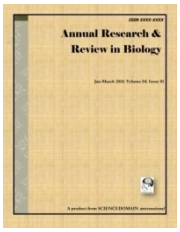

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A Species-Level of Morphological and Nut Micromorphology Study of the *Cyperus* Complex (*Cyperaceae*) in Northeast of Iran

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Authors' contributions

This work was carried out in collaboration between all authors. Author MP carried out the study, performed the statistical analyses, and wrote the first draft of the manuscript. The rest of the authors managed the analyses and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: The genus *Cyperus* with six subgenera and 45 species is the second largest genus of the Cyperaceae family based on Flora Iranica. This genus includes 23 species in Iran, of which five are located in Northeast of the country.
Aims: We determine the taxonomic boundaries among species of the genus *Cyperus* distributed in Northeast of Iran by using morphological and nut micro-morphological characters.
Study Design: Morphometric and nut micro-morphology studies of species of *Cyperus* in northeast of Iran.
Place and Duration of Study: Ferdowsi University of Mashhad Herbarium (FUMH), specimens collected during the growing season 2010 in Northeast of Iran (including three provinces of Khorassan Shomali, Khorassan Jonoubi, and Khorassan Razavi).
Methodology: We numerically examined 43 morphological characters, nine

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morphological and nut micro-morphological traits on 80 herbarium specimens in order to determine the taxonomic value of the features and also to better discriminate *Cyperus* species distributed in Northeast of Iran.
Results: The results revealed that morphological characters are somewhat useful to discriminate species of the genus in the region under study. Scatter plot displayed two distinct groups among samples. Individuals of *C. longus* are accommodated in the first group. Second group is divided into two subgroups. Individuals of *Cyperus rotundus* are located in first subgroup. Second subgroup is showed to be more complex by consisting of *C. laevigatus* subsp*. distachyos*, *C. fuscus* and *C. glaber.* The results obtained from stereomicroscope study indicated that nut of *C. laevigatus* subsp*. distachyos* is lenticular but nuts of other species are trigonous. The results of scanning electron microscope study revealed that *C. laevigatus* subsp*. distachyos* differentiated completely in terms of nut micro-morphology from the other species.
Conclusion: The results confirm fully results obtained from previously anatomical study of these species. Also the scanning electron microscope study indicated that three species including *C. rotundus*, *C. longus* and *C. glaber* are similar in terms of nut micro-morphology.

Keywords: Cyperus; Cyperaceae; principal component analysis; stereomicroscope study; scanning electron microscope study; northeast of Iran.

1. INTRODUCTION

Cyperaceae is the third largest family among the angiosperms (after Orchidaceae and Poaceae) [1] with 104 genera and 5000 species worldwide [2]. The word sedge is commonly used for plants of Cyperaceae [3]. Plants in this family are generally perennial, somewhat gramineous or rarely woody in their roots. Shoot or stems are solid, triangle, with no leaves or they may be leafy which are produced from growth of the rhizome buds [4]. Stem of members of this family is mainly rhizomatous. For instance, *Cyperus rotundus* L. has plagiotropic rhizome and *C. esculentus* L. has corm [5]. Leaves are tape-like and similar to those in Poaceae [4]. There are wind-pollinated flowers in plants of Cyperaceae where they are organized into spicate, paniculate, or umbellate inflorescences. Fruits are small single-seeded achenes [3].

Two achene-dispersal patterns are documented in plants of the family including wind (anemochory) and water (hydrochory) dispersals [6]. The achenes of *Cyperus colymbetes* Kotschy & Peyr., *C. pectinatus* Vahl and certain wetland *Carex* spp. are dispersed by moving water because these species have a spongy suberized pericarp that facilitates this type of dispersal [7]. Similarly, the achene of *Cyperus odoratus* L. remains in a buoyant corky rachilla and this achene is dispersed by moving water [8]. For instance, floodwaters disperse *Cyperus fuscus* L. along the Missouri River in the USA [9].

Westbrooks [10] reported that weeds cause severe economic losses, but placing an exact value on their impact worldwide is difficult, especially in natural and nonagricultural areas. In the U.S.A., economic losses due to invasive species (plants, animals and photogenes) were estimated to be more than $ 138 billion per year. In this country, it is estimated that cottons yields are reduced 8.5% by *Cyperus* L. weeds, a loss of about $ 40.5 million annually. Two famous *Cyperus* weeds in cotton and other row crops are *C. rotundus* L. (purple nutsedge) and *C. esculentus* L. (yellow nutsedge) [3]. Various species of *Cyperus* are able to support

extreme climatically conditions in different parts of Iran and some species may be used in traditional medicine [11].

After *Carex*, the genus *Cyperus* is the second largest genus with 709 species worldwide [12]. *Cyperus* belongs to the subfamily Cyperoideae and the tribe Cypereae [13]. The classification of this highly variable genus is still unstable. The genus is divided in Flora of Turkey [14] in to four subgenera while it is split into six in Flora of USSR [15]. In 1884, six subgenera of *Cyperus* were introduced by Clarke, but later he distinguished six genera and three subgenera [16]. However, according to Flora Iranica [16], *Cyperus* comprises six subgenera, eighteen sections, and 45 species of which 23 occur in Iran. Five species out of 23 are located in Northeast of Iran. In Flora Palaestina [17], this genus has 600 species. Most of them are hygrophyllus which are found in tropical and warm temperate regions.

According to Muasya et al. [18], the family Cyperaceae is monophyletic and divided into two clades including Mapanioideae and Cyperoideae. Moreover, the results of cladistic analyses by using the plastid *rbc*L gene, *rps*16 intron, *trn*L intron, and *trn*L-F intergenic spacer sequence data indicate that the genus *Cyperus s.s.* (Cyperoideae) is not monophyletic [19].

The first study of fruit epidermal silica bodies was done by Schuyler [20] on two species of the genera *Scirpus* L. and *Eriophorum* L. The results of this study caused to develop new characters that could reevaluate the systematics of Cyperaceae. Subsequent investigations showed that these characters are effective for species boundaries [21-24] and even sectional circumscriptions [21,22,25]. However, researches were clearly demonstrated that many groups delineated based on the silica body features conflicted greatly with taxa delimited based on morphology and other features [24,26,27,28,29,30].

The recognition of the some of *Carex* species using characters were evaluated of the fruit epidermal silica body can be confirmed but, because of partial knowledge of the effects of the environment and development on their structure and complexity of defining qualitative character states, use of the silica bodies in cladistic analyses was not successful [21]. Most of studies of nuts are done on the genus *Carex* in Cyperaceae. However, there have been no morphological studies of nuts within the genus *Cyperus*.

Many of *Cyperus* species were characterized by high intraspecific variability. Therefore, the status of some taxa and their taxonomic boundaries is ambiguous. Although some of *Cyperus* species are identified by morphological characters in the key of floras such as Flora Iranica [16], Flora Palaestina [17] and Flora of Turkey [14], identification and stringent discrimination of many of *Cyperus* species are difficult. In this study it is purpose to use qualitative and quantitative morphological (both vegetative and floral) and nut micro-morphological characters to determine the taxonomic boundaries among species of the genus *Cyperus* distributed in Northeast of Iran. Furthermore, we attempt to provide a comprehensive key to studied species based on significantly differentiated traits.

2. MATERIALS AND METHODS

2.1 Morphometric Study

2.1.1 Plant materials

In the initial study, we examined 50 herbarium specimens deposited in Ferdowsi University of Mashhad Herbarium (FUMH) as well as 110 fresh specimens collected during the growing season 2010 in Northeast of Iran (including three provinces of Khorassan Shomali, Khorassan Jonoubi, and Khorassan Razavi). All specimens were identified by using Flora Iranica [16], Flora Palaestina [17] and Flora of China [1]. According to Flora Iranica [16], four taxa including *C. rotundus* L., *C. longus* L., *C. laevigatus* subsp. *distachyos* (All.) Ball and *C. glaber* L. are reported for the region under study, but after an extensive field collection, we identified another species (*C. fuscus* L.) for the flora of Northeastern Iran. Of the 160 specimens examined in the initial study, 22 mature individuals were finally included in the subsequent investigations (Table 1). The specimens were selected from different populations to collect the maximum variations.

2.1.2 Data collection

We evaluated 43 morphological characters (Table 2), of which 31 were quantitative and 12 qualitative. In order to standardize the measurements, the specimens which have reached the full maturity were taken into considerations; however, due to the lack of these plants in some species, the measurements of plants with other stages were unavoidable. A ruler (with the precision of 1mm) was used for the measuring examinations of the quantitative traits.

2.1.3 Numerical methods

Univariate analysis was used to determine which morphological traits were significantly differentiated the species. The characters which could not significantly differentiate the species were excluded from final analyses. Initially, we separated qualitative from quantitative data. Then, the distribution of quantitative data was tested for normality using the Kolmogorov-Smirnov test (K–S test). Some quantitative data were not normally distributed. Therefore, normalization was applied on these variables using mean and standard deviation (Z-scores). Finally, in order to investigate significantly differentiating quantitative characters, the one-way parametric ANOVA was used. The Kruskal-Wallis H test, a nonparametric test, was applied to determine which qualitative traits could significantly differentiate the examined species. Moreover, the Mann-Whitney U test was applied to examine which qualitative characters significantly differentiate pairs of species. Similarly, independent samples T-test was conducted to investigate differentiating quantitative traits. All the statistical analyses were performed using the SPSS ver. 16 software [31].

The Principal Component Analysis (PCA) was conducted on the traits which significantly discriminate the species using the CANOCO software, ver. 4. 5 [32]. This analysis was used to evaluate the pattern of relationships between the individuals of the species under study as well as among the traits employed. In this analysis, the focus scaling on inter-species correlation was performed.

Table 1. List of herbarium and field-collected specimens including herbarium code and location information. Specimens marked with an asterisk are herbarium specimens (FUMH)

Herbarium voucher	Species	Location	Collection date	collectors
31010	*C. rotundus*	Gonabad- Sarasiab	23 Jun. 2011	Basiri-Hejazi
38057	*C. rotundus**	Inside the Ferdowsi University of Mashhad	22 Jul. 2003	Akramian
31011	*C. rotundus*	90 Km to Kalat	16 Jul. 2011	Basiri-Pashirzad
31012	*C. rotundus*	25Km to Mashhad- Kahoo	12 Aug. 2011	Pashirzad
31013	*C. rotundus*	Ecology garden of Ferdowsi University of Mashhad	23 Jun. 2011	Basiri-Hejazi
29465	*C. rotundus**	West Bojnord - Between Ashkhaneh and Mehmanak	16 Aug. 2007	Rafei- Zangouei
17979	*C. rotundus**	Boshruyeh- Fathabad dam	16 Sep. 2007	Faghihnia-Zangouei
31014	*C. longus*	Sarakhs-Mazdavand	23 Jun. 2011	Basiri-Hejazi
31015	*C. longus*	Between Esfarayen and Bojnord	17Jul. 2011	Basiri
31016	*C. longus*	90Km to Kalat	16 Jul. 2011	Basiri-Pashirzad
41085	*C. longus**	West Bojnord-Protected area of Ghorkhurd	24 Aug. 2008	Memariani-Zangouei
29164	*C. longus**	South of Dargaz-Between Col of allahoakbar and Dargaz	19 Jul. 1997	Rafei- Zangouei
22142	*C. longus**	Between Torbat heidarieh and Khaf	16Aug. 1992	Faghihnia- Zangouei
18914	*C. longus**	Mashhad to Kalat- Baze nakhrug	24 Aug. 1990	Faghihnia- Zangouei
31017	*C. laevigatus* subsp. *distachyos*	Abgarme Kalat	21 Jul. 2011	Basiri-Pashirzad
34508	*C. laevigatus* subsp. *distachyos**	East of Salehabad- Between Saghez Cheshmeh and Garmab	30 Jul. 1993	Zangouei -Joharchi
38444	*C. fuscus**	Southeast of Ferdowsi University of Mashhad	1 Oct. 1997	Akramian
19106	*C. fuscus**	Esfaraen- Cave of Anushiravan	28 Sep. 1998	Faghihnia- Zangouei
19053	*C. fuscus**	Torbate heidarieh- Roodmajan	30 Aug. 1990	Faghihnia- Zangouei
17977	*C. fuscus**	Boshrueh- Dam of Fathabad	16 Sep. 1989	Faghihnia- Zangouei
41383	*C. fuscus**	North Westhern of Bojnoord -5Km to Ghezel ghaleh	10 Sep. 1998	Zangouei -Joharchi
18910	*C. glaber**	Mashhad to Kalat- Baze nakhrug	24 Aug. 1990	Faghihnia- Zangouei

Table 2. List of the morphological characters used for the herbarium and field-collected specimens of the genus *Cyperus* in the present study. Characters marked with an asterisk are qualitative

Characters	Abbreviation
Life cycle of plant* (perennial 0/annual 1)	LICP
Plant height	LEPL
Length of stem	LEST
Diameter of stem	DIST
Length of inflorescence	LEIN
Length of leaf	LELF
Width of leaf	WILF
Number of bract*	NUBR
Length of bract	LEBR
Width of bract	WIBR
Type of inflorescence*	TYIN
Diameter of rhizome	DIRH
Ratio between length of bract and width of bract	RLWL
Length of style	LESL
Length of stigma	LESG
Number of stigma*	NUSG
Length of stamen	LESM
Length of anther	LEAN
Width of anther	WIAN
Length of filament	LEFI
Length of a floral scale near the top of a spikelet	LGTS
Length of a floral scale in the middle of a spikelet	LGMS
Length of a floral scale near the base of a spikelet	LGBS
Maximum width of a floral scale in the middle of a spikelet	MWGM
Length of a random spikelet	LESP
Maximum length of the rachis	MLER
Color of rachis*	CORA
Ratio between spikelet prophyll length and bract length	RPLB
Ratio between floral scale length at the base of a spikelet and mean floral scale length	RGBM
Maximum number of spikelets per spike	MNSP
Type of fruit *	TYFU
Length of fruit	LEFU
Width of fruit	WIFU
Color of glume*	COGL
Color of spike*	COSP
Color of midnerve*	COMD
Length of a prophyll of a spikelet	LEPR
Ratio between length of leaf and width of leaf	RLLW
Shape of Margin glume *	SHMG
Length of sheath	LESH
Type of rhizome*	TYRH
Number of stamen*	NUAN
Shape of glume	SHGM

2.2 Nut Micro-Morphological Study

In order to assess the taxonomic value of nut micro-morphological traits, we examined five herbarium specimens of the *Cyperus* species (Table 1) collected from Northeast of Iran. Ten to twenty nuts were sampled from each specimen. The nuts were cleaned in EtOH 50%. Then, the air dried nuts were examined for their size, type, and color using stereomicroscope Olympus-model BX-50 (Table 3). Micrographs were taken using Dino capture coupled with the Dino capture 2.0 software. In order to evaluate micro-morphological characters (Table 4), the mature nut was removed from the specimens. Then, achenes were acetolyzed in a 1:9 sulfuric acid-acetic anhydride solution. The achenes were vigorously shaken for 5 min, and then they were left for 24-48h in the solution. At the end of this period, achenes were shaken for 5 min, removed, and then they were washed in distilled water by shaking for a further 5 min. The specimens were dried overnight, mounted onto aluminum stubs with conductive carbon paint (SPI® Sup-plies), and coated with 100-200 nm of a gold- palladium alloy in an Edwards Sputter Coater S150B. Micrographs were taken along the median portion of the achenes using a Stereoscan120 scanning electron microscope (Cambridge Instruments; 20 KV accelerating voltage) that was connected to a Kontron Elektronik IBAS image analyzer. The silica bodies were described according to the terminology of Schuyler [20].

Table 3. A list of the nut morphological characters used for the herbarium specimens of the genus *Cyperus* in the stereo microscope study

Character	Abbreviation	*C. rotundus*	*C. longus*	*C. laevigatus* subsp. *distachyos*	*C. fuscus*	*C. glaber*
Length of achene (mm)	LECH	1.21	1.17	1.41	0.85	1.20
Width of achene (mm)	WICH	0.75	0.63	0.93	0.59	0.76
Color of achene	COCH	Reddish brown	Dark brown or black	Shiny yellow	Light brown	Brown or reddish brown
Shape of achene	TYCH	Trigonous	Trigonous obovoid	Lenticular	Trigonous ellipsoid	Triangular

Table 4. A list of micro-morphological characters (qualitative characters) used for the herbarium specimens of the genus *Cyperus* in the scanning electron microscope study

Character	Abbreviation
Shape of silica platform	SHSP
Shape of central bodies	SHCB
Margin of silica platform	MASP
Periclinal wall of silica platform	PWSP
Anticlinal wall of silica platform	AWSP

3. RESULTS AND DISCUSSION

3.1 Morphometric Study

3.1.1 Univariate analysis

Seven quantitative characters including LEFU, WIFU, RLWL, RLLW, MNSP, RGBM, and RPLB as well as two qualitative traits, COMD and MGSH, did not significantly differentiate the species. Therefore, these characters were excluded from the subsequent analyses. Discriminating qualitative characters among pairs of species are listed in Table 5. Moreover, results of the independent samples T-test on quantitative traits are presented in Table 6.

Table 5. Differentiating qualitative characters among the pairs of species under study obtained from mann-whitney U test

Number	*C. glaber* *C. laevigatus* subsp. *distachyos*	*C. fuscus* *C. laevigatus* subsp. *distachyos*	*C. glaber* *C. fuscus*	*longus C. rotundus C.*
1	LICP	LICP	NUSG	TYIN
2	NUBR	NUSG	NUAN	COGL
3	NUSG	NUAN	TYIN	COSP
4	TYFU	NUBR		SHMG
5	TYIN	TYIN		
6	SHMG	TYFU		
7		SHMG		

Table 6. Differentiating quantitative characters among the pairs of species under study obtained from independent samples T-test

Number	*C. glaber* *C. laevigatus* subsp. *distachyos*	*C. fuscus* *C. laevigatus* subsp. *distachyos*	*C. glaber* *C. fuscus*	*C. longus* *C.rotundus*
1	LEST	LELF	DIRH	LEPL
2	DIRH	LPRA	LESG	LEST
3	LEPR	WIBR	DIST	LEIN
4	WIAN	LESG	LESM	LESM
5	WIBR	LEIN	LEIN	MWGM
6	-	LEFI	WIAN	LEGH
7	-	MWGM	LEFI	-
8	-	LESP	LEPR	-

3.1.2 Principal Component Analysis (PCA)

In the PCA analysis (Fig. 1), the PC1 and PC2 axes account for 98.4% and 1.1% of the total variations, respectively. Therefore, it seems that the first axis is the most effective one in separation of the species. The results of the PCA reveal that the most loading and effective traits in distinction among the species belong to the qualitative and quantitative characters including LEPL, LEST, DIST, SHGM, LPRA, LEBR, LEIN, DERH, LELF, WILF, WIBR, and RLWL (Table 6). Of which, some characters such as LEPL, LEST, LEIN, DERH, and DIST are used to provide an identification key to the species.

The results of the PCA (Fig. 1) revealed that the species under study are divided into two main groups. The first group includes individuals of the species *Cyperus longus.* The characters such as LEPL, LEST, LEBR, LELF, LEAN, LESH, LESM, and WILF discriminate this species. The second group is divided into two subgroups. The first includes individuals of *C. rotundus* and the second consists of the taxa including *C. fuscus*, *C. laevigatus* subsp. *distachyos*, and *C. glaber.* The characters such as TYIN, LESL, and LEPL differentiate individuals of the species *C. rotundus* from the species of the second subgroup (Fig. 1).

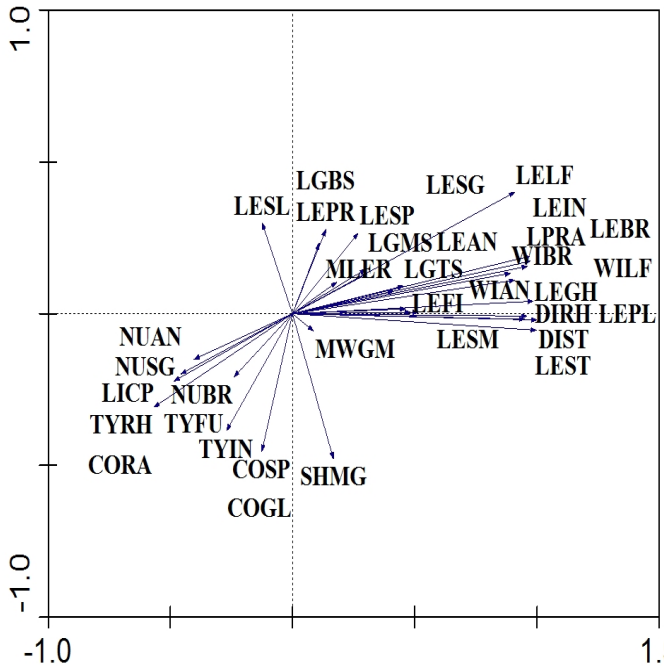

Fig. 1. Scatter plot of PC1 versus PC2 from the PCA of the morphometric data of 22 specimens of *Cyperus* in Northeast of Iran. Star: *C. fuscus*; square: *C. laevigatus* subsp*. distachyos*; circle: *C. glaber*; triangle:*C. longus*; diamond: *C. rotundus.* The character vectors are illustrated in the right. Character abbreviations are explained in Table 2

3.2 Nut Micro-Morphological Study

Results of the stereomicroscope study revealed that there are two different types of nut shape including lenticular and trigonous. All nut morphological characters differentiate *C. laevigatus* subsp. *distachyos* from the other studied species (Fig. 2 and Table 3). Type of nut shape (TYCH) in *C. laevigatus* subsp. *distachyos* is lenticular while it is trigonous in the others. Color of nut (COCH) in *C. laevigatus* subsp. *distachyos* is shiny yellow whereas it is almost brown in the other species. *Cyperus laevigatus* subsp. *distachyos* has longest and widest nut size (LECH and WICH) among the species included in the current study. Shortest nut size (LECH) belongs to *C. fuscus*. Nut in *C. glaber* is sharp trihedral whereas it is blunt trihedral in *C. longus* and *C. rotundus* (Fig. 2 and Table 3).

Results obtained from the nut micro-morphological traits (Fig. 3) indicate that *C. rotundus, C. longus,* and *C. glaber* possess convex silica platform (SHSP) whereas *C. laevigatus* subsp. *distachyos* and *C. fuscus* have concave silica platform. Margins of silica platform (MASP) in *C. rotundus*, *C. longus*, *C. laevigatus* subsp. *distachyos,* and *C. fuscus* are not thickened (Fig. 3b, d, f, h) while they are thickened in *C. glaber* (Fig. 3n). The periclinal wall (PWSP) is not persistent in *C. rotundus, C. glaber,* and *C. longu*s whereas it is persistent in *C. laevigatus* subsp. *distachyos* and *C. fuscus* (Fig. 3). *Cyperus rotundus* has no applicable anticlinal wall (AWSP) but there are thin and straight anticlinal walls in *C. longus* and *C. glaber*. Furthermore, *C. laevigatus* subsp. *distachyos* and *C. fuscus* have generated anticlinal wall. Shape of central bodies (SHCB) in *C. rotundus*, *C. longus,* and *C. glaber* are mucronate (Fig. 3b, d, n) but in *C. laevigatus* subsp. *distachyos* and *C. fuscus* they are not applicable (Fig. 3f, h). The central bodies are very short in *C. longus*, while the species *C. rotundus*, *C. longus,* and *C. glaber* are characterized by the apparently central bodies surrounded by a trough created by a thickened ridge at the margin (Fig. 3b, d, n). The beak of central bodies is sharp in *C. glaber* whereas it is blunt in *C. rotundus* and *C. longus*. The central bodies are bigger in *C. rotundus* than those in *C. longus* (Fig. 3b, d). Surface silica platform is larger in *C. longus* than that in other taxa. Furthermore, the margins of the silica platform in *C. laevigatus* subsp. *distachyos* and *C. fuscus* are appressed to the platforms of adjacent cells while this character is absent in the other species (Fig. 3f, h). Finally, the micro-morphological characters such as SHSP, PWSP, and SHCB (Table 4) are similar in three species including *C. rotundus*, *C. longus,* and *C. glaber. Cyperus rotundus* is differentiated from *C. longus* by the anticlinal wall of the silica platform character (AWSP) and the margin of the silica platform (MASP) separates *C. glaber* from the two species *C. longus* and *C. rotundus* (Fig. 3).

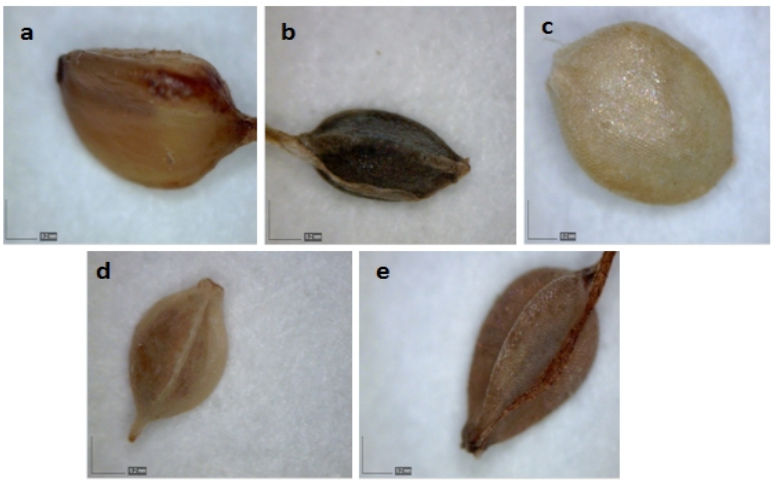

Fig. 2. Light photos of nuts of five species of *Cyperus* distributed in Northeast of Iran. a. *Cyperus rotundus*; b. *Cyperus longus*; c. *C. laevigatus* subsp. *distachyos*; d. *Cyperus fuscus*; e. *Cyperus glaber*

According to the results obtained from the morphometric and nut micro-morphology investigations, it is inferred that two species, *C. longus* and *C. rotundus* do not have any remarkable differences in most of characters except the characters such as plant height (LEPL), length of stem (LESM), and type of inflorescence (TYIN) (Tables 5, 6). The type of

inflorescence in this species is digitate while it is spicate in *C. rotundus*. Furthermore, the longest length of plant and length of stem belongs to *C. longus*.

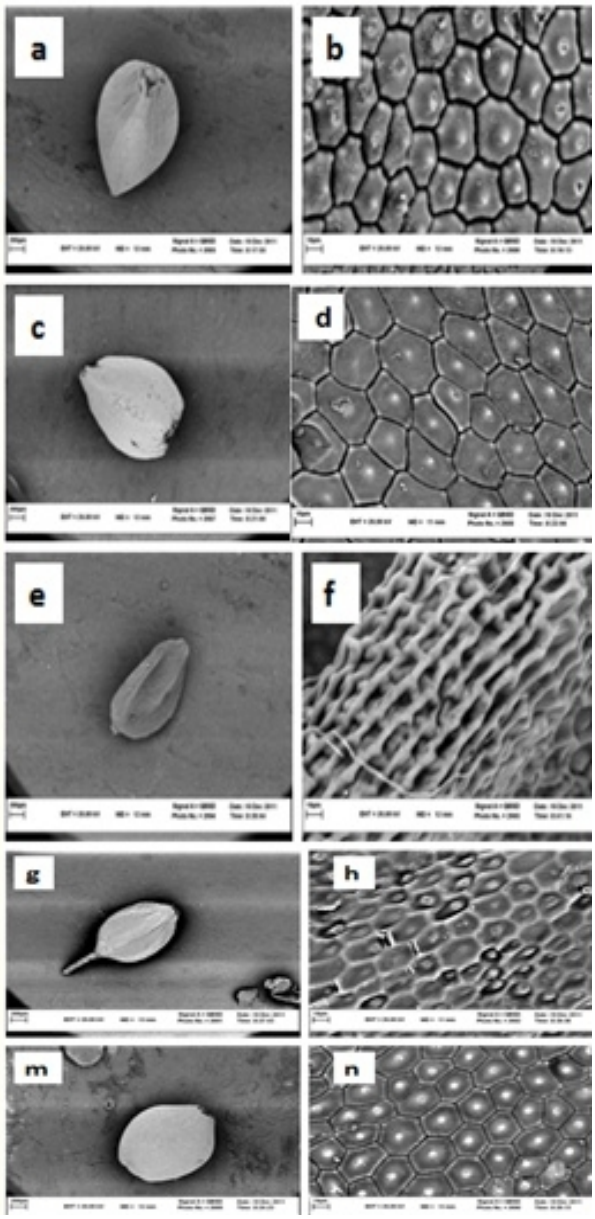

Fig. 3. SEM photos of nuts of five species of *Cyperus* included in the current study. a,b. *C. rotundus*; c,d. *C. longus*; e,f. *C. laevigatus* subsp. *distachyos*; g,h. *C. fuscus*; m,n. *C. glaber*

The characters such as shape of silica platform (SHSP) and periclinal wall of silica platform (PWSP) make two species, *C. longus* and *C. rotundus*, resemble to each other. It seems that these two species show more distinctions concerning the morphological characters

rather than those of the nut micro-morphology surface. Therefore, it may be resulted that in the case of these species the morphological characters have been more affected by environmental conditions. Concerning the nut micro-morphological similarities between *C. longus* and *C. rotundus*, our taxonomic interpretation is almost in agreement with the taxonomic treatment proposed by Pashirzad [33] and Amini Rad [13] based on anatomical features. Consequently, it is assumed that these two species are morphologically different, while the taxonomic distinction between these species seems to be difficult using anatomical and nut micro-morphology surface characters.

Results obtained from the morphological analysis indicate that it is difficult to discriminate the taxa including *C. fuscus, C. glaber* and *C. laevigatus* subsp*. distachyos* (Fig. 1). Although each species has the unique properties which make difference between them, they lack remarkably distinctive morphological characters. *Cyperus laevigatus* subsp*. distachyos* could be identified from the other two species by the characters such as number of bract (NUBR), type of inflorescence (TYIN), type of fruit (TYFU), number of stigma (NUSG) and life cycle of plant (LICP) (Table 5). This species usually has one bract, while the others have three or more bracts. The type of inflorescence in this species is capitate while the others have irregular, spreading multiple spike inflorescence. The type of fruit is lenticular, but it is triangle in the other species. Additionally, the number of stigma in this species is two while the other species have a three-branched stigma. Finally, this species is perennial whereas *C. fuscus* and *C. glaber* are annual.

Cyperus fuscus could be distinguished from the other species by its unique characters such as the number of anther (NUAN), number of stigma (NUSG) and type of inflorescence (TYIN). The number of anther in this species is two, while the others have three anthers. The number of stigma in this species is two or three, while the others have three stigmas.

Cyperus glaber is distinguished from *C. laevigatus* subsp. *distachyos* by the characters such as life cycle of plant (LICP), number of bracts (NUBR), type of fruit (TYFU), type of inflorescence (TYIN), and number of stigma (NUSG). This species is annual but *C. laevigatus* subsp*. distachyos* is perennial*.* In addition*,* the type of nutlet and inflorescence in this species is triangle and irregular, respectively, while in *C. laevigatus* subsp*. distachyos* the type of nutlet and inflorescence is lenticular and capitate, respectively. The number of stigma in *C. glaber* is three while it is two in *C. laevigatus* subsp*. distachyos.*

The results obtained from the PCA indicate that there is no conformity between our results and taxonomic classification obtained from Flora Iranica [16]. According to Flora Iranica [16], three species including *C. rotundus*, *C. longus* and *C. glaber* are placed in subgenus *rotundi* based on morphological similarities*.*

According to Flora Iranica [16] *C*. *laevigatus* subsp*. distachyos* is placed in the subgenus *Juncellus* while *C. fuscus* belongs to the subgenus *Pycnostachys.* The results obtained from the present study indicate that these two taxa seem to be a complex based on similarities of the nut micro-morphological traits. However, nut micro-morphological characters such as the shape of silica platform (SHAP), the periclinal wall of silica platform (PWSP), and the actinidial wall of silica platform (AWSP) distinguish *C*. *laevigatus* subsp*. distachyos* from the species including *C. rotundus*, *C. longus* and *C. glaber*.

Although the light microscope study showed some morphological similarities in nutlet between *C. fuscus* and the species including *C. rotundus*, *C. longus* and *C. glaber*, the scanning electron microscope study revealed that there is a clear distinction between *C.*

fuscus and the three above-mentioned species by using the characters such as the shape of silica platform (SHSP), the periclinal wall of silica platform (PWSP) and the shape of central bodies (SHCB). The shape of silica platform in *C. fuscus* is concave while it is convex in the others. Silica bodies are not applicable in *C. fuscus* while they are sharp in the other three species. The periclinal wall is not persistent in *C. fuscus* while it is persistent in another three species.

Identification key to the *Cyperus* species in northeast of Iran:

1. Bracts usually three or more; stamens three; stigmas three; nut dark brown or reddish brown; silica platform convex; periclinal wall not persistent…….....2

   Bracts usually three or less; stamens two or three; stigmas two or three; nut light brown or yellow; silica platform concave; periclinal wall persistent…….....……3
2. Inflorescence irregular, spreading multiple spike; spikes 3-15 × 1.5-2 mm; bracts two or three; stigmas two or three; nut ellipsoid, triangular (1.2 × 0.8 mm) ……..……..*C. fuscus*

   Inflorescence capitate; pseudo-lateral; spikes 7-20 × 2-3.5 mm; bracts usually one; stigmas two; nut ellipsoid or obovoid, lenticular (1.41 × 0.93 mm) ……...*C. laevigatus* subsp. *distachyos*

3. Plant annual; inflorescence irregular, spreading small anthelodium, glumes reddish brown; bracts usually three; margin of silica platform thickened ..... *C. glaber*

   Plant perennial; inflorescence regular; spreading large anthelodium, glumes brown or grey brown; bracts three or more; margin of silica platform not thickened.....4

4. Spikes digitately arranged; stem to 1000 mm; style length 0.2-1.2 mm; stigmas three (1-2.5 mm); glumes brown; nut trigonous; obovoid, anticlinal wall no applicable; beak of central body sharp…….....……...*C. longus*

   Spikes spicately arranged; stem 150-300 mm; style length 1.3-3 mm; stigmas three (3-4 mm); glumes dark reddish brown or grey- brown; nut triangular, ellipsoid or obovoid; anticlinal wall applicable; beak of central body blunt …….....*C. rotundus*

4. CONCLUSION

This research was accomplished to determine the taxonomic differentiations among species of the genus *Cyperus* distributed in Northeast of Iran by using morphological and nut micro-morphological features.

Results obtained from the morphological study indicated that *C. fuscus*, *C. laevigatus* subsp. *distachyos* and *C. glaber* were not completely differentiated by using micro-morphological characters but stereomicroscope study of nut and scanning electron microscope study seem to be useful to discriminate *C. glaber* from two others taxa. Although three species of *C.*

rotundus, *C. longus* and *C. glaber* were distinguished by morphological traits, nut micro-morphological characters revealed that these species are similar in characters of nut surface.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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