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Pollinarium morphology of *Vincetoxicum* (Apocynaceae: Asclepiadoideae) in Turkey

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Abstract

In this study, the pollen morphology of 20 representatives of ten taxa of *Vincetoxicum* from Turkey was observed under a scanning electron microscope and light microscope. Observations showed that each flower contained five pollinaria, including a pair of pollinia and caudicles attached to a central corpusculum. In the investigated taxa, the shape of the pollinium varied from ovate, elliptical, and obovate, to clavate, pollen cell surfaces exhibited gemmate or rugulate ornamentation, and the shape of the corpuscula was ovate or oblong. Numerical analysis showed that the shape of pollinia and corpuscula, size and surface ornamentation of pollen cells, and size of corpuscula and caudicles are valuable traits in delimiting the examined taxa. A key to Turkish *Vincetoxicum* based on pollinarium morphology is presented.

Key words: Anatolia, micromorphology, pollen morphology, pollinium

Introduction

Vincetoxicum Wolf (1776: 130) (Apocynaceae: Asclepiadoideae) comprises approximately 100 species, distributed from Europe and the Mediterranean, to eastern Asia (Yamashiro *et al.* 2008, Endress *et al.* 2014). *Vincetoxicum* consists of perennial herbs growing in dry, open rocky slopes, steppes, river valleys, shrubs, mountain slopes, and *Quercus* Linnaeus (1753: 994) dominated forests (Browicz 1978). Members of the genus are known to be poisonous to humans and some have been used in conventional and folk medicine (Mansoor *et al.* 2011).

Browicz (1978) reported six species and four subspecies, excluding doubtful records, in the first account of Turkish *Vincetoxicum*. More recently, Güner (2012) listed ten taxa of *Vincetoxicum* from Turkey, including three endemics *(V. canescens* (Willdenow) Decaisne [1844: 523] subsp. *pedunculata* Browicz [1975: 265], *V. fuscatum* Reichenbach [1854: 17] subsp. *boissieri* (Kusnezov) Browicz [1978: 171], and *V. parviflorum* Decaisne [1844: 525]*)*. A number of authors have investigated morphological (Liede 1996, Yamashiro *et al.* 2008), karyological (Albers & Meve 2001, Yamashiro *et al.* 2002), chemical (Stærk *et al.* 2005, Zaidi 2006, Mansoor *et al.* 2011), and molecular (Liede 2001, Liede & Kunze 2002, Yamashiro *et al.* 2004, Goyder *et al.* 2007, Liede-Schumann *et al.* 2012) properties of *Vincetoxicum* in general, while studies of Turkish *Vincetoxicum* specifically have focused on pharmaceutical (Özay 2013), and anatomical and seed micro-morphological properties (Ilçim *et al.* 2010).

Vincetoxicum is a systematically difficult genus, including many closely related species with complex and variable floral features (Browicz 1978). In order to solve systematic problems in a number of genera of Apocynaceae, pollinarium morphology has been investigated by several authors using light microscopy (Sinha & Mondal 2011, Gaykar *et al.* 2012, Sreenath *et al.* 2012) and scanning and transmission electron microscopy (Verhoeven & Venter 2001, Verhoeven *et al.* 2003, Wanntorp 2007, Mo *et al.* 2010). However, there are a very limited number of studies on the pollinarium morphology of members of *Vincetoxicum* (Shah & Ahmad 2014, Yaseen & Perveen 2014). Consequently, in order to contribute to an increased understanding of the genus, the aim of the present study is to describe the pollinarium morphology of ten taxa of Turkish *Vincetoxicum* using light microscopy and scanning electron microscopy.

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Material and methods

Specimens: Plant materials used in the present study were collected from natural habitats in Turkey over the period 2013–2014 (Table 1). All specimens were processed according to standard herbarium techniques and deposited in the Herbarium of Recep Tayyip Erdogan University, Department of Biology (RUB).

TABLE 1. Locality information of the examined taxa of *Vincetoxicum*.

Taxon	Locality
V. canescens subsp. *canescens*	Erzincan: From Erzincan to Kemah, 39º 40′ N, 39º 23′ E, 1130 m, 10 June 2013, *Güven 37*, RUB; Adana: From Gülek Boğazı to Pozantı, 37º 17′ N, 34º 47′ E, 1158 m, 24 May 2014, *Güven 91*, RUB
V. canescens subsp. *pedunculata*	Manisa: From Salihli to Kula, 38º 33′ N, 28º 34′ E, 843 m, 20 June 2013, *Güven 51*, RUB; Muğla: Yılanlı Mountain, 37º 12′ N, 28º 27′ E,1334 m, 21 June 2013, *Güven 56*, RUB
V. funebre	Ardahan: Posof, Erin Village, 41º 34′ N, 42º 43′ E, 1642 m, 17 June 2014, *Güven 126*, RUB
V. fuscatum subsp. *boissieri*	Erzincan: From Erzincan to Sakaltutan, 39º 52′ N, 39º 09′ E,1980 m, 10 June 2013, *Güven 35*, RUB; Bayburt : Bayburt-Değirmencik Village road, 40º 17′ N, 40º 13′ E, 1552 m, 29 June 2013, *Güven 73*, RUB
V. fuscatum subsp. *fuscatum*	Niğde: Ulukışla, Maden Village, 37º 26′ N, 34º 37′ E,1700 m, 24 May 2014, *Güven 93*, RUB; Tokat: Keçeci Village, 40º 32′ N, 36º 24′ E, 983 m, 27 May 2014, *Güven 106*, RUB
V. hirundinaria subsp. *hirundinaria*	Kırklareli: From Kızılağaç to Demirköy, 41º 42′ N, 27º 52′ E, 317 m, 18 July 2013, *Güven 19*, RUB; Kırklareli: From Demirköy to İğneada, 41º 52′ N, 27º 53′ E, 199 m, 19 May 2013, *Güven 28*, RUB
V. parviflorum	Tunceli: Ovacık, Karagöl Valley, 1508 m, 21 July 2013, 39º 22′ N, 39º 08′ E, *Güven 80*, RUB; Kayseri: Yahyalı-Kapuzbaşı Waterfalls road, 704 m, 25 May 2014, 37º 50′ N, 35º 28′ E, *Güven 95*, RUB
V. scandens	Trabzon: Maçka, Altındere Village, 928 m, 10 June 2013, 40º 43′ N, 39º 38′ E, *Güven 03*, RUB; Ordu: Ünye, İnkur-Akkuş road, near Tekneli, 906 m, 27 May 2014, 40º 55′ N, 37º 05′ E, *Güven 101*, RUB
V. speciosum	Tekirdağ: Saray, Safaalan-Binkılıç road, 206 m, 19 May 2013, 41º 25′ N, 28º 07′ E, *Güven 20*, RUB; Bursa: İnegöl, Sayfiye Village, 884 m, 26 June 2014, 40º 06′ N, 29º 20′ E, *Güven 131*, RUB
V. tmoleum	Denizli: Sarayköy, Babadağ, Taşoluk Plateau, 1431 m, 20 June 2013, 37º 47′ N, 28º 47′ E, *Güven 52*, RUB; Ankara: Beypazarı, Karagöl Plateau, 1570 m, 24 June 2013, 40º 18′ N, 31º 57′ E, *Güven 60*, RUB; Hatay: Amanos Mountains, 1584 m, 3 July 2013, 36º 79′ N, 36º 39′ E, *Güven 75*, RUB

Palynological studies: Polleniferous materials were obtained from the herbarium specimens or samples stored in FAA (Formaldehyde: Acetic Acid: Alcohol) for 24 hours and then preserved in ethanol (70%). Pollinaria were dissected from the flowers and fixed in 100% ethanol, following Verhoeven & Venter (2001). For light microscopy (LM), pollinaria were mounted in glycerine-gelatine (in order to obtain permanent slides) and photographed with an Olympus BX 50 research microscope Bs200Prop Image Processing and Analysis System. Measurements and observations were made on 15 fully developed pollinaria per sample. For scanning electron microscopy (SEM), the pollinaria were dried outdoors and mounted on stubs using double-sided tape, coated with a thin layer of gold for 3 minutes, and then examined and photographed using JEOL-JSM 6610 SEM at The Central Research Laboratories of Recep Tayyip Erdogan University. Pollen and pollinarium terminology primarily follows Newton (1984) and Punt *et al.* (2007).

Numerical analysis: Twenty samples representing all ten Turkish taxa of *Vincetoxicum* were assessed based on 11 palynological characters. Three characters were nominally scored as 0 or 1, and the remaining eight characters were quantitative, including linear measurements and digit numbers (Table 2). Palynological measurements used in the numerical analysis are provided in Appendix 1. Cluster analysis (CA) and principal components analysis (PCA) were performed by Syn-Tax PC 5.0 (Podani 1993). For CA (UPGMA), a pair-wise matrix of resemblance values was calculated from raw standardized data matrix, using Gower's coefficient of resemblance designed for mixed data sets (Sneath & Sokal 1973). A dendrogram was generated by the unweighted pair-group method by using arithmetic averages (UPGMA). Also, cophenetic correlation coefficient (rcs) was calculated (Sneath & Sokal 1973). For PCA, the raw mixed data (binary and continuous) were used to create a correlation matrix (for more on mixed data in Syn-Tax see Podani 1993). Two eigenvectors (a set of coordinates) were extracted by Eigen analysis from this correlation matrix and two coordinates (axes) were projected to give a two-dimensional plot of the taxa and the characters.

Key: Based on the results of the above analyses, a dichotomous key to Turkish *Vincetoxicum*, based on palynological characters, was developed (see Results below).

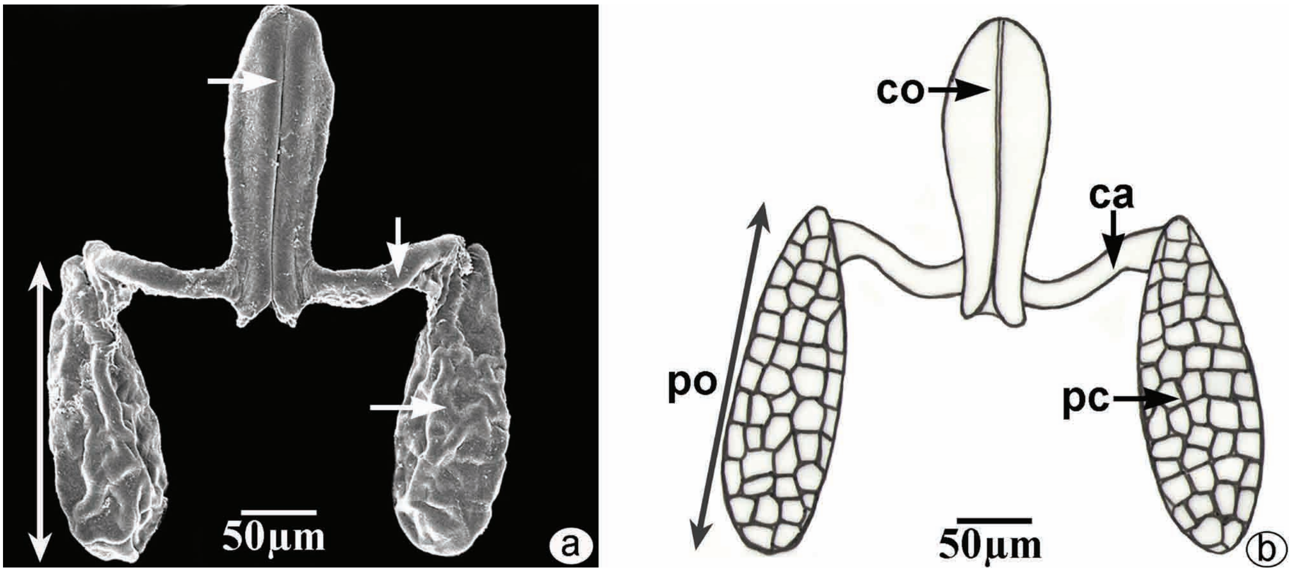

FIGURE 1. The general structure of a pollinarium. **a.** SEM; **b.** Outline drawing. ca: caudicle, co: corpusculum, pc: pollen cell, po: pollinium.

TABLE 2. Palynological characters used in numerical analysis.

Symbol	Characters
X_1	Width/ length of pollinium (µm)
X_2	Pollinium shape: ovate or elliptical: 0; clavate or obovate: 1
X_3	Width of pollen cell (µm)
X_4	Length of pollen cell (µm)
X_5	Pollen ornamentation: rugulate: 0; gemmate: 1
X_6	Width of corpusculum (µm)
X_7	Length of corpusculum (µm)
X_8	Width/ length of corpusculum (µm)
X_9	Corpusculum shape: ovate: 0; oblong: 1
X_{10}	Width of caudicle (µm)
X_{11}	Length of caudicle (µm)

Results

Palynological characterization

Asclepiad flowers are characterized by five anthers, each of which produces two pollinia derived from the two ventral pollen sacs (Endress & Bruyns 2000). The pollinia remain in the anther locules until they are removed by insects in the form of a characteristic structure called pollinarium (Verhoeven & Venter 2001). *A* pollinarium comprises a pair of pollinia derived from adjacent anthers, which are attached to a central corpusculum by two flexible arms (Liede 1996) (Fig. 1). All of the examined *Vincetoxicum* taxa exhibited five pollinaria (ten pollinia) per flower. The general pollinarium structure of the studied taxa is consistent with the members of Asclepiadoideae (Mo *et al.* 2010, Sinha & Mondal 2011, Gaykar *et al.* 2012, Shah & Ahmad 2014, Yaseen & Perveen 2014). We found that the dimensions of pollen cells were generally useful in distinguishing the examined taxa at the interspecific level (and for *V. canescens* also at the intraspecific level). Detailed characterizations of the pollinaria of the studied taxa are provided below.

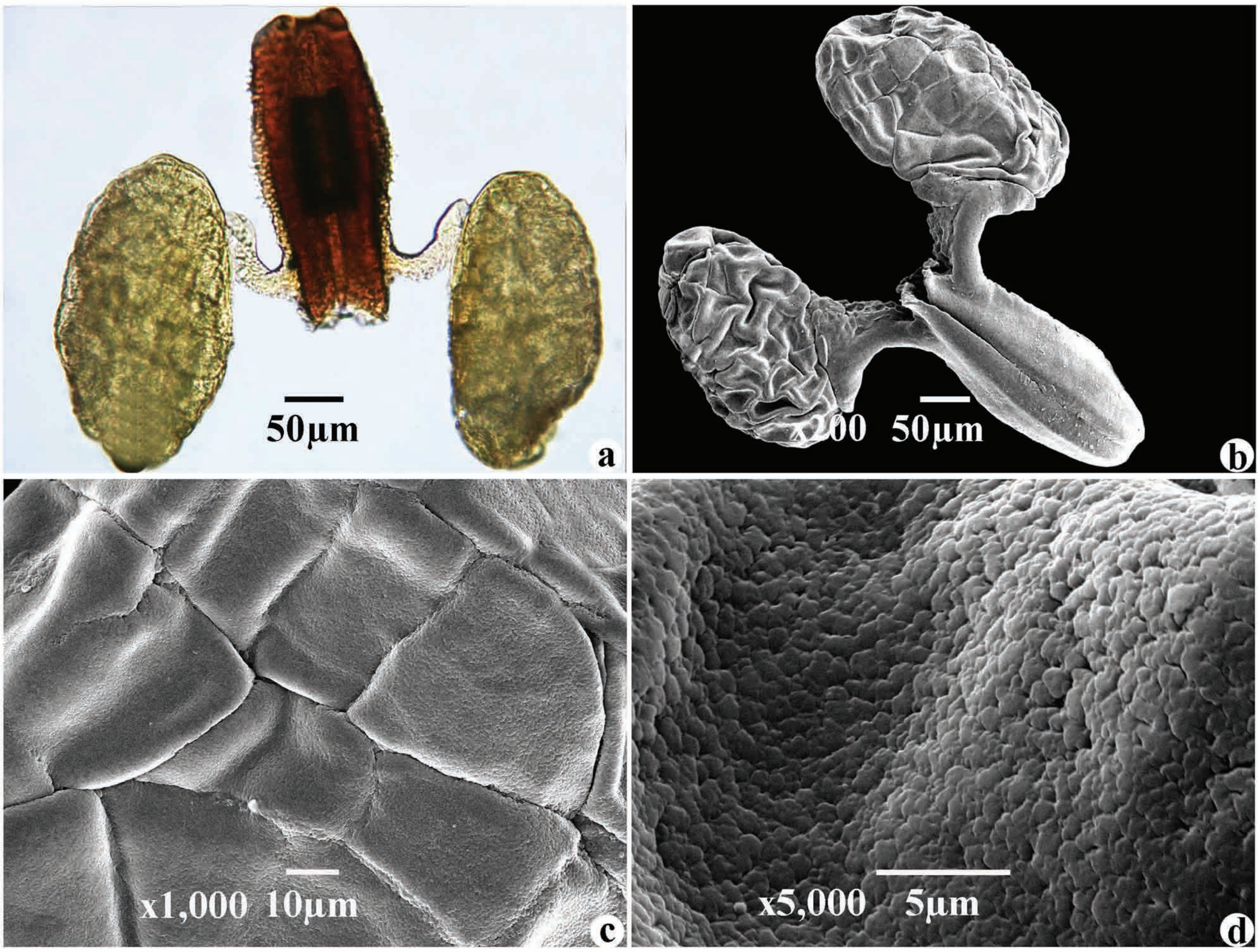

FIGURE 2. *Vincetoxicum canescens* subsp. *canescens* (*Güven 91*). **a.** Pollinarium (LM); **b.** Pollinarium (SEM); **c.** Pollen cells; **d.** Ornamentation.

***V. canescens* subsp. *canescens*:**
Pollinium ovate, 281.7 (±6.95) × 179.7 (±7.41) µm; pollen cells 45.1 (±2.71) × 34.1 (±2.42) µm; inaperturate; ornamentation tectate-perforate, rugulate. Corpusculum ovate, 301.9 (±4.55) × 122.5 (±3.16) µm. Caudicle attached to the corpusculum sub-apically and to the pollinium sub-basally, 114.7 (±2.42) × 24.8 (±2.07) µm (Fig. 2).

***V. canescens* subsp. *pedunculata*:**
Pollinium ovate, 253.9 (±3.86) × 154.5 (±3.52) µm; pollen cells 40.9 (±3.45) × 28.9 (±2.28) µm; inaperturate; ornamentation tectate-perforate, rugulate. Corpusculum ovate, 264.9 (±6.63) × 125.1 (±2.34) µm. Caudicle attached to the corpusculum sub-apically and to the pollinium sub-basally, 112.8 (±4.68) × 23.4 (±2.16) µm (Fig. 3).

***V. funebre* Boissier & Kotschy (1849: 79):**
Pollinium obovate, 257.9 (±4.46) × 100.1 (±2.96) µm; pollen cells 35.6 (±3.65) × 24.7 (±2.27) µm; inaperturate; ornamentation tectate-perforate, rugulate. Corpusculum oblong, 221.1 (±7.28) × 81.5 (±4.55) µm. Caudicle attached to the corpusculum sub-apically and to the pollinium basally to sub-basally, 113.7 (±2.02) × 18.3 (±1.74) µm (Fig. 4).

***V. fuscatum* subsp. *boissieri*:**
Pollinium clavate, 227.7 (±6.12) × 107.9 (±3.67) µm; pollen cells 32.2 (±1.84) × 22.1 (±1.64) µm; inaperturate; ornamentation tectate-perforate, gemmate. Corpusculum oblong, 195.7 (±3.81) × 61.4 (±1.54) µm. Caudicle attached to the corpusculum sub-apically and to the pollinium basally to sub-basally, 88.3 (±1.18) × 14.8 (±0.89) µm (Fig. 5).

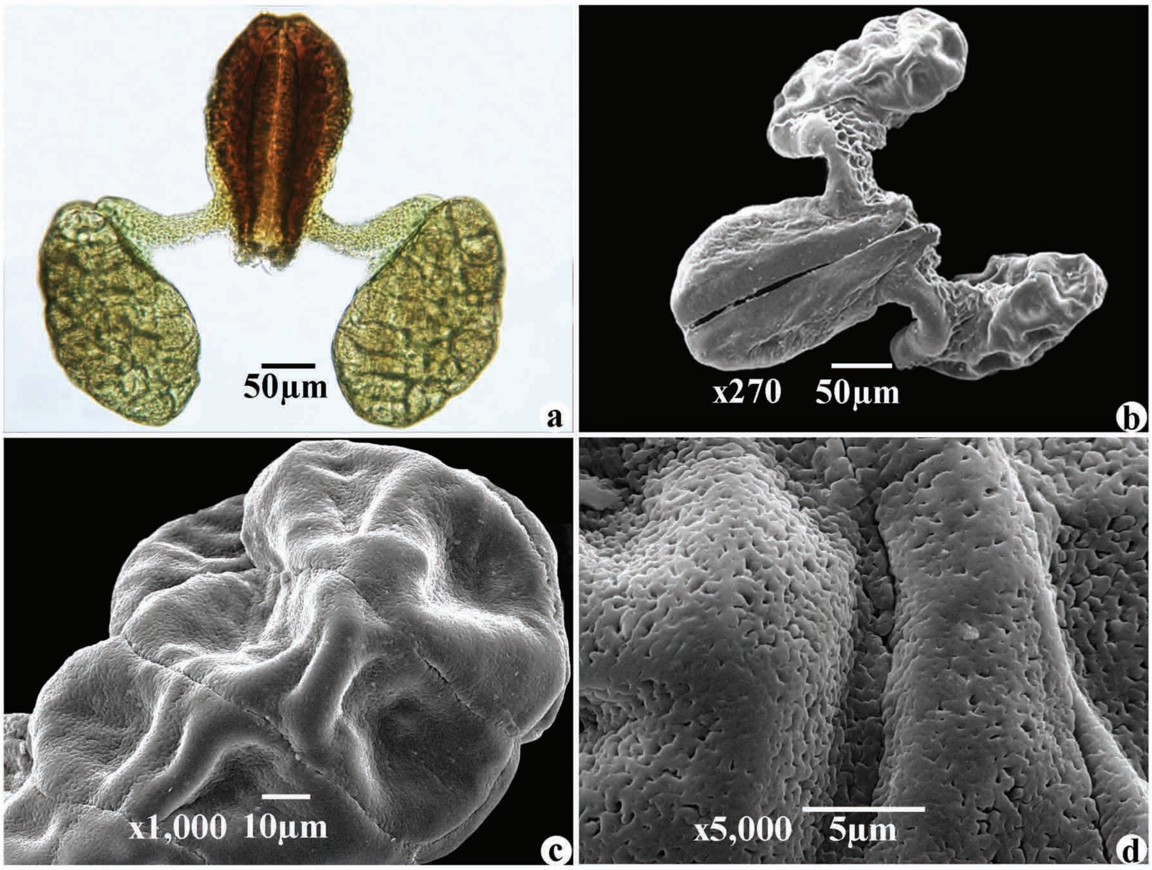

FIGURE 3. *Vincetoxicum canescens* subsp. *pedunculata* (*Güven 51*). **a.** Pollinarium (LM); **b.** Pollinarium (SEM); **c.** Pollen cells; **d.** Ornamentation.

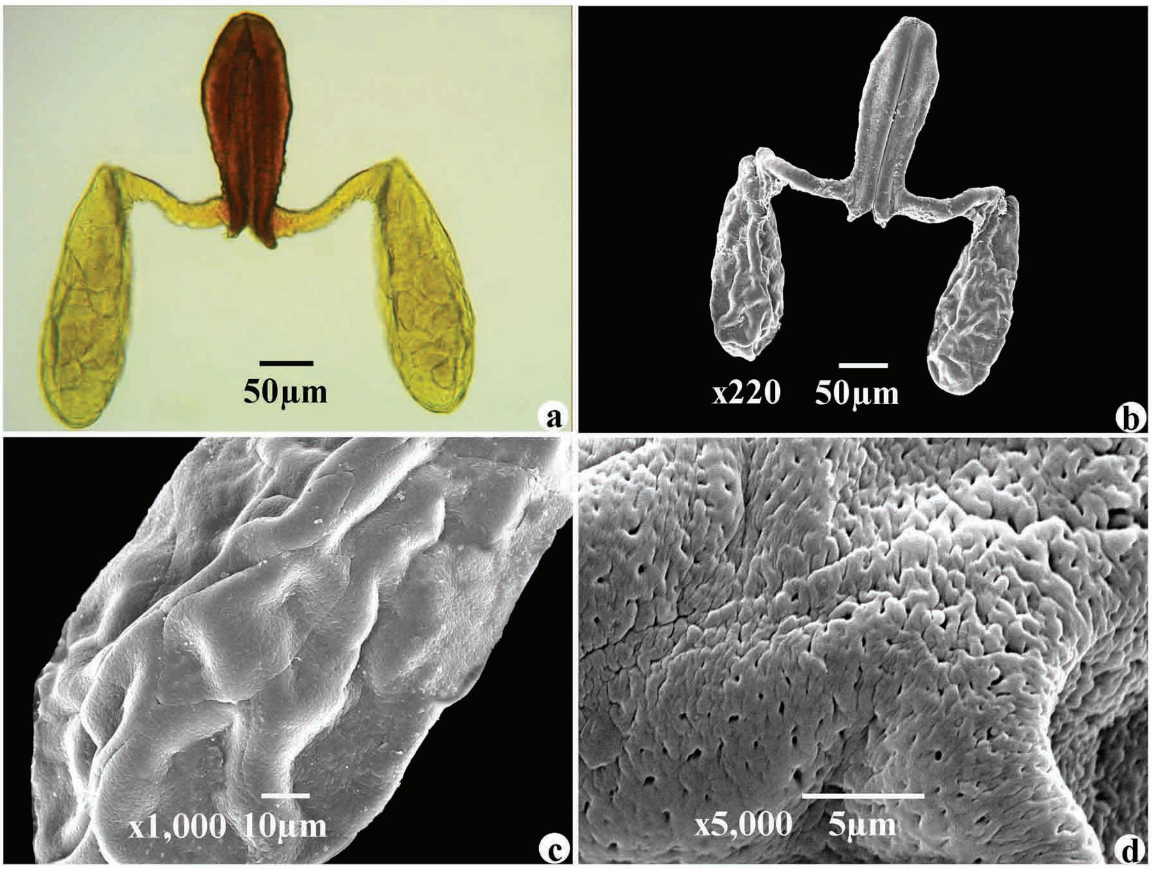

FIGURE 4. *Vincetoxicum funebre* (*Güven 126*). **a.** Pollinarium (LM); **b.** Pollinarium (SEM); **c.** Pollen cells; **d.** Ornamentation.

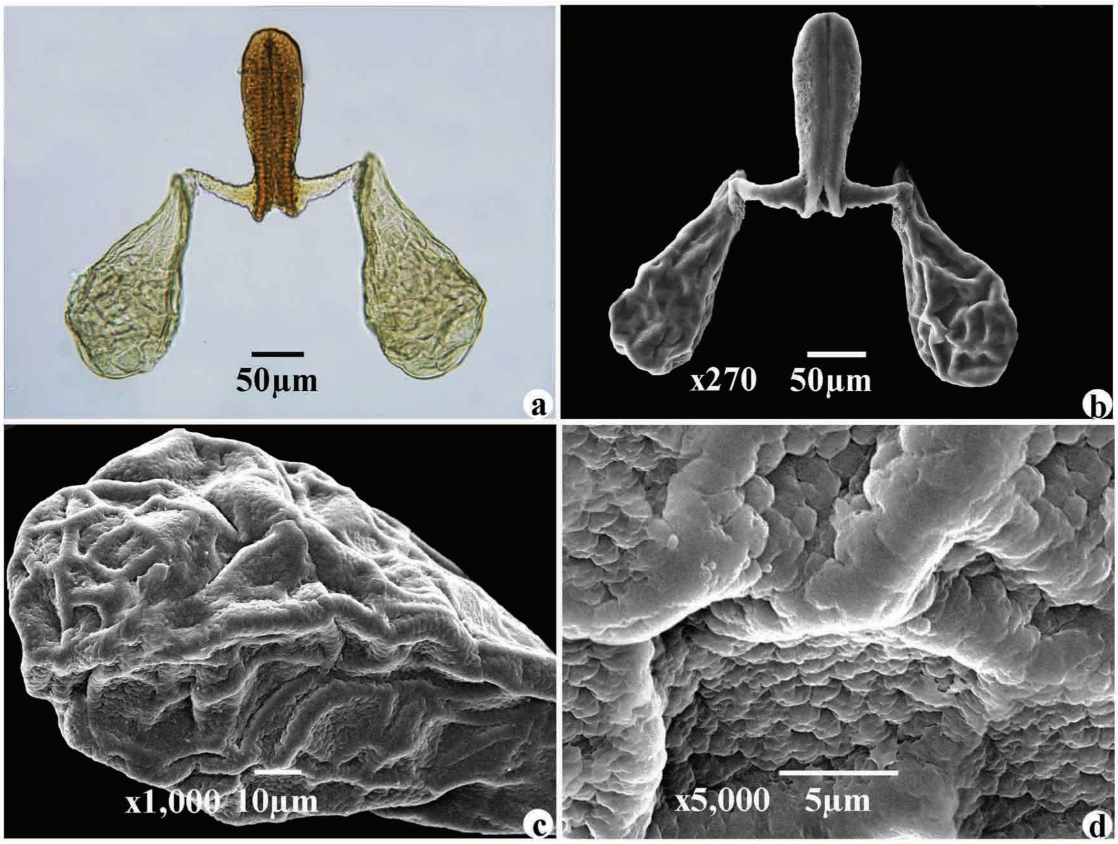

FIGURE 5. *Vincetoxicum fuscatum* subsp. *boissieri* (*Güven 35*). **a.** Pollinarium (LM); **b.** Pollinarium (SEM); **c.** Pollen cells; **d.** Ornamentation.

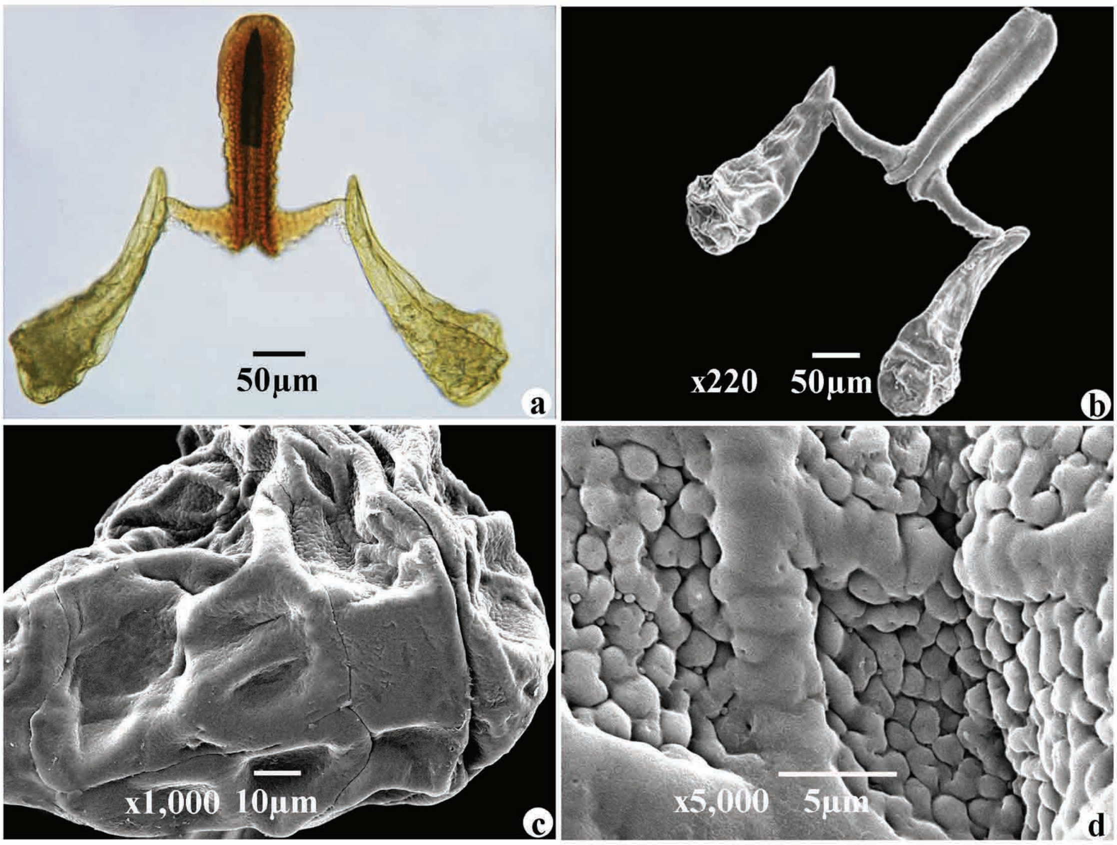

FIGURE 6. *Vincetoxicum fuscatum* subsp. *fuscatum* (*Güven 106*). **a.** Pollinarium (LM); **b.** Pollinarium (SEM); **c.** Pollen cells; **d.** Ornamentation.

***V. fuscatum* subsp. *fuscatum*:**
Pollinium clavate, 260.2 (±6.83) × 98.5 (±4.80) µm; pollen cells 33.6 (±2.55) × 22.4 (±2.01) µm; inaperturate; ornamentation tectate-perforate, gemmate. Corpusculum oblong, 227.3 (±5.28) × 70.1 (±4.00) µm. Caudicle attached to the corpusculum sub-apically and to the pollinium sub-basally, 97.4 (±1.43) × 14.6 (±0.82) µm (Fig. 6).

***V. hirundinaria* Medikus (1790: 404) subsp. *hirundinaria*:**
Pollinium obovate, 241.1 (±6.33) × 89.7 (±5.23) µm; pollen cells 35.3 (±2.62) × 23.9 (±2.20) µm; inaperturate; ornamentation tectate-perforate, rugulate. Corpusculum oblong, 210.6 (±6.75) × 89.1 (±5.37) µm. Caudicle attached to the corpusculum sub-apically and to the pollinium sub-basally, 96.1 (±5.01) × 21.2 (±1.52) µm (Fig. 7).

***V. parviflorum*:**
Pollinium elliptical, 159.1 (±7.05) × 102.4 (±6.34) µm; pollen cells 35.2 (±3.27) × 25.5 (±2.64) µm; inaperturate; ornamentation tectate-perforate, gemmate. Corpusculum oblong, 110.7 (±5.46) × 58.1 (±3.05) µm. Caudicle attached to the corpusculum sub-apically and to the pollinium basally to sub-basally, 101.3 (±6.39) × 13.8 (±1.78) µm (Fig. 8).

***V. scandens* Sommier & Levier (1892: 158):**
Pollinium obovate, 223.1 (±5.87) × 97.4 (±5.94) µm; pollen cells 34.6 (±2.31) × 24.6 (±2.44) µm, inaperturate; ornamentation tectate-perforate, gemmate. Corpusculum oblong, 189.4 (±6.97) × 67.5 (±4.52) µm. Caudicle attached to the corpusculum sub-apically and to the pollinium sub-basally, 81.8 (±4.55) × 17.3 (±0.84) µm (Fig. 9).

***V. speciosum* Boissier & Spruner (1844: 39):**
Pollinium obovate, 341.6 (±11.42) × 103.8 (±4.18) µm; pollen cells 37.4 (±2.67) × 28.2 (±2.79) µm, inaperturate; ornamentation tectate-perforate, gemmate. Corpusculum oblong, 235.5 (±8.49) × 75.9 (±3.54) µm. Caudicle attached to the corpusculum sub-apically and to the pollinium basally to sub-basally, 79.9 (±3.38) × 20.4 (±1.16) µm (Fig. 10).

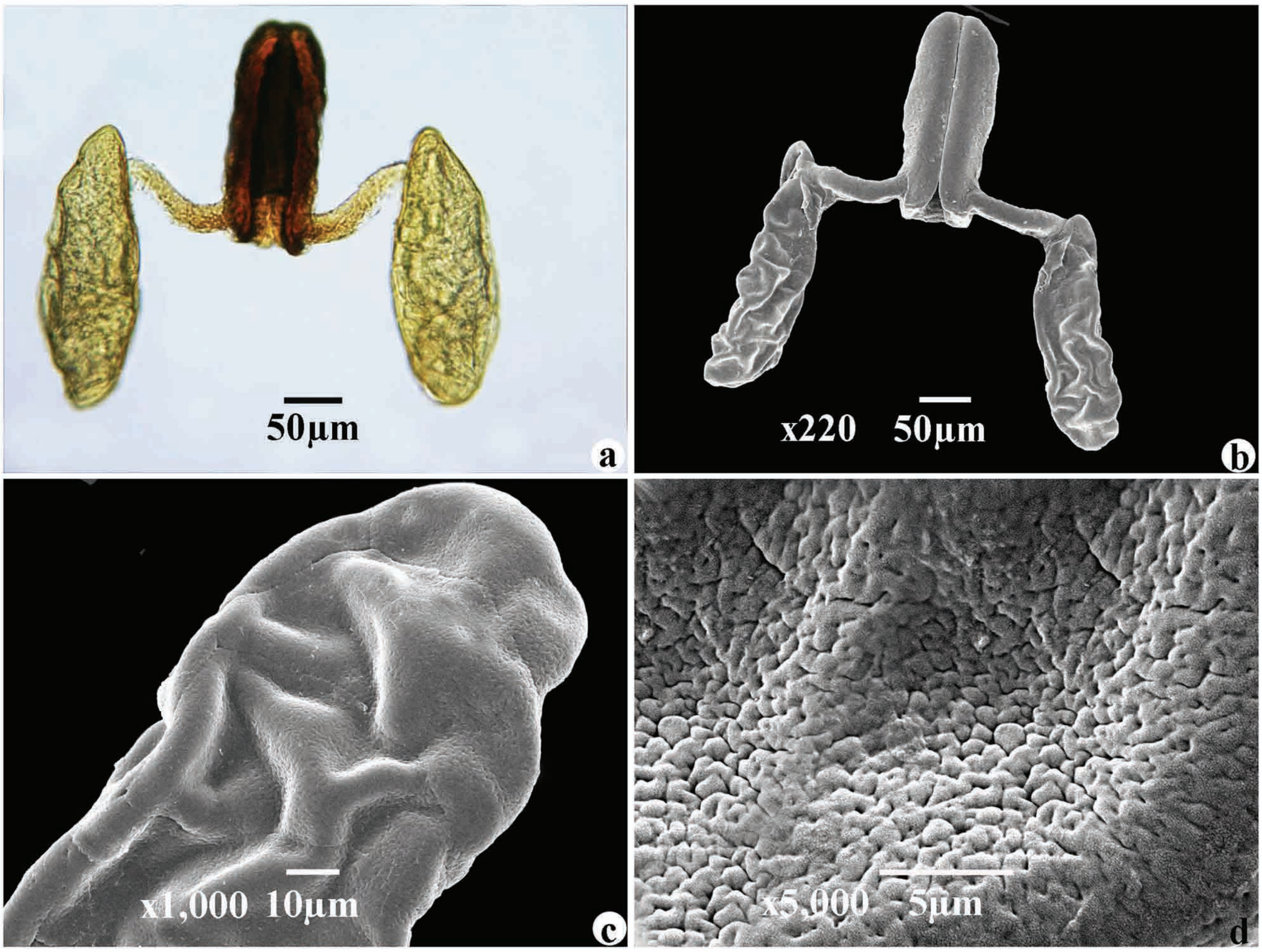

FIGURE 7. *Vincetoxicum hirundinaria* subsp. *hirundinaria* (*Güven 28*). **a.** Pollinarium (LM); **b.** Pollinarium (SEM); **c.** Pollen cells; **d.** Ornamentation.

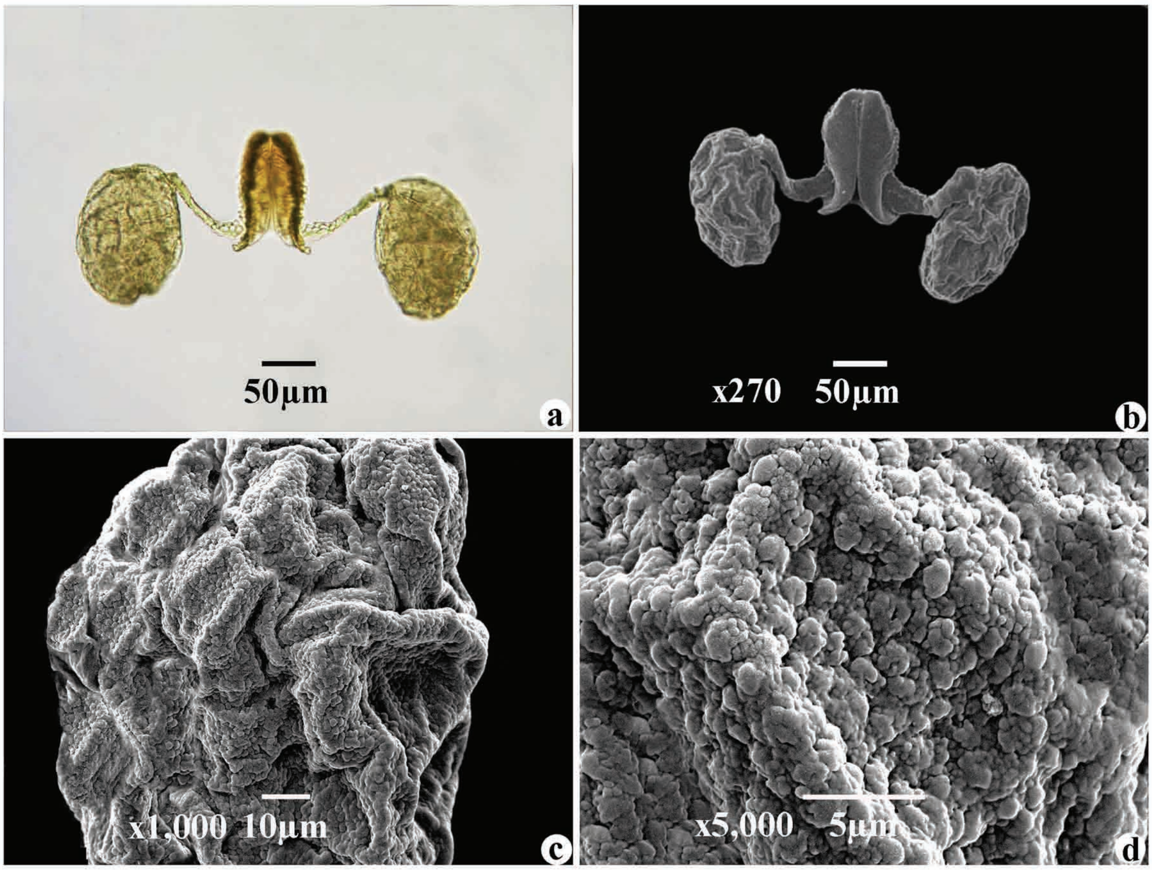

FIGURE 8. *Vincetoxicum parviflorum* (*Güven 80*). **a.** Pollinarium (LM); **b.** Pollinarium (SEM); **c.** Pollen cells; **d.** Ornamentation.

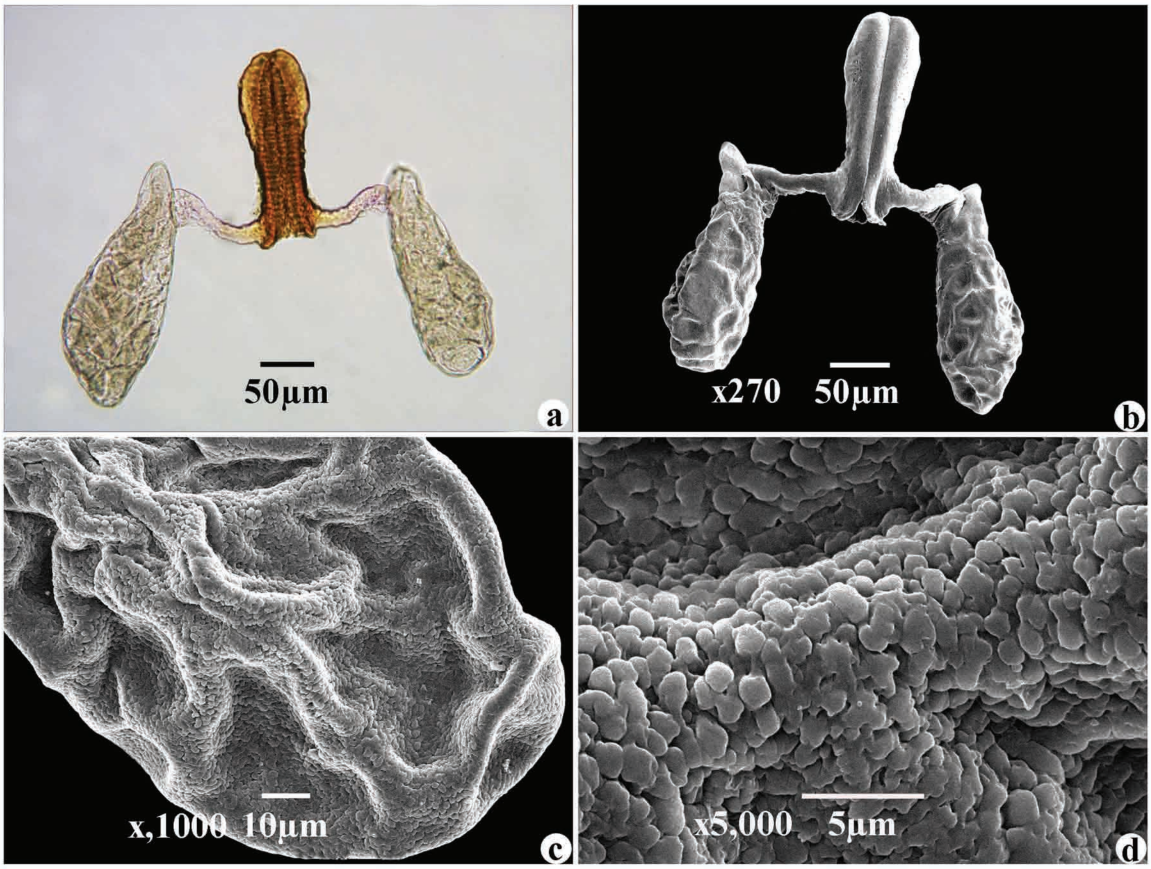

FIGURE 9. *Vincetoxicum scandens* (*Güven 03*). **a.** Pollinarium (LM); **b.** Pollinarium (SEM); **c.** Pollen cells; **d.** Ornamentation.

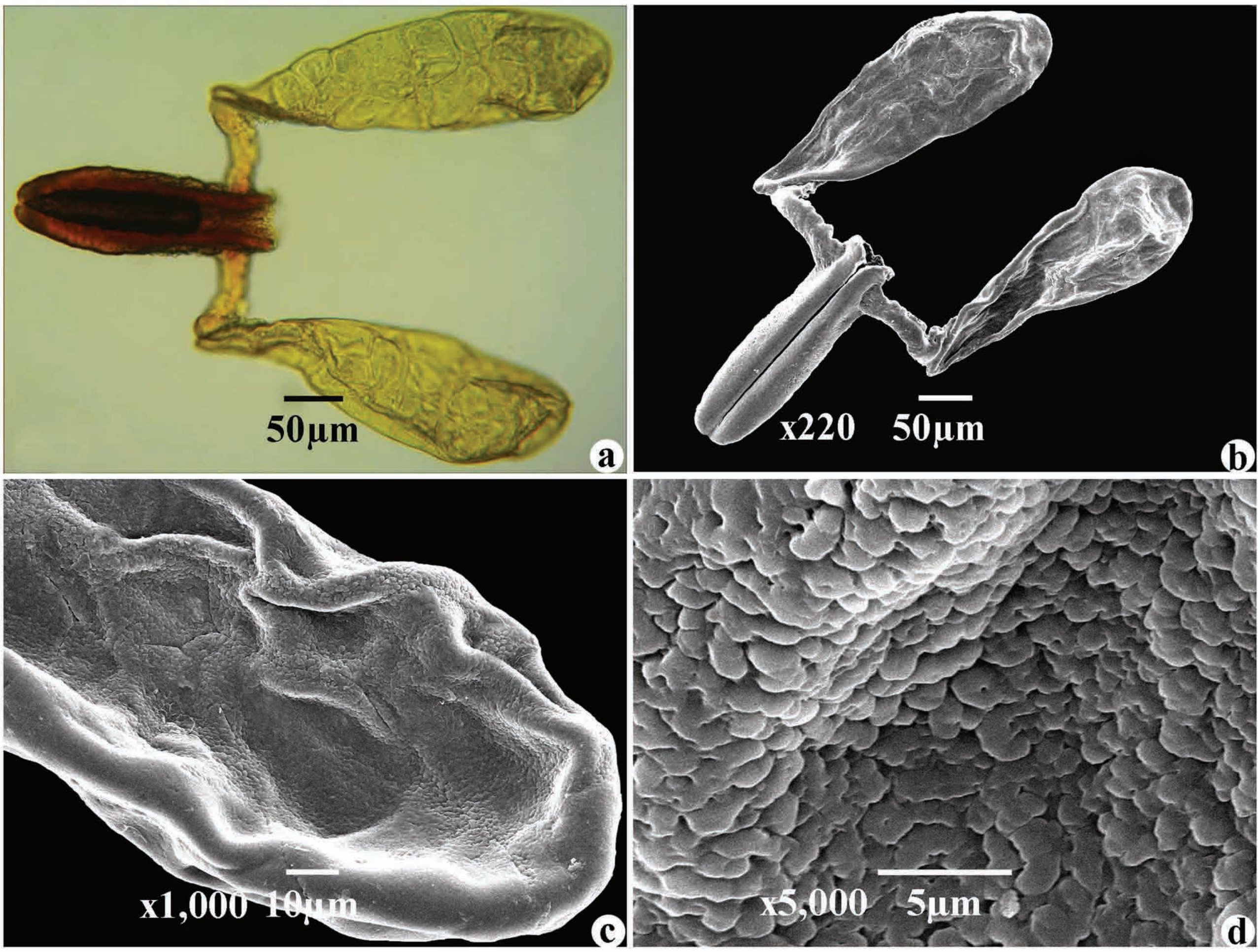

FIGURE 10. *Vincetoxicum speciosum* (*Güven 131*). **a.** Pollinarium (LM); **b.** Pollinarium (SEM); **c.** Pollen cells; **d.** Ornamentation.

V. tmoleum **Boissier (1844: 38):**
Pollinium ovate, 259.1 (±7.28) × 149.8 (±5.02) µm; pollen cells 40.5 (±2.76) × 27.8 (±3.07) µm, inaperturate; ornamentation tectate-perforate, rugulate. Corpusculum ovate, 223.7 (±6.73) × 108.6 (±5.21) µm. Caudicle attached to the corpusculum sub-apically and to the pollinium sub-basally, 113.4 (±4.63) × 19.4 (±1.30) µm (Fig. 11).

Numerical analysis
The dendrogram resulting from the cluster analysis (based on 11 palynological characters of 20 samples representing ten taxa) is given in Fig. 12. As seen in the dendrogram, all investigated taxa fall into two major clusters at 98.75% dissimilarity level. The first cluster, labeled "a", comprises nine samples belonging to *V. canescens* subsp. *canescens*, *V. canescens* subsp. *pedunculata*, *V. parviflorum*, and *V. tmoleum*. The second cluster, labeled "b", includes the remaining eleven samples (representing six taxa) of Turkish *Vincetoxicum*. The cophenetic correlation coefficient of the dendogram is 0.86. Cluster "a" is characterized by ovate or elliptical pollinia with width/length ratios ranging from 0.58 to 0.65. Cluster "b" is represented by clavate or obovate pollinia with width/length ratios ranging from 0.30 to 0.47.

Cluster "a" is divided into two subclusters, linked to each other at a 83.75% dissimilarity level. The first subcluster contains seven different populations in total, representing two subspecies of *V. canescens*, as well as *V. tmoleum*. *V. canescens* subsp. *canescens* and *V. canescens* subsp. *pedunculata* were noted as phenetically similar taxa by Browicz (1978). These two taxa, previously distinguished from one another based on peduncle length, are also similar in palynological traits, but can be distinguished by pollinium length (see key below). *V. canescens* and *V. tmoleum* share similar yellow or greenish flowers (Browicz 1978), but can be distinguished by stems decumbent and indumentum grey-tomentose in the former (Browicz 1978). Our results demonstrate that the two can also be distinguished by palynological characters, including the length of the corpusculum.

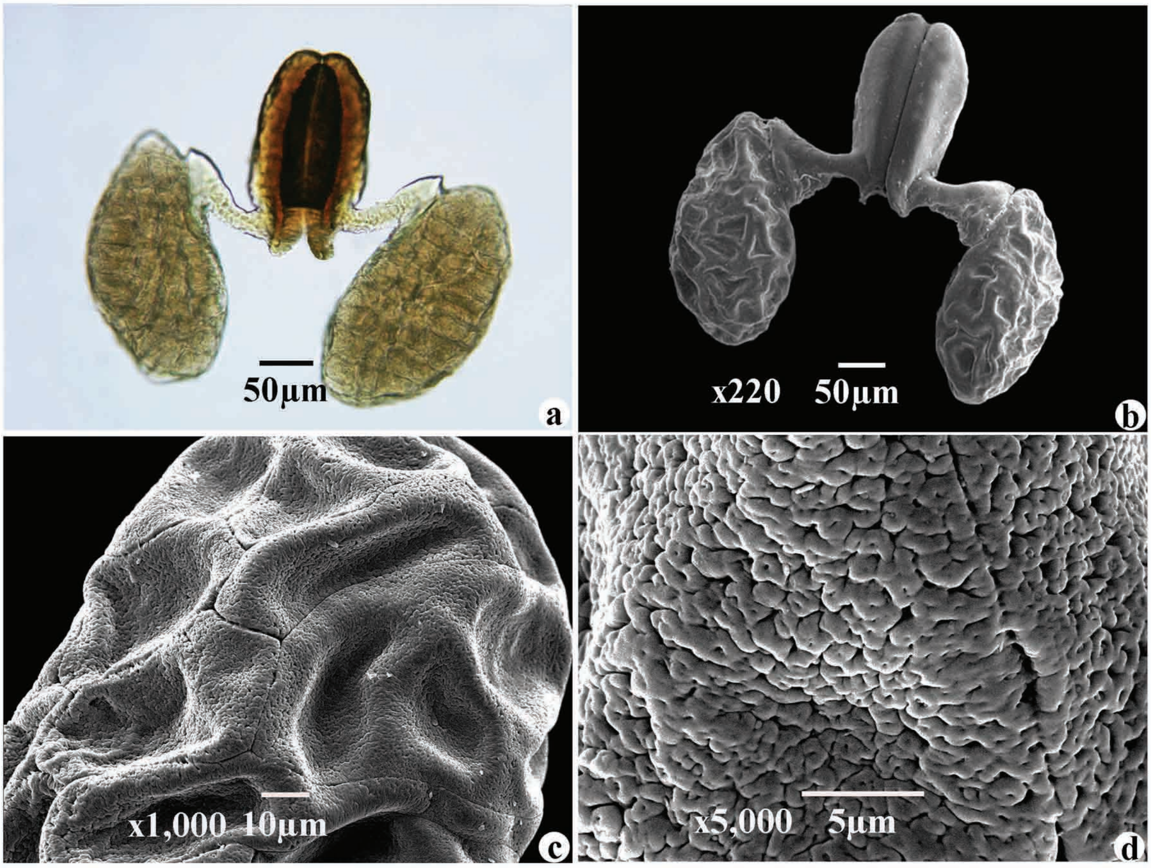

FIGURE 11. *Vincetoxicum tmoleum* (*Güven 52*). **a.** Pollinarium (LM); **b.** Pollinarium (SEM); **c.** Pollen cells; **d.** Ornamentation.

Vincetoxicum parviflorum, a member of the second subcluster of cluster "a", is easily distinguished from the rest of the three taxa in the cluster by the pollinaria smaller, the pollinia elliptical, the corpuscula oblong, and the pollen gemmate. Browicz (1978) suggested that *V. parviflorum* could be a variety of *V. fuscatum* with small-flowers, but our palynological results are not in accordance with this view, supporting instead Boissier (1875), who considered the taxa distinct at the species level. In fact, Boissier (1875) noted that these two taxa differ from each other in a number of morphological characters related to the stem and flower (Boissier 1875). For example, *V. fuscatum* is characterized by a cup-shaped, 5-parted corona, the scales of which exhibit intermediate short teeth and are nearly equal with the gynostegium length. In contrast, in the smaller flowers of *V. parviflorum*, the corona is deeply 5-parted and the corona scales are triangular-ovate and quite shorter than gynostegium. Additionally, while *V. parviflorum* exhibits densely branched stems, *V. fuscatum* exhibits simple or few-branched stems (Boissier 1875).

The second major cluster is also divided into two subclusters. Subcluster "d" consists of four populations belonging to *V. fuscatum* subsp. *boissieri* and *V. scandens,* while subcluster "c" includes *V. funebre, V. fuscatum* subsp. *fuscatum*, *V. hirundunaria* subsp. *hirundinaria*, and *V. speciosum.* Browicz (1978) recognized two phenetically similar subspecies of *V. fuscatum*—subsp. *boissieri* and subsp. *fuscatum*—distinguished only by corolla indumentum. In contrast to Browicz (1978), these two subspecies were recognized as separate species by Pobedimova (1952) (as *Antitoxicum boissieri* (Kusnezov) Pobedimova (1952: 680) [=*V. fuscatum* subsp. *boissieri*] and *Antitoxicum minus* (C.Koch) Pobedimova (1952: 687) [=*V. fuscatum* subsp. *fuscatum*]). Although the general appearance and corona structure of *V. fuscatum* subsp. *boissieri* and *V. fuscatum* subsp. *fuscatum* are very similar (Pobedimova 1952), Pobedimova (1952) suggested that these two taxa be classified under different series based on the pubescence of the inner surface of corolla. As seen in the dendrogram resulting from our cluster analysis, the two subspecies emerged in different subclusters based on palynological features, including the size of pollinia, corpuscula, and caudicles. Thus, our results support Pobedimova's (1952) view, but further morphological and molecular studies are required to better understand the evolutionary relationship of these phenetically similar taxa.

In subcluster "c", samples of *V. speciosum* differed from the other three taxa on the basis of the long pollinia (341.6 ±11.42 µm) and the low pollinium width/length ratio (0.30). Interestingly, the phenetically rather different species *V. funebre* (corollas dark purple and stems erect, to 70 cm) and *V. hirundinaria* subsp. *hirundinaria* (corollas white or pale yellow and stems twining, ca. 120 cm) (Browicz 1978) emerged in the same subcluster. This is likely the result of similarities in the size, shape, and surface ornamentation of their pollinia.

An ordination derived from PCA based on 11 palynological traits is given in Fig. 13. The eigenvalues of the first, second, and third component in percentages are 66.17%, 19.97%, 6.58%, respectively (Table 3). The first three components explain 92.72% of the total variation among the investigated taxa, suggesting that the shape of the pollinium and corpusculum, the size and surface ornamentation of pollen cells, and the size of the corpusculum and caudicle are valuable palynological characters for grouping the investigated taxa.

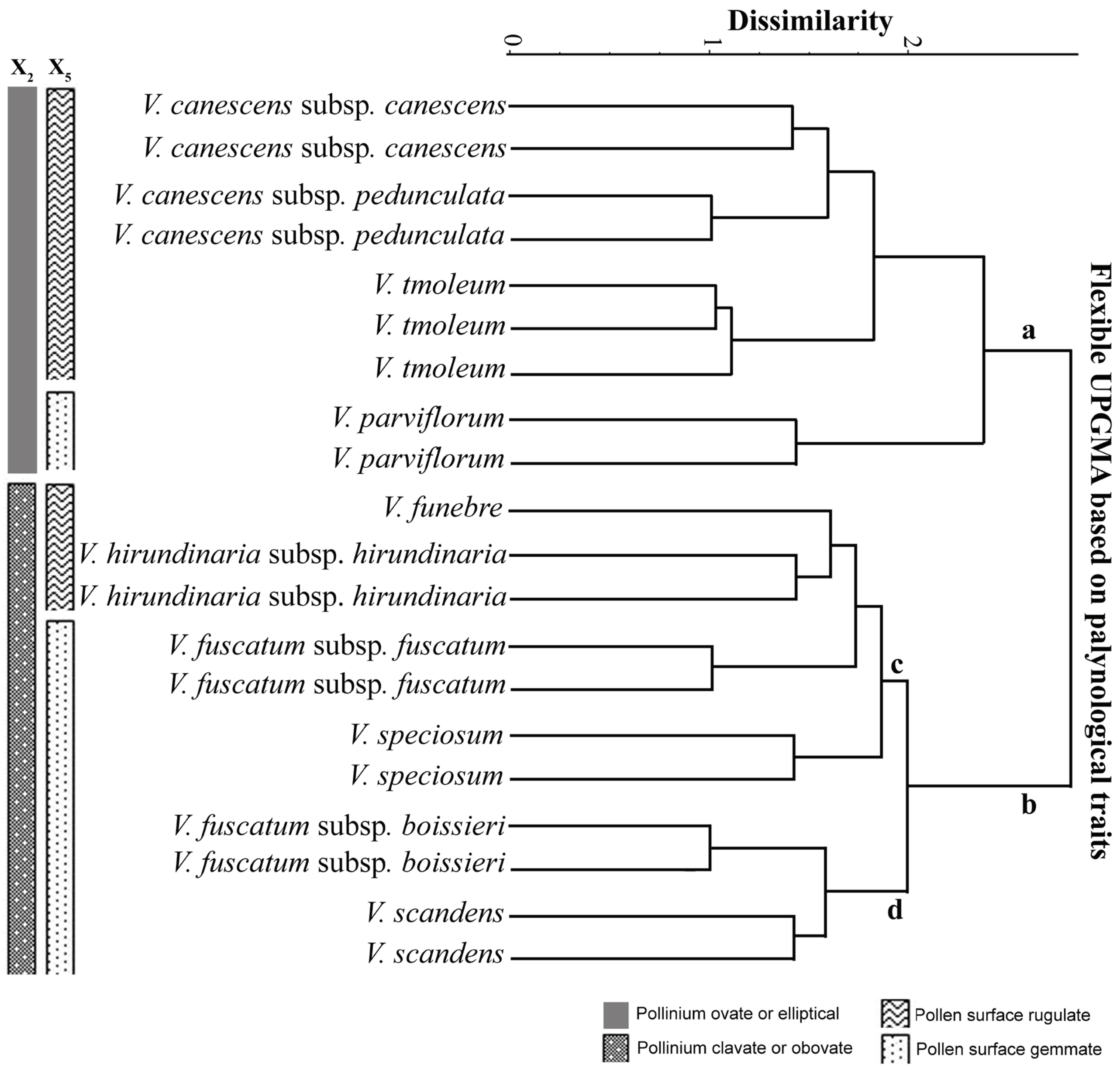

FIGURE 12. Dendrogram resulting from cluster analysis-UPGMA (X_2: Pollinium shape, X_5: Pollen ornamentation.

TABLE 3. Percentage of variance accounted for by the first three components.

	PC1	PC2	PC3
Percentage of variance explained	66.17	19.97	6.58
Cumulative of variance explained	66.17	86.14	92.72
Square roots of eigenvalues	2.697863	1.482095	0.850869

Key to Turkish *Vincetoxicum* based on pollinarium morphology

1.	Pollinium ovate or elliptical	2.
-	Pollinium clavate or obovate	5.
2.	Pollinium elliptical, corpusculum oblong, pollen surface gemmate	*V. parviflorum*
-	Pollinium ovate, corpusculum ovate, pollen surface rugulate	3.
3.	Corpusculum length 210–240 µm	*V. tmoleum*
-	Corpusculum length 250–310 µm	4.
4.	Pollinium length 270–295 µm	*V. canescens* subsp. *canescens*
-	Pollinium length 250–260 µm	*V. canescens* subsp. *pedunculata*
5.	Pollen surface rugulate	6.
-	Pollen surface gemmate	7.
6.	Pollinium length 250–270 µm and caudicle length 110–116 µm	*V. funebre*
-	Pollinium length 230–250 µm and caudicle length 90–105 µm	*V. hirundinaria* subsp. *hirundinaria*
7.	Pollinium clavate	8.
-	Pollinium obovate	9.
8.	Pollinium length 220–240 µm and corpusculum length 190–206 µm	*V. fuscatum* subsp. *boissieri*
-	Pollinium length 250–275 µm and corpusculum length 220–240 µm	*V. fuscatum* subsp. *fuscatum*
9.	Pollinium length 210–240 µm and corpusculum length 175–200 µm	*V. scandens*
-	Pollinium length 315–360 µm and corpusculum length 220–250 µm	*V. speciosum*

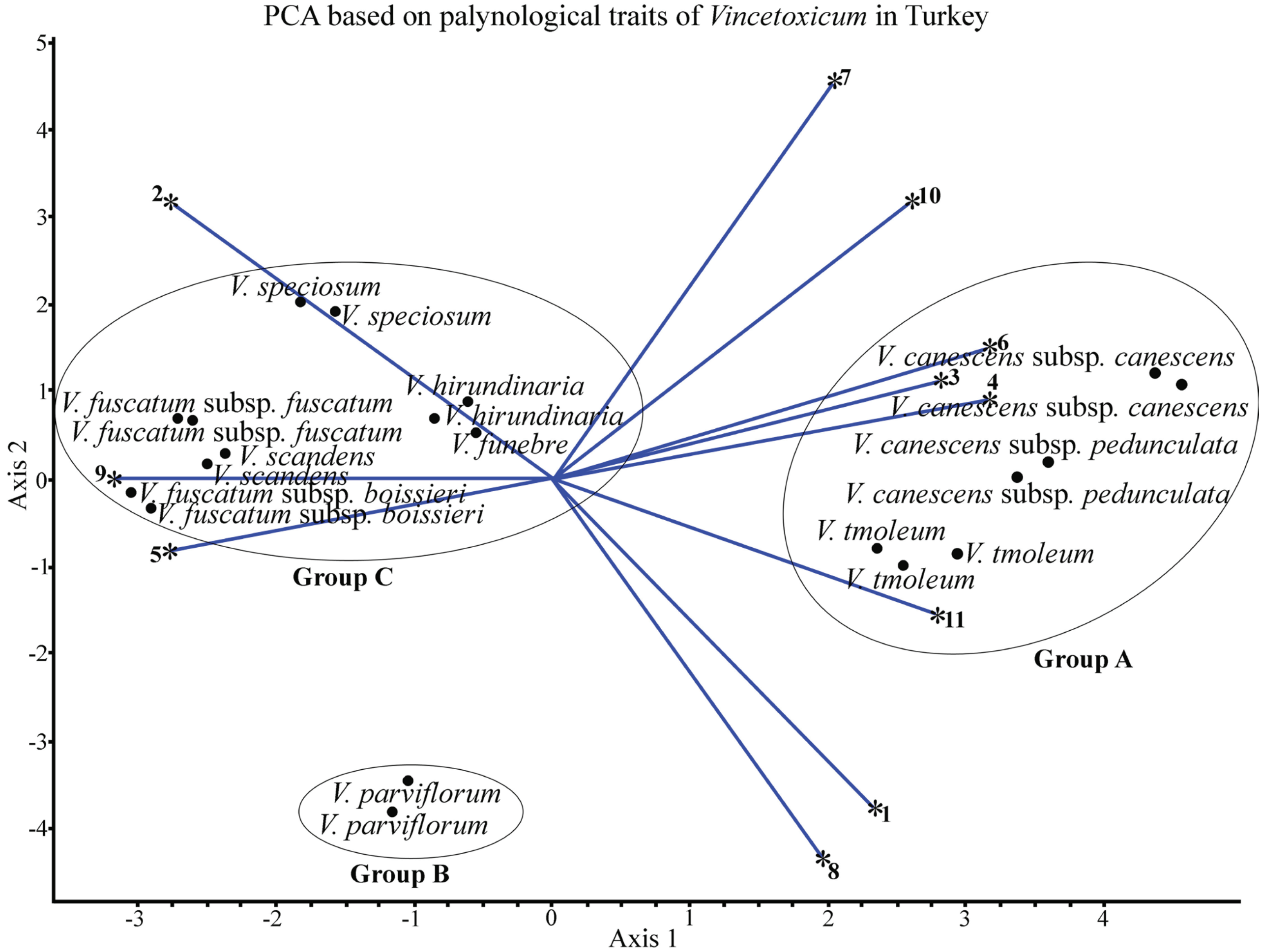

FIGURE 13. Ordination resulting from Principal Components Analysis (PCA) of 20 samples belonging to ten taxa of Turkish *Vincetoxicum*, based on 11 palynological traits.

Discussion

The Asclepiadoideae sensu Endress & Bruyns (2000) were formerly treated at the rank of family (Asclepiadaceae), however, morphological and molecular evidence suggested that the group was evolutionarily derived from the Apocynaceae, and thus it was sunk into the latter, as one of five subfamilies (Rauvolfioideae, Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae; Endress & Bruyns 2000, APG III 2009). Pollen morphology exhibits great variation among the subfamilies, representing the extreme of an evolutionary trend in the Apocynaceae (Endress & Bruyns, 2000).

In Rauvolfioideae and Apocynoideae, the most basal subfamilies, pollen cells occur as colporate or porate single pollen cells, or 3–6-porate tetrads (Fishbein 2001). However, in the more advanced Periplocoideae, pollen cells are generally arranged in sticky masses of multiporate tetrads, although in some genera, porate tetrads in each anther compose four free pollinia lacking an outer wall (Fishbein 2001). Both Secamonoideae and Asclepiadoideae, the most advanced subfamilies of Apocynaceae, are characterized by four and two pollinia per anther, respectively (Verhoeven & Venter 2001, Wyatt & Lipow 2007). In Secamonoideae, the pollinia composed of inaperturate calymmate tetrads are not surrounded by an outer wall. In contrast, the pollinia of Asclepiadoideae (except Fockea Endlicher [1839: 17]) consist of single inaperturate pollen cells and are surrounded by an outer wall (Verhoeven & Venter 2001, Wyatt & Lipow 2007). Our findings related to the pollinium structure in *Vincetoxicum* are in accordance with the studies of Verhoeven & Venter (2001) and Wyatt & Lipow (2007).

In higher plants, exine ornamentation of the pollen provides useful information for plant systematics (Hesse 1986). The surface ornamentation of the pollinia was also shown as an important trait in some members of the Asclepiadoideae (Vinckier & Smets 2002, Mo *et al.* 2010). According to Mo *et al.* (2010), smooth, sinuous, granulate and sulcate ornamentation were observed in a number of genera of the Asclepiadoideae. Vinckier & Smets (2002) reported a small amount of variation in the ornamentation types (psilate or pilate) among the Asclepiadoideae, including *V. nigrum* Moench (1802: 313). Vinckier & Smets (2002) also concluded that the orbicule typology was correlated with evolutionary trends in Apocynaceae. While orbicules were found in the majority of species belonging to the basal groups of Apocynaceae (Rauvolfioideae, Apocynoideae, and Periplocoideae), they are completely absent in Secamonoideae and the most advanced Asclepiadoideae (Vinckier & Smets 2002). Consistent with the findings of Vinckier & Smets (2002), we also did not observe any orbicule on the surfaces of pollen cells. The present study also showed that, the ornamentation type of pollen cells (rugulate and gemmate) is quite consistent at both intraspecific and interspecific levels within the studied taxa.

Recent studies on the Asclepiadoideae demonstrated that pollinium shape varies greatly at the intergeneric level (Verhoeven & Venter 2001, Mo *et al.* 2010, Sinha & Mondal 2011). Previous studies in *Vincetoxicum* reported pollinia that are either elliptical (e.g., *V. arnottianum* Wight [1850: plate 1614], Shah & Ahmad 2014) or ovate (e.g., *V. stocksii* Ali & Khatoon [1982: 65], Yaseen & Perveen 2014). In the present study, four different pollinium shapes were observed (ovate in *V. tmoleum* and both subspecies of *V. canescens*; elliptical in *V. parviflorum*; clavate in two subspecies of *V. fuscatum*; obovate in the rest of the taxa).

Measurements related to pollinium width and length have been used as a diagnostic character for systematic studies in many genera of the Apocynaceae (Wanntorp 2007, Sinha & Mondal 2011, Sreenath *et al.* 2012, Gaykar *et al.* 2012). However, comparative studies on pollinarium morphology are very limited in *Vincetoxicum*. Our results on the pollinium dimension in Turkish *Vincetoxicum* are consistent with previous studies of congeners elsewhere (Shah & Ahmad 2014, Yaseen & Perveen 2014). The ratio between width and length of pollinium was reported as 0.59 for *V. arnottianum* by Shah & Ahmad (2014). According to our findings (Appendix 1), while the highest ratio was exhibited by *V. canescens* subsp. *canescens* (0.65) and *V. parviflorum* (0.65), the lowest was observed in *V. speciosum* (0.30). PCA analysis showed that the ratio between width and length of the pollinium (X_1) was among the valuable features in grouping the investigated taxa.

Endress & Bruyns (2000) reported that the position of translators plays a significant role in the classification of the Asclepiadoideae. According to Swarupanandan *et al.* (1996), the caudicle is attached to the corpusculum either apically or basally in Asclepiadoideae. However, in the examined Turkish *Vincetoxicum*, we found the caudicle attached sub-apically to the corpusculum. It also has been reported that the shape of the corpusculum may exhibit valuable intergeneric variation in the Apocynaceae (Wanntorp 2007, Sreenath *et al.* 2012, Gaykar *et al.* 2012, Shah & Ahmad 2014), although only limited data was available for *Vincetoxicum*. While we did not address intergeneric variability, our results demonstrate that this trait is highly consistent at both interspecific and intraspecific levels among Turkish *Vincetoxicum*.

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References

Albers, F. & Meve, U. (2001) A karyological survey of Asclepiadoideae, Periplocoideae, and Secamonoideae, and evolutionary considerations within Apocynaceae s.l. *Annals of the Missouri Botanical Garden* 88 (4): 624–656. http://dx.doi.org/10.2307/3298637

Ali, S.I. & Khatoon, S. (1982) Genus *Vincetoxicum* von Wolf (Asclepiadaceae) in Pakistan. *Pakistan Journal of Botany* 14: 61–68.

APG III (2009) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* 161: 105–121. http://dx.doi.org/10.1111/j.1095-8339.2009.00996.x

Boissier, P.E. (1844) *Diagnoses plantarum Orientalium novarum*, vol. 4. Apud B. Hermann, Lipsiae, 86 pp.

Boissier, P.E. (1849) *Diagnoses plantarum Orientalium novarum*, vol. 11. Typis Marci Ducloux et Cons. Via École-de-Médecine, 80, Paris, 136 pp.

Boissier, P.E. (1875) *Vincetoxicum* Maench. *In:* Boissier, E.P. (Ed.) *Flora Orientalis*, vol. 4. H. Georg., Genevae & Basileae, pp. 51–56.

Browicz, K. (1975) *Vincetoxicum canescens* (Willd.) Decne. and *V. tmoleum* Boiss. Systematic problems and geographical distribution. *Fragmenta Floristica et Geobotanica* 21: 261–271.

Browicz, K. (1978) *Vincetoxicum* Wolf. *In:* Davis, P.H. (Ed.) *Flora of Turkey and East Aegean Island*, vol. 6. Edinburgh University Press, Edinburg, pp. 167–173.

Brown, R. (1810) *Prodromus florae Novae Hollandiae et Insulae Van-Diemen, exhibens characteres plantarum*, vol 1. Typis R. Taylor et socii, London, 590 pp.

Decaisne, J. (1844) *Vincetoxicum* Moench. *In*: Candolle, A.P. de (Ed.) *Prodromus systematis naturalis regni vegetabilis*, vol. 8. Treuttel & Würtz, Paris, pp. 523–526.

Endlicher, S.L. (1839) *Novarum stirpium decades*. Apud Fridericum Beck, Universitatis Bibliopolam, 90 pp.

Endress, M.E. & Bruyns, P.V. (2000) A revised classification of the Apocynaceae s. l. *Botanical Review* 66: 1–56. http://dx.doi.org/10.1007/BF02857781

Endress, M.E., Liede-Schumann, S. & Meve, U. (2014) An updated classification for Apocynaceae. *Phytotaxa* 159 (3): 175–194. http://dx.doi.org/10.11646/phytotaxa.159.3.2

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

Gaykar, B.M., Thombre, S. & Suryawanshi, S. (2012) Pollinarium diversity in Asclepiadaceae. *An International Journal of Environment and Biodiversity* 3 (1): 78–83.

Goyder, D.J., Nicholas, A. & Liede-Schumann, S. (2007) Phylogenetic relationships in subtribe Asclepiadinae (Apocynaceae: Asclepiadoideae). *Annals of the Missouri Botanical Garden* 94: 423–434. http://dx.doi.org/10.3417/0026-6493(2007)94[423:PRISAA]2.0.CO;2

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.

Hesse, M. (1986) Orbicules and the ektexine are homologous sporopollenin concretions in Spermatophyla. *Plant Systematics and Evolution* 153: 37–48. http://dx.doi.org/10.1007/BF00989416

Ilçim, 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.

Liede, S. (1996) *Cynanchum–Rhodostegiella–Vincetoxicum–Tylophora* (Asclepiadaceae): new considerations on an old problem. *Taxon* 45: 193–211. http://dx.doi.org/10.2307/1224660

Liede, S. (2001) Molecular considerations on the subtribe Astephaninae Endl. ex Meisn. (Apocynaceae-Asclepiadoideae). *Annals of the Missouri Botanical Garden* 88: 657–668. http://dx.doi.org/10.2307/3298638

Liede, S. & Kunze, H. (2002) *Cynanchum* and the *Cynanchinae* (Apocynaceae-Asclepiadoideae)—a molecular, anatomical and latex triterpenoid study. *Organisms Diversity and Evolution* 2: 239–269.
http://dx.doi.org/10.1078/1439-6092-00045

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.

Linnaeus, C. (1753) *Species plantarum, exhibentes plantas rite cognitas, ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas*. Impensis Laurentii Salvii, Holmiae, Stockholm, 1200 pp.

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

Medikus, F.K. (1790) Ueber den gynandrischen situs der staubfäden und pistille einiger pflanzen. *Historia et Commentationes Academiae Electoralis Scientiarum et Elegantiorum Litterarum Theodoro-Palatinae* 6: 374–414.

Mo, L.J., Zhuang, X.Y., Huang, J.X. & Li, B.T. (2010) Pollinarium morphology of seven Marsdenieae genera (Asclepiadaceae). *Journal of Wuhan Botanical Research* 28 (8): 529–535.

Moench, C. (1802) *Supplementum ad methodum plantas : a staminum situ describendi*. Officina Nova Libraria Academiae, Marburgi Cattorum.

Newton, L.E. (1984) Terminology of structures associated with pollinia of the Asclepiadaceae. *Taxon* 33 (4): 619–621.
http://dx.doi.org/10.2307/1220779

Özay, S.G. (2013) *Bazı Vincetoxicum* N.M. Wolf *(Asclepiadaceae) türleri üzerinde farmakognozik araştırmalar.* PhD Thesis, Institute of Health Sciences, University of Mersin, 260 pp.

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.

Podani, J. (1993) *Multivariate data analysis in ecology and systematic, a methodological guide to Syn-Tax 5.0.* Package SPB Academic Publishing, Netherlands.

Punt, W., Hoen, P.P., Blackmore, S., Nilsson, S. & Le Thomas, A. (2007) Glossary of pollen and spore terminology. *Review of Palaeobotany and Palynology* 143: 1–81.
http://dx.doi.org/10.1016/j.revpalbo.2006.06.008

Reichenbach, H.G. (1854) *Icones florae Germanicae et Helveticae*. Sumptibus Ambrosii Abel., Lipsiae, 113 pp.

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

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

Sneath, P.H.A. & Sokal, R.R. (1973) *Numerical taxonomy: The principles and practice of numerical classification.* WH Freeman and Company, San Francisco.

Sommier, S. & Levier, E. (1892) Decas plantarum novarum Caucasi. *In*: Tischutkin, N., Winkler, C., Herder, F., Hundrieser, R., Sommier, S., Levier, E. & Batalin, A. (Eds.) *Acta Horti Petropolitani*, vol. 12. Imperatorskii S.-Peterburgskii Botanicheskii Sad., Trudy, pp. 151–159.

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.

Stærk, D., Nezhad, K.B., Asili, J., Emami, S.A., Ahi, A., Sairafianpour, M. & Jaroszewski, J.W. (2005) Phenanthroindolizidine alkaloids from *Vincetoxicum pumilum*. *Biochemical Systematics and Ecology* 33: 957–960.
http://dx.doi.org/10.1016/j.bse.2005.01.004

Swarupanandan, K., Mangaly, J.K., Sonny, T.K., Kish-orekumar, K. & Basha, S.C. (1996) The subfamilial and tribal classification of the family Asclepiadaceae. *Botanical Journal of the Linnean Society* 120: 327–369.
http://dx.doi.org/10.1111/j.1095-8339.1996.tb00486.x

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

Verhoeven, R.L., Liede, S. & Endress, M.E. (2003) The tribal position of *Fockea* and *Cibirhiza* (Apocynaceae: Asclepiadoideae): evidence from pollinium structure and cpDNA sequence data. *Grana* 42: 70–81.
http://dx.doi.org/10.1080/00173130310012549

Vinckier, S. & Smets, E. (2002) Morphological and ultrastructural diversity of orbicules in relation to evolutionary tendencies in

Apocynaceae s.l. *Annals of Botany* 90: 647–662.
http://dx.doi.org/10.1093/aob/mcf243

Wanntorp, L. (2007) Pollinaria of *Hoya* (Marsdenieae, Apocynaceae) —shedding light on molecular phylogenetics. *Taxon* 56 (2): 465–478.

Wight, R. (1850) *Icones plantarum Indiae Orientalis :or figures of Indian plants*, vol. 4: t. 1614. J.B. Pharoah, Madras.

Wolf, N.M. (1776) *Genera plantarum: vocabulis characteristicis definita*. Müller, Dantisci, 178 pp.

Wyatt, R. & Lipow, S.R. (2007) A new explanation for the evolution of pollinia and loss of carpel fusion in Asclepias and the Apocynaceae s.l. *Annals of the Missouri Botanical Garden* 94: 474–484.
http://dx.doi.org/10.3417/0026-6493(2007)94[474:ANEFTE]2.0.CO;2

Yamashiro, T., Yokota, M., Denda, T., Tateishi, Y., Yokoyama, J. & Maki, M. (2002) Chromosome numbers of Japanese Asclepiadaceae. *Acta Phytotaxonomica et Geobotanica* 53 (2): 143–152.

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.I. (2006) Phytotoxicity and insecticidal activity of *Vincetoxicum stocksii. In:* Barak, S.K. (Ed.) *Plant ecology and biodiversity research.* Regency Publishers, New Delhi, pp. 193–197.

APPENDIX 1. Palynological measurements of the examined taxa of Turkish *Vincetoxicum*. See Table 2 for explanation of characters and units.

Characters	*V. canescens* subsp. *canescens*		*V. canescens* subsp. *pedunculata*		*V. funebre*	*V. fuscatum* subsp. *boissieri*		*V. fuscatum* subsp. *fuscatum*		*V. hirundinaria* subsp. *hirundinaria*		*V. parviflorum*		*V. scandens*		*V. speciosum*		*V. tmoleum*		
	Güven 37	*Güven 91*	*Güven 51*	*Güven 56*	*Güven 126*	*Güven 35*	*Güven 73*	*Güven 93*	*Güven 106*	*Güven 19*	*Güven 28*	*Güven 80*	*Güven 95*	*Güven 03*	*Güven 101*	*Güven 20*	*Güven 131*	*Güven 52*	*Güven 60*	*Güven 75*
X_1	0.65	0.63	0.61	0.61	0.39	0.47	0.47	0.38	0.38	0.37	0.38	0.65	0.64	0.44	0.43	0.30	0.31	0,58	0.58	0,58
X_2	0	0	0	0	1	1	1	1	1	1	1	0	0	1	1	1	1	0	0	0
X_3	33.8 ±2.54	34.2 ±2.37	28.7 ±2.28	29.2 ±2.34	24.7 ±2.27	22.1 ±1,83	21.9 ±1.49	22.9 ±1.92	21.9 ±2.03	23.9 ±2.37	23.9 ±2.10	24.9 ±2.46	26.1 ±2.76	24.2 ±2.70	25.1 ±2.15	27.6 ±2.69	28.9 ±2.83	26.4 ±2.95	27.5 ±2.88	29.6 ±2.61
X_4	45.8 ±2.43	44.4 ±2.87	40.7 ±3.68	41.1 ±3.33	35.6 ±3.65	32.2 ±1.78	32.2 ±1.90	33.3 ±2.47	33.9 ±2.70	34.9 ±2.77	35.8 ±2.46	34.6 ±3.25	35.8 ±3.30	34.2 ±2.48	34.9 ±2.15	37.7 ±2.72	37.1 ±2.69	41.1 ±2.89	40.1 ±2.67	40.3 ±2.82
X_5	0	0	0	0	0	1	1	1	1	0	0	1	1	1	1	1	1	0	0	0
X_6	124.5 ±2.33	120.4 ±2.47	124.9 ±1.62	125.3 ±2.94	81.5 ±4.55	60.9 ±1.33	61.9 ±1.64	69.7 ±4.39	70.4 ±3.68	88.3 ±5.84	89.7 ±4.95	58.9 ±2.94	57.3 ±304	67.2 ±4.16	67.9 ±4.98	74.9 ±3.44	77.1 ±3.39	110.1 ±3.76	103.3 ±3.87	112.5 ±2.75
X_7	302.1 ±4.35	301.9 ±4.90	263.5 ±7.61	266.3 ±5.35	221.1 ±7.28	196.3 ±4.39	195.1 ±3.15	226.1 ±5.87	228.5 ±4.47	207.8 ±6.47	213.5 ±5.94	108.3 ±5.02	113.1 ±4.92	188.2 ±8.17	190.6 ±5.55	231.7 ±9.75	239.3 ±4.83	221.4 ±6.29	223.7 ±6.76	226.1 ±6.73
X_8	0.41	0.40	0.47	0.47	0.37	0.31	0.32	0.31	0.31	0.43	0.42	0.55	0.51	0.36	0.36	0.32	0.32	0.50	0.46	0.50
X_9	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
X_{10}	24.5 ±2.61	25.2 ±1.32	22.7 ±2.06	24.2 ±2.04	18.3 ±1.74	14.7 ±0.98	14.9 ±0.80	14.5 ±0.92	14.7 ±0.72	20.9 ±1.46	21.6 ±1.55	13.9 ±2.03	13.8 ±1.57	17.3 ±0.72	17.3 ±0.96	20.9 ±1.10	19.8 ±0.94	19.5 ±1.41	19.1 ±1.39	19.6 ±1.12
X_{11}	114.9 ±3.20	114.5 ±1.36	112.3 ±5.88	113.3 ±3.22	113.7 ±2.02	87.9 ±0.83	88.8 ±1.32	96.9 ±1.39	97.8 ±1.37	94.6 ±4.66	97.5 ±5.10	100.2 ±6.52	102.3 ±6.30	82.1 ±2.97	81.4 ±5.80	77.6 ±3.27	82.1 ±1.41	111.9 ±4.19	113.3 ±5.46	115.1 ±3.81