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THE TAXONOMIC SIGNIFICANCE OF ACHENE MICRO- AND MACRO-MORPHOLOGY IN *CYPERUS* L. (CYPERACEAE)

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Abstract

A data matrix was compiled to accommodate 38 macro- and micro-morphological characters of the achenes recorded comparatively for 12 *Cyperus* species (with three subspecies) using Light (LM) and Scanning Electron Microscopy (SEM).The data matrix was analyzed numerically using Jaccard's coefficient as a similarity measure and the UPGMA clustering method. The recorded characters proved useful in two ways: (i) to delimit the taxa, and (ii) the dendrogram reflected the taxonomic relationships among the 15 taxa. Comparison between the taxonomic arrangement of the 15 taxa and previous classifications is provided.

Key words: Cyperaceae, *Cyperus*, Achenes, Macromorphology, Micromorphology.

Introduction

Cyperus L., the type genus of Cyperaceae, is a large genus of about 600 species of sedges, distributed throughout the continents in both tropical and temperate regions. They are annual or perennial and mostly aquatic plants, which grow in still or slow-moving water up to 0.5 m (Govaerts *et al*., 2007).

Cyperus is taxonomically complex and the status of its infra-generic taxa is widely disputed among taxonomists. The difficulties of classifying the sedges are attributed to their substantially reduced floral and vegetative parts (Guarise & Vegetti, 2008; Gonzalez & Lo´pez, 2010). Kunth (1837) classified the species of *Cyperus* with a trifid style into 19 groups of unspecified rank, now usually treated at sectional level. He discussed the species of *Cyperus* with a bifid style separately, also accepted *Mariscus* and *Kyllinga* as distinct genera. Nees (1842) was the first to apply a sectional division in the genus *Cyperus*, and created 8 sections. While, Väre & Kukkonen (2006) followed the sectional treatment of Nees (1842), which have often been incorrectly assigned to Kunth (1837).

Steudel (1854-1855) divided *Cyperus* into three unnamed sections. The first section included species with a bifid style and corresponds to the genus *Pycreus*. His "Sectio II", split into 17 groups of unspecified rank, comprised the species of *Cyperus* with a trifid style. Many of these groups are now treated at sectional level. The third section held the species with uncertain affinities.

Furthermore, Steudel (1854) recognised *Mariscus* and *Kyllinga* ("Kyllingia") as segregate genera. complete revision of *Cyperus* was published in the generic monograph of Kükenthal (1936). His infrageneric classification is the one most commonly used at present.

Clarke (1884) accepted *Cyperus* into six subgenera, *Cyperus* (including *Mariscus* as an independent subgenus) *Juncellus*, *Diclidium* (including *Torulinium*) *Pycnostachys* C.B. Clarke (=*Anosporum* (Nees) C.B.Clarke) and *Pycreus* (=*Fymbricyperus*). In the previous report of Nees (1842) and Clarke (1893, 1908) *Kyllinga*, *Pycreus* P. Beauv. and *Mariscus* were accepted as independent genera. In addition, *Diclidium* and *Papyrus* L. and *Juncellus* were also treated as independent genera (Clarke 1893, 1908). Clarke (1893) accepted the subgenera *Anosporum*, *Pycnostachys* (six section) and *Choristachys* (seven sections). The same author (1908) recognized only two subgenera *Pycnostachys* (with 13 sections, *Anosporum* being one of them), and *Choristachys* (11sections). *Cyperus* is commonly divided into two units, determined by the alternative character states of an anatomical and of an inflorescence character set. The presence of Kranz anatomy, correlated with C4 photosynthesis, has been used in the classification of *Cyperus* since Rikli (1895).

Similarly, Barros (1960), Koyama (1961), Engler (1964), Lye (1981a & b), Tucker & McVaugh (1993), Tucker (1994) and Tucker *et al.* (2002) accepted *Cyperus* also with six subgenera. Haines & Lye (1983) at the other extreme accepted no fewer than 15 subgenera. Goetghebeur (1989) discussed the problems in the lectotypification of names relating to the infrageneric classification of *Cyperus*. He specifically excluded all taxa with laterally compressed nutlets, i.e. *Kyllinga*, *Pycreus* and *Queenslandiella*, and several highly specialised taxa, i.e., *Alinula*, *Courtoisina*, *Kyllingiella*, *Oxycaryum*, *Remirea* and *Sphaerocyperus*, from *Cyperus* s.str. However, he included such segregates as *Anosporum, Galilea, Juncellus, Mariscus, Sorostachys* and *Torulinium*. Goetghebeur (1989, 1998) divided *Cyperus* into two subgenera *Cyperus* and *Anosporum*. Bruhl (1995) accepted the subgenus *Cyperus* and *Pycnostachys*, later accepted the same direction of the subgenus *Anosporum* since Goetghebeur (1989). Goetghebeur (1989) divided the genus *Cyperus* into two subgenera: subgenus *Cyperus* with the kranz syndrome and C4-type photosynthesis (chlorocyperoid anatomy) and subgenus *Anosporum* without the kranz cells and C3 (eucyperoid anatomy). The first group often with separately arranged spikelets, the second with spikelets digitately arranged or capitately condensed. Adams (1994) accepted *Kyllinga*, *Pycreus* and *Torulinium* Sev. as independent genera. Bruhl (1995) accepted these three and also *Anosporum* as independent genera .This shows that even after the proposal of Goetghebeur (1989), there is no consents among the authors regarding the constituency of *Cyperus*. However, species of the genus have been included in studies of phenetic, phylogenetic and molecular as given by Bruhl (1995), Soros & Bruhl (2000) and Muasya *et al.* (2002), Larridon *et al.* (2011) which support the acceptance of only two subgenera to *Cyperus*, called by Bruhl (1995) and Soros & Bruhl (2000) as subgenera *Cyperus* and *Pycnostachys*, the later corresponding the subgenus sensu *Anosporum,* Goetghebeur (1989) as already mentioned. A new classification of the giant paraphyletic genus *Cyperus* (Cyperaceae), phylogenetic relationships and generic delimitation in C4 *Cyperus* (Larridon *et al*., 2013). Taxonomic changes in C4 Cyperus (Cypereae, Cyperoideae, Cyperaceae): combining the sedge genera *Ascolepis, Kyllinga* and *Pycreus* into *Cyperus* s.l.was recognized by Larridon *et al*. (2014). In the present study we accept the most comprehensive classification of *Cyperus* as proposed by Goetghebeur (1989).

Oliveira (1980) examined the morphology of the achene of 31 genera of Cyperaceae using stereomicroscope, grouping them according to the presence or absence of associated structures, as estilopódios, bristles and utricles. Moreover, SEM studies allows the observation of general characteristics of shape and ornamentation of the fruit, and also show micro-morphological aspects as shape and orientation of epidermal cells, the shape of the cell lumen, the presence of silica bodies, etc. These studies are intended to assist in the delimitation of species and contribute to understanding the structure and function of diaspores (Lye, 2000). This tool has been used in taxonomic studies of various genera of Cyperaceae (Guaglianone, 1979; Araújo & Wagner, 1997; Abid *et al*., 2014). Morphological characters of the achene surface in scanning electron microscopy were used to differentiate 38 of 45 species of *Bulbostylis* Kunth (Cyperaceae) occurring in Brazil, allowing the recognition of three patterns of surface ornamentation achene: tubercular, and transversely rugose reticulated. In addition, other taxonomic characters of interest were observed by Prata *et al*. (2008) such as variation in the sculpture of primary achenes, the ornamentation of anticlinal walls, the presence or absence of silica bodies and contour of epidermal cells.

Gonzalez & Lo´pez (2010) provided a detailed documentation of the gynoecium and nutlet and described the ontogeny in two species of *Bulbostylis* with different achene micro-morphologies to resolve which structures determine the diverse patterns of the nutlet surface. The value of achene ornamentation in the taxonomy of *Eleocharis* was reported by Menapace (1991, 1993) and Jianqiu (1999). Menapace, (1991 found that the characters of the anticlinal and periclinal walls were sufficient to distinguish species such as *Eleocharis albida* and *E. melanocarpa*. In order to verify and extend the diagnostic value of the achene surface in the taxonomy of *Eleocharis*, achenes of 26 species of *Eleocharis* in Brazil were studied by SEM. Comparison of the silica wall ornamentation with recent phylogenetic studies were given by Silva *et al*. (2011). In *Cyperus*, achene ornamentation supported the separation of well determined species such as *Cyperus esculentus* and *C. giganteus* (Hefler & Wagner, 2008).

In order to verify and extend the diagnostic value of the achene surface in the taxonomy of *Cyperus,* in the current study achenes of species were collected from different geographical regions of Egypt or obtained from a certified herbarium and examined by SEM to delineate the patterns of ornamentation on the surface of the achene.

Material and Methods

This study is based on mature achenes of 12 species and three subspecies of *Cyperus* and collection data of specimens is presented in Table 1. This sample of taxa is admittedly small relative to the size of the genus but it represents only the two subgenera of Goetghebeur (1998) treatments. Mature achenes were examined under dissecting microscope (10–15 achenes for each taxon were chosen to cover the range of intra-taxon variation). For the study of macro-morphological characters by SEM, preliminary examination by light microscope (L.M.) were carried out. For the analysis of macro-and micro-morphology of the fruit were selected two replicates of each taxon. The achenes were mounted on SEM stubs, using double adhesive tape. The stubs were sputter-coated with gold for 5 min in an E1100 (Polaron Equipment). After coating, the specimens were examined with a Jeol JSM 5400 SEM, using accelerating voltages at 20–25 kV. The terminology for achene characters set by Denton (1983), Barthlott (1984) and Hefler & Wagner (2008) was adopted. Aspects of achene micro- and macro-morphology as defined in Table 2 are recorded and scored comparatively for the OTU's into a data matrix. In preparing the raw data matrix, multistate characters were transformed into two-state characters in coding and their presence or absence was coded 1 and 0 respectively (Table 2). The program NTSYS-pc 2.2 (Rohlf, 2005) was used in the data analysis as follows: the raw data matrix was standardized with STAND module; similarity matrix was generated by SIMQUAL module based on Jaccard coefficient. Clustering was performed using unweighted pair-group method with arithmetic average (UPGMA) and represented in phenogram (tree). The distortion between the tree and its related distance matrix (Rohlf & Sokal, 1981) was evaluated by computing the tree's cophenetic (ultramatric) value matrix using COPH and comparing them using MXCOMP modules.

Results and Discussion

The description of ornamentation of the achene surface in this study provided several distinctive characteristics for species of *Cyperus* as previously demonstrated by Lye (2000). Achenes morphological characters of the studied taxa of the genus *Cyperus* as shown by light microscopy and SEM are reviewed in Tables 1 and 2 and Figs. 1-3.

External achene morphology: Achenes are ovoid only in *C. maculatus*; obovoid in four species: *C. bulbosus, C. capitatus, C. conglomeratus* and *C. fuscus*; obovoid to rounded in two species: *C. alopecuroides* and C. *laevigatus*; elliptic in two species: *C. difformis* and *C. michelianus* and the remaining OTU's have oblong achene (Fig. 1). Lye (2000) reported that tropical and subtropical species show higher variation in shape than temperate achene. Achene colour varies in the studied taxa, it was light brown to yellow in four species; dark brown in 6 species and gray in the remaining 5 species (data matrix, Table 2). The achene length of 9 investigated OTU's is short (0.75-1.5mm), the medium length (1.6-2.8mm) is observed only in the achene of the two species: *C. bulbosus* and *C. capitatus*; the remaining 4 OTU's showed long distinct achene (2.9mm-10mm long) (see Table 2); at the same time 7 OTU's of the examined taxa have small seed width (0.3mm-0.7mm), 5 OTU's have seed width (0.8mm-1.8mm) (Table 2), but only two species: *C. difformis* and *C. fuscus* have the achene width (1.9-4mm) (Fig. 1). The criterion of achene dimensions was used by Araujo & Wagner (1997) to separate *Cyperus burkartii* from the other species of *Cyperus* subgenus A*nosporum.* During the analysis of basal portion of the fruit a constriction forming a short stipe was found in12 species (Fig. 1), while achenes of three species lack such short stipe (Table 2, Fig. 1).

Table 1. *Cyperus* taxa used for this study arranged in alphabetical order according to Boulos, (2005). Localities of voucher specimens provided, and herbarium acronyms given in parentheses.

Number	Taxon	Taxa abbreviations'	Voucher
1.	*C. alopecuroides* Rottb.	*C. alo*	Egypt: Fayium, 4.1.2011, Shalabi, L. s.n. (EdAS)
2.	*C. alternifolius* L. ssp. *flabelliformis* (Rottb.) Kuk.	*C. alt*	Egypt: Garden El-Saff, Giza,, 17.4.2003 Shalabi, L. s.n. (EdAS)
3.	*C. articulatus* L.	*C. art*	Egypt: Ismailia-Canal bank, 6.3.1994, Gazer. s.n. (SUEZ).
4.	*C. bulbosus* Vahl.	*C. bul*	Egypt: Gebel Elba, 17.2.1998, Tolba. s.n. (SHDR).
5.	*C. capitatus* Vand	*C. cap*	Egypt: Rafah, sand duns, 5.4.1994, Gazer s.n. (SUEZ).
6.	*C. conglomeratus* Rottb .	*C. con*	Egypt:Marsa Halaieb,14.3.1992, Tolba .s.n. (SHDR).
7.	*C. difformis* L.	*C. dif*	Egypt: Ismailia-canal bank, 6.3.1994, Gazer. s.n (SUEZ)
8.	*C. digitatus.* Roxb. ssp. *auricomus.* (Sieb. ex Spring.) Kuk.	*C. dig*	Egypt: Nile-Delta, Foua city, 8.11.2007 Shalabi, L. s.n. (EdAS)
9.	*C. fuscus* L.	*C. fus*	Egypt: Burg El Arab, El Dabaa, 7.4.1989, Gazer. s.n (SUEZ).
10.	*C. imbricatus* (L.) Delile	*C. imb*	Egypt: Menia, Mamdouh El-Amery, 5.7.2000, (CAI)
11.	*C. laevigatus* L.	*C. lae*	Egypt: Alexandria–Matrouh coastal road, 9.3.2001, Shalabi, L. s.n (EdAS)
12.	*C .maculatus* Boeckeler	*C. mac*	Egypt: Plant Island, Aswan, 13.5.1991, El-Hadidi (CAI)
13.	*C.michelinus* (L.) Delile ssp. *Pygmaeus* (Rottb.) Asch. & Graebn.	*C. mic*	Egypt: Nile-Bank Barag, 23.5.1992, Bolous (CAI)
14.	*C. papyrus* L.	*C. pap*	Egypt: Orman Garden, , 14.4.2001,Shalabi, L. s.n (EdAS)
15.	*C. rotndus* L.	*C. rot*	Egypt: Sinai Peninsula, 4.3.2001, Gazer. s.n. (SUEZ).

(CAI)= Herbarium of Cairo University, (EdAS)= Herbarium of Faculty of Education, Ain-shams, University, (SHDR)= Herbarium of Desert Research Centre, (SUEZ)= Herbarium of Suez Canal University

Table 2. Data matrix based on 38 macro- and micro-morphological character states of achenes, taxa numbers as defined in Table (1).

Characters	No.	Character States/Taxa no.	1	2	3	4	5	6	7`	8	9	10	11	12	13	14	15
Achene shape:	1.	Ovoid	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	2.	Obovoid	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0
	3.	Obovoid to rounded	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	4.	Oblong	0	1	1	0	0	0	0	1	0	1	0	0	0	1	1
	5.	elliptic	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
Achene colour:	6.	Light brown to yellow	0	1	0	0	0	1	0	0	0	1	0	1	0	0	0
	7.	Dark brown	0	0	1	0	0	0	1	1	1	0	1	0	0	0	1
	8.	Gray	1	0	0	1	1	0	0	0	0	0	0	0	1	1	0
Achene length:	9.	0.75-1.5 mm	1	1	1	0	0	1	0	0	0	1	1	1	1	0	1
	10.	1.6-2.8 mm	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
	11.	2.9-10 mm	0	0	0	0	0	0	1	1	1	0	0	0	0	1	0
Achene width	12.	0.3 – 0.7 mm	1	1	1	0	0	1	0	0	0	0	0	1	1	0	1
	13.	0.8 – 1.8 mm	0	0	0	1	1	0	0	1	0	1	1	0	0	0	0
	14.	1.9 – 4 mm	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0
Achene base:	15.	With small stipe	1	1	1	1	1	1	1	0	1	1	1	1	0	0	1
	16.	Without	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0
Overall exocarp pattern:	17.	Reticulate	0	0	1	1	0	1	0	0	0	1	1	1	0	0	1
	18.	Rugose	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0
	19.	Granulate	1	1	0	0	1	0	0	0	1	0	0	0	1	0	0
Homogeneity of exocarp cells	20.	Uniform	1	0	1	1	0	0	1	0	0	1	1	0	1	1	1
	21.	Gradually smaller toward achene ends	0	1	0	0	1	1	0	1	1	0	0	1	0	0	0
Exocarp cell shape:	22.	Angular	0	0	1	1	1	1	0	0	1	1	1	1	1	0	1
	23.	Irregular	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0
Anticlinal walls' level:	24.	Undulated	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
	25.	Raised	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1
Anticlinal walls' shape:	26.	Straight	1	0	1	1	1	1	0	0	1	1	1	1	0	0	1
	27.	Wavy	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
	28.	Variable	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0
Anticlinal walls' thickness:	29.	Thick	0	0	1	1	0	1	1	0	0	1	1	0	1	1	1
	30.	Thin	0	1	0	0	1	0	0	0	1	0	0	1	0	0	0
	31.	Variable	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Periclinal walls' level:	32.	± flat	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1
	33.	Concave	1	0	1	1	0	0	1	0	0	0	0	1	0	0	0
	34.	Convex	0	1	0	0	1	0	0	0	1	1	0	0	0	1	0
	35.	Variable	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
Superficial silica bodies:	36.	One per cell	1	1	0	0	1	0	0	0	1	1	0	1	1	0	1
	37.	Many per cell	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	38.	Not clear	0	0	1	1	0	1	0	1	0	0	1	0	0	1	0

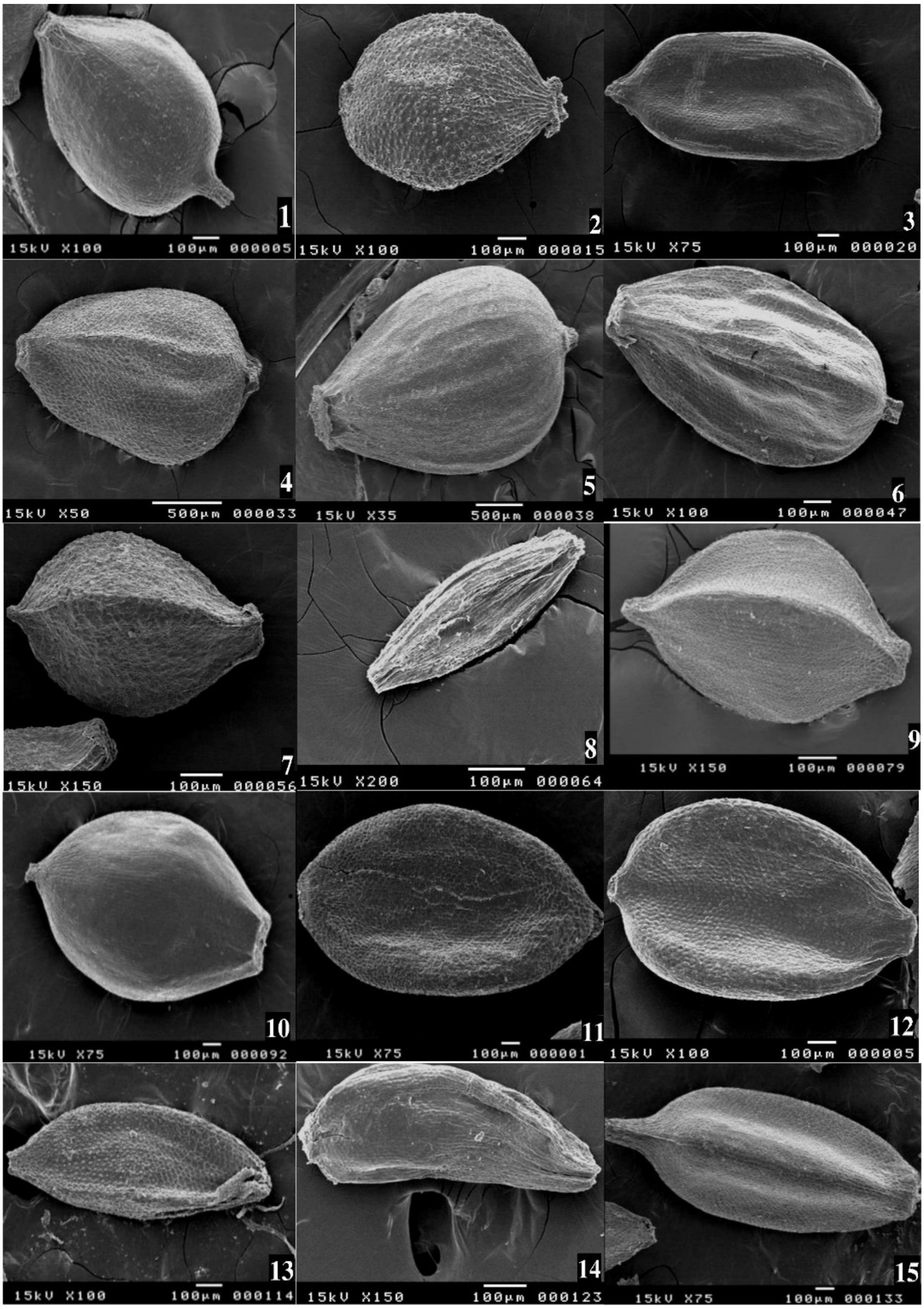

Fig. 1. SEM micrographs of mature achens showing the characters of external achene micromorphology and exocarp cell pattern, numbers refereeing to the taxa (Table 1).

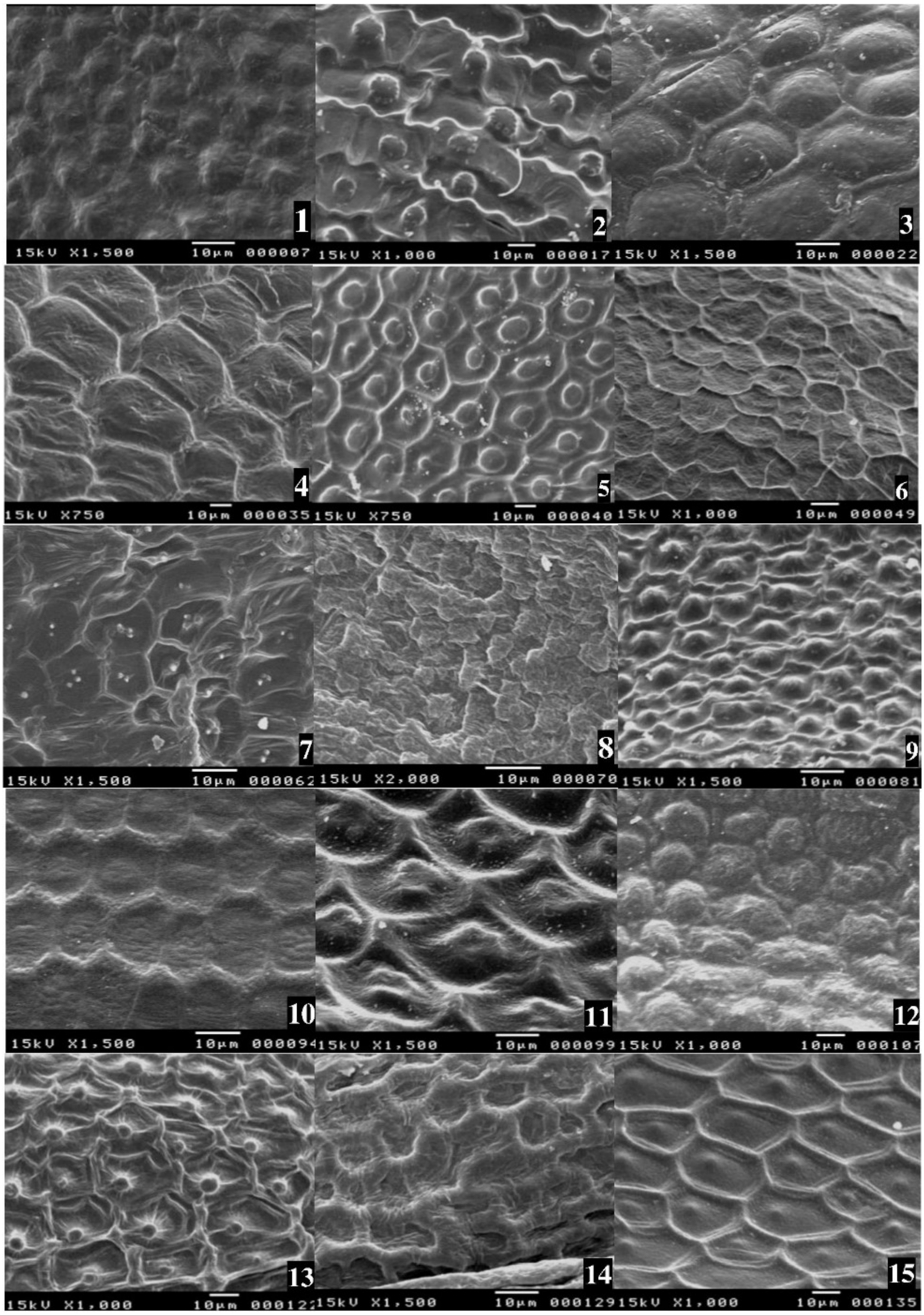

Fig. 2. SEM micrographs of cell shape, anticlinal and pereclinal characters, numbers refereeing to the taxa (Table 1).

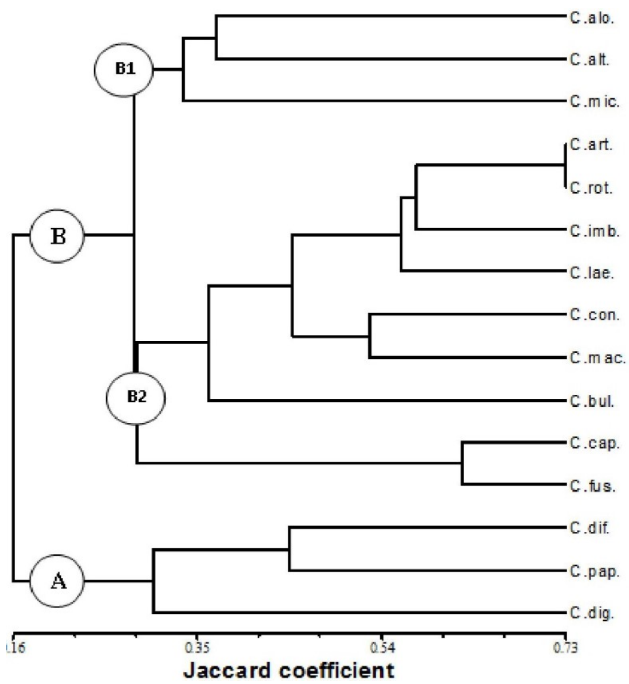

Fig. 3. UPGMA phenogram based on the achene micro- and macromorphology of 15 taxa of *Cyperus*, for taxa abbreviations' see Table 1.

Similar results were found by Tucker (1994) while studing the basis of the achenes of *Cyperus* species subgenus *Cyperus* occuring in Mexico, where he recognized that *C. imbricata* , *C. aggrigatus* and *C. rotndus* with short stipe. Hefler & Wagner (2008) was found that *C. imbricatus* with constriction, forming a short stipe, while *C. aggrigatus* and *C. rotundus* without constriction.

Exocarp cell pattern: Three patterns of overall exocarp are recognized: reticulate in *C. articulatus, C. bulbusus* , *C. conglmeratus, C. laevigatus* , *C. imbricatus, C. maculates and C. rotnddus* (Table 2, Fig. 2); rugose in three species: *C. difformis, C. digitatus* and *C. papyrus*; and granulate in the remaining 5 OTU's (Table 2, Fig. 2). The exocarp cells are variable in size that appeared gradually smaller towards achene ends in 6 OTU's (Table 2, Fig. 2); the remaining 9 taxa showed a uniform exocarp cells (Table 2, Fig. 2). Hefler & Wagner (2008) proved that the outer wall surface periclinal on the *Cyperus* subgenus *Cyperus* in Southern Brazil were uniform and without any short stipe, unlike Boechat & Wagner (2003) found that the exocarp cells were variable and appeared smaller towards the end in some species of *Eragrosis* Wolf (Poaceae).

Exocarp cells have angular shape in the majority of taxa (10 taxa); but the other 5 taxa represent an irregular exocarp cell shape (Table 2, Fig. 2). Haines & Lye (1983) and Lye (2000) recorded that most of taxa of family *Cyperaceae,* epidermal cells arranged in irregular longitudinal rows. On the other hand, the regular distribution of epidermal cells has been recorded in some species of *Cyperus* (Araújo & Wagner, 1997), few species of *Bulbostylis* and *Fimbristylis* (Haines & Lye, 1983).

Characteristics of anticlinal and periclinal cell walls of achene surface: The anticlinal walls of the epidermal cell of achenes have provided several diagnostic characteristics in *Cyperus* (Denton, 1983; Araújo & Longhi-Wagnar, 1997; Lye, 2000 and Hefler & Longhi-Wagnar, 2008). The anticlinal boundaries are undulated only in two taxa: *Cyperus bulbosus* and *Cyperus digitatus*, while the remaining 13 taxa have raised anticlinal wall borders (Table 2, Fig. 2). The majority of the observed taxa (10 species) have straight anticlinal walls, only the two taxa: *Cyperus alternifolius* and *Cyperus michelianus* have wavy anticlinal walls, while *Cyperus difformis, Cyperus digitatus* and *Cyperus papyrus* have variable anticlinal walls' shapes (Table 2, Fig. 2). Straight anticlinal wall seems to be the most common type in Cyperaceae.

Studies of Denton (1983), Luceno (1992) and Araujo & Wagner (1997), showed that there misinterpretation regarding the presence of silica bodies, when the walls are high or anticlines thick. Anticlinal walls are thick in the majority of taxa (9 taxa), and thin in only four (Table 2, Fig. 2), while only two taxa: *Cyperus alopecuroides* and *Cyperus digitatus* have a variable anticlinal walls' thickness.

Wall thickness anticlines were also used by Araújo & Wagner (1997) to separate *Cyperus incomtus* Kunth (with anticlinal walls very thick) and *Cyperus surinamensis* Rottb. (± thicker walls anticlines) of other species of *Cyperus* subgenus *Anosporum* studied, presenting anticlines thin walls. The pereclinal walls are flat in three taxa: *Cyperus difformis, Cyperus digitatus* and *Cyperus rotundus*; while 5 taxa have concave pereclinal walls; and another 5 taxa have convex pereclinal walls (Table 2, Fig. 2); but only 2 OTU's: *Cyperus laevigatus* and *Cyperus michelianus* appeared with variable pereclinal walls' level (Fig. 2). Silica bodies appeared superficially one per cell in 8 of the examined taxa, while *Cyperus difformis* is unique in having many silica bodies per cell, at the same time, it is not clear to see silica bodies in the remaining 6 taxa (Table 2, Fig. 2). Hefler & Wagnar (2008) separated three taxa depending on the characters of anticlinal walls, thick wall and straight in *Cyperus esculentus*, wavy in *Cyperus giganteus* and *Cyperus imbricatus* , he found that the pereclinal walls are concave only in *Cyperus corymbosus,* while the remaining taxa are flat .

Phenetic analysis: The 15 *Cyperus* taxa were grouped together at the phenogram in Fig. 1, it separates the 15 *Cyperus* taxa into two main groups A and B. Group A includes 3 taxa: *C. digitatus, C. papyrus* and *C. difformis* because of sharing the long achene (2.9-10 mm), rugose overall pattern of exocarp with irregular cell shape and variable shaped anticlinal walls, but *C. difformis* and *C. papyrus* are more related to each other and clustered together because of achene width (1.9 – 4 mm), uniform exocarp cells, raised level of anticlinal walls and thick anticlinal walls. At the same time, *C. digitatus* splits-off separately at the similarity level of 0.30 because of achene width (0.8 – 1.8 mm), exocarp cells gradually smaller toward achene ends, undulated anticlinal walls' level, variable thickness of anticlinal walls and flat periclinal walls. The remaining 12 taxa are clustered in the main group B, which is divided into two subgroups B1and B2

(Fig. 3). The B1 subgroup consists of three taxa: *C. alopecuroides, C. alternifolius* and *C. michelianus* on the basis of having small achene (0.75-1.5 mm in length and 0.3 – 0.7 mm in width), granulate overall exocarp pattern, raised anticlinal walls and the distribution of superficial silica bodies as one per cell. Furthermore, *C. alopecuroides* and *C. alternifolius* appear more related to each other and are grouped together because they have achenes with constructed base and irregular shaped exocarp cell.

The remaining 9 taxa constitute subgroup B2 (Fig. 3). *C. capitatus* and *C. fuscus* are clustered together due to obovoid achene with constructed base, granulate exocarp, angular exocarp cells which gradually smaller toward ends, raised straight thin anticlinal walls, convex periclinal walls and the distribution of superficial silica bodies as one per cell. In addition, *C. bulbosus* splits off from the other taxa (Fig. 3) because of gray achenes, with medium size (1.6-2.8 mm), and undulated anticlinal walls. The remaining 6 taxa (Fig. 3) are grouped together and subdivided into two branches, two taxa appear at the first branch: *C. conglomeratus* and *C. maculates* due to having light brown to yellow achenes, small sized (0.75-1.5 mm in length and 0.3-0.7 mm in width) with short stipe at base, reticulate exocarp with angular cells which gradually smaller toward ends and raised straight anticlinal walls. At the same time, four taxa clustered together consisting the second branch, *C. laevigatus* splits off from this branch because of obovoid to rounded achene shape and variable periclinal walls' level. In addition, *C. articulates, C. rotundus* and *C. imbricatus* are clearly clustered together due to having oblong short achenes (0.75-1.5 mm in length) with short stipe at base, reticulate exocarp with uniform angular cells and raised anticlinal walls; also *C. imbricatus* splits off separately from the two other species due to having dark brown achenes with small width (0.3-0.7 mm), despite of sharing the all other characters with them, at the same time, *C. articulates* and *C. rotundus* appeared related to each other that sharing the all characters together except the flat periclinal walls and silica bodies distributed one per cell in *C. rotundus*, while concave periclinal walls and the indistinct silica bodies in *C. articulates*.

Conclusion

The analysis of the surface micromorphology of achenes of *Cyperus* species revealed taxonomical characters such as the variation of primary sculpture with the proposition three ornamentation patterns: reticulate, rugose and granulate. Furthermore, it was observed that the variation of some characters such as the ornamentation of anticlinal walls, superficial silica bodies and the contour of the epidermal cells used as an aid to distinguish the species of the genus *Cyperus*.

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