

FOLIAR EPIDERMAL MORPHOLOGY OF THE GENERA *ANEILEMA* AND *COMMELINA* (COMMELINACEAE)

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ABSTRACT

A Comparative study of the foliar epidermal morphology of some members of the genera *Commelina* and *Aneilema* was undertaken. The aim was to study the structure of the foliar epidermis of five species belonging to the two genera and thus provide anatomical information that will help to possibly eliminate identification problems that exist among species of the genera using diagnostic characters of the foliar epidermal morphology. Epidermal peels of both the adaxial and abaxial surfaces of members of the genera were made using standard method and photomicrographs of the surfaces were made. Qualitative and quantitative characters were observed, measured and recorded. Results show that the preponderance of hexacytic stomata is classificatory of the two genera. Rectangular to polygonal epidermal cells on the adaxial lamina is diagnostic of *Aneilema aequinoctiale*. Result also reveals that simple hooked bicellular and unicellular non glandular trichome is diagnostic of *Commelina benghalensis* and *Aneilema aequinoctiale* respectively. Similarities exist in foliar epidermal characters like the trichome and stomata types among other characters between the genera *Commelina* and *Aneilema*. The low level of variability in the foliar epidermal morphology of the taxa studied show that foliar epidermal characters cannot stand alone in the separation of the taxa but need to be combined with characters from other lines of evidence.

Key words: *Aneilema*, Delimitation, Subtropical, Systematics, Trichome

INTRODUCTION

The family Commelinaceae R. Brown commonly called the Spiderwort family has c. 42 genera and 650 species (Cabezas et al. 2009). Members are widespread in tropical, subtropical and warm temperate regions (Faden, 1998). This family comprises genera which include- *Commelina* (170 species), *Tradescantia* (70 species), *Aneilema* (65 species), *Murdannia* (50 species), *Cyanotis* (50 species), *Dichorisandra* (30 species), *Zebrina* (4 species) and *Rheo* (1 species) (Faden, 2000). Hunt (1975 and 1986) reported that genera like *Zebrina*, *Rheo* and others have been reduced into synonyms under *Tradescantia* or *Callisia* and this same generic delimitation has been followed by Faden (1998). The family Commelinaceae has been divided into two subfamilies-Commelinoideae and Cartonematoidea (Faden, 2000). However, Faden and Hunt (1991) recognised three tribes representing the African genera of Commelinaceae. According to Faden & Inman (1996), the genera of Commelinaceae are readily distinguishable morphologically though their phylogenetic relationship is pending further research. The use of foliar anatomical characters in the family Commelinaceae is by no means new. Tomlinson (1966 and 1969) utilised anatomical characters in separating the genera *Aneilema* and

Murdannia that were previously taxonomically confused while Faden and Hunt (1991) similarly separated taxa that were wrongly grouped together using anatomical characters.

Stebbins and Jain (1960) gave a detailed description of the structure and development of the stomatal apparatus in leaves of *Commelina diffusa* while Kaushik (1971) provided information on the structure of the stomatal apparatus in five other species of *Commelina*. According to Stebbins and Jain (1960), the mature apparatus consists of the two guard cells whose aperture is “oriented parallel to the long axis of the leaf.” The guard cells, in turn, are surrounded by six subsidiary cells—a terminal subsidiary cell proximal (basal) to the guard cell pair, one distal (above) to the pair, and a pair of subsidiary cells (laterals) on either flank of the guard cells. According to Tomlinson (1966 and 1969), hairs on vegetative organs of members of Commelinaceae can be divided into non glandular microhairs (clavate trichomes), macrohairs and glandular hairs. In the latter group, the types found on *Commelina* are two-celled prickly hairs, hook hairs, and uniseriate hairs of differing numbers of cells. Members of the two genera have quite a lot of economic importance. Considerable research has confirmed that

[chimpanzees self-medicate](#) with *A. aequinoctiale* by swallowing the leaves whole. Though not normally a part of the chimpanzee's diet, this behaviour is most often witnessed during rainy seasons when the animals are most likely to be afflicted with the parasitic [nematodes](#) *Oesophagostomum stephanostomum* and other species of parasitic worms. Examination of the faecal matter of chimpanzees shows that the whole-swallowed leaves remain intact, along with multiple expelled worms (MacClancy *et.al*, 2009).

The main uses of *A. aequinoctiale* in Africa are medicinal, culinary, and domestic. In the Ondo regions of Nigeria, infants may be bathed in a wash made from the roots of *A. aequinoctiale*. In other regions, the roots may be used as feed for livestock, and leaves may be cooked like spinach. Ogbebor and Edeoga (2008) reported that *A. aequinoctiale* also has application in the treatment of children with Osteomalacia, thus helping them to walk. Similarly, the plant bodies of *Commelina benghalensis* and of several species of *Aneilema* are used in the treatment of leprosy and leucoderma (Mukerjee, 2006). According to Evans *et al.* (2000), though numerous taxonomic treatments have been produced for the family Commelinaceae, no consensus as to which characters should be used to define relationships among the genera have been reached.

In view of the general confusion associated with the identification of species of the two genera, a foliar epidermal study of some of the species in Nigeria was carried out to provide information on the epidermal morphology that could help in eliminating some of the identification and taxonomic problems that exist among the species and genera studied.

MATERIALS AND METHODS

The species of the family Commelinaceae used for this are *Commelina benghalensis* L., *C. diffusa* Burm.F., *C. lagosensis* C.B.C.L., *Aneilema aequinoctiale* (P.Beauv) Kunth and *A. umbrosum* (Vahl) Kunth. For the purpose of comparative anatomical work, small sizeable portions of the leaves (about 4mm x 4mm) were cut from the median parts of matured and well expanded leaves, that is, midway between the base and the apex.

Epidermal peels of the adaxial and abaxial surfaces of the leaves of the five species were also made. The required epidermis was obtained using the scrape method of Metcalfe (1989). The leaf samples of each species were placed on a clean glass slide with the interested surface turned face-down, the epidermis above being irrigated intermittently with distilled water placed in a Petri dish was carefully scraped with a sharp razor blade (or a dissecting knife) until the epidermis underneath was reached and the adhering long tissues were removed with a Carmel hair brush (Cutler, 1978). The epidermal peels were stained in 2% aqueous solution of Safranin O for about 3-5 minutes after which they were serially dehydrated using, 50%, 70% and 90% ethyl ethanol for 3-4 minutes interval each. The epidermal peels of the leaf specimens of the species were mounted in dilute glycerine solution after they were washed in 3-4 changes of water. For each of the species, about 20-25 measurements of the length and width of guard cells were taken from the adaxial and abaxial epidermal surfaces using a calibrated ocular micrometer at x40 objective lens. Stomata number/ frequency (which is the average number of stomata per square millimetre of leaf) was calculated. The epidermal peels were observed under a light microscope at x40 magnification. Features like types and distribution of trichomes, stomata, and shape of the epidermal cells were observed, measured and documented.

Also calculated was the stomata index.

The stomata index is expressed by the formula,

$$\text{Stomata index (S.I)} = 100S/(S+E)$$

Where

S.I. = Stomata Index,

S = number of stomata per unit area,

E = number of ordinary epidermal cells plus the subsidiary cells per field

Single Linkage Cluster Analysis (SCLA) was carried out on the quantitative data using Paleontological Statistics (PAST). ver. 1.75

RESULTS

The summary of both the qualitative and quantitative foliar epidermal characters of members of the two genera studied are presented in Tables 1 and 2 while the photomicrographs of the epidermal morphology of the taxa are presented in Figure 1(a-l). Clustered diagram of the taxa studied is presented in Figure 2. Details of the epidermal morphology of the species are presented.

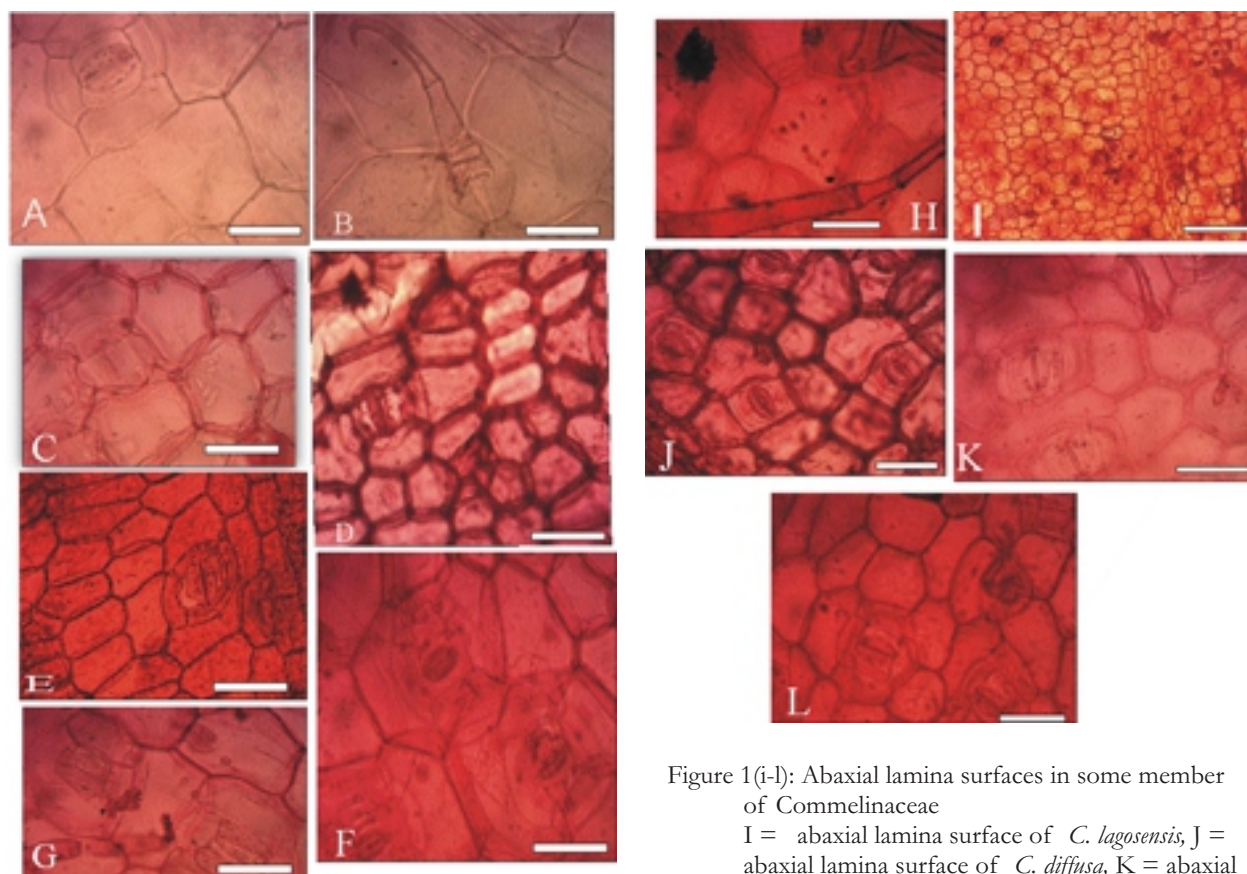


Figure 1(i-l): Abaxial lamina surfaces in some member of Commelinaceae
 I = abaxial lamina surface of *C. lagosensis*, J = abaxial lamina surface of *C. diffusa*, K = abaxial lamina surface of *Aneilema aequinoctiale*, L = abaxial lamina surface of *A. umbrosum*

Figure 1(a-h): Lamina surface view in some member of genera *Aneilema* and *Commelina*

Legend - A and B adaxial lamina surface of *C. benghalensis*, C- adaxial lamina surface of *C. lagosensis*, D- adaxial lamina of *C. diffusa*, E- adaxial lamina surfaces of *Aneilema aequinoctiale*, F = adaxial lamina surface of *A. umbrosum*. G and H- abaxial lamina surfaces - *C. benghalensis* (see right figure)

Table 1: Summary of Foliar Epidermal Features of the Adaxial Surface of the Species of *Commelina* and *Aneilema* Studied.

Character	<i>Commelina benghalensis</i>	<i>Commelina lagosensis</i>	<i>Commelina diffusa</i>	<i>Aneilema aequinoctiale</i>	<i>Aneilema umbrosum</i>
Epidermal cell shape	Polygonal	Polygonal	Polygonal	Rectangular to Polygonal	Polygonal
Anticlinal wall pattern	Straight	Straight	Straight	Straight	Straight
Stomata shape	Elliptic	Elliptic	Elliptic	Elliptic	Elliptic
Stomata type	hexacytic	hexacytic	hexacytic	hexacytic	hexacytic
Trichome type	bicellular, hooked, non-glandular	none	none	none	none
Epidermal size range (µm ²)	8.57-17.99	6.96-18.85	6.85-20.56	6.43-13.57	5.36-13.57
Stomatal index (%)	5.14-12.31	3.51-9.10	0.00-6.21	0.00-4.62	1.65-10.34

Table 2: Summary of Foliar Epidermal Features of the Abaxial Surface of the Species of *Commelina* and *Aneilema*.

Character	<i>Commelina benghalensis</i>	<i>Commelina lagosensis</i>	<i>Commelina diffusa</i>	<i>Aneilema aequinoctale</i>	<i>Aneilema umbrosum</i>
Epidermal cell shape	Polygonal	Polygonal	Polygonal	Polygonal	Polygonal
Anticlinal wall pattern	Straight	Straight	Straight	Straight	Straight
Stomata shape	Elliptic	Elliptic	Elliptic	Elliptic	Elliptic
Stomata type	hexacytic	hexacytic	hexacytic	hexacytic	hexacytic
Epidermal size range (μm^2)	1.79-23.92	17.85-33.20	15.17-35.34	1.61-13.57	8.35-17.85
Trichome type	Simple bicellular hooked non-glandular	none	none	unicellular, hooked, non-glandular	none
Stomatal index (%)	18.10-31.91	16.22- 20.00	16.39-18.56	16.92-26.09	8.86-17.71

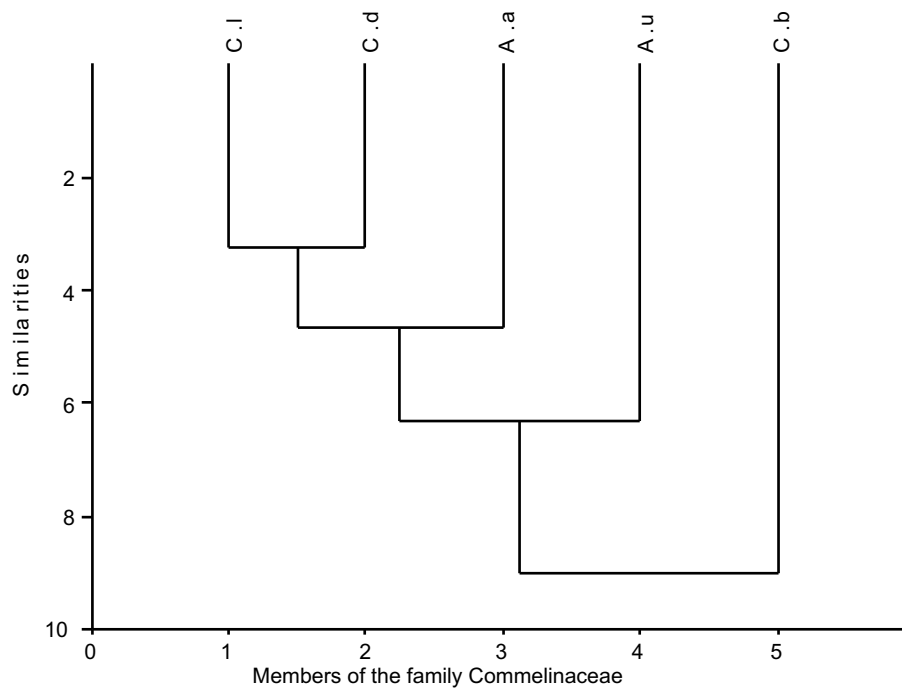


Figure 2: Single Linkage Cluster Analysis (SLCA) Dendrogram of Members of the Genera *Commelina* and *Aneilema* Species Studied Using the Mean Stomata Index

Legend

Cb- *Commelina benghalensis*, Cd- *Commelina diffusa*, Cl- *Commelina lagosensis*, Aa- *Aneilema aequinoctale*, Au- *Aneilema umbrosum*

Commelina benghalensis L. (Figures 1a, b, g and h)

Epidermal cells are generally polygonal with straight anticlinal wall on both the adaxial and abaxial surfaces, cell size varies, ranging from $6.49 \pm 1.43 \mu\text{m}$ long and $6.49 \pm 1.64 \mu\text{m}$ wide on the adaxial, $7.43 \pm 1.96 \mu\text{m}$ long to $7.50 \pm 1.57 \mu\text{m}$ wide on the abaxial lamina surface. Number of epidermal cells per field varies, 12-32 cells per field on adaxial, 18-42 cells per field on the abaxial. Stomata type is largely hexacytic on both surfaces. Mean stomatal index varies on both surfaces, ranging from 8.94 to 26.7% on the upper and lower surfaces respectively. The indumentum shows the presence of simple bicellular, hooked non glandular trichomes on both surfaces of *C. benghalensis* (Figures 1b and h)

Commelina lagosensis (Figures 1c and i)

In *C. lagosensis*, epidermal cells are polygonal and anticlinal wall is straight on both adaxial and abaxial lamina surfaces. Cell size variable, 6.57 ± 0.68 to $9.85 \pm 2.18 \mu\text{m}$ on adaxial and $6.14 \pm 2.11 \mu\text{m}$ long to $8.85 \pm 0.75 \mu\text{m}$ wide on the abaxial. Number of cells varies on both surfaces, 10-31 on adaxial and 49-67 cells per field on abaxial surface. Stomata type is hexacytic on both upper and lower surfaces. Stomata frequency ranges from 2-5 and 4-12 respectively on the upper and lower surfaces. Mean stomata index up to 6.81 and 14.22 % respectively. Trichome is absent on both adaxial and abaxial surfaces.

Commelina diffusa Burm. F. (Figures 1d and J)

Epidermal cells are polygonal, anticlinal wall straight on both adaxial and abaxial lamina surfaces. Cell size varies, $6.35 \pm 0.82 \mu\text{m}$ long to $6.85 \pm 0.57 \mu\text{m}$ wide on adaxial, $10.42 \pm 0.57 \mu\text{m}$ long and $8.50 \pm 1.07 \mu\text{m}$ wide on the abaxial lamina surface. Number of epidermal cells per field varies, 56-79 cells on the adaxial and 32-89 cells per field on the abaxial surface. On the upper and lower lamina surfaces, stomata type is hexacytic, stomata frequency per field ranges between 0-4 and 10-18 on the upper and lower surfaces respectively. Mean stomatal index varies between 3.32 and 18.9% on the adaxial, and abaxial surface respectively. Trichome is absent on both adaxial and abaxial surfaces.

Aneilema aequinoctiale (P. Beauv) Kunth (Figures 1e and k)

In *Aneilema aequinoctiale*, epidermal cells are rectangular to polygonal on the adaxial, largely polygonal on the abaxial. Cell size varies, $6.14 \pm 0.43 \mu\text{m}$ long and $5.14 \pm 0.54 \mu\text{m}$ wide on the adaxial lamina surface, $5.14 \pm 0.86 \mu\text{m}$ long and $5.14 \pm 0.99 \mu\text{m}$ wide on the abaxial. Number of epidermal cells per field on both surfaces is in the range of 52-86 and 39-69 cells on the upper and lower surface respectively. Similarly, stomata type is hexacytic on both surfaces, mean stomata index 1.45 % on adaxial and 20.33 % on the abaxial. Trichome only present on the abaxial and basically simple, unicellular non glandular (Figure 1K)

Aneilema umbrosum (Vahl) Kunth (Figures 1f and l)

Epidermal cells are polygonal with straight anticlinal walls on both the adaxial and abaxial surfaces. Cell size varies, about $5.21 \pm 0.71 \mu\text{m}$ long and $6.35 \pm 0.36 \mu\text{m}$ wide on the adaxial lamina surface, $6.07 \pm 2.64 \mu\text{m}$ long and $7.50 \pm 0.46 \mu\text{m}$ wide on the abaxial lamina surface. Stomata type on both surfaces is hexacytic, stomata frequency 1-6 on adaxial and 49-67 on the abaxial surface. Mean stomatal index about 4.81 % and 17.71% on the adaxial and abaxial surfaces. Trichome is absent on both surfaces.

The single linkage cluster analysis dendrogram of the quantitative foliar anatomical attributes of *Commelina* and *Aneilema* species shows that *C. benghalensis* is separated from the rest of the taxa on the first sub-cluster being connected to the other taxa at a lower phenon level. Other species of the genus *Aneilema* clustered with the remaining members of the genus *Commelina* (Fig.2).

DISCUSSION

The results of this study reveal that a lot of similarities in foliar epidermal characters exist at both intra- and inter-generic levels of the members of Commelinaceae. This may be an indication of the level of taxonomic relationship existing in the family. This corroborates the assertion of Evans *et al.* (2000) that though numerous taxonomic treatments have been produced for the family Commelinaceae, only little consensus as to which characters should be used to define relationships among the genera was reached. The high level of homology in

morphological characters (Evans *et al.* 2000) and anatomical characters has resulted in several discordant classification schemes for the family and invariably the genera.

Some inter-generic characters of note in the epidermal morphology of the taxa include hexacytic stomata, simple hooked non glandular trichome and the largely polygonal epidermal cells found in the two genera on both the adaxial and abaxial surfaces except in *Aneilema aequinoctiale* that possesses rectangular cells on the adaxial surface. This is a key diagnostic feature in *Aneilema aequinoctiale* which delimits it from the rest of the taxa. *Aneilema aequinoctiale* also possesses the smallest epidermal cells among all of the taxa studied in both surfaces while *Commelina diffusa* has the highest epidermal size range on both the adaxial and abaxial surfaces (i.e. 6.85-20.56 μm^2 on the adaxial surface and 15.17-35.34 μm^2 on the abaxial surface). The systematic values of epidermal characters have been documented and have been utilised in resolving quite a number of taxonomic problems at both the generic and specific levels (Fontenelle, *et al.*(1994); Kadiri (2006)). The presence of hexacytic stomata in the two genera shows it is an intergeneric character that has been reported previously by Faden (1991)

Dehgan (1980) used stomata type to differentiate between the subgenera of the genus *Jatropha*. According to Olatunji (1983) stomata index is highly constant for certain species and can be used for species delimitation. In this study, stomatal index is lowest in *Aneilema aequinoctiale* and highest in *Commelina benghalensis* on the adaxial surface and on the abaxial surface, *Commelina lagosensis* has the lowest while *Commelina benghalensis* has the highest but the considerable inter specific and inter generic overlap in the stomatal index in the taxa studied limits the diagnostic value of this character in the two genera

The bicellular hooked non glandular trichome observed in *Commelina benghalensis* and in *Aneilema aequinoctiale* is classificatory of the two species. This type of trichome constitutes important distinguishing feature that demarcate the two species from the rest of the taxa that possess largely glabrous leaves. Ramayya (1977) and Tomlinson (1966) provided a comprehensive

review of the systematic distribution of trichomes in the family Commelinaceae. The presence of hooked trichome in the genus *Commelina* has been previously reported by Tomlinson (1966 and 1969) and by Faden (1991) for the genus *Aneilema*.

The dendrogram reveals that the *C. benghalensis* is more closely related to the two *Aneilema* species than the remaining members of *Commelina* studied

CONCLUSION

From the comparative foliar epidermal morphology of the five species of the family Commelinaceae, anatomical study revealed close relationship in the species though with few distinguishing characters. This study therefore shows that foliar anatomical characters cannot stand alone but must be taken together with other anatomical characters in any taxonomic endeavour that hopes to show the level of taxonomic relationship within the taxa.

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