

RESEARCH ARTICLE

## Comparative study of foliage leaf and bract leaf anatomy of six species of *Cyperus* L. (Cyperaceae) from West Bengal

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### Abstract

Six species of *Cyperus* L. are anatomically characterized in an attempt to identify diagnostic characters and to find out the possible relationship among the studied species. From the present study, we observed that some anatomical features are alike to all the studied species (such as foliage leaf bulliform cell layer, form of foliage leaf sclerenchyma, bract leaf bulliform cell layer, form of bract leaf sclerenchyma and position of sclerenchyma in relation to vascular bundle in bract leaf), while others characters (like minor vascular bundle position in foliage leaf and bract leaf, foliage leaf epidermis layer, transverse septum between aerenchyma etc.) are variable. The studied taxa were separated on the basis of 14 inter-specifically variable characters (these are, presence of air cavities in foliage leaf, major vascular bundles position in relation to air cavities in foliar leaf, presence of minor vascular bundle, morphology of foliage leaf blade margins, total number of bulliform cells in foliage leaf, foliage leaf mestome sheaths form, angle of foliage leaf margin, bract leaf air cavities, major vascular bundles position in relation to air cavities in bract leaf, presence of minor vascular bundle in bract leaf, bract leaf blade margin morphology, bract leaf mestome sheaths form, depth of keel in bract leaf, maximum thickness at one half middle of bract leaf). The key to the identification of the studied taxa is also prepared from anatomical data.

**Keywords:** Anatomy, Cyperaceae, *Cyperus*, Foliage leaf, Bract leaf, Phenogram

### Introduction

With 109 genera and ca. 5500 species (Muasya *et al.* 2009), Cyperaceae is the third largest family among monocots (Lunkai *et al.* 2010). Comprising with about 600 species, *Cyperus* L. is the second largest genus of this family (Kukenthal 1936; Rad & Sonboli 2008). Approximate 70 species of *Cyperus* reported from India (Prasad & Singh 2002).

Due to morphological similarities and highly reduced flowers, the members of this family are quite difficult to identify (Reznicek 1990; Starr *et al.* 1999). So it is a major

concern to find out the additional diagnostic features for identification of the members.

The oldest and most comprehensive anatomical study of this family was conducted by Metcalfe (1971), comprising the anatomy of the leaves and stems of 280 species belonging to 90 genera, among them 18 species of *Cyperus* had been presented.

Several studies have demonstrated the utility of anatomical characters in the taxonomy of closely related species. For example, Govindarajalu (1974) employed anatomical characters to separate three subgenera of *Cyperus*. Similarly, Reznicek & Catling (1986) established the role of vegetative morphology and anatomy in the

taxonomy of *Carex* (Cyperaceae). Importance of anatomical study for taxonomy was emphasized by Standley (1990), who used anatomy of leaves for the taxonomical and phylogenetic classification within the genus *Carex*. Asper Hejazi *et al.* (2012) micro-morphological characters, especially qualitative characters are useful for *Carex* (Cyperaceae) identification. Based on the qualitative and quantitative morpho-anatomical characters Plunkett *et al.* (2013) recognized new species of *Lepidosperma* (Cyperaceae) from the mountain of Tasmania. Three species of *Mapania* (Cyperaceae) were taxonomically distinguished by Silva *et al.* (2014) based on basal leaf and involucre bract anatomy. Pashirzad *et al.* (2014) used morphological and nut micro-morphological data in identification of Iranian *Cyperus*.

Evaluation of the genus *Cyperus* in relation to the foliage leaf and bract leaf anatomical trait is still unknown. The present study aimed to identify the anatomically diagnostic character and find out the possible phenetic relationship based on foliage leaf and bract leaf anatomy of studied species, collected from of Burdwan (23.4595° N, 87.6186° E) and Nadia (23.4710° N, 88.5565° E) district, West Bengal.

## Materials and Methods

Three specimens of *Cyperus difformis* L., *Cyperus rotundus* L.; two of *Cyperus imbricatus* Retz., *Cyperus michelianus* subsp. *pygmaeus* (Rottb.) Asch. & Graebn and one of *Cyperus cyperoides* (L.) Kuntze, *Cyperus iria* L. were collected from three different locations (such as Churnipota (23.3903° N, 88.4486°E), Golapbag (23.2521° N, 87.8434°E), Nabagram (23.1136° N, 88.0578°E) for anatomical study. To minimize the risk, we collected two ramets for each sample from the same site.

The herbarium specimens were deposited at Burdwan University Herbarium (BURD), Department of Botany, West Bengal, India. Collection details are given in **Tab. 1**.

A fully developed foliage leaf and largest bract leaf were removed and placed in Formalin-Aceto-Alcohol (FAA),

from which sections of each leaf organ were made. Leaves were hand sectioned with a razor blade and sections were cleared in Lactic acid (50%). After that, the sections were stained with Safranin (0.1%) if needed. Then the section was mounted by Glycerol (10%). The mounted semi-permanent slides were micro-photographed with Leica DC295 attached with Leica DM1000 microscope and photographs were analyzed by using Digimizer (ver. 4.3.0), an image analyzing software.

Fifty qualitative characters (**Tab. 2**) of collected samples were compared and phenetic similarity was also determined using Past 3.14. The terminologies used to describe the anatomy of leaves were adopted from Metcalfe (1971), Bruhl (1995) & Bugg *et al.* (2013).

## Result and Discussion

The result obtained from the cluster analysis (**Fig. 1**) indicate that six species grouped in two major clusters (A and B) based on two characters, i.e. angle of foliage leaf margin and transverse septum morphology in bract leaf. The first cluster consisted of three species including *C. cyperoides*, *C. iria*, and *C. michelianus* subsp. *pygmaeus* while the other cluster, consisting of *C. difformis*, *C. imbricatus*, and *C. rotundus*. The analysis indicates that foliage leaf bulliform cells layer, foliage leaf sclerenchyma form, bract leaf bulliform cells layer, bract leaf sclerenchyma form and position of sclerenchyma in relation to vascular bundle in bract leaf are uniform across the studied species.

In cluster A (**Fig. 2**), *C. cyperoides* (**Fig. 3 J-L and Fig. 4 D-F**) can be distinguished from *C. iria* (**Fig. 4 G-H and Fig. 5 D-E**) and *C. michelianus* subsp. *pygmaeus* (**Fig. 4 B-C and Fig. 5 A-C**) on the basis of foliage leaf blades maximum width, foliage leaf to bract leaf length ratio, total number of vascular bundles in foliage leaf, foliage leaf blade margins morphology, depth of keel in foliage leaf, bract leaf blades maximum width, bract leaf air cavities, major vascular bundles position in relation to air cavities in bract leaf, major vascular bundles position in

**Table 1.** Collection details of the studied species.

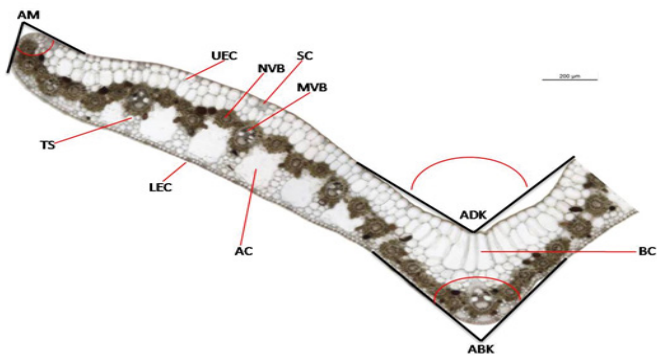
Name of the species	Accession No	Collection site with coordination	Collection date
<i>Cyperus imbricatus</i> Retz.	BURD12019	Churnipota (23.3903° N, 88.4486°E)	23-04-2017
	BURD12029	Golapbag (23.2521° N, 87.8434°E)	25-05-2017
<i>Cyperus difformis</i> L.	BURD12020	Churnipota (23.3903° N, 88.4486°E)	23-04-2017
	BURD12028	Golapbag (23.2521° N, 87.8434°E)	25-05-2017
	BURD12031	Nabagram (23.1136° N, 88.0578°E)	15-06-2017
<i>Cyperus rotundus</i> L.	BURD12021	Churnipota (23.3903° N, 88.4486°E)	23-04-2017
	BURD12024	Golapbag (23.2521° N, 87.8434°E)	25-05-2017
	BURD12040	Nabagram (23.1136° N, 88.0578°E)	15-06-2017
<i>Cyperus michelianus</i> subsp. <i>pygmaeus</i> (Rottb.) Asch. & Graebn.	BURD12025	Golapbag (23.2521° N, 87.8434°E)	25-05-2017
	BURD12042	Nabagram (23.1136° N, 88.0578°E)	15-06-2017
<i>Cyperus cyperoides</i> (L.) Kuntze	BURD12030	Golapbag (23.2521° N, 87.8434°E)	25-05-2017
<i>Cyperus iria</i> L.	BURD12039	Nabagram (23.1136° N, 88.0578°E)	15-06-2017

**Table 2.** Foliage leaf and bract leaf characters with character states of collected samples.

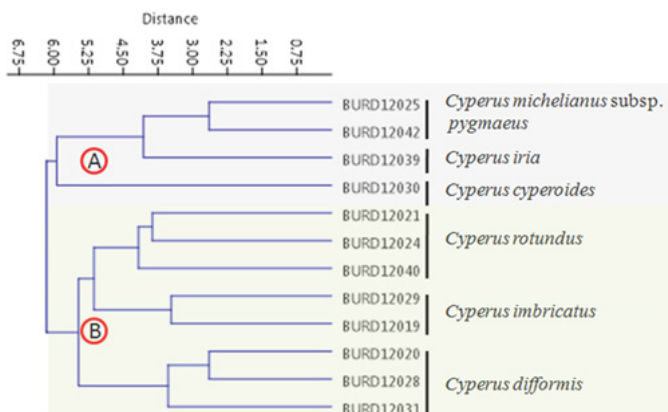
SL NO.	CHARACTERS AND CHARACTER STATES	<i>C. imbricatus</i>		<i>C. difformis</i>			<i>C. rotundus</i>		<i>C. michelianus</i> subsp. <i>Pygmaeus</i>		<i>C. cyperoides</i> <i>C. iria</i>		
		BURD12019	BURD12029	BURD12020	BURD12028	BURD12031	BURD12021	BURD12024	BURD12040	BURD12025	BURD12042	BURD12030	BURD12039
1	Foliage leaf blades (maximum length) 1. More than 10 cm; 2. Less than 10 cm	1	1	1	1	1	1	1	2	2	1	1	
2	Foliage leaf blades (maximum width in middle) 1. More than 2 mm; 2. 2 mm or less than 2 mm	1	1	1	1	2	2	1	1	2	2	1	2
3	Foliage leaf to bract leaf length ratio 1. Less than 1:1; 2. 1:1; 3. More than 1:1	2	3	1	1	1	1	1	3	3	1	3	
4	Foliage leaf to bract leaf width ratio 1. Less than 1:1; 2. 1:1; 3. More than 1:1	3	2	1	2	2	2	2	1	3	2	2	2
5	Foliage leaf blade trans-section (in middle) 1. V-shaped; 2. Flanged V-shaped (includes inversely W-shaped)	1	1	1	1	1	2	2	2	1	1	1	2
6	Total number of vascular bundles in foliage leaf 1. More than 50; 2. Less than 50	1	1	2	2	2	2	1	1	2	2	1	2
7	Presence of air cavities in foliage leaf 1. Present; 2. Absent	1	1	1	1	1	1	1	1	2	2	2	1
8	Major vascular bundles position in relation to air cavities in foliar leaf 1. Alternate; 2. Absent	1	1	1	1	1	1	1	1	2	2	2	1
9	Major vascular bundles position in foliage leaf 1. Closer to abaxial surface; 2. More or less middle; 3. Closer to adaxial surface	3	3	1	1	1	3	3	1	1	1	1	2
10	Presence of minor vascular bundles 1. Present; 2. Absent	1	1	2	2	2	1	1	1	1	1	1	1
11	Minor vascular bundles position in foliage leaf 1. Closer to abaxial surface; 2. More or less middle; 3. Closer to adaxial surface; 4. Not applicable	3	3	4	4	4	3	3	2	1	1	1	3
12	Total number of bulliform cells in foliage leaf 1. More than 7; 2. Less than 7	1	2	2	2	2	2	2	2	2	2	1	1
13	Foliage leaf bulliform cells morphology 1. Similar to epidermal cells; 2. Dissimilar to epidermal cells	2	2	2	2	1	2	2	2	1	1	2	2
14	Foliage leaf bulliform cells layer 1. Single layered; 2. Multiple layered	1	1	1	1	1	1	1	1	1	1	1	1
15	Foliage leaf blade margins morphology 1. Armed with prickle-hairs; 2. Without prickle-hairs	1	1	2	2	2	2	2	2	2	2	1	2
16	Foliage leaf epidermis layer 1. Continuously single-layered; 2. Continuously bi-layered; 3. mixed	1	1	1	1	1	2	1	3	1	1	3	1
17	Foliage leaf mestome sheaths form 1. Chlorenchymatous; 2. Non-chlorenchymatous	1	1	2	2	2	1	1	1	1	1	1	1
18	Foliage leaf sclerenchyma form 1. Forming strands (above and/or below the vascular bundles); 2. Forming (adaxial and/or abaxial) girders; 3. Forming caps (above and/or below the vascular bundles)	1	1	1	1	1	1	1	1	1	1	1	1

19	Depth of keel in foliage leaf 1. More than 300 µm; 2. Less than 300 µm	1	1	1	1	2	1	1	1	2	2	1	2
20	Ratio of adaxial to abaxial epidermal cells in foliar leaf 1. More than 3:1; 2. Less than 3:1	1	1	1	2	2	1	1	1	2	1	1	2
21	Position of sclerenchyma in relation to vascular bundles in foliage leaf 1. Opposite; 2. Alternate and opposite	1	1	1	1	1	2	1	1	1	1	1	1
22	Angle of foliage leaf margin 1. More than 60°; 2. Less than 60°	1	1	1	1	1	1	1	1	2	2	2	2
23	Angle of foliage leaf keel at adaxial side 1. More than 90°; 2. Less than 90°	1	2	2	1	2	1	1	1	1	2	1	2
24	Angle of foliage leaf keel at abaxial side 1. More than 90°; 2. Less than 90°	1	1	2	1	2	2	2	2	2	2	2	2
25	Maximum thickness at one half middle of foliage leaf 1. More than 300 µm; 2. Less than 300 µm	1	1	1	1	2	2	1	2	2	2	2	2
26	Transverse septum between air chamber in foliage leaf 1. Bicellular; 2. Multicellular; 3. Heterogenous; 4. Absent	2	2	2	2	2	1	3	3	4	3	2	3
27	Bract leaf blades (maximum length) 1. More than 10 cm; 2. Less than 10 cm	1	1	1	1	2	1	1	2	2	2	1	1
28	Bract leaf blades (maximum width in middle) 1. More than 2 mm; 2. 2 mm or less than 2 mm	1	1	2	1	2	2	1	2	2	2	1	2
29	Bract leaf blade trans-section (in middle) 1. V-shaped; 2. Flanged V-shaped (includes inversely W-shaped, and plicate)	1	2	1	1	1	2	2	2	1	1	1	1
30	Total number of vascular bundles in bract leaf	1	1	2	2	2	2	1	1	2	1	1	2
31	Bract leaf air cavities 1. Present; 2. Absent	1	1	1	1	1	2	2	2	2	2	1	2
32	Major vascular bundles position in relation to air cavities in bract leaf 1. Opposite; 2. Alternate; 3. Not applicable	2	2	2	2	2	3	3	3	3	3	2	3
33	Major vascular bundles position in bract leaf 1. Closer to abaxial surface; 2. More or less middle; 3. Closer to adaxial surface	3	3	2	2	1	2	2	2	2	2	1	2
34	Presence of minor vascular bundle in bract leaf 1. Present; 2. Absent	1	1	2	2	2	1	1	1	1	1	1	1
35	Minor vascular bundles position in bract leaf 1. Closer to abaxial surface; 2. More or less middle; 3. Closer to adaxial surface; 4. Not applicable	3	3	4	4	4	2	2	2	2	2	1	1
36	Total number of bulliform cells in bract leaf 1. More than 7; 2. Less than 7	2	1	2	1	2	1	1	1	2	1	1	2
37	Bract leaf bulliform cells morphology 1. Similar to epidermal cells; 2. Dissimilar to epidermal cells	1	1	1	1	2	2	2	2	2	2	2	2
38	Bract leaf bulliform cells layer 1. Single layered; 2. Doubled layered; 3. Multiple layered	1	1	1	1	1	1	1	1	1	1	1	1
39	Bract leaf blade margin morphology 1. Armed with prickles-hairs; 2. Without prickles-hairs	1	1	2	2	2	2	2	2	2	2	1	2
40	Bract leaf epidermis layer 1. Continuously single layered; 2. Continuously double layered; 3. Mixed	1	1	1	1	1	2	1	1	1	1	3	1
41	Bract leaf mestome sheaths form 1. Chlorenchymatous; 2. Non-chlorenchymatous	1	1	2	2	2	1	1	1	1	1	1	1

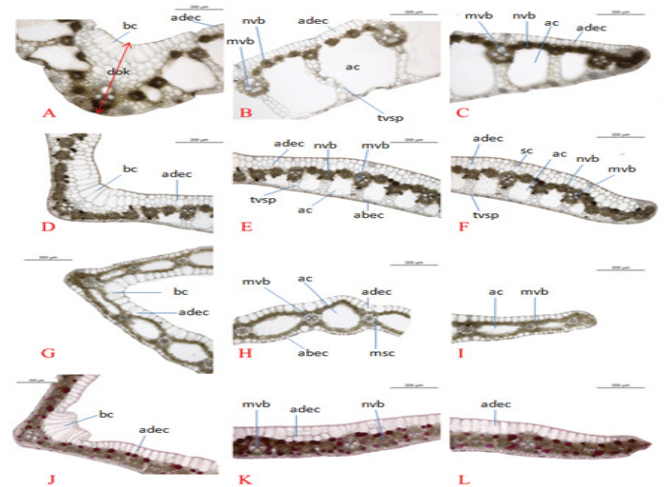
Bract leaf sclerenchyma form														
42	1. Forming strands (above and/or below the vascular bundles);	1	1	1	1	1	1	1	1	1	1	1	1	1
	2. Forming (adaxial and/or abaxial) girders;													
	3. Forming caps (above and/or below the vascular bundles)													
Depth of keel in bract leaf														
43	1. More than 300 µm;	1	1	1	1	1	1	2	2	2	1	2		
	2. Less than 300 µm													
Angle of bract leaf margin														
44	1. More than 60°;	2	1	2	2	1	1	2	2	2	2	1	2	
	2. Less than 60°													
Ratio of adaxial to abaxial epidermal cells in bract leaf														
45	1. More than 3:1;	1	1	2	2	2	1	2	1	1	1	1	2	
	2. Less than 3:1													
Position of sclerenchyma in relation to vascular bundle in bract leaf														
46	1. Opposite;	1	1	1	1	1	1	1	1	1	1	1	1	
	2. Alternate													
Angle of keel at adaxial side of bract leaf														
47	1. More than 90°;	2	2	2	2	1	2	1	1	2	2	1	2	
	2. Less than 90°													
Angle of keel at abaxial side of bract leaf														
48	1. More than 90°;	1	2	2	1	2	2	2	2	2	1	1	2	
	2. Less than 90°													
Transverse septum between air chamber in bract leaf														
49	1. Absent;	4	2	3	3	3	3	3	3	1	1	1	1	
	2. Bicellular;													
	3. Multicellular;													
	4. Heterogenous													
Maximum thickness at one half middle of bract leaf														
50	1. More than 300 µm;	1	1	2	2	2	2	2	2	2	2	2	2	
	2. Less than 300 µm													



**Figure 1.** Transverse section of foliage leaf, *Cyperus rotundus*: one half portion of lamina showing bulliform cells (BC), air chamber (AC), lower epidermal cell (LEC), transverse septum between air chambers (TS), upper epidermal cells (UEC), major vascular bundle (MVB), sclerenchyma cells (SC); angle of the leaf margin (AM), angle of the keel at a abaxial side (ABK).



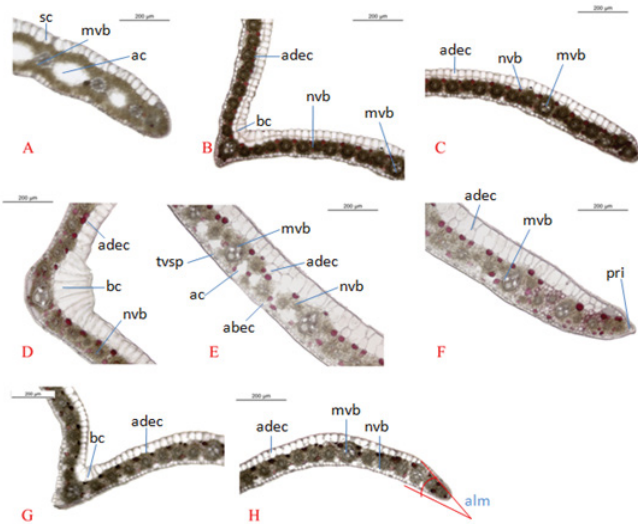
**Figure 2.** Phenogram resulting from cluster analysis of six species of *Cyperus* using Ward's method and correlation similarity index, Coph. Corr. 0.8741 (Table 2 for characters and character states).



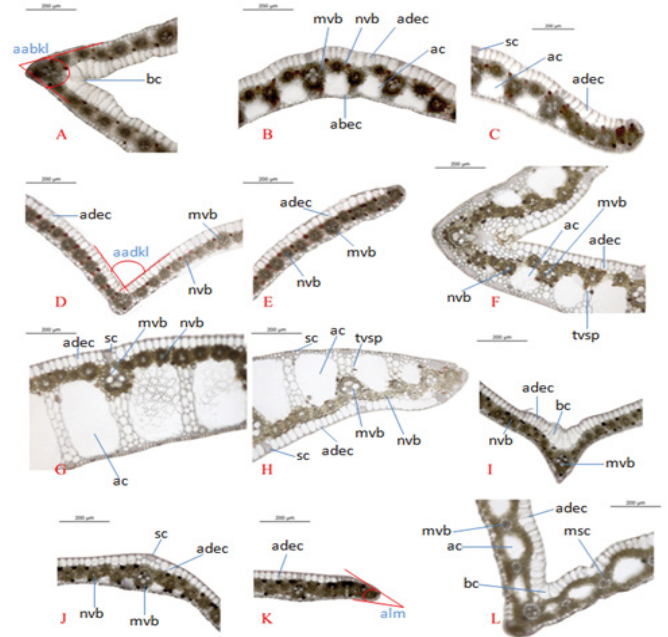
**Figure 3.** A-C trans-section of foliage leaf of *Cyperus imbricatus* showing keel region, middle portion and marginal portion; D-F trans-section of foliage leaf of *C. rotundus* showing keel region, middle portion and marginal portion; G-I trans-section of foliage leaf of *C. difformis* showing keel region, middle portion and marginal portion; J-L trans-section of bract leaf of *C. cyperoides* showing keel region, middle portion and marginal portion. (dok-depth of the keel, bc-bulliform cell, mvb-major vascular bundle, nvb-minor vascular bundle, adec- adaxial epidermal cell, abec- abaxial epidermal cell, ac-air chamber, sc-sclerenchyma).

bract leaf, bract leaf blade margin morphology, and angle of keel at adaxial side of bract leaf (**Tab. 2.**).

In cluster B, *C. imbricatus* (**Fig. 3. A-C and Fig. 5. F-H**) and *C. rotundus* (**Fig. 3. D-F and Fig. 5. I-K**) forms a subgroup of *C. difformis* (**Fig. 3. G-I and Fig. 4. A, and Fig. 5. L**). The separable characters of the species are presence minor vascular bundles, minor vascular bundles position in foliage leaf, foliage leaf mestome sheaths form, presence of minor vascular bundles in bract leaf, minor vascular



**Figure 4.** A trans section of bract leaf of *Cyperus difformis* showing marginal to middle portion; B-C trans-section of bract leaf of *C. michelianus* subsp. *pygmaeus* showing keel region, a middle portion, and marginal portion; D-F trans-section of bract leaf of *C. cyperoides* showing keel portion, middle portion to marginal portion; G-H trans-section of bract leaf of *C. iria* showing keel portion to middle portion and middle portion to marginal portion. (bc-bulliform cell, mvb-major vascular bundle, nvb- minor vascular bundle, ad-adaxial epidermal cell, adec-abaxial epidermal cell, ac-air chamber, sc-sclerenchyma, tvsp-transverse septum between air chambers, alm-anglr of leaf margin, pri-prickle hair base, msc- mestome sheath).



**Figure 5.** A-C trans-section of foliage leaf of *Cyperus michelianus* subsp. *pygmaeus* showing keel portion, middle portion and marginal portion; D-E trans-section of foliage leaf of *C. iria* showing keel portion to middle portion and middle portion to marginal portion; F-H trans-section of foliage leaf of *C. imbricatus* showing keel portion, middle portion and marginal portion; I-K trans-section of foliage leaf of *C. rotundus* showing keel portion, middle portion and marginal portion; L trans-section of foliage leaf of *C. difformis* showing keel portion, (aabkl- angle of keel at abaxial side, bc- bulliform cell, mvb-major vascular bundle, nvb-minor vascular bundle, abec- adaxial epidermal cell, abec-abaxial epidermal cell, ac- air chamber, sc-sclerenchyma, aadkl-depth of the keel at adaxial side, tvsp-transverse septum between air chambers, alm-angle of leaf margin, msc- mestome sheath).

**Table 3 (suppl).** Foliage leaf and bract leaf characters with character states of collected samples.

SL NO.	CHARACTERS	<i>C. imbricatus</i>			<i>C. difformis</i>			<i>C. rotundus</i>			<i>C. michelianus</i> subsp. <i>Pygmaeus</i>		<i>C. cyperoides</i>		<i>C. iria</i>	
		BURD12019	BURD12029	BURD12020	BURD12028	BURD12031	BURD12021	BURD 2024	BURD12040	BURD12025	BURD12042	BURD12030	BURD12039			
1	Foliage leaf blades (maximum length (in cm))	13.5	25.5	14	16.5	11	25.5	31.8	11.6	4.7	4.8	26.2	10.9			
2	Foliage leaf blades (maximum width (in mm) in mid-point)	4	4	4	3	2	2	3	3.5	1.5	1	3	2			
3	Foliage leaf (lamina) to bract leaf length ratio															
	1. Less than 1:1	2	3	1	1	1	1	1	1	3	3	1	3			
	2. 1:1															
4	Foliage leaf (lamina) to bract leaf width ratio															
	1. Less than 1:1	3	2	1	2	2	2	2	1	3	2	2	2			
	2. 1:1															
5	Foliage leaf blade trans-section (middle)															
	1. 'V-shaped'	1	1	1	1	1	2	2	2	1	1	1	2			
	2. 'flanged V-shaped' (includes inversely W-shaped, and plicate)															
6	Total number of vascular bundles (major + minor) in foliage leaf	81	90	15	13	11	39	78	66	28	43	60	46			
7	Foliage leaf air cavities (whether present)															
	1. present	1	1	1	1	1	1	1	1	2	2	2	1			
	2. absent															

8	Major vascular bundles position in relation to parenchyma in foliar leaf																			
	1. Alternate																			
	2. Not applicable																			
9	Major vascular bundles position in foliage leaf																			
	1. Closer to abaxial surface.																			
	2. More or less middle.																			
	3. Closer to adaxial surface																			
10	Minor vascular bundles 1. Present																			
	2. Absent																			
11	Minor vascular bundles position in foliage leaf																			
	1. Closer to abaxial surface.																			
	2. More or less middle.																			
	3. Closer to adaxial surface																			
	4. Not applicable																			
12	Total number of bulliform cells in foliage leaf																			
13	Foliage leaf bulliform cells morphology																			
	1. Similar to epidermal cells.																			
	2. Dissimilar to epidermal cells.																			
14	Foliage leaf bulliform cells layer 1. Single layered																			
	2. Multiple layered																			
15	Foliage leaf blade margins (whether armed with prickle-hairs)																			
	1. Armed with prickle-hairs																			
	2. without prickle-hairs																			
16	Foliage leaf epidermis layer 1. Continuously single-layered																			
17	Foliage leaf mestome sheaths (primary bundles; whether chlorenchymatous)																			
	1. chlorenchymatous																			
	2. 'non-chlorenchymatous' (never with abundant chloroplasts)																			
18	Foliage leaf sclerenchyma (form)																			
	1. Forming strands (above and/or below the vascular bundles)																			
	2. Forming (adaxial and/or abaxial) girders																			
	3. forming caps (above and/or below the vascular bundles)																			
19	Depth of keel in foliage leaf (in µm)																			
20	Ratio of adaxial to abaxial epidermal cells in foliar leaf																			
21	Position of sclerenchyma in relation to vascular bundle in foliage leaf																			
	1.opposite																			
	2.alternate and opposite																			
22	Angle of foliage leaf margin(°)																			
23	Angle of keel at adaxial side(°)																			
24	Angle of keel at abaxial side(°)																			
25	Maximum thickness at one half middle (in µm)																			
26	Transverse septum																			
	1. Bicellular																			
	2. Multicellular																			
	3. Heterogenous																			
	4. Absent																			
27	Bract leaf blades (maximum length (in cm))																			
28	Bract leaf blades (maximum width (in mm) in mid-point)																			
29	Bract leaf blade trans-section (middle)																			
	1. 'V-shaped' (with or without a median adaxial groove)																			
	2. 'flanged V-shaped' (includes inversely W-shaped, and plicate)																			
30	Total number of vascular bundles (major + minor) in bract leaf																			
31	Bract leaf air cavities (whether present)																			
	1. present																			
	2. absent																			

32	Major vascular bundles position in relation to aerenchyma in bract leaf 1. Opposite 2. Alternate 3. Not applicable	2	2	2	2	2	2	2	3	3	3	3	3	2	3
33	Major vascular bundles position in bract leaf 1. Closer to abaxial surface. 2. More or less middle. 3. Closer to adaxial surface.	3	3	2	2	1	2	2	2	2	2	2	2	1	2
34	Minor vascular bundle 1. Present 2. absent	1	1	2	2	2	1	1	1	1	1	1	1	1	1
35	Minor vascular bundles position in bract leaf 1. Closer to abaxial surface. 2. More or less middle. 3. Closer to adaxial surface. 4. Not applicable	3	3	4	4	4	2	2	2	2	2	2	2	1	1
36	Total number of bulliform cells in bract leaf	5	9	5	8	6	9	9	8	6	9	6	9	9	5
37	Bract leaf bulliform cells morphology 1. Similar to epidermal cells. 2. Dissimilar to epidermal cells.	1	1	1	1	2	2	2	2	2	2	2	2	2	2
38	Bract leaf bulliform cells layer 1. Single layered 2. Doubled layered 3. Multiple layered	1	1	1	1	1	1	1	1	1	1	1	1	1	1
39	Bract leaf blade margins (whether armed with prickle-hairs) 1. Armed with prickle-hairs 2. without prickle-hairs	1	1	2	2	2	2	2	2	2	2	2	2	1	2
40	Bract leaf epidermis layer (whether present) 1. continuously single-layered 2. continuously double layered 3. mixed	1	1	1	1	1	2	1	1	1	1	1	1	3	1
41	Bract leaf mestome sheaths (primary bundles; whether chlorenchymatous) 1. chlorenchymatous 2. 'non-chlorenchymatous' (never with abundant chloroplasts)	1	1	2	2	2	1	1	1	1	1	1	1	1	1
42	Bract leaf sclerenchyma (form) 1. Forming strands (above and/or below the vascular bundles) 2. Forming (adaxial and/or abaxial) girders 3. forming caps (above and/or below the vascular bundles)	1	1	1	1	1	1	1	1	1	1	1	1	1	1
43	Depth of keel in bract leaf (in $\mu\text{m}$ )	378.9	312.38	9618.1	342.12	415.28	353.72	380.88	271.51	226.97	131.87	318.52	245.8		
44	Angle of bract leaf margin( $^{\circ}$ )	55.5	65.2	41.6	55.5	78.6	80.1	25.2	47.6	35.8	28.6	81	28.2		
45	Ratio of adaxial to abaxial epidermal cells in bract leaf	5.5:1	4.33:1	2.4:1	1.97:1	2.20:1	3.8:1	2.94:1	3.89:1	4.4:1	3.2:1	6.47:1	2.56:1		
46	Position of sclerenchyma in relation to vascular bundle in bract leaf 1. opposite 2. alternate	1	1	1	1	1	1	1	1	1	1	1	1	1	1
47	Angle of keel at adaxial side( $^{\circ}$ )	40.4	21.58	86.37	55.72	95.98	71.38	106.9	124.1	79.86	84.76	92.36	63.34		
48	Angle of keel at abaxial side( $^{\circ}$ )	95.31	87.81	89.99	92.63	65.7	59.34	36.9	58.49	69.05	96.05	90.46	57.32		
49	Transverse septum 1. Absent 2. Bicellular 3. Multicellular 4. Heterogenous	4	2	3	3	3	3	3	3	1	1	1	1		
50	Maximum thickness at one half middle (in $\mu\text{m}$ )	560	550.3	284.7	254.8	233.4	278.9	151.3	197	157.6	127	267.7	156.9		

bundles position in bract leaf and bract leaf mestome sheaths form (Tab. 2).

Foliage leaf to bract leaf width ratio, major vascular bundles position in foliage leaf, minor vascular bundles position in foliage leaf, ratio of adaxial to abaxial epidermal cells in foliar leaf, angle of foliage leaf keel at adaxial side, maximum thickness at one half middle of

foliage leaf, total number of vascular bundles in bract leaf, total number of bulliform cells in bract leaf, angle of bract leaf margin, angle of keel at abaxial side of bract leaf (Tab. 2) varies between sample to sample of *C. imbricatus*, *C. difformis*, *C. rotundus*, and *C. michelianus* sub sp. *pygmaeus*.

Presence of air cavities in foliage leaf, major vascular



**Table 4.** Correlation of variation among studied samples.

	BURD12019	BURD12029	BURD12020	BURD12028	BURD12031	BURD12021	BURD12024	BURD12040	BURD12025	BURD12042	BURD12030	BURD12039
BURD12019	1	0.8	0.6	0.7	0.5	0.57	0.7	0.5	0.24	0.2	0.08	0.3
BURD12029	0.82	1	0.6	0.6	0.4	0.51	0.7	0.4	0.29	0.3	0.05	0.39
BURD12020	0.62	0.6	1	0.9	0.8	0.43	0.6	0.5	0.12	0.1	0	0.24
BURD12028	0.68	0.6	0.9	1	0.8	0.43	0.6	0.4	0.16	0.1	0	0.2
BURD12031	0.5	0.4	0.8	0.8	1	0.4	0.5	0.4	0.21	0.1	0	0.24
BURD12021	0.57	0.5	0.4	0.4	0.4	1	0.7	0.6	0.08	0.1	0.19	0.25
BURD12024	0.71	0.7	0.6	0.6	0.5	0.69	1	0.7	0.32	0.3	0.21	0.47
BURD12040	0.48	0.4	0.5	0.4	0.4	0.57	0.7	1	0.38	0.4	0.38	0.34
BURD12025	0.24	0.3	0.1	0.2	0.2	0.08	0.3	0.4	1	0.9	0.39	0.68
BURD12042	0.18	0.3	0.1	0.1	0.1	0.07	0.3	0.4	0.88	1	0.38	0.61
BURD12030	0.08	0.1	0	0	0	0.19	0.2	0.4	0.39	0.4	1	0.23
BURD12039	0.3	0.4	0.2	0.2	0.2	0.25	0.5	0.3	0.68	0.6	0.23	1

bundles position in relation to air cavities in foliar leaf, presence of minor vascular bundles, morphology of foliage leaf blade margins, total number of bulliform cells in foliage leaf, foliage leaf mestome sheaths form, angle of foliage leaf margin, bract leaf air cavities, major vascular bundles position in relation to air cavities in bract leaf, presence of minor vascular bundles in bract leaf, bract leaf blade margin morphology, bract leaf mestome sheaths form, depth of keel in bract leaf, maximum thickness at one half middle of bract leaf (**Tab. 2.**) are possible inter-specifically variable characters (**supp. Tab. 3.**). These characters can be used as the diagnostic character among the studied taxa.

As suggested by Standley (1987), the ratio of adaxial and abaxial epidermal cell is a taxonomically useful character for *Carex* sect. *Phacocystis* (Cyperaceae). But the present study shows that it is also useful for studied species. The value is always more than 2 and reached up to 7.99 in foliage leaf of *C. cyperoides*. But it shows variation at the species level (**supp. Tab. 3.**). It is constantly  $\geq 4.5$  in case of *C. imbricatus* (**supp. Tab. 3.**).

The presence and absence prickles on the margins of the foliage leaves show distinct difference among *Carex* sect. *Phyllostachys* (Starr & Ford 2001). In the present study, only *C. imbricatus* and *C. cyperoides* contain prickles and it is useful to separate the species among groups (**Tab. 4.**).

Govindarajalu (1974) reported that occurrence of 'V' shaped foliage leaf in trans-section, 5 celled bulliform cells in foliage leaf, the presence of air cavities in foliage leaf, 23 vascular bundles in foliage leaf were characteristic features of *C. pygmaeus* Rottb. In our study *C. michelianus* subsp. *pygmaeus* (it was previously treated as *C. pygmaeus* Rottb.) shows similar result though the number of bulliform cells varies from 5 to 6, number of the vascular

bundle in foliage leaf ranges from 28 to 43. Govindarajalu (1974) also reported that presence of prickles at leaf margin, 'V' shaped foliage leaf in trans-section, 75 vascular bundles in foliage leaf, 11-12 bulliform cells in *C. cyperoides* but present investigation shows that the number of vascular bundle in foliage leaf may reach up to 60 and number bulliform cells may vary from 10 to 11 in *C. cyperoides*.

The variability in a number of bulliform cells has already been reported in *Carex hirta* by Molina *et al.* (2006); Bugg *et al.* (2013) and is confirmed in the present studied species.

Rad & Sonboli (2008) observed *C. rotundus* having flanged 'V' shaped foliage leaf in trans-section, the presence of air cavities and bulliform cells in adaxial side of midrib. Dhyan (2017) also reported single layered bulliform cells are distributed in upper epidermis of *C. rotundus*. The data presented in this investigation shows bulliform cells are specifically distributed in keel region of adaxial epidermis; it also includes the width of the foliage leaf in trans-section may vary from 20-30 mm. Bugg *et al.* (2013) suggested that, shape of the transverse section of leaves are useful as taxonomic characters which supports by the present study but the other characters like, the relative size of adaxial and abaxial epidermal cells in foliage leaf, the number of layers of bulliform cells in foliage leaf, the size relationship between bulliform and epidermal cells in foliage leaf, the number of vascular bundle in foliage leaf, the depth of the keel in foliage leaf, suggested by them are not useful for identification of the studied species at species level due to variation.

#### Identification key to the *Cyperus* species

- 1a. The angle of foliar leaf margin more than 60°;

transverse septum between air chamber bicellular, multicellular or heterogeneous. **2**

**2a.** Foliage leaf and bract leaf margin armed with prickles-hairs, Foliage leaf to bract leaf length ratio 1:1, foliage leaf, width more than 20 mm, vascular bundle more than 50, depth of the keel more than 300µm; bract leaf, major vascular bundle closer to abaxial surface, angle of keel at adaxial side more than 90°. *Cyperus cyperoides*

**2b.** Foliage leaf and bract leaf margin without prickles-hairs, foliage leaf to bract leaf length ratio more than 1:1, Foliage leaf, width 20mm or less, vascular bundle less than 50, depth of the keel less than 300µm; bract leaf, major vascular bundle more or less middle of the leaf, angle of keel at adaxial side less than 90°. **3**

**3a.** Foliage leaf blades, more than 10cm long, flanged 'V' shaped in trans-section, air cavities present, minor vascular bundles closer to the adaxial surface, major vascular bundle more or less middle; bulliform cells more than 7, bulliform cells larger than epidermal cells. *Cyperus iria*

**3b.** Foliage leaf blades less than 10cm long, 'V' shaped in trans-section, air cavities absent, minor vascular bundles closer to the abaxial surface, major vascular bundle closer to the abaxial surface; bulliform cells less than 7, bulliform cells similar with epidermal cells *Cyperus michelianus* subsp. *pygmaeus*

**1b.** The angle of foliar leaf margin less than 60°; transverse septum between air chamber absent. **4**

**4a.** Mestome sheath non-chlorenchymatous in bract leaf and foliage leaf, minor vascular bundles absent *Cyperus difformis*

**4b.** Mestome sheath chlorenchymatous in bract leaf and foliage leaf, minor vascular bundles present. **5**

**5a.** Foliage leaf and bract leaf margin armed with prickles hairs; foliage leaf 'V' shaped in trans section, the angle of the keel at abaxial side more than 90°; bract leaf, air cavities present, bulliform cells similar with epidermal cells, the maximum thickness at one-half middle of lamina more than 300 µm *Cyperus imbricatus*

**5b.** Foliage leaf and bract leaf margin without prickles hairs; foliage leaf blade flanged 'V' shaped in trans-section, the angle of the keel at abaxial side less than 90°; bract leaf, air cavities absent, bulliform cells larger than epidermal cells, the maximum thickness at the one-half middle of lamina less than 300 µm *Cyperus rotundus*

*Cyperus rotundus*

## Conclusion

It can be concluded that out of 50 evaluated anatomical characters 14 characters of foliage leaf and bract leaf of

the studied species (like, presence of air cavities in foliage leaf, major vascular bundles position in relation to air cavities in foliar leaf, presence of minor vascular bundle, morphology of foliage leaf blade margins, total number of bulliform cells in foliage leaf, foliage leaf mestome sheaths form, angle of foliage leaf margin, bract leaf air cavities, major vascular bundles position in relation to air cavities in bract leaf, presence of minor vascular bundle in bract leaf, bract leaf blade margin morphology, bract leaf mestome sheaths form, depth of keel in bract leaf, maximum thickness at one half middle of bract leaf) provide valuable anatomical feature that is helpful for taxonomic delimitation of the studied species. It is also noted that the resolution of these characters for species identification is higher when all the character are used simultaneously rather than specific individual character.

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