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Palynological characteristics of the heterostylous subspecies of *Linum mucronatum* Bertol.

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Abstract

Palynological characteristics of the heterostylous subspecies of *Linum mucronatum* Bertol.— *Linum mucronatum* is a heterostylous species from sect. *Syllinum* with four subspecies in Iran. The present study examines palynological characteristics of the heterostylous subspecies of *Linum mucronatum*, pollen characters of brevistylous individuals (pins) as well as longistylous individuals (thrums) of these plants by scanning electron microscope and light microscope using the prolonged acetolysis procedure. Sixteen qualitative and quantitative characters were investigated. Pollen equatorial shapes varied between pin and thrum individuals of each subspecies with the exception of *L. mucronatum* subsp. *assyriacum*. Pollen sculptures varied between pin and thrum samples of each subspecies and were seen in the gemmate, clavate and baculate shapes. In addition, quantitative palynological characters differed between plants and ANOVA test showed significant variations for traits such as equatorial length, colpi width and apocolpium diameter. Hetrostylous individuals of each subspecies were separated from others in the UPGMA tree and also in the PCO and PCA plots. This study confirmed variations in pollen features between pin and thrum individuals of each subspecies.

Key words: heterostyly; *Linum mucronatum*; pollen characters; thrum/pin individuals.

Resumen

Características palinológicas de las subspecies de *Linum mucronatum* Bertol. con heterostilia.— *Linum mucronatum* es una especie con heterostilia, que pertenece a la sección *Syllinum* del género *Linum*, y tiene cuatro subespecies en Irán. En el presente estudio se examinan las características palinológicas de las subespecies heterostilas de *Linum mucronatum* Bertol., así como los caracteres polínicos de individuos de los morfos brevistilo (*pin*) y longistilo (*thrum*) de estas plantas, mediante microscopía electrónica de *scanning* y microscopía óptica usando el método de acetolisis prolongada. Se estudiaron un total de 16 caracteres cualitativos y cuantitativos. La forma ecuatorial del polen varía entre los morfos *pin* y *thrum* en todas las subspecies, excepto en *L. mucronatum* subsp. *assyriacum.* La ornamentación también varía entre las muestras de morfos *pin* y *thrum* de cada subespecie, en los que se puede observar polen gemado, clavado y baculado. En algunos caracteres palinológicos cuantitativos, se encontraron también diferencias entre morfos y el test de ANOVA muestra que son significativas en cuanto a la longitud ecuatorial, la anchura de los colpos y el diámetro del apocolpio. Los individuos heterostilos de cada susbespecie aparecen separados en el árbol UPGMA y también en los gráficos de PCO y PCA. Este estudio confirma las diferencias en las características del polen entre individuos *pin* y *thrum* de cada una de las subespecies.

Palabras clave: características polínicas; heterostilia; individuos *thrum*/*pin*; *Linum mucronatum*

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INTRODUCTION

Among diverse sexual systems in flowering plants, heterostyly has been one of the most attractive mating systems for researchers, although heterostylous plants are the minority within angiosperms (Naiki, 2012). In distylous taxa, two flower morphs are known as pin (long style/low anther) and thrum (short style/high anther). Ganders (1979) reviewed the taxonomic distribution of heterostyly and listed 164 genera in 24 families. Since then, new heterostylous taxa have been reported and based on the APG III classification system (APG III, 2009), in a way that 199 genera in 28 families in 15 orders are recognized as taxa that contain heterostylous species (Naiki, 2012).

Darwin provided the first functional interpretation of the adaptive significance of heterostyly and speculated on the evolutionary pathway leading to the evolution of distyly (Darwin, 1877; Barrett, 2010). A number of elaborate studies examining morphological, genetic, physiological, ecological, pollination, inheritance, phylogenetic reconstruction and evolutionary fields have been accumulated after Darwin's seminal work on heterostyly (Darwin, 1877; Dulberger, 1992; Pérez-Barrales *et al.*, 2006; Barrett & Shore, 2008; Weller, 2009; Cohen, 2010). Although morphological differences are found between species, several characteristics are shared among them. These morphological differences promote self-incompatibility to initiate outcrossing, which is essential for seed production since most heterostylous species are self-incompatible (Barrett, 1992; Richards, 1997).

Linum is the main genus of flax family (Linaceae) and it is widely distributed in the world with over 180 species (Heywood, 1993; McDill *et al.*, 2009). Several studies have described the breeding system of *Linum* species. These studies showed that heterostyly (distyly) is widespread in this genus (Ockendon, 1968; Dulberger, 1973; Rogers, 1979; Güvensen *et al.*, 2013). One of the first researchers of this genus was Darwin; he revealed the existence of distyly in several species such as *L. pubescens* Banks & Sol., *L. grandiflorum* Desf., *L. mucronatum* Bertol., *L. flavum* L., *L. perenne* L., *L. austriacum* L., and *L. maritimum* L. (Güvensen *et al.*, 2013). This feature exists in four of the five sections recognized by Ockendon & Walters (1968), namely, *Linum*, *Syllinum*, *Dasylinum* and *Linastrum*.

Heterostylous species display some morphological and micromorphological characteristics such as the number and size of pollen grains, stamens shape, and shape and color of stigma while its surface papillae were different in pin and thrum plants (Richards & Barrett, 1992). Similarly, in heterostylous species of *Linum* such as *L. perenne, L. grandiflorum* and *L. alpinum* morphological traits like exine sculpturing structure, the stigma size as well as the wall structure of the papillae, differ between the pin and thrum plants (Dulberger, 1981) and also in the case of nuclear genome size (Talebi *et al.*, 2012*a*). In other species of the genus, e.g. *L. album*, *L. austriacum* and *L. glaucum*, some morphological features and pollen sculpturing shape vary between thrum and pin populations (Talebi *et al.*, 2012*a*). Furthermore, Armbruster *et al.* (2006) found variation in distyly of *Linum suffruticosum* L., with styles and stamens bending and twisting, achieving a three-dimensional arrangement.

Linum mucronatum Bertol. belongs to sect. *Syllinum* Griseb. It is a heterostylous species with four subspecies in Iran (Sharifnia & Assadi, 2001). *Linum mucronatum* was reported to be a very variable species (Özcan & Zorlu, 2009), and a palynological study confirmed these interpretations (Talebi *et al.*, 2012*b*).

Pollen morphology in the genus *Linum* has an important taxonomic value (Saad, 1961; Erdtman, 1964). Characters such as exine sculpturing have been used as diagnostic at various taxonomic levels (Xavier & Rogers, 1963; Ockendon, 1968), as well as intraspecific exine polymorphism (Dulberger, 1981). In the present study in order to study the effects of heterostyly on palynological characters, pollen morphological traits of pin as well as thrum individuals of four *L. mucronatum* subspecies were investigated in Iran.

MATERIALS AND METHODS

Plant samples

Plant specimens of pin and thrum individuals of four *L. mucronatum* subspecies, namely *L. mucronatum* subsp. *armenum, L. mucronatum* subsp. *mucronatum, L. mucronatum* subsp. *orientale* and *L. mucronatum* subsp. *assyriacum* were collected from natural populations in different regions of

Table 1. Locality and herbarium voucher number of studied subspecies of *Linum mucronatum.*

Taxa	Locality	Voucher
L. mucronatum Bertol. subsp. *mucronatum* pin	Hamedan, Avaj, 2350 m	HSBU2011196
L. mucronatum Bertol. subsp. *mucronatum* thrum	Hamedan, Avaj, 2350 m	HSBU2011296
L. mucronatum subsp. *orientale* (Boiss.) P. H. Davis pin	Zanjan, 90 km Abhar to Zanjan, 1839 m	HSBU2011132
L. mucronatum subsp. *orientale* (Boiss.) P. H. Davis thrum	Zanjan, 90 km Abhar to Zanjan, 1839 m	HSBU2011232
L. mucronatum subsp. *armenum* (Bordzil.) P. H. Davis pin	Azerbaijan, Salmas, Ghoshchi, 1557 m	HSBU2011140
L. mucronatum subsp. *armenum* (Bordzil.) P. H. Davis thrum	Azerbaijan, Salmas, Ghoshchi, 1557 m	HSBU2011240
L. mucronatum subsp. *assyriacum* P. H. Davis pin	Khuzestan, Izeh, Atabaki Park, 350 m	HSBU2011164
L. mucronatum subsp. *assyriacum* P. H. Davis thrum	Khuzestan, Izeh, Atabaki Park, 350 m	HSBU2011264

Iran during spring 2010 and 2011. In each locality three to four individuals were collected randomly per each morph. Details of localities and voucher numbers are given in Table 1. Vouchers have been deposited in the herbarium of Shahid Beheshti University of Tehran, Iran (HSBU).

Palynological study

Pollen grains were obtained from mature buds of heterostyled individuals. For each subspecies, three specimens were used and from each specimen, at least three to four anthers were investigated and their pollen grains prepared for scanning electron microscope (SEM) and light microscopy (LM) using the prolonged acetolysis procedure of Erdtman (1960).

For LM, the pollen grains were mounted in glycerin jelly and sealed with paraffin. The polar (*P*) and equatorial (*E*) shape and length and *P*/*E* ratios were obtained under the light microscope (×1000). Three replicates were used for character measurements.

For SEM, the pollen grains were transferred directly to double-sided tape affixed stubs; they were then vacuum-coated with gold in Biorad E5200 auto sputter coater (Bio-Rad, Hercules, CA, USA) and were examined and photographed by a CamScan MV2300 scanning electron microscope at 10kV (Electron Optic Services Inc., Ottawa, Canada). The sculpturing types and dimensions, together with their fine structure, as well as colpi dimensions, apocolpium and mesocolpium length were studied (Table 2). The terminology in this paper corresponds to that the one used by Moore *et al.* (1991).

Statistical analyses

For grouping the studied heterostyled individuals, data were standardized (mean = 0, variance = 1) and used for the multivariate analyses including Unweighted Paired Group using Average (UPGMA), Principal Coordinate Ordination (PCO), and Principal Coordinate Analysis (PCA), *cf.* Podani (2000).

One-way ANOVA and t-test were employed to assess the significance of quantitative palynological difference between morph and among subspecies. Pearson's coefficient of correlation was used to ascertain the strength of correlations between quantitative palynological characters. NTSYS v2 (Rohlf, 1998) and SPSS v9 (1998) were used for statistical analyses.

RESULTS

In the present study pollen morphological and micromorphological characters of heterostyled individuals belonging to four subspecies of *L. mucronatum* were investigated. Four qualitative and eleven quantitative morphological traits (totally sixteen characters) were examined. In the formerly studied subspecies, pollen grains were trizonocolpate, with three long grooves in equatorial zone and had monomorphic or polymorphic processes on their surfaces.

Pollen polar shape was similar between pin and thrum individuals of each subspecies, while this character was alike among subspecies and was seen in the shape of circular. In contrast, pollen equatorial shape was different between pin and thrum individuals of each subspecies with the exception of *L.*

Table 2. Selected palynological characters of heterostylous individuals of *L. mucronatum* subspecies (all values are in μm). Pin: long style; Thrum: short style; *N*: sample size; SD: standard deviation.

Taxa	Statistical parameters	Equatorial shape	Equatorial length *(E)*	Polar shape	Polar length *(P)*	*P/E*	Aperture shape	Colpi length	Colpi width	Apocolpium length	Aperture length	Aperture width	Aperture distance	Mesocolpium	Colpi length / width
L. mucronatum subsp. *assyriacum* Thrum	Mean		34.86		65.00	1.86		52.24	3.88	13.40	1.37	1.35	0.81	26.34	13.02
	N	elliptic-emanate	25	circular	25	25	baculate and pilate	25	25	25	25	25	25	25	25
	SD		6.61		3.62	0.33		9.16	0.60	0.49	0.51	0.49	0.34	0.93	2.75
L. mucronatum subsp. *assyriacum* Pin	Mean		37.80		67.10	1.77		50.76	4.15	11.28	1.35	1.19	0.66	23.60	12.30
	N	elliptic-emanate	25	circular	25	25	gemmate and baculate	25	25	25	25	25	25	25	25
	SD		2.70		1.24	0.16		3.43	0.57	.0.78	0.11	0.09	0.03	0.82	1.66
L. mucronatum subsp. *armenum* Pin	Mean		43.25		42.40	0.98		31.45	3.69	13.25	1.41	1.23	0.64	28.76	8.33
	N	obtuse-truncate	25	circular	25	25	gemmate and baculate	25	25	25	25	25	25	25	25
	SD		2.87		1.19	0.09		1.61	0.80	2.18	0.07	0.06	0.12	1.90	1.92
L. mucronatum subsp. *armenum* Thrum	Mean		39.55		46.98	1.18		39.43	5.21	17.99	1.18	1.14	0.45	32.59	7.69
	N	elliptic-obtuse	25	circular	25	25	gemmate and clavate	25	25	25	25	25	25	25	25
	SD		6.17		3.87	0.11		3.57	0.93	0.36	0.082	0.11	0.04	0.53	1.61
L. mucronatum subsp. *mucronatum* Pin	Mean		37.50		52.00	1.38		42.97	2.79	15.39	1.12	1.10	.51	28.61	15.47
	N	obtuse-truncate	25	circular	25	25	gemmate and clavate	25	25	25	25	25	25	25	25
	SD		3.09		2.90	0.15		2.68	0.634	0.71	0.04	0.05	0.12	3.38	4.55
L. mucronatum subsp. *mucronatum* Thrum	Mean		43.28		46.98	1.08		40.91	7.69	39.79	1.51	1.41	0.58	29.92	5.27
	N	elliptic-obtuse	25	circular	25	25	small and large gemmate	25	25	25	25	25	25	25	25
	SD		1.48		1.36	0.045		2.12	1.67	0.48	0.18	0.13	0.16	1.93	1.33
L. mucronatum subsp. *orientale* Pin	Mean		39.89		59.88	1.50		45.99	2.67	16.47	1.41	1.36	0.76	27.89	17.31
	N	obtuse-truncate	25	circular	25	25	small and large gemmate	25	25	25	25	25	25	25	25
	SD		0.67		0.95	0.04		1.45	0.42	0.88	0.08	0.08	0.25	0.72	2.22
L. mucronatum subsp. *orientale* Thrum	Mean		52.01		46.97	0.90		39.30	13.87	50.62	1.41	1.29	0.78	34.65	2.84
	N	circular	25	circular	25	25	gemmate	25	25	25	25	25	25	25	25
	SD		1.02		0.49	0.01		1.97	2.08	2.02	0.14	0.15	.237	1.63	0.46

mucronatum subsp. *assyriacum*, the shape of which was elliptic-emanated and similar between pin and thrum individuals. Pollen equatorial shapes in pin individuals of subspecies *L. mucronatum* subsp. *armenum, L. mucronatum* subsp. *mucronatum* and *L. mucronatum* subsp. *orientale* were similar. These conditions were true in the case of thrum samples of *L. mucronatum* subsp. *armenum* and *L. mucronatum* subsp. *mucronatum* and were alike in the shape of elliptic-obtuse (Fig. 1). Pollen sculptures were different between pin and thrum samples of each subspecies and were seen in the gemmate and clavate shapes (*L. mucronatum* subsp. *armenum* thrum and *L. mucronatum* subsp. *mucronatum* pin), in the small and large gemmate (*L. mucronatum* subsp. *mucronatum* thrum, *L. mucronatum* subsp. *orientale* pin and *L. mucronatum* subsp. *orientale* thrum) and in the gemmate with baculate (*L. mucronatum* subsp. *armenum* pin and *L. mucronatum* subsp. *assyriacum* pin) (Fig. 2).

Pollen quantitative traits were different between heterostylous individuals and subspecies. Box and whisker plots are ideal for detecting and illustrating distributions pattern of quantitative data because the central, widespread and overall range are immediately apparent. In order to show the shape of data distribution, their central values as well as their variability and box and whisker plots were used for quantitative pollen data (Fig. 3). Largest length of equatorial axis (52.01 μm) was seen in *L. mucronatum* subsp. *orientale* thrum, while shortest equatorial axis length (34.86 μm) was found in *L. mucronatum* subsp. *assyriacum* thrum. The shortest (42.40 μm) and largest (67.10 μm) polar axis length were found in pin individuals of *L. mucronatum* subsp. *armenum* and *L. mucronatum* subsp. *assyriacum* respectively. The smallest (0.90) and also the highest (1.86) polar/equatorial axis (P/E) ratio occurred in *L. mucronatum* subsp. *orientale* thrum and *L. mucronatum* subsp. *assyriacum* thrum respectively. The longest colpi (52.24 μm) were seen in *L. mucronatum* subsp. *assyriacum* thrum, while the smallest (31.45 μm) was found in *L. mucronatum* subsp. *armenum* pin. ANOVA test showed significant variations ($P < 0.01$) of some quantitative traits such as equatorial length, colpi width, and apocolpium diameter (Table 3). Pollen sizes in each subspecies varied between thrum and pin individuals, while paired t-test analysis did not show significant variations ($P < 0.05$) in pollen size within thrum and pin individuals of the studied subspecies.

Heterostylous individuals of each subspecies were separated from each other in UPGMA tree (Fig. 4) and also PCA and PCO plots (Figs. 5 and 6). In addition, thrum individuals of *L. mucronatum* subsp. *mucronatum* and *L. mucronatum* subsp. *orientale* were placed far from others. This subject confirmed variations in pollen features between pin and thrum individuals of each subspecies. PCA biplot showed that some of heterostyled individuals had distinguished traits which became differentiated from others. For example, colpi width and apocolpium diameter were important characters in thrum samples of *L. mucronatum* subsp. *mucronatum* and *L. mucronatum* subsp. *orientale* or aperture shape was a distinct characteristic of *L. mucronatum* subsp. *armenum* thrum (Fig. 7).

DISCUSSION

Heterostyly is one of the most visible and fascinating examples of convergent evolution in plants, and it is present in all plant forms, from herbs to trees (Vuilleumier, 1967; Thompson, 2005). In this study, anther height in thrum flowers was larger than style height; the opposite was observed in pin flowers. In addition to the floral dimorphism, dimorphism in pollen size between thrum and pin flowers has often been reported, thrum pollen grains being larger (e.g. Baker, 1953; Dulberger, 1992; Ping & Johnston, 2001). In the present work, pollen grains traits were examined between pin and thrum individuals of four *L. mucronatum* subspecies.

Polar shape was similar between pin and thrum individuals of the studied taxa; this characteristic was also fixed among subspecies. In contrast, pollen equatorial shape varied between long and thrum flowers. Equatorial shapes were alike between pin individuals of some subspecies. These conditions were also found in thrum samples of some subspecies (for example, subsp. *mucronatum* and subsp. *armenum*); therefore equatorials shapes were analogous between thrum individuals of studied taxa. This has been described in other *Linum* species; for example, in *L. grandiflorum* pollen dimorphism was found (Saad, 1961; Erdtman, 1964; Dulberger, 1981). The numbers of pollen grains produced per flower and pollen size polymorphism between the

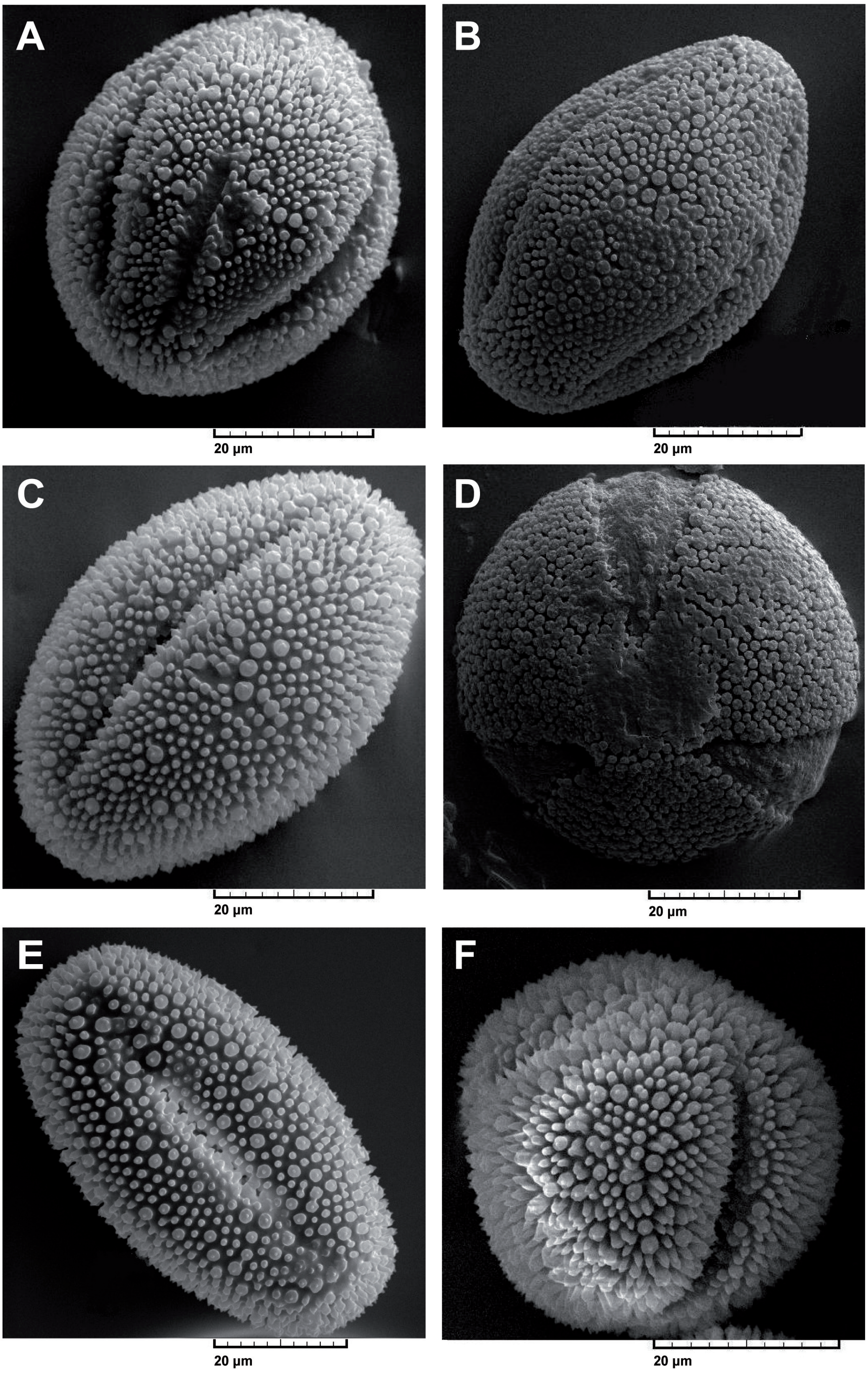

Figure 1. Electronic micrographs of pollen in studied subspecies of *Linum mucronatum*. Pin: long style; Thrum: short style. (A), subsp. *mucronatum* pin; (B), subsp. *mucronatum* thrum; (C), subsp. *orientale* pin; (D), subsp. *orientale* thrum; (E), subsp. *assyriacum* pin; (F), subsp. *armenum* pin. Scale bar: 20 µm.

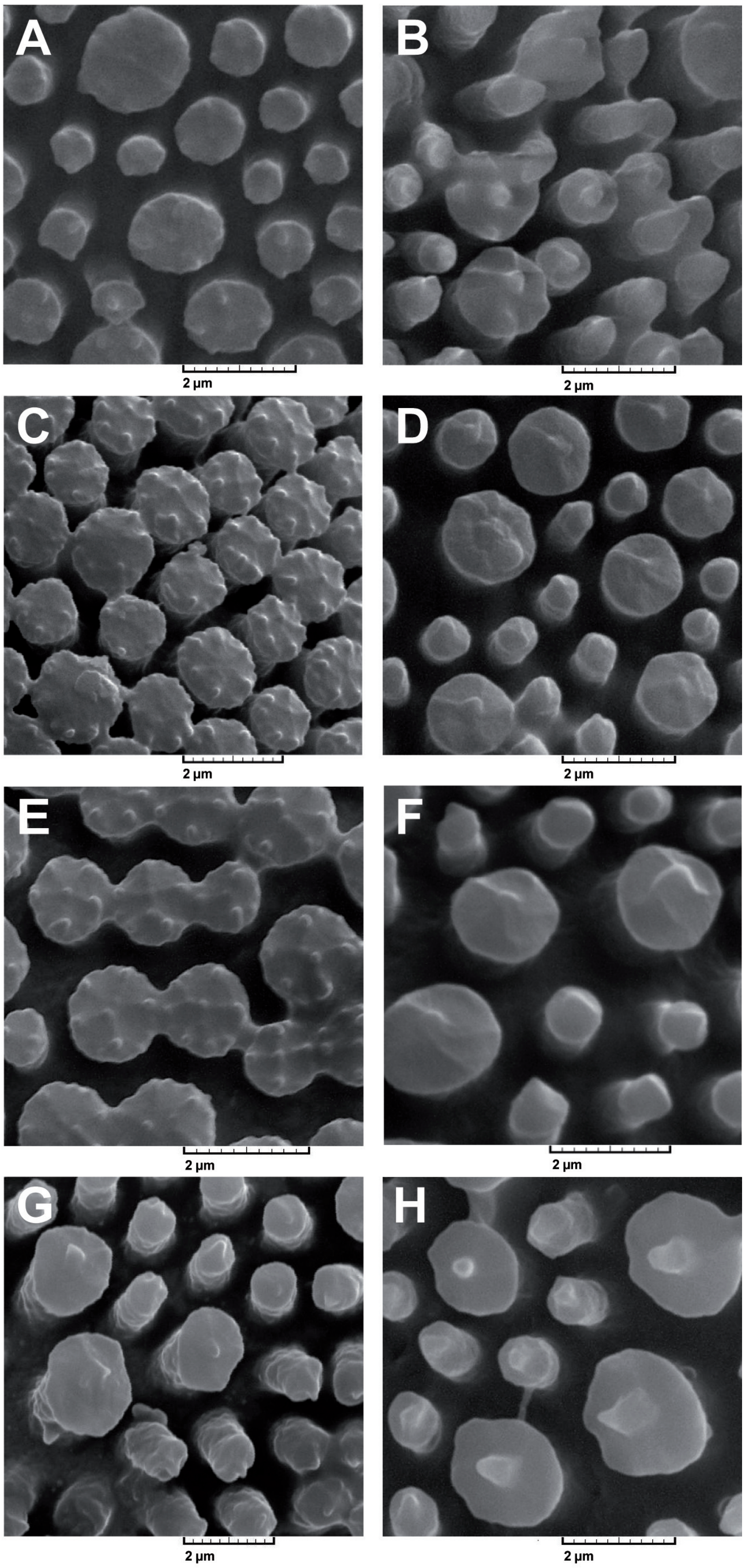

Figure 2. Electronic micrographs of exine surface sculpturing in the studied subspecies of *Linum mucronatum.* Pin: long style, Thrum: short style. (A), subsp. *armenum* thrum; (B), subsp. *armenum* pin; (C), subsp. *mucronatum* thrum; (D), subsp. *mucronatum* pin; (E), subsp. *orientale* thrum; (F) subsp. *orientale* pin; (G), subsp. *assyriacum* thrum; (H), subsp. *assyriacum* pin. Scale bar: 20 μm.

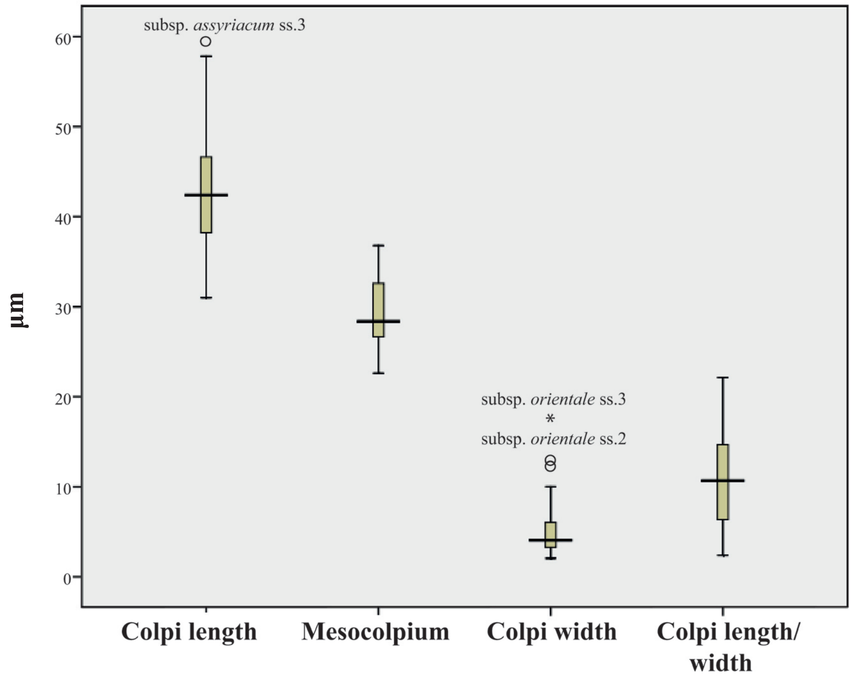

Figure 3. Box and whisker plots of quantitative palynological data.

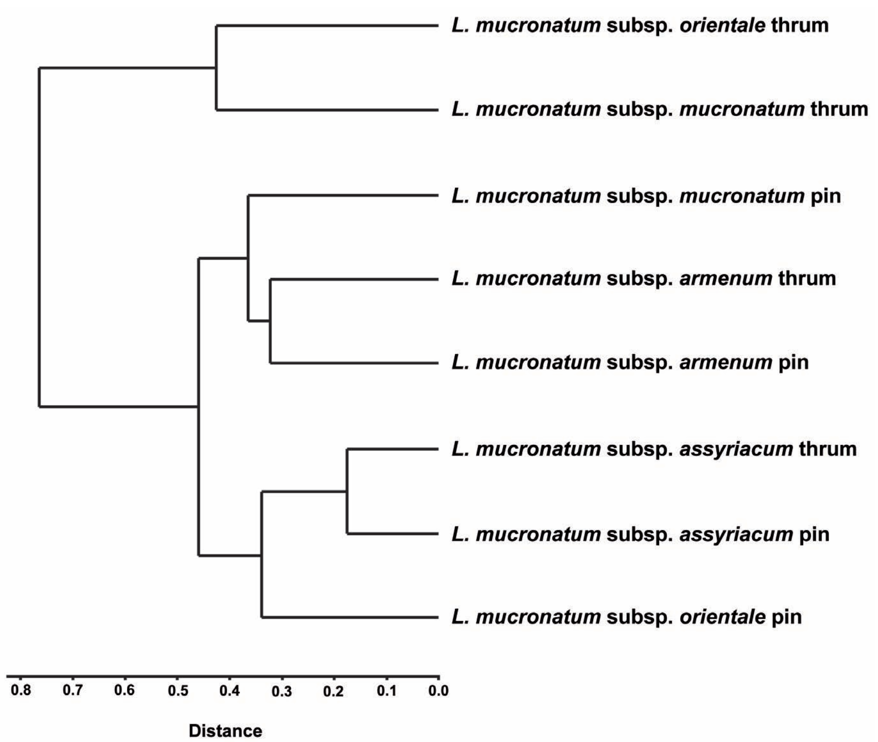

Figure 4. UPGMA tree of the heterostylous individuals of *Linum mucronatum* subspecies.

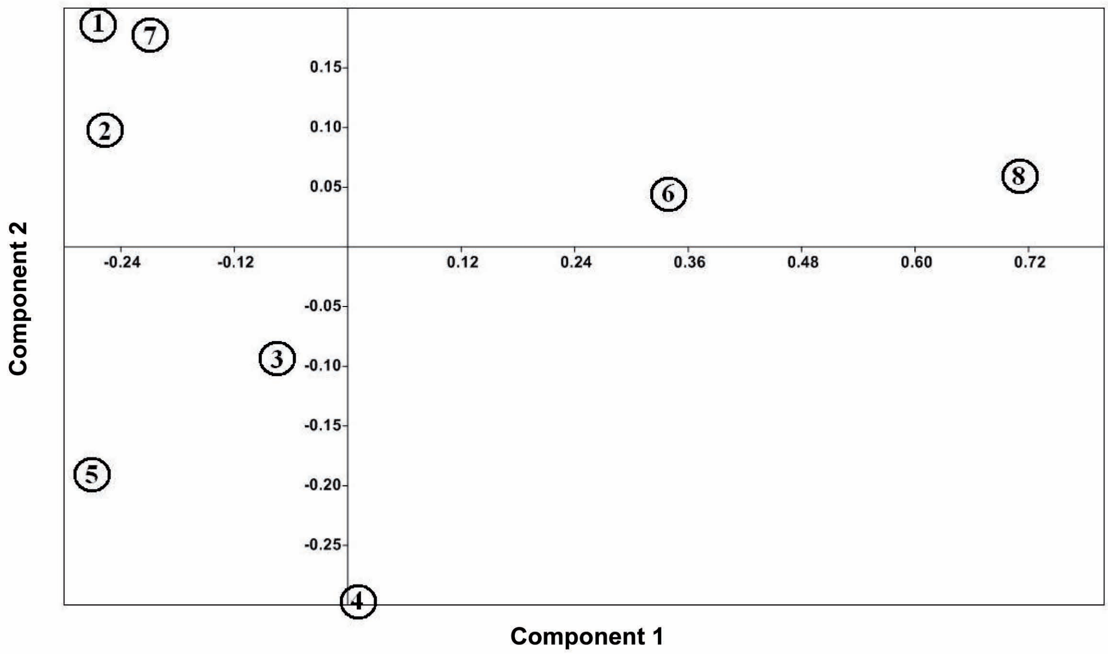

Figure 5. PCA plot of heterostylous individuals of the studied subspecies: (1), *L. mucronatum* subsp. *assyriacum* thrum; (2), *L. mucronatum* subsp. *assyriacum* pin; (3), *L. mucronatum* subsp. *armenum* pin; (4), *L. mucronatum* subsp. *armenum* thrum; (5), *L. mucronatum* subsp. *mucronatum* pin; (6), *L. mucronatum* subsp. *mucronatum* thrum; (7), *L. mucronatum* subsp. *orientale* pin; (8), *L. mucronatum* subsp. *orientale* thrum.

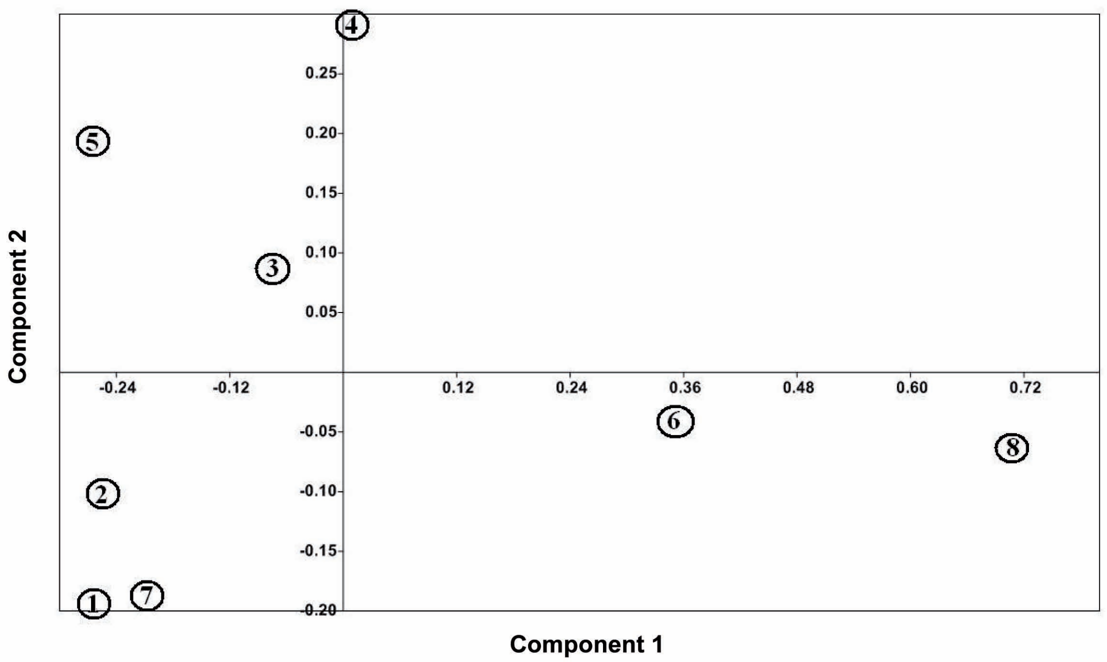

Figure 6. PCO plot of heterostylous individuals of the studied *L. mucronatum* subspecies: (1), *L. mucronatum* subsp. *assyriacum* thrum; (2), *L. mucronatum* subsp. *assyriacum* pin; (3), *L. mucronatum* subsp. *armenum* pin; (4), *L. mucronatum* subsp. *armenum* thrum; (5), *L. mucronatum* subsp. *mucronatum* pin; (6), *L. mucronatum* subsp. *mucronatum* thrum; (7), *L. mucronatum* subsp. *orientale* pin; (8), *L. mucronatum* subsp. *orientale* thrum.

Table 3. Results on the ANOVA analysis to assess for differences in quantitative palynological traits in *Linum mucronatum* subspecies. d.f.: degrees of freedom; *F*: *F*-statistic; *P*: probability.

Characters		Sum of Squares	d.f.	Mean Square	*F*	*P*
Equatorial length	Between Groups	4572.329	7	653.190	47.211	0.000
	Within Groups	2656.402	192	13.835		
	Total	7228.730	199			
Polar length	Between Groups	15,639.257	7	2234.180	421.262	0.000
	Within Groups	1018.278	192	5.304		
	Total	16,657.536	199			
Polar/equatorial axis length ratio	Between Groups	22.856	7	3.265	138.328	0.000
	Within Groups	4.532	192	0.024		
	Total	27.388	199			
Colpi length	Between Groups	7430.370	7	1061.481	65.921	0.000
	Within Groups	3091.642	192	16.102		
	Total	10,522.013	199			
Colpi width	Between Groups	2424.905	7	346.415	280.324	0.000
	Within Groups	237.267	192	1.236		
	Total	2662.172	199			
Colpi length/width ratio	Between Groups	4754.569	7	679.224	122.740	0.000
	Within Groups	1062.495	192	5.534		
	Total	5817.065	199			
Apocolpium diameter	Between Groups	2.082×10^7	7	2.975×10^6	2.218×10^6	0.000
	Within Groups	257.456	192	1.341		
	Total	2.082×10^7	199			
Mesocolpium width	Between Groups	2075.442	7	296.492	99.667	0.000
	Within Groups	571.166	192	2.975		
	Total	2646.607	199			

morphs are sometimes associated with heterostyly as ancillary characteristics (Naiki, 2012).

In the studied subspecies, pollen size in thrum individuals was larger than in pin ones, with the exception of subsp. *assyriacum*. This condition was also found in species of the genera *Damnacanthus* (Naiki & Nagamasu, 2003), *Plumbago* (Ferrero *et al*., 2009) and *Polygonum* (Chen & Zhang, 2010).

With the exception of subsp. *orientale*, pollen surface sculpturing types varied between pin and thrum flowers in each subspecies. Although, the kind of sculpture looked alike in thrum and pin flower in subsp. *orientale*, the size of surface ornamentation in pollen grains was unequal and presented in small and large parts. In some heterostylous species of *Linum* such as *L. austriacum*, *L. album* and *L. glaucum*, exine sculpturing structure differed between pin and thrum flowers (Talebi *et al.*, 2012*a*). Furthermore dimorphic exine ornamentation was observed in other *Linum* species for example, *L. grandiflorum*, *L. perenne*, *L. pubescens*, *L. mucronatum*, *L. flavum*, and *L. maritimum* (Dulberger, 1974). Pollen polymorphism associated to the morphs is a typical feature in heterostyly species. Exine pattern of pollen can interact with biotic and abiotic pollination vectors, and it can also interact with the surface area of the stigma interface and mediate stigma adhesions (Talebi *et al.*, 2012*a*).

Aperture size, number and complexity affect environmental vulnerability to desiccation, fungal invasion and mechanical stress, and serve as portals for pollen tube exit during germination (Edlund *et al*., 2004). Wang *et al.* (2009) found

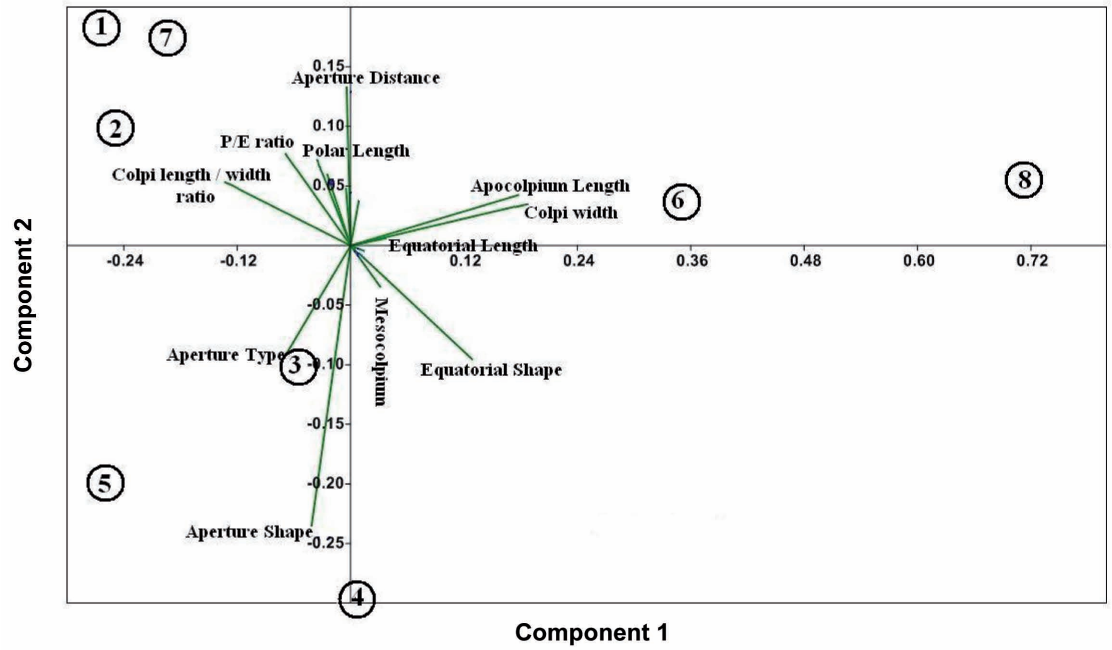

Figure 7. PCA biplot of palynological characters of *Linum mucronatum* subspecies: (1), *L. mucronatum* subsp. *assyriacum* thrum; (2), *L. mucronatum* subsp. *assyriacum* pin; (3), *L. mucronatum* subsp. *armenum* pin; (4), *L. mucronatum* subsp. *armenum* thrum; (5), *L. mucronatum* subsp. *mucronatum* pin; (6), *L. mucronatum* subsp. *mucronatum* thrum; (7), *L. mucronatum* subsp. *orientale* pin; (8), *L. mucronatum* subsp. *orientale* thrum.

that in *Pedicularis* (Orobanchaceae) there was a significant association between pollen aperture types and corolla types, as well as between pollination syndromes and corolla. There was a distinct correlation between exine ornamentation, floral morphology and pollination in *Bauhinia* (Ferguson & Pearce, 1986). Differences in surface ornamentation in pollen grains have also been reported in some distylous species (Baker, 1953, 1956; Dulberger, 1975). For example, pollen grains are reticulate or spinulose in *Waltheria* (Köhler, 1976), and smooth or granulate muri in *Damnacanthus* (Naiki & Nagamasu, 2003).

Heterostyly should be considered a morphological variation more than simple variations in anther and style height, because other characters, i.e. morphological and cytological traits show difference between pin and thrum individuals. This condition leads to intra and interpopulation variance. Talebi *et al*. (2012*a*) examined morphological, cytological and also palynological characters of three thrum and pin populations of three species of the genus *Linum*, namely *L. austriacum*, *L. glaucum* and *L. album*. Results showed a higher mean value of the plant height, size of the basal leaves width, flower leaves width, calyx length, sepal length and petal length occurred in the pin plants, while the mean value of branch number, basal leaves length, flower leaves length, calyx width, pedicel length and sepal length was higher in the thrum plant populations. In their study, t-test analyses of morphological characters showed significant difference for some of the studied characters. Principal coordinate analysis of pin and thrum plant populations based on all morphological characters also separated the two morphs of the three species studied. C-values obtained by flow cytometry, differed in the pin and thrum plants of the studied species and also the aperture shape differed between these populations (Talebi *et al.*, 2012*a*).

The arrangements of pin and thrum individuals of the studied taxa in plots and tree were very interesting as the heterostylous individuals of some subspecies were separated from each other. For example, thrum individuals of subsp. *orientale* and subsp. *mucronatum* were closely tougher.

Similarities in pollen can influence palynological taxonomic treatment in this genus. Pollen morphology of the genus *Linum* could not firmed any of the previously proposed arranging the species in sections (Grigoryevka, 1988) and also the obtained palynological data of fifteen *Linum* taxa cannot show the species relationship in sections and also infraspecific classification in the mentioned genus (Talebi *et al.*, 2012*b*).The reasons of these uncongenial may be related to heterostyly phenomenon which abundantly present in this genus and about 40% of these species are distylous and occurred in four out of five sections of the genus, namely *Linum*, *Syllinum*, *Dasylinum* and *Linastrum* (Rogers, 1979; Sharifnia & Assadi, 2001).

Two major models were considered for evolution of heterostyly (mainly distyly) in plants: change in flowers morphology or diallelic self-incompatibility designing by Charlesworth & Charlesworth (1979). Based on this model, under conditions of pollinator limitation, self-incompatibility first occurred within a homostylous population as a result of selection to prevent self-fertilization then stigma height polymorphism occurred to reduce intra-flower interference between males and females, and subsequently reciprocal herkogamy evolved to promote legitimate pollination. Another model proposed by Lloyd & Webb (1992). These authors proposed that the ancestral flowers showed approach herkogamy, and subsequent polymorphism in stigma height followed by invading and spreading a mutant that had shortened style length, and then reciprocal herkogamy was established by the appearance of reverse herkogamous mutants. Heteromorphic self-incompatibility may arise if selection restricts self-fertilization. The phylogenetic study of *Linum* and Linaceae subfamily Linoideae indicates that neither heterostyly nor homostyly can yet be confirmed as the ancestral state in Linoideae or Linaceae, but provide strong evidence that breeding system is evolutionarily labile in this group (McDill *et al.*, 2009).

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