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THE POLLEN MORPHOLOGICAL DIVERSITY OF *ZIZIPHORA CLINOPODIOIDES* (LAMIACEAE)

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A cogent medicinal and aromatic plant, *Ziziphora clinopodioides* (Lamiaceae) is a perennial herb, its aerial parts is used as a wild vegetable or additive in foods to proffer pleasant aroma and flavour. There are many discussions about the intraspecific classification of this species and several subspecies have been introduced for it in different flora. These subspecies are morphologically very similar and identification of them is very difficult and in some cases, impossible. Therefore, in the present study, the pollen grains morphology of nine subspecies (32 specimens) of *Z. clinopodioides* were probed and documented in details utilising the scanning electron microscopy (SEM). In total, eleven pollen morphological characteristics were investigated and analysed by PAST software. The obtained results showed that pollen grains were hexacolpate and their equatorial views were sub-oblate to prolate. The exine ornamentation types of the pollen grains are bireticulate, microreticulate, reticulate and bireticulate-reticulate. The ANOVA test did not show significant difference for the studied quantitative traits. Although, the results of the multivariate analysis revealed a high diversity amongst the specimens even in the specimens of a single subspecies; it did not confirm the separation of subspecies in *Z. clinopodioides.*

Key words: exine ornamentation, Lamiaceae, pollen diversity, SEM, *Ziziphora clinopodioides*

INTRODUCTION

Ziziphora L. (Lamiaceae) consists of 25 to 30 species in East and West Asia, Central Europe, North African (Keshavarzi *et al.* 2008). Four species of it, *Ziziphora clinopodioides* Lam., *Ziziphora capitata* L., *Ziziphora persica* Bunge and *Ziziphora tenuior* L. naturally grow in Iran (Jamzad 2012, Rechinger 1982). Furthermore the only perennial *Ziziphora* species is *Z. clinopodioides*, which is known as "Kakuti-e-kuhi" or "Taramoshk" in Iran. It is a medicinal and aromatic plant that is used as wild vegetable or additive in foods to offer aroma and flavour (Khodaverdi-Samani *et al.* 2015). In the Iranian tradition medicine, this species is used as a sedative or carminative, for/in stomachic, and

common cold, diarrhoea, coughing and wound healing material (Ghasemi Pirbalouti *et al.* 2013, Naghibi *et al.* 2005, Öztürk *et al*. 1995, Tarakci 2004).

Ziziphora clinopodioides Lam. has prostrated to erect stems and is mainly much branched from the base. The leaves vary in size and shape. Inflorescence is terminal with a compact capitulum. The calyx measures 4–7 mm and the corolla measures up to 8 mm. The flowers are glabrous to densely hirsute, purplish pink to white, with or without a peduncle. It grows in steppes and stony slopes (Keshavarzi *et al.* 2008).

There is consensus on the exact numbers of the subspecies of *Z. clinopodioides* that should be recognised. For example, it is divided into four subgroups (i.e. *rigida*, *glabrata*, *tomentosa* and *canescens*) based on large calyx and corolla structure, leaf morphology, nutlet and sexual characters (Hedge 1961). In the Flora Iranica (Rechinger 1982), nine subspecies have been introduced for Iran (subsp. *afghanica* (Rech. f.) Rech. f., subsp. *bungeana* (Juz.) Rech. f., subsp. *elbursensis* (Rech. f.) Rech. f., subsp. *filicaulis* (Rech. f.) Rech. f., subsp. *pseudodasyantha* (Rech. f.) Rech. f., subsp. *kurdica* (Rech. f.) Rech. f., subsp. *rigida* (Boiss.) Rech. f., subsp. *ronnigeri* (Nábělek) Rech. f., and subsp. *szowitsii* (Rech. f.) Rech. f.). This species in the Flora of Iran (Jamzad 2012) and Turkish flora (Edmondson 1982), however, is not divided into subspecies.

The pollen grain morphology in Lamiaceae has proven to be a quintessential purpose for the classification within this family (Abu-Asab and Cantino 1992). The pollen morphology has been adopted as a tool for demonstrating the taxonomic and phylogenetic importance of taxa in different genera of Lamiaceae, such as *Phlomis* L., *Marrubium* L., *Stachys* L., *Nepeta* L., *Mentha* L., *Ziziphora* L. and *Ajuga* L. (Abu-Asab and Cantino 1994, Celenk *et al.* 2008*a*, *b*, Harley *et al.* 1992, Jamzad *et al.* 2006, Kose *et al.* 2011, Moon *et al.* 2008*a*, *b*, Tabaripour *et al.* 2018, Wagstaff 1992). Little research has been conducted on the genus *Ziziphora.* Moreover, in the anatomical studies, just the cross sections taken mainly from the stems and leaves have been reported (Keshavarzi *et al.* 2008, Koca and Tümen 1996, Koca *et al.* 1995, Sezik and Tümen 1984, 1988). The micro-morphological and pollen grain studies have been focused on four subspecies of *Z. clinopodioides* (Keshavarzi *et al.* 2008). Selvi *et al.* (2013) investigated the pollen morphology of the five species from Turkey.

The palynological characters of *Z. clinopodioides* are variable even among several populations of the same subspecies (Keshavarzi *et al.* 2008). For this reason and lacking of comprehensive study on the subspecies, this study was conducted for the first time in the world; hence, the aims of the present paper were twofold: (i) to determine whether the pollen characters could differentiate the subspecies of *Z. clinopodioides* by scanning electron microscopy (SEM) and (ii) to assess the taxonomic significance of the pollen grain morphology.

MATERIAL AND METHODS

Plant material

In the current investigation, pollen grains morphology of nine subspecies (32 specimens) of *Ziziphora clinopodioides* were studied by scanning electron microscopy (SEM). The subspecies were identified based on the descriptions provided in the Flora Iranica (Rechinger 1982) and some samples were not identified in subspecies level, for the reason of overlapping in morphological characters of several subspecies.

The pollen samples were obtained mostly from herbarium materials. Voucher specimens have been deposited in Shahid Beheshti University Herbarium (HSBU), Herbarium Ministerii Iranici Agriculturae (IRAN) and Herbarium of Natural History Museum (W) (Table 1).

Scanning electron microscopy

Small quantities of pollen grains were transferred directly to aluminium stubs with double-sided cellophane tape and coated with gold. The specimens were examined using a Philips × L20 scanning electron microscopes (SEM). UTHSCSA Image Tool Version 3.0 was used to carry out required measurements. Pollen terminology follows Erdtman (1952), Punt *et al.* (2007) and Hesse *et al.* (2009).

Statistical analysis

Ten to 30 fully developed pollen grains were randomly selected for analysis. Eight quantitative and three qualitative palynological features were used for multivariate analysis including the shape (basically, was determined by ration of the equatorial length / polar length (P/E)), polar axis length (P), equatorial axis length (E), diameter of perforation (DP), diameter of muri (DM), apocolpium length (AI), length of colpus (LC), distance between colpi ends (T), number of perforation in 25 μm^2 area (NP) and pattern of sculpturing of pollen (SC) (Table 2).

The analysis of variance (ANOVA) test was performed to detect significant difference for palynological characters of the studied subspecies.

Multivariate analysis including MDS (multidimensional scaling), PCO (principal coordinates analysis) and UPGMA (unweighted pair group method with arithmetic mean) were performed using PAST ver. 2.17 software, for plotting variation among the populations (Hammer *et al.* 2001). For grouping

Table 1
Specimen information for populations of *Ziziphora clinopodioides* sampled, including herbarium vouchers for specimens

Pop. no.	Features	Locality	No.	Voucher number
1	*Z. clinopodioides* subsp.?*	Razavi Khorasan, Torbat-e Heydarieh	1	HSBU2014413
2	*Z. clinopodioides* subsp.?*	Razavi Khorasan, northern Kashmar	1	HSBU2014414
3	*Z. clinopodioides* subsp.?*	Tehran, Damavand	2	HSBU2014425
4	*Z. clinopodioides* subsp.?*	Mazandaran, Sorkh Geriveh village	2	HSBU2014426
5	*Z. clinopodioides* subsp.?*	Qazvin, Qastin Lar	4	HSBU2014411
6	*Z. clinopodioides* subsp.?*	Mazandaran, Kelardasht	1	HSBU2014419
7	*Z. clinopodioides* subsp.?*	Mazandaran, Jurband	1	HSBU2014415
8	*Z. clinopodioides* subsp.?*	Mazandaran, Rineh	1	HSBU2014423
9	*Z. clinopodioides* subsp.?*	Qazvin, Bahram Abad village	1	HSBU2014417
10	*Z. c.* subsp. *kurdica*	Typus	1	W1889-009018
11	*Z. c.* subsp. *pseudodasyantha*		1	W1966-0021503
12	*Z. c.* subsp. *pseudodasyantha*	Razavi Khorasan, Torbat-e Heydarieh, Sefid Darreh	1	26294(IRAN)
13	*Z. c.* subsp. *pseudodasyantha*	Gilan, Pakdeh village	1	26296/2(IRAN)
14	*Z. c.* subsp. *elbursensis*	Typus	1	W1956-0006985
15	*Z. c.* subsp. *elbursensis*		1	22286(IRAN)
16	*Z. c.* subsp. *elbursensis*	Tehran	1	26300/1(IRAN)
17	*Z. c.* subsp. *filicaulis*	Typus	1	W1956-0006986
18	*Z. c.* subsp. *filicaulis*	Semnan, kuh-e-Shahvar	1	26292(IRAN)
19	*Z. c.* subsp. *szowitsii*	Typus	1	W0031739
20	*Z. c.* subsp. *ronningeri*	East Azarbaijan, Khoy	1	W1984-0001938
21	*Z. c.* subsp. *ronningeri*	Azarbaijan	1	26357(IRAN)
22	*Z. c.* subsp. *ronningeri*	Azarbaijan	1	26358(IRAN)
23	*Z. c.* subsp. *afghanica*	Shahroud, Khvosh Yeylaq, gardaneh Olang	1	26289/1(IRAN)
24	*Z. c.* subsp. *afghanica*	Shahroud, Khvosh Yeylaq, gardaneh Olang	1	26289/3(IRAN)
25	*Z. c.* subsp. *bungeana*	North Khorasan, Shirvan, Namanlu village, Galil	1	55403(IRAN)
26	*Z. c.* subsp. *bungeana*	Shahroud	1	26290(IRAN)
27	*Z. c.* subsp. *rigida*	Tehran	1	26334/2(IRAN)

No. = number of specimens sampled;
* = These samples were not identified in subspecies level because of overlapping on morphological characters of several subspecies

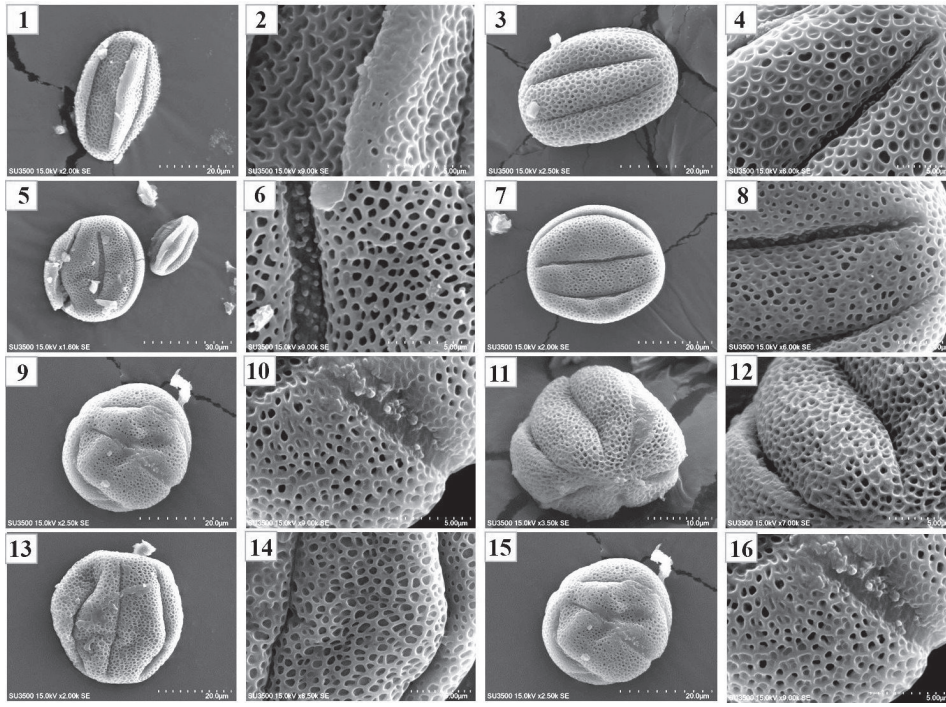
of the specimens, UPGMA (unweighted paired group with arithmetic average) and MDS plot were performed.

The data were standardised (mean = 0, variance = 1) for these analyses. Euclidean and taxonomic distance are used among the subspecies (Podani 2000).

RESULTS

General pollen grain features

Morphological characteristics of the pollen grains of *Ziziphora clinopodioides* subspecies are displayed in Tables 2 and Figures 1–64. Pollen grains are radially symmetrical, isopolar and hexacolpate monads. The shape of the pollen grains is sub-oblate (P/E = 0.82–0.88; Figs 1, 3), oblate-spheroidal (P/E =



Figs 1–16. SEM micrographs of *Z. clinopodioides* pollen grains. – Figs 1–2: *Z. clinopodioides* (Razavi Khorasan, Torbat-e Heydarieh); Figs 3–4: *Z. clinopodioides* (Razavi Khorasan, northern Kashmar); Figs 5–8: *Z. clinopodioides* (Tehran, Damavand); Figs 9–12: *Z. clinopodioides* (Mazandaran, Sorkh Geriveh village); Figs 13–16: *Z. clinopodioides* (Qazvin, Qastin Lar) (equatorial view: 1, 3, 5, 7, 9, 13 and 15; polar view: 11; exine ornamentation: 2, 4, 6, 8, 10, 12, 14 and 16)

Table 2
Some important pollen grain morphological characters of the studied *Ziziphora clinopodioides* populations

No.	Taxa	P	E	P/E	T	LC	DP	NP	DM	AI	Sh	SC	SC
1	*Z. clinopodioides*	36.10±0.23	22.03±0.25	1.64	7.00±0.19	30.34±0.06	0.42±0.09	< 2 0	0.43±0.06	0.63	4	II	I
3	*Z. clinopodioides*	33.52±1.90	24.11±0.35	1.39	9.16±1.21	27.15±1.88	0.86±0.17	< 20	0.42±0.03	0.55	4	I	I
4	*Z. clinopodioides*	31.86±7.50	28.03±9.75	1.14	7.46±4.03	20.34±3.24	0.78±0.20	≥ 20	0.48±0.01	0.52	5	I	I
4	*Z. clinopodioides*	36.54±0.34	31.94±0.81	1.14	11.27±1.65	31.72±0.29	0.73±0.11	≥ 20	0.44±0.10	0.53	5	II	I
5	*Z. clinopodioides*	25.72±1.14	29.05±0.68	0.88	9.58±0.52	22.41±1.22	0.44±0.14	≥ 20	0.34±0.07	0.55	2	II	I
5	*Z. clinopodioides*	28.75±0.37	21.94±0.24	1.31	8.21±0.20	22.45±0.46	0.43±0.09	≥ 20	0.34±0.07	0.69	3	II	IV
2	*Z. clinopodioides*	37.07±4.50	26.24±4.85	1.41	10.55±1.64	31.24±5.87	0.71±0.21	≥ 20	0.56±0.15	0.69	4	I	I
2	*Z. clinopodioides*	35.49±1.08	35.30±2.44	1.00	12.07±0.51	30.48±3.18	0.73±0.13	≥ 20	0.31±0.08	0.57	6	I	I
2	*Z. clinopodioides*	25.57±1.68	16.32±1.05	1.57	6.33±1.59	18.86±1.31	0.53±0.10	< 20	0.45±0.07	0.49	4	II	I
2	*Z. clinopodioides*	38.25±1.94	28.00±2.54	1.37	8.86±1.46	32.14±2.33	0.63±0.17	≥ 20	0.48±0.27	0.68	4	II	I
6	*Z. clinopodioides*	29.89±0.30	24.36±0.09	1.23	6.30±1.78	24.51±0.54	0.48±0.20	≥ 20	0.40±0.11	0.52	3	I	I
8	*Z. clinopodioides*	26.28±0.35	16.31±0.27	1.61	7.73±2.61	19.62±0.37	0.47±0.07	≥ 20	0.42±0.03	0.50	4	II	I
9	*Z. clinopodioides*	37.62±2.94	25.25±4.25	1.69	9.75±1.56	29.68±4.00	0.74±0.17	≥ 20	0.42±0.15	0.62	4	I	I
7	*Z. clinopodioides*	26.66±3.66	22.63±6.32	1.18	8.61±0.12	22.78±4.06	0.52±0.10	< 20	0.47±0.09	0.66	3	II	I
10	*Z. c.* subsp. *kurdica*	25.28±1.32	26.93±1.95	0.94	9.72±1.59	24.22±0.31	0.59±0.10	< 20	0.43±0.08	0.58	1	II	IV
11	*Z. c.* subsp. *pseudodasyantha*	40.30±1.25	33.88±2.66	1.19	10.72±1.22	35.20±1.82	0.91±0.16	≥ 20	0.54±0.18	0.55	3	I	I
11	*Z. c.* subsp. *pseudodasyantha*	39.67±1.82	31.94±1.89	1.24	11.30±0.60	32.75±2.59	0.92±0.16	< 20	0.57±0.13	0.52	3	I	I
11	*Z. c.* subsp. *pseudodasyantha*	37.18±2.33	29.44±4.77	1.26	10.37±1.88	32.36±1.66	0.66±0.14	≥ 20	0.51±0.14	0.49	3	I	IV
12	*Z. c.* subsp. *elbursensis*	21.50±0.10	26.04±1.55	0.82	9.93±0.60	21.05±0.35	0.40±0.07	< 20	0.34±0.08	0.55	2	II	III
12	*Z. c.* subsp. *elbursensis*	22.84±2.51	18.14±2.16	1.26	7.85±3.21	18.84±2.07	0.50±0.13	< 20	0.37±0.02	0.84	3	II	II
12	*Z. c.* subsp. *elbursensis*	37.76±2.08	29.41±3.79	1.28	8.79±1.24	31.34±3.01	0.77±0.20	≥ 20	0.46±0.18	0.64	3	III	IV
13	*Z. c.* subsp. *szowitsii*	31.12±2.67	26.65±2.36	1.17	9.96±1.68	27.69±1.90	0.42±0.03	≥ 20	0.37±0.11	0.60	3	I	I
16	*Z. c.* subsp. *filicaulis*	38.73±0.11	28.67±0.35	1.35	9.26±1.02	32.20±0.10	0.72±0.26	≥ 20	0.45±0.14	0.69	4	I	I

Table 2 (continued)

No.	Taxa	P	E	P/E	T	LC	DP	NP	DM	AI	Sh	SC	SC
16	*Z. c.* subsp. *filicaulis*	31.69±10.84	21.40±5.61	1.48	5.48±0.66	28.09±10.19	0.85±0.20	≥ 20	0.55±0.26	0.49	4	I	I
17	*Z. c.* subsp. *ronningeri*	25.36±0.41	20.33±0.81	1.25	7.01±0.85	20.27±1.18	0.39±0.06	< 20	0.41±0.06	0.41	3	III	IV
17	*Z. c.* subsp. *ronningeri*	28.35±1.88	27.48±2.85	1.03	10.08±0.81	24.87±2.17	0.32±0.04	< 20	0.40±0.03	0.51	5	II	III
17	*Z. c.* subsp. *ronningeri*	34.41±2.01	26.10±5.01	1.32	8.26±1.96	29.54±1.86	0.79±0.23	≥ 20	0.41±0.10	0.63	3	I	I
14	*Z. c.* subsp. *afghanica*	30.53±0.71	26.50±0.49	1.15	9.34±0.96	26.25±0.28	0.69±0.06	< 20	0.42±0.07	0.55	3	II	I
14	*Z. c.* subsp. *afghanica*	40.39±1.78	27.85±2.53	1.45	10.85±1.07	35.00±2.63	0.71±0.17	≥ 20	0.44±0.10	0.54	4	III	IV
15	*Z. c.* subsp. *bungeana*	32.50±0.40	28.24±0.48	1.15	9.19±0.20	27.44±0.36	0.57±0.09	≥ 20	0.46±0.18	0.52	3	I	I
15	*Z. c.* subsp. *bungeana*	34.99±5.33	30.05±2.82	1.16	10.69±0.63	31.80±5.10	0.68±0.22	≥ 20	0.55±0.16	0.52	3	II	I
18	*Z. c.* subsp. *rigida*	25.57±4.87	24.96±6.26	1.02	10.01±3.29	21.73±3.13	0.49±0.13	≥ 20	0.37±0.12	0.53	5	I	I

Sh = shape; P = polar axis length (μm); E = equatorial axis length (μm); P/E = ratio of polar axis to equatorial axis; T = mesocolpium; LC = length of colpus (μm); DP = diameter of perforation (μm); NP = number of perforation in 25 μm² area; DM = diameter of muri (μm); AI = apocolpium index (μm); Shape of pollen (P/E): 1) oblate-spheroidal, 2) sub-oblate, 3) sub-prolate, 4) prolate, 5) prolate-spheroidal, 6) spherical; SC = pattern of sculpturing of pollen. Numbers (No.) are based on Table 1

0.94; Figs 29–30), spherical (P/E = 1.00; Figs 1–2), prolate-spheroidal (P/E = 1.02–1.14; Figs 1, 4) and subprolate (P/E = 1.17–1.31, Figs 1–4) to prolate (P/E = 0.82–1.69; Figs 1–4) in the equatorial view. The polar axis (P) varies between 21.50 to 40.30 μm, and the equatorial diameter (E) is between 16.31 and 35.30 μm. The range of the colpus length of all the studied taxa is 18.86–35.20 μm. The length of the colpus is not correlated with the whole pollen size. The range of the distance between the colpi of all the studied populations is 6.30–12.07 μm. The apocolpium length (AI) is the ratio of the distance between the apices of two colpus to its equatorial diameter. The ranges of the apocolpium index vary between 0.41 to 0.84 μm. Colpi are narrow towards the poles and have coarsely granular membranes. The diameters of perforations are between 0.32–0.92 μm.

Exine ornamentation

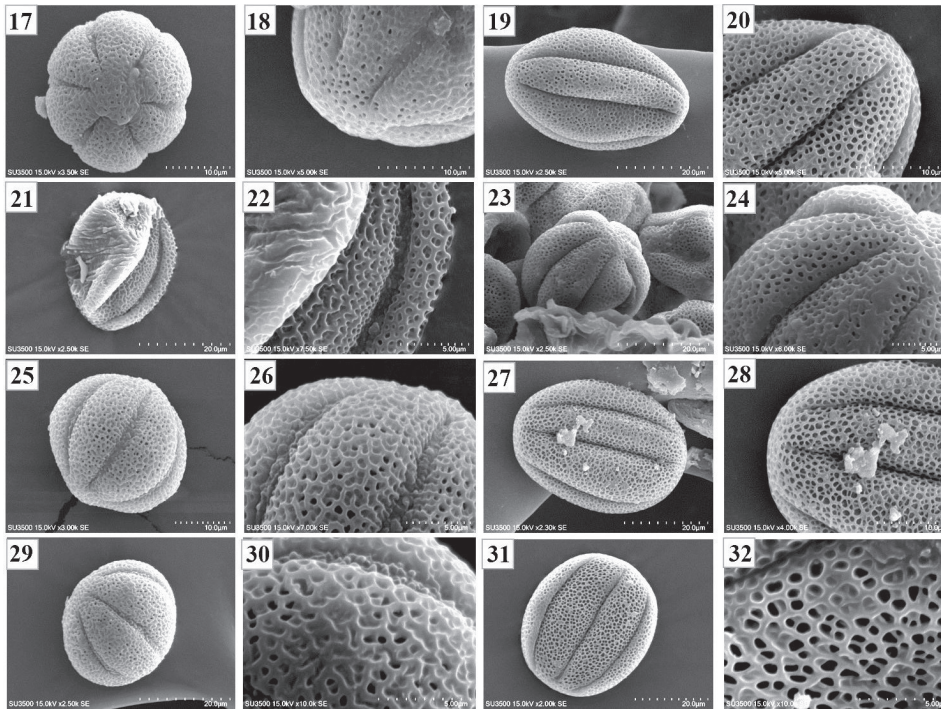
Variations are observed in the exine sculpturing ornamentations. The exine of the pollen grains is mainly bireticulate, microreticulate or a combination of these types. Based upon the different pattern of the exine sculpturing, three types can be recognised for *Ziziphora clinopodioides*:

Pollen type I: bireticulate ornamentation pattern is the common type of exine ornamentation amongst the studied populations (Figs 3–6, 13–16, 21–22, 25–26, 31–32, 33–36, 43–48, 53–54, 63–64). A bireticulate is comprised of two-layered-reticulum. The brochi of the primary reticulum are filled by a small secondary reticulum and are divided into 2–4 smaller pieces.

Pollen type II: microreticulate ornamentation (Figs 1–2, 7–12, 17–20, 23–24, 27–30, 37–40, 49–52, 55–56, 59–62).

Other exine ornamentation types

The other rare recognised ornamentations in *Ziziphora clinopodioides* is bireticulate-reticulate (type III) that was recorded in *Z. clinopodioides* subsp. *elbursensis* (Figs 40–41), *Z. clinopodioides* subsp. *ronningeri* (Figs 53–54) and *Z. clinopodioides* subsp. *afghanica*.

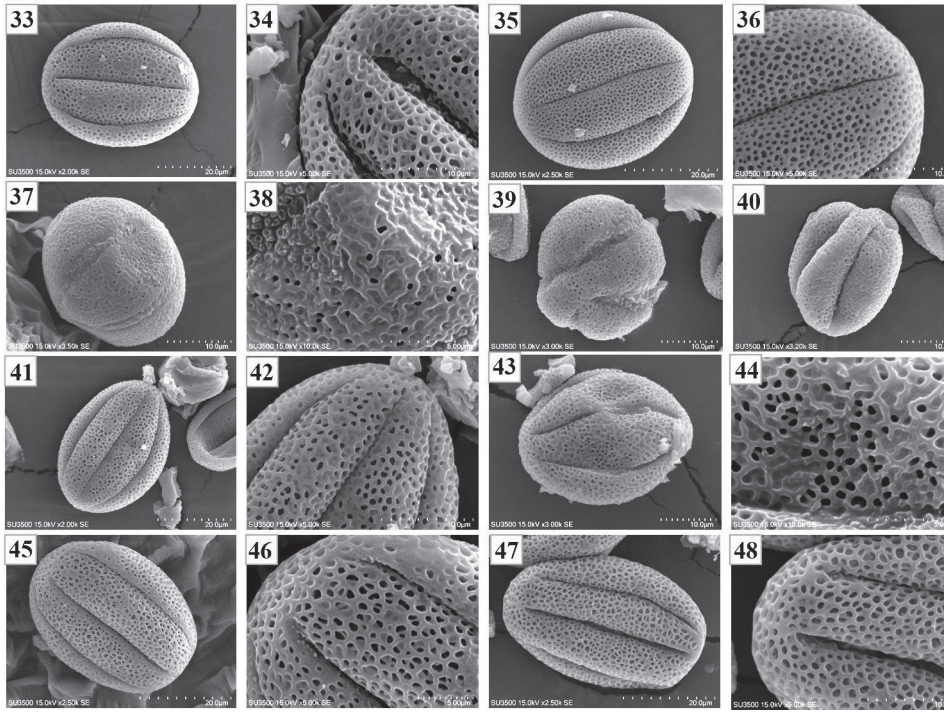


Figs 17–32. SEM micrographs of *Z. clinopodioides* pollen grains. – Figs 17–20: *Z. clinopodioides* (Qazvin, Qastin Lar); Figs 21–22: *Z. clinopodioides* (Mazandaran, Kelardasht); Figs 23–24: *Z. clinopodioides* (Mazandaran, Jurband); Figs 25–26: *Z. clinopodioides* (Mazandaran, Rineh); Figs 27–28: *Z. clinopodioides* (Qazvin, Bahram Abad village); Figs 29–30: *Z. clinopodioides* subsp. *kurdica* (Typus); Figs 31–32: *Z. clinopodioides* subsp. *pseudodasyantha* (equatorial view: 19, 21, 25, 27, 29 and 31; polar view: 17 and 23; exine ornamentation: 18, 20, 22, 24, 26, 28, 30 and 32)

Infraspecific variation

The results of ANOVA indicate that there is no significant difference in the quantitative traits of the pollen grains ($p > 0.05$). Moreover, different ordination methods produced similar results therefore, the MDS and PCO plot of the studied populations based on the palynological features are provided (Figs 65–66). The UPGMA, MDS and PCO plot analysis (Figs 65–67) did not support the subspecies classification of the studied populations of *Ziziphora clinopodioides*. The representatives of the presumed subspecies of the *Z. clinopodioides* were intermixed in the plot. Therefore, the subspecies were not differentiated by pollen data.

UPGMA tree (Fig. 67) revealed that the studied populations (representative of subspecies) in *Z. clinopodioides*, overlapped together and they did not form separate clusters; especially individuals were collected from the geo-

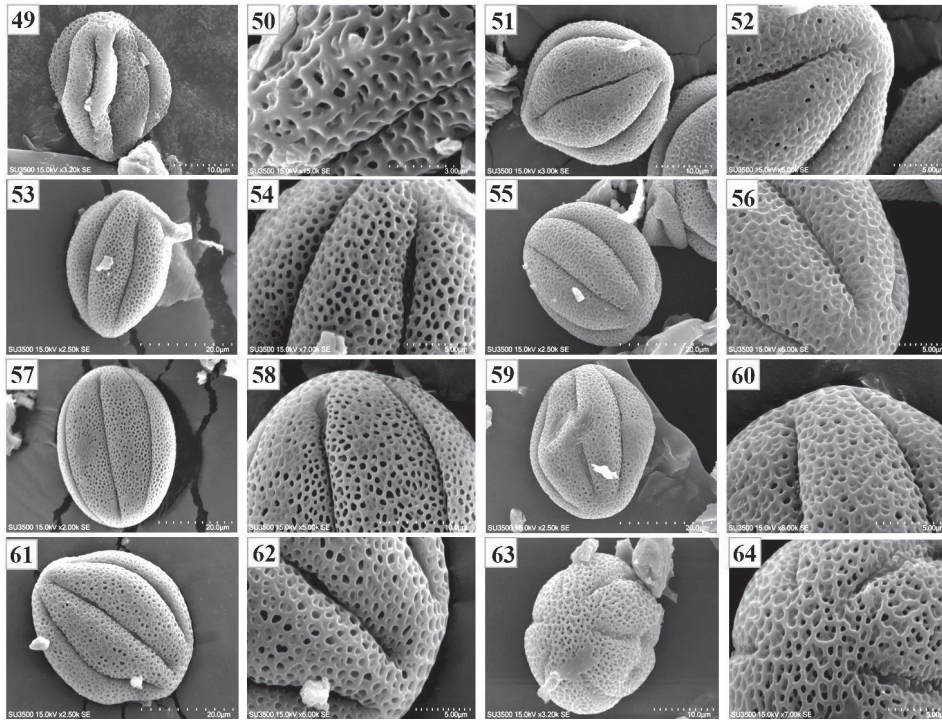


Figs 33–48. SEM micrographs of *Z. clinopodioides* pollen grains. – Figs 33–36: *Z. clinopodioides* subsp. *pseudodasyantha* (Razavi Khorasan, Torbat-e Heydarieh, Sefid Darreh; Gilan, Pakdeh village); Figs 37–42: *Z. clinopodioides* subsp. *elbursensis* (type: 39, 40); Figs 43–44: *Z. clinopodioides* subsp. *szowitsii* (type); Figs 45–48: *Z. clinopodioides* subsp. *filicaulis* (type; Semnan, kuh-e-Shahvar) (equatorial view: 33, 35, 37, 39, 40, 41, 43, 45 and 47; exine ornamentation: 34, 36, 38, 42, 44, 46 and 48)

graphical localities of Iran. Therefore, there is little support for the pollen morphology divergence amongst the subspecies.

DISCUSSION

Ziziphora clinopodioides has a large geographical distribution and represents high morphological diversity in Iran and adjacent countries. Our investigations have revealed that the pollen grain morphology can be useful in clarifying the infra-specific classification (Jamzad and Hasani-Nejad 2014, Keshavarzi *et al.* 2008, Selvi *et al.* 2013, Tabaripour *et al.* 2018), such as the ornamentation of the pollen grains have valuable morphological characteristics to solve taxonomic problems of some subspecies in *Ajuga*. The sculpture of



Figs 49–64. SEM micrographs of *Z. clinopodioides* pollen grains. – Figs 49–54: *Z. clinopodioides* subsp. *ronningeri* (East Azarbaijan, Khoy; Azarbaijan; Azarbaijan); Figs 55–58: *Z. clinopodioides* subsp. *afghanica* (Shahroud, Khvosh Yeylaq, gardaneh Olang); Figs 59–62: *Z. clinopodioides* subsp. *bungeana* (North Khorasan, Shirvan, Namanlu village, Galil; Shahroud); Figs 63–64: *Z. clinopodioides* subsp. *rigida* (Tehran) (equatorial view: 49, 51, 53, 55, 57, 59 and 61; polar view: 63; exine ornamentation: 50, 52, 54, 56, 58, 60, 62 and 64)

A. chamaepitys subsp. *chia* var. *chia* was granulate, but it was reticulate in *A. chamaepitys* subsp. *chia* var. *ciliata* (Kose *et al.* 2011).

In the present study it was observed that the shape of the pollen grains of *Z. clinopodioides* are sub-oblate, oblate-spheroidal, spherical, sub-prolate to prolate and the ornamentation is bireticulate, microreticulate, reticulate and bireticulate-reticulate. It was also found that there is a remarkable problem in the case of shape and ornamentation, even in different specimens of the same subspecies.

Moon *et al.* (2008*a*) have studied the pollen grain morphology of subtribe Menthinae. They have investigated 58 species from 42 genera including *Z. clinopodioides* and suggested that the pollen grains of this species are hexacolpate, oblate to oblate-spheroidal shape and exine ornamentation is bireticulate. Selvi *et al.* (2013) have suggested that the pollen grains of *Z. clinopodioides* are hexacolpate, symmetrical, isopolar, oblate spheroidal to prolate shape and

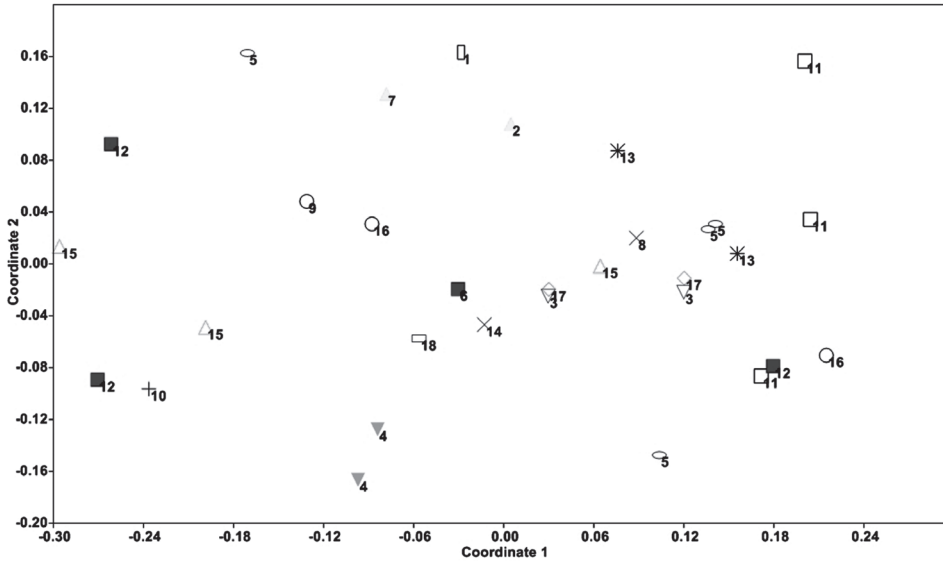


Fig. 65. MDS of the studied *Z. clinopodioides* populations of based on the palynological data. 1 = *Z. clinopodioides* (Razavi Khorasan, Torbat-e Heydarieh); 2 = *Z. clinopodioides* (Razavi Khorasan, northern Kashmar); 3 = *Z. clinopodioides* (Tehran, Damavand); 4 = *Z. clinopodioides* (Mazandaran, Sorkh Geriveh village); 5 = *Z. clinopodioides* (Qazvin, Qastin Lar); 6 = *Z. clinopodioides* (Mazandaran, Kelardasht); 7 = *Z. clinopodioides* (Mazandaran, Jurband); 8 = *Z. clinopodioides* (Mazandaran, Rineh); 9 = *Z. clinopodioides* (Qazvin, Bahram Abad village); 10 = *Z. clinopodioides* subsp. *kurdica*; 11 = *Z. clinopodioides* subsp. *pseudodasyantha*; 12 = *Z. clinopodioides* subsp. *elbursensis*; 13 = *Z. clinopodioides* subsp. *filicaulis*; 14 = *Z. clinopodioides* subsp. *szowitsii*; 15 = *Z. clinopodioides* subsp. *ronningeri*; 16 = *Z. clinopodioides* subsp. *afghanica*; 17 = *Z. clinopodioides* subsp. *bungeana*; 18 = *Z. clinopodioides* subsp. *rigida*.

exine sculpturing is bireticulate. Keshavarzi *et al.* (2008) have observed that the shape of the pollen grains of *Z. clinopodioides* is hexacolpate, prolate, and prolate spheroidal to spheroid and the sculpturing is bireticulate.

Variation in the pollen grains size, the exine ornamentation and aperture numbers occurs in many families, such as *Canna indica* L. (Chen *et al.* 1989), *Lythrum salicaria* L. (Mal and Hermann 2000), *Thymus capitatus* (Karabournioti *et al.* 2007), *Malus* (Nazeri 2008), *Pyrus* (Zamani *et al.* 2010) and *Acer* (Nikzat Siahkolaee *et al.* 2017). The pollen grain morphology in Rosaceae has a large differentiation in populations of one species. Bednorz *et al.* (2005) indicated that there are no important differences in the pollen morphology between the subspecies of *Sorbus aucuparia*.

Most of the *Mentha* species are characterised by vast morphological variations, as reflected by the large number of names at different taxonomic ranks attributed by the taxonomists to the mints during the past two centuries. Furthermore, the intra- and interspecific hybridisation occurs commonly when

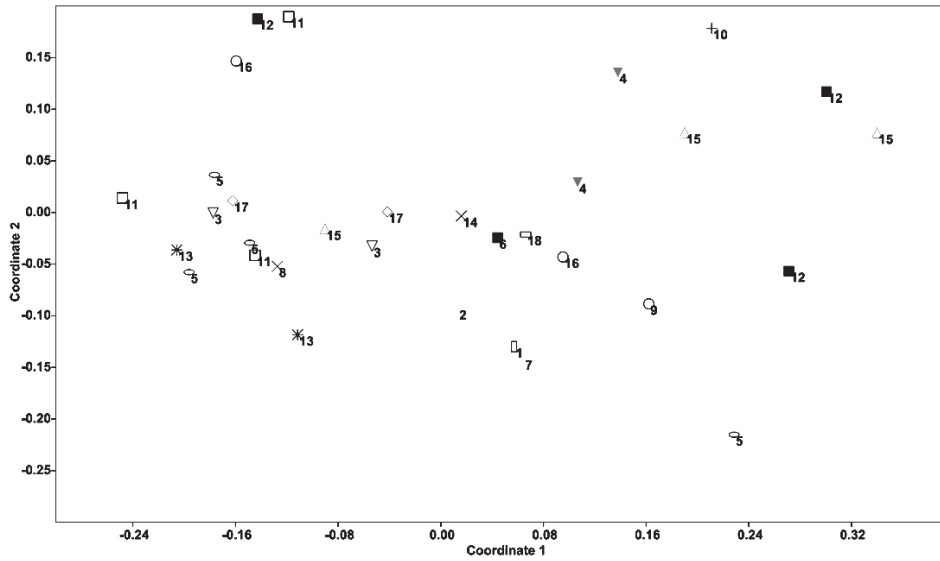


Fig. 66. PCO plot of the studied *Z. clinopodioides* populations based on the palynological data. 1 = *Z. clinopodioides* (Razavi Khorasan, Torbat-e Heydarieh); 2 = *Z. clinopodioides* (Razavi Khorasan, northern Kashmar); 3 = *Z. clinopodioides* (Tehran, Damavand); 4 = *Z. clinopodioides* (Mazandaran, Sorkh Geriveh village); 5 = *Z. clinopodioides* (Qazvin, Qastin Lar); 6 = *Z. clinopodioides* (Mazandaran, Kelardasht); 7 = *Z. clinopodioides* (Mazandaran, Jurband); 8 = *Z. clinopodioides* (Mazandaran, Rineh); 9 = *Z. clinopodioides* (Qazvin, Bahram Abad village); 10 = *Z. clinopodioides* subsp. *kurdica*; 11 = *Z. clinopodioides* subsp. *pseudodasyantha*; 12 = *Z. clinopodioides* subsp. *elbursensis*; 13 = *Z. clinopodioides* subsp. *filicaulis*; 14 = *Z. clinopodioides* subsp. *szowitsii*; 15 = *Z. clinopodioides* subsp. *ronningeri*; 16 = *Z. clinopodioides* subsp. *afghanica*; 17 = *Z. clinopodioides* subsp. *bungeana*; 18 = *Z. clinopodioides* subsp. *rigida*

the species of section Menthae section meets sympatrically with each other, leading to the complex variation patterns that characterise most of the wild populations (Celenk *et al*. 2008*b*).

Supporting the ANOVA results, the MDS, PCO plot and UPGMA tree did not separate the studied subspecies based on the qualitative and quantitative characters. Therefore, combination of the UPGMA and PCO plot indicated a palynological divergence amongst the probed subspecies.

Rechinger (1982) in the Flora Iranica has introduced this variety in the form of subspecies. In this paper, the pollen characters indicated that there are no subspecies in the studied populations of *Z. clinopodioides*. The findings of the present study confirm the previous study performed by Jamzad (2012) in the Flora of Iran; after thorough morphological investigation in *Z. clinopodioides*, Jamzad (2012) stated that due to the high degree of morphological variability and co-occurrence of many subspecies in one location, the number of subspecies within *Z. clinopodioides* cannot be determined with absolute cer-

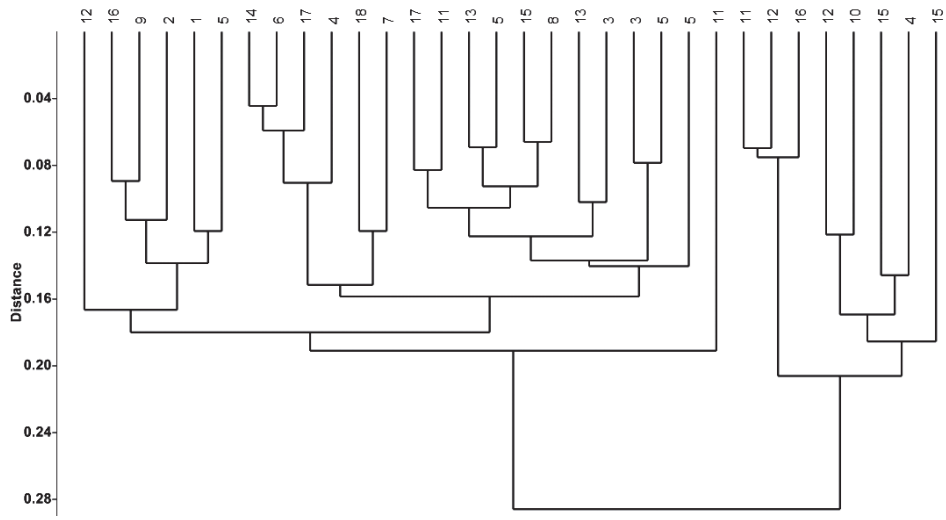


Fig. 67. UPGMA tree of the studied populations of *Z. clinopodioides* based on the palynological data. 1 = *Z. clinopodioides* (Razavi Khorasan, Torbat-e Heydarieh); 2 = *Z. clinopodioides* (Razavi Khorasan, northern Kashmar); 3 = *Z. clinopodioides* (Tehran, Damavand); 4 = *Z. clinopodioides* (Mazandaran, Sorkh Geriveh village); 5 = *Z. clinopodioides* (Qazvin, Qastin Lar); 6 = *Z. clinopodioides* (Mazandaran, Kelardasht); 7 = *Z. clinopodioides* (Mazandaran, Jurband); 8 = *Z. clinopodioides* (Mazandaran, Rineh); 9 = *Z. clinopodioides* (Qazvin, Bahram Abad village); 10 = *Z. clinopodioides* subsp. *kurdica*; 11 = *Z. clinopodioides* subsp. *pseudodasyantha*; 12 = *Z. clinopodioides* subsp. *elbursensis*; 13 = *Z. clinopodioides* subsp. *filicaulis*; 14 = *Z. clinopodioides* subsp. *szowitsii*; 15 = *Z. clinopodioides* subsp. *ronningeri*; 16 = *Z. clinopodioides* subsp. *afghanica*; 17 = *Z. clinopodioides* subsp. *bungeana*; 18 = *Z. clinopodioides* subsp. *rigida*

tainty. Keshavarzi *et al.* (2008) reported that *Z. clinopodioides* subsp. *elbursensis* has not been distinctly separated from subsp. *rigida*, subsp. *pseudodasyantha* and subsp. *szowitsii*, however, it was identified to be clearly separated. The present study, nonetheless, did not confirm this finding. It was found that all the subspecies are distributed among each other.

The delimitation of Z. *clinopodioides* into subspecies is not quite easy (Jamzad 2012). The major reason for the introgression or the introgressive hybridisation is the incorporation (usually via hybridisation and backcrossing) of alleles from one entity (species) into the gene pool of a second, divergent entity (species) (Anderson and Hubricht 1938, Harrison and Larson 2014).

CONCLUSIONS

There have been numerous discussions about intraspecific classification of *Ziziphora clinopodioides.* To this end, various subspecies have been definite for it in different flora. These subspecies are morphologically very similar; therefore, the palynological traits were used for the identification and delimitation of the subspecies. The present study unveiled that the palynological features that have been deemed useful for the idea of including the subspecies in the *Z. clinopodioides* are not approved. Therefore, these variables have little taxonomic value for the identification amongst the subspecies of *Z. clinopodioides.* It can be stated that the finding by Jamzad (2012) for the intraspecific classification and introducing all subspecies to be synonymous were confirmed in the present paper.

*

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