

Leaf epidermal morphology of *Diospyros* (*Ebenaceae*) in Nigeria

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Received: August 25, 2015 ▷ Accepted: March 7, 2016

Abstract. The genus *Diospyros* L. belongs to *Ebenaceae*, with 30 species in Nigeria, that are either trees or shrubs, characterized by simple, estipulate, entire-margin leaves, without latex. The heartwood yields some commercial black ebony. The observed variations in the comparative study of leaf epidermis included: cell shapes ranging from irregular, polygonal to isodiametric; anticlinal cell wall pattern which is straight, curved, slightly wavy, wavy, or very wavy; cell wall thickness between 1.30 µm to 5.50 µm; cell wall ornamentations, present or absent; cuticular striations, present or absent; coronulated papillae, present or absent; stomata types, anomocytic, polycytic, cyclocytic to staurocytic; and trichome types, glandular to non-glandular.

Key words: leaf epidermis, morphology, Nigeria, taxonomy

Introduction

The family *Ebenaceae* was first described by Ventenat in 1799, and subsequently revised by Jussieu (1804) and by Brown in 1810. However, Wallnöfer (2001) attributed the description of *Ebenaceae* to Gürke in 1891. The earliest infrafamilial classification of *Ebenaceae* s.s. on a worldwide scale was that of de Candolle (1844), who recognized eight genera: *Cargillia* R. Brown, *Diospyros*, *Euclea* L., *Gunisanthus* A.DC., *Maba* J.R. Forst & G. Forst., *Macreightia* A. DC., *Rospidios* A. DC., and *Royena* L. Hiern (1873) recognized five genera in the family viz: *Diospyros* L., *Euclea*, *Maba*, *Royena*, and *Tetraclis* Hiern. Meanwhile, the monogeneric family *Lissocarpaceae* has been formally included in *Ebenaceae* (Wallnöfer 2004). Duangjai & al. (2006) proposed a new infrafamilial classification based on a phylogenetic approach, consisting of two subfamilies, *Lissocarpoideae* and *Ebenoideae*, and four genera, *Lissocarpa* Benth., *Euclea*, *Royena*, and *Diospyros*.

Members of the *Ebenaceae* are trees, shrubs and seldom geoxylic subshrubs. Most species are evergreen,

but some are deciduous. The bark of tropical species is often black, charcoal-like and brittle. In some species heartwood is black, latex absent. Branchlets are spine-tipped in certain species; growth architecture of many species corresponds to Massart's model (Halle & al. 1978). The bark of roots is black in most species and several species produce root suckers. Most species have unisexual, usually markedly dimorphic flowers and are mostly dioecious, or quite seldom monoecious (Wright 1904; Yasui 1915; Ng 1971; Athaya & Mishra 1979; Oliveira 1996). The ebenaceous inflorescence is conventionally referred to as a cyme (Ng 1991). Flowers are actinomorphic and 3–5(-8) in number. The persistent calyx is usually gamosepalous and has valvate or imbricate lobes. Due to the absence of lobes in some species, the calyx appears distally more or less truncate. The number of floral parts is known to vary within species and cannot, therefore, be used effectively for their distinction, as has been done in the past (e.g., Candolle 1844; Hiern 1873). The fruit is a multilocular, 1–16-seeded, usually a berry subtended by a persistent calyx. Four different kinds of gray, brown-

ish, or often characteristically rusty-brown hairs can be found in *Ebenaceae*, usually on stem apices, young leaves, inflorescence axes, bracts, pedicels and calyces, but on older organs they are often shed.

Wallnöfer (2001) reported *Diospyros* with ca. 500–600 species, of which 200–300 species occur in Asia and the Pacific area, 98 species in Madagascar and the Comoro Islands, 94 species on the African mainland, ca. 100 species in the Americas, and 15 species in Australia. *Diospyros* is undoubtedly the largest genus in the family *Ebenaceae* and exhibits the greatest amount of variations (Hiern 1873).

In Nigeria, members of the genus *Diospyros* consist of trees and shrubs which are characterized by simple, exstipulate, entire-margin leaves, without latex; the heartwood is usually black, yielding commercial ebony. The inflorescence is cymose, solitary; the flowers are unisexual with 3–7 united sepals, 3–7 united petals and stamens, which vary from two to more than 100. The fruit is a berry, which is often surrounded at the base by a persistent calyx (Hutchinson & Dalziel 1963 and Keay 1989). *Diospyros* is the only genus of *Ebenaceae* represented in West Tropical Africa, including Nigeria. According to Hutchinson and Dalziel (1963), there are thirty-nine species in West Tropical Africa, out of which twenty-five and additional two imperfectly known species are found in Nigeria. The imperfectly known species have been designated with different names by some authors (Keay & al. 1964; Keay 1989). They are, however, designated *Diospyros* sp. 1 and *Diospyros* sp. 2. in this paper.

Most Nigerian species of *Diospyros* are forest trees, a few are found in the Savanna zone, but chiefly in forest outliers, while *D. tricolor* (Schum. & Thonn.) Heirn. is a coastal species found only near Lagos (Keay 1989). Three approaches have been adopted for adding data to the taxonomic complexity of the genus. They include field and herbarium studies, as well as laboratory analysis. The objective of this research was to assess the variations of the leaf epidermis within and between species of *Diospyros* for taxonomic purposes.

Material and methods

Both fresh and herbarium specimens of thirty taxa of the genus *Diospyros* in Nigeria were studied. The plants were identified by the authors with the assistance of experienced herbarium workers at the For-

est Herbarium Ibadan (FHI). References were made to the taxonomic and nomenclature information in Hutchinson & Dalziel (1963), White (1978), Keay (1989), and www.tropicos.org.

Epidermal preparations were obtained using the techniques of Olowokudejo & Obi-Osang (1993), Bakare (1991) and Olatunji & Bakare (1993). Ten (10) specimens of both fresh and herbarium samples were used per species. However, for some species, such as *Diospyros cinnabarina* (Gürke) Gürke, *Diospyros malabarica* (Desr.) Kostel., *Diospyros melocarpa* F. White, and *Diospyros platanooides* Letouzey & F. White, only herbarium specimens were available, hence, five samples were used per species. Samples (c. 5–8 mm²) of each taxon were taken from a standard central position, usually midway between the base and apex of the lamina. Boiling in water for 5–10 minutes rehydrated the herbarium samples. Each herbarium sample was macerated in concentrated trioxonitrate (V) acid for 2–24 hours, depending on the nature of the leaf. It was necessary to bleach the epidermises of all herbarium samples in 15% sodium hypochlorite, after clearing with concentrated trioxonitrate (V) acid. The sample was transferred to water in a Petri-dish, while the abaxial and adaxial epidermises were carefully separated using forceps and dissecting needle. The inner parts (mesophyllous tissue) of the leaves were carefully cleaned with camel hairbrush. The isolated epidermal layers were washed in several changes of water before transferring them into 50% ethyl alcohol for 1 or 2 minutes to harden. They were then stained in 1% safranin O for 5 minutes, before being dehydrated in 50%, 70%, 90%, and 100% ethyl alcohol series. Each membrane was mounted in glycerine. Observations were made with a M20 Wild microscope at different magnification. Statistical analysis (standard deviation, mean and standard error) was based on 25 randomly selected epidermal cells and stomata. Terminologies employed in this study were those of Dilcher (1974) and Metcalfe & Chalk (1979).

Results

Results of the investigation are given in detail in Table 1, while the light micrographs of the variations observed are shown in Figs 1-17. The shapes of the adaxial and abaxial epidermal cells are irregular in nine species, as

in *D. canaliculata* De Wild. (Figs 1 & 2), and polygonal or isodiametric in the remaining species. The anticlinal cell wall pattern is straight to curved in 17 species, as in *D. abyssinica* (Hiern) F. White (Fig. 3), *D. barteri* Hiern (Fig. 4), *D. mannii* Hiern (Fig. 9); slightly wavy to wavy in eight species, as in *D. conocarpa* Gurke & K. Schum (Figs 5, 6); and very wavy only in *D. physocalycina* Gurke (Fig. 7). In four species, the anticlinal cell patterns on the adaxial surface differ from those on the abaxial surface, as in *D. canaliculata* (Figs 1 & 2). In some taxa, the anticlinal cell wall of the abaxial surface may be indistinct being obscured by papillae, as e.g. in *D. tricolor* (Fig. 8). In some species, cuticular striations may also obscure the anticlinal cell wall, as in *D. mannii* (Fig. 9). The cell walls are ornamented in six species, e.g. *D. elliotii* (Hiern) F. White (Figs 10 & 11). The cell wall thickness varies from 1.30 mm in *D. monbuttensis* Gurke and *D. gabunensis* Gurke to 5.50 µm in *D. abyssinica*. Epidermal cells range in sizes from 8.50×7.80 µm² in *D. gabunensis* (Fig. 12) to 55.00×33.00 µm in *D. conocarpa* (Fig. 5), but there are enormous variations in cell sizes within and between the species. The leaves are hypostomatic, with stomata occurring only on the abaxial surface of the leaves. The stomata vary from anomocytic, polycytic and cyclocytic, to staurocytic in the genus. The subsidiary cells range from 4–9. A combination of these two types of stomata oc-

asionally occurs within the same species. The stomata are characteristically abundant in *D. abyssinica* (Fig. 3). The ratio of the mean epidermal cell length to that of the stomata separates *Diospyros* into two groups. The first group comprises 12 taxa, with a ratio of less than 1, while the second group of 18 species has a ratio of 1 or more (Table 1). Glandular and non-glandular trichomes are present in 22 species, but absent in others. Trichomes are either present on both surfaces, as in 15 species, e.g. *D. barteri*, *D. cinnabarina* (Gurke) F. White and *D. fragrans* Gurke, or on abaxial surface only in the remaining taxa, e.g. *D. iturensis* (Gurke) Letouzey & F. White, *D. canaliculata*, and *D. piscatorial* Gurke. Three types of trichomes were observed: the non-glandular, multicellular, uniseriate, and non-branched (Fig. 13); the non-glandular, simple, unicellular and non-branched trichomes (Fig. 14); and the glandular type as shown in Fig. 16. The other forms of trichomes, if any, could not be assessed because some had fallen off, leaving their bases, as e.g. *D. fragrans* (Fig. 15). The trichome bases are arranged radially, with their cell walls relatively thicker than the other epidermal cell walls. Coronulated papillae are characteristically present on the abaxial surfaces of eight species viz: *D. obliquifolia* (Hiern ex Gurke) F. White (Fig. 17), *D. barteri*, *D. cinnabarina*, *D. fragrans*, *D. gracilescens* Gurke, *D. mannii*, *D. suaveolens* Gurke, and *D. tricolor*.

Table 1. Summary of leaf epidermal characters of *Diospyros* in Nigeria.

S/N	Taxa	Anticlinal cell L/S wall pattern	CO	CS	TR	CP	Cell wall thickness	Epidermal cell size (range, mean ± se)		Stomatal size (range, mean ± se)		No. of stomata per field (×1000)	No. of subsidiary cells per stoma	EL		
								Length (µm)	Width (µm)	Length (µm)	Width (µm)			SL	ST	
1	<i>D. abyssinica</i>	Ad Straight, curve	-	-	-	-	4.50–6.00 5.50±0.99	12.00–25.00 18.00±0.88	10.00–22.00 14.05±0.54							
		Ab Straight, curve	-	-	-	-	3.50–4.50 3.70±0.45	12.00–24.00 18.00±0.76	10.00–22.00 13.70±0.71	20.00–30.00 22.43±0.69	15.00–27.00 20.40±0.55	20–30	5–12	0.80	P	
2	<i>D. barteri</i>	Ad Straight, curve	-	-	+	-	3.80–4.50 4.00±0.56	14.00–32.00 21.60±0.12	11.00–21.00 16.00±0.69							
		Ab Straight, curve	-	-	+	+	1.80–2.20 2.00±0.18	11.00–23.00 19.15±1.80	10.00–17.00 14.45±0.49	20.00–26.00 23.00±0.44	16.00–25.00 20.50±0.41	0–5	5–6	0.83	P,S	
3	<i>D. canaliculata</i>	Ad Slightly Wavy	-	-	-	-	1.50–2.50 2.00±0.06	18.00–23.00 20.50±0.40	10.00–19.00 15.75±0.49							
		Ab Wavy	-	-	+	-	1.20–2.00 1.80±0.11	17.00–30.00 23.50±0.59	13.00–21.00 18.01±0.38	20.00–35.00 26.50±0.50	20.00–26.00 24.20±0.26	4–8	5–7	0.89	A,S	
4	<i>D. cinnabarina</i>	Ad Straight, curve	-	+	+	-	4.50–5.30 5.00±0.24	23.40–33.80 27.60±0.55	15.60–23.40 18.50±0.37							
		Ab Straight, curve	-	+	+	+	1.80–2.50 2.30±0.80	23.40–39.00 30.20±0.79	16.90–29.90 22.40±0.45	32.00–38.40 36.20±0.32	12.80–32.00 21.40±0.38	0–4	5–7	0.83	C	
5	<i>D. conocarpa</i>	Ad Wavy	-	-	+	-	2.00–3.50 3.00±0.22	40.00–65.00 51.50±0.84	30.00–45.00 38.50±0.11							
		Ab Wavy	-	-	+	-	2.00–2.80 2.50±0.25	38.00–78.00 55.00±1.21	23.00–45.00 33.00±0.20	25.50–34.00 28.80±0.32	21.00–25.00 23.70±0.27	0–5	4–6	1.91	A	

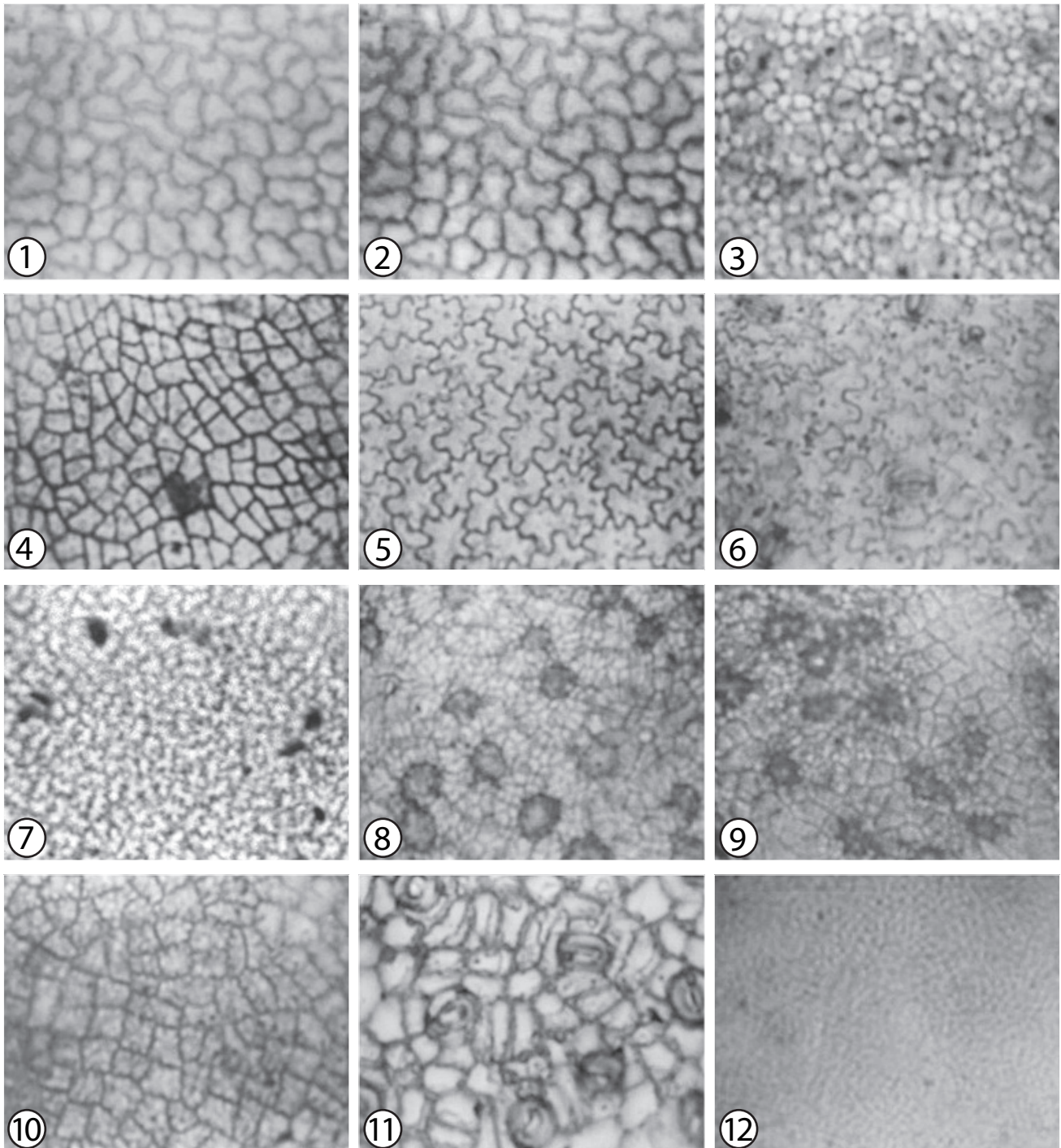
Table 1. Continuation.

S/N	Taxa	Anticlinal cell L/S wall pattern	CO	CS	TR	CP	Cell wall thickness	Epidermal cell size (range, mean ± se)		Stomatal size (range, mean ± se)		No. of stomata per field (×1000)	No. of subsidiary cells per stoma	EL SL ST	
								Length (µm)	Width (µm)	Length (µm)	Width (µm)				
6	<i>D. crassiflora</i>	Ad Straight, curve	-	-	-	-	3.00-4.50 4.00±0.39	22.10-35.10 26.60±0.74	13.00-27.30 19.20±0.59						
		Ab Straight, curve	-	-	-	-	2.50-4.00 3.60±0.50	22.10-49.40 34.80±0.37	13.00-28.60 20.40±0.36	19.50-26.00 21.80±0.32	18.20-22.10 19.30±0.18	3-5	4-7	1.60 S,P	
7	<i>D. dendo</i>	Ad Wavy	-	-	-	-	1.40-1.70 1.50±0.35	13.00-22.00 17.00±0.31	10.00-16.00 13.05±0.54						
		Ab Wavy	-	-	+	-	1.80-2.60 2.00±0.73	20.00-38.00 30.00±0.32	10.00-21.00 15.00±0.77	20.00-27.00 23.00±0.61	17.00-20.00 18.00±0.44	3-9	4-8	1.30 S,P	
8	<i>D. elliotii</i>	Ad Curve, straight	+	-	-	-	2.80-3.70 3.00±0.82	15.00-35.00 23.25±0.27	8.00-23.00 17.45±0.92						
		Ab Straight, curve	+	-	-	-	3.00-3.80 3.50±0.17	20.00-33.00 28.80±0.96	15.00-27.00 20.00±0.68	25.00-35.00 31.00±0.67	23.00-28.00 25.00±0.44	4-9	4-7	0.93 S,P	
9	<i>D. ferrea</i>	Ad Wavy	-	-	+	-	2.10-2.70 2.30±0.27	18.20-36.40 27.00±0.78	15.60-26.00 22.80±0.63						
		Ab Wavy	-	-	+	-	1.30-1.80 1.50±0.44	12.00-27.00 19.00±0.82	10.00-20.00 15.2±0.61	19.00-22.00 20.00±0.81	12.00-20.00 15.00±0.46	2-18	4-6	0.95 A	
10	<i>D. fragrans</i>	Ad Straight, curve	-	-	+	-	3.90-4.40 4.00±0.68	15.00-27.00 22.00±0.90	13.00-18.00 15.00±0.33						
		Ab Straight, curve	-	+	+	+	2.20-2.80 2.50±0.39	10.00-30.00 21.00±0.92	8.00-19.00 14.50±0.51	25.00-27.00 26.00±0.37	18.00-20.00 19.20±0.25	2-6	6-8	0.81 A	
11	<i>D. gabunensis</i>	Ad Straight, curve	-	-	+	-	1.40-2.00 1.60±0.46	5.20-10.40 8.50±0.76	5.20-10.40 7.80±0.55						
		Ab Very wavy	-	-	+	-	1.20-1.50 1.30±0.62	16.90-32.50 24.30±0.60	9.10-19.50 14.30±0.38	19.50-26.00 21.80±0.29	15.60-20.80 18.20±0.20	5-9	5-7	1.11 A	
12	<i>D. gracilencens</i>	Ad Straight, curve	-	-	+	-	2.00-2.20 1.70±0.07	19.20-40.00 30.00±0.28	13.00-21.00 17.00±0.59						
		Ab Straight, curve	-	+	+	+	2.00-2.70 2.20±0.78	17.00-40.00 28.00±0.22	17.00-25.00 21.50±0.72	15.00-20.00 16.50±0.33	12.00-14.00 13.10±0.21	0-6	5-8	1.70 S,P	
13	<i>D. hoyleana</i>	Ad Straight, curve	+	-	-	-	4.00-4.80 4.50±0.55	19.00-44.00 27.60±0.27	15.00-24.00 18.50±0.65						
		Ab Straight	+	-	-	-	3.10-3.80 3.30±0.23	19.00-44.00 30.00±0.43	17.00-25.00 20.65±0.54	18.50-24.00 21.80±0.32	15.00-17.00 16.00±0.15	1-6	4-6	1.38 S,A	
14	<i>D. iturensis</i>	Ad Straight, curve	+	-	-	-	2.80-3.20 3.00±0.26	17.00-35.00 27.00±1.09	15.00-23.00 18.00±0.57						
		Ab Straight, curve	+	-	-	-	3.00-3.80 3.50±0.51	20.00-38.00 30.00±0.19	17.00-26.00 20.50±0.58	21.00-25.00 23.05±0.33	16.00-20.00 18.50±0.21	5-11	4-6	1.30 S,A	
15	<i>D. mannii</i>	Ad Straight, curve	-	-	+	-	3.00-4.00 3.50±0.16	12.00-23.00 18.50±0.76	10.00-16.00 12.50±0.37						
		Ab Straight, curve	-	+	+	+	2.00-2.70 2.30±0.54	15.60-26.00 20.00±0.34	10.40-19.50 14.00±0.35	25.60-32.00 28.80±0.27	19.20-25.60 20.40±0.25	5-10	4-6	0.70 S	
16	<i>D. melabarica</i>	Ad Straight, curve	-	-	-	-	4.00-4.50 4.20±0.50	20.00-43.00 30.50±0.52	15.00-27.00 21.00±0.82						
		Ab Straight, curve	-	+	+	-	4.10-4.80 4.30±0.75	18.00-45.00 27.20±0.71	15.00-28.00 21.50±1.16	24.00-30.00 20.50±0.36	18.00-25.00 21.50±0.36	1-7	7-9	1.33 P	
17	<i>D. melocarpa</i>	Ad Curve, slightly wavy	-	+	-	-	1.30-1.80 1.60±0.94	16.00-28.80 21.40±0.24	9.60-19.20 14.20±0.19						
		Ab Curve, slightly wavy	-	+	-	-	2.20-2.80 2.30±0.09	19.50-39.00 28.30±0.78	11.70-23.40 17.20±0.35	16.90-24.70 20.10±0.13	16.90-24.70 20.10±0.13	2-5	4-6	1.43 S,P	
18	<i>D. mespiliformis</i>	Ad Wavy	-	-	-	-	3.20-3.70 3.50±0.28	15.00-26.00 20.80±0.91	12.00-24.00 17.00±0.82						
		Ab Wavy	-	-	+	-	2.30-2.80 2.50±0.37	15.00-27.00 19.45±0.36	10.00-18.00 13.81±0.59	22.4-32.00 27.00±0.26	15.00-25.60 22.40±0.12	9-14	4-5	0.93 S	

Table 1. Continuation.

S/N	Taxa	Anticlinal cell L/S wall pattern	CO	CS	TR	CP	Cell wall thickness	Epidermal cell size (range, mean ± se)		Stomatal size (range, mean ± se)		No. of stomata per field (×1000)	No. of subsidiary cells per stoma	EL SL	ST	
								Length (µm)	Width (µm)	Length (µm)	Width (µm)					
19	<i>D. monbuttensis</i>	Ad Wavy	-	-	-	-	1.30-1.80 1.50±0.05	15.00-20.00 17.00±0.39	13.00-18.00 15.00±0.52							
		Ab Slightly wavy	-	+	+	-	1.10-1.60 1.30±0.18	15.60-26.00 20.00±0.26	11.70-22.10 16.20±0.81	22.40-32.00 27.10±0.26	19.20-25.60 22.00±0.13	6-12	5-7	0.74	A	
20	<i>D. obliquifolia</i>	Ad Straight, curve	-	+	+	-	3.80-4.30 4.00±1.01	25.00-50.00 31.50±0.46	15.00-25.00 21.00±0.62							
		Ab Straight, curve	-	+	+	+	3.10-3.80 3.50±0.11	23.40-45.50 33.08±0.96	18.20-29.90 24.00±0.59	20.00-30.00 24.00±0.53	15.00-20.00 17.00±0.29	0-6	4-7	1.38	A,S	
21	<i>D. physocalycina</i>	Ad Very wavy	-	-	-	-	1.30-1.90 1.70±0.66	14.00-26.00 20.00±0.49	10.00-14.00 11.00±0.33							
		Ab Very wavy	-	-	+	-	1.20-1.60 1.50±0.32	13.00-26.00 19.00±0.50	9.00-20.00 14.00±0.47	18.00-26.00 22.00±0.15	17.00-22.00 19.00±0.19	3-7	5-8	0.86	P	
22	<i>D. piscatoria</i>	Ad Wavy	-	-	-	-	2.00-2.80 2.50±0.19	20.00-40.00 31.00±1.23	15.00-28.00 20.50±0.65							
		Ab Wavy	-	-	+	-	2.20-3.00 2.50±0.71	24.00-47.00 35.00±0.58	15.00-30.00 22.00±0.73	16.00-24.00 19.00±1.46	16.00-19.00 17.00±0.30	9-15	4-6	1.84	S	
23	<i>D. platanoides</i>	Ad Slightly wavy	-	+	+	-	2.20-2.50 2.00±0.24	13.00-29.00 21.00±0.71	10.00-23.00 16.00±0.57							
		Ab Slightly wavy	-	+	+	-	2.00-2.70 2.30±0.98	17.00-34.00 25.00±0.82	13.00-23.00 18.00±1.37	16.00-18.00 17.00±0.16	16.00-18.00 17.00±0.14	3-10	4-7	1.47	S,A	
24	<i>D. preussi</i>	Ad Slightly wavy	-	-	-	-	2.40-2.80 2.50±0.85	20.00-35.00 27.50±0.98	12.00-30.00 19.60±0.84							
		Ab Slightly wavy, curve	-	-	-	-	2.60-3.00 2.80±0.48	12.00-33.00 22.00±1.27	8.00-20.00 14.80±0.78	15.00-26.00 18.47±0.33	17.00-25.00 19.20±0.38	5-11	4-6	1.20	C,S	
25	<i>D. pseudomespilus</i>	Ad Straight, curve	+	-	+	-	3.80-4.30 4.00±0.31	15.00-28.00 21.50±0.98	12.00-20.00 15.60±0.48							
		Ab Straight, curve	+	-	+	-	3.10-3.50 3.00±0.58	17.00-43.00 32.50±1.54	14.00-25.00 19.00±0.73	20.00-28.00 25.00±0.33	18.00-21.00 20.45±0.21	4-11	4-7	1.30	C	
26	<i>D. soubreana</i>	Ad Slightly wavy	-	+	+	-	2.10-2.30 2.00±0.38	19.00-47.00 32.00±0.43	18.00-30.00 23.15±0.83							
		Ab Slightly wavy	-	+	+	-	1.50-2.10 1.80±0.57	21.00-49.00 32.75±0.38	15.00-31.00 20.95±0.88	18.00-25.00 21.00±1.01	18.00-21.00 19.00±0.81	3-10	4-6	1.56	S,A	
27	<i>D. suaveolens</i>	Ad Straight, curve	-	-	+	-	4.10-4.30 4.00±0.21	19.00-33.00 27.00±0.37	13.00-21.00 17.20±1.48							
		Ab Straight curve	-	-	+	+	2.10-2.70 2.50±0.34	15.60-26.00 20.80±0.66	13.00-18.20 15.60±0.34	18.00-23.00 21.00±0.30	15.00-18.00 16.00±0.25	6-12	5-7	1.00	P,S	
28	<i>D. tricolor</i>	Ad Straight, curve	-	+	+	-	3.80-4.70 4.00±0.33	18.00-33.00 25.00±1.11	13.00-21.00 16.80±0.70							
		Ab Straight, curve	-	+	+	+	1.90-2.30 2.00±0.54	15.60-26.00 20.80±0.82	13.00-18.90 16.20±0.43	23.40-32.50 27.00±0.58	18.20-26.00 22.00±0.76	2-6	7-8	0.77	P	
29	<i>D. viridicans</i>	Ad Straight, curve	+	-	+	-	3.70-4.30 4.00±1.02	23.00-52.00 32.00±1.40	13.00-23.00 17.50±0.48							
		Ab Straight, curve	+	-	+	-	3.30-4.10 3.50±0.97	20.00-38.00 29.00±0.20	15.00-25.00 20.00±0.71	17.00-21.00 18.00±0.29	16.00-19.00 17.00±0.24	4-9	4-9	1.61	S,P	
30	<i>D. zenkeri</i>	Ad Straight, curve	+	-	-	-	3.20-3.70 3.00±0.26	15.00-30.00 21.50±0.15	11.00-18.00 14.00±0.62							
		Ab Straight, curve	+	-	-	-	2.30-2.90 2.50±0.51	17.00-40.00 27.10±0.24	13.00-24.00 18.00±1.71	17.00-24.00 20.00±0.63	15.00-23.00 17.20±0.55	4-11	4-6	1.36	S,A	

L/S – leaf surface; CO – cell wall ornamentation; CS – cuticular striations; EL – epidermal cell length; SL – stomata length; TR – trichome; CP – coronulated papillae; Ad – adaxial; Ab – abaxial, se – standard error; ST – stomata type; P – polycytic; S – staurocytic; C – cyclocytic; A – anomocytic.



- Fig. 1. *D. canaliculata* (adaxial): epidermal cells irregular, anticlinal cell walls slightly wavy ($\times 400$).
 Fig. 2. *D. canaliculata* (abaxial): epidermal cells irregular, anticlinal cell walls wavy ($\times 400$).
 Fig. 3. *D. abyssinica* (abaxial): anticlinal cell wall straight to curved, stomata characteristically abundant ($\times 400$).
 Fig. 4. *D. barteri* (adaxial): anticlinal cell wall straight to curved ($\times 400$).
 Fig. 5. *D. conocarpa* (adaxial): anticlinal cell wall slightly wavy to wavy, epidermal cells the largest in the genus in Nigeria ($\times 400$).
 Fig. 6. *D. conocarpa* (abaxial): anticlinal cell wall slightly wavy to wavy, epidermal cells the largest in the genus in Nigeria ($\times 400$).
 Fig. 7. *D. physocalysina* (adaxial): anticlinal cell walls very wavy ($\times 400$).
 Fig. 8. *D. tricolor* (abaxial): anticlinal cell walls indistinct, being obscured by papillae ($\times 400$).
 Fig. 9. *D. mannii* (abaxial): cuticular striations obscured the anticlinal cell walls ($\times 400$).
 Fig. 10. *D. elliotii* (adaxial): the cell walls ornamented ($\times 400$).
 Fig. 11. *D. elliotii* (abaxial): the cell walls ornamented ($\times 400$).
 Fig. 12. *D. gabunensis* (adaxial): epidermal cells the smallest in the genus in Nigeria ($\times 400$).

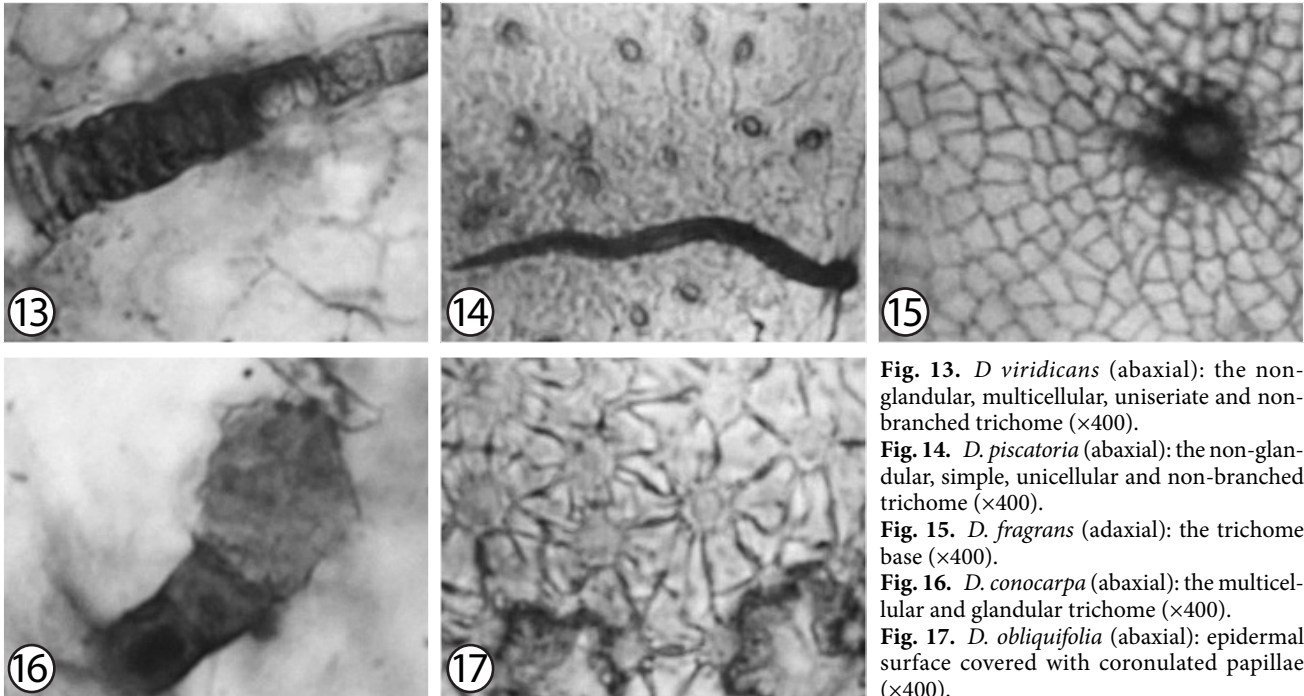


Fig. 13. *D. viridicans* (abaxial): the non-glandular, multicellular, uniseriate and non-branched trichome ($\times 400$).

Fig. 14. *D. piscatoria* (abaxial): the non-glandular, simple, unicellular and non-branched trichome ($\times 400$).

Fig. 15. *D. fragrans* (adaxial): the trichome base ($\times 400$).

Fig. 16. *D. conocarpa* (abaxial): the multicellular and glandular trichome ($\times 400$).

Fig. 17. *D. obliquifolia* (abaxial): epidermal surface covered with coronulated papillae ($\times 400$).

Discussion and conclusions

The taxonomic use of leaf epidermal characters has been reviewed by some researchers, namely Wilkinson (1979), Metcalfe & Chalk (1979), and Stace (1989), while the merits and demerits of using these features as taxonomic markers have been discussed by many authors, e.g. Olowokudejo (1993), Soladoye (1982), Edward (1935), Rowson (1943), Van Staveren & Bass (1973), Saheed & Illoh (2010), Ogunkunle (2013), Abdulrahman & al. (2014), etc. The systematic value of epidermal characters varies from one group of plants to another, e.g. the epidermal characters were found very useful in *Poaceae* (Prat 1932; Sorenson 1953; Ogundipe & Olatunji 1991); *Musaceae* (Tomlinson 1959); *Apiaceae* (Guyot 1971); *Combretaceae* (Stace 1965, 1980); and *Euphorbiaceae* (Kakkar & Paliwal 1974; Raju & Rao 1977; Dehgan 1980), but of less value in *Winteraceae* (Bailey & Nast 1944). The results obtained from leaf epidermal study of the Nigerian *Ebenaceae* clearly indicate that the micro-morphological characters are useful for identification of the species groups and seldom at species level. A survey of the laminar epidermises shows that the anticlinal cell walls vary in their degree of undulation, which could be used to separate a group of species of *Diospyros*. For example,

the anticlinal cell walls of *D. physocalycina* are very wavy; slightly to moderately wavy in eight species viz: *D. conocarpa*, *D. dendo* Welw. ex Hiern, *D. fereira* (Willd) Bakh., *D. melocarpa* F. White, *D. mespiliformis* Hochst. Ex A.DC., *D. piscatoria*, *D. soubreana* F. White, and *D. platanoides* Letouzey & F. White; while in five species, the anticlinal cell wall differs on both surfaces – *D. gabunensis*, *D. preussii* Gurke, *D. monbuttensis* Gurke, *D. canaliculata* and *D. hoyleana* F. White; and it is straight to curved in the remaining 16 species. *D. cinnabarina*, *D. fragrans*, *D. grascilenscens*, *D. mannii*, *D. obliquifolia*, and *D. tricolor* can be distinguished from other taxa by their possession of cuticular striations and coronulated papillae. In addition to these six species, *D. barteri* and *D. suaveolens* have coronulated papilla, but without cuticular striations. The ornamentation of the cell wall can be used to differentiate six species: *D. elliotii*, *D. hoyleana*, *D. iturensis*, *D. pseudomespilus* Milbre, *D. viridicans* Hiern, and *D. zenkeri* (Gurke) F. White.

The stomata are confined to the abaxial leaf surfaces. Wallnöfer (2001), reported that *D. mespiliformis* has stomata on the adaxial side, which was not observed in this study. The epidermal cell size, cell wall thickness and the stomata size are variable and overlapping within and between the species, so they may not be useful in characterization of the *Di-*

ospyros species. However, the extremely small size of epidermal cells (on adaxial surface) of *D. gabunensis* constitutes an important distinguishing feature. The number of stomata per field in the Nigerian *Diospyros* ranges between 0–30. The highest number within the range for all other taxa is 18, while the lowest for *D. abyssinica* is 20 and the highest 30. This factor is characteristically unique for *D. abyssinica*. The ratio of the mean epidermal cell length to the stomata length divides *Diospyros* into two groups. The first group comprises 12 taxa with a ratio of less than 1 viz: *D. abyssinica*, *D. barteris*, *D. canaliculata*, *D. cinnabarina*, *D. elliotii*, *D. ferrea*, *D. fragrans*, *D. mannii*, *D. mespiliformis*, *D. monbuttensis* and *D. physocalycina*, and *D. tricolor*, while the second group (18 species) has a ratio of 1 or more. Metcalfe & Chalk (1950) and Solereder (1908) reported anomocytic stomata for the family *Ebenaceae*. In this study, in addition to anomocytic stomata, polycytic, staurocytic and cyclocytic stomata were observed.

The investigations of the 30 taxa of *Diospyros* show some significant differences in the characters, which could be used to characterize the species even when they are sterile. The leaf anatomical investigation revealed some striking features, e.g. trichomes and stomata types, common to all species and of less taxonomic value at a specific generic level. Meanwhile, the differences observed in some features could be used to differentiate a group of species. For instance, the degree of undulation of epidermal cell wall; presence or absence of cell wall ornamentation, cuticular striations and coronulated papillae; stomata type, number of stomata per field and the ratio of epidermal cell length to stomata length are taxonomically important.

The species of *Diospyros* listed in the work of Duangjai & al. (2006) did not include most of the Nigerian species. Duangjai & al. (2006) reported a phylogenetic hypothesis for *Ebenaceae* with 11 clades. However, the groupings obtained from the results of leaf epidermal study are not congruent with the clades identified in this phylogeny, hence, homoplastic in the genus, with the exception of the clade comprising *D. fragrans* and *D. mannii*. The groupings in which *D. fragrans* and *D. mannii* are included, are based on the ratio of the mean epidermal cell length to the stomata length with a ratio of less than 1, their possession of cuticular striations and coronulated papillae.

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