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BIOSYSTEMATIC STUDY IN SOME *DRACOCEPHALUM* SPECIES (LAMIACEAE) BASED ON MORPHOLOGY AND ANATOMY IN IRAN

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The genus *Dracocephalum* L. (Lamiaceae) with about 60 to 70 species is a genus in the subtribe Nepetinae, tribe Mentheae of Lamiaceae family, native to temperate regions of the Northern Hemisphere. They are mostly perennial herbs, and rarely annual. Flora Iranica reports 8 *Dracocephalum* species and the Flora of Iran reports 10 *Dracocephalum* species in Iran out of which, 4 species are endemic. We collected 7 *Dracocephalum* species and studied species delimitation and species relationship by morphometric and anatomic results. The species were efficiently delimited by morphological and anatomical characters. Morphological and anatomical characters revealed closer affinity between *D. moldavica* and *D. subcapitatum* and *D. thymiflorum* were placed with distance from these species.

Key words: anatomy, *Dracocephalum*, morphology, species delimitation.

INTRODUCTION

Dracocephalum L. also named dragonhead with about 60 to 70 species is a genus in the subtribe Nepetinae, tribe Mentheae of Lamiaceae family, native to temperate regions of the Northern Hemisphere (Budantsev 1987, 1993, Kadereit 2004, Nixon 2006).

These species are mostly perennial herbs, and rarely annual taxa growing in alpine and semidry regions mainly in temperate Asia, with a few species occurring in Europe and only one in N America (Brach and Song 2006). The centre of distribution is presumably the alpine steppes of the Pamir Altai and Tian-Shan (Diklić 1999).

The members of this genus are well known as medicinal plants with several uses, such as antihyperlipidemic, analgesic, antimicrobial, antioxidant, anticancer and oxidative stress protective activity (Sajjadi *et al.* 1998, Sonboli *et al.* 2008, Zeng *et al.* 2010).

According to Budantsev (1987) *Dracocephalum* divided into three subgenera *Dracocephalum*, *Fedtschenkiella* (Kudr.) Schischk and *Ruyschiana* (Mill.) Briq. *Dracocephalum* subgenus with anthers glabrous and stamens included including seven sections.

Flora Iranica (Rechinger 1982) reports 8, the Flora of Iran reports 10 *Dracocephalum* species in Iran (Jamzad 2012), out of which 4 species (*D. ghahremanii* Jamzad, *D. kotschyi* Boiss., *D. polychaetum* Bornm. and *D. surmandinum* Rech. f.) are endemic that grow in north and central parts of the country.

According to morphology, *Dracocephalum* is a heterogeneous genus. They can be prostrate or erect. The stems are square and bear simple leaves arranged oppositely or in whorls. The plants are characterised by tubular two-lipped flowers, lobed at the base and the upper lip, which resemble fanciful heads of dragons. the calyx glabrous within and not-gibbous at base; sinuses between lobes of the calyx with swollen folds at base, bracteoles aristately toothed, nutlets elliptic to oblong and areole not curved.

The application of anatomy and its implication in the systematic of Lamiaceae are well known from various comprehensive works (Bokhari and Hedge 1971, Bosabalidis and Kokkini 1997, Laber 1954, Metcalfe and Chalk 1979, Ryding 1992, 1994, 1995, 2007, Salmaki *et al.* 2008, 2011).

The usefulness of the structure of the root, stem, leaf, and petioles for species identification, taxonomic significance, subgeneric classification and species delimitation in the family Lamiaceae has been demonstrated (Agbagwa and Ndukwu 2004, Celep *et al.* 2014, Eric *et al.* 2007, Kahraman *et al.* 2011, Kharazian 2007, Metcalfe and Chalk 1972, Olowokudejo 1987, Radford *et al.* 1974, Salmaki *et al.* 2008, Shaheen 2007, Simpson 2006, Stace 1984).

Dracocephalum moldavica is characterised by the presence of non-glandular, unbranched trichomes, a number of parenchyma and collenchyma layers of petiole and stomata frequency in adaxial and abaxial surface (Hatamneia *et al*. 2008).

In general, taxonomic and biosystematics studies performed on the genus *Dracocephalum* are very few. Therefore, the main purpose of this research is taxonomy, species delimitation, and species relationship in the genus *Dracocephalum* in Iran by using morphological and anatomical data.

MATERIAL AND METHODS

Plant materials

42 plant specimens were randomly collected of 7 *Dracocephalum* species (*D. kotschyi*, *D. thymiflorum*, *D. multicaule*, *D. aucheri*, *D. moldavica*, *D. subcapitatum,* and *D. lindbergii*) from their natural habitats during 2013–2015.

Morphometry

Morphological characters studied were: life-history strategy, habitat, plant height, shape of stem leaf, length of stem leaf, width of stem leaf, tip of

Table 1
Anatomical characters in *Dracocephalum*

Characters	No
Length of epidermis in stem	1
Length of collenchymas in stem	2
Length of parenchyma in stem	3
Length of sclerenchyma in stem	4
Length of upper phloem in stem	5
Length of xylem in stem	6
Length of lower phloem in stem	7
Length of pith in stem	8
Length of stem in transverse transects	9
Width of stem in transverse transects	10
Length of simple trichomes in stem	11
Length of glandular trichomes in stem	12
Number of layer in collenchymas in stem	13
Number of layer in parenchyma in stem	14
Number of layer in sclerenchyma in stem	15
Number of layer in xylem in stem	16
Length of upper epidermis in leaf	17
Length of lower epidermis in leaf	18
Length of collenchymas in leaf	19
Length of parenchyma in leaf	20
Length of mesophyll in leaf	21
Length of upper phloem in leaf	22
Length of xylem in leaf	23
Length of lower phloem in leaf	24
Length of simple trichomes in leaf	25
Length of glandular trichomes in leaf	26
Number of layer in collenchymas in leaf	27
Number of layer in parenchyma in leaf	28
Number of layer in xylem in leaf	29

steam leaf, stem leaf margin, shape of inflorescence , shape of bracteole, tip of bracteole, length of bracteole, width of bracteole, situation of bract arista, colour of calyx, length of calyx, colour of corolla, length of corolla, kind of middle lobe in corolla, situation of Corolla tube, shape of nutlet, length of nutlet, width of nutlet.

The species differences for morphological characters were investigated by ANOVA (Podani 2000). For multivariate morphological analyses, quantitative characters were divided into discrete groups and along with qualitative characters were coded as multistate characters. Grouping of the species was done by different clustering and ordination methods such as WARD PCA and MDS (Podani 2000). PCA was performed to identify the most variable morphological characters among the species studied (Podani 2000). PAST version 2.17 (Hammer *et al.* 2012) was used for multivariate analyses.

Anatomy

Embedded materials were prepared as follows: adult plant samples were excised and immediately fixed in formalin-acetic acid-alcohol (FAA) (formalin 5%: acetic acid 5% and 50% ethanol 90%) for 48 to 72 h, and stored at 4 °C until sectioning, then dehydrated in a graded ethanol series and embedded in 70% ethanol. After preparation of free transverse hand sections of the lamina and stem samples were washed with distilled water and placed in 5% sodium hypochlorite solution for 20 min for clearing and rinsed with distilled water. The sections were stained with methyl blue and carmine and mounted on the slides using Canada balsam. Thin cut sections were observed under a microscope fitted with a digital camera. Anatomical characters in stem and leaf are in Table 1.

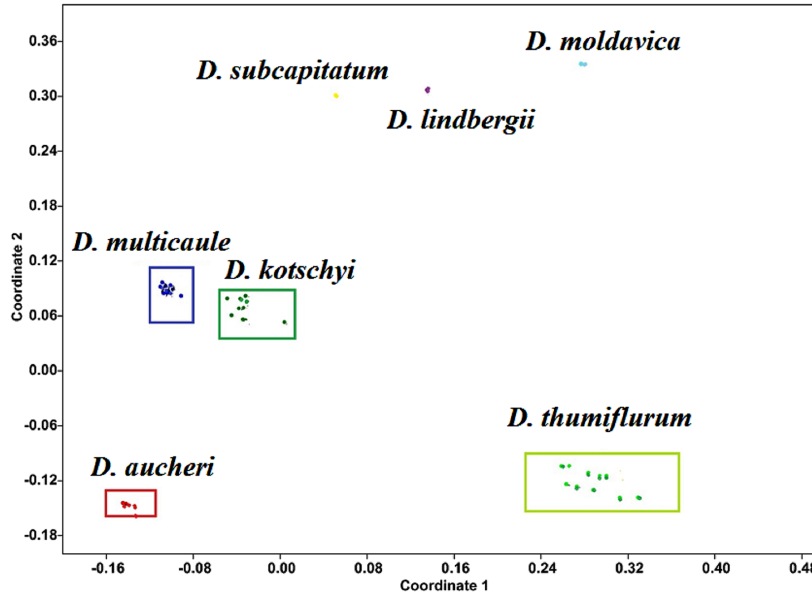

Fig. 1. PCA plot of morphological characters in *Dracocephalum* species

ANOVA was performed to show anatomical difference among the species. Anatomical characters were first standardised (mean = 0, variance = 1) and used to establish Euclidean distance among pairs of taxa (Podani 2000). For the grouping of the plant specimens, PCoA and UPGMA were used (Podani 2000). PAST version 2.17 (Hammer *et al.* 2012) was used for multivariate analyses.

RESULTS AND DISCUSSION

Morphometry

The ANOVA test showed a significant difference ($p < 0.05$) for quantitative morphological characters among *Dracocephalum* species. Different methods like PCA, MDS, and WARD produced similar results, Therefore, only PCA plot is presented here.

PCA analysis revealed that the first 2 components comprised about 70% of total morphological variability. In the first PCA components with about 50% of total variation, characters like habitat, shape of bracteole, shape of stem leaf, colour of corolla, stem leaf margin, and life-history strategy showed the highest positive correlation (> 0.80). The shape of nutlet and colour of calyx showed the highest positive correlation (> 0.80) with the second PCA component. These characters may be used in taxonomy of the genus and delimiting *Dracocephalum* species.

PCA plot of morphological characters (Fig. 1) separated the studied species from each other. In this plot *D. kotschyi* and *D. multicaule* like *D. subcapitatum*, *D. moldavica* and *D. lindbergii* were placed close to each other, while *D. aucheri* and *D. thymiflorum* were placed far from the others.

Anatomy

Representative anatomy of each species is illustrated (Fig. 2).

The ANOVA test showed a significant difference ($p < 0.05$) for quantitative anatomical characters among *Dracocephalum* species (Table 2). The anatomical studies on some of the species in *Dracocephalum* showed that they had the same anatomical characteristics like a number of layer epidermis, shape of transverse transects and ring-shaped in sclerenchyma (Kandemir 2003, Kaya *et al.* 2000, Uysal 2002, 2003). These characteristics are observed in all species as well.

Different methods like PCoA and UPGMA according to anatomical characters produced similar results; therefore, only PCoA plot is presented here. PCoA plot of anatomical characters (Fig. 3) separated the studied species from each other. In this plot *D. kotschyi* and *D. lindbergii* like *D. subcapitatum*, *D. mol-*

davica and *D. aucheri* were placed close to each other, while *D. multicaule* was placed far from the others.

The present study revealed that morphology and anatomy could delimit *Dracocephalum* species and had taxonomic value. The study found closer relationship between *D. moldavica* and *D. subcapitatum*, which supports the stud-

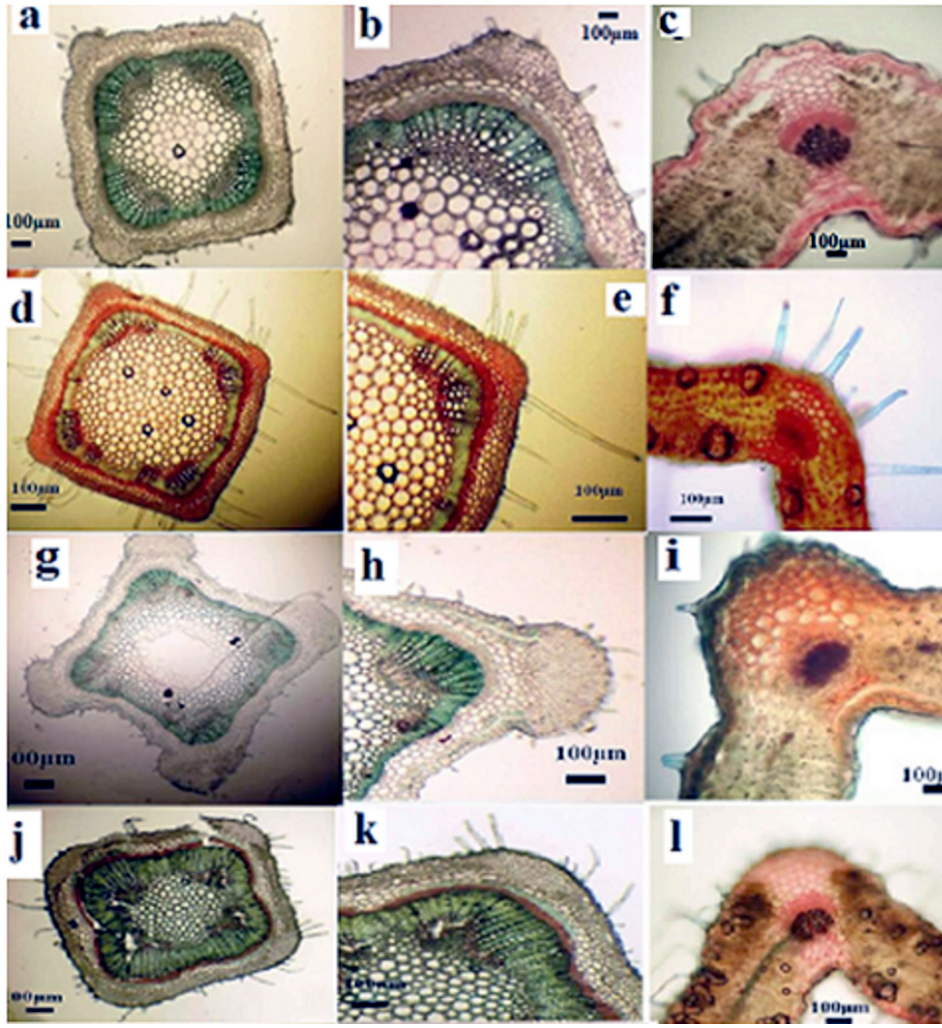

Fig. 2. Appearance of stem (a, b) and leaf (c) in *D. kotschyi*; appearance of stem (d, e) and leaf (f) in *D. moldavica*; appearance of stem (g, h) and leaf (i) in *D. thymiflorum*; appearance of stem (j, k) and leaf (l) in *D. lindbergi*; appearance of stem (m, n) and leaf (o) in *D. subcapitatum*; appearance of stem (p, q) and leaf (r) in *D. aucheri*; appearance of stem (s) and leaf (t) in *D. multicaule*

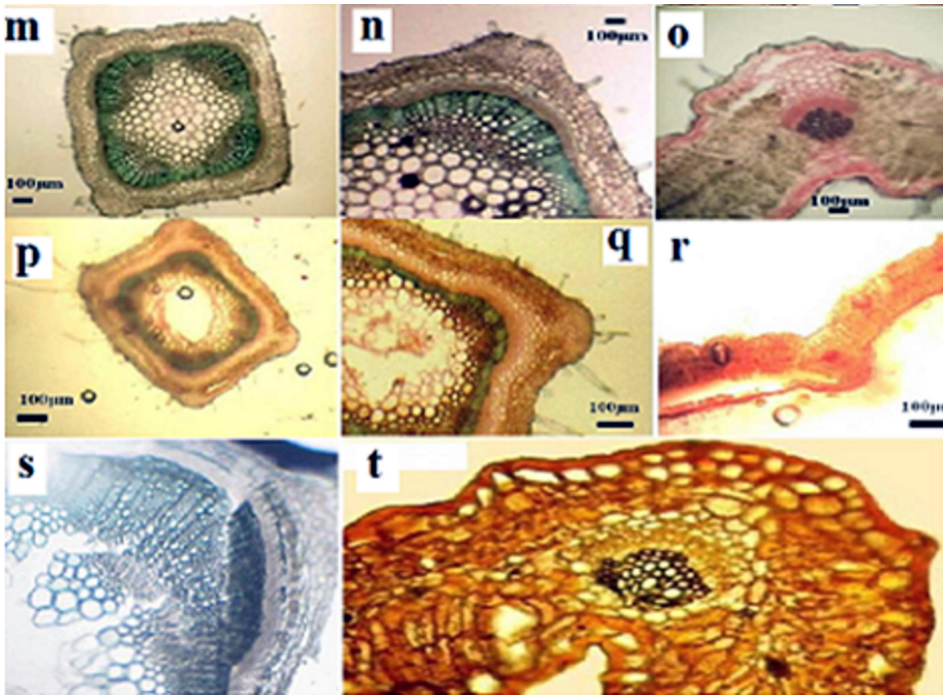

Fig 2 (continued)

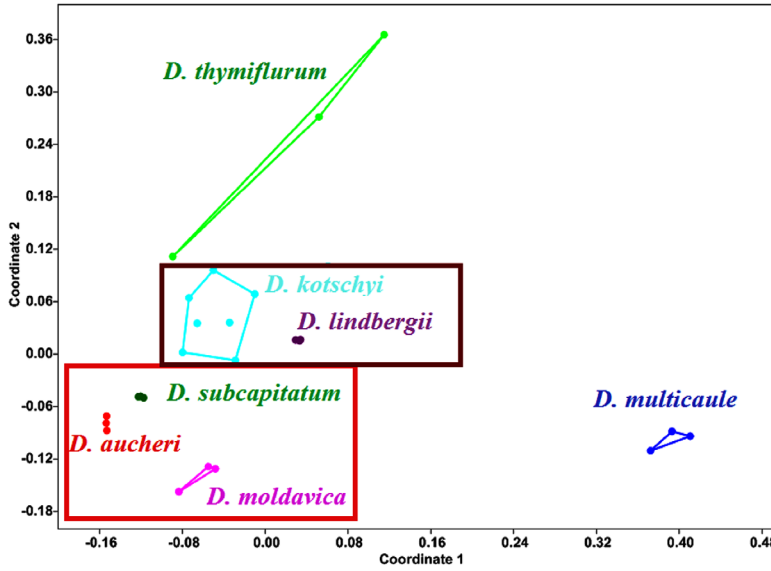

Fig. 3. PCoA plot of anatomical characters in *Dracocephalum* species

Table 2.
Quantitative anatomical characters among *Dracocephalum* species

Characters	*D. kotschyi*	*D. thymiflorum*	*D. lindbergii*	*D. subcapitatum*	*D. moldavica*	*D. multicaule*	*D. aucheri*
Length of epidermis in stem	20.86	34.88	25.71	13.17	12.94	57.66	18.63
Length of collenchymas in stem	109.19	243.3	132.36	71.66	76.3	159.5	98.41
Length of parenchyma in stem	61.86	92.73	80.05	25.75	53.79	100.05	50
Length of sclerenchyma in stem	24.45	25.75	30.77	15.78	26.95	33.47	15.71
Length of upper phloem in stem	46.73	38.88	50.55	20.80	40.86	74.97	20.80
Length of xylem in stem	140.57	154.37	220.96	93.39	144.46	364.39	63.25
Length of lower phloem in stem	49.57	51.02	75.43	35.34	40.91	152.01	37.50
Length of pith in stem	583.69	1256.32	628.88	304.43	769.34	1314.67	493.29
Length of stem in transverse transects	1098.31	1791.30	1332.45	657.81	1064.89	2168.20	827.02
Width of stem in transverse transects	1073.97	1713.94	1498.92	607.12	1100.30	1917.71	686.90
Length of simple trichomes in stem	95.22	64.59	101.46	62.02	173.25	56.13	70.35
Length of glandular trichomes in stem	18.88	25.34	31.94	40.86	53.38	48.49	31.72
Number of layer in collenchymas in stem	10	11	8	7	6	9	10
Number of layer in parenchyma in stem	4	4	5	3	3	3	4
Number of layer in sclerenchyma in stem	2	2	2	2	2	2	2
Number of layer in xylem in stem	20	19	16	8	14	22	14
Length of upper epidermis in leaf	65.22	68.365	30.77	36.37	38.86	21.24	52.31
Length of lower epidermis in leaf	61.26	50.665	44.63	23.03	43.71	18.29	65.42
Length of collenchymas in leaf	140.29	173.49	120.14	114.02	79.39	36.06	115.32
Length of parenchyma in leaf	195.0467	251.995	216.31	132.46	106.29	80.88	93.99
Length of mesophyll in leaf	712.5367	463.49	664.28	618.93	572.18	268.4	266.96

Table 2 (continued)

Characters	*D. kotschyi*	*D. thymiflorum*	*D. lindbergii*	*D. subcapitatum*	*D. moldavica*	*D. multicaule*	*D. aucheri*
Length of upper phloem in leaf	128.7867	109.6	138.21	88.92	69.16	35.14	76.3
Length of xylem in leaf	204.9933	146.405	178.61	131.32	86.9	53.15	87.94
Length of lower phloem in leaf	150.7833	107.64	160.1	67.16	56.71	35.83	42.86
Length of simple trichomes in leaf	189.755	172.965	335.39	175.43	394.3	38.39	338.9
Length of glandular trichomes in leaf	68.69	50.515	90.35	40.61	76.93	21.24	92.71
Number of layer in collenchymas in leaf	2	4	2	3	2	3	3
Number of layer in parenchyma in leaf	5	4	5	4	3	4	4
Number of layer in xylem in leaf	7	9	11	9	5	12	6

ies based on RAPD data (Sonboli *et al.* 2011) and pollen data (Naderifar *et al.* 2015). In this study *D. thymiflorum* was placed far from the other species.

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