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Pollen morphological traits analysis of eighteen *Nepeta* species in Iran

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Abstract. *Nepeta* is one of the largest genera of Lamiaceae, which is widely distributed in Iran. Several species of the genus are traditionally used as culinary, industrial, and medicinal plants. In the present study, we examined fourteen qualitative and quantitative pollen morphological characters of twenty-four populations of eighteen *Nepeta* using light and scanning electron microscope. The pollen grains are radially symmetric, isopolar, and hexacolpate in all species. We have found that the quantitative features vary among the studied taxa. According to PCA analysis, the colpus length and width are the most variable traits. Polar view shapes in all the studied taxa are circular, while the equatorial view is prolate-spheroidal, subprolate, perprolate, and prolate. We determined microreticulate and bireticulate exine sculpturing, and the bireticulate type was dominant. We revealed that polar and equatorial axes length of pollen grains varied between populations of the same species, and they, therefore, should be ignored in taxonomical identification of species. The polar view was similar in all the studied pollen grains and could not be considered as a diagnostic trait. The equatorial shape was stable between populations of the same species, and this was taxonomically important, while we registered some infraspecific variations in exine sculpture. Our cluster of taxa based on the pollen morphology is not in concordance with the traditional sectional classification of the genus.
Keywords. Iran, Lamiaceae; Mentheae; Nepetoideae; Palynology; Taxonomy; infraspecific variations.

Analisis polínico de dieciocho especies iraníes de *Nepeta*

Resumen. *Nepeta* es uno de los géneros más numerosos en especies de la familia Lamiaceae, ampliamente distribuida en Irán. Algunas especies del género se usan tradicionalmente como plantas culinarias, industriales y medicinales. En el presente trabajo hemos estudiado catorce caracteres cuali- y cuantitativos de la morfología polínica de veinticuatro poblaciones correspondientes a 18 especies de *Nepeta* mediante microscopía óptica y electrónica de barrido. Los granos de polen de todos ellos presentan simetría radial, son isopolares y hexacolpados, aunque se han observado diferencias en alguno de los caracteres cuantitativos entre diferentes taxones. El análisis de PCA revela que el carácter más variable es la longitud y la anchura de los colpos. La forma de la vista polar de los granos de polen es circular en todos los taxones estudiados. Sin embargo, la forma de la vista ecuatorial varía desde prolado-esferoidal, pasando por subprolado y perprolado hasta prolado. Hemos observado que la exina puede ser microreticulada o bireticulada, ésta presente en la mayoría de los taxones. La longitud de los ejes polares y ecuatoriales varía entre las poblaciones de la misma especie, por lo tanto, no representa un buen carácter para la identificación de las mismas. Además, la vista polar es muy parecida entre diferentes especies y no debería ser considerada como carácter diagnóstico. La forma de la vista ecuatorial es estable entre las diferentes poblaciones de la misma especie. Existe una variación infraespecífica en la ornamentación de la exina. El agrupamiento de los taxones basado en la morfología de los granos de polen no está en concordancia con la clasificación tradicional del género en secciones.
Palabras clave. Irán; Lamiaceae; Mentheae; Nepetoideae; palinología; taxonomía; variación infraespecífica.

Introduction

The genus *Nepeta* L. is the largest in Lamiaceae in Iran. It is included in the Nepetoideae subfamily, tribe Mentheae (Cantino, 1992), being the infrageneric classification highly controversial. Rechinger (1982) has listed 63 species in Iran grouped into 13 sections. Recently, Jamzad (2012) identified more than 79 species in the country and classified them into six sections.

Nepeta species are widely used in traditional medicine because of their expectorant, antispasmodic, antitussive, diuretic, antiseptic, and antiasthmatic activities, while the inflorescences of some species have also been used as a sedative drug (Baser *et al*., 2000). Some species were used as culinary plants, for example, *Nepeta cataria* L. had been used as a tea beverage in Europe before the real tea was imported (Newall *et al*., 1996; Baser *et al*., 2000). Sammataro & Avitabile (1998) reported that bees use several species as a source of pollen and nectar.

Previous studies proved that frequent hybridization and introgression associated with the substantial age-related or phenotypic plasticity make *Nepeta* be considerably complex genus (Talebi *et al*., 2018). Until now, various patterns of infrageneric classifications were constructed in different Flora by Boissier (1879), Pojarkova (1954), Rechinger (1982), Hedge & Lamond (1982), Budantsev (1997) and Dirmenci (2003). These patterns are mainly based on the different morphological

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features of vegetative and reproductive organs, like plant habit, leaf morphology, inflorescence, calyx and corolla structure, and nutlet traits. Jamzad *et al.* (2003) conducted a phylogenetic study of this genus and reported that *Nepeta* is a monophyletic genus. Moreover, they proposed the revision of the previous infrageneric classification.

Palynology is one of the important tools in the taxonomy of Lamiaceae, and it provides valuable data towards the classification of genera and species within the family. According to Erdtman (1945), family Lamiaceae is divided into two subfamilies based on the pollen: 1) Lamioideae with tricolpate and bi-nucleate pollen and 2) Nepetoideae with hexacolpate and tri-nucleate pollen. The classification based on a number of nuclei and apertures in the pollen grains is widely used (Harley *et al.*, 1992; Abu-Asab & Cantino, 1994).

The pollen morphological characteristics of some *Nepeta* species have been studied by several botanists (e.g. Ubera, 1982; Perveen & Qaiser, 2003). Moon *et al.* (2008) revealed that ornamentation of sexine is variable and could be significant at the generic level. Just a few authors studied pollen morphological features of *Nepeta* species in Iran, but these investigations restricted to members of one to two sections/groups, namely section *Psilonepeta* (Azizian *et al*., 2001), section *Cataria* (Ranjbari *et al.*, 2004), and groups 2 and 4 (Razavi *et al.*, 2017). Jamzad *et al*. (2000) studied pollen morphology of eleven annual species of two sections of the genus. Razavi *et al*. (2017) found variations in pollen characteristics among some taxa, and their species classification based on palynological traits were similar to Rechinger (1982) subgeneric classification of *Nepeta.*

The phylogenetic finding in the genus *Nepeta* has triggered the need to obtain phylogenetic patterns based on other features than inflorescence morphology and its sequence data. The highly diverse *Nepeta* flora of Iran shows a large coverage of the phenotypical variations and sections of the genus. While some species of the genus *Nepeta* growing in Iran have been poorly known palynologically, the present study confirmed the importance of pollen morphological traits and their infraspecific variations in the infrageneric classification.

Therefore, the main goal of this research was to: a) determine the infrageneric and infraspecific pollen morphological variations of eighteen Iranian *Nepeta* taxa including in five groups and b) to assess the implementation of the palynological data for classification at infraspecific and sectional levels within the genus.

Material and Methods

We examined the pollen grains of twenty-four populations representing eighteen recognized taxa of the genus *Nepeta* from Iran. Based on the geographical distribution, one to two population(s) of each taxon was collected during spring 2015-2016. The identification of collected samples was done according to descriptions provided in *Flora Iranica* (Rechinger, 1982) and *Flora of Iran* (Jamzad, 2012). The herbarium samples of each taxon were housed at the Herbarium of Research Center of Agricultural and Natural Resources of Mashhad (MRCH) and Institute of Medicinal Plants Herbarium (IMPH) (Table 1, Figure 1).

Table 1. Habitat locations of the studied *Nepeta* species (sectional classification is based on Jamzad 2012).

Sample N.	Section	Species	Habitat locality	Latitude	Longitude	Voucher code
1	2	*N. binaludensis* Jamzad	Khorasan Razavi province, Zoshk, Abdullah River, 2200 m asl	36° 30′ 15.20″ N	59° 28′ 02.11″ E	9026 – IMPH
2	4	*N. cataria* L. (Mazandaran population)	Mazandaran province, Siahbisheh, 2300 m asl	36° 14′ 33” N	51° 15′ 04.11” E	7046 – IMPH
3	4	*N. cataria* L. (Khorasan population)	Khorasan Razavi province, Kalat, Gharesoo, 1100 m asl	37° 00′ 05.20″ N	59° 45′ 27.01″ E	11116 – MRCH
4	4	*N. crassifolia* Boiss. & Buhse.	Semnan province, Semnan to Damghan road, Ahovan, 1300 m asl	37° 20′ 24.3″ N	57° 03′ 13.9” E	243 – IMPH
5	4	*N. fissa* Mey. (Albourz population)	Albourz province, Albourz mountain, Dizin,ʃ2664 m asl	36° 03’ 1.29” N	51° 24’ 85.7” E	7054 – IMPH
6	4	*N. fissa* Mey. (Polor population)	Mazandaran province, Haraz road, Polor, 2200 m asl	35° 51’ 50.8” N	52° 03’ 78.6” E	7064 – IMPH
7	1	*N. glomerulosa* Boiss. var. *carmanica* Bornm. (Jiroft population)	Kerman province, Jirof, 42 km to Kerman, Deh-Bakri, 2260 m asl	28° 93′ 85.04″ N	57° 46′ 27.27” E	5907– MRCH
8	1	*N. glomerulosa* Boiss. var. *carmanica* Bornm. (Gachsaran population)	Kohkiloyeh and Boier Ahmad, Gachsaran, Lar, 1400 m asl	30° 72′ 46.29″ N	50° 84′ 56.22” E	9087 – MRCH
9	4	*N. hausskneckhtii* Bornm.	Ardabil, Khalkhal to Asalem, 1870 m asl	37° 35′ 66” N	48° 41′ 18” E	7065 – IMPH
10	4	*N. heliotropifolia* Lam.	Markazi province, Arak, Sefidkhani mountain, 2180 m asl	33° 59′ 29.6” N	49° 34′ 10.02” E	7053 – IMPH
11	4	*N. kotschyi* Boiss. var. *persica* (Boiss.) Jamzad (Kashmar population)	Khorasan Razavi province, Kashmar, Norh Baharieh, 1350 m asl	35° 25′ 28.09” N	58° 50′ 45.13” E	7049 – IMPH

Sample N.	Section	Species	Habitat locality	Latitude	Longitude	Voucher code
12	4	*N. kotschyi* Boiss. var. *persica* (Boiss.) Jamzad (Nyshabur population)	Khorasan Province, Nyshabur, 1700 m asl	35° 25′ 28.09” N	58° 50′ 45.13” E	7049 – IMPH
13	5	*N. lasiocephala* Benth.	Kerman province, Rayen, Hezar mountain, 4243 m	29° 30’ 42” N	57° 16’ 18.10” E	1597 – IMPH
14	2	*N. menthoides* Boiss. & Buhse.	Ardabil, Khalkhal, 2500 m asl	37° 37’ 60” N	48° 32’ 59.99” E	1575 – IMPH
15	4	*N. meyeri* Benth. (Lasem population)	Mazandaran province, Haraz road, Polor, Lasem 2650 m asl	35° 35’ 77.8” N	52° 34’ 12.7” E	7051 – IMPH
16	4	*N. meyeri* Benth. (Bostan population)	East Azerbaijan, Tabriz, to Zanjan, Bostan Abad, 1841 m asl	37° 44’ 04. 6” N	46° 50’ 49” E	7055 – IMPH
17	5	*N. mirzayanii* Rech f. & Esfand.	Kerman province, Rabor, Naniz Olia, 2418 m asl	29° 19’ 01.9” N	56° 54’ 10.2” E	7056 – IMPH
18	2	*N. pogonosperma* Jamzad & Assadi	Qazvin province, Alamout, 3000 m asl	36° 08’ 81.16” N	49° 85’ 47.34” E	7050 – IMPH
19	4	*N. racemosa* Lam.	Qazvin province, Alamout to Moalem Kelayeh, 2515 m asl	36° 23’ 47.4” N	50° 45’ 57.1” E	7066 – IMPH
20	4	*N. saccharata* Bunge	Zanjan province, 20km Dandi to Zanjan 1513 m asl	36° 29’ 49.5” N	47° 44’ 54.4” E	7063 – IMPH
21	2	*N. satureioides* Bioss.	South Khorasan province, Ferdous, Galekooh slopes, 1600 m asl	33° 22′ 51.07” N	58° 28′ 34.51” E	4454– MRCH
22	6	*N. sessilifolia* Bunge (Arak population)	Markazi province, Arak, Sefidkhani mountain, 2180 m asl	33° 59′ 30.7” N	49° 34′ 12.09” E	7052 – IMPH
23	6	*N. sessilifolia* Bunge (Alvand population)	Isfahan province, Golpaygan, Alvand, 2000 m asl	33° 56’ 08.22” N	50° 43 08.33” E	7048 – IMPH
24	4	*N. wettsteinii* Heir. Braun	West Azerbaijan, Road of Urmia to Sero, 1680 m asl	37° 19’ 73.13” N	44° 76’ 53.34” E	7058 – IMPH

Figure 1. Distribution map of *Nepeta* species and populations studied. Location Numbers refer to those of Table 1.

Light microscopy

We prepared pollen grains for light microscopy (LM) according to the Acetolysis method (Erdtman, 1960). We measured twenty acetolyzed pollen grains per herbarium sheet with Olympus bx51 light microscope and digital camera dp71. Measurements include polar and equatorial axes length, colpus length and width, apocolpium and mesocolpium diameter. We used image tools (ver. 2) for the measurements.

Scanning Electron Microscopy

For scanning electron microscopy (SEM) examination, pollen grains were transferred directly to a stub covered with double–sided transparent tape. The studs were coated with gold in a JFC–1600 Auto Fine Coater and photographed in SU 3500 scanning electron microscope at 15 kV. We measured polar and equatorial shapes, pori diameter, lumen shape and dimensions by the electronic micrographs. Descriptive terms were, according to Moore *et al.* (1991) and Erdtman (1960). The pollen shape has been determined using the ratio between the polar axis and the equatorial diameter (P/E ratio). If P/E = ca. 1, the grains are termed spheroidal. If P/E > ca 1.2, the grains are termed prolate, if P/E < ca. 0.8, the grains are oblate. Moreover, the subcategories prolate-spheroidal and oblate-spheroidal were used for the grains that are slightly prolate or oblate, respectively. Pollen sculpturing is an external feature of the pollen wall. The pollen grains with reticulate sculpturing pattern present elements termed a murus, and space between muri is termed a lumen.

Statistical analysis

We applied one-way ANOVA test to assess the palynological characteristics and Pearson's coefficient of correlation to determine the relationships between the pollen features using SPSS ver. 15. The data for principal coordinates analysis (PCOA) and correspondence analysis (CA) were standardized. We performed the CA-joined plot represented pollen features similarity relationships among the species, linking the palynological traits using MVSP ver. 3.2 (Podani, 2000).

Results

Pollen morphology

Pollen grains of all examined taxa of *Nepeta* are radially symmetric hexacolpate monads. The shape of the polar view is circular, and it is very similar in all the studied taxa. However, the pollen equatorial views vary and are determined as prolate-spheroidal (*N. sessilifolia*, Arak population, *N. fissa*, Polor population, *N. meyeri*, Lasem population), subprolate (*N. glomerulosa* var. *carmanica*, Jiroft population, *N. cataria*, both populations, *N. lasiocephala*, *N. menthoides*, *N. mirzayanii*, *N. racemosa*, *N. saccharata*, *N. saturioides*, *N. sessilifolia*, Alvand population, *N. wettsteinii*, *N. binaludensis*), perprolate (*N. kotschyi* var. *persica*, Kashmar population), and prolate (in the rest taxa). The main features of the investigated pollen grains are summarized in Table 2, and the selected micrographs of the pollen grains are presented in Figures 2-5.

Table 2. Selected quantitative and qualitative palynological features of the studied *Nepeta* species (all values are in µm). Abbreviations are: S, Sample; CL, Colpus length; CW, Colpus width; CLW, Colpus length/ width ratio; P, Polar axis length; E, Equatorial Axis length; AD, Apocolpium diameter; M, Mesocolpium; PD, Pori diameter; PDi: Pori distance; EV, Equatorial view: P, prolate; SP, Subprolate; PP, Perprolate; P-S, Prolate-Spheroidal; PV, Polar view: C, circular; LS, Lumina shape: C, Circula; E, Elongate; P, Polygonal; EO, Exine ornamentation: BR, Bireticulate; MR, Microreticulate; PS: Pori shape.

S	CL	CW	CLW	P	E	P/E ratio	AD	M	PD	PDi	EV	PV	LS	EO	PS
1	12.12± 0.92	0.23± 0.21	52.69±2.94	15.37± 0.14	11.93± 0.57	1.28±0.10	1.44±0.07	4.03±0.14	0.83±0.13	0.31±0.6	SP	C	P	BR	Circular
2	10.66±1.02	0.9± 0.45	11.84±3.08	27.66± 0.24	23.95±0.63	1.15±0.13	2.68±0.08	9.11±3.60	0.86±0.15	0.52±0.12	SP	C	——	MR	Polygonal
3	18.87±1.09	0.77± 0.13	24.5±2.28	23.11±0.80	18.95±0.37	1.21±0.25	3.37±0.11	6.84±1.26	1.01±0.26	0.35±0.09	SP	C	P	BR	Elliptic
4	16.3±1.23	1.09±0.27	14.95±2.94	19.65±0.08	12.43±0.36	1.58±0.20	4.25±0.01	6.82± 0.35	0.94±0.29	0.45±0.07	P	C	P	BR	Elongate
5	14.63±1.62	1.29±0.03	10.87± 3.10	15.79±0.40	9.85±0.30	1.60±0.39	3.92±0.07	3.86± 0.89	1.01±0.19	0.50±0.09	P	C	P	BR	Polygonal
6	14.41±1.35	0.90±0.09	69.84±5.73	12.2±0.45	10.73±3.10	1.13±0.18	4.4±0.11	7.12± 1.10	1.01±0.11	0.61±0.03	P-S	C	P	BR	Trigonal
7	19.56±1.22	0.49±0.05	39.91±3.41	17.42±2.65	10.79±0.26	1.61±1.07	2.6±0.14	6.27±0.39	1.04±0.09	0.49±0.10	P	C	P	BR	Polygonal
8	14.96±1.15	0.52±0.10	25.38±2.97	19.23±0.21	15.04±0.11	1.27±0.99	3.51±0.1	4.96±1.29	0.92±0.17	0.44±0.15	SP	C	P	BR	Elongate
9	5.76±1.30	0.33±0.05	17.45±1.09	8.06±3.09	5.13±0.34	1.57±0.87	5.34±0.13	1.95±1.45	0.66±0.09	0.56±0.14	P	C	——	MR	Polygonal
10	32.82±0.95	0.38±0.02	92.12±7.89	28.74±4.96	18.84±3.10	1.52±1.19	4.65±0.05	5.66±3.35	0.61±0.07	0.54±0.71	P	C	——	MR	Elongate
11	15.92±0.68	0.61±0.07	27.57±1.18	8.17±5.48	5.71±7.36	1.43±1.94	5.1±0.13	4.9±1.96	0.92±0.16	0.33±0.70	P	C	P	BR	Polygonal
12	15.46±0.76	0.44	42.05±3.98	14.63±4.6	5.78±8.04	2.53±1.20	14.05±0.14	4.6±0.06	0.72±0.30	0.44±0.31	PP	C	——	MR	Elongate
13	14.85±0.64	0.39	38.07±4.05	15.96±3.28	12.63±1.85	1.26±1.85	2.04±0.10	4.35±0.79	0.9±0.27	0.46±0.16	SP	C	P	BR	Elongate
14	14.89±0.39	0.33	45.12±4.10	15.33±1.98	10.91±0.18	1.40±1.57	2.64±0.12	4.7±1.20	0.84±0.14	0.48±0.11	P	C	——	MR	Elongate
15	21.29±0.75	0.73	29.16±2.15	25.97±2.11	15.45±1.39	1.68±1.97	5.12±0.07	7.17±0.77	0.5±0.29	0.75±0.04	P	C	P	BR	Elliptic
16	13.04±1.58	0.88±0.09	14.81±1.16	13.25±0.11	11.54±0.10	1.14±0.90	6.3±0.17	6.99±2.13	0.5±0.09	0.48±0.15	P-S	C	P	BR	Elliptic
17	24.9±2.11	1.7±0.11	14.64±0.98	32.84±0.41	26.6±3.11	1.23±1.26	6.59±0.24	10.31±0.4	0.48±0.20	0.58±0.13	SP	C	E	BR	Elongate
18	22.13±1.62	0.53±0.07	41.75±2.04	21.43±4.21	14.86±0.37	1.44±1.39	11.6±0.02	11.38±0.14	1.00±0.27	0.56±0.18	P	C	P	BR	Elongate
19	19.25±1.47	1.7±1.02	11.32±1.00	25.8±5.70	19.75±0.52	1.30±1.92	4.03±0.28	9.03±0.39	0.57±0.16	0.39±0.09	SP	C	P	BR	Elongate
20	14.86±1.08	0.22±0.08	69.42±4.95	20.52±0.77	15.81±0.28	1.29±1.00	7.2±0.08	4.41±0.62	0.71±0.25	0.48±0.03	SP	C	E	BR	Circular
21	14.79±1.4.	0.4±0.10	36.97±2.98	18.45±1.15	15.93±3.13	1.15±0.98	3.3±0.59	4.33±0.46	0.9±0.12	0.56±0.15	SP	C	E	BR	Elongate
22	13.3±2.02	0.41± 0.21	39.65± 2.79	17.87± 0.14	16.03± 0.57	1.11±0.70	7.31±0.2	3.7±0.13	1.58±0.97	0.69±0.10	P-S	C	E	BR	Polygonal
23	13.19±1.91	1.54±1.36	30.07±3.10	15.38±0.11	12.46±0.35	1.23±0.94	10.41±0.19	9.53±1.16	1.21±0.99	0.5±0.16	SP	C	E	BR	Elongate
24	17.03±1.69	0.21±0.1.25	8.10±0.65	19.18±3.10	14.96±0.21	1.28±1.12	4.17±0.28	4.75±0.99	0.61±0.95	0.63±0.03	SP	C	C	BR	Circular

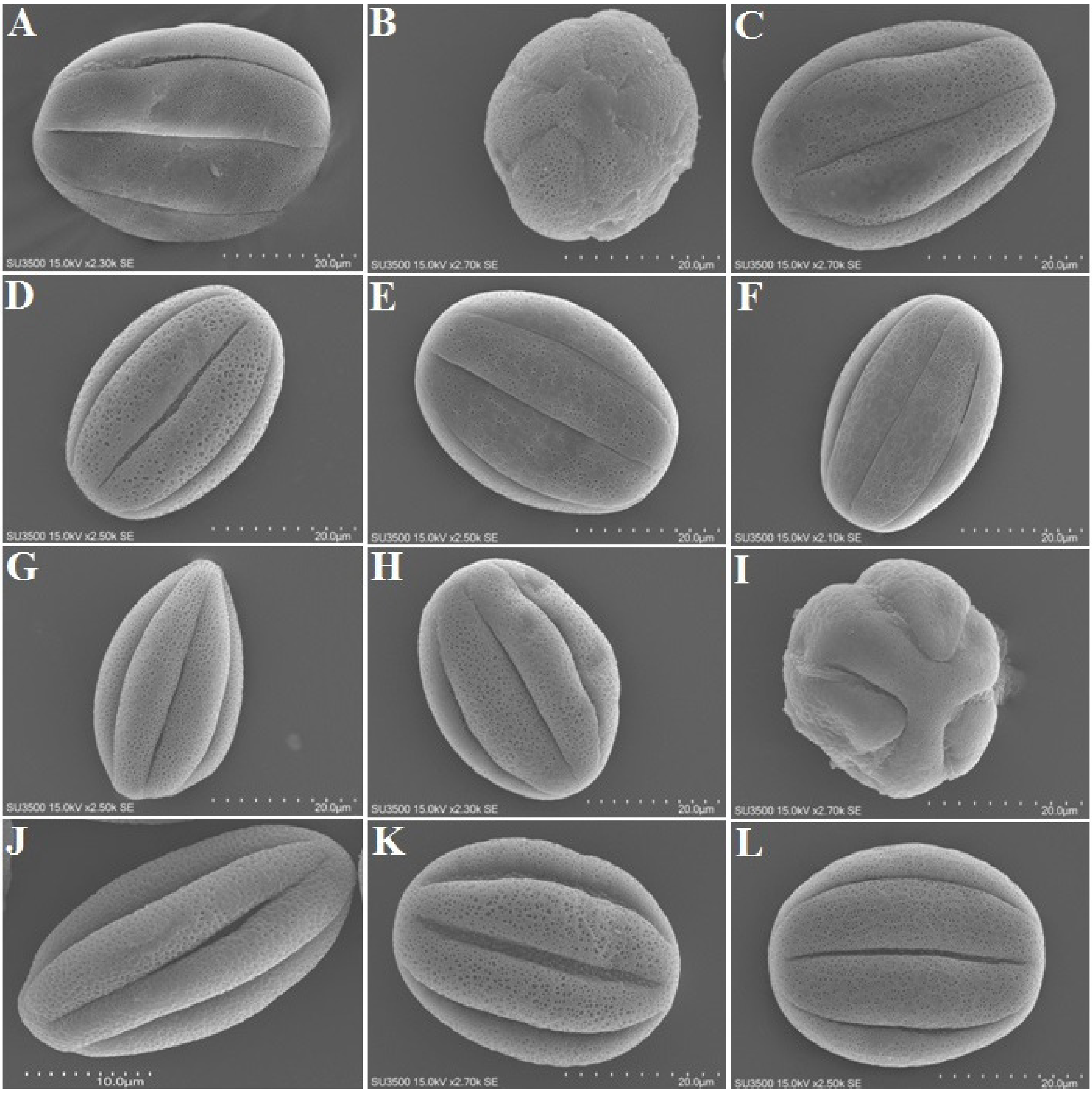

Figure 2. SEM micrographs of pollen grains in *Nepeta* spp.; polar view (B, I); equatorial view (A, C–H, J–L). A) *Nepeta binaludensis*; B) *N. cataria* (Khorasan population); C) *N. cataria* (Mazandaran population); D) *N. crassifolia*; E) *N. fissa* (Polor population); F) *N. fissa* (Albourz population); G) *N. glomerulosa* var. *carmanica* (Gachsaran population); H) *N. glomerulosa* var. *carmanica* (Jiroft population); I) *N. haussknecktii*; J) *N. heliotropifolia*; K) *N. kotschyi* var. *persica* (Nyshabur population); L) *N. lasiocephala.*

We observed two types of pollen exine ornamentation by means of scanning electron microscopy: bireticulate (*N. binaludensis, N. cataria*, Mazandaran population, *N. crassifolia, N. fissa*, both populations, *N. glomerulosa* var. *carmanica*, both populations, *N. kottschyi* var. *persica*, Nyshabur population, *N. lasiocephala, N. meyeri*, both populations, *N. mirzayanii*, *N. pogonosperma*, *N. racemosa*, *N. saccharata*, *N. saturioides*, *N. sessilifolia*, both populations and *N. wettsteinii* (and microreticulate (the rest populations). The pollen grains with bireticulate sculpture had various patterns with secondary tectal connections of round lumina with irregular secondary reticulums and primary lumina consist of different numbers of smaller units.

Bireticulate sculpture was further divided into three main subtypes based on the lumina shape and appearance of primary lumen: bireticulate ornamentation with (i) polygonal lumina in *N. binaludensis*, *N. cataria* (Mazandaran population), *N. crassifolia*, *N. fissa* both populations, *N. glomerulosa* var. *carmanica* both populations, *N. kottschyi* var. *persica* (Nyshabur population), *N. lasiocephala*, *N. meyeri* (both populations(, *N. pogonosperma* and *N. racemosa,*, (ii) elongate lumina in *N. sessilifolia* both populations, *N. saturioides*, *N. saccharata*, *N. mirzayanii*, and (iii) circular lumina in *N. wettsteinii*. Later we divided each of these main subtypes into different sub-types based on the muri shape (Figures 6 and 7).

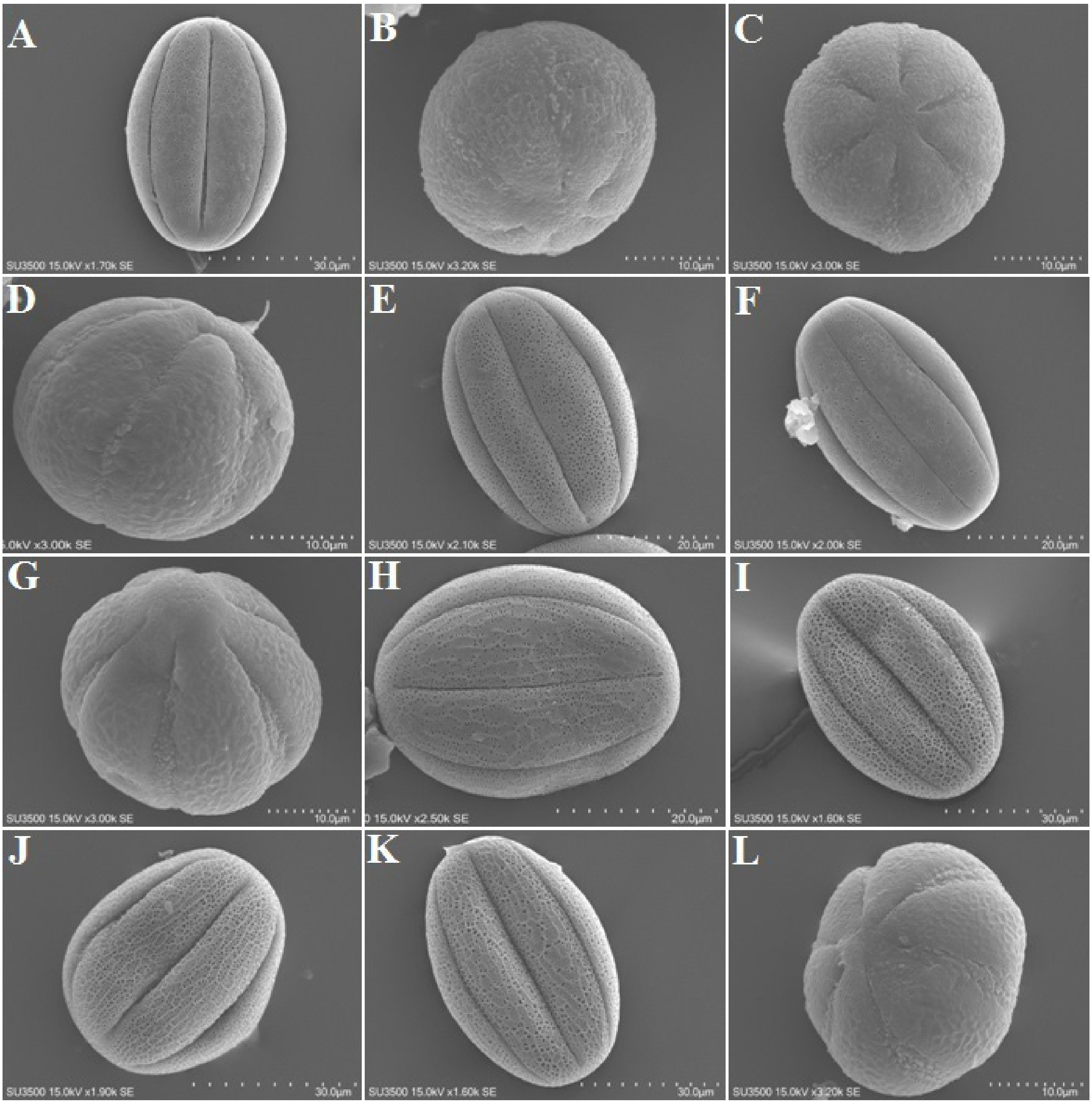

Figure 3. SEM micrographs of pollen grains in *Nepeta* spp.; polar view (B, C, G, L); equatorial view (A, D-F, H-K). A) *N. menthoides,* B) *N. meyeri* (Bostan population), C) *N. meyeri* (Lasem population), D) *N. mirzayanii*, E) *N. kotschyi* var. *persica* (Kashan population), F) *N. pogonosperma*, G) *N. racemosa*, H) *N. saccharata*, I) *N. satureioides* J) *N. sessilifolia* (Alvand population), K) *N. sessilifolia* (Arak population), L) *N. wettsteinii.*

Pollen measurements

Pollen size (P = 8.06-32.84 l µm, E = 5.13-26.60 µm) is highly variable among the studied taxa.

Nepeta mirzayanii has the largest polar axis length (32.84 µm), while *N. haussneckhtii* has the smallest length (8.06 µm). The largest (26.6 µm) and smallest (5.13 µm) values of equatorial axis length and colpus length are recorded in *N. mirzayanii* and *N. haussneckhtii*, respectively. *N. kotschyi* var. *persica* (Kashmar population) has the highest value (2.53) of polar/equatorial length ratio, while *N. sessilifolia* (Arak population) has the lowest value (1.11).

The largest (32.82 µm) and the shortest colpus length (5.76 µm) are registered in *N. heliotropifolia* and *N. haussknechtii*, respectively. The narrowest colpi (0.22 µm) are measured for *N. saccharata,* while *N. mirzayanii* and *N. racemosa* have the broadest colpi (1.70 µm). Besides, *N. kottschyi* var. *persica* (Kashmar population) and *N. glomerulosa* var. *carmanica* (Gachsaran population) have the largest (14.05 µm), and the smallest (2.6 µm) diameter of apocolpium, respectively (Table 2).

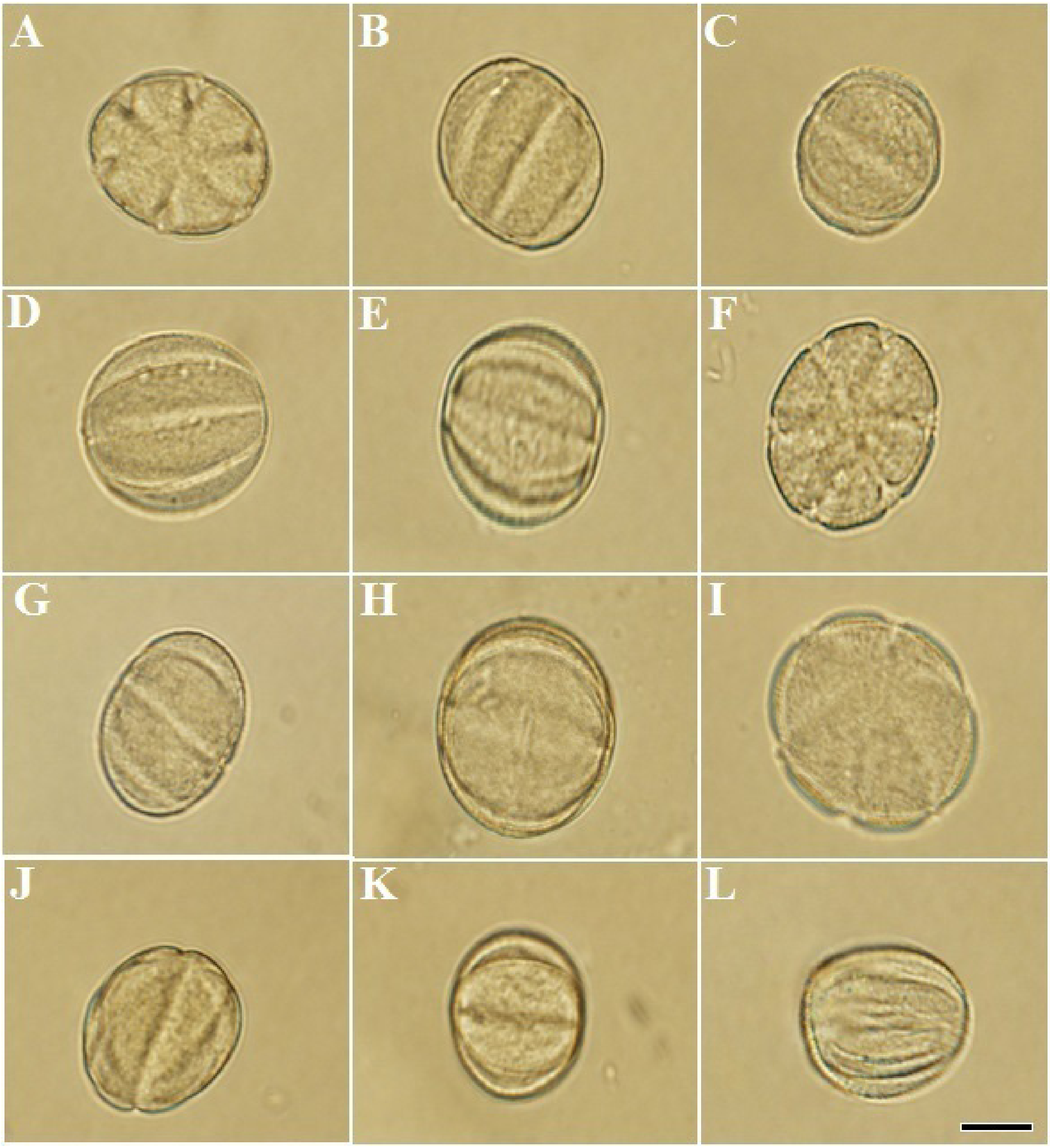

Figure 4. Light microscope images of pollen grains in *Nepeta* spp.; polar view (A, C, G, H, K, L); equatorial view (B, D-F, I, J). A) *N. fissa* (Polor population); B) *N. fissa* (Albourz population); C) *N. meyeri* (Bostan population); D) *N. meyeri* (Lasem population); E) *N. binaludensis*; F) *N. mirzayanii*; G) *N. haussknechtii*; H) *N. cataria* (Khorasan population); I) *N. cataria* (Mazandaran population); J) *N. glomerulosa* var. *carmanica* (Gachsaran population); K) *N. glomerulosa* var. *carmanica* (Jiroft population); L) *N. heliotropifolia.* Scale bar = 10 µm.

Statistical analysis

ANOVA test showed significant differences ($p \leq 0.05$) among all quantitative palynological traits, except colpus width, polar axis length, polar/equatorial length ratio, and mesocolpium (Table 3). Principal component analysis proved that five quantitative variables, namely colpus length, width and length/width ratio, polar axis length, and equatorial axis length, comprise 77.79 % of total variations (Table 4).

Table 3. Results on the ANOVA analysis to assess for variations in palynological features of the studied *Nepeta* taxa. d.f.: degrees of freedom; F: F-statistic; P: probability.

Characteristics		Sum of Squares	df	Mean Square	F	P
Colpus length	Between Groups	1343.327	23	58.406	2.160	0.024
	Within Groups	811.047	30	27.035		
	Total	2154.374	53			
Colpus width	Between Groups	13.555	23	0.589	1.601	0.108
	Within Groups	11.782	32	0.368		
	Total	25.337	55			
Colpus length/ width ratio	Between Groups	26465.907	23	1150.692	2.320	0.018
	Within Groups	13886.720	28	495.954		
	Total	40352.626	51			
Equatorial axis length	Between Groups	1529.657	23	66.507	2.245	0.042
	Within Groups	533.175	18	29.621		
	Total	2062.832	41			
Polar axis length	Between Groups	891.288	23	38.752	0.761	0.733
	Within Groups	865.611	17	50.918		
	Total	1756.899	40			
Polar/equatorial length ratio	Between Groups	1.573	23	0.068	0.410	0.975
	Within Groups	2.668	16	0.167		
	Total	4.240	39			
Apocolpium diameter	Between Groups	350.107	23	15.222	4.939	0.000
	Within Groups	80.129	26	3.082		
	Total	430.236	49			
Mesocolpium	Between Groups	318.093	23	13.830	1.149	0.345
	Within Groups	445.213	37	12.033		
	Total	763.306	60			
Pori diameter	Between Groups	4.863	23	0.211	8.753	0.000
	Within Groups	1.015	42	0.024		
	Total	5.878	65			
Pori distance	Between Groups	0.840	23	0.037	1.965	0.028
	Within Groups	0.780	42	0.019		
	Total	1.620	65			

Table 4. PCA results of quantitative palynological variables. Abbreviations are: IEV, Initial Eigenvalues of Variance (%); CPV, Cumulative percentage of Variance; ESL, Extraction Sums of Squared Loadings: T, Total; V, % Variance; C, Cumulative %.

Pollen Feature	Total	IEV	CPV	T	ESL V	C
Colpus length	2.921	24.343	24.343	2.921	24.343	24.343
Colpus width	2.134	17.782	42.125	2.134	17.782	42.125
Colpus length/ width ratio	1.711	14.262	56.387	1.711	14.262	56.387
Polar axis length	1.402	11.686	68.073	1.402	11.686	68.073
Equatorial axis length	1.166	9.719	77.793	1.166	9.719	77.793
Polar/equatorial length ratio	0.933	7.779	85.571			
Apocolpium diameter	0.650	5.418	90.989			
Mesocolpium	0.498	4.148	95.138			
Pori diameter	0.327	2.723	97.861			
Pori distances	0.015	0.127	100.00			

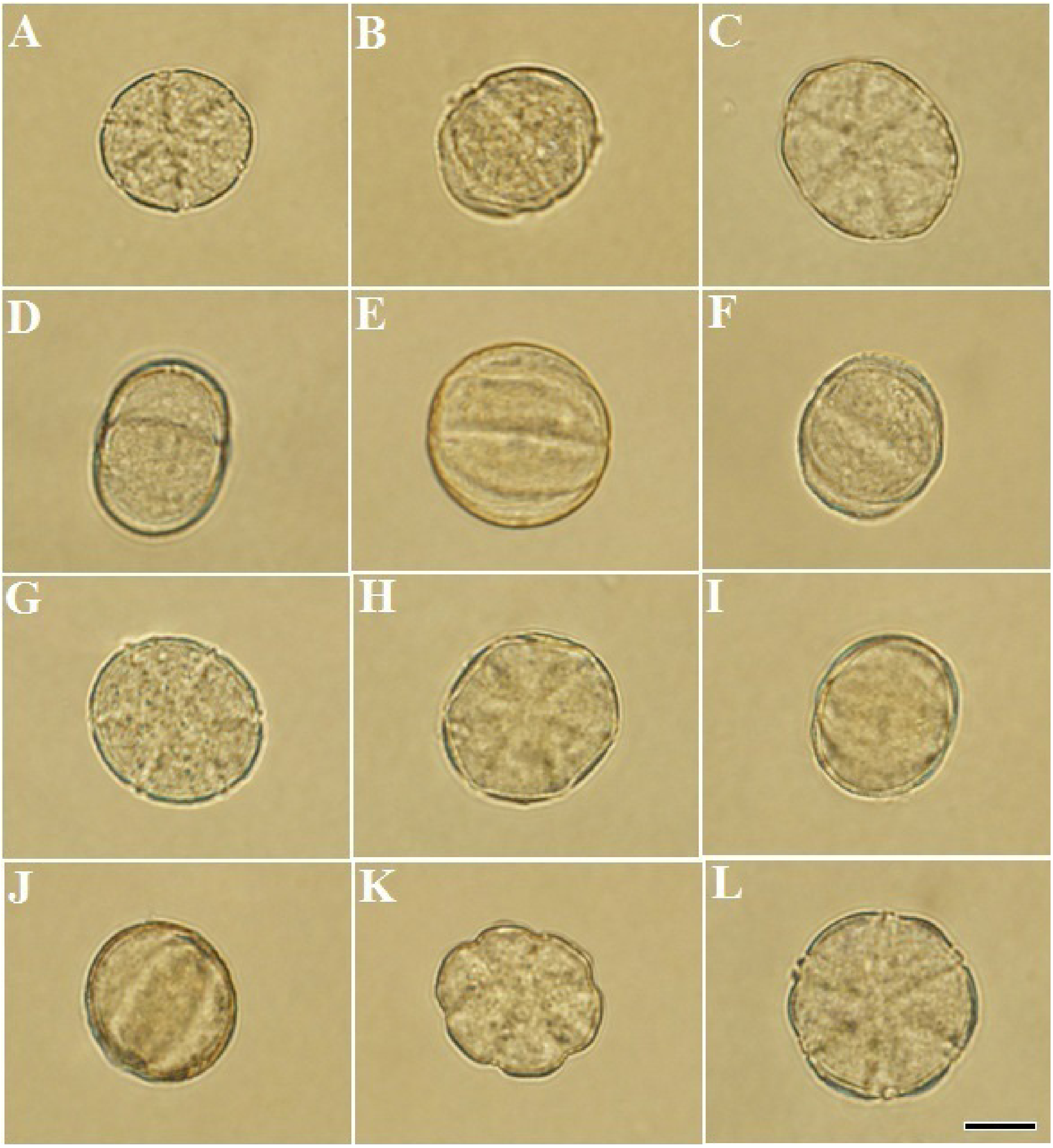

Figure 5. Light microscope images of pollen grains in *Nepeta* spp., polar view (A, F, I); equatorial view (B-E, G, H, J-L). A) *N. kottschyi* var. *persica* (Nyshabur population); B) *N. kottschyi* var. *persica* (Nyshabur population); C) *N. mirzayanii*; D) *N. pogonosperma*; E) *N. racemosa*; F) *N. saccharata*; G) *N. satureioides*; H); *N. sessilifolia* (Alvand population); I) *N. sessilifolia* (Arak population); J) *N. lasiocephala*; K) *N. menthoides*; L) *N. crassifolia.* Scale bar = 10 µm.

We registered significant positive/negative correlations between some quantitative palynological characteristics. For example, the colpus length had significant positive correlations ($p \leq 0.01$) with equatorial ($r = 0.54$) and polar ($r = 0.65$) axes length, but it had significant negative correlation with the pori diameter ($p \leq 0.05$, $r = -0.33$). We also revealed positive correlation ($p < 0.01$, $r = 0.45$) between the colpus width with mesocolpium. Polar axis length had positive correlation ($p < 0.01$, $r = 0.76$) with equatorial axis length, we also recorded negative correlation ($p < 0.05$, $r = -0.32$) between the pori diameter and equatorial axis length.

We separated the studied taxa and their populations by PCOA plot of palynological data. The populations of the same species placed considerably far from each other, moreover, the species of the same group did not cluster closely, while the species of different groups were clustered together. We revealed (Figure 8) that the species plotting did not coincide with the sectional classifications proposed by Jamzad *et al.* (2013).

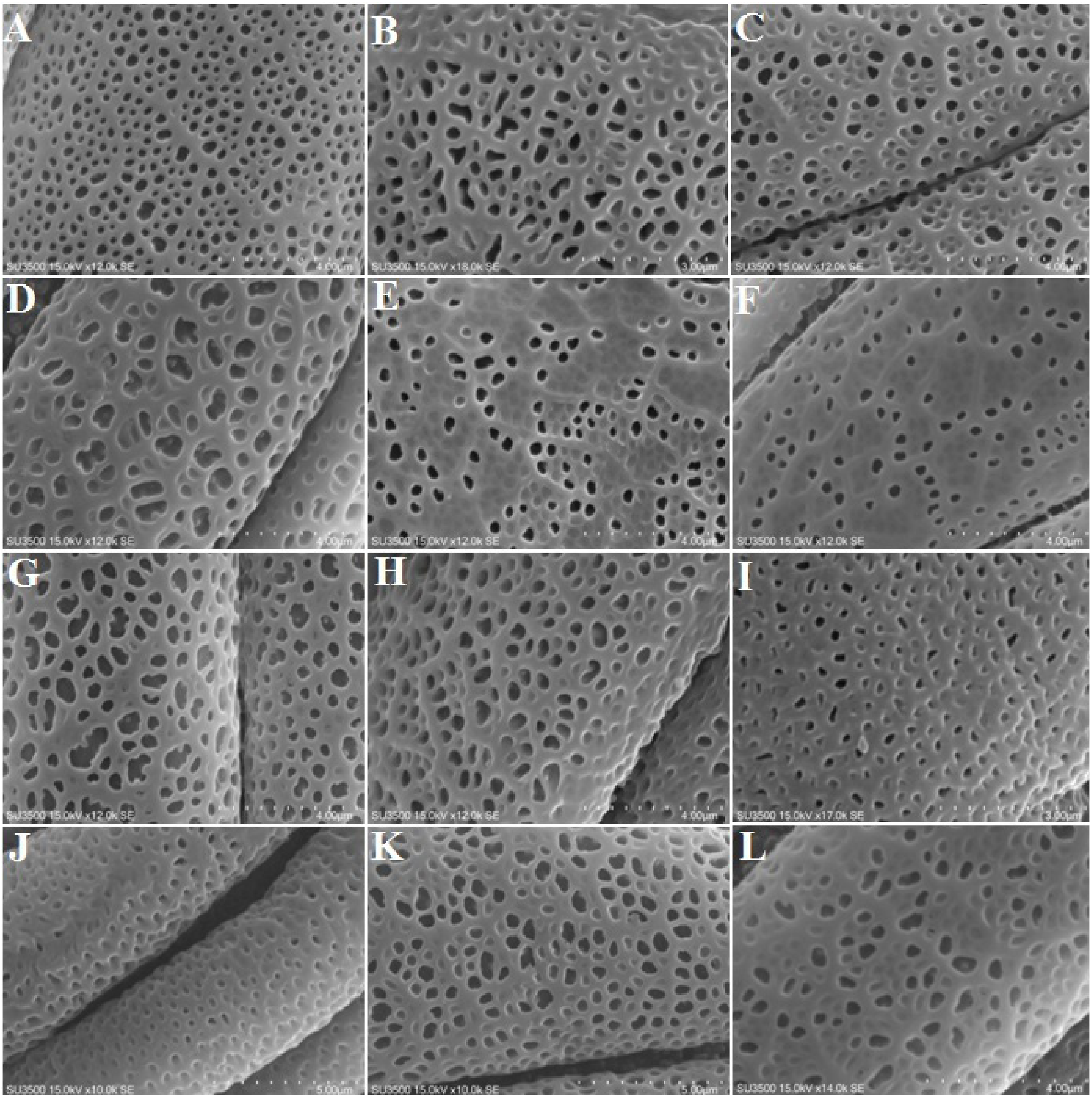

Figure 6. SEM micrographs of sexine ornamentation in *Nepeta*. A) *N. binaludensis*; B) *N. cataria* (Khorasan population); C) *N. cataria* (Mazandaran population); D) *N. crassifolia*; E) *N. fissa* (Polor population); F) *N. fissa* (Albourz population); G) *N. glomerulosa* var. *carmanica* (Gachsaran population); H) *N. glomerulosa* var. *carmanica* (Jiroft population); I) *N. haussknechtii*; J) *N. heliotropifolia*; K) *N. kotschyi* var. *persica* (Nyshabur population); L) *N. lasiocephala.*

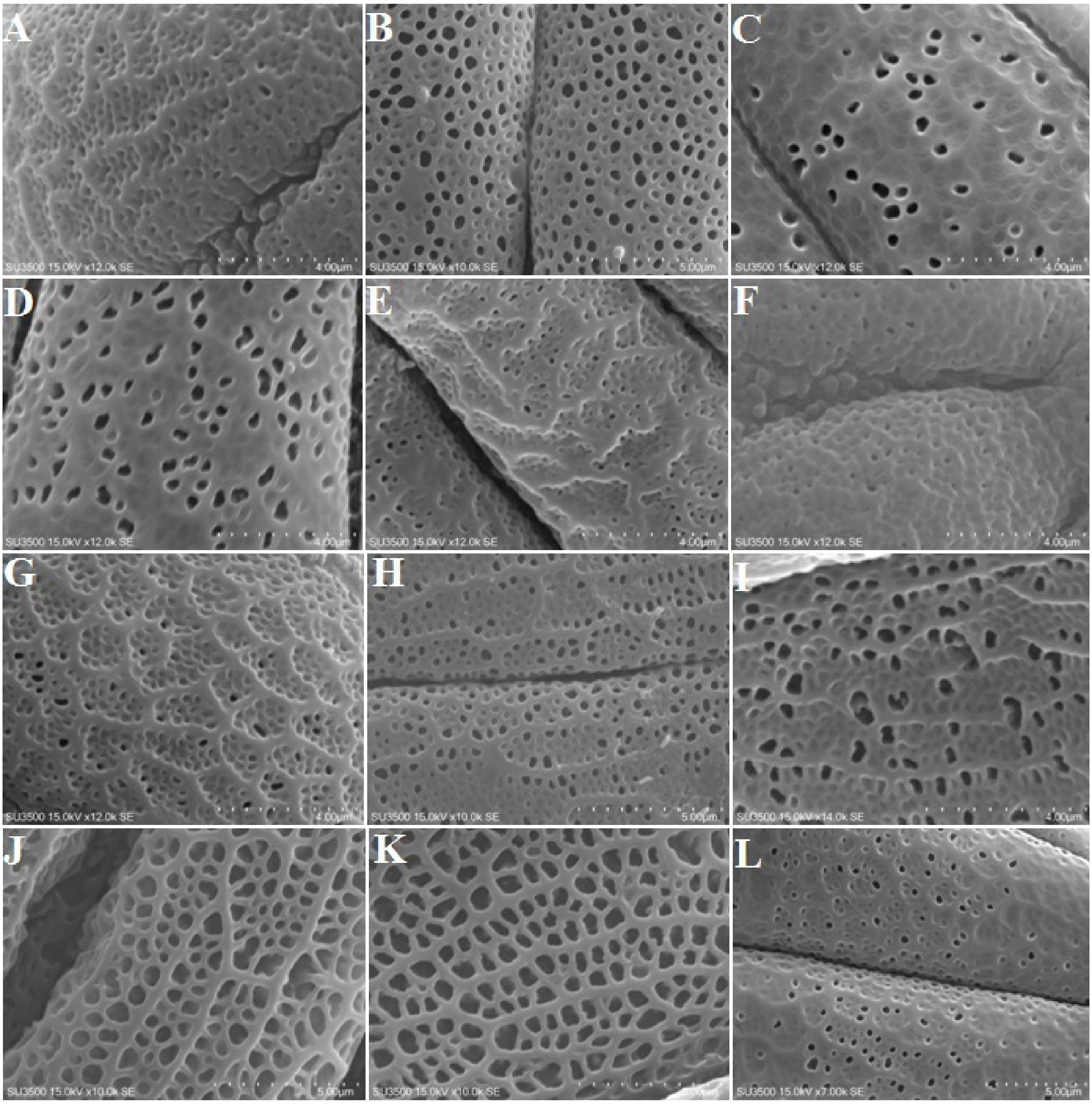

Figure 7. SEM micrographs of sexine ornamentation in *Nepeta*. A) *N. menthoides*; B) *N. meyeri* (Bostan population); C) *N. meyeri* (Lasem population); D) *N. mirzayanii*; E) *N. kotschyi* var. *persica* (Kashmar population); F) *N. pogonosperma*; G) *N. racemosa*, H) *N. saccharata*; I) *N. satureioides*; J) *N. sessilifolia* (Alvand population); K) *N. sessilifolia* (Arak population); L) *N. wettsteinii.*

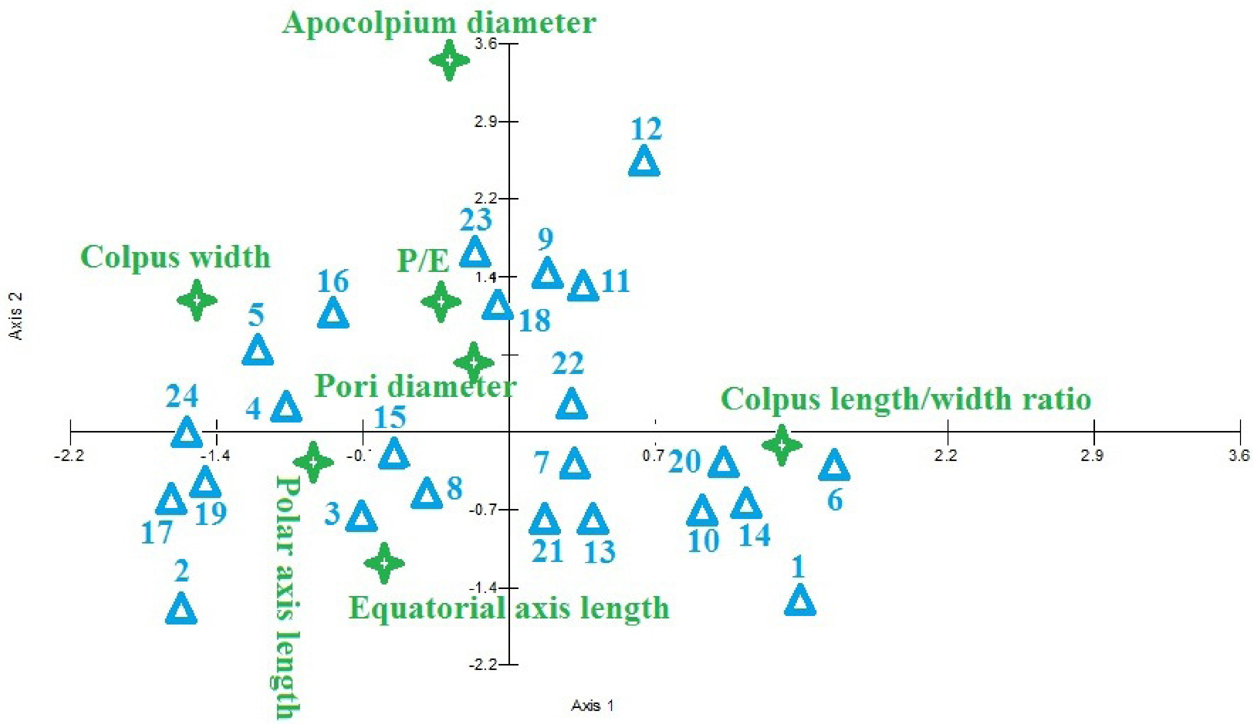

Figure 8. PCOA diagram of the *Nepeta* spp. and their populations. Numbers refer to those of Table 1.

CA-joined plot showed that some of the studied taxa and their populations were characterized by special trait(s), which could be useful in species identification. For example, the polar/equatorial (P/E) ratio was a reliable trait for the identification of *N. glomerosa* (Jiroft population). Polar and equatorial axe lengths should be considered as prompt diagnostic features for *N. cataria* (Khorasan population) and *N. cataria* (Mazandaran population), respectively. *N. fissa* (Albourz population) and *N. meyeri* (Lasem population) had different colpus width, whereas the apocolpium diameter was a specific characteristic for *N. sessilifolia* (Alvand population). *N. fissa* (Albourz population) was separated from other species by mesocolpium width value and *N. crassifolia* was identified by lumina specific shape (Figure 9).

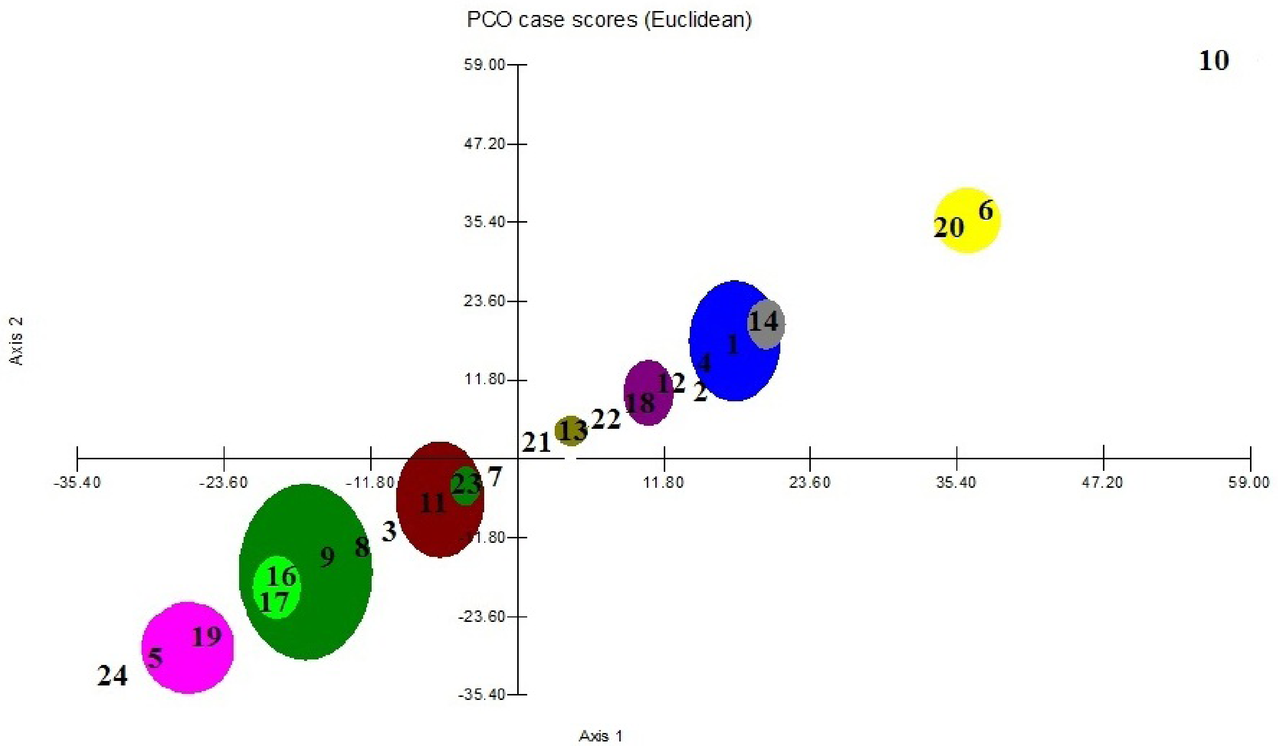

Figure 9. CA-joint plot of the studied taxa with their pollen morphological variables (see Table 1 for taxa numbers).

Discussion

Nepeta belongs to the subfamily Nepetoideae with hexacolpate pollen grains (Harley *et al.*, 1992; Abu-Asab & Cantino, 1994; Razavi *et al.*, 2017). We found that all the examined taxa present 6–zonocolpate monad pollen grain, this was also reported by Celenk *et al.* (2008a). Most of the species and their populations had hexacolpate pollen grains, whereas the tetracolpate and abnormal pollen grains have been rarely described before.

Variations in pollen size among the populations of the same species were observed in various Lamiaceae genera such as *Mentha* L. (Gocmen *et al.*, 1997; Celenk *et al.*, 2008b) and *Lycopus* (Moon & Hong, 2003; Trudel & Morton, 1992) and our results confirmed this. The pollen size cannot be used as a diagnostic character for the studied *Nepeta* species because the pollen size greatly varied among the populations (Table 5).

Table 5. Comparison of the results obtained in the present study with the previous investigations. Abbreviations are: P, Polar axis length (μm); E, Equatorial axis length (μm).

Species	P	E	References
N. meyeri	21.01	17.60	Razavi *et al.* (2017)
N. meyeri	32.81	29.09	Celenk *et al.* (2008a)
N. meyeri	36.00	37.00	Azizian *et al.* (2001)
N. meyeri (Azerbaijan population)	25.97	15.45	Current study
N. meyeri (Lasem population)	14.81	11.54	Current study
N. fissa	17.20	14.40	Razavi *et al.* (2017)
N. fissa	29.09	27.15	Celenk *et al.* (2008a)
N. fissa (Albourz population)	9.85	15.79	Current study
N. fissa (Polor population)	10.73	12.2	Current study
N. sessilifolia	39.08	28.91	Azizian *et al.* (2000)
N. sessilifolia (Arak population)	17.87	16.03	Current study
N. sessilifolia (Alvand population)	15.38	12.46	Current study

We observed different shapes of pollen grains derived from P/E ratio among the studied taxa, though the more frequent type was subprolate. *N. kotschyi* var. *persica* (Kashmar population) was the only species with the perprolate pollen grains. It broadly agrees with the results of early studies of *N. cataria*, *N. fissa, N. heliotropifolia*, *N. meyeri*, *N. racemosa*, *N. crassifolia*, *N. hausskneckhtii*, and *N. sessilifolia* (Azizian *et al*., 2001; Celenk *et al*., 2008a; Razavi *et al*., 2017).

N. sessilifolia (Arak population) and *N. fissa* (Albourz population) had completely different shape than the reported one in previous research (Jamzad *et al*., 2000). It seems that these variations could be related to the creation of infraspecific taxonomical ranks. Both species have a wide distribution in Iran. Previous studies on various populations of these species (Talebi *et al*., 2018, 2019) have revealed that environmental factors should determine the essential oil compositions and anatomical characteristics, and substantially lead to the infraspecific variations. We believed that these variations cause the creation of new infraspecific taxonomical ranks, which should be not morphologically identified. Nevertheless, the identification of these hide ranks needs an additional comprehensive molecular and cytological studies. Pollen grain shape is a nearly stable trait among the studied species and should be a valid taxonomical characteristic.

Polar views were circular in all the studied taxa of this genus and this was broadly in line with previous palynological studies (Celenk *et al*., 2008a, Razavi *et al*., 2017).

We registered microreticulate and bi-reticulate exine ornamentations among the studied taxa; moreover, the more frequent type was the bi-reticulate that was divided into several subtypes. Celenk *et al.* (2008a) found that the exine ornamentation of the majority *Nepeta* taxa examined was the bi-reticulate with different primary muri and secondary reticulum. This type can also be divided into different subtypes. Various studies (e.g., Wagstaff, 1992; Abu-Asab & Cantino, 1994; Jamzad *et al*., 2000; Celenk *et al*., 2008a; Moon *et al*., 2008) have confirmed that the presence of microreticulate or bireticulate sculpturing is a common feature in several *Nepeta* species like *N. aristate*, *N. baytopii* Hedge & Lemond, *N. betonicifolia* C.A. Meyer, *N. cadmea* Boiss., *N. caesarea* Boiss., *N. cilicia* Boiss., *N. concolor* Boiss., *N. conferta* Hedge & Lamond, *N. congesta* Fisch. & Mey., *N. crinita* Montbert & Aucher, *N. eremophila* Hausskn. & Bornm., *N. elymaitica* Bornm., *N. flavida* Hub.-Mor., *N. humulis* Benth., *N. latifolia* DC., *N. micrantha* Bunge, *N. pungens* (Bunge) Benth., *N. sibirica* L., *N. sorgerae* Hedge & Lamond, and *N. speciosa* Boiss.

Wagstaff (1992) reported that the pollen grains with a tectate-perforate sculpturing to reticulate exine structure and a surface ornamented with a network of supratectal ridges surrounding polygonal lumina is hypothesized to be a plesiomorphic condition in the different genera of subfamily Nepetoideae, such as *Nepeta*, *Perillula* Maxim., *Perilla* L., *Hyssopus* L., *Elsholzia* Willd., and *Monardella* Benth.

We compared the exine ornamentations between the populations of the same species and compared them with results of previous palynological studies. We observed infraspecific exine ornamentation variations for populations of *N. kotschyi* var. *persica* and *N. cataria*; moreover, the variations in exine ornamentation were found in different palynological study on the same species. For example, Celenk *et al*. (2008a) recognized the bireticulate exine in *N. fissa*, but Razavi *et al.* (2017) found that these species has microreticulate ornamentation.

The polygonal lumina shape was present in all most studied taxa. We registered such variations in lumina shape within the genus and populations. For example, two different shapes of lumina were registered in *N. meyeri* and *N. fissa* (Celenk *et al*., 2008a; Razavi *et al*., 2017). We observed some infraspecific variations in lumina shapes between populations of the same species; therefore it seems to be a semi-plastic character.

Therefore, in the current study, pollen grain morphology was investigated to examine the value of pollen features in the infrageneric taxonomy. We compared our results with sectional classification patterns proposed by Jamzad (2012) and revealed that group/section species do not occur together towards the species of different sections.

Nepeta pollen data are good taxonomical tool (Celenk *et al*., 2008a) and there is no similarity between the pollen types/subtypes and different sectional classification according to Boissier (1879), Pojarkova (1954), Rechinger (1982), Budantsev (1997), Hedge & Lamond (1982), and Dirmenci (2003).

Conclusion

The pollen morphological features of *Nepeta kotschyi* var. *persica*, *N. lasiocephala, N. menthoides*, *N. mirzayanii*, *N. pogonosperma*, *N. saccharata*, *N. satureioides,* and *N. wettsteinii* were described for the first time. The size of the pollen grains of the studied taxa varied greatly among the different species. The length of the polar and equatorial axes varied significantly between the populations of the same species. Nevertheless, the equatorial pollen view is a specific feature, while the polar view was similar in all species and cannot be used as a diagnostic trait.

We have found two types of exine sculpture in the *Nepeta* species and determined significant infraspecific variability. The analysis of variance (ANOVA) indicated that there was a significant difference in some quantitative characteristics, namely apocolpium diameter and pori diameter. Some of the studied taxa were characterized by special variable(s) such as P/E ratio by CA-joined plot. Finally, we conclude that the taxa arrangement in PCOA plot does not correspond with previously proposed sectional classification.

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