

ORIGINAL ARTICLE

Infra-generic morphological variations in some *Nepeta* L. taxa of Iran

Seyed Mehdi Talebi^{*1}, Majid Ghorbani Nahooji², Mahbobeh Yarmoohammadi¹

¹Department of Biology, Faculty of Sciences, Arak University
Arak, 38156-8-8349 Iran. Phone: 098-863-4173317. E-mail: seyedmehdi_talebi@yahoo.com

²Department of Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR,
Karaj, Iran.

Nepeta L. is one of the most important genera of the *Lamiaceae* family, which widely distributed all over the world. The members of this genus are use of traditional and modern medicine. *Nepeta* species growth naturally in various parts of Iran and recent years the number of its species is increasing. In the study, morphological characteristics of twenty four populations of seventeen *Nepeta* taxa were used for infra-generic classifications, because there were many discussions about infra-generic classification of this genus. For this, forty qualitative as well as quantitative morphological features of both vegetative and reproductive organs were examined. Data were analysed with MVSP and SPSS softwares. ANOVA test showed significant variations for all the studied quantitative traits, with the exception of flower number per cycle. Furthermore, most of qualitative characters such as basal and floral leaf shape and petal color differed between taxa. PCA-biplot and CA joint plot showed that some morphological characters had taxonomic value and were useful in identifications of species. In many cases, clustering of species in the UPGMA tree, PCA and PCO plots did not confirm species placements in groups/ sections according to modern classifications. Furthermore, populations of same species did not cluster closely. It seems that ecological conditions had strong effect on different features of this study and high infra-specific variations were found among the studied species.

Key words: *Nepeta*; morphology; taxonomy; infra-generic; sections

Abbreviations: ANOVA: analyses of variance test, CA: correspondence analyses, PCA: Principal Component Analysis, PCO: Principal Coordinates Analysis, UPGMA: Unweighted Pair Group Method.

Introduction

Lamiaceae is one of the most important plant families, because its members have ethno-medicine and medicinal values (Venkateshappa & Sreenath, 2013). Furthermore, different species of this family are highly aromatic and produce volatile oils. Various studies showed that the essential oil of the mentioned family has multiply usages in industries such as: pesticide, flavoring, pharmaceutical, fragrance, perfumery, and cosmetic (Venkateshappa & Sreenath, 2013).

The family has an almost cosmopolitan distribution with about 220 genera and almost 4000 species worldwide. Some genera such as *Nepeta* L., *Phlomis* L., *Eremostachys* Bunge, *Salvia* L. and *Lagochilus* Bunge have a great diversity in the Mediterranean as well as central and south west of Asia (Naghibi et al., 2005).

The common Persian name of the genus *Nepeta* is Pune-sa (Formisano et al., 2011). Jamzad et al., (2003) stated that the genus *Nepeta* has nearly 300 species, which widely distributed in different parts of Eurasia. The genus is one of the biggest genera of sub-family Nepetoideae in southwestern Asia. The former studies (Pojarkova, 1954; Jamzad, 2012; Mozaffarian, 2013) proved that Iran is considered as one of the main origins of the mentioned genus with several species. Most of these species (nearly 77%) are endemics.

The members of the genus *Nepeta* are used in folk as well as modern medicine. For example, recent studies (Bisht et al., 2010; Khanavi et al., 2012) reported that many species of the genus can be used as antispasmodic, diuretic, febrifuge, diaphoretic and for tooth trouble, kidney and also liver disease. Moreover, species of *Nepeta* have different properties as analgesic, anticancer, antiseptic, antilazheimeran, antitussive, carminative, digestive, antispasmodic, laxative as well as sedative (Nazemiyeh et al., 2009; Kumar et al., 2014).

The members of this genus are: annual or perennial herbs, generally aromatic, sporadically gynodioecious or gynomonoecious. Verticillasters in spikes or opposite cymes in panicles or racemes; floral leaves are bract-like; bracts narrow, shorter / longer than flowers. Calyx has 13-15 veins, tubular, slightly curved or straight, throat oblique or regular; teeth 5, equal or unequal, subulate or narrowly lanceolate to oblong-triangular, apex acuminate to spiny-acuminate. Corolla has 2 lips; tube basally

narrow, \pm abruptly dilated into an ample throat; upper lip \pm flat or concave, which has 2 lobes or emarginate; lower lip large and has 3 lobes, with the middle lobe larger, concave or \pm flat, margin undulate or dentate; lateral lobes small, ovate to semicircular. The stamens are 4, nearly parallel, glabrous, ascending under upper lip of the corolla, posterior 2 longer than anterior, included or exerted, fertile; stamens of pistillate flowers rudimentary, included; anther cells 2, ellipsoid, divaricate, apex not confluent. Style exerted, apex subequally 2-cleft (Turner, 1972).

Previous studies have confirmed that the infra-generic taxonomy of the genus has been problematic, so that various investigators had different, incongruent classifications. The last infra-generic classification of *Nepeta* is belonging to Jamzad et al., (2003). This classification is based on the molecular techniques and presented a new classification that could be connected with the architecture of the flower of the taxa. Phylogeny analysis proved that the genus is monophyletic and have five main monophyletic groups; most of them contain taxa were owned to more than one section in former classifications.

Although, Jamzad et al., (2003) conducted a comprehensive study of the genus and proposed a new classification system for it. As far as we could search, we cannot find any morphological study for comparison the recent classification system of the genus (Jamzad et al., 2003) with traditional ones (Rechinger, 1982). Therefore, in the present investigation, morphological characteristics of twenty four populations of seventeen *Nepeta* taxa were used for infra-generic classification of this genus and comparison of it with recent and also previous classifications. In addition, infra-specific variations in morphological traits were studied in some species and its role was examined in infra-generic variations.

Material and method

Ten qualitative and thirty quantitative morphological characteristics of twenty four populations of *Nepeta* were studied. These populations were related to sixteen species and one variety of the genus (table 1). These taxa connected to four taxonomical groups of the genus (according to Jamzad, 2012). These species were elected from natural populations of this genus during spring 2016 and identified based on the descriptions provided in Flora Iranica (Rechinger, 1982) and Flora of Iran (Jamzad, 2012). On the basis of distribution, one, two or three populations were selected for each species. In total, eighty individuals of twenty four populations selected randomly (3-4 individuals per population). The mean and also standard deviation was determined for quantitative characteristics (table 2).

In addition, these traits were subjected to Principal Component Analysis (PCA) in order to reduce dimensionality of multivariate data, while preserving most of the variance. Cluster analysis was performed for the average of each morphological trait of each population based on the Euclidean distances using the UPGMA (Unweighted Pair Group Method) algorithm. Visualization of PCA, PCO (Principal Coordinates Analysis) and CA (correspondence analyses) data was performed using MVSP software ver.2. Analysis of variance (ANOVA) was performed using SPSS software ver.16.

Table 1. Localities address and coding of studied *Nepeta* species (taxon classification are according to Jamzad 2012)

coding	Group	Taxa	Habitat address
1	VI	<i>N. sessilifolia</i> Bunge	Markazi province, Arak, Sefidkhani mountain, 2180 m.
2	VI	<i>N. sessilifolia</i> Bunge	Isfahan province, Golpaygan, Alvand, 2100 m.
3	IV	<i>N. racemosa</i> Lam.	Qazvin province, Alamout, 3000 m
4	II	<i>N. pogonosperma</i> Jamzad and Assadi	Qazvin province, Alamout, 3000 m
5	IV	<i>N. fissa</i> Mey.	Mazandaran province, Haraz road, Polor
6	IV	<i>N. saccharata</i> Bunge	Tehran province, Fasham, Zaygan, 2700 m.
7	IV	<i>N. haussknechtii</i> Bornm.	Ardabil, Asalem to Khalkhal, 2000 m.
8	IV	<i>N. heliotropifolia</i> Lam.	Markazi province, Ghargh Abad, Sangak, 2237 m.
9	IV	<i>N. heliotropifolia</i> Lam.	Markazi province, Arak, Sefidkhani mountain, 2180 m
10	IV	<i>N. heliotropifolia</i> Lam.	Qazvin province, Alamout, 3000 m
11	IV	<i>N. meyeri</i> Benth.	Mazandaran province, Haraz road, Polor, Lasem 2650m.
12	IV	<i>N. meyeri</i> Benth.	west Azerbaijan, Tabriz, to Zanjan, Bostan Abad 1841 m.
13	IV	<i>N. wettsteinii</i> Heir. Braun	West Azerbaijan, Urmia, Sero, 1680 m.
14	IV	<i>N. kotschyivar. persica</i> Jamzad	Khorasan Province, Nyshabur, 1700 m.
15	V	<i>N. ispahanica</i> Boiss.	West Azerbaijan, 60 km Salmas to Urmia, 1580 m.
16	II	<i>N. bracteata</i> Benth.	Khorasan Province, Nyshabur, 1700 m.
17	IV	<i>N. saccharata</i> Bunge	Zanjan province, 20km Dandi to Zanjan 1513m.
18	V	<i>N. mirzayanii</i> Rech f. & Esfand.	Kerman province, Rabor, Naniz Olyia, 2418 m.
19	IV	<i>N. fissa</i> Mey.	Mazandaran province, Albourz mountain, Protected area, 2250 m.
20	IV	<i>N. cataria</i> L.	Mazandaran province, Siahbisheh, 2300 m.
21	IV	<i>N. wettsteinii</i> Heir. Braun	Mazandaran province, Chalous road, between pole Zangooleh and Chalous, 2229 m.
22	IV	<i>N. crassifolia</i> Boiss. & Buhse.	Semnan province, Semnan to Damghan road, Ahovan, 1300 m.
23	V	<i>N. lasiocephala</i> Benth.	Kerman province, Raein, Hezar mountain 4443 m.
24	II	<i>N. menthoides</i> Boiss. & Buhse.	east Azerbaijan, Sahand mountain, 2500 m.

Table 2. Mean and standard deviations of some important morphological characteristics (all values are in mm).

species		Stem lengt h	stem leaf shape	stem leaf lengt h	stem leaf width	Basal leaf shape	Basal leaf length	Basal leaf width	Floral leaf shape	Flora leaf length	Floral leaf width	Calyx width	Calyx tooth length	Seed length	Seed width
<i>N. sessilifolia</i> (Sefidkhani)	Mean	382		43.00	28.75		18.00	12.50		28.50	13.75	1.00	1.00	2.21	1.22
	N	4	ovate	4	4	ovate	4	4	ovate	4	4	4	4	4	4
	Std. D.	4.92		1.02	6.29		8.08	6.13		4.50	1.50	0.00	0.00	0.00	0.00
<i>N. sessilifolia</i> (Alvand)	Mean	236		29.00	17.00		26.66	15.00		18.33	9.00	1.66	0.50	2.65	1.52
	N	3	ovate	3	3	ovate	3	3	ovate	3	3	3	3	3	3
	Std. D.	3.51		3.60	4.35		2.88	1.00		2.88	3.60	0.57	0.00	0.00	0.00
<i>N. racemosa</i>	Mean	270		13.00	6.00		7.66	4.33		10.00	5.66	1.00	0.50	2	1
	N	3	ovate	3	3	ovate	3	3	ovate	3	3	3	3	3	3
	Std. D.	1.00		2.00	3.46		0.57	.57		4.35	1.15	0.00	0.00	0.05	0.09
<i>N. fissa</i> (Albourz)	Mean	437		8.25	3.75		14.00	9.30		6.00	2.25	1.50	1.00	2.10	1.20
	N	4	ovate	4	4	lanceolate	4	4	lanceolate	4	4	4	4	4	4
	Std. D.	10.3		6.39	2.50		1.00	7.00		2.70	0.95	0.57	0.00	0.08	0.10
<i>N. fissa</i> (Polor)	Mean	663		14.33	9.00		16.00	10.00		4.6	1.66	1.00	1.00	2.00	1.00
	N	3	ovate	3	3	ovate	3	3	ovate	3	3	3	3	3	3
	Std. D.	9.29		1.25	7.93		1.38	8.66		5.03	1.52	0.00	0.00	0.10	0.04
<i>N. saccharata</i> (Fasham)	Mean	200		13.33	9.66		22.00	6.33		13.33	3.66	1.66	.50	1.50	0.80
	N	3	ovate	3	3	ovate	3	3	lanceolate	3	3	3	3	3	3
	Std. D.	3.00		2.88	4.50		2.43	1.52		1.01	3.78	0.57	0.00	0.12	0.11
<i>N. saccharata</i> (Dandi)	Mean	375		17.50	12.00		13.00	8.50		10.50	4.00	1.00	0.50	1.80	1.00
	N	2	lanceolate	2	2	lanceolate	2	2	linear	2	2	2	2	2	2
	Std. D.	2.12		2.12	1.41		1.41	2.12		4.94	0.00	0.00	0.00	0.00	0.05
<i>N. heliotropifolia</i> (Sangak)	Mean	452		21.80	5.60		30.60	6.80		14.40	3.20	1.60	1.00	3	2
	N	5	lanceolate	5	5	Linear-lanceolate	5	5	linear	5	5	5	5	5	5
	Std. D.	3.11		6.01	1.34		3.78	1.30		1.51	.44	.54	0.00	0.00	0.07
<i>N. heliotropifolia</i> (Sefidkhani)	Mean	330		26.60	7.80		20.20	7.20		22.00	6.00	1.00	0.60	2.99	1.98
	N	5	lanceolate	5	5	Linear-lanceolate	5	5	lanceolate	5	5	5	5	5	5
	Std. D.	360		4.82	1.92		6.22	1.78		2.12	1.00	0.00	0.22	0.10	0.11
<i>N. heliotropifolia</i> (Alvand)	Mean	510		25.00	15.00		9.00	7.00		24.00	9.00	4.00	1.00	3	2.10
	N	3	elliptic	3	3	ovate	3	3	elliptic	3	3	3	3	3	3
	Std. D.	3.12		2.51	2.36		1.97	1.02		2.89	2.49	0.63	0.19	0.11	0.09
<i>N. meyeri</i> (Lasem)	Mean	370		13.00	9.00	ovate	13.60	7.80	elliptic	8.40	3.20	1.80	0.70	1.50	0.90
	N	5	ovate	5	5		5	5		5	5	5	5	5	5
	Std. D.	4.52		3.80	2.00		4.82	3.34		2.30	1.09	0.44	0.27	0.02	0.13
<i>N. meyeri</i> (Bostan Abad)	Mean	566		24.33	13.66		14.33	10.00		18.00	11.00	1.66	0.40	1.83	1.12
	N	3	ovate	3	3	ovate	3	3	ovate	3	3	3	3	3	3
	Std. D.	3.51		1.00	3.21		3.05	2.00		3.46	4.58	0.57	0.00	0.04	0.08
<i>N. mirzayanii</i>	Mean	313		12.25	9.75		7.50	5.00		8.00	3.50	1.00	1.00	1.40	0.61
	N	4	ovate	4	4	ovate	4	4	elliptic	4	4	4	4	4	4
	Std. D.	5.58		2.87	2.62		1.73	1.63		1.41	1.73	0.00	0.00	0.06	0.13
<i>N. kotschyivar.</i> <i>persica</i>	Mean	292		20.25	16.25		13.75	12.50		9.25	7.00	1.00	0.50	1.45	1.25
	N	4	ovate	4	4	ovate	4	4	ovate	4	4	4	4	4	4
	Std. D.	4.78		.50	0.50		2.06	3.31		2.50	3.16	0.00	0.00	0.10	0.04
<i>N. isphahanica</i>	Mean	66		10.80	6.20		7.60	4.60		8.20	2.20	1.00	0.50	1.55	0.80
	N	5	ovate	5	5	ovate	5	5	oblong	5	5	5	5	5	5
	Std. D.	2.01		2.04	1.78		1.94	1.67		1.64	0.44	0.00	0.00	0.11	0.07
<i>N. bracteata</i>	Mean	112		9.50	3.50		7.50	3.00		6.00	3.50	1.00	1.00	1.35	0.65
	N	2	lanceolate	2	2	lanceolate	2	2	elliptic	2	2	2	2	2	2
	Std. D.	2.47		6.36	0.70		6.36	2.82		0.00	0.70	0.00	0.00	0.00	0.02
<i>N. wettsteinii</i> (Siahbisheh)	Mean	446		19.20	11.40		11.80	6.40	ovate-lanceolate	10.80	5.80	1.40	0.60	1.80	1.21
	N	5	ovate	5	5	ovate	5	5	lanceolate	5	5	5	5	5	5

	Std. D.	1.30		3.27	2.60		2.94	2.40		4.02	2.77	0.89	0.22	0.12	0.11
<i>N. wettsteinii</i> (Sero)	Mean	547		34.75	14.5		19	10.75		16	7.75	1.25	1	1.78	1.18
	N	3	lanceolate	3	3	lanceolate	3	3	lanceolate	3	3	3	3	3	3
	Std. D.	1.94		4.30	2.20		1.32	1.90		0.79	1.87	0.50	0.44	0.10	0.00
<i>N. haussknechtii</i>	Mean	210		10.80	7.80		5.40	3.60		13.80	10.20	1.80	0.50	1.45	2.50
	N	5	ovate	5	5	ovate	5	5	ovate	5	5	5	5	5	5
	Std. D.	2.23		2.58	2.04		1.51	1.67		1.64	0.44	0.83	0.00	0.00	0.00
<i>N. cataria</i>	Mean	420		44	20		26	15		23	10	2	1	1.75	1.00
	N	3	ovate	3	3	elliptic	3	3	lanceolate	3	3	3	3	3	3
	Std. D.	1.96		3.38	4.32		1.06	2.67		1.96	1.10	0.9	0.00	0.00	0.00
<i>N. crassifolia</i>	Mean	452		16.75	10.00		12.25	7.50		8.25	3.50	1.50	1.00	1.75	1.25
	N	4	elliptic	4	4	elliptic	4	4	elliptic	4	4	4	4	4	4
	Std. D.	1.78		1.69	5.41		9.17	7.04		5.25	3.00	0.57	0.00	0.00	0.00
<i>N. lasiocephala</i>	Mean	950		7.00	5.00		6.00	4.00		7.00	5.00	1.00	1.00	1.40	0.60
	N	3	ovate	3	3	ovate	3	3	ovate	3	3	3	3	3	3
	Std. D.	1.49		4.06	4.58		7.12	1.58		3.69	1.02	0.69	0.00	0.03	0.00
<i>N. menthoides</i>	Mean	433		25.66	5.66		10.33	2.00		20.00	5.00	2.00	1.00	2.25	1.25
	N	3	lanceolate	3	3	lanceolate	3	3	lanceolate	3	3	3	3	3	3
	Std. D.	5.68		6.02	1.15		9.60	2.00		0.00	0.00	0.00	0.00	0.02	0.05
<i>N. pogonosperma</i>	Mean	366		30.00	10.00		21.33	6.66		21.00	6.66	1.33	1.00	2.75	1.1
	N	3	elliptic	3	3	linear	3	3	ovate	3	3	3	3	3	3
	Std. D.	1.52		4.35	4.35		2.30	0.57		5.29	1.52	0.57	0.00	0.10	0.00

Results

In the study, forty qualitative and quantitative morphological features of vegetative as well as reproductive organs were examined for infra-generic classification of this genus.

Not only quantitative morphological traits differed between the studied taxa, but also ANOVA test showed significant variations ($p \leq 0.05$) for all of the studied characteristics, with the exception of flower number per inflorescence cycle (table 3).

It proved that floral number of each inflorescence cycle is at least stable between various populations of the studied species. Largest (28.5mm) and widest (13.75mm) floral leaves were recorded in *N. sessilifolia* (Sefidkhani population), in contrary, smallest values of these characters were reported of *N. fissa* (Polor population). In addition, *N. sessilifolia* (Sefidkhani population) had longest style (20.5mm) and shortest calyx (2.5mm). However, longest (15.6mm) and shortest corolla (4.5mm) were found in *N. menthoides* and *N. sessilifolia* (Sefidkhani population), respectively.

Moreover, most of the studied qualitative traits varied between the studied species. For example, on the base of position, three types of leaves were seen in each flowering stem; basal, stem and floral. The basal leaf shapes of most populations were ovate, while other shapes as lanceolate (*N. wettsteinii*, *N. bracteata*, *N. menthoides*, *N. heliotropifolia* Sefidkhani and Sangak populations, *N. saccharata*) and elliptic (*N. pogonosperma*, *N. crassifolia*, *N. heliotropifolia* Alvand) were found.

These conditions hold true of stem leaf and the most frequent shapes were ovate, lanceolate and linear-lanceolate, respectively. The petal color was another variable trait, while it was stable between different populations of each species.

Significant positive/negative correlations were found between different morphological traits. For example, significant positive correlations ($p < 0.05$) were found between corolla length with anther and style length.

Calyx tooth width had significant positive correlation ($p < 0.05$, $r = 0.35$) with stem length. Stem length has significant positive correlation ($p < 0.05$, $r = 0.34$) with stem leaf length. Moreover, significant negative correlations were seen between style length with basal leaf petiole length ($p < 0.05$, $r = -0.22$) as well as stem leaf petiole length ($p < 0.01$, $r = -0.30$). While, style length has positive significant correlations ($p < 0.01$) with floral leaf width and length, basal leaf width, stem leaf width and length.

Significant negative correlation ($p < 0.05$, $r = -0.26$) were recorded between anther length with stem leaf petiole length. In contrary, significant positive correlations ($p < 0.05$) were occurred between floral leaf width and length, basal leaf width, stem leaf width and length with style length. Petal length /width ratio has negative significant correlations ($p \leq 0.05$) with floral leaf width and length. Significant positive correlations were found between seed length with calyx width ($p < 0.01$, $r = 0.48$) and length ($p < 0.01$, $r = 0.42$). Calyx tooth length had significant positive correlations with seed length ($p < 0.05$, $r = 0.42$) and width ($p < 0.01$, $r = 0.52$). A significant positive correlation ($p < 0.01$, $r = 0.85$) was observed between seed length and width.

Floral leaf length had significant positive correlations with seed length ($p < 0.01$, $r = 0.66$) as well as width ($p < 0.01$, $r = 0.58$).

CA- biplot (fig. 1) and also CA joint plot (fig. 2) showed that some of studied taxa had prominent character(s), which was useful in identification of them. For example, the length of stem was a good trait for identification of *N. wettsteinii* (Siahbisheh population) and *N. meyeri* (Bostan Abad population) from the rest taxa. The pedicle was useful for *N. pogonosperma* and the ratio of leaf / stem length for both *N. fissa* populations and *N. crassifolia*. Moreover, both populations of *N. sessilifolia* and *N. cataria* were distinguished from others by means of basal and stem leaves width.

Table 3. Results on the ANOVA analysis to assess for differences in quantitative qualitative morphological traits of studied *Nepeta* species. d.f.: degrees of freedom; F: F-statistic; P: probability.

Characteristics		Sum of Squares	df	Mean Square	F	P
Flower no. Per cycle	Between Groups	90.309	22	4.105	.820	.689
	Within Groups	285.188	57	5.003		
	Total	375.497	79			
Pedicel length	Between Groups	199.438	22	9.065	11.295	.000
	Within Groups	45.750	57	.803		
	Total	245.188	79			
Floral leaf petiole length	Between Groups	72.393	21	3.447	3.802	.000
	Within Groups	51.683	57	.907		
	Total	124.076	78			
Basal leaf petiole length	Between Groups	1395.883	22	63.449	3.993	.000
	Within Groups	905.667	57	15.889		
	Total	2301.550	79			
Stem leaf petiole length	Between Groups	1052.450	22	47.839	2.272	.007
	Within Groups	1200.300	57	21.058		
	Total	2252.750	79			
Style length	Between Groups	1047.300	22	47.605	14.384	.000
	Within Groups	188.650	57	3.310		
	Total	1235.950	79			
Anther length	Between Groups	1192.504	22	54.205	15.574	.000
	Within Groups	198.383	57	3.480		
	Total	1390.887	79			
Calyx tooth width	Between Groups	4.480	22	.204	16.580	.000
	Within Groups	.700	57	.012		
	Total	5.180	79			
Calyx tooth length	Between Groups	19.521	22	.887	9.252	.000
	Within Groups	5.467	57	.096		
	Total	24.987	79			
Corolla width	Between Groups	31.804	22	1.446	7.786	.000
	Within Groups	10.583	57	.186		
	Total	42.388	79			
Corolla length	Between Groups	763.704	22	34.714	9.667	.000
	Within Groups	204.683	57	3.591		
	Total	968.387	79			
Calyx width	Between Groups	15.654	22	.712	3.042	.000
	Within Groups	13.333	57	.234		
	Total	28.987	79			
Calyx length	Between Groups	155.833	22	7.083	4.990	.000
	Within Groups	80.917	57	1.420		
	Total	236.750	79			
Floral leaf width	Between Groups	798.554	22	36.298	8.645	.000
	Within Groups	239.333	57	4.199		
	Total	1037.887	79			
Floral leaf length	Between Groups	3093.000	22	140.591	9.704	.000
	Within Groups	825.800	57	14.488		
	Total	3918.800	79			
Basal leaf width	Between Groups	941.383	22	42.790	3.406	.000
	Within Groups	716.167	57	12.564		
	Total	1657.550	79			
Basal leaf length	Between Groups	4402.988	22	200.136	3.683	.000
	Within Groups	3097.400	57	54.340		
	Total	7500.388	79			
Stem leaf width	Between Groups	2418.421	22	109.928	8.939	.000
	Within Groups	700.967	57	12.298		
	Total	3119.388	79			
Stem leaf length	Between Groups	6197.454	22	281.702	6.097	.000
	Within Groups	2633.733	57	46.206		
	Total	8831.188	79			
Stem length	Between Groups	1549883.438	22	70449.247	14.313	.000
	Within Groups	280551.250	57	4921.952		
	Total	1830434.688	79			

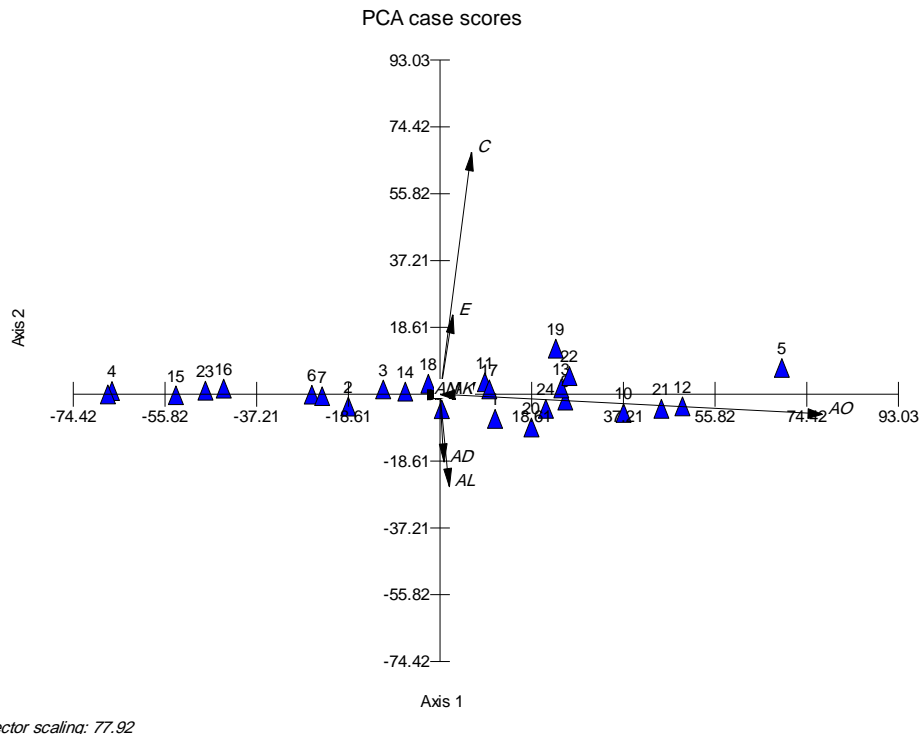


Fig. 1. PCA-biplot of the studied taxa and their morphological traits. Abbreviations; C: ratio of stem length / stem leaf length, E: ratio of stem length / floral leaf length, AO: stem length, AD: floral leaf length, AL: basal leaf shape, K: floral leaf margin shape. Numbers indicate species code 1-24 as in Table 1.

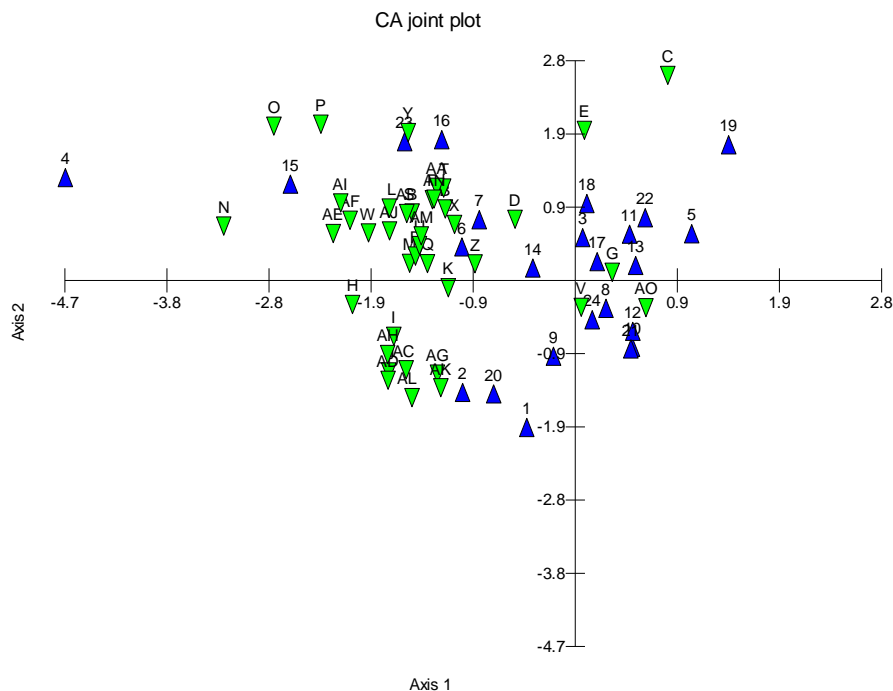


Fig. 2. CA joint plot of morphological characteristics and studied *Nepeta* taxa. Numbers indicate species code 1-24 as in Table 1. Abbreviations: C: ratio of stem length / stem leaf length, E: ratio of stem length / floral leaf length, F; Flower no. per cycle, G; Pedicle length, N; Floral leaf petiole length, O; Basal leaf petiole length, P; Stem leaf petiole length, Q; Style length, R; Anther length, T; Calyx tooth width, U; Calyx tooth length, W; Corolla width, X; Corolla length, Z; Calyx width, AC; Floral leaf width, AD; Floral leaf length, AG; Basal leaf width, AH; Basal leaf length, AK; Stem leaf width, AL; Stem leaf length, AO: Stem length

The studied species were separated from each other and clustered separately in the UPGMA tree (fig. 3), in addition PCO and PCA plots (figs. 4, 5) produced similar results. Therefore, taxa arrangements in the tree were discussed here: it had two big and small branches. In the smaller one, *N. isphahanica*, *N. bracteata*, *N. lasiocephala* and *N. pogonosperma* were found, which the

first three species clustered closely. In the bigger branch, *N. fissa* (Polor population) placed far from others in the separated sub-branch, while in the other sub-branch the rest taxa clustered into two main groups. In smaller group, *N. sessilifolia* (Alvand population), *N. saccharata* (Fasham population) and *N. haussknechtii* grouped closely, but *N. racemosa*, *N. heliotropifolia* (Sefidkhani population), *N. kotschy* var. *persica* and *N. mirzayanii* arranged together. The large group had two sections. In a section, *N. meyeri* (Bostan Abad population), *N. heliotropifolia* (Qazvin population) and *N. wettsteinii* (Siahbisheh population) were together. However, in other two sub-sections were observed. In one sub-section, species were in pairs: *N. sessilifolia* (Sefidkhani population) versus *N. cataria* and *N. saccharata* (Dandi population) versus *N. meyeri* (Lasem population). However, in the last sub-section, *N. fissa* (Albourz population) was aside. The rest species arranged in two pairs: *N. heliotropifolia* (Sangak population) with *N. menthoides* and *N. wettsteinii* (Sero population) with *N. crassifolia*.

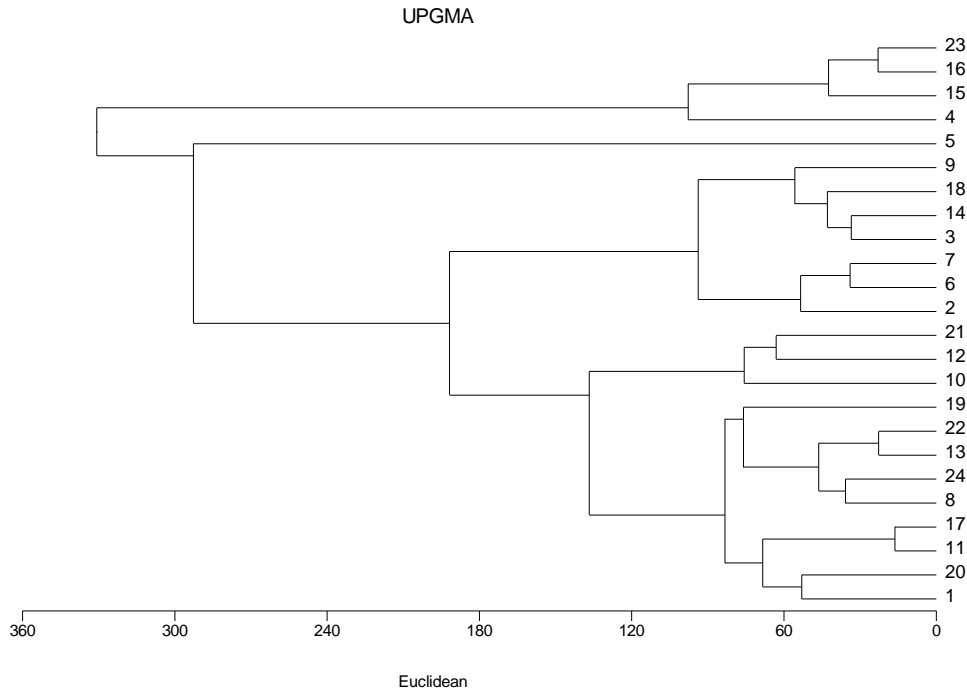


Fig. 3. UPGMA tree of *Nepeta* species on the bases of morphological features. Numbers indicate species code as in Table 1.

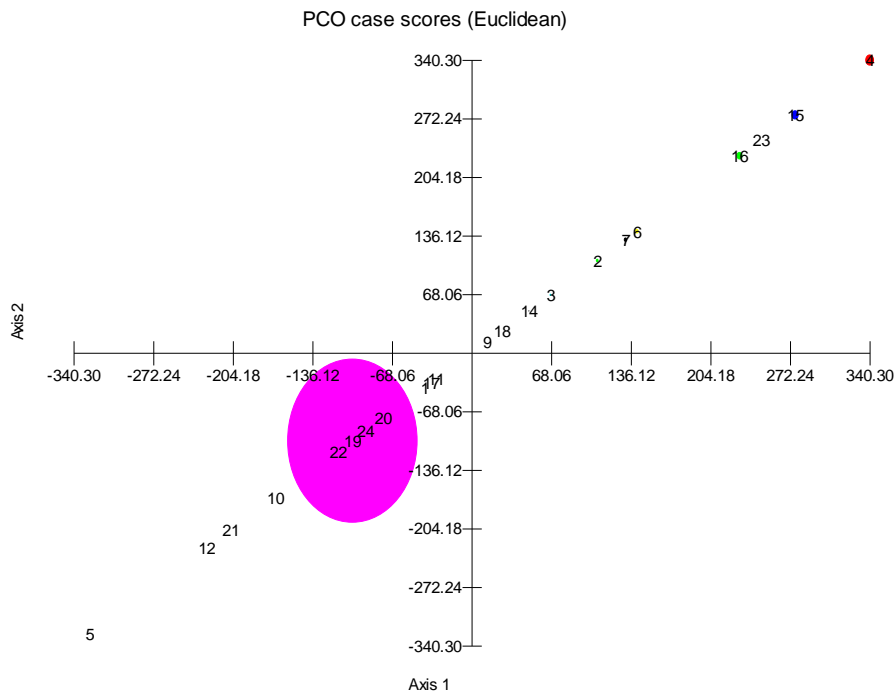


Fig. 4. PCO plot of the studied *Nepeta* taxa and their populations with use of morphological characteristics. Numbers indicate species code 1-24 as in Table 1.

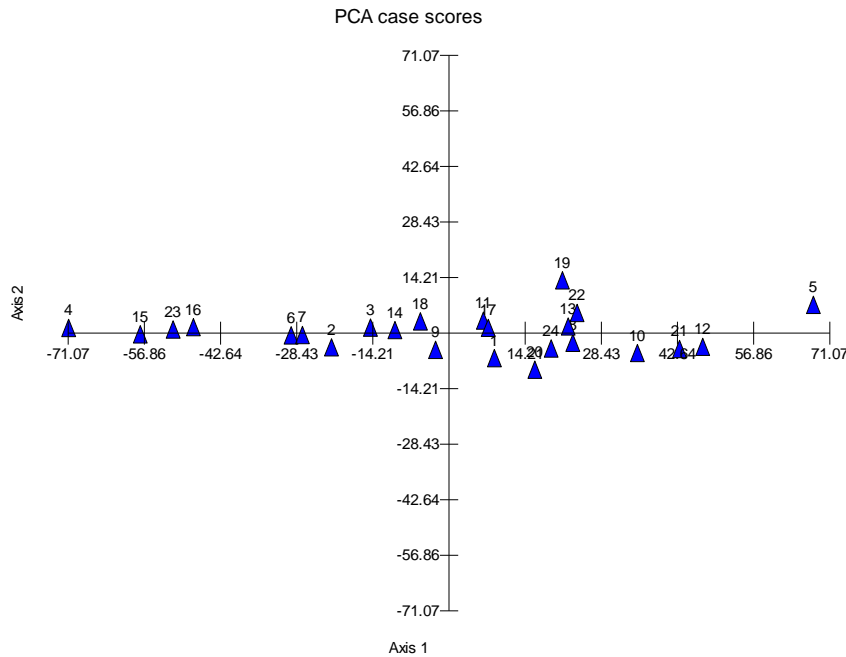


Fig. 5. PCA plot of the studied *Nepeta* species. Numbers indicate species code 1-24 as in Table 1.

Discussion

Nepeta is a particularly complex genus in Lamiaceae family, as yet different infra-generic classifications have been proposed for it, such as Bentham (1848), Briquet (1896), Pojarkova (1954), Budantsev (1993) and in recent years Jamzad et al. (2003). Bentham (1848) classified its species (109) into eight sections and five subsections. However, Briquet (1896) identified 150 species of *Nepeta* and clustered them into two sections and 15 subsections. The classification patterns of Budantsev and Pojarkova were very similar with minor changes. Budantsev (1993) revised the genus and recognized 19 sections and 13 subsections and listed 210 species in this genus.

As seen from above, the main problem was related to classification of species into groups/ sections. In this study, the morphological traits were used for infra-generic classification by using numerical taxonomy. Simpson (1961) has been defined systematics as the scientific study of organism's type and diversity and relationships between them. The existed relationship may be expressed in the phrase of likeness (as in numerical taxonomy) or assumed phylogenetic connection (as in phylogenetic taxonomy or cladistics). Irrespective of how relationship is observed, the basis for its evolution is generally a list of traits and trait state. Traditionally, the mentioned traits have been mainly morphology (Sattler & Rutishauser, 1997), because morphological features play a major role in both phonetic and phylogenetic classifications (Cronquist, 1988; woodland, 1996). High variations were observed among both qualitative and quantitative morphological traits between the studied species and also their populations. These findings were in agreement with former investigations. Pădure (2006) studies showed many variations in different morphological characteristics between *Nepeta* species such as: the color of flowers; corolla length related to calyx size; the middle lobe shape of the lower corolla lip; the shape of calyx, teeth and tube length ratio in calyx; type anastomosis of the veins in calyx; the presence or absence of bract in the central flower of cyme; the bracts and bracteoles shape; the type of inflorescence; bracts and calyx length ratio.

The species clustering in the UPGMA tree and also PCO and PCA plots did not confirm the Jamzad et al., (2003) proposed infra-generic classification in many cases. For example, of three studied species of group V, only *N. ispahanica* and *N. lasiocephala* clustered together, while other species, *N. mirzayanii*, placed far from others. These species in Flora Iranica belonged to three different sections, *Micronepeta*, *Cupituliferae* and *Micranthae*, respectively (Rechinger, 1982). Our obtained results confirmed placement of *N. ispahanica* and *N. lasiocephala* in a group, but did not prove clustering of *N. mirzayanii* in this group.

This condition held true for members of group II. In the study, three species of the group were investigated. Two of them, *N. bracteata* and *N. menthoides*, placed separately. In Flora Iranica (Rechinger, 1982) these species were members of two different sections *Cataria* (*N. menthoides*) and *Micronepeta* (*N. bracteata*).

Most of the studied species were the members of group IV. Our findings confirmed high morphological differences between these species, because they placed separately far from each other. In fact, these species in flora Iranica belonged to four sections. For instance, *N. racemosa*, *N. haussknechtii*, *N. kotschy* var. *persica* and *N. crassifolia* were members of section *Stenostegiae*. It is more important to know that with the exception of *N. crassifolia*, the rest species clustered nearly. However, section *Micranthae* consisted of *N. saccharata*, *N. meyeri*, and *N. wettsteinii* (Rechinger, 1982). In this study, two populations of these species were examined, but in all cases the populations of same species did not cluster closely. This holds true for *N. fissa*, *N. sessilifolia* as well as *N. heliotropifolia*.

N. fissa is the other member of section IV. Two studied populations of it were thrown away. Its Albourz population clustered with some members of this group (*N. heliotropifolia*, *N. wettsteinii* and *N. crassifolia*, but the other one (Polor population) placed away. *N. fissa* belonged to section *Schizocalyx* in flora Iranica. Therefore, if only Polor population of this species is examined,

the taxonomical pattern of Rechinger (1982) will be correct. These proved that high infra-specific morphological variations are presented among the studied species. Hedge and Lamond (1982) believed that frequent hybridization and introgression, associated with substantial age or habitat-linked difference, make *Nepeta* a particularly complex genus. These conditions were reported in different species of Lamiaceae family, such as *Phlomis olivieri* (Talebi, 2014), *Acinos graveolens* (M.B.) Link. (Talebi, 2015) *Stachys inflata* (Talebi et al., 2014a) and also species of other families, for example Linaceae (Afshar et al., 2015). Different reasons were proposed for infra-specific morphological variations, such as creation of ecotype or ecophene. In both states, morphological traits of populations differed under various ecological conditions (Talebi et al., 2014b).

Conclusion

The results of present investigations did not confirm the designed taxonomical pattern of Jamzad et al., (2003) in many cases. There are many possible reasons for these conditions; however, it seems that the main reason is the existence of high infra-specific variations. Jamzad et al., (2003) used only one population of each species and did not pay attention to infra-specific variations, which highly are seen between different populations of each species. Different studies proved that ecological conditions have strong effects on both morphological characteristics and also genetical structures of various populations of each species. These conditions were seen in different species of Lamiaceae and many other families. Therefore, for each taxonomical treatment, some populations of each taxon must be used.

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Infra-generic morphological variations in some *Nepeta* L. taxa of Iran

By: Talebi, SM (Talebi, Seyed Mehdi)^[1]; Nahooji, MG (Nahooji, Majid Ghorbani)^[2]; Yarmooammadi, M (Yarmooammadi, Mahbobeh)^[1]

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Abstract

Nepeta L. is one of the most important genera of the Lamiaceae family, which widely distributed all over the world. The members of this genus are used of traditional and modern medicine. *Nepeta* species grow naturally in various parts of Iran and recent years the number of its species is increasing. In the study, morphological characteristics of twenty four populations of seventeen *Nepeta* taxa were used for infra-generic classifications, because there were many discussions about infra-generic classification of this genus. For this, forty qualitative as well as quantitative morphological features of both vegetative and reproductive organs were examined. Data were analysed with MVSP and SPSS softwares. ANOVA test showed significant variations for all the studied quantitative traits, with the exception of flower number per cycle. Furthermore, most of qualitative characters such as basal and floral leaf shape and petal color differed between taxa. PCA-biplot and CA joint plot showed that some morphological characters had taxonomic value and were useful in identifications of species. In many cases, clustering of species in the UPGMA tree, PCA and PCO plots did not confirm species placements in groups/sections according to modern classifications. Furthermore, populations of same species did not cluster closely. It seems that ecological conditions had strong effect on different features of this study and high intra-specific variations were found among the studied species.

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Author Keywords: *Nepeta*; morphology; taxonomy; infra-generic; sections

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Author Information

Reprint Address: Talebi, SM (reprint author)

Arak Univ, Fac Sci, Dept Biol, Arak 3875688349, Iran.

Addresses:

[1] Arak Univ, Fac Sci, Dept Biol, Arak 3875688349, Iran

+ [2] ACECR, Inst Med Plants, Plants Res Ctr, Dept Med Plants, Karaj, Iran

E-mail Addresses: seyedmehdi_talebi@yahoo.com

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