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A Numerical-Taxonomic Study of the *Juncus* L. (Juncaceae) in North, Razavi and South khorassan provinces in Iran

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Abstract

The genus *Juncus* L. belongs to the family Juncaceae, which has over 250 species worldwide. In Flora Iranica, this genus has six subgenera: *Juncus*, *Genuini*, *Pseudotenagia*, *Poiophylli*, *Septati* and *Alpini*. In this study, we investigated seven spices of *Juncus* that belongs to four different subgenera and studied morphologically characters in the case of annual and perennial species of *Juncus* in the Northest of Iran. A total of 74 characters were examined on each specimen, comprising 37 quantitative and 28 qualitative characters. Cluster analysis with UPGMA method and principal component analysis were used to investigate the differences among species of *Juncus* in Khorassan province. The results explained that the morphological characters are useful to discriminate taxa. The results of CA and PCA analysis shows that the Cluster analysis was better than principal component analysis for species boundaries. In CA analysis, two main groups were distinguished that the first group includes perennials and second group includes annuals species. We concluded that micromorphological characters are somewhat but not fully useful for species boundaries.

Keywords: *Juncus*, Juncaceae, Cluster analysis, principal component analysis, Iran

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1. Introduction

Juncus and *Luzula* are the two large genera which belongs to the family Juncaceae. *Luzula* has a three-seeded capsule with basal placentation, closed sheaths and hairs, while *Juncus* has a many-seeded capsule with different degrees of marginal to central placentation, usually open sheaths and no hairs (Snogerup, 1993).

Juncus L. (Juncaceae) is a widely distributed genus of rushes with nearly 220 species around the world (Kirschner, 2002) and 20 species in Iran (Taheri, 1372). Plants of Juncaceae, commonly called rushes. These plants are found in diverse habitats and occupy areas of every continent except Antarctica (Knup and Naczi, 2008). Most diversity of the genus *Juncus* is in mesophyticand boreal regions of the word (Buchenau, 1906).

Plants of this genus are denoted by their nude leaves, which the leaves are more cylindrical and more or less flat and with a spongy pith. Many species are exceptionally hardy and some are considered weeds in gardening; a few are invasive weeds of significance. On the other hand, there is some use of rushes as ornamental plants, e.g. at garden ponds; more generally their tolerance of extreme environmental conditions (except drought) means that some species or another is likely suitable for ornamental purposes even where few other plants grow (Coffey, 1993). The people were used from the culms of this plants for making baskets, about 6000 years ago, and they were also used at that time for wrapping the bodies of the deceased (Tackholm *et al*.1950).

Linnaeus in 1753 described the genus *Juncus* for the first time. He reported 15 species of the *Juncus* and divided them into two groups, 1.nodus and 2.foliofis (according to stem type). Boissier, (1881) reported 26 species of the genus *Juncus* that divided into 8 sections. Buchenau (1875, 1890) divided 250 species of *Juncus* into eight subgenera and he published the most comprehensive systematic account of the Juncaceae ,however another classifications have been suggested by Kuntze (1903), Vierhapper (1930), Krechetovich and Goncharov (1935), Weimarck (1946), and others. Rechinger in the Flora Iranica (Rechinger, 1982) divided the genus *Juncus* into six subgenera: *Juncus*, *Genuini*, *Pseudotenagia*, *Poiophylli*, *Septati* and *Alpini*.

In 1990, 350-389 species of *Juncus* were distinguished and divided into two subgenera, four sections and 45 subsections by Novikov. He computed 75 species and 14–15 subspecies, composing tow subgenera, 12 sections, and 25 subsections in the former Flora of the USSR (Chernyakovaskaya, 1939).

According to the Kirshner in 2002, Juncaceae is a cosmopolitan family. This family includes seven genera and 442 species. He divided 300 species of genus *Juncus* into 10 sections. Centers of diversity of these species are in the temperate zones.

So far the chromosome morphology of the genus *Juncus*, has been much studied. Snogerup in 1963, 1985, 1993; Taylor and Mulligan in 1968; Zandee in 1981 and Cope and Stace in 1985 are studied in this field. The chromosome number in *Juncus* subgen. *Juncus* is 2n=48 but also 2n=46 has been reported for *Juncus rigidus* that belongs to this subgen. It is known whether x=24 or x=23 is the original basic number in *Juncus* subgen. *Juncus* (Snogerup,1993). The chromosome number in *Juncus* subgen. *Genuine* 2n=40, subgen. *Septati* 2n=80 and subgen. *Poiophylli* 2n= 100-110 (Rechinger, 1982).

The seed morphology of 15 species of *Juncus* from Kansas (USA) were studied using scanning electron microscopy (SEM) by Brooks and Kuhn in 1986 (Brooks, 1986). Furthermore Kadry in 2010 studied seed coat morphology of nine taxa of *Juncus* using light and SEM microscopy in Egypt (Kadry, 2010). Taxonomy, morphology and geographic distribution of three species of *Juncus* were studied by Knapp and Naczi in 2008, They concluded that the seeds of this species differ in shape and size (Knapp, 2008).

Molecular studies were explained that *Luzula* is monophyletic and *Juncus* is non-monophyletic. A cladistic analysis of rbcL nucleotide sequences for 58 species from most subgenera and sections of *Luzula* and *Juncus* was performed by Dr´abkov´a *et al*., (2003).

According to the results of Dr´abkov´a and Vl˘cek study in 2009, was presented a phylogenetic tree using mitochondrial and plastome genes that the main topology of this tree is similar to the separate rbcL tree (Dr´abkov´a *et al*., 2003). In this phylogenetic tree, the subgenus *Juncus* was divided into two clades.

As regards, studies on the morphology of this genus are limited in this study, we can try to separate species of this genus that common in the Northeast of Iran using the numerical taxonomy.

I will address the following questions:

1) Are the populations morphologically distinct, and are the distinguishing characteristics floral, vegetative or both?

2) Do vegetative and floral characters show similar patterns of geographic variation?

2. Materials and Methods

2.1 Plant materials

This research was based on herbarium material and collected plants. The majority of specimens are from FUMH (Ferdowsi University of Mashhad Herbarium) and some species belonging to the family Juncaceae were collected from different localities of Khorassan (Shomali, Razavi and Jonoubi Khorasan provinces), located in Northeast of Iran. Specimens were preserved in the herbarium of the Faculty of Science at Ferdowsi University of Mashhad and were identified by identification keys: Flora Iranica (Rechinger, 1982), Flora of Pakistan (Jafri, 1973), Flora of the U.S.S.R (Chernyakovaskaya, 1939), Flora of Turkey (Davis, 1965) and Flora Orientalis (Boissier, 1975).

Despite the species *J.rigidus*, *J.gerardi* subsp. *libanoticus*, *persicus*, *J.articulatus*, *J. fontanesii* subsp. *kotschyi* and *J. turkestanicus* have been noted in Khorasan in flora iranica, but *J. rigidus* and *J. turkestanicus* were not found in data collection. Also, in Flora of Iran, only two species: *J. articulates* and *J. inflexus* distributed in khorassan provinces.

Species studied in this research include: *J. articulates*, two subspecies of *J. fontanesii* (subsp. *kotschyii* and subsp. *pyramidatus*), *J. inflexus*, *J. gerardi*, *J. bufonius* and *J. rechingeri*. Of the 75 specimens examined, 27 were included in the statistical analysis. We used only mature and complete specimens from different population, in order to allow standardized measurements to be made and to collect the maximum variation. In some cases, because the specimens were incompleted in a particular character, the average measurement was replaced (Legendre & Legendre, 1998)

Table-1: A list of field and herbarium-collected specimens (FUMH) including herbarium code, sampeling location, collection date and collector name. Specimens marked with an asterisk are herbarium specimens (FUMH).

Herbarium Code	Species	Location	Collection Date	Collectors
36623	*J. bufonius**	Chenaran, Ferizi Region, Derme Valley	1384/3/28	Emadzadeh-Memariani-Zangooei
38004	*J. rechingeri**	SW of Bojnurd, reiin	1385/3/18	Memariani-Zangooei-Arjmandi
18035	*J. rechingeri**	North o mashhad, Aal	1368/4/3	Joharchi
742	*J. punctorius**	Tbas to Chiruk,	1360/2/10	Zokaei
36865	*J. punctorius**	Torbat Jam, Bezd Mount, East Valley	1384/4/18	Joharchi-Zangooei
15330	*J. fontanesii subsp. pyramidatus**	Road Mashhad to Kalat, Baze Nakhroo	1369/4/24	Fghihnia-Zangooei
15345	*J. fontanesii subsp. pyramidatus**	West of torbat Heydarieh, Sorkh Abad	1366/3/18	Joharchi-Zangooei
21860	*J. fontanesii subsp. kotschyii*	Cheshme Aal	1390/3/14	Vaezi
21861	*J. fontanesii subsp. kotschyii*	Kalat Spa	1390/2/31	Basiri-Ahmadpour
21862	*J. fontanesii subsp. kotschyii*	65km of Kalat to Mashhad	1390/2/31	Basiri-Ahmadpour
24152	*J. inflexus**	Kalat Naderi, Khanzoo, Otokand Village	11373/3/19	Fghihnia-Zangooei
36848	*J. inflexus**	Torbat Jam, Bezd Mount	1384/4/18	Joharchi-Zangooei
21863	*J. gerardi subsp. libanoticus*	Mashhad to Neyshabur, Sharif Abad	1390/3/24	Basiri
21864	*J. gerardi subsp. libanoticus*	Shirvan, Gelul Region	1390/3/25	Basiri
21865	*J. gerardi subsp. libanoticus*	Sarakhs Road, Mazdavand	1390/2/5	Basiri-Ahmadpour
21866	*J. gerardi subsp. libanoticus*	Chenaran, Ferizi Region, Derme Valley	1390/2/28	Baisiri-Ahmadpour
22845	*J. gerardi subsp. libanoticus**	Bajestan to Ghasem Abad	1372/2/1	Fghihnia-Zangooei

19943	*J. gerardi subsp. libanoticus**	SW of Torbat Heydarieh, Eskandar Abad	1370/2/14	Joharchi-Zangooei
39208	*J. articulates**	SW of Bojnurd, Reiin	1386/5/17	Zangooei-Arjmandi
21867	*J. articulatus*	Zoshk	1389/5/18	Basiri
21868	*J. articulatus*	Radekan Village	1390/2/28	Basiri-Ahmadpour
21869	*J. articulatus*	Kalat Spa	1389/6/11	Vaezi
21870	*J. articulatus*	Kalat Naderi Road	1390/3/25	Basir-Ahmadpour
21871	*J. articulatus*	Mashhad to Neyshabur, Sharif Abad	1390/3/24	Basiri
21872	*J. articulatus*	Sarakhs Road, Mazdavand	1390/2/5	Basiri-Ahmadpour
34339	*J. articulates**	Mashhad, Dehbar Village, Western Mountain Region	1381/5/2	Joharchi
33866	*J. articulates**	West of Bojnurd, between Darkesh and Havar	1380/4/18	Joharchi-Hossein Zadeh

2.2 Morphological characters

During this study, we studied morphologically characters in the case of annual and perennial species of *Juncus* in the Northeast of Iran. A total of 74 characters were examined on each specimen, comprising 37 quantitative and 28 qualitative characters.

Table - 1 was a list of the measurements used in the analysis. The qualitative characters were scored as binary or multi state characters were assessed.

Table -2. List of qualitative and quantitative characters used in numerical analysis in present study. *Traits marked with an asterisk were not included in final PCA analysis.

No Qualitative	Abbreviation	Characters
1	LICY	Plant Life Cycle
2	RHPL	Rhizome of Plant
3	SHCU	Sheath of Culm
4	SCCU	Sheath Color of Culm
5	GRST*	Groove of Stem
6	LEDE	Leaf Density
7	PULE*	Hardness of Leaves
8	LETY	Leaf Type
9	SHLE	Sheath of Leaves
10	LEAU	Leaf Auricle
11	LESH	Leaf Shape
12	LETW	Leaf Transvers Wall
13	GRLE	Grooves of Leaves
14	AUBR	Auricle of Bract
15	INSH	Inflorecens Shape
16	INDE	Inflorecens Density
17	GLSH	Glomerules Shape
18	OBIN	Length of Outer Bract Compared with Length of Inflorescence
19	IBFL	Involucre Bracteole of Flowers
20	OPAU	Outer Perianth Auricle
21	IPAU	Inner Perianth Auricle
22	OPSH	Outer Perianth Shape
23	IPSH	Inner Perianth Shape
24	CPOU	Outer Perianth Color
25	CPIN	Inner Perianth Color

No	Abbreviation	Characters
26	PESI	Perianth Size
27	ASFI	Anther Size Compared with Filament
28	SSPE	Stamen Size Compared with Perianth
29	STSH	Stigma Shape
30	CASH	Capsule Shape
31	CACO	Capsule Color
32	CATI	Capsule Tip
33	CSCP	Capsule size Compared with Perianth
34	SESH	Seed shape
35	SEAP	Seed Appendages
36	PLHE	Plant Height
37	CUHE	Culm Height
38	CWBA	Culm width at Base
39	MCWI	Mid-culm width
40	LESL	Leanght of Stem Leaf
41	LELB	Leanght of Leaf Base
42	WLEW	Widest Leaf Width
43	NUSL	Number of Stem Leaves
44	NULB	Number of Basal Leaves
45	INLE	Inflorecens Length
46	INWI	Inflorecens width
47	NGIN	Number of Glomeruls in Inflorecens
48	NFGL	Number of Flora in Glomerules
49	GLWI	Glomerules Width
50	GLLE	Glomerules Length
51	OBLE*	Outer Bract length
52	IBLE	Inner bract Length
53	ICAL*	Inflorecens Central Axis Length
54	IPLE	Inner Perianth Length
55	OPLE	Outer Perianth Length
56	OTIT	Ratio of Length of Outer Tepals to Length of Inner Tepals
57	IPWI*	Inner Perianth Width
58	OPWI	Outer Perianth Width
59	TMWI	Tepal Margin Width
60	NUST*	Number of Stamen
61	FILE*	Filament Length
62	ANLE	Anther Length
63	RAFL*	Ratio of Anther to Filament Length
64	ANWI	Anther Width
65	STLE	Style length
66	SILE	Stigma length
67	CALE	Capsule Length
68	CAWI	Capsule Width
69	NCSE*	Number of Capsule Section
70	CSWI	Capsule Section Width
71	ITLC	Ratio of length of Inner Tepals to Length of capsule
72	OTLC	Ratio of length of Outer Tepals to Length of capsule
73	SLWT	Seed Length with Testa
74	SLNT	Seed Length without Testa

The Kruskal-wallis H test was performed to significantly investigate which qualitative characters differentiate the species. All tests were performed using the software SPSS ver.16 (Carver and Gradwohl Nash, 2007). Significant difference was considered at $P<0.05$. Those characters with non-significant values (not effective in species delimitation) were excluded from final analyses.

2.3 Statistical Analysis

The normality of the distribution of 74 characters was tested using the Kolmorogov-smirnov test (K–S test).

This is a nonparametric test for the equality of continuous, one-dimensional probability distributions that can be used to compare a sample with a reference probability distribution (one-sample K–S test), or to compare two samples (two-sample K–S test). Then normalization (elimination the unit of measurement) using centering and standard deviation (Z-scores) were applied on variables that were not normally distributed. After normalization, analysis of variance (ANOVA) is used to examine the variance of a dependent variable. The dependent variable is measured at different levels of one or more factor variables. For the normally distributed characters with unequal variance ANOVA was performed using Games-Howell Post hoc test The Kruskal-wallis H test was performed to significantly investigate which qualitative characters differentiate the species. All tests were performed using the software SPSS ver.16 (Carver and Gradwohl Nash, 2007). Significant difference was considered at P<0.05. Those characters with non-significant values (not effective in species delimitation) were excluded from final analyses.

Principal Component Analysis (PCA) characterized the amount of morphological variations between specimens and determined the characters that are most diagnostic to species separating. We performed this analysis using the software CANOCO ver. 4.5 (Ter braak and Smilauer, 2002) for windows. Finally, Cluster analysis (CA) based on the UPGMA method was used as an exploratory method to establish if the data grouped the classes to which the specimens had been assigned. CA analysis was performed using NTSYS-pc Ver. 2 (Rohlf, 2000). It was impossible to measure all characters on all specimens, and hence some values are missing in the dataset. In case of missing values an average of measurements obtained from specimens from localities of Khorassan for each population. Prior to doing the PCA and CA, we standardized the data to a mean of 0 and a variance of 1 to remove the effects of characters with large variances (Knapp, 2008).

3. Results

3.1 PCA results

We performed the first PCA using all 74 characters, but these characters did not produce distinct groupings of the individuals. Therefore, univariate analysis carried out for total characters and the nine characters that comprises GRST, PULE, OBLE, ICAL, IPWI, NUST, FILE, NCSE and RAFL were removed from analysis.
A total 65 characters (28 qualitative characters and 37 quantitative characters) were used in subsequent analysis.

In the PCA analysis, the PC1 and PC2 axes account for 84.19% and 8.6% respectively. Therefore, the results show that the first axis (PC1) is the most in separation of the species. In the case of PC1, quantitative and qualitative characters that are including PLHE, CUHE, MCWI, INDE, CWBA, LICY,RHPL, INWI, NGIN, OPAU, STSH and SLNT had the highest loadings (Table 3).

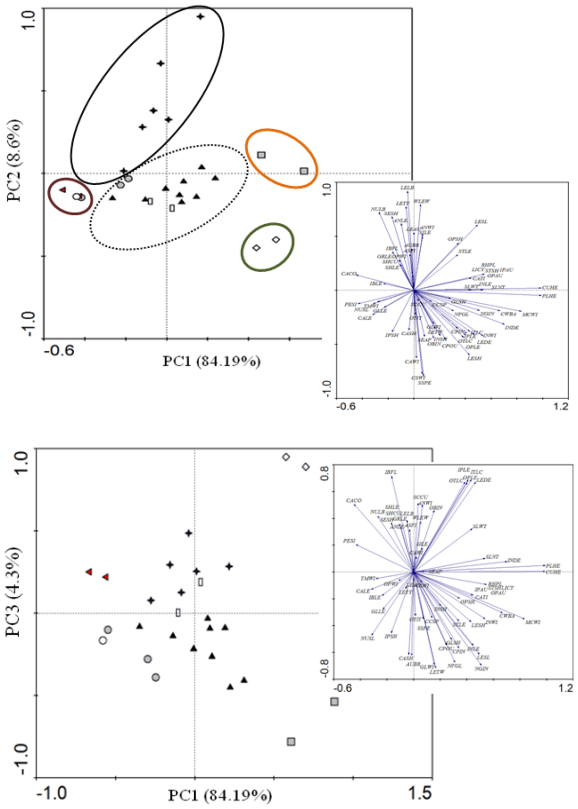

Fig. 1. Scotterplots of PCA analysis for the 27 individuals of the *Juncus* from different populations. Only axes 1 and 2 separated the species well✦ symbol of *J. gerardi*, ▨ symbol of *J.punctorius*, ▲symbol of *J. articulates*, ○ symbol of *J. bufonius*, ◍ symbol of *J. fontanesii* subsp. *kotschyi*, ▯ symbol of *J. fontanesii* subsp. *pyramidatus*, ◊ symbol of *J. inflexus* and ◀symbol of *J. rechingeri*.

The scatter plot of the results of PCA (Figure 1) shows that the species under study are divided into five groups. The first group includes individuals of the species *J. gerardi* that this genus belongs to the pseudotenagia subgenus. These species differentiate from another species with characters such as LELB, NULB, LETY, SESH, LEAU, SHCU and SHLE. The species of *J. punctorius* are comprises the second group. GLSH, INLE, STLE and OPSH are characters that differentiate individuals of this species from other species.
The third group includes individuals of *J. inflexsus*. The characters such as CUHE, PLHE, MCWI and CWBA are characteristics of this group. According to the results of the PCA analysis, the two complexes can be observed. The complex I consist of the species *J.articulatus*, *J. fontanesii* subsp. pyramidatus and *J. fontanesii* subsp. kotschyi . The members of the complex I are belonging to the septati subgenus. The

complex II includes of the species *J. bufonius* and *J. rechingeri* that are belonging to *poiophylli* subgenus and one of the individuals of *J. fontanesii* subsp. *kotschyi*.

Table -3: Results of the principal component analysis (PCA) for the individuals of *Juncus* and loadings of 65 characters for the three first principal components (PC1-PC3)

Sl.No	Character	PC1	PC2	PC3
1	LICY	0.5500	0.1506	-0.0935
2	PLHE	0.9920	-0.0478	0.0489
3	RHPL	0.5500	0.1506	-0.0935
4	CUHE	0.9885	0.0208	0.0052
5	CWBA	0.6709	-0.1904	-0.3049
6	MCWI	0.8358	-0.1949	-0.3511
7	SHCU	-0.1062	0.2393	0.3612
8	SCCU	0.0350	-0.1017	0.5158
9	LEDE	0.4677	-0.4095	0.6735
10	LETY	-0.0604	0.7735	-0.1249
11	SHLE	-0.1062	0.2393	0.3612
12	LEAU	0.0006	0.5351	-0.0710
13	LESH	0.4331	-0.6002	-0.3723
14	LETW	0.1662	-0.3575	-0.7161
15	LESL	0.4962	0.6059	-0.6154
16	LELB	-0.0491	0.9181	0.3879
17	WLEW	0.0528	0.7957	0.3773
18	GRLE	-0.1062	0.2393	0.3612
19	NUSL	-0.3162	-0.1249	-0.4732
20	NULB	-0.2703	0.7232	0.4192
21	AUBR	-0.0146	0.3856	-0.6237
22	INSH	0.2058	-0.4213	-0.2525
23	INDE	0.7043	-0.3157	0.0782
24	INLE	0.4500	0.0046	-0.5476
25	INWI	0.5388	-0.4233	-0.3500
26	NGIN	0.5115	-0.1873	-0.6974
27	NFGL	0.3114	-0.1916	-0.6754
28	GLWI	0.1564	-0.3099	-0.6936
29	GLLE	-0.2312	-0.1585	-0.2786
30	GLSH	0.2866	-0.0769	-0.5060
31	IBLE	-0.2382	0.0633	-0.1763
32	OBIN	0.1553	-0.4446	0.4611
33	IBFL	-0.1662	0.3575	0.7161
34	OPAU	0.5500	0.1506	-0.0935
35	IPAU	0.5500	0.1506	-0.0935
36	IPLE	0.4107	-0.3985	0.6897
37	OPLE	0.3873	-0.4368	0.6734
38	OTIT	0.0141	-0.2273	-0.3281
39	OPWI	-0.1141	0.2827	-0.0639
40	OPSH	0.3487	0.4385	-0.1975
41	IPSH	-0.1688	-0.3864	-0.4561
42	CPOU	0.2474	-0.4535	-0.5461
43	CPIN	0.3418	-0.3540	-0.5692
44	PESI	-0.4346	-0.1254	0.2053
45	TMWI	-0.2840	-0.1088	-0.0494
46	ANLE	-0.0970	0.6181	0.3389
47	ANWI	0.0683	0.5228	0.5026
48	ASFI	-0.0307	0.3349	0.3196
49	SSPE	0.0754	-0.7944	-0.3514
50	STLE	0.3427	0.3298	-0.3665
51	SILE	0.0670	0.4982	0.1818
52	STSH	0.5500	0.1506	-0.0935

Sl.No	Character	PC1	PC2	PC3
53	CALE	-0.3208	-0.2279	-0.1301
54	CAWI	0.0208	-0.6274	0.1158
55	CASH	-0.0378	-0.3700	-0.6176
56	CACO	-0.4481	0.1428	0.5066
57	CATI	0.4480	0.1136	-0.1726
58	CSWI	0.0654	-0.7747	-0.0768
59	CCSP	0.1390	-0.1052	-0.3434
60	ITLC	0.4349	-0.3816	0.6904
61	OTLC	0.4036	-0.4136	0.6689
62	SLWT	0.4423	0.0040	0.3244
63	SLNT	0.5394	0.0051	0.1028
64	SESH	-0.1701	0.6831	0.3690
65	SEAP	0.0846	-0.4291	0.0033

3.2 CA results

According to CA results, the phylogenetic tree obtained from the analysis is divided into two main clades. The first clade includes perennials species and second clades consists of annual species. These two groups separated from each other with LICY, CATI, NUSL characters. The firs clade is divided into two subgroups. One of the subgroups includes *J. inflexus* that distinguished from other subgroup with PLHE, LETY, LEDE and INSH characters.The other subgroup includes *J. gerardi*, *J. articulatus* and two sub species of *J. fontanesii*. The second clades also divided into two subgroups that includes *J. bufonius* and *J. rechingeri*. These species are annual.

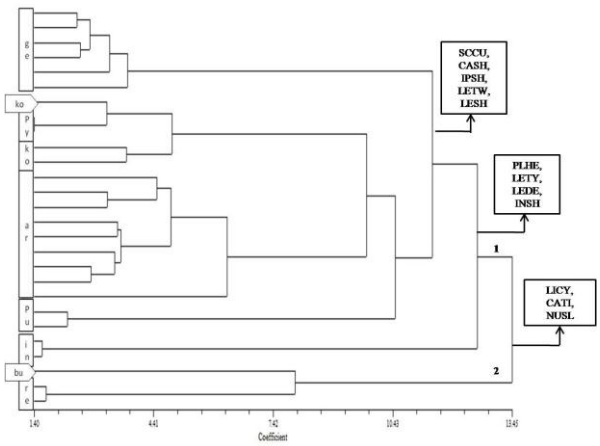

Fig. 2. Cluster analysis (UPGMA) of the 27 specimens measured. OTUs represented by ge = *J. gerardi*, ko = *J.fontanesii* subsp. *kotschyi*, py = *J. fontanesii* subsp. *pyramidatus*, ar = *J. articulatus*, pu = *J. punctorius*, in = *J. inflexus*, bu = *J. bufonius* and re = *J. rechingeri*.

4. Discussion

In the last decade, using the greater number of characters (morphometric study) has been proposed as a powerful tool in the species delimitation in plant systematic science. With the aid of morphometric analysis, the possible of more carefully species boundaries will be provided among taxa with high similarity.

In this study, the multivariate analyses show that the most species of *Juncus* in the Northest of Iran are as a complex and can not be found the characters that differentiate between species of this genus. The species *J. inflexus*, *J. punctorius* and *J. gerardi* have been separated better than the other species. These species are separated from other species with characters such as: LICY (plant life cycle), PLHE (plant height), CUHE (Culm height), INSH (Inflorecens Shape), and NULB (Number of Basal Leaves). But in the case of species *J. articulatus*, *J. fontanesii*, *J. bufonius* and *J. rechingeri* proximity of characters makes it difficult the separations of these species and probably individuals of these species are complex. However, can be observed some differentiation between populations of *J. bufonius*- *J. rechingeri* and *J. articulates*- *J. fontanesii* subsp. *Pyramidatus*- *J. fontanesii* subsp. *Kotschyi* via visual inspection.

In this study was observed that the characters such as: NCSE (Number of Capsule Section), NUST (Number of Stamen), IPWI (Inner Perianth Width) and GRST (Groove of stem) among the different species of *Juncus* are fixed, Therfore are not useful in identifying.

As shown in figure 1, individuals of *J. inflexus* and *J. rigidus* and annual species (*J. turkestanicus*, *J. rechingeri* and *J. bufonius*) and individuals of *J. articulatus*, *J.gerardii* and two sub-species of *J. fontanesii* do not make quite distinct species. This is possibly due to the somewhat their similar morphology.The results of this study indicate that the *J. inflexus* and *J. rigidus* are complex have high similarities (Figs.1). However there are some characters that could be differentiated them from each other (Table 3). The distinguishing traits of this group of other species are (CUHE) Culm Height, (PLHE) Plant Height, (OBLE) Outer Bract length, (SLWT)

Seed Length without Testa and (CAWI) Capsule Width. analysis do not makes theses quite distinct species.

The results of this study indicate that the *J. turkestanicus*, *J. bofunius* and *J. rechingeri* complex have high similarities (Fig. 1). However there are some characters that could be differentiated them from each other (Table 3). Plant Life Cycle (LICY), This is a good characteristic trait for separation of three species from the other species. Although the PCA cannot provide distinct boundaries between *J. turkestanicus*, *J. bofunius* and *J. rechingeri* complex species (Fig.1), however, cluster analysis is almost able to distinguish these three species from each other (Fig. 3). With regard to the results obtained from principal components and cluster analysis, micro- and macromorphological characters are somewhat able to differentiate the species in Northeast of Iran. The results of this study indicate that qualitative characters are useful in distinction among the species, whereas the quantitative traits due to overlapped size ranges could not be enough effective in the separation of species. Finally, in addition to the morphological study, anatomical, palynological and molecular studies may be useful to better delimit the species under study.

5. References

Abdel Khalik, K. 2010. Seed coat morphology and its systematic significance in *Juncus* L. (Juncaceae) in Egypt. *Journal of Systematics and Evolution*, 48(3): 215-223.

Balslev, H. 1996. Juncaceae. Flora Neotropica Monograph, 68: 1-168

Boissier, E. 1975. Flora Orientalis. Amsterdam, 5:346-362.

Boissier E. 1881. Flora Orientalis. Vol. 5. Geneva and Basel.

Brooks, R.E. and Kuhn, C. 1986. Seed morphology under SEM and light microscopy in Kansas *Juncus* (Juncaceae). *Brittonia*, 38(3): 201–209.

Buchenau, F. 1875. Monographia der Juncaceen vom Cap. Abbh. Naturwiss. *Verrine Bremen*, 4: 393–512.

Buchenau, F. 1890. Monographia Juncacearum. *Botanische Jahrb¨ucher f¨ur Systematik Pflanzengeschichte und Pflanzengeographie*, 12: 1–495.

Buchenau, F. 1906. Juncaceae. In: Engler A, Daz Pflanzenreich IV 36 (Heft 25). Leipzig: Verlag von Wilhelm Englemann. 1–284.

Buchenau, F. 1906. Juncaceae. In: Engler A, Daz Pflanzenreich IV 36 (Heft 25). Leipzig: Verlag von Wilhelm Englemann. 1–284.

Chernyakovskaya, E.G.1939. Flora of the U.S.S.R. Moskva-leningrad, 3:515-576.

Cullen J.1965. Flora of Turkey. Edinburgh, 9:1-32.

Dr´abkov´a, L., Kirschner, J., Seberg, O., Petersen, G. and Vlcˇek Cˇ . 2003. Phylogeny of the Juncaceae based on rbcL sequences, with special emphasis on *Luzula* DC. and *Juncus* L. *Plant systematic and Evolution*, 240: 133–147.

Jafri S.M.H. 1973. Flora of west Pakistan herbarium, Department of botany, University of Karachi, 138.

Kirschner, J. 2002. Juncaceae 2: *Juncus* subgen. *Juncus* Pp. 27-57 in *species plantarum*: *flora of the world* vol. 7, ed. A. E. Orchard, J. Bleyerveen, A. J. G. Wilson, and B. Kuchlmayr. Canberra: Australian Biological Resources Study.

Knapp, M.W. and Naczi, R. F. C. 2008. Taxonomy, Morphology and Geographic Distribution of *Juncus longii* (Juncaceae). *Systematic Botany*, 33(4): 685-694.

Krechetovich, V.I. and Goncharov, N.F. 1935. Juncaceae Vent. In: V. L. Komarov, editor. *Flora of the U.S.S.R.* 3:504-576.

Kuntze, O. 1903. *Juncus*. Pages 303-304. In: T. von Post & O. Kuntze, Lexicon Generum Phanerogamarum. Deutsche Vedags-Anstalt, Stuttgart.

Legendre, P. and Legendre, L. 1998. Numerical Ecology. Elsevier pp. 47-48.

Linnaeus C. 1753. Species Plantarum, 2 vols. Stockholm.

Nobvikov, V.S. 1990. Konspekt sistemy roda *Juncus* L. (Junaceae). Synopsis of the genus *Juncus* L. (Juncaceae). Bjulleten Moskovskogo Ispytalele Prirody, Biologiceskii 95(5): 111– 125.

Snogerup, S. 1993. A Revision of *Juncus* Subgen. *Juncus* (Juncaceae). Willdenowia 23: 23–73.

Snogerup, S. 1958. Studies in the genus *Juncus*. Some cytologica observations. *Bot. Not*., 111: 249-250.

Coffey, S.J. 1993. Juncaceae - Rush Family. In: The Jepson Manual. University of California.

Tackholm, V. and Drar, M. 1950. Flora of Egypt. Vol. 2. Bulletin of the Faculty of Science 28, Cairo University.

Vierhapper, F. 1930. Juncaceae. In: A. Engler & K. Prantl, Die natiirlichen Pflanzenfamilien, 2nd ed., 15A: 192-224.

Weimarck, H. 1946. Studies in Juncaceae, with special reference to the species in Ethiopia and the Cape. Svensk Bot. Tidskr. 40:141-178.