



## African Continent a Likely Origin of Family Combretaceae (Myrtales). A Biogeographical View

Jephris Gere<sup>1,2\*</sup>, Kowiyou Yessoufou<sup>3</sup>, Barnabas H. Daru<sup>4</sup>, Olivier Maurin<sup>2</sup>  
and Michelle Van Der Bank<sup>2</sup>

<sup>1</sup>Department of Biological Sciences, Bindura University of Science Education, P Bag 1020, Bindura Zimbabwe.

<sup>2</sup>Department of Botany and Plant Biotechnology, African Centre for DNA Barcoding, University of Johannesburg, P.O.Box 524, South Africa.

<sup>3</sup>Department of Environmental Sciences, University of South Africa, Florida campus, Florida 1710, South Africa.

<sup>4</sup>Department of Plant Science, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa.

### Authors' contributions

This work was carried out in collaboration between all authors. Author JG designed the study, wrote the protocol and interpreted the data. Authors JG, OM, MVDB anchored the field study, gathered the initial data and performed preliminary data analysis. While authors JG, KY and BHD managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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### ABSTRACT

**Aim:** The aim of this study was to estimate divergence ages and reconstruct ancestral areas for the clades within Combretaceae.

**Methods:** We utilized a comprehensive dataset of 144 species of Combretaceae with a worldwide sampling to reconstruct a dated phylogeny based on a Bayesian analysis of five gene regions (ITS, *rbcl*, *matK*, *psaA-yf3* and *trnH-psbA*). Bayesian phylogenetic tree was generated using a Bayesian MCMC approach implemented in BEAST v.1.7.5 to generate lineage dating. Two fossils *Dilcherocarpon* Combretoides (93.5-112 mya) and *Terminalioxylon* (28 mya) were used for

\*Corresponding author: E-mail: [gerejephris@gmail.com](mailto:gerejephris@gmail.com);

calibration. S-DIVA and DEC model analysis were used to estimate ancestral area ranges.

**Results:** Our results indicate that the earliest diversification of Combretaceae occurred ca. 110 mya. This was followed by the splitting of the family into two subfamilies, Combretoideae and Strephonematoideae during the Late Cretaceous period. This event followed the radiation of Combretoideae, ca 105.6 mya to give rise to two tribes, Combretaeae and Laguncularieae which diverged around 60.9 mya and 52.9 mya, respectively. The two main subtribes Combretineae and Terminaliinae, radiated at ca. 48.3 and 46.4 mya respectively. African continent is inferred as the origin of Combretaceae, with dispersal as the major event responsible for the intercontinental disjunct distribution observed in the tropical and subtropical regions.

**Main Conclusions:** Our results revealed that the crown age of Combretaceae is ca.110 mya, a time hypothesised to be marked by high angiosperm diversification. Two largest subtribes Combretineae and Terminaliinae, split occurred in the Late Cretaceous period with divergence estimated at the commencement of Eocene epoch. The African continent is hypothesised to have emerged from the split of the super continent Gondwana. Long distance dispersal is postulated as the major modeller, with vicariance and extinction playing marginal roles in shaping the current intercontinental disjunct distribution of Combretaceae in the tropical and subtropical regions of the world.

**Keywords:** Tropical; biogeography; divergence age estimates; dispersal; vicariance; BEAST analysis.

## 1. INTRODUCTION

The major thrust of biogeography has always been to understand the current distribution of organisms and trace their origins. However, unresolved genus and species-level phylogenies have led to controversy in the testing of hypotheses concerning intercontinental disjunct distribution in majority of tropical and subtropical plant families [1].

Three paradigms, long-distance dispersal, vicariance, and extinction have been postulated to explain plant disjunct distributions [2-8]. Vicariance is linked to fragmentation of widespread ancestors, with dispersal involving movement of species across pre-existing barriers from one region to another and extinction being responsible for the loss of species or populations in the intermediate zones of widespread taxa [9-12]. Nevertheless, the relevance of each paradigm may vary depending on the historical backgrounds and biological characteristics of the different plant species [6,13].

In most tropical plant disjunctions, dispersal has been recognised as an important process in the colonisation of oceanic islands [14-17]. Axelrod [18] suggested that shared, disjunct African–South American families indicate “the splitting apart of a more homogeneous flora by fragmentation of Gondwana.” In contrast, others [19-22] strongly argued for more recent over-water dispersal to account for many, if not all, of these intercontinental disjunctions. To address

satisfactorily issues involving vicariance of widespread taxa with subsequent formation of barriers or dispersal of taxa across pre-existing barriers, there is need to research on three major items namely: robust estimates of phylogenetic relationships, ages of relevant clade formation, and a geological time sequence of barrier formation. These aspects are covered in this study.

Here, the focus is on Combretaceae R Br., a tropical and subtropical family, with about 500 species of mainly trees, shrubs and sometimes lianas, in approximately 12 to 23 genera. Previous studies within Combretaceae have focused on inferring taxonomic relationships, with little attention on biogeography [23,24]. As yet, our understanding of the biogeographical history within this family remains poorly known with no extensive study addressing global disjunct distribution patterns. Members of the family occupy a wide range of habitats including rainforest, savannah, woodland, and mangrove ecosystems [23,25-28]. Geographically, the family is distributed between the New World (85 species) and the Old World, the latter having the bulk of the species richness [25,27,29,30]. The greatest diversity has been recorded in Africa for the largest genus *Combretum* Loefl. and Southeast Asia for *Terminalia* L. [27,31-33]. These two genera show the most prominent intercontinental disjunctions, with distributions in all continents.

In this study, the biogeographic history of the Combretaceae using a dated phylogeny of 144

species distributed across the globe was inferred. Firstly, the divergence times of different clades were estimated, followed by analyses of the current intercontinental disjunct distribution pattern in terms of dispersal and vicariance models, and lastly reconstruction of the ancestral distribution ranges for the different clades.

## 2. MATERIALS AND METHODS

### 2.1 Taxon Sampling and Outgroup Selection

In this study, we included 144 species and subspecies of Combretaceae sampled from all the continents, using DNA sequence data from four plastid regions (*rbcL*, *matK*, spacer's *trnH-psbA* and *psaA-ycf3*), and one nuclear gene, internal transcribed spacer (ITS). A total of 245 unpublished sequences were generated for the present study from samples newly extracted samples and these were added to the preexisting dataset of Maurin et al., [25]. Dataset comprises 14 genera of the family Combretaceae, 2 species of subfamily Strephonematoideae, 73 taxa of two subgenera (*Combretum* and *Cacoucia*), 17 sections of *Combretum*, 64 species of the subtribe Terminaliinae and 5 species of tribe Laguncularieae. Missing in our study sample are species of *Dansiea* and *Finetia*. Strephonematoideae, a subfamily in Combretaceae represented by two species (*Strephonema mannii* Hook.f. and *Strephonema pseudocola* A.Chev.) was used as outgroups in this study. Voucher information, name of herbarium, GenBank accession are listed in Table S1 (Supplementary Information).

### 2.2 DNA Extraction, Amplification, Sequencing and Alignment

Genomic DNA was extracted from silica gel-dried and herbarium leaf materials following a modification of Cetyltrimethyl Ammonium Bromide (CTAB) method of Doyle & Doyle, [34]. To ease the effects of high polysaccharide concentrations in the DNA samples, we added polyvinyl pyrrolidone (2% PVP). Purification of samples was done using QIAquick purification columns (Qiagen, Inc., Hilden, Germany) following the manufacturer's protocol.

PCR amplifications were performed using both the Applied Biosystems 9800 Fast Thermal Cycler and the GeneAmp PCR System 9700 machines. Amplification of *rbcL* was carried out in two overlapping fragments using the

following primer combinations: 1F-724R and 636F-1426R [35,36]. For *matK*, primer combination 390F and 1326R [37] was used for both amplification and sequencing. Spacers *trnH-psbA* and *psaA-ycf3* were amplified and sequenced using the primers 1F and 2R [38] and PG1F and PG2R [39] respectively. The nrITS was amplified in two non-overlapping pieces using two internal primers with a pair of external primers: 17SE-ITS2 and ITS3-26SE [40,41].

All PCR reactions were carried out using Ready Master Mix (Advanced Biotechnologies, Epsom, Surrey, UK). 4.5% of dimethyl sulfoxide (DMSO) was added to the reagents solution during the amplification of nrITS to reduce secondary structure problems common in ribosomal DNA [42]. The following programme was used to amplify *rbcL*: pre-melt at 94°C for 60 sec, denaturation at 94°C for 60 sec, annealing at 48°C for 60 sec, extension at 72°C for 60 sec (for 28 cycles), followed by a final extension at 72°C for 7 min; for *matK*, the protocol consisted of pre-melt at 94°C for 3 min, denaturation at 94°C for 60 sec, annealing at 52°C for 60 sec, extension at 72°C for 2 min (for 30 cycles), final extension at 72°C for 7 min. The amplification of *trnH-psbA* followed a pre-melt at 94°C for 3 min, denaturation at 94°C for 60 sec, annealing at 48°C for 60 sec, extension at 72°C for 1 min (for 28 cycles), final extension at 72°C for 7 min. However, for nrITS and spacer *psaA-ycf3* the protocol consisted of pre-melt at 94°C for 1 min, denaturation at 94°C for 60 sec, annealing at 48°C for 60 sec, extension at 72°C for 3 min (for 26 cycles), final extension at 72°C for 7 min.

Purification of the amplified products was done using QIAquick columns (QIAGEN, Germany) following the manufacturer's manual. The purified products were then cycle-sequenced with the same primers used for amplification using BigDye® V3.1 Terminator Mix from Applied Biosystems, Inc., ABI, Warrington, Cheshire, UK. The cleaning of the cycle-sequenced products was done using the EtOH-NaCl method provided by ABI, followed by sequencing on an ABI 3130xl genetic analyser.

Sequences were trimmed, assembled, and edited using Sequencher version 4.6 (Gene Codes Corp., Ann Arbor, Michigan, USA). Alignment was performed using Multiple Sequence Comparison by Log-Expectation (MUSCLE vs. 3.8.31; [43], with the subsequent manual adjustments to refine the alignments.

### 2.3 Tree Reconstruction and Estimation of Divergence Time

First, the most appropriate model of substitution for each gene partition (*rbcLa*, *matK*, *trnH-psbA*, *psaA-ycf3* and nrITS) was selected using Akaike information criterion implemented in MODELTEST v.3.06 [44]. The GTR+I+G was chosen as the best model for ITS, *rbcL*, *matK* and *psaA-ycf3* whereas TIM+G was chosen for *trnH-psbA*. Both models share similar parameters including; substitutions = 6, rates = gamma, base frequency = empirical and clock = unconstrained.

Congruence test between the plastid (*rbcL*, *matK*, *trnH-psbA* and *psaA-ycf3*) and nuclear (ITS) data sets was conducted. This was done using the partitioned Bremer support (PBS) test [44], with 1000 heuristic searches, and implemented in TreeRot v.3 [46]. A negative Bremer index is indicative of incongruence between plastid and nuclear genes, whereas a positive score indicates congruence, thus allowing the combination of plastid and nuclear genes as one partition.

An XML file of the combined matrix Using BEAUti implemented within the BEAST v.1.7.5 suite [47] was generated, followed by calibration of points using crown ages assigned to fossils of Combretaceae. Three calibration points were used for the dating of the tree although the use of single calibration point is widely used for datasets without enough fossil record. The choice of multiple fossils for calibration was influenced by the representation in the fossil record (richness) and how accurately each fossil could be ascribed to a taxonomic group. *Dilcherocarpon combretoides* [48], a recently described fin-winged fruits fossil was assigned to the tribe Combretaeae, to give a crown date of between 93.5 – 112 mya. Calibration of the clade consisting of closely related taxa belonging to the Asian *Terminalia* (*T. catappa* L., *T. litoralis* See. and *T. kaernbachii* Warb.) was done, with a date of 4 mya obtained from a fossil dated from the Pliocene to Pleistocene [49]. The other calibration point was also fixed on subtribe Terminallinae, of the genus *Terminalia*, for the three closely related species *T. arjuna* Roxb. ex DC. Wight & Arn., *T. myriocarpa* Decne. and *T. tomentosa* (Roxb.)Wight & Arn., (*Terminalioxylon*) at a minimum age ca. 28 mya.

A dated phylogeny of Combretaceae was reconstructed and divergence times were

estimated using a Bayesian MCMC approach implemented in BEAST v.1.7.5. A speciation model following a Yule process was selected as the tree prior, with an uncorrelated relaxed lognormal model for rate variation among branches. Further, simultaneous searches of topology and divergence times were conducted. Twenty million generations of the Markov Chain Monte Carlo (MCMC) chains were run, sampling every 1000 generations. These were chosen as they were found to be efficient to obtain ESS values above 200, which is the recommended threshold (when viewed in Tracer v.1.5) which is of sufficient sampling of parameter space recommended by Drummond & Rambaut [47]. Several trial runs of 20 million generations were done to optimise efficiency and operator parameters before final analysis which included four analysis which generated 900 000 trees. Subsampling was done in each of the analyses using LogCombiner v1.7.5 in BEAST [47]. In all the runs, we used an uncorrelated lognormal model. Posterior estimates were checked using the software Tracer v1.5 [47]. The first 2001 trees were treated as burn-in, and samples were summarised in the maximum credibility tree using TreeAnnotator v1.7.5 [47] with the PP limit set to 0 and summarising mean node heights. The results were visualised using Figtree v1.4.0

### 2.4 Biogeographic Analysis

Previous biogeographic histories of many taxa were hypothesised by the use of analytical methods, such as dispersal–vicariance analysis (DIVA) [50] and Lagrange (likelihood analysis of geographical range evolution) implementing dispersal-extinction cladogenesis (DEC) model [51,52]. However, DIVA method has been criticised for ignoring uncertainty in phylogenetic inference as ancestral ranges are reconstructed onto fixed topology assumed to be free from error [50,53,54]. To counter for the limitation identified in DIVA (version 1.2, Ronquist [55]), a recently modified DIVA (Reconstruct Ancestral State in Phylogenies (RASP) version 1.1, [56,57] was performed using two methods implemented in Statistical Dispersal-Vicariance Analysis (RASP, version 1.1; [56,57] to reconstruct the possible ancestral ranges of the Combretaceae. This program complements DIVA and implements the methods of Nylander et al. [53] and Harris and Xiang [58]. Statistical -DIVA uses the collection of trees from a Bayesian MCMC analysis and can handle optimization uncertainty in reconstructing biogeographic histories. These methods suggest possible ancestral ranges at

each node and also calculate probabilities of each ancestral range at nodes [59]. In these methods, the frequencies of an ancestral range at a node in ancestral reconstructions are averaged over all trees. In addition to S-DIVA method, we also performed Lagrange Analysis (DEC model) [52]. DEC model analysis allows for testing specific dispersal hypotheses through time, subject on the routes available during the historic interval(s) of interest [51,52]. We used a single MCMC tree obtained from BEAST analysis for both S-DIVA and DEC model analyses. Possible ancestral ranges at each node on the tree were obtained. We ran simultaneously the MCMC chains for 500 000 generations and sampling of the state were done every 100 generations. Fixed JC+G (Jukes and Cantor +Gamma) were used for Bayesian binary MCMC (BBM) analysis with null root distribution. The distribution of Combretaceae was coded unordered character states according to Buerki et al. [60] based on the literature on current distribution. As such the number of areas for this analysis was kept at four. These areas are: A (Africa including Madagascar), B (America including South American countries, e.g. Costa Rica, Brazil), C (Australia including Papua New Guinea), D (Asia including China, India and Pacific Islands).

### 3. RESULTS

#### 3.1 Beast Analysis and Lineage Dating

Combretaceae diversified at 110 mya (Fig. 1). This event followed the radiation of Combretaceae, ca 105.6 mya to give rise to two tribes, Combretaceae and Laguncularieae which diverged around 60.9 mya and 52.9 mya, respectively. The splitting of tribe Combretaceae gave rise to two subtribes, Combretineae and Terminaliinae, radiating at ca. 48.3 and 46.4 mya respectively. Major clades within subtribe Combretineae diverged. A summary of divergence times and HPD values are given in Table 1.

#### 3.2 Tribe Laguncularieae

This group is mainly comprised of mangroves, with two with subdivisions within it. It is sister to the rest of Combretaceae and diverged at age of ca. 52.9 mya. The tribe has subdivisions, true and associated mangroves. True mangroves are comprised of the genera *Laguncularia* and *Lumnitzera*, which diverged at ca. 2.5 mya and

ca. 7.2 mya, respectively. *Macropteranthes*, a mangrove associate, diverged from *Lumnitzera* ca. 32 mya and later radiations occurring at the age ca. 3.1 mya.

#### 3.3 Subtribe Terminaliinae

Divergence age estimates of Terminaliinae are 46 mya. The early diverging lineage within Terminaliinae is *Conocarpus*, which diversified at 3.3 mya with the rest of the group diversifying at ca. 39.6 mya. The largest genus, *Terminalia* is comprised of two distinct groupings, clade I and II. Clade I is comprised of New World species and diverged much earlier (ca.39 mya) than Clade II comprised of the Old World species (ca. 17 mya).

#### 3.4 Subtribe Combretineae

Within Combretineae, *Adans.*, and *Calycopteris* Lam., are sisters to the rest of the subtribe, and these diverged 48 mya and the splitting of both took place ca. 20 mya. *Thilao* Eichler is sister to the genus *Combretum*, with a divergence age of ca. 36.4 mya. Subgenus *Cacoucia*, diverged at 32 mya with the subsequent diversification which splits the subgenus into two main clades at 18 mya. Clade I consists of members of section *Conniventia*, *Megalantherum*, *Oxstachia* with a divergence date of ca. 15.3 mya. Clade II comprised of sections, *Poivreia* and the recently transferred *Quisqualis* (to *Combretum*), diverged at the age of ca. 14.2 mya. *Meistemon* separates the two subgenera, *Combretum* and *Cacoucia* and is sister to the subgenus *Combretum*, diverging ca. 28 mya. Within subgenus *Combretum*, section *Hypocrateropsis* is sister to the rest of the subgenus *Combretum*, and diverged ca. 23.9 mya. The most diverse section in this study, *Cilitipetala*, has a divergence age of ca.13.2 mya, and split into two main subclades comprised of mainly southern African species and the other with species from rest of Africa. These two subclades diverge at age of ca. 13.2 mya. The rest of the sections within subgenus *Combretum* have an average divergence age of ca. 23 mya.

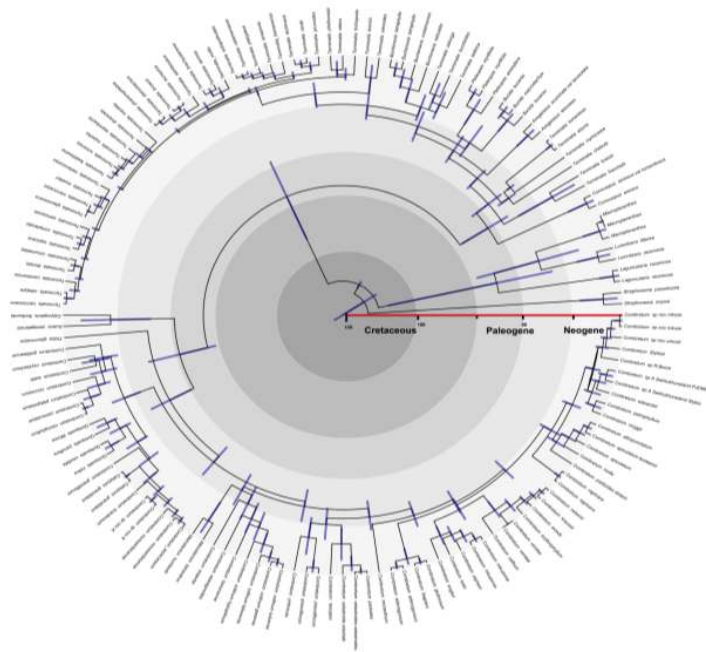
#### 3.5 Ancestral Area Reconstructions

Analyses were conducted using two methods implemented in RASP, S-DIVA and Bayesian Binary MCMC (BBM) analysis, as well as in DEC model (Lagrange). Results of reconstructions are congruent in almost all nodes except for a few

exceptions such as nodes: 4, 7, 15, 16, and 17. All analyses suggest a complex biogeographical history showing the vital role played by dispersal and vicariance to shape the current disjunctive distribution of Combretaceae. Africa is inferred as the ancestral origin of the family Combretaceae, node 17 in both BBM and Lagrange analyses (Figs. 2 and 3). Dispersal is inferred as the prime event of movement from all the analyses, Table 1, whilst vicariance is noted mostly on the tips with extinction being observed on only one node, in both S-DIVA and for Lagrange analyses. Congruence was observed on most of the nodes from all the three analyses with the nodes (1-15) producing the highest number of dispersal events. Noteworthy are some differences in the total number of events as reflected in Table 2.

Node 4 represents members of the subtribe Combretineae, and the possible ancestor range at this node Africa indicating one dispersal event with 56% and 22% marginal probability from Lagrange and BBM analyses respectively. In contrast, S-DIVA result suggests Africa and America as the origin of the ancestors for the members with a low marginal probability of 25%. This basal node suggests both dispersal and vicariance events.

Within subtribe Terminaliinae, a number of key nodes reveal an African and Asian origin. S-DIVA analysis for node 7 (Fig. 4) representing the two large clades of taxa distributed in Africa and Asia, suggest dispersal as the prime event with no vicariance being reported. BBM analysis (Fig. 2) suggests Africa as the ancestral origin with a 43.17% marginal probability and both dispersal and vicariance events highlighted. A total of four dispersal and three vicariance are noted for this node showing the highest number of events. The basal node 17 for the subtribe Terminaliinae in both BBM and Lagrange, suggests one possible ancestral range, Asia with two dispersal events from all the analyses except for Lagrange analysis which indicated only one event. Marginal probability for this result is low (BBM=31% and Lagrange 10%). Clades within the subtribe show different ancestral reconstruction areas, with Africa and Asia coming out most prominently suggesting both dispersal and vicariance as the dispersion events. Node 17 for S-DIVA result is ambiguous, showing all four possible ancestral reconstruction ranges, A,B,C, and D, suggesting trans-oceanic dispersal between all continents.



**Fig. 1. Maximum Clade Credibility (MCC) chronogram obtained from BEAST analysis of the combined analysis of plastids and nrITS. The bars reflect maximum/ minimum age range for divergence of Combretaceae lineages based on 95% confidence interval**

**Table 1. Estimated ages along with the 95% high posterior density interval (HPD) are presented. Node ages are given in million years (mya)**

Node	Description	Age	95% HPD
1	Crown age of Combretaceae	110	104; 126
2	Crown age of Strephonematoideae	8	1.9; 18.2
3	Crown age of Combretoideae	105	103.8; 107.6
4	Crown age of Combreteae	60	43; 82.8
5	Crown age of Laguncularieae	52.9	28.1; 101.3
6	Crown age of Terminaliinae (split between <i>Conocarpus</i> and rest of Terminaliinae)	46.3	36.1; 59.9
7	Crown age of <i>Combretineae</i>	48.2	34.2; 64.5
8	Crown age of <i>Conocarpus</i>	3.3	10; 12.1
9	Crown age of <i>Combretum</i> (Including <i>Guiera</i> ; <i>Calycopteris</i> and <i>Thiloa</i> )	48.2	34.2; 64.5
10	Crown age of <i>Guiera</i> and <i>Calycopteris</i>	20.5	7.6; 38
11	Crown age corresponding to split between subgenus <i>Combretum</i> and <i>Cacoucia</i>	32.7	24.9; 41.8
12	Crown age of Terminaliinae without <i>Conocarpus</i>	39.6	32.4; 48.8
13	Crown age of subgenus <i>Cacoucia</i>	32	27.5; 41.5
14	Crown age of split of subgenus <i>Cacoucia</i>	18.8	13.6; 26.5
15	Crown age of subgenus <i>Combretum</i> including <i>Meiostemon</i>	30.3	23.1; 38.4
16	Crown age of <i>Meiostemon</i>	28	23.6 34.1
17	Crown age of <i>Buchenavia</i> = <i>Terminalia</i> including American taxa	18.3	12.2; 25
18	Crown age of <i>Macropteranthes</i> split from <i>Lumnitzera</i>	32.6	14.6; 60.7
19	Crown age of Old World(African <i>Terminalia</i> )	17	12; 23.6
20	Crown age of New World ( <i>Terminalia</i> )	39	31.3; 51.6
21	Crown age of <i>Quisqualis</i> and <i>Poivreia</i>	14.2	9.8; 20.3
22	Crown age of section <i>Ciliatipetala</i>	13.2	9.3; 17.8
23	Crown age of <i>Conniventia</i> , <i>Megalantherum</i> and <i>Oxystachia</i>	15.3	10.7; 21.4

#### 4. DISCUSSION

This study presents the most extensive in depth biogeographical introspection of the family Combretaceae based on the largest diverse dataset ever assembled to investigate biogeographical histories. Only two genera (*Dansiea*, *Finetia* as well as the monotypic *Combretum* subgenus *Apetalanthum*) known to belong to Combretaceae were not included in this study.

Inference from BEAST analysis combined with the fossil record, suggest a crown date of ca. 110 mya for Combretaceae. Current results concur with most recent results of Berger [24] and slightly differ from those of Maurin [23]. Our results are within the range (ca 93.5 to 112 mya) with the most recent described fossil, *Dilcherocarpon combretoides* [48], which has been assigned to the tribe Combreteae. The assignment of the fossil to tribe Combreteae was based on shared fruit morphological features

reminiscent of those of extant *Combretum* and some *Terminalia* [24]. Previously assigned fossils such as *Esgueiria*, known to exist in Northern Hemisphere in Late Cretaceous deposits are ca 70 to 90 mya old. Studies on angiosperms have linked the Cretaceous period (from ca. 130 mya) to the start of evolution followed by major diversification up to ca. 90 mya [62,63]. However, the age of *Dilcherocarpon combretoides* is far much older than the age that was assigned to Combretaceae (ca 90 mya) in previous studies [23,64-68]. Nonetheless, molecular dating study [69], had suggested the evolution of angiosperms to have started in the mid-Jurassic (ca. 170 mya), with some major angiosperm lineages diversifying rapidly in the early Cretaceous period (ca. 140 mya). Given such a scenario, Combretaceae could have evolved in the Campanian epoch that is between end of Cretaceous and beginning of the Tertiary period. According to Sytsma & Berger [70], the crown age of Combretaceae is ca. 90 mya, with subfamily Strephonematoideae diverging first

followed by the rest of the family afterwards in the early Tertiary period.

The split of Combretaceae into two subfamilies (Combretoideae and Strephonematoideae) occurred ca. 110 mya with 95% HPD ranging from ca. 104 - 126 mya. The most recent similar study of Berger [24] estimates the age to be ca 106 mya. In contrast to Maurin [23], this age is much older than what was previously estimated (82 mya), but within range of Berger's [24] of ca. 106 mya. Our results may be close to the actual crown age for the splitting of the two families in that, a more robust phylogeny with five loci DNA regions was analysed, compared to previous study that used less number of DNA regions and smaller sample size. Subfamily Strephonematoideae, known to have a solitary genus, *Strephonema*, of about three species, is restricted to west tropical Africa, diverged ca. 8 mya. This event coincided with the Medieval warm period of the African Continent, that was characterised by the creation of savannah and woodland vegetation types, that happen to be the habitat for *Strephonema*.

#### 4.1 Tribe Laguncularieae

Tribe Laguncularieae, mainly comprised of mangrove species and distributed in the coastal tropical and subtropical regions, is comprised of true mangroves (*Laguncularia* and *Lumnitzera*) and mangrove associates (*Dansiea* and *Macropteranthes*) [71]. In Stace's [72] treatment of the Combretaceous mangroves, *Conocarpus* is included as one of mangrove genera. The placement of *Conocarpus* within Terminaliinae has been debatable; however, molecular data supports its placement in the group [23, 25, 73]. Morphologically, *Conocarpus* share a number of features with other mangrove genera such as water storage tissues [72].

Results suggest that Laguncularieae diverged from the rest of Combretoideae ca 105 mya, and diversified ca. 52.9 mya, reinforcing the results from the most recent similar study of Berger [24]. However, our results differ with Plaziat et al., [74] and Maurin [23], which depict the divergence age of mangroves at ca. 70.6 and 77 mya, respectively. An introspection of the age estimates of fossils assigned to mangroves in family Rhizophoraceae, *Bruguiera* and *Cerriops* which are known to occur from the early Eocene (ca. 33.9 – 55.8 mya) gives credibility to our findings [75]. In contrast, Ricklefs et al. [76] dated *Laguncularieae* to age of ca 23 mya. The discrepancy reflected in the crown age of

mangroves in different studies might be a reflection of differences in fossils used for calibrations in the data sets. Since current results have been obtained from analysis that was calibrated with fossils assigned to Combretaceae, our results are more robust compared to those that included other mangrove families. The position of *Macropteranthes* has also been contentious within the mangrove group, with previous studies suggesting that *Macropteranthes* has mangrove ancestral origin. Our MCMC tree topology support this proposal and reveal divergence from the rest of the tribe to have taken place ca. 32.6 mya. Despite the different habitats which *Macropteranthes* occupy at the present time, our results strongly support mangrove ancestral origin.

#### 4.2 Subtribe Combretineae

Combretineae embraces the largest genus of Combretaceae, *Combretum* Loeffl. and our results estimates the split with subtribe Terminaliinae ca. 60.9 mya, suggesting the event to have occurred during the Late Cretaceous period. Major diversification of both fauna and flora is known to have characterised this period [24]. Notable climatic changes during this period include the warming within the tropics restricted to equatorial regions and northern latitudes experiencing a markedly more seasonal climatic condition [75]. Positioned sister to Combretineae, is the clade of *Guiera* and *Calycopteris* which diverged ca. 48 mya and diversified ca. 20.5 mya. According to Maurin [23], the relationship between these two taxa is unclear, and it has been suggested that their grouping may reflect multiple lineages which arose following end of mass extinctions of both flora and fauna during the end of Cretaceous epoch. However, no tangible evidence has been found to support this view, hence the need for further investigation into the relationship of this clade with the rest of Combretineae.

Early diverging lineage from the rest of Combretineae is *Thiloa*, a genus restricted to South America, which diverged ca. 36.4 mya from Combretineae. It is difficult to make conclusions relating to its position since there is not much *Combretum* taxon sampled from the American continent. However, it can be hypothesised that may be its position may reflect that American *Combretum* species are sister to African species.

Two major clades within genus *Combretum*, are revealed, with the split corresponding to subgenus *Combretum* and *Cacoucia* occurring

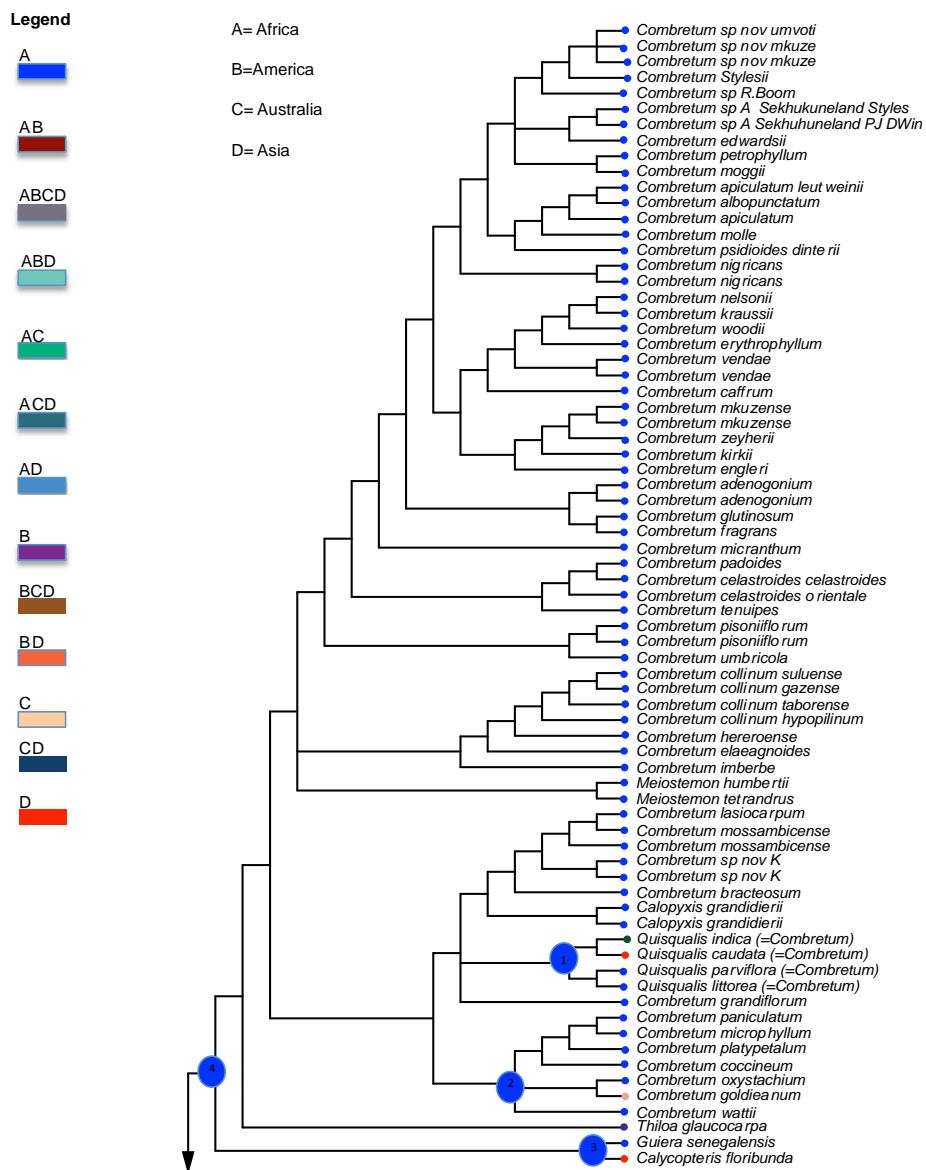


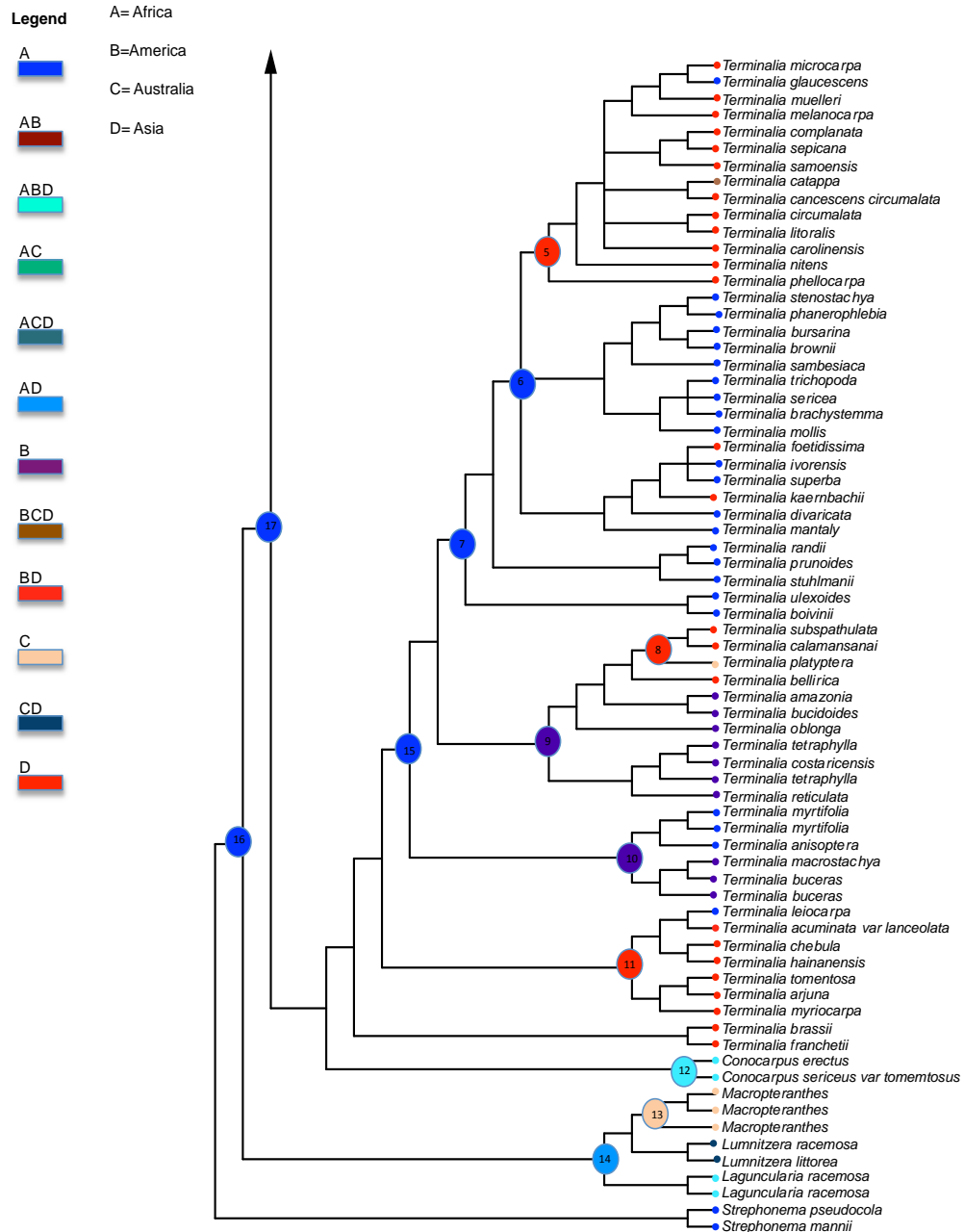
ca. 32.7 mya. Phylogeny of Combretaceae recognises these two subgroupings [25]. Subgenus *Combretum* radiated much earlier than its sister subgenus *Cacoucia* (ca. 30.3 and 18.8 mya respectively). Distinct morphological characters including presence or absence of

scales and trichomes, and floral structure distinguish the major two subgenera within genus *Combretum*. Evolution of these characters seems to have undergone a complex evolutionary path within the diversification of the different sections [23,77].

**Table 2. Global event matrix for S-DIVA, Bayesian Binary MCC and DEC Model (Lagrange) analyses showing total number of events per each analysis**

Event	S-DIVA	Bayesian Binary Method (BBM)	DEC Model (Lagrange)
Dispersal	42	62	58
Vicariance	19	18	15
Extinction	1	1	4





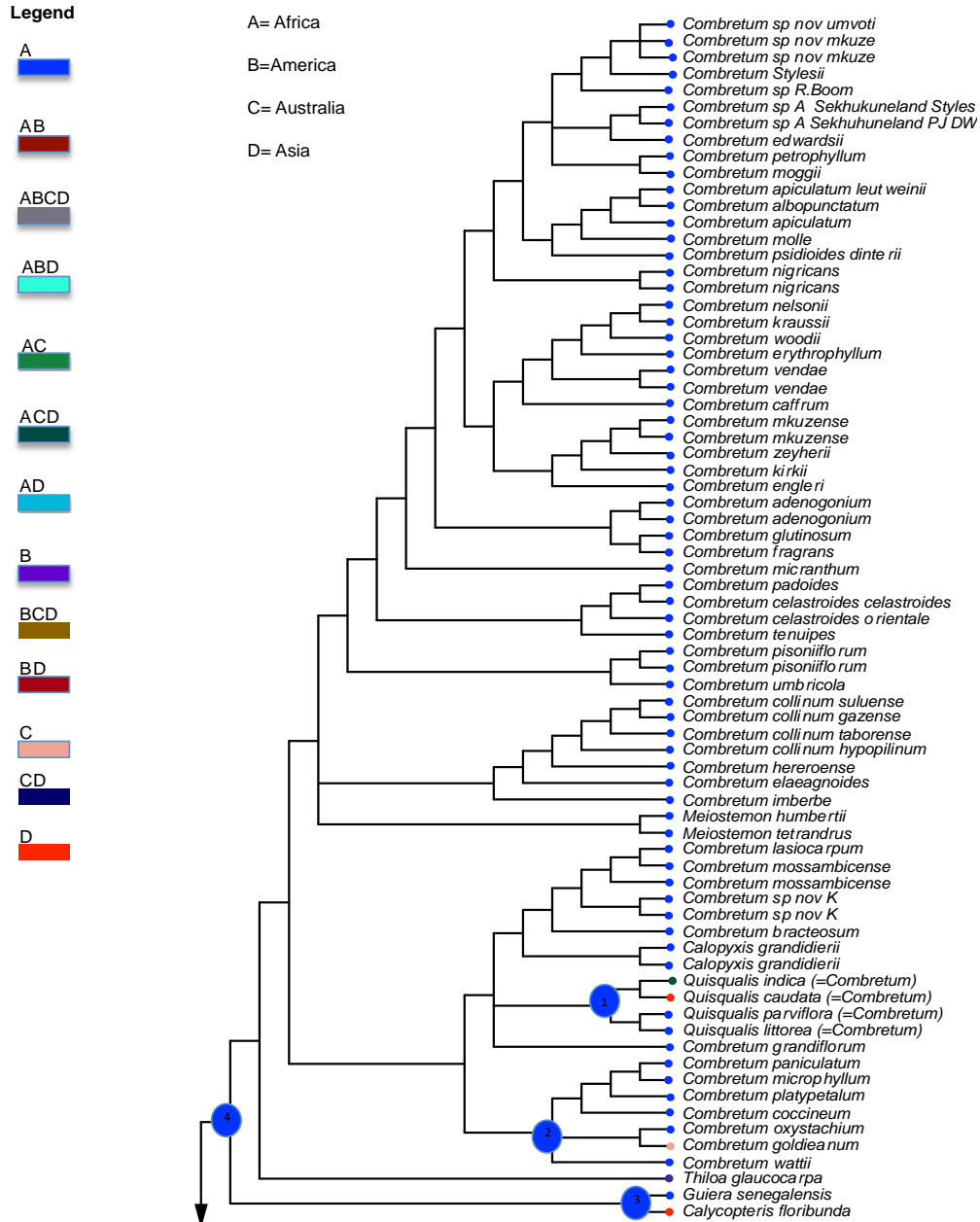
**Fig. 2. Results of the ancestral area reconstruction from Bayesian Binary MCC analysis (BBM), overlaid on the chronogram obtained from BEAST. Nodes numbered 1-17 represent important nodes with more than two key events**

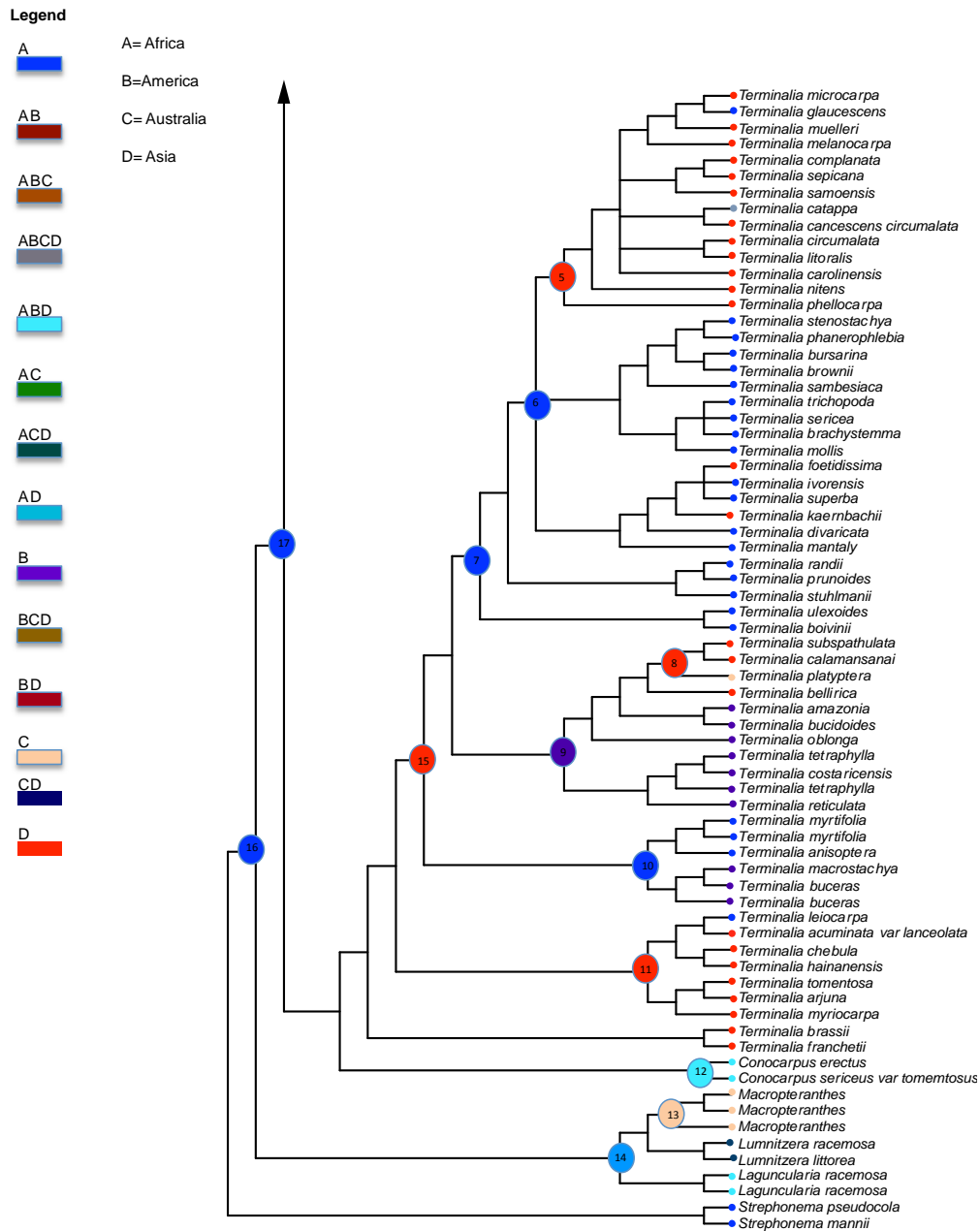
Early diverging lineage within subgenus *Combretum* is *Meiostemon* Exell & Stace that diverged ca. 28.4 mya, and is morphologically distinguished by its flattened upper hypanthium. *Meiostemon* is thought to separate the two main subgenera within genus *Combretum*. Our results suggest that diversification of subgenus

*Combretum* went through two different episodes. Firstly, during Meocene epoch, it underwent a decreased diversification and then followed a subsequent accelerated rate during Pliocene period with sections such as *Angustimarginata* (ca. 8.7 mya) and *Macrostigmaea* (9.8 mya) radiating during this epoch.

In contrast to subgenus *Combretum* as highlighted above, subgenus *Cacoucia* diversified at later age (ca.18.8 mya) splitting into two clades, with major diversification occurring at ca. 15.3 and 14. 2 mya. This event probably occurred during the Meiocene epoch, a period in which subgenus *Combretum* experienced a reduced diversification. An important floral feature observed in subgenus *Cacoucia*,

indicates that the inflorescence is much brighter compared to its sister subgenus *Combretum* [23,77]. It can be hypothesised that very bright inflorescence attract a wide range of insect pollinators, and hence give rise to high speciation within subgenus *Cacoucia*. Furthermore, the cooling climate during Miocene epoch may have also played a critical role in the speciation process of members of *Cacoucia*.





**Fig. 3. Results of the ancestral area reconstruction from Lagrange analysis (DEC model), overlaid on the chronogram obtained from BEAST. Nodes numbered 1-17 represent important nodes with more two key events**

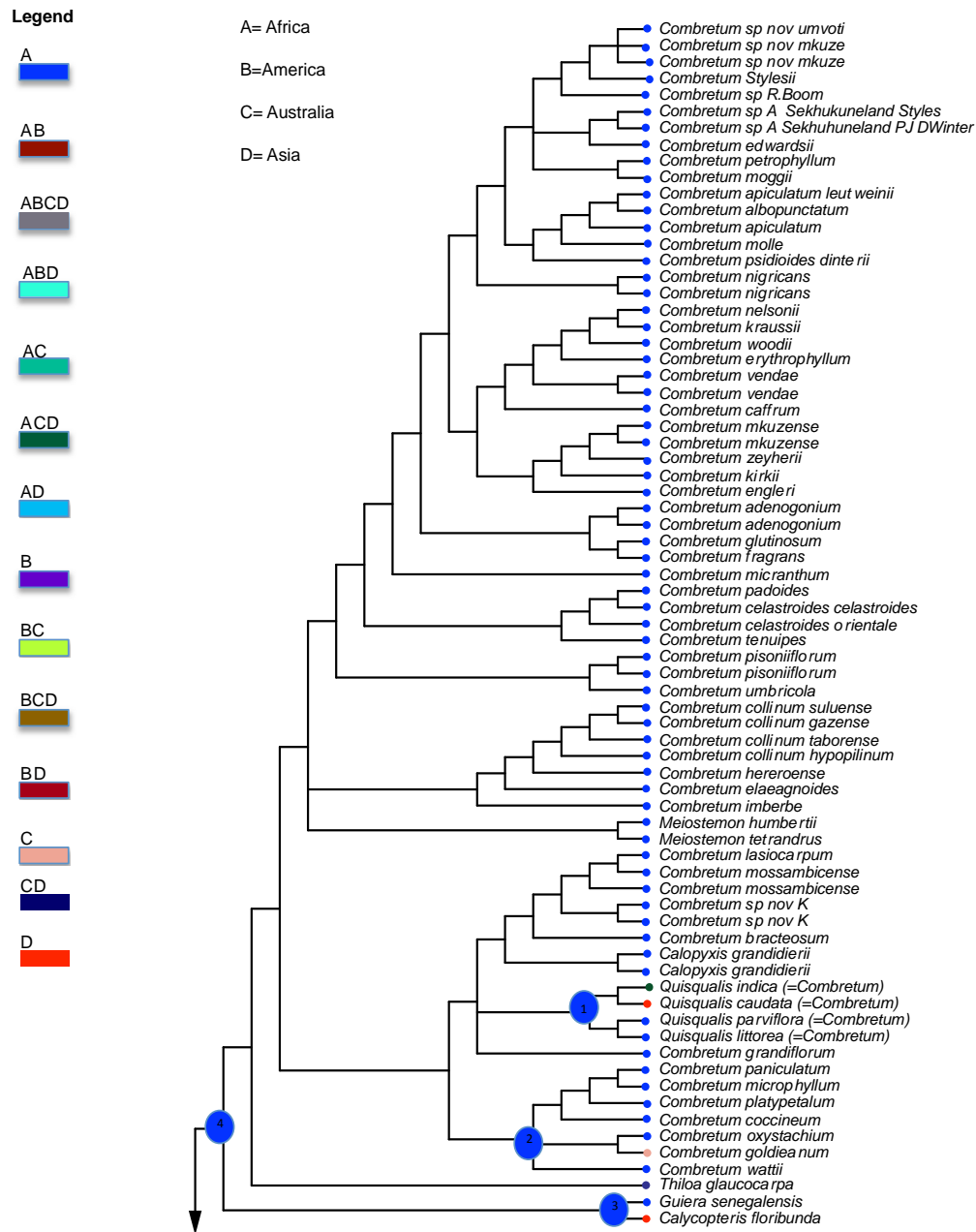
### 4.3 Subtribe Terminaliinae

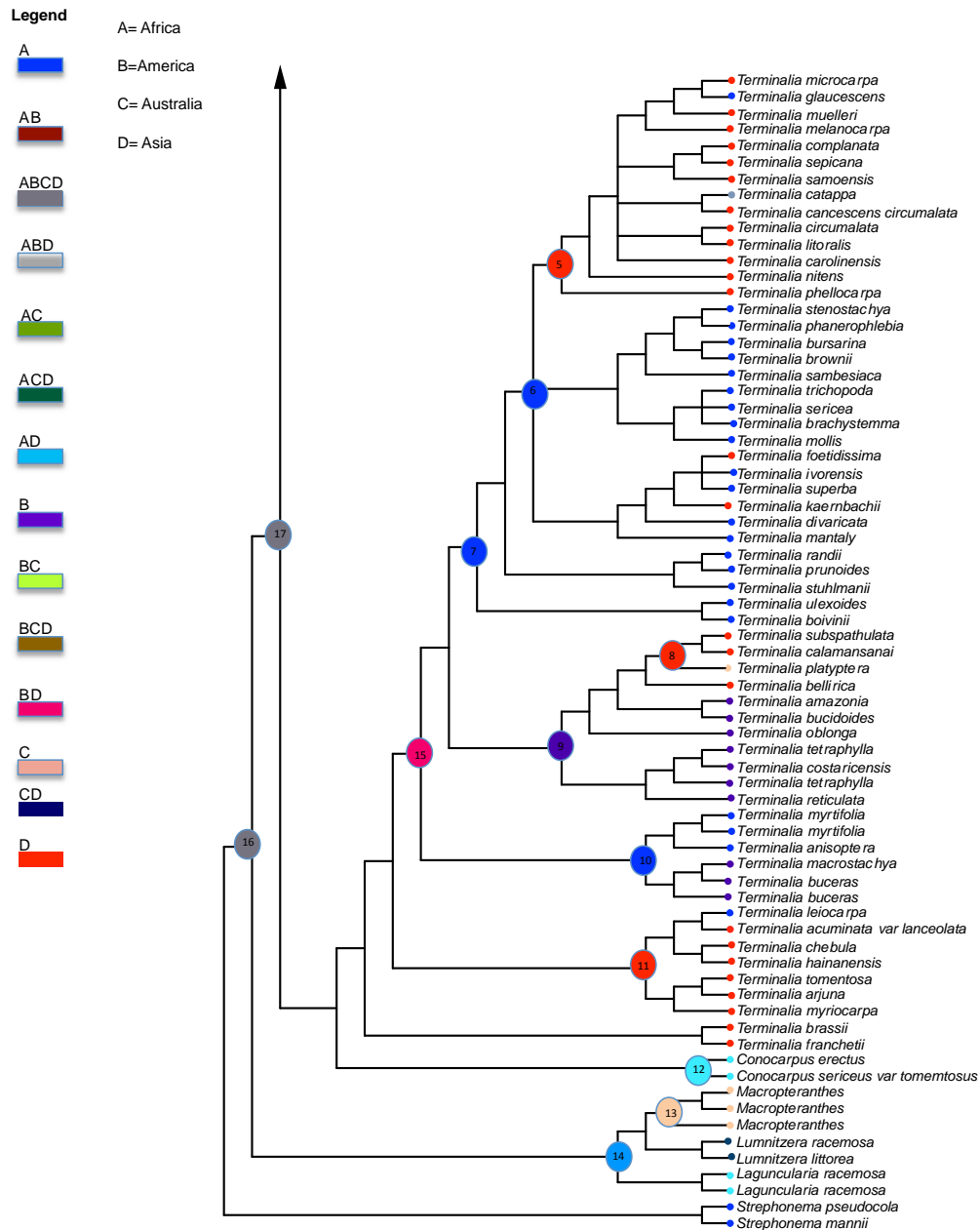
The split between Terminaliinae and Combretineae (ca. 60 mya) occurred during the Eocene epoch, which is regarded as the warmest period of the Tertiary. This age estimate is within range of previous studies [23,24]. It is worthwhile to note that Terminaliinae diversified

at a later age compared to its sister subtribe Combretineae. Within Terminaliinae, the first lineage to diverge is *Conocarpus* (ca. 46.3 mya) whose placement in Terminaliinae has been in doubt due to shared habitat with tribe Laguncularieae. However, molecular results support its current placement within Terminaliinae. Subsequent diversification

followed with Asian *Terminalia* (ca. 39.6 mya), *Anogeissus* (= *Terminalia*) (ca. 34.8 mya) and lastly with another clade of Asian *Terminalia* (ca. 4.5 mya). It can be hypothesised that much of the diversification within Terminaliinae occurred during the acidification of the African continent, a view shared by Berger [24]. Ancestral area reconstructions depict Asia as origin of Terminaliinae and the current disjunct distribution can be probably due to long-distance dispersal, a

phenomenon highlighted to have played a role in many tropical and subtropical disjunctions in angiosperms [6,61]. However, not much can be interpreted in current results as the phylogeny of this group requires further taxonomic attention with increased sampling of Asian and South American species. A much illuminated phylogeny is warranted for a better biogeographical understanding of this group.





**Fig. 4. Results of the ancestral area reconstruction from S-DIVA, overlaid on the chronogram obtained from BEAST. Nodes numbered 1-17 represent important nodes with more two key events**

#### 4.4 Ancestral Area Reconstructions

A number of similar biogeographic studies on families exhibiting similar intercontinental disjunct distribution as with the Combretaceae have suggested a Gondwanan origin for example, subfamily Chrysophylloideae (Sapotaceae), [6]. The most recent study by Berger [24] also

depicts the same origin, and current results reinforce previous findings. A number of biogeographical inferences can be made from current results analyses, notably, origin, event(s) involved and also likely geological times when these events took place as discussed above, enabling an in depth illumination of the biogeography of Combretaceae. A summary of

global events from the three analyses are shown in Table 2. Generally, dispersal event is shown to be most likely event to have shaped the current intercontinental disjunct distribution of Combretaceae corroborating previous similar studies as by of Berger [24].

The basal node 16 in all S-DIVA analysis, suggests all four possible ancestral reconstruction ranges ABCD (Africa, America, Asia and Australia). Nonetheless, results from Lagrange and Bayesian Binary Method (BBM) analyses suggest Africa as the most likely ancestral origin for the family with a 10% marginal probability suggesting dispersal from Africa to other continents. Previous studies [23,24,27,65], have earlier linked the origin of Combretaceae to African continent due to the breakup of the super continent Gondwana [18,78]. Aridification of the savanna region from the Miocene onward and the American–Australian disjunction might possibly explain the vicariant event obtained from the Lagrange result. Similarly, Ali et al. [59] made comparable observations for the subfamily Hyacinthoideae as well as Bartish et al. [6] for pantropical subfamily Chrysophylloideae (Sapotaceae) with disjunct distributions in the tropical and subtropical regions. Fossil discoveries assigned to Combretaceae, which have been reported in all continents, concur with current results, as ancestral reconstructions depict a worldwide distribution [48], though with strong bias towards Africa as the ancestral origin for the family Combretaceae.

Results for the node 4, in all the three analyses representing subtribe Combretaeae, gave limited insight about the biogeographic history of its members due to limited sampling with a strong bias towards the African Continent. Nonetheless, the inclusion of a few members from outside Africa such as species of *Quisqualis*, tend to reduce the marginal probability of Africa as the ancestor origin to a marginal probability of 56%.

Results from all the three analyses for node 1, (*Combretum* subgenus *Cacoucia* section *Quisqualis*), suggest Africa as the ancestral origin with a 100% marginal probability. The occurrence of *Q. caudata* Craib. in Asia alone and of *Q. Indica* L. in Africa, Asia and Australia may indicate a vicariance event as suggested by all the three analyses. Divergence estimates results for this clade (ca.14.2 mya) show that these species occurred much later after the splitting of Gondwana (ca. 105 mya), thereby casting some

doubt on responsibility of this event as the sole modeller for the current disjunct distribution.

Node 17 representing members of the subtribe Terminaliinae, in both Bayesian Binary Method (BBM) and Lagrange analysis suggest Africa as the ancestral range for this node, with marginal 31% and 10% probability, respectively. In contrast, S-DIVA analysis suggests a combination of Africa, America, Australia and Asia as ancestral origins of the members of subtribe Terminaliinae. In all the analyses, dispersal event is detected suggesting trans-oceanic movements and linking to the splitting of the super continent Gondwana. However, previous studies have suggested Asia as the centre for genetic diversity of *Terminalia*, the largest genus within this node, which may probably reflect the ancestral origin of the members of this node [27,31,32,79].

Asia has also been observed to be an ancestral origin of other plant families including *Uvaria* (Annonaceae) Richardson et al., [80], *Bridelia* (Phyllanthaceae) Li et al. [81] and *Macaranga* and *Mallotus* (Euphorbiaceae) Kulju et al. [82]. According to Ali et al. [59] a number of dispersal mechanisms could be responsible for the observed plant disjunctive distributions. Dispersal agents such as birds that are capable of long distance flight and monsoon trade winds coupled with oceanic currents are among the potential drivers of plant dispersal across barriers [83].

Current results reflect the great diversity observed within Terminaliinae, and this links with the rapid diversification that occurred during the Eocene period as discussed above. Diverging age estimates in the current study do not tally with the break-up of the super Gondwanan continent. This result puts into question the ages estimates assigned to fossils and the accuracy of molecular phylogenies in estimating evolutionary rate. However, similar results trend have been observed in studies [24].

## 5. CONCLUSION

The reconstructed dated phylogeny of Combretaceae based on the Bayesian analysis and calibrated with fossils *Dilcherocarpon combretoides* and *Terminalioxylon* revealed that the crown age of Combretaceae is ca.110 mya, a time hypothesised to be marked by high angiosperm diversification. The splitting of Combretaceae into two distinguished

subfamilies, Combretaceae and diverged early within the Combretaceae. Within tribe Combretaceae, subtribe Combretineae diverged earlier than Terminaliinae, and the split occurred in the Late Cretaceous period with divergence estimated at the commencement of Eocene epoch, which characterises an important event of radiations within the subtribes.

The African continent, which is hypothesised to have emerged from the split of the super continent Gondwana, is inferred as the origin of Combretaceae. Long distance dispersal is postulated as the major modeller, with vicariance and extinction playing marginal roles in shaping the current intercontinental disjunct distribution patterns of Combretaceae in the tropical and subtropical regions of the world.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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