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Pollen studies of some species in six genera of the family Cyperaceae from Ile-Ife, South West, Nigeria.

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Abstract

This study was undertaken to investigate the pollen diversity and possible delimitation of 24 species from six genera of the family Cyperaceae based on pollen morphology. The pollens were acetolysed using standard methods and viewed under the light microscope. The quantitative and qualitative pollen characters were documented. The quantitative data were subjected to PCA and SCLA analysis. From this study, it was documented that quantitative characters have little taxonomic value and cannot be solely relied upon for identifying a particular genus in the family Cyperaceae. However, species in a genus can be distinctly identified using qualitative characters such as pollen shapes and ornamentation though only a few of the species studied were ornamented. The study revealed the pollen diversity among the members of the family studied and concluded that even though palynological studies using the light microscope may not be used alone in delimiting the species, it can be used to identify distinct species in the family.

Keywords: Cyperus; Fimbristylis; Kyllinga; Mariscus; Pycreus; Ornamentation; Pollen shape

Introduction

The family Cyperaceae (sedges) consists of about 5500 species in 109 genera (Govaerts *et al.*, 2007; Pal and Choudhury, 2017) and the eleventh most populous among the Angiosperm families and the third among the monocotyledons (Goetghebeur, 1998; Simpson *et al.*, 2003). The family was grouped into two subfamilies, namely: Mapanioideae and Cyperoideae, based on DNA data (Simpson *et al.*, 2008; Muasya *et al.*, 2009; Coan *et al.*, 2010). However, Bruhl (1995) divided the family into two different subfamilies (Cyperoideae and Caricoideae) with 12 tribes, while it was classified by Goetghebeur (1998) into four subfamilies. In terms of ancestry, Cyperaceae is believed to have originated from Juncaceae (rushes) because of their close relationship (Dahlgren *et al.*, 1985, Duvall *et al.*, 1993; Plunkett *et al.*, 1995).

The members of the family are found in different habitats in the tropical and Arctic regions, although their preference for wetlands cannot be over-emphasized (Simpson *et al.*, 2003). Most of the time, they have restricted habitat preferences; hence they tend to be rare and endangered. However they are very important as phytoindicators of site properties since their tendency to have narrow ecological amplitudes makes them readily responsive to any change in environmental factors such as soil acidity or water chemistry (Simpson *et al.*, 2003).

There are many genera in the family Cyperaceae which have been delimited by their morphological and floral characters. However, these two characteristics are common among the members of the family such that identification may sometimes be difficult within a genus.

In a research work conducted by Padhye (1972), the morphological, embryological, and palynological results support the retention of *Kyllinga* as a genus distinct from *Cyperus*.

Palynological studies in Cyperaceae have been of interest for quite a while, mainly because of the occurrences of pseudomonads that result from simultaneous microsporogenesis, which is only found in one tribe, Styphelieae of the family Eriaceae (Smith-White, 1959; Kawarase and Kunjalwar, 2016a). Meanwhile, two pollen types were reported in Cyperaceae; mapania and pseudomonad (Simpson *et al.*, 2003). Rocha *et al.* (2017) carried out a study to understand the process of pollen formation in Cyperaceae. They surmised that the late meiosis cytokinesis and precocious pollen Mitosis I seem to be associated with pseudomonad formation after which vacuolation occurs with continuous autophagy of degenerative microspores. This suggests that the death of three microspores is an advantage such that the functional microspore that remains is fit. This is because reserves are accumulated as starch, depending on environmental conditions. The resulting pollens are therefore referred to as pseudomonads, and their establishment allows the formation of a longevous, rapidly germinating, tricellular pollen, which could be key to the great adaptive success of Cyperaceae (Coan *et al.*, 2010; Rocha *et al.* 2017).

Peripheral pollen arrangement has been confirmed to be predominant in Cyperaceae, and central pollen arrangement was also reported (Kirpes *et al.*, 1996). Different pollen morphologies have been described by various researchers in the family, which include spheroidal, oblate spheroidal, sub oblate, perprolate, apple and pear-shaped pollens (Nagels *et al.*, 2009; Kawarase and Kunjalwar, 2016a; 2016b; Sosam and Al-mayyahi, 2018). In addition, apolar, heteropolar and tetracolporate pollens have been recognized in the family (Nagels *et al.*, 2009; Kawarase and Kunjalwar, 2016a; 2016b; Butt *et al.*, 2018; Sosam and Al-mayyahi, 2018). Moreover, Cyperaceae pollens are characterized by orbicules (Nagels *et al.*, 2009). The pollen morphology in Cyperaceae was said to be taxonomically useful and has played a vital role in identification at species level up to subgeneric and generic levels. Furthermore, it has been employed in elucidating genetic evolutionary relationships among species of interest (Wronska-Pilaresk *et al.*, 2010; Butt *et al.*, 2018; Sosam and Al-mayyahi, 2018). There is a strong correlation between pollen types and phylogenetic relationships (Grimsson *et al.*, 2017).

Many authors have described the pollen morphologies of the family Cyperaceae and variations among them have been pointed out. However, there is no known report on the pollens of the members of this family from Nigeria. This study was carried out to assess the pollen diversity among some species in Cyperaceae from Ile-Ife, South-West, Nigeria.

Materials and Methods

Whole plants of 24 species from 6 genera were collected from different locations in Ile-Ife, Osun State, Nigeria (7°27" 7.49N, 4° 34" 4.56E) and were identified at IFE herbarium. The six genera are: *Cyperus* L. (12), *Kyllinga* Rottb. (5), *Mariscus* Vahl (2), *Fimbristylis* Vahl (2), *Rhynchospora* Vahl (1) and *Pycreus* P. Beauv (2). The species were planted in buckets and allowed to mature. Mature spikelets were collected from each species and stored in 70% ethanol for palynological studies.

Pollen acetolysis was carried out according to Erdtman's method used by Azeez *et al.* (2019). The polliniferous material in the 70% alcohol was crushed with a glass rod for 2-4 minutes, shaken well and sieved through a fine mesh into centrifuge tubes. Pollen material was centrifuged at 3000 rpm for 3 minutes, and the ethanol was decanted. Five (5) ml glacial acetic acid was added to the residue, again centrifuged and decanted. The acetolysis mixture was prepared by taking nine parts of acetic anhydride and one part concentrated sulphuric

acid, added drop by drop to the residue with constant shaking. The tubes with the acetolysis mixture were stirred well and kept in a water bath at 70 °C. The water was then allowed to boil for 2-4 minutes till the mixture in tubes turned golden brown. The acetolysis mixture in the tubes was again centrifuged, after cooling and completely decanted. Then, 5 ml of glacial acetic acid was added into the tubes, the mixture was centrifuged and the supernatant was decanted. The pollen material in the tubes was washed with distilled water and centrifuged again. Finally, 2 ml of dilute glycerine was added to the tubes and the mixture was shaken thoroughly to disperse the pollen equally. The pollen grains were mounted on the slides in glycerine, covered with a clean coverslip and then observed under the microscope.

Data were taken from 50 pollen grains randomly at the X40 objective lens of a light microscope. The characters assessed were pollen/equatorial diameter, length of the polar axis, the distance between the colpi, colpi depth, and pollen wall thickness. The axis ratio and pollen size were calculated using data from the polar axis and equatorial diameter. Photomicrographs of the different shapes of the pollens encountered were taken at the X40 objective. Pollen attributes such as pollen shape, type and ornamentation were described according to Hesse *et al.* (2009).

Statistical Analysis

The quantitative pollen data were subjected to one-way ANOVA and the means were separated by Duncan Multiple Range Tests. The data were further subjected to Principal Components Analysis (PCA) and Single Linkage Cluster Analysis (SLCA) using the Paleontology Statistics Software Package (PAST).

Results and Discussion

Results

The spheroidal pollen shape was common to all the 24 species of Cyperaceae studied (Table 1, Figure 1), while triangular, quadrangular, and polygonal were frequently encountered. Some other shapes were occasionally encountered such as prolate in R. corymbosa (L.) Britt., and K. squamulata Thorn. ex Vahl, oblate in R. corymbosa, lobate in C. haspan L., and arcus in C. compressus L., C. distans L. and K. erecta Schumach (Figure 2). No visible pollen ornamentation (psilate) was observed under the light microscope in the majority of the species studied. However, C. iria L. and F. dichotoma var. pluristriata (C. B. CL) Napper had granulum ornamentation and F. littoralis Gaud, and R. corymbosa had micro-reticulate ornamentation (Figures 1 and 2). All the species of Cyperaceae studied were aporate (no pore), whereas there was the presence of colpi (i.e colpate) in the majority of them, varying from one to four with tetracolpate being predominant among them (Table 1). Kyllinga bulbosa P. Beauv, K. squamulata and M. alternifolius Vahl were acolpate while C. tuberosus Rottb and C. sphacelatus Rottb were monocolpate. Cyperus haspans L., C. rotundus L., C. tuberosus, K. nemoralis (Forst.) Dandy ex Hutch, M. flabelliformis Kunth, P. polystachyos (Rottb) P. Beauv and R. corymbosa can either be monocolpate or bicolpate. Cyperus esculentus var. esculentus L, C. strigosus L. and K. pumila Michx had pollens which varied from monocolpate to tricolpate, while the others varied from monocolpate to tetracolpate. It was observed that the quantitative data of the genera studied overlapped as they could not be distinctly separated from each other (Table 2). The species grouping showed in the scatter diagram from the PCA analysis based on components 1 and 2 (Figure 3) gave similar species grouping with the SLCA (Figure 4). Pollen axis, equatorial diameter and pollen size loaded

on Component 1, axis ratio, colpi depth and distance between colpi loaded on Component 2

and pollen wall thickness loaded on Component 3. Component 1 contributed 43.79% of the total variation observed in this study; Component 2 contributed 27.08%, while Component 3 contributed 12.04% (Table 3). The SLCA (Figure 4) separated the species into two main clusters with no discrimination among the species in both groupings. Subjecting the Cyperus and Kyllinga species studied to SLCA, however, showed that the pollens of the species in each genus can be delimited based on their quantitative pollen attributes. The Kyllinga species studied were grouped into two main clusters with K. pumila in a cluster of its own. The second cluster was further subdivided into two groups with K. squamulata and K. bulbosa in one subdivision, while K. erecta and K. nemoralis were in the other subcluster (Figure 5A). In Figure 5B, the Cyperus species investigated were also divided into two main clusters. The first main cluster was subdivided into two, with C. rotundus in one group, and the second group was also subdivided into two groups. Cyperus imbricatus Retz and C. tuberosus clustered together at a 98.5% level of similarity and the second group was subdivided further into two, with C. iria and C. sphacelatus clustered at 98% similarity, while C. pseudovegetus Steud and C. strigosus grouped at a 99% level of similarity. The second main cluster is subdivided into two. The first subgroup subdivided into two with C. esculentus var. esculentus on one side, and C. haspans and C. compressus on the other. The second subgroup consisted of C. difformis L. and C. distans together at a 95% similarity level.

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S/N	Species	Species Shape Ornamentation		Predominant Pollen Type	
1	Cyperus compressus	spheroidal, triangular, arcus	psilate	tetracolpate	
2	Cyperus difformis	spheroidal, quadrangular, triangular	psilate	tetracolpate	
3	Cyperus distans	spheroidal, arcus	psilate	tetracolpate	
4	Cyperus esculentus var. esculentus	spheroidal, triangular, prolate, quadrangular	psilate	tricolpate	
5	Cyperus haspan	spheroidal, triangular, lobate	psilate	tricolpate	
6	Cyperus imbricatus	spheroidal, triangular, polygonal	psilate	bicolpate	
7	Cyperus iria	spheroidal, quadrangular	granulum	tetracolpate	
8	Cyperus pseudovegetus	spheroidal, triangular	psilate	tetracolpate	
9	Cyperus rotundus	spheroidal, triangular	psilate	bicolpate	
10	Cyperus sphacelatus	spheroidal	psilate	monocolpate	
11	Cyperus strignosus	spheroidal, triangular	psilate	tricolpate	
12	Cyperus tuberosus	spheroidal	psilate	bicolpate	
13	Fimbristylis dichotoma var. pluristriata	spheroidal, triangular	granulum	monocolpate	
14	Fimbristylis littoralis	spheroidal, triangular	micro-reticulate	tetracolpate	
15	Kyllinga bulbosa	spheroidal, triangular	psilate	acolpate	
16	Kyllinga erecta	spheroidal, triangular, quadrangular, arcus	psilate	tetracolpate	
17	Kyllinga nemoralis	spheroidal	psilate	bicolpate	
18	Kyllinga pumila	spheroidal, triangular	psilate	tricolpate	
19	Kyllinga squamulata	spheroidal, triangular, prolate	psilate	acolpate	
20	Mariscus alternifolius	spheroidal	psilate	acolpate	
21	Mariscus flabelliformis	spheroidal, ovate, quadrangular, polygonal	psilate	bicolpate	
22	Pycreus flavescens	spheroidal, triangular	psilate	tetracolpate	
23	Pycreus polystachyos	spheroidal, triangular, quadrangular	psilate	bicolpate	
24	Rhynchospora corymbosa	spheroidal, prolate, triangular, oblate, ovate	micro-reticulate	bicolpate	



Figure 1: Various Shapes of Pollen Encountered in the Cyperaceae Species Studied. (A) ovate shape in *Mariscus flabeliformis* (B) irregular shape in *Fimbristylis littoralis* (C,D,E) spherical, prolate and ovate shapes in *Rynchospora corymbosa* (F,G) pentagonal and hexagonal shapes in *Cyperus haspan* (H,I) quadrangular and irregular shapes in *Cyperus iria* (arrowed granulum ornamentation (J,K) triangular shapes in *Kyllinga erecta* (L) triangular and quadrangular shapes in *Cyperus esculentus var. esculentus*



Figure 2: Colpi and Other Shapes Encountered in the Cyperaceae Species Studied. (A) monocolpate in *C. difformis,* (B) bicolpate in *C. difformis,* (C) tricolpate in *C. difformis,* (D,E) tetracolpate in *C. difformis,* (F) oblate shape in *R. corymbosa,* (G) arcus in *K. erecta,* (H,I) lobate shape in *C. haspan,* (G) micro-reticulate exine ornamentation in *R. corymbosa* (arrowed)

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Table 2: Mean Values of the	Quantitative Pollen Characters in Sp	pecies of Cyperaceae Studied	d.
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S/N	Species	P.A	E.D	P.S	P/E	PWT	CD	DBC
1	Cyperus compressus	23.50±2.16gh	21.68±1.49def	204.51±28.98fg	2.71±0.20g	4.50±0.89a	2.50±0.00h	12.50±0.00b
2	Cyperus difformis	20.05±2.02j	16.40±2.011	131.95±24.61k	3.10±0.48bc	1.33±0.31m	2.08±1.06i	6.82±2.16i
3	Cyperus distans	20.18±2.34j	17.80±2.43k	144.41±31.52jk	2.88±0.48defg	2.71±1.25cd	4.20±1.10b	5.83±1.86k
4	Cyperus esculentus var. esculentus	22.30±2.80hi	18.70±1.96ijk	168.28±36.21ij	2.99±0.28bcde	2.96±1.14bc	2.50±0.00h	8.75±1.25f
5	Cyperus haspan	23.30±2.14gh	20.40±2.22fgh	191.15±32.58ghi	2.88±0.33defg	1.96±0.62hijkl	2.81±1.36g	8.75±2.80f
6	Cyperus imbricatus	25.50±2.30f	22.38±2.79de	229.64±43.24ef	2.88±0.31defg	2.08±0.59	2.50±0.00h	13.13±2.07a
7	Cyperus iria	25.69±2.15k	28.13±3.02ijk	289.75±45.14ghi	2.30±0.26h	2.29±0.47b	3.18±1.11f	10.00±3.99d
8	Cyperus pseudovegetus	27.88±2.09dc	24.53±1.71c	273.95±32.34d	2.85±0.23defg	1.83±0.62jkl	3.50±1.22d	10.80±3.05c
9	Cyperus rotundus	33.85±3.47b	27.60±3.31b	375.20±65.76c	3.10±0.41bc	1.75±0.61kl	5.00±0.00a	
10	Cyperus sphacelatus	28.68±2.44c	25.88±1.89c	297.60±39.83d	2.78±0.24fg	2.29±0.57efg	3.75±1.25c	13.39±4.57a
11	Cyperus strigosus	27.65±2.23dce	24.53±2.66c	272.43±44.85d	2.84±0.29defg	2.13±0.80fghij	2.75±0.94g	6.88±3.23i
12	Cyperus tuberosus	25.95±4.20fe	22.88±2.49d	238.89±51.48e	2.85±0.52defg	3.15±1.70b	2.72±0.48g	8.82±3.11f
13	Fimbristylis dichotoma var. pluristriata	30.20±2.15b	26.55±2.00b	320.74±33.02c	2.86±0.33bcd	3.17±1.57b	5.00±0.00a	
14	Fimbristylis littoralis	27.70±1.22dce	24.70±2.51c	273.55±28.91d	2.84±0.34defg	2.79±1.00bc	3.33±1.18e	12.50±2.50b
15	Kyllinga bulbosa	24.98±2.77fg	22.38±2.75de	225.45±49.81ef	2.81±0.30efg	2.25±0.50efgh	2.50±0.00h	7.50±0.00h
16	Kyllinga erecta	22.58±2.00hi	18.30±1.97jk	165.33±23.47ji	3.12±0.43b	2.17±1.02fghi	3.13±1.08f	6.56±2.48ij
17	Kyllinga nemoralis	22.15±2.68hi	19.70±1.72hij	175.45±31.93ghi	2.82±0.32defg	1.75±0.61kl		
18	Kyllinga pumila	34.45±6.74b	29.35±3.87b	412.85±133.77b	2.92±0.33cdef	2.04±0.60ghijk	3.13±1.08f	9.38±1.08e
19	Kyllinga squamulata	26.58±2.56def	22.38±1.74de	237.98±30.17e	2.99±0.38bcde	2.50±0.00de		
20	Mariscus alternifolius	40.25±10.03a	30.30±6.18a	500.35±206.07a	3.37±0.75a	2.39±0.36ef		
21	Mariscus flabelliformis	25.05±2.54fg	22.65±2.17de	228.70±39.17ef	2.77±0.18fg	2.13±0.57fghij	2.50±0.00h	10.00±0.00d
22	Pycreus flavescens	23.53±2.20gh	21.25±2.04efg	200.98±35.11fgh	2.78±0.25fg	1.92±0.62ijkl	2.50±0.00h	8.00±1.00g
23	Pycreus polystachyos	21.50±1.77ij	19.90±1.65ghi	171.66±24.82hij	2.71±0.25g	3.17±1.01b	3.33±1.18e	6.25±1.25j
24	Rhynchospora corymbosa	27.28±2.72dce	22.18±2.27de	242.21±35.91e	3.10±0.43bc	1.67 ± 0.591		-

Key: P.A = Polar Axis, E.D = Equatorial diameter, P/E = Axis ratio i.e. (P.A/E.D), P.S = Pollen size (P.A * E.D), PWT = Pollen Wall Thickness, CD = Colpi Depth, DBC = Distance Between Colpi. Values with different letters are significantly different from each other at $p \le 0.05$

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Table 3: Principal Components Analysis of Pollen Attributes of the Species Studied in the Family Cyperaceae.

Attributes	Component 1	Component 2	Component 3
P.A	0.56	0.08	0.03
E.D	0.50	0.32	-0.06
P.S	0.56	0.16	0.01
P/E	0.24	-0.50	0.12
PWT	-0.10	0.36	0.92
CD	-0.05	0.50	-0.25
DBC	-0.23	0.48	-0.27
Eigenvalue	3.07	1.90	0.84
Difference	1.17	1.05	0.16
Proportion	43.79	27.08	12.04
Cumulative	43.79	70.88	82.91

Key: P.A = Polar Axis, E.D = Equatorial diameter, P/E = Axis ratio i.e. (P.A/E.D), P.S = Pollen size (P.A * E.D), PWT = Pollen Wall Thickness, CD = Colpi Depth, DBC = Distance Between Colpi



Figure 3: Scatter Diagram of the Pollens of the Species Studied Based on Components 1 and 2.

Keys: CC = Cyperus compressus, CD = Cyperus difformis, CDi = Cyperus distans, CEE = Cyperus esculentus var. esculentus, CH = Cyperus haspan, CI = Cyperus iribricatus, CIr = Cyperus iria, CP = Cyperus pseudovegetus, CR = Cyperus rotundus, CS = Cyperus sphacelatus, CSt = Cyperus strignosus, CT = Cyperus tuberosus, FD = Fimbristylis dichotoma var. pluristata, FL = Fimbristylis littoralis, KB = Kyllinga bulbosa, KE = Kyllinga reecta, KN = Kyllinga nemoralis, KP = Kyllinga pumila, KS = Kyllinga squamulata, MA = Mariscus alternifolius, MF = Mariscus flabelliformis, PF = Pycreus flavescens, PP = Pycreus polystachyon, RC = Rynchospora corymbosa



Figure 4: Dendrogram of the Cyperaceae Species Studied Based on the Quantitative Characters of the Pollens.



Discussion

All the members of the family Cyperaceae studied have many similarities in their quantitative pollen characters such that it is difficult to delimit them based on those characters alone. However, the pollen shape seems to be useful in this regard as *R. corymbosa* had varying shapes of pollen, with oblate pollens being unique to this species. In the genus *Cyperus*, *C. haspan* is unique because of the lobate-shaped pollen it has which is different from the pollens of other members of the genus. This agrees with the findings of Moore and Web (1978), Arogundade and Adedeji (2009), and Arogundade *et al.* (2019) that pollen shape can be used to delimit groups of plants.

In addition, ornamentation on the exine of the pollen was only observed in four out of the 24 species studied, showing that pollen ornamentation can be used to delimit members of the family Cyperaceae. It is worthy of note here that both species of *Fimbristylis* studied are ornamented; *F. littoralis* with micro-reticulate ornamentation can be distinguished from *F. dichotoma var pluristriata*, which had granulum ornamentation. *Rhynchospora corymbosa* also had micro-reticulate ornamentation. *Cyperus iria* is distinguished from other members of the genus *Cyperus* because of its granulum ornamentation. Butt *et al.* (2018) reported similar ornamentation patterns as obtained in this study in the pollens of *C. iria, C. rotundus* and *F. dichotoma var. pluristriata*.

The only form of aperture observed in most of the species of Cyperaceae studied was the colpi. Pollen with a high number of colpi has been identified as being evolutionarily advanced (Adedeji, 2005; Adedeji and Akinniyi, 2015; Arogundade and Lawal, 2018; Arogundade *et al.*, 2019). Based on this, *K. bulbosa, K. squamulata* and *M. alternifolius* are more primitive than other members of the family Cyperaceae studied being inaperturate. The pollens of the species studied ranged from 20.05 μ m in *C. difformis* to 40.25 μ m in *M. alternifolius* in diameter. This means that the pollens ranged from minutae to mediae according to the classification of Erdtman (1945).

The result from SCLA showed that quantitative pollen characters alone cannot be used to delimit the members of the family. However, when each genus (*Cyperus* and *Kyllinga*) was subjected to cluster analysis, *K. pumila* was distinctly separated from other members of its genus. This means that the pollen morphology can be used to delimit members of the same genus rather than the family. Kawarase and Kunjalwar (2016b), in their study of pollen morphology of five species of *Cyperus* concluded that there is enough variation within the genus for the species to be delimited by pollen morphology. This assertion was in agreement with the results obtained in this study.

The results obtained from PCA analysis showed that pollen axis, equatorial diameter and pollen size are primarily responsible for the variations observed in the pollens studied. The axis ratio and colpi depth were of secondary importance, while pollen wall thickness was of tertiary importance.

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Conclusion

From this current study, most of the quantitative data overlapped, while many qualitative characters were common among the species of Cyperaceae studied. However, only the pollen shape and ornamentation seem to be of taxonomic value in delimiting members of the family.

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Conflict of interest

The authors declared that there is no conflict of interest

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