

NUMERICAL TAXONOMIC STUDY OF THE IRANIAN SPECIES OF *ALYSSUM* L. BASED ON MORPHOLOGICAL CHARACTERS

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Abstract

The genus *Alyssum* L. belongs to the subtribe Alyssinae, tribe Alyseae and family Cruciferae (Brassicaceae). This genus is one of the largest genera of the family of Cruciferae in Iran, and seems to be the most problematic genus in which the boundary of certain species is not completely clear due to the polymorphism of morphological characters. The main objective of this research is to study the species of the genus *Alyssum* using numerical taxonomic methods. Thirty-three Operational Taxonomic Units (OTUs) belonging to the twenty-nine species of *Alyssum* L. from Iran were examined and scored for thirty-six qualitative characters having two or more states. This work is based on field and different herbarium samples. The data were analysed by UPGMA clustering method. The results of this study suggest that there are basically four sections (*Meniocus*, *Odontarrhena*, *Alyssum* and *Psilonema*) which can be grouped under two subgenera, namely subgenus *Eualyssum* and subgenus *Meniocus*. The results of cluster analysis were compared with the classification obtained by conventional methods and the relations among the taxa have been discussed.

Introduction

The main objective of this research is to study Iranian species of *Alyssum* L. using numerical taxonomic methods, and to compare the results of UPGMA-clustering analysis with the conventional classification of K. H. Rechinger in the Flora Iranica [11]. In spite of recent progress in numerical taxonomy and the existence of many computer programs on this subject, few works have been done on Iranian flora by means of these methods.

According to the O.E. Schultz monography [16], the Cruciferae is one of the most important families of the dicotyledonous plants and contains about 351 genera and 3000 species. The number of species of this family in Iran (c. 350 species) is quite considerable with regard to the total flora of Iran (c. 7000-8000 species) [11].

Keywords: *Alyssum*; Cruciferae (Brassicaceae); Iran; Numerical taxonomy; UPGMA-clustering analysis

The biology and chemistry of this family has been the subject of much research by different authors [10]. In this study, the data obtained in cytotoxic research carried out by Ramak-Maassoumi [14], Aryavand [1-3] and others into Iranian Cruciferae have been used.

The genus *Alyssum* L. belongs to the subtribe Alyssinae, tribe Alyseae and family Cruciferae. This genus contains about 150 species which are indigenous to the Mediterranean, Near and Middle East. The genus *Alyssum* is closely related to *Clypeola*, *Farsetia* etc. *Alyssum campestre* L. and related species are recommended as pectorant and in the treatment of sore throats and hoarseness [5].

The genera *Erysimum* and *Alyssum* in Iran are the largest ones in this family [11]. These two genera seem to be the most problematic genera in which the boundary of certain species is not completely clear due to the

polymorphism of morphological characters. Thus, the identification of the different species of the genus *Alyssum* is somewhat difficult and controversial.

This genus contains thirty-six species in the Flora Iranica area, and thirty species have been found in Iran [11]. The different species of the *Alyssum* in Iran, like many other genera of this family in which floral structure is relatively constant, are uniform. The best diagnostic characters of this genus are the fruit and seed characters.

To the best of our knowledge, no numerical taxonomic study has yet been undertaken on the genus *Alyssum* and related genera throughout the world. However, some numerical taxonomic research has been carried out into other members of the Cruciferae, the most important of which are cited as follows: Hernandez Bermejo and Clemente Munoz [9] performed the numerical analysis of the tribe Brassicaceae from Spain. Takahata and Hinata [18] have also studied the relationships between species in the subtribe Brassicinae by way of cluster analysis of morphological characters. Borgen [4] carried out a

biosystematic study of the genus *Lobularia* in the Macaronesian region. Feliner [8] has made a multivariate and cladistic analysis of the purple-flowered species of *Erysimum* from the Iberian Peninsula. Marhold [13] studied a multivariate morphometric of the *Cardamine amara* group in the mountains of the Slovak. El Naggar [7] studied the tribe Lepidieae and some other genera of the Cruciferae from Egypt with respect to numerical taxonomy.

In the present paper, thirty-three taxa belonging to the twenty-nine species of *Alyssum* from Iran have been studied, among which four species have two varieties. *Alyssum alyssoides* (L.) L. var. *alyssoides* and *A. penjwinense* Dudley from Iran were reported on when the Flora Iranica was published; we studied specimens of these species from the herbarium of Tehran Botanical Garden (TARI). Unfortunately, we could not find good specimens of the three Iranian species of *Alyssum*: *A. baumgartnerianum* Bornm., *A. constellatum* Boiss. and *A. iranicum* Hausskn. ex Baumg. Therefore, these species are not included in this work.

Table 1. List of 33 OTUs studied for numerical analysis and the abbreviations adopted for each OTU.

1- <i>Alyssum alyssoides</i> (L.) L. var. <i>alyssoides</i>	(al)
2- <i>Alyssum bracteatum</i> Boiss. and Buhse.....	(br)
3- <i>Alyssum contemptum</i> Schott ex Ky.....	(co)
4- <i>Alyssum dasycarpum</i> Steph. ex Willd. var. <i>dasycarpum</i>	(da)
5- <i>Alyssum dasycarpum</i> Steph. ex Willd. var. <i>minus</i> Bornm. ex Dudley.....	(dm)
6- <i>Alyssum desertorum</i> Stapf var. <i>desertorum</i>	(de)
7- <i>Alyssum desertorum</i> Stapf var. <i>prostratum</i> Dudley.....	(dp)
8- <i>Alyssum heterotrichum</i> Boiss.....	(he)
9- <i>Alyssum hirsutum</i> M.B.....	(hi)
10- <i>Alyssum homolocarpum</i> (Fisch. and C.A. Mey.) Boiss.....	(ho)
11- <i>Alyssum inflatum</i> Nyarady.....	(in)
12- <i>Alyssum lanceolatum</i> Baumg.....	(lc)
13- <i>Alyssum lanigerum</i> DC.....	(lg)
14- <i>Alyssum linifolium</i> Steph. ex Willd. var. <i>linifolium</i>	(li)
15- <i>Alyssum linifolium</i> Steph. ex Willd. var. <i>teheranicum</i> Bornm.....	(lt)
16- <i>Alyssum longistylum</i> (Sommier and Levier). Grossh. and Schischk.....	(lo)
17- <i>Alyssum marginatum</i> Steud. ex Boiss.....	(ma)
18- <i>Alyssum menioides</i> Boiss.....	(me)
19- <i>Alyssum minus</i> (L.) Rothm. var. <i>micranthum</i> (C.A. Mey.) Dudley.....	(mc)
20- <i>Alyssum minus</i> (L.) Rothm. var. <i>minus</i>	(mi)
21- <i>Alyssum minutum</i> Schlecht ex DC.....	(mn)
22- <i>Alyssum mulleri</i> Boiss. and Buhse.....	(mr)
23- <i>Alyssum murale</i> Waldst. and Kit.....	(mu)
24- <i>Alyssum penjwinense</i> Dudley.....	(pn)
25- <i>Alyssum persicum</i> Boiss.....	(pr)
26- <i>Alyssum polycladum</i> Rech. f.....	(po)
27- <i>Alyssum repens</i> Baumg. subsp. <i>trichostachyum</i> (Rupr.) Hayek.....	(re)
28- <i>Alyssum stapfii</i> Vierh.....	(sf)
29- <i>Alyssum strictum</i> Willd.....	(sm)
30- <i>Alyssum strigosum</i> Banks and Soland.....	(st)
31- <i>Alyssum szowitsianum</i> Fisch. and C.A. Mey.....	(sz)
32- <i>Alyssum turgidum</i> Dudley.....	(tg)
33- <i>Alyssum turkestanicum</i> Regel and Schmalh.....	(tu)

Materials and Methods

This work is based on field studies and herbarium samples. Apart from my own herbarium, and the herbarium of the University of Isfahan (UI), specimens housed at the herbarium of Tehran Botanical Garden (TARI), the herbarium of the Plant Pests and Diseases Research Institute Ewin (PPDRIE), the Institute of Natural Resources Research of Isfahan (INRRI) and the herbarium at the Technical University of Isfahan (TUI) (Table 1) were examined and scored for thirty-six qualitative characters having two or more states (Table 2). All specimens were collected from Iran. Nomenclature is the same as that used in the Flora Iranica [11].

The characters used for numerical analysis are based on a detailed morphological study of vegetative organs, flowers, fruits and seeds as well as chromosome numbers. For all characters, at least five specimens were observed and the mean value of five measurements (for some of the characters) were calculated and the means were used as a single character statement. The data were analysed by UPGMA clustering method. The NTSYS program package written for IBM PC by Rohlf [15] was used in this analysis.

First of all, we made a raw matrix for thirty-three OTUs and thirty-six two or multistate qualitative characters using the Pe2 program package (Table 3). Then, the simple matching coefficient and average taxonomic distances between pairs of OTUs, for measuring taxonomic resemblance using the standardized scores, were computed.

For cluster analysis, the unweighted pair-group method with arithmetic average (UPGMA), which is more commonly used in numerical taxonomy, was employed. For more details refer to Sneath and Sokal [17]. UPGMA clustering of similarity matrices based on the simple matching coefficient and average taxonomic distances yielded cophenetic correlation of 0.81830 and 0.77014, respectively [17]. Therefore, the results based on the simple matching coefficient are presented in this paper.

We also computed a principal coordinates analysis by performing the following operations: the raw matrix is standardized by variables (characters), a similarity matrix between the OTUs is computed by using the simple matching coefficient. This matrix has been transformed to scalar product form by double-center program, so that its eigenvalues and eigenvectors can be computed. Then a plot was made showing the OTUs in a 3-dimensional space [15]. Finally, the minimum length of spanning tree was calculated using similarity matrix based on the simple matching coefficient. This is useful for showing the nearest neighbors of the OTUs based on their positions in a multidimensional space. The data are given in Table 4. Although it is often useful to draw the minimum spanning tree (MST) in on a plot of the projections of the OTUs onto the first few principal components axes, because of the

Table 2. Qualitative, two and multi-state characters and characters state used in the numerical analysis

Characters	Characters state
1. Life form	1: perennial or biennial 2: annual
2. Base of plant	1: suffruticose 2: not suffruticose
3. Length of plant	1: until 5 cm long 2: 6-30 cm long 3: more than 30 cm long
4. Stem form	1: branched 2: simple
5. Leaf form	1: linear-lanceolate 2: obovate-spathulate or oblongo lanceolate 3: + orbicular
6. Leaf apex	1: obtuse 2: apex with very small teeth (acute)
7. Leaf margin	1: conduplicate 2: entire
8. Leaf width	1: until 2 mm 2: more than 2 mm
9. Form of inflorescent (1)	1: initio capitato-congesta 2: always racem elongated or shortened
10. Form of inflorescent (2)	1: condensate and often with a few flowers 2: elongated and multiflore
11. Racem in fruit state	1: elongated or without change after fruiting 2: condensed after fruiting
12. Length of bract to stem leaves	1: greater 2: smaller
13. Base of pedicels	1: swollen 2: not swollen
14. Position of pedicel towards inflorescence axis	1: appressed 2: spread
15. Length of pedicel	1: until 2 mm long 2: more than 2 mm long
16. Duration of sepal	1: always persistent and often swollen after fruiting 2: persistent 3: deciduous
17. Indumentum of sepal	1: glabrous 2: glabrous or sparsely hairy 3: hairy
18. Form of petal	1: extremely long clawed 2: not above form
19. Colour of petal	1: nearly whitish 2: rosea 3: yellow, pale yellow or yellowish green
20. Length of petal	1: until 2 mm 2: 2-3.5 mm 3: more than 3.5 mm

(Note: See Table 2 continued on the next page.)

Table 2. Continued

Characters	Characters state
21. Width of petal	1: until 1 mm 2: 1-2 mm 3: more than 2 mm
22: Petal length to sepal length	1: petal nearly longer than sepal 2: petal completely longer than sepal
23. Structure of filament	1: filament without wing, tooth and appendix 2: filament free, unilaterally or bilaterally with wing, appendix or tooth or wing 3: long filament unilaterally winged and toothed and short filament with appendix free or on the base covering together 4: long filaments free, unilaterally or bilaterally winged, short filament with appendix free or connate and toothed the wing towards upwards gradually or abruptly contracted
24. Length of style	1: style until 1 mm long 2: style more than 1 mm long
25. Form of silicule valve	1: compressed or plane 2: the valve equally inflated 3: the valve unequally inflated 4: the valve convex with transversal section sigmoid
26. Form of silicule	1: orbicular 2: ovate 3: not above fom
27. Apex of silicule	1: obtuse 2: retuse, emarginate or truncate 3: not determinate form
28. Margin of silicule	1: smooth 2: papillose 3: hairy
29. Indumentum of silicule	1: glabrous 2: with normal appressed stellate hairs with many, equal and long rayed 3: with appressed and outspread stellate hairs with short and unequal rayed 4: with appressed and outspread stellate hairs with unequal rayed 5: with appressed stellate hairs with short and 7-rayed 6: with appressed stellate hairs and with breviter and multi rayed 7: with appressed and stellate hairs and simple or forked tuberculate hairs 8: stellate hairs with few (4-8) rayed and dot like 9: dense stellato-pilosae
30. Presence of tuberculate hairs in silicule	1: without tuberculate hairs 2: with tuberculate hairs with equal rayed 0.2-1 mm long 3: with simple tuberculate hairs 1-3 mm long or with forked hairs with unequal rayed

Table 2. Continued

Characters	Characters state
31. Presence of tubercule in indumentum of silicule	1: without tuberculate hairs 2: tubercule until 0.5 mm thick 3: tubercule more than 0.6 mm thick
32. Duration of indumentum of silicule	1: without hairs 2: quickly disappearing 3: persistent
33. Form of placentation	1: lateral 2: subapical
34. Number of ovule in each loculus	1: 2-4 (8) ovules 2: 2 ovules 3: 1 ovule
35. form of seed	1: seed without wing 2: seed with very narrow wing 3: seed with wing
36. Kind of moist seed	1: without mucilage 2: with mucilage
37. Chromosome number	1: 2n = 16 2: 2n = 32 3: 2n = 48

large number of the OTUs, it is impossible to demonstrate this plot using the NTSYS program [15].

Results

The phenogram resulting from UPGMA clustering of similarity matrix is presented in Figure 1. A line across the phenogram at 0.640 similarity level results in two subgroups or phenons corresponding to the sections of *Psilonema* (C. A. Mey.) Hooker, *Alyssum* and *Odontarrhena* (C.A. Mey.) Hooker on the one hand, and *Meniocus* (Desv.) Hooker, on the other. The latter is considered an independent genus named *Meniocus DC.* in the Flora of the U.S.S.R. [12]. This genus is separated from the *Alyssum* by four ovules in each loculus of ovary, while in the genus *Alyssum* there are only one-two ovules in each loculus. Therefore, the phenogram separates the section *Meniocus* from the other sections. This section contains four OTUs under study as follows:

Alyssum linifolium var. *linifolium*
Alyssum linifolium var. *teheranicum*
Alyssum meniocoides and *Alyssum heterotrichum*

Also, the phenon line at 0.720 similarity level creates three sections namely sect. *Psilonema*, sect. *Alyssum* and sect. *Odontarrhena*.

Phenon no. 1, which contains four OTUs as follows: *Alyssum alyssoides*, *A. dasycarpum* var. *dasycarpum*, *A. dasycarpum* var. *minus* and *A. homolocarpum* corresponds with the section *Psilonema* (C. A. me.) Hooker of the Flora Iranica and the Flora of U. S. S. R.

Phenon no. 2, which is the largest group in the genus

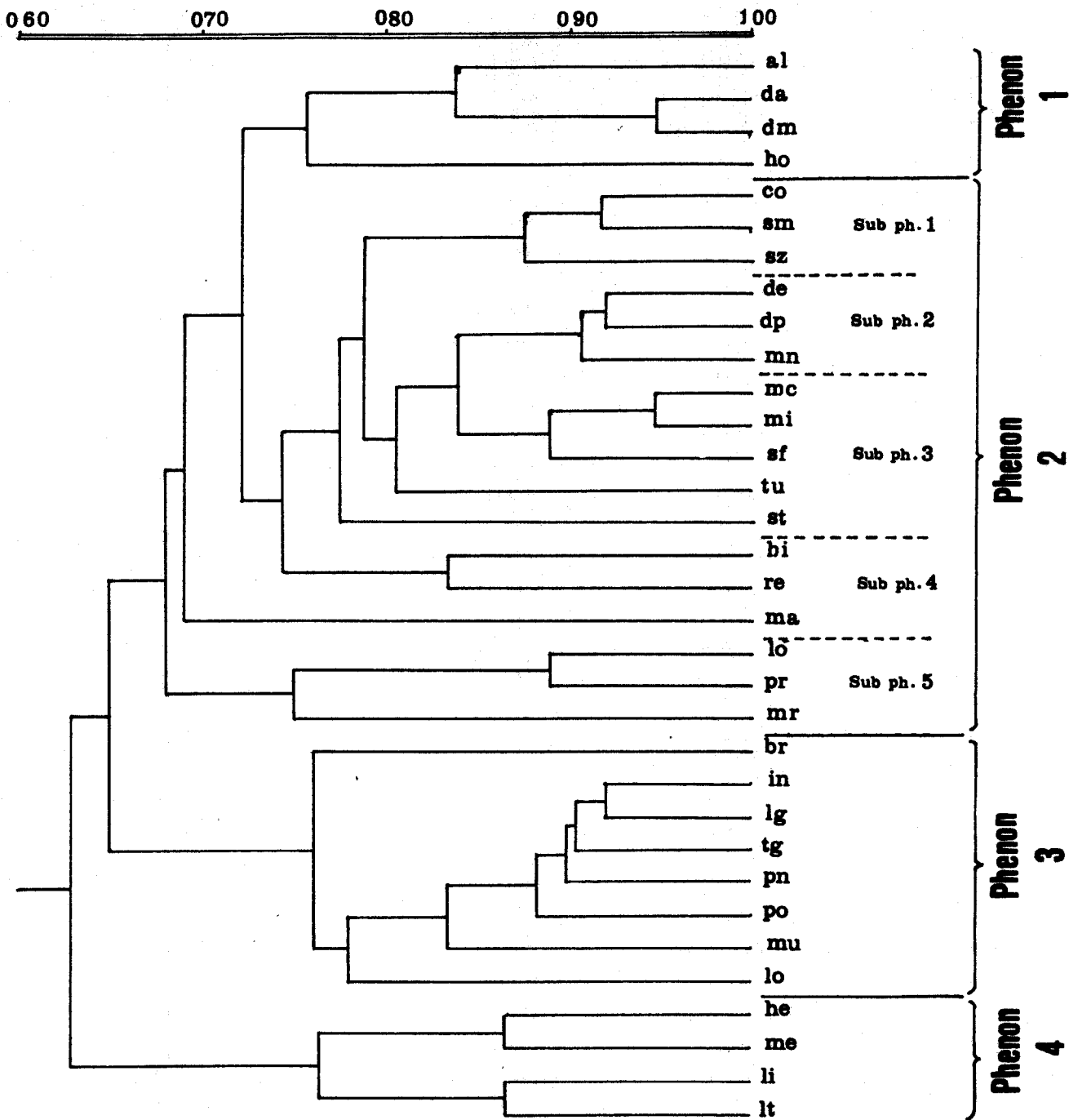


Figure 1. Phenogram for UPGMA-clustering analysis of thirty-three OTUs belonging to the Iranian species of *Alyssum* L. Scored for thirty-six qualitative characters having two or more states.

Table 4. The minimum length of spanning tree from a similarity matrix based on the simple matching coefficient for the thirty-three OTUs belonging to the Iranian species of *Alyssum* L. For abbreviations of the OTUs refer to Table 1.

i	j	length
al	da	0.493197
da	dm	0.164399
al	mi	0.493197
mi	mc	0.284747
mc	sf	0.434959
sf	re	0.434959
mi	ma	0.464991
mi	mn	0.464991
mn	dp	0.328798
dp	de	0.232495
ma	sm	0.464991
sm	sz	0.402694
sz	he	0.434959
he	me	0.434959
sm	co	0.434959
me	li	0.519875
li	lt	0.434959
mn	ho	0.519875
he	lg	0.569495
lg	mu	0.569495
mu	lo	0.545250
lg	br	0.569495
br	in	0.493197
in	tg	0.434959
mc	tu	0.592749
mc	pr	0.636715
pr	lc	0.328798
pr	po	0.615125
po	pn	0.592749
pr	hi	0.657596
hi	st	0.569495
st	mr	0.677834

Alyssum and possesses seventeen OTUs from thirty-three OTUs under study, corresponds to the subgenus *Eualyssum* of Davis in the Flora of Turkey [6]. The phenogram indicates five subgroups or subphenons in this section which are as follows:

Subphenon no. 1: *A. contemptum*, *A. strictum*,
A. szowitsianum.

Subphenon no. 2: *A. desertorum*, *A. minutum*.

Subphenon no. 3: *A. minus*, *A. stapfii*, *A. turkestanicum*,
A. strigosum.

Subphenon no. 4: *A. hirsutum*, *A. repens*, *A. marginatum*.

Subphenon no. 5: *A. lanceolatum*, *A. persicum*, *A. mulleri*.

This classification is relatively similar to the order of description of species in the Flora Iranica except *A. marginatum* with 0.68 similarity coefficient that is less

than phenon line 0.72 to attach to *A. repens*.

Also phenon no. 3 which contains eight OTUs under study as follows: *Alyssum bracteatum*, *A. inflatum*, *A. lanigerum*, *A. turgidum*, *A. penjwinense*, *A. polycladum*, *A. murale* and *A. longistylum* corresponds with the section *Odontarrhena* (C. A. Mey.) Hooker of the Flora Iranica and *Odontarrhena* (C. A. Mey.) Koch of the Flora of U. S. S. R.. At the phenogram, *A. bracteatum* was separated from other OTUs of the section and relies on the group at the similarity level of 0.76. This situation is due to the special morphological characters of this species.

As mentioned above, we also computed a principal coordinates analysis. The results show that it is possible to divide the OTUs into four separate groups as obtained from clustering method. However, considering that the figure of the two dimensional plot is not sufficiently clear, we were obliged to demonstrate the results obtained from the clustering method which is more common and advantageous.

Discussion

As was mentioned above, up to now, little numerical taxonomic research has been carried out on Iranian Flora. With this kind of work, we can analyse and assess the exact meaning of any characters and also examine these characters in all specimens. Furthermore, we can study phylogenetic relationships between the taxa. Finally, we can compare the results of the numerical taxonomy with results obtained through other biosystematic methods, such as cytotoxicity, chemotaxonomy and so on.

We suggest that at least in the Flora Iranica area and the Middle East, the genus *Alyssum* can be divided into two subgenera namely: subgen. *Meniocus* (including sect. *Meniocus*) and subgen. *Eualyssum* (including other sections of the genus *Alyssum*).

It is possible that the subgen. *Meniocus*, all species of which are annual and have four ovules in each loculus of ovary, is more advanced than the subgen. *Eualyssum*. Despite the fact that the phenogram shows the relationships among the taxa under study more precisely, the phylogeny and branching patterns throughout the course of evolution of all the species have to be determined using cladistics analysis based on structural, chromosomal banding patterns, as well as amino-acid sequences of cytochrome B and other characters. In this study, between all numerical methods, the UPGMA-clustering method gave more information than other methods.

The present study is somewhat limited because only the Iranian species were examined. A comprehensive study of the whole *Alyssum* species including a comparison of seed proteins analysis is necessary before a satisfactory infrageneric classification of this genus can be constructed. Recognition and classification would be better obtained

through this method than through classical studies. Nevertheless, the classification obtained by numerical taxonomic methods seems to give more information than those of conventional methods on the relationship between OTUs.

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