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Numerical Taxonomy of *Combretum* Loefl. from Southeastern Nigeria

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ABSTRACT

Numerical taxonomic study of eleven *Combretum* Loefl. species common in southeastern Nigeria was carried out based on morphological and anatomical characters. PAST software was used to calculate the Euclidean distances and the coefficients were clustered by unweighted pair-group clustering method (UPGCM). From the 107 characters employed in the numerical analysis, the species were separated into two broad clusters (C1 represented by *C. zenkeri* and C2-the remaining 10 species). The clustering further suggested that there were groups within the genus hence C2 separated into two subgroups with *C. glutinosum*, a lone sub-cluster and the other nine species, which separated into four other sub-clusters. The similarity coefficient among the *Combretum* species studied ranged from 4.69 between *C. mooreanum* and *C. bracteatum* to 8.72 between *C. glutinosum* and *C. zenkeri*. Generally, results obtained favour species arrangement and clustering based on phylogenetic relationship as in *C. mooreanum*, *C. bracteatum* and *C. platypterum* and habitat preference. The unknown species, *Combretum* sp.1 is more closely related to *C. bracteatum* than any other species studied. However, *C. constrictum*, *C. dolichopetalum*, *C. glutinosum*, *C. hispidum*, *C. paniculatum*, *C. platypterum*, *C. racemosum* and *C. zenkeri* have been confirmed as individual taxa based on previous classifications. Though each Operational Taxonomic Unit (OTU) is confirmed species, we assert that the closeness observed amongst the OTUs imply that introgression as a mechanism of speciation in the wild, is at play in the genus.

Key words: *Combretum*, Combretaceae, introgression, Nigeria, numerical taxonomy, OTU

INTRODUCTION

Combretaceae is family of plant with 20 genera and 600 species, of tropical and subtropical regions of the world (Gill, 1988). In West Africa, the family Combretaceae is represented by 9 genera with 72 species and the genus *Combretum* Loefl. is the largest genus with 49 species (Gill, 1988). Keay (1989) reported 25 species of the genus *Combretum* Loefl. which are mainly straggling shrubs or lianes in Nigeria. Hutchinson and Dalziel (1954) showed that a number of species are indeterminate and occur in the South-Eastern Nigeria. The ethnopharmacological importance which include anti-inflammatory, anthemintic, anti-biharzia (anti-schistosomal), treatment of malaria, pain, dermatitis, diarrhea, Pneumonia, gonorrhea, syphilis, hypertension and cancer have been reported (Coulidiati *et al*., 2009; Fyhrquist *et al*., 2002; Baba-Moussa *et al*., 1999; Simon *et al*., 2003; Martini *et al*., 2004; Batawila *et al*., 2005).

The existing information on these species in Nigeria is strictly based on morphological descriptions by Hutchinson and Dalziel (1954). These information were however, sourced from

herbarium specimens. Literature search revealed neither recent description nor taxonomic treatment of Nigerian members of this genus. Hutchinson and Dalziel (1954) reported indeterminate species and noted that much taxonomic gaps needed to be filled with the identified species in tropical West Africa. This underscores the need for a study approach that utilizes different taxonomic lines of evidence to confirm the identity of species in southeastern Nigeria and other parts of tropical West Africa. In the wild forest of southeastern Nigeria were the species occur, there are conflicting identities with morphological signs of introgression. Thus the study is aimed at resolving difficulties with identifying taxonomic species from this section of Nigeria. It seeks to support the great taxonomic work of Hutchinson and Dalziel (1954), which unarguably is the taxonomic Bible of tropical West Africa. The numerical taxonomy approach adopted for this study has been applied in the elucidation of complex genera by Sornsuwan *et al.* (2006), Khalik *et al.* (2008), Dogan *et al.* (2009) and Osman *et al.* (2011).

MATERIALS AND METHODS

Plant material: Different specimens of *Combretum* were collected from southeastern part of Nigeria (Fig. 1) between January 2009 and December 2011. By painstaking reference to the Flora of West Tropical Africa (Hutchinson and Dalziel, 1954), these specimens were sorted into ten known taxa and an indeterminate species (Table 1). Further authentication of the taxonomic identity of

Table 1: Species name, locality, coordinates and date of collection of *Combretum* species studied

Species name	Locality	Coordinates/ elevation	Date of collection
C. bracteatum (Laws.) Engl. and Diels	Along Aba Port Harcourt express way, by Asa High Secondary School, Abia State	N04 55' 45.40", E007 14' 28.70"/ 34 m	February 5, 2011
C. constrictum (Benth.) Laws	Bank of Taylor Creek, Zarama, Bayelse State	N05 10' 55.20", E006 13' 36.20"/ 23 m	December 12, 2010
C. dolichopetalum Engl. and Diels	Elikpokwuodu/Mgbuchi road by Obio-Akpor Modern Market, Port Harcourt, Rivers State	N04 53' 43.00", E006 59' 49.00"/30 m	May 2, 2011
C. paniculatum Vent.	Ogbogu, Rivers State	N05 13' 17.10", E006 39' 28.60"/ 20 m	November 20, 2010
C. hispidum Laws	East/West Road Choba, opposite University of Port Harcourt main gate, Port Harcourt	N04 53' 24.80", E006 54' 59.80"/19 m	January 8, 2009
C. mooreanum Exell.	University of Port Harcourt Teaching Hospital Road, Alakahia, Rivers State	N04 58' 24.40", E006 55' 35.00"/ 17 m	December 31, 2010
C. platypterum (Welw.) Hutch and Dalz	Opposite Choba Police Station, along East/West Road, Port Harcourt.	N04 53' 32.20", E006 54' 57.20"/16 m	September 4, 2010
C. racemosum P. Beauv.	Beside Choba borrow pit along East/West road, Rivers State	N04 53' 24.20", E006 55' 09.40"/18 m	December 31, 2011
C. zenkeri Engl and Diels	Mgbirichi Community along Owerri-Port Harcourt Road, by Hausa settlement, Imo State	N05 21' 49.20", E006 57' 30.00"/ 44 m	August 25, 2010/September 10, 2010/May 8, 2011
Combretum sp.1	Ikot Ekwere, Akwa Ibom State	N04 45' 46.80", E007 59' 55.60"/ 17 m	August 30, 2011
C. glutinosum Perr. ex DC.	Zarama community, Bayelsa State	N05 10' 52.20", E006 13' 34.20"/ 15 m	November 10, 2010

the species was achieved by sending representative samples of each identified taxon to Forestry Herbarium Ibadan (FHI). These specimens were compared with species from the study area deposited at FHI. Morphological and epidermal studies were carried out on these species and characters and their character states (Table 2) deduced from the study were used for numerical analysis. Taxa were deposited at the University of Port Harcourt Herbarium (UPH).

Character observation: In this study, the species were treated as the Operational Taxonomic Units (OUT). Eleven OTUs (Table 1) and one hundred and seven (107) characters derived from the morphology (anther colour, flower colour, stem colour, colour of receptacle plus calyx tube, stem hair, habit, number of wings, average leaf length, average leaf breath, average petiole length, average internode length, leaf type, leaf apex, leaf base, leaf shape, leaf surface and leaf arrangement) and anatomy (crystal type, crystal distribution, number of palisade layers, No. of spongy layers, stomata types/complex/occurrence, trichome type, anticlinal cell wall pattern and epidermal cell wall shape) (Table 2) of the species were used in constructing the dendrogram. These characters were selected without prejudice. Ten samples of each authenticated OUT were either qualitatively observed or quantitatively scored to deduce the results summarized in Table 2.

Numerical analysis: Numerical taxonomic analysis was based on Sneath and Sokal (1973). The hierarchical cluster analysis for qualitative and quantitative characters was performed using binary Euclidean distance; the dendrogram was drawn using average distance linkage (between groups). The similarity coefficient/Euclidean distance were measured using PAST and clustered by Unweighted Pair-group Clustering Method (UPGCM). Data generated from the different morphological and anatomical characters were scored and converted to binary No. 1 or 0 i.e., present or absent and fed into a computer which generated clusters of the OTUs according to their overall similarity/Euclidean distance using PAST software. Dendrogram was constructed, which represented the diagrammatic illustration of the relationship among the species based on their degree of similarity in which the OTUs are linked together at various levels of resemblance.

RESULTS

The characters and character states generated and used for the cluster analysis is presented in Table 2. From the 107 characters employed in the numerical analysis, the dissimilarity coefficients among the *Combretum* species studied ranged from 4.69% between *C. mooreanum* and *C. bracteatum* to 8.72% between *C. racemosum* and *C. zenkeri* (Table 3). At 11.54% dissimilarity as shown in Fig. 1, the OTUs separated into two broad clusters. Cluster C1 contains only one species, *C. zenkeri* while cluster C2 contains 10 species. However at 11.11% dissimilarity, cluster C2 further separated into two sub-clusters (C3 with only *C. glutinosum* and C4). Sub-cluster 4 gave rise to C5 and C7 each with two species and C6 with five species (*C. mooreanum*, *C. paniculatum*, *C. bracteatum*, *C. platypterum* and *Combretum* sp.1) as shown in Fig. 1.

The unknown species *Combretum* sp.1 is closest to *C. bracteatum* and together with *C. mooreanum* (all three in C9) occur in marshy environment. *C. mooreanum*, *C. bracteatum* and *C. platypterum* in C6 are phylogenetically related in Hutchinson and Dalziel (1954) classification scheme.

Table 2: Character and character states used for cluster analysis of *Combretum* species

Character	Charater state	Character	Charater state
Crystal type	Druse	Stem hair	Glabrous/glabrescent
	Cyltolith		Sparsely Hairy
Crystal distribution	Sub-epidermis adaxial	Densely hairy	
	Sub-epidermis abaxial	Habit	Climbing shrub
	Spongy mesophyll		Shrub
	Palisade mesophyll		Small tree
No. of palisade layers	1 layer	No. of wings	Five wings
	1-2 layers		Four wings
No. of spongy layers	1-4 layers	Anticlinal cell wall pattern	Arched/Curved
	5-8 layers		Straight
	9-12 layers		Wavy/Undulating
Stomata types/complex/occurrence	Tetracytic	Epidermal cell wall shape	Oval
	Brachyparacytic		Rectangular
	Anomocytic		Polygonal
	Staurocytic		Irregular
	Anomotetracytic	Average Leaf length (cm)	0-10
	Actinocytic		>10
	Isotricytic		
	Anisocytic	Average Leaf breath (cm)	0-5
	Anisotricytic		10-May
	Diacytic		
	Contiguous	Average petiole length (cm)	0.1-0.5
	Amphistomatic		0.6-1.0
	Hypostomatic		1.1-1.5
			>1.5
Trichome type	Gland head unicellular/uniseriate stalk	Average Internode length (cm)	0-5
	Uniseriate cylindrical clavate trichome		5.1-10
	Unicellular gland head/uniseriate stalk		>10
	Nulticellular glandhead/uniseriate stalk		
	Peltate gland head	Leaf type	Acute
	Eglandular conical trichome	Leaf apex	Acuminate
Anther colour	Red		Cuspidate
	Pink		Mucronate
	Purple	Leaf base	Cordate
	Yellow		Rounded
	Cream		Obtuse
			Cuneate
Flower colour	Red		Acute
	Pink	Leaf shape	Obovate
	Purple		Ovate
	Yellow		Lanceolate
	Cream		Elliptic
	White		Oblong
	Green		
Stem colour	Purple	Leaf surface	Glabrous/glabrescent
	Dark brown		Sparsely hairy
	Brown		Densely hairy

Table 2: Continue

Character	Charater state	Character	Charater state
	Red	Leaf arrangement	Opposite
	Grey		Alternate
	Rusty brown		Whorl
	Black		
Receptacle+calyx tube	Yellow		
	Pale green		
	Cream		
	White		
	Red		
	Brown		

Table 3: Range of coefficient of dissimilarity matrix among the eleven *Combretum* species

0	1	2	3	4	5	6	7	8	9	10	11
1	0.00										
2	6.25	0.00									
3	6.25	6.93	0.00								
4	6.25	7.21	7.35	0.00							
5	5.29	5.74	6.40	6.40	0.00						
6	4.69	5.92	6.25	6.40	6.00	0.00					
7	5.74	6.78	6.63	6.32	6.40	6.25	0.00				
8	5.00	6.48	6.93	6.63	5.92	6.08	5.66	0.00			
9	6.00	6.56	6.40	7.28	6.48	6.32	6.25	6.40	0.00		
10	7.00	7.07	7.62	8.49	6.08	7.42	7.35	7.07	8.43	0.00	
11	5.66	6.08	6.56	6.25	6.16	5.29	5.92	6.08	6.48	7.14	0.00

1: *C. bracteatum* (Laws.) Engl. and Diels., 2: *C. constrictum* (Benth.) Laws, 3: *C. dolichopetalum* Engl. and Diels., 4: *C. glutinosum* Perr. ex DC., 5: *C. hispidum* Laws, 6: *C. mooreanum* Exell., 7: *C. paniculatum* Vent., 8: *C. platypterum* (Welw.) Hutch. and Dalz., 9: *C. racemosum* P. Beauv., 10: *C. zenkeri* Engl. and Diels. and 11: *Combretum* sp.1

DISCUSSION

The close morphological similarities of members of the genus *Combretum* in Nigeria oftentimes lead to confusion in species identification and collection. Existing taxonomic information on the genus in the country is based on morphological characters documented in Hutchinson and Dalziel (1954), which reported some indeterminate species. Reassessment of the phylogenetic relationship of members of this genus is therefore worthwhile. We have begun this by subjecting species within the southeastern region of the country to numerical analysis and phylogenetic interpretation.

Dendrogram based on morphological and anatomical information grouped these eleven southeastern species into two major clusters C1 and C2. Cluster C1 is distinct containing only *C. zenkeri*. This indicates that *C. zenkeri* is distinct from all the other southeastern species studied. The remaining ten species clustered in C2. The unknown species *Combretum* sp.1 is found midway among these other species. In cluster C2, *C. glutinosum* is distinct among the species in this cluster. *C. racemosum* and *C. dolichopetalum* are closely related supporting Hutchinson and Dalziel (1954). The relative closeness between these species is attributed to their similar anatomical features. However, *C. hispidum* and *C. dolichopetalum* did not follow the sequence. The divergence

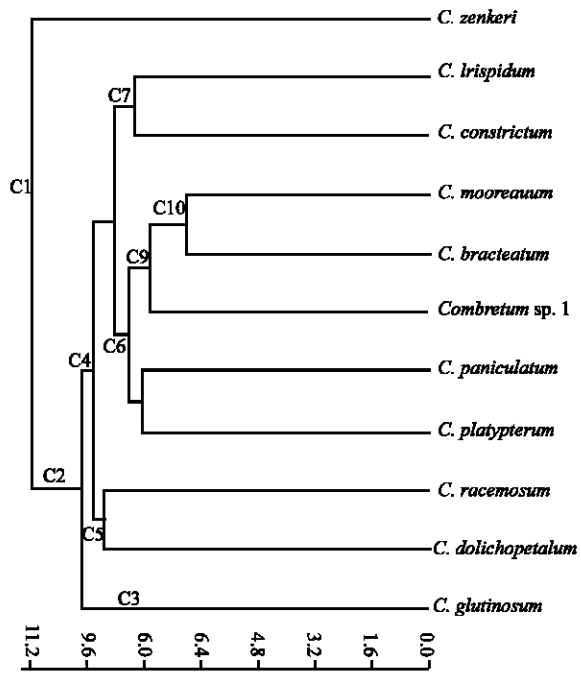

Fig. 1: Dendrogram for the hierarchical cluster analysis of 11 southeastern Nigeria *Combretum* species using Euclidean distance among the taxa

between these two species is as result of their anatomical differences. Similarly, *C. zenkeri* and *C. glutinonum* are the most diverged species in the dedrogram. On the other hand, the unknown species *Combretum* sp.1 is more closely related to *C. bracteatum* and *C. paniculatum* than any other species in the dendrogram. Based on their percentage dissimilarity, *Combretum* sp.1 is a new unnamed species but more closely related to *C. bracteatum*.

It is important to reemphasize the fact that current information is lacking on members of the genus *Combretum* in Nigeria. A closer comparative assessment of the results obtained from this study with existing information in Hutchinson and Dalziel (1954) confirm the phylogenetic relationship between *C. mooreanum*, *C. bracteatum* and *C. platypterum*. In fact, *C. bracteatum* is phylogenetically next to *C. mooreanum* in Hutchinson and Dalziel (1954) classification key. These species also have similar habitat preference occurring in marshy environments. *C. constrictum*, a low shrubby liana also prefers damp habitats. However, *C. glutinosum*, which has been previously reported as savannah small tree, was collected within the region. So also was *C. paniculatum*, which reportedly thrive at the extreme of the forest zone into the savannah region of the country. These may not be unconnected with human dispersal of reproductive structures.

The close affinity observed in morphological and anatomical characters employed in this study across species is worthy of note. These observations were highest amongst members in C9 (*C. mooreanum*, *C. bracteatum* and *Combretum* sp.1). We therefore assert that introgression as a mechanism for speciation is at work on members of the genus *Combretum* in southeastern Nigeria. Further work is ongoing for the authentication of this assertion.

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