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Micromorphological studies on inflorescence and seeds of some plantain (*Plantago* L.) taxa in Turkey

Original Article

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Abstract:

Plantago L. (Plantaginaceae) is one of the largest genera of the Plantaginaceae family. There are relatively fewer morphological studies on the genus. Seed surface micromorphology has become popular in plant taxonomy in recent years and found useful in most groups. The inflorescence and seed surface characteristics of 22 *Plantago* taxa collected from Turkey were examined using a Scanning Electron Microscope (SEM). Cluster analysis performed in R. We observed that the micromorphology of the inflorescence parts and seeds of genus *Plantago* are very variable, and do not support the classification based on general morphology. This study shows that these characteristics are highly variable and micromorphological characteristics of inflorescence parts are not good characters for making a specific distinction between different taxa. Additionally, some groups have been to possess a stable seed surface characteristics. However, these characters are not reliable among all taxa, which means that they should be used carefully, especially when identifying widespread taxa.

Key words:

inflorescence, micromorphology, *Plantago*, Plantaginaceae, seed

Apstract:

Mikromorfološka studija cvast i semena nekih taksona bokvica (*Plantago* L.) u Turskoj

Plantago L. (Plantaginaceae) je jedan od najvećih rodova porodice Plantaginaceae. Postoji relativno malo morfoloških studija o ovom rodu. Mikromorfologija površine semena postala je popularna u biljnoj taksonomiji poslednjih godina i smatra se korisnom u većini grupa. Karakteristike cvasti i površine semena 22 taksona roda *Plantago* sakupljenih iz Turske ispitane su pomoću skenirajućeg elektronskog mikroskopa (SEM). Klaster analiza je izvršena u R. Primetili smo da su mikromorfologija delova cvasti i semena roda *Plantago* veoma promenljive i ne podržavaju klasifikaciju zasnovanu na opštoj morfologiji. Ova studija pokazuje da su ove karakteristike veoma promenljive, a mikromorfološke karakteristike delova cvasti nisu dobri karakteri za pravljenje posebne razlike između različitih taksona. Pored toga, neke grupe su pokazale da imaju stabilne karakteristike površine semena. Međutim, ovi karakteri nisu pouzdani kod svih taksona, što znači da ih treba pažljivo koristiti, posebno pri identifikaciji rasprostranjenih vrsta.

Ključne reči:
cvasti, mikromorfologija, *Plantago*, Plantaginaceae, seed

Introduction

Plantaginaceae is a very heterogeneous family with diverse evolutionary trends, and *Plantago* L. is one of the greatest genera of the Plantaginaceae family with 200 species, 56 subspecies, 188 varieties and 9 subvarieties according to Albach et al. (2005). It is probably the most widespread genus of the family Plantaginaceae (Albach et al., 2005).

Plantago genus is divided into nineteen sections and two subgenera (Pilger, 1937). 9 of these sections including 23 taxa, two of which are endemic to Turkey, have been recorded in the Flora of Turkey and the East Aegean Islands (Tutel, 1982).

Although Rahn (1996) claimed that *Plantago* can be divided into six genera, he divided the genus into six subgenera and twelve sections in order to prevent synonym pollution. These two classification suggestions for the genus are summarized in **Tab. 1**. Here in this study, we used Pilger's (1937) classifica-

Table 1. Classification of the Turkish *Plantago* taxa according to Pilger [2] and Rahn [4]

Sensu Pilger			**Sensu Rahn**	
Subgen.	**Sect.**	**Taxa**	**Sect.**	**Subgen.**
Euplantago	*Plantago*	*Plantago major* subsp. *major* *P. major* subsp. *intermedia*	*Plantago*	*Plantago*
	Gentianoides	*P. gentianoides*		
	Lamprosantha	*P. media*		
	Coronopus	*P. crassifolia* *P. coronopus* *P. weldenii* *P. holosteum*	*Coronopus*	*Coronopus*
		P. maritima	*Maritima*	
	Oreades	*P. atrata*	*Montana*	*Albicans*
	Leucopsyllium	*P. albicans* *P. loeflingii*		
	Arnoglossum	*P. lanceolata* *P. argentea* *P. lagopus*	*Lanceifolia*	
	Hymenopsyllium	*P. cretica* *P. bellardii*	*Hymenopsyllium*	
Psyllium	*Psyllium*	*P. squarrosa*	*Psyllium*	*Psyllium*
		P. afra *P. scabra* *P. sempervirens* *P. euphratica*		

tion in order to be able to follow the ranking in the Flora of Turkey (Tutel, 1982).

Plantago taxa are annual or perennial herbs or dwarf shrubs with alternate rosettes or opposite leaves. The inflorescence is a spike with 4-merous flowers. The sepals are imbricate and keeled. The flowers have bracts. The corolla is sympetalous and forms a cylindrical tube (Tutel, 1993).

Even though various studies have been made with *Plantago* taxa, morphological and systematic studies on *Plantago* taxa are relatively limited (Sagar & Harper, 1964; Sharma et al., 1990; Tutel et al., 2005). Micromorphological studies have been made in China (Jun-Zhe et al., 1992) and Poland (Klimko et al., 2004) which were both limited to the taxa in their region. The most extensive study on micromorphology of *Plantago* seeds composed by Shehata and Loutfy (2006) included 31 taxa.

Seed surface micromorphology has become very popular in plant taxonomy in recent years. One of the main reasons for that is that structure of the seed surface seems to be the most stable character due to less contact with environment. Flower elements are also one of the most stable characters in flowering plants (Endress, 2001). For this reason, we decided to include the surface investigations of inflorescence elements in this study.

Considering the lack of the studies about *Plantago* taxa, this study aims to clarify the relationships within *Plantago* taxa in Turkey; using seed and inflorescence features. Moreover, SEM was used to investigate further the micromorphology of the seed and the inflorescence surfaces of these taxa in Turkey for the first time.

Materials and Methods

22 Turkish *Plantago* taxa including four varieties, which were not presented in the Flora of Turkey and East Aegean Islands (Tutel, 1982), are examined in this study (**Tab. 2**). These taxa were obtained mostly from ISTF (Istanbul University Faculty of Sciences Herbarium) as well as from ANK (Ankara University Faculty of Science Herbarium). We used 15 individuals' inflorescence elements and seeds from each

Table 2. The examined taxa in the study with voucher specimens

Taxa	Voucher No	Location
P. major L. subsp. *major*	ISTF 24301	Rize
P. major L. subsp. *intermedia* (Gilib) Lange	ISTF 23423	İstanbul
P. coronopus L. subsp. *coronopus*	ISTF 557	İstanbul
P. weldenii Rchb.	ISTF 2306	İstanbul
P. crassifolia Forsskal	ISTF 40190	Istanbul
P. maritima L.	ISTF 26779	Kirklareli
P. holosteum Scop.	ISTF 27341	Ankara
P. media L.	ISTF 23998	Erzurum
P. atrata Hoppe	ISTF 23710	Erzurum
P. gentianoides SM. subsp. *gentianoides*	ISTF 32242	Bursa
P. lanceolata L.	ISTF 23372	Istanbul
P. argentea Chaix	ISTF 63	Bursa
P. lagopus L.	ISTF 22271	Istanbul
P. albicans L.	ANK	Ankara
P. loeflingii L.	ISTF 35420	Sanliurfa
P. cretica L.	ISTF 24743	Antalya
P. bellardii All.	ISTF 27212	İstanbul
P. squarrosa Murray	ISTF 9790	Antalya
P. scabra Moench	ISTF 32258	İstanbul
P. afra L.	ISTF 36362	Mersin
P. sempervirens Crantz	ALM 27	Tekirdağ
P. euphratica Decne. ex Barnéoud	ISTF 37227	Malatya

taxon when possible.

Apart from the other studies (Jhun-Zhe et al., 1992; Klimko et al., 2004; Shehata & Loutfy, 2006) on *Plantago* micromorphology, this study includes some other areas from the inflorescence parts (e.g. bract, anterior and posterior sepals, and corolla) (**Fig. 1**). For the examination of the inflorescence characteristics, the lowest and the most mature flowers of the inflorescences have been chosen, and the macro morphological observations have been made using Olympus SZX7 stereomicroscope. The micromorphology of the inflorescence parts and seeds

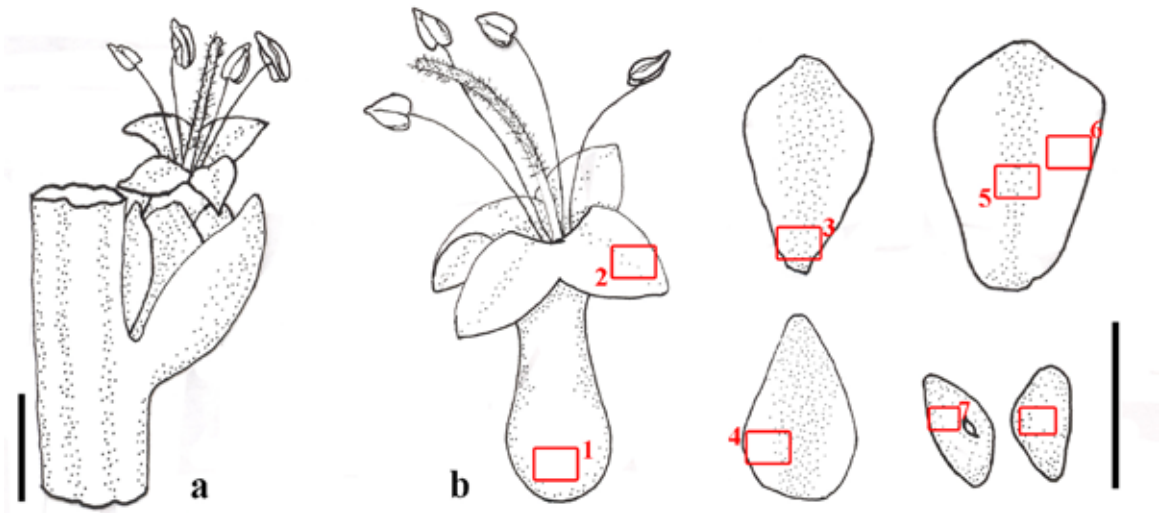

Fig. 1. General view of a *Plantago* flower (a). The observed areas- corolla tube (1), petal (2), anterior sepal base (3), bract margin (4), posterior sepal carina (5) and margin (6), seed ventral (7) and dorsal (8) surfaces- are shown with squares (b). Scale bars 1 mm.

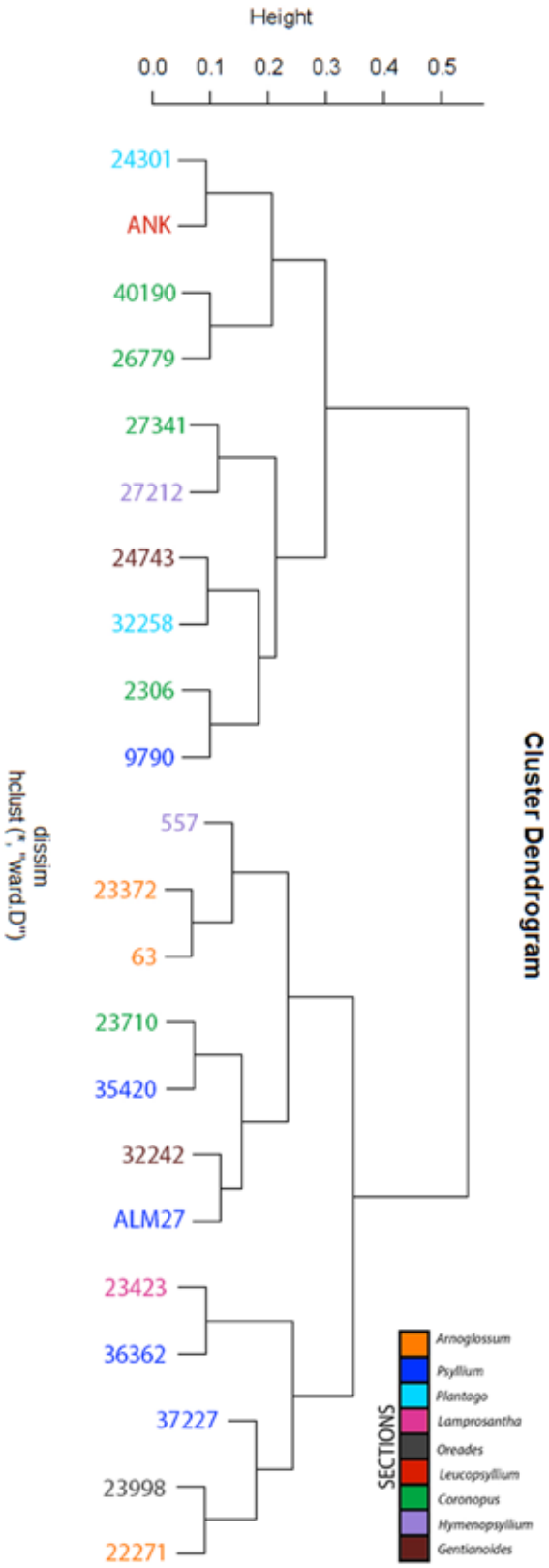

Fig. 2. Cluster dendrogram of *Plantago* taxa. The sections marked with different colors

have been observed and photographed by a JEOL Benchtop Scanning Electron Microscope JCM-5000 under 10 kV of voltage. The nomenclature of the surface characteristics is adapted from Stearn (1991) and Barthlott et al. (1998).

The categorical variables of surface characters in various anatomical compartments of *Plantago* were used as variables in the cluster analysis. Hierarchical cluster analysis of 26 categorical variables across 22 taxa was performed using a clustering algorithm via ward.D method. In brief, a mean distances matrix was generated and subjected to clustering with the ward.D method of the hclust function. Following the clustering, the relationships between the sections were visualised as dendrograms using the tree functions in R v3.4.3.

Results

The dissimilarity cluster analyses formed two main groups and four subgroups according to the surface characters (**Fig. 2**). Both of the groups included different taxa from different sections.

The macro-morphological observations showed that the seed shapes and sizes of *Plantago* taxa are very variable. The micromorphological aspects of the examined parts using SEM are shown in **Tab. 3**. The SEM photos of all the examined areas are given in **Fig. 3**. It can be seen from the **Fig. 3** that hairs, and epidermal cell arrangements are variable among the studied taxa.

Observations showed that the surface type of corolla tube was the most stable feature appearing to be lineate in all of the examined taxa but only differing in terms of cell size and presence of hairs (**Tab. 3, Fig. 3**). This result shows that the surfaces of the petals and the posterior sepals seem to have more or less stable features through sections (**Tab. 3, Fig. 3**). Being the outermost part of a flower structure, bract has shown a great variety in terms of surface micromorphology among all the examined parts (**Tab. 3, Fig. 3**).

In Section *Coronopus* bract surfaces are differing among the taxa whereas the seed surface characteristics are very stable. All taxa in this section has reticulate seed surface ornamentation and all but one taxa (*P. holosteum*) has undulated anticlinal walls. Section *Coronopus* also show stability in terms of inflorescence parts' surface characters generally except anterior sepal and bract surfaces (**Tab. 3**). Similarly, taxa in Section *Arnoglossum* have very stable seed surface characteristics (smooth). The smooth seed surfaces have also been observed in some taxa of Section *Psyllium*. Section *Psyllium*, has the most variable seed surface characteristics. The inflorescence surface characters vary among these taxa, as well (**Tab. 3**). Sections *Lamprosantha*, *Oreades*

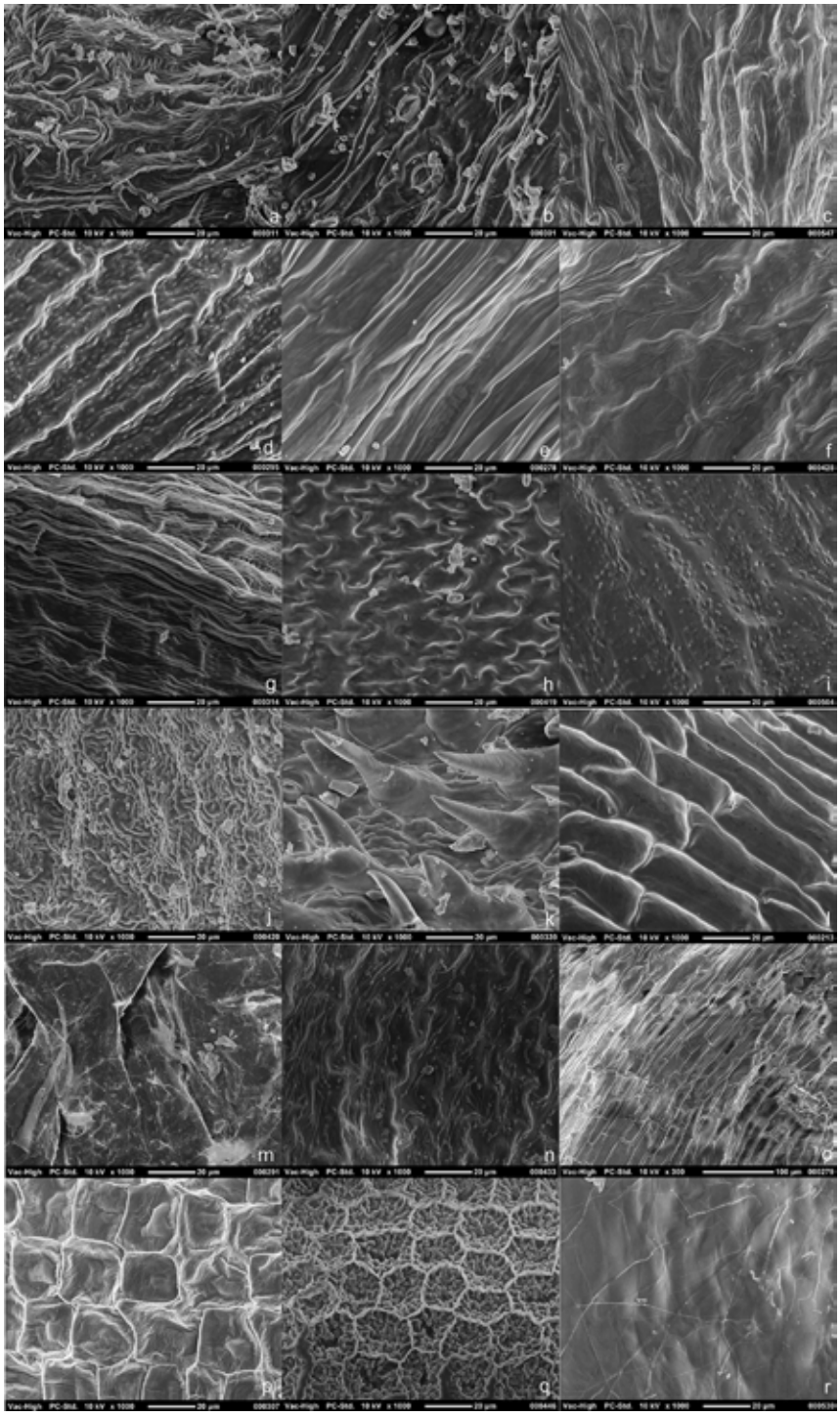

Fig. 3. SEM micrographs of studied areas. Posterior sepal carina surfaces of (a) Reticulate, *Plantago major* subsp. *major*; (b) Rugose, *P. weldenii*; (c) Striate, *P. gentianoides* subsp. *gentianoides*; Petal surfaces of (d) Granulate, *P. weldenii*; (e) Striate, *P. gentianoides* subsp. *gentianoides*; (f) Rugose, *P. sempervirens*; Anterior sepal base surfaces of (g) Rugose, *P. major* subsp. *major*; (h) Rugulose, *P. coronopus* subsp. *coronopus*; (i) Granulate, *P. albicans*; Bract margin surfaces of (j) Reticulate, *P. coronopus* subsp. *coronopus*; (k) Rugose, *P. maritima*; (l) Smooth, *P. argentea*; *P. squarrosa*; (m) Fissured, *P. afra*; (n) Granulate, *P. sempervirens*; Corolla tube surface of (o) Lineat, *P. gentianoides* subsp. *gentianoides*; Seed surfaces of (p) Rugose, *Plantago major* subsp. *major*; (q) Reticulate, *P. crassifolia*; (r) Smooth, *P. lagopus*.

and *Gentianoides* are represented by one taxon each, and these taxa have similar characteristics with each other, and in the terms of seed surface characters they resemble Section *Plantago*.

In *P. major* subsp. *major* and in *P. major* subsp. *intermedia* seed surfaces are rugose. Other examined parts (i.e. anterior sepal, posterior sepal carina, corolla tube, petal, and dorsal and ventral sides of seeds) show parallel characteristics within these two taxa except anterior sepals. The bases of anterior sepals are both rugose, but cell shapes and anticlinal wall nature is different (**Tab. 3**).

The seed surfaces are reticulate in Section *Leucopsyllium* and Section *Hymenopsyllium*. Section *Leucopsyllium* has diverse inflorescence surface characters. Section *Hymenopsyllium* taxa have more or less stable characters on micromorphological level with rugose posterior sepal carina surfaces, striate corolla tube, and striate petal surfaces but there is only two taxa available in this study.

Discussion

The two subspecies of *P. major* appear in two different main groups. Concurrently, the observation of the seed surfaces of *P. major* were controversial in previous studies focusing on the seed micromorphology of *Plantago* taxa (Klimko et al., 2004; Shehata & Loutfy, 2006; Hoghoughi et al., 2016; Verma & Bharti, 2017). Klimko et al. (2004) reported the seed surfaces of *P. major* as "not slightly rugose as it has been reported in previous studies" (Rymkiewicz, 1979; Rothmaler et al., 1984). On the contrary, other authors identified the same area as reticulate (Shehata & Loutfy, 2006), tuberculate (Verma & Bharti, 2017), and areolate with wrinkled surface (Hog-

houghi et al, 2016). In this study, seed surfaces of both *P. major* subsp. *major* and *P. major* subsp. *intermedia* were identified as rugose. *Plantago lanceolata* is another taxon which shows a great variety in the sense of seed micromorphology like *P. major*. Verma et al. (2017) reported the seed surface of *P. lanceolata* as scalariform to reticulate with spindle shaped striations whereas Hoghoughi et al. (2016) identifies it as negative reticulate, and the samples in this study showed no ornamentation (smooth surface). These taxa are widespread and adapted to a variety of habitats, therefore it is an expected outcome.

Similarly, *P. maritima* and *P. gentianoides* were reported as having regulate or areolate ornamentation and reticulum cristatum respectively (Hoghoughi et al., 2016), but in this study they are identified as reticulate and rugose.

It has been known that *Plantago* taxa are very variable in morphology, and that this variation may be due to environmental differences (Meudt, 2012). However, some taxa show very consistent surface characteristics. For instance, *P. coronopus* has found to be reticulate in both this study and others (Hoghoughi et al., 2016). *Plantago major* is a widespread species with capability to adapt in a variety of habitats, thus its positioning in the clustering may not be considered meaningful.

Section *Psyllium* taxa have representatives in both main groups in dendrogram. Some of these taxa have shown different seed surface characters than previously studied (Shehata & Loutfy, 2006). The comparison of these results shows that seed surfaces may show variation on an intraspecific level among Sect. *Psyllium* and also some other taxa (*P. major* and *P. lanceolate*)

Section *Coronopus* taxa have a very distinct surface structure. All of the studied taxa in this section have shown undulated anticline membranes and ruminate seed surfaces. These findings are in accordance with the previous studies (Klimko et al., 2004; Shehata & Loutfy, 2006). However, the classification of the *Coronopus* section, including *P. maritima*, is problematic. Rahn (1978); Dietrich (1980) and Klimko et al. (2004) have divided the section *Coronopus* into two sections as Section *Coronopus* and Section *Maritima*. Shehata & Loutfy (2006) have separated the *P. maritima* taxon from subgenus *Coronopus*. In this study, *P. maritima* emerged in the second main group with the other Section *Coronopus* (*P. weldenii*, *P. crassifolia* and *P. holosteum*) except *P. coronopus*. The dendrogram separated the taxa of Sect. *Coronopus* into four subgroups; *P. maritima* grouped with *P. crassifolia*; *P. weldenii* which was formerly assigned as a subspecies of *P. coronopus*. However, these findings don't agree with the molecular data completely (Serrano et al., 2017).

In Tutel et al. (2005), it has been suggested that *Hymenopsyllium* section in subgenus *Euplantago* is closer to subgenus *Psyllium* based on morphological traits. In this study Sect. *Hymenopsyllium* is clustered completely in first main group, whereas most of the Sect. *Psyllium* taxa are placed in the second main group. Therefore, this study does not support the initial statement. However, the results showed the latter section is very diverse in terms of micromorphologic traits and the study of molecular markers already showed *P. bellardii* and *P. cretica* are in the Subgenus *Psyllium* (Ronsted et al., 2002). This shows the micromorphology of inflorescence parts of these taxa are not very reliable in this subgenus.

The *Plantago* taxa are known to be wind polinated but we observed that the flowers attract some insects (Syrphidae) in field and Alfred Heilbronn Botanical Garden. By the reason of the fact that they do not have flashy flowers, it may be due to the UV reflection characteristics of the epicuticular waxes (Whitney et al., 2009). Some taxa having more ability to draw the insects (*P. maritima*, *P. lanceolata*, *P. argentea*) have granulate and striate petal surfaces. However, this finding needs further investigations.

In the overall results of the examinations, we have pointed out that the inflorescence parts' surfaces are variable among taxa except for the corolla tube, and the differences are probably not distinctive characteristics for these taxa. This may be due to its protective position.

This work shows that hairs, epidermal cell arrangements and surface characters are very variable across the genus. Therefore, analysis of inflorescence parts is not conclusive to make a specific distinction between different taxa. Although some groups have very stable seed surface characteristics, they are not reliable among all taxa, which means it should be used with extreme care especially with the widespread taxa. Further studies may be conducted to understand how variable the seed characteristics of the widespread taxa, and whether it is a result of different habitat types.

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