# A morphometric study of the annual species of *Alyssum* (*Brassicaceae*) in Iran based on their macro- and micro-morphological characters

**Saman Bolourian & Maneezhe Pakravan**

Biology Dept., Faculty of Science, Alzahra University, Vanak, Tehran, Iran, email: saman.bolourian@yahoo.com (corresponding author)

Received: June 14, 2011 ▷ Accepted: November 15, 2011

**Abstract.** Phenetic traits of nine species and three varieties belonging to the genus *Alyssum* (*Brassicaceae*) were studied in 55 populations from different localities of Iran. Macro- and micro-morphological characters were analyzed quantitatively and qualitatively and the results of UPGMA and WARD clustering methods were compared. The factor analysis of morphological characters has shown that the reproductive structure is more important than the vegetative characters. Contrary to the morphological analysis, the anatomical studies of stem cross sections in four problematic species have proved unable in distinguishing *Alyssum contemptum*, *A. marginatum*, *A. strictum* and *A. szovitsianum* from each other.

**Key words:** *Alyssum*, *Brassicaceae*, Iran, phenetic traits

## Introduction

*Brassicaceae* (*Cruciferae*) is one of the largest Angiosperm families, comprising 338 genera and 3709 species (Warwick & al. 2006). *Alyssum* L. is one of many bi-regional genera that gives the impression that the Irano-Turanian territories are its main developmental area and the Mediterranean region its secondary area (Hedge 1976). *Flora Iranica* has reported 30 species of the genus *Alyssum* from Iran (Rechinger 1968). Since its publication, new species and records have been added to the Iranian flora. There have also been some nomenclature changes and reductions in synonymy. Altogether, the changes have led to an increase in the total number of species to *ca*. 42 (Akhani 2003). Due to the polymorphism of morphological characters, *Alyssum* seems to be one of the most problematic genera of this family in Iran, in which the boundaries of certain species are not quite distinct (Aryavand 1996). The *Alyssum* species collected from Iran have always shown complications and consequently the herbarium specimens might be renamed by later researchers. Most researches in Iran have been limited so far to reporting new species in this genus. There have been few comprehensive studies of the *Alyssum* genus and its species boundaries in Iran. The current study was conducted in order to delimit the species from different sections by means of macro- and micro-morphological characters. The number of characters and populations used has increased the accuracy of methods, and has helped in finding out possible overlapping characters or high levels of variety. Also, we have compared by stem cross sections the anatomical relationship between *A. contemptum*, *A. marginatum*, *A. strictum*, and *A. szovitsianum*. Combining stem cross sections with other methods have proved helpful in the earlier studies of this genus (Pakravan & al. 2011).

## Material and methods

Herbarium specimens, or freshly collected plants from 55 populations belonging to 12 annual taxa (*A. alyssoides* (L.) L., *A. contemptum* Schott & Kotschy, *A. dasycarpum* Stephan ex Willd., *A. desertorum* Stapf, *A. linifolium* Stephan ex Willd., *A. marginatum* Steud., *A. minus* (L.) Rothm. var. *minus*, -var. *micranthum* (C.A. Mey.) T.R. Dudley, -var. *mazandaranicum* Pakravan & Bolourian, -var. *strigosum* (Banks & Soland) Zohary, *A. strictum* Willd. and *A. szovitsianum* Fisch. & C.A. Mey.) from different localities were studied out (Fig. 1, Table 1). Each population was encoded as a single Operational Taxonomic Unit (OTU). Ten plants were taken from each locality and used for morphological studies. Voucher specimens of the collected plants are deposited in Alzahra University Herbarium (ALUH).

Twenty-six qualitative/quantitative morphological characters, consisting of vegetative and reproductive (flower and silicule) structures, were assessed (Table 2). The qualitative characters were coded as binary/multi-state characters and the quantitative characters means were also coded as multi-state characters.

In order to group the OTUs with morphological similarities and to find the species relationships, a cluster analysis with minimum variance spherical clusters (Ward) and Unweighted Paired Group Mean Average (UPGMA) was performed (Ingrouille 1986). A factor analysis based on the Principle Component Analysis (PCA) was used to identify the most variable characters among the studied species. SPSS ver.17 (2008) software was applied for statistical analysis.

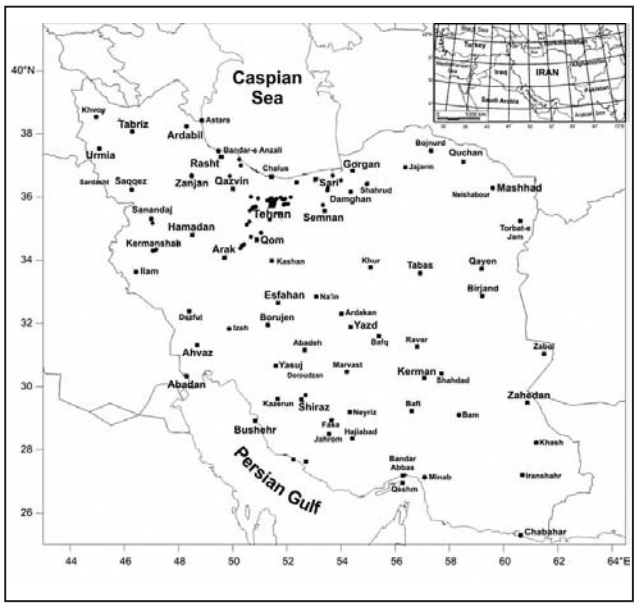



**Fig. 1.** Geographical distribution of the 55 investigated populations.

Specimens for anatomical studies were soaked in boiling water and glycerol. Transverse sections of stem were prepared by hand cutting. Bleaching, safranin and methyl green staining were used to distinguish the tissues during microscopic examinations and the sections were mounted in glycerine. All microscopic observations were made with an Olympus B×51 light microscope.

**Table 1. Collection data of populations of *Alyssum* L. species used in this study (ALUH: Alzahra University Herbarium, TUH: Tehran University Herbarium).**

| Collector | Locality | Voucher No | Taxa |
|---|---|---|---|
| El-Shehbaz | Iran, Qom province, Salafchegan towards Araq | 12555-TUH | *Alyssum alyssoides* |
| Bolourian | Iran, Tehran province, Darabad, 1800m | 5026-ALUH | *A. contemptum* |
| Bolourian-Rastipisheh | Iran, Tehran province, Tochal, 2890m | 5034-ALUH | |
| Keshavarzi | Iran, Tehran province, Kan, 2116m | 5045-ALUH | |
| Falaturi | Iran, Markazi province, Tehran to Saveh, 40km before Saveh, 1260m | 5048-ALUH | *A. dasycarpum* |
| Bolourian | Iran, Qom province, Qom road to Araq, after Imamzadeh Jafar, 1740m | 5015-ALUH | |
| Falaturi | Iran, Tehran province, Mahdasht to Eshtehard road, 26km before Eshtehard, 1090m | 5049-ALUH | |
| Nataj | Iran, Mazandaran province, Galugah, 9km after Sorkhe geriveh | 5054-ALUH | *A. desertorum* |
| Bolourian | Iran, Qom province, Qom road to Arak, After Imamzadeh Jafar, 1740m | 5016-ALUH | |
| Bolourian | Iran, Tehran province, Shahre Jadeed Pardis, 1600m | 5003-ALUH | |
| Bolourian | Iran, Tehran province, Shahre Jadeed Pardis, 1600m | 5009-ALUH | |
| Bolourian | Iran, Tehran province, Chalous, Rineh | 5011-ALUH | |

**Table 1. Continuation.**

| Collector | Locality | Voucher No | Taxa |
|---|---|---|---|
| Bolourian | Iran, Tehran province, Darabad, 1800m | 5027-ALUH | |
| Bolourian | Iran, Tehran province, Lavasan, Naran, 1780m | 5029-ALUH | |
| Bolourian | Iran, Tehran province, Lavasan to Afjeh, 1800m | 5033-ALUH | |
| Keshavarzi | Iran, Tehran province, Kan, 2116m | 5041-ALUH | |
| Falaturi | Iran, Tehran province, Haraz road, after Pardis, Kamard, 1460m | 5043-ALUH | |
| Falaturi | Iran, Tehran province, Karaj, Mahdasht, 1120m | 5051-ALUH | |
| Falaturi | Iran, Tehran province, Mahdasht road, before Eshtehard, 1400m | 5052-ALUH | |
| Falaturi | Iran, Tehran province, 15km to Abe-ali | 5053-ALUH | |
| Rastipisheh | Iran, Fars province, 35km before Marvdasht, Pasargad | 5039-ALUH | *A. linifolium* |
| Bolourian | Iran, Gillan province, Kuhgir Sofla, 800m | 5001-ALUH | |
| Nataj | Iran, Mazandaran province, Galugah, 9km after Sorkhe geriveh | 5056-ALUH | |
| Bolourian | Iran, Qom province, Qom road to Arak, Jafarieh, 1080m | 5014-ALUH | |
| Bolourian | Iran, Qom province, Qom road to Arak, after Imamzadeh Jafar, 1740m | 5017-ALUH | |
| Bolourian | Iran, Tehran province, Shahre Jadeed Pardis, 1600m | 5002-ALUH | |
| Bolourian | Iran, Tehran province, Lavasan to Afjeh, 1850m | 5005-ALUH | |
| Bolourian | Iran, Tehran province, Lavasan to Afjeh, 1840m | 5007-ALUH | |
| Bolourian | Iran, Tehran province, Sohanak natural Park, 2020m | 5008-ALUH | |
| Bolourian | Iran, Tehran province, Shahre Jadeed Pardis, 1600m | 5010-ALUH | |
| Falaturi | Iran, Tehran province, Mahdasht to Eshtehard, 1090m | 5050-ALUH | |
| Falaturi | Iran, Tehran province, Ziaran to Taleghan, 2000m | 5061-ALUH | |
| Bolourian | Iran, Markazi province, Tehran to Saveh, 1480m | 5021-ALUH | *A. marginatum* |
| Bolourian | Iran, Qom province, Tehran to Qom motorway, 80km before Qom, 1160m | 5013-ALUH | |
| Pakravan | Iran, Semnan province, Shahmirzad | 5035-ALUH | |
| Bolourian | Iran, Tehran province, Lavasan to Afjeh, 1800m | 5030-ALUH | *Alyssum minus* var. *minus* |
| Gholami | Iran, Tehran province, Shahrestanak | 5062-ALUH | |
| Jahandideh | Iran, Kermanshah province, Kouhestan Park | 5038-ALUH | -var. *mazandaranicum* |
| Nataj | Iran, Mazandaran province, Galugah, Sorkhegeriveh village, 1917m | 5058-ALUH | |
| Nataj | Iran, Gillan province, Espili road, Rudsar | 5059-ALUH | -var. *micranthum* |
| Bolourian | Iran, Tehran province, Darabad, 1800m | 5023-ALUH | |
| Bolourian | Iran, Tehran province, Darabad, 1800m | 5025-ALUH | |
| Bolourian | Iran, Tehran province, Lavasan, Naran road, 1780m | 5028-ALUH | |
| Bolourian | Iran, Tehran province, Lavasan, 1800m | 5031-ALUH | |
| Bolourian | Iran, Tehran province, Lavasan to Afjeh, 1800m | 5032-ALUH | |
| Bolourian | Iran, Tehran province, Abe-ali, 2140m | 5037-ALUH | |
| Falaturi | Iran, Tehran province, Ziaran to Taleghan, 2100m | 5060-ALUH | |
| Bolourian, Hariri, Noghanian | Iran, Gillan province, Garmabdasht, 700m | 5000-ALUH | -var. *strigosum* |
| Nataj | Iran, Mazandaran province, Sari road, Kiasar | 5057-ALUH | |
| Jahandideh | Iran, Kordestan province, Sannandaj, Abidar | 5047-ALUH | *A. strictum* |
| Falaturi | Iran, Tehran province, Lavasan, Tapehsorkh road, 1700m | 5022-ALUH | |
| Bolourian | Iran, Tehran province, Lavasan, Naran road, 1780m | 5046-ALUH | |
| Bolourian | Iran, Qom province, Qom-Arak road, after Imamzadeh Jafar, 1740m | 5018-ALUH | *A. szowitsianum* |
| Bolourian | Iran, Qom province, Qom-Arak road, after Dizijan, Besharat Abad, 1780m | 5020-ALUH | |
| Bolourian | Iran, Tehran province, Tehran-Qom road, before Hasan Abad, 1260m | 5012-ALUH | |

**Table 2. Macro- and micro-morphological characters and their coding range (silicule length – sl, silicule width – sw, style length – stl).**

| Characters | Code | Characters | Code |
|---|---|---|---|
| 1 – life form | 1 – Annual<br>2 – Biennial | 14 – silicule length – sl (mm) | 1 – 2 ≥ sl<br>2 – 2.5 ≥ sl > 2<br>3 – 3 ≥ sl > 2.5<br>4 – 3.5 ≥ sl> 3<br>5 – 4 ≥ sl > 3.5<br>6 – 4.5 ≥ sl > 4<br>7 – sl > 4.5 |
| 2 – base of plant | 1 – not herbaceous<br>2 – herbaceous | | |
| 3 – stem form | 1 – simple<br>2 – branched<br>3 – both | 15 – silicule width – sw (mm) | 1 – 2 ≥ sw<br>2 – 2.5 ≥ sw > 2<br>3 – 3 ≥ sw > 2.5<br>4 – 3.5 ≥ sw > 3<br>5 – 4 ≥ sw > 3.5<br>6 – 4.5 ≥ sw > 4<br>7 – sw > 4.5 |
| 4 – form of inflorescence | 1 – raceme<br>2 – raceme-cylindrical | | |
| 5 – raceme length change | 1 – elongated after fruiting<br>2 – without change<br>3 – condensed after fruiting | 16 – silicule apex | 1 – obtuse<br>2 – retuse<br>3 – acute<br>4 – obliterate |
| 6 – duration of sepal | 1 – always persistent<br>2 – persistent<br>3 – deciduous | 17 – silicule indumentum | 0 – absent<br>1 – present<br>2 – sometimes present |
| 7 – form of petal | 1 – gradually attenuated<br>2 – oblong<br>3 – constricted in middle<br>4 – spathulate | 18 – simple stellate trichome | 0 – absent<br>1 – present |
| | | 19 – intermediate stellate trichome | 0 – absent<br>1 – present |
| 8 – petal tip | 1 – truncate<br>2 – emarginate<br>3 – cleft | 20 – compound stellate trichome | 0 – absent<br>1 – present |
| 9 – simple filament | 0 – absent<br>1 – present | 21 – dentritic trichome | 0 – absent<br>1 – present |
| 10 – filament teeth | 0 – absent<br>1 – present | 22 – stigma | 1 – diffuse<br>2 – lobed |
| 11 – filament wings | 0 – absent<br>1 – present | 23 – style length – stl (mm) | 1 – 0.5 ≥ stl<br>2 – 1 ≥ stl > 0.5<br>3 – 1.5 ≥ stl > 1<br>4 – stl > 1.5 |
| 12 – form of silicule | 1 – orbicular<br>2 – ovate<br>3 – obovate<br>4 – elliptic<br>5 – rotund-turgid | 24 – style indumentum | 0 – absent<br>1 – covering the style<br>2 – present at the base<br>3 – scattered |
| 13 – silicule valve | 0 – not inflated<br>1 – one valve inflated<br>2 – both valves inflated | 25 – placentation form | 1 – lateral<br>2 – subapical |
| | | 26 – wing of seed | 0 – absent<br>1 – present<br>2 – sometimes present |

## Results

Depending on the ecology of the area the plants were collected from, most vegetative characters were too variable between populations. Therefore, in order to lower possible errors, characters dependant on ecological factors or altitude change (i.e. plant height, leaf size) were excluded from the final analysis. Factor analysis based on PCA of morphological characters among the species has revealed that the first three factors comprise about 63 % of the total variance. The first factor (comprising about 27.7 % of the total variance) that includes silicule width, style indumentum, form of silicule valve, structure of filament, and placentation form showed the highest correlation. The second factor (comprising about 21.3 % of the total variance) includes petal tip and silicule indumentums, and the third factor (comprising about 15.3 % of the total variance) is the presence of sepal. Thus, we may consider the characters mentioned in the first three factors as the most variable morphological characters among the studied annual *Alyssum* taxa. These morphological characters delimit the studied annual species. Ordination of the species collected from various localities based on the first two axes of principal components of their studied characters is presented in Fig. 2. Two observable groups consisting of *A. minus* varieties (previously studied by Pakravan & al. 2011), and the four species *A. marginatum*, *A. szovitsianum*, *A. contemptum*, and *A. strictum* were distinguished from other annual species of *Alyssum*.

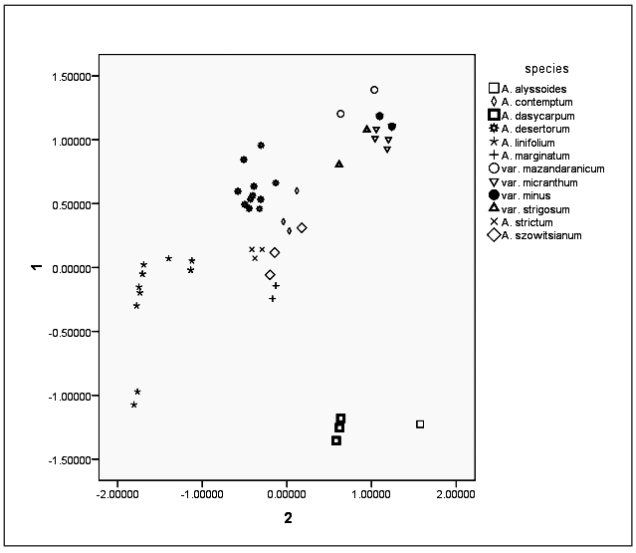



**Fig. 2.** Ordination of *Alyssum* specimens from 55 populations on the first two principal components axes.

The UPGMA method showed better results in major clusters, when compared with Ward. Despite the differences observed in the major clusters in each analysis, the species were delimited similarly by both methods. The UPGMA method produced three clusters. The first UPGMA cluster (Fig. 3) comprised all species populations classified in the section *Alyssum*. The second cluster was formed by *A. linifolium* populations that belong to the section *Meniocus*. A large number of intermediate specimens were found in each population of *A. linifolium* and the characters defined for each variety overlapped. The third cluster included *A. dasycarpum* and *A. alyssoides*, two species from the *Psilonema* section.

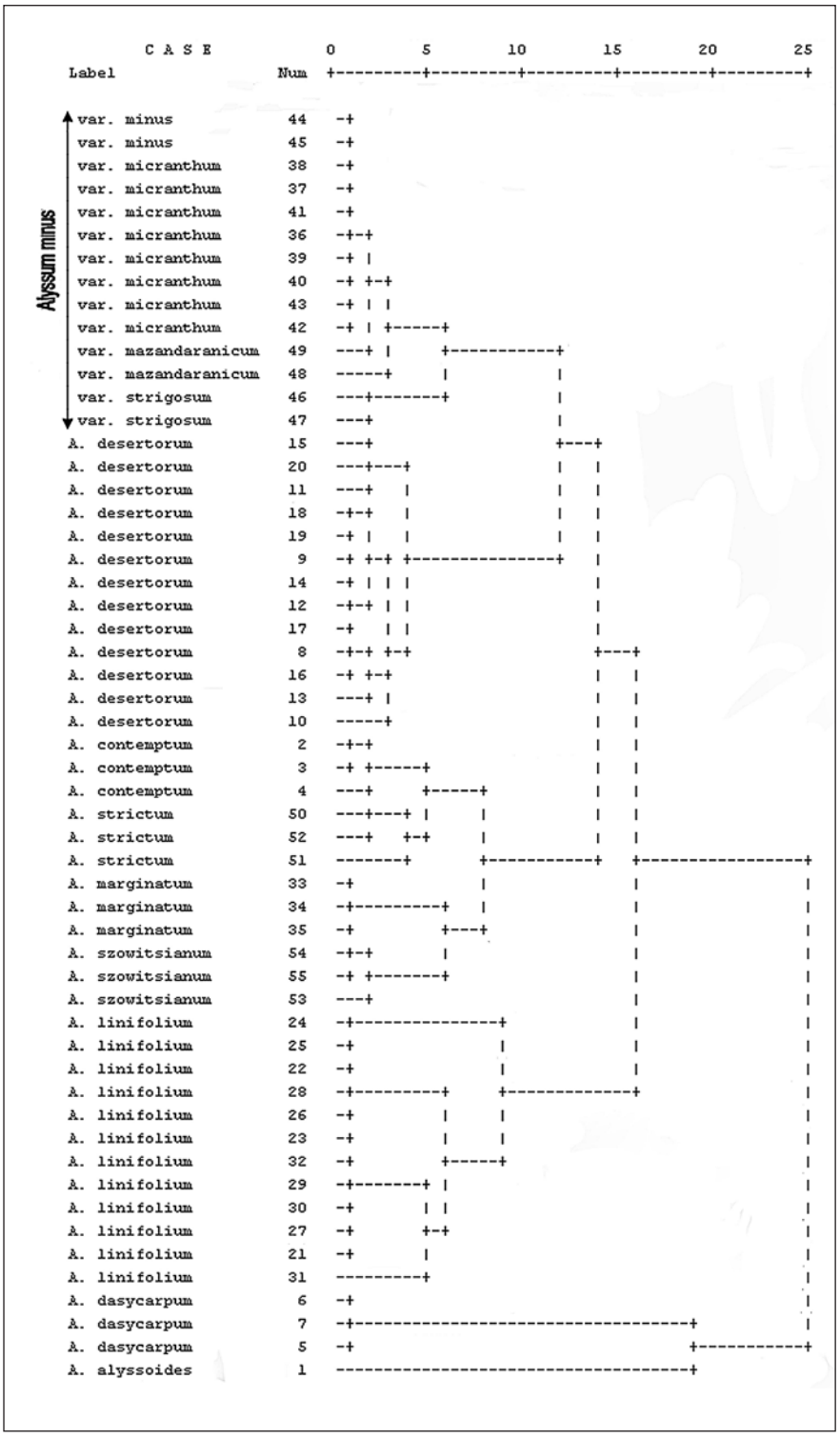
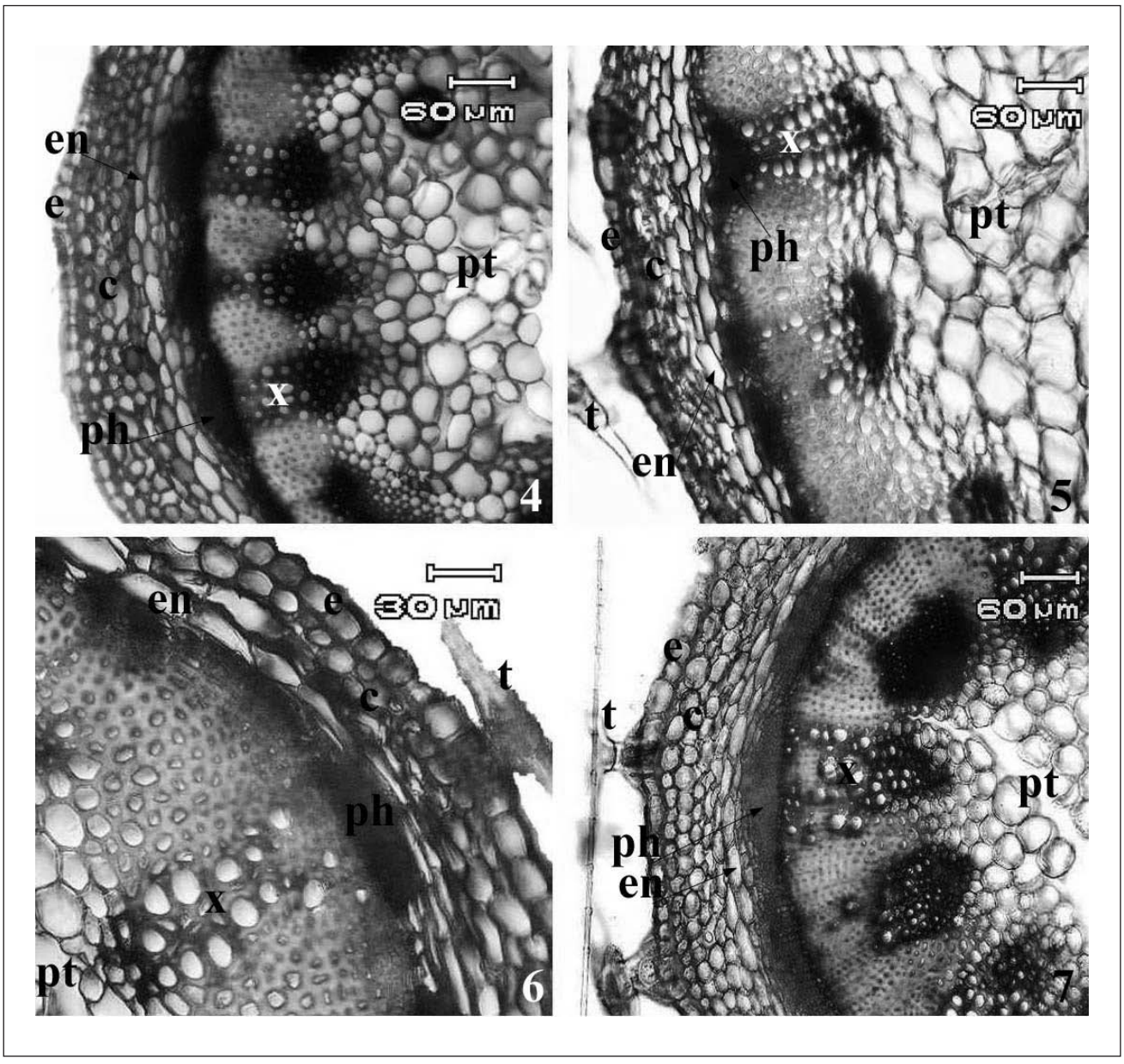



**Fig. 3.** Phenogram using the UPGMA method based on macro- and micro-morphological characters in nine annual *Alyssum* species.

In minor clusters during both analyses the four species *A. marginatum*, *A. szovitsianum*, *A. contemptum*, and *A. strictum* were delimited from other annual species of *Alyssum*, showing their extremely close morphological and micromorphological resemblance. In order to find possible diagnostic characters so as to delimit these close species, the anatomical characteristics were also studied. The species stem cross sections showed similarity in their epidermis, endodermis, lack of sclerenchyma cells, approximate number of vascular bundles, approximate cortex thickness (80–100 µm with a minimum of 40 µm in *A. strictum*), and parenchyma cell size in the pith zone (Figs 4-7; Table 3). The stamens and petals structure were similar in all four studied species and were not suitable as diagnostic characters. On the contrary, the fruit shape helped delimit *A. strictum* and *A. marginatum* from the other two species and the fruit indumentums could delimit *A. contemptum* and *A. szovitsianum* which were similar in shape.




**Figs 4-7.** Cross section of the stem (**4**) *A. contemptum*, (**5**) *A. marginatum*, (**6**) *A. strictum*, (**7**) *A. szovitsianum*, **t**: trichome, **e**: epidermis, **c**: cortex, **en**: endodermis, **ph**: phloem, **x**: xylem, **pt**: pith.

**Table 3. Comparison between the anatomical characters of stem cross sections in *A. contemptum*, *A. marginatum*, *A. strictum*, and *A. szovitsianum*.**

| Taxon | Approximate cortex thickness (µm) | Number of vascular bundles | Sclerenchyma cells in cortex layer | Number of epidermis layers |
|---|---|---|---|---|
| *A. contemptum* | 80–100 | 10–16 | – | one |
| *A. marginatum* | 80–100 | 10–16 | – | one |
| *A. strictum* | 80–100 | 8–12 | few scattered cells | one |
| *A. szovitsianum* | 80–100 | 10–12 | – | one |

## Discussion

According to the statistical analysis, a combination of reproductive structures, especially the characters concerning the silicule (e.g. silicule valve inflation, silicule apex) and filaments (e.g. simple, winged), have proved particularly important in delimiting the annual species. On the other hand, due to strong variation (e.g. leaf width and length), or to complete lack of variation (life form and base of plant), there were only a few vegetative structures that could be used. As a result, the presence of at least one fruit and flow-

er is necessary so as to identify correctly the annual species of *Alyssum*.

Three large clusters were observed in the UPGMA phenogram. These clusters delimited the sections *Psilonema*, *Meniocus* and *Alyssum*, which have been used in the different floras (Rechinger 1968; Townsend 1980). During earlier researches made by Aryavand (1996), on the grounds of delimitation of species in the section *Meniocus* from other sections he suggested the division of genus *Alyssum* into two subgenera, *Meniocus* (including sect. *Meniocus*) and *Eualyssum* (including other sections of the genus *Alyssum*). On the other hand, the two major clusters in the phenogram resulting from the present study separated the sections *Meniocus* and *Alyssum* from *Psilonema* and did not support these subgenera.

Although two varieties (– var. *linifolium* and – var. *teheranicum* Bornm.) have been defined for *A. linifolium* (Rechinger 1968), the characters used to distinguish them overlapped in the populations and did not show sufficient variation to delimit them.

The presence of *A. minus* var. *strigosum* in a subgroup with other varieties of *A. minus* also supported an earlier classification by Zohary (1966), where *A. strigosum* (Banks & Soland) was reduced to a variety of *Alyssum minus* (L.) Rothm.

The extremely high similarity between *A. contemptum*, *A. marginatum*, *A. strictum*, and *A. szovitsianum* has led to discussions of the possibility of reducing some species. This problem especially concerns *A. marginatum* and *A. szovitsianum* that have been considered earlier as synonyms (Botschantzev 1978). Due to their obvious similarities, these species were referred to one subgroup. However, even though stem anatomy did not prove helpful, the present study was able to delimit each species on the basis of its morphological characters.

## References

**Akhani, H.** 2003. Notes on the flora of Iran: 4. Two new records and synopsis of the new data on Iranian *Cruciferae* since *Flora Iranica*. – Candollea, **58**(2): 369-385.

**Aryavand, A.** 1996. Numerical Taxonomic Study of the Iranian species of *Alyssum* L. based on morphological characters. – J. Sci. Iran, **7**(3): 129-136.

**Botschantzev, V.P.** 1978. De Cruciferis notae criticae – In: **Akhani, H.** 2003. Notes on the flora of Iran: 4. Two new records and synopsis of the new data on Iranian *Cruciferae* since *Flora Iranica*. – Candollea, **58**(2): 369-385.

**Hedge, I.C.** 1976. A systematic and geographical survey of the old world *Cruciferae*. – In: **Vaughan, J.G., Macleod, A.J. & Jones, B.M.G.** (eds), The Biology and Chemistry of the *Cruciferae*. pp. 1-46. Academic Press, New York.

**Ingrouille, M.J.** 1986. The construction of cluster webs in numerical taxonomic investigation. – Taxon, **35**: 541-545.

**Pakravan, M., Nazem Bokaee, Z. & Bolourian, S.** 2011. A biosystematic study on the four varieties of *Alyssum minus* (L.) Rothm. (*Brassicaceae*) in Iran. – Iran. J. Bot., **17**(1): 55-62.

**Rechinger, K.H.** 1968. *Alyssum* L. – In: **Rechinger, K.H.** (ed.), *Flora Iranica*. Vol. **57**, pp. 146-170. Akad. Druck-u. Verlagsanstalt, Graz.

**Townsend, C.C.** 1980. *Alyssum* L. – In: **Townsend, C.C. & Guest, E.** (eds.), Flora of Iraq. Vol. **4**, pp. 959-984. Ministry of Agriculture, Baghdad and Bentham-Moxon Trust.

**Warwick, S.I., Francis, A. & Al-Shehbaz, I.A.** 2006. *Brassicaceae*: Species checklist and database on CD-Rom. – Pl. Syst. Evol., **259**: 249-258.

**Zohary, M.** 1966. *Cruciferae*. – In: **Zohary, M.** (ed.), Flora Palaestina. Vol. **1,** pp. 246-288. Israel Academy of Sciences and Humanities, Jerusalem.