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Taxonomic Significance of Glume Morphology and Leaf Epidermal Characteristics in some Taxa of Tribe *Aveneae* (*Poaceae*)

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Abstract

The numerical classification of tribe *Aveneae* (*Poaceae*) is discussed regarding the glume morphology and silica skeleton morphologies. The present study dealt with 18 species belonging to 10 genera of the tribe to cover as many groups as possible within *Aveneae*. The total of 31 structural characters and 71 character states were scored comparatively. The resulted data matrix was analyzed under a combination of Euclidean distance measure and Ward's clustering method included in the program package PC-ORD version 5. The resulted dendrogram separated the tribe into five basic sub-ordinate groups created from three major groups A, B and C. The taxonomic significance of these results was discussed. The results showed congruence between the clustering and PCA method, in suggesting three major groups and 5 sub-ordinate groups.

Keywords: *Aveneae*, morphology, numerical analysis, phytoliths, *Poaceae*, silica bodies, taxonomy

Introduction

Tribe *Aveneae* [Dumort., Observ. Gramin. Belg., 82:1824] including Agrostideae [Dumort., Observ. Gramin. Belg., 83:1824] is the second largest tribe in subfamily *Pooideae* Benth., and is one of the main groups of the grass family *Poaceae* (R. Br.) Barnhart. It includes about 73 genera and 1050 species (APG III, 2009) mainly found in temperate regions of both hemispheres and extends to mountainous regions of the tropics (Clayton, 1975, 1981; Clayton and Renvoize, 1986; MacFarlane, 1987; MacFarlane and Watson, 1980, 1982; Mitra and Mukherjee, 2005; Stebbins, 1956; Stebbins and Crampton, 1961; Watson and Dallwitz, 1992). It characterized by laterally compressed spikelets with one to several fertile florets, rachilla usually disarticulating above glumes; glumes persistent, often equal to spikelet or at least longer than first floret.

In Egypt, *Aveneae* includes 14 genus and 33 species (Boulos, 2005; Täkholm, 1974), mostly herbaceous, growing on wide range of habitats such as desert, wetlands, farmlands and salt marshes. *Phalaris* is the largest genus (6 specie), followed by *Avena* and *Rostraria* (5 species for each). Seven monospecific genera include *Holcus, Agrostis, Ammophila, Triplanche, Gastridium, Lagurus* and *Alopecurus*.

Aveneae is a large heteromorphous tribe, in which different genera show morphological variations, but species within the genus are quite similar morphologically, e.g the genus *Polypogon* and *Avena*. In addition, *Agrostis viridis* seems quite similar to genus *Polypogon* and there is confusion in identifying *Polypogon monospeliensis* from *P. fugax* and *Avena fatua* from *A. ludoviciana*, so has posed many problems to the taxonomists using gross morphology alone (Strivastava, 1978). So, in this work, glume morphology beside foliar epidermal characters, especially silica skeleton, will assist to elucidate taxonomic relationships at different levels in tribe *Aveneae*.

Clayton and Renvoize (1986) employed the first tribal name *Aveneae,* with four recognized subtribes *Duthieinae, Aveninae, Phalaridinae* and *Alopecurinae*. *Aveneae* classification and its taxonomical borders with its sister tribe *Poeae* R. Br. have varied historically depending on an author's interpretations of the tribe's morphologic heterogeneity; consequently, the a description of many of its genera has been problematical (Tab. 1).

In different classifications, *Aveneae* have been separated from *Poeae* based on the floral traits cited (Clayton and Renvoize, 1986; MacFarlane and Watson, 1982; Tzvelev, 1976; Watson and Dallwitz, 1992). Tzvelev (1989), however, did not recognize *Aveneae* but transferred their members to the large tribe *Poeae*, although *Phleeae* Dumort. (Including *Phalarideae* Kunth) was separated from *Poeae*.

An increasing number of numerical classification studies in recent decades have helped to clarify taxonomic relationships within the subfamily *Pooideae* including tribe *Aveneae* (Catalan *et al*., 1997; Davis and Soreng, 1993; Grit and Röser, 2006; Grit *et al*., 2009; GPWG, 2001; Hsiao *et al*., 1995; Nadot *et al*., 1994; Quintanar *et al*, 2007; Soreng *et al*., 1990). However, the classification of *Aveneae*

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has remained largely problematic, some taxonomic treatments related to the avenoids have focused on particular genera, like *Trisetum* (Edgar, 1998; Randall and Hilu, 1986), *Helictotrichon* (Grebenstein *et al*., 1998), *Avena* (El-Rabey, 2008; Peng *et al*., 2010; Rodionov *et al*., 2005), *Deschampsia* (Chiapella, 2007), *Anthoxanthum* (Pereira *et al*., 2007), *Alopecurus* (Dogan, 1999), *Phleum* (Scholz, 1999), and *Calamagrostis* (Hai-Ying *et al*., 2006).

Foliar epidermal characters as an aid to the identification and classification of tribe *Aveneae* was the subject of many works (Ahmad, *et al*., 2011; Hai-Ying *et al*., 2006; Xinming *et al*., 1998), but focusing on silica skeleton morphologies of the epidermis have never been studied previously in tribe *Aveneae*. Silica in the form of bodies or particles deposited within or on cells of living plant leaves, in addition to silica incorporated in cell walls or completely filling hairs and other plant tissues composed silica skeleton of the plant (Arimura and Kanno, 1958). The silica skeleton may represent a potentially significant taxonomic character and may be diagnostic to subfamilies and genera, that a strong genetic influence governs formation of silica bodies. In other words, families and orders of plants show strong tendencies to silicify or not silicify their tissues (Barthlott *et al.*, 1998; Brown, 1984; Cai and Wang, 1994; Davila and Clark, 1990; Ellis, 1979; Fredlund and Tieszen, 1994; Mejia and Bisbey, 2003; Metcalfe, 1960; Mulholland, 1989; Mulholland and Rapp, 1992; Palmer and Tucker, 1981; Palmer *et al*., 1985; Piperno, 1988; Stebbins, 1956; Twiss *et al*., 1969). So this work aimed to study the glumes macromorphology combined with silica skeleton morphology as taxonomic tools in the numerical classification of tribe *Aveneae* in the flora of Egypt.

Material and methods

Taxon sample

On the basis of previous classifications (Tab. 1) and molecular studies focused on *Pooideae* or *Aveneae* (Catalan *et al.,* 2004; Davis and Soreng, 2007; Soreng and Davis, 2000), the taxon sample was selected to cover as many groups as possible within *Aveneae* (i.e. subtribes *Aveninae, Agrostidinae, Phalaridinae* and *Alopecurinae*). The study dealt with 18 species belonging to 10 genera of the tribe (Tab. 2), specimens were collected as dried materials from the Cairo University Herbarium (CAI).

Observations of characters

The total of 31 structural characters and 71 character states were scored, of which 8 were morphological with 25 character states and 23 were anatomical with 63 character states. For morphometric analyses, 5-10 individuals from each taxon were studied. The morphological characters were concerned with glumes, chosen due to experience of authors and previous studies. The anatomical characters were covered the silica skeleton features of the leaf epidermis (Tab. 3), characters were selected based on those reported by Piperno and Pearsall (1998), Bowdery *et al*. (2001), Madella *et al*. (2005), and Honaine *et al*. (2006). The character states were recorded in the data matrix (Tab. 4) to show the distribution of characters among the species examined from tribe *Aveneae*, to be ready for numerical analyses.

Leaf blades of the specimens have been prepared for describing the silica skeleton morphology found in tissues of central part of the middle of leaf. There are several methods currently established for investigating the pattern of plant silica skeleton. The theory is: silica is acidic in nature, very resistant to oxidation (unlike pollen, but quite like diatoms). Wet, dry, or combined oxidation techniques are commonly used (Clark, 1960; Theunissen, 1994). Before oxidation, leaves and culms of each species have to be cut into small pieces and pre-washed to remove dust particles. After digestion of organics with strong oxidizing agent, hydrogen peroxide, (for 24 hours), residue has to be treated with hydrochloric acid (for 2 hours) to remove carbonates, and washed by distilled water. The obtained samples were stained using safranin, light green dyes or both in double staining technique. Then samples were mounted onto microscopic slides in canada balsam medium for photomicrography. Light photomicrography at × 400 magnification was used to describe silica skeleton.

Data analysis

In order to group the species having structural similarities, the data matrix (Tab. 4) was subjected to numerical analysis under four different combinations of two dissimilarity assessment methods (Euclidean and Relative Euclidean) and two clustering methods with high clustering intensity (Ward's method and Flexible Beta -0.25) included in the program package PC-ORD version 5 for Windows (McCune, 1997). Other combinations in this package were either mathematically incompatible or yielded taxonomically unacceptable dendrograms with obvious tailing problems.

Principal component analysis (PCA) was performed for the studied samples based on the examined 31 characters using Multivariate Statistics Package (MVSP) version 3.13 for Windows (Kovach, 1999).

Result and discussion

Fig. 1 shows the dendrogram based on analysis of 31 characters listed in (Tab. 3) and recorded comparatively for 18 species belonging to 10 genera of tribe *Aveneae* and analyzed under a combination of Euclidean distance measure and Ward's clustering method. The tree was separated into five basic sub-ordinate groups (1, 2, 3, 4 and 5). The sub-ordinate groups 1 and 2 were resulted from the first major group A, while the sub-ordinate group 3 was resulted from the second major group B, but the sub-ordinate groups 4 and 5 were resulted from the third major group C (Fig. 1)

Tab. 1. Placement of the *Aveneae* representatives included in our study in selected classification systems

Ascherson and Graebner (1898-1902)	Schaffner (1912)	Maire *et al.* (1953)	Prat (1960)	Tzvelev (1976)	Tutin *et al.* (1980)	Conert (1983-1992)	Clayton and Renvoize (1986)	Watson and Dallwitz (1992)	GPWG (2001)	Soreng *et al.* (2003-2007)	Mabberley (2008)
									Poeae	*Poeae*	*Poeae*
											Polypogon
Aveneae	*Aveneae*	*Aveneae*	*Aveneae*	*Aveneae*	*Aveneae*	*Aveneae*	*Aveneae*	*Aveneae*			*Aveneae*
		Aveninae		*Aveninae*		*Aveninae*				*Aveninae*	*Aveninae*
Avena	*Avena*	*Avena*	*Avena*	*Avena*	*Avena*	*Avena*	*Avena*	*Avena*	*Avena*	*Avena*	*Avena*
		Rostraria	*Rostraria*	*Rostraria*	*Rostraria*		*Rostraria*	*Rostraria*	*Rostraria*	*Rostraria*	
	Trisetaria					*Trisetaria*				*Trisetaria*	*Trisetaria*
										Lagurus	
Agrostideae	*Agrostideae*	*Agrostideae*	*Agrostideae*								
		Agrostidinae		*Agrostidinae*		*Agrostidinae*	*Alopecurinae*			*Agrostidinae*	*Alopecurinae*
Agrostis	*Agrostis*	*Agrostis*	*Agrostis*	*Agrostis*	*Agrostis*	*Agrostis*	*Agrostis*	*Agrostis*	*Agrostis*	*Agrostis*	*Agrostis*
	Ammophila	*Ammophila*	*Ammophila*	*Ammophila*	*Ammophila*		*Ammophila*	*Ammophila*	*Ammophila*	*Ammophila*	
Lagurus		*Lagurus*	*Lagurus*	*Lagurus*	*Lagurus*	*Lagurus*	*Lagurus*	*Lagurus*			
Polypogon	*Polypogon*	*Polypogon*	*Polypogon*	*Polypogon*	*Polypogon*	*Polypogon*	*Polypogon*	*Polypogon*	*Polypogon*	*Polypogon*	
				Phleeae							
Phleinae		*Phleinae*		*Alopecurinae*						*Alopecurinae*	
Alopecurus	*Alopecurus*	*Alopecurus*	*Alopecurus*	*Alopecurus*	*Alopecurus*		*Alopecurus*	*Alopecurus*	*Alopecurus*	*Alopecurus*	*Alopecurus*
				Phleinae							
Phleum	*Phleum*	*Phleum*	*Phleum*	*Phleum*	*Phleum*		*Phleum*	*Phleum*	*Phleum*	*Phleum*	*Phleum*
Phalarideae	*Phalarideae*	*Phalarideae*	*Phalarideae*	*Phalarideae*		*Phalarideae*					
				Phalaridinae			*Phalaridinae*			*Phalaridinae*	*Phalaridinae*
Phalaris	*Phalaris*	*Phalaris*	*Phalaris*	*Phalaris*	*Phalaris*	*Phalaris*	*Phalaris*	*Phalaris*	*Phalaris*	*Phalaris*	*Phalaris*

Tab. 2. The taxon sample and taxa abbreviations used in the numerical analysis of tribe *Aveneae*, Poeaceae (According to Boulos, 2005)

No.	Taxa	Abbreviations
1	*Agrostis stolonifera* L.	Agrost
2	*Alopecurus myosuroides* Huds.	Alopec
3	*Ammophila arenaria* (L.) Link	Ammoph
4	*Avena barbata* Pott ex Link	A barb
5	*A. fatua* L.	A fatu
6	*A. sativa* L.	A sati
7	*A. sterilis* L.	A ster
8	*Lagurus ovatus* L.	Laguru
9	*Phalaris minor* Retz.	P mino
10	*P. paradoxa* L.	P para
11	*Phleum pratense* L.	Ph pra
12	*Ph. subulatum* (Savi) Asch. & Graebn.	Ph sub
13	*Polypogon maritimus* Willd.	Po mar
14	*P. monospeliensis* (L.) Desf.	Po mon
15	*P. viridis* (Gouan) Breistr.	Po vir
16	*Rostraria cristata* (L.) Tzvelev	Rostra
17	*Trisetaria glumacea* (Boiss.) Maire	T glum
18	*T. linearis* Forssk.	T line

The sub-ordinate group 1 comprised three species; *Agrostis stolonifera*, *Rostraria cristata* and *Trisetaria glumacea*. These species were quite similar in unequal lanceolate glumes with acute apex and one-nerved lower glumes, at the same time, the three species have a trapezoid silica bodies with flat ends and length more than 3 times as broad (Plate1; 1, 16 and 17). But *Agrostis stolonifera* differ in some characters from the other two species, which made it separated monophyletically from them, i.e. having 1-flowered inflorescence, absence of macrohairs and presence of irregular bilobate and trilobite silica bodies (Plate 1, 1).

The sub-ordinate group 2 included the two *Phalaris* species; *P. minor* and *P. paradoxa* as a monophyletic group, these species were similar in all recorded characters except the glumes shape oblanceolate with acuminate apex in *P. minor,* but glumes were lanceolate with acute apex in *P. paradoxa*. The monophyletically aspect of this group reflects the separation of genus *Phalaris* in a separate subtribe *Phalaridineae* under tribe *Aveneae* or *Poeae*, while other classifications placed genus *Phalaris* in a separate tribe *Phalarideae* as illustrated in Tab. 1. This result goes with that reported by Quintanar *et al*. (2007) through using the Plastid trnT-F and nuclear ribosomal ITS sequences to reconstruct the phylogeny of the Aveneae–Poeae–Seslerieae complex, and another similar study by Saarela *et al*. (2010).

The sub-ordinate group 3 encompasses six species; *Lagurus ovatus*, the three *Polypogon* species, *P. maritimus, P. monospeliensis* and *P. viridis* and the two *Phleum* species, *Ph. pratense* and *Ph. subulatum*. The placement of these species in one group agrees with the majority of classification systems as shown in (Tab. 1). These species were grouped according to the relative similarity in the 1-flowered inflorescence, the hairy awened glumes, as well as the trapezoid silica bodies with flat ends with length more than 3 times as broad (Plate 1; 8 and 11-15). It is clear from the dendrogram (Fig. 1) that *Polypogon maritimus* and *P. monospeliensis* appeared very close to each other and seemed to be one species, that they were similar in all recorded characters, which needs further study using wider characters. At the same time, the two *Phleum* species (*Ph. pratense* and *Ph. subulatum*) appeared in a close inner-group far from the other four species of the sub-ordinate group 3; this is due to the similarity between each other and the differences with other species within the same sub-group, such as unequal 3-nerved glumes and presence of irregular bilobate silica bodies.

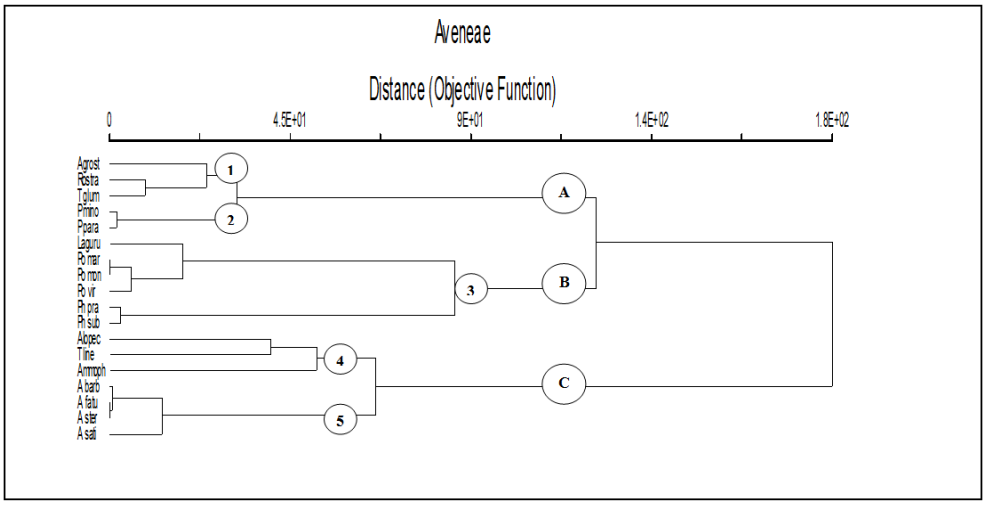


Fig. 1. Dendrogram of *Aveneae* based on analysis of characters recorded in Tab. 4 under a combination of Euclidean distance measure and Ward's clustering method. % chaining = 3.19%

Tab. 3. Characters and character states used in numerical analysis of tribe *Aveneae*

No	Characters	Character states	Code
C1	Number of flowers in spikelets	1-flowered	1
		several-flowered	0
C2	Similarity of glumes in length	equal	1
		unequal	0
C3	Glumes shape	lanceolate	1
		oblanceolate	2
		elliptic	3
		oblong	4
C4	Glume apex	acute	1
		acuminate	2
		aristulate	3
		awned	4
C5	Glume surface	glabrous	1
		hairy	2
		spiny	3
C6	Glume length (mm)	1.5-3.9	1
		4-10.9	2
		11-30	3
C7	Number of veins in lower glume	1	1
		2	2
		3	3
		7-9	4
C8	Number of veins in upper glume	1	1
		3	2
		7-9	3
C9	Spiral thickening of xylem vessels	present	1
		absent	0
C10	Annular thickening of xylem vessels	present	1
		absent	0
C11	Epidermal long cells: parallel walls	thickened	1
		not thickened	0
C12	Epidermal long cells; parallel walls shape	undulate	1
		straight	0
C13	Bulliform cells	silicified	1
		not silicified	0
C14	Stomatal subsidiary cells	oblong	1
		triangular	0
C15	Epidermal papillae	present	1
		absent	0
C16	Hooked prickle hairs	present	1
		absent	0
C17	Straight prickle hairs	present	1
		absent	0
C18	Macro hairs	present	1
		absent	0
C19	Irregular bilobate silica bodies	present	1
		absent	0
C20	Trilobate silica bodies	present	1
		absent	0
C21	Elongate smooth silica bodies	present	1
		absent	0

Tab. 3. Characters and character states used in numerical analysis of tribe *Aveneae* (cont.)

C22	Trapezoid silica bodies	present	1
		absent	0
C23	Narrow elliptic silica bodies	present	1
		absent	0
C24	Side walls of silica bodies	sinuous-wavy	1
		straight	0
C25	Silica bodies with concave ends	present	1
		absent	0
C26	Silica bodies with convex ends	present	1
		absent	0
C27	Silica bodies with flat ends	present	1
		absent	0
C28	Silica bodies with pointed ends	present	1
		absent	0
C29	Silica bodies > 3 times as long as width	present	1
		absent	0
C30	Silica bodies < 3 times as long as width	present	1
		absent	0
C31	Silica-cork cells	present	1
		absent	0

Tab. 4. Distribution of characters among *Aveneae* species for species abbreviations, see Tab. 2

Species	Character states																													
Abbreviations	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Agrost	1	0	1	1	3	1	1	1	1	1	0	0	0	1	0	1	1	0	1	1	0	1	0	1	1	0	1	1	1	1	0
Alopec	1	0	1	1	2	1	4	2	1	0	0	0	1	1	1	1	1	0	0	0	1	0	0	0	0	0	1	0	1	0	0
Ammoph	1	0	1	1	2	3	2	2	0	1	0	1	1	0	0	1	1	1	0	0	0	0	1	0	0	0	0	1	0	1	1
A barb	0	1	1	1	1	3	4	3	0	1	0	1	1	1	0	1	1	1	0	0	0	1	0	1	0	0	1	0	1	0	0
A fatu	0	1	1	1	1	3	4	3	0	1	0	1	1	1	0	1	1	0	0	0	0	1	0	1	0	0	1	0	1	0	0
A sati	0	1	3	2	1	3	4	3	0	1	0	1	1	1	0	1	1	1	0	0	0	1	0	1	0	0	1	0	1	0	0
A ster	0	1	1	1	1	3	4	3	0	1	0	1	1	1	0	1	1	0	0	0	0	1	0	1	0	0	1	0	1	0	0
Laguru	1	0	1	4	2	2	1	1	1	0	0	0	1	1	0	1	1	1	0	0	0	1	0	1	1	0	1	0	1	0	0
P mino	0	1	2	1	3	2	1	1	0	1	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	0	1	0	1	0	0
P para	0	1	1	2	3	2	1	1	0	1	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	0	1	0	1	0	0
Ph pra	1	0	4	4	2	1	3	2	0	1	0	0	1	1	0	0	1	0	1	1	0	1	0	1	1	0	1	0	1	1	0
Ph sub	1	0	3	4	2	1	3	2	0	1	0	0	1	1	0	1	1	0	1	1	0	1	0	1	1	0	1	0	1	1	0
Po mar	1	1	1	4	2	1	1	1	1	1	0	0	0	1	0	1	1	0	0	1	0	1	0	1	1	1	1	0	1	1	0
Po mon	1	1	1	4	2	1	1	1	1	1	0	0	0	1	0	1	1	0	0	1	0	1	0	1	1	1	1	0	1	1	0
Po vir	1	1	1	4	2	1	1	1	1	1	0	0	1	1	0	0	1	0	0	1	0	1	0	1	0	0	1	0	1	1	0
Rostra	0	0	1	1	2	1	1	2	0	1	1	0	0	1	0	1	1	1	0	0	0	1	0	0	1	0	1	0	1	0	0
T glum	0	0	1	1	3	2	1	1	0	1	0	1	0	1	0	1	1	1	0	0	0	1	0	1	0	0	1	0	1	0	0
T line	1	0	1	3	3	2	4	3	0	1	1	1	0	1	0	1	1	1	0	0	0	1	0	1	0	0	1	0	1	0	0

The sub-ordinate group 4 comprised three species; *Alopecurus myosuroides*, *Trisetaria linearis* and *Ammophila arenaria*. These species were moderately similar in unequal lanceolate glumes, 1-flowered spikelet and the presence of both straight and hooked hairs, while *Ammophila arenaria* differ in some characters from the other two species, which made it separated monophyletically from them, i.e. having 2-nerved lower glumes, the triangular stomatal subsidiary cells, presence of narrow elliptic silica bodies with pointed ends, in addition to the silica-cork cells (Plate 1; 3). The placement of *Alopecurus myosuroides* and *Ammophila arenaria* in one sub-group matches with previous classification systems (Tab. 1), while the appearance of *Trisetaria linearis* with them in one group is unusual. It is important to study the scattering of the two species of genus *Trisetaria* in two different sub-groups (1 and 4) using more characters.

The sub-ordinate group 5 included the four *Avena* species; *A. barbata, A. fatua, A. sterilis* and *A. sativa*. It is obvious from the dendrogram (Fig. 1) and the data matrix

Tab. 5. Factor loadings of the 31 characters (C1-C31) on the first three principal components axes. Highest loadings in bold

Characters	Characters	PCA Axes		
		1	2	3
	Eigenvalue	0.392	0.229	0.080
C1	No. of flowers in spikelet	**-0.562**	0.418	-0.026
C2	Similarity of glumes in length	0.135	-0.005	-0.459
C3	Glume shape	-0.020	**0.644**	**0.621**
C4	Glume apex	**-0.664**	**0.673**	-0.230
C5	Glume surface	**-0.545**	**-0.462**	0.374
C6	Glume length	**0.800**	-0.140	-0.300
C7	Number of veins in lower glume	**0.820**	**0.516**	0.065
C8	Number of veins in upper glume	**0.875**	0.391	-0.029
C9	Spiral thickening of xylem vessels	**-0.607**	0.0198	-0.350
C10	Annular thickening of xylem vessels	0.050	-0.009	0.072
C11	Epidermal long cells: parallel walls	0.081	-0.090	0.044
C12	Epidermal long cells; parallel walls shape	**0.806**	-0.025	-0.284
C13	Bulliform cells	**0.439**	**0.546**	-0.073
C14	Stomatal subsidiary cells	-0.148	0.219	0.030
C15	Epidermal papillae	0.163	-0.032	0.235
C16	Hooked prickle hairs	0.320	-0.443	-0.149
C17	Straight prickle hairs	0.119	**0.463**	-0.296
C18	Macro hairs	0.332	-0.173	-0.206
C19	Irregular bilobate silica bodies	-0.286	0.423	**0.633**
C20	Trilobate silica bodies	**-0.703**	**0.465**	0.075
C21	Elongate smooth silica bodies	-0.001	-0.410	0.394
C22	Trapezoid silica bodies	-0.228	0.183	-0.149
C23	Narrow elliptic silica bodies	0.150	-0.219	-0.030
C24	Side walls of silica bodies	-0.058	**0.597**	-0.385
C25	Silica bodies with concave ends	**-0.663**	0.289	0.111
C26	Silica bodies with convex ends	**-0.495**	0.118	-0.440
C27	Silica bodies with flat ends	-0.150	0.219	0.030
C28	Silica bodies with pointed ends	-0.035	-0.335	0.163
C29	Silica bodies > 3 times as long as width	-0.150	0.219	0.030
C30	Silica bodies < 3 times as long as width	**-0.609**	0.347	0.058
C31	Silica-cork cells	0.150	-0.219	-0.030

(Tab. 4) that *A. fatua* and *A. sterilis* appeared very close to each other and seemed to be one species, that they were similar in all recorded characters, which needs further study using wider characters. At the same time, *A. barbata* appeared closer to them due to the characters similarity, except the presence of macrohairs, while, *A. sativa* differ from the other three species by the presence of macrohairs and elliptic acuminate glumes, so it appeared as monophyletic species.

The placement of all *Avena* representatives in one group matches with the classification systems constructed by Loskutov (2007), by studying the relationships of genomes of different *Avena* species at each ploidy level, and (Peng *et al.,* 2010) through studying the evolution pattern of rDNA ITS in *Avena* and phylogenetic relationship of the *Avena* species.

PCA based on the 31 characters (Fig. 2) explained 68.2% of the total variation. Axis one explained 34.1%, axis two 20.7%, and axis three 13.4%. It was evident that members of sub-ordinate groups 1 and 2 that comprised of *Agrostis stolonifera*, *Rostraria cristata* and *Trisetaria glumacea*, *Phalaris minor* and *P. paradoxa* were strongly correlated to differences in glume surface (C5). Members of sub-ordinate group (3) that comprised of *Lagurus ovatus*, *Polypogon maritimus, P. monospeliensis* and *P. viridis*, and the *Phleum pratense* and *Ph. subulatum* showed highly correlated to spiral thickening of xylem vessels (C9), silica bodies with concave ends (C25) and silica bodies with convex ends (C26). On the other hand, members of sub-ordinate groups (4) and (5) showed high correlations with

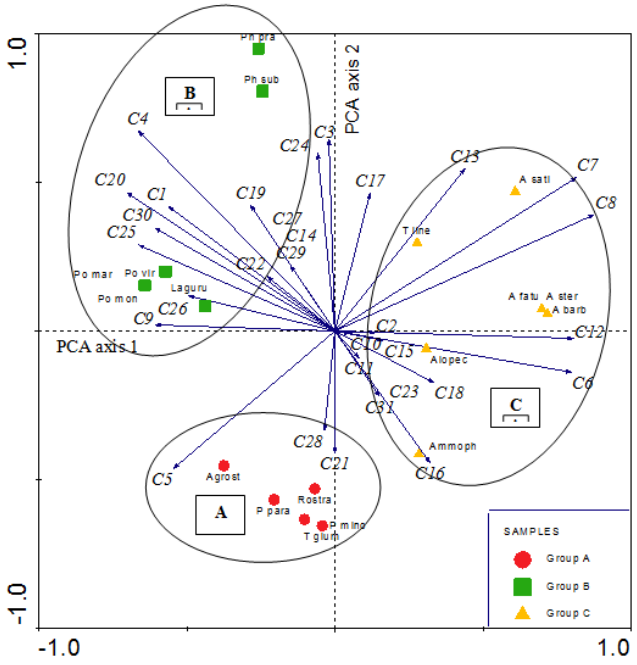
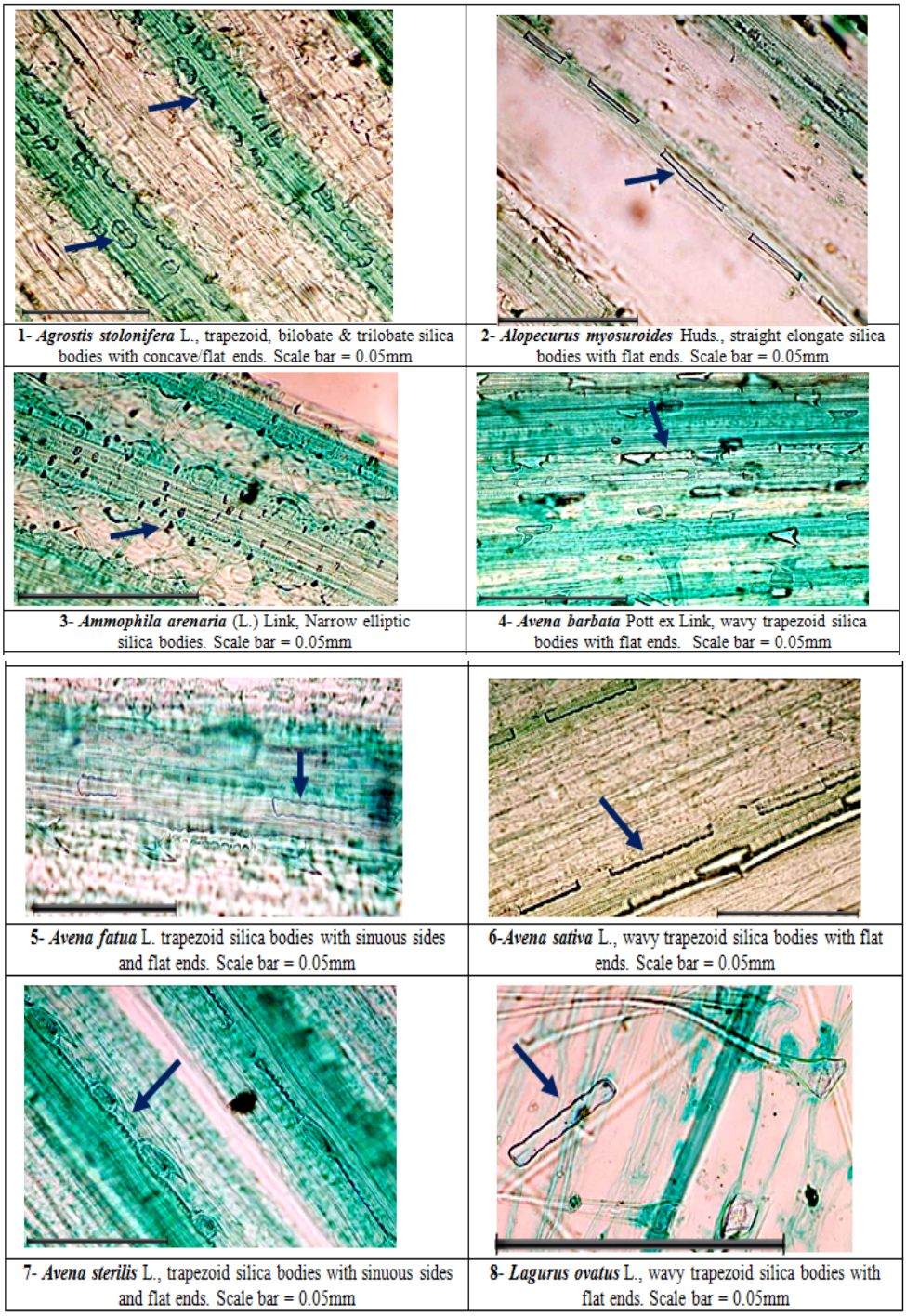


Fig. 2. Principal component analysis (PCA) of the studied species of Aveneae based on 31 morphological characters (arrows), together with their clusters (A, B and C). See Tab. 2 for abbreviations of species, and Tab. 3 for character abbreviations



1- ***Agrostis stolonifera*** L., trapezoid, bilobate & trilobate silica bodies with concave/flat ends. Scale bar = 0.05mm

2- ***Alopecurus myosuroides*** Huds., straight elongate silica bodies with flat ends. Scale bar = 0.05mm

3- ***Ammophila arenaria*** (L.) Link, Narrow elliptic silica bodies. Scale bar = 0.05mm

4- ***Avena barbata*** Pott ex Link, wavy trapezoid silica bodies with flat ends. Scale bar = 0.05mm

5- ***Avena fatua*** L. trapezoid silica bodies with sinuous sides and flat ends. Scale bar = 0.05mm

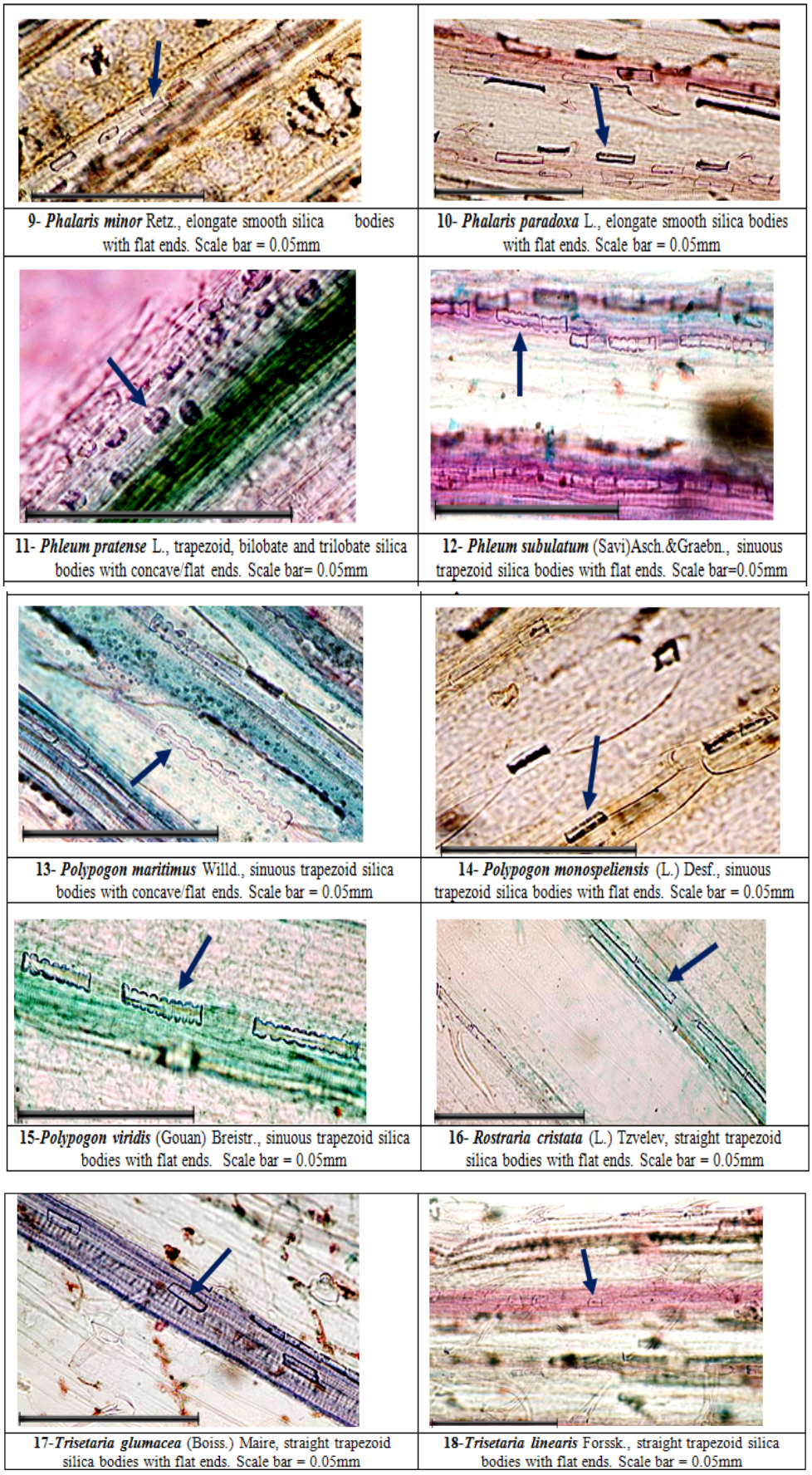
6-***Avena sativa*** L., wavy trapezoid silica bodies with flat ends. Scale bar = 0.05mm

7- ***Avena sterilis*** L., trapezoid silica bodies with sinuous sides and flat ends. Scale bar = 0.05mm

8- ***Lagurus ovatus*** L., wavy trapezoid silica bodies with flat ends. Scale bar = 0.05mm

Plate 1. Light micrographs of the studied taxa of tribe *Aveneae,* showing their silica bodies structure

number of veins in lower glume (C7), epidermal long cells; parallel walls shape (C12) and bulliform cells (C13). Significant negative loadings in relation to PCA axis 1 were number of flowers in spikelets, glume apex and glume, spiral thickening of xylem vessels, trilobate silica bodies, silica bodies with concave ends, silica bodies with convex ends and silica bodies less than 3 times as long as width, while significant positive loadings included glume length, number of veins in lower glume, number of veins in upper glume, epidermal long cells; parallel walls shape and bulliform cells (Tab. 5). Along PCA axis 2, the significant negative loadings included glume surface, while the significant positive loadings included glume shape, glume apex, number of veins in lower glume, bulliform cells, straight prickle hair, trilobite silica bodies and side walls of silica bodies.



9- ***Phalaris minor*** Retz., elongate smooth silica bodies with flat ends. Scale bar = 0.05mm

10- ***Phalaris paradoxa*** L., elongate smooth silica bodies with flat ends. Scale bar = 0.05mm

11- ***Phleum pratense*** L., trapezoid, bilobate and trilobate silica bodies with concave/flat ends. Scale bar= 0.05mm

12- ***Phleum subulatum*** (Savi)Asch.&Graebn., sinuous trapezoid silica bodies with flat ends. Scale bar=0.05mm

13- ***Polypogon maritimus*** Willd., sinuous trapezoid silica bodies with concave/flat ends. Scale bar = 0.05mm

14- ***Polypogon monospeliensis*** (L.) Desf., sinuous trapezoid silica bodies with flat ends. Scale bar = 0.05mm

15-***Polypogon viridis*** (Gouan) Breistr., sinuous trapezoid silica bodies with flat ends. Scale bar = 0.05mm

16- ***Rostraria cristata*** (L.) Tzvelev, straight trapezoid silica bodies with flat ends. Scale bar = 0.05mm

17-***Trisetaria glumacea*** (Boiss.) Maire, straight trapezoid silica bodies with flat ends. Scale bar = 0.05mm

18-***Trisetaria linearis*** Forssk., straight trapezoid silica bodies with flat ends. Scale bar = 0.05mm

Plate 1. Light micrographs of the studied taxa of tribe *Aveneae,* showing their silica bodies structure (Continuos)

Conclusions

In the present study, the total 31 characters recorded the morphology of glumes and the features of silica skeleton among 18 species of tribe *Aveneae*. Numerical methods were applied to study the relationship and the level of variation within and among these species. The relationships between silica skeleton morphologies and the taxa of tribe *Aveneae* have never been studied previously.

Metcalfe (1960) described several kinds of specialized cells (short cells) as: cork-cells containing silica bodies, stomata, and dermal appendages. The shape of the silica bodies formed in the short cells over the veins can be used to identify the subfamilies of the Gramineae and also some of the tribes (Piperno and Pearsall, 1998; Twiss *et al*., 1969). This evidence was confirmed here through the separation of the two *Phleum* species in an inner-group due to the presence of irregular bilobate silica bodies. In another case, the appearance of *Ammophila arenaria* as a monophyletic species in the sub-ordinate group 4, due to the differences in some characters from the other species, such as, the triangular silicified stomatal subsidiary cells and the presence of narrow elliptic silica bodies with pointed ends, in addition to the silica-cork cells.

The application of characteristics of leaf epidermis to classification and system evolution of tribe *Aveneae* was conducted by Xinming *et al*. (1998). The results of that study showed that characteristics of essential cells, such as size and shape of long cells, shape of short cells, as well as the distributions and shapes of macro hairs and prickle hairs, possess important value for classification of *Aveneae*. This evidence appeared obviously in our study by the presence of both straight and hooked hairs in *Ammophila arenaria,* which may be the reason of grouping it with the other two species in sub-group 4, despite of its monophyletic aspect.

Past intertribal hybridization events were advocated as a plausible explanation for the traditional misclassifications and the present existence of certain *Avenae* taxa with *Poeae* plastid genomes and vice versa (Soreng and Davis, 2000). The first phylogenetic study with a large sampling of *Aveneae* taxa was by Soreng and Davis (2000). Their combined analysis of plastid restriction site data and structural data resulted in a consensus topology suggested the placement of *Aveneae* within *Poeae* and recognized a series of subtribes of *Aveneae* that were later expanded by Soreng *et al.* (2003). The unstable and problematic classification of tribe *Aveneae* into a set of subtribes, may explains the unusual events in this study, such as: the placements of *Agrostis stolonifera* far from the sub-ordinate group 3, where included the majority of the *Agrostideae* representatives, that characterized by many characters as the 1-flowered spikelets. Another case is the scattered species of genus *Trisetaria* in two different sub-groups (1 and 4), which requires an extra study using wider range of parameters.

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