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Asperula pseudochlorantha var. *antalyensis comb. et stat. nov.* (Rubiaceae)

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Asperula pseudochlorantha Ehrend. and *Asperula antalyensis* Ehrend. from Turkey were described in 1958. General characteristics, seed structure and micromorphology, pollen measurements and other pollen characteristics were examined to determine the taxonomic status of these taxa. Morphologically, the two taxa cannot be easily distinguished, and the seeds and pollen grains are similar. The chromosome number is 2n = 22 for both taxa. Based on morphological, palynological and cytological similarities, and the fact that both taxa are found in the same habitats, we propose that *A. antalyensis* should be recognized as *A. pseudochlorantha* Ehrend. var. *antalyensis* (Ehrend.) Minareci & K. Yıldız, *comb. et stat. nov.*

Key words: *Asperula*, nomenclature, Rubiaceae, SEM, taxonomy

Most taxa of the large family Rubiaceae are tropical but a number occurs in temperate regions, and there are also a few arctic species (Robbrecht *et al.* 2007). *Asperula*, 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).

The most comprehensive information on the distribution of *Asperula* in Turkey is in the *Flora of Turkey and East Aegean Islands* (Ehrendorfer & Schönbeck-Temesy 1982). In the Turkish flora there are 51 taxa in six sections, 26 of the taxa being endemic. *Asperula pseudochlorantha* and *A. antalyensis* belong in sect. *Thlipthisa*, which has three further taxa in Turkey: *A. brevifolia*, *A. serotina* and *A. purpurea* subsp. *apiculata*.

Describing *A. pseudochlorantha* and *A. antalyensis*, Ehrendorfer and Schönbeck-Temesy (1982) noted that they are very similar and sympatric, but the seed and pollen characters were not described by them. However, palynological characters have a high taxonomic value in the Rubiaceae (Lobreau-Callen 1978, Van Campo 1978, Huysmans *et al.* 1998, 1999, Dessein *et al.* 2000, 2002, Piesschaert *et al.* 2000). On the other hand, as Ehrendorfer and Schönbeck-Temesy (1980) indicated, the floral morphology in *Asperula* is not taxonomically very important. In this study, we aimed to define the similarities and differences of the two taxa in detail and to determine the taxonomic positions accurately.

Material and methods

Asperula pseudochlorantha Ehrend. var. *pseudochlorantha*.

A. pseudochlorantha Ehrend., Notes Royal Bot. Garden Edinburgh 22: 351 f. 3a (1958). — TYPE: Turkey C3. Antalya distr. Kemer (Lycia): Kesme bogaz near Kemer, 60–100 m, 14.VIII.1949 *Davis 14053* (holotype E!, isotype W!).

Asperula pseudochlorantha var. *antalyensis* (Ehrend.) Minareci & K. Yıldız, *comb. et stat. nov.*

A. antalyensis Ehrend., Notes Royal Bot. Garden Edinburgh 22: 352 f. 3b (1958). — TYPE: Turkey C3. Antalya distr. Kemer (Lycia): Kesme bogaz near Kemer, 14.VIII.1949 *Davis 14053* bis (holotype W!).

Plant samples were collected from natural populations in Antalya (Turkey) between May 2004 and July 2007 from the following locations: Kemer, Kesme Boğazı, under *Pinus brutia* forest [50–100 m, 7.VIII.2005 *Minareci E. 452-2*, *453-2* (36°35´341´´N, 30°30´311´´E)]; Göynük, west slope of town, rocky area, forest limit [60 m, 11.VI.2006 *Minareci E. 452-49*, *453-49* (36°40'306´´N, 30°32´209´´E)]; Kurşunlu waterfall, under *Pinus*, rocky area [11.VI.2006 *Minareci E. 452-50*, *453-50* (37°00´125´´N, 30°49´081´´E)]. Geographical distributions of these two taxa are similar.

The specimens were identified using the key in Ehrendorfer and Schönbeck-Temesy (1982). Owing to their taxonomic significance in *Asperula*, characters such as plant length, defoliation, leaf size, inflorescence, corolla length, colour, shape and internode were used. Seed morphology terminology follows Prentice (1979) and Stearn (1996), and was assessed using microphotographs obtained from SEM images. Thirty specimens of each taxon and 50 seeds from each individual were used.

For palynological studies, the pollen grains were acetolysed as described by Erdtman (1960). At least 50 pollen grains from each taxon were measured under a compound microscope for their diameter, colpus length, colpus width, mesocolpium, microperporate diameter and exine thickness. The descriptive terminology in Punt *et al*. (1994) and Moore *et al*. (1997) was employed. For shape classes (*P*/*E*), we adopted the definitions of Nilsson and Praglowski (1992). 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.

The seeds were germinated on filter paper placed inside Petri dishes. Root tips with lengths of 0.5 mm–1 cm were removed and pretreated with 0.5% colchicine for 1–5 hour. The aceto-orcein squash method (Elci 1982) was applied to the root tips. Olympus CX21FS1 microscope with D plan 1.00–1.25 160/0.17 oil immersion objective and NFKx3.3 LD 125 lens were used and the microphotographs, taken by Pentax Optio S camera, were used for the cytological studies.

Graphad Prism for Windows software package and Minitab (ver. 15) were used for statistical analyses. Analysis of variance (one-way ANOVA) was used to determine whether significant differences in the morphological and palynological characters existed among the taxa, and Tukey test was used to determine the statistically significant differences (Peicheto *et al*. 2007).

Results and discussion

In this study, morphological and palynological data from *Asperula pseudochlorantha* and *A. antalyensis* were compared with such data from other taxa in sect. *Thlipthisa* distributed in Turkey. All studied taxa are perennial semi-shrubs. All taxa except for *A. purpurea* subsp. *apiculata* are endemic and have a narrow distribution (Fig. 1).

Morphological characteristics

Sixteen parameters of five taxa belonging to sect. *Thlipthisa* were evaluated (Table 1). Morphological differences occurred in only flower characteristics in *A. pseudochlorantha* and *A. antalyensis*.

The seeds of *Asperula* species are very similar in size and shape (Table 2). Although differ-

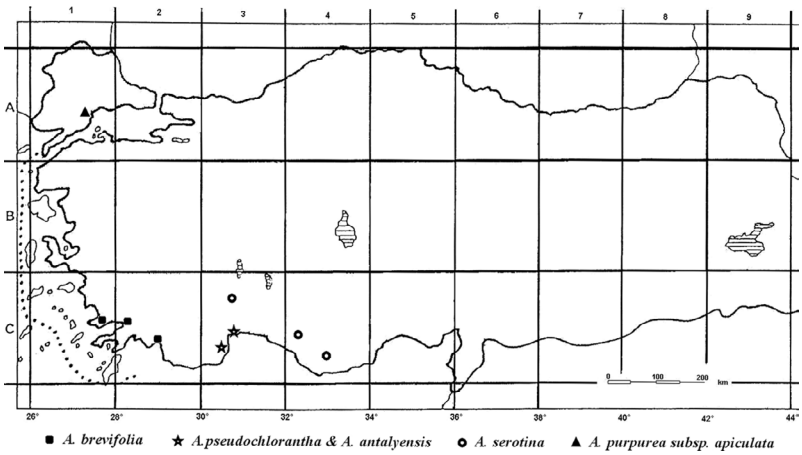

Fig. 1. The geographical distributions of taxa in *Asperula* section *Thlipthisa* in Turkey.

ences are observed in morphology of seeds from different sections, such differences in general appearance of species in the same section are usually minimal (Minareci 2007). This is also true for sect. *Thlipthisa*. Thus, details were scrutinized by examining the seed surface using SEM (Fig. 2). Differences and similarities based on these data were evaluated. *Asperula antalyensis* and *A. pseudochlorantha*, which are closely related (Ehrendorfer & Schönbeck-Temesy 1982), had similar properties in terms of seed size, general seed appearance and testa cells. In contrast, although *A. serotina* and *A. purpurea* are also closely related to each other, they do not have such similarities in the seed characteristics, and the seeds of *A. brevifolia* differ from all of the other taxa.

Pollen characteristics, cytology and conclusions

All pollen grains were spheroidal in shape, exines were tectate and the ornamentation was microechinate-perforate (Fig. 3). The examined pollen grains, as well as those belonging to other taxa in the Rubiaceae (Robbrecht 1982, Vinckier *et al*. 2000) were closely similar (Table 3). Huysmans *et al*. (2003) noted that species in the Rubiaceae cannot be distinguished by pollen size, shape or colpus numbers. Furthermore, it was noted that the ornamentation of sexine and nexine, rather than pollen shape and size, are important in separation of species.

One-way ANOVA test showed that the morphological and palynological characteristics differed significantly among the taxa (Table 4). The Tukey test (Table 4) revealed that *A. pseudochlorantha* and *A. antalyensis* do not differ significantly from each other while all the other taxa do (Table 4). Results of a cluster analysis performed using an unweighted pair-group method using the arithmetic average (UPGMA) and the Manhattan distance coefficient are presented in Fig. 4.

The chromosome numbers of *A. pseudochlorantha* and *A. antalyensis* were found to be 2n = 22 (Fig. 5). The metaphase chromosomes were small and their centromeres and arms could not be distinguished clearly; also their distribution in the cells were not clear for each taxon. Therefore, it was very difficult to determine the karyograms and idiograms. No differences in the chromosome numbers or abnormalities in the chromosomes, or other cytological differences were observed in *A. pseudochlorantha* and *A. antalyensis*.

When all findings are considered, it is clear that the similarities between the sympatric taxa *A. pseudochlorantha* and *A. antalyensis* were numerous and the differences were few. Their morphological features showed very little differences, such as the colour of the corolla and also the anther shape and size. Also the pollen and seed features were similar (Tables 2 and 3), and the chromosome number was 2n = 22 for both taxa.

When all similarities are taken into consideration, it becomes clear that we do not have

Table 1. Morphological comparison of *Asperula pseudochlorantha*, *A. antalyensis* and other taxa in section *Thlipthisa*.

	A. brevifolia	*A. pseudochlor-antha*	*A. antalyensis*	*A.serotina*	*A.purpurea* subsp. *apiculata*
Habit	perennial	perennial	perennial	perennial	perennial
Height of plant (cm)	10–60	20–45	20–50	20–45	15–45(–50)
Stem properties	numerous, erect	numerous, erect	numerous, erect	numerous, erect	numerous, erect
İnternod (mm)	15–30	5–40	5–35(–45)	20–45	5–20
Leaf persistent or deciduous	deciduous	deciduous	deciduous	deciduous	persistent
Leaf number of nodes	(4–)6	4–6(–8)	(4–)6(–8)	4–6	6–8(–11)
Leaf size Lenght × width (mm)	15 × 1.3	8–15 × 1–2	8–18 × 1–2	6–13 × 0.6–1	5–17 × 1–2
Vein thickness	1/3 × leaf breadth	1/10–1/12 × leaf breadth	1/10–1/12 × leaf breadth	1/7–1/10 × leaf breadth	1/5 × leaf breadth
Corolla lenght (mm)	3.5–6	1.5–2.5	0.6–1.8	2–2.5	1.5–2.5
Corolla colour	yellowish green, reddish green	red-green, usually red	yellowish white, pinkish	greenish red, red	greenish red, red
Corolla lobe number	4	4	4	4–5	4
Pedicel lenght (mm)	nonexistent	0.3–0.8	0.3–0.6	0–0.2–0.4	0.5–4
Anther shape and size (mm)	ellipsoid, 0.5	oblong, 0.5–0.7	subglobose, 0.2	subglobose, 0.2	subglobose, 0.15
Habitat	lLimestone rocks, scree, hill slopes	limestone rocks in *Pinus brutia* forest	limestone rocks in *Pinus brutia* forest	limestone rocks	scree, hill slopes, dry places
Flowering time (month)	6–7	6–8	6–8	6–7	6–7
Altitude (m)	20–1600	1–100	1–100	500–2000	100

Table 2. Comparison of the seed characteristics in *Asperula* section *Thlipthisa*.

Seed characteristics (n = 50)	*A. brevifolia*	*A. pseudochlorantha*	*A. antalyensis*	*A. serotina*	*A. purpurea* subsp. *apiculata*
Seed length × width (μm)	1987 × 1415	1387 × 1063	1405 × 1081	1009 × 672	1803 × 1368
Hilum length × width (μm)	1248 × 142	865 × 104	871 × 224	718 × 175	1389 × 152
Max. testa cell length × width (μm)	71 × 54	43 × 38	49 × 45	81 × 41	84 × 40
Length/width ratio	1.2–1.45	1.36–1.45	1.41–1.5	1.37–1.94	1.39–1.41
Mean distance between testa cells (μm)	15.3	1.15	1.49	7.12	10.2
Number of testa cell per 0.1 mm^2	17	50	45	23	30
Seed type	obovoid-reniform	obovoid-reniform	obovoid-reniform	obovoid-reniform	obovoid-reniform
Surface type	concavo-convex	concavo-convex	concavo-convex	concavo-convex	concavo-convex
Seed back	convex-rounded	convex-rounded	convex-rounded	convex-rounded	convex-rounded
Hylar zone type shape	slightly recessed	slightly recessed	slightly recessed	slightly recessed	slightly recessed
Surface suture	rugose-ribbed	sinuous-reticulate	sinuous-reticulate	clavate-reticulate	lineate-sulcate
Suture outline	flat	flat	flat	flat	flat
Colour	dark brown, black	light brown	light brown	yellowish brown	brown

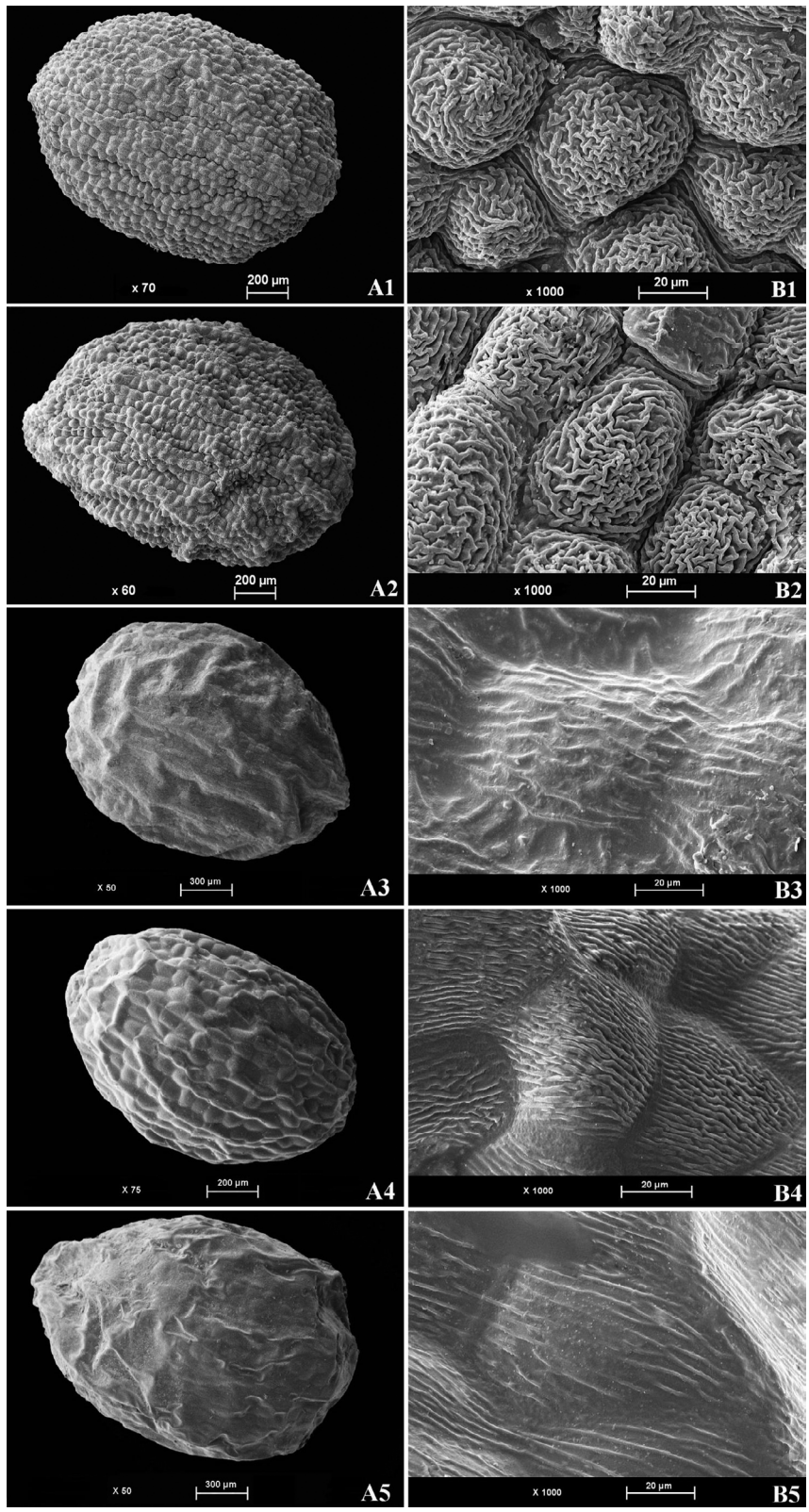

Fig. 2. SEM micrographs of seed morphology. — **A**: general appearance, — **B**: surface structure. (1) *Asperula pseudochlorantha*, (2) *A. antalyensis*, (3) *A. brevifolia*, (4) *A. serotina*, (5) *A. purpurea* subsp. *apiculata.*

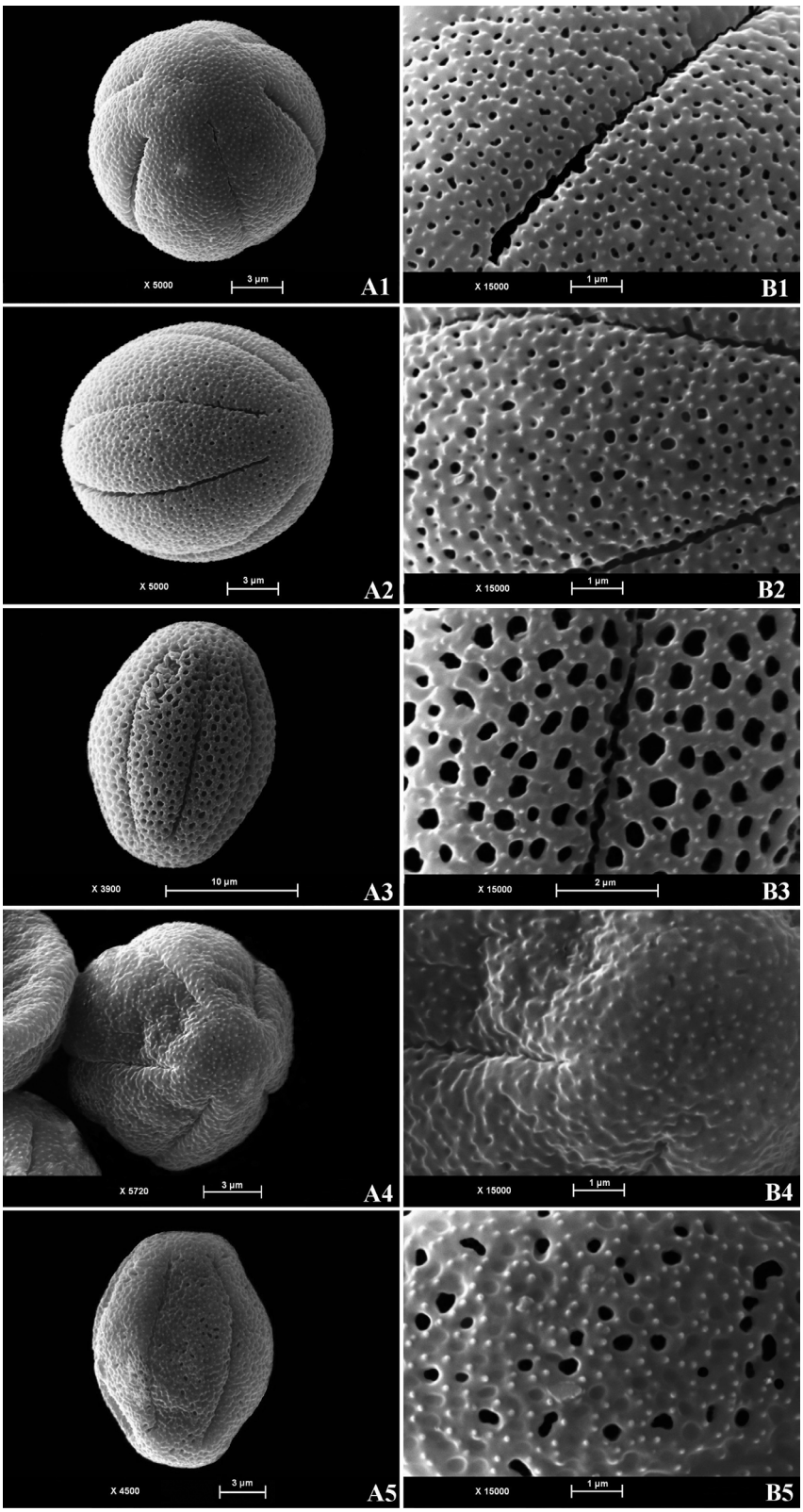

Fig. 3. SEM micrographs of pollen. — **A**: general appearance, — **B**: detail of sexine ornamentation. (1) *Asperula pseudochlorantha*, (2) *A. antalyensis*, (3) *A. brevifolia*, (4) *A. serotina*, (5) *A. purpurea* subsp. *apiculata.*

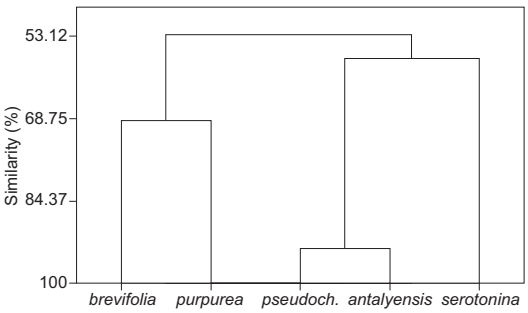

Fig. 4. Cluster analysis dendogram of the relationships between *Asperula* taxa.

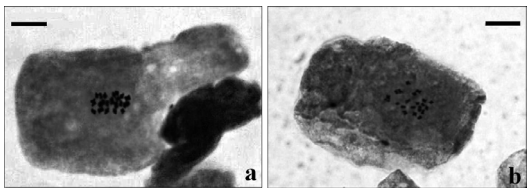

Fig. 5. Mitotic metaphase chromosomes in root tip cells. — **a**: *Asperula pseudochlorantha*. — **b**: *A. antalyensis*. Scale bar = 10 μm.

Table 3. Palynological comparison within *Asperula* sect. *Thlipthisa.*

Pollen characteristics (mean ± SD, *n* = 50)	*A. brevifolia*	*A. pseudochlorantha*	*A. antalyensis*	*A. serotina*	*A. purpurea* subsp. *apiculata*
Diameter polar (*P*, μm)	17.7 ± 1.4	18 ± 0.8	19.3 ± 0.8	16.3 ± 0.8	18.0 ± 0.5
Diameter equatorial (*E*, μm)	17.9 ± 1.3	17 ± 1.2	18.5 ± 0.8	17.2 ± 0.9	18.8 ± 1.3
P/*E*	1.00	1.04	1.00	0.94	0.98
Colpus length (Clg, μm)	11.4 ± 1.0	12.6 ± 0.4	13.8 ± 0.5	11.3 ± 0.5	13.4 ± 1.2
Colpus width (Clt, μm)	0.31 ± 0.08	0.27 ± 0.05	0.25 ± 0.06	0.18 ± 0.09	0.21 ± 0.10
Mesocolpium (*M*)	5.1 ± 0.6	6 ± 0.7	6.4 ± 0.5	6.6 ± 0.4	6.4 ± 0.5
Exine thickness (μm)	1.4 ± 0.1	1.7 ± 0.3	1.5 ± 0.2	1.4 ± 0.2	1.4 ± 0.2
Microperporate diameter(μm)	0.56 ± 0.08	0.23 ± 0.05	0.22 ± 0.05	0.14 ± 0.02	0.33 ± 0.04
Number of perforations per 5 μm^2	16	36	33	20	12
Number of spines per 5 μm^2	35	58	55	42	30
Apocolpium index	0.51	0.47	0.48	0.46	0.50
Colpus	6–7–8	6–7	7	6–7	6–7
Pollen type	spheroidal	spheroidal	spheroidal	spheroidal	spheroidal
Sexine pattern	perforate	perforate	perforate	perforate	perforate
Supratectal elements	microechinate	microechinate	microechinate	microechinate	microechinate

Table 4. Results of one-way ANOVA and Tukey test for some quantitative seed and pollen characters.

	Number of testa cell per 0.1mm²	Between testa cells distance	Number of perforate per 5 μm²	Microperporate diameter	Number of spines per 5 μm²
ANOVA					
Number of groups	5	5	5	5	5
F	2437	2088	1159	1611	482
Significance level	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Tukey's multiple comparison test					
A. brevifolia vs. A. pseudochlorantha	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
A. brevifolia vs. A. antalyensis	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
A. brevifolia vs. A. serotina	$p < 0.001$	$p < 0.001$	$p < 0.01$	$p < 0.001$	$p < 0.001$
A. brevifolia vs. A. purpurea	$p < 0.001$	$p < 0.001$	ns	$p < 0.001$	$p < 0.01$
A. pseudochlorantha vs. A. antalyensis	ns	ns	ns	ns	ns
A. pseudochlorantha vs. A. serotina	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
A. pseudochlorantha vs. A. purpurea	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
A. antalyensis vs. A. serotina	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
A. antalyensis vs. A. purpurea	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
A. serotina vs. A. purpurea	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

two distinct species here, but the taxa should be recognized at the variety level. It was also seen that the taxa constituted separate populations in their distribution area, and no other *Asperula* taxa or intermediate forms were observed in the area. Therefore, it is not likely that the taxa are hybrids.

According to the priority rule (McNeill *et al*. 2006), the correct name of *Asperula pseudochlorantha* is *A. pseudochlorantha* var. *pseudochlorantha*, and the correct name of *A. antalyensis* is *A pseudochlorantha* var. *antalyensis*. The varieties can be distinguished by the following key:

1. Corolla reddish-green, anthers oblong, 0.5–0.7 mm .... var. *pseudochlorantha*
1. Corolla yellowish-white, anthers subglobose, 0.2 mm ... . var. *antalyensis*

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