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Micro-morphological studies in the genus *Balanites* Del. in West Africa

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Abstract. We examined the two species of the genus *Balanites* in West Africa for their foliar and pollen micro-characters. Fresh and herbarium specimens were used for this study. Results showed that the species have overlapping characteristics. Epidermal cells were generally anisodiametric or polygonal; anticlinal walls straight-curved while stomata were anomocytic and surrounded by large guard cells in both species. Trichomes were tectorial and only observed in *B. aegyptiaca*. Pollen grains were generally single, isopolar, tricolporate, and oblate spheroidal in shape, ranging from 23-23.5µm in *B. aegyptiaca* and 22-23µm in *B. wilsoniana,* in length. The exine was generally finely reticulate with indistinct lumina and thin in both species while pollen shape was oblate spheroidal in *B. aegyptiaca* and prolate spheroidal in *B. wilsoniana*. Ecological data showed that the species are allopatric but sometimes may be found in same ecological zone. Although the present study supports the co-existence of the taxa as sister species, the overlapping characters as observed also suggest the need for further taxonomic studies to ascertain beyond reasonable doubt, the recent infra-generic classification within the Zygophyllaceae.

Keywords: *Balanites,* conservation, leaf anatomy, pollen, West Africa, Zygophyllaceae.

INTRODUCTION

Balanites Del. is a genus of flowering plants in the family Zygophyllaceae (Dresler et al. 2014) comprising deciduous or semi-evergreen spiny trees or shrubs with simple or branching spines which are derived from the distal of two or more buds, axillary or at a varying distance above the subtending leaf (Sands 2001). Studies on several species in the genus have been published by many authors based on floral characters (Van Tieghen 1906; Sprague 1913; Mildbraed and Schlechter, 1914) and revised by Engler (1931). Sands (2001) recognised 9 species and 11 infra-specific taxa based on bud and inflorescence position, as well as characters associated with spines, which remain on the plants even when the fruits, flowers, and leaves are absent. In West Africa, the genus comprises only two species - *Balanites aegyptiaca* (L.) Del. and *B. wilsoniana* Dawe & Sprague (Hutchinson and Dalziel 1958; Keay 1989).

B. aegyptiaca popularly called 'desert date' is a highly drought-tolerant, evergreen, multi-branched, small tree up to l0 m high, with greenish stems and flexible drooping branches which bears long alternately set thorns; and yellow edible fruits. On the other hand, *B. wilsoniana* is described as an upper canopy tree reaching about 38 m in height and over 3 m in girth, and produces fruits that are greenish-brown in colour (Hutchinson and Dalziel 1958; Hall and Walker 1991; Chapman et al. 1992; Arbonnier 2002; Orwa et al. 2009). Keay (1989) reported that *B. aegyptiaca* is a savanna species easily recognized by long straight greenish spines arranged spirally along all the branches, either bearing flowers or not, while *B. wilsoniana* is a large forest tree distinguished by its larger leaves and lack of spine on the flowering branches. Sands (2001) noted that in *B. aegyptiaca,* the spines are simple or with subordinate branches and fruits usually less than 5 cm long, whereas in *B. wilsoniana* the spines are forked or branching several times and fruits 8-12 cm long. In his studies, Sands (2001*)* further separated the latter into 3 varieties: *glabripetala*, *mayumbensis,* and *wilsoniana*) based on pedicel length and hairiness of petals.

Balanites species have diverse uses in West Africa. The fruits of *B. aegyptica* are rich in edible oil (Newinger 1996) and used to brew alcoholic drinks, while the flowers are used as an ingredient in dawadawa (Hausa); and a food prepared from *Parkia filicoidea* Welw. ex Oliv. and the young leaves of *B. aegyptiaca* is eaten as vegetable in Chad, Nigeria and Sudan (Burkill 1985). As a thorny tree, *B. aegyptiaca* is useful for fencing while the wood is easily worked and made into spoons, handles, stools, and combs. In traditional medicine, the roots are used to treat malaria, oedema, chest pain, heart burn, etc. Although, the species is used as firewood, it is considered one of the most neglected tree species in arid regions (Burkill 1985; Hall and Walker 1991; Orwa et al. 2009). The seeds of *B. wilsoniana* are edible and oil bearing (Burkill 1985) while the wood is suitable for general construction (Irvine 1961). The bark contains a copious quantity of scented gum which is used in Ghana in the production of cosmetics; the ointment is also applied to newborn babies.

Over the years, foliar micro-morphological characteristics such as epidermal cell length, stomatal size, absence or presence of trichome etc., have provided valuable supplementary evidences and are of prospective taxonomic value (Soladoye and Crane 1985; Baronova 1992; Chukwuma et al. 2014; Chukwuma et al. 2017). Morphological characters of pollen grains have also been useful in taxonomic studies of plants (Erdtman 1952, 1969; Soladoye and Crane 1985; Pehlivan et al. 2009), and the ability to identify plants from their pollen has enabled botanists and ecologists to reconstruct past assemblages of plants and identify periods of environmental change (Faegri and Iversen 1989).

Despite the numerous information available on *Balanites* species including their ecology, distribution and uses, the current taxonomic placement of the genus has triggered a lot of interest among taxonomists. Sarma and Rajo Rao (1991) suggested a total separation of *Balanites* from Simourabaceae and a further creation of Balanitoideae as a subfamily within the Zygophyllaceae. This argument supports earlier opinion of Parvathi and Narayana (1978), who had noted that although *Balanites* differs from Zygophyllaceae and Simourabaceae, the genus should rather be retained within the Zygophyllaceae but as a sub-family Balanitoideae based on chemical evidences. In furtherance, studies had earlier reported the micromorphological characteristics (Ndoye et al. 2004; Usama 2007; Bhupendra et al. 2017; Mohammed et al. 2020) and genetics (Ram et al. 2008) of *B. aegyptiaca*, but non has provided similar details for *B. wilsoniana.* Comparative study on these species is also lacking. Consequently, our study focuses on the foliar and pollen micro-characters of the two West African species of *Balanites*, with a view to providing additional details which would complement existing diagnostic characters for identification of the taxa. We also provide information as to their current distribution within the region based on herbarium records, available literatures and online resources.

MATERIALS AND METHODS

Plant material

Fresh and herbarium specimens of *B. aegyptiaca* were used for the present study. The fresh specimens were collected from the arboretum of the Forestry Research Institute of Nigeria (FRIN), Ibadan and Usmanu Danfodiyo University, Sokoto, Nigeria, and carefully identified at the Forest Herbarium Ibadan (FHI) (Holmgren et al. 1990) prior to micro-morphological examinations. On the other hand, only herbarium specimens were used for *B. wilsoniana* because fresh samples where difficult to obtain at the time of this study. All herbarium specimens studied were those deposited at Forest Herbarium Ibadan (FHI) and University of Ibadan Herbarium (UIH) (Appendix I).

Leaf epidermal study

We examined 4 representatives of *B. aegyptiaca* and 5 samples of *B. wilsoniana* for their leaf epidermal charac-

teristics (Tables 2 and 3). Specifically, pieces of 2–5 cm^2 of the leaves of each representative (Chukwuma et al. 2017) were cut and soaked in concentrated trioxonitrate (v) acid (HNO_3) in well covered Petri dishes for about two to three hours to macerate the mesophyll. Upon the disintegration of tissues as indicated by the presence of bubbles on the leaves, the specimens were carefully transferred unto clean Petri-dish and rinsed thoroughly with distilled water before the epidermal surfaces were separated using forceps. Tissue debris was carefully cleared off the epidermis with fine Carmel® hair brush, and the isolated epidermal layers were adequately rinsed in distilled water. The epidermises were then transferred in to another Petri-dish containing 50% ethanol for 1–2 minutes, thereby allowing hardening of cells. Afterwards, tissues were stained with Safranin O for five minutes and then rinsed again in distilled water to remove excess stain. They were thereafter mounted in 25% glycerol on clear microscopic glass slides, covered with cover-slip and the edges of the cover slip were ringed with nail varnish to prevent dehydration. Five slides per specimen were prepared for each epidermis of the two species. Leaf epidermal descriptions followed those of Radford et al. (1974), Khatijah and Zaharina (1998) and Adedeji (2004) while stomata architecture was described following Carpenter (2005).

Pollen morphology

Fresh flowers of *B. aegyptiaca* and dried samples from herbarium specimens of *B. wilsoniana* were used for this purpose following acetolysis method described by Erdtman (1960). Pollen descriptions are in accordance with Erdtman (1943), Sowunmi (1973) and Sowunmi (1995).

All prepared slides were examined under Olympus® light microscope with ×40 objective lens. All photo micrographic images were taken using an attached ScopeImage® 9.0 camera mounted on the same microscope, at the Forest Herbarium Ibadan (FHI) while all measurements were obtained with a micrometer eyepiece. Each character was measured in twenty-five replicates.

Scanning electron microscopy

Small pieces (about 6 mm^2) of the dried leaf samples were fixed on a Aspex 3020 scanning electron microscope stubs with a double-sided tape and sputter coated with gold. The structural patterns of the leaf surfaces were carefully observed and photo-micrographic images were taken at an accelerating potential of 20.0kV at the Department of Material Science and Engineering, Faculty of Engineering, Kwara State University, Malete, Nigeria.

Species distribution

Records from Global Biodiversity Information Facility (GBIF) database (GBIF.org) and previously collected specimens of *Balanites* species deposited at FHI and UIH were utilized for this aspect. In addition, fresh specimens of *B. aegyptiaca* collected during the present study were also included. Geographic locations and coordinates of these specimens were carefully retrieved and thereafter used to produce a distributional map of the species in West Africa (Figure 1), using ArcGis 10.3.1.

Statistical analysis

All quantitative data were subjected to descriptive statistics and further analysed using PAST (PAlaeontological STatistics) version 4.02 (Hammer et al. 2001) to generate a UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) based dendrogram.

RESULTS AND DISCUSSION

The quantitative and qualitative foliar micro-characters of all examined specimens of *Balanites* species are presented in Tables 1–4. Comparatively, epidermal cells are generally anisodiametric or polygonal with thick, straight-curved anticlinal walls (Table 1, Figure 5). The species are amphistomatic, but more abundant on the adaxial surfaces than on the abaxial. However, stomata were fewer in specimens of *B. wilsoniana* collected from Cameroon (Tables 2 & 3). On the average, stomatal size in *B. aegyptiaca* were larger (271.9μm^2 – abaxial; 313.0μm^2 – adaxial) than the epidermal cells (104.3μm^2 – abaxial; 122.2μm^2 – adaxial), whereas in *B. wilsoniana,* the stomata were smaller, measuring 153.9μm^2 on the abaxial and 203.3μm^2 on the adaxial, while epidermal cells were averaged 156.7μm^2 on the abaxial and 206.3μm^2 on the adaxial surface (Table 4). All specimens of *B. aegyptiaca* examined showed similar epidermal characteristics (Figures 2 A–H) and they have thicker epidermal cell walls than those of *B. wilsoniana* (Figures 3 A–J). Trichomes were generally tectorial and unicellular (Table 1; Figure 4), up to 259.2μm in length and 22.4μm in width as observed on the adaxial surface of *B. aegyptiaca* collected in Ibadan, Nigeria while specimens obtained from Freetown in Sierra Leone and Sokoto in Nigeria, were void of trichomes (Table 2).

Sands (2001) treated Kennedy 1658 (Figures 3 A & B), Kennedy 1949 (Figures 3 C & D) and Odedoyin's 1959 collection (Figures 3 E & F) as *B. wilsoniana* var.

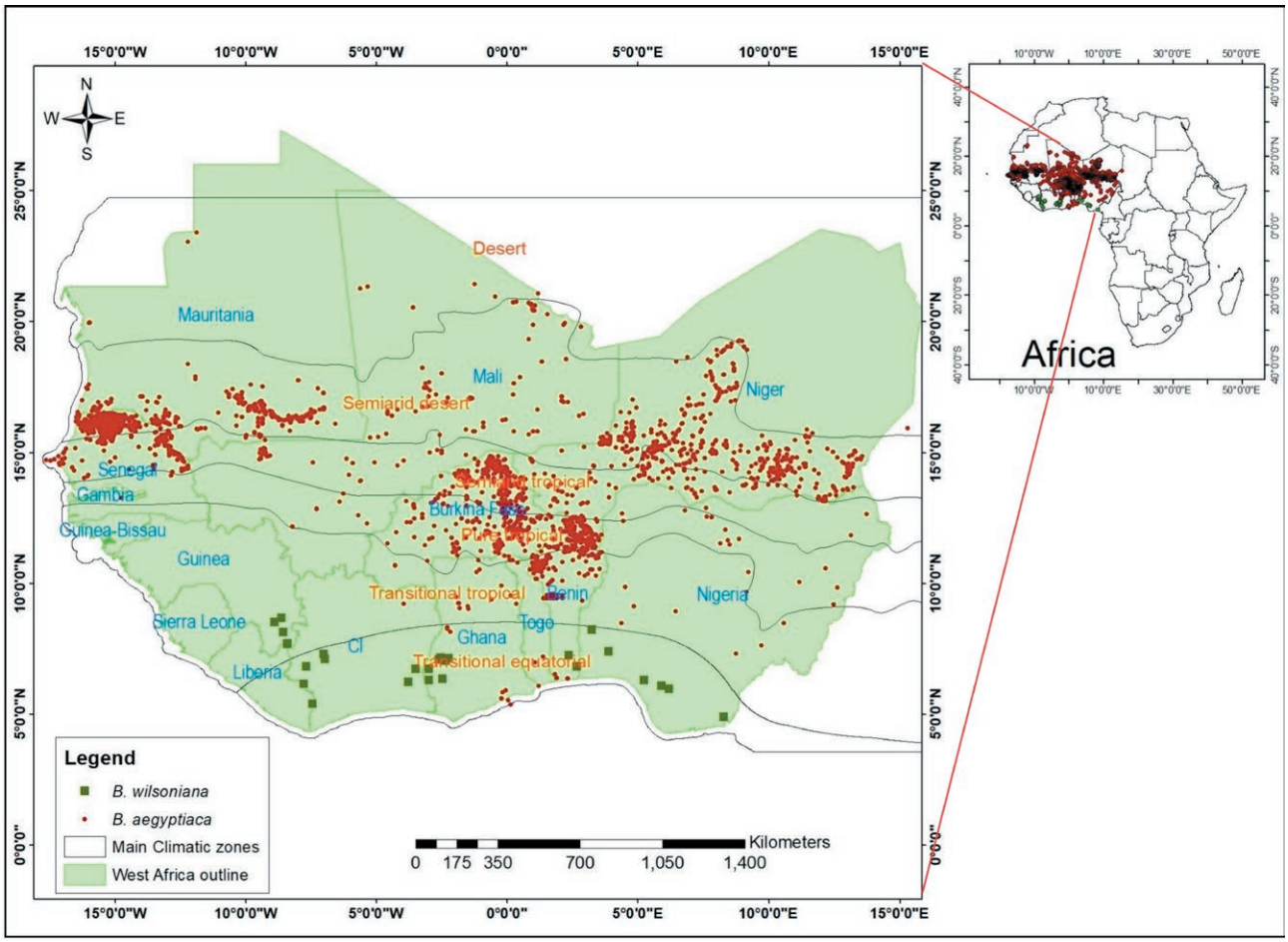

Figure 1. Distribution of *Balanites* species in West Africa.

grabipetala (loc cit. Pg 29); and Keay's 1950 collection (Figures 3 G & H) as *B. wilsoniana* var. *wilsoniana*. In our study, there was little or no difference in the foliar epidermises of Odedoyin's collection of 1959 (Figures 3 E & F) and that of Keay in 1950 (Figure 3 G & H). However, Kennedy's collections (FHI 9055 & 9056) showed distinct epidermal features as shown in Figure 3 (A-D). Following this observation, further study may reveal Odedoyin's collection as var. *wilsoniana* similar to that of Keay (FHI 28198). Additionally, a specimen from Cameroon (Figures 3 I & J) showed similar features with those of Odedoyin (Figures 3 E & F) and Keay (Figures 3 G & H) from Nigeria, suggesting that they could be of the same variety within *B. wilsoniana* complex.

As reflected through the dendrogram, variation even among same species is possible, and such variation may be brought about by alterations in ecological and climatic conditions. While specimens of *B. aegyptiaca* collected from Savane Palmraie, Burundi (FHI 101464) and Sokoto, Nigeria (FHI 111228) appear to be distinctly unique in epidermal characteristics, others share some attributes. For instance, abaxial surfaces of *B. wilsoniana* collected from Sapoba, Nigeria (sample F; FHI48385) and Kumba, Cameroon (sample G; FHI8496) are the most similar surfaces but also share some resemblance with abaxial surface of the one collected from Ikom, Nigeria (Sample E; FHI28198). More specifically, the studied taxa are divided into three main clusters (Figure 6a). As illustrated, both surfaces of samples F, G and the abaxial of E appear as the first goup; surfaces of A, B, C, H and I formed the second group in the central position, while the two surfaces of sample D and adaxial surface of E occupy the extreme end of the dendrogram as the third group. While *B. aegyptiaca* collected from Sokoto (FHI 111228) appears to occupy an isolated position of the scatter plot (Figure 6b) within component 1, others are clustered together at the other end of the plot within component 2. This further reflects the phylogenetic relationship between the species understudied and the overlapping of taxonomic characters within collections.

Table 1. Summary of qualitative foliar characteristics of *Balanites* species studied.

Species	Surface	Epidermal cell type/shape	Anticlinal cell wall pattern	Stomata type (s)	Trichome type (s)
B. aegyptiaca	Abaxial	Anisodiametric / polygonal	Straight - curved	Anomocytic	Tectorial, unicellular
	Adaxial	Anisodiametric / polygonal	Straight – curved	Anomocytic	Tectorial, unicellular
B. wilsoniana	Abaxial	Anisodiametric / polygonal	Straight – curved	Anomocytic, cyclocytic	Absent
	Adaxial	Anisodiametric / polygonal	Straight – curved	Anomocytic	Absent

Table 2. Quantitative foliar characteristics of *B. aegyptiaca* studied.

Specimen (location/voucher nos.)	Surfcae	ECL (µm)	ECW (µm)	ECS (µm^2)	CWT (µm)	No. St (per mm^2)	St. L (µm)	St. W (µm)	ST.S (µm^2)	No. tr (per mm^2)	Tr. L (µm)	Tr. W (µm)	Tr. D
Savane Palmraie, Burundi/ FHI 101464	Abaxial	6.5-10.8 8.5±0.4	2.9-6.6 4.8±0.3	25.9-61.1 41.1±3.6	1.0-1.3 1.1±0.0	61-91 72.4±2.6	11.7-20.1 15.5±0.9	8.4-18.1 11.6±1.1	98.4-364.6 187.8±29.0	3-13 7.6±0.9	45.4-160.5 78.7±10.3	8.8-16.7 11.9±0.8	15.3-66.2 38.7±4.8
	Adaxial	7.1-9.6 8.4±0.3	3.2-6.8 5.3±0.4	27.4-61.2 44.4±4.0	0.7-1.7 1.3±0.1	61-92 77.6±2.9	15.0-19.2 18.0±0.5	11.7-16.0 15.4±0.2	185.0-307.0 250.1±13.2	6-10 7.9±0.6	35.4-113.9 73.1±7.9	8.1-14.6 11.9±0.6	30.6-56.0 40.2±2.9
Freetown, Sierra Leone/ UIH 10260	Abaxial	7.7-15.2 12.0±0.8	4.8-11.7 7.3±0.6	49.3-151.7 88.2±10.9	0.5-2.4 1.7±0.2	51-78 68.8±2.3	10.1-19.5 16.5±0.8	11.2-15.2 13.3±0.4	153.3-262.6 217.4±11.0	Absent	Absent	Absent	Absent
	Adaxial	8.5-11.7 10.1±0.3	4.3-7.0 5.9±0.3	36.3-69.6 59.7±3.2	0.8-2.3 1.7±0.2	54-76 65.1±2.1	16.4-19.6 17.9±0.3	12.2-16.8 14.9±0.5	207.7-317.9 267.4±12.6	Absent	Absent	Absent	Absent
Sokoto, Nigeria/ FHI111227	Abaxial	9.7-15.7 13.7±0.6	7.2-9.4 7.9±0.4	71.7-127.1 107.5±7.0	0.9-1.7 1.3±0.1	54-61 56.7±0.8	12.5-18.0- 15.9±0.5	10.6-15.6 13.5±0.4	158.5-280.8 215.5±11.6	Absent	Absent	Absent	Absent
	Adaxial	11.8-18.6 14.6±0.8	7.2-11.6 8.9±0.4	85.1-179.2 130.6±8.7	0.5-2.0 1.3±0.1	40-47 43.7±0.7	14.9-19.1 16.7±0.5	11.7-16.0 14.1±0.5	191.8-284.4 235.2±10.1	Absent	Absent	Absent	Absent
Ibadan, Nigeria/ FHI111228	Abaxial	16.0-19.2 17.0±0.5	9.6-12.8 10.6±0.5	153.6-245.8 180.2±12.3	1.9-5.2 3.8±0.3	40-64 50.8±2.2	22.4-25.6 24.3±0.5	16.0-22.4 19.2±0.5	409.6-573.4 466.9±15.8	0-3 1.6±0.3	67.2-240.0 109.1±16.1	6.4-12.8 8.6±0.7	0-10.2 8.1±1.7
	Adaxial	16.0-22.4 19.5±0.7	9.6-16.0 13.1±0.7	184.3-358.4 254±14.4	2.3-3.6 3.0±0.2	46-58 50.0±1.2	22.4-25.6 23.7±0.5	16.0-25.6 21.1±1.1	409.6-655.4 498.7±25.4	1-5 2.9±0.4	108.8-259.2 160.0±16.9	9.6-22.4 14.7±1.2	5.1-25.5 14.8±1.9

Key: ECL- epidermal cell length; ECW- epidermal cell width; ECS- Epidermal Cell Size; CWT- epidermal cell wall thickness; No. St- number of stomata: St.L- stomata length; St. W- stomata width; ST.S- Stomatal Size; No. Tr- number of trichome; Tr.L- trichome length; Tr.W- trichome width; Tr.D- trichome density.
All measurements expressed as minimum – maximum above, mean ± standard error beneath.

Table 3. Quantitative foliar characteristics of *B. wilsoniana* studied.

Specimen (Location / voucher nos.)	Surfcae	ECL (μm)	ECW (μm)	ECS (μm²)	CWT (μm)	No. St (per mm²)	St. L (μm)	St. W (μm)	ST.S (μm²)
Cross-Rivers, Nigeria/ FHI 28198	Abaxial	16.4-24.4 20.7±0.9	7.5-14.6 10.9±0.7	175.5-270.6 220.0±3.6	0.8-3.3 2.0±0.2	31-59 44.6±2.9	12.2-15.4 13.9±.4	9.8-13.8 11.2±0.4	130.3-211.6 157.0±8.5
	Adaxial	20.6-30.5 26.7±1.1	12.9-20.7 16.8±0.7	364.4-552.8 446.0±22.5	2.3-5.1 3.9±0.3	5-17 9.5±1.2	19.6-22.8 21.2±0.4	17.4-18.6 17.8±0.2	324.5-418.5 377.0±8.5
Sapoba, Nigeria/ FHI 48385	Abaxial	13.0-25.2 18.4±1.2	7.0-12.9 10.6±0.5	142.0-236.7 192.0±10.7	1.1-3.2 2.0±0.3	40-60 49.0±2.0	11.1-18.4 13.7±0.6	8.1-12.4 9.7±0.5	107.4-228.3 134.0±12.1
	Adaxial	11.5-25.6 18.2±1.2	7.6-12.8 10.4±0.6	87.2-227.8 165.0±19.8	1.1-2.7 2.0±0.2	7-15 10.7±0.9	12.8-16.9 15.1±0.4	10.4-12.8 11.6±0.3	142.3-212.3 175.0±7.2
Kumba, Cameroon/ FHI 8496	Abaxial	12.4-23.5 18.8±1.2	9.0-13.7 10.7±0.5	131.4-265.7 198.0±12.7	0.7-2.4 1.4±0.2	33-44 38.7±1.1	12.5-18.4 14.5±0.7	8.4-11.7 10.1±0.3	105.3-209.9 148.0±10.8
	Adaxial	13.1-25.7 20.4±1.1	10.0-14.2 12.0±0.5	167.0-301.3 245.0±15.3	1.1-2.7 2.1±0.2	0-2 1.0±0.2	12.7-17.7 15.4±0.5	9.3-12.5 10.5±0.3	132.5-222.2 162.0±8.0
Sapoba, Nigeria/ FHI9056	Abaxial	9.9-14.6 12.8±0.4	1.1-9.4 6.6±0.8	10.7-120.2 86.2±10.4	1.4-2.5 1.8±0.1	79-114 102.2±10.1	13.4-24.1 16.1±1.0	11.1-14.1 12.0±0.3	149.2-338.8 194.0±17.7
	Adaxial	9.6-17.0 12.3±0.7	7.2-9.7 7.9±0.4	54.4-116.1 96.0±6.5	0.7-1.7 1.2±0.1	50-70 59.9±2.0	9.8-15.9 12.4±0.5	9.6-13.3 10.9±0.3	93.4-162.5 136.0±7.7
Sapoba, Nigeria/ FHI9055	Abaxial	10.9-14.9 13.0±0.5	4.9-9.8 6.7±0.5	59.1-116.1 86.5±6.8	0.6-1.7 1.4±0.1	71-110 89.3±4.0	11.9-16.4 13.4±0.5	8.4-12.4 10.1±0.3	98.9-203.6 136.0±8.7
	Adaxial	10.6-14.3 12.1±0.3	4.5-8.4 6.6±0.4	52.4-106.9 79.7±5.8	1.0-2.6 1.6±0.1	46-62 53.8±1.8	10.9-15.0 13.0±0.4	11.3-14.8 12.7±0.4	138.1-213.7 166±7.6

Key: ECL- epidermal cell length; ECW- epidermal cell width; ECS- Epidermal Cell Size; CWT- epidermal cell wall thickness; No. St- number of stomata: St.L- stomata length; St.W- stomata width; ST.S- Stomatal Size. All measurements expressed as minimum – maximum above, mean ± standard error beneath.

Table 4. Summary of quantitative foliar micro-characters of *Balanites* species studied (mean ± standard error).

Characters	*B. aegyptiaca*		*B. wilsoniana*	
	Abaxial	Adaxial	Abaxial	Adaxial
ECL (μm)	*12.8±0.6	**13.2±0.7	*16.7±0.6	**17.9±0.9
ECW (μm)	7.6±0.4	8.3±0.5	9.1±0.4	10.5±0.6
ECS (μm²)	*104.3±9.1	**122.2±13.9	*156.7±9.4	**206.3±20.2
CWT (μm)	2.0±0.2	1.8±0.1	1.7±0.1	2.1±0.2
No. St (per mm²)	*62.2±1.7	**59.1±2.3	*64.8±4.3	**26.9±3.6
St. L (μm)	*18.1±0.7	**18.9±0.5	*14.3±0.3	**15.4±0.5
St. W (μm)	*14.4±0.6	**16.1±0.6	*10.6±0.2	**12.7±0.4
St.S (μm²)	*271.9±20.2	**313.0±18.9	*153.9±6.0	**203.3±13.0
No. tr (per mm²)	2.3±0.6	2.7±0.5	Absent	Absent
Tr. L (μm)	6.9±8.9	58.3±11.5	Absent	Absent
Tr. W (μm)	5.1±0.9	6.6±1.1	Absent	Absent
Tr. D	11.7±2.8	13.8±2.8	Absent	Absent

P≤ 0.05.

* = significance in abaxial surface; ** = significance in adaxial surface?

Key: ECL: epidermal cell length; ECW: epidermal cell width; ECS: epidermal cell size; CWT: epidermal cell wall thickness; No. St: number of stomata: St.L: stomata length; St. W: stomata width; St.S: stomatal size; No. Tr: number of trichome; Tr. L: trichome length; Tr. W: trichome width; Tr. D: trichome density.

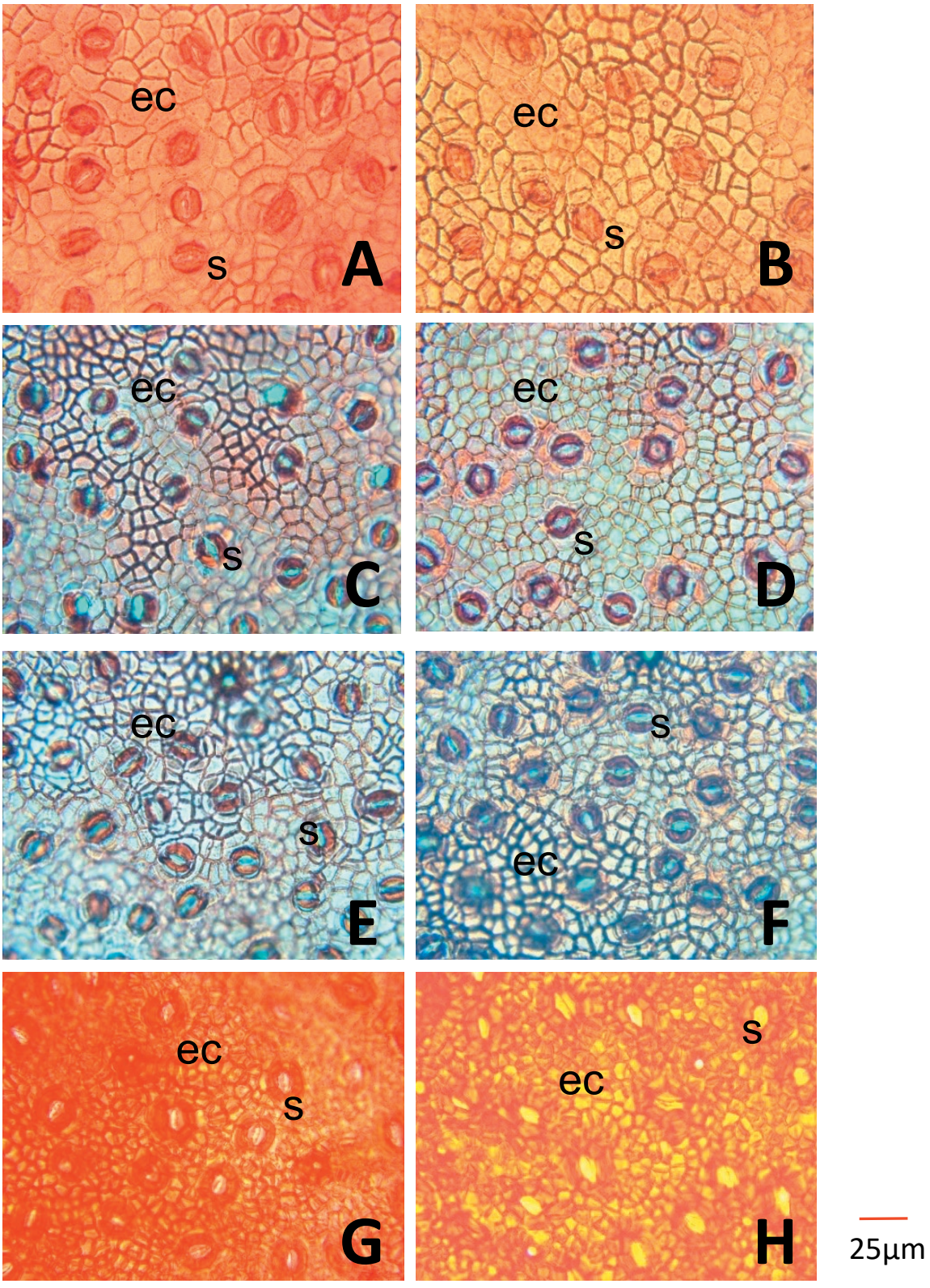

Figure 2. Photomicrographs (Light microscope) of the foliar epidermises of *B. aegyptiaca* studied. Mg. X400. **A&B** - Ibadan, Nigeria; **C&D** - Sokoto, Nigeria; **E&F** - Freetown, Sierra Leone; **G&H** - Savane Palmraie, Burundi. **A, C, E, G**: abaxial surface; **B, D, F, H**: adaxial surface. ec- epidermal cell; s- stoma.

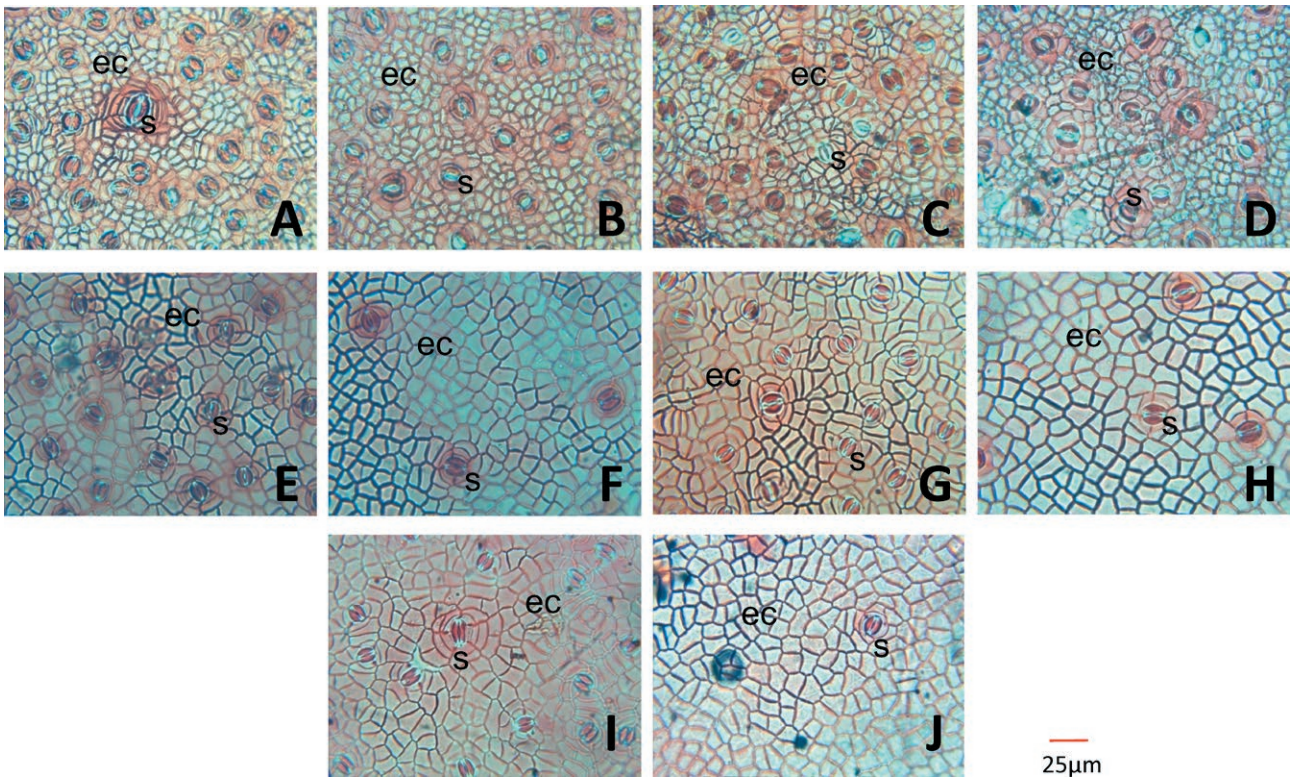

Figure 3. Photomicrographs of the foliar epidermis of *B. wilsoniana* studied. Mg. X400. **A&B** - Sapoba, Edo State, Nigeria; **C&D** - Sapoba, Edo State, Nigeria; **E&F** - Sapoba, Edo State, Nigeria; **G&H** - Ikom, Cross-Rivers State, Nigeria; **I&J** - Kumba division, Cameroon. **A, C, E, G, I**: abaxial surface; **B, D, F, H, J**: adaxial surface. ec- epidermal cell; s- stoma.

Sama and Rajo Rao (1991) noted a combination of polygonal and anisodiametric epidermal cells in *B. aegyptiaca* with anomocytic stomata. This was also the case as observed in our study. Stomata were mostly anomocytic, with some associated cyclocytic ones (as noticed in Keay's collections and that from Cameroon), and densely distributed on abaxial and adaxial epidermal surfaces of the species studied. Variation in stomatal size as observed in the present study could be linked to water stress, which has been reported to be responsible for the reduction in leaf size and also a reduction in the proportion of epidermal cells responsible for the formation of stomata and increased trichomes (Quarrie and Jones 1977; Usama 2007). Quantitative genetic studies have indicated ample genetic variation for trichome number and trichome density (Mauricio and Rausher 1997; Roy et al. 1999; Clauss et al. 2006). Perez-Estrada et al. (2000) in their study noted that trichome density decreased during the rainy season and increased during the dry season; and further opined that plants growing in sun exposed areas tend to have higher trichome densities than those in shady environment. It is also noteworthy that, the number of trichomes and density may also vary genetically within and among species on one hand, and even within populations of the same species on the other hand; since evolution does not take place in the same organ at the same time or even at the same rate. Hence, the presence or absence of trichome in certain species or specimens as noticed in the present study could be attributed to environmental factors. For instance, the specimen collected in Ibadan (fruiting and flowering every year), a rain forest zone, contradicts earlier reports that *B. aegyptiaca* is a typical savanna species. There was also little significant difference in the trichome density which was only recorded in *B. aegyptiaca* and absent in *B. wilsoniana*. However, the trichome type observed in our study is in tandem with the submissions of previous authors (Sarma and Rajo Rao 1991; Usama 2007; Bhupendra et al. 2017). Although, in their study on Simaroubaceae-Zygophyllaceae complex, Sarma and Rajo Rao (1991) recorded a total of eight trichome types, mostly unicellular in Zygophyllaceae and a combination of unicellular and uniseriate in Simourabaceae, they clearly reported that *Balanites* possess some unique epidermal characteristics including only one trichome type (unicellular). Likewise, Usama (2007) and Bhupen-

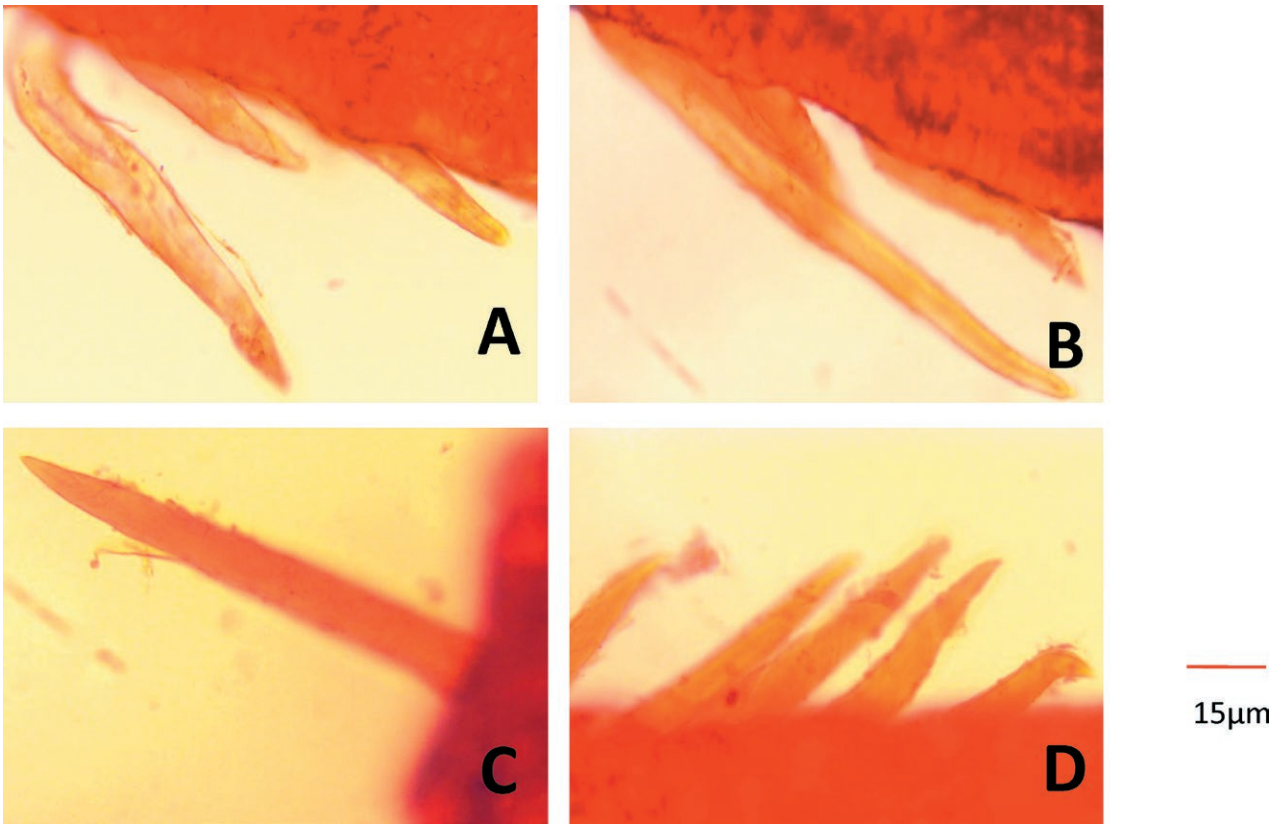

Figure 4. Trichomes in *B. Aegyptiaca.* Mg. x400. **A, B :** abaxial surfaces; **C, D :** Adaxial surfaces.

Table 5. Pollen characteristics of *Balanites* species studied.

	B. aegyptiaca	*B. wilsoniana*
Exine Pat.	Finely reticulate	Finely reticulate
Exine (µm)	2.1-2.5	2.0-2.3
Ora size	Large	Large
Ora size (h x w)	5.2-8.0 X 5.0-7.0	7.1-8.1 x 7.0
Apertures	3-colporate	3-colporate
Polar diameter (µm)	25.0-28.0	26.0-30.0
Equatorial diameter (µm)	26.0-30.0	22.5-29.0
Colpi (µm)	23.0-23.5	22.0-23.0
Shape	Oblate spheroidal	Prolate spheroidal

dra et al. (2017) also reported this trichome type in *B. aegyptiaca*.

Pollen description

Balanites aegyptiaca: Pollen grains are single, isopolar and oblate spheroidal in shape. Polar diameter ranged from 25.0µm to 28.0µm with an average of 26.5µm, while equatorial diameter ranged from 26.0µm to 33.0µm with an average of 28.0µm. Pollen grains are 3-colporate; colpi are with margo 23-23.5µm in length, and taper towards the poles. Ora are lolongate, spheroidal in shape, 5.2-8.0µm long and 5.0-7.0µm wide. Exine is generally thin; 2.1-2.5µm thick; exine is finely reticulate (Table 5, Figure 7 A & B).

Balanites wilsoniana: Pollen grains generally similar to those of *B. aegyptiava*. They are single, isopolar and prolate spheroidal in shape. Polar diameter 26.0-30µm with an average of 28.0µm; equatorial diameter 22.5-29.0µm with an average of 25.7µm. Pollen grains 3-colporate; colpi are also with margo and about 22-23µm in length, also taepering towards the poles. Ora are lolongate, oblate in shape; 7.1-8.1µm long and 7.0µm wide. Exine is generally thin; 2.0-2.3µm thick; exine is also finely reticulate with indistinct lumina (Table 5, Figure 7 C & D).

Species distribution

Although, herbarium specimens studied support that the species are allopatric in distribution – *B. aegyp-*

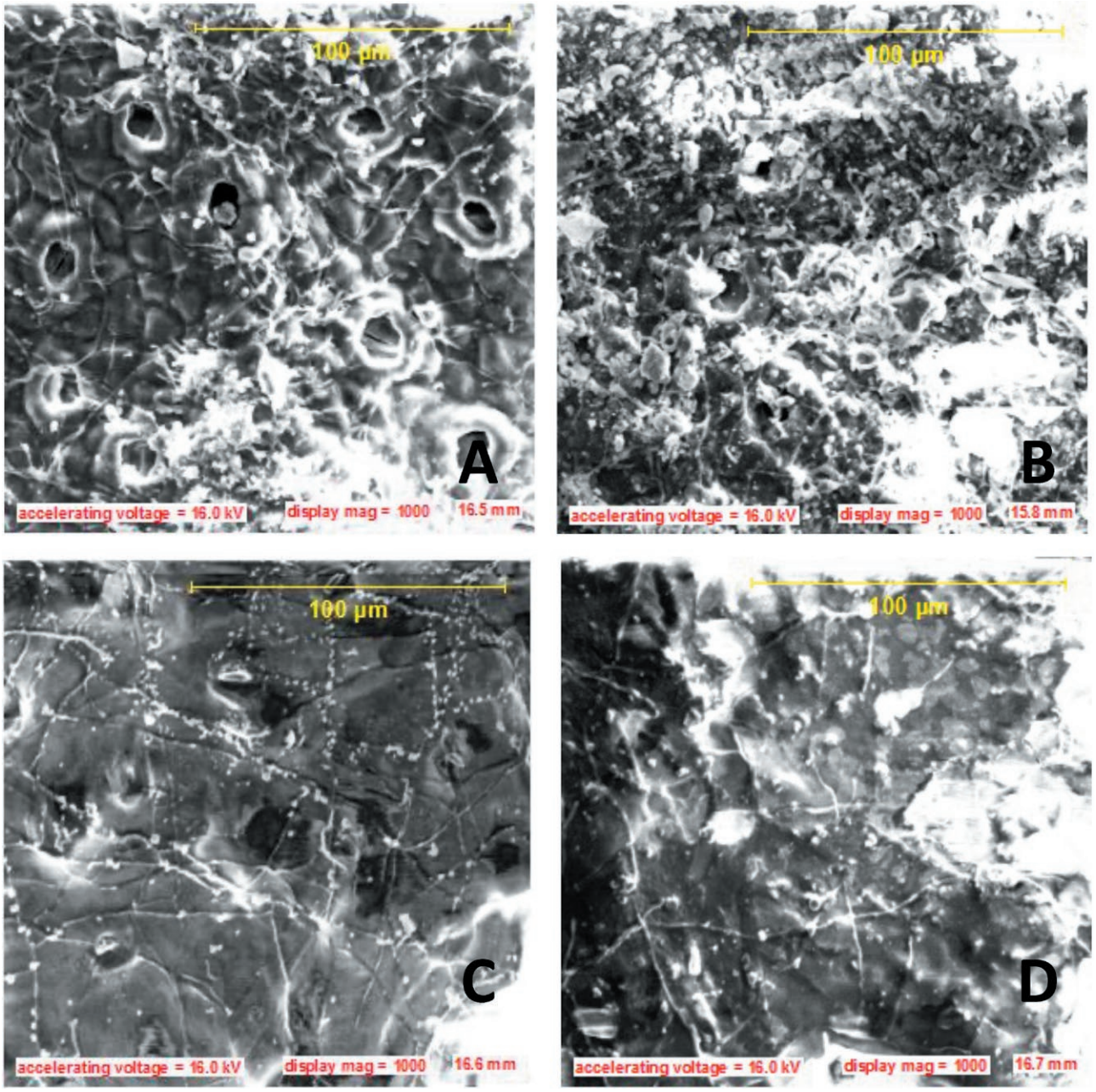

Figure 5. Photomicrographs (Scanning Electron Microscopy) of epidermal surfaces. Mg. x1000. **A**: *B. aegyptiaca* (Abaxial); **B**: *B. aegyptiaca* (Adazial); **C**: *B. wilsoniana* (Abaxial); **D**: *B. wilsoniana* (Adaxial)

tiaca is widespread in the dry areas of West Africa and *B. wilsoniana* occurs in the forest zones, further observations during the collection of plant materials for this study showed that the former can also thrive in forest areas. Hutchinson and Dalziel (1958) had earlier reported *B. aegyptiaca* to be found in 8 West African countries (Benin, Ghana, Guinea-Bissau, Mali, Mauritania, Nigeria, Senegal and Togo), while *B. wilsoniana* occurred only in 4 (Benin, Cote D' Ivoire, Nigeria and Ghana). A recent study by Hassler (2017) reported 12 countries for *B. aegyptiaca,* and maintained those 4 countries for *B. wilsoniana*. In our study however, based on herbarium assessments, field visits, and online data sourced from GBIF, we identified that *B. aegyptiaca* is widespread across the semiarid desert, semiarid tropical, pure tropical and transitional tropical climatic zones of West Afri-

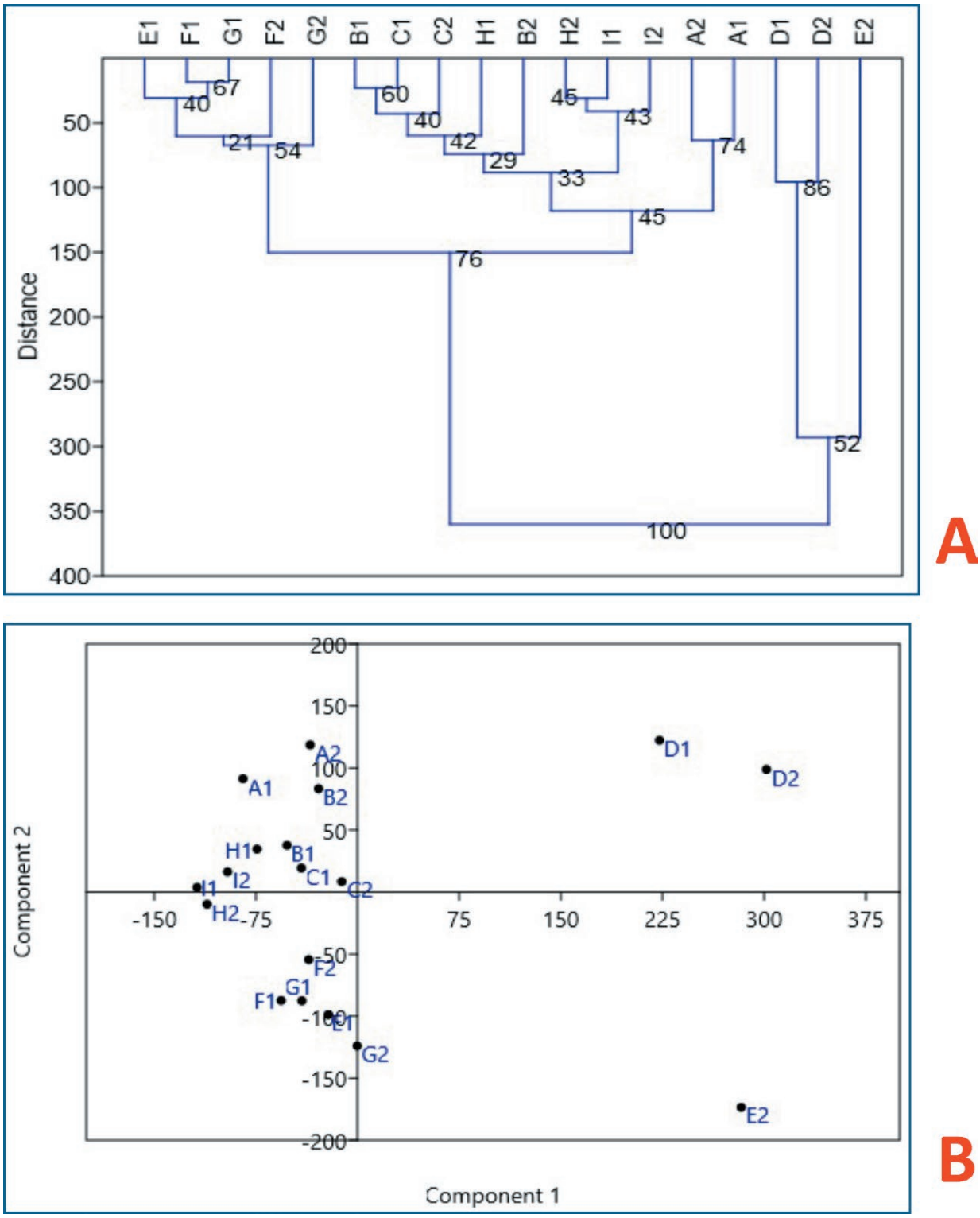

Figure 6. A - Dendrogram (UPGMA, Euclidean distance) showing degree of resemblance within *Balanites* collections. **B** - Component plot of species in rotated space. Samples **A-D**: *B. aegyptiaca*; **E-I**: *B. wilsoniana*. **A** - FHI101464; **B** – UIH10260; **C** – FHI111227; **D** – FHI111228. **E** - FHI28198; **F** – FHI48385; **G** – FHI8496; **H** – FHI9056; **I** – FHI9055. **1**- abaxial surface ; **2** – adaxial surface.

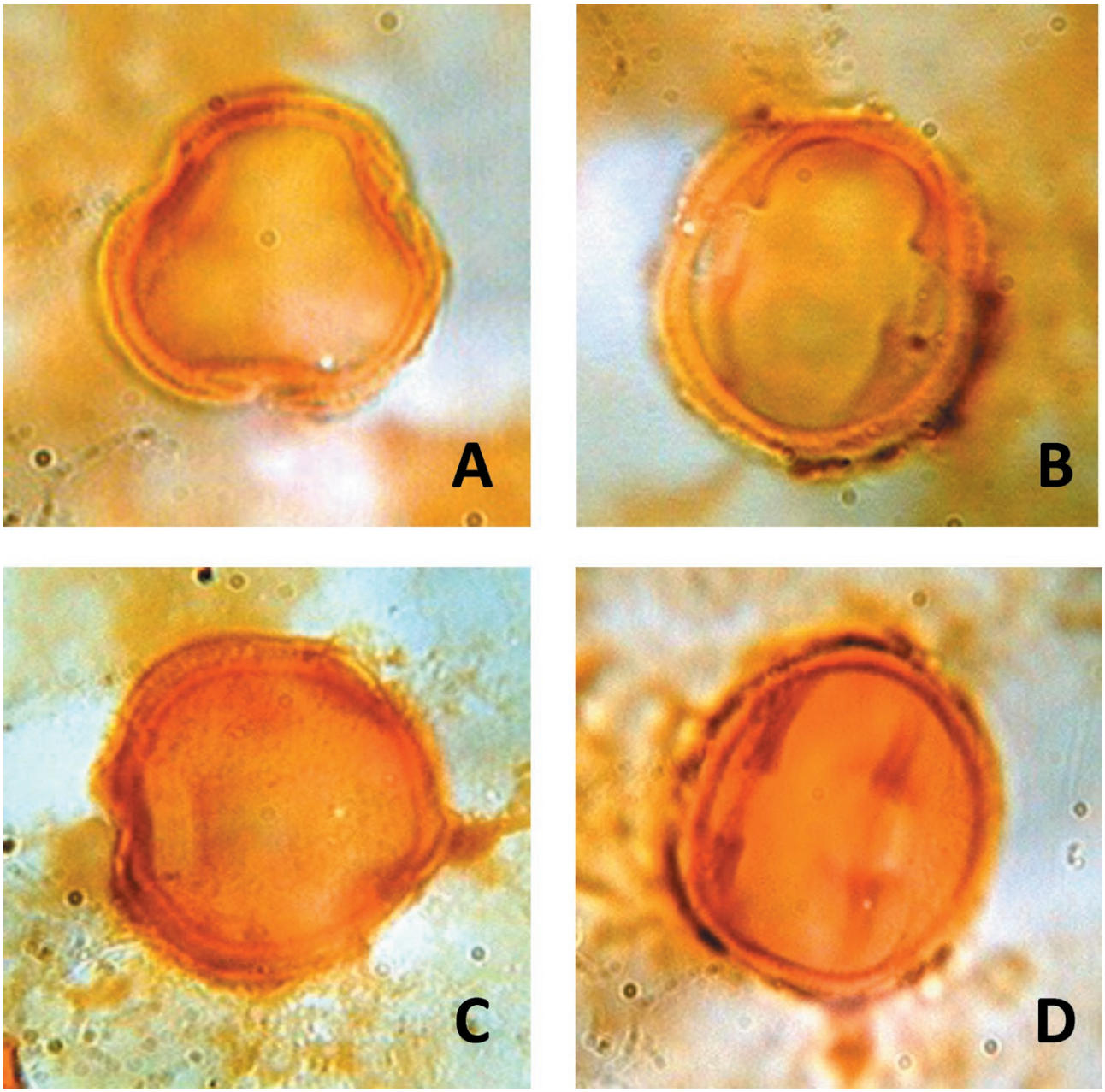

Figure 7. Photomicrographs of the pollen of *Balanites* species studied X400. **A&B** - *B. aegyptiaca*; **C&D** - *B. wilsoniana*. **A&C**- equatorial view; **B&D** - polar view.

ca while *B. wilsoniana* occupies the transitional equatorial belt and extends into the transitional tropical areas (Figure 1), yet their conservation status is poorly known. This trend thus calls for immediate attention towards protecting the species and many others whose statuses are also unknown, as a way of ensuring their sustainable collection and use, and also a step towards the restoration of our degraded ecosystems.

CONCLUSIONS

The present study has shown that the West African *Balanites* species share a number of overlapping anatomical characteristics, yet they can be distinguished from each other using some foliar and pollen microcharacters such as number of stomata, presence or absence of trichome, pollen/equatorial ratio etc. Field studies also opposed previous reports that the spe-

cies are allotropic in distribution, but suggest that the species may either be sympatric or allopatric depending on the region of occurrence. Although, the present study agrees with the co-existence of the two species, it also suggests a further re-evaluation of the present day Zygophyllaceae in an attempt to ascertain the current infra-generic re-classification of *Balanites* and other related species.

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Supplementary file. Voucher specimens used for the foliar microscopic study of *Balanites* species.

Species	Voucher number	Collector	Locality	Habitat	Collector's number	Date
B. aegyptiaca	FHI94202	Ekwuno	School of Wildlife, New-Bussa, Niger state, Nigeria	Savanna	-	27/01/1981
	FHI101464	Reekman, M	Bubanza, Plaine Rusizi, Km 14, Savane Palmraie, Burundi	Savanna	Reekman 9416	10/08/1980
	FHI31341	Chapman, J.D	Jimeta G.R.S, Benue Valley, Niger State, Nigeria	Savanna	Chapman 2604	14/11/1971
	UIH10260	Gledhiel, D.	Tower hill, Freetown, Sierra Leone	Savanna	-	Jan. 1967
	FHI111228	Chukwuma, E.C	Forestry Research Inst. of Nigeria, Ibadan, Nigeria	Secondary forest	-	21/06/2017
	FHI111227	Chukwuma, E.C	Usmanu Danfodiyo University, Sokoto, Nigeria	Savanna	-	05/07/2017
B. wilsoniana	FHI8496	Dundas	South of Banga between R Mungo & Kumba-Victoria, Kumba division, Cameroon	Old high forest	-	27/11/1945
	FHI48385	Odedoyin, R.O	Compartment 121, Sapoba Reserve, Sapob, Edo State, Nigeria	Rain forest	-	06/03/1959
	FHI9056	Kennedy, J.D	Sapoba, Edo State, Nigeria	Rain forest	Kennedy 1658	
	FHI48386	Odedoyin, R.O	Jameson river, 7 miles from Sapoba labour camp, Sapoba, Edo State, Nigeria	Rain forest	-	11/03/1959
	FHI9055	Kennedy, J.D	Sapoba, Edo State, Nigeria	Rain forest	Kennedy 1949	02/12/1931
	FHI44149	Adebusuyi, J.K	Oban Group Forest Reserve, Calabar, Cross-Rivers state, Nigeria	Rain forest	-	15/03/1961
	FHI28198	Keay, R.W.J	Afi River Forest Reserve, near Aboabam, Ikom, Cross-Rivers state, Nigeria	Rain forest	-	09/12/1950

FHI- Forest Herbarium Ibadan; UIH – University of Ibadan Herbarium.