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Application Morphometric and taxonomic study of the genus Carex L. (Cyperaceae) in Northeast of Iran

Jinus Hejazi[1*], Ahmad Reza Bahrami[2], Jamil Vaezi[3], Farshid Memariani[4], Mohammad Reza Joharchi[4]

[1] Department of Biology, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran

[2] Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran

[3] Department of Biology, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran

[4] Research Centre for Plant Sciences, Ferdowsi University of Mashhad, Mashhad, Iran

*correspondence should be addressed to Jinus Hejazi, Department of Biology, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran; Tell: +98; Fax: +98; Email: jina_hejazi66@yahoo.com.

ABSTRACT

The genus *Carex* L. is one of the largest (2000 spp.) of all flowering plant genera. There are 85 species of *Carex* in Iran plateau with approximately half of them are present in Iran. We investigated morphologically nine species of *Carex* from two subgenera, *Vignea* and *Carex,* in Northeast of Iran. In this study, 102 characters were assessed including 53 quantitative and 49 qualitative characters. Principal component analysis and cluster analysis (UPGMA) were used to examine the relationships between taxa included in this study. Consequently, two major groups were identified; first group consists of *C. pachystylis* J. GAY., *C. divisa,* HUDS., *C. physodes* M.B., *C. divulsa* STOKES., and *C. cuprina (Sándor ex Heuff.) Nendtv.ex A.Kern.*; and second group includes *C. sylvatica* HUDS., *C. songorica* KAR. &KIR., *C. distans* L., and *C. diluta* M. B. In the PCA, two species *C. diluta* and *C. distans* were not well separated while the cluster analysis seemed to be better for distinguishing these two species. We concluded that micro-morphological characters are somewhat, but not fully, useful for species boundaries. Finally we provided revised key for the identification of these nine species.

Key words: Carex, Cyperaceae, principal component analysis (PCA), cluster analysis (CA), Northeast of Iran

1. INTRODUCTION

Cyperaceae (known as sedge) is a cosmopolitan family of monocotyledons (1). The members of this family are distinguished from grasses or rushes by features such as triangular stem in cross-section and spirally arrangement of leaves in three ranks (2). The Cyperaceae family generally has bisexual flowers with highly reduced or absent perianth parts. The two- to three-carpellate ovary matures into an achene (3, 4). Goetghebeur (1985) (5) recognized four subfamilies, *Cyperoideae, Sclerioideae, Caricoideae,* and *Mapanioideae*. Bruhl (1995) (6) revised this classification to two subfamilies *Cyperoideae* and *Caricoideae.* Morphological characteristics in combination with anatomical features, phytochemical analysis, and embryological examinations led to the classification of the ca. 5500 species and 109 genera (7). Many advances in molecular sciences have led to an increased understanding of phylogenetic relationships within this large family. The use of the plastid *rbc*L, *trn*L-F, *mat*K, ribosomal ITS and morphological studies led to identifying two subfamilies, *Cyperoideae* and *Mapanioideae* (8). Also, Thorne and Reveal (2007) (9) consider the number of genera to be 104 with 5010 species with the following internal breakdown: *Mapanioideae* (13 genera and 140 species) and *Cyperoideae* (91 genera and 4870 species, including *Carex*L.) (10). In Flora Iranica (11) two subfamilies, *Cyperoideae* and *Caricoideae*, and four tribes, *Scirpeae, Cypereae, Rhynchosporeae,* and *Cariceae*, are introduced for Cyperaceae family. According to this Flora, the genus *Carex* L. belongs to *Caricoideae* subfamily and *Cariceae* tribe. The genus is divided into four sub-genera (*Psyllophora, Indocarex, Vignea,* and *Carex*) and 33 sections, mainly based on the number of stigmas, arrangement of spikes and whether the spikes are unisexual or bisexual. The genus *Carex* with approximately 2000 species (12, 13) is one of the largest genera of vascular plants (14) that equals in species richness only by

Euphorbia L. and *Piper* L. (15). *Carex* includes the majority of species within the *Caricoideae* subfamily (16). The genus is separated from other genera in the *Caricoideae* by an entire closed perigynia (utricle); it is typically extended into a "rostrum" or beak, which is often divided at the tip (bifid) into two teeth (7). The shape, venation, and vestiture (hairs) of the perigynium are important structures for distinguishing *Carex* species. Almost all *Carex* species are monoecious; each flower is either male (staminate) or female (pistillate) (17). Sedges show diverse arrangements of male and female flowers. Often, the lower and upper spikes are entirely pistillate spikes staminate respectively, with one or more spikes in between having pistillate flowers near the base and staminate flowers near the tip. In other species, all spikes are similar. In this case, they may have male flowers above and female flowers below (androgynous) or female flowers above and male flowers below (gynecandrous) (7).

1.1. Distribution

The species diversity in this genus is greatest at high latitudes and altitudes in the Northern Hemisphere. Sedges occur in a wide range of humid to dry habitats (including flooded wetlands, tundra, alpine grasslands, rocky mountain habitats, coniferous woods, mixed or deciduous forests, steppes, meadows, pastures and salt marshes) and have a rather weak affinity with man-made habitats (13, 17, 18). It is also of global importance as one of the few truly cosmopolitan plant genera with centers of diversity in the temperate regions of Asia, Europe, and the Americas (19). *Carex* species often indicate a high degree of habitat specificity, making them some of the best indicator plants for characterizing habitat types e.g., (20-27).

1.2. Cytological studies

The chromosome number in *Carex* varies almost continually from x=6 in *C. siderosticta* (28) to x=62 in *C. roraimensis* (29). Considering the almost continual variation in chromosome numbers and the lack of a positive correlation between the DNA content and the chromosome number, an important role for agmatoploidy or symploidy relative to polyploidy in karyotype evolution in *Carex* could be expected. Whereas polyploidy is frequent in some other genera of *Cyperaceae* (e.g., *Rhynchosporeae* Vahl (30, 31); *Eleocharis* R. Br.: (32, 33) in *Carex*, it has been confirmed only in *Carex siderosticta*, *C. dolichostachya*, *C. parciflora,* and *C. roraimensis* (34).

1.3. Taxonomic problem

Evolutionary relationships within *Carex* are poorly understood, despite the global distribution and ecological importance of this genus (16). This lack of understanding can be attributed to the nature of morphological and anatomical characters in *Carex.* The repeated events of parallelism and reversal (16, 35), floral reduction (36) and uniform vegetative morphology and anatomy (37) have obscured phylogenetic trends and have led to the recognition of many artificial taxa at the sectional and sub-generic level (35). Many species of *Carex* are characterized by high intraspecific variability and the ability to produce hybrids within and, rarely, between sections. Thus, the status of some taxa is ambiguous, causing heterogeneity in taxonomic descriptions. The majority of the studies published so far, particularly the older ones referring to the taxonomies of the genus *Carex*, have been mainly based on observations of the morphological and anatomical traits of specific organs e.g., (13, 38-41). However, many more recent papers indicate that micro-morphological, molecular or biochemical analyses present a wide range of taxonomic relationships e.g. (42) in the genus *Carex* and in the entire family Cyperaceae (Poales sensu (42, 43). According to Flora Iranica (11), there are 85 species of *Carex* in Iran plateau of which approximately half of them exist in Iran. We investigated, morphologically, nine species of *Carex* from subgenera *Vignea* and *Carex* in Northeast of Iran (Khorasan provinces) including *C.diluta* M. B., *C. distans* L., *C. songorica* KAR. & KIR., *C. divisa* HUDS., *C. divulsa* STOKES., *C. sylvatica* HUDS., *C. cuprina* (Sándor ex Heuff.) Nendtv.ex A.Kern., *C. pachystylis* J. GAY., and *C. physodes* M. B. Two species, *C. diluta* and C. *distans*, are not easily distinguished from each other due to having overlapped characters such as shape and red-brown punctate between veins of utricles, color and shape of spikes and traits of nutlets. In this study we try to find morphological characters that are effective for species boundaries and also evaluate taxonomic position of each species. The objectives of our study were to investigate: (1) whether quantitative characters are useful for species differentiation. In other words, whether we find reliable quantitative characters for species boundaries; (2) what is taxonomic position of the two species *C. diluta* and *C. distans*; (3) whether *C. diluta* and *C. distans* should be considered as a complex within the subgenus *Carex*; and (4) whether exclusively morphological characters are able to differentiate species in the Northeast of Iran.

2. MATERIALS AND METHODS

2.1. Sample collection

We sampled 350 individuals (30-40 per species) including field-collected and herbarium specimens of Ferdowsi University of Mashhad Herbarium (FUMH)from different populations throughout Northeast of Iran including, North Razavi and South Khorasan provinces (Table 1). Within each population we randomly selected one individual using the ignorant man method (44). The populations of each species were selected with significantly geographical distances to collect more intra-specifically morphological variations. In order to identify the species correctly, only specimens with the firm and mature nutlets were considered. All voucher specimens were deposited at the herbarium of Faculty of Sciences of Ferdowsi University of Mashhad. We tried to identify the specimens using different floras such as: Flora Iranica (11), Flora of

Pakistan (45), Flora Orientalis (46) Flora of Iraq (47), Flora of Palestine (48), Flora of USSR (49), and Flora of Turkey (50). In total, 28 field-collected and herbarium specimens were evaluated and entered in final analyses.

Table 1. List of field-collected and herbarium specimens of the genus *Carex* used in the current study. The vouchers that are denoted by asterisks are duplicate samples of Ferdowsi University of Mashhad Herbarium (FUMH)

Voucher ID	*Taxon*	*Locality*	*Collection date*	*Collector(s)*
60111	*C. pachystylis*	Mashhad, Ferdowsi University	April 2011	Hejazi
60112	*C. pachystylis*	Mashhad, Khorshid Park	May 2011	Basiri
26170*	*C. pachystylis*	Tabas, Deyhook	March 1996	Rafei, Zangouei
10700*	*C. pachystylis*	South of Mashhad, Bidak Kaal	April 1984	Joharchi, zangouei
38279*	*C. pachystylis*	Southwest of Bojnord, Zoyreiin	May 2006	Joharchi, Memariani
70111	*C. sylvatica*	Southwest of Bojnord, Sarigiv Valley	June 2011	Hejazi, Basiri
37252*	*C. sylvatica*	Southwest of Bojnord, Delav Valley	April 2006	Memariani, Zangouei
26209*	*C. physodes*	Tabas, Deyhook	April 1996	Rafei, Zangouei
28263*	*C. physodes*	Southeast of Birjand	April 1998	Rafei, Zangouei
28346*	*C. physodes*	Northwest Of Nehbandan	April,1997	Rafei,Zangouei
29813*	*C. physodes*	Boshroye	April,1998	Hojjat,Zangouei
80111	*C. cuprina*	Southwest of Bojnord	May 2011	Hejazi
43792*	*C. cuprina*	West of Bojnord	May 2007	Joharchi, Memariani
90111	*C. divisa*	Gonabad, Sarasiab	March 2011	Sokhanvar
90112	*C. divisa*	Mashhad, Freizei	April 2011	Hejazi, Basiri
90113	*C. divisa*	Sarakhs, Mazdavand	May 2011	Hejazi, Basiri
10011	*C. diluta*	Mareshk	April 2011	Basiri
10012	*C. diluta*	Frazei, Derme Valley	May 2011	Hejazi, Basiri
34463*	*C. diluta*	Northwest of Ghaen	May 2003	Joharchi
17720*	*C. distans*	Birjand, Bagheran Mountain	June 1989	Joharchi, Zangouei
18932*	*C. distans*	Kalat, Gharesoo	July 1990	Faghihnia, Zangouei
34661*	*C. distans*	South of Mashhad	June 2003	Ajenni, Zangouei
12011	*C. divulsa*	Torghabe, Dehbar	June 2011	Hejazi, Basiri
12012	*C. divulsa*	Shandiz	June 2011	Ansari
12013	*C. divulsa*	Zoshk	May 2011	Basiri
13011	*C. songorica*	Bojnord, Reiin	April 2011	Hejazi, Basiri
13012	*C. songorica*	Frazei, Derme Valley	May 2011	Hejazi, Basiri
13013	*C. songorica*	Shirvan, Galol and Sorani	June 2011	Basiri

2.2. Morphological characters

In total, 102 characters were measured including 53 quantitative and 49 qualitative (Table 2). Large quantitative characters were measured in millimeter scale with a ruler, and smaller characters increments using a stereomicroscope (OLYMPUS SZH10DFplanapo). Qualitative characters were evaluated by introducing visual indices that are related to character states in different floras. For standardization, measurements were performed on mature individuals. In order to minimize errors, missing data were replaced with mean of measurements for each character within same species (51).

Table 2. List of quantitative (QN) and qualitative (QL) characters used in the current study. The characters that denoted with asterisks were used in the PCA

No.	*Characters*	*Abbreviation*	*Character type*
1	Length of plant	PLLG	QN
2	Width of leaf in widest part	LWWP	QN
3	Length of leaf from top of sheath*	LLTS	QN
4	Comparison of leaf length to length of plant	CLPL	QL
5	Leafs margin	LFMG	QL

6	Leafs channel	LFCH	QL
7	Smooth or rough state of adaxial leaf surface	SRAD	QL
8	Smooth or rough state of abaxial leaf surface	SRAB	QL
9	Smooth or rough state of stem	SRST	QL
10	Type of triangular stem*	TTRS	QL
11	Rhizomatous or stoloniferous plant*	RSPL	QL
12	Size of ligule	LISZ	QN
13	Length of leaf sheath*	LLSH	QN
14	Type of sheath	TSHT	QL
15	Type of leafs	TLEF	QL
16	Mean of bracts length*	BRLG	QN
17	Length of lower bract*	LBLG	QN
18	Length of upper bract	UBLG	QN
19	Width of widest bract*	BRWD	QN
20	Ratio of BRLG to BRWD*	RTLW	QN
21	Type of bracts*	TBRC	QN
22	Bract sheath	BRSH	QL
23	Length of bract sheath	BSHL	QN
24	Ciliate bracts	CLBR	QL
25	Comparison of bracts length to length of inflorescence*	CLIL	QL
26	Comparison of bracts length to length of their spike*	CBLS	QL
27	Colour of bracts*	BRCO	QL
28	Length of inflorescence*	INFL	QN
29	Number of (bisexual) spikes*	NUAS	QN
30	Number of spikelet in androgynous (bisexual) spikes*	NSBS	QN
31	Sex of spikes*	SXSP	QL
32	Length of staminate spike	SSPL	QN
33	Maximum width of staminate spikes	MWSS	QN
34	Shape of staminate spike*	STSS	QL
35	Colour of staminate spikes*	COSS	QL
36	Colour of androgynous (bisexual) spikes*	CBIS	QL
37	Length of glume of staminate spike	GLSP	QN
38	Width of glume of staminate spike	GWSP	QN
39	Ratio of GLSP to GWSP	RALW	QN
40	Apex shape of staminate glume	SGAS	QL
41	Colour of staminate glume of terminal spike*	SGCT	QL
42	Length of staminate glume awn of terminal spike*	LSGA	QN
43	Number of staminate spike	NSSP	QN
44	Number of pistillate spikes	NPSP	QN
45	Average length of pistillate spikes*	ALPS	QN
46	Average length of androgynous spikelets*	ALAS	QN
47	Maximum width of pistillate spikes	MWPS	QN
48	Maximum width of androgynous spikelets	MWAS	QN
49	Ratio of ALPS to MWPS	RAAM	QN
50	Ratio of ALAS to MWAS	RALM	QN
51	Shape of pistillate spike*	PISS	QL
52	Distance between terminal staminate spikes from uppermost pistillate spikes	DTSP	QN
53	Average distance between pistillate spikes*	ADPS	QN
54	Average distance between androgynous spikelets	ADAS	QN
55	Presence of peduncle in pistillate spikes	PPPS	QL
56	Length of lowermost pistillate spike peduncle*	LPSP	QN
57	Average length of uppermost pistillate spikes peduncle	ALPP	QN
58	Length of glume of pistillate spikes	GLPS	QN
59	Length of glume of androgynous spikelets	GLAS	QN
60	Width of glume of pistillate spikes	GWPS	QN
61	Width of glume of androgynous spikelets	GWAS	QN
62	Shape of glume of pistillate spike*	PGLS	QL
63	Shape of glume of staminate spike*	SGLS	QL
64	Shape of glume of androgynous spike*	ANGS	QL
65	Glume apex shape of lower pistilate spikes*	GASP	QL
66	Apex shape of glume of androgynous spikelets	GASA	QL
67	Length of awn of pistillate glume*	LPGA	QN
68	Length of awn of androgynous glume	LAGA	QN
69	Ratio of GLPS to GWPS	RAGG	QN
70	Ratio of GLAS to GWAS	RASS	QN
71	Colour of glume of androgynous spikes*	GCAS	QL
72	Colour of glume of lower pistillate spikes*	GCPS	QL
73	Length of anther	ANTL	QN
74	Suspension state of peduncle of lower pistillate spike	SPSP	QL
75	Length of utricle*	UTCL	QN
76	Widest point of utricle*	WUTC	QN
77	Ratio of UTCL to WUTC	RUTV	QN
78	Length of beak*	BEKL	QN
79	Presence of nerves on utricle surface*	PNUT	QL

80	Width of utricle in stipitate base	WUTS	QN
81	Length of utricle in stipitate base	LUTS	QN
82	Texture of utricle	TXUT	QL
83	Location of widest point of utricle	LWUT	QL
84	Colour of utricle*	UTCO	QL
85	Colour of beak*	BECO	QL
86	Location state of glumes to utricles*	LGLU	QL
87	Type of beak*	TBEK	QL
88	Length of achene to apex of style*	LAAS	QN
89	Length of achene to base of style	LABS	QN
90	Widest point of achene	WPAC	QN
91	Ratio of LABS to WPAC	RASC	QN
92	Length of style	LSTY	QN
93	Surface texture of achene	ACST	QL
94	Colour of achene*	ACHC	QL
95	Shape of achene*	ACHS	QL
96	Location of widest point of achene	LWAC	QL
97	Shape of utricle*	UTSH	QL
98	Condensity of spikes*	CNDS	QL
99	Colour of pistillate spikes*	COPS	QL
100	Diameter of stem	DIST	QN
101	Number of stigma*	NUST	QN
102	Length of stigma*	LGST	QN

2.3. Data analysis

2.3.1. Univariate analysis

To determine which characters are more effective in differentiating the nine species under study, Univariate analysis was conducted. All quantitative characters were evaluated to determine the normality distribution of data 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) was used to examine the variance of a dependent variable. The dependent variable was 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 (52). Significant difference was considered at $P<0.05$. Those characters with non-significant values (not effective in species delimitation) were excluded from final analyses.

2.3.2. Mann-Whitney U test

This test (also called the Mann–Whitney–Wilcoxon (MWW) or Wilcoxon rank-sum test) is a non-parametric statistical hypothesis test for evaluating distinguishing qualitative characters between pair of the species. The Wilcoxon-Mann-Whitney test is a non-parametric analog to the independent samples t-test and can be used when one do not assume that the dependent variable is a normally distributed interval variable (you only assume that the variable is at least ordinal).It can also be used when the variable being recorded is measured using an arbitrary scale which cannot be measured accurately (e.g. a color scale measured by eye). This test was performed using the software SPSS ver.16 (52).

2.3.3. Independent samples t-test

An independent samples t-test can be used to compare two small sets of quantitative data when samples are collected independently of one another. It was also used for comparing the means of a normally distributed interval dependent variable for two independent groups. Also Levene's test was conducted for equality of variances. The discriminative characters obtained from two latest tests were then applied for creating identification key. This test was conducted using the software SPSS ver.16 (52).

2.4. Multivariate analysis

2.4.1. Principal component analysis (PCA)

The PCA (principal component analysis) was conducted based on a correlation matrix of standardized traits and specimens to elucidate relationships among the taxa. This analysis was performed using the software CANOCO ver. 4.5 (51).

2.4.2. Cluster analysis (CA)

A dendrogram was constructed with a cluster analysis of the matrix by using the UPGMA (a simple agglomerative or hierarchical clustering) method. This analysis was performed in NTSYS-PC ver.2 software (52). In this method after data standardization, the algorithm evaluates

the structure present in a pair wise distance matrix (or a similarity matrix) to then construct a rooted tree (dendrogram) (53).

3. RESULTS

3.1. Univariate analysis

In this test, two characters, LABS and WUTS, did not significantly (P<0.05) differentiate the species and were excluded from the subsequent analyses. Therefore we entered 100 characters in the multivariate analyses. When all of these characters were used in the PCA, species differentiation was not performed. Since measurement range of quantitative characters is very close in most of the species of *Carex*, using the large number of characters does not lead to species boundaries within *Carex* species in Northeast of Iran. Logically, characters with a significant difference of P<0.01 were selected for the principal components and cluster analyses. Therefore, characters were reduced to almost half. On the other hand, the analyses were performed using 49 characters of which 29 were qualitative and 20 quantitative.

3.2. Mann-Whitney U test and independent samples t-test

The discriminative characters among nine studied species were outlined in Table 3 . As results shown, the numbers of distinguishing characters between species pairs with more similarity were much greater than species with distinct morphological differences.

Table 3 . List of distinguishing characters between pair of some species and two main groups obtained from Mann-Whitney U test and independent samples t- test. The species name represented by abbreviation: phy = *C. physodes*, pach = *C. pachystylis*, dvi = *C. divisa*, otr = *C. cuprina*, dvu = *C. divulsa*, sy = *C. sylvatica*, dil = *C. diluta*, dis = *C. distans*, son = *C. songorica*, F = first group, and S = second group

NO.	*phy and pach*	*pach and dvi*	*phy and dvi*	*otr and dvu*	*otr and dvi*	*sy and dil*	*dil and dis*	*dil and son*	*F and S*
1	RSPL	LLTS	LLTS	TTRS	TTRS	RSPL	RSPL	RSPL	NUST
2	LLSH	ALPS	RSPL	RSPL	TBRC	STSS	COSS	CLIL	SXSP
3	BRLG	CBLS	ALPS	CLIL	CLIL	COSS	SGCT	STSS	NSBS
4	RTLW	BRCO	BRLG	CBLS	CBLS	SGCT	PISS	COSS	ALAS
5	CBLS	INFL	BRWD	ANGS	BRCO	PISS	PGLS	SGCT	CBIS
6	BRCO	NSBS	BRCO	GCAS	CBIS	SGLS	SGLS	PISS	ANGS
7	INFL	CBIS	NSBS	PNUT	ANGS	GCPS	GASP	PGLS	GCAS
8	NSBS	ALAS	CBIS	UTCO	GCAS	PNUT	LPGA	SGLS	NUAS
9	ALAS	UTCL	ANGS	TBEK	PNUT	UTCO	GCPS	GASP	INFL
10	ANGS	BEKL	GCAS	ACHC	UTCO	BECO	UTCL	LPGA	TBRC
11	GCAS	WUTC	UTCL	ACHS	BECO	LGLU	UTCO	GCPS	ADPS
12	UTCL	UTCO	WUTC	INFL	LGLU	TBEK	BECO	PNUT	UTSH
13	WUTC	BECO	PNUT	LLSH	TBEK	CNDS	TBEK	UTCO	UTCO
14	PNUT	LGLU	UTCO	NSBS	ACHC	BEKL	ACHC	BECO	
15	BECO	ACHC	BECO	NUAS	UTSH	LGST	LAAS	TBEK	
16	LGLU	UTSH	LGLU		NSBS	ALPS	WUTC	UTSH	
17	TBEK	LGST	TBEK		LGST	UTCL	COPS	COPS	
18	LAAS		LAAS		LLTS		LLSH	LGST	
19	ACHC		UTSH				LGST	INFL	
20	UTSH		LGST				LPGA		
21	LGST						LPSP		

3.3. Principal component analysis

In the PCA (Figure1, A & B), the three axes including PC1, PC2 and PC3 account for 64.9%, 24.8%, and 7.6%, respectively. Therefore, the highest percentage of total variance is related to the first axis. The characters, INFL, SXSP, NUST, BRLG, LBLG, RTLW, ADPS, LPSP, and UTSH due to having higher loading (> 0.7) on axis 1 were more effective in species separation. Thus, these characters have possessed the highest attribute in species differentiation along the X (PC1) axis (list of eigenvectors of the characters is shown in Table 4).

Table 4. The eigenvectors of studied characters obtained from the PCA

No	*Characters*	*AX1*	*AX2*	*AX3*	*AX4*
1	LLTS	0.0437	0.9962	-0.0738	-0.0052
2	TTRS	0.0284	0.0704	-0.2572	-0.2689
3	RSPL	0.3037	-0.0444	-0.0818	0.2909
4	LLSH	0.0938	0.6220	0.4404	0.1713
5	BRLG	0.8981	0.0831	0.3687	0.0421

6	LBLG	0.8559	0.0654	0.5023	-0.0991
7	BRWD	0.2115	-0.4833	0.3245	0.2368
8	RTLW	0.8696	0.1368	0.3553	-0.0088
9	TBRC	0.7463	0.5163	-0.0576	-0.2240
10	CLIL	-0.0533	0.6486	0.3104	0.1772
11	CBLS	-0.2878	0.4798	-0.4435	-0.1727
12	BRCO	-0.6189	-0.2722	-0.0240	0.1588
13	INFL	0.9755	-0.0583	-0.2101	-0.0226
14	NUAS	-0.2097	0.4662	-0.4635	-0.2829
15	NSBS	-0.7218	0.0682	-0.1850	0.1644
16	SXSP	-0.9335	0.0358	-0.3038	0.0710
17	STSS	0.7560	0.1060	0.5696	-0.1288
18	COSS	0.6941	0.1231	0.5858	-0.1426
19	CBIS	-0.7901	-0.1915	-0.1242	0.1438
20	SGCT	0.6763	0.1686	0.5987	-0.0335
21	LSGA	0.5663	-0.0863	-0.3379	0.5603
22	ALPS	0.7589	0.1861	0.5330	0.2042
23	ALAS	-0.7833	-0.0699	-0.2272	0.1131
24	PISS	0.7800	-0.2035	0.1059	-0.3897
25	ADPS	0.8896	0.0841	0.2776	0.3098
26	LPSP	0.8035	-0.1831	-0.2895	0.3126
27	PGLS	0.7082	0.1793	0.6350	-0.0122
28	SGLS	0.7800	-0.2035	0.1059	-0.3897
29	ANGS	-0.7137	-0.0735	-0.1900	0.1412
30	GASP	0.7082	0.1793	0.6350	-0.0122
31	LPGA	0.7928	0.1675	0.4900	0.2119
32	GCAS	-0.5861	0.4895	-0.4027	-0.0248
33	GCPS	0.6941	0.1231	0.5858	-0.1426
34	UTCL	-0.2984	-0.2255	-0.0794	0.1351
35	BEKL	0.3392	0.3284	-0.5007	0.3542

36	WUTC	-0.3323	-0.1851	-0.0023	0.1095
36	WUTC	-0.3323	-0.1851	-0.0023	0.1095
37	PNUT	0.2247	0.6156	0.4263	-0.1783
38	UTCO	0.4807	0.6780	0.0486	-0.0560
39	BECO	0.1590	0.5578	0.3641	0.0853
40	LGLU	-0.0675	-0.1989	-0.2060	0.4447
41	TBEK	0.2637	0.2894	0.3009	-0.1617
42	LAAS	-0.2970	-0.3216	-0.0996	0.0336
43	ACHC	0.5746	-0.0728	0.2174	-0.1157
44	ACHS	0.7626	-0.2610	0.4498	0.0928
45	UTSH	0.8221	-0.3116	0.0690	-0.0945
46	CNDS	0.5644	-0.0872	-0.3299	0.5347
47	COPS	0.7082	0.1793	0.6350	-0.0122
48	NUST	-0.9335	0.0358	-0.3038	0.0710
49	LGST	-0.6328	-0.3127	0.1078	0.3203

According to the PC1 (Figure 1A), two major groups are identified; the first group consists of *C.pachystylis, C. divisa, C. physodes, C. divulsa,* and *Cuprina*. The second group includes *C. sylvatica, C. songorica, C. distans,* and *C. diluta.* These two groups are separated from each other with three main characters including NUST, SXSP, and INFL. The specimens of *C. pachystylis* and *C. physodes* are close together (Figure 1A). However, some characters such as LAAS and UTCL make distinction between them. These species are differentiated from *C. divisa* by the characters including CBIS, ANGS, BRCO, and LGST (Table 3). The specimens of *C. divisa* are isolated from those of *C. divulsa* and *C. cuprina* by the trait GCAS (Table 3). The individuals of *C. divulsa* and *C. cuprina* could be different from each other by the traits CBLS and NUAS (Table 3). Furthermore the number of androgynous spikes (NUAS) is one the most important trait in differentiating between *C. divulsa* (15-20 spikes) and *C. cuprina* (single spike) (Table 3). The bracts of *C. cuprina* are as long as the inflorescence, but shorter in *C. divulsa*. The leaf length relative to length of plant (CLPL) in *C. divulsa* is longer than that of in *C. cuprina*. The average distance between androgynous spikes (ADAS) in *C. divulsa* is 13-16 mm, whereas this distance between androgynous spikelets is reduced to 1-2 mm in *C. cuprina*. The types of leaf (TLEF) are basal and alternate in *C. cuprina*, but basal only in *C. divulsa*. The type of underground organ (RSPL) in *C. cuprina* is different from that of in *C. divulsa.* The first is rhizomatous whereas the second is developed by stolon. Stem in *C. divulsa* is entirely scabrid but in *C. cuprina* it is scabrid only near the tip (top) of the stem. Other distinct characters between two species *C. divulsa* and *C. cuprina* include ACHS, ACHC, TBEK, UTCO, PNUT, and INFL (Table 3). In the second group (Figure 1A, right hand side); three subgroups of species are detectable. First subgroup includes the specimens of the species *C. songorica*; second subgroup consists of the individuals of the species *C. sylvatica*; and third subgroup comprises a complex of the specimens of the two species *C. distans* and *C. diluta*. The individuals of *C. songorica* are easily identifiable from those of the species *C. sylvatica* by the characters such as, UTCO, TBRC, BEKL, TBEK, ALPS, GASP, BRLG, and LBLG (Table 3). The specimens of *C. sylvatica* are differentiated from the third sub group by the characters such as, INFL, CNDS, and LSGA (Table 3). The species of the third subgroup are distinct from each other with a few characters including PISS, UTSH, ACHS, ACHC, and SGLS (Table 3).

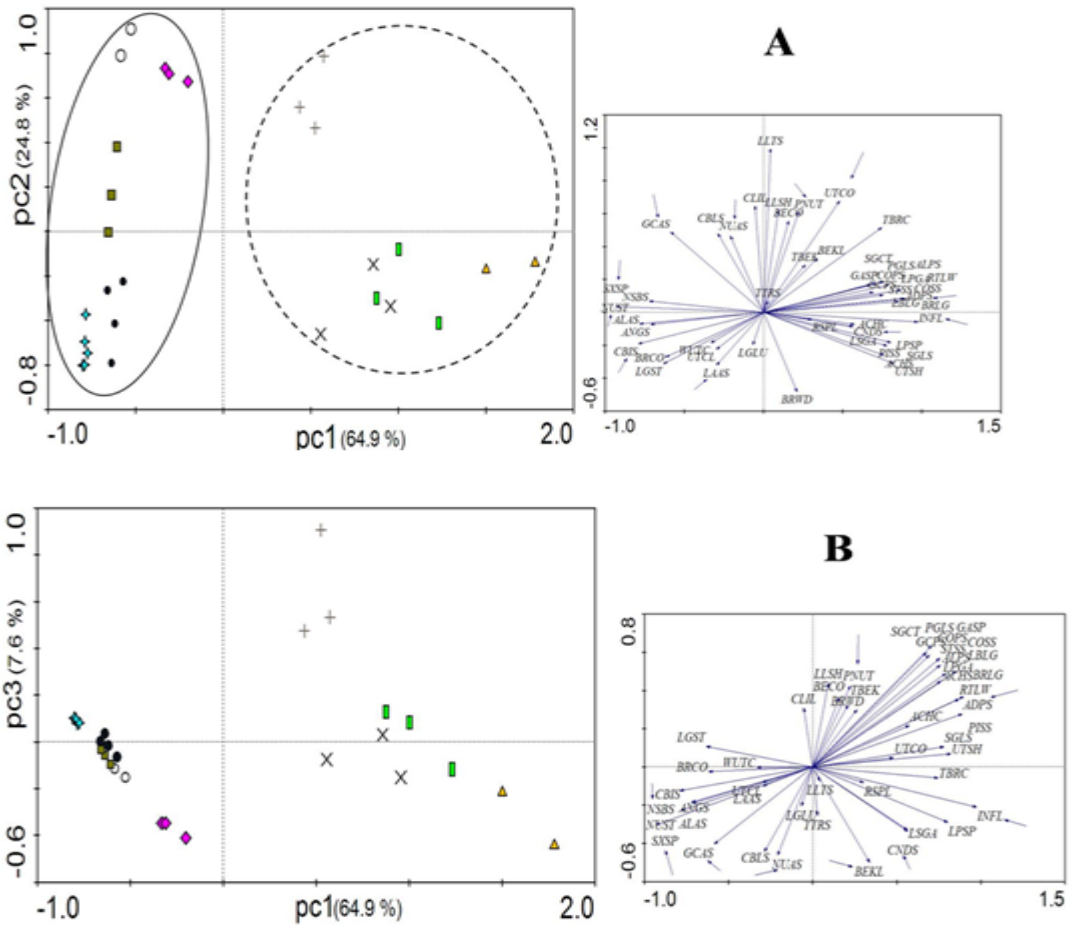

Figure 1. Principal component analysis- Scatter diagrams of specimens and characters. A. position of taxa based on the first and second principal components. B. position of taxa based on the first and third principal components. The following symbols representing the species; black circle: *C. physodes*; white circle: *C. cuprina*; 4-point star: *C. pachystylis*; rectangle: *C. distans*; triangle: *C. sylvatica*; diamond: *C. divulsa*; square: *C. divisa*; plus sign: *C. songorica*; multiplication sign: *C. diluta*. The characters that are effective in species differentiation marked with arrows. Continuous and stipple-bordered ellipses represents first and second group, respectively

3.4. CA results

The results (Figure 2) obtained from the cluster analysis greatly overlapped with the results of the PCA (Figure 1, A & B). However, the cluster analysis provided more obvious species boundaries. According to this analysis (Figure 2), nine species in Northeast of Iran were placed in two major clusters (indicated by the numbered arrows in Figure 2). First cluster consists of species of subgenus *Vignea* which are distinct from those of the second one with androgynous and distigmatic flowers. The first cluster is indicated by five species including *C. pachystylis, C. divisa, C. physodes*, *C. divulsa,* and *C. cuprina*. The second cluster consists of species of the subgenus *Carex* including *C. sylvatica, C. dilutea , C. distans,* and *C. songorica* (Figure 2). The species *C. sylvatica* is located as a sister group in relation to the remaining species. This species is specialized by having the "female lax spike" character. The results obtained from the cluster analysis (Figure 2) indicate that the two species, *C. distans* and *C. diluta*, are obviously differentiated. However, the PCA (Figure 1, A & B) could not clearly separate these two species. The individuals of the species *C. songorica* are placed as a sister to the last two species. This species is differentiated by the characters such as colour and size of the utricle.

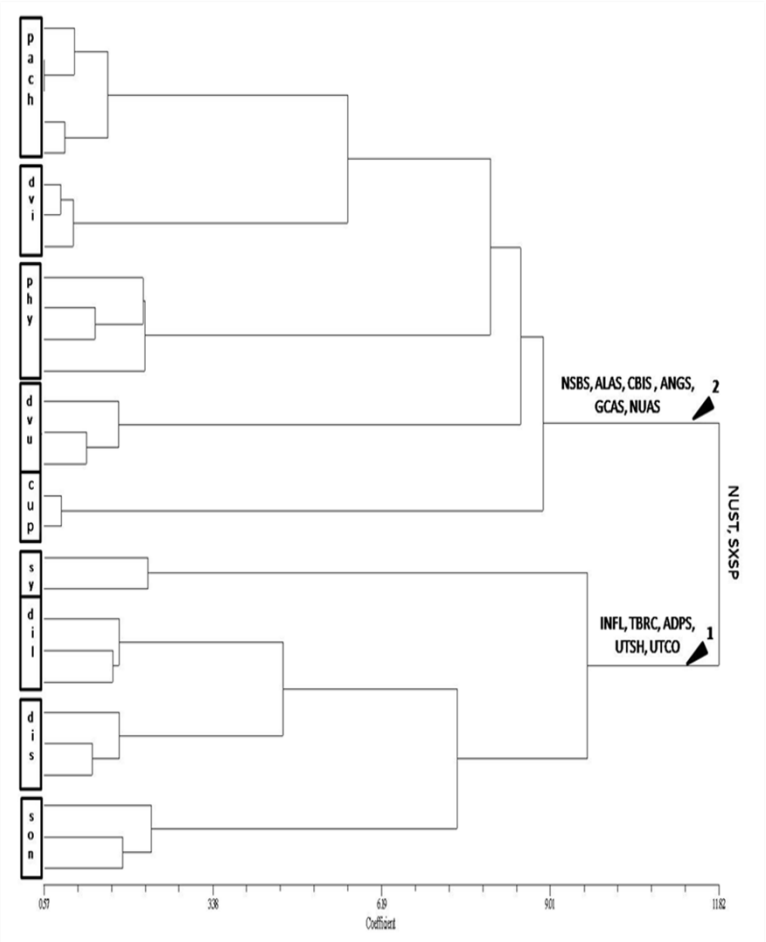

Figure 2. Phenogram resulting from the UPGMA method of *Carex* species in Northeast of Iran. The scientific name of species is shown with acronyms. OTU's presented by pach = *C. pachystylis*, dvi = *C. divisa*, phy = *C. physodes*, dvu = *C. divulsa*, cup = *C. cuprina*, sy = *C. sylvatica*, dil = *C. diluta*, dis = *C. distans*, son = *C. songorica*. The numbers 1 and 2 representing second and first groups, respectively

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 (54, 55). With the aid of morphometric analysis, the possiblity of more species boundaries will be provided among taxa with high similarity (56). The univariate analysis indicated that 49 characters are effective in differentiation among nine species in Northeast of Iran. Reproductive traits which are relevant to inflorescence, utricles and nutlets have dominant proportion in species boundaries (Table 3). In the present study, multivariate analyses of morphological characters indicate that the species are classified in two main groups (Figures 1A & 2). These groups belong to two subgenera including *Vignea* and *Carex*. The first group consists of the species *C. divisa, C. divulsa, C. pachystylis, C. physodes,* and *C. cuprina*. The second group includes the species *C. songorica, C. diluta, C. distans,* and *C. sylvatica*. This initial grouping is based on three distinctive characters ofNUST, SXSP, and INFL (shown in Figure 2 & Table 4). These characters have the highest loading (>0.9) on PC1. Accordingly, the two subgenera *Vignea* and *Carex* are easily distinct from each other (Figures 1A & 2). Almost all species of the subgenus *Vignea* have androgynous and distigmatic flowers, whereas the subgenus *Carex* often has unisexual spike and tristigmatic flowers.

4.1. First group

As shown in Figure 1 (parts A & B), individuals of *C. pachystylis* and *C. physodes* do not make quite distinct species. This is possibly due to their similar morphology. Some of overlapped characters are: TTRS, BRWD, TBRC, CLIL, CBIS, BEKL, UTCO, ACHS, and CNDS. Nevertheless, there are characteristic traits causing distinction between the two species. Some of these traits are: UTSH, LAAS, UTCL, WUTC, BRCO, LGST, ANGS, ALAS, and CBIS (Table 3). The two species are similar together in terms of vegetative traits. Some of these characters are: PLLG, SRST, TSHT (these characters not entered in the analyses but they were evaluated during the initial examination). It should be noted that the two species in terms of phylogenetic affinities mentioned in Flora Iranica (11), Flora of Pakistan (45), Flora of Turkey (50), and Flora of USSR (49), are close to each other and placed in the same section called *physodeae*. In addition, the results obtained from cluster analysis also indicate that the two species are grouped together (Figure 2). Individuals of *C. divisa* are placed between the *C. pachystylis*, *C. physodes* group and the *C. cuprina, C. divulsa* group (Figure 1A). There are distinctive traits separating *C.*

divisa from these two groups. Some of these characteristics are: NSBS, GCAS, RSPL, TSHT, CBIS, ANGS, ALAS, CBLS, PNUT, TXUT, TBEK, and UTSH (Figure 1A). The cluster analysis shows that *C. divisa* is located within the *C. pachystylis* and *C. physodes*clade (Figure 2). *Carex divisa* is morphologically more similar to *C. pachystylis*. This similarity is confirmed in the cluster analysis (Figure 2). The distinctive characters such as BRLG**,** CBIS**,** TBRC, ANGS, and GCAS differentiated *C. divisa* from the other species of the subgenus *Vignea* (Figure 1A).

Individuals of *C. divulsa* and *C. cuprina* are placed close together (Figures 1A, 1B & 2). This proximity is due to having similarly morphological characters such as BECO, UTSH, and CBIS. However, there are morphologically distinct differences between these two species (Table 3).

4.2. Second group

In the second group (subgenus *Carex*), marked with stipple-bordered ellipse, (Figure 1A), individuals of four species *C. songorica*, *C. sylvatica, C. diluta,* and *C. distans* are recognized by having male and female spikes. The vein on utricle (PNUT) in C. *songorica* is more conspicuous than those of the other three species. The color of the utricle and the type of beak are dark brown to dark-red and simple bifid in this species, respectively. The Average length of female spikes is 55 mm which is much longer than that of other species. The length of arista (awn) in female glumes is almost 1.5 mm which has maximum length among the other species. The length of the lowermost bracts is larger than that of the others. The mentioned characters are able to differentiate *C. songorica* from three other species in the subgenus *Carex*. As it can be concluded from Figure 1 (A, B parts), *C. songorica* can be easily separated from others and placed in a certain position in ordination graphs. Some of the other distinct characters obtained from Mann-Whitney U test are listed in Table 3 . The species *C. sylvatica* is a rare species in Northeast of Iran (Table 1). The lax female spikes identify this species from the species *C. diluta* and *C. distans*. Furthermore, in this species connection of the utricle to rachillae is loose and can be easily separated from rachillae. On the other hand, the length of inflorescence (INFL) has highest loading (Figure 1A). This is a good characteristic trait for separation of this species from the other species. The length of beak in *C. sylvatica* is longer than that of the other species and reaches to 2 mm. The average distance between female spikes is almost 130 mm which is the maximum range. The characters such as shape of utricle, colour of female spikes, colour and shape of achene and type of leaves make it difficult to distinguish this species from *C. diluta* and C. *distans* complex. The results of this study indicate that the*C. diluta* and *C. distans* complex have high similarities (Figures 1 & 2). However, there are some characters that could be used for their differentiation from each other (Table 3). The average distance between middle female spikes from lowermost ones is much longer in *C. distans* than that of *C. diluta*. The most obvious characteristic between these two species is glume apex shape of pistillate spikes. Glume shape of female spikes in *C. dilutai*s ovate but in *C. distans* is deltate. Shape of the pistillate spikes in *C. diluta* is orbicular to cylindrical but in *C. distans* is ovate to cylindrical. *Carex diluta* develops by rhizome but the growth organ in *C. distans* is stolon. The lowermost length of pistillate spike peduncle in *C. diluta* is almost 47 mm which is longer than that of *C. distans*. Although the PCA cannot provide distinct boundaries between *C. diluta* and *C. distans* (Figure 1, A & B), however, cluster analysis is almost able to distinguish these two species from each other (Figure 2).

5. CONCLUSION

With regard to the results obtained from the principal components and cluster analysis, micro- and macro-morphological characters are somewhat able to differentiate the species of the genus *Carex* in Northeast of Iran. The results of this study reveal that the qualitative characters are useful in distinction among the species, whereas the quantitative traits, due to overlapped size ranges, could not be effective enough in separation of the species. All species of the genus *Carex* in Northeast of Iran, excluding *C. diluta* and *C. distans,* could be separated from each other with help of various micro- and macro morphological traits, especially reproductive characters. Despite the high morphological similarity between two species *C. diluta* and *C. distans*, there are several distinctive characters between them. Finally, in addition to the morphological study, anatomical, palynological and molecular studies may be useful to better delimit the species under study.

****Attachment**

Revised identification key to the*Carex* species in Northeast of Iran Key to subgenera

1a. Spikes bisexual, androgynous, male and female flowers in the same spike, female flowers distigmatic ……...…… subgen. *Vignea*

b. Inflorescence with multiple spikes, male and female spikes distinct and separate, male spike terminal on main axis, female flowers tristigmatic……..… subgen. *Carex*

Key to species of subgen.*Vignea*

1a. Tall plants, 50-70 cm, lower bracts filiform, longer than spike, stem sharply trigonous…… ……... *C.cuprina*

b. Medium plants, 15-45 cm, bracts glume-like, or lowest occasionally foliar…….… 2

2a. Inflorescence elongated, spikes mostly remote, green, average distance between spikes 12-20 mm, utricle 4-5 mm, palnoconvex, nut 1.5-2.5 mm ……..…… *C. divulsa*

b. Inflorescence condensed, all spikes overlapping, brown to yellowish brown, stem obtusely trigonous ……..…… 3

3a. Utricle rounded, much inflated, to 25 mm, beak 0.7-1.5 mm, conical or cylindrical, nut 3.5-5 mm, plants of semi-desert ……..……..…..*C. physodes*

b. Utricle not rounded, nut biconvex, glume apex shape of androgynous spikelets acute, colour of glume reddish brown with scarious margin …….…… 4

4a. Small plants of semi desert, leaves 1.5-2 mm wide, inflorescence light brown, utricle 3-4 mm, nut 2-2.5 mm, yellowish brown……..…… *C.pachystylis*

b. Plant rather slender, leaves shorter than stem, 18-25 cm, terminal spike

smaller than the other, utricle 4.8-5.6 mm…… *C. divisa*

Key to species of subgen.*Carex*

1a. Leaves 4.5-5.5 mm wide; Pistillate spikes lax, green; glume apex shape of staminate spike rotundatis; rhizomatous-stoloniferous plants, utricle 4.5-5.1 mm, with beak C. 2 mm, nerveless, trigonous, green to light brown…….. *C. sylvatica*

b. Leaves 3-4 mm, pistillate spikes compact, nerves conspicuous, glume apex shape of staminate spike acute 2

2a. Leaves longer than inflorescence, 25-32 cm; stoloniferous plants; bracts equalling inflorescence or longer, ca. 12 cm; staminate spikes dark brown, pistillate spike dark to light brown, utricle 3.5-4.2 mm, brownish dark red, inflated, ellipsoid ..... *C. songorica*

b. Leaves shorter than inflorescence, 3-4 mm wide, bracts shorter than inflorescence, staminate spikes 15-30 mm, cylindrical or club-shape..... 3

3a. Rhizomatous plants, stem smooth, staminate spikes pale light brown, distance between pistillate spikes 25-45 mm, glume apex shape of pistillate spike acute, utricle green ..... *C.diluta*

b. Stoloniferous plants, stem slightly scabrous above, staminate spikes light to dark brown, distance between pistillate spikes 60-115 mm,glume apex shape of pistillate spike acuminate, utricle green to light brown ..... *C.distans*

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AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

REFERENCES

1.Govaerts R, Simpson D, Bruhl J, Egorova T, Goetghebeur P, Wilson K. Word Checklist of Cyperaceae: Sedges. Kew: Royal Botanic Gardens. 2007:765.
2.Marloth R. The flora of South Africa: with synopical tables of the genera of the higher plants: Darter bros. & co.; 1915.
3.Standley LA. Systematics of the Acutae group of Carex (Cyperaceae) in the Pacific Northwest. Systematic Botany Monographs. 1985:1-106.
4.Simpson DA, Muasya AM, Alves MV, Bruhl JJ, Dhooge S, Chase MW, et al. Phylogeny of Cyperaceae based on DNA sequence data-a new rbcL analysis. Aliso. 2007 (23):72-83.
5.Goetghebeur P. Studies in Cyperaceae 6. Nomenclature of the Suprageneric Taxa in the Cyperaceae. Taxon. 1985:617-32.
6.Bruhl JJ. Sedge genera of the world: relationships and a new classification of the Cyperaceae. Australian Systematic Botany. 1995;8(2):125-305.
7.Unit EF, Council NC. Handbook for Phase 1 habitat survey− a technique for environmental audit: Joint nature Conservation Committee, Peterborough; 2007.
8.ABDEL KHALIK KN. Seed coat morphology and its systematic significance in Juncus L.(Juncaceae) in Egypt. Journal of Systematics and Evolution. 2010;48(3):215-23.
9.Thorne RF, Reveal JL. An updated classification of the class Magnoliopsida ("Angiospermae"). The Botanical Review. 2007;73(2):67-181.
10.McLaughlin DC. Synopsis of the morphology and taxonomy of Carex section Glaucescentes in North America: Texas A&M University; 2004.
11.Noroozi J, Willner W, Pauli H, Grabherr G. Phytosociology and ecology of the high-alpine to subnival scree vegetation of N and NW Iran (Alborz and Azerbaijan Mts.). Applied Vegetation Science. 2014;17(1):142-61.
12.Vrijdaghs A, Goetghebeur P, Muasya A, Smets E, Caris P, Goldblatt P. The nature of the perianth in Fuirena (Cyperaceae). South African Journal of Botany. 2004;70(4):587-94.
13.Egorova TV. The sedges (Carex L.) of Russia and adjacent states (within the limits of the former USSR). St Petersburg: St Petersburg State Chemical-Pharmaceutical Academy. 1999.
14.WRONSKA-PILAREK D, Janyszek M, Jagodzinski AM. Pollen morphology of selected Central European species from subgenera Vignea and Carex (Carex, Cyperaceae) and its relation to taxonomy. Botanical Journal of the Linnean Society. 2010;164(4):422-39.
15.Frodin DG. History and concepts of big plant genera. Taxon. 2004;53(3):753-76.
16.Reznicek A. Evolution in sedges (Carex, Cyperaceae). Canadian Journal of Botany. 1990;68(7):1409-32.
17.Bouby L, Leroy F, Carozza L. Food plants from late Bronze Age lagoon sites in Languedoc, southern France: reconstruction of farming economy and environment. Vegetation history and archaeobotany. 1999;8(1-2):53-69.
18.Bertin RI. Sex allocation in Carex (Cyperaceae): effects of light, water, and nutrients. Botany. 2007;85(4):377-84.
19.Axelrod D, Raven P. Late Cretaceous and Tertiary vegetation history of Africa. Biogeography and ecology of southern Africa: Springer; 1978. p. 77-130.
20.Magee DW. Freshwater wetlands: a guide to common indicator plants of the Northeast: Univ of Massachusetts Press; 1981.
21.Ceska A, Scagel A. Indicator plants of coastal British Columbia: UBC Press; 2011.
22.Anderson DS, Davis RB, Rooney SC, Campbell CS. The ecology of sedges (Cyperaceae) in Maine peatlands. Bulletin of the Torrey Botanical Club. 1996:100-10.
23.Azeria ET, Bouchard M, Pothier D, Fortin D, Hébert C. Using biodiversity deconstruction to disentangle assembly and diversity dynamics of understorey plants along post-fire succession in boreal forest. Global Ecology and Biogeography. 2011;20(1):119-33.
24.Vellend M, Lechowicz MJ, Waterway MJ. Germination and establishment of forest sedges (Carex, Cyperaceae): tests for home-site advantage and effects of leaf litter. American Journal of Botany. 2000;87(10):1517-25.
25.Karlsen SR, Elvebakk A. A method using indicator plants to map local climatic variation in the Kangerlussuaq/Scoresby Sund area, East Greenland. Journal of Biogeography. 2003;30(10):1469-91.
26.Gignac L, Gauthier R, Rochefort L, Bubier J. Distribution and habitat niches of 37 peatland Cyperaceae species across a broad geographic range in Canada. Canadian Journal of Botany. 2004;82(9):1292-313.
27.Levkovskaya G, Filatenko A. Palaeobotanical and palynological studies in South Arabia. Review of palaeobotany and palynology. 1992;73(1):241-57.
28.田中信徳. かやつりぐさ科ノ染色體研究 XIII. 植物学雑誌. 1941;55(652):181-6.
29.Hipp AL. Nonuniform processes of chromosome evolution in sedges (Carex: Cyperaceae). Evolution. 2007;61(9):2175-94.
30.Luceño M, Vanzela AL, Guerra M. Cytotaxonomic studies in Brazilian Rhynchospora (Cyperaceae), a genus exhibiting holocentric chromosomes. Canadian journal of botany. 1998;76(3):440-9.
31.Vanzela A, Luceño M, Guerra M. Karyotype evolution and cytotaxonomy in Brazilian species of Rhynchospora Vahl (Cyperaceae). Botanical Journal of the Linnean Society. 2000;134(4):557-66.
32.Bureš P. A high polyploidEleocharis uniglumis SL (Cyperaceae) from Central and Southeastern Europe. Folia Geobotanica. 1998;33(4):429-39.
33.da Silva CRM, González-Elizondo MS, de Almeida LdNA, Torezan JMD, Vanzela ALL. Cytogenetical and cytotaxonomical

analysis of some Brazilian species of Eleocharis (Cyperaceae). Australian Journal of Botany. 2008;56(1):82-90.
34.Hipp AL, Rothrock PE, Roalson EH. The evolution of chromosome arrangements in Carex (Cyperaceae). The Botanical Review. 2009;75(1):96-109.
35.Starr JR, Bayer RJ, Ford BA. The phylogenetic position of Carex section Phyllostachys and its implications for phylogeny and subgeneric circumscription in Carex (Cyperaceae). American Journal of Botany. 1999;86(4):563-77.
36.Smith D, Faulkner J. The inflorescence of Carex and related genera. The Botanical Review. 1976;42(1):53-81.
37.Metcalfe CR. Anatomy of the monocotyledons: Oxford: V. Cyperaceae. Clarendon Press; 1971.
38.Baluška F. Cell-Cell Channels, Viruses, and Evolution. Annals of the New York Academy of Sciences. 2009;1178(1):106-19.
39.Reznicek A, Camelbeke K. Carex porrecta (Cyperaceae), a Distinctive New Species from Northern South America and Costa Rica. Novon. 1996:423-5.
40.Cayouette J, Catling PM. Hybridization in the genusCarex with special reference to North America. The Botanical Review. 1992;58(4):351-438.
41.Väliranta M, Kaakinen A, Kuhry P. Holocene climate and landscape evolution east of the Pechora Delta, East-European Russian Arctic. Quaternary Research. 2003;59(3):335-44.
42.Molina A, Acedo C, Llamas F. Taxonomy and new taxa of the Carex divulsa aggregate in Eurasia (section Phaestoglochin, Cyperaceae). Botanical Journal of the Linnean Society. 2008;156(3):385-409.
43.Bremer B, Bremer K, Chase M, Fay M, Reveal J, Soltis D, et al. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Botanical Journal of the Linnean Society. 2009.
44.Baker WL. A bibliography of Colorado vegetation description. The Great Basin Naturalist. 1983:45-64.
45.Khan MA, Qaiser M. Halophytes of Pakistan: characteristics, distribution and potential economic usages. Sabkha ecosystems: Springer; 2006. p. 129-53.
46.Jayawardene S, Lawes J. Biographical notices of historians of science: a checklist. Annals of Science. 1979;36(4):315-94.
47.der Tschechischen Republik E. Biographies of Contributors. Globalization and Environmental Challenges. 2005;437:1087.
48.Kukkonen I, editor A nomenclatural correction to Flora Iranica: Cyperaceae. Annales Botanici Fennici; 1998: Helsinki: Societas Biologica Fennica Vanamo, 1964-.
49.Naqinezhad A, Jalili A, Attar F, Ghahreman A, Maasoumi A. Two New Records from Wetland Habitats of the Central Alborz Mountains, Iran. Turkish Journal of Botany. 2008;32(3):249-53.
50.Escudero M, Valcárcel V, Vargas P, Luceño M. Evolution in Carex L. sect. Spirostachyae (Cyperaceae): a molecular and cytogenetic approach. Organisms Diversity & Evolution. 2008;7(4):271-91.
51.Ozinga WA, Schaminée JH, Bekker RM, Bonn S, Poschlod P, Tackenberg O, et al. Predictability of plant species composition from environmental conditions is constrained by dispersal limitation. Oikos. 2005;108(3):555-61.
52.Purdue JR, Smith MH, Patton JC. Female philopatry and extreme spatial genetic heterogeneity in white-tailed deer. Journal of Mammalogy. 2000;81(1):179-85.
53.Shepard RN, Arabie P. Additive clustering: Representation of similarities as combinations of discrete overlapping properties. Psychological Review. 1979;86(2):87.
54.Ekrt L, Štech M. A morphometric study and revision of the Asplenium trichomanes group in the Czech Republic. Preslia. 2008;80:325-47.
55.Soladoye MO, Onakoya MA, Chukwuma EC, Sonibare MA. Morphometric study of the genus Senna Mill. in South-western Nigeria. African Journal of Plant Science. 2010;4(3):044-52.
56.Henderson A. Traditional morphometrics in plant systematics and its role in palm systematics. Botanical Journal of the Linnean Society. 2006;151(1):103-11.