

INTERSPECIFIC SEPARATION OF *GNETUM* L. (GNETACEAE) BASED ON MACRO AND MICRO MORPHOLOGICAL FEATURES.

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ABSTRACT

The study confirmed the occurrence and distribution of two species of *Gnetum* (*G. africanum* and *G. buchholzianum*) in southeastern Nigeria. A new collection, *Gnetum* species, which is clearly distinguishable from the other two by the peculiar structure of its male strobili is reported. Macro morphological variation was observed in characters like leaf form, leaf margin, inflorescence type, fruit shape and colour. Entire and lanceolate leaves are peculiar to *G. buchholzianum* while bead-like inflorescence of the new collection distinguishes it from the other species. Morphometric features like length of leaf, length of internode and length of spike varied among the species. However, analysis of variance on these features showed no significant difference ($P < 0.05$) in their mean values. The species are hypostomatic with amphibrachy-paracytic type of stomata. Pollens are generally spherical and acolpate. Pollen fertility values were high in the three species with the new collection having the highest value of 96%. Though difference was observed in pollen diameter, the generality of micro morphological characters suggest close affinity of the species. These results are discussed with a view to providing good description and taxonomic separation of the species.

KEY WORDS: *Gnetum*, male-strobili, pollen, stomata, vegetative-character

INTRODUCTION

Gnetum L. is a monotypic genus of the family Gnetaceae, which is found in the tropics (Hutchinson and Dalziel 1954; Burkill, 1994). In West Africa, two species *G. africanum* Welw. and *G. buchholzianum* Engl. were reported by Hutchinson and Dalziel (1954) to occur in Cameroun and Gabon. Lowe (1984) confirmed and reported the occurrence of *G. africanum* and *G. buchholzianum* in Nigeria, and separated the two species on the basis of their leaf dimensions and male inflorescence. Burkill (1994) noted that *G. buchholzianum* was not even known in Kew Herbarium to occur in Nigeria till 1971 when its leaves were observed in a local market in England, whence it had been air-freighted from Nigeria. However, this is a common wild vegetable among the south - eastern Nigerians, which has received some attention in local domestication. Due to their high nutritive values the leaves of these species are used as vegetables and masticatories (Okafor, 1975; Okigbo, 1975, 1980). They also occur in various forms in local salads. Though these species of immense economic importance are widely distributed in south - eastern Nigeria, except for Lowe (1984), taxonomic recognition of their status is lacking.

The usefulness of morphological characters in taxonomy (Metcalf & Chalk, 1950, Davis & Heywood, 1973, and Stace, 1980) has been highlighted by. These characters have been employed at various hierarchical levels (Akpabio & Olorode, 1988; Koek-Noorman *et al.*, 1990;

Ndukwu & Okoli, 1992; Thulin, 1994; Bhandari & Mukhopadhyay, 1997) for separation of taxa. Micromorphological (epidermal) characters including pollen morphology (Gill & Karatella, 1985; Okoli & Nyananyo, 1988; Faegri & Iversen, 1992; Edeoga & Osawe, 1996; Abubakar & Yunusa, 1998; Obute & Omotayo, 1999; Ogundipe & Akinrinade, 1999; Agbagwa & Ndukwu 2001; Agbagwa, 2001) have been effectively utilized in comparative anatomy and taxonomy. These authors utilized information from the structure, development and types of stomata, trichome and trichome types, and various other quantitative and qualitative leaf epidermal characters to achieve taxa elucidation. Cutler (1978) reported that apart from the usefulness of these characters in taxonomy, they are also effectively used in the identification of fossil leaf impressions in paleobotany and authentication of foliar drugs in pharmacognosy. So far basic taxonomic information and literature on the Nigeria species of *Gnetum* is scarce. In these days of constant deforestation and increased need of the species as vegetable, *Gnetum* species in Nigeria, which are all climbers face threats of extinction. This study uses quantitative and qualitative approach to describe the macro and micro morphological characters of *Gnetum* species in Nigeria with a view to providing a good description of these species. A new collection of this genus that has not been reported in West Africa is described alongside the existing two species. This collection is undergoing confirmation.

Table 1: Macro-morphological characters of *Gnetum* species studied

Character	<i>G. africanum</i>	<i>G. buchholzianum</i>	<i>Gnetum</i> species.
Habit	Tough twining shrub with green and red nodes	Tough twining shrub	Tough twining slender shrub
Stem colour	Brown, occasionally green	Always green	Green
Degree of branching	Absent or remotely branched	Much branched	Absent or remotely branched
Leaf architecture			
i. Organization	Simple and opposite	Simple and opposite	Simple and opposite
ii. form	Ovate or ovate-oblong	Elliptic or lanceolate	Ovate-elliptic or obovate
iii. margin	Remotely subcrenate	Entire	Serrated
iv. apices	Abruptly acuminate or mucronate	Acuminate or caudate	Caudate
v. bases	Rounded or slightly cuneate	Attenuate	Attenuate
vi. venation	Pinnate; camptodromous, brochidodromous	Pinnate; camptodromous, brochidodromous	Pinnate; camptodromous, brochidodromous
Inflorescence (Strobilus)			
i. type	Catkin-like spike	Catkin-like spike	bead-like spike
ii. position of spike	Borne laterally and terminally	Borne terminally	Borne laterally and terminally
iii. colour	Brown or green	Brown	Green
iv. number of spike per leaf axil	One or two	One	Three
v. internode	Same diameter through the spike	Thicker at the base	Same diameter
Fruit			
i. shape	Oval	Oval and slightly elongated	Round
ii. colour	Green when unripe, red when ripe	Green when unripe, dark-brown and marked with rings when ripe	Green when unripe, brown with no rings when ripe

MATERIALS AND METHODS

All materials used in this investigation were collected during field trips to different parts of south-eastern Nigeria between 1999 to 2001, from private gardens or secondary forest and galleried forests.

Macromorphological Studies

Observations on vegetative and floral characteristics were made on ten different stands of each species (*G. africanum*, *G. buchholzianum* and the new collection, *Gnetum* species). Thirty mature leaves and fruits were randomly selected from each species and quantified for

length and breadth dimensions. The lengths of the petiole, peduncle, and spike, and leaf branching angles were measured. The habit, stem colour, leaf type, arrangement, margin and fruit colour were visually assessed and scored. Photographs of relevant features were taken for each species.

Micromorphological Studies

Rectangular cuttings were obtained from mid-portions of mature leaves and soaked inside concentrated nitric acid in glass petri-dishes. Appearance of bubbles indicated that the epidermis could be carefully separated from each other. Adaxial and abaxial surfaces were carefully removed with forceps and mounted under a stereo microscope. Such strips were mounted in a drop of aqueous glycerin after staining in 1% safranin for 1 minute. The epidermal cells and stomata were enumerated from 50 such strips for each surface at x 20 objective magnification for 10 field views per strip. Length and breadth dimensions of the stomata were measured with ocular micrometer for 100 stomata for 20 views. Photomicrographs were taken with Leitz Diplan (Light microscope) fitted with Leica WILD MPS 52 camera. Stomata description is according to Dilcher (1974) and Stace (1980).

Pollen grains were collected from matured anthers and treated with a drop of absolute alcohol for 2 minutes, mounted in glycerine: acetocarmine 1:1 (V/V) on glass

slide and left to stand for two hours at 60°C (Obute and Omotayo, 1999). Stained and unstained pollen grains were enumerated at x 40 objective for 30 views for 10 flowers from each species. Pollen grain diameter was measured with an ocular micrometer. Pollen description is according to Faegri and Iversen (1992).

RESULTS

A summary of the macromorphological features and quantitative characters of the three species of *Gnetum* studied are presented in Tables 1 and 2. The species are generally strong, scandent dioecious lianas. *G. buchholzianum* is much branched (2-5 branches per plant) than *G. africanum* and *Gnetum* species where branching is either absent or very remote (0-2 per plant). Leaves were observed to be simple and opposite in the species. However, the leaf form varied being ovate or ovate – oblong with remotely subcrenate margins, abruptly acuminate or mucronate apex and rounded or slightly cuneate base in *G. africanum* (Fig. 1a), lanceolate with entire margins, acuminate or caudate apex and attenuate base in *G. buchholzianum* (Fig. 1c), and ovate elliptic or obovate with slightly serrated margins, caudate apex and attenuate base in the new collection, *Gnetum* species. (Fig. 1e). Other features of the leaves are in Table 2. The variations in petiole and internode lengths presented in Table 2 show

Table 2: Macromorphometric characters of *Gnetum* species studied

Character	<i>G. africana</i>	<i>G. buchholzianum</i>	<i>Gnetum</i> species
1. Number of branches per plant	0 - 2	2 - 5	0 - 5
2. Leaf dimensions (cm)			
i. length			
Range	6.00 - 9.50	3.50 - 5.00	7.50 - 12.00
Mean	7.60 ± 2.97	4.24 ± 2.81	9.41 ± 3.10
ii. breadth			
Range	2.40 - 4.80	2.46 - 4.60	2.55 - 4.40
Mean	3.61 ± 1.95	3.40 ± 1.89	3.40 ± 1.87
3. Petiole length (cm)			
Range	0.60 - 1.50	0.40 - 1.20	0.30 - 1.22
Mean	0.88 ± 0.97	0.75 ± 0.90	0.79 ± 0.89
4. Internode length (cm)			
Range	2.70 - 11.67	3.02 - 5.60	4.00 - 7.50
Mean	8.78 ± 2.40	4.10 ± 1.80	5.30 ± 2.11
5. Inflorescence (cm)			
i. spike length			
Range	2.00 - 3.48	1.50 - 2.50	4.05 - 7.00
Mean	2.90 ± 1.80	1.80 ± 1.62	5.12 ± 4.60
ii. number of internode per spike			
range	6 - 7	9	7 - 10
iii. peduncle length			
Range	0.26 - 0.64	0.48 - 0.87	0.70 - 1.26
Mean	0.35 ± 0.61	0.65 ± 0.70	1.06 ± 1.01
6. Fruit dimension (cm)			
i. length	0.50 - 1.20	1.40 - 2.00	0.5 - 1.00
ii. breadth	0.40 - 0.70	0.30 - 0.50	0.5 - 0.70

that *G. africanum* and *G. buchholzianum* are within the same range of petiole length but are separated from the new collection, *Gnetum* species, while *G. buchholzianum* and *Gnetum* species are within the same internode length range but separated from *G. africanum*.

The inflorescence in the three species vary from a bead – like jointed spike in the new collection, *Gnetum* species, to catkin – like spike in *G. africanum* and *G. buchholzianum* (Figs. 1a, c & e). They are borne either laterally in leaf axils or terminally. The strobili occur mostly singly in *G. buchholzianum*, singly for male and

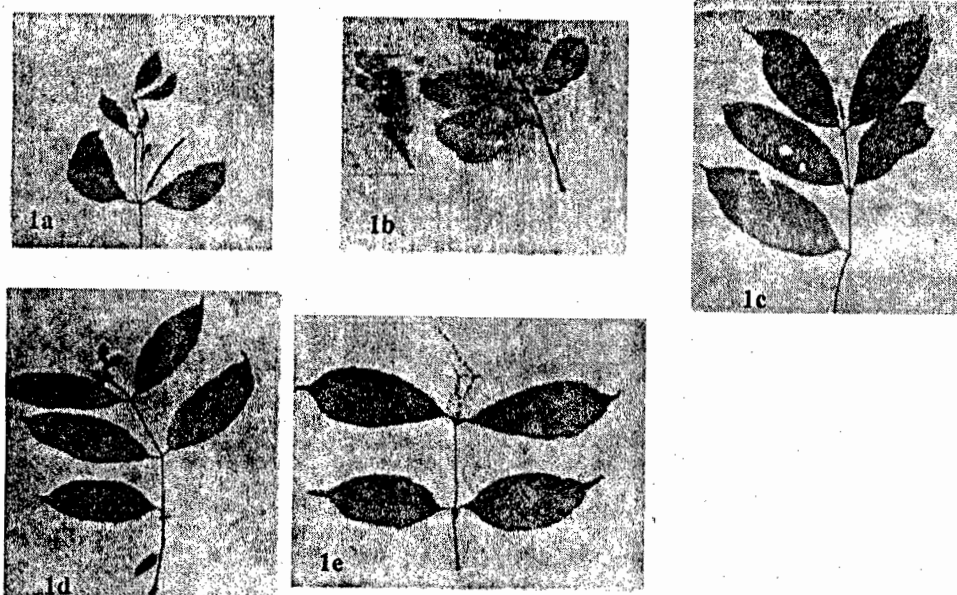


Figure 1: (a – e): (a) Part of shoot of *G. africanum* with male spike. (b) Mature female spike of *G. africanum* with fruits. (c) Habit of *G. buchholzianum* showing female spike. (d) Mature female spike with fruits. (e) Part of shoot of *Gnetum* species showing habit of plant and the bead-like inflorescence.

Table 3: Summary of micromorphometric character, which separates the species of *Gnetum* in Nigeria.

Character	<i>G. africanum</i>			<i>G. buchholzianum</i>			<i>Gnetum</i> species		
	Min.	x ± s.d.	Max.	Min.	x ± s.d.	Max.	Min.	x ± s.d.	Max.
1. Stomatal index (Abaxial)		29.10			33.12			30.71	
2. Stomatal dimensions (Abaxial)									
i. length (µm)	33.00	36.50 ± 6.36	42.00	26.40	29.20 ± 3.61	31.50	18.10	21.10 ± 2.76	24.00
ii. breadth (µm)	17.50	18.62 ± 2.12	20.50	14.00	16.42 ± 1.46	16.00	9.80	10.62 ± 1.61	12.00
3. Pollen grain									
i. diameter (µm)	13.40	16.50 ± 4.74	20.10	14.50	19.00 ± 5.64	22.47	13.00	14.60 ± 2.33	16.30
ii. fertility (%)		94.00			91.00			96.00	

Key to the species

- 1. Inflorescence is a bead – like spike, fruits brown at maturity, leaves obovate or ovate – elliptic and serrated *Gnetum* species
- 1' Inflorescence is a catkin – like spike 2
- 2 Leaves ovate and elliptic, fruits oval and red when ripe *G. africanum*
- 2' Leaves elliptic or lanceolate, fruits oval and slightly elongated, dark-brown and marked with rings at maturity *G. buchholzianum*

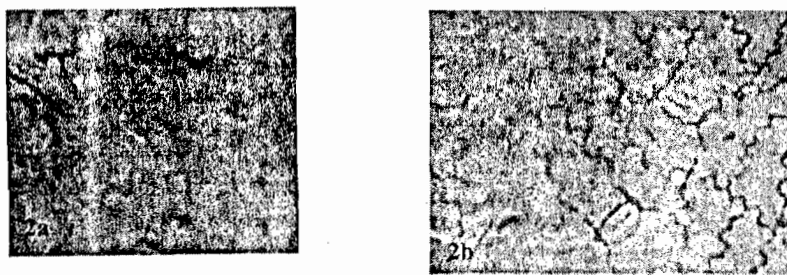


Figure 2: (a) Adaxial epidermis of *Gnetum africanum*, (b) Abaxial Epidermis of *Gnetum africanum*. Note that the irregular and wavy anticlinal wall pattern and amphibrachy-paracytic in *Gnetum africanum* is similar to those in *Gnetum buchholzianum* and the new collection.

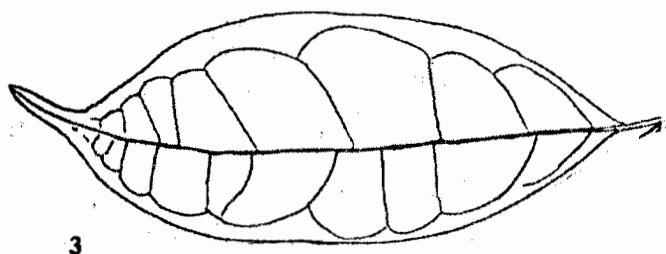


Figure 3: Venation pattern in *Gnetum* species

singly or in pairs for female *G. africanum* and in threes in the new collection, *Gnetum* species. The mean strobili (spike) lengths were 2.90 ± 1.80 cm in *G. africanum* 1.80 ± 1.62 cm in *G. buchholzianum* and 5.12 ± 4.60 cm in *Gnetum* species. Other features of the inflorescence are summarized in Tables 1 and 2. The fruits are oval, $0.90 - 1.1$ cm x $0.40 - 0.70$ cm, red when ripe in *G. africanum*, oval and slightly elongated, $1.40 - 2.00$ cm x $0.30 - 0.50$ cm, dark brown with ring marks when ripe in *G. buchholzianum* (Figs. c & d). They are roundish, $0.5 - 1.00$ x $0.5 - 0.7$ cm in *Gnetum* species.

The pollen of the three species were generally spherical and acolpate. Pollen fertility values were high in the three species with *Gnetum* species, showing the highest value of 96% (Table 3). Significant difference under analysis of variance ($P < 0.05$) was observed in pollen diameter. The new collection, *Gnetum* species, was observed to have the least mean value of $14.60\mu\text{m}$ while *G. buchholzianum* had $22.00\mu\text{m}$ (Table 3).

The epidermal walls on both adaxial and abaxial leaf surfaces of the species are irregular in shape with loosely wavy anticlinal walls (Fig. 2). The species are hypostomatic with amphibrachy-paracytic type of stomata. Stomatal indices and dimensions varied among the species (Table 3). However, the length and breadth dimensions of *G. buchholzianum* and the new collection, *Gnetum* species, are closely related when compared with *G. africanum*.

Venation pattern in the three species (Fig. 3) is pinnate, camptodromous and brochidodromous. Primary vein is of moderate size, straight and unbranched with

secondary veins diverging at $45^\circ - 60^\circ$. Secondary veins unbranched, forming loops with other secondaries towards the margin.

DISCUSSION

Qualitative and quantitative morphological (micro and macro) characters of species of *Gnetum* occurring in Nigeria have been studied for taxonomic purposes. Though some of these characters clearly demonstrate that the species are separate species, there were also overlaps and similarities in characters among them, which hitherto confirm their close affinity as members of one genus (Tables 1 & 2). For instance, the habit of the species is the same while the mode of branching and leaf margins were diagnostic for species separation. The climbing habit and evergreen leaves with reticulate venation observed in these species are similar to the ones used by Dutta (1979) to describe *Gnetum*. These characters of *Gnetum* are hardly distinguishable from those of angiosperms.

Leaf morphological characters are indispensable tools in taxonomy (Metcalfe and Chalk, 1950; Stace, 1980). Apart from species differences in form, margin and dimensions, other leaf characters were either similar or overlapped. While the differences observed in leaf characters serve for species delimitation, the similar and overlapping characters suggests similarities within the genus. Although pollen grain features are hardly adequate for taxa separation even at the generic level (Heslop - Harrison, 1971), it could be of diagnostic value at interspecific level. The different ranges and mean values of pollen diameter among the three species could separate them. Similar findings of variations in pollen diameter at the species and varietal levels have been made previously (Ugborogho and Oyelana, 1992; Obute and Omotayo, 1999). Pollen fertility in the species was generally high. The new collection *Gnetum* species, recorded the highest pollen fertility of 96%. Apart from the diagnostic importance of the differences in percentage pollen fertility per species, the high pollen fertility encountered in the three species may be a reproductive strategy of these anemophilous species aimed at circumventing entomophilous exigencies.

The inflorescence and fruit morphology of the species were very diagnostic and constitutes good characters for

their separation. For instance, *Gnetum* species, with its bead-like male strobilus (Fig.1e) and 7-10 internodes, is clearly separated from the catkin-like strobili with 6-7 internodes in *G. africanum* and mostly 9 internodes in *G. buchholzianum*. Lowe (1984) also recognized the taxonomic importance of the inflorescence of these species and used differences in male strobili of *G. africanum* and *G. buchholzianum* as one out of the two characters for separating them. The inflorescence characters in fact constitutes the mostly used morphological characters in taxonomy (Hutchinson and Dalziel, 1954; Gbile, 1979; Akpabio and Olorode, 1988). The variations in fruit shape and especially the differences in fruit colour at ripening reported in this study are useful for separating the species.

The hypostomatic distribution in the three species together with the strict adherence to amphibrachyparacytic stomata type confirms the close affinity of the species. On the other hand, differences observed in stomatal dimensions, index and distribution (number per area per view) in the three species could be used in their delimitation. This also confirms their existence as separate species. This conforms to similar use of such characters in the separation of taxa by Edeoga and Osawe (1996) in *Senna*, Abubakar and Yunusa (1998) in *Acacia*, Ogundipe and Akinrinlade (1999) in *Albizia*, and Agbagwa and Ndukwu (2001) in *Cucurbita*.

The new collection (*Gnetum* species) is distinct from *G. africanum* and *G. buchholzianum* on account of the characters of the inflorescence, pollen, fruit, leaf and stomata dimensions. This new collection is undergoing confirmation and further authentication. *G. buchholzianum* and *G. africanum* are themselves different from each other in the above characters with overlaps and similarities. The two species including the new collection are widely distributed within the southeastern states of Nigeria

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