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LEAF ANATOMY AND POLLEN MORPHOLOGY: SOURCES OF ADDITIONAL TAXONOMIC CHARACTERS IN DELIMITING SPECIES OF *DUBOSCIA* BOCQ. (MALVACEAE *S.L*) OCCURRING IN PARTS OF SOUTHERN NIGERIA

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ABSTRACT

Duboscia is a genus of two species native to tropical Africa. It belongs to the Malvaceae family. The composition of the genus has been a subject of controversy since its establishment. Leaf anatomy and pollen characters of the *Duboscia* species were investigated using light microscopy with a view to provide additional taxonomic characters in delimiting species boundary in the genus. Dried leaves rehydrated in boiled water were used for the anatomical studies while flower buds were used for pollen morphological studies. Epidermal cells were isodiametric, polygonal to irregular with straight to wavy anticlinal cell walls. Leaves were bifacial and hypostomatic with anisocytic and staurocytic stomata types. Pollen grains were small (21.3 – 23.5 μm) and tricolporate. A combination of characters such as type of trichomes, number of glandular trichome head cells in leaves and petioles, shape of the midrib and petiole vascular bundle, petiole vascular bundle isolates, petiole outline, number of chlorenchyma cell layers and secretory ducts in midrib and petiole, exine surface, Amb and colpi length can be used as additional taxonomic characters to distinguish the two species and to determine their phylogenetic relationship between related genera in the tribe Grewieae. A key is provided for the identification and delimitation of the species.

Keywords: *Duboscia,* Leaf anatomy, Pollen, Taxonomy

INTRODUCTION

Duboscia Bocq. is a small genus of trees in the tribe Grewieae Endl. (subfamily Grewioideae) (Bayer and Kubitzki, 2003; Brunken and Muellner, 2012),), along with *Colona* Cav., *Goethalsia* Pittier., *Luehea* Wild., *Lueheopsis* Burret., *Mollia* Mart., *Trichospermum* Blume., *Hydrogaster* Kuhlm., *Tetralix* Griseb., *Visivaea* Baill., *Desplatsia* Bocq., *Microcos* Linn. and *Grewia* Linn. in the expanded Malvaceae (APG III, 2009).

The genus was first established by Bocquillon (1866) and later treated as *Diplanthemum* K. Schum. in Engl. & Prantl. (1897). However, current classifications retained the name *Duboscia* as established by Bocquillon (1866). Members of the genus are forest trees with pubescent stems and leaves. The leaf margin is often entire to undulate. The inflorescence is an axillary cyme located opposite to the leaves with few to many flowers enclosed in an involucre of bracts. The inflorescences, however, consist of 0.5–3 cm long peduncles subtending a cluster of bracts below the pedicels (which are much shorter than the peduncles) and flowers (Roger *et al*., 2012). The style is as long as or longer than the stamens with a lobed stigma. Androgynophore is also present with free stamens (Burret, 1926). The fruits are woody and ribbed with stellate hairs (Hutchinson and Dalziel, 1954). The genus is represented in West Tropical Africa and in Nigeria by 2 species: vis *Duboscia macrocarpa* Bocq. and *Duboscia viridiflora* (K. Schum.) Mildbr. (Hutchinson and Dalziel, 1954; Burkill, 1985; Roger *et al*., 2012).

The genus *Duboscia* has been controversial in terms of composition since its establishment. Hawthorne and Jongkind (2006) and Cheek *et al.* (2011) considered *Duboscia macrocarpa* as the only species in the genus recognizing

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Duboscia viridiflora as a synonym of *D*. macrocarpa. However, Lebrun and Stork (2003) recognised *D. macrocarpa* and *D. polyantha* as the two separate species in *Duboscia*. Burret (1926), Hutchinson and Dalziel (1954), Sosef *et al*. (2006), Harris and Wortley (2008) recognized *D. macrocarpa* and *D. viridiflora* as the two species in the genus. In all these treatments, only Louppe *et al*. (2008) adopted *D. polyantha* but added *D. viridiflora and D. macrocarpa* earlier recognised by Lebrun and Stork (2003) in addition to Burret (1926), Hutchinson and Dalziel (1954), Airy Shaw (1985), Sosef *et al*. (2006) and Harris and Wortley (2008) to bring the number of species to three. The most recent treatment of the genus by Roger *et al*. (2012) after a critical scrutiny of previous works and available herbarium specimens recognized two species which are *Duboscia macrocarpa* and *Duboscia viridiflora*. The previous treatments of *Duboscia* were based mainly on floral, fruit and a few vegetative morphological characters most especially the type of indumentum on the leaf, stem and petiole. Recently, Brunken and Muellner (2012), based on morphological phylogeny, reported that *Duboscia* is a closer relative to *Grewia* when compared with other genera in the tribe Grewieae. However, no report has been presented on molecular phylogeny of the *Duboscia*.

The taxonomic significance of leaf anatomy in providing characters of taxonomic value has been extensively demonstrated by several workers (Carlquist, 1961; Metcalfe and Chalk, 1979). Many workers have shown that the shape of epidermal cells, types and arrangement of stomata as well as the size, type of mesophyll, outline of midrib and petiole, number, shape and type of vascular bundle, distribution and number of cells in trichomes are important taxonomic characters which can be used in delimiting species boundary (Stace, 1984; Ogundipe and Wujek, 2004). Also of taxonomic importance are the pollen features which have been utilized over the years in resolving phylogenetic relationship problems (Judd and Olmstead, 2004; Perveen *et al.*, 2004 ; Stuessy, 2009). The present study was aimed at investigating the leaf anatomy and pollen morphology of the two species of *Duboscia* to provide additional taxonomic characters to further elucidate species boundary and to aid understanding of the taxonomic similarities within the species.

MATERIALS AND METHODS

Source of Plant Materials

Dried herbarium specimens from the Forest Herbarium Ibadan (FHI) were used for the study (Table 1).

Table 1 List of specimens studied

S/n	Taxa	Locality	Collectors	Voucher number	Date
1.	*Duboscia macrocarpa*	Ikom, Ogoja,Cross River	R.W.J Keay	**FHI** 28256	14-12-50
		Okomu Forest Reserve, Edo	C.F.A. Onochie	34610	23-6-55
		Ikom, Ogoja, Cross River	M.G. Latilo	30995	23-5-52
2.	*Doboscia viridiflora*	Ikom, Ogoja, Cross River	M.G. Latilo	**FHI** 31824	11-6-52

Leaf micro-morphological study

Preparation of Epidermal Peel

The dried herbarium specimens of the species of *Duboscia* (Table 1) were first rehydrated by soaking in boiled water for about 20 minutes before use. The specimens were cut at the median portions and soaked in concentrated Trioxonitrate (VI) acid for about 8 hours depending on the texture of the leaves according to the procedure described by Ibrahim *et al.* (2009, 2012). The appearance of air bubbles indicated the readiness of the epidermises to be separated. The samples

were then transferred to Petri dishes containing water and with the use of fine forceps and dissecting needle, the upper and lower epidermises were separated. These were cleared with camel hair brush in water to remove residual mesophyll layers. The peels were stained with toluidine for 3 to 5 minutes, rinsed in water and mounted in glycerol on clean glass slides. The slides were observed and studied using the light microscope. Twenty-five measurements of each of the characters were made at random. The Stomata Index (SI) was calculated using the formula of Salisbury (1927).

Leaf Anatomy

Leaf and Petiole sectioning: Transverse sections of dried herbarium specimens were used for the study. The leaves and petioles were first irrigated in boiling water until all the leaves sank in the water. These were then transferred to 50% ethanol ready for sectioning. A 3 cm^2 portion of the leaves was cut at the median position while a 3 cm portion of the petioles was cut at the median position for sectioning. Transverse sections of the leaf and petiole were cut at 20 µ thickness using Reichert Sledge Microtome and preserved in 50% ethanol. The sections were stained in 1% aqueous solution of Safranin O for 5 minutes, washed in 3 changes of water to remove excess stain counter-stained in 1% solution of Alcian blue for 5 minutes and then washed in three changes of water followed by dehydration by passing through series of ethyl alcohol: 50, 70, 80, 90 and 100% alcohol. Excess stain was then removed (differentiation process). The dehydrated and differentiated sections were cleared in xylene to remove the last traces of water and clear the sections (making it more transparent) and to remove last traces of ethanol. The sections were mounted in DPX according to Akinloye *et al.* (2012). Photomicrographs of the leaf and petiole sections were taken with Leica CM E with Digital Microscope eyepiece attachment and Photo Explorer 8.0 SE Basic software. Assessments were based on 20 observations from leaf lamina and petiole sections chosen randomly from each specimen. Identification of cells and tissues was done following the work of Metcalfe and Chalk (1989) and Ayodele and Olowokudejo (1997).

Pollen Morphology

The dried flower and floral buds from herbarium specimens were assessed for pollen morphology using the acetolysis method of Erdtman (1952, 1960). The floral buds were crushed with a glass rod in centrifuge tube. Three millilitres of freshly prepared acetolysis mixture (9 parts acetic anhydride to 1 part concentrated Tetraoxosulphate VI acid) was added to the content in the tubes. The content was heated in a water bath from 70˚C to boiling point and stirred occasionally. The centrifuge tubes and content were left in boiling water for 3 minutes and then centrifuged at 4000 r.p.m. for 5 minutes while still hot. The supernatant was decanted into acetolysis waste bottles before the addition of some water to the sediments in the tubes, and was shaken vigorously using a whirl mixer. Few drops of methylated spirit were added to remove the foam that formed and centrifuged again. The supernatant was decanted, washed with water and centrifuged repeatedly four times. Fifty percent glycerin was added and left standing for two hours. The tubes were shaken vigorously using a whirl mixer and centrifuged at 4000 r.p.m. for 10 minutes. The supernatant was finally decanted off, and the tube was inverted over filter paper and left overnight. One hundred percent glycerol was added to the tubes, shaken and poured into labeled storage vials. The pollen grains were mounted in unstained glycerin jelly. The slides were examined with a Fisher Scientific illumination microscope under (E 40; 0.65) oil immersion (E 100; 1.25) using x10 eye piece. The pollen measurements were based on 20 records from each specimen. Photomicrographs were taken using Leica CME with Digital Microscope Eyepiece attachment and Photo Explorer. Terminologies used were based on Erdtman (1952), Moore *et al.* (1991) and Perveen *et al.* (2004). For pollen sizes, Kremp's (1965) method was used (< 10 µ=very small pollen; 10-25 µ= small pollen; 25-50 µ= medium pollen; 50-100 µ= large pollen; 100-200 µ= very large pollen; >200µ=giant pollen). All slides were deposited in the Herbarium, Department of Botany, University of Ibadan, Ibadan, Nigeria (UIH).

RESULTS

Duboscia macrocarpa

Leaf epidermal surface

Epidermal cells were isodiametric, polygonal to irregular with either straight to curve or wavy anticlinal cell walls on the adaxial surface (Fig. 1a). The trichomes were 4-armed, e-glandular and 3-celled head multicellular glandular, sparsely distributed on the adaxial surface (Fig.1b; Table. 2). The number of epidermal cells/mm^2 on this surface ranged from 400 to 490 and was 10-15μm wide. Stomata and crystals were absent (Table 3). The leaf was hypostomatic with anisocytic and staurocytic stomata types (Fig. 1c). The abaxial epidermal cells were irregular with curved to wavy anticlinal cell walls covered by dense distribution of stellate and 3-celled head multicellular glandular trichomes (Fig. 1c & d; Table 2). The cell width ranged from 7.5 - 12.5 μm; stomata size 18.1 x 12.5 μm while the number of stomata ranged from 50-78/mm^2 (Table 3).

The lamina anatomy

The adaxial epidermis of the lamina transverse sections in *D. macrocarpa* was uniseriate and consisted of rectangular to almost round cells with thin non-striated cuticle. The adaxial cells were densely covered with stellate and glandular trichomes. The leaf was bifacial with the mesophyll region well differentiated into palisade and spongy parenchyma tissues. The palisade tissue consisted of 2 layers of undivided cylindrical cells compactly arranged while the spongy tissue was made up of 3 to 4 layers of loosely arranged oval to polygonal cells with large intercellular cavities (Fig.2a). In the trans-current lamina, the bundle sheaths extension to both epidermises ended in a groove with stellate trichomes on the abaxial surface (Fig.2a). Druses and prismatic crystals were distributed in the mesophyll region of the lamina.

Midrib anatomy

The midrib was knot-like on the adaxial surface consisting of sclerenchyma cells and a secretory duct but protruded with wavy round base at the abaxial surface. The epidermises were uniseriate and consisted of oval to rectangular cells. The thin cuticle was densely covered with 3-4 armed stellate, non-glandular trichomes and multicellular glandular trichomes (Fig. 2b). The vascular bundle was amphicribral, U-shaped and surrounded by 3 to 5 layers of sclerenchyma cells associated with 2-3 medullary bundles at the pith region (Table 4). Sclerenchyma and parenchyma cells were also present in the pith. The ground tissue was composed of 4 to 6 layers of collenchyma cells and 3 to 5 layers of parenchyma cells with secretory cells distributed on both the adaxial and abaxial regions. Styloid, prismatic and druse crystals were distributed in the cortex region while druses were associated with the pith in the medullary region.

Petiole anatomy

The median petiole outline in transverse section was boat-shaped with dense but short stellate trichomes, 2-celled head uniseriate and 4-6 celled head multicellular glandular trichomes (Fig. 3a & b). The epidermis was uniseriate and consisted of oval to rectangular small cells of equal sizes surrounded by thin cuticle. The cortex was made up of 1 to 2 layers of collenchyma cells on the outermost region close to the epidermis and 10 to 14-layers of parenchyma cells (Table 5). Sclerenchyma cells were sparsely or rarely observed around the vascular bundle. The amphicribral vascular bundle had 3 arc-shaped incurved isolated bundles (Fig. 3a). Parenchyma cells formed the ground tissue in which 8 to 13 secretory ducts were observed (Fig. 3b). Starch grains and druse crystals were abundant in both the cortex and medullary region (Fig. 3b).

Duboscia viridiflora

Leaf epidermal surface

The epidermal cells on the adaxial surface were polygonal to irregular with straight to curved anticlinal cell walls (Fig. 1e & f). The cell width ranged from 10 – 15 µm while the number of epidermal cells/mm^2 ranged from 496 to 500. Stellate, 2-armed e-glandular and 4-6 celled head multicellular glandular trichomes were sparsely distributed on the surface (Fig. 1e & f; Table 2 & 3). On the abaxial surface, the cells were irregular with curved to wavy anticlinal cell walls (Fig. 1g; Table 2). Stellate, 2 to 4-armed e-glandular trichomes and 2-3 celled head uniseriate and 3-4 celled head multicellular glandular trichomes were present on the abaxial surface (Table 2). The hypostomatic leaf of *Duboscia viridiflora* had abundance of anisocytic and staurocytic stomata, 16.5 x 12.1 µm in size on its abaxial surface. The stomatal index was 23.8% while the number of stomata was 60 - 124/mm^2. The number of epidermal cells/mm^2 ranged from 204 to 280 while the cell width ranged from 10-15µm (Table 3).

The lamina anatomy

The lamina epidermises were uniseriate with round and rectangular cells (Fig. 2c). The cuticle was non-striated. The mesophyll region of the bifacial lamina consisted of 2 layers of divided cylindrical palisade cells compactly arranged and 3 to 4 layers of oval to polygonal spongy cells loosely arranged with large intercellular cavities (Table 4). The lamina was trans-current with the bundle sheaths ending in a groove (Fig. 2c). Druses, styloid and prismatic crystals were abundant in the mesophyll.

Midrib anatomy

The midrib was knot-like on the adaxial surface composed of collenchyma cells (Fig. 2d) with protruded round, wavy abaxial surface (Fig. 2d). The epidermis was uniseriate and consisted of oval to rectangular cells. The thin cuticle was densely covered with 2-armed, stellate, non-glandular trichomes and multicellular glandular trichomes (Table 4). The amphicribral vascular bundle was arc-shaped with 2-3 smaller medullary bundles which were surrounded by 3 to 4 layers of sclerenchyma cells. Sclerenchyma and parenchyma cells were present in the pith (Fig. 2d). The ground tissue consisted of 3 to 4 layers of collenchyma cells and 10 to 11 layers of parenchyma cells with secretory cells found only on the adaxial surfaces (Table 4). Styloid, prismatic and druse crystals were distributed in the cortex region while druses were recorded in the medullary region.

Petiole anatomy

The median petiole outline was circular with uniseriate epidermis surrounded by a thin layer of cuticle (Fig. 3C). The epidermal cells were oval, rectangular-shaped on both adaxial and abaxial surfaces. The cortex consisted of 5-6 layers of collenchyma cells on the outermost region, followed by 12 - 15-layers of parenchyma cells and 5-6 layers of sclerenchyma cells which surrounded a ring-shaped vascular bundle located at the medullary region. In the pith were isolates of 6 - 8 medullary bundles (Fig. 3c). The petiole was densely covered with short stellate, 3-4 armed e-glandular and 3-4 celled head multicellular glandular trichomes (Fig. 3d; Table 5). About 15 - 17 secretory ducts were distributed in clusters of two to three and in some cases solitary in the cortex region (Fig. 3c). Druses and starch grains were distributed sparsely in both the cortex and medullary region.

Pollen morphology

Duboscia macrocarpa

Pollen grains were generally 3-colporate, small, single, isopolar and sub-prolate about 21.1 µm (20-22.5 µm) in polar view and 17.9 µm (16.8-20 µm) in equatorial diameter. The P/E ratio was 117. The amb was triangular/round; colpi 17.9 µm (17.5-18.7 µm) long with sharp ends at the poles but lacked margo. Ora were la-longate, 2.5-3.0 µm wide and 8.7-10.2 µm long. Exine was thin (1.2-1.5 µm) with reticulations and perforations (Table 6).

Duboscia viridiflora

Pollen grains were 3-colporate (Fig.); single, isopolar and sub-prolate; about 23.3 µm (22.5-24.5 µm) in polar view and 19.1 µm (18.7-20 µm) in equatorial diameter; P/E ratio was 122. Amb was round; colpi 19.8 µm (19.5-20 µm) with sharp ends becoming pointed at the poles and lack margo (Fig. 4b). Ora were la-longate, 2.7-3.5 µm wide and 9.5-10.0 µm long (Fig. 4b). Exine was thin (1.2-2.0µm); exine pattern reticulate-rugulate; not perforated (Table 6).

Table 2: Qualitative leaf epidermal characters of *Duboscia* species in Nigeria

S/n	Taxa	Cell shape		Anticlinal wall		Types of trichome		Type of stomata
		Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	
1.	*Duboscia macrocarpa*	Isodiametric/ Polygonal	Irregular	Straight/curved	Undulate	3 celled mct	3celled mct, ss & st	anisocytic, staurocytic
2.	*D. viridiflora*	Polygonal/ Irregular	Irregular	Curved/wavy	Undulate	2-armed, ss, 4-6celled mct	2,3,4-armed, 2-4 celled ust & mct, ss	anisocytic, staurocytic

ust = uniseriate glandular trichome, mct = multicellular glandular trichome, ss = sessile stellate, st = stellate trichome

Table 3: Quantitative epidermal and stomata characters of some *Duboscia* species in Nigeria

S/n	Taxa	Epidermal cells						Stomata			
		Number/mm^2		Cell width		Thickness(µm)		Number per mm^2	Size (µm)		Index %
		Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial		Length	Width	
1.	*Duboscia macrocarpa*	400-490	N.A	10.0-15.0	7.50-12.5	2.5	2.5	50.0-78.0	15.0-25.0	10.0-15.0	
		414±19.8		14.2±1.37	10.0±0.05	2.5±0.0	2.5±0.0	64.0±9.91	18.1±1.64	12.5±0.71	N.A
2.	*Duboscia viridiflora*	496-500	208-280	10.0-15.0	10.0-15.0	2.5	2.5	60.0-124	15.0-17.5	10.0-15.0	23.8
		498±2.83	230±35.8	13.3±0.82	12.9±0.75	2.5±0.00	2.5±0.00	72.0±13.2	16.5±0.55	12.1±0.75	

N. A=`Not available

Table 4: Anatomical characters of lamina of *Duboscia* species in Nigeria

S/n	Taxa	Lamina					Midrib			
		Type of leaf	Number of palisade cells layers	Number of spongy cells layers	Vascular bundle shape	Number of collenchyma cell layers	Number of Parenchyma cell layers	Number of sclerenchyma cell layers	Type of trichomes	Type of crystal
1.	*Duboscia macrocarpa*	Bifacial	2	3-4	U-shape	4-6	3-5	3-5	3-4 armed, ss, mct	Druses, styloid, prismatic
2.	*Duboscia viridiflora*	Bifacial	2 subdivided	3-4	Arc-Shape	3-4	10-11	3-4	2-armed, ss	Druses, styloid, prismatic

Table 5: Anatomical characters of the petiole of *Duboscia* species in Nigeria

S/n	Taxa	Petiole outline	Vascular bundle		Number of medullary bundles	Number of collenchyma cell layers	Number of parenchyma cell layers	Number of sclerenchyma cell layer	Secretory ducts			Type of trichome
			Shape	Isolates					Number	In cortex	In pith	
1.	*Duboscia macrocarpa*	Boat-shape	Arc	3	Absent	1-2	10-14	1	8-13	Present	Present	Ss, 2-celled ust, 4-6 celled mct
2.	*Duboscia viridiflora*	Circular	Ring	1	6-8	5-6	12-15	5-6	15-17	Present	Absent	3-4 armed, ss 3-4celled mct

Table 6: Qualitative and quantitative pollen morphological characters of *Duboscia* species in Nigeria

S/n	Taxa	Exine pattern	Pollen shape	Exine surface	AMB	Polar view (µm)	Equator view (µm)	Exine (µm)	Colpi Length (µm)	Ora size (µm) width	Ora size (µm) length	PA/ED %
1.	*Duboscia macrocarpa*	Reticulate	Subprolate	Perforated	Round/ triangular	22.5-24.5 23.5±1.0	18.7-20.0 19.4±0.65	1.2-2.0 1.6±0.4	19.5-21.0 20.0±0.25	2.5-3.0	8.7-10.2	121
2.	*Duboscia viridiflora*	Reticulate-rugulate	Subprolate	Absent	Round	20.0-22.5 21.3±1.25	16.8-20.0 18.4±1.6	1.2-1.5 1.4±0.2	17.5-18.7 18.1±0.60	2.7-3.5	9.5-10.0	116

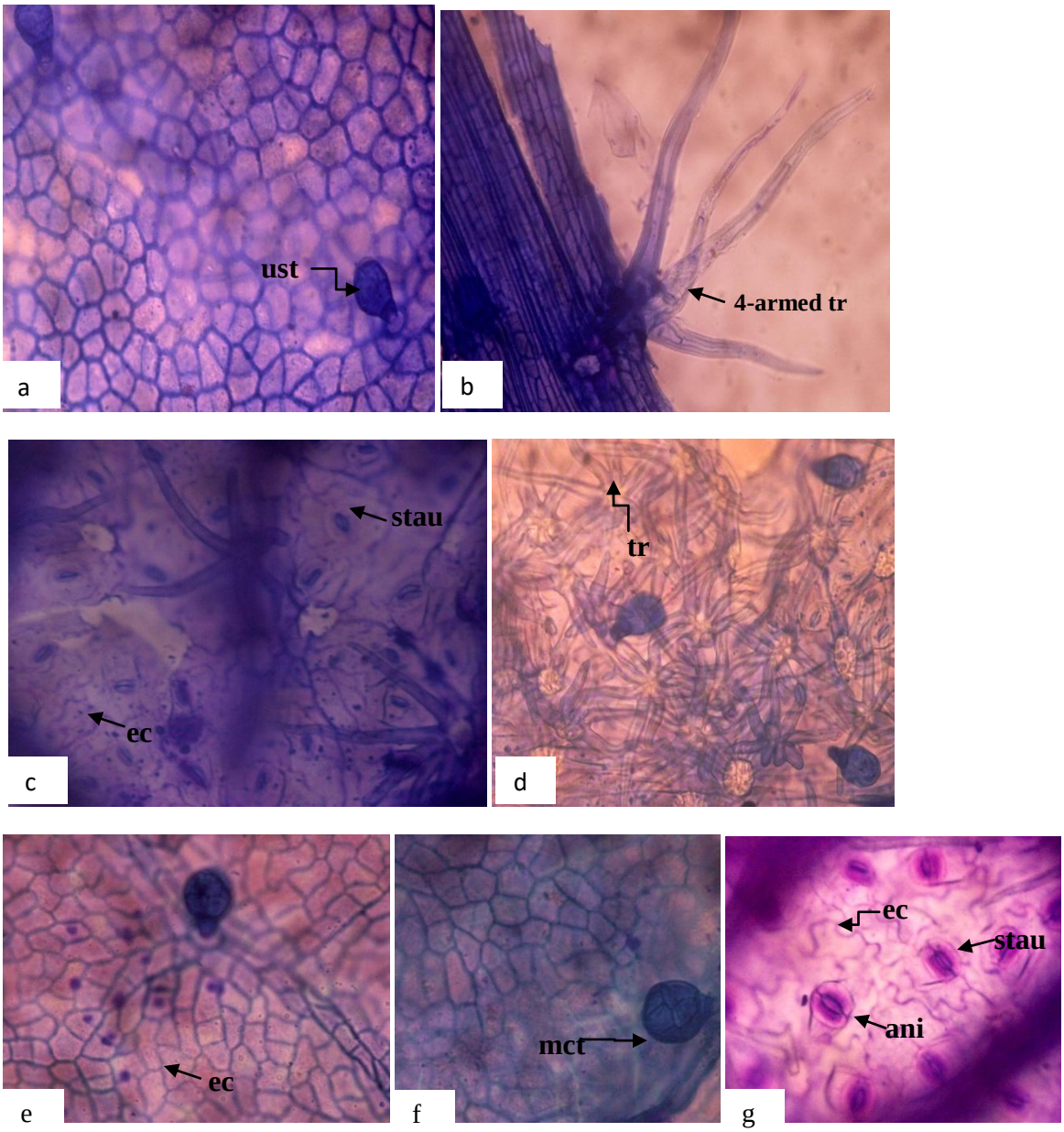

Fig. 1: Photomicrograph of the leaf epidermal surfaces of Nigerian species of *Duboscia*

(a) *Duboscia macrocarpa* Adaxial surface showing straight, curved and wavy anticlinal cell wall; (b) adaxial surface showing 4-armed e-glandular trichome and a multicellular glandular trichome; (c) abaxial surface showing staurocytic stomata, polygonal and irregular epidermal cells (d) abaxial surface densely covered by trichomes. (e &f) *Duboscia viridiflora* adaxial surface showing polygonal/irregular epidermal cells and multicellular glandular trichomes, (g) abaxial surface showing wavy anticlinal walls, anisocytic and staurocytic stomata types.

stau = staurocytic stomata, ani = anisocytic stomata, ust = uniseriate glandular trichome, mct = multicellular glandular trichome, tr = trichome, ec = epidermal cells.

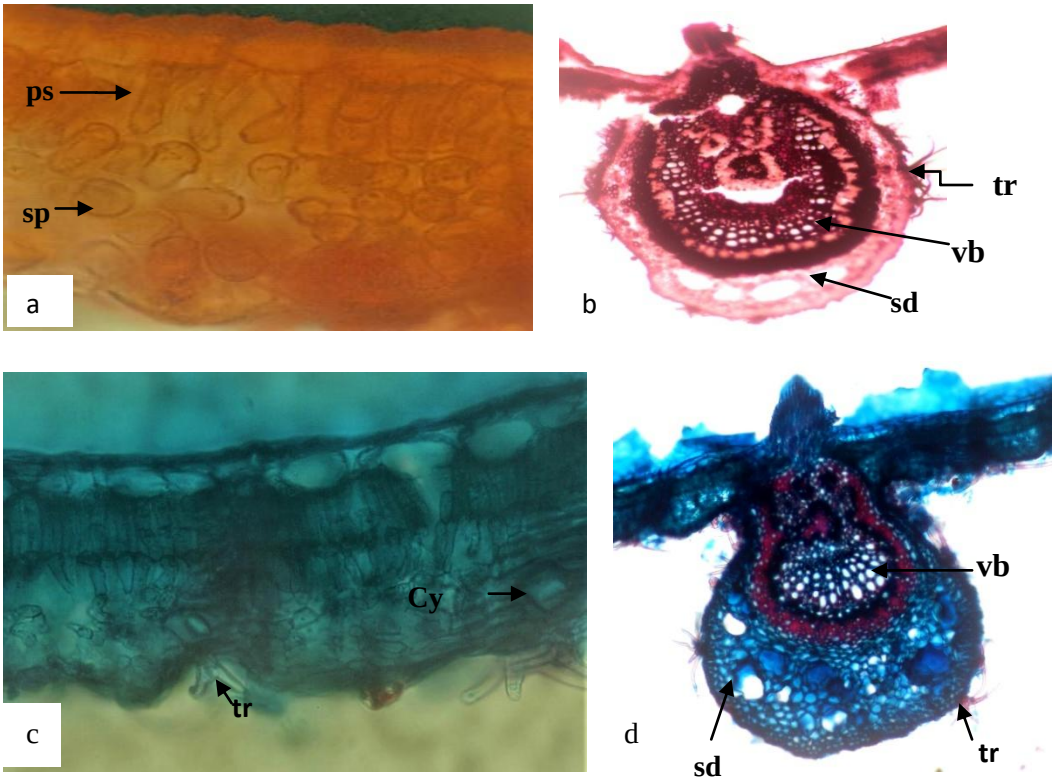

Fig. 2: Photomicrograph of Transverse sections of the lamina and midrib of Nigerian species of *Duboscia*

(a): Transverse section of *Duboscia macrocarpa* showing palisade and spongy cells. x 400;
(b): Transverse section of *Duboscia macrocarpa* showing a knot-shaped adaxial outline, secretory duct, U-shaped vascular bundle and trichomes on the midrib x 40.
(c): Transverse section of *Duboscia viridiflora* showing prismatic crystal and trichomes at the abaxial side of the bundle sheath extension. x 400;
(d): Transverse section of *Duboscia viridiflora* showing a knot-shaped adaxial outline, arc-shaped vascular bundle and secretory duct. x 40
cy = crystal, vb= vascular bundle, sd=secretory duct, tr= trichome, pc = pallisade cells, sp = spongy cells.

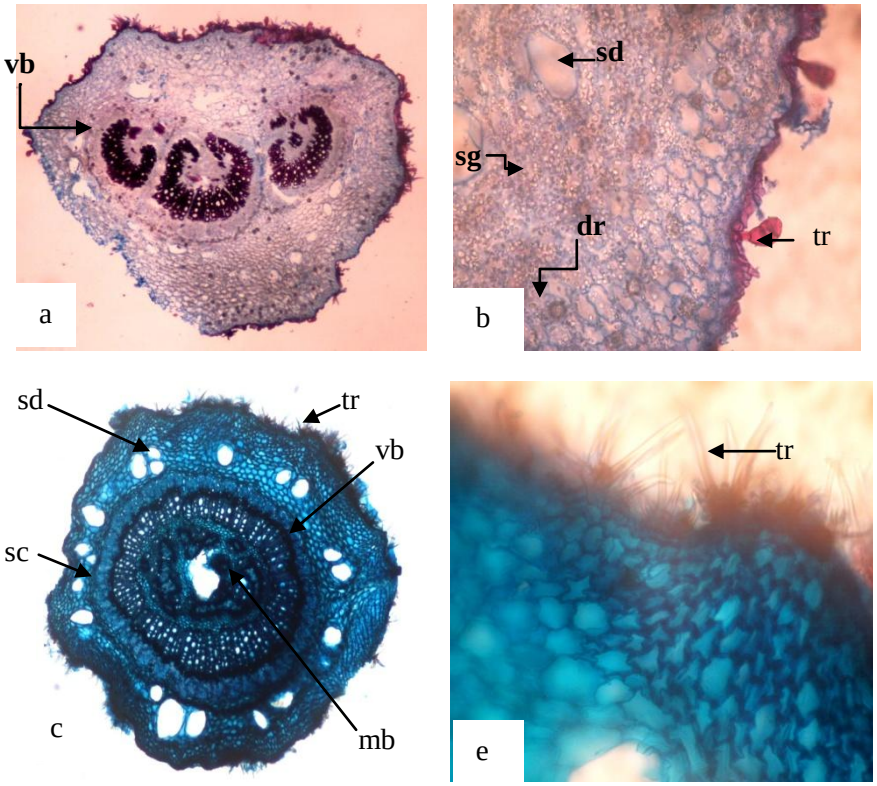

Fig. 3: Transverse sections of the petiole of Nigerian species of *Duboscia*.

(a): Transverse section of *Duboscia macrocarpa* petiole median region showing boat-shaped outline with three Arc-incurved vascular bundles. x 40;
(b): Transverse section of *Duboscia macrocarpa* petiole showing 3-celled head uniseriate glandular trichomes on the epidermis, druses, secretory ducts and starch grains in the cortex region. x 100
(c): Transverse section of *Duboscia viridiflora* petiole median region showing ring-shaped vascular bundle with medullary bundles in the pith, clustered secretory ducts and sclerenchyma cells. x 40
(d): Transverse section of *Duboscia viridiflora* showing dense distribution of stellate trichomes on the epidermis. x100
vb = vascular bundle, ust = uniseriate glandular trichome, dr = druse crystal, sg = starch grains, mb = medullary bundle, sd = secretory duct, sc = sclerenchyma cells and tr= trichome

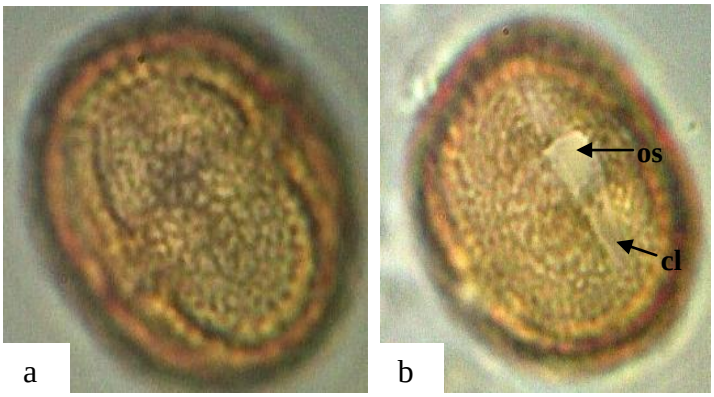

Fig. 4: Photomicrograph of the pollen grains of Nigerian species of *Duboscia*

a: *Duboscia macrocarpa*: equatorial view. x 1000
b: *Duboscia viridiflora*: equatorial view showing the colpi(cl) and ora (os). x 1000
cl = colpi length; os = ora

DISCUSSION

The *Duboscia* species studied are lowland forest trees found in the southern parts of Nigeria. The results obtained from most of the foliar epidermal characters overlap in both *Duboscia* species. However, few distinguishing features were observed such as the type of trichome on both epidermal surfaces of the species with reference to the number of cell heads of the glandular trichomes. This corroborates reports of the previous workers who stated that the only character variation in sterile specimens of both species is the type of hair present on the leaves (Hutchinson and Dalziel, 1954). The foliar micro-morphological characters observed in this study conform to the report of Metcalfe and Chalk (1979) on the general features of the genera in the former Tiliaceae. Lamina anatomical characters like the foliar epidermal characters overlap in both species. However, characters of taxonomic significance in the lamina that may be used to distinguish both *Duboscia* species were shape of the vascular bundle as well as number of collenchyma and parenchyma cell layers in the midrib.

The petiole anatomical study provided considerable variations which are of taxonomic significance in separating both species in the present investigation. These diagnostic features are the petiole outline, shape of the vascular bundle, number of vascular bundle isolates, presence or absence of medullary bundles in the pith region of the petiole. Nurul-Aini *et al.* (2013) used petiole outline to identify certain species of *Microcos* L. while taxonomic importance of petiole vascular bundle structure in species identification has also been well documented by Hare (1942), Metcalfe and Chalk (1950 and 1979), Olowokudejo (1987) and Rojo (1987). Soladoye (1982) and Akcin (2011) have also reported the taxonomic importance of the shape and number of vascular bundle isolates in delimitation and classification of taxa. The number of chlorenchyma cell layers has been reported to be of diagnostic value in some genera (Struwig *et al*., 2011). The numbers of parenchyma, sclerenchyma and collenchyma cell layers in the petiole of the studied taxa are of diagnostic value which can be employed in distinguishing the species.

Trichomes have been most employed taxonomically to compare species within a genus (Olowokudejo and Ayodele, 2007) but they can also be useful at higher levels such as within subfamilies (Batterman and Lammers, 2004) or families (Hoot, 1991). Glandular and e-glandular trichomes are usually present on the adaxial and abaxial surfaces in

the species studied. However, the presence of 2-armed e-glandular and 4-6 celled head multicellular glandular trichomes on the leaf abaxial surface in *D. viridiflora* separate it from *D. macrocarpa* in which 3-celled head multicellular glandular trichomes are predominant. Furthermore, the occurrence of 2-celled head uniseriate glandular trichomes only on the petiole of *D. macrocarpa* is diagnostic for the species.

The significance of pollen morphology in the taxonomy of angiosperms have been exemplified in several works where it was used either to suggest relationship in the family, to determine variation at the specific level or to solve problems at the generic or sub-generic levels (Blackmoore, 1981; Stuessy, 1990; Ayodele, 2005). Palynological evidence from this work shows similarity in the pollen shape of both species. David and Heywood (1973) and Moore *et al*. (1991) cautioned that the shape of pollen grains is of less taxonomic value as it varies considerably within a species as a result of the effects of the choice of extracting methods and embedding media used during preparation. However, both *Duboscia* species can be separated based on the variations observed in exine sculpture, colpi length, ora size, Amb and pollen size. The overlapping of both anatomical and palynological characters documented in this investigation could be utilized to further understand the affinity between both species in the genus.

This investigation has provided detail information on foliar epidermal, leaf anatomy and pollen characters which can be employed in delimiting species boundary in the two Nigerian species of *Duboscia*. The leaf anatomical and palynological characters obtained in the study could also be utilized in addition to other taxonomic characters to determine the phylogeny of the genus in relation to other genera in the tribe Grewieae. A dichotomous key is presented for Nigeria species of *Duboscia* based on the observed features from light microscopy

CONCLUSION

The leaf anatomical and palynological characters which include subdivided palisade cells, vascular bundle shape and number of chlorenchyma cell layers in the midrib and petiole, petiole median outline, vascular bundle shape, number of vascular bundle isolates, presence or absence of medullary bundles, the type of trichomes present on the lamina and petiole coupled with pollen characters such as exine sculpturing, Amb and colpi length are characters of taxonomic significance that have been used as additional characters in delimiting species boundary for the Nigerian species of *Duboscia*.

Key to the species

1. Epidermal cell isodiametric/polygonal; number of adaxial cells/mm^2 400-490; number of stomata 50-78mm^2; midrib vascular bundle U-shaped; petiole outline boat-shaped; petiole vascular bundle Arc- shaped incurved, medullary bundle absent; 2-celled uniseriate trichomes present in petiole; exine pattern reticulate, perforated; colpi 19.5-21 µm long……..*Duboscia macrocarpa*

1. Epidermal cell polygonal/irregular; number of adaxial cells/mm^2 496-500; number of stomata 60-124mm^2; midrib vascular bundle Arc-shaped; petiole outline circular; petiole vascular bundle ring-shaped, medullary bundle present; 2-celled uniseriate trichomes absent in petiole; exine pattern reticulate-rugulate, not perforated; colpi 17.5-18.7µm long…… ……..*Duboscia viridiflora*

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