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Original scientific article

PALYNO-MORPHOLOGY OF *Hedera* L. (the Ivy genus, Araliaceae) AND THEIR SYSTEMATICS IMPLICATIONS

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The palynomorphological characteristics of 5 species belonging to *Hedera* of the Araliaceae family were studied in detail. These plant species were collected from various phytogeographical regions of Iran and Hungary. The palynological investigation was accomplished using Transmission Electron Microscope (TEM) and Scanning Electron Microscope (SEM). Pollen grains of the species are reticulate, prolate, sub-prolate, tricolporate, isopolar, radially symmetrical, and monad. The largest pollen grain was in the *Hedera colchica* species (72.24±1.6 μm) and the smallest pollen grain was in *Hedera helix* (28.63±2.1 μm). The research carried out by the TEM showed that the species were different in terms of exine thickness, tectum thickness, foot layer thickness, the diameter and length of the Columella, the thickness and shape of the Caput, the tectum to foot layer (T/F) ratio, the absence or presence of the Endexine and the thickness of the Intine layers. The main purpose of this study was the importance of the relationship between pollen grain size and the number of chromosomes and ploidy level in *Hedera* species.

Keyword: Pollen Morphology, Systematics, TEM, SEM, *Hedera*

INTRODUCTION

The genus *Hedera* L. occupies riparian vegetation and forest understories in temperate latitudes throughout Asia, N Africa, and Europe (MEUSEL *et al*., 1965; MABBERLEY, 2018). The taxonomical treatments of *Hedera* published through the second half of the twentieth century identified between 3 and 19 species (SEEMANN, 1868; TOBLER, 1912; LAWRENCE and SCHULZE, 1942; POJARKOVA, 1951; FALLAH and GHAHREMANINEJAD, 2021). Previous cytogenetic and molecular investigations found western and eastern parts of the Mediterranean region as the main diversity centres for *Hedera*. The key traits were analyzed to assess the ancestral character states

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and geographical patterns of ivy lineages. Interestingly, the western Mediterranean has the greatest *Hedera* diversity (LOWRY *et al*., 2001; WEN *et al*., 2001; VALCÁRCEL *et al*., 2003; PLUNKETT *et al*., 2004). Since McAllister and Rutherford's, treatment the delimitation and identification of *Hedera* species have mostly been on the basis of the combination of trichome morphology (stellate-multangular, stellate-rotate, and scale-like hairs (SEEMANN, 1868; LUM and MAZE, 1989; MCALLISTER and RUTHERFORD, 1990; ACKERFIELD and WEN, 2002; VALCÁRCEL and VARGAS, 2010), juvenile leaf morphology from entire to 3–7 lobate (RUTHERFORD *et al*., 1993; ACKERFIELD and WEN, 2002; VALCÁRCEL**,** 2008; VALCÁRCEL and VARGAS, 2010), and ploidy levels (from 48 to 196; VARGAS *et al*., 1999). Also, the *Hedera hibernica* status has been discussable (ROSE, 1996). Several authors (e.g., STACE, 1997; KENT, 1991; POYARKOVA, 1973; LAWRENCE and SCHULZE, 1942) have treated this taxon as *H. helix* subsp. *hibernica*, whereas others identified it as a distinct species (ROSE, 1996; MCALLISTER, 1990; BEAN, 1915). The number of chromosomes is different between between *H. hibernica* and *H. helix* subsp. *helix* (MCALLISTER, 1990). The Asian species of *Hedera* have received comparatively little attention taxonomically (RUTHERFORD *et al*., 1993). Most workers have recognized four taxa from Asia: *H. nepalensis* var. *nepalensis*, *H. nepalensis* var. *sinensis*, *H. pastuchovii*, and *H. rhombea*. Trichome morphology has been widely used to delimit taxa in *Hedera* (SEEMANN, 1868; HIBBERD, 1893; TOBLER, 1912; LAWRENCE and SCHULTZE, 1942; MCALLISTER, 1981; ROSE, 1996). ACKERFIELD and WEN (2002) hypothesized the maternal parents for several allopolyploid taxa based on cpDNA evidence. The ITS phylogeny (VARGAS *et al*., 1999) suggests that the hexaploid *H. maderensis* subsp. *iberica* (2*n*=144) originated as the result of hybridization between a diploid taxon with reddish- scale hairs (probably *H. canariensis* as the maternal ancestor) and the tetraploid *H. hibernica* (2n=96). Micromorphological characters are good diagnostic value to recognize many taxa, fundamentally at the species level. The pollen morphological analysis is successfully used as an additional document for delimitation of the taxa (AMINI *et al*., 2018; 2019). In the Northwest European Pollen Flora mentioned that pollen class of *Hedera* is 3-zonocolporate, sub-erect to semi-erect with reticulate ornamentation (VAN HELVOORT and PUNT, 1984). In Scandinavian pollen flora, apocolpium diameter of pollen grains is about 10 p. Colpi is narrow; exine thickness is about 2.5 p and sometimes distinctly thicker at poles (ERDTMAN *et al*., 1961).

Pollen studies in recent years on different plant families in Iran have been increasing significantly (e.g., ATAZADEH *et al*., 2020; MOHSENZADEH *et al.*, 2020; RANJBAR *et al.*, 2020); but the only study on pollen morphology of Iranian *Hedera* species related to the findings of AMINI *et al*. (2019) by scanning electron microscopy that studied 11 accessions of two species of *Hedera* (*H. helix* and *H. pastuchovii*) have been considered to evaluate the relationships in *Hedera*. They observed the pollen grains of the studied species revealed some variations and separated two species of *Hedera*. There is no comprehensive palynological investigation of *Hedera* species in Iran. The main aim of the present study is to use the pollen grain features as a source of diagnostic characters to distinguish and relationship between pollen size and ploidy level in different Iranian and Central European *Hedera* species.

MATERIAL AND METHOD

Pollen sampling

Totally 14 populations were collected from 5 species (*H. helix*, *H. crebrescens*, *H. hibernica*, *H. pastuchovii*, and *H. colchica*) of *Hedera* genus from different habitats in Iran and Central Europe and the Soroksár Botanical Garden of Budapest for study pollen grain features. (Table 1). For SEM morphology, ten individuals from each location were examined for 2 qualitative characteristics, 6 quantitative characteristics (Table 2), and 13 quantitative characteristics of 3 species for TEM (Table 3). For the palynological terminology we followed ERDTMAN (1952), FAEGRI and IVERSEN (1964) and PUNT *et al*. (1994).

Table 1. List of species used in the palynological studied

No	Taxon name	Locality
1	*Hedera helix* L.	Hungary: Budapest; Gellért Hill
2	*H. helix*	Iran: Golestan; Ziyarat Village
3	*H. helix*	Iran: Kermanshah; Paveh
4	*H. crebrescens* M. Bényei-Himmer et M. Höhn	Hungary: Budapest; Gellért Hill
5	*H. hibernica* (G.Kirchn.) Bean	Hungary: Budapest; Soroksár Botanical Garden Botanical Garden
6	*H. hibernica*" Variegata"	Hungary: Budapest; Soroksár Botanical Garden Botanical Garden
7	*H. pastuchovii* Woronow	Iran: Golestan; Naharkhoran
8	*H. pastuchovii*	Iran: Mazandaran; Savadkuh
9	*H. pastuchovii*	Iran: Mazandaran; Nowshahr; Mashalak
10	*H. pastuchovii*	Iran: Gilan; Talesh; Khotbesara
11	*H. colchica* (K.Koch) K.Koch	Iran: Gilan; Talesh; Kargan Rud
12	*H. colchica*	Iran: Gilan; Astara; Lisar Rud
13	*H. colchica*	Iran: Gilan; Asalem to Khalkhal
14	*H. colchica*	Iran: Mazandaran; Ramsar; Neidasht

SEM techniques.

For SEM studies, Pollen grains of 14 populations of *Hedera* species followed the standard methods by MOORE and WEBB (1978), suspended in a drop of water were directly transferred by a fine pipette to a metallic stub using double sided cello tape and then coated with gold in a sputtering chamber (Sputter Coater BALTEC, SCDOOS). Coating with gold by the physical vapor deposition method (PVD) was restricted to 100 Å. Samples were obtained with electron microscopy (SEM) of Tarbiat Modares University (XL30 model and Philips Company from the Netherlands).

For transmission electron microscopy (TEM), fresh pollen grains of three taxa (*Hedera helix*, *H. hibernica* and *H. pastuchovii*) were maintained for ca. 50 hours in TAG-solution (1% tannic acid + 1 glutaralde-hyde in 0.1 M phosphate buffer, pH = 7.4). Following dehydration in alcohol solutions (RUZIN, 1999), grains were embedded in araldite resin using a rapid embedding technique described by SKVARLA (1966). Sections were cut with a Sovall MT 6000 ultramicrotome, stained with 1% aqueous uranyl acetate for 20 min at room temperature and with lead citrate for 5 min. The sections, on copper grids, were examined in a Hitachi H7100. Measurements of the exine and intine layers of the pollen wall were made in nanometers using a TEM with an ''image measuring system.'' The thickness of the exine, intine, and total pollen wall was determined on 10 pollen grains at three different regions each.

Data analysis

To detect significant differences in the studied characters among the studied species, an analysis of variance (ANOVA) and mean comparisons were carried out using Duncan's Multiple Range Test (α =0.05). Then Squared Euclidean Distances used as a measure of dissimilarity (WARD, 1963) and using Euclide Principal Components Analysis (PCA) were performed among the species to determine palynological characters useful for separating the species. To group, the species were cluster analyzed using (Ward's methods).

ANOVA and mean comparisons were carried out using were conducted using SAS (ver 9.1) software while clustering and principal component analysis (PCA) were performed using SPSS (ver16). The evaluated and measured characters of the species studied of *Hedera* are summarized in Table 2.

Table 2. Evaluated SEM characters of pollen grains in Hedera species studied (values M ± SD μm)

No.	Taxon name	Polar axis length (μm)	Equatorial axis length (μm)	P/E ratio	Colpus length (μm)	Colpus with (μm)	Pore diameter(μm)	Pollen shape	Exine ornamentation type
1	*H. helix* L. (Gellért Hill)	28.63±2.1	24.41±2.2	1.17±0.04	17.21±3.6	2.55±1.5	0.57±0.15	Sub-prolate	reticulate
2	*H. helix* (Ziyarat Village)	39.00±3.4	33.44±2.9	1.17±0.06	35.83±4.1	2.07±0.63	0.78±0.34	Sub-prolate	reticulate
3	*H. helix* (Paveh)	40.59±1.6	35.37±1.4	1.15±0.04	27.98±4.7	3.70±1.41	0.87±0.22	Sub-prolate	reticulate
4	*H. crebrescens* (Gellért Hill)	38.42±4.1	31.92±1.9	1.20±0.12	33.83±2.8	3.25±0.55	1.44±0.54	Sub-prolate	reticulate
5	*H. hibernica* (Soroksár Botanical Garden)	49.25±1.6	39.11±2.8	1.27±0.11	41.55±5.9	4.13±0.72	1.40±0.31	Sub-prolate	reticulate
6	*H. hibernica* "Varigata" (Soroksár Botanical Garden)	43.72±2.9	35.91±1.4	1.25±0.11	38.27±4.4	4.23±2.4	1.32±0.11	Sub-prolate	reticulate
7	*H. pastuchovii* (Naharkhoran)	60.71±3.9	47.28±2.4	1.31±0.23	55.93±1.6	3.31±1.3	1.45±0.33	prolate	reticulate
8	*H. pastuchovii* (Savadkuh)	59.56±3.9	39.00±3.6	1.53±0.09	58.89±4.3	3.82±1.5	1.32±0.12	prolate	reticulate
9	*H. pastuchovii* (Mashalak)	56.01±2.4	44.01±2.9	1.31±0.21	61.72±3.7	5.71±1.3	1.63±1.1	prolate	reticulate
10	*H. pastuchovii* (Khotbesara)	60.49±2.8	41.30±4.2	1.48±0.12	63.97±3.5	6.81±1.5	1.67±0.56	prolate	reticulate
11	*H. colchica* (Kargan Rud **)**	70.79±3.0	51.55±4.5	1.38±0.24	67.75±5.2	6.35±1.11	1.57±0.53	prolate	reticulate
12	*H. colchica* (Lisar Rud)	69.25±2.8	49.34±2.2	1.42±0.02	64.24±4.9	5.65±1.16	1.64±0.41	prolate	reticulate
13	*H. colchica* (Asalem to Khalkhal)	72.24±1.6	48.84±1.3	1.48±0.07	67.02±3.2	4.27±1.03	1.26±0.16	prolate	reticulate
14	*H. colchica* (Neidasht)	69.78±2.8	49.48±2.2	1.42±0.14	64.30±3.7	5.42±0.52	1.50±07	prolate	reticulate

RESULTS

General pollen grain features

All palynological structures and measurements for the examined species concerning pollen type from polar view, polar (P) and equatorial (E) measurements, P/E ratio, colpus length and width, pollen shape and exine ornamentation were shown in Table 2. Selected SEM micrographs of the pollens and their surfaces are shown in Figs. 1 and 2. Generally, type of pollen grain aperture is observed tricolporate among studied species (Figs. 1 and 2). The shape classes are based on the ratio between the length of polar axis (P) and equatorial diameter (E) (ERDTMAN, 1952). The pollen type ranged from sub-prolate in *H. helix* (Gellért Hill), *H. crebrescens* (Gellért Hill) and *H. hibernica* (Soroksár Botanical Garden) (Figs. 1 a, d, g), to prolate in *H. pastuchovii* (Naharkhoran) and *H. colchica* (Asalem to Khalkhal) (Figs. 2 m, p) in equatorial view and spheroidal all species in polar view. Also AMINI *et al.* (2019) stated that the pollen shape is subprolate in *H. helix* but prolate in *H. pastuchovii* which is congruent with present findings.

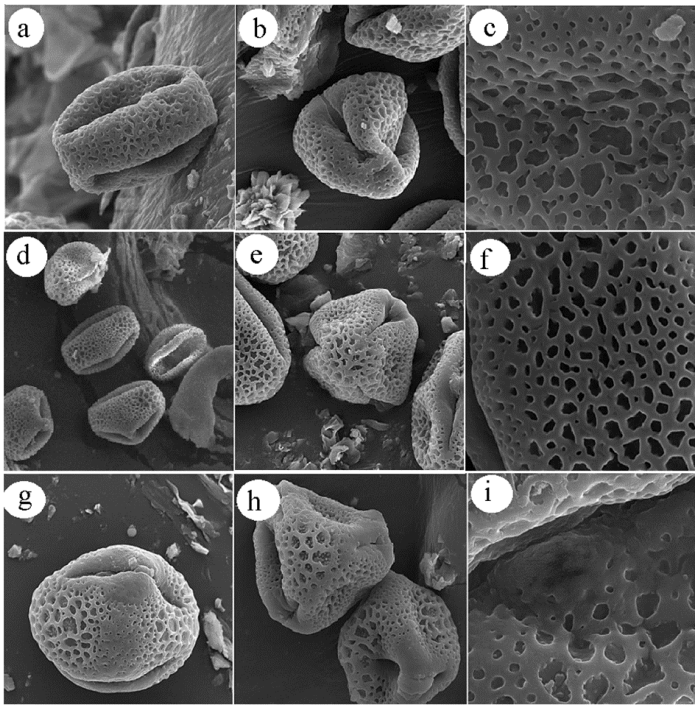

Figure 1. Pollen SEM micrographs of *Hedera* species: a-c: *H. helix* (Gellért Hill), d-f: *H. crebrescens* (Gellért Hill), g-i: *H. hibernica* (Soroksár Botanical Garden), a,d,g – equatorial view; b,e,h - polar view; c,f,i -exine sculpture.

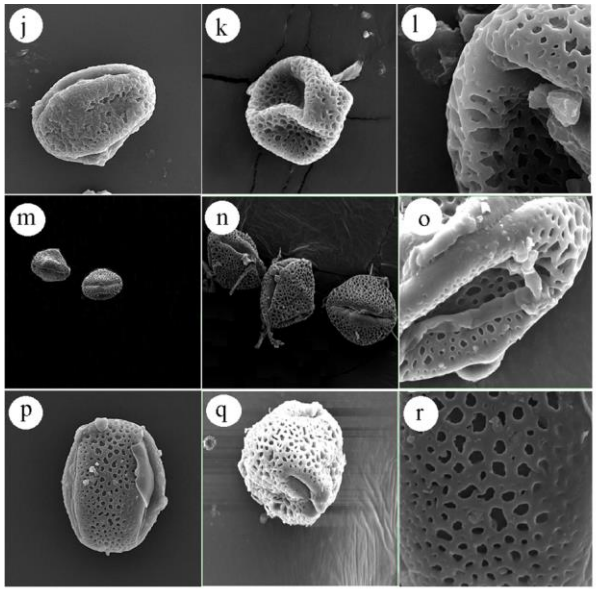

Figure 2. Pollen SEM micrographs of *Hedera* species: j-l: *H. hibernica* "Varigata" (Soroksár Botanical Garden), m-o: *H. pastuchovii* (Naharkhoran), p-r: *H. colchica* (Asalem to Khalkhal), j,m,p– equatorial view; k,n,q - polar view; l,o,r -exine sculpture.

Analysis has shown that *H. colchica* (Asalem to Khalkhal) had the largest (Table 2, Figs. 2 m-o), while *Hedera helix* (Gellért Hill) had the smallest pollen grains (Table 2, Figs. 1 a-c).

Mean polar axis length varied from 28.63±2.1 μm, *Hedera helix* (Gellért Hill) to 72.24±1.6 μm *H. colchica* (Asalem to Khalkhal), while mean of the equatorial axis length varied from 24.41±2.2 μm (*H. helix* (Gellért Hill)) to 51.55±4.5 μm (*H. colchica* (Kargan Rud)). The main colpus length varied from 17.21±3.6 μm (*H. helix* (Gellért Hill)) to 67.75±5.2μm (*H. colchica* **(**Kargan Rud)). In all the 5 analyzed, species the P/E ratio varied from 1.15±0.04μm (*H. helix* (Paveh)) to 1.53±0.09μm (*H. pastuchovii* (Savadkuh)). The main features of the investigated pollen grains are summarized in Table 2. The basic ornamentation of exine surface in the *Hedera* species studied was reticulate.

The *Hedera* species pollen wall consisted of two layers: the outer exine layer and the inner intine layer (Fig. 3). The pollen walls of all *Hedera* species were of similar appearance in cross-section. The exine is subdivided into two components, sexine and nexine, and is of the tectate type (Fig. 3 a, c, e). The tectum is continuous and the thickness of these layers varied significantly among the cultivars, as shown in Table 3. For each pollen grain, the thickness of the intine layer was variable. Around the aperture region, the intine layer was found to be thicker, whereas the exine layer was thinner (Fig. 3).

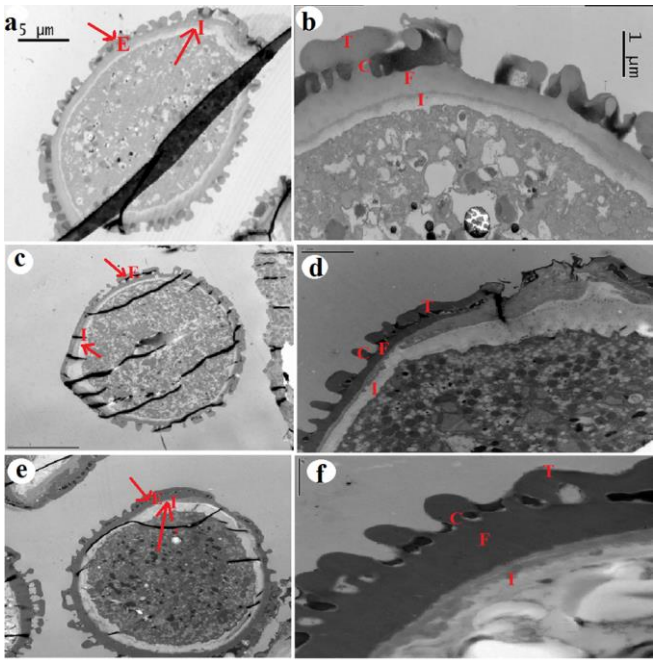

Figure 3. Transmission electron micrographs (TEM) of pollen grain and pollen wall in *H. helix* (Gellért Hill) (a-b) and *H. pastuchovii* (Naharkhoran) (c-d) and *H. hibernica* (Soroksár Botanical) (e-f). E: Exine; T: Tectum; F: Foot Layer; C: Columella; I: intin.

Infrageneric variation

The analysis of variance (ANOVA) showed that all populations were significantly determined by the length equatorial and polar axes, P/E ratio, Colpus length, Colpus with, and pore diameter (Table 4). ANOVA analysis and group comparison between diploid species, *H. helix* and *H. crebrescens* showed significant difference only for pore diameter. In group comparisons between *H. helix* (diploid) and *H. hibernica* (tetraploid) species showed significant difference for all quantitative characters except equatorial axis length and P/E ratio. Group comparison between *H. helix* (diploid) with *H. pastuchovii* (hexaploid) and *H. colchica* (octaploid) species showed significant differences for all pollen grains traits. Therefore, the result of group comparison between *Hedera* polyploid species showed a significant difference in pollen grains traits and relationship between pollen size and ploidy level (Table 4). Means within the same column followed by the same letter(s) are not significantly different at ($\alpha = 0.05$) according to Duncan's Multiple Range Test (Table 5).

Table. 3. Evaluated TEM characters of pollen grains in Hedera species studied (values M ± SD µm).

Taxon name	Exine thickness	Tectum thickness	Foot-layer	T/F	Columellae width	Columellae length	Caput width	Caput length	Endexine	Intine	Ectintine	Mesintine	**Endintine**
H. helix (Gellért Hill)	9.42±1.79	0.39±0.06	0.362±0.02	1.07±0.18	0.21±0.07	0.28±0.09	0.47±0.05	0.48±0.07	0.11±0.02	1.35±0.30	0.15±0.02	0.19±0.05	0.16±0.01
H.hibernica (Soroksár Botanical Garden-Botanical Garden)	1.39±0.10	0.95±0.15	0.55±0.07	1.78±0.47	0.45±0.12	0.72±0.11	0.72±0.16	0.74±0.19	0.06±0.01	2.11±0.72	0.12±0.06	0.17±0.08	0.13±0.07
H. pastochovii (Naharkhoran)	2.66±0.64	0.78±0.11	1.06±0.04	0.73±0.12	0.67±0.13	0.87±0.15	0.82±0.14	0.87±0.35	0.17±0.07	1.73±0.23	0.17±0.03	0.26±0.02	0.39±0.04

M- Mean value; SD- Standard deviation

Table 4. ANOVA analysis and Group comparison for the estimated traits of pollen grains in Hedera species studied

S.O.V	Df	Mean Square					
		Polar axis length (µm)	Equatorial axis length (µm)	P/E ratio	Colpus length (µm)	Colpus width (µm)	Pore diameter (µm)
Species	4	75.80*	71.66	0.01	26.90	2.81	0.07
population	13	892.70**	301.78**	0.07**	1308.46**	9.39**	0.53**
Q_1	1	2.03	0.23	0.00	113.14	0.30	1.62**
Q_2	1	465.31**	171.59	0.04	802.73**	8.83*	2.05**
Q_3	1	3965.58**	987.07**	0.46**	8658.60**	32.29**	4.70**
Q_4	1	8853.19**	2838.47**	0.41**	11845.02**	56.23**	5.19**
Q_5	1	w	104.42	0.01	122.98	2.87	0.02
Q_6	1	1726.24**	482.29**	0.17**	2764.76**	10.98**	0.03
Q_7	1	3944.59**	1361.17**	0.15**	4014.87**	20.74**	0.06
Q_8	1	1075.77**	193.21*	0.16**	2724.71**	3.53	0.16
Q_9	1	3629.21**	1100.82**	0.13**	4371.63**	12.12**	0.25
Q_{10}	1	1561.50**	458.46**	0.03	1376.87**	25.96**	0.28
Error	52	27.51	45.97	0.02	35.40	1.47	0.14
CV %		9.66	16.47	9.40	11.88	27.27	27.91

Q_1: Group comparison between *H. helix* and *H. crebrescens* species
Q_2: Group comparison between *H. helix* and *H. hibernica* species
Q_3: Group comparison between *H. helix* and *H. pastuchovii* species
Q_4: Group comparison between *H. helix* and *H. colchica* species
Q_5: Group comparison between *H. crebrescens* and *H. hibernica* species
Q_6: Group comparison between *H. crebrescens* and *H. pastuchovii* species
Q_7: Group comparison between *H. crebrescens* and *H. colchica* species
Q_8: Group comparison between *H. hibernica* and *H. pastuchovii* species
Q_9: Group comparison between *H. hibernica* and *H. colchica* species
Q_{10}: Group comparison between *H. pastuchovii* and *H. colchica* species

Table 5. Mean comparison with Duncan's multiple range test (α = 0.05.), Minimum, maximum, mean values, and standard deviation for the estimated traits of pollen grains in Hedera taxa, studied

No.	Taxon name	Polar axis length (µm)	Equatorial axis length (µm)	P/E ratio	Colpus length (µm)	Colpus with (µm)	Pore diameter(µm)
1	*H. helix* (Gellért Hill)	28.63^{e}	24.41^{f}	1.17^{de}	17.21^{f}	2.55^{ef}	0.57^{c}
2	*H. helix* (Ziyarat Village)	39.00^{de}	33.44^{ef}	1.17^{e}	35.83^{de}	2.07^{f}	0.78^{c}
3	*H. helix* (Paveh)	40.59^{de}	35.37^{def}	1.15^{e}	27.98^{ef}	3.70^{def}	0.87^{bc}
4	*H. crebrescens* (János Hill)	38.42^{de}	31.92^{ef}	1.20^{de}	33.83^{de}	3.25^{ef}	1.44^{a}
5	*H. hibernica* (Soroksár Botanical)	49.25^{c}	39.11^{cde}	1.27^{cde}	41.55^{d}	4.13^{cde}	1.40^{a}
6	*H. hibernica Varigata* (Soroksár Botanical Garden)	43.72^{cd}	35.91^{def}	1.25^{cde}	38.27^{d}	4.23^{cde}	1.32^{ab}
7	*H. pastuchovii* (Naharkhoran)	60.71^{b}	47.28^{abc}	1.31^{bcde}	55.93^{c}	3.31^{ef}	1.45^{a}
8	*H. pastuchovii* (Savadkuh)	59.56^{b}	39.00^{cde}	1.53^{a}	58.89^{bc}	3.82^{de}	1.32^{ab}
9	*H. pastuchovii* **(**Mashalak**)**	56.01^{b}	44.01^{abcd}	1.31^{bcde}	61.72^{abc}	5.71^{abc}	1.63^{a}
10	*H. pastuchovii* (Khotbesara)	60.49^{b}	41.30^{cde}	1.48^{ab}	63.97^{abc}	6.81^{a}	1.67^{a}
11	*H. colchica* (Kargan Rud)	70.79^{a}	51.55^{a}	1.38^{abcd}	67.75^{a}	6.35^{ab}	1.57^{a}
12	*H. colchica* (Lisar Rud)	69.25^{a}	49.34^{ab}	1.42^{abc}	64.24^{abc}	5.65^{abc}	1.64^{a}
13	*H. colchica* (Asalem to Khalkhal)	72.24^{a}	48.84^{a}	1.48^{abcd}	67.02^{ab}	4.27^{bcde}	1.26^{a}
14	*H. colchica* (Neidasht)	69.78^{a}	49.48^{ab}	1.42^{abc}	64.30^{abc}	5.42^{abcd}	1.50^{a}
	Maximum	72.24	51.55	1.53	67.75	6.81	1.67
	Minimum	28.63	24.41	1.17	17.21	2.07	0.57
	Average	54.17	40.78	1.32	49.89	4.37	1.31
	Standard deviation	14.24	8.04	0.12	16. 84	1.42	0.34

PCA analysis performed on these characters revealed that the first three factors components about 95% of the total observed variation in the studied pollen grains. Studying the component matrix for each factor showed that equatorial axis length, polar axis length, P/E ratio, colpus length, and pore diameter, are the most variable pollen characteristics in the first factor with about 81% of the total variation (Table 6, Fig. 4). Principal component analysis (PCA) based on quantitative traits of pollen grains confirms the results of clustering analysis by Ward's method (Ward 1963) (Fig. 5).

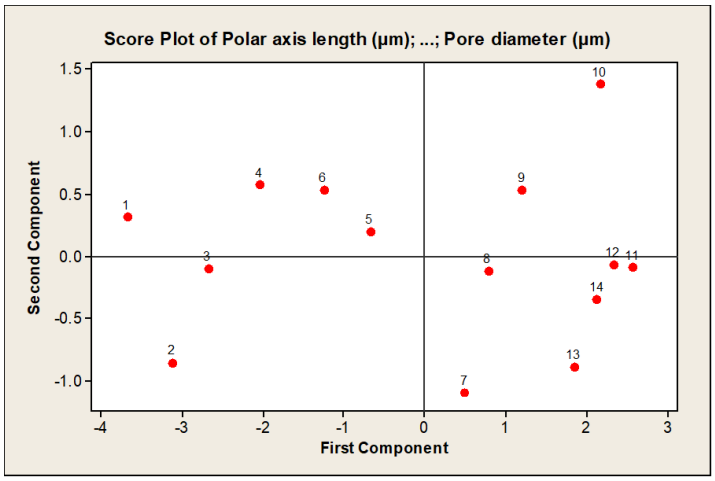

Figure 4. PCA plot pollen grain characters in *Hedera* species: 1. *H. helix* (Gellért Hill), 2. *H. helix* (Ziyarat Village), 3. *H. helix* (Paveh), 4. *H. crebrescens* (János Hill), 5. *H. hibernica* (Soroksár Botanical Garden), 6. *H. hibernica* "Varigata" (Soroksár Botanical Garden), 7. *H. pastuchovii* (Naharkhoran), 8. *H. pastuchovii* (Savadkuh), 9. *H. pastuchovii* (**Mashalak**), 10. *H. pastuchovii* (Khotbesara), 11. *H. colchica* (**Kargan Rud**), 12. *H. colchica* (Lisar Rud), 13. *H. colchica* (Asalem to Khalkhal), 14. *H. colchica* (Neidasht).

Table 6. Evaluation of traits by used Principal Component Analysis

	PC1	PC2	PC3	PC4	PC5
Polar axis length (µm)	0.44	-0.36	-0.04	0.15	-0.33
Equatorial axis length (µm)	0.41	-0.52	0.34	0.16	-0.22
P/E ratio	0.38	0.17	-0.83	-0.06	-0.23
Colpus length (µm)	0.44	-0.17	-0.08	-0.07	0.87
Colpus width (µm)	0.38	0.66	0.27	0.59	0.01
Pore diameter (µm)	0.40	0.33	0.34	-0.77	-0.17
Eigenvalue	4.900	0.446	0.391	0.226	0.035
Proportion of variance	0.817	0.074	0.065	0.038	0.006
Cumulative variance	0.817	0.891	0.956	0.994	1.000

WARD cluster analysis separated the samples within the studied species in a distinct cluster (Fig. 5). Two major clusters were formed in the WARD tree (Fig. 5). The first major cluster contained two sub-clusters: the diploid samples of *Hedera helix* (Ziyarat Village), *Hedera helix* (Paveh), *H. crebrescens* (Gellért Hill), *Hedera helix* (Gellért Hill), and tetraploid samples of *H. hibernica* (Soroksár Botanical Garden) and *H. hibernica* "Variegata" (Soroksár Botanical Garden) were placed close to each other due to pollen morphological similarity and characterized by a sub-prolate pollen grain. The second major cluster contained two sub-clusters characterized by prolate pollen type: the hexaploid samples of *H. pastuchovii* (Naharkhoran), *H. pastuchovii* (Savadkuh), *H. pastuchovii* (Mashalak), *H. pastuchovii* (Khotbesara), and octaploid samples of *H. colchica* (Kargan Rud **),** *H. colchica* (Lisar Rud), *H. colchica* (Asalem to Khalkhal) and *H. colchica* (Neidasht) comprised the second major cluster. Principal component analysis (PCA) based on quantitative traits of pollen grains confirms the results of cluster analysis.

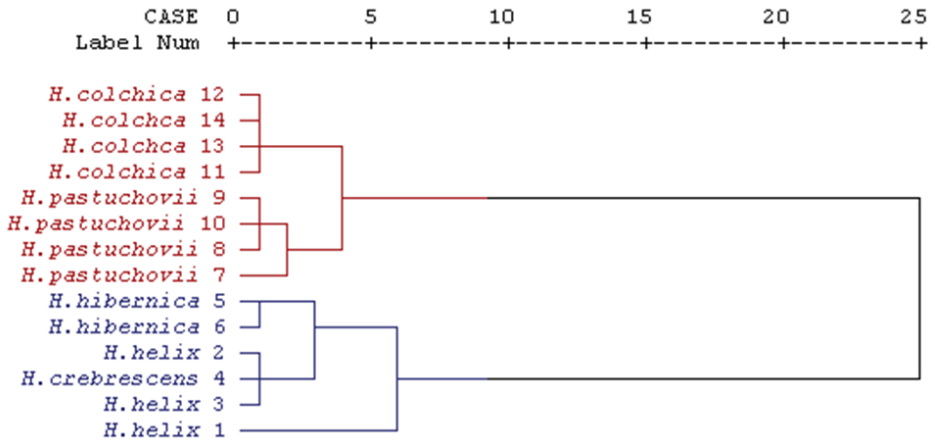

Figure 5. Cluster analysis (ward's method and squared Euclidean distance) 14 populations of 5 *Hedera* species studied, based on pollen features.

1. *H. helix* (Gellért Hill), 2. *H. helix* (Ziyarat Village), 3. *H. helix* (Paveh), 4. *H. crebrescens* (János Hill), 5. *H. hibernica* (Soroksár Botanical Garden), 6. *H. hibernica* "Varigata" (Soroksár Botanical Garden), 7. *H. pastuchovii* (Naharkhoran), 8. *H. pastuchovii* (Savadkuh), 9. *H. pastuchovii* (Mashalak), 10. *H. pastuchovii* (Khotbesara), 11. *H. colchica* (Kargan Rud), 12. *H. colchica* (Lisar Rud), 13. *H. colchica* (Asalem to Khalkhal), 14. *H. colchica* (Neidasht).

DISCUSSION

The pollen grains of *Hedera* species analyzed here are monad, medium-sized (28-72 µm), colporate, isopolar, spheroidal, polar view circular, interapertural area sunken, tricolporate The colpi are long and narrow with slightly thickened margins and end tapering or blunt. The sexine is thick and reticluate, the intine is thickened below the apertures.

AMINI *et al*. (2019) stated that there was significant difference between the *H. helix* and *H. pastuchovii* in pollen grains. They observed the pollen shape is subprolate in *H. helix* but prolate in *H. pastuchovii*. The ornamentation of tectum is microperforate and in *H. helix* is reticulate in *H. pastuchovii* while in present study exine ornamentations were reticulate. Their results showed that these taxa differ in taxonomically important micromorphological, anatomical and molecular characteristics and these data provide reliable evidence for separation of these species. The largest pollen grain was in the *Hedera colchica* species (72.24±1.6 μm) and the smallest pollen grain was in *Hedera helix* (28.63±2.1 μm).

This is consistent with their different ploidy level that *H. helix* is diploid (2n = 2x = 48), whereas *H. colchica* is octoploid (2n = 8x =192) (VARGAS *et al*., 1999). Pollen grain size varies extremely in plants, from about 13 to 130 μm in diameter (KNIGHT *et al*., 2010). Pollen size per has been found to present some taxonomic value in some plant families at the genus or species level (EL NAGGAR and AWAY, 2008; TODERICH *et al*., 2010). A direct relation between nuclear DNA content and pollen volume was found across grasses (BENNETT, 1987). A positive correlation is between pollen size with ploidy level in species of *Chenopodium* (UOTILA, 1974). The study by KNIGHT *et al*. (2010) between the plant kingdom found an established correlation between pollen size and C-values and they discovered a tendency of larger pollen with increasing genome size.

KIZILPINAR TEMIZER (2019) examined pollen morphology of *H. helix* belonging to the genus *Hedera* (Araliaceae) with light microscopy (LM). Their results showed that pollen grains of the species are monad, radially symmetrical, isopolar, tricolporate, oblate-spheroidal or prolate-spheroidal, colporate, reticulate, regulate and the palynological features of the taxon might be helpful to investigate the taxa various palynological, taxonomical, melissopalynological and pharmaceutical research.

KAYA *et al*. (2005) determined that pollen grains of *H. helix* are dominant in Kırklareli. ÖZLER (2018) determined *H. helix* pollen in honey from Ereğli-Namık Kemal neighborhood. *H. helix* pollens are 28 (26.2-31) μm (medium), tricolporatae and reticulate, microreticulate or fossulate, triangular, spheroid (0.96), isopolar, heterobrochate, lumens are getting small toward the colpus. Aperture membranes are no ornamentation, Pore wide is 5.7 (5.2-6.4) μm, apocolpium is broad (KAYA *et al*., 2005).

H. helix pollens are monad, medium-sized (26-50 μm), isopolar, spheroidal, outline in polar view: circular, shape (dry pollen): prolate, outline in polar view (dry pollen): triangular, aperture 3 colporate, ornamentation reticulate, heterobrochate (ÖZLER, 2018). *Hedera helix* (Ivy) is an evergreen dioecious wood and liana. It is one of the best species of the *Hedera* (Araliaceae), in traditional uses, many parts of this plant have been used to treat various diseases (LUTSENKO *et al*., 2010).

Present study showed that pollen features are capable of distinguishing different species in *Hedera*. There is also a directed relationship between pollen grain size and ploidy level so that *H. helix* pollen grain is the smallest size and *H. colchica* had the largest pollen grains. Further studies should be focused on indumentum and seed and fruit micro-morphological features.

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PALINO-MORFOLOGIJA *Hedera* L. (rod Bršljana, Araliaceae) I NJIHOVE SISTEMATSKE IMPLIKACIJE

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Izvod

Detaljno su proučene palinomorfološke karakteristike 5 vrsta koje pripadaju *Hedera* iz familije Araliaceae. Ove biljne vrste su sakupljene iz različitih fitogeografskih regija Irana i Mađarske. Palinološko ispitivanje je izvršeno primenom prenosnog elektronskog mikroskopa (TEM) i skenirajućeg elektronskog mikroskopa (SEM). Polenova zrna vrste su mrežasta, prolatasta, subprolatna, trikoporativna, izopolarna, radijalno simetrična i monadna. Najveće zrno polena bilo je u vrsti *Hedera colchica* (72,24 ± 1,6 mm), a najmanje zrno polena kod *Hedera helix* (28,63 ± 2,1 mm). Istraživanje koje je sprovedeno TEM-om pokazalo je da su se vrste razlikovale u pogledu debljine eksena, debljine tektuma, debljine sloja stopala, prečnika i dužine Columellae, debljine i oblika Caput-a, sloja tektuma do stopala (T/F) odnos, odsustvo ili prisustvo Endexina i debljina slojeva Intine. Glavna svrha ovog istraživanja bila je važnost veze između veličine zrna polena i broja hromozoma i nivoa ploidnosti kod vrsta *Hedera*.

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