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Epidermal morphology of the genus *Dialium* (Fabaceae: Caesalpinoideae)

With 3 Figures and 2 Tables

Summary

Epidermal morphology of adaxial and abaxial leaflet surfaces of the five species in the genus *Dialium* (Fabaceae: Caesalpinoideae) were studied by light microscopy. The epidermises are composed of cells which are isodiametric, irregular or both types in outline. Anticlinal walls are either straight, curved or undulate. Leaflets of all taxa are hypostomatic. Paracytic stomata occur in all species except *D. guineense* that posses both paracytic and staurocytic stomata. Other features of the epidermis that show variation include stomatal size and density, size and density of epidermal cells, and type, size, shape, frequency and basal cells of the trichomes. An indented, dichotomous key for identifying the species is presented.

Zusammenfassung

Die Morphologie der Epidermis von Blattober- und Blattunterseiten von fünf Arten der Gattung *Dialium* (Fabaceae: Caeasalpinoideae) wurde unter dem Lichtmikroskop untersucht. Die Epidermen sind aus isodiametrischen Zellen zusammengesetzt oder irregulär oder aus Zellen beider Typen aufgebaut. Die antiklinalen Zellwände sind gerade, gebogen oder gewellt. Die Blättchen aller Arten sind hypostomatisch. Parazytische Stomata finden sich bei allen Arten außer bei *D. guineense*, das sowohl parazytische als auch staurozytische Stomata besitzt. Andere variable Merkmale der Epidermis sind Stomatagröße und -dichte, Größe und Dichte der Epidermiszellen, ferner Typ, Größe, Form, Häufigkeit und Basalzellen der Trichome. Ein dichotomer Schlüssel zur Artbestimmung ist beigefügt.

Introduction

The genus *Dialium* L. (Fabaceae: Caesalpinoideae) is presented by five species in West Africa (HUTCHINSON & DALZIEL 1954). Two of the species, *D. guineense* WILLD. and *D. dinklagei* HARMS, are of considerable economic importance in African traditional medicine because of their culinary and medicinal usefulness. An infusion of the leaves and fruits of *D. guineense* is used for fever, and a decoction of the bark with spices is used for stomach-ache in Sierra Leone (DALZIEL 1956). In Nigeria, a bark decoction of *D. guineense* is used as a gargle for sore throat and as a mouthwash while the leaf decoction is used for tumours (DALZIEL 1956). In Cote d'Ivoire, the leaf sap is used to ease labour in pregnant women (IRVINE 1961).

A leaf decoction of *D. dinklagei* is used as a beverage for fever, jaundice, and yellow fever (IRVINE 1961). It is considered a sovereign remedy for haematuria, with retention of urine; the bark is used as purgative (KERHARD & BOUQUET 1950). Also, the leaves of both *D. guineense* and *D. dinklagei* are used for febrifuge, astringent, diarrhoea, cough, bronchitis, fever and diuretic (OLIVER 1960).

In many West African countries including Nigeria, medicinal species of *Dialium* and other popular medicinal plants are sold in local markets and street corners in sterile or fragmentary conditions. This common practice usually renders crude drug plants highly susceptible to substitution and adulteration. The problems of accurate identification of, and dearth of information about, the numerous medicinal plant species in a coun-

try like Nigeria whose flora is not well-documented, have hampered the optimal utilization of these crude drugs. These have also discouraged the conduct of phytochemical and pharmacological research into the efficacy of these drug plants. This is especially the case when dealing with closely related species of the same genus which contain both medicinal and non-medicinal taxa.

This work is part of a wider programme of research (OLOWOKUDEJO 1987; OLOWOKUDEJO & PEREIRA-SHETEOLU 1988, 1992; OLOWOKUDEJO & NYANANYO 1990; OGUNDIPE 1992; PEREIRA-SHETEOLU 1992; OGUNDIPE & OGUNTADE 1993; OLOWOKUDEJO & OBI-OSANG 1993) into the taxonomy of medicinal plants in West Africa being undertaken at the University of Lagos. The purpose is to provide, through a detailed systematic evaluation of epidermal features of the leaf, reliable taxonomic characters that would facilitate an accurate and rapid identification of these samples. This paper reports the leaflet epidermal characters of five taxa of *Dialium* as observed by light microscopy. It describes the significance of, and discusses the extent to which, these valuable features may be used for identifying sterile plants of each species which are otherwise indistinguishable.

Material and methods

Plant material used for this investigation was obtained from the herbaria of the Forestry Research Institute of Nigeria, Ibadan (FHI), and Department of Biological Sciences, University of Lagos, Nigeria (LUH). Five specimens of each taxon from wide geographical and ecological range were examined.

Epidermal preparation: An area about one cm. square was cut out from the median portion of the leaflet lamina near the mid-rib. Each sample was transferred to a beaker containing 5% sodium hypochlorite (Commercial bleach). The beaker was then placed in a water bath at 100 °C and boiled for 10–20 minutes. By this method, each sample was bleached and the epidermises separated from the mesophyll. This was indicated by bubbles appearing in the leaflet pieces. The beaker was brought out of the water bath and the leaflet samples carefully removed from the beaker and washed thoroughly in distilled water to which two drops of glacial acetic acid has been added.

Upper and lower epidermises were completely isolated from the mesophyll using a pair of fine forceps and dissecting needles. Remainders of mesophyll tissues were brushed off with a camel hair brush before washing each membrane thoroughly in distilled water. Membranes were later transferred to 50% ethanol for 5 minutes to harden and then stained in aqueous safranin for 5 minutes. Each membrane was dehydrated by passing through different grades of alcohol, viz. 50%, 70%, 90%, absolute ethanol series and a mixture of equal parts of absolute ethanol and xylene (about 5 minutes in each). The membranes were cleared in xylene for 2 minutes and mounted in Canada balsam and the slides were dried on hot plate.

For statistical analysis, 50 epidermal cells and 30 stomata were chosen randomly from each taxon and measured using a micrometer eye-piece. For each quantitative character, the range, mean, standard deviation and standard error were determined for all taxa. The stomatal index (SI) was calculated using the formula of SALISBURY (1927): $\frac{S}{S+E} \times 100$, where S denotes the number of stomata per unit area, and E the number of epidermal cells of the same area. Photomicrographs were made using Reichert Microstar IV microscope to which a camera is attached while drawing were made from Wild M12 microscope fitted with a camera lucida.

Results

The observed epidermal characteristics of all species of *Dialium* examined are summarized in Tables 1 and 2 where taxa are listed according to the classification of HUTCHINSON & DALZIEL (1954). Photomicrographs depicting various micromorphological features of the adaxial and abaxial epidermises in each of the five species are shown in Figures 1 and 2. Trichome types found in the genus are drawn from the camera lucida and are shown in Fig. 3. Descriptive terminology is based on STACE (1965), DILCHER (1974) and WILKINSON (1979).

The epidermises are composed of variously shaped cells which in surface view are either isodiametric or irregular in outline but both types may occur within the same surface. All isodiametric cells have straight or curved walls while irregular cells have undulate walls except in *D. guineense* with straight or curved walls (Figs. 1, 2). The cells are generally thickwalled and those on the upper surface are slightly wider than those on the lower surface except in *D. aubrevillei* PELLEGR. and *D. dinklagei* (Table 1). Anticlinal walls of epidermal cells are either straight, curved or undulate. All species with

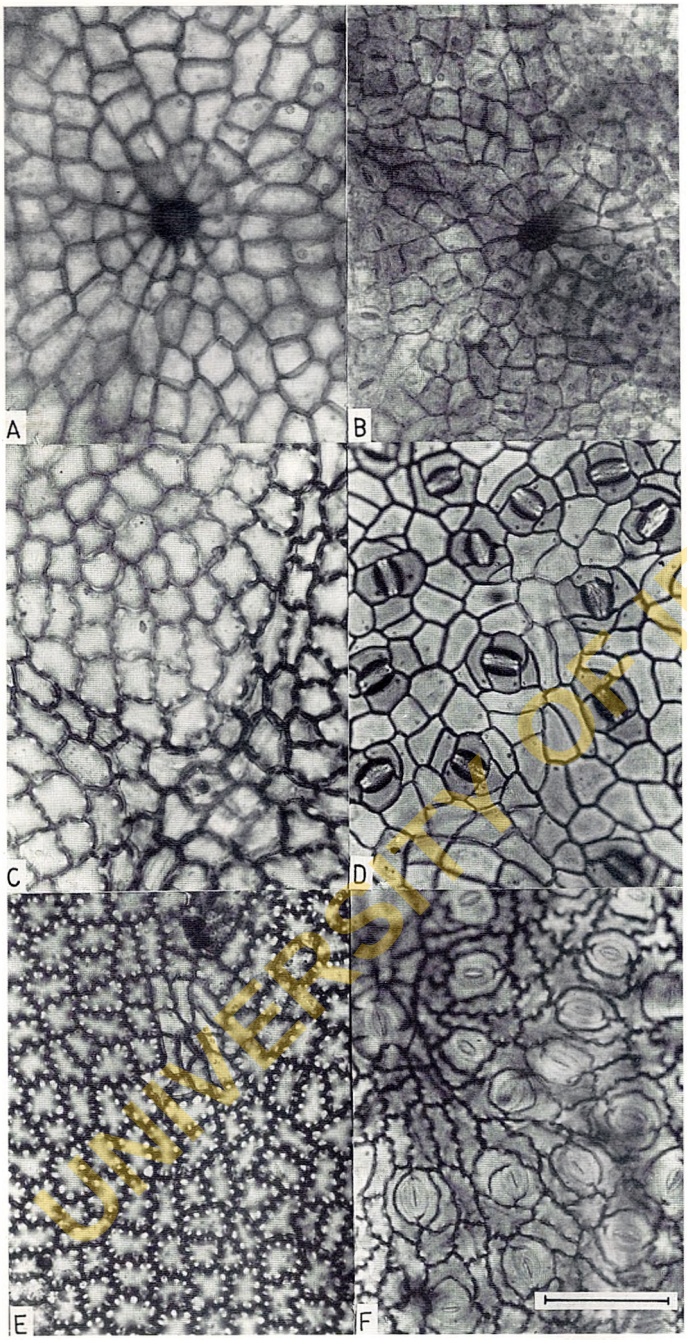

Fig. 1
Photomicrographs of leaflet epidermal features of *Dialium* species
A – *D. pobeguinii,* adaxial surface showing simple, unicellular trichome seated on a pedestal of relatively smaller basal cells; B – *D. pobeguinii*, abaxial surface showing paracytic stomata and trichome base, C – *D. guineense*, adaxial surface with undulate anticlinal walls; D – *D. guineense*, abaxial surface with both paracytic and staurocytic stomata, E – *D. aubrevillei*, adaxial surface with undulate anticlinal walls: F – *D. aubrevillei,* abaxial surface with paracytic stomata.
All same scale = 50 μm

isodiametric cells have straight or curved walls, both types occurring together (Fig. 1A, B, D, 2C) except in *D. guineense* which also has irregular cells with straight or curved walls (Fig. 1D). Other species with irregular cells have undulate walls (Figs. 1C, E, F, 2A–D).

The length and width of the epidermal cells on the adaxial and abaxial surfaces of each species were measured. On the adaxial surface, the epidermal cell length varies from 22.95 µm in *D. aubrevillei* to 25.40 µm in both *D. pachyphyllum* HARMS and *D. dinklagei* while the width ranges from 18.95 µm in *D. aubrevillei* to 23.85 µm in *D. pobeguinii* PELLEGR. (Table 1). On the abaxial surface, the epidermal cell length varies from 22.20 µm to 25.15 µm in *D. aubrevillei* and *D. pachyphyllum* respectively while the epidermal cell width ranges from 19.05 µm in *D. pachyphyllum* to 23.50 µm in *D. dinklagei* (Table 1).

Leaflets of all species are hypostomatic. Paracytic stomata which are enclosed on either side by a subsidiary cell parallel to the long axis of the pore and guard cells occur in all taxa (Figs. 1B,

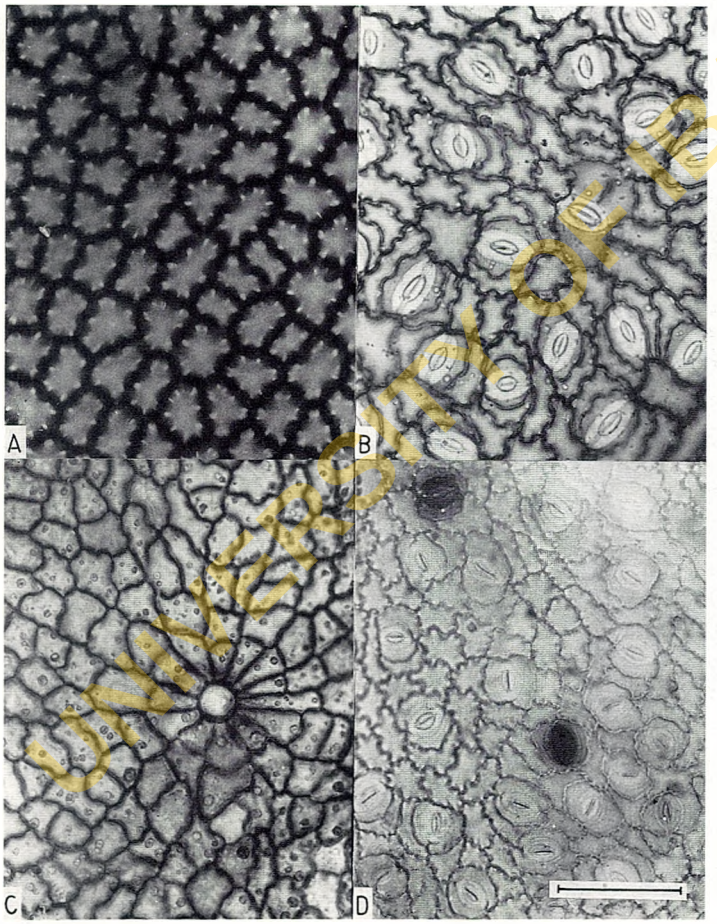

Fig. 2
Photomicrographs of leaflet epidermal features of *Dialium* species
A – *D. pachyphyllum*, adaxial surface with relatively thick, undulate anticlinic walls; B – *D. pachyphyllum,* abaxial surface with paracytic stomata; C – *D. dinklagei*, adaxial surface showing trichome base, and undulate and curved anticlinal walls, D – *D. dinklagei*, abaxial surface with paracytic stomata
All same scale = 50 µm

Table 1
Epidermal cell characters of *Dialium*

Taxa	Leaf Surface	Cell Shape	Anticlinal cell wall pattern	Epidermal cell size (μm)					
				Length			Width		
				min	(mean ± std. error)	max	min	(mean ± std. error)	max
D. pobeguinii	Adaxial	Isodiametric	Straight/ Curved	20.00	(24.15 ± 0.37)	30.00	20.00	(23.85 ± 0.34)	30.00
	Abaxial	Isodiametric	Straight/ Curved	20.00	(24.05 ± 0.37)	32.50	17.50	(22.05 ± 0.37)	27.50
D. guineense	Adaxial	Irregular	Undulate	20.00	(25.30 ± 0.36)	32.50	15.00	(22.60 ± 0.42)	30.00
	Abaxial	Irregular/ Isodiametric	Straight/ Curved	17.50	(23.55 ± 0.42)	30.00	15.00	(20.45 ± 0.35)	25.00
D. aubrevillei	Adaxial	Irregular	Undulate	15.00	(22.95 ± 0.51)	32.50	12.50	(18.95 ± 0.54)	25.00
	Abaxial	Irregular	Undulate	15.00	(22.20 ± 0.51)	32.50	12.50	(21.13 ± 0.52)	30.00
D. pachyphyllum	Adaxial	Irregular	Undulate	20.00	(25.40 ± 0.43)	32.50	12.50	(22.40 ± 0.44)	27.50
	Abaxial	Irregular	Undulate	17.50	(24.15 ± 0.49)	32.50	12.50	(19.05 ± 0.50)	25.00
D. dinklagei	Adaxial	Isodiametric/ Irregular	Curved/ Undulate	20.00	(25.40 ± 0.52)	35.00	17.50	(21.40 ± 0.39)	25.00
	Abaxial	Irregular	Undulate	20.00	(24.05 ± 0.37)	30.00	20.00	(23.50 ± 0.25)	25.00

D, F; 2B, D). Although, *D. guineense* has both paracytic and staurocytic, i.e. each stoma is surrounded by three to five similar subsidiary cells with anticlinal walls arranged crosswise to the guard cells (Fig. 1B). The mean number of stomata or square millimeter varies from 23 in *D. pobeguinii* and *D. pachyphyllum* to 31 in *D. dinklagei* (Table 2).

There are usually more cells per unit area on the adaxial than the abaxial side. For the adaxial surface, the range of mean cell per square millimetre, is from 146 in *D. pachyphyllum* to 171 in

Table 2
Epidermal features of *Dialium*

Taxa	Leaf Surface	No. of stomata (sq. mm)			No. of epidermal cells (cells/sq. mm)			Stomatal index (%)	Stomatal complex length × with (μm)	Trichome
		min	(mean)	max	min	(mean)	max			
D. pobeguinii	Adaxial	–			137	(152)	162	–	–	Present
	Abaxial	19	(23)	29	119	(137)	150	14.38	(21.92 ± 0.42) × (15.50 ± 0.34)	Present
D. guineense	Adaxial	–			154	(164)	174	–	–	Absent
	Abaxial	20	(25)	30	122	(145)	173	14.70	(20.92 ± 0.38) × (15.08 ± 0.32)	Present
D. aubrevillei	Adaxial	–			156	(166)	176	–	–	Absent
	Abaxial	21	(26)	32	130	(141)	150	15.57	(17.75 ± 0.32) × (16.50 ± 0.38)	Absent
D. pachyphyllum	Adaxial	–			120	(146)	165	–	–	Absent
	Abaxial	15	(23)	35	112	(139)	160	14.20	(23.25 ± 0.34) × (14.33 ± 0.31)	Present
D. dinklagei	Adaxial	–			165	(171)	177	–	–	Present
	Abaxial	27	(31)	36	127	(131)	135	19.14	(21.83 ± 0.48) × (15.33 ± 0.31)	Present

D. dinklagei. On the abaxial surface, the range is from 131 in *D. dinklagei* to 145 in *D. guineense* (Table 2). The stomatal index varies from 14.20% in *D. pachyphyllum* to 19.14% in *D. dinklagei*. The stomatal complex varies considerable among the five taxa studied. The stomatal size of 17.75 × 16.50 µm found in *D. aubrevillei* is the smallest while the largest stomate of 23.25 × 14.33 µm is recorded in *D. pachyphyllum* (Table 2).

The presence or absence and nature of trichomes in each species of the genus are summarized in Table 2 and Fig. 3. All taxa are pubescent except *D. aubrevillei* that is completely glabrous. *D. pobeguinii* and *D. guineense* possess both glandular and non-glandular hairs on the same surface (Fig. 3). Other species have non-glandular, unicellular trichomes with either a long or short gradual taper (Figs. 3A, E, H, J, N, O, P). Some trichomes have swollen base as in *D. pobeguinii* and *D. dinklagei* (Figs. 3A, B, C. D). Some are partically curved or bend e.g. *D. pobeguinii* and *D. guineense* (Figs. 3E, F, M), curved as in *D. pobeguinii* and *D. dinklagei* (Figs. 3C, D) or hooked as found in *D. pobeguinii* (Fig. 3 G). Other trichome types found in the genus include cylindrical types as recorded in

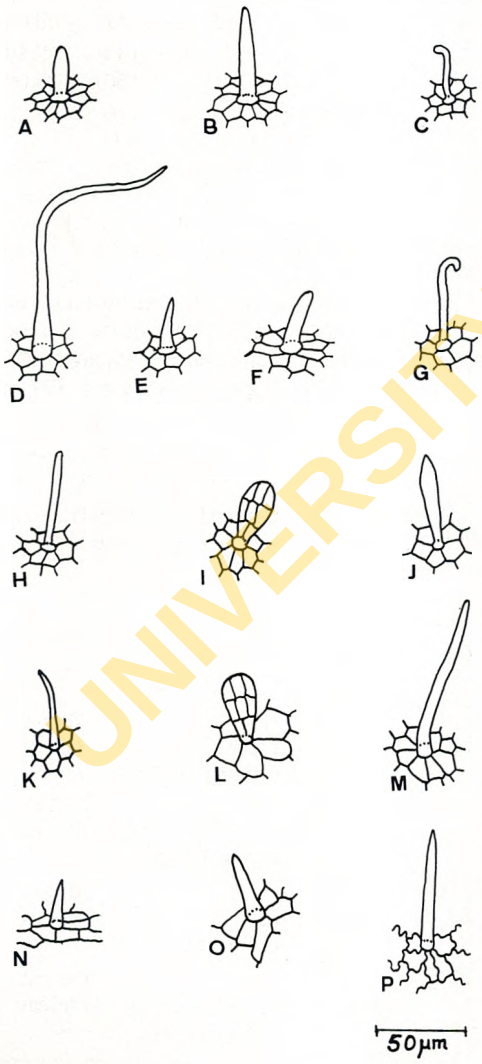

Fig. 3
Types of trichomes in the genus *Dialium*

A – simple, short, unicellular trichome with swollen base in *D. pobeguinii*; B – simple, long, unicellular trichome with swollen base in *D. pobeguinii*; C – simple, unicellular trichome with curved apex e.g. *D. pobeguinii* and *D. dinklagei*; D – simple, unicelluar, tapering trichome with bent mid-portion e.g. *D. pobeguinii* and *D. dinklagei*; E – simple, unicellular trichome with a short taper in *D. pobeguinii*; F – simple, unicelluar, semifalcate hair in *D. pobeguinii*; G – simple, unicellular trichome with hooked tip and swollen base in *D. pobeguinii*; H – simple, unicellular, cylindrical trichome e.g. *D. pobeguinii* and *D. dinklagei*; I – glandular, multicellular trichome seated on relatively smaller cells in *D. pobeguinii*; J – simple, unicellular trichome with a swollen base e.g. *D. guineense*; K – simple, unicellular tapering trichome slightly bent at the tip in *D. guineense*; L – glandular, multicellular trichome seated on relatively large cells e.g. *D. guineense*; M – simple, unicellular trichome slightly bent at the base in *D. guineense*; N, O – simple, unicellular tapering trichome seated on higher order vein with basal cells conspicuously different from the epidermal cells in *D. pachyphyllum*; P – simple, unicellular trichome seated on epidermal cells in *D. pachyphyllum*

D. pobeguinii and *D. dinklagei* (Fig. 3H), semi-falcate e.g. *D. pobeguinii* (3F), and glandular, multicellular trichomes are found on the abaxial surface of *D. pobeguinnii* and *D. guineense* only (Figs. 3I, L). Some of these trichomes are seated on pedestals of several epidermal cells as in *D. pobeguinii* (Figs. 1A; 3A, B, F) and those seated on higher order veins of the leaf with basal cells conspicuously different from other epidermal cells as recorded in *D. pachyphyllum* (Figs. 3N, O).

Discussion

The preceding observations and the summaries of character variation in Tables 1 and 2 show that the diversity of epidermal morphology in *Dialium* has taxonomic applications. The cell shape and cellwall pattern vary considerably within the genus. Based on these two characters, the genus can be conveniently divided into three groups. *D. pobeguinii* which has isodiametric shape with straight or curved wall on both surfaces can be separated easily from other species. Also *D. aubrevillei* and *D. pachyphyllum* with irregular cell shape and undulate wall pattern on both epidermises are distinctly different from other species. However, different combinations of cell shape and anticlinal wall pattern occur in the remaining two species – *D. guineense* and *D. dinklagei*.

The occurrence of curved walls in some of the species agreed with the suggestion of STACE (1965) that curved wall is a mesomorphic character. However, since the species are mostly forest trees growing in the moist forests and river banks of the tropical and subtropical regions of West Africa (HUTCHINSON & DALZIEL 1954), the presence of straight and undulate walls in members of the genus does not agree with the suggestion of STACE (1965). Furthermore, the epidermal cell size shows that the species are homogenous. On both surfaces, the range for the epidermal cell length is from 22.20 µm to 25.40 µm while the epidermal cell width varies from 18.95 µm to 23.85 µm. Based on these two characters, the species cannot be easily separated.

The relative abundance and variation of the trichomes is of taxonomic importance in the genus. Out of 16 different types recorded, *D. pobeguinii* has nine types thereby making the species difficult to separate from other species having trichomes. However, *D. pobeguinii* and *D. guineense* can be separated easily from other species due to the presence of glandular, multicellular trichomes on their abaxial surface. Also, *D. aubrevillei* which is completely glabrous can be distinguished from other species with relative ease. METCALFE & CHALK (1979) hold that trichome frequency and size are environmentally controlled, while STACE (1965) believes that hairs are constant in a species when present, and showed a constant range of form and distribution useful in diagnosis.

The presence of both paracytic and staurocytic stomata in *D. guineense* distinguishes it from other species. Stomatal type is of no diagnostic importance in other species because they all have paracytic stomata. However, stomatal index could be used to separate the species into two groups. Those with stomatal index less than 15% include *D. pobeguinii*, *D. guineense* and *D. pachyphyllum* while *D. aubrevillei* and *D. dinklagei* have stomatal index greater than 15%. *D. dinklagei* with 31 stomata per square millimeter can be separated from the remaining species with 23 to 26 stomata per square millimeter. Also, stomatal length values are found to be reliable in distinguishing between the species. *D. pobeguinii*, *D. guineense*, *D. pachyphyllum* and *D. dinklagei* have values that vary from 20.92 µm to 23.25 µm while relatively low value of 17.75 µm is recorded in *D. aubrevillei*. The importance of stomatal complex in taxonomy especially in the identification of small leaf fragments, the state at which most plant specimens occur in West African herbal markets, has been emphasized by STACE (1965). Based on the features observed with the light microscope, and indented dichotomous key separating the species is presented.

Key to the species of *Dialium*

1. Trichomes absent, mean stomatol length less than 18.00 µm . . . . . *aubrevillei*
1. Trichomes present at least on one surface; mean stomatol length greater than 20.00 µm 2
   2. Cell wall of abaxial surface undulate; epidermal cell length of adaxial surface 25.40 µm 3

3. Stomatal index 19.14%; mean no. of epidermal cells on adaxial surface 171 . . . . . *dinklagei*
3. Stomatal index 14.20%; mean no. of epidermal cells on adaxial surface 146. . . . . *pachyphyllum*

2. Cell wall of abaxial surface straight or curved; epidermal cell length of adaxial surface less than 25.04 µm 4
4. Cell shape of adaxial surface isodiametric, cell wall of adaxial surface straight or curved . . . . . *pobeguinii*
4. Cell shape of adaxial surface irregular; cell wall of adaxial surface undulate . . . . . *guineense*

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