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# 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 quantitative and 28 qualitative characters were examined on 98 herbarium specimens. The results explained morphological treats are useful to discriminate taxa. Phenogram and scatter gram 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 detailed morphological study in field and molecular analysis could solve the problem of theses complexes.

**Key words:** Cluster Analysis, Principal Component Analysis, Acanthophyllum, Caryophyllaceae, Khorassan provinces



## 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 ($2n =$

2*x* = 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 karyotype 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 (2*n* = 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 (2*n* = 6*x* = 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* (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 quantitative and 28 qualitative

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). 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.

**Table 1. 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 color at neck (PECN) [white (1), pink (2), lilac (3), purple (4)] |
| 30. *Petal's color 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 color (LECO) [Pallid (0), Green (1), brown (2), Grayish Green (3)]
51. Gemma length (GELE)
52. Internodes length (INLE)
53. *Stem color (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 apriority 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).

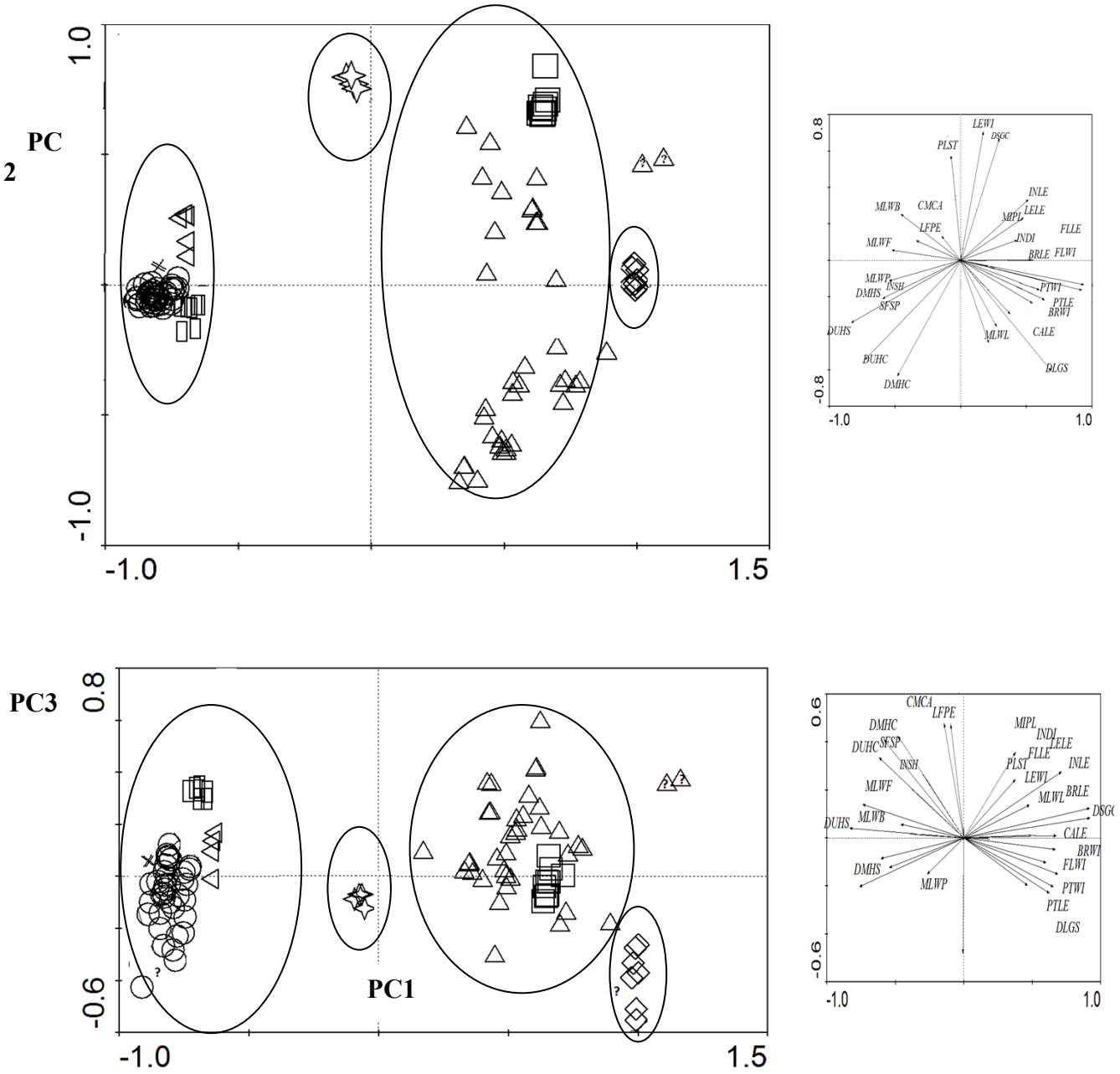
## 3. RESULTS AND DISCUSSION

*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 (Figure 1); 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 (Figure 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 (Figure 2) *A. korshinskyi* has been fallen between Adena complex individuals. Two individuals of Adena complex 36220 (FUMH) and 36810 (FUMH) are misplace.

**Table 2. 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 Khorasan provinces. The ellipses encompass the suggestion series for this section in Khorasan province**

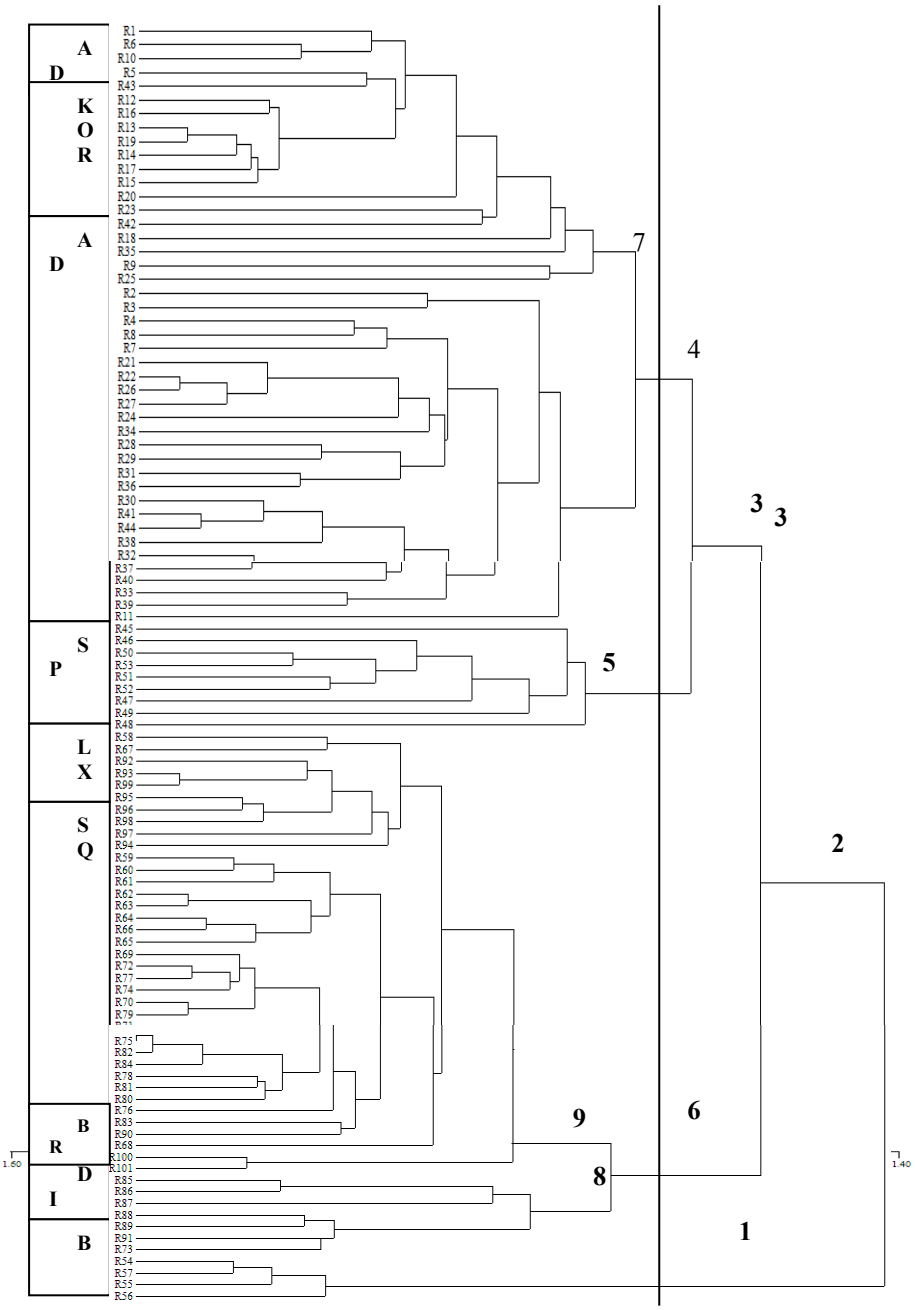



**Figure 2. Phenogram resulting from the UPGMA of the *Acanthophyllum* sect. *Oligosperma* in khorassan provinces. OTU'S represented by AD= complex Adena, KOR= *A. korshinskyi*, SP= *A. speciosum*, DI= *A. diezianum*, SQ= complex Squarra, BR= *A. brevibracteatum*, LX= *A. laxiusculum*, B= *A. borsczowii***

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 Figure 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). The clusters clearly confirmed the obtained results from Principal Component Analysis. Multivariate analysis in this investigation (Figure 1 and Figure 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 (Figure 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 Khorasan provinces in less dry climate and are growing in mountainous region. On the other hand, *Acanthophyllum* species which grow in southern parts of Khorasan 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 (Figure 1). This replacement is the result of conflict in feature of this species in flora USSR and flora Iranica. We faced 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 (Figure 1), 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 high interspecies diversity and intermediate traits in *Acanthophyllum* genus, especially within Adena and Squarra complxes may be due to the influence of hybridization, polyploidy, B-chromosome 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).

## 4. CONCLUSION

### Taxonomic conclusion

Identification key for *Acanthophyllum* sect. *Oligosperma* in Khorasan 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 covered 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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The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

## AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

### 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 Sabzevar, Cheshmeh Avish | 1155 | 21/5/2007 | *A. korshinskyi* 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. & |

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|---|---|---|---|---|
| | | | | 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. |

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| 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 |

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