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Phylogenetic relationships and biogeographic distribution of *Termitomyces* (Lyophyllaceae, Basidiomycota)

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ABSTRACT. *Termitomyces* (Lyophyllaceae, Basidiomycota) is a group of edible fungi that developed a mutualistic relationship exclusively with all members of Macrotermitinae, especially the *Macrotermes*. The phylogeny of the genus and its symbionts has already been established, but its relation to their biogeographic distribution needs to be better understood. In this paper, partial sequences of the small subunit ribosomal RNA (SSU rRNA) gene from the mitochondrial DNA (mitDNA) of 20 *Termitomyces* spp. were analyzed to infer its phylogeny and biogeographic distribution using maximum parsimony (MP), maximum likelihood (ML), Bayesian inference (BI), and statistical dispersal-vicariance analysis (S-DIVA). The constructed MP and ML trees show that all *Termitomyces* spp. are monophyletic with a bootstrap percentage of 91% and 63%, respectively, with several variations in the branching of other taxa, notably the polyphyly of the three formae of *T. striatus*. A few monophyletic groups in the MP tree were reconstructed as either polyphyletic or paraphyletic in the ML tree. The maximum clade credibility (MCC) tree inferred from BI split *Termitomyces* into two major clades (posterior probability, 1.00), unlike the topologies inferred from MP and ML. The BI also indicates that *Termitomyces* and its sister clade diverged from their most recent common ancestor (MRCA) around 36.17 M years ago (Ma) (posterior probability, 1.00). The divergence time dating of *Termitomyces* and its sister clade, *Lyophyllum*, shows strong support for the previously established divergence time. Results from the S-DIVA imply that all extant species of *Termitomyces* emerged from their MRCA in Africa through rapid adaptive radiation, primarily via dispersal rather than vicariance around 17 Ma. This was most likely driven by the Oligocene-Miocene transition and Cenozoic deformations in Asia, causing the genus to disperse across the Oriental region.

Keywords: dispersal, molecular phylogeny, taxonomy, vicariance

INTRODUCTION

Termitomyces (Lyophyllaceae, Basidiomycota) is a group of edible paleotropical fungi that developed obligate symbiosis exclusively with all members of Macrotermitinae, a subfamily of Termitidae (Mossebo *et al.*, 2017). Although Macrotermitinae can internally decompose organic matter like the rest of Termitidae, the subfamily has adapted to culturing *Termitomyces* selectively on combs they build (Tang *et al.*, 2015). These combs are composed of predigested plant substrates that the fungi consume, providing the termites with asexual spores that they use as food and, consequentially, to maintain the nest (Aanen *et al.*, 2002). The spores of *Termitomyces* are transmitted by their symbionts either horizontally, from sibling to sibling when new colonies are formed, or vertically, when the termites inherit the fungi from the alates (Aanen *et al.*, 2002; Boomsma & Aanen, 2006; de Fine Licht *et al.*, 2006; Nobre & Aanen, 2010).

The genus *Termitomyces* was first characterized by Heim in 1942 when it was originally thought to have no symbiotic relationship with termites. The genus was characterized by Pegler & Vanhaecke (1994) as having an expanded pileus attached to a solid stipe, free lamellae that are densely crowded, pink spore deposit, presence of hymenial cystidia, presence of siderophilous granulation in the basidia, divergent hyphae in the basidiomatal primordia, and its pluteoid habit. The obligate symbiosis of the *Termitomyces* with Macrotermitinae has been attributed to the lignocellulosic enzymes that can degrade lignin, which termites do not usually eat as a nutrient source but used to efficiently utilize cellulose through their symbionts (Hyodo *et al.,* 2003; Gomathi *et al.,* 2019). The fruiting bodies of *Termitomyces*, especially *T. letestui, T. schimperi, T. eurrhizus,* and *T. globulus* are widely consumed as food and seasoning throughout equatorial and southern Africa, as well as in Southeast Asia (Sangvichien & Taylor-Hawksworth, 2001). Because of its economic importance, *Termitomyces* are often cultivated by indigenous groups.

Recent dating studies show that the symbiosis between *Termitomyces* and Macrotermitinae originated in the rainforests of Africa around 31 M years ago (Ma) (Nobre *et al.,* 2011; Wisselink *et al.,* 2020). The host and the symbiont also do not show strict interaction specificity. Fossil evidence of termite agriculture found in southwestern Tanzania has also provided a solid basis to the estimated divergence time of 31 Ma during the early Oligocene (Roberts *et al.*, 2016), during which most of the landscape changes in Africa occurred (Séranne, 2002).

Today, *Termitomyces* are widely distributed in the paleotropical region. Several *Termitomyces* species have been recorded across the Philippine islands (De Leon *et al.,* 2013; Reyes *et al.,* 2016; Tantengco & Ragragio, 2018; Guerrero, 2020), Japan (Mau *et al.,* 2004; Choi *et al.,* 2012; Ono *et al.,* 2018), Indochinese Peninsula (Loganathan *et al.,* 2010; Sawhasan *et al.*, 2011; Khaing *et al.,* 2019; Anwar *et al.,* 2020; Jannual *et al.,* 2020; Seelan *et al.*, 2020), India (Karun & Sridhar, 2013), China (Wei *et al.*, 2009; Ye *et al.*, 2019; Tang *et al.*, 2020), Middle East (Enshasy *et al.*, 2013), and all across the African tropical forests (Rouland-Lefevre *et al.*, 2002; Aanen *et al.*, 2007; Mossebo *et al.*, 2017; Koné *et al.*, 2018).

Current molecular phylogenetic analyses of *Termitomyces* provide an incomplete picture of relationships between species within its genus. Several studies have attempted to establish the molecular phylogenetic relationships of *Termitomyces* and its related taxa (Aanen *et al.,* 2002; Moncalvo *et al.,* 2002; Hoffstetter *et al.,* 2002; Rouland-Lefevre *et al.,* 2002; Froslev *et al.*, 2003; Sawhasan *et al.*, 2011; Oyetayo, 2012; Saré *et al.,* 2014; Mossebo *et al.*, 2017). However, among these studies, only Froslev *et al.* (2003) and Mossebo *et al.* (2017) compared the strains from both Africa and Asia, the main regions where *Termitomyces* are distributed, and only Mossebo *et al.* (2017) included a considerable number of 28 taxa. Moreover, there is no literature as of writing that establishes the relation of the phylogenetic relationships of the genus and its symbionts with other taxa to the biogeographic distribution of *Termitomyces* in paleotropical region. In this paper, the phylogenetic relationships and divergence of *Termitomyces* have been inferred, and the results have been used as the basal information in determining the biogeographic distribution of the genus. This information broadens our understanding of the taxonomy and distribution of *Termitomyces* which could contribute to the worldwide biodiversity conservation and ecological management of economically important fungi species.

MATERIALS AND METHODS

Acquisition of mitochondrial DNA sequences from GenBank

Partial sequences of small subunit ribosomal RNA (SSU-rRNA) gene from the mitochondrial DNA was used to compare *Termitomyces* from its sister clade, *Lyophyllum* (Lyophyllaceae), and to other representative taxa of older Basidiomycota (*Cryptococcus* and *Phanerochaete*), Ascomycota (*Candida* and *Saccharomyces*), and the ancestral *Rozella allomycis*. SSU-rRNA is a reliable fungal marker prominently used in the hierarchical classification of fungal taxa and cryptic fungal species due to its species-level variability in intron insertions and large availability in public databases (Papaioannou *et al.*, 2014; Yarza *et al.*, 2017). It is also the only common gene marker available for all species studied.

Twenty-nine SSU-rRNA gene from mitochondrial DNA, each coming from a single specimen, and a complete mitochondrial genome of the outgroup, *Rozella allomycis*, were taken from GenBank. Twenty species of *Termitomyces* were used in the study, namely, *T. aurantiacus, T. brunneopileatus, T. bulborhizus, T. cartilagineus, T. clypeatus, T. eurrhizus, T. globulus, T. heimii, T. le-testui, T. mboudaeinus, T. medius, T. microcarpus, T. robustus, T. schimperi, T. striatus f. bibasidiatus, T. striatus f. subclypeatus, T. striatus f. subumbonatus, T. striatus, T. subumkowaan,* and *T. titanicus.* Five species of *Lyophyllum* were used to build a sister clade, namely, *L. caerulescens, L. decastes, L. favrei, L. semitale,* and *L. sykosporum*. Two representative taxa of old Basidiomycota (*Cryptococcus neoformans* var. *grubii* and *Phanerochaete chrysosporium*) and Ascomycota (*Candida chilensis* and *Saccharomyces pastorianus*) were used to estimate the divergence times of all taxa involved. The summary list of all taxa studied, accession numbers, and the numbers of base pairs, is shown in **Table 1**.

Phylogenetic analyses

The sequences were set to follow mold mitochondrial genetic code and aligned using the ClustalW alignment tool in MEGA X version 10.2 (Kumar *et al.,* 2018). The aligned sequences were analyzed using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI).

The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm (Nei & Kumar, 2000) with search level 1. Initial trees were obtained by randomly adding sequences with 10 replicates across

Table 1. Strains of *Termitomyces*, *Lyophyllum*, *Rozella*, and other reference taxa from Basidiomycota and Ascomycota used in the phylogenetic study. Except for *Rozella*, the accession numbers of all taxa are taken from partial sequences of mitochondrial small subunit ribosomal RNA (mtSSU-rRNA) genes and mitochondrial 12S ribosomal RNA (mt12S-rRNA) genes (*Lyophyllum*). The full mitochondrial genome of *Rozella* was used as an outgroup.

No.	Taxon name	Voucher number	GenBank accession number	Sequence length (bp)
1	*Termitomyces aurantiacus*	DM152E	KY809186	354
2	*T. brunneopileatus*	DM392	KY809225	354
3	*T. bulborhizus*	KM128338	KY809213	352
4	*T. cartilagineus*	KM109565	KY809211	354
5	*T. clypeatus*	KM128340	KY809214	354
6	*T. eurrhizus*	KM142419	KY809218	354
7	*T. globulus*	DM770	KY809204	354
8	*T. heimii*	KM16528	KY809205	354
9	*T. le-testui*	DM150D	KY809224	354
10	*T. mboudaeinus*	DM223	KY809226	354
11	*T. medius*	KM16685	KY809206	357
12	*T. microcarpus*	DM268E	KY809191	349
13	*T. robustus*	DM436	KY809223	354
14	*T. schimperi*	DM24E	KY809181	352
15	*T. striatus f. bibasidiatus*	DM280B	KY809193	352
16	*T. striatus f. subclypeatus*	DM370B	KY809220	354
17	*T. striatus f. subumbonatus*	DM208	KY809187	354
18	*T. striatus*	KM142436	KY809219	354
19	*T. subumkowaan*	DM260B	KY809227	354
20	*T. titanicus*	KM142416	KY809216	354
21	*Lyophyllum caerulescens*	HC80140	AF357128	531
22	*L. decastes*	Lc42(T5P)	AF357137	547
23	*L. favrei*	HC96cp4	AF357104	537
24	*L. semitale*	HC85/13	AF357125	562
25	*L. sykosporum*	HCM3	AF357127	555
26	*Cryptococcus neoformans var. grubii*	CBS 8710	FJ534894	585
27	*Phanerochaete chrysosporium*	BKM-F-1767	U27058	532
28	*Candida chilensis*	NRRL Y-7790	EU018554	529
29	*Saccharomyces pastorianus*	NRRL Y-1525	AY130314	637
30	*Rozella allomycis*	-	NC_021611	12055

30 nucleotide sequences. The 1st+2nd+3rd+Noncoding codon positions were included in the analysis, resulting in 12,095 positions in the final dataset.

Before analyzing the aligned sequences for ML, the best-fit substitution model was inferred using MEGA X. The analysis obtained an initial tree for the heuristic search using Neighbor-Join and BioNJ algorithms since there were 30 nucleotide sequences. The branch lengths of the tree were adjusted based on the best-fit Tamura 3-parameter (T92+G) model, which included a discrete gamma distribution to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.3479)). Topology variants are created to search for a topology with a superior log likelihood value.

For BI, the substitution model was chosen based on the results of the BEAST Model Test (bModelTest) from BEAST2 (Bayesian Evolutionary Analysis Sampling Trees) version 2.6.3, which utilizes reversible jump Markov chain Monte Carlo (rjMCMC) (Bouckaert & Drummond, 2017). The best substitution model, TN93 (Tamura-Nei, 93), was used to analyze the previously aligned sequences and to create a phylogenetic tree inferred from Calibrated Yule Model.

To identify divergence times of all taxa, several priors were set in Bayesian Evolutionary Analysis Utility (BEAUTi2) version 2.6.3. The phylogenetic tree was inferred using the Calibrated Yule Model as the tree prior, with rates of AG/CT transitions determined from the bModelTest. The clock model was calibrated using Relaxed Clock Exponential (Drummond *et al.*, 2006). The estimated divergence of the representative taxa from Basidiomycota and Ascomycota and the fossil age of *Rozella* were taken from the study of Chang *et al.* (2015). To make the resulting tree more stable, all *Termitomyces* spp. were set as monophyletic, as established by Frøslev *et al.* (2003), with divergence time estimated at 31 Ma (Roberts *et al.*, 2016).

Biogeographic analysis

The maximum clade credibility (MCC) tree produced from BI was used to infer the biogeographical distribution of *Termitomyces*. The MCC tree was pruned to focus on *Termitomyces* spp., excluding the widely distributed outgroups from the analysis. To reconstruct the MCC tree for statistical dispersal-vicariance analysis (S-DIVA), RASP (Reconstruct Ancestral State in Phylogenies) version 4.2 was used (Yu *et al.*, 2015), with the settings for event cost set to default (Hosaka *et al.*, 2008). Considering the divergence time of *Termitomyces* at 31 Ma (Roberts *et al.*, 2016) when the Oligocene-Miocene transition established the paleotropical regions through plate convergence (Horowitz, 2002), the areas of endemism were fixed at four standard regions, namely, Africa (A), South Asia (B), Southeast Asia (C), and East Asia (D) based on the presence of the identified endemic species. These distribution states were used to predict the most parsimonious distribution events in the studied genus. Occurrence data of *Termitomyces* spp. were taken from the Global Biodiversity Information Facility (2020) and other literature (Morris, 1986; Pegler & Vanhaecke, 1994; Frøslev *et al.*, 2003; Karun & Sridhar, 2013; Mossebo *et al.*, 2017; Nacua *et al.*, 2018; Ye *et al.*, 2019). The final tree was then rooted to the MRCA of all *Termitomyces* based on the final MCC tree.

RESULTS AND DISCUSSION

Phylogenetic relationships

The maximum likelihood tree (MLT) inferred the best topology of all taxa based on the probability of the character states observed in the extant species rather than the common ancestors' characters as required in the maximum parsimony tree (MPT), through the T92+G model. The resulting MPT and MLT analyses showed several nodes with very low bootstrap support (<60%). The original bootstrap consensus trees have been truncated and condensed, showing only the nodes with at least 60% bootstrap support, making the condensed MPT and MLT multifurcated. However, the interpretations of the phylogenetic relationships of the internal branches or subclades of *Termitomyces* and, especially, the tips with strong bootstrap support are still presented in the subsequent discussion provided that some major clades of the group need to be better supported in the two analyses.

The condensed MPT in **Figure 1** shows strong support for the monophyly of all extant species of *Termitomyces* studied, with a bootstrap percentage of 91%. Since a few *Lyophyllum* spp. were used to stabilize the topology of *Termitomyces* in both MPT and MLT in the presence of the ancestral fungus *R. allomycis* and other distant representative taxa of Basidiomycota and Ascomycota, it is important to note the high bootstrap support in the major clade formed by *Termitomyces* and its sister group in both MPT (97%) and MLT (100%). These results coincide with the findings of Frøslev *et al.* (2003).

Similar to the condensed MPT, all *Termitomyces* spp. showed monophyly in the condensed MLT (**Figure 2**)

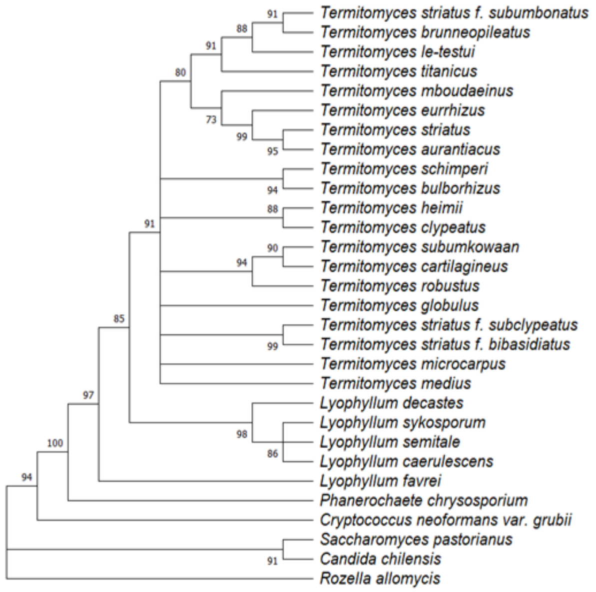

Figure 1. The condensed maximum parsimony tree (MPT) inferred from the Subtree-Pruning-Regrafting (SPR) of 1,000 replicates with search level 1 (Nei & Kumar, 2000) using MEGA X. The bootstrap percentage is shown next to the node. Interior branches with a bootstrap percentage of less than 60% are eliminated, reducing branch lengths to 0. The tree was rooted to *Rozella allomycis*.

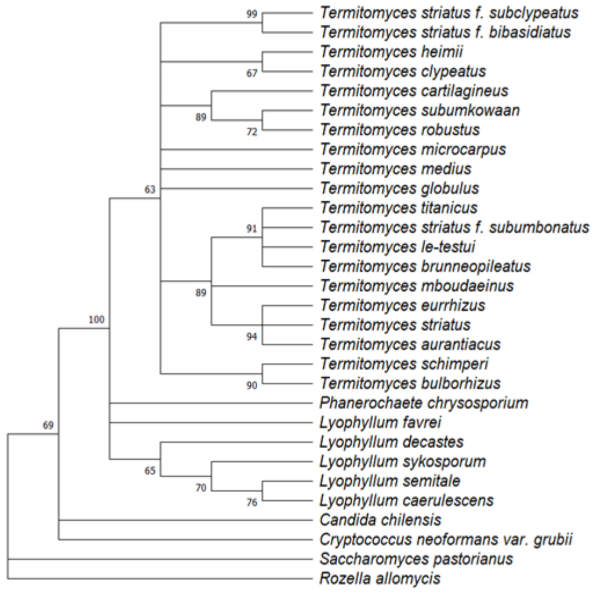

Figure 2. The condensed maximum likelihood tree (MLT) inferred using the Tamura 3-parameter model with a discrete Gamma distribution (+G) of 0.3479 through Neighbor-Join and BioNJ algorithms (Tamura, 1992) from 1,000 replicates. The bootstrap percentage is shown next to the node. Interior branches with a bootstrap percentage of less than 60% are eliminated, reducing branch lengths to 0. The tree was rooted to *Rozella allomycis*.

but with a comparatively lower bootstrap percentage of 63%. However, several differences were also observed in the phylogeny of *Termitomyces*. In the MLT, both *T. schimperi* and *T. bulborhizus* have formed a monophyletic group with a bootstrap percentage of 90% that directly descended from the main node of the group, forming a major subclade, unlike in MPT, where the subclade branched from a pruned internal node. In the MPT, *T. medius* emerged close to the sister clade of all *Termitomyces*, the *Lyophyllum*; unlike in MLT, it diverged from an ancestor distantly related to *Lyophyllum*.

Another notable difference observed in the MLT was the new multifurcated topology of the clade formed by *T. striatus f. subumbonatus* with *T. titanicus*, *T. le-testui*, and *T. brunneopileatus* as opposed to the well-supported bifurcations in the subtree formed by the aforementioned taxa in the MPT. Moreover, among the three other forms of *T. striatus*, only *T. striatus f. subclypeatus* and *T. striatus f. bibasidiatus* showed stability in both MP and ML trees, with a bootstrap percentage of 99%.

A few monophyletic groups in MPT were reconstructed as either polyphyletic or paraphyletic in MLT. For instance, the monophyly of *T. subumkowaan* and *T. cartilagineus* (bootstrap percentage, 90%) in MPT was not supported in MLT; instead, a paraphyly was observed.

One key similarity between MPT and MLT is the polyphyly of *T. striatus f. subumbonatus* from the other forms of *T. striatus* (Hofstetter *et al.*, 2002; Mossebo *et al.*, 2017). Moreover, in both trees, the representative taxa of Basidiomycota (*Phanerochaete* and *Cryptoccoccus*) did not form a clade, which concurred with the studies of Hibbett (2006) and Wang *et al.* (2009) but is contrary to the phylogenomic analysis conducted by Chang *et al.* (2015). This incongruence in results is expected due to the difference in the principles and algorithms of the two methods.

Divergence dating

Several most recent common ancestor (MRCA) priors were set to estimate divergence times to constrain the phylogenetic tree. To set *Rozella* as the outgroup, all the other taxa were grouped as an MRCA prior, relative to the age of *Rozella*, based on the data from Chang *et al.* (2015). The divergence times of Ascomycota, Basidiomycota, and Termitomyces were set as MRCA priors, based on the data from Roberts *et al*. (2016). The AG/CT transition rates were based on the results of the bModelTest. The Calibrated Yule Model was used to infer the phylogenetic tree with the values for MCMC set to default. The tree was annotated using TreeAnnotator version 2.6.3 with a burn-in percentage of 5%, removing 500 trees.

The time tree or maximum clade credibility (MCC) tree in **Figure 3** shows that *Termitomyces* and *Lyophyllum* diverged from their common ancestor 36.17 Ma (credibility interval 6.64 – 65.04) at the end of the Eocene epoch, with a high posterior probability of 1.00. This means the divergence has a high probability based on the priors set and the likelihood model. The 20 taxa of *Termitomyces* diverged from the earliest *Termitomyces* 17.47 Ma (credibility interval 3.22 – 36.65), forming two major clades (A1, A2) with a posterior probability of 1.00. The topology of the MCC tree is different from the ML tree, where *T. schimperi* and *T. bulborhizus* formed a major clade opposite the remaining *Termitomyces* spp. The corresponding node ages, posterior probabilities, and 95% HPD (height posterior density) of the nodes in **Figure 4** are listed in **Table 2**. The large credibility interval in all calibrations can be attributed to the independent estimates for all three groups of reference taxa and the estimate for the *Termitomyces* group.

The two major clades (A1, A2) of the studied *Termitomyces* spp. have subsequently split into two subclades: clade A1 split into subclades B1 and B2 9.62 Ma (credibility interval 1.78 – 14.38; posterior probability 0.54); clade A2 split into subclades B3 and B4 9.90 Ma (credibility interval 1.98 – 14.332; posterior probability 0.53). The average posterior probabilities of the branching of the B1, B2, B3, and B4 subclades may improve if new molecular data are established. The overlapping credibility intervals of the split imply that the major clades split simultaneously. Moreover, the subsequent radiation of the genus occurred in the past 8 Ma, forming 19 of the 20 extant species studied.

Among the extant species, eight species diverged from a node with a posterior probability value of less than 0.50, specifically in nodes 12, 20, 24, 25, and 26. *T. robustus* and *T. subumkowaan* diverged from their MRCA 2.17 Ma with a posterior probability of 0.40 (credibility interval 0.15–3.58). *T. aurantiacus* and *T. eurrhizus* diverged from their MRCA 0.48 Ma with a posterior probability of 0.34 (credibility interval 0.00–1.17). *T. brunneopileatus* and *T. titanicus* diverged from their MRCA 0.54 Ma with a posterior probability of 0.23 (credibility interval 0.00–1.30). In contrast, *T. le-testui* and *T. striatus f. subumbonatus* diverged from their MRCA 0.52 Ma with a posterior probability of 0.22 (credibility interval 0.00–1.17). This suggests a need for more molecular data to establish the divergence of species in these nodes at acceptable posterior probabilities. However, the remaining 22 nodes showed high posterior probabilities (**Table 2**), implying that the MCMC algorithm sampling reached an acceptable topology.

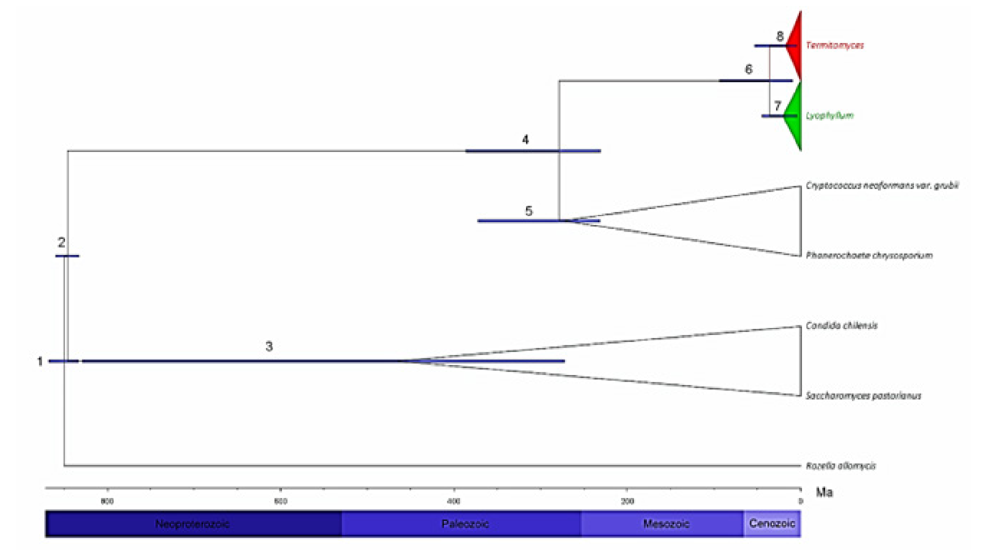

Figure 3. The maximum clade credibility (MCC) tree inferred from the Calibrated Yule Model using the TN93 substitution model (rateAG:1.706; rateCT:1.701). The Bayesian inference of phylogeny was analyzed using BEAST2. The node number is shown next to the node, corresponding to the node numbers listed in **Table 2**, while the node bars represent the tree height at 95% HPD (height posterior density) interval for each branch. The estimated divergence time of the representative taxa from Basidiomycota (*Cryptococcus* and *Phanerochaete*), Ascomycota (*Candida* and *Saccharomyces*), and *Rozella* were taken from Chang *et al.* (2015).

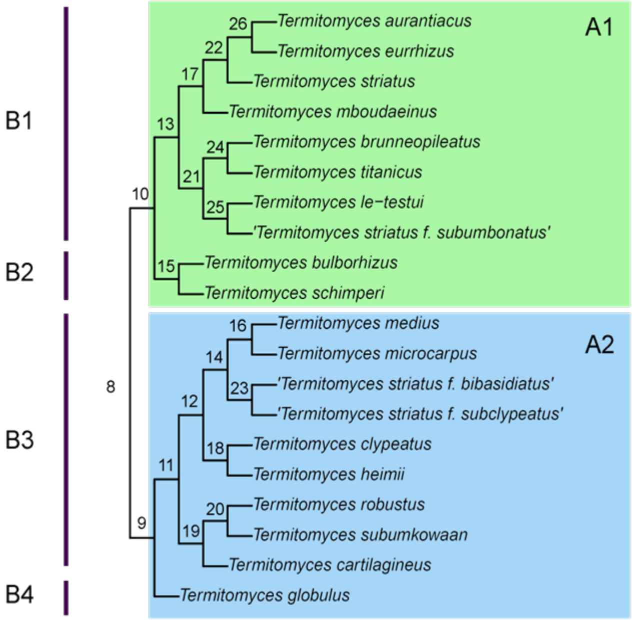

Figure 4. Cladogram from the maximum clade credibility (MCC) tree pruned to only show *Termitomyces* using RStudio. Branch lengths are set equal to 1. Numbers at each node represent the node numbers in **Table 2**. The two major clades (A1, A2) have subsequently split into two subclades: clade A1 split into subclades B1 and B2 9.6 Ma (credibility interval 1.78 – 14.38); clade A2 split into subclades B3 and B4 9.9 Ma (credibility interval 1.98 – 14.32).

The bootstrap consensus MLT produced using the Tamura 3-parameter substitution model showed similarities with the chronogram developed from the Bayesian inference (BI) of the mitochondrial gene sequences using MCMC. The resulting divergence time of *Termitomyces* is congruent with the current estimates from another fungal phylogeny study (Roberts *et al.*, 2016) and several phylogenetic studies of fungus-termite symbiosis (Rouland-Lefevre *et al.*, 2002; Nobre *et al.*, 2011; Zhao *et al.*, 2017).

Table 2. Node ages, posterior probabilities, and 95% HPD (height posterior density) generated from the Bayesian inference of the maximum clade credibility (MCC) tree using BEAST2. The node numbers correspond to those presented in **Figures 3** and **4**. High posterior probability values mean a branch is more likely to be correct based on the priors and the likelihood model. The 95% HPD intervals show the divergence time estimates with the highest density.

Node no.	Node age (Ma)	Posterior probability	95% HPD
1	850.00	1.00	580.77–604.85
2	845.71	1.00	580.51–599.25
3	466.61	1.00	189.79–578.06
4	278.63	1.00	161.04–269.15
5	273.04	1.00	161.60–259.32
6	36.17	1.00	6.64–65.04
7	20.62	1.00	3.03–30.90
8	17.47	1.00	3.22–36.65
9	9.90	0.53	1.98–14.32
10	9.62	0.54	1.78–14.38
11	7.88	0.92	1.55–11.40
12	6.42	0.43	1.17–8.95
13	5.50	1.00	0.69–8.39
14	5.04	0.78	0.82–7.14
15	4.65	1.00	0.48–7.32
16	3.73	0.67	0.50–5.53
17	3.32	0.63	0.22–5.22
18	3.33	0.56	0.08–5.51
19	3.29	0.97	0.25–5.17
20	2.17	0.40	0.15–3.58
21	1.92	1.00	0.04–3.56
22	1.23	1.00	0.01–2.46
23	0.73	1.00	0.00–1.71
24	0.54	0.23	0.00–1.30
25	0.52	0.22	0.00–1.17
26	0.48	0.34	0.00–1.17

Dispersion-vicariance analysis

The reconstructed S-DIVA tree (**Figure 5**) shows that all extant species of *Termitomyces* studied originated from Africa (A). Among all states or areas specified, the most dispersion events originated from Africa (A), producing 16 speciation events in the area alone, while the least occurred in Southeast Asia (C), with only two speciation events. Moreover, the highest number of dispersal events occurred in Node 21 (*T. aurantiacus* and *T. eurrhizus*), which radiated eastward from Africa (A), spanning the entire paleotropical region. *T. striatus*, *T. robustus*, *T. clypeatus*, and *T. microcarpus* also radiated in all four areas (ABCD) eastward from Africa (A), while *T. mboudaeinus*, *T. brunneopileatus*, *T. titanicus*, *T. striatus f. subumbonatus*, *T. subumkowaan*, *T. striatus f. bibasidiatus*, and *T. striatus f. subclypeatus* emerged from local speciation events in Africa (A). When all species are considered, the most dispersal events occurred 3.3 Ma (**Figure 6**).

Among all nodes presented in **Figure 5**, Node 36 had the least probability of 0.025, showing three dispersal events, two of which involved local speciation and an in-situ diversification in Africa that gave rise to *T. striatus f. bibasidiatus* and *T. striatus f. subclypeatus*. This low probability value for the node from the S-DIVA did not coincide with the node's high posterior probability value of 0.92 from the MCC tree. This could

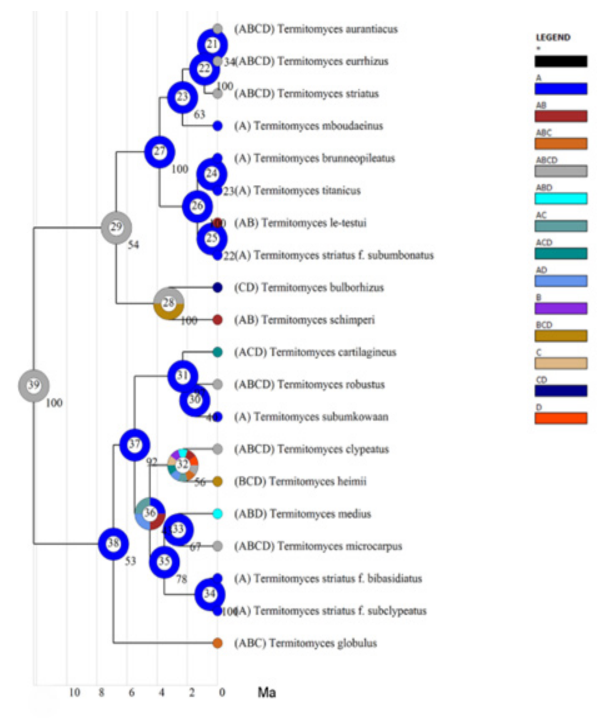

Figure 5. Phylogenetic tree inferred from the MCC tree's statistical dispersal-vicariance analysis (S-DIVA). The donut chart at each node represents the marginal probabilities for each area of endemism. The numbers outside each node indicate the node frequency. The areas of endemism are indicated before each species (A = Africa, B = South Asia, C = Southeast Asia, and D = East Asia). The legend shows the color key to possible ancestral ranges at different nodes. The corresponding events and probabilities for each node are listed in **Table 3**.

be mediated if more occurrence data were available for all extant species that diverged from Node 36.

Biogeographical analysis of the MCC tree revealed that the distribution of *Termitomyces* is mainly a result of dispersal events and not vicariance. That is, ancestral species have migrated into new areas across preexisting geographic barriers. Compared to the global dispersal events of 31, only two vicariance events occurred specifically in Nodes 28 and 29. In Node 28, a dispersal event occurred from South Asia (B), Southeast Asia (C), and East Asia (D), which eventually led to a vicariance event that resulted in the speciation of *T. bulborhizus* (CD) and *T. schimperi* (AB) from their common ancestor around 3.24 Ma. The vicariance event in Node 28 can be traced back to Node 29, which had a vicariance event that isolated Africa (A) from the rest of the paleotropical region (BCD) around 6.71 Ma. The vicariance events and, consequentially, the dispersal events around this time could be attributed to the Cenozoic deformations that occurred in Asia, as detailed by Yin (2010). However, more occurrence data and additional specific areas of endemism may provide a better insight into the underlying causes of the only two vicariance events in **Figure 5**.

Nevertheless, the 31 dispersal events traced back to Node 39 can be associated with the Oligocene-Miocene transition (around 23 Ma), when the African continent established a direct connection to Asia after the Levantine land bridge was established (Horowitz, 2002). Most members of Macrotermitinae are condensed in West and Central Africa, but its most recent genera (*Macrotermes*, *Odontotermes*, *Microtermes*) are widely distributed in the paleotropical region (Eggleton, 2000; Inward *et al.*, 2007; Roberts *et al.*, 2016). This allowed *Termitomyces* to be dispersed across the Oriental region, as is inferred from the S-DIVA tree (**Figure 5**), which shows strong support for dispersal events.

Table 3. Dispersal, vicariance, extinction events, and probability for each node represented in **Figure 5**.

Node no.	Event			Probability
	Dispersal	Vicariance	Extinction	
21	6	0	0	1.000
22	3	0	0	1.000
23	0	0	0	1.000
24	0	0	0	1.000
25	1	0	0	1.000
26	0	0	0	1.000
27	0	0	0	1.000
28	1	1	0	0.500
29	0	1	0	0.500
30	3	0	0	1.000
31	2	0	0	1.000
32	3	0	0	0.100
33	5	0	0	1.000
34	0	0	0	1.000
35	0	0	0	1.000
36	3	0	0	0.025
37	1	0	0	0.250
38	2	0	0	1.000
39	1	0	0	1.000

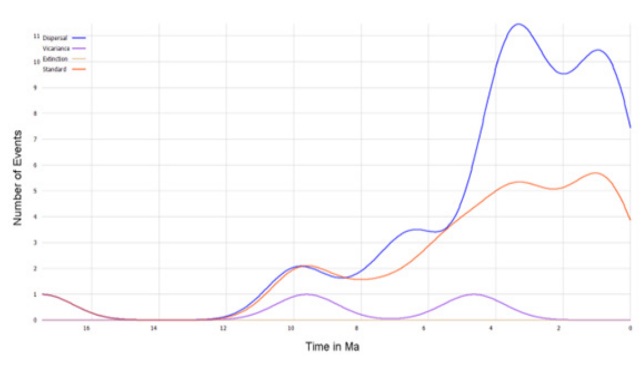

Figure 6. Graph detailing the changes in the rate of dispersal (blue), vicariance (violet), extinction (yellow), and standard events (orange) against time (Ma). Dispersal is the most dominant event, which peaked at 3.3 Ma.

CONCLUSION

Building phylogenetic trees of *Termitomyces* without fossil data has remained elusive. Even using several representative taxa of outgroups, stable clades and branching patterns are challenging to establish. Several monophyletic groups in MPT remained monophyletic in MLT; however, most of the taxa in both trees did not conform. This is expected since the two methods rely on different principles and algorithms. Nevertheless, the strict monophyly of the genus shown in MPT and MLT has resulted in a Bayesian inference that is congruent with the current divergence data of their symbionts – the members of *Macrotermes*. This congruence is most likely due to the obligate symbiosis of the two groups. Since the MCC tree relies on the appropriate evolutionary clock model, nucleotide substitution model, and fossil data priors, it can provide better inferences on the phylogeny and divergence of the studied genus than MP and ML. Compared to MPT and MLT, the major clades of *Termitomyces* showed higher probabilities in MCC that coincided with the probabilities from the result of the dispersal-vicariance analysis.

Moreover, the increasing availability of occurrence data, molecular data, and previously established phylogenetic relationships of *Termitomyces*, and their symbionts with other taxa proved useful in understanding their biogeography. Further analysis of the phylogeny of the symbionts of *Termitomyces* may provide a basis for establishing congruent maximum parsimony and maximum likelihood trees.

Future studies may consider doing a larger multi-locus phylogenetic and biogeographic analysis with more nucleotide sequences for *Termitomyces* and its symbionts. The availability of more molecular data could improve the topologies inferred from maximum parsimony and maximum likelihood, preventing multifurcations in the trees, as presented in this study. Using Bayesian binary MCMC (BBM) analysis with the appropriate state frequency model and Gamma distribution for a larger dataset, together with S-DIVA, can also provide a better picture of the biogeography and the symbiotic interactions of *Termitomyces* and *Macrotermes* that are not included in this study.

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LITERATURE CITED

Aanen, D. K., Eggleton, P., Rouland-Lefèvre, C., Guldberg-Frøslev, T., Rosendahl, S., & Boomsma, J. J. (2002). The evolution of fungus-growing termites and their mutualistic fungal symbionts. *PNAS*, *99*(23), 14887–14892.

Aanen, D., Ros, V., de Fine Licht, H., Mitchell, J., de Beer, Z., Slippers, B., & Boomsma, J. (2007). Patterns of interaction specificity of fungus-growing termites and *Termitomyces* symbionts in South Africa. *BMC Evolutionary Biology*, *7*(115). DOI:https://doi.org/10.1186/1471-2148-7-115

Anwar, K., Sudirman, L., & Nandika, D. (2020). Comb establishment of fungus-growing termites species Macrotermitinae (Isoptera: Termitidae) with *Termitomyces cylindricus* (Basidiomycota: Agaricales) basidiospores. *Oriental Insects*, *54*(4), 591–606.

Bouckaert, R. R. & Drummond, A. J. (2017). bModelTest: Bayesian phylogenetic site model averaging and model comparison. *BMC Evolutionary Biology*, *17*(1), 42. DOI:https://doi.org/10.1186/s12862-017-0890-6

Chang, Y., Wang, S., Sekimoto, S., Aerts, A. L., Choi, C., Clum, A., & Berbee, M. L. (2015). Phylogenomic analyses indicate that early fungi evolved digesting cell walls of algal ancestors of land plants. *Genome Biology and Evolution*, *7*(6), 1590–1601.

Choi, J. H., Maeda, K., Hirai, H., Harada, E., Kawade, M., Qi, J., & Kawagishi, H. (2012). Novel Cerebroside, *Termitomycesphin I*, from the Mushroom, *Termitomyces titanicus*. *Bioscience, Biotechnology, and Biochemistry*, *76*(7), 1407–1409.

de Fine Licht, H. H., Boomsma, J. J., & Aanen, D. K. (2006). Presumptive horizontal symbiont transmission in the fungus-growing termite *Macrotermes natalensis*. *Molecular Ecology*, *15*(11), 3131–3138.

De Leon, A., Luangsa-ard, J., Karunarathna, S., Hyde, K., Reyes, R., & dela Cruz, T. (2013). Species listing, distribution, and molecular identification of macrofungi in six Aeta tribal communities in Central Luzon, Philippines. *Mycosphere*, *4*(3), 478–494.

Drummond, A. J., Ho, S. Y., Phillips, M. J., & Rambaut, A. (2006). Relaxed phylogenetics and dating with confidence. *PLoS Biology*, *4*(5): e88. DOI:https://doi.org/10.1371/journal.pbio.0040088

Eggleton, P. (2000). Global Patterns of Termite Diversity. *In*: Abe, T., Bignell, D., & Higashi, M. *Termites: Evolution, Sociality, Symbioses, Ecology*, pp. 25–51. Dordrecht: Springer. DOI:https://doi.org/10.1007/978-94-017-3223-9_2

Enshasy, H. E., Elsayed, E. A., Aziz, R., & Wadaan, M. A. (2013). Mushrooms and truffles: Historical biofactories for complementary medicine in Africa and in the Middle East. *Evidence-Based Complementary and Alternative Medicine*, 2013(620451). DOI:https://doi.org/10.1155/2013/620451

Frøslev, T. G., Aanen, D. K., Læssøe, T., & Rosendahl, S. (2003). Phylogenetic relationships of *Termitomyces* and related taxa. *Mycological Research*, *107*(11), 1277–1286.

GBIF [The Global Biodiversity Information Facility]. (2020). *What is GBIF?* Retrieved on 13 January 2020 from: The Global Biodiversity Information Facility: <https://www.gbif.org/what-is-gbif>.

Gomathi, V., Esakkiammal, M., Thilagavathi, S., & Ramalakshmi, A. (2019). Lignocellulosic enzyme production by *Termitomyces* spp. from termite garden. *Universal Journal of Agricultural Research*, *7*(2), 100–111.

Guerrero, J. J. (2020). Insights and prospects toward the undergraduate mycological researches of Bicol University. *Philippine Journal of Science*, *149*(2), 405–413.

Hibbett, D. S. (2006). A phylogenetic overview of the Agaricomycotina. *Mycologia*, *98*(6), 917–925.

Hofstetter, V., Clémençon, H., Vilgalys, R., & Moncalvo, J.-M. (2002). Phylogenetic analyses of the Lyophylleae (Agaricales, Basidiomycota) based on nuclear and mitochondrial rDNA sequences. *Mycological Research*, *106*(9), 1043–1059.

Horowitz, A. (2002). Elephants, horses, humans, and others: Paleoenvironments of the Levantine land bridge. *Israel Journal of Earth Sciences*, *51*(3): 203–209.

Hosaka, K., Castellano, M. A., & Spatafora, J. W. (2008). Biogeography of *Hysterangiales* (Phallomycetidae, Basidiomycota). *Mycological Research*, *112*(4), 448–462.

Hyodo, F., Tayasu, I., Inoue, T., Azuma, J.-I., Kudo, T., & Abe, T. (2003). Differential role of symbiotic fungi in lignin degradation and food provision for fungus-growing termites (Macrotermitinae: Isoptera). *Functional Ecology*, *17*(2), 186–193.

Inward, D., Vogler, A., & Eggleton, P. (2007). A comprehensive phylogenetic analysis of termites (Isoptera) illuminates key aspects of their evolutionary biology. *Molecular Phylogenetics and Evolution*, *44*(3), 953–967.

Jannual, N., Nipitwattanaphon, M., Hasin, S., & Kaewgrajang, T. (2020). Morphological and molecular characterization of *Termitomyces* (Lyophyllaceae, Agaricales) in Thailand. *Biodiversitas Journal of Biological Diversity*, *21*(6). DOI:https://doi.org/10.13057/biodiv/d210620

Karun, N. & Sridhar, K. (2013). Occurrence and distribution of *Termitomyces* (Basidiomycota, Agaricales) in the Western Ghats and on the west coast of India. *Czech Mycology*, *65*(2), 233–254.

Khaing, A. A., Aung, S. S., & Htwe, O. (2019). Taxonomic study on ten wild mushrooms from Loikaw Township in Kayah State. *Journal of the Myanmar Academy of Arts and Science*, *17*, 1–13.

Koné, N. A., Soro, B., Vanié-Léabo, L. P., Konaté, S., Bakayoko, A., & Koné, D. (2018). Diversity, phenology and distribution of *Termitomyces* species in Côte d'Ivoire. *Mycology*, *9*(4), 307–315.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549.

Loganathan, J. K., Gunasundari, D., Hemalatha, M., Shenbhagaraman, R., & Kaviyarasan, V. (2010). Antioxidant and phytochemical potential of wild edible mushroom *Termitomyces reticulatus*: individual cap and stipe collected from south eastern part of India. *International Journal of Pharmaceutical Sciences and Research*, *1*(7), 62–72.

Mau, J.-L., Chang, C.-N., Huang, S.-J., & Chen, C.-C. (2004). Antioxidant properties of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia. *Food Chemistry*, *87*(7), 111–118.

Moncalvo, J. M., Vilgalys, R., Redhead, S. A., Johnson, J. E., James, T. Y., Aime, M. C., & Miller Jr, O. K. (2002). One hundred and seventeen clades of euagarics. *Molecular Phylogenetics and Evolution*, *23*(3), 357-400.

Morris, B. (1986). Notes on the genus *Termitomyces* Heim in Malawi. *The Society of Malawi Journal*, *39*(1), 40–49.

Mossebo, D., Essouman, E., Machouart, M., & Gueidan, C. (2017). Phylogenetic relationships, taxonomic revision and new taxa of *Termitomyces* (Lyophyllaceae, Basidiomycota) inferred from combined nLSU- and mtSSU-rDNA sequences. *Phytotaxa*, *321*(1), 71–102.

Nacua, A., Pacis, H., Manalo, J., Soriano, C., Tosoc, N., Padirogao, R., & Deocaris, C. (2018). Macrofungal diversity in Mt. Makiling Forest Reserve, Laguna, Philippines: with floristic update on roadside samples in Makiling Botanic Gardens (MBG). *Biodiversitas*, *19*(4), 1579–1585.

Nei, M. & Kumar, S. (2000). *Molecular Evolution and Phylogenetics*. New York: Oxford University Press.

Nobre, T. & Aanen, D. K. (2010). Dispersion and colonisation by fungus-growing termites. *Communicative & Integrative Biology*, *3*(3), 248–250.

Nobre, T., Koné, N.A., Konaté, S., Linsenmair, K. E., & Aanen, D. K. (2011). Dating the fungus-growing termites' mutualism shows a mixture between ancient codiversification and recent symbiont dispersal across divergent hosts. *Molecular Ecology*, *20*(12), 2619–2627.

Ono, K., Yoshimura, T., & Kinjo, K. (2018). Screening of strains and media of *Termitomyces eurrhizus* collected from Japan toward nutritional analyses. *In*: McLellan, B. (ed.), Sustainable Future for Human Security, pp. 53–59. Springer, Singapore. DOI:https://doi.org/10.1007/978-981-10-5430-3_5

Oyetayo, V. O. (2012). Wild *Termitomyces* species collected from Ondo and Ekiti States are more related to African species as revealed by ITS Region of rDNA. *The Scientific World Journal*, 2012: 1–5.

Papaioannou, I. A., Dimopoulou, C. D., & Typas, M. A. (2014). "Cryptic" group-I introns in the nuclear SSU-rRNA gene of Verticillium dahliae. *Current Genetics*, *60*(3), 135–148.

Pegler, D. & Vanhaecke, M. (1994). *Termitomyces* of Southeast Asia. *Kew Bulletin*, *49*(4), 717–736.

Reyes, R., Undan, J., Dulay, R., Kalaw, S., & JR, U. (2016). The first report on the molecular identification of *Termitomyces* of Central Luzon, Philippines. *International Journal of Pharmaceutical Research & Allied Sciences*, *5*(1), 140–145.

Roberts, E. M., Todd, C. N., Aanen, D. K., Nobre, T., Hilbert-Wolf, H. L., O'Connor, P. M., & Stevens, N. J. (2016). Oligocene termite nests with in situ fungus gardens from the Rukwa Rift Basin, Tanzania support a Paleogene African origin for insect agriculture. *PLoS ONE*, *11*(6), 1–17.

Rouland-Lefevre, C., Diouf, M. N., Brauman, A., & Neyra, M. (2002). Phylogenetic relationships in *Termitomyces* (Family Agaricaceae) based on the nucleotide sequence of ITS: A first approach to elucidate the evolutionary history of the symbiosis between fungus-growing termites and their fungi. *Molecular Phylogenetics and Evolution*, *22*(3), 423–429.

Sangvichien, E., & Taylor-Hawksworth, P. (2001). *Termitomyces* mushrooms: A tropical delicacy. *Mycologist*, *15*(1), 31–33.

Saré, I. C., Ndir, M. S., Manga, A., Ndiaye, M., Barroso, G. & Diop, T. A. (2014). Molecular characterization of wild *Senegalese* voucher belonging to the *Agaricus*, *Ganoderma* and *Termitomyces* genera. Evidences of new species phylogenetically distant from described ones. *Journal of Biological & Scientific Opinion 2*(2), 124–131. [ISSN 2321-6328]

Sawhasan, P., Worapong, J., & Vinijsanun, T. (2011). Morphological and molecular studies of selected *Termitomyces* species collected from 8 districts of Kanchanaburi Province, Thailand. *Thai Journal of Agricultural Science*, *44*(3), 183–196.

Seelan, J. S., Yee, C. S., Fui, F. S., Dawood, M., Tan, Y. S., Kim, M.-J., & Lim, Y. W. (2020). New species of *Termitomyces* (Lyophyllaceae, Basidiomycota) from Sabah (Northern Borneo), Malaysia. *Mycobiology*, *48*(2), 95–103.

Séranne, M. (2002). Early Oligocene stratigraphic turnover on the west Africa continental margin: a signature of the tertiary greenhouse-to-icehouse transition? *Terra Nova*, *11*(4), 135–140.

Tamura, K. (1992). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. Molecular Biology and *Evolution, 9*, 678–687.

Tang, S., He, M., Raspé, O., Luo, X., Zhang, X., YJ Li, K. S., & Hyde, K. (2020). Two new species of *Termitomyces* (Agaricales, Lyophyllaceae) from China and Thailand. *Phytotaxa, 439*(3), 231–242.

Tang, X., Mi, F., Zhang, Y., He, X., Cao, Y., Wang, P., & Xu, J. (2015). Diversity, population genetics, and evolution of macrofungi associated with animals. *Mycology, 6*(2), 94–109.

Tantengco, O. & Ragragio, E. (2018). Ethnomycological survey of macrofungi utilized by Ayta communities in Bataan, Philippines. *Current Research in Environmental & Applied Mycology, 7*(1), 104–108.

Wei, T., Tang, B., & Yao, Y. (2009). Revision of *Termitomyces* in China. *Mycotaxon, 108*(1), 257–285.

Wisselink, M., Aanen, D., & van 't Padje, A. (2020). The longevity of colonies of fungus-growing termites and the stability of the symbiosis. *Insects, 11*(8),1–15.

Yarza, P., Yilmaz, P., Panzer, K., Glöckner, F. O., & Reich, M. (2017). A phylogenetic framework for the kingdom Fungi based on 18S rRNA gene sequences. *Marine Genomics, 36*, 33–39.

Ye, L., Karunarathna, S., Li, H., Xu, J., Hyde, K., & Mortimer, P. (2019). A survey of *Termitomyces* (Lyophyllaceae, Agaricales), including a new species, from a subtropical forest in Xishuangbanna, China. *Mycobiology, 47*(4), 391–400.

Yin, A. (2010). Cenozoic tectonic evolution of Asia: A preliminary synthesis. *Tectonophysics, 488*(2010), 293–325.

Yu, Y., Harris, A., Blair, C., & He, X. (2015). RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. *Molecular Phylogenetics and Evolution, 87*, 46–49.

Zhao, R.-L., Li, G.-J., Sánchez-Ramírez, S., Stata, M., Yang, Z.-L., Wu, G., & Hyde, K. D. (2017). A six-gene phylogenetic overview of Basidiomycota and allied phyla with estimated divergence times of higher taxa and a phyloproteomics perspective. *Fungal Diversity, 84*, 43–74.