has an erect stem and lanceolate fruits (Browicz, 1978; Güven et al., 2021a). The present micro-morphological studies also supported this distinction, and it was observed that the indumentum of the stems and leaves is densely canescent-tomentose in *V. canescens* while it is in densely crisped hairy in *V. tmoleum*. *V. canescens* subsp. *canescens* and *V. canescens* subsp. *pedunculata*, which could be distinguished from each other only by the peduncle length, were also found to be similar in terms of palynological (Güven et al., 2015), cytological and molecular data (Güven et al., 2019). Similarly, the present micro-morphological characters did not contribute to the distinction of these two subspecies.

V. coskuncelebiana (=*V. anatolicum*), which was recently published as a new species from Türkiye, was clustered under the same sub-branch with *V. funebre* and *V. scandens* species in the molecular analyses carried out by Güven et al. (2021a). Although these three *Vincetoxicum* species show similarities in terms of some characteristics such as general corona structure (cup-shaped), pollinium shape (ovate), and corpusculum shape (oblong), they can be easily distinguished from each other morphologically. *V. scandens* is characterized by its twining stem up to 2 m and dark purplish-black flowers densely pilose on the upper surface, whereas *V. funebre* has an erect and 40-135 cm high stem, and dark purplish-black flowers sparsely crisped hairy on the upper surface. Compared to these two highly structured plant species, *V. coskuncelebiana* is distinguished by its shorter stem structure (25–68 cm), and yellow to light brown flowers crisped hairy on the adaxial surface (Güven et al., 2021a). In addition, the present data revealed that the stem pubescence is biserially in *V. funebre*, while it is uniserially in *V. coskuncelebiana* and *V. scandens*.

CONCLUSION

In conclusion, the present work provided the first detailed micro-morphological descriptions of the stem, petiole, and lamina of all the *Vincetoxicum* taxa naturally distributed in Türkiye. The superficial appearance and shape of epidermal cells, ornamentation of epicuticular wax, and pattern of pubescence on stem, petiole, and lamina, and the type and distribution of stomata on both adaxial and abaxial leaf surfaces were examined in detail using SEM, and the obtained data was evaluated in a taxonomic perspective. The micro-morphological characters such as (1) indumentum pattern on stem, petiole, and lamina (grayish white, matted, long wool-like trichome; long, wooly, curled or wavy trichome; short curly trichome), (2) trichome distribution on stem (all around; uniserially; biserially), (3) trichome distribution on lamina (all around; along margins and veins), and (4) stomata distribution on leaves (hypostomatic; amphistomatic) were found to be useful in distinction of the Turkish *Vincetoxicum* taxa. Micro-morphological observations were carried out on plant samples taken from at least two different populations

for each taxon, except for *V. funebre*, which is known from a single locality in our country. The presence or absence of both trichomes and stomata and their density were considered to be affected by various ecological factors (Song et al., 2020). However, the pattern of pubescence and their surface ornamentation, and stomata type and distribution exhibited a stable structure among the taxa examined taxa and were found to be useful for the delimitation and identification of the present taxa collected from different habitats. Although this research included a limited number of individuals, the obtained data provided additional insights into comprehensive SEM-based taxonomical studies of the genus *Vincetoxicum*.

ACKNOWLEDGMENTS

The authors would like to thank Prof. Dr. Serdar MAKBUL, who allowed the stem and leaf materials used in the study to be obtained from the samples of the examined species. This study was supported by Scientific and Technological Research Council of Turkey (TUBITAK-2209A) under Grant Number 1919B012004568. The authors thank TUBITAK for their support.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors have declared no conflict of interest.

REFERENCES

Abere, T. A., & Onwukaeme, D. N. (2012). Pharmacognostic evaluation of the leaves of *Secamone afzelii* (Schult) K.Schum (Asclepiadaceae). *Tropical Journal of Pharmaceutical Research February, 11*(1), 125–131. http://dx.doi.org/10.4314/tjpr.v11i1.16

Abeysinghe, P. D., & Scharaschkin, T. (2022). Comparative anatomy of two forms of Sri Lankan *Calotropis gigantea* (L.) R. Br. (Family Apocynaceae s.l.–Subfamily Asclepiadoideae) -Taxonomic implications. *Ceylon Journal of Science, 51*(3), 307–318. http://doi.org/10.4038/cjs.v51i3.8038

Aybeke, M. (2022). Bazı Orkide taksonlarında labellum mikromorfolojisi. *KSÜ Tarım ve Doğa Derg, 25*(1), 42–48. https://doi.org/10.18016/ksutarimdoga.vi.882554

Barthlott, W., Neinhuis, C., Cutler, D., Ditsch, F., Meusel, I., Theisen, I., & Wilhelmi, H. (1998). Classification and terminology of plant epicuticular waxes. *Botanical Journal of the Linnean Society, 126*(3), 237–260. https://doi.org/10.1111/j.1095-8339.1998.tb02529.x

Bashir, K., Sohail, A., Ali, U., Ullah, A., Ul Haq, Z., Gul, B., Ullah, I., Sunera, & Asghar, M. (2020). Foliar micromorphology and its role in identification of the Apocynaceae taxa. *Microscopy Research and Technique, 83*, 755–766.

Behnke, H. D., & Barthlott, W. (1983). New evidence from the ultrastructural and micromorphological fields in angiosperm classification. *Nordic Journal of Botany, 3*, 43–66. https://doi.org/10.1111/j.1756-1051.1983.tb01444.x

Browicz, K. (1978). Asclepiadaceae. In Davis, P.H. (ed) *Flora of Turkey and the East Aegean Islands, Vol. 6.* Edinburgh University Press, Edinburgh, pp 163–174.

Carpenter, K. J. (2005). Stomatal architecture and evolution in basal angiosperms. *American Journal of Botany, 92*, 1595–1615. https://doi.org/10.3732/ajb.92.10.1595

Carvalho, R., Pellissari, L. C. O, Pace, M. R., Scremin-Dias, E, Arruda, R. D. O., & Farinaccio, M. A. (2017). Leaf morphoanatomy of *Araujia* and *Morrenia* (Asclepiadoideae, Apocynaceae): phylogenetic implications and species key. *Botanical Journal of the Linnean Society, 20*, 1–14. https://doi.org/10.1093/botlinnean/bow004

Chang, Y. H., Tzeng, H. Y., & Tseng, Y. H. (2012). Pollinaria morphology of *Cynanchum* L. (Asclepiadaceae) in Taiwan. *Quarterly Journal of Forestry Research, 34*(4), 251–262.

Civeyrel, L., Thomas, A. L., Ferguson, K., & Chase, M. W. (1998). Critical reexamination of palynological characters used to delimit Asclepiadaceae in comparison to the molecular phylogeny obtained from plastid *mat*K sequences. *Molecular Phylogenetics and Evolution, 9*(3), 517–527. https://doi.org/10.1006/mpev.1997.0510

Davis, P. H. (1965). *Flora of Türkiye and the East Aegean Islands, Vol. 1.* Edinburgh: Edinburgh University Press.

Dilcher, D. L. (1974). Approaches to the identification of angiosperm leaf remains. *Botanical Review, 40*(1), 1–160. https://doi.org/10.1007/BF02860067

El-Taher, A. M., Gendy, A. E. -N. G. E., Alkahtani, J., Elshamy, A. I., & Abd-ElGawad, A. M. (2020). Taxonomic implication of integrated chemical, morphological, and anatomical attributes of leaves of eight Apocynaceae taxa. *Diversity, 12*(9), 334. https://doi.org/10.3390/d12090334

Feng. Y., Qin. X., Huang. J., & Hong, W. (2012). Pollinarium morphology of 17 species and 1 subspecies of *Cynanchum* (Asclepiadaceae). *Acta Botanica Boreali-Occidentalia Sinica, 32*(9),1758–1762.

Freire, S. E., Arambarri, A. M., Bayón, N.D., Sancho, G., Urtubey, E., Monti, C., Novoa, M.C., & Colares, M. N. (2005). Epidermal characteristics of toxic plants for cattle from the Salado river basin (Buenos Aires, Argentina). *Boletín de la Sociedad Argentina de Botánica, 40*, 241–281.

Fishbein, M. (2001). Evolutionary innovation and diversification in the flowers of Asclepiadaceae. *Annals of the Missouri Botanical Garden, 88*, 603–623. http://dx.doi.org/10.2307/3298636

Gabr, D. G., Khafagi, A. A. F., Mohamed, A. H., & Mohamed, F. S. (2015). The significance of leaf morphological characters in the identification of some species of Apocynaceae and Asclepiadaceae. *Journal of American Science, 11*, 61–70.

Grossheim, A. A. (1967). *Flora of the Caucasus, Vol. 7*. Academy of Science of the USSR, pp 221–225.

Güner, A. (2012). Apocynaceae. In Güner, A., Aslan, S., Ekim, T., Vural, M., & Babaç, M. T. (eds.) *Türkiye bitkileri listesi (Damarlı bitkiler)*. Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, Istanbul, pp 83–86.

Güven, S. (2017). Türkiye *Vincetoxicum* (Apocynaceae Asclepiadoideae) cinsinin taksonomik revizyonu. PhD Thesis, Recep Tayyip Erdoğan Üniversitesi, Rize.

Güven, S., Makbul, S., Coskuncelebi, K., & Pınar, N. M. (2015). Pollinarium morphology of the genus *Vincetoxicum* Wolf (Apocynaceae: Asclepiadoideae) in Turkey. *Phytotaxa, 230*(1), 22–38. https://doi.org/10.11646/phytotaxa.230.1.2

Güven, S., Makbul, S., & Coşkunçelebi, K. (2019). Contribution to chromosome numbers and phylogeny of Turkish *Vincetoxicum* wolf (Apocynaceae, Asclepiadoideae). *European Journal of Taxonomy, 590*, 1–19. https://doi.org/10.5852/ejt.2019.590.

Güven, S., Coşkunçelebi, K., & Makbul, M. (2021a). A new *Vincetoxicum* (Apocynaceae) species from Eastern Anatolia and its relationship based on biosystematic properties. *Nordic Journal of Botany, 39*(12), e03405. https://doi.org/10.1111/njb.03405.

Güven, S., Makbul, S., & Çoşkunçelebi, K. (2021b). *Vincetoxicum tmoleum* Boiss. taksonunun genişletilmiş morfolojik özellikleri ve Türkiye'deki yayılışı. *Recep Tayyip Erdogan University Journal of Science and Engineering, 2*(2), 1–12. https://doi.org/10.53501/rteufemud.945076

Güzel, M. E. (2021). Kültür Marulu (*Lactuca sativa* L.) ile yabani akrabalarının yaprak mikromorfolojileri. *Bağbahçe Bilim Dergisi*, *8*(1), 121–133. https://doi.org/10.35163/bagbahce.866561

İlçim, A., Özay, S. G., & Kökdil, G. (2010). Exomorphic seed characters and anatomy of leaf and stem of some *Vincetoxicum* (Asclepiadaceae/Apocynaceae) species from Turkey. *Journal of Faculty of Pharmacy of Ankara University, 39*(1), 1–16. https://doi.org/10.1501/Eczfak_0000000550

Jaleel, C. A., Gopi, R., Azooz, M. M., & Panneerselvam, R. (2009). Leaf anatomical modifications in *Catharanthus roseus* as affected by plant growth promoters and retardants. *Global Journal of Molecular Sciences, 4*(1), 01–05.

Joubert, L. (2007). A taxonomic study of *Cryptolepis* (Apocynaceae) in Southern Africa. [Master's Thesis, Faculty of Natural and Agricultural Sciences, Department of Plant Sciences (Botany), University of the Free State Bloemfontein]. South Africa.

Koch, K., Bhushan, B., & Barthlott, W. (2008). Diversity of structure, morphology and wetting of plant surfaces. *Soft Matter*, *4*, 1943–63.

Liede, S. (1996). *Cynanchum – Rhodostegiella – Vincetoxicum – Tylophora* (Asclepiadaceae): new considerations on an old problem. *Taxon, 45*, 193–211.

Liede, S., & Täuber, A. (2002). Circumscription of the genus *Cynanchum* (Apocynaceae–Asclepiadoideae). *Systematic Botany, 27*(4), 789–800.

Liede, S., Täuber, A., & Schneidt, J. (2002). Molecular considerations in the Tylophorinae K.Schum. (Apocynaceae-Asclepiadoideae). *Edinburgh Journal of Botany, 59*(3), 377–403. https://doi.org/10.1017/S0960428602000239

Liede-Schumann, S., Kong, H., Meve, U., & Thiv, M. (2012). *Vincetoxicum* and *Tylophora* (Apocynaceae: Asclepiadoideae: Asclepiadeae) – two sides of the same medal: independent shifts from tropical to temperate habitats. *Taxon, 61*, 803–825. https://doi.org/10.1002/tax.614007

Liede-Schumann, S., Khanum, R., Mumtaz, A. S., Gherghel, I., & Pahlevani, A. (2016). Going west – a subtropical lineage (*Vincetoxicum*, Apocynaceae: Asclepiadoideae) expanding into Europe. *Molecular Phylogenetics and Evolution, 94*, 436–446. https://doi.org/10.1016/j.ympev.2015.09.021

Liede-Schumann, S., & Meve, U. (2018). *Vincetoxicum* (Apocynaceae—Asclepiadoideae) expanded to include Tylophora and allies. *Phytotaxa, 369*, 129–184. https://doi.org/10.11646/phytotaxa.369.3.1

Makbul, S., & Güven, S. (2022). A new replacement name for the Turkish *Vincetoxicum anatolicum* S. Güven, S. Makbul & Coskunç. (Apocynaceae: Asclepiadoideae). *Phytotaxa, 547*(2), 217–218. https://doi.org/10.5281/zenodo.6575118

Malaspina, P., Ingegneri, M., Betuzzi, F., Di Gristina, E., Cornara, L., Trombetta, D., & Smeriglio, A. (2024). Beyond the scent: new evidence about micromorphological, phytochemical and biological features of *Plumeria rubra* 'Tonda Palermitana' (Apocynaceae). *Plants, 13*, 2479. https://doi.org/10.3390/plants13172479

Mansoor, A., Ibrahim, M. A., Zaidi, M. A., & Ahmed, M. (2011). Antiprotozoal activities of *Vincetoxicum stocksii* and *Carum copticum*. *Bangladesh Journal of Pharmacology, 6*(1), 51–54. https://doi.org/10.3329/bjp.v6i1.8442

Markgraf, F. (1972). *Flora Europaea, Vol. 3*, Cambridge University Press, ISBN: 978-0521084895, pp 71–73.

Martin, J. T., & Juniper, B. E. (1970). *The cuticles of plants*. New York: St. Martins.

Medina, M. C., Sousa-Baena, M. S., Capelli, N. d. V., Koch, R., & Demarco, D. (2021). Stinging trichomes in Apocynaceae and their evolution in angiosperms. *Plants, 10*(11), 2324. https://doi.org/10.3390/plants10112324

Metcalfe, C. R., & Chalk, L. (1950). *Anatomy of dicotyledons: leaves, stem and wood in relation to taxonomy with notes on economic uses, Vol. II.* Oxford Clarendon Press.

Omino, E. (1996). *A contribution to the leaf anatomy and taxonomy of Apocynaceae in Africa*. Backhuys Publishers, ISBN: 9073348544, 178 s.

Pobedimova, G. (1952). *Antitoxicum*. In Shishkin, B. K. & Bobrov, E. G. (eds.) *Flora of USSR Vol. 18*. Academy of Science of the USSR Press, Leningrad-Moscow, pp 674–691.

Prüm, B., Seidel, R., Bohn, H. F., & Speck, T. (2012). Plant surfaces with cuticular folds are slippery for beetles. *Journal of the Royal Society Interface*, *9*:127–35.

Rechinger, K. H. (1970). *Flora Iranica, vol. 73:10*. Akademische Druck und Verlagsanstalt, p 21.

Ridzuan, K., & Kalu, M. (2023). Comparative micromorphology leaf surface of selected *Hoya* spp. (Apocynaceae) from Sarawak, *Reinwardtia, 22*(2), 69–77.

Sennblad, B., & Bremer, B. (2002). Classification of Apocynaceae s.l. according to a new approach combining linnaean and phylogenetic taxonomy. *Systematic Biology, 51*(3), 389–409. https://doi.org/10.1080/10635150290069869.

Shah, S. A., & Ahmad, M. (2014). Reassessing palynological characters in the subfamilies Periplocoideae and Asclepiadoideae (Apocynaceae): taxonomic and evolutionary implications. *Palynology, 38*(2), 224–234. http://dx.doi.org/10.1080/01916122.2014.893264

Shah, S. A., Sultan, A., Wen, J., Nisa, S.U., Su, X., Iqbal, J., & Mumtaz, A.S. (2018). *Vincetoxicum luridum* (Asclepiadeae, Asclepiadoideae, Apocynaceae), a new and long misunderstood endemic species from Balochistan, Pakistan. *Phytotaxa, 357*(2), 117–125. https://doi.org/10.11646/phytotaxa.357.2.4

Shah, S. A., Sultan, A., Wen, J., Ullah, Z., Nisa, S. U., Ren, Z., Alam, M. M., Iqbal, J., & Mumtaz, A. S. (2021) Taxonomy of *Vincetoxicum* s.str. (Asclepiadoideae, Apocynaceae) from southern Asia including three new species and resurrected names. *PhytoKeys, 179*, 35–73. https://doi.org/10.3897/phytokeys.179.62514

Sheeley, S. E., & Raynal, D. J. (1996). The distribution and status of species of *Vincetoxicum* in eastern North America. *Bulletin of the Torrey Botanical Club, 123*(2), 148–156. https://doi.org/10.2307/2996072

Sinha, S., & Mondal, A. K. (2011). The morphological diversification of pollinia of some members of Asclepiadaceae. *African Journal of Biotechnology, 10*(41), 7981–7986.

Sivarajan, V. V. (1991). *Introduction to the principles of plant taxonomy*. Cambridge University Press, Cambridge.

Song, J. H., Yang, S., & Choi, G. (2020). Taxonomic implications of leaf micromorphology using microscopic analysis: A tool for identification and authentication of korean piperales. *Plants, 9*(5), 566. https://doi.org/10.3390/plants9050566

Sreenath, K. P., Ramakrishna, T. M., & Babu, T. P. (2012). Perspective on polinial apparatus and carriers of Asclepiadaceae sensu lato. *Global Journal of Bio-Science and Biotechnology, 1*(1), 45–53.

Stearn. W. T. (1992). *Botanical Latin.* London, David and Charles Publication.

Stace, C. A., Heywood, V. H., & Moore, D.M. (1984). T*he taxonomic importance of the leaf surface. Current Concepts in Plant Taxonomy*. Academic Press, London, pp 67–94.

Theisen, I., & Barthlott, W. (1994). Mikromorphologie der epicuticularwachse und die systematik der Gentianales, Rubiales, Dipsacales und Calycerales. *Tropische und Subtropische Pflanzenwelt, 89*, 1–62.

Theobald, W. L., Krahulik, J. L., & Rollins, R. (1979). Trichome description and classification. In Metcalfe, C. R., & Chalk, L., (eds). *Anatomy of the dicotyledons*. Oxford, Clarendon Press.

Tomaszewski, D., & Zieliński, J. (2014). Epicuticular wax structures on stems and comparison between stems and leaves–A survey. *Flora, 209*(5–6), 215–232. https://doi.org/10.1016/j.flora.2014.03.001

Verhoeven, R. L., & Venter, H. J. T. (2001). Pollen morphology of the Periplocoideae, Secamonoideae, and Asclepiadoideae (Apocynaceae). *Annals of the Missouri Botanical Garden, 88*, 569–582. http://dx.doi.org/10.2307/3298634

Xin-sheng, Q. (2010). Studies on the micromorphology of leaf epidermis in *Cynanchum* (Asclepiadaceae) from China under environmental scanning electron microscope (ESEM). *Journal of South China Agricultural University, 31*(3), 47–51.

Yamashiro, T., Fukuda, T., Yokoyama, J., & Maki, M. (2004). Molecular phylogeny of *Vincetoxicum* (Apocynaceae-Asclepiadoideae) based on the nucleotide sequences of cpDNA and nrDNA. *Molecular Phylogenetics and Evolution, 31*, 689–700. http://dx.doi.org/10.1016/j.ympev.2003.08.016

Yamashiro, T., Yamashiro, A., Yokoyama, J., & Maki, M. (2008). Morphological aspects and phylogenetic analyses of pollination systems in the *Tylophora–Vincetoxicum* complex (Apocynaceae-Asclepiadoideae) in Japan. *Biological Journal of the Linnean Society 93*, 325–341. http://dx.doi.org/10.1111/j.1095-8312.2007.00896.x

Yaseen, S., & Perveen, A. (2014). Pollinia morphology to some members of Asclepiadaceae of Pakistan. *Bangladesh Journal of Botany, 43*(3), 249–253.

Zaidi, M. A., & Crow Jr., S. A. (2005). Biologically active traditional medicinal herbs from Balochistan, Pakistan. *Journal of Ethnopharmacology, 96*(1-2), 331–334. https://doi.org/10.1016/j.jep.2004.07.023.

Appendix. Comparison of distinguishing stem, petiole and lamina characteristics of Turkish *Vincetoxicum* taxa.

Ek. Türkiye *Vincetoxicum* taksonlarının ayırt edici gövde, yaprak sapı ve lamina özelliklerinin karşılaştırılması.

	Stem indumentum			**Petiole indumentum**			**Adaxial and abaxial lamina indumentum**			**Leaf**
	distribution	**density**	**pubescence pattern**	**distribution**	**density**	**pubescence pattern**	**distribution**	**density**	**pubescence pattern**	
V. canescens subsp. *canescens*	all around	dense	canescent-tomentose hairy	all around	dense	canescent-tomentose hairy	all around	dense	canescent-tomentose hairy	amphistomatic
V. canescens subsp. *pedunculata*	all around	dense	canescent-tomentose hairy	all around	dense	canescent-tomentose hairy	all around	dense	canescent-tomentose hairy	amphistomatic
V. coskuncelebiana	along one side (uniserially)	sparse	crisped hairy	all around	sparse	crisped hairy	along margins and veins	sparse	crisped hairy	hypostomatic
V. funebre	along two sides (biserially)	sparse	crisped hairy	all around	dense	crisped hairy	along margins and veins	sparse	crisped hairy	hypostomatic
V. fuscatum subsp. *fuscatum*	along two sides (biserially)	sparse	crisped hairy	all around	sparse	crisped hairy	along margins and veins	sparse	crisped hairy	hypostomatic
V. fuscatum subsp. *boissieri*	along two sides (biserially)	sparse	crisped hairy	all around	sparse	crisped hairy	along margins and veins	sparse	crisped hairy	hypostomatic
V. hirundinaria subsp. *hirundinaria*	along two sides (biserially)	sparse	crisped hairy	all around	sparse	crisped hairy	along margins and veins	sparse	crisped hairy	hypostomatic
V. parviflorum	all around	sparse	crisped hairy	all around	sparse	crisped hairy	all around	sparse	crisped hairy	amphistomatic
V. scandens	along one side (uniserially)	sparse	crisped hairy	all around	sparse	crisped hairy	along margins and veins	sparse	crisped hairy	hypostomatic
V. speciosum	all around	dense	velutinous-lanate hairy	all around	dense	velutinous-lanate hairy	all around	dense	velutinous-lanate hairy	hypostomatic
V. tmoleum	all around	dense	crisped hairy	all around	dense	crisped hairy	all around	dense	crisped hairy	amphistomatic