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Macro and micromorphological studies and numerical analysis on the nutlet of some Cyperoideae-Cyperaceae taxa from Egypt and their taxonomic significances

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Abstract: Nutlets of 13 taxa of subfamily Cyperoideae (Cyperaceae) from Egypt were investigated using light and scanning electron microscopes to ascertain the taxonomic utility of the nutlet in corroborating and elaborating on distinctions between closely related genera and species. The studied taxa included representatives of tribes Fuireneae, Cypereae, Cariceae, Abildgaardieae and Schoeneae. Nutlet shape, size, color, surface, epidermal cell shape, anticlinal boundaries, outer periclinal cell wall and relief of outer cell walls were provided. It was found that the epidermis has useful microscopic characters to help in the taxonomic assessment of Cyperoideae. Seven different types of anticlinal cell wall boundaries were described, four types of outer periclinal cell walls, and five different forms of the secondary cell walls were recognized. A diagnostic key for the studied taxa based on nutlet characters was presented. The results showed congruence between the UPGMA dendrogram and principal component analysis in suggesting two major groups and six subgroups. The most important characters with the highest factor loadings along the first two PCA axes included nutlet shape, nutlet apex, surface topography and the presence of silica bodies. The results of this study confirmed the heterogeneity of the Cyperoideae and indicated that Fuireneae is the most heterogeneous tribe.

Key words: Cyperaceae, Cyperoideae, Egypt, morphology, nutlets, PCA, UPGMA

1. Introduction

Cyperaceae Juss. (sedge family) is the third-largest monocot family after Orchidaceae Juss. (orchids) and Poaceae Barn., with approximately 90 genera and 5500 species (Semmouri et al., 2018). It is a cosmopolitan family (except for Antarctica) of mainly perennial third-largest herbs, with trigonous, solid stem and 3-ranked leaves. Members of this family are especially abundant in damp, wet, or marshy regions of the temperate and subarctic zones, where species can dominate the vegetation (Heywood et al., 2007). Due to the reduced structure of flowers and fruits as well as the considerably uniform morphological characters of their vegetative organs, the identification of taxa of Cyperaceae is difficult (Schuyler, 1971).

The microscopic cellular structure has proven to be useful for taxonomic classification of the family at both generic and specific levels (Schuyler, 1971). Generally, the achene morphology in sedges shows considerable diversity in their color, size, shape, ornamentation and epidermal sculpturing (Patil and Prasad, 2016). The nutlet morphological characters of Cyperaceae are of taxonomic significance, and can be used in its classification (Patil and Prasad, 2016). As termed by Schuyler (1971), nutlets in the family Cyperaceae show a significant amount of cell diversity which provides systematically valuable character. Achene features have been successfully used for the taxonomic classification of a wide range of Cyperaceae taxa; Menapace (1991) investigated the forms of epidermal surface of the nutlets in *Eleocharis* R. Br. Govindarajalu (1990) studied the nutlets of *Pycreus* sect. Muricati using scanning electron microscope (SEM). Wujek et al. (1992) described the nutles micromorphology of some species from India i.e. *Fimbristylis* Vahl, *Cyperus* L., *Pycreus* P. Beauv., *Scleria* P. J. Bergius, and *Scirpus* L. In addition, the nutlet micromorphology was used as a possible taxonomic method for the systematic recognition of various sections in genus *Fimbristylis* (Menapace et al., 2003; Patil and Prasad, 2016). The surface can be smooth or with different types of ornamentations, i.e. puncticulate, verrucose, trabeculate, pitted, zonate or tessellate (Patil and Prasad, 2016).

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Modern classification of the Cyperaceae (Bruhl, 1995) was based on several lines of evidence, i.e. traditional morphological in addition to anatomical, embryological and physiological data. Muasya et al. (1998) proposed some modifications to Bruhl's classification, based on evidence from rbcL DNA sequences. Recent investigations revealed the presence of two subfamilies within Cyperaceae: Cyperoideae Beilschmied and Mapanioideae CB Clarke[1].

Many cladistic studies of subfamily Cyperoideae, based on a wide range of morphological, anatomical, phytochemical, physiological and embryological characters, proved the heterogeneity of the Cyperoideae even at tribal level (Bruhl, 1995; Goetghebeur, 1998). *Scirpus* was originally described in its broadest sense by Linnaeus (1753, 1754). The genus then split into four groups and 40–50 species based on the type of spike diameter, and shape of the culm, as well as the panicle leafiness (Govaerts and Simpson, 2007; Léveillé-Bourret et al., 2015). However, the genus has long been proved problematic in the Cyperaceae, some authors treated it as a single, heterogeneous genus (De Filipps, 1980), while others treated it in a strict sense and divided it into separate genera (*Blysmus* Panz., *Scirpus* L. *s.s.*, *Bolboschoenus* (Rchb.) Palla, *Schoenoplectus* (Rchb.) Palla, *Scirpoides* Ség., *Isolepis* R.Br. and *Trichophorum* Pers.), with *Scirpus s.s.* being recognized as a small genus of about nine species (Lye, 1973; Goetghebeur, 1998). This interpretation is mainly based on arguments from micromorphology (Schuyler, 1971), embryology (Van der Veken, 1965), and molecular biology (Muasya et al., 1998; Muasya et al., 2001).

The number of species and genera of Cyperaceae in Egypt has varied over time. Forsskål (1775) recognized 13 species within three genera, while Muschler (1912) recorded 35 species belonging to 7 genera, Täckholm (1974) enumerated 44 species of the Cyperaceae under eight genera. El Habashy et al. (2005) updated this number as 47 species in 12 genera including 6 subspecies and 2 varieties. The genus *Scirpus s.l.* was represented by 9 species by Täckholm (1974). After the segregation of the genus *Scirpus* (*s.l.*), El Habashy et al. (2005) reported eight taxa belonging to 4 genera viz.: *Bolboschoenus*, *Schoenoplectus*, *Scirpoides*, and *Isolepis*. Recently, and according to the database of The Plant List (2013)[2], a new genus namely *Schoenoplectiella* is derived from the genus *Schoenoplectus* which raises the number of genera in Egypt that segregated from the genus *Scirpus s.l.* to five. The aim of this study is to evaluate the taxonomic significance of nutlet macro- and micromorphological characters in distinguishing between closely related taxa. To achieve this purpose, the nutlets of 13 taxa of subfamily Cyperoideae (Cyperaceae) from Egypt were studied using light and scanning electron microscope (SEM).

2. Materials and methods

The current study was mainly based on herbarium specimens kept in the following Egyptian herbaria: Cairo University (CAI), Assiut University (ASTU), and Sohag University (SUG). Acronyms of herbaria were ??? made according to the Index Herbariorum[3]. In this study, healthy and fully mature nutlets of 13 taxa included in ten genera were selected to study the morphological characters using a stereomicroscope (LM) and scanning electron microscope (Table 1). Mostly 15–20 nutlets per plant and five plants per species were selected for each taxon to cover the range of nutlet morphological variation. The samples were examined by an Olympus SZ61 stereo dissecting microscope and photographed by a digital Olympus camera SC100 located in ASTU. For scanning electron microscopy, dry nutlets were directly mounted on metallic stubs using the double adhesive tape and coated with gold for a period of 4–6 min in a sputtering chamber using JOEL JFC1100E ion-sputtering device. The specimens were examined using a JOEL JSM 5400 LV SEM, operated at an accelerated voltage of 15 kV. All photomicrographs were taken at the Scanning Electron Microscope Unit, Assiut University, Egypt.

Aspects of nutlet micro-and macromorphology based on 66 characters were recorded and scored for the OTU's in a data matrix. Nutlet characters were transformed into two-state coding characters, their presence or absence was coded 1 and 0, respectively. Cluster analysis was constructed based on similarity matrix data by using the unweighted pair-group clustering (UPGMA) and represented in a phenogram tree. Principal components analysis (PCA) was performed using the product-moment correlation as a coefficient. The cluster analysis was carried out by using NTSYS-pc 2.0 software according to Rohlf (1988), while PCA was performed using PAST (Paleontological Statistics version 3.15) (Hammer, 2001).

The terminology used here essentially followed (Barthlott, 1990; Stearn, 1992) with some modifications by the authors. Results of nutlet shape, size, color, apex, surface sculpturing, beak, epidermal cell shape, anticlinal boundaries, periclinal cell wall and absence or presence of silica bodies were examined and summarized for each taxon. The LM photomicrographs of the studied nutlets

[1] Stevens P (2001 onwards). Angiosperm Phylogeny Website, version 14 [online]. Website http://www.mobot.org/MOBOT/research/APweb/ [accessed 23 February 2018].

[2] The Plant List (2013). Version 1.1. [online]. Website http://www.theplantlist.org [accessed 01 January 2013].

[3] Thiers B (2019). New York Botanical Garden's Virtual Herbarium. Index Herbariorum. A global directory of public herbaria and associated staff [online]. Website http://sweetgum.nybg.org/science/ih [accessed 25 February 2019].

Table 1. List of plant specimens used in light and scanning electron microscopes studies.

No.	Taxon	Voucher
1	*Bolboschoenus glaucus* (Lam.) S.G. Smith	Faiyum; Sennuris 08. 12. 1967. (CAI)
2	*Schoenoplectus litoralis* (Schrad.) Palla subsp. *thermalis* (Trab.) S. Hooper	El Mattaria, Dakahlia 21. 05. 1967. (SUG)
3	*Schoenoplectiella praelongata* (Poir.) Lye	Beheira Province, Itai El Barud 18. 09. 1987. (SUG)
4	*S. supina* (L.) Lye	Weed in rice fields near the animal breeding sections, Kharga, 04.11.1966. (SUG)
5	*Fuirena ciliaris* (L.) Roxb.	In Rice fields, El Baramoun, Nile Delta, 27. 10. 1967. (CAI)
6	*F. pubescens* (Poir.) Kunth	Lake Umm Risha, Wadi Natroun, 12.06.1968. (CAI)
7	*Scirpoides holoschoenus* (L.) Soják subsp. *australis* (L.) Soják	Southern Sinai, Farsh Kanesat Al-Hamar, 28.54644 N 33.96768 E, Alt.: 2038 m., 04.05.2010 (ASTU)
8	*Carex distans* L.	St. Catherina, Wadi Halfa, 23.04.1983. (CAI)
9	*C. extensa* Good.	Rossetta, Abu-mandure, 15.06.1974. (CAI)
10	*Fimbristylis bisumbellata* (Forssk.) Bubani	El-Esawia, Sohag, 26.02.1982 (ASTU)
11	*F. sieberiana* Kunth	Markaz Bilbeis, 10.09.1966. (CAI)
12	*Schoenus nigricans* L.	Gebel Serbal region; Sharafat Qoleib, 28. 63087 N 33. 658900 E, Alt.: 1503 m, 25.04.2004 (ASTU)
13	*Cladium mariscus* (L.) Pohl subsp. *mariscus*	Siwa, 24. 04. 1962. (CAI)

and the SEM micrographs of nutlet outline and surface microstructures were illustrated.

3. Results

3.1. Nutlet color

The color of the nutlets was of significant diagnostic and systematic value among the studied taxa (Figures 1A–1M, Table 2). Four main categories of nutlet color were observed: white, yellow, brown, and gray to blackish. The majority of studied taxa showed brown nutlets with some degrees of variation from light to dark. Yellowish nutlets recorded in both taxa of *Fimbristylis* as well as *Fuirena ciliaris* (L.) Roxb. (Figures 1F, IG and 1I). White color recognized to the nutlets of *Fuirena pubescens* (Poir.) Kunth and *Schoenus nigricans* L. (Figures 1H and 1J). While *Scirpoides holoschoenus* (L.) Soják subsp. *australis* (L.) Soják was the only studied taxa that have a gray-blackish nutlet (Figure 1E). Our results revealed that all taxa under investigation have glossy nutlets except *F. ciliaris* and *Carex distans* L. which have dull nutlets (Figures 1I and 1L).

3.2. Nutlet shape

The shape of the nutlet is of high diagnostic and systematic value among the studied taxa. The results revealed four categories of nutlet shape: obovate, suborbicular, ellipsoid and fusiform. Obovate nutlets observed in *Bolboschoenus glaucus* (Lam.) S.G. Smith, *Schoenoplectus litoralis* (Schrad.) Palla subsp. *thermalis* (Trab.) S. Hooper, *Schoenoplectiella praelongata* (Poir.) Lye, *Fimbristylis bisumbellata* (Forssk.) Bubani, *Fuirena pubescens*, *F. ciliaris*, and *Carex distans* (Figures 1A, 1B, 1C, 1F, 1H, 1I and 1L) respectively. Suborbicular shape observed in *Schoenoplectiella supina* (L.) Lye (Figure 1D) and *Fimbristylis sieberiana* Kunth (Figure 1G). Ellipsoid nutlet is recognized to *Schoenus nigricans* (Figure 1J), *Carex extensa* Good. (Figure 1M) and *Scirpoides holoschoenus* subsp. *australis* (Figure 1E). However, fusiform nutlet shape is unique to *Cladium mariscus* (L.) Pohl subsp. *mariscus* (Figure 1K). The shape of nutlet sides showed variations: digonous, biconvex in *Schoenoplectus litoralis* subsp. *thermalis*, *Schoenoplectus supina*, *Fimbristylis bisumbellata*, and *F. sieberiana* (Figures 1B, 1D, 1F and 1G); trigonous plano-convex in *Bolboschoenus glaucus* (Figure 1A). Yet, *Schoenoplectiella praelongata* was easily recognized by having trigonous concave nutlet (Figure 1C). The nutlets of the remaining taxa were characterized by trigonous convex sides. The presence or absence of stipe was of less diagnostic and systematic value. However, the nutlet was stipitate in the taxa of *Scirpoides holoschoenus* subsp. *australis*, *Fimbristylis bisumbellata*, *Fuirena pubescens*, *Fuirena ciliaris*, *Cladium mariscus* subsp. *mariscus*, *Carex distans* and *Carex extensa* (Figures 1E, 1F, 1H, 1I, 1K, 1L, 1M and Table 2).

3.3. Nutlet size

The nutlet dimensions (L × W) exhibited a wide range of variations. *Cladium mariscus* had the largest size with 3.5 × 1.5 mm, while the smallest size was found in *Fimbristylis bisumbellata* with 0.7 × 0.5 mm (Table 2).

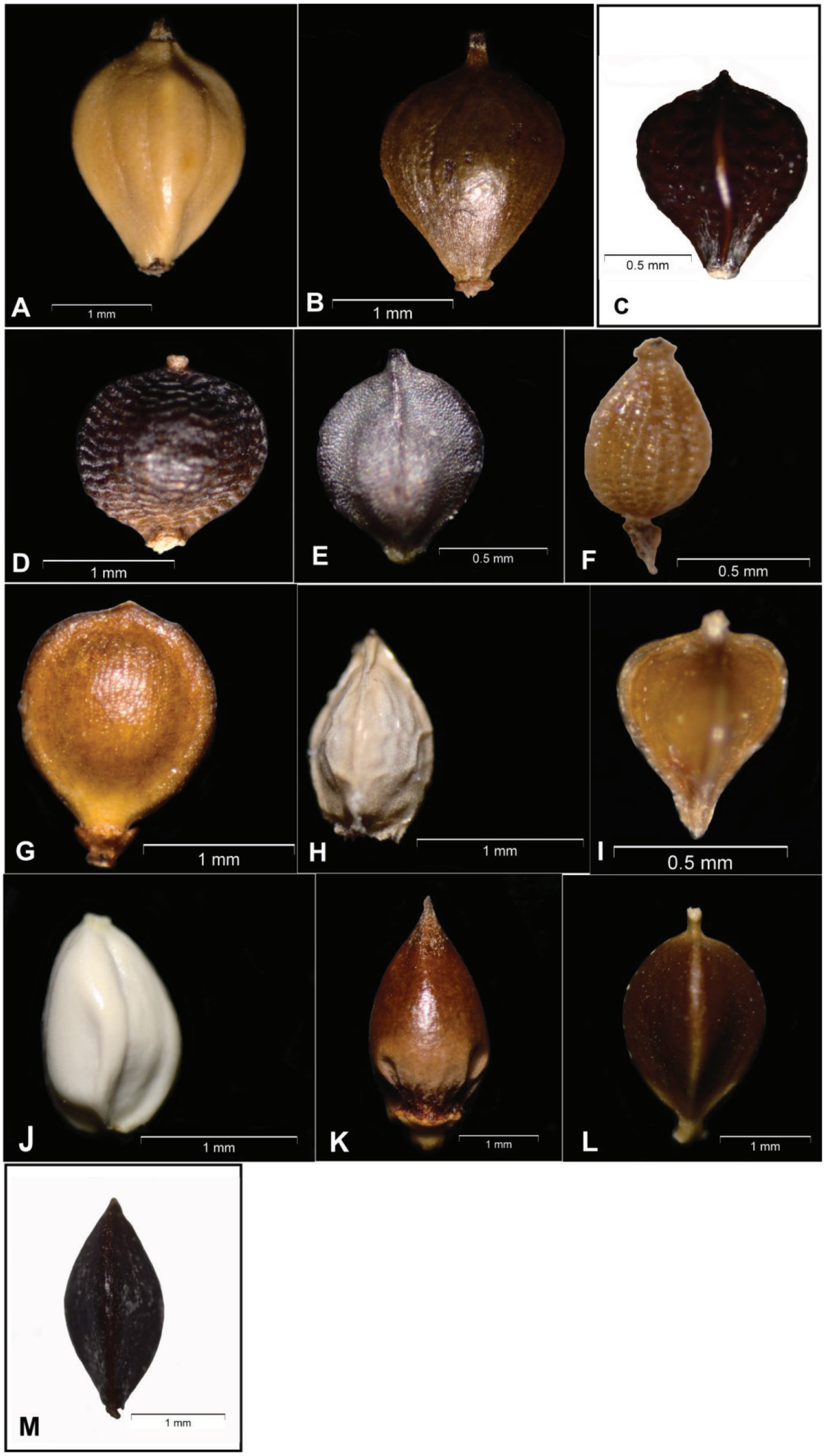

Figure 1. LM micrographs of nutlet outline. A) *Bolboschoenus glaucus*; B) *Schoenoplectus litoralis* subsp. *thermalis*; C) *Schoenoplectiella praelongata*; D) *Schoenoplectiella supina*; E) *Scirpoides holoschoenus* subsp. *australis*; F) *Fimbristylis bisumbellata*; G) *Fimbristylis sieberiana*; H) *Fuirena pubescens*; I) *Fuirena ciliaris*; J) *Schoenus nigricans*; K) *Cladium mariscus* subsp. *mariscus*; L) *Carex distans*; M) *Carex extensa*.

Table 2. Nutlets macro- and micromorphological characters of the studied taxa using light and scanning electron microscopes.

No.	Taxon	Shape	Dimensions (mm)	Colour	Apex	Surface topography	Beak	Epidermal cell			
								Shape	Anticlinal boundaries	Periclinal cell wall	Silica body
1	*Bolboschoenus glaucus*	Obovate, trigonous, plano-convex	2.5 × 1.5	Light brown, glossy	Attenuate	Smooth	Short, narrow	Undifferentiated			Absent
2	*Schoenoplectus litoralis* subsp. *thermalis*	Obovate; digonous, biconvex	2.0 × 1.5	Dark brown, glossy	Apiculate	Reticulate, foveate	Long, narrow	Polygonal in longitudinal rows	Raised, straight, thick, fine verrucose	Flat to concave, fine verrucose	Absent
3	*Schoenoplectiella praelongata*	Obovate, trigonous, concave	1.3 × 1.0	Dark brown, glossy	Attenuate	Rugulose, wavy rows bearing furrows and prominent ridges	Short, narrow	Narrowly linear in longitudinal rows;	Raised, straight, thin, with very fine folded	Flat, smooth	Absent
4	*Schoenoplectiella supina*	Sub orbicular, digonous, biconvex	1.3 × 1.1	Black brown, glossy	Apiculate	Rugulose, wavy rows bearing furrows and prominent ridges	Short, narrow	Narrowly oblong in longitudinal rows;	Raised, wavy, thick, with very fine ridges	Flat, rough	Absent
5	*Fuirena ciliaris*	Obovate, stipitate, trigonous, convex	0.8 × 0.6	Yellow, dull	Apiculate	Rugulose	Short, narrow	Oblong, 5, 6 gonals transversely elongated	Raised, wavy, thick, smooth.	Convex, fine verrucose	Absent
6	*Fuirena pubescens*	Obovate, stipitate, trigonous, convex	1.3 × 0.7	White to yellowish, glossy	Obtuse	Cracked	Short, broad	Oblong, 5, 6 gonals transversely elongated	Raised, straight, thin, smooth	Flat, smooth	Absent
7	*Scirpoides holoschoenus* subsp. *australis*	Ellipsoid, stipitate, trigonous, convex	1.0 × 0.6	Gray to blackish, glossy	Mucronate	Reticulate, foveate	Long, narrow	Polygonal in longitudinal rows	Raised, straight, thick, smooth	Deeply concave, fine verrucose	Absent
8	*Carex distans*	Obovate, stipitate, trigonous, convex	2.3 × 1.3	Brown, dull	Apiculate	Reticulate, foveate	Long, narrow	6-8 gonals, mostly hexagonal, isodiametric	Raised, straight, thick, rough	Deeply concave with colliculate projection	Present
9	*Carex extensa*	Ellipsoid, stipitate; trigonous, convex	2.0 × 1.0	Dark brown, glossy	Conical	Reticulate, foveate	Long, broad	Polygonal, isodiametric	Raised, straight, thick, smooth	Deeply concave with colliculate projection	Present
10	*Fimbristylis bisumbellata*	Obovate, stipitate, digonous, biconvex	0.7 × 0.5	Yellow, glossy	Attenuate	Trabeculate, sclariform between ribs	Short, broad	Oblong, 5-6 gonals, transversely elongated	Raised, straight, thick, wavy ribs between walls	Concave, smooth	Absent
11	*Fimbristylis sieberiana*	Sub-orbicular, digonous, biconvex	1.4 × 1.2	Yellow, glossy	Mucronate	Reticulate, foveate	Short, broad	Hexagonal, reticulate	Raised, straight, thick, smooth	Flat, smooth	Absent
12	*Schoenus nigricans*	Ellipsoid, trigonous, convex	1.5 × 0.9	Creamy-white, glossy	Obtuse	Smooth	Absent	Stare-shape	Sunken, wavy, thin, smooth	Flat to slightly convex, smooth	Absent
13	*Cladium mariscus* subsp. *mariscus*	Fusiform, stipitate, trigonous, convex	3.5 × 1.5	Dark brown, glossy	Conical	Reticulate, foveate	Long, narrow	Polygonal, elongated, reticulate	Raised, straight, thick, smooth	Flat, smooth	Absent

3.4. Nutlet apex

Five types of nutlet apex were recorded: apiculate, attenuate, obtuse, mucronate, and conical shape. Apiculate apex was observed in the nutlets of *Schoenoplectus litoralis* subsp. *thermalis*, *Schoenoplectiella supina*, *Fuirena ciliaris*, and *Carex distans* (Figures 1B, 1D, 1I, and 1L). Attenuate apex was recorded in *Bolboschoenus glaucus*, *Schoenoplectiella praelongata,* and *Fimbristylis bisumbellata* (Figures 1A, 1C, and 1F). Mucronate nutlet apex was found in *Scirpoides holoschoenus* subsp. *australis* and *Fimbristylis sieberiana* (Figures 1E and 1G). Obtuse apex characterized both *Fuirena pubescens* and *Schoenus nigricans* (Figures 1H and 1J), while the conical shape was reported in the nutlet of *Cladium mariscus* subsp. *mariscus* and *Carex extensa* (Figures 1K and 1M).

3.5. Nutlet surface topography

The results indicated that *Bolboschoenus glaucus* and *Schoenus nigricans* had a smooth surface pattern (Figures 2A and 2J). While *Schoenoplectus litoralis* subsp. *thermalis*, *Scirpoides holoschoenus* subsp. *australis*, *Fimbristylis sieberiana*, *Cladium mariscus* subsp. *mariscus*, *Carex distans*, and *Carex extensa* have reticulate, foveate sculpturing (Figures 2B, 2E, 2G, 2K, 2L, and 2M) respectively. The rugulose surface was observed in *Schoenoplectiella praelongata*, *Schoenoplectiella supina*, and *Fuirena ciliaris* (Figures 2C, 2D, and 2I) respectively. In addition, *Fimbristylis bisumbellata* and *Fuirena pubescens* can easily be recognized by having tuberculate and cracked surfaces, (Figures 2F and 2H), respectively.

3.5.1. Epidermal cell shape

SEM results indicated the variations of nutlet epidermal cell shape which can be considered a valuable diagnostic and systematic character. Out of 13 studied taxa, the nutlet of *Bolboschoenus glaucus* has an undifferentiated epidermal cell (Figure 2A2). Five main shapes of epidermal cells were recognized: polygonal, hexagonal, oblong, narrowly linear to oblong, and stare shape. The polygonal type was observed in four taxa i.e. *Schoenoplectus litoralis* subsp. *thermalis*, *Scirpoides holoschoenus* subsp. *australis*, *Cladium mariscus* subsp. *mariscus*, and *Carex extensa* (Figures 2B2, 2E2, 2K2, and 2M2), respectively. Hexagonal epidermal cells shape was recorded in *Fimbristylis sieberiana* (Figure 2G2) and *Carex distans* (Figure 2L2). In addition to *Fimbristylis bisumbellata*, all members of the genus *Fuirena* showed the oblong epidermal cell shape (Figures 2H2, 2I2, and 2F2). The nutlets of the genus *Schoenoplectiella* were characterized by its narrowly linear to oblong longitudinal rows epidermal cells (Figures 2C2 and 2D2). Yet, *Schoenus nigricans* was the only species that have a stare-shape epidermal cell (Figure 2J2).

3.5.2. Anticlinal cell wall boundaries

Apart from *Bolboschoenus glaucus* which has undistinguishable anticlinal cell boundaries, the anticlinal cell wall boundaries were mostly well developed among other taxa (Figure 2A2). Seven types of cell wall boundaries were recognized: 1) Raised, straight to slightly sinuous, two-layered in thickness, protrusions found at the junction of walls in *Fimbristylis bisumbellata* (Figure 2F2); 2) Channeled, sinuous, smooth, protrusions found at the junction of walls in *Schoenus nigricans* (Figure 2K2); 3) Raised, straight, more or less evident, fine folded in *Fuirena ciliaris* (Figure 2I2); 4) Raised, wavy, thick, with very fine ridges found in *Schoenoplectiella supina* (Figure 2D2); 5) Raised, straight, thin, smooth or with very fine folded in *Schoenoplectiella praelongata* and *Fuirena pubescens* respectively (Figures 2C2 and 2H2); 6) Raised, straight, thick, fine verrucose in *Schoenoplectus litoralis* subsp. *thermalis* (Figure 2B2); and 7) Raised, straight, thick, smooth or rough in the remaining taxa.

3.5.3. Outer periclinal cell walls

As a diagnostic character for the lowest taxonomic ranks, characters of the outer periclinal cell walls can be used. The investigated taxa exhibited three forms of outer periclinal cell walls (Table 2): concave, convex and flat. Concave type observed in *Schoenoplectus litoralis* subsp. *thermalis*, *Scirpoides holoschoenus* subsp. *australis*, *Fimbristylis bisumbellata*, *Carex distans*, and *Carex extensa* (Figures 2B2, 2E2, 2F2, 2L2, and 2M2), respectively. While the convex type characterized *Schoenus nigricans* and *Fuirena ciliaris* (Figures 2J2 and 2I2), the remaining taxa exhibited a flat periclinal boundary.

3.5.4. Secondary cell wall sculpture

Three different types of cell wall surfaces were recognized: verrucose, rough, and smooth. The verrucose surface was observed in the genera of *Schoenoplectus* (Figure 2B2), *Fuirena* (Figures 2H2 and 2I2), *Scirpoides* (Figure 2E2), and *Carex* (Figures 2L2 and 2M2). The rough surface was recognized in *Schoenoplectiella supina* (Figure 2D2). The rest of the studied taxa exhibited a smooth periclinal surface.

3.5.5. The silica bodies

The presence or absence of silica bodies can be used as a diagnostic and systematic character for supraspecific, specific and infraspecific taxa within Cyperaceae. In this study, the silica bodies were identified in the members of the genus *Carex*, as pointed central projection (Figures 2L2 and 2M2).

3.6. Numerical analysis

3.6.1. Cluster analysis

Based on 66 micro-and macromorphological characters of the nutlets, cluster analyses were carried out. From the cumulative data presented in (Tables 2–4). The similarity matrix was determined, and UPGMA dendrogram was illustrated (Figure 3). At the similarity level of 12.25%, two major clusters were separated: C1 and C2. The first

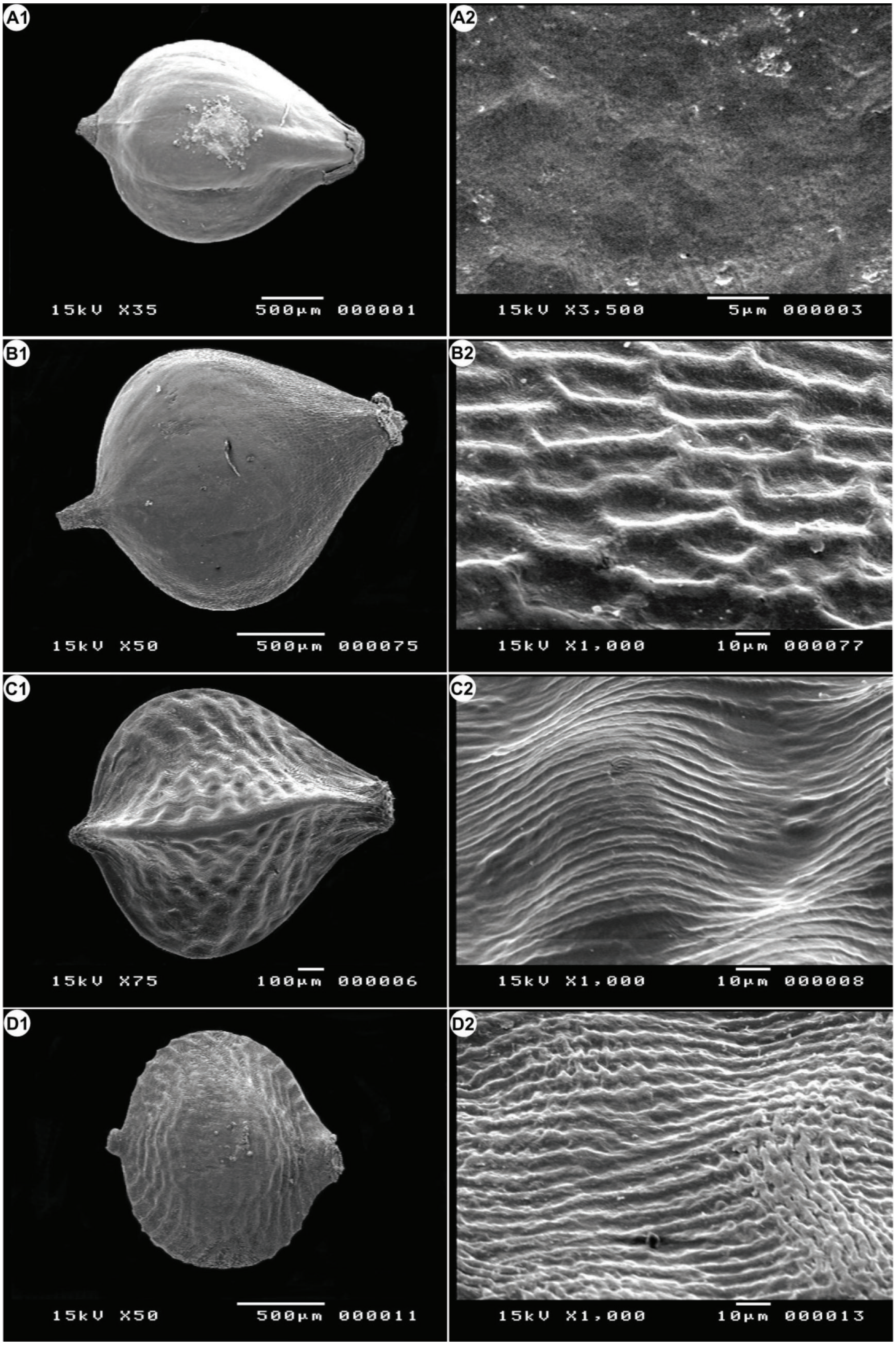

Figure 2. SEM micrographs of and bars indicate size outline. A) *Bolboschoenus glaucus*; B) *Schoenoplectus litoralis* subsp. *thermalis*; C) *Schoenoplectiella praelongata*; D) *Schoenoplectiella supina*.

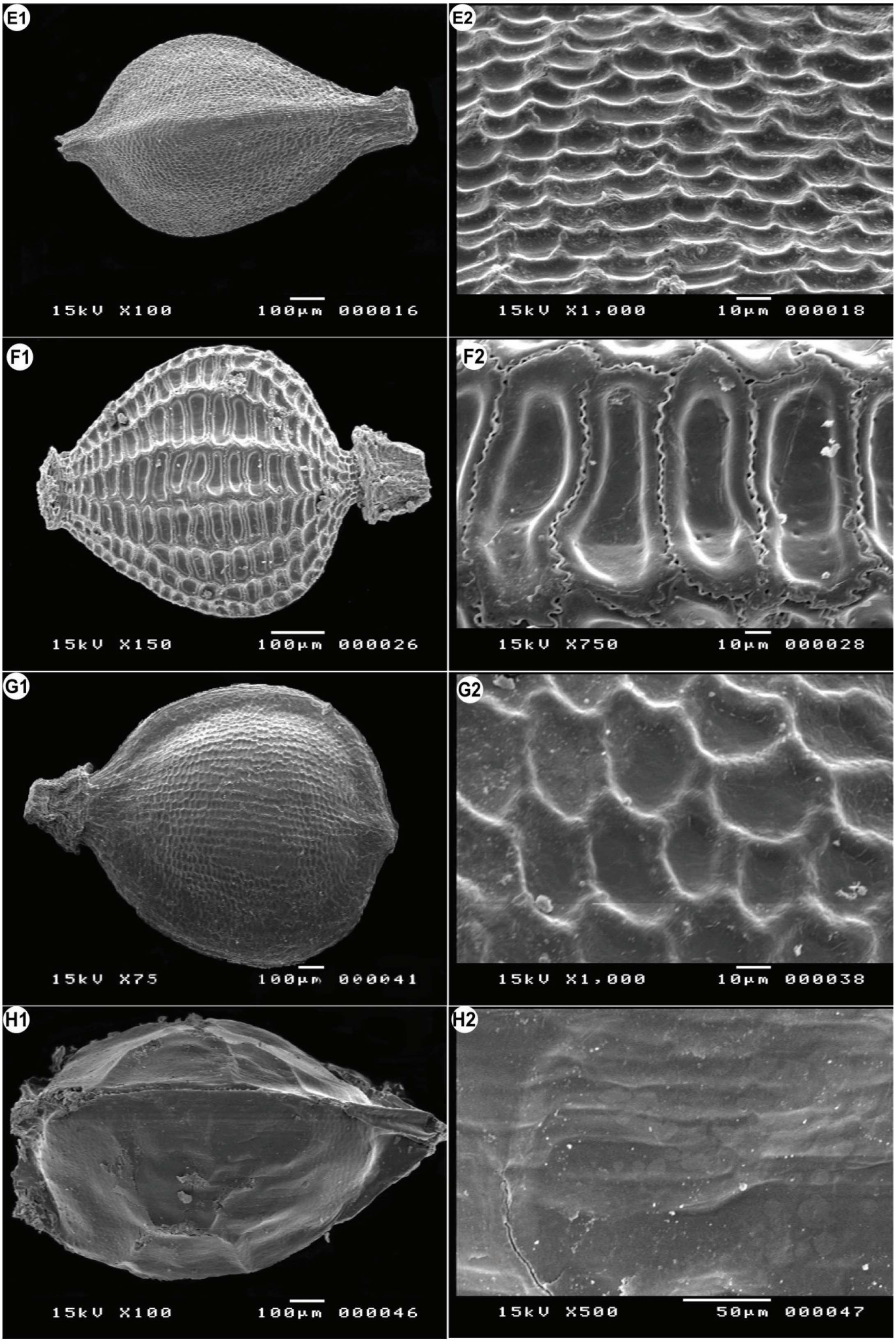

Figure 2 (cont.). SEM micrographs of nutlet. Figures E–H, 1) entire outline of nutlet, 2) enlargement of nutlet coat and bars indicate size outline. E) *Scirpoides holoschoenus* subsp. *australis*; F) *Fimbristylis bisumbellata*; G) *Fimbristylis sieberiana*; H) *Fuirena pubescens*.

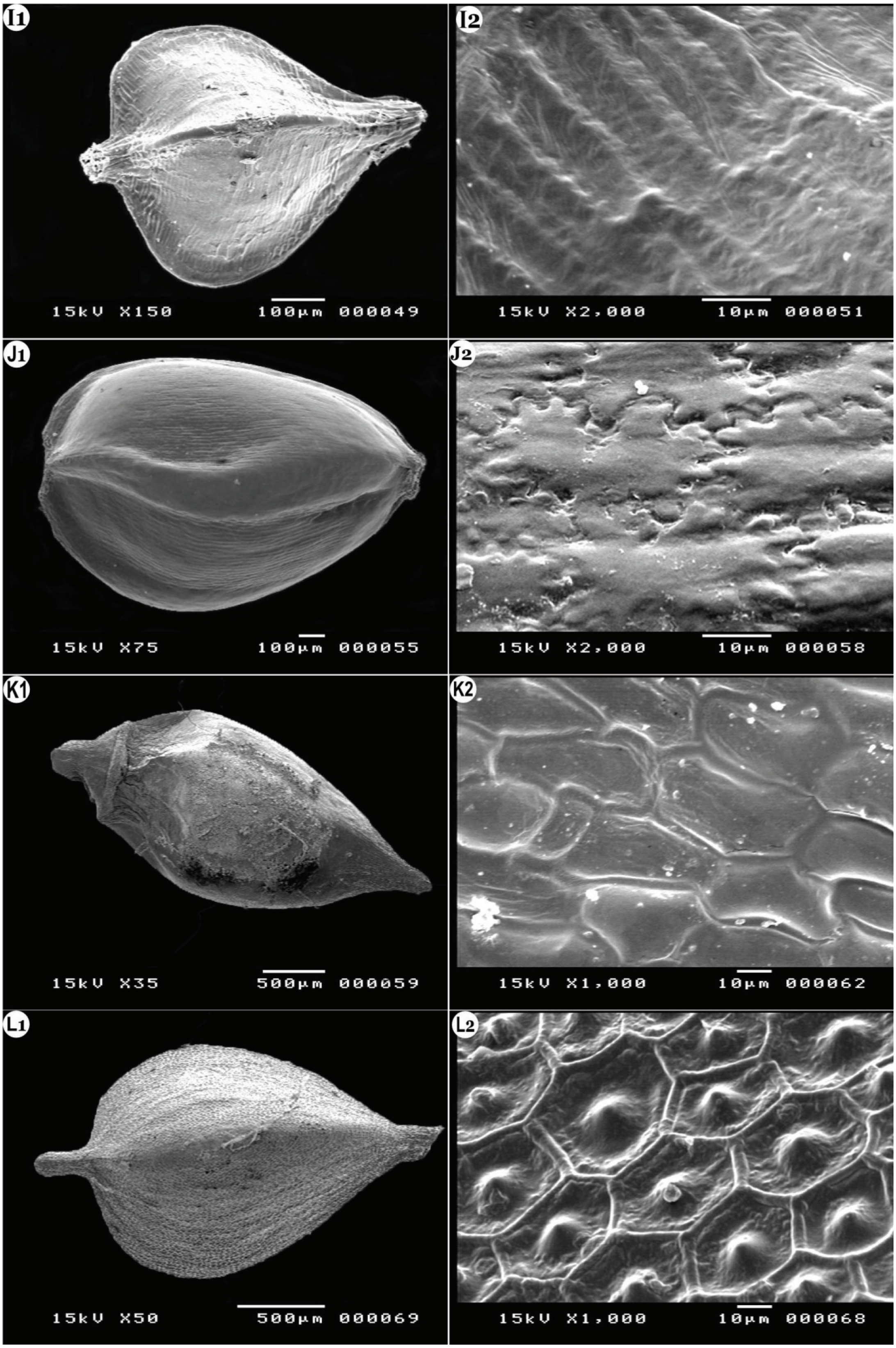

Figure 2 (cont.). SEM micrographs of nutlet. Figures E–H, 1) entire outline of nutlet, 2) enlargement of nutlet coat and bars indicate size outline. I) *Fuirena ciliaris*; J) *Schoenus nigricans*; K) *Cladium mariscus* subsp. *mariscus*; L) *Carex distans*.

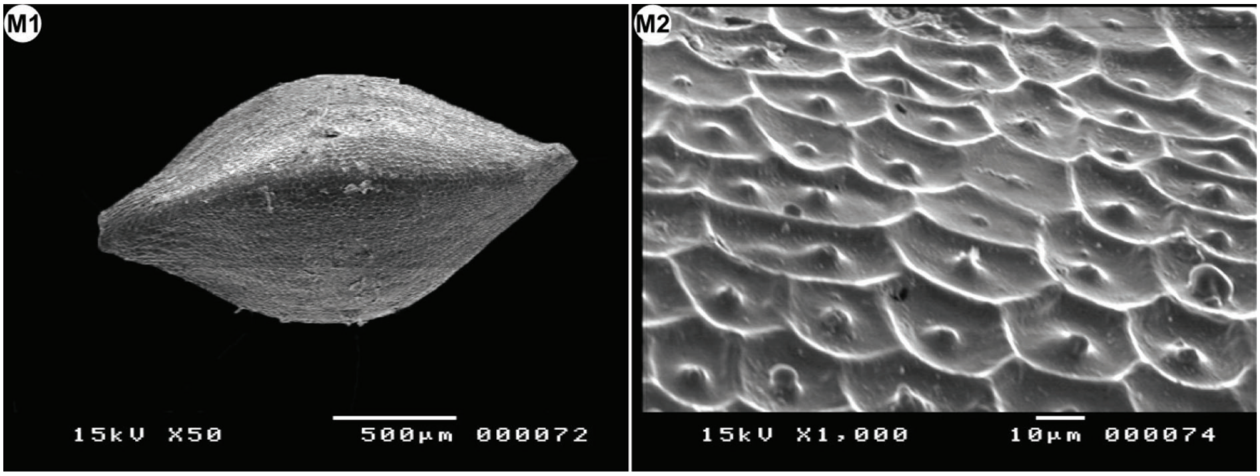

Figure 2. (Continued).

cluster C1 included 2 main groups (A and B) separated at a similarity level of 20.5%. Group A comprised of *Fimbristylis bisumbellata* and *F. sieberiana*; tribe Abildgaardieae) that was separated at a similarity level of 31.5%. Group B was represented by *Schoenoplectus litoralis* subsp. *thermalis* and *Schoenoplectiella supina* (tribe Fuireneae) with a similarity index 33%. The second cluster C2 comprised of four groups: C, D, E, and F. Group (C) included *Scirpoides holoschoenus* subsp. *australis* (tribe Cypereae), group (D) contained two subgroups: the first one included *Schoenus nigricans* and *Cladium mariscus* subsp. *mariscus* (tribe Schoeneae), the second subgroup comprised of *Carex distans*, and *C. extensa* (tribe Cariceae). Group (E) comprised of the members of *Fuirena* (*F. ciliaris* and *F. pubescens*; tribe Fuireneae) with a similarity index of 32.5%, while group (F) included *Bolboschoenus glaucus* and *Schoenoplectiella praelongata* (tribe Fuireneae).

3.6.2. Principal component analysis (PCA)

The plot of 13 OTU's on the first two principal component axes as shown in (Figure 4), explaining 41.43% of the total observed variation. On the first axis (21.16% of the total variation, Figure 4) segregation was clear between four groups. 1) Group of *Bolboschoenus glaucus, Schoenoplectiella praelongata, Fuirena ciliaris* and *F. pubescens.* 2) *Cladium mariscus* and *Schoenus nigricans.* 3) *Carex distans and C. extensa.* 4) *Scirpoides holoschoenus*. 5) *Fimbristylis bisumbellata* and *F. sieberiana.* The main characters explaining this segregation (characters with high factor loading ≥± 0.6) are ellipsoid digonous, trigonous, convex and biconvex nutlets; reticulate foveate nutlet surface; conical nutlet apex and deeply concave with colliculate projection periclinal cell wall (Table 5). On the second axis (20.27% of the total variation, Figure 4) revealed a split of *Schoenoplectus litoralis* subsp. *thermalis, Schoenoplectiella supina.* This group characterized by trigonous, digonous and biconvex nutlets; smooth or reticulate foveate nutlet surface; mucronate nutlet apex; short and narrow nutlet beak; raised, straight, thick, smooth anticlinal boundaries.

Key to the studied taxa based on nutlet characters

1a. Nutlet obovate 2
1b. Nutlet suborbicular, ellipsoid or fusiform 8
2a. Nutlet trigonous 3
2b. Nutlet digonous 7
3a. Nutlet length 2–2.5 mm 4
3b. Nutlet length 0.8–1.3 mm 5
4a. Nutlet glossy, surface smooth; epidermal cells undifferentiated; silica body absent .... *Bolboschoenus glaucus*
4b. Nutlet dull, surface reticulate-foveate; epidermal cells 6–8 gonals, mostly hexagonal, isodiametric; silica body present .... *Carex distans*
5a. Nutlet stipitate, convex, white to yellowish; epidermal cells oblong, 5–6 gonals, transversely longated 6
5b. Nutlet not stipitate, concave, dark brown; epidermal cells narrowly linear in longitudinal rows .... *Schoenoplectiella praelongata*
6a. Nutlet apex broadly obtuse; surface cracked; anticlinal wall straight, thin; periclinal wall flat, smooth ... . *Fuirena pubescens*
6b. Nutlet apex narrowly apiculate; surface rugulose; anticlinal wall undulate, thick; periclinal wall convex, fine verrucose .... *Fuirena ciliaris*
7a. Nutlet stipitate, dark brown, up to 2 mm long; .... *Schoenoplectus litoralis* subsp. *thermalis*
7b. Nutlet not stipitate, yellow, not exceed 1 mm long .... *Fimbristylis bisumbellata*
8a. Nutlet ellipsoid 9
8b. Nutlet suborbicular or fusiform 11
9a. Nutlet stipitate, gray to dark brown; beak present 10
9b. Nutlet not stipitate, white; beak absent .... *Schoenus nigricans*

Table 3. Characters and character states used in nutlets statistical analysis.

Character	Character states	Character	Character states
Achene shape	1. Obovate	Epidermal cell Shape	36. Undifferentiated
	2. Trigonous		37. Polygonal in longitudinal rows
	3. Plano-convex		38. Narrowly linear in longitudinal rows
	4. Digonous		39. Narrowly oblong in longitudinal rows
	5. Biconvex		40. Oblong, 5–6 gonals, transversely elongated
	6. Concave		41. Hexagonal, reticulate
	7. Suborbicular		42. Stare-shape
	8. Ellipsoid		43. Polygonal, elongated, reticulate
	9. Stipitate		44. 6–8 gonals, mostly hexagonal, isodiametric
	10. Convex		45. Polygonal, isodiametric
	11. Fusiform	Anticlinal boundaries	46. Undifferentiated
Surface topography	12. Smooth		47. Raised, straight, thick, fine verrucose
	13. Reticulate foveate		48. Raised, straight, thin, with very fine folded
	14. Rugulose		49. Raised, wavy, thick, with very fine ridges
	15. Trabeculate		50. Raised, straight, thick, smooth
	16. Cracked		51. Raised, straight, thick, rough
	17. Rugulose		52. Raised, straight, thick, wavy ribs between walls
Achene colour	18. Light brown, glossy		53. Raised, straight, thin, smooth
	19. Dark brown, glossy		54. Raised, wavy, thick. smooth
	20. Brown, dull		55. Sunken, wavy, thin, smooth
	21. Gray to blackish, glossy	Periclinal cell wall	56. Undifferentiated
	22. Yellow, glossy		57. Flat to concave, fine verrucose
	23. White to yellowish, glossy		58. Flat, smooth
	24. Yellow, dull		59. Flat, rough
	25. Creamy-white, glossy		60. Deeply concave, fine verrucose
Achene apex	26. Attenuate		61. Concave, smooth
	27. Apiculate		62. Convex, fine verrucose
	28. Mucronate		63. Flat to slightly convex, smooth
	29. Obtuse		64. Deeply concave with colliculate projection
	30. Conical	Silica body	65. Present
Achene beak	31. Short, narrow		66. Absent
	32. Long, narrow		
	33. Short, broad		
	34. Long, broad		
	35. Absent		

10a. Nutlet not exceeding 1 mm long; silica body absent .... *Scirpoides holoschoenus* subsp. *australis*

10b. Nutlet 1.5–2 mm long; silica body present .... *Carex extensa*

11a. Nutlet fusiform, stipitate, trigonous, convex; up to 3.5 mm length .... *Cladium mariscus* subsp. *mariscus*

11b. Nutlet suborbicular, not stipitate, digonous, biconvex; not exceeds 1.5 mm length 12

12a. Nutlet dark brown, rugulose with wavy rows bearing furrows and prominent ridges .... *Schoenoplectiella supina*

12b. Nutlet yellow, reticulate-foveate .... *Fimbristylis sieberiana*

Table 4. Data matrix based on 66 macro- and micromorphological character states of nutlets, taxa numbers as defined in Table 1.

Character	No.	Character state	Taxa												
			1	2	3	4	5	6	7	8	9	10	11	12	13
Achene shape	1	Obovate	1	1	1	0	0	1	0	1	1	0	0	1	0
	2	Trigonous	1	0	1	0	1	0	0	1	1	1	1	1	1
	3	Plano-convex	1	0	0	0	0	0	0	0	0	0	0	0	0
	4	Digonous	0	1	0	1	0	1	1	0	0	0	0	0	0
	5	Biconvex	0	1	0	1	0	1	1	0	0	0	0	0	0
	6	Concave	0	0	1	0	0	0	0	0	0	0	0	0	0
	7	Suborbicular	0	0	0	1	0	0	1	0	0	0	0	0	0
	8	Ellipsoid	0	0	0	0	1	0	0	0	0	1	0	0	1
	9	Stipitate	0	0	0	0	1	1	0	1	1	0	1	1	1
	10	Convex	0	0	0	0	1	0	0	1	1	1	1	1	1
	11	Fusiform	0	0	0	0	0	0	0	0	0	0	1	0	0
Surface topography	12	Smooth	1	0	0	0	0	0	0	0	0	1	0	0	0
	13	Reticulate foveate	0	1	0	0	1	0	1	0	0	0	1	1	1
	14	Rugulose, wavy rows bearing furrows and prominent ridges	0	0	1	1	0	0	0	0	0	0	0	0	0
	15	Trabeculate, sclariform between ribs	0	0	0	0	0	1	0	0	0	0	0	0	0
	16	Cracked	0	0	0	0	0	0	0	1	0	0	0	0	0
	17	Rugulose	0	0	0	0	0	0	0	0	1	0	0	0	0
Achene colour	18	Light brown, glossy	1	0	0	0	0	0	0	0	0	0	0	0	0
	19	Dark brown, glossy	0	1	1	1	0	0	0	0	0	0	1	0	1
	20	Brown, dull	0	0	0	0	0	0	0	0	0	0	0	1	0
	21	Gray to blackish, glossy	0	0	0	0	1	0	0	0	0	0	0	0	0
	22	Yellow, glossy	0	0	0	0	0	1	1	0	0	0	0	0	0
	23	White to yellowish, glossy	0	0	0	0	0	0	0	1	0	0	0	0	0
	24	Yellow, dull	0	0	0	0	0	0	0	0	1	0	0	0	0
	25	Creamy-white, glossy	0	0	0	0	0	0	0	0	0	1	0	0	0
Achene apex	26	Attenuate	1	0	1	0	0	1	0	0	0	0	0	0	0
	27	Apiculate	0	1	0	1	0	0	0	0	1	0	0	1	0
	28	Mucronate	0	0	0	0	1	0	1	0	0	0	0	0	0
	29	Obtuse	0	0	0	0	0	0	0	1	0	1	0	0	0
	30	Conical	0	0	0	0	0	0	0	0	0	0	1	0	1
Achene beak	31	Short, narrow	1	0	1	1	0	0	0	0	1	0	0	0	0
	32	Long, narrow	0	1	0	0	1	0	0	0	0	0	1	1	0
	33	Short, broad	0	0	0	0	0	1	1	1	0	0	0	0	0
	34	Long, broad	0	0	0	0	0	0	0	0	0	0	0	0	1
	35	Absent	0	0	0	0	0	0	0	0	0	1	0	0	0

Table 4. (Continued).

Epidermal cell shape	36	Undifferentiate	1	0	0	0	0	0	0	0	0	0	0	0	0
	37	Polygonal in longitudinal rows	0	1	0	0	1	0	0	0	0	0	0	0	0
	38	Narrowly linear in longitudinal rows	0	0	1	0	0	0	0	0	0	0	0	0	0
	39	Narrowly oblong in longitudinal rows	0	0	0	1	0	0	0	0	0	0	0	0	0
	40	Oblong, 5–6 gonals, transversely elongated	0	0	0	0	0	1	0	1	1	0	0	0	0
	41	Hexagonal, reticulate	0	0	0	0	0	0	1	0	0	0	0	0	0
	42	Stare-shape	0	0	0	0	0	0	0	0	0	1	0	0	0
	43	Polygonal, elongated, reticulate	0	0	0	0	0	0	0	0	0	0	1	0	0
	44	6–8 gonals, mostly hexagonal, isodiametric	0	0	0	0	0	0	0	0	0	0	0	1	0
	45	Polygonal, isodiametric	0	0	0	0	0	0	0	0	0	0	0	0	1
Anticlinal boundaries	46	Undifferentiate	1	0	0	0	0	0	0	0	0	0	0	0	0
	47	Raised, straight, thick, fine verrucose	0	1	0	0	0	0	0	0	0	0	0	0	0
	48	Raised, straight, thin, with very fine folded	0	0	1	0	0	0	0	0	0	0	0	0	0
	49	Raised, wavy, thick, with very fine ridges	0	0	0	1	0	0	0	0	0	0	0	0	0
	50	Raised, straight, thick, smooth	0	0	0	0	1	0	1	0	0	0	1	0	1
	51	Raised, straight, thick, rough	0	0	0	0	0	0	0	0	0	0	0	1	0
	52	Raised, straight, thick, wavy ribs between walls	0	0	0	0	0	1	0	0	0	0	0	0	0
	53	raised, straight, thin, smooth	0	0	0	0	0	0	0	1	0	0	0	0	0
	54	Raised, wavy, thick, smooth.	0	0	0	0	0	0	0	0	1	0	0	0	0
	55	Sunken, wavy, thin, smooth	0	0	0	0	0	0	0	0	0	1	0	0	0
Periclinal cell wall	56	Undifferentiate	1	0	0	0	0	0	0	0	0	0	0	0	0
	57	Flat to concave, fine verrucose	0	1	0	0	0	0	0	0	0	0	0	0	0
	58	Flat, smooth	0	0	1	0	0	0	1	1	0	0	1	0	0
	59	Flat, rough	0	0	0	1	0	0	0	0	0	0	0	0	0
	60	Deeply concave, fine verrucose	0	0	0	0	1	0	0	0	0	0	0	0	0
	61	Concave, smooth	0	0	0	0	0	1	0	0	0	0	0	0	0
	62	Convex, fine verrucose	0	0	0	0	0	0	0	0	1	0	0	0	0
	63	Flat to slightly convex, smooth	0	0	0	0	0	0	0	0	0	1	0	0	0
	64	Deeply concave with colliculate projection	0	0	0	0	0	0	0	0	0	0	0	1	1
Silica body	65	Present	0	0	0	0	0	0	0	0	0	0	0	1	1
	66	Absent	1	1	1	1	1	1	1	1	1	1	1	0	0

4. Discussion

Despite recent advances in molecular phylogenetic investigations, deep evolutionary relationships in Cyperaceae are still not entirely resolved. Table 6 showed the most effective and important taxonomic treatment carried out on the studied taxa either on the morphological level (Bruhl, 1995; Goetghebeur, 1998) or on DNA sequencing data (Muasya et al., 2009). Generally, Bruhl (1995) recognized two subfamilies, Cyperoideae and Caricoideae, while Goetghebeur (1998) recognized two other subfamilies, Sclerioideae and Mapanioideae, both of which were previously included in Caricoideae by Bruhl (1995). Muasya et al. (2009) did not support the recognition of Caricoideae and Sclerioideae as subfamilies separated from Cyperoideae *sensu* Goetghebeur (1998). According to Stevens[4], Simpson et al. (2003), and Muasya et al. (2009), the family Cyperaceae included two subfamilies: Mapanioideae CB Clarke and Cyperoideae Beilschmied.

[4] Stevens P (2001 onwards). Angiosperm Phylogeny Website, version 14 [online]. Website http://www.mobot.org/MOBOT/research/APweb/ [accessed 23 February 2018].

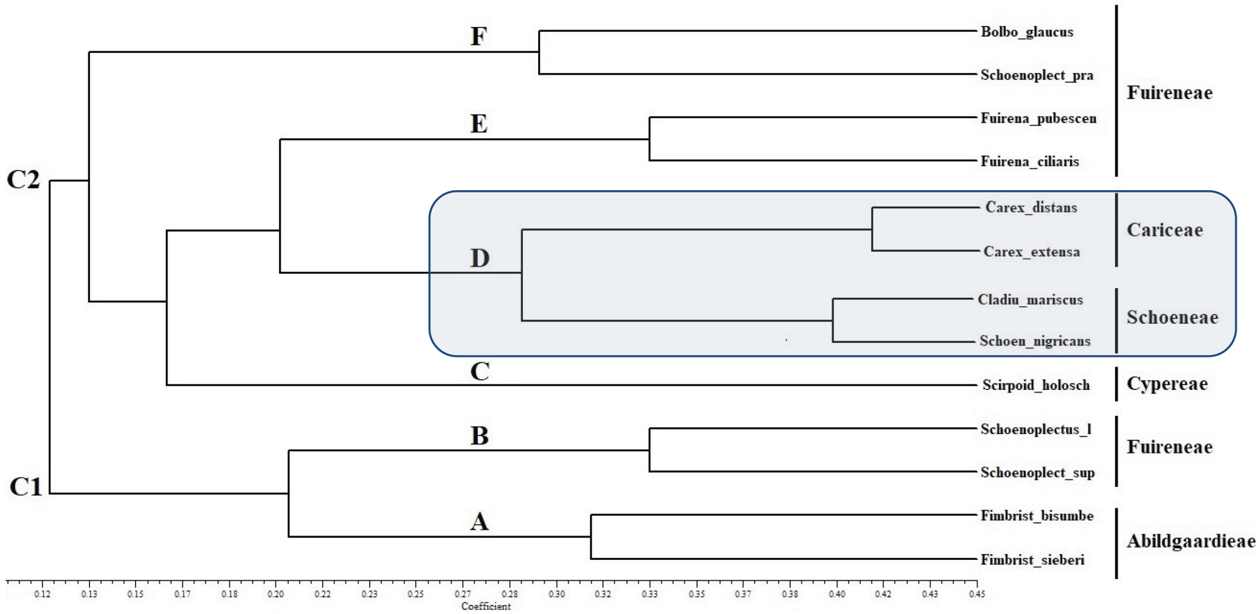

Figure 3. UPGMA dendrogram of the 13 taxa of Cyperoideae based on 66 nutlet morphological characters.

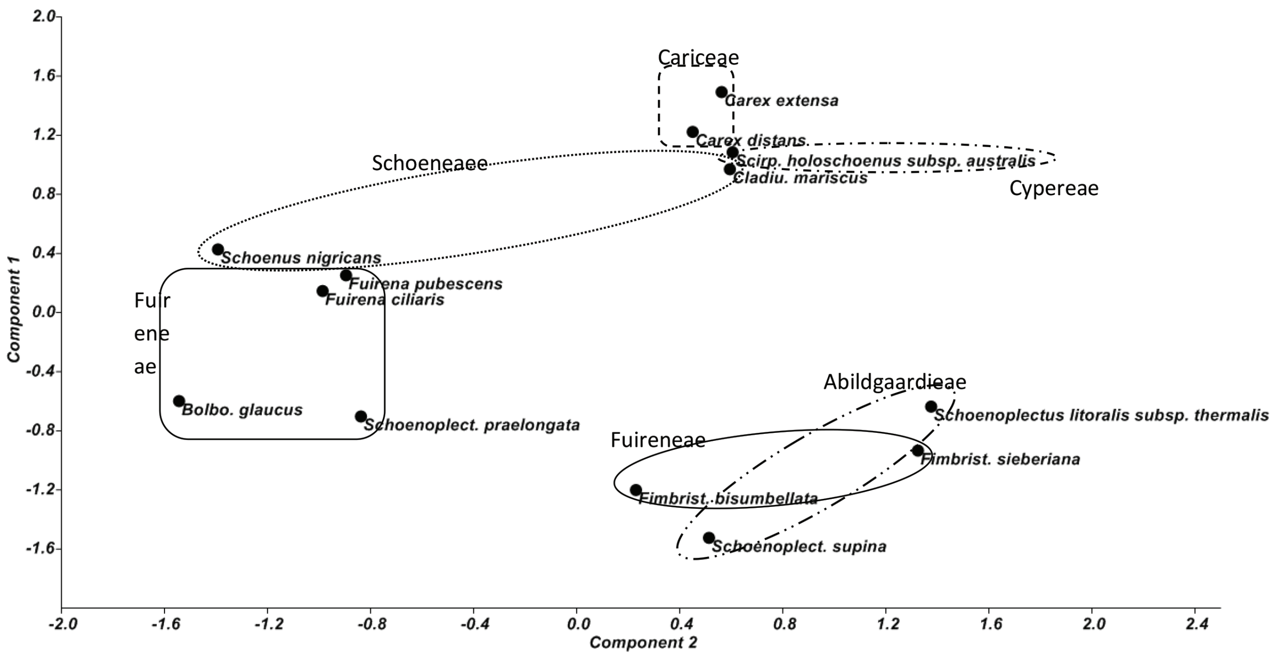

Figure 4. Scatter plot of the 13 OTUs of Cyperoideae plotted against the first principal component by the second principal component based on 66 nutlet morphological characters.

The treatments also differed in tribal circumscription; tribal concepts in subfamily Cyperoideae have changed over the years. Many legitimate tribal names have been reported in Cyperoideae, including Scirpeae, Cypereae, Fuireneae, Schoenoplecteae, Ficinieae, Abildgaardieae, Eleocharideae, and Lipocarpheae (Goetghebeur, 1985).

Bruhl (1995) recognized 12 tribes and treating Scirpeae broadly to include taxa classified in tribes Dulicheae, Fuireneae, Eleocharideae and Cypereae *sensu* Goetghebeur (1998); while Stevens[5] recognized 19 tribes within Cyperoideae. In the current study, we followed the taxonomic treatment of Muasya et al. (2009) in which 9

[5] Stevens P (2001 onwards). Angiosperm Phylogeny Website, version 14 [online]. Website http://www.mobot.org/MOBOT/research/APweb/ [accessed 23 February 2018].

Table 5. Macro and micromorphological nutlet characters on the first two principal components axes showing highest factor loading, factor loading values ≥ ± 0.6 are indicated with an asterisk.

No.	Character	Character states	Principal components Factor loading PC1	 PC2
1	Nutlet shape	Obovate	−0.38	−0.40
2		Trigonous	0.69*	−0.66*
3		Plano−convex	−0.21	−0.49
4		Digonous	−0.69*	0.66*
5		Biconvex	−0.69*	0.66*
6		Concave	−0.24	−0.29
7		Suborbicular	−0.44	0.46
8		Ellipsoid	0.62*	−0.04
9		Stipitate	0.48	0.41
10		Convex	0.87*	−0.19
11		Fusiform	0.31	0.18
12	Surface topography	Smooth	−0.03	−0.63*
13		Reticulate foveate	0.60*	0.70*
14		Rugulose, wavy rows bearing furrows and prominent ridges	−0.47	−0.14
15		Trabeculate, sclariform between ribs	−0.41	0.22
16		Cracked	0.01	−0.17
17		Rugulose	−0.01	−0.31
18	Nutlet colour	Light brown, glossy	−0.21	−0.49
19		Dark brown, glossy	−0.02	0.22
20		Brown, dull	0.34	−0.01
21		Gray to blackish, glossy	0.34	0.19
22		Yellow, glossy	−0.44	0.55
23		White to yellowish, glossy	0.01	−0.17
24		Yellow, dull	−0.01	−0.31
25		Creamy-white, glossy	0.16	−0.36
26	Nutlet apex	Attenuate	−0.54	−0.35
27		Apiculate	−0.15	0.04
28		Mucronate	−0.17	0.60*
29		Obtuse	0.13	−0.39
30		Conical	0.60*	0.22
31	Nutlet beak	Short, narrow	−0.49	−0.60*
32		Long, narrow	0.46	0.38
33		Short, broad	−0.37	0.37
34		Long, broad	0.49	0.11
35		Absent	0.16	−0.36

Table 5. (Continued).

36	Epidermal cell shape	Undifferentiate	−0.21	−0.49
37		Polygonal in longitudinal rows	0.10	0.36
38		Narrowly linear in longitudinal rows	−0.24	−0.29
39		Narrowly oblong in longitudinal rows	− 0.40	0.10
40		Oblong, 5–6 gonals, transversely elongated	−0.26	−0.17
41		Hexagonal, reticulate	−0.19	0.53
42		Stare-shape	0.16	−0.36
43		Polygonal, elongated, reticulate	0.31	0.18
44		6–8 gonals, mostly hexagonal, Isodiametric	0.34	−0.01
45		Polygonal, isodiametric	0.49	0.11
46	Anticlinal boundaries	Undifferentiate	−0.21	−0.49
47		Raised, straight, thick, fine verrucose	−0.19	0.29
48		Raised, straight, thin, with very fine folded	−0.24	−0.29
49		Raised, wavy, thick, with very fine ridges	−0.40	0.10
50		Raised, straight, thick, smooth	0.54	0.60*
51		Raised, straight, thick, rough	0.34	−0.01
52		Raised, straight, thick, wavy ribs between walls	−0.41	0.22
53		Raised, straight, thin, smooth	0.01	−0.17
54		Raised, wavy, thick, smooth.	−0.01	−0.31
55		Sunken, wavy, thin, smooth	0.16	−0.36
56	Periclinal cell wall	Undifferentiate	−0.21	−0.49
57		Flat to concave, fine verrucose	−0.19	0.29
58		Flat, smooth	−0.16	0.30
59		Flat, rough	−0.40	0.10
60		Deeply concave, fine verrucose	0.34	0.19
61		Concave, smooth	−0.41	0.22
62		Convex, fine verrucose	−0.01	−0.31
63		Flat to slightly convex, smooth	0.16	−0.36
64		Deeply concave with colliculate projection	0.61*	0.08
65	Silica body	Present	0.61*	0.08
66		Absent	−0.61*	−0.08
Percentage per PCO			21.16	20.27

Percentage total variation for the first two principal components is 41.43 %.

genera within 6 tribes belonging to subfamily Cyperoideae were presented (Table 7).

4.1. Tribe Abildgaardieae Lye (1973), (group A)

Tribe Abildgaardieae (subfamily Cyperoideae) was first recognized informally as Fimbristylideae by Reichenbach (1828) which was published formally by Raynal (1978), but in the meantime, name Abildgaardieae was adopted by Lye (1973) for the tribe. According to Stevens[6], the tribe composed of seven genera and about 540 species of which *Fimbristylis* includes 315 spp.

According to Vahl (1805), genus *Fimbristylis* was segregated from the genus *Scirpus*. Different authors carried out the nutlet micromorphological studies of *Fimbristylis*, as a potential systematic method to the taxonomic recognition of different sections in the genus. The genus *Fimbristylis* represented in the flora of Egypt by two species: *F. sieberiana* and *F. bisumbellata* (El Habashy et al., 2005). Based on 66 morphological characters of the nutlet, our UPGMA results (Figure 3) placed the genus *Fimbristylis* in the base of the dendrogram with a close

[6] Stevens P (2001 onwards). Angiosperm Phylogeny Website, version 14 [online]. Website http://www.mobot.org/MOBOT/research/APweb/ [accessed 23 February 2018].

Table 6. The studied genera in the previous works.

<table>
<tr><td rowspan="2">Studies Genus</td><td colspan="2">Bruhl (1995)</td><td colspan="2">Goetghebeur (1998)</td><td colspan="2">Muasya et al. (2009)</td></tr>
<tr><td>Subfamily</td><td>Tribe</td><td>Subfamily</td><td>Tribe</td><td>Subfamily</td><td>Tribe</td></tr>
<tr><td>Bolboschoenus</td><td rowspan="6">Cyperoideae</td><td rowspan="5">Scirpeae</td><td rowspan="8">Cyperoideae</td><td rowspan="4">Fuireneae</td><td rowspan="9">Cyperoideae</td><td rowspan="4">Fuireneae</td></tr>
<tr><td>Schoenoplectus</td></tr>
<tr><td>Schoenoplectiella</td></tr>
<tr><td>Fuirena</td></tr>
<tr><td>Scirpoides</td><td>Cypereae</td><td>Cypereae</td></tr>
<tr><td>Fimbristylis</td><td>Abildgaardieae</td><td>Abildgaardieae</td><td>Abildgaardieae</td></tr>
<tr><td>Schoenus</td><td rowspan="3">Caricoideae</td><td rowspan="2">Schoeneae</td><td rowspan="2">Schoeneae</td><td rowspan="2">Schoeneae</td></tr>
<tr><td>Cladium</td></tr>
<tr><td>Carex</td><td>Cariceae</td><td>Caricoideae</td><td>Cariceae</td><td>Cariceae</td></tr>
</table>

relationship to some members of tribe Fuireneae. Inside the cluster (group A), the two species of *Fimbristylis* were recognized with approximately 31% morphological similarity, which can be clearly defined on the basis of some nutlet characters: glossy yellow, bigonous, biconvex, and broadly short beak (Figures 1F and 1G). Our results were matching to those of Patil and Prasad (2016), where they indicated that the color of the fully matured nutlets is dependable in the identification of *Fimbristylis* species. The surface topography of the *Fimbristylis* nutlets differs from species to species or at an infraspecific level (Wujek et al., 1992; Menapace et al., 2003).

Within the taxa of *Fimbristylis*, nutlet surface was reticulate when the epidermal cells were hexagonal or roundish, and lineolate when the epidermal cells were linear (Patil and Prasad, 2016). Our SEM results proved that the micromorphological characters of the nutlets were sufficient to discriminate *F. sieberiana* from *F. bisumbellata* in *F. sieberiana*, the nutlet was suborbicular, not stipitate, apex mucronate, epidermal cells hexagonal, reticulate, periclinal wall flat; while in *F. bisumbellata* was obovate, stipitate, apex attenuate, epidermal cells 5–6 gonals, oblong; periclinal wall concave (Figures 2F and 2G).

4.2. Tribe Cypereae Dumort (1829), (group C)

Cypereae forms one of the most diverse lineages of Cyperaceae, its members are cosmopolitan, occur in almost tropical wetland habitats (Reynders et al., 2011). It comprises 900 species in 19 genera (Muasya et al., 2009).

Based on the morphological data, the two latest classifications of Cyperoideae, have differed in assignment of genera in Cypereae: Goetghebeur (1998) placed all taxa characterized by *Cyperus*-type embryo in Cypereae, while Bruhl (1995) classified the genera with spiral glume order (i.e. *Ficinia, Isolepis*, *Scirpoides*, *Desmoschoenus*, *Oxycaryum* and *Kyllingiella*) in Scirpeae. Sojak (1972) segregate the genus *Scirpoides* from the genus *Scirpus,* but it has not received a full acceptance, for example, De Filipps (1980) maintained the use of genus *Scirpus* ignoring the segregation of Sojak (1972). Nevertheless, Goetghebeur (1986) and Bruhl (1990), reviewed the generic limits within Cyperaceae, recognized *Scirpoides*, but with the reservation, that additional study of its limits and constituent species is required. The genus *Scirpoides* represented in the flora of Egypt by one taxon: *S. holoschoenus* subsp. *australis*. The nomenclature of *S. holoschoenus,* has been subjected to considerable confusion in the literature, as too much weight being given to the number of flower heads (Desfayes, 2004). While Goetghebeur (1986) and Bruhl (1990) utilized the combination *S. holoschoenus* and used this species to help exemplify characteristics of the genus. Our UPGMA results (Figure 3) placed *S. holoschoenus* in a separate subclade (group C) with a close relationship to some members of tribes Cariceae, Schoeneae (group D) and Fuireneae (group E). According to Pignotti and Mariotti (2004), the micromorphological characters such as the reproductive structures are considered significant in taxonomic revisions on *Scirpus*. Our SEM results (Figures 1E and 2E) showed that the micromorphological characters of *S. holoschoenus* nutlets were matching with the studies of (Pignotti, 2003; Pignotti and Mariotti, 2004).

4.3. Tribe Cariceae Kunth ex Dumort (1827), (group D)

Tribe Cariceae includes approximately 2100 species of worldwide distribution[7], comprises nearly half of all the species found in the large and taxonomically complex Cyperaceae (Starr et al., 2008). Because of the numerous taxonomic problems that surround the genus

[7] Stevens P (2001 onwards). Angiosperm Phylogeny Website, version 14 [online]. Website http://www.mobot.org/MOBOT/research/APweb/ [accessed 23 February 2018].

Table 7. Tribes and synonyms of the studied taxa in subfamily Cyperoideae.

Tribe According to Muasya et al. (2009)	Taxon	Synonyms
Fuireneae	*Bolboschoenus glaucus* (Lam.) S.G. Smith	*Scirpus tuberosus* Desf. *S. glaucus* Lam. *S. maritimus* L. *S. maritimus* L. var. *tuberosus* (Desf.) Roem. & Schult.
	Schoenoplectus litoralis (Schrad.) Palla subsp. *thermalis* (Trab.) S. Hooper	*Scirpus litoralis* Schrad. *S. litoralis* Schrad. subsp. *thermalis* (Trab.) Murbeck. *S. thermalis* Trab. *S. subulatus* Vahl *S. pectinatus* Roxb. *S. wardianus* J. R. Drummond
	Schoenoplectiella praelongata (Poir.) Lye	*Schoenoplectus praelongatus* (Poir.) J. Raynal *Scirpus praelongatus* Poir.
	Schoenoplectiella supina (L.) Lye	*Schoenoplectus supinus* (L.) Palla *Scirpus supinus* L. *Isolepis supine* (L.) R. Br.
	Fuirena ciliaris (L.) Roxb.	*Scirpus ciliaris* L.
	Fuirena pubescens (Poir.) Kunth	*Carex pubescens* Poir. *Scirpus pubescens* (Poir.) Lam.
Cypereae	*Scirpoides holoschoenus* (L.) Soják subsp. *australis* (L.) Soják	*Scirpus holoschoenus* L. *S. australis* L. *S. holoschoenus* L. var. *australis* (L.) Sm. *Holoschoenus australis* (L.) Rchnb. *H. vulgaris* Link var. *australis* Nyman *H. romanus* (L.) Fritsch var. *australis* (L.) Bech.
Cariceae	*Carex distans* L.	*Carex sinai* Boott *C. sinaica* Nees in Steud.
	Carex extensa Good.	*C. arcuata* Wahlenb. *C. nervosa* Desf.
Abildgaardieae	*Fimbristylis bisumbellata* (Forssk.) Bubani	*Scirpus bisumbellatus* Forssk. *Fimbristylis dichotoma* (L.) Vahl
	Fimbristylis sieberiana Kunth	*Fimbristylis ferruginea* (L.) Vahl var. *sieberiana* (Kunth) Boeck. *F. ferruginea* (L.) Vahl subsp. *sieberiana* (Kunth) Lye
Schoeneae	*Schoenus nigricans* L.	*Chaetospora nigricans* (L.) Kunth
	Cladium mariscus (L.) Pohl subsp. *mariscus*	*Schoenus mariscus* L. *Cladium mariscus* (L.) Pohl subsp. *martii* (Roem. & Schult.) Egor. *Isolepis martii* Roem. & Schult. *Cladium mariscus* (L.) Pohl subsp. martii (Roem. & Schult.) Kük. *C. grossheimii* Pobed.

Carex, several authors studied the role of nutlets surface ornamentation so as to distinguish among the species (Starr and Ford, 2001; Jin and Zheng, 2013).

In addition, wealth of studies demonstrated the significance of silica body features that can be used to identify sections, subsections and species of the genus *Carex* (Menapace et al., 1986; Wujek and Menapace, 1986). Our results coincide with previous studies and showed the presence of *Carex* members (*C. distans*, and *C. extensa*) in a subgroup of cluster C2, group D (Figure 3). The genus *Carex* is distinguished from other studied taxa by its silica bodies on the nutlet surface (Figures 2L2 and 2M2). The similarity index between both species *C. distans*, and *C. extensa* reached 41.5%, where they shared some morphological characters such as raised anticlinal cell boundaries, deeply concave periclinal cell wall, and reticulate foveate surface topography. On the other hand, *C. distans* differed mainly from *C. extensa* in the shape and apex of the nutlets: obovate, apiculate in the former; and ellipsoid, conical in the latter (Figures 1L and 1M), respectively.

4.4. Tribe Schoeneae Dumort (1827), (group D)

The generic boundaries within tribe Schoeneae have long been contentious due to the high levels of its morphological variation Bruhl (1995). There have been several modifications in generic circumscription and new genera described in Schoeneae since Goetghebeur (1998) who provided generic concepts for the family Cyperaceae. According to Govaerts and Simpson (2007) tribe Schoeneae (approximately 450 species) is distributed throughout the southern continents, with particularly high endemism in South Africa and Australia. The Schoeneae, that previously known as Rhynchosporeae (Hooper, 1973) was treated (as Rhynchosporeae) in the subfamily of Scirpeae by Bentham (1883), while Pax (1887) moved the tribe to the subfamily Caricoideae based on the sympodial structure of the spikelet. Later, Goetghebeur (1998) and Muasya et al. (2009) placed tribe Schoeneae in subfamily Cyperoideae based on some morphological and molecular characters. However, Rhynchosporeae (with only one genus) was treated as a separate tribe by Stevens[8]. The molecular studies of (Verboom, 2006; Muasya et al., 2009) showed that Schoeneae, as defined by both Bruhl (1995) and Goetghebeur (1998), is not monophyletic (Viljoen et al., 2013). Tribe Schoeneae represented in the flora of Egypt by two genera: *Cladium* and *Shoenus* (El Habashy et al., 2005). Our results revealed the clustering of both genera together in a subgroup of cluster C2 (Group D) with a close clustering to the members of tribe Cariceae. The similarity index between both taxa (*Cladium mariscus* subsp. *mariscus* and *Schoenus nigricans*) reached 40%; they share some morphological characters such as convex trigonous nutlet (Figures 1J and 1K). On the other hand, *S. nigricans* was the only species that have a stare-shape epidermal cell (Figure 2J2). In addition, *C. mariscus* had the largest nutlet size with 3.5 × 1.5 mm. Bruhl (1995) placed tribes Schoneae and Cariceae in subfamily Caricoideae, while Goetghebeur (1998) transferred tribe Schoneae to subfamily Cyperoideae. Later, Muasya et al. (2009) moved both tribes Schoneae and Cariceae to subfamily Cyperoideae ignoring the presence of subfamily Caricoideae. In our UPGMA results (Figure 3), the taxa of Schoeneae are placed as a clade sister to Cariceae members (Group D). Therefore, the authors highly support the classification of Bruhl (1995) in which the tribes Schoneae and Cariceae should be placed in subfamily Caricoideae.

4.5. Tribe Fuireneae Reichenb. ex Fenzl (1836), (groups B, E, and F)

Bruhl (1995) studied the phylogeny of Cyperaceae based on some morphological and anatomical characters, yet he did not recognize the tribe Fuireneae that was previously proposed by (Goetghebeur, 1986). Later, Goetghebeur (1998), Muasya et al. (2009) and Glon et al. (2017) recognized six genera within tribe Fuireneae (Govaerts et al., 2015). The tribe Fuireneae is represented in the flora of Egypt by four genera i.e. *Bolboschoenus*, *Fuirena*, *Schoenoplectus* and *Schoenoplectiella* (El Habashy et al., 2005). Morphometric analysis based on nutlet morphological characters revealed that the studied taxa of tribe Fuireneae are not clade together. Whereas, *Schoenoplectus litoralis* subsp. *thermalis* and *Schoenoplectiella supina* placed together (cluster 1, group B); *Fuirena ciliaris* and *F. pubescens* (cluster 2, group E), *Bolboschoenus glaucus* and *Schoenoplectiella praelongata* (cluster 2, group F). Our results were in line with all the recent molecular phylogenetic studies (Shiels et al., 2014; Spalink et al., 2016; Semmouri et al., 2018) which suggested that Fuireneae is a paraphyletic tribe. As shown in UPGMA dendrogram, some members of Fuireneae being sister to Abildgaardieae members (groups A and B), others close to members of Cypereae (groups C, E, F). Many molecular and morphological accounts suggested the paraphyletic status of Fuireneae to be closely related to Abildgaardieae and Cypereae (Goetghebeur, 1986; Muasya et al., 2009). In general, the genus *Schoenoplectus* has been widely accepted in different taxonomic accounts (Pignotti, 2003; Egorova, 2005). On the other hand, Lye (2003) described a new genus *Schoenoplectiella*, to accommodate species which was formerly placed in *Schoenoplectus*. Both *Schoenoplectiella* and *Schoenoplectus* can be easily distinguished from each other by some nutlet morphological characters, whereas, the surface topography was reticulate to foveate in *Schoenoplectus* and rugulose with wavy rows bearing furrows ridges in *Schoenoplectiella*. In addition, the epidermal cells were polygonal in *Schoenoplectus* and being narrowly oblong to linear in *Schoenoplectiella* (Figures 2B–2D) respectively. These results were in line with those of Hayasaka (2012). The numerical analysis showed that the two taxa of *Schoenoplectiella* (*S. supina* and *S. praelongata*) were placed apart from each other, where *S. supina* was grouped with *Schoenoplectus litoralis* subsp. *thermalis* (cluster 1, group B), and *S. praelongata* with *Bolboschoenus glaucus* (cluster 2, group F). According to Wollstonecroft et al. (2011), nutlets of *B. glaucus* were obovate to elliptic in outline, plano-convex to subtrigonous in cross-section, and have a smooth surface. Our SEM results supported

[8] Stevens P (2001 onwards). Angiosperm Phylogeny Website, version 14 [online]. Website http://www.mobot.org/MOBOT/research/APweb/ [accessed 23 February 2018].

these findings. Unlike other genera of Fuireneae, the morphological characters of *Fuirena* have been considered so distinct and homogeneous within Cyperaceae (Van der Veken, 1965). In the present study, *Fuirena ciliaris* and *F. pubescens* clustered together (group E) with a similarity index reached 32.5%.

5. Conclusion

The nutlets of subfamily Cyperoideae have adequate epidermal micromorphological characteristics to warrant their use as a taxonomic aid. Characters of systematic interest included the epidermal cells shape, configuration of the anticlinal cell boundaries, the periclinal of the cell wall as well as the presence or absence of silica bodies. Unquestionably, these characters will be of assistance at the species level. Their significant value, however, may be greater in assessing the infrageneric classification. The results of this study approved the heterogeneity of the subfamily Cyperoideae, and provided additional support to the existing recognition as natural taxa of the genera *Scirpus*, *Bolboschoenus*, *Scirpoides*, *Schoenoplectus,* and *Schoenoplectiella*. The developmental variations of nutlets were worthy to be taken into account, not only because it gives a better understanding of sculpture development, but also for preparing an identification key. It would be very useful if further studies using pollen grains, stem and leaf anatomy, and molecular data as tools to clarify the infrageneric, specific and infraspecific classification of the Egyptian taxa.

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