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Research Article

Multivariate analysis of morphological variation in *Acanthophyllum* Sect. *Oligosperma* (*Caryophyllaceae*) from NE of Iran

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Abstract

Cluster analysis and principal component analysis were used to investigate the differences among species of *Acanthophyllum* sect. *Oligosperma* in Khorassan provinces (NE Iran). In this study, a total of 60 including 32 quantitatives and 28 qualitatives characters were examined on 98 herbarium specimens. The results explained morphological treats are useful to discriminate taxa. Phenogram and scattergram displayed four distinct groups among samples which suggest four series for this section in NE Iran. These series are agreement with previous series considered for this section in Flora of USSR. In addition, here, a new series named "Speciosa" is introduced for the first time for the Flora of Iran. Our analysis didn't provide any distinguishing pattern between *A. squarrosum* Boiss., and *A. heratense* Schiman-Czeika, and also between *A. pachystegium* Rech. f., *A. adenophorum* Freyn, and *A. lilacinum* Schischk. It seems more ditailed morphological study in field and molecular analysis could solve the problem of theses complexes.

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1. Introduction

Acanthophyllum C.A. Mey. (*Caryophyllaceae*, *Caryophylloideae*, *Caryophylleae*) with about 60 species is distributed in the Irano-Turanian region [1-2]. The genus distributes in Iran with 33 species from which 23 species are endemic. However, Basiri *et al*. [3] suggested seven synonyms and five reductions to the rank of variety for the genus; accepting 21 species in Iran in their recent investigation on Iranian *Acanthophyllum* species. Traditionally, the root of this genus, have been used as detergent [4]. Other investigations demonstrated that the genus due to presence gypsoside has the positive effects on the cardio-vascular systems [5-6]. *Acanthophyllum* species are adapted to deserts, mountains and temperate areas [7]. Distribution of this genus is in Iran, Afghanistan, Pakistan, Kazakhstan, Tajikistan, Uzbekistan, Turkmenistan, Western China, Armenia, Iraq, Turkey and Syria. The northeast of Iran and adjacent regions in Afghanistan and Turkmenistan, are considered as the most important center of diversity of the genus [8]. The number of species reduces from the east of Afghanistan to China and the west of Turkey to Syria, so as *Acanthophyllum pungens* (Bunge) Boiss and *A. verticillatum* (Willd.) Hand-Mzt., only have been reported in China and Syria, respectively [9]. It has not been reported any *Acanthophyllum* species from Palestine and Europe [10]. Based on Flora Iranica [2], the genus has been divided to seven sections. Of these, four sections including *Oligosperma* Schischk., *Macrostegia* Boiss., *Acanthophyllum* and *Plesiosperma* Boiss have been reported for the flora of Iran. The sect, *Oligosperma* with 23 species worldwide is the largest section of the genus, of which 16 occur in Iran. This section was first described by Shishkin [11] in Flora of the USSR. The members of the section are identified by dense flowers, spherical terminal heads, (4) 6–12 mm long calyx, 1–2 mm long calyx-teeth and 4-ovuled ovary [2, 11]. Basic chromosome number in this genus is $x = 14$ and $x = 15$. Three levels of ploidy have been reported for the genus by Ghaffari [9]. Most species in the sect *Oligosperma* and *Macrostegia* are diploid ($2n = 2x = 30$), except for *A. cespitosum* (sect. *Oligosperma*) which is different in chromosome number (2n=2x=28) and in morphology of inflorescence. Ghaffari and Corgues [8] recognized two karyotypic variants in populations of A. *laxiusculum*: variant A (2n=30) and variant B (2n=30) with 0 to 3 B-chromosomes. The comparison of behaviors in two variants showed an increase in pollen stainability and seed production; it seems these effects were due to presence of B-chromosomes. The species in sect *Acanthophyllum* are uniformly tetraploid ($2n = 4x = 60$) and distributed in central and western parts of the Irano-Turanian region. The third ploidy level of the genus is found in the sect. *Plesiosperma* which is hexaploid ($2n = 6x = 90$). The species of this section are found in the eastern and northern parts of Irano-Turanian region [8-9]. Meratan *et al.* [12] investigated tolerance mechanisms against salinity and water stress among three species of *Acanthophyllum* with three different ploidy levels. Their study showed that the hexaploid species in contrast with diploid and tetraploid species has better mechanisms against salinity and water stresses and shows greater tolerance. These results

provide a support for distribution pattern of *Acanthophyllum* species. In the current study, morphological variations have been investigated within sect. *Oligosperma* in the northeast of Iran (including Khorassan provinces). According to Flora Iranica [2], this section represents in Khorassan provinces by 13 species namely *A. borsczowii* Litw., *A. speciosum* Rech. f. & Schiman-Czeika, *A. korshinskyi* Schischk., *A. pachystegium* Rech. f., *A. adenophorum* Freyn., *A. lilacinum* Schischk., *A. brevibracteatum* Lipsky., *A. diezianum* Hand.-Mzt., *A. laxiusculum* Schiman-Czeika, *A. squarrosum* Boiss., *A. heratense* Schiman- Czeika, *A. elatius* Boiss., *A. andersenii* Rech. f. & Schiman-Czeika. *A. lilacinum* firstly described by Shishkin [11] in Flora USSR as a species covered with simple hairs, lilac petals and obliquely ascending leaves which were placed in series *squarrosa*. But Samples which identified by distinguishing characters in Flora Iranica [2] as *A. lilacinum* have stems and leaves covered with glandular hairs and in inflorescence multicellular simple hairs intermix with glandular hairs. Davis [13] studied the anatomy of *Caryophyllaceae* and reported that the glandular and eglandular hairs have diagnostic value in this family. Also other workers [2, 11], used this character in separation of species in this genus. Therefore it seems the concept of *A. lilacinum* needs to be revised. During the survey on specimens, we observed samples 43817 (FUMH) and 40772 (FUMH) (appendix 1) fully match with the original description of *A. lilacinum* [11]. These specimens have been previously named as *A. brevibracteatum* based on Flora Iranica. The latter species have been described in Flora USSR [11] as a plant with simple hairs intermixed with glandular hairs on stem and leaf, and calyx length of 7.5–8.5 mm, placed in series *Adenophora*. But Schiman-Czeika [2] in Flora Iranica has recognized this species as a plant with simple hairs on calyx and with 6 mm long calyx but hairs type on stem and leaves is unclear. Therefore, these difficulties indicate a need for a clarification of the specific boundaries of these two species. Schiman-Czeika [2] separated *A. adenophorum* from the closest species, namely *A. pachystegium* and *A. lilacinum*, using calyx character length (calyx longer or shorter than 8 mm). Our preliminary investigation indicated that this character could not establish a sharp boundary between the species. It should be noted that Schiman-Czeika [2] notes under the description of *A. adenophorum* the similarity among this species and two species *A. lilacinum* and *A. pachystegium*. This delimitation has led to confusion and several specimens have been misidentified. Hence, we have a complex group including *A. adenophorum*, *A. lilacinum*, and *A. pachystegium* namely Adena to be investigated. The diagnostic character for identification of *A. speciosum* is "petals longer than 20 mm" based on Schiman-Czeika [2]. In this study, the petal length of the specimens 18690 (FUMH) and 35634 (FUMH) (appendix 1) was measured ca.15 mm, but other characters were similar to *A. speciosum*. Are these specimens' new taxa or relate to *A. speciosum*? If these specimens are *A. speciosum*, what characters are suitable to establish a distinguishing boundry?. Ghazanfar & Nasir [14] characterized two variates for *A. squarrosum* as *A. squarrosum* var. *squarrosum* and *A. squarrosum* var. *stocksianum*. The var. *stocksianum* has been reduced under the synonymy of *A. stocksianum* and Itch introduced a form for this species as nana. The species *A. heratense* has close similarity to *A. squarrosum* var. *squarrosum* which led to confusion to delimit these taxa. Hence, we have other complex with these two species which in this study has been named as Squarra. Our objectives in the current study are to investigate the morphological relationships among the *Acanthophyllum*

species within the sect. *Oligosperma* and find distinguishing characters and finally provide an identification key.

2. Materials and Methods

2.1. Plant material

The materials included in this study were matched to descriptions of *A. borsczowii*, *A. speciosum*, *A. korshinskyi*, *A. pachystegium*, *A. adenophorum*, *A. lilacinum*, *A.brevibracteatum*, *A. diezianum*, *A. laxiusculum*, *A. squarrosum*, *A. heratense*. Only specimens with fully open flowers and mature leaves were included to allow standardized measurements to be made. Selected morphological characters were studied on 98 herbarium specimens. The majority of specimens are from Ferdowsi University of Mashhad Herbarium (FUMH), Herbarium of Mashhad School of Pharmacy and a few of them collected from around Khorassan provinces (appendix 1). Specimens borrowed from Herbarium of Mashhad School of Pharmacy, were without herbarium number.

2.2. Morphological characters

A total of 60 including 32 quantitatives and 28 qualitatives characters were examined on each specimen (Table 1). Qualitative characters were scored as binary or multistate characters. The published keys and descriptions of the genus [2, 11, 14], checked to establish characters that had previously been considered to be of taxonomic importance and some of characters were used in this investigation have not been employed in previous studies. Some characters such as stem length, despite of their taxonomic importance, were not measurable in herbarium sheets (e. g. stem length in *A. borsczowii* reaches to ca. 50 cm and herbarium sheets were incomplete in this feather). Instead of this character, the flowering branched length measured. In this investigation missing data replacement were made with the means of variables [15]. Table1. Qualitative and quantitative characters used in multivariate analysis of *Acanthophyllum* sect. *Oligosperma* followed by their abbreviations in brackets. The qualitative characters denoted by asterisks and character states and considered score are given in square brackets. All quantitative characters are in mm

Table1. Missing data replacement were made with the means of variables

Characters
1. Flowering branch height (FBHI)
2. *Plant state (PLST) [erect (0) / cushion-shape (1)]
3. Inflorescence diameter (INDI)
4. Lateral flower pedicel length (LFPE)
5. Style length (STLE)
6. Middle inflorescence pedicel length (MIPL)
7. Lateral inflorescence pedicel length (LIPL)
8. *Inflorescence shape (INSH) [bowl shape (0) / umbrella like (1)]
9. *Density of long glandular hairs on inflorescence pedicel (DLGP)

10. *Density of short glandular hairs on inflorescence pedicel (DSGP)
11. *Density of unicellular hair on pedicel (DUHP)
12. *Density of multicellular hair on pedicel (DMHP)
13. Floral leaves length (FLLE)
14. Floral leaves width (FLWI)
15. Maximum length/width ratio flower leaves (MLWF)
16. *Floral leaves shape (FLSH) [linear (1), lanceolate (2), ovate-lanceolate (3)]
17. Bract length (BRLE)
18. Bract width (BRWI)
19. Maximum length/width ratio bract (MLWB)
20. *Bract shape (BRSH) [linear (1), lanceolate (2), ovate-lanceolate (3)]
21. Bract length (BRLE)
22. Bracteole length (BTLE)
23. Bracteole width (BTWI)
24. Maximum length/width ratio bracteole (MLWT)
25. Petal length (PTLE)
26. Petal width (PTWI)
27. Maximum length/width ratio petals (MLWP)
28. Petals extra calyx length (PECL)
29. *Petal's colour at neck (PECN) [white (1), pink (2), lilac (3), purple (4)]
30. *Petal's colour at base (PECB) [white (1), pink (2), lilac (3), purple (4)]
31. Calyx length (CALE)
32. Calyx teeth length (CATL)
33. Calyx mucronum length (CAML)
34. *Calyx teeth shape (CATS) [triangular (1), narrow triangular (2)]
35. Calyx teeth size (CATE)
36. *Density of Long glandular hairs on calyx (DLGC)
37. *Density of short glandular hairs on calyx (DSGC)
38. *Density of unicellular hairs on calyx (DUSE)
39. *Density of multicellular hairs on calyx (DMSE)
40. Superior Leaves length (LELE)
41. Leaves width (LEWI)
42. Maximum length/width leaves ratio (MLWL)
43. Lower leaves length (LLEL)
44. *Density of Long glandular hairs on leaves (DLGL)
45. *Density of short glandular hairs on leaves (DSGL)
46. *Density of unicellular hairs on leaves (DUSL)
47. *Density of multicellular hairs on leaves (DMHL)
48. Superior leaves angle (SLAN)
49. Lower leaves angle (LLAN)
50. *Leaves colour (LECO) [Pallid (0), Green (1), brown (2), Grayish Green (3)]
51. Gemma length (GELE)
52. Internodes length (INLE)

53. *Stem colour (STCO) [Pallid (0), Green (1), brown (2), Grayish Green (3)]
54. *Density of long glandular hairs on stem (DLGS)
55. *Density of short glandular hairs on stem (DSGS)
56. *Density of unicellular hairs on stem (DUHS)
57. *Density of multicultural hairs on stem (DMHS)
58. * Swollen in the middle of calyx (SMCA) [clearly (1), unclearly (2)]
59. *Bracts spreading (BRSP) [horizontal (1), ascending (2), recurved (3), erect (4)]
60. *Superior flower leaves spreading (SFSP) [horizontal (1), ascending (2), recurved (3), erect (4)]

2.3. Measurement

All measurements except for hairs density were made on dried herbarium materials. For each specimen, three measurements were taken from mature flowers and calyces in the middle of the inflorescence. Mature leaves were measured from the second nodes of the shoot apex and internodes considered between the second and third leaves of the shoot apex. The second leaves from top were taken to measure superior leave's angle and for lower leaves were taken on the leaves at the base of the flowering branches, which defined on the scale of 0°–180°. In this survey, hairs density was considered as three states including $1<d<5$ scored as 1, $5<d<10$ scored as 2 and $d>10$ scored as 3, where "d" is the number of hairs in 1 mm² for the same magnification of all measurements.

2.4. Numerical methods

Ordination and cluster analysis are two keys approaches have been used in the numerical analyses [16]. The results of these investigations are based on Principal Component Analysis (PCA) and Cluster Analysis (CA). To ascertain evaluation relationship among taxa prior to doing PCA and CA, the data matrix was standardized to eliminate the results of characters with large variance.

2.5. PCA

Principal Component Analysis is an R-mode type of analysis, in which the relationship among characters is, assessed [17]. This technique was performed using CANOCO software version 4.5 [18]. For collection the characters that found to be functional in separating an apriori group several runs of PCA were carried out. In this analysis OTU'S were a total of 98 herbarium specimens and only those characters allow to be contributed that variability of the first three axes of the PCA ($r>0.5$) and had the least correlation coefficient ($r<0.5$) were used to differentiate specimens from each other.

2.6. CA

This analysis is a Q-mode type of analysis, in which the relationship between specimens is being, assessed [17]. This techniques first developed by the school of numerical taxonomic and numerical ecologists, later improved by other researchers in the physical sciences and humanities [15]. This technique carried out based on UPGMA method and Euclidian distance and was performed using Ntsys-pc software version 2 [19].

3. Results

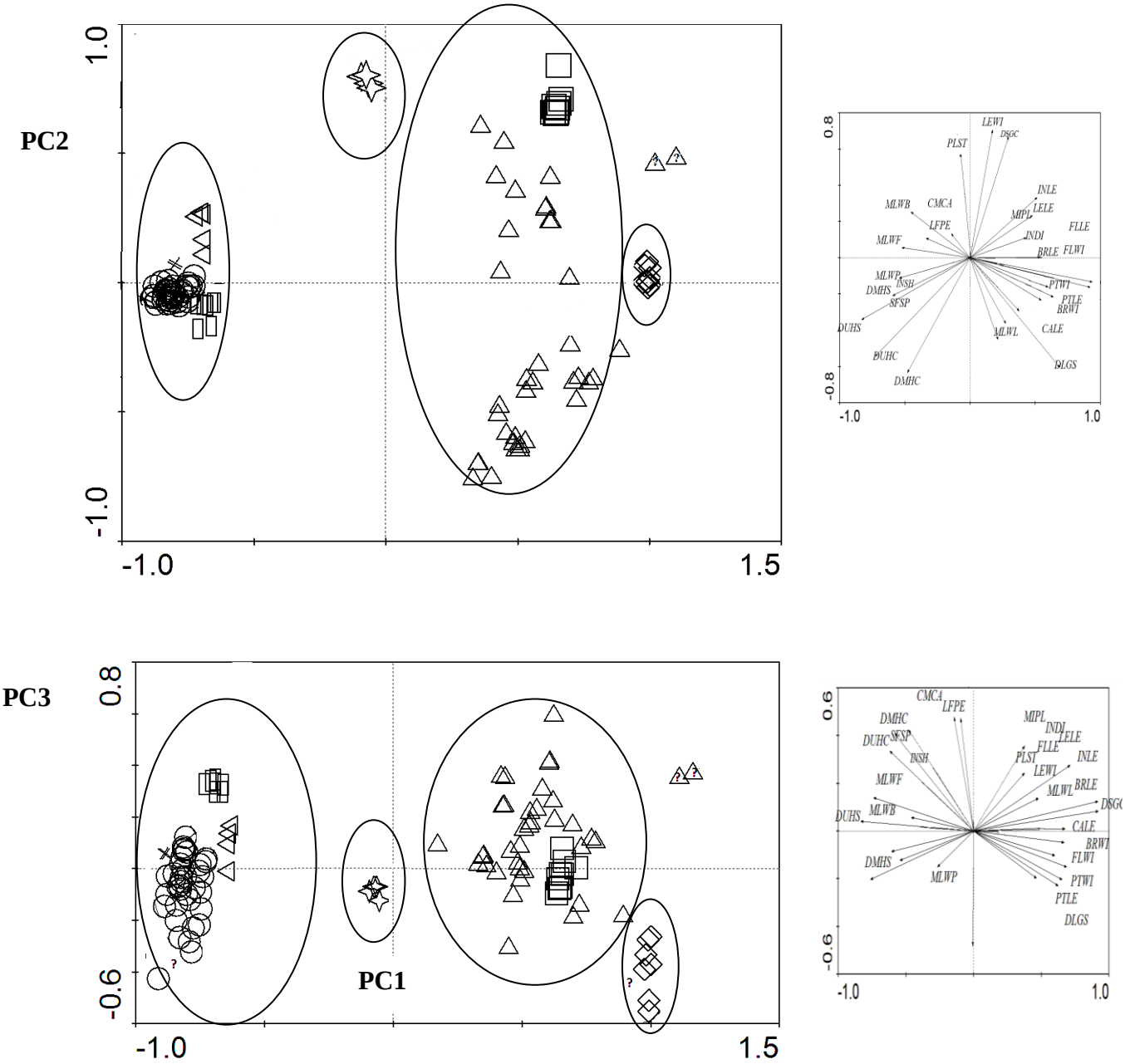
3.1. PCA

Using all 60 characters didn't produce distinct grouping between samples (data not shown). For establishing differentiation between OTU's, some characters that have no role in grouping were logically eliminated. Only characters that have high Eigen value on the first three Principal component (r>0.5) and had the least correlation coefficient (r< 0.5) were selected to separate OTU's (Table 2). The first three components explain 72.8 % of the total character variation 58 %, 11.7 % and 3.1 % for the respective axes. In a plot of the first and second PCs (Fig. 1 A); one group comprising OTU's of *A. korshinskyi*, *A. speciosum* and complex Adena have been formed an assemblage at the positive end of axis 1. While individuals of *A. laxiusculum*, *A. diezianum*, *A.lilacinum* and Squarra complex occupied the negative end. OTU's of *A. borsczowii* have an intermediate position between two axes (Fig. 1). However, Adena complex *A. korshinskyi*, *A. speciosum*, *A. laxiusculum*, *A. diezianum*, *A.lilacinum* had the greatest separation along the second PC. *Acanthophyllum korshinskyi* separated from Adena complex in first PC but along the third axes (Fig. 1B) *A. korshinskyi* has been fallen between Adena complex individuals. Two individuals of Adena complex 36220 (FUMH) and 36810 (FUMH) are misplace.

Table 1. Eigen vectors of the characters have been used on the first three axes in PCA1

NAME	AX1	AX2	AX3	AX4

5	FLWI		-0.4903	-0.1325	0.9412	-1.3364
6	MLWF	1.1692		0.5237	0.2170	0.9958
7	BRLE		0.5320	0.2509	0.6215	0.3858
8	BRWI		-0.3946	0.2128	1.1762	-1.0871
9	MLWB	1.1956		0.3275	0.0685	0.8789
10	BTLE		0.5516	0.4474	0.4311	0.5428
11	BTWI		-0.2636	-0.1427	-0.5087	-2.4745
12	MLWT	1.0580		0.5606	1.4102	1.4353
13	PTLE		0.1862	0.3679	0.8119	0.1192
14	PTWI		-0.0949	0.3692	0.4877	-0.6266
15	MLWP	0.7642		0.3671	0.8150	0.5069
16	PECL		0.0716	0.2121	0.4962	-0.2433
17	CALE		0.2357	0.4087	0.4866	0.3859
20	LELE	0.1270	-0.1924	0.6769	-0.2198	
21	LEWI		0.2929	-2.2073	0.6206	-1.0975
22	MLWL	0.2126		0.6268	0.5635	0.3058
23	PECO	0.6350	0.7725	0.3050	-0.0831	
26	INLE		-0.2424	-0.8046	0.5028	0.7967
29	DLGS		-4.3609	5.2887	-0.4606	1.7070
30	DSGS		-3.5313	0.2507	-1.2146	-2.4684
31	DUHS		4.6343	2.4232	-0.6074	1.0208
32	DMHS	7.8896		0.4151	-0.7624	0.2910
33	DLGC		-4.2392	4.4092	-2.3766	4.4756
34	DSGC		-3.5908	-4.9555	-0.2072	-1.7109
35	DUHC	2.6377		2.7565	-2.5798	-2.3570
36	DMHC		2.6553	2.9506	-3.3263	0.4804
37	INSH		1.0534	0.4115	0.2912	0.7692
39	PLST		0.8886	0.5739	0.4709	-0.5714
41	FBHI	0.7047	0.2679	0.0461	0.3358	
42	INDI		0.4986	0.1579	0.3711	0.1960
45	BRSP	0.1060	0.4653	0.4721	0.5013	
47	SFSP	0.0596	0.0801	0.4025	0.4026	
51	CMCA		0.1189	0.5057	0.5407	0.7391



△= Adena Complex, ◁ = *A. laxiusculum,* □= *A.diezianum,* ×= *A. brevibracteatum*

✧= ***A. borsczowii,*** ○ = **Squarra *complex*,** ◇= ***A. speciosum.***

Figure 1. Principal Component Analysis-Scatter diagram of specimens and characters from 98 specimens of *Acanthophyllum* sect. *Oligosperma* in Khorassan provinces. The ellipses encompass the suggestion series for this section in Khorrasan province

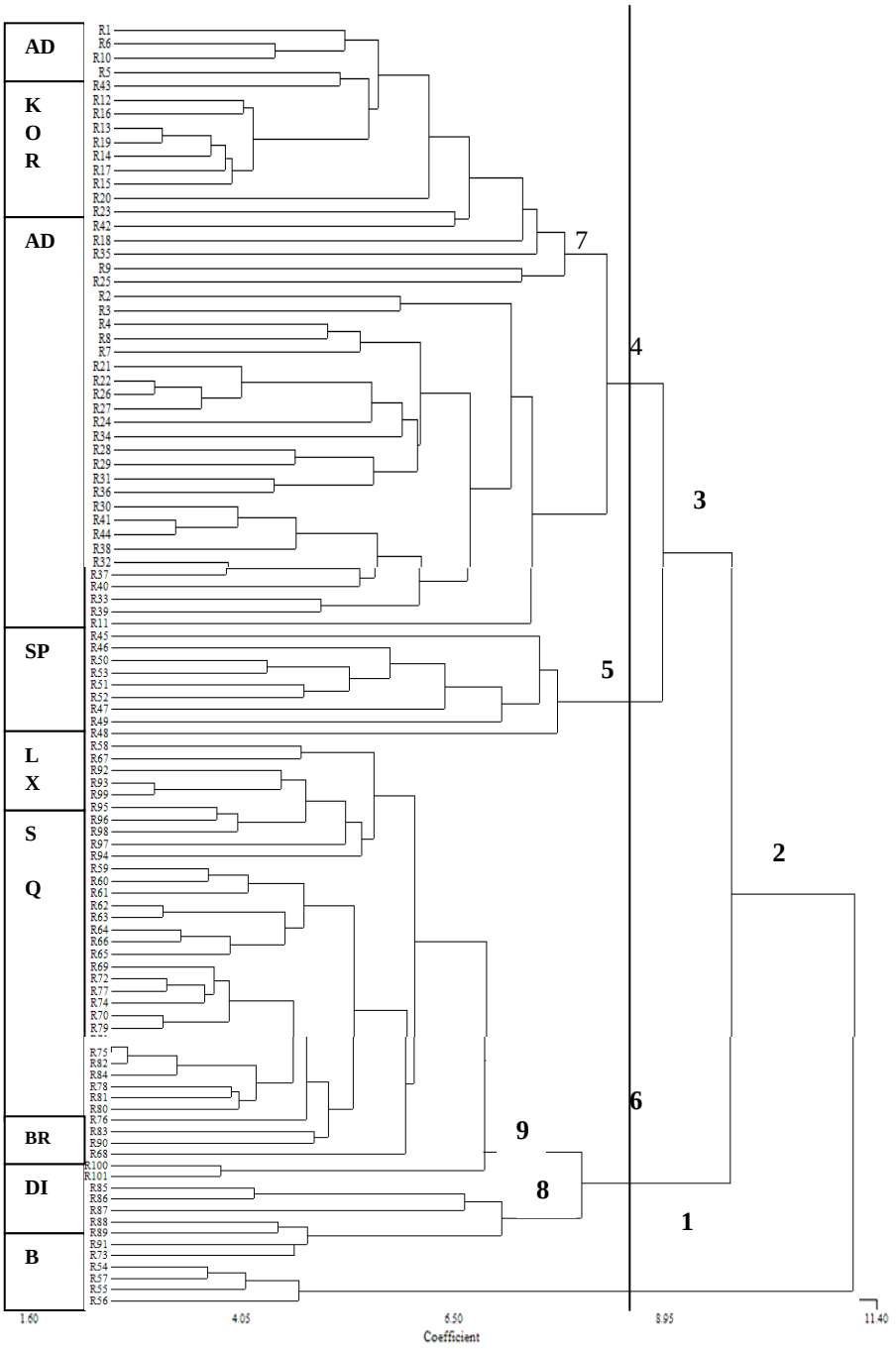


Figure 2. Phenogram resulting from the UPGMA of the *Acanthophyllum* sect. *Oligosperma* in khorassan provinces. OTU'S represented by AD= complex Adena, KOR= *A. korshinskyi*, SP= *A. speciosum*, DI= *A. diezianum*, SQ= complex Squarra, BR= *A. brevibracteatum*, LX= *A. laxiusculum*, B= *A. borsczowii*.

These samples have robust stature and are similar to *A.stenostegium* Freyn which belong to the flora of Afghanistan (the neighbor of Iran) and with having glandular hairs distinct from

that. Elements of Square complex formed a continuous range and samples have overlapped each other. In ordination between characters, elements of *A. speciosum* are well isolated by DLGS (Density of Long glandular hairs on stem), PTLE (Petal length), CALE (calyx length), FLWI (floral leaves width) and BRWI (bract width) (Table 1). Specimens of *A. borsczowii*, as was expected, sharply isolated from the rest by PLST (plant state) character. DSGC (density of short glandular hairs on calyx) character caused *A. korshinsky* separate from Adena complex. Characters LFPE (lateral flower pedicel lenght), SMCA (swollen in the middle of calyx) have been distinctive for *A. laxiusculum* from Squarra complex. *Acanthophyllum diezianum* have been separated by characters INSH (inflorescence shape), DMHC (density of multicellular hairs on calyx) from Square complex.

3.2. CA

The UPGMA of the OTU'S used in this study is shown in Fig 2. A small cluster of four elements including *A. borsczowii* emerges as a branch off the two primary groups (labeled1). *Acanthophyllum borsczowii* considered as sister group for the rest. In addition, the bigger cluster (labeled 2) divided to two branches (labeled 3and 6). Middle branch (labeled 5) excluded the elements of *A. speciosum* from the rest. The outcome indicates samples 18690 (FUMH) and 35634 (FUMH) that had a doubtful position fall between elements of *A. speciosum*. Specimens of *A. korshinskyi* added to three elements of Adena complex formed a subgroup that joined to individuals of complex Adena (labeled 7). OUT'S of *A. diezianum* (labeled 8) joined to elements of *A. brevibracteatum* (samples 43817 (FUMH) and 40772 (FUMH), that seems to be real *A. lilacinum*), *A.laxiusculum* and Squarra complex (labeled 9).

4. Conclusion and Discussion

The clusters clearly confirmed the obtained results from Principal Component Analysis. Multivariate analysis in this investigation (Fig. 1 and Fig. 2) with the exception of *A. borsczowii* explained two assemblages of species including Adena complex, *A. korshinskyi* and *A. speciosum* in the positive end of PC1 and bigger branch in CA (labeled 3). While negative end of PC1 and smaller branch in CA (labeled 6) occupied by Squarra complex, *A. laxiusculum*, *A. diezianum* and *A. lilacinum*. *Acanthophyllum borsczowii* in PCA (Fig. 1) contains intermediate position between two assemblages, and in CA excluded from the rest by having erect state, ovate-lanceolate leaves, glabrous leaves and stem that have not seen in the other species in this section except for *A. elatius* Bunge ex Boiss which didn't participate in this analysis. Based on Schiman-Czeika [2, 11], *A. borsczowii* is distinguished from *A. elatius* due to leaves width and length. With respect to the whole attempts that have been made to collect *A.elatius*; this species has not been reported from anywher, after the typus specimen reported, and existence of this species is doubtful. However, *A. borsczowii* is quite different from the rest, as Boissier [20] and Parsa [21] placed it in the sect. *Euacanthophyllum.* In *Acanthophyllum* genus appears character glandular hairs are linked with broader bract, bracteole, floral leaves and petals followed by longer calyces and petals. Species with these characters are often found in Northern parts of Khorassan provinces in less

dry climate and are growing in mountainous region. On the other hand, *Acanthophyllum* species which grow in southern parts of Khorassan provinces with drier climate and in desert regions possess simple hairs, narrower leaves, floral leaves, bracts, bracteoles and petals which followed by shorter calyces and petals. As have been shown by Meratan *et al*. [12], water and salt stresses are effective on seedling and growing parameters. Hence, ecological condition seems to have an important role in isolation and diversion in *Acanthophyllum* species. *Acanthophyllum lilacinum* in flora USSR placed in *squarra* series but in this study placed in *Adena* series (Fig. 1). This replacement is the result of conflict in feathure of this species in flora USSR and flora Iranica. We faced with this problem in *A. brevibracteatum* too. *Acanthophyllum brevibracteatum* in this investigation placed in *Squarrosa* series but in flora USSR sited in *Adenophora* series. In order to solve problems we need to observe the type specimens of these species. However, location of these species still remained uncertain. Although samples [(35634) (FUMH), (18690) (FUMH) identified as *A. speciosum*], in this analysis have fallen inter *A. speciosum* elements but, as clear in first and second PC (fig. 1B), they are a little apart from the other *A. speciosum* individuals. Likely they are in the initial stage of speciation way. Distinctive value of some morphological traits (such as swollen in the middle of calyx, flower and cympartial pedicel, width and length of leaf, type of hairs and etc.) that used in species delimitation is unclear, isolating range of these traits are ambiguous. As the results of the analysis have shown, close morphological traits are there in Adena complex and also in Squarra complex individuals. Despite the morphological homoplasy has been reported for *Caryophyllaceae* familly by Fior *et al.* [22], we have seen the high interspecies diversity and intermediate traits in *Acanthophyllum* genus, especially within Adena and Squarra complxes may be due to the influence of hibridization, poliploidy, B-choromosome or ecological stress, Similar evidences have been reported for other genera in the family *Caryophyllaceae* (e.g. Weiss *et al*. [23], Minder *et al*. [24].

5. Taxonomic conclusion

Identification key to *Acanthophyllum* sect. *Oligosperma* in Khorassan provinces

1. Erect, leaves shorter than internodes ……..series 1. ***Elatiora*** Schischk.

2. Shrub, leaves longer than internodes, plant covered with simple as well as glandular hairs or short glandular only……..…….series 2. ***Adenophora*** Schischk.

3- Shrub, leaves longer than internodes, petals longer than 16 mm (often longer than 20), plant coverd only with long glandular hairs (not papillose)……..……..….series 3 ***Speciosa*** Mahmoudi.

4- Shrub, leaves longer than internodes, plant coverd only with simple hairs …….....series 4**. *Squarrosa*** Schischk.

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Appendix 1

Herbarium No.	Locality	Altitude (m)	Date of collection	Taxa
31542	Khorassan: South East of Bojnurd, Nodeh to Esfidan	1500	10/6/1998	*A. adenophorum* Freyn
23578	Khorassan: North East of Bojnourd, between Ali Muhammad and Robat	1500	4/7/1993	*A. adenophorum* Freyn
40541	Khorassan: South of Bojnourd, Esfarayen road, 14 km from Bash Ghardash to Asadly	1737	31/5/2008	*A. adenophorum* Freyn
23088	Khorassan: Dargaz, south hills of Hesar	250	16/5/1993	*A. adenophorum* Freyn
SN	Khorassan: South west Bojnurd, Rein	1970	28/5/2010	*A. adenophorum* Freyn
23546	Khorassan: South of Bojnurd, Rakhtian to Hesar	1600	4/7/1993	*A. adenophorum* Freyn
38225	Khorassan: South West of Bojnourd	1970	3/7/2006	*A. adenophorum* Freyn
2405	Khorassan: Mashhad, East of Kalat-e Naderi	800	25/5/1994	*A. adenophorum*

				Freyn
36810	Khorassan: North of Mashhad, Kalat-e Naderi	1000	3/7/2005	*A. adenophorum* Freyn
24455	Khorassan: Bojnourd, Bdranlu	1900	10/7/1994	*A. adenophorum* Freyn
23428	Khorassan: Ghoochan, Bajgiran	1700	23/6/1993	*A adenophorum* Freyn
13079	Khorassan: Mashhad, North East of Kalat-e Naderi	1000	20/5/1985	*A. adenophorum* Freyn
40360	Khorassan: East of Bojnourd, Sisab	1362	27/5/2008	*A. adenophorum* Freyn
20553	Khorassan: South of Sabzevar, Hares Abad	800	1/6/1991	*A. borsczowii* Bunge ex Boiss.
38868	Khorassan: Sout East of Sabzevar between Shamakan and Yahya Abad	1097	21/5/2007	*A. borsczowii* Bunge ex Boiss.
32456	Khorassan: South West of Sabzevar, Parvand	800	17/5/1999	*A. borsczowii* Bunge ex Boiss.
34546	Khorassan: South West of Sabzevar, Parvand	900	21/5/2003	*A. borsczowii* Bunge ex Boiss.
25602	Khorassan: North East of Ghaen, Tikab	1200	28/5/1995	*A. korshinskyi* Schischk.
30825	Khorassan: East of Ghaen, Verzg to Verzgh	1600	20/5/1998	*A. korshinskyi* Schischk.
34249	Khorassan: East of Ghaen, Ghaleh Ahangaran mountain	1100	18/6/2002	*A. korshinskyi* Schischk.
SN	Khorassan: Mashhad, Ferdowsi University, Mashhad	---	10/6/2010	*A. korshinskyi* Schischk.
18586	Khorassan: Sarakhs, Madan darband road	500	20/5/1990	*A. korshinskyi* Schischk.
15359	Khorassan: Torbat Heydarieh, Sagh village	1800	9/6/1987	*A. korshinskyi* Schischk.
32422	Khorassan: South of Sabzevar, Dowlat Abad,	1000	16/5/1999	*A. korshinskyi* Schischk.
28826	Khorassan: Kashmar, Zarmehr to Ghaleh Jugh	1300	21/5/1991	*A. korshinskyi* Schischk.
38877	Khorassan: South East of	1155	21/5/2007	*A. korshinskyi*

	Sabzevar, Cheshmeh Avish			Schischk.
24041	Khorassan: Mashhad, North East of Kalat-e Naderi	1200	24/5/1994	*A. speciosum* Rech. f. & Schiman-Czeika
35448	Khorasan: Dargaz National Park, Tandureh, Chehel Mir	1032	31/5/2004	A. *speciosum* Rech. f. & Schiman-Czeika
35634	Khorassan: Dargaz, Tandureh National Park, Shekarab to Chehel Mir	1700–1900	26/6/2004	A. *speciosum* Rech. f. & Schiman-Czeika
28993	Khorassan: Mashhad, Kalat-e Naderi to Archangan	1100	1/6/1997	A. *speciosum* Rech. f. & Schiman-Czeika
18690	Khorassan: South of Dargaz, Doab	1400	29/5/1990	A. *speciosum* Rech. f. & Schiman-Czeika
27635	Khorassan: South West of Dargaz, Sanghez	1500–1800	1/7/1996	A. *speciosum* Rech. f. & Schiman-Czeika
27500	Khorassan: East of Ghochan, Goganlu mountain	1650	20/6/1997	A.*speciosum* Rech. f. & Schiman-Czeika
24337	Khorassan: 10 km from Imam Ghuli to Dargaz	1800	6/7/1994	A. *speciosum* Rech. f. & Schiman-Czeika
15422	Khorassan: Ghochan, Dargaz, Aghmazar hill	1850	30/6/1987	A. *speciosum* Rech. f. & Schiman-Czeika
30525	Khorassan: East of Bajestan, Hojat Abad to Helali	1250	9/5/1998	*A. laxiusculum* Schiman-Czeika
23505	Khorassan: Between Torbat Jam and Fariman, North of Zharf mountain	1950	25/6/1993	*A. laxiusculum* Schiman-Czeika
1085	Khorassan: 201 km from Zabol to Sefidabeh	550	10/5/1986	*A. laxiusculum* Schiman-Czeika
10943	Khorassan: Ozbako mountains	1000	1/5/1985	*A. laxiusculum* Schiman-Czeika
17703	Khorassan: Birjand, Ghaleh mountains	2000	12/6/1989	A. *laxiusculum* Schiman-Czeika
28575	Khorassan, South West of Gonabad, 4 km from Sano road	1400	20/5/1997	*A. laxiusculum* Schiman-Czeika

14246	Khorassan: Birjand, Shokra mountain	2000	18/5/1986	*A. laxiusculum* Schiman-Czeika
SN	Khorassan: South west of Bojnurd, Rein	1700	25/5/2002	*A. pachystegium* Rech. f.
27190	Khorassan: North of Bojnurd, 8 km from Chudar	1600	11/6/1996	*A. pachystegium* Rech. f.
11247b	Khorassan: East Sabzevar, Mount Baghjar	1400	27/5/1984	*A. pachystegium* Rech. f.
40660	Khorassan: South West Bojnurd, 13 km from Bash Ghardash	1785	2/6/2008	*A. pachystegium* Rech. f.
20758	Khorassan: North East Bojnurd, Gifan	1300–1400	16/6/1991	*A. pachystegium* Rech. f.
34771	Khorassan: North West Bojnurd, Turkmenistan border	900	16/6/2003	*A. pachystegium* Rech. f.
38975	Khorassan: North East Bojnurd, 8 km from Gifan Road	900	29/5/2007	*A. pachystegium* Rech. f.
SN	Khorassan: Dargaz, Aghmazar	1600	1/5/2002	*A. pachystegium* Rech. f.
40477	Khorassan: West Bojnourd, Badranloo	1633	28/5/2008	*A. pachystegium* Rech. f.
33994	Khorassan: South of Digrostam, Lut desert	900	16/4/2002	*A. pachystegium* Rech. f.
31026	Khorassan: Bojnourd, 75 km from Bojnourd to Almeh	1250	28/5/1997	*A. pachystegium* Rech. f.
33702	Khorassan: North West of Bojnourd, between Goinik and Baghlagh	1400	11/6/2001	*A. pachystegium* Rech. f.
26895	Khorassan: North West Gonabad	1300	14/5/1997	*A. heratense* Schiman-Czeika
28506	Khorassan: Bajestan, 16 km from Bajestan to Ghasem Abad	1700	19/5/2002	*A. heratense* Schiman-Czeika
34124	Khorassan: 25 km from Bajestan to Ferdows	1700	19/5/2002	*A. heratense* Schiman-Czeika
34148	Khorassan: Between Gonabad and Kakhak	1250	21/5/2002	*A. heratense* Schiman-Czeika
11247a	Khorassan: East of Sabzevar, Baghjar mountain	1400	27/5/1984	*A. heratense* Schiman-Czeika
11353	Khorassan: 5 km from Jajarm to Garmeh	1000	30/5/1989	*A. heratense* Schiman-Czeika
21891	Khorassan: Taibad, Polband Barrier	1050	26/5/1992	*A. heratense* Schiman-Czeika
30561	Khorassan: Neyshabor, Sabzevar	1200	1/6/1976	*A. heratense* Schiman-Czeika

23513	Khorassan: Between Torbat Heydarieh and Fariman, Kallehmanar	1850	25/6/1993	*A. heratense* Schiman-Czeika
32019	Khorassan: West of Tabas, Darin	900	14/4/1999	*A. heratense* Schiman-Czeika
11255	Khorassan: East Sabzevar, Baghjar mountains	1400	27/5/1984	*A. heratense* Schiman-Czeika
32225	Khorassan: South East of Gonabad	1400	2/5/1999	*A. heratense* Schiman-Czeika
37462	Khorassan: South East of Gonabad, Kabotarkoh	1533	12/5/2006	*A. heratense* Schiman-Czeika
17664	Khorassan: Ghaen, Birjand after Khonik	1850	11/6/1989	*A. heratense* Schiman-Czeika
16580	Khorassan: South West of Bojnourd, between Sankhast and Khorashah	850	15/5/1988	*A. heratense* Schiman-Czeika
SN	Khorassan: Torbat Heydarieh, Robatsefid	1100	18/5/2002	*A. heratense* Schiman-Czeika
24197	Khorassan: North East of Birjand, Rack to Tazian	1900	14/6/1994	*A. heratense* Schiman-Czeika
23505	Khorassan: Between Torbat Heydarieh and Fariman, Zharf mountains	1950	25/6/1993	*A. heratense* Schiman-Czeika
43145	Khorassan: Torbat Jam, Saleh Abad, North of Kalkrab	1200	17/6/2009	*A. maimanense* Rech. f. & Schiman-Czeika
5311G	Khorassan: North East of Bojnurd, Naveh to ghatlish, 3 km to Izman	1900	3/6/1991	*A.lilacinum* Schischk.
42945	Khorassan: Dargaz, 2 km from Dihesar to Lotf Abad	1000	21/5/2006	*A.lilacinum* Schischk.
40779	Khorassan: North of Bojnurd, Ghezelghan	1430	11/6/2008	*A.lilacinum* Schischk.
15422	Khorassan: North West of Bojnourd, Garglan region	1850	30/6/1987	*A.lilacinum* Schischk.
31158	Khorassan: Ghochan, Dargaz, Aghmazar	1500	31/5/1998	*A.lilacinum* Schischk.
30997	Khorassan: Bojnourd, Aghtapeh	1600	28/5/1998	*A.lilacinum* Schischk.
30692	Khorassan: Bojnourd, Aghtapeh	1500	12/5/1998	*A.lilacinum* Schischk.
23405	Khorassan: East of Ferdows, Sarand to Bajestan	1800	20/7/1993	*A. squarrosum* Boiss.

30751	Khorassan: Between Gonabad and Ferdows, Cheshmehmahi	1300	13/5/1985	*A. squarrosum* Boiss.
30764	Khorassan: 18 km from Gonabad to Kakhak	1700	19/5/1985	*A. squarrosum* Boiss.
SN	Khorassan: North West of Ghaen Road, 6 km from Karghnd village	1200	12/5/2002	*A. squarrosum* Boiss.
12818	Sistan, Chahekhorma	1200	13/5/1985	*A. squarrosum* Boiss.
35412	Khorassan: Gonabad, between Zibod and Darsofeh	850	25/5/2004	*A. squarrosum* Boiss.
32465	Khorassan: East of Jajarm, between Khorashah and Jorbat	1000	17/5/1999	*A. squarrosum* Boiss.
11113	Khorassan: South West Sabzevar, Parvand mountain	1600	16/5/1985	*A. squarrosum* Boiss.
289G	Khorassan: Torbat Heydarieh, Segholle mountain	1100	19/5/1981	*A. squarrosum* Boiss.
38931	Khorassan: Kalat-e Karchaki	1500	25/5/2007	*A.Diezianum* Hand-Mzt.
30872	Khorassan: Kashmar, Chalpoo	1750	22/5/1998	*A. Diezianum* Hand-Mzt.
20667	Khorassan: North West of Ghaen, 12 km from Grymnij to Behyod	2000	9/6/1991	*A. Diezianum* Hand-Mzt.
32615	Khorassan: Between Mashhad and Torbat Heydarieh, Robat Sefid mountains	1700	25/5/1999	*A. Diezianum* Hand-Mzt.
23879	Khorassan: North of Kashmar, 10 km from South Ataieh	1200	11/5/1994	*A. Diezianum* Hand-Mzt.
20706	Khorassan: North of Torbat Jam, between Timnak and Dosangeh	2000	12/6/1991	*A. Diezianum* Hand-Mzt.
34622	Khorassan: North West of Torbat Heydarieh, Kadkan, Burs mountain	1500	4/6/2003	*A. Diezianum* Hand-Mzt.
43817	Khorassan: South of Mashhad, 10 km from Robat Sefid	1534	7/6/2010	*A. Diezianum* Hand-Mzt.
40772	Khorassan: West of Bojnurd, Ghorkhord Protected Area	1430	11/6/2008	*A. brevibracteatum* Lipsky
31542	Khorassan: North West Bojnurd, Jargalan area	1500	10/6/1998	*A. brevibracteatum* Lipsky