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Taxonomic Relationships of Ten *Fritillaria* Species of Subgenera *Fritillaria* and *Theresia* Based on Analysis of Flower Qualitative and Quantitative Morphological Characters

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Abstract

Flower morphology of 10 *Fritillaria* species of subgenera *Theresia* and *Fritillaria* growing wild in Iran is studied using seventy morphological characters. Qualitative and quantitative datasets separated taxa into subgenera, sectional and groups. Three enigmatic species *F. caucasica*, *F. zagrica* (endemic to Zagros Mountains) and *F. pinardii* (recently reported from Zagros Mountains) appeared as closely related, distinct taxa. *F. chlorantha* (endemic to central Zagros Mountains) was put in an intermediate position between members of sections *Trichostyleae* and *Olostyleae*. Two distinct groups of taxa corresponding to Caucasian group and sect. *Trichostyleae*, were clustered in subgenus *Fritillaria*, mainly by three quantitative characters, *i.e.* lengths of the petal, sepal, and the stamen. Exploratory analyses of both quantitative and qualitative characters produced consistent results and showed the application of quantitative characters of flower in refining the taxonomy of this genus.

Key words: Taxonomy; Flower of *Fritillaria*; Morphology

Introduction

Genus *Fritillaria* L. (Liliaceae) comprises of approximately 170 taxa (130-140 species) distributed through the temperate regions of the northern hemisphere (Day *et al.*, 2014; Metin *et al.*, 2013). They are characterized by bisexual nodding flowers, campanulate to cupulate perianth of six tepals marked with light/dark colored squares or with longitudinal stripes or fascia, and with nectaries at the base, or at the inflection (Rechinger, 1990; Rix, 1997). Most of taxa being described from Turkey (Rix, 1984; Ozhatay, 2000), and the Zagros mountains of Iran as the center of diversity above the species level (Rix, 1997). *Fritillaria* species in Iran are represented by diploid (2n= 24) taxa (Jafari *et al.*, 2014; Bakhshi Khaniki, 2002a-c; Bakhshi Khaniki, 2005). Recent phylogenetic studies (Day *et al.*, 2014; Ronsted *et al.*, 2005) support the monophyly of *Fritillaria*. *F. zagrica* Staff. was proposed as a synonym for *F. pinardii* Boiss. based on morphological and molecular data (Celebi *et al.*, 2008), and was confirmed in the revision on the genus in the Mediterranean region of Turkey, where the floral morphology

(anther color, tip of tepal color, style divisions, and color of back of the tepals) of the two species was stressed (Teksen and Aytac, 2011). Incongruent datasets reported by most recent studies (Day *et al.*, 2014; Ronsted *et al.*, 2005) also suggest more studies are needed. Multivariate analysis of quantitative morphological data is an outstanding technique useful in numerically classification of a group of related taxa (Christensen *et al.*, 2014). Multivariate morphometric methods, were firstly considered as extensions of allometry, but were later highly stimulated by numerical phenetic concepts, and in particular by ordination techniques (Sneath, 1995). In the present paper, quantitative floral morphology of ten *Fritillaria* species collected from natural populations of Iran are studied to assess the relationships within species of this genus in Iran and to evaluate the application of these characters in the taxonomy of the genus. In this morphometric study, only the flower morphology was investigated, and the vegetative organs were excluded due to the possible environmental-derived variations in vegetative characters.

Materials and Methods

Fresh plant material was collected from natural habitats and identified using relevant identification keys (Rechinger, 1990; Rix, 1997). Vouchers are deposited in the herbarium of the faculty of science at Shahrekord University (Table 1). Floral parts of at least five separate specimens in different accessions are studied either *in situ* or after specimen dried for herbarium preparations (Fig. 1). Newly dried material was macerated shortly before floral parts being studied. Specimens (Table 1) belonged to two subgenera *Theresia* (C. Koch) Engler and *Fritillaria*; including two sections *Trichostyleae* Boiss. and *Olostyleae* Boiss. Twenty-six qualitative and 44 quantitative morphological characters relating to flowers and inflorescent, were selected (Table 2) and measured using the image processing software

Image-J ver. 1.49 (Schneider *et al.*, 2012). Digital images were calibrated by the millimeter scale in each image. Measurements entered into a standard raw data matrix and analyzed using Clustering (uncorrected P-distance), PCO (Cosine for quantitative and Dice for qualitative data sets) and MDS (agglomerative method and Cosine for quantitative data, Correlation coefficient for qualitative data) implemented in NTSYSpc ver. 2.11 (Rohlf, 2000), SplitsTree ver. 4 and Cluto ver. 2 software packages (Huson and Bryant, 2006; Karypis, 2003).

Table 1. Geographical information for species investigated in this study

Taxa	Specimens (accession no., GPS coord., Alt.)
Subgen. *Theresia*	
F. persica L.	183: 37 18 33.79 N, 45 09 44.04 E, 1890m
	1281: 37 17 47.71 N, 45 09 56.31 E, 2070m
	15: 32 32 36.68 N, 50 13 50.20 E, 2378m
	189: 32 07 35.88 N, 50 21 45.96 E, 2853m
	113: 35 16 26.66 N, 46 12 36.02 E, 1827m
	135: 35 13 15.14 N, 46 17 49.56 E, 1646m
	1280: 35 19 10.76 N, 46 15 07.44 E, 3519m
Subgen. *Fritillaria*, sect. *Trichostyleae*, group crassifolia	
F. reuteri Boiss.	1125: 37 28 50.46 N, 45 1 5.12 E, 1784m
	3: 32 28 19.18 N 50 30 37.28 E, 2521m
	598: 32 24 45.10 N, 50 6 37.75 E, 2498m
	1315: 32 09 47.97 N, 50 47 30.81 E, 2515m
F. crassifolia Boiss. & Huet	1189: 38 24 00.54 N, 46 51 44.03 E, 1685m
	162: 37 28 57.77 N, 45 1 4.67 E, 1756m
	181: 37 19 21.10 N, 45 9 14.04 E, 1581m
	565: 35 17 32.79 N, 46 12 10.75 E, 2136m
	1179: 35 16 56.65 N, 47 07 09.21 E, 1842m
F. poluninii (Rix) Bakhshi-Khaniki & Persson	206-213: 35 12 2.56 N, 46 16 52.15 E, 2380m
	1289-1292: 35 17 20.89 N, 46 13 21.89 E, 2190m
Subgen. *Fritillaria*, sect. *Olostyleae*, group caucasica	
F. assyriaca Baker	173: 37 28 50.14 N, 45 0 48.15 E, 1862m
	1252: 35 13 09.72 N, 46 22 16.98 E, 1995m
	1264: 35 16 01.96 N, 46 19 57.10 E, 2280m
	1235: 35 17 02.26 N, 47 07 05.63 E, 1865
F. uva-vulpis Rix	1131: 37 28 50.46 N, 45 1 5.12 E, 1784m
	126: 35 16 10.56 N, 46 12 56.35 E, 1674m
	1293: 35 16 09.19 N, 46 12 58.42 E, 1680m
	1302: 35 12 58.95 N, 46 16 58.35 E, 1800m
	1311: 35 19 23.88 N, 46 14 47.31 E, 2123m
F. caucasica Adams	1284-1288: 38 23 20.73 N, 46 52 27.07 E, 2115m
F. chlorantha Hausskn. & Bornm.	1229-1234: 35.97 N, 4594 E, 2400m
F. zagrica Stapf.	46: 32 11 12.18, N 50 45 46.54 E, 2530m
	60: 32 11 13.15 N, 50 45 39.00 E, 2505m
	1163: 34 45 38.66 N, 48 26 3.98 E, 2350m
	103: 35 36 28.18 N, 46 55 28.02 E, 2656m
F. pinardii Boiss.	168: 37 28 50.46 N, 45 1 5.12 E, 1784m
	1275-1277: 37 17 00.07 N, 45 10 22.37 E, 2175m

Results

Qualitative morphological characters

Three clusters of taxa were obtained from cluster analysis of qualitative data (Fig. 2). Cluster A consisted of members of sect. *Trichostyleae*, group crassifolia. Cluster B consisted of members of sect. *Olostyleae* (Caucasian group) and *F. persica* was located in a separate cluster C. *F. pinardii*, *F. zagrica* and *F. caucasica* were grouped close together in cluster C and *F. chlorantha* was distantly grouped to other members of this cluster. In cluster A, *F. poluninii* and *F. crassifolia* were closely related. Results are concordant to the classification of the taxa proposed by (Rix *et al.*, 2001). Results of PCO analysis of qualitative characters is shown in Fig. 3. There are four groups of taxa (members of sect. *Olostyleae* and members of sect. *Trichostyleae* group *crassifolia*) were the two main groups and *F. chlorantha* (group 3) which was distantly clustered with Caucasian group (in the cluster analysis, Fig. 2), was also separated from other members of this group along both axes 1, 2 of PCO analysis (Fig. 3). The fourth group was *F. persica*. PCO plot of qualitative characters showed that *F. zagrica* and *F. pinardii* are closely related taxa which did not separate along the main axes 1, 2; they were just separated along the third axis. Cumulated percent of variation in first four axes in PCO analysis (Fig. 3) is presented in Table 3, denoting 68.76 percent of the variation is summarized in the first 3 axes. PCO plot in Fig. 3 also shows that members of sect. *Trichostyleae* group crassifolia (Table 1) are separated from other groups along axis 1, moderately separated from each other along axis 2, and they remain constant along the third axis.

Quantitative morphological characters

Two main groups of taxa appeared in the plot of PCO analysis of quantitative characters (Fig. 4). Group a consisted of four species of sect. *Olostyleae* and group b consisted of all members of sect. *Trichostyleae* group crassifolia. A third small group, *F. caucasica* and *F. zagrica* were distantly separated from sect. *Olostyleae*. *F. persica* itself was the fourth group (Fig. 4). PCO plot of quantitative characters showed that *F. zagrica* and *F. pinardii* did not show close

relationships, however, *F. poluninii* and *F. crassifolia* were closely related. Cumulated percent of variation in first four axes in PCO analysis (Fig. 4) is presented in Table 4 denoting that 78.43 percent of variation is summarized in the first 3 axes. PCO plot in Fig. 4 also shows that members of sect. *Trichostyleae* group crassifolia (Table 1) are separated from other groups along axis 1, remained constant along axis 2, and very slightly separated from each other along axis 3. This cluster (sect. *Trichostyleae* group crassifolia) is well supported by the results obtained from analysis of both qualitative and quantitative characters.

Exploratory analysis of morphological characters

Clustering algorithms are used to divide data into meaningful clusters, in a way that the intra-group similarity tends to be maximized and the inter-group similarity be minimized. Clusters are useful in explaining the characteristics of the data and their distribution; serving as the basis of various data mining and analyses techniques (Karypis, 2003). An important aspect of partitional-based criterion-driven clustering algorithms implemented in Cluto is the method used to optimize this criterion function. Cluto uses a randomized incremental optimization algorithm that is greedy in nature and has been shown to produce high-quality clustering solutions (Zhao and Karypis, 2001). As the interpretation of the number of resultant groups (clusters) may become readily subjective, k-means clustering methods are used for exploratory data analysis to cope with this problem. Results of MDS analysis (Fig. 5) showed that different fritillary species in this study were effectively grouped in four or five clusters according to qualitative or quantitative characters, respectively. The intra-group similarity was highest and inter-group similarity was lowest for these K values, and further partitioning of taxa into K=5 or K=6 (respectively for qualitative and quantitative character), led to incomplete separation of the extra cluster (Fig. 5). Measures of internal similarity (ISim) and external similarity (Esim) for K=4, K=5 (for qualitative and quantitative characters, respectively) along with the membership of each taxon in K clusters are presented in Table 5. Results confirmed the close

relationships between members of sect. *Trichostyleae* group *crassifolia*. Both qualitative and quantitative characters, grouped taxa of this section in a distinct robust cluster. This cluster was also produced in clustering and PCO analyses (Figs. 2-4).
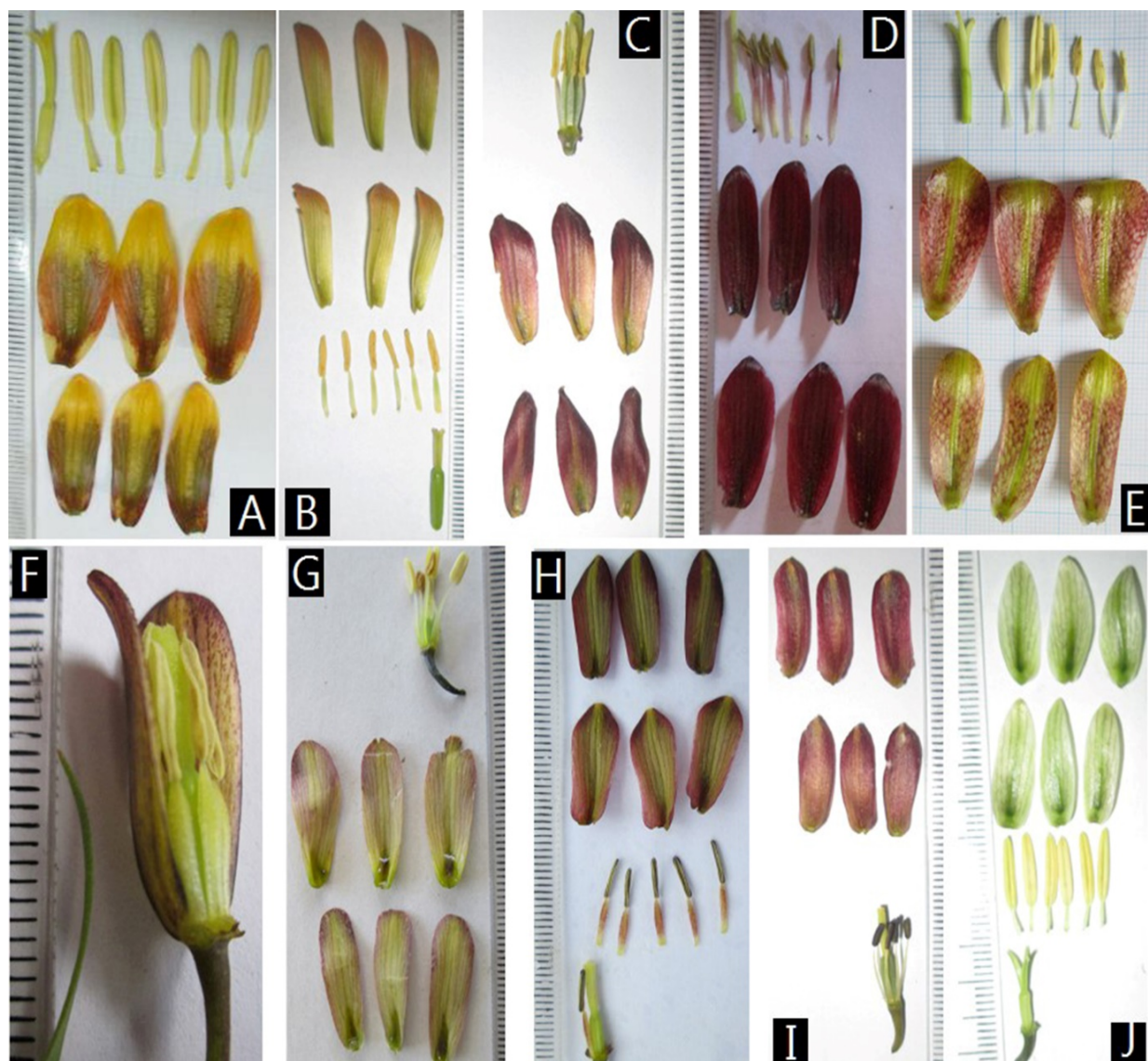
Fig. 1. Flower parts in studied *Fritillaria* species. Specimen and character lists are presented in Tables 1, 2. A: *F. reuteri*, B: *F. chlorantha*, C: *F. assyriaca*, D: *F. caucasica*, E: *F. crassifolia* ssp. *kurdica*, F: *F. uva-vulpis*, G: *F. persica*, H: *F. pinardii*, I: *F. zagrica*, J: *F. poluninii*

Table 2. Qualitative and quantitative morphological characters observed/measured on specimens. Qualitative (0/1) characters are shown as 15 multistate characters. Freu: *F. reuteri*, Fchl: *F. chlorantha*, Fass: *F. assyriaca*, Fcau: *F. caucasica*, Fcra: *F. crassifolia* ssp. *kurdica*, Fuva: *F. uva-vulpis*, Fper: *F. persica*, Fpin: *F. pinardii*, Fzag: *F. zagrica*, Fpol: *F. poluninii*

Characters	Freu	Fchl	Fass	Fcau	Fcra	Fuva	Fper	Fpin	Fzag	Fpol
Qualitative										
Sigma cleft (0), lobed (1), entire (2)	1	1	2	2	0	2	2	2	2	0
Anther color dark	no	no	no	no	no	no	no	yes	yes	no
Filament papillose	no	no	no	no	no	no	no	yes	no	no
Anth. larger than fil. (0)/ shorter (1)/ equal (2)	0	2	2	1	0	2	1	2	1	0
Filament threadlike (0), thick (1), normal (2)	2	2	2	1	2	1	2	1	0	2
Filament color yellow (0), green (1), red (2)	0	0	1	2	0	0	0	2	1	0
Style longer than ovary (0), shorter (1)	1	1	1	0	1	1	1	1	1	1
Style thinner than ovary (0), thicker (1)	0	0	1	0	1	1	1	1	1	1
Ovary color darker than style	no	yes	no	no	no	no	yes	no	yes	no
Perigon segments symmetrical	yes	no	yes	yes	yes	yes	yes	yes	yes	yes
Perigon tessellate	no	no	no	no	yes	yes	no	no	no	no
Perigon tip of different color	yes	yes	yes	yes	yes	no	no	no	no	no
Sepals width shorter than petals	yes	no	no	no	yes	no	no	no	no	no
Infl. raceme (0), solitary (1), bi-flower (2)	2	1	1	1	2	1	0	1	1	2
Perigon narrow campanulate (0), wide (1)	1	0	0	0	1	0	1	0	0	1
Quantitative										
Length of stamen (c1)	18.75	14.02	12.50	16.32	21.61	15.53	14.67	12.40	10.71	13.10
Length of anther (c2)	12.46	6.38	6.02	5.20	14.08	7.57	5.04	5.96	3.87	9.62
Width of anther (c3)	3.03	1.32	1.00	1.07	2.69	1.46	1.93	0.97	0.96	1.72
Width od filament (c4)	1.11	1.04	0.95	0.80	0.90	1.43	1.08	1.40	0.49	0.62
Length of ovary (c5)	6.95	10.29	7.01	4.38	9.77	8.12	4.57	6.01	6.67	5.40
Width of ovary (c6)	2.05	2.53	2.17	1.73	2.03	2.20	1.92	2.00	2.15	1.94
Length of style (c7)	10.14	6.67	7.84	12.07	11.20	7.99	6.17	7.86	6.21	6.32
Width of style (c8)	1.81	1.78	1.89	1.00	2.29	1.97	1.07	1.85	1.20	1.79
Length of sepal (c9)	17.84	20.54	15.41	22.15	29.49	21.04	20.36	16.55	13.94	16.92
Width of sepal (c10)	7.45	5.77	5.15	6.54	10.80	3.09	5.90	6.52	4.78	6.37
Length of petal (c11)	22.43	21.92	16.84	22.38	30.64	18.25	21.60	16.98	15.33	17.89
Width of petal (c12)	10.78	5.92	5.92	9.21	13.87	7.01	8.02	7.65	5.49	6.42
Length of filament (c13)	6.29	7.64	6.48	11.12	7.53	7.97	9.64	6.44	6.85	3.49
Length of filament/ Length of stamen (c14)	0.34	0.55	0.52	0.68	0.35	0.51	0.66	0.52	0.64	0.27
Length of anther/ Length of stamen (c15)	0.66	0.45	0.48	0.32	0.65	0.49	0.34	0.48	0.36	0.73
Length of anther/ Length of filament (c16)	1.98	0.83	0.93	0.47	1.87	0.95	0.52	0.93	0.56	2.76
Length of filament/ Length of anther (c17)	12.46	6.38	6.02	5.20	14.08	7.57	5.04	5.96	3.87	9.62
Width of anther/ Width of filament (c18)	2.74	1.28	1.06	1.34	3.00	1.03	1.79	0.69	1.96	2.77
Width of filament/ Width of anther (c19)	0.37	0.78	0.94	0.75	0.33	0.97	0.56	1.45	0.51	0.36
Length of anther/ Width of anther (c20)	4.11	4.83	6.01	4.86	5.23	5.17	2.62	6.16	4.04	5.59
Width of anther/ Length of anther (c21)	0.24	0.21	0.17	0.21	0.19	0.19	0.38	0.16	0.25	0.18
Length of filament/ Width of filament (c22)	5.69	7.38	6.85	13.90	8.40	5.59	8.94	4.58	14.00	5.62
Width of filament/ Length of filament (c23)	0.18	0.14	0.15	0.07	0.12	0.18	0.11	0.22	0.07	0.18
Length of gynoecium (c24)	17.09	16.96	14.85	16.45	20.97	16.12	10.74	13.87	12.89	11.71
Length of ovary/ Length of style (c25)	0.69	1.54	0.89	0.36	0.87	1.02	0.74	0.76	1.07	0.85
Length of style/ Length of ovary (c26)	1.46	0.65	1.12	2.75	1.15	0.98	1.35	1.31	0.93	1.17
Length of style/ Length of gynoecium (c27)	0.59	0.39	0.53	0.73	0.53	0.50	0.57	0.57	0.48	0.54
Length of gynoecium/ Length of style (c28)	1.69	2.54	1.89	1.36	1.87	2.02	1.74	1.76	2.07	1.85
Length of ovary/ Length of gynoecium (c29)	0.12	0.15	0.15	0.11	0.10	0.14	0.18	0.14	0.17	0.17
Length of gynoecium/ Length of ovary (c30)	2.46	1.65	2.12	3.75	2.15	1.98	2.35	2.31	1.93	2.17
Length of ovary/ Width of ovary (c31)	3.39	4.07	3.23	2.53	4.81	3.69	2.38	3.01	3.10	2.78
Width of ovary/ Length of ovary (c32)	0.30	0.25	0.31	0.40	0.21	0.27	0.42	0.33	0.32	0.36
Length of style/ Width of style (c33)	5.60	3.74	4.15	12.04	4.90	4.06	5.77	4.26	5.17	3.52
Width of style/ Length of style (c34)	0.18	0.27	0.24	0.08	0.20	0.25	0.17	0.23	0.19	0.28
Length of gynoecium/ Width of ovary (c35)	8.33	6.71	6.85	9.50	10.33	7.32	5.59	6.95	5.99	6.03
Width of ovary/ Length of gynoecium (c36)	0.12	0.15	0.15	0.11	0.10	0.14	0.18	0.14	0.17	0.17
Length of sepal/ Width of sepal (c37)	2.39	3.56	2.99	3.39	2.73	6.80	3.45	2.54	2.92	2.66
Width of sepal/ Length od sepal (c38)	0.42	0.28	0.33	0.30	0.37	0.15	0.29	0.39	0.34	0.38
Length of petal/ Width of petal (c39)	2.08	3.70	2.85	2.43	2.21	2.60	2.69	2.22	2.79	2.79
Width of petal/ Length od petal (c40)	0.48	0.27	0.35	0.41	0.45	0.38	0.37	0.45	0.36	0.36
Length of sepal/ Length of petal (c41)	0.80	0.94	0.91	0.99	0.96	1.15	0.94	0.97	0.91	0.95
Length of petal/ Length of sepal (c42)	1.26	1.07	1.09	1.01	1.04	0.87	1.06	1.03	1.10	1.06
Width of sepal/ width of petal (c43)	0.69	0.97	0.87	0.71	0.78	0.44	0.74	0.85	0.87	0.99
Width of petal/ Width of sepal (c44)	1.45	1.03	1.15	1.41	1.28	2.27	1.36	1.17	1.15	1.01

Table 3. Cumulated percent of variation in axes 1-4 in PCO analysis.

i	Eigenvalue	Percent	Cumulative
1	2.197	39.40	39.40
2	0.882	15.81	55.21
3	0.756	13.55	68.76
4	0.495	8.87	77.64

**Table 4.** Eigenvalues for PCO of quantitative characters

| i	Eigenvalue	Percent	Cumulative
1	0.138	47.90	47.90
2	0.050	17.37	65.27
3	0.038	13.23	78.49
4	0.029	9.94	88.43
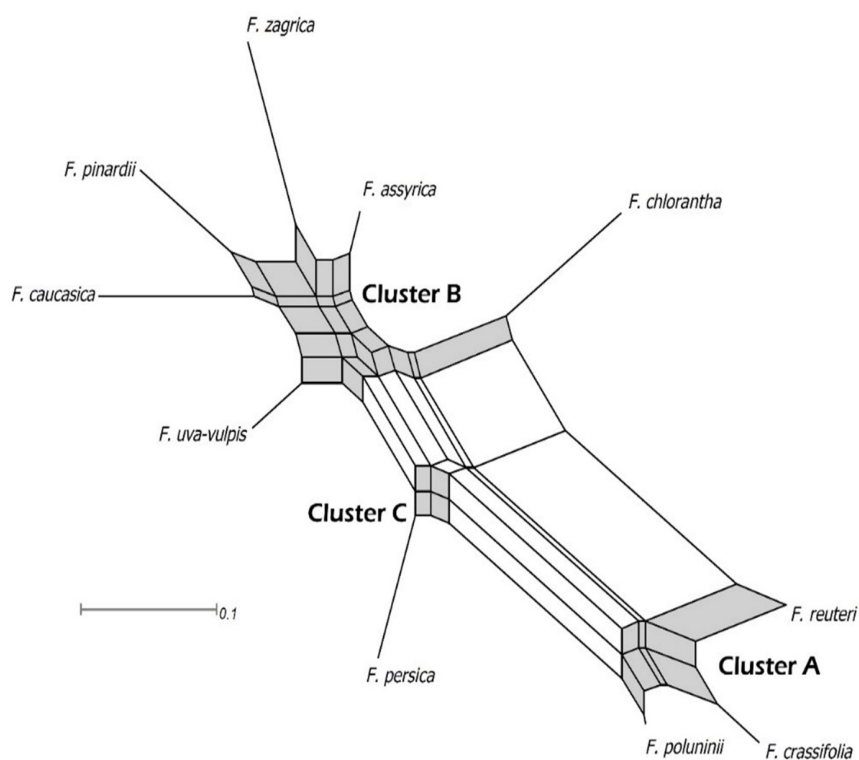
Fig. 2. Dendrogram resulted from cluster analysis of qualitative morphological characters. Three clusters are recognized corresponding to group crassifolia of sect. *Trichostyleae*, sect. *Olostyleae*, and subgen. *Theresia*.

Fritillaria chlorantha had an intermediate position between clusters A (sect. *Trichostyleae*) and B (sect. *Olostyleae*) based on qualitative characters' dataset, but was definitively put in cluster A of the quantitative character set. The intermediate position of *F. chlorantha* was also evident in Figs. 2, 3 resulted from clustering and PCO of qualitative character. Separation of *F.* *chlorantha* as a distinct cluster is supported by two qualitative characters (3-lobed stigma and non-symmetric shape of perigon segments). Section *Olostyleae* was mainly supported by four descriptive qualitative characters (non-branching stigma, narrow campanulate perigon, few flowered inflorescent, and symmetry of perigon segments).

Qualitative Characters
Dice Coefficient Dcentered
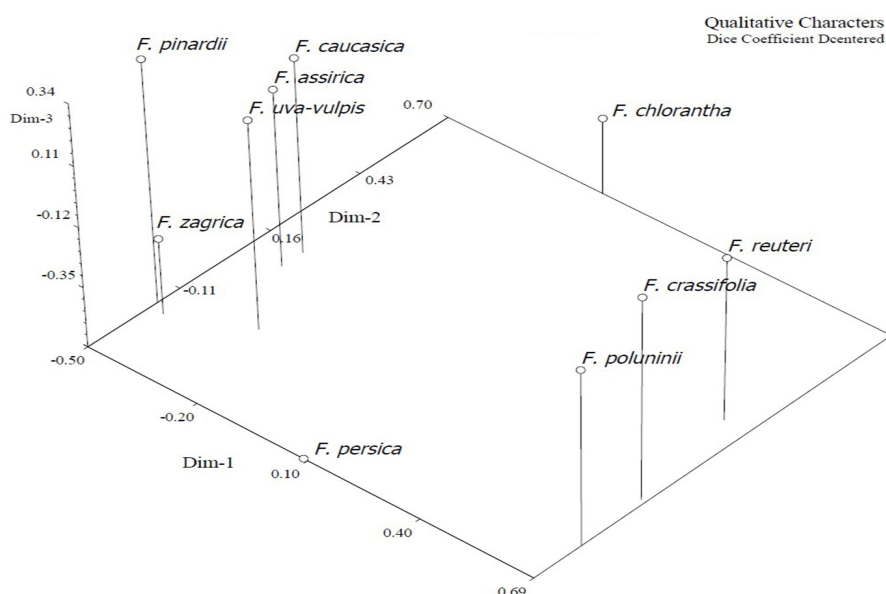
Fig. 3. Plot of PCO analysis of qualitative morphological characters. Resultant groups similar to Fig. 2 but *F. chlorantha* distantly grouped with members of sect. *Olostyleae*.

quantitative characters
Cosine distance coefficient, Dcentered
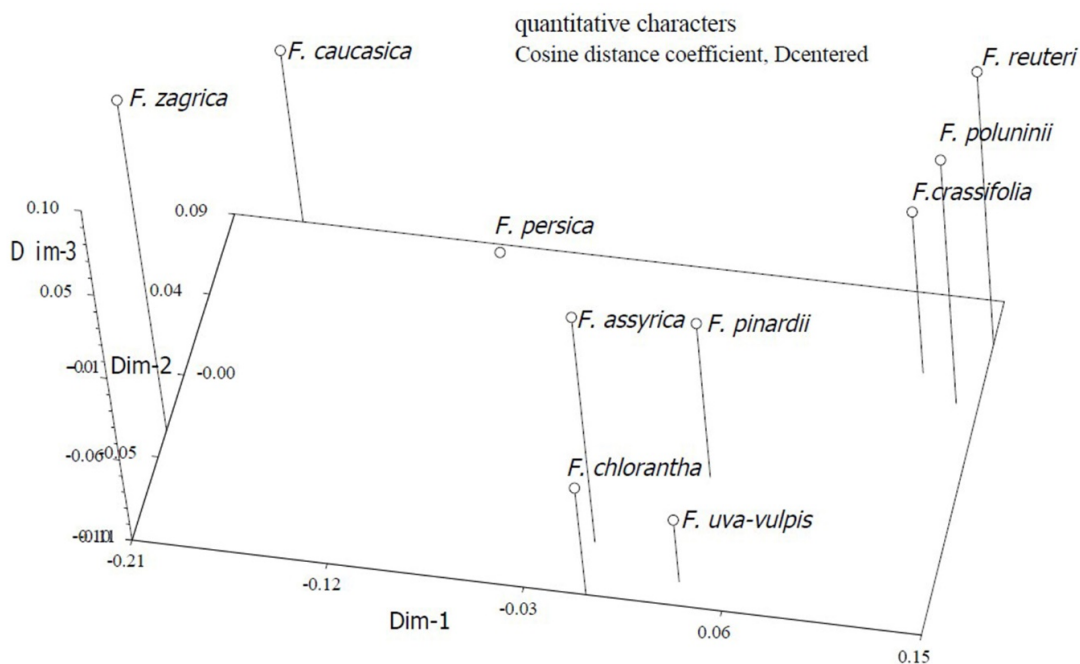
Fig. 4. Plot of PCO analysis of quantitative morphological characters. Four clusters are recognized corresponding to a: major members of group crassifolia of sect. *Trichostyleae*, b: (*F. zagrica* + *F. caucasica*), c: sect. *Olostyleae*, and d: subgen. *Theresia*.

Table 5. K means clustering. Internal and external similarity measures of groups and membership of taxa in each cluster corresponding to Fig. 5.

Data Set	Cluster	N	Members	ISim	Isdev	Esim	ESdev
Qualitative, K=4							
	A	3	*F. poluninii*, *F. crassifolia* ssp. *kurdica*, *F. reuteri*	0.785	0.030	-0.140	0.027
	B	5	*F. zagrica*, *F. assyriaca*, *F. uva-vulpis*, *F. pinardii*, *F. caucasica*	0.549	0.037	-0.077	0.114
	C	1	*F. chlorantha*	1.0	0.000	0.028	0.000
	D	1	*F. persica*	1.000	0.000	0.101	0.000
Quantitative, K=5							
	A	4	*F. chlorantha*, *F. uva-vulpis*, *F. assyriaca*, *F. pinardii*	0.991	0.002	0.969	0.004
	B	3	*F. crassifolia*, *F. poluninii*, *F. reuteri*	0.993	0.001	0.962	0.005
	C	1	*F. zagrica*	1.000	0.000	0.951	0.000
	D	1	*F. caucasica*	1.000	0.000	0.958	0.000
	E	1	*F. persica*	1.000	0.000	0.966	0.000
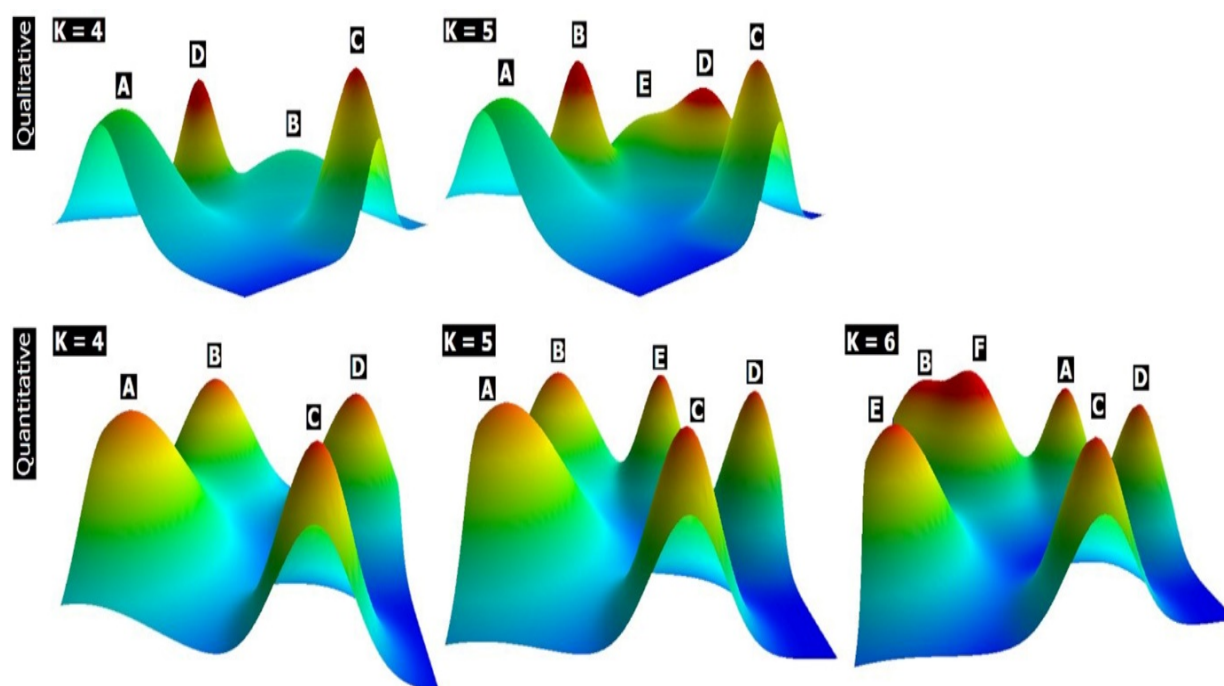
Figure 5. Mountain visualization of k-means clustering analysis combined with multidimensional scaling. Membership of each species in clusters are presented in Table 5. Taxa are grouped in 4 or 5 clusters using qualitative or quantitative datasets, respectively, and groupings concordant to clustering and PCO analyses.

Fritillaria persica (subgenus *Theresia*; cluster D) was mainly supported by two qualitative characters; non-branching stigma and raceme inflorescent.

Results based on quantitative characters showed that there were two main groups of taxa in the subgenus *Fritillaria*, corresponding for so-called Caucasian group and sect. *Trichostyleae* which were described mainly by four quantitative characters; lengths of petal, sepal, and stamen.

The length of gynoecium and the proportion of lengths of filament to its width, consisted the main discriminating characters in each group.

Discussion

Relationships within genus *Fritillaria* is an interesting field attracting many researchers. Species of this genus contain the largest plant genomes ever discovered (Bennett and Leitch,

2012) and the relationships between species especially in the largest subgenus *Fritillaria* is not clearly understood, remaining open for further investigation. Baker (1874) wrote: 'In these petaloid monocotyledons we may safely lay it down as a general rule that all descriptions not drawn up from living specimen are more or less unsatisfactory, so that a synopsis must of necessity be far more imperfect than in those orders in which herbarium specimens show the distinctive characters more clearly; and a good synopsis must be of gradual growth and the work of observers in different countries uniting together the result of their studies' (Baker, 1874). The importance of fresh living specimens for floral morphology in *Fritillaria* is also emphasized by Rix (1974). Sing-Chi and Yi-Bo (1996) in their taxonomic revision of the genus *Fritillaria* in Xinjiang (China), where more than 30 species were reduced to nine previously recognized species and 21 subspecies to synonyms, mainly by using herbarium specimens. Morphology of the genus is yet not straightforward and could be easily confusing (Sing-chi and Yi-bo, 1996). Vegetative characters could be misleading in some species when the specimen is young, or the number of flowers in older specimens is increased (Rix, 1974).

In our study, vegetative characters or those floral characters with a tendency to be confused (like nectary, phylotaxy, etc.) are omitted. Our results are congruent with the generally accepted classification by Rix (Rix *et al.*, 2001), supporting sectional and groups within subgenus *Fritillaria*. In a recent study by Day and co-workers (Day *et al.*, 2014), evolutionary relationships in the genus *Fritillaria* was evaluated using 11 LCNGs (low-copy nuclear genes) and three cpDNA regions (*mat*K, *rbc*L, *rpl*16). This was the most comprehensive molecular phylogenetic study to encompass most of the currently recognized species in this genus, including representatives from all subgenera and groups. Results showed that the latest proposed taxonomy by Rix (Rix *et al.*, 2001) is generally supported by molecular datasets.

Infra-generic classification of *Fritillaria* proposed by Ronsted and co-workers using ITS and three cpDNA regions (Ronsted *et al.*, 2005) showed that the classification of *Fritillaria* by Rix (Rix *et al.*, 2001) was supported and that *Fritillaria* was monophyletic. However,

relationships within the main subgenus *Fritillaria* was of low resolution. Resolution in this subgenus was also low in Day and co-worker's report, denoting the complexity of taxa in this group (Day *et al.*, 2014). Khourang and co-workers studied the phylogenetic relationship in eight Iranian species of *Fritillaria* using ITS and *trn*L-F sequence data and showed that subgenus *Fritillaria* was supported (72% BS and 100%BS) by *trn*L-F and ITS sequences, respectively, but relationships between taxa were obscured under subgeneric level (Khourang *et al.*, 2014). *Fritillaria zagrica* (section *Olostyleae*) was clustered with 100% BS with *F. reuteri* and nested within the main cluster containing the rest of taxa in sect. *Trichostyleae* (Khourang *et al.*, 2014). Although molecular and morphological datasets are congruent for the taxonomy and relationships at subgeneric and sectional levels, this is not the case under the sectional level. Our results are drawn from multivariate analyses of qualitative and quantitative datasets, however, are congruent with previous findings by above-mentioned molecular and morphological classifications. Consistent results show that the subgenus *Fritillaria* consists of two main clusters of taxa namely a) the so-called Caucasian group and b) group crassifolia, the latter in which *F. zagrica*, *F. caucasica* and *F. pinardii* are related but obviously separate taxa. Our results, therefore, did not support the synonymy of these taxa (Celebi *et al.*, 2008). One other consistent result drawn from our study was the intermediate position of *F. chlorantha* between the major clusters of the subgenus *Fritillaria*. This was interesting since this species is a recently identified endemic to Zagros Mt. of Iran and may merit to be considered as a separate cluster. This remains to be confirmed with more investigations. Results from *trn*H-*psb*A sequences (Sharifi-Tehrani and Advay, 2015) showed that *F. chlorantha* was distantly related to other members of sect. Trichostyleae and had an intermediate position between subgenus *Fritillaria* and (*Petilium+Theresia*). This Study also showed that the multivariate analysis of floral morphology in *Fritillaria* species was robust in separating the species and applicable for elucidating the relationships in the genus at the species level.

Acknowledgments

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