Morphological diversity of phytolith structures in six species of *Carex* L. and *Cyperus* L. (Cyperaceae Juss.) from West Bengal, India

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Abstract. Majumder S, Mallick T, Ghosh A. 2020. Morphological diversity of phytolith structures in six species of *Carex* L. and *Cyperus* L. (Cyperaceae Juss.) from West Bengal, India. Biodiversitas 21: 3471-3486. Cyperaceae is a taxonomically challenging group due to its cosmopolitan distribution, similar vegetative structure, and reduced reproductive morphology. The present study focused on the characterization and description of phytolith morphotypes based on shape, structure, and ornamentation. Six species of *Carex* L. and *Cyperus* L. (three species each) were considered for the present study. From the investigation, we found 56 phytolith morphotypes, among which the principle ones were conical, elongate bulbous margin, lanceolate psilate, tabular concave with verrucate, tabular concave columellate, ovate granulate, and orbicular concave. The conical morphotype was most commonly found. The elongate bulbous margin, lanceolate psilate, and tabular concave with verrucate phytolith morphotypes were specific to the genus *Carex*, while the tabular concave columellate, ovate granulate, and orbicular concave morphotypes are constant at the genus level and may play a role in taxonomic identification in the family Cyperaceae.

Keywords: *Carex*, Cyperaceae, *Cyperus*, phytolith morphotypes, silica body

INTRODUCTION

With 98 genera and approximately 5500 species, Cyperaceae is the third largest family of monocotyledons (WCSP 2020), In India, Cyperaceae is the sixth largest family, with 38 genera and 545 species (Arisdason and Lakshminarasimhan 2017), Karthikeyan et al. (1989) reported 163 species of *Carex* from India and Prasad and Singh (2002) reported 70 species of *Cyperus* from India. Due to its cosmopolitan distribution, short life cycle pattern, similarity in vegetative morphology and highly reduced reproductive structure, the taxonomy of Cyperaceae is difficult (Reznicek and Catling 1986; Reznicek 1990; Starr et al. 1999; Simpson and Inglis 2001; Starr and Ford 2001; Pal and Choudhury 2014).

In monocotyledons, especially the members of the families Cyperaceae and Poaceae are the most important phytolith accumulators in the clade Poales (Naskar and Bera 2018; Fernández Honaine et al. 2009).

Silica deposition occurs in epidermal cells and vascular strands of the plant body (Hodson et al. 2005; Piperno 2006; Eksambekar 2009; Liszt-szabo et al. 2015; Naskar and Bera 2018). The shape and size, ornamentation patterns, and structure of phytoliths depend upon the cell structure on which the silica bodies are deposited (Naskar and Bera 2018), The structure of phytolith may vary at the family level and in some cases at the genus or species level (McNamee 2013; Naskar and Bera 2018). The formation of phytoliths, the shape and size of morphotypes are not only controlled by environmental conditions but also by genetic influence (Prychid et al. 2004).

Fernández Honaine et al. (2009) reported that the conical silica body type with or without a variable number of satellite bodies is the dominant morphotype among different tribes of Cyperaceae. However, this conical morphotype is not unique to the family Cyperaceae and is also present in Orchidaceae and Arecaceae (Ollendorf 1992). In the case of Orchidaceae and Arecaceae, the conical silica bodies are never found in the leaf epidermis, and they never form a plate-like structure (i.e., present singly) (Ollendorf 1992), Epidermal micro-morphology of the leaf, culm, and achem was used to delimit the sections of *Carex*, Cyperaceae (Star and Ford 2001).

In the last few decades, phytolith morphotypes concerning systematics of different taxa of angiosperms have been evaluated by different authors in different ways. Netolitsky (1929) revised phytolith morphotypes and attempted to identify marker phytolith morphotypes in different taxa of flowering plants. Subsequently, phytolith characteristics were used effectively by several authors in the characterization and identification of different members of grasses (Rosen 1992; Wang and Lu 1993; Pearsall et al. 1995; Houyouan et al. 1997; Zhao et al. 1998; Fahmy 2008; Shakoor et al. 2014); Mudassir et al. (2018) effectively and efficiently used phytolith signatures (elemental composition) to identify three species of *Setaria* (Poaceae). Prychid et al. (2004) summarized the silica morphotypes in the tribe Cyperae of the subfamily Cyperoideae and in the tribe Cariceae of the subfamily Caricoideae. Though, Masuya et al. (2009) divided the...
family Cyperaceae into two subfamilies: Cyperoideae and Mapanioideae, based on molecular data.

Phytoliths were added to the taxonomy of Cyperaceae by Toivonen and Timonen (1976) and Browning and Gordon Gray (1995). The Cyperaceae family can be distinguished from other major phytolith-producing angiosperms families (except Orchidaceae and Arecaceae) based on its characteristic conical morphotype (Piperno 1988; Mulholland et al. 1989; Olendorf 1992; Kondo et al. 1994; Wallis 2003; Fernández Honaine et al. 2009, 2013). The most recent work on sedge phytoliths was conducted by Bobrov et al. (2016), Murungi (2017), and Murungi and Bamford (2020), emphasizing the achen conical morphotype and non-conical morphotypes.

According to Fernández Honaine et al. (2013), the papilae morphotype (referred to as conical in the present treatment) found in Cyperaceae is a major morphotype, whereas the family Poaceae is characterized by the bilobate morphotype (Naskar and Bera 2018).

Piperno (1988) considered the pointed or blunted apices and smooth to nearly smooth peripheral surface as conical-shaped morphotypes, whereas Mulholland et al. (1989) categorized the conical structure into two types: one is long, sharply hollow pointed, and the other is short, solid, and resembles Hershey’s kisses. Eventually, many researchers applied different terms, such as Cyperaceous type (Mehra and Sharma 1965), cones (Metcalfe 1971), conical-shaped (Piperno 1985), hat-shaped (Piperno 1988), and papilae (Stevanato et al. 2019), all of which are synonymous.

Phytolith characteristics are considered ready references for systematic treatments in different taxa of Poaceae (Twiss et al. 1969; Piperno 1998, 2006; Gallego and Distel 2004; Naskar and Bera 2018). This is possible because there is a reference standard of these morphotypes in Poaceae.

The present investigation was undertaken to study the phytolith morphotypes found in different parts of the plant and to understand the effectiveness of phytolith morphotypes for taxonomic identification and differentiation of the genera Carex and Cyperus of Cyperaceae.

MATERIALS AND METHODS

Materials

Six species of Cyperaceae were collected from different locations in the Burdwan and Darjeeling districts, West Bengal, India. For phytolith analysis, leaf blades, leaf sheaths, and culms were collected separately and dried using silica gel and brought to the laboratory for oxidation treatment. Voucher specimens are deposited in BURD (Thiers 2019), and details of the collection are given in Table 1.

Methods

Phytoliths were extracted using the method of Lu and Liu (2003) and Dhoooge (2005) with some modifications. Five samples were considered for each studied species. First, 1 gm of plant parts (leaf blade, leaf sheath, and culm treated separately) were soaked in distilled water overnight and rinsed with distilled water five to six times. Then, the plant parts were dried properly and treated with 1:1 sulfuric acid and nitric acid, and heated at 150°C for 10 to 15 minutes. Next, the mixture was centrifuged at 5000 rpm for 5 minutes, and the pellet was collected. The centrifugation process was repeated five times to remove all the acid residues. Subsequently, the washed materials were diluted in 1 ml of distilled water. One drop of the suspension was placed on a slide and covered with a cover glass and observed under a Magnus MLX light microscope (model no: 527955) and a Leitz LABORLUX S (model no: 512859/102299) microscope.

For confirmation, we considered a morphotype when it occurred at least five times in a particular sample. We observed all the morphotypes present in plant parts. Images were captured using a Magcam DC14 (S/N C: 1804044093), Length of the conical, height of conical, height of apex, the width of apex, and inter-apical area distance were measured from the captured images, using Digimizer software (version 4.6.1). We measured characters of conical morphotype seven to twenty-one times for a particular species. The data were used to determine the mean value with standard deviation.

The description and characterization of the phytolith morphotypes were performed following ICPN 1.0 (Madella et al. 2005) and ICPN 2.0 (Neumann et al. 2019); Fernández Honaine et al. (2009); Mercader et al. (2010); Liszttes-Szabo et al. (2014, 2015) and Biswas et al. (2016).

The detailed phytolith micromorphological study was conducted using FE-SEM analysis for two selected phytolith morphotypes (conical and elongate bulbous margin). For FE-SEM sample preparation, diluted extractions were dried in a hot-air oven for 10 minutes and then put on a carbon-coated stab. Then, the samples were gold coated for 3 minutes. High-resolution photography was performed using a ZEISS Gemini Field-emission Scanning Electron Microscope (Model no. Sigma 300), and the images were analyzed with the help of Digimizer software (version 4.6.1).

Table 1. Collection details of the studied species

| Name of the species          | Collection area with coordination | Date of collection | Voucher No.  |
|-----------------------------|-----------------------------------|--------------------|--------------|
| Carex cruciata Wahlend.     | Zoological park, Darjeeling (27.2907°N 88.3134°E) | 25.11.2017         | BURD12148    |
| Carex filicata Nees.        | Gayabari, Darjeeling (26.8567°N 88.3754°E)     | 15.05.2015         | BURD12149    |
| Carex setigera D. Don        | Gairidwla, Darjeeling (27.0131°N 88.2515°E)    | 16.05.2015         | BURD12150    |
| Cyperus distans L.f.        | Golapbag, Burdwan (23.2532°N 87.8465°E)        | 25.10.2017         | BURD12151    |
| Cyperus exaltatus Retz.     | Durgapur, Burdwan (23.2907°N 88.3134°E)       | 15.11.2017         | BURD12152    |
| Cyperus imbricatus Retz.    | Golapbag, Burdwan (23.2514°N 87.8460°E)       | 15.09.2017         | BURD12153    |
RESULTS AND DISCUSSION

Results

During the present investigation, 56 phytolith morphotypes were observed (Table 2; Figures 1 and 2). Among them, 15 phytolith morphotypes are common in both the genera Carex and Cyperus; 26 morphotypes are specific to the genus Carex (Table 3) and 15 morphotypes are specific to Cyperus (Table 3), whereas 27 morphotypes are unclassified. Of the studied morphotypes, the most common are conical (observed in all the studied species), elongate bulbous margin, lanceolate psilate, tabular concave with verrucate, tabular concave columellate, ovate granulate, orbicular concave, favose, elongate form, stomatal complex, scutiform, tabular form, tower, and tower wide. Details of the morphotypes (plant part wise) and descriptions are provided in Table 2 and Figures 1 and 2. FE-SEM analyses of two selected phytolith morphotypes are presented in Figures 3 and 4.

In Carex, elongate bulbous margin, trapezoidal, tabular concave with verrucate morphotypes are found in leaf blades and leaf sheath. Whereas elongate laminate found in culm and lanceolate form is found in leaf blade only (Table 2, Figure 1).

In Cyperus, orbicular concave morphotype found in culm, tabular concave columellate morphotype found in leaf blade and ovate granulate morphotype found in both leaf blade and leaf sheath (Table 2, Figure 2).

Though, tower wide and conical morphotypes are found in both leaf blade and leaf sheath, and stomatal complex, favose are found only in leaf blade, whereas tower morphotype is found in leaf sheath of all the studied species (Table 2).

Elongate bulbous morphotype (characterized by rectangular shape with two times longer arms than width, margin with series of balloon or bulging like structure) found only in studied species of Carex. In C. cruciata base or stalk of the bulbous structure is narrow to short and the surface is smooth (Figure 3.A). Besides this, C. filicina and C. setigera shape of the bulbous structure is quite similar; though, in C. setigera the stalk is broad and short, and the bulbous surface is wavier (Figure 3.B, C).

Among the characters of the conical phytolith, margin of conical, form of conical, arrangement of conical (platelet/individual), apex structure, apex format, the arrangement of peripheral satellites, and inter apical area (zone) with or without satellite are also presented in Table 4 and Figure 5 as qualitative variations of the conical morphotype.

Silica bodies are present singly in several bodies per unit (Figures 1 and 2). The variation in the structures is mainly found in the number of peripheral satellites (Figure 3). These small cones or satellites around the main conical body are variable from species to species.

In all three studied species of Carex, the conical length was more than 11 μm (Figures 5.C, E; Figure 6), while in the three studied Cyperus species, the conical length was less than 11 μm (Figures 5.C, E; Figure 6). The height of the conical morphotypes in Carex species was more than 6 μm (Figures 5.C and 6), while it was less than 6 μm in species of Cyperus (Figures 5.C and 6). Similarly, the apex width in species of Carex was more than 5 μm and in Cyperus was less than 5 μm. The apex height in species of Carex was more than 3 μm, while that in Cyperus species was less than 3 μm (Figures 5 and 6).

Discussion

Among the 56 phytolith morphotypes, elongate morphotypes are found in all the studied Carex and Cyperus species, and the morphotypes were previously recorded by Fernández Honaine et al. (2009) and Stevanato et al. (2019) from different species of the genera Carex and Cyperus of Cyperaceae. Elongate forms of morphotypes were also described by Twiss et al. (1969), and Lisztesszabo et al. (2015) in Poaceae, Prychid et al. (2004) in Orchidaceae, Ebigwai et al. (2015) in Cucurbitaceae; Collura and Neumann (2017) in Anacardiaceae and Sapotaceae.

The elongate bulbous margin phytolith morphotype was found in species of Carex (C. cruciata, C. filicina, C. setigera) (Figures 1.A–E; 4.A–C). Previously, Murungi (2017) described elongate crenate or psilate morphotype from the leaf of Bulbostylis, Fuirena, and Scleria, and also reported that elongate crenate plate as the most dominant phytolith morphotype in F. pubescens.

Meanwhile, tabular (Fernández Honaine et al. 2009; Mercader et al. 2010) phytolith morphotypes were found in all the studied species, and the morphological variation was low for genus-level identification.

The term tower phytolith morphotype was first introduced by Lu and Liu (2003). Subsequently, tower wide morphotypes were reported by Mercader et al. (2010). In the present study, a tower form of phytolith morphotypes [Like- tower (Figures 1.AQ, AR; 2.Z), tower wide (Figures 1.BB; 2.AG, AH)], were found in almost all the studied species of Carex and Cyperus.

From a comprehensive observation of the studied species of Cyperus and Carex, Ollendorf (1992) found a rounded, individual, psilate cone base with a pointed apex, without peripheral satellites. With the similar objective of Ollendorf (1992), Fernández Honaine et al. (2009) and Stevanato et al. (2019) reported conical or papillae morphotypes in fruits and leaf respectively. Variations were also described in ornamentation, apex format, base form, and base shape of conical (polygonal in fruit; square, rectangular, rounded, hexagonal, oblong in leaf). The present results are similar to those of Ollendorf (1992), except for the arrangement of the satellites and conical. This study clarified and improved the characterization of conical structures. Here, conicals are arranged in a platelet form, and satellites are surrounding the central apex with an oblong conical base shape (Figures 3 and 5). The studied species showed two distinct sub-morphotypes: (i) the oblong-shaped conical base is found in all three species of Carex (Figures 3 and 5.B), and (ii) the square-shaped conical base is evident in all the studied species of Cyperus (Figures 3 and 5.A).
| First descriptor | Second descriptor | Studied species | Studied plant parts | Description of morphotypes |
|------------------|-------------------|----------------|--------------------|---------------------------|
| Elongate (Twiss et al. 1969) | Bulbous margin (this study) | *Carex cruciata* Wahlend.  
*Carex filicina* Nees.  
*C. setigera* D. Don | Leaf blade  
Leaf blade  
Leaf sheath | Much longer than wide; margin with globular or enlarge bulb. (Figure 1.A-E) |
|               |                    | *C. cruciata* | Leaf blade | Stomata with guard cell. (Figure 1.F-H) |
|               |                    | *C. filicina* | Leaf blade | Having the outline of a trapeziform, with four unequal sides, none of them parallel, and margins with uneven concavities and convexities. (Figure 1.J) |
|                |                   | *C. setigera* | Leaf blade | Having the outline of a trapeziform, with four unequal sides, none of which are parallel. (Figure 1.K) |
|               |                   | *C. cruciata* | Leaf sheath | Shape like a lance head, longer than wide, with a broad base and blunted tip. (Figure 1.L) |
|                |                   | *C. cruciata* | Leaf blade | Lance head-like body; longer than wide, with a broader base and slightly curved. (Figure 3.M) |
|               | Articulated (ICPN 2.0) | *C. cruciata* | Leaf blade | Much longer than wide; surface with minute or acute protuberances. (Figure 1.N, O) |
| Trapezoid (ICPN 2.0) | Sinuate (ICPN 2.0) | *C. cruciata* | Leaf blade | They show rounded or intermediate forms in the top and bottom view and concave in the side view. (Figure 1.P, Q) |
| Trapezoid (ICPN 2.0) |                   | *C. cruciata* | Leaf sheath | Much longer than wide with a smooth surface. (Figure 1.R) |
| Lanceolate (ICPN 1.0) | Psilatate (ICPN 2.0) | *C. cruciata* | Leaf blade | Honeycombed structure, arranged in a parallel form. (Figure 1.S) |
|               | Reflexed (ICPN 1.0) | *C. filicina* | Leaf blade | Cone-shaped structure, base, and cones are not differentiated, and the tips of the cones are pointed. (Figure 1.T-Y) |
|               | Papillate (ICPN 2.0) | *C. cruciata* | Leaf sheath | Much longer than wide; lance-shaped head base is broader than the apex. (Figure 1.Z) |
| Rondel (ICPN 2.0) | Tenus reflexed (this study) | *C. cruciata* | Leaf sheath | Having four sides more or less at a 90° angle, each side with the same length as the one opposite, with a smooth surface. (Figure 1.AA) |
| Scutiform (ICPN 1.0) | Triangular (this study) | C. cruciata | Leaf sheath | Shield-shaped structure, tri-angled. (Figure 1.AB, AC) |
|---|---|---|---|---|
| Tabular (ICPN 2.0) | Scrobiculate (ICPN 2.0) | C. cruciata | Leaf sheath | Table-like flat pitted surface. (Figure 1.AD) |
| Elongate (Twiss et al. 1969) | Sinuate (ICPN 2.0) | C. cruciata | Leaf sheath | Much longer than wide, having a margin with alternating but uneven concavities and convexities. (Figure 1.AE) |
| Tracheary (ICPN 2.0) | | | | Cylindrical and elongate bodies, relatively straight surface covered with ring to helical-shaped ridges arranged perpendicular to the long axis. (Figure 1.AF, AG) |
| Tabular (ICPN 2.0) | Psilate (ICPN 2.0) | C. setigera | Leaf sheath | Table-like surface, flat and smooth. (Figure 1.AH) |
| Elongate (Twiss et al. 1969) | Concave with verrucate (this study) | C. cruciata | Leaf blade | Much longer than wide, both ends are concave with irregularly shaped, wart-like processes. (Figure 1.AI) |
| Acute bulbosus (ICPN 2.0) | | C. filicina | Leaf sheath | Solid-body with a narrower apex and a spherical to fusiform-shaped base. (Figure 1.AJ) |
| Trichome (Carnelli et al. 2004) | | C. filicina | Leaf sheath | A crescent-shaped structure with a smooth surface. (Figure 1.AL) |
| Crescenti (Twiss et al. 1969) | | C. filicina | Leaf sheath | Elongated, and the margins are very convex. (Figure 1.AM) |
| Tabular (ICPN 2.0) | Gibbous (ICPN 1.0) | C. filicina | Leaf sheath | Much longer than wide and margins with prickles. (Figure 1.AN) |
| Elongate (Twiss et al. 1969) | Echinate (ICPN 2.0) | C. filicina | Leaf sheath | Wedge-shaped bulliform cell. (Figure 1.AP) |
| Crescenti (Twiss et al. 1969) | Convex (ICPN 2.0) | C. filicina | Leaf sheath | Conical tall body with a flat base and flat apex. (Figure 1.AQ, AR) |
| Cuneiform (ICPN 1.0) | | C. filicina | Leaf sheath | Elongated tabular shaped, margins with uneven concavities and convexities. (Figure 1.AS, AT) |
| Tower (Lu and Liu 2003) | Sinuate (ICPN 2.0) | C. setigera | Leaf sheath | Much longer than wide, and the surface is covered with layers. (Figure 1.AU) |
| Elongate (Twiss et al. 1969) | Laminate (ICPN 1.0) | C. filicina | Leaf sheath | Elongated structure and both ends are curved inward; stomata are attached to this curved portion. (Figure 1.AV) |
| Tabular (ICPN 2.0) | Concave echinate with stomata (this study) | C. setigera | Leaf blade | Elongate tabular body with curved ends; surface with prickles. (Figure 1.LAV) |
| Tabular (ICPN 2.0) | Concave echinate (this study) | C. setigera | Leaf blade | Elongate tabular body with curved ends; surface with prickles. (Figure 1.LAX) |
| Tabular (ICPN 2.0) | Concave with verrucate (this study) | C. cruciata | Leaf blade | Elongate tabular body with curved ends. (Figure 1.LY) |
| Tabular (ICPN 2.0) | Concave (ICPN 2.0) | C. setigera | Leaf blade | More or less square-shaped structure, with four arms; margin with uneven concavities or convexities. (Figure 1.LZ) |
| Semi square (this study) | Sinuate (ICPN 2.0) | C. setigera | Leaf blade | Elongate bodies with straight rod- or pillar-like projections are found. (Figure 1.BA) |
| Tabular (ICPN 2.0) | Columellate (ICPN 1.0) | C. setigera | Leaf blade | Cone-shaped tall body with a flat apex and a wider base than apex. (Figure 1.BB) |
| Tabular (ICPN 2.0) | Favose castelate (ICPN 1.0) | C. setigera Leaf blade | Elongate, honeycomb-like ornamentation and margins with square or rectangular processes. (Figure 1.BC) |
|-------------------|---------------------------|------------------------|--------------------------------------------------------------------------------------------------|
| Tabular (ICPN 2.0) | Crenate (ICPN 2.0)        | C. setigera Leaf blade | Elongate body, dented, with teeth-like margins. (Figure 1.BD) |
| Acuminate (ICPN 2.0) | Hollow (this study)      | C. setigera Leaf blade | Globe-shaped structure with a wavy surface. (Figure 1.BG) |
| Unclassified      |                           | C. setigera Leaf blade | Cone-shaped structure, wider at the base and tapering to the apex. (Figure 1.BH) |
|                   |                           | C. setigera Leaf blade | Shield-like structure with one end wider than the other. (Figure 1.BL) |
|                   |                           | C. cruciata Leaf blade | More or less circular to ovulate body with articulation. (Figure 1.BN-BQ) |
|                   |                           | C. setigera Leaf blade | Star-shaped structure with five arms. (Figure 1.BR) |
|                   |                           | C. cruciata Leaf blade | Bubble-shaped cell with a truncated appearance. (Figure 1.BS) |
|                   |                           | C. cruciata Leaf blade | Crescent-form structure with a smooth surface and deeply convex inner portion. (Figure 1.BT) |
|                   |                           | C. distans Leaf blade | Longer than wide, both margins acute, and concave elongated ends. (Figure 1.BU) |
|                   |                           | C. imbricatus Leaf blade | Elongate; having minute rounded papillae or acute protuberances. (Figure 1.BV) |
|                   |                           | C. exaltatus Leaf blade | Table-shaped structure, and one end curves inward. (Figure 1.BW) |
|                   |                           | C. cruciata Leaf blade | Conical tall with a curved cylindrical body. (Figure 1.BZ) |
|                   |                           | C. cruciata Leaf blade | Table-like surface; the ends of the long cells are concave, and the outer surface has rod or pillar-like processes. (Figure 2.B) |
| Elongate (Twiss et al. 1969) | Sinuate (ICPN 2.0)      | C. setigera Leaf blade | Much longer than wide, sometimes with inclusions; both sides beset with prickles, although one side is more frequently arranged than the other side. (Figure 2.C) |
| Tabular (ICPN 2.0) | Concave columellate (this study) | C. distans Leaf blade | Cones have a much thicker rounded base, and the tips are pointed. (Figure 2.D-I) |
| Elongate (Twiss et al. 1969) | Echinate (ICPN 2.0)      | C. distans Leaf blade | Much longer than wide, sharply pointed, and terminating very quickly. (Figure 2.J) |
| Conical (Ollendorf 1992) |                           | C. exaltatus Leaf blade | Table-like surface; the ends of the long cells are concave, and the margins are sinuate or echinate. (Figure 2.K) |
| Tabular (ICPN 2.0) | Concave acute (this study) | C. distans | Leaf blade | Table-like surface; both margins are acute, and the elongated ends are concave. (Figure 2.L) |
|-------------------|---------------------------|------------|------------|-----------------------------------------------------------------------------------------------|
| Tabular (ICPN 2.0) | Tabular (ICPN 2.0)        | C. distans | Leaf blade | Elongate with a flat table-like surface. (Figure 2.M, N)                                      |
| Tabular (ICPN 2.0) | Papillate (ICPN 2.0)      | C. distans | Leaf blade | Having a flat, table-like surface with sharp acute or minute rounded margin papillate. (Figure 2.O, P) |
| Ovate (ICPN 2.0)  | Granulate (ICPN 2.0)      | C. distans | Leaf blade | Oblong but broader at one base; surface with granules. (Figure 2.Q)                           |
| Elongate (Twiss et al. 1969) | Psilate (ICPN 2.0) | C. distans | Leaf blade | Much longer than wide with a smooth surface and margins. (Figure 2.R-U)                        |
| Tabular (ICPN 2.0) | Rectangular (ICPN 2.0)    | C. exaltatus | Leaf blade | Elongate, having four sides with 90° angles, and each side has the same length. (Figure 2.V) |
| Scutiform (ICPN 1.0) | Triangular (this study) | C. distans | Leaf blade | Shield-shaped structure, tri-angled. (Figure 2.W)                                              |
| Tabular (ICPN 2.0) | Crenate (ICPN 2.0)        | C. distans | Leaf blade | Table-like surface, notched or scalloped; dented, with rounded to flat teeth. (Figure 2.X)    |
| Tabular (ICPN 2.0) | Crenate (ICPN 2.0)        | C. imbricatus | Leaf blade | Table-like surface, notched or scalloped; dented, with rounded to flat teeth. (Figure 2.Y)    |
| Tower (Lu and Liu 2003) | Sulcate (ICPN 1.0) | C. exaltatus | Leaf blade | Cone-shaped, wide at the base, and tapering or slender to the apex. (Figure 2.Z)               |
| Tracheary (ICPN 2.0) | Echinate verrucate (this study) | C. exaltatus | Leaf blade | Cylindrical and elongate bodies with a relatively straight surface covered with ring- to helical-shaped ridges arranged perpendicular to the long axis. (Figure 2.AA-AD) |
| Tabular (ICPN 2.0) | Cuneiform (ICPN 1.0)      | C. exaltatus | Leaf blade | Elongated and beset with prickles in one margin, while the other has irregularly shaped wart-like processes. (Figure 2.AE) |
| Favose (ICPN 1.0) | Tabular (ICPN 2.0)        | C. exaltatus | Leaf blade | Honeycomb-like structure; parallel arrangement. (Figure 2.AF)                                  |
| Tower wide (Mercader at al. 2010) | Sinuate (ICPN 2.0) | C. exaltatus | Leaf blade | Cone-shaped tall body with flat apex and the base is much wider than the apex. (Figure 2.AG, AH) |
| Elongate (Twiss et al. 1969) | Sulcate (ICPN 1.0) | C. imbricatus | Leaf sheath | Body is much longer than wide, and the surface is furrowed. (Figure 2.AI)                      |
| Square (ICPN 1.0) | Tabular (ICPN 2.0)        | C. exaltatus | Leaf blade | Having more or less four sides with 90° angles. (Figure 2.AJ, AK)                             |
| Tabular (ICPN 2.0) | Crenate (ICPN 2.0)        | C. exaltatus | Leaf blade | Elongated body that is much longer than wide; margins with alternating but uneven concavities and convexities. (Figure 2.AL) |
| Cuneiform (ICPN 1.0) | Scrobiculate (ICPN 2.0) | C. exaltatus | Leaf blade | Elongate body with a pitted surface. (Figure 2.AN)                                            |
| Tabular (ICPN 2.0) | Scrobiculate (ICPN 2.0)  | C. exaltatus | Leaf blade | Smooth surface with depressed double edges. (Figure 2.AO)                                     |
| Short saddle (ICPN 2.0) | Concave (ICPN 2.0)       | C. distans | Leaf blade | Circular body and the surface is curved inwardly in the middle. (Figure 2. AP)               |
| Bulliform (ICPN 1.0) | Parallelepipedal articulated (ICPN 1.0) | C. exaltatus | Culm | Bubble-shaped large cell with parallel joints and a more or less four-sided structure. (Figure 2.AQ) |
|---------------------|----------------------------------------|--------------|------|-------------------------------------------------------------------------------------------------|
| Tabular (ICPN 2.0)  | C. imbricatus | Leaf blade | Much longer than wide with a smooth surface with rod-like processes and concave sides. (Figure 2.AR) |
| Polylobate (ICPN 2.0) | C. imbricatus | Leaf blade | More than one lobe linearly arranged. (Figure 2.AS) |
| Stomatal complex (Carnelli et al. 2004) | C. imbricatus | Leaf blade | Stomata with guard cells. (Figure 2.AT) |
| Unclassified        | C. distans | Leaf blade | Table-shaped base; lance-shaped head with a wider base and pointed tip. (Figure 2.AU) |
|                     | C. distans | Leaf sheath | Bubble-shaped epidermal cells. (Figure 2.AY-BB) |
|                     | C. exaltatus | Leaf blade | Conical tall body with a flat base and a slender apex. (Figure 2.BF) |
|                     | C. imbricatus | Leaf blade | Conical tall body with a slender, curved apex; base is much wider than the apex. (Figure 2.BG) |
|                     | C. distans | Leaf sheath | Conical tall body with a slender base that is much wider than the apex. (Figure 2.BH, BI) |
|                     | C. exaltatus | Leaf sheath | Tall body, flat apex, and a broad base, which is more or less three times wider apex. (Figure 2.BJ, BK) |
|                     | C. imbricatus | Leaf blade | Tall cylindrical lobes, jointed with other lobes. (Figure 2.BM) |
|                     | C. exaltatus | Leaf sheath | Having cylindrical three-lobed structures with a thick-walled smooth surface. (Figure 2.BN) |
|                     | C. imbricatus | Leaf blade | Spindle-shaped; swollen in the middle and narrowing towards the edges; lobes are present. Middle two lobes are broader than the two edges. (Figure 2.BP) |
|                     | C. imbricatus | Leaf sheath | Cone-shaped, wide at the base, and tapering to the apex; apex with two blunted horns. (Figure 2.BQ) |
|                     | C. exaltatus | Leaf sheath | Having a table-like surface that is curved inward in the middle. (Figure 2.BR) |
|                     | C. imbricatus | Leaf blade | Lance-shaped with a very long, pointed tip; one margin is curved, and the base is narrow. (Figure 2.BS) |
|                     | C. imbricatus | Leaf sheath | The base is narrower than the apex; club-shaped, gradually thickening from a slender base. (Figure 2.BT) |
Table 3. Unique and common phytolith morphotypes found in the studied species of Carex and Cyperus at the genus level, including their count and specificity.

| Number of observed phytolith morphotypes | Phytolith morphotypes observed in all the three studied species of Carex | Phytolith morphotypes observed in all the three studied species of Cyperus | Phytolith morphotypes common in both Carex and Cyperus |
|-----------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------|
| 1                                       | Acute bulbous                                                            | Bulliform parallelepiped articulated                                     | Conical                                                |
| 2                                       | Acuminate hollow                                                         | Elongate acute                                                           | Cuneiform                                              |
| 3                                       | Clavate                                                                  | Elongate sulcate                                                         | Elongate echinate                                      |
| 4                                       | Crescenti                                                                | Polylobate                                                               | Elongate psilate                                        |
| 5                                       | Crescenti convex                                                         | Orbicular concave                                                        | Elongate sinuate                                       |
| 6                                       | Elongate articulated                                                     | Ovate granulate                                                         | Favose                                                 |
| 7                                       | Elongate bulbous margin                                                  | Short saddle                                                             | Scutiform triangular                                   |
| 8                                       | Elongate concave with verrucate                                          | Square                                                                  | Stomatal complex                                       |
| 9                                       | Elongate lanceolate                                                      | Tabular                                                                  | Tabular concave echinate                              |
| 10                                      | Elongate lanceolate                                                      | Tabular concave acut                                                               |
| 11                                      | Elongate papillate                                                       | Tabular concave columnate                                                | Tabular scrobiculate                                   |
| 12                                      | Lanceolate psilate                                                       | Tabular echinate verrucate                                                | Tabular sinuate                                        |
| 13                                      | Lanceolate reflected                                                     | Tabular papillate                                                        | Tower                                                  |
| 14                                      | Rectangle psilate                                                        | Tabular pilate                                                           | Tower wide                                             |
| 15                                      | Rondel tenuis reflexed                                                   | Tabular rectangular                                                      | Tracheary                                              |
| 16                                      | Semi square sinuate                                                      |                                                                         |
| 17                                      | Tabular columnellate                                                     |                                                                         |
| 18                                      | Tabular concave                                                          |                                                                         |
| 19                                      | Tabular concave echinate with stomata                                    |                                                                         |
| 20                                      | Tabular concave with verrucate                                           |                                                                         |
| 21                                      | Tabular favose castelate                                                 |                                                                         |
| 22                                      | Tabular gibbous                                                          |                                                                         |
| 23                                      | Tabular pilate                                                           |                                                                         |
| 24                                      | Trapezoid                                                                |                                                                         |
| 25                                      | Trapezoid sinuate                                                        |                                                                         |
| 26                                      | Trichome                                                                 |                                                                         |

Table 4. Qualitative data of conical morphotypes (secondary descriptor) in the studied Carex and Cyperus species

| Conical character                        | Carex cruciata | Carex filicina | Carex setigera | Cyperus distans | Cyperus exaltatus | Cyperus imbricatus |
|------------------------------------------|----------------|----------------|----------------|-----------------|-------------------|-------------------|
| Shape of conical                         | Oblong         | Oblong         | Oblong         | Square          | Square            | Square            |
| Margin of conical (entire/undulate)      | Entire          | Entire         | Entire         | Entire          | Entire            | Entire            |
| Form of conical (concave/convex)         | Convex          | Convex         | Convex         | Convex          | Convex            | Convex            |
| Arrangement form of conical (platelet/individual) | Platelet      | Platelet       | Platelet       | Platelet        | Platelet          | Platelet          |
| Apex structure                           | Straight        | Straight       | Straight       | Straight        | Straight          | Straight          |
| Apex format                              | Acuminate       | Acuminate      | Acuminate      | Acuminate       | Acuminate         | Acuminate         |
| Arrangement form of peripheral satellite (continuous/discontinues) | Discontinues   | Discontinues   | Discontinues   | Discontinues    | Discontinues      | Discontinues      |
| Inter-apical area with/without satellite | Without         | Without        | Without        | With            | With              | With              |
|                                          | satellite       | satellite      | satellite      | satellite       | satellite         | satellite         |
|                                          | (rarely with few satellites) | (rarely with few satellites) | (rarely with few satellites) | (rarely with few satellites) | (rarely with few satellites) | (rarely with few satellites) |

These morphotypes are taxon-specific, and this pattern of distinct attributes among the different species under different genera remains ill-defined, as predicted by Stevanato et al. (2019). Whereas Stevanato et al. (2019) used leaf samples of different species of Cyperaceae, the present study used not only leaf blades but also leaf sheaths and culms. However, remarkable variations were not found in different parts of the same species, although interspecific variation was prominent. Recently, Murungi and Bamford (2020) emphasized the morphology, surface texture, and ornamentation of achene cones (conical in the present study) as a taxonomic characteristic rather than the size of the morphotypes.
Figure 1. Phytolith morphotypes observed in the studied species of Carex. A. Elongate bulbous margin in Carex cruciata (lb), B. Elongate bulbous margin in C. cruciata (ls), C. Elongate bulbous margin in Carex filicina (lb), D. Elongate bulbous margin in C. filicina (ls), E. Elongate bulbous margin in Carex setigera (lb), F. Stomatal complex in C. cruciata (lb), G. Stomatal complex in C. filicina (lb), H. Stomatal complex in C. setigera (lb), I. Elongate articulated in C. cruciata (lb), J. Trapezoid sinuate in C. cruciata (lb), K. Trapezoid in C. cruciata (ls), L. Lanceolate psilate in C. cruciata (lb), C. filicina (lb), M. Lanceolate reflexed in C. setigera (lb), N. Elongate
According to Fernández Honaine et al. (2009), a polygonal shape of the conical base was observed in the fruit of the species of both Carex and Cyperus, but in presently investigated species of Carex and Cyperus, there is no evidence of such a polygonal shape of conical base morphotypes, because only vegetative samples were analyzed.

Among conical second descriptors, the number of peripheral satellites, length of the conical, height of the conical, height of the apex, the width of the apex, and inter-apical area distance were also measured (Figures 5 and 6; Table S1). Of the quantitative characteristics, the inter-apical area distance (Figure 5.A–B) is an important characteristic that was previously suggested by Ollendorf (1992), and it is also applicable for the studied species. Here, the inter-apical area was without satellites (or rarely with satellites), and the inter-apical distance in the Carex species was more than 4 μm (Figures 3, 6). Meanwhile, in Cyperus species, the inter-apical area had distinct satellites, and the distance was less than 4 μm (Figures 3, 6). Fernández Honaine et al. (2009) and Stevanato et al. (2019) considered the length of the conical to be a significant character. Murungi (2017) illustrated leaf cone morphotypes of three species of Cyperus (Cyperus congestus, C. haematocephalus, and C. semitriquitosus), However, these qualitative and quantitative characteristics were not considered.

From their quantitative and qualitative analysis of nine South African Cyperus species, Murungi and Bamford (2020) concluded that cone phytoliths were found in most species of Cyperaceae but were not the dominant morphotype. They also inferred that the variation in leaf cones in terms of size and presence of satellites are generally less important taxonomic characteristics for the family Cyperaceae. In addition, Murungi and Bamford (2020) did not find any genus-specific (Cyperus type) phytoliths in Cyperus. However, they did not evaluate any of the species of Carex s.s. in their studies. The current study reports a considerable difference between the conical phytolith structures, based on which we can distinguish the studied genera.

Furthermore, Murungi and Bamford (2020) observed that achene cone phytoliths are not always cone-shaped in lateral view (i.e., they may be polygonal or isodiametric or sometimes elongate in structure), and leaf cone features are not consistent within a single genus, indicating its taxonomic applicability. The current study also supports this finding. Other morphotypes, such as stemal complexes and tabular and/or blocky parallelepiped morphotypes, are the most dominant (Murungi and Bamford 2020). In the present work, stemal complexes and tabular morphotypes were also reported.

In recent phylogenetic studies on different taxa of monocotyledons, the presence and absence of different phytolith morphotypes have been considered for taxonomic treatment (Prychid et al. 2004). Variations of morphotypes or sub-morphotypes were not considered for particular taxon delimitation. But this study shows that the value of conical length for Carex and Cyperus species is also important. Other important characteristics are the height of the apex (Stevanato et al. 2019), the height of the conical, and the width of the apex (for the first time reported in this study). However, from a taxonomic point of view, the qualitative characteristics of phytolith morphotypes show more notable value than the quantitative characteristics.

This study demonstrates that the presence or absence of some phytolith morphotypes (e.g., elongate bulbous margin, Figure 1.A–E; lanceolate psilate, Figure 1.L; tabular cone with verrucate, Figure 1.AX; tabular cone columellate, Figure 2.B; ovate granulate, Figure 2.Q; and orbicular cone, Figure 2.AP) are genus-specific and can provide further support for taxonomists to confirm morphological and phylogenetic classifications of the genera in Cyperaceae, as stated by Murungi (2017).
Figure 2. Phytolith morphotypes observed in the studied species of Cyperus. A. Elongate sinuate in *Cyperus distans* (lb), B. Tabular concave columnellate in *C. distans* (lb), *C. imbricatus* (lb), C. Elongate echinate in *C. distans* (lb), D. Conical in *C. distans* (lb), E. Conical in *C. distans* (ls), F. Conical in *C. exaltatus* (lb), G. Conical in *C. exaltatus* (ls), H. Conical in *C. imbricatus* (lb), I. Conical in *C. imbricatus* (ls), J. Elongate acute in *C. distans* (lb), K. Tabular concave echinate in *C. distans* (lb), L. Tabular concave acute in *C. distans* (lb), M. Tabular in *C. distans* (lb), N. Tabular in *C. distans* (cu), O. Tabular papillate in *C. distans* (lb), P. Tabular papillate in *C. exaltatus* (lb), Q. Ovate granulate in *C. distans* (lb), *C. exaltatus* (lb), *C. imbricatus* (ls), R. Elongate psilate in *C. distans* (lb), S. Elongate psilate in *C. distans* (ls), *C. imbricatus* (ls), T. Elongate psilate in *C. distans* (cu), *C. imbricatus* (cu), U. Elongate psilate in *C. exaltatus* (lb), V. Tabular rectangular in *C. distans* (lb), W. Scutiform triangular in *C. distans* (ls), X. Tabular crenate in *C. distans* (ls), Y. Tabular crenate in *C. imbricatus* (lb), Z. Tower in *C. distans* (ls), AA. Tracheary in *C. distans* (cu), AB. Tracheary in *C. exaltatus* (lb), AC. Tracheary in *C. exaltatus* (cu), AD. Tracheary in *C. imbricatus* (cu), AE. Tabular echinate verrucate in *C. exaltatus* (lb), AF. Favose in *C. exaltatus* (lb), AG. Tower wide in *C. exaltatus* (lb), AH. Tower wide in *C. imbricatus* (ls), AI.
Elongate sulcate in *C. exaltatus* (lb), AJ. Square in *C. exaltatus* (lb), AL. Tabular sinuate in *C. exaltatus* (lb), AM. Cuneiform in *C. exaltatus* (lb), AN. Tabular scrobiculate in *C. exaltatus* (lb), AO. Short saddle in *C. exaltatus* (lb), AP. Orbicular concave in *C. distans* (lb), AQ. Bulliform parallelepipedal articulated in *C. exaltatus* (lb), AR. Tabular pilate in *C. imbricatus* (lb), AS. Polylobate in *C. imbricatus* (lb), AT. Stomatal complex in *C. imbricatus* (lb), AU. Unclassified in *C. distans* (lb), AV. Unclassified in *C. distans* (lb), AW. Unclassified in *C. distans* (lb), AX. Unclassified in *C. distans* (lb), AY. Unclassified in *C. distans* (lb), AZ. Unclassified in *C. exaltatus* (lb), BA. Unclassified in *C. imbricatus* (lb), BB. Unclassified in *C. imbricatus* (lb), BC. Unclassified in *C. distans* (lb), BD. Unclassified in *C. distans* (lb), BE. Unclassified in *C. distans* (lb), BF. Unclassified in *C. distans* (lb), BG. Unclassified in *C. exaltatus* (lb), BH. Unclassified in *C. exaltatus* (lb), BI. Unclassified in *C. distans* (lb), BJ. Unclassified in *C. distans* (lb), BK. Unclassified in *C. distans* (lb), BL. Unclassified in *C. distans* (lb), BM. Unclassified in *C. exaltatus* (lb), BN. Unclassified in *C. exaltatus* (lb), BO. Unclassified in *C. exaltatus* (lb), BP. Unclassified in *C. imbricatus* (lb), BQ. Unclassified in *C. imbricatus* (lb), BR. Unclassified in *C. imbricatus* (lb), BS. Unclassified in *C. imbricatus* (lb), BT. Unclassified in *C. imbricatus* (lb), BU. Unclassified in *C. imbricatus* (lb), BV. Unclassified in *C. imbricatus* (lb), (lb=leaf blade, ls=leaf sheath, cu=culm; scale bar=10 µm)

Figure 3. FE-SEM and LM images of conical morphotype and arrangement of satellites in the studied species of *Carex* and *Cyperus*. A, B. *Carex cruciata*; C, D. *Carex filicina*; E, F. *Carex setigera*; G, H. *Cyperus distans*; I, J. *Cyperus exaltatus*; K, L. *Cyperus imbricatus*.

Figure 4. FE-SEM images of elongate bulbous margin phytolith morphotypes in the studied species of *Carex*. A. *Carex cruciata*; B. *Carex filicina*; C. *Carex setigera*.
Figure 5. Diagrammatic representation and characterization of different conical morphotypes. A. Square shape of conical. B. Oblong shape of conical. C. Acuminate type of conical and different measurement area of conical (length of conical, height of conical, height of apex), D. Blunt type of conical. E. Arrangement form of satellite and measurement area for length of conical (following Stevanato et al. 2019)

Figure 6. Bar graph comparing the quantitative data of conical morphotypes in the studied species of Carex and Cyperus (data are presented in Table S1)

Table S1. Measurements of different quantitative characters of conical in studied species of Carex and Cyperus

| Conical characters                  | Carex cruciata | Carex filicina | Carex setigera | Cyperus distans | Cyperus exaltatus | Cyperus imbricatus |
|------------------------------------|----------------|----------------|----------------|-----------------|-------------------|--------------------|
| Number of peripheral satellite     | 9 ±1.4         | 10 ±2.04       | 9.1 ±1.46      | 14 ±1.33        | 13 ±2.61          | 14 ±1.33           |
| Length of conical (µm)             | 11.2 ±0.92     | 12 ±0.64       | 11.6 ±1        | 10.7 ±1.17      | 10.4 ±1.01        | 10.5 ±0.94         |
| Height of conical (µm)             | 6.7 ±0.42      | 7 ±0.69        | 7 ±0.58        | 5 ±0.37         | 5.3 ±0.43         | 4.6 ±0.36          |
| Height of apex (µm)                | 3.4 ±0.37      | 3.8 ±0.27      | 3.7 ±0.26      | 2 ±0.37         | 2 ±0.30           | 2 ±0.25            |
| Width of apex (µm)                 | 5.7 ±0.40      | 5.5 ±0.97      | 6.3 ±1.07      | 3.9 ±0.54       | 3.3 ±0.56         | 3.7 ±0.54          |
| Inteapical area distance (µm)      | 4.1 ±0.42      | 4.4 ±0.92      | 4.3 ±1.26      | 3.7 ±0.87       | 3.6 ±1.02         | 2.3 ±0.86          |

Note: Calculation was executed separately for each of the conical characters; total n = 80
In conclusion, the present investigation describes the phytolith morphotypes in six species of Cyperaceae (under two genera), and some distinct morphotypes are found for the genera Carex and Cyperus. Of all the studied morphotypes, the conical morphotype has important characteristics with taxonomic applicability. In Carex (C. cruciata, C. filicina, C. setigera), elongate bulbous margin, lanceolate psilate, and tabular concave verrucate type of phytolith were commonly found, whereas, in Cyperus (C. distans, C. exaltatus, C. imbricatus), tabular concave columnellate, ovate granulate, and orbicular concave morphotypes were constantly present. Both qualitative and quantitative data are helpful in the study of patterns and differentiation of phytolith morphotypes among the studied species of Cyperaceae, although quantitative data are more useful in the analysis of conical morphotypes while qualitative data are more useful for other morphotypes. As some morphotypes are sometimes phenotypically plastic, common to other botanical families, further extensions of this work covering a considerable number of species would help in the identification of species and infraspecific taxa of Cyperaceae. From these data, it can be concluded that some of the morphotypes are found throughout the studied species, and some are constant for the genera. Thus, some morphological differences, such as the height, length, or inter-apical area of conical phytoliths, could be important for the identification of Cyperaceous taxa. However, a detailed study of the phytolith morphotypes in other taxa of Cyperaceae is needed to provide permission for the CAL herbarium consultation.

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