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# Phylogenetic Study of African *Combretaceae* R. Br. Based on *rbcL* Sequence

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*Abstract*

*Combretaceae* R. Br. is an angiosperm family of high economic value. However, there is dearth of information on the phylogenetic relationship of the members of this family using ribulose biphosphate carboxylase (*rbcL*) gene. Previous studies with electrophoretic-based and morphological markers revealed that this family is phylogenetically complex.

In the present study, 79 sequences of *rbcL* were used to study the phylogenetic relationship among the members of *Combretaceae* of African origin with a view to provide more information required for the utilization and management of this family. Multiple Sequence alignment was executed using the MUSCLE component of Molecular Evolutionary Genetics Version X Analysis (*MEGA X*). Transition/Transversion ratio, Consistency index, Retention Index and Composite Index were also determined. Phylogenetic trees were constructed using Maximum parsimony (MP) and Neighbor joining methods.

The alignment of *rbcL* in the family *Combretaceae* shows 0.59 for both variation and parsimony sites with the overall mean distance of 0.71. Result shows that the genera *Combretum, Terminalia* are polyphyletic, while *Conocarpus*, *Quisqualis* and *Meiostemon* are monophyletic.

Findings from this study can be applied to the scientific classification of the African *Combretaceae*, especially where morphological and electrophoretic-based molecular delimitations have failed. *rbcL* from this family can also be used as barcodes against drug adulteration of the medicinal species.

**Keywords:** Phylogeny, *rbcL gene, Combretum, Terminalia*

## Introduction

*Combretaceae* R. Br. is one of the major families of angiosperm, which belongs to the order *Myrtales*. It comprises of two subfamilies, *Combretoideae* and *Strephonematoideae,* of which the former is monogeneric while the latter owns most of the genera in the family. The family consists of about 20 genera and approximately 600 species and subspecies world-wide (Tan et al. 2002, APG III 2009, Krachai and Pornpongrungrueng 2015, Kiew 2019). The genera of this family (Combretaceae) include *Calopyxis, Combretum, Conocarpus, Guiera, Laguncularia, Quisqualis, Strephonema, Terminalia, Buchenavia, Bucida, Getonia, Lumnitzera, Myrobalanus, Pteleopsis, Thiloa, Dansiea, Meiostemon, Vicentia* and *Anogeissus*. According to Gere et al. (2015), *Calopyxis, Combretum, Conocarpus, Guiera, Laguncularia, Quisqualis, Strephonema, Terminalia, Pteleopsis* and *Meiostemon* are originated from African continent, but the number of the species in Africa are still unknown. However, a total of 200 and 250 species have been reported for *Terminalia* and *Combretum,* respectively, all over the world, which are the largest genera of the family. About 54 species of *Terminalia* are known to originate from Africa (Smith et al. 2004) while the exact number of *Combretum* of African origin is not known, but are more represented than *Terminalia.*

Most of the species of *Combretaceae*, especially *Terminalia* and *Combretum* species have economic value. Virtually all the Terminalia are woody species and have been variously used as timber resources, leather and pharmaceutical industries (Srivastav 1993, Tan et al. 2002). Studies have proved that members of the family are highly concentrated with antioxidant compounds, which possess anti-inflammatory and antiviral activities (Cai et al. 2003, Cheng et al. 2003). For instance, *Terminalia chebula* and *Terminalia ivorensis* have been successfully used for the treatment of urinary and liver diseases (Masoko and Eloff 2007, Ansah et al. 2016). However, there is limited information on phylogenetic relationships of this important family. Previous phylogenetic and taxonomic findings on members of *Combretaceae* revealed that they are closely related morphologically (Uzoechina 1978, El-Ghazali 1998, De-Ridder 2013, Sanjeewa et al. 2013, Santos et al. 2016, Sarkar et al. 2016). Members of *Combretaceae* have also been reported to have unique curative effect on some diseases and as a result recommended for drug development (Mosango 2013, Jesus et al. 2015, Zhang et al. 2015, Salih et al. 2018). In some parts of Sudano-Sahelian Africa for instance, *Terminalia brownii* and *Terminalia laxiflora* are essentially used for the treatment of cough, fever, chest pain and many other symptoms that are connected to tuberculosis (Mosango 2013). According to Fyhrquist (2007), anticancer compounds that could be utilized for anticancer drug development were produced successfully from *Combretum* species. However, many of these *Combretaceae* taxa with unique bioactive properties are still having taxonomic delimitation issues due to their close relatedness. Therefore, it become very difficult and o set boundaries among such closely related group of plants for critical purpose like monitoring of drug adulteration using morphological markers. This is because morphological variables are affected by changes in environmental conditions, which result to seemingly unique differences among closely related taxa. Sometimes, there is also an overlap in morphological characters among the taxa under study.

It is, therefore, advantageous to authenticate morphological phylogenies with molecular data to arrive at natural, accurate and reliable taxonomic classification (Endress 2003). Out of the few scientific findings that are available on molecular evaluation of *Combretaceae,* none has been focused on the molecular phylogeny of taxa indigenous to Africa at the taxonomic point of view. Additionally, most of the available phylogenetical studies are electrophoretic-based and were mainly focused on taxa at the specific levels (Demenou et al. 2013, Intharuksa et al. 2016, Nithaniyal and Parani 2016). These electrophoretic based molecular studies have however revealed that the family *Combretaceae* is phylogenetically complex. Intharuksa et al*.* (2016) emphasized the fact that AFLP was not efficient to determine the molecular differences among some selected species of *Terminalia* from Thailand, which is one of the major representatives of *Combretaceae*.

Over the years, phylogenetic analysis using the *rbcL* gene has not only been effectively utilized as core plant barcodes for the amelioration taxonomic problems and problems related to the use of wrongly identified plants for medicinal purposes among taxa of angiosperms but has also addressed some critical issues relating to ecology of higher plants (Angiosperms APG III 2009, Vamosi et al. 2009, Cavender-Bares et al. 2009). Ecological issues like conservation, plant invasion, phytosociology, climate change and species evolution are important to phylogenetic studies (Schaefer et al. 2011).

This study, therefore, assessed the possibility of the conserved region of Chloroplast *rbcL* gene in determining the taxonomic differences among the African *Combretaceae* plants.

## Materials and Methods

Chloroplast DNA sequence of *rbcL* gene of *Combretaceae* representing ten genera and seventy-nine species of African origin were retrieved from the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov) (Table 1). All the plant species were each represented by single sequence except for *Combretum* subsp. *Sekhukhuneland*, which had two sequences for the analysed gene in the database. The scope of the data used was therefore limited by the available sequences of *Combretaceae* taxa of African origin in the database. Data analysis was performed for two groups of datasets and was made using Molecular Evolutionary Genetics Version X Analysis (*MEGA X*) by Kumar et al. (2018). The first group included all the plant species in the family while the second group was to determine the phylogenetic relationships within each of the two genera (*Combretum* and *Terminalia*) that are well represented in the family and available in the database.

Multiple sequence alignment was performed using the MUSCLE component of MEGA X. Statistics such as Transition/Transversion ratio, Consistency index (CI), Retention index (RI), the number of variable sites, etc. were determined. Maximum Parsimony analysis and different clades among the species were also determined. Phylogenetic trees were constructed according to Yessoufou (2012), using Maximum parsimony (MP) and the Neighbour joining method. The MP tree was obtained

**Table 1.** List of the species used for the study

| S/N | Species | Length | Accession |
|---|---|---|---|
| 1 | *Combretum adenogonium* | 1407 | EU338151.1 |
| 2 | *Combretum albopunctatum* | 1436 | EU338141.1 |
| 3 | *Combretum apiculatum subsp. apiculatum* | 1412 | EU338142.1 |
| 4 | *Combretum apiculatum subsp. leutweinii* | 1438 | EU338143.1 |
| 5 | *Combretum bracteosum* | 1439 | EU338128.1 |
| 6 | *Combretum caffrum* | 1437 | EU338167.1 |
| 7 | *Combretum celastroides subsp. celastroides* | 1430 | EU338152.1 |
| 8 | *Combretum celastroides subsp. orientale* | 1444 | EU338153.1 |
| 9 | *Combretum mkuzense* | 1445 | EU338164.1 |
| 10 | *Combretum coccineum* | 1328 | FJ381800.1 |
| 11 | *Combretum collinum subsp. gazense* | 1444 | EU338158.1 |
| 12 | *Combretum collinum subsp. hypopilinum* | 1411 | FJ381790.1 |
| 13 | *Combretum collinum subsp. suluense* | 1435 | EU338159.1 |
| 14 | *Combretum collinum subsp. taborense* | 522 | JX572435.1 |
| 15 | *Combretum edwardsii* | 1402 | EU338144.1 |
| 16 | *Combretum elaeagnoides* | 552 | KC158540.1 |
| 17 | *Combretum englerii* | 1444 | EU338161.1 |
| 18 | *Combretum erythrophyllum* | 552 | JX572439.1 |
| 19 | *Combretum fragrans* | 1445 | FJ381788.1 |
| 20 | *Combretum glutinosum* | 1399 | FJ381789.1 |
| 21 | *Combretum grandiflorum* | 1448 | FJ381797.1 |
| 22 | *Combretum hereroense* | 650 | EU213458.1 |
| 23 | *Combretum imberbe* | 1206 | KU761906.1 |
| 24 | *Combretum kirkii* | 1414 | EU338162.1 |
| 25 | *Combretum kraussii* | 1435 | EU338134.1 |
| 26 | *Combretum lasiocarpum* | 1370 | KF753905.1 |
| 27 | *Combretum micranthum* | 1295 | FJ381793.1 |
| 28 | *Combretum microphyllum* | 1444 | EU338130.1 |
| 29 | *Combretum moggii* | 1444 | EU338145.1 |
| 30 | *Combretum molle* | 1407 | EU338146.1 |
| 31 | *Combretum mossambicense* | 1446 | EU338131.1 |
| 32 | *Combretum nelsonii* | 1409 | EU338135.1 |
| 33 | *Combretum nigricans* | 1360 | KF753900.1 |
| 34 | *Combretum oxystachyum* | 1436 | EU338127.1 |
| 35 | *Combretum padoides* | 1361 | EU338156.1 |
| 36 | *Combretum paniculatum* | 1441 | EU338132.1 |
| 37 | *Combretum petrophilum* | 1430 | EU338148.1 |
| 38 | *Combretum pisoniiflorum* | 1435 | EU338139.1 |
| 39 | *Combretum platypetalum* | 552 | KC158506.1 |
| 40 | *Combretum psidioides subsp. dinterii* | 1420 | EU338149.1 |
| 41 | *Combretum sp.* | 1374 | KF753897.1 |
| 42 | *Combretum subsp. Sekhukhuneland* | 1374 | KF753898.1 |
| 43 | *Combretum subsp. Sekhukhuneland.* | 1374 | KF753898.1 |
| 44 | *Combretum tenuipes* | 1419 | EU338157.1 |
| 45 | *Combretum umbricola* | 1215 | KF753906.1 |
| 46 | *Combretum vendae* | 1443 | EU338136.1 |
| 47 | *Combretum wattii* | 1433 | EU338126.1 |
| 48 | *Combretum woodii* | 552 | JX572459.1 |
| 49 | *Combretum zeyheri* | 1403 | EU338166.1 |
| 50 | *Terminalia boivinii* | 1374 | KF753912.1 |
| 51 | *Terminalia brachystemma* | 1446 | FJ381810.1 |
| 52 | *Terminalia brownii* | 1374 | KF753924.1 |
| 53 | *Terminalia bursarina* | 1369 | KF753925.1 |
| 54 | *Terminalia catappa* | 567 | GU135220.1 |
| 55 | *Terminalia divaricata* | 1374 | KF753919.1 |
| 56 | *Terminalia glaucescens* | 1374 | KF753929.1 |
| 57 | *Terminalia ivorensis* | 1199 | KU761918.1 |
| 58 | *Terminalia mantaly* | 1420 | FJ381815.1 |
| 59 | *Terminalia mollis* | 1433 | EU338118.1 |
| 60 | *Terminalia phanerophlebia* | 546 | JF265624.1 |
| 61 | *Terminalia prunioides* | 552 | JF265625.1 |
| 62 | *Terminalia randii* | 552 | JX573039.1 |
| 63 | *Terminalia sericea* | 552 | JF265626.1 |
| 64 | *Terminalia stuhlmannii* | 552 | KU568099.1 |
| 65 | *Terminalia superba* | 1317 | KF753920.1 |
| 66 | *Terminalia trichopoda* | 552 | JX573043.1 |
| 67 | *Quisqualis indica* | 1430 | FJ381798.1 |
| 68 | *Quisqualis littorea* | 552 | KT208372.1 |
| 69 | *Quisqualis parviflora* | 1445 | FJ381799.1 |
| 70 | *Calopyxis grandidieri* | 1447 | FJ381796.1 |
| 71 | *Conocarpus erectus* | 1187 | KU761908.1 |
| 72 | *Conocarpus sericeus* | 1430 | FJ381822.1 |
| 73 | *Guiera senegalensis* | 1448 | FJ381803.1 |
| 74 | *Laguncularia racemosa* | 1203 | KU761909.1 |
| 75 | *Meiostemon humbertii* | 1448 | FJ381794.1 |
| 76 | *Meiostemon tetrandrus* | 1284 | EU338122.1 |
| 77 | *Pteleopsis anisoptera* | 1444 | EU338115.1 |
| 78 | *Strephonema mannii* | 1198 | KU761913.1 |
| 79 | *Strephonema pseudocola* | 1207 | KU761914.1 |

using the Subtree-Pruning-Regrafting (SPR) algorithm with search level 0 in which the initial trees were obtained by the random addition of sequences (10 replicates). This analysis involved 79 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions with less than 95 % site coverage were eliminated, i.e., fewer than 5 % alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option).

## Results

Recent development in molecular biology, nucleotide sequencing and various analyses have made it possible and easy to characterize the genomes of many plant species. This has invariably provided some important information regarding the identification as well as taxonomic delimitation of various taxa for appropriate utilization purposes. In the present study, sequences of conserved chloroplast *rbcL* gene region were analysed to determine the molecular differences at both generic and specific levels.

The multiple sequence alignment indicated that there are variable number of deletion and insertion in the chloroplast genome *rbcL*. The alignment of *rbcL* in the family *Combretaceae* shows 0.59 for both variation and parsimony sites with the overall mean distance of 0.71 (Figure 1). The combined Phylogenetic tree of the family *Combretaceae* (Figure 2) indicates three major groups (I, II, III). Group I has two branches comprising of six clades and three monotaxa. Group II is also bifurcated into two clusters, which consists of two clades and a mono clade. Group III being the largest is segmented into two big clusters; each one with many sub clusters. This group has a total of sixteen clades and twenty-seven monoclades.

The evolutionary divergence between sequences at the generic level varies from 0.37 to 0.76 (Table 2) with *Meiostemon* and *Calopyxis* having the highest similarity of about 63 % identity. The pair of genera that are

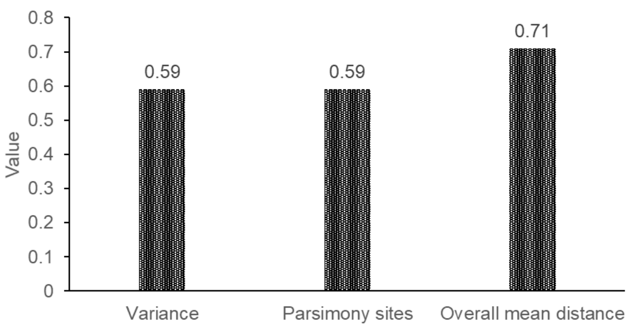


**Figure 1.** Variance, Parsimony sites and overall mean distance of African *Combretaceae*

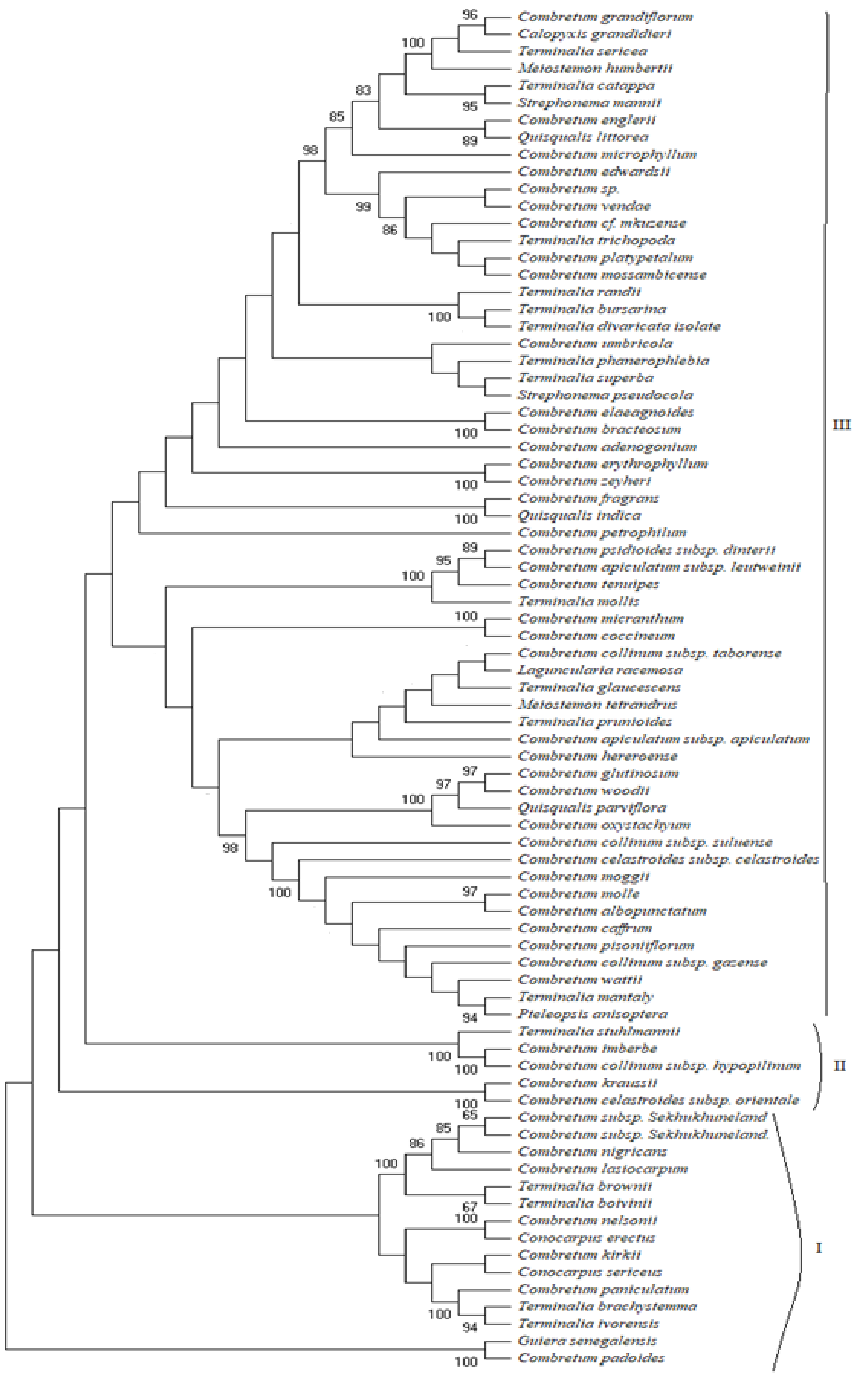


**Figure 2.** Combined Phylogenetic Tree of the Family *Combretaceae* of African origin (N.B.: Bootstrap values are shown under branches)

**Table 2.** Estimates of Evolutionary Divergence between Sequences at the generic level

| | Genera | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *Combretum* | 0.00 | | | | | | | | | |
| 2 | *Quisqualis* | 0.71 | 0.00 | | | | | | | | |
| 3 | *Meiostemon* | 0.73 | 0.75 | 0.00 | | | | | | | |
| 4 | *Calopyxis* | 0.71 | 0.75 | 0.37 | 0.00 | | | | | | |
| 5 | *Terminalia* | 0.71 | 0.74 | 0.71 | 0.67 | 0.00 | | | | | |
| 6 | *Pteopsis* | 0.61 | 0.72 | 0.75 | 0.74 | 0.70 | 0.00 | | | | |
| 7 | *Guiera* | 0.73 | 0.75 | 0.76 | 0.77 | 0.74 | 0.74 | 0.00 | | | |
| 8 | *Strephonema* | 0.75 | 0.74 | 0.74 | 0.75 | 0.74 | 0.74 | 0.74 | 0.00 | | |
| 9 | *Conocarpus* | 0.73 | 0.76 | 0.74 | 0.76 | 0.74 | 0.75 | 0.74 | 0.74 | 0.00 | |
| 10 | *Laguncularia* | 0.74 | 0.74 | 0.72 | 0.71 | 0.74 | 0.76 | 0.76 | 0.75 | 0.75 | 0.00 |

more distantly related among all the genera in the family with each pair having about 76 % variants include *Conocarpus/Quisqualis, Conocarpus/Calopyxis, Laguncularia/Pteopsis* and *Laguncularia/Guirea.* According to the ancestral relationship among the different genera in *Combretaceae*, it can be said that *Combretum* and *Terminalia* are polyphyletic, while *Conocarpus*, *Quisqualis* and *Meiostemon* are monophyletic.

Comparison at the specific level of the two largest genera shows that *Combretum* is the largest genus in the Family *Combretaceae* and consists of 49 species, with overall average distance of 0.704, variable sites of 58.94 % and the transition/transversion range of 0.71 (Table 3). The analysis shows that the tree has 58.04 most parsimonious site (Table 3). The consistence index is 0.25, the composite index is 0.16 while the retention index is 0.64 (Table 3). There were a total of 1225 positions in the final dataset. The following pairs of *Combretum* species appeared to have 100 % identity: *Combretum psidioides* subsp *dinterii*/*Combretum apiculatum* subsp. *Leutweinii, Combretum elaeagnoides/Combretum bracteosum*, *Combretum mole*/*Combretum albopunctatum*, *Combretum tenuipes*/*Combretum apiculatum* subsp. *leutweinii, Combretum woodii*/*Combretum glutinosum*, *Combretum imberbe*/*Combretum collinum* subsp. *hypopilinum*, *Combretum platypetalum*/ *Combretum mossambicense*, *Combretum Sekhukhuneland* subsp. *Sekhukhuneland*/*Combretum nigricans* and *Combretum tenuipes*/*Combretum psidioides* subsp. d*interii* (Figure 3).

**Table 3.** Phylogenetic parameters between *Combretum* and *Terminalia*

| Characteristics | *Combretum* | *Terminalia* |
|---|---|---|
| Transition/Transversion | 0.71 | 0.59 |
| Variable sites (%) | 58.94 | 57.39 |
| Parsimony information sites (%) | 58.04 | 55.55 |
| Consistency index | 0.25 | 0.48 |
| Retention index | 0.64 | 0.54 |
| Composite index | 0.16 | 0.26 |
| Positions in final dataset | 1225 | 443 |
| Overall average distance | 0.704 | 0.714 |

*Terminalia* consists of about 54 species that are of African origin, out of which gene sequence of *rbcL* is available for 17 species in the database. The overall average distance of the genus estimated in this study is 0.714 with variable sites of 57.39 and the Transition/ Transversion ratio is 0.59 (Table 3). The parsimony-in-

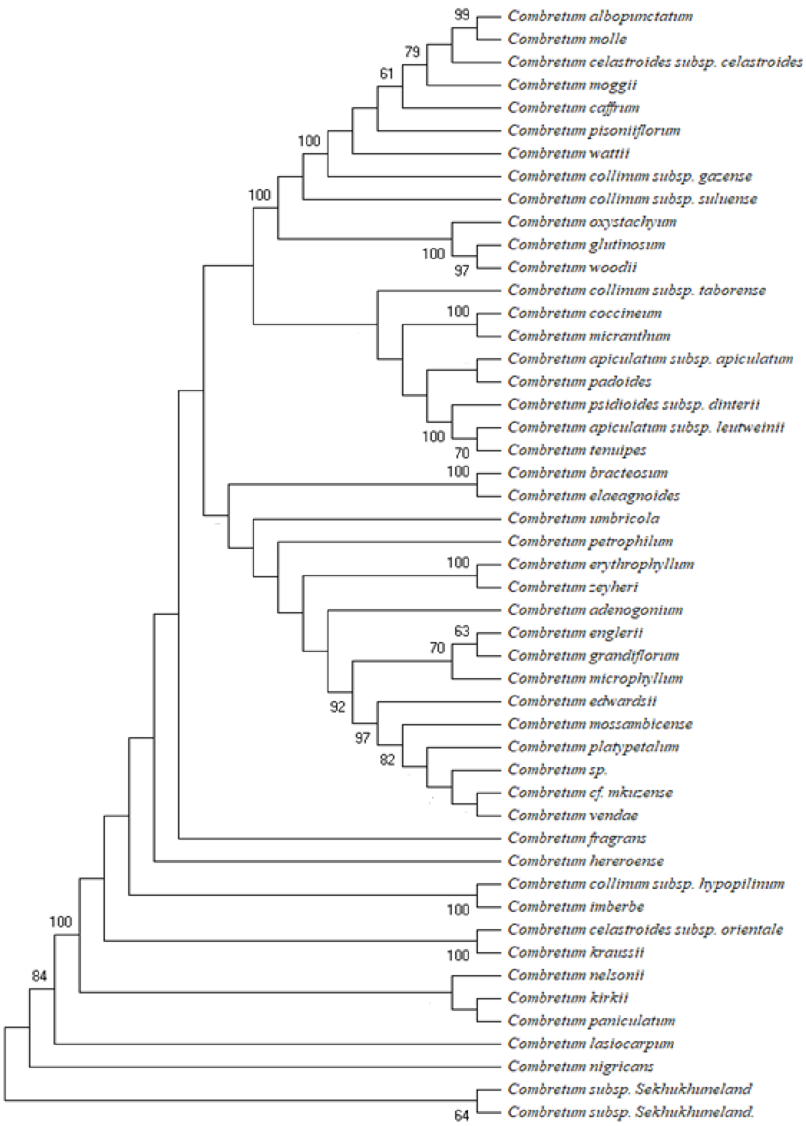


**Figure 3.** Phylogenetic Tree of the genus *Combretum* of African origin (N.B.: Bootstrap values are shown under branches)

formation site is 55.55 %, with 0.48 consistency index while the retention and composite indices are 0.54 and 0.26, respectively. Relationship between the species indicates that *T. prunioides* and *T. boivinii* as well as *T. prunioides* and *T. brownii* had the highest dissimilarity of about 80 % (Table 4) while the species pairs such as *T. brownii/ T. boivinii, T. ivorensis/T. brachystemma, T. randii/T. bursarina, T. divaricata/T. bursarina, T. randii/T. divaricata,* and *T. sericea* and *T. catappa* are 100 % similar (Table 4, Figure 4). There was a total of 443 positions in the final dataset.

variation was more pronounced compared to the specific levels.

The polyphyletic relationship observed for *Terminalia* and *Combretum* genera conforms to the findings of authors in the literature (Maurin et al. 2010, Nithaniyal and Parani 2016). Maurin et al. (2010) emphasized that *Terminalia is* broadly categorized into two groups which include African and Asian species, this is equally confirmed by the phylogenetic tree produced from this study. On the other hand, monophyletic relationship exhibited by *Conocarpus*, *Quisqualis* and *Meiostemon* this study

**Table 4.** Estimates of Evolutionary Divergence between Sequences of the *Terminalia* species of African origin

| | Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *T. boivinii* | 0.00 | | | | | | | | | | | | | | | | |
| 2 | *T. brachystemma* | 0.72 | 0.00 | | | | | | | | | | | | | | | |
| 3 | *T. brownii* | 0.00 | 0.72 | 0.00 | | | | | | | | | | | | | | |
| 4 | *T. bursarina* | 0.77 | 0.72 | 0.77 | 0.00 | | | | | | | | | | | | | |
| 5 | *T. catappa* | 0.76 | 0.77 | 0.76 | 0.72 | 0.00 | | | | | | | | | | | | |
| 6 | *T. divaricata* | 0.77 | 0.72 | 0.77 | 0.00 | 0.72 | 0.00 | | | | | | | | | | | |
| 7 | *T. glaucescens* | 0.75 | 0.74 | 0.75 | 0.77 | 0.73 | 0.77 | 0.00 | | | | | | | | | | |
| 8 | *T. ivorensis* | 0.72 | 0.00 | 0.72 | 0.72 | 0.77 | 0.72 | 0.74 | 0.00 | | | | | | | | | |
| 9 | *T. mantaly* | 0.75 | 0.77 | 0.75 | 0.71 | 0.77 | 0.71 | 0.77 | 0.77 | 0.00 | | | | | | | | |
| 10 | *T. mollis* | 0.74 | 0.77 | 0.73 | 0.76 | 0.72 | 0.76 | 0.70 | 0.77 | 0.77 | 0.00 | | | | | | | |
| 11 | *T. phanerophlebia* | 0.77 | 0.75 | 0.77 | 0.76 | 0.75 | 0.76 | 0.74 | 0.75 | 0.77 | 0.74 | 0.00 | | | | | | |
| 12 | *T. prunioides* | 0.80 | 0.74 | 0.80 | 0.73 | 0.78 | 0.73 | 0.74 | 0.74 | 0.74 | 0.73 | 0.76 | 0.00 | | | | | |
| 13 | *T. randii* | 0.77 | 0.72 | 0.77 | 0.00 | 0.72 | 0.00 | 0.77 | 0.72 | 0.71 | 0.76 | 0.76 | 0.73 | 0.00 | | | | |
| 14 | *T. sericea* | 0.76 | 0.77 | 0.77 | 0.72 | 0.00 | 0.72 | 0.73 | 0.77 | 0.77 | 0.72 | 0.75 | 0.78 | 0.72 | 0.00 | | | |
| 15 | *T. stuhlmannii* | 0.79 | 0.79 | 0.79 | 0.74 | 0.76 | 0.74 | 0.79 | 0.79 | 0.71 | 0.79 | 0.73 | 0.77 | 0.74 | 0.76 | 0.00 | | |
| 16 | *T. superba* | 0.73 | 0.76 | 0.73 | 0.75 | 0.72 | 0.75 | 0.74 | 0.76 | 0.72 | 0.76 | 0.72 | 0.75 | 0.75 | 0.72 | 0.75 | 0.00 | |
| 17 | *T. trichopoda* | 0.75 | 0.71 | 0.75 | 0.72 | 0.72 | 0.72 | 0.74 | 0.71 | 0.73 | 0.78 | 0.76 | 0.75 | 0.72 | 0.71 | 0.72 | 0.74 | 0.00 |

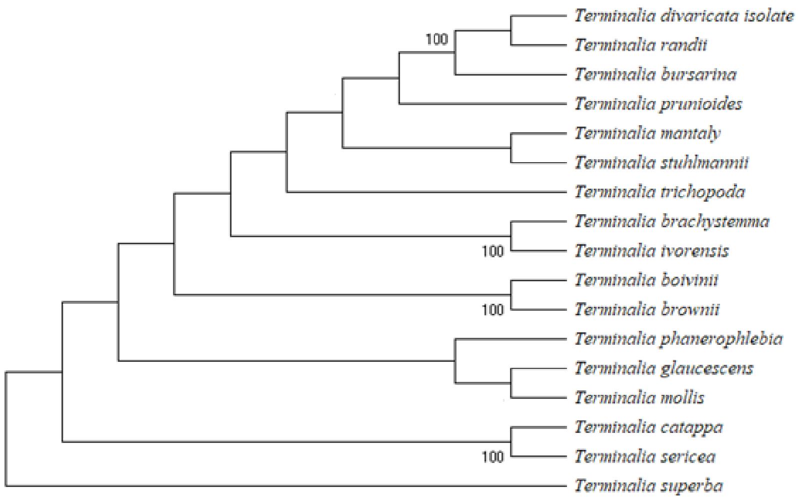


**Figure 4.** Phylogenetic Tree of the genus *Terminalia* of African origin (N.B.: Bootstrap values are shown under branches)

## Discussion and Conclusions

Findings from this study has revealed that there are considerable Inter-generic, inter-specific and intra-specific variations at the conserved region of *rbcL* within African *Combretaceae* plants. Previous studies (Lahaye et al. 2008, Maurin et al. 2010, Nithaniyal and Parani, 2016) revealed that divergence at the generic and specific levels are very significant to correct plant identification vis-à-vis its utilization. As expected, intergeneric

is inconclusive due to the few available *rbcL* nucleotide sequences in the database that representative of the members of these genera. There is therefore need for more robust sequences to further confirm this finding.

At the specific level, however, *Combretum* was discovered with a higher phylogenetic variation than the counterpart *Terminalia*. This is due to the fact that there are more significant variable sites in *Combretum* than *Terminalia* which displayed inter-specific divergence and discrimination of the species, this agrees to the finding by Nithaniyal and Parani (2016). Deshmukh et al. (2009) reported that *Terminalia* is highly associated with various taxonomic problem as a result of the presence of complex species, especially in *Catappa* and *Pentaptera* sections. The delimitation of taxa such as *Quisqualis*, *Calopyxis* and *Pteleopsis* within the family *Combretaceae* are yet to be resolved. For instance, the taxonomic position of *Quisqualis* and *Calopyxis* has previously been contented, hence Jongkind (1990) suggested their transfer to the genus *Combretum*. This change of position was agreed by the study of Maurin (2010). In the present finding, all the *Quisqualis* and *Calopyxis* species analyzed had a strong support with *Combretum* which equally buttresses the fact that the genera should be merged with *Combretum*. Plant species in *Pteleopsis* were described to have shrubby growth habit without scales or glands on the stalk. They often have conspicuous small, pink, conical buds in the axis of the leaf or leaf scars (Hyde et al. 2019). The report by Stace (2007) emphasized that the genus is taxonomically distinct from *Terminalia* due to the fact that the male flowers are located at the base of the inflorescence contrary to that of *Terminalia,* which are attached to the apex of the inflorescence. Conversely, Wickens (1973) was of the opinion that *Pteleopsis* should be an intermediate genus between *Terminalia* and *Combretum*, as a result of the characteristics shared with the two taxa. However, Maurin (2010) proposed that *Pteleopsis* should be united with *Terminalia.* The current study, therefore, corroborated the finding of Maurin (2010), because *Pteleopsis* appeared to be sister clade to *Terminalia mantaly* with a strong branch support.

Most of the existing literatures on morphological description of the taxa *Combretaceae* (Uzoechina 1978, El-Ghazali 1998, De-Ridder 2013, Sanjeewa et al. 2013, Santos et al. 2016, Sarkar et al. 2016) considered single species with different populations except for Uzoechina (1978) and El-Ghazali (1998). Morphological comparative study of *Terminalia glaucescens* and *Terminalia ivorensis* by Uzoechina (1978), revealed that the two species were closely related, however, reverse is the case in the present study where at least 74 % taxonomic distance was discovered between the species using *rbcL.* According to El-Ghazali (1998), the genus *Laguncularia* and *Strephonema* are meant to be categorized in the same taxonomic group because of the presence of tricolporate pollen in the two genera. This grouping somehow corresponds to the finding of the current study, because the only available representative of the taxa clustered at the same phylogenetic group. *Terminalia ivorensis* and *Terminalia superba* are also known to be closely related morphologically, but phylogenetic association within the genus considered them to be distantly related in which *Terminalia superba* is placed at the root as the ancestor of all the 17 *Terminalia* investigated. However, further studies that will compare these taxa using more robust sequences is needed to affirm this finding.

It is possible to phylogenetically distinguish the different taxa within the African *Combretaceae* using the Ribulose bisphosphate carboxylase (*rbcL*) gene sequences retrieved from the NCBI database. Results from this study could however be sufficiently applied to the scientific classification of the African *Combretaceae*, especially where morphological and electrophoretic-based molecular studies have failed. It can also be used as barcodes for monitoring appropriate use of members of this family for medicinal application and drug development. However, other genera apart from the monospecific genera, *Combretum* and *Terminalia* included in this family that are scarcely represented in the database for *rbcL* requires further studies to confirm the findings obtained in this study. It should also be well noted that there is low branch support in the phylogenetic relationship among some of the taxa in the present study, which may be as result of limited representation of the taxonomic units in the family. The repositioning of the unresolved African *Combretaceae* genera such as *Quisqualis*, *Calopyxis* and *Pteleopsis* supported by this study may, therefore, be inconclusive until further studies on the African taxa of *Combretaceae* with more robust sequences will be conducted to confirm the finding.

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