

Systematic implications of fruits and seeds of *Plantaginaceae* and some related genera of *Scrophulariaceae*

Azza A. Shehata¹, Wafaa K. Taia², Manaser K. Ibrahim³ & Islam M. El-Shamy⁴

¹ Botany and Microbiology Department, Faculty of Science, University of Alexandria, 21511 Moharrem Bey, Alexandria, Egypt. e-mail: dr_azzashehata@yahoo.com (corresponding author)

² Botany and Microbiology Department, Faculty of Science, University of Alexandria, 21511 Moharrem Bey, Alexandria, Egypt. e-mail: taitaxonmy88@gmail.com

³ Botany and Microbiology Department, Faculty of Science, University of Alexandria, 21511 Moharrem Bey, Alexandria, Egypt. e-mail: manaser99@yahoo.com

⁴ Botany and Microbiology Department, Faculty of Science, University of Alexandria, 21511 Moharrem Bey, Alexandria, Egypt. e-mail: eslamelshamy510@yahoo.com

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Abstract. Fruit and seed macro- and micromorphological analyses were carried out of 18 *Plantago* species representing two subgenera (*Euplantago* and *Psyllium*), and 18 taxa representing nine genera related to *Scrophulariaceae* sensu lato (s.l.) were investigated by light microscopy (LM) and scanning electron microscopy (SEM). The main aim of this study was by studying the fruit and seed morphological characters to investigate the phenetic relationships among *Plantago* species, as well as between certain taxa of *Plantaginaceae* sensu lato (s.l.) formerly assigned to the family *Scrophulariaceae*. The phenetic analysis of fruit and seed morphological characters of all studied taxa produced a phenogram that showed two main series: the first comprising the *Plantago* species [*Plantaginaceae* sensu stricto (s.str.)] and the second including the studied *Scrophulariaceae* taxa. The resulting phenogram supported the retaining of the studied *Scrophulariaceae* taxa within the family *Scrophulariaceae* s.l. and *Plantago* in a distinct monogeneric family *Plantaginaceae* s.str., and was incompatible with the new circumscription of *Plantaginaceae* s.l. Moreover, the present findings were in line to a certain extent with Shipunov's infrageneric classification of *Plantago*.

Key words: Fruit, morphology, numerical analysis, *Plantago*, *Plantaginaceae*, *Scrophulariaceae*, seed, SEM

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Introduction

Most traditional taxonomic treatments have considered the family *Plantaginaceae* s.str. as including

three genera: *Plantago*, *Littorella* and *Bougueria*, with about 275 species distributed in different habitats across the world (Bentham and Hooker 1876; Cronquist 1981; Heywood 1993; Takhtajan 1997). On the

other hand, Rahn (1996) considered it a monogeneric family, only with *Plantago* in it. *Plantaginaceae* was represented in the flora of Egypt by the genus *Plantago*, which includes 21 species and seven varieties (Täckholm 1974). El Hadidi & Fayed (1995), and Boulos (2002) reported the occurrence of 22 species.

The affinity of the *Plantaginaceae* has been doubtful; Bentham & Hooker (1876) considered the family as representing an anomalous group. Gaebel (1933) assigned it close to the family *Verbenaceae*. Several authors have emphasized a close relationship between the *Plantaginaceae* and *Scrophulariaceae* (Takhtajan 1980; Heywood 1993; Mabberley 1997). Ever since, there has been an ongoing controversy about their taxonomic relationships. Based on the limited molecular, anatomical and chemical evidence, Judd & al. (1999) treated the two families as one.

The concept of *Plantaginaceae* s.str. has changed considerably since the application of molecular approaches in plant systematics (Olmstead & al. 2001). It has been greatly expanded in the Angiosperm Phylogeny Group classifications (APG II 2003, APG III 2009) to include many genera that have been previously assigned to the family *Scrophulariaceae* s.l. (*Anarrhinum*, *Antirrhinum*, *Digitalis*, *Kickxia*, *Linaria*, *Scoparia*, *Russelia*, *Veronica* among others). The expanded *Plantaginaceae* is a heterogeneous family with different evolutionary trends, comprising about 92 genera and 2000 species classified under 12 tribes (APGII 2003). The newly demarcated *Plantaginaceae* family is challenging and the inter- and intraspecific relationships between its genera, as well as with the nearby families need to be elucidated, while several important problems remain obscure and unsolved (Albach & al. 2004).

Plantago is one of the greatest and most widespread genera of the family *Plantaginaceae* s. str., with over 200 species, mostly distributed in the tropical and subtropical regions (Hoggard & al. 2003). Some species of the genus *Plantago* have been used in both conventional and traditional systems of medicine across the world, because of their different medicinal properties (Goncalves & Romano 2016). The taxonomy of this important genus is complex. Its species delimitation is challenging due to reduced morphology and lack of useful taxonomic characters for species identi-

fication and classification. Moreover, it has a number of challenging nomenclature issues that have not been resolved yet (Ishikawa & al. 2009; Hassemer 2018).

In terms of infrageneric classification, Pilger (1937), subdivided the genus *Plantago* into two subgenera, *Plantago* and *Psyllium*, whereas, Rahn (1978) had initially divided it into three, namely *Plantago*, *Cornopus* and *Psyllium*. Subsequently, Rahn (1996) proposed a new infrageneric classification that included six subgenera: *Plantago*, *Cornopus*, *Albicans*, *Psyllium*, *Littorella*, and *Bougueria*. In spite of the multiple molecular phylogenetic studies, disagreement still persisted regarding its species delimitation. Sectional position of some *Plantago* species has been ambiguous and its infrageneric classification controversial, inadequate and in a great need of taxonomic reconsideration (Di Pietro & al. 2013a; Shipunov 2021; Hassemer 2018).

Fruit and seed morphology has been long recognized as important for classification and can be successfully used in identification, taxonomic circumscription and phylogenetic implication (Arambarri 2000; Mazur & al. 2021; among others). Both macro- and micromorphological seed characters have been proven of essential systematic importance within and among the genera of the traditional *Scrophulariaceae* and *Plantaginaceae* families (Shehata & Loutfy 2006; Ahedor & Elisens 2015; Mohsenzadeh & al. 2020; Al Masoudi & al. 2021), where seed morphological characters have been widely used to differentiate the various taxa or to trace the affinities between them. Seed morphology is valuable for understanding the taxonomic and phylogenetic relationships of the *Plantago* species (Shehata & Loutfy 2006; Verma & al. 2017; Mohsenzadeh & al. 2023).

The aim of this study was to demonstrate and document variability in fruit and seed morphological traits in the genus *Plantago* and some genera of the family *Scrophulariaceae* in an attempt to evaluate their taxonomic implications for the systematics of *Plantaginaceae* s.l., with a focus on the systematics of genus *Plantago*. The results have been discussed in the light of the current systems of classification of *Plantaginaceae* (sensu Judd & al. 1999; Albach & al. 2004; APG III 2009) and infrageneric classifications of the genus *Plantago* (Pilger 1937; Rahn 1978, 1996).

Material and methods

Plant materials

The present study has been conducted on mature fruits and seeds of 36 taxa that were freshly collected from different places in Egypt. A list of the plants and their localities is presented in Table 1. The studied material included 18 taxa belonging to nine genera of *Scrophulariaceae* s.l., viz. *Anarrhinum*, *Antirrhinum*, *Digitalis*, *Kickxia*, *Linaria*, *Russelia*, *Scrophularia*, *Verbascum*, *Veronica*, and 18 species of *Plantago* corresponding to six sections of

two subgenera, (*Euplantago* Harms and *Psyllium* (Juss.) Harms), according to Pilger (1937). The collected taxa represented 32 wild and four cultivated species; the wild species were identified after Täckholm (1974) and Boulos (2002), while the cultivated species were identified after Bailey (1949). Nomenclature of the investigated taxa was updated according to the World Flora Online (WFO) (<https://www.worldfloraonline.org/classification>). Voucher specimens of the studied taxa were kept in the herbarium of Botany and Microbiology Department, University of Alexandria, Alexandria, Egypt.

Table 1. List of the studied taxa and their localities.

No.	Taxa	Localities and Geographical Coordinates
1	<i>Anarrhinum forskahlii</i> subsp. <i>pubescens</i> (Fresen.) D.A.Sutton. Rev. Antirrh. : 258 (1988)	Wadi Al Talah, Saint Katherine, Egypt. (28°55'20.71" N // 33° 93'45.55" E).
2	<i>Antirrhinum majus</i> L., Sp. Pl. 617 (1753).	Botanical Garden, Faculty of Science, Alexandria University. (31°12'26" N // 29° 55'08" E).
3	<i>Digitalis lanata</i> Ehrh., Beitr. Naturk. [Ehrhart] 7: 152 (1792).	Mashtal Faculty of Pharmacy, Cairo University, Saft Al-Laban, Cairo, Egypt. (30° 01'49" N // 31°11'42" E).
4	<i>Nanorrhinum acerbianum</i> (Boiss.) Betsche, Courier Forschungsinst. Senckenberg 71: 132 (1984); (1984).	Wadi Al Talah, Saint Katherine, Egypt. (28°57'13.66" N // 33°93'21.19" E).
5	<i>Kickxia aegyptiaca</i> (L.) Nábelek, Publ. Fac. Sci. Univ. Masaryk Brno 70: 31 (1926).	Borg El Arab, Alexandria, Egypt. (31°00'22.11" N // 29°43'54.94" E).
6	<i>Linaria haelava</i> (Forssk.) Delile, Descer, Egypte, Hist.Nat.66 (1814)	Khashm Al-Aish Plateau, OmayedMarsa Matroh road, Egypt. (30°45'18" N//29°12'17" E).
7	<i>Linaria micrantha</i> (Cav.) Hoffmanns. & Link. Fl. Portug. 1: 258 (1811).	Khashm Al-Aish Plateau, OmayedMarsa Matroh road, Egypt. (30°45'18" N//29°12'17" E).
8	<i>Linaria simplex</i> Desf., Tabl. École Bot. 65 (1804).	Borg El Arab, Alexandria, Egypt. (31°00'22.11" N // 29°43'54.91" E).
9	<i>Plantago afra</i> L., Sp. Pl., ed. 2. 1: 168 (1762).	Wadi El-Arbeen, Saint Katherine, Egypt. (28°32'66.63" N// 33°56'02.72" E).
10	<i>Plantago albicans</i> L., Sp., Pl., ed.1, 114 (1753).	Wadi Al-Aqara, Matrouh, Egypt. (31°21'18.22" N//27°02'51.55" E).
11	<i>Plantago amplexicaulis</i> Cav., Icon. [Cavanilles] ii. 22. t. 125.	Al King Maryout, Amreya, Alexandria, Egypt. (30°00'84.87" N//29°73'33.01" E).
12	<i>Plantago indica</i> L. Syst. Nat., ed. 10. 2: 896 (1759).	Edko, Beheira, Egypt. (31°16'35.86" N//30°13'31.90" E).
13	<i>Plantago ciliata</i> Desf., Fl. Atlant. 1: 137, t. 39 (1798).	Wadi Al-Aqara, Matrouh, Egypt. (31°21'18.21" N// 27°02'51.55" E).
14	<i>Plantago weldenii</i> Rchb. Fl. Germ. Excurs. 1: 396 (1831).	Italian Cemetery at Al Alamein. (30°54'25.25" N// 28°50'26.26" E).

No.	Taxa	Localities and Geographical Coordinates
15	<i>Plantago coronopus</i> L., Sp. Pl., ed. 1, 115 (1753).	Italian Cemetery at Al Alamein. (30°54'25.25" N // 28°50'26.26" E).
16	<i>Plantago crassifolia</i> Forssk., Fl. Aegypt.-Arab. 31. (1775).	Al King Maryout, Amreya, Alexandria, Egypt. (30°00'84.87" N // 29°73'33.01" E).
17	<i>Plantago crypsoides</i> Boiss., Fl. Orient. 4: 888 (1879).	Italian Cemetery at Al Alamein. (30°54'25.25" N // 28°50'26.26" E).
18	<i>Plantago cylindrica</i> Forssk., Fl. Aegypt. -Arab. 31. (1775).	Al King Maryout, Amreya, Alexandria, Egypt. (30°00'84.87" N // 29°73'33.01" E).
19	<i>Plantago exigua</i> Murray, Novi Comment. Soc. Regiae Sci. Gott. (1778) 94. t. 5.	Edko, Beheira, Egypt. (31°16'35.86" N // 30°13'31.90" E).
20	<i>Plantago lagopus</i> L., Sp. Pl., ed. 1, 114 (1753).	Gamal AbdEl Naser Hospital, Alexandria, Egypt. (31°20'59.47" N // 29°92'62.86" E).
21	<i>Plantago lanceolata</i> L., Sp., Pl., ed. 1, 113 (1753).	Gamal AbdEl Naser Hospital, Alexandria, Egypt. (31°20'59.47" N // 29°92'62.86" E).
22	<i>Plantago major</i> L., Sp. Pl., ed. 1, 112 (1753).	Victoria, Alexandria, Egypt. (31°24'77.24" N // 29°97'87.20" E).
23	<i>Plantago notata</i> Lag., Gen. Sp. Pl. [Lagasca] 7 (1816).	Al King Maryout, Amreya, Alexandria, Egypt. (30°00'84.87" N // 29°73'33.01" E).
24	<i>Plantago ovata</i> Forssk., Fl. Aegypt. -Arab. 31. (1775).	Al King Maryout, Amreya, Alexandria, Egypt. (30°00'84.87" N // 29°73'33.01" E).
25	<i>Plantago phaeostoma</i> Boiss. & Heldr., Diagn. Pl. Orient. ser. 2, 4: 71 (1859).	Wadi Al-Aqara, Matrouh, Egypt. (31°21'18.21" N // 27°02'51.51" E).
26	<i>Plantago sinaica</i> (Barn.). Decne., Prodr. 13(1):733 (1852).	Wadi El-Arbeen, Saint Katherine, Egypt. (28°32'66.66" N // 33°56'02.71" E).
27	<i>Russelia equisetiformis</i> Schldtl. & Cham., Linnaea 6: 377 (1831).	Botanical Garden, Faculty of science, Ain Shams University, Abbassia, Egypt. (30°07'80.19" N // 31°28'40.66" E).
28	<i>Scrophularia deserti</i> Delile, Derscr. Egypte, Hist. Nat. 240, t.33, f.1 (1813).	Wadi El-Arbeen, Saint Katherine, Egypt. (28°54'80.07" N // 33°95'20.26" E).
29	<i>Scrophularia libanotica</i> Boiss., Diagn. Pl. Orient. ser. 1, 12: 36 (1853).	Abo-Haman, Saint Katherine, Egypt. (28°55'56.95" N // 33°94'86.94" E).
30	<i>Verbascum letourneuxii</i> Asch., in Aschers. & Schwein., Ill. Fl. Egypte (in Mem. Inst. Egypt., 2: 189 & 114 (1887).	Borg El Arab, Alexandria, Egypt. (31°00'22.11" N // 29°43'54.91" E).
31	<i>Verbascum sinaiticum</i> Benth. in A.DC., Prodr. 10: 236 (1846).	Wadi El-Arbeen, Saint Katherine, Egypt. (28°53'50.69" N // 33°96'57.56" E).
32	<i>Verbascum sinuatum</i> L., Sp., Pl., ed. 1, 178 (1753).	Monument Valley, Saint Katherine, Egypt. (28°49'58.56" N // 34°00'26.36" E).
33	<i>Verbascum thapsus</i> L., Sp. Pl. 1: 177 (1753).	Mashtal Faculty of Pharmacy, Cairo University, Saft Al-Laban, Egypt. (30° 01'49" N // 31°11'42" E).
34	<i>Veronica anagallis-aquatica</i> L., Sp. Pl., ed. 1, 12 (1753).	Around water irrigation canal, Kafr El-Sheikh, Egypt. (31°01'46.91" N // 30°49'43.22" E).
35	<i>Veronica anagalloides</i> subsp. <i>taeckholmiorum</i> Chrtek & Osb. -Kos., Folia Geobot. Phytotax. 16(4): 426 (1981).	Around water irrigation canal, Kafr El-Sheikh, Egypt. (31°01'46.91" N // 30°49'43.22" E).
36	<i>Veronica polita</i> Fr., Novit. Fl. Suec. Alt. 1 (1819).	Borg El Arab, Alexandria, Egypt. (31°00'22.11" N // 29°43'54.91" E).

Fruit and seed observation and measurements

At least five individuals for each taxon were studied; 10-15 mature fruits and seeds were macromorphologically examined under LM, and at least five seeds were micromorphologically examined under SEM. Seed measurements were taken with the Image Tool software. For the SEM study, cleaned and dry seeds were mounted on copper stubs and then coated with gold-palladium for 10 minutes with a sputter coater. The coated seeds were examined and photographed at various magnifications by a JEOL JSM-200IT SEM, at 20 kV acceleration. Ventral and dorsal seed faces were investigated. The seed coat surface sculpturing pattern was described in terms of the dorsal face of the seed. Photomicrographs were taken at the Electron Microscopy Unit of Alexandria University, Egypt. Descriptive terminology for fruit and seed characters followed Stearn (1983), Barthlott (1984), Verma & al. (2017), Mazur & al. (2021).

Phenetic analysis

A total of 23 comparative morphological charac-

ters of fruits and seeds, divided into 103 characters states, were scored and coded in a unified data matrix. Cluster analysis was applied to assess the phenetic relationships between the investigated taxa using the PAST program, version 3.12 (Paleontological Statistics), software package (Hammer & al. 2013). Clustering was performed using Unweighted Pair-Group Method with Arithmetic Average (UPGMA) and presented in a phenogram. The generated phenogram expressed the phenetic relationships between the studied taxa based on their degree of similarity, in which the OTUs have been linked together at various levels of resemblance. Comparisons were made between the produced classification and the earlier taxonomic treatments of the investigated taxa.

Results

The fruit and seed morphological characters of the studied taxa, as shown by LM and SEM microscopy, are presented in Tables 2 and 3. The LM and SEM micrographs of seeds are illustrated in Figs 1- 6.

Table 2. Macro-morphological characteristics of the fruits and seeds of the studied taxa.

Taxa no.*	Capsule						Seed						
	Shape	D	Tex	Seed Number/ Capsule	Color	Length (mm)	Width (mm)	L/W Ratio	Shape	Margin	TF	Hilum	
												P	Le
1	Globose	AP	G	Many	DB	1.5-1.8 (1.65±0.17)	0.8-1 (0.9±0.011)	1.83	Ellipsoid	Indistinct	A	ST	Su
2	Ovate	PS	H	Many	B	0.8-1.1 (0.95±0.11)	0.5-0.9 (0.7±0.23)	1.35	Rhomboid	Indistinct	A	ST	Su
3	Ovate - Conical	S	H	Many	DB	1.1-1.4 (1.25±0.17)	0.5-0.9 (0.7±0.23)	1.78	Trigonus- Prismatic	Indistinct	A	ST	Su
4	Broadly Ovate	L L	H	Many	YB	0.7-0.9 (0.86±0.08)	0.5-0.6 (0.55±0.05)	1.56	Ovoid	Indistinct	A	M	F
5	Broadly Ovate	L L	H	Many	DB	0.4-0.5 (0.45±0.05)	0.2-0.4 (0.3±0.11)	1.5	Prismatic	Indistinct	A	M	F
6	Subglobose	AV	H	Many	SG	0.5-.06 (0.55±0.05)	0.3-0.45 (0.37±0.08)	1.4	Reniform	Indistinct	A	M	F
7	Subglobose	AV	G	Many	SG	1.5-2 (1.75±0.28)	1.3-1.9 (1.6±0.34)	1.09	Discoid	distinct	A	M	F
8	Subglobose	AV	G	Many	SG	1.8-2.6 (2.2±0.4)	1.5-1.9 (1.7±0.23)	1.29	Discoid	distinct	A	M	F
9	Ovate	AL	G	2	B	2-3 (2.5±0.57)	0.7-1 (0.9±0.03)	2.77	Cymbiform	Indistinct	A	M	Su

10	Ovate	AL	G	2	Br	1.9-2.3 (2±0.08)	1-1.32 (1.2±0.17)	1.67	Cymbiform	Indistinct	A	M	Su
11	Ovate	AL	G	2	b	4.85-6 (5.5±0.37)	2-3.3 (2.96±0.25)	1.85	Cymbiform	Indistinct	A	SM	Su
12	Ovate	AL	G	2	B	2-3 (2.5±0.5)	0.8-1 (0.97±0.07)	2.57	Cymbiform	Indistinct	A	SM	Su
13	Ovate	AL	G	2	YB	2-2.3 (2.12±0.57)	1.2-1.4 (1.25±0.1)	1.69	Cymbiform	Indistinct	A	M	Su
14	Ovate	AL	H	3	Br	1.3-1.5 (1.4±.11)	0.7-0.9 (0.85±0.05)	1.64	Lenticular	Indistinct	P	SM	Su
15	Ovate	AL	H	3-4	B	1.2-1.5 (1.35±0.12)	0.6-0.9 (0.65±0.05)	1.69	Lenticular	Indistinct	P	SM	Su
16	Ovate	AL	H	2	Br	1.1-1.55 (1.38±.12)	0.7-0.8 (0.75±0.05)	1.84	Lenticular	Indistinct	P	SM	Su
17	Ovate	AL	H	3	B	1-1.5 (1.3±0.28)	0.6-0.8 (0.7±0.05)	1.85	Lenticular	Indistinct	P	SM	Su
18	Ovate	AL	G	2	YB	2-2.5 (2.25±0.28)	1.2-1.4 (1.3±0.1)	1.74	Cymbiform	Indistinct	A	M	Su
19	Ovate	AL	G	2	B	2-2.6 (2.3±0.6)	0.9-1.1 (1±0.11)	2.3	Cymbiform	Indistinct	A	M	Su
20	Ovate	AL	G	2	B	1.2-1.8 (1.6±0.57)	0.6-0.8 (0.65±0.11)	2.4	Cymbiform	Indistinct	A	SM	Su
21	Ovate	AL	G	2	B	1.9-2.3 (2.2±0.75)	0.9-1.3 (1.1±0.11)	2	Cymbiform	Indistinct	A	M	Su
22	Ovate	AL	G	Many	DB	0.8-1.3 (1.1±0.02)	0.4-0.7 (0.59±0.05)	1.84	Ellipsoid	Indistinct	A	M	Su
23	Ovate	AL	G	2	B	1.8-2.2 (1.8±0.11)	0.6-0.85 (0.75±0.17)	2.40	Cymbiform	Indistinct	A	M	Su
24	Ovate	AL	G	2	YB	2.2-2.9 (2.5±0.24)	1.2-1.55 (1.32±0.1)	1.89	Cymbiform	Indistinct	A	M	Su
25	Ovate	AL	G	2	DB	3-3.5 (3.25±0.28)	1-1.5 (1.25±0.28)	2.6	Cymbiform	Indistinct	A	SM	Su
26	Ovate	AL	G	2	B	2.5-3.5 (3 ±0.5)	1-1.5 (1.25±0.28)	2.45	Cymbiform	Indistinct	A	SM	Su
27	Globose	S	G	Many	M	0.3-0.4 (0.35±0.05)	0.4-0.5 (0.45±0.05)	0.77	Ovoid	Indistinct	A	Te	R
28	Subglobose	S	G	Many	B	1.3-1.5 (1.4±0.5)	0.4-0.6 (0.5±0.1)	2.8	Ovoid	Indistinct	A	Te	R
29	Globose	S	G	Many	B	1-1.2 (1.1±0.2)	0.4-0.6 (0.5±0.1)	2.2	Ovoid	Indistinct	A	Te	R
30	Oblong/ Cylindrical	S	T	Many	D	1.35-1.5 (1.44±0.03)	0.6-0.7 (0.65±0.01)	2.21	Ovoid	Indistinct	A	Te	R
31	Ovate	S	T	Many	DB	1.1-1.3 (1.19±0.1)	0.65-0.74 (0.69±0.06)	1.7	Ovoid	Indistinct	A	Te	R
32	Subglobose	S	T	Many	DB	0.9-1 (0.98±0.1)	0.5-0.6 (0.56±0.05)	1.75	Ovoid	Indistinct	A	Te	R
33	Ovate	S	T	Many	DB	0.7-0.9 (0.82±0.1)	0.4-0.45 (0.43±0.02)	1.9	Ovoid	Indistinct	A	Te	R
34	Orbicular	L	G	Many	YB	0.5-0.8 (0.65±0.1)	0.4-0.55 (0.47±0.1)	1.4	Ellipsoid	Indistinct	A	ST	F
35	Orbicular	L	G	Many	YB	0.5-0.6 (0.55±0.05)	0.3-0.5 (0.4±0.1)	1.37	Ovoid	Indistinct	A	ST	F
36	Reniform	L	H	Many	PY	0.9-1.6 (1.3±0.4)	0.8-1.3 (1.1±0.2)	1.2	Cymbiform	Indistinct	A	ST	Su

* = See table 1 for taxa names

Absent=A, Apical Lid=AL, Apical One Pore=AP, Apical Valves=AV, Black=B, Brown=Br, Dark Brown=DB, Dehiscence=D, Flat=F, Glabrous=G, Hairy=H, Lateral Lid=LL, Level=Le, Like Skull=PS, Loculicidal=L, Median=M, Mustard=M, Pale Yellow=PY, Position=P, Present=P, Raised=R, Silvery-Grey=SG, Sub-Median=SM, Sub-Terminal=ST, Sunken=Su, Terminal=Te, Texture=Tex, Septicidal=S, Tomentose=T, Transverse Furrow=TF, Yellowish-Brown=YB.

Table 3. Micro-morphological characteristics of the seeds of the studied taxa.

Taxa no.*	Seed Micro-Morphological Characteristics													
	SF	Ventral Face					Overall Pattern	Cells Outline	Dorsal Face					
		Cavity				Shape			Anticlinal Cell Walls			Periclinal Cell Walls		
		Shape	Nature	Edges	Ends				Shape	Thic	Level	Sc	Level	Sculpture
1	Bi	-	-	-	-	Reticulate-Tuberculate	P	S	Th	Ra	S	F	Rugulate / Verrucate	
2	Bi	-	-	-	-	Reticulate	P	S	V Th	Ra	BB	F	Granulate	
3	Bi	-	-	-	-	Reticulate	P	S	T	Ra	S	F	Smooth	
4	Bi	-	-	-	-	Tuberculate / Verrucate	P	S	T	Ra	-	Cx	Rugulate	
5	Bi	-	-	-	-	Tuberculate / Verrucate	P	S	T	Ra	R/S	Cc	Rugulate / Verrucate	
6	Bi	-	-	-	-	Reticulate / Tuberculate	P	S	T	Ra	S	F	Tuberculate/Papillate	
7	F	-	-	-	-	Reticulate	P	S	T	Le	S	F	Tuberculate/Papillate	
8	F	-	-	-	-	Reticulate / Tuberculate	P	S	T	Ra	S	F	Tuberculate/Papillate	
9	Co	BS	Na	F		Scalariform	P	S	T	Le	TS	F	Undulate	
10	Co	E	B	F	OTS	Reticulate / Scalariform	P	S	Th	Ra	Ru	F	Rugulate	
11	Co	BS	B	R	OOS	Scalariform	P	S	Th	Le	Ru	F	Rugulate	
12	Co	BS	B	F	OOS	Scalariform	P	S	T	Le	TS	F	Undulate	
13	Co	E	B	F	OTS	Reticulate / Scalariform	P	S	T	Su	-	F	Rugulate	
14	CP	-	-	-	-	Reticulate	R	U	Th	Ra	No	Cc	Ruminate	
15	CP	-	-	-	-	Reticulate	R	U	Th	Ra	No	Cc	Ruminate	
16	CP	-	-	-	-	Reticulate	R	S	Th	Le	LS	F	Ruminate	
17	CP	-	-	-	-	Reticulate	R	S/U	Th	Ra	No	Cc	Ruminate	
18	Co	E	B	F	OTS	Reticulate / Scalariform	P	S	T	Ra	Ru	F	Rugulate	
19	Co	BS	B	F	OOS	Reticulate	P	S	T	Le	TS	F	Undulate	
20	Co	E	Na	R	OOS	Reticulate / Scalariform	P	S/U	Th	Ra	LS	Cc	Undulate	
21	Co	E	B	R	OOS	Reticulate / Scalariform	P	S	Th	Le	TS	F/Cx	Undulate	
22	Bi	-	-	-	-	Reticulate	P	U	Th	Ra	S	Cc	Rugulate	
23	Co	N	B	R	OTS	Reticulate / Scalariform	P	S	T	Le	Ru	F	Rugulate	
24	Co	N	B	F	OTS	Reticulate / Scalariform	P	S	T	Le	Ru	F	Rugulate	
25	Co	BS	B	R	OOS	Scalariform	P	S	T	Le	TS	F/Cx	Undulate	
26	Co	BS	B	F	OOS	Scalariform	P	S	T	Le	TS	F/Cx	Undulate	
27	Bi	-	-	-	-	Reticulate	R	U	Th	Ra	R / S	Cc	Rugulate / Verrucate	

28	Bi	-	-	-	-	Deeply Alveolate	H	S	Th	Ra	No	F	Transversely Striated
29	Bi	-	-	-	-	Shallow Alveolate	H	S	Th	Ra	No	F	Transversely Striated
30	Bi	-	-	-	-	Shallow Alveolate / Apiculate Beak	H	S	Th	Ra	No	F	Transversely Striated
31	Bi	-	-	-	-	Shallow Alveolate / Apiculate Beak	H	S/U	Th	Ra	No	F	Transversely Striated
32	Bi	-	-	-	-	Shallow Alveolate / Apiculate Beak	H	S/U	Th	Ra	No	F	Transversely Striated
33	Bi	-	-	-	-	Deeply Alveolate	H	S/U	Th	Ra	No	F	Transversely Striated
34	CP	-	-	-	-	Reticulate	P	S	Th	Ra	S	Cc	Smooth
35	CP	-	-	-	-	Reticulate	P	S	Th	Ra	S	Cc	Smooth
36	Co	O	B	R	OOS	Strongly Ridged	P	U	Th	Ra	S	Cc	Domate

* = See table 1 for taxa names

Biconvex=Bi, Bottle Shaped=BS, Broad=B, Buttressed to Beaded= BB, Cochlidiospermous=Co, Concave=Cc, Convex=Cx, Convexo-plane=CP, Elliptic=E, Flat=F, Hexagonal=H, Leveled=Le, Longitudinally Striate=LS Narrow=Na, Naviculoid=N, Nodulated=No, Opened One Side=OOS, Opened two Sides=OTS, Ovoid=O, Polygonal=P, Raised=Ra, Rounded=R, Rugulate / Striated=R/S, Ruminant=Ru, Seed Faces=SF, Sculpture=Sc, Smooth=S, Straight=S, Straight /Undulate =S/U, Undulate =U, Sunken=Su, Thick= Th, Thickness=Thic, Thin=T, Transversely Striate=TS, Very Thick=V Th,

I. General description of the fruit and seed morphology

A. Fruit macromorphological characters

a. Fruit shape and dehiscence

A capsule fruit type distinguishes all studied taxa. Regarding fruit shapes, seven types have been recognized in the investigated species: ovate in *Plantago*, *Antirrhinum majus*, *Digitalis lanata* *Verbascum sinaiticum* and *Verbascum thapsus*; broadly ovate (*Nanorrhinum acerbianum* and *Kickcia aegyptiaca*); sub-globose (*Linaria haelava*, *L. micrantha*, *L. simplex*, *Scrophularia deserti* and *Verbascum sinuatum*); globose (*Anarrhinum forskaohlii* subsp. *pubescens*, *Russelia equisetiformis* and *Scrophularia libanotica*); oblong to cylindrical (*Verbascum letourneuxii*); orbicular (*Veronica anagallis-aquatica* and *V. anagalloides* subsp. *Taeckholmiorum*), and *V. polita* was the only species with a reniform capsule.

The capsules dehisced into four different modes, viz. apical lid (circumscissile) in all *Plantago* species; apical pores in *Anarrhinum forskaohlii* subsp. *pubes-*

cens and *Antirrhinum majus*; apical valves in the examined *Linaria* species; septicial in *Digitalis lanata*, *Russelia equisetiformis*, *Scrophularia deserti*, *Scrophularia libanotica*, *Verbascum letourneuxii*, *Verbascum sinaiticum*, *Verbascum sinuatum*, and *Verbascum thapsus*; and, finally, loculicidal fruits in *Veronica anagallis-aquatica*, *Veronica anagalloides* subsp. *taeckholmiorum* and *Veronica polita*.

b. Fruit surface

Most investigated taxa had glabrous capsule surfaces. *Veronica polita*, *Nanorrhinum acerbianum*, *Kickcia aegyptiaca*, *K. commutata*, *Plantago weldenii*, *P. coronopus*, *P. crassifolia*, *P. crypsoides*, and *Antirrhinum majus* had hairy surfaces, while *Verbascum letourneuxii*, *V. sinaiticum*, *V. sinuatum*, and *V. thapsus* had tomentose surfaces (Fig.1).

c. Seed number per capsule

The recorded number of seeds produced per capsule in almost all investigated species of *Plantago* ranged from two to four, except for *Plantago major*;

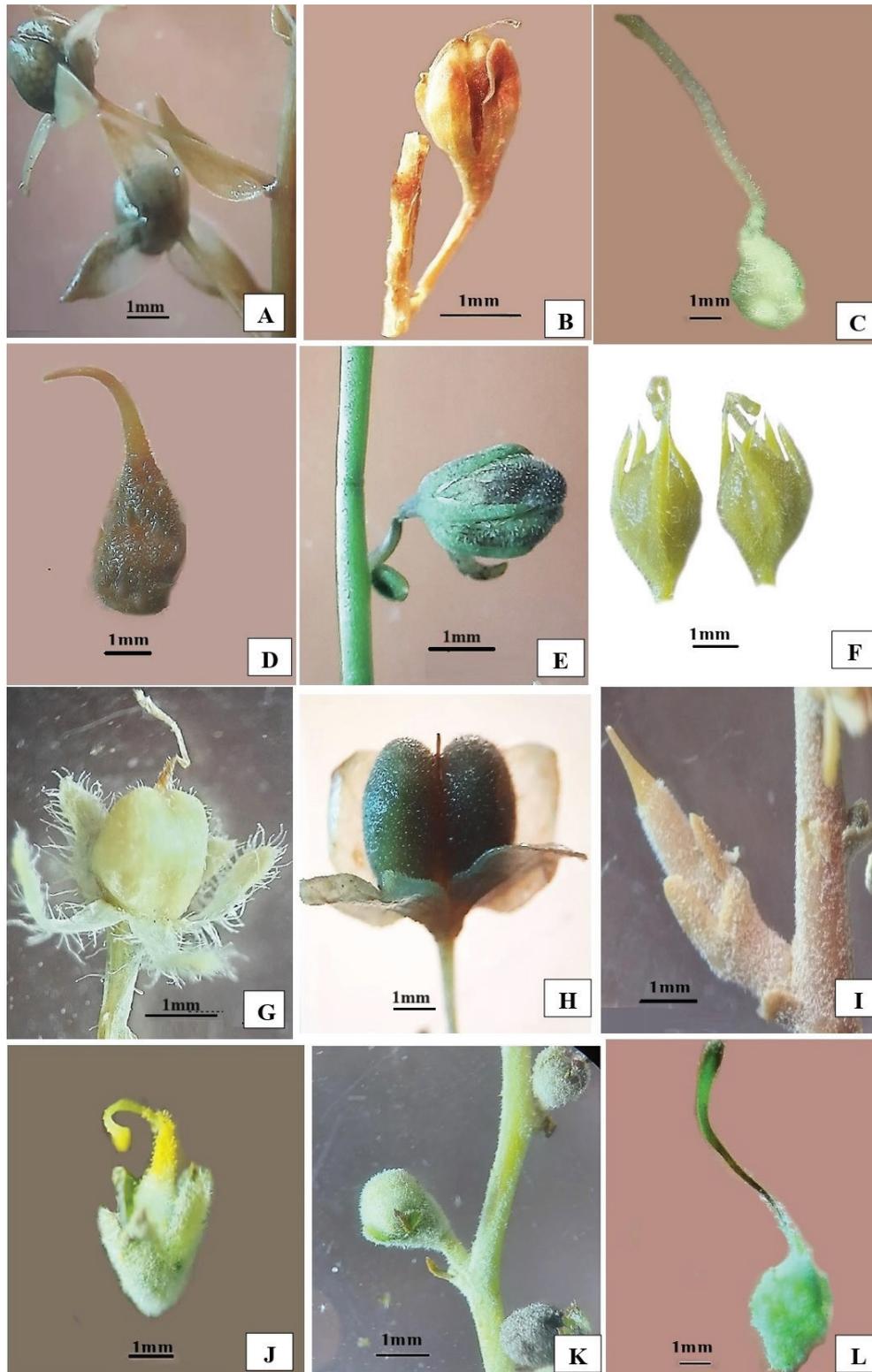


Fig. 1. Capsule surface of some studied taxa of Scrophulariaceae, as revealed by stereo microscope: A, Glabrous surface (*Veronica analallis-aquatica*); B-H: Hairy surface (*Anarrhinum forskaoalii*, *Antirrhinum majus*, *Digitalis lanata*, *Nanorrhinum acerbianum*, *K. aegyptiaca*, *Veronica polita*); I-L, Tomentose surface (*Verbascum letournenxii*, *V. sinaiticum*, *V. sinuatum*, *V. thapsus*).

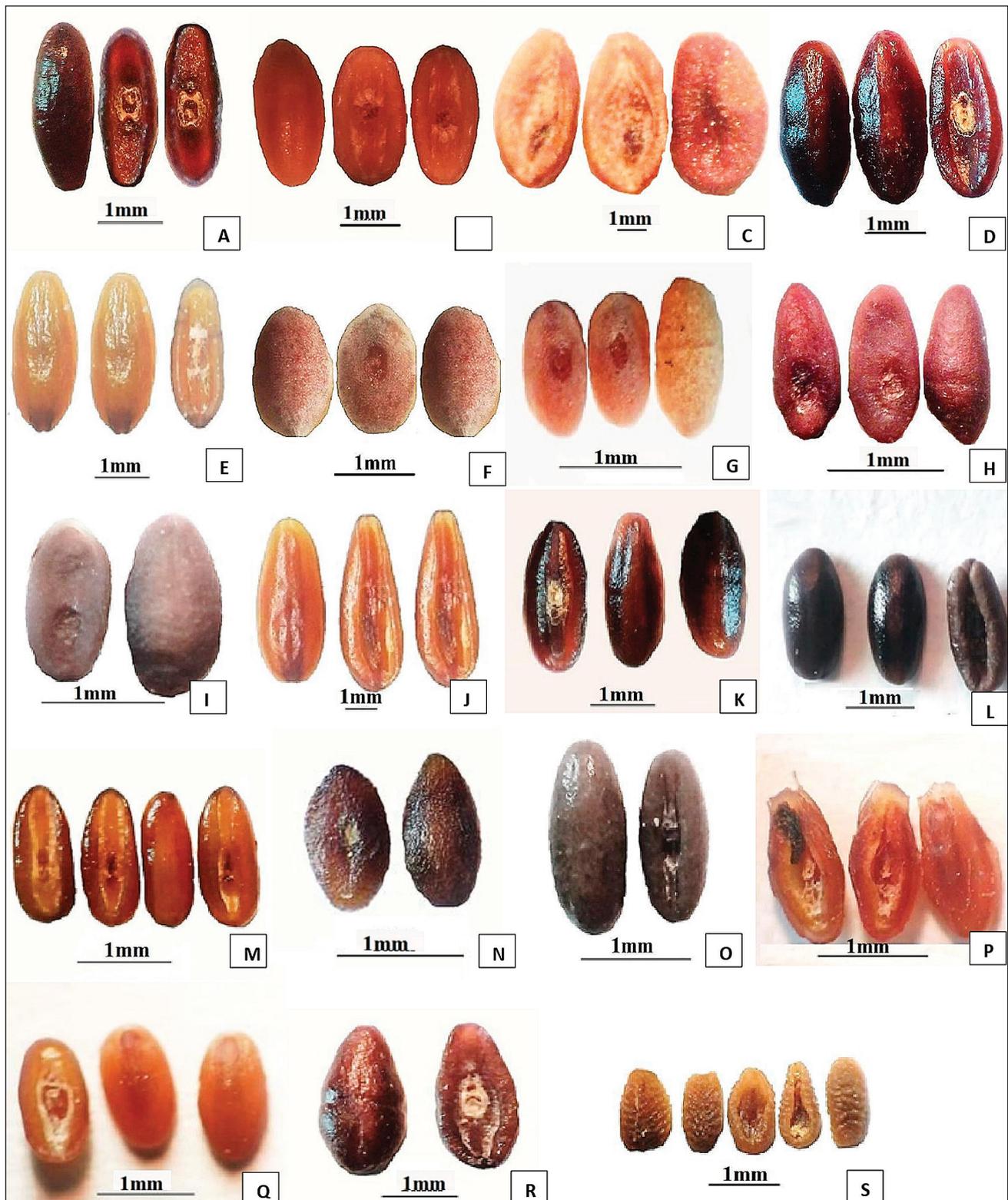


Fig. 2. Seeds of the studied *Plantago* and *Veronica* species showing dorsal & ventral faces. A, *Plantago afra*; B, *P. albicans*; C, *P. amplexicaulis*; D, *Plantago indica*; E, *P. ciliata*; F, *Plantago weldenii*; G, *P. coronopus*; H, *P. crassifolia*; I, *P. crypsoides*; J, *P. cylindrica*; K, *P. exigua*; L, *P. lagopus*; M, *P. lanceolata*; N, *P. major*; O, *P. notata*; P, *P. ovata*; Q, *P. phaeostoma*; R, *P. sinaica*; S, *Veronica polita*.

in the other investigated taxa, many seeds have been produced per capsule.

B. Seed macro- and micromorphological characters

The seed size, shape, color, margin, hilum features, faces, surface sculpturing pattern, anticlinal cell wall and periclinal cell wall were described (Tables 2, 3 and Figs 2-6).

a. Seed size

Seed dimensions (L×W) showed a high degree of variation. *Russelia equisetiformis* had the smallest seeds, with 0.3-0.4 × 0.4-0.5 mm, while *Plantago phaeostoma* had the largest seeds of 3-3.5 × 1-1.5 mm.

b. Seed shape

The shape of seeds varied greatly. Most studied *Plantago* species had cymbiform seeds; there were lenticular seeds in *Plantago coronopus*, *P. crassifolia*, *P. crypsoides* and *P. weldenii*; rhomboid seeds in *Antirrhinum majus*; prismatic seeds in *Digitalis lanata* and *Kickxia aegyptiaca*; ellipsoid seeds in *Anarrhinum forskahlii* subsp. *pubescens* and *Veronica anagallis-aquatica*; wide ellipsoid seeds in *Plantago major*; discoid seeds in *Linaria micrantha* and *L. simplex*; reniform seeds in *L. haelava*, and ovoid seeds in the rest of the studied taxa.

c. Seed color

Most of the investigated taxa exhibited brown seeds, with variations from light brown to dark brown. *Plantago afra*, *P. amplexicaulis*, *P. coronopus*, *P. crypsoides*, *P. lagopus*, *P. indica*, *P. lanceolata*, *P. notata*, and *P. sinaica* had black seeds. Mustard-colored seeds were seen in *Russelia equisetiformis*, *Verbascum sinaiticum* and *Verbascum sinuatum*, and silvery grey in *Linaria haelava*, *L. micrantha* and *L. simplex*.

d. Seed faces (sides)

Scanning electron micrographs revealed four types of seed faces: cochlidospermous (ventrally concave), flat, convexoplane, and biconvex seed faces. Cochlidospermous seeds were found in most species of *Plantago* and *Veronica polita*. Cavities of variable shapes

were seen on the ventral side of the cochlidospermous seeds, viz. naviculoid, ovoid, bottle-shaped, and elliptic, with either rounded or flattened edges. Flat seeds were observed in *Linaria haelava*, convexoplane seed faces in *Plantago coronopus*, *P. crassifolia*, *P. crypsoides* and *P. weldenii*, *Veronica anagallis-aquatica*, and *Veronica anagalloides*, and the remaining taxa showed biconvex seed faces.

e. Seed edges

Linaria micrantha and *L. simplex* were the only species with winged seeds among the studied taxa, whereas the others had wingless seeds.

f. Seed hilum features

Four hilum positions were recognized: median, submedian, terminal, and subterminal. The median type predominated among the studied taxa. Moreover, three levels of hila were recorded: flat in *Kickxia aegyptiaca*, *Linaria haelava*, *L. micrantha*, *L. simplex*, *Nanorrhinum acerbianum*, *Veronica anagallis-aquatica*, and *V. anagalloides* subsp. *Taeckholmiorum*; raised in *Russelia equisetiformis*, *Scrophularia deserti*, *S. libanotica*, *Verbascum letourneuxii*, *V. sinaiticum*, *V. sinuatum*, and *V. Thapsus*; and sunken hilum in the remaining investigated taxa.

g. Overall seed coat pattern (primary sculpture)

A scanning electron microscope examination of the studied taxa revealed eight seed-coat sculpture patterns. *Antirrhinum majus*, *Digitalis lanata*, *Linaria micrantha*, *Plantago coronopus*, *P. crassifolia*, *P. crypsoides*, *P. exigua*, *P. major*, *P. weldenii*, *Russelia equisetiformis*, *Veronica anagallis-aquatica*, and *Veronica anagalloides* subsp. *taeckholmiorum* exhibited a reticulate pattern. A scalariform pattern was observed in *Plantago afra*, *P. amplexicaulis*, *P. indica*, *P. phaeostoma*, and *P. sinaica*. Reticulate to scalariform was the predominant seed-coat pattern among the *Plantago* species. *Anarrhinum forskahlii* subsp. *pubescens*, *Linaria haelava* and *L. simplex* showed reticulate to tuberculate pattern. Species of *Scrophularia* and *Verbascum* exhibited an alveolate surface pattern. *Kickxia* species and *Nanorrhinum acerbianum* displayed a tuberculate to verrucate seed-coat sculpture pattern, while *Veronica polita* had a strongly ridged pattern.

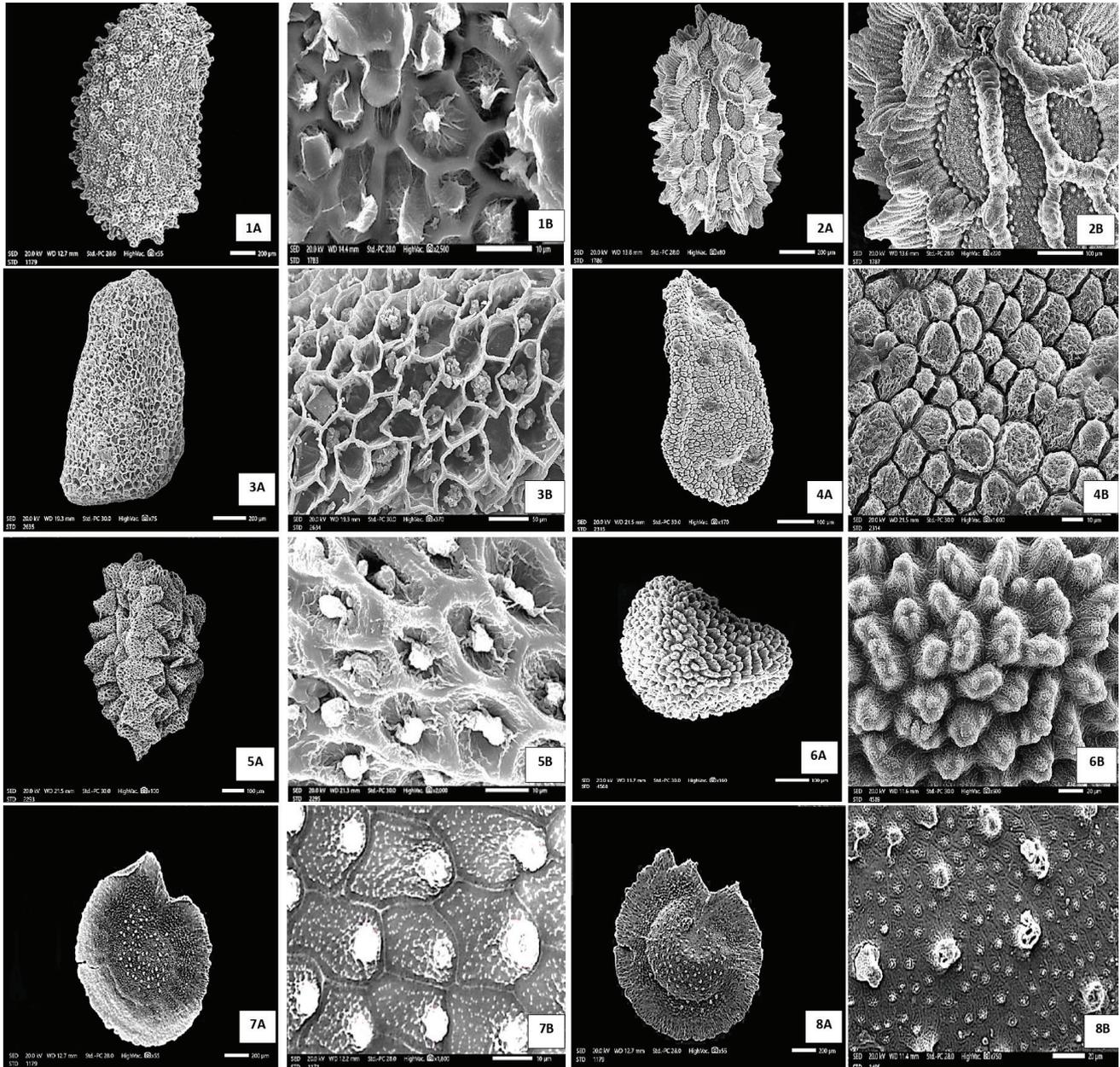


Fig. 3. Seeds micro-morphological characteristics of the studied Scrophulariaceae taxa. (1A, 1B), *Anarrhinum forskahlii*; (2A, 2B), *Antirrhinum majus*; (3A,3B), *Digitalis lanata*; (4A, 4B), *Nanorrhinum acerianum*; (5A, 5B), *Kickxia aegyptiaca*; (6A, 6B), *Linaria haelava*; (7A, 7B), *Linaria micrantha*; (8A,8B), *Linaria simplex*.

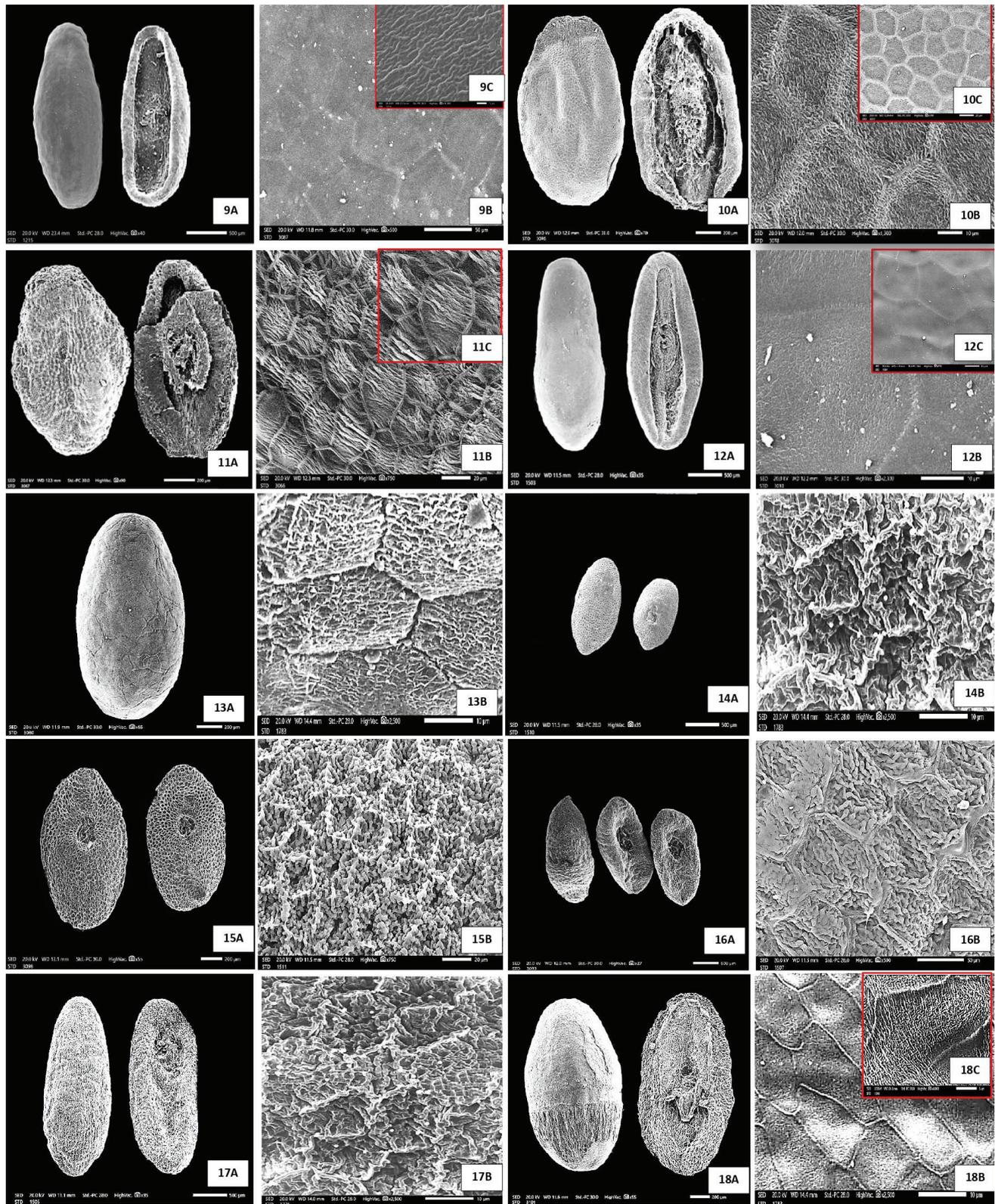


Fig. 4. Seeds micro-morphological characteristics of the studied *Plantago* species. (9A-9C), *Plantago afra*; (10A-10C), *Plantago albicans*; (11A-11C), *Plantago amplexicaulis*; (12A-12C), *Plantago indica*; (13A, 13B), *Plantago ciliata*; (14A, 14B), *Plantago weldenii*; (15A, 15B), *Plantago coronopus*; (16A, 16B), *Plantago crassifolia*; (17A, 17B), *Plantago crypsoides*; (18A-18C), *Plantago cylindrica*.

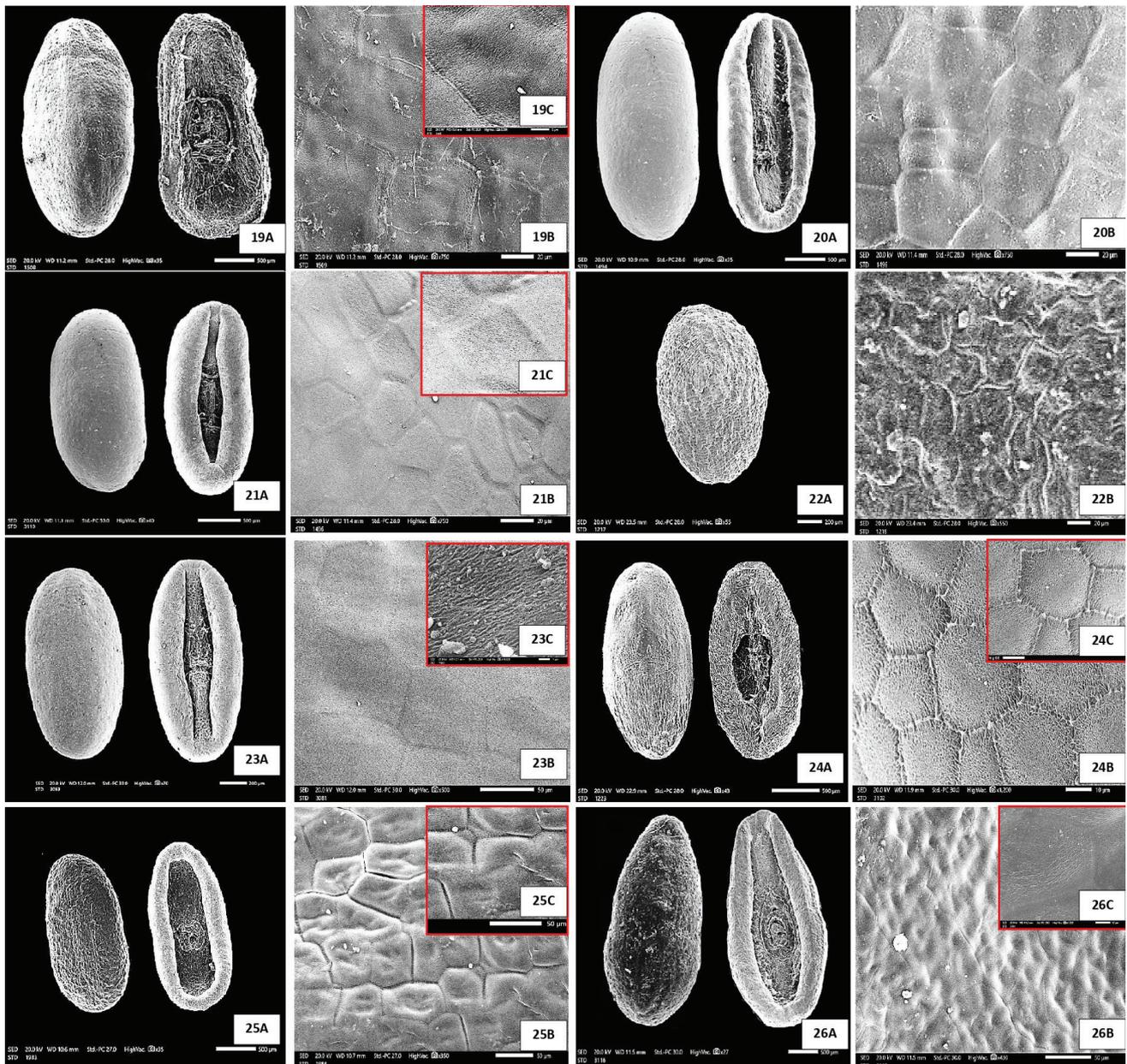


Fig. 5. Seeds micro-morphological characteristics of the studied *Plantago* species. (19A-19C), *Plantago exigua*; (20A, 20B), *Plantago lagopus*, (21A-21C), *Plantago lanceolata*; (22A, 22B), *Plantago major*; (23A-23C), *Plantago notata*, (24A-24C), *Plantago ovata*; (25A-25C), *Plantago phaeostoma*; (26A-26C), *Plantago sinaica*.

h. Anticlinal cell wall boundaries

In terms of thickness of the anticlinal walls, very thick, thick, and thin cell walls could be observed. *Antirrhinum majus* showed very thick anticlinal walls; most investigated taxa (20 in number) had thick anticlinal cell walls, while another 15 had thin anticlinal walls. The most common shape of the anticlinal walls was straight. However, straight to undulate walls were recorded in *Plantago crypsoides*, *P. lagopus*, *V. sinaiticum*, *V. sinuatum*, and *V. thapsus*. Undulate anticlinal walls were identified in *Plantago coronopus*, *P. major*, *P. weldenii*, *Russelia equisetiformis*, and *Veronica polita*. The surface of anticlinal walls (secondary sculpture) was smooth in most investigated taxa; nodulate in *Plantago coronopus*, *P. weldenii*, *Scrophularia deserti*, *Scrophularia libanotica*, *Verbascum le-tourneuxii*, *Verbascum sinaiticum*, *Verbascum sinuatum*, and *Verbascum thapsus*; buttressed to beaded in *Antirrhinum majus*, and ruminant in *Plantago afra*, *P. albicans*, *P. amplexicaulis*, *P. exigua*, *P. indica*, *P. lanceolata*, *P. notata*, *P. phaeostoma*, and *P. sinaica*. Anticlinal walls were sunken in *Plantago ciliata* and *Nanorrhinum acerbianum*, leveled in *Linaria micrantha*, *P. afra*, *P. amplexicaulis*, *P. indica*, *P. crassifolia*, *P. exigua*, *P. lanceolata*, *P. notata*, *P. ovata*, *P. phaeostoma*, and *P. sinaica*, and raised in the remaining investigated taxa.

i. Periclinal cell walls

Four types of periclinal cell wall levels were recognized: concave, flat to convex, convex, and flat. *Kickxia aegyptiaca*, *Plantago coronopus*, *P. crypsoides*, *P. lagopus*, *P. major* and *P. weldenii*, *Russelia equisetiformis* and *Veronica* species had concave periclinal cell walls. Flat to convex were the walls in *Plantago lanceolata*, *P. phaeostoma* and *P. sinaica*, convex in *Nanorrhinum acerbianum*, and flat in the remaining investigated taxa. The periclinal walls displayed eight ornamentation patterns: ruminant in *Plantago coronopus*, *P. crypsoides* and *P. crassifolia*; undulate in *Plantago afra*, *P. exigua*, *P. indica*, *P. lanceolata*, *P. lagopus*, *P. phaeostoma* and *P. sinaica*, and *P. weldenii*; rugulate in *Nanorrhinum acerbianum*, *P. albicans*, *P. ciliata*, *P. cylindrica*, *P. major*, *P. notata*, and *P. ovata*

; transversely striated in the *Verbascum* species and *Scrophularia deserti* and *S. libanotica*; tuberculate and papillate in the investigated *Linaria* species; rugulate to verrucate in *Anarrhinum forskahlii* subsp. *pubescens*, *Kickxia aegyptiaca* and *Russelia equisetiformis*; granulate in *Antirrhinum majus*, and domate domed??? in *Veronica polita*. *Digitalis lanata*, *Veronica anagallis-aquatica* and *Veronica anagalloides* subsp. *Taeckholmiorum* had smooth periclinal cell walls.

II. Data analysis

Cluster analysis has been used to determine the contribution of fruit and seed morphological characters in clarifying the phenetic relationships between the studied taxa. The constructed phenogram, based on coding of 103 characters states belonging to 23 characters from the fruit and seed morphology, revealed separation of the investigated taxa at similarity level of 0.19 into two main series (SI & SII). Series I comprised all studied taxa of *Scrophulariaceae* s.l. (18 taxa), while Series II included the investigated species of *Plantago* (*Plantaginaceae* s.str.). Series II contained two clusters (C1 & C2): Cluster1 comprising only *Plantago major*, and Cluster 2 including the remaining *Plantago* species (17 taxa). Cluster 2 was divided into two groups: group 1 included 13 species of *Plantago* and group 2 included *Plantago coronopus*, *P. crassifolia*, *P. crypsoides*, and *P. weldenii*.

Discussion

Phenetic studies of the morphological characters can yield an accurate estimate of the relationships between the taxa (El-Hadidy & al. 2018). The relationship between the *Plantaginaceae* and the *Scrophulariaceae* is rather murky and needs to be clarified (Heywood 1993, Albach & al. 2004). In this study, variations of fruit and seed morphological characters were recorded comparatively, and subsequently subjected to numerical analysis in an attempt to inspect the relationship among the *Plantago* species, as well as between some taxa of *Plantaginaceae* s.l. formerly assigned to the *Scrophulariaceae* s.l. The interspecific relationships

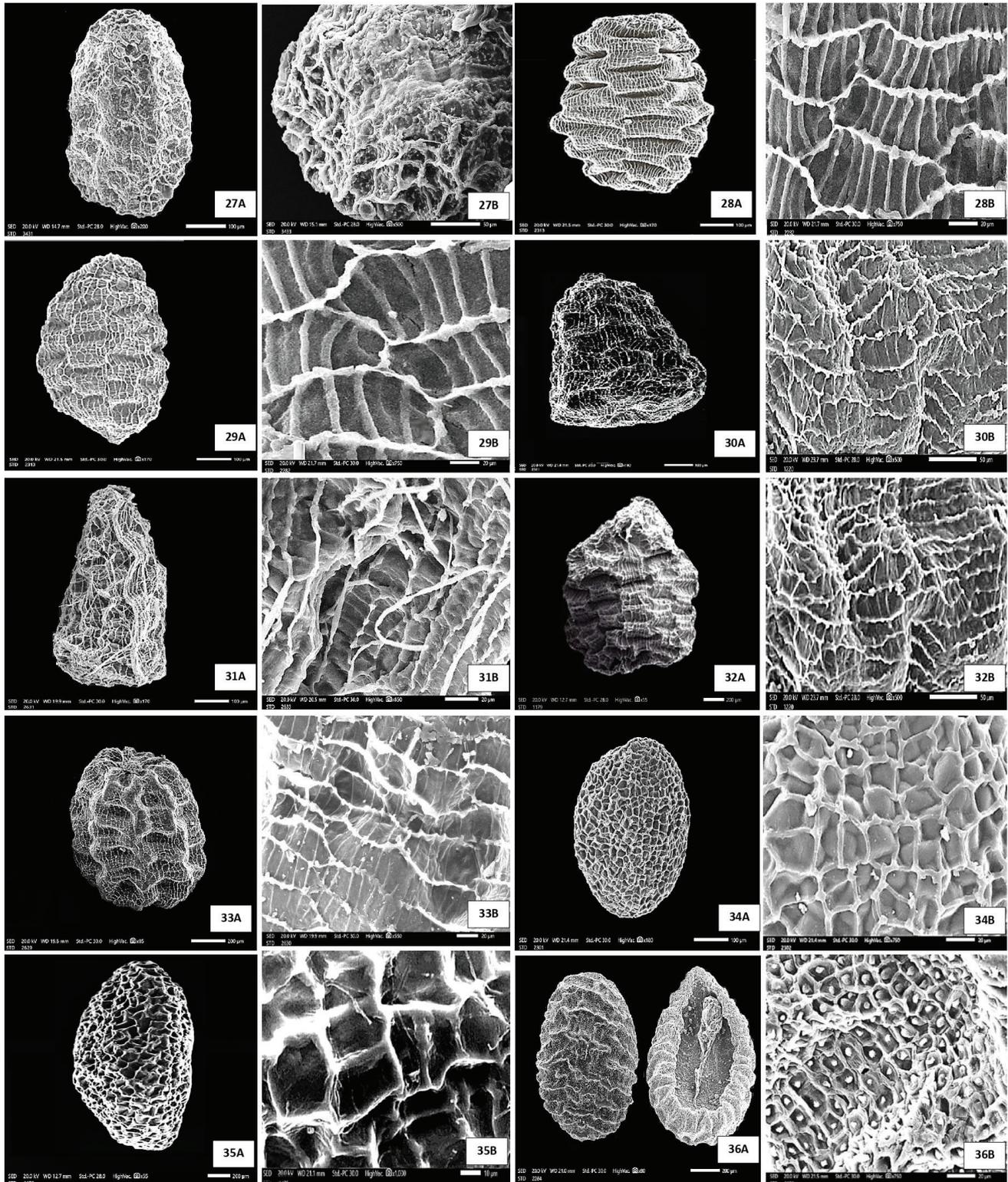


Fig. 6. Seeds micro-morphological characteristics of the studied scrophulariaceae taxa. (27A, 27B), *Russelia equisetiformis*; (28A, 28B), *Scrophularia deserti*; (29A, 29B), *Scrophularia libanotica*; (30A, 30B), *Verbascum letourneuxii*; (31A, 31B), *Verbascum sinaiticum*; (32A, 32B), *Verbascum sinuatum*; (33A, 33B), *Verbascum thapsus*; (34A, 34B), *Veronica anagallis-aquatica*; (35A, 35B), *Veronica anagalloides* subsp. *taekholmiorum*; (36A, 36B), *Veronica polita*.

will be discussed and compared with the earlier taxonomic treatments of the investigated taxa.

The constructed phenogram (Fig.7), based on the phenetic analysis of fruit and seed morphological characters, has clarified and indicated the following:

1. The investigated taxa have been divided into two main series (S I and S II) at a similarity level of 0.19. Series I include the Scrophulariaceae taxa and is characterized by a septicidal, loculicidal lateral lid, pore and apical valves capsule dehiscence; many seeds have been produced per capsule; biconvex, convexo-plane and flat seeds (except for *Veronica polita* which has shown cochlidiospermous seeds). Series II comprise all studied *Plantago* species. The characters that separate the *Plantago* species from others are the apical lid dehisced fruit, few seeds produced per capsule, except in *P. major*, cochlidiospermous seed, median to sub-median hilum, sunken hilum, and undulate periclinal cell walls with ruminant sculpture pattern.

In general, segregation of the investigated taxa into two main groups (SI & SII) in the phenogram is compatible with the views of several other authors (Bentham and Hooker 1876; Cronquist 1981; Heywood 1993; Takhtajan 1980; Hamed & al. 2014; Al Masoudi & al. 2021), who have set apart the *Plantago* species in a separate monogeneric family of *Plantaginaceae*

s. str. However, the present findings run contrary to those of Olmstead & Reeves (1995) and the phylogenetic classifications proposed by Judd & al. (1999); APG II (2003) and APGIII (2009), which broadened the concept of the *Plantaginaceae* s.str. to include many genera that have been formerly assigned to the *Scrophulariaceae* s.l. Also, they disagree with an earlier suggestion of Olmstead & Reeves (1995) and Wagstaff & Olmstead (1997), who have used??? cpDNA, that *Plantaginaceae* s.l. (including the added genera of the *Scrophulariaceae*) is a monophyletic family.

2. In the phenogram, the studied *Veronica* species occupy an intermediate position between the Scrophulariaceae taxa and *Plantago* species, thus supporting Warming's (1913) prognoses that *Veronica* represents a link between *Scrophulariaceae* and *Plantaginaceae*. Using data from seed morphology, Shehata & Loutfy (2006) have highlighted the prevalence of some particular affinities of *Veronica* with the *Plantago* species. Furthermore, the current outcome corroborated the reports of Olmstead & Reeves (1995), and Olmstead & al. (2001), who viewed *Veronica* as a sister group of *Plantago* on the basis of DNA sequence data.

3. Concerning the studied *Plantago* species (SII), a notable finding was the splitting off of *Plantago major* into an independent phenon line (C1), away from the

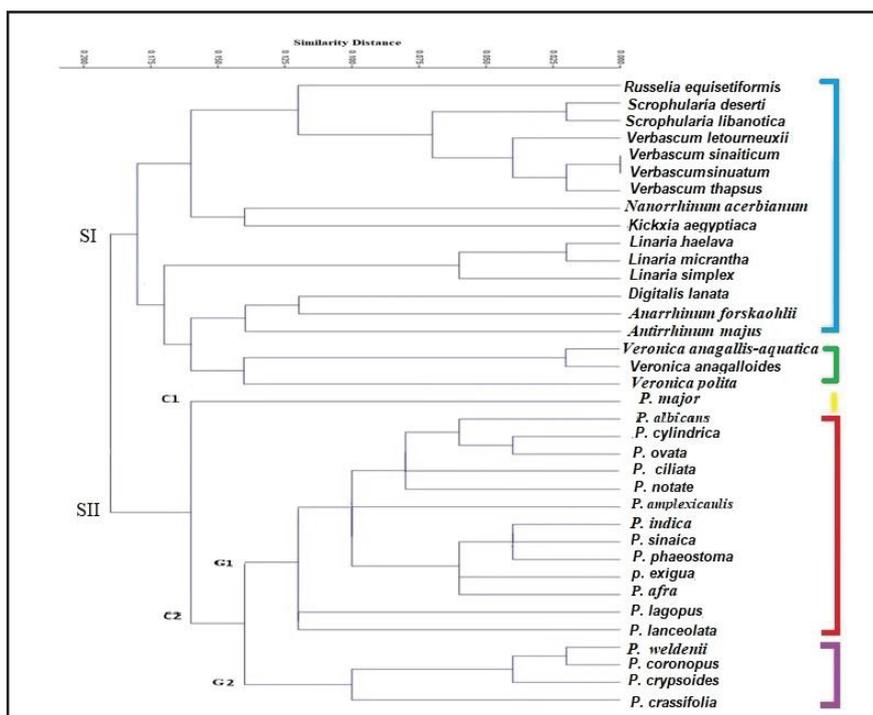


Fig. 6. Phenogram showing the relationships between the studied taxa based on fruit and seed morphological characters, expressed as average taxonomic similarity distance, and based on UPGMA method of analysis using PAST program version 3.

remaining species, based on distinguishing characters such as the presence of many seeds per capsule, angular/ elliptic seed shape and smooth anticlinal cell walls, and biconvex seed faces. The seed coat pattern of *P. major* has been variously described: as being of rugose type (Rothmaler & al. 1982; Ciftçi & Erol 2020), reticulate type (Shehata & Loutfy 2006), tuberculate type (Verma Bharti 2017), and areolate type with a wrinkled surface, (Houghoughi & al. 2016). The present data, along with the findings of Mohsenzadeh & al. (2023), have revealed a reticulate seed coat pattern. Differences in the reporting of seed coat patterns for a species may be due to injuries of the seed coat cells, thus leading to inaccuracy in identification, or presence of a cryptic species, especially in the subgenus *Plantago*, which has low morphological diversity (Mohsenzadeh & al. 2023).

Isolation of *Plantago major* from the other species favors its placement in the subgenus *Plantago*, as defined by Pilger (1937), Rahn (1978, 1996) and Shipunov (2021). *Plantago major* is considered an ancient species, from which all other representatives of the genus had derived (Good 1947). The present findings are consistent with those of Shehata & al. (2023), who have used morphological data and have shown that *Plantago major* (subgenus *Plantago*) has a different evolutionary line than the species of subgenera *Cornopus*, *Psyllium*, and *Albicans*. Nevertheless, the present findings contradict with those of Mohsenzadeh & al. (2023), who on the basis of data from seed morphology have reported high affinities between the subgenus *Plantago* and subgenus *Cornopus*, as compared to the subgenera *Albicans* and *Psyllium*.

The remaining 17 *Plantago* species are grouped in Cluster 2 (C2), which is further subdivided into two groups (G1 and G2), based on the similarity distance 0.141. Group G1 (*Plantago afra*, *P. albicans*, *P. amplexicaulis*, *P. ciliate*, *P. cylindrical*, *P. exigua*, *P. indica*, *P. lanceolata*, *P. lagopus*, *P. notata*, *P. ovata*, *P. phaeostoma*, and *P. sinaica*) corresponds to the subgenera *Albicans* and *Psyllium sensu* Rønsted & al. (2002). This finding is consistent with that of Mohsenzadeh & al. (2023), who have mentioned a strong affinity between the species of subgenera *Albicans* and *Psyllium* on the basis of morphological data and seed morphology, respectively.

Plantago afra, *P. exigua*, *P. indica*, *P. phaeostoma*, and *P. sinaica*, are grouped together at the level of similarity 0.63, due to their sharing two seeds per capsule, bottle-shaped cavity, scalariform overall seed coat sculpture pattern, straight anticlinal cell walls, and undulate periclinal cell walls. These five species correspond to the subgenus *Psyllium* section *Psyllium*, as proposed by Pilger (1937), or subgenus *Albicans* section *Psyllium sensu* Shipunov (2021). In the meanwhile, *Plantago albicans*, *P. cylindrical*, *P. ciliate*, *P. ovata*, and *P. notata* are linked at the similarity level of 0.085, due to sharing black seed color, cymbiform seed shape, elliptic cavity shape with rounded cavity edges, reticulate to scalariform overall seed coat pattern, thick anticlinal cell walls, and ruminant periclinal cell walls. The classification of these species corroborates that of Rahn (1996), and Shipunov's (2021), an infrageneric classification which places the above species among others under subgenus *Albicans*.

Plantago amplexicaulis is distinguished from the other studied species by the seed macro-morphological features (shape, color and size). This finding is supported by the results of Mohsenzadeh & al. (2020, 2023), who have reported that *Plantago amplexicaulis* is distinguished from the other species of subgenus *Albicans* by seed morphological characters. Furthermore, separation of *P. amplexicaulis* into a distinct phenon line in the produced phenogram is consistent with Shipunov's classification (2021), which has placed it in a separate section (*Bauphula*) within the subgenus *Albicans*.

Plantago coronopus, *P. crassifolia*, *P. crypsoides*, and *P. weldenii* have valuable taxonomic traits, viz. ovate fruit, lenticular seed, submedian and sunken, convexoplane seed faces, reticulate seed coat pattern, rounded outline cells, and thick undulated anticlinal cell walls with ruminant secondary sculpture. The current findings are consistent with the earlier research of Ciftçi and Erol (2020), who have found that the species under *Cornopus* section exhibited undulated anticlinal cell walls and reticulate seed surface sculpture pattern. The grouping of *Plantago coronopus*, *P. crypsoides*, *P. crassifolia*, and *P. weldenii* corresponds with Rahn's (1978, 1996) and Shipunov's (2021) taxonomic treatments, which have placed

these four species under subgenus *Coronopus* section *Coronopus*, based on both molecular and morphological approaches. *Plantago weldenii* and *P. coronopus* clustered at a similarity level of 0.022. The present findings agree with Tutel & al. (2005), who suggested a close relationship between them based on morphological and ecological approaches.

Moreover, grouping *Plantago lanceolata* and *P. lagopus* at the same similarity level of 0.123 convincingly supports the close relationship between them. They are characterized by black-colored cymbiform seed, elliptic cavity shape, rounded cavity edges, reticulate to scalariform overall seed coat pattern, thick anticlinal cell walls and finely undulate periclinal cell walls. These results agree with those of Rhan (1978) and Shipunov (2021), in the light of taxonomic treatments, who have assigned them to the subgenus *Albicans* (section *Lanceifolia*); they also agree with the finding of Taskova & al. (2002), who reported close relations between *Plantago lagopus* and *P. lanceolata* on the basis of chemical evidence. In terms of morphological and molecular approaches, Hamed & al. (2014) and Mohsenzadeh & al. (2020) emphasized a good relationship between *Plantago lagopus* and *P. lanceolata*.

Finally, regardless of the limited range of considered taxa, the present study supports the placement of the investigated Scrophulariaceae taxa in the family *Scrophulariaceae* s.l. and the maintenance of genus *Plantago* in a separate monogeneric family *Plantaginaceae*. However, it does not support the monophyly of the *Plantaginaceae* s.l. (including the added genera of the *Scrophulariaceae*). Meanwhile, the current study supports an earlier view that the *Plantaginaceae* s.str. and *Scrophulariaceae* s.l. families are linked by the genus *Veronica*. Furthermore, the obtained data have provided more information about the infrageneric classification of *Plantago* and have corroborated to some extent Shipunov's (2021) infrageneric classification system. Nonetheless, a more comprehensive study of more taxa by using other parameters seems to be needed in order to achieve more precise and concrete results regarding the transposition of certain genera of the family *Scrophulariaceae* into the *Plantaginaceae* family and in order to enhance the proper

taxonomic characterization of the genus *Plantago* followed by a more satisfactory classification.

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