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Taxonomic significance of lamina epidermal characteristics in some taxa of the genus *Plantago* L. (Plantaginaceae Juss.) from Egypt

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Abstract

Plantago L. is widely considered to be a challenging genus from a taxonomic perspective. The lamina epidermal characters have great value in taxonomic studies. The lamina epidermal features of 17 species and one subspecies of Egyptian *Plantago* were investigated. The specific object of the present study was to throw light on the micro-morphological features of the lamina surfaces and assess their taxonomic relevance. The lamina epidermal characters were examined for both the ad/abaxial surfaces using LM and SEM. The data were numerically analyzed to detect the phenetic relationships between the studied taxa. Stomatal type, epidermal cell walls, cuticular ornamentation, and trichome characteristics varied among the taxa studied. Trichomes exhibited the most noticeable characteristics of all the studied taxa. The generated dendrogram showed a segregation of *Plantago major* subspecies *major*, in a distinct phenon from the remaining *Plantago* species, which is subsequently divisible into smaller groups. It is concluded that the phenetic analysis supports Rahn's sectional classification of *Plantago*. Additionally, the lamina epidermal features can help identify *Plantago* species or groups of species by combining character states.

Keywords: Lamina; LM; Morphology; Plantaginaceae; *Plantago*; SEM; Taxonomy

Introduction

The genus *Plantago* L. (Plantaginaceae Juss.) comprises more than 200 annual and perennial herbs and subshrubs distributed worldwide (Rønsted *et al*., 2002). In the Egyptian flora, the reported number of *Plantago* species varies among authors: Boulos (2002) recorded 20 species; Hosny and Waly (2001) reported 19; El Hadidi and Fayed (1994/1995) documented 22; and Täckholm (1974) listed 21 species and seven varieties.

Many *Plantago* species are widely known in both conventional and traditional medicine systems across the world (Gonçalves and Romano, 2016; Weryszko-Chmielewska *et al*., 2012). In addition, the genus holds considerable ecological and conservation importance due to its role in biodiversity and habitat stability (Samuelsen, 2000). The taxonomy of *Plantago* is notoriously complex, with numerous unresolved nomenclatural and systematic problems (Rahn, 1996; Hassemer, 2018). Species delimitation within the genus is hindered by reduced morphological differentiation, a scarcity of diagnostic characters, morphological convergence among unrelated species, frequent hybridization and introgression, and pronounced phenotypic plasticity under variable environmental conditions. These factors have complicated the classification of *Plantago*, leading to persistent ambiguities in the delimitation of sections and subgenera (Rahn, 1996; Rønsted *et al*., 2002;

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Ishikawa *et al*., 2009; Tay *et al*., 2010; Meudt, 2011; Hassemer, 2018; Hassemer *et al*., 2019; Di Pietro *et al*., 2013).

The infrageneric classification of *Plantago* has undergone several revisions. Pilger (1937) divided the genus into two subgenera: *Euplantago* Harms (subgenus *Plantago*) and *Psyllium* (Juss.) Harms. Later, Rahn (1978) proposed three subgenera: *Plantago*, *Cornopus* (Lam. & DC.) Rahn, and *Psyllium* (Juss.). Subsequently, Rahn (1996) introduced a more detailed classification comprising six subgenera: *Plantago*, *Cornopus* (Lam. & DC.) Rahn, *Albicans* Rahn, *Psyllium* (Juss.) Harms & Reiche, *Bougueria* (Decne.) Rahn, and *Littorella* (P.J. Bergius) Rahn. Based on molecular evidence from nuclear ribosomal ITS and plastid *trnL–F* sequences, Rønsted *et al.* (2002) redefined the genus into five subgenera: *Plantago*, *Coronopus*, *Psyllium*, *Bougueria*, and *Littorella*. According to Rahn (1996) and Rønsted *et al*. (2002), the Egyptian *Plantago* species are assigned to four subgenera.

Historically, species identification and assessment of relationships were primarily based on reproductive traits, which are not always readily available. In contrast, foliar macro- and micro-morphological features provide abundant and reliable taxonomic information for distinguishing taxa at both intra- and interspecific levels (Paul and Chowdhury, 2021). Leaf micro-morphological characteristics have proven to be valuable supplementary tools for systematic studies in numerous vascular plant families (Carlquist, 1957/1991).

The taxonomic utility of foliar epidermal characteristics in *Plantago* has been emphasized by several researchers. Metcalfe and Chalk (1950) provided fundamental data on the leaf anatomy of Plantaginaceae. Andrzejewska-Golec (1992) and Andrzejewska-Golec and Swiçtoslawski (1987) examined trichome morphology in *Plantago* section *Cornopus* DC., supporting the division of this section into lower taxa as proposed by Rahn (1978).

Filippa *et al.* (1999) developed an identification key for ten *Plantago* species from Central Argentina based on leaf anatomy. Andrzejewska-Golec and Swiçtoslawski (1993) described trichome types in seven *Plantago* species of subgenus *Psyllium*, identifying three types of headless and three types of headed hairs, while disputing Rahn's (1978) proposal to expand *Psyllium* by including six sections from *Plantago* sensu Pilger (1937). Bahadar *et al.* (2018) analyzed leaf anatomy in 17 Pakistani *Plantago* species, and Mohsenzadeh *et al.* (2020) investigated 20 Iranian species, concluding that morphological data were more informative than molecular data at sectional and subgeneric levels. Butt *et al.* (2025) examined foliar epidermal and pollen morphology in several Plantaginaceae genera, reinforcing their taxonomic importance within the family.

In Egypt, several studies have addressed the morphology and anatomy of *Plantago*. Hosny and Waly (2001) conducted a systematic revision of the family, providing a detailed morphological review and identification key for 19 *Plantago* species. Shalabi and Abou-El-Enain (2013) examined 20 species based on variations in vegetative and floral morphology, emphasizing the need for further studies incorporating additional characters to clarify infrageneric relationships. Hamed *et al.* (2014) investigated six wild species, and more recently, Shehata *et al.* (2023a) presented a comprehensive account of the macro-morphological features of 18 Egyptian *Plantago* species.

Lamina epidermal features, including epidermal cell patterns, cuticular ornamentation, epicuticular waxes, stomatal types, and trichomes, are well recognized for their diagnostic and taxonomic significance, particularly in resolving relationships among morphologically similar or problematic taxa (Hardin, 1979; Barthlott *et al.,* 1998; Wang *et al.,* 2021; El-Gazzar and Moustafa, 2021; Ragab *et al.,* 2022; Tantawy *et al.,* 2023; Sonia *et al.,* 2025). Nevertheless, information on lamina epidermal characteristics within *Plantago* remains scarce. Therefore, the present study investigates the epidermal traits of the lamina surfaces in 18 *Plantago* taxa distributed in Egypt using scanning electron microscopy (SEM) and light microscopy (LM), aiming to elucidate variations in these traits and evaluate their taxonomic significance.

Materials and Methods

a- Morphological characters investigation

Three to five replicates of fresh samples from 18 *Plantago* species representing four subgenera were collected from their natural habitats across Egypt. Species identification was conducted following Täckholm (1974) and Boulos (2002), and nomenclature was updated according to Plants of the World Online (POWO, 2025). The investigated taxa are listed in Table 1, arranged systematically in accordance with Rahn's (1996) classification.

Scanning Electron Microscopy (SEM)

For SEM examination, small segments of the cleaned and air-dried lamina were mounted adaxially and abaxially onto copper stubs using double-sided adhesive tape. The mounted samples were sputter-coated with gold using a JEOL-JFCL 1100E sputter coater. Both adaxial and abaxial lamina surfaces were scanned and photographed with a JEOL JSM-200IT scanning electron microscope operated at an accelerating voltage of 20 kV. The SEM work was carried out at the Electron Microscopy Unit, Faculty of Science, Alexandria University, Egypt.

Light Microscopy (LM)

For LM observations, thin strips were peeled from the adaxial and abaxial lamina surfaces. The epidermal strips were stained with 1% aqueous safranin solution for 4-8 minutes (Mishra, 1977), then rinsed gently with distilled water to remove excess stain and mounted in 10% glycerol on clean glass slides. The prepared slides were examined under a Krűss light microscope at ×40 magnification, and images were captured using a digital camera attached to the microscope.

Terminology

The terminology used to describe epidermal characteristics follows Metcalfe and Chalk (1950), Payne (1978), Barthlott (1981), Stace (1984), Yoshida *et al.* (1997), and Beentje (2010).

b- Phenetic Analysis

A data matrix was constructed by converting multistate lamina epidermal characters into binary form (1 and 0). The phenetic relationships among the studied taxa were assessed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering algorithm and Minimum Spanning Tree (MST) analysis, implemented in the PAST software package (Paleontological Statistics, version 3.12; Hammer *et al.,* 2013). The resulting dendrogram and MST plot were interpreted and discussed in the context of the infrageneric classification systems of the genus *Plantago* proposed by Pilger (1937) and Rahn (1978/ 1996).

Table 1. Data collection of the studied taxa of the genus *Plantago* collected from Egypt

No.	Taxa	Subgenus/ Section	Locality
1	*Plantago major* L. subsp. *major*	*Plantago / Plantago*	Alexandria, Victoria (31°24'77.24" N//29°97'87.20" E)
2	*P. coronopus* L.	*Coronopus /Coronopus*	Alexandria, Italian Cemetery at Al Alamein (30°54'25.25" N// 28°50'26.26" E)
3	*P. crassifolia* Forssk.	*Coronopus /Coronopus*	Alexandria, King Maryout, Amreya, Egypt. (30°00'84.87" N//29°73'33.01" E)
4	*P. crypsoides* Boiss.	*Coronopus /Coronopus*	Alexandria, Italian Cemetery at Al Alamein (30°54'25.25" N// 28°50'26.26" E).
5	*P. weldenii* Rchb. subsp. *weldenii* Guss.	*Coronopus /Coronopus*	Alexandria, Italian Cemetery at Al Alamein (30°54'25.25" N// 28°50'26.26" E)
6	*P. amplexicaulis* Cav. subsp. *bauphula* (Edgew.)Rech.f.	*Albicans / Bauphula*	Alexandria, King Mariout, Amreya, (30°00'84.87" N//29°73'33.01" E)
7	*P. lagopus* L.	*Albicans / Lanceifolia*	Alexandria, Gamal Abd-El Naser Hospital, (31°20'59.47" N//29°92'62.86" E)
8	*P. lanceolata* L.	*Albicans / Lanceifolia*	Alexandria, Gamal AbdEl Naser Hospital (31°20'59.47" N//29°92'62.86" E)
9	*P. albicans* L.	*Albicans / Albicans*	Mersa Matruh, Wadi Al-Aqara (31°21'18.22" N//27°02'51.55" E)
10	*P. ciliata* Desf.	*Albicans / Albicans*	Mersa Matruh, Wadi Al-Aqara (31°21'18.21" N// 27°02'51.55" E)
11	*P. cylindrica* Forssk.	*Albicans / Albicans*	Alexandaria, King Mariout, Amreya (30°00'84.87" N// 29°73'33.01" E)
12	*P. notata* Lag.	*Albicans / Montana*	Alexandria, King Mariout, Amreya (30°00'84.87" N//29°73'33.01" E)
13	*P. ovata* Forssk.	*Albicans / Albicans*	Alexandria, King Mariout, Amreya (30°00'84.87" N//29°73'33.01" E)
14	*P. afra* L.	*Psyllium /-*	Saint Katherine, Wadi El-Arbeen (28°32'66.63" N// 33°56'02.72" E)
15	*P. exigua* Murry.	*Psyllium /-*	Beheira, Idku (31°16'35.86" N // 30°13'31.90"E)
16	*P.indica* L. *(=P. arenaria* Waldst. & Kit.)	*Psyllium /-*	Beheira, Idku (31°16'35.86" N//30°13'31.90" E).
17	*P. phaeostoma* Boiss.& Heldr.	*Psyllium /-*	Mersa Matruh, Wadi Al-Aqara (31°21'18.21" N //27°02'51.51" E).
18	*P. sinaica (*Barnéoud) Decne.	*Psyllium /-*	Saint Katherine, Wadi El-Arbeen, (28°32'66.66" N ///33°56'02.71" E)

Results

The micro-morphological characteristics of the adaxial and abaxial lamina epidermal surfaces are summarized in Table 2&3 and illustrated in the scanning electron micrographs (SEMs) and selected light micrographs (LMs) (Figs 1-6). The examined taxa shared several common features, including a random distribution of stomata, elliptic stomatal apertures, and the presence of granular epicuticular wax deposits of varying densities on both lamina surfaces. However, considerable interspecific variation was observed in stomatal type, cuticular ornamentation, epidermal cell wall configuration, and trichome morphology. A detailed description of the diagnostic and distinctive characters of each species is provided below.

A- Lamina epidermal micro-morphological characters of the studied taxa of *Plantago*

1. Subgenus: *Plantago*
Section*: Plantago*
Species*: Plantago major* L. **subsp. *Major.*** Fig.1 (1A&B)

Leaves amphistomatic with anomocytic, diacytic, and anisocytic stomata. The ad/abaxial lamina surfaces revealed a ruminate ornamentation pattern. The anticlinal cell walls channeled, and the periclinal cell walls convex. Two types of trichomes recognized: short capitate glandular (brevicollate) and bristle-like non-glandular. The former trichomes are being more prevalent than later. The glandular trichome characterized by a unicellular stalk, a small square basal cell, a two-celled vertically divided head, and covered by a smooth cuticle. The non-glandular trichome characterized by a multicellular uniseriate stalk and a large basal cell.

2. Subgenus: *Coronopus*
Section: *Coronopus*
Species: *Plantago coronopus* L., ***P. crassifolia*** Forssk., ***P. crypsoides*** Boiss., ***P. weldenii*** Rchb. **subsp. *weldenii*** Guss. Figs. 1&2 (2-5)

Leaves amphistomatic with diacytic, anisocytic, and anomocytic stomata. The ad/abaxial lamina surfaces showed rugulate sculpture pattern, with raised anticlinal and flat outer periclinal cell walls. Two distinct forms of non-glandular trichome recognized: bristle and web–like. The bristle non-glandular trichome characterized by a unicellular or bi-cellular uniseriate stalk, and a bulgy basal cell, while the web–like trichome characterized by a multicellular uniseriate stalk, a bulgy basal cell, and covered by nodulated cuticle. Glandular trichome bottle-shaped, characterized by a unicellular stalk, a small basal cell, and multicellular head (4-7 cells).

3. Subgenus *Albicans*
a- Section. *Bauphula*
Species: *Plantago amplexicaulis* Cav. **subsp. *bauphula*** (Edgew.)Rech.f. Figs.2 (6A&B)

Leaves amphistomatic with diacytic, anomocytic, and anisocytic stomata. The ad/abaxial lamina surfaces revealed rugulate ornamentation pattern, with raised anticlinal and flat outer periclinal cell walls. Non-glandular trichomes are web–like with a uniseriate stalk, composed of multicellular jammed cells, where the initial stalk cell is small, and the subsequent cells are considerably elongated. The cuticle covering the non-glandular is striated. Glandular trichomes are scarcely dispersed, brevicollate shape, with a unicellular stalk, a small square basal cell, and two-celled vertically divided head.

b- Section. *Lanceifolia*

Species: *Plantago lagopus* L., and ***P. lanceolata*** L. Figs. 2 (7-8)

Leaves amphistomatic with diacytic, anomocytic, and anisocytic stomata. The ad/abaxial lamina surfaces exhibited a rugulate sculpture pattern, with raised anticlinal and flat outer periclinal cell walls. Both ad/abaxial lamina surfaces are sub-glabrescent to sparsely hairy. Two types of trichomes were identified: long, web-like non-glandular and short, morel-like, glandular trichomes. The non-glandular trichome characterized by a uniseriate stalk built of multicellular jammed cells, where the initial stalk cell is square and shorter than the remaining cells, and covered by striated cuticle. The glandular trichome characterized by a unicellular stalk, a small square basal cell, and a head made up of 4-5 cells.

c- Section. *Albicans*

Species: *Plantago albicans* L., ***P. ciliata*** Desf., ***P. cylindrica*** Forssk., ***P. notata*** Lag., ***P. ovata*** Forssk. Figs. 3&4 (9-13)

Leaves amphistomatic, with diacytic, anomocytic, and anisocytic stomata. The ad/abaxial lamina surfaces exhibited a striate ornamentation pattern, with raised anticlinal and flat outer periclinal cell walls. Both ad/abaxial lamina surfaces are densely villous, having both non-glandular and glandular trichomes. The former is more prevalent than glandular trichomes. Non-glandular trichome is very long, web–like with a digger-shaped tip, a bi-cellular stalk, a small bulgy basal cell, and covered by a striated cuticle. Glandular trichome scarcely spread, brevicollate-like with a short, unicellular stalk, a square basal cell, and a two-celled, vertically divided head.

4-Subgenus: *Psyllium*

Species: *Plantago afra* L., ***P. exigua*** Murry., ***P.indica*** L., ***P. phaeostoma*** Boiss.& Heldr., ***P. sinaica*** (Barnéoud) Decne. Figs. 4&5 (14-18)

Leaves amphistomatic, with anomocytic, diacytic, and anisocytic stomata. The ad/abaxial lamina surfaces revealed a rugulate and nodulate ornamentation pattern, with raised anticlinal and flat periclinal cell walls. Both ad/abaxial lamina surfaces of *Plantago afra*, *P. indica, P. phaeostoma*, and *P.sinaica* are densely hairy, covered by non-glandular and glandular trichomes. However, the ad/abaxial surfaces of *Plantago exigua* covered by glandular trichomes. The non-glandular trichomes bristle-like, with a stalk that is either unicellular or multicellular, a bulgy basal cell, and covered by a verrucate cuticle. Glandular trichomes have two

shapes: clavate and capitate (Fig.6). Clavate glandular trichomes characterized by a unicellular stalk, a normal basal cell, and a two-celled horizontally divided head. Capitate trichome characterized by a unicellular head, a multicellular stalk, and a bulgy basal cell.

B-Phenetic analysis

The obtained dendrogram resulting from the cluster analysis of ٥١ lamina epidermal character states showed that *Plantago major* subspecies *major* (GI) segregated at a similarity level of 0.50 (Fig.7).

The remaining 17 *Plantago* species (GII) were further subdivided at a similarity level of 0.57 into three subgroups: SG1, SG2, and SG3. Subgroup (SG1) included *Plantago albicans*, *P. ciliate*, *P. cylindrica*, *P. notata*, *P. ovata*, *P. amplexicaulis*, *P. lagopus,* and *P. lanceolata*, while subgroup (SG2) included *Plantago coronopus*, *P. crassifolia*, *P. crypsoides,* and *P. weldenii*. SG1 is further divided into two clusters (C1 and C2) at a similarity level of 0.69. Cluster 1 gathered *Plantago albicans, P. ciliate, P. cylindrica, P. notata, P. ovata*, and *P. amplexicaulis,* while cluster 2 included *Plantago lagopus and P. lanceolata*. SG2 comprised *Plantago coronopus, P. crassifolia, P. crypsoides,* and *P. weldenii*. SG3 comprised *P. afra, P. exigua*, *P. indica, P. phaeostoma,* and *P. sinaica.*

MST analysis is performed to reveal the morphological affinity among the studied taxa of *Plantago*. The MST plot shown in Fig.8 indicates that the studied *Plantago* taxa are divisible into four groups: G1 comprised *Plantago afra, P. exigua*, *P. indica, P. phaeostoma,* and *P. sinaica* (subgenus *Psyllium*); G2 included *Plantago coronopus*, *P. crassifolia*, *P. crypsoides,* and *P. weldenii* (subgenus *Coronopus*)*;* G3 comprised *Plantago albicans, P. ciliate, P. cylindrica, P. notata, P. ovata*, and *P. amplexicaulis* (subgenus *Albicans*), and G4 comprised *Plantago major* (subgenus *Plantago*).

Discussion

The present study provides additional insights into the lamina epidermal micro-morphological characteristics of Egyptian *Plantago* taxa. The lamina epidermal features namely: stomatal type, the configuration of anticlinal and periclinal epidermal cell walls, cuticular ornamentation, and trichome morphology showed notable variation among the examined taxa.

All studied taxa were found to be amphistomatic. SEM observations revealed that stomata varied in level, ranging from superficial to slightly sunken. Several types of mature stomata were identified on both the adaxial and abaxial surfaces, including anomocytic, anisocytic, and diacytic types. These findings are consistent with those of Bahadar *et al.* (2018) and Mesquita *et al.* (2017), who reported the coexistence of different stomatal types on the same lamina surface in *Plantago* species. Four distinct cuticular ornamentation patterns were observed on the adaxial and abaxial surfaces (ruminate, rugulate, striate, and rugulate/nodulate).

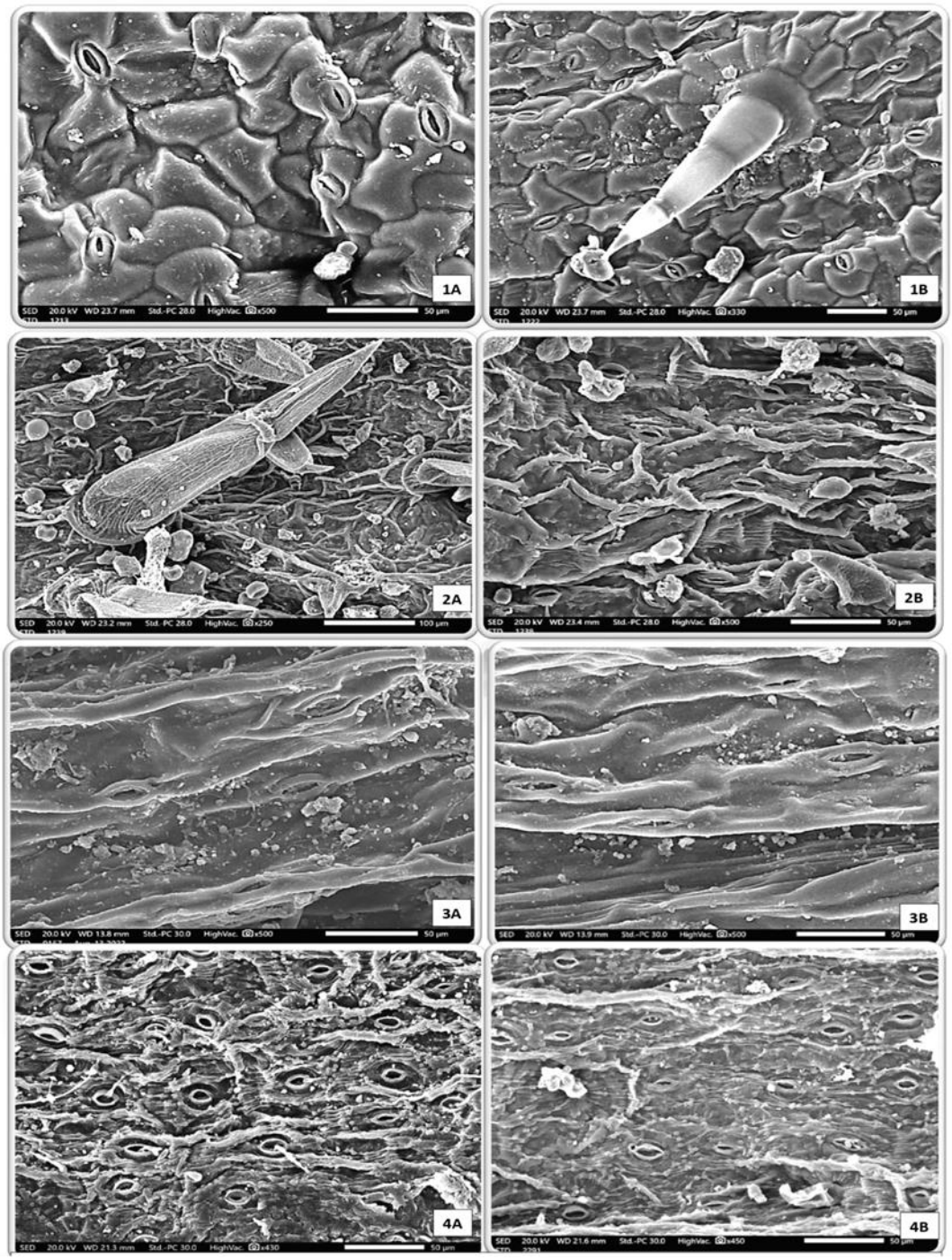

Fig. 1. SEM micrographs of adaxial (A) and abaxial (B) leaf surfaces (١A, ١B) *Plantago major* (2A, 2B*) Plantago coronopus;* (3A, 3B) *Plantago crassifolia;* (4A, 4B) *Plantago crypsoides.*

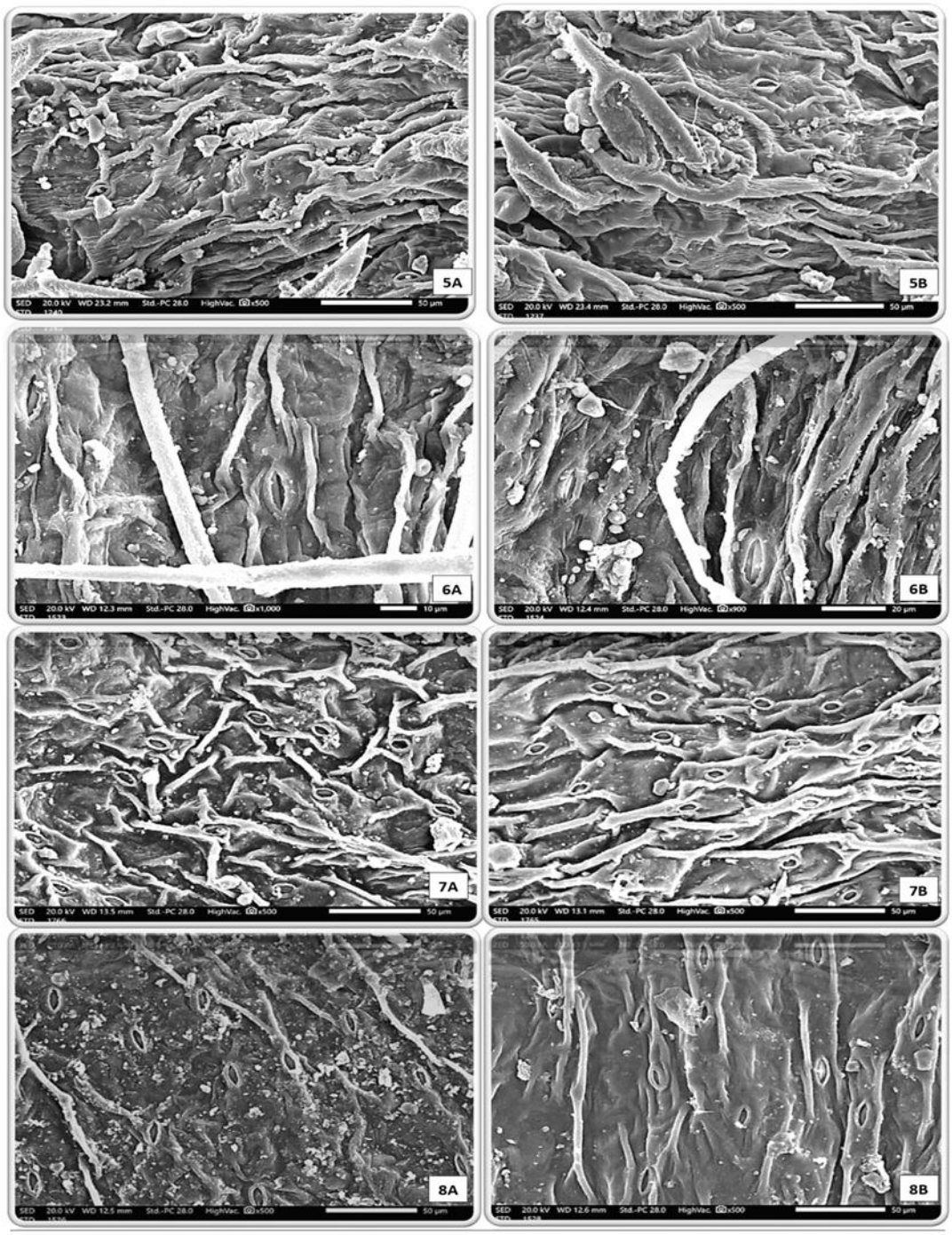

Fig.2. SEM micrographs of adaxial (A) and abaxial (B) leaf surfaces. ((5A, 5B), *Plantago weldenii*; (6A, 6 B) *Plantago amplexicaulis;* (7A, 7B) *Plantago lanceolata*; (8A, 8B) *Plantago lagopus.*

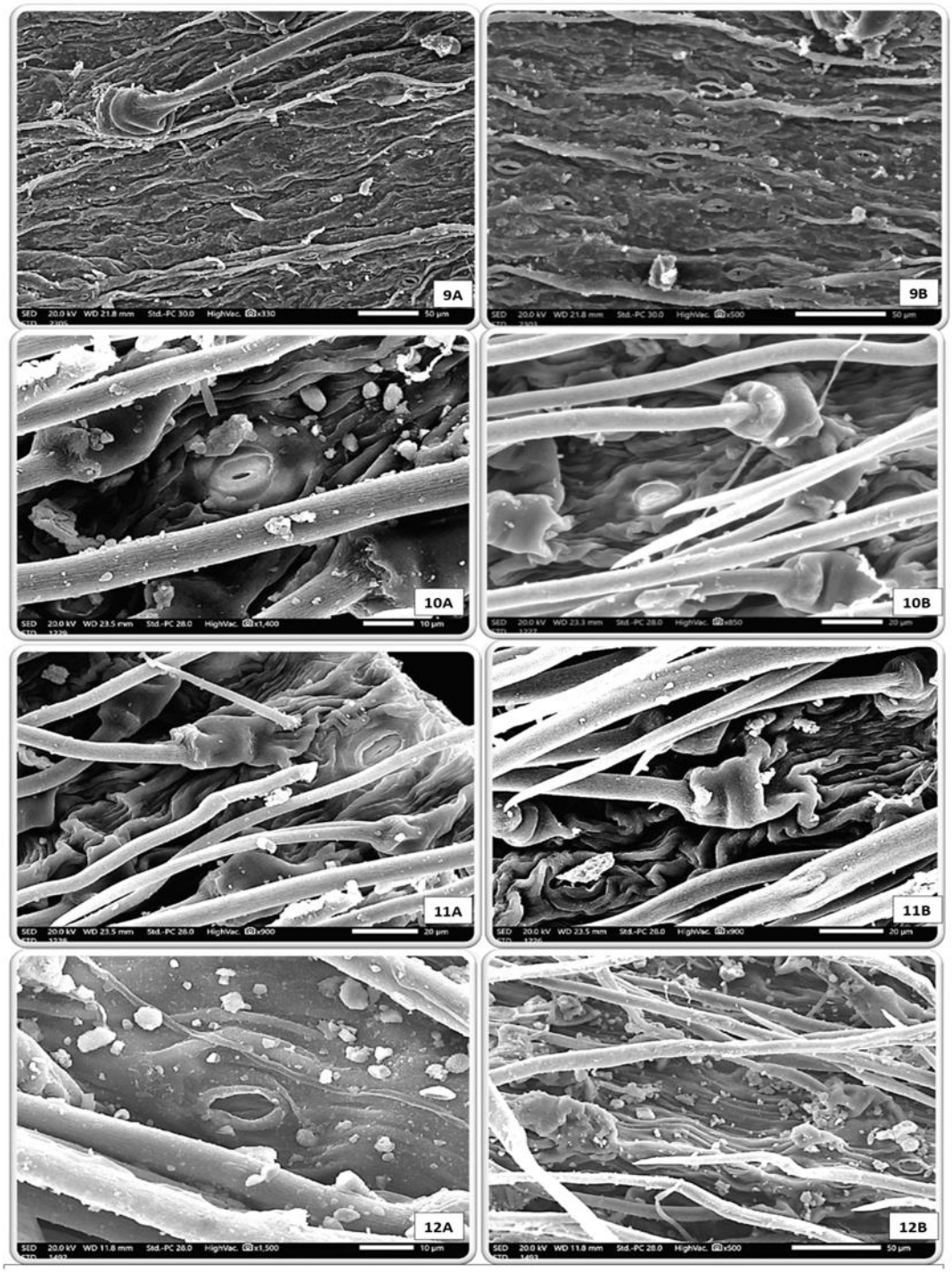

Fig.3. SEM micrographs of adaxial (A) and abaxial (B) leaf surfaces. (9A, 9B), *Plantago notata;* (10A,10B) *Plantago albicans*; (11A, 11B) *Plantago ciliata;* (12A, 12 B) *Plantago cylindrica*.

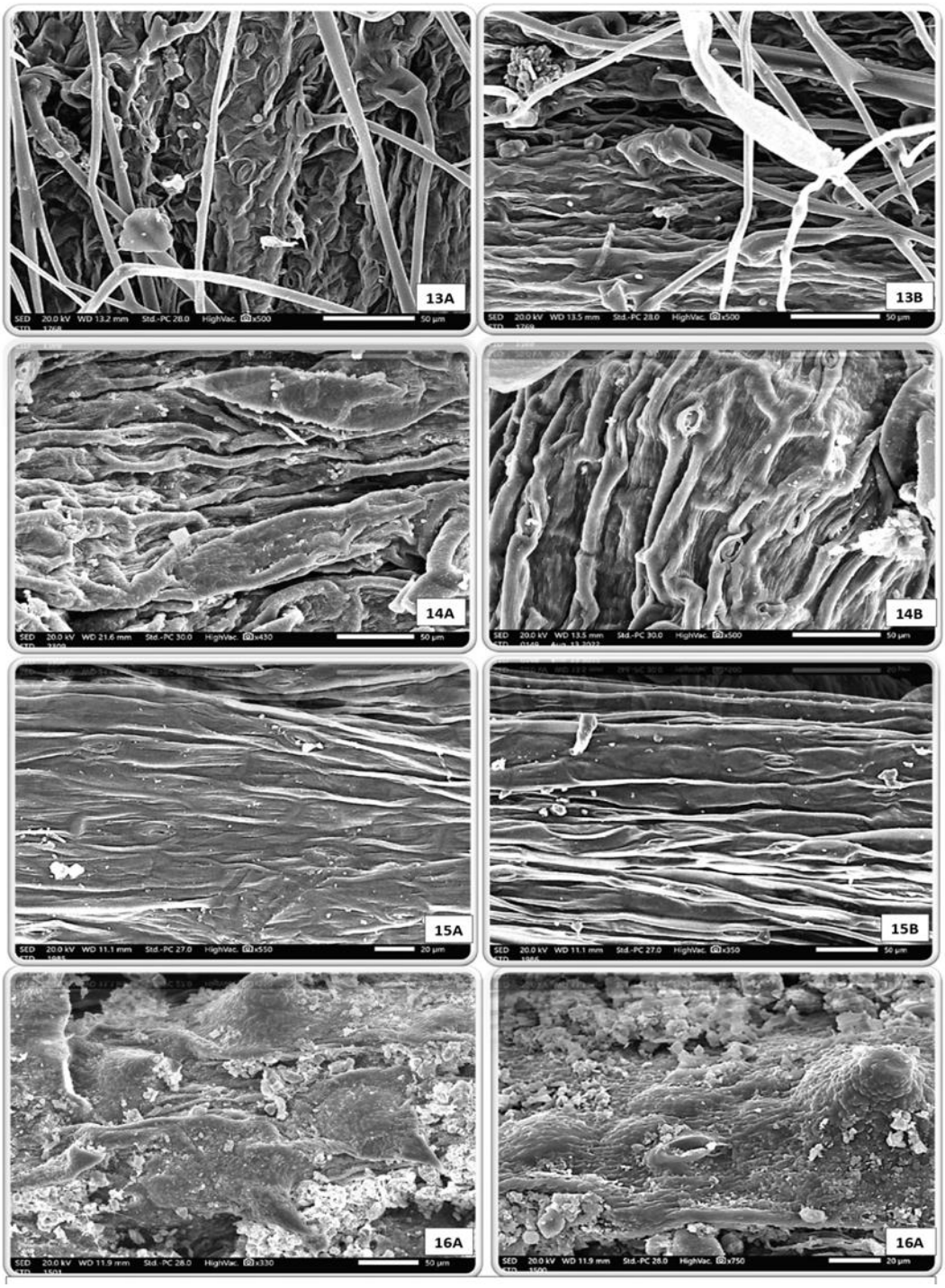

Fig. 4. SEM micrographs of Adaxial (A) and Abaxial (B) leaf surfaces. (13A, 13B) *Plantago ovata*; (14A, 14B) *Plantago afra*; (15A, 15B), *Plantago exigua; (*16A, 16B) *Plantago indica.*

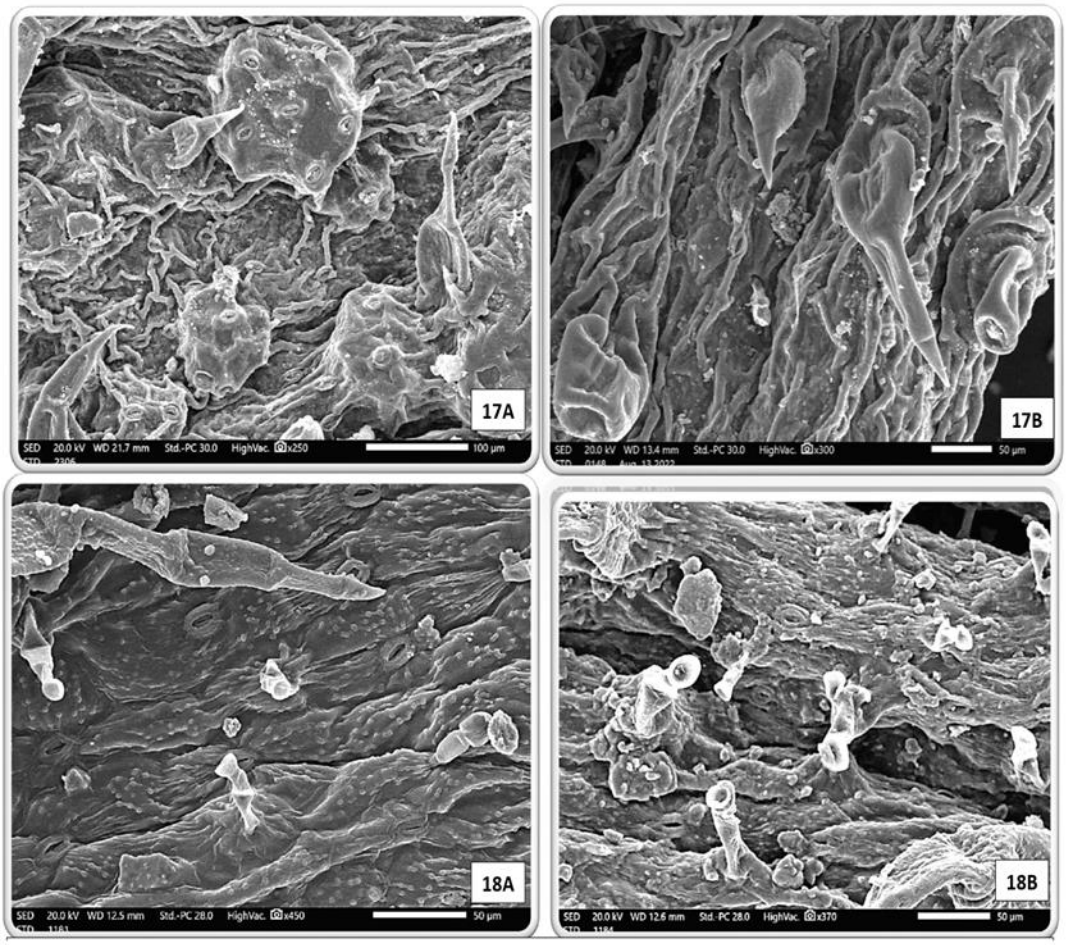

Fig. 5. SEM micrographs of adaxial (A) and abaxial (B) leaf surfaces. (17A, 17B) *Plantago phaeostoma*; (18A,18 B) *Plantago sinaica*.

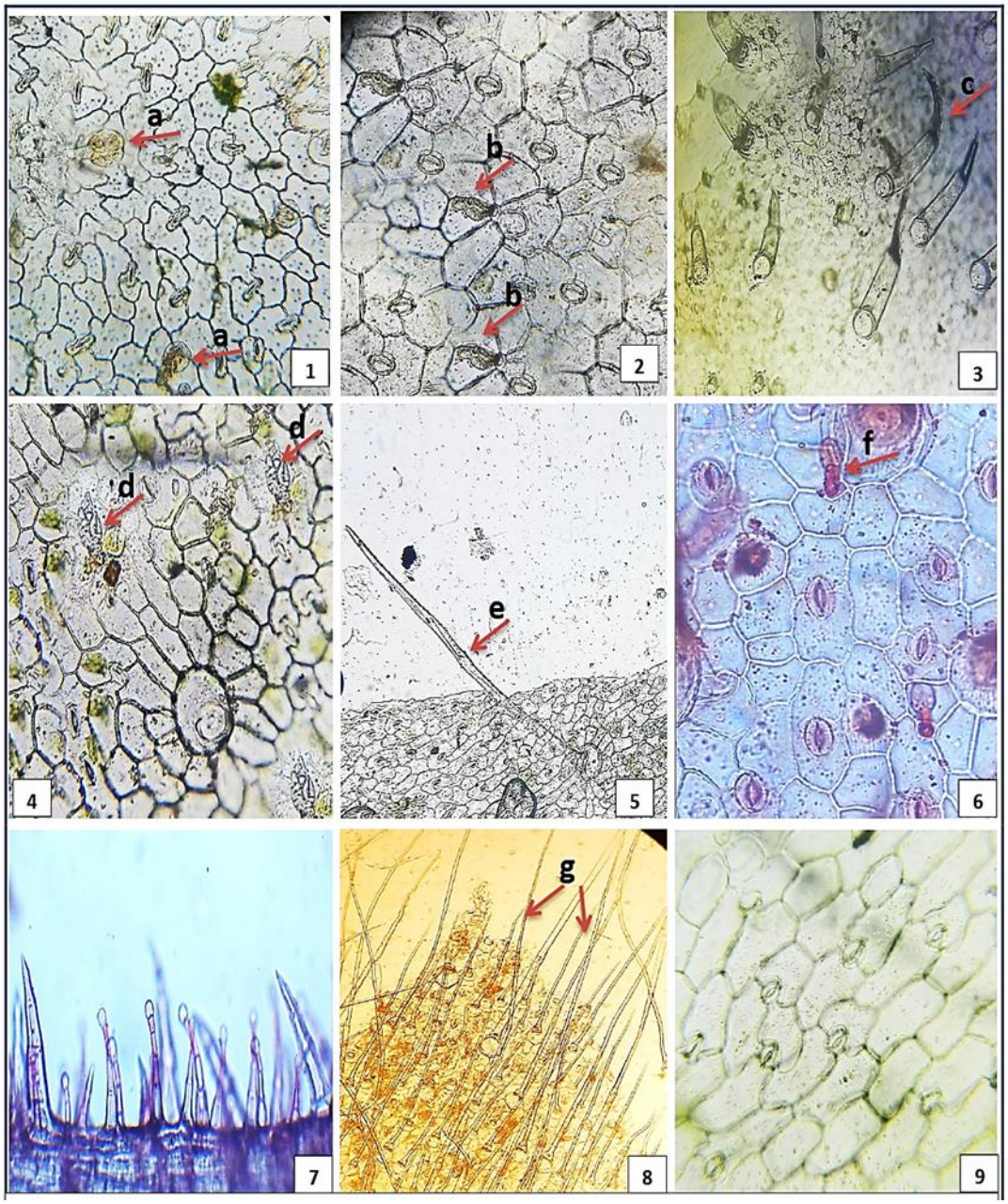

Fig. 6. Light micrograph of lamina epidermis, showing stomata types and trichomes. (1), *Plantago major;* (2, 3) *Plantago coronopus;* (4, 5) *Plantago lanceolata*; (6, 7) *Plantago sinaica*; (8, 9) *Plantago ciliata.* a, Brevicollate glandular trichomes; b, Bottle-shaped glandular trichomes; c, Bristle-shaped non-glandular trichomes; d, Morel-shaped with several head cell glandular trichomes; e, Web-like1 multicellular non-glandular trichomes with Jammed cells; f, Clavate shape glandular trichomes; g, Web-like glandular trichomes.

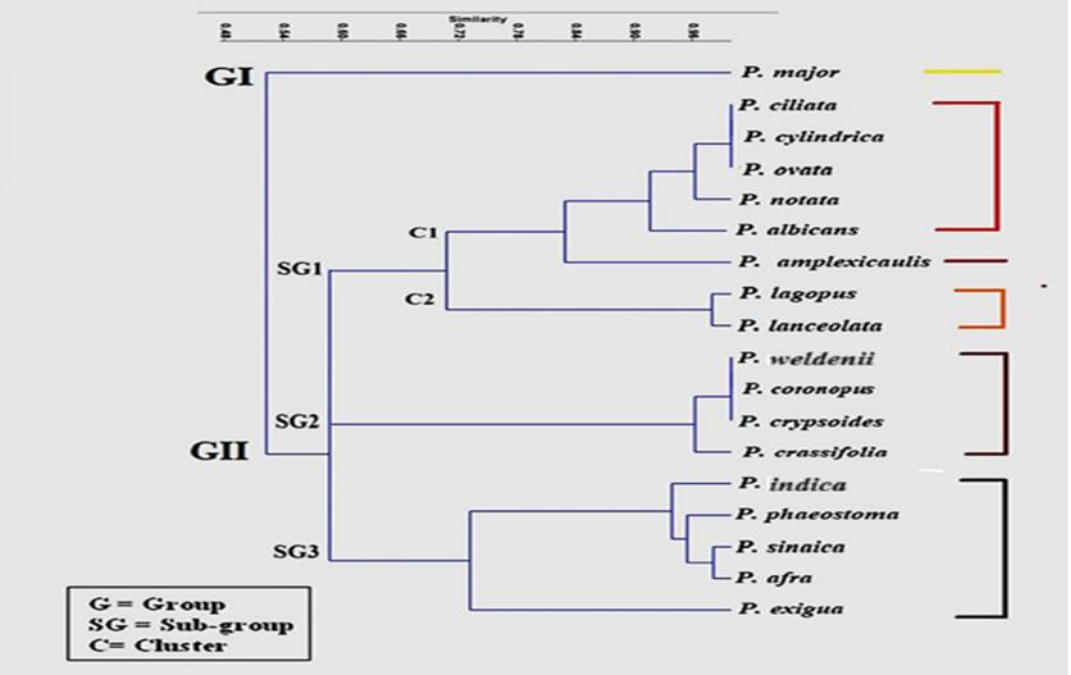

Fig. 7. A hierarchical clustering dendrogram shows the relationship between the 18 *Plantago* taxa.

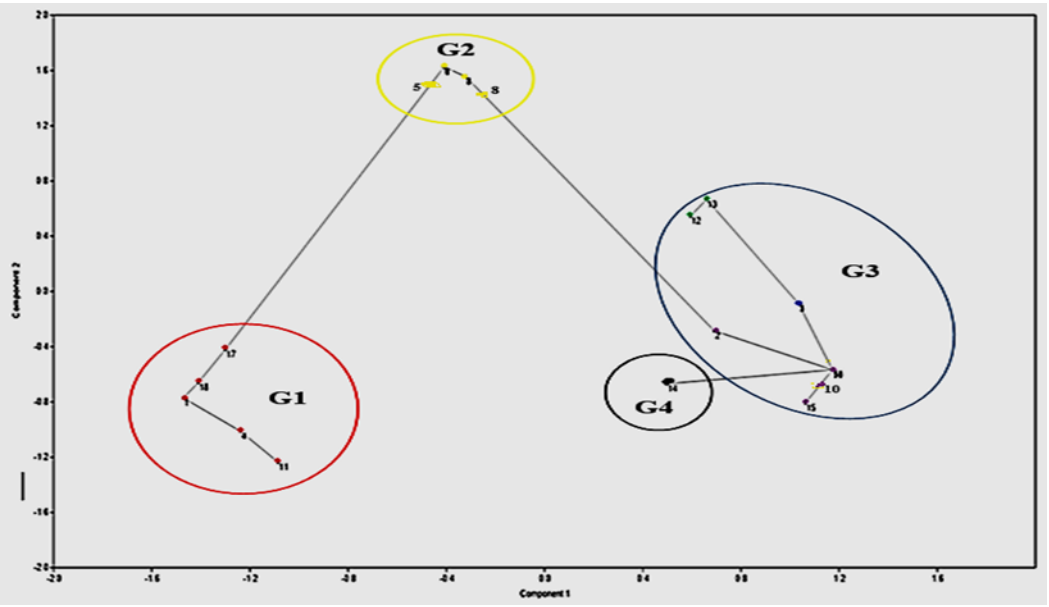

Fig. 8. A hierarchical clustering dendrogram shows the relationship between the 18 *Plantago* taxa.

Table 2. Lamina micro-morphological characters of the studied *Plantago* taxa. Abbreviations: Anisocytic=Ans, Anomocytic=Ano, Diacytic=Di, Sub-glabrescent/ Sparsely Hairy=Sg/Sh.

Characters / Taxa	Stomata Type (Adaxial &Abaxial)			Wax Density	Overall Cuticular pattern	Anticlinal cell walls	Periclinal cell Walls	Hairiness	Trichomes Types
	Ans	Ano	Di						
Plantago major	++	+++	+	Sparse	Ruminate	Channeled	Convex	Sparsely Hairy	Glandular/Non-Glandular
P. coronopus	+	++	+++	Dense	Rugulate	Raised	Flat	Densely Hairy	Glandular/Non-Glandular
P. crassifolia	+	++	+++	Dense	Rugulate	Raised	Flat	Sparsely Hairy	Glandular/Non-Glandular
P. crypsoides	+	++	+++	Dense	Rugulate	Raised	Flat	Densely Hairy	Glandular/Non-Glandular
P. weldenii	+	++	+++	Dense	Rugulate	Raised	Flat	Densely Hairy	Glandular/Non-Glandular
P. amplexicaulis	+	++	+++	Dense	Rugulate	Raised	Flat	Densely Hairy	Glandular/Non-Glandular
P. lagopus	+	++	++	Dense	Rugulate	Raised	Flat	Sg/Sh	Glandular/Non-Glandular
P. lanceolata	+	++	++	Dense	Rugulate	Raised	Flat	Sg/Sh	Glandular/Non-Glandular
P. albicans	+	++	+++	Dense	Striate	Raised	Flat	Villous	Glandular/Non-Glandular
P. ciliata	+	++	+++	Dense	Striate	Raised	Flat	Villous	Glandular/Non-Glandular
P. cylindrica	+	++	+++	Dense	Striate	Raised	Flat	Villous	Glandular/Non-Glandular
P. notata	+	++	+++	Dense	Striate	Raised	Flat	Villous	Glandular/Non-Glandular
P. ovata	+	++	+++	Dense	Striate	Raised	Flat	Villous	Glandular/Non-Glandular
P. afra	+	+++	++	Dense	Rugulate/ Nodulate	Raised	Flat	Densely Hairy	Glandular/Non-Glandular
P. exigua	+	+++	++	Sparse	Rugulate / Nodulate	Raised	Flat	Sparsely Hairy	Glandular
P. indica	+	+++	++	Dense	Rugulate/ Nodulate	Raised	Flat	Densely Hairy	Glandular/Non-Glandular
P. phaeostoma	+	+++	++	Dense	Rugulate / Nodulate	Raised	Flat	Densely Hairy	Glandular/Non-Glandular
P. sinaica	+	+++	++	Dense	Rugulate / Nodulate	Raised	Flat	Densely Hairy	Glandular/Non-Glandular

Table 3. Micro-morphological characteristics of Lamina trichomes of the studied *Plantago* taxa.
Abbreviations: Abundance =A**,** Bicellular=Bi, Multicellular= Mu, Unicellular= Un., *= Rare, +

Characters / Taxa	Trichomes (Adaxial &Abaxial)										
	Glandular Trichomes						Non-Glandular Trichomes				Cuticle Pattern
	A	Shape	Basal Cell	Stalk Cells	Head Cell		A	Shape	Stalk Cells	Basal Cell	
					No.	Division					
Plantago major	++	Brevicollate	Normal	Un.	2	Vertical	+	Bristle	Mu.	Normal	Smooth
P. coronopus	+	Bottle Shape	Normal	Un.	Several	-	+++	Bristle Web -like	Uni/ Bi Mu.	Bulgy	Nodulate
P. crassifolia	+	Bottle Shape	Normal	Un.	Several	-	+	Bristle Web -Like	Uni/ Bi Mu.	Bulgy	Nodulate
P. crypsoides	+	Bottle Shape	Normal	Un.	Several	-	+++	Bristle Web -like	Uni/ Bi Mu.	Bulgy	Nodulate
P. weldenii	+	Bottle Shape	Normal	Un.	Several	-		Bristle Web -Like	Uni/ Bi Mu.	Bulgy	Nodulate
P. amplexicaulis	*	Brevicollate	Normal	Un.	2	Vertical	+++	Web- like	Mu.	Square	Striate
P. lagopus	+	Morel Shaped	Normal	Un.	Several	-	+	Web- like	Mu.	Square	Striate
P. lanceolata	+	Morel Shaped	Normal	Un.	Several	-	+	Web Like	Mu.	Square	Striate
P. albicans	*	Brevicollate	Normal	Un.	2	Vertical	+++	Web Like	Bi	Bulgy	Striate
P. ciliata	*	Brevicollate	Normal	Un.	2	Vertical	+++	Web Like	Bi	Bulgy	Striate
P. cylindrica	*	Brevicollate	Normal	Un.	2	Vertical	+++	Web Like	Bi	Bulgy	Striate

Table 3. Continued.

Characters / Taxa	Trichomes (Adaxial &Abaxial)										
	Glandular Trichomes						Non-Glandular Trichomes				
	A	Shape	Basal Cell	Stalk Cells	Head Cell		A	Shape	Stalk Cells	Basal Cell	Cuticle Pattern
					No.	Division					
P. notata	*	Brevicollate	Normal	Un.	2	Vertical	+++	Web Like	Bi	Bulgy	Striate
P. ovata	*	Brevicollate	Normal	Un.	2	Vertical	+++	Web Like	Bi	Bulgy	Striate
P. afra	++	Clavate	Normal	Un.	2	Horizontal	+++	Bristle	Un./ Mu.	Bulgy	Verrucate
		Capitate	Barrel	Mu.	1	-					
P. exigua	+	Clavate	Normal	Un.	2	Horizontal	-	-	-	-	-
		Capitate	Barrel	Mu.	1	-					
P. indica	++	Clavate	Normal	Un.	2	Horizontal	+++	Hispid / Bristle	Un.	Bulgy	Verrucate
		Capitate	Barrel	Mu.	1	-			Mu.		
P. phaeostoma	++	Clavate	Normal	Un.	2	Horizontal	+++	Hispid/ Bristle	Un.	Bulgy	Verrucate
		Capitate	Barrel	Mu.	1	-		Web Like	Mu.		
P. sinaica	++	Clavate	Normal	Un.	2	Horizontal	+++	Hispid/ Bristle	Un.	Bulgy	Verrucate
		Capitate	Barrel	Mu.	1	-		Web Like	Mu.		

The configuration of the epidermal cell walls also showed taxonomically informative differences, in agreement with previous studies emphasizing their diagnostic significance at the specific level (Barthlott, 1981; Bello *et al.,* 2017). The lamina epidermis of the studied *Plantago* taxa displayed slightly undulate to straight anticlinal walls and flat to convex periclinal walls. *Plantago major* subsp. *major* was clearly delimited from the other taxa by its straight, channeled anticlinal walls and convex periclinal walls.

Trichomes, recognized as key taxonomic characters in anatomical studies (Metcalfe & Chalk, 1950; Cutler, 1969; Hardin, 1979), exhibited marked diversity among the examined taxa. All investigated taxa possessed both glandular and non-glandular trichomes on the adaxial and abaxial surfaces, except *P. exigua*, which bore only glandular trichomes. Glandular trichomes exhibited a range of morphologies including brevicollate, bottle-shaped, clavate, and capitate forms, whereas non-glandular trichomes were either bristle-like or web-like. These observations agree with the findings of Andrzejewska-Golec and Świętosławski (1987, 1989 a/b, 1991, 1993), who described similar trichome diversity across different *Plantago* sections.

In addition to type, trichomes differed considerably in length and surface ornamentation. Their lengths ranged from very short to relatively long, and SEM analysis revealed four ornamentation patterns: smooth, striate, nodulate, and verrucate. *Plantago major* subsp. *major* was distinguished by its smooth trichomes. The taxonomic importance of trichome ornamentation has previously been demonstrated in *Fagonia* taxa (Gabr *et al*., 2013), and the present findings reinforce its diagnostic value for *Plantago*.

Taxonomic Implications of Lamina Epidermal Micro-Morphological Traits

Cluster analysis based on lamina epidermal features was employed to elucidate phenetic relationships among the studied *Plantago* taxa. The resulting dendrogram (Fig.7) and MST plot (Fig.8) were interpreted in relation to the infrageneric classification systems of Pilger (1937) and Rahn (1978, 1996). The analysis revealed the following main groupings:

1. Separation of *Plantago major* subsp. *major* (G1). This taxon was clearly segregated from all other species (G2) as a distinct phenon. It is characterized by ruminate cuticular ornamentation, channeled anticlinal and convex periclinal walls, and short capitate glandular (brevicollate) and bristle-like non-glandular trichomes. This distinctiveness supports its placement in subgenus *Plantago*, as proposed by Pilger (1937), Rahn (1978, 1996), and Rønsted *et al.* (2002).

2. Cluster of *P. albicans*, *P. ciliata*, *P. cylindrica*, *P. notata*, *P. ovata*, and *P. amplexicaulis*. These species form a coherent group characterized by raised anticlinal and flat periclinal walls, villous adaxial and abaxial surfaces, brevicollate glandular trichomes with a normal basal cell, unicellular stalk, and two-celled vertically divided head, and web-like striated non-glandular trichomes. This grouping supports their inclusion within subgenus *Albicans* (Rahn, 1996), in

agreement with Shalabi and Abou-El-Enain (2013) and Shehata *et al.* (2023b), but contrasts with Rønsted *et al.* (2002), who regarded subgenus *Albicans* as paraphyletic.

3. Distinct position of *P. amplexicaulis*. Although previously grouped with *Albicans* species, *P. amplexicaulis* was separated based on its rugulate cuticular ornamentation and web-like non-glandular trichomes with multicellular jammed stalks. This supports Rahn's (1996) classification, which treated it as the sole member of the monotypic section *Bauphula* of subgenus *Albicans*, but contradicts Rahn (1978), who placed it, in subgenus *Psyllium*. Similar conclusions were reached by Mohsenzadeh *et al.* (2020) and Shehata *et al.* (2023b).

4. Association of *P. lanceolata* and *P. lagopus*. The clustering of these two species reflects their close morphological affinity. They share sub-glabrescent to sparsely hairy leaves, rugulate ornamentation, raised anticlinal and flat periclinal walls, long web-like non-glandular trichomes with striated cuticles, and short, morel-like glandular trichomes with smooth cuticles. Their grouping supports their placement in subgenus *Albicans*; section *Lanceifolia*, following Rahn (1996). This relationship is further supported by chemical data (Taskova *et al.*, 2002), morphological and molecular data (Mohsenzadeh *et al*., 2020), and fruit and seed morphology (Shehata *et al*., 2023a/b).

5. Cluster of *P. coronopus*, *P. crassifolia*, *P. crypsoides*, and *P. weldenii*. These taxa share rugulate cuticular ornamentation, raised anticlinal and flat periclinal walls, bottle-shaped glandular trichomes with unicellular stalks and multicellular heads, and bristle-like and web-like non-glandular trichomes with nodulate surfaces. This cluster corresponds to subgenus *Coronopus*, section *Coronopus*, as delimited by Rahn (1978/ 1996) and Rønsted *et al.* (2002).

6. Cluster of *P. afra*, *P. exigua*, *P. indica*, *P. phaeostoma*, and *P. sinaica*. These species are characterized by rugulate/nodulate cuticular ornamentation, short clavate glandular trichomes with unicellular stalks and two-celled horizontally divided heads, and capitate glandular trichomes with barrel-like basal cells and multicellular stalks. Their grouping supports their placement within subgenus *Psyllium* sensu Rahn (1996).

The MST analysis (Fig.8) further confirmed the organization of the studied taxa into four principal groups corresponding to the four subgenera: *Plantago*, *Coronopus*, *Albicans*, and *Psyllium*, in strong agreement with Rahn's (1996) classification.

Conclusion

The present study demonstrates that lamina epidermal micro-morphological features, particularly trichome characteristics which provide valuable diagnostic information for distinguishing *Plantago* species and species groups when considered in combination. Despite the limited number of taxa analyzed, the phenetic analyses support Rahn's (1996) infrageneric classification, especially at the sectional level. Further research incorporating additional taxa and

supplementary data sources (e.g., anatomical, palynological, and molecular evidence) is recommended to achieve a more comprehensive understanding of the infrageneric relationships within the genus *Plantago.*

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