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Comparative morphological and palynological study on poorly known *Asperula serotina* and its closest relative *A. purpurea* subsp. *apiculata*

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Morphological and palynological features were examined in the closely related Turkish endemic taxa *Asperula serotina* and *A. purpurea* subsp. *apiculata*. Their morphological features showed very minor difference such as internodes length and the vein thickness of leaf, however, the seed and pollen characteristics clearly appear to have taxonomic value. The clavate-reticulate seed surface suture of *A. serotina* clearly differs from that lineate-sulcate of *A. purpurea* subsp. *apiculata*. Similarly, the pollen perforations and number of spines differ in each taxon. Moreover, the two taxa are geographically isolated from each other. Ecological features are also briefly discussed. The seed and pollen micromorphological properties could be used for intra/interspecific classification of *Asperula* which is taxonomically critical.

Key words: *Asperula*, taxonomy, morphology, seed, pollen, SEM.

INTRODUCTION

The Rubiaceae (coffee family) are the fourth largest family of flowering plants after Asteraceae, Orchidaceae and Fabaceae. It contains approximately 13,200 species in 620 genera, of which 26 genera have more than 100 species (Robbrecht et al., 2007). *Asperula* L., with a total of 183 species, is one of the most important genera in the family. The total number of *Asperula* taxa is 230 with subspecies and varieties (Minareci, 2007).

Asperula includes 51 taxa in six sections in the Turkish flora, 26 of which are endemic (Ehrendorfer and Schönbeck-Temesy, 1982). *Asperula serotina* (Boiss. et Heldr.) Ehrend. and *Asperula purpurea* (L.) Ehrend. subsp. *apiculata* (Sibth. et Sm.) Ehrend. belong to sect. *Thlipthisa* (Griseb.) Ehrend., which includes three more taxa in Turkey: *Asperula brevifolia* Vent., *Asperula pseudochlorantha* Ehrend. var. *pseudochlorantha* and *A. pseudochlorantha* var. *antalyensis* (Ehrend.) Minareci et Yildiz. The taxa in sect. *Thlipthisa* are all perennials. Their flowers are shortly pedicellate to sessile, subtended by short bracts and bracteoles; the corollas are mostly 4-

merous, usually without filiform appendages.

According to Ehrendorfer and Schönbeck-Temesy (1982), *Asperula serotina* and *A. purpurea* subsp. *apiculata* are morphologically very similar and closely related. However, these authors did not describe seed and pollen features, although these types of characters have been proved to be of high taxonomic value in the Rubiaceae (Lobreau-Callen, 1978; Van Campo, 1978; Huysmans et al., 1998; Huysmans et al., 1999; Dessein et al., 2000; Dessein et al., 2002; Piesschaert et al., 2000; Khalik et al., 2008). On the other hand, as Ehrendorfer and Schönbeck-Temesy (1980) indicated, the floral morphology in *Asperula* is not taxonomically very important.

The aim of this paper is to present a detailed description of *A. serotina* and to define similarities and differences of the *A. serotina* and *A. purpurea* subsp. *apiculata* based on morphological and palynological features in order to determine their taxonomic identities accurately.

MATERIALS AND METHODS

Plant samples of the two taxa were collected from natural populations between May 2004 and July 2007. Some samples of *A. serotina* and *A. purpurea* subsp. *apiculata* were fixed in 70%

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alcohol. One herbarium specimen for each species is stored at the Celal Bayar University Herbarium. The collected data of the two taxa were:

1. *A. serotina* [Antalya, Turkey]: Akseki – Seydisehir road 6 km, limestone rocks, 1300 m, 15.07.2006, (37°05'139" N, 31°46'039" E).
2. *A. purpurea* subsp. *apiculata* [Tekirdağ, Turkey]: Şarköy, 7 km from Uçmakdere to Yeniköy, Dry places, 250 m, 14.06.2007, (40°50'175" N, 27°23'077 E).

Taxonomical identification of the plants was done according to Flora of Turkey (Ehrendorfer and Schönbeck-Temesy, 1982). Owing to their significance in the determination of *Asperula* species, diagnostics such as plant length, defoliation, leaf size, inflorescence, corolla length, color, shape and internodes were used. Seed morphology of each taxon was assessed using microphotographs obtained from SEM images according to Prentice (1979) and Stearn (1996). Thirty specimens from each taxon and 50 seeds from different individuals for each taxon were used for morphological examinations.

For palynological studies, the pollen grains were acetolysed as described by Erdtman (1960). At least 50 pollen grains representing each taxon were used to measure the pollen features such as diameter, colpus length, colpus width, mesocolpium, microperporate diameter and exine thickness. Pollen samples were analyzed with an Olympus triocular microscope with D-plan 100-1.25 160/0.17 oil immersion objective and NFK X 3.3 LD 125 lens. Pollen terminology follows (Punt et al., 1994; Moore et al., 1997). For pollen shape types (P/E), we followed Nilsson and Praglowski (1992): Spheroidal: 0.88 - 1.14. For SEM investigations, seeds and pollen grains were directly mounted on stubs using double-sided adhesive tape. The samples were coated with gold/palladium in POLARON SC 7620 ion-sputter and then observed by standard techniques using a LEO 440 scanning electron microscope.

Graphpad Prism for Windows software package and Minitab (version 15) were used for statistical analyses. Multivariate analysis of variance (MANOVA) was used to determine whether significant difference which exist between taxa by morphological and palynological data (Rosenberg, 2001).

RESULTS

Morphological characteristics

The studies on the specimens collected from Akseki in Antalya province led to the conclusion that they are identical with that described by Ehrendorfer as *A. serotina*.

A. serotina (Boiss. et Heldr.) Ehrend. in P.H. Davis (ed.), Fl. Turkey 7: 766 (1982). Syn: *Galium serotinum* Boiss. et Heldr. in P.E.Boissier, Diagn. Pl. Orient. 10: 71 (1849). Type: [Antalya, Turkey] in rupibus Tauri Pamphylici inter Akseki et Antalya, 1220 m, vii, Heldreich (holotype G!).

Description of Ehrendorfer: Stems 20 - 45 cm, erect to ascending, ± virgate, quadrangular with ridges distinctly projecting, scarcely scabrid; internodes 20 - 45 mm, shorter towards ± puberulent base. Leaves caducous, in whorls of 4 - 6, 6 - 13 × 0.6 - 1.0 mm, narrowly oblanceolate subobtuse to acute, with revolute, somewhat scabrid margins; vein thin, 1/7 - 1/10 × leaf-

breadth, not projecting below. Inflorescence ± paniculate, broadly pyramidal, many flowered, with linear bracts. Peduncles 0 - 3 mm, glabrous. Bracteoles c. 0.5 mm, lanceolate with smooth margins. Pedicels glabrous, (0 -) 0.2 - 0.4 mm, usually erect in fruit. Corolla tube part is yellowish green, outside of lamina is greenish purple, inside of lamina are purple, rotate, 2 - 2.5 mm diameter with ovate, filiform-apiculate lobes. Anthers subglobose, c. 0.2 mm. Style exerted with globose stigmas. Mericarps 0.85 - 1.25 mm, glabrous. Fl. 6 - 7. Limestone rocks, 500 - 2000 m.

Morphological differences between *A. serotina* and *A. purpurea* subsp. *apiculata* occurred only in internodes length and the vein thickness of leaf (Figure 1). A series of 15 parameters obtained for the two taxa are shown in Table 1.

Seeds of studied two taxa are very similar in size and shape. Thus, details were revealed by examining seed surface of taxa using SEM (Figure 2). Comparison of the seed morphological characters of studied taxa is shown in Table 2.

Pollen characteristics

The pollen grains of *A. serotina* and *A. purpurea* subsp. *apiculata* are spheroidal in shape, exines are tectate with microechinate-perforate ornamentations (Figure 3). Table 3 summarizes the quantitative pollen characters for the two taxa investigated and measurements on SEM images (that is, apocolpium index, density of perforations and spines, and size of perforations).

Based on the results of MANOVA test, the differences between given taxa were found to be significant ($p < 0.05$) for seed and pollen features, although, morphological differences between taxa were not to be significant ($p > 0.05$) for morphological characters (Table 4). This indicates that seed micro-morphological and palynological data can be used in statistical separation of taxa.

DISCUSSION

The Akseki Jungles on which *A. serotina* grows are an interesting and isolated area ascending from sea level to approximately 1300 - 1800 m. The area is one of the Global 200 and Terrestrial Ecoregions of the World (Olson and Dinerstein, 2002) and one of the main centers of endemism in Turkey (Davis, 1971) and a shelter ca. 163 Anatolian endemics (Duran, 2002). There are two other *Asperula* species in this region: *A. arvensis* L. and *A. setosa* Jaub. et Spach. On the contrary, *A. purpurea* subsp. *apiculata* grows in Tekirdag-Sarkoy located at a distance of about 860 km from Akseki. *A. serotina* is thus geographically isolated from its closest relative. But, *A. serotina* is not seasonally isolated from *A. purpurea* subsp.



Figure 1. 1a. Herbarium specimen of *A. serotina* (Heldreich, G 74268, holotype), 1b - e. Flowers of *A. serotina*, 2a. Herbarium specimen of *A. purpurea* subsp. *apiculata* (N et E. Ozhatay, ISTE 33757), 2b-e. Flowers of *A. purpurea* subsp. *apiculata*.

apiculata. Both studied taxa bloom from June to July and fruity in the end of August. *A. purpurea* subsp. *apiculata* grows at a lower altitude compared to *A. serotina*. Therefore, the generative development period of *A. purpurea* subsp. *apiculata* is faster and shorter

compared to *A. serotina*.

A. serotina has only been known from a few gatherings since 1982. As earlier described, the given leaf size was 10 - 18 × 0.6 - 1.2 mm and the corolla color purplish (Ehrendorfer and Schönbeck-Temesy, 1982). The present

Table 1. Comparison of *A. serotina* and *A. purpurea* subsp. *apiculata*.

	<i>A. serotina</i>	<i>A. purpurea</i> subsp. <i>apiculata</i>
Habit	Perennial	Perennial
Height of plant (cm)	20 - 45	15 - 45(- 50)
Stem properties	Numerous, erect	Numerous, erect
Internod (mm)	20 - 45	5 - 20
Leaf number of nodes	4 - 6	6 - 8(- 11)
Leaf size length x width (mm)	6 - 13 × 0.6 - 1	5-17 × 1-2
Vein thickness	1/7 - 1/10 × leaf breadth	1/5 x leaf breadth
Corolla length (mm)	2 - 2.5	1.5 – 2.5
Corolla colour	Greenish red, red	Greenish red, red
Corolla lobe number	4-5	4
Pedicel length (mm)	0 - 0.2 - 0.4	0.5 - 4
Anther shape and size (mm)	Subglobose, 0.2	Subglobose, 0.15
Habitat	Limestone rocks	Scree, hill slopes, dry places
Flowering time (month)	6 - 7	6 - 7
Altitude (m)	500 - 2000	100

study showed that the leaf size is 6 - 13 × 0.6 - 1.0 mm and corolla tube was yellowish-green, lamina was greenish-red outside and red inside (Figures 1b - e). Similarly, in the earlier description *A. purpurea* subsp. *apiculata* corolla color were given greenish-yellow (Ehrendorfer and Krendl, 1974). In this study, it was determined that tube part was yellowish-green and lamina part was greenish-red and purplish outside and inside, respectively (Figure 1b - e).

Seeds of *Asperula* species are very similar in size and shape. Although differences were observed in the morphologies of the seed from different sections, differences in general appearance of species in the same section are usually minimal (Minareci, 2007). This is also true for *Thlipthisa* section. Thus, differences and similarities based on SEM data were evaluated. Minareci (2007) worked on general appearance and surface micromorphological properties of seeds of *Asperula pseudochlorantha* var. *pseudochlorantha* and *A. pseudochlorantha* var. *antalyensis* in *Thlipthisa* section. Minareci (2007) determined similar properties in terms of seed size, general appearance and testa cells. Also, he found that the of the color of seed surface was light brown, surface suture was sinuous-reticulate, number of testa cells/0.1mm² to be 45 - 50 and distance between testa cells was 1.15 - 1.49 in seeds of the of studied taxa. These reported values are different from our studied taxa in this study (Table 2). According to the results obtained from our study, *A. serotina* and *A. purpurea* which are closely related in the Turkish flora do not have such similarities in terms of seed characteristics. The two taxa are different in terms of color, surface suture, number of testa cells/0.1 mm² and between testa cells distance of seeds.

Pollen of different species of Rubiaceae is very similar in general appearance and size (Robbrecht, 1982;

Vinckier et al., 2000). Gücel (2005) described the pollen of *Asperula daphneola* O. Schwarz as stephanocolpate, spheroidal, P/E ratio 1.06, 6-colpate, tectate and with granulate exine ornamentation. In a different palynological study (Minareci, 2007), the pollen features of *A. pseudochlorantha* var. *pseudochlorantha* and *A. pseudochlorantha* var. *antalyensis* were: Polar diameter (P) 18 - 19 µm, equatorial diameter 17 - 18.5 µm, colpus length 12.6 - 13.8 µm, colpus number 6 - 7, sexine pattern perforate and microechinate suprategal elements. Our pollen findings (Table 3) were quite similar to the findings of earlier studies for other species of *Asperula*. The pollen grains of *A. serotina* and *A. purpurea* subsp. *apiculata* showed similar characteristics of pollen type, polar and equatorial diameter, exine thickness, colpus number, although exine perforations and spines (number/µm²) clearly differ from each other. Huysmans et al. (2003) studied palynological features of 29 species belonging to *Asperula*, *Crucianella* L., *Cruciata* Miller, *Galium* L., *Rubia* L. and *Sherardia* L. from Rubiaceae. Huysmans et al. (2003) noted that species in the Rubiaceae family cannot be evaluated by size, shape and colpus numbers. Furthermore, it has been noted that sexine and nexine ornamentation is a more diagnostic character for separation of species with pollen shape and size (Huysmans et al., 2003).

As a result of the study, the situation of the taxa, which were expressed to be close to each other and problematic, has been clarified. When all findings are considered, it was observed that the differences between the two allied taxa *A. serotina* and *A. purpurea* subsp. *apiculata* were much, whereas the similarities were fewer. In addition, these two taxa have the different distribution. The present seed and pollen micromorphological data, points out that the seed surface and exine ornamentation may be used for intra/interspecific

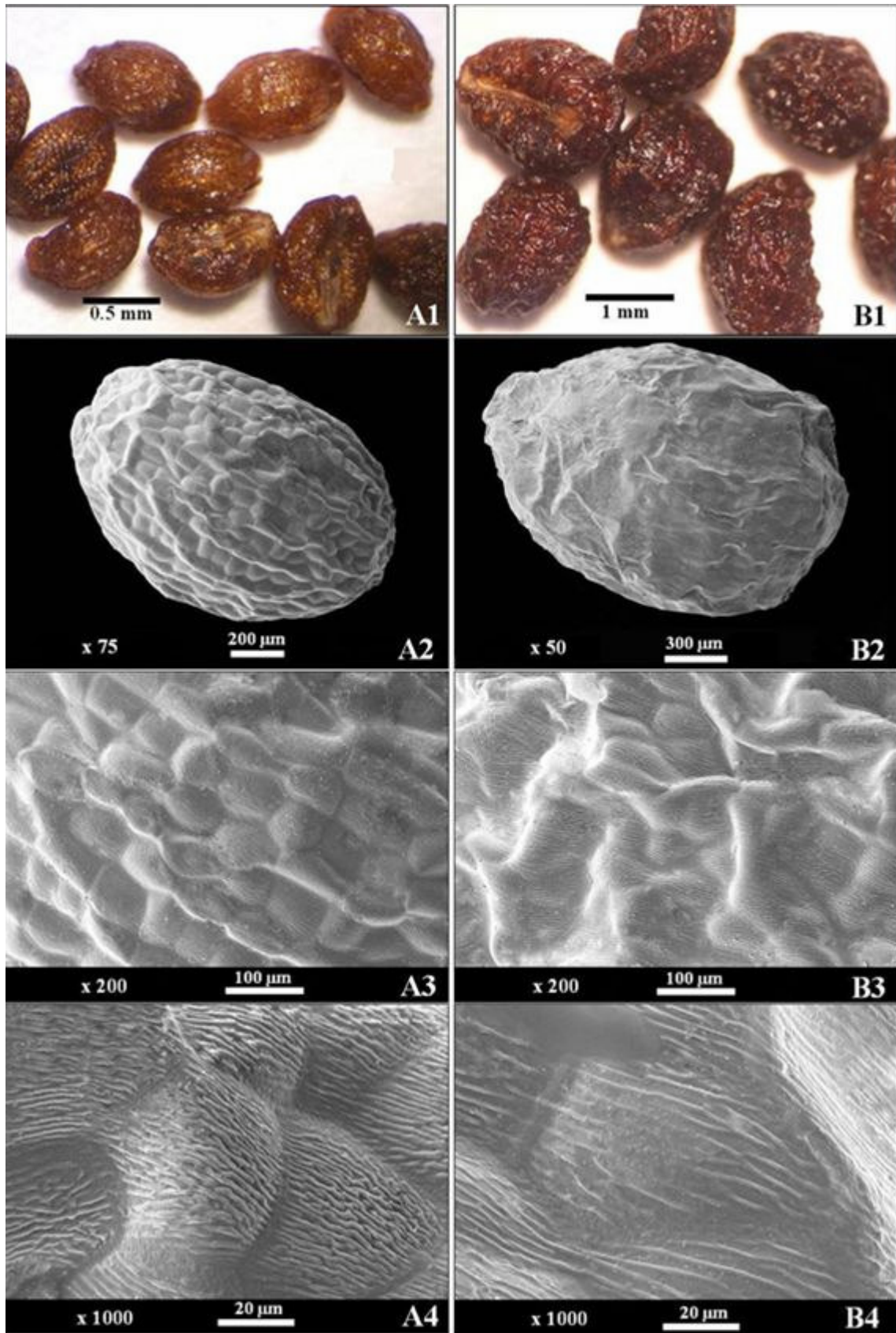


Figure 2. Seed morphology of A— *A. serotina* B— *A. purpurea* subsp. *apiculata*. 1— General appearance (observed with LM), 2— General appearance (observed with SEM), 3,4— Surface structure (observed with SEM).

Table 2. Some characteristics of seed of *A. serotina* and *A. purpurea* subsp. *apiculata*.

Seed characteristics (n = 50)	<i>A. serotina</i>	<i>A. purpurea</i> subsp. <i>apiculata</i>
Seed length x width (μm)	1009 x 672	1803 x 1368
Hilum length x width (μm)	718 x 175	1389 x 152
Testa cell length x width (μm) (max.)	81 x 41	84 x 40
Length / width ratio	1.37 - 1.94	1.39 - 1.41
Between testa cells distance Mean (μm)	7.12	10.2
Number of testa cell / 0.1mm^2	23	30
Seed type	Obovoid-reniform	Obovoid-reniform
Surface type	Concavo-convex	Concavo-convex
Seed back	Convex-rounded	Convex-rounded
Hylar zone type shape	Slightly recessed	Slightly recessed
Surface suture	Clavate-reticulate	Lineate-sulcate
Suture outline	Flat	flat
Colour	Yellowish brown	Brown

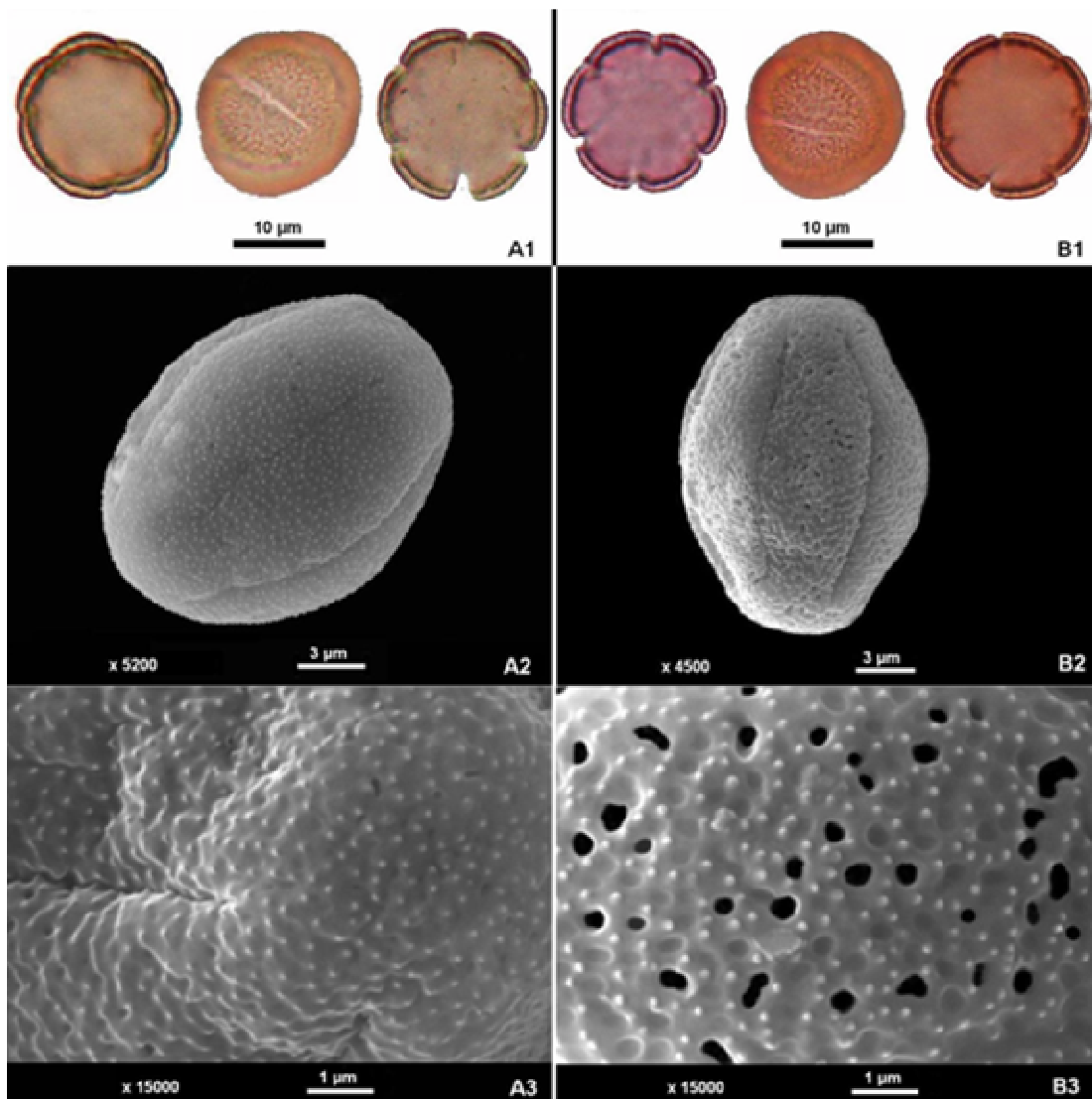
**Figure 3.** Pollen microphotographs of A— *A. serotina* and B— *A. purpurea* subsp. *apiculata*. 1, 2— general appearance (observed with LM and SEM), 3— sexine patterns in detail (observed with LM and SEM).

Table 3. Pollen morphological characters for *A. serotina* and *A. purpurea* subsp. *apiculata*.

Pollen characteristics (n=50)	<i>A. serotina</i>	<i>A. purpurea</i> subsp. <i>apiculata</i>
Diameter polar (P) Mean ± SD (µm)	16.3 ± 0.8	18.0 ± 0.5
Diameter equatorial (E) Mean ± SD (µm)	17.2 ± 0.9	18.8 ± 1.3
P / E ratio	0.94	0.98
Colpus length (Clg) Mean ± SD (µm)	11.3 ± 0.5	13.4 ± 1.2
Colpus width (Clt) Mean ± SD (µm)	0.18 ± 0.09	0.21 ± 0.10
Mesocolpium (M) Mean ± SD	6.6 ± 0.4	6.4 ± 0.5
Exine thickness Mean ± SD	1.4 ± 0.2	1.4 ± 0.2
Microperporate diameter Mean ± SD	0.14 ± 0.02	0.33 ± 0.04
Number of perforations / µm ²	20	12
Number of spines / 5µm ²	42	30
Apocolpium index	0.46	0.50
Colpus	6 – 7	6 – 7
Polen type	Spheroidal	Spheroidal
Sexine pattern	perforate	Perforate
Supratectal elements	Microechinate	microechinate

Table 4. Results of multivariate analyses of variance on determined data, conducted on all traits combined ("All") and separately for morphological, seed and pollen traits, testing for differences between *A. serotina* and *A. purpurea* subsp. *apiculata*.

Comparison	Type of traits	Wilks λ	Approximate F	Df	P
Between taxa	All	0.772	2.865	30, 934	< 0.005
	Morphological	0.974	0.838	10, 318	0.593
	Seed	0.671	2.154	36, 158	0.001
	Polen	0.827	2.125	15, 152	0.011

classification of *Asperula* which is taxonomically critical.

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REFERENCES

- Davis PH (1971). Distribution patterns in Anatolia with particular reference to endemism. In: Davis PH, Harper PC, Hedge IC (eds.), Plant Life of South-West Asia, 15–27. Aberdeen, Botanical Society of Edinburgh, Great Britain.
- Dessein S, Huysmans S, Robbrecht E, Smets E (2002). Pollen of African Spermaceae species (Rubiaceae) Morphology and evolutionary aspects. *Grana*, 41: 69-89.
- Dessein S, Scheltens A, Huysmans S, Robbrecht E, Smets E (2000). Pollen morphological survey of *Pentas* (Rubiaceae-Rubioideae) and its closest allies. *Rev. Palaeobot. Palyno.*, 112: 189-205.
- Duran A (2002). Flora of Tuzaklı, Otluk, Gidefi Mountains and Surroundings (Akseki). *Turk. J. Bot.*, 26: 303-349.
- Ehrendorfer F, Krendl F (1974). Notes on *Rubiaceae* in Europe. *Bot. J. Linn. Soc.*, 68: 268-272.
- Ehrendorfer F, Schönbeck-Temesy E (1980). 71. Rubiaceae. In: Townsend CC, Guest E (Eds.), Flora of Iraq, 4(1), Cornaceae to Rubiaceae, 564-628. Ministr. Agric. Agr. Reform, Baghdad.
- Ehrendorfer F, Schönbeck-Temesy E (1982) *Asperula* L. In: Davis PH (ed.), Flora of Turkey and the East Aegean Islands, Edinburgh University Press, Edinburgh, 7: 734-767.
- Erdtman G (1960). The Acetolysis Method, A Revised Description. *Svensk Botanisk Tidskrift*, 51: 561–564.
- Gücel S (2005). Conservation Biology, *Ex-Situ* Conservation Methods/Applications and *In-Situ* Conservation Strategies of *Minuartia nifensis* Mc Neill and *Asperula daphneola* O. Schwarz's. PhD Thesis, Ege University, Institute of Natural and Applied Sciences, Bornova, İzmir.
- Huysmans S, Dessein S, Smets E, Robbrecht E (2003). Pollen morphology of NW European representatives confirms monophyly of *Rubieae* (Rubiaceae). *Rev. Palaeobot. Palyno.* 127: 219-240.
- Huysmans S, Robbrecht E, Delprete P, Smets E (1999). Pollen morphological support for the Catesbaeeae-Chiococceae-Exostemac-complex (Rubiaceae). *Grana*, 38: 325-338.
- Huysmans S, Robbrecht E, Smets E (1998). A collapsed tribe revisited: pollen morphology of the *Isertieae* (Cinchonoideae-Rubiaceae). *Rev. Palaeobot. Palyno.*, 104: 85-113.
- Khalik KA, El-Ghani MA, El-Kordy A (2008) Fruit and seed morphology in *Galium* L. (Rubiaceae) and its importance for taxonomic identification. *Acta Bot. Croat.*, 67: 1-20.
- Lobreau-Callen D (1978). l'Aperture composee des Rubiaceae. *Extrait d'Annales des Mines Belgique*, 2: 167-173.
- Minareci E (2007). The Revision of the Section *Thliptis* (Griseb.) Ehrend. of the Genus *Asperula* L. (Rubiaceae) Spreading in Turkey. PhD Thesis, Celal Bayar University, Institute of Natural and Applied Sciences, Manisa.
- Moore PD, Webb JA, Collinson ME (1997). An Illustrated Guide to

- Pollen Analysis. Blackwell Scientific Publications, London.
- Nilsson S, Pragowski J (1992). Erdtman's Handbook of Palynology, 2nd edition. Munksgaard, Copenhagen.
- Olson DM, Dinerstein E (2002). The Global 200: Priority ecoregions for global conservation. *Ann. Mo. Bot. Gard.*, 89: 199-224.
- Piesschaert F, Huysmans S, Jaimes I, Robbrecht E, Smets E (2000). Morphological evidence for an extended tribe Coccocypseleae (Rubiaceae-Rubioideae). *Plant Biol.*, 2: 536-546.
- Prentice HC (1979). Numerical Analysis of Intraspecific Variation in European *Silene alba* and *S. dioica* (Caryophyllaceae). *Bot. J. Linn. Soc.*, 78: 181-212.
- Punt W, Blackmore S, Nilsson S, Le Thomas A (1994). Glossary of Pollen and Spore Terminology. LPP Foundation, Utrecht, p. 71.
- Robbrecht E (1982). Pollen morphology of the tribes Anthospermeae and Paederieae (Rubiaceae) in relation to taxonomy. *Bull. Jard. Bot. Nat. Bel.*, 52: 349-366.
- Robbrecht E, De Block P, Degreef J, Stoffelen P (2007). Monographic and systematic studies in Rubiaceae. National Botanic Garden of Belgium. <http://www.br.fgov.be/research/projects/-rubiaceae.php>
- Rosenberg MS (2001). PASSAGE. Pattern Analysis, Spatial Statistics, and Geographic Exegesis. Department of Biology, Arizona State University, Tempe, Arizona.
- Stearn WT (1996). Botanical Latin, David and Charles Newton, London.
- Van Campo M (1978). La face interne de l'exine. *Rev. Palaeobot. Palyno.*, 26: 301-311.
- Vinckier S, Huysmans S, Smets E (2000). Morphology and ultrastructure of orbicules in the subfamily Ixoroideae (Rubiaceae). *Rev. Palaeobot. Palyno.*, 108: 151-174.