

A PALYNOLOGICAL STUDY OF SOME *HELICHRYSUM* (ASTERACEAE) SPECIES IN IRAN

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In this study, palynological characteristics of 23 specimens of *Helichrysum* Mill., representing 12 Iranian species were investigated. We evaluated pollen grains (25 per specimens) and tested them by 15 qualitative and quantitative characteristics using Scanning Electron Microscopy (SEM). Pollen grain descriptions of most species were presented for the first time in the world. The pollen grains were tricolporate, radially symmetrical and isopolar in all examined samples. They were small, and rarely medium in size. The polar outline view (amb) was the same among the species. The overall views of pollen grains were prolate-spheroidal, oblate-spheroidal, subprolate, prolate and spheroidal. However, the more abundant type was prolate-spheroidal. The exine sculpture was echinate, and tectum is perforate. The ANOVA test revealed a significant variation ($P < 0.001$) for all the quantitative characteristics. In PCA analysis, the colpus length, width, and length/width ratio traits, polar and equatorial axes length, were the most variable features and some species were characterised by these characters. Results indicated that a few qualitative characteristics such as polar outline view and exine sculpture lack taxonomic importance via their stability among species. Also, the palynological traits had enough potential to separate the taxonomic boundaries of *H. rubicundum* and *H. globiferum*, but approximately the species clustering pattern did not agree with Flora Iranica.

Key words: *Helichrysum*, Iran, pollen, population, SEM, taxonomy

INTRODUCTION

Helichrysum Mill. with about 600 species in the world, is one of the largest genera in Gnaphalieae tribe of Asteraceae family (Bayer *et al.* 2007, Galbany-Casals *et al.* 2009). The genus is distributed throughout the African continent, Madagascar, the Mediterranean Basin, Macaronesia, Western and Central Asia, and India (Anderberg 1991). Approximately 41 species are distributed in the Mediterranean region, European, western and Central Asian (Galbany-Casals *et al.* 2004). *Helichrysum* species are divided into three sections: sect. *Helichrysum*, sect. *Stoechadina* (DC.) Gren. et Godr. and sect. *Virginea* (DC.) Gren. et Godr. (Galbany-Casals *et al.* 2006). Based on the latest achievements,

Iranian *Helichrysum* species belong to section *Helichrysum* (Galbany-Casals *et al.* 2004). Georgiadou *et al.* (1980) listed 19 species in Iran. Mozaffarian *et al.* (2018) divided it into 16 species in Flora of Iran.

Many species of this genus have ornamental and medicinal values (Viegas *et al.* 2014). For example, *H. italicum* (Roth) G. Don, *H. leucocephalum* Boiss., *H. artemisioides* Boiss. et Hausskn. bearing essential oils (Javidnia *et al.* 2009, Tzanova *et al.* 2018), and *H. compactum* Boiss. and *H. italicum* contain flavonoids with antioxidant and antibacterial activities (Kramberger *et al.* 2021, Maksimovic *et al.* 2017). Due to extreme morphological variation and fragmented distribution, *Helichrysum* is a problematic genus (Akaberi *et al.* 2019, Galbany-Casals *et al.* 2009) and identification of its species is often obscured (Puglia *et al.* 2018), and a great number of taxa have been described at various taxonomic ranks (Galbany-Casals *et al.* 2006).

Palynology is one of the important taxonomic tools in the Asteraceae family, which is employed for delimitation of taxa at the subfamily, tribe, and subtribe levels (Azizi *et al.* 2021, Bolick and Keeley 1994, Cancelli *et al.* 2007, Coutinho and Dinis 2007, Coutinho *et al.* 2011, El-Deen Osman 2006, 2011, Erdtman 1952, Perveen 1999, Skvarla *et al.* 1977, Wortley *et al.* 2008). In this regard, Zafar *et al.* (2007) reported a great diversity in pollen morphological characteristics in this family, especially in shape, size, colpi length, and morphology of spines. In Gnaphalieae tribe, pollen morphological features have been used to better identify taxonomic position of several taxa (Blackmore *et al.* 2007, El-Deen Osman 2011, Elkiran *et al.* 2017, Pinheiro *et al.* 2003, Wagenitz and Hellwig 2000, Wortley *et al.* 2012, 2015). For example, in a study, Breitwieser and Sampson (1997) evaluated the palynological traits of New Zealand Gnaphalieae taxa, and observed that the pollen grains of all taxa were spheroidal and tricolporate. Additionally, they could resolve some complexities among *Helichrysum* species, using pollen grain characteristics. In another study, the pollen grain morphological features of two *Helichrysum* species, *H. foetidum* (L.) Moench and *H. italicum*, were investigated by Coutinho and Dinis (2009), and detected the pollens grains are radially symmetrical, sub-oblate to prolate, 3-zonocolporate and echinate-perforate.

The palynomorphological characteristics of six taxa, *H. chionophilum* Boiss. et Balansa, *H. noeanum* Boiss., *H. arenarium* subsp. *aucheri*, *H. graveolens* (M. Bieb.) Sweet, *H. plicatum* DC. subsp. *plicatum* DC., and *H. plicatum* subsp. *polyphyllum* were examined (Elkiran *et al.* 2017, Hayta *et al.* 2015). Results indicated the pollen grains shape, aperture type and ornamentations in these taxa are similar. However, some quantitative traits differed among species (Elkiran *et al.* 2017). Pinar and Oybak Donmez (2000) investigated the palynology of six endemic *Helichrysum* species of Turkey. The pollen grains of all taxa were similar in that they are tricolporate with spines and rugulate exine sculpture. Although several investigations, including phylogenetic, genetic diversity and cytology have been conducted on *Helichrysum* taxa (Elkiran *et al.*

2017, Galbany-Casals and Romo 2008, Galbany-Casals *et al.* 2004, 2006, 2010, 2011, 2014, Sabetta *et al.* 2006, Salmeri *et al.* 2014, Scialabba *et al.* 2008), but the relationships among species in this genus are still confused as well as the palynological studies have not been sufficiently in this genus.

Therefore, we aimed this study to investigate general pollen morphological characters and to assess the implementation for classification and delimitation in *Helichrysum*.

MATERIAL AND METHODS

Plant samples – In this work, the pollen materials were obtained from herbarium specimens of the Shahid Beheshti University herbarium (HSBU) and herbarium of Research Institute of Forests and Rangeland (TARI). Voucher specimens are listed in Table 1. The pollen samples were obtained mostly from freshly collected specimens and also from dry herbarium materials. Five individuals were randomly selected from each population, and five buds were picked up from each individual.

Light microscopy (LM) – Pollen grains were acetolysed according to Erdtman method (1960). We used Olympus CH2 light microscope for the measurement of polar and equatorial axes length and determining the shape of pollen grains and polar outline view. Image Tools (ver. 2) was used for measurements.

Scanning electron microscopy (SEM) examination – For SEM study, the pollen grains in 23 *Helichrysum* growing in Iran, were coated with approximately 25 nm of gold-palladium in a sputter coater. The specimens were examined and photographed with a Phillips $\times$ L20 SEM. We used SEM micrographs to evaluate the pollen grains morphological traits. The examined palynological characters were the polar and equatorial axes length and their ratio, colpi length, width and length/width ratio, the polar and equatorial outline views, the aperture type and exine ornamentations pattern and dimensions. The size of pollen grains was determined based on Erdtman (1971). We determined the pollen grains shape as peroblate ($P/E < 0.50$), oblate ($0.51 < P/E < 0.75$), suboblate ($0.76 < P/E < 0.88$), oblate-spheroidal ($0.89 < P/E < 0.99$), spheroidal (1.00), prolate-spheroidal ($1.01 < P/E < 1.14$), subprolate ($1.15 < P/E < 1.33$) and prolate ($1.34 < P/E < 2.00$) based on Erdtman (1960) description. We used the following terminology for qualitative morphological traits: Halbritter *et al.* (2018), Hesse *et al.* (2009) and Punt *et al.* (2007).

Statistical analysis – Analysis of variance (ANOVA) test and average amounts were calculated for quantitative palynological features using SPSS v. 15.0. We standardised the data (mean = 0, variance = 1) and processed them by Unweighted Paired Group Using Average method (UPGMA) and Principal Components Analysis (PCA) and PCA-biplot using MVSP v. 2.0 (Higgs 1991, Podani 2000).

Table 1
The localities address and voucher no. of the studied *Helichrysum* species (Pop. = population no)

No	Species	Localities	Altitude (m)	Voucher No
1	<i>H. araxinum</i> Takht. ex Kürp.	Hamadan province: Hamadan, Virai	1,700	HSBU-2012200
2	<i>H. armenium</i> DC. (Pop. 1)	Hamadan province: Hamadan, Asad Abad	1,795	HSBU-2012215
3	<i>H. armenium</i> DC. (Pop. 2)	West Azerbaijan province: Uremia, Mavana	1,670	HSBU-2012202
4	<i>H. armenium</i> DC. (Pop. 3)	West Azerbaijan province: Uremia, Razhan	1,800	HSBU-2012247
5	<i>H. armenium</i> DC. (Pop. 4)	Hamadan province: 10 km Asad Abad	1,740	HSBU-2012214
6	<i>H. armenium</i> DC. (Pop. 5)	Kurdistan province: Divandareh, Zagheh	1,950	HSBU-2012212
7	<i>H. armenium</i> DC. (Pop. 6)	East Azerbaijan province: 2 km Tabriz to Ahar	1,380	HSBU-2012208
8	<i>H. davisianum</i> Rech. f.	Yazd province: Mehriz, Damgahan	2,100	TARI-77715
9	<i>H. glanduliferum</i> Schultz-Bip.	Kurdistan province: Sanandaj, Ariz Col.	2,200	HSBU-2012211
10	<i>H. globiferum</i> Boiss. (Pop. 1)	East Azerbaijan province: Miab	2,400	HSBU-2012211
11	<i>H. globiferum</i> Boiss. (Pop. 2)	East Azerbaijan province: Payam Village	2,000	HSBU-2012226
12	<i>H. globiferum</i> Boiss. (Pop. 3)	West Azerbaijan province: Khoy, Chaldoran	900	HSBU-2012225
13	<i>H. globiferum</i> Boiss. (Pop. 4)	East Azerbaijan province: Marand	1,450	HSBU-2012224
14	<i>H. graveolens</i> (M. B.) Sweet.	Mazandaran province: East of Ramsar, Lapasar	2,950	TARI-21658
15	<i>H. leucocephalum</i> Boiss.	Fars province: Neyriz, Palangan	1,600	HSBU-2012230
16	<i>H. oligocephalum</i> DC.	Fars province: Neyriz, Palangan	1,650	HSBU-2012230
17	<i>H. pallasi</i> (Spreng.) Ledeb.	East Azerbaijan province: Haris, 30 km after Inche village	2,100	TARI-469
18	<i>H. polyphyllum</i> Ledeb. (Pop. 1)	West Azerbaijan province: Uremia, Solouk	1,350	HSBU-2012239
19	<i>H. polyphyllum</i> Ledeb. (Pop. 2)	East Azerbaijan province: Mishoo Dagh	2,600	TARI-29836
20	<i>H. pseudoplicatum</i> Nab.	West Azerbaijan province: Uremia, Razhan	1,800	HSBU-2012241
21	<i>H. rubicundum</i> (C. Koch) Bomm. (Pop. 1)	East Azerbaijan province: 22 km Tabriz- Ahar	1,300	HSBU-2012244
22	<i>H. rubicundum</i> (C. Koch) Bomm. (Pop. 2)	East Azerbaijan province: 25 km Tabriz- Ahar	1,375	HSBU-2012245
23	<i>H. rubicundum</i> (C. Koch) Bomm. (Pop. 3)	East Azerbaijan province: Ahar	1,400	HSBU-2012246

RESULTS

Qualitative traits

The pollen morphological traits of the studied *Helichrysum* species are summarised in Table 2. The pollen grains in all species and their populations were isopolar, tricolporate, and radially symmetrical.

The pollen grains shape differed as oblate-spheroidal (*H. polyphyllum* populations no. 1, *H. davisianum*, *H. armenium* populations no. 2, *H. araxinum*), spheroidal (*H. oligocephalum*), prolate-spheroidal (*H. rubicundum* populations no. 1 and 3, *H. polyphyllum* populations no. 2, *H. pallasii*, *H. leucocephalum*, *H. graveolens*, *H. globiferum* populations no. 2 and 4, *H. glanduliferum*, *H. armenium* populations no. 1, 4 and 6), subprolate (*H. pseudoplicatum*, *H. globiferum* populations no. 1), and prolate (*H. globiferum* populations no. 3). The polar outline shape was the same (circular) among the studied species and their populations (Figs 1–2).

The exine surface sculpturing pattern was echinate- perforate in all examined species and their populations (Figs 3–4).

Quantitative variables

Although, the size of pollen grains differed among the studied samples, all of them were classified as small, except for *H. globiferum* population no. 3, which was considered medium. The length of polar axis (P) varied from 20.21 μm (*H. armenium* population no. 4), to 29.28 μm (*H. globiferum* population no. 4). Furthermore, the length of equatorial axis (E) ranged from 19.37 μm (*H. pseudoplicatum*) to 24.72 μm (*H. armenium* population no. 1). The ratio of polar axis length to equatorial axis length (P/E) from 0.97 (*H. davisianum*) to 1.36 (*H. globiferum* population no. 3). The largest (3.36 μm) and smallest (1.37 μm) spines length (SL) were detected in *H. glanduliferum* and *H. globiferum* population no. 3, respectively. Colpi margin shape was granulated in all the examined populations. The shortest (13.75 μm) colpus length was recorded in *H. armenium* population no. 4, while the largest one (22.14 μm) in *H. globiferum* population no. 3. Colpus width ranged from 2 μm (*H. glanduliferum*) to 4.71 μm (*H. davisianum*). Distances between spines ranged from 0.42 μm (*H. graveolens*) to 1.37 μm (*H. leucocephalum*). Additionally, the exine thickness ranged from 1.04 μm (*H. pseudoplicatum*) to 2.49 μm (*H. armenium* population no. 1).

Results of ANOVA test revealed a significant variation ($P < 0.001$) for all the quantitative characteristics of pollen grains. In PCA analysis, we observed the first three components comprised more than 62% of total differences (PCA1 = 25.07, PCA2 = 21.7, PCA3 = 15.28), with colpus length, width, and length/width ratio, polar and equatorial axes length, as the more variable features.

Table 2
Selected quantitative and qualitative palynological features of the studied *Helichrysum* species

Taxa	P (µm)	E (µm)	P/E ratio	SL (µm)	SW (µm)	SL/ SW	CL (µm)	CW (µm)	CL/ CW	DSB (µm)	ET (µm)	PN	PSh	PS	PV
<i>H. araxinum</i>	21.23	21.68	0.98	2.05	2.8	0.73	14.96	3.8	3.94	0.51	1.94	1	oblate-spheroidal	1	circular
<i>H. armenium</i> (Pop. 1)	24.89	24.72	1.01	3.13	3.39	0.92	18.3	4.31	4.25	0.72	2.49	1	prolate-spheroidal	1	circular
<i>H. armenium</i> (Pop. 2)	21.62	22.19	0.97	2.29	2.52	0.91	15.58	4.04	3.86	0.48	1.69	1	oblate-spheroidal	1	circular
<i>H. armenium</i> (Pop. 3)	-	-	-	-	-	-	16.67	2.67	6.24	0.59	-	3	-	1	circular
<i>H. armenium</i> (Pop. 4)	20.21	19.9	1.02	1.74	2.5	0.7	13.75	3.57	3.85	0.59	1.69	2	prolate-spheroidal	1	circular
<i>H. armenium</i> (Pop. 5)	23.92	22.57	1.06	2.84	3.48	0.82	19.47	3.93	4.95	0.73	2.19	1	prolate-spheroidal	1	circular
<i>H. armenium</i> (Pop. 6)	25.81	23.23	1.11	2.71	3.53	0.77	17.59	4.3	4.09	0.58	1.79	3	prolate-spheroidal	2	circular
<i>H. davisianum</i>	23.29	24.63	0.95	2.56	3.42	0.75	16.44	4.71	3.49	0.74	1.58	3	oblate-spheroidal	1	circular
<i>H. glanduliferum</i>	22.68	20.45	1.11	3.36	3.01	1.12	17.52	2.00	8.76	0.44	1.24	3	prolate-spheroidal	1	circular
<i>H. globiferum</i> (Pop. 1)	26.70	21.36	1.25	3.06	3.11	0.98	18.77	2.69	6.98	0.55	2.12	2	subprolate	1	circular
<i>H. globiferum</i> (Pop. 2)	24.26	23.64	1.03	-	-	-	17.00	4.26	3.99	0.53	-	2	prolate-spheroidal	1	circular
<i>H. globiferum</i> (Pop. 3)	29.26	21.48	1.36	1.37	2.26	0.61	22.14	3.45	6.42	0.82	1.8	3	prolate	1	circular
<i>H. globiferum</i> (Pop. 4)	23.00	22.78	1.01	2.66	3.00	0.89	16.12	3.50	4.61	0.47	1.9	2	prolate-spheroidal	1	circular

Table 2 (continued)

Taxa	P (μm)	E (μm)	P/E ratio	SL (μm)	SW (μm)	SL/ SW	CL (μm)	CW (μm)	CL/ CW	DSB (μm)	ET (μm)	PN	PSh	PS	PV
<i>H. graveolens</i>	20.49	19.76	1.04	2.78	2.95	0.94	14.87	3.39	4.38	0.42	1.47	3	prolate-spheroidal	1	circular
<i>H. leucocephalum</i>	25.88	23.4	1.11	1.71	1.73	0.99	21.61	2.65	8.15	1.37	1.84	1	prolate-spheroidal	1	circular
<i>H. oligocephalum</i>	21.13	21.17	1	1.97	2.58	0.76	14.82	3.73	3.97	0.45	–	2	spheroidal	1	circular
<i>H. pallasi</i>	24.45	24.2	1.01	2.13	2.47	0.86	18.51	4.32	4.28	0.58	1.88	1	prolate-spheroidal	1	circular
<i>H. polyphyllum</i> (Pop. 1)	24.43	24.62	0.99	2.42	2.91	0.83	13.80	3.76	3.67	0.74	1.60	2	oblate-spheroidal	–	circular
<i>H. polyphyllum</i> (Pop. 2)	23.64	21.83	1.08	2.13	3.05	0.70	17.65	–	–	0.67	1.71	1	prolate-spheroidal	1	circular
<i>H. pseudoplicatum</i>	23.52	19.37	1.21	1.91	3.11	0.61	17.35	3.75	4.63	0.71	1.04	1	subprolate	2	circular
<i>H. rubicundum</i> (Pop. 1)	23.66	20.75	1.14	1.77	2.55	0.69	16.47	2.73	6.03	0.42	1.51	1	prolate-spheroidal	1	circular
<i>H. rubicundum</i> (Pop. 2)	24.35	20.92	1.16	2.25	2.7	0.83	18.8	–	–	0.56	1.58	2	subprolate	1	circular
<i>H. rubicundum</i> (Pop. 3)	24.38	23.86	1.02	2.25	2.53	0.89	16.04	3.42	4.69	0.48	1.67	3	prolate-spheroidal	1	circular

Pop = population no, P = polar axis length, E = equatorial axis length, SL = spine length, SW = spine width, SL/W = spine length/ width ratio, CL = colpus length, CW = colpus width, CL/W = colpus length/ width ratio, DSB = distance between spines; ET = exine thickness, PN = perforation number, PSh = pollen shape, PS = perforations on spine, PV = polar outline view

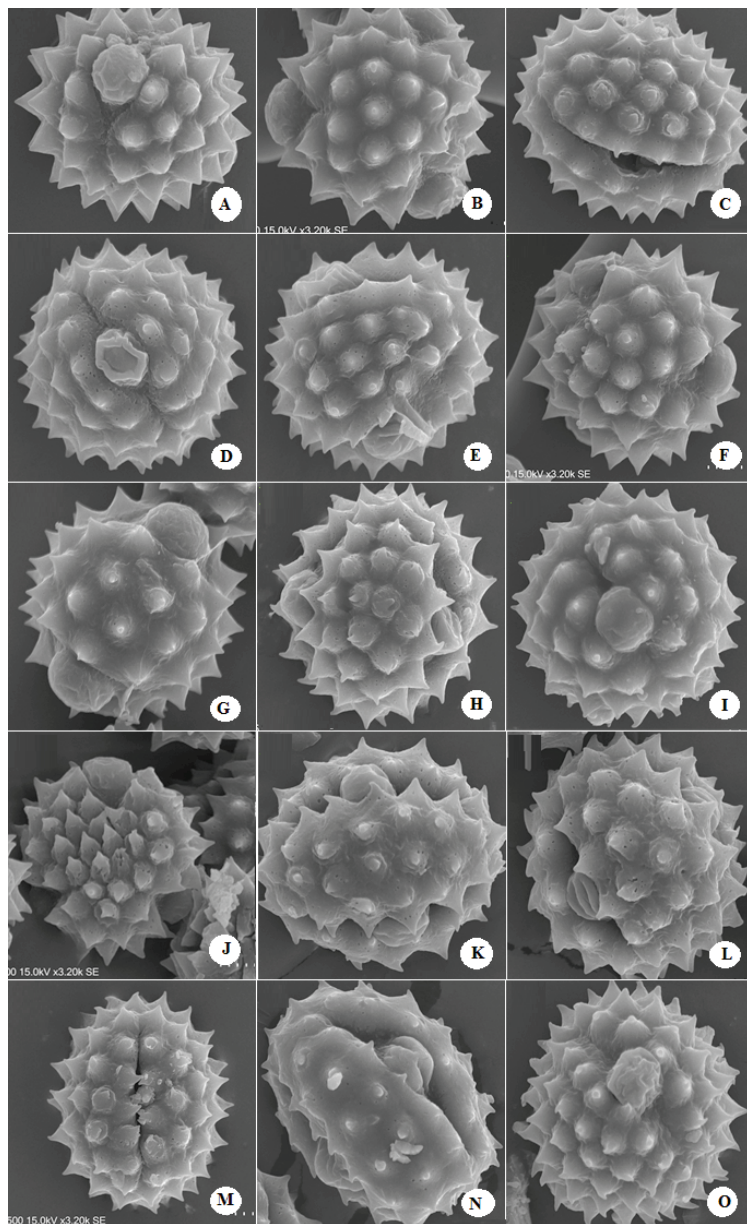


Fig. 1. Scanning micrographs of *Helichrysum* species. – A = *H. araxinum*, B = *H. armenium* population no. 1, C = *H. armenium* population no. 2, D–E = *H. armenium* population no. 3, F = *H. armenium* population no. 4, G = *H. armenium* population no. 5, H = *H. armenium* population no. 6, I = *H. davisianum*, J = *H. glanduliferum*, K–L = *H. globiferum* population no. 1, M = *H. globiferum* population no. 2, N = *H. globiferum* population no. 3, O = *H. globiferum* population no. 4 (scale bars = 10 μ m)

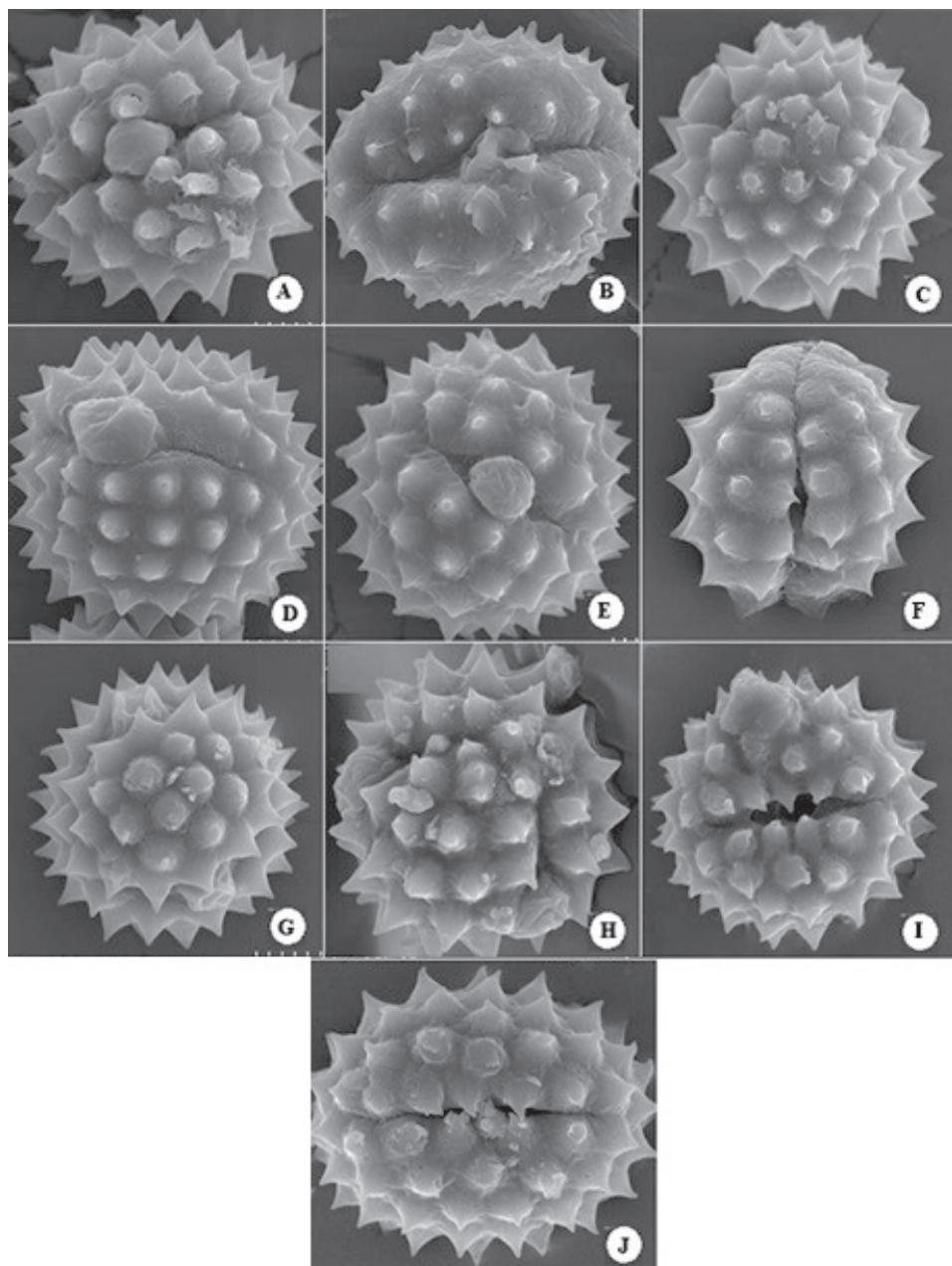


Fig. 2. Scanning micrographs of *Helichrysum* species. – A = *H. graveolens*, B = *H. leucocephalum*, C = *H. oligocephalum*, D = *H. pallasii*, E = *H. pseudoplicatum*, F = *H. polyphyllum* population no. 1, G = *H. polyphyllum* population no. 2, H = *H. rubicundum* population no. 1, I = *H. rubicundum* population no. 2, J = *H. rubicundum* population no. 3 (scale bars =10 μ m)

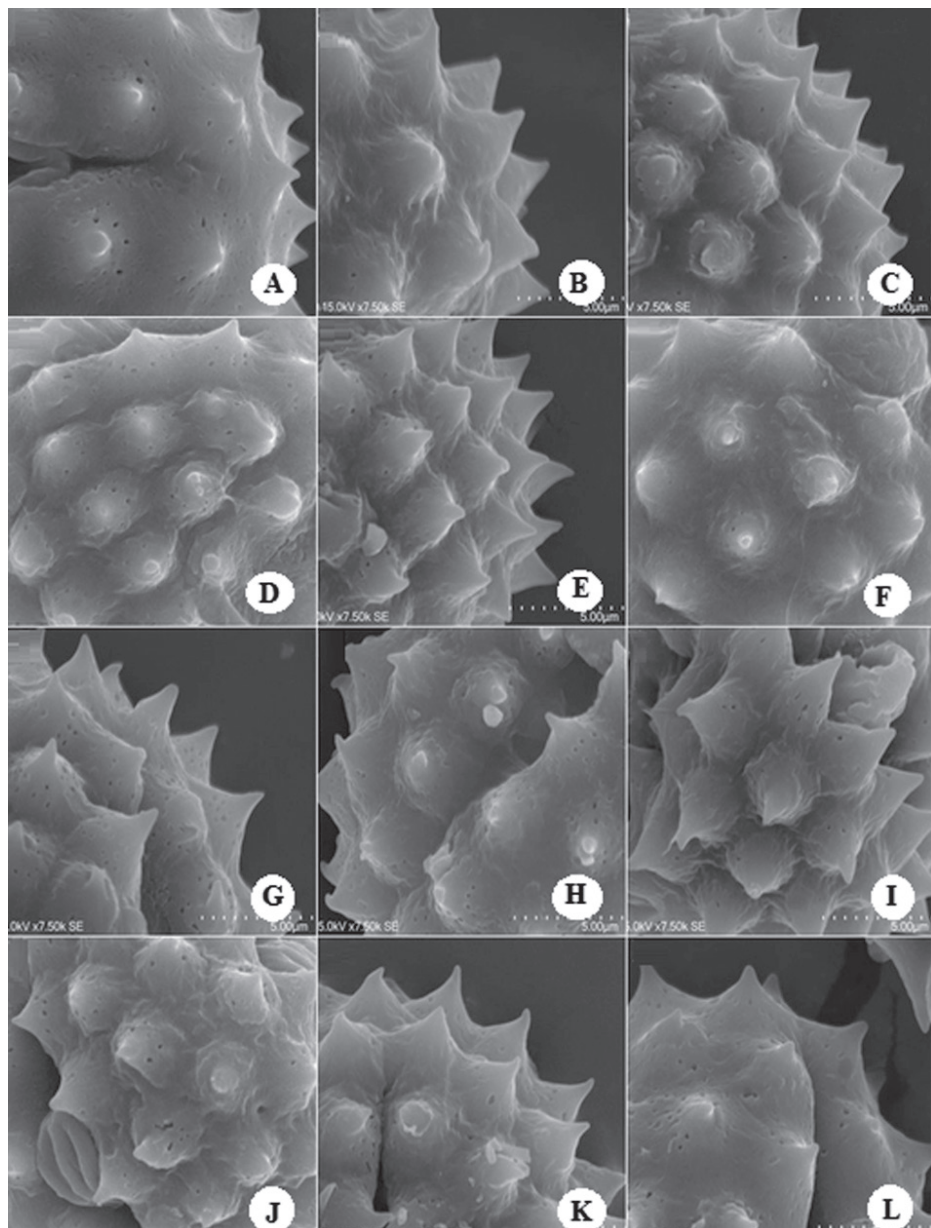


Fig. 3. Scanning micrographs of exine surface sculpturing in *Helichrysum* species. – A = *H. araxinum*, B = *H. armenium* population no. 1, C = *H. armenium* population no. 2, D = *H. armenium* population no. 3, E = *H. armenium* population no. 4, F = *H. armenium* population no. 5, G = *H. armenium* population no. 6, H = *H. davisianum*, I = *H. glanduliferum*, J = *H. globiferum* population no. 1, K = *H. globiferum* population no. 2, L = *H. globiferum* population no. 3 (scale bars = 2 μ m)

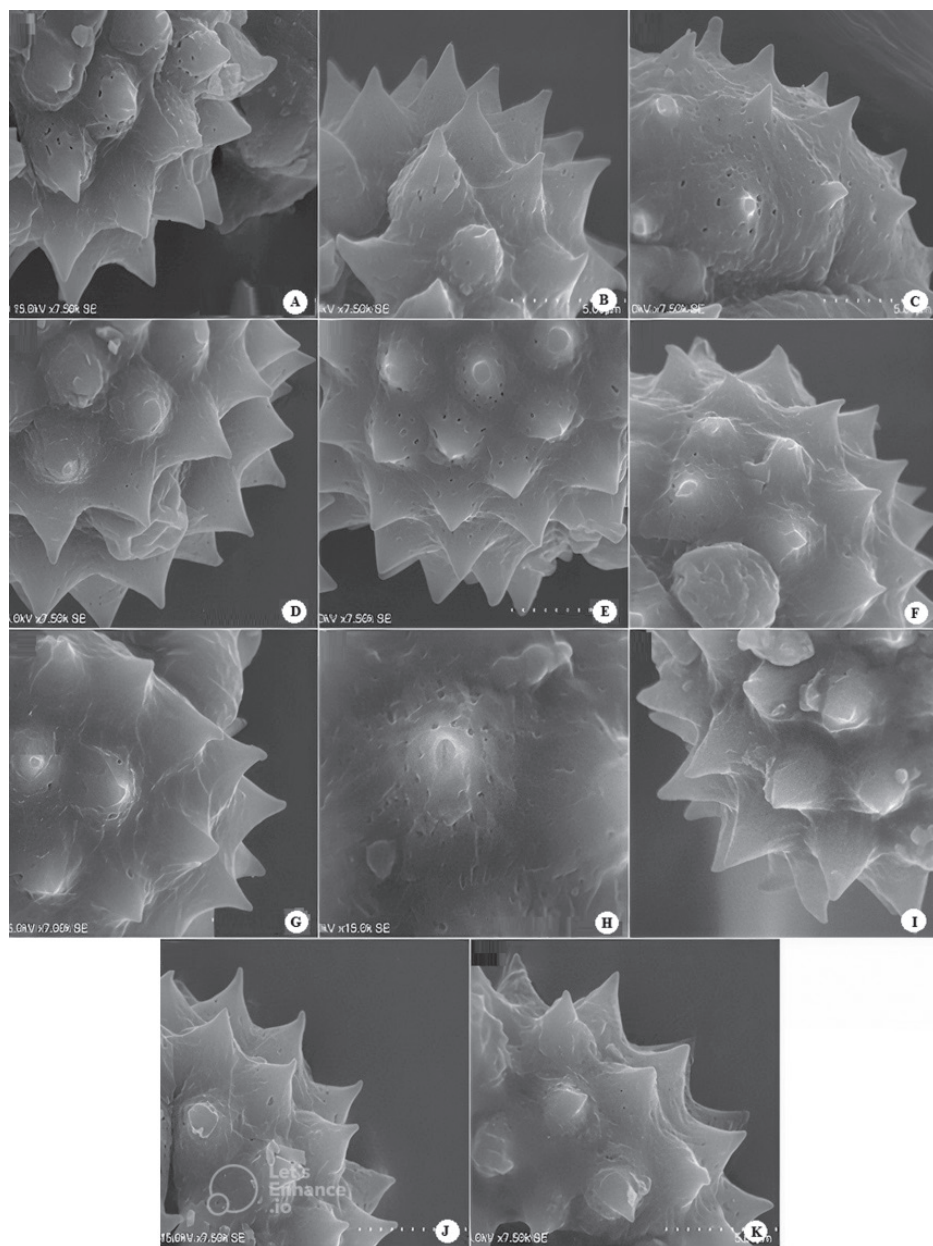


Fig. 4. Scanning micrographs of exine surface sculpturing in *Helichrysum* species. – A = *H. globiferum* population no. 4, B = *H. leucocephalum*, C = *H. oligocephalum*, D = *H. pallasii*, E = *H. pseudoplicatum*, F = *H. polyphyllum* population no. 1, G = *H. polyphyllum* population no. 2, H = *H. rubicundum* population no. 1 (scale bar = 3 μ m), I = *H. rubicundum* population no. 2, J = *H. rubicundum* population no. 3 (scale bars = 5 μ m)

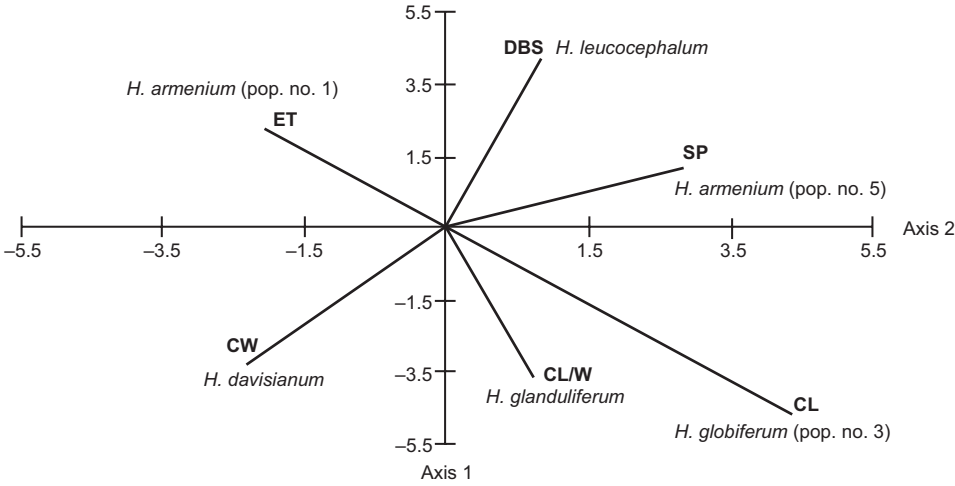


Fig. 5. PCA-biplot of the studied species and their populations with pollen morphological characteristics (CW = colpi width, CL/W = colpi length/width ratio, CL = colpi length, SP = spine width, DBS = distance between spines, ET = exine thickness)

Populations clustering

Based on PCA-biplot, some of the species and their populations had a special trait(s) that are useful in identification of species / populations. For example, the colpus length/width ratio was the characteristic trait for identification of *H. glanduliferum* from the rest. Moreover, we considered distance

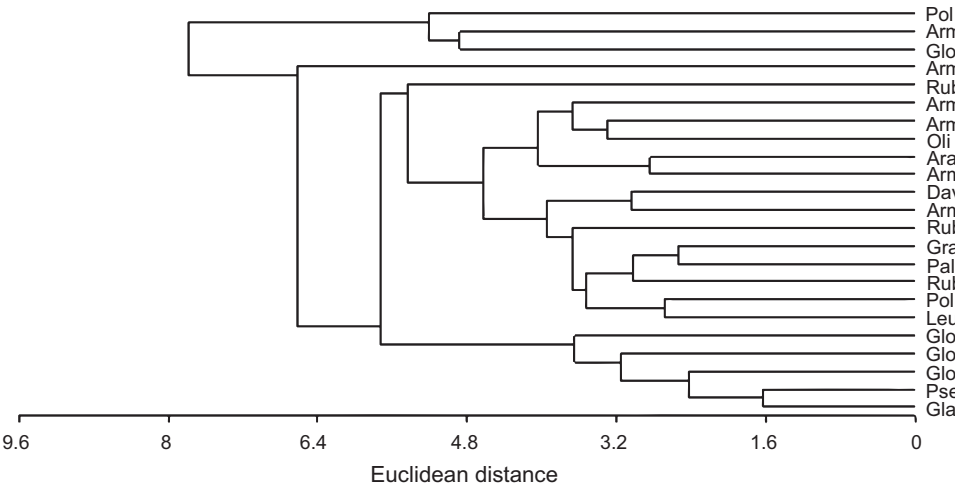


Fig. 6. UPGMA tree of the studied taxa of *Helichrysum* based on pollen grains features

between spines (DBS) as a distinct feature for *H. leucocephalum*. Spine width was a diagnostic characteristic for *H. armenium* population no. 5 (Fig. 5).

In the UPGMA tree (Fig. 6), the examined species and their populations were clustered into two branches. The first branch contained: *H. polyphyllum* population no. 1, *H. armenium* population no. 4, and *H. globiferum* population no. 3. Another branch composed of the rest populations that was divided into two sub-branches: a sub-branch contained *H. armenium* population no. 3, and another sub-branch was divided into three groups. *H. rubicundum* population no. 1 created a group, in addition, *H. globiferum* populations no. 1, 2 and 4, *H. pseudoplicatum* and *H. glanduliferum* were clustered together as a group. Furthermore, another group was divided into two sub-groups: A sub-group had *H. armenium* populations no. 2 and 5, *H. oligocephalum*, *H. araxinum* and *H. rubicundum* populations no. 3 and the rest the species/populations *H. armenium* populations no. 1, *H. rubicundum* populations no. 1 and 3, *H. davisianum*, *H. graveolens*, *H. polyphyllum* populations no. 2, *H. leucocephalum* and *H. pallasii*. In some cases, the pattern of species clustering was not similar to species clustering pattern according to Flora Iranica.

DISCUSSION

Due to high morphological variability, we have a complex group of species in the genus *Helichrysum*, therefore detection of species border is very difficult. Carstens *et al.* (2013) believed that the delimitation species in such complex groups, with varying degrees of morphological overlap is a challenging task. In these situations, it is suggested to use other taxonomic tools such as palynology to determine the species boundaries.

We evaluated the pollen grains morphological characteristics in between and within species. In this study, the quantitative characters showed highly differed within the populations. In this regard, Torres (2000) believed that the ecological condition of habitat and physiological state of plant can affect the size of pollen grains. Therefore, such variation in quantitative traits among various populations of each species is expected. A few qualitative traits such as the pollen polar view shape and exine surface ornamentations were the same among the populations. Meanwhile, we detected some variations in pollen grain overview shape among the populations of all the species. Such variations have been reported for different genera such as *Onobrychis* Mill. (Talebi *et al.* 2020a), *Hymenocrater* Fisch. et Mey. (Tabaripour *et al.* 2022) and *Nepeta* L. (Talebi *et al.* 2020b). We think these variations look natural and affect taxonomic importance of these characteristics.

The populations of *H. armenium* did not cluster closely, which indicates high intra-specific variability in their pollen grains characteristics. These results are in accordance with the previous morphological and molecular in-

vestigations (Azizi *et al.* 2019). In addition, populations *H. rubicundum* were placed far from each other. Similar results were recorded in genetic molecular diversity study of *H. rubicundum* populations using ISSR markers (Azizi *et al.* 2019). According to former evaluations (Azizi *et al.* 2019, Taban *et al.* 2019), the taxonomic boundaries of *H. rubicundum* and *H. globiferum*, based on morphological characteristics, are unclear. But populations of these species were clustered separately that indicates pollen morphological traits have enough potential to separate these species.

The results revealed all species have the isopolar and tricolporate pollen grains. These findings agreed with Elkiran *et al.* (2017) and Pinar and Oybak Donmez (2000). Breitwieser and Sampson (1997) evaluation on the pollen grains in eight New Zealand *Helichrysum* species. Although the mentioned studies were conducted on various species of the genus, the same findings indicated that these characteristics (isopolar and tricolporate) are stable in the genus.

The shape of pollen grains differed as prolate-spheroidal, oblate-spheroidal, subprolate, prolate and spheroidal. These findings agreed with Pinar and Oybak Donmez (2000) study. However, our findings, in some cases, did not agree with previous palynological evaluations, in which Elkiran *et al.* (2017) detected the pollen shape of *H. graveolens* as oblate-spheroidal, while we registered it as prolate-spheroidal. In our studied samples the more abundant shape was prolate-spheroidal. The abundant shape for Turkish taxa was oblate-spheroidal (Elkiran *et al.* 2017). However, the pollen shape for all examined New Zealand species was spheroidal (Breitwieser and Sampson 1997). Due to these variations, the pollen shape has a limited taxonomic value for the genus.

The pattern of exine surface ornamentation was the same (echinate-perforate) among these species. Similar findings were reported in former palynological studies. These findings revealed the characteristic lack taxonomic value at inter-specific level (Breitwieser and Sampson 1997, Elkiran *et al.* 2017). The polar outline view in all samples was the same. For Turkish species same result was reported by Elkiran *et al.* (2017). Meanwhile, our detected shape was circular that differed from Turkish taxa (triangular).

We detected the pollen grains size in most of taxa as small, which was in agreement with the previous study on *Helichrysum* taxa (Elkiran *et al.* 2017). However, these findings did not agree with Breitwieser and Sampson (1997) study, which detected most of their studied species have pollen grains in medium size. Results of these investigations suggested that the pollen grain size has a taxonomic value at the species level.

According to previous investigations (Coutinho and Dinis 2009, Skvarla *et al.* 1977) the characteristics of spine are taxonomically important in Gnaphalieae, Asteroidae, and Lactuceae. For example, the spine length and width are two important characteristics for identification of species in the genus. We detected significant variations in spine size among the species.

The studied species were clustered separately in UPGMA tree of palynological features. The clustering patterns mostly did not agree with those, which were detected in previous studies (Georgiadou *et al.* 1980). It seems that high morphological variety in *Helichrysum* species arises from high inter-specific variation in its species.

CONCLUSIONS

We described the pollen grains morphological features in 23 populations of 12 species of *Helichrysum* for the first time in the world, except those for *H. graveolens*. The pollen grains were tricolporate, and mainly small in size. The overall view of pollen grains differed, meanwhile, the more abundant shape was prolate-spheroidal. Polar outline view of the pollen grains was stable among the species. Exine surface sculpturing pattern was echinate-perforate. Therefore, most of evaluated qualitative characteristics lack taxonomic value. All quantitative traits varied among the species and their populations, furthermore the ANOVA test and PCA analysis indicated a significant difference for all of them. According to PCA-biplot, some of species were characterised by a diagnostic trait. Based on UPGMA tree of palynological traits, high inter-specific diversity was observed. But, in general, some palynological features could help to resolve problems related to the taxonomy of *Helichrysum*. For example, in determining the taxonomic boundaries of complex species with overlapping niches.

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